Bacterial Fruit Blotch of Watermelon: Association of the Pathogen with Seed

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ABSTRACT

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Bacterial fruit blotch, a recently described disease of watermelon, resulted in severe losses to Indiana watermelon growers in 1989. Strains of the pathogen recovered from symptomatic fruit and seedlings exhibited similar phenotypic characteristics. Seedling symptoms (water-soaked lesions becoming necrotic on cotyledons and true leaves) and fruit symptoms (large, water-soaked blotches on the exposed rind surface) were induced by strains from both sources. Seed transmission was demonstrated in both seed from symptomatic fruit and seed soaked in a suspension of the bacterium. Treatment of naturally infested and laboratory-infested seed with 0.525% NaOCl for 20 min, 50-C H_2O for 20 min, or 1.8% HCl for 5 min reduced disease incidence but did not eradicate the pathogen. The bacterium was recovered from seed coats and embryos of both naturally infested and laboratory-infested seed. The pathogen was not recovered from peduncles or stems of plants with symptomatic fruit. No transmission of the pathogen occurred in seed from inoculated, asymptomatic fruit. The pathogen was recovered in one seedling of nearly 9,000 grown from a commercial seed source.

An unusual bacterial disease of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) appeared for the first time in southern Indiana in 1989. Initial symptoms on commercially grown greenhouse seedlings included water-soaked and necrotic cotyledon lesions and necrotic spots with or without chlorotic halos on true leaves. The most striking symptom, however, was a large water-soaked blotch on the upper surface of maturing fruit in the field. Loss of marketable fruit approached 90% in some commercial production fields. A nonfluorescent, gram-negative bacterium was recovered from both fruit and leaves (6,8).

A similar bacterial disease was reported from Florida in 1969 (2), but the causal agent was not identified. Wall and co-workers (17,18) discovered the disease in the Mariana Islands and published a report suggesting a link between fruit blotch and *Pseudomonas pseudoalcaligenes* subsp. *citrulli* Schaad et al. Severe epidemics of bacterial fruit blotch also occurred in Florida and other states in 1989 (5,11). The Florida researchers found that the pathogen was similar but not identical to *P. p. citrulli*. The taxonomy of both the fruit blotch bacterium and *P. p. citrulli* remains uncertain (3,11,15).

We examined bacterial strains from symptomatic fruit and seedlings and investigated the nature of the association of the bacterium with watermelon seeds. Preliminary reports of this research have been published (6,8).

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MATERIALS AND METHODS

Strains. Bacterial strains were isolated from symptomatic fruit of the cultivar Prince Charles (five strains) and diseased seedlings from seed removed from naturally infected fruit from commercial fields (six strains) in the summer and fall of 1989. All strains were subjected to several biochemical tests using the methods outlined by Schaad (9). The strains also were tested for pathogenicity in watermelon cotyledons by injecting a 10^7 cfu/ml cell suspension in sterile distilled water into the abaxial leaf surface with a tuberculin syringe. The seedlings were incubated under intermittent mist for 24 hr after inoculation.

Two strains were used for further pathogenicity tests. Strain 8908, isolated from diseased rind tissue, and strain 9016, recovered from a diseased seedling, were maintained on King's medium B (KB) at 28 C for all studies.

Cultivars. Seeds of Jubilee watermelon (Rogers NK Seed Company, Woodland, CA) that had not been treated with protectant fungicide were used for seedling pathogenicity and seed treatment studies. Field studies were conducted with the cultivar Charleston Elite (Rogers NK Seed Company).

Isolations. Recovery of the causal bacterium from inoculated plants was accomplished by placing 3- to 5-mm^2 tissue pieces in 0.1 ml of sterile distilled water for 5 min. The resulting suspension was streaked onto KB. The bacterial colonies were convex, opaque, creamy white, and approximately 0.7 mm in diameter after 48 hr at 28 C. Selected colonies were transferred to KB, and 24-hr-old cultures were tested for oxidase, gram reaction, and pathogenicity on watermelon cotyledons.

In addition, a modification of sorbitol neutral red agar (13) (10% ethanol substituted for sorbitol) was used for isolations. In preliminary studies on selective media, local strains of the pathogen developed distinctive colonies with raised centers and appressed margins on the ethanol neutral red agar (ENR). The morphology was best observed under a stereomicroscope at ×30 using oblique transmitted light (Fig. 1). ENR did not suppress nontarget bacteria compared with KB, but the characteristic morphology of the watermelon pathogen was easily distinguished from the convex colonies of other plant-associated bacteria. Characteristic colonies were selected from ENR plates and tested for oxidase, gram reaction, and pathogenicity as described above.

