Susceptibility of Fourteen New Cucurbit Species to Gummy Stem Blight Caused by *Stagonosporopsis citrulli* Under Field Conditions

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Abstract

At least 24 species of cucurbits from 13 genera are known to be susceptible to gummy stem blight, caused by three species of *Stagonosporopsis*. Cankers that are formed on crowns and stems play an important role in the disease cycle and the survival of the pathogen. Fourteen cucurbit species of unknown susceptibility representing 12 genera, four taxonomic tribes, and four geographic origins were inoculated with *Stagonosporopsis citrulli* in Charleston, SC, in spring 2015, spring 2016, and fall 2016 to evaluate their level of susceptibility to gummy stem blight and the ability of the pathogen to reproduce on crown cankers. An additional species, *Cucumis melo*, was included as a reference due to its known high susceptibility. Data sets of area under the disease progress curve (AUDPC) for foliar severity and crown cankers, final percentage of diseased leaf area, final percentage of plants with cankers, final percentage of plants with fruiting bodies, and rates of increase in canker incidence were analyzed to evaluate susceptibility. Results were similar for datasets of AUDPC and final ratings but there were more differences for AUDPC. In all experiments, *Apodanthera sagittifolia*, *Ecballium elaterium*, and *Kedrostis leloja* were at least as susceptible to foliar blight as the reference *C. melo*. *K. leloja* was as susceptible to crown cankers as *C. melo* in all experiments and *A. sagittifolia* and *E. elaterium* were among the species most

susceptible to crown cankers in two experiments. *Coccinia grandis* was highly resistant to gummy stem blight and had a few cankers only in fall 2016. *Sicana odorifera* and *Zehneria pallidinervia* also consistently grouped with the most resistant species. Incidence of crown cankers on *Cucumis melo* and *K. leloja* increased at the fastest rate of all species in all experiments and had, along with *E. elaterium*, the highest incidence of crowns with fruiting bodies. In general, the most susceptible species also were most suitable for reproduction of the pathogen and had the fastest disease progression. The tribes Benincaseae and Cucurbitaceae had consistently lower levels of foliar blight than Bryonieae and Coniandreae. The tribe Benincaseae had a consistently lower AUDPC for canker incidence than Bryonieae and Coniandreae. The species originating from Europe (*E. elaterium*) was consistently most susceptible to both symptoms, while African species grouped with the least susceptible species in all experiments. To the best of our knowledge, this is the first report of susceptibility to gummy stem blight of 14 species and the first report of susceptibility of the cucurbit tribes Coniandreae and Gomphogyneae. This expands the host range of *Stagonosporopsis citrulli* to 37 species representing 21 genera and seven tribes in the family Cucurbitaceae. This study demonstrates the importance of crown cankers as reproductive sites for *S. citrulli*.

The plant family Cucurbitaceae comprises about 960 species in 115 genera that are mainly tropical and subtropical tendril-bearing climbers. Approximately 50 genera in the family contain only one species (Kocyan et al. 2007). Its center of origin is Asia, from which cucurbits spread around the world via long-distance dispersal events. The majority of species (40%) is endemic to the American continent, 28% occur in Africa, and 26% in Asia. The remaining species are found in Australia (2%) and Europe (1%) (Schaefer et al. 2009). Cucurbitaceae is one of the most genetically diverse families in the plant kingdom and among the most important ones for human utilization, even though only about 0.5% of the species are used commercially (Robinson and Decker-Walters 1997). In 2014, the annual worldwide production of the main cucurbit crops—cucumber, melon, gourd, pumpkin, squash, and watermelon—was 240.81×10^6 tons, of which 3.96×10^6 tons were produced in the United States. The total worldwide vegetable production in the same year, 1,169.45 $\times 10^6$ tons, puts these numbers in perspective (FAO 2017) and illustrates the importance of cucurbits as commercial crops (Kocyan et al. 2007; Zhang et al. 2006).

Gummy stem blight (GSB), caused by three morphologically indistinguishable but genetically distinct species of *Stagonosporopsis* (Stewart et al. 2015), is a major disease of cucurbits with a worldwide distribution that occurs in all cucurbit-producing areas (Keinath

2011). Two of the three species, *S. citrulli* and *S. cucurbitacearum* (syn. *Didymella bryoniae*), are host specific to cucurbits, whereas *S. caricae* also infects papaya (*Carica papaya*) (Stewart et al. 2015). The most common and widespread species, *S. citrulli*, is also the predominant species in the southeastern United States (Brewer et al. 2015; Stewart et al. 2015). Symptoms of GSB are circular, tan to brown water-soaked lesions on cotyledons, leaves, petioles, hypocotyls, and stems. The disease is referred to as black rot on fruit, which can have variable symptoms depending on the affected fruit. If the cortical tissue of stems and crowns are infected, cankers can develop, which may lead to the production of a reddish-brown gummy exudate (Chiu and Walker 1949; Grossenbacher 1909; Keinath 2008, 2017; Zitter 2017). Cankers are commonly formed on crowns and stems of pumpkin (*Cucurbita pepo*), squash (*C. maxima*), bottle gourd (*Lagenaria siceraria*), cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), and muskmelon (*Cucumis melo*) (Chiu and Walker 1949; Keinath 2008, 2014a; Schenck 1968). High levels of resistance to crown cankers were found in *Cucurbita moschata* and *C. pepo* ‘Gray Zucchini’ (Chiu and Walker 1949; Grossenbacher 1909; Keinath 2014a).

At least 24 species of cucurbits from 13 genera are known to be susceptible to GSB (Jiang et al. 2015; Keinath 2011). Among these are all major cucurbit crops. To this day, no commercially acceptable cultivars of any cucurbit crop with resistance to GSB are available (Keinath 2017). Susceptibility to GSB varies greatly within the family Cucurbitaceae. Generally, *Citrullus lanatus* (watermelon) and *Cucumis melo* (melon) are the most susceptible species (Chiu and Walker 1949; dos Santos et al. 2009; Keinath 2014a). Under conducive conditions, yield losses in cantaloupe can reach 100% and average above 40% in watermelon (Keinath and Duthie 1998; Zhang et al. 1999). In previous studies where the susceptibility of various cucurbits to GSB was compared, *Cucurbita* spp. were consistently less susceptible than watermelon and melon (Bala and Hosein 1986; Chiu and Walker 1949; dos Santos et al. 2009; Grossenbacher 1909; Keinath 2014a). The only exception to this is the high susceptibility of *C. argyrosperma* reported by Keinath (2014a). Apart from

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a study by Wehner and St. Amand (1993), who tested six species of *Cucumis*, no experiments to test cucurbits for their susceptibility to GSB have included minor crops or wild species. Because of the high potential to find resistance in wild, untested cucurbits, there is a need to investigate these unstudied plant species.

The survival of *S. citrulli* in colonized cucurbit debris, which acts as a source of inoculum for subsequent crops, was investigated in several studies (Chiu and Walker 1949; Keinath 2002, 2008; Sitterly 1969; Van Steekelenburg 1983). On buried, naturally infected watermelon vines, *S. citrulli* survived for up to 30 weeks, while the survival time on buried muskmelon crowns was extended to 103 weeks (Keinath 2002, 2008). The crown of a cucurbit plant can be described as the part where the short main stem divides into two or more primary vines. It is the thickest and one of the most lignified parts of the plant (Keinath 2008). Therefore, crowns decompose slowly and are of particular importance as a reservoir for the survival of inoculum into a consecutive crop. The difference in survival time on watermelon vines as opposed to muskmelon crowns illustrates this aspect (Keinath 2002, 2008).

As a homothallic fungus, *S. citrulli* produces both pycnidia (asexual fruiting bodies) and pseudothecia (sexual fruiting bodies) on suitable hosts (Keinath 2014b; Punithalingam and Holliday 1972). The requirements for the formation of fruiting bodies are free moisture and temperatures of $>5^{\circ}\text{C}$ (Van Steekelenburg 1983). When these conditions are met, the fungus produces pycnidia and pseudothecia on cucurbit debris remaining on fields, which subsequently release the inoculum for a new disease cycle (Chiu and Walker 1949; Keinath 2002; Schenck 1968). The two spore types produced by pycnidia and pseudothecia play different roles in the epidemiology of the pathogen. Although conidia released from pycnidia mainly spread from plant to plant, ascospores released from pseudothecia can act as primary inoculum and as units of further dispersal (Schenck 1968). Reports of fruiting bodies of *S. citrulli* on crowns and stems are scarce. In the first account of GSB on watermelon, Chester (1891) reported pycnidia on vines. Later, Schenck (1968) described both types of fruiting bodies specifically on crowns of watermelon as lesions that started on the cotyledons of seedlings and spread to the hypocotyl and crown. After inoculation experiments, Grossenbacher (1909) and Jensen et al. (2011) found both pycnidia and pseudothecia on vines and stems of *Cucurbita maxima*. The only study that examined the frequency of fruiting bodies on a large selection of cucurbits did not include crowns. Sporulation of the pathogen was reported on leaves, pedicels, peduncles, petioles, tendrils, and vines of nine species from five genera (Keinath 2014b). The formation of cankers and reproduction of *S. citrulli* has not been studied on minor crop and wild cucurbit species.

The objectives of this study were to (i) characterize the level of susceptibility of 14 species of cucurbits to foliar blight and crown

cankers caused by *S. citrulli*, (ii) compare the relative susceptibility of cucurbits in different geographic origins and tribes within the family Cucurbitaceae, (iii) compare the rate of canker development among the species, and (iv) compare the reproduction of the pathogen on different hosts and test whether more susceptible species also are more suitable for reproduction of the pathogen. The species included in this study were chosen to represent the taxonomic and geographic diversity of the cucurbit family. Species initially considered for inclusion were limited to those with seed readily available commercially.

