Wound Predisposition of Maize to Anthracnose Stalk Rot as Affected by Internode Position and Inoculum Concentration of *Colletotrichum graminicola*

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ABSTRACT

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A 2-yr study was carried out to ascertain the nature of wound predisposition of maize to anthracnose stalk rot (ASR) relative to internode position and inoculum level. Stalks of hybrids Cornell 281 (susceptible) and CM174 × LB31 and B37 × LB31 (resistant) were inoculated with 1-ml suspensions of 10^2, 10^4, 10^6, 10^7, and 10^8 conidia of *Colletotrichum graminicola* per milliliter into wounds at midwhorl or anthesis and immediately or 6 hr after wounding. For each inoculation regime, the most severe ASR developed in the susceptible hybrid. At each inoculum level, the most severe ASR resulted from inoculation at anthesis and immediately after wounding. Disease severity increased with increasing inoculum up to 10^7 conidia per milliliter in greenhouse-grown plants and up to 10^6 conidia per milliliter in field-grown plants. Genotypic, ontogenic, and "wound healing" resistances to ASR were expressed in maize even at high inoculum levels of *C. graminicola*. Systemic ASR resulted from inoculation immediately after wounding of any of the first five internodes above the brace roots, but wound healing restricted considerably systemic ASR after inoculation of aged wounds in the lowermost stem internodes.

Additional keyword: *Zea mays*

Anthracnose stalk rot (ASR), caused by the fungus *Colletotrichum graminicola* (Ces.) G. W. Wils., has become one of the predominant stalk rot diseases of maize (*Zea mays* L.) in the United States, including New York State, during the past few decades (1,5,12,27,32). Once considered economically unimportant, ASR has been shown in recent years to cause considerable yield losses in maize (15,26,33,34). ASR-related yield reduction is attributable to reduction in grain weight as well as to increased stalk lodging, which reduces harvestability.

The most economical method to control ASR is the deployment of host resistance genes. Maize genotypes with polygenic resistance to ASR have been reported (8,16,34). An inbred line, LB31, and its related hybrids have been reported to carry a major gene that confers a high level of ASR resistance (2). Although ASR may occur at different ontogenic stages of maize (34), the disease is more severe when it results from infection at reproductive stages (14,34). Keller and Bergstrom (14) observed that ASR development was restricted in susceptible genotypes inoculated at vegetative stages in a manner that closely resembled symptom restriction in resistant genotypes inoculated at reproductive stages. Wounds that breach the stem rind have been shown to be a significant factor in the predisposition of maize to ASR (13,22,24). However, this predisposition is transitory, suggesting that a rapid wound healing mechanism(s) reduces establishment of *C. graminicola* at wound sites. Bostock and Stermer (7) discuss several plant/pathogen systems where wound-induced chemical and morphological responses interfere with pathogen ingress. In addition to genotype- and ontogeny-related ASR resistance in maize, wound healing has been shown to substantially reduce ASR (22,24).

Inoculation methods, including inoculum concentrations, are among the important factors to consider in evaluating resistance to plant pathogens. A number of studies on ASR have used wound inoculation of maize in the first elongated internode above the brace roots with about 10^5 conidia of *C. graminicola* per milliliter. However, maize stalk injuries such as those inflicted by the European corn borer (ECB), *Ostrinia nubilalis* (Hübner), through which *C. graminicola* may enter the plants (6,15,20,33), often occur in different internodes. Whether wound predisposition varies with inoculum level of *C. graminicola* and in different plant internodes is not known. Information on ASR development relative to changes in the level of the fungus inoculum over maize ontogeny also is not available.

In the present investigation, we report on the nature of wound predisposition of maize to ASR relative to internode position and inoculum level of *C. graminicola*. We also document possible interactions of these factors with maize genotype and ontogenic stage. A preliminary report of this work has been published (23).

MATERIALS AND METHODS

Effects of inoculum concentration and host ontogenic stage. Seeds of hybrids Cornell 281 and CM174 × LB31 (Department of Plant Breeding and Biometry, Cornell University), susceptible and resistant to ASR, respectively, were sown on 28 May and 20 August 1988 in a greenhouse with ambient temperatures of 18–37 C and relative humidities of 40–99% during the growing period. Plants were grown in 30-cm-diameter plastic pots (two plants, one of each hybrid, per pot) filled with a soil mixture of peat moss and autoclaved compost (12:6, v/v), and a 5-10-5 NPK fertilizer. Every week, plants were watered with a 20-20-20 NPK fertilizer and sprayed with a mixture of 1.26 g of resmethrin (SBP-1382 24.3EC) and 0.59 g of dienochlor (Pentac 50W) per liter of water for control of mites, thrips, and aphids. Under these greenhouse conditions, nearly all plants set seed, although the numbers of kernels per ear were less than from those grown under field conditions.

The experimental design, which comprised three blocks, was a randomized split-split plot with inoculation at two ontogenic stages (midwhorl and anthesis) as main plots, four different inoculum concentrations (10^2, 10^4, 10^6, and 10^7 conidia per milliliter) as subplots, and the two hybrids as sub-subplots. Each sub-subplot treatment had three pots each containing two plants, one of each hybrid, so that three plants per block were used to compute the mean of the treatment. Host ontogenic stages were described by the scheme of Ritchie and Hanway (29), in which the stages are defined when 50% or more of the plants are at or beyond midwhorl (collar of the eighth leaf visible) and anthesis (silks emerging and pollen shedding).

