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thesis entitled

Field and Laboratory Performance of Winter Wheat Seed Affected by Pre-Harvest Sprouting and Storage

presented by

Marcelo Queijo

has been accepted towards fulfillment
of the requirements for

Master of Science degree in Crop and Soil Sciences

L. O. Copeland

Major professor

Date January 23, 1998

FIELD AND LABORATORY PERFORMANCE OF WINTER WHEAT SEED AFFECTED BY PRE-HARVEST SPROUTING AND STORAGE

By

Marcelo Queijo

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Crop and Soil Sciences

1998

ABSTRACT

FIELD AND LABORATORY PERFORMANCE OF WINTER WHEAT SEED AFFECTED BY PRE-HARVEST SPROUTING AND STORAGE

By

Marcelo Queijo

Field and laboratory experiments were conducted on two winter wheat varieties representing different imbibition periods and storage levels to measure the effects of nonvisible incipient sprouting (NVIS) on germination, storability and field performance. Warm germination, tetrazolium and accelerated aging test performance were evaluated in the laboratory experiments. Seed lots with higher NVIS resulted in the lowest germination, especially under longer periods of storage. A strong interaction between imbibition period and storage occurred in both laboratory and field tests. Germination of visibly sprouted seed decreased more rapidly than for nonvisibly sprouted seed over time. No differences in field emergence, spring quantitative regrowth index, and yield occurred among varieties. Highly significant differences in field performance occurred between NVIS seed and that with visible sprouting damage. Generally, damage from NVIS affected the quality of seed as well as its field performance if seed lots were stored more than 2 months.

TO THE MEMORY OF

Don José A. Queijo to whom I owe

my love and dedication to agriculture

ACKNOLEDGEMENTS

I will always be thankful to Dr. Lawrence O. Copeland, my major professor, for his continuous support, patience, dedication and advice. His encouragement and guidance were very important in the completion of this work.

I am also grateful to the members of my committee, Dr. James Kells and Dr. Perry Ng, for their suggestions and cooperation during this work.

A sincere thanks goes to Mr. Hongyu Liu, for his assistance, suggestions and help along these two years. Also, I am thankful to the members of the Wheat crew for their help and collaboration, especially to Mr. Larry Fitzpatrick.

I would like to express my appreciation to all the members of Michigan Crop Improvement Association for helping me so much during all this time, especially Mrs. Ann Tucker whose support and assistance were very important to make this program successful.

To my wife, Gabriela, and my parents and sister, thank you very much for your love, patience and support.

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INTRODUCTION

Preharvest sprouting in cereals is the precocious germination of the grain while still in the ear in the field, usually in response to unfavorable weather conditions (e.g., rain and high relative humidity) (Olered, 1967). This phenomenon is a perennial problem in many parts of the world where there is a high probability of rain during the harvest period (MacKey, 1976). Changes in the chemical constituents of the wheat kernel which accompany germination and which have deleterious effects on the subsequent commercial utilization of the wheat are collectively known as preharvest sprouting.

It is clear that the most important aspect of preharvest sprouting is the germinability of the grain at particular stages of maturity under different environmental conditions (Mares, 1985). Susceptibility to preharvest sprouting in wheat is dependent on genotype (Belderok, 1968; Bhatt et al., 1981; Bingham and Whitmore, 1966; McCrate et al., 1981) and environmental conditions such as moisture and temperature (Lalluka, 1976; Nielsen et al., 1984; Olson and Mattson, 1976).

In some areas sprout damage in wheat occurs in climates with persistent rain and cool temperatures before harvesting. In other areas, periods of rain accompanied by high temperatures and high humidity before harvest increase sprouting in wheat. High temperatures during the soft dough stage of grain development have been reported to shorten the period of dormancy after physiological maturity (Nielsen, 1980). Nielsen et al. (1984) also reported

preharvest sprouting to be more severe when large diurnal temperature fluctuations and high precipitation occurred during and after physiological maturity of the grain. Karkoven et al. (1991) noted that an average relative humidity of over 80 percent and a maximum daily temperature of below 13°C during grain filling decrease falling number levels to below 120 seconds (the lower limit for commercially acceptable starch quality). On the other hand, if the average relative humidity falls below 70 percent and the average maximum temperature exceeds 16°C, falling number readings often go over 230 seconds. It should be noted that wheat flour with falling number values below 120 seconds are unacceptable for milling and can only be used for animal feed.

Following moisture imbibition, the germination sequence of the wheat grain is sprouting, endosperm degradation, and alpha-amylase response. Gordon et al. (1977) noted that other enzymes (other than alpha-amylase) associated with germination also appear to contribute substantially to endosperm degradation.

Sprouting in wheat occurs first by the penetration of the pericarp of the kernel by the radicle, or primary root. During sprouting, the seed's enzyme systems are mobilized and translocated to the interior of the endosperm where they catalyze the breakdown of starches into soluble sugars that can be translocated to growth areas of the embryo. The reduction of starch content and increase in sugars has serious consequences on the use of sprouted wheat for milling (Copeland et al., 1980).

Breeding for Sprouting Resistance

When wheat resists germination in intact spikes when subjected to favorable conditions, it is said to be sprouting tolerant. Selection against sprouting damage in wheat is usually based on lack of embryo growth or low activity of alpha-amylase at some time after ripeness (Gordon et al., 1977).

Attempts to associate specific grain characteristics with resistance to preharvest sprouting have been moderately successful. Seed coat characteristics other than pigmentation have been reported to be associated with decreased germinability of wheat seed (Nielsen, 1980). Chang (1943) concluded that differences in sprouting resistance are in most cases due to differences in length of dormancy and in some cases may be due to rapidity of germination. Gordon et al. (1979) have shown that maturity also may contribute to sprouting susceptibility.

The presence of awns hastens water loss from wheat ears during drying and could enhance uptake during wetting (Pool and Patterson, 1958). King and Chadim (1983) stated that a wheat variety bred for reduced preharvest sprouting should be awnless.

It is generally accepted that the long-term solution to the preharvest sprouting problem in wheat lies in the development of cultivars which are able to tolerate or resist the damaging effects of rain during the period between ripeness or maturity and the completion of harvest. For sprouting resistance, it is not sufficient for the grain to be unable to germinate at morphological ripeness;

Belderok (1968) claimed that the crop must be able to stand in the field one or two weeks longer without starting to sprout under prolonged rainy conditions.

D. J. Mares (1992) found considerable evidence to suggest that at least part of the control of germination and presprouting resides in the embryo and is mediated by abscisic acid (ABA) and/or other endogenous inhibitors.

Seed Dormancy

Seed dormancy is the failure of the seed to germinate when subjected to favorable conditions. Dormancy is very important for the survival of many plant species, however, it is considered to be a negative characteristic of a seed lot being tested for germination. The positive effects of seed dormancy, however, are more important than the negative ones for both cereal production and the cereal industry. Inhibition of the enzyme systems that are active during germination is of fundamental importance for conserving cereal crop quality under unfavorable harvest conditions (Ringlund, 1992).

Preharvest sprouting is closely related to the release of the seed from dormancy. Dormancy normally disappears with storage, and this process is temperature dependent (Belderok, 1961). Although seed dormancy is recognized to be under genetic control, this control is also influenced by environmental conditions during seed formation (Takahashi, 1979). Takahashi (1967) showed that induction of dormancy was strictly affected by high temperature and high moisture during the vegetative stage.

The association between red seed coat color and dormancy is well established, but it has only been recently that white seeded genotypes with significant levels of dormancy have been identified (Bhatt and Derera, 1980; De Pauw and McCaig, 1983 and 1990; Mares, 1987; Morris and Paulsen, 1987). But as of today it has not been possible to transfer, in its entirety, the dormancy associated with some red wheats into a white cultivar.

Seed Coat

Observations on the germination of wheat in the ear have shown that varieties with white caryopses are more likely to sprout before harvest than those with red caryopses (Wellington, 1953). Wellington (1956) proposed that the dormant, red grained wheat have a more restrictive seed coat than the white seeded, nondormant wheat. He found that initial (24 hr) water absorption by the embryo was similar for both dormant and nondormant grain. However, further water uptake by the embryo was delayed in the dormant variety unless the embryo was uncovered or the distal half removed.

Paulsen et al. (1984), in their research on agronomic and quality attributes of sibling white and red winter wheat lines, cited several advantages for white over red wheat regarding flour extraction, flour protein concentration, and other desirable milling qualities; but, they also mentioned the greater susceptibility to preharvest sprouting as the major disadvantage of white wheat over red wheat.

Collectively, the red seeded cultivars have higher initial seed dormancy, lower tendency to sprout, greater capacity to maintain test weight, less visible sprout damage to the grain, and higher seed viability under sprouting conditions than similar cultivars with white seed (McEwan, 1975).

Position

The position in the ear can also affect the time when a particular wheat seed is first able to germinate. Wellington (1953) noted that the first seeds to germinate were those at the top of the ear which had started to change color. Hardesty et al. (1956) found that sprouting starts in the top of the ear, follows soon after at the base, and finally proceeds to the center.

Ripeness

In both red and white wheat varieties it appears that seeds are unable to germinate as long as the pericarp remains green, but as soon as the covering layers change color, a high proportion of the white seeds in the middle and top spikelets become able to germinate. At the same time, very few of the seeds in similar spikelets of red varieties are able to germinate, nor many in the lowest spikelets in the ears of both white and red varieties (Wellington, 1953).

Wellington (1956) reported that no seeds are able to germinate while still green, but with the disappearance of the chlorophyll and the rupture of the layer containing chloroplasts during ripening, germination was able to occur.

Enzymes

Preharvest sprouting causes physiological and biochemical changes in the grains which render them unsuitable for many end-uses. Enzyme activity is known to increase greatly during cereal seed germination. The increased activity of the amylases, particularly alpha-amylase, adversely affects grain quality of wheat. Studies have shown that high levels of alpha-amylase in wheat flour result in a sticky crumb, poor bread texture, and discolored crust (Morris and Paulsen, 1985; Mares, 1985; Greenaway, 1969; Buchanan and Nicholas, 1979; Lorenz, Roewe-Smith, Kulp, and Bates, 1984; Nielsen, 1980). Excess levels of natural alpha-amylase activity produce a highly colored loaf with a sticky crumb resulting from the production of potentially sticky substances with high molecular weight dextrines and sugars during baking (Buchanan and Nicholas, 1979). However, a small amount of alpha-amylase activity in flour has been shown to enhance bread quality (Nielsen, 1980).

Olered (1964) showed that there are 2 types of alpha-amylases, one mainly found in the pericarp of premature seeds, and another in germinating seeds. Later it was shown that there were many isozymes for both types, both of

which are coded by 2 different genes, and dormancy is related to the gibberellic acid insensitivity found in semi-dwarf wheats (Gale, 1983).

When cereals germinate, the amylases in the grain increase rapidly and hydrolyze the starch in the endosperm into sugars needed for seedling growth. The consequences for the milling industry are that flour and meal from sprouted wheat or rye contain increased amounts of alpha-amylase which adversely affect baking quality; the ensuing breakdown of a proportion of the starch during baking leads to sticky crumb texture and undesirable crust color of the bread. The most important factor to the flour miller is the performance of the flour during baking, hence the preference of the miller for alpha-amylase determination rather than visual determinations of sprouting (Belderok, 1968). Thus, measurement of the falling number has proved very suitable for this purpose. Mathewson and Pomeranz (1977) considered alpha-amylase activity an appropriate estimate of sprout damage as the enzyme activity reflected actual end-use properties of the grain. Also, Hagemann and Ciha (1984) noted that germination tests are better in predicting sprouting susceptibility whereas enzymatic tests are better in quantifying actual end-use damage. The tetrazolium test was used in these studies to measure the effect of nonvisible incipient sprouting on seed quality, storability and performance.

Economic Impact

Preharvest sprouting may seriously reduce the agronomic and milling and baking quality of wheat grain, as well as its use as a thickening agent in cream soups and gravy mixes.

As early as the 1930's, Harrington (1932) noted that sprouted grain has distinctly less value for seed purposes or for milling than undamaged grain.

Visible sprouting occurs when the root-shoot axis breaks through the pericarp cover. Such kernels are considered damaged by grain inspectors, often resulting in substantial grower discounts.

Belderok (1961) stated that in northwest Europe, weather conditions result in the occurrence of sprouting once in every three years. In Michigan, the probability of serious sprouting damage due to unfavorable weather conditions is about once in every four years, although some sprouting occurs almost every year. It is clear that this phenomenon presents a serious problem to agriculture. The incidence of sprout damage varies with the incidence of untimely rain at harvest. Most of the world's major wheat producing regions are affected by sprout damage to some degree.

Stoy (1983) noted the great economic losses that occur because of sprouting damage to wheat in various regions of the world, and Weilenmann et al. (1976) cited the substantial economic losses from decreased grain quality caused by sprouting. Belderok (1968) estimated that a 10 percent yield reduction due to preharvest sprouting would seem by no means exceptional.

Bhatt et al (1981) also noted that grain quality is adversely affected by preharvest sprouting. In 1980, Copeland, Freed and Helsel estimated that as much as 50 to 60 percent of the acreage of soft white wheat in Michigan was affected by severe sprouting in any given year. They noted the serious consequences of sprouted wheat to the milling industry as well as its use for seed. Sprouting affects the quality desired by end-users of wheat products because the enzymes that determine crumb structure and the shape of baked products are affected. Wahl and O'Rourke (1992) stated that the market value of sprouted wheat is reduced because of its lower value to end-users, which results in economic losses to farmers.

Regarding wheat seed quality, Copeland et al. (1980) noted that any degree of sprouting will lower the seed quality to some extent, but may not destroy its germination potential if sprouting is not too severe and if the moisture content is brought down to safe levels (about 13.5 - 14.0 percent). Elias (1987) showed that although seed with minimum presprouting can retain its capacity for a period of time, presprouted wheat seed will lose germination capacity more rapidly than non-sprouted seed. The time required for the loss of germination depends on the extent of the presprouting and the storage conditions.

It's not easy to obtain reliable figures of the total damage that may be caused during one year to the world's grain crops. However, there are many examples from various regions where 30 to 50 percent or even more of the grains harvested may be so severely damaged that they are unsuited for human consumption.

Nonvisible Sprouting Damage

A rise in amylase activity in grain may cause the starch to begin breaking down into sugars before sprouting in the ear can be observed visually (Sumeola, 1965; LaCroix et al, 1976). Gold and Duffus (1992) denoted the production of alpha-amylase by pre-ripe grains in the absence of visible sprouting as pre-maturity alpha-amylase (PMAA). Gale and Lenton reported the first widespread damage due to PMAA in 1987. In 1992, Mares and Mrva reported that several wheat cultivars produced (during the later stages of grain ripening) significant levels of alpha-amylase that reduce falling number levels below commercially acceptable levels.

Nonvisible sprouting injury has been detected by seed analysts at Michigan Crop Improvement Association with the tetrazolium test. They have observed critical unstained portions of the embryo (which are an indicator of dead tissue) in wheat seeds not showing visible sprouting damage. The term "weak seed" has been applied to this phenomenon denoting that those caryopses have undergone the very first stages of sprouting. We suggest that this phenomenon should be denoted as nonvisible incipient sprouting (NVIS) damage.

Tetrazolium Test

The tetrazolium test is widely recognized as a valuable means of estimating seed viability. In Germany in the early 1940s Dr. George Lakon discovered tetrazolium as a good chemical in determining seed viability. The tetrazolium chloride or TZ test is often called a quick germination test. The main function of the tetrazolium test is to distinguish between viable and nonviable seeds.

In the United States, the TZ test is often used as a complement of the standard germination test because it allows faster estimates of viability. Historically the test has been around for many years with early research and development performed by Lakon (1942), Bulat (1961) and Lindenbein (1961; 1965). This led to the formation of the Tetrazolium committee within International Seed Testing Association. In 1983, R.P Moore presented a handbook to the ISTA Congress. This manual is the standard that most official seed laboratories use.

Today the test is used throughout the world as a highly regarded method of estimating seed viability and is a routine test in many seed testing laboratories. It is often referred to as a "quick test" since it can be completed in 24 to 48 hours compared to regular germination tests, which may require up to seven days.

