Field and Greenhouse Evaluations of Cucurbit Rootstocks to Improve *Verticillium* Resistance for Grafted Watermelon

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Abstract. Grafting watermelon (*Citrullus lanatus* Thunb.) onto resistant rootstocks is used in many areas of the world to overcome soilborne disease losses including verticillium wilt caused by *Verticillium dahliae* Kleb. Currently, this disease poses a serious risk to watermelon growers in Washington State. To identify resistant rootstocks, the verticillium wilt reactions (chlorosis, necrosis, and wilting) of 14 nongrafted PI accessions including *Benincasa hispida* Thunb., *Cucurbita moschata* Duchesne ex Poir., and *Lagenaria siceraria* Molina Standl. from the U.S. Department of Agriculture National Plant Germplasm System (USDA NPGS); 11 nongrafted commercially available rootstocks; and, nongrafted ‘Sugar Baby’ watermelon (verticillium wilt–susceptible control) were visually assessed in a field naturally infested with *V. dahliae* at a rate of 17 colony-forming units (cfu) per gram of soil. Typical symptoms of verticillium wilt were observed on all entries. ‘Sugar Baby’ had the highest relative area under disease progress curve (RAUDPC) value (26.80), which was not significantly different from ‘64-19 RZ’, ‘Marvel’, PI 368638, PI 634982, and PI 642045 (average = 10.16). PI 419060 (1.46) had the lowest RAUDPC value, which was not significantly different from ‘Miniature Bottle Gourd’, PI 326320, PI 419016, PI 536494, PI 636137, ‘Strong Tosa’, ‘Strongtosa’, and ‘TZ 148’ (average = 3.36). The mean RAUDPC value of PI accessions (5.49) did not differ significantly from the mean value of the commercial rootstocks (5.68). Microsclerotia typical of *Verticillium* spp. were observed in the stems of all but one entry (PI 181913). In a greenhouse study, a subset of 12 entries were inoculated with *V. dahliae*, and by 22 days after inoculation (DAI), ‘Sugar Baby’ had a significantly higher disease rating than all entries except PI 419060, PI 438548, and ‘Titan’. A strong positive correlation was observed between the field and greenhouse studies. Results indicate that commercial rootstocks as well as PI accessions could be used to successfully manage verticillium wilt in Washington; however, grafting compatibility with watermelon must first be ascertained for the promising PI accessions. Although greenhouse-based verticillium wilt assays can be used to help predict rootstock performance in the field, accurate assessment may require manipulating environmental conditions (e.g., temperature and humidity) to approximate field conditions.

Throughout the 20th century, watermelon (*C. lanatus* Thunb.) grafting was adopted in many areas of the world to manage soilborne

diseases, primarily fusarium wilt caused by *Fusarium oxysporum* Schlecht. (Davis et al., 2008; King et al., 2008; Miguel et al., 2004; Sakata et al., 2007). Other soilborne diseases of watermelon can also be controlled by grafting, such as monosporascus vine decline (caused by *Monosporascus cannonballus* Pollack & Uecker), phytophthora crown rot (caused by *Phytophthora capsici* Leonian), and verticillium wilt (caused by *Verticillium dahliae* Kleb.) (Louws et al., 2010). In Washington, verticillium wilt is problematic for watermelon growers due to the widespread distribution of the pathogen (Sunseri and Johnson, 2001). To complicate matters,

V. dahliae has a wide host range and can persist in the soil for long periods (Berlanger and Powelson, 2000). Farmers have traditionally used soil fumigation to control this pathogen, but some products (e.g., methyl bromide) are no longer available, whereas others have questionable efficacy (Klosterman et al., 2009; U.S. Environmental Protection Agency, 2013; Woodward et al., 2011). Thus, grafting onto disease-resistant or -tolerant rootstocks may be the only feasible way to manage severe infestations.

