Powdery Mildew Resistant Rootstocks that Impart Tolerance to Grafted Susceptible Watermelon Scion Seedlings

Chandrasekar S. Kousik,[†] U.S. Vegetable Laboratory, USDA, ARS, Charleston, SC 29414; Mihir Mandal, ORISE participant at U.S. Vegetable Laboratory, USDA, ARS, Charleston, SC 29414; and Richard Hassell, Clemson University, Coastal Research and Education Center (CREC), Charleston, SC 29414

Abstract

Powdery mildew (PM) is a major foliar disease causing serious economic losses of cucurbit crops grown in the United States. The pathogen *Podosphaera xanthii*, which causes PM, is known to infect seedlings, stems, foliage, petioles, and fruit of cucurbit crops. In recent years, grafting watermelon on resistant rootstocks for managing soilborne diseases has been gaining popularity in the U.S.A. However, grafting for managing foliar diseases has not yet received adequate attention. Three greenhouse experiments were conducted during the summer months of 2012, 2013, and 2014 to determine if PM-resistant rootstocks could impart resistance to a susceptible watermelon scion. Susceptible watermelon scion 'Mickey Lee' seedlings were grafted onto 25 watermelon (*Citrullus lanatus*, *C. amarus*, *C. mucosopersicus*) and four bottle gourd (*Lagenaria siceraria*) rootstocks. Grafted

plants were inoculated with a 2×10^4 conidia ml⁻¹ suspension of *P. xanthii* conidia and disease severity was rated 14 days after inoculation. Mickey Lee grafted on six PM-resistant watermelon rootstocks had significantly ($P \leq 0.05$) lower PM severity on cotyledons, 2nd true leaf, and upper leaves (rating for 3rd to 7th or 8th true leaf) compared with Mickey Lee grafted on susceptible watermelon USVL677-PMS or bottle gourd USVL848-PMS rootstocks. However, some of the resistant watermelon rootstocks did not impart significant resistance to the scion. The resistant bottle gourd rootstocks USVL482-PMR and USVL351-PMR provided significantly greater levels of resistance, compared with many of the resistant watermelon rootstocks. Grafting watermelon on resistant rootstocks may help mitigate the effects of PM on susceptible scion seedlings.

Podosphaera xanthii (Castagne) U. Braun & Shishkoff (syn. *Sphaerotheca fuliginea*), the causal agent of cucurbit powdery mildew (PM), is a major foliar pathogen that can severely limit production of cucurbit crops in greenhouses and fields (Jarvis 1992; Keinath and DuBose 2004, 2012). The pathogen is known to infect hypocotyl, cotyledons, stems, petioles, leaves, and fruit (Cohen et al. 2004; Davis et al. 2001; Jahn et al. 2002; Keinath and DuBose 2004, 2012; Kousik et al. 2008, 2011; McGrath 2017). Infection of young seedlings by *P. xanthii* can lead to reduced vigor and, in extreme cases, death of seedlings (Jarvis 1992). In addition, *P. xanthii* infection is known to predispose cucurbit plants to other foliar diseases (McGrath 2010). Powdery mildew can result in significant yield reduction of watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) in conventional and organic agriculture (Keinath 2015; Keinath and DuBose 2004; McGrath 2010). Powdery mildew has been reported to occur more frequently on watermelon in recent years (Keinath 2015; Keinath and Rennberger 2017; Kousik et al. 2011; Mercier et al. 2014), and hence the Watermelon Research and Development Group (WRDG) has considered it an important research priority (Kousik et al. 2016).

Most of the cultivated cucurbit crops including watermelon are susceptible to PM (Hammett 1977; Jahn et al. 2002; McGrath 2017). Furthermore, isolates of *P. xanthii* collected from one cucurbit crop have been shown to infect many other cucurbit crops (Hammett 1977; Lebeda et al. 2011). Cohen et al. (2004) and Jahn et al. (2002) have described

the cucurbit PM pathogen and its race classification system in extensive detail. The current race classification is based on melon (*Cucumis melo*) differentials and so far, seven *P. xanthii* races have been described (Cohen et al. 2004; Jahn et al. 2002; McGrath 2017; Pitrat et al. 1998). The possibility for existence of races based on other cucurbit differentials including watermelon has also been suggested (Kousik and Ikerd 2014). Similarly, several other race classification schemes for melon and other cucurbits have been put forth (Lebeda et al. 2008, 2011, 2012, 2016; McCreight 2006). However, races of *P. xanthii* have not been classified based on other cucurbit crops except melon, because of the lack of highly resistant germplasm and the need for well characterized differentials (Cohen et al. 2004). Very few commercial watermelon varieties and pollenizers with resistance to PM are currently available (Kousik and Ikerd 2016). However, resistance has been identified in watermelon germplasm resources (Davis et al. 2007; Tetteh et al. 2010) and the potential for existence of races based on watermelon differentials has been proposed (Kousik and Ikerd 2016). Apart from the potential for existence of races, *P. xanthii* has been known to rapidly evolve and develop resistance to fungicides and hence integrated strategies, including the appropriate use of fungicides, will be needed to manage PM (McGrath 2010, 2017). Currently, several conventional fungicides are available to effectively manage PM on cucurbits (Keinath 2015; Keinath and DuBose 2012; Keinath et al. 2010). However, the PM pathogen is known to rapidly develop on the underside of the leaves and many of the currently available fungicides, especially those approved for organic production, do not have translaminar activity to help manage this situation (McGrath 2010; 2017). In addition, it is difficult to deliver these products to the underside of the leaves where the pathogen can infect. Therefore, alternative management strategies are needed.

Grafting provides a new and quickly implemented option to incorporate important traits such as resistance to soilborne diseases and rootstock vigor, thus enabling and increasing production of important high-value vegetables including watermelon, other melon types, and tomato (Bruton et al. 2009; Cohen et al. 2007; Davis et al. 2008; Louws et al. 2010). In different regions of the world, watermelon is grafted on different types of rootstocks (Cohen et al. 2007; Lee and Oda 2003; Oda 1993, 2002). In the U.S.A., grafting watermelon on various rootstocks primarily as an alternative soilborne disease management strategy has been gaining popularity in recent years. This technique, however, has been utilized for many years in a number of Asian countries for soilborne disease management (Bruton et al. 2009; Cohen et al. 2007; Davis et al. 2008; Lee 1994). The use of gourd rootstocks for grafting watermelon in commercial

[†]Corresponding author: C. S. Kousik, E-mail: shaker.kousik@ars.usda.gov

Funding: This research was funded in part by a SCRI Vegetable Grafting grant award 2016-1498-08 and the SCRI CuCAP grant award 2015-51181-24285.

The use of trade, firm or corporation names in this publication is for the convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agriculture Research Service or Clemson University of any product or service to the exclusion of others that may also be suitable.

Accepted for publication 30 December 2017.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2018.

production to manage soilborne diseases first started in Japan and Korea in the 1920s (Lee 1994). The primary benefit of using bottle gourd (*Lagenaria siceraria*) and other gourds as rootstock for grafting watermelon is to manage Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (Cohen et al. 2007; Keinath and Hassell 2014; Yetisir et al. 2007). However, the majority of rootstocks that are currently being tested and used in the U.S.A. are relatively old rootstocks that were developed in Asia (King et al. 2010). In theory, grafting a susceptible scion on a resistant rootstock has the ability to rapidly provide resistance against soilborne diseases, thus potentially removing the necessity for a prolonged program to breed rootstocks (Cohen et al. 2007). Thus, from a conventional plant breeding perspective, a few well-developed rootstocks with the ability to provide important and desirable traits can be used with many different scion cultivars (Cohen et al. 2007). However, the appropriate rootstock-scion combination needs to be determined before large-scale use (Cohen et al. 2007; Davis et al. 2008; King et al. 2008). Even though the schematics of grafting for disease management seem to be very straightforward, numerous factors will have to be considered in selecting a suitable rootstock that will eventually be successful. These factors can include resistance to diseases prevailing in the region, adaptation of grafted plants to local conditions, and the appropriate rootstock-scion combination for a given region (Cohen et al. 2007; King et al. 2008; Yetisir and Sari 2003).

