GENETIC STUDIES OF "COLD TEST" REACTION IN MAIZE(1)

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In northern areas, such as Wisconsin, soil conditions are often unfavourable for germination when maize is planted. High quality seed of strong vitality therefore is of paramount importance. Further, freezing temperatures frequently occur before seed maize is fully developed, and matured thus reducing, if not seriously damaging, seed germination and vitality. The consequences of early freezing temperatures can be especially significant in seasons when development of the crop is retarded by unfavourable growing conditions. That the ability of seed maize to resist frost injury is influenced by the genetic constitution has been emphasized by a number of workers (Holbert and Burlison, 1929; Pinnell, 1949; Hooker and Dickson, 1952). The results of Rossman (1949) showed that the tolerance of seed maize to forest injury was inherited in crosses, and that "the maternal seed characteristics were more important than embryo constitution". But Haskell and Singleton (1949) reported that the cold resistance of seeds was primarily determined by the genetic constitution of the embryo.

Hoppe and Middleston (1953) have shown that various pathogenic organisms were capable of causing kernel rot and seedling blight in cold, wet soils. Genetic differences in reaction to attack by soil-borne organisms have been found by Torfasson and Nonnecke (1954). Genetic constitution of the kernel was shown to affect "cold test" perfomance, with wide differences existing among inbred lines and hybrids (Neptune and Rossman, 1953; Pinnell, 1949 and Rush 1950). In a substantial measure, these differences were dependent on the maternal parent, the pollen parent having relatively little influence on the "cold test" reaction of the seed. These results were explained in several ways: (a) on a basis of complementary gene action proposed by Pinnell (1949), the nature of endosperm being responsible for the maternal correlation, (b) on the basis of

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multiple factors functioning in the pericarp and endosperm suggested by Rush (1950) either independently or in association and (c) in part due to cytoplasmic effect mentioned by Wortman (1950) and by Toffasson and Nonnecke (1954). Data presented by Hooker (1952) and by Hooker and Dickson (1952) indicated that differences in the embryos of the corn strains determined the major course of seedling reaction to *Pythium* at low soil temperatures.

Materials and Methods

The basic materials involved in this study, comprising six inbred lines, (W153R, W23, A374B, W22R, W64A and M14) and some single and double hybrids of which they were the parents. Seed of the double hybrids was produced in commercial seed fields and that of the inbred lines and single hybrids by hand pollination.

In order to simulate preharvest frosts, but under controlled conditions, representative unhusked ears of the respective seed classes were harvested at successive stages of maturity, precooled to 34°F, and then given freezing treatments of varying intensities and duration in a specially equipped freezing cabinet, after which the ears were husked, dried and shelled in the conventional manner.

Spargue's (1936) investigations indicated that kernel moisture content usually was between 50% and 60% within 30 days after pollination. Accordingly, in this study, it was decided to make the first harvest of each strain approximately at that stage of maturity. Thereafter, successive harvests were made at 9 or 10-day intervals. Approximately fifty unhusked ears of each hybrid, and sixty ears of each inbred line were harvested at each date. For the first to third harvests, freezing treatments at 26°F, 21°F, 16°F and 11°F were given respectively for 2, 4, 6 and 8 hours each.

Hoppe's (1959) "cold test" germination procedure was employed and the seed protectant used throughout was Arasan (tetramethyl thiuram disulphide).

During the course of the investigation, it became evident that moisture content of the kernels would be an important factor in determining the degree of freezing injury and its influence in respect to the attack of seed decaying organisms under conditions unfavourable for germination. Therefore, in comparing strains in these respects, it is important that freezing treatments be given at comparable moisture levels. Usually, however, it is not possible to obtain this degree of control. Under these circumstances, the "sign test" is most useful (Steel and Torrie, 1960).

Experimental Results

In all cases, as would be expected, the use of a fungicidal seed protectant substantially improved germination under "cold test" conditions. Prevention of attack by soil-borne seed decaying organisms permits an evaluation of the extent of freezing injury. Without a seed protectant, the lower germination results are a consequence of both freezing injury and the relative inherent resistance, or susceptibility to the attack of seed decaying organisms.

