CORRELATION BETWEEN COLD TEST GERMINATION AND THE OPTICAL MEASUREMENT OF LEACHED MATERIALS FROM THE SEED OF *ZEA MAYS*

by

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TABLE OF CONTENTS

INTRODUCTION

It has long been recognized that there are many factors which either singly or together influence corn yield. Of primary importance is that the corn seeds germinate and establish a perfect or near-perfect stand. Frequently, especially in northern areas, cold, wet weather prevails after spring planting. This is usually accompanied by a reduction in stand. The reduced stands under these environmental conditions have been attributed to the invasion by soil fungi of the germinating corn kernel, causing pre-emergence or post-emergence killing or reduced vigor in the plants which survive infection.

"Cold testing", a laboratory simulation of germination in cold, wet, disease infested soils, has shown that strains of corn and ears within inbred strains often differ in their ability to germinate under these unfavorable conditions. The cold test reaction of hybrids is often similar to the reaction of its maternal parent. Factors such as age of seed, degree of maturity, preharvest exposure to frost, artificial drying and pericarp damage also appear to influence cold test response.

The above factors are of such a physical or physiological nature as to indicate that the structure of the maternal tissue (pericarp and testa) and/or the vitality of the protoplast could be involved in the cold test reaction. For example, immature corn seed could be associated with both a less differentiated maternal tissue and a weakened protoplast condition. A less differentiated maternal tissue might be less likely to inhibit penetration by the soil fungi, and a weakened protoplast might slow down resistance

development, giving the soil fungi a longer period to become established.

Assuming that a weakened protoplast and/or a less differentiated maternal tissue is associated with increased permeability, the vitality of the protoplast and/or the maternal tissue structure can be studied indirectly by determining the amount of soluble solids and colloidal materials leached from steeped corn seed.

The studies described in this thesis have attempted to develop a usable technique for the leaching of solid materials from the seed of Zea mays, and show the relationship between seed permeability, as determined by the optical measurement of leached solid materials from steeped corn seed, and the reduction in germination due to cold testing.

REVIEW OF LITERATURE

Because of the nature of the studies described here the literature has been divided into two topics: cold testing, and permeable membranes and permeability.

Cold Testing

Cold testing of corn has been defined as a laboratory method of determining the vitality of seed germinated under unfavorable growing conditions, i.e., cold, wet, disease infested soils (56). Dickson (8) was the first investigator to report cold testing of corn.

That microorganisms, and not the adverse growing conditions per se, are primarily responsible for the reduced germination has

been shown by Hoppe and Middleton (25), Haskell (12), Livingston (38), Rush (47), Haltiwanger, et al. (11), Ho (17), and others. The various organisms commonly associated with seedling blight have been reported by numerous investigators; Hoppe (22), Hoppe and Middleton (25), Curme (7) and others. In summarizing the literature on cold testing of corn, Wernham (57) stated that 17 organisms have been associated with seedling blight. Eight of these organisms were Pythium spp. In Iowa, Wisconsin, and Kansas soils, Pythium spp. appear to be the most common and probably the most destructive (7, 17, 25).

High soil moisture has been shown by Svien (49) and Hooker (20) to increase the severity of seedling blight. Haltiwanger et al. (11) reported that the pathogenicity of different organisms was greatly affected by the incubation period and temperature of the cold test experiments. Rush (47) and Haskell (12) also reported that the incubation period was an important factor. Low temperatures between 10° and 12° C. were shown by Haskell (12) and Hoppe (26) to increase seedling blight. Instances of seedling blight in soils at high temperatures (20° to 24° C.) and low soil moisture have been reported by Hoppe (24) and Koehler (36) respectively. Cold testing procedures have for the most part included high soil moisture and low soil temperature; however, the cold tests have not been standardized and different investigators have employed slightly different techniques, temperatures, and incubation periods (13, 21, 26, 27, 28, 42, 48, 51).

Various factors associated with seed condition have been shown to be important in influencing cold test germination. Damage to

the pericarp which ruptures and exposes the endosperm and/or embryo has been reported to increase seedling blight (1, 30, 35, 39, 40, 51, 58). Rush (48), conversely reported that pericarp damage did not increase seedling blight. Immature seed as compared to mature seed has been shown by Hooker (20), Hoppe et al. (23), Livingston (39), and Rush and Neal (48) to be more susceptible to seedling blight. Artificial drying increases the susceptibility of the seed to cold test germination (38). Pre-harvest frost injury was demonstrated by Rush and Neal (48) to increase the severity of the disease. Seed shape, i.e., round versus flat, and moisture content prior to actual germination apparently do not effect cold test response (Haskell 14, 15).

The genotype of the embryo appears to influence germination under adverse conditions. Seed produced on plants which were highly resistant to cold in the fall, were observed by Holbert (18) and Holbert and Burlison (19) to be resistant to seedling blight when planted the following spring. Hoppe (22) reported that F_1 hybrids between resistant (R) by susceptible (S) parents, and S by S parents were as resistant as the resistant parent and susceptible as the susceptible parent respectively. He contended that hybrid vigor did not mask susceptibility. Tatum (50) showed that parental inbreds did not germinate as well under cold test conditions as single crosses between the two parental inbreds. He concluded that there was a relationship between hybrid vigor and resistance.

Rush (47) observed that single crosses and double crosses gave slightly higher germination than inbreds, but the differences were

insignificant when compared to the differences between lines. Pinnell (42) found that on the average, double crosses germinated best, followed by single crosses and inbreds in that order. Stand performance and seedling vigor in the cold test of selfed F_2 and F_3 ear progenies from very early single crosses were positively correlated. There was considerable variation between individual ears of selfed inbred lines in the cold test germinations. He attributed this high degree of variability to unknown environmental influences. Hooker (20) reported that there were significant differences in cold test germination within ears of longtime inbred lines. He was able to isolate sublines that were more resistant. Further isolation from sublines was not effective. Hooker and Dickson (21) demonstrated that with excised embryos in vitro, resistance to Pythium debaryanum developed faster in lines previously proven resistant than in lines previously proven to be susceptible. Embryos of single crosses acquired resistance faster than did embryos of inbreds.

Various investigators have reported that the maternal parent is important in determining cold test reaction. Reciprocal crosses of a single cross were shown by Tatum (50) and Pinnell (42) to differ in their ability to germinate under cold test conditions. Rush (47) reported R by S crosses to be resistant and S by R crosses to be susceptible, indicating the importance of the maternal parent. In general, Hooker and Dickson (21) found that reciprocal crosses showed only small differences. However, in some cases there was an association between pistillate parent and cold test germination. They contended that this may be a nutritional re-

lationship.

Embryonic tissues in resistant plants had a much higher acidity than similar tissues in susceptible plants, according to Dickson et al. (9). The presence of uronic acids was positively correlated with the acidity. Dilute solutions of the uncombined uronic acids reduced fungus growth when added in low concentrations to the culture solutions. From histological studies, Hooker (20) observed that intra-cellular mycelium spread extensively in the scutellum of susceptible embryos, and that the cells in advance of and adjacent to the mycellium appeared dead. The spread of the mycelium in the scutellum of resistant embryos was limited. The cell walls of susceptible embryos were dissolved by a warm dilute sulfonic acid solution, while those of resistant embryos remained intact. From these results, and those of Dickson et al. (9), Hooker concluded that the embryo was the major cause of resistance to Pythium spp. Haskell and Singleton (13) also concluded that the genetic constitution of the embryo was the most important factor in resistance to seedling blight. Differences between ears within inbred lines were attributed by Hooker (20) and Wernham (57) to residual heterozygosity.

Rush (47) concluded that the genetic nature of the reaction to soil fungi seemed best explained on the basis of the action of a series of multiple factors in the endosperm and/or pericarp rather than in the embryo. Pinnell (42) also postulated that the endosperm may be the part of the kernel which conditions resistance or susceptibility.

Alberts (1) contended that pericarp injury upset the stabil-

izing function of the seed coat. Enzymes, which are secreted by the epithelial cells of the scutellum, diffuse through the endosperm and are utilized by the invading fungi. Pericarp injury was also thought by Tatum and Zuber (51) to be of primary importance in determining cold test response.

Permeable Membranes and Permeability

The fundamental semipermeable membrane in the maize caryopsis was attributed by Beeskow (6) to several layers of partially disorganized cells between the pericarp and endosperm. These cells were primarily fatty or lipoid in character. Randolph (44) observed that the suberized membrane was derived from the epidermis of the nucellus and that this layer plus the pericarp constituted the sole covering of the corn caryopsis. Conversely, Johann (32) demonstrated that the suberized membrane was derived from both the inner integument and the epidermis of the nucellus. Although the origin of the semipermeable membrane was not studied, Kiesselbach and Walker (33) stated that this membrane unquestionably functions as a protective structure.

According to Pugh, et al. (43), the suberized and cutinized layer and the testa of the wheat kernel are important in preventing the entrance and spread of Gibberella saubinetii. In corn, Johann (31) concluded that the suberized layer and the closing layer of the hilar orifice were not associated with resistance to kernel rot in corn. She suggested that a chemical resistance associated with the anatomical stage of development might be operating.

The permeability of the portion of the corn seed coat covering

the embryo by mercury compounds was shown by Orton (41) to be slower than such permeability in that portion covering the endosperm. Also this permeability varied with each variety. Tharp (53) found that the permeability of the seed coat by trichloroacetic acid was faster over the embryo than over the endosperm.

Distilled water has been reported to have a toxic effect on the protoplast of seeds and seedling roots (2, 19, 34, 55). Eyster (10) found that proteins, digestive enzymes, and growth promoting substances had diffused from steeped bean seeds, and that subsequent germination was proportional to the loss of these materials. Germination was reduced less at 25°C. than at temperatures above or below this point. The injurious after-effects of soaking corn seeds was attributed by Robinson (45) to the depletion of the oxygen supply by bacterial growth. Tilford et al. (54) reported that bacterial growth per se was the primary cause of devitalization of bean seeds soaked in distilled water. According to Barton (4), oxygen bubbled through the steeping medium (distilled water) increased the injurious after-effects of soaking various seeds, while carbon dioxide reduced or completely prevented injury to the seeds. In a later paper, Barton (5) reported that carbon dioxide protected steeping corn seeds against the adverse effects of nutrient salt solutions, prevented toxicity to the embryo by selenium salts and 2-4 dichlorophenoxyacetic acid, and increased the resistance of corn seed when germinated in soil at low temperatures. She did not mention whether or not the low temperature soil was infested with seedling blight organisms. She concluded that the protective action of carbon dioxide was in preventing excessive

absorption of water.

Leaching of total soluble solids and colloidal materials from sweet corn seed physiologically, but not physically, injured was observed by Hottes and Huelson (29). They reported that the amount of leached materials from steeped sweet corn seed could serve as an index of the vitality of the protoplast and thus the vigor of the seed. A similar relationship was demonstrated by Robinson (49) for dent corn seed. He found that more material was leached from immature dent corn seed than from mature dent corn seed. Curme (7) and Tatum (52) observed that the amount of material leached from steeped field corn seed was associated with the reduction in germination due to cold testing.

LEACHING TECHNIQUE

Introduction

Previous investigations by Hottes and Huelson (29), Curme (7), and Tatum (52) indicated that there was a need for a standardized technique for the leaching of soluble solids and colloidal materials from seed of Zea mays. From their work it appeared that the following problems needed to be studied: (1) microbial growth in the steep solution; (2) time and temperature of steeping; (3) proportion of corn seed to the amount of distilled water; and (4) replications. The following experiments have attempted to study each of these problems in order that a standard leaching technique might be developed.

