Potential Yield Reductions in Maize Associated with an Anthracnose/European Corn Borer Pest Complex in New York

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ABSTRACT

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In a 2-yr field study, the individual and combined effects of *Colletotrichum graminicola* and *Ostrinia nubilalis* (European corn borer [ECB]) on grain yield and stalk rot development in a maize hybrid susceptible to both organisms were determined. In 1983, a year favorable for development of anthracnose leaf blight and stalk rot, plants infested with ECB and/or inoculated with *C. graminicola* at the whorl stage of development showed average grain reductions of 13.5% (12 q/ha), 35.2% (31.2 q/ha), and 46.5% (41.2 q/ha) in association with ECB injury, anthracnose development, and both ECB injury and anthracnose, respectively. The same treatments at the silk stage resulted in grain reductions of 6.4% (5.4 q/ha), 16.5% (13.8 q/ha), and 12.2% (10.2 q/ha), respectively. Inoculation and infestation of plants in the dough stage resulted in no yield reductions. In 1984, a year less favorable for anthracnose development, grain yield was reduced (10.7%, 9.6 q/ha) in plants inoculated with *C. graminicola* at the whorl stage and infested with ECB at the kernel blister stage but was not reduced in plants inoculated and/or infested at later growth stages. Even minimal stalk damage by ECB significantly predisposed plants to anthracnose stalk rot development. Anthracnose stalk rot-induced grain reductions in New York consistently have been associated with early or midseason ECB infestations.

Additional key words: *Zea mays*.

Stalk-boring insects and stalk rots are among the major factors limiting maize (*Zea mays* L.) production in the world (7,8). Stalk rots are incited by several fungi and bacteria; the predominant pathogens vary with geography and climate. *Colletotrichum graminicola* (Ces.) Wils., causal fungus of maize anthracnose, has become recognized as one of the predominant stalk rot pathogens in North America during the past few decades (1,13,18,22,27). Significant yield reductions caused by anthracnose stalk rot (ASR) have been reported in the major corn production areas in north central United States (20,22,23,25). Yield reductions induced by ASR may result from physiological reduction in grain weight, premature plant death, and/or stalk breakage, which reduces harvestability.

European corn borer (ECB) (*Ostrinia nubilalis* Huebner) is one of the most prevalent maize pests in North America (8) and is generally acknowledged as the number one insect problem of maize in the northeastern states. ECB infestations may lead to decreases in maize yield by reducing grain weight, by increasing the incidence of lodging and ear drop, and by providing entry sites for stalk and ear pathogens (6,9,12,16,17). Yield reductions depend on many factors, including environmental conditions and the stage of growth of the plant when infested (11,12,21).

Although an association between stalk rot and ECB infestation was first reported in 1950 by Christensen and Schneider (6), there have been few detailed investigations of this relationship until recent years. Incidence and severity of stalk rots have been shown to increase with the presence of ECB (2,6). Hybrids normally resistant to stalk rots incited by *Diplodia* and *Fusarium* spp. were readily infected when injured by ECB (5,15–17). Jarvis et al (16) reported that yield reduction resulting from the presence of ECB and three common stalk rot pathogens, *Diplodia maydis* (Berk.) Sacc., *Gibberella zeae* (Schw.) Petch, and *Fusarium moniliforme* (Sheld.) Snyd. & Hans., was a summation of the individual reductions from ECB and stalk rot.

In Illinois, White and Humy (26) observed an increase in ASR associated with ECB wounds, whereas Carson and Hooker (4) did not. During an anthracnose epidemic in New York in 1981, increased incidence and severity of ASR were associated with ECB infestation (1). Although Bergstrom et al (2) experimentally confirmed a synergism between *C. graminicola* and ECB in the development of stalk rot, no thorough study was done on the interactive effects of these two pests on maize yield. Therefore, the following study was undertaken to determine the potential individual and combined effects of *C. graminicola* and ECB on yield and stalk rot development in maize.

