Relationship Between Kernel Pericarp Thickness and Susceptibility to Fusarium Ear Rot in Field Corn

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ABSTRACT

Hoenisch, R. W., and Davis, R. M. 1994. Relationship between kernel pericarp thickness and susceptibility to Fusarium ear rot in field corn. Plant Dis. 78:517-519.

In a 2-yr study, the thickness of kernel pericarp and aleurone layers was compared among 12 dent corn hybrids representing a range of resistance to ear rot caused by *Fusarium moniliforme*. The pericarp layer on the cap of the eight hybrids with high or intermediate levels of resistance (determined by the percentage of kernels visibly infected with the fungus) was thicker than the pericarp of the four susceptible hybrids. In contrast, the aleurone layer of susceptible hybrids was thicker than the aleurone layer of the intermediate and resistant hybrids. It is suggested that the relatively thin pericarp layer of the susceptible hybrids allows access of the fungus into the kernels, especially through insect wounds.

Additional keyword: maize

In California, Fusarium ear rot of corn (*Zea mays* L.), caused by *Fusarium moniliforme* J. Sheld. (teleomorph = *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura), is associated with insect damage to kernels. Farrar and Davis (3) demonstrated that the severity of ear rot was positively correlated with intraear populations of western flower thrips (*Frankliniella occidentalis* Pergande) and loose husks surrounding the developing ears. Husks of resistant hybrids, but not those of susceptible hybrids, physically excluded the entry of thrips through the silk channel openings during the period of ear development when kernels were highly susceptible to infection. Presumably, the feeding activity of thrips allowed entrance of the fungus through the pericarp of the kernels. An insecticide application to the ears of susceptible hybrids at green silk stage of development almost completely eliminated Fusarium ear rot in California (2,3).

Scott and King (7) showed that resistance to ear rot is under the genetic control of the female plant, which is consistent with the relationship between tight husks and relatively high resistance. However, they suggested that the site of resistance may be the pericarp of the kernels, which also is maternal tissue (7). The objective of this study was to examine the thickness of the pericarp and aleurone layers of kernels from many of the same hybrids included in the earlier study on thrips, husk configuration, and severity of ear rot (3). The dent corn hybrids, which ranged from distinctly susceptible to highly resistant to ear rot, were chosen from the University of California Cooperative Extension field screen for kernel resistance to Fusarium ear rot (2). For purposes of class comparisons, the hybrids were grouped into susceptible, intermediate, and resistant reactions to ear rot based on the percentage of kernels visibly infected with the fungus.

Accepted for publication 22 January 1994.

MATERIALS AND METHODS

Field experimental design and cultural practices. Twelve corn hybrids were planted on the campus of the University of California, Davis, in May of each year in randomized complete block designs with five replications in 1990 and six replications in 1992. Each plot was four rows in 1990 and two rows in 1992 and was 8 m long planted on 75-cm centers. The rows were planted at 8.3 seeds per meter. Nitrogen (168 kg/ha) was applied as ammonia before sowing each year. Each planting received a single cultivation for weed control and was irrigated by furrow every 2 wk. No fungicide or insecticide was applied.

Kernel measurements. Two healthy ears were collected at random from each plot at dough and early dent stages of ear development each year. The ears were broken about two-thirds down from the tip. Two to four undamaged kernels were removed from each ear, cut in half longitudinally (parallel to the flattened side), and placed in a 10-ml aqueous solution containing 1% chromic acid, 10% acetic acid, 37% formaldehyde, and a drop of Tween 20 (5). After vacuum infiltration for 10 min, the solution was replaced with fresh solution (when necessary, the samples were stored in this solution). The kernels were then rinsed in distilled water and were dehydrated in a series of solutions of 10% ethanol to 100% tertiary butyl alcohol at 15% increments in 1-hr intervals. The samples were then embedded in TissuePrep 2 (Fisher Scientific, Fair Lawn, NJ) paraffin according to Jensen (4). Embedded kernels were sectioned with a rotary microtome at 12 μm and fixed onto glass slides on a slide warmer at 45 C for 12 hr. Paraffin was removed from the tissues by transferring the slides through a series of three baths of 50% xylene, 25% clove oil, and 25% tertiary butyl alcohol, followed by three baths of 100% xylene. The tissue was stained and counterstained with safranin and fast green (4). Coverslips were mounted over the specimens with Permount (Fisher Scientific).