Seedling pathogenicity tests. Seedlings of the cultivar Jubilee were grown in 50-cell plastic trays in a commercial potting medium and inoculated when the first true leaf had begun to expand (10–12 days after planting). A cell suspension in sterile distilled water containing approximately 10^7 cfu/ml was prepared from 48-hr-old cultures of each strain. Groups of 10 seedlings were inoculated with either strain 8908 or strain 9016 in one of three ways—infiltrating the abaxial cotyledon surface with inoculum, piercing the hypocotyl approximately 1 cm below the apex with a dissecting needle that had been previously immersed in inoculum, and spraying inoculum over the surface of the plant to runoff with a hand-held sprayer. Control seedlings (five per inoculation method) received sterile distilled water in place of the bacterial suspension. All plants were kept under intermittent mist for 24 hr after inoculation and then observed daily in the greenhouse for symptom development. The pathogen was reisolated from symptomatic tissue by soaking tissue pieces in a drop of sterile distilled water and streaking a loopful of the resulting suspension onto KB and ENR plates.

Field pathogenicity tests. Charleston Elite watermelons were raised according to commercial specifications at the Purdue University Horticultural Farm, West Lafayette, IN. Two to 5 wk after pollination, 0.1 ml of a 10^7 cfu/ml suspension was spread across the upper sur-

face of fruit measuring 15–40 cm from stem to blossom end with a sterile gauze pad. Forty-six fruit were inoculated with strain 8908. All of these fruit were enclosed in plastic bags and then covered with brown paper bags to maintain high humidity while avoiding excessively high temperatures. Bags were removed after 48 hr. Forty-one fruit were inoculated with strain 9016. Ten of these fruit were not enclosed in bags after inoculation; the remaining fruit were enclosed in plastic bags for 48 hr postinoculation. Ten fruit were treated with sterile distilled water and bagged as controls. The field plot was observed daily for 3 wk. Isolations as described above were performed with sections of lesions on fruit and leaves. Stems and peduncles were sampled from inoculated plants and tested for presence of the pathogen. Seeds were removed by hand from several symptomatic and inoculated, asymptomatic fruit for further study.

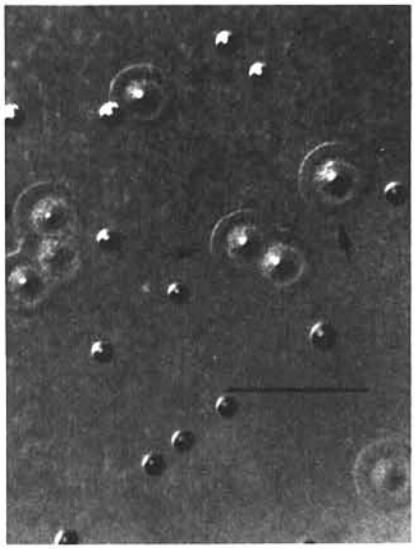

Fig. 1. Colonies of the watermelon fruit blotch bacterium (arrows) and other seed-associated bacteria grown for 48 hr on ethanol neutral red agar at 28 C. Bar = 1 mm.

Seed transmission. Seeds were collected by hand from several naturally infected Prince Charles fruit obtained from commercial fields in July and September 1989 and from four symptomatic inoculated Charleston Elite fruit from the field pathogenicity tests. Seeds of Jubilee were soaked in a cell suspension of strain 8908, 10^9 cfu/ml, for 5 min and then air-dried overnight. Four 25-seed samples from each source were planted in 49-cell plastic trays containing a commercial potting mix and arranged in the greenhouse in a randomized complete block design. The trays were placed on larger trays and watered from below to avoid splash dispersal of the pathogen. Diseased seedlings were removed at the onset of symptoms, and randomly selected seedlings were sampled to confirm the presence of the causal organism by isolation on KB and ENR. The experiment was terminated 3 wk after planting. Seeds removed from three inoculated asymptomatic Charleston Elite fruit were planted in the greenhouse and observed for 3 wk for symptom development.

Unopened containers of Prince Charles seed purchased by an Indiana grower in 1988 were examined for the presence of the fruit blotch pathogen. Seeds were planted in 72-cell plastic trays in a greenhouse containing no other fruit blotch experiments. Groups of approximately 1,100 seeds were planted at 1-wk intervals for 8 wk and seedlings were observed for 3 wk for symptom development. During the 8-wk period, the greenhouse temperature ranged from 21 to 25 C and relative humidity ranged from 20 to 40%.

