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# Bacterial fruit blotch of melon: screens for disease tolerance and role of seed transmission in pathogenicity

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**Abstract** Bacterial fruit blotch (BFB) of cucurbits, caused by *Acidovorax avenae* subsp. *citrulli*, is a serious threat to the watermelon and melon industries. To date, there are no commercial cultivars of cucurbit crops resistant to the disease. Here we assessed the level of tolerance to bacterial fruit blotch of various commercial cultivars as well as breeding and wild lines of melon, using seed-transmission assays and seedling-inoculation experiments. Selected cultivars were also tested in a greenhouse experiment with mature plants. All tested cultivars/lines were found to be susceptible to the pathogen, and most of them showed different responses (relative tolerance vs. susceptibility) in the different assays; however, some consistent trends were found: cv. ADIR339 was relatively tolerant in all tested assays, and cv. 6407 and wild lines BLB-B and EAD-B were relatively tolerant in seed-transmission assays. We also provide evidence supporting a strong correlation between the level of susceptibility of a cultivar/line and the ability of the pathogen to adhere to or penetrate the seed. To the best of our knowledge, this is the first attempt to assess melon cultivars/lines for bacterial fruit blotch response.

**Keywords** *Acidovorax* · *citrulli* · *Cucumis melo* · Seed transmission

**Abbreviations**

| | |
|---|---|
| BFB | bacterial fruit blotch |
| PI | plant introduction |

## Introduction

Bacterial fruit blotch (BFB), caused by the Gram-negative bacterium *Acidovorax avenae* subsp. *citrulli* (Schaad et al. 1978; Willems et al. 1992), is a destructive disease, which has been responsible for significant economic losses of cucurbit crops world-wide since the late 1980s. Most economic losses due to BFB have been reported in watermelon (Wall and Santos 1988; Wall et al. 1990; Somodi et al. 1991; Latin and Hopkins 1995; Schaad et al. 2003), and in recent years, in melon (Isakeit et al. 1997, 1998; O'Brien and Martin 1999; Burdman et al. 2005). Nevertheless, other cucurbit crops, such as cucumber, squash and pumpkin, are also susceptible to the pathogen (Langston et al. 1999; Martin and O'Brien 1999; Walcott et al. 2004).

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*Acidovorax avenae* subsp. *citrulli* can affect different organs of the plant, at different developmental stages. The bacterium is seedborne and the BFB cycle generally begins with contaminated seeds, which are transferred to transplant-production facilities where warm and humid conditions favour its proliferation (Latin and Hopkins 1995). Emerging seedlings are highly susceptible to the bacterium, and usually suffer from cotyledon infections and seedling blight. If weak infection occurs, symptoms might not be detected, and the seedlings are transplanted into the field. Mature plants are relatively resistant to the pathogen and may have inconspicuous symptoms. These apparently healthy plants may serve as a pathogen reservoir for later infection of the fruits, which, as the name of the disease infers, are highly susceptible. Under optimal conditions for BFB, near-total losses can occur in affected fields, because symptomatic fruits are unmarketable (Latin and Hopkins 1995).

Due to the destructive nature of *A. avenae* subsp. *citrulli* and the fact that BFB losses are often associated with infested seeds or transplants, continued crop losses have resulted in litigation against several seed companies by watermelon growers in the US (Schaad et al. 2003). Therefore, many attempts have been made to develop effective means of coping with BFB, including seed treatments (Rane and Latin 1992; Hopkins et al. 2003), detection of the pathogen in contaminated seeds (Walcott et al. 2006; Bahar et al. 2008), and chemical treatments in the field (Hopkins 1991, 1995). Despite their contribution to combating the disease, the efficiency of these approaches is limited, and BFB still poses a serious threat to the watermelon and melon industries.

Resistance is an efficient means of combating plant diseases. However, to date there are no commercial cucurbit cultivars with reliable BFB resistance. Several screens for BFB resistance have been performed with plant introductions (PIs) and commercial cultivars of watermelon, but inconsistencies are often found, due mainly to differences in experimental conditions (Hopkins and Thompson 2002). For instance, several PIs that appeared to be resistant in assays performed during the winter were clearly susceptible in summer assays, and vice versa. Another factor that is likely to contribute to these inconsistencies is the high diversity among *A. avenae* subsp. *citrulli* isolates used in screens. As an example, Sowell and Schaad (1979) reported two PIs apparently resistant to BFB. However, when these PIs were re-examined in 1989–1990 using a different isolate, they were found to be as susceptible as the other PIs (Hopkins et al. 1993).

*Acidovorax avenae* subsp. *citrulli* isolates have been shown to differ on the basis of DNA-fingerprinting profiles, whole-cell fatty-acid composition, carbon-source utilization and pathogenicity assays. Two genetically distinct groups have been identified: group I includes strains isolated mainly from non-watermelon hosts, while group II includes strains isolated mainly from watermelon seedlings and fruits (Walcott et al. 2000, 2004). In agreement with this classification, group I isolates are generally more virulent than group II isolates on non-watermelon cucurbits, while the opposite is generally observed on watermelon (Walcott et al. 2004; Burdman et al. 2005).

The need for BFB-resistant sources is indisputable, but so are the difficulties in finding them. This is reflected by the fact that over 1,000 watermelon PIs have already been (unsuccessfully) screened (Hopkins and Thompson 2002). No screens for resistance or tolerance have been published to date for any other cucurbit, despite the serious threat posed by BFB to these crops, particularly melon. With this as our rationale, the objective of the present work was to assess various commercial cultivars, breeding lines and wild lines of melon for BFB resistance or tolerance using different inoculation techniques, including seed transmission, seedling inoculation and a greenhouse assay with mature plants. As these assays represent different stages of plant growth and timings of infection, we aimed to elucidate any potential connections among them in terms of cultivar/line response to infection. We also present evidence that bacterial seed adhesion or penetration plays a critical role in virulence by seed transmission.

## Materials and methods

### Bacterial strains, growth conditions and inoculum preparation

*Acidovorax avenae* subsp. *citrulli* strains M1, M3, M5 and M6 (group I strains), and W1, W2, W3, W4 and W5 (group II strains) have been previously described (Burdman et al. 2005). All strains were grown at 28°C on nutrient agar (NA; Becton, Dickinson and Co., Sparks, MD, USA) or nutrient broth (NB; Becton,

Dickinson and Co.), supplemented with 100 μg $ml^{-1}$ ampicillin. For inoculation, cultures were washed from NA plates using 5 ml of sterile distilled water (SDW). Bacterial suspensions were adjusted to an $OD_{600}$ of 0.6, corresponding to ~$3\times10^8$ colony-forming units (cfu) $ml^{-1}$, using a spectrophotometer. This suspension was diluted to final concentrations of ~1 or ~$3\times10^6$ cfu $ml^{-1}$ (verified by dilution plating), which were used for inoculation. For selection purposes in experiments performed to assess bacterial seed adhesion (see below), the pDSK519 plasmid (Keen et al. 1988), conferring kanamycin (Km) resistance, was introduced into electrocompetent *A. avenae* subsp. *citrulli* cells, using an Eppendorf 2510 electroporator (Hamburg, Germany). Electrocompetent cells were prepared according to standard procedures (Sambrook et al. 1989).