The first step in developing a successful management strategy for verticillium wilt in watermelon via the use of grafted plants is to evaluate rootstocks for resistance to *V. dahliae*. Paplomatas et al. (2000) are the only researchers to date that have carried out such an experiment. These researchers screened 33 cucurbit rootstocks for resistance to *V. dahliae* in Greece and found some degree of tolerance in zucchini, pumpkin, and bottle gourd, but none of the tested rootstocks were resistant. Of the many commercial watermelon rootstocks on the market, none have been sufficiently tested for verticillium wilt resistance or tolerance in Washington. The USDA NPGS contains over 1000 PI accessions in the Cucurbit family, including several genera and species that are used for commercial rootstocks for watermelon. Among these are *B. hispida* Thunb., *Cucurbita maxima* Duchesne, *C. moschata* Duchesne ex Poir., and *L. siceraria* Molina Standl. There are additional genera and species that may be suitable for use as rootstocks, but are so far untested. An extensive search for PI accessions with resistance or tolerance to specific pathogens is much needed and could result in the development of affordable and regionally adapted rootstocks in the United States (Lee et al., 1998).

Although Paplomatas et al. (2002), Paroussi et al. (2007), Buller et al. (2013), and Wimer et al. (2015) have demonstrated that grafting can significantly reduce verticillium wilt of watermelon, no commercial growers in Washington are currently using grafted plants to manage this disease. Adoption of this practice is hindered by the increased costs of grafted seedling production attributed to the extra (and often expensive) seed needed to grow the rootstock, the labor needed to perform the grafting and care for the grafted seedlings, and the special facilities that are required for proper graft union formation (Boughalleb et al., 2007; King et al., 2010; Lee et al., 2010). In addition, there is currently a lack of published information regarding the performance of commercially available rootstocks in specific environments (Kubota et al., 2008). For large-scale growers in Washington (each of whom requires tens of thousands of plants), adoption of this practice is contingent on the resolution of these issues.

To identify verticillium wilt–resistant rootstocks, an efficient disease evaluation system is needed. Field assays can be used for this purpose, but they tend to be lengthy and confined to each growing season’s

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constraints (Bae et al., 2011). Greenhouse assays, however, are rapid and can be conducted most times of the year. Bae et al. (2011) developed a greenhouse assay to screen potato seedlings for verticillium wilt and demonstrated that resistance, tolerance, and susceptibility to this disease can be accurately assessed. The protocol used in the Bae et al., study involved submerging exposed roots into a *V. dahliae* suspension. Dervis et al. (2009), Boughalleb et al. (2007), Paplomatas et al. (2000), and others used a similar procedure to test the pathogenicity of various pathogens on multiple cucurbit crops in the greenhouse. Although such greenhouse studies can be useful in determining resistance, tolerance, or susceptibility, the correlation with field studies can vary considerably (Zhang et al., 1997). Thus, greenhouse assays that can be used to predict field performance need to be developed to ensure cost-effective and efficacious rootstock development.

One objective of this study was to identify commercially available rootstocks and PI accessions that could be used to manage verticillium wilt of watermelon in Washington. The second objective was to compare the verticillium wilt reactions of entries in field and greenhouse assays. To achieve these objectives, plants were first evaluated in a field naturally infested with *V. dahliae*, then in the greenhouse using artificial inoculation.

Materials and Methods

Field conditions. The field study was conducted during the summer of 2013 at Washington State University (WSU) Mount Vernon. The soil type is Skagit silt loam, a fine-silty mixed nonacid mesic Typic Fluvaquents (University of California, 2010). The soil pH was 6.1 (saturated paste) and the organic matter content was 29 g·kg⁻¹ soil (loss of ignition). The summer climate is cool and humid, with an average daily temperature of 16 °C (22 °C maximum and 11 °C minimum, on average), and 81% relative humidity (RH). Total precipitation during this study was 94 mm (AgWeatherNet, 2013).

Soil assay for *V. dahliae*. The study site had a history of natural infestation of *V. dahliae* at a rate of 18.0 cfu/g soil (Buller et al., 2013). To measure the current infestation level, 20 random samples were taken from the experimental field to a depth of 15 cm. Samples were bulked and placed in the greenhouse to dry (Harris et al., 1993). Once dry, 1 g subsamples were uniformly distributed onto 10 plates of NP-10 media using a salt shaker, a method that is a modification of the Butterfield and DeVay (1977) method which distributes soil onto plates via an Anderson Sampler. Following incubation, colonies of *V. dahliae* were counted with the aid of a dissecting microscope (×40) and the population density was calculated to be 17.0 cfu/g of soil.