Materials and Methods

Field experiments. Three experiments were conducted at the Clemson University Coastal Research and Education Center in Charleston, SC. The experiments were done in spring 2015, spring 2016, and fall 2016. A different field was used for each experiment. The field used in spring 2015 was cropped to muskmelon the previous spring, followed by a cover crop of rye and clover, which was disked into the soil 3 months before transplanting. In the field used in spring 2016, sunflower was grown the previous year, followed by a cover crop of crimson clover. The cover crop was disked 3 months before transplanting. The field used in fall 2016 was cropped to corn and sorghum in the spring of the same year and disked twice after mowing the sorghum. Fourteen species of cucurbits were selected to represent 12 genera and five tribes originating from six different geographical regions (Table 1). In 2015, all species were seeded at the same time. In 2016, seeding was staggered based on germination in 2015 to achieve similar developmental stages of species. *Coccinia grandis*, *Cucumis anguria*, *Ecballium elaterium*, *Gynostemma pentaphyllum*, *Melothria scabra*, and *Zehneria pallidinervia* were seeded first. Four days after that, *Acanthosicyos horridus*, *Apodanthera sagittifolia*, *Kedrostis leloja*, *C. melo* ‘Athena’, and *Sicana odorifera* were seeded. The last group of species—*C. zambianus*, *Cucurbita digitata*, *C. ecuadorensis*, and *L. sphaerica*—was seeded 6 days after the second group.

All plots consisted of a single-row raised bed that was 0.9 m wide on a 3.6-m center. Rows of plastic mulch were arranged in blocks of three, with the middle row remaining unplanted. Beds were covered with white-on-black polyethylene mulch in spring 2015 and fall 2016. In spring 2016, black polyethylene mulch was used. Seedlings were transplanted on 4 May 2015, 26 April 2016, and 11 August 2016. Plots were arranged as a randomized complete block design with four replications and seven plants per plot. In 2015, plots were 8.5 m long with 1.2-m spacing between plants within the row. Unplanted space between plots within rows was 4.6 m. In 2016, plots were 10.7 m long, with 9.1 and 6.1 m of unplanted space between plots in spring and fall, respectively. In 2016, spacing between plants

Table 1. Cucurbit species tested for gummy stem blight susceptibility in the field in South Carolina in three experiments 2015 and 2016

Genus	Species	Common name	Tribe ^x	Origin	Cultivated	Native status in the United States ^y	Seed source
<i>Acanthosicyos</i>	<i>horridus</i>	Nara melon	Benincaseae	Africa	Yes	Not native	rarepalmseeds.com
<i>Apodanthera</i>	<i>sagittifolia</i>	Argentinian melon	Coniandreae	South America	No	Not native	kcb-samen.ch
<i>Coccinia</i>	<i>grandis</i>	Ivy gourd	Benincaseae	Africa	Yes	Invasive	rarepalmseeds.com
<i>Cucumis</i>	<i>anguria</i>	Bur gherkin	Benincaseae	Africa	Yes	Invasive	sunshine-seeds.de
<i>Cucumis</i>	<i>melo</i> ‘Athena’	Cantaloupe	Benincaseae	Africa	Yes	Introduced	siegers.com
<i>Cucumis</i>	<i>zambianus</i>	None	Benincaseae	Africa	Yes	Not native	horizonherbs.com
<i>Cucurbita</i>	<i>digitata</i>	Fingerleaf gourd	Cucurbitieae	North America	No	Native	horizonherbs.com
<i>Cucurbita</i>	<i>ecuadorensis</i>	None	Cucurbitieae	South America	No	Not native	rarepalmseeds.com
<i>Ecballium</i>	<i>elaterium</i>	Squirting cucumber	Bryoniae	Europe	Yes	Introduced	horizonherbs.com
<i>Gynostemma</i>	<i>pentaphyllum</i>	Jiaogulan	Gomphogyneae	Asia	Yes	Not native	horizonherbs.com
<i>Kedrostis</i>	<i>leloja</i>	None	Coniandreae	Africa	No	Not native	kcb-samen.ch
<i>Lagenaria</i>	<i>sphaerica</i>	None	Benincaseae	Africa	Yes	Not native	rarepalmseeds.com
<i>Melothria</i>	<i>scabra</i>	Mouse melon	Benincaseae	North America	Yes	Not native	sunshine-seeds.de
<i>Sicana</i>	<i>odorifera</i>	Casabanana	Cucurbitieae	South America	Yes	Introduced ^z	rarepalmseeds.com
<i>Zehneria</i>	<i>pallidinervia</i>	Bat wing vine	Benincaseae	Africa	No	Not native	...

^x Classification based on Schaefer and Renner (2011).

^y Based on United States Department of Agriculture (USDA)–Natural Resources Conservation Service (NRCS), <https://plants.usda.gov/java/>.

^z Introduced only in Puerto Rico (USDA-NRCS).

within plots was adjusted according to the growth habit of the species. In spring 2016, *Cucumis zambianus* and *Coccinia grandis* were spaced 1.5 m apart and all other species 1.2 m. In fall 2016, spacing between plants was 0.6 m for *Acanthosicyos horridus* and *Z. pallidinervia*, 1.5 m for *Cucumis anguria* and *C. zambianus*, 0.9 m for *E. elaterium*, and 1.2 m for all other species. In all experiments, weeds, insects, anthracnose, downy mildew, and powdery mildew were managed with recommended pesticides (Keinath et al. 2010).

In all experiments, crowns of all plants were inoculated with a suspension of two isolates of *S. citrulli* that were originally recovered from watermelon in South Carolina (HD10 and W764). Isolates of this species were used because of its local and global distribution. Fungal cultures were grown on quarter-strength potato dextrose agar (QPSDA) for 2 weeks with 12 h of light. Inoculum was harvested by flooding plates with 7 ml of a solution of 0.05% casein and 0.1% sucrose (Svedelius 1990) and scraping with sterile microscope slides. In all, 20 plates of each isolate were prepared in 2015 and 30 plates in 2016. The volume was brought up to 500 ml with additional casein-sucrose solution and blended for 1 min. The blended inoculum suspension was diluted to a volume of 1.3 liter and contained spores and mycelial fragments. Inoculum densities in the three experiments, as determined by plating of serial dilutions on QPSDA, were 6.7×10^6 , 9×10^6 , and 6×10^5 CFU/ml, respectively. Handheld spray bottles were used to apply 3 ml of inoculum suspension on the crowns of all plants. Inoculations were done on 10 June 2015, 15 June 2016, and 8 August 2016 in the evening at 7 to 8 P.M. The middle unplanted row of the blocks of three rows was used to set up a misting system with overhead, low-pressure, mini-wobbler sprinklers (Senninger Irrigation) to supply additional leaf wetness before inoculation and then throughout the entire duration of the experiments. In spring 2015 and in fall 2016, the misting system was turned on for 15 min prior to inoculation. In spring 2016, heavy rain just prior to inoculation provided optimum conditions and rendered additional misting unnecessary. After inoculation, the misting system was run twice per day for 30 min at 6 A.M. and 8 P.M. in spring 2015 and 2016. In fall 2016, it was run four times for 15 min at 3 A.M., 6 A.M., 8 P.M., and 12 P.M. due to below-average precipitation.

Severity of GSB on foliage was rated on each third of each plot using a percent scale from 0 to 100% with 5% increments, plus 1% for minor symptoms. In spring 2015, severity was rated seven times. In spring 2016, severity was rated nine times, of which the first rating was 5 days prior to inoculation because early symptoms of GSB were observed on six species. In fall 2016, severity was rated five times. Four symptomatic leaves were collected from each plot on 10 July 2015, 7 August 2016, and 26 October 2016. One piece of tissue from each collected leaf was surface disinfested for 1 min in a solution of 0.825% sodium hypochlorite and transferred to petri dishes with QPSDA amended with chloramphenicol at 0.1 g/liter, streptomycin at 0.1 g/liter, and mefenoxam (Ridomil Gold SL; Syngenta AG) at 45.3 μ l/liter to recover the pathogen (Keinath 2008).

Incidence of crown cankers and presence of fruiting bodies of *S. citrulli* was determined by closely examining each crown for symptoms and signs of the pathogen. Crown cankers were counted three, four, and two times in the three field experiments (spring 2015, spring 2016, and fall 2016, respectively). Presence of fruiting bodies of the pathogen was recorded every time, whenever incidence of crown cankers was rated, with the exception that it was omitted the first time canker incidence was determined in 2015. After the last rating in each experiment, four symptomatic crowns were collected from each plot. The pathogen was recovered from two pieces of tissue from each crown as described for leaves.

Statistical analyses. The three severity ratings per plot were averaged together. The area under the disease progress curve (AUDPC) of foliar severity ratings and of crown incidence ratings was calculated with PROC MEANS (version 9.4; SAS Institute Inc.) (Shaner and Finney 1977). Relative AUDPC was calculated by dividing data from each experiment by the corresponding number of days between the first and the last rating. Values for AUDPC of foliar severity in 2015 and fall 2016 were square-root transformed and log transformed in spring 2016. Disease severity data for last ratings were

square-root transformed in spring 2015 and spring 2016. Based on residual plots, no transformation was necessary in fall 2016. The effects of species, experiment, and their interaction were included as fixed effects and block within years was included as a random effect in the model used in PROC GLM. When a species–experiment interaction was present, data sets from experiments were compared separately, and the SLICE statement of the LSMEANS option of PROC GLM was used to analyze differences among experiments for each species. Values presented in the tables were back transformed.

Values for AUDPC of crown canker incidence were square-root transformed for 2015 and arcsine square-root transformed for spring 2016. Based on residual plots, no transformation was necessary for fall 2016. The effects of species, experiment, and their interaction were included as fixed effects and block within years was included as a random effect in the model used in PROC GLM. When an interaction was present, data sets from experiments were compared separately, and the SLICE statement of the LSMEANS option of PROC GLM was used to analyze differences among experiments for each species. Values presented in the tables were back transformed. The data of fruiting body incidence based on total number of plants was analyzed in the same manner but only data from 2015 were arcsine square-root transformed.