## Seed-transmission assays

Fifteen commercial cultivars/breeding lines and 20 wild lines of melon (*Cucumis melo*; Table 1) were assayed. Twenty-four seeds of each cultivar/line were incubated for 2 h with gentle agitation in 50 ml Falcon tubes containing 10 ml suspensions of strain M6 at ~$3\times10^6$ cfu $ml^{-1}$. Control treatments consisted of seeds incubated with SDW. Following incubation, seeds were collected, air-dried and sown in 600 ml pots (six seeds per pot, four pots per cultivar/line) containing equal parts of vermiculite, peat and sand. Emerging seedlings were grown in a greenhouse (25–28°C) for 10–14 days, and then disease severity was scored on a scale of 0 to 7, based on shoot-weight values of inoculated plants relative to the average shoot weight of non-inoculated controls: 0, weight higher than 90% of average control weight; 1 to 5, weight equal to 76–90%, 61–75%, 46–60%, 31–45% and 16–30% of average control weight, respectively; 6, weight equal to or lower than 15% of average control weight; 7, seedling dead. This experiment was carried out twice for each cultivar/line and analysed by Tukey–Kramer HSD test using JMP software (Cary, NC, USA).

**Table 1** Melon cultivars/lines used in this study

| Melon | Type[a] | Source |
|---|---|---|
| Cultivars/breeding lines | | |
| 6405 | Ananas | HG |
| 6407 | Ananas | HG |
| 6801 | Cantaloupe | HG |
| 6812 | Cantaloupe | HG |
| 6802 | Cantaloupe | HG |
| 6815 | Cantaloupe | HG |
| 6813 | Cantaloupe | HG |
| ADIR339 | Ananas | ZG |
| YC-17016 | Yellow Canary | ZG |
| MG-10183 | Galia | ZG |
| MG-10267 | Galia | ZG |
| OR-027 | Charentais | ZG |
| 350 | Ananas | ZG |
| OPHIR | Ananas | ZG |
| IDEAL | Galia | CTS |
| Wild lines | | |
| BLN-B | Cantaloupe | GCNY |
| ARA-B | Cantaloupe | GCNY |
| EAD-B | Honeydew | GCNY |
| ANDb-B | Cantaloupe | GCNY |
| A-1-B | Ha'ogen | GCNY |
| BUL-B | Charentais | GCNY |
| BLM-B | Galia | GCNY |
| EAR1-B | Cantaloupe | GCNY |
| EMJ | Mixture | GCNY |
| ENQ-4 | Ananas | GCNY |
| BIGA-B | Mixture | GCNY |
| BAY-B | Ha'ogen | GCNY |
| ARJ-OP | Indian Exotic | GCNY |
| GOK-1 | Yellow Canary | GCNY |
| AMBC-B | Galia | GCNY |
| BDG-B | Casaba | GCNY |
| FRN-3 | Mixture | GCNY |
| BKU-B | Mixture | GCNY |
| BRO-B | Casaba (Branco) | GCNY |
| BDR-B | Casaba (Branco) | GCNY |

*HG* Hazera Genetics, Mivchor, Israel; *ZG* Zeraim Gedera, Gedera, Israel; *CTS* CTS, Petach Tikvah, Israel; *GCNY* Germplasm Collection at the Newe Ya'ar Research Centre, Agricultural Research Organisation, Israel.

[a] In types: 'Mixture' means mixture between different melon types.

## Assessment of bacterial adhesion to/penetration of seeds

To evaluate the potential correlation between bacterial seed adhesion or penetration and cultivar/line susceptibility in the seed-transmission assays, 25 seeds of each of the selected cultivars/lines were inoculated as described for the seed-transmission assay. For selection purposes, M6 carrying pDSK519 ($Km^R$) was used. After inoculation, bacterial suspensions were poured through a 1 mm mesh screen and seeds were rinsed for 30 s with SDW. Seeds were then divided into five groups of five seeds each, and each group was ground separately by mortar and pestle with

1.5 ml of SDW. The ground suspensions were transferred to 2 ml Eppendorf tubes and centrifuged at 100 g for 2 min to pellet the seed debris. Supernatant was transferred to a new tube and centrifuged at 2,000 g for 10 min. Bacterial pellets were resuspended in SDW, serially diluted and plated on NA plates with 50 μg $ml^{-1}$ Km to determine number of cfu per seed. Additional assays were performed in the same manner using cv. OPHIR to assess differences in seed adhesion/penetration ability between group I and II *A. avenae* subsp. *citrulli* strains. Strains M1, M3, M5 and M6 (group I), and W2, W3, W4 and W5 (group II), all carrying plasmid pDSK519, were used. All experiments were carried out twice and were analysed by Tukey–Kramer HSD test using the JMP software.

### Seedling-inoculation assays

Melon cultivars/lines were further examined by seedling inoculation. Fifteen seeds of each cultivar/line were sown in propagation trays containing 20 pots (6×6× 7 cm; one seed per pot) filled with the same soil mixture used for the seed-transmission assays. Seeds of each cultivar/line were planted in groups of three in five different rows. Seedlings were grown in a greenhouse (25–28°C) for 10–14 days, and then sprayed until runoff with a ~$10^6$ cfu $ml^{-1}$ suspension of strain M6. Following inoculation, seedlings were kept on a greenhouse table covered with plastic curtains. After the first 4 h, a fogger system was activated for 8 s at 30 min intervals during the daylight hours (10 h). The high-humidity regime was maintained for 48 h. The plastic curtains were then removed and seedlings were allowed to grow for another 7–10 days. Disease severity was assessed on a scale of 1 to 9 (Hopkins and Thompson 2002): 1, no symptoms; 2, a few small, necrotic lesions on the cotyledons; 3, small, necrotic lesions on <20% of the cotyledon; 4, small, necrotic lesions on >20% of the cotyledon; 5, necrotic lesions with chlorosis on 20% to 50% of the cotyledon; 6, necrotic lesions on 20 to 50% of the cotyledon with restricted lesions on the true leaf; 7, large spreading necrotic lesions on >50% of the cotyledon with restricted lesions on true leaves; 8, large spreading necrotic lesions on >50% of the cotyledon with lesions and chlorosis on the true leaves; 9, >90% necrosis of the cotyledon and large spreading lesions on the true leaves, or plant dead. Selected cultivars/lines were further assayed in a second experiment that was carried out similarly, but with 30–40 seedlings per cultivar/line. The two experiments were analysed separately by Tukey–Kramer HSD test using the JMP software.

