Transmission of *Erwinia stewartii* through Seed of Resistant and Susceptible Field and Sweet Corn

A. Khan, S. M. Ries, and **J. K. Pataky,** Department of Crop Sciences, University of Illinois, Urbana 61801

ABSTRACT

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.

Plant-to-seed and seed-to-seedling transmission of *Erwinia stewartii* was evaluated while considering the influence of resistant and susceptible seed parents. Leaves of 20 sweet corn (*Zea mays*) hybrids and eight dent corn inbreds were inoculated weekly from the three- to 10-leaf stage in 1992 by the pinprick method with a rifampicin-resistant strain or wild-type strains of *E. stewartii*. In 1993, leaves of four sweet corn hybrids and eight dent corn inbreds were inoculated by the pinprick method, and inoculum also was injected into ear shoots, ear shanks, or silk channels of selected plants. Seed from inoculated plants were harvested at maturity and assayed for *E. stewartii*. Seed and seed leachates were plated on nutrient broth yeast extract (NBY) agar amended with rifampicin, and leachates were inoculated into the susceptible sweet corn hybrid Jubilee in a greenhouse assay. *Erwinia stewartii* was detected in seed produced in 1992 on four hybrids, 89-3889, 88-2757, 91-1209, and 91-1574, which had systemic Stewart's wilt following leaf inoculation. The bacterium was not detected in seed from hybrids or inbreds with nonsystemic Stewart's wilt. In 1993, *E. stewartii* was isolated from cob and shank tissues and from seed of plants inoculated in ear shoots, ear shanks, or silk channels. *Erwinia stewartii* was recovered in 2 to 5% of leachates from individual seed of Jubilee inoculated in ear shanks and silk channels and from 1 to 16% of leachates of individual seed of FR632 inoculated in ear shanks and silk channels. There was no evidence of seed-to-seedling transmission of *E. stewartii* when over 75,000 seed from infected plants were planted in field or greenhouse trials. This may have been due, in part, to low rates of plant-to-seed transmission in seed produced on plants that were not systemically infected.

Additional keyword: seed pathology

Stewart's bacterial wilt, caused by *Erwinia stewartii*, is an important disease of corn, causing a severe early season wilt of susceptible sweet corn and a late season leaf blight of susceptible sweet corn and field corn. The bacterium overwinters in the corn flea beetle (*Chaetocnma pulicaria* Melsh.) (6,18).

Evidence of seed transmission of *E. stewartii* was reported in the early literature (7,8,17,19–21). Stewart (21) suggested that seed may be a primary method by which the pathogen is spread. Smith (20) reported low rates of seed-to-seedling transmission. Rand and Cash (17) isolated *E. stewartii* from the endosperm of seed produced on infected plants and from the interior of seed 5 months after harvest (19). In greenhouse studies in which secondary sources of inoculum were controlled, incidence of infected seedlings ranged from 2 to 13% among plants grown from seed harvested from infected plants (17). Frutchey (7) isolated *E. stewartii* from infected plants and reported 10.6% seedling infection when surface sterilized seed were grown on sterile agar slants. Frutchey (7) also reported that a greater percentage of plants were infected when the chalazal region of the corn kernel was injured after germination than when it was not injured. Ivanoff (8) observed seed-to-seedling transmission when an avenue for entrance of the bacterium was provided by injuring seedling roots.

Recently, Block et al. (2) recovered *E. stewartii* from only nine of 4,058 seedlings grown from seed produced on Stewart's wilt–infected plants. In a subsequent trial, Block et al. (3) observed only 29 infected seedlings grown from nearly 53,600 seed from infected plants. Transmission of *E. stewartii* from seed to seedlings has not been observed in several hundred seed lots certified for domestic use and export by the Illinois Crop Improvement Association even though *E. stewartii* was detected in leaves of plants sampled from the fields in which the seed were produced (N. R. Pataky, personal communication). Similarly, Biddle et al. (1) detected *Clavibacter michiganensis* subsp. *nebraskensis*, the causal agent of Goss's wilt, in 17 to 31% of seed from a highly susceptible inbred, A632*Ht*, but transmission of the bacterium to seedlings did not occur among 13,000 samples. Rates of seed-to-seedling transmission were 0.1 to 0.4% when seed were inoculated with *C. michiganensis* subsp. *nebraskensis* by vacuum infiltration.

Differences in rates of transmission of *E. stewartii* reported in the early literature and in recent observations may be due, in part, to improved levels of Stewart's wilt resistance in modern corn inbreds and hybrids. Resistance in many sources is due to restricted systemic movement of *E. stewartii* in the host (4), which reduces the probability of *E. stewartii* infecting ear shoots, cobs, and kernels. Block et al. (3) concluded that the risk of spreading *E. stewartii* through maize seed is essentially nil for seed obtained from plants with less than 50% of the leaf tissue diseased. Our research reevaluates plant-to-seed and seed-to-seedling transmission of *E. stewartii,* considering the influence of resistant and susceptible seed parents. Preliminary results have been reported (9).

