AN EVALUATION OF CORN LINES AND SEED CONDITION IN THE COLD TEST

by

JOHN HENRY CURME

A. B., Harvard College, 1949

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
and
Department of Botany and Plant Pathology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1951

TABLE OF CONTENTS

INTRODUCTION

Cold and wet field conditions have been shown to reduce the stand of corn. In order to estimate stands under these conditions various tests indicating reaction of plants to low temperatures have been used. For best results such a test should be conducted with a specific genetic type of corn grown under the least favorable temperatures and moisture conditions which may be expected, and in soils infested with organisms known to be pathogenic to corn kernels and seedlings. Previous tests have shown that there are distinct differences among hybrids and inbreds in their response to low temperatures. These differences may be compared directly with resistance and susceptibility to disease producing organisms of corn. Further, it has been shown that in "cold testing" certain fungi affect and even prevent germination in some lines of corn.

A means of resistance has been sought to improve the stands of corn under cold, wet conditions. The fundamental cause of resistance to such conditions is not definitely known. Two hypotheses to explain why certain varieties are resistant have been suggested. The arguments for mechanical resistance have had some support, since tests have shown that when the pericarp is broken or damaged germination is generally low when seeds are exposed to low temperatures and high moisture. There is also evidence to support a chemical means of resistance. Probably both chemical and mechanical factors are involved.

It has been a problem then to discover how fungi invade the kernel, and conversely to discover the mechanism of resistance of

the host, and then to breed specifically for this means of resistance in corn.

The studies described here have attempted to show the organisms which attack corn seed and which are present in Kansas soils, the "cold test" response of some inbred lines exposed to these organisms, and to correlate the "cold test" response with the permeability of the pericarp.

REVIEW OF LITERATURE

The literature having a bearing on cold testing covers a wide range of approaches none of which offers a perfect solution to the problem. Dickson (2) was among the first to recognize the problem which has been given since and is being given considerable attention.

It was first thought that a simplification could be made by identifying the organism responsible for "seedling blight" which occurs in wet, cold soils, commonly associated with late spring and short growing seasons. Gibberella zeae (G. saubinetii) has been frequently mentioned as shown by papers by Dickson (2), Hoppe (10), Hoppe, Holbert, and Dickson (12), McIndoe (26), Hayes, Johnson, and Stakman (7), Manns and Adams (25), Koehler (19), and Koehler, Dungan, and Burlison (20). Penicillium oxalicum has been identified often with the seedling disease and is mentioned by Johann, Holbert, and Dickson (17), Diachun (1), Leng (22), and possibly by Melchers and Brunson (27). Other organisms identified with the disease are Fusarium

moniliforme by Leonian (23), Manns and Adams (25), Melchers and Brunson (27), and Koehler, Dungan, and Burlison (20). Aspergillus species have been mentioned by Koehler (18), and by Melchers and Brunson (27), and Cephalosporium species by Manns and Adams (25), and by Koehler, Dungan, and Burlison (20). Recently attention has been given to Pythium species by Johann, Holbert, and Dickson (16), and by Hoppe and Middleton (13). Ho (8) has reported that Pythium debaryanum and Pythium graminicola along with Gibberella zeae are highly destructive, that Rhizoctonia solani, Helminthosporium sativum, Diplodia zeae, and Penicillium oxalicum are moderately destructive, and that Aspergillus niger, Fusarium moniliforme, Trichoderma viride (T. lignorum), Rhizopus species, and other Fusarium species are slightly destructive. Any one of these organisms may occur in one or more combinations and still produce symptoms of the disease.

That the organisms found are responsible for low percentage germination and stunted growth was shown by Livingston (24), Rush (32), Ho (8), Johann, Holbert, and Dickson (16), and by Hoppe and Middleton (13). The host-parasite relation is complex. Dickson (3) reported that pentose sugars and pentosans are efficient nutrients in parasite metabolism. Dickson and Holbert (4) concluded that the influence of low temperature of seedling blight of corn is primarily a host response. Seedlings grown at temperatures above 24 degrees C. were found to have cellulose cell walls impregnated with suberin and to have hexose sugars which are inhibitory to fungus growth. Johann, Holbert, and Dickson (17) reported that Penicillium oxalicum injured or

killed host cells with oxalic acid in advance of fungus penetration. Diachun (1) agreed with this analysis. Manns and Adams (25) found that fungus mycelia entered the tip cap and the tissue not protected by the dark layer of the scutellum, sometimes penetrated under the pericarp and entered the embryo. Leach (21) found that the ratio between the coefficient of velocity of seedling emergence and the growth rate of the pathogen is inversely related to the severity of infection. Preemergence infection was most severe at temperatures that were relatively less favorable to the host than to the pathogen as measured by their growth rates. Hoppe (11) reported that low temperature in itself was not injurious but served merely to predispose unprotected, slowly germinating kernels to attack by soil fungi. Dickson, Link, and Dickson (6) found that resistand seedlings contained 20 percent more uronic acids, associated with relatively higher acidity and with polyuronides, especially polyglucuronides, than did susceptible seedlings. Hottes and Huelson (14) found that the permeability of the protoplast can be measured by the colloidal index of leaching solutions. They found that the condition of the protoplast determined the vigor and subsequent growth of seedlings.