Comparison Between Inbred Lines

Figure 1 shows the behavior of inbreds, W153R, W23 and M14 under "cold test" conditions with, and without a seed protectant, when frozen at 16°F for four hours. In respect to the three inbred lines, it is observed that with a seed protectant, inbreds W23 and W153R sustained less damage than M14 (table 1 and figure 1) from freezing at all stages of maturity when given freezing treatments of varying intensity and duration. Without a seed protectant, it is clear that inbred W153R shows a low level of resistance to seed-decaying organisms, W23 a comparatively a high level of resistance when mature, and M14 shows an intermediate reaction. It is to be noted, however, that W23 is relatively susceptible when immature.

Table 1. *Comparisons between inbred lines in "cold test" germination of treated (T) and nontreated (N. T) seed maize harvested at successive stages of maturity and exposed to freezing temperatures of varying intensity and duration*

Comparisons		No. of pairs of observations	No. of times left entry greater than right entry	Level of significance
W23 vs W153R	(T)	16	10	n. s.
W23vs M14	(T)	20	20	1%
W153R vs M14	(T)	20	20	1%
W23 vs W153R	(N. T)	22	22	1%
W23 vs M14	(N. T)	22	22	1%
M14 vs W153R	(N. T)	22	22	1%

Table 1 shows the results of an analysis of comparisons between inbred lines by the sign test, for "cold test" germination with, and without a seed protectant. These comparisons clearly demonstrate that ability to resist damage to germination by freezing is not necessarily correlated with inherent resistance to attack by seed decaying organisms. For example, the germination of inbred W153R with a seed protectant was substantially higher than that of M14 but in the absence of protectant, M14 was significnantly better.