MATERIALS AND METHODS

In this study, field-grown sweet corn and field corn plants were inoculated with *Erwinia stewartii,* seed produced on infected plants were assayed for *E. stewartii,* and the transmission of *E. stewartii* from seed to seedlings was evaluated in field and greenhouse plantings.

Corn inbreds and hybrids. Four sweet corn hybrids, (Challenger, Jubilee, Summer Sweet 7710, and Summer Sweet 8701) and eight dent corn inbreds (B68*Ht1*, FR21, FR22, FR36, FR602, FR632, FR819, and FR902) were planted at the University of Illinois pomology farm on 18 May 1992 and 19 May 1993. Sixteen additional sweet corn hybrids (Miracle, More, Reveille, 88-2757, 88-3012, 89-2628, 89-3889, 89-4948, 91-1018, 91-1209, 91-1573, 91-1574, 91-3730, 91-3783, and XPH 3030) were planted at the University of Illinois Agronomy/Plant Pathology South Farm on 6 May 1992. Hybrids and inbreds were planted at the pomology farm in single rows spaced 76 cm apart and about 30 m in length with 150 plants per row. Hybrids were planted at the south farm in 3-m-long rows with about 15 plants per row. Inbreds and hybrids were selected to represent a range of reactions to *E. stewartii,* from

Corresponding author: J. K. Pataky
E-mail: j-pataky@UIUC.EDU

Portion of a thesis submitted by the first author in partial fulfillment of the requirements for the M.S. degree.

Accepted for publication 15 December 1995.

Publication no. D-1996-0126-06R

highly susceptible to resistant. There were two replicates of hybrids and inbreds.

Inoculation. Inbreds and hybrids at the pomology farm were inoculated in both years with a single rifampicin-resistant strain (24) of *E. stewartii*. Hybrids at the south farm in 1992 were inoculated with a mixture of about 10 different wild-type strains of *E. stewartii* collected from various locations in southern and central Illinois. Strains of *E. stewartii* were maintained at –80°C in a 15% glycerol solution and also on corn plants grown in the greenhouse. In the greenhouse, strains were transferred alternately from resistant to susceptible hybrids (i.e., Crisp n Sweet 710 and Jubilee) to maintain virulence. For inoculation of inbreds and hybrids in field plots, inocula were produced from frozen cultures and from isolations from greenhouse-grown infected plants. Inocula were produced overnight in nutrient broth shake cultures. Concentration of inocula from shake cultures was determined to be 10^6 to 10^7 CFU/ml (A_{590} = 0.05) (16). In 1992, leaves forming the whorls of plants were inoculated by the pinprick method (5) at weekly intervals beginning at the three- to five-leaf stage. Corn at the south farm was inoculated three times, and corn at the pomology farm was inoculated four times. In 1993, the rifampicin-resistant strain of *E. stewartii* and the preparation and concentration of inocula were the same as in 1992; however, the inoculation procedure was amended from 1992 as follows: leaves of all plants were inoculated by the pinprick method beginning at the three- to five-leaf stage and continuing at weekly intervals until emergence of tassels. After tasseling, each row was divided into four sections with equal numbers of plants. A hypodermic needle was used to inject 0.1 ml of inoculum into the ear shoots of plants in one section of each row 1 or 2 days after shoot emergence. In two other sections of each row, plants were inoculated at anthesis by injecting 0.1 ml of inoculum into either the ear shank or the silk channel. Plants in the fourth section of each row were inoculated only by the pinprick method. In both years, Sevin (carbaryl) was applied weekly at the pomology farm beginning at seedling emergence and continuing until seed maturity in order to reduce natural infection associated with corn flea beetle feeding.

Disease severity and seed harvest. Severity of Stewart's wilt was rated 2 August 1992 at both farms and 8 August 1993 at the pomology farm using a 1 to 9 rating scale (22) in which 1 represented no symptoms and 9 represented plant death due to Stewart's wilt. Several plants representative of the predominate severity category in each row were tagged, and ears were harvested from these plants at seed maturity following the formation of black-layers. Ears were bulked by genotype in 1992 and by genotype and method of inoculation in 1993. Ears were dried with forced, ambient air on a drying table. After moisture content reached 15%, kernels were removed from cobs using an individual ear sheller. The sheller was cleaned between shellings to minimize contamination. Bulked lots of shelled seed were stored in paper bags at 4°C. Samples of seed and plant tissues also were harvested, as described below, when seed was at 40% moisture as measured with a Dickey-John GAC II moisture meter (Dickey-John Corp., Auburn, IL).

