Characterization of Leaf Spot Pathogens from Several Spinach Production Areas in the United States

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Abstract

Leaf spot diseases have become a major concern in spinach production in the United States. Determining the causal agents of leaf spots on spinach, their prevalence and pathogenicity, and fungicide efficacy against these pathogens is vital for effective disease management. Spinach leaves with leaf spots were collected from Texas, California, Arizona, and South Carolina from 2016 to 2018, incubated in a moist chamber, and plated on potato dextrose and tryptic soy agar media. Fungal and bacterial colonies recovered were identified based on morphology and sequence analysis of the internal transcribed spacer rDNA and 16S rRNA, respectively. Two predominant genera were isolated: (i) *Colletotrichum* spp., which were identified to species based on sequences of both introns of the glutamate synthetase (*GS-I*) and glyceraldehyde-3-phosphate dehydrogenase (*gapdh-I*) genes; and (ii) *Stemphylium* spp., identified to species based on sequences of the *gapdh* and calmodulin (*cmdA*) genes. Anthracnose (*Colletotrichum spinaciae*) and Stemphylium leaf spot

(*Stemphylium vesicarium* and *S. beticola*) were the predominant diseases. Additional fungi recovered at very limited frequencies that were also pathogenic to spinach included *Colletotrichum coccodes*, *C. truncatum*, *Cercospora beticola*, and *Myrothecium verrucaria*. All of the bacterial isolates were not pathogenic on spinach. Pathogenicity tests showed that *C. spinaciae*, *S. vesicarium*, and *S. beticola* caused significant leaf damage. The fungicides Bravo WeatherStik (chlorothalonil), Dithane F-45 (mancozeb), Cabrio (pyraclostrobin), and Merivon (fluxapyroxad and pyraclostrobin) were highly effective at reducing leaf spot severity caused by an isolate of each of *C. spinaciae* and *S. vesicarium*, when inoculated individually and in combination.

Keywords: *Stemphylium vesicarium*, *Stemphylium beticola*, leaf spot survey, morphological and molecular identification, pathogenicity tests, fungicide testing, leaf spot pathogens

Spinach (*Spinacia oleracea* L.) is an important leafy vegetable crop grown on approximately 25,140 ha in the United States with an annual production of approximately 424,000 metric tons (USDA-NASS 2018). Approximately 1,500 ha of spinach are grown in the Winter Garden Area of Texas, west of San Antonio and near Uvalde, where conditions are favorable for production in the winter months (Dainello and Morelock 2005). Moreover, >17,960 ha of fresh market and processing spinach is grown in California annually, making California the leading producer of spinach in the United States (USDA-NASS 2018).

Fresh market spinach production has changed dramatically in the last two decades, with exceptionally high seeding rates used in most production areas of California, Arizona, Texas, and other states where baby leaf spinach is grown (1.9 to 8.75 million seed/ha) (Fresh Express 2019; Reed et al. 2010). The high-density plantings create a dense crop canopy, prolonged periods of leaf wetness (because the crop typically is overhead irrigated), poor air circulation, and high humidity that favor the development of common foliar diseases (Gullino et al. 2019) such as downy mildew, caused by *Peronospora effusa* (= *P. farinosa* f. sp. *spinaciae*); anthracnose, caused by *Colletotrichum spinaciae* (*C. dematium* f. sp. *spinaciae*) (Correll et al. 1994; Gilardi et al. 2018; Kurt et al. 2016); *Cercospora* leaf spot,

caused by *Cercospora beticola* (Dechmani and Goode 1972); white rust, caused by *Albugo occidentalis* (Correll et al. 1994); and Stemphylium leaf spot, caused by *Stemphylium botryosum* (du Toit and Derie 2001; Hernandez-Perez and du Toit 2006; Koike et al. 2001; Reed et al. 2010), *Stemphylium* sp. (Misawa et al. 2017), and *S. beticola* (Gilardi et al. 2018). In addition, several other fungi can cause leaf spot diseases on spinach, including *Cladosporium* leaf spot (*Cladosporium variabile*) (Hernandez-Perez and du Toit 2006) and *Myrothecium* leaf spot (*Myrothecium verrucaria*) (Garibaldi et al. 2016; Matic et al. 2019).

Leaf spot diseases can reduce spinach quality and yield, particularly for fresh markets (Correll et al. 1994; Gullino et al. 2019). Determining the causal agents of various leaf spot diseases on spinach, their relative frequency, pathogenicity on commercial spinach cultivars, and fungicide efficacy against these pathogens is essential for effective disease management. The purpose of this research was to determine the major and minor fungal and bacterial pathogens causing leaf spot diseases on spinach in Texas, California, Arizona, and South Carolina, the relative frequency and virulence of the pathogens, and efficacy of fungicides to control the two major leaf spot diseases in the United States, anthracnose and Stemphylium leaf spot. In addition, the species of *Colletotrichum* and *Stemphylium* causing leaf spots on spinach in the United States were re-examined relative to previous reports of these leaf spot pathogens. A preliminary report of this work has been published (Liu et al. 2017).

Materials and Methods

Sample collection and pathogen identification. Spinach leaves with leaf spot symptoms were collected from 74 locations in Texas (50), California (7), Arizona (4), and South Carolina (2) between November 2016 and November 2018 (Table 1). Due to the prevalence of spinach leaf spots, the focus of the study was spinach crops in Texas, although a number of opportunistic samples were received from the other states. The sample locations were based on reports from commercial growers, extension personnel, or spinach breeders. A

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subsample of leaves (leaves selected randomly from symptomatic leaves collected from a given field) for each sample was incubated in a moist chamber for 1 to 5 days at 22°C to evaluate microbial growth. The leaves were then examined using both a dissecting microscope (Olympus SZH) and a compound microscope (Olympus BX41) at 500× magnification to observe asexual or sexual fungal structures. Fungal and bacterial isolations also were performed on a subset of symptomatic leaves from each sample by plating lesions onto potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) or tryptic-soy agar (TSA) (Difco Laboratories), respectively. Fifty lesions were evaluated for each of 50 of the 74 samples that had an adequate number of leaves collected. However, for 24 samples, only 10 to 20 lesions were available for examination. For each sample, small sections of the diseased leaf tissue (3 × 3 mm) were surface sterilized in 10% household bleach (0.525% sodium hypochlorite) for 2 min, rinsed in sterilized water, air dried, and placed into Petri dishes containing PDA or TSA. Plates were incubated on a 12-h light/dark-cycle at 22°C. After 7 to 14 days, fungal and bacterial colonies were transferred to obtain pure cultures, although *Myrothecium* colonies were only transferred after they were first observed after 21 days of incubation. Fungal and bacterial isolates were identified to species based on morphology and DNA sequencing. For morphological characterization, *Colletotrichum* spp. and *Cercospora* spp. were cultured on PDA for 2 weeks, and *Stemphylium* spp. were cultured on V8 agar for 2 weeks. Conidia (25 per isolate) were collected from the center of the Petri dish for each isolate, and the length and width were measured at a magnification of 500× with a compound microscope (Olympus BX41).

Molecular identification. For all of the fungi recovered, including isolates pathogenic or nonpathogenic to spinach, the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was examined by polymerase chain reaction (PCR) amplification with the universal primers ITS1 and ITS4 (White et al. 1990). Two additional target regions were examined for sequence diversity of the *Colletotrichum* spp. recovered. The introns of the glutamine synthetase (*GS-I*) and glyceraldehyde-3-phosphate dehydrogenase (*gapdh-I*) genes were amplified by PCR assay with the primers GSF1 and GSR-1 for *GS-I*, and GDF1 and GDR1 for *gapdh-I*, respectively (Guerber et al. 2003; Liu et al. 2007). Likewise, two additional DNA targets were sequenced for the *Stemphylium* spp. recovered, *gapdh* and calmodulin (*cmdA*) genes, using the primers and PCR assay described by Woudenberg et al. (2017). All bacterial isolates were identified to genus and species by sequencing the 16S rRNA gene using the universal primers 310F and 516R (Liu et al. 2016).

Total DNA was extracted for fungal isolates using the DNeasy Plant Mini Kit following the manufacturer's instructions (Qiagen, Valencia, CA). The PCR template for bacteria was prepared as follows: each bacterial colony (6 days old on TSA) was picked up with a toothpick, and the cells were put into a PCR tube with 50 µl of distilled water and incubated at 95°C in a PCR thermal cycler (Bio-Rad T100, Applied Biosystems, Foster City, CA) for 5 min. The bacterial suspension was then diluted by adding a 2-µl bacterial suspension into 48 µl of distilled water as the template for the PCR assay. The PCR conditions for bacteria were the same as for the fungal PCR assays. One bacterial isolate from each location was chosen for DNA sequence analysis, with three individual isolates from different geographic locations, representing white (*Pseudomonas fluorescens*), red (*Pantoea vagans*), and yellow (*Erwinia persicina*) colonies, respectively, sequenced. The PCR products amplified with the ITS rDNA, *GS-I*, *gapdh-I*, *cmdA*, and 16S rRNA primers were purified using the Quick PCR purification kit (Qiagen) and were sequenced directly using the same primers by Eton Bioscience (Research Triangle Park, NC). Sequencing reactions were carried out with the ABI PRISM Dye Terminator Cycle Sequence Ready Reaction Kit (Applied Biosystems).