### Greenhouse-inoculation experiment with mature plants

Eight commercial cultivars/breeding lines were selected for an inoculation experiment in which plants were grown, according to standard agricultural practice, to fruit maturation. Eighty to one hundred seeds of each cultivar/line were sown at Hishtil Nurseries (Ashkelon, Israel) and grown until the second true leaf stage. Transplants were then transferred to a greenhouse where they were planted in 10 l pots filled with a commercial soil mixture containing 50% peat, 30% tuff, 20% compost and 2 kg $m^{-3}$ Osmocote (Shacham Givat Ada, Givat Ada, Israel). The experiment was organised in five randomised blocks, with each block containing 10 to 20 plants per cultivar/line. The greenhouse temperature ranged from 15°C on cold nights to 37°C on hot days. Plants were inoculated at three different time points: one week after seedlings were transplanted into the greenhouse (5 weeks after sowing); at the beginning of flowering (9 weeks after sowing); and after fruit set (13 weeks after sowing). Symptom severity on plant foliage was assessed 10 days after inoculation (d.a.i.). For the first inoculation, the 1 to 9 scale described above for seedling-inoculation experiments (Hopkins and Thompson 2002) was used. For the second inoculation, a scale based on percentage of symptomatic leaves was used. This scale ranged from 1 to 6 as follows: 1, 10% or less symptomatic leaves; 2 to 5, 11–25%, 26–50%, 51–75% and 76–90% symptomatic leaves, respectively; 6, >90% symptomatic leaves. Fruit was harvested close to maturation. Fruits were evaluated by observation of visible BFB symptoms, and by assessing the percentage of fruits containing infected seeds, which was determined by sowing ~20 seeds per fruit (as described above for seed-transmission assays) and assessing seedlings showing typical blight symptoms.

## Results

### Seed-transmission assays

Two experiments were carried out to assess tolerance/susceptibility of melon commercial cultivars/breeding

lines. The results of both experiments were similar and effects of the experiments on BFB severity were not significant ($P$=0.34); data from the two experiments were therefore pooled for one analysis. Results showed significantly high variability ($P$=0.05) among the different cultivars/lines in their sensitivity to *A. avenae* subsp. *citrulli*, as determined by disease-severity values (Table 2). In both experiments, cvs 6407 and ADIR339 were numerically the most tolerant, although the ratings were not significantly different from some of the other lines. Cultivars 6812 and OPHIR, appeared to be the most susceptible, although they did not differ significantly from some of the other lines.

Two seed-transmission assays were performed to assess differential responses of wild melon lines to *A. avenae* subsp. *citrulli*. In contrast to the assays with commercial cultivars/breeding lines, these experiments differed significantly ($P$=0.0025), and were therefore analysed separately. Differences in light conditions between the two experiments (the first experiment was conducted in the autumn, when skies were generally overcast, whereas the second experiment was conducted in the spring, with most days being sunny) could have contributed to the observed differences between the experiments. A probable effect of ambient conditions on cultivar tolerance to BFB has been previously suggested for watermelon (Hopkins and Thompson 2002).

**Table 2** Seed-transmission assays of commercial melon cultivars/breeding lines inoculated with *Acidovorax avenae* subsp. *citrulli* strain M6 at ~$3\times10^6$ cfu $ml^{-1}$

| Cultivar | Disease severity[a] | Statistical significance[b] |
|---|---|---|
| 6812 | 6.5 | A |
| OPHIR | 6.3 | AB |
| 6813 | 5.9 | ABC |
| OR-027 | 5.1 | ABCD |
| 6801 | 4.8 | ABCDE |
| 6802 | 4.7 | BCDE |
| MG-10267 | 4.4 | CDE |
| IDEAL | 3.9 | DE |
| 6405 | 3.9 | DEF |
| 6815 | 3.8 | DEFG |
| 350 | 3.7 | DEF |
| MG-10183 | 3.4 | EFG |
| YC-17016 | 3.0 | EFG |
| ADIR339 | 2.0 | FG |
| 6407 | 2.0 | FG |

[a] Disease severity scale based on shoot weight of inoculated seedlings as compared with non-inoculated controls: 0, seedling weight > 90% of control seedling average weight; 1 to 5, seedling weight is equal to 76–90%, 61–75%, 46–60%, 31–45% and 16–30% of control seedling average weight, respectively; 6, seedling weight is equal or lower than 15% of control seedling average weight; 7, seedling dead. Results represent averages from two experiments that did not differ statistically ($P$=0.34).

[b] Cultivars/lines not connected by the same letter are significantly ($P$=0.05) different according to Tukey–Kramer HSD test.

Significant differences ($P$=0.05) in tolerance/susceptibility were observed among the tested wild lines, particularly in the first experiment. In the second experiment, disease-severity values were collectively much lower than in the first, and less significant differences were observed among lines (Table 3). Despite the differences between the two experiments, some consistent trends were observed, especially among the most tolerant and the most susceptible lines. For instance, lines BLN-B and EAD-B, although not differing significantly from most of the other lines, were numerically among the three most tolerant lines in both experiments. In contrast, lines BDR-B and BKU-B were numerically among the three most susceptible lines in both experiments and differed significantly ($P$=0.05) from lines BLN-B and EAD-B (Table 3).

## *Acidovorax avenae* subsp. *citrulli* adhesion to/penetration of melon seeds

Seed-adhesion/penetration assays were carried out to examine whether a correlation exists between the capability of *A. avenae* subsp. *citrulli* to adhere to or penetrate the seeds of different cultivars/lines and their susceptibility in seed transmission. Six cultivars/lines showing high susceptibility (6812, 6813 and OPHIR) or high tolerance (ADIR339, 6407 and 6405) in seed-transmission assays (Table 2) were selected for these experiments.

Following incubation of seeds with the pathogen and bacterial extraction, values of cfu per seed obtained from susceptible cultivars/lines were two to six times higher than those obtained from tolerant ones (Fig. 1). Differences among susceptible and tolerant cultivars/lines were statistically significant ($P$=0.05). These results suggest that the level of melon susceptibility to *A. avenae* subsp. *citrulli* at the earliest stage of infection is associated with the ability of the pathogen to adhere to or penetrate the seed.

**Table 3** Seed transmission assays of wild melon lines inoculated with *A. avenae* subsp. *citrulli* strain M6 at ~$3\times10^6$ cfu $ml^{-1}$

| Experiment I | | | Experiment II | | |
|---|---|---|---|---|---|
| Line | Disease severity[a] | Statistical significance[b] | Line | Disease severity[a] | Statistical significance[b] |
| BDR-B | 7.0 | A | BDR-B | 5.6 | A |
| BRO-B | 6.6 | A | BKU-B | 4.8 | A |
| BKU-B | 6.5 | A | ARJ-OP | 4.2 | A |
| BDG-B | 6.4 | A | AMBC-B | 4.2 | A |
| FRN-3 | 6.1 | A | BDG-B | 4.0 | A |
| ARJ-OP | 6.1 | AB | ARA-B | 3.8 | AB |
| BIGA-B | 6.1 | AB | ENQ-4 | 3.5 | ABC |
| AMBC-B | 5.8 | ABC | BIGA-B | 3.4 | ABC |
| GOK-1 | 5.7 | ABC | BLM-B | 3.2 | ABC |
| BAY-B | 5.2 | ABCD | BRO-B | 3.1 | ABC |
| ENQ-4 | 4.8 | ABCDE | FRN-3 | 3.1 | ABC |
| EMJ | 4.6 | ABCDEF | BAY-B | 3.0 | ABC |
| EAR1-B | 4.4 | ABCDEF | EAR1-B | 3.0 | ABC |
| BLM-B | 4.3 | ABCDEF | ANDb-B | 2.8 | ABC |
| BUL-B | 3.2 | BCDEF | EMJ | 2.5 | ABC |
| A-1-B | 3.1 | CDEF | GOK-1 | 2.5 | ABC |
| ANDb-B | 2.5 | DEF | BUL-B | 2.2 | ABC |
| EAD-B | 2.0 | EF | EAD-B | 1.1 | BC |
| ARA-B | 2.0 | EF | BLN-B | 1.1 | C |
| BLN-B | 1.8 | F | A-1-B | 1.0 | C |

[a] Disease-severity scale: see Table 2 for details.