Tetrazolium test results can be extremely valuable for providing information for immediate quality information on a seed lot followed by a warm germination test and/or a cold germination test.

The tetrazolium test distinguishes between viable or dead tissues of the embryo on the basis of their relative respiration rate in the hydrated state. Although many enzymes are active during respiration, the test utilizes the dehydrogenase enzymes as an index to the respiration intensity and seed viability. The highly reduced state of the dehydrogenases enables them to give off hydrogen ions to oxidize colorless tetrazolium salt solution that is changed into red formazan as it is reduced by hydrogen ions. Seed viability is interpreted according to the staining pattern of the embryo.

Preparation: Two 100-seed replications are soaked in water overnight. They are then dissected longitudinally so that the embryo is exposed to the tetrazolium chloride solution. Tetrazolium solution must come in contact with the embryo. A solution of 2,3,5-triphenyltetrazolium chloride (a salt) is added to water to form a colorless solution. The seeds are placed in a one percent solution for approximately two hours at room temperature.

Evaluation: Upon penetration into living cells, the tetrazolium chloride is reduced by dehydrogenase enzymes present in living tissue to formazan, which is a reddish, water-insoluble compound. The reaction occurs within or near living cells which are releasing hydrogen in respiration processes. Sound tissues produce a normal red color. Such tissues resist the rate of penetration of tetrazolium. The rate of hydrogen release in sound tissues is slow in comparison to that in partially weakened tissues. Weak living tissues produce an abnormal color. Such tissues have lost some of the initial resistance to the penetration of tetrazolium. Respiration is accelerated and formazan is produced rapidly.

During early stages of deterioration, these tissues become darker red (bruised tissue) more rapidly than sound, healthy tissues. Dead tissues do not stain, usually remaining white (aged tissue) because lack of respiration prevents formazan production by embryo tissues. The accuracy of results depends largely upon the training, experience and background of the analysts.

Summary

Over the last few years, seed analysts at Michigan Crop Improvement Association have explored several methods to detect nonvisible incipient sprouting damage. They have observed a relationship between embryo staining and incipient (nonvisible) sprouting damage, indicating that damage can occur well before any sprouting effects are visible. In commercial practice, the seed quality of cereals is judged by the ability of the seed to germinate. Very little has been reported about the detection of nonvisible incipient sprouting damage and its consequences on seed quality. It is mainly in this area where this work was conducted.

OBJECTIVES OF STUDY

The objectives of the following studies were to determine the effects of: 1) storage and 2) imbibition period on the laboratory and field performance of wheat seed.

MATERIALS AND METHODS

Laboratory and field experiments were conducted with two wheat (Triticum aestivum L.) varieties: one soft white winter variety, Lowell, and the soft red variety, Mendon.

LABORATORY EXPERIMENT:

Three sets of experiments were conducted in 1995, 1996 and 1997. During the first year, lots of Lowell and Mendon wheat obtained from Michigan Crop Improvement Association were induced to produce different nonvisible sprouting levels described in Table 1.

Table 1. Description of sprouting levels obtained after periods of imbibition in all experiments during 1995 and 1997

Imbibition period	Description of sprouting levels
0 hr	Nonsprouted wheat seed (control); no exposure to moisture imbibition.
15 hr	5-10 percent of seed with nonvisible incipient sprouting.
24 hr	20-30 percent of seed with nonvisible incipient sprouting.
40 hr	Visibly sprouted wheat seed, in which the root-shoot axis has just broken through the pericarp.

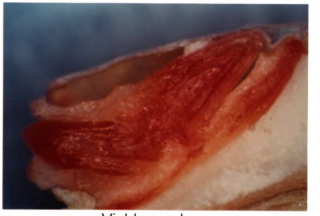
Seeds were induced to sprout by placing them on moistened blotter paper in a germination chamber at 22°C and near 100 percent of relative humidity until the desired sprouting level was obtained. The amount of seed induced was large enough for both laboratory and field experiments.

After induction, seed lots were air-dried for one week and stored at room temperature for periods of 0, 2, 4, and 6 months to study the effects of storage on seed quality when affected by nonvisible incipient sprouting.

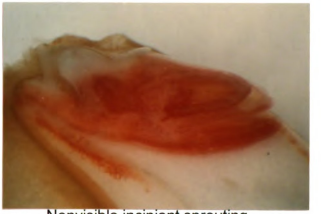
At the end of each storage period, seeds were evaluated by the standard warm germination test, the tetrazolium test, and the accelerated aging test.

Four 100-seed replications from each treatment were evaluated by the standard warm germination test following the Association of Official Seed Analysts Rules for Testing Seeds (AOSA, 1994) at 25°C for seven days on moist blotter paper.

Two 100-seed replications for each treatment were evaluated by the tetrazolium test (AOSA, 1970). Treatments were placed in water overnight to allow enough time for imbibition, after which seeds were sliced and placed in a 1-percent solution of 2,3,5-triphenyl tetrazolium chloride (TTC) for a period of two hours at room temperature. The tetrazolium test was conducted according to a classification method developed by Michigan Crop Improvement Association, which is described in Table 2. Close-up photographs with a microscope (at 30x and 50x) were taken of stained embryos in the tetrazolium test to illustrate the appearance of different levels of incipient sprouting. This pictorial representation is shown in Figure 1. Normal red color in tetrazolium testing develops when hydrogen ions from respiration processes of living cells are accepted by the colorless 2,3,5- triphenyl tetrazolium chloride and reduces it to the red dye formazan. White flaccid tissues are evidence that the unstained tissues are dead.



Viable seed



Nonvisible incipient sprouting

Dead seed

Figure 1. Pictorial representation of tetrazolium test evaluation

Table 2. Description of classification criteria used for the tetrazolium test

Levels	Description of levels
Viable seed	Embryo showing complete staining.
NVIS	Embryo showing small unstained areas (usually less than 1/3).
Dead seed	Embryo showing large, critical unstained areas (usually more than 1/3).

Finally, four 100-seed replications for each treatment were subjected to accelerated aging for 72 hours at 41°C following the ISTA Seed Testing Rules (ISTA, 1995). Afterward, seed lots were germinated at 25°C for seven days on moist blotter paper.

Tests were repeated in 1996 and 1997 following the same procedures as in 1995, including germination and vigor evaluation.

FIELD EXPERIMENT:

First Year (1995):

In the Fall of 1995, observations were made on the number of hours of moisture imbibition required to obtain different levels of nonvisible sprouting on two winter wheat varieties grown in Michigan: Lowell (a soft white wheat) and Mendon (a soft red wheat).

The first set of experiments was planted in late fall of 1995. Four replications of Lowell representing all treatments described in Table 1 were planted in 2x2 m boxes in greenhouse soil at the Michigan State University Hancock Turf Center. The objective of this experiment was to study the effects of different levels of sprouted seed lots on soil emergence, perhaps the most important consequence of preharvest sprouting. Field emergence was measured two weeks after planting. Unfortunately, due to late planting and a severe winter none of the plots survived the winter.

Second Year (1996):

Field studies were conducted at the Crop and Soil Sciences Research Farm on the M.S.U. campus and at Clarksville Horticulture Research Farm, 60 miles west of East Lansing. Preparation of the different seed lots was timed so the storage period for each ended at the normal planting time (October 1) for wheat.

Once the different seed lots were removed from storage, four replications for each treatment (Table 1) were planted at both locations for both Lowell and Mendon. Each replication consisted of 7 rows 6 meters long.

The field experiments were evaluated for field emergence, quantitative index of spring regrowth potential, and grain yield.

Field emergence was measured 21 days after planting at both locations. Two rows of plants (one meter per row) were counted for each plot to determine plant density. Before spring green-up, 90 kg/ha of nitrogen were applied at both the East Lansing and Clarksville locations. In April 1997, all plots were evaluated and given a quantitative regrowth index. A visual index from 0 to 10 was created, where 0 indicated no regrowth (no plants emerged and/or surviving) and 10 indicated normal regrowth (all plants emerged and surviving the winter). The same index was used for both locations.

During July 1997, all plots were combine harvested, the grain air-dried and moisture levels adjusted to 13.5 percent.

Field experiments were analyzed with a completely randomized design. All data in these studies were analyzed with the SAS Statistical Software package (SAS Institute Inc., 1997). Because of interactions that occurred, a procedure called "slicing" was performed. This allowed the study of significant interactions by analyzing the levels of one factor across all levels of other factors. All tables describing the statistical analyses and the slicing procedures are placed in the Appendix and tables numbered A1, A2, etc.

Results and discussion

1. Laboratory experiments

a) Tetrazolium viability

In 1995, highly significant differences were obtained in tetrazolium viability among treatments ($p > 0.0001$). Variety and imbibition period (IP), as well as the interaction among them showed significant differences in TZ viability ranging from 73 to 98 percent for Lowell and 63.0 to 98.5 percent for Mendon (Tables 3 and Appendix A1). Fifteen hours of moisture imbibition did not appear to be enough to reduce viability (measured by the TZ test) in any of the seed lots. Differences became significant as the imbibition period (IP) increased from 15 to 24 hr, and even larger differences in viability occurred as IP reached 40 hr.

Table 3. Tetrazolium test results (TZ viability) and nonvisible incipient sprouting (NVIS) of Lowell and Mendon, 1995

Variety	Imbibition period	TZ viability (%)	NVIS (%)
Lowell	0 hr	98.00 a	2.50 a
	15 hr	96.00 a	10.00 b
	24 hr	77.00 b	29.50 c
	40 hr	73.00 c	62.00 d
Mendon	0 hr	98.50 a	2.00 a
	15 hr	94.50 a	10.00 b
	24 hr	76.50 b	22.00 c
	40 hr	63.00 c	35.50 d

$LSD_{TZ\ viability} = 3.44$
$LSD_{NVIS} = 3.13$

Because of interactions that occurred, the SAS slicing procedure was performed. High levels of significance ($p > 0.0001$) occurred in TZ viability across IP when slicing analysis was performed by variety, e.g., the white variety Lowell showed highly significant differences across all imbibition periods (Table A2). No differences in viability occurred among nonvisible levels of sprouting (0, 15, and 24 hr) across the two varieties, while significant differences occurred for the visibly sprouted level (40 hr) between Lowell (73 percent) and Mendon (63 percent).

In 1996, highly significant differences occurred in TZ viability among treatments (Tables 4 and A3). Variety, imbibition period (IP) and storage all showed highly significant differences in TZ viability ($p > 0.0001$), while the interactions between variety and IP, and between storage and IP were also highly significant (Table A3). TZ viability ranged from 19.0 to 97.0 percent for Lowell and 34.5 to 97.5 for Mendon (Table 4). Viability decreased for both Lowell and Mendon as IP increased, while little variation occurred in viability across storage periods, even though significant differences occurred between 0-2 months and 4-6 months of storage. Finally, TZ viability decreased as IP and storage period increased, especially for seed lots with higher NVIS (24 and 40 hr of moisture imbibition).

Commercially acceptable levels of germination/viability were still obtained after 15 hr of imbibition, with a rapid decrease in quality as the imbibition period

continued. Incipient sprouted seed of both Lowell and Mendon with up to 15 hr imbibition retained viability even if stored as long as 6 months, while visibly sprouted seed lost viability after 2 months (Fig. 2).

Table 4. Percent tetrazolium (TZ) viability of Lowell and Mendon in 1996

	Imbibition period	Storage period				
Lowell		0 months	2 months	4 months	6 months	mean
	0 hr	94.50	97.00	95.50	97.00	96.00 a
	15 hr	97.00	95.50	91.00	96.00	94.88 a
	24 hr	63.00	76.00	70.50	71.00	70.13 b
	40 hr	61.50	24.00	22.00	19.00	31.63 c
	Mean	79.00 a	73.13 b	69.75 c	70.75 c	
Mendon		0 months	2 months	4 months	6 months	mean
	0 hr	93.50	97.50	95.50	97.00	95.88 a
	15 hr	96.00	97.00	87.50	97.00	94.38 a
	24 hr	69.00	77.00	71.00	71.00	72.13 b
	40 hr	74.00	48.50	41.00	34.50	49.50 c
	Mean	83.13 a	80.00 b	73.75 c	75.00 c	

$LSD_{(Imbibition)} = 2.22$
$LSD_{(Storage)} = 2.22$

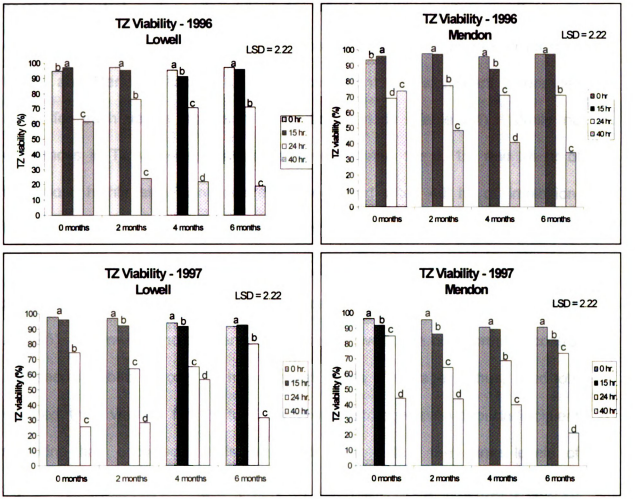


Figure 2. The effect of imbibition period and storage on the TZ viability of Lowell and Mendon in 1996 and 1997

No significant differences in TZ viability occurred between the non-sprouted control and seed imbibed for 15 hr, while significant differences occurred between these two levels and those imbibed for 24 and 40 hr for both Lowell and Mendon (Table 5). This is consistent with significant differences noted for each variety across all imbibition periods (Table A4). While no differences in TZ viability occurred between varieties for 0, 15, and 24 hr of imbibition, highly significant differences occurred after 40 hr of imbibition. Mendon had higher TZ viability (49.5 percent) than Lowell (31.6 percent).

Highly significant differences in TZ viability occurred among imbibition periods (IP) for each storage level as analyzed by the slicing procedure (Tables 6 and A5). Meanwhile, no differences occurred between the non-sprouted control and seed imbibed for only 15 hr across all levels of storage. Thus, seed lots retained their viability for up to 6 months of storage even though incipient sprouting had occurred. It is important to note that the same levels of significance occurred among IP (Table 6), showing that significant deterioration occurred during storage, the extent of which depended on the length of imbibition.

Table 5. Least square means for TZ viability of Lowell and Mendon in 1996

Variety	Imbibition period	TZ viability (%)	NVIS (%)
Lowell	0 hr	96.00 a	2.38 a
	15 hr	94.88 a	8.63 b
	24 hr	70.13 b	37.25 c
	40 hr	31.63 c	26.38 d
Mendon	0 hr	95.88 a	3.00 a
	15 hr	94.38 a	9.50 b
	24 hr	72.13 b	34.13 c
	40 hr	49.50 c	36.13 c

$LSD_{TZ\ viability} = 2.22$
$LSD_{NVIS} = 2.17$

Table 6. Least square means for the interaction between imbibition period and storage on TZ viability of Lowell and Mendon in 1996

Storage	Imbibition period	Tetrazolium Viability (%)	Nonvisible incipient sprouting (%)
0 months	0 hr	94.00 b	3.75 c
	15 hr	96.50 a	5.75 c
	24 hr	66.00 c	41.50 b
	40 hr	67.75 c	50.50 a
2 months	0 hr	97.25 a	2.25 d
	15 hr	96.25 a	10.00 c
	24 hr	76.50 b	36.50 a
	40 hr	36.25 c	26.00 b
4 months	0 hr	95.50 a	2.25 d
	15 hr	89.25 b	11.75 c
	24 hr	70.75 c	34.25 a
	40 hr	31.50 d	26.75 b
6 months	0 hr	97.00 a	2.50 d
	15 hr	96.50 a	8.75 c
	24 hr	71.25 b	30.50 a
	40 hr	26.75 c	21.75 b

$LSD_{TZ\ viability} = 2.22$
$LSD_{NVIS} = 2.17$

In 1997, TZ viability decreased with increased storage and imbibition periods (IP) for both Lowell and Mendon. Highly significant differences in TZ viability occurred among seed lots (Tables 7 and A6) due to the effects of IP and storage, whereas no significant differences occurred between Lowell and Mendon. TZ viability ranged from 31.5 to 98.0 percent for Lowell and 21.5 to 96.5 percent for Mendon. The interaction among varieties, IP and length of storage was significant as determined by the slicing procedure.

