Transmission of *Erwinia stewartii* from Plants to Kernels and Reactions of Corn Hybrids to Stewart's Wilt

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ABSTRACT

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Stewart's wilt reactions of 98 food-grade, white corn hybrids, 3 yellow dent corn hybrids, and 23 sweet corn hybrids and infection of kernels by *E. stewartii* were evaluated in 1998, 1999, and 2000. Stewart's wilt symptoms were rated from 1 (no appreciable spread of symptoms) to 9 (dead plants) following inoculation. The mean Stewart's wilt ratings for the food-grade, white corn and yellow dent corn hybrids were 1.9, 2.4, and 2.9 in 1998, 1999, and 2000, respectively. The mean Stewart's wilt ratings for the sweet corn hybrids were 3.8, 4.2, and 4.6 in 1998, 1999, and 2000, respectively. Hybrids with ratings less than 3 were classified as resistant. Hybrids with ratings between 3 and 4.5 were classified as moderate. Hybrids with ratings greater than 4.5 were classified as susceptible. Ears harvested from each row in 1998 and 1999 were assayed for *E. stewartii* using an enzyme-linked immunosorbent assay (ELISA)-based seed health test. Kernels from 16 hybrids were positive for *E. stewartii* in 1998. Kernels from 11 hybrids were positive for *E. stewartii* in 1999. Kernel infection by *E. stewartii* was affected considerably by the level of host resistance (i.e., reactions of seed parent plants). For hybrids classified as resistant, estimates of kernel infection were 0.024 and 0.0007% in 1998 and 1999, respectively. For hybrids with moderate reactions to Stewart's wilt, estimates of kernel infection were 0.19 and 0.07% in 1998 and 1999, respectively. For hybrids with susceptible reactions to Stewart's wilt, estimates of kernel infection were 11.6 and 7.8% in 1998 and 1999, respectively. Based on high levels of Stewart's wilt resistance in food-grade, white corn hybrids, and low rates of kernel infection by *E. stewartii* in resistant and moderate hybrids, there is an exceedingly low probability of introducing *E. stewartii* to areas where it does not occur by transmitting the bacterium in grain of the food-grade, white corn hybrids evaluated in this study. Although all of the kernels harvested in these experiments were produced as grain on open-pollinated F1 hybrids, the rates of kernel infection observed for hybrids with resistant, moderate and susceptible reactions to Stewart's wilt are applicable to seed produced on inbred lines with equivalent Stewart's wilt reactions.

Stewart's bacterial wilt, caused by *Erwinia stewartii* (Smith) Dye (Syn. *Pantoea stewartii*), occurs primarily in the Mid-Atlantic states, the Ohio River Valley, and the southern part of the U.S. Corn Belt. The corn flea beetle, *Chaetocnema pulicaria* Melsh., is the main vector and overwintering host of *E. stewartii* (8,14). Based on average daily temperatures across the U.S. Corn Belt, the winters of 1991 to 1992, 1997 to 1998, 1998 to 1999, and 1999 to 2000 were 4 of the 10 warmest in the past 100 years. Consequently, flea beetles overwintered farther north and Stewart's wilt occurred in many areas where seed was produced.

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There are two phases of Stewart's wilt. During the seedling wilt phase, plants can be infected systemically if *E. stewartii* moves within the vascular system. Systemic infection of sweet corn hybrids with moderate to susceptible Stewart's wilt reactions reduces ear weight and size; and in some instances, ears are not produced (19). If infection occurs prior to the two- to three-leaf stage, the main stalk may be killed if the growing point is infected. The leaf blight phase occurs when plants are infected near or after anthesis, and can result in long, linear, necrotic lesions on leaves. Yield losses in sweet corn are primarily the result of the seedling wilt phase of Stewart's wilt (19).

Many countries prohibit or place restrictions on importation of maize grain or seed produced in the United States to prevent the introduction of *E. stewartii*. Often, maize seed produced in the United States must be certified free of *E. stewartii* based on field inspections or greenhouse growouts. An enzyme-linked immunosorbant assay (ELISA) also was developed for detecting *E. stewartii* in kernels (11). Quarantine restrictions are implemented in spite of recent research in which seed-to-seedling transmission of *E. stewartii* was approximately 0.02 to 0.14% for seed produced on susceptible dent corn inbreds that were naturally-infected or inoculated, respectively (1). Plant-to-seed transmission (i.e., infection of kernels by *E. stewartii*) was approximately 5% or less in highly susceptible maize lines and nearly nondetectable in maize with moderate to high levels of Stewart's wilt resistance (2,10).

Block et al. (1) maintain that the risk of introducing *E. stewartii* on good quality seed produced on a moderately resistant seed parent plant has been exaggerated. Reports of seed transmission of *E. stewartii* that appeared in literature from approximately 1900 to 1940 were based on evaluations of highly susceptible germplasm or on trials in which dissemination of the bacterium by corn flea beetles was overlooked (9,15,16,18,21). Smith (18) reported 9% seedling infection in 1909 and Thomas (21) reported transmission of *E. stewartii* to 85% of plants grown in a greenhouse in 1924. Neither of these reports considered the possibilities of corn flea beetle vectors of *E. stewartii* nor fungal pathogens that cause seedling wilts with symptoms that vaguely resemble Stewart's wilt (1). In 1933, Rand and Cash (16) reported 2% (one infected seedling of 54) and 13% (3 infected seedlings of 23) seedling infection in greenhouse studies of highly susceptible cultivars. When "2 quarts of the worst-looking seed…from which the preceding season's crop had been almost a total failure due to bacterial wilt" were grown in the greenhouse, Rand and Cash (16) observed 2% seedling infection. Following the identification of the corn flea beetle as the primary vector of *E. stewartii*, seed transmission of *E. stewartii* was reported to be a limited but potentially important source of introducing the bacterium into new areas (13). The 2% rate of seed transmission observed by Rand and Cash (16) was reported in several subsequent monographs on Stewart's wilt (7,13,17).

