Project Title: Title: Identification and determination of disease incidence and severity of leaf spot pathogens of spinach
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Principal investigator:

Jim Correll
Department of Plant Pathology
University of Arkansas
Fayetteville, Arkansas 72701
479-283-1628
jcorrell@uark.edu

Cooperating personnel: Richard Smith (UC Cooperative Extension). Dr. Chunda Feng (Research Specialist, University of Arkansas), Dr. Ainong Shi (spinach breeder, University of Arkansas). Bayer Seed, Bejo Seed, Enza Seed, Monsanto Seed, Pop Vriend Seed, Rijk Zwaan Seed, Sakata Seed, Syngenta Seed, Gowan Seed Co., Holiday Seed Co. various growers and extension personnel in California and Arizona.

ABSTRACT

Spinach leaf spot diseases are becoming much more common in both conventional and organic spinach production systems throughout the U.S. including California's central coast production areas and the Imperial and the Yuma Valley of Arizona. The leaf spots exhibit a wide range of symptoms and diagnosis of the various pathogens based on symptoms has been a challenge. The increase in the number of leaf spot symptoms represents a significant concern to the spinach industry. In addition, a number of abiotic disorders also have been observed that complicate accurate diagnosis. The fungal pathogens known to be involved in the leaf spot symptoms include (*Stemphylium* spp., *Colletotrichum spinaciae*, *Cladosporium viriabile*, and *Cercospora beticola*, but also include several new pathogens newly identified in the U.S. (*Colletotrichum truncatum, Colletotrichum beticola*, and *Myrothecium verrucaria*). The accurate identification of these pathogens is critical for implementing proper and effective disease management strategies. A wide range of isolations, pathogenicity tests, and fungicide efficacy were performed. In addition, a molecular diagnostic test to differentiate the two species of *Stemphylium* known to cause leaf spot on spinach is underway. The species-specific primers based on gapdh and cmdA genes to differentiate *Stemphylium vesicarium* and *S. beticola* were evaluated for isolates of *Stemphylium* and shown to be specific for each species. In addition, several fungicides were demonstrated to be effective to control leaf spots diseases caused by S. *vesicarium* and *S. beticola*.

OBJECTIVES

1. Determine the incidence and severity of leaf spot pathogens of spinach in conventional and organic spinach production systems.

2. Evaluate select isolates of the identified leaf spot pathogens for resistance to commonly used fungicides in conventional spinach production systems.

3. Develop a quick and accurate PCR-based identification method to determine which pathogen may be causing the leaf spot or if the symptoms are due to an abiotic disorder (for example, spray damage).

Objectives done

1. Determine the incidence and severity of leaf spot pathogens of spinach in conventional and organic spinach production systems.

The sampling of leaf spot symptoms determined which fungal leaf-spot pathogens are present in California and the Yuma Valley, and which pathogens predominate and represent a major concern in these areas. Leaf samples with symptoms of leaf spots were collected from growers, shippers, seed company personnel, PCAs and extension personnel. Passport information on the samples with regard to the production system (organic versus conventional), geographic location, variety, spray history, etc. were recorded. Symptomatic leaves were incubated under conditions that will favor the sporulation of the various leaf spot fungal pathogens. Fungal species were identified based on sporulation on incubated leaves as well as isolation of the fungal pathogens from 20-50 lesions per sample. A collection of isolates of the various pathogens was stored for analysis of various traits including molecular identification, pathogenicity, and fungicide resistance in greenhouse tests.

Table 1. Fungi recovered from leaf spots of spinach collected from 2017 to 2020 in California and Arizona.

Sample date	Sample identifier	Sender	Cultivar	Pathogens			Genus and species identification	State
				Stemphylium vesicarium	***Stemphylium beticola***	***Cladosporium variabile***		
1/18/2017	Maya-untreated	/	/	12/50*	0/50	0/50	Abiotic and *S. vesicarium*	CA
1/24/2017	PV1237 Sakata 236	/	/	0/20	20//20	0/20	*S. beticola*	Yuma, AZ
10/11/2017	UCCE-Monterey Co.	Richard Smith	/	0/50	50/50	0/50	*S. beticola*	CA
10/11/2017	CA, Francisco	Richard Smith	/	50/50	0/50	0/50	*S. vesicarium*	CA
11/17/2017	D. mist Rivara, 922-B, Carmel	/	/	50/50	0/50	0/50	*S. vesicarium*	CA
11/29/2017	Richard Smith	Richard Smith	/	50/50	0/50	0/50	*S. vesicarium*	CA
3/6/2018	Richard Smith	Richard Smith	/	0/20	20/20	0/20	*S. beticola*	AZ
3/15/2018	Yellow spot	Dr. Correll	/	10/10	0/10	0/10	*S. vesicarium***	CA
9/7/2018	/	Richard Smith	/	20/20	0/20	0/20	*S. vesicarium***	AZ
9/11/2018	BV207 B3 Alvor Heavy	Holaday Seed	Alvor Heavy	20/20	0/20	0/20	*S. vesicarium***	AZ
9/11/2018	Upper Sorenson Lot 6 C2 Algr	Holaday Seed	Algr	20/20	0/20	0/20	*S. vesicarium***	AZ
9/11/2018	Upper Sorenson Lot 6 AZ-4 Banobo	Holaday Seed	Banobo	20/20	0/20	0/20	*S. vesicarium***	AZ