MATERIALS AND METHODS

Cultural practices recommended for New York were used in planting Cornell 281, a commonly grown maize hybrid susceptible to *C. graminicola* and *O. nubilalis*. Planting dates were 3 June 1983 and 5 June 1984 at Freeville, NY. A herbicide mixture of atrazine (Aatrax) at about 0.67 kg/ha, alachlor (Lasso) at about 1.75 L/ha, and Booster (an oil-based surfactant) at about 4.68 L/ha was applied before planting. Fertilizer (3 kg of 15-15-15 NPK per 100-m row) was applied at sowing. Fields were side-dressed with 1.4 kg of urea per 100-m row when plants were in the early whorl stage (14). Soil was a Howard gravelly loam that had been planted to potatoes in the previous year. Fields of about 2 ha were planted with a 91-cm, two-row planter at a rate of about 56,000 seeds per hectare, and the final stand was 51,000 plants per hectare.

Field experiments. The experiment was a 2×2×3 factorial. The design was randomized blocks with five replicates in 1983 and six replicates in 1984. Subplots were five rows wide and about 2.4 m long in 1983 and three rows wide and about 3.4 m long in 1984. Individual subplots within each replicate were separated by two guard rows lengthwise and about 6 m widthwise to minimize

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interplot interference caused by ECB larval migration and *C. graminicola* dispersal.

Subplots were treated at three growth stages: midwhorl (6–7 wk after sowing), midsilk (10 wk after sowing), and middough (13 wk after sowing). Background populations of ECB (<5% of field infested) and *C. graminicola* at Freeville were very low, so noninoculated and noninfested plants served as adequate controls. Treatments were inoculation with *C. graminicola* alone, infestation with ECB alone, inoculation with *C. graminicola* and infestation with ECB, and untreated controls. Thus, the 1983 experiment consisted of three levels of plant maturity at treatment, two levels of *C. graminicola*, and two levels of ECB for a total of 60 subplots. In 1984, the dough-stage treatment was dropped from the experiment because there was no yield reduction or stalk rot development resulting from this treatment in 1983. There were 48 subplots in the 1984 experiment.

Insect culture. ECB larvae were collected from overwintering sites (field corn stubble) near Batavia, NY, in 1982 and incubated individually to allow culling of parasitized and diseased specimens. The procedure of Guthrie (10) was used to rear larvae on meridic diet. Test insects were three to seven generations removed from field collections. In 1983, ECB were applied as one or two egg masses per plant. Egg masses were applied to the undersides of emerging leaves at the whorl stage and to the undersides of the leaves at and below ear level on older plants. The insects were applied as individual larvae in 1984 because of a shortage of egg masses. Individual larvae (one to three per plant) were placed in the collar area of the leaves below the ear. The collar area lies next to the maize stalk and joins the sheath and blade tissue of the leaf. Fields were monitored after infestation dates, and when insects failed to establish, plants were infested with supplementary applications of ECB. This delayed field establishment of ECB until the kernel blister and kernel milk stages in the 1984 season.

Fungus culture. An isolate of *C. graminicola* was obtained from naturally infected maize plants in Tompkins County, NY, in 1982. The fungus was maintained on oatmeal agar (24) under 12 hr of fluorescent light (230 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 24 C, was periodically inoculated into susceptible maize plants, and was reisolated to ensure minimal change in pathogenicity. Inoculum was prepared by culturing the fungus on autoclaved oat grains. Infested oat grain inoculum was supplemented by spore suspensions prepared by flooding 2-wk-old oatmeal agar cultures with distilled water, then scraping off the conidia and associated matrix and filtering suspensions through cheesecloth. The resulting inoculum was diluted with distilled water, and the spore concentration was adjusted to 10^5 spores per milliliter. Plants were inoculated with 10 ml of infested oat grain, then wetted with 10 ml of the spore suspension of *C. graminicola*. Inocula were placed in the whorl and lower leaves of plants in the whorl stage and near the collar of leaves below the ear of plants in the silk and dough stages. This method of inoculation ensured that fungal ingress into the stalk would simulate that in nature—either active penetration by the fungus or entry via ECB wounds.

