Proc. Fla. State Hort. Soc. 131:151–158. 2018.

Stemphylium Leaf Spot in Spinach: Chemical and Breeding Solutions for This Threatening Disease in Florida

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Additional index words. *Stemphyllium botryosum*, Stemphylium Leaf Spot, carboxamides, fungicide resistance, strobilurins, systemic acquired resistance, plant resistance, screening

Stemphylium Leaf Spot (SLS) caused by the fungus *Stemphylium botryosum* f. sp. *spinacia* is a growing threat to baby spinach (*Spinacia oleracea*) production in Florida and regions with a hot humid climate. To date there are no approved fungicides to control the disease. The pathogen seems to be seed-borne and transported on contaminated seed; however, the disease is not detected in seed production fields as the climate locations where spinach seed is produced are not conducive for disease development. The efficacy of broad-spectrum fungicides was tested in the field with fungicides having strobilurin, carboxamide and systemic acquired resistance (SAR) active ingredients. None of the fungicides tested alone provided a complete preventive control of SLS. However, when combined, mixes of SARs with phosphites seem to offer an immediate solution to decrease the disease in spinach. Hybrids currently used by the baby spinach industry in Florida were tested with locally collected isolates of *S. botryosum* f.sp. *spinacia*. Three hybrids 'Shelby,' 'Perentie,' and 'Goldeneye' had consistently less disease incidence in field trials but were not immune to the pathogen. As no current hybrid is completely resistant to the disease, additional putatively resistant Plant Introductions (PIs) and landraces from the USDA were tested for resistance against the fungus. Nine landraces and PIs had significantly lower disease than the controls but none of them were immune to the mix of isolates used. An integrated disease management of SLS should be in place that combines, seed health, fungicide treatments and tolerant hybrids to help growers to mitigate the threat of the disease. In the long term, breeding schemes that intermate the possible resistant landraces and PIs should be started.

Spinach (*Spinacia oleracea* L.) grown for baby leaf and spring mix production has become an increasingly important leaf crop in the Everglades Agricultural Area (EAA) of South Florida. It is by far the most important leafy component in spring mix and it comprises nearly 60% of total spring mix production. The baby spinach crop is relatively short-term compared to full-season spinach, grown for its much larger but still tender leaves. Baby leaf spinach takes only 21–28 d for development, depending upon temperatures and other environmental conditions. This is advantageous, since it reduces the time of field exposure to insect pests and diseases. However, baby leaf spinach is planted at extremely high densities (> 3 million plants per acre) and the cost of seed alone may exceed $1,200 per acre, so significant crop losses can be prohibitively expensive.

In 1997, a new leaf spot disease of spinach was first identified in California (Koike et al., 2001). Referred to as Stemphylium Leaf Spot (SLS) (Fig. 1A), the causal pathogen was identified as *Stemphylium botryosum* f. sp. *spinacia*. Disease symptoms initially appear as small, grayish circular leaf spots measuring 0.1 to .25 inches in diameter. Over time, leaf spots enlarge and turn tan in color. Older lesions frequently coalesce, dry up, and become papery in texture (Fig. 1B). Fungal signs exterior to the leaf surface are generally absent, differentiating this disease from other spinach leaf spots, such as Cladosporium leaf spot (*Clado-*

Fig. 1. Spinach infected with Stemphylium Leaf Spot. (**A**) A close-up image of highly infected plant and a (**B**) patch of infected spinach in field conditions.

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sporium variabile), or anthracnose (*Colletotrichum dematium* f. sp. *spinaciae*) (Koike et al. 2007). While this disease is reported to be of minor importance in arid spinach production regions, such as California (Koike et al. 2007), environmental conditions in Florida are much more conducive for SLS. The state's high relative humidity and frequent precipitation events serve to enhance spore germination, infection, and multiplication, greatly increasing the potential for catastrophic losses.

DuToit and Derie (2001) reported *S. botryosum* as a pathogen on spinach grown for seed production in the state of Washington, a major seed production area, in 2001. This was the same year SLS was reported for the first time in Florida (Raid et al. 2001). Other first reports soon followed (Everts and Armentrout 2001a; Koike et al. 2005). In an early Florida efficacy trial (Raid 2001), it was reported that fungicides belonging to the strobilurin class (FRAC 11), which are Quinone Outside Inhibitors (QoI), were among those most efficacious with regard to SLS management. Based upon this evidence and additional studies (Everts and Armentrout 2001b, Raid et al. 2017), many spinach growers came to rely on strobilurin fungicides as the basis for their chemical management programs, dutifully rotating them with fungicides of dissimilar modes of action, as recommended. However, in recent years, Florida growers have had to increase their application numbers to manage SLS, and even then, have had more difficulty in controlling this particular disease. With fungicide insensitivity to the strobilurins being suspected, additional research on chemical control is needed.

Confirmed seed-borne disease efforts are currently underway (Hernandez-Perez and duToit 2006) to discover effective seed-treatments to lessen the impact of SLS. Hot-water treatment, fungicides, and antibiotics are all being investigated with some promising results (du Toit et al., 2018a; du Toit et al., 2018b). However, due to its extremely high seeding rate, and the very low tolerance for any leaf spot (< 1% incidence on marketable product), seed treatments must be exceedingly effective. At a seeding rate of 3 million seeds per acre, even a 1% seed lot infection would represent almost one infected seed per ft^2 of planted bed. Such high inoculum densities beg for additional control measures, such as host plant resistance.