No differences in TZ viability occurred from nonimbibed (control) seed lots of either Lowell or Mendon when tested across storage periods (Table 7), indicating that nonimbibed seed was able to maintain its viability for at least 6 months. Lowell also showed no differences in viability across storage periods for up to 15 hr of moisture imbibition, indicating that even though incipient sprouting had occurred, viability was retained for up to 6 months.

Highly significant differences in TZ viability occurred for both Lowell and Mendon with increased storage periods as the imbibition period increased from 15 to 24 and 40 hr (Table A7). Though the overall viability at each storage period did not appear to decrease for Lowell, it did decrease for Mendon, clearly indicating that different genotypes react differently when exposed to adverse conditions or stored for different length of time.

Table 7. Percent tetrazolium (TZ) viability showing the effects of imbibition period (IP) and storage on Lowell and Mendon in 1997

	Imbibition period	Storage Period				
Lowell		0 months	2 months	4 months	6 months	Mean
	0 hr.	98.00	97.00	94.00	91.50	95.13 a
	15 hr.	96.00	92.00	91.50	92.50	93.00 a
	24 hr.	74.50	63.50	65.00	80.00	70.75 b
	40 hr.	25.50	28.50	56.50	31.50	35.50 c
	mean	73.50 b	70.25 c	76.75 a	73.88 b	
Mendon		0 months	2 months	4 months	6 months	Mean
	0 hr.	96.50	95.50	91.00	91.00	93.50 a
	15 hr.	92.00	86.50	89.50	82.50	87.63 b
	24 hr.	85.00	64.50	69.00	73.50	73.00 c
	40 hr.	44.50	44.00	40.00	21.50	37.50 d
	mean	79.50 a	72.63 b	72.38 b	67.13 c	

$LSD_{(imbibition)} = 2.22$
$LSD_{(storage)} = 2.22$

Highly significant differences in TZ viability occurred for Lowell and Mendon for different imbibition periods (averaged across storage) (Table A7 and A8). TZ viability of Lowell and Mendon decreased sharply from 90 percent to 70 percent as imbibition increased from 0 and 15 to 24 hr and especially from 24 to 40 hr where the lowest viability occurred (35.5 percent for Lowell and 37.5 percent for Mendon) (Table 7).

Slicing analyses between length of imbibition and period of storage showed highly significant differences in TZ viability between Lowell and Mendon after 40 hr of imbibition for all storage periods (Table A9). At 6 months of

storage, significant differences in TZ viability between Lowell and Mendon also occurred after 15 and 24 hr of imbibition, with Lowell performing better than Mendon at all imbibition periods.

b) Nonvisible incipient sprouting

In 1995, highly significant differences occurred in the level of nonvisible incipient sprouting (NVIS) among treatments (Tables 3 (page 19), A10 and A11). Again, differences in NVIS were highly significant among varieties and IP, as well as their interaction ($p > 0.0001$), with NVIS ranging from 2.5 to 62.0 percent for Lowell and 2.0 to 35.5 for Mendon. A visual indication of the sprouting levels used in all these experiments is shown in Fig. 1. Lowell was the most affected by sprouting damage, supporting the well-known association between sprouting susceptibility and the white seed coat color. However, red wheat will also sprout if high moisture conditions persist long enough.

The percent NVIS increased and TZ viability decreased as the duration of moisture imbibition increased, indicating that damage due to sprouting had occurred even though actual damage was not yet visible. This phenomenon has been described by Sumeola (1965), La Croix et al. (1976) and Sawada et al. (1995). Gold and Dufus (1995) described it as "pre-maturity alpha amylase activity," indicating that important levels of alpha-amylase are produced, reducing falling number values before any sprouting damage could be observed visually.

Slicing analyses were also performed on the interaction between variety and imbibition periods (IP) when levels of NVIS were measured by the TZ test (Table A11). Highly significant differences in NVIS occurred for both varieties with increased time of imbibition. The non-sprouted control (0 hr) and the seed lot imbibed for 15 hr showed no differences in the NVIS across the two varieties, while significant differences occurred at both the 24 hr (29.5 percent for Lowell vs. 22.0 percent for Mendon) and at the 40 hr (62.0 percent for Lowell vs. 35.5 percent for Mendon) imbibition periods. Fifteen hr of moisture imbibition did not appear sufficient to cause any damage due to incipient sprouting for either of the two varieties.

In 1996, highly significant differences occurred among treatments when the levels of NVIS were analyzed (Tables 8 and A12). Variety, IP and length of storage showed highly significant differences in NVIS level when measured by the TZ test. The 3-way interaction among them was also significant. NVIS ranged from 1.0 to 54.5 percent for Lowell and 2.5 to 46.5 percent for Mendon. NVIS damage increased as the IP increased and decreased as the storage period continued. Some deterioration occurred during imbibition and become accentuated as the storage period was prolonged. It was clear that most of the seed with NVIS died during storage, especially that receiving longer periods of imbibition (Tables 4, 8 and Fig. 4).

Table 8. Percent nonvisible incipient sprouting (NVIS) for different imbibition periods and storage of Lowell and Mendon in 1996

	Imbibition period	Storage period				
Lowell		0 months	2 months	4 months	6 months	mean
	0 hr	5.00	2.00	1.00	1.50	2.38 d
	15 hr	6.50	10.00	9.50	8.50	8.63 c
	24 hr	52.00	36.50	30.00	30.50	37.25 a
	40 hr	54.50	17.50	19.00	14.50	26.38 b
	mean	29.50 a	16.50 b	14.88bc	13.75 c	
Mendon		0 months	2 months	4 months	6 months	mean
	0 hr	2.50	2.50	3.50	3.50	3.00 c
	15 hr	5.00	10.00	14.00	9.00	9.50 b
	24 hr	31.00	36.50	38.50	30.50	34.13 a
	40 hr	46.50	34.50	34.50	29.00	36.13 a
	mean	21.25 a	20.88 a	22.63 a	18.00 b	

$LSD_{(Imbibition)} = 2.17$
$LSD_{(Storage)} = 2.17$

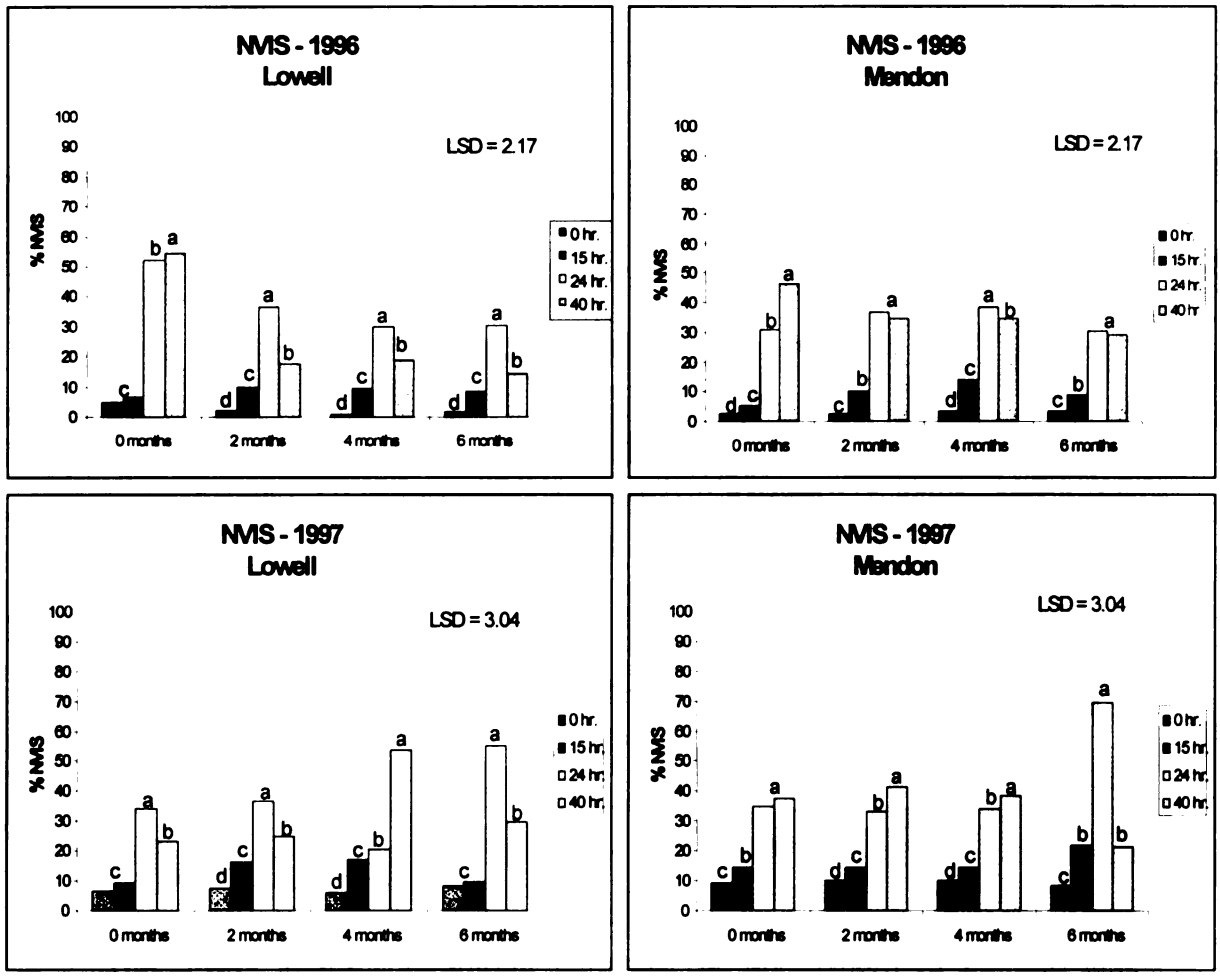


Figure 3. The effect of imbibition period and storage on nonvisible incipient sprouting (NVIS) of Lowell and Mendon in 1996 and 1997

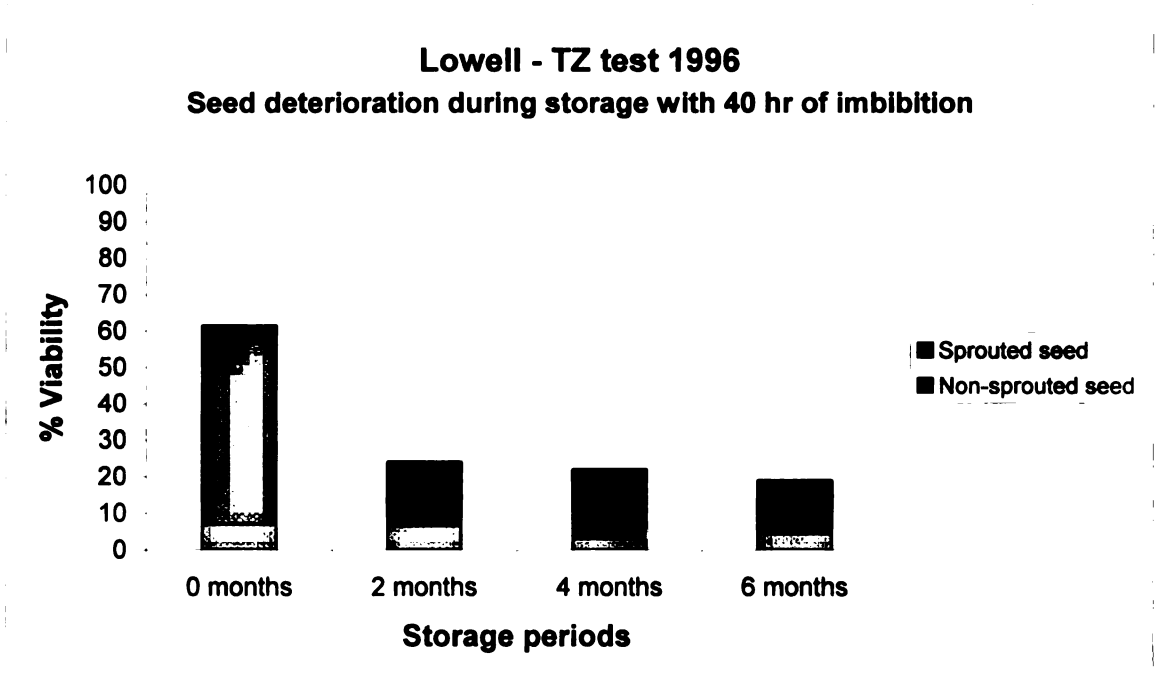


Figure 4. Effect of seed storage on decline of nonvisible incipient sprouting (NVIS)

The 3-way interaction among varieties, IP and length of storage was significant ($p > 0.0013$). The performance of each variety was analyzed across all IP and levels of storage (Tables A13, A14 and A15). Lowell showed no differences in NVIS with little or no exposure to moisture imbibition across storage periods, with values ranging from 1 to 5 percent for the non-sprouted control (0 hr) and 6.5 to 10.0 percent for seed with 15 hr of imbibition (Table A13). Significant differences occurred for the 24 and 40 hr of imbibition across storage periods, with NVIS values ranging from 30 to 52 percent for 24 hr of

imbibition and 14.5 to 54.5 for 40 hr of imbibition. For both 24 and 40 hr of moisture imbibition, NVIS of Lowell significantly decreased after 0 months of storage, indicating that highly sprouted seed loses its viability within days. Mendon also showed no significant differences in NVIS across different storage periods when seed lots were not exposed to moisture, but significant differences occurred for exposures of 15, 24, and 40 hr across storage periods. No sharp decreases in TZ viability during storage (as was observed for Lowell) occurred for Mendon, although the decrease became greater as imbibition period increased. Highly significant differences in NVIS occurred for all periods of storage across IP for both Lowell and Mendon (Table A14). As IP increased, the level of NVIS increased and viability decreased with time of storage. Mendon showed greater NVIS than Lowell as well as increased viability (Tables 4 and 8), especially after 24 hr of imbibition and 2 months of storage. It was noted earlier that red wheat has higher sprouting resistance than white wheat. Though these data (Table 8 and Figure 3) might appear to contradict those reports, they actually support them. As the storage period increased, both TZ viability and NVIS decreased for almost all imbibition periods, indicating that seeds continued to deteriorate during storage. Mendon seed lots did not deteriorate as fast as those of Lowell, explaining why a substantial level of sprouted seeds still remained after 4 and 6 months of storage (Table 8). After 40 hr of imbibition and 0 months of storage, Lowell had 54.5 percent NVIS compared to 46.5 percent for Mendon. But 2 months later, NVIS level of Lowell dropped to 17.5 percent while

that of Mendon rose to 34.5 percent, indicating that the amount of damage and/or deterioration was larger for Lowell than for Mendon.

Lowell and Mendon showed no differences in NVIS across all periods of storage for imbibition periods of 0 and 15 hr (Table A15), but highly significant differences occurred between varieties for 40 hr of imbibition (visible sprouting) at all periods of storage. At 0 and 4 months of storage, NVIS between varieties also showed significant differences after 24 hr of imbibition.

Highly significant differences occurred in NVIS among IP across all times of storage, with the nonimbibed control (0 hr imbibition) having the lowest NVIS. Unstored seed (0 months) imbibed for 40 hr, had the highest NVIS, but with extended storage periods (2, 4, and 6 months) seed imbibed for 24 hr had the highest NVIS. This may be due to gradual death of visibly sprouted seed with prolonged storage. At the same time, NVIS decreased after 4 months of storage, possibly due to loss in viability.

In 1997, the percentage of sprouted seed increased with increased length of imbibition. As damage from sprouting increased (because of more exposure), nonvisible incipient sprouting (NVIS) decreased with prolonged storage due to seeds dying during storage.

Highly significant differences in NVIS occurred due to the effects of variety, storage and imbibition period (IP), as well as the interaction among them (Tables 9 and A16). NVIS ranged from 6.0 to 55.0 percent for Lowell and 9.0 to 69.5 percent for Mendon.