9/11/2018	Upper Sorenson Lot 6 B2-1 Dozer	Holaday Seed	Dozer	20/20	0/20	0/20	*S. vesicarium***	AZ
10/9/2018	Bakersfield	Richard Smith	/	10/10	0/10	0/10	*S. vesicarium***	Bakersfield, CA
10/25/2018	Volans	Richard Smith	Volans	10/10	0/10	0/10	*S. vesicarium***	CA
11/8/2018	Field: Brosie 120, Organic	Tyler Shaddy	Virgo	0/10	10/10	0/10	*S. beticola***	Wellton, Yuma, AZ
11/9/2018	Bonobo Untreated	Joe Phelps	Bonobo	4/10	0/10	0/10	Abiotic and *S. vesicarium***	Imperial Valley, CA
11/25/2018	Richard Smith	Richard Smith	/	10/10	0/10	0/10	*S. vesicarium***	CA
12/18/2018	Volans	Chris Nelson	Volans	8/10	0/10	0/10	*S. vesicarium***	CA
1/10/2019	Fresh Farm Occident	/	Occident	10/10	0/10	0/10	*S. vesicarium*** and abiotic	Imperial County, CA
1/10/2019	Pomelo	/	/	0/10	0/10	0/10	Physical damage	Imperial County, CA
1/10/2019	Pine26, organic	/	/	10/10	0/10	0/10	*S. vesicarium***	Imperial County, CA
1/15/2019	Scott	Joe Phelps	/	10/10	0/10	0/10	*S. vesicarium***	CA
10/14/2019	Regor	Dr. Feng	Regor	10/10	0/10	0/10	*S. vesicarium*	CA
10/14/2019	Nevada	Dr. Feng	Nevada	8/10	2/10	0/10	*S. vesicarium* and *S. beticola*	CA
10/14/2019	COCO PAH	Dr. Feng	/	3/10	0/10	2/10	Abiotic mixed with *S. vesicarium*, *C. variabile* and *P. effusa*	CA
10/14/2019	Showcase Trial (Holaday Sculptur)	Dr. Feng	/	0/10	5/10	2/10	*S. vesicarium* and *C. variabile*, and mixed with leaf miner damage	CA
12/12/2019	Gowan seed	Joe Phelps	Sioux	18/20	0/20	0/20	*S. beticola***	Yuma, AZ

12/19/2019	/	Joe Phelps	/	0/10	4/10	0/10	*S. beticola* mixed with unknown fungi	Imperial valley, CA
1/2/2020	Finwhale	Tyler Shaddy	Finwhale	0/10	0/10	10/10	*Cladosporium. variabile*	Scottsdale, AZ
1/2/2020	Colusa	Derek McKelvey	Colusa	0/10	0/10	10/10	*C. variabile*	Scottsdale, AZ
1/2/2020	Nevada	Derek McKelvey	Nevada	0/10	0/10	10/10	*C. variabile*	Scottsdale, AZ
1/2/2020	07E1	Jerry Rava	/	0/10	0/10	0/10	Unknown fungus mixed with *Alternaria* spp.	Imperial, CA
1/2/2020	04E1	Jerry Rava	/	0/10	0/10	0/10	Unknown fungus mixed with *Alternaria* spp.	Imperial, CA

*: Number of lesions confirmed / number evaluated.
**: Identification was also based on DNA sequence analysis of the ITS region for further confirmation.
/: not tested

Three major pathogens were found from spinach samples with leaf spots from California and Arizona, which are *Stemphylium vesicarium* and *S. beticola*, and *Cladosporium variabile*. Saprophyte fungi were also isolated from diseased leaves, which are *Alternaria* spp. and *Cladosporium* spp. (Table 1).

2. Evaluate fungicide efficacy and select isolates of the identified leaf spot pathogens for resistance to commonly used fungicides in conventional spinach production systems.

Major leaf spot pathogens (*Stemphylium vesicarium* and *S. beticola*) identified were evaluated in the greenhouse tests for fungicide resistance to commonly used conventional fungicides.

2a: Evaluate the effectiveness of Strobilurin fungicides (Tables 2 and 3) to *Stemphylium* spp. including *S. vesicarium* and *S. beticola* on spinach cv. Viroflay (*Spinacia oleracea*).