P. xanthii is known to infect rootstocks and watermelon scion seedlings before and after grafting (Keinath and DuBose 2012; Kousik et al. 2008). Kousik and Hassell (2009) reported high level of susceptibility of most of the commercial interspecific hybrid (*Cucurbita moschata* × *C. maxima*) and bottle gourd rootstocks evaluated in greenhouse trials. In addition, PM severity was shown to increase on rootstock seedlings when they were placed in a healing humid chamber soon after grafting and prior to transplanting (Hassell et al. 2008; Keinath and DuBose 2012). In order

for a rootstock seedling to be effective in a vegetable grafting project, the hypocotyls and cotyledons should be healthy and free of disease so they can provide the initial carbohydrates for rapid growth and development of the scion (Kousik et al. 2008). Therefore, we identified bottle gourd plant introductions (PIs) with resistance to PM and have developed highly resistant lines from these PIs by successive screening and selection for use in rootstock breeding programs (Kousik et al. 2008). Similarly, we selected PM-resistant watermelon PIs identified in previous studies (Davis et al. 2007; Kousik and Ikerd 2016; Tetteh et al. 2010; Thomas et al. 2005) and developed highly resistant germplasm by successive screening and selections. The objective of this study was to determine if PM-resistant bottle gourd and watermelon germplasm lines used as rootstocks for grafting will impart resistance to a susceptible watermelon seedling used as the scion.

Materials and Methods

Three experiments were conducted during the summer months of 2012, 2013, and 2014 at the U.S. Vegetable Laboratory (USVL) greenhouse in Charleston, SC, to determine if grafting seedlings of a PM-susceptible cultivar (scion) onto watermelon and bottle gourd rootstocks with resistance to PM would provide resistance to foliage of the scion.

Rootstocks. PM-resistant selections from watermelon (*Citrullus* sp.) PIs, bottle gourd (*L. siceraria*) PIs, and a susceptible watermelon line (USVL677-PMS) derived from PI 269677 were used as rootstocks. Original seeds of the PI were provided by the Plant Genetic Resources and Conservation Unit (PGRCU), Griffin, GA (<https://www.ars-grin.gov/>). Each resistant USVL line used in this study was obtained by self-pollinating by hand the most resistant single plant selections after screening plants from the original PI seed for three to four generations in a greenhouse. Details of the germplasm lines used in this study are presented in Table 1.

Table 1. Details of the U.S. Vegetable Laboratory (USVL) developed watermelon and bottle gourd germplasm lines used as rootstocks in this study

Rootstock ^w	Rootstock type ^x	Derived from accession ^y	Origin of germplasm collection (country)	Resistance to PM ^z
USVL225-PMR	<i>Citrullus amarus</i>	PI 189225	Zaire	R
USVL326-PMR	<i>C. amarus</i>	PI 482326	Zimbabwe	R
USVL331-PMR	<i>C. amarus</i>	PI 500331	Zambia	R
Mickey Lee	<i>Citrullus lanatus</i>	Cultivar	U.S.A.	S
USVL677-PMS	<i>C. lanatus</i>	PI 269677	Belize	S
USVL545-PMR	<i>C. lanatus</i>	PI 270545	Sudan	R
USVL074-PMR	<i>C. lanatus</i>	PI 459074	Botswana	R
USVL248-PMR	<i>C. lanatus</i>	PI 482248	Zimbabwe	R
USVL278-PMR	<i>C. lanatus</i>	PI 482278	Zimbabwe	R
USVL313-PMR	<i>C. lanatus</i>	PI 482313	Zimbabwe	R
USVL318-PMR	<i>C. lanatus</i>	PI 482318	Zimbabwe	R
USVL341-PMR	<i>C. lanatus</i>	PI 482341	Zimbabwe	R
USVL323-PMR	<i>C. lanatus</i>	PI 500323	Zambia	R
USVL443-PMR	<i>C. lanatus</i>	PI 508443	Korea	MR
USVL516-PMR	<i>Citrullus mucosospermus</i>	PI 326516	Ghana	R
USVL528-PMR	<i>C. mucosospermus</i>	PI 494528	Nigeria	R
USVL531-MDR	<i>C. mucosospermus</i>	PI 494531	Nigeria	R
USVL738-PMR	<i>C. mucosospermus</i>	PI 532738	Zaire	R
USVL002-PMR	<i>C. mucosospermus</i>	PI 560002	Nigeria	R
USVL003-PMR	<i>C. mucosospermus</i>	PI 560003	Nigeria	R
USVL005-PMR	<i>C. mucosospermus</i>	PI 560005	Nigeria	R
USVL006-PMR	<i>C. mucosospermus</i>	PI 560006	Nigeria	R
USVL008-PMR	<i>C. mucosospermus</i>	PI 560008	Nigeria	MR
USVL010-PMR	<i>C. mucosospermus</i>	PI 560010	Nigeria	MR
USVL020-MDR	<i>C. mucosospermus</i>	PI 560020	Nigeria	R
USVL024-PMR	<i>C. mucosospermus</i>	PI 560024	Nigeria	R
USVL482351-F1	<i>Lagenaria siceraria</i>	F1 hybrid	U.S.A.	R
USVL351-PMR	<i>L. siceraria</i>	PI 271351	India	R
USVL848-PMS	<i>L. siceraria</i>	PI 381848	India	S
USVL482-PMR	<i>L. siceraria</i>	PI 487482	Israel	R

^w USVL: U.S. Vegetable Laboratory-developed germplasm lines, each derived by successive screening and single plant selections for resistance to powdery mildew (PM) for four generations.

^x The rootstock type is the species based on the descriptions given in the germplasm resources information network (GRIN, <https://npgsweb.ars-grin.gov/>).

^y The plant introduction (PI) from which the germplasm was derived by successive screening and selection.

^z Reaction to powdery mildew race 1W. R: resistant, S: susceptible, and MR: moderately resistant based on evaluations at USVL and by other researchers (Davis et al. 2007; Tetteh et al. 2010; Thomas et al. 2005).

In 2012, 25 watermelon rootstocks and two bottle gourd rootstocks were evaluated (Table 1). Of the 25 watermelon rootstocks, three belonged to *Citrullus amarus* (syn: *C. lanatus* var. *citroides*, also known as cow melon or pickling melon), 10 were cultivated type watermelon that belonged to *C. lanatus*, and 12 were Egusi type watermelon that belonged to *C. mucospermus* (syn: *C. lanatus* var. *lanatus*) from the continent of Africa (Chomicki and Renner 2015; Paris 2015). In 2013, a total of 17 rootstocks were evaluated, and of these, 13 were watermelon and two were bottle gourd, selected based on the results of the 2012 study. In addition, two bottle gourd rootstocks, of which one was susceptible to PM (USVL848-PMS) and one a resistant F₁ hybrid (USVL482351-PMR), were also included in 2013. In 2014, a total of 10 watermelon rootstocks were selected for evaluation based on the results of 2013. The commercial watermelon rootstock from Syngenta, 'Ojjakyo,' was also included in 2014. Five bottle gourd rootstocks were evaluated in 2014, including a commercial rootstock 'Emphasis.' Sufficient seeds of the commercial rootstocks were not available to us in the previous years and hence they were not included in the 2012 and 2013 studies.

Susceptible watermelon scion and grafting. Plants of the PM-susceptible watermelon cultivar 'Mickey Lee' (Clifton Seed Company, Faison, NC) were used as the scion. Mickey Lee seedlings were grafted onto the PM-resistant or -susceptible rootstocks using the single cotyledon method described by Hassell et al. (2008). Immediately after grafting, the plants were allowed to heal in a humid chamber for a week (Hassell et al. 2008). The grafted plants and controls were transplanted into 7.6-cm square pots (3" Kord TRAD SQ Green, Kord Products, Toronto, Canada) 2 weeks after grafting and then placed on greenhouse benches. The susceptible scion Mickey Lee grafted on itself was also used as a susceptible check in 2012 and 2014.

A randomized complete block design (RCBD) with five replications of four plants for each rootstock was used to evaluate the grafted plants in 2012. In 2013, an RCBD with eight replications of four plants for each rootstock was evaluated. In 2014, an RCBD with six replications of four plants for each rootstock was evaluated. The variable number of replications each year was due to the amount of rootstock seed that was available and the grafting efficiency.

