Relationship of endosperm exhaustion to growth and survival of orchardgrass seedlings (Dactylis glomerata L.) subjected to cold stress
by Glen A Murrary

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
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Abstract:
Endosperm exhaustion of two, populations of orchardgrass (Dactylis glomerata L.) was determined for temperatures of 10, 15, and 20°C. using percent dry weight loss and seedling length measurements as criteria.

Endosperm exhaustion occurred between 14-16 days at a temperature of 20°C., between 16-18 days at 15°C., and between 22-24 days at 10deg;C.

Rate of shoot elongation increased relative to root elongation with increasing temperature but root elongation and shoot elongation were identical at endosperm exhaustion for all temperatures.

Seedling length and weight loss were significantly correlated at all temperatures, with a weight loss associated with an increase in seedling length. For a given unit of weight loss, seedling length increase was constant regardless of temperature.

Seedling injury to. cold stress was significantly dependent on seedling age, rate of endosperm exhaustion, and population.

Injury was progressively greater to seedlings at emergence, endosperm exhaustion, and post-endosperm exhaustion stages of development.

Seedlings having a faster rate of endosperm exhaustion exhibited more leaf injury by cold stress than seedlings having a slower rate of endosperm exhaustion. Seedling survival was greater when seedlings had a faster endopserm exhaustion rate than when seedlings had a slower endosperm exhaustion rate.

The population best able to germinate against a moisture stress recieved more cold injury than the population least able to germinate against a moisture stress.

# RELATIONSHIP OF ENDOSPERM EXHAUSTION TO GROWTH AND SURVIVAL OF ORCHARDGRASS SEEDLINGS (*DACTYLIS GLOMERATA* L.) SUBJECTED TO COLD STRESS

by

Glen A. Murray

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Approved:

A. W. Post
Head, Major Department

Robert J. Eslick
Chairman, Examining Committee

Louis D. Smith
Dean, Graduate Division

MONTANA STATE COLLEGE
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TABLE OF CONTENTS

# LIST OF TABLES

LIST OF FIGURES

## ABSTRACT

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# INTRODUCTION

Growth and survival of orchardgrass (Dactylis glomerata L.) (7) seedlings subjected to cold stress is dependent upon environmental and physiological conditions before and after the cold stress. Environmental conditions such as extreme temperature changes are probably most influential in regulating plant survival. At the same time, growth conditions before and after cold stress occurs are important. Seedlings germinated and grown under warm conditions may be different structurally and physiologically from seedlings germinated and grown under cool conditions. Because of these differences, they may react differently when subjected to a temperature stress.

Seedling age at the time of cold stress occurrence is important. Seedling age may be divided into three stages: (1.) pre-endosperm exhaustion or emergence, (2.) endosperm exhaustion, and (3.) post-endosperm exhaustion. Endosperm exhaustion was considered to be the point at which weight loss or seedling length increase ceased.

The objectives of this study were (1.) to determine the effect of temperature upon rate and time of occurrence of endosperm exhaustion, (2.) to determine the effect of rate of endosperm exhaustion upon seedling growth and differentiation, and (3.) to determine the effect of rate of endosperm exhaustion and physiological seedling age upon survival of seedlings following cold stress.

Information from objective (1.) is prerequisite to initiation of objectives (2.) and (3.), and as a result of many problems encountered with technique, a major portion of the following discussion concerns

development of techniques necessary to proceed with objectives (2.) and (3.).

# LITERATURE REVIEW

Endosperm exhaustion studies are somewhat limited in number and a majority of those reported utilized respiration measurements as the basis for determining endosperm exhaustion. Endosperm exhaustion determinations utilizing dry weight loss or seedling length as a criterion for evaluation were found only for large seeded plants.