Genetic resistance to SLS has been researched to a limited extent. In a screening of the USDA germplasm collection and 22 commercial varieties, significant differences in resistance were found among genotypes. Two USDA accessions were found to be partially resistant to a strain of SLS isolated from the spinach variety 'Cheetah' in Arizona, but unfortunately, no varieties were found to be immune (Mou et al., 2008). The genetic basis of variation in SLS resistance was further validated by the identification of eigiht single nucleotide polymorphisms (SNPs) associated with partial resistance to SLS (Shi et al., 2015) caused by *S. botryosum* f. sp. *spinacia*, is an important fungal disease of spinach. Progress in determining which varieties may be resistant to Florida SLS isolates and fungicides that control SLS in spinach are crucial to preventing devastating losses from this disease.

These investigations were intended to lay the groundwork for finding short-term and long-term solutions to control SLS in South Florida. First, we tested a broad range of fungicides to find chemistries which may control SLS. Second, we screened commercial spinach hybrids to identify any that may be genetically resistant. Potentially varietal resistance, used in combination with fungicidal control, will provide growers a management strategy that is both efficacious and economical.

Materials and Methods

Fungicide trials. Four fungicide trials are included in these investigations. The first compared the efficacy of a number of different strobilurin fungicides against a broad-spectrum protectant. The second compared fungicide programs rotating a strobilurin fungicide with chemistries with dissimilar modes-of-action. A third fungicide trial compared various carboxamide fungicides of FRAC Class 7 for SLS efficacy, and a fourth compared two systemic acquired resistance (SAR) inducers, used alone and in combination, for their potential as rotational partners.

All fungicide trials were conducted on spinach planted in a commercial leafy production field located six miles east of Belle Glade, FL. The soil was a Histosol with a soil pH of 7.2 and it was fertilized according to soil test recommendations. Seed of the commercial hybrid 'Stanton', previously determined to be infested with the SLS pathogen, was directed-seeded on 29 Nov. 2017 on 8-inch raised beds that were 5-ft wide on 6-ft centers. Seed were planted at a depth of 0.5 inches using a 0.5-inch in-row spacing and a 2-inch between-row spacing. Allowing for 22 rows across the top of the bed, this produced a seeding density typical of baby spinach production, resulting in approximately 3.2 million plants per acre.

Each field experiment consisted of a randomized complete block design with three replications of each treatment. Experimental units were 10-ft sections of sprayed bed with 2-ft unsprayed buffers on each end. Fungicide treatments were applied in a volume of 63 gal/acre of water using a CO_2 backpack sprayer equipped with a single TeeJet 11004 nozzle delivering spray at 30 psi. Two overlapping passes were made to each experimental unit to ensure coverage. Applications were made on a 4–5 d schedule due to the short duration of the crop and the explosive nature of the disease. All fungicides in these trials were applied without the use of a surfactant (spreader/sticker) to avoid phytotoxicity issues, which frequently occur on baby spinach due to its tender foliage. Application dates in all listed trials were 12, 17, 21, and 26 Dec. with the first application occurring at the first true leaf stage. Untreated checks in all trials were sprayed with tap water alone to serve as experimental controls.

All field trials relied on natural infection for disease pressure and SLS developed primarily from infested seed. Disease incidence was assessed on 22 and 25 Dec. by counting the number of leaves displaying symptoms of SLS, regardless of lesion size, within a 1 ft^2 area located near the center of each experimental unit. Disease severity was assessed by visually estimating the percentage of visible leaf area turned necrotic by the pathogen within a 4 ft^2 area central to each plot on 28 and 31 Dec.

Experiment 1. In experiment one, six strobilurins were compared for SLS efficacy, including: pyraclostrobin (Cabrio WG), azoxystrobin (Quadris SC), fenamidone (Reason SC). picoxystrobin (Aproach SC), fluoxystrobin (Evito SC), and trifloxystrobin (Flint WG). All were compared with the broad-spectrum protectant mancozeb (Manzate 4F). Strobilurins were not rotated in this experiment since the objective was to compare efficacy. Fungicide treatments and rates for the respective treatments are listed in Table 1 and are expressed in rates of product applied per acre in this and all other tables.

Experiment 2. This trial compared the efficacy of various fungicides rotated with a single strobilurin fungicide, pyraclostrobin. Treatments included: pyraclostrobin alone, and rotations of pyraclostrobin with acibenzolar-S-methyl (Actigard WG), cymoxanil + famoxadone (Tanos DF), boscalid (Endura WDG),

Table 1. Disease incidence and disease severity of Stemphylium leaf spot 28, 31, 34, and 37 d after planting on the spinach hybrid 'Stanton' following treatment with strobilurin fungicides during Winter 2017 in Belle Glade, FL.