Because transformations could not correct for nonnormality and inequality of variances for the canker incidence ratings, data were analyzed with PROC CATMOD for categorical data analysis. Using the logit as response function, the proportions of plants with crown cankers were analyzed with the maximum-likelihood method to estimate linear model parameters. Because data sets that contain percentage values of 0 or 100 cannot converge in this procedure, percentage values were replaced with a Bayes estimator with the following formula: $x = (ct + k/r) \times n/(n + k)$, where x = the transformed percentage, ct = number of plants with crown cankers, $r = 2$ (for a binary response), k = a constant which was set equal to r , and n = total number of plants (Santner and Duffy 1989). In tables, back-transformed values >84.0 and values of *E. elaterium* correspond to percentages of 100.0 and values ≤ 14.0 correspond to percentages of 0.0 (no cankers present). Species had a significant effect in all three experiments ($P \leq 0.0002$). Therefore, linear contrasts were written to compare species. The block factor was excluded from the model because it was not significant in any experiment ($P \geq 0.13$).

Slopes of the canker progress were calculated for the interval between inoculation and the first postinoculation rating, except for four species in spring 2016, which had low levels of crown cankers before the research plots were inoculated. Therefore, the first rating in spring 2016 was done prior to inoculation and slopes for these species were calculated for the interval between the first and second rating. The slopes of canker progress were analyzed with PROC GLIMMIX. The effects of species, experiment, and their interaction were included as fixed effects and block within years was included as a random effect. The Gaussian distribution was used as the response distribution and the identity was used as the link function. Due to a significant species–experiment interaction, the datasets were analyzed separately for each experiment. To adjust for nonnormality and inequality of variances, the data from spring 2016 was square-root transformed before analysis. Using PROC CORR, Pearson correlation coefficients between incidence of crown cankers and incidence of fruiting bodies were calculated for each individual experiment and for data combined from all three experiments.

Results

Severity of foliar blight. GSB was significantly more severe in fall 2016 than in spring 2015 and 2016 ($P < 0.0001$, from preliminary analysis of combined datasets). Foliar severity was $>70\%$ on 6 of the 14 species in fall 2016 whereas, in the two previous seasons, severity was $\leq 65\%$ on all species (Table 2). There were species–experiment interactions for both foliar severity and canker incidence based on AUDPC values and final percentages ($P < 0.0001$). Therefore, all datasets were analyzed separately for each experiment. Symptoms of foliar blight were found on all species in all experiments except on *Z. pallidinervia* in 2015 (Table 2). Lesions with fruiting bodies of *S. citrulli* were present on thorns of *A. horridus* (Fig. 1A).

Three species (*E. elaterium*, *A. sagittifolia*, and *C. melo*) were among the three most susceptible species across all experiments based on AUDPC and final severity, except for *C. melo* in 2015 (Fig. 1B and C). The AUDPC of *E. elaterium* was consistently among the two highest values and significantly higher than all species, with the exception of *A. sagittifolia* and *C. melo* (Table 3). The AUDPC of *A. sagittifolia* was significantly higher than the AUDPC of *C. melo* in 2015 and in fall 2016. In each experiment, *K. leloja* was one of the five most susceptible species and, in 2016, it was at least as susceptible as *C. melo*. *K. leloja*, however, had a lower AUDPC than *E. elaterium* in each experiment. By the end of the season in fall 2016, about half of the plants of *C. melo*, *E. elaterium*, and *K. leloja* and almost all plants of *A. sagittifolia* were killed by GSB.

C. grandis and *Z. pallidinervia* were consistently least affected by *S. citrulli*. The two species had a significantly lower AUDPC and percent severity based on final ratings than all other species in 2015 and spring 2016. In fall 2016, these two species and *S. odorifera* had the lowest AUDPC values, although AUDPC of *Z. pallidinervia* was not significantly different from AUDPC for *C. anguria* and *M. scabra*. In spring 2015 and fall 2016, *S. odorifera* was among the three species with the lowest AUDPC but, in spring 2016, the AUDPC value was only significantly lower than *E. elaterium*. The AUDPC of *C. anguria* was consistently the fifth lowest and its severity rating was among the four lowest. *C. grandis* and *Z. pallidinervia* had lower levels of foliar severity than *C. anguria* in all three experiments and, in fall 2016, *S. odorifera* was also less susceptible than *C. anguria*. The AUDPC for *L. sphaerica* was less than the AUDPC for *C. melo* in each experiment.

Based on severity of foliar blight at the end of the experiments, *C. grandis* and *Z. pallidinervia* were consistently among the three least susceptible species although, in fall 2016, *Z. pallidinervia* also grouped with four moderately susceptible species (*C. anguria*, *C. ecuadorensis*, *L. sphaerica*, and *M. scabra*) (Table 2). In general, the data sets for AUDPC and percentages at the end of the experiment are in correspondence. The four species *A. sagittifolia*, *C. melo*, *E. elaterium*, and *K. leloja* were consistently among the species most susceptible to foliar blight, whereas *C. grandis* and *Z. pallidinervia* were consistently highly resistant to the foliar blight phase of GSB in this study.

S. citrulli was successfully recovered from leaves of all included species in at least one experiment. Recovery levels in 2015 were lower than in the 2016 seasons. Overall, there were few differences

among species in pathogen recovery. In 2015, recovery was lowest for *A. sagittifolia* and *M. scabra* whereas, in spring 2016, it was lowest for *M. scabra* and *Z. pallidinervia*. The pathogen was not recovered from leaves of *C. grandis*, *L. sphaerica*, *S. odorifera*, and *Z. pallidinervia* and thorns of *A. horridus* in 2015 because no symptomatic leaves could be found at the time of sampling.

In fall 2016, symptoms of powdery mildew caused by *Podosphaera xanthii* were observed on *C. digitata*, *C. zambianus*, and *M. scabra* (Rennberger et al. 2018). The occurrence of powdery mildew did not affect the results of the experiment because the results were in general agreement with the results of the preceding two experiments.

Incidence of crown cankers. Cankers formed on crowns of most species, except *C. grandis*, *S. odorifera*, and *Z. pallidinervia* in 2015 and *C. grandis* in spring 2016 (Table 2). At the end of each experiment, cankers were present on all plants of *A. sagittifolia*, *C. melo*, *C. digitata*, *E. elaterium*, and *K. leloja*. On *A. horridus* and *C. digitata*, cankers developed on all plants in two experiments. The characteristic amber-brown gummy exudate was observed on stems of *A. sagittifolia*, *C. digitata*, *C. melo*, *C. zambianus*, *E. elaterium*, *K. leloja*, and *L. sphaerica*.

In fall 2016, the percentage of crowns with cankers was significantly ($P < 0.0001$) greater than in the previous two experiments and, in 2015, it was significantly ($P \leq 0.0405$) less than in the 2016 seasons (Table 2). Despite significant interactions across experiments for most species, species had clear susceptibility patterns to crown cankers. As expected, *C. melo* was among the species most susceptible to crown cankers in all three experiments for both AUDPC and final incidence. The AUDPC of *K. leloja* was statistically equal to that of *C. melo* in all three experiments (Table 3). *E. elaterium* had an equally high AUDPC as *C. melo* and *K. leloja* in 2015 and fall 2016. *A. sagittifolia* and *C. zambianus* were among the four most susceptible species in fall 2016 and grouped with *E. elaterium* in all experiments.

C. grandis and *S. odorifera* were the species least susceptible to crown cankers in all experiments. In 2015, these two species plus *L. sphaerica* and *Z. pallidinervia* had significantly lower AUDPC values for canker incidence than any other species (Table 3). In spring 2016, AUDPC for *C. grandis* was significantly smaller than for *L. sphaerica* and, in fall 2016, it was smaller than for *Z. pallidinervia* and *L. sphaerica*. AUDPC values did not differ significantly

Table 2. Percentage incidence and severity of gummy stem blight compared within three seasons in 2015 and 2016 for 15 cucurbit species inoculated with *Stagonosporopsis citrulli*^w

Species	Foliar severity			Incidence of crown cankers ^x		
	7 August 2015	5 August 2016	20 October 2016	23 July 2015	6 August 2016	16 October 2016
<i>Acanthosicyos horridus</i>	65.1 a	38.9 b	95.2	84.8 a	83.5 ab	71.4 abcd ^y
<i>Apodanthera sagittifolia</i>	46.6 bc	60.4 a	96.3	88.3 a	84.4 ab	80.6 abc
<i>Coccinia grandis</i>	0.7 f	8.6 e	15.4	11.1 b	11.8 c	30.6 d
<i>Cucumis anguria</i>	10.9 e	16.6 d	31.7	73.9 a	75.0 ab	83.3 abc
<i>Cucumis melo</i> 'Athena'	42.8 c	54.2 a	87.1	88.9 a	88.6 a	88.6 a
<i>Cucumis zambianus</i>	28.1 d	36.1 b	47.1	66.7 a	86.1 a	88.9 a
<i>Cucurbita digitata</i>	44.3 bc	24.0 cd	73.8	88.9 a	59.1 ab	87.5 ab
<i>Cucurbita ecuadorensis</i>	29.2 d	21.7 cd	33.3	25.0 b	77.5 ab	88.9 a
<i>Ecballium elaterium</i>	54.1 abc	54.2 a	87.7 ab	87.6 a	77.8 ab	79.0 abc
<i>Gynostemma pentaphyllum</i> ^z	51.7	29.2	n.d.	23.5	20.0	n.d.
<i>Kedrostis leloja</i>	57.8 ab	54.4 a	80.4 bc	88.9 a	80.7 ab	86.8 ab
<i>Lagenaria sphaerica</i>	14.0 e	29.5 bc	35.4 e	14.7 b	41.7 b	69.4 abc
<i>Melothria scabra</i>	41.5 c	17.1 d	34.6 e	88.9 a	76.8 ab	74.7 abc
<i>Sicana odorifera</i>	14.9 e	28.6 bc	20.4 f	11.1 b	39.1 bc	58.9 c
<i>Zehneria pallidinervia</i>	0.0 f	3.6 f	25.4 ef	13.8 b	25.0 c	62.3 bc

^w Each value is the mean of four replications of the final rating. Means with the same letters within seasons do not differ significantly; Fisher's least significant difference test, $P = 0.05$; n.d. = no data due to exclusion of *Gynostemma pentaphyllum* after the first two seasons.