Detection of *E. stewartii* in seed and plant tissues. Seed and plant tissues were assayed for *E. stewartii*. One hundred seed from each bulked seed lot were surface sterilized as described below and plated directly on either nutrient broth yeast extract (NBY) agar or NBY agar containing rifampicin (50 mg per liter) to determine incidence of seed harboring *E. stewartii*. Plates were incubated at 25°C. Each lot of seed was assayed three times in 1992 and four times in 1993.

Leachates from 100 seed of each seed lot also were assayed following a seed-soaking procedure (15,23). Leachates were assayed from dried seed (15% moisture) in both years and from seed harvested at about 40% in 1993. Seed were surface sterilized for 90 to 120 s in 0.5% sodium hypochlorite and for 90 to 120 s in 70% ethyl alcohol before rinsing four times in sterile distilled water. Seed then were placed in 50 ml of sterile phosphate buffer in flasks that were stored in a refrigerator for 24 h at 4°C, after which time they were shaken vigorously for 3 min (15,23). Seed were removed by filtering the buffer through cheesecloth. The filtrate was centrifuged at 27,138 × *g* for 20 min at 4°C. The supernatant was discarded and the pellet was suspended in 9 ml of sterile distilled water (10). The suspension was serially diluted in sterile distilled water to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. Three 0.1-ml aliquots of each dilution were individually plated on NBY agar or NBY agar amended with rifampicin and cycloheximide (40 mg per liter). Each seed lot was assayed three separate times using this procedure.

The undiluted suspension also was tested in a seedling assay by inoculating 2-week-old greenhouse-grown seedlings of the susceptible sweet corn hybrid Jubilee. Three replicates of approximately 10 plants were injected with 0.1 ml of the undiluted extract using a hypodermic syringe. Plants were rated for the presence or absence of Stewart's wilt symptoms 2 weeks after inoculation. Assays were done in the winter when insect vectors were not present in fields.

In 1993, 100 dried seed were assayed individually from seed lots of Jubilee and FR632 inoculated by the shank and silk channel methods. Seed were surface sterilized as described previously and individual kernels were soaked at 4°C in 1 ml of sterile phosphate buffer in test tubes for 24 h. The resulting suspensions were diluted in sterile distilled water to 10^{-1}, and 0.1-ml aliquots of suspensions and dilutions were plated in duplicate on NBY agar containing rifampicin (50 mg per liter) and cycloheximide (40 mg per liter). Colonies were counted after plates were incubated at 25°C for 48 h. A 0.1-ml aliquot of undiluted seed leachate from individual kernels was used to inoculate seedlings as described previously. Seedlings were observed for symptoms of Stewart's wilt after 2 weeks.

In 1993, approximately 20 g of cob tissue, 15 g of shank tissue, and 100 kernels were collected when seed was at 40% moisture. These also were assayed for *E. stewartii*. Cob and shank tissue were cut into cross sections using pruning shears. Pieces of tissue and seed were surface sterilized as described above, placed in 15 ml of sterile saline buffer and incubated at 4°C for 24 h. Seed and pieces of tissue were removed by filtration through cheesecloth, and the filtrate was centrifuged as described above. The pellet was suspended, serially diluted, and plated in triplicate as described previously. All plates were incubated at 25°C and colonies of *E. stewartii* were counted after 48 h. Assays of *E. stewartii* from shank and cob tissues and seed at 40% moisture were not repeated.

Transmission to seedlings. Transmission of *E. stewartii* from seed to seedlings was evaluated in field and greenhouse trials. Field trials were done at the Northern Illinois Agronomy Research Center, DeKalb, IL, where corn flea beetles typically do not overwinter because of cold winter temperatures. Seed harvested and bulked from each inoculated hybrid and inbred in the pomology and south farm trials in 1992 and from each hybrid and inbred leaf-inoculated by the pinprick method in 1993 were stored overwinter at 4°C. Seed were planted 11 June 1993 and 15 June 1994. There were three replicates of each seed lot arranged in a randomized complete block design with approximately 400 kernels per experimental unit. All plants were examined 1 week after emergence for symptoms of Stewart's wilt. Plots were rated four additional times at weekly intervals. Plants with any symptoms that resembled Stewart's wilt were collected and assayed for *E. stewartii*. Bacteria isolated from collected plants were identified and characterized by the methods described by Marello and Bochner (12), by culturing on NBY agar containing rifampicin, and by inoculation of greenhouse-grown corn seedlings. Commercial seed of the susceptible hybrid Jubilee also was planted at DeKalb as a control to measure the occurrence of natural infection.

