# Developmental Predisposition of Maize to Anthracnose Stalk Rot

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**ABSTRACT**

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The influence of maize developmental stage at time of inoculation with *Colletotrichum graminicola* on the development of anthracnose stalk rot (ASR) was investigated in a 3-year field study. Maize hybrids were inoculated by stalk injection with conidia of *C. graminicola* at various plant growth stages from midwhorl to late kernel dent. Plants of each hybrid inoculated at whorl vegetative stages developed little stalk rot by physiological maturity, as evidenced by internal stalk discoloration. Cornell 281 and Pioneer 3901 plants (susceptible to ASR) inoculated at the late whorl stage showed increased susceptibility to the pathogen, but pronounced levels of ASR did not develop until plants had reached anthesis. Severe ASR developed in plants inoculated at all subsequent growth stages through physiological maturity. In hybrid RD6501 × A632 (moderately resistant to ASR), significant ASR developed only in plants inoculated after the kernel dough stage. Rating of hybrids for ASR prior to kernel dent reduces misinterpretation due to the development of other stalk rots.

Stalk rot has long been recognized as a major factor limiting maize production in the world (4,12,15). Several fungi are consistently associated with stalk rots, although the prevalent pathogens vary with environment and time. Over the last 25 years, *Colletotrichum graminicola* (Ces.) Wils., causal agent of anthracnose stalk rot (ASR), has been cited increasingly as a major stalk rot pathogen in the United States (13,17,19–22) and, recently, has become a prominent stalk rot pathogen in New York maize fields (2,10,11).

A sizeable proportion of stalk rot literature has emphasized the development of Fusarium and Diplodia stalk rots in maturing plants, and ASR has been assumed by many to follow this pattern of invasion of senescent tissue. During an investigation of the interactive effects of the European corn borer and *C. graminicola* on maize production in New York (10,11), field observations suggested a relationship between maize developmental stage and susceptibility to ASR not confined to senescing stalk tissue. Previous studies (22,23) showed that ASR development was cultivar dependent and that symptoms were generally not visible until just prior to senescence in both maize and sorghum (8,23), but no published work has defined the relationship between host age and ASR development.

The effects of host age on disease severity are known for many host-parasite interactions including that of anthracnose leaf blight (ALB) on maize, also caused by *C. graminicola*. Although leaves may be infected by *C. graminicola* at any time in the season, there is a decrease in susceptibility to ALB between seedling and mid to late whorl stages (3,14). Stalk rots incited by *Fusarium* spp. and *Stenocarpella maydis* (Berk.) Sutton (syn. *Diplodia maydis* (Berk.) Sacc.) are not known to have extensive leaf blight phases and are opportunistic saprophytes that flourish in senescing tissue. The deterioration of pith in senescing stalks, which may be associated with large kernel sink size or close spacing of plants, has been linked to the development of these stalk rots (5,7,24). Although one investigation has reported that *C. graminicola* was inhibited by living sorghum stalk tissue in a manner like that of other stalk rot pathogens (9), in other instances *C. graminicola* was shown to be pathogenic on living maize tissue (16,20), suggestive of a hemibiotrophic mode of pathogenesis. The objective of this study was to define the pattern of ASR development in hybrids with different genetic backgrounds and to determine the effect of host developmental stage on susceptibility to ASR.

## MATERIALS AND METHODS

Cornell 281, a maize hybrid susceptible to *C. graminicola*, was planted 3 June 1983 and 4 June 1984 at Freeville, NY. Pioneer 3901 and experimental hybrid RD6501 × A632, susceptible and moderately resistant to ASR, respectively, were planted 28 May 1985 at the same location. A herbicide mixture of Aatrex (atrazine) at 0.67 kg/ha, Lasso (alachlor) at 1.75 L/ha, and Booster (an oil-based surfactant) at 4.7 L/ha was applied prior to planting. Fertilizer (3 kg 15-15-15 NPK/100 m row) applied at planting totaled 56 kg/ha of each element. Fields were sidedressed with 1.4 kg urea/100 m row when plants were in the early whorl stage. Soil was a Howard gravelly loam that had been planted to potatoes in the year preceding each experiment. Fields of approximately 2 ha were planted with a 91 cm spaced 2-row planter at a rate of 56,000 seeds/ha, and the final stand was approximately 51,000 plants/ha in 1983 and 1984. Fields were hand-planted in 1985: 12 hills of 2–3 plants each per 4 m. Hills were thinned to two plants each at seedling stage.