Methods and Materials

Strains of double cross corn harvested by hand and shelled by machine at the Kansas station in 1949 were used throughout the experiments. The seeds were steeped in bottles of approximately 75 milliliters capacity. After the various treatments the liquid in which the seed was steeped was decanted and passed through coarse filter paper to remove extraneous pieces of chaff, silk, etc. The amount of leached material was then determined by using a Coleman Universal spectrophotometer at a wave length of 400 millimicrons. The values reported are the amount of light transmitted through the solution containing the leached materials expressed as a percentage of the amount of light transmitted through a distilled water reference. Thus the values reported are inversely proportional to the optical density of the solution containing the leached materials. In this case the optical density is equal to the concentration of the leached materials.

A qualitative analysis of the leached materials was not made. According to Hottes and Huelson (29), the leached materials are organic and inorganic, crystalline and colloidal in nature, i.e., soluble solids and colloidal materials.

Distilled water was used as the steeping medium. If leaching is assumed to be a measure of protoplast vitality (29) and/or maternal tissue structure, the steeping medium should be such that it has no adverse effects on either the protoplast or maternal tissue. The use of distilled water could be questioned, since several investigators had reported that distilled water had a toxic effect on the protoplast of seeds. However, for uniformity of

results and freedom from salts, distilled water seemed preferable to tap water or other solutions.

Details of methods that pertain to particular experiments are given along with the results and discussions of the individual experiments.

Experimental Results

Microbial Growth in the Steep Solution. The Effect of Time and Temperature on Microbial Growth in the Steep Solutions. Preliminary experiments have shown that when corn seeds were steeped in open beakers at room temperature, large amounts of microbial growth appeared both in and on the steeping media. When seeds were steeped in capped bottles there was also considerable development of microorganisms. Since microbial growth interfered with the spectrophotometer measurements, it was deemed important that this variable be eliminated.

Microbial growth in the steep solutions was also encountered by Hottes and Huelson (29). They suggested that the microorganisms might be controlled by steeping the seed at low temperatures for relatively long periods of time. An experiment was conducted to determine the effect of time and temperature on microbial growth in the steep solution.

Ten gram samples of seed from seven dent corn hybrids (K1892, K1907, NC7520, K1897, K1936, NC8909 and K1910) were immersed in 50 ml. of distilled water. The bottles were capped. Triplicate samples of each strain were steeped at 10°, 18°, and 38° C. for 24, 48, 72, and 96 hours.

Since an actual determination of the amount of microbial growth was beyond the limits of the experiment, it could be determined only in part by visual observation of cloudiness or clearness of the steeping solutions. This did not seem entirely suitable and other ways of determining whether the microorganisms were interfering with the spectrophotometer readings were sought. It was observed that with increasing cloudiness (increasing microbial growth) the spectrophotometer readings were lower and the variability of triplicate samples increased. This suggested that variability within triplicate samples could be indicative of whether or not the microorganisms were interfering with the spectrophotometer readings. Thus variability within triplicate samples was also used to evaluate microbial development in the steep solutions.

The mean spectrophotometer readings and the error variances for this experiment are reported in Table 1.

Differences among the mean spectrophotometer readings for the solutions at 10° C. for 24, 48, 72, and 96 hours were small. The amount of leached materials at 10° C. was considerably lower than at the higher temperature. All of the 24 and 48 hour solutions at 10° C. were clear. A few of the 72 and 96 hour solutions at 10° C. were cloudy. Error variances at 72 and 96 hours were larger than those for the 24 and 48 hour periods.

The mean spectrophotometer readings at 18° C. showed a slight decrease with increased steeping periods. The amount of leached materials at 18° C. for 24 hours was the same as the corresponding solutions at 10° C. The spectrophotometer readings for the solutions at 18° C. for 48, 72 and 96 hours were lower than the corre-

Table 1. Effect of time and temperature on the amount of leaching.

Steeping treatment		Mean spectrophotometer readings of triplicate samples of strains:[a]								Variance[b]
Temp. (°C.)	Time (hours)	K1892	K1907	NC7520	K1897	K1936	NC8909	K1910	Mean	
10	24	95	90	94	92	95	95	94	93.6	4.5
	48	92	90	93	90	94	94	93	92.3	4.0
	72	93	88	94	90	94	94	91	92.0	8.5
	96	92	88	91	89	94	93	91	91.1	10.0
	Mean								92.3	
18	24	93	90	95	91	95	95	93	93.1	2.5
	48	85	81	88	84	88	90	87	86.1	3.0
	72	86	79	85	83	89	88	83	84.7	12.7
	96	86	82	83	82	85	86	85	84.1	8.3
	Mean								87.0	
38	24	87	81	86	82	90	88	86	85.7	15.7
	48	78	75	77	75	84	77	71	76.7	56.3
	72	62	48	67	69	83	83	76	69.7	221.3
	96	68	44	53	62	67	67	49	58.6	525.7
	Mean								72.7	

(a) Values are the amount of light transmitted through the steep solution expressed as a percentage of the amount of light transmitted through a distilled water reference.
(b) Variance within triplicate samples.

sponding readings at 10° C. A few of the solutions at 18° C. for 72 and 96 hours were cloudy. The error variances at 72 and 96 hours were slightly higher than those at 24 and 48 hours.

At 38° C. the mean spectrophotometer readings decreased sharply with an increased period of steeping. The spectrophotometer readings for all the steeping periods were lower than the corresponding readings at 10° and 18° C. Most of the solutions were cloudy at 38° C. and the error variances were high for all the steeping periods.

There was little microbial growth at 10° C. However, it was concluded that leaching for longer periods at this temperature was not suitable since the leaching process was retarded. The low spectrophotometer readings at 38° C. can be attributed to two factors: (1) increased leaching at this higher temperature; and (2) increased microbial growth due to the favorable high temperature and increased amount of leached materials.

Leaching at 18° C. for 48 hours appeared to be suitable with respect to the amount of leaching (see page 27) and microbial growth. However, because there was some microbial development at the 72 and 96 hour periods for this temperature, there was reason to suspect that a better method of eliminating the microbial variable could be found.

The Effect of Phenol on Microbial Growth in the Steep Solution. The use of a germicide in the steeping medium as a means of controlling microbial growth was suggested by Hottes and Huelson (29). Toluene in the steeping medium was observed by Robinson (45) to be effective in removing the microorganisms, but tended to have an

adverse effect on subsequent germination. If leaching of corn seed is an indication of protoplast vitality, chemicals should be used with caution lest they have an injurious effect on the protoplast.

Another important factor must be considered with respect to the use of germicides in the steeping media. If the chemical agent has color, a standard reference solution would have to be maintained for the spectrophotometer readings. Preliminary tests with various commercial seed protectants (dusts and liquids) indicated that it was not feasible to maintain a standard reference solution using these types of compounds. Other preliminary tests indicated that phenol (carbolic acid) was suitable with respect to the reference solutions for the spectrophotometer measurements.

An experiment was conducted to determine whether phenol was effective in inhibiting microbial growth and to determine if phenol was injurious to the protoplast of corn seed. Ten gram samples of seed from six dent corn hybrids (K1870, K1839, K1835, NC8917WI, NC8917WII, and K1925) were each immersed in 50 ml. solutions containing 0.01, 0.001, 0.0008, 0.0006, 0.0004, 0.0002 and 0.0001 percent phenol by weight. The bottles were capped. Triplicate samples of each variety with each steeping media were steeped at 25° C. for 66 hours. After steeping, the seeds were washed with distilled water. They were placed on moist germination towels, rolled into "paper dolls" and placed in a germinating chamber in the greenhouse. After six days, germination percentages were determined. (See page 35 for criteria for determining germination.)

The mean spectrophotometer readings, mean percent germinations,

and error variances for this experiment are reported in Table 2.

The 0.01, 0.001, 0.0008 and 0.0006 percent phenol solutions were clear. The error variances at these concentrations were small. A few of the 0.0004 phenol solutions were cloudy and the error variance was larger than those for any of the above four concentrations. Most of the 0.0002 and 0.0001 percent phenol solutions and the distilled water check solutions were cloudy. The error variances were considerably increased over the 0.01, 0.001, 0.0008, 0.0006 and 0.0004 percent phenol solutions.

The mean spectrophotometer readings of all seven strains in the 0.01 percent phenol solution were lower than those of the 0.0001, 0.0008, 0.0006 and 0.0004 percent phenol solutions. Differences between the mean spectrophotometer readings of all seven strains of the 0.0001, 0.0008, 0.0006 and 0.0004 percent phenol solutions were small. The mean spectrophotometer readings of all strains in the 0.0002 and 0.0001 percent phenol solutions and the distilled water check solutions were considerably lower than the 0.01, 0.001, 0.0008, 0.0006 and 0.0004 percent phenol solutions.

All of the seeds steeped in the 0.01 percent phenol solution were killed. The mean germination of all seven strains was increased with each successive decrease in phenol concentration from the 0.01 percent solution up to the 0.0006 percent solution. Differences in germination between the 0.0006, 0.0004, 0.0002 and 0.0001 percent solutions and the distilled water checks were small.

Phenol concentrations of 0.01, 0.0001 and 0.0008 were apparently toxic to the protoplast as indicated by the reduced germination. The remaining phenol solutions did not appear to be toxic

Table 2. Effect of various phenol concentrations on the amount of leaching and germination.

Phenol concentration[a]	Mean spectrophotometer readings (S) and percent mean germination (G) of triplicate samples of strains:														Variance[b]
	K1830		K1839		K1835		NC8917W1[c]		NC8917W2[c]		K1925		Mean		
	G	S	G	S	G	S	G	S	G	S	G	S	G	S	
0.01%	0	84	0	82	0	84	0	85	0	85	0	84	0	84	1.8
0.001%	76	91	59	90	66	90	80	91	74	92	87	88	74	90	3.5
0.0008%	73	94	60	92	68	92	90	95	81	93	94	91	78	93	3.2
0.0006%	78	92	77	89	89	89	95	91	84	93	95	91	86	91	2.9
0.0004%	86	89	79	87	94	85	92	88	84	91	100	90	89	88	13.5
0.0002%	87	71	75	75	91	68	91	73	91	79	95	77	88	74	40.1
0.0001%	87	72	79	75	93	81	96	79	88	70	95	84	90	77	38.6
Distilled water check	85	70	71	81	87	80	92	76	97	86	96	82	88	79	46.1
Mean	72	83	63	84	74	84	80	85	74	86	83	86			
Normal germination[d]	100		100		100		100		100		100				

(a) Percent phenol concentration by weight
(b) Variance within triplicate samples
(c) Same strains but from different sources
(d) Mean germination of duplicate 50 seed samples

when compared to the distilled water check. Microbial growth was inhibited in the 0.01, 0.001, 0.0009 and 0.0006 percent phenol solutions but not in the 0.0004, 0.0002 and 0.0001 percent phenol solutions and the distilled water check. The low spectrophotometer readings of the latter three solutions could be attributed to microbial growth. The low spectrophotometer readings of the 0.01 percent phenol solutions could be attributed to the toxic effect of the phenol on the protoplast, causing increased leaching. Although the mean spectrophotometer readings of the 0.0001 and 0.0008 percent phenol readings showed no adverse effect on the protoplast of the seeds, the mean germination indicated that both concentrations were harmful. Thus it appeared that all of the phenol concentrations that inhibited microbial growth except the 0.0006 percent solution, were toxic to the protoplast, while the phenol concentrations that did not inhibit microbial growth had no effect on the protoplast.

