Texas A&M AgriLife Extension

Wintergarden Spinach Producers Board

Tiro Tres Farms

2020 Spinach Field Day

February 19, 2020

10:00 a.m. - 2:00 p.m.

Tiro Tres Farms - Crystal City, TX

AGENDA

10:00 a.m. - 2:00 p.m.

Welcome & Introductions

Marcel Valdez, Zavala CEA-AG, Crystal City, TX
Ed Ritchie,
President of Wintergarden Spinach Producers Board
Larry Stein, Uvalde, TX
Texas A&M AgriLife Extension Service

Mike Phillips,
Cargile Consulting, Uvalde, TX

Kimberly Cochran, Uvalde, TX
Texas A&M AgriLife Extension Service

Lindsey Du Toit and Kayla Spawton,
Washington State University,
Department of Plant Pathology

Field Tour

Lunch

You are welcome to stay after 2:00 pm

2019 White Rust Nursery Photo provided by: Julia Paige Ritchie, Tiro Tres Farms

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Winter Garden Soil & Water Conservation District #326

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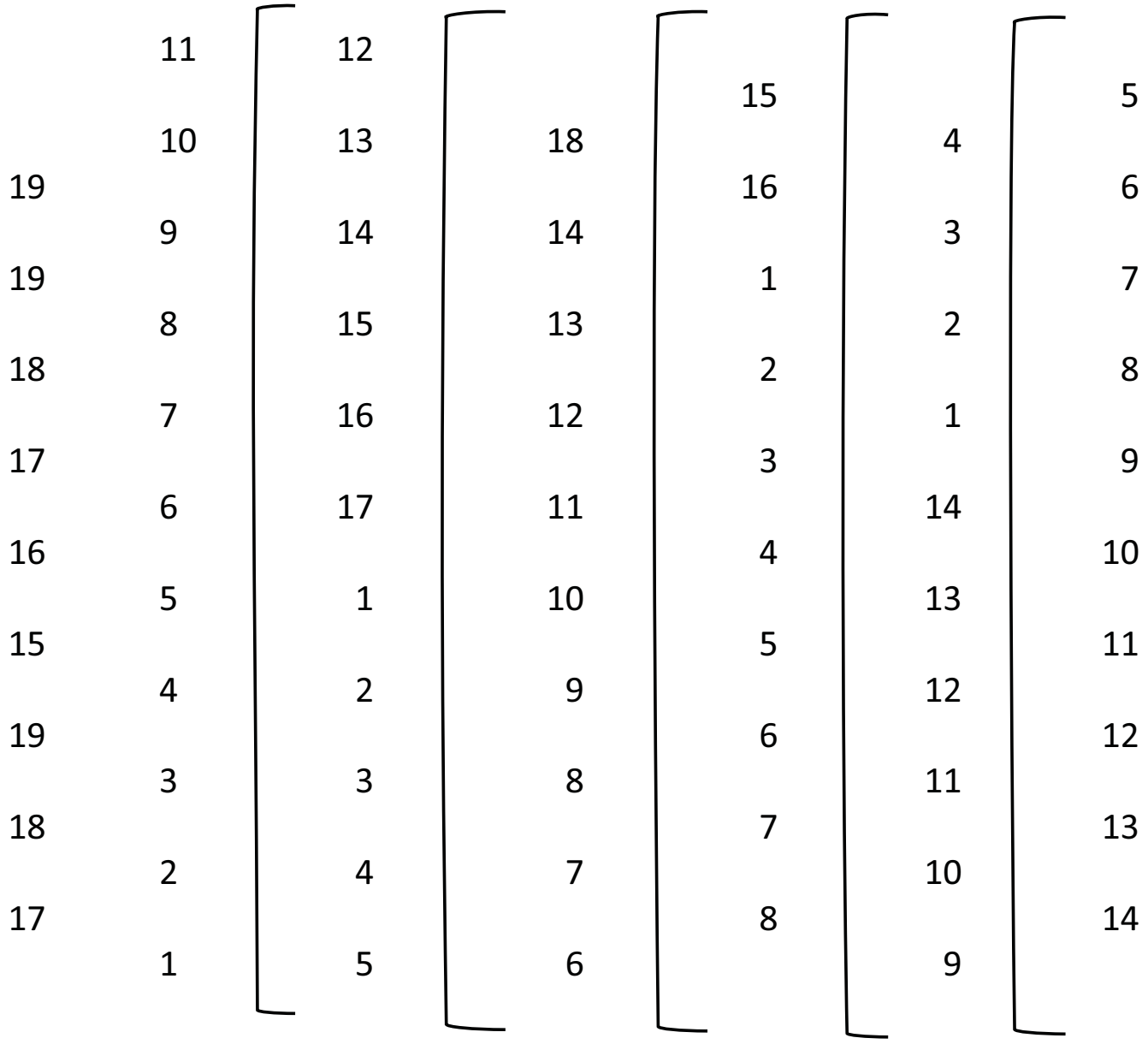
2019-2020 Spinach Fungicide Trial