Pope (21) determined ensosperm exhaustion of two varieties of barley based upon dry weight loss and shoot length measurements. The period during which the weight-growth rate and length-growth rate tapered off was considered endosperm exhaustion. Endosperm exhaustion occurred in approximately 25 days for the Hannchen variety and 22 days for the Tennessee Winter variety. These barley varieties were grown in soil under natural conditions with a mean temperature of 52°F.

Stiles and Leach (31), using $CO_2$ as a measure of respiration, divided seedling growth into five phases. These phases were: (1.) a fairly rapid increase in respiration rate as seed absorb water, (2.) a period of constant respiration rate which continues until the seed coat ruptures, (3.) a very rapid rise in respiration rate following rupture of the testas, (4.) a period of approximately constant respiration rate, and (5.) a slowly diminishing respiration rate.

James and James (10) gave a similar breakdown of a respiratory curve of barley. Starting with the seed which showed low carbon dioxide output, their curves were divided into five phases. A gradual rise in respiratory intensity characterized the first phase in which water was absorbed, the embryo develops rapidly, and the embryonic reserves are

used. In the second phase, carbohydrate reserves were mobilized and reached a maximum on the seventh day as shown by a respiratory rise. Greatest respiration intensity was reached between the second and third phase. The third phase showed a decline in respiration rate due to decreased endosperm reserves. Respiration was further decreased as a mixture of reserves were utilized in the fourth phase. The fifth phase showed a slight respiratory increase due to action of saprophytic organisms on seedling material. Earlier work by Rishcarvi (22) showed that wheat seedling reached a maximum respiration rate on the 12th day at 21°C.

Mayer (16) measuring respiration in terms of oxygen uptake, found that respiration rate increased to a maximum and then declined. Maximum respiration rate was reached in 15 days at a temperature of 11.8°C. and in 7 days at 23.8°C.

In general, respiration rate increased from the time of water uptake by the seed to a maximum, which is temperature dependent. As reserve material was less available to actively growing regions, the respiration rate gradually diminished. Upon exhaustion of endosperm reserves respiration rate from the seedling itself was very low. These seed are similar chemically to orchardgrass, and one might expect a similar respiratory curve.

During seed germination heat is released and extensive transformation and movement of materials from storage organs to the growing seedling occurs (3). Both processes involve energy.

Terroine et al. (34) found that energy efficiency of germination for

seeds rich in starch was 73 percent, for seed high in protein, 63 percent, and for seed high in fats, 53 percent. Energy efficiency was defined as $E/E_1 - E_2$ in which, E was the heat of combustion of the seedling, $E_1$ heat of combustion of the ungerminated seed, and $E_2$ heat of combustion of the seed at a given stage of germination. These authors concluded that energy efficiency for a given seed was determined by chemical composition. A temperature increase hastened germination but did not change the energy efficiency for a given stage of germination. The same energy efficiency was shown at every stage of germination, providing storage reserves were not limiting.

James (9) and Toole (35) have shown that reserves of the embryo are utilized first. This was followed by utilization of endosperm immediately surrounding the embryo. The transformation of material in the seed depends on chemical composition. Much of the fat was oxidized to carbohydrate and transported to various active regions. The carbohydrates, principally starch, in orchardgrass are transformed to soluble carbohydrates and translocated. In barley, sucrose was found to be the primary sugar utilized for respiratory energy, (9).

Although temperature apparently does not affect energy efficiency of a given seed, Loomis (15), Sprague (29), and Went (37), have shown that temperature does have a pronounced effect on shoot and root growth.

Loomis (15) suggested that root growth tends to be limited by supplies of carbohydrates and other growth materials from the top, and growth of the shoot limited by supplies of water and minerals obtained through

the root.

Sprague (29) found as temperature increased to the optimum range, dry matter of tops and roots of orchardgrass increased. Rate of increase of top growth was more rapid than the rate of increase of root growth.

Work done by Roberts and Struckmeyer (23) showed that most but not all species tested had more roots in relation to tops in cooler temperatures as compared to warm. These authors suggested, as did Went (37), that the composition and reserve conditions within the top, control production of roots and thus shoot-root ratios.