With regard to both effect on the protoplast and control of microorganisms, the 0.0006 percent phenol solution appeared to be suitable for use in the leaching test. However, since slightly higher concentrations of phenol were toxic to the protoplast, it was thought that the 0.0006 percent phenol solution might also affect the protoplast even though it was not apparent from this experiment. Therefore the use of phenol in the steep solution to remove the microbial varieble was considered unsuitable.

The Source and Identification of the Microorganisms Contaminating the Steep Solutions. Since steeping at low temperatures and the use of phenol in the steep solution were found to be un-

suitable for control of the microorganisms, it was thought that the microorganisms might be utilized in a quantitative bio-assay of the amount of leached materials. That is, the amount of microbial growth might be dependent upon the amount of leached materials in the solutions. The microorganisms contaminating the solutions would therefore be the logical ones to use in a bio-assay.

An experiment was conducted to identify the microorganisms in the steep solutions and to locate the source of microorganisms. Seven dent corn hybrids (K1925, K1839, K1835, NC8917WI, NC8917WII, K1910, and K1830) were each subjected in triplicate ten gram samples to three treatments: (1) The seeds were disinfected with a one percent chlorox (active ingredients, sodium hypochlorite, 5.25 percent by weight) solution for one minute, and then rinsed twice in distilled water. They were placed in bottles that had previously been disinfected with a 25 percent chlorox solution for five minutes. Fifty milliliter of sterile distilled water were added to each bottle. (2) The seeds were not disinfected and were placed in bottles that had been disinfected as described above. Fifty milliliter of non-sterile distilled water were added to each bottle. (3) Neither the seeds nor the bottles were disinfected. Fifty milliliters of non-sterile distilled water were added to each bottle.

The bottles were capped and the seeds were steeped for 66 hours at 25° C.

At the end of the steeping period, one milliliter of each steep solution was plated out on approximately ten milliliters of potato-dextrose agar. The plates were incubated and examined at

the end of 48, 72 and 96 hours to identify the microorganisms present. No attempt was made to determine the actual number of microorganisms.

The mean spectrophotometer readings and the error variances of this experiment are reported in Table 3.

The solutions from treatments one and two were all clear. The error variances of the triplicate samples of both treatments were small. There was a small difference between the mean spectrophotometer readings of treatments one and two. Differences between strains within treatments one and two were small.

All of the solutions of treatment three were cloudy. The variance of the triplicate samples of treatment three was high compared to the error variances of treatments one and two. The mean spectrophotometer readings of all seven strains of treatment three were lower than the same readings for treatments one and two. Differences between strains in treatment three were larger than differences between strains in treatments one and two.

Organisms were isolated from all the solutions of all three treatments. A bacterium, probably a Bacillus species, was the most abundant microorganism, followed in order by the following species of fungi: Rhizopus, Aspergillus, Penicillium, Fusarium and Alternaria. Although no attempt was made to deterine the number of bacteria and fungi per solution, it was evident from the amount of growth on the plates that there were fewer organisms in the solutions of treatments one and two than in treatment three.

Since treatments one and two were clear and the spectrophotometer readings of treatments one and two were higher than treat-

Table 3. Effect of three disinfecting and sterilizing procedures on the amounts of leached material.

Treatment	Mean spectrophotometer readings of triplicate samples of strains:								Variance[a]
	K1925	K1835	K1839	NC8917w1[b]	NC8917w2[b]	K1830	K1910	Mean	
(1) Bottles, caps disinfected; sterile distilled water.	90	90	89	92	91	91	91	90.6	11.7
(2) Bottles, caps disinfected.	92	90	91	92	93	93	--	92.2	6.0
(3) No sterility or disinfection measures.	75	80[c]	74	83	78	75	75	77.1	102.7

(a) Variance within triplicate samples.
(b) Same strain but from different sources.
(c) Spectrophotometer reading obtained on only one sample.

ment three, it was obvious that the microorganisms were coming primarily from the bottles. Although the microorganisms were not completely inhibited in treatments one and two, the low error variances of these treatments indicated that the microorganisms were probably not interfering with the spectrophotometer readings. Thus disinfecting the bottles with clorox eliminated the microorganisms to an extent that would justify the use of this method in reducing the microbial variable. Obviously a more complete elimination of the organisms on the bottles could be accomplished by autoclaving, but facilities were not available for this type of sterilization.

Since there were only small differences between strains where the microbial variable was reduced (treatments one and two) the differences between strains in treatment three could be attributed to microbial growth and not to seed permeability differences.

Estimation of the Amount of Leached Materials from Steeped Corn Seed by a Quantitative Bio-assay Method. Although the microorganisms could be effectively reduced by clorox disinfection of the bottles, it was thought advisable to investigate the possibility of using the microorganisms in a quantitative bio-assay of the leached materials. As pointed out above, the amount of microbial growth might be indicative of the amount of leached materials. Since Bacillus species were the predominant microorganisms found in the steep solutions it seemed logical that Bacillus organisms be used in a bio-assay of the leached materials.

The previous experiment had shown that with the microbial variable removed there were only small differences between the

permeabilities of the seed stocks being used. In the following experiment, corn strains with presumably different protoplast vitalities and/or maternal tissue structures were used in order to partially evaluate the relation between these characteristics and the amount of leaching.

Four strains of corn (open pollinated K2234 and sibbed or selfed (wh38-11 x 33-16), (B3 x K155) and (K44 x 33-16)) grown in 1952 at the K.S.C. agronomy farm were harvested by hand at seven day intervals for five weeks. The strains were shelled by hand to avoid damage to the pericarp. A summary of the harvest dates and the condition of the corn at each harvest is shown in Table 10.

Triplicate ten gram samples of each strain at each harvest date were surface disinfected with a five percent clorox solution for one minute. The seeds were placed in bottles that had previously been disinfected with a 25 percent clorox solution for five minutes. Fifty milliliters of sterile distilled water were added to each bottle. Each strain at each harvest date was subjected to two treatments: (1) one milliliter of a broth culture containing Bacillus subtilus was added to each solution and (2) no solution of Bacillus was added to the steep solution. Three checks of distilled water plus broth solution without seed were included. The bottles were capped and the seeds were steeped for 72 hours at 25° C.

The mean spectrophotometer readings for this experiment are reported in Table 4.

The mean spectrophotometer readings of the distilled water

Table 4. Effect of adding Bacillus subtilus to the steep solution.

Strain	Harvest		Mean spectrophotometer readings of triplicate samples of treatments:	
	no.	days[a]	Bacillus	No Bacillus
K2234	1.	106	73	80
	2.	113	77	87
	3.	120	85	90
	4.	127	84	92
	5.	134	84	92
Mean			80.7	88.2
(B3XK155)	1.	88	12	27
	2.	95	75	82
	3.	102	74	82
	4.	109	74	82
	5.	116	77	83
Mean			62.5	71.3
(K44X33-16)	1.	98	67	73
	2.	105	75	83
	3.	112	83	92
	4.	119	80	89
	5.	127	80	88
Mean			77.1	85.1
(Wh38-11X33-16)	1.	116	80	88
	2.	123	87	90
	3.	130	83	92
	4.	137	84	92
	5.	144	80	90
Mean			82.8	90.6
Total Mean			75.8	83.8
Distilled water checks			92	99

(a) Number of days from planting to harvest.

checks of treatments one and two were 92 and 99 respectively, i.e., the one milliliter of broth culture of Bacillus subtilus lowered the transmittance by seven percent.

The total mean spectrophotometer readings of treatments one and two for all four strains at each harvest date were 75.8 and 83.8 respectively. The average differences between the total means of treatments one and two was eight percent. This approximates fairly closely the difference in transmittance that could be attributed to the one milliliter of broth solution.

The relation between the amount of leached materials and the degree of maturity was the same in both treatments one and two. The mean spectrophotometer readings for harvests one, two and three of strains K2234 and (K44 x 33-16) showed a decrease in the amount of leached materials with each successive increase in maturity, while there were only small differences between harvests three, four and five. The mean spectrophotometer readings of the first two harvests of strain (B3 x K155) showed a decrease in the amount of leached materials with each successive increase in maturity, while there were only small differences between harvests two, three, four and five. The mean spectrophotometer readings of all five harvests of strain (wh38-11 x 33-16) showed only small differences.

The error variance of treatment two was 38.4 (Table 12c). This was high when compared to the error variances of the previous experiments. However, a large portion of this variance can be attributed to the triplicate samples of the first harvest of strains K2234 and (B3 x K155). The triplicate sample readings

were 84, 50 and 85 percent and 24, 14 and 43 percent respectively. At harvest these seeds were so immature that there was considerable mold growth before they were completely dry. As a consequence it was impossible to completely disinfect these seeds and microbial growth developed in the solutions. Microbial growth was observed in only two other samples.

Since the distilled water checks of both treatments showed that the broth culture of Bacillus subtilus lowered the transmittance by seven percent, the 8.05 percent difference between the total mean spectrophotometer readings of treatments one and two could be attributed to the addition of the broth culture in treatment one. Since the results were the same for each harvest of each strain in both treatments it was concluded that the addition of Bacillus subtilus to the solutions was of no advantage in the determination of the amounts of leached materials.

A comparison between the seed condition at each harvest for each strain (Table 10) and the mean spectrophotometer readings at each harvest for each strain in treatment two showed that the maximum amount of leaching occurs in the milk stage. The amount of leached materials is reduced in the dough stage as compared to the milk stage and is usually at a minimum in the glaze, dent and fully mature stages. This gradient in leached materials between harvests was similar to that reported by Robinson (45). It appeared then that the amount of leached materials is related to the physiological condition of the protoplast and/or the maternal tissue differentiation.

Time and Temperature of Steeping. In leaching materials from corn seed two factors need to be considered. First, it was important that a point be determined where pronounced differences in the amounts of leached materials are obtained. Second, in measuring transmittances greater than 95 percent the accuracy of the spectrophotometer is reduced. Thus, the amounts of leached materials should be such that the maximum transmittance of any steep solution does not exceed 95 percent.

The time and temperature of steeping would be important with respect to both of the above factors. With this in mind, the experiment in which the data in Table 1 were obtained was also designed to study the effect of time and temperature on the amount of leaching.

Microbial growth was so abundant at 38° C. that no evaluation of the effect of temperature on the amount of leaching could be made. The differences between the 10° C. and 18° C. readings indicated, as was expected, that the amount of leaching is increased with an increase in temperature. At 10° C. leaching was considerably reduced for all time periods, while at 18° C. the amount of leached materials was increased over 10° C. for the 48, 72 and 96 hour periods but not for the 24 hour period. Thus slightly higher temperatures and longer steeping periods should be effective in increasing the amounts of leached materials.

Since a 25° C. constant temperature room is maintained at the Kansas Station, 25° C. was selected as the steeping temperature. Although 48 hours at 18° C. seemed adequate with respect to the

amount of leaching and the spectrophotometer accuracy, it was thought that 72 hours would be more effective in bringing out pronounced differences. Therefore 72 hours was selected as the steeping time.