Larry Stein and Marcel Valdez

1.	UTC
2.	Lifegard 2 oz/A
3.	Regalia 2 qts/A
4.	Lifegard 2 oz/A alt. Lifegard 1 oz/A + Presidio 3oz/A alt. Merivon 8 oz/A alt. Miravis Prime 13.7 oz/A
5.	Lifegard 2 oz/A alt. Lifegard 1 oz/A + Presidio 3 oz/A alt. InspireSUPER 20 oz/A alt. Cabrio 16 oz/A
6.	Lifegard 2 oz/A alt. Lifegard 1 oz/A + Merivon 8 oz/A alt. Fontelis 20oz/A alt. Cabrio 16 oz/A
7.	Lifegard 2 oz/A alt. Lifegard 1 oz/A + InspireSUPER 20 oz/A alt. Presidio 3 oz/A alt. Cabrio 16 oz/A
8.	Regalia2 qts/A alt. Luna Tranquility 21.5 oz/A alt. Regalia 2 qts/A alt. Luna Tranquility 21.5 oz/A
9.	Lifegard 2 oz/A alt. Lifegard1 oz/A + Merivon 8 oz/A alt. Miravis Prime 13.7 oz/A
10.	Switch 14 oz/A alt. Switch 14 oz/A alt. Orondis Ultra 8 oz/A alt. Switch 14 oz/A alt. Switch 14 oz/A
11.	Miravis Prime 13.7 oz/A alt. Switch 14 oz/A alt. Orondis Ultra 8 oz/A alt. Miravis Prime 13.7 oz/A alt. Switch 14 oz/A
12.	Oso (polyoxin D) 8.5 oz/A
13.	Terraneem 48 fl.oz/A alt. Rango 46 fl.oz/A alt. Terraneem 48 fl.oz/A alt. Rango 46 fl.oz/A
14.	Rhyme 6 oz/A
15.	Inspire SUPER 20 oz/A plus permup 5 oz/A
16.	Miravis Prime 13.7 oz/A plus permup 5 oz/A
17.	Inspire SUPER 20 oz/A plus permup 5 oz/A plus NIS 0.25% vv
18.	Miravis Prime 13.7 oz/A plus permup 5 oz/A plus NIS 0.25% vv
19.	Reason 8.2oz/A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17 6 17 11 3 14 9
16 5 1 10 4 13 10
15 4 2 9 5 12 11
19 3 3 8 6 11 12
18 2 4 7 7 10 13
17 1 5 6 8 9 14

	VARIETY	SEED Co
1	Lakeside	Sakata
2	Magnetic	Seminis
3	Nembus	Nunhems
4	C2-606	Sakata
5	Countryside	Sakata
6	51-722	Rijk Zwaan
7	Banjo	Pop Vriend
8	2157	Seminis
9	PV 1449	Pop Vriend
10	Riverside	Sakata
11	Sunangel	Rijk Zwaan
12	1470	Seminis
13	PV 1526	Pop Vriend
14	Seaside	Sakata
15	2146	Seminis
16	RZ 51-723	Rijk Zwaan
17	Sculptur	Nunhems
18	PV 1512	Pop Vriend
19	PV 1435	Pop Vriend
20	PV 1513	Pop Vriend
21	Capybarra	Rijk Zwaan
22	Hammerhead	Rijk Zwaan
23	1042	Enza
24	Patton	Bejo
25	Minkar	Nunhems
26	Regor	Nunhems
27	3719	Seminis

Crystal City Test Plot - Commerical Field Day Trial
Plant Date: 12/19/19
Population: 2 mil
30 lines