		Disease incidence		Disease severity	
Fungicide	Rate/acre	22 Dec.	25 Dec.	28 Dec.	31 Dec.
Untreated check	---	17.0 a[z]	38.7 a	18.3 a	27.0 a
Pyraclostrobin	1.6 oz	14.7 a	27.0 ab	12.3 b	20.0 bc
Azoxystrobin	4.2 fl oz	9.7 ab	23.3 ab	13.3 b	20.0 bc
Fenamidone	3.52 fl oz	11.0 ab	17.3 b	12.3 b	19.3 bc
Picoxystrobin	1.8 fl oz	8.0 ab	23.7 b	17.3 b	23.3 ab
Fluoxystobin	0.9 fl oz	9.0 ab	26.3 ab	10.0 b	16.0 c
Trifloxystrobin	1.36 oz	8.3 ab	31.7 ab	13.0 b	23.3 ab
Mancozeb	1.5 qt	2.7 b	2.0 c	1.0 c	2.3 d

[z]Values in a column followed by the same letter are not significantly different ($P < 0.05$) as determined by Fisher's least significant difference test.

Table 3. Disease incidence and disease severity of Stemphylium leaf spot 28, 31, 34, and 37 d after planting on the spinach hybrid 'Stanton' following treatment with carboxamide fungicides during Winter 2017 in Belle Glade, FL.

		Disease incidence		Disease severity	
Fungicide	Rate/acre	22 Dec.	25 Dec.	28 Dec.	31 Dec.
Untreated heck	---	18.7 a[z]	58.0 a	25.0 a	32.3 a
Fluopyram	2.1 fl oz	1.3 c	3.0 e	1.0 d	2.0 d
Solatenol	4 fl oz	13.7 a	23.0 c	4.3 c	7.0 cd
Pydiflumetofen	2.5 fl oz	4.0 c	5.0 de	1.3 d	1.3 d
Boscalid	6.3 oz	11.7 ab	36.0 b	10.0 b	22.0 b
Penthiopyrad	4.9 fl oz	3.3 c	14.7 cd	3.7 cd	10.0 c
Fluxapyroxad	1.3 fl oz	5.3 bc	12.0 de	2.3 cd	6.0 cd

[z]Values in a column followed by the same letter are not significantly different ($P < 0.05$) as determined by Fisher's least significant difference test.

cyprodinil + fludioxonil (Switch WG), and fluopyram (Luna Privilege SC). Fungicide treatments and rates for this experiment (Expt. 2) are listed in Table 2. Cabrio was applied on the first and third application dates, and rotational products were applied on the second and fourth application dates.

Experiment 3. Six different carboxamide fungicides were compared for efficacy in this trial. Treatments included fluopyram (Luna Privilege SC), solatenol (Solatenol SE), pydiflumetofen (Miravis SC), boscalid (Endura WG), penthiopyrad (Fontelis SC), and fluxapyroxad (Xemium EC). They were not rotated in this trial since the objective was to compare relative efficacy. Fungicide treatments and rates for Expt. 3 are listed in Table 3.

Experiment 4. Two SAR compounds, acibenzolar-S-methyl (Actigard WG) and *Bacillus mycoides* (LifeGard DF), were compared in this trial, alone and in combination with potassium phosphite (K-Phite SL) or copper hydroxide (Kentan DF). These treatments were contrasted with a pyraclostrobin + fluxapyroxad (Merivon SC) pre-mixture. Fungicide treatments and product rates per acre for Expt. 4 are listed in Table 4.

Genetic Resistance Trials

Pathogen isolation. *S. botryosum* f.sp. *spinacia* was isolated from diseased spinach leaf tissue collected in commercial spinach fields in the EAA during the 2017–18 field season. Leaves were surface sterilized in 20% bleach for 3 s, then rinsed twice in sterile water to remove the bleach. Disease lesions were cut from the leaf tissue then plated on V8 agar media (Koike et al. 2001) supplemented with 50 μg/mL gentamicin using sterile technique. Fungal colonies developed after 3 d on the plated leaf tissue and were examined under a light microscope to visually confirm the presence of *S. botryosum* f.sp. *spinacia* conidia.

All screening trials were inoculated with a mixture of three pathogenic isolates collected from the spinach hybrid 'Stanton', named Stanton-4, Stanton-14, and Stanton-25. Inoculum for screening trials was prepared from pathogen colonies grown on V8 media. The fungus was physically removed from petri plates using a sterile spatula and was then transferred to a beaker containing sterile water to create a spore suspension. Conidia were quantified using a hemocytometer before being adjusted to a final concentration of approximately 1×10^4 spores/mL. Inoculations were performed by spraying a spore suspension evenly across all accessions using a 2-gal pump sprayer with sufficient volume to thoroughly wet the foliage. Inoculations were performed at the end of the day to ensure leaf wetness duration for germination and infection.

Screening trials. To test for genetic variation of SLS resistance in cultivated hybrids and USDA plant introductions (PIs), we performed screening trials in both the field and in a glasshouse equipped to encourage disease development. The four field experiments were conducted in different months over Winter 2018. In the field, spinach was grown using standard production practices in 9.4-m sections on 1.8-m wide raised plots on muck soil in the EAA. Plots were inoculated when plants had 4–6 true leaves. Experiment 1 had 16 hybrids (Fig. 2) and was planted on 18 Jan. 2018 and inoculated on 7 Feb. Experiments 2 and 3 consisted of 25 and 26 hybrids, respectively, and were planted on 14 and 17 Feb., respectively. These hybrids are shown in Fig. 3 and were inoculated on 10 and 16 Mar, respectively.