Plant material. There were 26 entries planted in the field study (Table 1). Eleven

of the entries were commercially available watermelon rootstocks, 14 were PI accessions obtained from the USDA NPGS, and one was ‘Sugar Baby’, a verticillium wilt-susceptible watermelon control (Elena, 2000). All entries were grown from seed at WSU Mount Vernon. Seeds were sown in potting mix (Sunshine #3 N and O; Sun Gro Horticulture, Agawam, MA) in 72-celled trays between 28 May and 3 June (the different sowing dates were based on receipt of seeds).

Field experiment establishment. On 3 June, fertilizer (Wil-Gro Organic fertilizer 8N–0.9P–3.3K; Wilbur-Ellis, Mount Vernon, WA) was broadcast at a rate of 78 kg N/ha over bed centers and raised beds were then formed using a bed shaper (Rain-Flo 2600; Rain-Flo Irrigation, East Earl, PA). The beds were 0.25 m tall, 0.9 m wide, and 3 m center to center and covered with black plastic mulch (0.025 mil embossed; Pliant Corporation, Washington, GA). Irrigation drip tape (low flow, 16 mm diameter, 0.2 mm wall thickness, 20 cm emitter spacing; John Deere, San Marcos, CA) was located on the center of each bed underneath the plastic.

Transplanting into the field occurred between 12 and 21 June when the plants had 2–4 true leaves, with the three replications of any given entry being planted on the same day. Drip irrigation commenced on 1 July and continued at a rate of 25 mm of water per week through July and every 3 weeks thereafter to promote the onset of disease symptoms (Cappaert et al., 1994). Fertilizer

(5N–0.9P–0.8K; Converted Organics, Gonzales, CA) was applied through the irrigation system at a rate of 4–5 kg N/ha per week through July and every 3 weeks thereafter for the remainder of the growing season.

Plant disease ratings and pathogen detection. Field plots were rated visually for verticillium wilt severity on a weekly basis from 2 Aug. to 18 Sept. Disease severity was recorded as the percent of the plot canopy displaying chlorosis, necrosis (in the form of V-shaped lesions), and wilting. Stem samples were assayed for *Verticillium* spp. using the techniques outlined by Buller et al. (2013). Following incubation, samples were analyzed for microsclerotia and pure cultures were obtained from infested stems using NP-10 agar medium (Kabir et al., 2004). Seven of the cultures based on rootstock diversity were selected for species identification at WSU Molecular Biosciences Laboratory (Table 2).

Greenhouse assays. Twelve entries were selected for a greenhouse inoculation study based on species diversity and variation in verticillium wilt reactions. The entries included ‘Sugar Baby’ as the verticillium wilt-susceptible watermelon control; the commercial rootstock cultivars Tetsukabuto, Titan, Marvel, and Strongtosa; and NPGS accessions PI 326320, PI 419016, PI 419060, PI 438548, PI 536494, PI 636137, and PI 181913. This study was carried out from Mar. to May in 2014 (average daily temperature 22 °C, 24 °C maximum and 20 °C

Table 1. Species and source of each cucurbit cultivar and accession included in the verticillium wilt field screening study at Washington State University Mount Vernon Northwestern Washington Research and Extension Center in 2013.