Approximately 900 seed from each lot of seed harvested in 1992 and 540 seed from each lot of seed harvested in 1993

also were evaluated for disease in the greenhouse during the winter. For each lot, three flats were used planted with 100 seed per flat in 1992 and approximately 60 seed per flat in 1993. The group of three flats was an experimental unit and three replicates for each seed lot were arranged in a randomized complete block design. All seedlings were examined weekly for 6 weeks for symptoms of Stewart's wilt. Commercial seed of the susceptible hybrid Jubilee was included as a control.

RESULTS

Reactions of inbreds and hybrids to *E. stewartii*. Sweet corn hybrids and dent corn inbreds inoculated with wild-type strains and a rifampicin-resistant strain of *E. stewartii* displayed a range of reactions (Table 1). Sweet corn hybrids 89-4948, 91-1209, and Reveille were most severely infected, and 91-3783, Miracle, and 91-4361 were least severely infected by the wild-type strains. Among the sweet corn hybrids inoculated with the rifampicin-resistant strain, Jubilee was the most severely infected and Summer Sweet 7710 was the least severely infected. The dent corn inbreds FR36 and FR22 had the lowest ratings among lines inoculated with rifampicin-resistant strain in both years. Ratings were highest on FR602 in 1992 and FR21 in 1993. Symptoms indicative of systemic movement of *E. stewartii* throughout the plant, i.e., ratings above 4.5 on a 1 to 9 scale (22), occurred on half of the sweet corn hybrids inoculated with the wild-type strains in 1992 and on Jubilee and FR21 inoculated with the rifampicin-resistant strain in 1993.

Detection of *Erwinia stewartii* in seed by direct plating. Colonies of *E. stewartii* were not detected from direct plating of 8,400 seed at 15% moisture content from field inoculated plants in 1992 or from plating of 4,800 seed at both 15 and 40% moisture from field-inoculated plants in 1993. Neither wild-type strains nor the rifampicin-resistant strain were observed on either NBY agar or NBY agar amended with rifampicin, respectively.

Detection of *Erwinia stewartii* in leachates from seed, cobs, and shanks. *Erwinia stewartii* was detected occasionally in 1992 when greenhouse-grown plants were inoculated with seed leachates. Stewart's wilt symptoms were not observed on plants inoculated with leachates of seed harvested from plants inoculated with the rifampicin-marked strain of *E. stewartii* in 1992 nor on plants inoculated with leachates from seed of 12 of the 16 sweet corn hybrids inoculated with the wild-type strains in 1992. Seed leachates from 91-1574, 88-2757, 89-3889, and 91-1209 inoculated with the wild-type strains in 1992 produced typical symptoms of Stewart's wilt on 100, 66, 66, and 25% of the assay plants, respectively, in the first trial of the seedling assay. However, in the second and third trials, which were done sequentially as soon as each previous trial was finished, seed leachates from these four hybrids failed to produce symptoms

Table 1. Stewart's wilt ratings for sweet corn hybrids and dent corn inbreds inoculated with wild-type strains or a rifampicin-resistant strain of *Erwinia stewartii*[a]

Hybrids and inbreds	1992	1993
Wild-type strains		
89-4948	7.2	. . .
91-1209	7.2	. . .
Reveille	6.0	. . .
91-1574	5.8	. . .
91-1573	4.8	. . .
88-2757	4.7	. . .
88-3012	4.7	. . .
89-3889	4.7	. . .
91-3730	3.7	. . .
XPH 3030	3.5	. . .
89-2628	3.2	. . .
91-1018	3.2	. . .
More	3.2	. . .
91-4361	3.0	. . .
Miracle	2.5	. . .
91-3783	2.3	. . .
Rfampicin-resistant strains		
Jubilee	4.2	6.8
Summer Sweet 8701	2.7	3.3
Challenger	2.7	3.1
Summer Sweet 7710	1.7	2.6
FR 602	4.0	3.5
FR 632	3.0	3.5
FR 902	2.8	3.7
B68 Ht_1	2.2	3.0
FR 819	2.0	3.1
FR 21	2.0	5.9
FR 22	1.4	1.0
FR 36	1.2	1.3
BLSD[b]	1.2	

[a] Stewart's wilt ratings are means of two replicates, on a 1 to 9 scale in which 1 = no symptoms and 9 = death of plants (22). In 1993, ratings describe plants leaf-inoculated by the pinprick method only.
[b] Bayesian least significant difference (k = 100) for comparison of wild-type strains only.

Table 2. Assay of leachates from 100 seed at 15% moisture harvested from plants infected by *Erwinia stewartii* in 1993

	Method of field inoculation[a]			
Hybrids and inbreds	Pinprick	Prinprick and ear shoot	Pinprick and ear shank	Pinprick and silk channel
Jubilee	0%[b]	17%	9%	12%
Summer Sweet 8701	0	0	0	0
Challenger	0	0	0	0
Summer Sweet 7710	0	2	5	3
FR 602	0	0	0	0
FR 632	0	0	0	30
FR 902	0	10	0	21
B68 Ht_1	NA[c]	0	0	0
FR 819	0	10	0	0
FR 21	0	10	4	0
FR 22	0	36	0	80
FR 36	0	0	0	0

[a] Plants inoculated by the leaf pinprick method (5) and by injection into ear shoots, ear shanks, or silk channel with a syringe.
[b] Percentage of approximately 90 assay plants (cv. Jubilee) with typical symptoms of Stewart's wilt. Plants inoculated with seed leachates.
[c] Data not available.

