This is to certify that the

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Low temperature priming and pregermination
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Celery seeds and onion seeds

presented by

Sheldon Chris Furutani

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Bernard H. Zandstra
Major professor

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LOW TEMPERATURE PRIMING AND PREGERMINATION OF CELERY AND ONION SEEDS

By

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ABSTRACT

LOW TEMPERATURE PRIMING AND PREGERMINATION
OF CELERY AND ONION SEEDS

By

Sheldon Chris Furutani

Two systems were developed to pregerminate celery and onion seeds: (1) celery seeds were germinated at 10°C in H_2O; (2) onion seeds were primed for 8 days in -1.1 MPa mannitol solution at 10°C and germinated for 2 days in H_2O at 10°C. Both systems produced pregerminated seeds with short enough radicles to prevent radicle injury when sowing with a fluid drill.

Section I

Low Temperature Pregermination of Celery Seeds for Fluid Drilling

Celery seeds (Apium graveolens L. var. dulce (Miller) Pers. cvs. Florimart 19 and Florida 683) pregerminated at 10°C for 14 days produced 0 to 2 mm radicles. Pregermination at 24°C for 9 days resulted in 0 to 7 mm radicles for 'Florimart 19' and 0 to 10 mm for 'Florida 683.' Germination at the lower temperature resulted in shorter, more uniform radicles, but time to 50% germination increased from 6 to 13 days. Total germination was not reduced for either cultivar at either temperature.

At 10°C, light and seed leachate removal during priming increased germination 30% over seeds germinated in dark with leachates.

Seeds primed for 2 days in -1.1 MPa NaCl or $C_6H_{14}O_6$ respired over twice as fast as raw seeds. Seeds primed for 6 days did not enhance respiration over 2-day-primed seeds. There were no differences in respiration of seeds primed in NaCl or $C_6H_{14}O_6$ solutions.

Quantitative analysis of carbohydrates, alpha-amino acids, lipids, and reducing sugars were similar between primed and raw seeds during 8 days of priming and 10 days of germination.

To my wife Claire

ACKNOWLEDGMENTS

To my friend and advisor, Bernie Zandstra goes my "aloha" for his support throughout every step of my graduate study. His guidance has given me a deep appreciation for horticulture. I'll always remember those trips to Sodus and coffee stops at Battle Creek that we shared.

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INTRODUCTION

Onion and celery are economically important crops that germinate slowly and emerge from the soil nonuniformly. At harvest, these crops are at various stages of maturity, which results in non-uniform onion bulbs and celery stalks. In these crops, bulb and stalk size determines the value of the crop.

The conventional method to sow most vegetables is direct seeding. In this method, seed germination in the field can be a slow process especially under cold soil conditions. As a result, plant emergence is nonuniform and delayed, producing nonuniform crop maturity.

Pregerminating the seeds before planting in the field increases the speed and uniformity of seedling emergence. Crops from pregerminated seeds also mature earlier and more uniformly than raw seeds.

Fluid drilling has been successfully used to sow pregerminated seeds in crops such as tomatoes and peppers. This system of sowing suspends pregerminated seeds in a fluid gel carrier and extrudes them into the soil. The advantages of such a system are: (1) faster and more uniform emergence; (2) and more uniform crop size and maturity. Fluid drilling, however, is limited to sowing seeds with short, uniform radicles.

Since onion and celery seeds germinate nonuniformly, they produce long and short nonuniform radicles. The pregerminated seeds with long radicles are injured during the fluid drilling operation.

The objectives of this study were: (1) to develop systems to pregerminate onion and celery seeds with short radicles; (2) to determine the effects of priming onion seeds; (3) and to investigate physiological and biochemical changes which occur during and after priming of onion seeds.

CHAPTER I

REVIEW OF LITERATURE

Factors Affecting Germination

The germination of seeds is, in most cases, dependent on external environmental factors such as water, temperature, and light. The level of each factor required for germination varies with seed and species.

Water

Availability of water is probably the most important factor in seed germination. Normally, when a nondormant seed comes in contact with water, the seed imbibes water and germinates. However, some seeds are unable to imbibe water due to impermeable seed coats.

Several methods are used to overcome resistance to water uptake through impermeable seed coats: mechanical abrasion, chemical scarification, and impaction (removal of the strophiolar cleft) (70).

The amount of water taken up by seeds is dependent upon internal food reserve composition. Of the food reserves found in seeds, protein has the greatest capacity for water uptake. Starches and fats have a low capacity for water uptake (70). Therefore, seeds that swell upon imbibition contain large quantities of protein. Seeds that do not swell during imbibition are low in protein composition (70).

After water has entered the seed, membranes and other cellular components begin to hydrate and biochemical processes leading to germination are initiated. Within the seed, the embryo elongates and the radicle eventually penetrates the seed coat; this penetration is commonly referred to as seed germination (7).

Temperature

Seeds of different species have different temperature requirements for germination. Some seeds, such as celery and lettuce, become dormant above a critical temperature. This condition is called thermodormancy (110). Seeds such as watermelon and okra germinate well above 35°C, but do not germinate at temperatures below 15°C (47, 90). Other seeds, such as onions, germinate well at both high and low temperatures (116), but the speed of germination is much slower at lower temperatures.

Wagenvoot et al. (116) described the optimum germination temperature of seeds as a function of heat units. They contended that optimal heat units improve the germination of nonuniform germinating seeds such as onions, carrots, and celery.

Light

Seeds of some species germinate better in light than in dark. Pressman et al. (87) reported that celery seeds incubated in the dark did not germinate at 25°C, whereas those in the light (white light) germinated. The celery seeds in light germinated as light elevated the critical temperature of thermodormancy (110).

The response of celery seeds to light is mediated by phytochrome (110). Lettuce seeds have a similar phytochrome-mediated response (45, 48, 115). Thus, uniform germination of celery and lettuce seeds is dependent upon optimum light and temperature levels.

The light requirement of celery seeds can be replaced, to a degree, by growth regulators and inorganic salts. Combinations of gibberellins and cytokinins have been reported to raise the temperature at which celery seeds become thermodormant (8, 11, 12, 110). Solvents, inorganic salts, and growth regulators have also been shown to overcome the light requirement of seeds of several species (55, 70, 113).

Improved Speed and Uniformity of Germination

Seed germination is often a slow and erratic process, resulting in nonuniform crop stands. Poor or slow germination is caused by a number of factors including suboptimal environmental conditions, seed dormancy, and nonuniform maturity of seed embryos. Several techniques can be used to improve germination such as soaking, hardening, priming, and pregermination.

Soaking

Soaking is the process in which seeds are placed in water and allowed to imbibe for a period of time before planting. Tincker (112), Chippendale (16), Taylor (108), and Haight and Grabe (39) reported that soaking seeds in water prior to planting resulted in accelerated germination of several crop species. Chippendale (16),

working with cocksfoot (Dactaylis glomerata L.), speculated that soaking seed increased water uptake through the palea, thereby accelerating germination. Later, Haight and Grabe (39) measured the rate of water uptake of soaked and nonsoaked cocksfoot seeds and found no differences. However, soaked seeds consumed more oxygen during the first 12 hr of imbibition than did nonsoaked seeds.

Extended periods of soaking in water may injure seeds of some species. Kidd and West (58) observed that soaking periods of 12 hr or less decreased the time to emergence of pea, dwarf bean, barley, and sunflower seeds without injury (58). However, soaking the seeds for 24 hr inhibited root growth. Increasing the soaking period to 48 hr seriously inhibited root growth and injured the cotyledons. Similar observations have been reported with peas by Larson and Lwang (62) and Perry and Harrison (83).

Several methods to reduce injury during extended periods of soaking in water have been reported. Eyster (28) reduced soaking injury in beans and peas by allowing the seeds to imbibe slowly on wet filter paper before soaking in water. Perry and Harrison (83), Powell and Matthews (85, 86), and Woodstock and Tao (119) also reduced soaking injury by partially submerging pea seeds in an osmotic solution prepared with polyethylene glycol 4000. The polyethylene glycol solution created an osmotic tension surrounding the seed, lowering the osmotic gradient between the soaking solution and the interior of the seed. Reducing the osmotic gradient decreased the rate of water uptake and prevented seed injury.

Orphanos and Heydecker (80) hypothesized that oxygen deficiency in the interior of the seed cavity between the cotyledons is the major cause of soaking injury. During soaking, the seed cavity becomes flooded and excess water is trapped within the cavity. The flooded seed cavity swells, creating high internal pressure, thus lowering gas diffusion into the seed. The seed is injured by anaerobiosis. In contrast, Barton (4, 5) demonstrated that supplying oxygen during imbibition of pea seeds is more lethal than supplying air.

Hardening

Hardening refers to the process of imbibing a seed for a given period of time and then redrying it back to a given level of storage moisture. The redried seed is said to be "hardened." Seeds may be soaked and redried ("hardening cycles") several times before planting.

May et al. (69) speculated that hardening preconditions seeds to germinate and develop more rapidly under dry conditions. Hafeez and Hudson (38), however, reported faster germination and development of hardened radish seeds under optimal moisture and fertility conditions, but no increased growth under dry conditions. Austin et al. (3) and Currah and Salter (19) found that 3 cycles of hardening were optimal for carrot seed germination in the field. The effect of hardening on carrot seed was further improved by limiting the volume of water taken up during each cycle to 70% of seed weight. Longden (65) reported similar results with sugar beet seeds using 3 hardening

cycles. Hardening seeds of forage grasses (1), wheat (66), and pepper (56), has also increased the speed of germination over non-hardened seeds.

Hardening allows morphological development of the embryo to proceed without radicle penetration through the seed coat. Austin et al. (3) demonstrated that the embryo in carrot seeds actually increased in size with each hardening cycle. They observed that after 6 hardening cycles, the size of the embryo doubled and the number of embryonic cells increased 6 times over nonhardened seeds.

Hardening does not always increase the speed of germination of seeds. Hegarty (42) reported slower germination of corn seeds when they were soaked for 16 hr and redried. Berrie and Drennan (6) observed radicle and plumule injury in oats and tomatoes after 3 hardening cycles. Taylor (108) found a serious decline in germination of celery seeds after redrying. More recently, work by Biddington et al. (12) has shown that the rate of redrying imbibed celery seeds can seriously affect percent germination. When imbibed celery seeds are dried too quickly, cell membranes appear to become disrupted. Sen and Osborne (101), and McKersie and Tomes (72) reported damage to newly synthesized ribosomal RNA in embryos of imbibed wheat, wild oats and birdsfoot trefoil upon dehydration of imbibed seed.

Priming

Priming is the process of soaking seeds in an osmotic solution instead of water in order to decrease the rate of imbibition.

Organic and inorganic salt solutions have been used for priming seeds. Kotowski (59) observed a stimulation of germination of pepper seeds after priming for 48 hr in 0.2% and 1.5% salt solutions. Solutions of $NaNO_3$, $MgCl_2$, or $MnSO_4$ gave the best results in increasing the total germination of pepper seeds. Doyle et al. (23) obtained increased germination of freshly harvested wheat, oats, barley, and flax by priming the seeds in a dilute KNO_3 solution prior to planting. However, priming of 6-month stored seed did not improve germination.

Some early work by Hashimoto (41) with photoblastic tobacco seeds showed an enhancement of germination with NH_4^+ and NO_3^- solutions. The addition of gibberellin to the NO_3^- solution was synergistic in promoting tobacco seed germination. Gibberellin replaced the tobacco seeds' requirement for light and the NO_3^- complex promoted germination. Later, Roberts (88) elucidated the mechanism of the nitrate-light replacement effect on weed seeds. Currently, the International Seed Testing Association (54) recommends using KNO_3 to "break" dormancy of seeds of many plant species. Sachs (90) revealed that replacing the light requirement of some seeds is not the only beneficial response of priming in salt solutions. He demonstrated a reduction of the heat requirement for germination of watermelon seeds with KNO_3 + K_3PO_4 or KCl osmotic solutions.

Levitt and Hamm (63) found increased speed of germination when seeds of Taraxacum kok-sahgyz were primed in an osmotic solution. They further reported that the speed of germination could be altered

by regulating the concentration of inorganic salt in the osmotic solution. Ells (25) reported similar results with tomato seeds in osmotic solutions formulated with NaCl or KNO_3 + K_3PO_4.

Ells (25) and Levitt and Hamm (63) hypothesized that regulating the moisture level within the seed increased the speed of germination. The seeds' moisture level can be kept low with a strong osmotic solution. The low moisture level permits biochemical germination processes to proceed, but prevents radicle protrusion and complete germination of the seed. This hypothesis is supported by Woodstock's work, in which primed tomato seeds consumed more O_2 than nonprimed seeds (118). Oyer and Koehler (81) demonstrated with tomato seeds that the advancement of germination produced by priming does not occur in the absence of oxygen. More recently, Coolbear and Grierson (17) and Coolbear et al. (18) measured large accumulations of nucleic acids, especially ribosomal RNA in osmotically primed tomato seeds, further supporting Ells and Levitt and Hamm's early hypotheses.

Excessive salt concentrations in priming solutions can be detrimental to subsequent seedling growth. Sugar beet seeds primed in 0.4 to 1.0 molar NaCl solutions exhibited severely stunted radicle growth and seedling development (24). High concentrations of ions such as sodium may be toxic to seeds being primed (47).

The speed and uniformity of germination has been increased by priming seeds with lower concentrations of NaCl. Ells (25) and Malnassy (67) did not observe any injury or decrease in germination

with tomato seeds primed in 1.5 to 2.0% NaCl or KNO_3 + K_3PO_4 solutions. The tomato seeds primed in either salt solution had greater speed and uniformity of germination than nontreated and hardened tomato seeds.

Polyethylene glycol (PEG) has been used extensively for priming seeds at low temperatures. Several reports (43, 45, 49, 50, 51, 52, 120) indicate that there is a more rapid and uniform germination and emergence of seedlings when vegetable and ornamental seeds are primed in a PEG solution. Similarly, Akalehiywot and Bewley (13) found that oats and wheat germinated more rapidly and uniformly when primed at 10°C than at 24°C. Priming at low temperatures extended the period to radicle protrusion, permitting biochemical advancement to proceed beyond priming at higher temperatures. Salter and Darby (95) prevented emergence of celery seed radicles for 21 days by soaking in a -10 bar PEG solution at 15°C. They observed that seeds primed at 15°C reached 50% germination in 1.4 days, compared with 13.7 days for nonprimed seeds.

Some primed seeds lose most of the benefits of priming when they are redried to their initial moisture content. Heydecker (43) and Gibbins (49), and Heydecker et al. (50, 51) observed that redrying onion seeds primed with a -10 bar PEG solution for 23 days at 10°C resulted in a 50% reduction in the benefits of priming. The primed and redried onion seeds germinated more slowly than primed and nonredried seeds. Heydecker and Gibbins (49) explained that the time difference between the germination of redried and nonredried primed

onion seeds is far greater than the time needed for rehydration. They suggested that degradation of ribosomal RNA and other newly synthesized protein products occurs during redrying, resulting in delayed germination for redried-primed seeds.

The PEG solution, as an osmoticum for priming seeds, must not allow the seed to germinate completely, yet provide sufficient water to initiate germination processes. PEG 6000 is a chemically inert molecule which does not enter or react with seed tissue. Unlike PEG, smaller nonelectrolites such as glycerol, sucrose, and mannitol enter and react with seed tissues (68). Other desirable properties of PEG include long-term osmotic stability (111), and a quadratic relationship between concentration and osmotic potential (74). However, the availability of oxygen in PEG solutions is limited. Heydecker et al. (44) found a decline in total germination of seeds when soaked with increasing concentrations of PEG. Mexal et al. (73) reported a 50% reduction in oxygen diffusivity in a -13 bar PEG solution. Attempts to increase oxygen levels in PEG solutions by aeration have had little success due to their high viscosity (84, 97).

Pregermination

Pregermination or "chitting" is similar to soaking, but the process is continued until the radicle emerges. The seeds are then planted using a "fluid drilling" system. Pregermination of seeds produced faster and more uniform germination than soaking, hardening, or priming (20, 36, 92, 93, 94). Biddington et al. (10) demonstrated that fluid-drilled pregermination celery seeds emerged 25 days

earlier with greater percent emergence than fluid-drilled nonpregerminated celery seeds. In similar work, Currah (21, 22), Currah et al. (20), Entwistle (26), Steckel and Gray (105) and Lipe and Skinner (64) increased the speed and uniformity of emergence of onion seeds with pregermination.

Research on fluid drilling of pregerminated seeds with long radicles has shown varying results (36). Radicles longer than 5 mm may be mechanically injured during the fluid drilling operation (95). Damage to the radicles reduces the speed, uniformity, and total emergence of the crop. Maintenance of radicle lengths of 1 to 2 mm has reduced injury of fluid-drilled pregerminated lettuce seeds (34, 35). More uniform emergence has resulted from fluid drilling tomato seeds having 1 to 2 mm radicles than with longer or shorter radicles (14). In some crops, such as carrot, onion, celery, and leek, morphological development of the seeds is irregular. Nonuniform embryo maturity results from irregular morphological development. Therefore, these seeds germinate sporadically with large variability in radicle lengths (47). Salter and Darby (95) have shown that priming celery seeds in a -10 bar PEG or -10 bar KNO_3 + K_3PO_4 solution at 15°C for 21 days reduced the variability in radicle length of celery. The improvement in uniformity of radicle length was attributed to the greatly increased uniformity of germination attained by priming the celery seed.

CHAPTER II

PREGERMINATION AND INCUBATION OF CELERY SEEDS

Introduction

Germination of celery seeds in the field is slow and sporadic resulting in delayed and nonuniform stand establishment, especially under cold soil conditions (10). Therefore, most celery crops are started in greenhouses and transplanted outdoors. Greenhouses provide more favorable germinating conditions for celery seeds than direct seeding in the field. However, even under greenhouse conditions, celery seed germination can be nonuniform. Attempts to improve the germination of celery seeds with growth regulators have been only partially successful in overcoming the phytochrome-mediated dormancy in celery seeds (9,11,109).

An alternative method to improve the uniformity and speed of emergence of celery seeds is to pregerminate them prior to planting. The pregerminated seeds are then sown by suspending the seeds in a fluid gel and extruding them into the soil with a fluid drill. Currah et al. (20) and Biddington et al. (10) established earlier and more uniform celery stands with fluid drilling using partially pregerminated (mixture of imbibed and pregerminated) seeds than with nonpregerminated (raw) seeds.

Radicles of pregerminated seeds must be short and of uniform length in order for fluid drilling to provide the greatest improvement

in rapid and uniform emergence. However, uniform radicle length is difficult to attain due to the nonsynchronous nature of celery seed germination (36, 47). Nonsynchronous germination results from irregular embryo maturity due to time differences in initiation of umbels, pollination of florets, and embryo development (47). Furthermore, attempts to fluid drill pregerminated celery seeds with radicles longer than 5 mm have resulted in radicle injury (95). Celery seed germination is more uniform when seeds are primed with polyethylene glycol or inorganic salts (95, 96).

The objectives of this study were: (1) to determine the effect of low temperature germination on celery seeds; (2) to compare the emergence of pregerminated and raw celery seeds under optimal (20°C) and high (32°C) temperatures; and (3) to compare the uniformity of plants gorwn from pregerminated and raw celery seeds.

Materials and Methods

Pregerminated

The Effect of Seed Leachate, Light, and Temperature on Germination

Celery (Apium graveolens L. var. dulce (Miller) Pers. cv. Florimart 19)[1] seeds were germinated in glass columns containing 2 g seed and 400 ml distilled water. The water in the columns was aerated continuously, with an air stone placed in the bottom of each column.

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[1]Celery seeds were obtained from the Keystone Seed Co., Inc., Hollister, California. 'Florida 683' Lot no. 19-031-005 dated November 1978. 'Florimart 19' Lot no. 19-027-005 dated November 1980.