Table 2. Disease incidence and disease severity of Stemphylium leaf spot 28, 31, 34, and 37 d after planting on the spinach hybrid 'Stanton' following treatment with fungicide treatments alternated with strobilurin fungicides during Winter 2017 in Belle Glade, FL.

		Disease incidence		Disease severity	
Fungicide	Rate/acre	22 Dec.	25 Dec.	28 Dec.	31 Dec.
Untreated check	---	23.7 a[z]	65.7 a	22.0 a	33.3 a
Pyraclostrobin	1.6 oz	5.3 b	22.7 bc	14.7 b	26.7 ab
Pyraclostrobin alt. w/Acibenzolar-S-methyl	1.6 oz/0.375 oz	5.3 b	21.0 bc	7.0 cd	15.7 cd
Pyraclostrobin alt. w/Cymoxanil + Famoxadone	1.6 oz/4 + 4 oz	6.0 b	29.0 b	14.0 b	21.3 bc
Pyraclostrobin alt. w/Boscalid	1.6 oz/6.3 oz	10.0 b	29.7 b	11.7 bc	18.7 c
Pyraclostrobin alt. w/Cyprodinil + Fludioxonil	1.6 oz/4.1 + 2.7 oz	9.7 b	19.0 bc	7.3 cd	12.7 d
Pyraclostrobin alt. w/ Fluopyram	1.6 oz/2.1 fl oz	3.7 b	9.7 c	3.0 d	4.7 e

[z]Values in a column followed by the same letter are not significantly different ($P < 0.05$) as determined by Fisher's least significant difference test.

Table 4. Disease incidence and disease severity of Stemphylium leaf spot 28, 31, 34, and 37 d after planting on the spinach hybrid 'Stanton' following treatment with systemic acquired resistance inducers, alone and in combination, during Winter 2017 in Belle Glade, FL.

		Disease incidence		Disease severity	
Fungicide	Rate/acre	22 Dec.	25 Dec.	28 Dec.	31 Dec.
Untreated Check	---	17.3 a[z]	46.7 a	14.7 a	25.0 a
Acibenzolar	0.375 oz	9.0 b	18.3 c	7.3 b	11.3 c
Acibenzolar + Potassium Phosphite	0.375 oz + 1.65 pt	2.0 c	.3 de	1.3 c	3.0 e
Acibenzolar + Copper Hydroxide	0.375 oz +1. 2 lb	5.0 bc	7.0 de	3.3 bc	9.3 cd
Bacillus mycoides	2.4 oz	7.0 bc	33.0 b	7.7 b	18.7 b
Bacillus mycoides + Potassium Phosphite	2.4 oz + 1.65 pt	3.3 bc	3.0 e	1.0 c	3.3 de
Bacillus mycoides + Copper Hydroxide	2.4 oz + 1.2 lb	6.3 bc	7.0 de	2.7 bc	9.3 cd
Pyraclostrobin + Fluxapyroxad	1.2 + 1.2 fl oz	4.7 bc	11.0 cde	2.0 c	2.3 e

[z]Values in a column followed by the same letter are not significantly different ($P < 0.05$) as determined by Fisher's least significant difference test.

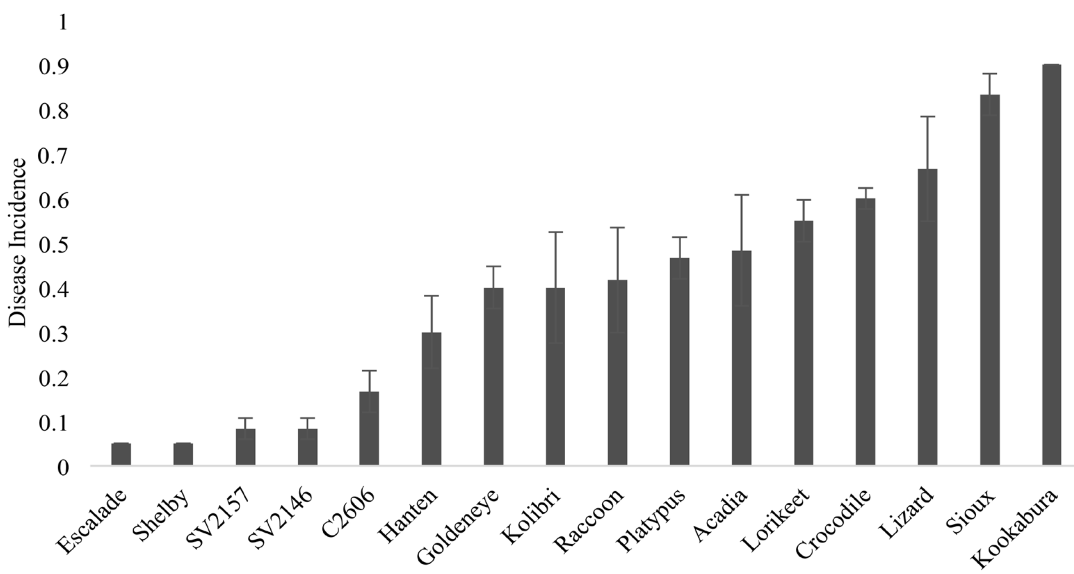

Fig. 2. Disease Incidence of 16 commercial hybrids inoculated in the field with a mix of three isolates of *Stemphyllium botryosum* f.sp. *spinacia* in Belle Glade, FL 2018.