Went (38) reported evidence for reduced translocation of sugar as temperature increased. There is some speculation as to what range of temperature is influential, however.

Hewitt and Curtis (6), working with tomatoes, found that translocation rate decreased with a temperature rise above 25°C. Other workers found that translocation of sugar decreased as temperature increased above 2°C. (8, 38).

The above data tend to show that maximum root production occurred at lower temperatures than shoot production. Went (37), stated that each morphological and physiological process seems to have an optimum temperature. Stem and root elongation and translocation rate may and apparently do differ in optimum temperature ranges. The effect of soil temperature on emergence in relation to root and shoot production is thus important.

Dubetz et al. (4) tested the rate and percentage emergence of 19 native and cultivated herbaceous species at soil temperatures of 6, 13,

18, and 24°C. The emergence of all species was greater at a temperature of 18°C. than at 6°C. Orchardgrass emergence was significantly higher at 18°C. than at 24°C.

References on the effect of soil temperature on emergence force were not found. Williams (40) evaluation of emergence force of small seeded legumes provides a technique for such a determination. By the use of glass push rods, Williams evaluated emergence force by determining the weight moved upwards by germinating seedlings. A correlation of 0.999 between seed weight and emergence force was found.

Freezing temperatures influence seedling growth. The tolerance of plants to freezing temperatures was influenced by temperature before, during, and after freezing; soil moisture; light; and mineral nutrition. Physiological factors, such as seedling age, growth rate, and hardening ability are important (13). Two types of freezing can occur in plant tissues, intracellular and extracellular. Intracellular ice formation almost always results in death of the tissue (26). This type of freezing does not commonly occur in nature. Siminovitch and Scarth (26) found intracellular ice formation to occur only when rapid freezing occurs. They found intracellular ice formation in potted plants when temperature dropped from 0 to -10°C. in one-half hour. Intracellular ice formation was found to occur less frequently in hardy tissues because of increased permeability of protoplasm to water (25, 27).

Extracellular ice formation was more common than intracellular ice formation and was induced by slowly lowered temperatures. This type of

freezing can occur in all cells and was nearly always fatal in non-hardy cells. Stucky and Curtis (32) state that injury in this case seems not to be due to the quantity of ice formed but to an indirect effect of water removal. Levitt (13) suggests that water removal from cells results in two possible explanations of dehydration injury: (1.) salt precipitation of proteins and (2.) mechanical injury of the protoplasm. Mechanical injury is thought to be the most important form of injury caused by frost dehydration. According to Levitt, two factors are involved in mechanical injury: (1.) frost dehydration leads to a progressive increase in protoplasmic consistency until under extreme dehydration it becomes brittle, and (2.) due to water removal, from the cell as a whole, contraction subjects the protoplasm to tensions.

Scarth (25) listed three main types of freezing injury: (1.) intracellular freezing, (2.) mechanical effects of freezing and thawing when ice was extracellular and (3.) physico-chemical effect of dehydration. The first occurs at the moment of rapid freezing of the tissue. The second occurs during temperature fluctuations. The third occurred at the critical low temperature which marked the limit of frost endurance of the cell and was probably the most common cause of death.

Resistance to these types of injury are summarized below. Intracellular freezing tends to be prevented by increased permeability to water. Thus water moves out of the cell more easily, leaving carbohydrates and other solutes behind, and promotes ice growth outside of cells. Mechanical injury during freezing and thawing is principally prevented by the reduced structural viscosity of the cytoplasm. Proto-

plasmic hardening results in increased bound water, reduced coagulability, and an increase in protein. These changes may do much to prevent dehydration injury (25).

