Development of *Phytophthora* Fruit Rot Caused by *Phytophthora capsici* on Resistant and Susceptible Watermelon Fruit of Different Ages

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Abstract

Watermelon is an important crop grown in 44 states in the United States. *Phytophthora* fruit rot caused by *Phytophthora capsici* is a serious disease in the southeastern U.S.A., where over 50% of the watermelons are produced. The disease has resulted in severe losses to watermelon growers, especially in Georgia, South Carolina, and North Carolina during the past few years. Several fruit rot-resistant watermelon germplasm lines have been developed for use in breeding programs. To evaluate the development of *Phytophthora* fruit rot on fruit of different ages, plants of fruit rot-resistant and susceptible lines were planted at weekly intervals for five consecutive weeks in experiments conducted over three years (2011 to 2013). Flowers were routinely inspected and hand pollinated

to ensure having fruit of different ages. In each year, different aged fruit were harvested on the same day and inoculated with a 5-mm agar plug from an actively growing colony of *P. capsici*. Inoculated fruit were maintained in a room set to conditions conducive for disease development (>95% relative humidity, $26 \pm 2^\circ\text{C}$). After 5 days, lesion diameter and intensity of sporulation was recorded for each fruit. Lesion diameter and sporulation intensity were significantly greater on fruit of susceptible lines compared with resistant lines. Fruit age did not have an effect on either measurement on susceptible (Sugar Baby) or resistant lines (PI 560020 and PI 595203). Our results showed that resistance to *Phytophthora* fruit rot in watermelon was not correlated with fruit age.

Watermelon is an important vegetable crop grown in 44 states in the U.S. on 51,597 ha with a total production of approximately 2 million tons (USDA ERS 2012). *Phytophthora* fruit rot of watermelon caused by *Phytophthora capsici* was first reported in the U.S. in the 1940s in Colorado (Wiant and Tucker 1940). However, the disease is now prevalent in most watermelon growing areas, and particularly in the eastern United States, where about 53% (27,599 ha) of the U.S. watermelon crop is produced (Gevens et al. 2008; Hausbeck and Linderman 2016; Kousik et al. 2011, 2014a, 2016; McGrath 2017; USDA ERS 2012). *Phytophthora* fruit rot on watermelons can be a pre- or postharvest problem. If the disease occurs early during fruit formation, it could result in total yield loss. For example, between 2003 and 2008 and again in 2013, many watermelon growers in Georgia, South Carolina, and North Carolina did not harvest the crop due to severe preharvest fruit rot. In other instances, fruits rotted postharvest during and after shipping, resulting in rejection of loads and loss of revenue (Kousik et al. 2011, 2014a, 2016). Because of these situations, the National Watermelon Association (NWA) listed *Phytophthora* fruit rot as their top research priority in 2006 (Morrissey 2006) and again from 2013 to 2015 (Kousik et al. 2014a, 2016).

P. capsici has been reported to cause disease on plants belonging to 23 different families and is particularly serious and devastating on vegetable crops in the Solanaceae, Cucurbitaceae, and Fabaceae families (Erwin and Ribeiro 1996; Granke et al. 2012; Hausbeck and Lamour 2004; Hausbeck and Quesada-Ocampo 2017; McGrath 2017; Sanogo and Ji 2012). The pathogen is known to cause a variety of symptoms including crown rot, foliar blight, and fruit rot (Granke

