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COLD TOLERANCE STUDIES WITH HYBRID SEED CORN

Thesis for the Degree of Ph. D.

MICHIGAN STATE COLLEGE

Gerard Neptune

1953

THESIS

This is to certify that the

thesis entitled

Cold Tolerance Studies with Hybrid

Seed Corn

presented by

Gerard Wertune

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Farm Crops

E. E. Rosaman
Major professor

Date December 17, 1923

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COLD TOLERANCE STUDIES WITH HYBRID SEED CORN

By

Gerard Neptune

A THESIS

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Farm Crops

1953

ACKNOWLEDGMENTS

The writer wishes to express his sincere thanks to Dr. E. C. Rossman for his guidance and appreciable help throughout the experiments and preparation of this thesis.

He also deeply appreciates the Alumni fellowship and the scholarship provided by Michigan State College which made it possible for him to complete this investigation.

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INTRODUCTION

A good stand of corn is essential for successful production since grain yields are markedly affected by the number of plants per acre. Much emphasis is currently being placed on the production of maximum yields by adjusting planting rates to soil fertility.

Cold wet weather frequently follows corn planting, particularly in northern areas, kernels and seedlings become susceptible to attack by various soil pathogens, and stands are reduced. Injury varies from pre-emergence killing to blighting and root-rotting of the seedlings.

Corn hybrids and the seed for these hybrids adapted to northern areas should produce satisfactory stands under adverse conditions, and retain high yielding potentialities.

The ability of hybrid seed corn to produce good stands under cold wet conditions has been shown to be affected by genetic constitution of the seed, maturity of the seed when harvested, frost injury to the seed before harvest, care during processing, amount and type of seed coat injury, seed treatment, and age of the seed when planted.

Corn seed may be subjected to a cold test germination prior to planting in order to ascertain its probable germination and stand when subjected to field conditions. Such tests are run in flats in the greenhouse. Soil from a corn field is placed in flats, the seed planted and the soil thoroughly soaked before placing in a cold chamber at 45-50° F for 8 to 12 days.

Since little or no information is available on several phases of cold test germination, this study was designed to study:

(a) the effects of temperature and length of exposure on seed in various stages of germination;

(b) the effect of previous crop and soil type on cold test germination, and

(c) the effectiveness of early generation testing in selecting cold tolerant inbred lines of corn.

REVIEW OF LITERATURE

Several species of microorganisms are responsible for the diseases that affect seed corn at low soil temperatures. Dickson (3) and Valleau (26) showed that Gibberella saubinetii may cause seedling blight. Johann, Holbert and Dickson (12) reported that Pythium arrhenomanes caused rot of the embryo prior to emergence, seedling blight after emergence, and root rot that reduced size, vigor, and yield of the maturing corn plant.

Hoppe (9) pointed out that Pythium injury varies from severe stunting of the seedlings to complete pre-emergence killing. Of 138 Pythium isolates made from decayed corn kernels, Hoppe and Middleton (11) found that 58, falling into six species, were capable of causing pre-emergence killing of corn at low temperatures.

Ho (7) studied the soil pathogens which attack the roots of maize and found that Pythium debaryanum and Pythium graminicola, together with Gibberella saubinetii, were the most destructive, when the soil temperature was below 16° C and moisture was abundant.

Diplodia zeae has been reported as a seedling blight pathogen under adverse conditions (21). Ho (7) classified the organism as only moderately destructive and according to Raleigh (21), Diplodia injury is more serious at high (20°-24°C) than at low (15°-19°C) temperatures.

Various species of Fusarium, notably F. moniliforme and F. Succisee, have been isolated from rotting corn roots and decayed kernels. They caused little injury and appeared to be only secondary organisms (7).

Five species of Helminthosporium i.e., H. maydis, H. bicolor, H. carbonum, H. sativum, and H. turcicum, have been reported as seedling blight pathogens (2, 22). In general, these pathogens caused blight at 20°-28°C, though one of them, H. carbonum race II was pathogenic at temperatures as low as 10° C.

Several other organisms i.e., Penicillium oxalicum, Trichoderma lignorum, Rhizoctonia solani, Rhizopus spp., and Aspergillus niger, have been mentioned in relation to cold germination injury (7), but their pathogenicity has not been demonstrated.

According to Dickson (3), soil temperature is the most important factor which determines the extent of seedling blight. Dickson pointed out that while temperatures of 12° - 18° C favor the blighting of wheat, maize seedlings are more subject to attack between 8° and 20° C. He attributed the difference in reaction between wheat and corn to the changes produced in their metabolism by the differences in temperatures. The influence of temperature upon seedling blight development appeared to be primarily a host response. Dickson and Holbert (4) concluded that the differences in resistance among inbred lines of corn were due to different influences of temperature upon the metabolism of the young seedlings.

Additional evidence that some relationship might exist between the metabolism of the corn seedling and the development of blight at low temperatures is found in the work of Smith (24). He planted two contrasting inbreds: RYD_4 and GG_{26} at high and at low temperatures. At high temperature (24° C) both inbred were normally green. But at low temperatures (16° - 19° C) the seedlings of RYD_4 were of a normal green

color, while those of GG_{26} were almost devoid of chlorophyll. At both temperatures endosperm utilization was more rapid in inbred GG_{26} than in RYD_4. Seedlings of GG_{26} were very susceptible to Gibberella blight whereas those of RYD_4 were highly resistant.

Johann, Holbert and Dickson (12) found that soil temperatures, 16° C or lower, together with high soil moistures were so favorable for disease infection that either germination of corn kernels was prevented or seedling blight developed. Data presented by Ho (7), Hoppe (9), and Livingston (16) support the findings of Johann et al.

Flor (5) reported that Pythium injury to germination of corn decreased with rise in temperature. At 35° C, Pythium did not injure corn. At 30° C, there was an appreciable amount of injury and as the temperature was lowered to 11° C, injury became more severe. He also found that injury increased with the moisture content of the soil, but was less severe in warm wet soils than in cold wet soils.

Haskell (6) planted five inbred lines of sweet corn in a soil known to contain pathogenic organisms and held the flats for various intervals up to 32 days at 40° F (4° C) and 50° F (10° C). Germination was reduced as the duration of exposure to cold increased. The treatments at 40° F were not as injurious as the corresponding treatments at 50° F. Eight days of exposure to 50° F were necessary to reduce germination by 50 percent. Haskell concluded that cold injury to the seed was in part responsible for the reduction in germination at 40° F, since the activity of the pathogens was partially inhibited. He indicated that the 40° F temperature gave a truer picture of cold hardiness of the inbreds

whereas the 50° F temperature was more critical and constituted a better test for disease resistance.

Hoppe (10) also found that disease level is higher at 11° C than at 4° C. His results indicated that low temperature in itself was not injurious but served merely to predispose unprotected, slowly germinating kernels to attack by soil fungi.

Pericarp injury has been found to affect germination and yield of corn. In general, germination decreases as the amount of injury increases. Under field conditions, Meyers (18) obtained a significant reduction in both stand and vigor of seedlings when broken seeds were planted. The difference in vigor became less evident as the season progressed and finally disappeared. There was no reduction in yield, except in the plots planted with broken seed inoculated with spores of Penicillium spp. In these plots stand was reduced from a possible 837 to 396 plants.

Alberts (1) pointed out that when the pericarp was injured, various soil pathogens readily penetrated the kernels and caused a rapid decomposition of the endosperm. Seedlings developing from such kernels grew slowly and their mesocotyl was subject to attack by soil pathogens.

Koehler (13) studied the pathologic significance of seed coat injury in dent corn. He found that a slight puncture in the seed coat at the crown caused a 12 to 16 percent loss in yield of grain, while removal of the seed coat from the whole crown resulted in a loss of 18 to 23 percent. A cut through the seed coat on the side of the kernel caused no injury to stand or yield. Injury from Giberella and other

soil pathogens was most pronounced in plots planted with seed injured at the crown.

Tatum and Zuber (25) presented data indicating that a close relationship exists between inconspicuous pericarp injury over the germ and stand which in turn affects yield of corn in the field.