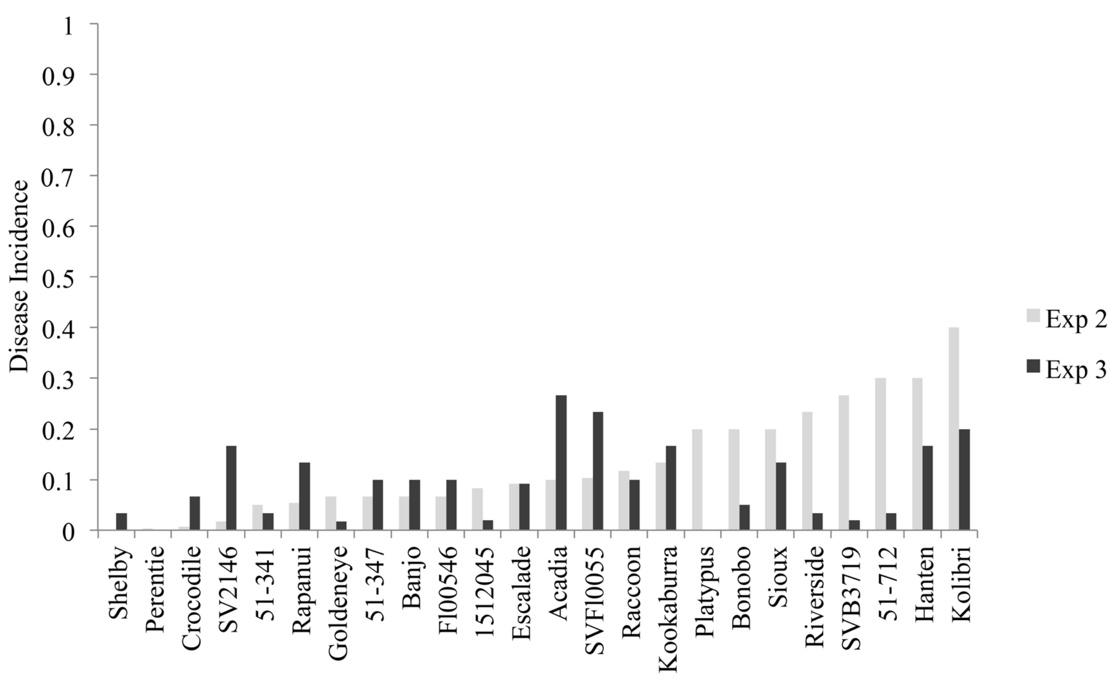

Fig. 3. Disease incidence of 25 spinach hybrids in two separate field trials planted 3-d apart and inoculated with a mix of three isolates of *Stemphyllium botryosum* f.sp. *spinacia*. The earlier planting is represented in gray, the later planting is represented in black. Belle Glade, FL 2018.

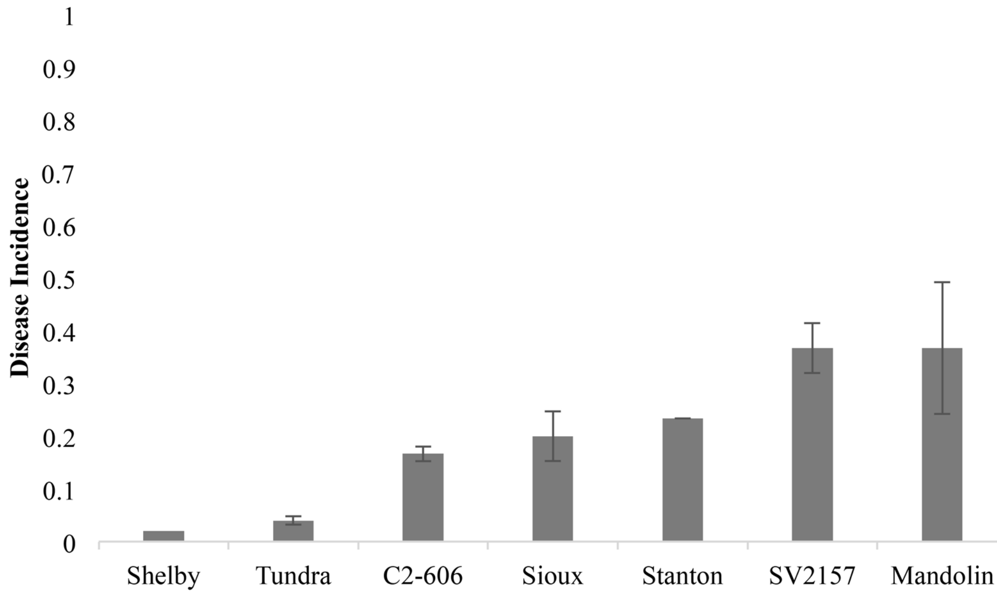

Fig. 4. Disease incidence of seven spinach hybrids inoculated in the field with a mix of three isolates of *Stemphyllium botryosum* f.sp. *spinacia* in Belle Glade, FL 2018.