Inoculation and PM ratings. A PM isolate collected from Mickey Lee growing in the greenhouse was routinely maintained on 'Early Prolific Straight Neck' squash and Mickey Lee watermelon plants. The isolate was confirmed as *P. xanthii* using specific PCR primers as previously described (Kousik et al. 2011; Takamatsu and Kano 2001). The PM isolate occurring in the greenhouse in Charleston was classified as Race 1 based on melon (*C. melo*) differentials (Cohen et al. 2004; McGrath 2017; Pitrat et al. 1998). *P. xanthii* conidia collected from severely infected watermelon leaves was used to prepare a conidial suspension (2×10^4 conidia/mL) in sterile water containing 0.02% Tween 20. Inoculum suspension of conidia was then sprayed on grafted plants using a handheld pump sprayer 3 weeks after grafting. Preparation of the inoculum and inoculation was done within 30 min of dislodging the conidia from the leaves. Powdery mildew naturally occurs in the greenhouse; however, plants were inoculated for uniformity. In 2012, grafted plants were inoculated on 11 June. In 2013, grafted plants were inoculated on 31 May, and in 2014, on 9 June. Plants were carefully watered daily to prevent splashing onto the leaves. To prevent whitefly and aphid infestation, plants were treated 2 days after transplanting with insecticide Merit 2.5 G (a.i. imidacloprid). The greenhouse was maintained at $26 \pm 2^\circ\text{C}$ without supplemental lighting. Disease severity ratings for PM were recorded using a modified Horsfall-Barrett scale of increasing disease severity (0 to 10 scale) about 2 weeks after

Table 2. Development of powdery mildew (PM) on nongrafted or on susceptible watermelon scion 'Mickey Lee' (ML) grafted on PM mildew resistant and susceptible watermelon and bottle gourd rootstocks, 2012

Rootstock	Rootstock type ^w	PM ^x on rootstock cotyledon	PM ^y on scion (ML) 2nd true leaf	PM ^y on scion (ML) upper leaves
Mickey Lee	CL	81.2 a ^z	87.8 a	42.5 abc
USVL677-PMS	CL	90.1 a	87.1 a	49.7 a
Mickey Lee, nongrafted	CL	52.9 b	82.2 ab	46.9 ab
USVL005-PMR	CM	4.0 def	75.7 abc	35.1 a-e
USVL341-PMR	CL	0.0 f	70.7 a-d	37.6 a-d
USVL010-PMR	CM	9.8 c-f	70.6 a-d	34.5 a-e
USVL318-PMR	CL	3.0 def	67.5 b-e	37.1 a-e
USVL024-PMR	CM	1.2 ef	67.5 b-e	37.1 a-d
USVL313-PMR	CL	6.3 def	66.0 b-e	33.2 a-e
USVL006-PMR	CM	22.8 cde	65.8 b-e	31.5 a-e
USVL008-PMR	CM	37.9 bc	65.7 b-e	30.7 a-e
USVL4437-PMR	CL	33.2 bc	64.3 b-e	17.0 e-h
USVL326-PMR	CA	2.3 ef	62.1 b-e	20.3 d-g
USVL002-PMR	CM	7.0 def	61.6 cde	32.6 a-e
USVL020-MDR	CM	4.6 def	59.1 c-f	31.8 a-e
USVL278-PMR	CL	29.2 cd	58.2 c-f	28.0 b-f
USVL545-PMR	CL	0.3 f	57.9 c-f	30.5 a-f
USVL003-PMR	CM	1.8 ef	57.8 c-f	21.8 d-g
USVL225-PMR	CA	12.5 c-f	56.3 c-f	26.6 b-f
USVL074-PMR	CL	1.1 ef	55.2 c-f	23.2 c-g
USVL738-PMR	CM	0.4 f	53.9 def	29.6 b-f
USVL516-PMR	CM	2.3 def	53.8 def	18.7 d-h
USVL331-PMR	CA	3.0 def	51.5 def	8.6 gh
USVL323-PMR	CL	7.8 def	48.6 efg	17.3 e-h
USVL248-PMR	CL	3.0 def	47.5 efg	19.2 e-h
USVL531-MDR	CM	2.4 def	46.7 efg	20.3 e-f
USVL528-PMR	CM	11.1 def	40.5 fgh	13.1 fgh
USVL351-PMR	LS	0.2 f	28.8 gh	8.1 gh
USVL482-PMR	LS	0.2 f	22.5 h	5.0 h

^w CL: *Citrullus lanatus*, CM: *C. mucospermus*, CA: *C. amarus*, LS: *Lagenaria siceraria*.

^x PM ratings for the rootstock cotyledons based on a 0-10 scale where; 0 = no PM to 10 = 98 to 100% cotyledon or leaf area covered with PM conidia.

^y PM ratings for the 2nd true leaf and the upper leaves (combined rating for 3rd to 7th or 8th true leaf) of susceptible ML scion. PM ratings are on a 0 to 10 scale (0 = no PM to 10 = 98 to 100% leaf area covered with PM and leaf dying).

^z Means within a column with the same letters do not differ significantly based on Fisher's Protected LSD ($\alpha = 0.05$).

inoculation, where 0 = no visible PM, 1 = very sparse mycelial growth on leaves or cotyledons with very few to no visible conidia (0 to 3%), 2 = 4 to 6% of leaf area covered with PM and sparse development of conidia, 3 = 7 to 12%, 4 = 13 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, 7 = 76 to 87% leaf area covered with PM and presence of abundant conidia, 8 = 88 to 94%, 9 = 95 to 97% and 10 = 98 to 100% of leaf or cotyledon area covered with abundant conidia and leaf dying or dead.

Statistical analysis. Data on PM severity was recorded using the 0 to 10 scale for the single rootstock cotyledons, the scion cotyledons (if present), the 2nd true leaf of the Mickey Lee scion, and the upper leaves (rating for 3rd to 7th or 8th true leaf on each grafted plant) of the scion. One rating for upper leaves was given for the 3rd to 7th or 8th true leaf for each grafted plant that was similar to rating a whole plant. However, the scion cotyledons or the 2nd true leaf was not considered for this rating. Data were not recorded on the first true leaves as in most cases the first true leaf had senesced. Data were converted to midpoint percentages. The PROC GLIMMIX procedure of SAS was used to analyze all the data and means were separated using the PDIF option ($P = 0.05$). Pearson's correlation coefficients among the disease ratings on rootstock cotyledon, scion cotyledon, 2nd true leaf, and the upper leaves were calculated using the PROC CORR procedure of SAS using the data for each replication for each year. In addition, for 2013 and 2014, correlations between the bottle gourd or watermelon rootstock cotyledons and the Mickey Lee scion cotyledon, 2nd true leaf, and upper leaves was also analyzed separately. For all experiments, disease reduction (%) was calculated based on PM severity on the 2nd true leaf of the scion on each rootstock relative to the PM severity on the 2nd true leaf of the scion on the highly susceptible rootstock USVL677-PMS that was used as a susceptible check. Disease severity on the 2nd true leaf of Mickey Lee grafted on USVL677-PMS was considered as 100% for each year and the relative percent disease reduction was calculated. Only rootstocks that were evaluated in 2014 and in at least one other year were included in the analysis of percent disease reduction compared with Mickey Lee grafted on USVL677-PMS. For statistical analysis, the data from each year was considered as a replication. Means for overall disease reduction was analyzed using the PROC GLIMMIX procedure of SAS. Means separation was done using the PDIF option ($P \leq 0.05$) on arcsine transformed percentages. Specific contrasts to compare disease severity on the Mickey Lee scion grafted on PM-resistant watermelon and resistant bottle gourd rootstocks was conducted using the PROC GLIMMIX and the LSMEANS procedure of SAS for each experiment. The susceptible and moderately resistant rootstocks as described in Table 1 were not included in this analysis.

Results

In 2012, significantly ($P \leq 0.0001$) less PM colonization was seen on the rootstock cotyledons of 18 PM-resistant watermelon and two bottle gourd rootstocks (Table 2, Fig. 1). In contrast, severe PM was observed on the rootstock cotyledon of Mickey Lee and USVL677-

Fig. 1. Grafting on a powdery mildew (PM)-resistant rootstock (USVL482-PMR, right) imparts resistance/tolerance to a susceptible scion ('Mickey Lee') cotyledons compared with grafting on a susceptible rootstock (left). Both the rootstock (large cotyledon on right) and the two scion cotyledons are relatively free of PM compared with the dying rootstock and scion cotyledon on the left.