In 1983, starting 6.5 wk after planting, 20 plants each week were inoculated with 2 ml of a $10^5$ conidial suspension of *C. graminicola* for a total of 8 wk. The conidial suspension was injected into the second internode above the brace roots using a 50-ml syringe as previously described (22). Early inoculations were attempted only if the growing tip was above ground. In 1984, times of inoculation were 6, 7, 7.5, 8, 8.5, 12, and 14 wk after planting. Plants within subplots in the 1985 randomized block experiment were inoculated either with 2 ml of the conidial suspension or 2 ml of distilled water at 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, or 14 wk after emergence. Plant developmental stage was assessed using Ritchie and Hanway's revised developmental scheme that presented the vegetative and reproductive stages of maize development (18). The vegetative stages are: VE, leaf emergence; V1, first leaf; V2, second leaf (early whorl and midwhorl); V(n), nth leaf (late whorl); and VT, tasseling. The reproductive stages are: R1, silking; R2, kernel blister; R3, kernel milk; R4, kernel dough; R5, kernel dent; and R6, physiological maturity. Each stage is defined when 50% or more plants in a field are in or beyond that stage. There were no replications in 1983, three replications in 1984, and four replications in 1985. Individual subplots within each replicate were separated by two guardrows lengthwise and 6 m widthwise to minimize interplot interference due to *C. graminicola* dispersal.

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An isolate (Cg111NY82) of *C. graminicola* was obtained in 1982 from naturally infected maize plants in Tompkins Co., NY. The fungus was maintained on oatmeal agar under a 12-hr photoperiod of fluorescent light at 24 C. Susceptible maize plants were inoculated periodically and the fungus was then reisolated to ensure minimal change in pathogenicity. Conidial suspensions were prepared by flooding 2-wk-old oatmeal agar cultures, scraping off the conidia and associated matrix, and filtering suspensions through cheesecloth. The resulting inoculum was diluted with distilled water, and the conidial concentration was adjusted to $10^5$ conidia/ml.

Plant stalks were split from the internode above the adventitious roots up through eight consecutive internodes and were rated for visible discoloration due to stalk rot. Data were taken on 30 September (after kernel dent stage) in 1983. In 1984, disease severity was rated at harvest (24–28 October). Five ratings, 11 August, 18 August, 1 September, 6–7 October, and at harvest (24 October to 4 November), were taken in the 1985 season. Subplots were harvested randomly with regard to treatment to minimize harvest date effects on yield or stalk rot development. Each individual internode was rated on a visual scale of 0–5 for stalk rot (percentage of internal stalk tissue discolored): 0 = none, 1 = 0–5%, 2 = 5–25%, 3 = 25–75%, 4 = 75–100%, and 5 = 100%. The use of analysis of variance, dependent on a continuous scale of values, dictated the conversion of integer data points into percentage midpoints. The percentage midpoint for each range in the rating scale was determined for each internode and was totaled for each plant. The total was used to express percentage discoloration for each stalk, and the mean percentage value represented disease severity.

In the 1985 season, ears were hand-harvested in the field and labeled for later identification. Ears were dried at 60 C to constant weight and dry ear wt, dry shelled wt, and 1,000-kernel wt were recorded. Data from 20 randomly harvested plants were averaged for each subplot. Data were analyzed using SAS (Statistical Analysis System, SAS Institute, Inc., Cary, NC) for analysis of variance.