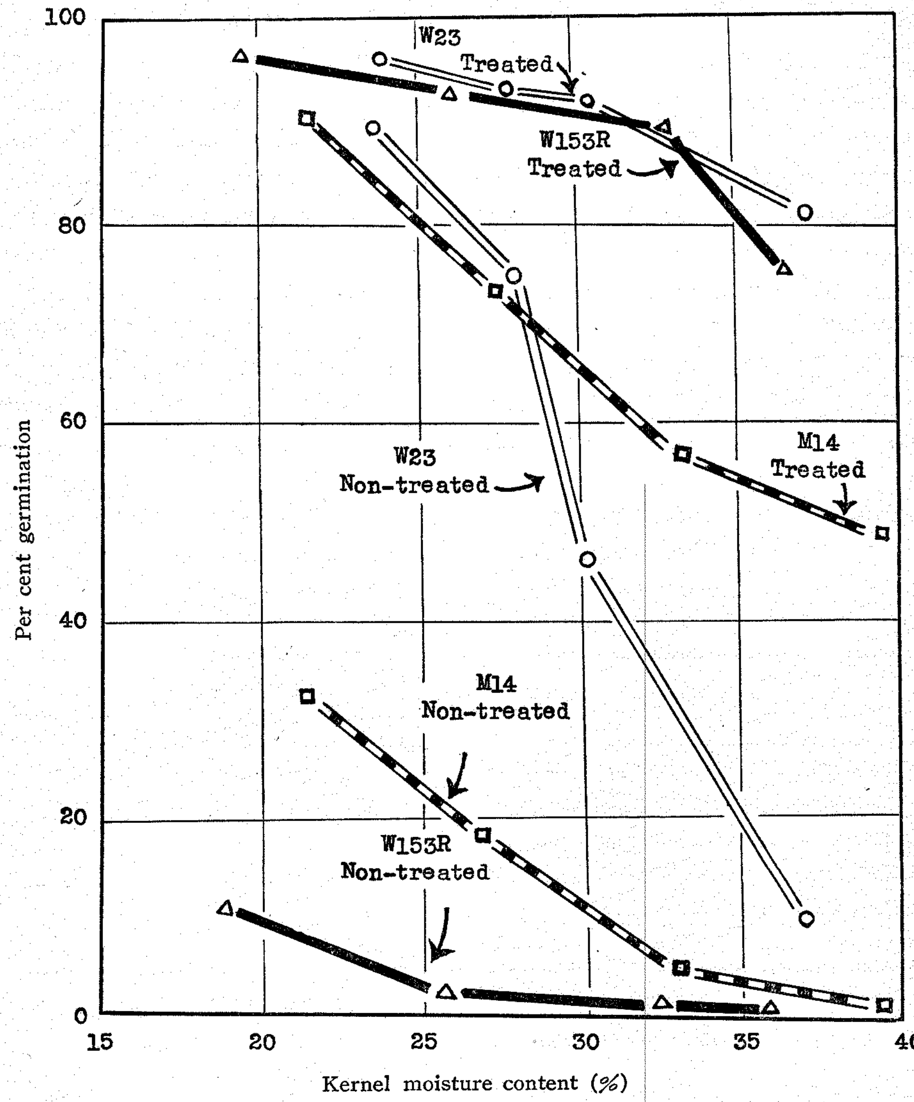

Figure 1. Comparative "cold test" germination results with, and without, a seed protectant of seed of inbreds W23, W153R and M14, harvested at successive stages of maturity and exposed to a freezing temperature of 16°F for 4 hours.

Comparisons Between Single Hybrids

Similar information of the "cold test" germination without a seed protectant for the three single hybrids, M14×W64A, W153R×A374B and W23×W22R as shown in Table 2 and figure 2, closely parallels that for the inbred lines. These comparisons clearly demonstrate the importance of the influence of the maternal seed parent for the ability to resist attack by seed decaying organisms. But in the case of those for the "cold test" germinations with a seed protectant, which is considered as a measure of frost injury in this investigation, it appears that the ability of single hybrids to give good stands was not as strongly influenced by the maternal parents as with nontreated seed. For example, with a protectant, there was no significant difference between inbred W23 and W153R, but the difference between the single hybrids W23×W22R and W153R×A374B reached statistical significance at the 5% level. In contrast, inbred W153R was highly superior to M14, but there was no significant difference between their single hybrids W153R×A374B and M14×W64A.

Table 2. *Comparisons between single hybrids in "cold test" germination of treat (T) and nontreated (N. T) seed maize harvested at successive stage of maturity and exposed to freezing temperatures of varying intensity and duration*

Comparisons		No. of pairs of observations	No. of times left entry greater than right entry	Level of significance
(W23×W22R) vs (M14×W64A)	(T)	15	14	1%
(W23×W22R) vs (W153R×A574B)	(T)	15	12	5%
(M14×W64A) vs (W153R×574B)	(T)	17	10	n. s.
(W23×W22R) vs (M14×W64A)	(N. T)	13	12	1%
(W23×W22R) vs (W153R×A374B)	(N. T)	17	17	1%
(M14×W64A) vs (W153R×A374B)	(N. T)	17	17	1%

Comparison Between Open-Pollinated Single Hybrid Progenies

For seed of open-pollinated progenies of the same three single hybrids, a similar relationship prevails as shown in table 3. It is to be noted however, that the comparison of (W23×W22R) OP* with (W153R×A374B) OP was significantly different at the 1% level whereas that involving single hybrid seed was significantly different at the 5% level. Possibly the difference suggests that tolerance to freezing injury might also be influenced by the pollen parents.

* OP=Open-pollinated.