Entry	Species	Source ²
Watermelon cultivar		
Sugar Baby	<i>Citrullus lanatus</i>	Osborne Seed
Commercial Rootstock		
64–19 RZ	<i>Cucurbita maxima</i>	Rijk Zwaan
Emphasis	<i>Lagenaria siceraria</i>	Syngenta
Marvel	<i>Cucurbita</i> hybrid	Takii Seed
Miniature Bottle Gourd	<i>L. siceraria</i>	Osborne Seed
Rampart	<i>Lagenaria</i> sp.	Takii Seed
RS 841	<i>Cucurbita</i> hybrid	De Ruiter
Strongtosa	<i>Cucurbita</i> sp.	Osborne Seed
Strong Tosa	<i>Cucurbita</i> hybrid	Syngenta
Tetsukabuto	<i>Cucurbita</i> hybrid	Takii Seed
Titan	<i>Cucurbita</i> hybrid	Takii Seed
TZ 148	<i>Cucurbita</i> hybrid	Clause Seeds
PI accession		
PI 181913	<i>L. siceraria</i>	PGRU (Syria, 1949)
PI 326320	<i>Cucurbita moschata</i>	PGRU (United States, 1968)
PI 368638	<i>L. siceraria</i>	PGRU (Macedonia, 1971)
PI 381820	<i>C. moschata</i>	PGRU (India, 1973)
PI 419016	<i>Benincasa hispida</i>	PGRU (China, 1977)
PI 419060	<i>B. hispida</i>	PGRU (China, 1977)
PI 419123	<i>B. hispida</i>	PGRU (China, 1977)
PI 438548	<i>C. moschata</i>	PGRU (Belize, 1979)
PI 482490	<i>C. moschata</i>	PGRU (Zimbabwe, 1983)
PI 536494	<i>C. moschata</i>	PGRU (Maldives, 1986)
PI 604505	<i>C. moschata</i>	PGRU (Pennsylvania, 1961)
PI 634982	<i>C.a moschata</i>	PGRU (United States, 1980)
PI 636137	<i>L. siceraria</i>	PGRU (India, 1993)
PI 642045	<i>L. siceraria</i>	PGRU (Georgia, United States, 2006)

²Source is a commercial supplier or the National Plant Germplasm System located at the Plant Genetic Resources Unit in Geneva, NY, or the PGRU located in Griffin, GA. Country of origin and collection date given in parentheses for PI accessions.

Table 2. Final verticillium wilt severity ratings and relative area under disease progress curve (RAUDPC) values for each cucurbit cultivar and accession included in the verticillium wilt field screening study at Washington State University Mount Vernon Northwestern Washington Research and Extension Center in 2013.

Entry	Severity (%) ²	RAUDPC ³
Watermelon cultivar		
Sugar Baby	98	26.80 a
Commercial rootstock		
64-19 RZ	28	7.14 abc
Emphasis	17	5.20 cde
Marvel	33	14.57 a
Miniature Bottle Gourd	12	2.26 fg
Rampart	20	5.66 bcd
RS 841	28	5.85 bcd
Strong Tosa	28	3.84 cdefg
Strongtosa	33	4.00 cdefg
Tetsukabuto	28	5.15 cde
Titan	23	4.82 cde
TZ 148	20	3.97 cdefg
Germplasm accession		
PI 181913	19	5.43 bcd
PI 326320	12	4.27 cdefg
PI 368638	67	10.84 ab
PI 381820	24	5.49 bcd
PI 419016	4	2.39 fg
PI 419060	6	1.46 g
PI 419123	16	5.71 cdef
PI 438548	11	4.73 cdef
PI 482490	35	5.89 bcd
PI 536494	13	3.33 defg
PI 604505	38	6.21 bcd
PI 634982	32	6.85 abc
PI 636137	8	2.84 efg
PI 642045	65	11.42 ab
Average	28	6.39
P value ⁴	<0.0001	<0.0001

²The field site was naturally infested with *Verticillium dahliae* at a rate of 18.0 cfu/g of soil. Final verticillium wilt severity ratings were taken on 18 Sept.; severity was recorded as the percent of the plot canopy displaying typical verticillium wilt symptoms: chlorosis, necrosis, and wilting.

³RAUDPC values were calculated by dividing area under disease progress curve (AUDPC) values by the maximum potential AUDPC. The maximum potential AUDPC was calculated as (final rating date – transplant date) × 100 (Fry, 1978).