Table 3. Assay of leachates from 100 seed at 40% moisture harvested from plants infected with *Erwinia stewartii* in 1993

	Method of field inoculation[a]			
Hybrids and inbreds	Pinprick	Pinprick and ear shoot	Pinprick and ear shank	Pinprick and silk channel
Jubilee	37%[b]	52%	10%	82%
Summer Sweet 8701	0	10	0	22
Challenger	0	21	0	0
Summer Sweet 7710	0	10	0	0
FR 602	0	0	75	22
FR 632	0	0	0	36
FR 902	0	25	11	0
B68 Ht_1	NA[c]	NA	0	0
FR 819	0	0	0	10
FR 21	NA	0	0	0
FR 22	0	22	0	13
FR 36	0	0	0	50

[a] Leaves inoculated by the leaf pinprick method (5) and by injection into ear shoots, ear shanks, or silk channel with a syringe.
[b] Percentage of approximately 90 assay plants (cv. Jubilee) with typical symptoms of Stewart's wilt. Plants inoculated with seed leachates.
[c] Data not available.

except for that from 91-1574 in the third trial for which 83% of the assay plants were symptomatic.

Inoculations with leachates from seed at 15 and 40% moisture from the 1993 trials frequently produced symptoms on assay plants (Tables 2,3). The method by which plants were inoculated in the field had a considerable influence on assay results. When leaves were the only tissues inoculated by the pinprick method, none of the leachates from seed at 15% moisture produced symptomatic assay plants (Table 2), and only leachates from Jubilee yielded symptomatic plants (37%) when seed at 40% moisture were tested (Table 3). When emerging ear shoots, ear shanks, or silk channels were inoculated in conjunction with the pinprick inoculation method, leachates of seed at 15 and 40% moisture produced up to 82% symptomatic assay plants (Tables 2,3). The percentage of symptomatic assay plants was not consistently different among the methods of supplemental inoculation or among seed from hybrids or inbreds with different levels of resistance to Stewart's wilt.

Erwinia stewartii was isolated on NBY agar amended with rifampicin in leachates from seed, and cob and shank tissue (Tables 4,5). The method of inoculation influenced the recovery of *E. stewartii* from seed and plant tissues. *Erwinia stewartii* was not isolated in leachates from seed of Challenger, Summer Sweet 7710, FR632, B68*Ht1*, FR21, FR22, and FR36 when leaves of plants were inoculated by the pinprick method (Table 5). Similarly, isolation from cob and shank tissues occurred less frequently when only leaves of plants were inoculated (Table 5). The lack of systemic infection on plants inoculated by the pinprick method appeared to be weakly associated with the inability to isolate the bacterium from leachates. *Erwinia stewartii* was not isolated in leachates from inbreds and hybrids with the lowest Stewart's wilt ratings, i.e., FR36, FR22, B68*Ht1*, and Summer Sweet 7710, when those plants were inoculated only by the pinprick method (Tables 1,4,5).

Recovery of *E. stewartii* in leachates from individual seed of Jubilee or FR632 inoculated in the ear shanks or silk channels ranged from 2 to 16% in two trials (Table 6). Recovery was highest in leachates from seed from FR632 inoculated through the silk channel.

Transmission of *Erwinia stewartii* to seedlings. Typical symptoms of Stewart's wilt were not observed at DeKalb, IL, in 1993 on 29,072 seedlings grown from seed harvested from plants infected with Stewart's wilt in 1992, nor in 1994 on approximately 12,000 seedlings grown from seed harvested from plants infected with Stewart's wilt in 1993. Plants grown from commercial seed of the susceptible check, Jubilee, also were free of Stewart's wilt at DeKalb in both years. A few plants had symptoms that vaguely resembled Stewart's wilt, but *E. stewartii* was not detected by any of the three assay methods. Similarly, symptoms were not seen in the greenhouse on 27,612 seedlings grown in 1992, nor on nearly 7,200 seedlings grown in 1993.