Table 2. Strobilurin fungicides recommended dose and dose used.

Product	FRAC codes[a]	Active ingredients	Recommended dose (ml/0.40 ha)[b]	Dose used (ml/50 ml)[c]
Bravo WeatherStik	M5	Chlorothalonil	947.2	0.83
Cabrio	11	Pyraclostrobin	453.6	0.40 gram
Merivon	7 + 11	Fluxapyroxad + Pyraclostrobin	324.5	0.29
Top Guard EQ	3 + 11	Flutriafol + Azoxystrobin	236.8	0.21
Trilogy	2 + 3 + 11	Iprodione + Triticonazole + Trifloxystrobin	3,788.8	3.33
Tanos	27 + 11	Cymoxanil + Famoxadone	296.0	0.26
Quadris	11	Azoxystrobin	457.25	0.40
Luna sensation	7 + 11	Fluopyram + Trifloxystrobin	224.96	0.20
Water	/[d]	Water	/	50.0

[a] 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).

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

[c] Dose determined based on the volume (ml) of fungicide by volume (ml) of carrier (water).

[d] / = no information available.

Table 3. Mean disease severity of spinach leaf spot on the cv. Viroflay treated with different strobilurin fungicides prior to inoculation with *Stemphylium* spp.[x]

	Mean disease severity					
	S. vesicarium					*S. beticola*
Fungicide	**St430**	**St480**	**Sb-1-St001**	**TX-3**	**TX-1**	**TX-8**
Water control	2.5 ± 0.1 a[y]	2.0 ± 0.1 a	1.8 ± 0.1 a	2.0 ± 0.1 a	2.2 ± 0.1 a	1.8 ± 0.1 a
Top Guard EQ	1.8 ± 0.1 b	1.5 ± 0.1 b	1.0 ± 0.1 b	0.5 ± 0.1 e	0.8 ± 0.1 c	0.3 ± 0.1 c
Luna sensation	1.8 ± 0.1 b	1.5 ± 0.1 b	1.0 ± 0.1 b	1.0 ± 0.1 b	1.0 ± 0.1 b	0.3 ± 0.1 c
Cabrio	1.3 ± 0.1 c	1.2 ± 0.1 c	0.0 d	0.5 ± 0.1 e	0.5 ± 0.1 e	0.0 d
Merivon	1.2 ± 0.1 d	1.0 ± 0.1 d	0.8 ± 0.1 c	0.5 ± 0.1 e	0.5 ± 0.1 e	0.0 d
Trilogy	1.2 ± 0.1 d	1.2 ± 0.1 c	1.0 ± 0.1 b	1.0 ± 0.1 b	0.8 ± 0.1 c	0.3 ± 0.1 c
Tanos	1.2 ± 0.1 d	1.0 ± 0.1 d	1.0 ± 0.1 b	0.8 ± 0.1 c	0.8 ± 0.1 c	0.5 ± 0.1 b
Quadris	1.0 ± 0.1 e	1.0 ± 0.1 d	1.0 ± 0.1 b	0.7 ± 0.1 d	0.7 ± 0.1 d	0.3 ± 0.1 c
Bravo WeatherStik	0.0 f	0.0 e	0.0 d	0.0 f	0.0 f	0.0 d
LSD	0.2	0.5	0.1	0.1	0.1	0.2
Factor	***Pr* > F[z]**	***Pr* > F**	***Pr* > F**	***Pr* > F**	***Pr* > F**	***Pr* > F**
Repeat	0.6574	0.4269	1.0000	0.1531	0.4771	0.4211
Fungicide	< 0.001	0.3428	< 0.001	< 0.001	< 0.001	0.0501
Fungicide x repeat	0.9934	0.4908	0.3704	0.9931	0.9955	0.6954

[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 – 25%; 2 = 26 – 50%; 3 = 51 – 75%; and 4 = 76 – 100% leaf area infected. One 45-day-old plant in each of three replicate pots was inoculated with a spore suspension of 1 x 10^5 spores/ml. Inoculated plants were incubated at 20 to 23°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. 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.
[y] Means with different letters in the column were significantly different at $P < 0.05$ based on Fisher's protected least significant difference.
[z] Significance level (P) from analysis of variance (ANOVA) for disease severity with a repeated experiment.

Fungicide testing result showed that Bravo WeatherStik was completely effective at preventing leaf spot symptoms from developing on Viroflay plants inoculated with both *S. vesicarium* and *S. beticola* (Tables 2 and 3). All strobilurin fungicides can reduce the disease severity of leaf spots in a certain degree. All strobilurin fungicides are highly effective at preventing leaf spot symptoms from developing on plants inoculated with *S. beticola* (TX-8), however, only certain strobilurin fungicides are highly effective to certain isolates of *S. vesicarium.* For example, Top Guard EQ, Luna sensation, Cabrio, Merivon, Trilogy, Tanos, and Quadris were highly effective to the following isolates of Sb-1-St001, TX-3 and TX-1. Cabrio, Merivon, Trilogy, Tanos and Quadris were highly effective to the following isolates of St430 and St480. The remaining fungicides were either ineffective or weakly effective at reducing disease severity of the isolates of *S. vesicarium* (Tables 2 and 3).