Many corn hybrids and inbreds grown in the midwestern United States have relatively high levels of Stewart's wilt resistance that restrict the movement of *E. stewartii* in infected plants (3). Restricted movement of bacteria should limit or inhibit kernel infection. Nevertheless, phytosanitary certificates based on visual inspections of fields do not differentiate between systemic infection and infection that is restricted by host resistance. Therefore, grain from resistant hybrids and seed from resistant inbreds may be quarantined in spite of the resistance of the plants on which kernels are produced. Rates of plant-to-kernel transmission of *E. stewartii* determine levels of *E. stewartii*-infection of grain or seed produced in areas where Stewart's wilt occurs. Rates of seed-to-seedling transmission determine the number of seedlings with Stewart's wilt when infected seed is planted. These rates of transmission appear to be different for seed produced on plants with different reactions to Stewart's wilt (1,2,10).

Production of food-grade white corn has increased in the United States in recent years due to an increase in demand for products from this corn (i.e., corn chips, tortillas, etc.) (20). An increase in global demand for food-grade white corn and its products may make it an economically important export crop. Knowledge of the Stewart's wilt reactions of food-grade white corn hybrids will be useful in determining if grain of these hybrids present a risk of introducing *E. stewartii* to areas where it is not endemic. This information also can be used to examine the risk of introducing *E. stewartii*.

The objectives of this research were to evaluate Stewart's wilt reactions of food-grade white corn hybrids grown in the midwestern United States where Stewart's wilt is endemic and to compare rates of plant-to-seed transmission of *E. stewartii* when kernels are produced on plants with different reactions to Stewart's wilt.

MATERIALS AND METHODS

Stewart's wilt reactions of corn hybrids. Stewart's wilt reactions of 124 corn hybrids were evaluated in 1998, 1999, and 2000. The trials included 98 white, food-grade corn hybrids, 3 yellow dent corn hybrids, and 23 sweet corn hybrids included as checks (Table 1). Ninety-two hybrids were common to all three trials. Stewart's wilt reactions of sweet corn checks were known previously (12). Ninety-six hybrids were planted 17 May 1998; 106 hybrids were planted 30 April 1999; and 119 hybrids were planted 28 April 2000 at the University of Illinois South Farms, Urbana. The experimental design for each trial was a randomized complete block with four replicates. Each experimental unit was a single 4-m row with approximately 16 plants. Plants at the three- to six-leaf stages were inoculated three or four times with *E. stewartii* by the pinprick method (4). Plants were inoculated 11, 16, and 23 June 1998, 1, 3, and 10 June 1999, and 22, 26, and 30 May, and 6 June 2000. Inocula were produced from a mixture of *E. stewartii* isolates collected in the spring from naturally infected plants and isolates collected the previous year that were maintained as a bulk population on plants in the greenhouse. Several pieces of symptomatic leaf tissue were surface sterilized in 95% ethanol for about 10 s and rinsed with deionized water. Leaf tissue was cut into approximately 2.5-cm squares and placed in 1.5 liter of sterile nutrient broth. Cultures were placed on benchtop shakers for approximately 16 h. The nutrient broth cultures were diluted 1:10 with a 0.1 M solution of NaCl prior to inoculation. All experimental units were inoculated in the same manner, but concentration of inocula and purity of cultures were not determined.

Hybrids were rated for Stewart's wilt symptoms 2 July and 13 August 1998, 22 June and 21 July 1999, and 20 June 2000. At each rating time, each row was given three ratings in order to account for variation among plants within rows. The mean of the three scores was calculated for each experimental unit. Symptoms were rated on a 1 to 9 scale: where 1 = no appreciable spread of symptoms from pinpricks, 2 = limited water-soaking, chlorosis, or necrosis within 3 cm of pinpricks, 3 = limited spread from pinpricks, chlorosis, or necrosis predominantly towards tips of leaves, 4 = abundant spread from pinpricks, chlorosis, or necrosis towards both ends of leaves, 5 = minimal systemic infection, a few symptomatic streaks on noninoculated leaves, 6 = moderate systemic infection, symptoms on 5 to 25% of leaf area, minimal stunting, 7 = abundant systemic infection, symptoms on 25 to 50% leaf area, distinct stunting, 8 = 50 to 90% leaf area symptomatic, severe system infection and stunting, 9 = 90 to 100% leaf tissue symptomatic or dead plants. Early and late ratings in 1998 and 1999 were highly correlated so their means were used in analyses. Ratings of hybrids common to all three trials were compared in a combined analysis of variance (ANOVA). Hybrids also were compared within years using FLSD values for mean separation. Hybrid mean ratings also were compared among years by correlation. Hybrids were considered to be resistant, moderate, or susceptible when rated <3, from 3 to 4.5, or >4.5 respectively. These hybrid reactions were assigned based on separation of means by FLSD values and the relationship between ratings and the level of systemic infection. The midpoint rating of each reaction category was more than one FLSD value from the highest or lowest rating of the next reaction category. Rows of hybrids with resistant reactions typically did not contain any systemically infected plants. Rows of hybrids with moderate reactions contained an occasional systemically infected plant. Rows of hybrids with susceptible reactions typically contained several systemically infected plants.

