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Effect of Symptomless Fungal Infection of Maize Seed on Germination in the Presence and Absence of *Pythium debaryanum*.[1]

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Seeds from ears of *Zea mays* infected by *Gibberella zeae* and *Nigrospora oryzae* frequently failed to show these fungi when germination took place in the absence of *Pythium debaryanum*. *Diplodia zeae* emerged from symptomless, infected seeds in both the presence and absence of *P. debaryanum*. Symptomless infection by *Fusarium moniliforme* evidently did not reduce germination in the absence of *Pythium*. The effect of *F. moniliforme* in the presence *Pythium* could not be tested because of inadequate controls. Symptomless infection by *D. zeae*, *G. zeae*, and *N. oryzae* always reduced the ability of seedlings to resist *Pythium*.
INDEX DESCRIPTORS: *Diplodia zeae*, *Fusarium moniliforme*, *Gibberella zeae*, *Nigrospora oryzae* and *Zea mays*.

Seeds of Southern Dent types of *Zea mays* L. are subject to infection by a large number of fungi, among them are *Diplodia zeae*, *Gibberella zeae*, and *Nigrospora oryzae* (Koehler 1959, Messiaen and Lafon 1957). Despite years of selection in breeding programs for resistance, these fungi continue to cause damage. One reason that selection has not been more effective is that infections by these fungi tend to be symptomless. In studying the performance of seed exposed to *Pythium debaryanum*, the importance of symptomless infection became evident. The failure of seed of resistant cultivars and ear progenies from entries previously selected for resistance to *Pythium* to survive the pythium test was strongly correlated with the presence of another organism within the seed.

With these preliminary observations in mind, a study was initiated to investigate the effect of symptomless infection by several commonly occurring fungi on the ability of maize seeds to resist infection by *Pythium debaryanum*.

MATERIALS AND METHODS

One host cultivar was a synthetic made by combining eight inbred lines (B7, B10, B14, Hy, L317, OhO7, WF9, and 38-11). Other cultivars, tested as inbred lines were C103, Os520, Hy, K757, M14, I153, and W64. The seed, grown at Ames, Iowa, was hand-shelled after discard of all ears showing symptoms of ear rot or cob rot. Seed infection was the result of natural inoculation.

A culture of *Pythium debaryanum* Hesse, originally isolated by P. Hoppe, University of Wisconsin, provided the inoculum for the pythium test. The test medium consisted of agar, 17g; glucose, 5g; NH_4NO_3, 0,32g; $CaCl_2$, 0.285g; KC1, 0.285g; KH_2PO_4, 0.285g; $MgSO_4.7H_2O$, 0.427g; biotin, 5μg; thiamine 100μg; and trace amounts of $MgCl_2.4H_2O$, H_3PO_3, $ZnSO_4$, and MnO_3, dissolved in 1 liter of solution (Foley 1980). Petri dishes (9-cm diam) containing the test medium were seeded with a *Pythium* inoculum disc (0.5-cm diam) and incubated 4 days at 23-27C. Each test consisted of 25 maize seeds from a single ear. These were put into a small bottle and surface-sterilized for less than 1 min with sodium hypochlorite (1.3%). The solution was decanted, and the bottles were plugged with nonabsorbent cotton and placed in a forced-air drying oven for 4 days at 35C. The seeds then were placed directly on the 4-day-old *Pythium* cultures and incubated 14 days at 12C, followed by 6 days at 23-27C (pythium test).

After incubation, seedlings with green plumules were classified as "germinated" while those whose plumules were necrotic, translucent, water-soaked, or were not emergent were classified as dead.

The selection of ears, used in this study, as having symptomless infection was done by examining seeds exposed to *Pythium*. *Diplodia zeae* was identified on the seeds by pycnidia, conidia, and white mycelium. *Gibberella zeae* did not sporulate on the seeds and, thus, was identified by the carmine color of the mycelium on the agar medium (Booth 1971, Toussoun and Nelson 1968). Some of the cultures tentatively identified by color were isolated and grown on sterilized peas to induce sporulation, so that identification could be confirmed by macroconidia. *Nigrospora oryzae* was identified by black spherical conidia. These three fungi were selected for reporting in the study because they were the most commonly associated with reduced germination in the pythium test. Seeds that did not yield a fungus or yielded organisms that were rare, or unidentified, or too common to correlate with reduced germination (ex. *Fusarium moniliforme*) were classified as "remainder" and used as the control.

Seed samples from all ears of the inbred lines (that were germinated in the pythium test) also were germinated in the absence of *Pythium* at 12C and 25C. For *D. zeae*, *G. zeae*, and *N. oryzae*, the results represent mean germination percentages of tests of 5, 6, and 4 ears, respectively. For "remainder", the figures represent means of 9 ears tested.

The data for the seed samples of the synthetic population, germinated in the presence of *Pythium* in 7 years are expressed as the means of germination percentages of all ears found infected with the respective fungi in each year. The number of ears of the synthetic tested each year was 2042, 1118, 1166, 1925, 858, 578, and 558. For each of the organisms, the number of ears classified as infected but symptomless were: for *D. zeae* 34, 59, 16, 6, 8, 0, and 0; for *G. zeae* 36, 56, 118, 77, 75, 17, and 28; and for *N. oryzae* 10, 5, 4, 23, 10, 5, and 3.

Analysis of variance was calculated for years and organisms by using a randomized design.

