Weight Loss of Mature Corn Stalk Tissue Induced by Twelve Fungi

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ABSTRACT

The capability of 12 fungi to cause decay in mature corn stalks of four single crosses harvested at two dates was investigated using a modified culture media technique developed to study wood rots. Decomposition was measured by dry weight changes during treatment.

The single crosses differed in their abilities to resist decay by various organisms. The differences were inconsistent between sampling dates and among organisms. *Diplodia maydis* and *Gibberella zeae*, organisms that commonly cause stalk rots of living corn plants, were less effective than *Trichoderma viride, Penicillium urticae,* and *Helminthosporium pedicellatum* in decomposing dead stalk tissue. In nature, these latter organisms and others may play a significant role in causing loss of stalk strength and thereby increase lodging. Phytopathology 60: 1790-1793.

Additional key words: corn stalk rot, *Zea mays,* saprophytic decay.

Diplodia maydis (Berk.) Sacc., *Gibberella zeae* (Schw.) Petch, *Fusarium moniliforme* Sheldon, and other organisms are known to cause stalk rot in senescent corn (*Zea mays* L.) plants. These organisms accentuate loss of physiological vigor, and are believed to continue their activities as saprophytes in dead plants leading to loss of structural strength. This contributes to plant lodging (3, 4, 7, 8, 10).

Other fungi which normally do not invade the living stalk prior to loss of physiological vigor may also rot mature stalks before harvest. The importance of these secondary invaders and weak parasites in the rotting of the mature stalk has not been determined.

Resistance to stalk rot of the living plant has been found to be related to sugar concn (5, 13), soluble solids content (15), tissue density (5, 11, 12, 14), level of glycoside (2), and other attributes. Selection for stalk rot resistance should also take cognizance of the firmness of the mature stalk. Various techniques have been developed to measure the mechanical strength of rotted and nonrotted corn stalks (4, 7, 8, 16).

Rotted stalks after they are mature and dead lose structural strength, but little work has been done to study the role of different microorganisms or of different plant genotypes in this phase of the stalk rot problem. The ease of decay of any tissue will depend on the proportion and amount of decay-resistant constituents present in it. Hence, in the wood industry the value of woods is based on their ability to resist deterioration and actions of wood-destroying fungi. Standardized tests have been established to measure these attributes.

In this report, a modification of a standard culture media technique (1) used in the study of wood rots was used to study the capability of several fungi to cause decay in mature corn stalks.

Materials and Methods.—Four single crosses, B14 × C103, WF9 × 38-11, OS420 × R109B, and W23 × L289, ranging in stalk rot reaction from resistant to susceptible, as measured in living plants, were grown in randomized complete block designs in 1966 and 1967. Each single cross was replicated 3 times. In both years, stalk samples at the second and third internodes above the brace roots were taken 2 and 4 weeks after anthesis. Stalk sections were dried immediately after sampling in a forced air-dryer for 48 hr at 65 C and stored for laboratory investigation.

Seven fungal organisms were used in 1966. Five others were added in 1967. The organisms used were *Cephalosporium acremonium* Cda., *Chaetomella* sp., *Colletotrichum graminicola* (Ces) Wils., *Diplodia maydis* (Berk.) Sacc., *Gibberella zeae* (Schw.) Petch, *Helminthosporium pedicellatum* Henry, *H. sorokinianum* Sacc. ex Sorok., *Penicillium urticae* Bainier, *Penicillium* sp. (isolate MOP 50), *Sclerotium bataticola* Taub, *Trichoderma viride* Pers. ex Fr., and an unidentified chlamydospore forming Ascomycete fungus (isolate As.22), herein designated as Ascomycete 22. With the exception of *H. sorokinanium,* all were isolated from diseased or dead corn stalks.

The wt loss of stalk sections inoculated with each fungus and incubated in closed containers for 1 month was used as a measure of stalk decomposition. The second internodes of the harvested stalks were cut into 1-inch pieces, labeled and oven-dried to constant wt at a temp of 68 ± 2 C. The stalk pieces were kept in a desiccator containing calcium chloride for 30 min before weighing. Water was added in separate beakers up to 1.5 times the wt of each individual 1-inch stalk section. The beakers were covered with parafilm, and the water allowed to equilibrate for 48 hr. The stalks were then autoclaved twice at 15 lb. psi for 30 min, 48 hr apart. Forty cc of potato-dextrose agar (PDA) was dispensed into individual 230-cc AC bottles fitted with plastic screw lids. The bottles were then autoclaved for 15 min at 15 lb. psi. The different bottles were inoculated with discs of the same size as the circumference of a No. 2 cork-borer taken from week-old

cultures of each organism, and the fungal cultures allowed to grow at room temp for 1 week. Single-stalk pieces were then aseptically placed on the cultures in the bottles. Control stalk pieces were placed on sterile PDA. Lids were placed on the bottles, tightened and then released one-fourth turn from the tightened position. The bottles were incubated in an incubator for 1 month at 20 ± 1 C. Rotted stalk pieces were then removed from the bottles and the surface mycelium removed. The stalk pieces were dried to constant wt, as was done prior to incubation with fungi, and weighed.