Transmission of *E. stewartii* from plants to kernels. Ears were harvested at maturity from each row and dried at ambient air temperatures. Ten and six consecutive ears were sampled from each experimental unit in 1998 and 1999, respectively. Kernels were shelled from ears and bulked by experimental unit. Infection of kernels by *E. stewartii* was determined from an ELISA-based seed health test (11) that uses a commercially available compound, direct ELISA reagent set (Agdia, Inc. Elkhart, IN) that is sensitive to about 10^5 CFU per ml. A two-step approach to sampling was followed. Experimental units with *E. stewartii*-infected kernels were identified from four samples of 25 kernels after which the proportion of kernels infected was estimated for each hybrid from 92 5-kernel samples.

Four hundred kernels were tested initially for each hybrid. Four samples of 25 kernels were selected randomly from each experimental unit for a total of 16 samples of 25 kernels per hybrid. ELISA plates were coated with antibody and incubated overnight at 4°C. Kernel samples were soaked in 50 ml of phosphate-buffered saline with Tween-20 (PBST) solution for 16 to 18 h at room temperature. After soaking, samples were ground in a commercial blender for 15 s. The supernatant was decanted. Prior to loading samples, the coating solution was hand-washed from the ELISA plates four times with PBST. A 100 µl sample of the supernatant was pipetted into a single ELISA plate-well and incubated for 2 to 2.5 h in a humid box at room temperature. Plates were washed four times with PBST. The enzyme conjugate solution was added, and plates were incubated for 2 h in a humid box at room temperature. The enzyme conjugate solution was washed four times with PBST, and the peroxidase substrate solution was added to each well. Positive-control sample wells became distinctly colored in approximately 15 to 20 min. Absorbance was measured with a Dynatech MR4000 plate reader at 490 nm.

All hybrids with at least one *E. stewartii*-positive 25-kernel sample were assayed again. Ninety-two 5-kernel samples, two positive controls, and two negative controls were assayed for each hybrid on one ELISA plate in order to estimate the proportion of infected kernels for each hybrid. An equal sample was taken from all replicates with at least one positive 25-kernel sample. Five-kernel samples were prepared and assayed in the same manner as 25-kernel samples.

The proportion of *E. stewartii*-infected kernels (p) was estimated for each experimental unit from an equation for group testing of binomial experiments:

$$p = 1 - (1 - y/n)^{1/k}$$

where k = number of kernels per sample, y = number of positive samples, and n = total number of samples (5,6). Experimental units without positive 25-kernel samples were assumed to have no infected kernels. If any 25-kernel sample from an experimental unit was positive, p was calculated from the assays of 5-kernel samples. In one

Table 1. Stewart's wilt ratings[a] of hybrids inoculated with *Erwinia stewartii* in 1998, 1999, and 2000