Seed treatments. Seeds of one symptomatic Charleston Elite fruit from the field pathogenicity test were separated into 12 groups of 40 seeds each. Each group was soaked in one of four treatments. The treatments included 20 min in sterile distilled water (control), 20 min in 0.525% NaOCl, 20 min in a 50-C water bath, or 5 min in 1.8% HCl. After treatment, the seeds were air-dried overnight and planted the next morning in soilless media in plastic growing trays arranged in a randomized complete block design in the greenhouse. Diseased seedlings were removed at the onset of symptoms, and isolations were made as described above. The experiment was terminated 3 wk after planting. The treatments were rated for percent emergence and percent emerged plants with disease. In a similar experiment, seeds of Jubilee watermelon were infested with strain 8908 as described above and exposed to the four seed treatments with three replications of 25 seeds per treatment.

Seed isolations. Twenty-five seeds from the same inoculated, symptomatic fruit used in the seed treatment study were split along the longitudinal axis using two pairs of surface-disinfested pliers. The exposed embryo pieces were removed from the seed coat with a sterile needle. The embryo pieces did not come into contact with the outer seed coat surface during removal, and no fragments of seed coat were visible on the extracted embryos. Embryos and seed coats were incubated separately overnight at 21 C in flasks containing 100 ml of sterile phosphate buffer (pH 7.1). Serial dilutions were made from the liquid phase, and 0.1-ml aliquots were plated on KB and ENR. Plates were incubated at 30 C for 48 hr, and characteristic colonies were tested as described above. Twenty-five seeds of Jubilee infested with strain 8908 also were subjected to this isolation procedure.

Statistical analysis. Data were recorded as percent emergence and percent emerged plants infected with the pathogen for seed transmission and seed treatment experiments. The arcsine-square root transformation was used before analysis of variance and mean

Table 1. Phenotypic characteristics of watermelon fruit blotch bacterial strains from fruit and seedlings in Indiana and *Pseudomonas pseudoalcaligenes* subsp. *citrulli* (Ppc)

Characteristic	Fruit strains[w]	Seedling strains[x]	Ppc[y]
Flagella	Single polar	Single polar	Single polar
Fluorescent pigment	−	−	−
Oxidase	+	+	+
Arginine dihydrolase	−	−	−
Lipase	+	+	+
Gelatin liquefaction	−	−	±
Growth at 41 C	+	+	+
Growth at 4 C	−	−	−
Growth on MacConkey agar	+	+	+
Watermelon cotyledon lesions	+	+	ND[z]
Tobacco hypersensitivity	+	+	−
Utilization			
β-alanine	+	+	+
L-arabinose	+	+	ND
Ethanol	+	+	+
Fructose	+	+	+
Glycerol	+	+	ND
Glucose	+	+	−
Galactose	+	+	ND
L-leucine	+	+	+
Trehalose	+	+	ND
Sucrose	−	−	−
Lactose	−	−	ND
Sorbitol	−	−	ND
Mannitol	−	−	ND
Rhamnose	−	−	−
Cellobiose	−	−	−
myo-Inositol	−	−	−

[w] n = 5.
[x] n = 6.
[y] Data from Schaad et al, 1978 (10).
[z] ND = not determined.

separation procedures (SuperAnova, Abuscus Concepts, Inc., Berkeley, CA).

RESULTS

Strains. All strains were similar in phenotype for the characteristics evaluated (Table 1). Our strains differed from the original description of *P. p. citrulli* in glucose utilization and tobacco hypersensitivity. All strains, regardless of their source, caused lesions when infused into watermelon cotyledons. The lesions were first visible as water-soaked areas 24 hr after inoculation and became necrotic 48–72 hr after inoculation.

Seedling pathogenicity tests. Both strains 8908 and 9016 induced lesions in all seedlings inoculated by cotyledon infusion. In contrast, water-soaked streaks resulted on eight of 10 and seven of 10 hypocotyls inoculated with 8908 and 9016, respectively. Symptoms first were observed 48 hr after inoculation. Occasionally, water-soaked areas became necrotic and extended into cotyledons, and after 1 wk, the hypocotyls of several seedlings collapsed. Necrotic lesions with narrow chlorotic halos were first observed on developing true leaves 48 hr after spray inoculation with both strains. Lesions expanded to 3–6 mm in diameter in 5 days. Fewer seedlings developed symptoms with foliar spray than with either cotyledon infusion or hypocotyl inoculation (six of 10 with strain 8908 and five of 10 with strain 9016). No symptoms developed on cotyledons of plants receiving foliar spray inoculation. No symptoms developed on seedlings inoculated with sterile distilled water.

