Kernel Infection, Seedling Blight, and Wilt of Maize Caused by Colletotrichum graminicola

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ABSTRACT

Anthracnose caused by *Colletotrichum graminicola* was observed on corn kernels. In field soil in the greenhouse, kernels of corn severely infected with *C. graminicola* had the highest disease severity index and lowest seedling dry weight. In the field, seedling emergence was severely affected. Six percent of the seedlings emerged from extensively damaged kernels, 37% from kernels with few infection sites, and 90% from healthy kernels. Seed lots containing kernels lightly infected with *C. graminicola* were treated with nine fungicides or combinations thereof. Seedling stand was best, and the disease severity index was least, when kernels were treated with Terra-Coat L-205.

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Additional key words: anthracnose, corn, fungicides.

Anthracnose caused by *Colletotrichum graminicola* Ces.) G. W. Wils. is considered a minor leaf-spotting disease on corn (*Zea mays* L.) in the U.S. (2, 10). However, recent severe outbreaks of anthracnose on dent corn in Maryland (5), Indiana (8) and Kentucky (9) have been reported. Severe leaf and stalk damage have been reported outside the United States (1, 3, 4, 7), and it has caused severe damage to sweet corn stalks in the U.S. (8), but not to dent corn stalks.

Anthracnose on dent corn kernels was shown by Messiaen and Lafon (4) when ears were artificially inoculated with *C. graminicola*, but volunteer and dent corn kernels in adjacent fields were not infected.

During the past 2 years, lots of dent and sweet corn kernels were visually observed for acervuli of *C. graminicola* from areas reporting anthracnose. A low percentage of both dent and sweet corn kernels was infected by *C. graminicola* (Warren, *unpublished*). Although anthracnose-infected kernels have not become a serious economic threat, the potential for increased damage exists, especially in foundation and seed-production fields. This report is concerned with the effects on stand and the results of planting naturally infected kernels of dent corn and with possible chemical control.

MATERIALS AND METHODS.—Kernels from nearly 200 seed lots of corn from various hybrids and inbreds were examined for damage caused by *C. graminicola*. These seed lots were hand-harvested in September and October and examined 4 to 8 weeks after storage at 4 C. One hundred kernels of each lot were surface-disinfested with a 1% solution of NaOCl for 3 minutes, then plated on oatmeal or potato-dextrose agar (PDA). The plates were incubated at 24 C in an incubator for 10 days, and the percentage of infected kernels was determine.

Kernels examined visually were placed into four grades: 1 = apparently healthy kernels; 2 = small black streaks near the base of the kernel; 3 = black streaks, elongated with none to a few acervuli of *C. graminicola*; and 4 = long black streaks with many acervuli. *C. graminicola* was isolated from all lots of graded kernels except the apparently healthy lot.

Corn kernels were sown in field soil in 15.2 cm (6-inch) diameter clay pots and placed in a greenhouse at 24 C (range 22-27 C) to test for wilt or seedling blight, and to determine the effect of infected kernels on emergence, stand count, wilt, and dry weight. Five kernels were planted per pot, replicated five times and each test repeated three times. Dry weight of shoots and roots was obtained by placing the plant material in paper bags, drying them for 48 hours in an oven at 70 C, and then weighing the material.

Nine fungicides, or combinations, were applied to kernels in glass jars at the rates recommended on the labels. Fungicides not labeled for seed treatment were used at the rate of 0.8 g/kg (2 oz per 100 lb) of seeds. Kernels with fungicide were sown in the greenhouse in field soil. Emergence, stand count, and wilt symptoms were recorded.