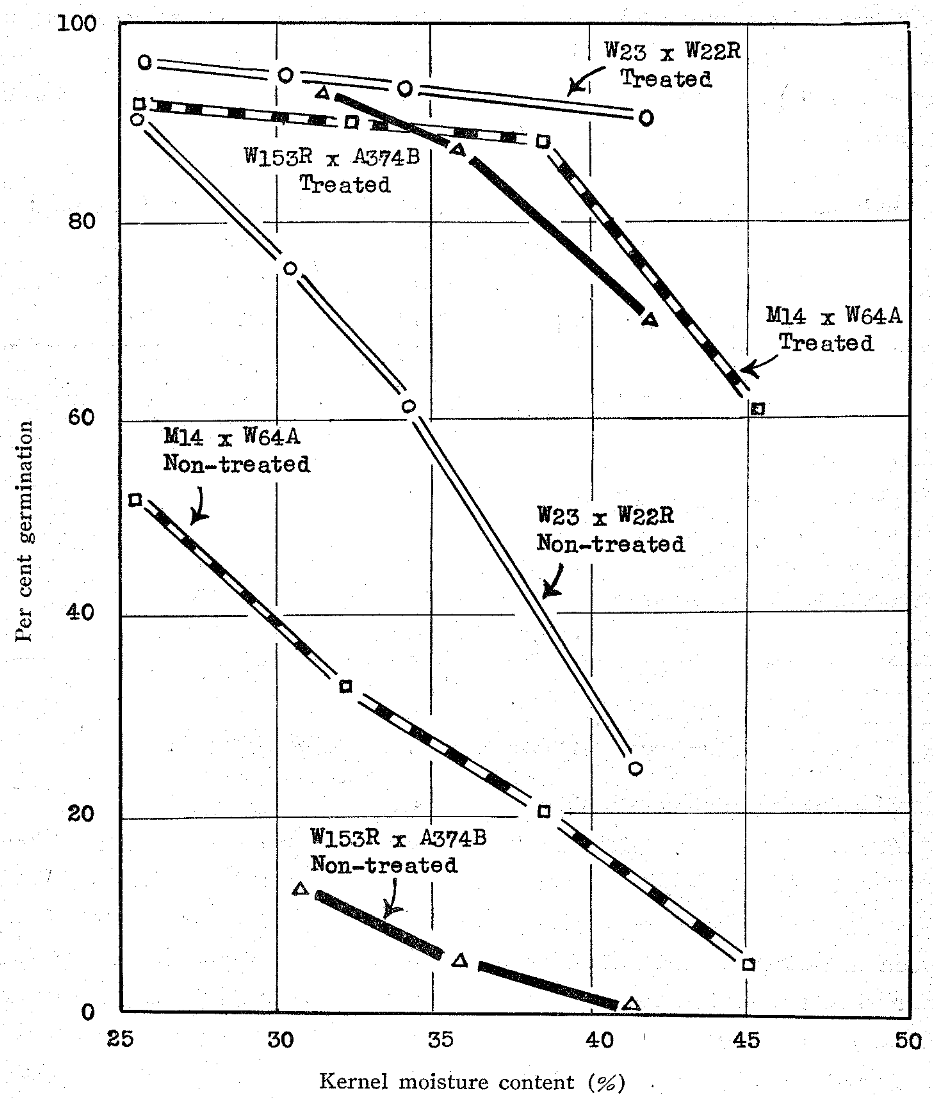

Figure 2. Comparative "cold test" germination results with, and without, a seed protectant of seed of three single hybrids W23 × W22R, W153R × A374B and M14 × W64A harvested at successive stages of maturity and exposed to a freezing temperature of 16°F for 4 hours.

The data presented in table 1, 2, and 3 provide a general conclusion that hybrids with inbred W23 as the seed parent given good stands both with, and without a fungicidal seed protectant under "cold test" conditions; the hybrids having inbred M14 as seed parent showed an intermediate reaction for both "cold test" germinations; the hybrids having inbred W153R as seed parent showed a low level of resistance to seed decaying organisms, but a high level of tolerance to freezing injury.

Table 3. *Comparisons between three open-pollinated single hybrid progenies in "cold test" germination of treated (T) and nontreated (N.T) seed maize harvested at successive stages of maturity and exposed to freezing temperatures of varying intensity and duration*

Comparisons	No. of pairs of observations	No. of times left entry greater than right entry	Level of significance
(W23×W22R) OP vs (M14×W64A) OP (T)	15	13	1%
(W23×W22R) OP vs (W153R×A374B) (T)	15	14	1%
(W153R×A374B) OP vs (M14×W64A) OP (T)	13	7	n. s.
(W23×W22R) OP vs (M14×W64A) OP (N.T)	21	16	5%
(W23×W22R) OP vs (W153R×A374B) OP (N.T)	16	16	1%
(M14×W64A) OP vs (W153R×A374B) (N.T)	16	16	1%

Comparision Between Inbreds and Their Hybrids

The data presented in table 4 show that:

Inbred M14 group. "Cold test" germinations of seed with, and without treatment with a fungicidal seed protectant of hybrid (M14×W64A) OP were better than those of single hybrid M14×W64A, and the differences between them were significant at the 10% level, though both were highly superior to inbred M14 in respect to resistance to seed decaying organisms and for tolerance to freezing injury at all stages of maturity as shown in figure 3.