Data collection and analysis. Plant stalks were split open from the internode above the adventitious roots up eight consecutive internodes and were rated for stalk rot development and ECB injury at physiological maturity. Harvest was 3–17 October in 1983 and 19–28 October in 1984. 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 tissue discolored): 0 = none, 1 = 0–5%, 2 = 5–25%, 3 = 25–75%, 4 = 75–100%, and 5 = 100%. The percentage midpoint for each range in the rating scale of each internode was totaled for each plant. The total was used to express discoloration for each stalk, and the mean percentage value represented disease severity. ECB injury was rated by measuring the length (in centimeters) of tunneling in each internode, then adding these numbers over eight internodes.

Ears were hand-harvested in the field and labeled for future identification. Whole ear weight was recorded on the day of harvest. Ears were then dried at 60 C to constant weight; dry ear weight and dry shelled kernel weight were recorded. Yield data were adjusted to quintals per hectare at 15.5% moisture before analysis.

Data from 20 randomly harvested plants were averaged for each subplot. The SAS statistical package (Statistical Analysis System, SAS Institute, Inc., Cary, NC) was used for analysis of variance. The Minitab statistical package (Pennsylvania State University, University Park) was used to compute regression equations.

RESULTS

1983 Season: yield assessment and stalk rot development. Yield was influenced significantly by the interaction between host developmental stage at treatment and the presence of *C. graminicola*. The analysis of variance of grain yield and total ASR severity for all three main effects (*C. graminicola*, ECB, and maturity stage of plant when inoculated/infested) and their interactions is summarized in Table 1. Because there was a significant interaction between plant maturity stage and the other two factors, the effects of *C. graminicola* and ECB were considered separately for each maturity stage.

Both *C. graminicola* and ECB individually reduced yields of plants treated at the whorl stage, but only ECB reduced yields of plants treated at the silk stage (Table 2). Both organisms significantly increased stalk rot severity when applied at the whorl and silk stages (Table 2). The interactive effect of both organisms was additive for both yield reduction and stalk rot severity (Table 2). Inoculation and infestation of plants at middough did not affect yields or stalk rot development.

In plants treated at the whorl stage, the presence of ECB alone resulted in a 13.5% (12 q/ha) reduction of grain yield. This yield reduction was associated with a mean larval tunneling length of 9 cm/stalk. Reductions of 35.2% (31.2 q/ha) and 46.5% (41.2 q/ha) were recorded for plants treated with *C. graminicola* alone and with *C. graminicola* and ECB, respectively. The mean tunneling length associated with this last treatment was 8.3 cm/stalk. The yield reduction attributed to the *C. graminicola* treatment at the whorl stage was associated with both anthracnose leaf blight (ALB) and ASR development in these plants.

In plants treated at the silk stage, ECB alone reduced yield 16.5% (13.8 q/ha) and *C. graminicola* in combination with ECB reduced yield 12.2% (10.2 q/ha). Mean tunneling lengths associated with these treatments were 4.7 and 5.5 cm, respectively.

1984 Season: yield assessment and stalk rot development. In 1984, when data were separated by maturity stage, yield was significantly affected only by *C. graminicola* in plants treated at the whorl stage (Table 3). Development of stalk rot, however, was influenced at all maturity stages by *C. graminicola*, by ECB, and by their interaction (Table 3).

Yield was reduced 8.1% (7.2 q/ha) in plants inoculated with *C. graminicola* alone and 10.7% (9.6 q/ha) in plants inoculated with

TABLE 1. Analysis of variance of the main and interactive effects of *Colletotrichum graminicola*, European corn borer (ECB), and host maturity stage on yield and stalk rot severity in Cornell 281 maize plants over 2 yr

Source[a]	df[b]	Yield		Stalk rot	
		1983	1984	1983	1984
Cg	1	19.6***[c]	6.6*	18.4***	58.1***
ECB	1	43.5***	0.6	44.0***	33.5***
M	2(1)	23.6***	2.0	48.7***	4.0
M + Cg	2(1)	27.5***	4.8*	12.0***	4.1*
M + ECB	2(1)	2.0	0.1	7.9**	2.5
Cg + ECB	1	0.4	0.0	0.0	35.8***
M + Cg + ECB	2(1)	0.1	2.4	0.9	3.1
Pooled st. error		9.5	8.3	1.4	0.9

[a]The sources of variation are *C. graminicola* (Cg), European corn borer (ECB), and maturity stage (M) of maize at time of inoculation and/or infestation.