^x Percentages of crowns with cankers were back-transformed from categorical data analysis in PROC CATMOD (SAS). Values > 84.0 and values of *E. elaterium* correspond to percentages of 100.0 and values ≤ 14.0 correspond to percentages of 0.0 (no cankers present) because a Bayes estimator was used in the analysis instead of the actual percentages.

^y Not significantly different from *C. grandis* due to missing values of *A. horridus*.

^z Excluded from analyses due to large number of plants killed by *Pythium* spp.

among *L. sphaerica*, *S. odorifera*, and *Z. pallidinervia* in any experiment. *Z. pallidinervia* had significantly fewer cankers than *M. scabra* in spring 2015 and 2016 but not in fall 2016.

Overall, there were fewer differences for the percentages of plants with crown cankers on the last rating dates than for AUDPC based on canker incidence. *C. grandis*, *S. odorifera*, and *Z. pallidinervia* had consistently lower percentages of crowns with cankers compared with *C. melo* and *C. zambianus* (Table 2). In addition, *L. sphaerica* had significantly fewer cankers than *C. melo* in 2015 and spring 2016. Although *C. ecuadorensis* was grouped with the three least susceptible species in 2015, in fall 2016, canker incidence was

greater than on those three species (Table 2). The four species *A. sagittifolia*, *C. melo*, *C. zambianus*, and *K. leloja* grouped with the species showing the highest incidences of crown cankers in all experiments and were the only species with final incidence values >80%, except for *C. zambianus* in 2015. *A. horridus*, *A. sagittifolia*, *C. anguria*, *C. melo*, *E. elaterium*, and *K. leloja* had consistently high incidences of cankers that did not differ among experiments (species-experiment interaction $P \geq 0.23$, from combined analysis). With few exceptions, the five species *A. sagittifolia*, *C. melo*, *C. zambianus*, *E. elaterium*, and *K. leloja* were the most susceptible to crown cankers (Fig. 2), and the three species *C. grandis*, *S. odorifera*, and

Fig. 1. Typical symptoms of gummy stem blight on leaves of new cucurbit host species. **A**, Diseased thorns of *Acanthosicyos horridus* colonized by *Stagonosporopsis citrulli*; thorn on the left with fruiting bodies of the pathogen. **B**, Three water-soaked lesions on a leaf of *Apodanthera sagittifolia*. **C**, Lesion with abundant sporulation of *S. citrulli* on a leaf of *Ecballium elaterium*.

Fig. 2. Typical symptoms of gummy stem blight caused by *Stagonosporopsis citrulli* on crowns of new cucurbit host species. **A**, Canker and abundant gummy exudate on crown of *Apodanthera sagittifolia*. **B**, Cankers and necrotic lesions on crown and stems of *Ecballium elaterium*. **C**, Corky cankers on crown of *Cucumis zambianus*.

Z. pallidinervia were the least susceptible, showing only minor symptoms across all three experiments.

S. citrulli was successfully recovered from crowns with cankers of all species in at least one experiment. Recovery was significantly lower in 2015 than in 2016 ($P < 0.0001$) but there was no difference between the two experiments in 2016 ($P = 0.46$). The lowest percent recovery was from *C. grandis*, which was significantly less than from all other species except *M. scabra*, which had the second lowest recovery. Otherwise, there were few significant differences in recovery from crowns.

There were several notable differences among species within the genera *Cucurbita* and *Cucumis*. Within the genus *Cucurbita*, final disease severity of *C. ecuadorensis* was lower than that of *C. digitata* in 2015 and fall 2016 (Table 2). Both *Cucurbita* spp. had higher AUDPC values for crown cankers than the three most resistant species and lower AUDPC values than the three most susceptible species in 2015 and spring 2016. Within the genus *Cucumis*, foliar severity of *C. anguria* at the end of the experiments was consistently the lowest of the three species. *C. zambianus* was consistently more susceptible than *C. anguria* but less susceptible than *C. melo* (Table 2). *C. anguria* had a lower AUDPC of crown canker incidence than *C. melo* in 2015 and spring 2016 but final incidences of crown cankers did not differ in any experiment.

Comparisons among geographic origins and tribes. The four tribes and four geographic origins represented by the cucurbit species included in this study were compared using linear contrasts. AUDPC for foliar disease was significantly less severe for the tribes Benincaseae and Cucurbitae than for Bryonieae and Coniandreae in all experiments, except that the AUDPC values of Coniandreae and Cucurbitae were not significantly different in spring 2016. Benincaseae had the

lowest AUDPC in all experiments, with the exception that Cucurbitae was equally low in fall 2016. In all experiments, Cucurbitae and Benincaseae also were the least susceptible tribes based on AUDPC calculated from crown incidence data (Table 4). In 2015 and spring 2016, Cucurbitae had a significantly lower crown incidence AUDPC than all other tribes (single-degree-of-freedom contrasts, $P \leq 0.05$).

The AUDPC for percent severity among African species was among the statistically lowest AUDPC values in all experiments (Table 5). South American species consistently had the second lowest severity. The European species *E. elaterium* was the most susceptible species based on geographic origins (Table 5). Severity of North American species was intermediate in 2015 and spring 2016 and low in fall 2016.

E. elaterium, the only species originating from Europe, had the highest AUDPC based on crown canker incidence in all three experiments but, in 2015, North American species were statistically equal and, in fall 2016, South American species had an equally high AUDPC (Table 5). African species consistently had intermediate AUDPC values. South American species had a significantly lower AUDPC value than African, European, and North American species in 2015. In fall 2016, species split into two groups by geographic origins as African and North American species had statistically lower AUDPC of canker incidence than European and South American species (Table 5).

Development of crown cankers. The data for increase in canker incidence between the first and second ratings (approximately 2 weeks) were analyzed separately for each experiment because there was a species–experiment interaction ($P \leq 0.0001$). The slopes of crown canker incidence in spring 2016 were significantly lower than in 2015 and fall 2016. ($P < 0.0001$, from preliminary analyses of combined datasets). On *C. grandis* and *S. odorifera*, there was a

Table 3. Area under the disease progress curve values calculated from incidence and severity of gummy stem blight on 15 cucurbit species inoculated with *Stagonosporopsis citrulli* compared within three seasons in 2015 and 2016^y

Species	Foliar severity			Incidence of crown cankers		
	2015	Spring 2016	Fall 2016	2015	Spring 2016	Fall 2016
<i>Acanthosicyos horridus</i>	1,005 c	488 cd	699 ef	1,764 e	3,014 abc	1,012 ef
<i>Apodanthera sagittifolia</i>	1,492 ab	716 bc	1,771 a	2,646 abc	1,265 cd	2,650 a
<i>Coccinia grandis</i>	12 g	148 e	240 j	0 g	0 f	659 f
<i>Cucumis anguria</i>	429 d	502 cd	418 gh	1,868 de	1,648 bcd	2,112 abc
<i>Cucumis melo</i> ‘Athena’	979 c	945 ab	1,211 bc	3,066 ab	5,208 a	2,555 a
<i>Cucumis zambianus</i>	934 c	852 bc	785 de	2,100 cde	3,195 ab	2,650 a
<i>Cucurbita digitata</i>	1,064 c	816 bc	920 d	2,317 cd	761 de	2,360 ab
<i>Cucurbita ecuadorensis</i>	921 c	624 bcd	554 fg	348 f	1,696 bcd	2,511 a
<i>Ecballium elaterium</i>	1,796 a	1,593 a	1,234 b	2,596 bc	3,138 ab	2,650 a
<i>Gynostemma pentaphyllum</i> ^z	1,715	510	n.d.	530	47	n.d.
<i>Kedrostis leloja</i>	1,385 b	761 bc	987 cd	3,253 a	2,361 bc	2,337 ab
<i>Lagenaria sphaerica</i>	227 e	527 cd	533 fg	18 g	440 de	1,709 bcd
<i>Melothria scabra</i>	823 c	352 d	372 hi	2,412 c	1,154 cd	1,023 ef
<i>Sicana odorifera</i>	106 f	558 bcd	278 ij	0 g	123 ef	1,191 def
<i>Zehneria pallidinervia</i>	0 g	64 f	352 hij	0 g	94 ef	1,627 cde

^y Each value is the mean of four replications. Means with the same letters within experiments are not significantly different; Fisher’s least significant difference test, $P = 0.05$; n.d. = no data due to exclusion of *Gynostemma pentaphyllum* after the first two experiments.

^z Excluded from analyses due to large number of plants killed by *Pythium* spp.

Table 4. Contrasts among cucurbit tribes for area under the disease progress curve calculated from incidence of crown cankers and severity of foliar blight^w

Tribe (N) ^z	Incidence of crown cankers ^x						Severity of foliar blight ^y					
	2015	SE	Spring 2016	SE	Fall 2016	SE	2015	SE	Spring 2016	SE	Fall 2016	SE
Benincaseae (8)	987 b	425	1,326 b	647	1,669 b	261	413 d	154	369 c	108	543 b	111
Bryonieae (1)	2,596 a	0	3,138 a	0	2,650 a	0	1,796 a	0	1,593 a	0	1,235 a	0
Coniandreae (2)	2,942 a	304	1,773 ab	548	2,493 a	157	1,438 b	54	738 b	23	1,350 a	392
Cucurbitae (3)	496 c	14	710 c	457	2021 ab	417	596 c	298	657 b	77	553 b	186

^w Each value is the mean of four replications. Means with the same letters within seasons do not differ significantly; single-degree-of-freedom contrasts, $P = 0.05$. SE = standard error, calculated from back-transformed values of individual species.

^x Number of ratings in 2015, spring 2016, and fall 2016 were three, four, and two, respectively.

^y Number of ratings in 2015, spring 2016, and fall 2016 were seven, nine, and five, respectively.

