Genetically Distinct *Acidovorax citrulli* Strains Display Cucurbit Fruit Preference Under Field Conditions

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ABSTRACT

Strains of *Acidovorax citrulli*, the causal agent of bacterial fruit blotch (BFB) of cucurbits, can be assigned to two groups, I and II. The natural association of group I and II strains with different cucurbit species suggests host preference; however, there are no direct data to support this hypothesis under field conditions. Hence, the objective of this study was to assess differences in the prevalence of group I and II *A. citrulli* strains on cucurbit species in the field. From 2017 to 2019, we used group I and II strains to initiate BFB outbreaks in field plots planted with four cucurbit species. At different times, we collected symptomatic tissues and assayed them for group I and II strains using a group-specific PCR assay. Binary distribution data analysis revealed that the odds of melon, pumpkin, and squash foliage infection by group

I strains were 21.7, 11.5, and 22.1 times greater, respectively, than the odds of watermelon foliage infection by the group I strain ($P < 0.0001$). More strikingly, the odds of melon fruit infection by the group I strain were 97.5 times greater than watermelon fruit infection by the same strain ($P < 0.0001$). Unexpectedly, some of the group II isolates recovered from the 2017 and 2019 studies were different from the group II strains used as inocula. Overall, data from these experiments confirm that *A. citrulli* strains exhibit a preference for watermelon and melon, which is more pronounced in fruit tissues.

Keywords: *Acidovorax citrulli*, bacteriology, host preference, watermelon fruit blotch

Bacterial fruit blotch (BFB) is an economically important seedborne and seed-transmitted disease of cucurbitaceous plant species including watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) and melon (*Cucumis melo* L.). *Acidovorax citrulli*, the causal agent of BFB, was first isolated by Webb and Goth in 1965 at the Regional Plant Introduction Station in Griffin, Georgia, U.S.A. (Webb and Goth 1965). However, it was not until 1987 that the first natural BFB outbreak occurred in the Mariana Islands on watermelon (Wall and Santos 1988). In 1989 the disease was observed in commercial watermelon fields in Florida (Somodi et al. 1991) and Indiana (Latin and Rane 1990). Subsequently, BFB outbreaks have occurred in many regions around the world, primarily on watermelon and melon crops, resulting in significant economic losses (Burdman and Walcott 2012).

A. citrulli can survive to varying degrees on plant tissues and substrates, e.g., weeds, volunteer seeds, plant and fruit debris, and planting trays. In the greenhouse, high plant densities, high relative humidity, and overhead irrigation are highly conducive for the development and spread of BFB. Generally, cucurbit seedlings are inspected for BFB symptoms before transplanting to the field. However, symptomless but latently infected seedlings may escape detection and serve as inoculum sources in the field. In general, warm humid conditions favor BFB development under field conditions and prolonged leaf wetness, and overhead irrigation or

frequent rainfall promote pathogen dispersal and bacterial ingress into plant tissues via open stomata or wounds (Zhao and Walcott 2018).

BFB has a wide host range in the family Cucurbitaceae (Zhao and Walcott 2018). Watermelon and melon are highly susceptible, and as mentioned above, most BFB outbreaks have been reported on these species. However, other hosts include cucumber (*Cucumis sativus* L.) (Martin et al. 1999), grammar (*Cucurbita moschata*) (Martin and Horlock 2002), squash and pumpkin (*Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata*) (Langston et al. 1999), citron melon (*Citrullus lanatus* var. *citroides*) (Isakeit et al. 1998), prickly paddymelon (*Cucumis myriocarpus* subsp. *myriocarpus*) (O'Brien and Martin 1999), and several types of gourds.

A. citrulli strains can be divided into two major groups, I and II, based on carbon substrate utilization (Walcott et al. 2004), DNA fingerprint analyses by pulse field gel electrophoresis (PFGE) (Burdman et al. 2005; Walcott et al. 2000; Yan et al. 2013), repetitive extragenic palindromic-polymerase chain reaction (rep-PCR) (Walcott et al. 2004), multilocus sequence analysis (MLSA) (Feng et al. 2009; Yan et al. 2013), and fatty acid methyl ester analysis (Walcott et al. 2000). The diversity of *A. citrulli* was initially reported in an Australian population by O'Brien and Martin (1999). Carbon substrate utilization profiles and pathogenicity assays were employed to characterize strains recovered from several cucurbit hosts including watermelon, rockmelon, honeydew (*Cucumis melo* var. *indorus* Jacq.), and prickly paddymelon. Carbon substrate utilization profile analysis separated the strains into two groups. One group recovered from rockmelon and honeydew failed to utilize L-leucine, but utilized 2-amino ethanol. The other group, recovered from watermelon, utilized L-leucine, but were unable to utilize 2-amino ethanol.