PMS. PM was severe on the 2nd true leaf of the susceptible Mickey Lee scion grafted onto itself, nongrafted Mickey Lee, and grafted on USVL677-PMS (Fig. 2). In contrast, PM severity was significantly ($P \leq 0.001$) less on the 2nd true leaf of Mickey Lee plants grafted on 14 of the PM-resistant watermelon rootstocks (Table 2, Fig. 2). Similarly, PM was significantly less on the 2nd true leaf and the upper leaves (one combined rating for 3rd to 7th or 8th true leaf) of Mickey Lee grafted on the PM-resistant bottle gourd rootstocks (USVL482-PMR and USVL351-PMR). Several PM-resistant watermelon rootstocks (e.g., USVL331-PMR, USVL531-PMR, and USVL528-PMR) also conferred varying levels of resistance to the upper leaves of the susceptible scion. Though some watermelon rootstocks imparted resistance to the susceptible scion, there were some PM-resistant rootstocks that did not. For example PM on plants of Mickey Lee grafted on to the highly resistant rootstocks USVL005-PMR or USVL341-PMR were not statistically different from Mickey Lee grafted on to itself or Mickey Lee grafted on USVL677-PMS (Table 2).

In 2013 as well, PM severity was significantly ($P \leq 0.001$) less on the cotyledons of the resistant rootstocks (Table 3) compared with the susceptible checks USVL677-PMS and USVL848-PMS. Cotyledons of seven of the watermelon rootstocks had no observable PM on them and the cotyledons of the two bottle gourd rootstocks had minimal to no PM. A hybrid bottle gourd rootstock USVL482351-F1 that was a cross of USVL482-PMR X USVL351-PMR, also had no observable PM on its cotyledons. In contrast, PM was severe on the rootstock cotyledons of the highly susceptible watermelon check USVL677-PMS and Mickey Lee. Cotyledons of Mickey Lee scion grafted onto the susceptible watermelon USVL677-PMS and the bottle gourd rootstock USVL848-PMS were severely diseased and covered with PM. In contrast, significantly less PM was observed on cotyledons of the susceptible Mickey Lee scion grafted on all resistant watermelon and bottle gourd rootstocks, except USVL443-PMR and USVL331-PMR compared with Mickey Lee grafted on USVL677-PMS. Similarly, significantly less PM was seen on the 2nd true leaf of Mickey Lee grafted on all resistant rootstocks (e.g., USVL225-PMR, USVL531-PMR) compared with those on the susceptible rootstocks ($P \leq 0.0001$). Grafting Mickey Lee on the PM-resistant bottle gourd rootstocks USVL351-PMR and USVL482-PMR and the hybrid rootstock, USVL482351-PMR-F1, resulted in significantly less PM on the 2nd true leaf and the upper leaves compared with Mickey Lee grafted on the susceptible check USVL848-PMS. In 2013, significant differences in levels of PM were observed on the upper true leaves of the susceptible Mickey Lee grafted on PM-resistant rootstocks ($P \leq 0.001$). However, the severity was not reduced as much as observed on the 2nd true leaf. Powdery mildew severity was significantly lower on upper leaves

Fig. 2. A, Reduced development of powdery mildew (PM) on the second true leaf of a susceptible watermelon scion ('Mickey Lee') grafted on a PM-resistant rootstock (leaf on right) in contrast to severe PM and conidial formation on second true leaf of Mickey Lee grafted on a susceptible rootstock (leaf on left). B, Reduced PM development on second and upper true leaves of a susceptible watermelon scion (Mickey Lee) grafted on a PM-resistant rootstock USVL482-PMR (plant on right) in contrast to severe PM and conidial formation on the second true leaf and upper leaves of Mickey Lee grafted on a susceptible rootstock USVL677-PMS (plant on left).

of Mickey Lee scion grafted on bottle gourd hybrid (USVL482351F1-PMR) rootstock and the parents (USVL482-PMR and USVL351-PMR) compared with Mickey Lee on susceptible bottle gourd check USVL848-PMS. In 2013, the Mickey Lee plants grafted on itself did not survive and hence could not be included as a part of the evaluation. However, the Mickey Lee plants grafted on all the other scions, including the one on the PM-susceptible rootstock USVL677-PMS, remained vigorous after grafting and performed well.

The results from 2014 followed a similar trend as in 2013 on the rootstock cotyledon, PM-susceptible scion cotyledon, 2nd true leaf, and upper leaves. In 2014, the three PM-resistant bottle gourd rootstock cotyledons had minimal to no PM (Table 4). Similarly, the seven PM-resistant watermelon rootstocks had sparse PM and some

had no PM (e.g., USVL225-PMR). In contrast, the susceptible check USVL677-PMS cotyledons had severe PM. Similarly, Mickey Lee grafted onto itself or the nongrafted Mickey Lee also had severe PM on the scion cotyledon, the 2nd true leaf, and the upper leaves, and were not significantly different from Mickey Lee grafted on susceptible USVL677-PMS. The cotyledons of commercial rootstocks Emphasis and Ojjakyo as well as those of the susceptible bottle gourd rootstock USVL848-PMS had significantly higher levels of PM compared with the PM-resistant rootstocks. Powdery mildew on the cotyledons of the susceptible Mickey Lee scion grafted on the three resistant bottle gourd rootstocks was significantly less severe compared with when grafted on the susceptible rootstock USVL848-PMS ($P < 0.0001$). Similarly, the cotyledons of Mickey

Table 3. Development of powdery mildew (PM) on a susceptible watermelon scion ‘Mickey Lee’ (ML) grafted on PM-resistant watermelon and bottle gourd rootstocks, 2013

Rootstock	Rootstock type ^w	PM ^x on rootstock cotyledon	PM ^y on scion (ML) cotyledon	PM ^y on scion (ML) 2nd true leaf	PM ^y on scion (ML) upper leaves
USVL677-PMS	CL	78.4 a ^z	47.9 ab	64.1 a	12.6 a-d
USVL848-PMS	LS	71.0 a	60.0 a	58.1 a	18.1 ab
USVL443-PMR	CL	50.4 b	44.2 ab	41.0 b	7.3 def
USVL323-PMR	CL	8.9 cd	20.6 cde	37.5 b	15.5 ab
USVL341-PMR	CL	0.0 cd	6.1 g	36.5 b	6.9 def
USVL020-MDR	CM	2.7 cd	10.5 efg	36.1 b	11.5 b-e
USVL516-PMR	CM	0.0 d	9.8 fg	34.3 bc	10.5 b-e
USVL248-PMR	CL	0.0 d	20.2 c-f	32.7 bcd	12.7 a-e
USVL738-PMR	CM	0.0 d	9.3 efg	28.1 bcd	12.6 a-e
USVL331-PMR	CA	0.0 d	33.7 bc	27.6 bcd	21.3 a
USVL003-PMR	CM	5.8 cd	11.7 d-g	27.5 bcd	19.7 a
USVL528-PMR	CM	14.7 c	24.6 cd	25.1 b-e	14.3 abc
USVL531-MDR	CM	0.0 d	21.2 c-f	21.1 cde	6.7 def
USVL225-PMR	CA	0.0 d	3.0 g	20.4 cde	8.4 cde
USVL351-PMR	LS	0.4 cd	19.9 c-f	20.0 de	6.5 ef
USVL482351-F1	LS	0.0 d	9.6 d-g	18.2 de	2.4 f
USVL482-PMR	LS	0.1 cd	4.0 g	12.1 e	6.5 ef

^w CL: *Citrullus lanatus*, CM: *C. mucospermus*, CA: *C. amarus*, LS: *Lagenaria siceraria*.

^x PM ratings for the rootstock cotyledons based on a 0 to 10 scale where 0 = no PM to 10 = 98 to 100% cotyledon or leaf area covered with PM conidia.

^y PM ratings for the scion cotyledon, 2nd true leaf and the upper leaves (combined rating for 3rd to 7th or 8th true leaf) of susceptible ML scion. PM ratings are on a 0 to 10 scale (0 = no PM to 10 = 98 to 100% leaf area covered with PM and leaf dying).

^z Means within a column with the same letters do not differ significantly based on Fisher’s Protected LSD ($\alpha = 0.05$).