Proportion of Corn Seed to Distilled Water and Number of Replications. The proportion of corn seed to distilled water is important in that it determines to some extent the concentration of the leached materials. Fifty milliliters was selected as the amount of distilled water because this was the maximum amount for the size of the bottles available for steeping and it was the maximum amount necessary for the spectrophotometer readings. Thus the amount of corn and not the amount of water was varied.

Two factors had to be considered with regard to the weight of the corn sample. First, the sample weight had to be such that it could be measured accurately within plus or minus one kernel. Secondly, in the correlation studies which follow only small quantities of certain lots of seed were available. The size of the sample used in the leaching test had to be small enough so that enough corn was left for normal and cold test germinations. It was determined that the maximum amount of seed available for steeping was 30 grams.

With a maximum of 30 grams of seed for steeping purposes the number of replications and sample size was considered. Thirty grams of seed would leave three possibilities: six, five gram samples, three, ten gram samples or two, fifteen gram samples from each seed lot. Since five gram samples were hard to weigh accurately

i.e., within plus or minus one kernel, these were eliminated.

An experiment was conducted to study the differences between three 10 gram samples and two 15 gram samples. Seed of nine corn strains in triplicate 10 gram samples and duplicate 15 gram samples were placed in bottles that had previously been disinfected with a 25 percent clorox solution for five minutes. The seed had also been previously disinfected with a five percent clorox solution for one minute. Fifty milliliters of distilled water were added to each bottle and the seeds were steeped at 25° C. for 72 hours.

The mean spectrophotometer readings of this experiment are reported in Table 5.

The means of the spectrophotometer readings of the triplicate, 10 gram samples except in strain NC8917WII, were higher than the means of the duplicate 15 gram samples. This difference in the amounts of leached materials between the 10 and 15 gram samples was expected since the ratio of corn to distilled water was higher in the 15 gram samples (0.33 grams per ml.) than in the 10 gram samples (0.2 grams per ml.). The fact that the amount of leached materials was the same in strain NC8917WII for both the 10 and 15 gram samples could be attributed to microbial growth in one of the triplicate 10 gram samples which lowered the mean spectrophotometer reading.

The difference between the error variance of the three 10 gram samples and the two 15 gram samples was small. The spectrophotometer readings were all either 95 percent or below for both types of sample-size replications. It appeared then that either

Table 5. Effect of sample size replication on the amount of leached materials.

Gms. corn per 50 ml. distilled water	Replications	Mean spectrophotometer readings of strains: K1907	K1897	NC8909	K1892	NC8917-WI[a]	NC8917-WII[a]	K1925	K1910	K1830	Mean	Variance[b]
10	3	83	87	91	91	95	90	92	88	94	86.7	7.7
15	2	81	80	88	88	88	90	86	84	90	85.9	8.2

(a) Same strain but from different sources.

(b) Variance within triplicate 10 gram and duplicate 15 gram samples.

type of sample-size replication would probably be adequate. However, since even after clorox disinfection procedures microbial growth did occasionally occur, it was thought advisable to use the three 10 gram samples. For example, if one of the solutions became contaminated it would not affect the mean spectrophotometer reading as much as in the duplicate 15 gram samples because the spectrophotometer readings of the other two solutions would tend to balance the one low reading.

Summary

In the development of a usable leaching technique for the leaching of soluble solids and colloidal materials from the seed of Zea mays four factors were studied: (1) microbial growth in the steep solution; (2) time and temperature of steeping; (3) proportion of corn seed to distilled water; and (4) number of replications.

Microbial growth in the steep solution could not be controlled adequately by steeping the corn seed for long periods at temperatures unfavorable for microbial development. Phenol in the steep solutions appeared to be effective in controlling microbial growth only in concentrations toxic to the protoplast of the seed. Since the amount of leached materials is probably determined to some extent by the protoplast vitality of the seeds, the use of phenol for controlling the microorganisms was considered unsatisfactory. It was found that the primary source of the microorganisms was the bottles and that the microorganisms were primarily bacteria, probably Bacillus species. Disinfection of the bottles and seed

with clorox (active ingredients, sodium hypochlorite, 5.25 percent by weight) solutions of 25 percent for five minutes and five percent for one minute respectively, was effective in reducing microbial growth of the microorganisms in the steep solution. It was thought that the growth of the microorganisms in the steep solutions might be indicative of the amounts of leached materials. A quantitative bio-assay of the leached materials using Bacillus subtilus showed that the addition of a microorganism to the steep solution was of no advantage in determining the amount of leached materials. Therefore, clorox disinfection of the bottles used for steeping and clorox disinfection of the seed were selected as the means of reducing microbial growth in the steep solutions.

An attempt was made to determine the optimum time and temperature of steeping. It was concluded that a temperature slightly higher than 18° C. and a period of either 48 or 72 hours would be effective in obtaining the optimum amount of leaching. Therefore, 25° C. and 72 hours were selected as the time and temperature for steeping.

Fifty milliliters of distilled water was selected as the amount of distilled water to be used for the steeping medium. A comparison was made between the mean spectrophotometer readings of triplicate 10 gram samples and duplicate 15 gram samples to determine which sample-size and replication number should be used. The data indicated that either sample-size replication combination would be satisfactory. For reasons discussed, triplicate 10 gram samples were selected as the number of replications and sample-

size to be used in the leaching test.

A usable leaching technique would consist of the following: (1) disinfection of the steeping bottles and the seed with a 25 percent clorox solution for five minutes and a five percent clorox solution for one minute respectively; (2) steeping for 72 hours at 25° C.; (3) triplicate samples of each seed lot; and (4) a ratio of 0.2 grams of seed per milliliter of distilled water, i.e., ten grams of seed per 50 milliliters of distilled water.

The spectrophotometer readings of four strains of corn harvested at seven day intervals for five weeks and leached as described above, indicated that differences in the amounts of leached materials could probably be attributed in part to the physiological condition of the protoplast and/or the maternal tissue differentiation of the corn seed.

CORRELATION BETWEEN SEED PERMEABILITY AND COLD TEST GERMINATION IN *ZEA MAYS*

Introduction

These studies have attempted to determine if the differences in the reduction in germination due to cold testing between reciprocal crosses, between ears within a strain, between seed of different ages, between seed of different degrees of maturity, and between normal and frosted seed, are related to corn seed permeability as determined by the optical measurement of leached materials from steeped corn seed.

Methods and Materials

The steeping procedure was determined in the technique studies. Triplicate ten gram samples of each seed lot were surface disinfected with a five percent clorox (active ingredients, sodium hypochlorite, 5.25 percent by weight) solution for one minute. Each ten gram sample was placed in a 75 ml. bottle that had previously been disinfected with a 25 percent clorox solution for five minutes. Fifty milliliters of distilled water were added to each bottle. The bottles were capped and the seeds were steeped for 72 hours at 25° C. After steeping, the liquid was decanted and passed through porous filter paper to remove any extraneous pieces of chaff, silk, etc. The amount of leached material was determined with a Coleman Universal spectrophotometer at a wave length of 400 millimicrons. The values reported are the amount of light transmitted through the steep solution expressed as a percent of the amount of light transmitted through the distilled water reference. The values are inversely proportional to the optical density or turbidity of the solution, which in this case is the concentration of the leached materials.

The cold testing procedure was essentially the same as that described by Hoppe (28). Soil for the cold test germinations was obtained from a field at the Kansas State agronomy farm where corn was being grown at that time. The soil was mixed with sand in the ratio of two-thirds soil to one-third sand. Water was added so that the soil-sand mixture was at approximately 60 percent water holding capacity. Moist paper germination towels, five by seven and one-half inches, were covered with the soil sand mixture so

that the mixture was approximately three-fourths of an inch deep. Fifty seeds of each seed lot were equally spaced throughout the soil-sand mixture. Another moist paper germination towel was placed over the seeds and soil. The "paper doll" was then rolled in waxed paper to minimize the loss of water. The "paper dolls" were placed in the cold room for a specific period as explained below. After the cold treatment they were removed to a germination chamber in the greenhouse where germination was completed. The method of determining germination was as follows. The "paper doll" was unrolled and the sprouted kernels were separated from the soil. An estimation of the average length of the radicals and plumules was made. The seeds were counted as germinated if the radical and plumule were approximately equal to or larger than the average length. Duplicate 50 seed samples of each seed lot were tested. The cold test germination for each duplicate sample was obtained from the number of germinated kernels out of 50. The mean cold test germination of each seed lot was then arrived at by averaging the duplicate samples.

Normal germination was determined as follows. Fifty seeds of each seed lot were equally spaced on moist paper germinating towels, five by seven and one-half inches in size. Another moist germinating towel was placed on top of the seed and they were rolled in wax paper to minimize the loss of water. The "paper dolls" were placed in a germinating chamber in the greenhouse for a specific period of time as shown below. Germination was determined as described above. The number of seed germinated out of fifty was used as an estimate of normal germination.

The cold test data were derived as follows. For each seed lot the mean cold test germination of the duplicate 50 seed samples was subtracted from the normal germination. This reduction in germination due to cold testing was then divided by the normal germination and multiplied by 100 to obtain a percentage. Thus the data reported are the reduction in germination due to cold testing expressed as a percent of the normal germination. For convenience this has been referred to as the "cold index". Since normal germination was not always 100 percent the cold index was used rather than the reduction in cold test germination so that all the data would be on the basis of 100 percent normal germination.

Cold test and normal test germination periods were varied depending on the seed conditions and on the weather conditions respectively. If the seed was presumed to be in a good, physiological state it was subjected to a more severe cold test in order to accentuate the differences in germination more fully. The time of germination in the greenhouse of both the normal and cold test germinations was varied depending on the weather conditions, i.e., the period was shortened during warm weather.

Details of harvesting and handling procedures and the cold test and normal test schedules for the various seed lots are given along with the results of the individual experiments.

Experimental Results

Reciprocal Crosses. Seed of 11 double cross hybrids made reciprocally in 1952 was harvested and shelled by hand to minimize pericarp damage. They were cold tested at 9° C. for 21 days; germination was completed in the greenhouse in five days. Normal germination was completed in the greenhouse in four days.

The spectrophotometer readings and the cold indices for the 11 double cross hybrids made reciprocally are reported in Table 6a. The analyses of variance of the cold indices and the spectrophotometer readings are reported in Tables 6b and 6c.

A highly significant negative correlation coefficient of 0.593 was shown by the cold indices and the spectrophotometer readings. The analysis of variance of the cold indices showed highly significant differences between the reciprocal single crosses composing each double cross hybrid and among the 11 double cross hybrids. The coefficient of variation of the cold indices was 34.9 percent. The analysis of variance of the spectrophotometer readings showed no significant differences between the reciprocal crosses and highly significant differences among the double cross hybrids. The coefficient of variation of the spectrophotometer readings was 2.7 percent. Using an LSD, only four strains (K2460, K2465, K2489 and K2492) showed significant differences between the cold indices of their respective reciprocals. In the spectrophotometer readings only one strain (K2460) showed a significant difference between its reciprocals.

Table 6a. Spectrophotometer readings and cold indices of eleven double cross hybrids made reciprocally.