McIndoe (26) found that inheritance of resistance to seedling blight caused by Gibberella zeae is probably quantitative, conditioned by multiple factors. Hayes, Johnson, and Stakman (7) reported that the conditions under which an ear develops determines in some way the manner of reaction of the progeny to

the same organism. Previous to this Dickson and Holbert (4) found first generation hybrids of resistant and susceptible inbred parents gave only a susceptible reaction to seedling blight, while Hoppe (10) found that progeny of a cross between resistant and susceptible parents were as resistant as the resistant parent. Pinnell (30) stressed the importance of maternal inheritance in determining the final stand of both single and double crosses, with certain effects best explained by complementary action of genes. Tatum (34) found that the influence of the maternal parent sometimes obscured the genetic effect of the zygote. Rush (32) reported that R x S crosses produced resistant (R) progeny while S x R crosses produced susceptible (S) progeny. Hooker (9) with excised embryos, sterilized and recultured in vitro, found that resistance or susceptibility to Pythium infestation was expressed by the embryo. Rush (32) concluded that inheritance of response to cold is best explained on the basis of multiple factors functioning in the pericarp and/or the endosperm rather than in the embryo. Pinnell (30) concluded that the nature of the endosperm may be a governing factor in cold test performance.

Koehler, Dungan, and Burlison (20) reported that field stand varied directly with vigor of seed planted. Pinnell (30) found highly significant correlations of cold test stands with seedling vigor. Hoppe (10) reported that hybrid vigor does not necessarily mask susceptibility. Hayes, Johnson, and Stakman (7) reported no significant relationship between reaction to Gibberella zeae and indices of plant vigor. Tatum (34) found no

evidence for the relation between vigor and resistance.

Koehler, Dungan, and Burlison (20) found that immature kernels were susceptible to seedling diseases. Rush (32) found that stand in general improved and seed coat damage decreased with progressive maturity up to frost. Frost reduced cold test germinations. Rossman (31) found that physiological maturity increased freezing tolerance. A standard index for cold test response has not been determined.

MATERIALS AND METHODS

Isolation Studies

Isolations were made to determine organisms present in Kansas soils and in or on Kansas grown seed corn. Five genetic types of corn, inbred WF9 grown in Ames, Iowa in 1950, and inbred CI7, single cross 38-11 x CI7, and inbreds 38-11 and K10 all grown on the Agronomy Farm of the Kansas Agricultural Experiment Station in Manhattan in 1950 were used as hosts to the fungi. The corn types were designated A, B, C, D, and E respectively. Soil was taken from a field of the Agronomy Farm where corn had been grown the previous season. The seed was planted in wet soil according to the glass tumbler technique described by Hoppe (11) and incubated for seven days at approximately 45 degrees F. At the close of the incubation period the seed was separated from the soil, cleaned of soil particles and washed in three successive baths of distilled water. One half of the seed was soaked then in a 1/1000 solution of mercury bichloride for one minute for surface sterilization. Three kernels of each genetic type which had been

surface sterilized and three which had not been treated with the bichloride solution were plated on potato dextrose agar (P.D.A.) and on malt agar (Malt) in petri dishes. Then the fungal growths were transferred to pure culture and identified. A second group of isolations was made which followed the same procedure and in addition the procedure was modified to exclude the incubation of kernels as a check.

Cold Tests

The cold test described by Tatum (34) and used by Tatum and Zuber (35) was used. Corn types tested for effect of age of storage were inbreds K41 (white), K150, and K201 provided by the Kansas Hybrids Association. The various types were open pollinated in isolated fields, and shelled and graded by machine. Samples of K41 tested were produced in 1944, 1945, 1946, 1947, and 1948. Those of K150 were produced in 1945, 1947, and 1950. Those of K201 were produced in 1944, 1949, and 1950. Freezing pretreatments were given 1950 Iowa produced inbreds WF9 and L289. These treatments were ten freezings, three freezings, and one freezing in a household refrigerator held to 0 to 4 degrees F. These freezings were approximately 4 hours long each alternated by a 4 hour period of thawing at 45 degrees F. Previous to the freezings, the kernels had been soaked for 4 hours in distilled water. Following the freezing pretreatments the samples were germinated in the cold test. A third set of seed tested consisted of 1949 grown Pioneer 359, a hybrid which is characterized by low cold test germination and by a high percentage of missing tip

caps. This seed was divided into four groups, those with the tip cap missing, those whose pericarp was broken or scratched, normal appearing kernels, and kernels mixed in the original proportion. A fourth group of seed corn tested came from two sources, the Agronomy Farm of the Iowa Agricultural Experiment Station at Ames and the Soil Conservation Service Nursery in Manhattan, Kansas. The following inbreds were tested: I233, I198, Hy, 187-2, WF9, and L289. Germinations at greenhouse temperatures on all seed cold tested were made for controls. The soil used was from the same source as that used in the isolation studies.

Colloidal Indexes of Leaching Solutions

After the method of Hottes and Huelson (14) seed corn of known cold test germination was soaked in distilled water in 125 ml. Ehrlenmeyer flasks and incubated for approximately 48 hours at 85 degrees F. Five grams of kernels weighed to the nearest kernel were placed in 50 cc of distilled water. After incubation solutions were filtered with coarse filter paper to remove fungal growth. Then readings of the colloidal filtrates were made using a Coleman Universal spectrophotometer at a wave length setting of 400 nu.