The data were analyzed statistically. Least significant difference (LSD) values for making comparisons between means of isolates within each single cross were calculated by the modified Duncan multiple range test (6). In these calculations, K was set at 100, a value which approx equals the .05 level of significance.

RESULTS.—The results obtained in 1966 and 1967 were similar, therefore only 1967 data are presented in this report. All 12 fungi and four corn single crosses were studied in each experiment for each sampling date. Each experiment was repeated. The data were examined statistically first as separate experiments and then combined. Conclusions reached from the analysis of data from separate experiments were essentially the same as those seen in the combined analyses.

First sampling date.—Highly significant differences occurred among the different fungal isolates in ability to cause decay and among the single crosses in ability to resist decay (Table 1).

The fungi can be classified according to their abilities to cause decay as weak, moderate, or efficient saprophytes. Weight losses for weakly saprophytic fungi *D. maydis*, *G. zeae*, and *Chaetomella* sp. were either not significantly or slightly significantly different from the control. The moderately saprophytic organisms are *Colletotrichum graminicolum*, *H. sorokinanium*, *Cephalosporium acremonium*, *Penicillium* sp., and *S. bataticola*. These fungi were inconsistent among hybrids and experiments in the amount of decay they caused in mature stalk tissue. The remaining fungi were consistent in causing large amounts of tissue deterioration. These, in decreasing order of their abilities, are *T. viride*, *P. urticae*, *H. pedicellatum*, and Ascomycete 22.

When ranked according to the amount of decay they caused, the ordinal positions of the 12 fungi fluctuated within different single crosses. This resulted in a highly significant interaction between the fungal isolates and single crosses. This interaction was typified by decay activities of *Penicillium* sp., *C. acremonium*, and Ascomycete 22 on the four hybrids. *Penicillium* sp. was more effective in decomposing tissues of B14 × C103 and OS420 × R109B, and less effective on WF9 × 38-11. But *C. acremonium* was more active in rotting tissues of WF9 × 38-11 and less active in rotting those of B14 × C103 and OS420 × R109B. Moreover, *C. acremonium* was more effective in decomposing W23 × L289 stalk tissue and less effective in decomposing OS420 × R109B stalk tissue when compared with the action of Ascomycete 22 on the same two hybrids.

In considering mean total of tissue decomposition caused by all fungi, B14 × C103 appeared to be the most resistant and W23 × L289 the least resistant single cross to decomposition.

In the combined analysis, the interactions of isolates × experiments and hybrids × experiments were both statistically significant at the .01 level. The mean sq values for the interactions were small in comparison to the values for isolates or for hybrids. Although the ranking of the isolates in the two experiments was similar, it was not identical. The ranking of hybrids was also inconsistent between the two experiments.

Second sampling date.—The data obtained from samples harvested during the second sampling date (Table 2) were very similar to those of the first sampling date; however, in the second sampling date when the susceptible single cross W23 × L289 was tested, the wt loss of only a few isolates differed significantly from the control. Moreover, in stalk tissue collected during the first sampling date, the amount of decay

TABLE 1. Mean wt loss as a percentage of original wt of stalk section of four corn single crosses sampled 2 weeks after anthesis and incubated for 1 month with 12 fungi in closed containers

Fungus	Single cross hybrid				Mean for all hybrids
	B14 × C103	WF9 × 38-11	OS420 × R109B	W23 × L289	
None—control	20.33[a]	24.00	24.79	26.39	23.88
Penicillium urticae	30.94	36.39	37.39	36.87	35.40
Trichoderma viride	33.77	37.16	35.40	34.83	35.29
Ascomycete 22	33.36	37.65	36.08	33.09	35.04
Helminthosporium pedicellatum	29.48	33.39	33.96	36.34	33.29
Colletotrichum graminicola	30.51	34.65	32.63	34.69	33.12
H. sorokinianum	28.26	33.88	32.14	34.14	32.11
Sclerotium bataticola	30.19	32.76	29.83	33.98	31.69
Cephalosporium acremonium	27.72	35.37	28.96	33.25	31.32
Penicillium sp.	28.43	27.99	30.13	32.22	29.69
Diplodia maydis	24.42	29.98	28.40	29.74	28.14
Gibberella zeae	25.44	25.50	27.41	29.10	26.86
Chaetomella sp.	19.84	25.64	23.37	28.92	24.44
Mean for all fungi	28.53	32.53	31.31	33.10	
Modified LSD[b]	2.83	3.16	2.67	2.74	

[a] Each value is the mean of three replicates in each of two experiments, except the control, which is the mean of nine replicates in each of two experiments.

[b] Duncan modified LSD; K = 100 approximates .05 level of significance.