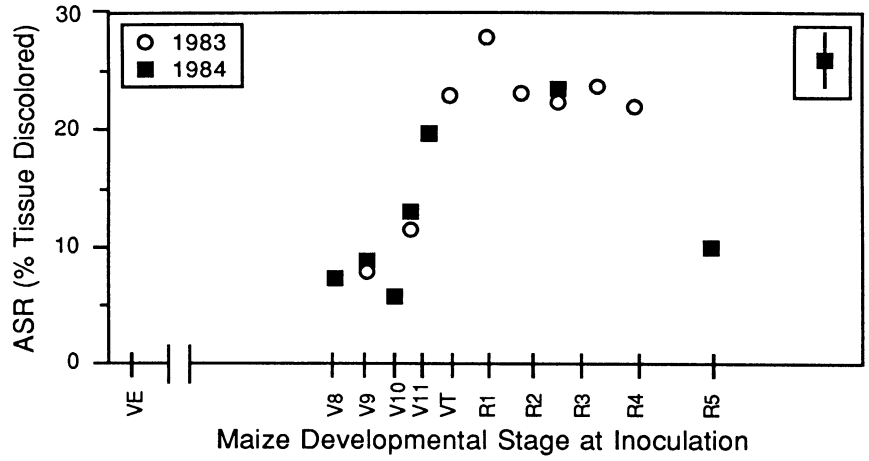


**Fig. 1.** Anthracnose stalk rot development in Cornell 281 maize plants rated at harvest. The abscissa is divided chronologically into host developmental stages denoting time of inoculation with *Colletotrichum graminicola*. The vertical line represents Fisher's protected LSD (4.5) for the 1984 season for $P = 0.05$.

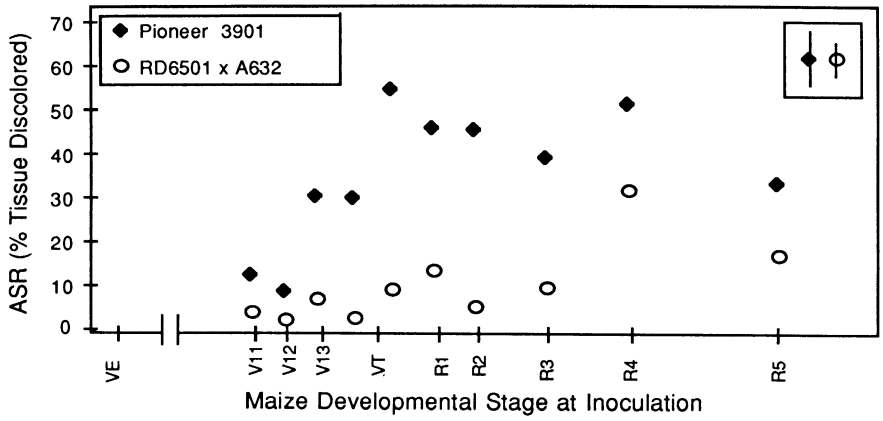


**Fig. 2.** Anthracnose stalk rot development in Pioneer 3901 and RD6501 × A632 maize plants rated at harvest. The abscissa is divided chronologically into host developmental stage(s) denoting time of inoculation with *Colletotrichum graminicola*. The vertical lines represent Fisher's protected LSD for the two hybrids for $P = 0.05$. Pioneer 3901 LSD = 13.4 and RD6502 × A632 LSD = 7.7.

## RESULTS

There was a significant difference in ASR development between Cornell 281 plants inoculated at midwhorl (V8) through late whorl stages (V9, V10), and those inoculated at all later stages for each year (Fig. 1). The first noticeable increase in ASR development occurred in plants inoculated at the late whorl stage (V11), and a greater extent of ASR development was observed in plants inoculated at tasseling. This higher level of host susceptibility to ASR was maintained throughout anthesis and the grain filling period, although there was some suggestion of decreased susceptibility towards kernel dent stage. In plants inoculated at earlier vegetative stages, stalk rot symptoms were confined to the inoculated internode throughout the season.

The susceptible hybrid Pioneer 3901 showed a similar change in pattern of ASR development as Cornell 281 (Fig. 2). Increased susceptibility to ASR was first observed just prior to tasseling. As with Cornell 281, the highest ASR ratings were observed in plants inoculated at the anthesis stage. However, moderately resistant RD6501 × A632 maintained a low level of susceptibility to ASR over all dates of inoculation. The stalk rot rating for RD6501 × A632 did not reach the level of Pioneer 3901, and the highest ASR ratings did not occur until the R4 stage.

Figure 3 presents the results of the five-stage rating schedule of RD6501 × A632 and Pioneer 3901 for all inoculation treatments over the 1985 season. Relatively little ASR developed in the first two late whorl inoculations (V11 and V12), regardless of host developmental stage at rating. Time of inoculation had no significant effect on symptom development at tasseling, but by silking, a significant increase in ASR development was observed in Pioneer 3901 plants inoculated at the very late vegetative stage (V13). This apparent change in tissue susceptibility of Pioneer 3901 during the short period between late whorl and tasseling was also observed at all subsequent rating periods.