Table 9. Percent nonvisible incipient sprouting (NVIS) for different imbibition periods and storage of Lowell and Mendon in 1997

Lowell	Imbibition period	Storage period				
		0 months	2 months	4 months	6 months	mean
	0 hr.	6.50	7.50	6.00	8.50	7.13 d
	15 hr.	9.00	16.00	17.00	9.50	12.88 c
	24 hr.	34.00	36.50	20.50	55.00	36.50 a
	40 hr.	23.00	25.00	53.50	29.50	32.75 b
	Mean	18.13 c	21.25 b	24.25ab	25.63 a	
Mendon		0 months	2 months	4 months	6 months	mean
	0 hr.	9.00	10.00	10.00	8.50	9.38 d
	15 hr.	14.50	14.50	14.50	22.00	16.38 c
	24 hr.	35.00	33.00	34.00	69.50	42.88 a
	40 hr.	37.50	41.50	38.50	21.50	34.75 b
	Mean	24.00 b	24.75 b	24.25 b	30.38 a	

$LSD_{(imbibition)} = 3.04$
$LSD_{(storage)} = 3.04$

No differences in NVIS occurred for either Lowell or Mendon across storage periods when seed lots were exposed to 0 or 15 hr of imbibition (Table A17). Highly significant differences in NVIS occurred among storage periods at the 24 and 40 hr of imbibition for both Lowell and Mendon. As in previous years, seed lots damaged by NVIS decreased in viability during storage.

Lowell and Mendon showed significant differences in NVIS among storage levels across imbibition periods (IP) (Table A18). NVIS increased with increased moisture imbibition, with the largest levels occurring between 24 and 40 hr of imbibition, depending on the variety. No differences in NVIS occurred between Lowell and Mendon at all storage periods for nonimbibed seed (0 hr) (Table

A19). Neither variety showed differences in NVIS when imbibed for 15 hr at 0, 2 and 4 months of storage. Highly significant differences occurred after 40 hr of imbibition at all storage periods, with Mendon having higher levels at 0 and 2 months of storage and Lowell having more after 4 and 6 months.

c) Standard Warm Germination Test

In 1996, highly significant differences in germination occurred among treatments for seed at 25°C on moist blotter paper (Table 10). Variety, imbibition period (IP), and storage level caused significant differences, as well as the 3-way interaction among them. There were no differences in germination between Lowell (84.5 percent) and Mendon (87.3 percent). Germination ranged from 26.3 to 97.0 for Lowell and 52.0 to 96.7 for Mendon (Tables 10 and A20).

No differences in germination occurred for Lowell and Mendon across IP for unstored seed (0 months) (Table A21), while highly significant differences occurred for seed stored for 2, 4, and 6 months. Differences in germination became larger as the IP increased and as storage continued (Table 10).

Lowell showed no differences in germination for the 0 and 15 hr of imbibition across storage periods (Table A22), while Mendon showed no differences as storage increased following 0, 15, and 24 hr of imbibition. Damage caused by moisture imbibition was not adequate to reduce germination with increased storage, even after longer periods of imbibition. Mendon appeared to have resisted imbibition damage, probably due to a lower rate of

moisture imbibition by the red seed coat. However, significant germination differences occurred across storage periods after seed lots of Lowell were imbibed for 24 hr and for both Lowell and Mendon after 40 hr of imbibition. Greater damage from moisture imbibition occurred after sprouting became visible and reduced germination occurred with increased storage.

Table 10. Percent germination following different imbibition period and storage of Lowell and Mendon in 1996

	Imbibition period	Storage Period				
Lowell		0 months	2 months	4 months	6 months	Mean
	0 hr	95.33	95.00	94.00	97.00	95.33 a
	15 hr	93.67	96.67	92.67	93.67	94.17 a
	24 hr	87.00	86.00	92.67	80.67	86.58 b
	40 hr	91.00	80.00	49.67	26.33	61.75 c
	mean	91.75 a	89.42 b	82.25 c	74.42 d	
Mendon		0 months	2 months	4 months	6 months	Mean
	0 hr	94.33	96.00	96.67	95.00	95.50 a
	15 hr	94.00	93.00	91.67	94.67	93.33 b
	24 hr	86.67	92.00	93.33	88.33	90.08 c
	40 hr	88.67	74.00	67.00	52.00	70.42 d
	mean	90.92 a	88.75 b	87.17 b	82.50 c	

$LSD_{(Imbibition)} = 2.00$
$LSD_{(Storage)} = 2.00$

Significant germination decreases occurred only for the longest imbibition period (40 hr) after 4 months of storage, and for 24 and 40 hr of imbibition after 6 months as detected by the slicing procedure across varieties (Table A23). No differences in germination occurred between Lowell and Mendon across all imbibition periods after 0 and 2 months of storage and across 0 and 15 hr after 4 and 6 months. At 4 months of storage, differences between the two varieties became significant and were maintained after 6 months, with Mendon having higher germination than Lowell. The maximum differences in germination occurred after 40 hr of moisture imbibition in which sprouting damage became visible.

In 1997, seed lots of Lowell and Mendon had reduced germination values as imbibition and storage were prolonged (Table 11). Highly significant differences in germination occurred due to the effects of imbibition and storage, however, no differences occurred between Lowell and Mendon. The interactions between variety and IP, and storage and IP were also significant (Table A24).

Highly significant differences in germination across IP occurred for both Lowell and Mendon (Table A25). Germination decreased rapidly after 15 hr of imbibition, reaching values below 50 percent when imbibed for 40 hr. Nonimbibed Lowell and Mendon showed no differences in germination among storage periods. Significant differences occurred between both varieties after

some imbibition, with Lowell reporting higher germination than Mendon at 15 and 24 hr of imbibition.

Highly significant differences in germination occurred at all storage periods across imbibition periods (Table A26), indicating that NVIS seed lost germination potential with increased imbibition. No differences occurred between nonimbibed seed lots stored for different amounts of time. Differences became significant as IP increased, with germination decreasing sharply as storage was prolonged. Lots imbibed for 40 hr and stored for up to 6 months had the lowest germination, a clear indication of the deleterious effect of storage on NVIS seed.

Table 11. Percent germination of Lowell and Mendon after different periods of imbibition and storage in 1997

	Imbibition period	Storage Period				
Lowell		0 months	2 months	4 months	6 months	Mean
	0 hr.	95.25	94.25	90.50	93.50	93.38 a
	15 hr.	95.25	91.25	91.00	89.75	91.81 a
	24 hr.	91.25	87.75	84.50	72.50	84.00 b
	40 hr.	58.00	50.25	42.25	14.25	41.19 c
	mean	84.94 a	80.88 b	77.06 c	67.50 d	
Mendon		0 months	2 months	4 months	6 months	Mean
	0 hr.	93.00	92.75	90.00	91.75	91.88 a
	15 hr.	88.25	84.00	85.50	83.75	85.38 b
	24 hr.	84.00	85.50	81.50	68.75	79.94 c
	40 hr.	65.00	59.75	56.50	21.25	50.63 d
	mean	82.56 a	80.50 b	78.38 c	66.38 d	

$LSD_{(imbibition)} = 1.81$
$LSD_{(storage)} = 1.57$

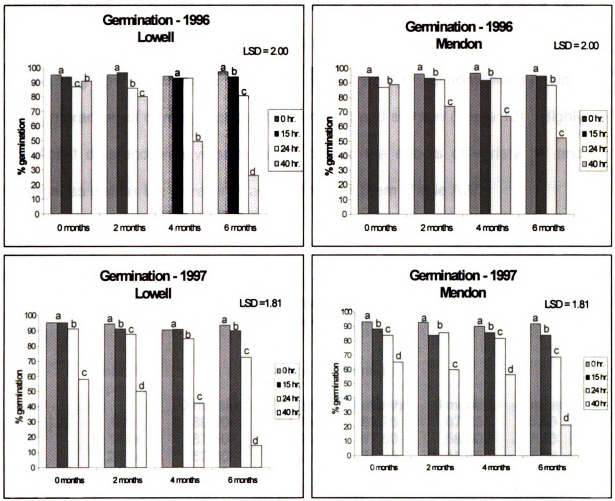


Figure 5. The effects of imbibition period and storage on the germination of Lowell and Mendon in 1996 and 1997

d) Accelerated aging test

In 1997, the accelerated aging (AA) germination of both Lowell and Mendon tended to decrease dramatically after imbibition, reaching almost zero as sprouting became visible following 40 hr of imbibition for unstored seed. Decreasing vigor occurred with increased time of storage, demonstrating again a clear interaction of damage caused by sprouting and storage (Table 12). Highly significant differences in vigor occurred due to the effects of variety, IP and storage, as well as the 3-way interaction between them (Table A27).

Table 12. Percent accelerated aging germination for different imbibition period (IP) and storage of Lowell and Mendon in 1997

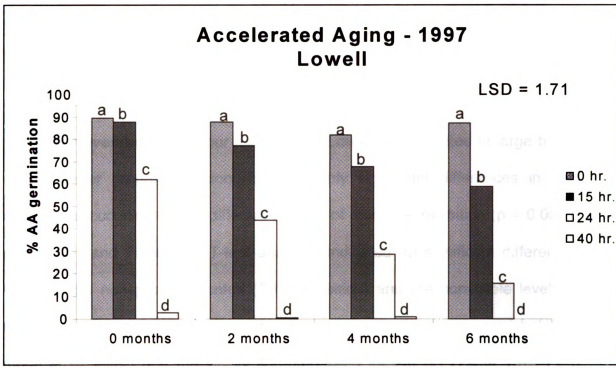
	Imbibition period	Storage period				
Lowell		0 months	2 months	4 months	6 months	mean
	0 hr.	89.50	87.75	81.75	87.25	86.56 a
	15 hr.	87.50	77.00	67.50	58.50	72.63 b
	24 hr.	62.00	43.50	28.25	15.75	37.38 c
	40 hr.	2.75	0.50	1.00	0.00	1.06 d
	Mean	60.44 a	52.19 b	44.63 c	40.38 d	
Mendon		0 months	2 months	4 months	6 months	Mean
	0 hr.	83.00	76.00	69.75	69.00	74.44 a
	15 hr.	80.25	54.75	47.50	47.50	57.50 b
	24 hr.	46.25	23.00	27.00	12.25	27.13 c
	40 hr.	2.00	0.50	0.50	0.00	0.75 d
	Mean	52.88 a	38.56 b	36.19 c	32.19 d	

$LSD_{(imbibition)} = 1.706$
$LSD_{(storage)} = 1.706$

Slicing analyses revealed highly significant differences in vigor for both Lowell and Mendon at the 0, 15 and 24 hr imbibition periods across all storage periods. Thus, when NVIS occurred (0, 15 and 24 hr of imbibition), vigor levels for both varieties sharply decreased during storage (Table A28). For example, Mendon seed imbibed for 15 hr decreased in germination from 80.3 percent at 0 months of storage to 47.5 percent after 6 months. Meanwhile, no differences occurred in vigor across storage periods for either variety after 40 hr of imbibition; their vigor levels were already very low without storage.

Lowell and Mendon showed highly significant differences in vigor at every period of storage across different imbibition periods (Table A29). Vigor readings decreased almost to zero as IP increased from 0 to 15 hr and then to 24 and 40 hr. The amount of damage from NVIS appears to have a larger effect than storage on vigor, although storage effects were still able to reduce vigor following sprouting.

Lowell and Mendon showed significant differences in accelerated aging vigor at every storage period when the amount of moisture received was 24 hr or less. No differences in vigor occurred for seed imbibed for 24 hr after 4 and 6 months of storage. Also, no differences in vigor occurred between varieties for seed imbibed up to 40 hr following any storage period (Table A30).



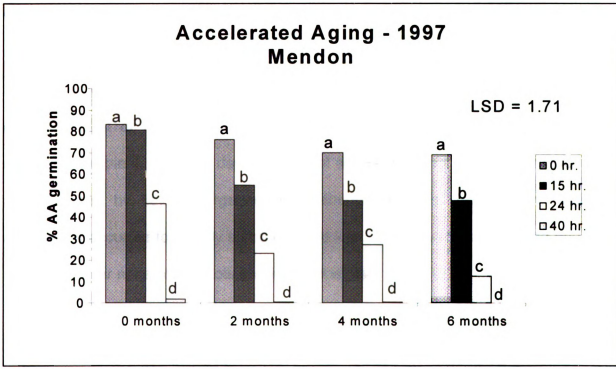


Figure 6. The effect of imbibition and storage period on accelerated aging germination of Lowell and Mendon in 1997

2. Field experiments

a) Percent field emergence

In November 1995, four replicates of Lowell were planted in large boxes of soil under greenhouse conditions. Highly significant differences in field emergence occurred for the different periods of moisture imbibition ($p > 0.0001$) (Table A31 and Figure 4). T-test analyses indicated no significant differences between the nonsprouted control (0 hr imbibition) and the nonvisible levels of sprouting (15 and 24 hr), while highly significant differences occurred between those three levels and the visibly sprouted seed lot (40 hr) (Table 13). Field emergence (FE) values ranged from 51.2 to 75.8 percent. Regression analyses showed a significant correlation between nonvisible incipient sprouting (NVIS) and field emergence, with emergence decreasing 0.445 percent per unit increase in the amount of NVIS ($r^2 = 0.445$). Based on these preliminary studies, nonvisible incipient sprouting damage did not appear to affect field performance as measured by field emergence, even though a large decrease in field emergence occurred for visibly sprouted seed lots. Due to very late planting and a severe winter none of the plots survived the winter.

Table 13. Field emergence 21 days after planting for Lowell in 1995

Imbibition period	Field emergence (%)
0 hr	70.57 a
15 hr	75.80 a
24 hr	68.78 a
40 hr	51.23 b

$LSD_{0.05} = 7.26$

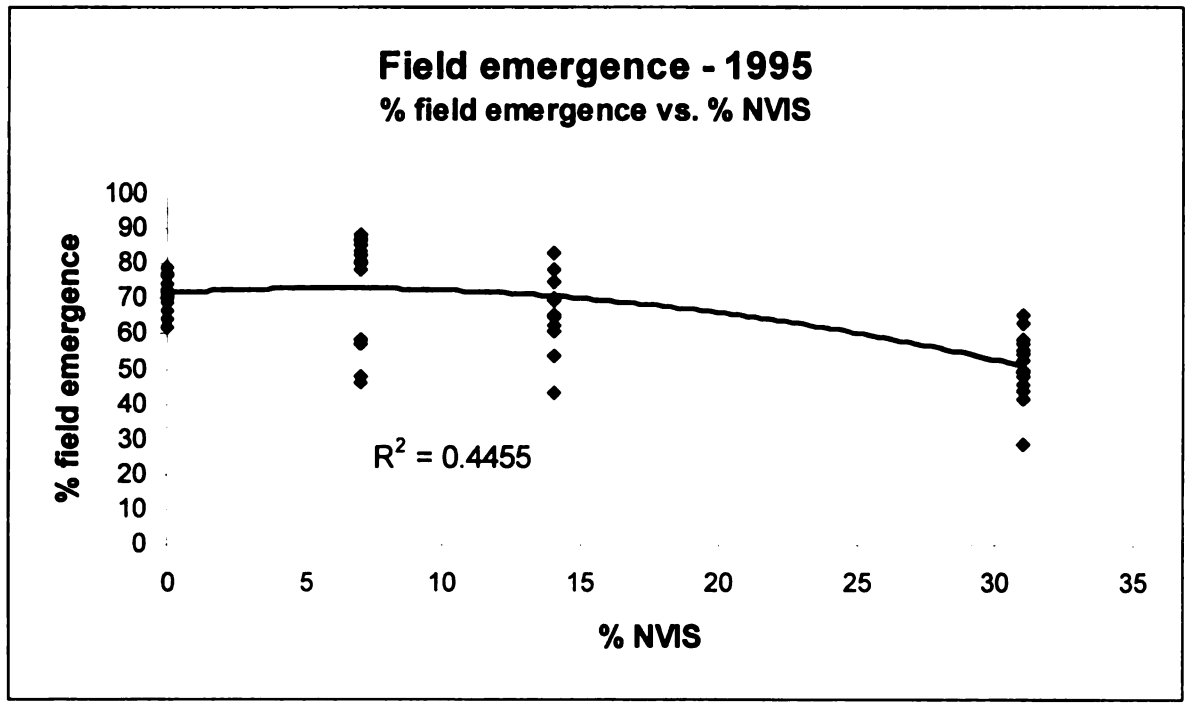


Figure 7. The effect of nonvisible incipient sprouting on field emergence of Lowell in 1995

In 1996, field emergence was largely affected by the duration of moisture imbibition, damage by sprouting and seed deterioration during storage. Highly significant differences in emergence occurred in 1996 due to variety, imbibition period (IP) and storage, but none between locations. Thus, emergence levels for variety, storage and IP were averaged across the two locations (Table 15). Significant differences occurred for the interactions between variety and IP and between IP and storage.