Walcott et al. (2000) first reported two genetically distinct groups of *A. citrulli* among 120 strains collected from a broad range of geographical origins and a range of cucurbitaceous plants. In 2005,

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Yan et al. (2017) recently developed a detached melon fruit assay that clearly distinguished representative group I and II *A. citrulli* strains based on symptom development. Using this assay, they observed a consistent difference in pathogenicity between group I and II *A. citrulli* strains in the fruit (Yan et al. 2017). Based on these observations, we hypothesized that *A. citrulli* host preference manifests more strongly in cucurbit fruits than in foliar tissues. Consequently, the objective of this study was to assess differences in cucurbit host preference in foliar and fruit tissues between group I and II *A. citrulli* strains under field conditions.

Vegetable Park farm in Tifton, GA in 2017 (one plot), 2018 (two plots), and 2019 (two plots).

Development of a duplex PCR assay to rapidly distinguish group I and II *A. citrulli* strains. To rapidly detect the presence of *A. citrulli* in symptomatic plant tissues and to determine the group to which each strain belonged, we developed a duplex PCR assay with group-specific primer sets (Table 1). Primers for this assay were designed using the Primer 3 program (Kõressaar et al. 2018) based on genes *APS58_3303* (gene ID according to the M6 annotation; GenBank accession CP029373.1 (Yang et al. 2019a)) and *Aave_2708* (gene ID according to the AAC00-1 annotation; GenBank accession CP000512.1), which were present only in group I and II strains, respectively (Eckshtain-Levi et al. 2014; Jiménez Guerrero et al. 2019). PCR was conducted using *OneTaq* DNA polymerase according to the manufacturer's instructions (New England Biolabs Inc., Ipswich, MA). The sensitivity of the duplex PCR assay was evaluated using DNA from 10-fold serial dilutions of boiled cells of *A. citrulli* group I strain AAC203-1RR1 and group II strain AAC94-21SR1. The specificity of the PCR assay was evaluated using DNA from boiled cells (10^8 CFU/ml) of group I ($n = 9$) and group II ($n = 10$) *A. citrulli* strains (Table 2). Cell suspensions of each strain were generated by suspending bacterial cells from overnight cultures in sdH₂O. The concentration of each cell suspension was adjusted to OD₆₀₀ = 0.3 ($\sim 10^8$ CFU/ml) and a 1-ml aliquot was lysed by incubating at 100°C for 10 min to release genomic DNA. Samples of 2.5 µl of lysed cells were used as templates for PCR assays with the following thermal profile: 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 68°C for 30 s; and then a final extension of 68°C for 5 min. Products were separated by electrophoresis on 1% agarose gels stained with ethidium bromide (0.5 µl /ml) and in 1× Tris-acetate-EDTA buffer for 30 min at 120 V.

DNA fingerprinting of representative field isolates. Bacterial isolation was performed from selected leaf and fruit tissues on King's B medium (King et al. 1954) to confirm the presence and viability of the *A. citrulli* isolates detected by PCR. To test if the group I and II *A. citrulli* isolates causing BFB symptoms in the field were identical to those used for inoculation, DNA restriction digestion with *SpeI* (NEB) followed by PFGE was conducted on representative field isolates (Table 3) from 2017 ($n = 8$), 2018 ($n = 6$), and 2019 ($n = 8$) as previously described (Walcott et al. 2000).

Statistical analysis. For each cucurbit species, the data were expressed as the presence of group I and II *A. citrulli* members detected from symptomatic foliar and fruit lesions. We used the GLIMMIX (SAS V9.4; SAS Institute, Cary, NC) procedure on the leaf and fruit tissue types for binary distribution data, and the odds ratios were calculated using a logit link function. As BFB-infected squash fruits were detected only in 1 year, data from watermelon and melon fruits were used for comparisons at the fruit tissue type level.