Type of corn and hybrid	2000	1999	1998
White corn hybrids			
AgriGold A6530W	2.8	2.1	2.0
AgriGold A6680W	1.9	1.7	1.5
Asgrow RX776W	3.3	2.6	2.0
Asgrow RX792W	3.1	...	...
Asgrow RX901W	3.7	3.2	2.2
Asgrow XP7308W	3.7	3.5	2.8
Asgrow XP8118W	3.0	2.3	1.7
Dekalb DK555W	3.3	3.3	3.1
Dekalb DK631W	3.5	3.6	2.6
Dekalb DK665W	2.6	2.3	2.0
Dekalb DK703W	2.2	1.6	1.5
Dekalb DK739W	3.2	3.2	2.1
Dekalb DK742W	2.4	2.1	1.5
Dekalb EXP866W	2.8	2.7	2.3
Dekalb EXP868W	2.3	2.8	1.9
Diener D 114W	2.8	2.1	1.8
Diener D 115W	2.8	...	...
Exsegen ES241W	2.4	1.9	...
Garst 8277W	2.9	2.6	...
Garst 8419W	2.6	1.8	1.4
Garst 8490W	2.3	1.7	1.3
Garst 8527W	2.8	2.3	1.8
Garst N6278W	2.5	2.0	1.7
IFSI 90-1	2.3	2.0	1.4
IFSI 95-1	2.6	1.8	1.6
IFSI 95-2	2.7	2.0	1.8
IFSI 97-1	2.3	...	...
IFSI 98-1	2.4	1.6	1.5
IFSI 98-2	1.9	2.3	1.6
IFSI 98-3	2.8	2.6	2.0
IFSI 98-4	2.9	2.6	2.1
IFSI 98-5	2.5	2.2	1.9
LG Seeds LG2558W	3.1	3.3	2.8
LG Seeds LG2596W	3.0	3.2	2.4
LG Seeds NB749W	2.3	1.9	1.4
NC+ 4089W	2.8	...	...
NC+ 4950W	3.0	...	...
NC+ 5633W	2.8	2.3	1.9
NC+ 6989W	3.1	2.0	1.5
NC+ RE372W	...	...	4.5
NC+ 6990W	2.8	...	...
NC+ RE652W	3.2	2.8	2.2
Novartis N71-T7	2.9	2.4	1.8
Pioneer 3203W	3.2	2.5	2.0
Pioneer 3281W	2.8	2.8	2.0
Pioneer 3283W	...	1.9	1.5
Pioneer 3287W	3.3	3.0	2.4
Pioneer 32H39	3.5	2.9	2.0
Pioneer 32K72	3.3	2.8	...
Pioneer 32Y52	2.8	2.3	...
Pioneer 32Y65	3.6	3.3	2.9
Pioneer 3392W	3.1	2.7	2.2
Pioneer 33T17	3.5	...	...
Pioneer 3443W	3.3	2.9	2.7
Pioneer 3463W	3.3	2.9	2.0
Pioneer 34P93	3.7	3.0	2.4
Pioneer X1127DW	3.7	2.5	1.8
Pioneer X1127FW	3.4	2.8	2.4
Pioneer X1128BW	3.8	3.1	2.1
Pioneer X1138AW	4.2	3.1	...
Pioneer X1167BW	3.1	2.2	...
Pioneer X1177PW	2.8	2.0	1.8
Tennessee TN 98-1	2.8	2.3	1.7
Trislers 4113W	2.6	1.8	1.3
Trislers 4211W	2.5	2.1	1.5
Trislers 4214W	2.4	2.0	1.5
White corn hybrids (cont.)			
Vineyard V413W	3.1	2.7	2.1
Vineyard V414W	3.1	2.3	2.0
Vineyard V424W	2.8	2.1	1.6
Vineyard V433W	3.1	...	...
Vineyard V438W	2.8	2.4	1.8
Vineyard V448W	3.3	2.2	2.0
Vineyard V449W	2.9	2.3	2.1
Vineyard V453W	3.3	2.2	1.7
Vineyard V455W	2.8	...	...
Vineyard V462W	2.8	...	...
Vineyard Vx4319W	2.5	...	...
Vineyard Vx4337	3.3	2.4	1.8
Vineyard Vx4359W	3.3	...	...
Vineyard Vx4517	3.0	2.3	1.4
Vineyard Vx4548W	2.9	2.2	...
Vineyard Vx4618W	2.5	2.2	1.6
Vineyard Vx4596	2.8	1.8	...
Whisnand 50AW	2.4	1.7	1.7
Whisnand 51AW	2.5	1.7	1.3
Wilson 1780W	2.7	1.5	1.7
Wilson 1790W	2.7	2.2	1.8
Wilson E8051	3.1	2.3	1.7
Zimmerman 1780W	2.7	...	...
Zimmerman 1790W	2.7	...	...
Zimmerman 1851W	3.1	...	...
Zimmerman N71-T7	2.8	...	...
Zimmerman NX7208	2.9	2.2	...
Zimmerman Z62W	2.6	1.5	1.5
Zimmerman Z64W	3.1	2.6	1.8
Zimmerman Z74W	3.2	2.3	1.6
Zimmerman Z75W	3.2	2.2	1.9
Zimmerman Z76W	3.0	2.6	...
Yellow dent corn hybrids			
B73 x Mo17	3.1	2.2	2.0
Pioneer 3245	3.6	2.8	2.7
Pioneer 3394	3.8	2.8	1.9
Sweet corn hybrids			
Accolade	...	3.3	...
Alpine	3.9	3.7	3.2
Argent	3.2	3.2	2.7
Bonus	2.6	3.0	1.6
Candy Corner	...	4.6	...
Early Cogent	4.8	4.3	2.9
Even Sweeter	4.0	3.6	4.1
Fantasia	5.4	5.4	4.1
Frontier	4.6	4.9	3.9
How Sweet It Is	4.1	3.8	3.5
Jubilee	6.6	5.7	5.7
Pegasus	5.4	5.2	4.8
Platinum Lady	...	6.5	5.4
Pristine	4.0	3.2	1.9
Silver Dollar	3.5	3.0	3.0
Silver Queen	4.0	3.4	2.8
Snow White	7.2	4.6	5.3
Snowbelle	5.4	...	4.6
Summer Sweet 7631	3.7	3.5	2.3
Summer Sweet 781 Ultra	3.4	3.3	2.5
Treasure	3.5	4.0	3.8
Viva	4.5	4.1	3.6
WSS 3681	6.8	5.4	5.0
Mean	3.2	2.8	2.3
FLSD (p = .05)	0.6	0.5	0.4
CV%	13.2	14.8	14.6

[a] Stewart's wilt ratings on a 1 to 9 scale (1 = no appreciable spread of symptoms from pinpricks, 2 = limited water-soaking, chlorosis, or necrosis within 3 cm of pinpricks, 3 = limited spread from pinpricks, chlorosis, or necrosis predominantly towards tips of leaves, 4 = abundant spread from pinpricks, chlorosis, or necrosis towards both ends of leaves, 5 = minimal systemic infection, a few symptomatic streaks on noninoculated leaves, 6 = moderate systemic infection, symptoms on 5 to 25% of leaf area, minimal stunting, 7 = abundant systemic infection, symptoms on 25 to 50% leaf area, distinct stunting, 8 = 50 to 90% leaf area symptomatic, severe system infection and stunting, 9 = 90 to 100% leaf tissue symptomatic or dead plants).

instance when all 5-kernel samples from an experimental unit were positive (i.e., one replicate of cv. Pegasus in 1999), the upper limit of p (i.e., $y = n - 1$) for the 5-kernel samples was used as the level of kernel infection for that experimental unit. When none of the 5-kernel samples were positive, the best estimate of kernel infection was considered to be a value that was less than the lower limit of p (i.e., $y = 1$) for 5-kernel samples (e.g., <0.05% when all 92 5-kernel samples were negative and only one of four experimental units tested positive). Kernel infection for each hybrid was calculated as the mean of the four experimental units. Kernel infection was estimated for three classes of hybrid reactions to Stewart's wilt (resistant, moderate, or susceptible) as the mean of all hybrids in each class.