Field pathogenicity tests. Both strains caused fruit blotch symptoms on watermelon fruit in the field. Water-soaked lesions were first observed 4 days after inoculation. The lesions expanded to cover the entire upper surface of the fruit within 8–12 days. Thirty-seven percent of fruit inoculated with strain 8908 developed fruit blotch. Bagging fruit to maintain high humidity after inoculation was unnecessary for symptom development with strain 9016. Nearly 20% of fruit that were enclosed in a plastic bag after inoculation with strain 9016 expressed symptoms, whereas 60% of the fruit that were not bagged became diseased. Necrotic leaf spots developed on foliage near large fruit with advanced blotch symptoms, and the pathogen was recovered from these lesions. No symptoms developed on control fruit or on any uninoculated fruit during this 3-wk field study. The pathogen was recovered from margins of fruit blotches but not from peduncles of symptomatic fruit or stems of plants with symptomatic fruit.

Seed transmission. Diseased seedlings grew from all seed samples whether from naturally infected fruit or experimentally inoculated fruit or seed (Table 2). The amount of infection ranged from 7% of seedlings from seed collected from naturally infected Prince Charles fruit in September 1989 to nearly 100% from one diseased Charleston Elite fruit that was inoculated with strain 9016. Symptom development on seedlings from infested Jubilee seed (strain 8908) was similar to seedlings grown from seed from symptomatic fruit. Water-soaked lesions, often originating at the cotyledon apex, were first visible 1–3 days after seedling emergence. No symptoms were observed on any seedlings from the 1,245 seed harvested from three inoculated, asymptomatic fruit.

One Prince Charles seedling out of nearly 9,000 that developed from a commercial seed lot expressed typical fruit blotch seedling symptoms. The pathogen was recovered from both cotyledon and true leaf lesions on this seedling, and its identity was confirmed by phenotypic and pathogenicity tests.

Seed treatments. All treatments significantly reduced the number of diseased seedlings that developed from seed harvested from both inoculated, symptomatic Charleston Elite fruit and laboratory-infested Jubilee seed, but no treatment prevented all disease (Table 3). Germination was not adversely affected by the seed treatments, but certain seedlings from the HCl treatment were chlorotic and slightly stunted.

Seed isolations. The pathogen was recovered from both seed coats and embryos of seed from inoculated, symptomatic fruit and from Jubilee seed infested with strain 8908. The pathogen was de-

Table 2. Seed transmission of the fruit blotch pathogen from naturally infected and inoculated, symptomatic watermelon fruit and laboratory-infested seed

Seed lot[y]	Seed source	Emergence (%)	Plants with symptoms (%)
PC 1	Prince Charles September 1989	97.0[z]	7.1
PC 2	Prince Charles July 1989	86.0	10.8
FS 8	Charleston Elite inoculated with 8908 September 1990	89.0	40.1
FS 12	Charleston Elite inoculated with 8908 September 1990	98.0	62.3
SS 34	Charleston Elite inoculated with 9016 September 1990	99.0	65.6
SS 22	Charleston Elite inoculated with 9016 September 1990	99.0	96.0
JF 1	Jubilee, seed infested with 8908 September 1990	97.0	75.3

[y] For PC 1 and PC 2, seeds were collected from naturally infected fruit from commercial fields in September and July 1989, respectively. FS 8 and 12 and SS 34 and 22 were removed from individual inoculated, symptomatic fruit from a field plot in West Lafayette, IN. JF 1 seed was soaked in a 10^9 cfu/ml suspension of strain 8908 for 5 min and air-dried overnight before planting.

[z] Mean of four replications, 25 seeds per replication.