The nucleotide sequences of representative isolates were searched using the Basic Local Alignment Search Tool (BLAST) against the database of the National Center for Biotechnology Information for species identification (Altschul et al. 1997). For *Colletotrichum* spp., the ITS rDNA and *gapdh-I* from representative isolates in this

survey were deposited in GenBank (Table 2). For *Stemphylium* spp., the *gapdh* and *cmdA* sequences of representative isolates, and those of isolates from earlier work (Hernandez-Perez and du Toit 2006; Koike et al. 2001), were deposited in GenBank (Table 2).

Species identification of *Colletotrichum* spp. and *Stemphylium* spp. based on phylogenetic analyses. The ITS and *gapdh-I* sequences of isolates of *Colletotrichum* spp. obtained in this survey, and an isolate (JG13) from a prior study (Correll et al. 1993; Liu et al. 2007), were compared with representative sequences of *Colletotrichum* species published by Damm et al. (2009) for phylogenetic analysis. The analysis was run for the individual gene sequences and the concatenated gene sequences. Related sequences of *C. lindemuthianum* (CBS151.28), published by Damm et al. (2009), were used as an outgroup for each analysis. The sequences of ITS rDNA, *gapdh*, and *cmdA* from isolates of *Stemphylium* spp. obtained in this survey and from isolates in earlier studies (Hernandez-Perez and du Toit 2006; Koike et al. 2001) were compared with representative sequences of *Stemphylium* species published by Woudenberg et al. (2017) for phylogenetic analysis. The analysis was run with the individual gene sequences and the concatenated gene sequences. The relevant sequences of *Alternaria alternata* (GV14-634a1), published by Woudenberg et al. (2017), were used as an outgroup in each analysis.

Sequences of each isolate of *Colletotrichum* and *Stemphylium* were entered into the SeqPup DNA sequence editor (Gilbert 1996) and the data converted to a NEXUS file. Phylogenetic analyses, as well as basic statistics, were performed using PAUP* 4.0 beta 10 (Swofford 2003). Three methods of tree building were used: maximum parsimony (MP), maximum likelihood (ML), and neighbor joining (NJ). Bootstrap analysis was performed using 1,000 replicate data sets. The *Colletotrichum* species used by Damm et al. (2009) for phylogenetic analysis included *C. spinaciae*, *C. circinans*, *C. dematium*, and the outgroup *C. lindemuthianum* (Fig. 1). The diverse *Stemphylium* species examined by Woudenberg et al. (2017), including *S. vesicarium*, *S. simmonsii*, *S. beticola*, *S. lycopersici*, *S. solani*, and *S. botryosum*, along with the outgroup species *A. alternata*, were included in this study for phylogenetic analysis (Fig. 2). In addition, Bayesian analyses were performed with MrBayes 3.2.7-WIN (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The Markov chain Monte Carlo analysis used four chains and started from a random tree topology. The sample frequency was set at 1,000, and the temperature value of the heated chain was set at 0.1. The run stopped when the average standard deviation of split frequencies reached below 0.01. Burn-in was set to 25%, after which the likelihood values were stationary.

Pathogenicity tests. Pathogenicity tests were conducted for a subset of fungal and bacterial isolates obtained from the spinach samples. Two types of pathogenicity tests were conducted for the fungal isolates. The first test involved comparing multiple species of each genus for virulence. The second test involved evaluating isolates of two species of *Stemphylium*, *S. vesicarium* and *S. beticola*, for relative virulence.

All fungal isolates of *Colletotrichum*, *Stemphylium*, and *Cercospora* (Table 3) tested for pathogenicity on spinach were cultured on PDA for 14 days before collecting spores for inoculation of 30-day-old spinach seedlings (cultivar Viroflay, Pop Vriend Seed Co., Andijk, the Netherlands). In addition, three randomly selected isolates of all other fungal genera recovered were tested for pathogenicity. Conidia on PDA plates were washed with distilled water by scraping the colonies with a sterilized glass spreader. The conidia were then filtered through two layers of cheesecloth and quantified using a hemocytometer (Propper Lumicyte Improved Neubauer), and the inoculum concentration was adjusted to 1 × 10⁶ spores/ml. Ten spinach seeds were planted in each pot (10 cm diameter, 10 cm height) containing the potting mix LC1 (SunGro Horticulture Distribution, Agawam, MA). Plant stands were thinned to three per pot, and three replicate pots per treatment were used in each trial. Plants were inoculated with the relevant spore suspension, and non-inoculated control plants were sprayed with distilled water and subjected to the same conditions. Eighteen pots of plants were sprayed with a total volume of 50 ml of inoculum using a Badger Basic Spray

Table 1. Fungi recovered from leaf spot on spinach leaf samples collected from 2016 to 2018 in the United States^u

Sample date	Pathogens			Genus and species identification	State
	<i>Colletotrichum spinaciae</i>	<i>Stemphylium vesicarium</i> or <i>S. beticola</i>	<i>Cercospora beticola</i>		
7/12/2016	50/50 ^v	0/50	0/50	<i>Colletotrichum spinaciae</i>	TX
7/12/2016	40/50	0/50	0/50	<i>C. spinaciae</i> (and <i>C. coccodes</i> [5/50]) ^w	TX
7/12/2016	3/5	0/5	0/5	<i>C. spinaciae</i>	TX
7/12/2016	50/50	0/50	0/50	<i>C. spinaciae</i>	TX
12/12/2016	6/10	0/10	0/10	<i>C. spinaciae</i>	TX
12/12/2016	45/50	0/50	0/50	<i>C. spinaciae</i> (and <i>C. truncatum</i> [5/50]) ^x	TX
12/12/2016	50/50	0/50	0/50	<i>C. spinaciae</i>	TX
12/12/2016	2/8	0/8	0/8	<i>C. spinaciae</i>	TX
20/12/2016	20/20	0/20	0/20	<i>C. spinaciae</i>	TX
6/1/2017	48/50	0/50	0/50	<i>C. spinaciae</i>	TX
9/1/2017	50/50	0/50	0/50	<i>C. spinaciae</i>	TX
28/2/2017	50/50	0/50	0/50	<i>C. spinaciae</i>	TX
28/2/2019	44/50	0/50	0/50	<i>C. spinaciae</i> (and <i>C. coccodes</i> [6/50]) ^w	TX
28/2/2017	41/50	0/50	0/50	<i>C. spinaciae</i> (and <i>C. truncatum</i> [5/50]) ^x	TX
28/2/2017	50/50	0/50	0/50	<i>C. spinaciae</i>	TX
28/2/2017	50/50	0/50	0/50	<i>C. spinaciae</i>	TX
18/1/2018	50/50	0/50	0/50	<i>C. spinaciae</i>	TX
18/1/2018	50/50	0/50	0/50	<i>C. spinaciae</i>	TX
12/1/2016	0/10	5/10	0/10	<i>Stemphylium</i> spp. ^y	TX
12/12/2016	0/20	20/20	0/20	<i>Stemphylium</i> spp.	TX
6/1/2017	0/50	48/50	0/50	<i>Stemphylium</i> spp.	TX
9/1/2017	50/50	0/50	0/50	<i>Stemphylium</i> spp.	TX
10/1/2017	0/50	50/50	0/50	<i>Stemphylium</i> spp.	TX
18/1/2017	0/50	50/50	0/50	<i>Stemphylium</i> spp.	TX
18/1/2017	0/10	4/10	0/10	<i>Stemphylium</i> spp.	TX
18/1/2017	0/10	5/10	0/10	<i>Stemphylium</i> spp. (and <i>M. verrucaria</i> [5/10]) ^z	TX
31/1/2017	0/10	10/10	0/10	<i>Stemphylium</i> spp.	TX
31/1/2017	0/10	10/10	0/10	<i>Stemphylium</i> spp.	TX
31/1/2017	0/10	5/10	0/10	<i>Stemphylium</i> spp.	TX
8/3/2017	0/50	50/50	0/50	<i>Stemphylium</i> spp.	TX
22/3/2017	0/50	48/50	0/50	<i>Stemphylium</i> spp.	TX
20/12/2017	0/50	31/50	0/50	<i>Stemphylium</i> spp.	TX
20/12/2017	0/50	32/50	0/50	<i>Stemphylium</i> spp.	TX
20/12/2017	0/50	33/50	3/50	<i>Stemphylium</i> spp.	TX
18/1/2018	0/50	25/50	0/50	<i>Stemphylium</i> spp.	TX
24/1/2017	0/20	20/20	0/20	<i>Stemphylium</i> spp.	AZ
17/11/2017	0/50	50/50	0/50	<i>Stemphylium</i> spp.	CA
29/11/2017	0/50	50/50	0/50	<i>Stemphylium</i> spp.	CA
30/11/2017	0/50	50/50	0/50	<i>Stemphylium</i> spp.	CA
6/3/2018	0/20	20/20	0/20	<i>Stemphylium</i> spp.	AZ
15/3/2018	0/10	10/10	0/20	<i>Stemphylium</i> spp.	CA
13/5/2018	0/20	20/20	0/20	<i>Stemphylium</i> spp.	AZ
9/10/2018	0/10	10/10	0/10	<i>Stemphylium</i> spp.	CA
25/10/2018	0/10	10/10	0/10	<i>Stemphylium</i> spp.	CA
8/11/2018	0/10	10/10	0/10	<i>Stemphylium</i> spp.	AZ
15/11/2018	0/10	10/10	0/10	<i>Stemphylium</i> spp.	CA
19/10/2018	0/30	20/30	0/10	<i>Stemphylium</i> spp.	SC
23/10/2018	0/30	22/30	0/30	<i>Stemphylium</i> spp.	SC
28/11/2016	0/50	0/50	31/50	<i>Cercospora beticola</i>	TX
30/11/2016	0/20	0/20	12/20	<i>C. beticola</i>	TX
12/12/2016	0/10	0/10	7/10	<i>C. beticola</i>	TX
31/11/2017	0/50	0/50	50/50	<i>C. beticola</i>	TX
28/11/2016	8/50	39/50	0/50	<i>C. spinaciae</i> and <i>Stemphylium</i> spp.	TX
29/11/2016	3/50	5/50	37/50	<i>C. spinaciae</i> , <i>Stemphylium</i> spp. and <i>C. beticola</i>	TX
18/1/2017	10/50	35/50	0/50	<i>C. spinaciae</i> and <i>Stemphylium</i> spp.	TX
28/2/2017	25/50	18/50	0/50	<i>C. spinaciae</i> and <i>Stemphylium</i> spp.	TX
8/3/2017	26/50	24/50	0/50	<i>C. spinaciae</i> and <i>Stemphylium</i> spp.	TX
22/3/2017	10/50	18/50	0/50	<i>C. spinaciae</i> and <i>Stemphylium</i> spp.	TX
22/3/2017	10/50	18/50	0/50	<i>C. spinaciae</i> and <i>Stemphylium</i> spp.	TX
19/12/2017	9/50	6/50	0/50	<i>C. spinaciae</i> and <i>Stemphylium</i> spp.	TX
19/12/2017	9/50	8/50	0/50	<i>C. spinaciae</i> and <i>Stemphylium</i> spp.	TX
30/11/2016	6/10	0/10	3/10	<i>C. spinaciae</i> and <i>C. beticola</i>	TX

