Wintergarden
Spinach
Producers
Board

2023 Spinach Field Day
February 22, 2023
10:00 a.m. – 1:00 p.m.
Tiro Tres Farms – Crystal City, TX

2022-2023 White Rust Trial

Coordinated by: Larry Stein, Paige Ritchie and Maribel Alonzo
Texas A&M AgriLife Extension Service
All photos provided by Paige Ritchie, Tiro Tres Farms

Agenda

10:00 a.m. - 1 :00 p.m.

Larry Stein	Introductions Texas A&M AgriLife Extension Service, Uvalde
Ed Ritchie	Welcome President of Wintergarden Spinach Producers Board
Leslie Dominguez	CEUs Zavala County, CEA Agriculture Texas A&M AgriLife Extension Service
Larry Stein	Tour Overview of the Research Trials, Cone Planter, White Rust Control Trial
Mike Phillips	Overview of Fungicide Control Trial Stemphylium Cargile Consulting
Lindsey Du Toit and Kayla Spawton	Overview of Stemphylium Screening Trials Washington State University
Kimberly Cochran	Overview of Anthracnose Screening Texas A&M AgriLife Extension Service, Uvalde
Carlos Avila	Potential Spinach Seed for Grain Texas A&M AgriLife Research, Weslaco

Field Tour of research plots

Lunch

Recognition of Sponsors

Group Photo

Evaluation

You are welcome to stay after 1:00 pm

2023 White Rust Nursery

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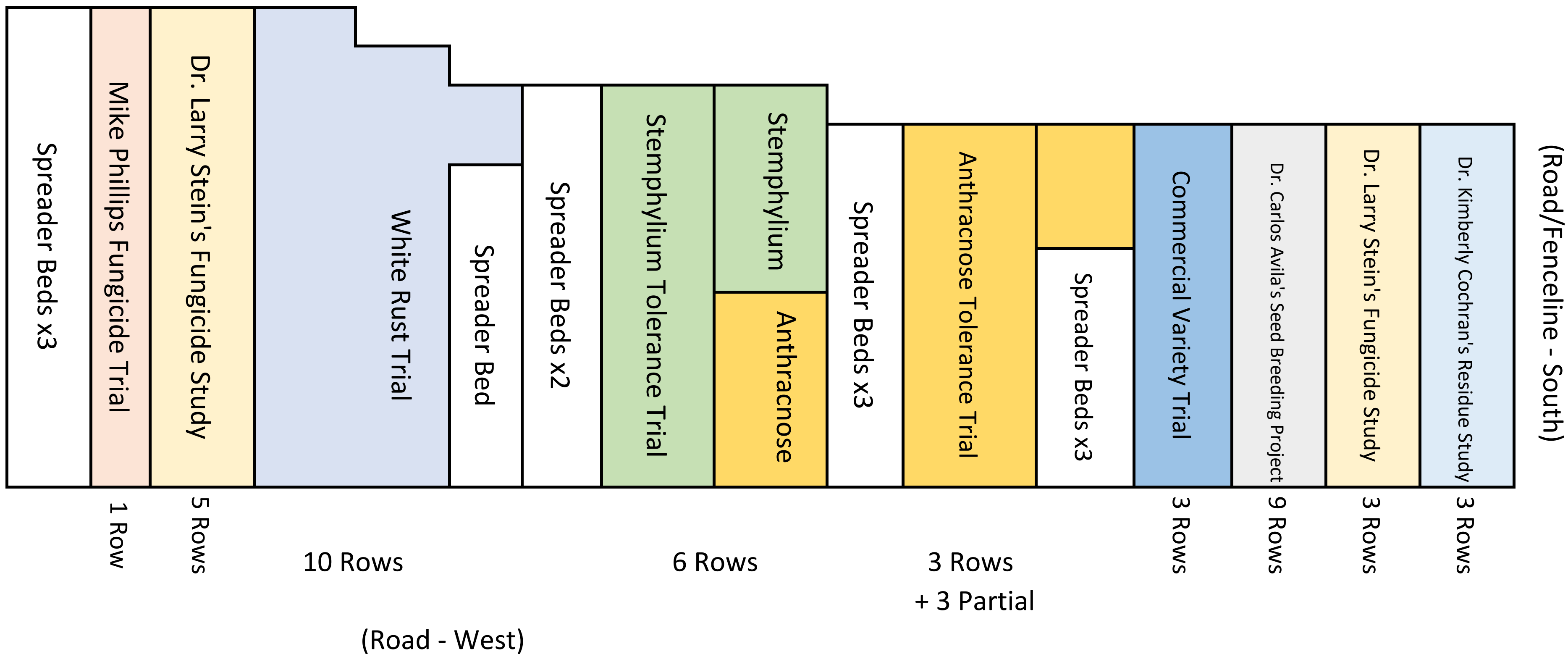
Research Plot Layout 2022-23