Bravo WeatherStik can completely control leaf spot diseases caused by both *S. vesicarium* and *S. beticola*. Certain strobilurin fungicides can effectively control leaf spot diseases caused by certain isolates of *S. vesicarium*, but certain strobilurin fungicides cannot effectively control leaf spot diseases caused by certain isolates of *S. vesicarium*. Moreover, all strobilurin fungicides can effectively control leaf spot diseases caused by the isolate of *S. beticola* (Tables 2 and 3).

2b: Evaluate the effectiveness of various fungicides (Tables 4 and 5) to *Stemphylium* spp. including *S. vesicarium* and *S. beticola* on spinach cv. Viroflay (*Spinacia oleracea*).

Table 4. Fungicides recommended dose and dose used.

Product	FRAC codes[a]	Active ingredients	Recommended dose (ml/0.40 ha)[b]	Dose used (ml/50 ml)[c]
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 gram
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 gram
Fontelis	7	Penthiopyrad	708.0	0.63
Prophyt +	/[d]	Potassium phosphate +	2,131.2	1.88
Presidio	43	Fluopicolide	88.8	0.08
Revus	40	Mandipropamid	236.6	0.26
Trilogy	2 + 3 + 11	Iprodione + Triticonazole + Trifloxystrobin	3,788.8	3.33
Serenade	Biological	*Bacillus subtilis*	3,788.8	3.33
Double Nickel	Biological	*Bacillus amyloliquefaciens*	1,894.4	1.66
PurGrow 100%	/	Hypochlorous acid	/	50.0
Tanos	27 + 11	Cymoxanil + Famoxadone	296.0	0.26
Quadris	11	Azoxystrobin	457.25	0.50
Cueva	M1	Copper octanoate	3,788.8	3.33
Ridomil Gold	4	Mefenoxam	354.0	0.31
Luna Sensation	7 + 11	Fluopyram + Trifloxystrobin	224.96	0.25
Rhyme	3	Flutriafol	206.99	0.18
Orondis Ultra	49 + 40	Oxathiapiprolin + Mandipropamid	236.8	0.21
Actigard	P1	Acibenzolar-S-methyl	59.14	0.07
Zampro	45 + 40	Ametoctradin + Dimethomorph	414.4	0.46
Curzate	27 + M3	Cymoxanil + Mancozeb	148.0	0.16 gram
Aliette	33	Fosetyl-aluminium	2267.9	2.54 gram
Miravis Prime	12 + 7	Pydiflumetofen + Fludioxonil	404.85	0.45
Foli-R-Plus (Ridez)		Potassium Phosphate + Microbial metabolites	1894.4	1.66
Water	/	Water	/	50.0

[a] 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).

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

[c] Dose determined based on the volume (ml) of fungicide by volume (ml) of carrier (water).

[d] / = no information available.

Table 5. Mean disease severity of spinach leaf spot on the cv. Viroflay treated with different fungicides prior to inoculation with *Stemphylium* spp.[z]

	Mean disease severity	
	S. vesicarium	***S. beticola***
Fungicide	**RS-1[y]**	**JP-6[y]**
Water	3.0 a[x]	3.2 a
Actigard	3.0 a	3.2 a
Double Nickel	2.8 a	2.5 b
Trilogy	2.2 b	1.8 cd
Serenade	2.0 b	2.0 cb
Ridomil Gold	2.0 b	1.3 e
Cueva	1.8 cb	2.5 b
Aliette	1.8 cb	1.8 cd
Prophyt + Presidio	1.5 cd	1.5 cde
PurGrow 100%	1.5 cd	1.7 cd
Quadris	1.2 ed	1.0 fge
Luna Sensation	1.2 ed	1.5 cde
Switch	1.0 ef	0.5 igh
Tanos	0.8 efg	0.5 igg
Foli-R-Plus (Ridez)	0.8 efg	0.7 gh
Fontelis	0.7 hfg	0.8 fgh
Zampro	0.5 hig	0.7 gf
Revus	0.3 hij	1.3 fde
Curzate	0.3 hij	0.3 ih
Top Guard EQ	0.2 hij	0.0 i
Orondis Ultra	0.2 hij	0.8 fgh
Rhyme	0.2 j	0.7 g
Miravis Prime	0.2 j	0.3 ih
Merivon	0.0 j	0.0 i
Cabrio	0.0 j	0.0 i
Dithane F-45	0.0 j	0.0 i
Bravo WeatherStik	0.0 j	0.0 i
Factor	***Pr* > F[z]**	***Pr* > F**
Repeat	0.4277	0.1735
Fungicide	< 0.001	< 0.001
Fungicide x repeat	0.9932	0.7911

[z] 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 – 25%; 2 = 26 – 50%; 3 = 51 – 75%; and 4 = 76 – 100% leaf area infected. One 45-day-old plant in each of three replicate pots was inoculated with a spore suspension of 1 x 10^5 spores/ml. Inoculated plants were incubated at

20 to 23°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. 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. The standard error and least significant difference (LSD) for both RS-1 and JP-6 treated with different fungicides are 0.