No differences in field emergence occurred among nonvisible incipient sprouting (NVIS) levels (15 and 24 hr). Seed imbibed for 40 hr emerged significantly lower than that imbibed for 0, 15 and 24 hr. Emergence ranged from 37 to 90 percent for Lowell and 46 to 88 percent for Mendon. Field emergence did not decrease as the IP increased from 0 to 24 hr, but decreased sharply when imbibed for 40 hr (Tables 15 and 16). However, it decreased significantly as the storage period increased from 0 to 6 months. Significant differences in emergence occurred among different times of seed storage.

Table 14. Field emergence 21 days after planting following imbibition period and storage of Lowell and Mendon in 1996 at East Lansing and Clarksville, MI

Location: East Lansing, MI
Data in percent field emergence

	Imbibition period	Storage period				
Lowell		0 months	2 months	4 months	6 months	Mean
	0 hr	73.96	75.74	78.75	87.86	79.08
	15 hr	87.95	91.82	83.04	80.63	85.86
	24 hr	87.05	90.03	70.45	84.64	83.04
	40 hr	78.13	50.74	32.95	35.63	49.36
	mean	81.77	77.08	66.29	72.19	74.33
Mendon		0 months	2 months	4 months	6 months	Mean
	0 hr	82.59	84.67	73.13	81.43	80.45
	15 hr	81.10	75.74	67.50	77.41	75.44
	24 hr	88.69	72.77	74.46	79.82	78.94
	40 hr	69.79	57.44	39.91	44.73	52.97
	mean	80.54	72.66	63.75	70.85	71.95

Location: Clarksville, MI
Data in percent field emergence

	Imbibition period	Storage period				
Lowell		0 months	2 months	4 months	6 months	Mean
	0 hr	89.58	79.76	84.38	84.11	84.46
	15 hr	83.33	88.69	80.09	92.68	86.20
	24 hr	87.65	81.70	77.95	86.79	83.52
	40 hr	78.13	56.70	38.57	38.04	52.86
	mean	84.67	76.71	70.25	75.40	76.76
Mendon		0 months	2 months	4 months	6 months	Mean
	0 hr	73.96	81.70	76.34	69.11	75.28
	15 hr	80.80	82.74	76.61	79.29	79.86
	24 hr	86.31	84.97	73.66	73.39	79.58
	40 hr	78.87	55.95	51.16	58.66	61.16
	mean	79.99	76.34	69.44	70.11	73.97

Table 15. Field emergence 21 days after planting following imbibition period and storage of Lowell and Mendon in 1996

Average of two locations
Data in percent field emergence

	Imbibition period	Storage period				
Lowell		0 months	2 months	4 months	6 months	mean
	0 hr	81.8	77.8	81.6	86.0	81.8 b
	15 hr	85.6	90.3	81.6	86.7	86.0 a
	24 hr	87.4	85.9	74.2	85.7	83.3 ab
	40 hr	78.1	53.7	35.8	36.8	51.1 c
	mean	83.2 a	76.9 b	68.3 c	73.8 b	
Mendon		0 months	2 months	4 months	6 months	Mean
	0 hr	78.3	83.2	74.7	75.3	77.9 a
	15 hr	81.0	79.2	72.1	78.4	77.6 a
	24 hr	87.5	78.9	74.1	76.6	79.3 a
	40 hr	74.3	56.7	45.5	51.7	57.1 b
	mean	80.3 a	74.5 b	66.6 d	70.5 c	

$LSD_{(imbibition)} = 3.62$
$LSD_{(storage)} = 3.62$

Highly significant interactions occurred between variety and IP, and between IP and storage (Table A33). Slicing analyses for the interaction between variety and IP showed high levels of significance for both varieties across all imbibition periods, with values ranging from 51.1 to 86.0 percent for Lowell and 57.1 to 79.3 percent for Mendon (Tables 16 and A34).

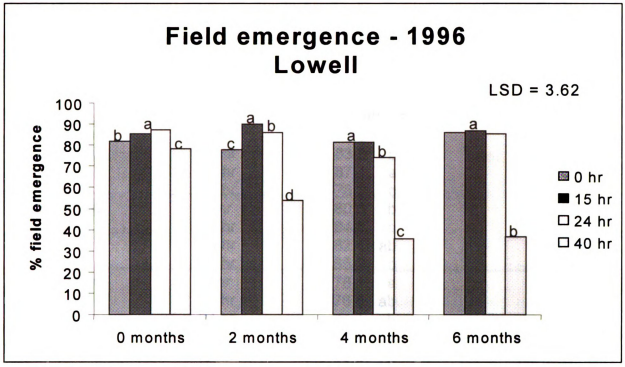
The interaction between imbibition period (IP) and length of storage was highly significant. Significant differences in emergence also occurred among the 0, 15, 24, and 40 hr of moisture imbibition at each storage period (Tables 17 and A35). Highly significant differences occurred in field emergence between the 24 and 40 hr of imbibition across all storage periods, and decreased sharply as

storage increased from 0 to 6 months. However, the 0 and 15 hr imbibition periods showed no differences in emergence across storage periods, indicating that both nonsprouted seed and nonvisible incipient sprouted seed maintained emergence capacity in the field.

Table 16. Field emergence following imbibition periods (IP) of Lowell and Mendon in 1996

Variety	IP	Field emergence (%)
Lowell	0 hr	81.8 b
	15 hr	86.0 a
	24 hr	83.3 ab
	40 hr	51.1 c
Mendon	0 hr	77.9 a
	15 hr	77.6 a
	24 hr	79.3 a
	40 hr	57.1 b

$LSD_{(emerge.)} = 3.62$



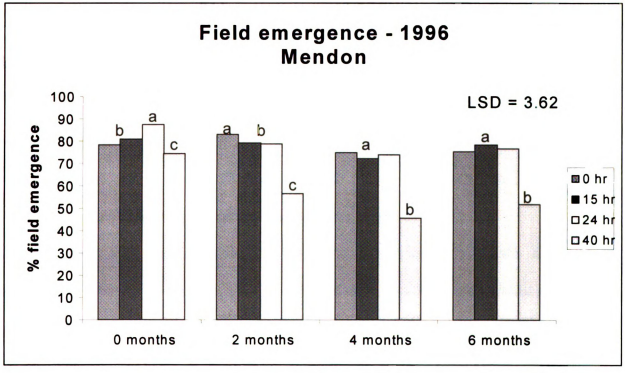


Figure 8. The effects of imbibition period and storage on the field emergence of Lowell and Mendon in 1996

Table 17. Least square means for the interaction between imbibition period and storage for field emergence of Lowell and Mendon in 1996

Storage	Imbibition period	Percent field emergence	
0 months	0 hr	80.0	b
	15 hr	83.3	b
	24 hr	87.4	a
	40 hr	76.2	c
2 months	0 hr	80.5	b
	15 hr	84.8	a
	24 hr	82.4	ab
	40 hr	55.2	c
4 months	0 hr	78.1	a
	15 hr	76.8	ab
	24 hr	74.1	b
	40 hr	40.6	c
6 months	0 hr	80.6	a
	15 hr	82.5	a
	24 hr	81.2	a
	40 hr	44.3	b

$LSD_{0.05} = 3.62$

b) Quantitative regrowth index potential

During the months of March and April 1997 an index was used to indicate the potential of seed lots to establish a crop. With further deterioration, incipient sprouted seed lots lose the capacity to emerge and produce normal vigorous stands under field conditions. The reduced seed/seedling vigor, therefore, results in a reduced stand with reduced vegetative growth with less potential for winter survival and productivity.

Similar trends as those observed for field emergence occurred in the quantitative regrowth index. The index decreased with increasing imbibition periods and prolonged storage (Tables 18, 19 and 20). Highly significant differences occurred among seed lots due to variety, imbibition period (IP) and length of storage, but none between locations. Thus, index levels for variety, storage and IP were averaged across the two locations (Table 19). Highly significant differences in the index also occurred for the interactions between variety and IP and between IP and storage levels. Indexes ranged from 1.3 to 8.4 for Lowell and 2.1 to 8.2 for Mendon, with 0 indicating minimum stand and vigor level and 10 with maximum stand and vigor level.

Lowell and Mendon showed significant decreases in regrowth indexes across all periods of storage (Tables 20 and A37). Lowell had index values ranging from 7.84 for unstored seed lots compared to 3.94 for storage periods of 6 months. However, no significant differences in regrowth index occurred between Lowell and Mendon at any storage period. At 0 months of storage, the index was 7.84 and 7.75 of Lowell and Mendon, respectively.

Table 18. Quantitative index of regrowth potential in the spring of 1997 following imbibition period and storage of Lowell and Mendon at East Lansing and Clarksville, MI

Location: East Lansing, MI

	Imbibition period	Storage period				
Lowell		0 months	2 months	4 months	6 months	mean
	0 hr	8.00	8.75	5.00	6.00	6.94
	15 hr	8.50	8.75	5.75	5.75	7.19
	24 hr	8.75	8.25	6.00	5.75	7.19
	40 hr	7.75	5.00	1.75	1.50	4.00
	mean	8.25	7.69	4.63	4.75	
Mendon		0 months	2 months	4 months	6 months	mean
	0 hr	8.38	8.50	4.63	4.75	6.56
	15 hr	8.25	8.25	5.25	4.63	6.59
	24 hr	8.00	8.13	4.50	4.75	6.34
	40 hr	7.38	5.75	2.25	3.13	4.63
	mean	8.00	7.66	4.16	4.31	

Location: Clarksville, MI

	Imbibition period	Storage period				
Lowell		0 months	2 months	4 months	6 months	mean
	0 hr	7.50	7.50	6.00	5.50	6.63
	15 hr	7.75	8.00	4.00	4.25	6.00
	24 hr	7.75	8.25	4.25	4.00	6.06
	40 hr	6.75	4.75	1.25	1.00	3.44
	mean	7.44	7.13	3.88	3.69	
Mendon		0 months	2 months	4 months	6 months	mean
	0 hr	6.75	6.00	3.25	4..50	5.13
	15 hr	7.75	6.75	3.75	2.75	5.25
	24 hr	7.25	8.25	2.75	2.75	5.25
	40 hr	8.25	4.75	2.00	2.00	4.25
	mean	7.50	6.44	2.94	3.00	

Table 19. Quantitative index of regrowth potential in the spring of 1997 following imbibition period and storage of Lowell and Mendon

Average of two locations
Data in index of regrowth potential

	Imbibition period	Storage period				
Lowell		0 months	2 months	4 months	6 months	mean
	0 hr	7.75	8.13	5.50	5.75	6.78 a
	15 hr	8.13	8.38	4.88	5.00	6.59 a
	24 hr	8.25	8.25	5.13	4.88	6.63 a
	40 hr	7.25	4.88	1.50	1.25	3.72 b
	Mean	7.84 a	7.41 b	4.25 c	4.22 c	
Mendon		0 months	2 months	4 months	6 months	Mean
	0 hr	7.56	7.25	3.94	4.63	5.84 a
	15 hr	8.00	7.50	4.50	3.69	5.92 a
	24 hr	7.63	8.19	3.63	3.75	5.80 a
	40 hr	7.81	5.25	2.13	2.56	4.44 b
	Mean	7.75 a	7.05 b	3.55 c	3.66 c	

$LSD_{(imbibition)} = 0.368$
$LSD_{(storage)} = 0.368$

Table 20. Least square means for the interaction between storage and variety for the quantitative index of regrowth potential of Lowell and Mendon in the spring of 1997

Variety	Storage level	Quantitative index of regrowth potential
Lowell	0 months	7.84 a
	2 months	7.41 b
	4 months	4.00 c
	6 months	3.94 c
Mendon	0 months	7.75 a
	2 months	7.05 b
	4 months	3.55 c
	6 months	3.66 c

$LSD_{index} = 0.368$

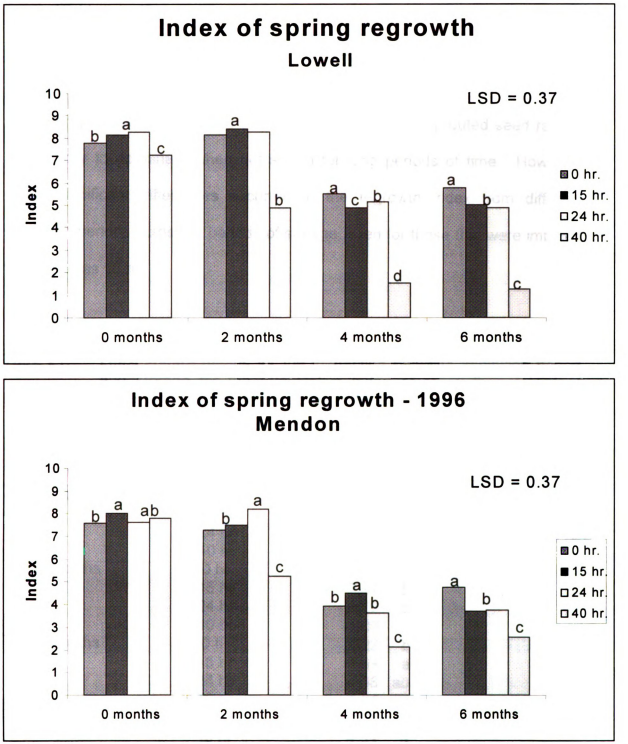


Figure 9. Index of regrowth potential following imbibition and storage periods of Lowell and Mendon in 1997

Highly significant differences in quantitative regrowth index occurred across imbibition periods after 2 months of storage and continued with further storage (Tables 21 and A38). No differences in the index occurred among imbibition periods for nonstored seed, demonstrating that sprouted seed retains its capacity to germinate when not stored for long periods of time. However, highly significant differences occurred in the regrowth index from different imbibition periods across all periods of storage, even for those that were imbibed for as little as 15 hr.

Table 21. Least square means for the interaction between imbibition period and storage for the quantitative index of regrowth potential of Lowell and Mendon in the spring of 1997

Storage Level	Imbibition Period	Quantitative index of regrowth potential	
0 months	0 hr	7.66	bc
	15 hr	8.06	a
	24 hr	7.94	ab
	40 hr	7.53	c
2 months	0 hr	7.69	b
	15 hr	7.94	b
	24 hr	8.22	a
	40 hr	5.06	c
4 months	0 hr	4.22	b
	15 hr	4.69	a
	24 hr	4.38	ab
	40 hr	1.81	c
6 months	0 hr	4.63	a
	15 hr	4.34	a
	24 hr	4.31	a
	40 hr	1.91	b

$LSD_{0.05} = 0.368$

c) Grain Yield

The 1997 wheat crop was combine harvested during the month of July. Data are presented in kilograms of dry wheat per hectare (Tables 22 and 23).

Highly significant differences occurred among the yield of plots planted with seed from different imbibition periods stored for different lengths of time. Imbibition periods and duration of storage, as well as the interaction between them, resulted in significant differences in grain yields (Tables 22 and A39) which ranged from 2887 to 4884 kg per ha for Lowell and 3052 to 4416 kg per ha for Mendon.

No differences in yield occurred between the two varieties, with Lowell averaging 4027 kg per ha and Mendon 3933 kg per ha across all locations (Table A39). Meanwhile, highly significant differences occurred in yield of plots planted from seed with different levels of sprouting caused by different periods of imbibition. Seed lots imbibed for 0, 15 and 24 hr did not perform significantly different, but differed significantly from those imbibed for 40 hr. Seed lots not showing visible sprouting produced an average of 4118 kg per ha, while those with visible sprouting averaged 3568 kg per ha. This is consistent with that of field emergence and the quantitative regrowth index, demonstrating that nonvisible sprouted seed retains its capacity to perform well under field conditions.