[b] Lines not connected by the same letter in each experiment are significantly ($P$=0.05) different according to Tukey–Kramer HSD test.

To further address this question, we assessed differences among group I and group II strains (four strains from each group) for their ability to adhere to melon seeds. All group I strains (M strains) showed significantly ($P$=0.05) higher values of cfu per seed than group II (W) strains (Fig. 2). These results further support the strong correlation between seed adherence/penetration by the bacteria and susceptibility at the earliest stage of infection.

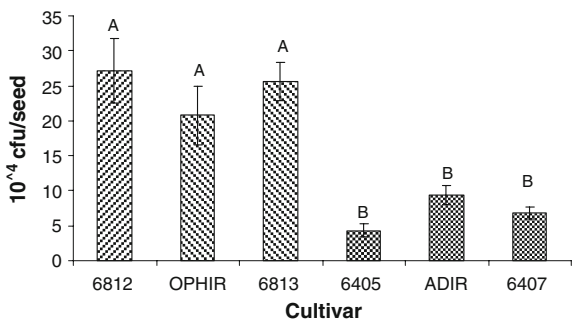


**Fig. 1** Extraction of *Acidovorax avenae* subsp. *citrulli* cells from inoculated seeds of different melon cultivars. Seeds of different cultivars were incubated with a $3\times10^6$ cfu $ml^{-1}$ suspension of strain M6 carrying pDSK519 for 2 h. After washing, seeds were grounded, and serial dilutions were plated on nutrient agar supplemented with 50 μg $ml^{-1}$ kanamycin for bacterial counts. Different letters indicate significant differences ($P$=0.05) between treatments by Tukey–Kramer HSD test. Results from one out of two experiments with similar results are shown

## Seedling inoculations of melon cultivars/breeding lines

To determine whether a genotype is tolerant or susceptible to a particular disease, its response to the pathogen needs to be assessed at different growth stages. Therefore, the melon cultivars/lines were also assessed for their tolerance/susceptibility to *A. avenae* subsp. *citrulli* by seedling-inoculation experiments. Two week-old seedlings were inoculated with strain M6 at ~$10^6$ cfu $ml^{-1}$ by spray inoculation. Disease severity was assessed 9 to 12 d.a.i. To better illustrate the results, a photographed description of the used scale (Hopkins and Thompson 2002) is shown in Fig. 3.

The first experiment involved 12 commercial cultivars/breeding lines. Disease-severity ratings ranged from 3.1 for ADIR339 to 7.0 for YC-17016,

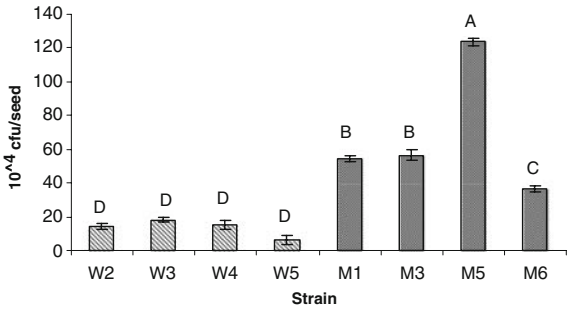


**Fig. 2** Extraction of *A. avenae* subsp. *citrulli* cells from inoculated melon (cv. OPHIR) seeds. Seeds were incubated with $3\times10^6$ cfu $ml^{-1}$ suspensions of group I (M) and II (W) strains carrying pDSK519 for 2 h. After washing, seeds were grounded, and serial dilutions were plated on nutrient agar supplemented with 50 μg $ml^{-1}$ kanamycin for bacterial counts. Different letters indicate significant differences ($P$=0.05) between treatments by Tukey–Kramer HSD test. Results from one out of two experiments with similar results are shown

that were numerically the most tolerant and susceptible cultivars/lines, respectively (Table 4). The three most tolerant cultivars/lines, ADIR339, 6812 and OR-027, differed significantly ($P$=0.05) from the two most susceptible ones, YC-17016 and 6813. In the second experiment, which included eight selected cultivars/lines, a lower variation in disease severity was observed relative to the first experiment. In fact, the only significant ($P$=0.05) differences were between the two most tolerant cultivars/lines and the most susceptible one. Disease-severity scores ranged from 5.3 for ADIR339 (numerically the most tolerant, as in the first experiment) to 8.2 for MG-10267 (Table 4).

A correlation was found for a few commercial cultivars/breeding lines between their tolerance/susceptibility in seed-transmission and seedling-inoculation assays. The clearest example was cv. ADIR339, which was consistently ranked among the most tolerant cultivars/lines in both assays. Most cultivars/lines, however, showed a relatively high susceptibility or tolerance in one of the assays and an intermediate level in the other. For instance, cv. OPHIR was highly susceptible in seed-transmission assays (Table 2 and data not shown); however, this cultivar, although clearly susceptible to the pathogen, was not among the most susceptible lines following seedling inoculation (Table 4). An extreme case is illustrated by line YC-17016, which was shown to be relatively tolerant to the bacterium in seed-transmission assays (Table 2) but highly susceptible in seedling inoculation (Table 4).

The same pattern of seedling inoculation was used to evaluate wild melon lines, in two experiments. In the first experiment, the disease severity range (3.7–7.2; Table 5) was similar to that of the commercial cultivars/breeding lines (3.1–7.0; Table 4), but no significant differences were observed among the different lines. In both experiments, most wild lines showed a wider distribution of disease scores than commercial cultivars/breeding lines. Moreover, for some wild lines, disease severity of the replicates varied from as low as 1 to as high as 9. Nevertheless, in the second experiment, which included selected wild lines with more replicates than in the first experiment, significant differences were observed. Lines EAR1-B and ANDb-B were numerically the most tolerant, although they only differed significantly ($P$=0.05) from the two most susceptible lines, ARA-B and A-1-B.

No correlations were found between seed-transmission and seedling-inoculation results of wild melon lines. These lines followed the dominant pattern described above for commercial cultivars/breeding lines, in which lines that were highly susceptible or tolerant in one of the assays exhibited an intermediate rank in the other.

## Greenhouse-inoculation experiment with mature plants

A greenhouse experiment to assess tolerance/susceptibility of selected cultivars/lines grown to maturity was conducted. Plants were inoculated with strain M6 at three time points as described in ‘Materials and methods’.