^u Fifty lesions were evaluated for each sample, except for samples with a limited number of leaves, for which 10 to 20 lesions were evaluated. In total, 74 samples were processed, of which 11 samples had leaf spots attributed to an abiotic disorder because either no microorganisms were isolated from the lesions or the microbes isolated were not pathogenic to spinach (latter are not included in this table). Dates are given in day/month/year format.

^v Number of lesions confirmed/number of lesions evaluated.

^w *Colletotrichum coccodes* was also isolated from this sample.

^x *Colletotrichum truncatum* was also isolated from this sample.

^y *Stemphylium* spp. were *S. vesicarium*, *S. beticola*, or a mixture of both.

^z *Myrothecium verrucaria* was also isolated from this sample. In addition, isolates of this species were obtained from a spinach leaf sample collected on 7 December 2016 in Texas, which is not listed in this table.

Gun (Model 250). Tween 20 (0.01%) was added to the conidial suspension and the water control treatment.

Bacteria were isolated from spinach leaves from 10 samples from Texas. A total of nine isolates from four locations were tested for pathogenicity on the spinach cultivar Viroflay. The nine isolates included three isolates of *Pseudomonas fluorescens* (white to transparent colony on TSA), three isolates of *Pantoea vagans* (red colony on TSA), and three isolates of *Erwinia persicina* (yellow colony on TSA). The leaves were wounded with an autoclaved needle prior to the inoculations by punching 10 tiny holes in each spinach leaf. Plants were inoculated with the relevant bacterial suspension (1×10^8 CFU/ml), which was quantified using a hemocytometer (Propper Lumicyte Improved Neubauer), and noninoculated control plants were sprayed with distilled water and subjected to the same conditions. Eighteen pots of plants were sprayed with a total volume of 50 ml of inoculum using a Badger Basic Spray Gun (Model 250).

Tween 20 (0.01%) was added to the bacterial cell suspension and the water control treatment in one experiment, but Tween 20 was not added to the bacterial suspension and the water control treatment in another experiment.

All inoculated plants were incubated in a mist chamber (the mist chamber is shaded in ambient daylight) for 48 h at 24 to 25°C, and then they were moved to a greenhouse (20 to 30°C) to observe disease development. Foliar disease severity was recorded 5 to 14 days after inoculation. Inoculation tests were repeated. The pathogenicity tests with isolates of the two species of *Stemphylium* (Table 4) were done similarly to the tests conducted with multiple fungal genera (Table 3) as described above. However, in the latter tests, spinach plants were 40 days old when inoculated with each spore suspension (1×10^5 spores/ml) and were incubated for 48 h in the dark at 24 to 25°C in a dew chamber. Leaf spot severity was scored on a scale of 0 to 4 as follows: 0 = no symptoms on leaves; 1 = 1 to 25% leaf area

Table 2. *Colletotrichum* and *Stemphylium* isolates obtained from spinach leaf spot samples from Texas, Arizona, California, and South Carolina, and used for pathogenicity tests on spinach as well as phylogenetic analyses for species determination^x

Species	Isolate	Host	Geographical origin	Year isolated	Source of strain	Original designation	Reference	GenBank accession number			
								ITS rDNA	gapdh	cmdA	gapdh-I
<i>C. spinaciae</i>	Cd-1^y	Spinach	TX	2016	L. Stein	/ ^z	/	MK920415	/	/	MK920422
<i>C. spinaciae</i>	Cd-2	Spinach	TX	2016	L. Stein	/	/	MK920416	/	/	MK920423
<i>C. spinaciae</i>	Cd-3	Spinach	TX	2017	L. Stein	/	/	MK920417	/	/	MK920424
<i>C. spinaciae</i>	Cd-4	Spinach	TX	2017	L. Stein	/	/	MK920418	/	/	MK920425
<i>C. spinaciae</i>	Cd-5	Spinach	TX	2017	L. Stein	/	/	MK920419	/	/	MK920426
<i>C. spinaciae</i>	Cd-6	Spinach	TX	2017	L. Stein	/	/	MK920420	/	/	MK920427
<i>C. spinaciae</i>	JG13	Spinach	AR	1993	J. Correll	/	Correll et al. 1993	MK920421	/	/	MK920428
<i>S. vesicarium</i>	Sb-1-St001	Spinach	WA	2013	L. du Toit	St001	Hernandez-Perez and du Toit 2006	MK091749	MK868641	MK868613	/
<i>S. vesicarium</i>	Sb-2-St026	Spinach	OR	2013	L. du Toit	St026	Hernandez-Perez and du Toit 2006	MK091750	MK868642	MK868614	/
<i>S. vesicarium</i>	Sb-3-St033	Spinach	WA	2013	L. du Toit	St033	Hernandez-Perez and du Toit 2006	MK091751	MK868643	MK868615	/
<i>S. vesicarium</i>	A-St416	Spinach	AZ	2013	L. du Toit	St416	Hernandez-Perez and du Toit 2006	MK091740	MK868650	MK868622	/
<i>S. vesicarium</i>	B-St431	Spinach	TX	2013	L. du Toit	St431	Hernandez-Perez and du Toit 2006	MK091741	MK868651	MK868623	/
<i>S. vesicarium</i>	D-St432	Spinach	TX	2013	L. du Toit	St432	Hernandez-Perez and du Toit 2006	MK091743	MK868652	MK868624	/
<i>S. vesicarium</i>	E-St430	Spinach	TX	2013	L. du Toit	St430	Hernandez-Perez and du Toit 2006	MK091744	MK868653	MK868625	/
<i>S. vesicarium</i>	I-St428	Spinach	CA	2013	L. du Toit	St428	Hernandez-Perez and du Toit 2006	MK091748	MK868654	MK868626	/
<i>S. vesicarium</i>	TX-1	Spinach	TX	2016	L. Stein	/	/	MK091761	MK868644	MK868616	/
<i>S. vesicarium</i>	TX-2	Spinach	TX	2016	L. Stein	/	/	MK091762	MK868645	MK868617	/
<i>S. vesicarium</i>	TX-3	Spinach	TX	2016	L. Stein	/	/	MK091763	MK868646	MK868618	/
<i>S. vesicarium</i>	VSP05-42A1	Onion	WA	2005	L. du Toit	Stv001	/	MK091777	MK868647	MK868619	/
<i>S. vesicarium</i>	VSP05-43-1	Onion	WA	2005	L. du Toit	Stv009	/	MK091778	MK868648	MK868620	/
<i>S. vesicarium</i>	VSP06-67D	Onion	WA	2006	L. du Toit	Stv013	/	MK091779	MK868649	MK868621	/
<i>S. beticola</i>	Sb-4-St037	Spinach	WA	2009	L. du Toit	St037	Hernandez-Perez and du Toit 2006	MK091752	MK868655	MK868627	/
<i>S. beticola</i>	Sb-5-St030	Spinach	WA	2009	L. du Toit	St030	Hernandez-Perez and du Toit 2006	MK091753	MK868656	MK868628	/
<i>S. beticola</i>	Sb-6-St257	Spinach	AZ	2009	L. du Toit	St257	Hernandez-Perez and du Toit 2006	MK091754	MK868657	MK868629	/
<i>S. beticola</i>	CA-1	Spinach	CA	2001	L. du Toit	St006	Koike et al. 2001	MK091770	MK868662	MK868634	/
<i>S. beticola</i>	CA-2	Spinach	CA	2001	L. du Toit	St007	Koike et al. 2001	MK091771	MK868663	MK868635	/
<i>S. beticola</i>	CA-3	Spinach	CA	2001	L. du Toit	St008	Koike et al. 2001	MK091772	MK868664	MK868636	/
<i>S. beticola</i>	CA-4	Spinach	CA	2001	L. du Toit	St009	Koike et al. 2001	MK091773	MK868665	MK868637	/
<i>S. beticola</i>	CA-5	Spinach	CA	2001	L. du Toit	St010	Koike et al. 2001	MK091774	MK868666	MK868638	/
<i>S. beticola</i>	CA-6	Spinach	CA	2001	L. du Toit	St011	Koike et al. 2001	MK091775	MK868667	MK868639	/
<i>S. beticola</i>	CA-7	Spinach	CA	2001	L. du Toit	St012	Koike et al. 2001	MK091776	MK868668	MK868640	/
<i>S. beticola</i>	C-St004	Spinach	WA	2009	L. du Toit	St004	Hernandez-Perez and du Toit 2006	MK091742	MK868659	MK868631	/
<i>S. beticola</i>	F-St005	Spinach	WA	2009	L. du Toit	St005	Hernandez-Perez and du Toit 2006	MK091745	MK868661	MK868632	/
<i>S. beticola</i>	G-St406	Spinach	OR	2009	L. du Toit	St406	Hernandez-Perez and du Toit 2006	MK091746	MK868660	MK868633	/
<i>S. beticola</i>	TX-8	Spinach	TX	2016	L. Stein	/	/	MK091768	MK868658	MK868630	/

^x All the isolates listed were used for phylogenetic analyses to aid in species determination.