The relationship of seedling age and growth conditions to freezing injury prior to freezing has been widely studied. Laude (12), working with several grasses including orchardgrass, found that seedling emergence was reduced by a freezing temperature of 20°F. As seedlings approached emergence, they were more susceptible to cold injury. Increasing delay of emergence was also associated with freezing at progressively later pre-emergence stages. Arakeri and Schmid (1) and White and Horner (39) found grasses tested were more susceptible to injury in early growth stages. Early growth stage was defined as plumule emerged. After emergence, grasses were again found to be more resistant to freezing injury. After the seedlings have attained the 2-3 leaf stage, some of the grasses again become more susceptible to freezing injury. The same type of result was obtained by Peltier and Kiesselbach (19) who attributed exhaustion of reserves as the factor influencing susceptibility in the 2-3 leaf stage. Suneson and Peltier (33) report the youngest winter wheat plants regardless of hardening appear to be the most cold resistant. They noted one exception: wheat in the 3-4 leaf stage had increased susceptibility to cold stress. They attributed this to wheat seedlings being on the verge of endosperm exhaustion.

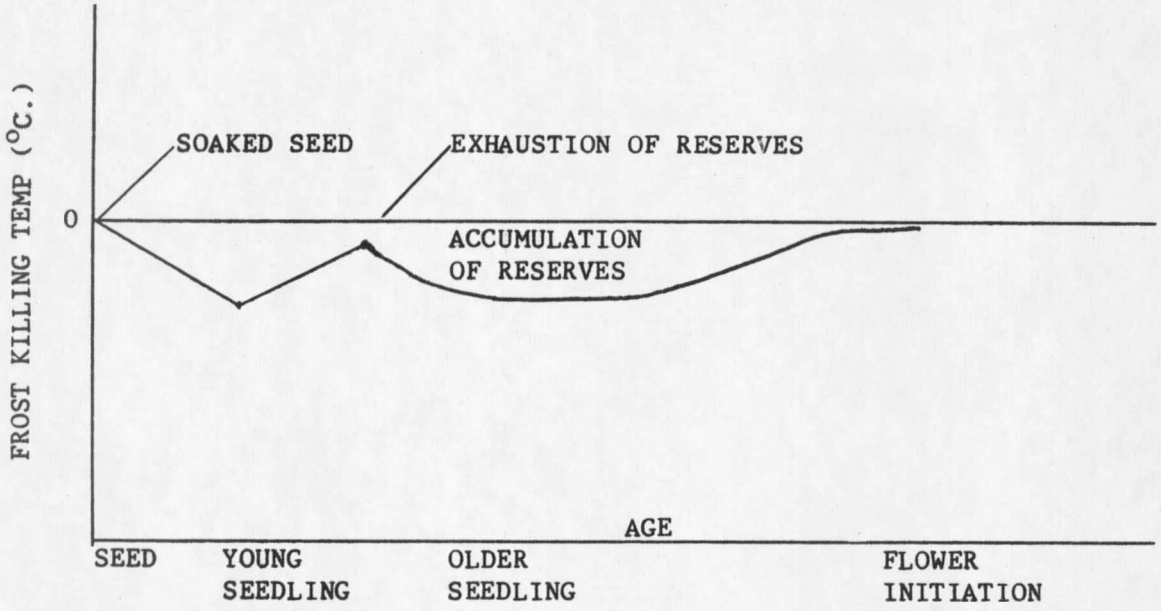
Worzella and Cutler (41), on the other hand, show that germinated seed or seedlings of wheat in the one leaf stage or coleoptile stage are

quite susceptible to cold. Winter wheat seedlings having 2-4 leaves show an increase in winter survival; those possessing 5-15 leaves per plant are the most resistant.

Peltier and Kiesselbach (19), working with seedlings of oats, barley, and spring wheat found that (1.) seedlings were more resistant to cold injury when emerging from the soil than in the 2-3 leaf stage, and (2.) seedlings manifest the least cold endurance when food reserves of the endosperm are depleted. They further state resistance to cold decreases progressively with increasing age and size of the seedling until a minimum was reached at endosperm exhaustion. After this time, cold resistance again increased.

