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## Anthracnose Kernel Rot of Maize Caused by *Colletotrichum graminicola* (Ces.) Wils.: Mode of Entrance into and Disease Progression in Ears<sup>1</sup>

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The mode of establishment of *Colletotrichum graminicola* in maize ears to cause kernel infection was studied for two types of infective propagules and three sites of penetration. Shank inoculations with either oat-grain inoculum or spores induced a greater percentage of infected kernels than inoculations of the husk or kernel area with an infected oat grain or inoculations of the silks with an oat grain or a spore suspension in 1985. But in 1986, a spore suspension in the kernels ranked first among all the treatments based on the basis of disease severity on the kernels. However, a large percentage of kernel infection, in general, came from shank infections, the pathogen progressing from the vascular tissue of the cobs into the kernels. The date of inoculation did not significantly affect disease development. Infection in the maize ear was more severe than indicated by symptoms.

INDEX DESCRIPTORS — Anthracnose, *Colletotrichum graminicola*, ear rot, kernel rot, maize, seed quality

Anthracnose caused by *Colletotrichum graminicola* (Ces.) Wils. gradually has become severely damaging both as a leaf and a stalk disease of maize (*Zea mays* L.) in the warmer, more humid areas of the Corn Belt (2, 3, 6, 9, 17). Within the last two decades, accumulated evidence has demonstrated the ability of the pathogen to cause yield losses in dent maize, which is of concern to U.S. maize producers, breeders, and seedsmen.

The report of ear infection by *C. graminicola* (9, 14, 15) justifies this concern, because ear infection may lead to kernel infection and seedling blight (11, 12). No information is available on resistance to kernel infection (13).

Miles et al. (7) reported a positive, significant genetic correlation between anthracnose and *Diplodia* stalk rots in two maize populations. Koehler (5) reported that most of the ear infections by *Diplodia zeae* came through the point of attachment of the ear to the shank. Presumably, *D. zeae* can invade progressively from the stalk to the shank and to the ear. Knowledge of the actual course or path of kernel infection by *C. graminicola* is important in designing a practical and economical control program for this phase of maize anthracnose.

The primary objective of this study was to determine the common paths of kernel infection and to determine the progress of ear and kernel infection after inoculation by various methods.

### MATERIALS AND METHODS

Four hybrids (B73HtxLH38, B73HtxLH24, FR29xLH51, and B73HtxLH22), ranging from early to late maturity, were used as the main plots in a split-plot design in 1985; five methods of inoculation were the subplot treatments: (a) infected oat grain in shank, (b) infected oat grain in kernels, (c) spore suspension in shank, (d) spore suspension in silks, and (e) control.

In 1986, two hybrids, one susceptible to anthracnose stalk rot (C123xA619Ht) and the other resistant (W64AHtxW117Ht), were the whole-plot treatments of a split-split-plot design. Eight methods of inoculation were the subplot treatments: (f) infected oat grain in the shank, (g) infected oat grain in the kernels, (h) spore suspension in the shank, (i) spore suspension in the ear tip, (j) spore suspension in the kernels, (k) infected oat grain in the ear tip, (l) spore suspension behind the leaf sheath, and (m) control. Two dates of inoculation (at anthesis and 2 weeks later) were the sub-subplot treatments.

The hybrids in 1985 were machine-planted in a randomized block design, replicated three times. Six weeks after planting, the plants were thinned to about 60,000 plants/ha.

The 1986 experiment was hand-planted by using a jab-planter in a 5-m single-row/date/sub-treatment, replicated four times. Six weeks after planting, the plants were thinned to about 50,000 plants/ha.

A sterile oat medium was prepared by placing 1000 cm<sup>3</sup> of oat grain in a 3-L flask with 600 ml of distilled water and autoclaving twice (24-h interval) at 118-121 C for 30 min. The grains were then seeded with a 16-day-old culture of an isolate of *C. graminicola* isolated from locally grown maize. During incubation, the flask was shaken daily to avoid clumping. Unseeded sterile oat grain was the control.