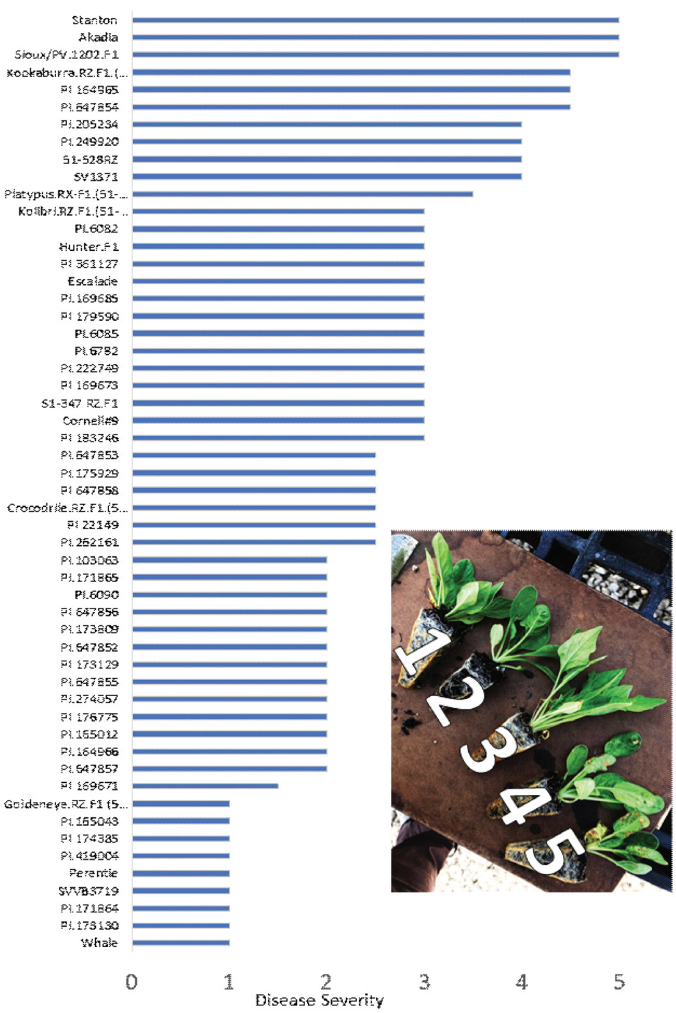

Fig. 5. Disease severity (DS) of 62 hybrids, landraces and plant introductions (PI) of spinach inoculated with a mix of three isolates of *Stemphyllium botryosum* f.sp. *spinacia* in glasshouse conditions. Median DS is represented on a 0–5 rating scale as described in the right bottom picture where 0 = no spots present and 5 = plant completely colonized.

and four landraces were also tested. Twenty-three commercial hybrids previously planted in the field were also tested here for comparison. Seeds of each accession were planted in a row of 8 in 8 × 24 nursery trays on 6 Feb. Trays were grown outdoors until plants had four true leaves, transferred into a glasshouse and inoculated 9 Apr. using 1×10^4 spores/ mL inoculum.

In the field screening experiments, disease incidence (DI) was recorded in three random 1-ft^2 sections in each plot. Time after inoculation varied between experiments because variation in climate throughout the season affected the rate of disease development. In the first experiment, DI was scored 27 s after planting on 14 Feb. Experiment 2 and 3 occurred in dry and cool months that were less conducive to disease development, thus DI was not recorded until 23 Mar. (five weeks later) for both experiments. Experiment 4 was conducted in a warmer month, so DI was collected 16 Apr., 7 d after inoculation.

The glasshouse trial was inoculated and scored for disease on the same dates as the fourth field experiment. Unlike the field experiments, here disease severity (DS) was recorded for each individual plant in the row of 8. DS ratings were on a 0-5 scale where individuals with no SLS symptoms were rated as 0 and individuals with extreme SLS were rated as 5 (Fig. 5).

Statistical analysis. The fungicide trials were analyzed as a randomized complete block design using a PROC GLM of SAS Institute. Mean separation was done according to the protected Least Significance Difference of Fisher at the 0.05 level. The same procedures were used for the screening of hybrids in field tests. The experiment in the glasshouse with 62 different PIs and hybrids was analyzed as an augmented randomized design. Blocks were used as random effects and the tested hybrids and PIs were compared to both controls ('Riverside' and 'Platypus') using a *t*-test of Dunett.

Results

Fungicide Trials

Experiment 1. Disease incidence (DI) and severity (DS) as influenced by six different strobilurin fungicides are shown in

Table 1. None of the six strobilurins tested resulted in management levels that would be commercially acceptable. Final DI ranged from a low of 16% for fluoxystrobin, to 23.3% for picoxystrobin and trifloxystrobin, as compared to 27% in the untreated check. The levels of control with the strobilurins observed in this trial are in sharp contrast to those reported early on regarding SLS control in Florida (Raid 2001; Raid 2004; Raid et al. 2017), and are even significantly lower than the broad-spectrum protectant mancozeb, included for comparison.