Assessment of disease severity on foliage after the first inoculation revealed relatively weak infection of the plants. Disease scores ranged from 1.5 and 1.6 for YC-17016 and 6812, respectively, to 2.5 and 4.2 for OPHIR and MG-10267, respectively. The two most tolerant cultivars/lines differed significantly ($P$=0.05) from the two most sensitive ones (Table 6, first evaluation).

Disease appeared more severe after the second inoculation. Significant differences among cultivars/lines after this inoculation were generally in agreement with results following the first inoculation: YC-17016 and 6812 were again numerically the most tolerant cultivars/lines. Cultivar YC-17016 significantly ($P$=0.05) differed from the other lines in this evaluation. As in the first evaluation, OPHIR was numerically among the most susceptible cultivars/lines, although it did not differentiate significantly from most of the other lines (Table 6, second evaluation).

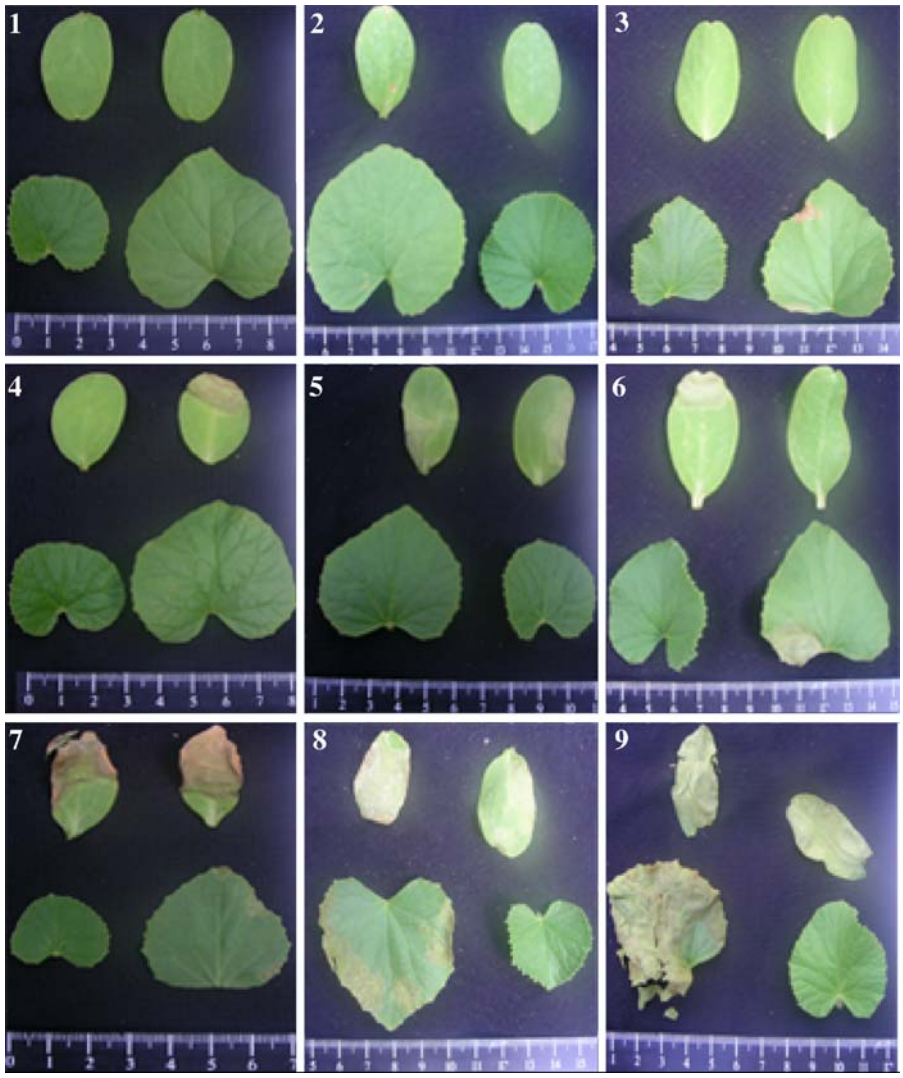


**Fig. 3** A photographed description of the 1 to 9 disease severity scale (Hopkins and Thompson 2002) used in seedling inoculation assays of melon with *A. avenae* subsp. *citrulli* strain M6. Pictures were taken 10 d.a.i.

No significant differences were observed among cultivars/lines in terms of disease parameters on the fruit. Symptomatic fruits were sporadically seen in all examined cultivars/lines: only ~2% of the harvested fruits showed typical BFB symptoms, thus making it difficult to quantitatively assess fruits affected by the disease. However, in all cultivars/lines, >50% of the fruits contained infected seeds (as observed by seed-transmission assays of extracted seeds), regardless of symptom appearance.

## Discussion

BFB is a threatening disease of cucurbits worldwide. The disease started to gain importance after severe outbreaks in watermelon fields in the USA during the late 1980s/early 1990s (Wall and Santos 1988; Wall et al. 1990; Somodi et al. 1991). Since then, BFB has been reported in other cucurbits, such as melon, pumpkin and cucumber, and has spread to many parts of the world, including the Americas, Asia, Europe, the Middle East and Australia (Isakeit et al. 1997, 1998; Langston et al. 1999; Martin and O'Brien 1999; O'Brien and Martin 1999; Schaad et al. 2003; Walcott et al. 2004; Burdman et al. 2005).

It is well accepted that an efficient means of coping with BFB is through disease resistance; however, to date there are no commercial cultivars of cucurbits resistant to the disease. Several screens for BFB resistance of watermelon have been performed (Sowell and Schaad 1979; Goth and Webb 1981;

**Table 4** Seedling inoculation of commercial melon cultivars/breeding lines inoculated with *A. avenae* subsp. *citrulli* strain M6 at ~$10^6$ cfu $ml^{-1}$

| Experiment I | | | Experiment II | | |
|---|---|---|---|---|---|
| Cultivar | Disease severity[a] | Statistical significance[b] | Cultivar | Disease severity[a] | Statistical significance[b] |
| YC-17016 | 7.0 | A | MG-10267 | 8.2 | A |
| 6813 | 6.8 | AB | YC-17016 | 6.8 | AB |
| MG-10183 | 5.7 | ABC | 6407 | 6.5 | AB |
| 6405 | 5.6 | ABC | 6813 | 6.3 | AB |
| 6815 | 5.4 | ABC | 6405 | 6.0 | AB |
| OPHIR | 5.3 | ABC | 6812 | 5.8 | AB |
| 6407 | 5.0 | ABCD | OPHIR | 5.4 | B |
| IDEAL | 4.8 | BCD | ADIR339 | 5.3 | B |
| MG-10267 | 4.7 | BCD | | | |
| OR-027 | 4.6 | CD | | | |
| 6812 | 4.4 | CD | | | |
| ADIR339 | 3.1 | D | | | |

[a] Disease-severity scale based on symptom appearance on cotyledons and true leaves as described by Hopkins and Thompson (2002) and illustrated in Fig. 3.

[b] Cultivars/lines not connected by the same letter in each experiment are significantly ($P$=0.05) different according to Tukey–Kramer HSD test.