^z N = number of species.

consistent lack of canker development at the beginning of the disease progress curve between the first and second ratings ($P \geq 0.11$). *C. grandis* had no cankers throughout the experiments in 2015 and spring 2016 but cankers were observed starting 24 days after inoculation (dai) in fall 2016. No cankers developed on *S. odorifera* over the course of the experiment in 2015 but appeared 37 dai in spring 2016 and 24 dai in fall 2016. Despite the earlier onset of canker development in fall 2016, these two species still had the fewest cankers at the end of the experiment. In all three experiments, *C. melo* and *K. leloja* had the fastest increase of canker incidence per day (Table 6). *E. elaterium* grouped with the species with the highest rates of increase in 2016. Two additional species (*A. sagittifolia* and *C. zambianus*) grouped with the five species that had the highest rate of canker increase in 2015 and fall 2016 (Table 6).

Incidence of fruiting bodies on crown cankers. The percentage of crowns with fruiting bodies of *S. citrulli* in fall 2016 was significantly higher ($P \leq 0.0011$) than in 2015 and spring 2016. No fruiting bodies were found on *C. ecuadorensis*, *L. sphaerica*, *G. pentaphyllum*, and *Z. pallidinervia* in 2015; *C. grandis* and *S. odorifera* in 2015 and spring 2016; or *M. scabra* in fall 2016. The data for incidence of fruiting bodies on crown cankers were analyzed separately for each experiment because there was an overall species–experiment interaction ($P \leq 0.0001$). The species–experiment interaction was significant for *A. sagittifolia*, *C. anguria*, *C. digitata*, *C. ecuadorensis*, *C. zambianus*, and *E. elaterium* ($P \leq 0.0096$) but not for the remaining eight species ($P \geq 0.0547$). The main reasons for the interaction were the absence of fruiting bodies on crowns of *C. ecuadorensis* in 2015 and low incidence of fruiting bodies on *A. sagittifolia* in spring 2016.

Among all 15 species, *C. melo* consistently had the highest incidence of fruiting bodies. Fruiting bodies were present on $\geq 92\%$ of its crowns in every experiment (Table 7). Several other species had similar levels of fruiting body incidence. *A. sagittifolia*, *E. elaterium*, and *K. leloja* consistently grouped with the five species with the highest incidence of fruiting bodies, except for *A. sagittifolia* in spring 2016 and *K. leloja* in fall 2016. Five species—*C. grandis*, *L. sphaerica*, *M. scabra*, *S. odorifera*, and *Z. pallidinervia*—consistently were among the species with the lowest incidence of fruiting bodies on crowns. *C. anguria* also had fewer fruiting bodies than *C. melo* in all experiments.

There was a significant ($P < 0.0001$) correlation (Pearson's r) between the incidence of crown cankers and the incidence of fruiting bodies on cankers in all experiments. The correlation was moderate to high: Pearson's $r = 0.7062$, 0.6262 , and 0.5550 in 2015, spring 2016, and fall 2016, respectively. There also was a significant ($P < 0.0001$) moderately high correlation between the two variables across experiments (Pearson's $r = 0.6373$).

Discussion

To the best of our knowledge, this field study is the first confirmation of susceptibility to both phases of GSB, foliar blight and crown cankers, caused by *S. citrulli* on 14 new cucurbit species. Thus, the host range of *S. citrulli* is wider than previously reported (Keinath 2011). Susceptibility to GSB had been reported on 24 species in 13 genera of cucurbits (Jiang et al. 2015; Keinath 2011). This study

expands the known list of affected plants to 37 species in 22 genera (Table 6). Because *S. citrulli* infected all 14 species tested, its host range might be much wider than what is known currently. Immunity against GSB appears to be extremely rare or even absent in the family Cucurbitaceae. The 14 new host species reported in this study represent 12 genera and four taxonomic tribes.

The family Cucurbitaceae comprises 15 tribes, of which 8 are now known to include species susceptible to GSB. This study included species in three of the six tribes with species known previously to be susceptible to GSB: Benincaseae, Bryoniaeae, and Cucurbitaeae. No members of the tribes Momordiceae, Sicyoaceae, and Siratieae were included; in part, because existing documentation of their susceptibility was considered to be sufficient (Keinath 2011; Schaefer and Renner 2011). Susceptibility was confirmed for two new tribes, Coniandreae and Gomphogyneae, from which susceptibility had not been reported previously. The two species from Coniandreae, *A. sagittifolia* and *K. leloja*, were highly susceptible to both phases of the disease (foliar blight and crown cankers). *G. pentaphyllum*, a cucurbit native to moist forests in Asia and member of the tribe Gomphogyneae (Schaefer and Renner 2011), was initially included in this study but had to be excluded because of poor germination, lack of plant vigor due to the hot climate and wet soil, and the number of transplants that were killed by *Pythium* spp. Nonetheless, the species was successfully inoculated and the pathogen was recovered from crowns and leaves in two experiments.

Muskmelon was very susceptible to GSB in this study, as reported previously (Chiu and Walker 1949; Crinò et al. 2007; Keinath 2008, 2014a). Although 8 other species had incidences of crown cankers that did not differ from muskmelon in all three experiments, muskmelon was the most susceptible species of the 15 species included in this study because incidence was 100% in each experiment. These findings confirm earlier studies where similar differences in susceptibility were observed among various cucurbit hosts. Chiu and Walker (1949) reported formation of cankers on bottle gourd, cucumber, muskmelon, and five species of *Cucurbita*, with muskmelon being most susceptible. In the study by dos Santos et al. (2009), *Cucumis* was among the most susceptible genera, while *Luffa* and *Cucurbita* were the least susceptible. The fact that there were fewer significant differences in susceptibility to crown cankers in the percentage data compared with the AUDPC was caused by differences among the cucurbit species in the onset of crown cankers and the speed at which they developed and spread (Table 6). Fry (1978) also compared AUDPC and final disease ratings and found that AUDPC generally was a better measure to evaluate late blight resistance of potato cultivars.

In contrast to the experiments done by Grossenbacher (1909), in which *C. anguria* was highly resistant to stem cankers, this species was susceptible to crown cankers in our study. The pathogen colonized crowns and was successfully isolated in all three experiments. Grossenbacher inoculated *C. anguria* 10 times but did not observe any symptoms. A possible explanation for the failure of infection throughout Grossenbacher's experiments is that he used mycelium from cultures that were grown on PDA to inoculate through stem wounds. He reported bacterial contamination of the cultures, which might have prevented successful infection by *Stagonosporopsis* spp.

Table 5. Contrasts among geographic origins of cucurbits for area under the disease progress curve calculated from incidence of crown cankers and severity of foliar blight^w

Continent (N) ^z	Incidence of crown cankers ^x						Severity of foliar blight ^y					
	2015	SE	Spring 2016	SE	Fall 2016	SE	2015	SE	Spring 2016	SE	Fall 2016	SE
Africa (8)	1,051 b	470	1,463 b	642	1,833 b	207	458 d	184	407 c	111	615 c	117
Europe (1)	2,596 a	0	3,138 a	0	2,650 a	0	1,796 a	0	1,593 a	0	1,235 a	0
North America (2)	2,364 a	1	947 b	197	1,691 b	668	871 b	121	536 bc	232	616 c	274
South America (3)	546 c	15	859 b	469	2,117 a	465	698 c	402	629 b	46	753 b	459

^w Each value is the mean of four replications. Means with the same letters within seasons do not differ significantly; single-degree-of-freedom contrasts, $P = 0.05$. SE = standard error, calculated from back-transformed values of individual species.

^x Number of ratings in 2015, spring 2016, and fall 2016 were three, four, and two, respectively.

^y Number of ratings in 2015, spring 2016, and fall 2016 were seven, nine, and five, respectively.

^z Geographic origin. N = number of species.

despite the wounding. It is also possible that Grossenbacher used isolates of *S. cucurbitacearum*, which have been identified from New York (Somai et al. 2002, Stewart et al. 2015). Isolates of *S. cucurbitacearum* might have a host preference for *Cucurbita* spp. because *S. cucurbitacearum* was recovered most frequently from this genus. However, Stewart et al. (2015) did not find a host specialization in experiments with *C. moschata*.

The two isolates of *S. citrulli* used for inoculation in this study were both originally recovered from watermelon, a genus that was not included in this study. The fact that these isolates successfully caused disease on all tested species shows the small or nonexistent effect of host origin on pathogenicity of *S. citrulli*, which confirms previous observations that isolate virulence is not correlated with

host origin (Wiant 1945; Zúniga 1999). It is possible, however, that there were slight effects on pathogen virulence on the different included cucurbits, as was reported in other studies (Chiu and Walker 1949; dos Santos et al. 2009; Keinath 2014a).

In general, all species except *C. anguria*, *M. scabra*, and *G. pentaphyllum* were equally susceptible or resistant to foliar blight and crown cankers. *C. anguria* and *M. scabra* were moderately resistant to foliar blight but clearly susceptible to crown cankers. The reverse appears to be true for *G. pentaphyllum*, which was resistant to crown cankers but susceptible to foliar blight. Due to problems with severe *Pythium* root rot on this species, this observation, however, could not be verified statistically. In a previous study, watermelon was found to be less susceptible to crown cankers than to the foliar blight phase of GSB. This was attributed to the potential existence of genes that confer partial resistance to crown cankers (Keinath 2014a). The finding that two species in this study had a resistance pattern the reverse of watermelon infers that there also might be genes encoding for specific resistance to foliar blight.

Differences in susceptibility were observed among the tribes and geographic origins included in this study. The tribe Bryoniaeae and the European origin grouping each included only one species, the very susceptible *E. elaterium*; therefore, the results for the comparisons among taxonomic tribes and geographic origins are similar (Tables 4 and 5) even though the single species was compared with different taxonomic and geographical groupings. The foliar blight phase on *E. elaterium*, *A. sagittifolia*, and *K. leloja*, which reached 100% severity in fall 2016, was as severe as on *C. melo*. These four most susceptible species represent three taxonomic tribes (Benincaseae, Bryoniaeae, and Coniandreae). Nevertheless, Benincaseae was consistently the least susceptible tribe, and three of the four most resistant species—*C. grandis*, *L. sphaerica*, and *Z. pallidinervia*—are members of this tribe. Because seven of the eight species in Benincaseae were from Africa, this convergence explains why African species were the most resistant geographic grouping. In contrast to this, muskmelon also belongs to this tribe. Likewise, the three species from South America were susceptible (*A. sagittifolia*), intermediately resistant (*C. ecuadorensis*), and resistant (*S. odorifera*). In addition, the differences between pairs of species in *Cucumis* and *Cucurbita* included in our study, of which one species in both genera was very susceptible and the other moderately resistant to resistant, show the great variability of GSB resistance within genera. Thus, membership in a genus, taxonomic tribe, or geographic origin has no bearing on the resistance level of a given cucurbit species. Similarly, Keinath (2014b) found variations of fungal reproduction on different hosts within the genera *Citrullus*, *Cucumis*, and *Cucurbita* and concluded that generalizations about differential susceptibility of cucurbits to GSB should be restricted to differences among species or horticultural types.