Experiment 2. In general, alternations of different fungicides with the strobilurin pyraclostrobin resulted in improved control, although the various chemistries differed in terms of significance (Table 2). Of the products tested, fluopyram was significantly better than all others, followed by the cyprodinil + fludioxonil premixture, acibenzolar-S-methyl, and boscalid, in that order. Cymoxanil + famoxadone was not significantly better than pyraclostrobin alone, which was not significantly different than the untreated check. The poor performance of pyraclostrobin in experiment 2 mirrors that observed in Expt. 1.

Experiment 3. Table 3 shows the results of the comparison of carboxamide fungicides applied alone for SLS management. All fungicides tested provided significant disease control when compared to the untreated check. Pydiflumetofen and fluopyram provided the highest level of control, followed by fluxapyroxad, solatenol, and penthiopyrad. Boscalid was the poorest performing carboxamide tested, although resultant disease severities were still lower than in the untreated check.

Experiment 4. The two SAR inducing compounds acibencilar-S-methyl and the biological *B. mycoides* both provided significant SLS suppression, even when applied alone at tested rates (Table 4). Performance of each was improved when combined with potassium phosphite, which reputedly also has SAR inducing properties. Combinations of the SARs with potassium phosphite were the statistical equivalent of the pyraclostrobin + fluxapyroxad premixture tested in this particular experiment for comparison sake. Combinations of the SARs with copper hydroxide were generally better than the SARs alone, but not as efficacious as the SAR + potassium phosphite combinations.

Besides efficacy of SLS management, phytotoxicity was also noted in the trials. All treatments with acibenzolar-S-methyl at the rate used here (0.75 oz/acre) developed leaf cupping. Treatments receiving azoxystrobin SC showed some stunting and slight leaf cupping, with both anomalies being more severe in treatments receiving picoxystrobin. Mancozeb treatments exhibited enhanced growth compared to the untreated check, presumably due to the presence of manganese, an element frequently at suboptimal levels in some EAA soils.

Screening trials. Initially, hybrids currently used by baby spinach growers in FL were the first to be screened against *S. botryosum* f.sp. *spinacia* with the hope of making a recommendation on which hybrids may be planted. In the first experiment, significant differences ($P < 0.0001$) for disease incidence (DI) were found among the16 hybrids commonly used in the EAA. No hybrids were immune to the disease (0% DI) but 'Escalade' and 'Shelby' had the lowest (5%) DI after inoculation. 'Sioux' and 'Kookaburra' had the highest DI at 83% and 90%, respectively (Fig. 2).

Two additional experiments were conducted in cooler and drier months with 24 hybrids that included 14 hybrids used in the initial experiments. As both experiments were planted two days apart, an initial analysis was calculated with the two experiments together. Using this procedure, however, showed that the experiments were significantly different ($P = 0.0200$); therefore, individual analysis by experiment was later computed. In both experiments, significant differences ($P < 0.0001$) were detected among the 24 hybrids. 'Shelby' again had a low DI, 0% and 0.3% , and a hybrid not tested in the first experiment, 'Perentie,' was equally low with DI of 0.3% and 0%, in first and second experiments, respectively. Poorly performing hybrids 'Acadia,' 'Hanten,' and 'Kolibri' had the highest DI of 10% and 26%, 30% and 17%, then 40% and 20% respectively. In those two experiments, SLS development was slower and less severe as the climate was not conducive to disease development during the execution of the trials (Fig. 3). A fourth experiment comprised of hybrids 'Shelby', 'C2-606' and 'SV2157' which initially had the lowest DI in previous experiments were planted with other hybrids never tested before such as 'Tundra', Stanton' and 'Mandolin' jointly with 'Sioux' used as susceptible control in a warmer environment (Fig. 4). Differences ($P = 0.0002$) among these 7 hybrids were found. The hybrids 'Shelby' and 'Tundra' had the least DI with 2% and 4%, while 'Sioux,' 'SV2157,' and 'Mandolin' had the highest DI with 23%, 37%, and 37% respectively. Although, the experiment was conducted in a warmer month, DI was not as high as previously detected in the first field experiment.

Because Expt. 2 and Expt. 3 occurred in cool, dry months most of the hybrids used in those experiments were tested again in a warm and humid glasshouse to better test resistance under higher disease pressure. The glasshouse study also included USDA accessions including Plant Introductions (PIs) known to be partially resistant to one isolate collected in Arizona, *S. botryosum*. This experiment found significant differences among the new tested cultivars/PIs/landraces ($P = 0.0332$). Because of the limited sample size (8 or less individuals) disease severity (DS) was scored on each individual plant. Medians of the different genotypes ranged in DS from 1–5. Several USDA accessions and hybrids had an average DS of 1, including CPPSH 3 09 ('Whale'), PI 171330, PI 171864, 'SVVB3719,' 'Perentie,' PI 419004, PI 174385, PI 165043, and 'Goldeneye.' The genotypes, PI 165043, 'Goldeneye,' and 'Whale' were significantly ($P > 0.001$) less diseased than 'Platypus,' the susceptible control. Commercial hybrids with the worst DS were 'Kookaburra' (4.5), 'Sioux' (5), 'Acadia' (5), and 'Stanton' (5). No genotype was found to be immune under this high disease pressure (Fig. 5).