RESULTS

Stewart's wilt reactions of white corn hybrids. Although Stewart's wilt ratings of hybrids were highly correlated among years (Fig. 1), the hybrid by year interaction was significant in the combined ANOVA. Therefore, hybrids were compared within years.

Since early (2 July) and late (13 August) Stewart's wilt ratings in 1998 were highly correlated ($r = 0.96$), means of the two ratings were used to compare hybrids. Hybrid means ranged from 1.3 to 5.7 with a grand mean of 2.3 (Table 1). Stewart's wilt ratings for 72 food-grade, white corn hybrids and 3 yellow dent corn hybrids ranged from 1.3 to 4.5 with a mean of 1.9. Ratings for 21 sweet corn hybrids ranged from 1.6 to 5.7 with a mean of 3.8. Stewart's wilt ratings were below 3 for 80 of 96 hybrids. Ratings were from 3 to 4.5 for 10 hybrids. Ratings for 6 sweet corn hybrids were above 4.5. Rows of hybrids rated above 4.5 typically included several systemically infected plants. Some plants of hybrids rated 3 to 4.5 were systemically infected.

In 1999, early (22 June) and late (21 July) Stewart's wilt ratings were highly correlated ($r = 0.95$), so means of the two ratings again were used to compare hybrids. Hybrid means ranged from 1.5 to 6.5 with a grand mean of 2.8 (Table 1). Ratings for 81 food-grade, white corn hybrids and 3 yellow dent corn hybrids ranged from 1.5 to 3.6 with a mean of 2.4. Ratings for 22 sweet corn hybrids ranged from 3.0 to 6.5 with a mean of 4.2. Twenty-one hybrids considered highly resistant were rated 2.0 or below and were not significantly different from the hybrid with the lowest rating, 1.5. Fifty-one hybrids rated between 2.0 and 3.0 also were considered resistant because movement of *E. stewartii* was restricted to a few centimeters from inoculation wounds. Twenty-six hybrids rated from 3.0 to 4.5 had moderate reactions to Stewart's wilt. Eight sweet corn hybrids were rated above 4.5 and were considered susceptible.

In 2000, Stewart's wilt ratings ranged from 1.9 to 7.2 with a grand mean of 3.2 (Table 1). Ratings for 96 food-grade, white corn hybrids and 3 yellow dent corn hybrids ranged from 1.9 to 4.2 with a mean of 2.9. Twenty sweet corn hybrids were rated from 2.6 to 7.2 with a mean of 4.6. Nineteen hybrids considered highly resistant were rated 2.5 or less, which was not significantly different from the hybrid with the lowest rating, 1.9. An additional 36 hybrids rated below 3.0 were considered resistant. Fifty-six hybrids rated between 3.0 and 4.5 were considered moderate. Eight sweet corn hybrids rated above 4.5 were considered susceptible.

Transmission of *E. stewartii* from plants to kernels. In 1998, kernels from 16 hybrids were positive for *E. stewartii* in at least one replicate when four 25-kernel samples were assayed (Table 2). For the five resistant hybrids that tested positive for *E. stewartii* and for four of five hybrids with moderate reactions that tested positive, *E. stewartii* was detected in kernels from only one replicate. Kernels from one hybrid with a moderate reaction to Stewart's wilt were positive for *E. stewartii* in two replicates. Kernels from six hybrids with susceptible reactions to Stewart's wilt were positive for *E. stewartii* in three or four replicates.

When kernels from all 16 hybrids that were positive in at least one replicate were tested using 92 5-kernel samples, kernels from 14 hybrids were positive for *E. stewartii* (Table 2). Kernels from two resistant hybrids (Summer Sweet 781 Ultra and Vineyard V448W) did not test positive for *E. stewartii* when 5-kernel samples were assayed. Kernels from one moderate hybrid (Even Sweeter), which was positive in two replicates of 25-kernel sample assays, was positive in only one replicate of 5-kernel sample assays. Kernels from all other hybrids were positive for the same replicates of 5-kernel sample and 25-kernel sample assays.

Kernel infection in 1998 ranged from 3.1 to 17.9% for hybrids considered susceptible to Stewart's wilt, from 0 to 0.8% for hybrids with moderate reactions, and from 0 to 0.9% for hybrids with resistant reactions (Table 2). Kernel infection for all hybrids in the resistant, moderate, and susceptible classes was approximately 0.024, 0.19, and 11.58%, respectively in 1998 (Table 3).