There was a slight increase in ASR development at some rating periods in RD6501 × A632 plants after the V12 stage, but no consistent increase was observed until plants were inoculated at kernel dough stage. In both hybrids, the highest level of discoloration due to stalk

rot was recorded by kernel dent regardless of stage of inoculation. Yield was not affected by stalk inoculation with *C. graminicola* (*data not shown*), although previous studies have suggested otherwise (10,11,17,19,23).

## DISCUSSION

Anthracnose is manifested as seedling blight early in the season, and as stalk rot and leaf blight on postanthesis plants; rarely have symptoms been reported between early whorl stage and anthesis (3,6,13,14). Lesions develop early in the summer on the lower leaves of young plants and then are not noticeable until later in the season on the upper leaves of mature plants even though midsummer environmental conditions are nearer the optimum for lesion development, as determined by greenhouse studies on the cultivar Gaspe Flint (14). This work, and a similar study using a susceptible sweet and susceptible dent cultivar (3), suggested that the pattern of early and late-season infection described above was due to a host developmental change in susceptibility to ALB.

Our results demonstrated that a host developmental predisposition to ASR also occurs in susceptible maize hybrids. The stalks of susceptible hybrids were resistant to stalk rot development when inoculated with a conidial suspension of *C. graminicola* prior to the phase of rapid stalk elongation (late whorl stage). This phenomenon was observed repeatedly in field and greenhouse experiments (10). Stalk rot symptoms were confined to the inoculated internode of these plants. The inoculated internode was often distorted but the rest of the plant grew normally with no further ASR symptoms, even though viable *C. graminicola* could be recovered from nonsymptomatic, upper internodes of these plants (1). A transition to susceptibility occurred during the rapid stalk elongation period beginning just prior to tasseling and extending through anthesis.

This susceptibility of young maize tissue to ASR development is not constant in all hybrids. We found that ASR development was delayed and reduced in RD6501 × A632, a moderately resistant hybrid. A different mechanism may govern the resistance to ASR spread in susceptible cultivars inoculated prior to the rapid stalk elongation period as compared with the resistance exhibited by the mature tissues of resistant cultivars. Since differences in disease severity between resistant and susceptible cultivars may be obscured dependent on developmental stage at time of infection, inoculation time may have a significant role in screening for resistance. Our data also indicated that maximal ASR developed by the kernel dent stage. This suggests that this stage be used for rating for resistance to ASR, rather than at harvest, so as to minimize the confounding effects of other naturally invading stalk rot organisms present at the end of the season.

Previous research showed that early to midseason infections of maize with *C. graminicola* resulted in the most severe ASR development, yield reductions, and decline in standability under New York conditions (10,11). Furthermore, stalk ingress by *C. graminicola* was associated with injuries caused by insects such as the European corn borer (10,11). Integrated pest management strategies for maize stalk pests should stress the value of protecting stalk tissue from injury in the weeks immediately encompassing anthesis, when host susceptibility to *C. graminicola* is great and much of the season remains for further disease development.

Our finding that maize is predisposed to ASR development prior to grain filling stages suggests that, while ASR has much in common with other stalk rots, the interaction of *C. graminicola* with maize may differ in significant ways from the interactions of maize with such stalk rot fungi as *Stenocarpella maydis* and *Fusarium* spp. Several investigators have reported that plants inoculated with *S.*