RESULTS

Development of a duplex PCR assay to distinguish group I and II *A. citrulli* strains. We developed a new duplex PCR assay to distinguish the two groups of *A. citrulli* in a single reaction. The detection thresholds of the group I- and II-specific PCR assays based on primer sets, 550-34F/550-626R and 2708-225F/2708-433R were 10^7 and 10^6 CFU/ml, respectively (Fig. 1). With regards to specificity, the group I primer set amplified DNA from all nine group I strains tested, but yielded no amplicons with DNA from boiled cells from 10 group II strains (Table 2). In contrast, the group II primer set amplified DNA from all 10 group II strains tested, but yielded no amplicon with DNA from the nine group I strains (Table 2).

TABLE 1. List of primers used in this study

Primer designation	Sequence (5' – 3')	Group	Target gene	Amplicon size (bp)
550-34F	TCATCGCCAACCGTTACACA	I	<i>APS58_3303</i> ^a	593
550-626R	CTCCACGAGTCGAGAACCAC			
2708-225F	GCTGTTAGCCAACCTCCCTGT	II	<i>Aave_2708</i> ^b	209
2708-433R	TAGGCGAGTCAAAGTGCTGG			

^a Based on the annotation of the *Acidovorax citrulli* M6 chromosome (GenBank accession CP029373.1).

^b Based on the annotation of the *A. citrulli* AAC00-1 chromosome (GenBank accession CP000512.1).

TABLE 2. Specificity of *Acidovorax citrulli* group I- and II-specific oligonucleotide primers as determined by PCR assay^a

Strain	Group	<i>SpeI</i> PFGE haplotype	PCR assay results with	
			550-34F/550-626R	2708-225F/2708-433R
92-300	I	B3	+	–
92-301	I	B1	+	–
201-14	I	B11	+	–
203-16	I	B13	+	–
208-1	I	B14	+	–
209-108	I	B5	+	–
209-128	I	B21	+	–
IACANT58-1	I	B8	+	–
AU-2	I	B4	+	–
92-2	II	A1	–	+
92-3	II	A4	–	+
92-303	II	A2	–	+
94-39	II	A7	–	+
96-6	II	A6	–	+
209-118	II	A30	–	+
211-2	II	A5	–	+
211-8	II	A14	–	+
211-29	II	A24	–	+
211-36	II	A17	–	+

^a + and – indicate the presence or absence of a 593- or a 209-bp band resulting from conventional PCR using primer sets 550-34F/550-626R and 2708-225F/2708-433R, respectively. Strain source: R. Walcott collection.

Field experiments to investigate *A. citrulli* cucurbit host preference. *2017 field study.* During the field study, total precipitation was 20.85 cm and average rainfall per day was 0.41 cm. The average temperature during the field study was 26.1°C (Table 4). In total, 37% (11/30) of watermelon leaf samples that exhibited visible BFB symptoms were infected with the group I strain, while 60% (15/25) of symptomatic melon leaf samples were infected with the group I strain. Additionally, 67% (4/6) of symptomatic pumpkin and 71% (10/14) of symptomatic squash leaf samples were infected with the group I strain (Fig. 2A). For fruits, none of the pumpkin or squash fruit samples with putative BFB lesions were determined to be positive for *A. citrulli* by PCR assay. On the other hand, 100% (9/9) of the symptomatic watermelon fruit samples were determined to be infected with the group II strain. With regards to symptomatic melon fruits, 86% (6/7) were determined to be infected with the group I strain (Fig. 2B).

2018 field study. During the field study, total precipitation was 26.59 cm and average rainfall per day was 0.44 cm. The average temperature during the field study was 25.6°C (Table 4). In total, 42% (28/67) of the watermelon leaf samples that exhibited BFB symptoms were infected with the group II strain, while 100% of symptomatic melon ($n = 80$), pumpkin ($n = 2$), and squash ($n = 19$) leaf samples were infected with the group I strain (Fig. 3A). For fruit samples, no putative pumpkin or squash fruit lesions were determined to be positive for *A. citrulli* by PCR assay. On the other hand, 100% ($n = 20$) of the symptomatic melon fruit samples were infected with the group I strain. Interestingly, in 2018, only two infected watermelon fruits were observed and both were infected with the group I strain (Fig. 3B).