Strain	Maternal Parent	Cold index[a]	Spectrophotometer reading[b]
US523W	(K 55 x K 64)	12	95
	(Ky 27 x Ky 49)	21	93
K2248	(K 41 x wh38-11)	19	92
	(whHy x wh205)	9	93
K2249	(K41 x wh38-11)	35	90
	(R 30 x 33-16)	21	90
US547W	(K 64 x 33-16)	11	94
	(K 55 x R 30)	17	95
K2460	(wh38-11 x K 694)	57 **[d]	86 **[c]
	(K 696 x K 699)	21	93
K2465	(K 694 x K 699)	34 **[d]	90
	(K 696 x whHy)	14	92
K2487	(whHy x wh187-2)	21	92
	(K 693 x K 699)	20	90
K2489	(whL 299 x whL)	12 **[d]	94
	(K 693 x K 698)	48	94
K2492	(whHy x whL)	3 **[d]	92
	(K 699 x K698)	29	92
K2495	(H 24 x H 25)	21	91
	(whHy x wh38-11)	19	92
K2497	(H22 x H55)	11	94
	(whHy x Mo 917W)	9	93

(a) Reduction in cold test germination expressed as a percentage normal germination.

(b) Mean spectrophotometer readings of triplicate subsamples.

(c) L.S.D. for between reciprocal crosses in the spectrophotometer readings: .05=4.08 percent; .01=5.45 percent.

(d) L.S.D. for between reciprocal crosses in cold indices: .05= 14.84 percent; .01= 20.17 percent.

Table 6b. Analysis of variance of cold indices of 11 double cross hybrids made reciprocally.

Source	d.f.	SS	MS	F
Double cross hybrid	10	2914	291.40	5.69**
Reciprocal cross	11	4090	371.82	7.27**
Error	22	1128	51.18	
Total	43	8132		

Table 6c. Analysis of variance of spectrophotometer readings of 11 double cross hybrids made reciprocally.

Source	d.f.	SS	MS	F
Double cross hybrid	10	181	18.10	2.94**
Reciprocal cross	11	111	10.09	1.64ns
Error	44	271	6.16	
Total	65	563		

The highly significant negative correlation suggested that differences between reciprocal crosses of each strain in the cold indices might show a relationship with the differences between their spectrophotometer readings. This was the case only in strain K2460. The differences between strains were significant in both the cold indices and the spectrophotometer readings. Thus, it is probable that most of the correlation between the cold indices and the spectrophotometer readings was due to strain differences rather than differences between reciprocal crosses.

The differences between the reciprocal crosses of strains K2465, K2489 and K2492 in the cold indices could possibly be attributed to uncontrolled factors in the cold tests, as discussed below. The differences between the reciprocal crosses of K2460 in the cold indices might be explained on the same basis. However, since there was a difference between the seed permeabilities of the reciprocals of this hybrid, it is believed that differences in cold test germination were due to actual differences between the two reciprocal crosses. This could probably be attributed to the effect of the maternal parent.

In summary, it was believed that the negative correlation between the cold indices and the spectrophotometer readings was probably due to two factors. First, the variation between strains in the cold indices was associated with the variation between strains in the spectrophotometer readings. Second, in one case the variation between the two reciprocal crosses of K2460 was associated with the variation in their spectrophotometer readings. The data suggest that differences between strains and between

reciprocal crosses in the reduction in germination due to cold testing were associated with corresponding differences in seed permeability.

Individual Ears of Open Pollinated Single Cross Hybrids. Five individual ears of each of ten open pollinated single crosses grown in 1952 were harvested and shelled by hand. They were cold tested at 10° C. for 14 days; germination was completed in the greenhouse in five days. Normal germination was completed in the greenhouse in four days.

The spectrophotometer readings and cold indices for the five individual ears of each of ten open pollinated single crosses are reported in Table 7a. The analyses of variance of the cold indices and spectrophotometer readings are reported in Tables 7b and 7c respectively.

A highly significant negative correlation coefficient of 0.627 was shown by the cold indices and the spectrophotometer readings. The analysis of variance of both the cold indices and the spectrophotometer readings showed highly significant differences both between strains and within strains. The coefficient of variance for the cold indices and the spectrophotometer readings were 25.9 percent and 4.9 percent respectively. All three of the steep solutions of strain eight, ear one, were yellow and did not appear to be contaminated with microorganisms. In a few other solutions at least one of the triplicate subsamples was cloudy, indicating microbial growth.

The highly significant negative correlation between the cold

Table 7a. Spectrophotometer readings and cold indices of individual ears of ten open pollinated single cross hybrids.

Strain	Ind. ear	Cold index[a]	Spec. read[b]	Strain	Ind. ear	Cold index[a]	Spec. read[b]
1	1	3	77	6	1	25	86
	2	0	82		2	35	85
	3	2	91		3	11	89
	4	2	88		4	9	91
	5	13	86		5	9	88
	mean	4	85		mean	18	88
2	1	19	82	7	1	9	88
	2	8	87		2	0	91
	3	4	88		3	17	83
	4	22	84		4	27	91
	5	0	80		5	9	89
	mean	11	84		mean	10	88
3	1	56	84	8	1	64	43
	2	32	84		2	46	70
	3	50	76		3	52	74
	4	20	85		4	48	86
	5	28	87		5	20	85
	mean	37	83		mean	46	67
4	1	17	92	9	1	64	69
	2	2	84		2	23	80
	3	0	90		3	8	84
	4	8	86		4	13	87
	5	4	90		5	22	75
	mean	8	88		mean	26	79
5	1	35	77	10	1	26	79
	2	13	81		2	10	88
	3	45	83		3	26	85
	4	42	85		4	52	74
	5	36	81		5	36	84
	mean	34	81		mean	30	76

(a) Reduction in cold test germination expressed as percent of normal germination.
(b) Mean spectrophotometer readings of triplicate subsamples.

Table 7b. Analysis of variance of cold indices of individual ears of ten open pollinated single cross hybrids.

Source	d.f.	SS	MS	F
Strains	9	18363	2040.31	60.5**
Ears within a strain	40	13722	343.04	10.2**
Error	50	1686	33.72	
Total	99	33771		

Table 7c. Analysis of variance of spectrophotometer readings of individual ears of ten open pollinated single cross hybrids.

Source	d.f.	SS	MS	F
Strains	9	3544	393.82	24.2**
Ears within a strain	40	5574	139.34	8.4**
Error	98	1608	16.41	
Total	147	10726		

indices and the spectrophotometer readings suggests that the differences between strains and among ears of open pollinated single cross strains in reduction in germination due to cold testing were associated with differences in their seed permeability.

It should be pointed out that since these ears were from open pollinated single crosses, the results obtained are not comparable to the differences in the reduction in germination due to cold testing between ears of "long time" inbred lines (20,42).

It was of interest to note that yellow steep solutions, similar to the ones obtained for ear one, strain eight, were reported by Hottes and Huelson (29). The cause of the "yellowing" is unknown since the solutions did not appear to be contaminated with microorganisms.

Individual Ears of a Composite of Open Pollinated Varieties. Ten individual open pollinated ears of a composite of open pollinated varieties grown in 1952 were harvested and shelled by hand. They were cold tested at 10° C. for ten days; germination was completed in the greenhouse in six days. Normal germination was completed in the greenhouse in five days.

The spectrophotometer readings and the cold indices of the ten individual ears of a composite of open pollinated varieties are reported in Table 8a. The analyses of variance of the cold indices and the spectrophotometer readings are reported in Tables 8b and 8c respectively.

Table 8a. Spectrophotometer readings and cold indices of ten individual ears from a composite of open pollinated varieties.

Individual ear	Cold index[a]	Spect. reading[b]
1	6	91
2	12	86
3	10	88
4	50	78
5	3	86
6	10	86
7	8	86
8	2	88
9	9	88
10	2	90

(a) Reduction in cold test germination expressed as percent of normal germination.
(b) Mean spectrophotometer readings of triplicate subsamples.

Table 8b. Analysis of variance of cold indices of ten individual ears from a composite of open pollinated varieties.

Source	d.f.	SS	MS	F
Ind. ears	9	3598	399.78	13.60**
Error	10	294	29.40	
Total	19	3892		

Table 8c. Analysis of variance of spectrophotometer readings of ten individual ears from a composite of open pollinated varieties.

Source	d.f.	SS	MS	F
Ind. ears	9	352	39.11	5.18**
Error	20	151	7.55	
Total	29			

A significant (five percent level) negative correlation of 0.666 was shown by the spectrophotometer readings and the cold indices. The analysis of variance of both the cold indices and spectrophotometer readings showed highly significant differences between individual ears. The coefficients of variation for the cold indices and the spectrophotometer readings were 49.2 percent and 3.2 percent respectively.

The significant negative correlation suggests that differences existing between ears of a composite of open pollinated varieties in reduction in germination due to cold testing were associated with differences in seed permeability. The differences between ears in the cold indices of this experiment are not analagous to the differences in reduction in germination due to cold testing between ears of "long time" inbred lines (20,42) because of the presumably high heterozygosity of the open pollinated varieties used here.

Age of Storage. Strains of corn harvested by hand and shelled by machine in 1945, 1948, and 1949 were used in the age of storage study. The 1945 seed consisted of top crosses of open pollinated lines by single crosses. The 1948 seed consisted of single cross seed and the 1949 seed consisted of double cross seed. They were cold tested for 16 days at 9° C.; germination was completed in the greenhouse in five days. Normal germination was completed in the greenhouse in four days.

The spectrophotometer readings and the cold indices for the

various strains of corn stored three, four and seven years are reported in Table 9a. The analyses of variance of the cold indices and the spectrophotometer readings are reported in Tables 9b and 9c respectively.

The strains of corn used in this study were different for each storage age. Therefore the analyses of variance of the cold indices and of the spectrophotometer readings have been determined for each storage age, since if they were analyzed together, strain differences would be confounded with seed ages.

A highly significant negative correlation coefficient of 0.797 was shown by the spectrophotometer readings and the cold indices over all seed ages.

A significant (five percent level) negative correlation coefficient of 0.805 was shown by the spectrophotometer readings and cold indices of the seed stored seven years. The analyses of variance of the spectrophotometer readings and the cold indices for the seven year seed showed significant differences at the five percent level among strains. Strains 1514, 1577 and 1587 were completely susceptible to the cold test. The coefficients of variation for the cold indices and the spectrophotometer readings were 21.4 percent and 12 percent respectively. Microbial growth was present in at least one or more of the subsamples of each strain.

A non-significant negative correlation coefficient of 0.533 was shown by the four year seed. The analysis of variance of the cold indices for the four year seed showed differences among strains to be significant at the five percent level. The analysis

Table 9a. Spectrophotometer readings and cold indices of corn stored three, four and seven years.

Year	Strain	Cold index[a]	Spect. readings[e]
1945	1414[b]	50	62
	1420	51	61
	1436	46	73
	1513	68	53
	1514	100	56
	1577	100	53
	1587	100	50
	mean	74	58
1948	(K 10 x K 55)[c]	72	52
	(38-11 x Ky 21)	70	37
	(38-11 x K 155)	75	35
	(K 148 x 38-11)	64	47
	(38-11 x CI 7)	89	33
	(K 10 x CI 7)	66	52
	(38-11 x La 44)	81	48
	mean	74	43
1949	NC 8917W1[d]	15	93
	NC 8917W2[d]	16	90
	K 1830	19	69
	K 1907	27	65
	K 1897	17	67
	K 1919	10	74
	K 1936	18	85
	mean	17	78

(a) Reduction in cold test germination expressed as percent of normal germination.
(b) 1945 field numbers.
(c) Single crosses.
(d) Same strain but from different sources.
(e) Mean spectrophotometer readings of triplicate subsamples.