et al. 2012; Hausbeck and Lamour 2004; Keinath 2007; Kousik et al. 2011). In severely infested cucurbit fields, *P. capsici* has been known to cause complete loss and cessation of production (Babadoost 2004; Babadoost and Zitter 2009; Hausbeck and Lamour 2004; Kreutzer et al. 1940). Various strategies for managing *P. capsici* have been developed and because no single strategy can provide a commercially acceptable level of control, they are often used in combination. The most common practices are crop rotation, soil solarization, application of fungicides, and practices that ensure well-drained soils to prevent pathogen growth and eliminate free-standing water to reduce splash dispersal (Babadoost 2004; Granke et al. 2012; Hausbeck and Lamour 2004; Kousik et al. 2011, 2014a; McGrath 2017; Sanogo and Ji 2012). Several commercial fungicides that are effective in managing *Phytophthora* fruit rot of watermelon have been identified (Kousik et al. 2011, 2014a, 2017). However, *P. capsici* isolates insensitive to fungicides, particularly mefenoxam and cyazofamid, have also been documented (Granke et al. 2012; Hausbeck and Lamour 2004; Jackson et al. 2012; Keinath 2007; Kousik and Keinath 2008). Furthermore, the application of fungicides is not very effective when disease pressure is high (Granke et al. 2012; Kousik et al. 2011, 2014a; McGrath 1994). Alternative strategies for managing *Phytophthora* fruit rot such as host resistance are needed, but are not yet available in commercial watermelon varieties.

Host plant resistance is the cornerstone of an integrated disease management (IPM) program, and watermelon cultivars with resistance to *P. capsici* would be very useful for the management of *Phytophthora* fruit rot. It has been extremely challenging to identify and develop resistance to *Phytophthora* fruit rot in cucurbits. Gevens et al. (2006) identified cucumber varieties that limit development of *P. capsici*; however, none of the varieties tested had complete fruit rot resistance. More importantly, resistance in cucumber was related to the developmental stage of the fruit, increasing with increasing age and size of the fruit (Ando et al. 2009, 2015; Gevens et al. 2006). Ando et al. (2009) also observed that watermelon fruit of cultivar Crimson Sweet were susceptible at all stages of growth. However, they did not evaluate any resistant watermelon lines as these were not yet identified. For watermelon, Kousik et al. (2012) identified several watermelon plant introductions (PIs) with high levels of resistance to *Phytophthora* fruit rot and developed germplasm lines for use in breeding programs (Kousik et al. 2014b). In those studies, all the evaluations were done on mature fruit (Kousik et al. 2012) because mature watermelon fruit are thought to be the most susceptible

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to fruit rot. The objective in this study was to determine if resistance to *Phytophthora* fruit rot varies in fruit of differing ages.

Materials and Methods

Seeds. The original seeds of U.S. watermelon PIs belonging to the core collection were obtained from Plant Genetic Resources Conservation Unit (PGRU), Griffin, GA (<https://npgsweb.ars-grin.gov/gringlobal/crop.aspx?id=151>). In the current study, we evaluated fruit of germplasm lines derived from PI 560020, PI 595203, and PI 306782 that were found to be resistant in the previous study (Kousik et al. 2012). Additionally, we included a highly susceptible line PI 536464 and the commercial watermelon variety Sugar Baby as susceptible controls. Seeds for Sugar Baby were purchased from Willhite Seeds (Poolville, TX).

Pathogen. *P. capsici* isolate RCZ-11 was used as the source of inoculum and was provided by Dr. A. P. Keinath, Clemson University. The isolate was collected in 2003 from zucchini (*Cucurbita pepo*) plants in South Carolina and belongs to mating type A2. It is highly aggressive on watermelon fruit and causes severe rot (Kousik et al. 2012, 2014 a, b). The isolate was stored for long term on autoclaved hemp seed placed in sterile water as described before (Jackson et al. 2012; Lamour and Hausbeck 2002). The isolate was cultured and maintained on V8 juice agar amended with antibiotics (PARP) as described by others (Jackson et al. 2012; Keinath 2007; Quesada-Ocampo et al. 2009) and was inoculated onto watermelon or cucumber fruit and reisolated 2 weeks to a month prior to studies conducted each year to maintain its virulence.