Patton			Nembus	Magnetic	Lakeside
Sunangel	Hammerhead	2146	51-722	Countryside	C2-606
Minkar	Regor	3719	PV1449	2157	Banjo
Hammerhead	1042	Patton	1470	Sunangel	Riverside
PV1435	PV1513	Capybarra	2146	Seaside	PV1526
51-723	Sculptur	PV1512	PV1512	Sculptur	51-723
PV1526	Seaside	2146	Capybarra	PV1513	PV1435
Riverside	Sunangel	1470	Patton	1042	Hammerhead
Banjo	2157	PV1449	3719	Regor	Minkar
C2-606	Countryside	51-722	2146	Hammerhead	Sunangel
Lakeside	Magnetic	Nembus			

North Farm Field Day
Plant Date: 12/20/19
Population: 2 mil
42 Lines

N Nembus	SM Magnetic	SK Lakeside	N Minkar	N Regor	SM 3719
RZ 51-722	SK Countryside	Banjo	RZ Hammerhead	EZ 1042	BE Patton
PV 1449	SM 2157	SK C2-606	PV 1435	PV 1513	RZ Capybarra
SM 1470	RZ Sunangel	SK Riverside	RZ 51-723	N Sculptur	PV 1512
SM 2146	SK Seaside	PV 1526	PV 1526	SK Seaside	SM 2146
PV 1512	N Sculptur	RZ 51-723	SK Riverside	RZ Sunangel	SM 1470
RZ Capybarra	PV 1513	PV 1435	SK C2-606	SM 2157	PV 1449
BE Patton	EZ 1042	RZ Hammerhead	Banjo	SK Countryside	RZ 51-722
SM 3719	N Regor	N Minkar	SK Lakeside	SM Magnetic	N Nembus

Crystal City Test Plot
Plant Date: 1/31/2020
Population: 2 mil
30 Lines

N Formax	Parakeet (T)	EXP 1 (T)	N Lacerta	Cabezon	EXP 2	EZ 1042	Patton	N Crater	N Tabit	EXP 3	EXP 4	N Eridanus	Capybarra (T)	N Octans	EXP 5	Kookaburra (T)	Viceroy (F300)

Feb-4-2020 (2020 SPINACH)

Mike Phillips

Cargileconsulting74@outlook.com

Reps: 4 Appl Code: _ Plots: 1 by 5 meters
Appl. Amount: 20 GAL/AC Mix Size: 0.3742 L (total for 4 plots; minimum=0.3742 L)

Trt No.	Treatment Name	Rate Unit	Appl Timing	Rep 1	Rep 2	Rep 3	Rep 4
1	DURIVO UNTREATED	10 fl oz/a	A	101	201	306	402
2	DURIVO SWITCH	10 fl oz/a 14 oz wt/a	A BCD	102	203	303	403
3	DURIVO MIRAVIS PRIME	10 fl oz/a 13.7 fl oz/a	A BCD	103	205	307	405
4	DURIVO INSPIRE SUPER	10 fl oz/a 20 fl oz/a	A BCD	104	204	308	406
5	DURIVO LUNA TRANQUILITY	10 fl oz/a 24 fl oz/a	A BCD	105	208	302	401
6	DURIVO MERIVON	10 fl oz/a 8 fl oz/a	A BCD	106	202	304	408
7	DURIVO REGALIA	10 fl oz/a 64 fl oz/a	A BCD	107	207	301	404
8	DURIVO OSO POLY OXIN D	10 fl oz/a 13 fl oz/a	A BCD	108	206	305	407
9	DURIVO MIRAVIS PRIME UNTREATED	10 fl oz/a 13.7 fl oz/a	A A	109	209	314	410
10	DURIVO MIRAVIS PRIME SWITCH	10 fl oz/a 13.7 fl oz/a 14 oz wt/a	A A BCD	110	211	311	411
11	DURIVO MIRAVIS PRIME MIRAVIS PRIME	10 fl oz/a 13.7 fl oz/a 13.7 fl oz/a	A A BCD	111	213	315	413
12	DURIVO MIRAVIS PRIME INSPIRE SUPER	10 fl oz/a 13.7 fl oz/a 20 fl oz/a	A A BCD	112	212	316	414
13	DURIVO MIRAVIS PRIME LUNA TRANQUILITY	10 fl oz/a 13.7 fl oz/a 24 fl oz/a	A A BCD	113	216	310	409
14	DURIVO MIRAVIS PRIME MERIVON	10 fl oz/a 13.7 fl oz/a 8 fl oz/a	A A BCD	114	210	312	416
15	DURIVO MIRAVIS PRIME REGALIA	10 fl oz/a 13.7 fl oz/a 64 fl oz/a	A A BCD	115	215	309	412
16	DURIVO MIRAVIS PRIME OSO POLY OXIN D	10 fl oz/a 13.7 fl oz/a 13 fl oz/a	A A BCD	115	215	309	412