Mechanical processing of the seed has been found to cause injury to the pericarp. Koehler and Dungan (14) compared bin-dried and hanger-dried seed corn. There was more seed coat injury in the seed that had been bin-dried and machine processed than in the grain that had been hanger-dried and shelled by hand. In field tests, hanger-dried hybrid seed averaged 3.2 bu. better in yield over a three year period. This difference was statistically significant. Similar results on mechanical injury were reported by Wortmann and Rinke (28) who found also that hybrids differ in their susceptibility to mechanical injury.

Tatum and Zuber (25) reported that seed samples which were hand shelled and not subjected to any mechanical treatment were free from injury and germinated approximately the same in a cold test as in a normal test. The amount of pericarp injury in commercial seed depended upon processing methods and the care during processing. Pericarp injury was reflected in lower cold test germinations. Neptune and Rossman (19) found that cold test germination of the same hybrid varied depending on the seed producer.

Age of the seed was found to be a factor in cold test germination (19). New seed consistently gave higher cold test stands than old seed

though all seed lots were capable of satisfactory germination in the standard warm tests.

Seed treatment has been reported as capable of reducing cold germination injury. Rush and Neal (23) obtained better stands with Arasan treated seed than with non-treated seed, but seed treatment did not give complete protection in cold tests. Normal emergence was obtained by Livingston (16) under cold test conditions when the seed was treated with Arasan, irrespective of the drying method or moisture content of the seed at harvest time.

Wortman and Rinke (28) found reductions in stand from samples treated with Arasan in a slurry treater when compared with seed treated by hand with dust fungicide. Tatum and Zuber (25) presented data indicating that dust treatment was not an adequate means of preventing poor stands in the field. The use of seed with an intact pericarp was more effective.

Maturity of the seed and frost injury prior to harvest have been reported as factors affecting cold germination of seed corn. Data presented by Johann, Holbert and Dickson (12) showed that different degrees of maturity of the seed influenced the reaction to Giberelle seedling blight and the stand and yield of the crop. Resistance to Giberella increased with the maturity of the seed.

Koehler, Dungan and Burlison (15) harvested seed corn at different stages of maturity. They found that field stand varied directly with the vigor of the seed and the acre yield varied directly with the stand; the more mature stages produced the best stands and yields.

Rush and Neal (23) obtained large differences in germination between seed harvested at 10-day intervals when planted in soil at low temperatures (10° C). In general, stands improved with maturity of the seed. Seed harvested after a light frost gave significantly lower cold germination than unfrosted seed while both types were equal in warm tests.

Genetic differences in cold test performance have been reported. Dickson and Holbert (4) pointed out that resistance of inbred lines to Giberella seedling blight was an inhertiable characteristic. The expression of resistance was constant over a given temperature range. First generation hybrids between resistant lines and susceptible lines at temperatures of 12° to 32° C were susceptible at all temperatures, indicating that susceptibility was dominant.

Hoppe (8) observed a wide range of relative resistance to Giberella among long-time inbred lines at 16° C. The behavior of these lines was very consistent, suggesting a high degree of homozygosity for the genetic factors involved in resistance. He found that first generation hybrids between resistant and susceptible lines were as resistant as the resistant parent while crosses between susceptible lines were as susceptible as either parent. Analysis of F_3 generations gave evidence of transgressive segregation since families were isolated which were more resistant or more susceptible than the parent strains. One F_3 family, practically immune, maintained its resistance in the F_4 while results in other families indicated that the F_3 families were very heterozygous.

McIndoe (17) studied the inheritance of reaction to Giberella in 27 selfed lines, all the possible crosses between these lines, and some F_3 generations of the F_1 hybrids. Crosses of susceptible and resistant lines gave conflicting results in the F_1 generation. Resistance was neither dominant nor recessive to susceptibility. The results were best explained when inheritance of disease reaction was assumed to be quantitative in nature. Further evidence of a quantitative inheritance was obtained from the behavior of the F_3 progeny. A definite segregation of the F_3 lines occurred in conformity with a Mendelian explanation of such inheritance. Where the parental lines differed widely in reaction, highly resistant and susceptible lines appeared in the F_3 generation, in sufficient proportion to suggest that, perhaps relatively few factors were involved for resistance.

Pinnel (20) found that, at low temperatures, double crosses germinated best, followed by single crosses and inbreds in that order. The higher stands for double crosses and single crosses over inbreds were best explained on the basis of complementary gene action. Among the inbreds, he obtained wide differences which appeared to be heritable in crosses. Differences among hybrids were largely determined by the maternal parent, in both single and double crosses. There was no relation between the performance of an inbred as a female in crosses and its performance as a male. A very high ear to ear variation was obtained in some long-time inbred lines.

PART I

Influence of Temperature, Stage of Germination, and Length of Exposure on Cold Test Germination

MATERIALS AND METHODS

Five double-cross hybrids were used: Michigan 480, Michigan 350, Michigan 250, Nosco N_4 and Gries 201A. In previous cold tests conducted during 1950 and 1951 at 45° F, these hybrids germinated on the average: 92.3, 92.0, 89.6, 58.3 and 46.0 percent respectively. On the basis of these results, the three Michigan hybrids were rated as tolerant and the other two as susceptible to injury during germination under adverse conditions.

All seed, as it was obtained from commercial companies, germinated 95 percent or better in standard germination tests conducted under the ideal conditions of the laboratory. All seeds were treated with a seed protectant. The seed was planted in flats filled with a Hillsdale sandy loam obtained from a corn field. The soil was watered to saturation and the flats subjected to various treatments.

There were five different pre-cold treatments and four different exposures to cold. Pre-cold treatments consisted of keeping the flats in a 75° - 80° F greenhouse for 0, 1, 2, 3, or 5 days. At the end of the five-day treatment, the seedlings had emerged and reached a maximum height of 1/2 in.

After pre-cold treatment, the flats were moved to a walk-in cold chamber for 4, 8, 12, or 16 days. They were then placed in a warm greenhouse for 12 days and the strong seedlings were counted.

The design of the experiment was a double split-plot in which the five pre-cold treatments constituted the main plots; the four lengths of exposure to cold were the sub-plots, and the five hybrids constituted the sub-sub$_6$plots. Two replications of 25 seeds each were used. The entire procedure was duplicated at 32° and 40° F.

The temperature in the 32° chamber varied from 30° to 34° F, and twice went up to 38° F for about four hours. This was due to the accumulation of ice on the pipes of the cooling system. The temperature variation in the 40° chamber was only plus or minus one degree.

EXPERIMENTAL RESULTS AND DISCUSSION

Tables I and II present the average cold test germination of five corn hybrids following various periods of exposure to 32° and 40° F at different stages of germination. Analyses of variance for the data are given in Tables III and IV. Since the five day pre-cold treatment coupled with the 32° cold treatment resulted in an almost complete killing it was not included in the analysis of variance.

At both temperatures, there were differences among the pre-cold treatments in their effect upon germination. Exposing the seed to cold immediately or one day after planting resulted in nearly the same decreases in stand. Germination was less as the time interval preceding exposure to cold increased beyond one day. Apparently in the more advanced stages of germination the seed became more susceptible to attack by the soil pathogens responsible for cold germination injury. Three and five days of favorable conditions followed by 8, 12, or 16 days of cold treatment seriously reduced the stand of all hybrids.