Table 9b. Analysis of variance of cold indices of strains of corn stored three, four and seven years.

Source	d.f.	SS	MS	F
1945:Strain	6	7959	1326.50	5.32*
Error	7	1746	249.43	
Total	13	9705		
1948:Strain	6	911	151.83	5.01*
Error	7	212	30.29	
Total	13	1123		
1949:Strain	6	315	52.50	3.28ns
Error	7	112	16.00	
Total	13	427		

Table 9c. Analysis of variance of spectrophotometer readings of strains of corn stored three, four and seven years.

Source	d.f.	SS	MS	F
1945:Strain	6	1121	186.83	3.4*
Error	14	768	54.85	
Total	20	1889		
1948:Strain	6	1194	199.00	1.8*
Error	14	1529	109.21	
Total	20	2723		
1949:Strain	6	2438	406.33	8.8*
Error	14	641	45.79	
Total	20	3079		

of variance of the spectrophotometer readings showed no significant difference among strains. The coefficients of variation for the cold indices and the spectrophotometer readings were 7.4 percent and 24.1 percent respectively. The high coefficient of variation of the spectrophotometer readings could be attributed to microbial growth in one or more of the subsamples of each strain.

A non-significant negative correlation coefficient of 0.432 was obtained for the three year seed. The analysis of variance of the cold indices showed differences among strains to be non-significant, while the analysis of variance of the spectrophotometer readings showed differences among strains to be significant at the one percent level. The coefficient of variation for the cold indices and spectrophotometer readings were 22.9 percent and 8.7 percent respectively. Microbial growth was present in at least one or more of the steep solutions.

Even though strains and seed age were confounded it appeared that the seven and four year seed were on the average more susceptible to germination under adverse conditions than was the three year seed. The mean spectrophotometer readings for each seed age showed that on the average the permeability of the seven and four year seed was greater than that of the three year seed. Thus the means of the cold indices and spectrophotometer readings for each seed age, plus the highly significant negative correlation (0.797) did suggest that the differences between seed of different ages in reduction in germination due to cold testing might be associated with corresponding differences in seed permeability. Further experiments using different seed ages of the same strain

would be needed to verify this association.

The significant strain differences in the cold indices and spectrophotometer readings of the seven year seed plus the highly significant negative correlation coefficient (0.805) suggested that differences between the seven year strains in reduction in germination due to cold testing were associated with corresponding differences in seed permeability.

Three and four year seed showed no association between reduction in germination due to cold testing and seed permeability. The results of the four year seed might be partially reconciled if the microbial growth in the steep solutions was considered. As indicated by the coefficient of variation (24 percent) the microbial growth evidently increased the variability of the spectrophotometer readings. Thus the precision of the spectrophotometer readings in estimating the amounts of leached materials might have been reduced.

Maturity. Four strains of corn grown in 1952 were selected for the maturity study. These were open pollinated K2234, and sibbed or selfed (B3 x K155), (wh38-11 x 33-16) and (K44 x 33-16). The last three strains were considered sibbed or selfed since they were obtained from isolated double cross seed production fields in which they were the male parents. Ears of each strain were harvested by hand at seven day intervals for five weeks. After harvesting they were hung in the greenhouse to dry. They were shelled by hand to minimize pericarp damage. The dates of

planting, number of days from planting till harvest and condition of the seed at each harvest are summarized in Table 10. All the strains at each harvest were cold tested for 16 days at 9° C.; germination was completed in the greenhouse in five days. Normal germination was completed in the greenhouse in four days.

The spectrophotometer readings and cold indices of the four strains of corn harvested at seven day intervals are reported in Table 11a. The analyses of variance of the cold indices and the spectrophotometer readings are reported in Tables 11b and 11c respectively.

A negative correlation coefficient, significant at the five percent level, of 0.451 was found between the spectrophotometer readings and the cold indices. There was a highly significant strain-harvest interaction for the cold indices and spectrophotometer readings. Therefore harvests within strains were analyzed for the cold indices and the spectrophotometer readings. There were highly significant differences between harvests for strains K2234 and (wh38-11 x 33-16) and significant (five percent level) differences between harvests for strain (B3 x K155) in the cold indices. Strain (K44 x 33-16) showed no significant differences between harvests in the cold indices. In the spectrophotometer readings, strains (B3 x K155) and (K44 x 33-16) showed highly significant differences between harvests while there were no significant differences between harvests for strains K2234 and (wh38-11 x 33-16). The coefficients of variation for the cold indices and the spectrophotometer readings were 20.3 percent and 7.4 percent respectively.

Table 10. Summary of harvest dates and seed condition in maturity study.

Strain	1952 planting date	Number of days from planting and seed condition				
		Harvest no.				
		1.	2.	3.	4.	5.
K2234	May 5	106 milk	113 dough	120 glaze	127 dent	134 mature
(wh30-11 x 33-16)	April 25	116 dough	123 glaze	130 dent	137 mature	144 mature
(B 3 x K155)	May 23	88 milk	95 dough	102 glaze	109 dent	116 mature
(K 44 x 33-16)	May 13	98 milk	105 dough	112 glaze	119 dent	127 mature

Table 11a. Spectrophotometer readings and cold indices of four strains of corn harvested at seven day intervals.

Strain	Harvest[a]	Cold index[b]	Spect. readings[c]
K 2234	106	72	80
	113	57	87
	120	35	90
	127	11	92
	134	7	92
	Mean	42	88
(B 2 x K 155)	88	77	27
	95	69	82
	102	46	82
	109	80	82
	116	60	83
	Mean	66	71
(K 44 x 33-16)	98	43	73
	105	58	83
	112	65	92
	119	56	89
	126	57	88
	Mean	56	85
(wh38-11 x 33-16)	116	66	88
	123	38	90
	130	43	92
	137	25	92
	144	24	90
	Mean	39	99

(a) Number of days from planting till harvest.
(b) Reduction in cold test germination expressed as percent of normal germination.
(c) Mean spectrophotometer readings of triplicate subsamples.

Table 11b. Analysis of variance of cold indices of four strains of corn harvested at seven day intervals.

Source	d.f.	S.S.	M.S.	F
Harvests	4	3732	933.00	1.36 ns
Harvests within strains				
K2234	4	7575	1893.75	17.82 **
(B3 x K 155)	4	1510	377.50	3.55 *
(K44 x 33-16)	4	536	134.00	1.26 ns
(wh38-11 x 33-16)	4	2308	577.00	5.43 **
Strains	3	4712	1570.67	2.29 ns
S x H	12	8197	683.08	6.43 **
Error	20	2126	106.30	
Total	39	18767		

Table 11c. Analysis of variance of spectrophotometer readings of four strains of corn harvested at seven day intervals.

Source	d.f.	S.S.	M.S.	F
Harvests	4	4433	1108.25	3.33 ns
Harvests within strains				
K2234	4	337	84.25	2.19 ns
(B3 x K 155)	4	7375	1843.75	47.98 **
(K44 x 33-16)	4	674	168.50	4.39 **
(wh38-11 x 33-16)	4	44	11.00	- ns
Strains	3	3342	1114.00	3.34 ns
S x H	12	3997	333.08	8.67 **
Error	40	1537	38.45	
Total	59	13309		

The strain by harvest interaction in the cold indices suggested that different strains react differently to varied harvest periods. This was expected since there were differences in the rate of maturity and planting dates of the four hybrids. Even with a strain by harvest interaction, it was expected that the cold indices of each strain should show a progressive decrease with an increase in maturity. In general this was the case in strains K2234 and (wh38-11 x 33-16), but not in strains (B3 x K155) and (K44 x 33-16). Strains K2234 and (wh38-11 x 33-16) showed significant differences between harvests, indicating that immature seed was more susceptible than mature seed to germination under adverse conditions. The lack of significant differences in the cold indices of (K44 x 33-16) and (B3 x K155) might possibly be attributed to uncontrolled factors in the cold tests as discussed below.

From Robinson's (45) investigation, non-significant differences between harvests of each of the strains in the spectrophotometer readings might be expected. In his experiments the steeping medium was evaporated and the amount of leached materials was weighed. He found large differences in the amounts of leached material between the dough and glaze stages, but progressively smaller differences between the glaze, dent and fully mature stages. Since the spectrophotometer is obviously not as exact in determining the amounts of leached materials, non-significant differences between the spectrophotometer readings in the later harvests might be expected. This was the case in strains K2234 and (wh38-11 x 33-16). The increased microbial growth in the first

harvest of strain (B3 x K155), which undoubtedly lowered the mean spectrophotometer reading, would account for the significant differences between harvests in that strain. The differences between the first, second and third harvests of (K44 x 33-16) were apparently enough to show a significant difference between harvests.

Differences in the rates of maturity and planting dates of the four hybrids would also account for the strain-harvest interaction in the spectrophotometer readings.

Considering the expected cold test response and the spectrophotometer readings there probably wouldn't be a high degree of association between the cold indices and spectrophotometer readings in this experiment. The correlation coefficient ($r = -0.451^*$) was of the magnitude expected. Since the correlation was significant it did indicate that differences between harvests in reduction in germination due to cold testing were associated with corresponding differences in seed permeability.

Normal versus Frosted Seed. Three strains were used in the normal versus frosted seed study; open pollinated K2234 and US523W, and sibbed or selfed ears of (K44 x 33-16). They were all harvested by hand two days after harvest number two (Table 10) in the maturity study. Thus the number of days from planting till harvest was 115, 115, and 107 respectively. A representative number of ears for normal samples were hung in the greenhouse to dry. The rest of the ears were placed in a refrigerator at an average temperature of -9° C. Three

ears from each strain were removed at intervals of 1.5, 2.0, 2.5, 3.0 and 3.25 hours. They were then hung in the greenhouse to dry. Cold testing consisted of a 10 day period at 10° C. and germination was completed in the greenhouse in six days. Normal germination was completed in seven days.

The spectrophotometer readings and cold indices of the artificially frosted individual ears of three corn hybrids are reported in Table 12a. The analyses of variance for the cold indices and the spectrophotometer readings are shown in Tables 12b and 12c respectively.

A non-significant negative correlation coefficient of 0.038 was found for the cold indices and the spectrophotometer readings. The analysis of variance for both the cold indices and the spectrophotometer readings showed highly significant differences between individual ears of each treatment for each strain. There were highly significant differences among "frost" treatments for both the cold indices and the spectrophotometer readings. However, the mean cold indices and the mean spectrophotometer readings of the three ears of each strain at each "frost" treatment showed no trend in reduction in germination and amount of leached materials respectively, with increased exposure to "frost". For example, the mean cold index of the three check ears of K2234 was greater than the mean cold indices of any of the five "frost" treatments, while the mean spectrophotometer readings of the three check ears of K2234 was lower than the mean spectrophotometer readings of the 1.5, 2.0, 2.5 and 3.25 hour treatments but higher than those of the 3.0 hour treatment. The coefficients of variation for the

Table 12a. Spectrophotometer readings and cold indices of artificially frosted individual ears of three corn hybrids.