2019 field study. During the field study, total precipitation was 18.62 cm and average rainfall per day was 0.31 cm. The average temperature during the field study was 25.4°C (Table 4). Seven percent (5/69) of infected watermelon leaf samples were determined to be infected with the group I strain, while 82% (75/92) of symptomatic melon leaf samples were infected with the group I strain. Additionally, 100% (5/5) of the BFB-infected squash leaf samples were infected with the group I strain (Fig. 4A). No pumpkin leaves or fruit samples tested positive for *A. citrulli* by PCR assay. In contrast, 95% (36/38) of the symptomatic watermelon fruit samples were determined to be infected with group II by PCR assay. With regards to symptomatic melon fruits, 78% (7/9) were infected with the group I strain (Fig. 4B). For the first time in 2019, we observed

squash fruits with BFB symptoms and 100% (5/5) of these fruits were infected with the group I strain.

Genetic analysis of *A. citrulli* isolates recovered from field plots. All group I *A. citrulli* field isolates had *SpeI*-PFGE profiles that were identical to the strain used for inoculation (AAC203-1RR1) (Fig. 5). Additionally, the recovered isolates were resistant to rifampicin as expected. In contrast, the 2017 group II field isolates had two *SpeI*-PFGE profiles that were both different from that of the group II strain used for inoculation (AAC94-21SR1) (Fig. 5). Moreover, these isolates were sensitive to streptomycin, indicating that they occurred naturally in the environment. The 2018 group II field isolates recovered were genetically identical to the strain used for inoculation (AAC94-21SR1) based on *SpeI*-PFGE analysis (Fig. 5). The 2019 group II field

Fig. 1. Detection sensitivity of group I (550-34F/550-626R)- and II (2708-225F/2708-433R)-specific PCR assays for *Acidovorax citrulli* strains based on 10-fold serial dilutions of bacterial cell suspensions. Lane M: GeneRuler 1 kb Plus DNA Ladder; lane 1: AAC203-1RR1, 10^8 CFU/ml; lane 2: AAC203-1RR1, 10^7 CFU/ml; lane 3: AAC203-1RR1, 10^6 CFU/ml; lane 4: AAC203-1RR1, 10^5 CFU/ml; lane 5: AAC203-1RR1, 10^4 CFU/ml; lane 6: AAC94-21SR1, 10^8 CFU/ml; lane 7: AAC94-21SR1, 10^7 CFU/ml; lane 8: AAC94-21SR1, 10^6 CFU/ml; lane 9: AAC94-21SR1, 10^5 CFU/ml; lane 10: AAC94-21SR1, 10^4 CFU/ml; and lane 11: H₂O negative control.

TABLE 3. List of *Acidovorax citrulli* isolates analyzed by *SpeI*-digested pulse field gel electrophoresis (PFGE)

Isolate	Sample date	Host	Tissue	PFGE group	PFGE profile	Rifampicin resistance	Streptomycin resistance
17-1	7/4/2017	Watermelon	Fruit	II	2	+	–
17-2	7/4/2017	Melon	Fruit	II	2	+	–
17-3	6/19/2017	Melon	Leaf	I	1	+	–
17-4	6/19/2017	Watermelon	Leaf	I	1	+	–
17-5	7/12/2017	Watermelon	Fruit	II	3	+	–
17-6	7/12/2017	Watermelon	Fruit	II	2	+	–
17-7	7/12/2017	Watermelon	Fruit	II	3	+	–
17-8	7/20/2017	Melon	Fruit	I	1	+	–
18-1	5/22/2018	Melon	Leaf	I	1	+	–
18-3	5/22/2018	Watermelon	Leaf	I	1	+	–
18-8	5/22/2018	Watermelon	Leaf	II	4	+	+
18-18	6/1/2018	Watermelon	Leaf	II	4	+	+
18-19	6/22/2018	Watermelon	Fruit	I	1	+	–
18-20	6/22/2018	Melon	Fruit	I	1	+	–
19-2	6/1/2019	Watermelon	Fruit	II	2	+	–
19-3	6/1/2019	Melon	Fruit	I	1	+	–
19-4	6/1/2019	Melon	Leaf	II	2	+	–
19-5	6/1/2019	Watermelon	Leaf	II	5	+	–
19-7	6/1/2019	Melon	Leaf	I	1	+	–
19-8	6/1/2019	Melon	Leaf	I	1	+	–
19-10	6/13/2019	Melon	Fruit	I	1	+	–
19-13	6/21/2019	Watermelon	Fruit	II	2	+	–

isolates ($n = 4$) were identical to the strain used for inoculation (17-6), with one exception (AAC strain 19-5) (Fig. 5).