Levitt (13) cites a considerable amount of evidence showing younger tissues being more resistant to cold injury than older tissues and also much evidence to the contrary. He lists the following reasons for some of the apparent contradictions involving seedling age: (1.) young seedlings to one author may be old to another, (2.) uniform freezing conditions which are needed to compare injuries of different studies are lacking, and (3.) plant mechanisms differ in response to cold. An example of the latter point; some woody plants apparently translocate water to older tissues as freezing occurs, thus protecting the youngest tissues from rapid freezing.

This diagram by Levitt (13), was based on a summary of frost injury in relation to seedling age.




An often overlooked factor influencing seedling survival at time of freezing is soil moisture content. Platt (20) and Tysdal (36) found soil moisture to have an important effect on freezing injury of grain and alfalfa, respectively.

Good correlations have been found between frost and drought hardiness in many plant species. Levitt (13) lists the following reasons for these correlations:

1. When plants become drought hardy due to reduced water supply, they also become frost hardy.

2. When plants become frost hardy due to low temperature exposure, they also become drought hardy.

3. Changes in hardiness with development are similar.

4. At least many of the same physiological changes occur during frost and drought hardening.

5. Small cell size is correlated with frost and drought resistance.

Poor correlations between frost and drought hardiness may arise from poor terminology (17). Drought evasive, drought enduring, and drought resistant plants must be first properly segregated before true comparisons between drought and frost hardiness can be made. Field measurements on cold injury may measure all possible effects of frost injury, which, unless due to dehydration, can not be comapred to drought injury.

For good artificial freezing trials, standardization of procedures are needed before results of various studies can be accurately evaluated. Levitt (13) proposes the following conditions for artificial freezing trials: (1.) plants must be frozen, not undercooled, (2.) freezing must be at a standard rate, (3.) a single freeze must be used for a standard length of time, (4.) thawing must be at a standard rate, and (5.) the conditions after thawing must be standardized.

## MATERIALS AND METHODS

### Seed Source and Preparation

Seed from two populations of orchardgrass were used. One population is made up of plants procurred from seed that had the ability to germinate against a mannitol moisture stress. Plants of the other population were from seed that did not have the ability to germinate against a moisture stress. Seed from these plants were selected that gave the most extreme response for their respective populations.

Seed were harvested at maturity from individual plants of each population in July and August, 1962. Endosperm exhaustion trials began in October, 1962 and were completed in October, 1963. All seed not being used were stored in metal cans at a temperature of approximately 15°C.

For all endosperm exhaustion trials, seed were prepared using the following method. Seed were cleaned, halved in a Gamet divider until the desired weight of seed was obtained and blown by a South Dakota blower to remove all inert material. All light weight seed were removed from inert material and thoroughly mixed with heavier seed. Multiple florets were removed to reduce error due to the weight of sterile florets.

Rate of endosperm exhaustion was measured using dry weight loss and length of seedlings grown in the darkness as measurement criteria. The point at which weight loss or seedling growth ceased was considered to be endosperm exhaustion. Since technique difficulties were encountered almost immediately, three successive trials were conducted. Procedures were varied in each succeeding trial in an attempt to overcome difficulties previously encountered.

Endosperm Exhaustion Trials

Trial 1.

Following seed preparation, seed from each population were counted into 10 lots of 100 seed. Each seed lot was weighed to the nearest ten-thousandth of a gram and placed on filter paper.

Since roots of germinating seedlings penetrate filter paper, it was expected that a source of error would occur when seedlings were removed from it. To avoid this error each sheet of paper was weighed. When seed were removed from the germinator, both filter paper and seed were weighed. The weight of the germinated seed were obtained by subtracting the original weight of the paper.