Characterization of *Stemphylium* spp. from Spinach Based on Molecular Data, Host Response, and Azoxystrobin Sensitivity

K.A. Spawton[1], M.L. Derie[1], J.C. Correll[2], L.A. Stein[3], G. Olaya[4], R.N. Raid[5], G.V. Sandoya[5], T.L. Peever[6], and L.J. du Toit[1]

[1]Washington State University, Mount Vernon, WA; [2]University of Arkansas, Fayetteville, AR; [3]Texas A&M University, Uvalde, TX; [4]Syngenta Crop Protection, Vero Beach, FL; [5]University of Florida, Belle Glade, FL; [6]Washington State University, Pullman, WA

ABSTRACT (APS annual meeting, Cleveland, OH, USA, 3-7 Aug. 2019)

Stemphylium leaf spot of spinach causes losses under warm, humid conditions. The causal agent was described originally as *Stemphylium botryosum* based on anamorph and teleomorph morphology. As a result of increasing losses to this disease and evidence of diversity among *Stemphylium* isolates from spinach, molecular characterization, pathogenicity tests, and azoxystrobin resistance screening were performed on isolates sampled from symptomatic plants in Arizona, California, Florida, Oregon, Texas, and Washington as well as isolates from seeds grown in six countries, including the USA. The internal transcribed spacer 1 and 2 regions of ribosomal DNA, and regions of *glyceraldehyde-3-phosphate dehydrogenase* and *calmodulin* were sequenced. Two spinach cvs., Mandolin and Viroflay, were inoculated with each isolate. Isolates clustered into two phylogenetic lineages: 1) isolates most similar to *S. vesicarium*, and 2) isolates previously identified as *S. botryosum*. The former caused small (≤ 5 mm diameter) lesions on Mandolin 2 days after inoculation, but no symptoms on Viroflay, even after 21 days. The latter produced larger (5 to 15 mm diameter) lesions on both cultivars within 10 to 14 days. Isolates resistant to azoxystrobin (EC_{50} > 10 mg/liter) were detected, and mutations conferring resistance are being characterized. Differences in the biology, epidemiology, and management of these two causal agents of Stemphylium leaf spot of spinach are being investigated.

INTRODUCTION

- In 2001, the causal agent of Stemphylium leaf spot of spinach was first described in California and identified as *S. botryosum* based on morphology (5). Soon after, the disease was reported in Washington, Oregon, Delaware, Maryland, Florida, Arizona, and Texas (1,2,5,6,8,9). The fungus is seedborne and occurs in all regions of spinach seed production (4).
- In 2017 and 2018, other species of *Stemphylium* were reported to be pathogenic on spinach in Japan and Italy, including *S. beticola* (3,6).
- Recently, growers in Texas and Florida have reported reduced efficacy of QoI fungicides against Stemphylium leaf spot.
- The objectives of this study were to identify the causal agent causing Stemphylium leaf spot of spinach through molecular analyses, screen spinach cultivars for susceptibility to *Stemphylium* isolates, and assess the sensitivity of isolates to azoxystrobin.

Fig. 1. Typical symptoms caused by lineage 1 (A) and lineage 2 (B) on the spinach cv. Mandolin.

MATERIALS AND METHODS

- Isolates of *Stemphylium* from symptomatic plants and seed lots were selected to represent a range of years, locations, and cultivars of spinach production.
- **Causal agent identification:** ITS rDNA, *gapdh*, and *cmdA* loci were sequenced. Phylogenetic lineages were determined based on BLAST results and phylogenetic analyses.
- **Pathogenicity:** Each isolate was inoculated onto two spinach cvs., Mandolin and Viroflay.
- **Azoxystrobin sensitivity:** Isolates were grown on medium amended with azoxystrobin at 0, 0.001, 0.01, 0.1, 0.1, 1, and 10 μg/ml (with 100 μg SHAM/ml) to assess sensitivity of mycelial growth and conidial germination to the fungicide. Some isolates were also tested at 100 μg azoxystrobin/ml.