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Effects of inoculum concentration and wound healing. Seeds of the same hybrids were sown, as described earlier, on 28 June and 14 August 1988 in a greenhouse with ambient temperatures of 15–31 C and relative humidities of 40–95% during the growing period. Pots were filled with a soil mixture of peat moss, perlite, and autoclaved compost (8:12:6, v/v/v), and 1.75 L of steamed bonemeal, 5 L of dolomitic lime, 0.75 L of P_2O_5, 0.30 L of 10-10-10 NPK, 57 g of fritted trace elements, and 454 g of KNO_3. Three times a week, plants were watered with either 473 or 536 ppm solutions of fertilizer (peatlite special) (Peters fertilizer, W. R. Grace & Co., Fogelsville, PA) in the form of 20-19-18 and 15-16-17 NPK, respectively. Plants were sprayed with bifenthrin (Talstar 10WP) at rates of 0.59 g/L of water 1 mo after planting and a mixture of 0.32 ml of abamectin (Avid 0.15 EC) and 0.16 ml of fluvalinate (Mavrik 23.3 EC) per liter of water 2 mo after planting. The experimental design, comprising three blocks, was a randomized split-split plot with the two hybrids as main plots, two time intervals (0 and 6 hr) between stalk wounding and inoculation as subplots, and the four inoculum concentrations as sub-subplots. Each sub-subplot treatment had three pots each containing two plants (one of each hybrid) so that three plants per block were used to compute the mean of the treatment. Plants were inoculated at anthesis.

Seeds of Cornell 281 and a resistant hybrid B37 × LB31 (Cornell University), were sown by hand in two-row plots on 22 May 1989 in Ithaca, NY. Row spacing was 0.76 m and plant density was 55,000 plants per hectare. A 10-10-10 NPK (224 kg/ha) and additional nitrogen (280 kg/ha 46-0-0 urea) fertilizers were applied at planting. A herbicide mixture of 16.6 L of Sutazine + (2.15 kg of sutan 57E and 0.50 kg of atrazine 13W) and 2.25 kg of metolachlor (Dual 8E) per hectare was also applied before planting. Every 2 wk from midwhorl to anthesis, plants were sprayed with 1.71 kg/ha of carbaryl (Sevin 4F) using a polyethylene hand sprayer. An experimental design similar to that in the greenhouse was used except each sub-subplot comprised 20 plants, inoculum concentrations were 0, 10^2, 10^4, 10^6, 10^7, and 10^8 conidia per milliliter, and there were four blocks separated by two rows of B37 × LB31.

Effects of internode position and wound healing. Seeds of Cornell 281 were sown with a two-row plateless maize planter on 12 May 1988 and 22 May 1989 at the same location as described earlier. Row spacing and plant density were also as described earlier. In 1988, 181.6 kg/ha of a 10-10-10 NPK fertilizer was applied at planting and 56.8 kg/ha of nitrogen (NH_4NO_3) fertilizer was sidedressed at midwhorl stage. A herbicide mixture of 2.27 kg of atrazine (AAtrex 4L), 1.14 kg of metolachlor, and 2.37 L of bentazone (Basagran 4WS) per hectare was also applied before planting. The 1989 field was treated as described in the preceding section.

The experimental design was a randomized complete block comprising three blocks for each combination of time interval (0, 1, and 6 hr between stalk wounding and inoculation) and internode position (inoculation of plants into a wound in the first through fifth elongated internodes above the brace roots). Each treatment combination had 15 plants inoculated at anthesis. The three blocks were separated by two rows of Cornell 281. In 1989, additional plants of B37 × LB31 were wounded and inoculated (five plants per set) in the first, third, or the fifth internodes immediately or 6 hr after wounding.

Inoculation procedures. The isolate of *C. graminicola* used was Cg151NY82, originally collected in 1982 from infected leaves of sweet maize in Tioga County, NY. It was grown on oatmeal agar (31) at room temperature (22 ± 1 C) under a 12-hr regime of fluorescent light supplied by cool-white Sylvania F40CW lamps. Periodically, the fungus was inoculated onto leaves of Cornell 281 greenhouse-grown seedlings and reisolated to minimize loss of virulence. Inoculum was produced by dislodging colonies from the surface of 14- to 18-day-old cultures with sterile (autoclaved) distilled water, filtering the suspension through four layers of cheesecloth, and determining conidial concentrations with a hemacytometer. Unless otherwise noted, final concentration was adjusted to 5×10^5 conidia per milliliter with sterile distilled water, and one drop of Tween 20 was added as a wetting agent per 200 ml of inoculum. Maize stalks were wounded in various internodes with a pistol grip vaccinator (Ideal Instru-

Table 1. Analysis of variance for anthracnose stalk rot severity in susceptible and resistant maize hybrids inoculated at midwhorl and anthesis with various inoculum concentrations of *Colletotrichum graminicola*

Source of variation	Test 1 df	Test 1 Sums of squares	Test 1 Mean squares[y]	Test 2 Sums of squares	Test 2 Mean squares	Pooled df	Pooled Sums of squares	Pooled Mean squares
Block (B)	2	41.7	20.9	44.3	22.2	...	...	...
Experiment (E)	...	...	...	...	...	1	23.4	23.4
Growth stage (GS)	1	456.4	456.4*[z]	446.7	446.7*	1	903.1	903.1***
E × B	...	...	...	...	...	4	86.1	21.5
E × GS	...	...	...	...	...	1	0.03	0.03
E × B × GS	...	...	...	...	...	4	51.3	12.8
B × GS	2	40.4	20.2	10.8	5.4	...	...	...
Inoculum level (I)	3	2,959.7	986.6***	954.7	318.2***	3	3,561.4	1,187.1***
E × I	...	...	...	...	...	3	352.9	117.6***
B × I	6	105.4	17.6	38.5	6.4	...	...	...
GS × I	3	21.4	7.1	23.4	7.8	3	37.1	12.4
E × GS × I	...	...	...	...	...	3	7.7	2.6
B × GS × I	6	41.6	6.9	29.8	4.9	...	...	...
E × B × GS × I	...	...	...	...	...	24	215.3	8.9
Hybrid (H)	1	539.0	539.0***	300.2	300.2***	1	821.8	821.8***
E × H	...	...	...	...	...	1	17.4	17.4
GS × H	1	50.3	50.3	63.1	63.1***	1	113.1	113.1***
E × GS × H	...	...	...	...	...	1	0.4	0.4
I × H	3	198.7	66.2*	14.2	4.7	3	147.9	49.3**
E × I × H	...	...	...	...	...	3	64.9	21.6
GS × H × I	3	60.5	20.2	22.6	7.5	3	75.3	25.1
E × GS × H × I	...	...	...	...	...	3	7.7	2.6
Residual error	16	258.5	16.2	41.1	2.6	32	299.6	9.4
Total	47	4,773.6		1,989.3		95	6,786.3	