EXPERIMENTAL RESULTS

Isolation Studies

A complete list of organisms isolated and identified is given in Tables 1 and 2. In general these organisms correspond to isolations made previously and listed in the review of literature. Pythium species and especially P. debaryanum was the most common organism isolated from soil incubated seed. However it was not found once growing on those seed which were not incubated in soil. It appears to be definitely a soil borne organism. The perfect stage of Gibberella zeae was not found, however, the conidial stage was identified and is termed here Fusarium graminearum. A new species to be associated with seedling blight was isolated and identified as Curvularia lunata. The virulence of this organism remains to be tested. There was no association of organisms with a specific host. Alternaria species were found on all genetic types but D, inbred 38-11. Trichoderma viride which was isolated only twice was found both times on this inbred. This was not considered sufficient evidence for a specific host-parasite relation. Dickson (3) found that in resistant strains of corn the same types of fungus penetration occurred but at lower temperatures than in susceptible strains. It should be determined if two or more organisms can penetrate the same host with equal or different ease at a fixed temperature. Rush (32) concluded that the nature of cold tolerance is associated with the ability of a strain to resist attack by soil organisms rather than the ability to germinate at low temperatures. Koehler (19) found

Table 1. Fungi isolated, Group I, all seed incubated in soil.

Inbred and medium	Seed treatment: Washed with water: No. isolated	Organism	Washed with $HgCl_2$: No. isolated	Organism
A (P.D.A.)	1	Pythium debaryanum	1	Penicillium
	3	Pythium	1	Moniliaceae type
	2	Fusarium		
	-	Mucor corticclus		
	2	Penicillium		
	1	Alternaria		
	1	Helminthosporium		
	1	Rhizopus		
A (Malt)	1	Fusarium graminearum	1	Pythium (?)
			1	Helminthosporium
	1	Fusarium		
	2	Penicillium		
	1	Pythium		
	1	Diplodia (?)		
	1	Mucor corticolus		
B (P.D.A.)	1	Pythium	1	Pythium debaryanum
	1	Fusarium	1	Pythium (other?)
			1	Dematiaceae type with brown mycelium
B (Malt)	1	Fusarium poae	1	Alternaria tenuis
	1	Fusarium	1	Alternaria
	2	Alternaria		
	1	Pythium		
	1	chlamydospore type		
C (P.D.A.)	2	Helminthosporium sativum	1	Fusarium
	1	Alternaria tenuis		
	2	Fusarium poae		
	2	Pythium		
	2	Alternaria		
	2	Fusarium		

Table 1. (concl.)

Inbred and medium	Seed treatment: Washed with water: No. isolated	Organism	Washed with $HgCl_2$: No. isolated	Organism
C (Malt	1	Fusarium oxysporum	1	Fusarium poae
	1	Fusarium	2	Fusarium
	1	Curvularia lunata		
	2	Alternaria		
	2	Pythium		
	1	Penicillium		
D (P.D.A.			1	Rhizoctonia, with wide mycelium
D	1	Pythium debaryanum	1	Pythium (?)
	2	Pythium		
	3	Rhizopus nigricans		
(Malt	2	Rhizopus		
	1	Trichoderma viride		
	3	Fusarium		
E	1	Pythium		
(P.D.A.	1	Fusarium		
	1	Alternaria		
	1	Dematiaceae type		
	4	Fusarium graminearum, i.e., Gibberella zeae		
(Malt	1	Penicillium variabile		
	1	Penicillium oxalicum		

Table 2. Fungi isolated, Group II.

Inbred and medium	Seed treatment: Washed with water: No. isolated	Organism	Washed with $HgCl_2$: No. isolated	Organism
		Seed incubated in soil		
A (P.D.A.)	2	Pythium debaryanum	1	Pythium debaryanum
	1	Pythium	4	Pythium
	1	Fusarium graminearum	1	Fusarium poae
A (Malt)	1	Penicillium variabile	2	Pythium debaryanum
			1	Pythium
	1	Penicillium	1	Helminthosporium
B (P.D.A.)	2	Pythium debaryanum		
	1	Pythium		
B (Malt)	1	Fusarium graminearum	1	Penicillium oxalicum
	1	Fusarium oxysporum	1	Mucor corticolus
	1	Fusarium	1	Pythium
	1	Pythium		
C (P.D.A.)	2	Pythium debaryanum	1	Penicillium tardum
	1	Pythium	2	Pythium
	1	Alternaria tenuis		
	1	Fusarium		
C (Malt)	1	Pythium debaryanum	1	Pythium debaryanum
	2	Pythium	1	Pythium
	1	Alternaria tenuis	2	Fusarium poae
	1	Mucor corticolus	1	Fusarium
D (P.D.A.)	1	Pythium	1	Pythium debaryanum
			3	Pythium
			1	Pythium (?)
			1	Trichoderma viride
D (Malt)	1	Pythium debaryanum	2	Pythium debaryanum

Table 2 (concl.)

Inbred and medium	Seed treatment: Washed with water: No. isolated	Organism	Washed with $HgCl_2$: No. isolated	Organism
E (P.D.A.)	1	Fusarium graminearum	1	Pythium
	1	Fusarium oxysporum		
	2	Pythium		
E (Malt)	1	Mucor corticolus	1	Fusarium graminearum
	1	Pythium		
	1	Fusarium		
	1	Helminthosporium		
Seed started in water				
A (P.D.A.)				
A (Malt)	1	Rhizopus nigricans		
B (P.D.A.)	1	Penicillium oxalicum		
B (P.D.A.)	1	Fusarium		
B (Malt)				
C (P.D.A.)	1	Fusarium poae	3	Fusarium poae
C (Malt)	1	Helminthosporium	2	Fusarium poae
D (P.D.A.)	1	Fusarium oxysporum		
D (P.D.A.)	1	Fusarium		
D (Malt)				
E (P.D.A.)	1	Mucor corticolus	3	Fusarium culmorum
E (Malt)	1	Fusarium poae	2	Fusarium culmorum

that infection in the pericarp is easily killed by ordinary surface disinfection, but infection in the tip cap and internal parts persists. Those isolations which were made from kernels which had been surface sterilized are assumed to have grown from mycelia which had already penetrated the pericarp before sterilization. Other infection which passed the bichloride treatment may have been lodged in crevices in and around the tip cap or in cracks and markings in the pericarp.