Hybrid	Cold exposure[a]	Individual ears 1 Cold index[b]	1 Spect. reading[c]	2 Cold index	2 Spect. reading	3 Cold index	3 Spect. reading	Mean Cold index	Mean Spect. reading
US 523W	1.5	49	83	12	89	7	90	23	87
	2.0	16	93	1	81	27	87	21	87
	2.5	39	85	23	89	36	90	30	88
	3.0	36	79	3	88	28	86	20	84
	3.25	72	82	70	82	37	87	60	84
	0.0	6	92	32	86	15	87	18	88
K 2234	1.5	20	91	10	87	36	87	23	89
	2.0	23	90	3	89	45	79	28	87
	2.5	6	93	22	90	30	89	19	90
	3.0	26	88	5	68	10	86	14	83
	3.25	9	93	54	84	5	90	23	89
	0.0	54	87	60	85	58	86	57	86
(K 44 x 33-16)	1.5	13	93	31	90			22	92
	2.0	15	90	33	92			24	91
	2.5	5	93	15	92			10	93
	3.0	35	92	5	79			20	87
	3.25	43	84	17	92			30	90
	0.0	23	91	23	93			23	92

(a) Hours of exposure to -9° C.
(b) Reduction in cold test germination expressed as percent of normal germination.
(c) Mean spectrophotometer reading of triplicate subsamples.

Table 12b. Analysis of variance of cold indices of artificially frosted individual ears of three corn hybrids.

Source	d.f.	S.S.	M.S.	F
Treatment	17	4492	264.24	3.49**
Individual ears within a treatment for each strain	30	25382	846.07	11.18**
Error	46	3481	75.67	
Total	93	33355		

Table 12c. Analysis of variance of spectrophotometer readings of artificially frosted individual ears of three corn hybrids.

Source	d.f.	S.S.	M.S.	F
Treatment	17	948	55.76	21.36**
Individual ears within a treatment for each strain	30	1878	62.60	23.95**
Error	85	222	2.61	
Total	132	2948		

cold indices and the spectrophotometer readings were 32.7 percent and 1.8 percent respectively.

From Table 12a it can be seen that either the spectrophotometer readings were exceptionally high for the three hour "frost" period of ear two, strain K2234, and for the two hour period of ear two, strain US523W. Since microbial growth was not observed in the steep solutions, it appeared that it was probably the cold indices which were exceptionally high. The high cold indices might have been due to uncontrolled factors in the cold tests.

Since there was no treatment gradient for each strain either in the cold indices or spectrophotometer readings, a question arises as to what effect the different cold exposures had on the strains. This might be explained by the fact that there were significant differences between ears within a treatment for each strain in both the cold indices and the spectrophotometer readings. The cold treatment evidently had a different effect on each ear.

The negative correlation suggested that there was no relationship between seed permeability and cold test germination of the artificially frosted strains used in this experiment. However, this might not be a true picture of the relationship between these two variables in this experiment since the three exceptionally high cold indices mentioned above obviously had a pronounced effect on the correlation obtained.

Discussion

The coefficients of variation of the cold indices were high as compared to those of the spectrophotometer readings. It

appeared then that the greatest source of error was in the cold tests. Tatum (52) had previously reported a high degree of variability (coefficient of variation equaled 11 percent) in the cold test germination. His method consisted of using large wooden flats in the cold testing procedure. The method of Hoppe (28) was adapted for these experiments with the expectation of lowering the cold test variability. The experimental unit in Hoppe's test is the "paper doll". This should have been a more homogenous unit with respect to soil fungi populations than were the large flats used by Tatum. However, the "paper dolls" apparently were not, since the coefficients of variation in all the experiments reported, except one, were greater than those reported by Tatum. The variability obtained by using the "paper dolls" could possibly be attributed to an uneven distribution of the soil pathogens throughout the soil-sand mixture so that each "paper doll" did not have the same kind or amount of microorganisms. This could account for some of the cold test results discussed above. A more homogenous experimental unit could possibly be obtained by using sterile sand and adding a certain kind and amount of microorganisms.

Since in all the experiments except one there was a correlation between the reduction in germination due to cold testing and seed permeability, the question arises as to how the two phenomena are related. Tatum (52) pointed out three ways they could be associated: (1) Seed permeability might be indicative of the degree of differentiation of the maternal tissues (pericarp and testa). The degree of differentiation might determine the

extent to which the microorganisms can penetrate the kernel. This would be a purely mechanical type of resistance. (2) The permeability might determine the amount of materials that leach from the seed during the germination process. The leached materials might provide a source of nutrient from which the soil pathogens can become established and infect the germinating corn kernel. (3) Seed permeability is associated with the protoplast vitality which is in turn associated with increased susceptibility in the cold test reaction.

These experiments have made no attempt to determine which one(s) if any of these hypotheses are valid. Obviously, each of the three, or a combination of the three factors could contribute to the cold test reaction, depending on which of various seed conditions were being studied. The results and conclusions of Dickson (8), Leach (37), Hooker and Dickson (21) and Hooker (20) suggest which of the three factors might be operating under specific seed conditions.

Dickson (8) pointed out that corn is a warm weather crop with an optimum growth between 24° C. and 28° C., while the pathogen Gibberella saubinetii grows at temperatures from 3° C. to 32° C. Seedling blight occurs only at temperatures below 24° C. He concluded that temperature is the greatest single environmental factor affecting the development of seedling blight, and that its effect is on the host rather than the pathogen.

Leach (37) reported that with other factors constant, the relative growth rates of the host and pathogen at certain temperatures determine the severity of preemergence damping-off.

The development of resistance in excised corn embryos was observed by Hooker and Dickson (21). Corn embryos were excised at three day intervals during germination at 12° C. and subjected to Pythium organisms. Resistance to Pythium at this temperature was increased as the period of germination was increased, and resistance developed faster in lines previously proven resistant than in lines previously proven susceptible. They concluded that the effects of temperatures below 10° C. and above 24° C. were on the pathogen, while temperatures between 10° C. and 24° C. had their effect primarily on the host.

Sprouted kernels were observed by Hooker (20) to be more resistant in the cold test than non-sprouted kernels. The degree of resistance was directly related to the germination.

The above investigations indicate that resistance to soil pathogens causing a reduction in germination under adverse conditions is controlled by hereditary characters. Any factors which tend to slow down the germination process would also slow down the development of resistance and would therefore predispose the germinating corn kernel to attack by soil pathogens.

Differences in the cold test germination between strains could be attributed primarily to genetic differences. From the experiments reported above, differences between strains in cold test germination could be partially attributed to differences in seed permeability of the strains.

The increase in resistance to cold test germination of single crosses over inbreds might be attributed to hybrid vigor, i.e., resistance might develop faster in single crosses. The increase

in resistance of double crosses over single crosses could not be attributed to hybrid vigor, but might be due to differences in the maternal parents making up the crosses. Differences in cold test germination between reciprocal crosses could be due to a nutritional relationship between the embryo and endosperm. For example, in the maturing process the endosperm develops at the expense of the embryo, and therefore the protoplast of the mature seed is weakened. Upon germination under cold test conditions, resistance would develop more slowly in those seeds in which the protoplast was weakened.

Differences between ears within a strain have been attributed to residual heterozygosity by Hooker (20) and Wernham (57). Pinnell (42) attributed them to unknown environmental factors. As pointed out above, the association between seed permeability and cold test reaction of individual ears within a strain provides no critical evidence for either explanation. However, it could be that individual ears differ slightly in their physiological maturity when harvested, and subsequently some of the ears are of lower protoplast vitality than others. This could be due either to genetic factors controlling the physiological maturing process or to environmental factors.

The cold test reaction of seed of different maturities, of seed exposed to a preharvest frost, and of seed artificially dried, could be attributed to different protoplast vitalities and/or maternal tissue differentiation. The high negative correlation in the maturity study would seem to support this while the correlation in the preharvest frost study would not.

If pericarp damage (1) upsets the enzyme system of the germinating corn kernel, then germination might be impeded, and resistance would develop more slowly, increasing susceptibility.

Differences between age of seed in the reduction in germination due to cold testing could probably be attributed to the lower protoplast vitality of older seeds.

It appears that the cold test reaction consists of a complex of factors operating together. The effect of seed permeability is probably important; however it is only one of many variables that must be considered when determining the cold test response of various seed lots.

Summary

Experiments were conducted to determine if the differences in reduction in cold test germination between reciprocal crosses, between ears within a strain, between seed of different ages, between seed of different degrees of maturity and between normal and frosted seed are related to corn seed permeability as determined by the optical measurement of leached materials from steeped corn seed.

A summary of the correlation coefficients between the "cold indices" (the reduction in germination due to cold testing expressed as a percent of normal germination) and the spectrophotometer readings for all the relationships studied is given in Table 13.

A highly significant negative correlation (0.593) was shown between the reduction in germination due to cold testing and seed

Table 13. Summary of correlation coefficients between cold indices and spectrophotometer readings.

Experiment	Correlation coefficient
Reciprocal crosses.	-0.593 **
Individual ears of open pollinated single cross strains.	-0.627 **
Individual ears of a composite of open pollinated varieties.	-0.666 *
Age of storage.	
Three years	-0.432 ns
Four years	-0.533 ns
Seven years	-0.805 *
Combined	-0.797 **
Maturity.	-0.451 *
Normal versus frosted seed.	-0.038 ns

permeability of eleven double cross hybrids made reciprocally. This suggested that differences between strains and between the reciprocals of each strain in the cold test germination were associated with seed permeability.

Differences between strains and between ears of a strain of ten open pollinated single cross hybrids in the reduction in germination due to cold testing were associated (r= -0.627**) with differences in seed permeability. Differences between ears of a composite of open pollinated varieties in the cold test germination were associated (r= -0.666*) with seed permeability. Further experiments would be needed to determine if the differences between individual ears of long time inbreds in cold test germi-

nation are associated with seed permeability.

The relation between the reduction in germination due to cold testing and seed permeability of seed stored three, four and seven years could not be determined accurately since each seed age was composed of different strains, i.e., seed age was confounded with strains. However, the means of the cold test germinations and spectrophotometer readings for each seed age, plus a highly significant negative correlation of 0.797 suggested that the differences between seed of different ages in the reduction in germination due to cold testing might be associated with seed permeability.

There was a significant negative correlation of 0.451 between the cold test germination and seed permeability of four strains of corn harvested at seven day intervals for five weeks. Considering the cold test response and the spectrophotometer analysis of the amount of leached materials, the correlation was of the magnitude expected. Therefore, the significant negative correlation suggested that differences between immature and mature seed in reduction in germination due to cold testing were associated with seed permeability.

Individual ears of three corn strains exposed to various cold periods in order to simulate "frost" injury showed no association between the reduction in germination due to cold testing and seed permeability. The reduction in cold test germination of three ears was unusually low. It appeared that this influenced the correlation obtained.

Coefficients of variation of the cold tests and the spectro-

photometer readings indicated that the cold tests were the greatest source of error in the experiments. This could have been due to an uneven distribution of microorganisms throughout the soil-sand medium used for the cold tests.

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LITERATURE CITED

(1) Alberts, H. W.
Effect of pericarp injury on moisture absorption, fungus attack, and vitality of corn. Amer. Soc. of Agron. Jour. 19:1021-1030, 1927.

(2) Bailey, W. M.
Structural and metabolic after effects of soaking seeds of Phaseolus. Bot. Gaz. 94:688-713, 1933.

(3) Barton, A. W.
The effect of long continued soaking on seed germination. Amer. Jour. Bot. 14:632, 1927.