To determine the effects of seed leachate, light, and temperature, the seeds were germinated, as described above, under light or dark with 3 intervals of leachate removal at 10 or 24°C. The leachates were removed at either 24 or 48 hr intervals or not at all, to remove potential germination inhibitors (96, 108). Leachates were removed by decanting-off the water in the columns and replacing it with distilled water.

For the light requirement, columns were illuminated with 2 G.E. 40 watt cool, white fluorescent lights, 1.2 m in length, placed 2 m transverse to the sides of the columns. For the dark treatment, columns were wrapped with a single layer of aluminum foil to prevent light penetration. Germination counts were taken daily on an aliquot taken from each column. A green safe light was used for viewing germination in the dark treatment. Each treatment was replicated 3 times (3 columns) in a completely randomized design.

The Effect of Temperature on Pregermination

Seeds of 2 celery cultivars, 'Florida 683' and 'Florimart 19' were germinated in aerated columns as described above, with 24 hr leachate removal, at 10 and 24°C. The seeds were illuminated during the entire experiment.

Radicle lengths were measured at approximately 80% germination by sampling 100 seeds of each cultivar at 10 or 24°C. Six by nine inch photographic prints from slides (1:1 enlargement) of the germinated seeds were used to measure the radicle lengths. Germination counts were recorded daily using a dissecting microscope at 7 X.

The treatment combinations were 2 cultivars x 2 germinating temperatures. Each treatment was replicated 4 times (4 columns).

Incubation

The Effect of Temperature on the Emergence of Raw and Pregerminated Seeds

'Florida 683' celery seeds were pregerminated at 10°C in light for 14 days, as described above. After reaching 80% germination, they were sown 2 mm deep in flats containing moistened No. 2 grade vermiculite. Raw celery seed was planted as a control. The flats were placed in temperature-controlled growth chambers in the dark at 20 or 32°C. The flats were watered by subirrigation. Seedling emergence was recorded daily. Each treatment was replicated 4 times using 30 seeds per replicate. The treatments were arranged in a completely randomized design.

Transplants

A Comparison of Transplants Grown from Raw and Pregerminated Seeds

Seeds of 'Florida 683' were pregerminated at 10°C, as described above. Pregerminated and raw seeds were sown 2 mm deep in flats (Speedling trays No. 100 A) containing peat and vermiculite (1:1). Each treatment was replicated 8 times using 20 seeds per replicate. The celery seeds were grown in a greenhouse with 22°C day and 16°C night temperatures. Seedling emergence was recorded daily.

Plant measurements were taken 28 days after sowing, to determine the growth differences between plants grown from pregerminated and raw seeds. Number of leaves, plant heights, and shoot dry weights were recorded. Coefficient of variability (CV) within treatments was used to compare plant uniformity (106). The coefficient of variability was calculated using the following formula:

$$CV = \frac{(EMS)^{\frac{1}{2}}}{\bar{x}}$$

CV = coefficient of variability

EMS = error mean square

$\bar{x}$ = treatment mean

Days to 50% emergence and percent emergence were also calculated for the treatments (79). The formula used to calculate days to 50% emergence is as follows:

$$D = \Sigma\, fx/\Sigma f$$

D = days to 50% emergence

f = number of seeds germinated on day x

x = days after sowing

Spread of germination ($T_{90} - T_{10}$) was calculated by probit analysis (109). Duncan's multiple range test was used in mean separations.

Results

Pregermination

Effect of Light, Seed Leachate, and Temperature on Germination

Over 80% of the celery seeds germinated in light at 24°C when seed leachates were removed. Without leachates removed, about 25% of the seeds germinated in light (Figure 1). Seeds incubated for 8 days at 24°C in the dark did not germinate, regardless of whether or not the leachate was removed. At 10°C, more than 90% of the seeds germinated in light with leachates removed, while only 72% germinated without leachates removed (Figure 2). More than 60% of the seeds germinated in the dark, regardless of the presence of leachates.

Effect of Temperature on Pregermination

Temperature affected the time to total germination (Figure 3). At 24°C, seeds of 'Florida 683' attained total germination in 7 days, while 'Florimart 19' reached total germination in 8 days. At 10°C both cultivars required 15 days for total germination. Total germination at 10°C took 6 to 9 days longer than at 24°C. Temperature did not affect total percent germination of seeds of either cultivar (Figure 4).

Temperature did not affect days to 50% germination (Figure 4). The time required to reach 50% germination at 10°C was approximately twice that required at 24°C. There was no difference in cultivar response to germination temperature.

Figure 1. Germination of 'Florimart 19' after 8 days at 24°C. Seed leachate was removed every 24 hr, 48 hr, or not removed (NR) for 8 days. Vertical lines on bars represent standard deviations.

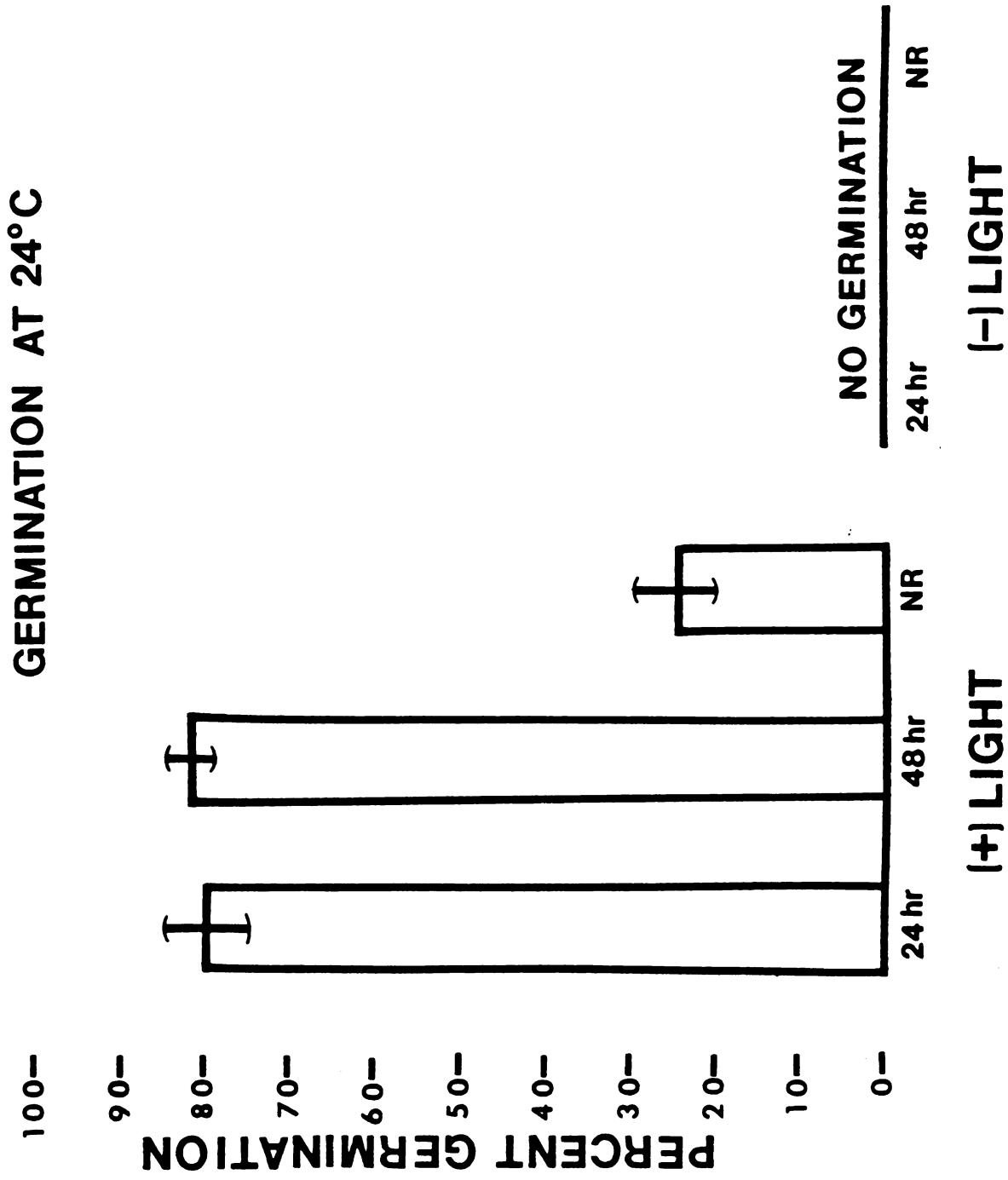

GERMINATION AT 24°C
PERCENT GERMINATION
100
90
80
70
60
50
40
30
20
10
0
24 hr
48 hr
NR
(+) LIGHT
NO GERMINATION
24 hr
48 hr
NR
(-) LIGHT

Figure 2. Germination of 'Florimart 19' after 20 days at 10°C. Seed leachate was removed every 24 hr, 48 hr, or not removed (NR) for 8 days. Vertical lines represent standard deviations.

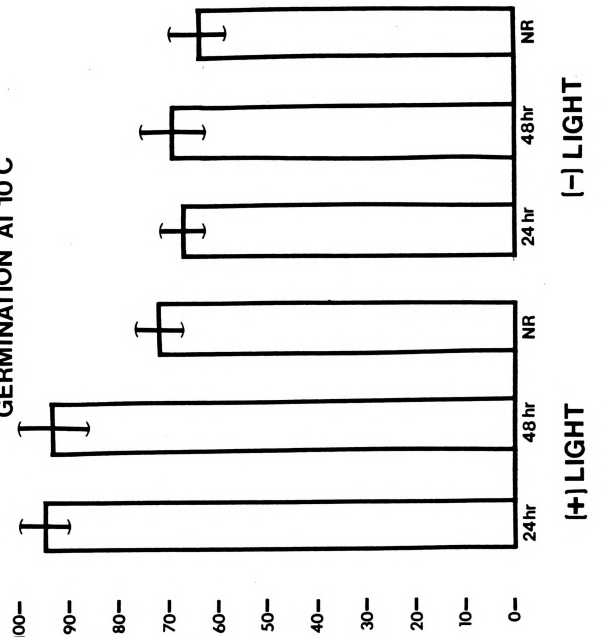

GERMINATION AT 10°C
PERCENT GERMINATION
100
90
80
70
60
50
40
30
20
10
0
24hr
48hr
NR
24hr
48hr
NR
[+] LIGHT
[-] LIGHT

Figure 3. Germination of 'Florimart 19' and 'Florida 683' at 10 and 24°C in aerated columns.

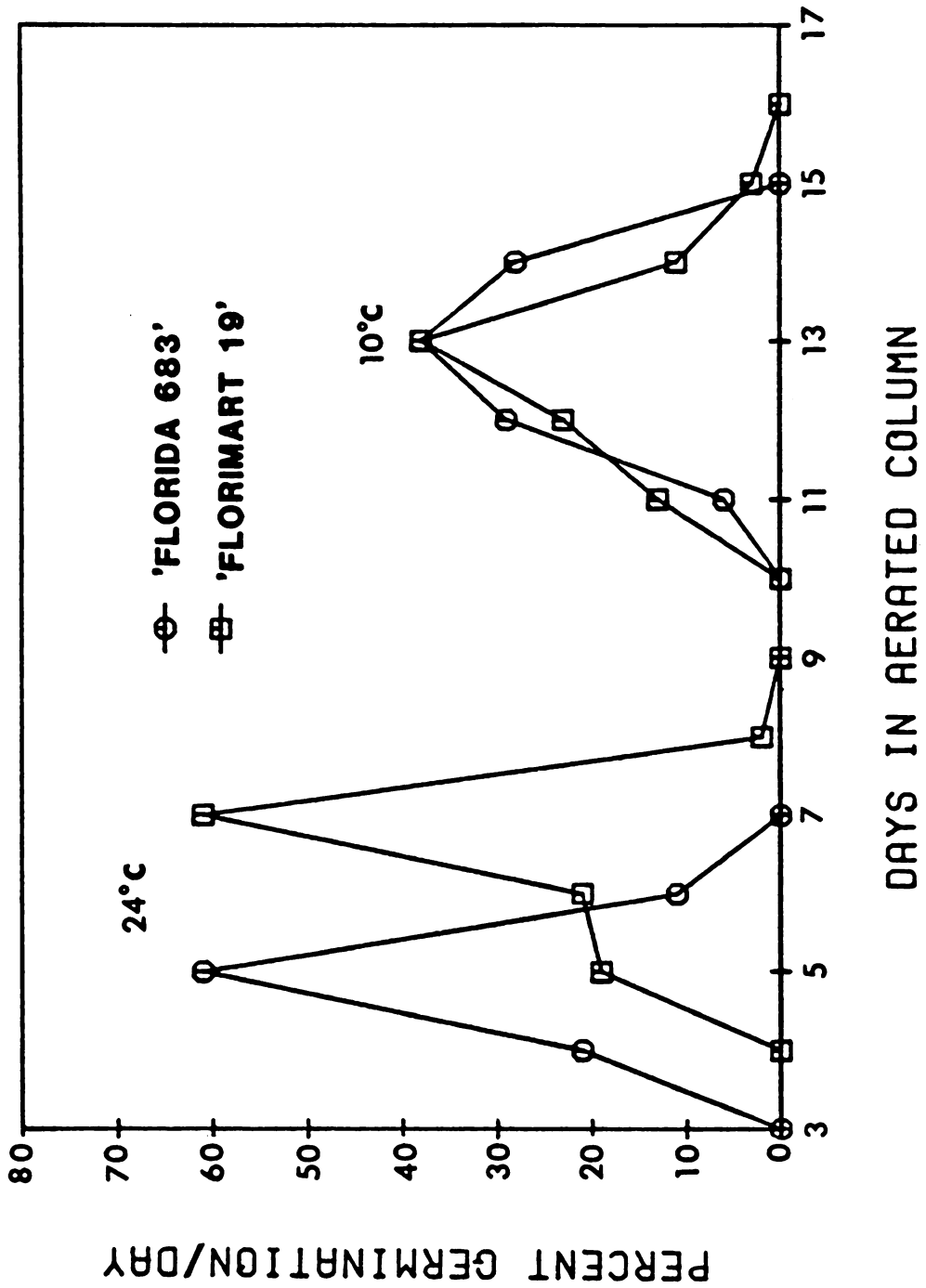
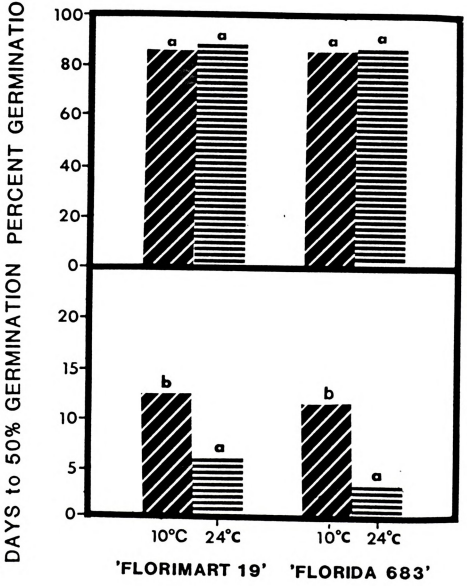

PERCENT GERMINATION/DAY
80
70
60
50
40
30
20
10
0
3
5
7
9
11
13
15
17
DAYS IN AERATED COLUMN
24°C
10°C
'FLORIDA 683'
'FLORIMART 19'

Figure 4. Percent germination and days to 50% germination of 'Florimart 19' and 'Florida 683' celery seeds germinated at 10 and 24°C in aerated columns. Mean separation by Duncan's multiple range test, at 5% level.


DAYS to 50% GERMINATION PERCENT GERMINATION
100
80
60
40
20
0
20
15
10
5
0
a
a
a
a
b
a
b
a
10°C 24°C
10°C 24°C
'FLORIMART 19'
'FLORIDA 683'

The radicle lengths of pregerminated celery seeds were shorter and more uniform when germinated at 10°C than at 24°C (Figure 5). The range of radicle lengths for pregerminated seeds of 'Florida 683' was 5 times longer at 24°C (0-10 mm) than at 10°C (0-2 mm) (Figure 6). Radicles of 'Florimart 19' pregerminated seeds were 0 to 2 mm (10°C) and 0 to 7 mm (24°C). The mean length of radicles was less than 1 mm for both cultivars when germinated at 10°C.

Incubation

The Effect of Temperature on the Emergence of Raw and Pregerminated Seeds

Fifty percent of the pregerminated seeds emerged in 9 days, while raw seeds emerged in 17 days (Figure 7). There was no difference in days to 50% emergence between either cultivar. Emergence at 32°C was not analyzed due to the low number of emerged seedlings attained from the raw seed treatment (less than 5%).

Emergence of seedlings was affected by presowing treatment (raw and pregerminated) and temperature (20 and 32°C) (Figure 8). Sixty to 70% of the seedlings from raw seeds emerged at 20°C, while only 5% emerged at 32°C. In contrast, over 80% of the seedlings from pregerminated seeds emerged at 20 and 32°C.

Transplants

A Comparison of Transplants Grown from Raw and Pregerminated Seeds

Fifty percent of the pregerminated seeds emerged in 3.5 days, while raw seeds required 11.8 days to reach 50% emergence (Figure 9).

Figure 5. Pregerminated seeds of 'Florimart 19' and 'Florida 683' after 80% germination at 10 and 24°C in aerated columns.

A. 'Florimart 19' germinated at 24°C for 7.5 days.

B. 'Florida 683' germinated at 24°C for 6 days.

C. 'Florimart 19' germinated at 10°C for 14 days.

D. 'Florida 683' germinated at 10°C for 14 days.

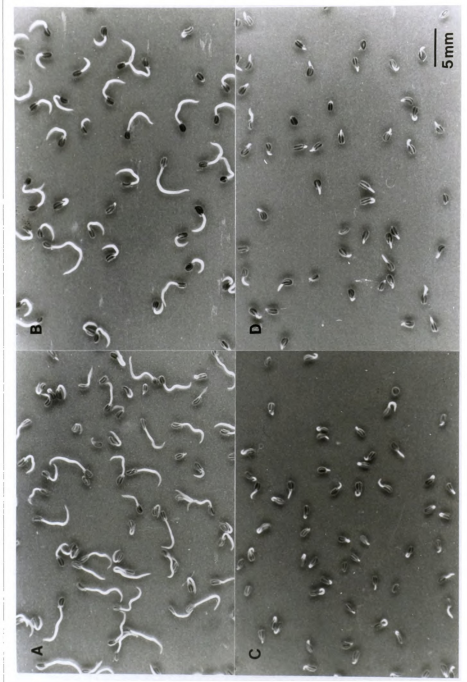

A
B
C
D
5mm

Figure 6. Effect of germination temperature on radicle lengths of seeds of 2 celery cultivars pregerminated in aerated columns. Radicle lengths of pregerminated seeds were measured at 80% germination, which required 6 days for 'Florida 683' and 7.5 days for 'Florimart 19' at 24°C and 14 days for both cultivars at 10°C.