In 1999, kernels of 11 hybrids were positive for *E. stewartii* in at least one replicate when four 25-kernel samples were assayed (Table 2). Kernels from one hybrid with a resistant reaction, Vineyard V453W, were positive for *E. stewartii* in

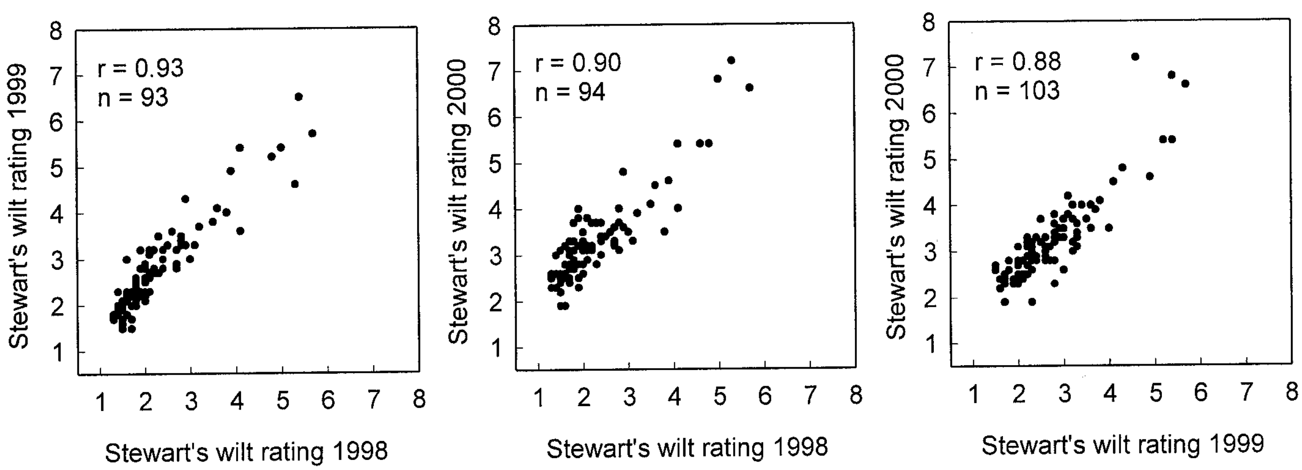

Fig. 1. Stewart's wilt ratings (1 = no appreciable spread of symptoms from pinpricks, 2 = limited water-soaking, chlorosis, or necrosis within 3 cm of pinpricks, 3 = limited spread from pinpricks, chlorosis, or necrosis predominantly towards tips of leaves, 4 = abundant spread from pinpricks, chlorosis, or necrosis towards both ends of leaves, 5 = minimal systemic infection, a few symptomatic streaks on noninoculated leaves, 6 = moderate systemic infection, symptoms on 5 to 25% of leaf area, minimal stunting, 7 = abundant systemic infection, symptoms on 25 to 50% leaf area, distinct stunting, 8 = 50 to 90% leaf area symptomatic, severe system infection and stunting, 9 = 90 to 100% leaf tissue symptomatic or dead plants) for corn hybrids inoculated with *Erwinia stewartii* in 1998, 1999, and 2000.

one replicate. Kernels from three hybrids with moderate reactions to Stewart's wilt were positive for *E. stewartii* in one or two replicates. Kernels from three susceptible hybrids were positive in all four replicates, and kernels from four hybrids were positive in one replicate. When 92 5-kernel samples of these 11 hybrids were assayed, kernels from 10 hybrids were positive for *E. stewartii* (Table 2). Kernels from the resistant hybrid that were positive in one replicate of the assays of 25-kernel samples were not positive in the assays using 5-kernel samples.

In 1999, kernel infection ranged from 0 to 26.8% for hybrids considered susceptible to Stewart's wilt, and from 0 to 0.8% for hybrids with moderate reactions. Kernel infection was not detected from resistant hybrids except in one of four 25-kernel samples from one replicate of Vineyard V453W. Based on the lower limit of group testing for binomial experiments and 92 5-kernel samples that were negative, the best estimate of kernel infection for this hybrid is less than 0.05%. *E. stewartii* was not detected in kernels from 23 of 26 hybrids with moderate reactions and from 71 of 72 resistant hybrids. Kernels of 7 of 8 susceptible hybrids were infected with *E. stewartii*. Kernel infection for all hybrids in the resistant, moderate, and susceptible classes was approximately 0.0007, 0.07, and 7.80%, respectively (Table 3).

Based on our data and most probable number estimates from binomial probabilities, approximately 1 in 4,166 (0.024%) or 1 in 142,857 (0.0007%) kernels from all hybrids with resistant reactions were infected with *E. stewartii* in 1998 and 1999, respectively. Approximately 1 in 526 (0.19%) or 1 in 1,429 (0.07%) kernels from all hybrids with moderate reactions were infected with *E. stewartii* in 1998 and 1999, respectively. For all hybrids with susceptible reactions, approximately 1 in 9 (11.58%) or 1 in 13 (7.80%) kernels were infected with *E. stewartii* in 1998 and 1999, respectively.

DISCUSSION

The probability of introducing *E. stewartii* to areas where it does not occur by transmitting the bacterium in grain of the food-grade, white corn hybrids evaluated in this study is exceedingly low. We observed high levels of Stewart's wilt resistance in food-grade, white corn hybrids accompanied by low rates of kernel infection by *E. stewartii* in resistant and moderate hybrids. Although all of the kernels harvested in these experiments were produced as grain on open-pollinated F1 hybrids, the rates of kernel infection we observed for hybrids with resistant, moderate, and susceptible reactions to Stewart's wilt are applicable to seed produced on inbred lines with equivalent Stewart's wilt reactions. As noted by others (1,2,10), we conclude from our data that infection of seed by *E. stewartii* is exceptionally low when seed is produced on resistant or moderately resistant seed parent plants.