Field planting and harvesting. The study was conducted over the course of three summers (2011, 2012, and 2013) at the U.S. Vegetable Laboratory Farm in Charleston, SC. The studies were conducted in fields with no history of *P. capsici*. The soil was Yonges loamy fine sand in the fields where the watermelon plants were grown. In 2011, three resistant germplasm lines derived from PIs (PI 560020, PI 595203, and PI 306782) and two susceptible checks (Sugar Baby and PI 536464) were evaluated. In 2012 and 2013, only two of the resistant PIs (PI 560020 and PI 595203) and the check Sugar Baby were evaluated. This was done primarily to accommodate more fruits of each line in the trials. Plants of each of the lines were seeded in 50-cell Jiffy trays (Jiffy Products of America, Norwalk, OH) filled with Metro Mix (Sun Gro Horticulture, Bellevue, WA) and allowed to germinate and grow in a greenhouse for 4 weeks. To harvest fruit of different ages, watermelon transplants were grown by seeding once every week for a total of five consecutive weeks in each of the three years. Four-week-old plants were transplanted every week for a total of five consecutive weeks onto 96-cm wide and 20-cm high raised beds covered with white plastic mulch. Transplanting was started on 14 July in 2011, 12 July in 2012, and 23 June in 2013. In 2011, two sets of five plants of each germplasm line or check was planted each week for 5 weeks for a total of 50 plants for each germplasm line. In 2012 and 2013, two sets of 10 plants each for each germplasm line was planted each week for 5 weeks for a total of 100 plants for each line. Plants were spaced 46 cm apart within rows. Distance between each plot was 4.6 m. Standard watermelon irrigation and weed management practices were followed (Kemble 2010). After bedding but before planting, the row middles were sprayed with Roundup Pro (1.17 liter/ha) and Strategy (2.24 liter/ha) for weed management. Weeds between beds were controlled during the season with spot applications of Roundup and hand cultivation. Plants were sprayed twice with Oberon 2SC (490 ml/ha) for management of whiteflies and with Cabrio EG (1.12 kg/ha) for management of anthracnose during each season. Female flowers were hand pollinated and each developing fruit was tagged by monitoring the plots regularly. Female flowers were pollinated once every 4 days in each year. When most of the fruit from the first and about half from the second planting were mature, all the fruit from all the plots were harvested. This provided fruit of differing ages for the study. All the harvested fruit were assessed for resistance to *Phytophthora* fruit rot as described below.

Fruit rot assessment. Watermelon fruit of varying sizes and ages were labeled accordingly and maintained in a walk-in-humidity chamber (4 × 3 × 3.7 m, length × width × height) with wire shelves

as described previously (Kousik et al. 2012, 2014a, b). The labeled fruit were then placed in a completely randomized design on the wire shelves within the humid chamber. Fruit were surface disinfested with 10% Clorox bleach solution (0.6% sodium hypochlorite) prior to inoculation (Gevens et al. 2006; Kousik et al. 2014a, b). Each fruit was inoculated by placing a 5-mm V8 agar plug from a 4-day-old actively growing isolate of *P. capsici* in the middle of the fruit as described before (Ando et al. 2009; Gevens et al. 2006; Kousik et al. 2012, 2014a, b; Krasnow et al. 2014; Krasnow and Hausbeck 2016). The agar plug was placed delicately without injuring the fruit so that the mycelium side touched the fruit. After inoculation, high relative humidity (>95%) was maintained in the room using a humidifier and the temperature was maintained at 26 ± 2°C. The room was continuously illuminated with fluorescent lights to enhance sporulation. Five days after inoculation, the lesion diameter and the diameter of the area with visible pathogen growth and sporulation was measured (pathogen growth diameter) on each fruit. The intensity of sporulation within the lesion was recorded on a 0 to 5 scale, where 0 = no visible sporulation and 5 = abundant sporangia, very dense and covering most (>85%) of the lesion area as described before (Kousik et al. 2012, 2014a, b).

Statistical analysis. Data from each year was analyzed separately using the PROC GLIMMIX procedure of SAS software (version 9.4, Cary, NC) and means were separated using the lsmeans option ($P = 0.05$). Pearson's correlation coefficients between age of fruit in days and lesion diameter, pathogen growth diameter, and sporulation intensity were determined using the PROC CORR procedure of SAS. Since hand pollinations of female flowers were done once every 4 days, the fruit were grouped into 4-day age groups and the median age and mean fruit rot lesion size and standard error for each age group was determined.