Table 4. Development of powdery mildew (PM) on a susceptible watermelon scion ‘Mickey Lee’ (ML) grafted on powdery mildew resistant watermelon and bottle gourd rootstocks, 2014

Rootstock	Rootstock type ^w	PM ^x on rootstock cotyledon	PM ^y on scion (ML) cotyledon	PM ^y on scion (ML) 2nd true leaf	PM ^y on scion (ML) upper leaves
Mickey Lee	CL	47.0 b ^z	72.1 ab	81.8 a	34.1 a
Mickey Lee, nongrafted	CL	-	84.7 a	78.9 ab	34.1 a
USVL677-PMS	CL	79.8 a	81.0 a	68.7 abc	31.3 ab
USVL848-PMS	LS	31.5 b	48.3 c	67.0 abc	22.5 b-e
Emphasis	LS	25.3 b	53.9 bc	66.3 abc	15.5 def
Ojjakyo	CA	23.8 b	76.3 a	63.9 bcd	26.6 abc
USVL443-PMR	CL	34.4 b	45.3 cd	53.3 cde	23.9 a-d
USVL323-PMR	CL	7.4 c	17.0 efg	51.9 cde	18.0 cde
USVL248-PMR	CL	2.3 cd	32.6 c-f	48.8 de	16.0 de
USVL531-MDR	CM	4.3 cd	28.8 def	44.1 ef	14.5 efg
USVL003-PMR	CM	2.4 cd	17.6 efg	42.9 efg	16.4 de
USVL225-PMR	CA	0.0 d	13.6 efg	41.2 efg	16.4 cde
USVL528-PMR	CM	3.0 cd	32.9 cde	40.7 e-h	16.6 cde
USVL331-PMR	CA	0.9 d	17.5 efg	29.5 f-i	18.0 cde
USVL482-PMR	LS	0.0 d	5.8 g	26.6 ghi	6.7 gh
USVL351-PMR	LS	0.0 d	10.0 fg	23.5 hi	7.2 fgh
USVL482351-F1	LS	0.1 d	11.4 eg	20.8 i	4.7 h

^w CL: *Citrullus lanatus*, CM: *C. mucospermus*, CA: *C. amarus*, LS: *Lagenaria siceraria*.

^x PM ratings for the rootstock cotyledons based on a 0 to 10 scale where 0 = no PM to 10 = 98 to 100% cotyledon or leaf area covered with PM conidia.

^y PM ratings for the scion cotyledon, 2nd true leaf and the upper leaves (combined rating for 3rd to 7th or 8th true leaf) of susceptible ML scion. PM ratings are on a 0 to 10 scale (0 = no PM to 10 = 98 to 100% leaf area covered with PM and leaf dying).

^z Means within a column with the same letters do not differ significantly based on Fisher’s Protected LSD ($\alpha = 0.05$).

Lee had less severe PM when grafted on the seven resistant watermelon rootstocks compared with Mickey Lee grafted on itself or the susceptible rootstock USVL677-PMS. The 2nd true leaf of the Mickey Lee scion grafted on six resistant watermelon rootstocks (e.g., USVL225-PMR, USVL528-PMR) had significantly less severe PM compared to when grafted on USVL677-PMS or self-grafted on Mickey Lee. Mickey Lee grafted on the three PM-resistant bottle gourd rootstocks had significantly less severe PM on the 2nd true leaf compared with Mickey Lee grafted on the susceptible check USVL848-PMS or the commercial rootstock Emphasis. A similar trend was observed on the upper leaves of the susceptible Mickey Lee scions and severity was significantly lower when grafted on resistant rootstocks compared with susceptible rootstocks.

Highly significant positive correlations ($P \leq 0.0001$ to $P = 0.05$) were observed between ratings recorded for rootstock cotyledon, the grafted scion cotyledon, the 2nd true leaf, and the upper leaves in all three years (Table 5). In short, the scion response was correlated with the response of the rootstock to PM. In 2012, highly significant ($P \leq 0.0001$) correlations between the rootstock cotyledon, the 2nd true leaf, and the upper leaves was observed. Similarly, in 2013, significant correlations ($P \leq 0.05$) between the rootstock cotyledon, the 2nd true leaf, and the upper leaves was observed. However, in 2013, the correlation coefficients were relatively low ($r = 0.270$, $P \leq 0.05$) for the correlations between the rootstock cotyledon and the upper leaves and the scion cotyledon and the upper leaves ($r = 0.294$, $P \leq 0.05$). In 2013, highly significant ($P \leq 0.0001$) correlations between the disease severity values for only the bottle gourd rootstock cotyledons and the Mickey Lee scion cotyledons ($r = 0.877$), 2nd true leaf ($r = 0.843$), and the upper leaves ($r = 0.782$) were observed. Similarly, highly significant correlation ($P \leq 0.0001$) between the watermelon rootstock cotyledon and the scion cotyledon ($r = 0.746$) and the 2nd true leaf ($r = 0.524$) was observed. However, the watermelon rootstock cotyledon and the scion upper leaves were not significantly correlated. In 2014, highly significant ($P \leq 0.0001$) correlations between the rootstock cotyledon, the scion cotyledon, 2nd true leaf, and the upper leaves were observed. Similarly, a highly significant correlation between the scion cotyledon and the 2nd true leaf was observed in 2014. The bottle gourd rootstock cotyledon ratings were highly correlated ($P \leq 0.0001$) with the scion cotyledon, 2nd true leaf, and the upper leaves ($r = 0.808$ to 0.886). Similarly, highly significant correlations ($P \leq 0.0001$) between the watermelon rootstock cotyledon and the scion cotyledon, 2nd true leaf, and the upper leaves ($r = 0.587$ to 0.744) were observed (Table 5).

Overall, grafting the susceptible Mickey Lee scion on the PM-resistant bottle gourd rootstocks resulted in 61 to 81% disease reduction

on the 2nd true leaf when compared with grafting on the susceptible watermelon rootstock USVL677-PMS that was used as the check in all three years of the study (Table 6). All eight PM-resistant watermelon rootstocks evaluated over three years also imparted significant levels of disease reduction (22 to 69%) to the susceptible Mickey Lee scion compared with USVL677-PMS. The three PM-resistant bottle gourd rootstocks reduced PM severity on the grafted susceptible scion by 60 to 79% in 2013 and 2014 relative to the disease severity on the susceptible bottle gourd check USVL848-PMS (data not presented). There were no significant differences between the rootstock cotyledons of the resistant watermelon or bottle gourd in 2012 and 2013 as expected

Table 6. Percent disease reduction on the 2nd true leaf of the powdery mildew (PM)-susceptible 'Mickey Lee' (ML) scion grafted on various PM-resistant watermelon and bottle gourd rootstocks over three years

Rootstock ^w	Rootstock type ^x	Percent disease reduction ^y			Overall mean ^z
		2012	2013	2014	
USVL482-PMR	LS	74	61	81	72 a
USVL482351-F1	LS	-	70	72	69 ab
USVL351-PMR	LS	67	66	69	67 ab
USVL528-PMR	CM	54	41	61	52 bc
USVL331-PMR	CA	41	57	57	52 bc
USVL531-MDR	CM	46	36	67	50 bc
USVL225-PMR	CA	35	40	68	48 c
USVL003-PMR	CM	34	38	57	43 c
USVL443-PMR	CL	22	66	36	42 c
USVL248-PMR	CL	45	29	49	41 c
USVL323-PMR	CL	44	24	41	38 c
USVL848-PMS	LS	-	2	9	5 d
Mickey Lee	CL	0	-	0	0 e
USVL677-PMS	CL	0	0	0	0

^w Seedlings of Mickey Lee were grafted onto various PM-resistant or susceptible rootstocks.

^x CL: *Citrullus lanatus*, CM: *C. mucospermus*, CA: *C. amarus*, LS: *Lagenaria siceraria*.

^y Percent disease reduction was calculated based on PM severity on the 2nd true leaf of the ML scion grafted on the susceptible watermelon rootstock USVL677-PMS compared with ML scion grafted on the resistant watermelon or bottle gourd rootstocks. USVL677-PMS was selected as the susceptible watermelon rootstock and was included in all three years of the study. Only rootstocks included in 2014 and evaluated for at least two years were included in the analysis.

^z Means within the overall mean column followed by the same letter are not significantly different ($\alpha = 0.05$). Year was considered as a random variable in the analysis.