[b]The degrees of freedom of the maturity stage were 2 in 1983 and 1 in 1984, respectively.

[c]* = $P<0.05$, ** = $P<0.01$, and *** = $P<0.001$ based on the F statistic from the analysis of variance.

the fungus at the whorl stage and infested with ECB at the kernel blister stage. Smaller yield reductions were associated with *C. graminicola* in 1984, a year in which ALB development was minimal and ASR development was delayed. ECB establishment was also delayed and caused no yield reductions.

Unlike 1983, no significant ASR developed in plants inoculated with *C. graminicola* alone; rather, ASR developed only in plants inoculated with both fungus and insect (Table 3). In plants treated at the whorl or silk stages, stalk rot development was enhanced by the presence of ECB. The interaction between the fungus and insect resulted in a synergistic increase in stalk rot development.

Regression analyses. The value of stalk rot severity at harvest as a predictor of yield reduction was tested in both the 1983 and 1984 seasons. Figure 1 shows the regression lines from plants treated at the whorl and silk stages in 1983. Both lines are highly significant, although the correlation coefficient for the whorl regression line is greater than that for the silk regression line. Plants treated at the whorl stage show a steeper decline in yield with increasing stalk rot than do plants treated at the silk stage. The yields of inoculated plants were not greatly decreased by anthracnose in 1984. Consequently, there was not a significant regression line from the 1984 season.

TABLE 2. Means of yield, percentage of stalk rot, and length of larval tunneling in maize plants inoculated with *Colletotrichum graminicola* and/or infested with European corn borer (ECB) at three stages of plant development in 1983

Growth stage at treatment with[a]				
C. graminicola	ECB	Yield (q/ha)	Stalk rot (%)	Tunneling (cm/stalk)
...	...	88.6	4.3	0.0
Whorl	...	57.4	26.3	0.0
...	Whorl	76.6	19.0	9.0
Whorl	Whorl	47.4	52.7	8.3
$LSD_{0.05}$[b]		7.4	7.9	3.6
...	...	83.8	5.4	0.0
Silk	...	78.4	14.4	0.0
...	Silk	70.0	22.0	4.7
Silk	Silk	73.6	38.1	5.5
$LSD_{0.05}$		4.9	10.3	0.9
...	...	82.6	6.2	0.0
Dough	...	81.4	8.4	0.0
...	Dough	78.4	12.0	0.9
Dough	Dough	77.2	11.2	1.1
$LSD_{0.05}$		4.7	5.8	0.7

[a] Maize plants were treated at three growth stages: whorl, silk, and dough. The treatments were inoculation with *C. graminicola* alone, infestation with ECB alone, inoculation and infestation with both pests, or no treatment.

[b] Means were analyzed using Fisher's protected LSD.

TABLE 3. Means of yield, percentage of stalk rot, and length of larval tunneling in maize plants inoculated with *Colletotrichum graminicola* and/or infested with European corn borer (ECB) at four stages of plant development in 1984

Growth stage at treatment with[a]				
C. graminicola	ECB	Yield (q/ha)	Stalk rot (%)	Tunneling (cm/stalk)
...	...	89.7	1.2	0.0
Whorl	...	82.5	4.2	0.0
...	Kernel blister	92.2	9.5	5.9
Whorl	Kernel blister	80.1	38.4	6.5
$LSD_{0.05}$[b]		6.0	6.2	0.7
...	...	92.2	1.3	0.0
Silk	...	86.2	2.4	0.0
...	Kernel milk	88.0	9.9	4.9
Silk	Kernel milk	91.6	24.5	5.8
$LSD_{0.05}$		7.3	6.3	0.9

[a] Maize plants were treated at four growth stages: whorl, silk, kernel blister, and kernel milk. Treatments were inoculation with *C. graminicola* alone, infestation with ECB alone, inoculation and infestation with both pests, or no treatment.