[y] Mean squares of disease severity in three maize plants per treatment 21 days after inoculation.
[z] Values are significantly different from zero at $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***).

ment, Inc., Damon Company, Chicago, IL) fitted with a stainless steel needle, and plants were inoculated using procedures described previously (24).

Data collection and analysis. In greenhouse experiments, all three stalks per treatment were collected 21 days after inoculation (DAI), whereas in the field, five stalks were collected 21 DAI and 10 stalks were collected at normal grain harvest time. They were split longitudinally from the eighth internode to the ground level. The extent of pith discoloration in each internode attributable to ASR was then rated based on a 0–5 visual scale for stalk rot (15) where 0 = no pith discoloration, 1 = 1–5, 2 = 6–25, 3 = 26–75, 4 = 76–99, and 5 = 100% pith discoloration. The percent midpoint of each range was then determined and scores were averaged for six (inoculum level experiment) or eight (internode position experiment) internodes to give ASR severity of the plant. In addition, the number of internodes discolored and with 50% or more pith discoloration were assessed for the study of effects of inoculum concentration and wound healing in the field.

Data for each test in the greenhouse and for each year in the field were subjected to analysis of variance (ANOVA) using the SAS PROCANOVA or PROCGLM procedures (SAS Institute, Inc., Cary, NC). Two-tailed F tests were performed between error mean squares of the treatment interaction terms, and the data for each set of experiments were combined and analyzed again to ascertain that error variances between experiments as well as experiment × treatment interaction allowed pooling the data. Sums of squares of the interaction terms were further partitioned into single df components to account for significant trends in ASR development. Regression analysis was done using the PROCREG procedure (SAS Institute), and Fisher's protected least significant difference (LSD) test was used to compare the treatment means (18). Because of similarity in disease development, only observations of ASR at normal grain harvest time are reported for the field studies.

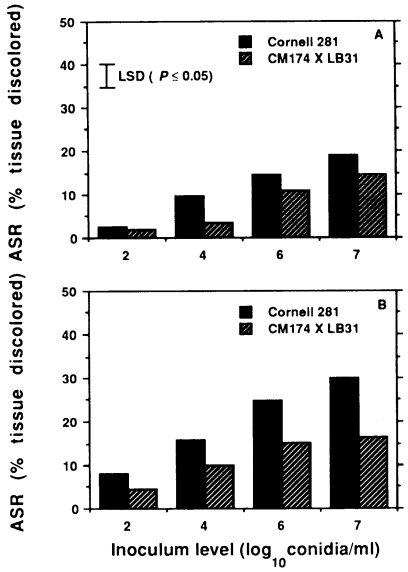

Fig. 1. Anthracnose stalk rot (ASR) (averaged for six internodes per plant and three plants for each of three replicates) in maize hybrids Cornell 281 and CM174 × LB31 inoculated in the greenhouse at **(A)** midwhorl or **(B)** anthesis with 1-ml suspensions of 10^2, 10^4, 10^6, and 10^7 conidia of *Colletotrichum graminicola* per milliliter (average of two experiments). Inoculation was into a wound in the first elongated internode above the brace roots. Vertical line represents Fisher's protected LSD ($P \leq 0.05$) for comparison of the disease severities 21 days after inoculation between hybrids at the same combination of maize ontogenic stage and fungus inoculum concentration (LSD = 5.10).

Table 2. Anthracnose stalk rot severity (ASR) in maize hybrids Cornell 281 and CM174 × LB31 inoculated with various inoculum concentrations of *Colletotrichum graminicola*

Inoculum level (conidia/ml)	ASR (%) in hybrid[y]	
	Cornell 281	CM174 × LB31
10^2	5.2	3.2
10^4	12.8	6.9
10^6	19.7	13.2
10^7	24.5	15.6
LSD ($P \leq 0.05$)[z]	3.58	

[y] Anthracnose stalk rot severities (percent tissue discolored) averaged for all maize ontogenic stages are means of six internodes per plant and three plants for each of three replicates. Plants were inoculated into a wound in the first internode above the brace roots at midwhorl and anthesis with 1-ml suspensions of 10^2, 10^4, 10^6, and 10^7 conidia per milliliter. Disease ratings were taken 21 days after inoculation.

[z] LSD ($P \leq 0.05$) for comparison of different inoculum levels of *C. graminicola* (over all maize ontogenic stages) at the same or different combinations of maize hybrids.