Table 22. Yield (kg/ha) of seed lots following imbibition period and storage of Lowell and Mendon in 1997. Average of two locations

	Imbibition period	Storage Period				
Lowell		0 months	2 months	4 months	6 months	Mean
	0 hr.	4335	4242	3911	4026	4129 a
	15 hr.	4294	4547	3952	4154	4237 a
	24 hr.	4884	4281	3725	4199	4272 a
	40 hr.	4399	3652	2938	2887	3469 b
	mean	4478 a	4181 b	3631 d	3817 c	
Mendon		0 months	2 months	4 months	6 months	Mean
	0 hr.	4339	4013	3911	3636	3975 a
	15 hr.	4416	4256	3828	3851	4088 a
	24 hr.	4276	4105	3831	3809	4005 a
	40 hr.	4205	3954	3452	3052	3666 b
	mean	4309 a	4082 b	3756 c	3587 d	

$LSD_{(imbibition)} = 175$
$LSD_{(storage)} = 175$

Table 23. Least square means for the interaction between imbibition period (IP) and storage on yield (kg/ha) in 1997

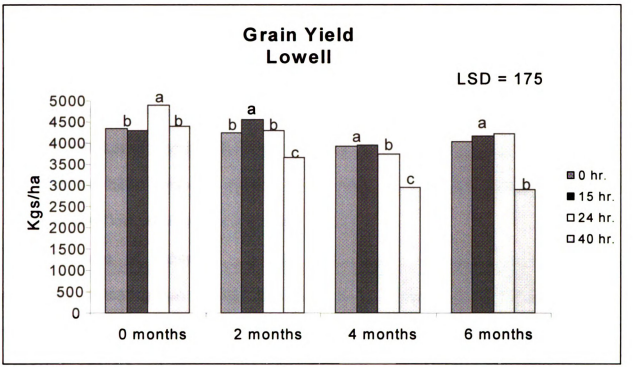
Storage level	Imbibition period	Yield (kg/ha)
0 months	0 hr.	4337 b
	15 hr.	4355 b
	24 hr.	4580 a
	40 hr.	4302 b
2 months	0 hr.	4128 b
	15 hr.	4402 a
	24 hr.	4193 b
	40 hr.	3803 c
4 months	0 hr.	3911 a
	15 hr.	3890 ab
	24 hr.	3778 b
	40 hr.	3195 c
6 months	0 hr.	3831 b
	15 hr.	4002 a
	24 hr.	4004 a
	40 hr.	2969 c

$LSD_{0.05} = 175$

Highly significant differences in yield also occurred from seed lots stored for different periods of time, with yield decreasing with increased time of storage (Table 23). Unstored lots resulted in the highest yields (4394 kg per ha), followed by those stored for 2 months (4131 kg per ha). Seed lots stored for 4 and 6 months showed no difference in yield at 3698 kg per ha.

In general, NVIS damage did not affect the yield from seed across all storage periods, though yields decreased rapidly with storage for visibly sprouted seed. Slicing analyses (Tables 23 and A40) showed that no differences in yield occurred among periods of imbibition for unstored seed, while highly significant differences resulted from different storage periods. The results indicate that sprouted seed could be used for planting if stored for no longer than 2 months. However, as storage increased from 0 to 2, 4 and 6 months, differences in yield became significant, with the seed lot imbibed for 40 hr having the lowest yield at all storage levels. Clearly, the effects of storage on visibly sprouted seed seriously affected its yield potential.

Highly significant yield differences resulted from seed exposed to different periods of moisture imbibition across storage periods. Seed lots rapidly deteriorated after 2 months of storage, including those with NVIS. Differences in yield increased with increased NVIS and prolonged storage, supporting previously noted evidence that sprouted seed has reduced performance with longer storage.



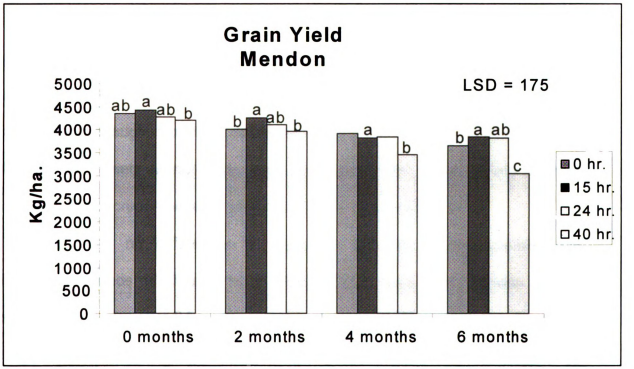


Figure 10. The effects of imbibition period and storage on the grain yield of Lowell and Mendon in 1997

Discussion and recommendations

Commercially acceptable levels of germination/viability were still obtained after 15 hr of imbibition, although a rapid decrease in quality occurred thereafter. Seed of both Lowell and Mendon with nonvisible incipient sprouting (NVIS) after 15 hr of imbibition retained viability for as long as 6 months, while visibly sprouted seed lost viability after 2 months (Fig. 3).

As time of imbibition increased, TZ viability decreased and NVIS increased, indicating that damage had occurred even though sprouting was not yet visible. NVIS damage increased with prolonged imbibition and storage, especially after 24 and 40 hr.

The white variety Lowell was most affected by both nonvisible and visible damage, supporting the well known association between sprouting and the white seed coat color. However, red wheat will also sprout if high moisture conditions persist long enough. No sharp decrease in TZ viability during storage occurred for the red variety Mendon (as was observed for the white variety Lowell), although the decrease became greater with prolonged imbibition.

Although no differences in storability occurred among varieties for imbibition periods of 0 and 15 hr, our studies show that Mendon stored significantly better after 24 hr of imbibition. These differences may be due to its red seed coat color allowing slower imbibition than for Lowell. Consequently, Mendon was less affected by both nonvisible and visible sprouting damage.

Since red varieties incur less damage, the level of deterioration during storage was smaller than for the white variety Lowell.

Damage caused by moisture imbibition was not adequate to reduce germination of unstored seed lots, although germination decreased as storage was prolonged and imbibition increased. Mendon appeared to resist damage caused by prolonged imbibition more than Lowell, possibly due to its slower imbibition rate. Imbibition damage largely affected seed performance after sprouting became visible and increased with prolonged storage.

Accelerated aging vigor of unstored Lowell and Mendon decreased dramatically with increasing time of imbibition, reaching almost zero as sprouting became visible after 40 hr of imbibition. Decreasing vigor occurred during storage, demonstrating again a clear interaction between damage caused by imbibition and storage (Table 11). However, the amount of damage caused by NVIS seems to indicate that imbibition influences vigor more than storage.

Laboratory performance decreased as NVIS increased. TZ viability and standard warm germination decreased with longer imbibition, although no measurable differences in performance occurred in the field.

NVIS did not affect field emergence, even though a large decrease in emergence occurred from visibly sprouted seed, indicating that seed with NVIS could be used for planting if not stored for long periods of time.

Although seed with NVIS retain germinability, field performance (measured by the spring regrowth index) was reduced, even when imbibed for as little as 15 hr and stored for 2 months or more.

Yield results are consistent with those for field emergence and the spring quantitative regrowth index. Thus, nonvisibly sprouted seed retained the capacity to perform well under field conditions if not stored for more than 2 months. Yield was not affected by NVIS, but only from stored, visibly sprouted seed.

These results indicate that sprouted seed could be used for planting if stored for no longer than 2 months. However, as storage increases to 4 and 6 months, differences in yield potential become significant, especially for lots imbibed for 40 hr. Clearly, the effects of storage on visibly sprouted seed seriously impaired its yield potential.

Seed lots rapidly deteriorated after 2 months of storage, including those with NVIS. Differences in yield increased with increased NVIS and prolonged storage, supporting previously cited evidence that the use of sprouted seed results in reduced performance after longer storage.

The interaction between imbibition period and variety confirms the influence of genotype on the susceptibility of a variety to preharvest sprouting. It is clear that the duration of imbibition greatly affects germination. This interaction has been reported many times in the literature. Though not specifically shown, our vigor and field emergence data suggest that performance of seed with NVIS may suffer under adverse field conditions.

Visible sprouting damage occurred after approximately 30 hr of moisture imbibition, but NVIS was detected with the tetrazolium test after only 15 hr. While imbibition period greatly influenced the level of NVIS, it did not

substantially affect the TZ test results. This is consistent with the report of Elias (1987) that sprouted seed maintains its capacity for germination under favorable conditions.

Performance of seed lots was seriously affected by both the length of imbibition and storage. However, unstored seed lots maintained their potential for acceptable laboratory and field performance even though visible sprouting had occurred. That potential rapidly decreased if storage was prolonged more than 2 months, especially for visibly sprouted seed.

Recommendations

To assure the maintenance of quality (germination and vigor) of wheat seed that has been exposed to sprouting conditions, the following recommendations should be considered:

1. Seed of both white and red varieties can be stored for up to two months even if incipient visible sprouting has occurred. Growers should be aware that visibly sprouted seed lots have dramatically reduced vigor capacity, which can severely reduced field emergence under adverse conditions.
2. Wheat seed with NVIS will also have reduced vigor, which could also reduce field emergence, especially during storage periods of 6 months or more.

Appendix

Table A1. Analysis of variance for the effect of imbibition period (IP) on TZ viability of Lowell and Mendon, 1995

Dependent Variable: TZ viability

Source	DF	Mean Square	Pr > F
Model	7	378.63	0.0001
Error	8	4.44	
Corrected Total	15		

Source	DF	Mean Square	Pr > F
VARIETY	1	33.06	0.0259
IP	3	849.23	0.0001
VARIETY * IP	3	23.23	0.0273

Table A2. Slicing procedure for the interaction between imbibition period (IP) and variety for TZ viability of Lowell and Mendon in 1995

Sliced by Variety

Variety	Df	Mean Square	Pr > F
Lowell	3	329.33	0.0001
Mendon	3	543.13	0.0001

Sliced by Imbibition period

Imbibition period	df	Mean Square	Pr > F
0 hr	1	0.25	0.818
15 hr	1	2.25	0.497
24 hr	1	0.25	0.818
40 hr	1	100	0.0015

Table A3. Analysis of variance for the effect of imbibition period (IP) and storage on TZ viability of Lowell and Mendon in 1996

Dependent Variable: TZ viability

Source	DF	Mean Square	Pr > F
Model	31	1235.80	0.0001
Error	32	9.50	
Corrected Total	63		

Source	DF	Mean Square	Pr > F
VARIETY	1	370.56	0.0001
IP	3	10790.46	0.0001
STORAGE	3	282.71	0.0001
VARIETY * IP	3	308.19	0.0001
IP * STORAGE	9	408.58	0.0001
VARIETY*STORA	3	7.60	0.5028
VARI * IP * STOR	9	10.56	0.3827

Table A4. Slicing procedure for the interaction between imbibition period (IP) and variety on TZ viability in 1996

Sliced by Variety

Variety	Df	Mean Square	Pr > F
Lowell	3	7273.53	0.0001
Mendon	3	3825.11	0.0001

Sliced by Imbibition period

Imbibition period	Df	Mean Square	Pr > F
0 hr	1	0.06	0.936
15 hr	1	1.00	0.748
24 hr	1	16.00	0.204
40 hr	1	1278.06	0.0001

Table A5. Slicing procedure for the interaction between imbibition period and levels of storage on TZ viability in 1996

Sliced by Storage Level

Storage level	Df	Mean Square	Pr > F
0 months	3	1079.73	0.0001
2 months	3	3254.23	0.0001
4 months	3	3321.83	0.0001
6 months	3	4360.42	0.0001

Sliced by Imbibition period

Imbibition period	df	Mean Square	Pr > F
0 hr	1	9.07	0.426
15 hr	1	51.42	0.004
24 hr	1	73.75	0.001
40 hr	1	1374.23	0.0001

Table A6. Analysis of variance for the effect of imbibition period (IP) and storage on the TZ viability of Lowell and Mendon in 1997

Dependent Variable: TZ viability

Source	DF	Mean Square	Pr > F
Model	31	1178.74	0.0001
Error	32	9.53	
Corrected Total	63		

Source	DF	Mean Square	Pr > F
VARIETY	1	7.56	0.3797
IP	3	11131.79	0.0001
STORAGE	3	123.38	0.0001
VARIETY * IP	3	51.60	0.0040
IP * STORAGE	9	163.17	0.0001
VARIETY*STORA	3	139.27	0.0001
VARI * IP * STOR	9	80.76	0.0001

Table A7. Slicing procedure for the 3-way interaction among variety, imbibition period (IP) and storage on TZ viability of Lowell and Mendon in 1997

Effects of VARIETY * IP * STORAGE sliced by Variety * IP

Variety	Imbibition period	df	Mean Square	Pr > F
Lowell	0 hr.	3	17.46	0.1613
	15 hr.	3	8.33	0.4646
	24 hr.	3	123.50	0.0001
	40 hr.	3	404.00	0.0001
Mendon	0 hr.	3	17.00	0.1701
	15 hr.	3	33.46	0.0263
	24 hr.	3	155.00	0.0001
	40 hr.	3	235.67	0.0001

Table A8. Slicing procedure for the 3-way interaction among variety, imbibition period and storage on nonvisible incipient sprouting (NVIS) of Lowell and Mendon in 1997

Effects of VARIETY * IP * STORAGE sliced by Variety*Storage

Variety	Storage	df	Mean Square	Pr > F
Lowell	0 months	3	2274.33	0.0001
	2 months	3	1984.83	0.0001
	4 months	3	708.83	0.0001
	6 months	3	1660.46	0.0001
Mendon	0 months	3	1133.67	0.0001
	2 months	3	1067.46	0.0001
	4 months	3	1133.13	0.0001
	6 months	3	1952.46	0.0001

Table A9. Slicing procedure for the 3-way interaction among variety, imbibition period (IP) and storage on nonvisible incipient sprouting (NVIS) in 1997

Effects of VARIETY * IP * STORAGE sliced by Storage x IP

Storage	Imbibition period	df	Mean Square	Pr > F
0 months	0 hr.	1	2.25	0.6304
	15 hr.	1	16.00	0.2044
	24 hr.	1	110.25	0.0018
	40 hr.	1	361.00	0.0001
2 months	0 hr.	1	2.25	0.6304
	15 hr.	1	30.25	0.0843
	24 hr.	1	1.00	0.7481
	40 hr.	1	240.25	0.0001
4 months	0 hr.	1	9.00	0.3385
	15 hr.	1	4.00	0.5217
	24 hr.	1	16.00	0.2044
	40 hr.	1	272.25	0.0001
6 months	0 hr.	1	0.25	0.8724
	15 hr.	1	100.00	0.0028
	24 hr.	1	42.25	0.0432
	40 hr.	1	100.00	0.0028

Table A10. Analysis of variance for the effect of imbibition period (IP) on nonvisible incipient sprouting (NVIS) of Lowell and Mendon in 1995

Dependent Variable: Nonvisible incipient sprouting.

Source	DF	Mean Square	Pr > F
Model	7	830.28	0.0001
Error	8	3.69	
Corrected Total	15		

Source	DF	Mean Square	Pr > F
VARIETY	1	297.56	0.0001
IP	3	1684.40	0.0001
VARIETY * IP	3	153.73	0.0001

Table A11. Slicing procedure for the interaction between imbibition period (IP) and variety on nonvisible incipient sprouting (NVIS) of Lowell and Mendon in 1995

Sliced by Variety

Variety	Df	Mean Square	Pr > F
Lowell	3	1411.00	0.0001
Mendon	3	427.13	0.0001

Sliced by Imbibition period

Imbibition period	Df	Mean Square	Pr > F
0 hr	1	0.25	0.801
15 hr	1	0.00	1.000
24 hr	1	56.25	0.0045
40 hr	1	702.25	0.0001

Table A12. Analysis of variance for the effect of imbibition period and storage on nonvisible incipient sprouting (NVIS) of Lowell and Mendon in 1996

Dependent Variable: Nonvisible incipient sprouting.