Thickness of pericarp and aleuorone layers was measured at 400× with an eyepiece micrometer on a compound microscope. Although measurements were initially made from many locations on the kernel, pericarp and aleurone measurements were finally limited to the two shoulders, or high points, of the caps of the kernels. Measurements at locations other than the shoulders were discontinued because of extreme variability. The two measurements from each kernel were averaged, and the means of measurements from each kernel from an individual ear were averaged. Data analysis was performed on the means of the two ears from each replication.

Disease assessment. When the plants were fully senescent at late dent, about 20 ears per plot were evaluated for ear rot severity. Husks were peeled back and ears were rated for ear rot severity on a calibrated pictorial scale based on five increments of a 90° angular transformation, where 0 = no visual disease, 1 = 10%, 2 = 35%, 3 = 65%, 4 = 90%, and 5 = 100% of the kernels visually affected. Data analysis was performed on the mean ratings for each plot. Ears damaged by common smut or corn earworm (*Helicoverpa zea* (Boddie)) were not included in the assessment.

Statistical analysis. Data were analyzed in MSTATC (Michigan Statistics Group, Michigan State University, East Lansing) by analysis of variance and contrast analysis.

RESULTS

The thickness of the pericarp of kernels from the intermediate and resistant groups of hybrids was significantly greater ($P \leq 0.05$) than the thickness of the pericarp of the susceptible hybrids (Table 1). The pericarp thickness was not significantly different between the intermediate and resistant groups of hybrids.

There were no significant differences in pericarp thickness among the susceptible hybrids, but in both years, significant differences occurred among the hybrids with intermediate resistance to ear rot.

The thickness of the aleurone layer of kernels from the susceptible hybrids was significantly greater ($P \leq 0.05$) than that of the intermediate and resistant groups of hybrids (Table 1). The thickness of the aleurone layer was not significantly different between the intermediate and resistant groups of hybrids. In 1992, there were no significant differences in the thickness of the aleurone layer among hybrids within the susceptible, intermediate, or resistant groups.

In general, the trend of thinner pericarp and thicker aleurone layers in the susceptible hybrids also occurred at dough stage of development, but the variability in the measurements was greater at dough than at early dent (*data not presented*).

The susceptible hybrids as a group were distinctly more susceptible to ear rot than the intermediate and resistant hybrids (Table 2). In both years, the percentage of affected kernels from ears of susceptible hybrids was significantly greater ($P \leq 0.05$) than that of the intermediate and resistant hybrids, and the percentage of affected kernels in the intermediate hybrids fell between the severity of ear rot of the other two groups. There was significant variability in disease incidence among the hybrids within the susceptible and intermediate groups in both years.

DISCUSSION

The thickness of the pericarp and aleurone layers of the kernels at early dent could be used to separate hybrid susceptibility to Fusarium ear rot in California. As a group, the susceptible hybrids possessed thinner pericarps, but thicker aleurone layers, than the hybrids with an intermediate or high degree of resistance to ear rot. The thickness of the pericarp and aleurone layers did not differ between intermediate and resistant hybrids, and the incidence of ear rot was similar between the two groups. In contrast, the susceptible hybrids were heavily infected with *F. moniliforme* and possessed pericarps that were distinctly thinner than those of the intermediate and resistant hybrids selected for this study. The thickness of the pericarp and aleurone layers at dough stage was highly variable and was not as reliable as measurements at early dent for determining trends among hybrids.

If *F. moniliforme* enters the kernels through damage caused by insects, as suggested by several researchers (3,6,8), the thickness of the pericarp may be an important factor in resistance to ear rot. All parts of the pericarp in the later stages of kernel development are composed of dead cells that are cellulosic tubes (9). The overlapping, interlocking arrangements of these thick-walled cells account for considerable strength of the pericarp. A thin pericarp might give the fungus greater access to the kernel, especially through wounds caused by the feeding of insects. In California, relatively high intraear populations of thrips and, presumably, the damage they cause by feeding are associated with hybrids susceptible to ear rot (3).

In contrast to the pericarp layer, the aleurone layer was thicker in the susceptible hybrids than in the intermediate and resistant hybrids. The significance of this in relation to susceptibility to ear rot, if any, is unknown. The aleurone layer develops from the endosperm and is composed of living cells. Unlike the pericarp, which is maternal tissue, both male and female genes contribute to the development of the aleurone layer. In corn, the aleurone layer is usually one cell thick, and it is pushed up against the pericarp during the expansion of the endosperm (1). By early dent, the aleurone layer is mature and measurements are reliable. Although resistance to ear rot is governed by maternal tissue, the consistent differences in the thickness of the aleurone layer between susceptible and resistant hybrids warrant further study.