**Table 5** Seedling inoculation of wild melon lines inoculated with *A. avenae* subsp. *citrulli* strain M6 at ~$10^6$ cfu $ml^{-1}$

| Experiment I | | | Experiment II | | |
|---|---|---|---|---|---|
| Line | Disease severity[a] | Statistical significance[b] | Line | Disease severity[a] | Statistical significance[b] |
| GOK-1 | 7.2 | A | ARA-B | 5.0 | A |
| ARA-B | 6.3 | A | A-1-B | 4.6 | AB |
| BUL-B | 6.2 | A | EMJ | 4.2 | ABC |
| BLN-B | 6.1 | A | BUL-B | 3.5 | ABCD |
| FRN-3 | 6.0 | A | BLN-B | 3.5 | ABCD |
| ARJ-OP | 5.8 | A | EAD-B | 3.5 | ABCD |
| BDG-B | 5.6 | A | BLM-B | 3.4 | BCD |
| ANDb-B | 5.3 | A | ENQ-4 | 3.2 | BCD |
| EMJ | 5.3 | A | ANDb-B | 3.0 | CD |
| BKU-B | 5.2 | A | EAR1-B | 2.2 | D |
| ENQ-4 | 5.0 | A | | | |
| A-1-B | 4.9 | A | | | |
| EAR1-B | 4.6 | A | | | |
| EAD-B | 4.5 | A | | | |
| AMBC-B | 4.5 | A | | | |
| BAY-B | 4.3 | A | | | |
| BDR-B | 4.1 | A | | | |
| BRO-B | 4.0 | A | | | |
| BIGA-B | 3.8 | A | | | |
| BLM-B | 3.7 | A | | | |

[a] Disease-severity scale based on symptom appearance on cotyledons and true leaves as described by Hopkins and Thompson (2002) and illustrated in Fig. 3.

[b] Lines not connected by the same letter in each experiment are significantly ($P$=0.05) different according to Tukey–Kramer HSD test.

**Table 6** Foliar evaluation of disease severity from the greenhouse experiment (mature plants until harvest)

| First evaluation[a] | | | Second evaluation[b] | | |
|---|---|---|---|---|---|
| Cultivar | Disease severity | Statistical significance[c] | Cultivar | Disease severity | Statistical significance[c] |
| MG-10267 | 4.2 | A | 6813 | 3.4 | A |
| OPHIR | 2.5 | B | OPHIR | 3.3 | A |
| 6813 | 2.4 | BC | 6407 | 3.3 | A |
| 6405 | 2.1 | BC | 6405 | 3.1 | A |
| 6407 | 2.0 | BC | ADIR339 | 3.0 | AB |
| ADIR339 | 1.9 | BC | MG-10267 | 2.8 | AB |
| 6812 | 1.6 | C | 6812 | 2.5 | B |
| YC-17016 | 1.5 | C | YC-17016 | 1.9 | C |

[a] Evaluation of disease severity 10 days after the first inoculation, conducted one week after plantlets were transferred to the greenhouse. Disease-severity scale was based on symptom appearance on cotyledons and true leaves, as described by Hopkins and Thompson (2002) and illustrated in Fig. 3.

[b] Evaluation of disease severity 10 days after the second inoculation, conducted at the beginning of flowering (9 weeks after sowing). Disease-severity scale was based on percentage of symptomatic leaves on the plant: 1, 10% or less symptomatic leaves; 2 to 5: 11–25%, 26–50%, 51–75% and 76–90% symptomatic leaves, respectively; 6, >90% symptomatic leaves.

[c] Cultivars/lines not connected by the same letter in each evaluation are significantly ($P$=0.05) different according to Tukey–Kramer HSD test.

Hopkins et al. 1993; Hopkins and Thompson 2002). However, to the best of our knowledge, there are no published studies assessing melon cultivars for BFB resistance or tolerance.

One of the obstacles of BFB-resistance screens is the fact that the plant is highly susceptible at both the earliest and latest stages of growth (young seedlings and fruits, respectively). From an economic point of view, fruits are the most crucial organ affected by the disease; however, the seedborne nature of *A. avenae* subsp. *citrulli* often leads to seedling blight in the nursery or in the field (Webb and Goth 1965; Latin and Hopkins 1995). Occurrence of a few symptomatic seedlings in the nursery leads to the destruction of all seedlings from that lot (often hundreds of thousands) to avoid the risk of transplanting symptomless infected seedlings in the field. This, along with the fact that the vegetative parts of the plant serve as an inoculum reservoir for further fruit infection, make screens based on seed transmission and seedling inoculation as relevant as fruit assays, even if the results of the former do not fully correlate with the latter. Therefore, in this study we used several inoculation techniques, applied at the different growth stages of the plant at which *A. avenae* subsp. *citrulli* infection occurs naturally. The rationale was that by combining data from different techniques, we would be able to more reliably determine the nature of a certain cultivar response to BFB.

In seed-transmission assays of both commercial cultivars/breeding lines and wild lines of melon, significant differences were observed among cultivars/lines. Apart from their genetic differences, this may be attributed to the selection of an appropriate scale to assess disease severity. Instead of using a small-range, subjective scale based on a qualitative evaluation of symptoms, we used a wide-range scale, which is also based on quantitative measures (seedling shoot weight), in order to make the evaluation more objective. Furthermore, in these assays, a high level of consistency was found for the most tolerant cultivars/lines (cvs 6407 and ADIR339, and wild lines BLN-B and EAD-B), as well as for the most susceptible ones (cvs 6812 and OPHIR, and wild lines BDR-B and BKU-B), among the different experiments, thus supporting the accuracy and reproducibility of this assay.

Since disease severity is often related to the initial bacterial inoculum, we examined whether the amount of *A. avenae* subsp. *citrulli* present in melon seeds after inoculation correlates with disease severity. Indeed, a positive correlation was found between the amount of bacteria present in the seeds of the tested cultivars/lines and their susceptibility at early stages of growth. On the one hand, these results have an important, although not unexpected implication for growers and seed companies—the higher the number of contaminated seeds, the higher the possibility of

vast seedling blight in the field. On the other hand, this correlation may be problematic for resistance/tolerance screens based solely on this kind of assay, as it implies that disease severity of a cultivar may be, at least in part, a consequence of the amount of bacteria that can adhere to and/or penetrate the seed: this is more likely to be a result of seed-coat structure and composition, rather than a reflection of purely tolerance/resistance-related genetic traits. Nevertheless, this feature is important because from a practical point of view, reduced seed adhesion/penetration by the pathogen will lead to a reduced disease rate, especially under conditions that are less than optimal for BFB development.

Group I strains have been shown to be generally more virulent than group II strains in seed-transmission assays with different cucurbit hosts, including watermelon (Burdman et al. 2005). Based on this, and on results from the bacterial seed-adhesion/penetration assays on tolerant and susceptible cultivars/lines, we further hypothesized that differences in aggressiveness between the two types of strains in seed transmission may be due to their differential ability to adhere to the seed coat and/or to penetrate the seeds. Indeed, we found that significantly higher amounts of bacteria can be extracted from melon seeds incubated with group I strains relative to group II strains. In contrast to group I strains, which are highly aggressive in seed-transmission assays on melon, all tested group II strains failed to induce clear disease symptoms in seed-transmission assays with susceptible melon cultivars (not shown), thus supporting a high correlation between seed adherence and/or penetration and disease severity.