^y The isolates in bold font were used for pathogenicity tests on the spinach cultivar Viroflay.

^z / = no information available.

infected; 2 = 26 to 50% leaf area infected; 3 = 51 to 75% leaf area infected; and 4 = 76 to 100% leaf area infected.

Fungicide evaluation. The spinach cultivar Viroflay was grown in pots (10 seeds per pot) in a greenhouse as described above. Stands were thinned to three plants per pot. A virulent isolate of each of *C. spinaciae* (Cd-1) and *S. vesicarium* (TX-3) were used for the fungicide efficacy assay. The isolates were cultured on PDA for 14 days before collecting spores for inoculation. A total of 19 fungicides and biofungicides was examined. Water was used as a negative control treatment. The 19 fungicides evaluated, along with their respective properties, including active ingredient(s) and recommended commercial dose, are summarized in Table 5.

Thirty-day-old spinach plants were treated with the various fungicides 24 h prior to being inoculated. Plants were then inoculated with Cd-1 (*C. spinaciae*), TX-3 (*S. vesicarium*), or a mixture of an equal spore concentration and volume of both, incubated in a mist chamber for 48 h, and then moved to a greenhouse with the same conditions described previously. Foliar disease severity was recorded 5 to 14 days after inoculation using the disease rating scale of 0 to 4 described previously.

Experimental design and statistical analysis. The two pathogenicity tests and the fungicide trials were each set up in a randomized complete block design with three replications (pots) per treatment. Each experiment was repeated once. The scoring of disease severity for each plant was converted to a percentage value (midpoints, whereby 0 = 0%, 1 = 12.5%, 2 = 25%, 3 = 62.5%, and 4 = 87.5%). The percentage scores of the severity ratings were used for statistical analysis (Villarreal-Zaballos et al. 2012). All analyses were performed using Statistical Analysis Systems software (PC-SAS 8.0; SAS Institute, Cary, NC). Variances of the raw data for the repeat experiments of disease severity were compared using Levene's test for homogeneity under the general linear model procedure (PROC GLM) of SAS version 9.3 (SAS Institute). Variances of the two experiments were not statistically different for each test; therefore, the raw data for the two repeats of each experiment were combined for subsequent analysis. In addition, data were subjected to analyses of variance (ANOVA) and means comparisons using Tukey's multiple comparison test ($P = 0.05$) with PROC GLM of SAS with main effects of trials (experimental repeats), replications, and isolates or fungicides and the interaction of trials with isolates or fungicides.

Fig. 1. A, Maximum-parsimony (MP) tree based on combined ITS rDNA and *gapdh-I* sequences of *Colletotrichum* spp. obtained from leaf spots on spinach samples collected from Texas, Arizona, California, and South Carolina. MP tree scores were as follows: 653 total characters, tree length = 811, consistency index = 0.9470, and retention index = 0.9892. Bootstrap values are shown above tree branches. The scale bar represents 10 nucleotide substitutions. **B**, Maximum-likelihood tree based on combined ITS rDNA and *gapdh-I* sequences of *Colletotrichum* spp. obtained from leaf spots on spinach samples collected from Texas, Arizona, California, and South Carolina. JG13 is a spinach isolate from prior research (Correll et al. 1993; Liu et al. 2007). Isolates listed in bold font were collected in the United States from this study, and isolates without bold font were from the research of Damm et al. (2009).

Isolates and fungicides were considered fixed effects, whereas repeats of the trials, replications, and the interactions of trials with isolates or fungicides were considered random effects.

Results

Disease survey. In total, 74 spinach leaf spot samples were examined, from which fungal and/or bacterial colonies were recovered for 70 samples. Four of the 74 samples had symptoms that likely were caused by abiotic disorders, including spray damage, nutritional deficiencies, or weather-related stress (hail damage). Of the 70 samples from which isolates were recovered, a known fungal pathogen or a suspected fungal pathogen was recovered from 63 samples. Bacterial colonies were recovered from only 10 samples. Anthracnose (*Colletotrichum spinaciae*) and Stemphylium leaf spot (*Stemphylium* spp.)

were the most common diseases identified, with each genus recovered from 28 (44%) and 39 (62%) of the 63 samples, respectively (Tables 1 and 6). Additional suspected fungal pathogens were recovered from leaf spot lesions at a limited frequency, including five (8%) of *Cercospora* leaf spot (*Cercospora* spp.), four (6%) of anthracnose (*C. coccodes* or *C. truncatum*), and two (3%) of *Myrothecium* leaf spot (*M. verrucaria*) (Table 6).

Fungal and bacterial isolations, and morphological and molecular species identification. The following pathogenic fungi were isolated and identified from spinach leaves: *C. spinaciae*, *C. coccodes*, and *C. truncatum*; *S. vesicarium* and *S. beticola*; *C. beticola*; and *M. verrucaria*. The following nonpathogenic species were identified based on a pathogenicity test of three representative isolates of each of the species: *Cercospora citrullina* (n = no. of isolates recovered:

Fig. 2. A. Maximum-parsimony (MP) tree based on combined ITS rDNA, *gapdh*, and *cmdA* sequences of *Stemphylium* spp. obtained from leaf spots on spinach samples collected from Texas, Arizona, California, and South Carolina. MP tree scores were as follows: 1,799 total characters, tree length = 4,759, consistency index = 0.7651, and retention index = 0.9694. Bootstrap values are shown above tree branches. The scale bar represents 100 nucleotide substitutions. **B.** Maximum-likelihood tree based on combined ITS rDNA, *gapdh*, and *cmdA* sequences of *Stemphylium* spp. obtained from leaf spots on spinach samples collected from Texas, Arizona, California, and South Carolina. Isolates listed in bold font were collected in the United States from this study, and isolates without bold font were from the research of Woudenberg et al. (2017).

n = 3) and *C. apii* (*n* = 4); *Nigrospora oryzae* (*n* = 22) and *N. spheerica* (*n* = 3); *Phoma eupyrena* (*n* = 3); *Alternaria alternata* (*n* = 26) and *A. porri* (*n* = 4); *Cladosporium cladosporioides* (*n* = 25), *C. oxysporum* (*n* = 3), and *C. tenuissimum* (*n* = 3); *Fusarium solani* (*n* = 3) and *F. oxysporum* (*n* = 3); and *Verticillium* spp. (*n* = 3). Molecular identification was based on ITS rDNA >99.0% sequence similarity to ITS rDNA sequences deposited in GenBank for both pathogenic and non-pathogenic fungal species. Molecular identification for *C. spinaciae*, *C. coccodes*, and *C. truncatum* was also based on *GS-I* and *gapdh-I* >99.0% sequence similarity to *GS-I* and *gapdh-I* sequences deposited in GenBank. Moreover, molecular identification for *S. vesicarium* and *S. beticola* was based on *gapdh* and *cmdA* >99.0% sequence similarity to *gapdh* and *cmdA* sequences deposited in GenBank. Six isolates were identified as *C. spinaciae*, three isolates as *C. coccodes*, and three isolates as *C. truncatum*. Identification for *S. vesicarium* and *S. beticola* was based on 14 isolates. The identifications for the rest of the species were based on sequence analysis for three representative isolates.

Bacteria were isolated from spinach leaves from 10 samples from Texas with molecular identification to genus and species based on 16S rRNA >99.0% similarity to 16S rRNA sequences deposited in GenBank. Representative isolates of each of the following species were identified: *Pseudomonas fluorescens* (19 isolates), *Pantoea vagans* (23 isolates), and *Erwinia persicina* (23 isolates). These bacterial isolates were not pathogenic on spinach leaves (*data not shown*).

C. spinaciae. The isolates of *Colletotrichum* identified as *C. spinaciae* (Table 2) produced dark gray-olivaceous to olivaceous-black colonies with a white margin on PDA. Conidia were hyaline, smooth-walled, aseptate, minimally curved with both sides of the conidia gradually tapering toward the round apex and round or truncate base, and were 18 to 21 × 3.5 to 4 μm. These morphological characteristics were consistent with the description of *C. spinaciae* (Damm et al. 2009).

Phylogenetic analysis of *Colletotrichum* spp. MP, ML, NJ, and Bayesian analyses produced similar statistically supported clades and tree topologies for each of the DNA regions sequenced and for the combined dataset from the ITS rDNA and *gapdh-I* for the *Colletotrichum* isolates. Therefore, only the combined phylogenetic trees (MP and ML) are shown (Fig. 1). A phylogenetic tree based on the Bayesian analyses is provided as supplementary material (Supplementary Fig. 1). Phylogenetic analysis with the two combined

sequences showed that the spinach *Colletotrichum* isolates from Texas, isolate JG13 from Arkansas, and *Colletotrichum* isolates from the Netherlands (Damm et al. 2009) were in the same clade as *C. spinaciae* with high bootstrap value support and phylogenetic indices (Table 2; Fig. 1). The clade with *C. spinaciae* was separated distinctly from those with other closely related *Colletotrichum* species, including *C. circinans* and *C. dematium* (Fig. 1).