The fungicides used in the greenhouse trials were: benomyl (50% active), methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate; Dithane M45 (50% active) coordination product of zinc ion and manganous ethylenebis (dithiocarbamate); thiram (50% active) bis(dimethylthiocarbamoyl) disulfide; Demosan T (43% chloroneb plus 22.4% thiram) 1,4-dichloro-2,5-dimethoxybenzene plus bis(dimethylthiocarbamoyl) disulfide; Terra-Coat L-205 (PCNB 23.2% plus ETMT 5.8) pentachloronitrobenzene plus 5 - ethoxy 3 - (trichloromethyl) - 1,2,4 - thiadiazole; Terra-Coat LT-2 (50% active) pentachloronitrobenzene; captan (80% active) N- (trichloromethyl) thio -4-cyclohexene - 1,2 - dicarboximide; captan plus PCNB (42% captan plus 42% PCNB), and captan plus thiram, (16.67% captan plus 16.67% thiram active).

In field plots, 38 kernels not surface-disinfested were sown singly about 25 cm apart in three replicated rows with a hand planter. Seedling emergence data were taken 3 weeks after planting and wilt symptoms 4 and 8 weeks after planting. Anthracnose symptoms were classified

using a disease severity index (DSI) of 0 to 4 where: 0 = healthy plants; 1 = chlorotic streaks or inward rolling of lower leaves; 2 = chlorotic streaks on lower and upper leaves; 3 = necrotic streaks on lower leaves, plants stunted, and leaves twisted; and 4 = necrotic streaks on all leaves, plants severely stunted, or plants killed.

RESULTS.—Of 192 samples (100 kernels per sample) of sweet and dent corn plated on PDA or oatmeal agar, 14 percent were infected with *C. graminicola*. Five of 12 sweet corn samples and 23 of 180 dent corn samples were infected. The percentage of kernels infected within the samples ranged from less than 1 percent to 5.6 percent. Dent corn samples had fewer infected kernels than sweet corn samples, never having more than 2% infected kernels.

Seedling blight and wilt from infected kernels.—Dent corn kernels graded on the basis of extent of damage to the pericarp, are shown in Fig. 1. In the greenhouse, there were no differences in plant emergence. Kernels judged to be infected with *C. graminicola* produced diseased plants 7-14 days after planting. Plants grown from infected kernels were stunted as compared with those from healthy kernels (Fig. 2). Significant differences were found among, and between, grades of infected kernels (Table 1). Most seedlings produced from infected kernels developed wilt symptoms. Seedlings produced from heavily infected kernels had the highest DSI and the lowest dry weight. For example, the DSI was 0.2 for the control (grade 0) and 3.42 for the heavily infected kernels (grade 4). The small percentage of diseased seedlings observed in the apparently healthy lot indicates that some infected kernels may go unnoticed when observed visually. Thus, it is possible to have infected kernels without extensive damage to the pericarp.

The rate of symptom development and frequency of necrotic lesions on leaves from heavily infected kernels (grade 4) indicate that seedlings can be damaged severely when infected kernels are planted. When roots of seedlings grown from diseased kernels were examined 14

TABLE 1. Effect of sowing maize kernels infected with *Colletotrichum graminicola* on emergence, plant height, seedling blight, and dry weight in the greenhouse 12 days after planting

Kernel rating	Emergence	Plant height (cm)	Disease severity index[y]	Mean dry weight[y] of seedling (g)
Control	70	40.7 a[z]	0.2 a	42.95 a
Light	100	31.1 b	1.50 b	31.03 b
Moderate	93	24.1 b	2.58 c	25.27 c
Heavy	93	14.2 c	3.42 d	16.81 d

[y]Disease severity index based on 0 to 4 scale, in which 0 = healthy seedlings and 4 = severe necrosis or plants killed.

[z]Numbers followed by the same letter in a column are not different according to Duncan's multiple-range test $P = 0.05$.