TABLE 2. Mean wt loss as a percentage of original wt of stalk sections of four corn single crosses sampled 4 weeks after anthesis and incubated for one month with 12 fungi in closed containers

Fungus	Single cross hybrid				Mean for all hybrids
	B14 × C103	WF9 × 38-11	OS420 × R109B	W23 × L289	
None—control	27.71[a]	27.29	25.10	22.51	25.65
Trichoderma viride	35.86	36.73	37.50	28.88	34.74
Cephalosporium acremonium	35.19	35.68	34.43	28.02	33.33
Ascomycete 22	37.89	34.53	32.22	27.66	33.07
Helminthosporium pedicellatum	35.38	33.43	33.69	26.81	32.33
Penicillium sp.	32.95	35.44	33.26	27.59	32.31
Penicillium urticae	32.43	33.12	34.40	29.25	32.30
Colletotrichum graminicola	34.53	33.04	29.32	26.58	30.87
Diplodia maydis	32.05	29.76	31.03	27.88	30.18
H. sorokinianum	28.53	31.74	31.38	27.86	29.88
Gibberella zeae	30.51	29.68	24.94	24.37	27.38
Sclerotium bataticola	29.17	30.55	26.18	23.40	27.32
Chaetomella sp.	25.25	24.85	24.18	26.32	25.15
Mean for all fungi	32.48	32.38	31.04	27.05	
Modified LSD[b]	2.79	2.28	2.64	3.68	

[a] Each value is the mean of three replicates in each of two experiments except the control, which is the mean of nine replicates in each of two experiments.

[b] Duncan's modified LSD; K = 100 approximates .05 level of significance.

caused by all isolates was greater in the susceptible single cross W23 × L289 than in the resistant single cross B14 × C103; the reverse was the case on stalk tissue from the second sampling date. This difference was significant at the .01 level.

Helminthosporium pedicellatum and *D. maydis* were more efficient in decomposing B14 × C103 stalk tissues and less efficient in decomposing WF9 × 38-11 stalk tissue when compared with the decomposition due to *Penicillium* sp. and *H. sorokinianum* on the same single crosses (Table 2).

As was true for the first sampling date, the interactions of isolates × experiments and hybrids × experiments were statistically significant at the .01 level.

DISCUSSION.—Although wt losses in corn stalk tissue were measured, the role of secondary invaders, as reported in this study, does not necessarily reflect the relative importance of secondary organisms in the decomposition and weakening of corn stalks after maturity as it happens in nature. The system used in this study is highly artificial, because of the concn of inoculum and the environment under which each organism grew. In the field, a combination of several organisms is probably involved in the decay of corn stalks. Furthermore, the activity of these organisms may follow a prior invasion by one or more parasites that have killed the plant. The results show, however, that several fungi can decompose corn stalk tissue, and that variation in resistance to decomposition exists in stalk tissue of different hybrids. The results also show that two common stalk-rotting organisms, *D. maydis* and *G. zeae*, are less effective in rotting mature corn stalks than are *T. viride, P. urticae, H. pedicellatum,* and Ascomycete 22. It should be noted that *T. viride*, the most efficient organism in rotting mature stalks, has been reported to be present in large numbers in root rhizospheres at the time of plant senescence (9).

In comparing organisms for ability to decay stalk tissue before harvest, hybrids that bring out differences between organisms are needed. Single crosses of the OS420 × R109B and W23 × L289 type do not satisfy this requirement and therefore should not be used. Hybrids like B14 × C103 and WF9 × 38-11 are good differential hybrids for measuring the relative ability of fungi to cause decay. B14 × C103, however, appeared to be more resistant to decay by the various fungi used in this study than WF9 × 38-11. Therefore, intermediate reacting hybrids like WF9 × 38-11 might be the best type to use.

The importance of the level of resistance of the different single crosses in limiting the saprophytic activity of the different secondary invaders is not clear. In tissue of the single cross B14 × C103, which is resistant to stalk rot caused by *D. maydis* in the growsignificantly lower than wt losses in tissue of the susing plant, wt losses due to saprophytic decay were ceptible single cross W23 × L289 in samples taken 2 weeks after anthesis. The converse was true in samples taken 4 weeks after anthesis.

The highly significant interaction of fungi with hybrids also points out some difficulties in determining hybrid reaction to saprophytic decay by the wt-loss method. Certain hybrids appear to have a greater resistance to decay by some fungi than by the others. This could have important implications to field lodging. Therefore, in selecting corn hybrids for resistance to stalk tissue decay after maturity but before harvest, several organisms including *T. viride, P. urticae, H. pedicellatum, Colletotrichum graminicola* and *Cephalosporium acremonium* might be needed in laboratory tests. It would be inadequate to use *T. viride* alone, though it is the most efficient rotter, since in this study this fungus selectively rotted certain hybrids more than others.

For unknown reasons, stalk samples collected 2 weeks after anthesis seem superior to samples collected later in the season for studies on saprophytic decay. In this study, the ability of different organisms to decompose stalk tissue was better evaluated on samples harvested 2 weeks after anthesis than on samples har-

vested 4 weeks after anthesis. Frequently, at the later date, there were no significant differences in the ability of the fungi to decompose stalk tissue. The failure of different hybrids to rank the same when tests were repeated or when stalk samples tested were collected on different harvest dates is another important problem that needs to be solved.

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