Figures 1 and 2 illustrate the effect of the cold treatments at 32° and 40° on germination. In general, increase in duration of exposure to cold resulted in lower survival. The interactions, temperature x pre-cold treatment, temperature x cold treatment, and temperature x pre-cold treatment x cold treatment, were significant (Table IV) indicating that the two temperatures failed to affect germination in the same manner in the various treatments. Pre-cold treatments of 0 and one day

TABLE I

GERMINATION PERCENTAGES FOR HYBRID SEED CORN WITH EITHER 0, 1, 2, 3, OR 5 DAYS OF PRE-COLD TREATMENT FOLLOWED BY EITHER 4, 8, 12, OR 16 DAYS OF COLD TREATMENT AT 32° F

Days of Pre-cold Treatment	Days of Cold Treatment	Nosco N_4	Gries 201A	Mich. 250	Mich. 350	Mich. 480	Average
0	4	66	86	94	94	92	86.4
	8	42	68	88	96	96	78.0
	12	44	70	86	82	98	76.0
	16	36	38	76	78	94	66.0
	Average	46.8	65.6	86.0	87.2	94.8	76.0
1	4	82	78	96	92	100	89.6
	8	62	54	94	96	96	80.4
	12	42	20	68	78	86	58.8
	16	42	32	72	70	84	60.0
	Average	56.8	46.0	82.4	84.0	91.2	72.0
2	4	78	68	90	84	90	82.0
	8	58	58	70	92	84	72.4
	12	40	24	20	48	52	36.8
	16	10	6	10	26	20	14.4
	Average	46.4	38.8	47.2	62.4	61.4	51.2
3	4	64	44	84	88	94	74.8
	8	4	4	4	12	12	7.2
	12	0	0	2	8	6	3.2
	16	2	2	6	10	6	5.2
	Average	17.6	12.4	24.0	29.6	29.6	22.4
5	4	24	20	40	44	52	36.0
	8	0	0	0	0	0	0
	12	0	0	0	0	0	0
	16	0	0	0	0	0	0
	Average						
	Grand Average	42.0	40.8	60.0	65.6	69.2	55.6

TABLE II

GERMINATION PERCENTAGES FOR HYBRID SEED CORN WITH EITHER 0, 1, 2, 3, OR 5 DAYS OF PRE-COLD TREATMENT FOLLOWED BY EITHER 4, 8, 12, OR 16 DAYS OF COLD TREATMENT AT 40° F.

Days of Pre-cold Treatment	Days of Cold Treatment	Hybrid					Average
		Nosco N_4	Gries 201A	Mich. 250	Mich. 350	Mich. 480	
0	4	50	44	88	86	90	71.6
	8	20	24	80	86	92	60.4
	12	42	32	72	80	94	64.0
	16	18	10	72	72	90	52.4
	Average	32.4	27.2	78.0	80.8	91.2	62.0
1	4	66	86	96	88	94	86.0
	8	38	44	84	86	88	68.0
	12	32	14	76	84	94	60.8
	16	8	16	36	72	80	42.4
	Average	36.0	40.0	72.8	83.2	88.8	64.4
2	4	30	50	94	94	98	73.2
	8	14	24	86	86	94	60.8
	12	16	10	60	78	86	50.0
	16	20	6	38	52	66	36.4
	Average	20.0	22.4	69.6	77.4	86.0	55.2
3	4	48	60	96	86	92	76.4
	8	14	22	82	80	80	59.2
	12	16	18	36	38	64	34.4
	16	6	6	36	38	36	24.4
	Average	20.8	26.4	62.4	60.4	72.4	48.4
5	4	58	58	92	84	100	78.4
	8	38	30	60	54	88	54.0
	12	2	2	26	30	32	18.4
	16	2	0	22	28	38	18.0
	Average	24.8	28.0	50.0	48.8	64.4	42.0
	Grand Average	26.8	28.0	66.4	70.4	80.8	54.4

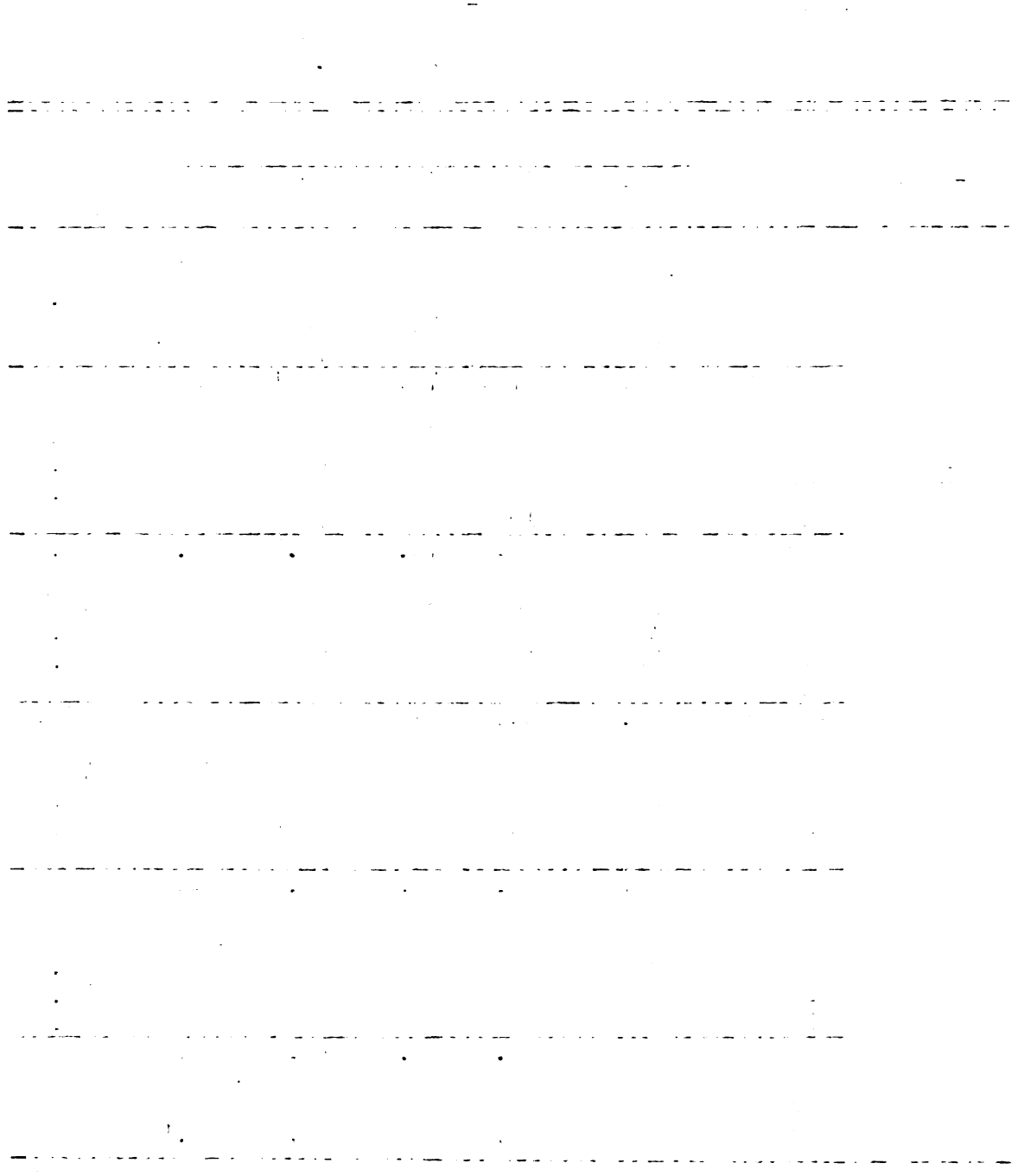


TABLE III

ANALYSES OF VARIANCE FOR COLD TEST GERMINATION AT 32° F. AND 40° F.

Source	32° F.		40° F.	
	Degrees of Freedom	Mean Square	Degrees of Freedom	Mean Square
Total	159		199	
Replications	1	57.6	1	5.5
Pre-cold	3	1,505.4**	4	212.6**
Error (a)	3	49.2	4	10.4
Cold	3	1,085.6**	3	1,061.8**
Pre-cold x Cold	9	162.1**	12	56.2**
Error (b)	12	8.2	15	14.0
Hybrid	4	360.0**	4	1,598.2**
Pre-cold x Hyb.	12	35.2**	16	19.1**
Cold x Hyb.	12	4.2	12	24.0**
P-c x C x Hyb.	36	10.1*	48	11.1
Error (c)	64	4.1	80	8.0

* Significant at the 5 percent level of probability.
** Significant at the 1 percent level of probability.