Statistical analysis. Statistical analysis of binary distribution data over the 3-year field study showed that the odds of melon, pumpkin, and squash foliage being infected by the *A. citrulli* group I strain were 21.7, 11.5, and 22.1 times greater than the odds of watermelon foliage being infected by the group I strain, respectively ($P < 0.0001$). However, the odds of melon, pumpkin, and squash foliage being infected by *A. citrulli* group I strain were not significantly different. More strikingly, the odds of melon fruits being infected by the

group I *A. citrulli* strain were 97.5 times greater than watermelon fruits being infected by the group I strain ($P < 0.0001$) (Table 5).

DISCUSSION

To rapidly determine if a BFB lesion from a plant sample was caused by a group I or II *A. citrulli* strain, we developed a group-specific duplex PCR-based assay. Primers for this assay were designed to amplify group-specific type III-secreted effectors (T3Es) (Eckshtain-Levi et al. 2014; Jiménez Guerrero et al. 2019). As expected, the two

TABLE 4. Temperature and precipitation for the Coastal Plain Experiment Station in Tifton GA during the field trials in 2017 to 2019

Year	Mean maximum temperature (°C)	Mean minimum temperature (°C)	Mean average temperature (°C)	Total precipitation (cm)	Mean precipitation (cm)/day
2017	31.0	21.2	26.1	20.85	0.41
2018	31.0	20.1	25.6	26.59	0.44
2019	31.5	19.4	25.4	18.62	0.31

Fig. 2. The proportion of group I and II *Acidovorax citrulli* PCR-positives detected in symptomatic cucurbit A, foliage and B, fruit samples from the 2017 field trial to investigate cucurbit host preference. The percentages of group I (yellow) and II (green) *A. citrulli* isolates are shown in the bar charts.

Fig. 3. The proportion of group I and II *Acidovorax citrulli* PCR-positives detected in symptomatic cucurbit A, foliage and B, fruit samples from the 2018 field trial to investigate cucurbit host preference. The percentages of group I (yellow) and II (green) *A. citrulli* isolates are shown in the bar charts.

primer sets successfully discriminated group I and II strains. The detection sensitivity of this assay was low (10^6 to 10^7 CFU/ml) relative to previously developed PCR-based assays for *A. citrulli* (Ha et al. 2009; Yang et al. 2019b). We are aware of the possibility of occurrence of false-negative results from the field samples. However, since our goal was to detect the presence of *A. citrulli* in well-established leaf and fruit lesions, and we expected pathogen populations in lesions to be relatively high (Yan et al. 2017; Zivanovic and Walcott 2017), we determined that the PCR assays were suitable for this study.

In 2017, we observed no substantial difference in the proportion of group I and II *A. citrulli* detected on the foliage of the four cucurbit

species. Foliage from non-watermelon hosts (melon, pumpkin, and squash) showed slightly higher percentages (60, 67, and 71%, respectively) of samples infected with group I strains, while watermelon foliage showed a slightly higher percentage (63%) of samples infected with group II strains. On the other hand, there appeared to be a difference in susceptibility of cucurbit fruits to different *A. citrulli* strains. More specifically, all watermelon fruit samples were infected by the group II strain, while all melon fruits, except one, were infected by the group I strain. The fruits of the pumpkin and squash cultivars tested did not appear to be susceptible to infection by the *A. citrulli* strains used under the tested conditions.

Fig. 4. The proportion of group I and II *Acidovorax citrulli* PCR-positives detected in symptomatic cucurbit **A**, foliage and **B**, fruit samples from the 2019 field trial to investigate cucurbit host preference. The percentages of group I (yellow) and II (green) *A. citrulli* isolates are shown in the bar charts.

Fig. 5. Dendrogram indicating the relationship among representative field isolates of *Acidovorax citrulli* based on polymorphisms among restriction fragments obtained following *SpeI* digestion of whole-cell genomic DNA and separation of fragments by pulse field gel electrophoresis (PFGE). The distance matrix for the dendrogram was generated by Dice's coefficient of similarity and the dendrogram was generated based on the unweighted pairwise group method with arithmetic mean algorithm. The numbers in parentheses represent the PFGE types recovered from the field trials.