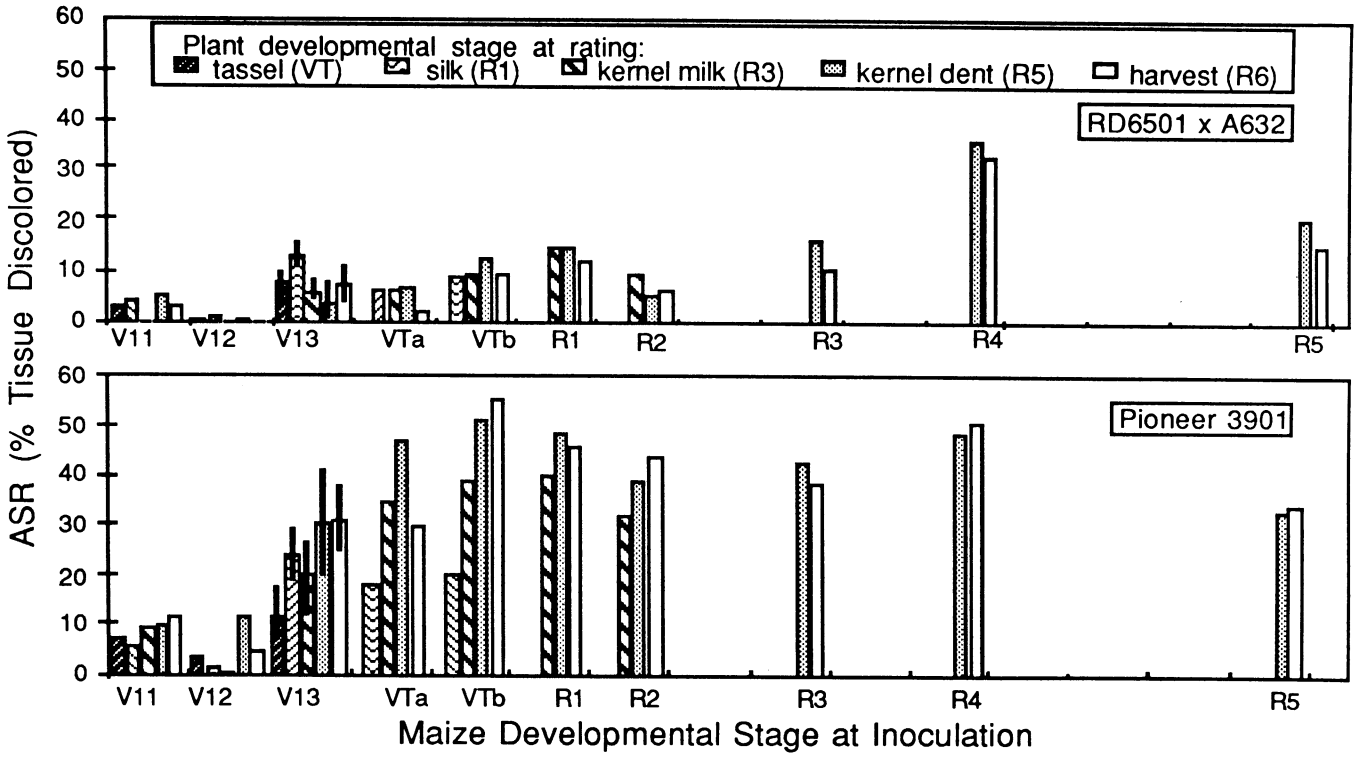


**Fig. 3.** Anthracnose stalk rot development in hybrids RD6501 × A632 and Pioneer 3901 at the tassel, silk, kernel milk, and kernel dent stages of host development and at harvest. The abscissa is divided chronologically into host developmental stages at time of inoculation with *Colletotrichum graminicola*. VTa represents the period where 50% of the plants were tasseling and VTb represents the period where >50% of the plants were tasseling, but <50% were silking. At $P = 0.05$, Fisher's protected LSD values for the five rating times are 6.2, 5.0, 4.4, 7.8, 7.7 for RD6501 × A632, and 9.8, 10.0, 14.8, 21.6, and 13.4 for Pioneer 3901, respectively. These values are represented visually at the V13 stage of inoculation.

*maydis* and *Gibberella zeae* (Schw.) Petch prior to pollination seldom develop much stalk rot before pith senescence (5,12,24). One study reported that maize stalks were susceptible to Diplodia and Fusarium stalk rots prior to pollination, but that stalk rot was never as extensive in these plants as in those inoculated later in the season (15).

It has been postulated that stalk rot develops when root and stalk pith cells senesce prematurely due to deprivation of sugars caused by photosynthetic stresses or an imbalance in source/sink relations, i.e., the photosynthetic stress-translocation balance (PS-TB) concept of Dodd (7). These factors influence the general deterioration of stalk quality and may account for the pattern of late season stalk rots in mature maize and sorghum (5,7,9,12,24). However, hybrid susceptibility, pathogen isolate, and time of infection affect the development of specific interactions such as anthracnose stalk rot (10,16,22). The PS-TB concept was based largely on observations of stalk rot associated with the opportunistic fungi *S. maydis* and *Fusarium* spp. Our data suggest that *C. graminicola* exhibits a hemibiotrophic mode of pathogenesis in maize stalks, a suggestion corroborated by previous observations (10,11,16,20). These observations suggest that *C. graminicola* may have a different nutritional relationship with its host, which may affect its response to source/sink disturbances. The pattern of stalk rot development that we report in this paper with *C. graminicola* on susceptible hybrids warrants a critical test of the PS-TB hypothesis with this pathogen.

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