RESULTS

Effects of inoculum concentration and host ontogenic stage (greenhouse experiment). Although error variances between the two tests differed statistically, mean squares of the experiment × treatment interaction term were not significantly different from zero (Table 1). Therefore, data on ASR severity across the tests were pooled. Maize ontogenic stage (GS), hybrid (H), and inoculum concentration (I) of *C. graminicola* each had a significant effect ($P \leq 0.001$) on ASR development (Table 1). The GS × H × I interaction term was not significantly different from zero ($P \leq 0.05$), but GS × H and H × I terms were different ($P \leq 0.01$ and 0.001, respectively). ASR increased in both hybrids and at each ontogenic stage with increasing inoculum concentration. Significantly more severe symptoms developed in plants of the susceptible Cornell 281 than in plants of the resistant CM174 × LB31 only when inoculation was at anthesis and with inoculum levels from 10^4 to 10^7 conidia per milliliter (Fig. 1). The disease severity resulting from inoculation at anthesis (averaged for all the inoculum levels) was significantly greater in both hybrids (mean severity = 19.7 and 11.7% for Cornell 281 and CM174 × LB31, respectively) compared with the disease severity resulting from inoculation at midwhorl (mean severity = 11.4 and 7.7% for Cornell 281 and CM174 × LB31, respectively). There was no significant difference ($P \leq 0.05$) between ASR in plants of Cornell 281 inoculated at midwhorl and plants of CM174 × LB31 inoculated at anthesis. Inoculation of either hybrid with 10^2 conidia per milliliter resulted in significantly less ASR ($P \leq 0.05$) than did inoculation with the other levels of inocula (Table 2).

Effects of inoculum concentration and wound healing (greenhouse experiment). Neither error variances of the two tests nor mean squares of the experiment × treatment interaction term were significantly different from zero ($P \leq 0.05$). Thus, data on ASR severity across the tests were pooled (Table 3). From ANOVA (Table 3), H, I, and time interval (Ti) each had a significant effect ($P \leq 0.001$) on disease development. The H × Ti and H × Ti × I interaction terms were not significantly different from zero ($P \leq 0.05$), but H × I and Ti × I terms were different ($P \leq 0.05$). When inoculation was immediately after wounding, mean disease severity increased rapidly with increasing level of inoculum, but ASR was always significantly greater ($P \leq 0.05$) in the susceptible Cornell 281 than in the resistant CM174 × LB31. There was a highly significant ($P \leq 0.001$) linear relationship between inoculum concentration and symptom development in each hybrid at each time interval. Regression analysis of disease severity vs. level of inoculum revealed significantly higher slopes of the ASR regression lines for plants of Cornell 281 than for plants of CM174 × LB31 inoculated immediately vs. 6 hr after wounding (Table 4). For every 10-fold increase in inoculum concentration, ASR increased 2.11 and 1.41% in Cornell 281 inoculated immediately and 6 hr after wounding, respectively, compared with 1.42 and 1.04% increases in ASR in CM174 × LB31 inoculated immediately and 6 hr after wounding, respectively.

Effects of inoculum concentration and wound healing (field experiment). The range of disease symptoms in Cornell 281 and B37 × LB31 inoculated in the field with various inoculum levels of *C. graminicola* is shown in Figure 2A–D. As in the greenhouse experiment, H, Ti, and I significantly influenced ASR development (Table 3). The H × Ti × I interaction term was not significantly

different from zero ($P \leq 0.05$), but H × Ti, H × I, and Ti × I terms were significant ($P \leq 0.01$ or 0.001) (Table 3). ASR severity resulting from Ti × I interaction also increased rapidly with inoculum concentration up to 10^6 conidia per milliliter. There was a highly significant ($P \leq 0.001$) quadratic relationship between inoculum concentration and ASR development. In both hybrids and at each time interval, ASR increased with increasing inoculum level up to 10^6 conidia per milliliter, after which ASR decreased (Fig. 2A–D). However, ASR was always greater in plants of Cornell 281 than in those of B37 × LB31.

To determine whether ASR resulting from inoculation at the two time intervals tested was statistically different, disease severity in each plant for each treatment combination was regressed against inoculum concentration. Differentials of the regression equations provided inoculum levels that caused maximum disease development. By fitting the resulting fungus inoculum levels and their squares in the SAS PROCREG procedure, the maximum ASR severities were then estimated for each hybrid and time interval. Although inoculum levels that caused maximum disease development were about equal in each hybrid and at each time interval between wounding and inoculation, inoculation of either hybrid immediately after stalk wounding always resulted in significantly more severe symptoms than did inoculation 6 hr after wounding (Table 4). However, more severe ASR developed in Cornell 281 than in B37 × LB31.

In addition to overall ASR severity, there also were significant differences ($P \leq 0.05$) in the total number of internodes discolored and the number with 50% or more pith discoloration between plants of each hybrid inoculated immediately vs. 6 hr after wounding (Table 5). Furthermore, these estimates of disease development were always significantly greater ($P \leq 0.05$) in each hybrid inoculated immediately than in those inoculated 6 hr after wounding, and they increased with inoculum concentration up to 10^6 conidia per milliliter, after which they decreased. In plants of either hybrid, no more than two internodes sustained 50% or more pith discoloration when inoculation was 6 hr after wounding (Table 5).

Effects of internode position and wound healing (field experiment). ASR resulting from inoculation of Cornell 281 in different internodes and at various time intervals after stalk wounding is shown in Figure 3A and B. Error variances of the 1988 and 1989 experiments and mean squares of the experiment × treatment interaction term were all significantly different from zero ($P \leq 0.001$). Therefore, data on disease severity were analyzed separately for each year.