Inbred W153R group. Smaller, but significant difference existed between hybrid (W153R×A374B) OP and its maternal single cross W153R×A374B for "cold test" germinations respectively with, and without, a seed protectant. Both were superior to inbred W153R in "cold test" without a seed protectant, and all differences were statistically significant at the 1% level. But no significant difference was found between the hybrids and their maternal inbred W153R for the "cold test" germination with a seed protectant.

Inbred W23 group. The results for the comparison between open-pollinated single hybrid progenies and their parental single hybrids of the inbred W23 group were similar to those obtained with the inbred W153R group. Hybrid (W23×W22R) OP was significantly better than inbred W23 with a seed protectant, but gave somewhat poorer results without a protectant. The "cold test" germinations of single hybrids with, and without a seed protectant were both better than inbred W23 and were significant at the 10% and 5% levels respectively.

Table 4. *Comparisons between inbred lines and the hybrids of which they are the maternal pvrents in "cold test" germination of treated (T) and nontreated (N. T) seed maize, harvested at successive stages of maturity and exposed to freezing temperatures of varying intensity and duration*

Comparisons		No. of pairs of observations	No. of times left entry greater than right entry	Level of significance
GROUP I. M14				
(M14×W64A) OP vs (M14×W64A)	(T)	14	11	10%
(M14×W64A) OP vs (M14×W64A)	(N. T)	13	10	10%
(M14×W64A) OP vs (M14)	(T)	16	16	1%
(M14×W64A) OP vs (M14)	(N. T)	16	16	1%
(M14×W64A) vs (M14)	(T)	16	16	1%
(M14×W64A) vs (M14)	(N. T)	16	16	1%
GROUP II. W153R				
(W153R×A374B) OP vs (W153R×A374B)	(T)	13	10	10%
(W153R×A374B) OP vs (W153R×A374B)	(N. T)	12	9	25%
(W153R×A374B) OP vs (W153R)	(T)	19	12	n. s.
(W153R×A374B) OP vs (W153R)	(N. T)	22	22	1%
(W153R×A374B) vs (W153R)	(T)	14	9	n. s.
(W153R×A374B) vs (W153R)	(N. T)	13	13	1%
GROUP III. W23				
(W23×W22R) OP vs (W23×W22R)	(T)	16	12	10%
(W23×W22R) OP vs (W23×W22R)	(N. T)	15	11	25%
(W23×W22R) OP vs (W23)	(T)	22	22	1%
(W23×W22R) OP vs (W23)	(N. T)	19	12	n. s.
(W23×W22R) vs (W23)	(T)	16	12	10%
(W23×W22R) vs (W23)	(N. T)	19	15	5%