[b] Means were analyzed using Fisher's protected LSD.

DISCUSSION

ASR caused by *C. graminicola* has increased in prevalence and severity in New York maize fields in recent years, in tandem with infestations by ECB. An important factor influencing the pathology of this pest complex was the timing of both insect infestation and pathogen colonization during the growing season. Our data showed that the largest yield reductions associated with *C. graminicola* and its interaction with ECB were observed when plants were inoculated and infested at the midwhorl stage. The 46.5% (41.2 q/ha) grain reduction from the combined effect of *C. graminicola* and ECB in 1983 was approximately a summation of the grain reductions induced by the pests individually. A similar summation was seen in plants treated at the silk stage in 1983. This agrees with the work of Jarvis et al (16) on an ECB plus *Gibberella zeae, Fusarium moniliforme,* and *Diplodia maydis* stalk rot complex.

The greater yield reduction in 1983 (35.2%, 31.2 q/ha from inoculation with *C. graminicola* alone) compared with the yield reduction in 1984 (8.1%, 7.2 q/ha) was probably due to differential anthracnose development as influenced by the environment for the 2 yr. Nevertheless, yield reductions over the 2 yr were within the range reported by other investigators. Smith (23) reported an

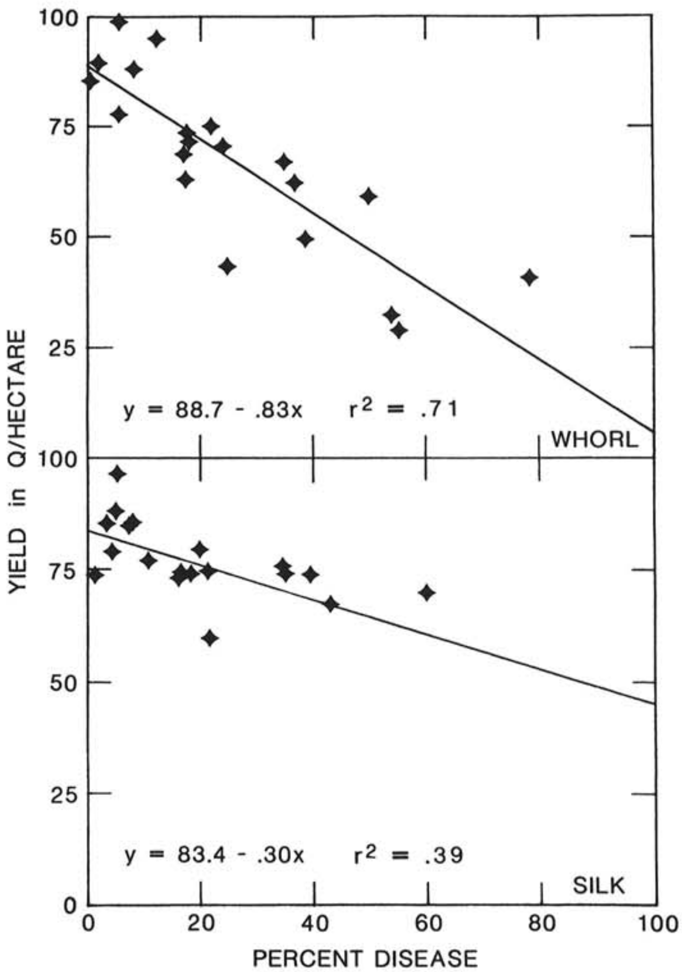

Fig. 1. Relationship between grain yield (q/ha) and stalk rot (mean percentage) at harvest in Cornell 281 maize plants. Data points are averaged plot values of plants either inoculated with *Colletotrichum graminicola*, infested with European corn borer, inoculated and infested with both pests, or left as controls at the whorl and silk stages in 1983. Each regression equation is based on the F statistic and has a P value of <0.01.