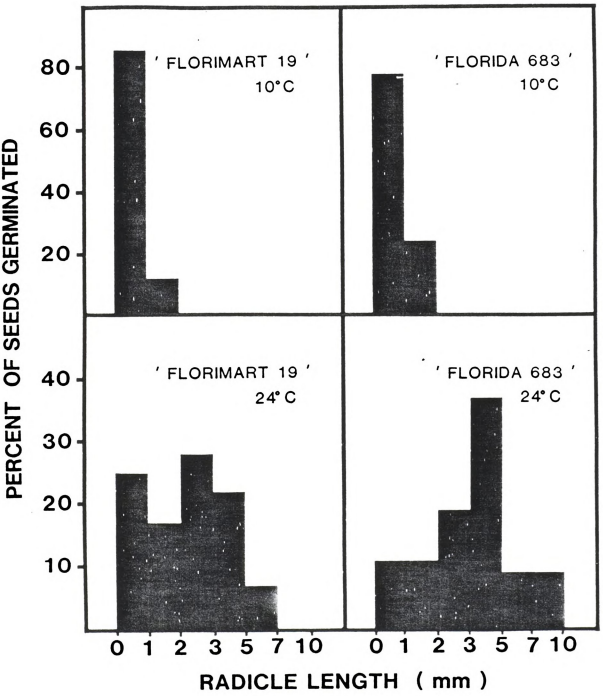

' FLORIMART 19 '
10°C
' FLORIDA 683 '
10°C
' FLORIMART 19 '
24°C
' FLORIDA 683 '
24°C
80
60
40
20
40
30
20
10
PERCENT OF SEEDS GERMINATED
0 1 2 3 5 7 10
0 1 2 3 5 7 10
RADICLE LENGTH (mm)

Figure 7. Days to 50% emergence of celery seedlings from raw (R) and pregerminated (P) seeds incubated in dark growth chambers at 20°C. Mean separation by LSD, at 5% level.

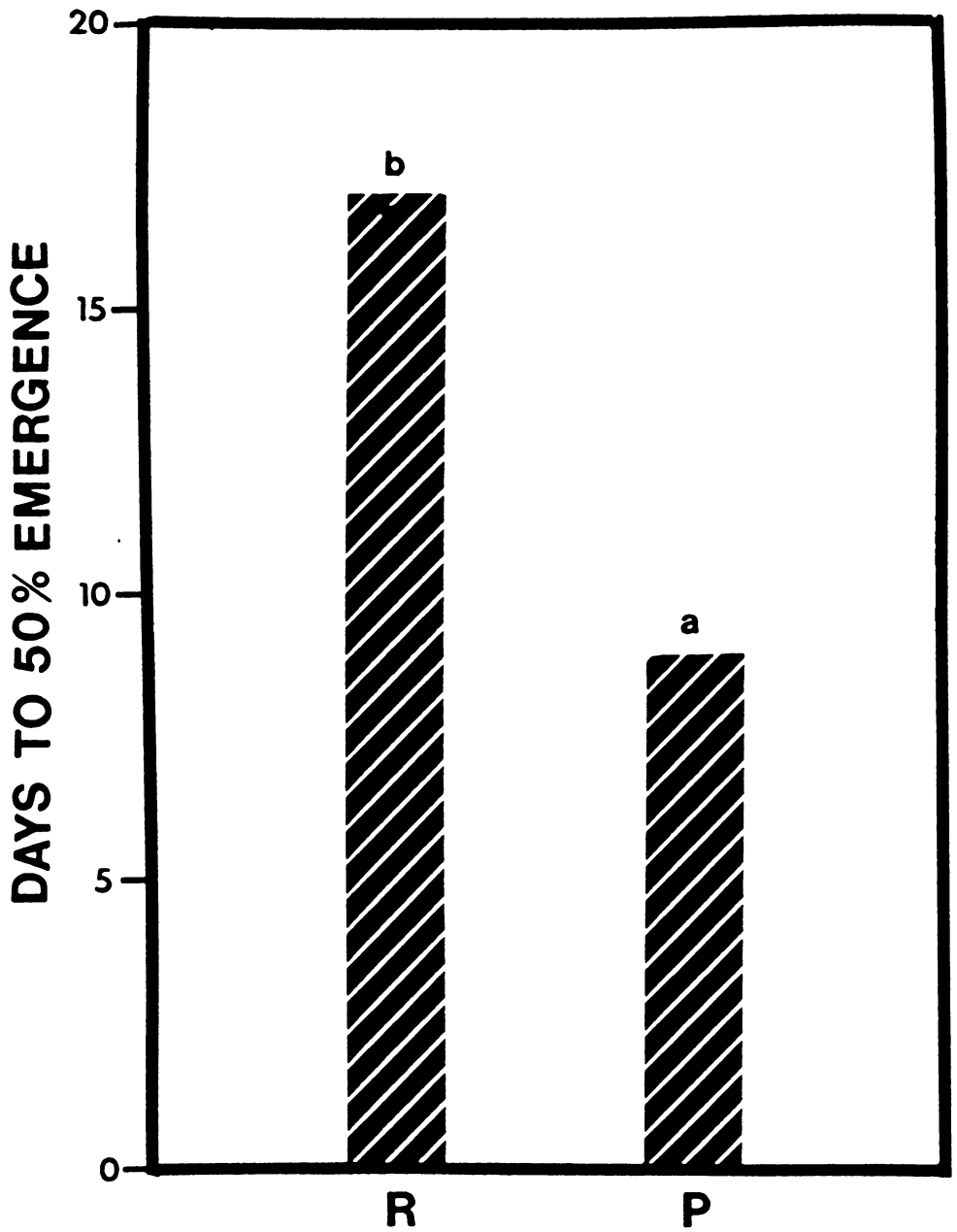

20
15
10
5
0
DAYS TO 50% EMERGENCE
b
a
R
P

Figure 8. Emergence of 'Florimart 19' and 'Florida 683' celery plants from raw (R) and pregerminated (P) seeds sown in flats and placed in dark growth chambers at 20 or 32°C. Mean separation by Duncan's multiple range tests at 5% level.

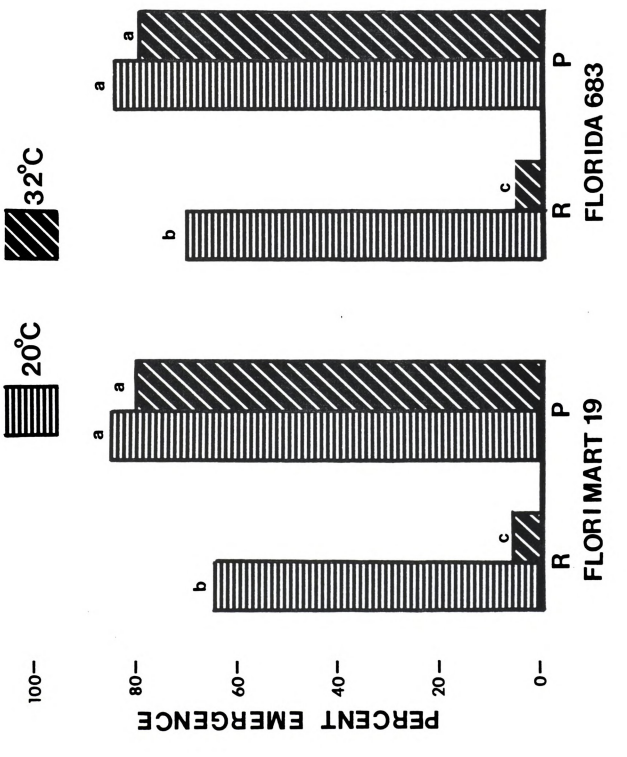
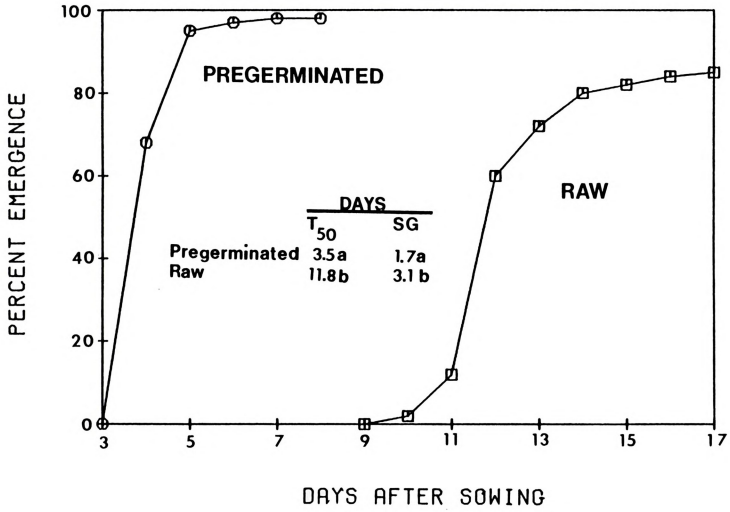

20°C
32°C
PERCENT EMERGENCE
100
80
60
40
20
0
a
a
b
c
R
P
FLORIMART 19
FLORIDA 683

Figure 9. Emergence of 'Florida 683' celery plants from raw and pregerminated seeds sown in flats and placed in a greenhouse with 22°C day and 16°C night temperatures. T_{50} = time to 50% germination; SG = spread of germination. Means were separated within columns by LSD, at 5% level.


PERCENT EMERGENCE
100
80
60
40
20
0
3
5
7
9
11
13
15
17
DAYS AFTER SOWING
PREGERMINATED
RAW
DAYS
T50
SG
Pregerminated 3.5a 1.7a
Raw 11.8b 3.1 b

In addition, the spread of germination (time difference to 90 and 10% emergence) (109) of seedlings from pregerminated seeds (1.7 days) was more concentrated than raw seeds (3.1 days). Ninety-five percent of the pregerminated seeds emerged compared to 85% of raw seeds.

Twenty-eight-day-old plants from pregerminated seeds grew larger and were more uniform (lower coefficient of variation) in height, number of leaves, and dry weights than plants grown from raw seeds (Table 1).

Table 1.--Height, number of leaves, and weight of 28-day-old celery plants grown in the greenhouse from raw and pregerminated seeds. The figures are means of 8 replicates of 20 plants each.

Treatment	Celery Plants					
	Plant Height (cm)		No. of Leaves		Dry Weight (mg)	
	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV
Raw	5.5	7.2	4.2	2.8	21.3	10.0
Pregerminated	6.6	5.6	5.1	2.2	40.7	7.1

Discussion and Conclusion

The most striking effect of low temperature (10°C) pregermination on celery seeds is the reduction of radicle length. Injury to radicles greater than 5 mm in length has been a common problem when fluid drilling pregerminated seeds (95). Prior to this study, there have been no reports on radicle length reduction using low temperature during the pregermination process.

The biochemical processes that lead to radicle protrusion might not be as strongly affected by the low temperature (10°C) as radicle elongation. Thus, while a population of seeds might exhibit a rather large spread in time to germination (radicle protrusion), further growth of those seeds that germinate quickly is held in check by the low temperature while the rest of the population is germinating. The result is that the later germinating seeds "catches up" with the early germinators. At 24°C, radicle elongation begins immediately after germination and the spread of emergence is maintained.

Low temperature also increased the time to 50% germination. At 10°C, it took twice as long to reach 50% germination as at 24°C, but radicles were shorter and more uniform (Figures 3, 4). In contrast, Salter and Darby (95) produced shorter radicles from celery seeds by priming in an osmotic solution of KNO_3 + K_3PO_4 at 15°C for 23 days, thereby reducing the number of days to 50% germination. They contended that reducing the time to 50% germination resulted in shorter radicles of pregerminated seeds.

Differences between our results and that of Salter and Darby (95) may be due to the difference in response of celery seeds to low temperature pregermination and priming. Primed seeds are soaked in an osmotic solution and redried before radicle emergence. In low temperature pregermination, the seeds are germinated in water without redrying. Physiological and biochemcial processes such as cellular and subcellular membranal organization and enzyme formation occurring under these 2 systems may account for these differences. Biddington recently reported that redrying celery seeds may injure

cellular and subcellular membranes (12). It is possible that Salter and Darby (95) observed shorter radicles resulting from injury caused by redrying primed seeds.

Our studies have shown that light, seed leachate, and temperature are important factors affecting germination of celery seeds in aerated columns. Light enhances the germination of celery seeds. Seeds incubated in the dark at 24°C failed to germinate, while those in light germinated (Figure 1). At 10°C, however, both light- and dark-incubated seeds germinated (Figure 2). At 24°C, seeds became thermodormant and did not germinate in the dark, but did germinate in light, as the temperature threshold for thermodormancy was elevated (110). Pressman et al. (87) and Thomas et al. (110) also reported decreased celery seed germination with increased temperature, and light elevated the temperature at which germination inhibition occurred. However, Taylor (108) and Harrington (40) found no differences in the germination level between dark- and light-incubated celery seeds at low temperatures (10 and 15°C). Possible explanations for these differences in response to light are: (1) 'Florimart 19' and 'Florida 683' seeds used in this experiment may have greater sensitivity to light than the celery cultivars used by Taylor and Harrington (108); (2) the dark-treated seeds were exposed to short periods of light during daily germination counts which may have been sufficient to overcome the thermodormancy. Pressman et al. (87) have shown that brief periods of light irradiations are sufficient to obtain a light response in seed germination; (3) Taylor and Harrington (108) did not remove seed leachates in the light-incubated seeds.

This study indicates that the presence of leachates in light-incubated seeds decreases the germination response to light.

Removing seed leachates increased germination of light-incubated seeds by removing germination inhibitors or by decreasing osmotic potential of the water in the aerated columns (Figures 1, 2). Celery seed leachates are known to contain coumarin compounds, which may inhibit germination (27, 30, 31, 32, 33). It is possible that these compounds became concentrated in the water. If so, replacing the water in the columns may have removed the coumarin compounds and improved germination. Secondly, increased osmotic potential has been reported to inhibit the hydration of cellular membranes and the phytochrome-receptor pigments of celery seeds (27). If this is true, the phytochrome-receptor pigments might have been unable to respond to light, thereby reducing germination.

Seed leachate removal in light at 24°C improved germination over nonremoval more than removal at 10°C (Figures 1, 2). More inhibitors may have leached-out of the seed coat at 24°C than at 10°C. The greater concentration of germination inhibitors in the solution at 24°C may have reduced germination with no removal. Alternatively, the higher leakage rate may have increased the osmotic potential. The stronger osmotic potential (24°C) may have affected the hydration of the phytochrome-receptor pigments more than the lower osmotic potential at 10°C.

Clearly, the system of seed leachate removal, light, and low temperature pregermination in aerated columns produces shorter and more uniform radicles. Most previous work has been conducted with

petri dishes, which limits capacity for pregerminating seeds. This system uses aerated columns capable of pregerminating commercial size seed lots. In addition, this study shows that celery seeds pregerminated in aerated water columns respond similarly to seeds pregerminated in petri dishes (87, 110).

Having established that this system of pregerminating seeds produces uniform, short radicles, and is capable of commercial scale production, the pregerminated seeds were tested in the greenhouse. These findings demonstrate that plants grown from pregerminated seeds are more uniform than plants grown from raw seeds (Figure 9, Table 1). Moreover, pregerminated seeds emerged more quickly with less spread of germination than raw seeds (Figure 9). The greater uniformity is demonstrated by the lower coefficient of variation (CV) of plants grown from pregerminated seeds compared with those grown from raw seeds (Table 1). Welch and Inman (117) determined that uniform transplants produce more uniform celery stalks, an important goal in the production of a celery crop.

These findings also show that pregerminated seeds are not susceptable to thermodormany as are raw seeds (Figure 8). Pregerminated seeds are not affected by high temperatures in the plant bed, since they had germinated already and were thus past the stage of thermodormancy. Pregerminating seeds prior to planting overcomes the thermodormancy problems encountered when raw seed is sown directly into the soil.

The major conclusions in this study are summarized as follows.

Effect of Light, Seed Leachate, and Temperature on Germination

Higher germination percentages are attained when seeds are germinated under light with seed leachate removed either at 24 or 48 hr intervals.

Effects of Temperature on Pregermination

Celery seeds pregerminated at 10°C have more uniform radicles than seeds pregerminated at 24°C. All radicles of seeds pregerminated at 10°C were 0 to 2 mm long after 14 days, while those of seeds pregerminated at 24°C were 0 to 10 mm long.

Incubation

Pregerminated seeds emerged from the plant bed at 32°C but raw seeds did not. Pregerminated seeds reached 50% emergence 10 days earlier than raw seeds at 20°C. Plants grown from pregerminated seeds were more uniform at transplanting than plants from raw seeds.

CHAPTER III

PRIMING AND PREGERMINATION OF ONION SEEDS

Introduction

Onions (Allium cepa L.) are an important vegetable crop in Michigan. They are sown as soon as the soil dries out in the spring, usually in April and early May. Soils at that time of the year are cold, usually 3 to 5°C. Under cold soil conditions, seeds germinate slowly and emerge nonuniformly, which delays emergence and lengthens crop maturity (95).

Priming and pregermination improve the speed and emergence and increase the uniformity of emergence in cold soils (36). Primed seeds emerge faster than raw seeds and can be sown with conventional seeders. Pregerminated seeds emerge faster and more uniformly than raw or primed seeds. A disadvantage is that pregerminated seeds must be sown with a special planter such as a fluid drill. In this system, the seeds are suspended in a fluid gel, then extruded into the soil. Seeds must have short radicles (less than 5 mm) and must be uniform in length to avoid damage during this operation (95). Uniform length is difficult to attain with nonuniform germinating seeds such as onions (47). Onion seeds are not uniform because initiation of cymes occur at different times among flowering plants and florets within the cymes are not pollinated simultaneously (47).

Since priming improves the speed and uniformity of germination, I studied leakage, food reserve utilization and respiration of onion seeds after priming to obtain a better understanding of the priming process.

In cold soils, emergence is delayed and seeds leak large quantities of food reserves, which increases their susceptability to microbial infections (94, 98, 99, 100). Research on leakage from primed onion seeds has not yet been reported.

The level of food reserves is important for seeds to have enough resources to emerge and develop seedlings (70). Measurements of food reserve utilized during priming and subsequent germination has not been reported.

Seeds accumulate biochemical compounds such as enzymes needed for germination during priming (17, 18). These accumulated enzymes increase the speed of germination. Respiration measurements on oxygen uptake may be an important assay for the advancement of these biochemical substances after priming (119).

The hypotheses of this study were:

Hypothesis 1: Priming and pregermination would affect radical lengths of pregerminated onion seeds.

Hypothesis 2: Sowing of primed or pregerminated seeds would speed up emergence and improve uniformity of seedling emergence.

The objective of this study was to develop a system of priming and pregermination of onion seeds that would improve uniformity of germination and emergence under low temperatures.

Materials and Methods

Priming

General Methods Used in Priming Onion Seeds

Onion cv. Cima seeds were obtained from the Keystone Seed Co. Inc., Hollister, California. In all the priming experiments described below, the seeds were primed in glass columns containing aerated osmotic solutions, as described by Salter and Darby (96). The osmotic solutions were replaced every 24 hr with freshly prepared solutions to maintain a constant osmotic pressure.

Five priming solutions were prepared from organic and inorganic solutes which were reported to stimulate germination and emergence of seeds after priming. These solutes were sodium chloride (NaCl) (24, 25, 67), potassium nitrate + tripotassium phosphate (KNO_3 + K_3PO_4) (14, 25, 37, 67, 81, 95), $C_6H_{14}O_6$ (mannitol) (67), and $H(OCH_2)_nOH$ polyethylene glycol (PEG 6000) (13, 43, 44, 45, 46, 47, 49, 50, 51, 95, 96). The nominal osmotic potentials of the 5 solutes were obtained from the literature and checked with a dew point psychrometer (Table 2).

The osmotic potential of NaCl solutions was obtained from a report by Lang (60). KNO_3 + K_3PO_4 phosphate concentrations were calculated by dew point psychrometer using NaCl standards. The osmotic potential of mannitol was obtained from Michael and Kaufmann (74) and by calculation with Van't Hoff's equation (91):

Table 2.--Priming solutions used in these experiments.

Osmotica	Temperature of Priming °C	Osmotic potential (MPa)[x]		
		-1.1	-1.3	-1.7
Mannitol	0	0.49	--	--
	2.5	0.49	--	--
	5	0.48	--	--
	10	0.47	0.56	0.73
	24	0.45	0.53	0.69
NaCl	10	0.26	0.30	0.40
	24	0.24	0.28	0.37
PEG 6000	10	310.0[z]	--	--
KNO_3 + K_3PO_4	10	0.12, 0.12[y]	--	--

[x]Nominal osmotic potentials

[y]pH adjusted to 7.0 with 5.4 ml H_3PO_4/liter of solution

[z]Figures expressed in molal concentrations, except PEG 6000 in g/kg H_2O.

$$\pi = mRT$$

π = osmotic potential

m = molarity of solute

R = gas constant 0.0812 liter bars per mole degree.