In 1988, only Ti had a significant effect on ASR ($P \leq 0.001$). Inoculation of plants immediately after wounding induced significantly greater ASR than did inoculation 1 or 6 hr after wounding. The severity ratings in plants inoculated immediately after wounding (averaged for all inoculated internode positions) were 20.7% compared with 12.5 and 11.7% in plants inoculated at 1 or 6 hr time intervals, respectively. In 1989, on the other hand, Ti, internode position (IP), and IP × Ti interaction each influenced the disease development

Table 3. Analysis of variance for anthracnose stalk rot severity (ASR) in susceptible and resistant maize hybrids inoculated at anthesis with various inoculum concentrations of *Colletotrichum graminicola* immediately or 6 hr after wounding

Source of variation	Pooled ASR (%) in greenhouse[x] df	Sum of squares	Mean squares	ASR (%) in field[y] df	Sum of squares	Mean squares
Block (B)	...	...	...	3	155.1	51.7
Experiment (E)	1	1,349.6	1,349.6	...	...	...
Hybrid (H)	1	209.9	209.9***[z]	1	2,637.3	2,637.3**
E × B	4	41.9	10.9	...	...	...
E × H	1	25.5	25.5	...	...	...
E × B × H	4	17.3	4.3	...	...	...
B × H	...	...	...	3	115.9	38.6
Time interval (Ti)	1	183.0	183.0***	1	3,478.7	3,478.7***
E × Ti	1	45.5	45.5*	...	...	...
B × Ti	...	...	...	3	130.3	43.5
H × Ti	1	8.9	8.9	1	806.7	806.7**
E × H × Ti	1	2.0	2.0	...	...	...
B × H × Ti	...	...	...	3	28.6	9.5
E × B × H × Ti	8	57.9	7.2	...	...	...
Inoculum level (I)	3	800.1	266.7***	5	8,073.2	1,614.6***
E × I	3	52.7	17.6***	...	...	...
H × I	3	28.4	9.5*	5	1,080.0	216.0***
E × H × I	3	7.3	2.4	...	...	...
Ti × I	3	29.2	9.7*	5	1,447.2	289.5***
E × Ti × I	3	18.4	6.1	...	...	...
H × Ti × I	3	7.1	2.4	5	353.9	70.7
E × H × Ti × I	3	3.1	1.0	...	...	...
Residual error	48	123.9	2.6	60	1,898.5	31.6
Total	95	3,011.7		95	20,205.4	

[x] Anthracnose stalk rot expressed as mean percentage of tissue discoloration in three maize plants per treatment (averaged for six internodes per plant) for each of three replicates 21 days after inoculation.

[y] Anthracnose stalk rot expressed as percentage of tissue discoloration in 10 maize plants per treatment (averaged for six internodes per plant) for each of four replicates at normal grain harvest time.

[z] Values are significantly different from zero at $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***).

Table 4. Regression statistics for the relationship between inoculum concentration of *Colletotrichum graminicola* and anthracnose stalk rot severity (ASR) in susceptible and resistant maize hybrids inoculated at various time intervals after wounding (greenhouse) and the inoculum levels that caused maximum disease in the field

Hybrid	Time interval (hr)[y]	Greenhouse b[w]	Greenhouse R^2[x]	Field Log_{10} inoculum level (conidia/ml)[y]	Field Maximum ASR (%)[z]
Cornell 281	0	2.11 ± 0.22	0.98	5.70 ± 0.23	47.82 ± 7.72
	6	1.41 ± 0.21	0.95	6.08 ± 0.41	18.14 ± 3.12
CM174 × LB31	0	1.42 ± 0.07	0.99	...	...
	6	1.04 ± 0.12	0.98	...	...
B37 × LB31	0	...	...	6.00 ± 0.24	22.26 ± 3.65
	6	...	...	7.72 ± 0.84	11.12 ± 1.81

[y] Plants were inoculated at anthesis into a wound in the first internode above the brace roots with 1-ml suspensions of 10^2, 10^4, 10^6, and 10^7 conidia per milliliter in greenhouse or the above inocula including 10^8 conidia per milliliter and injection with distilled water (check) in field. Inoculation was immediately or 6 hr after wounding.

[w] Slopes and standard deviations of regression lines of mean disease severities (averaged for three plants per treatment for each of three replicates) 21 days after inoculation and inoculum concentrations.

[x] Coefficients of determination.

[y] Log inoculum levels causing maximum ASR in each hybrid and at each time interval between stalk wounding and inoculation in the field.

[z] Maximum ASR after which the severity decreased with increasing inoculum level.

significantly ($P \leq 0.05$ or 0.001). The significant IP × Ti interaction indicated that ASR resulting from inoculation of plants at various time intervals varied with the internode position inoculated (Fig. 4).

Inoculation immediately after wounding caused more severe ASR regardless of internode position, but ASR resulting from inoculation in the third internode was slightly less than ASR resulting from inoculation in the other internodes. Although symptom development from inoculation 1 or 6 hr after wounding in either internode position was reduced markedly, inoculation of the third through fifth internodes resulted in slight systemic ASR development (Fig. 4). A similar trend in the disease development also occurred in the resistant B37 × LB31 inoculated in the first, third, or fifth internodes, but symptoms were always less severe than symptoms in the susceptible Cornell 281.

DISCUSSION

Maize growth stage, hybrid, and inoculum concentration of *C. graminicola* each had a significant effect on disease development. Furthermore, host ontogenic stage and hybrid, as well as hybrid and inoculum level, also had significant interactive effects on ASR. Generally, the differences in ASR between the two hybrids were obvious only after inoculation at anthesis and with high inoculum levels (Fig. 1). Maize stalks have been reported to become more susceptible to ASR during the transition from vegetative to reproductive stages (14,34). Bergstrom and Bergstrom (3) also reported that when maize was inoculated with *C. graminicola* at late-whorl stage, systemic colonization of plants by the fungus was reduced until silking. The results of the present study were consistent with these observations and our previous findings (22,24).