Cold Tests

The tests which involved the inbreds K41, K150, and K201 were intended to show the effect of age of seed on cold test germinations. The test was composed of ten replications each consisting of the eleven possible combinations of inbred variety and year of harvest. Fifty kernels of an inbred were planted in each entry and the entries or treatments assorted at random within each replication. Analyses of variance showed a significant difference in treatments which might be expected among the inbreds. There was a significant difference at the five percent level among replications only in the cold test which was read at seven days.

When the cold tests were read at seven days it was obvious that the expectation of lower germinations for the older kernels had been reversed, at least in part. Rush (32) and other workers have made their readings at ten days after removal from the cold chamber. A second reading of germinations of cold test and control material was made at ten days. Comparison of the readings

have been listed in Tables 8, 9, 10, 11, and 12. Table 8 shows that there is a significant difference in germination counts between K41, 1944, and K41, 1946, between K41, 1944, and K41, 1947, and between K41, 1944, and K41, 1948. K41, 1944, had the highest germination count and germinations of this inbred generally became progressively lower as age of storage decreased. There were significant differences between the germinations of K150 for the three years of harvest with K150, 1950, having the highest count and K150, 1947, having the lowest count. Inbred K201 showed comparative germinations similar to those of K41. The seed which had been the longest in storage gave the highest germination; that which had been most recently harvested gave the poorest germination. Table 9 shows comparisons of germinations among the years and inbreds used as a control. Again there were significant differences between K41, 1944, and K41, 1947, and between K41, 1944, and K41, 1948. The comparison of K41, 1944, and K41, 1946, no longer gave a significant comparison. K41, 1945, and K41, 1947, and K41, 1945, and K41, 1948, were comparisons of significant difference at the five percent level. Again the differences between K150, 1945 and K150, 1950, and between K150, 1947, and K150, 1950, were significant. K201 showed a significant difference only between the years 1944 and 1949 and between 1944 and 1950. Table 10 shows the cold test readings made at seven days compared with the seven day control readings. Significant differences were found only with K150, 1947, which had germinated poorly in the cold test, and with K201, 1950, which had not had the fungicide treatment.

Table 3. Analysis of variance in 7 day cold test germinations, age of storage study.

Sources	D/F	S. S.	M. S.	F
replications	9	213	23.667	2.126 *
treatments	10	3,756	375.600	33.738 **
error	90	1,002	11.133	
total	109	4,971		

Table 4. Analysis of variance in 7 day control germinations, age of storage study.

Sources	D/F	S. S.	M. S.	F
replications	9	211	23.444	1.519
treatments	10	2,377	237.700	15.402 **
error	90	1,389	15.433	
total	109	3,977		

Table 5. Analysis of variance of 10 day cold test germinations, age of storage study.

Sources	D/F	S. S.	M. S.	F
replications	9	81	9.000	--
treatments	10	3,286	328.600	31.427 **
error	90	941	10.456	
total	109	4,308		

Table 6. Analysis of variance of 10 day control germinations, age of storage study.

Sources	D/F	S. S.	M. S.	F
replications	9	102	11.333	--
treatments	10	1,688	168.800	8.230 **
error	90	1,846	20.511	
total	109	3,636		

Table 7. Treatment totals for cold test and control germinations, age of storage study.

Inbred and year of harvest	Cold test germinations		Control germin't'ns	
	7 day	10 day	7 day	10 day
K41 1944	429	450	433	444
K41 1945	401	411	426	438
K41 1946	378	393	409	429
K41 1947	396	410	382	398
K41 1948	381	398	380	399
K150 1945	327	361	325	367
K150 1947	248	271	312	351
K150 1950	454	464	466	476
K201 1944	412	427	407	425
K201 1949	369	379	365	379
K201 1950	299	310	332	349

Table 8. Comparison of germinations within the 7 day cold test, age of storage study.

Comparison of inbreds	Difference of treatment totals	Mean difference	t value
K41 1944 and K41 1945	429 - 401	2.8	1.877
K41 1944 and K41 1946	429 - 378	5.1	3.418 **
K41 1944 and K41 1947	429 - 396	3.3	2.212 *
K41 1944 and K41 1948	429 - 381	4.8	3.217 **
K41 1945 and K41 1946	401 - 378	2.3	1.542
K41 1945 and K41 1947	401 - 396	0.5	0.335
K41 1945 and K41 1948	401 - 381	2.0	1.340
K41 1946 and K41 1947	378 - 396	1.8	1.206
K41 1946 and K41 1948	378 - 381	0.3	0.201
K41 1947 and K41 1948	396 - 381	1.5	1.005
K150 1945 and K150 1947	327 - 248	7.9	5.295 **
K150 1945 and K150 1950	327 - 454	12.7	8.512 **
K150 1947 and K150 1950	248 - 454	20.6	13.81 **
K201 1944 and K201 1949	412 - 369	4.3	2.882 **
K201 1944 and K201 1950	412 - 288	12.4	8.311 **
K201 1949 and K201 1950	369 - 288	8.1	5.429 **

$s_{\bar{x}} = 1.492$, $t_{.95} = 1.987$, $t_{.99} = 2.632$

Table 9. Comparison of germinations within 7 day control, age of storage study.