T = temperature in degrees Kelvin

The osmotic potential of PEG 6000 was calculated from an equation reported by Michael and Kaufmann (74):

$$\Psi = -(1.8 \times 10^{-2})\ C - (1.18 \times 10^{-4})\ C^2 + (2.67 \times 10^{-4})\ C^2T$$

Ψ = osmotic potential in bars

C = concentration of PEG 6000 in g/kg H_2O

T = temperature in degrees centigrade

After priming, the seeds were removed from the columns and rinsed 3 times with 300 ml distilled water. The seeds were then spread 1-seed-thick on shallow plastic trays at 22°C and kept under continuous air movement for 24 hr. Final drying to 6 to 7% moisture was attained by $CaSO_4$ dessication at 22°C for 5 days.

Primed and raw (control) seeds were tested for germination by placing 40 seeds from each treatment into 9 cm petri dishes containing a single disc of Whatmann No. 2 filter paper. The dishes were covered and placed into a clear plastic container. Two water evaporation wicks were placed into the plastic container to prevent evaporation of water from the petri dishes. The evaporation wicks were constructed with 12 cm Whatmann No. 3 filter paper folded into a fan and inserted into a 50 ml beaker of distilled water. The plastic

container was placed into a dark growth chamber at 10°C. The 10°C incubation of the seeds simulated stress conditions encountered during early season plantings into cold soil. The seeds were checked daily for germination. Visual radicle protrusion constituted germination. Days to 50% germination (T_{50}) (79), percent germination, and spread of germination ($T_{90} - T_{10}$) (109) by probit analysis were calculated. The formula used to calculate days to 50% germination is as follows:

$$D = \Sigma fx/\Sigma x$$

D = days to 50% germination

f = number of seeds germinated on day x

x = days after sowing

Square root of arcsin $(\arcsin)^{\frac{1}{2}}$ transformation were utilized on percentage data and tested for significance by Bartlet's test (106). Means were compared to LSD or Duncan's multiple range test. Each treatment was replicated 4 times in a completely randomized design.

Effect of Solute Composition on Germination

All 4 solutes were adjusted to an osmotic potential of -1.1 megapascals (MPa) (-1.0 MPa ≈ -10.0 bars) (Table 2). The seeds were primed at 10°C for either 24 or 48 hr and germinated as described above.

Effect of Osmotic Potential and Temperature of Priming on Onion Seed Germination

Mannitol solutions were prepared to osmotic potentials of -1.1, -1.3, and -1.7 MPa (Table 2). The seeds were primed at 10 or 24°C until 3 to 4% of the seeds were germinated. Mannitol is metabolically inert (74), and therefore should not stimulate or inhibit germination, or participate in chemical reactions during priming. This inertness facilitated the study of osmotic potential and temperature on germination. The levels of osmotic potentials, -1.1, -1.3, and -1.7 MPa, and temperature of priming, 10 and 24°C, were selected because they stimulated germination of onions, as well as other seed species after priming (13, 14, 46, 50, 67, 95).

The 3 to 4% germination range was arbitrarily selected to standardize the seeds' germination advancement between osmotic potential and temperature treatments.

Effect of Priming Duration and Solute on Germination

Mannitol and NaCl solutions were prepared to a nominal osmotic potential of -1.1 MPa. The seeds were primed in either osmotic solution for 0, 1, 2, 4, 6, or 8 days. (From the experiment above, we knew that the seeds would germinate after 8 days under these conditions.) Regression analyses were used in determining the relationship between priming duration and germination. Mannitol and NaCl were selected to compare priming in a metabolically inert (mannitol) and reactive (NaCl) solution.

Physiological Effects of Priming on Onion Seeds

Leakage Studies

Leakage of alpha-amino acids, 262 mμ absorbing substances, and reducing sugars from raw and primed seeds. Onion seeds were primed for 8 days in -1.1 MPa NaCl solution at 10°C, as described above. NaCl was used in this experiment instead of mannitol for the following reasons: it is a good solute for priming onion seeds; it did not react with the chemical assays used in this study, as did mannitol; and it was as effective as mannitol in increasing the speed and uniformity of germination.

Two g primed and raw seeds were placed in 10 cc disposable plastic syringes. Serum caps were used to seal the openings of the hypodermic receptacles on the syringes. Four ml distilled water was pipetted into each syringe, which were then stoppered with syringe plungers. The syringes were agitated for 30 seconds to remove air trapped around the seeds, and incubated at 10°C. Each hr, up to 5 hr, seed leachates were collected from the syringes by removing the serum cap and pumping the plunger until all leachates were removed. The serum cap was replaced and the addition of water and collection of seed leachate procedure was repeated. Seed leachates were centrifuged at 2000 g's for 15 minutes to remove suspended particles. Two 1 ml aliquots of the supernatant were removed by pipet and immediately analyzed for optical density, alpha-amino acids, and reducing sugars.

The nondiluted leachate samples were analyzed with a Beckman DU spectrophotometer. The samples were pipetted into 0.5 ml cuvettes and optical density measurements at 262 mμ were recorded. This wave length, 262 mμ, is generally absorbed by carboxyl, ester, and other bonds, and many of these bonds are prevalent in substances that leak from seeds. Thus, the 262 mμ absorbance readings obtained from seed leachates provided a quantification of these substances. Equal volumes of distilled water served as blanks (standard for 0% absorbance). Readings were expressed as relative absorbance.

Alpha-amino acid levels in the seed leachates were determined by the Ninhydrin test, as described by Yemm and Cocking (122). In this assay, amino acids undergo oxidative deamination forming ammonia. Ninhydrin is reduced to hydrindatin and ammonia condenses with hyrindatin to form diketohydrindylindenediketohydrindamine anion (DYDA), which has an intense blue color. Peak absorbance for DYDA is 570 mμ. Samples with high amino acid concentrations were diluted with 70% ethanol. D, L-aspartic acid was used as a standard at 0, 6.25, 12.5, and 25 mg/ml. Alpha-amino acids were expressed as mg D, L-aspartic acid equivalents/g oven-dried seed/hr. Equal volumes of 70% ethanol were utilized as blanks.

Reducing sugar analysis was conducted, as described by Nelson (78) and Somogi (104). The assay determines the presence of free-reducing groups in carbohydrates by their capacity to reduce Cu^{++} in an alkaline solution. The amount of Cu^{++} reduced is directly proportional to the amount of reducing sugars present in the carbohydrate being analyzed. The Cu^{+} formed in the reaction precipitates as

rust-colored Cu_2O. Arsenomolydic acid is added to the solution, and is quantitatively reduced to arsenomolybdous acid by Cu^+. Arsenomolybdous acid is emerald green with peak absorbance at 500 mμ. D-glucose was used as a standard at concentrations of 0, 12.5, 25, 50, and 100 ug/ml. For sample dilutions, 70 percent ethanol was used. Reducing sugars was expressed as mg D-glucose equivalents/g oven-dried seed/hr.

Rates of water uptake were measured by placing a 1 g sample of seed from each treatment into 10 cc disposable syringes. The hypodermic receptacles on the syringes were stoppered with serum caps and filled with 6 ml distilled water. Five sets of each treatment were prepared; a treatment set was sampled each hr for 5 hr. After each hr, the serum caps were removed and the water drained from the syringes. The seeds were surface-dried on Whatmann No. 2 filter paper and weighed, then oven-dried at 100°C for 16 hr and reweighed for moisture determinations (121). Water was drained from the remaining treatment sets every hr and replaced with freshly distilled water to maintain a constant osmotic potential. Water uptake was expressed as mg/water/g oven-dried seed/hr. Each treatment was replicated 4 times (4 syringes).

Effect of hydration and dehydration on leakage of alpha-amino acids, 262 mμ absorbing substances, and reducing sugars from raw seeds. Seed leachates from 1 g samples of raw seeds, containing 6.5 ± 0.1% moisture, were extracted at 10°C and analyzed, as described above. Seed moisture levels were determined by weight loss after

oven-drying for 16 hr at 110°C (121). Standard deviation of seed moisture (6.5 ± 0.1%) was calculated using 4 replicates.

After the fifth hour extraction, the seeds were removed from the syringes and placed in aluminum weighing pans. The seeds were dried for 24 hr at room temperature in a dessicator containing $CaSO_4$, until the original moisture level (6.1 ± 0.5%) was attained. The redried seeds were then rehydrated at 10°C and the seed leachate was collected and analyzed again, as described above. Each treatment was replicated 4 times (4 syringes).

Effect of osmotic potential and temperature on leakage of 262 mμ absorbing substances. Three g of raw seeds, 6.5 ± 0.1% moisture was primed at 2 osmotic potentials, as described above. The osmotic potentials were -0.1 MPa (H_2O) or -1.1 MPa (NaCl solution). The seeds were primed in 100 ml of either osmotic solution for 5 hr at 10 and 24°C. At 1 hr intervals, 2 ml of seed leachates were pipetted from each column and analyzed for optical density, as described above. Water and -1.1 MPa NaCl solution were used as blanks. Rates of water uptake were measured for each treatment, as described above. Each treatment was replicated 4 times (4 columns).

Food Reserve Utilization Studies

The utilization of food reserves during priming. Six g samples of raw onion seeds were placed in glass columns containing 400 ml of aerated -1.1 MPa NaCl solution at 10°C and primed, as described above. One g samples of seed were removed from the columns

at 0, 2, 4, 6, and 8 days to determine the utilization of food reserves during priming. Each seed sample was rinsed 3 times with 300 ml distilled water, wrapped with a single layer of aluminum foil, and placed in a Dewar flask containing dry ice. After 60 minutes, the aluminum foil packets were removed, unwrapped, transferred into aluminum weighing pans, and freeze-dried for 48 hr at -30°C. The freeze-dried seed samples were ground to a fine powder using a motorized mortar and pestal (Torson Balance Co.). One g of ground seed sample was extracted with 20 ml each of ethanol for the first 2 extractions and 10 ml for the final extraction. During each 30 minute extraction, the flasks were agitated with a wrist-action shaker. All 3 extracts were collected, combined, filtered through glass wool under vacuum, and centrifuged at 2000 x g's for 10 minutes. The extracted supernatant was pipetted into 10 ml test tubes, stoppered, and stored at -30°C until analyzed for carbohydrates, reducing sugars, and alpha-amino acids.

Carbohydrates were quantified with Dreywood's anthrone reagent, as described by Morris (76). The anthrone test is based on the hydrolyzing and dehydrating action of concentrated H_2SO_4 on carbohydrates. H_2SO_4 catalyzes the hydrolysis of any glycosidic bond present in the sample and dehydrates these groups to furfural (pentoses) or hydroxymethyl furfural (hexoses). These furfurals then condense with anthrone to form a greenish-colored liquid with peak absorbance at 620 mμ. Sucrose was used as a standard at 0, 12.5, 25, 50, 100, and 200 μg/ml. Carbohydrates were expressed as mg sucrose

equivalents/g oven-dried seed. Reducing sugars and alpha-amino acid analyses were conducted, as described above.

Lipids were extracted by the Folch method (29). One g samples of finely-ground freeze-dried seeds were placed in 500 ml separatory funnels and extracted 3 times with 2:1 chloroform-methanol (v/v) for 45 minutes at 20°C. Twenty ml were used for the first 2 extractions and 10 ml for the final extraction. After each extraction, the supernatant was decanted, collected, combined, and washed with 10 ml 0.9% NaCl solution. The aqueous phase was drained-off and discarded. The lipid-containing phase was washed twice with Folch's solution and the chloroform-methanol solution was removed by vacuum evaporation at 20°C. The lipid-chloroform extract was reevaporated in weighing vials under N_2 atmosphere. Total lipids were quantified as lipid wt/g oven-dried seed.

Food reserve utilization during germination. Seeds were primed for 8 days in -1.1 MPa NaCl solution at 10°C, as described above. Two and one-half g of seed from either 8-day-primed or raw seeds were placed in plastic petri dishes containing 20 g of acid washed silica sand and 9 ml distilled water. The seeds were incubated in dark growth chambers at 10°C. Seeds were sampled at 0, 5, and 10 days after sowing. The seeds were separated from the silica sand by sieving with a wire mesh screen, freeze-dried, and analyzed for food reserves, as described above. Duplicate treatments were prepared for determination of weight loss of seeds after

germination. The weight loss values were used as a correction factor for food reserve measurements.

Respiration Studies

Effect of solute on respiration. A Gilson differential respirometer (General Medical Engineering Co.) was used to measure seed respiration. One-and one-half g of raw onion seeds were placed into the outer well of a single-side-arm respirometer flask. Two-tenths ml of 20% KOH (w/w) was pipetted into the center well, which was fitted with a wick, constructed from Whatmann No. 2 filter paper. The wick increased the surface area of the KOH solution, which "scrubs" CO_2 evolving from the seeds. Two ml water, -1.1 MPa NaCl or -1.1 MPa mannitol solutions were pipetted into the side arm of the respirometer flask. The side arms were stoppered and the flasks fitted onto the respirometer. The air valves in the reference flasks were adjusted to the total volume of the respirometer flasks by water displacement.

With the respirometer's air vent valve left open, all flasks were lowered into the water bath at 10°C and agitated for 60 minutes. The solution in the side arm was then combined with the seeds by tilting the flasks; this allowed the fluid to flow into the outer well. The air vent valve was left open for an additional 10 minutes to allow the escape of trapped gases evolving from the seeds. The air vent valve was closed and respiration (oxygen uptake) measured every hr for 5 hr.

Oxygen uptake measurements were corrected to standard temperature and pressure (STP) by the following equation (114):

$$O_2 = \frac{(273)(Pb\text{-}3\text{-}Pw)}{(t + 273)(760)}$$

O_2 = volume of oxygen at STP

t = water bath temperature in degrees Kelvin

Pb = barometric pressure of the atmosphere in mmHg

Pw = water vapor pressure of water bath in mmHg

Respiration rates were expressed as µl oxygen utilized/g of oven-dried seed/hr. Each treatment was replicated 3 times. Water uptake measurements were determined for these treatments, as described above.

Respiration of primed onion seeds. Onion seeds were primed in H_2O or -1.1 MPa mannitol, as described above, at 10°C for 2 days. Raw seeds were utilized as controls. Respiration and water uptake at 10°C were analyzed, as described above.

Effect of duration of priming on respiration. Raw seeds were placed in -1.1 MPa NaCl, or -1.1 MPa mannitol solutions and primed, as described above. The seeds were primed in mannitol at 10°C for 0, 2, or 6 days. Only 0- and 6-day durations were used for the NaCl priming treatment. Primed seeds were reimbibed in water at 10°C and analyzed for respiration and water uptake, as described above.

Pregermination

The Effect of Temperature and Duration of Priming, and Temperature of Pregermination on Onion Seed Radicle Length

Seeds were primed in -1.1 MPa mannitol solution for 0, 5, 10, or 20 days at 0, 2.5, 5, or 10°C, as described above. In the 10°C treatment, seeds were primed for 0, 5, and 8 days, since radicles emerged after 8 days. Primed seeds were germinated at 2.5, 5, and 10°C, as described above, without redrying the seeds after soaking. Redrying has been shown to slightly decrease the priming effect on onion seeds (50). Germination was recorded daily and radicle length measurements were taken on each treatment at 80% germination.

Emergence of Seedlings from Raw, Primed, and Pregerminated Onion Seeds

Seeds were pregerminated, as described above (primed for 8 days in -1.1 MPa mannitol and germinated at 10°C for 2 days) or primed, as described above (primed for 8 days in -1.1 MPa mannitol and redried). Pregerminated, primed, and raw seeds were sown 3 mm deep in flats containing moistened No. 2 grade vermiculite and placed in dark growth chambers at 10°C. Raw seeds were the controls. Seedling emergence was recorded daily. There were 4 flats for each treatment.

Results

Priming

Effect of Solute Composition on Germination

Of the solutes tested, seeds primed in PEG 6000 took the longest time to reach 50% germination and had the lowest germination (Table 3). Seeds primed in the other solutes were not different from raw seeds in percent germination and time to 50% germination.

Effect of Osmotic Potential and Temperature of Priming on Onion Seed Germination

Osmotic potential and temperature affected the number of days to 3 to 4% germination in the priming solution (Table 4). Seeds primed in -1.7 MPa at 10°C required 12 days to reach 3 to 4% germination, the longest priming duration of any treatment. In contrast, seeds primed in -1.1 MPa at 24°C required only 4 days to reach 3 to 4% germination, the shortest duration of any treatment. For each priming temperature, increasing the osmotic potential increased the time to reach 3 to 4% germination.

Days to 50% germination of primed seeds were reduced significantly in comparison to raw seeds, except for -1.7 MPa at 24°C (Table 4). Generally, 10°C-primed seeds had greater reduction in days to 50% germination and higher percent germination than 24°C-primed seeds.

Table 3.--Germination of onion seeds at 10°C after priming for 24 and 48 hr in various solute solutions.

Osmotica (-1.1 MPa)	Duration of Priming (hr)	Days to 50% Germination	Percent Germination[x]
PEG 6000	24	7.2 b[y]	83.8 bc
NaCl	24	6.8 a	90.6 ab
KNO_3 + K_3PO_4	24	6.9 a	90.6 ab
Mannitol	24	6.8 a	85.0 ab
PEG 6000	48	7.3 b	80.6 c
NaCl	48	6.8 a	92.5 a
KNO_3 + K_3PO_4	48	6.7 a	92.5 a
Mannitol	48	6.7 a	92.5 a
Raw (control)	--	6.9 a	91.9 ab

[x]Statistical analysis conducted with $(\arcsin)^{\frac{1}{2}}$ transformation

[y]Means within columns separated by Duncan's multiple range test, at 5% level.

Table 4.--Germination on onion seeds at 10°C after priming with mannitol solutions

Mannitol Osmotic Potential (MPa)	Temperature of Priming C	Days to 3 to 4% Germination[x]	Days to 50% Germination[y]	Percent Germination
-1.1	10	8	4.4 ab[z]	81 ab
-1.3	10	10	4.4 ab	78 abc
-1.7	10	12	4.0 a	69 bc
-1.1	24	4	5.2 bc	65 cd
-1.3	24	6	5.5 bc	64 cd
-1.7	24	8	5.6 bcd	56 d
Raw Seeds (Control)	--	--	6.4 d	83 a

[x]The germination "marker" used to equilibrate the priming advancement between treatments during priming in the aerated columns

[y]Statistical analysis conducted with $(\arcsin)^{\frac{1}{2}}$ transformation

[z]Means within columns separated by Duncan's multiple range test, at 5% level

Effect of Priming Duration and Solute on Germination

Increasing priming duration at 10°C reduced the seeds' days to 50% germination and spread of germination (Table 5). Priming duration and days to 50% germination were negatively correlated ($r = -0.96^{**}$) (Figure 10). Priming duration and spread of germination was also negatively correlated ($r = -0.81^{*}$) (Figure 11).

There were no differences in days to 50% germination, percent germination, or spread of germination between mannitol and NaCl primed seeds (Table 5). There was no interaction between priming duration and solute.

Physiological Effects of Priming Onion Seeds

Leakage Studies

Leakage of alpha-amino acids, 262 mμ absorbing substances, and reducing sugars from raw and primed seeds. Leakage of alpha-amino acids, reducing sugars, and 262 mμ absorbing substances from raw seeds was approximately 4 times greater than from primed seeds during the first hour of imbibition (Figure 12). After the second hour of imbibition, leakage from raw seeds declined, and paralleled the leakage from primed seeds. However, leakage of alpha-amino acids (d,l-aspartic acid equivalents) decreased more slowly than reducing sugars and 262 mμ absorbing substances. There were no differences in water content between primed and raw seeds at 10°C after 5 hr imbibition (Figure 13).

