Wet Seed Treatments for the Control of Bacterial Fruit Blotch of Watermelon

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ABSTRACT

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The bacterium causing fruit blotch of watermelon was transmitted in seeds from fruit that had the typical water-soaking symptom; from fruit that had small, restricted, necrotic lesions; and from symptomless fruit adjacent to typically symptomatic fruit. Storage of seed at 12°C for 12 months did not reduce the level of seed transmission. Fermentation of seeds in watermelon juice and debris prior to washing and drying reduced the level of seed transmission from symptomatic fruit from 61% to less than 1%. Treatment of washed seeds from symptomatic fruit with 1% $CaOCl_2$ for 15 min was relatively ineffective in reducing seed transmission, but 1% HCl for 15 min was as effective as fermentation. Fermentation of seeds for 24 to 48 h followed by 1% HCl or 1% $CaOCl_2$ treatment for 15 min prior to washing and drying were the most effective treatments for eliminating bacterial contamination of watermelon seeds. These treatments did not adversely affect seed germination.

Additional keywords: *Acidovorax avenae* subsp. *citrulli, Citrullus lanatus*

In 1989, a new bacterial fruit rot of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) occurred in commercial watermelon in Florida and other southeastern, mid-Atlantic, and midwestern states (1,4,8). In some commercial fields, loss of marketable fruit approached 90%. Bacterial fruit blotch (BFB) of watermelon has occurred in one or more of the watermelon-producing states in the eastern United States every year since 1989. Fortunately, in most years, BFB has occurred in a limited number of fields, but the disease has been devastating in many of these fields. BFB was especially widespread in Georgia in 1992 and throughout the eastern United States in 1994. Thousands of hectares of watermelon in at least 10 states were affected in 1994.

Symptoms of BFB occur on seedlings, leaves, and fruit. On seedlings, water-soaked lesions occur on hypocotyls and cotyledons, sometimes resulting in collapse and death of the seedling. Foliar symptoms can develop throughout the watermelon season but may be relatively inconspicuous. Leaf lesions are light brown to reddish brown and often spread along the midrib of the leaf. Leaf lesions do not usually result in defoliation but are important as reservoirs of bacteria for fruit infection. Fruit symptoms begin as small water-soaked areas with irregular margins that may expand to cover the upper surface of the melon. Eventually, the lesions turn brown, and cracks may appear, resulting in fruit decay.

The bacterium that causes watermelon fruit blotch, *Acidovorax avenae* subsp. *citrulli* (formerly *Pseudomonas pseudoalcaligenes* subsp. *citrulli*) (7,13), was first reported in 1965 to cause a watermelon seedling blight (12). In 1987, BFB of watermelon was first reported in Guam and Tinian (11). The bacterium has been reported to be seed-transmitted (6,9,10). Watermelon seeds appeared to be contaminated both internally and externally, but the bacterium did not appear to systemically invade the watermelon seeds through the plant vascular system (2,6). Openings in the watermelon seed coat at the hilum region could be ports of entry into the seeds by the bacterium during the seed extraction process. Watermelon seeds have been reported to be more difficult to disinfest than other vegetable seeds (5), and Rane and Latin (6) concluded that the seed treatments for BFB that they evaluated did not appear practical.

The purpose of this study was to evaluate the effect of fermentation in the seed extraction process and of various wet seed treatments prior to seed drying on seed transmission of the watermelon fruit blotch bacterium.

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MATERIALS AND METHODS

Bacterial strains and production of infested seed. Two strains of *A. a.* subsp. *citrulli* that had been isolated from commercial watermelon fields in Florida in 1989 were used in this study (WFB89-1 and WFB89-2). For inoculation of plants or fruit in the field, bacteria were grown on nutrient agar (Difco) for 48 h and washed from the agar surface with sterile, deionized water. These bacterial suspension concentrations were adjusted to A_{600nm} = 0.25 using a spectrophotometer and diluted 200-fold with sterile water to approximately 10^6 CFU/ml. Approximately 0.1 ha of the cultivar Charleston Gray was grown for the production of BFB-infested seeds. Fourteen to 21 days prior to fruit maturation, watermelons were inoculated with the bacterial suspension by misting the upper rind surface until runoff.