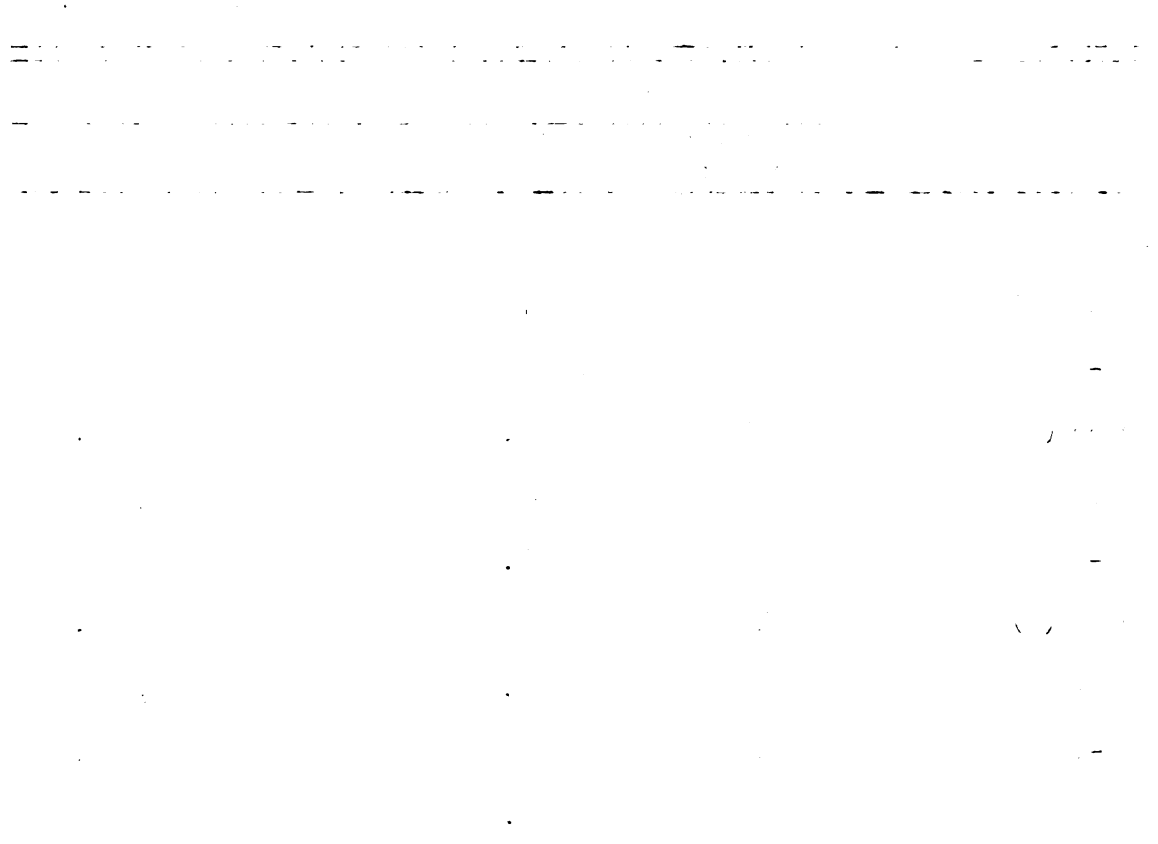
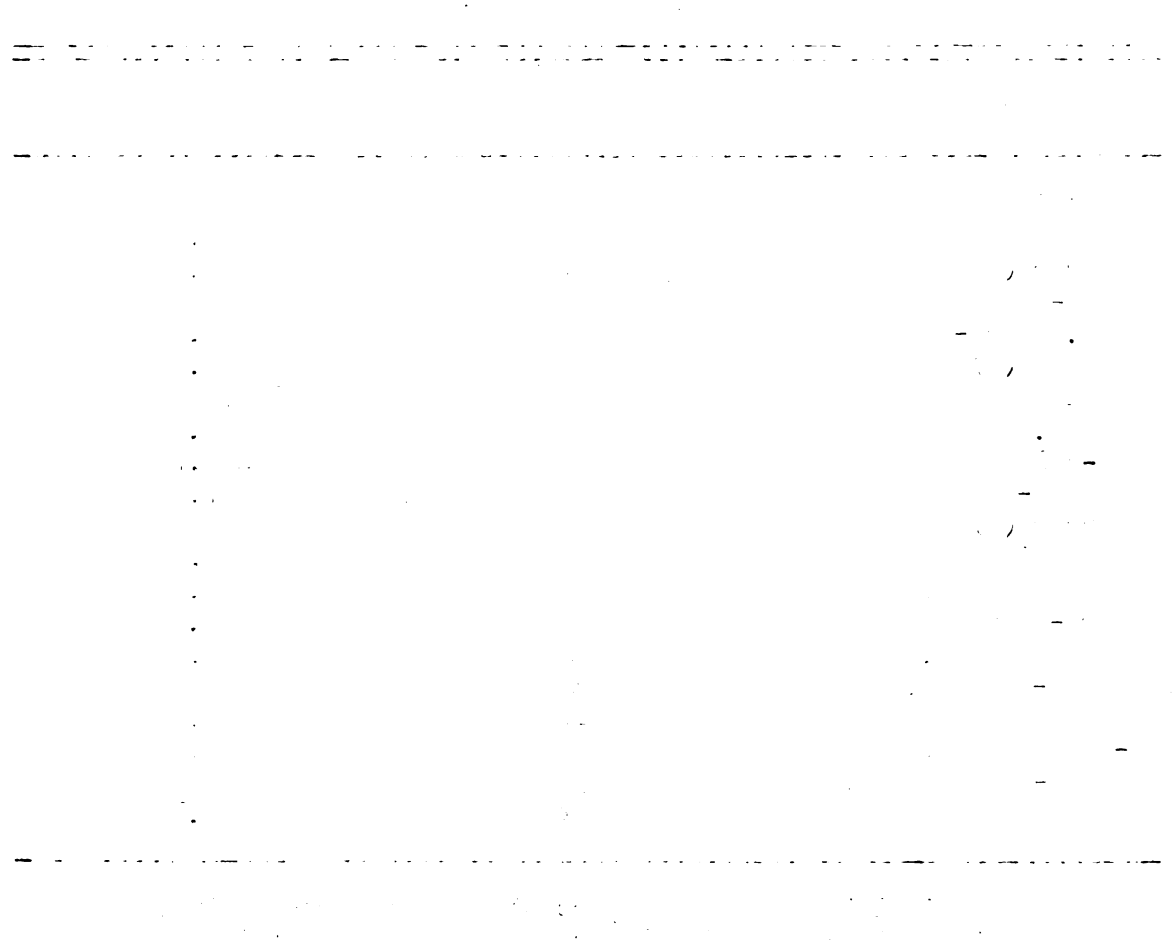


TABLE IV

COMBINED ANALYSIS OF VARIANCE FOR COLD TEST GERMINATION AT 32° AND 40° F.

Source	Degrees of Freedom	Mean Square
Total	319	
Temperature	1	18.5
Error (a)	2	31.1**
Pre-cold	3	1,242.4**
Temp. x Pre-cold	3	390.1**
Error (b)	6	31.5
Cold	3	1,676.4**
Temp. x Cold	3	48.0*
Pre-cold x Cold	9	129.7**
T x P-C x C	9	67.7**
Error (c)	24	12.2
Hybrids	4	1,645.3**
Temp. x Hyb.	4	194.4*
Pre-cold x Hyb.	12	20.0
Cold x Hyb.	12	24.2
T x P-C x Hyb.	12	26.3
T x C x Hyb.	12	9.5
P-C x C x Hyb.	36	15.5
T x P-C x C x Hyb.	36	6.5
Error	128	60.1

* Significant at the 5 percent level of probability.
** Significant at the 1 percent level of probability.



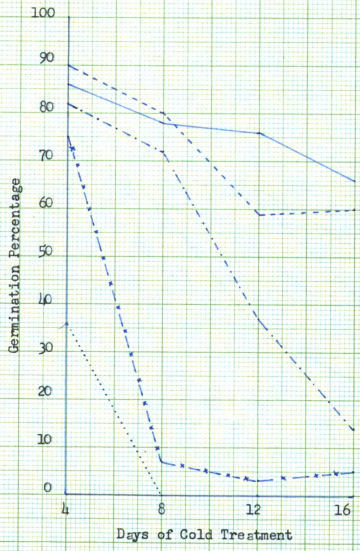


Fig. 1 - Effect of cold treatment at 32° F on germination of hybrid seed corn.