REFERENCES

1. du Toit, L.J., and Derie, M.L. 2001. Plant Dis. 85:920.
2. Everts, K.L., and Armentrout, D.K. 2001. Plant Dis. 85:1209.
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RESULTS

- **Causal agent identification:** Based on both *gapdh* and *cmdA* sequences, all isolates grouped in two phylogenetic lineages: isolates in lineage 1 (*n* = 147) most closely matched *S. vesicarium* and isolates in lineage 2 (*n* = 44) most closely matched *S. beticola* in the NCBI database with 99 to 100% identity scores. However, ITS rDNA sequences had 100% identity score to *S. vesicarium* or *S. globuliferum*. The latter is not a recognized species in recent studies (10). Therefore, spinach isolates previously identified morphologically as *S. botryosum* appear to be more closely related to *S. beticola*, as supported by phylogenetic analyses (*data not shown*).
- **Pathogenicity**:
  - Isolates of lineage 1 produced leaf spots on Mandolin only, while isolates of lineage 2 produced leaf spots on both cultivars (**Table 1**).
  - Isolates of lineage 1 produced numerous small lesions (≤ 5 mm diameter) that each developed a dark margin (**Fig. 1A**). Isolates of lineage 2 produced fewer, larger lesions (5 to 10 mm diameter), each with a diffuse, rapidly expanding margin (**Fig. 1B**).
- **Azoxystrobin sensitivity**: Mycelial growth of isolates of lineage 1 was sensitive to azoxystrobin at ≥ 10 μg/ml whereas isolates of lineage 2 were sensitive at ≥ 0.1 μg/ml (**Fig. 2**). Conidial germination of isolates of lineage 1 was reduced at 100 μg/ml compared to 1 μg/ml for isolates of lineage 2 (**Fig. 3**).

Table 1. *Stemphylium* isolates sequenced and tested for pathogenicity.

				Pathogenicity	
Isolate	Year	Location	DNA-based ID	Mandolin	Viroflay
St009	2001	WA, USA	lineage 2	+	+
St018	2002		lineage 2	+	+
St030	2002	WA, USA	lineage 2	+	+
St125	2004		lineage 2	+	+
St257	2004	AZ, USA	lineage 2	+	+
St354[a]	2005	Netherlands	lineage 2	+	+
St406	2012	OR, USA	lineage 2	+	+
St416	2014	AZ, USA	lineage 1	+	-
St428	2016	CA, USA	lineage 1	+	-
St430	2018	TX, USA	lineage 1	+	-
St468	2017	FL, USA	lineage 1	+	-
St469	2017	FL, USA	lineage 1	+	-
St480		FL, USA	lineage 1	+	-
St481	2019	TX, USA	lineage 1	+	-
St485	2019	TX, USA	lineage 1	+	-
St510	2018	FL, USA	lineage 1	+	-
St511	2018	FL, USA	lineage 1	+	-
St522	2019	TX, USA	lineage 1	+	-
St525	2019	TX, USA	lineage 1	+	-
St535	2019	TX, USA	lineage 1	+	-
St542	2019	TX, USA	lineage 1	+	-
St544	2019	TX, USA	lineage 1	+	-
St554	2019	TX, USA	lineage 1	+	-
St562	2019	TX, USA	lineage 1	+	-
St567	2019	TX, USA	lineage 1	+	-
St571	2019	TX, USA	lineage 1	+	-

[a]St354 was the only non-USA isolate tested to date for pathogenicity and sensitivity to azoxystrobin. Isolates of lineage 1 (*n* = 24) and lineage 2 (*n* = 6) were from seed lots grown in the European Union, Denmark, the Netherlands, Chile, Italy, China, and France ~ 15 years ago (4).