In previous work, a comparison of several strains revealed that group I strains possess significantly higher motility than group II strains (Burdman et al. 2005). In agreement with this, transmission electron microscopy revealed a much higher abundance of polar flagella attached to group I than group II strains (O. Bahar and S. Burdman, unpublished results). While it is not likely that motility per se confers an advantage to the bacteria in terms of seed adherence under the experimental conditions used in the reported assays (characterised by high bacterial concentration and shaking), it could contribute to bacterial penetration into the seeds. The seed assays conducted in this study did not discriminate between bacteria adhering to the seed coat and those penetrating the seeds. Evidence that *A. avenae* subsp. *citrulli* can penetrate cucurbit seeds has been provided (Rane and Latin 1992). However, polar flagella could also contribute to a stronger attachment to the seed coat, as it is well-established that bacterial flagella play an important role in the attachment and adhesion of several bacteria (Moens and Vanderleyden 1996). Therefore, the role of the polar flagella, as well as of other bacterial extracellular components, in seed infestation warrants further investigation.

Seedling inoculation is the most commonly used assay for the assessment of BFB resistance (Sowell and Schaad 1979; Goth and Webb 1981; Hopkins et al. 1993; Hopkins and Thompson 2002; Walcott et al. 2003). It does not take up too much space and it does not take too much time for completion. As in seed-transmission assays, seedling inoculations revealed significant differences among the different melon cultivars/lines. However, results from different experiments were less reproducible than with the seed-transmission assays, as reflected by the distribution of most cultivars/lines in two independent experiments. Nevertheless, some commercial cultivars/breeding lines, mainly those that were distributed at the top or bottom of the disease-severity scale, did behave similarly in the different experiments, implying that their scores are reliable.

Seedling-inoculation experiments involving wild melon lines showed more inconsistencies than those with commercial cultivars/breeding lines. It is likely that the high variability in disease severity observed among replicates in these assays was due to the relatively high genetic heterogeneity within the wild lines compared to commercial cultivars/breeding lines. This phenomenon was also observed in watermelon screens performed by Hopkins and Thompson (2002). In those screens, as in our study, disease severity of seedling inoculations varied from 1 to 9 among replicates of some of the plant introductions.

In the experiment involving inoculation of mature plants, disease evaluation of the foliage following two inoculations showed very similar patterns, especially for those cultivars/lines that were located at the top or bottom of the disease-severity scale. Symptomatic fruits were very few in number and appeared sporadically throughout the greenhouse. Therefore, we were unable to detect differences in fruit susceptibility among the examined cultivars/lines. It is possible that the low relative humidity limited the development of visible symptoms in fruits. However,

seed-transmission assays with collected seeds revealed that most fruits, although symptomless, were infected by the pathogen. The pathogen was isolated from the resulting seedlings and confirmed by PCR (not shown). These results are in agreement with a previous study in which directly inoculated watermelon blossoms yielded symptomless fruits bearing infected seeds (Walcott et al. 2003). They also emphasise the difficulties encountered by seed companies in producing *A. avenae* subsp. *citrulli*-free seeds, especially under conditions that do not favour symptom development, which cannot ensure that the seeds are not infected.

Altogether, none of the tested cultivars/lines was found to be resistant to *A. avenae* subsp. *citrulli*, but the level of susceptibility among them varied in the different assays. Several trends could be observed for some cultivars/lines: for instance, OPHIR was among the most susceptible cultivars/lines in seed-transmission assays, it was moderately susceptible in the seedling inoculations, and was among the cultivars/lines showing the highest disease-severity scores in the greenhouse experiment. In contrast, the combined data from the three assays revealed that cv. ADIR339 possesses a relatively high degree of tolerance to BFB. As stated above, most cultivars/lines were shown to be relatively tolerant or susceptible in some of the assays, and to exhibit an intermediate rank in others. Moreover, a few cultivars/lines showed opposite patterns in the different assays. For example, YC-17016 was relatively tolerant in seed-transmission assays and was the most tolerant cultivar/line in the greenhouse experiment. However, it was among the most susceptible cultivars/lines in seedling inoculations. These results emphasise the limitations of screening for tolerance against pathogens that affect different plant organs at different growth stages, as is the case for BFB. A classical example of this limitation can be found in the screening for early blight of potato (*Phytophthora infestans*), as it is known that many cultivars that are highly resistant in the foliage are highly susceptible in the tuber, and vice versa (Kadish et al. 1990). To these limitations, one can add the effects of ambient conditions on the response to BFB (Hopkins and Thompson 2002), and the high genetic variability within *A. avenae* subsp. *citrulli* (Walcott et al. 2000, 2004; Burdman et al. 2005).

It is important to mention that despite the economic importance of the disease, very little is known about basic aspects of BFB pathogenesis. In this regard, the recent release of the genome sequence of an *A. avenae* subsp. *citrulli* strain (AAC00-1; http://genome.jgi-psf.org/finished_microbes/aciav/aciav.home.html) represents a significant contribution to the investigation of these aspects. Sequence analysis has revealed the presence of several genes encoding a typical Hrp (hypersensitive response and pathogenicity)-type III secretion system and putative avirulence (Avr) proteins. As for most plant-pathogenic Gram-negative bacteria, it has been shown that mutation of *hrp* genes in group I and II *A. avenae* subsp. *citrulli* strains leads to the bacterium's inability to cause disease on susceptible hosts, and to elicit a hypersensitive response (HR) in non-host plants such as tomato and tobacco (O. Bahar & S. Burdman, unpublished results; and R. Walcott, personal communication). It is expected that the availability of the *A. avenae* subsp. *citrulli* genome sequence together with established experimental techniques will contribute to the attempts to screen for and develop BFB resistance in cucurbits.

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## References

Bahar, O., Efrat, M., Hadar, E., Dutta, B., Walcott, R. R., & Burdman, S. (2008). New subspecies-specific polymerase chain reaction-based assay for the detection of *Acidovorax avenae* subsp. *citrulli*. *Plant Pathology* doi:10.1111/j.1365-3059.2008.01828.x.

Burdman, S., Kots, N., Kritzman, G., & Kopelowitz, J. (2005). Molecular, physiological, and host-range characterization of *Acidovorax avenae* subsp *citrulli* isolates from watermelon and melon in Israel. *Plant Disease*, *89*, 1339–1347. doi:10.1094/PD-89-1339.

Goth, R. W., & Webb, R. E. (1981). Resistance of commercial watermelon (*Citrullus lanatus*) to *Pseudomonas pseudoalcaligenes* subsp. *citrulli*. *Plant Disease*, *65*, 671–672.

Hopkins, D. L. (1991). Control of bacterial fruit blotch of watermelon with cupric hydroxide. *Phytopathology*, *81*, 1228.