Source	DF	Mean Square	Pr > F
Model	31	528.79	0.0001
Error	32	9.05	
Corrected Total	63		

Source	DF	Mean Square	Pr > F
VARIETY	1	66.02	0.0110
IP	3	4221.77	0.0001
STORAGE	3	260.06	0.0001
VARIETY * IP	3	119.31	0.0001
IP * STORAGE	9	176.77	0.0001
VARIETY*STORA	3	198.43	0.0001
VARI * IP * STOR	9	37.45	0.0013

Table A13. Slicing procedure for the interaction among variety, imbibition period (IP) and storage on NVIS of Lowell and Mendon in 1996

Effects of VARIETY * IP * STORAGE sliced by Variety * IP

Variety	IP	df	Mean Square	Pr > F
Lowell	0 hr	3	6.458	0.5509
	15 hr	3	4.792	0.6652
	24 hr	3	210.333	0.0001
	40 hr	3	710.125	0.0001
Mendon	0 hr	3	0.667	0.974
	15 hr	3	27.333	0.044
	24 hr	3	31.792	0.0261
	40 hr	3	109.125	0.0001

Table A14. Slicing procedure for the interaction among variety, imbibition period (IP) and storage on NVIS of Lowell and Mendon in 1996

Effects of VARIETY * IP * STORAGE sliced by Variety*Storage

Variety	Storage	df	Mean Square	Pr > F
Lowell	0 months	3	1507.00	0.0001
	2 months	3	435.67	0.0001
	4 months	3	311.46	0.0001
	6 months	3	305.83	0.0001
Mendon	0 months	3	898.83	0.0001
	2 months	3	590.46	0.0001
	4 months	3	555.46	0.0001
	6 months	3	379.00	0.0001

Table A15. Slicing procedure for the interaction among variety, imbibition period (IP) and storage on NVIS in 1996

Effects of VARIETY * IP * STORAGE sliced by Storage * IP

Storage	IP	df	Mean Square	Pr > F
0 months	0 hr	1	6.25	0.4120
	15 hr	1	2.25	0.6214
	24 hr	1	441.00	0.0001
	40 hr	1	64.00	0.0121
2 months	0 hr	1	0.25	0.8690
	15 hr	1	0.01	0.9999
	24 hr	1	0.01	0.9999
	40 hr	1	289.00	0.0001
4 months	0 hr	1	6.25	0.4120
	15 hr	1	20.25	0.1444
	24 hr	1	72.25	0.0081
	40 hr	1	240.25	0.0001
6 months	0 hr	1	4.00	05109
	15 hr	1	0.25	0.8690
	24 hr	1	0.01	0.9999
	40 hr	1	210.25	0.0001

Table A16. Analysis of variance for the effect of imbibition period and storage on nonvisible incipient sprouting (NVIS) of Lowell and Mendon in 1997

Dependent Variable: Percent NVIS

Source	DF	Mean Square	Pr > F
Model	31	518.20	0.0001
Error	32	17.77	
Corrected Total	63		

Source	DF	Mean Square	Pr > F
VARIETY	1	199.52	0.0021
IP	3	3611.14	0.0001
STORAGE	3	136.89	0.0005
VARIET *IP	3	16.10	0.4489
IP * STORA	9	379.92	0.0001
VARIETY*STORA	3	25.93	0.2440
VARI * IP * STOR	9	119.46	0.0001

Table A17. Slicing procedure for the 3-way interaction among variety, imbibition period and storage on nonvisible incipient sprouting (NVIS) of Lowell and Mendon in 1997

Effects of VARIETY * IP * STORAGE sliced by Variety * IP

Variety	Imbibition period	Df	Mean Square	Pr > F
Lowell	0 hr.	3	2.46	0.9363
	15 hr.	3	35.46	0.1344
	24 hr.	3	403.00	0.0001
	40 hr.	3	397.50	0.0001
Mendon	0 hr.	3	1.13	0.9788
	15 hr.	3	28.13	0.2127
	24 hr.	3	631.46	0.0001
	40 hr.	3	161.83	0.0002

Table A18. Slicing procedure for the 3-way interaction among variety, imbibition period (IP) and storage on nonvisible incipient sprouting (NVIS) of Lowell and Mendon in 1997

Effects of VARIETY * IP * STORAGE sliced by Variety*Storage

Variety	Storage	df	Mean Square	Pr > F
Lowell	0 months	3	329.46	0.0001
	2 months	3	308.83	0.0001
	4 months	3	836.83	0.0001
	6 months	3	954.13	0.0001
Mendon	0 months	3	412.33	0.0001
	2 months	3	447.50	0.0001
	4 months	3	397.50	0.0001
	6 months	3	1438.79	0.0001

Table A19. Slicing procedure for the 3-way interaction among variety, imbibition period (IP) and storage on nonvisible incipient sprouting (NVIS) of Lowell and Mendon in 1997

Effects of VARIETY * IP * STORAGE sliced by Storage x IP

Storage	Imbibition period	df	Mean Square	Pr > F
0 months	0 hr.	1	6.25	0.5573
	15 hr.	1	30.25	0.2012
	24 hr.	1	1.00	0.8140
	40 hr.	1	210.25	0.0016
2 months	0 hr.	1	6.25	0.5573
	15 hr.	1	2.25	0.7243
	24 hr.	1	12.25	0.4125
	40 hr.	1	272.25	0.0004
4 months	0 hr.	1	16.00	0.3497
	15 hr.	1	6.25	0.5573
	24 hr.	1	182.25	0.0031
	40 hr.	1	225.00	0.0012
6 months	0 hr.	1	0.01	0.9999
	15 hr.	1	156.25	0.0057
	24 hr.	1	210.25	0.0016
	40 hr.	1	64.00	0.0667

Table A20. Analysis of variance for the effect of imbibition period and storage on germination of Lowell and Mendon in 1996

Dependent Variable: Percent germination

Source	DF	Mean Square	Pr > F
Model	31	773.41	0.0001
Error	64	16.30	
Corrected Total	95		

Source	DF	Mean Square	Pr > F
VARIETY	1	198.38	0.0009
IP	3	4406.49	0.0001
STORAGE	3	771.63	0.0001
VARIETY * IP	3	110.04	0.0005
VARIETY*STORA	3	115.18	0.0004
IP * STORA	9	750.48	0.0001
VARI * IP * STOR	9	90.33	0.0001

Table A21. Slicing procedure for the interaction among variety, imbibition period and storage on germination of Lowell and Mendon in 1996

Effects of VARIETY * IP * STORAGE sliced by Variety*Storage

Variety	Storage	df	Mean Square	Pr > F
Lowell	0 months	3	39.64	0.0731
	2 months	3	184.08	0.0001
	4 months	3	1416.75	0.0001
	6 months	3	3231.64	0.0001
Mendon	0 months	3	44.31	0.0519
	2 months	3	298.75	0.0001
	4 months	3	555.22	0.0001
	6 months	3	1268.56	0.0001

Table A22. Slicing procedure for the interaction among variety, imbibition period and storage on germination of Lowell and Mendon in 1996

Effects of VARIETY * IP * STORAGE sliced by Variety x IP

Variety	Imbibition period	Df	Mean Square	Pr > F
Lowell	0 hr	3	4.67	0.8351
	15 hr	3	9.00	0.6486
	24 hr	3	72.53	0.0067
	40 hr	3	2588.97	0.0001
Mendon	0 hr	3	3.22	0.8976
	15 hr	3	5.11	0.8155
	24 hr	3	28.97	0.1604
	40 hr	3	696.75	0.0001

Table A23. Least square means for the interaction among variety, imbibition period and levels of storage on germination of Lowell and Mendon in 1996

Effects of VARIETY*SPROUTING*STORAGE sliced by Storage x Sprouting

Storage	Sprouting	df	Mean Square	Pr > F
0 months	0 hr	1	1.50	0.7626
	15 hr	1	0.17	0.9198
	24 hr	1	0.17	0.9198
	40 hr	1	8.17	0.4816
2 months	0 hr	1	1.50	0.7626
	15 hr	1	20.17	0.2702
	24 hr	1	54.00	0.0734
	40 hr	1	54.00	0.0734
4 months	0 hr	1	10.67	0.4216
	15 hr	1	1.50	0.7626
	24 hr	1	0.67	0.8404
	40 hr	1	450.67	0.0001
6 months	0 hr	1	6.00	0.5462
	15 hr	1	1.50	0.7626
	24 hr	1	88.17	0.0232
	40 hr	1	988.17	0.0001

Table A24. Analysis of variance for the effect of imbibition period (IP) and storage on the germination of Lowell and Mendon in 1997

Dependent Variable: Percent germination

Source	DF	Mean Square	Pr > F
Model	31	1799.41	0.0001
Error	96	10.14	
Corrected Total	127		

Source	DF	Mean Square	Pr > F
VARIETY	1	11.28	0.2941
IP	3	14620.30	0.0001
STORAGE	3	1692.80	0.0001
VARIETY * IP	3	390.30	0.0001
VARIETY*STORA	3	18.59	0.1460
IP * STORA	9	616.85	0.0001
VARI * IP * STOR	9	5.86	0.8117

Table A25. Slicing procedure for the interaction between variety and imbibition period (IP) on germination of Lowell and Mendon in 1997

Sliced by Variety

Variety	df	Mean Square	Pr > F
Lowell	3	9694.27	0.0001
Mendon	3	5316.33	0.0001

Sliced by Imbibition period

Imbibition period	df	Mean Square	Pr > F
0 hr.	1	18.00	0.1858
15 hr.	1	331.53	0.0001
24 hr.	1	120.13	0.0009
40 hr.	1	712.53	0.0001

Table A26. Slicing procedure for the interaction between imbibition period (IP) and storage on germination of Lowell and Mendon in 1997

Sliced by Storage Level

Storage level	df	Mean Square	Pr > F
0 months	3	1817.92	0.0001
2 months	3	2419.71	0.0001
4 months	3	2931.20	0.0001
6 months	3	9302.03	0.0001

Sliced by Imbibition period

Imbibition period	df	Mean Square	Pr > F
0 hr.	1	23.08	0.0845
15 hr.	1	38.45	0.0128
24 hr.	1	466.71	0.0001
40 hr.	1	3015.11	0.0001

Table A27. Analysis of variance for the effects of imbibition period (IP) and storage on accelerated aging germination of Lowell and Mendon in 1997

Dependent Variable: Percent accelerated aging germination

Source	DF	Mean Square	Pr > F
Model	31	4422.31	0.0001
Error	96	11.82	
Corrected Total	127		

Source	DF	Mean Square	Pr > F
VARIETY	1	2859.57	0.0001
IP	3	40204.42	0.0001
STORAGE	3	2482.32	0.0001
VARIETY * IP	3	329.32	0.0001
IP * STORA	9	62.97	0.0019
VARIETY*STORA	3	468.17	0.0001
VARI * IP * STOR	9	86.83	0.0001

Table A28. Slicing procedure for the 3-way interaction among variety, imbibition period (IP) and storage on accelerated aging germination of Lowell and Mendon in 1997

Effects of VARIETY * IP * STORAGE sliced by Variety * IP

Variety	Imbibition period	df	Mean Square	Pr > F
Lowell	0 hr.	3	44.90	0.0127
	15 hr.	3	621.58	0.0001
	24 hr.	3	1593.08	0.0001
	40 hr.	3	5.73	0.6936
Mendon	0 hr.	3	169.73	0.0001
	15 hr.	3	966.83	0.0001
	24 hr.	3	805.42	0.0001
	40 hr.	3	3.00	0.8584

Table A29. Slicing procedure for the 3-way interaction among variety, imbibition period (IP) and storage on accelerated aging germination of Lowell and Mendon in 1997

Effects of VARIETY * IP * STORAGE sliced by Variety*Storage

Variety	Storage	df	Mean Square	Pr > F
Lowell	0 months	3	6543.06	0.0001
	2 months	3	6169.90	0.0001
	4 months	3	5430.42	0.0001
	6 months	3	6349.75	0.0001
Mendon	0 months	3	5718.75	0.0001
	2 months	3	4472.73	0.0001
	4 months	3	3483.23	0.0001
	6 months	3	4030.90	0.0001

Table A30. Slicing procedure for the 3-way interaction among variety, imbibition period (IP) and storage on accelerated aging germination of Lowell and Mendon in 1997

Effects of VARIETY * IP * STORAGE sliced by Storage * IP

Storage	Imbibition period	df	Mean Square	Pr > F
0 months	0 hr.	1	84.50	0.0088
	15 hr.	1	105.13	0.0036
	24 hr.	1	496.13	0.0001
	40 hr.	1	1.13	0.7583
2 months	0 hr.	1	276.13	0.0001
	15 hr.	1	990.13	0.0001
	24 hr.	1	840.50	0.0001
	40 hr.	1	0.01	0.9999
4 months	0 hr.	1	288.00	0.0001
	15 hr.	1	800.00	0.0001
	24 hr.	1	3.13	0.6082
	40 hr.	1	0.50	0.8374
6 months	0 hr.	1	666.13	0.0001
	15 hr.	1	242.00	0.0001
	24 hr.	1	24.50	0.1531
	40 hr.	1	0.01	0.9999.

Table A31. Analysis of regression for the effect of imbibition period (IP) on field emergence for Lowell in 1995

Dependent Variable: Percent field emergence

Source	DF	Mean Square	Pr > F
Model	1	3385.20	0.0001
Error	62	135.52	
Corrected Total	63		

R-square = 0.287
C.V. = 17.48 percent

Table A32. Field emergence 21 days after planting following imbibition period and storage of Lowell and Mendon in 1996 at East Lansing and Clarksville, MI

Location: East Lansing, MI
Data in plants per meter.

	Imbibition period	Storage period				
Lowell		0 months	2 months	4 months	6 months	mean
	0 hr	62.13	63.63	66.15	73.80	66.43
	15 hr	73.88	77.13	69.75	67.73	72.12
	24 hr	73.13	75.63	59.18	71.10	69.76
	40 hr	65.63	42.63	27.68	29.93	41.46
	Mean	68.69	64.75	55.69	60.64	
Mendon		0 months	2 months	4 months	6 months	mean
	0 hr	69.38	71.13	61.43	68.40	67.58
	15 hr	68.13	63.63	56.70	65.03	63.37
	24 hr	74.50	61.13	62.55	67.05	66.31
	40 hr	58.63	48.25	33.53	37.58	44.49
	Mean	67.66	61.03	53.55	59.51	

Location: Clarksville, MI
Data in plants per meter.