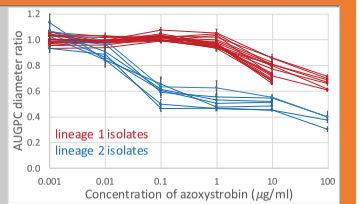


Fig. 2. Mycelial growth of *Stemphylium* isolates on azoxystrobin amended medium. AUGPC diameter ratio is the area under the growth progress curve of the mycelial diameter measured at 3, 5 and 7 days of growth on azoxystrobin amended medium divided by the AUGPC of the mycelial diameter on non-amended medium.

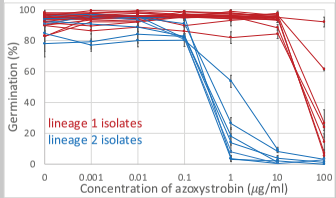


Fig. 3. Conidial germination of *Stemphylium* isolates on azoxystrobin amended medium.

CONCLUSIONS

- Symptoms caused by isolates of lineage 2 were typical of those previously described as being caused by *S. botryosum*. Molecular analyses suggest isolates previously described as *S. botryosum* from spinach are more closely related to *S. beticola* than *S. botryosum*.
- *cmdA* and *gapdh* sequences gave consistent lineages while ITS rDNA was inadequate.
- At least two phylogenetic lineages of *Stemphylium* cause Stemphylium leaf spots of spinach, although representatives of the two lineages caused different symptoms.
- Isolates of lineage 1 only caused leaf spots on Mandolin, but isolates of lineage 2 caused leaf spots on Mandolin and Viroflay. Additional cultivars need to be evaluated for susceptibility to both lineages.
- Isolates of lineage 1 were less sensitive to azoxystrobin than isolates of lineage 2, both in terms of conidial germination and mycelial growth. All isolates from spinach crops in southern states collected in 2016-19 were in lineage 1. This might account for the poor control of Stemphylium leaf spot with azoxystrobin reported by spinach growers in Texas and Florida.

Acknowledgments:

Puget Sound Seed Growers Association
Northwest Agricultural Research Foundation
Texas Wintergarden Spinach Producers Board

Investigations into Changes in Stemphylium Leaf Spot of Spinach in the USA.

Kayla Spawton, PhD student
kayla.spawton@wsu.edu
(562) 650-6191

Lindsey du Toit, Professor & Extension Plant Pathologist
dutoit@wsu.edu
(360) 848-6140

Washington State University Mount Vernon NWREC

Table 1: Severity of leaf spot (mean ± standard error of the % of leaf area with spots) 18 days after inoculation of spinach cultivars from the seed company Rijk Zwaan with *Stemphylium* isolates of lineage 1 (isolate St523) and lineage 2 (isolate St354).

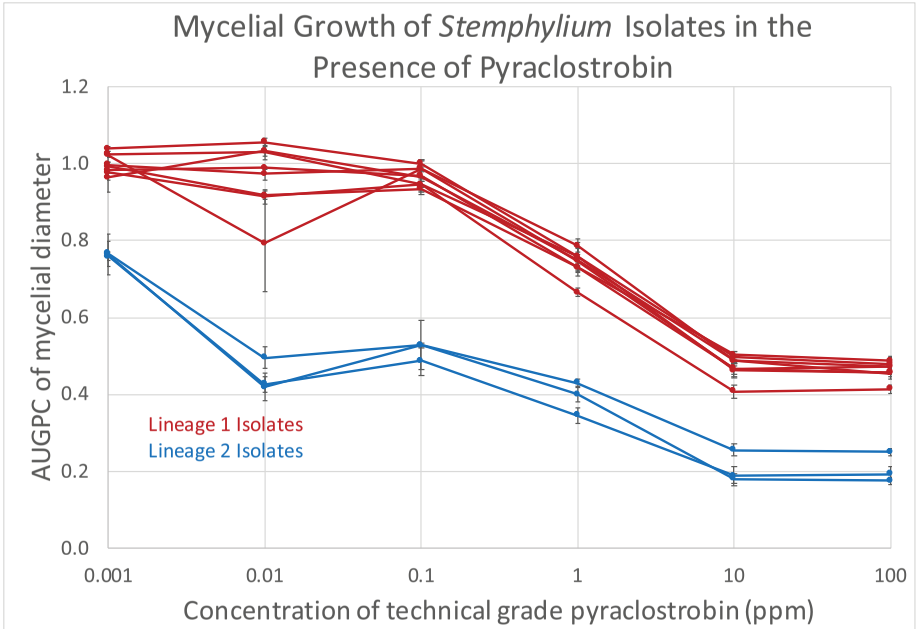
Spinach cultivar	*Stemphylium* isolate	
	St523, Lineage 1 (*S. vesicarium*)	St354, Lineage 2 (*S. beticola*)
Mandolin	43 ± 8	5 ± 0
Viroflay (Sakata)[a]	0	4 ± 1
Viroflay (Condor)[a]	0	5 ± 0
Viroflay (PopVriend)[a]	0	5 ± 0
Meerkat	23 ± 5	5 ± 0
Woodpecker	11 ± 3	1 ± 1
Parakeet	0	0
Perentie	0	5 ± 0
Baboon	0	0
Galah	0	3 ± 1
Bandicoot	0	0
Capybara	0	3 ± 1