Results

Phytophthora fruit rot developed very rapidly on the susceptible checks (Sugar Baby and PI 536464). By 5 days after inoculation, severe rot was observed on susceptible fruit of all ages (Fig. 1). Sporulation was very intense on fruit of PI 536464 at all fruit ages (Fig. 1) and similar results were observed for 'Sugar Baby.' Fruit of PI 536464 were highly susceptible and in some instances completely collapsed due to fruit rot. In 2011, fruit from all the resistant PIs, i.e., PI 560020, PI 595203, and PI 306782, had significantly smaller (mean <1 cm) to no lesions compared with fruits of PI 536464 and Sugar Baby that had lesions of about 10 cm in diameter (Table 1 and Fig. 2). Similarly, sporulation was very intense on fruit of the susceptible cultivars at all ages and covered over 85% of the lesion area on most fruit by 5 days after inoculation. In 2012, fruit of Sugar

Fig. 1. Intense sporulation and mycelial growth by the fruit rot causing pathogen *Phytophthora capsici* on fruit of susceptible variety PI 536464 of different ages (top row of fruits) compared with no sporulation on fruit of PI 560020 (bottom row) in an experiment in 2011. The figure indicates that susceptible fruit are susceptible to *P. capsici* at all ages of development. In contrast, resistant fruit are resistant at all ages.

Baby were again found to be susceptible at all ages with mean lesion diameter of 8 cm compared with <1 cm for the two resistant germplasm lines (PI 560020 and PI 595203). Similarly in 2013, Sugar Baby fruits were highly susceptible to *P. capsici* with intense sporulation visible on the rotting surface compared with the two resistant lines (Table 1). The small lesions that formed on the resistant lines were generally black in color and the tissue remained hard. No internal rot of the flesh was observed for the resistant lines. However, tissue on the susceptible checks was very soft and collapsed easily upon gentle pressure due to severe internal rot.

The fruit of PI 536464 and Sugar Baby were susceptible to *P. capsici* at all ages and a significant positive correlation between fruit age and lesion diameter was observed as expected because fruit size was also related to fruit age (Table 2). Similarly, pathogen growth was significantly correlated with age for the two susceptible checks. The lesions were generally largest on the large fruits. There was no correlation between the sporulation intensity and fruit age of the susceptible checks Sugar Baby and PI 536464. For most of the susceptible fruit, the sporulation intensity was rated as a 5 on the 0 to 5 scale as the lesions were heavily covered with *P. capsici* sporangia (Fig. 1). The results were similar in 2012 on the susceptible check Sugar Baby. However, in 2013 no correlation between fruit age and lesion development was observed. Results for the visible pathogen growth (pathogen growth diameter) on the fruit surface followed the same pattern as the lesion diameter and sporulation intensity.

The fruit of the resistant lines appeared resistant at all ages, with no lesions to very small lesions forming near or under the agar plug that was used as the inoculum (Table 1 and Fig. 2). There was no significant correlation between fruit age and *Phytophthora* fruit rot development (lesion diameter, visible pathogen growth, and sporulation intensity) for germplasm lines derived from PI 306782, PI 560020, and PI 595203 in 2011. In 2012, a low level of significant correlation ($r = 0.26$) was observed between age and lesion diameter for PI 560020. This could be because large numbers of fruit were evaluated during this season and small dark lesions next to the agar plug were noticed on some fruits. However, there was no correlation between sporulation intensity, pathogen growth, and fruit age. Similarly in 2013, a slight correlation between lesion diameter and fruit age for

PI 595203 was noticed; however, there was no correlation between sporulation intensity or pathogen growth and fruit age. Dark lesions next to the agar plug were observed on some fruit of PI 595203 as well.