S. citrulli attacked and sporulated on thorns of *A. horridus*. This constitutes the first time that lesions and fruiting bodies were found on this type of plant part. This observation confirms that the pathogen attacks all aboveground vegetative and reproductive plant parts of cucurbits (Chester 1891; Keinath 2011, 2013).

Conditions more conducive to disease development in fall 2016 resulted in higher levels of foliar severity, canker incidence, and sporulation on cankers, despite the fact that the inoculum density was lowest in that experiment. On watermelon, GSB epidemics were reported to begin sooner in fall seasons, which led to higher final levels of disease (Keinath 2000). In fall 2016, dew in the early morning hours and more frequent misting prolonged leaf wetness, which favored disease progress. The lower levels of foliar severity at the end of the spring 2015 experiment compared with spring 2016 likely were due to the shorter season in 2015. The ratings in 2016 that correspond to the time period from inoculation to the final rating in 2015 were lower than or comparable with the 2015 ratings. In addition, in spring 2016, the overall speed at which canker incidence increased was significantly lower than in the other two experiments, although the highest inoculum density was applied in that experiment. This can be attributed to the drier conditions with higher temperatures in spring 2016 (data not shown). The seasonal variations in susceptibility to GSB observed in this study confirm previous reports of

Table 6. Daily increase of incidence of crown cankers caused by *Stagonosporopsis citrulli* compared within experiments for 15 cucurbit species^x

Species	2015	Spring 2016	Fall 2016
<i>Acanthosicyos horridus</i>	0.0 e	0.8 ab ^y	2.5 bcd
<i>Apodanthera sagittifolia</i>	5.0 bc	0.1 b	4.2 a
<i>Coccinia grandis</i>	0.0 e	0.0 b	0.9 e
<i>Cucumis anguria</i>	0.0 e	0.0 b ^y	3.1 abc
<i>Cucumis melo</i> 'Athena'	8.2 a	3.4 a ^y	4.0 a
<i>Cucumis zambianus</i>	5.7 b	0.1 b ^y	4.2 a
<i>Cucurbita digitata</i>	2.5 d	0.1 b	3.6 ab
<i>Cucurbita ecuadorensis</i>	0.0 e	0.8 ab	3.9 a
<i>Ecballium elaterium</i>	4.6 bc	1.7 a	4.2 a
<i>Gynostemma pentaphyllum</i> ^z	0.0	0.0	n.d.
<i>Kedrostis leloja</i>	9.6 a	2.0 a	3.5 abc
<i>Lagenaria sphaerica</i>	0.0 e	0.1 b	2.5 bcd
<i>Melothria scabra</i>	3.2 cd	0.1 b	0.9 e
<i>Sicana odorifera</i>	0.0 e	0.0 b	1.6 de
<i>Zehmeria pallidinervia</i>	0.0 e	0.0 b	2.5 cd

^x Each value is the mean of four replications of the final rating. Means with the same letters within experiments do not differ significantly; Fisher's least significant difference test, $P = 0.05$; n.d. = no data due to exclusion of *Gynostemma pentaphyllum* after the first two experiments. Rates were calculated for the interval between inoculation and the first rating.

^y Slopes for species with cankers before inoculation were calculated between the first two ratings.

^z Excluded from analyses due to large number of plants killed by *Pythium* spp.

Table 7. Incidence of fruiting bodies of *Stagonosporopsis citrulli* compared within three experiments in 2015 and 2016 for 15 cucurbit species^x

Species	23 July 2015	6 August 2016	16 October 2016
<i>Acanthosicyos horridus</i>	90.4 a	54.5 bc	17.2 de ^y
<i>Apodanthera sagittifolia</i>	95.5 a	10.4 de	83.3 ab
<i>Coccinia grandis</i>	0.0 c	0.0 e	3.5 e
<i>Cucumis anguria</i>	5.7 bc	17.8 de	53.8 bc
<i>Cucumis melo</i> 'Athena'	93.3 a	92.3 a	96.5 a
<i>Cucumis zambianus</i>	23.6 b	60.8 bc	82.3 ab
<i>Cucurbita digitata</i>	27.0 b	13.5 de	78.3 ab
<i>Cucurbita ecuadorensis</i>	0.0 c	38.3 cd	67.8 ab
<i>Ecballium elaterium</i>	96.2 a	43.8 c	85.5 ab
<i>Gynostemma pentaphyllum</i> ^z	0.0 c	2.4 e	n.d.
<i>Kedrostis leloja</i>	89.1 a	74.3 ab	58.3 bc
<i>Lagenaria sphaerica</i>	0.0 c	7.3 e	24.8 cde
<i>Melothria scabra</i>	14.4 bc	4.3 e	0.0 e
<i>Sicana odorifera</i>	0.0 c	0.0 e	23.3 de
<i>Zehmeria pallidinervia</i>	0.0 c	3.5 e	30.0 cde

^x Ratings include both pycnidia and pseudothecia. Each value is the mean of four replications of the final rating. Means with the same letters within experiments do not differ significantly; Fisher's least significant difference test, $P = 0.05$; n.d. = no data due to exclusion of *Gynostemma pentaphyllum* after the first two experiments.

^y Not significantly different from *C. grandis* due to missing values of *A. horridus*.

^z Excluded from analyses due to large number of plants killed by *Pythium* spp.

substantial variations in susceptibility caused by environmental conditions (Lou et al. 2013; St. Amand and Wehner 2001; Wolukau et al. 2007; Zhang et al. 2017).

Both types of fruiting body, which are readily produced by the homothallic fungus *S. citrulli*, were found on crowns of all cucurbits. We did not quantify the ratio of the two types but, in an earlier study, Keinath (2014b) found that pseudothecia were the predominant type of fruiting body. Species that were most susceptible to crown cankers also had the highest incidence of fruiting bodies and the fastest rates of canker development. This pattern was especially consistent for *C. melo* and *K. leloja*. Conversely, the most resistant species—*C. grandis*, *S. odorifera*, and *Z. pallidinervia*—consistently had the lowest incidence of fruiting bodies and the slowest rates of increase for cankers. Keinath (2014a) reported that cucurbit cultivars with higher levels of severity or incidence of foliar GSB also had more leaves with fruiting bodies; however, he did not compare disease progress rates among the included cultivars. These observations were confirmed in this study. The rate of sporulation of a pathogen may be regarded as a general indicator of susceptibility. Thomas and Jourdain (1992) and Thomas et al. (2017) evaluated the virulence of *Pseudoperonospora cubensis*, an oomycete causing downy mildew of cucurbits, based on the level of sporulation on different hosts. Hosts that facilitated lower rates of sporulation were considered less susceptible owing to their interaction with virulence factors involved in pathogen sporulation. Analogously, cucurbit species that consistently had lower incidences of fruiting bodies of *S. citrulli* in this study might impair sporulation by the pathogen through interacting with virulence factors specific to the formation of fruiting bodies.

The cucurbit species included in this study can serve as inoculum sources for *S. citrulli* in regions where they occur. Schenck (1968) reported that fruiting bodies on crowns of watermelon produced inoculum, particularly ascospores, that contributed significantly to the secondary spread of GSB. In North America, the native *C. digitata* and *M. scabra* and the introduced *E. elaterium* could be reservoirs of initial inoculum due to their high levels of susceptibility.

C. anguria had significantly less severe foliar blight than the other two *Cucumis* spp. in each experiment. However, in contrast to the experiments done by Wehner and St. Amand (1993), in which *C. anguria* was highly resistant to the foliar blight phase of GSB, this species was only moderately resistant to foliar blight in our study. The pathogen colonized leaves and was successfully isolated in all three

experiments. Wehner and St. Amand did not use prepared inoculum solution immediately and did not provide additional nutrients in the spore solution, as recommended by Svedelius (1990); therefore, GSB development may have been restricted in their study. Although *C. anguria* and *C. zambianus* are closely related and even cross compatible, the difference in susceptibility between them was substantial. In 2015 and spring 2016, the AUDPC of *C. zambianus* was as high as that of *C. melo*, a species to which it is only distantly related (Widrechner et al. 2008). It is unlikely that *C. anguria* could serve as a source of resistance to foliar blight, due to lack of crossability with other *Cucumis* spp. (Robinson and Decker-Walters 1997).

Resistance to GSB in cucurbits has been studied mainly in cucumber and melon. Although previous research identified different single dominant genes conferring resistance to GSB in various plant introductions (Frantz and Jahn 2004; Norton 1979; Prasad and Norton 1967; Wako et al. 2002; Wolukau et al. 2007; Zúñiga et al. 1999), recent studies found resistance to be quantitative and controlled by two quantitative trait loci (QTL) or three pairs of additive epistatic major genes (Lou et al. 2013; Zhang et al. 2017). The variability of GSB resistance and susceptibility in this study reflects the wide range of results found in previous work on GSB resistance and the underlying inheritance. Collectively evaluated, it seems most likely that resistance to this disease of cucurbits is controlled by multiple genes, each contributing to the overall level of resistance.