Discussion

Restricted by the highly specific environmental conditions necessary for spinach flowering, seed producers are under increasing pressure to produce more seed on limited acreages of land; this could exacerbate Stemphylium leaf spot problems. Hernandez-Perez and Du Toit (2006) reported *Stemphylium* infestations exceeding 50% in several commercial lots. Such levels mean that baby leaf spinach producers are likely to battle the disease from the cotyledon stage, drastically increasing disease management problems.

In the experiment comparing strobilurin fungicides (Table 1), all Qo1 compounds were found to be far less efficacious than they had been in previous trials (du Toit et al., 2003; Everts and Armentrout, 2001b; Raid, 2001), and even as recently as one year ago (Raid et al., 2017). This suggested insensitivity to strobilurin in the pathogen population. A later independent testing by a chemical company confirmed insensitivity to 16 of 2018 Florida

S. boytrosum isolates to the strobilurin azoxystrobin (G. Olaya, personal communication). Previous strobilurin efficacy may be explained by the fact that the pathogen population in previous years may not have been the same as the one collected in 2018. Little is known on *S. botryosum* population genetics at this point and there is not a defined race classification of this pathogen.

The fungicide trial comparing the alternation of products with pyraclostrobin, with carboxamide and SAR indicate that prospects for resistance management are currently available. Pydiflumetofen appeared to offer some control and can be use in rotations; a pre-mixture of this compound with fludioxonil (Miravis Prime SC) has just received registration and therefore it could be a good fit in a resistance management program. The combination of potassium phosphite with the SAR biological *B. mycoides* could also be an effective rotation. Future tests to find an optimal dosage of acibenzilar-S-methyl on baby leaf spinach will be necessary since rates tested produced levels of phytotoxicity that threatened marketability. Lower rates could potentially solve this problem without sacrificing efficacy.

The SARs Actigard 50DWG and LifeGard DF showed efficacy when used alone, but both greatly benefitted from combination with potassium phosphite compounds to the point of being comparable with some strobilurin/carboxamide pre-mixtures, like Merivon SC. Although SARs and potassium phosphite compounds have very different chemistries, they both may elicit a host plant defense through the salicylic acid pathway (Herman et al., 2008; Eshraghi et al., 2007). This combination with the same modes-of-action raises the level of host response sufficiently to curb SLS. By contrast, carboxamide fungicides used alone provided better control than the strobilurins, and pre-mixtures of the two classes proved superior efficacy at reducing the disease, demonstrating the benefits of multiple modes-of-action.

Spinach growers in the EAA observed that SLS outbreaks were more problematic with some hybrids than others (Villegas, personal communication). Our findings indicate that those observations are likely due to genetic variation between hybrids. The most resistant hybrids were 'Shelby' from Enza Zadem and 'Perentie' from Rijk Zwaan, which were nearly immune to SLS with little disease incidence. Finding a relatively high degree of resistance in hybrids from two different breeding programs is promising and indicates that partial or even complete resistance to SLS may be available in more commercial hybrids that have yet to be tested. While testing more hybrids to find a source of resistance may be fruitful, better disease management systems would be helpful to control this disease. Processes such as seed treatment and seed testing to minimize the amount of pathogen in the field remain to be studied. A combination of sprays, partial resistance, and clean seed may be effective at decreasing SLS disease to a threshold not economically important.

In fact, it is not certain if seed used in field trials had contamination with *S. boytrosum* pathogen, which is known to be seed-borne (Hernandez-Perez and duToit 2006). Although, SLS was not observed in the field until after inoculation, having the pathogen in the seed may be causing an overestimate of susceptibility in some cases. Regardless, the hybrids with high disease severity and disease incidence are certainly susceptible to SLS and may not be ideal for cultivation in warm and humid climates.

When the commercial hybrids were tested in a controlled glasshouse experiment, the results were largely consistent with field trials. However, some USDA accessions found to be partially resistant in previous experiments using an isolate from Arizona (Mou et al. 2008) were found to be susceptible. The variation in disease observed between FL isolates and the isolate from AZ may be attributable to strain differences. Unfortunately, there is no information in the literature about the population structure of *S. boytrosum* or host-pathogen reaction between different strains of *S. boytrosum* in spinach. A screening of the ~350 accessions of spinach in the USDA germplasm collection should be carried out as genetic variation was identified in a sample of 60 PIs and landraces tested in this research.

Identification of efficacious sprays and partially resistant commercial hybrids is important for controlling this disease. The spray combinations presented here may be useful to growers to repress SLS development and the hybrids found to be most resistant may be preferred for planting in Florida. However, these results fall short of identifying a spray that controls SLS to an acceptable level or an immune genotype of spinach. Future studies using effective sprays to control SLS on spinach hybrids with varying degrees of partial resistance may be productive towards finding a combined method of control SLS completely. Furthermore, studies that find the source of *S. boytrosum* in the growers' fields and the relative virulence of *S. boytrosum* from different sources will be needed to find measures to maintain clean seed for the control of SLS.

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