The corn hybrids included in this study were chosen from a large number screened in northern California for resistance to ear rot. The performance of these hybrids in other environments is unknown. The relative abundance of thrips within ears of corn grown in California, which apparently influences hybrid susceptibility to Fusarium ear rot, may not occur in other corn-producing

Table 1. Thickness of pericarp and aleurone layers (μm) on the cap of kernels from corn ears in early dent stage of development[a]

Hybrids		1990		1992	
		Pericarp	Aleurone	Pericarp	Aleurone
Susceptible (S)					
Pioneer 3540		35.9	21.8	44.2	27.6
Pioneer 3569		30.1	17.3	40.6	24.8
Pioneer 3603		25.2	12.6	44.2	26.4
Pioneer 3779		33.5	18.2	43.5	24.8
Intermediate (I)					
Asgrow Rx749		56.2	14.1	46.7	23.5
Pioneer 3295		37.9	14.1	52.9	22.8
Pioneer 3733		49.8	20.1	57.5	22.1
Pioneer 3267		44.6	15.1	66.2	21.0
Resistant (R)					
Asgrow 947		50.5	18.4	47.3	22.2
NK S4590		41.1	17.6	53.8	20.2
Pioneer 3343		37.8	10.9	53.2	21.9
Pioneer 3377		45.5	14.0	53.2	22.2
Contrast	df	Sums of squares[b]			
S vs. (I+R)	1	2,706.3*	49.4*	1,846.9*	244.6*
I vs. R	1	116.0	4.4	185.9	6.3
Within S	3	319.7	216.5*	53.4	33.6
Within I	3	908.0*	123.9*	1,213.1*	20.7
Within R	3	452.1*	181.1*	169.8	17.5

[a] Values are the means of five and six replications (1990 and 1992, respectively) of two measurements on each of two to four kernels from each of two ears per replication.
[b] * = Significant at $P \leq 0.05$.

Table 2. Severity of ear rot of corn hybrids at late dent stage of development

Hybrids		Percentage of visually affected kernels per ear[a] 1990	1992
Susceptible (S)			
Pioneer 3540		73.9	70.4
Pioneer 3569		81.2	49.7
Pioneer 3603		92.9	86.2
Pioneer 3779		58.7	73.6
Intermediate (I)			
Asgrow Rx749		5.4	1.0
Pioneer 3295		14.2	3.7
Pioneer 3733		25.6	19.8
Pioneer 3267		9.8	17.1
Resistant (R)			
Asgrow 947		0.5	0.5
NK S4590		0.3	1.0
Pioneer 3343		4.0	2.6
Pioneer 3377		2.1	8.8
Contrast	df	Sums of squares[b]	
S vs. (I+R)	1	95.43*	96.60*
I vs. R	1	6.40*	2.23*
Within S	3	4.84*	5.13*
Within I	3	2.38*	5.60*
Within R	3	0.72	1.86

[a] Values are the means of five and six replications (1990 and 1992, respectively) of about 20 ears from each replication. When the plants were fully senescent at late dent, husks were peeled back and ears were rated for ear rot on a 0–5 pictorial scale based on five increments of a 90° angular transformation, where 0 = no disease, 1 = 10%, 2 = 35%, 3 = 65%, 4 = 90%, and 5 = 100% of kernels affected.
[b] * = Significant at $P \leq 0.05$. Analysis performed on mean ratings from each replication. The percentage of affected kernels was calculated by: % = 100 (sine (rating × 18))^2.

areas in the United States.

In general, the susceptible hybrids were early maturing, or short-season hybrids, relative to the resistant hybrids included in the study. Loose husks in the early maturing hybrids allow for quick drying of the grain but may inadvertently increase susceptibility to ear rot by providing entry for insects through the silk channel opening. Relatively thin pericarps also may play a role in the quick drying of these hybrids and may further increase susceptibility to insect damage and ear rot. Thus, the thickness of the pericarp may be one of several factors that play a role in resistance to ear rot. These factors need to be studied in a larger number of hybrids to confirm their involvement in corn resistance to Fusarium ear rot.

ACKNOWLEDGMENTS

We thank Pioneer Hi-Bred International Inc., Des Moines, Iowa, for support for this project, and F. R. Kegel, K. D. Marshall, and H. Wang for assistance.

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