Discussion

The fruit of the resistant germplasm lines were resistant at all ages with very minimal to no lesions, pathogen growth, or sporulation observed on most fruits. In contrast, fruit of the susceptible checks had large lesions with extensive pathogen growth and abundant sporangia on the fruit surface. The fruit rot resistant germplasm lines are all egusi-type watermelon with white flesh and low sugars (Kousik

Table 1. Mean lesion diameter, pathogen growth diameter, and sporulation intensity of *Phytophthora* fruit rot on resistant and susceptible watermelon fruit of different ages over three years, 2011 to 2013^v

Germplasm line	N ^w	Lesion diameter (cm) ^x	Pathogen growth diameter (cm) ^y	Sporulation (0 to 5) ^z
2011				
PI 536464	24	10.7 a	10.6 a	4.8 a
Sugar Baby	18	10.8 a	9.9 a	4.9 a
PI 306782	18	0.5 b	0.1 b	0.0 b
PI 595203	24	0.5 b	0.0 b	0.0 b
PI 560020	23	0.5 b	0.2 b	0.2 b
2012				
Sugar Baby	31	8.1 a	7.6 a	4.0 a
PI 595203	60	0.8 b	0.2 b	0.2 b
PI 560020	61	0.4 b	0.1 b	0.0 b
2013				
Sugar Baby	39	10.4 a	9.6 a	4.7 a
PI 595203	84	2.4 b	1.0 b	0.7 b
PI 560020	46	1.0 b	0.6 b	0.4 b

^v Means followed by the same letter in a column for each year are not significantly different ($P = 0.05$).

^w N indicates the number of fruit based on which the mean for each germplasm line is based on. The mean was calculated on fruit of all ages ranging from 5 to 45 days after pollination.

^x Lesion diameter was measured in two perpendicular directions on each fruit and the mean of the two was used in the analysis.

^y Pathogen growth diameter was measured as pathogen growth visible to the naked eye and sporulation on the fruit surface.

^z Sporulation intensity was measured on a 0 to 5 scale with 0 = no sporulation observed and 5 = >85% of the lesion covered with abundant *Phytophthora capsici* sporangia.

Fig. 2. Lesion development (lesion diameter) on watermelon fruit of *Phytophthora* fruit rot resistant germplasm lines PI 560020, PI 595203, and a susceptible cultivar Sugar Baby of different ages in studies conducted in 2011 (A), 2012 (B), and 2013 (C). Lesion diameter was recorded 5 days after inoculation. Fruit were grouped into 4-day age groups as female flowers were pollinated once every 4 days in each year. Each point in the graph represents the mean fruit rot lesion diameter and the bars represent the standard error for the mean in each age group.

et al. 2012, 2014b). The resistance from these lines is currently being introgressed into high quality watermelon cultivars to develop resistant breeding lines and cultivars for use by growers and the seed industry. The results of the current study suggest that once fruit rot-resistant cultivars are developed, they will likely express resistance at all ages. This could lead to a reduction in the number of fungicide applications necessary to manage fruit rot, but this will have to be evaluated prior to release of such resistant cultivars.

Despite the fruits being equally susceptible at all ages, there was a positive correlation between lesion diameter and fruit age. This was most likely because the pathogen had greater area to colonize as the size of fruit increased with age. Similarly sporulation intensity on fruit of the susceptible lines was generally high (rated >4.5 on 0 to 5 scale) and covered >85% of the lesion area irrespective of the fruit age, further confirming their susceptibility at all ages. A slight positive correlation ($r < 0.26$) between lesion diameter and fruit age was observed for germplasm lines derived from PI 560020 in 2012 and PI 595203 in 2013. This could be because of the small dark lesions that were observed next to that agar plug that was used as the inoculum and the large sample size. In these instances, the outer fruit rind had a dark black lesion and the fruit tissue was still hard. The lesion did not spread within the fruit on the resistant lines and the symptoms appeared similar to a hypersensitive reaction as described before (Kousik et al. 2014b). Further studies to determine the actual nature of this reaction are being pursued. In contrast, on the susceptible checks, on smaller fruits the entire inside of smaller fruit were rotted, and on larger fruits the rot extended to the middle of the fruit below the inoculation point. In addition, we observed greater colonization of the susceptible fruit by *P. capsici* away from the agar plug compared with the resistant fruit using specific primers and real-time qPCR (Kousik et al., unpublished results) and this was very similar to our qPCR results described previously (Kousik et al. 2012).