Comparison of inbreds and years	Treatment totals	Mean difference	t value
K41 1944 and K41 1945	433 - 426	0.7	0.398
K41 1944 and K41 1946	433 - 409	2.4	1.366
K41 1944 and K41 1947	433 - 382	5.1	2.903 **
K41 1944 and K41 1948	433 - 380	5.3	3.017 **
K41 1945 and K41 1946	426 - 409	1.7	0.968
K41 1945 and K41 1947	426 - 382	4.4	2.504 *
K41 1945 and K41 1948	426 - 380	4.6	2.618 *
K41 1946 and K41 1947	409 - 382	2.7	1.537
K41 1946 and K41 1948	409 - 380	2.9	1.651
K41 1947 and K41 1948	382 - 380	0.2	0.114
K150 1945 and K150 1947	325 - 312	1.3	0.740
K150 1945 and K150 1950	325 - 466	14.1	8.025 **
K150 1947 and K150 1950	312 - 466	15.4	8.765 **
K201 1944 and K201 1949	407 - 365	4.2	2.390 *
K201 1944 and K201 1950	407 - 332	7.5	4.269 **
K201 1949 and K201 1950	365 - 332	3.3	1.878

$s_{\bar{x}} = 1.757$, $t_{.95} = 1.987$, $t_{.99} = 2.632$

Table 10. Comparison of germinations in the 7 day cold test and 7 day controls, age of storage study.

Inbred and year of harvest	Treatment totals	Mean difference	t value
K41 1944	429 - 433	0.4	0.245
K41 1945	401 - 426	2.5	1.534
K41 1946	378 - 409	3.1	1.900
K41 1947	396 - 382	1.4	0.859
K41 1948	381 - 380	0.1	0.061
K150 1945	327 - 325	0.2	0.123
K150 1947	248 - 312	6.4	3.926 **
K150 1950	454 - 466	1.2	0.736
K201 1944	412 - 407	0.5	0.307
K201 1949	369 - 365	0.4	0.245
K201 1950	288 - 332	4.4	2.699 **

$s_{\bar{x}} = 1.630$, $t_{.95} = 1.972$, $t_{.99} = 2.601$

Table 11. Comparison of germinations within the 10 day cold tests, age of storage study.

Comparison of years and inbreds	Treatment totals	Mean difference	t value
K41 1944 and K41 1945	450 - 411	3.9	2.697 **
K41 1944 and K41 1946	450 - 393	5.7	3.942 **
K41 1944 and K41 1947	450 - 410	4.0	2.766 **
K41 1944 and K41 1948	450 - 398	5.2	3.596 **
K41 1945 and K41 1946	411 - 393	1.8	1.245
K41 1945 and K41 1947	411 - 410	0.1	0.069
K41 1945 and K41 1948	411 - 398	1.3	0.899
K41 1946 and K41 1947	393 - 410	1.7	1.176
K41 1946 and K41 1948	393 - 398	0.5	0.346
K41 1947 and K41 1948	410 - 398	1.2	0.830
K150 1945 and K150 1947	361 - 271	9.0	6.224 **
K150 1945 and K150 1950	361 - 464	10.3	7.123 **
K150 1947 and K150 1950	271 - 464	19.3	13.35 **
K201 1944 and K201 1949	427 - 379	4.8	3.320 **
K201 1944 and K201 1950	427 - 310	11.7	8.091 **
K201 1949 and K201 1950	379 - 310	6.9	4.772 **

$s_{\bar{x}} = 1.446$, $t_{.95} = 1.987$, $t_{.99} = 2.632$

Table 12. Comparison of germinations in 7 day and 10 day cold tests, age of storage study.

Year and inbred	Treatment totals	Mean difference	t value
K41 1944	429 - 450	2.1	1.430
K41 1945	401 - 411	1.0	0.681
K41 1946	378 - 393	1.5	1.021
K41 1947	396 - 410	1.4	0.953
K41 1948	381 - 398	1.7	1.157
K150 1945	327 - 361	3.4	2.314 *
K150 1947	248 - 271	2.3	1.566
K150 1950	454 - 464	1.0	0.681
K201 1944	412 - 427	1.5	1.021
K201 1949	369 - 379	1.0	0.681
K201 1950	299 - 310	1.1	0.749

$s_{\bar{x}} = 1.469$, $t_{.95} = 1.972$, $t_{.99} = 2.601$

Table 13. Analysis of variance of Pioneer 359 cold test germinations.

Sources	D/F	S. S.	M. S.	F
replications	4	223.7	55.925	2.904
treatments	3	120.4	40.133	2.084
error	12	231.1	19.258	
total	19	575.2		

Table 14. Analysis of variance of Pioneer 359 control germinations.

Sources	D/F	S. S.	M. S.	F
replications	4	1.7	0.425	2.214
treatments	3	1.2	0.400	2.083
error	12	2.3	0.192	
total	19	5.2		

The seed of K41, 1946, and of K201, 1949, had not been treated with fungicidal dust; by visual inspection all other combinations appeared to have been treated with a fungicide, possibly Spergon. Table 11 shows the comparison of germinations in the cold test when the counts were made ten days after removal from the cold chamber. There were highly significant differences between K41, 1944, and K41, 1945, between K41, 1944, and K41, 1946, between K41, 1944, and K41, 1947, and between K41, 1944, and K41, 1948. The differences between other years and inbred K41 were not significant. Again the higher germinations were with the seed which had been in storage longer. All comparisons of years within inbreds K150 and K201 showed significant differences. The ranking of years of these inbreds for counts made at ten days was the same as for the counts made at seven days. Table 12 lists the comparisons of cold test germinations read at seven days and at ten days. There were significant differences only with K41, 1946, non-treated, and with K150, 1947, and with K201, 1950, which was non-treated also. This comparison may have indicated more clearly than the visual inspection that K150, 1947, did not receive seed treatment and that K201, 1949, did have a fungicidal treatment. The variance of control germination counts made at ten days was not homogeneous with the variance of cold test germination counts made at ten days.