⁴The data for both severity and RAUDPC violated the assumptions of ANOVA and therefore required transformation. PROC RANK in SAS (Version 9.2, SAS Institute, Cary, NC) was used in all cases, and ranked data from each site was compared separately using PROC GLM with $\alpha = 0.05$. However, the means presented in the table are nontransformed. Fisher's LSD test was used to locate differences in RAUDPC values among treatments. RAUDPC values followed by the same letter within a column are not significantly different at $\alpha = 0.05$.

minimum; RH 40%) and repeated from Jan. to Mar. in 2015 (average daily temperature 21 °C, 24 °C maximum and 20 °C minimum; RH 42%). All plants were started from seed following the methods described above. Seedlings were inoculated at the 1–2 true leaf stage on 9 Apr. in 2014 and 2 Feb. in 2015 with the *V. dahliae* isolate 'JAW13-127' (isolated from 'Sugar Baby' in the field study) using the root-dip method and a conidial suspension of 10⁶/mL (Dervis et al., 2009). The inoculated seedlings were transplanted into 4-inch pots filled with moist Sunshine N & O potting mix. Noninoculated reference control plants of each entry also were included, and were dipped in deionized water.

Disease ratings were taken on 10, 16, and 22 DAI using the following rating scale: 1 = healthy, asymptomatic plant; 2 = one symptomatic leaf; 3 = two symptomatic leaves; 4 = three symptomatic leaves; 5 = four symptomatic leaves; and 6 = dead plant. The symptoms used for the ratings included chlorosis, necrosis, and wilting; the cotyledons were included as "leaves" in the rating. On 12 May in 2014 and 3 Mar. in 2015, stems from each

entry were harvested, surface sterilized, and plated on NP-10 agar for *V. dahliae* recovery.

Experimental design and statistical analysis. The field study was arranged as a randomized complete block design with three replications and five plants per plot. Verticillium wilt severity was measured over time and area under disease progress curve (AUDPC) values were calculated for each entry (Fry, 1978). Due to differences in planting dates, the AUDPC values were converted to RAUDPC values by dividing the AUDPC values by the maximum potential AUDPC. The maximum potential AUDPC was calculated as (final rating date – transplant date) × 100. Both RAUDPC and final severity data violated the assumptions of analysis of variance (ANOVA), and rank transformation was required for analysis (Kirk, 1982). Ranked RAUDPC values and final severities were analyzed using PROC GLM in SAS (Version 9.2; SAS Institute, Cary, NC) with $\alpha = 0.05$. Fisher's least significant difference (LSD) test was used to determine differences in RAUDPC values among entries ($\alpha = 0.05$).

The greenhouse inoculation study was a completely randomized design with six single-plant replicates of each entry and an additional reference control plant of each entry. Data were analyzed using PROC RANK and PROC GLM in SAS ($\alpha = 0.05$). Fisher's LSD test was used to determine which of the entries differed significantly on each rating date ($\alpha = 0.05$). Final disease ratings from the two greenhouse studies were averaged and correlated with RAUDPC values from the field study using PROC CORR in SAS.

Results

Field assay. Verticillium wilt symptoms were observed on all entries in the field study. At season's end (18 Sept.), significant differences in disease severity were observed among entries ($P = 0.0001$). 'Sugar Baby' had the highest final disease severity (98%), whereas PI 419016 had the lowest (4%) (Table 2). 'Sugar Baby' also had the highest RAUDPC value (26.80) but the value was not significantly different from 'Marvel' (14.57), PI 642045 (11.42), PI 368638 (10.84), '64-19 RZ' (7.14), and PI 634982 (6.85) ($P = 0.0001$). PI 419060 had the lowest RAUDPC value (1.46) but RAUDPC was not significantly different from 'Miniature Bottle Gourd' (2.26), PI 419016 (2.39), PI 636137 (2.84), PI 536494 (3.33), 'Strong Tosa' (3.84), 'TZ 148' (3.97), 'Strongtosa' (4.00), and PI 326320 (4.27). There was no significant difference between the mean RAUDPC values of the commercial cultivars (5.68) and PI accessions (5.49) ($P = 0.63$).

Microsclerotia were observed on incubated stem samples of all entries except PI 181913 (Table 3). Of the 25 entries that yielded microsclerotia, typical *Verticillium* isolates were recovered from 16. Of these, seven were selected and identified to the species level. Isolates obtained from 'Sugar Baby' and PI 419060 were identified as *V. dahliae*, whereas the other five isolates (obtained from '64-19 RZ', PI 604505, 'Rampart', 'Tetsukabuto', and 'Titan') were identified as *V. isaacii* Inderbitzin et al.