Seed source and bacterial fruit blotch transmission. Seeds were collected from individual fruit into 4-liter buckets. Seeds were washed in tap water by filling the buckets and floating debris out of the buckets, followed by collecting the seeds on a separate wire screen for each fruit and rinsing thoroughly with a garden hose. Fruit used as seed sources in 1993 included Charleston Gray fruit with typical fruit blotch symptoms, symptomless Charleston Gray fruit adjacent to symptomatic fruit, Sugar Baby fruit with restricted lesions and rotting flesh, and Sangria fruit from a field that did not have bacterial fruit blotch. In 1994, Charleston Gray seeds were used from fruit with typical fruit blotch symptoms, fruit with atypical restricted lesions and normal flesh, and symptomless fruit adjacent to symptomatic fruit, as well as commercial Charleston Gray seeds.

For assay, seeds were planted in 28 × 52 cm plastic trays filled with commercial potting mix. Two rows of 25 seeds were planted in each tray, with 14 cm between rows and 2 cm between seeds within the rows. When plants emerged (5 to 7 days after planting), all flats were covered with plastic domes for 48 h. The assays were run in July, and greenhouse temperatures ranged from 24 to 35°C. Disease evaluations were made 10 days after planting. In the 1993 assays, five replications of 100 seeds per treatment were planted, and in 1994, four replications of 100 seeds per treatment were planted. The disease incidence was calculated based on the number of emerged seedlings.

Wet seed treatments, 1992 tests. Seeds were collected by hand from fruit with symptoms ranging from obvious water-

soaking symptoms to cracking of the rind surface and internal decay. Seeds were collected into a 208-liter barrel. Approximately half a barrel of seeds, juice, and small pieces of watermelon flesh tissue was collected on June 23, and one-third barrel on June 30. The first batch was separated into seven equal groups and the second batch into four equal groups. Each group was placed into two 4-liter buckets for treatment. The treatments with batch one were: (1) wash seeds in tap water by filling buckets and floating debris out of the buckets, followed by collecting the seeds on a wire screen and rinsing thoroughly with a garden hose; (2) ferment seeds, juice, and watermelon debris for 24 h at air temperature (23 to 33°C) with periodic stirring, then wash seeds thoroughly; (3) ferment for 48 h then wash seeds; (4) ferment for 72 h, then wash seeds; (5) wash seeds, soak in 1% HCl for 15 min, and wash thoroughly; (6) ferment for 24 h, soak in 1% HCl for 15 min, and wash thoroughly; and (7) ferment for 48 h, soak in 1% HCl for 15 min, and wash thoroughly. Treatments with batch two were: (1) wash seeds and rinse thoroughly; (2) wash seeds, soak in 1% $CaOCl_2$ for 15 min, and wash thoroughly; (3) ferment for 24 h, soak in 1% $CaOCl_2$ for 15 min, and wash thoroughly; and (4) ferment for 48 h, soak in 1% $CaOCl_2$ for 15 min, and wash thoroughly. Soluble solids and pH readings were taken after 0, 24, and 48 h of fermentation. After treatment, seeds were air-dried for 3 days and stored in a seed storage room at 12°C.

Duplicate seed assays were conducted in Leesburg, FL and Woodland, CA. In the Leesburg assays, seeds were planted in 28 × 52 cm plastic trays filled with commercial potting mix. Two rows of 25 seeds were planted in each tray, with 14 cm between rows and 2 cm between seeds within the rows. Four replications with 100 seeds per replication of each treatment were planted. In the Leesburg test no. 1, seedlings were covered with a moist chamber for one night after emergence, and in test no. 2, seedlings were left on the greenhouse bench uncovered. Greenhouse temperatures were 24 to 35°C. In the Woodland assays, seeds were planted in flats consisting of 200 cells per flat. Sterile soil mix was preirrigated, and seeds were planted and covered with a thin layer of vermiculite. Four replications of 200 seeds were planted for each treatment. Seedlings were grown in a temperature-controlled greenhouse at 24 to 30°C. In all tests, symptoms were evaluated 10 days after planting.

Wet seed treatments, 1993 test. Seeds were collected from 59 infected watermelons as described for 1992, resulting in three-fourths barrel of seeds, juice, and watermelon tissue. The seeds were separated into eight equal groups. Treatments were: (1) wash seeds thoroughly in tap water; (2) ferment for 24 h and wash seeds; (3) ferment for 48 h and wash; (4) ferment for 72 h and wash; (5) wash seeds, soak in 1% HCl for 15 min, and wash thoroughly; (6) ferment for 24 h, soak in 0.5% HCl for 15 min, and wash; (7) ferment for 48 h, soak in 1% HCl for 15 min, and wash; and (8) ferment for 72 h, soak in 1% HCl for 15 min, and wash thoroughly. After treatment, the seeds were air-dried for 3 days and stored in a seed storage room.