For oat grain inoculations of "infected oat grain in shank" and "oat grain in kernels", a 0.5-cm hole was made in the ear shank or through the husks into the kernels, and, with the use of forceps, an oat grain was inserted into the hole. For the treatment "infected oat grain in ear tip" an oat grain was placed in the silk channel.

For treatments using spore suspensions in 1985, a 30-cm<sup>3</sup> volume of infected oat grain was blended in sterile distilled water and filtered with a double layer of gauze. In 1986, isolate 120, isolated from infected kernels of the 1985 experiment, was used to seed the sterile oat-grain medium, and the spore suspension was obtained by washing a 7 to 10-day-old culture with sterile distilled water, then filtered with double gauze. In both years, the spore concentration was determined with a haemocytometer and adjusted to about 2-2.5x10<sup>5</sup> spores/ml. Two ml of spore suspension was injected into the shank, onto silks at the tip of ears, in the kernels through the husks, or sprayed behind the leaf sheath, by using a pistol-grip syringe (Ideal Instruments, Inc. Chicago, IL).

At harvest, five ears were sampled in order of occurrence (one every two plants) in each treatment. Stalk internodes above and below the ear nodes were included and checked for discoloration (stalk rot). Harvested ears and the attached nodes were dried at 30-35 C for about 7-days.

Husk and shank infections were determined by examining them for presence of acervuli and/or discoloration.

One-third of the ear was shelled, depending on the inoculation treatment, the upper third for silk inoculations, the lower third for shank inoculations, and the middle for husk inoculations, and grains were kept separately. A random sample of 100 kernels/plot was sterilized in 0.5% sodium hypochlorite for 2-3 min and then rinsed in sterile distilled water. The samples were then plated on oatmeal agar amended after autoclaving with dicloran (40ug/ml), presterilized in a 30% ethanol solution (25 kernels/petri dish), and incubated at room temperature under ultraviolet lights to enhance sporulation and to retard floccose growth (10). After 3 days of incubation, acervuli of *C.*

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*graminicola* could be seen on and around infected kernels, which were then counted and recorded before contamination by *Fusarium moniliforme* masked the acervuli or before germination of kernels.

In 1986, a sample of each treatment was collected from the susceptible hybrid and examined for development and progression of the disease. The extent of conspicuous surface rot on kernels and of discoloration of vascular tissue in the cob was measured. The frequency of isolation of *C. graminicola* from the nondiscolored vascular tissue was determined by seeding dishes of oatmeal agar with pieces of nondiscolored and discolored vascular tissue, the latter being the control.

## RESULTS

The 1985 and 1986 growing seasons were nearly diametric opposites. Although 1986 had an abundance of rain throughout the maize-growing season, 1985 was dry.

Highly significant variation ( $P=0.0001$ ) occurred among methods of inoculation and a significant interaction of hybrid x method for shank, husk, and kernel inoculations occurred in both years. Also, estimates of hybrid differences were significant ( $P=0.05$ ) for kernel infection in both years.

Disease incidence on the shank and kernels was greater on hybrids that had a susceptible parent than on the other hybrids. Inbred lines B73 and C123 have been reported susceptible to anthracnose stalk rot (1, 16). In 1985, hybrids with the greatest shank infection had the greatest kernel infection. Date of inoculation (not tested in 1985) did not affect the response of hybrids to ear infection.

In general, disease severity for each method of inoculation was greater on the susceptible hybrid than on the resistant one. However, in 1986, three inoculation treatments: (g) oat grain in kernel, (k) oat grain in silk, and (i) spore suspension in silks induced more disease on kernels of the resistant hybrid than on the susceptible one.

The shank inoculation treatments (a) and (c) in 1985 had the greatest shank and kernel infections (Table 1). The treatment with infected oat grain in the shank ranked first in percentage of shank and kernel infection.