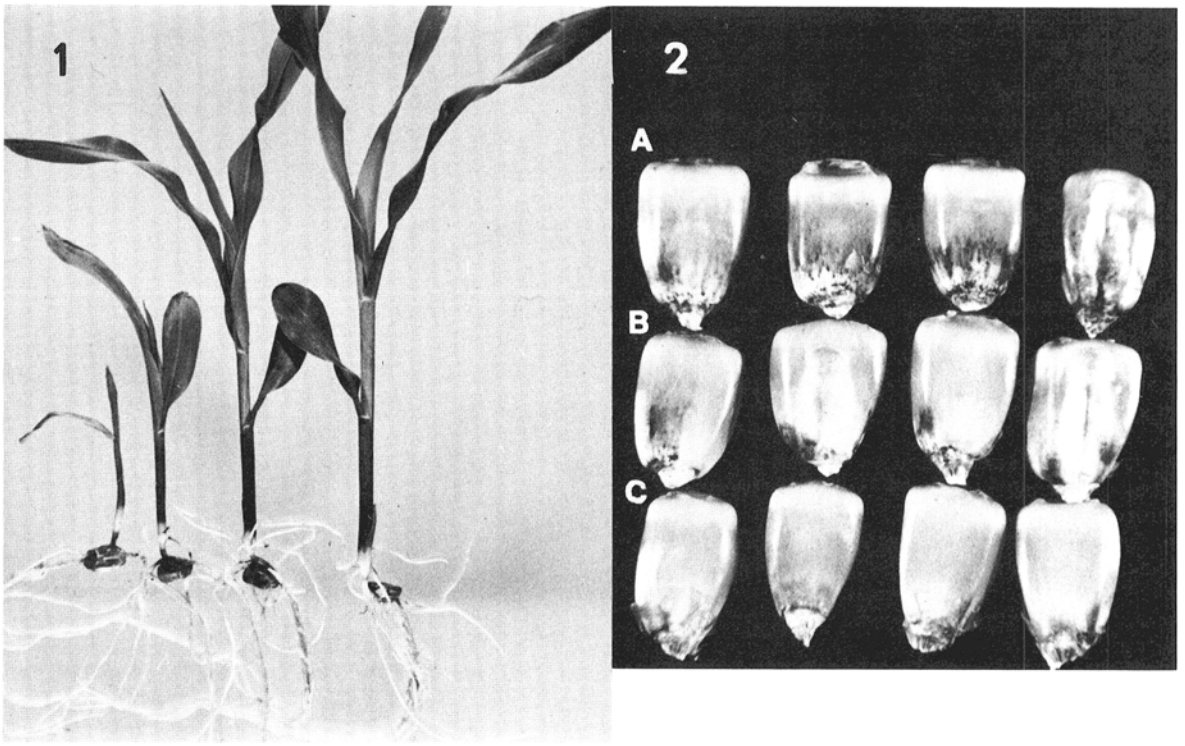

Fig. 1-2. 1) Dent corn kernels showing symptoms of anthracnose: A = restricted infected kernels; B = intermediately infected kernels; and C = extensively infected kernels. 2) Twelve-day-old corn seedlings grown from kernels infected with *Colletotrichum graminicola*. From right to left are healthy, restricted infected, intermediate infected, and extensively infected kernels.

TABLE 2. Emergence, wilt, and seedling blight of maize seedlings grown from *Colletotrichum graminicola*-infected kernels in the field

Grade of kernel infection	Emergence[a] (%)	Wilt[a] (%)	DSI[b]
Healthy	96	0	0
Restricted	37	52	3.0
Extensive	6	0	3.7

[a]Emergence and wilt data taken 21 and 28 days after planting, respectively.

[b]Disease severity index (DSI) is based on 0-4 scale, in which 0 = healthy plants and 4 = plant severely diseased or killed. Average disease severity index 28 days after planting.