Table 4. Number of assays in which *Erwinia stewartii* was isolated on nutrient broth yeast extract agar from leachates from 100 seed at 15% moisture harvested from plants infected with *E. stewartii* in 1993

	Method of inoculation[a]			
Hybrids and inbreds	Pinprick	Pinprick and ear shoot	Pinprick and ear shank	Pinprick and silk channel
Jubilee	0[b]	2	2	2
Summer Sweet 8701	0	1	1	2
Challenger	0	2	1	2
Summer Sweet 7710	0	3	2	3
FR 602	0	2	0	3
FR 632	0	0	3	3
FR 902	0	1	1	2
B68 Ht_1	0	1	0	NA[c]
FR 819	2	1	1	1
FR 21	1	1	2	1
FR 22	0	1	2	3
FR 36	0	1	0	1

[a] Plants inoculated by the leaf pinprick method (5) and by injection into ear shoots, ear shanks, or silk channel with a syringe.
[b] Number of assays (total of three) in which *E. stewartii* was isolated or nutrient broth yeast extract agar amended with rifampicin (50 mg per liter).
[c] Data not available.

DISCUSSION

Erwinia stewartii was not transmitted to seedlings in our trials. Although this result appears to be in contrast with those of others (2,3,7,8,17,19–21), the lack of *E. stewartii* transmission to seedlings in our studies may have been due to the limited extent to which seed-parent plants were infected from the pinprick inoculation. In 1992, systemic infection (i.e., ratings of 4.5 and above) did not occur on plants inoculated with the rifampicin-resistant strain of *E. stewartii* except for an occasional plant of the sweet corn hybrid Jubilee. Similarly, systemic infection occurred only in plants of Jubilee and the dent corn inbred FR21 inoculated with the rifampicin-resistant strain in 1993. Systemic infection was observed in eight of the 16 sweet corn hybrids inoculated with wild-

Table 5. Presence of *Erwinia stewartii* in leachates from 100 seed at 40% moisture, 20 g of cob tissue, and 15 g of shank tissue from plants infected with *E. stewartii* in 1993

	Method of inoculation[a]											
	Pinprick			Pinprick and ear shoot			Pinprick and ear shank			Pinprick and silk channel		
Hybrids and inbreds	Seed	Cob	Shank	Seed	Cob	Shank	Seed	Cob	Shank	Seed	Cob	Shank
Jubilee	+[b]	+	+	+	+	+	+	+	+	+	+	+
Summer Sweet 8701	+	+	+	+	+	+	+	+	+	+	–	–
Challenger	–	–	+	+	+	+	+	+	+	+	+	+
Summer Sweet 7710	–	–	–	+	+	+	+	+	+	+	–	+
FR 602	+	–	+	–	–	+	+	+	+	+	+	+
FR 632	–	–	–	+	+	+	+	+	+	+	+	–
FR 902	+	+	+	+	+	+	+	+	+	+	+	+
B68 Ht_1	–	–	–	–	–	–	–	–	+	+	+	+
FR 819	+	+	+	–	+	+	+	–	+	+	+	+
FR 21	–	–	–	–	+	+	+	+	+	+	+	+
FR 22	–	–	–	+	+	+	+	+	+	+	+	+
FR 36	–	–	–	+	+	+	+	–	+	+	–	+

[a] Plants inoculated by the leaf pinprick method (5) and by injection into ear shoots, ear shanks, or silk channel with a syringe.
[b] + = presence, – = absence of *E. stewartii* on nutrient broth yeast extract agar amended with rifampicin (50 mg per liter).

type strains of *E. stewartii* in 1992, but the amount of symptomatic leaf area on those plants was less than 50% (ratings below 7) for all but two hybrids, 89-4948 and 91-1209. Thus, our results corroborate the conclusion of Block et al. (3) that the risk of transmitting *E. stewartii* to seedlings is nearly nil for seed obtained from plants with less than 50% leaf area diseased. The rate of plant-to-seed transmission of *E. stewartii* probably is low for seed produced on most of the dent and sweet corn inbreds currently used as seed parents because levels of resistance to Stewart's wilt in these inbreds prevent systemic movement of *E. stewartii* when infection occurs as a result of leaf wounds by flea beetles. Rates of seed-to-seedling transmission of *E. stewartii* also may be low and similar to the rates (0.1 to 0.4%) for *C. michiganensis* subsp. *nebraskensis* (1). In contrast, many of the open pollinated sweet corn cultivars used in previous studies of seed transmission of *E. stewartii* (7,8,17,19) are among the maize cultivars most susceptible to Stewart's wilt, e.g., Golden Bantam. Higher rates of plant-to-seed transmission for these susceptible cultivars probably occurred largely because of the systemic movement of *E. stewartii* in these plants.