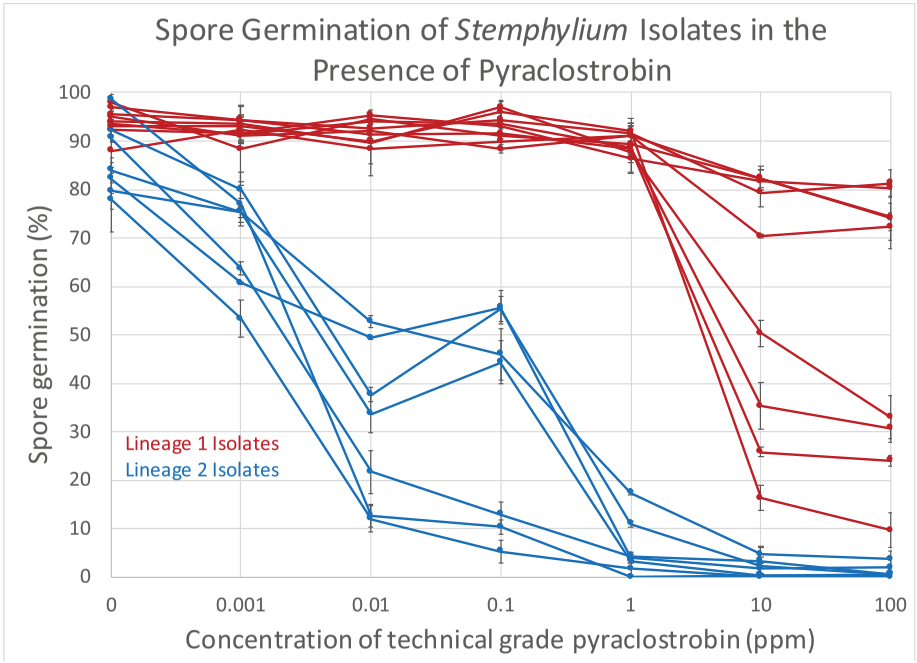
[a] Three sources of Viroflay, an open-pollinated cultivar, were used to assess whether plants grown from different lots produced similar results.

Table 2: Severity of Stemphylium leaf spot (mean ± standard error of the % of leaf area with spots) 21 days after inoculation of spinach cultivars used by Texas growers with *Stemphylium* isolates of lineage 1 (isolate St523) and lineage 2 (isolate St618). Cultivar L was part of a separate trial.