The freezing pretreatment experiments gave negative results. The inbreds WF9 and L289 which received both ten and three freezings did not germinate in either the cold test or control germinations. Of the inbreds which received one freezing only, L289 germinated 84 of a possible 265 in the controls germination,

i.e., those which did not pass through the cold test, and WF9 germinated only 4 in the controls out of a possible 270. In the cold test of L289 34 out of a possible 270 germinated and in a test of WF9 there was no germination. Improvements in this prefreezing technique must be made before it can yield useful results. It is likely that the freezings were too severe and killed the embryos. The object was to preserve the viability of the embryo and test the effect of artificial freezings on the condition of the pericarp as it affects cold test germinations.

The classification of Pioneer 359 into four groups was made by visual selection. The characters determining selection were presence or absence of the tip cap and condition of the pericarp. It was thought that both of these characters might affect the entrance of fungus mycelia into the kernel from the soil. A cold test germination showed marked differences in the ability of the four groups to survive cold test conditions, with those kernels which had either tip caps missing or scratched or marked pericarps showing susceptibility to cold test killing. However, the sample was not large enough to show significant differences either in replications or treatments. An analysis of variance of cold test germinations is given in Table 13 and an analysis of variance of control germinations is given in Table 14. The controls showed almost perfect germination. The seed was not dusted with a fungicide.

Seed of six inbreds both grown in 1950 in Kansas and in Iowa was cold tested. Table 15 shows an analysis of variance of the

split plot design. The size of the experiment was not large and significance is not indicated in the difference between germinations of Iowa and Kansas produced seed. However, there were differences in response by the same strains grown in the two different localities. It was indicated particularly that WF9 produced in Iowa is favored over Kansas produced seed of the same inbred. The germination test showed that I233 seed produced in Kansas is slightly favored over its Iowa produced counterpart, and that Iowa produced I198 is favored over Kansas produced I198.

Table 15. Analysis of variance of cold test germinations of inbreds from two sources.

Sources	D/F	S. S.	M. S.	F
replications	2	1.17	0.585	
source of seed	1	10.03	10.03	4.579
error (a)	2	4.38	2.19	
treatments	5	67.58	13.516	13.666 **
source x treatment interaction	5	81.81	16.362	16.544 **
error (b)	20	19.78	0.989	
total	35	184.75		

Colloidal Indexes of Leaching Solutions

Table 16 shows in part the results of the colloidal index studies using a Coleman spectrophotometer. A reading was made on each of the five solutions. The mean of these readings is given

and the sum of squares is recorded. Each solution was made by leaching five grams of seed from a different ear which may account for the wide range of variance among the different inbreds. Comparison by the method of least significant differences showed these differences: K41, 1944, had a lower reading than K41, 1945, significant at the one percent level, and had a higher reading than K41, 1946, significant at the five percent level. K41, 1944, was not significantly different from K41, 1947, or from K41, 1948. The readings of K41, 1945, were larger than those of K41, 1946, and K41, 1947, at the one percent level of significance, but not larger than those of K41, 1948. The readings of K41, 1947, were lower than those of K41, 1948, at the five percent level of significance. The readings of K41, 1946, 1947, and 1948, conform to the general expectation of increasing colloidal index with decrease in rate of germination.

Inbred K150 had no significant differences in its spectrophotometer readings and little variation in the mean readings. K150, 1950, which had the highest cold test germination, had the lowest mean reading of inbred K150. Inbred K201 also showed no significant difference among the colloidal index readings.

The samples of inbreds L289 and WF9 which did not have freezing pretreatments showed a difference significant at the one percent level in their colloidal indexes. L289 which had the lower mean reading had the higher germination rate in the cold test. Similar samples of the two inbreds taken from the same ears were frozen and thawed alternately ten times, then leached and the solutions tested. These solutions were not significantly

different although that of L289 still had the lower colloidal index. When colloidal indexes of solutions of WF9 which had been frozen in pretreatment were compared to solutions of this inbred which had not been frozen the difference was found to be significant at the five percent level. There was no significance in a similar comparison of inbred L289.

Pioneer 359 seed was separated into two groups one of which had the tip caps missing from the kernels and the other consisting of kernels with sound pericarp and good appearance. The colloidal index of the solutions of the group in good condition was lower than that of the group with tip caps missing by a difference significant at the five percent level. These differences were in accord with the trend of other colloidal index-cold test germination rate associations.

Lastly five inbreds produced in Kansas and in Iowa were compared for colloidal index. None of the five comparisons showed a significant difference by the method of least significant differences. This evidence supports directly the results of the cold test. There is no significant difference in cold response of the inbreds tested for place of origin.

Table 16. Means and variances of spectrophotometer readings.