In general, severe fruit rot is typically noticed in commercial fields as fruits start to mature. In many instances, this also coincides with heavy rainfall in the Southeast. Because of this, it has been suggested that the fruit may be more susceptible due to increase in sugars as fruit mature. However, in the present study, the fruit of Sugar Baby and PI 536464 were susceptible even during the early developmental stages when sugars tend to be very low compared with mature fruit. The mature fruit of resistant PI have low sugar content (brix ≤ 2) (Kousik et al. 2014b) compared with the susceptible checks (brix ≥ 9.5). In our recent studies to determine the genetics of resistance, we observed several resistant fruit with higher sugar content

(brix ≥ 8) approaching that of a commercial watermelon in a segregating F_2 population, compared with the lower levels in the resistant parent (Kousik et al., unpublished results), suggesting that the resistance or susceptibility response is not a function of sugar content.

With cucumbers, it was observed that infection generally occurred on the blossom end of the fruit in field and laboratory experiments (Ando et al. 2009; Gevens et al. 2006). However, we observed no difference in the level of infection when watermelon fruit were inoculated at the peduncle, middle, or the blossom end with respect to lesion diameter or sporulation intensity on resistant or susceptible accessions (Kousik et al., unpublished). Therefore, as in our other studies (Kousik et al. 2012, 2014a, b), we inoculated in the middle of the fruit (Fig. 1). This is beneficial, because it was easier to measure the disease parameters in the middle of the fruit and the agar plug does not slide and fall off the fruit despite the slippery surface because of high relative humidity in the room necessary to perform these studies. Inoculations with zoospores can be considered more natural unlike using an agar plug. Recently a *Phytophthora* fruit rot-resistant cucumber breeding line was developed and released using the zoospore inoculation procedure (Grumet and Colle 2017). We have attempted a similar zoospore inoculation procedure by placing a drop on the surface of a watermelon fruit. However, unlike cucumber fruit, the water droplet slides quickly off the watermelon fruit due its more spherical surface. Recently we have developed a zoospore inoculation procedure and the mature fruit from resistant lines used in this study were resistant to zoospore inoculations as well (Kousik et al., unpublished). Because the agar plug inoculation technique has been used extensively and effectively by many researchers to assay fruit rot (Ando et al. 2009; Enzenbacher and Hausbeck 2012; Gevens et al. 2006; Krasnow et al. 2014; Krasnow and Hausbeck 2016; Meyer and Hausbeck 2013), we too used V8 agar plugs from actively growing colonies of *P. capsici*, for inoculating the fruit in these studies instead of zoospores.

Age-related resistance to *P. capsici* has been reported in cucumber, pumpkin, winter squash, acorn squash, and butternut squash fruits (Ando et al. 2009; Gevens et al. 2006; Meyer and Hausbeck 2013; Krasnow and Hausbeck 2016; Krasnow et al. 2014). Similarly age-related resistance has been observed on some genotypes in crown rot of peppers caused by *P. capsici* (Hwang et al. 1996; Kim et al. 1989). More recently, new cucumber accessions with resistance to *P. capsici* at the young fruit stage were identified based on a large scale screening of over 1,200 accessions (Colle et al. 2014) and a cucumber breeding line was released (Grumet and Colle 2017). In watermelon, we observed that fruit of resistant lines were resistant at all ages