The filter paper and seed were placed in covered germination boxes, moistened, and randomly placed in darkened germinators at 15 and 30°C. Filter paper and seed and/or seedlings were placed in weighing bottles, oven dried at 70°C., cooled in a desiccator, and dry weight loss determined. All seed were weighed during the first six days, after this time non-germinated seed were counted and removed. Dry weight loss was determined by adjusting the weight of the germinated seed to a 100 seed weight basis. Root and shoot measurements were made on the tenth and twentieth day.

This trial had much variation in weight loss which was thought to be due to (1.) a large percentage of the weight (85%) being filter paper, making small weight losses of seedlings subject to weighing errors of the filter paper, (2.) extra weighings involved in weighing filter paper, (3.) variation in seedling growth due to location within germinator, and

(4.) weight adjustments made to compensate for germination percentage.

Trial 2.

In order to remove the error encountered in Trial 1 attributed principally to the use of filter paper substrate, a germination medium enabling seedlings to be removed without loss of roots was needed. Experiments with several media showed that blotters wrapped with heavy paper towel permitted seedling removal without loss of roots. This medium was used in subsequent trials.

In an effort to reduce location effect within the germinators, sample size was decreased to reduce space required within the germinators.

Correcting weight of germinated seed to a 100 seed basis was thought to give variations in seed weights. It was not known whether the lightweight seed or heavier seed germinated first. If heavier seed germinates first, correcting the weight of seed to a 100 seed basis results in an over correction. If lightweight seed germinated first, under correction of seed weight results. Results from other trials have shown that correlations between seed weight and rapidity of germination variable.

Weighing non-germinated seed resulted in other problems. Seed, although not germinated, were still undergoing respiration. In the later portion of trials, these seed were often covered with mold, and weight loss can be attributed to respiration of these organisms. Since the weight of these non-germinated seed does not remain constant, the weight of these seed could not be subtracted from the original oven dry weight to give weight of germinating seed. To remove this probable source of error, all seed were weighed regardless of germination.

With these modifications, Trial 2 was started at temperatures of 7 and 15°C. Temperatures were reduced because of poor germination encountered at 30°C. in Trial 1. Seed from each population were divided into two weight classes, and each weight class germinated at two temperatures thus providing 8 treatments. Seed lots of 10 represented the experimental unit. Eight lots, one of each treatment, were removed in duplicate on the fourth and eighth day, and at 2-day intervals following the eighth day.

Root and shoot measurements were made on days 10, 15, 17, 19, and 22 on 20 seedlings from each treatment.

Results were again variable and attributed mainly to small sample size. Root and shoot measurements were more consistent than those of the first trial.

Trial 3.

A third trial was started using larger seed samples and employing rotation of boxes within the germinator to remove location effect. Since both populations previously showed similar weight loss patterns, only seed from the population least able to germinate against a moisture stress were used in this trial. Seed were counted into 120 lots of 100 seeds each for germination at 10°C., 50 lots of 100 seeds each for germination at 15°C., and 80 lots of 100 seeds each for germination at 20°C. Each lot of seed was placed on blotters wrapped with heavy paper towel, put into covered germination boxes, and moistened. Boxes were randomly placed in germinators at 10, 15, and 20°C. The lots were randomly removed in groups of five at 2-day intervals for the first 10 days at

10°C., and first 8 days at 20°C., and at one-day intervals for the remainder of the trials (18 and 12 days, respectively). Seed lots were removed in groups of five at 2-day intervals for the 15°C. trial for 20 days. Blotters were watered periodically to insure sufficient moisture.

Weight loss was determined by weighing the entire 100 seed instead of removing non-germinated seed as in Trial 1. This practice resulted in less variation in weight loss.

All seed and/or seedlings were dried at 105°C. in Trial 3. Calculations of weight loss were made as follows:

Initial weight of seed x % oven dry matter = Initial oven dry weight

Initial oven dry weight - oven dry weight of germinated seed and/or seedlings = dry weight loss

(Dry weight loss / Initial oven dry weight) x 100 = % dry weight loss.