Greenhouse assays. In the greenhouse assays, there were interactions between year and DAI ($P = 0.005$) and between year and entry ($P < 0.0001$); thus, data for the two studies were analyzed separately. In both years, there were significant differences in disease ratings among entries for every rating date (Table 4). At 10 DAI in 2014, one entry (PI 438548) had a significantly higher disease rating than all others ($P = 0.01$), whereas seven entries were asymptomatic. At 16 DAI, PI 438548 again had the highest disease rating (2.67) but the rating was not significantly different compared with 'Sugar Baby' (2.50), 'Titan' (1.83), 'Marvel' (1.83), PI 419016 (1.67), or PI 536494 (1.83) ($P = 0.01$). Two entries—PI 636137 and PI 181913—remained asymptomatic at 10 DAI. At 22 DAI, 'Sugar Baby' surpassed PI 438548 with the highest disease rating (4.33 and 3.00, respectively), but these entries did

Table 3. Results from stem incubation assays for *Verticillium* sp., including detection of microsclerotia, recovered isolates, and species identification for selected entries from the verticillium wilt field screening study at Washington State University (WSU) Mount Vernon Northwestern Washington Research and Extension Center in 2013.

Entry	MS ^z	Isolate ^y	Species ^{xw}
64-19 RZ	Yes	JAW13-108	<i>Verticillium isaacii</i>
Emphasis	Yes	—	—
Marvel	Yes	JAW13-224	—
Miniature Bottle Gourd	Yes	—	—
PI 181913	No	—	—
PI 326320	Yes	—	—
PI 368638	Yes	JAW13-121	—
PI 381820	Yes	—	—
PI 419016	Yes	—	—
PI 419060	Yes	JAW13-301	<i>Verticillium dahliae</i>
PI 419123	Yes	JAW13-228	—
PI 438548	Yes	—	—
PI 482490	Yes	JAW13-322	—
PI 536494	Yes	JAW13-223	—
PI 604505	Yes	JAW13-113	<i>V. isaacii</i>
PI 634982	Yes	JAW13-123	—
PI 636137	Yes	JAW13-327	—
PI 642045	Yes	—	—
Rampart	Yes	JAW13-226	<i>V. isaacii</i>
RS 841	Yes	JAW13-311	—
Strongtosa	Yes	JAW13-202	—
Strong Tosa	Yes	—	—
Sugar Baby	Yes	JAW13-127	<i>V. dahliae</i>
Tetsukabuto	Yes	JAW13-306	<i>V. isaacii</i>
Titan	Yes	JAW13-110	<i>V. isaacii</i>
TZ 148	Yes	JAW13-222	—

^zMS refers to microsclerotia.

^y“—” indicates that a pure isolate was not obtained.

^xIsolates were identified to the species level at WSU Molecular Biosciences Laboratory in Puyallup, WA.

^w“—” indicates that the isolate was not sent to the laboratory for identification.

not differ significantly from ‘Titan’ and PI 419060 ($P = 0.002$). Only entry PI 636137 remained asymptomatic 22 DAI. In addition, all reference control plants remained asymptomatic, with the exception of ‘Titan’ and ‘Marvel’, which each received a rating of 2.00 on the final rating date. In 2015, ‘Sugar Baby’ had a significantly higher disease rating than all other entries at 10, 16, and 22 DAI ($P = 0.003, 0.003, \text{ and } 0.007$, respectively) and had a final rating of 4.33. At 22 DAI, none of the other entries differed significantly from one another, and all showed some symptoms of verticillium wilt. The incubation assay yielded *V. dahliae* on all inoculated entries in both greenhouse studies, whereas no *V. dahliae* was recovered from the stems of the noninoculated reference control plants. The final disease ratings from the greenhouse studies and RAUDPC values from the field study were found to be positively correlated ($r^2 = 0.81$; $P = 0.001$).