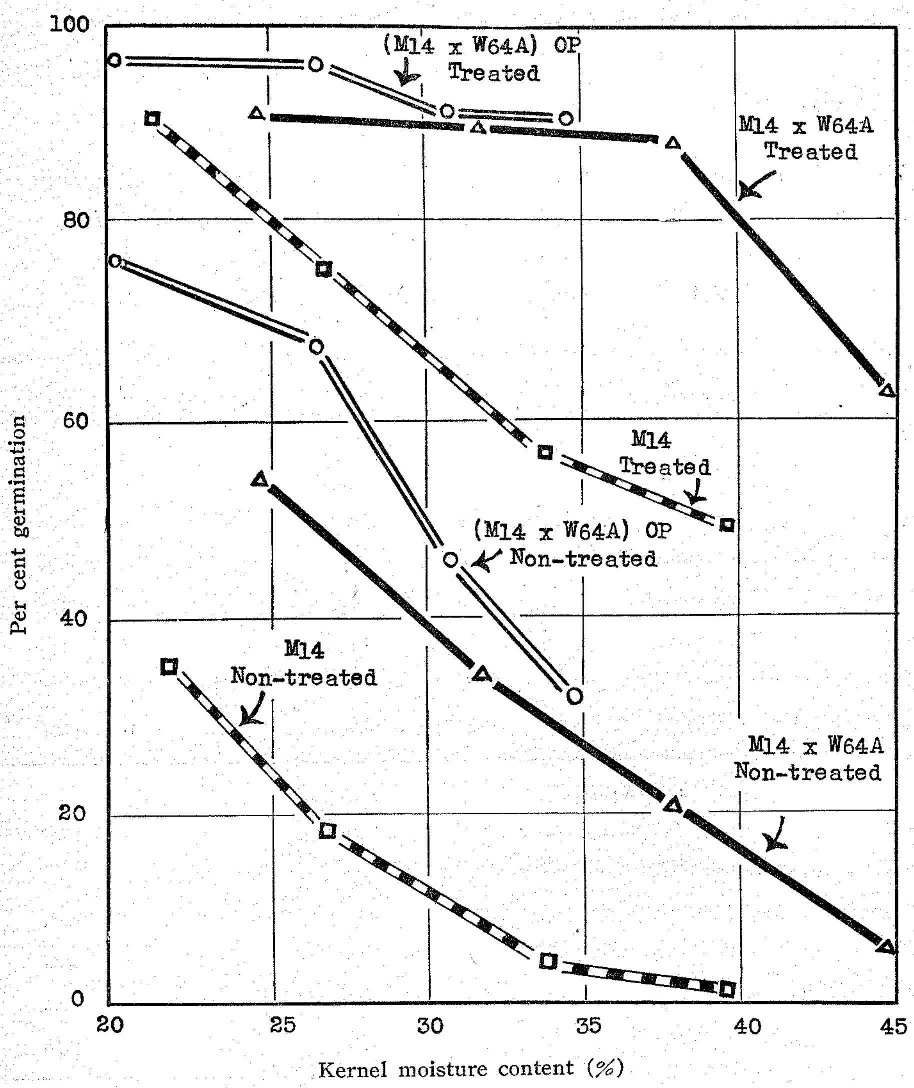

Figure 3. Comparative "cold test" germination results with and without a seed protectant, on seed of inbred M14, single hybrid M14 × W64A, and open-pollinated single hybrid progeny (M14 × W64A) OP, harvested at successive stages of maturity and exposed to a freezing temperature of 16°F. for 4 hours.

Discussion

In the case of seed not given freezing treatments, the stage of maturity was shown to have only a minor influence on germination under standard laboratory conditions, as well as in "cold test" conditions with a seed protectant. This is in agreement with the views expressed by Rush and Neal (1951). However, the results of "cold test" germinations without a seed treatment were found to be strongly associated with seed maturity, due to the fact that immature seed has a higher permeability than the mature as reported by Tatum (1954), as well as being more susceptible to soil-borne pathogens as shown by Rush and Neal (1951). In this investigation, the standard laboratory germination test without a seed protectant as well as the "cold test" germination with a protectant, serve as a measure of the potential germination capacity. On the other hand, "cold test" germination results without a seed treatment, indicate the degree of resistance to attack by soil-borne, seed-decaying organisms.

With seed given freezing treatments and germinated without a seed protectant under "cold test" conditions, the lower germination results in this study are a consequence of both freezing injury and of attack by seed-decaying organisms. The importance of the effect of the maternal parent upon the reaction of hybrids to be attacked by soil-borne pathogens under "cold test" conditions has been discussed in detail by other investigators (Pinnell, 1949: Rossman, 1949; Rush, 1950; Tatum, 1954; and Wortman, 1950). Pinnell (1954) has stated that "attributing differences between reciprocal crosses to differences in the nature of the endosperm seems logical on the basis of the double contribution from the female side." However, somewhat contrary presented by Wortman (1950) show that the backcross progeny (A354×A21)×A21 was significantly more tolerant than (A357×A21)×A357 indicating that A21 probably contributed genes for "cold test" performance. His data also showed that the superiority of A21, both as seed and as pollen parent in crosses, might be considered as further evidence supporting the contention of Hooker and Dickson (1952) that gene action in the embryo is of major importance.

In studying tolerance of seed corn to freezing injury, Rossman (1949) found a similar influence of the maternal parent and stated that "maternal effects would be found in the pericarp and in the endosperm". He did not find any relationship between pericarp thickness and freezing tolerance, and concluded that endosperm characteristics are of importance. The results of the present study are not wholly in agreement. In the case of "cold test" germination with a protectant of seed given freezing treatment, no significant difference existed between inbeds W23 and W153R but single hybrid W23×W22R was significantly superior to W153R×A374B. Further the difference between hybrids (W23×W22R) OP and (W153R×A374B) OP reached statistical significance at the 1% level, suggesting that the pollen parent could influence the effects of exposure to frost. The data also show that inbred W153R was significantly more tolerant to forest injury than M14, but no significant differences existed between either the single hybrids W153R×A374B and M14×W64A or between their open pollinated progenies. These results suggest that the maternal parent is not as important in determining tolerance to freezing injury as in tolerance to attack by soil-borne pathogens in cold, wet soils. The ability of seed corn to resist damage by freezing is not necessarily correlated with resistance to attack by seed-decaying organisms.