Our results from assays of seed leachates provide further evidence that inbred seed parents with high levels of resistance to Stewart's wilt are unlikely to transmit the bacterium through seed because movement of the bacterium is restricted in the host. *Erwinia stewartii* was not detected in seed obtained from inbreds or hybrids with Stewart's wilt ratings below 3 when these plants were inoculated by the pinprick method. However, the bacterium was isolated from seed, ear shanks, and cobs of all hybrids and inbreds when *E. stewartii* was injected into ear shoots, shanks, or silk channels. Braun (4) and Woods and Sherf (25) observed that the growth rate of *E. stewartii* was similar in leaves of resistant and susceptible genotypes, whereas leaf lesions expanded less rapidly in resistant plants. Braun (4) described the ultrastructural appearance and histochemical staining reactions of materials, presumably of host origin, that may have functioned in the localization of *E. stewartii* in resistant hosts. Conceivably, resistance that localizes the pathogen could not prevent kernel infection when inoculum was injected into ear shanks, ear shoots, and silk channels in our trials.

Because more than 50 countries have phytosanitary restrictions for seedborne *E. stewartii* (11), export of seed requires field inspections and/or laboratory grow-out tests as outlined by the Iowa State University Seed Health Testing Laboratory (13). An immunosorbent assay for seedborne *E. stewartii* also has been developed and could be used to detect the pathogen in seed lots (11). Nevertheless, seed-to-seedling transmission is unlikely because the bacterium is located within the vascular system and parenchymatous tissue of the chalazal region and in the endosperm rather than in the embryo (8). As shown by Ivanoff (8) and Frutchey (7), injury of the kernel in the chalazal region is necessary for seed-to-seedling transmission. Frutchey (7) concluded that transmission of *E. stewartii* from the endosperm to the embryo of seed during germination is probably due to larval feeding activities of various insects.

When seed leachates from individual kernels were assayed in our studies, plant-to-seed transmission was about 2 to 3% for seed from Jubilee and 1 to 16% for seed from FR632 when plants were inoculated in the ear shank or silk channel. These rates are similar to the rates of detection of *E. stewartii* from individual kernels of a dent corn inbred, A632, and a sweet corn hybrid, Home Pride of Canada (11). These four lines, A 632, FR632, Jubilee, and Home Pride of Canada, are some of the maize germ plasm that is most susceptible to Stewart's wilt.

We did not detect *E. stewartii* in seed by direct plating on NBY agar probably because the bacteria do not reside on the seed surface and they did not move from within dry, intact seed. Similar results were reported in the early literature (19). Ivanoff isolated *E. stewartii* only from crushed seed (8). We recovered *E. stewartii* from seed leachates after seeds were soaked for 24 h at 4°C, a procedure used by Kuan et al. to detect *Xanthomonas campestris* pv. *carotae* (10). The 24-h extraction time allowed for the release of *E. stewartii* from seed. The low incubation temperature of 4°C was selected to reduce the growth of saprophytes.

Although our trials were not designed to make direct comparisons of the frequency of recovery of *E. stewartii* from seed at 15 and 40% moisture, it is interesting that assays of leachates from seed at 40% yielded a higher percentage of assay plants infected with Stewart's wilt than did leachates from seed at 15% (Tables 2,3). Possibly, drying seed corn from about 35% moisture at harvest to 12.5% moisture for storage will reduce populations of *E. stewartii* when seed transmission occurs on susceptible inbreds. Further study is needed.

Based on our results, those of Block et al. (2,3), Lamka et al. (11), observations by seed health testing laboratories, and results for a similar bacterial pathogen of maize, *C. michiganensis* subsp. *nebraskensis* (1), it appears that the quarantine restrictions for seedborne *E. stewartii* based on reports from the early literature need to be modified. Restrictions on shipment of seed produced on Stewart's wilt–resistant inbred seed parents probably are unnecessary, as the bacteria are not systemic in these plants. When seed is produced on susceptible parents, *E. stewartii* may reach the kernels, but it is unlikely to move to the embryo. Further study of the conditions under which the *E. stewartii* is transmitted to seedlings may be warranted, including the role of host plant resistance in limiting or preventing plant-to-seed transmission. Inspection of seed production fields is based on the assumption that incidence of infected plants and seeds are related (14). This relationship may differ between Stewart's wilt resistant and susceptible corn.

Table 6. Recovery of *Erwinia stewartii* in leachates from individual seed of Jubilee and FR 632 inoculated in the ear shank and silk channel in 1993

Trial	Jubilee		FR 632	
	Ear shank	Silk channel	Ear shank	Silk channel
1	2[a]	3	1	3
2	3	3	5	16

[a] Percentage of seed from which *E. stewartii* was recovered in leachates from 100 individual kernels of Jubilee or FR 632 plated on nutrient broth yeast extract agar amended with rifampicin (50 mg per liter).

ACKNOWLEDGMENTS

We thank Lyle Paul, Mark Hurt, Kurt Nofftz, Rich Paoli, and John Gantz for assistance with field plots in DeKalb and Urbana, IL, and Arden Howey (Illinois Foundation Seeds, Inc., Tolono, IL), Michele Gardiner (Rogers Seeds, Nampa, ID), Steve Marshall (Asgrow Seed Co., Nampa, ID), George Crookham (Crookham Co., Caldwell, ID), and Bryant Long (Abbott and Cobb, Inc., West Palm Beach, FL) for seed of dent corn inbreds and sweet corn hybrids.