Table 5.--Main effects of solute and priming duration on onion seed germination

Duration (days)	Days to 50% Germination	Percent Germination[x]	Spread of Germination (Days)
Main Effect of Priming Duration*			
0	6.9 d[y]	91.9 ns	3.1 c
1	6.9 d	87.8	2.7 b
2	6.7 d	92.5	3.1 c
4	5.6 c	89.7	2.9 bc
6	5.1 b	90.0	2.7 b
8	3.7 a	90.0	2.1 a
Main Effect of Solute*			
NaCl	5.8 ns	89.9 ns	2.6 ns
Mannitol	5.8	90.7	2.9

[x]Statistical analysis conducted with $(\text{arcsin})^{\frac{1}{2}}$ transformation

[y]Means within columns separated by Duncan's multiple range tests, at 5% level

ns = Not significant by F test, at 5% level

*Interaction of priming duration and solute was not significant (ns)

Figure 10. Relationship between days to 50% germination and days of priming at 10°C on onion seeds.

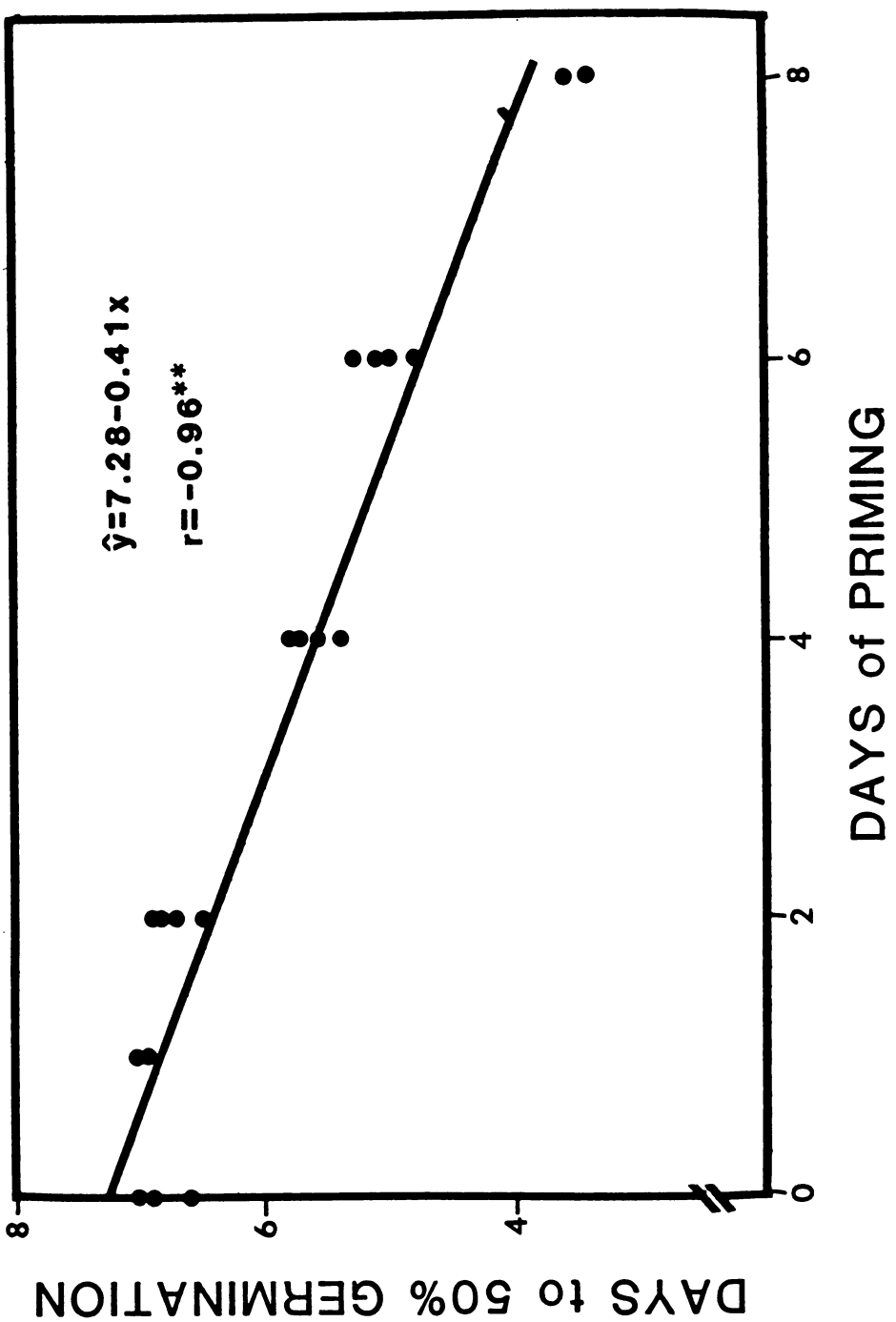

ŷ=7.28-0.41x
r=-0.96**
DAYS to 50% GERMINATION
DAYS of PRIMING
0
2
4
6
8
4
6
8

Figure 11. Relationship between days of priming at 10°C and spread of germination. Each point is the mean of 4 replicates.

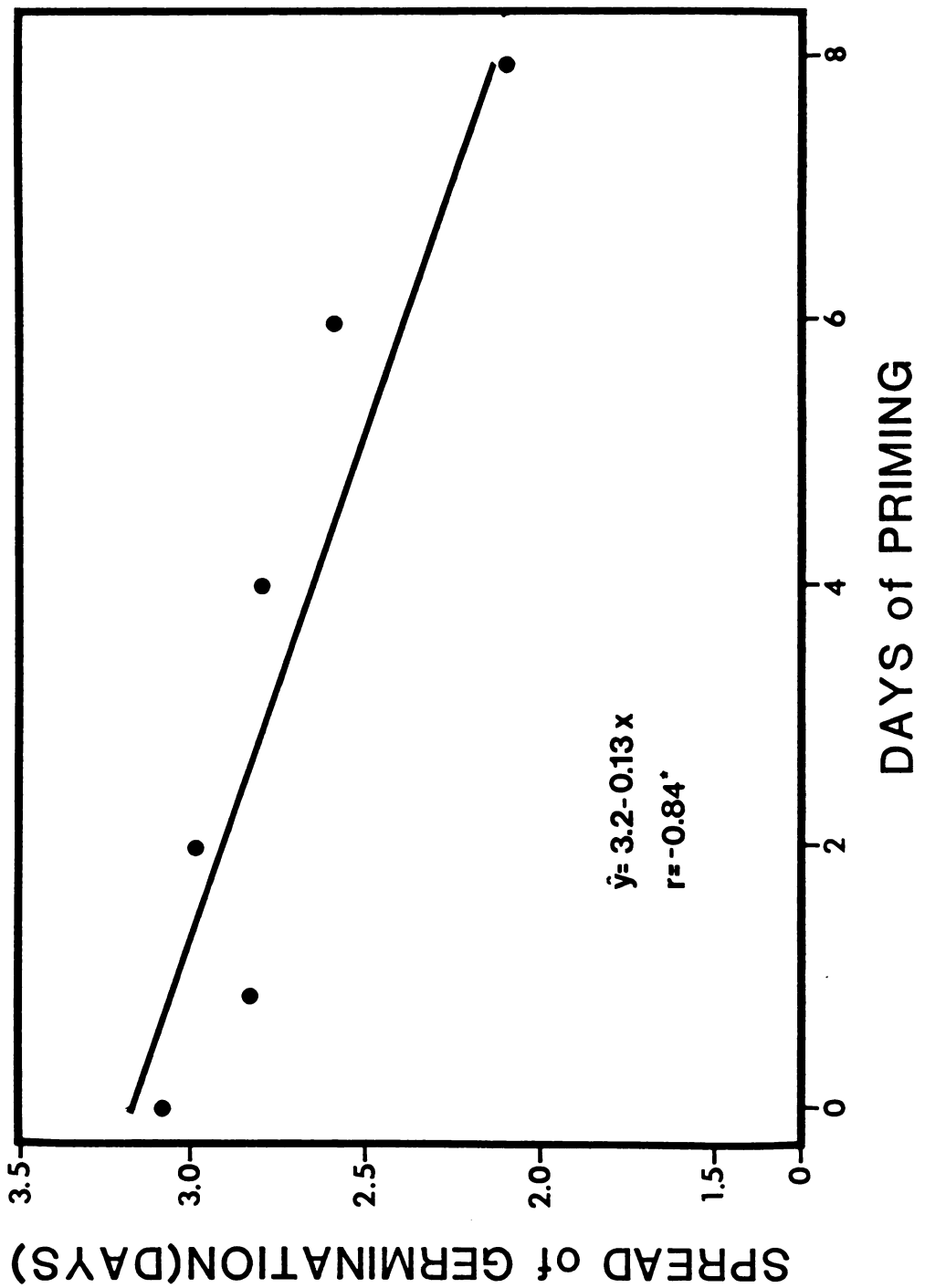

SPREAD of GERMINATION(DAYS)
3.5
3.0
2.5
2.0
1.5
0
0
2
4
6
8
DAYS of PRIMING
ŷ= 3.2-0.13 x
r=-0.84'

Figure 12. Leakage of alpha-amino acids, reducing sugars, and 262 mμ absorbing substances from raw and primed onion seeds during 5 hr imbibition in water at 10°C. Values are means of 4 replicates.

Key for $LSD_{0.05}$

LOD = Leachate optical density

RD = d-glucose equivalents

AA = d,l-aspartic acid equivalents

LEACHATE OPTICAL DENSITY 262 mμ △
3.0
2.0
1.0
0.0
.... Raw seeds
—— Primed for 9 days
LSD 0.05
LOD RS AA
di-ASPARTIC ACID EQUIVALENTS (μg/hr/g dry wt) ■
33
30
27
24
21
18
15
12
9
6
3
0
d-GLUCOSE EQUIVALENTS (μg/hr/g dry wt) ●
250
225
200
175
150
125
100
75
50
25
0
0
1
2
3
4
5
HOURS of IMBIBITION

Effect of hydration and dehydration on leakage of alpha-amino acids, 262 mμ absorbing substances, and reducing sugars from raw seeds. Alpha-amino acids, reducing sugars and 262 mμ absorbing substances leaked more during the first hydration cycle than during the second hydration cycle (Figure 14). Leakage of seeds during the first and second hydration cycles were parallel, although leakage during the second cycle was 50% lower than during the first cycle.

Effect of osmotic potential and temperature on leakage of 262 mμ absorbing substance. At 24°C, seeds imbibed in H_2O exhibited the greatest leakage; at 10°C, seeds imbibed in -1.1 MPa NaCl leaked the least (Figure 15). Seeds primed in H_2O at 10 and 24°C and in -1.1 MPa NaCl at 24°C contained a comparable level of H_2O (Figure 16). However, after 2 hr of imbibition, seeds primed in -1.1 MPa NaCl at 10°C contained less water than seeds primed in the other treatments.

Food Reserve Utilization Studies

The utilization of food reserves during priming. The quantity of food reserves (reducing sugars, carbohydrates, alpha-amino acids, and lipids) was constant over 8 days of priming in -1.1 MPa NaCl at 10°C (Figure 17).

Food reserve utilization during germination. During germination, there were only slight differences in the quantity of food reserves used by primed and raw seeds (Figure 18). Food reserves of primed and raw seeds decreased at a similar rate as germination progressed.

Figure 13. Water content of raw and primed onion seeds imbibing in water at 10°C.

ns = not significant by F test at 5% level.

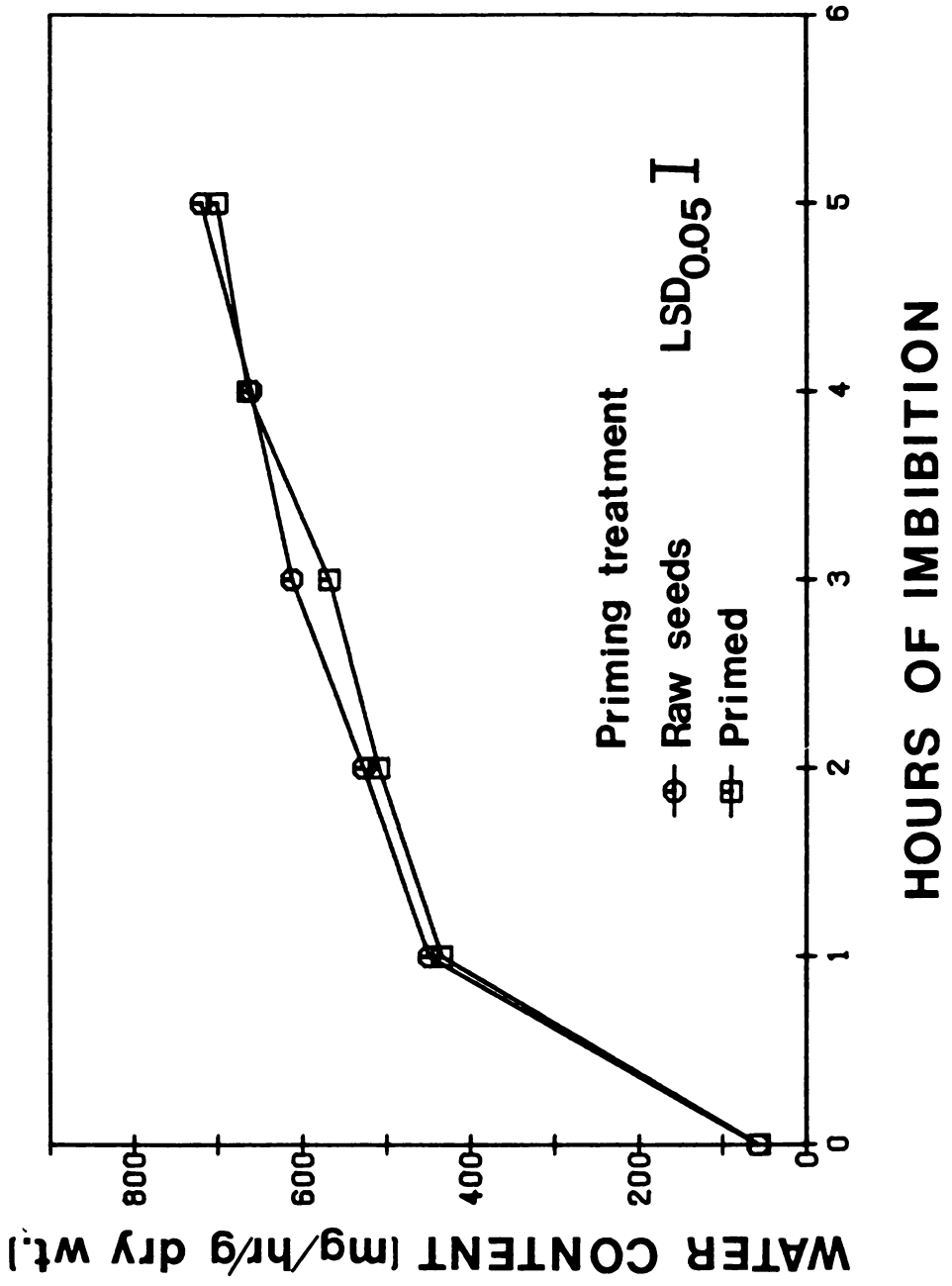

WATER CONTENT (mg/hr/g dry wt.)
800
600
400
200
0
0
1
2
3
4
5
6
HOURS OF IMBIBITION
Priming treatment
Raw seeds
Primed
LSD0.05

Figure 14. Leakage rates of alpha-amino acids, 262 mμ absorbing substances, and reducing sugars from onion seeds at 24°C after 2 hydration-dehydration cycles. Mean separation by LSD at 5% level.

Key for $LSD_{0.05}$

LOD = leachate optical density

RD = d-glucose equivalents

AA = d, l-asparatic acid equivalents

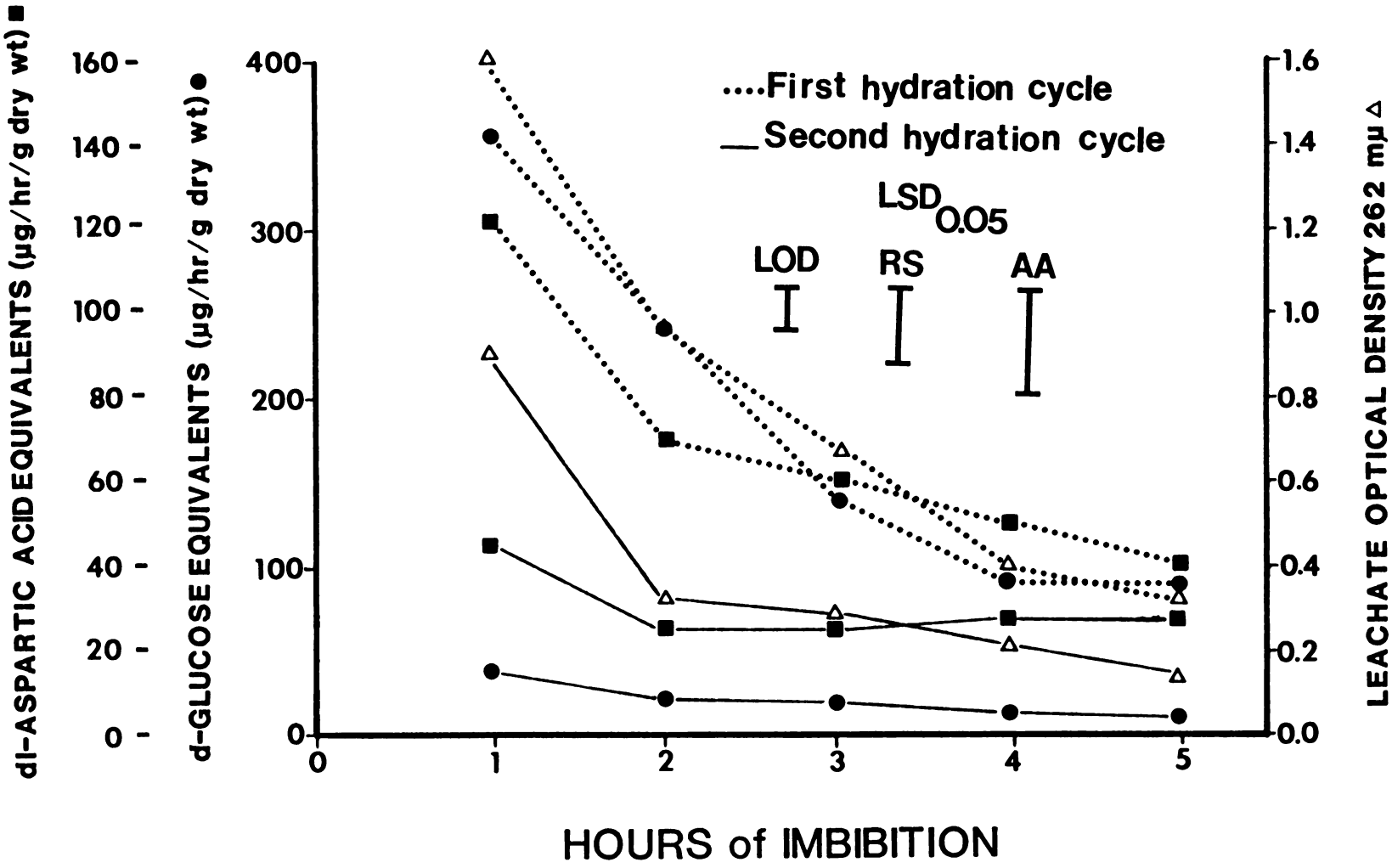

dl-ASPARTIC ACID EQUIVALENTS (µg/hr/g dry wt) ■
d-GLUCOSE EQUIVALENTS (µg/hr/g dry wt) ●
LEACHATE OPTICAL DENSITY 262 mµ △
160
140
120
100
80
60
40
20
0
400
300
200
100
0
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
....First hydration cycle
__Second hydration cycle
LSD0.05
LOD
RS
AA
HOURS of IMBIBITION

Figure 15. Leachate optical densities of imbibing solution after priming of raw onion seeds at 10 and 24°C with NaCl and H_2O. Values are means of 4 replicates.