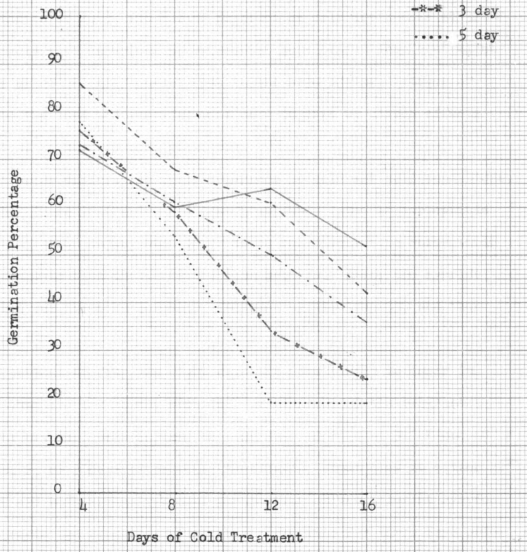


Fig. 2 - Effect of cold treatment at 40° F on germination of hybrid seed corn.

followed by cold treatments at 32° F were less injurious than similar pre-cold treatments followed by 40° F cold treatments. It is likely that the soil pathogens were more active at 40° F than at 32° F. Pre-cold treatments of 2, 3, and 5 days followed by cold treatments at 32° F were generally more injurious to subsequent germination than equivalent treatments at 40° F. It appears that in the more advanced stages of germination the seed and seedling were more injured by direct effects of cold at 32° F than by soil pathogens.

The hybrids differed in their tolerance to cold wet soil conditions. Nosco N_4 and Gries 201A which had been previously rated as susceptible to cold germination injury were found to be relatively more susceptible in all treatments than the three Michigan hybrids. Among the hybrids, Michigan 480 was the most tolerant, followed by Michigan 350 and then by Michigan 250.

Cold periods of four and eight days are more likely to occur in the field than 12- and 16-day periods. Germination of the tolerant hybrids was not seriously injured by four days of cold at 32° F and 40° F after 0, 1, 2, or 3 days of pre-cold treatment. Eight days at either 32° F or 40° F did not seriously reduce germination of the tolerant hybrids until the seeds had been under favorable conditions for three days prior to cold treatment. Germination of the susceptible hybrids was reduced by all treatments at all stages of germination. The relative ranking of the hybrids was generally consistent with all treatments. In most seed testing and corn breeding programs, cold tests are conducted with eight

to twelve days of cold treatment immediately after planting or one or two days after planting. On the basis of these results, this appears to be a satisfactory treatment for cold test evaluation.

PART II

Influence of Previous Crops and Soil Type Upon Cold Test Germination

MATERIALS AND METHODS

Michigan 480, Michigan 350, Michigan 250, Gries 201A, and Nosco N_4 were used. All seed was capable of 95 percent or better germination in standard tests. All seed was treated with a seed fungicide. Two types of soil: A Brookston silt-loam and a Hillsdale sandy loam, were used. Samples of the Brookston soil were taken in May from seven different plots which had been planted to wheat, corn, barley, beans, alfalfa, sweet clover, and sugar beets, the previous year. The Hillsdale soil samples were taken in early July from three plots on which corn, wheat, and sweet clover respectively had been grown the previous year.

Seed of each hybrid was planted in flats containing the various soils. The soil was watered to saturation and the flats transferred to a walk-in cold chamber for 12 days and maintained at 40° F. Germination was completed in a warm greenhouse and strong seedlings were counted after 21 days.

Experiment I - Three replications of 25 seeds each were planted in the Brookston soil samples. The experimental design was a split-plot in which the previous crops grown on the soil constituted the main plots and the five hybrids made the sub-plots.

Experiment II - In early July, the Brookston samples used for the previous experiment were mixed with the remainder of the original soil dug in May. Both samples had been kept in the greenhouse and were completely dry.

Half of the dry Brookston soil and half of the freshly dug Hillsdale soil was sterilized in the following manner. Two flats were filled with soil from each sample and placed cross-wise in a steam sterilizer for seven hours and thirty minutes.

After sterilization, the flats were removed to a greenhouse for 24 hours. Two replications of 25 seeds each were then planted in both the sterilized and the nonsterilized soils. For each soil type, the design was a double split-plot where the two treatments - sterilization and no sterilization - constituted the main-plots; the previous crops made up the sub-plots, and the five hybrids constituted the sub-sub-plots.

EXPERIMENTAL RESULTS AND DISCUSSION

Influence of Previous Crops

Table V contains the cold test germinations for five double-cross hybrids in samples of a Brookston silt-loam on which different crops had been grown previously. Analysis of variance is given in Table VI.

There were significant differences in the effects derived from the previous crops upon germination. The average stand on the soil which had previously grown sweet clover was 36.4 percent as compared to 66.4 percent on the soil which had grown wheat. The results for the other crops were intermediate between these two extremes. The legumes tended to be more detrimental than the cereals, corn excepted. The interaction, previous crop x hybrid, was not significant, indicating that the previous crops affected the germination of the five hybrids in the same manner.

Reduction in germination of seed corn under cold wet soil conditions has been shown to be caused primarily by soil pathogens. Among these pathogens, Pythium spp. appear to be the most common and the most destructive (11). Pythium can also cause a root rot of the corn plant in the field (12). The same group of organisms has been found to be pathogenic to several other crops under greenhouse conditions (22). Reaction to Pythium varied from normal growth of the plant in oats, rye and soybeans, to chlorosis of the leaf tips in wheat, severe

TABLE V

GERMINATION PERCENTAGES FOR HYBRID SEED CORN WHEN TESTED AT 40° F IN A BROCKSTON SILT-LOAM ON WHICH DIFFERENT CROPS HAD PREVIOUSLY GROWN

Previous Crop	Gries 201A	Nosco N_4	Mich. 250	Mich. 350	Mich. 480	Average
Wheat	28.0	48.0	81.2	84.0	90.4	66.4
Barley	37.2	32.0	54.4	76.0	76.0	57.6
Beans	14.4	37.2	50.4	77.2	92.0	54.4
Alfalfa	20.0	36.0	40.0	54.4	84.0	46.8
Sugar beets	13.2	25.2	45.2	58.4	78.4	44.4
Corn	12.0	20.0	42.4	50.4	74.4	40.0
Sweet clover	10.4	25.2	29.2	52.0	65.2	36.4
Average	19.2	32.0	51.2	64.8	80.0	49.6

TABLE VI

ANALYSIS OF VARIANCE FOR COLD TEST GERMINATION IN A BROOKSTON SILT-LOAM

Source	Degrees of Freedom	Mean Square
Total	104	
Replications	2	5.5
Previous crops	6	105.0*
Error (a)	12	25.2
Hybrids	4	783.5**
Crop x Hybrid	24	10.5
Error	56	6.9

* Significant at the 5 percent level of probability.
** Significant at the 1 percent level of probability.

damping off in alfalfa, sugar beets and timothy, and stunting of growth in corn.

The results obtained in this experiment suggest that parasitism may have been involved in the detrimental effects derived from the various crops tested. The growth of sweet clover, corn, sugar beets and alfalfa may have favored the multiplication of the pathogens responsible for cold injury, while the growth of wheat and barley may have altered the balance of microorganisms in the soil and reduced the quantity of microorganisms pathogenic to seed corn. The previous crops may also have caused some change in the soil, favorable or unfavorable to the development of the pathogens.

The differences in the effects from the previous crops do not warrant any generalization of their significance under field conditions. It is probable that the influence of previous crops upon cold germination will vary with the type of soil and its composition, as well as the climatic conditions, since these factors influence greatly the nature and abundance of the soil micro-biological population.

Large differences were obtained among hybrids. The Michigan hybrids were more tolerant than Nosco N_4 and Gries 201A. This is in agreement with the previous rating of the hybrids.

Influence of Soil Type

Table VII presents the average cold test germinations for the seed of five corn hybrids in sterilized and nonsterilized samples of a Brookston

silt-loam and a Hillsdale sandy loam, on which three different crops had been previously grown. Analyses of variance are given in Tables VIII and IX.

Considering both sterilized and unsterilized soils, soil type did not have any influence on cold germination. On the average, 86.2 percent of the seed planted in the Hillsdale soil produced strong seedlings compared to 82.0 percent in the Brookston soil. The difference, 4.2 percent, was not significant (Table IX).

