Effect of Time of Inoculation on Diplodia Stalk and Ear Rot of Maize in South Africa

K. R. CHAMBERS, Senior Plant Pathologist, Grain Crops Research Institute, Private Bag X1251, Potchefstroom, 2520, Republic of South Africa

ABSTRACT

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The effect of time of inoculation of maize (*Zea mays*) stalks and ears with *Diplodia maydis* on the severity of infection was studied. There was no significant difference between stalk pith rot ratings resulting from inoculations at midsilk and 18 days later. Similarly, there was no significant difference in yield loss (grain weight per plant and grain fill). This was explained in terms of vascular bundle distribution. Correlations between stalk pith rot ratings and yield indicated a nonsignificant relationship between these variables. Ear rot decreased sharply with a later inoculation date after midsilk. This was associated with a corresponding decrease in kernel moisture. A negative linear relationship existed between ear rot and yield loss (grain weight per plant) ($r = -0.977$, $P = 0.001$). Yield loss (grain weight per plant) was as high as 97% from inoculations made 10 days after midsilk. Plant attributes possibly involved in ear rot development showed a significant correlation between ear rot and starch accumulation in the kernels ($r = -0.854$, $P = 0.05$) and kernel moisture ($r = 0.974$, $P = 0.001$). No significant levels of β-glucoside (DIMBOA) were found in the kernels, nor were there significant correlations between ear rot and sugar concentration.

Additional keywords: premature death

Stalk and ear rot are widespread diseases of maize (*Zea mays* L.). Ear rot reduces plant yield directly by destroying the grain. Stalk rot, however, frequently causes stalk break and plant yield is reduced (5). The effect of stalk rot on yield has been studied by many workers (3,5). Wilcoxson (8) investigated yield loss due to stalk rot in relation to time of inoculation, but his results were inconsistent. Michaelson (5) and Hooker (2) studied the effect of time of inoculation on stalk rot development. Michaelson (5) found maize to be susceptible from several weeks before pollen shed to the hard dough stage. He reported that infections from early inoculations did not become as extensive as those from later inoculations. Hooker (2), however, concluded that time of inoculation had little effect on the rate of disease development. Little effort has been given to determine the effect of ear rot on yield loss in relation to time of inoculation.

The aim of this study was to compare 1) the development of stalk and ear rot resulting from inoculation at different times after midsilk, 2) changes in sugar, starch, glucoside, and moisture in the ear to ear rot development, and 3) yield loss (grain weight per plant and grain fill) caused by stalk and ear rot.

MATERIALS AND METHODS

Stalk and ear rot. Two trials (planted 1 November 1981 and 17 November 1982) were identical except that in one trial stalks were inoculated and in the other trial ears were inoculated. The soil was fertilized with 2:3:2 NPK (22%) and superphosphate, each at a rate of 175 kg/ha. A single cross hybrid, AX305W, was planted in both trials. The trials were laid out in a randomized complete block design with four replications consisting of four rows of 25 plants. Rows were 8 m long and 1 m apart. Plant population was 30,000 plants/ha. A further difference between the trials was an additional uninoculated row added to each plot in the ear rot trial for moisture, β-glucoside (DIMBOA), sugar, and starch determinations.

Stalks were inoculated at midsilk (0), 4, 6, 10, 14, and 18 days after midsilk, and ears were inoculated 10, 14, 16, 20, 24, 28, and 32 days after midsilk.

An isolate of *Diplodia maydis* (Berk.) Sacc (syn. *D. zeae* (Schw.) Lév.) from a culture collection maintained at the Grain Crops Research Institute at Potchefstroom, and known from studies to be aggressive on maize, was used for the inoculations. Inoculum was prepared using a modification of the toothpick method described by Young (10). Toothpicks were washed five times in boiling water, air-dried, then soaked in potato-carrot broth (infusion of 20 g of each of freshly skinned potato and carrot in 1 L of distilled water) for 24 hr. The toothpicks were placed into jars, covered with presoaked (24 hr) sorghum seeds, autoclaved, and then inoculated. Once the fungus had grown through the medium, the mycelium-covered toothpicks were removed and air-dried at room temperature for 2 days. Stalks were inoculated by inserting a fungus-covered toothpick into the first fully extended internode above ground level. Ears were inoculated by inserting a toothpick into the ear through the sheath leaves midway between the butt and the tip. Sterile, noninoculated toothpicks were inserted into the stalks and ears of plants in the control plots. On the same day, five randomly selected ears were collected for moisture, DIMBOA, sugar, and starch determinations. Moisture loss was determined after heating a 100 g random sample of homogenized kernels for 24 hr at 80 C. DIMBOA was determined according to Tipton et al (7) and sugars were assayed according to Yemm and Willis (9). Starch was determined from subsamples using iodine as an indicator.