In 1986, under favorable environmental conditions, the spore suspension was the best inoculum. The most severe husk and kernel infections were obtained from methods using a spore suspension, which also had high levels of shank infection (Table 1).

Only the treatment with oat grain in kernel induced a considerable level of husk colonization (63%) in 1985; nevertheless, kernel infection level induced by the oat grain in kernel treatment was not significantly different from that induced by spore suspension in shanks (14% versus 21%).

The greater means of extent of rot on kernels occurred for the first date of inoculation (2.6 cm) and for the treatment with spore suspension in kernels (6.5 cm).

Significant differences were obtained among treatments and hybrids for number of discolored shank internodes, but the date of inoculation had no effect on the progression of the disease in the shank. The highest means for the number of discolored shank internodes was obtained with C123xA619Ht, and for the treatment involving infected oat grain in shank. In general, discoloration was limited to the inoculated shank internode, on the resistant hybrid.

When husked ears were split, it was observed that the level of discoloration in the interior of the cob extended beyond the rot on kernels as viewed from the exterior. The length of discoloration was greatest in treatments involving spore suspension inoculations in shanks and kernels.

Cultures of *C. graminicola* were recovered 22% of the time from nondiscolored vascular tissue of the cob versus 89% from the adjacent discolored tissue.

## DISCUSSION

In 1986, the difference in reaction between susceptible and resistant hybrids was greater for shank infection (74% versus 28% respectively) than for kernel infection (28% versus 22%). The levels of infection in kernels in both years were in the range of infection obtained by Warren (11). On the susceptible inbred E43-25, he found 39% of kernels infected. By placing 1 ml of spore suspension near the tip of the ear of inbreds susceptible and resistant to kernel infection, Warren and Shepherd (13) found more than 50% of the kernels infected.

The high level of shank and kernel infection (28% and 22%) versus the very low percentage of stalk rot (0.32%) on the hybrid W64AHt x W117Ht suggests either that: (a) resistance to ear infection and resistance to stalk rot are not conditioned by the same genetic system or (b) that resistance may be overcome in the ear if ingress occurs before kernels mature and the moisture content of the kernels is very high.

In both 1985 and 1986, estimates of variance significantly differed among methods of inoculation for all the traits tested. This indicated the importance of type of inoculum used and site of inoculation in the process of disease establishment.

In 1985, the shank-inoculation treatments of infected oat grain and spore suspension had the greatest shank and kernel infections, as compared with the silk and husk inoculations. Plants from the treatment with infected oat grain in the shank ranked first in percentage of shank and kernel infections, but the difference in kernel infection was not significant from that induced by the treatment of spore suspension in the shank. This may have been due in part to the type of infective propagules present in the two types of inoculum. The dry conditions could have been, in part, responsible for the poor initial infection observed in 1985, with conidia failing to germinate. Wu and Warren (18, 19) reported that desiccation stress caused fluorescence of conidia of *C. graminicola* and that the fluoresced conidia did not germinate.

The infective propagules of infected oat grain placed in the shank are the mycelia, which, already established in the oat grain, use it as a

Table 1. Relative damage<sup>1</sup> (%) on shanks, husks, and kernels from different methods of inoculation from the pooled means of four hybrids and three replications in 1985 and the pooled means of two hybrids, two dates of inoculation, and four replications in 1986.

Inoculation Methods	Shanks	Husks	Kernels
	----- 1985 -----		
(a) Oat grain/shank	88.3a	0.0a	29.3a
(b) Oat grain/kernels	1.9b	63.3b	14.2b
(c) Spore suspension/shank	70.0c	0.0a	20.8abc
(d) Spore suspension/silks	0.0b	0.0a	8.8cd
(e) Control	0.0b	0.0a	0.0d
	----- 1986 -----		
(f) Oat grain/shank	83.8a	8.8a	4.9a
(g) Oat grain/kernels	28.8b	78.8b	23.8b
(h) Spore suspension/shank	90.0a	25.0c	25.9b
(i) Spore suspension/silks	42.5c	46.3d	37.5c
(j) Spore suspension/kernels	45.0c	97.5e	70.4d
(k) Oat grain/silks	40.0bc	13.8ac	17.3bc
(l) Spore suspension/sheath	43.8c	10.0a	13.3ae
(m) Control	35.0bc	5.0a	7.6ae