Table 2. Hybrids for which *Erwinia stewartii* infected kernels were detected in 1998 and 1999

Stewart's wilt reaction, hybrid, and year	Stewart's wilt rating (1-9)	Estimate of kernels infected with *E. stewartii*		Experimental units from which *E. stewartii* was detected	Positive 25K[b] samples	Positive 5K[c] samples	Total kernels sampled
		25K samples	5K samples				
1998							
Resistant							
Vineyard V438W	1.8	0.3	0.5	1	1	9	860
Vineyard V448W	2.0	1.3	<0.05[a]	1	3	0	860
Asgrow RX901W	2.2	0.7	0.9	1	2	16	860
SS 781 Ultra	2.5	0.3	<0.05[a]	1	1	0	860
Dekalb DK631W	2.6	0.3	0.3	1	1	6	860
75 other hybrids	<3	0	…	0	0	…	30000
Moderate							
How Sweet It Is	3.5	0.7	0.2	1	2	4	860
Treasure	3.8	0.3	0.3	1	1	5	860
Even Sweeter	4.1	1.0	0.1	2	3	1	860
Fantasia	4.1	25.0	0.5	1	4	9	860
NC+ RE372W	4.5	1.3	0.8	1	3	13	860
5 other hybrids	3-4.5	0	…	0	0	…	2000
Susceptible							
Snowbelle	4.6	52.0	12.5	4	14	42	860
Pegasus	4.8	26.4	3.1	3	8	17	860
WSS 3681	5.0	27.0	7.9	3	9	38	860
Snow White	5.3	52.7	17.2	4	14	53	860
Platinum Lady	5.4	52.0	10.8	4	13	42	860
Jubilee	5.7	76.4	17.9	4	15	53	860
1999							
Resistant							
Vineyard V453W	2.2	0.3	<0.05[a]	1	1	0	860
71 other hybrids	<3	0	0	0	0	…	28400
Moderate							
Accolade	3.3	1.0	0.7	2	3	6	860
Even Sweeter	3.6	0.6	0.1	2	2	1	860
Treasure	4.0	0.7	0.8	1	1	14	860
23 other hybrids	3-4.5	0	…	0	0	…	9200
Susceptible							
Fantasia	5.4	0.7	1.1	1	3	18	860
Frontier	4.9	0.7	0.5	1	2	9	860
Jubilee	5.7	51.4	26.8	4	13	67	860
Pegasus	5.2	75.7	17.1	4	14	43	860
Platinum Lady	6.5	0.7	2.3	1	2	12	860
Snow White	4.6	0.7	1.1	1	2	19	860
WSS 3681	5.4	27.8	13.4	4	11	39	860
Candy Corner	4.6	0	…	0	0	…	400

[a] Estimate based on no positive five-kernel samples but *E. stewartii* present in one 25-kernel sample.
[b] 25-kernel samples positive for *E. stewartii* based on an enzyme-linked immunosorbent assay (ELISA)-based seed health assay. Total of 16 samples per hybrid.
[c] 5-kernel samples positive for *E. stewartii* based on an ELISA-based seed health assay. Total of 92 samples per hybrid.

Table 3. Estimate of *Erwinia stewartii*-infected kernels for hybrids with resistant, moderate, and susceptible reactions to Stewart's wilt in 1998 and 1999

	Hybrid reaction		
	Resistant	Moderate	Susceptible
Stewart's wilt rating	<3	3-4.5	>4.5
1998			
Number of hybrids	80	10	6
Total kernels sampled in 25- and 5-kernel samples	34300	6300	5160
Estimated[a] number of infected kernels	8.2	12.0	597.5
Estimated[a] kernel infection	0.024%	0.19%	11.58%
1999			
Number of hybrids	72	26	8
Total kernels sampled in 25- and 5-kernel samples	29660	11780	6420
Estimated[a] number of infected kernels	0.2	8.1	500.6
Estimated[a] kernel infection	0.0007%	0.07%	7.80%

[a] Based on best estimate of kernels infected by *E. stewartii* using most probable number binomial probability of 5-kernel samples.

Most of the food-grade, white corn hybrids in these trials were highly resistant or resistant to Stewart's wilt. A few food-grade, white corn and sweet corn hybrids had moderate reactions to Stewart's wilt. Systemic Stewart's wilt infection was not observed in resistant hybrids and was rare among hybrids with moderate reactions. Previously, Braun (3) reported that *E. stewartii* was localized in infected leaves of a resistant corn inbred when compared histologically to a susceptible inbred. Since Stewart's wilt infection of plants of resistant and moderate hybrids was not systemic in our trials, it is not surprising that kernels produced on these hybrids were not infected by *E. stewartii*. In fact, some of the kernel samples of resistant and moderate hybrids that were positive for *E. stewartii* probably were due to experimental errors such as seed carry over between plots at planting, harvest error in rows that were lodged, or mixture of samples during shelling. Therefore, we believe that our estimates of infection of kernels from resistant and moderate hybrids may be slightly higher than the actual rates of kernel infection for these hybrids.