Stemphylium species. The isolates of *Stemphylium* identified as *S. vesicarium* (Table 2) produced dense aerial mycelium that was blackish to green-brown in color and formed conidia by 14 days on PDA under fluorescent lights. Conidia were oblong, rounded at the ends or broadly ellipsoidal, pale to medium brown, verrucose, with up to six transverse and several longitudinal septa, often constricted at the three major transverse septa, and 22 to 36 × 16 to 24 μm. The morphological characteristics were consistent with the description of *S. vesicarium* (Ellis 1971; Woudenberg et al. 2017).

The isolates of *Stemphylium* identified as *S. beticola* (Table 2) produced dense aerial mycelium that was greenish in color and formed conidia by 14 days on PDA under lights. Conidia were solitary, pale olive brown, verrucose, ellipsoid to cylindrical, and 20 to 30 × 10 to 20 μm, with two to four transverse septa and one to three longitudinal or oblique septa per transverse septum. The morphological characteristics were consistent with the description of *S. beticola* (Woudenberg et al. 2017).

BLAST searches showed that the *Stemphylium* isolates collected in this study, and isolates from earlier studies (du Toit and Derie 2001; Koike et al. 2001) in the United States, belonged to two species, *S. vesicarium* and *S. beticola*. The ITS rDNA, *gapdh*, and *cmdA* sequences of isolates from Texas (TX-1 to TX3, E-St430, D-St432, and B-St431), Washington (Sb-3-St033 and Sb-1-St001), Oregon (Sb-2-St026), and Arizona (A-St416) had 99 to 100% similarity to *S. vesicarium* sequences (KU850555 [ITS rDNA], KU850702 [*gapdh*], and KU850845 [*cmdA*], respectively) deposited in GenBank by Woudenberg et al. (2017) (Table 2). Other isolates from Texas (TX-8), California (CA-1 to CA-7), Washington (C-St004, F-St005, Sb-5-St030, and Sb-4-St037), and Oregon (Sb-6-St257) had 99 to 100% similarity to *S. beticola* sequences (KU850519 [ITS rDNA], KU850666 [*gapdh*], and KU850807 [*cmdA*], respectively) deposited in GenBank by Woudenberg et al. (2017) (Table 2).

MP, ML, NJ, and Bayesian analyses for *Stemphylium* isolates produced similar, statistically supported clades and tree topologies for

Table 3. Mean disease severity caused by various pathogenic fungi recovered from leaf spots on spinach samples in the United States^x

Species	Isolate	Geographic origin	Year collected	Mean disease severity ^y
<i>Colletotrichum spinaciae</i>	Cd-1	TX	2016	4.0 ± 0.2 a
<i>C. spinaciae</i>	Cd-2	TX	2016	4.0 ± 0.2 a
<i>C. spinaciae</i>	Cd-3	TX	2017	4.0 ± 0.2 a
<i>Stemphylium vesicarium</i>	TX-1	TX	2016	3.2 ± 0.2 b
<i>S. vesicarium</i>	TX-2	TX	2016	3.1 ± 0.2 b
<i>S. vesicarium</i>	TX-3	TX	2016	3.4 ± 0.2 b
<i>Cercospora beticola</i>	Cb-1	TX	2016	2.2 ± 0.2 c
<i>C. beticola</i>	Cb-2	TX	2016	2.1 ± 0.2 c
<i>C. beticola</i>	Cb-3	TX	2016	2.2 ± 0.2 c
Water control				0 d
LSD				0.28
Factor	df	<i>Pr</i> > <i>F</i> ^z		
Repeat	1	0.5599		
Isolate	9	<0.0001		
Isolate × repeat	10	0.9995		

^x Disease scale: 0 = no symptoms on leaves; 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; and 4 = 76 to 100% leaf area symptomatic. Three 30-day-old plants in each of three replicate pots were inoculated with a spore suspension of 1 × 10⁶ spores/ml, incubated at 24 to 25°C in a mist chamber for 48 h, and then moved to a greenhouse for observation. Final disease severity was recorded 14 days after inoculation. The midpoint percentage of the disease severity was used for statistical comparison. Data from repeated experiments were not significantly different based on a test of homogeneity of variances (Levene's test). Therefore, the data were combined across repeats for statistical analyses.

^y Mean disease severity ± standard deviations for leaf spot from two repeats of the experiment. Leaf spot severity was rated 14 days after inoculation. Means with different letters were significantly different at *P* < 0.05 based on Fisher's protected least significant difference (LSD).

^z Significance level (*P*) from the analysis of variance for disease severity, with the main effects of repeats of the experiment and isolates of the fungus, as well as the interaction between the two main effects.

each DNA region and for the combined dataset from three DNA regions (ITS rDNA, *gapdh*, and *cmdA*). Therefore, only the combined MP and ML phylogenetic trees are shown (Fig. 2). The phylogenetic tree based on Bayesian analyses is in the supplementary materials (Supplementary Fig. 2). Several distinct molecular clades were identified among the isolates of *Stemphylium* analyzed, with two clades of *Stemphylium* identified from spinach in the United States with high bootstrap value support and phylogenetic indices (Fig. 2). Isolates of *Stemphylium* from spinach, including isolates from Texas (TX-1 to TX-3, E-St430, D-St432, and B-St431), Washington (Sb-3-St033 and Sb-1-St001), Oregon (Sb-2-St026), and Arizona (A-St416), clustered with concatenated sequences of *S. vesicarium* deposited in GenBank by Woudenberg et al. (2017), indicating these isolates belong to *S. vesicarium*. Other isolates of *Stemphylium* spp., including the isolates from spinach in Texas (TX-8), California (CA-1 to CA-7), Washington (C-St004, F-St005, Sb-5-St030, and Sb-4-St037), and Oregon (Sb-6-St257), clustered consistently with the concatenated sequences of *S. beticola* deposited in GenBank by Woudenberg et al. (2017), indicating these isolates belong to *S. beticola* (Fig. 2).

Pathogenicity test for various fungi. Based on the ANOVA, there were no significant differences in disease severity between the two repeats of this experiment ($P = 0.5599$), and there was no significant isolate $\times$ repeat experiment interaction on disease severity ($P = 0.9995$). However, there was a significant main effect of isolates on disease severity ($P < 0.0001$) (Table 3). The anthracnose pathogen, *C. spinaciae*, caused significant leaf damage with 76 to 100% of the inoculated leaves (mean severity category of 4.0). Isolates of *S. vesicarium* also caused severe damage to inoculated spinach leaves, with 56 to 75% lesion coverage of leaves (mean severity category of 3.1 to 3.4). The isolates of *C. beticola* caused intermediate

severity, with 26 to 50% lesion coverage of leaves (mean severity category of 2.0) (Table 3). Disease severity was significantly greater on plants inoculated with *C. spinaciae* (mean severity category of 4.0) than *S. vesicarium* (3.1 to 3.4) (Table 3).

Pathogenicity test for *Stemphylium* spp. Isolates of *S. vesicarium* and *S. beticola* from this research and from earlier research caused leaf spots on spinach leaves of the cultivar Viroflay (Table 4). There was no significant main effect on disease severity of the two repeats of the experiment ($P = 0.1682$), and there was no significant isolate $\times$ repeat trial interaction effect ($P = 0.1724$). However, there was a significant main effect of isolates on disease severity ($P < 0.001$) (Table 4). Isolate Sb-1-St001 was the most virulent among the isolates of *S. vesicarium*, causing 75 to 100% of the inoculated leaf area to develop leaf spots 14 days after inoculation (mean severity category of 3.3). The other isolates of *S. vesicarium* caused intermediate leaf spot severity with 26 to 50% leaf area covered (mean severity category of 1.0 to 2.5). Isolates Sb-5-St030 and Sb-6-St257 were the most virulent among the isolates of *S. beticola*, causing 75 to 100% disease severity 14 days after inoculation (mean severity category of 3.8). The other isolates of *S. beticola* caused intermediate leaf damage of 26 to 50% (mean severity category of 1.5 to 2.5) (Table 4). In addition, the isolates of *S. vesicarium* from onion also caused leaf spots on spinach, but the damage was limited (mean severity category of 1.0 to 1.8) (Table 4). There were differences in lesion type among isolates tested on Viroflay. All isolates of *S. vesicarium* caused smaller, necrotic lesions that did not form brown ring(s) within the lesions, but most of the isolates of *S. beticola* caused spots that developed brown ring(s) within the lesions, except Sb-5-St030 and CA-1 (Table 4).