[y] RS-1 (*S. vesicarium*) (collected in Bakersfield, CA, by Richard Smith on 10/9/2018) and JP-6 (*S. beticola*) (collected in Imperial valley, CA, by Joe Phelps on 12/19/2019).
LSD for RS-1 is 0.2 for all fungicides, and for JP-6 is 0.3 for all the fungicides.

[x] Means with different letters in the column were significantly different at $P < 0.05$ based on Fisher's protected least significant difference (LSD).

Fungicide testing result showed that both *Stemphylium* species caused severe leaf spotting on Viroflay in the fungicide efficacy trial (3.0 severity category and 50 to 75% leaf damage) for the control plants with no fungicide treatment and the planted treated with Actigard (Acibenzolar-S-methyl) (Tables 4 and 5). Ridomil Gold (Mefenoxam), Serenade (*Bacillus subtilis*), Trilogy (Iprodione + Triticonazole + Trifloxystrobin), and Double Nickel (*Bacillus amyloliquefaciens*) were weakly effective at reducing disease severity on Viroflay plants inoculated with both *S. vesicarium* and *S. beticola*, Luna Sensation (Fluopyram + Trifloxystrobin), Quadris (Azoxystrobin), PurGrow 100%, Prophyt + Presidio (Potassium phosphate + Fluopicolide), Aliette (Fosetyl-aluminium), and Cueva (Copper octanoate) were medium-level effective at reducing disease severity, and Bravo WeatherStik (chlorothalonil), Dithane F-45 (mancozeb), Cabrio (pyraclostrobin), and Merivon (fluxapyroxad and pyraclostrobin), Miravis Prime (Pydiflumetofen + Fludioxonil), Rhyme (Flutriafol), Orondis Ultra (Oxathiapiprolin + Mandipropamid),Top Guard EQ (Flutriafol + Azoxystrobin), Curzate (Cymoxanil + Mancozeb), Zampro (Ametoctradin + Dimethomorph), Revus (Mandipropamid), Foli-R-Plus (Ridez) (Potassium Phosphate + Microbial metabolites),Tanos (Cymoxanil + Famoxadone), Fontelis (Penthiopyrad), and Switch (Fludioxonil + Cyprodinil) were highly effective at preventing leaf spot symptoms from developing on Viroflay plants (Tables 4 and 5).

In general, the synthetic fungicides are more effective to control Stemphylium leaf spots than bio-fungicides. In addition, there are some differences in disease severities of leaf spots on spinach leaves inoculated with different *Stemphylium* species under different fungicide treatments.

3. Develop a quick and accurate PCR-based identification method to determine which pathogen may be causing the leaf spot or if the symptoms are due to an abiotic disorder (for example, spray damage).

A PCR-based test was developed to identify and differentiate the leaf spot pathogens on spinach. Stemphylium species-specific primers were based on the sequence alignment of the glyceraldehyde-3-phosphate dehydrogenase (gapdh) and calmodulin (cmdA) genes, four sets of species-specific primers were designed and evaluated for their species-specificity to *Stemphylium vesicarium* and *S. beticola*.