TABLE 3. The effect of fungicidal treatment of corn kernels on seedling survival and disease index from a hybrid infected with *Colletotrichum graminicola* in the greenhouse[w]

Fungicide	Emergence[x]	DSI[x]
Control (noninfected)	90	0.0
Terra-Coat L-205	80 a	0.46 a
Thiram	60 d	0.60 a
Benomyl	70 c	0.78 a
Dithane M45	47 e	1.11 b
Captan and thiram[y]	50 de	1.25 b
Demosan T	67 c	1.50 bc
Captan and Terrachlor[z]	63 cd	1.79 c
Captan	63 cd	2.00 d
Terra-Coat LT-2	83 b	2.10 d
Control (infected, nontreatment)	70 c	2.23 d

[w]Values are averages of three tests, with three replicates, and five plants per replicate.

[x]Numbers followed by the same letter in a column are not different according to Duncan's multiple-range test. DSI (= Disease Severity Index) is based on 0-4 scale in which 0 = healthy plants and 4 = severely diseased or killed plants.

[y]Mixtures consist of 16.67% active ingredient of each chemical.

[z]Mixtures consist of 42% active ingredient of each chemical.

days after planting, the subcrown mesocotyl of some plants was extensively rotted, some had superficial anthracnose lesions, and still others appeared healthy. Isolations made from roots and subcrown mesocotyl tissue yielded *C. graminicola*.

Seedling blight caused by C. graminicola in the field.—Kernels separated into extensive infection, restricted infection, and healthy lots were sown in the field. Seedling emergence from infected kernels was much lower than from healthy kernels (Table 2). We dug up the kernels from which no seedling had emerged and found acervuli, setae, and pink spore masses on the pericarp, with discoloration extending into the endoderm of all lots of infected kernels. Seedlings derived from either grade of infected kernels had a DSI of 3 or higher. Wilt symptoms subsided in plants that survived more than 5 weeks in the field. These plants resumed "normal" growth and appearance. Examination of root systems of surviving plants showed formation of new lateral roots.

Seed treatment of infected kernels.—Emergence of kernels treated with two of the nine fungicides was better than from the nontreated infected control (Table 3). All of the fungicide-treated kernels had a lower DSI than the control. However, low seedling emergence would preclude the use of many of these chemicals as effective fungicides. The DSI with seven of the fungicides was significantly lower than that with the nontreated control.

DISCUSSION.—We believe this to be the first report of dent corn kernels naturally infected with *C. graminicola*. Previously, Messiaen and Lafon (4) reported results of artificially inoculated ears and described symptoms on corn kernels.

Reduced seedling stand appears to be the most serious problem caused by anthracnose-infected kernels. Under field conditions, some seedlings failed to germinate, some wilted then recovered, and still others grew normally to maturity. In the greenhouse, most infected kernels produced emergent seedlings that died of wilt 2-3 weeks after planting. Favorable conditions for rapid germination (3-5 days) may account for better emergence in the greenhouse than under field conditions. Kernels planted in cold soil early in the growing season may not emerge for 10-12 days. Delayed emergence would allow the pathogen more time to infect the germinating kernel.

Kernels infected with *C. graminicola* may not be detected, or may be confused with those infected with other pathogens and saprophytes. In the limited number of seed lots examined, the low percentage of infected kernels would not cause a serious decrease in plant population.

The ability of *C. graminicola* to attack both sweet and dent corn kernels suggests that additional problems with anthracnose could arise on these crops. Evidence of *C. graminicola* on sweet and dent corn kernels indicates that the population of the organism may be different from those reported elsewhere (1, 2, 3, 4, 5, 9, 10). Isolates found in Ohio (10) attacked only wounded tissue, and those reported from Arkansas (2) and Kentucky (6, 9) also attacked nonwounded leaf and stalk tissues. In another study (8) we reported foliar and stalk infection and production of spore masses on the seed coat of sweet corn. This report gives an account of kernel infection.

Because Terra-Coat L-205, gave the best emergence and the lowest DSI, it might be considered as an improved substitute seed treatment chemical than the more generally used captan seed treatment, if the seed lot is suspected to contain a high percentage of kernels infected with *C. graminicola*.

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