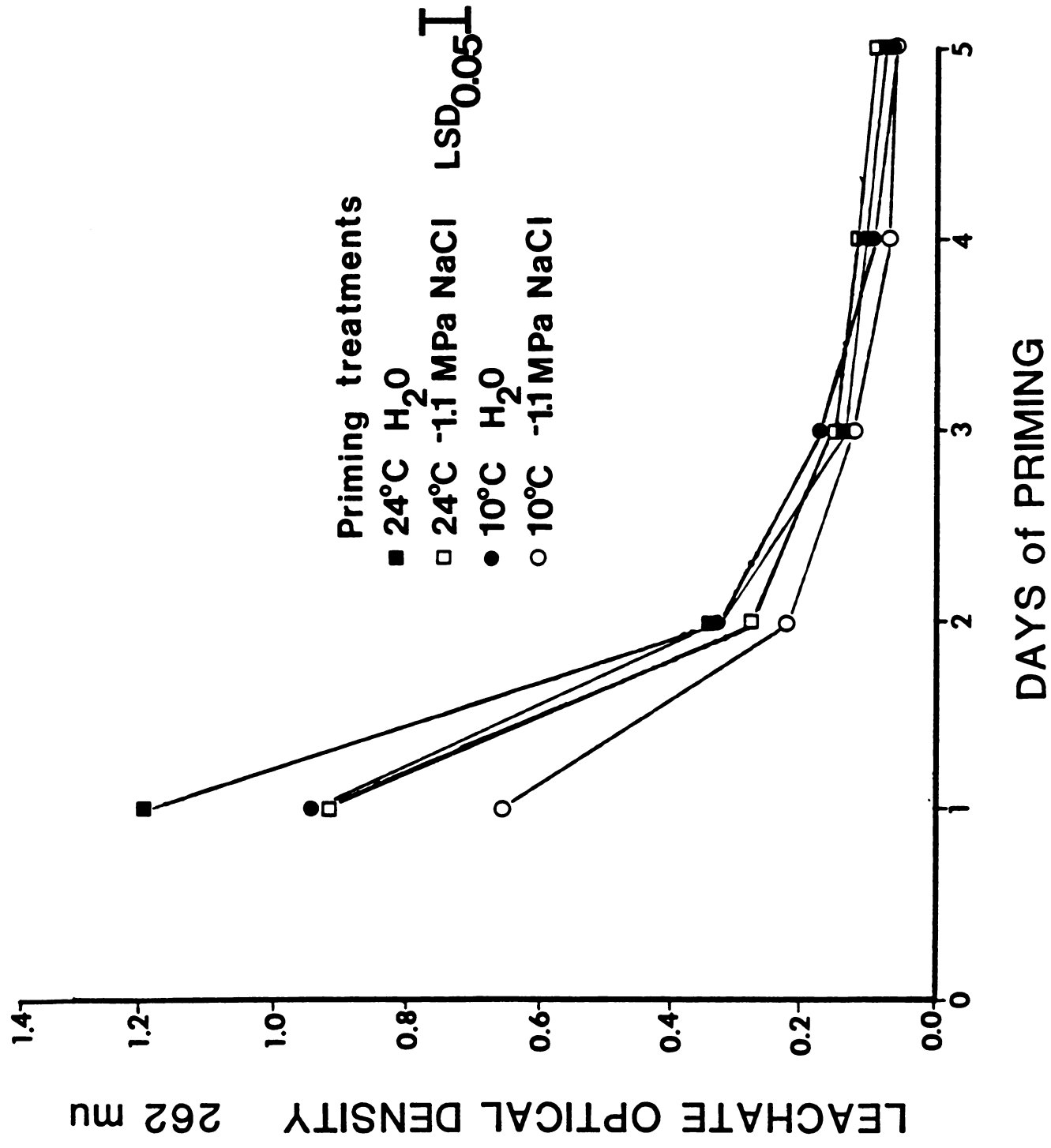

LEACHATE OPTICAL DENSITY 262 mu
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
DAYS of PRIMING
Priming treatments
24°C H2O
24°C -1.1 MPa NaCl
10°C H2O
10°C -1.1 MPa NaCl
LSD0.05

Figure 16. Water content of onion seeds after various treatments. Values are means of 4 replicates.

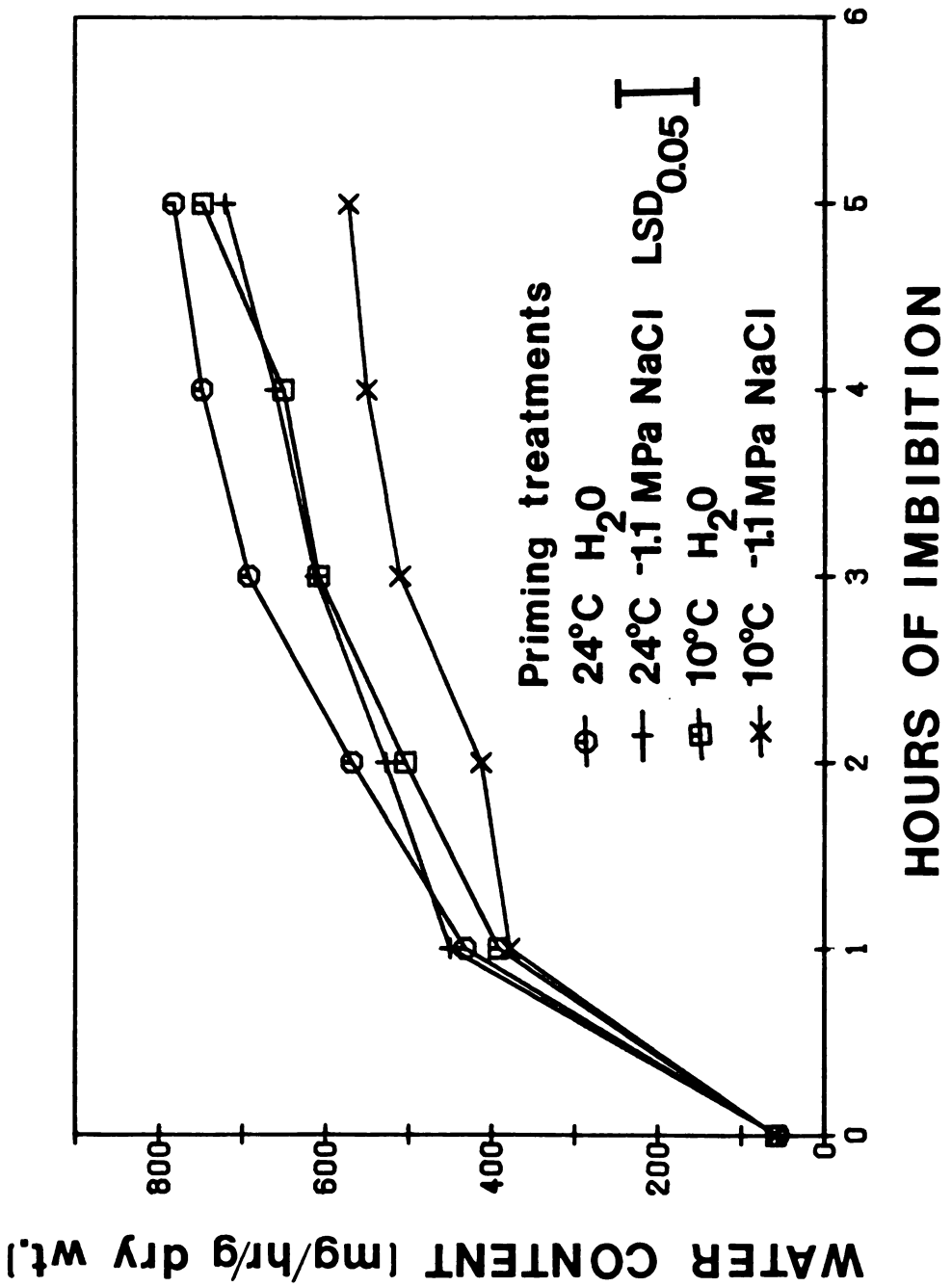
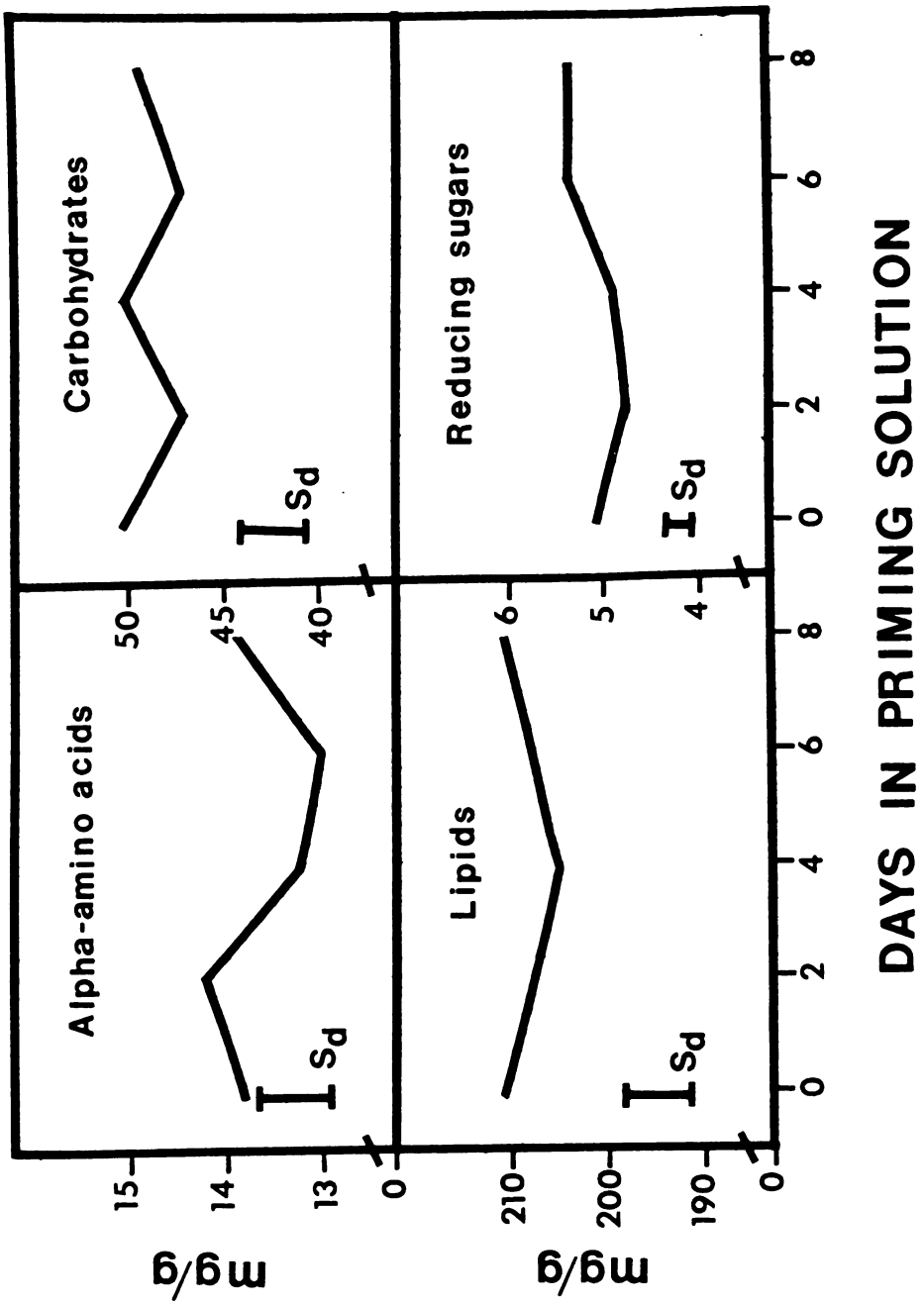

WATER CONTENT (mg/hr/g dry wt.)
800
600
400
200
0
0
1
2
3
4
5
6
HOURS OF IMBIBITION
Priming treatments
24°C H2O
24°C -1.1 MPa NaCl
10°C H2O
10°C -1.1 MPa NaCl
LSD0.05

Figure 17. Food reserve levels of onion seeds during priming in -1.1 MPa NaCl solution at 10°C. Bars represent standard deviations (S_d).


Alpha-amino acids
Carbohydrates
Lipids
Reducing sugars
mg/g
mg/g
S_d
DAYS IN PRIMING SOLUTION

Primed seeds lost more seed weight and gained more radicle dry weight than raw seeds, 5 days after sowing. At 10°C, primed seeds germinated in 3.7 days, while raw seeds took 6.9 days to germinate (Table 5). This accounted for the differences in seed and radicle weight. Raw seeds had lost 13% and primed seeds lost 10% of their weight, 10 days after sowing (Figure 18). The difference in radicle weight between raw and primed seeds was less than 10 mg on the tenth day after sowing.

Respiration Studies

Effect of solute on respiration. Respiration rates of seeds imbibed in all solutes were the same until 0 to 400 minutes. Thereafter, H_2O imbibed seeds respired significantly more than seeds imbibed in all other solutes (Figure 19). There were no differences in respiration between NaCl and mannitol imbibed seeds.

There were no difference in water content of the seeds between imbibing solutions (Figure 20).

Respiration of primed onion seeds. Seeds primed for 2 days in H_2O, -1.1 MPa NaCl or mannitol solutions had significantly higher respiration rates compared to raw seeds (Figure 21). Respiration of seeds primed in H_2O was approximately 2 times greater than respiration of raw seeds. There were no significant differences in respiration (Figure 21) and water content (Figure 22) between mannitol and NaCl primed seeds.

Effect of duration of priming on respiration. Primed seeds respired more than raw seeds (Figure 23). Increasing the priming

Figure 18. Food reserve levels of raw (dotted line) and primed (solid line) onion seeds after sowing and incubation at 10°C. Bars represent standard deviations (S_d).

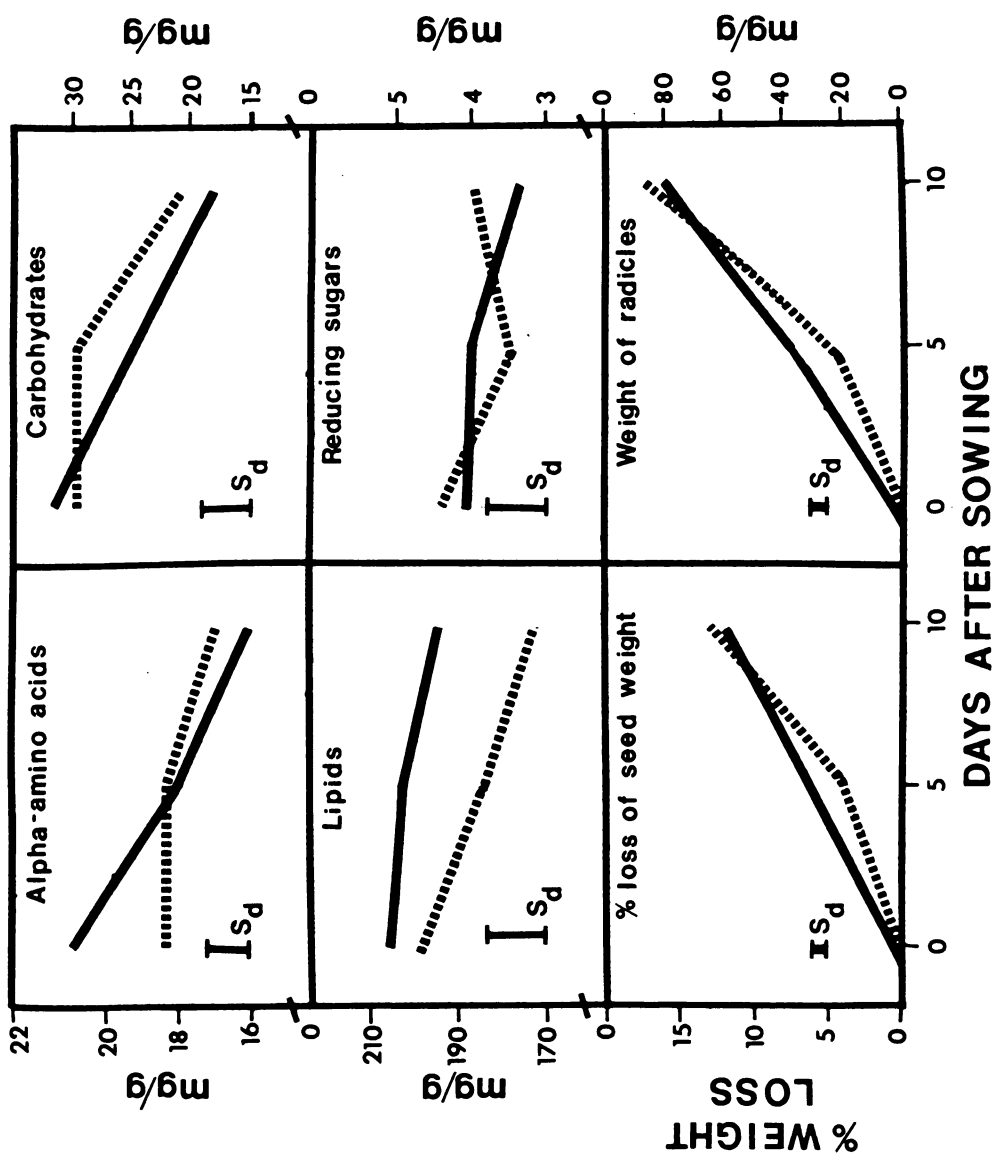

Carbohydrates
Reducing sugars
Weight of radicles
Alpha-amino acids
Lipids
% loss of seed weight
mg/g
mg/g
mg/g
% WEIGHT LOSS
DAYS AFTER SOWING
I_{S_d}

Figure 19. Oxygen uptake of onion seeds during imbibition in various solutions at 10°C. Values are means of 4 replicates.