Seedling assays were run immediately, 6 months, and 12 months after harvest in Leesburg and Woodland. In the Leesburg assays, five replications of 78 seeds per treatment were planted. When plants emerged (5 to 7 days after planting), all flats were covered with plastic domes for 48 h, and in the harvest and 12-month assays, symptoms were evaluated 10 days after planting. In the 6-month assay run in January, temperatures were lower and seedling emergence was slower; therefore, symptoms were evaluated 12 days after planting. Greenhouse temperatures ranged

Table 1. Seed transmission of bacterial fruit blotch of watermelon to seedlings

Seed source[y]	Disease incidence (%)[z]
Spring 1993 seeds	
Charleston Gray fruit with typical fruit blotch symptoms	45.2 a
Sugar Baby fruit with localized lesions and decaying flesh	34.4 a
Charleston Gray symptomless fruit, but adjacent to symptomatic fruit	0.2 b
Sangria fruit from a field with no fruit blotch symptoms	0.0 b
Spring 1994 Charleston Gray seeds	
Fruit with typical fruit blotch symptoms	80.0 a
Fruit with atypical restricted lesions	2.3 b
Symptomless fruit, but adjacent to symptomatic fruit	0.8 b
Commercial seeds	0.0 b

[y] Seeds were collected by hand from fruit into a 4-liter bucket. Seeds were washed on a wire screen and rinsed thoroughly with a garden hose followed by air-drying. In the greenhouse seedling assays, seedlings were covered with a moist chamber for two consecutive nights after emergence, and symptoms were evaluated 10 days after planting.

[z] The 1993 test values are the means of five replications of 100 seeds per replication, and 1994 values are the means of four replications of 100 seeds. Percent disease was calculated as number of diseased seedlings divided by the number of emerged seedlings. Means in columns followed by the same letter are not different significantly ($P = 0.05$) according to Duncan's multiple range test. Data were analyzed after arcsine square root transformation.

Table 2. Effect of fermentation and acid wash of watermelon seeds on seedling emergence and bacterial fruit blotch incidence, 1992 seeds

	Leesburg test 1[y,z]		Leesburg test 2[y,z]		Woodland test[y,z]	
Seed treatment[x]	Emerged (%)	Infected (%)	Emerged (%)	Infected (%)	Emerged (%)	Infected (%)
Batch 1 seeds						
Wash and dry	87 c	39.3 a	87 b	15.6 a	89 a	19.9 a
24 h ferm., wash, dry	95 ab	0.0 b	94 a	0.0 b	89 a	0.0 b
48 h ferm., wash, dry	92 abc	0.3 b	93 ab	0.0 b	82 b	0.0 b
72 h ferm., wash, dry	70 d	0.0 b	86 b	0.0 b	78 b	0.0 b
1% HCl, wash, dry	94 abc	0.0 b	92 ab	0.0 b	94 a	0.0 b
24 h ferm., 1% HCl, wash, dry	97 a	0.5 b	97 a	0.0 b	94 a	0.0 b
48 h ferm., 1% HCl, wash, dry	90 bc	0.0 b	88 b	0.0 b	91 a	0.0 b
Batch 2 seeds						
Wash and dry	92 a	61.8 a	92 bc	23.7 a	89 b	39.6 a
1% $CaOCl_2$, wash, dry	96 a	4.0 b	96 ab	5.1 b	93 b	4.7 b
24 h ferm., 1% $CaOCl_2$, wash, dry	98 a	0.0 c	96 a	0.0 b	97 a	0.0 c
48 h ferm., 1% $CaOCl_2$, wash, dry	94 a	0.0 c	91 cd	0.0 b	88 bc	0.0 c

[x] Treatments included washing on a wire screen using a garden hose and air-drying; fermenting seeds, juice, and watermelon debris for various lengths of time prior to washing and drying; soaking seeds in 1% HCl prior to washing and drying; soaking seeds in 1% $CaOCl_2$ prior to washing and drying; fermenting followed by soaking in HCl prior to washing and drying; and fermenting followed by soaking in 1% $CaOCl_2$ prior to washing and drying.