Tissue type	Host	Host	Odds ratio estimate	Degrees of freedom	95% confidence limits	
Leaf	Melon	Watermelon	21.662	401	11.48	40.88
Leaf	Pumpkin	Watermelon	11.488	401	1.94	68.15
Leaf	Squash	Watermelon	22.052	401	6.66	72.99
Fruit	Melon	Watermelon	97.538	79	13.72	693.44

Interestingly, the group II *A. citrulli* isolates recovered from the 2017 field samples differed from the inoculated strain, AAC94-21SR1, in that they were sensitive to streptomycin. *SpeI*-PFGE analysis of the 2017 group II field isolates revealed two distinguishable haplotypes, that were both different to the profile of AAC94-21SR1. Based on this observation, we conclude that the group II isolates responsible for the 2017 BFB epidemic originated from the local environment. On the other hand, the group I isolates recovered from the 2017 study were rifampicin-resistant and yielded identical PFGE profiles as the inoculum strain, AAC203-1RR1. Like other foliar Gram-negative bacteria, *A. citrulli* can survive epiphytically on host and nonhost plants under field conditions for certain time. These results suggest that group II isolates may be prevalent at the Tifton Vegetable Park farm, while group I isolates likely do not have localized sources of inoculum.

In 2018, group II *A. citrulli* strains were only observed on infected, symptomatic watermelon foliage. In contrast, we detected group I *A. citrulli* strains in leaf lesions of all cucurbit hosts, including watermelon. With regards to fruit samples, we only detected group I *A. citrulli* strains in BFB symptomatic melon and watermelon fruits. These data suggest that while the group II strain tested was specific to watermelon foliage, the group I strain could infect the foliage of all cucurbits tested under natural field conditions. As expected, the 2018 field isolates that were recovered had *SpeI*-PFGE profiles that were identical to their respective inocula. However, since we did not recover AAC94-21SR1 from infected watermelon fruits, we suspected this group II strain was weakly virulent under tested conditions. We also suspected that the local field isolate from the 2017 field trial was more virulent than AAC94-21SR1 under field conditions. Since this strain (called 17-6) occurred naturally, we considered that it might better allow us to test our hypothesis regarding cucurbit host preference of *A. citrulli* groups under field conditions. Hence, for the 2019 field study, we included both AAC94-21SR1 and the field isolate 17-6 as group II inocula.

For the 2019 field study, we observed a substantial difference in the proportion of group I and II *A. citrulli* strains detected on the foliage of the four cucurbit species. While all pumpkin leaf samples were negative for *A. citrulli*, symptomatic melon and squash leaf samples were infected with high percentages (82 and 100%, respectively) of group I strains. Additionally, 93% of the infected watermelon leaf samples were infected with group II strains. Unlike data from the 2017 and 2018 studies, these data strongly suggested *A. citrulli* host preference on cucurbit foliage, with the group II strains showing a strong preference for watermelon and the group I strain showing a strong preference for melon and squash foliage. It is possible that the specific microclimate environment during the 2019 field trial, with the combination of the used strains, resulted in a stronger host preference on cucurbit foliage in 2019.

In 2019, the *A. citrulli* strains showed strong host preference on the fruits of different cucurbits. More specifically, 95% of the symptomatic watermelon fruits were infected with the group II strains, while 78% of melon fruits were infected with the group I strain. Interestingly, for the first time in 2019, we observed natural BFB squash fruit infection and 100% (5/5) of these fruits were infected with the group I strain. Remarkably, lesions of all five infected squash fruits were associated with injury caused by pickleworms (*Diaphania nitidalis*). We speculate that wounds created by insects possibly facilitated the entry of *A. citrulli* into fruit tissues. We observed pickleworm damage on melon fruits in 2017 and 2018 trials, and on melon, squash, and pumpkin fruits in 2019 trials. Despite this, throughout this study, pumpkin fruits did not develop BFB symptoms. Hence, we suspect that it is likely that insect damage increases the likelihood of BFB fruit infection for some cucurbit fruits.