	Imbibition period	Storage period				
Lowell		0 months	2 months	4 months	6 months	mean
	0 hr	75.25	67.00	70.88	70.65	70.94
	15 hr	70.00	74.50	67.28	77.85	72.41
	24 hr	73.63	68.63	65.48	72.90	70.16
	40 hr	65.63	47.63	32.40	31.95	44.40
	Mean	71.13	64.44	59.01	63.34	
Mendon		0 months	2 months	4 months	6 months	mean
	0 hr	62.13	68.63	64.13	58.05	63.23
	15 hr	67.88	69.50	64.35	66.60	67.08
	24 hr	72.50	71.38	61.88	61.65	66.85
	40 hr	66.25	47.00	42.98	49.28	51.38
	Mean	67.19	64.13	58.33	58.89	

Table A33. Analysis of variance for the effect of imbibition period and storage on field emergence of Lowell and Mendon in 1996

Dependent Variable: Percent field emergence

Source	DF	Mean Square	Pr > F
Model	63	0.179	0.0001
Error	448	0.022	
Corrected Total	511		

Source	DF	Mean Square	Pr > F
VARIETY	1	0.086	0.0477
IP	3	2.323	0.0001
STORAGE	3	0.466	0.0001
VARIETY * IP	3	0.118	0.0011
VARIETY*STORA	3	0.002	0.9732
IP * STORA	9	0.164	0.0001
VARIE * IP * STORAG	9	0.038	0.0796

Table A34. Slicing procedure for the interaction among imbibition period and variety on germination across two locations for Lowell and Mendon in 1996

Sliced by Variety

Variety	df	Mean Square	Pr > F
Lowell	3	1.718	0.0001
Mendon	3	0.722	0.0001

Sliced by Imbibition period

Imbibition period	df	Mean Square	Pr > F
0 hr	1	0.049	0.1349
15 hr	1	0.225	0.0014
24 hr	1	0.052	0.1234
40 hr	1	0.116	0.0227

Table A35. Slicing procedure for the interaction between imbibition period and storage on field emergence across two locations for Lowell and Mendon in 1996

Sliced by Storage Level

Storage level	df	Mean Square	Pr > F
0 months	3	0.073	0.0192
2 months	3	0.607	0.0001
4 months	3	1.029	0.0001
6 months	3	1.107	0.0001

Sliced by Imbibition period

Imbibition period	df	Mean Square	Pr > F
0 hr	3	0.004	0.9019
15 hr	3	0.039	0.1495
24 hr	3	0.096	0.0044
40 hr	3	0.819	0.0001

Table A36. Analysis of variance for the effect of imbibition period and storage on the field performance measured by a quantitative index of regrowth potential of Lowell and Mendon in 1996

Dependent Variable: Index of regrowth potential

Source	DF	Mean Square	Pr > F
Model	63	20.905	0.0001
Error	192	1.114	
Corrected Total	255		

Source	DF	Mean Square	Pr > F
VARIETY	1	5.641	0.0256
SPROUTING	3	70.664	0.0001
STORAGE	3	299.737	0.0001
VARIETY*SPROUT	3	7.820	0.0002
SPROUT*STORA	9	6.014	0.0001
VARIETY*STORA	3	0.372	0.8005
VARIE*SPROUT*STORAG	9	1.194	0.3850

Table A37. Slicing procedure for the interaction between storage and variety for quantitative index of regrowth potential across two locations of Lowell and Mendon in 1996

Sliced by Variety

Variety	df	Mean Square	Pr > F
Lowell	3	143.64	0.0001
Mendon	3	156.47	0.0001

Sliced by Storage Level

Imbibition period	df	Mean Square	Pr > F
0 months	1	0.141	0.7228
2 months	1	2.066	0.1748
4 months	1	3.285	0.0875
6 months	1	1.266	0.2878

Table A38. Slicing procedure for the interaction between imbibition period and storage for quantitative index of regrowth potential across two locations of Lowell and Mendon in 1996

Sliced by Storage Level

Storage level	Df	Mean Square	Pr > F
0 months	3	0.964	0.4603
2 months	3	34.056	0.0001
4 months	3	27.952	0.0001
6 months	3	25.734	0.0001

Sliced by Imbibition period

Imbibition period	Df	Mean Square	Pr > F
0 hr	3	56.776	0.0001
15 hr	3	65.108	0.0001
24 hr	3	74.598	0.0001
40 hr	3	121.297	0.0001

Table A39. Analysis of variance for the effect of variety, imbibition period and storage on yield (kg/ha) in 1997

Dependent Variable: Yield (kg/ha)

Source	DF	Mean Square	Pr > F
Model	32	333.49	0.0001
Error	219	54.88	
Corrected Total	251		

Source	DF	Mean Square	Pr > F
VARIETY	1	123.28	0.1354
IP	3	1043.06	0.0001
STORAGE	3	1627.90	0.0001
VARIET * IP	3	139.24	0.0576
IP * STORA	9	139.34	0.0086
VARIETY*STORA	3	79.62	0.2289
VARI * IP * STOR	9	46.62	0.5714

Table A40. Slicing procedure for the interaction between imbibition period (IP) and storage on yield (kg/ha) of Lowell and Mendon in 1997

Sliced by Storage Level

Storage level	df	Mean Square	Pr > F
0 months	3	54.82	0.3942
2 months	3	217.97	0.0088
4 months	3	353.18	0.0003
6 months	3	807.90	0.0001

Sliced by Imbibition period

Imbibition period	df	Mean Square	Pr > F
0 hr.	1	182.79	0.0204
15 hr.	1	229.15	0.0067
24 hr.	1	407.72	0.0001
40 hr.	1	1213.96	0.0001

LITERATURE CITED

LITERATURE CITED.

1. American Association of Cereal Chemists. 1988. Approved methods of the AACC (7^{th} edition). Falling Number determination, method 56-81. The Association, St. Paul, MN (1962)
2. Association of Official Seed Analysts. 1970. Tetrazolium Testing Handbook for agricultural seeds. The Tetrazolium testing committee of the AOSA. Ed: Don F. Grabe. Contribution No.29 to the Handbook on Seed Testing, pp.1-62.
3. Association of Official Seed Analysts. 1994. Rules for testing seeds. Journal of Seed Technology, Volume 16, Number 3, pp. 1-113.
4. Belderok, B. 1961. Studies on dormancy in wheat. Proceedings of the International Seed Testing Association, 26(4):697-760.
5. Belderok, B. 1968. Seed dormancy problems in cereals. Field Crop Abstracts, 21:203-211.
6. Bhatt, G. and N. Derera. 1980. Potential use of Kenya 321 - type dormancy in a wheat breeding program aimed at evolving varieties tolerant to preharvest sprouting. Cereal Research Communications, 8:291-295.

7. Bhatt, G. M., G. M. Paulsen, K. Kulp, and, E. G. Heyne. 1981. Preharvest sprouting in hard winter wheats: assessment of methods to detect genotypic and nitrogen effects and interactions. Cereals Chemistry, 58:300-302.

8. Bingham, J., and E. T. Whitmore. 1966. Varietal differences in wheat in resistance to germination in the ear and alpha-amylase content of the grain. Journal of Agric. Science, 66:197-201.

9. Briggle, L. W.. 1980. Preharvest sprout damage. Cereal Research Communications, 8:245-250.

10. Buchanan, A. M., and, E. M. Nicholas. 1979. Sprouting, alpha-amylase, and breadmaking quality. Cereal Research Communications, 8:23-28.

11. Bulat, H. and W. Lindenbein. 1961. Twenty years of experience with the tetrazolium method on seeds of woody plants. A review. Proc. Int. Seed Test. Assoc. 26(3):453-471.

12. Chang, S. C. 1943. Length of dormancy in cereal crops and its relationship to after harvest sprouting. Journal of the American Society of Agronomy, 35:482-489.

13. Copeland, L. O., R. Freed, and Z. Helsel. 1980. Crop and Soil Sciences News, 6(25):1-2. Dept. of Crop and Soil Sciences, Michigan State University, East Lansing.

14. De Pauw, R. and T. McCaig. 1983. Recombining dormancy from RI4137 with white seed color. Proceedings of the Third International Symposium on Preharvest Sprouting in Cereals. Ed. J.E. Kruger and D. La Berge. Westview Press, Boulder, CO, USA. pp. 251-259.

15. De Pauw, R. and T. McCaig. 1990. Advances in the development of sprouting resistant white wheats. Proceedings of the Fifth International Symposium on Preharvest Sprouting in cereals. Ed. Ringlund, K.M., Mosleth, E. and Mares, D. pp. 241-247.

15. De Pauw, R., T. McCaig, R. Baker, and J. Clarke. 1992. Constructing a sprouting tolerance index. Proceedings of the Sixth International Symposium on Preharvest Sprouting in Cereals. Ed: M.K. Walker-Simmons and J.L. Ried. pp. 47-53.

16. Elias, S. G. 1987. The effects of preharvest sprouting on seed germination, storability and field performance of 4 winter wheat varieties grown in Michigan. Master of Sciences Thesis. Michigan State University, pp. 1-128.

17. Gale, M. 1983. Alpha-amylase genes in wheat. Proceedings of the Third International Symposium on Preharvest Sprouting in Cereals. Ed. J.E. Kruger and D. La Berge. Westview Press, Boulder, CO, USA. pp. 105-110.

18. Gale, M. and J. Lenton. 1987. Preharvest sprouting in wheat - a complex genetic and physiological problem affecting bread making quality of UK wheats. Aspects of applied biology. The association of applied biology, UK, 15:115-124.

19. Gold, C. and C. Duffus. 1992. The effect of gibberellic acid-insensitive dwarfing genes on pre-maturity alpha-amylase and sucrose relationships during grain development in wheat. Proceedings of the Sixth International Symposium on Preharvest Sprouting damage in Cereals. Ed: M.K. Walker-Simmons and J.L. Ried. pp. 171-177.

20. Gordon, I. L., N. Derera, L. Balaan, 1977. Selection against sprouting damage in wheat. I. Germination of unthresed grain, with a standard wetting procedure. Aust. J. Agric. Res. 28:583-596.

21. Gordon, I. L. 1979. Selection against sprouting damage in wheat. III. Dormancy, Germinative alpha-amylase, grain redness and flavanols. Aust. J. Agric. Res. 30:387-402.

22. Greenaway, W. T. 1969. The sprouted wheat problem: the search for a solution. Cereal Science Today, 14(12):390-395.

23. Hagemann, M. G., and A. J. Ciha. 1984. Evaluation of methods used in testing winter wheat susceptibility to preharvest sprouting. Crop Science, 24:249-254.

24. Hardesty, B., and F. C. Elliot. 1956. Differential post-ripening effects among seeds from the same parental wheat spike. Agronomy Journal, 48:406-409.

25. Harrington, J. B., 1932. The comparative resistance of wheat varieties to sprouting in the stock and windrow. Scientific Agriculture, Vol. XII, 11:635-646.

26. International Seed Testing Association. 1995. Handbook of Vigour Test Methods. 3rd Edition, 1995. Edited by J. G. Hampton and D. M. Tekrony. Published by The International Seed Testing Association, 1995. P.O. Box 412, 8046 Zurich, Switzerland. pp. 1-117.

27. Karkoven, T., J. Peltonen, and E. Kivi. 1991. The effect of northern climatic conditions on sprouting damage of wheat grains. Acta Agric. Scand. 41: 55-64.

28. King, R. W., and H. Chadim. 1983. Ear wetting and preharvest sprouting of wheat. Proceedings of the Third International Symposium on Preharvest Sprouting in Cereals. Ed. J.E. Kruger and D. La Berge. Westview Press, Boulder, CO, USA. pp. 36-42.

29. Kruger, J. E. 1975. Biochemistry of preharvest sprouting in cereals and practical applications in plant breeding. Cereal Research Communications, 4:187-193.

30. La Croix, L., P. Waikakul, and G. Young. 1976. Seasonal and varietal variation in dormancy of some spring wheat. Cereal Research Communications, 4:139-146.

31. Lakon, G. 1942. Topographical detection of the viability of cereal seeds by tetrazolium salts. Ber. Deut. Bot. Ges, 60:434-444.

32. Lalluka, U. 1976. The effect of the temperatures during the period prior to ripening on sprouting in the ear in wheat and rye varieties grown in Finland. Cereal Research Communications, 4:93-96.

33. Lindebein, W. 1961. The development and importance of the biochemical methods for testing viability in seed investigation. Saatgut-Wirtsch. 13:38-39.

34. Lindebein, W. 1965. Tetrazolium testing. Proc. Int. Seed Test. Assoc. 30(1):89-97.

35. Lorenz, K., P. Roewe-Smith, K. Kulp, and L. Bates. 1984. Preharvest sprouting of winter wheat II. Amino acid composition and functionality of flour and flour fractions. Cereal Chemistry, 60(5):360-366.

36. Mackey, J. 1976. Seed dormancy in nature and agriculture. Cereal Research Communications, 4:83-91.

37. Mares, D. J. 1985. Temperature dependence of germinability of wheat. Grain in relation to preharvest sprouting. Australian Journal Agric. Res., 35:115-128.

Mares, D. J. 1987. Preharvest sprouting tolerance in white-grained wheat. Proceedings of the Fourth International Symposium on Preharvest Sprouting in cereals. Ed. Mares, D. J. pp. 64-74.

38. Mares, D. J. 1992. Genetic studies of sprouting tolerance in red and white wheats. Preharvest sprouting in cereals. Proceedings of the sixth international symposium on preharvest sprouting in cereals. Ed: M.K. Walker-Simmons and J.L. Ried. pp. 21-29.

39. Mares, D. J. and K. Mrva. 1992. Late maturity alpha-amylase in wheat. Preharvest sprouting in cereals. Proceedings of the sixth international symposium on preharvest sprouting in cereals. Ed: M.K. Walker-Simmons and J.L. Ried. pp. 178-184.

40. Mathewson, P. R., and Y. Pomeranz. 1977. Detection of sprouted wheat by a rapid colorimetric determination of alpha-amylase. Journal of A.O.A.C., 60:16-20.

41. McCrate, A. J., M. T. Nielsen, G. M. Paulsen, and E. G. Heyne. 1981. Preharvest sprouting and alpha-amylase activity in hard red and hard white winter wheat cultivars. Cereal Chemistry, 58:424-428.

42. McEwan, J. M. 1975. Relative sprouting resistance of closely related wheats differing in grain color. Proceedings of the First International Symposium on Preharvest Sprouting Damage in cereals. Sweden. Pp. 151-156.

43. Moore, R.P. 1985. Handbook on Tetrazolium testing. Published by The International Seed Testing Association, 1995. P.O. Box 412, 8046 Zurich, Switzerland. pp. 1-117.

44. Morris, C. F., and G. M. Paulsen. 1985. Preharvest sprouting of hard winter wheat as affected by nitrogen nutrition. Crop Science, 25:1028-1031.

45. Morris, C. and G. Paulsen. 1987. Development of preharvest sprouting resistant germplasm from "Clark's Cream" hard white winter wheat. Cereal Research Communications, 15:229-235.

46. Nielsen, M. T. 1980. The influence of climatic factors, floral parts, and gibberellic acid on sprouting in wheat. Ph.D. Thesis. Kansas State University.

47. Nielsen, M. T., A. J. McCrate, E. G. Heyne, and G. M. Paulsen. 1984. Effect of weather variables during maturation on preharvest sprouting of hard white winter wheat. Crop Science, 24:779-782.

48. Olered, R. 1964. Studies on the development of alpha-amylase activity in ripening wheat. Ark. Kemi, 22:175-183.

49. Olered, R. 1967. Development of alpha-amylase and falling number in wheat and rye during ripening. Plant Husbandry Publ. N° 23, p. 106.

50. Olsson, G., and, B. Mattson. 1976. Seed dormancy in wheat under different weather conditions. Cereal Research Communications, 4:181-185.

51. Paulsen, G., E. Heyne, T. Walter, and R. Hoseney. 1984. Agronomic and quality attributes of sibling hard white and hard red winter wheat. Crop science, 23:859-862.

52. Pool, M., and F. L. Patterson. 1958. Moisture relations in soft red winter wheats. Agronomy Journal, 50:158-160.

53. Ringlund, K. 1992. The importance of preharvest sprouting research. Preharvest sprouting in cereals. Proceedings of the sixth international symposium on preharvest sprouting in cereals. Ed: M.K. Walker-Simmons and J.L. Ried. pp. 3-7.

54. Stoy, V. 1983. Proceedings of the Third International Symposium on Preharvest Sprouting in Cereals. Ed. J.E. Kruger and D. La Berge. Westview Press, Boulder, CO, USA. pp. 3-7.

55. Sumeola, H. 1965. On the factors affecting quality of wheat produced in Finland. Acta Agric. Fenn. 107:300-307.

56. Takahashi, N. 1967. Environmental variation of germination behavior in rice. Japanese Journal of Breeding, 17(1):62-63.

57. Takahashi, N. 1979. Effect of environmental factors during seed formation on preharvest sprouting. Cereal Research Communications, 8(1):175-183.

58. Wahl, T. and D. O'Rourke. 1992. The economics of sprout damage in wheat. Preharvest sprouting in cereals. Proceedings of the sixth international symposium on preharvest sprouting in cereals. Ed: M.K. Walker-Simmons and J.L. Ried. pp. 10-17.

59. Weilenmann, F. 1976. Physiologische und genetische Grundlagen des Auswuchsgeschehens im Hinblick auf die Züchtung neuer Weizensorten. Schweiz, Landwirtsch, Forsch, 15:411-421.

60. Wellington, P. S. 1953. Studies on the germination of cereals. 1. The germination of wheat grains in the ear during development. Annals of Botany, 20:105-120.

61. Wellington, P. S. 1956. Studies on the germination of cereals. 2. Factors determining the germination behavior of wheat grains during maturation. Annals of Botany, 20:481-500.