Inbred and year	D/F	Mean	s^2
K41 1944	4	64.1	286.56
K41 1945	3	75.5	19.49
K41 1946	4	44.5	661.12
K41 1947	4	54.9	264.88
K41 1948	4	69.8	245.22
K150 1945	4	69.7	233.91
K150 1947	4	69.5	380.54
K150 1950	4	63.2	1,600.94
K201 1944	4	63.8	406.95
K201 1949	0	71.8	--
K201 1950	4	74.8	257.46
L289, unfrozen	4	38.6	1,191.87
L289, frozen	4	51.5	436.46
WF9, unfrozen	4	74.9	282.78
WF9, frozen	4	58.9	405.84
Pioneer 359			
tip caps missing	4	52.5	54.52
in good condition	4	39.2	481.54
Kansas produced inbreds:			
L289	3	51.6	1,624.80
WF 9	3	75.2	2,445.10
187-2	2	81.2	1,513.73
I198	1	36.2	486.72
Hy	2	64.7	437.25
US523-W, a double cross	4	16.4	686.82
Iowa produced inbreds:			
L289	4	38.6	1,191.87
WF 9	4	74.9	282.78
187-2	4	65.3	972.64
I198	4	45.2	802.34
Hy	4	41.7	745.15
I233	4	65.4	72.26

DISCUSSION

The problem of seedling blight is complicated by many biological variables. This thesis has attempted to bring together three main factors, the causal organisms, factors which influence the physical nature of the pericarp, and an index of the permeability of the pericarp. The phase of the work dealing with seedling blight organisms is in agreement with previous published work. It extends a confirmation that Pythium debaryanum, Gibberella zeae, and Penicillium oxalicum are the principle species causing death of the embryo and seedling blight in cold test conditions in Kansas. One new species, Curvularia lunata which may be of importance, has been added to the group of organisms associated with the disease.

The results of low temperature germinations described here indicate that cold testing is not a sufficient means of ranking genetic strains of corn in resistance or susceptibility to seedling blight. It was expected from previous work by Tatum (34), Pinnell (30), and by Rush (32) that significant differences would be found between the so-called normal or control germinations and the corresponding cold tested material such that the genetic lines could be reclassified on the basis of their cold test germinations. It is difficult to account for the failure of this expectation and the lack of agreement with previous work. Further, in the age of storage tests which show a direct relation of germination with length of storage period where an inverse

relation is expected, factors beyond the control of this experiment affected the results. The growing season as it affects the condition of the pericarp and the viability of the embryo may be an important factor. Dickson (20) reported that the extent of seedling blight is directly proportional to the amount of infestation. The effectiveness of the cold test should thus be proportional to the concentration of blighting organisms in the soil. The isolation studies showed that this should not be a limiting factor. Leonian (23) concluded that even the most vigorous strains of Fusarium moniliforme exhibited their pathogenicity in cycles. It may be that the organisms concerned in this study are also subject to cyclic effects. Another suggestion has been that the cold tests were not severe enough either in low temperature or in length of time of cold exposure or both. This has not been true in previous work which used the same time period and temperature as was used here. It was thought that planting in the germination flats may have been too crowded to permit normal germinations, although this possibility is not fully substantiated. The cold test results presented here have not disproven previous results but did fail to confirm them.

The colloidal index study related directly with the results of Hottes and Huelson (14). The technique should be developed further to include standard procedures and nephelometer readings to decrease variance of readings and increase the accuracy of significant differences. This technique offers promise for a quick and accurate method of estimating cold response of corn lines. It is appropriate in that it measures indirectly the

permeability of the pericarp which is thought to be a limiting factor in fungus penetration of the corn kernel.

CONCLUSIONS

Isolation studies have shown that Pythium debaryanum, Fusarium graminearum (the conidial stage of Gibberella zeae), Penicillium oxalicum, Mucor corticolus, Curvularia lunata, Trichoderma viride, and species of Helminthosporium, Fusarium, Alternaria, Rhizoctonia, and Rhizopus are associated with seedling blight and the death of kernels in cold testing conditions. Of these the soil borne Pythium debaryanum is the most common and believed to be the most destructive in Kansas.

Results of cold testing indicated that there is no significant difference between germinations at 45 degrees F. and at normal greenhouse temperatures. Results described here indicated that cold tolerance improves with age of seed, although this is contrary with familiar concepts of seed viability. There is no significant difference in place of origin as it affects cold response of corn lines. There were no results which support the correlation of missing tip caps and scratched pericarps with low cold tolerance, although this is believed to be a reliable assumption. Freezing pretreatments at 0 to 4 degrees F. reduced all germinations and were believed to have killed the embryos.

Tests involving colloidal indexes of leaching solutions showed that there are significant differences between inbred lines and between years of harvest and storage within inbred

lines. These indexes were in agreement with the results of cold test germinations. These results offer an indication that the permeability of the corn kernel pericarp is directly correlated with the penetration of the kernel by fungus organisms. Generally it was found that a high colloidal index, which indicates high pericarp permeability, is correlated with low cold test germination . A breeding program for reduction of pericarp permeability should be effective in increasing tolerance to cold. Further work for the more complete understanding of pericarp permeability in relation to cold testing is in order.

ACKNOWLEDGEMENT

The author wishes to express his gratitude and sincere appreciation of the help given by Dr. Lloyd A. Tatum and Dr. Clark T. Rogerson in the development and writing of this thesis. Assistance and advice was given freely by many members of the Kansas State College faculty to whom the author is deeply indebted.

REFERENCES

(1) Diachun, S.
The effect of some soil factors on Penicillium injury to corn seedlings. Phytopath. 29:231-241, 1939.

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

(3) Dickson, J.G.
The relation of plant physiology and chemistry to the study of disease resistance in plants. Amer. Soc. Agron. Jour. 17:676-695, 1925.

(4) Dickson, J.G. and J.R. Holbert
The influence of temperature upon the metabolism and expression of disease resistance in selfed lines of corn. Amer. Soc. Agron. Jour. 18:314-322, 1926.