Perfect stands were not obtained in the sterilized soils, suggesting that either sterilization of the soils was not complete or the cold temperature was in itself injurious to germination. Haskell (6) has presented evidence of cold injury to seed corn at 40° F.

The interaction soil x treatment was highly significant, indicating that the two treatments (sterilization and no sterilization) failed to affect germination in the same manner in the two types of soil. Soil sterilization improved germination in the Brookston soil, but had no beneficial effect in the Hillsdale soil (Table VII). Hoppe and Middleton (11) pointed out that Pythium spp. which are among the most common pathogens responsible for cold germination injury, were associated with soil type. Possibly, the Hillsdale soil contained fewer pathogens capable of causing injury to seed corn at low temperatures. It is also possible that the drought period which preceded the collection of the soil in July had adversely influenced the pathogens in the Hillsdale soil, since climatic conditions can affect the nature and abundance of the soil microbial population.

TABLE VII

GERMINATION PERCENTAGES FOR HYBRID SEED CORN IN A BROOKSTON SILT LOAM AND A HILLSDALE SANDY LOAN AT 40° F

Soil Type	Hybrid	Treatment: Sterilization, Previous Crop: Wheat	Corn	Sweet Clover	Average	Treatment: No Sterilization, Previous Crop: Wheat	Corn	Sweet Clover	Average
Brookston	Gries 201A	64	82	78	74.4	68	30	66	54.4
	Nosco N_4	66	84	90	80.0	62	50	46	52.8
	Mich. 250	86	92	90	89.2	94	92	94	93.2
	Mich. 350	90	92	90	90.4	86	96	88	90.0
	Mich. 480	98	98	96	97.2	98	98	96	97.2
	Average	80.8	89.6	88.8	86.4	81.6	73.2	78.0	77.6
Hillsdale	Gries 201A	74	66	84	74.4	84	66	78	76.0
	Nosco N_4	80	86	86	84.0	68	56	72	65.2
	Mich. 250	98	96	86	93.2	94	96	90	93.2
	Mich. 350	94	90	88	90.4	94	96	86	92.0
	Mich. 480	96	98	88	94.0	100	100	98	99.2
	Average	88.4	87.2	86.4	87.2	88.0	82.8	84.8	85.2

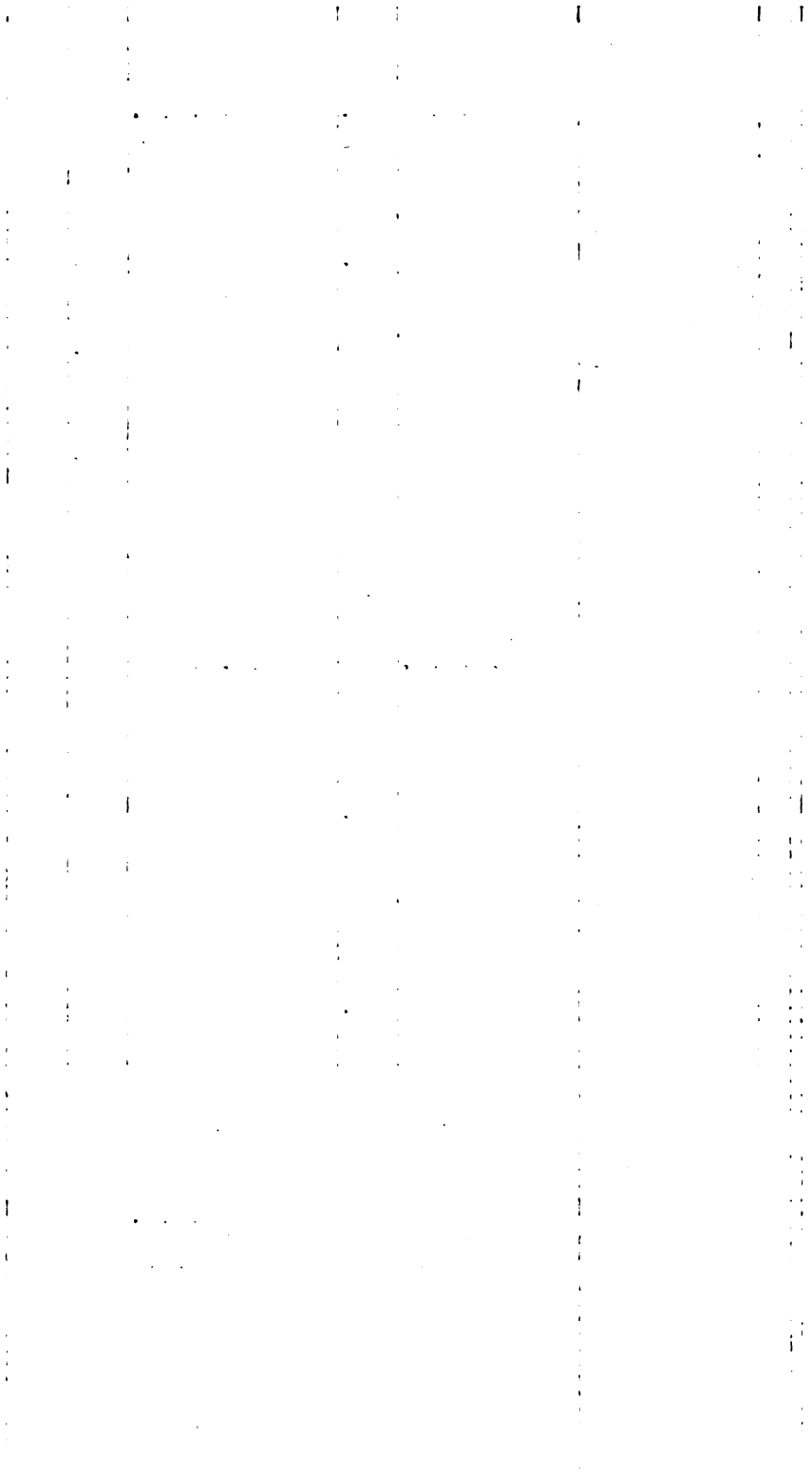


TABLE VIII

ANALYSIS OF VARIANCE FOR COLD TEST GERMINATION IN A BROOKSTON SOIL AND A HILLSDALE SOIL

	Degrees of Freedom	Brookston Mean Square	Hillsdale Mean Square
Total	59		
Replications	1	26.6	3.3
Treatment	1	72.6*	4.3
Error (a)	1	0.3	4.2
Crop	2	1.8	3.6
Treat. x Crop	2	24.0*	1.3
Error (b)	4	3.3	4.6
Hybrids	4	176.3**	82.1**
Treat. x Hyb.	4	35.8**	16.8**
Crop x Crop	8	4.4**	7.1**
Treat. x Crop x Hyb.	8	14.5**	3.0*
Error (c)	24	3.6	2.2

*Significant at the 5 percent level of probability.
**Significant at the 1 percent level of probability.