Discussion

‘Sugar Baby’ had the highest verticillium wilt rating in both the field and greenhouse studies confirming that it is highly susceptible to this disease (Elena, 2000). Of the 25 nonwatermelon entries included in the field study, 20 had significantly lower RAUDPC values than ‘Sugar Baby’. In the first greenhouse study, all but three of the entries had significantly lower disease ratings than ‘Sugar Baby’ at 22 DAI, whereas in the second greenhouse study all entries had significantly lower disease ratings than

‘Sugar Baby’ at 22 DAI. Furthermore, all species included in this study (*C. moschata*, *B. hispida*, *L. siceraria*, and *Cucurbita* hybrid) had entries with significantly lower verticillium wilt ratings than ‘Sugar Baby’. Thus, there is a wide diversity of species available that potentially could be used as rootstocks to manage verticillium wilt of watermelon in Washington.

Although there was a strong positive correlation between field and greenhouse studies, it is important to note that some entries performed differently in the two contrasting environments. For instance, PI 419060 had the lowest RAUDPC in the field, but was not significantly different from non-grafted ‘Sugar Baby’ in the greenhouse. The RAUDPC observed for ‘Marvel’ was not significantly different from that of ‘Sugar Baby’ in the field; however, in the greenhouse, the final disease rating of this rootstock was significantly lower than that of ‘Sugar Baby’. Similarly, the RAUDPC observed for ‘Titan’ was significantly lower than that of ‘Sugar Baby’ in the field, whereas this rootstock did not have a significantly lower final disease rating than ‘Sugar Baby’ in the greenhouse. Although it has been reported that *V. dahliae* assays performed in greenhouse conditions can be used to predict field performance, results from this study suggest that rootstock reactions to this pathogen in the field may not always be consistent with reactions in the greenhouse. Factors contributing to the observed differences between field and greenhouse studies may include the differing light, water, and

temperature levels in the two environments, as well as the presence of additional disease organisms in the field (Bae et al., 2011). In this study, temperatures during the field study were 16 °C on average, with 22 °C maximum and 11 °C minimum, and RH was 81%, whereas in the greenhouse, average temperature was 22 and 21 °C in 2014 and 2015, respectively (24 °C maximum and 20 °C minimum for both years), and RH was 40% and 42%, respectively. Attempting to harmonize environmental conditions between the field and greenhouse may partially overcome this problem, although replicating the biological diversity of the field in the greenhouse would prove difficult.

Despite the inherent differences between greenhouse and field studies, both testing environments can nonetheless be used to assess levels of verticillium wilt resistance or tolerance among potential rootstocks. In the field study, all but one entry (PI 181913) were found to be infected with at least one *Verticillium* sp. and all entries displayed at least some verticillium wilt symptoms. In the first greenhouse study, PI 636137 demonstrated no verticillium wilt symptoms; however, all entries were found to be infected. In the second greenhouse study, all entries displayed verticillium wilt symptoms and all were found to be infected. Thus, none of the entries included in these studies could be classified as completely resistant; instead, entries were determined to have varying levels of tolerance to *V. dahliae* (Shurtleff and Avere, 1997). A similar finding was reported by Paplomatas et al. (2000), who found many rootstocks (zucchini, pumpkin, and bottle gourd varieties) to be tolerant of but not entirely resistant to *V. dahliae*. Follow-up studies conducted by Paplomatas et al. (2002) and Wimer et al. (2015) showed that grafting onto such tolerant rootstocks could delay verticillium wilt onset and increase yield. Relying exclusively on tolerant rootstocks should be avoided, however, as this practice could ultimately increase (or at least maintain) the *V. dahliae* soil population density (Louws et al., 2010; Robb, 2007).

The PI accessions included in this study had comparable verticillium wilt resistance to the commercial rootstock cultivars. This result suggests that these PI accessions could be used directly as rootstocks to manage verticillium wilt in Washington, or they could be used in domestic rootstock breeding programs to develop new verticillium wilt-tolerant rootstock cultivars. The prospect of developing domestic rootstocks is of interest because the majority of commercial rootstocks are currently developed in Asia, and many cultivars (as well as breeding material) are not widely available in the United States or necessarily adapted to U.S. field conditions. Thus, PI accessions may present an avenue for the development of rootstocks that are better suited to regional conditions. In addition, domestically produced rootstocks would likely be more widely available and affordable compared with what is currently imported into the U.S. market.