(5) Dickson, J.G. and J.R. Holbert
The relation of temperature to the development of disease in plants. Amer. Nat. 62:311-333, 1928.

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

(7) Hayes, H.K., I.J. Johnson, and E.C. Stakman
Reaction of maize seedlings to Gibberella saubinetii. Phytopath. 23:905-911, 1933.

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

(9) Hooker, A.L.
The corn embryo as a factor in resistance in Pythium during germination. Phytopath. 41:17 (abs.), 1951.

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

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

(12) 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.

(13) 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, 1950.

(14) 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.

(15) Johann, H.
Histology of the caryopsis of yellow dent corn, with reference to resistance and susceptibility to kernel rots. Jour. Agr. Res. 51:855-883, 1935.

(16) Johann, H., J.R. Holbert, and J.G. Dickson
A Pythium seedling blight and root rot of dent corn. Jour. Agr. Res. 37:443-464, 1928.

(17) Johann, H., J.R. Holbert, and J.G. Dickson
Further studies on Penicillium injury to corn. Jour. Agr. Res. 43:757-790, 1931.

(18) Koehler, B.
Pathologic significance of seed coat injury to corn seedlings. Phytopath. 25:24 (abs.), 1935.

(19) Koehler, B.
Natural mode of entrance of fungi into corn ears and some symptoms that indicate infection. Jour. Agr. Res. 64:421-442, 1942.

(20) Koehler, B., G.H. Dungan, and W.L. Burlison
Maturity of seed corn in relation to yielding ability and disease infection. Amer. Soc. Agron. Jour. 26: 262-274, 1934.

(21) Leach, L.D.
Growth rates of host and pathogen as factors determining the severity of preemergence damping-off. Jour. Agr. Res. 75:161-178, 1947.

(22) Leng, E.R.
Differential reaction to Penicillium seedling disease of corn. Agron. Jour. 41:435-439, 1949.

(23) Leonian, L.H.
The pathogenicity and the variability of Fusarium moniliforme from corn. W.Va. Agr. Expt. Sta. Bul. 248:2-16, 1932.

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

(25) Manns, T.F. and J.F. Adams
Parasitic fungi internal of seed corn. Jour. Agr. Res. 23:495-524, 1923.

(26) McIndoe, K.G.
The inheritance of the reaction of maize to Gibberella saubinetii. Phytopath. 21:615-639, 1931.

(27) Melchers, L.E. and A.M. Brunson
Effect of chemical treatments of seed corn on stand and yield in Kansas. Amer. Soc. Agron. Jour. 26: 909-916, 1934.

(28) Melhus, I.E.
Some recent contributions to our understanding of corn diseases. Iowa Acad. Sci. Proc. 38:89-101, 1931.

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

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

(31) Rossman, E.C.
Freezing injury of inbred and hybrid maize seed. Agron. Jour. 41:575-583, 1949.

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

(33) 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.

(34) 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.

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

(36) Wernham, C.C.
The pure line or variety concept with respect to disease resistance in plants. Plant Disease Reporter. 32:479-481, 1948.

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

(38) Wortman, L.S., Jr.
The effect of seed corn injury upon cold test performance. Unpub. M.S. thesis, Univ. of Minnesota, 1948.

AN EVALUATION OF CORN LINES AND SEED CONDITION IN THE COLD TEST

by

JOHN HENRY CURME

A. B., Harvard College, 1949

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
and
Department of Botany and Plant Pathology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1951

Purpose. This thesis was undertaken to identify the organisms present in local Kansas soils which are associated with seedling blight, to determine the response to cold testing of locally available lines of corn, and to learn something of the mechanism of the host's resistance to the blighting organisms.

General method. For identification standard isolation techniques were used on seed samples which had been incubated in soil under cold test conditions and on similar samples which were germinated without exposing them to the soil. Identification was made of organisms isolated from these samples. For response to cold, four groups of seed were tested. One group consisted of three Kansas inbreds of known harvest date and age of storage; a second group was comprised of six inbreds produced in two locations to be compared for effect of their geographic source; a third group was of seed of a commercial hybrid, separated by visual selection into four groups on the basis of pericarp condition; and the last group was composed of two widely used inbreds subjected to three freezing treatments intended to affect the condition of the pericarp. Leaching solutions were prepared by soaking kernels in distilled water. The colloidal index of these solutions was determined using a spectrophotometer and the readings compared with cold test germinations.

Principle results. Pythium debaryanum was found most commonly in isolations and concluded to be the most destructive organism. Isolations of species of Penicillium, Fusarium, Helminthosporium, Alternaria, Mucor, and Rhizopus were common.

A species identified as Curvularia lunata was associated for the first time with seedling blight. Cold testing showed these results: under conditions of this experiment there was no consistent significant difference between germination of seed of different ages in cold tests and normal germinations of the corresponding seed samples; secondly, there was no relation between the age of the seed up to seven years and its response to cold; thirdly, the effect of geographic origin did not produce significant differences in the response to cold of lines tested; fourthly, the classes of seed of the commercial hybrid based on the condition of the pericarp showed marked but not significant differences in response to cold; and lastly, the freezing treatments were concluded to be too severe as most of the tested seed did not germinate.

Colloidal indexes correlated well with cold test germinations of the same seed. A low index indicated good germination and a high index indicated poor germination in the cold test. This was concluded to be a promising method of estimating the response to cold of corn lines quickly and accurately and to offer a possible means of explanation of the host mechanism of resistance to seedling blight organisms.