Table 4. Mean severity of leaf spot on the spinach cultivar Viroflay caused by *Stemphylium* spp. that were recovered from spinach samples from listed states and previous work published^w

Species	Isolates	Host	Geographical origin	Mean disease severity ^x	Presence of a brown ring inside the lesions	
					Test 1	Test 2
<i>S. vesicarium</i>	Sb-1-St001	Spinach	WA	3.3 $\pm$ 0.2 b	No	No
<i>S. vesicarium</i>	Sb-2-St026	Spinach	OR	2.3 $\pm$ 0.2 dc	No	No
<i>S. vesicarium</i>	Sb-3-St033	Spinach	WA	2.2 $\pm$ 0.2 dc	No	No
<i>S. vesicarium</i>	TX-3	Spinach	TX	2.5 $\pm$ 0.2 c	No	No
<i>S. vesicarium</i>	VSP05-42A1	Onion	WA	1.0 $\pm$ 0.2 h	No	No
<i>S. vesicarium</i>	VSP05-43-1	Onion	WA	1.8 $\pm$ 0.2 efg	No	No
<i>S. vesicarium</i>	VSP06-67D	Onion	WA	1.8 $\pm$ 0.2 efg	No	No
<i>S. beticola</i>	Sb-5-St030	Spinach	WA	3.8 $\pm$ 0.2 a	No ^y	No ^y
<i>S. beticola</i>	Sb-6-St257	Spinach	AZ	3.8 $\pm$ 0.2 a	Yes	Yes
<i>S. beticola</i>	CA-1	Spinach	CA	1.8 $\pm$ 0.2 efg	Yes	No ^y
<i>S. beticola</i>	CA-2	Spinach	CA	1.7 $\pm$ 0.2 fg	Yes	Yes
<i>S. beticola</i>	CA-3	Spinach	CA	1.5 $\pm$ 0.2 g	Yes	Yes
<i>S. beticola</i>	CA-4	Spinach	CA	1.8 $\pm$ 0.2 efg	Yes	Yes
<i>S. beticola</i>	CA-5	Spinach	CA	1.7 $\pm$ 0.2 fg	Yes	Yes
<i>S. beticola</i>	CA-6	Spinach	CA	1.8 $\pm$ 0.2 efg	Yes	Yes
<i>S. beticola</i>	CA-7	Spinach	CA	2.0 $\pm$ 0.2 cde	Yes	Yes
<i>S. beticola</i>	TX-8	Spinach	TX	2.5 $\pm$ 0.2 c	Yes	Yes
Water control				0 i		
LSD				0.49		
Factor	df	$Pr > F^z$				
Repeat	1	0.1682				
Isolate	17	<0.0001				
Isolate $\times$ repeat	36	0.1724				

^w Disease scale: 0 = no symptoms on leaves; 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; and 4 = 76 to 100% leaf infected. Three 40-day-old plants in each of three replicate pots were inoculated with a spore suspension of 1×10^5 spores/ml, incubated at 24 to 25°C in a dew chamber for 48 h, and then moved to a greenhouse for observation. Symptoms appeared 5 days after inoculation; final disease severity was recorded 14 days after inoculation. The midpoint percentage of the disease severity was used for statistical comparison. Data from repeated experiments were not significantly different based on a test of homogeneity of variances based on Levene's test. Therefore, the data were combined across repeats of the experiment for statistical analyses.

^x Mean disease severity $\pm$ standard deviations for leaf spot of two repeat experiments rated at 14 days after inoculation. Different letters indicate treatments were significantly different at $P < 0.05$ based on Fisher's protected least significant difference (LSD).

^y All *S. beticola* isolates except Sb-5-St030 (first and second tests) and CA-2 (second test) had brown ring(s) inside of the lesions.

^z Significance level (P) from analysis of variance for disease severity with a repeated experiment.

Fungicide efficacy. Isolate Cd-1 of *C. spinaciae* and TX-3 of *S. vesicarium*, and the combination of the two pathogens, caused severe leaf spotting on Viroflay in the fungicide efficacy trial (4.0 ± 0.1 , 2.0 ± 0.1 , and 4.0 ± 0.1 severity categories (76 to 100, 26 to 50, and 76 to 100% leaf damage, respectively) for the control plants with no fungicide treatment. There was no significant effect on disease severity for the main effect of repeated experiments ($P = 0.1345$), and there was no significant interaction effect of fungicide $\times$ repeat experiment on disease severity ($P = 0.1593$). However, there was a significant fungicide main effect ($P < 0.0001$) (Tables 5 and 7). Bravo WeatherStik (chlorothalonil), Dithane F-45 (mancozeb), Cabrio (pyraclostrobin), and Merivon (fluxapyroxad and pyraclostrobin) were completely effective at preventing leaf spot symptoms from developing on Viroflay plants inoculated with *C. spinaciae*, *S. vesicarium*, or the combination of both pathogens (Tables 5 and 7). Switch (fludioxonil and cyprodinil), Fontelis (penthiopyrad), ProPhyt and Presidio (potassium phosphate and fluopicolide), and Revus (mandipropamid) were also highly effective at reducing disease development on plants inoculated with *C. spinaciae*, *S. vesicarium*, or the combination of both pathogens. Lesions were observed but were reduced in severity (0.8 ± 0.1 to 1.2 ± 0.1 , 0.7 ± 0.1 to 1.0 ± 0.1 , and 0.8 ± 0.1 to 1.2 ± 0.1 , respectively) for these treatments compared with the control plants. The remaining fungicides were either ineffective or weakly effective at reducing disease severity. However, Top Guard EQ (flutriafol and azoxystrobin), Fontelis (penthiopyrad), Serenade (*Bacillus subtilis*), Double Nickel (*Bacillus amyloliquefaciens*), and Quadris (azoxystrobin) were highly effective at reducing disease severity on plants inoculated with *S. vesicarium* compared with control plants (Tables 5 and 7).

Discussion

Of the 74 spinach leaf spot samples examined in this study from four states in the United States from 2016 to 2018, 63 had lesions that were attributed to one of several fungal pathogens. The remaining 11 samples were attributed to abiotic disorders because either no microorganisms were isolated from the lesions or the microbes isolated were not pathogenic to spinach. None of the bacterial isolates recovered was pathogenic to spinach. Stemphylium leaf spot, caused by *S. vesicarium* or *S. beticola*, and anthracnose, caused by *C. spinaciae*,

were the most common diseases observed on the spinach samples. Stemphylium leaf spot was found on 26 of 49 samples from Texas and on all samples collected in California, Arizona, and South Carolina. Anthracnose was found on 28 of 49 samples from spinach plants in Texas but not on the samples from the three other states.

Anthracnose caused by *C. spinaciae* was the predominant disease observed from the leaf spot samples recovered from Texas, with 28 of 49 of the leaf samples (57%) having lesions attributed to *C. spinaciae*. Based on both morphological and molecular characterization, the isolates fit the description of *C. spinaciae* (Damm et al. 2009), which previously was classified as *C. dematium* f. sp. *spinaciae* (Ellis 1971). Two additional *Colletotrichum* spp. were recovered from samples from Texas, including *C. coccodes* from two fields and *C. truncatum* from two fields. Pathogenicity tests showed that isolates of *C. spinaciae*, *C. coccodes*, and *C. truncatum* were all pathogenic on the spinach cultivar Viroflay and caused significant damage to leaves. The anthracnose pathogens *C. coccodes* and *C. truncatum* have not been reported previously as causing a leaf spot of spinach in the United States and, thus, detailed characterization, pathogenicity tests, and fungicide efficacy assays are in progress. Anthracnose caused by *C. spinaciae* is widespread in spinach production areas of the United States (Correll et al. 1994; Koike and Correll 1993; Kurt et al. 2016; B. Liu et al., unpublished data). *C. spinaciae* has a wide host range, including *Medicago sativa*, *Portulaca oleracea*, *Chenopodium album* (Damm et al. 2009), and *Vicia sativa* (Wang et al. 2019). Thus, other hosts could serve as a potential source of inoculum for spinach.

The original reports of anthracnose of spinach were attributed to *C. dematium* (Pers.) Grove f. sp. *spinaciae* (Koike and Correll 1993). However, based on a reclassification by Damm et al. (2009), the taxonomic identity of the pathogen recovered from spinach is *C. spinaciae*. Examination of isolates obtained in this study, based on ITS rDNA and *gapdh*-I sequences, was consistent with the identity of the predominant isolates from spinach being *C. spinaciae*.

Stemphylium leaf spot (*S. vesicarium* or *S. beticola*) was also common in this survey, having been isolated from 39 of the 63 (62%) spinach samples examined from Arizona, California, South Carolina,

Table 5. Fungicides evaluated for control of *Colletotrichum spinaciae* and *Stemphylium vesicarium* inoculated on the spinach cultivar Viroflay

Product	FRAC codes ^w	Active ingredients	Recommended dose (ml/0.40 ha) ^x	Dose used (ml/50 ml) ^y
Bravo WeatherStik	M5	Chlorothalonil	947.2	0.83
Dithane F-45	M3	Mancozeb	1,894.4	1.66
Cabrio	11	Pyraclostrobin	453.6	0.40 g
Merivon	7 + 11	Fluxapyroxad + pyraclostrobin	324.5	0.29
Top Guard EQ	3 + 11	Flutriafol + azoxystrobin	236.8	0.21
Switch	12 + 9	Fludioxonil + cyprodinil	396.9	0.35 g
Fontelis	7	Penthiopyrad	708.0	0.63
ProPhyt + Presidio	/ ^z + 43	Potassium phosphate + fluopicolide	2,131.2 + 88.8	1.88 + 0.08
Revus	40	Mandipropamid	236.6	0.21
Trilogy	2 + 3 + 11	Iprodione + triticonazole + trifloxystrobin	3,788.8	3.33
Serenade	Biological	<i>Bacillus subtilis</i>	3,788.8	3.33
Double Nickel	Biological	<i>Bacillus amyloliquefaciens</i>	1,894.4	1.66
PurGrow 100%	/	Hypochlorous acid	/	50.0
PurGrow 10%	/	Hypochlorous acid	/	50.0
Tanos	27 + 11	Cymoxanil + famoxadone	296.0	0.26
Quadris	11	Azoxystrobin	457.25	0.40
Cueva	M1	Copper octanoate	3,788.8	3.33
Ridomil Gold	4	Mefenoxam	354.0	0.31
Luna	7 + 11	Fluopyram + trifloxystrobin	224.96	0.20
Water	/	Water	/	50.0

^w Fungicide Resistance Action Committee (FRAC) group. Products in the same FRAC group have the same or similar mode of action, with potential for pathogens to develop cross resistance to those products (except for products with the letter "M", which have multiple modes of action with no record of fungicide resistance developing in pathogen populations).