Premature death (senescence before normal ripening) of stalks and pith rot served as criteria for stalk rot. Pith rot was determined after splitting the stalks of 40 plants from the treatments and control 28 days after inoculation. Rot was quantified by visually estimating percentage of pith rot of all the internode cross sections.

Pith was considered rotten when the tissue was discolored and had started to degenerate. In addition, the distribution of the vascular bundles in a sample of stalk cross sections was determined at flowering to ascertain the effect of pith rot on translocation to the ear. The distribution was determined by counting vascular bundles in a 0.5-cm strip of stalk cross section. Ear rot was rated at harvest by visually estimating the proportion of rotted to healthy kernels. Yield consisted of grain weight on a single plant basis and 100 kernel weight obtained from three random seed samples for each plot of both the stalk and ear rot trials. Yield data were adjusted to 14% grain moisture.

Analysis of variance (Student's *t* test) was used to analyze the data. Least significant differences (LSD) are presented where variance ratios were significant.

RESULTS

Vascular bundle distribution. In the inner two-thirds of the stalk pith, only 32.1% of the vascular bundles were found. More than 44% of the vascular

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bundles were found in the outer rind-cortex of the stalk.

Stalk rot. Stalk pith rot and premature death ratings are presented in Table 1. Significantly more rot occurred in all the inoculated internodes than in the controls. All times of inoculation of the preinternode (internode below inoculated internode), except 18 days after midsilk, and the second fully extended internode, inoculated at midsilk and 4 days later, had significantly more rot than their controls. Spread of the fungus to the preinternode and the second internode occurred in all treatments. Except for plants inoculated at midsilk, rot consistently spread more into the preinternode than to the higher internodes. There was no significant correlation between time of inoculation and invasion of the preinternode. The greatest spread from the inoculated to adjacent internodes occurred at midsilk, with the rot extending to the third internode above the inoculation site. There was no significant difference between premature death recorded in the different treatments and the control (Table 1).

Although control plants consistently had higher grain weight per plant and 100 kernel weights than inoculated plants, differences were nonsignificant (Table 2). A positive correlation between grain weight per plant and 100 kernel weight and stalk pith rot existed for the internodes above the inoculation site (Table 3).

Table 1. Effect of inoculation time on stalk pith rot and premature deaths resulting from inoculation of the first fully extended internode of maize stalks with *Diplodia maydis*

Time of inoculation (days)	Stalk pith rot (%)[a]					Premature death (%)
	pre-I[b]	I1[c]	I2	I3	I4	
0 (midsilk)	19.2	81.7	23.8	5.3	1.2	18.3
4	19.0	82.7	17.4	1.2	0.0	20.6
6	17.4	80.3	14.8	0.9	0.0	23.4
10	27.7	89.2	11.1	0.1	0.0	26.6
14	16.9	89.3	10.2	0.3	0.0	23.0
18	9.7	81.4	5.3	0.0	0.0	23.5
Control	0.0	0.0	0.1	0.0	0.0	18.6
LSD ($P = 0.05$)	13.1	13.8	14.9	5.6	2.9	15.9

[a]Mean pith rot of 40 plants over two seasons (1981–1982 and 1982–1983).
[b]I = Internode from below the inoculation site (pre-I) to the fourth fully extended internode (I4).
[c]Inoculated internode.

Table 2. Effect of time of inoculation on yield of maize plants inoculated with *Diplodia maydis* in the first fully extended internode at various times after midsilk

Time of inoculation (days)	Yield (g)[a]	
	Grain wt per plant	100 kernel wt
0 (midsilk)	61.7	20.3
4	56.4	19.0
6	54.7	20.3
10	50.2	19.8
14	51.1	19.9
18	47.3	20.1
Control	62.0	21.5
LSD ($P = 0.05$)	21.1	2.8

[a]Yield data represent the mean of 40 plants over two seasons (1981–1982 and 1982–1983).

Table 3. Correlations between stalk rot as a result of inoculation with *Diplodia maydis* and yield loss over two consecutive seasons (1982–1983 and 1983–1984)

Stalk pith rot	Yield loss	
	Grain wt per plant (g)	100 kernel wt (g)
pre-I[a]	0.2221	−0.3872
I1[b]	−0.3643	−0.4487
I2	0.9503***[c]	0.5856
I3	0.9384***	0.7639*
I4	0.8787**	0.8421

[a]I = Internode from below the inoculation site (pre-I) to the fourth fully extended internode (I4).
[b]Inoculated internode.
[c]$P = 0.05$, ** $P = 0.01$, *** $P = 0.001$.