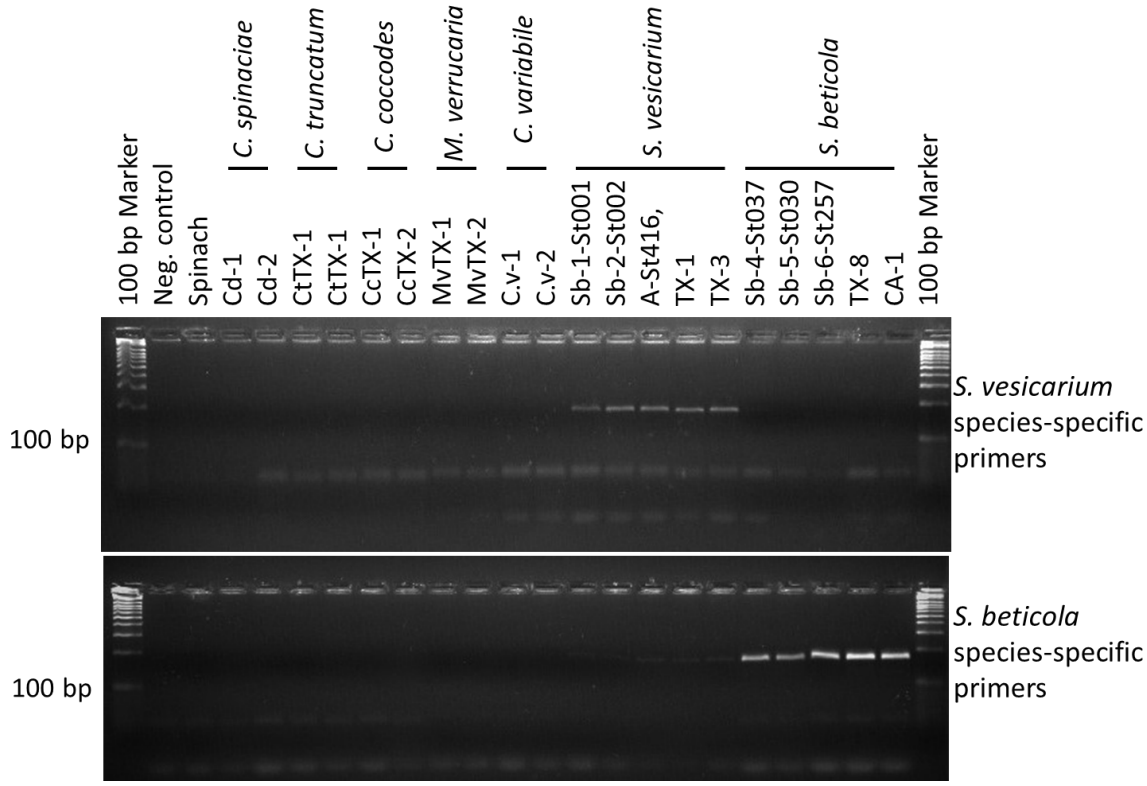

Fig.1. PCR using Stemphylium-specific primers (based gapdh gene) for the following isolates of *Stempylium vesicarium* (Sb-1-St001, Sb-2-St002, A-St416, TX-1, and TX-3), *Stemphylium beticola* (Sb-4-St037, Sb-5-St030, Sb-6-St257, TX-8, and CA-1), water and Viroflay DNA were used as the controls**.**