Hopkins, D. L. (1995). Copper-containing fungicides reduce the spread of bacterial fruit blotch of watermelon in the greenhouse. *Phytopathology*, *85*, 510.

Hopkins, D. L., & Thompson, C. M. (2002). Evaluation of *Citrullus* sp. germ plasm for resistance to *Acidovorax avenae* subsp. *citrulli*. *Plant Disease*, *86*, 61–64. doi:10.1094/PDIS.2002.86.1.61.

Hopkins, D. L., Thompson, C. M., & Elmstrom, G. W. (1993). Resistance of watermelon seedlings and fruit to the fruit blotch bacterium. *HortScience*, *28*, 122–123.

Hopkins, D. L., Thompson, C. M., Hilgren, J., & Lovic, B. (2003). Wet seed treatment with peroxyacetic acid for the control of bacterial fruit blotch and other seedborne diseases of watermelon. *Plant Disease*, *87*, 1495–1499. doi:10.1094/PDIS.2003.87.12.1495.

Isakeit, T., Black, M. C., Barnes, L. W., & Jones, J. B. (1997). First report of infection of honeydew with *Acidovorax avenae* subsp. *citrulli*. *Plant Disease*, *81*, 694. doi:10.1094/PDIS.1997.81.6.694C.

Isakeit, T., Black, M. C., & Jones, J. B. (1998). Natural infection of citronmelon with *Acidovorax avenae* subsp. *citrulli*. *Plant Disease*, *82*, 351–351. doi:10.1094/PDIS.1998.82.3.351D.

Kadish, D., Grinberger, M., & Cohen, Y. (1990). Fitness of metalaxyl-sensitive and metalaxyl resistant isolates of *Phytophthora infestans* in susceptible and resistant potato cultivers. *Phytopathology*, *80*, 200–206. doi:10.1094/Phyto-80-200.

Keen, N. T., Tamaki, S., Kobayashi, D., & Trollinger, D. (1988). Improved broad-host-range plasmids for DNA cloning in gram-negative bacteria. *Gene*, *70*, 191–197. doi:10.1016/0378-1119(88)90117-5.

Langston Jr, D. B., Walcott, R. R., Gitaitis, R. D., & Sanders Jr., F. H. (1999). First report of a fruit rot of pumpkin caused by *Acidovorax avenae* subsp. *citrulli* in Georgia. *Plant Disease*, *83*, 199. doi:10.1094/PDIS.1999.83.2.199B.

Latin, R. X., & Hopkins, D. L. (1995). Bacterial fruit blotch of watermelon: the hypothetical exam question becomes reality. *Plant Disease*, *79*, 761–765.

Martin, H. L., & O'Brien, R. G. (1999). First report of *Acidovorax avenae* subsp. *citrulli* as a pathogen of cucumber. *Plant Disease*, *83*, 965. doi:10.1094/PDIS.1999.83.10.965D.

Moens, S., & Vanderleyden, J. (1996). Functions of bacterial flagella. *Critical Reviews in Microbiology*, *22*, 67–100. doi:10.3109/10408419609106456.

O'Brien, R. G., & Martin, H. L. (1999). Bacterial blotch of melons caused by strains of *Acidovorax avenae* subsp *citrulli*. *Australian Journal of Experimental Agriculture*, *39*, 479–485. doi:10.1071/EA98172.

Rane, K. K., & Latin, R. X. (1992). Bacterial fruit blotch of watermelon: association of the pathogen with seed. *Plant Disease*, *76*, 509–512.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.

Schaad, N. W., Postnikova, E., & Randhawa, P. S. (2003). Emergence of *Acidovorax avenae* subsp. *citrulli* as a crop threatening disease of watermelon and melon. In N. S. Iacobellis, A. Collmer, S. W. Hutcheson, J. W. Mansfield, C. E. Morris, J. Murillo, N. W. Schaad, D. E. Stead, G. Surico, & M. S. Ullrich (Eds.), *Pseudomonas syringae and related pathogens* (pp. 573–581). Dordrecht: Kluwer.

Schaad, N. W., Sowell, G., Goth, R. W., Colwell, R. R., & Webb, R. E. (1978). *Pseudomonas pseudoalcaligenes* subsp. *citrulli* subsp-Nov. *International Journal of Systematic Bacteriology*, *28*, 117–125.

Somodi, G. C., Jones, J. B., Hopkins, D. L., Stall, R. E., Kucharek, T. A., Hodge, N. C., et al. (1991). Occurrence of a bacterial watermelon fruit blotch in Florida. *Plant Disease*, *75*, 1053–1056.

Sowell, G., & Schaad, N. W. (1979). *Pseudomonas pseudoalcaligenes* subsp. *citrulli* on watermelon: seed transmission and resistance of plant introductions. *Plant Disease Reporter*, *63*, 437–441.

Walcott, R. R., Castro, A. C., Fessehaie, A., & Ling, K. (2006). Progress towards a commercial PCR-based assay for *Acidovorax avenae* subsp. *citrulli*. *Seed Science and Technology*, *34*, 101–116.

Walcott, R. R., Fessehaie, A., & Castro, A. C. (2004). Differences in pathogenicity between two genetically distinct groups of *Acidovorax avenae* subsp. *citrulli* on cucurbit hosts. *Journal of Phytopathology*, *152*, 277–285. doi:10.1111/j.1439-0434.2004.00841.x.

Walcott, R. R., Gitaitis, R., & Castro, A. C. (2003). Role of blossoms in watermelon seed infestation by *Acidovorax avenae* subsp. *citrulli*. *Phytopathology*, *93*, 528–534. doi:10.1094/PHYTO.2003.93.5.528.

Walcott, R. R., Langston Jr, D. B., Sanders Jr, F. H., & Gitaitis, R. D. (2000). Investigating intraspecific variation of *Acidovorax avenae* subsp. *citrulli* using DNA fingerprinting and whole cell fatty acid analysis. *Phytopathology*, *90*, 191–196. doi:10.1094/PHYTO.2000.90.2.191.

Wall, G. C., & Santos, V. M. (1988). A new bacterial disease on watermelon in the Mariana Islands. *Phytopathology*, *78*, 1605.

Wall, G. C., Santos, V. M., Cruz, F. J., Nelson, D. A., & Cabrera, I. (1990). Outbreak of watermelon fruit blotch in the Mariana Islands. *Plant Disease*, *74*, 80. doi:10.1094/PD-74-0080D.

Webb, R. E., & Goth, R. W. (1965). A seedborne bacterium isolated from watermelon. *Plant Disease Reporter*, *49*, 818–821.

Willems, A., Goor, M., Thielemans, S., Gillis, M., Kersters, K., & De Ley, J. (1992). Transfer of several phytopathogenic *Pseudomonas* species to *Acidovorax* as *Acidovorax avenae* subsp. *avenae* subsp. nov., comb. nov., *Acidovorax avenae* subsp. *citrulli*, *Acidovorax avenae* subsp. *cattleyae*, and *Acidovorax konjaci*. *International Journal of Systematic Bacteriology*, *42*, 107–119.