[y] In the Leesburg test 1, seedlings were covered with a moist chamber for one night after emergence, and in test 2, seedlings were left on the greenhouse bench uncovered. In the Woodland test, seedlings were grown in a greenhouse at 24 to 30°C uncovered. Symptoms were evaluated 10 days after planting.

[z] Values are means of four replications with 100 seeds per replication in the Leesburg tests and 200 seeds per replication in the Woodland test. Means in columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Data were analyzed after arcsine square root transformation.

from 20 to 35°C. Randomly selected seedlings were sampled to confirm the presence of the fruit blotch bacterium by culturing and hypersensitivity on tobacco. In the Woodland seedling assays run at harvest and 6 months later, six replications of 78 seeds were planted. There was not enough seed to replicate the 12-month assay; therefore, a total of 88 to 164 seeds were planted per treatment. At emergence, seedlings were covered with a plastic dome moist chamber for 48 h, and symptoms were evaluated 13 days after planting. Greenhouse temperature was maintained at 24 to 30°C.

RESULTS

Seed source and seed transmission of bacterial fruit blotch. As reported previously (6,10,11), BFB of watermelon developed on seedlings grown from seeds that had been collected from watermelon fruit with typical fruit blotch symptoms (Table 1). Seed transmission of fruit blotch also occurred with seeds collected from fruit that had no typical symptoms but only small, restricted, necrotic lesions 1 to 3 mm in diameter. In 1993, Sugar Baby fruit with restricted lesions had nearly as much seed transmission as did seeds from Charleston Gray with typical symptoms. However, the interior flesh of this Sugar Baby fruit had been invaded from one of the restricted surface lesions and was decaying. In a second test in 1993 with Sugar Baby fruit having restricted lesions and normal firm flesh, there was only 0.3% seed transmission (data not shown). There was also a small amount of seed transmission in symptomless fruit that were located next to symptomatic fruit in the field in both the 1993 and 1994 tests. No symptoms were observed in seedlings grown either from seeds of fruit in a fruit blotch–free field or from commercial seeds.

Wet seed treatments, 1992 tests. All seed treatments reduced significantly the incidence of fruit blotch symptoms in seedlings that developed from treated seeds when compared with seeds that were washed and dried (Table 2). There were significant differences in emergence rates among the various treatments. In some cases, shorter fermentation times increased the emergence rates over the untreated controls; whereas fermentation for 72 h decreased emergence. In both batches of infested seeds that were washed and dried, approximately twice as many seedlings developed symptoms after being covered with a moist chamber for one night as developed symptoms after being left uncovered on the greenhouse bench in either Leesburg or Woodland.

When the seedlings in batch 1 were covered overnight with a moist chamber, symptoms developed on less than 1% of the seedlings from the 24-h fermentation plus 1% HCl treatment and the 48-h fermentation treatment (Table 2). Symptoms were not observed on seedlings that were covered in any of the other four treatments. No symptoms were seen on any of the six treatments when the seedlings were left uncovered. Assays done on batch 2 seeds showed no symptoms on any of the seedlings from treatments that included fermentation. Infected seedlings were observed in all of the 1% $CaOCl_2$ treatments whether or not the seedlings were covered during the assay.

In the extract of batch 1 seeds, the pH was 4.7 prior to fermentation and 3.9 after 24- or 48-h fermentation. The soluble solids was 7.8 prior to fermentation and 6.5 after fermentation. In the extract of batch 2 seeds, the pH was 5.7 prior to fermentation, 4.0 after 24-h fermentation, and 3.9 after 48 h. The soluble solids was 8.8 prior to fermentation, 7.6 after 24-h fermentation, and 7.5 after 48 h.

Wet seed treatments, 1993 tests. In the seedling assays conducted immediately after harvest, all wet seed treatments significantly reduced the number of seedlings with BFB symptoms when compared with the wash and dry control treatment (Tables 3 and 4). After 6 months of seed storage, the number of seedlings developing symptoms in the assays was considerably lower than at harvest. However, the assays con-

Table 3. Effect of fermentation and acid wash of watermelon seeds on seedling emergence and bacterial fruit blotch incidence under Florida greenhouse assay conditions at seed harvest and after seed storage for 6 and 12 months

	Germination (%)[z]			Disease incidence (%)[z]		
Seed treatment[y]	Harvest	6 months	12 months	Harvest	6 months	12 months
Wash and dry	88 a	68 b	93 a	51.0 a	3.0 a	88.0 a
24 h ferm., wash, dry	91 a	85 a	95 a	0.3 b	0.0 b	0.0 b
48 h ferm., wash, dry	89 a	85 a	96 a	0.3 b	0.0 b	0.0 b
72 h ferm., wash, dry	87 a	86 a	92 a	0.0 b	0.0 b	0.0 b
1% HCl, wash, dry	92 a	81 ab	96 a	0.3 b	0.0 b	0.0 b
24 h ferm., 0.5% HCl, wash, dry	90 a	91 a	96 a	0.0 b	0.0 b	0.0 b
48 h ferm., 1% HCl, wash, dry	88 a	92 a	93 a	0.0 b	0.0 b	0.0 b
72 h ferm., 1% HCl, wash, dry	85 a	87 a	92 a	0.0 b	0.0 b	0.0 b

[y] Treatments included washing on a wire screen using a garden hose and air drying; fermenting seeds, juice, and watermelon debris for various lengths of time prior to washing and drying; soaking seeds in 1 or 0.5% HCl prior to washing and drying; and fermenting followed by soaking in HCl prior to washing and drying. In the seedling assays run at harvest and 12 months later, seedlings were covered with a moist chamber for two consecutive nights after emergence and symptoms were evaluated 10 days after planting. In the 6-month assay run in January, temperatures were lower and seedling emergence was slower; therefore, symptoms were evaluated 12 days after planting.

[z] Values are means of five replications with 78 seeds per replication. Means in columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Data were analyzed after arcsine square root transformation.

Table 4. Effect of fermentation and acid wash of watermelon seeds on seedling emergence and bacterial fruit blotch incidence under Woodland, CA, greenhouse assay conditions at seed harvest and after seed storage for 6 and 12 months

	Germination (%)[z]			Disease incidence (%)[z]		
Seed treatment[y]	Harvest	6 months	12 months	Harvest	6 months	12 months
Wash and dry	99 a	99 a	100 a	29.6 a	11.7 a	48.9 a
24 h ferm., wash, dry	99 a	95 bc	98 a	0.4 b	0.0 b	0.0 a
48 h ferm., wash, dry	97 a	96 ab	99 a	0.0 b	0.0 b	0.8 a
72 h ferm., wash, dry	95 a	88 d	99 a	0.0 b	0.2 b	0.0 a
1% HCl, wash, dry	99 a	99 a	98 a	0.0 b	0.0 b	0.0 a
24 h ferm., 0.5% HCl, wash, dry	98 a	93 bc	95 a	0.0 b	0.0 b	0.0 a
48 h ferm., 1% HCl, wash, dry	99 a	96 ab	98 a	0.0 b	0.0 b	0.0 a
72 h ferm., 1% HCl, wash, dry	94 a	91 cd	92 a	0.0 b	0.0 b	0.0 a

[y] Treatments included washing on a wire screen using a garden hose and air drying; fermenting seeds, juice, and watermelon debris for various lengths of time prior to washing and drying; soaking seeds in 1 or 0.5% HCl prior to washing and drying; and fermenting followed by soaking in HCl prior to washing and drying. In the seedling assays, seedlings were covered with a plastic dome moist chamber for 48 h at emergence, and symptoms were evaluated 13 days after planting.

[z] Values for seedling assays run at harvest and 6 months are means of six replications with 78 seeds per replication. There were not enough seeds to replicate the 12-month assay; this data is based on 88 to 164 total seeds in the treatments. Means in columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Data were analyzed after arcsine square root transformation.

ducted 1 year after harvest had the highest number of infected seedlings. There were no significant differences between treatments in emergence rates in the assays done at harvest or after 12 months of storage. The assays run on seed stored for 6 months did have significant differences in emergence.

In the Leesburg assays, a single seedling developed symptoms in the 24- and 48-h fermentation treatments and in the 1% HCl treatment, when tested immediately after seed harvest (Table 3). In the 6- and 12-month seedling assays, symptoms were observed only in the wash and dry control seedlings. In the Woodland seedling assays, symptoms were observed in the 24-h fermentation treatment immediately after harvest, in the 72-h fermentation treatment 6 months after harvest, and in the 48-h fermentation treatment 12 months after harvest (Table 4). None of the seedlings grown from seeds treated by fermentation for 24, 48, or 72 h followed by soaking in HCl developed symptoms in any of the assays in Leesburg or Woodland.

DISCUSSION

Under certain environmental conditions, the fruit blotch bacterium can spread rapidly from very few primary infection sites and destroy an entire field of watermelon (3). The ideal control of BFB of watermelon would be to prevent the introduction of the causal agent into the field. Infested seed represents one mechanism through which the bacterium may be introduced into a watermelon field (6; D. L. Hopkins, *unpublished*). Other means of introduction would be infected transplants, contaminated volunteer watermelons, and possibly, infected wild cucurbits. The fruit blotch pathogen has been shown to be seed-transmitted on seeds harvested from fruit with the typical water-soaking symptom (6,12). If infested seeds are obtained only from symptomatic fruit, careful culling of symptomatic fruit in the seed production field would provide a means of producing clean, noninfested watermelon seeds. However, in this study, seed transmission also occurred with seeds collected from fruit with atypical, restricted, necrotic lesions and from symptomless fruit adjacent to typically symptomatic fruit. Culling of fruit with typical symptoms of fruit blotch in a seed production field would not necessarily eliminate all infested seeds. The atypical, restricted lesions on watermelon fruit also can be produced by other pathogens or by physical damage, and the lesions can be very small, making the detection and culling of these fruit very difficult. Of course, there is no means of detecting and culling the fruit that are symptomless carriers of the fruit blotch bacterium. Therefore, we would recommend not harvesting seeds from fruit in the vicinity of symptomatic fruit. The safest approach would be to harvest seeds only from fields that had no fruit blotch symptoms.

Since it is difficult to be absolutely sure that harvested seeds are not contaminated with the fruit blotch bacterium, a wet seed treatment to eradicate the pathogen from the seeds at harvest is another approach to obtaining noninfested seeds. Fermentation of the seeds in watermelon juice and debris was effective in reducing the amount of seed transmission; however, the bacterium was not eradicated from all of the seeds. There was still a very low level of seed transmission (<1%) after fermentation. There did not seem to be any difference in the effectiveness of 24, 48, or 72 h of fermentation under our conditions, but with cooler temperatures, fermentation could proceed more slowly and 24 h may not be long enough. Emergence was sometimes reduced with 72-h fermentation.

The mechanism of action of seed fermentation is not known, but fermentation results in seeds that are cleaner on the surface and do not have the sticky sugar coating that occurs on nonfermented seeds. Possibly, the bacteria are surface contaminants in the sugar and debris that stick to the seeds, and fermentation simply cleans them from the seed surface. The seed-contaminating bacteria also could be killed by bactericidal compounds or the lower pH produced during fermentation. Occasionally, some bacteria survived fermentation treatments. It is possible that fermentation has less than 100% efficiency in killing the pathogenic bacteria on watermelon seeds. Bacteria also could penetrate the seed coat through openings at the hilum region (6) and escape exposure to toxic substances produced during fermentation. Previous results indicate that the bacterium does not systemically invade watermelon plants and infest the seeds (2,6). There is also the possibility that the few infected seedlings resulted from errors in the fermentation procedure. They could have been seeds that escaped complete fermentation by sticking to another seed or floating on the surface of the slurry during fermentation due to inadequate stirring. Recontamination of seeds after fermentation could also explain the low level of infection. Because of the care that was taken in fermentation and the repeatability of the results, we believe that fermentation greatly reduces seed contamination but does not eradicate it.

Calcium hypochlorite treatment was relatively ineffective, as was sodium hypochlorite in another study (6). In contrast to the other study, we found HCl to be as effective as fermentation, with only one seedling developing symptoms in the 2 years of tests. The concentration of HCl and the time of treatment were different in the two studies and may account for the slightly different conclusions. Fermentation followed by HCl or $CaOCl_2$ treatment was most effective in eliminating bacterial contamination of watermelon seeds. Neither of these chemical treatments adversely affected seedling emergence.

The level of bacterial contamination of the seeds did not appear to decrease after 12 months of storage. Contamination of seeds with the fruit blotch bacterium probably would not be eliminated in long-term storage under dry conditions. The lower level of seedling symptoms in the assay after 6 months of seed storage than in the assay after 12 months probably was a result of the low temperature and low humidity in the greenhouse during the 6-month assay. However, there also could be a seasonal effect due to light levels or day length. Further work is underway on the effect of environmental conditions on seedling assays for bacterial fruit blotch of watermelon.

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