Table 5. Pearson's correlation coefficients between powdery mildew (PM) severity ratings on rootstock cotyledon, susceptible 'Mickey Lee' (ML) scion cotyledon, 2nd true leaf, and upper leaves

	PM on scion cotyledon	PM on scion second true leaf	PM on scion upper leaves
2012			
PM on rootstock cotyledon	— ^x	0.476** ^y	0.392**
PM on scion second true leaf			0.674**
2013			
PM on rootstock cotyledon	0.793**	0.613**	0.270*
PM on scion cotyledon		0.568**	0.294*
PM on scion second true leaf			0.414**
Bottle gourd rootstock cotyledon ^z	0.877**	0.843**	0.782**
Watermelon rootstock cotyledon	0.746**	0.524**	0.076 ^{NS}
2014			
PM on rootstock cotyledon	0.775**	0.644**	0.660**
PM on scion cotyledon		0.759**	0.665**
PM on scion second true leaf			0.640**
Bottle gourd rootstock cotyledon	0.886**	0.852**	0.808**
Watermelon rootstock cotyledon	0.744**	0.587**	0.612**

^x PM severity data were not recorded on the scion (ML) cotyledons in 2012 as the cotyledons had senesced on many scion plants.

^y **indicates significance at $P = 0.0001$, * indicates significance at $P = 0.05$, and NS = not significant.

^z Correlations between the bottle gourd or watermelon rootstock cotyledons and ML scion cotyledons, 2nd true leaf, and upper leaves were also analyzed separately for 2013 and 2014.

(Table 7). However, even though the disease severity on the cotyledons of the resistant watermelon rootstocks were low (3.2%) and will be considered resistant, it was significantly different than the bottle gourd cotyledon (0%). Overall, the PM-resistant bottle gourd rootstocks significantly ($P \leq 0.0001$) imparted greater levels of resistance to the 2nd true leaf and the upper leaves of the susceptible scion Mickey Lee compared with the resistant watermelon rootstocks in all the experiments (Table 7).

Discussion

Severe PM can occur and reduce the vigor of watermelon and other cucurbit seedlings in greenhouses in the southeastern U.S.A. (Keinath and DuBose 2012; Kousik and Hassell 2009; Kousik et al. 2008). In such situations, grafting watermelon on PM-resistant rootstocks will be beneficial and the ability of the resistant rootstocks to impart resistance to susceptible scions will be an added advantage. PM-resistant watermelon and bottle gourd rootstocks imparted resistance to the susceptible scion Mickey Lee in all three years of this greenhouse study. Overall, the disease reduction obtained by grafting the susceptible Mickey Lee seedlings on PM-resistant rootstocks ranged from 22 to 81% over the three years on the 2nd true leaf. Sakata et al. (2006) also reported high levels (15 to 93%) of PM reduction in susceptible cucumber scions grafted on resistant rootstocks. Highly significant correlations were observed among the ratings for the rootstock cotyledons and the ratings on susceptible scion cotyledons and the true leaves, suggesting a direct effect of the rootstocks on response of scion to PM. Despite the significant correlations, the correlation coefficients were relatively low, especially in 2012 and with upper leaves in 2013. Similarly, when the data for watermelon rootstocks were analyzed separately, there was no significant correlation between

the rootstock cotyledon and the upper leaves. This could be because of the large number of replications and rootstocks evaluated and the variability in the levels of resistance imparted by the different rootstocks (22 to 81% disease reduction over check). In addition, in 2012 and 2013, some of the rootstocks, though they were resistant, did not impart significant levels of resistance to the scion, and overall the disease levels on upper leaves was relatively low in 2013 compared with 2014. However, when the data for only the bottle gourd rootstocks were analyzed, the correlation coefficients ranged from $r = 0.782$ to 0.886 ($P \leq 0.0001$) for 2013 and 2014, indicating the effectiveness of bottle gourd rootstocks in imparting PM resistance to the scion. In general, the disease severity on the upper true leaves (rating for 3rd to 7th or 8th true leaf) was less than on the 2nd true leaf regardless of watermelon or bottle gourd rootstock. This could be because a combined rating for five to six leaves was made and some of the upper leaves would have developed after the inoculation. In addition, the latent period required for development of powdery mildew prior to the rating on the newer leaves would also affect the ratings.

The three PM-resistant bottle gourd rootstocks effectively reduced PM on the susceptible scion over the three years of the study when compared with the susceptible watermelon check USVL677-PMS (Table 6). Similarly, the three PM-resistant bottle gourd rootstocks reduced PM severity on the grafted susceptible scion by 60 to 79% in 2013 and 2014 when compared with the susceptible bottle gourd check USVL848-PMS. In the current study, the PM-resistant bottle gourd rootstocks imparted greater levels of resistance to 2nd and upper true leaves of the susceptible Mickey Lee scion compared with the resistant watermelon rootstocks (Table 7). In other studies, resistant bottle gourd (*L. siceraria*) rootstocks imparted resistance to a susceptible *Cucurbita* scion against carmine spider mites (Edelstein et al. 2000). Grafting has also been reported to affect other diseases of the foliage in several crops by decreasing, or in some instances, increasing disease development (Guan et al. 2012; Louws et al. 2010). In addition, grafting onto interspecific hybrid squash rootstocks was shown to increase phenolic contents in watermelon scions and this increase in secondary metabolites was thought to reduce incidence of foliar pests (Evrenosoglu et al. 2010).

The two PM-resistant *C. amarus* rootstocks USVL225-PMR and USVL331-PMR significantly reduced PM (percent disease reduction: 35 to 68%, Table 6) on the grafted Mickey Lee scion compared with the susceptible check USVL677-PMS over the three years of the study. In metabolomics analysis conducted on Mickey Lee grafted on USVL225-PMR using NMR spectroscopy, it was shown that certain metabolites were significantly different quantitatively in Mickey Lee and USVL225-PMR (Mahmud et al. 2015). However, the metabolites were qualitatively similar (Mahmud et al. 2015) between the rootstock (USVL225-PMR) and the scion (Mickey Lee). Grafting Mickey Lee on USVL225-PMR significantly increased metabolites choline, 5-hydroxyindole-3-acetate, and melatonin levels in Mickey Lee scions (Mahmud et al. 2015). Melatonin has been reported to trigger or mediate plant defense response against bacterial and fungal plant pathogens (Lee et al. 2014; Qian et al. 2015; Wei et al. 2017; Yin et al. 2013). Therefore, we are currently investigating the role of melatonin on development of PM on watermelon and other cucurbits including rootstocks used in this study. In the present study, a race 1 isolate of *P. xanthii* classified based on melon differentials was used for inoculating the grafted plants. In a preliminary study, USVL531-MDR and USVL225-PMR were found to be resistant to 11 isolates of both race 1 and race 2 from different states in the U.S.A. and in contrast, USVL677-PMS was susceptible to all the isolates (Kousik and Ikerd 2014; Kousik, unpublished results). It would be interesting to determine if the PM-resistant bottle gourd and watermelon rootstocks will also provide similar levels of disease reduction to susceptible scion against other races, other than race 1 and strains from different regions of the U.S.A.

In this study, the resistance imparted by the various watermelon rootstocks was variable. Furthermore, some of the highly resistant watermelon rootstocks did not impart resistance to the scion. This was the primary reason we evaluated a larger number of rootstocks in 2012 and narrowed it down in the next two years. Sakata et al.

Table 7. Contrasts between disease severity on scion cotyledon, 2nd true leaf and upper leaves of the powdery mildew (PM)-susceptible 'Mickey Lee' (ML) scion grafted only on the PM-resistant watermelon and resistant bottle gourd rootstocks in experiments conducted from 2012 to 2014

	Powdery mildew severity (%) ^x		
	Watermelon rootstock ^y	Bottle gourd rootstock ^y	P-value ^z
2012			
PM on rootstock cotyledon	5.5	0.2	0.1280
PM on scion (ML) 2nd true leaf	58.3	25.6	<0.0001
PM on scion (ML) upper leaves	26.3	6.5	<0.0001
2013			
PM on rootstock cotyledon	3.6	0.2	0.1441
PM on scion (ML) cotyledon	15.0	11.2	0.5644
PM on scion (ML) 2nd true leaf	29.7	16.8	<0.0001
PM on scion (ML) upper leaves	12.7	5.1	<0.0001
2014			
PM on rootstock cotyledon	3.2	0.0	<0.0001
PM on scion (ML) cotyledon	22.6	9.0	0.0055
PM on scion (ML) 2nd true leaf	42.7	23.6	<0.0001
PM on scion (ML) upper leaves	16.5	6.2	<0.0001

^x PM ratings are based on a 0 to 10 scale (0 = no PM to 10 = 98 to 100% leaf area covered with PM and leaf dying).