Root and shoot measurements were made at 2-day intervals for all temperatures. Seedlings, five from each box, were removed, placed on a moistened table top, pressed flat, and length of roots and of shoots measured with a compass. This distance was scribed on graph paper. This process was repeated for each measurement and average length of seedlings was determined from the total scribed distance on paper.

Correlations between seedling length and weight loss were made to determine usefulness of total seedling length as a measure of weight loss.

Cold Stress Trial.

Seed fractions were segregated from each population using a South Dakota blower to insure uniform seed weights between populations. Seed

from each population were planted one-half inch deep in vermiculite placed in 2" x 2" jiffy pots. Ten seed were planted per pot. The pots were placed in growth chambers at 10 and 20°C. under 600 foot-candles illumination. Under each temperature regime, there were 72 pots of seed, 36 with seed best able to germinate against a moisture stress and 36 with seed least able to germinate against a moisture stress. Within each population there were three age groups each having two rates of temperature controlled endosperm exhaustion. Each treatment was replicated four times.

The three ages of seedlings were established by watering seed with a standard nutrient solution beginning at pre-determined intervals. Seedling age at both temperatures correspond in terms of endosperm utilized; that is, age one of the 10°C. temperature corresponds to age one of the 20°C. temperature at time of cold stress application. This same relationship holds for ages two and three. The age of seedlings at time of cold stress application were: Age 1-emergence or pre-endosperm exhaustion, Age 2-endosperm exhaustion, and Age 3-post-endosperm exhaustion (four days after endosperm exhaustion).

Different rates of endosperm exhaustion were accomplished by growing seedlings at two temperatures, 10 and 20°C.

When seedlings reached desired ages, they were placed in a pre-conditioning room at 35°F. for 24 hours prior to cold stress application. All pots were watered just prior to placing in the preconditioning room and allowed to drain during the 24 hour preconditioning period to insure as uniform water conditions as possible. Following preconditioning,

seedlings were transferred immediately to a freezing chamber at a temperature of 20-22°F. The severity of cold stress was regulated by duration of exposure to this temperature. Exposures to cold stress were 2 and 4 hours. After exposure, seedlings were transferred to the preconditioning room immediately and allowed to thaw slowly. When complete thawing of vermiculite and seedlings had occurred, seedlings were transferred to the greenhouse. Survival and plant height measurements were made one day after freezing and at two weeks following freezing. Seedlings were transplanted into soil in the greenhouse and will be grown to maturity.

For testing hypothesis of statistical results, accepted methods described by Snedecor (28) and Steel and Torrie (30) were used.

## RESULTS

### Endosperm Exhaustion and Seedling Length Measurements.

All results reported are based on the last endosperm exhaustion trial, Trial 3, described in materials and methods, with the exception of root-shoot data presented on the 7 degree C. temperature.

Germination of orchardgrass seed was 66, 77, 80, and 67 percent at temperatures of 7, 10, 15, and 20°C., respectively.

Endosperm exhaustion was found to occur between 14-16 days at a temperature of 20°C., between 16-18 days at 15°C., and between 22-24 days at 10°C. using dry weight loss as a measure (Figure 1). Endosperm exhaustion based on seedling length measurements gave similar results (Figure 2). Time required for endosperm exhaustion increased as temperature decreased.

Correlation coefficients between seedling length and percent dry weight were found to be 0.978, 0.994, and 0.856 for temperatures of 10, 15, and 20°C., respectively. These coefficients were significant at the .01 probability level. All data were combined and the calculated correlation coefficient was 0.928. The $r^2$ value or coefficient of determination shows that 86 percent of the variation in weight loss is accounted for by seedling length. These correlations indicate that seedling length is a useful measure of endosperm exhaustion.

The number of seedlings needed to be sampled to estimate within $\pm 5$ and $\pm 10$ percent of the mean length was determined for a probability level of .05 (Table I). Calculations of sample size were based on the formula outlined by Snedecor (28). This formula was: