Occurrence of Foliar Pathogens of Watermelon on Commercial Farms in South Carolina Estimated with Stratified Cluster Sampling

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Abstract

A survey of foliar pathogens of watermelon based on two-stage cluster sampling was conducted on commercial farms in South Carolina in spring 2015, spring and fall 2016, and fall 2017. In total, 60 fields from 27 different growers in seven counties representing the main watermelon-producing areas in the state were sampled, using a stratified two-stage cluster sampling approach. In the sampling design, counties corresponded to strata, growers to first-stage clusters, and fields to second-stage clusters. In each field, 100 symptomatic leaves were collected at five equidistant sampling points along four transects encompassing a square shape of 2,500 m². After collection, pathogens were identified based on reproductive structures formed on leaves during >12 h incubation. Estimates were obtained for the statewide probability of pathogen occurrence and associations between pathogen pairs. Six fungal pathogens, *Stagonosporopsis* spp., *Podosphaera xanthii*, *Cercospora citrullina*, *Colletotrichum orbiculare*, *Myrothecium sensu lato* (s.l.), and *Corynespora cassicola*; the oomycete *Pseudoperonospora cubensis*; and three viral pathogens were identified on the examined leaves. With the exception of fall 2017, *Stagonosporopsis* spp. was the

most prevalent pathogen in every season, followed by *P. xanthii*. The highest occurrence of *P. cubensis* was in spring 2015; it did not occur in 2016. The highest occurrence of *C. orbiculare* was in spring 2016; it did not occur in spring 2015. *Myrothecium* s.l. was the most common pathogen in fall 2017 and the second most common pathogen occurring by itself in fall 2016. The third most common pathogen in fall 2017, *Corynespora cassicola*, was not observed in any other season. Eight of the 80 isolates of *Stagonosporopsis* spp. collected were identified as *S. caricae*, the rest as *S. citrulli*. All isolates of *S. caricae* were found in spring 2015 and originated from two fields in different counties. A total of three positive and five negative associations were found between pathogen pairs co-occurring on the same leaf. A positive association between *Stagonosporopsis* spp. and *C. citrullina* was the only significant association between pathogens found in two seasons, spring 2015 and spring 2016. Based on estimates of probability of pathogen occurrence across seasons, *Stagonosporopsis* spp. and *P. xanthii* are the most common pathogens on watermelons in South Carolina. This is the first report of *C. cassicola*, *S. caricae*, and *Myrothecium* s.l. on watermelon in South Carolina.

Watermelon is an important vegetable crop in South Carolina, which ranks fifth in the United States in watermelon production (USDA 2014). With 2,298 ha grown across all 46 counties of the state, the majority of the production is concentrated in the southern parts of the central and coastal plains of the state. The most common production system is raised beds covered with polyethylene mulch and drip irrigation. The main harvest season is in June and July, but an additional crop may be harvested in September and October. In the southeastern United States, the crop takes approximately 78 to 91 days from transplanting to harvest (Kemble et al. 2018).

On cucurbits, 13 foliar diseases are caused by fungi and oomycetes (Keinath et al. 2017).

Gummy stem blight (GSB) is caused by three genetically distinct but morphologically indistinguishable fungal species of *Stagonosporopsis*. *S. citrulli* and *S. cucurbitacearum* (syn. *Didymella bryoniae*) are host-specific to cucurbits, whereas *S. caricae* also infects papaya (*Carica papaya*). *S. citrulli* has the widest distribution, whereas *S. cucurbitacearum* occurs only in temperate regions (Stewart et al. 2015). The disease can affect all aboveground plant parts. On leaves, typical symptoms are circular to triangular, tan to dark brown, water-soaked lesions. *Stagonosporopsis* spp. produce tan to brown pycnidia and brown to black pseudothecia. A temperature

of 16–28°C and leaf wetness of >1 h are necessary for infection, and lesion expansion requires continuous leaf wetness. Moisture is generally more important than temperature for disease development (Keinath 2017b).

Powdery mildew is a major cucurbit disease with worldwide distribution mainly caused by the fungi *Podosphaera xanthii* and *Golovinomyces cichoracearum*, with *P. xanthii* being the most commonly reported pathogen in the southeastern United States. Cucurbit powdery mildew fungi are obligate parasites that are mainly dispersed via airborne conidia. Symptoms can develop on both leaf surfaces, petioles, and stems, and are characterized by white powdery growth (McGrath 2017).

Cercospora leaf spot is caused by the fungus *Cercospora citrullina*, a taxon that may be a species complex (Everts 2017; Groenewald et al. 2013). The disease is commonly found on watermelon and other cucurbits (Everts et al. 2016). Tan to brown, small, circular leaf spots are the main symptom of this disease. Crop debris and cucurbitaceous weeds serve as overwintering sites, and spores of the pathogen can be transported long distances when airborne. Conidiophores of *C. citrullina* are pale brown, not branched, and mostly form in groups of 2–5 stalks. Conidia are hyaline, multiseptate, up to 240 µm in length, and wider at their base (Everts 2017).

The oomycete *Pseudoperonospora cubensis* is the causal agent of downy mildew, one of the most important diseases of cucurbits. In the United States, its resurgence in 2004 caused substantial crop losses in cucumber production, despite the use of cultivars that were resistant prior to the disease outbreak in 2004. Symptoms of downy mildew can differ greatly depending on the affected cucurbit species. General symptoms and signs on cucurbits other than cucumber manifest themselves as small, irregular-shaped lesions on the upper leaf surface accompanied by sporulation on the lower leaf surface (Hausbeck 2017; Holmes et al. 2015). Characteristic for *P. cubensis* are dichotomously branched, hyaline sporangiophores and purplish, ovoid to ellipsoid sporangia (Hausbeck 2017). As a biotrophic pathogen, *P. cubensis* is unable to survive winters in regions where host

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plants are absent (north of the 30th latitude). The presence and spread of the disease is therefore dependent on the yearly long-distance transport of spores from source regions in the southern United States. Whether a crop in a given field is infected depends on the number of viable spores in the air surrounding the field, which is dependent on the number of spores that are released from the source field and their dilution and survival during transport (Neufeld et al. 2018). Recently, isolates of both mating types of the pathogen collected in the southern United States were shown to be capable of producing oospores on cucumber and cantaloupe. Considering this finding, it appears possible that *P. cubensis* might also overwinter as oospores in the southern United States (Thomas et al. 2017b).

Anthrachnose is a common disease of watermelon caused by the fungus *Colletotrichum orbiculare*. There are three races of *C. orbiculare*, of which race 2 infects all watermelon cultivars. Open-pollinated and heirloom cultivars are also susceptible to races 1 and 2B (Keinath 2015a; Wasilwa et al. 1993). On leaves, anthracnose lesions are dark brown, angular, and can appear as typical “shot-holes.” Anthracnose lesions may also affect petioles, vines, and fruits of susceptible cucurbits. Conidia appear as typical salmon-colored spore masses on acervuli and are disseminated predominantly by rain splashes and wind. A minimum of 2 h of leaf wetness is required for infection, but no additional leaf wetness is necessary for lesion expansion (Keinath 2017a).

Target spot is a plant disease caused by the fungus *Corynespora cassiicola*. The host range of this pathogen is very wide, with more than 530 plant species and even reports of infected human skin and corneas (Dixon et al. 2009; Mahgoub 1969; Yamada et al. 2013). Among cucurbits, cucumbers are most commonly affected, and symptoms on leaves appear as small, angular, water-soaked lesions. *C. cassiicola* overwinters on crop debris and is disseminated through airborne conidia. The conidiophores of *C. cassiicola* appear iridescent and pale gray or brown. Conidia are born singly or in short chains, pale brown to brown, characteristically pseudoseptate and with a distinct hilum on the end. Conditions of high humidity, temperatures of 25 to 35°C, and free moisture on plants promote infection by *C. cassiicola* and disease development (Williams and Vallad 2017).

Myrothecium leaf spot is a disease caused by one or more species in the species complex *Myrothecium sensu lato* (s.l.) (Bruton and Fish 2017; Lombard et al. 2016). In addition to the foliar phase, the disease can affect crowns, stems, and fruits, then referred to as crater rot. Lesions on leaves are round to irregular-shaped and often form concentric rings with abundant olive green to black spore masses on sporodochia (Bruton and Fish 2017).

The literature on surveys of cucurbit diseases is scarce. The majority of surveys have been conducted on viruses that affect cucurbitaceous crops (Ali et al. 2012; Coutts and Jones 2005; Köklü and Yilmaz 2006; Papayiannis et al. 2005; Sammons et al. 1989; Ullman et al. 1991). Only two of these studies were conducted on the United States mainland, and none of the results were analyzed statistically (Ali et al. 2012; Sammons et al. 1989). In the past 20 years, only one survey of foliar diseases of a cucurbit crop was conducted in the southeastern United States. Over the course of three seasons in the late 1990s, Peterson and Campbell (2002) examined 5,400 leaves with leaf spots collected from cucumber fields from 11 counties in North Carolina. Although the number of examined leaves was sufficient and the collection of leaves within a given field was appropriate, there were some shortcomings in their survey. The sampled fields were not selected systematically based on probability, and it is not clear how many fields per county were sampled. The results of pathogen occurrence were not analyzed statistically (Peterson and Campbell 2002).

Despite the lack of accurate field data of pathogen occurrence, watermelon diseases and their management were ranked as top priorities in a recent opinion survey of researchers and extension specialists. Fusarium wilt and GSB were regarded as most pressing issues in watermelon production, followed by anthracnose, Cucumber green mottle mosaic virus, and powdery mildew (Kousik et al. 2016). Due to the fact that different fungicides and management strategies are needed to control different cucurbit pathogens, accurate

information about their occurrence on commercial farms is crucial to improve recommendations for growers (Keinath 2018; Kemble et al. 2018).

In stratified sampling, the population is subdivided into disjoint subpopulations called strata that encompass the entire population. A simple random sample (SRS) is then taken from each stratum. Each sampling unit is a member of only one stratum. Stratified sampling has the advantage of avoiding unrepresentative samples and providing generally more precise estimates than SRS, especially when there are substantial differences among strata. In cluster sampling, the population is divided into groups of units, which are called clusters. Often, there are secondary sampling units within the clusters. In 1-stage-cluster sampling, a SRS of clusters is taken, and all units within the chosen clusters are observed. In 2-stage-cluster sampling, a SRS of units within the chosen first-stage clusters is taken, but not all units within the chosen clusters are observed. Cluster sampling is used for geographically widespread sampling units, because collection time and costs can be limited. However, the precision of estimators in cluster sampling is typically decreased compared with SRS. Cluster sampling is most efficient when the clusters are relatively homogeneous. Stratification and clustering can be combined in sampling designs to fit specific circumstances (Rao 2000).

The objectives of this study were to i) determine the most prevalent foliar pathogens of watermelons in South Carolina, ii) compare the occurrence of foliar pathogens between the selected counties and fields and characterize the differences between them, and iii) investigate associations between pathogens. By using a complex sampling design with stratification, clustering, and appropriate statistical analysis methods specifically designed for survey data, representative statewide estimates of pathogen occurrence were obtained.

Materials and Methods

Sampling design. A stratified two-stage cluster sampling design was used to sample symptomatic watermelon leaves from commercial fields in 2015 and 2016. In 2016, both spring and fall crops were sampled. The strata were preselected counties with the highest recorded watermelon acreage in South Carolina (USDA-NASS 2014). Six counties were sampled in 2015, seven in the spring of 2016, and six in the fall of 2016 (Table 1). In fall 2017, only two growers in Beaufort County grew watermelons. Two fields from each of those two growers were sampled. Thus, the sampling in fall 2017 lacked stratification and a second layer of clustering and was therefore a simple cluster sample among the fields of each of the two growers.

Growers, who were selected randomly from a compiled list obtained from Clemson Cooperative Extension agents, represented the first level of clustering within each county. Sixteen, 17, 7, and 2 different growers were included in spring 2015, spring 2016, fall 2016, and fall 2017, respectively. In total, fields from 27 different growers were sampled, of which one was sampled in all four seasons; one was sampled in 2015, spring 2016, and fall 2016; eight were sampled in both spring 2015 and 2016; one was sampled in both seasons of 2016; one was sampled in fall 2016 and fall 2017; six growers were sampled only in 2015; six growers were sampled only in spring 2016; and three growers were sampled only in fall 2016 (Fig. 1).

The randomly selected fields represented the second level of clustering in the sampling design. In 2015 leaves were collected from a total of 21 fields, in the spring of 2016 from 24 fields, and in the fall of 2016 from 11 fields (Table 1). The number of fields sampled per county was assigned using proportional allocation based on the watermelon acreage per county (Rao 2000). If a grower had one watermelon field, that field was sampled; if the grower had two fields, one was chosen randomly; if the grower had three or four fields, two were chosen randomly; and if the grower had five or more fields, three were chosen randomly. All fields were sampled within 1 week of harvest starting or ending.

A square shape consisting of four perpendicular transects 50 m in length that encompassed an area of 2,500 m² (0.62 ac) was sampled in each field. The size of the sampled fields ranged from 0.81 to 22.67 ha. The location within each field was chosen away from the edges of the field if possible. Along each transect, five leaves with spots were

collected at each of five sampling points spaced 10 m apart, resulting in a total of 20 sampling points and 100 leaves collected per field (Fig. 2). Additional information about cultivar and fungicides applied within 7 days and within 7 to 14 days prior to sampling was obtained from growers. GPS coordinates for each field were determined and saved prior to sampling. To prevent confounding of the factor county with sampling date and plant age, samples from at least two counties were taken on any given sampling day in spring and fall of 2016.

Leaf processing. Leaves from each sampling point were placed in a separate plastic bag and transferred to a cooler after sampling of a field was completed. Upon arrival at the laboratory, bags with leaves from 1 to 2 fields were incubated under constant light and high relative humidity for 12 to 25 h. Remaining samples were stored at 8°C until initial samples were processed, incubated, and examined, so that all leaves collected on an individual sampling day were processed within 72 h after collection. Leaves were examined with a stereomicroscope at 7× to 45× magnification, and reproductive structures observed were used for identification of pathogens. Presence of pathogens was recorded for each leaf. When no pathogens could be identified on any leaf from a sampling point, one lesion from each of four leaves per sampling point was surface disinfested in 0.825% sodium hypochlorite for 60 s, rinsed in autoclaved deionized water, and placed onto quarter-strength potato dextrose agar (QPD) amended with 0.1 g/liter chloramphenicol, 0.1 g/liter streptomycin, and 45.3 µl/liter mefenoxam (Ridomil Gold SL, Syngenta) for pathogen recovery (Keinath 2008). Four leaves from each sampling point were cultured for all four fields that were sampled in fall 2017. Because of the large number of leaves with typical symptoms of GSB but no signs of the pathogen in spring 2015 and spring 2016, the number of leaves in this category were recorded in fall 2016 and fall 2017. For identification of powdery mildew fungi, conidia of three isolates from each of spring 2015 and spring 2016 were mounted in 3% aqueous potassium hydroxide and examined microscopically for the presence of fibrosin bodies (Kable and Ballantyne 1963).

Molecular identification. In fall 2016, in fields where symptoms of viral diseases were found, a subsample of 10 leaves per field was

sent to a commercial laboratory (Agdia Incorporated, Elkhart, IN) for ELISA testing with specific antibodies for 14 viruses pathogenic to watermelon and a general antibody for the Potyvirus group. Identification of the begomovirus found in fall 2017 is in progress (Keinath et al., unpublished). Isolates of *Stagonosporopsis* spp. obtained as described above were stored on dried filter paper at –20°C in the dark until further use. In total, 80 isolates representing five fields, four growers, and three seasons (Table 2) were saved for species identification with a multiplex polymerase chain reaction (PCR) assay (Brewer et al. 2015). Briefly, agar plugs of actively growing cultures were transferred to plastic containers containing autoclaved 2.4% potato dextrose broth and incubated in the dark for 7 days at 22°C. Mycelium was harvested and added to 2-ml tubes containing a ceramic bead, two scoops of fine sand, and 500 µl of lysis buffer (400 mM Tris [pH 8], 60 mM EDTA [pH 8], 150 mM NaCl). Tissue was lysed for two cycles of 20 s in a bead beater (FastPrep-24, M.P. Biomedicals, Irvine, CA), after which 50 µl of 10% sodium dodecyl sulfate was added and vortexed. After 10 min at room temperature, 150 µl of potassium acetate (pH 4.8) was added to the sample, vortexed, and centrifuged at 10,000 × g for 1 min. The supernatant was removed, 10 µl of RNase A was added, and samples were incubated at 37°C for 20 min. Subsequently, samples were centrifuged again at 10,000 × g for 1 min, and the supernatant (600 µl) was added to 600 µl of isopropanol and gently mixed by inversion for 1 min followed by centrifuging at 10,000 × g for 2 min. The supernatant was discarded, and the DNA pellet was rinsed with 70% ethanol, dried, and resuspended in 50 µl of sterile water. The concentration of the extracted DNA was measured using the Qubit dsDNA BR Assay Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. The extracted DNA was adjusted to a concentration of 10 ng/µl and used as template in the PCR reactions.

The three primer pairs Db01F (5'-CGGTCCGGTCAACCTACTAC-3') and Db01R (5'-CACGCCAGCAATCTCACTA-3'), Db05F (5'-TATGACGTTGGGCAAGTGAG-3') and Db05R (5'-TTTGCTGGGATGGTGTGTA-3'), and Db06F (5'-GGTGACATCTTGGTGAATG-3') and Db06R (5'-TGGTTGTTTGGTTGTTTGA-3')

Table 1. Watermelon fields sampled in four seasons from 2015 to 2017 in South Carolina

Season, year	County	Acreage (ha)	Number of growers in county	Number of growers selected ^a	Number of fields of growers selected	Number of fields sampled ^b
Spring 2015	Bamberg	262	6	4	13	4
	Barnwell	379	6	5	7	5
	Beaufort	273	3	2	11	4
	Clarendon	103	1	1	1	1
	Colleton	285	4	2	14	4
	Hampton	211	5	3	3	3
Subtotal			25	17	49	21
Spring 2016	Allendale	102	4	1	3	1
	Bamberg	262	17	3	13	4
	Barnwell	379	9	5	8	6
	Beaufort	273	2	2	4	4
	Clarendon	103	4	2	4	2
	Colleton	285	4	3	23	4
Fall 2016	Hampton	211	6	3	7	3
	Subtotal		46	19	62	24
	Allendale	102	1	1	3	1
	Bamberg	262	4	2	2	2
	Barnwell	379	1	1	1	1
	Beaufort	273	2	1	5	2
Fall 2017	Colleton	285	2	2	6	3
	Hampton	211	1	1	3	2
	Subtotal		11	8	20	11
	Beaufort	273	2	2	20	4

^a The growers were chosen randomly from a list of all growers in a county.

^b The total number of fields to be sampled in a season was predefined at the beginning of each sampling season. The number of fields sampled in a county was calculated using proportional allocation based on the watermelon acreage in the county. Fields were chosen randomly from all fields of a selected grower.

were used in the multiplex PCR. These primers produce specific bands for each of the three species of *Stagonosporopsis*. The *Db01* locus is only amplified in *S. citrulli*, *Db06* amplifies fragments of *S. citrulli* and *S. cucurbitacearum*, and *Db05* amplifies fragments in all three species (Brewer et al. 2015). A total volume of 15 μ l was used for the PCR reactions. The reactions consisted of 3 μ l of 10 $\times$ buffer, 1.5 μ l dNTPs, 0.09 μ l of GoTaq (Promega, Madison, WI), 0.75 μ l of each primer, and 1 μ l of DNA template. In each PCR, reactions with DNA of isolates with known species identity were included as controls. Isolate RV16 served as control for *S. caricae*, isolate GSB19 for *S. cucurbitacearum*, and isolate C68 for *S. citrulli* (Brewer et al. 2015; Stewart et al. 2015). Thermal cycling was initiated by denaturation at 94°C for 2 min. This was followed by 35

Fig. 1. Locations of the 60 fields sampled in four seasons from 2015 to 2017. Black circles = 21 fields sampled in spring 2015. Dark gray squares = 24 fields sampled in spring 2016. Light gray diamonds = 11 fields sampled in fall 2016. White triangles = 4 fields sampled in fall 2017.

Fig. 2. Sampling scheme within a selected field. Five symptomatic leaves were collected at each of five sampling points along four transects 50 m in length perpendicular to one another. Sampling points were equally distanced from one another with a sampling interval of 10 m between points. Transects start at the corners of a square encompassing 2,500 m² (0.62 ac). Numbers 1 to 4 indicate order of transects.

cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. A final step of 72°C for 5 min completed the PCR. The entire volume of amplified product was separated on a 2.5% (wt/vol) agarose gel containing 1 $\times$ SYBR Safe nucleic acid gel stain (Invitrogen, Thermo Fisher Scientific), which was run at 95 V for 2 h and visualized with a molecular imager (Gel Doc XR+, Bio-Rad Laboratories Inc., Hercules, CA).

Data analysis. SAS (SAS Institute Inc., Cary, NC) statistical software was used for all analyses. PROC SURVEYMEANS was used to obtain estimates of the overall proportions of pathogen occurrence on leaves with spots. The county a given sample was collected from was specified as stratum variable, and the grower was specified as cluster variable. The incidence of a pathogen was the response variable, and proportions were estimated separately for each pathogen. Sampling weights were specified to account for the probability of choosing a particular field. They were calculated with the following formula: $w = 1/((a*b)/(c*d))$, where a = the number of selected growers within a county, b = number of selected fields within the county in a , c = total number of growers of the county in a , and d = total number of fields of the county in a (Table 1). In a combined analysis, data from all seasons were analyzed together by including each county-season combination as a separate stratum. In order to compare the estimates of overall pathogen occurrence, SAS PROC SURVEYMEANS was used to calculate 95% confidence intervals for the differences in occurrence between pathogen pairs. These differences were computed separately for each field. Associations between pathogen pairs on each leaf were calculated with PROC SURVEYFREQ for each season. Strata and cluster variables and sampling weights were accounted for in the analysis as described for estimation of overall means. The CHISQ option was used to calculate Rao-Scott Chi-Square tests for each pathogen combination (Rao and Scott 1981, 1984, 1987).

Results

Environmental conditions during sampling. The average temperatures prevailing in the months during which the sampling was carried out were all elevated compared with the 30-year average of the region (Table 3). In July and September 2016, the mean monthly temperature was more than 2°C higher than normal (National Weather Service Forecast Office 2018). In the other months, June and July 2015, June and October 2016, and September 2017, the mean temperatures were elevated from 0.6 to 1.8°C compared with the 30-year average. Spring 2016 was an unusually dry season. In June and July, the total precipitation was 115.5 and 84.8 mm below that of the previous year, which amounts to 63.8 and 54.6 mm below the 30-year average, respectively. In contrast to that, the fall of 2016 was wetter than normal. Both September and October received ≥ 266 mm of total precipitation, which was 156.7 and 170.9 mm above the average for these months. June and July 2015 and September 2017 received precipitation only slightly above the average, ranging from 14.2 to 51.8 mm precipitation above the average (Table 3).

Occurrence of pathogens. In spring 2015, spring 2016, and fall 2017, the highest percentage of leaves had no pathogen structures. The percentage of leaves without pathogens ranged from 24.1% in fall 2016 to 38.3% in fall 2017. Among leaves with only a single pathogen, the most common pathogen was by far *Stagonosporopsis* spp., which occurred at the highest frequency of all pathogens in spring 2015, spring 2016, and fall 2016. In fall 2016, signs of

Table 2. Isolates of *Stagonosporopsis* spp. collected throughout sampling of watermelon fields in 2015 to 2017

Season, year	County	<i>S. citrulli</i>	<i>S. caricae</i>	Total number of isolates
Spring 2015	Barnwell	15	5	20
Spring 2015	Beaufort	11	3	14
Spring 2016	Hampton	8	0	8
Fall 2017	Beaufort	35	0	35
Fall 2017	Beaufort	3	0	3
Total	3	72	8	80

Stagonosporopsis spp. were present on 64.0% of all examined leaves ($n = 1,100$), and typical symptoms of GSB were found on an additional 19.6% of leaves. Based on the condition that leaves were only cultured when no pathogens could be identified on any of the five leaves from a given sampling point, 10.4% of the 48 leaves cultured in fall 2016 yielded *Stagonosporopsis* spp., while the rest yielded no plant-pathogenic fungi. In fall 2017, 61.5% ($n = 400$) of the collected leaves exhibited symptoms of GSB, of which 42.7% showed signs of *Stagonosporopsis* spp. In order to obtain additional isolates of *Myrothecium* s.l. for future studies, all 400 leaves collected in fall 2017 were cultured. *Stagonosporopsis* spp. was recovered from 9.5% of those leaves.

Fibrosin bodies were present in conidia of all isolates of powdery mildew examined in 2015 and 2016, which confirmed their identity as *P. xanthii* (McGrath 2017). In spring 2015 and 2016, *P. xanthii* was the second most frequent pathogen observed. *P. xanthii*, however, occurred only about a third as often as *Stagonosporopsis* spp. in 2015. Out of the 21 fields sampled in spring 2015, *Stagonosporopsis* spp. was the most prevalent pathogen in 13 fields, and *P. cubensis* and *P. xanthii* each were the predominant pathogen in four of the remaining eight fields. In spring 2016, however, there were almost as many leaves with *P. xanthii* as with *Stagonosporopsis* spp. As in the previous season, *Stagonosporopsis* spp. was the most prevalent pathogen in 13 of the 24 fields, but *P. xanthii* was the most frequent in nine fields. In the other two fields, *C. orbiculare* was found most frequently. In fall 2016, *Stagonosporopsis* spp. was the most common pathogen occurring by itself, followed by *Myrothecium*

s.l. In fall 2016, *Stagonosporopsis* spp., *P. xanthii*, or *Myrothecium* s.l. was the most prevalent pathogen in eight, two, and one field out of the 11 fields sampled, respectively. By itself, however, *P. xanthii* occurred on less than 2.5% of leaves in fall 2016, and in fall 2017 it was one of the two least frequent pathogens occurring without other pathogens. The occurrence of *P. cubensis* was highest in spring 2015 and rare in fall 2017. It was not observed at all in the two 2016 seasons. The percentage of leaves with only *C. orbiculare* was highest in spring 2016 and very low in fall 2016 and 2017. *C. orbiculare* was not observed in spring 2015. *Myrothecium* s.l. was the second most common pathogen occurring by itself in fall 2016 and the most common in fall 2017. It was not detected in 2015 and was infrequent in spring 2016. The third most common pathogen in fall 2017, *Corynespora cassiicola*, was not observed in any other season. *Stagonosporopsis* spp. and *Myrothecium* s.l. each were the most prevalent pathogens in two of the four fields that were sampled in fall 2017. In fall 2016, viral pathogens were found in two fields, one in Bamberg County and one in Barnwell County. Based on ELISA testing, watermelon plants in those two fields were coinfecting by Papaya ringspot virus (PRSV) and Zucchini yellow mosaic virus (ZYMV). In fall 2017, a begomovirus was detected in two of the four fields sampled at low frequency (2 to 4%).

Estimates for probability of statewide pathogen occurrence. *Stagonosporopsis* spp. was the pathogen most likely to occur in all seasons except in fall 2017, when *Myrothecium* s.l. had an equally high probability of occurrence (Fig. 3). From spring 2015 to spring

Table 3. Sampling intervals and monthly environmental conditions during sampling of watermelon leaves

Season, year	Month	Sampling dates	Max (°C) ^a	Min (°C) ^a	Total rainfall (mm) ^a
Spring 2015	June	23 June (first)	33.1	22.7	195.3
	July	27 July (last)	33.6	23.4	196.1
Spring 2016	June	13 June (first)	33.0	22.8	79.8
	July	14 July (last)	35.3	24.9	111.3
Fall 2016	September	20 September (first)	31.3	21.8	311.7
	October	3 October (last)	27.1	15.4	266.2
Fall 2017	September	26 September	30.4	20.3	171.5

^a Weather data for Charleston, SC, from National Weather Forecast Service Office, available at: weather.gov

Fig. 3. Estimated probability of statewide pathogen occurrence in South Carolina by season 2015 to 2017. Error bars give standard errors calculated with SAS, PROC SURVEYMEANS.

2016, there was a marked decrease in the estimated probability of occurrence for *Stagonosporopsis* spp., which then increased to its highest level in fall 2016. The estimates for *Stagonosporopsis* spp. from the four fields that were sampled in fall 2017 were the lowest of all seasons. *P. xanthii* had the second highest estimates of occurrence in spring 2015 and spring 2016. In fall 2016, *Myrothecium* s.l. was as likely to occur as *P. xanthii*. With the exception of three leaves with *Pseudoperonospora cubensis* in fall 2017, this pathogen only occurred in spring 2015. That season, its likelihood of occurrence was comparable to that of *P. xanthii*. *Cercospora citrullina* and *C. orbiculare* both occurred in all seasons with very low probabilities of $\leq 7\%$. In fall 2017, *Myrothecium* s.l. were the pathogens with the highest probability of occurrence. The causal agent of target spot of cucurbits, *C. cassiicola*, occurred only in fall 2017. Its probability of occurrence was low, but nonetheless it was the third highest among all observed pathogens that season. Viral pathogens were only found in fall 2016 and fall 2017. Both seasons, their probability of occurrence was among the lowest (Fig. 3).

Across seasons, the probability of occurrence for *Stagonosporopsis* spp. was significantly higher than for all other pathogens that were found throughout this survey (based on 95% confidence intervals) (Fig. 4). Only leaves with no pathogens were as likely to occur as leaves with *Stagonosporopsis* spp. Overall, *P. xanthii* was the pathogen second most likely to occur on watermelon leaves in South Carolina. It was significantly more likely to occur than any other pathogen except *Stagonosporopsis* spp. There was no statistical difference in the probability of occurrence among the four pathogens, *C. citrullina*, *C. orbiculare*, *Myrothecium* s.l., and *P. cubensis*, except that *C. citrullina* was significantly more likely to occur than *C. orbiculare* and *P. cubensis*. Their overall probabilities of occurrence ranged from 3.8 to 6.0%. These pathogens were much less likely to occur than both *Stagonosporopsis* spp. and *P. xanthii*.

The remaining pathogens, *C. cassiicola*, PRSV, ZYMV, and begomovirus, were found in low frequencies in only one season and therefore were excluded from the combined analysis across seasons.

Species of *Stagonosporopsis* spp. Of the 80 isolates of *Stagonosporopsis* spp. identified to species during this study, eight were identified as *S. caricae*. The remaining 72 isolates were identified as *S. citrulli*. *S. caricae* was found in Barnwell and Beaufort counties in spring 2015 but not in 2016 or 2017 (Table 2). All isolates collected in Hampton County were identified as *S. citrulli*. Although in spring 2015, the incidence of *S. caricae* was 21.4% in the field sampled in Beaufort County, all 38 isolates collected in fall 2017 in Beaufort were *S. citrulli*. The fields sampled in these two seasons were from different growers. The incidence of *S. caricae* in the field in Barnwell County in spring 2015 was 25.0%.

Associations between pathogens. A total of 7, 10, 7, and 11 different combinations of two pathogens occurred together on the same leaf in spring 2015, spring 2016, fall 2016, and fall 2017, respectively (Tables 4 and 5). The most common pathogen pair occurring together on the same leaf was *P. xanthii* and *Stagonosporopsis* spp. in all seasons. Combinations of three pathogens occurring together on the same leaf were observed on $<5\%$ of sampled leaves in all seasons. There were 4, 6, 2, and 14 different combinations of three pathogens that occurred on the same leaf in the four seasons. A few leaves with four different pathogens were observed with one combination in spring 2015 and two different combinations in spring 2016 and fall 2017. Over the course of the entire study, there were a total of 19, 21, and 5 combinations of two, three, and four pathogens co-occurring on the same leaves (Tables 4 and 5).

Statistical analyses of the frequencies of pathogen occurrence revealed a number of significant associations between pathogen pairs (Table 6). In two seasons, spring 2015 and spring 2016, there was a positive association between *Stagonosporopsis* spp. and *C. citrullina*

Fig. 4. Estimated probability of statewide pathogen occurrence across all four seasons 2015 to 2017. Error bars give one standard error calculated with SAS, PROC SURVEYMEANS. Mean separation is based on 95% confidence intervals. *Corynespora cassiicola* and the viruses found in fall 2016 and fall 2017 were excluded from the combined analysis because each occurred in one season only.

($P < 0.0001$). When *Stagonosporopsis* spp. were present on a watermelon leaf, it was more likely for *C. citrullina* to be present than on leaves where *Stagonosporopsis* spp. was not present. In fall 2016, *Stagonosporopsis* spp. and *P. xanthii* also were positively associated with each other ($P < 0.0001$), meaning that when *Stagonosporopsis* spp. were present on a watermelon leaf, it was more likely for *P. xanthii* to be present. In spring 2016, there was a negative association between *Stagonosporopsis* spp. and *C. orbiculare*. It was less likely for *C. orbiculare* to occur on a leaf when *Stagonosporopsis* spp. was present on that leaf. In fall 2016, *Stagonosporopsis* spp. and *Myrothecium* s.l. were dissociated with one another ($P < 0.0001$), and there was a positive association between *Stagonosporopsis* spp. and viral pathogens ($P = 0.0002$).

Apart from *Stagonosporopsis* spp., *P. xanthii* also had several significant associations with other pathogens throughout the survey. In spring 2016, there were dissociations with *C. citrullina* ($P = 0.0001$) and *C. orbiculare* ($P = 0.0394$), and in fall 2016 there was a dissociation with *Myrothecium* s.l. ($P = 0.0002$) in addition to the already mentioned positive association with *Stagonosporopsis* spp. (Table 6).

Table 4. Occurrence of pathogens in combinations on watermelon leaves in spring 2015 and 2016

Pathogen combination ^a	Percentage of leaves	
	2015	2016
None	28.06	30.21
<i>Stagonosporopsis</i>	25.63	21.83
<i>Podosphaera</i>	8.00	20.42
<i>Pseudoperonospora</i>	6.19	0.00
<i>Cercospora</i>	1.29	1.12
Virus	0.10	0.00
<i>Myrothecium</i>	0.05	0.08
<i>Colletotrichum</i>	0.00	6.04
<i>Podosphaera</i> + <i>Stagonosporopsis</i>	9.96	14.08
<i>Pseudoperonospora</i> + <i>Stagonosporopsis</i>	7.29	0.00
<i>Cercospora</i> + <i>Stagonosporopsis</i>	3.14	1.75
<i>Pseudoperonospora</i> + <i>Podosphaera</i>	2.57	0.00
<i>Cercospora</i> + <i>Pseudoperonospora</i>	0.52	0.00
<i>Cercospora</i> + <i>Podosphaera</i>	0.48	0.67
<i>Colletotrichum</i> + <i>Stagonosporopsis</i>	0.05	1.21
<i>Colletotrichum</i> + <i>Podosphaera</i>	0.00	0.33
<i>Myrothecium</i> + <i>Podosphaera</i>	0.00	0.21
<i>Myrothecium</i> + <i>Stagonosporopsis</i>	0.00	0.17
<i>Cercospora</i> + <i>Colletotrichum</i>	0.00	0.08
<i>Cercospora</i> + <i>Myrothecium</i>	0.00	0.04
<i>Colletotrichum</i> + <i>Myrothecium</i>	0.00	0.04
<i>Pseudoperonospora</i> + <i>Podosphaera</i> + <i>Stagonosporopsis</i>	4.76	0.00
<i>Cercospora</i> + <i>Podosphaera</i> + <i>Stagonosporopsis</i>	0.86	1.08
<i>Cercospora</i> + <i>Pseudoperonospora</i> + <i>Stagonosporopsis</i>	0.48	0.00
<i>Cercospora</i> + <i>Pseudoperonospora</i> + <i>Podosphaera</i>	0.14	0.00
<i>Colletotrichum</i> + <i>Podosphaera</i> + <i>Stagonosporopsis</i>	0.00	0.25
<i>Myrothecium</i> + <i>Podosphaera</i> + <i>Stagonosporopsis</i>	0.00	0.17
<i>Cercospora</i> + <i>Colletotrichum</i> + <i>Stagonosporopsis</i>	0.00	0.04
<i>Cercospora</i> + <i>Myrothecium</i> + <i>Stagonosporopsis</i>	0.00	0.04
<i>Cercospora</i> + <i>Colletotrichum</i> + <i>Podosphaera</i>	0.00	0.04
<i>Cercospora</i> + <i>Pseudoperonospora</i> + <i>Podosphaera</i> + <i>Stagonosporopsis</i>	0.48	0.00
<i>Cercospora</i> + <i>Podosphaera</i> + <i>Myrothecium</i> + <i>Stagonosporopsis</i>	0.00	0.04
<i>Cercospora</i> + <i>Colletotrichum</i> + <i>Podosphaera</i> + <i>Stagonosporopsis</i>	0.00	0.04
Total number of leaves	2099	2400

^a Combination of pathogens that were present on a leaf.

Fungicides applied in fall seasons. On the 11 fields sampled in fall 2016, a total of 16 different fungicides was applied within 2 weeks prior to sampling. Seven of these were effective against GSB. In fall 2016, all but one field received at least two fungicides effective against GSB. On the four fields sampled in fall 2017, a total of 12 different fungicides was applied within the 2 weeks prior to sampling. Of these fungicides, three were effective against GSB. All four fields sampled in fall 2017 received at least three fungicides effective against GSB. All four were treated with cyprodinil, a FRAC

Table 5. Occurrence of pathogens in combinations on watermelon leaves in fall 2016 and 2017

Pathogen combination ^a	Percentage of leaves	
	2016	2017
<i>Stagonosporopsis</i>	38.82	9.25
None	24.09	38.25
<i>Myrothecium</i>	7.18	16.50
<i>Podosphaera</i>	2.36	0.75
<i>Cercospora</i>	0.82	0.00
<i>Colletotrichum</i>	0.45	3.25
Virus	0.27	1.00
<i>Corynespora</i>	0.00	5.50
<i>Pseudoperonospora</i>	0.00	0.25
<i>Podosphaera</i> + <i>Stagonosporopsis</i>	14.91	3.75
<i>Stagonosporopsis</i> + Virus	3.82	0.25
<i>Myrothecium</i> + <i>Stagonosporopsis</i>	3.18	3.50
<i>Cercospora</i> + <i>Podosphaera</i>	0.73	0.00
<i>Colletotrichum</i> + <i>Stagonosporopsis</i>	0.73	1.00
<i>Cercospora</i> + <i>Stagonosporopsis</i>	0.45	0.00
<i>Colletotrichum</i> + <i>Myrothecium</i>	0.09	1.25
<i>Corynespora</i> + <i>Myrothecium</i>	0.00	3.25
<i>Corynespora</i> + <i>Stagonosporopsis</i>	0.00	3.25
<i>Colletotrichum</i> + <i>Corynespora</i>	0.00	0.50
<i>Colletotrichum</i> + <i>Podosphaera</i>	0.00	0.50
<i>Corynespora</i> + <i>Podosphaera</i>	0.00	0.25
<i>Myrothecium</i> + <i>Pseudoperonospora</i>	0.00	0.25
<i>Cercospora</i> + <i>Podosphaera</i> + <i>Myrothecium</i>	1.91	0.00
<i>Myrothecium</i> + <i>Podosphaera</i> + <i>Stagonosporopsis</i>	0.18	0.75
<i>Corynespora</i> + <i>Podosphaera</i> + <i>Stagonosporopsis</i>	0.00	1.75
<i>Colletotrichum</i> + <i>Corynespora</i> + <i>Stagonosporopsis</i>	0.00	0.75
<i>Colletotrichum</i> + <i>Corynespora</i> + <i>Myrothecium</i>	0.00	0.75
<i>Corynespora</i> + <i>Myrothecium</i> + <i>Podosphaera</i>	0.00	0.50
<i>Cercospora</i> + <i>Corynespora</i> + <i>Myrothecium</i>	0.00	0.25
<i>Cercospora</i> + <i>Corynespora</i> + <i>Stagonosporopsis</i>	0.00	0.25
<i>Colletotrichum</i> + <i>Corynespora</i> + <i>Pseudoperonospora</i>	0.00	0.25
<i>Colletotrichum</i> + <i>Corynespora</i> + <i>Podosphaera</i>	0.00	0.25
<i>Colletotrichum</i> + <i>Myrothecium</i> + <i>Podosphaera</i>	0.00	0.25
<i>Colletotrichum</i> + <i>Myrothecium</i> + <i>Stagonosporopsis</i>	0.00	0.25
<i>Colletotrichum</i> + <i>Podosphaera</i> + <i>Stagonosporopsis</i>	0.00	0.25
<i>Corynespora</i> + <i>Myrothecium</i> + <i>Stagonosporopsis</i>	0.00	0.25
<i>Corynespora</i> + <i>Stagonosporopsis</i> + Virus	0.00	0.25
<i>Stagonosporopsis</i> + <i>Podosphaera</i> + <i>Colletotrichum</i> + <i>Corynespora</i>	0.00	0.50
<i>Stagonosporopsis</i> + <i>Podosphaera</i> + <i>Corynespora</i> + <i>Myrothecium</i>	0.00	0.25
Total number of leaves	1100	400

^a Combination of pathogens that were present on a leaf. Virus = Papaya ring-spot virus and Zucchini yellow mosaic virus in 2016 and a begomovirus in 2017.

group 9 fungicide, and fludioxonil, a FRAC group 12 fungicide (Table 7).

Discussion

In this study, a total of 5,999 symptomatic leaves from 60 fields in the main watermelon growing counties in South Carolina were examined for foliar pathogens in four seasons from spring 2015 to fall 2017. To the best of our knowledge, this study is the first multiseason survey of foliar diseases of watermelon in the United States with an appropriate underlying sampling design and statistical analysis. The overall most prevalent pathogen was *Stagonosporopsis* spp., the causal agent of GSB. The fact that an additional 20 to 35% of leaves had typical symptoms of GSB in the fall seasons of 2016 and 2017 indicates that *Stagonosporopsis* spp. was even more prevalent than could be estimated in this study. The lack of sporulation of *Stagonosporopsis* spp. as observed in the fall seasons in 2016 and 2017 might have been caused by the application of fungicides. The fact that the majority of fields received three or more applications of fungicides with activity against *Stagonosporopsis* spp. within a 2-week period

prior to sampling supports this explanation (Rennberger et al. unpublished). Inhibitory effects of fungicides on the sporulation of fungal pathogens have been shown in previous studies. Quinone-outside-inhibitor fungicides were found to reduce sporulation of the brown rot pathogen *Monilinia fructicola* of peaches. The sporulating area on fruits and twig cankers was reduced 50 to 70%, while the colony growth reduction was only $\leq 12\%$ (Burnett et al. 2010). Similarly, tricyclazole and azoxystrobin were shown to reduce the sporulation of *Magnaporthe oryzae*, the rice blast fungus (Kunova et al. 2013). Based on the observations in this study, we are examining the effects of fungicides on sporulation by *Stagonosporopsis* spp.

The overall second most prevalent pathogen was *P. xanthii*, which causes powdery mildew of cucurbits. The high occurrence of *P. xanthii*, especially in the more humid fall seasons was somewhat unexpected but an important observation. In spring 2016, the frequency of leaves with *P. xanthii* and the estimated probability of occurrence of this pathogen were almost as high as for *Stagonosporopsis* spp. Occurrence of *P. xanthii* was comparable to *Stagonosporopsis* spp. in spring 2016. The majority of the watermelon

Table 6. Associations between pathogen pairs on watermelon leaves collected in 2015 and 2016

Pathogen	Season, year		<i>Stagonosporopsis</i> spp.			<i>P. xanthii</i>		
			Absent	Present	<i>P</i> value ^a	Absent	Present	<i>P</i> value
<i>Cercospora citrullina</i>	Spring 2015	Absent	944 ^b	1000	<0.0001	1413	531	0.6511
		Present	51	104		114	41	
<i>Cercospora citrullina</i>	Spring 2016	Absent	1376	905	<0.0001	1430	851	0.0001
		Present	47	72		74	45	
<i>Colletotrichum orbiculare</i>	Spring 2016	Absent	1266	940	0.0360	1326	880	0.0394
		Present	157	37		178	16	
<i>Podosphaera xanthii</i>	Fall 2016	Absent	362	517	<0.0001	n.a. ^c	n.a.	n.a.
		Present	34	187		n.a.	n.a.	n.a.
<i>Myrothecium</i> s.l.	Fall 2016	Absent	316	667	<0.0001	764	219	0.0002
		Present	80	37		115	2	
Virus	Fall 2016	Absent	393	662	0.0002	834	221	n.c. ^d
		Present	3	42		45	0	

^a The probability of a greater Rao-Scott χ^2 for the proportions of the two pathogens.

^b Numbers are total numbers of leaves in designated category in that season.

^c n.a. = not applicable.

^d n.c. = not computable. This pathogen pair never occurred together on the same leaf.

Table 7. Fungicides applied within 2 weeks prior to sampling in watermelon fields sampled in fall 2016 and fall 2017^a

Fungicide	FRAC code ^b	Effective against GSB ^c	Number of fields	
			Fall 2016	Fall 2017
Azoxystrobin	11	No	1	2
Boscalid	7	No	2	2
Copper	M	No	2	0
Chlorothalonil	M	Yes	2	0
Cyazofamid	21	No	6	2
Cyflufenamid	U6	No	2	0
Cyprodinil	9	Yes	3	4
Difenoconazole	3	Yes	3	0
Fludioxonil	12	Yes	1	4
Fluopyram	7	Yes	6	0
Mancozeb	M	Yes	9	2
Mefenoxam	4	No	0	2
Oxathiapiprolin	49	No	0	2
Phosphonate	33	No	2	0
Propamocarb	28	No	0	2
Pyraclostrobin	11	No	2	4
Quinoxifen	13	No	2	0
Tebuconazole	3	Yes	7	3
Thiophanate-methyl	1	No	0	2
Zoxamide	22	No	2	0

^a Eleven and four fields were sampled in fall 2016 and 2017, respectively.

^b FRAC = Fungicide Resistance Action Committee.

^c GSB = Gummy stem blight. Classification based on Kemble et al. (2018).

cultivars planted in the fields sampled in this study were triploid cultivars, which were found to be less susceptible to powdery mildew than diploid cultivars (Keinath and Hassel 2000). Nonetheless, powdery mildew consistently was one of the most prevalent diseases found in this survey.

Overall, the estimates for the probability of occurrence in the state were low to very low for the other six pathogens that were found in this survey. Apart from *C. citrullina*, which occurred in every season, all pathogens other than the two most prevalent ones were absent in at least one season. *Cercospora* leaf spot was reported to occur along with anthracnose, GSB, and downy mildew on watermelon in research plots in Maryland in 2012 and 2013 (Everts et al. 2016). The occurrence of *P. cubensis* in this study was low and can be characterized as sporadic, since it was not found in the two seasons in 2016 and only at a very minor level in fall 2017. Apart from four fields where *P. cubensis* was the predominant pathogen, it also occurred infrequently on cucumbers in North Carolina. However, the survey by Peterson and Campbell in the late 1990s was done before the introduction of the A1 mating type, which is very virulent on cucumber (Holmes et al. 2015; Thomas et al. 2017c). Consistent with the suggestion by Peterson and Campbell (2002), this sporadic occurrence that is difficult to predict is most likely due to the reliance on windborne dissemination and the lack of overwintering in the Carolinas by the pathogen (Hausbeck 2017; Holmes et al. 2015). Another possible explanation for the infrequent occurrence of this pathogen might be that there are two distinct, genetic lineages of *P. cubensis* with host specialization. A distinct genetic cluster within lineage 1 was predominantly found on *C. lanatus* (Thomas et al. 2017a, c). This particular cluster may not be as common as isolates pathogenic on other cucurbits.

Peterson and Campbell (2002) examined 5,400 cucumber leaves with leaf spots in North Carolina from 1996 to 1998. In contrast to the results of the present survey, *C. orbiculare* was the most prevalent pathogen in two of the three seasons (1996 and 1998) and in a majority of the fields included in their study, while *Stagonosporopsis* spp. (formerly *D. bryoniae*) was the predominant pathogen in 1997 only. Overall, *Stagonosporopsis* spp. was the second most frequent pathogen in the survey in North Carolina. It was the most prevalent pathogen in 2 of 6, 7 of 18, and 6 of 24 sites sampled. The greater occurrence of *Stagonosporopsis* spp. in the current study compared with Peterson and Campbell's (2002) survey can be explained, in part, by the fact that watermelon generally is a more susceptible crop than cucumber (Keinath 2014). The occurrence of *C. orbiculare* in South Carolina throughout the present study was very low; the <4% estimated probability on watermelon fields in the state is significantly lower than that of *Stagonosporopsis* spp. and *P. xanthii*. With regards to *P. xanthii*, there is a stark difference between Peterson and Campbell's study and the present one. While there was no observation of this pathogen on cucumbers in North Carolina, it was a major pathogen in our survey, second only to *Stagonosporopsis* spp. The likely explanation for this is that cucumber growers in North Carolina planted cultivars resistant to powdery mildew (Peterson and Campbell 2002). On the other hand, there is currently only a single commercial watermelon cultivar resistant to powdery mildew, Lemon Krush, and it was not planted in any of the 60 fields sampled for this survey (Kemle et al. 2018).

The prevalence of *Stagonosporopsis* spp., a pathogen dependent on humid conditions, and *P. xanthii*, a pathogen capable of development under drier conditions, was clearly influenced by the environmental conditions that prevailed during sampling (Keinath 2017b; McGrath 2017). In spring 2016, the combined rainfall in June and July was less than half of that of the previous year, while fall 2016 was the most humid season with somewhat lower temperatures (Table 2). The estimated occurrence of *Stagonosporopsis* spp. dropped almost 15% from spring 2015 to spring 2016 and then rose more than 20% in fall 2016, above its level in spring 2015. The estimated occurrence of *P. xanthii*, however, followed a reverse trend. From spring 2015 to spring 2016, it rose almost 9% from where it dropped more than 13% below its level in spring 2015. The drier conditions in spring 2016 thus favored *P. xanthii*.

In the survey by Peterson and Campbell (2002), *C. cassiicola* occurred in every season and was the most prevalent pathogen in four fields that were sampled in 1997. In contrast, the present study is the first report of *C. cassiicola* on watermelon in South Carolina. Apart from this study, there is only one other U. S. state, North Carolina, where watermelon was reported as a host of this pathogen (Grand 1985; Farr and Rossman 2018). This indicates that the isolates of *C. cassiicola* present in the Carolinas might have a unique host adaptation that enables them to utilize watermelon as an additional host. Considering the wide host range of more than 530 plant species, and the fact that there are many different host range and host specificity patterns among isolates of *C. cassiicola*, it appears possible that only a small subset of isolates in a small geographic region would be adapted to infect watermelon (Cutrim and Silva 2003; Dixon et al. 2009; Farr and Rossman 2018; Onesiroso et al. 1974).

Peterson and Campbell (2002) tested associations between pathogen pairs using a 2-by-2 contingency table χ^2 analysis whenever pathogens occurred together at least five times in a field. No such restriction was used in the present study; instead, all leaves were included to test pathogen associations, separately for each season. Peterson and Campbell (2002) found significant associations for six different pathogen pairs. A negative association between *C. orbiculare* and *Stagonosporopsis* spp. accounted for more than half of them and occurred in all three seasons. In the current study, associations between pathogen pairs were analyzed using a Rao-Scott χ^2 test, which includes adjustments necessary to account for effects of clustering in the survey design, which can otherwise impact the significance level of the test (Rao and Scott 1984, 1987). Turechek and Madden (2000) used the Jaccard similarity index to analyze the association between leaf blight caused by *Phomopsis obscurans* and leaf spot caused by *Ramularia grevilleana* (syn. *Mycosphaerella fragariae*) on strawberries. They argued that the method of the Jaccard similarity index is more suitable to analyze associations between plant pathogens, because it does not use information of sampling units when neither pathogen occurs and because it does not assume that the pathogens are randomly distributed. The Rao-Scott test used in this study, however, has the advantage of accounting for the sampling design and a more straightforward estimation method of standard errors compared with the Jaccard similarity index. Turechek and Madden (2000) found that *P. obscurans* and *R. grevilleana* were distributed independently and that there was a general lack of co-occurrence between the two pathogens. Many studies have found aggregation of plant pathogens, but random spatial patterns of diseases also are not uncommon. Oerke et al. (2010) studied the spatial patterns of Fusarium head blight pathogens in wheat fields and found that most species of *Fusarium* spp. were distributed randomly. Their results indicate that spatial distributions of plant pathogens are influenced by many factors such as inoculum source, spore type, kind of dispersal, host availability, and microclimate. Species that depended on splash dispersal tended to be more aggregated, whereas species that relied on wind dispersal had a predominantly random distribution (Oerke et al. 2010). Especially for wind-dispersed foliar pathogens, such as *Stagonosporopsis* spp., *P. cubensis*, *P. xanthii*, and *C. citrullina*, and aphid-transmitted viruses, the assumption of a random distribution within the sampled fields seems therefore adequate.

In spring 2016, we observed the same association Peterson and Campbell (2002) encountered so frequently. *Stagonosporopsis* spp. and *C. orbiculare* were dissociated with one another, meaning that it was less likely for the former to occur on the same leaf, if the latter was present on that leaf or vice versa (Table 6). Our finding of a negative association between these two pathogens in spring 2016 agrees with the report from North Carolina. Peterson and Campbell (2002) suggested that the two pathogens might occupy distinct ecological niches in the plant canopy. However, considering the great overlap of the environmental conditions required for development of the two pathogens, this seems unlikely (Monroe et al. 1997; van Steekelenburg 1982). With short leaf wetness periods, *C. orbiculare* may be more likely than *Stagonosporopsis* spp. to infect a leaf. In fall 2016, there was a positive association between *Stagonosporopsis* spp. and *P. xanthii*. Bergstrom et al. (1982) suggested that infection

by *P. xanthii* predisposes cucurbits to infection by *Stagonosporopsis* spp. by facilitating nutrient release from the host that supports conidia germination. Although it has been shown that exogenous nutrients enhance spore germination and infection rates by *Stagonosporopsis* spp., the only positive association between the two most common pathogens in the present study, *P. xanthii* and *Stagonosporopsis* spp., was in fall 2016. It is possible that environmental conditions favorable for the development of both pathogens promoted their co-occurrence rather than a direct synergism between the two (Table 2) (Chiu and Walker 1949; Keinath and DuBose 2012; Svedelius and Unestam 1978). On the other hand, *P. xanthii* was negatively associated with three pathogens that require leaf wetness for infection, namely *C. citrullina*, *C. orbiculare*, and *Myrothecium* s.l., which implies that powdery mildew was less likely to occur in wet microenvironments that favored these pathogens. In general, a significant positive association does not allow for interpretation about which pathogen infected a leaf first nor which pathogen yields the main influence on the association.

Of the 80 isolates of *Stagonosporopsis* spp. cultured and identified to species level, 10% were identified as *S. caricae*, while the remaining isolates were identified as *S. citrulli*. *S. cucurbitacearum* was not found. This constitutes the first report of *S. caricae* in South Carolina. Among this subset of isolates, the frequency of *S. caricae* was much higher than previously reported in the southeastern United States. Brewer et al. (2015) genotyped 600 isolates of *Stagonosporopsis* spp. from Georgia and identified only 1% as *S. caricae*. All isolates were collected from a single field in Tift County in 2013. The following year, Li and Brewer (2016) reported a slightly higher occurrence of *S. caricae*, 3.6% of 528 isolates. These isolates originated from Tift and Cook counties in Georgia in 2013 and 2014, respectively, in which they represented 4 and 20% of the sampled isolates, respectively (Li and Brewer 2016). *S. caricae* was not found in two fields in Colleton County, South Carolina, that were sampled in 2012 and 2013 (Li and Brewer 2016). With 25 and 21.4% of isolates identified as *S. caricae* in Barnwell and Beaufort Counties in spring 2015, the proportion of isolates that were identified as that species was somewhat higher than in the two Georgia counties. On the other hand, *S. caricae* was not found in the other seasons and counties from which isolates were saved and tested (Hampton County in spring 2016 and Beaufort County in fall 2017). As with the sampling done by Li and Brewer (2016), it appears that the distribution of *S. caricae* in the southeastern United States is quite heterogeneous, and larger numbers of samples would be necessary to determine more accurately the extent of its occurrence and distribution. Analogous to the detection of isolates of a fungal plant pathogen resistant to a fungicide, the probability of detecting *S. caricae* in a sampling is dependent on the true frequency of *S. caricae* in the sampled population and the sample size. For instance if the true frequency is 1% and a sample size of 10 is chosen, the chance of not detecting *S. caricae* would be 90.4%. For the current example of an assumed true frequency of *S. caricae* of 2.2% (based on the average between Brewer et al. 2015 and Li and Brewer 2016) and a sample size of 80, the chance of detecting at least one isolate of *S. caricae* is 83.1% (Russell 2003).

We detected no apparent effect of applications of triazole fungicides in South Carolina watermelon fields on the occurrence of *S. caricae*. Li and Brewer (2016) reported isolates of *S. caricae* highly resistant to the triazole fungicide tebuconazole in Georgia and suggested that selection of resistant isolates driven by continuous use of tebuconazole in the southeastern United States could lead to a change in the species composition of *Stagonosporopsis* spp. causing GSB in the region. Our findings do not support such a trend, because the field in Barnwell County where 25% of *Stagonosporopsis* spp. were identified as *S. caricae* in 2015 was treated twice with tebuconazole within 2 weeks prior to sampling, while the field in Beaufort County where 27% of isolates were found to be *S. caricae* in 2015 did not receive any triazole fungicide treatment prior to sampling. No isolates of *S. caricae* were found in the field in Hampton County in spring 2016, which was not treated with tebuconazole, nor from two fields in Beaufort County, which were sprayed with

tebuconazole once before the fields were sampled. Although the sample size in this study was small to draw strong conclusions, the results do not indicate that isolates of *S. caricae* occur more frequently in fields that were treated with tebuconazole. A possible explanation for this could be the general low efficacy of tebuconazole in controlling GSB (Keinath 2015b, Keinath et al. 2001).

Work is underway to identify the species of Stachybotriaceae, referred to as *Myrothecium* s.l., found on watermelon leaves in this study, determine their virulence on cucurbits, and characterize the genetic variability among the collected isolates. Since this family of fungi is notorious for its genetic diversity and the difficulty associated with the identification of its members, the conundrum of false identifications and assigned names will be addressed (Lombard et al. 2016).

In conclusion, this survey shows that GSB and powdery mildew are the main foliar diseases on watermelon in South Carolina. Management programs for watermelon should therefore focus on these two diseases. Changes to grower recommendations based on our results have been implemented. This survey is the first report of *C. cassicola* and *S. caricae* on watermelon in South Carolina and of one or more species within *Myrothecium* s.l. Appropriately designed field surveys have the potential to unravel new and emerging diseases and improve management practices for many other crops as well.

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