(4) Barton, L. V.
Relation of different gases to the soaking injury of seed. Contrib. Boyce Thompson Inst. 10:55-71, 1950.

(5) Barton, L. V.
Relation of different gases to the soaking injury of seeds. II. Contrib. Boyce Thompson Inst. 17: 7-34, 1952.

(6) Beeskow, H. C.
The selective permeability of the seed coat of corn. Unpub. M.S. thesis, Uni. of Chicago, 1924.

(7) Curme, J. H.
An evaluation of corn lines and seed condition in the cold test. Unpub. M.S. thesis, Kan. State College, 1951.

(8) Dickson, J. G.
Influence of soil temperature on the development of seedling blight of wheat and corn caused by Gibberella saubinetii. Jour. Agr. Res. 23:837-870, 1923.

(9) Dickson, J. G., K. P. Link, and A. D. Dickson
Nature of resistance of corn to seedling blight. Phytopath. 23:9 (abs.), 1933.

(10) Eyster, H. C.
The cause of decreased germination of bean seeds soaked in water. Amer. Jour. Bot. 27:652-659, 1940.

(11) Haltiwanger, W. H., C. C. Wernham, and L. L. Huber
The effect of temperature, incubation period, disease organisms, and cultural media on the germination of six corn hybrids. Report of the Eighth Northeastern Corn Conference. New York City. Jan. 1953.

(12) Haskell, G.
Effect of low temperatures on the germination of inbred lines of sweet corn. Science. 107:150, 1948.

(13) Haskell, G. and W. R. Singleton
Use of controlled low temperatures in evaluating the cold hardiness of inbred and hybrid maize. Agron. Jour. 41:34-40, 1949.

(14) Haskell, G.
Presoaking and cold hardiness in maize. Plant and Soil 1:342-345, 1949.

(15) Haskell, G.
Cold hardiness and seed shape in maize. Nature 163: 907, 1949.

(16) Hibbard, R. P.
The question of toxicity of distilled water. Amer. Jour. Bot. 2:389-401, 1951.

(17) Ho, Wen-chun
Soil inhabiting fungi attacking roots of maize. Iowa Agr. Expt. Sta. Res. Bul. 332, 1944.

(18) Holbert, J. R.
Cold resistance and susceptibility in corn. Phytopath. 20:117-118 (abs.), 1930.

(19) Holbert, J. R. and W. I. Burlison
Studies of cold resistance and susceptibility in corn. Phytopath. 19:105-106 (abs.), 1929.

(20) Hooker, A. L.
Variation in heritability and nature of resistance to a Pythium seedling disease of corn. Unpub. Ph.D. thesis, Uni. of Wisconsin, 1952.

(21) Hooker, A. L. and J. G. Dickson
Resistance to Pythium manifest by excised corn embryos at low temperatures. Agron. Jour. 44:443-447, 1952.

(22) Hoppe, P. E.
Inheritance of resistance to seedling blight of corn caused by Gibberella saubinetii. Phytopath. 19: 79-80 (abs.), 1929.

(23) Hoppe, P. E., J. R. Holbert, and J. G. Dickson
The relation of maturity of seed to seedling blight susceptibility in dent corn. Phytopath. 22:12 (abs.), 1932.

(24) Hoppe, P. E.
Differences in Pythium injury to corn seedlings at high and low soil temperature. Phytopath. 39:77-84, 1949.

(25) Hoppe, P. E. and J. T. Middleton
Pathogenicity and occurrence in Wisconsin soils of Pythium species which cause seedling disease in corn. Phytopath. 40:13 (abs.) 1950.

(26) Hoppe, P. E.
A new technique for incubating seed corn in cold soil for disease tests. Phytopath. 41:18 (abs.), 1951.

(27) Hoppe, P. E.
A glass tumbler-paper doll technique for seed corn incubation and germination tests. Phytopath. 41: 856-858, 1951.

(28) Hoppe, P. E.
A paper doll technique for disease tests of seed corn in cold soils, Phytopath. 42:11 (abs.), 1952.

(29) Hottes, C. F. and W. A. Huelson
The determination of quality in sweet corn seed by means of the optical measurement of leached materials. Jour. Agr. Res. 35:147-166, 1927.

(30) Huelson, W. A. and W. N. Brown
Physical damage to sweet corn seed caused by mechanical harvesting and subsequent processing. Ill. Agr. Expt. Sta. Bul. 561, 1952.

(31) Johann, H.
Histology of the caryopsis of yellow dent corn with reference to resistance and susceptibility of kernel rots. Jour. Agr. Res. 51:855-888, 1935.

(32) Johann, H.
Origin of suberized semipermeable membrane in the caryopsis of maize. Jour. Agr. Res. 64:275-282, 1942.

(33) Kiesselbach, T. A. and E. R. Walker
Structure of certain specialized tissues in the kernel of corn. Amer. Jour. Bot. 39:561-569, 1952.

(34) Kidd, E. and C. West
Physiological pre-determination: The influence of the physiological condition of the seed upon course of subsequent growth and upon yield. I. The effects of soaking seeds in water. Ann. Appl. Bot. 5:1-10, 1918.

(35) Koehler, B.
Pathological significance of seed coat injury in dent corn. Phytopath. 25:24 (abs.), 1935.

(36) Koehler, B.
Corn seedling blight in dry soil. Phytopath. 43:477 (abs.), 1953.

(37) Leach, L. D.
Growth rate of host and pathogen as factors determining the severity of preemergence damping off. Jour. Agr. Res. 75:161-179, 1947.

(38) Livingston, J. E.
Effect of controlled low temperature on the emergence of seedlings from artificially dried seed corn. Phytopath. 41:24 (abs.), 1951.

(39) Livingston, J. E.
Injury and drying of seed corn in relation to emergence. Phytopath. 42:221-222, 1952.

(40) Meyers, M. T.
The influence of broken pericarp on the germination and yield of corn. Amer. Soc. of Agron. Jour. 16:540-550, 1924.

(41) Orton, C. R.
The permeability of the seed coat of corn to mercury compounds. Phytopath. 17:51 (abs.), 1927.

(42) Pinnel, E. L.
Genetic and environmental factors affecting corn seed germination at low temperatures. Agron. Jour. 41:562-568. 1949.

(43) Pugh, G., H. Johann, and J. G. Dickson
Relations of the semipermeable membranes of the wheat kernel to infection by Gibberella saubinetii. Jour. Agr. Res. 45:609-626, 1932.

(44) Randolph, L. F.
Developmental morphology of the caryopsis in maize. Jour. Agr. Res. 53:881-916, 1936.

(45) Robinson, J. L.
Physiologic factors affecting germination of seed corn. Iowa Agr. Expt. Sta. Bul. 176, 1934.

(46) Rossman, E. C.
Freezing injury of inbred and hybrid maize seed. Agron. Jour. 41:574-583, 1949.

(47) Rush, G. E.
Factors affecting stands of corn at low temperatures. Unpub. Ph.D. thesis, Uni. of Wisconsin, 1950.

(48) Rush, G. E. and N. P. Neal
The effect of maturity and other factors on stands of corn at low temperatures. Agron. Jour. 43:112-116, 1951.

(49) Svien, T. A.
Factors affecting germination in the cold test. Unpub. Ph.D. thesis, Iowa State College, 1951.

(50) Tatum, L. A.
The effect of genetic constitution and processing methods on the ability of maize seed to germinate in cold soil. Unpub. Ph.D. thesis, Iowa State College, 1942.

(51) Tatum, L. A. and M. S. Zuber
Germination of maize under adverse conditions. Amer. Soc. of Agron. Jour. 35:48-59, 1943.

(52) Tatum, L. A.
Seed permeability and "cold test" reaction in Zea mays. Agron. Jour. 46:8-10, 1954.

(53) Tharp, W. H.
Developmental anatomy and relative permeability of barley seed coats. Bot. Gaz. 97:240-271, 1935.

(54) Tilford, P., C. F. Able, and R. P. Hibbard
An injurious factor affecting the seeds of Phaseolus vulgaris soaked in water. Papers Michigan Acad. Sci., Arts and Letters. 4:345-356, 1924.

(55) True, R. H.
The harmful action of distilled water. Amer. Jour. Bot. 1:255-273, 1914.

(56) Vander-Muelen, E. and F. Henke
"Cold testing" of seed corn. The Canner. 107:10-12, 1948.

(57) Wernham, C. C.
Cold testing of corn- a chronological and critical review. Penn. Agr. Expt. Sta. Progress Report 47, 1951.

(58) Wortman, L. S. and E. H. Rinke
Seed corn injury at various stages of processing and its effect upon cold test performance. Agron. Jour. 43: 299-305, 1951.

CORRELATION BETWEEN COLD TEST GERMINATION AND THE OPTICAL MEASUREMENT OF LEACHED MATERIALS FROM THE SEED OF ZEA MAYS

by

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Various factors known to affect germination of corn seeds in cold, wet, disease infested soils are of such a physiological and/or physical nature as to suggest that corn seed permeability might be involved in the reaction to germination under these adverse conditions. Corn seed permeability can be indirectly studied by determining the amount of soluble solids and colloidal material leached from steeped corn seed.

These studies have attempted to (1) develop a technique for the leaching of solid materials from the seed of Zea mays; and (2) determine if differences in reduction in germination between reciprocal crosses, ears within a strain, seed of different ages, seed of different maturities and normal and frosted seed in the cold test are related to corn seed permeability as determined by the optical measurement of leached materials from steeped corn seed.

In the development of a standard leaching technique four problems were studied: (1) microbial growth in the steep solution; (2) time and temperature of steeping; (3) proportion of corn seed to distilled water; and (4) replications. It was concluded that the leaching test would consist of the following: (1) disinfection of the steeping bottles and seed with a 25 percent clorox (active ingredients, sodium hypochlorite, 5.25 percent by weight) solution and a five percent clorox solution respectively; (2) steeping for 72 hours at 25° C.; (3) a ratio of ten grams of seed per 50 milliliters of distilled water; and (4) triplicate samples of each seed lot. After steeping the liquid should be passed through coarse filter paper to remove extraneous pieces of chaff, etc. The

amount of leached materials is then determined using a spectrophotometer at a wave length of 400 millimicrons. Distilled water is used as the reference.

The cold test germinations were obtained using the "paper doll" technique. A "cold index" was established in order that the reduction in germination due to cold testing would be on the basis of 100 percent normal germination.

Highly significant (one percent level) negative correlation coefficients were obtained between the cold indices and the spectrophotometer readings of (1) eleven double cross hybrids made reciprocally ($r = -0.593$); (2) individual ears of ten open pollinated single cross strains ($r = -0.627$); and (3) strains of corn stored three, four and seven years ($r = -0.797$). Significant (five percent level) negative correlation coefficients were obtained for (1) individual ears of a composite of open pollinated varieties ($r = -0.666$); and (2) strains of corn harvested at seven day intervals for five weeks ($r = -0.451$). A non-significant negative correlation coefficient was obtained for strains of corn artificially frosted ($r = -0.039$).

The greatest source of experimental error was in the cold test experiments. It was believed that this might be attributed to an uneven distribution of the soil pathogens in the soil-sand medium used in the cold tests.

The data suggested that differences between the cold indices of double cross hybrids and their reciprocals, individual ears of a strain, seed of different ages and seed of different maturities are associated with seed permeability. There was no apparent re-

lation between the two variables in seed that had been artificially frozen.