The reduced ASR development resulting from inoculation at midwhorl stage, even in the presence of high inoculum levels of *C. graminicola*, warrants further investigation of factors that affect disease development at vegetative stages. However, the shift from resistance to stalk rotting fungi in maize during the vegetative stages to susceptibility at reproductive stages has been attributed to the onset of pith senescence resulting from the depletion of sugars in the stalks for grain filling (10,21,26). Keller and Bergstrom (14) claimed that this was insufficient to explain the *C. graminicola*-maize interaction because *C. graminicola*, unlike opportunistic maize stalk rot organisms such as *Stenocarpella maydis* (Berk.) Sutton (syn. *Diplodia maydis* (Berk) Sacc.) and *Gibberella zeae* (Schwein.) Petch, causes ASR during the period just before tasseling extending through anthesis. Furthermore, *C. graminicola* has been shown capable of parasitizing living pith tissues (25), and sucrose has been demonstrated to considerably increase conidial germination in the fungus (4). Moreover, in a 3-yr field experiment, removal of ears 3 wk after anthesis from plants of a susceptible hybrid to prevent development of a carbohydrate sink significantly increased plant susceptibility to ASR while decreasing the development of stalk rot due to *Gibberella* in the same plants (G. C. Bergstrom, *unpublished*). Ear removal has been associated with accumulation of a high level of sugars in the stalks and resistance to stalk rots caused by *S. maydis* and *G. zeae* (9,21,30).

Increase of inoculum concentration of *C. graminicola* in the field resulted in increase of ASR up to 10^6 conidia per milliliter, after which the severity decreased (Fig. 2). In the greenhouse experiment, by contrast, the disease increased linearly with the level of inoculum. However, the symptoms were always less severe than in the field at each level of inoculum. The greater ASR observed in the field, compared with that in the greenhouse, may be attributable to differential disease development as influenced by the environmental condi-

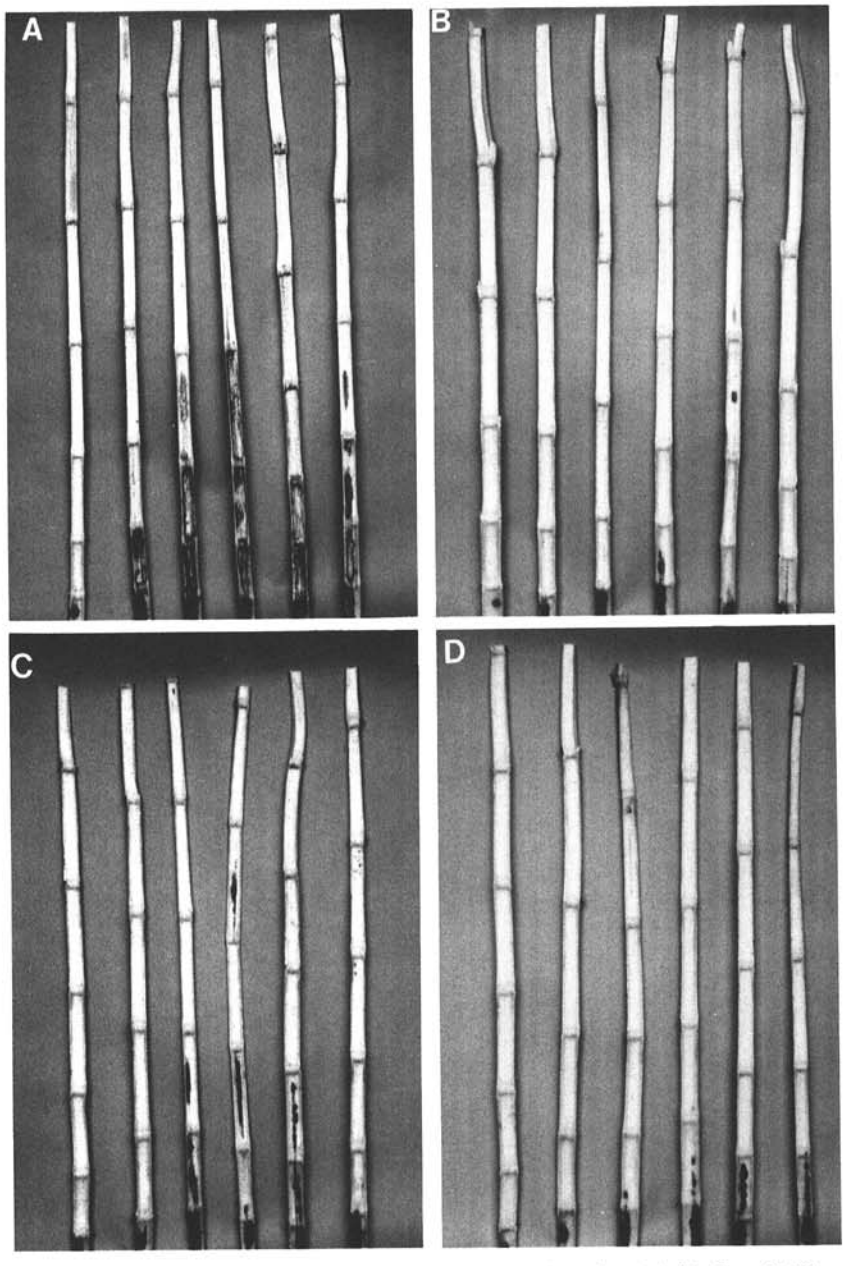

Fig. 2. Anthracnose stalk rot at normal grain harvest time in maize hybrids Cornell 281 and B37 × LB31 inoculated at anthesis in the field with 1-ml suspensions of *Colletotrichum graminicola*. Plants of Cornell 281 inoculated in the first elongated internode above the brace roots with (from left to right) distilled water (0) or 10^2, 10^4, 10^6, 10^7, and 10^8 conidia per milliliter **(A)** immediately or **(B)** 6 hr after wounding. Plants of B37 × LB31 inoculated in the first elongated internode above the brace roots with (from left to right) distilled water (0) or 10^2, 10^4, 10^6, 10^7, and 10^8 conidia per milliliter **(C)** immediately or **(D)** 6 hr after wounding.

tions and differences in plant morphology. That H × I, H × Ti, and Ti × I interactions were statistically significant suggests that ASR in each hybrid varied with time intervals between stalk wounding and inoculation, as well as with the level of fungus inoculum. Nevertheless, in both environments, there was a marked decrease in ASR each time inoculation was delayed after stalk wounding, and the symptoms were always more severe in the susceptible Cornell 281 than in the resistant CM174 × LB31 and B37 × LB31 (Fig. 2 and Table 4).