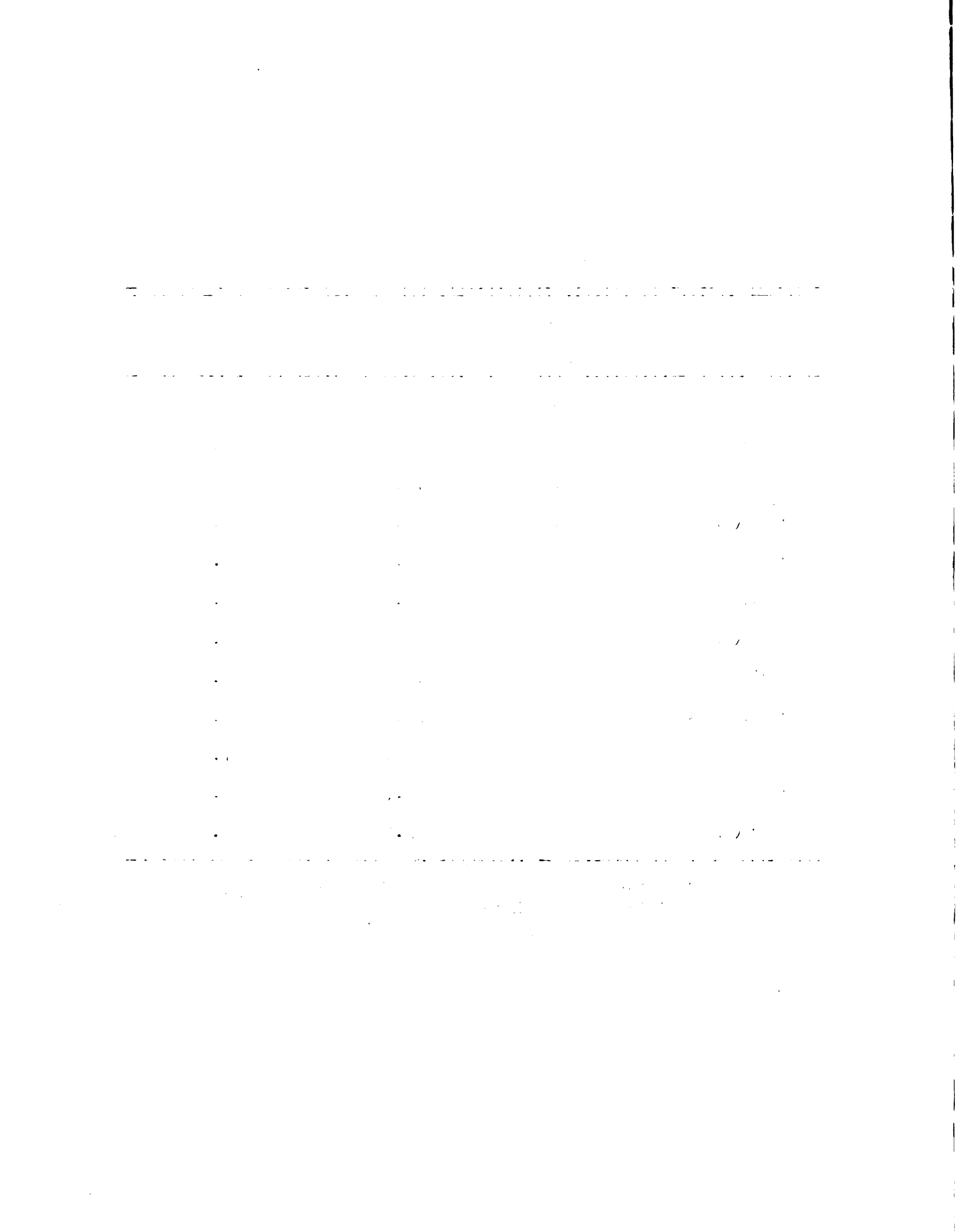


TABLE IX

COMBINED ANALYSIS OF VARIANCE FOR COLD TEST GERMINATION IN A BROOKSTON SOIL AND A HILLSDALE SOIL

Source	Degrees of Freedom	Mean Square
Total	119	
Replication	1	9.7
Soil type	1	34.1
Error (a)	1	17.6
Soil treatment	1	56.1*
Soil x Treat.	1	20.8**
Error (b)	2	0.2
Previous Crops	2	1.6
Soil x Crop	2	3.8
Treat. x Crop	2	17.8
Soil x Treat. x Crop	2	7.5
Error (c)	8	4.8
Hybrid	4	248.8**
Soil x Hyb.	4	9.2*
Treat. x Hyb.	4	44.7**
Crop x Hyb.	8	10.6*
Soil x Treat. x Hyb.	4	8.8*
Soil x Crop x Hyb.	8	1.2
Treat. x Crop x Hyb.	8	10.2*
Soil x Treat. x Crop x Hyb.	8	6.8
Error	48	3.0

* Significant at the 5 percent level of probability.
** Significant at the 1 percent level of probability.

In this connection, the average germination of all hybrids in the unsterilized Brookston soil, though lower than in the sterilized soil, was considerably higher than in the preceding experiment (Table V) conducted in May immediately after taking the soil from the field. The large differences in germination level between the two experiments suggest that the pathogens may have been adversely affected by the drying-out of the soil in the greenhouse after the first experiment.

Additional indication of the detrimental effect of drying-out of the soil on the pathogens is found in the fact that the previous crops had no influence upon germination. In the preceding experiment (Table V), significant differences were obtained among the effects of the same previous crops on cold germination. It appears that the soil to be used in cold test studies should not be allowed to dry out.

Large differences were found among the germinations of the hybrids. The three Michigan hybrids were again more tolerant to cold injury than Gries 201A and Nosco N_4, in both soil types, sterilized and unsterilized. The consistency of the relative ranking of these hybrids under so widely different cold test conditions indicates that, in corn breeding programs, the cold test method can be effectively used to determine the relative tolerance of inbreds and hybrids to cold germination injury.

PART III

Early Generation Testing in Selecting Cold Tolerant Inbred Lines

MATERIALS AND METHODS

S_0, S_1, and S_2 seed for 41 families from a cold tolerant double-cross hybrid, Pioneer 373, was cold tested at the same time in one experiment. The S_0 seed was produced in 1950, the S_1 seed in 1951, and the S_2 seed in 1952. Random samples of the seed indicated good germination under standard laboratory conditions. No seed fungicide was used.

Each family was composed of seven lines developed from a single S_0 plant selected on the basis of desirable agronomic characteristics. These lines represented three generations of inbreeding. There were: one S_0 line, two S_1 lines, and four S_2 lines in each family. Seed for each line was obtained from a single ear.

Three replications of 25 seeds each were planted in flats of Hillsdale sandy loam soil obtained from a corn field. The soil was watered to saturation and the flats transferred to a walk-in cold chamber at 40° F for 12 days. Germination was completed in a warm greenhouse and strong seedlings counted 21 days later.

EXPERIMENTAL RESULTS AND DISCUSSION

Table X presents the average cold test germination percentages for the 41 families. Analysis of variance is given in Table XI.

The mean square for replications was highly significant indicating that there was great variability within the lines even though the seed for each line was obtained from a single ear.

There were significant differences in average germination for the three generations. The average stand for the S_0 seed was 39.5 percent compared to 44.4 percent for the S_1 seed and 29.3 percent for the S_2 seed. The lower germination of the S_0 seed as compared to the S_1 seed may have been due to the fact that the S_0 seed was one year older than the S_1 seed at the time of testing. Cold test germination decreases as the age of the seed increases (19). The lower average for the S_2 seed was probably a result of inbreeding and consequent reduction in general vigor. The frequency distribution of the families in each generation (Table XII) shows that in the S_0 and S_1 generations, 16 and 18 families, respectively, had an average germination greater than 50 percent compared to only five families in the S_2.

Large differences were found among the families. Their average stands ranged from 80.4 percent to 5.1 percent. Germination in the S_0 varied from 0.0 percent to 92.0 percent. Variability within the lines increased with inbreeding as indicated by the larger mean square for "within lines in S_2" than for "within lines in S_1" (Table XI). However,