The two most resistant species found in this study were *C. grandis* and *Z. pallidinervia*. Because of their consistently high levels of resistance to *S. citrulli*, these species could be utilized to identify new QTL associated with GSB resistance of cucurbits. Lou et al. (2013) used introgression lines from a cross between *Cucumis hystris* and *C. sativus* to identify two QTL contributing to GSB foliar resistance. It would be interesting to test several different species of *Coccinia* and *Zehneria* for their susceptibility to GSB to identify the genetic background of resistance in these genera. Because the genus *Coccinia* comprises 27 species adapted to numerous different habitats (Holstein and Renner 2011), a species with significantly higher susceptibility to GSB than *C. grandis* is likely to exist. A more susceptible *Coccinia* sp. could be used in a cross with *C. grandis* to map QTL responsible for GSB resistance, similar to Lou et al. (2013). The genus *Zehneria*, which comprises approximately 65 species from different habitats in Africa, Asia, and Oceania, could be utilized in a similar way (Renner and Pandey 2013; Schaefer and Renner 2011). Before utilization of the

Table 8. Updated list of cucurbit genera known to be susceptible to gummy stem blight caused by *Stagonosporopsis* spp.

Genus (N) ^y	Common name	Tribe ^z	Reference
<i>Acanthosicyos</i> (1)	Nara melon	Benincaseae	This study
<i>Apodanthera</i> (1)	Argentinian melon	Coniandreae	This study
<i>Benincasa</i> (1)	Wax gourd	Benincaseae	Wiant 1945
<i>Bryonia</i> (3)	Bryony	Bryonieae	Gonnermann and Rabenhorst 1869
<i>Citrullus</i> (2)	Watermelon	Benincaseae	Chester 1891
<i>Coccinia</i> (1)	Ivy gourd	Benincaseae	This study
<i>Cucumis</i> (5)	Cucumber, melon	Benincaseae	Saccardo 1891
<i>Cucurbita</i> (7)	Squash, pumpkin	Cucurbiteae	Grossenbacher 1909
<i>Cyclanthera</i> (1)	Wild cucumber	Sicyoeae	Mendes et al. 1998
<i>Ecballium</i> (1)	Squirting cucumber	Bryonieae	This study
<i>Gynostemma</i> (1)	Jiaogulan	Gomphogyneae	This study
<i>Kedrostis</i> (1)	None	Coniandreae	This study
<i>Lagenaria</i> (2)	Bottle gourd	Benincaseae	Grossenbacher 1909
<i>Luffa</i> (2)	Loofah	Sicyoeae	Grossenbacher 1909
<i>Melothria</i> (1)	Mouse melon	Benincaseae	This study
<i>Momordica</i> (2)	Bitter melon	Momordiceae	Wiant 1945
<i>Sechium</i> (1)	Chayote	Sicyoeae	Wiant 1945
<i>Sicana</i> (1)	Cassabanana	Cucurbiteae	This study
<i>Sicyos</i> (1)	Bur-cucumber	Sicyoeae	Greene 1953
<i>Siraitia</i> (1)	Luohanguo	Siraitieae	Jiang et al. 2015
<i>Trichosanthes</i> (1)	Snake gourd	Sicyoeae	Punithalingam and Holliday 1972
<i>Zehneria</i> (1)	Batwing vine	Benincaseae	This study

^y N = number of species.

^z Classification based on Schaefer and Renner (2011).

resistance genes of *C. grandis* or *Z. pallidinervia*, which are the most promising candidates for use in advanced cucurbit breeding, it would be necessary to test them in different environments and with different isolates of the three species of *Stagonosporopsis* causing GSB on cucurbits. It has been shown that germplasm found to be highly resistant to GSB in one particular environment has to be retested under different conditions for successful use in breeding programs (Wolukau et al. 2007).

A potential use of *C. grandis* and *S. odorifera*, which are resistant to both the canker and foliar blight phases of GSB, might be as rootstocks for grafting cucurbit crops. Grafting is used in cucurbit production to manage Southern root-knot nematode (*Meloidogyne incognita*) and Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* in watermelon, vine decline of melon caused by *Monosporascus cannonballus*, and other diseases (Cohen et al. 2012; Keinath and Hassell 2014a,b; King et al. 2008; Thies et al. 2015). The cucurbits mainly utilized as rootstocks are bottlegourd (*L. siceraria*) and interspecific hybrid squash (*C. maxima* × *C. moschata*) (King et al. 2008; Lee 1994). However, all commonly used rootstocks are as susceptible to GSB as seedless watermelon, and the conditions needed for the healing of the graft union are very conducive to disease development (Keinath 2013). Crinò et al. (2007) tested eight interspecific hybrid squash rootstocks to manage GSB on melon and found four rootstocks with high resistance. The development of rootstocks from *C. grandis* and *S. odorifera* could supply growers with a rootstock that is resistant to this important cucurbit disease. Grafting commercial cucurbits on rootstocks of these two species should be possible, because grafts are generally successful with other rootstocks that are members of the tribe Benincaseae, to which most commercially important cucurbits belong (e.g., cucumber, melon, and watermelon). Moreover, grafting watermelon on members of the tribe Cucurbitaeae, to which pumpkin, squash, and *S. odorifera* belong, is now common practice in many production regions (King et al. 2008; Lee 1994). Although *C. grandis* is, as a member of the same taxonomic tribe, more closely related to watermelon and muskmelon, *S. odorifera* might be more suitable as a GSB-resistant rootstock because of its similar stem morphology. The hypocotyl of *C. grandis* thickens at an early developmental stage and expands to a diameter of about 3 cm to form a caudiciform stem, which is a swollen perennial storage stem at or above ground level (Simpson 2010). This stem morphology could lead to problems in grafting success and compatibility.

In summary, 2 of 14 new species tested, *C. grandis* and *Z. pallidinervia*, were consistently highly resistant to the foliar blight phase of GSB in this study, whereas *A. sagittifolia*, *C. melo*, *E. elaterium*, and *K. leloja* were among the most susceptible species. *S. odorifera* was consistently highly resistant to the crown canker phase of GSB in this study. *C. grandis* was the only species that was consistently resistant to both the foliar blight and crown canker phases of GSB. In all, 14 species from 12 genera were identified as new hosts of *S. citrulli*, information which could have future applications in resistance breeding and management of GSB on cucurbit crops (Table 8).

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Literature Cited

- Bala, G., and Hosein, F. 1986. Studies on gummy stem blight disease of cucurbits in Trinidad. *Trop. Agric. Trinidad* 63:195-197.
- Brewer, M. T., Rath, M., and Li, H.-X. 2015. Genetic diversity and population structure of cucurbit gummy stem blight fungi based on microsatellite markers. *Phytopathology* 105:815-824.
- Chester, F. D. 1891. Notes on three new or noteworthy diseases of plants. *Bull. Torrey Bot. Club* 18:371-374.
- Chiu, W. F., and Walker, J. C. 1949. Physiology and pathogenicity of the cucurbit black-rot fungus. *J. Agric. Res.* 78:589-615.
- Cohen, R., Pivonia, S., Crosby, K. M., and Martyn, R. D. 2012. Advances in the biology and management of *Monosporascus* vine decline and wilt of melons and other cucurbits. *Hortic. Rev. (Am. Soc. Hortic. Sci.)* 39:77-120.
- Corlett, M. 1981. A taxonomic survey of some species of *Didymella* and *Didymella*-like species. *Can. J. Bot.* 59:2016-2042.
- Crinò, P., Lo Bianco, C., Roupheal, Y., Colla, G., Saccardo, F., and Paratore, A. 2007. Evaluation of rootstock resistance to Fusarium wilt and gummy stem blight and effect on yield and quality of a grafted 'Inodorus' melon. *HortScience* 42:521-525.
- dos Santos, G. R., Álvares da Silva Velloso Ferreira, M., Caldas de Pinho Pessoa-Filho, M. A., Ferreira, M. E., and Café-Filho, A. C. 2009. Host specificity and genetic diversity of *didymella bryoniae* from Cucurbitaceae in Brazil. *J. Phytopathol.* 157:265-273.
- FAO. 2017. FAOSTAT. Online publication. <http://www.fao.org/faostat>
- Farr, D. F., and Rossman, A. Y. 2017. Fungal Databases, U.S. National Fungus Collections. Online publication. United States Department of Agriculture–Agricultural Research Service. <https://nt.ars-grin.gov/fungaldbatabases>
- Frantz, J. D., and Jahn, M. M. 2004. Five independent loci each control monogenic resistance to gummy stem blight in melon (*Cucumis melo* L.). *Theor. Appl. Genet.* 108:1033-1038.
- Fry, W. E. 1978. Quantification of general resistance of potato cultivars and fungicide effect for integrated control of potato late blight. *Phytopathology* 68:1650-1655.
- Gonnemann, W., and Rabenhorst, L. 1869. *Mycologia europaea: Abbildungen sämtlicher Pilze Europas*, Vol. 5/6 Pyrenomyceten, p. 15.
- Greene, H. C. 1953. Notes on Wisconsin parasitic fungi. XIX. *Amer. Midl. Naturalist* 50:501-508.
- Grossenbacher, J. G. 1909. A *Mycosphaerella* wilt of melons. Pages 193-229 in: N. Y. (Geneva) Agric. Exp. Stn. Tech. Bull. 9.
- Holstein, N., and Renner, S. S. 2011. A dated phylogeny and collection records reveal repeated biome shifts in the African genus *Coccinia* (Cucurbitaceae). *BMC Evol. Biol.* 11:28.
- Jensen, B. D., Massawe, A., and Swai, I. S. 2011. First report of gummy stem blight caused by *Didymella bryoniae* on watermelon and confirmation of the disease on pumpkin in Tanzania. *Plant Dis.* 95:768.
- Jiang, N., Hu, F. Y., Ye, Y. F., Jiang, Sh. Y., and Huang, X. Y. 2015. First report of leaf spot caused by *Stagonosporopsis cucurbitacearum* on Luohanguo (*Siraitia grosvenorii*) in China. *Plant Dis.* 99:1645.
- Keinath, A. P. 2000. Effect of protectant fungicide application schedules on gummy stem blight epidemics and marketable yield of watermelon. *Plant Dis.* 84:254-260.
- Keinath, A. P. 2002. Survival of *Didymella bryoniae* in buried watermelon vines in South Carolina. *Plant Dis.* 38:32-38.
- Keinath, A. P. 2008. Survival of *Didymella bryoniae* in infested muskmelon crowns in South Carolina. *Plant Dis.* 92:1223-1228.
- Keinath, A. P. 2011. From native plants in central Europe to cultivated crops worldwide: The emergence of *Didymella bryoniae* as a cucurbit pathogen. *HortScience* 46:532-535.
- Keinath, A. P. 2013. Diagnostic guide for gummy stem blight and black rot on cucurbits. Online publication. *Plant Health Prog.* doi.org/10.1094/PHP-2013-1024-01-DG
- Keinath, A. P. 2014a. Differential susceptibility of nine cucurbit species to the foliar blight and crown canker phases of gummy stem blight. *Plant Dis.* 98:247-254.
- Keinath, A. P. 2014b. Reproduction of *Didymella bryoniae* on nine species of cucurbits under field conditions. *Plant Dis.* 98:1379-1386.
- Keinath, A. P. 2017. Gummy stem blight. Page 59 in: *Compendium of Cucurbit Diseases and Pests*, 2nd ed. A. P. Keinath, W. M. Wintermantel, and T. A. Zitter, eds. American Phytopathological Society, St. Paul, MN.
- Keinath, A. P., and Duthie, J. A. 1998. Yield and quality reductions in watermelon due to anthracnose, gummy stem blight, and black rot. Pages 77-90 in: *Recent Research Developments in Plant Pathology*, Vol. 2. Research Signpost, Trivandrum, India.
- Keinath, A. P., Fillippelli, E. L., Baccari, G. V., and DuBose, V. B. 2010. Susceptibility of 15 cultivars of watermelon, melon, squash, pumpkin, and bottle gourd to gummy stem blight, 2009. Online publication. *Plant Dis. Manage. Rep.* 4:V120.
- Keinath, A. P., and Hassell, R. L. 2014a. Control of Fusarium wilt of watermelon by grafting onto bottlegourd or interspecific hybrid squash despite colonization of rootstocks by *Fusarium*. *Plant Dis.* 98:255-266.
- Keinath, A. P., and Hassell, R. L. 2014b. Suppression of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* race 2 on grafted triploid watermelon. *Plant Dis.* 98:1326-1332.
- King, S. R., Davis, A. R., Liu, W., and Levi, A. 2008. Grafting for disease resistance. *HortScience* 43:1673-1676.
- Kocyan, A., Zhang, L.-B., Schaefer, H., and Renner, S. S. 2007. A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. *Mol. Phylogenet. Evol.* 44:553-577.
- Lee, J.-M. 1994. Cultivation of grafted vegetables I. Current status, grafting methods, and benefits. *HortScience* 29:235-239.
- Lou, L., Wang, H., Qian, C., Liu, J., Bai, Y., and Chen, J. 2013. Genetic mapping of gummy stem blight (*Didymella bryoniae*) resistance genes in *Cucumis sativus*-hystrix introgression lines. *Euphytica* 192:359-369.
- Mendes, M. A. S., da Silva, V. L., Dianese, J. C., Ferreira, M. A. S. V., dos Santos, C. E. N., Gomes Neto, E., Urben, A. F., and Castro, C. 1998. Fungos em plantas no Brasil. Embrapa-SPI/Embrapa-Cenargen, Brasília, Brazil.
- Norton, J. D. 1979. Inheritance of resistance to gummy stem blight caused in watermelon. *HortScience* 14:630-632.
- Prasad, K., and Norton, J. D. 1967. Inheritance of resistance to *Mycosphaerella citrullina* in muskmelon. *Proc. Am. Soc. Hortic. Sci.* 91:396-400.
- Punithalingam, E., and Holliday, P. 1972. *Didymella bryoniae*. CMI Description of Pathogenic Fungi and Bacteria, No. 332. Commonwealth Mycological Institute, Kew, UK.