White et al (34) found that severe ASR could be caused by fungus inoculum concentrations as low as 2×10^4 conidia per milliliter. They also noted that the severity of the disease did not increase with concentrations above 2×10^4 conidia per milliliter, an observation similar to the decrease in ASR severity at inoculum above 10^6 conidia per milliliter noted in our field study (Fig. 2). A possible explanation has been offered by R. L. Nicholson (*personal communication*), who has observed that at high inoculum concentrations, *C. graminicola* often secretes chemical compounds that are inhibitory to its conidial germination. Furthermore, Louis et al (19) recorded increased germination of *C. capsici* (Syd.) E. J. Butler & Bisby, the causal fungus of pepper anthracnose, with decreased spore concentrations.

It is not clear how 10^6 conidia per milliliter, which induced the greatest symptom severity in our study, compares with the actual level of the fungus that causes severe ASR outbreaks under natural field conditions. Several studies have demonstrated that stalk wounding by ECB, as well as the level of genotypic susceptibility, are important preconditions for ASR development (6,20,33,34), although it has also been suggested that conidia produced from blighted maize leaves and washed behind the leaf sheaths may directly penetrate the stalks (4,20,35). ECB larvae not only provide a site for ingress of *C. graminicola* into maize, but they can also vector the fungus (A. Muimba-Kankolongo and G. C. Bergstrom, *unpublished*). However, there are no reports indicating the actual level of the fungus that is brought to infection courts by ECB. Conidial suspensions ranging from 16,000 to 20,000 conidia per water droplet have been recovered in water flowing over acervuli of *C. graminicola* on sorghum leaves (28). We suggest that acervuli on blighted maize leaves may yield inoculum suspensions in excess of 10^7 conidia per milliliter that may be washed behind leaf sheaths by rain water. More studies are needed to determine the amount and form of fungal inocula that may be carried to wound sites and that will cause subsequent maize stalk infection.

Inoculation immediately after stalk wounding always resulted in the most severe ASR at each level of inoculum (Fig. 2 and Table 3). It was also found that the number of internodes discolored or 50% or more discolored increased with an increase in inoculum concentration up to 10^6 conidia per milliliter and that the number of internodes discolored was significantly lower from inoculation 6 hr compared with immediately after wounding in both hybrids. The decrease in ASR development concurrent with time intervals between stalk wounding and inoculation was referred to as a "wound healing" response (13,22,24), which has been implicated in resistance to diseases in many other plant species (7,17). "Wound healing" occurred in each hybrid and at each inoculum level of *C. graminicola* tested. The manner in which the wound sites affect ASR development is not well understood. Our previous study (24) suggested a strong resistance mechanism induced at wound sites. Wound sites that were rewounded or regularly wetted with water before inoculation still exhibited the wound healing effect on ASR (24). Reduced symptom development concomitant with a delay in inoculation after stalk wounding, even in response to high inoculum concentrations, reinforces our hypothesis of an active resistance mechanism at or near wound sites.

White and Humy (33) observed that

Table 5. Number of internodes[w] discolored or with 50% or more pith discoloration attributable to anthracnose stalk rot in maize hybrids Cornell 281 and B37 × LB31 inoculated at anthesis with various inoculum concentrations of *Colletotrichum graminicola* at different time intervals after wounding[x]

Inoculum level (conidia/ml)	Discolored				50% or more discolored			
	Cornell 281		B37 × LB31		Cornell 281		B37 × LB31	
	0 hr	6 hr	0 hr	6 hr	0 hr	6 hr	0 hr	6 hr
10^2	3.3	2.2	2.6	2.1	1.5	0.4	0.3	0.1
10^4	4.3	2.9	3.7	2.3	2.5	1.0	1.3	0.6
10^6	5.9	3.7	4.6	3.0	4.2	1.7	2.3	1.2
10^7	4.9	3.4	4.0	2.6	2.9	1.3	1.6	0.9
10^8	4.1	2.8	3.7	2.7	2.1	1.1	1.3	0.9
Control[y]	1.9	1.5	1.7	1.5	0.0	0.0	0.0	0.0
LSD ($P \leq 0.05$)[z]	0.61		0.61		0.63		0.63	

[w] Based on means of 10 plants and four replicates at normal grain harvest time.
[x] Plants were inoculated into a wound in the first internode above the brace roots immediately (0 hr) or 6 hr after wounding.
[y] Plants in the control treatment were injected with sterile distilled water.
[z] LSD ($P \leq 0.05$) for comparison between time intervals at same combination of hybrid and inoculum level.