TABLE X

AVERAGE COLD TEST GERMINATION PERCENTAGES FOR 41 FAMILIES IN THE S_0, S_1, AND S_2 GENERATIONS

Family	Lines							Average
	S_1			S_2				
	S_0	S_{1-1}	S_{1-2}	S_{2-1-1}	S_{2-1-2}	S_{2-2-1}	S_{2-2-2}	
1	82.6	60.0	85.3	69.3	98.6	89.3	77.3	80.4
2	85.3	73.3	61.3	72.0	58.6	70.6	46.6	66.8
3	92.0	76.0	61.3	1.3	88.0	25.3	60.0	60.6
4	56.0	68.0	57.3	80.0	33.3	69.3	54.6	59.8
5	42.6	36.0	61.3	76.0	50.6	50.6	61.3	54.6
6	22.6	46.6	73.3	93.3	50.6	80.0	9.3	53.7
7	62.6	50.6	80.0	1.3	0.0	84.0	96.0	53.5
8	53.3	45.3	68.0	62.6	34.6	34.6	44.0	48.9
9	21.3	52.0	52.0	21.3	27.3	66.6	49.3	45.7
10	68.0	65.3	48.0	36.0	12.0	46.6	34.6	44.4
11	37.3	66.6	20.0	37.3	78.6	20.0	48.0	44.0
12	58.6	61.3	73.3	22.6	53.3	12.0	17.3	42.6
13	21.3	82.6	48.0	14.6	40.0	61.3	29.3	42.4
14	53.3	28.0	29.3	1.3	29.3	89.3	58.6	41.3
15	42.6	42.6	73.3	40.0	1.3	30.6	45.3	39.4
16	52.0	14.6	52.0	26.6	28.0	69.3	28.0	38.6
17	61.3	37.3	56.0	33.3	17.3	26.6	34.6	38.1
18	17.3	44.0	72.0	18.6	37.3	36.0	38.6	37.7
19	57.3	57.3	58.6	9.3	20.0	14.6	41.3	36.9
20	24.0	44.0	40.0	44.0	84.0	6.6	12.0	36.4
21	76.0	42.6	73.3	1.3	1.3	53.3	4.0	36.0
22	0.0	50.6	50.6	86.6	14.6	9.3	28.0	34.2
23	38.6	26.6	69.3	1.3	62.6	29.3	6.6	33.5
24	56.0	28.0	52.0	12.0	28.0	1.3	54.6	33.1
25	8.0	38.6	2.6	62.6	46.6	29.3	17.3	29.3
26	18.6	54.6	82.6	1.3	1.3	29.3	16.0	29.1
27	17.3	66.6	24.0	29.3	36.0	12.0	1.3	26.6
28	72.0	40.0	9.3	20.0	30.6	1.3	12.0	26.4
29	54.6	21.3	36.0	14.6	12.0	34.6	12.0	26.4
30	22.6	33.3	49.3	1.3	30.6	22.6	14.6	24.9
31	9.3	36.0	28.0	36.0	17.3	17.3	26.6	24.4
32	40.0	26.6	26.6	8.0	4.0	29.3	26.6	23.0
33	29.3	70.6	33.3	1.3	0.0	0.0	4.0	19.8
34	48.0	45.3	1.3	13.3	14.6	5.3	10.6	19.8
35	33.3	17.3	13.3	18.6	22.6	2.6	12.0	17.1
36	16.0	33.3	28.0	13.3	5.3	10.6	6.6	16.2
37	12.0	28.0	40.0	5.3	2.6	0.0	20.0	15.4
38	26.6	5.3	25.3	2.6	9.3	5.3	28.0	14.6
39	12.0	29.3	29.3	0.0	0.0	8.0	6.6	12.2
40	2.6	26.6	17.3	0.0	5.3	2.6	17.3	10.2
41	16.0	9.3	0.0	0.0	10.6	0.0	0.0	5.1
Average	39.5		44.4				29.3	

TABLE XI

ANALYSIS OF VARIANCE FOR COLD TEST GERMINATION OF 41 INBRED FAMILIES IN THE S_0, S_1, AND S_2 GENERATIONS

Source	Degrees of Freedom	Mean Square
Total	860	
Replications	2	10,877.2**
Lines	286	1,931.8**
Families	40	5,539.6**
Generations	2	19,999.1**
Families x Generations	80	1,215.9**
Between lines in S_0	40	1,774.2**
Between lines in S_1	40	1,878.9**
Between lines in S_2	40	4,318.4**
Within lines in S_1	41	876.4**
Within lines in S_2	123	1,282.2**
Error	572	261.2

** Significant at the 1 percent level of probability.

TABLE XII

FREQUENCY DISTRIBUTION OF THE 41 FAMILIES IN S_0, S_1, and S_2 GENERATIONS

Percent Germination	S_0	S_1	S_2
0-10	4	1	6
10-20	7	2	10
20-30	7	8	7
30-40	3	4	7
40-50	4	8	6
50-60	8	9	3
60-70	3	8	1
70-80	2	1	0
80-90	2	0	1
90-100	1	0	0

TABLE XIII

INTERGENERATION CORRELATION COEFFICIENTS

	r	Degrees of Freedom
$S_0 - S_1$	0.42**	40
$S_0 - S_2$	0.42**	40
$S_1 - S_2$	0.57**	40

** Significant at the 1 percent level of probability.

variability between lines increased more with continued inbreeding. In S_1 and S_2 generations there was considerably more variation between lines than within lines indicating that possibilities for selection were greater between lines than within lines.

Intergeneration correlation coefficients for germination were highly significant (Table XIII), indicating that cold test germination in the S_0 and S_1 generations was a fair indication of the relative tolerance in the S_2. The correlation of S_0 with S_1 was 0.42^{**} and the correlation of S_0 with S_2 was 0.42^{**}. Therefore only 17.6 percent of the variation in cold tests in S_0 could be accounted for by inheritance in S_1 and S_2. Correlation of S_1 with S_2 was 0.57^{**} and 32.5 percent of the variability in S_1 cold tests could be accounted for by inheritance in S_2. There is an indication here that the S_1 generation would be a better place to start selection for cold tolerance than the S_0.

In the S_0 material, three lines germinated 80 percent or better. Among the 12 S_2 lines from these three tolerant S_0 selections, there were six lines (50 percent of these 12 S_2 lines) that germinated 70 percent or better and three lines (25 percent of the 12 S_2 lines) that germinated 80 percent or above. From the 38 S_0 lines that germinated below 80 percent, there were only ten out of the 152 S_2 lines (6.5 percent) that germinated 70 percent or above. It appears that early generation testing can be effectively used in breeding corn for cold tolerance.

SUMMARY

Commercial seeds for two susceptible and three tolerant double-cross corn hybrids were cold tested in wet soil at 32° F and 40° F. All samples were treated with a seed disinfectant and were capable of highly satisfactory germination under standard laboratory conditions. After pre-cold treatments of 0, 1, 2, 3, or 5 days of warm temperature, seeds of each of the five hybrids were given cold treatments of 4, 8, 12, or 16 days at 32° F or 40° F.

At both temperatures, exposing the seed to cold immediately or one day after planting resulted in nearly the same decreases in stand. Germination decreased as the pre-cold treatments increased beyond one day. Apparently in the more advanced stages of germination the seed became more susceptible to attack by the soil pathogens responsible for cold germination injury. Three days of favorable conditions prior to cold treatment reduced the stand of all hybrids by 50 percent or more.

In general, increase in duration of exposure to cold resulted in lower germination. Pre-cold treatments of 0 and one day followed by cold treatments at 32° F were less injurious than similar pre-cold treatments followed by 40° F cold treatments. It is likely that the soil pathogens were more active at 40° F than at 32° F. In the more advanced stages of germination, cold treatments at 32° F were more injurious than similar treatments at 40° F and apparently the seed was more injured by cold itself at 32° F than by soil pathogens.

The hybrids differed in their tolerance to cold wet soil conditions. Seed of the tolerant hybrids consistently gave higher germinations than seed of the susceptible hybrids. Germination of the tolerant hybrids was not seriously injured by four days of cold at 32° F and 40° F regardless of the stage of germination. Eight days at either 32° F or 40° F did not seriously reduce germination of the tolerant hybrids until the seeds had been under favorable conditions for three days prior to cold treatment. Germination of the susceptible hybrids was reduced by all cold treatments at all stages of germination. Exposing seed corn to cold temperatures for eight to twelve days, immediately or one or two days after planting appears to be a satisfactory method for evaluating cold tolerance.

Seeds for the same five hybrids were cold tested at 40° F in samples of a Brookston silt-loam soil and a Hillsdale sandy loam soil from crop rotation experiments. Cold test germination percentages differed depending on the soil type and the previous crop grown in the soil. In the Brookston soil, germination was best in soil that had previously been in wheat and decreased progressively in soils that had previously been planted to barley, beans, alfalfa, sugar beets, corn, and sweet clover. In the Hillsdale soil there were no significant differences in germination in the soil samples that had previously grown corn, wheat, and sweet clover.

Soil sterilization decreased injury to germination but did not result in perfect stands. Part of the reduction in germination may have been caused by cold injury at 40° F. Drying out of the soil before its

use for cold test germination seemed to have an adverse effect on the soil pathogens responsible for cold germination injury.

S_0, S_1, and S_2 seeds for 41 families from a cold tolerant double-cross hybrid were cold tested at the same time in one experiment. Cold test germination decreased with inbreeding. Variability was greater between lines than within lines, indicating that selection would be relatively more effective between lines than within lines. Intergeneration correlations for germination indicated that early generation testing may be effectively used in selecting cold tolerant inbred lines.

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