<sup>1</sup>Means with the same letter are not significantly different on the basis of the least significant difference test.

food base for extended growth and penetration of and establishment in the host. Conidia, in the method with spore suspension in the shanks, rely on their food reserve to germinate and penetrate host tissue. Being without their spore matrix that plays an important role in their survival (8), spore viability and inoculum potential are seriously affected, mostly if dry weather prevails after inoculation. Wu and Warren (19) demonstrated that, under dry conditions, germination of conidia of *C. graminicola* was highly reduced and that fluorescence increased.

Under the favorable conditions of 1986, the treatments with spore suspension in the shank and infected oat grain in the shank induced the highest level of infection in the shank (90% and 84%). The 90% shank infection of the treatment with spore suspension in the shanks resulted in 26% kernel infection, whereas the treatment with the infected oat grain in the shank induced only 5% infection of the kernels (Table 1).

The silk treatments (spore suspension and infected oat grain) induced 43% and 40% infection in the shank and 38% and 17% kernel infection, respectively. At this site of inoculation, it was observed that; at early anthesis, an oat grain placed in the silk channel was extruded by silk growth, and this shortened the inoculum exposure period. This was not the case at the second date of inoculation when silks had reached their mature length at the time of inoculation.

Warren and Shepherd (13) demonstrated the protective role of the husk against penetration of *C. graminicola*. This explains, in part, the poor initial infection observed in 1985 when the infected oat grain was placed under the first husk. Yet, infection on kernels may take place as a result of fungal penetration through husk if it is wounded (mechanical and insect damages). Such a penetration, at a state of ear development at which kernel moisture content is greater than 22% (4), will result in a high percentage of infected kernels, as it did for the treatment with spore suspension in grains (Table 1).

These results support the opinion that *C. graminicola* frequently infects the ear through the shank, with the source of inoculum being either the infected stalk or the sporulating lesions on the ear leaf, with spores being washed behind the leaf sheath. Later in the season, if there is development of top dieback in the field, the silk channel may also be a path for disease establishment in the ear. Disease incidence in this case is low because top dieback usually develops late when kernels are mature and probably more resistant to infection (13).

For ear rot caused by *Diplodia zeae*, Koehler (5) found that the pathogen usually developed through the butt of the cob and less often through the tip of the ear.

The extent of disease in the vascular tissue of the cob spread beyond the area of visibly rotted kernels and was greater, in most of the treatments, for inoculation done at anthesis than that done 14 days after anthesis. This was in agreement with Katsanos et al. (4), who found that maize ears were more susceptible to inoculation with ear rot pathogens 10 to 20 days after silking.

The study of the disease progression in the ear indicated that, from the inoculation site, the organism progressed and colonized the vascular tissue of the cob, mainly the outer cylinder formed of sclerenchyma and lignified parenchyma. The pith of the cob, composed of a soft parenchyma without vascularization, was discolored only in advanced stages of the disease. The pedicels, which link kernels to the outer cylinder of the vascular tissue, were then infected, and through them, the organism invaded the kernels. When seem-

ingly healthy kernels from the discolored zones of the vascular tissue were plated on oatmeal agar, acervuli of *C. graminicola* developed on the tips of kernels. The pathogen was recovered from pieces of nondiscolored tissue adjacent to the discolored vascular tissue. Thus, infection was more severe than symptoms indicated. Because *Colletotrichum graminicola* is seed transmitted (12) these observations support an opinion that, in seed fields, if evidence of ear infection is found (shank completely darkened or dark streaks on kernels at the butt or at the tip of the ears), then all ears showing symptoms should be discarded.

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