Hopkins and Thompson (2002b) reported that in a field study using a single *A. citrulli* group II strain, fruit symptoms were not observed on the fruits of acorn squash cultivar Tay Belle, pumpkin

cultivar Ichabod, or butternut squash cultivar Butterboy. However, they observed a few small lesions on zucchini squash cultivar Elite 828 and yellow squash cultivar Crescent in 1 of 2 years (Hopkins and Thompson 2002b). Based on these results and our direct observations, we conclude that under natural field conditions, pumpkin and squash fruits are at low risk for *A. citrulli* infection relative to watermelon and melon fruits. However, *A. citrulli* infection of pumpkin fruits was reported to occur in a commercial field in Georgia in 1998 (Langston et al. 1999). To date, only watermelon and melon cultivars and germplasm collections have been extensively screened for BFB resistance (Bahar et al. 2009; Carvalho et al. 2013; Hopkins and Thompson 2002a; Hopkins et al. 1993; Wechter et al. 2011). It would be interesting to screen pumpkin germplasm for BFB resistance.

Host preference within a pathogen species has been demonstrated in other pathosystems. For example, *Erwinia tracheiphila*, the causal agent of bacterial wilt of cucurbits, infects two genera of cucurbit crop plants, *Cucurbita* spp. (pumpkin and squash) and *Cucumis* spp. (muskmelon and cucumber) (Rojas et al. 2015). Based on whole genome sequence analysis, Shapiro et al. (2018) found that 88 *E. tracheiphila* strains from different geographic ranges were divided into three clusters, Et-melon, Et-C1, and Et-C2. Strains from the three clusters were detected in field-infected cucumber plants, while muskmelon was infected only by the Et-melon strains, and squash was only infected by the Et-C1 and Et-C2 strains (Shapiro et al. 2018). *E. tracheiphila* strains also varied in their virulence in a host-dependent pattern. The authors suggested that the introduced and highly susceptible cucumber cultivars were driving *E. tracheiphila* lineage diversification.

Determining the host preference of a pathogen is important, as it helps to estimate the risk of disease outbreaks. While previous anecdotal observations suggested differences in cucurbit host preference of group I and II *A. citrulli* strains, no empirical data were generated to directly test this hypothesis under field conditions. In the current study, we demonstrated that the two *A. citrulli* groups differed in their ability to infect the foliage of different cucurbit species. More importantly, we demonstrated that this host preference manifested itself more strongly in cucurbit fruit tissues. We observed differential susceptibility of host tissue types (foliage versus fruit) to *A. citrulli* strains. Importantly, most of the fundamental investigations on model plant pathogenic bacterial species, such as *Pseudomonas syringae* pv. *tomato*, have involved foliar tissue inoculations (Xin et al. 2018). Fruit tissue represents a unique niche for plant and human pathogens that may require different adaptations compared with other host tissue types. Therefore, exploring pathogen–fruit interactions has biological and economic significance for improving disease management. From an economic standpoint, fruit infection is the most important phase of BFB epidemiology. Studying the mechanisms of fruit infection is also important for improving BFB management. *A. citrulli* cells have been shown to penetrate watermelon (Frankle and Hopkins 1993) and melon (Silva Neto et al. 2006) fruits via stomata. It would be interesting to test the factors that permit or promote entry of *A. citrulli* into plant tissues to initiate infection, and to investigate differences in the mechanisms of fruit infection by different *A. citrulli* strains in combination with fruits from distinct cucurbit crops.

Elucidating variations in virulence and host preference determinants among *A. citrulli* strains could set the stage for detection of genetic markers of host resistance. Currently, no BFB-resistant cucurbit cultivars are commercially available. Thus, in addition to field studies to investigate *A. citrulli* host preference, it is necessary to understand the genetic factors that differentiate group I and II strains and to determine their roles in virulence and host preference. Eckshain-Levi et al. (2014) reported that three effectors in the XopJ family were present in group II strains, but were absent or nonfunctional in group I strains. In addition, Jiménez Guerrero et al. (2019) identified 10 unique T3Es that were present in the *A. citrulli*

group I strain M6, but absent in the group II strain AAC00-1. The variability in T3E arsenals between *A. citrulli* group I and II strains may contribute to the differences in the host-preferential association between the groups. Despite this, more research is needed to test this hypothesis and to understand the mechanisms of host preference of *A. citrulli*. The current study demonstrates host preference of *A. citrulli* under natural field conditions, but additional research is critical for the development of more effective strategies for BFB management.

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