^y Data for only the resistant watermelon and bottle gourd rootstocks were included in the analysis. The moderately resistant and susceptible rootstocks were not included.

^z P-values ≤ 0.05 indicate a significant difference between the disease severity on the resistant watermelon or bottle gourd rootstocks and the grafted ML scion.

(2006) also observed a similar phenomenon whereby not all PM-resistant rootstocks evaluated imparted resistance to the susceptible cucumber scion. Grafting on resistant rootstocks does not always impart resistance to the scions. For example, grafting a susceptible scion on *Papaya ringspot* (PRSV) and *Squash vein yellowing virus* (SqVYV) resistant rootstocks was not effective in managing either of these diseases (Webster et al. 2012). However, grafting was shown to be effective in reducing virus in some studies (Park et al. 2005; Wang et al. 2002). There are numerous examples of grafting imparting varying levels of resistance to the scion against foliar diseases (Guan et al. 2012; Louws et al. 2010; Sakata et al. 2006). Therefore, PM-resistant rootstock will have to be selected by careful screening with various scions prior to large-scale use. Furthermore, even when PM-resistant rootstocks are used, it is appropriate to use an integrated disease management strategy to effectively manage the disease and prevent breakdown of the resistance, as *P. xanthii* is known to rapidly evolve.

Sakata et al. (2006) demonstrated that resistance to PM was imparted to adult cucumber plants grafted on PM-resistant rootstocks up to the 17th leaf. In field studies, grafting susceptible scions on *Phytophthora* fruit rot-resistant watermelon rootstocks imparted some level of tolerance to *Phytophthora capsici* development on the fruit (Kousik et al. 2012), suggesting occurrence of long-distance translocation of resistance traits in the scion. However, the level of resistance imparted was not sufficient to completely prevent *Phytophthora* fruit rot on grafted susceptible plants (Kousik et al. 2012). In the present study, complete resistance to PM was not imparted to the susceptible scion. Further experiments will be needed to determine if combining resistance imparted by grafting on resistant rootstocks with foliar applications of a systemic resistance activator (e.g., Actigard) or increasing the spray intervals of conventional fungicides might effectively manage PM on susceptible but popular watermelon varieties. The current study was conducted on seedlings in the greenhouse; however, similar field studies will be needed to determine if PM-resistant rootstocks can impart resistance to susceptible scions, especially during fruit development stages when more severe PM is often observed.

Acknowledgments

The authors wish to acknowledge the technical assistance provided by Jennifer Ikerd, Kim Alford, and Mark Schaffer in conducting many of these experiments. We also thank Drs. William Rutter and Patrick Wechter for critical review and suggestions to improve the manuscript prior to submission.

Literature Cited

- Bruton, B. D., Fish, W. W., Roberts, W., and Popham, W. 2009. The influence of rootstock selection on fruit quality attributes of watermelon. *Open Food Sci. J.* 3:15-34.
- Chomicki, G., and Renner, S. S. 2015. Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museonomics. *New Phytol.* 205:526-532.
- Cohen, R., Burger, Y., Horev, C., Koren, A., and Edelstein, M. 2007. Introducing grafted cucurbits to modern agriculture. The Israeli experience. *Plant Dis.* 91: 916-923.
- Cohen, R., Burger, Y., and Katzir, N. 2004. Monitoring physiological races of *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*), the causal agent of powdery mildew in cucurbits: Factors affecting race identification and the importance for research and commerce. *Phytoparasitica* 32:174-183.
- Davis, A. R., Bruton, B. D., Pair, S. D., and Thomas, C. E. 2001. Powdery mildew: An emerging disease of watermelon in the United States. *Cucurbit Genet. Coop.* 24:42-48.
- Davis, A. R., Levi, A., Tetteh, A., Wehner, T. C., Russo, V., and Pitrat, M. 2007. Evaluation of watermelon and related species for resistance to race 1W Powdery mildew. *J. Am. Soc. Hortic. Sci.* 132:790-795.
- Davis, A. R., Perkins-Veazie, P., Sakata, Y., Lopez-Galarza, S., Maroto, J. V., Lee, S.-G., Huh, Y.-C., Sun, Z., Miguel, A., King, S. R., Cohen, R., and Lee, J.-M. 2008. Cucurbit grafting. *Crit. Rev. Plant Sci.* 27:50-74.
- Edelstein, M., Tadmor, Y., Abo-Moch, F., Karchi, Z., and Mansour, F. 2000. The potential of *Lagenaria* rootstock to confer resistance to the carmine spider mite, *Tetranychus cinnabarinus* (Acari: Tetranychidae) in Cucurbitaceae. *Bull. Entomol. Res.* 90:113-117.
- Evrenosoglu, Y., Alan, O., and Ozdemir, N. 2010. Leaf phenolic content of some squash rootstocks used on watermelon (*Citrullus lanatus* (thunb.) Matsum and Nakai) growing and phenolic accumulation on grafted cultivar. *Afr. J. Agric. Res.* 5:732-737.
- Guan, W., Zhao, X., Hassell, R., and Thies, J. 2012. Defense mechanisms involved in disease resistance of grafted plants. *HortScience* 47:164-170.
- Hammett, K. R. W. 1977. Taxonomy of Erysiphaceae in New Zealand. *N. Z. J. Bot.* 15:687-711.
- Hassell, R., Memmott, F., and Liere, D. 2008. Grafting methods for watermelon production. *HortScience* 43:1677-1679.
- Jahn, M., Munger, H. M., and McCreight, J. D. 2002. Breeding cucurbit crops for powdery mildew resistance. Pages 239-248 in: *The Powdery Mildews: A Comprehensive Treatise*. R. R. Belanger, W. R. Bushnell, A. J. Dik, and T. L. W. Carver, eds. APS Press, St. Paul, MN.
- Jarvis, W. R. 1992. *Managing Diseases in Greenhouse Crops*. APS Press, St. Paul, MN.
- Keinath, A. P. 2015. Efficacy of fungicides against powdery mildew on watermelon caused by *Podosphaera xanthii*. *Crop Prot.* 75:70-76.
- Keinath, A. P., Baccari, G. V., and DuBose, V. B. 2010. Evaluation of fungicides for control of powdery mildew on acorn squash, 2009. *Plant Dis. Manage. Rep.* 4: V014.
- Keinath, A. P., and DuBose, V. B. 2004. Evaluation of fungicides for prevention and management of powdery mildew on watermelon. *Crop Prot.* 23:35-42.
- Keinath, A. P., and DuBose, V. B. 2012. Controlling powdery mildew on cucurbit rootstock seedlings in the greenhouse with fungicides and biofungicides. *Crop Prot.* 42:338-344.
- Keinath, A. P., and Hassell, R. L. 2014. Suppression of *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *niveum* race 2 on grafted triploid watermelon. *Plant Dis.* 98:1326-1332.
- Keinath, A. P., and Rennberger, G. 2017. Powdery mildew on watermelon. Fact sheet Hor 02. Clemson Cooperative Extension. Accessed 12 September 2017: <https://www.clemson.edu/extension/publications/files/horticulture/HOR02%20Powdery%20Mildew%20on%20Watermelon.pdf>
- King, S. R., Davis, A. R., Liu, W., and Levi, A. 2008. Grafting for disease resistance. *HortScience* 43:1673-1676.
- King, S. R., Davis, A. R., Zhang, X. P., and Crosby, K. 2010. Genetics, breeding and selection of rootstocks for Solanaceae and Cucurbitaceae. *Sci. Hortic. (Amsterdam)* 127:106-111.
- Kousik, C. S., Brusca, J., and Turechek, W. W. 2016. Diseases and disease management strategies take top research priority in the Watermelon Research and Development Group members survey (2014 to 2015). *Plant Health Prog.* 17:53-58.
- Kousik, C. S., Donahoo, R. S., Webster, C. G., Turechek, W. W., Adkins, S. T., and Roberts, P. D. 2011. Outbreak of cucurbit powdery mildew on watermelon fruit caused by *Podosphaera xanthii* in southwest Florida. *Plant Dis.* 95:1586.
- Kousik, C. S., and Hassell, R. L. 2009. Evaluation of commercial watermelon rootstocks for tolerance to crown rot and powdery mildew, 2007. *Plant Dis. Manage. Rep.* 3:V144.
- Kousik, C. S., and Ikerd, J. L. 2014. Evidence for cucurbit powdery mildew pathogen races based on watermelon differentials. Pages 32-34 in: *Proceedings of Cucurbitaceae 2014*. M. Havey, Y. Weng, B. Day, and R. Grumet, eds. American Society of Horticultural Science, Alexandria, VA.
- Kousik, C. S., and Ikerd, J. L. 2016. Evaluation of watermelon varieties for tolerance to powdery mildew and *Phytophthora* fruit rot, 2014. *Plant Dis. Manage. Rep.* 10:V082.
- Kousik, C. S., Ikerd, J. L., and Hassell, R. 2012. Effect of grafting onto resistant rootstocks on the development of *Phytophthora* fruit rot on susceptible scions, 2011. *Plant Dis. Manage. Rep.* 6:V012.
- Kousik, C. S., Levi, A., Ling, K. S., and Wechter, W. P. 2008. Potential sources of resistance to cucurbit powdery mildew in U.S. plant introductions of bottle gourd. *HortScience* 43:1359-1364.
- Lebeda, A., Křístková, E., Sedlakova, B., McCreight, J. D., and Coffey, M. D. 2008. New concept for determination and denomination of pathotypes and races of cucurbit powdery mildew. Pages 125-134 in: *Cucurbitaceae 2008*. Proceedings of the 9th EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae. Avignon, France.
- Lebeda, A., Kristkova, E., and Sedlakova, B. 2011. Gaps and perspectives of pathotype and race determination in *Golovinomyces cichoracearum* and *Podosphaera xanthii*. *Mycoscience* 52:159-164.
- Lebeda, A., Kristkova, E., Sedlakova, B., McCreight, J. D., and Coffey, M. D. 2016. Cucurbit powdery mildews: Methodology for objective determination and denomination of races. *Eur. J. Plant Pathol.* 144:399-410.
- Lebeda, A., Sedláková, B., Křístková, E., Vajdová, M., and McCreight, J. D. 2012. Application of a new approach for characterization and denomination of races of cucurbit powdery mildews – a case study on the Czech pathogen population. Pages 172-180 in: *Cucurbitaceae 2012*, Proceedings of the Xth EUCARPIA meeting on genetics and breeding of Cucurbitaceae, October 15-18, 2012, Antalya, Turkey. N. Sari, I. Solmaz, and V. Aras, eds.
- Lee, H. Y., Byeon, Y., and Back, K. 2014. Melatonin as a signal molecule triggering defense responses against pathogen attack in Arabidopsis and tobacco. *J. Pineal Res.* 57:262-268.
- Lee, J. M. 1994. Cultivation of grafted vegetables. 1. Current status, grafting methods and benefits. *HortScience* 29:235-239.
- Lee, J. M., and Oda, M. 2003. Grafting of herbaceous vegetable and ornamental crops. *Hortic. Rev. (Am. Soc. Hortic. Sci.)* 28:61-124.
- Louws, F. J., Rivard, C. L., and Kubota, C. 2010. Grafting fruiting vegetables to manage soil-borne pathogens, foliar pathogens, arthropods and weeds. *Sci. Hortic. (Amsterdam)* 127:127-146.