Table 3. Effect of seed treatment on emergence and disease occurrence in watermelon seed naturally infested with the fruit blotch pathogen and seed artificially infested with the pathogen

	Naturally infested seed[x]		Laboratory-infested seed[y]	
Treatment[w]	Emergence (%)	Plants with symptoms (%)	Emergence (%)	Plants with symptoms (%)
H_2O	50.8	87.7 a[z]	90.7	79.8 a
Heat	69.2	18.7 b	90.7	3.0 b
NaOCl	68.3	13.4 b	89.3	1.5 b
HCl	74.2	11.2 b	88.0	1.5 b

[w] Treatments were as follows: H_2O, soaked in sterile distilled H_2O (approximately 21 C) for 20 min; heat, soaked in 50-C water bath for 20 min; NaOCl, soaked in 0.525% NaOCl for 20 min; and HCl, soaked in 1.8% HCl for 5 min.

[x] Values are means of three replications with 40 plants per replication. Seeds were recovered from a symptomatic Charleston Elite fruit from field pathogenicity test.

[y] Values are means of three replications with 25 plants per replication. Seeds of Jubilee were soaked in a 10^9 cfu/ml suspension of strain 8908 for 5 min and air-dried overnight before treatment.

[z] Values in columns followed by the same letter are not significantly different (LSD, $P = 0.01$).

tected on ENR only, because of the large number of nontarget bacteria that also were recovered.

DISCUSSION

Before the fruit blotch epidemics of the late 1980s occurred, *P. p. citrulli* was considered to be primarily a pathogen of watermelon seedlings (4,10,12). Injection of the bacterium into watermelon fruit resulted in discoloration of the flesh, but no blotch symptom was reported (12). Wall and co-workers (17,18) suggested that *P. p. citrulli* caused fruit symptoms as well, based on phenotypic characteristics of their fruit blotch isolates. Our preliminary reports of this research included a similar suggestion. However, Somodi et al (11) found sufficient differences between the fruit blotch bacterium and the type strain of *P. p. citrulli* to refrain from concluding that they are the same organism. Indiana fruit blotch strains differed from *P. p. citrulli* in tobacco hypersensitivity, as did Florida strains (11). We found no phenotypic differences between strains from seedlings and fruit, and strains from both sources were able to cause typical symptoms in both fruit and seedling inoculations. In Florida (11), strains recovered from fruit were able to cause both foliar and fruit symptoms.

The fruit blotch pathogen has been reported to be seed-transmitted (16,17), and our research demonstrates seed transmission from both naturally diseased and inoculated, symptomatic fruit. The recovery of the pathogen from both seed coats and embryos of seeds from symptomatic fruit indicates that seeds are both internally and externally contaminated. The inability of our seed treatments to eradicate the causal bacterium also supports this contention. A previous report indicated that heat treatment was effective in controlling this disease in seed (16) but did not state whether the disease was completely eliminated by the treatment. Mundt and Hinkle (7) reported that watermelon seed was more difficult to disinfest than seed from other vegetables. They concluded that bacteria applied to the seed could penetrate the seed surface to a depth not achieved by a sodium hypochlorite solution. Based on our results, treatment of seed does not appear to be a practical control measure for this disease.

Although seed is internally contaminated, our results do not indicate that the fruit blotch pathogen systemically invades watermelon plants. We did not recover the bacterium from nonsymptomatic plant tissues nor from vascular tissues in peduncles of symptomatic fruit. Seed transmission occurred only in seed from fruit with symptoms, where wounding of lesioned areas to remove seed would allow seed to come in contact with the pathogen. No symptoms developed on any seedling grown from seed removed from inoculated, nonsymptomatic fruit. No vine collapse was observed either in naturally infected fields or our field plot. Finally, treatment of healthy watermelon seed by soaking in a suspension of the pathogen resulted in both internal and external contamination and a seedling disease syndrome similar to that occurring in seed from naturally infected fruit. There are openings in the watermelon seed coat at the hilum region visible with scanning electron microscopy (K. Rane, *unpublished*) through which microorganisms could enter during the seed extraction process. A similar mode of seed transmission has been found for two bacterial pathogens of bean, *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye (1) and *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al (14), where the pathogens were shown to contaminate bean seed internally through lesion development on pods rather than systemic infection.

The 1989 outbreak of fruit blotch in Indiana appeared to be initiated by contaminated seed. Watermelon fields are not continuous and bacteria are not spread by wind like some fungal spores. Initial observations of diseased seedlings in commercial greenhouses were limited to distinct foci and were associated with a certain lot number of Prince Charles watermelon. Also, we confirmed the presence of the fruit blotch pathogen in one commercial sample of Prince Charles seed. This evidence supports the contention that the original source of the pathogen was contaminated seed. Careful inspection of seed fields may be an effective method of controlling this disease, because our results indicate that seed contamination is associated with the development of a distinctive fruit symptom.

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