The comparative "cold test" germination data of inbreds and the hybrids of which they are maternal parents, showed that inbred lines, in general, are more susceptible than their hybrid offspring to injury by freezing treatments, as well as to attack by seed-decaying organisms. The superiority of the hybrids probably can be attributed to heterosis, resulting in somewhat larger kernal size, more rapid growth and earlier physiological maturity than is the case for their parental inbred lines. Magee (1947) showed that embryonic organ initiation was more rapid for the former than for the latter.

Another interesting fact observed in these investigations is that inbred W23 possessed a high level of tolerance to damage by both freezing injury and to attack by seed-decaying pathogens; inbred M14 showed an intermediate reaction in both respects; while inbred W153R possessed a low level of resistance seed-decaying organisms, but a high degree of tolerance to freezing injury. It is evident that inbred lines respond differently in respect to freezing injury and to attack by soil-borne pathogens.

Summary

The effects of the freezing treatments on germination, tests being made at room temperature and under "cold test" conditions, both with and without a seed protectant were investigated.

The following conclusions can be drawn from these studies:

1. In all instances, as would be expected, "cold test" germination of seed treated with a fungicidal protectant, gave better results than without it. The use of a protectant provides a means of evaluating the injury due to freezing treatments, whereas without such, germination results are influenced additionally by the relative inherent resistance, or susceptibility, to seed-decaying pathogens.

2. Significant differences between inbred lines and between hybrids of which they are the parents are shown to exist in respect to tolerance to freezing injury as well as to attack by pathogenic fungi attacking the germinating seed. Inbred W23 and the hybrids of which it is the seed parent

relatively good stands both with and without a protectant under "cold test" conditions, inbred W153 and its hybrid offspring show a high level of tolerance to freezing injury, but are highly susceptible to attack by soil pathogens and inbred M14, and its hybrids progeny, are intermediate in both respects.

3. In general, hybrids are more tolerant in frost injury effects on germination and in attack by soil-borne seed-decaying pathogens than are their parental inbred lines.

4. The ability of seed corn to resist damage to germination by freezing treatments is not necessarily correlated with inherent resistance to attack by soil-borne seed-decaying organisms. The tolerance of hybrids to freezing injury was not as strongly affected by the maternal parent as was resistant to attack by soil-borne organisms under "cold test" conditions.

玉米對"寒冷試驗"反應的遺傳研究

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本試驗的目的在研究低溫處理的玉米種子，在溫室內和在"寒冷試驗"情形下及使用殺菌劑處理和無處理之間對發芽能力的影響。試驗結果如下：

1. "寒冷試驗"情形下，用殺菌劑處理過的種子，發芽率都比沒處理的種子高。殺菌劑處理的種子，發芽率減低的原因完全是低溫的傷害，相反的，無處理的種子，發芽率低減的原因是由於低溫和菌類侵害共同的結果。如是即可研究不同品種對抗寒性和抗菌性的遺傳性。

2. 自交系和用這些自交系作親本所產生的雜種後代，對抵抗低溫傷害與菌類侵害發芽種子的能力有顯著的差異。在"寒冷試驗"的情形下，自交系 W 23 及用其做母本的雜種，不管用與不用殺菌劑處理，都有較高的發芽率。自交系 W153R 及用其做母本的雜種，抗低溫傷害性很高，但是抗菌類侵害性却很低。自交系M14及其雜種的後裔，對抗寒性與抗菌性都呈中度。

3. 一般講，雜種對抵抗低溫傷害與土壤中菌類的侵害都較其親本的自交系爲强。

4. 玉米種子對抵抗低溫傷害的能力與抵抗土壤中侵害種子的菌類的遺傳性不一定有相關。在"寒冷試驗"情形下，雜種抵抗低溫傷害的能力不像抵抗菌類侵害能力受其母本遺傳性的影響那樣强。

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