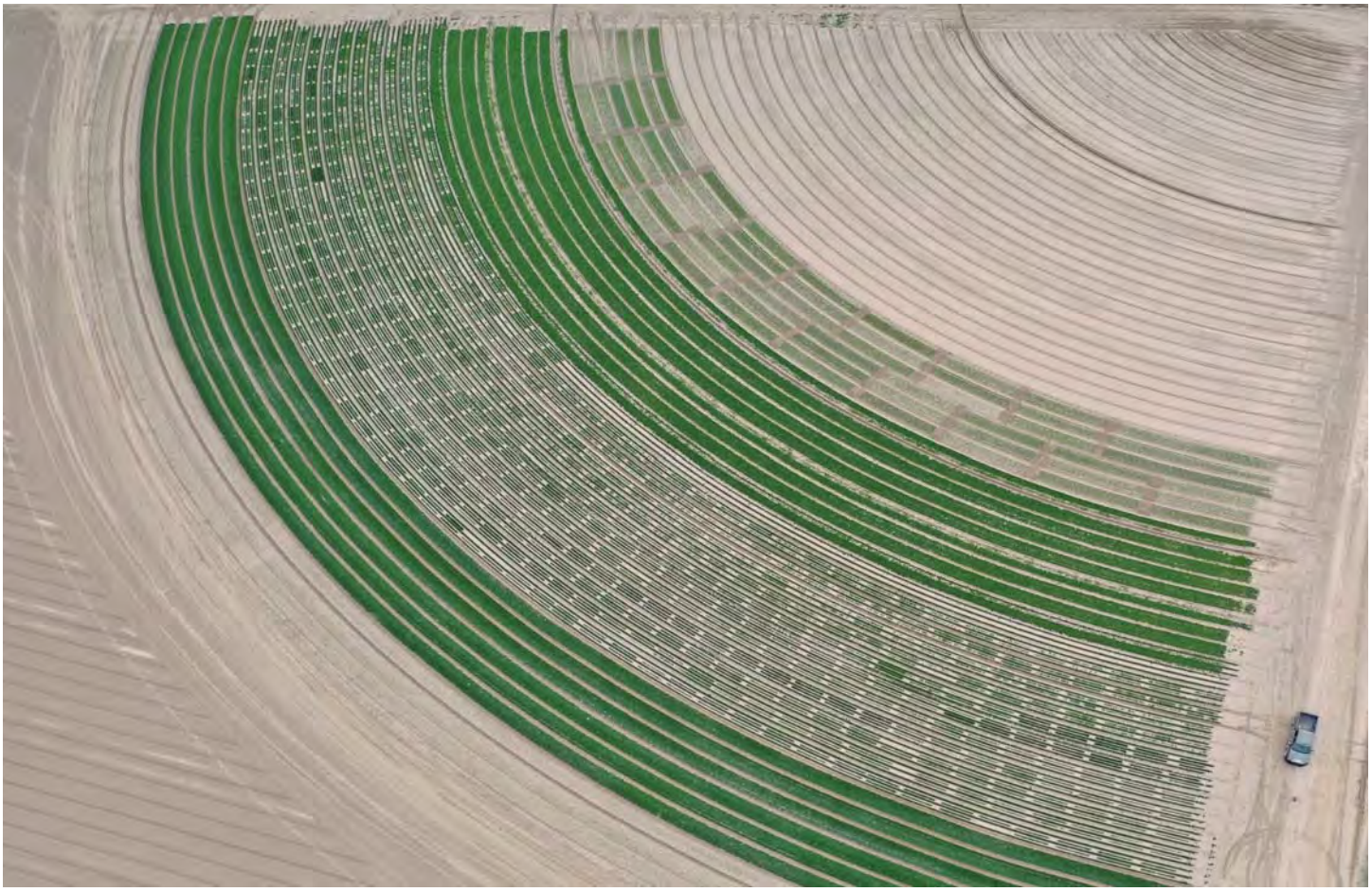
Spinach cultivar[a]	*Stemphylium* isolate	
	St523, Lineage 1 (*S. vesicarium*)	St618, Lineage 2 (*S. beticola*)
Mandolin	55 ± 3	15 ± 6
Viroflay	0	10 ± 4
A = Perentie (Rijk Zwaan)	0	5 ± 0
B	0	5 ± 0
C	40 ± 14	6 ± 1
D	0	9 ± 4
E = Hammerhead (Rijk Zwaan)	65 ± 3	6 ± 1
F = Kookaburra (Rijk Zwaan)	50 ± 6	7 ± 2
G = Platypus (Rijk Zwaan)	43 ± 9	5 ± 0
H	0	6 ± 1
I = Patton (Bejo)	45 ± 3	5 ± 0
J	0	14 ± 4
K	0	10 ± 4
L	0	7.5 ± 2

[a] Cultivars received from Texas growers with letter codes. Some names were provided after the trial was completed.





January 15, 2020—637 Spinach Cultivars in White Rust/Disease Nursery



Photos provided by: Julia Paige Ritchie, Tiro Tres Farms

January 15, 2020—637 Spinach Cultivars in White Rust/Disease Nursery

Photos provided by: Julia Paige Ritchie, Tiro Tres Farms

NOTES

*A **HUGE** thank you to all who helped us get ready for this field day, especially the sponsors listed in your program. No doubt, without their help, this would not be possible!!*