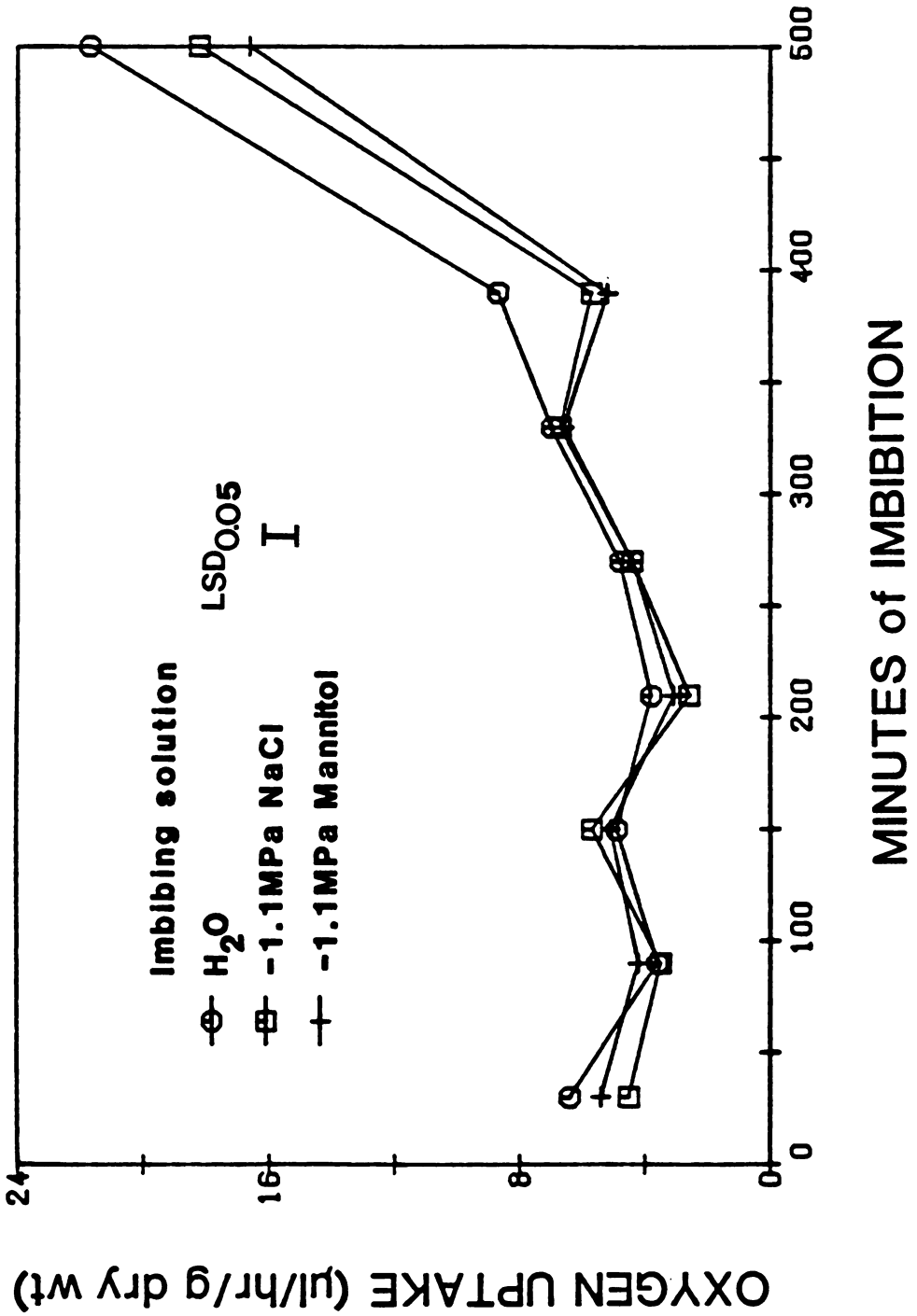

OXYGEN UPTAKE (µl/hr/g dry wt)
24
16
8
0
0
100
200
300
400
500
MINUTES of IMBIBITION
Imbibing solution
H2O
-1.1MPa NaCl
-1.1MPa Mannitol
LSD0.05

Figure 20. Water content of onion seeds during imbibition in various imbibing solutions at 10°C. Values are means of 4 replicates.

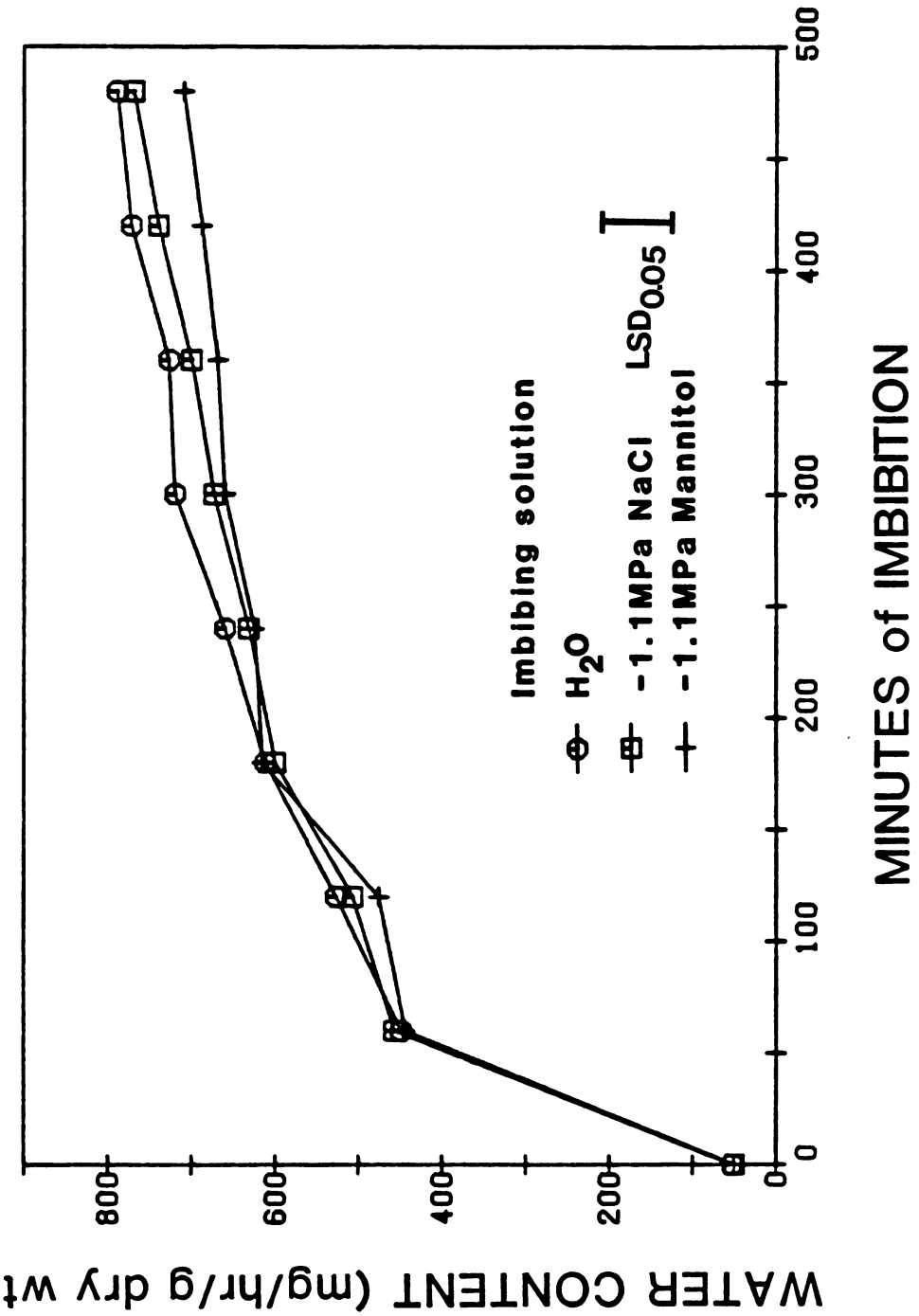

WATER CONTENT (mg/hr/g dry wt)
800
600
400
200
0
0
100
200
300
400
500
MINUTES of IMBIBITION
Imbibing solution
H2O
-1.1MPa NaCl
-1.1MPa Mannitol
LSD0.05

Figure 21. Oxygen uptake of onion seeds at 10°C after various priming treatments. Values are means of 3 replicates.

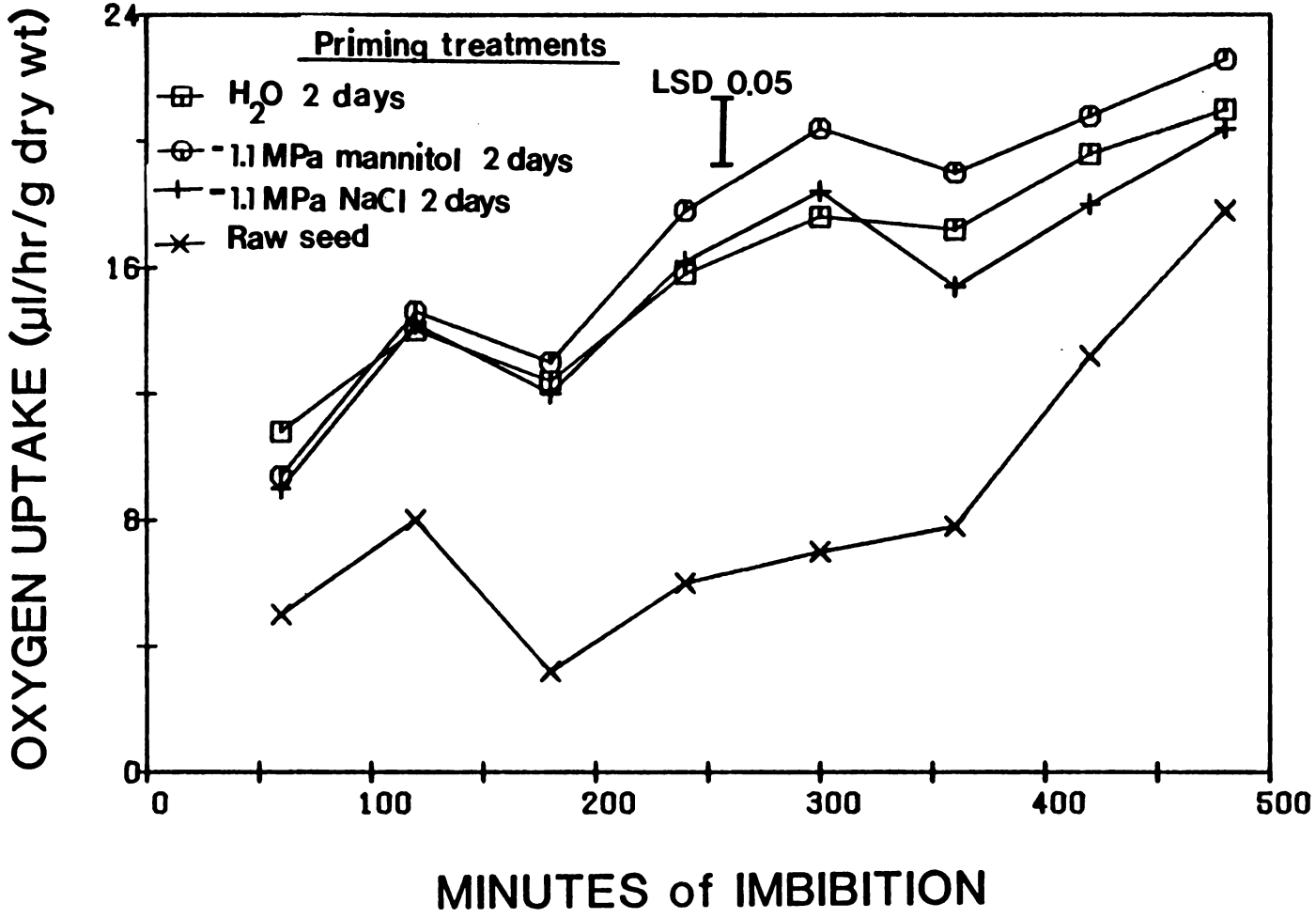

Priming treatments
H2O 2 days
-1.1 MPa mannitol 2 days
-1.1 MPa NaCl 2 days
Raw seed
LSD 0.05
OXYGEN UPTAKE (µl/hr/g dry wt)
0
8
16
24
0
100
200
300
400
500
MINUTES of IMBIBITION

Figure 22. Water content of onion seeds at 10°C after various priming treatments. Values are means of 4 replicates.

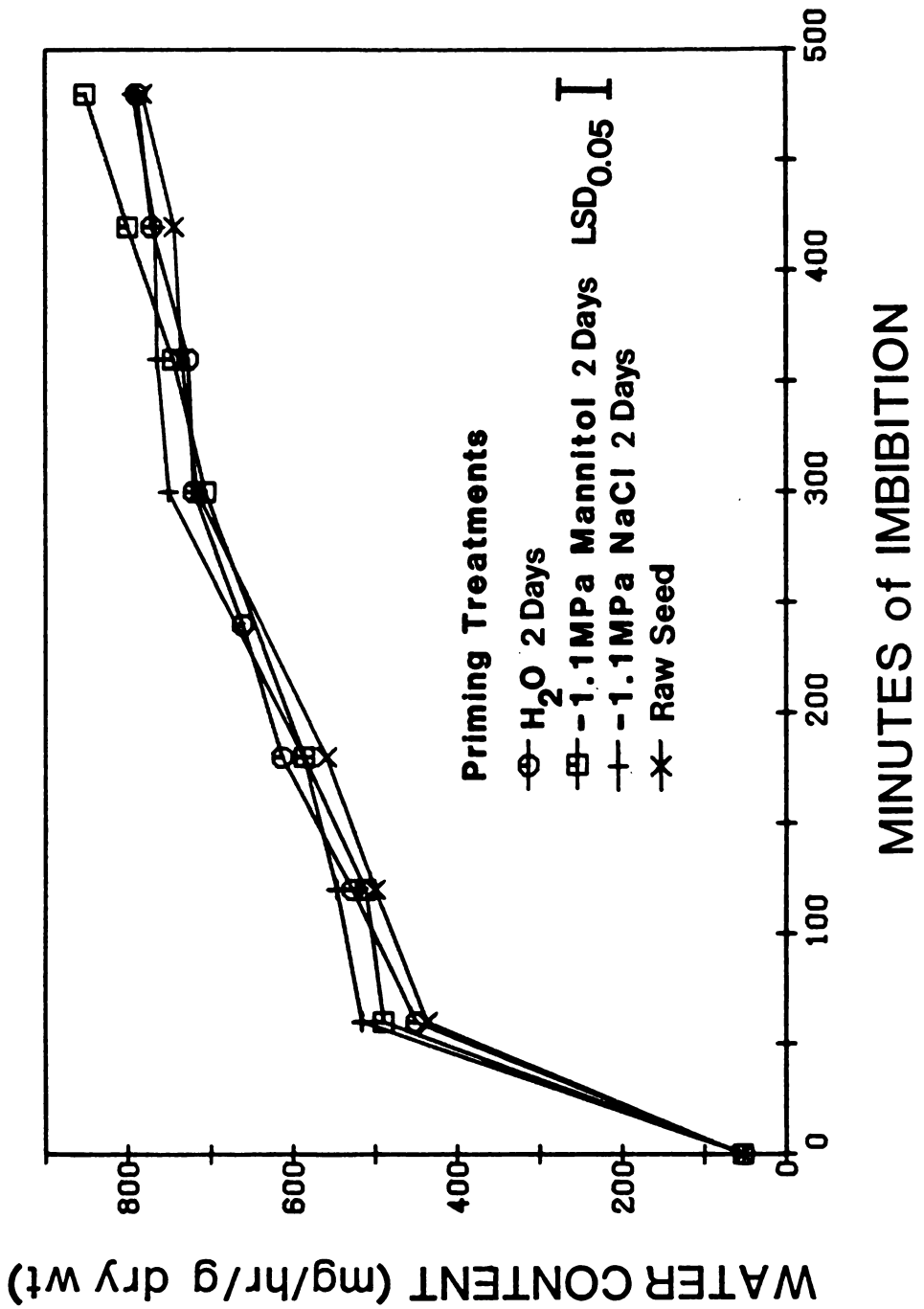
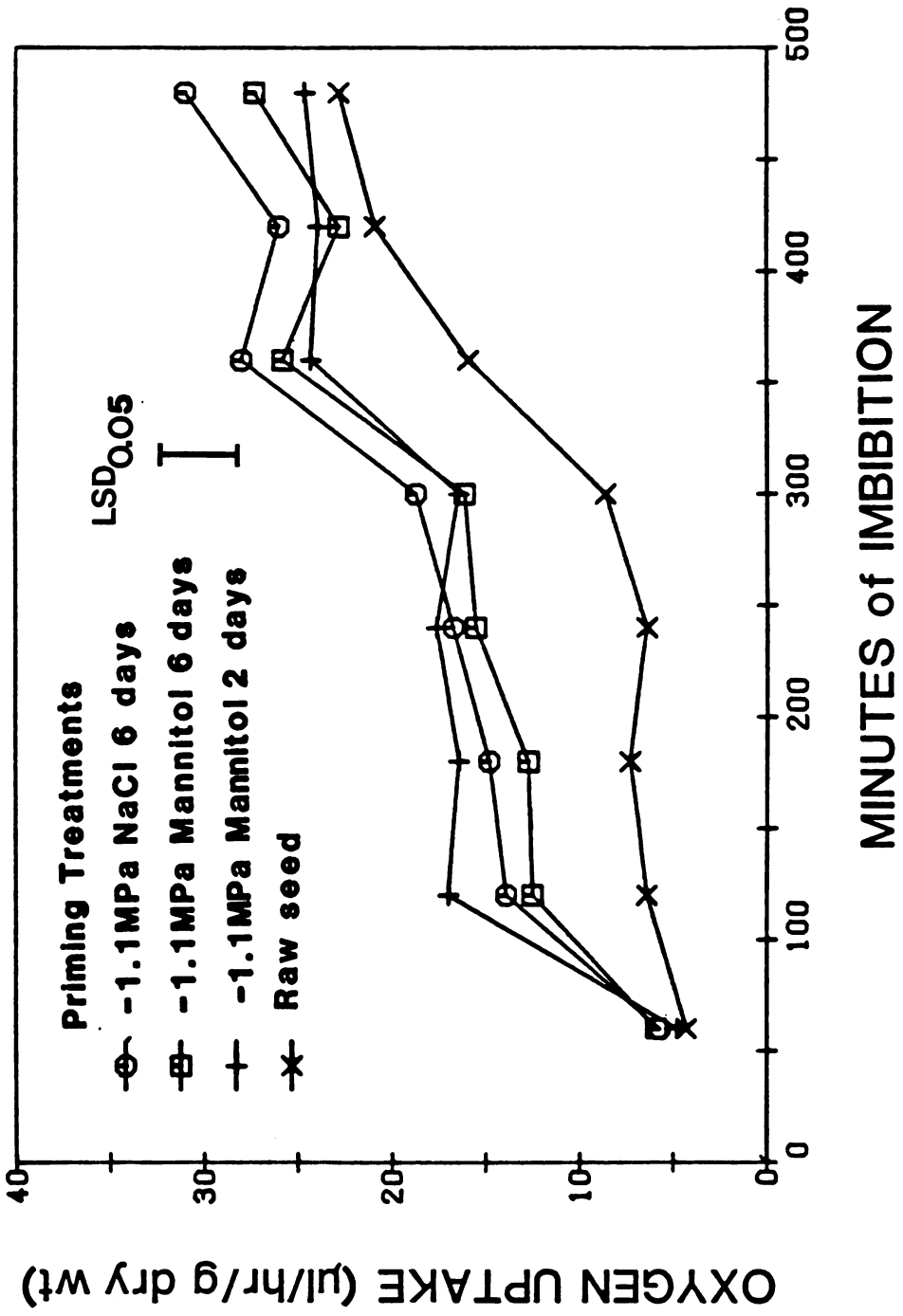

WATER CONTENT (mg/hr/g dry wt)
800
600
400
200
0
0
100
200
300
400
500
MINUTES of IMBIBITION
Priming Treatments
H2O 2 Days
-1.1MPa Mannitol 2 Days
-1.1MPa NaCl 2 Days
Raw Seed
LSD0.05

Figure 23. Oxygen uptake of onion seeds at 10°C after various priming treatments. Values are means of 3 replicates.


OXYGEN UPTAKE (µl/hr/g dry wt)
MINUTES of IMBIBITION
Priming Treatments
-1.1MPa NaCl 6 days
-1.1MPa Mannitol 6 days
-1.1MPa Mannitol 2 days
Raw seed
LSD0.05
0
10
20
30
40
0
100
200
300
400
500

duration from 2 to 6 days did not increase the respiration of mannitol primed seeds. There were no differences in respiration between seeds primed in NaCl or mannitol solutions (Figure 23) or in water uptake among treatments (Figure 24).

Pregermination

The Effect of Temperature and Duration of Priming and Temperature of Pregermination on Onion Seed Radicle Length

The temperature x duration of priming x temperature of germination interaction was significant for radicle length (Figure 25). Seeds primed at 10°C for 8 days had shortest radicles of seeds from all treatments. Generally, increasing the priming duration and temperature produced pregerminated seeds with shorter radicles.