Systemic Stewart's wilt infection was common among a few sweet corn hybrids. Levels of kernel infection of these hybrids were higher than those for moderate and resistant hybrids.

Block et al. (2) recently reported that kernels were not infected on plants with less than 25% severity of Stewart's wilt. Seed lots with greater than 5% infected kernels came from plants with greater than 50% severity. Our results corroborate these observations and further differentiate the effect of host resistance on kernel infection. Most estimates of *E. stewartii* seed transmission (i.e., percentage of infected seedlings from a seed lot) reported in literature (7,9,13,16,17,18) range from approximately 2 to 13%. These estimates are similar to our estimates of plant-to-seed transmission (i.e., kernel infection) in susceptible sweet corn hybrids. These estimates, however, do not consider rates of seed-to-seedling transmission. Block et al. (1) observed rates of seed-to-seedling transmission of *E. stewartii* approximately 0.02 and 0.14%. The estimates of seed transmission from earlier studies are much greater than what would be expected from the susceptible hybrids in this study. If rates of seed-to-seedling transmission (0.02 and 0.14%) reported by Block et al. are applied to our highest level of kernel infection (i.e., plant-to-seed transmission) from a single experimental unit (approximately 50%), only 0.07% of the seedlings grown from those kernels would be expected to be infected with *E. stewartii*. Although infection of kernels by *E. stewartii* is unequivocal, the rate of plant-to-seed transmission (i.e. kernel infection) appears to be influenced considerably by levels of host resistance. Rates of seed transmission reported in literature from the first half of the 20th century are not applicable to seed or grain produced on plants with levels of resistance that are common among most commercial hybrids and inbreds grown today in the U.S. Corn Belt.

LITERATURE CITED

1. Block, C. C., Hill, J. H., and McGee, D. C. 1998. Seed transmission of *Pantoea stewartii* in field and sweet corn. Plant Dis. 82:775-780.
2. Block, C. C., Hill, J. H., and McGee, D. C. 1999. Relationship between late-season severity of Stewart's bacterial wilt and seed infection in maize. Plant Dis. 83:527-530.
3. Braun, E. J. 1982. Ultrastructure investigation of resistant and susceptible maize inbreds infected with *Erwinia stewartii*. Phytopathology 72:159-166.
4. Chang, C. M., Hooker, A. L., and Lim, S. M. 1977. An inoculation technique for determining Stewart's bacterial leaf blight reaction in corn. Plant Dis. Rep. 61:1077-1079.
5. Chen, C. L., and Swallow, W. H. 1990. Using group testing to estimate a proportion, and to test the binomial model. Biometrics 46:1035-1046.
6. Chiang, C. L., and Reeves, W. C. 1962. Statistical estimation of virus infection rates in mosquito vector populations. Am. J. Hyg. 75:377-391.
7. Elliott, C. 1941. Bacterial wilt of corn. U.S. Dep. Agric. Farmers' Bull. 1878.
8. Elliott, C., and Poos, F. W. 1934. Overwintering of *Aplanobacter stewartii*. Sci. 80:289-290.
9. Frutchey, C. W. 1936. A study of Stewart's disease of sweet corn caused by *Phytomonas stewarti*. Mich. Agric. Exp. Sta. Tech. Bull. 152.
10. Khan, A., Ries, S. M., and Pataky, J. K. 1996. Transmission of *Erwinia stewartii* through seed of resistant and susceptible field and sweet corn. Plant Dis. 80:398-403.
11. Lamka, G. L., Hill, J. D., McGee, D. C., and Braun, E. J. 1991. Development of an immunosorbent assay for seedborne *Erwinia stewartii* in corn seeds. Phytopathology 81:839-846.
12. Pataky, J. K. 2000. Reactions of sweet corn hybrids to prevalent diseases. Pages 177-192 in: Midwest Food Processors Assocation Processing Crops Manual and Proceedings.
13. Pepper, E. H. 1967. Stewart's bacterial wilt of corn. Am. Phytopathol. Soc. Monogr. 4.
14. Poos, F. W., and Elliott, C. 1936. Certain insect vectors of *Aplanobacter stewarti*. J. Agric. Res. 52 585-608.
15. Rand, F. V., and Cash, L. C. 1921. Stewart's disease of corn. J. Agric. Res. 21:263-264.
16. Rand, F. V., and Cash, L. C. 1933. Bacterial wilt of corn. U.S. Dep. Agric. Tech. Bull. No. 362.
17. Robert, A. L. 1955. Bacterial wilt and Stewart's leaf blight of corn. U.S. Dep. Agric. Farmers' Bull. 2092.
18. Smith, E. F. 1909. Seed corn as a means of disseminating *Bacterium stewarti*. Sci. 30:223-224.
19. Suparyono, and Pataky, J. K. 1989. Influence of host resistance and growth stage at time of inoculation on Stewart's wilt and Goss's wilt development and sweet corn hybrid yield. Plant Dis. 73:339-345.
20. Thomas, D. J. 1992. White corn: a world market overview. Page 9 in: Occasional paper series OP-14. University of Kentucky Center for Agricultural Export Development, Lexington.
21. Thomas, R. C. 1924. Stewart's disease or bacterial wilt of sugar corn. Ohio Agric. Exp. Stn. Monthly Bull. 9:81-84.