- Mahmud, I., Kousik, C. S., Hassell, R., Chowdhury, K., and Boroujerdi, A. 2015. NMR Spectroscopy identifies metabolites translocated from powdery mildew resistant rootstocks to susceptible watermelon scions. *J. Agric. Food Chem.* 63:8083-8091.
- McCreight, J. D. 2006. Melon-powdery mildew interactions reveal variation in melon cultigens and *Podosphaera xanthii* races 1 and 2. *J. Am. Soc. Hortic. Sci.* 131:59-65.
- McGrath, M. T. 2010. Managing Cucurbit powdery mildew organically. Accessed 8 March 2012: <http://articles.extension.org/pages/30604/managing-cucurbit-powdery-mildew-organically>
- McGrath, M. T. 2017. Powdery mildew. Pages 62-64 in: *Compendium of Cucurbit Diseases and Pests*, 2nd Ed., A. P. Keinath, W. M. Wintermantel, and T. A. Zitter, eds. APS Press, St. Paul, MN.
- Mercier, J., Muscara, M. J., and Davis, A. R. 2014. First Report of *Podosphaera xanthii* race 1W causing powdery mildew of watermelon in California. *Plant Dis.* 98:158.
- Oda, M. 1993. Present state of vegetable production using grafted plants in Japan. *Agr. Hortic.* 63:1190-1196.
- Oda, M. 2002. Grafting of vegetable crops. *Sci. Rep. Agric. Biol. Sci. Osaka Prefer. Univ.* 53:1-5.
- Paris, H. S. 2015. Origin and emergence of the sweet dessert watermelon, *Citrullus lanatus*. *Ann. Bot. (Lond.)* 116:133-148.
- Park, S. M., Lee, J. S., Jegal, S., Jeon, B. Y., Jung, M., Park, Y. S., Han, S. L., Shin, Y. S., Her, N. H., Lee, J. H., Lee, M. Y., Ryu, K. H., Yang, S. G., and Harn, C. H. 2005. Transgenic watermelon rootstock resistant to CGMMV (*cucumber green mottle mosaic virus*) infection. *Plant Cell Rep.* 24:350-356.
- Pitrat, M., Dogimont, C., and Bardin, M. 1998. Resistance to fungal diseases of foliage in melon. Pages 167-173 in: *Cucurbitaceae '98*. J. D. McCreight, ed. ASHS Press, Alexandria, VA.
- Qian, Y., Tan, D., Reiter, R. J., and Shi, H. 2015. Comparative metabolomic analysis highlights the involvement of sugars and glycerol in melatonin-mediated innate immunity against bacterial pathogen in Arabidopsis. *Sci. Rep.* 5:15815.
- Sakata, Y., Sugiyama, M., Ohara, T., and Morishita, M. 2006. Influence of rootstocks on the resistance of grafted cucumber (*Cucumis sativus* L.) scions to powdery mildew (*Podosphaera xanthii* U. Braun & N. Shishkoff). *J. Jpn. Soc. Hortic. Sci.* 75:135-140.
- Takamatsu, S., and Kano, Y. 2001. PCR primers useful for nucleotide sequencing of rDNA of the powdery mildew fungi. *Mycoscience* 42:135-139.
- Tetteh, A. Y., Wehner, T. C., and Davis, A. R. 2010. Identifying resistance to powdery mildew race 2W in the USDA-ARS watermelon germplasm collection. *Crop Sci.* 50:933-939.
- Thomas, C. E., Levi, A., and Caniglia, E. 2005. Evaluation of U.S. plant introductions of watermelon for resistance to powdery mildew. *HortScience* 40:154-156.
- Wang, J., Zhang, D. W., and Fang, Q. 2002. Studies on antiviral disease mechanism of grafted seedless watermelon. *J. Anhui Agr. Univ.* 29:336-339.
- Webster, C. G., Kousik, C. S., Hassell, R. L., Ling, K. S., Turechek, W. W., and Adkins, S. 2012. Virus movement within grafted watermelon plants. *Phytopathology* 102:S4.133 (Abstract).
- Wei, Y., Hu, W., Wang, Q., Zeng, H., Li, X., Yan, Y., Reiter, R. J., He, C., and Shi, H. 2017. Identification, transcriptional and functional analysis of heat-shock protein 90s in banana (*Musa acuminata* L.) highlight their novel role in melatonin-mediated plant response to Fusarium wilt. *J. Pineal Res.* 62:e12347.
- Yetisir, H., Kurt, S., Sari, N., and Tok, F. M. 2007. Rootstock potential of Turkish *Lagenaria siceraria* germplasm for watermelon: Plant growth, graft compatibility, and resistance to Fusarium. *Turk. J. Agric. For.* 31:381-388.
- Yetisir, H., and Sari, N. 2003. Effect of different rootstock on plant growth, yield and quality of watermelon. *Aust. J. Exp. Agric.* 43:1269-1274.
- Yin, L., Wang, P., Li, M., Ke, X., Li, C., Liang, D., Wu, S., Ma, X., Li, C., Zou, Y., and Ma, F. 2013. Exogenous melatonin improves *Malus* resistance to Marssonina apple blotch. *J. Pineal Res.* 54:426-434.