Table 4. Mean verticillium wilt ratings^a for 11 rootstock entries and 'Sugar Baby', a verticillium wilt-susceptible watermelon control, inoculated with *Verticillium dahliae*^b isolate 'JAW13-127'^c in a verticillium wilt greenhouse screening study at Washington State University (WSU) Mount Vernon Northwestern Washington Research and Extension Center (NWREC) in 2014 (and repeated in 2015).

Entry	Days after inoculation (DAI)			Reference control ^w (22 DAI)
	10	16	22	
2014				
Sugar Baby	1.67 b	2.50 ab	4.33 a	1.00
Tetsukabuto	1.00 b	1.17 bc	1.50 def	1.00
Titan	1.33 b	1.83 ab	2.67 abc	2.00
Marvel	1.33 b	1.83 ab	2.00 bcde	2.00
Strongtosa	1.00 b	1.17 bc	1.33 def	1.00
PI 326320	1.00 b	1.33 bc	1.50 def	1.00
PI 419016	1.00 b	1.67 abc	2.17 bcde	1.00
PI 419060	1.00 b	1.83 bc	2.50 abcd	1.00
PI 438548	2.50 a	2.67 a	3.00 ab	1.00
PI 536494	1.50 b	1.83 abc	2.00 cdef	1.00
PI 636137	1.00 b	1.00 c	1.00 f	1.00
PI 181913	1.00 b	1.00 c	1.17 ef	1.00
<i>P</i> value ^v	0.01	0.03	0.002	
2015				
Sugar Baby	3.50 a	4.33 a	4.33 a	1.00
Tetsukabuto	1.17 bc	1.33 bc	1.33 b	1.00
Titan	1.83 b	1.83 b	1.83 b	1.00
Marvel	1.83 b	1.83 b	2.00 b	1.00
Strongtosa	1.00 c	1.00 c	1.17 b	1.00
PI 326320	1.17 bc	1.17 bc	1.17 b	1.00
PI 419016	1.50 bc	1.67 b	1.83 b	1.00
PI 419060	1.33 bc	1.33 bc	1.33 b	1.00
PI 438548	1.40 bc	1.80 bc	1.80 b	2.00
PI 536494	1.40 bc	1.40 bc	1.40 b	1.00
PI 636137	1.67 bc	1.83 b	1.83 b	1.00
PI 181913	1.17 bc	1.17 bc	1.17 b	1.00
<i>P</i> value	0.003	0.003	0.007	

^aRatings were conducted using a nonparametric rating scale: 1 = healthy, asymptomatic plant; 2 = one symptomatic leaf; 3 = two symptomatic leaves; 4 = three symptomatic leaves; 5 = four symptomatic leaves; and 6 = dead plant. The symptoms used for the ratings included chlorosis, necrosis, and wilting, and the cotyledons were included as "leaves" in the rating.

^bSeedlings at the 1–2 true leaf stage were inoculated using the root-dip method and a conidial suspension of 10⁶/mL (Dervis et al., 2009).

^c'JAW13-127' was isolated from 'Sugar Baby' during the 2013 field study at WSU Mount Vernon NWREC.

^wOne reference control plant was included for each entry. These control plants were inoculated with deionized water rather than the spore suspension.

^vThe data were analyzed using PROC RANK and PROC GLM in SAS (Version 9.2, SAS Institute, Cary, NC) with $\alpha = 0.05$. However, the means presented in the table are nontransformed. Fisher's LSD test was used to locate differences in treatment means. Treatment means followed by the same letter within a column are not significantly different at $\alpha = 0.05$.

Although results from these field and greenhouse studies suggest that it may be possible to manage verticillium wilt of watermelon with the use of tolerant rootstocks, field studies with grafted plants are needed in Washington to further characterize this potential. Such grafted studies would elucidate any interactions that might exist between these rootstocks and the commonly used watermelon cultivars in Washington's diverse production regions. In addition, the compatibility of the PI accessions with watermelon needs to be assessed before they could be considered for commercial use. Lastly, considering data from the rootstock screening studies (both field and greenhouse) as well as grafted field studies would allow for a more accurate assessment of rootstock performance and utility in Washington.

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