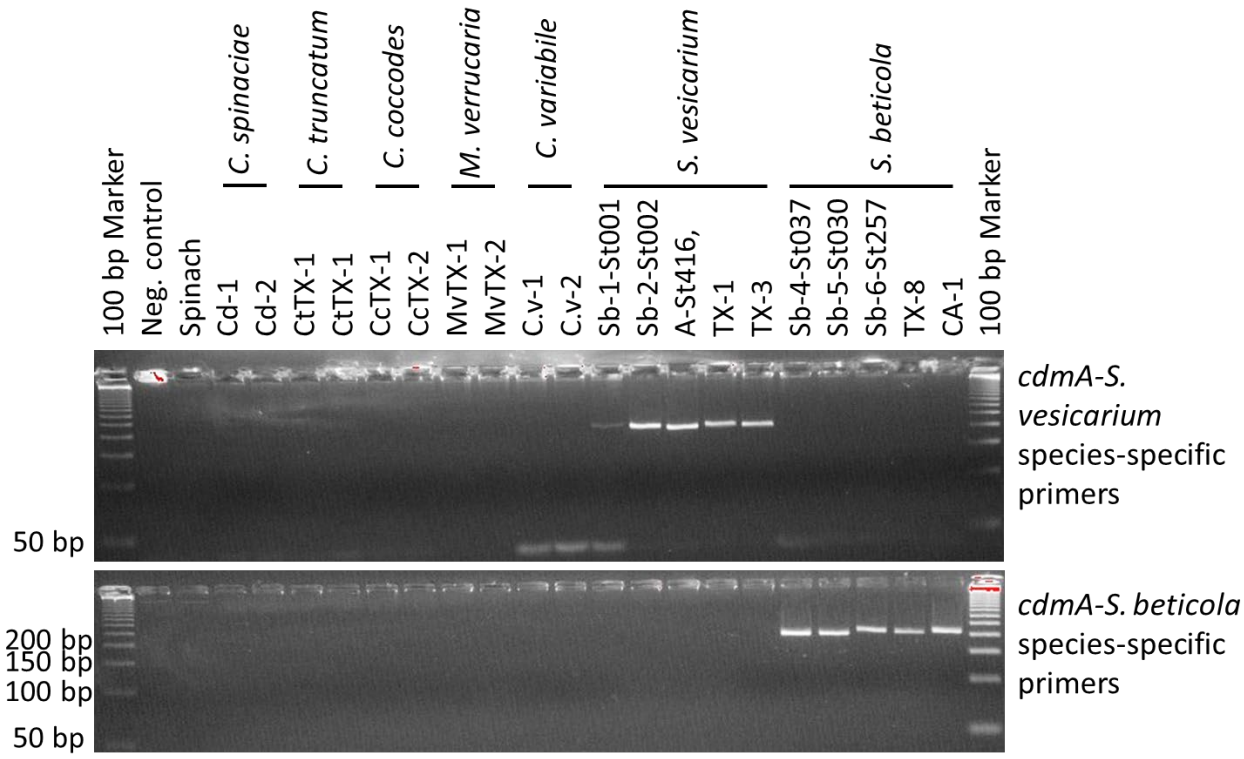

Fig. 2. PCR using Stemphylium-specific primers (based cdmA gene) for the following isolates of *Stempylium vesicarium* (Sb-1-St001, Sb-2-St002, A-St416, TX-1, and TX-3), *Stemphylium beticola* (Sb-4-St037, Sb-5-St030, Sb-6-St257, TX-8, and CA-1), water and Viroflay DNA were used as the controls.

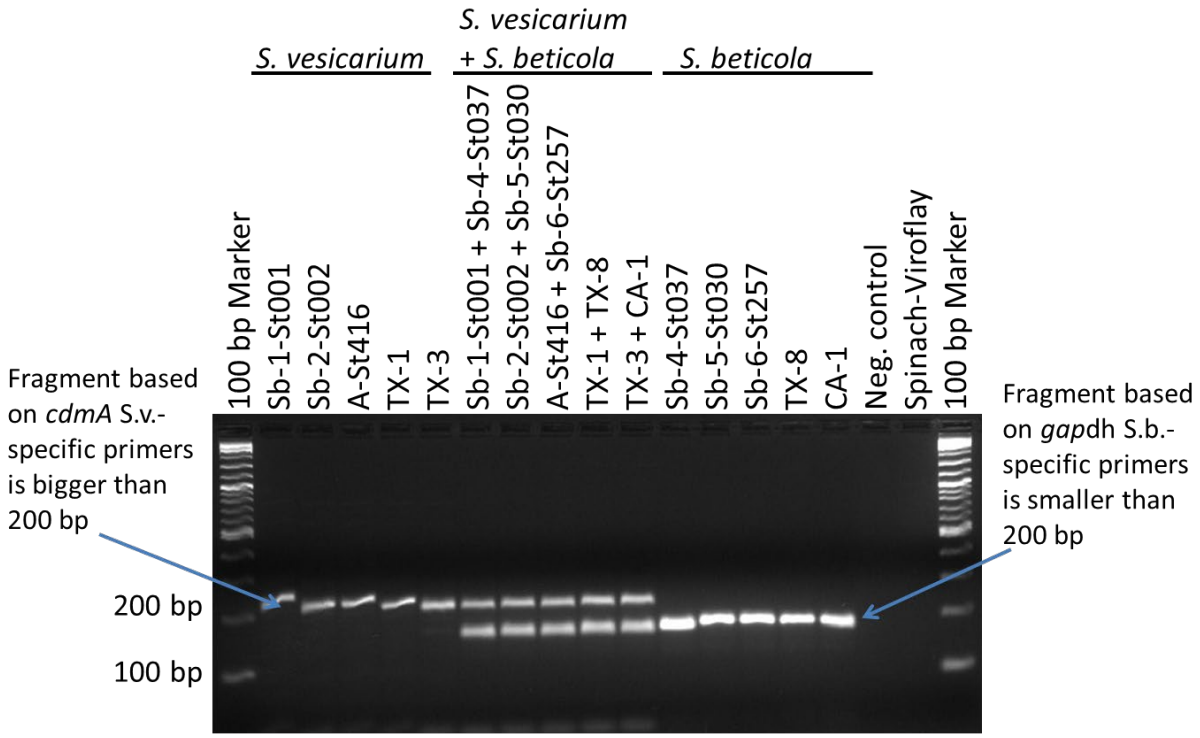

Fig. 3. PCR using Stemphylium-specific primers (based on gapdh and cdmA genes) for the following isolates of *Stempylium vesicarium* (Sb-1-St001, Sb-2-St002, A-St416, TX-1, and TX-3), the mixture of *Stempylium vesicarium* + *Stemphylium beticola* (Sb-1-St001 + Sb-4-St037, Sb-2-St002 + Sb-5-St030, A-St416 + Sb-6-St257, TX-1 + TX-8, and TX-3 + CA-1), *Stemphylium beticola* (Sb-4-St037, Sb-5-St030, Sb-6-St257, TX-8, and CA-1), water and Viroflay were used as the controls.