LITERATURE CITED

1. Biddle, J. A., McGee, D. C., and Braun, E. J. 1990. Seed transmission of *Clavibacter michiganense* subsp. *nebraskense* in corn. Plant Dis. 74:908-911.
2. Block, C. C., McGee, D. C., and Hill, J. H. 1992. Seed transmission of *Erwinia stewartii* in corn under field conditions. (Abstr.) Phytopathology 82:1154.
3. Block, C. C., McGee, D. C., and Hill, J. H. 1994. Assessing risk of seed transmission of *Erwinia stewartii* in maize. (Abstr.) Phytopathology 84:1153.
4. Braun, E. J. 1982. Ultrastructural investigation of resistant and susceptible maize inbreds infected with *Erwinia stewartii*. Phytopathology 72:159-166.
5. 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.
6. Elliott, C., and Poos, P. W. 1934. Overwintering of *Aplanobacter stewartii*. Science 80: 289-290.
7. Frutchey, C. W. 1936. A study of Stewart's disease of sweet corn caused by *Phytomonas stewartii*. Mich. Agric. Exp. Stn. Tech. Bull. 152.

8. Ivanoff, S. S. 1933. Stewart's wilt disease of corn, with emphasis on the life history of *Phytomonas stewartii* in relation to pathogenesis. J. Agric. Res. 47:749-770.
9. Khan, A., Ries, S. M., and Pataky, J. K. 1993. Transmission of *Erwinia stewartii* through seed of resistant and susceptible maize. (Abstr.) Phytopathology 83:885.
10. Kuan, T.-L., Minsavage, G. V., and Gabrielson, R. L. 1985. Detection of *Xanthomonas campestris* pv. *carotae* in carrot seed. Plant Dis. 69:758-760.
11. Lamka. G. L., Hill, J. H., 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. Marello, T. A., and Bochner, B. R. 1989. Biolog Reference Manual. Metabolic Reactions of Gram-Negative Species. Biolog, Inc., Hayward, CA.
13. McGee, D. C. 1988. Maize Diseases: A Reference Source for Seed Technologists. American Phytopathological Society, St. Paul, MN.
14. McGee, D. C. 1995. Epidemiological approach to disease management through seed technology. Annu. Rev. Phytopathol. 33:445-466.
15. Mohan, S. K., and Schaad, N. W. 1987. An improved agar plating assay for detecting *Pseudomonas syringae* pv. *syringae* and *P. s.* pv. *phaseolicola* in contaminated bean seed. Phytopathology 77:1390-1395.
16. Pataky, J. K. 1985. Relationships among reactions of sweet corn hybrids to Goss' wilt, Stewart's bacterial wilt, and northern corn leaf blight. Plant Dis 69:845-848.
17. Rand, V. F., and Cash, L. C. 1921. Stewart's disease of corn. J. Agric. Res. 21:263-264.
18. Rand, V. F., and Cash, L. C. 1924. Further evidence of insect dissemination of bacterial wilt of corn. Science 59:67-69.
19. Rand, V. F., and Cash, L. C. 1933. Bacterial wilt of corn. USDA Tech. Bull. 362.
20. Smith, E. F. 1909. Seed corn as a means of disseminating *Bacterium stewartii*. Science 30:223-224.
21. Stewart, F. C. 1897. A bacterial disease of sweet corn. N.Y. Agric. Expt. Stn. Bull. 13: 423-439.
22. Suparyono, and Pataky, J. K. 1989. Influence of host resistance and growth stage at the time of inoculation on Stewart's wilt and Goss's wilt development and sweet corn hybrid yield. Plant Dis. 73:339-345.
23. Van Vuurde, J. W. L., Van Den Bovenkamp, G. W., and Birnbaum, Y. 1983. Immunofluorescence microscopy and enzyme linked immunosorbent assay as potential routine test for the detection of *Pseudomonas syringae phaseolicola* and *X. c.* pv. *phaseoli* in bean seed. Seed Sci. Technol. 11:547-559.
24. Weller, M. D., and Saettler, A. W. 1978. Rifampin-resistant *Xanthomonas phaseoli* var. *fuscans* and *Xanthomonas phaseoli*: Tools for field study of bean blight bacteria. Phytopathology 68:778-781.
25. Woods, T., and Sherf, A. F. 1979. Population dynamics of *Erwinia stewartii* in leaves of sweet and field corn hybrids tolerant and susceptible to Stewart's disease. Abstr. 226. Proc. Int. Congr. Plant Prot., 9th.