Ear rot. Percentage of ear rot decreased nonlinearly with increase in days from midsilk to inoculation date and correlated significantly ($r = -0.977$, $P = 0.001$) with grain weight per plant (Table 4). Sugars, however, showed a rapid increase with date of inoculation. This variable was not significantly correlated with ear rot. Starch, on the other hand, increased steadily over the time of inoculation and correlated significantly with percentage of ear rot ($r = -0.854$, $P = 0.05$). Kernel moisture, like ear rot, decreased with time of inoculation and correlated significantly with percentage of ear rot ($r = 0.974$, $P = 0.001$). DIMBOA, however, correlated poorly with percentage of ear rot and, in fact, quantities of this substance were so low it could be ignored.

Table 4. Effect of time of inoculation on mean ear rot, sugar and starch concentration, kernel moisture, and grain weight per plant of ears inoculated with *Diplodia maydis* at various times after midsilk over two consecutive seasons (1982–1983 and 1983–1984)

Time of inoculation (days)	Ear rot (%)	Grain wt per plant (g)	Sugars (% T)[a]	Starch (g)	Kernel moisture (%)
10	97.1	0.0	55.6	0.166	89.9
14	94.3	0.4	60.8	0.189	90.6
16	93.7	6.3	66.2	0.198	91.4
20	82.4	8.8	73.9	0.175	83.5
24	73.9	18.2	73.9	0.206	75.4
28	38.7	31.0	75.2	0.212	66.5
32	16.9	59.2	75.8	0.234	61.1
Control	0.5	92.2	...	...	...
LSD ($P = 0.05$)	28.3	19.7	...	...	...

[a]Light transmission obtained spectrophotometrically according to Yemm and Willis (9).

DISCUSSION

Stalk pith rot decreased with an increase in the inoculation date only in the preinternode and second internode above the inoculation site. Severity of stalk pith rot in the third and fourth internodes above the inoculation site was too low for a similar trend to be evident. The preinternode inoculation 10 days after midsilk, for an unknown reason deviated from this trend. Hooker (2) inoculated stalks at weekly intervals over a period of four weeks starting 1 wk after pollen shed, and recorded least spread of the pathogen in the inoculated internode 1 wk after inoculation and the greatest 3 wk later. He concluded that date of inoculation had little effect on the rate of disease development in stalks. The results of this study agree with those of Hooker (2) for inoculations made up to 18 days after midsilk. Similarly, Michaelson (5) found earlier inoculations to be less effective than those made at a later date. It appears that this finding was largely influenced by inoculations made before pollen shed. His data indicate that had he inoculated the plants only after pollen shed, he could have reached the same conclusion as Hooker (2). There was no significant correlation between stalk rot at the different inoculation dates and yield loss. Other workers (5,6,8), however, have reported varying degrees of yield loss due to stalk rot. Wilcoxson (8) studied the effect on yield of inoculation of 14 hybrids at various times before harvest. He found the relationship between disease and yield loss to be inconsistent and, in some instances, hybrids inoculated with *D. maydis* had greater yields than the controls. Stalk rot does not appear to have a direct physiological effect on yield, but is an important factor in stalk breakage (5). However, in this study only pith infections were investigated and the effect on yield was thus limited by the fact that most of the vascular bundles, in excess of 44%, occur in the outermost cortex close to the rind of the maize stalk. Therefore, translocation to the ear is affected only to a limited extent with pith rot. It is possible that the distribution patterns of vascular bundles vary from genotype to genotype. This would account for the inconsistency in Wilcoxson's (8) findings. This reasoning is consistent with the photosynthetic stress-translocation balance concept proposed by Dodd (1). As the ear develops, the maize plant becomes committed to grain fill. When such plants are water-stressed the pith, including the vascular bundles in these tissues, senesces. Depending on the translocation flow, plants may rapidly wilt and the pith is invaded by stalk rot pathogens. This tendency to rot varies from genotype to genotype (1).

Maize ears were most susceptible to *D. maydis* from midsilk to approximately 24 days later. This corresponded with a kernel moisture level range of more than 90% to 75.4%. Koehler, cited by Katsanos et al (4), reported ears were most susceptible 10–20 days after midsilk, but resistant after kernel moisture dropped below 22%. In this study, however, resistance became apparent 28 days after midsilk, at which time kernel moisture approximated 66%. A drop of only 5% moisture leading to a kernel moisture of 61% was associated with nearly 22% decrease in ear rot. Thus, the 22% kernel moisture reported by Koehler probably refers to ear immunity. Sugars and DIMBOA had no apparent role in ear rot development.

In a program for breeding resistance to ear rot, the time of inoculation for germ plasm evaluation is critical. Since there was a rapid decrease in kernel moisture from 20 days after midsilk, ear inoculation should be made at or shortly after this date. Inoculation at this time would best differentiate resistant and susceptible germ plasm. Stalk inoculations for stalk breakage improvement, however, can be made any time from midsilk to approximately 18 days later.

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