^x Recommended dose (fungicide label) based on applying the product in water at the rate of 15 gallons/acre, which is equal to 142.5 liters/ha.

^y Dose determined based on the volume (ml) of fungicide by volume (ml) of carrier (water). Two doses are specifically listed in grams.

^z / = no information available.

and Texas. *Stemphylium* leaf spot was first reported in California (Koike et al. 2001) and subsequently has been found in Arizona (Koike et al. 2005), Washington (du Toit and Derie 2001), Texas (Reed et al. 2010), Florida (Raid 2004), and Maryland and Delaware (Everts and Armentrout 2001). However, *Stemphylium* spp. found in this survey and isolates re-examined in this study from earlier research (Hernandez-Perez and du Toit 2006; Koike et al. 2001) were verified as two species, *S. vesicarium* and *S. beticola*, based on symptoms, conidial morphology, and DNA sequences. Pathogenicity tests showed that both species were pathogenic on spinach and caused significant damage to spinach leaves. This was consistent with an earlier report that leaf spot of spinach was caused by *Stemphylium* sp. subgroups C2 and E3. Based on the symptoms and conidial morphology, we suspect that subgroup C2 was likely to be *S. vesicarium* and subgroup E3 was likely to be *S. beticola* (Misawa et al. 2017). Moreover, field observations of the typical symptoms caused by *S. beticola* showed that one or more brown ring(s) was often observed inside the lesions, but not for leaf spots caused by isolates of *S. vesicarium* (personal observation, Correll's lab members). Based on the presence of distinct brown ring(s) within the well-developed lesions in field visits, leaf spots caused by *S. beticola* (with the presence of a brown ring) or *S. vesicarium* (absence of a brown ring) can sometimes be distinguished on leaves. These symptoms were consistent with greenhouse trial observations (Table 4), in which 45-day-old plants inoculated with isolates of *S. vesicarium* produced lesions that did not develop one or more brown ring(s) inside the lesions, whereas the majority of isolates of *S. beticola* caused leaf spots that each developed one or more brown ring(s) within the lesions. However, this distinction was much less evident on young spinach leaves.

The conidial length of *S. vesicarium* isolates (22 to 36 µm) was generally greater than that of *S. beticola* (20 to 30 µm), and the conidial width of *S. vesicarium* isolates (16 to 24 µm) was generally greater than that of *S. beticola* (10 to 20 µm), which can be used to differentiate the two species. However, conditions under which the conidia are produced can affect conidial size (Correll lab members, personal observation). In addition, for some spinach leaf samples, both species were recovered from a given leaf sample, suggesting mixed infections by *S. vesicarium* and *S. beticola* can occur.

Phylogenetic analyses based on multilocus sequence analysis of three DNA regions (ITS rDNA, *gapdh*, and *cmdA*) showed that *Stemphylium* species associated with spinach leaf spots from this survey, and from earlier work, were caused by two species, *S. vesicarium* and *S. beticola*. The isolates identified as *S. vesicarium* clustered with *S. vesicarium* with 99 to 100% similarity for the three DNA sequences of *S. vesicarium* deposited in GenBank by Woudenberg et al. (2017). Likewise, the isolates identified as *S. beticola* clustered with *S. beticola* with 99 to 100% similarity to the three DNA sequences of *S. beticola* deposited in GenBank by Woudenberg et al. (2017).

Compared with the leaf spot diseases caused by *C. spinaciae*, *S. vesicarium*, and *S. beticola*, *Cercospora* leaf spot was not common in this survey, because only 8% of the symptomatic leaf samples were demonstrated to be caused by *C. beticola*. Pathogenicity tests

also indicated that *C. beticola* was less virulent than *C. spinaciae*, *S. vesicarium*, and *S. beticola* under the conditions evaluated. Several new leaf spot pathogens were identified from the samples examined, but at limited frequencies. This included *Myrothecium* leaf spot caused by *M. verrucaria*, which was previously reported in Italy (Garibaldi et al. 2016; Matic et al. 2019), and anthracnose caused by *C. truncatum* and *C. coccodes*. Detailed characterization, pathogenicity tests, and evaluation of fungicide efficacy against these pathogens are in progress.

Other fungal species isolated from the spinach leaves included *S. solani*, *C. citrullina*, *C. apii*, *N. oryzae*, *N. sphaerica*, and *P. eupyrena*. These fungi did not cause lesions on Viroflay leaves, even when inoculated at 1×10^6 conidia/ml. However, these isolates did cause some symptoms on spinach leaves when inoculated using PDA plugs colonized with mycelia (data not shown). A number of other fungal and bacterial isolates recovered from the spinach leaves, including *A. alternata*, *A. porri*, *C. cladosporioides*, *C. oxysporum*, *C. tenuisimum*, *F. solani*, *F. oxysporum*, *Verticillium* spp., *P. fluorescens*, *P. vagans*, and *E. persicina*, did not cause lesions on leaves of Viroflay,

Table 7. Mean disease severity of spinach leaf spot on the cultivar Viroflay treated with different fungicides prior to inoculation with *Colletotrichum spinaciae*, *Stemphylium vesicarium*, or a combination of the two pathogens^x

Fungicide	Mean disease severity ^y		
	<i>C. spinaciae</i> (Cd-1)	<i>S. vesicarium</i> (TX-3)	<i>C. spinaciae</i> + <i>S. vesicarium</i> (Cd-1 + TX-3)
Bravo WeatherStik	0 a	0 a	0 a
Dithane F-45	0 a	0 a	0 a
Cabrio	0 a	0 a	0 a
Merivon	0 a	0 a	0 a
Top Guard EQ	0.8 ± 0.1 b	0.7 ± 0.1 b	0.8 ± 0.1 b
Switch	1.0 ± 0.1 b	1.0 ± 0.1 c	1.0 ± 0.1 b
Fontelis	1.0 ± 0.1 b	0.7 ± 0.1 b	1.0 ± 0.1 b
ProPhyt + Presidio	1.2 ± 0.1 c	1.0 ± 0.1 c	1.2 ± 0.1 c
Revus	1.0 ± 0.1 b	1.0 ± 0.1 c	1.2 ± 0.1 c
Trilogy	2.7 ± 0.1 e	1.0 ± 0.1 c	1.5 ± 0.1 d
Serenade	2.0 ± 0.1 d	0.5 ± 0.1 b	2.0 ± 0.1 e
Double Nickel	2.0 ± 0.1 d	0.7 ± 0.1 b	2.0 ± 0.1 e
PurGrow 100%	2.5 ± 0.1 e	1.0 ± 0.1 c	2.0 ± 0.1 e
PurGrow 10%	2.5 ± 0.1 e	1.2 ± 0.1 c	2.3 ± 0.1 f
Tanos	3.7 ± 0.1 g	1.0 ± 0.1 c	2.7 ± 0.1 g
Quadris	3.0 ± 0.1 f	0.7 ± 0.1 b	3.0 ± 0.1 h
Cueva	2.0 ± 0.1 d	1.0 ± 0.1 c	3.0 ± 0.1 h
Ridomil Gold	4.0 ± 0.1 h	1.0 ± 0.1 c	3.0 ± 0.1 h
Luna	3.0 ± 0.1 f	1.0 ± 0.1 c	3.0 ± 0.1 h
Water	4.0 ± 0.1 h	2.0 ± 0.1 d	4.0 ± 0.1 i
LSD	0.31	0.29	0.3
Factor	Pr > F ^z	Pr > F	Pr > F
Repeat	0.7496	0.5237	0.1345
Fungicide	<0.001	<0.001	<0.001
Fungicide × repeat	0.9967	0.8754	0.1593

^x Water was used as a negative control treatment; there were no symptoms on spinach leaves for control plants. Disease scale: 0 = no symptoms on leaves; 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; and 4 = 76 to 100% leaf area infected. Three 30-day-old plants in each of three replicate pots were inoculated with a spore suspension of 1×10^6 spores/ml. Inoculated plants were incubated at 24 to 25°C in a mist chamber for 48 h and then moved to a greenhouse for observation. Symptoms appeared 5 days after inoculation; final disease severity was recorded 14 days after inoculation. Data from repeated experiments were not significantly different based on a test of homogeneity of variance (Levene's test). Therefore, the data were combined across repeats for statistical analyses. The midpoint percentage of the disease severity was used for statistical comparison.

^y Mean disease severity ± standard deviations for leaf spot of two repeat experiments. Treatment means with different letters were significantly different at $P < 0.05$ based on Fisher's protected least significant difference (LSD).

^z Significance level (P) from analysis of variance for disease severity with a repeated experiment.

Table 6. Summary of predominant pathogens isolated from spinach leaf spot samples collected from four states in the United States from 2016 to 2018

Pathogen	Samples positive for pathogen/ total number of samples	Incidence (%)
<i>Colletotrichum spinaciae</i>	28/63 ^z	44
<i>Stemphylium vesicarium</i> and <i>S. beticola</i>	39/63	62
<i>Cercospora beticola</i>	5/63	8
<i>Colletotrichum coccodes</i> and <i>C. truncatum</i>	4/63	6
<i>Myrothecium verrucaria</i>	2/63	3

^z Samples identified with pathogens/total samples identified with pathogens.

even when using an elevated conidial concentration, PDA plugs, or a concentrated fungal spore or bacterial cell suspension.