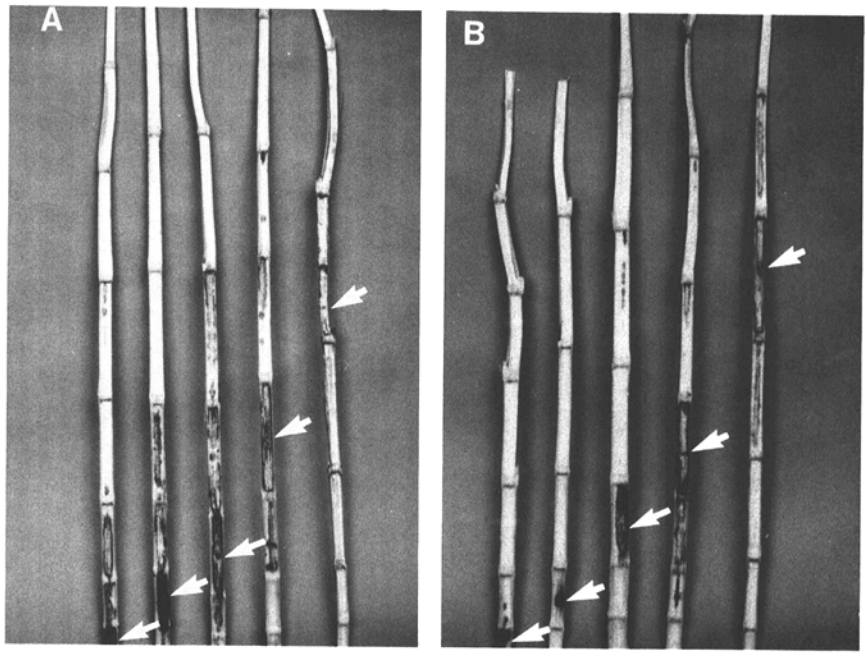

Fig. 3. Anthracnose stalk rot at normal grain harvest time in maize hybrid Cornell 281 inoculated at anthesis in (from left to right) the first through fifth elongated internodes above the brace roots with 1-ml suspensions of 5×10^5 conidia of *Colletotrichum graminicola* per milliliter **(A)** immediately or **(B)** 6 hr after wounding. Arrows show the respective internode positions into which the inoculum was introduced.

a greater stalk pith discoloration resulted from inoculation with *C. graminicola* in the first elongated internode above the brace roots than in the second internode below the ear. On the other hand, the incidence of premature plant death (i.e., top dieback) was equivalent in plants inoculated at either internode position. The present study provides the first evaluation of the effect of wound predisposition of maize to ASR in different maize internodes. Inoculation immediately after wounding resulted in systemic ASR development regardless of the internode inoculated. However, it was consistently observed that ASR was confined markedly to the lower plant internodes if inoculation was 1 or 6 hr after wounding in the first or second internodes, whereas the symptoms progressed to other internodes when inoculation was in the third through fifth internodes. But, overall, disease severity in plants inoculated after a time delay between wounding and inoculation was reduced relative to that in plants inoculated immediately after wounding (Figs. 3 and 4). This finding was consistent with our observation in Cornell 281 naturally infected by ASR (*data not shown*) where more severe symptoms were found in plants with ECB injuries in the uppermost than in the lower internodes. Similarly, Hooker (11) observed the greatest spread of stalk rot caused by *S. maydis* in maize when plants were inoculated in the fifth internode above the brace roots.

The fact that mild ASR developed from inoculation 1 or 6 hr after wounding in the lowermost plant internodes justifies further studies on factors affecting ASR development from infection in different plant parts. This observation might explain variable ASR development that has been observed between maize plants under field conditions (8,34). Neither internode position nor the interaction of internode position and time interval had a significant influence on ASR development in 1988 as they did in 1989. This may have been the result of less symptom development in 1988 attributable to the drought that prevailed from late July to mid-August in New York State. Nevertheless, inoculation immediately after wounding resulted in more severe ASR irrespective of the internode inoculated. Moreover, the finding that symptom development resulting from inoculation of plants 1 or 6 hr after wounding was reduced in both years suggested that ASR may be curtailed whenever a short interval is allowed between plant wounding and inoculation with *C. graminicola*.

Throughout the investigation, ASR was significantly greater in the susceptible than in the resistant hybrids. The extent of pith discoloration was considerably less in the resistant hybrids, even at high inoculum levels of *C. graminicola* and during a crop stage (i.e., anthesis) most conducive for severe ASR development. Resistance to ASR in the inbred line LB31 and its related hybrids is controlled by a single, dominant gene that confers a high level of resistance (2). Whether the reaction observed to ASR development in these hybrids would also occur in maize lines with polygenic resistance to ASR is not known. However, the observation that delaying inoculation after wounding also decreases ASR severity in the susceptible Cornell 281 strongly suggests that "wound healing" may commonly reduce ASR in maize.

Until recently, no information was available regarding the nature of wound predisposition of maize to ASR. Keller and Bergstrom (13) and we (22,24) showed not only that wound predisposition of maize to ASR was transitory but also that wound healing contributed significantly to the reduction of the disease in maize. The results of the present investigation demonstrated that less ASR developed in resistant maize hybrids, when plants were inoculated at vegetative stages, or when a time interval was allowed between wounding and inoculation. Wound healing occurred regardless of the internode inoculated or the inoculum level of *C. graminicola*, but its effect was most pronounced in the lowermost maize internodes. As mentioned earlier, genotypic resistance remains an effective method to control ASR. In screening germ plasm for resistance to ASR, precautions should be taken to inoculate maize plants at the most appropriate ontogenic stage for satisfactory symptom development and in the same internode at a uniform time (e.g., immediately) after wounding to obtain reliable ratings. Otherwise, considerable variation may occur between plants, making interpretation of the reactions difficult.

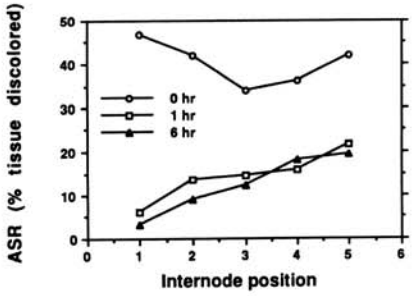

Fig. 4. Anthracnose stalk rot (ASR) at normal grain harvest time in maize hybrid Cornell 281 (averaged for eight internodes per plant and 10 plants for each of three replicates) inoculated at anthesis in 1989 with 1-ml suspensions of 5×10^5 conidia of *Colletotrichum graminicola* per milliliter. Inoculation was immediately (0 hr) or 1 or 6 hr after wounding in the first through fifth elongated internodes above the brace roots.

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