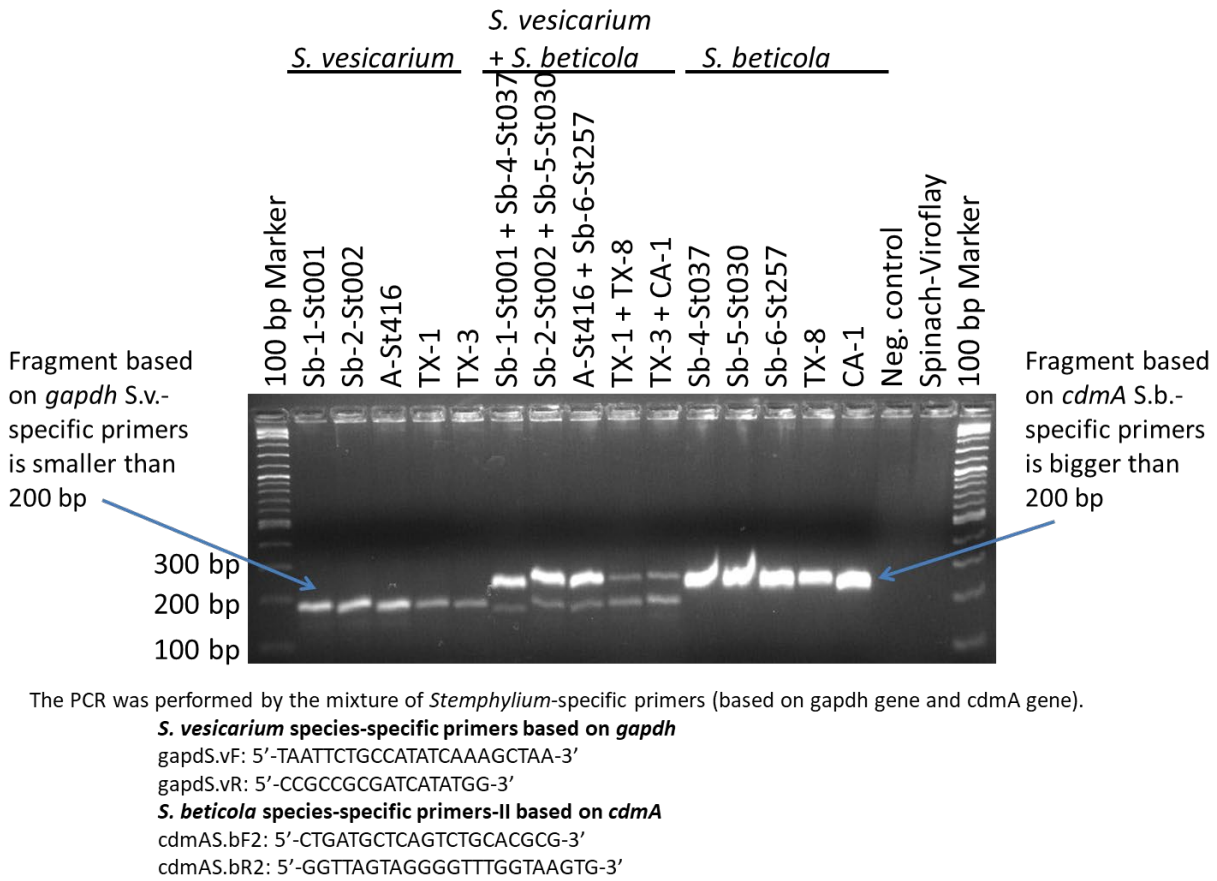

Fig. 4. PCR using Stemphylium-specific primers (based on gapdh and cdmA genes) for the following isolates of *Stempylium vesicarium* (Sb-1-St001, Sb-2-St002, A-St416, TX-1, and TX-3), the mixture of *Stempylium vesicarium* + *Stemphylium beticola* (Sb-1-St001 + Sb-4-St037, Sb-2-St002 + Sb-5-St030, A-St416 + Sb-6-St257, TX-1 + TX-8, and TX-3 + CA-1), *Stemphylium beticola* (Sb-4-St037, Sb-5-St030, Sb-6-St257, TX-8, and CA-1), water and Viroflay were used as the controls.

Results showed that the Stemphylium species-specific primer pairs from each species based on either gapdh or cmdA genes were highly sensitive and specific for detection and differentiation of each species based on DNA isolations from the cultured isolates (Figs. 1, 2, 3, and 4). Both Stemphylium species-specific primers cannot amply the isolates of *Colletotrichum spinaciae*, *Colletotrichum coccodes*, *C. truncatum*, *Cladosporium variabile*, *Myrothecium verrucaria*, and spinach (Figs. 1 and 2).

Efforts are underway to evaluate the robustness of the Stemphylium species-specific primers with a broader set of isolates of each species and from field samples. The PCR based diagnostic analysis can be completed within 24 h, compared with 1-2 weeks for doing isolations and molecular identification.

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