Fungicides remain an important approach for the management of leaf spot diseases on spinach under conventional production conditions (Dainello and Morelock 2005; du Toit et al. 2007, 2018). Fungicide efficacy testing in this study showed that Bravo WeatherStik (chlorothalonil), Dithane F-45 (mancozeb), Cabrio (pyraclostrobin), and Merivon (fluxapyroxad and pyraclostrobin) were highly effective at preventing leaf spot symptoms caused by *C. spinaciae* and *S. vesicarium*, or the combination of both pathogens. Although the strobilurin fungicides (Fungicide Resistance Action Committee group 11) were demonstrated to be highly effective for the control of leaf spot diseases in this experiment, as is consistent with earlier reports (du Toit et al. 2004, 2005, 2007), a single isolate of each of *C. spinaciae* and *S. vesicarium* were evaluated in this study, so more isolates of these pathogens should be used for evaluating the efficacy of strobilurin fungicides. There have been reports that isolates of *S. vesicarium* from onion have developed resistance to azoxystrobin (Hay et al. 2019), and many isolates of *S. vesicarium* obtained from baby leaf spinach crops in Texas and Florida in 2018 to 2019 proved to be resistant to azoxystrobin in vitro (Spawton et al. 2019).

Stemphylium spp. can be seedborne and seed-transmitted in spinach (du Toit and Hernandez-Perez 2005; Hernandez-Perez and du Toit 2006). Early assays of spinach seed lots from the United States, Denmark, the Netherlands, and New Zealand showed that *Stemphylium* was present in all seed lots examined (Hernandez-Perez and du Toit 2006). *S. beticola* isolates (identified previously as *S. botryosum*) were demonstrated to be able to infect the pericarps and embryos of seeds (du Toit et al. 2018; Hernandez-Perez and du Toit 2006), which makes *Stemphylium*-infected seeds more recalcitrant to eradication of the pathogen by treatment with fungicides, chlorine, or hot water. Chlorine (1.2% NaOCl for 60 min) or hot water (50°C for ≤30 min or 55 or 60°C for ≤10 min) treatments of spinach seeds reduced the incidence of *Stemphylium* in spinach seeds without damaging seed germination, but could not eradicate this genus from the seeds. Hot water treatments at higher temperatures or longer durations damaged the embryos significantly (Hernandez-Perez and du Toit 2006).

Managing leaf spot pathogens in high-density spinach production will continue to be a challenge, particularly under organic production systems for which there are no highly effective organic fungicides available for disease management. Information on the identification of leaf spot pathogens present in various spinach production areas, the prevalence of the pathogens, their fungicide sensitivities, and sources of primary inoculum is valuable for managing these increasingly important pathogens of spinach.

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In the article “Characterization of Leaf Spot Pathogens from Several Spinach Production Areas in the United States” by Bo Liu et al., the GenBank accession numbers, isolate designation, and DNA sequences of *gapdh* and *cmdA* genes were not properly matched. The correct table is shown below:

Table 2. *Colletotrichum* and *Stemphylium* isolates obtained from spinach leaf spot samples from Texas, Arizona, California, and South Carolina, and used for pathogenicity tests on spinach as well as phylogenetic analyses for species determination^x

Species	Isolate	Host	Geographical origin	Year isolated	Source of strain	Original designation	Reference	GenBank accession number			
								ITS rDNA	<i>gapdh</i>	<i>cmdA</i>	<i>gapdh-I</i>
<i>C. spinaciae</i>	Cd-1^y	Spinach	TX	2016	L. Stein	/ ^z	/	MK920415	/	/	MK920422
<i>C. spinaciae</i>	Cd-2	Spinach	TX	2016	L. Stein	/	/	MK920416	/	/	MK920423
<i>C. spinaciae</i>	Cd-3	Spinach	TX	2017	L. Stein	/	/	MK920417	/	/	MK920424
<i>C. spinaciae</i>	Cd-4	Spinach	TX	2017	L. Stein	/	/	MK920418	/	/	MK920425
<i>C. spinaciae</i>	Cd-5	Spinach	TX	2017	L. Stein	/	/	MK920419	/	/	MK920426
<i>C. spinaciae</i>	Cd-6	Spinach	TX	2017	L. Stein	/	/	MK920420	/	/	MK920427
<i>C. spinaciae</i>	JG13	Spinach	AR	1993	J. Correll	/	Correll et al. 1993	MK920421	/	/	MK920428
<i>S. vesicarium</i>	Sb-1-St001	Spinach	WA	2013	L. du Toit	St001	Hernandez-Perez and du Toit 2006	MK091749	MK868641	MK868613	/
<i>S. vesicarium</i>	Sb-2-St026	Spinach	OR	2013	L. du Toit	St026	Hernandez-Perez and du Toit 2006	MK091750	MK868642	MK868614	/
<i>S. vesicarium</i>	Sb-3-St033	Spinach	WA	2013	L. du Toit	St033	Hernandez-Perez and du Toit 2006	MK091751	MK868643	MK868615	/
<i>S. vesicarium</i>	A-St416	Spinach	AZ	2013	L. du Toit	St416	Hernandez-Perez and du Toit 2006	MK091740	MK868650	MK868622	/
<i>S. vesicarium</i>	B-St431	Spinach	TX	2013	L. du Toit	St431	Hernandez-Perez and du Toit 2006	MK091741	MK868651	MK868623	/
<i>S. vesicarium</i>	D-St432	Spinach	TX	2013	L. du Toit	St432	Hernandez-Perez and du Toit 2006	MK091743	MK868652	MK868624	/
<i>S. vesicarium</i>	E-St430	Spinach	TX	2013	L. du Toit	St430	Hernandez-Perez and du Toit 2006	MK091744	MK868653	MK868625	/
<i>S. vesicarium</i>	I-St428	Spinach	CA	2013	L. du Toit	St428	Hernandez-Perez and du i	MK091748	MK868654	MK868626	/
<i>S. vesicarium</i>	TX-1	Spinach	TX	2016	L. Stein	/	/	MK091761	MK868644	MK868616	/
<i>S. vesicarium</i>	TX-2	Spinach	TX	2016	L. Stein	/	/	MK091762	MK868645	MK868617	/
<i>S. vesicarium</i>	TX-3	Spinach	TX	2016	L. Stein	/	/	MK091763	MK868646	MK868618	/
<i>S. vesicarium</i>	VSP05-42A1	Onion	WA	2005	L. du Toit	Stv001	/	MK091777	MK868647	MK868619	/
<i>S. vesicarium</i>	VSP05-43-1	Onion	WA	2005	L. du Toit	Stv009	/	MK091778	MK868648	MK868620	/
<i>S. vesicarium</i>	VSP06-67D	Onion	WA	2006	L. du Toit	Stv013	/	MK091779	MK868649	MK868621	/
<i>S. beticola</i>	Sb-4-St037	Spinach	WA	2009	L. du Toit	St037	Hernandez-Perez and du Toit 2006	MK091752	MK868655	MK868627	/
<i>S. beticola</i>	Sb-5-St030	Spinach	WA	2009	L. du Toit	St030	Hernandez-Perez and du Toit 2006	MK091753	MK868656	MK868628	/
<i>S. beticola</i>	Sb-6-St257	Spinach	AZ	2009	L. du Toit	St257	Hernandez-Perez and du Toit 2006	MK091754	MK868657	MK868629	/
<i>S. beticola</i>	CA-1	Spinach	CA	2001	L. du Toit	St006	Koike et al. 2001	MK091770	MK868662	MK868634	/
<i>S. beticola</i>	CA-2	Spinach	CA	2001	L. du Toit	St007	Koike et al. 2001	MK091771	MK868663	MK868635	/
<i>S. beticola</i>	CA-3	Spinach	CA	2001	L. du Toit	St008	Koike et al. 2001	MK091772	MK868664	MK868636	/
<i>S. beticola</i>	CA-4	Spinach	CA	2001	L. du Toit	St009	Koike et al. 2001	MK091773	MK868665	MK868637	/
<i>S. beticola</i>	CA-5	Spinach	CA	2001	L. du Toit	St010	Koike et al. 2001	MK091774	MK868666	MK868638	/
<i>S. beticola</i>	CA-6	Spinach	CA	2001	L. du Toit	St011	Koike et al. 2001	MK091775	MK868667	MK868639	/
<i>S. beticola</i>	CA-7	Spinach	CA	2001	L. du Toit	St012	Koike et al. 2001	MK091776	MK868668	MK868640	/
<i>S. beticola</i>	C-St004	Spinach	WA	2009	L. du Toit	St004	Hernandez-Perez and du Toit 2006	MK091742	MK868659	MK868631	/
<i>S. beticola</i>	F-St005	Spinach	WA	2009	L. du Toit	St005	Hernandez-Perez and du Toit 2006	MK091745	MK868661	MK868632	/
<i>S. beticola</i>	G-St406	Spinach	OR	2009	L. du Toit	St406	Hernandez-Perez and du Toit 2006	MK091746	MK868660	MK868633	/
<i>S. beticola</i>	TX-8	Spinach	TX	2016	L. Stein	/	/	MK091768	MK868658	MK868630	/

^x All the isolates listed were used for phylogenetic analyses to aid in species determination.

^y The isolates in bold font were used for pathogenicity tests on the spinach cultivar Viroflay.

^z / = no information available.