- Rennberger, G., Kousik, C. S., and Keinath, A. P. 2018. First Report of Powdery Mildew on *Cucumis zambianus*, *Cucurbita digitata*, and *Melothria scabra* Caused by *Podosphaera xanthii* in the United States. *Plant Dis.* 102:246.
- Renner, S. S., and Pandey, A. K. 2013. The Cucurbitaceae of India: Accepted names, synonyms, geographic distribution and information on images and DNA sequences. *PhytoKeys* 20:53-118.
- Robinson, R. W., and Decker-Walters, D. S. 1997. Cucurbits. CAB International, New York.
- Saccardo, P. A. 1891. *Didymella melonis*. Syll. fung. addit. Vol. 1-4:86.
- Santner, T. J., and Duffy, D. E. 1989. The Statistical Analysis of Discrete Data. Springer-Verlag, New York.
- Schaefer, H., Heibl, C., and Renner, S. S. 2009. Gourds afloat: A dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events. *Proc. R. Soc. B.* 276:843-851.
- Schaefer, H., and Renner, S. S. 2011. Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae). *Taxon* 60:122-138.
- Schenck, N. C. 1968. Epidemiology of gummy stem blight (*Mycosphaerella citrullina*) on watermelon: Ascospore incidence and disease development. *Phytopathology* 58:1420-1422.
- Shaner, G., and Finney, R. E. 1977. the effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67: 1051-1056.
- Simpson, M. G. 2010. Plant Systematics, 2nd ed. Academic Press, Burlington, MA.
- Sitterly, W. R. 1969. Effect of crop rotation on cucumber gummy stem blight. *Plant Dis. Rep.* 53:417-419.
- Somai, B. M., Dean, R. A., Farnham, M. W., Zitter, T. A., and Keinath, A. P. 2002. Internal transcribed spacer regions 1 and 2 and random amplified polymorphic DNA analysis of *Didymella bryoniae* and related *Phoma* species isolated from cucurbits. *Phytopathology* 92:997-1004.
- St. Amand, P. C., and Wehner, T. C. 2001. Heritability and genetic variance estimates for leaf and stem resistance to gummy stem blight in two cucumber populations. *J. Am. Soc. Hortic. Sci.* 126:90-94.
- Stewart, J. E., Turner, A. N., and Brewer, M. T. 2015. Evolutionary history and variation in host range of three *Stagonosporopsis* species causing gummy stem blight of cucurbits. *Fungal Biol.* 119:370-382.
- Svedelius, G. 1990. Effects of environmental factors and leaf age on growth and infectivity of *Didymella bryoniae*. *Mycol. Res.* 94:885-889.
- Thies, J. A., Ariss, J. J., Hassel, R. L., Buckner, S., and Levi, A. 2015. Accessions of *Citrullus lanatus* var. *citrroides* are valuable rootstocks for grafted watermelon in fields infested with root-knot nematodes. *HortScience* 50:4-8.
- Thomas, A., Carbone, I., Lebeda, A., and Ojiambo, P. S. 2017. Virulence structure within the populations of *Pseudoperonospora cubensis* in the United States. *Phytopathology* 107:777-785.
- Thomas, C. E., and Jourdain, E. L. 1992. Host effect on selection of virulence factors affecting sporulation of *Pseudoperonospora cubensis*. *Plant Dis.* 76: 905-907.
- van Steekelenburg, N. A. M. 1983. Epidemiological aspects of *Didymella bryoniae*, the cause of stem and fruit rot of cucumber. *Neth. J. Plant. Pathol.* 89:75-86.
- Wako, T., Sakata, Y., Sugiyama, M., Ohara, T., Ishiuchi, D., and Kojima, A. 2002. Identification of melon accessions resistant to gummy stem blight and genetic analysis of the resistance using an efficient technique for seedling test. *Acta Hortic.* 588:161-164.
- Wehner, T. C., and St. Amand, P. C. 1993. Field tests for cucumber resistance to gummy stem blight in North Carolina. *HortScience* 28:327-329.
- Wiant, J. S. 1945. Mycosphaerella black rot of cucurbits. *J. Agric. Res.* 71:193-213.
- Widrechner, M. P., Kirkbride, J. H., Jr., Ghebretinsae, A. G., and Reitsma, K. R. 2008. *Cucumis zambianus* (Cucurbitaceae), a new species from northwestern Zambia. *Syst. Bot.* 33:732-738.
- Wolukau, J. N., Zhou, X., Li, Y., Zhang, Y., and Chen, J. 2007. Resistance to gummy stem blight in melon (*Cucumis melo* L.) germplasm and inheritance of resistance from plant introductions 157076, 420145, and 323498. *HortScience* 42:215-221.
- Zhang, J. X., Bruton, B. D., Miller, M. E., and Isakeit, T. 1999. Relationship of developmental stage of cantaloupe fruit to black rot susceptibility and enzyme production by *Didymella bryoniae*. *Plant Dis.* 83:1025-1032.
- Zhang, L.-B., Simmons, M. P., Kocyan, A., and Renner, S. S. 2006. Phylogeny of the Cucurbitales based on DNA sequences of nine loci from three genomes: Implications for morphological and sexual system evolution. *Mol. Phylogenet. Evol.* 39:305-322.
- Zhang, S., Liu, S., Miao, H., Shi, Y., Wang, M., Wang, Y., Li, B., and Gu, X. 2017. Inheritance and QTL mapping of resistance to gummy stem blight in cucumber. *Mol. Breed.* 37:49.
- Zitter, T. A. 2017. Black Rot. Page 92 in: Compendium of Cucurbit Diseases and Pests, 2nd ed. A. P. Keinath, W. M. Wintermantel, and T. A. Zitter, eds. American Phytopathological Society, St. Paul, MN.
- Zúniga, T. L. 1999. Gummy stem blight (*Didymella bryoniae*) of cucurbits: Pathogen characterization and inheritance of resistance in melon (*Cucumis melo*). Ph.D. dissertation, Department of Plant Pathology, Cornell University, Ithaca, NY.
- Zúniga, T. L., Jantz, J. P., Zitter, T. A., and Jahn, M. M. 1999. Monogenic dominant resistance to gummy stem blight in two melon (*Cucumis melo*) Accessions. *Plant Dis.* 83:1105-1107.