18.7% (19.8 q/ha) grain reduction in one susceptible hybrid inoculated at the whorl stage with *C. graminicola* although several other hybrids showed no yield reduction. Perkins and Hooker (22) reported an average 9.5% ASR-induced grain reduction in susceptible hybrids per individual plant and a 3.1% ASR-induced grain reduction per plot. Natti (20) reported ASR reductions ranging from 6.4% (5.8 q/ha) to 12.3% (12.8 q/ha) in individual plants of susceptible hybrids and an overall 2.4% field reduction from ASR. Mduruma (19) reported up to 12.3 q/ha yield reductions in susceptible and moderately resistant plants inoculated at silking.

Our results suggest grain reductions caused by ASR often depend on the time of entry of *C. graminicola* into maize stalks. Fungal ingress is commonly associated with ECB injury sites. The presence of one borer alone, though not enough to cause significant damage per se to the maize plant, is enough to allow early entry of the fungus into the stalk, and yield reduction from stalk rot may occur. The delayed ECB establishment and decreased larval tunneling in 1984 would account for both the lack of direct yield reduction from ECB injury and the lack of associated yield reduction from ASR, because the ECB provided entry holes too late in the season for the fungus to cause grain yield reductions. Also, the ability of *C. graminicola* to actively penetrate stalks, as well as the timing of penetration, is very dependent on weather conditions. Under conditions favorable to ASR development, fungus penetration may occur early enough in the season to result in yield reduction as the data of 1983 suggest. Nevertheless, our observations over 4 yr in New York State indicate that ingress by *C. graminicola* into stalk tissue, occurring early enough to cause yield reduction, is almost exclusively associated with ECB wounds.

We found that ECB not only provides a site of ingress for *C. graminicola* but the larvae can vector the fungus (*unpublished*), although the relative occurrence of vector transmission is unknown. Regardless of vector incidence, the most significant role of ECB in stalk rot development is in wound predisposition of stalks. Research concerning the effects of ECB insecticides on ASR development demonstrates that it only takes one entry site to let the fungus inside the stalk (R. I. Carruthers and G. C. Bergstrom, *unpublished*). A decrease in numbers of ECB per plant associated with insecticide application resulted in little decrease in stalk rot symptoms. Only treatments that reduce the number of plants affected by ECB are likely to significantly reduce ASR in a field.

The yield reduction associated with ECB alone was dissimilar for the two seasons. There was a significant reduction in yield caused by ECB in plants infested at the whorl and silk stages in 1983, yet no relationship was found between the length of stalk tissue tunneled and yield reduction. This differs from the results of several other studies where ECB-induced yield reductions were reported in terms of a unit length of tunnel per plant (3). In those experiments, yield reductions caused by ECB ranged from 2.8–3% (12) to 4.2–8% (2.4–2.3 q/ha) (3) per cavity. Guthrie et al (12) reported total field grain yield reductions ranging from 2.2 to 39.7 q/ha. The range was dependent on infestation levels of ECB. Patch et al (21) found total yield reductions of 3.0–3.9% (about 2.8 q/ha) from ECB over 2 yr. The 12.2–16.5% (12.0–13.8 q/ha) grain yield reductions from direct ECB damage reported in this paper are quantitatively similar to those mentioned above. The lack of yield reduction from the delayed infestation dates in 1984 suggests that relatively early-season ECB infestations are needed for damage manifestation.

Our results demonstrate the interactive effects of ECB and *C. graminicola* in stalk rot symptomatology and in reducing maize yield; others (2,5,6,15–17) have reported similar interactions of ECB with other stalk rot pathogens. Collectively, this evidence suggests that the presence of stalk rot pathogens and ECB in maize is so intimately linked that it constitutes a commonly and naturally occurring maize pest complex. Consequently, any yield reduction studies, breeding programs, or other control strategies focusing on a single fungus or insect would be incomplete without detailed consideration of the concomitant role of other pests.

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