RESULTS

Seeds from ears shown in the pythium test to contain *G. zeae* frequently failed to show this fungus when germinated in the absence of *Pythium*. *Nigrospora oryzae*, too, occasionally failed to emerge, in the absence of *Pythium* from seeds known to contain *N. oryzae*. *Fusarium moniliforme* frequently emerged from symptomless seed when exposed to *Pythium*; however, it emerged only occasionally from seeds placed on the same culture medium without *Pythium*. *Diplodia zeae* emerged from symptomless seeds in both the presence and absence of *Pythium*.

Results from the inbred lines regarding the effect of symptomless infection on germination in the absence of *Pythium* are given in Table 1. *Fusarium moniliforme* probably did not reduce germination at either 12 C or 25 C because the seed lots in which *F. moniliforme* had killed the embryos (ear rot) were excluded from this study. *Diplodia zeae* reduced germination to 25% at 12 C and 42% at 25 C. *Gibberella zeae* was not as deleterious as *D. zeae*, but still reduced germination to 74% and 65% at 12 C and 25 C, respectively. Fewer seeds per test were

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Table 1. Mean germination percentages in a *Pythium* test of seed of seven inbred lines of maize infected with the indicated seed-borne fungus; all seeds were from symptomless ears.

Inoculum	Temperature	Seed-borne fungus			
		Diplodia zeae	*Gibberella zeae*	*Nigrospora oryzae*	"Remainder"
	(°C)	(%)	(%)	(%)	(%)
None	25	42.0	65.7	60.0	93.3
None	12	24.8	74.0	11.0	98.2
Pythium	12	6.4	2.7	6.0	44.9

covered with mycelium of *G. zeae* than were covered with mycelium of *D. zeae*. *Nigrospora oryzae* showed a marked response to temperature, destroying 89% of seedlings at 12 C, but only 40% of them at 25 C.

The effect of symptomless infection on germination in the presence of *Pythium* is indicated in tables 1 and 2. It would have been desirable to have a control consisting of seeds having no internal fungi, but this was impossible, mainly because of the frequent occurrence of *F. moniliforme*. Thus, the effect of *F. moniliforme* on seedling resistance to *Pythium* could not be determined, and the effect of each of the other fungi on seedling resistance to *Pythium* must be regarded as the effect of both *F. moniliforme* and the other fungus on the ability of the infected seeds to germinate in the presence of *Pythium*. Results from the inbred lines and the synthetic population showed that survival of seedlings in the pythium test always was less if the seeds were previously infected with any of the three organisms than if they were not so infected. The pythium treatment used in these experiments resulted in, for all practical purposes, complete destruction of the germinating seeds. This was true even for lines normally considered resistant to attach by *Pythium*.

DISCUSSION

This study may help to explain why selection for resistance to *Pythium* has given erratic results. Seeds infected with any of the pathogens studied are not suitable for testing reaction to *Pythium*, not only because they may show reduced germination, but also because the infection may obscure resistance to *Pythium* by causing low ratings in resistance tests. Thus, ears discarded because of failure in the test may be of genotypes with potentially useful resistance to *Pythium*. Because susceptibility to *Pythium* is common, improvement in seed performance can be made by increasing resistance to *Pythium*. The accuracy of the pythium test can be increased by excluding the results of tests of infected ears in the evaluation of phenotypes.

Diplodia zeae used to be considered the cause of the most serious ear rot in maize while *Gibberella zeae* was considered less important (Koehler 1959, Koehler and Holbert 1930). In this study, *G. zeae*, from symptomless ears, usually did not emerge from the seed unless *Pythium* was present, whereas *D. zeae* was readily noted in the *Pythium*-free germination tests. Thus, the increased awareness of *D. zeae* may be due to its ease of isolation. One of the results of this study was the discovery that a highly virulent soil pathogen such as *P. debaryanum* can be an effective tool in the detection of seed-borne pathogens difficult to isolate (Kulik 1973).

A possible explanation of the interactions among these fungi is they attack different plant systems. Thus the host plant may be able to compensate for or tolerate the effect of one pathogen but it is not able to resist the effects of having two or more systems assaulted. *Pythium* has an affinity for root tissue and preferentially attacks the roots and root initials (Foley 1980). *Gibberella* attacks stem tissue as well as the developing seeds.

Internal pathogens of the seed interact differently from surface inhibitants, which often are protective to seeds (Chang and Kommedahl 1963). There was no indication of antagonism of the internally borne pathogens to *P. debaryanum*. Symptomless infection may not be detected in a standard germination test, and these infections usually reduce the survival of maize seeds placed under adverse conditions such as low temperatures and the presence of soil pathogens. Thus, symptomless infections can be important causes of low seed quality.

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Table 2. Mean germination percentages in a Pythium test, of seed infected with three species of fungi from symptomless ears of a maize synthetic tested over 7 years.

Fungus[a]	Year						
	1959	1960	1961	1962	1963	1967	1968
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Diplodia zeae	1.7	10.7	1.8	2.7	5.0	N F[b]	N F
Gibberella zeae	5.1	13.6	11.8	9.9	6.2	10.8	6.1
Nigrospora oryzae	14.8	27.2	1.0	9.7	15.2	21.6	28.0
"Remainder"	38.4	30.3	34.7	27.2	27.6	35.1	38.3

[a] Differences among fungi significant at 1% level; differences among years not significant.

[b] N F = not found in indicated year.

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