Increasing the priming temperature and duration and pregermination temperature significantly decreased the time to 50% germination (Figure 26). Seeds took the shortest (1.5 days) to germinate when primed for 8 days at 10°C and pregerminated at 10°C. In contrast, nonprimed, raw seeds pregerminated at 2.5°C took 40 days to reach 50% germination.

Percent germination of seeds was not affected by priming duration and temperature and pregermination temperature.

Emergence of Seedlings from Raw, Primed, and Pregerminated Onion Seeds

Seedlings from pregerminated seeds reached 50% emergence 9.2 days after sowing (Table 6). Primed and raw seeds took 13 and

Figure 24. Water content of onion seeds at 10°C after various priming treatments. Values are means of 4 replicates.

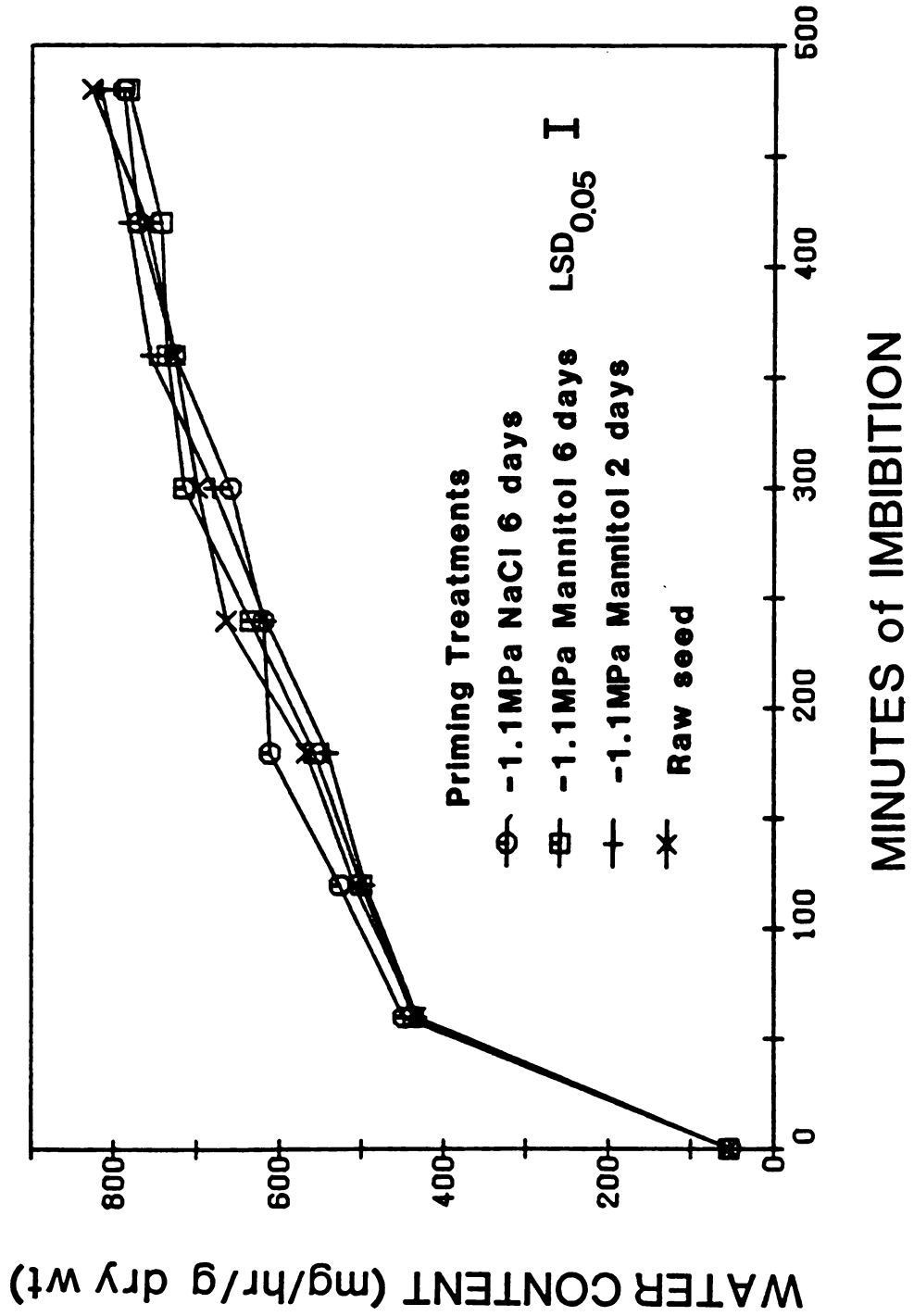

WATER CONTENT (mg/hr/g dry wt)
800
600
400
200
0
0
100
200
300
400
600
MINUTES of IMBIBITION
Priming Treatments
-1.1MPa NaCl 6 days
-1.1MPa Mannitol 6 days
-1.1MPa Mannitol 2 days
Raw seed
LSD0.05

Figure 25. Three-way interaction of temperature of priming by days primed by germination temperature for radicle length. Germination temperatures were 10 or 2.5°C Five°C treatment is not shown due to similar response to 2.5°C.

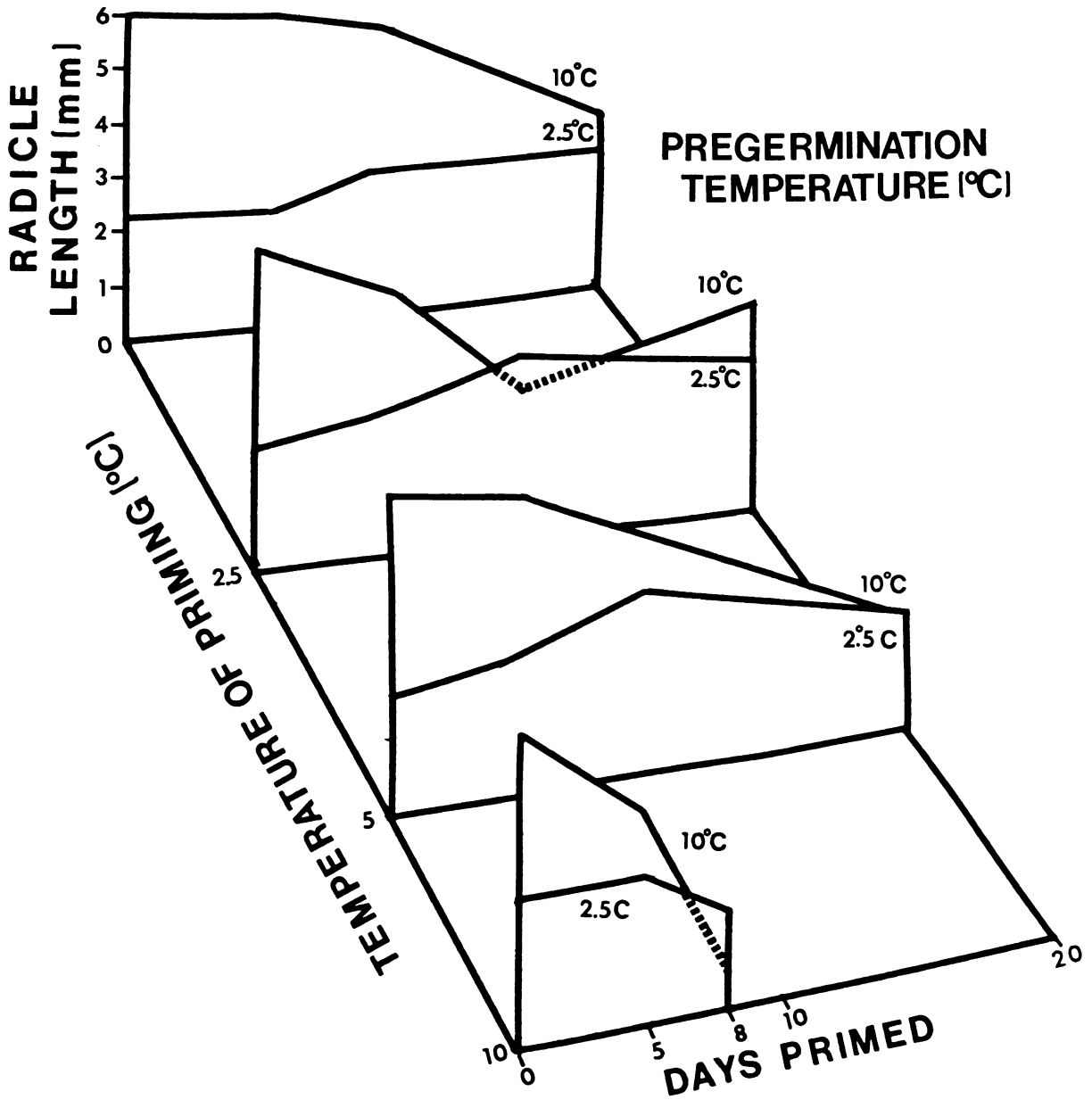

RADICLE LENGTH (mm)
6
5
4
3
2
1
0
10°C
2.5°C
PREGERMINATION TEMPERATURE (°C)
10°C
2.5°C
10°C
2.5°C
10°C
2.5C
TEMPERATURE OF PRIMING (°C)
2.5
5
10
0
5
8
10
20
DAYS PRIMED

Figure 26. Three-way interaction of temperature of priming x days primed x germination temperature for days to 50% germination. Germination temperatures were 2.5, 5, and 10°C.

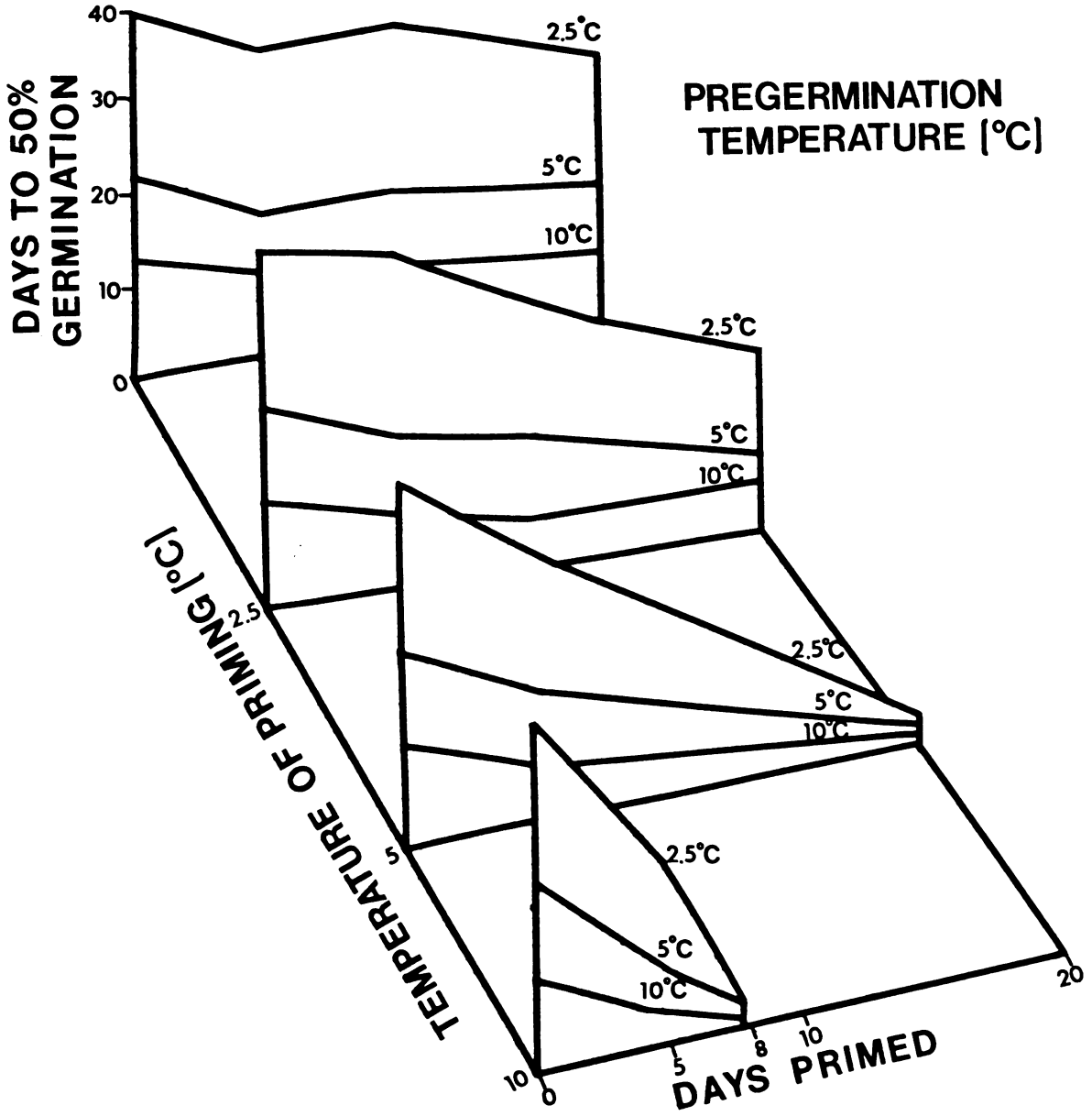

DAYS TO 50% GERMINATION
40
30
20
10
0
PREGERMINATION TEMPERATURE (°C)
2.5°C
5°C
10°C
2.5°C
5°C
10°C
2.5°C
5°C
10°C
2.5°C
5°C
10°C
TEMPERATURE OF PRIMING (°C)
2.5
5
10
0
5
8
10
20
DAYS PRIMED

Table 6.--Emergence of seedlings from raw, primed, and primed-pregerminated onion seeds.

Treatments	Days to 50% Emergence	Spread of Emergence (days)	Percent[y] Emergence
Primed-pregerminated	9 a[x]	2.4 a	77 a
Primed	13 b	2.4 b	75 a
Raw seed	15 c	4.0 c	74 a

[x]Means within columns separated by Duncan's multiple range test, at 5% level.

[y]$(\arcsin)^{\frac{1}{2}}$ transformation used for analysis of variance.

15 days to 50% emergence, respectively. Pregerminated seeds produced the most uniform seedling emergence (lowest spread of germination) compared to primed and raw seeds. Pregermination and priming had no effect on percent emergence.

Discussion and Summary

Seeds primed in -1.1 MPa mannitol or NaCl for 8 days at 10°C germinated faster and more uniformly than seeds primed with other treatments, without reducing total germination (Table 5). However, PEG 6000 was not as safe as mannitol or NaCl for priming onion seeds, since total germination was reduced after 48 hr of priming (Table 3). The reduction in germination of onion seeds primed in PEG 6000 may have been due to an insufficient oxygen supply in the priming solution. At 10°C, oxygen is 69% less available in -1.1 MPa PEG 6000

than in water (73). This low oxygen level may have induced anaerobiosis in some seeds. Anaerobic seeds accumulate ethanol which is toxic to the cells of the seed and may eventually lead to seed death (70). Furthermore, the high viscosity (4.91 centipose) of -1.1 MPa PEG 6000 makes it difficult to aerate, resulting in poor oxygen dispersion (73). Osmotic potentials of -1.1, -1.3, and -1.7 MPa did not affect the number of days to 50% germination or total germination of onion seeds (Table 4). However, increasing the osmotic potential of the priming solution lengthened the number of days to 3 to 4% germination. Thus, the lowest osmotic potential, -1.1 MPa, was utilized priming the onion seed.

Seeds primed at 10°C in NaCl or mannitol at all osmotic potentials and durations germinated faster and more uniformly than seeds primed at 24°C (Table 4). At 10°C, seeds required 8 days of priming to reach 3 to 4% germination, but required only 4 days of priming at 24°C. However, 10°C-primed seeds germinated faster and more uniformly, with greater total germination than 24°C-primed seeds after drying and rehydrating (Table 4). A possible explanation for these germination differences is that 24°C-primed seeds may have been exposed to anaerobic conditions. Seeds primed at 24°C apparently respire at a greater rate than at 10°C and oxygen availability for the seeds may have been inadequate (91). Thus, seeds primed at 24°C may have been injured or killed during priming by anaerobiosis which resulted in slower and reduced total germination in comparison to 10°C-primed seeds (Table 4).

Priming seeds for 8 days resulted in faster and more uniform germination after drying and rehydration than priming for shorter

periods (Figures 10, 11). Many seeds germinated on the ninth day, indicating that most of the seeds had reached maximum priming in 8 days. Priming durations less than 8 days may not have brought the seeds up to a point just prior to germination, and upon rehydration they germinated slower and less uniformly than 8-day-primed seeds.

Seeds primed for 2 days used more oxygen than raw seeds during 5 hr of imbibition at 10°C (Figure 21). Priming may have caused seeds to accumulate biochemical compounds required for germination, thus speeding the respiration process after imbibition. For instance, primed seeds are known to contain greater quantities of ribosomal RNA than raw seeds; ribosomal RNA is essential in the production of various compounds such as enzymes necessary for seed germination. Increasing the priming duration to 6 days, however, did not significantly increase oxygen uptake (Figure 23). Evidently, oxygen uptake of seeds was at maximum after 2 days of priming.

Primed seeds emerged faster than raw seeds and therefore would be expected to utilize more food reserves during germination (Table 6). However, there were no differences in food reserve utilization between primed and raw seeds during priming and 10 days of germination (Figures 17, 18). It appears that food reserve depletion is not a limiting factor in priming or pregermination processes.

Leakage studies confirmed that increased speed and uniformity of germination of primed seeds was not a result of weakening the seed coat. If seed coats were weakened by priming, leakage rates would have been greater during reimbibition. However, primed seeds leaked at a lower rate than nonprimed seeds (Figure 12). Two explanations

are plausible: (1) cellular and subcellular membranes may have become more organized during priming, thereby reducing solute leakage during rehydration; (2) leachable solutes were leached-out during priming so that solute leakage was reduced during rehydration.

In addition to priming temperature, duration, and solute, temperature of germination affects radicle length (Figure 23). Germination of primed seeds at 10°C resulted in short radicles and reduced the number of days to 50% germination in comparison to germination at 2.5 and 5.0°C (Figures 25, 26). Germination at 10°C appeared to have continued the same process as priming at 10°C, which produced faster and more uniformly germinating seeds.

This study shows that 2 methods could be used to improve the speed and uniformity of emergence of onion seedlings under cold soil conditions in the field: (1) priming seeds in -1.1 MPa NaCl or mannitol for 8 days at 10°C offers the advantage of greater speed and uniformity of emergence of seedlings in comparison to raw seeds (Table 6). An added advantage of using primed seeds is that a conventional seeder could be used; (2) these primed seeds could be germinated at 10°C in H_2O for 2 days to obtain pregerminated seeds with short, uniform radicles. Pregerminated seeds emerge with even greater speed and uniformity than primed and raw seeds (Table 6). A fluid drill planter could be used to sow these pregerminated seeds, since the seeds' radicles are short and would not be damaged during sowing.

The major findings of this study are summarized as follows.

Priming Onion Seeds

There were no differences between solutes used for priming, except for PEG 6000, which reduced germination. Osmotic potentials of -1.1 to -1.3 MPa for 8 days at 10°C were optimal for priming onion seeds.

Leakage Studies

Leakage from onion seeds was reduced after priming.

Food Reserve Utilization Studies

There were no changes in food reserves of onion seeds during priming. Food reserve utilization was similar for primed and raw seeds during germination.

Respiration Studies

Primed seeds had higher respiration rates than raw seeds after inbibition in water at 10°C. There is a limit to the increase in respiration obtained by priming.

Pregermination Studies

Temperature and duration of priming and temperature of pregermination affected radicle length. Priming for 8 days at 10°C and germinating at 10°C reduced radicle length fourfold over 10°C germinated controls. Pregerminated seeds emerged earlier and more uniformly at 10°C than primed and raw seeds.

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