Table 2. Correlations between age of fruit (days) and fruit diameter and *Phytophthora* fruit rot measurements (lesion diameter, pathogen growth diameter, and sporulation intensity) on resistant and susceptible watermelon fruit of different ages over three years, 2011 to 2013

	N	Correlation with fruit age (days)			Correlation with fruit size (cm)		
		Lesion diameter	Pathogen growth	Sporulation	Lesion diameter	Pathogen growth	Sporulation
2011							
PI 306782	18	0.1905	0.0146	−0.1071	−0.0105	−0.2541	−0.1271
PI 560020	23	0.3613	−0.1647	−0.1647	0.2711	−0.1280	−0.1280
PI 595203	24	0.3645	— ^y	—	0.3807	—	—
PI 536464	24	0.8398** ^z	0.8186**	−0.1762	0.9778**	0.9602**	−0.6117
Sugar Baby	18	0.7240**	0.7672**	0.20444	0.7827**	0.6578**	−0.3046
2012							
PI 560020	61	0.2612*	0.0885	0.0885	0.3377*	0.1394	0.1394
PI 595203	60	0.1275	0.0764	0.0609	0.2695*	0.1660	0.0620
Sugar Baby	31	0.6307**	0.5692**	0.2577	0.4335*	0.4078*	−0.0059
2013							
PI 560020	46	0.0729	−0.0509	−0.1088	−0.2113	−0.2307	−0.3609
PI 595203	84	0.2354*	0.0216	−0.0926	−0.0486	−0.1957	−0.2965
Sugar Baby	39	0.0192	−0.0507	0.0994	0.7052**	0.5723**	0.2722
Three years							
PI 560020	130	0.0794	−0.0875	−0.1153	0.0425	−0.0402	−0.1212
PI 595203	168	0.1805*	0.0264	−0.0457	0.1269	−0.0248	−0.1112
Sugar Baby	88	0.4716**	0.4127**	0.2445*	0.6207**	0.5489**	0.1887

^y No pathogen growth or sporulation was observed on PI 595203 in 2011 and hence no correlations are reported.

^z * indicates significant correlation at $P = 0.05$ and ** at $P = 0.01$.

and the susceptible fruit were susceptible independent of age. Similarly, in the study by Ando et al. (2009), watermelon fruit of the cultivar Crimson Sweet were susceptible at all ages with a slight decrease in lesion size as the fruit size increased; however, this difference was not statistically significant. In the same study, melon, zucchini, and summer squash fruit evaluated were susceptible at all ages similar to what was observed on susceptible watermelon check Sugar Baby. In contrast, the level of *P. capsici* infection in cucumber dramatically decreased with increasing fruit size (Ando et al. 2009). Using transcriptomic analysis, Ando et al. (2015) demonstrated that defense-related genes expressed in the peel were responsible for the age-related resistance in cucumber. Studies to determine if similar defense-related genes are playing a role in watermelon rind or fruit at different fruit age may provide additional information as to why watermelon fruit of these resistant lines show resistance at all ages.

The results of this study also have implications on fungicide usage on susceptible watermelon varieties. In previous studies, fungicides were generally applied when most fruits were 7 to 10 cm in diameter (Kousik et al. 2011, 2014a, 2017). However, based on the results of this study, fungicide sprays may have to be initiated earlier in fields with history of *P. capsici*, particularly when prevailing weather conditions favor *P. capsici* infection during early phases of fruit development. Such situations may result in one to two extra fungicide applications. In the study by Ando et al. (2009), watermelon fruit of the cultivar Crimson Sweet were susceptible at all developmental stages, similar to Sugar Baby and PI 536464 in our study, and the authors reached a similar conclusion for when to start applying fungicides for managing fruit rot. Results also suggest that once fruit rot-resistant watermelon varieties from the lines described in this study are developed, fruit will likely be resistant at all ages. However, it would be prudent to use an integrated approach to manage *Phytophthora* fruit rot of watermelon when resistant varieties become available to minimize selection pressure on the pathogen to overcome genetic resistance or develop fungicide resistance.

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