WHITE RUST TRIAL 2022-23

Plant Date: 12/1/2022

Row 1

D15	D16
D13	D14
D11	D12
D9	D10
D5/7	D8
D5/7	D6
D3	D4
D1	D2
C5	0
C3	C4
C1	C2
B75	0
B73	B74
B71	B72
B69	B70
B67	B68
B65	B66
B63	B64
B61	B62
B59	B60
B57	B58
B55	B56
B53	B54
B51	B52
B49	B50
B47	B48
B45	B46
B43	B44
B41	B42
B39	B40
B37	B38
B35	B36
B33	B34
B31	B32
B29	B30
B27	B28
B25	B26
B23	B24
B21	B22
B19	B20
B17	B18
B15	B16
B13	B14
B11	B12
B9	B10
B7	B8
B5	B6
B3	B4
B1	B2
A5	0
A3	A4
A1	A2

Row 2

D18	D17
D20	D19
D22	D21
D24	D23
D26	D25
D28	D27/29
D30	D27/29
D32	D31/33
D34	D31/33
D36	D35
D38	D37
D40	D39
D42	D41
D44	D43
D46	D45
D48	D47
D50	D49
E2	E1
E4	E3
E6	E5
E8	E7
E10	E9
0	E11
F2	F1
F4	F3
F6	F5
F8	F7
F10	F9
F12	F11
F14	F13
F16	F15
F18	F17
F20	F19
F22	F21
F24	F23
F26	F25
F28	F27
F30/32	F29/31
F30/32	F29/31
F32/34	F31/33
F36	F35
F38	F37
F40	F39
F42	F41
F44	F43
F46	F45
F48	F47
F50	F49
F52	F51
F54	F53
F56	F55

Row 3

B45	B46
B43	B44
B41	B42
B39	B40
B37	B38
B35	B36
B33	B34
B31	B32
B29	B30
B27	B28
B25	B26
B23	B24
B21	B22
B19	B20
B17	B18
B15	B16
B13	B14
B11	B12
B9	B10
B7	B8
B5	B6
B3	B4
B1	B2
A5	0
A3	A4
A1	A2
I1	0
H19	H20
H17	H18
H15	H16
H13	H14
H11	H12
H9	H10
H7	H8
H5	H6
H3	H4
H1	H2
G7	0
G5	G6
G3	G4
G1	G2
F75	0
F73	F74
F71	F72
F69	F70
F67	F68
F65	F66
F63	F64
F61	F62
F59	F60
F57	F58

Row 4

B48	B47
B50	B49
B52	B51
B54	B53
B56	B55
B58	B57
B60	B59
B62	B61
B64	B63
B66	B65
B68	B67
B70	B69
B72	B71
B74	B73
0	B75
C2	C1
C4	C3
0	C5
D2	D1
D4	D3
D6	D5
D8	D7
D10	D9
D12	D11
D14	D13
D16	D15
D18	D17
D20	D19
D22	D21
D24	D23
D26	D25
D28	D27
D30	D29
D32	D31
D34	D33
D36	D35
D38	D37
D40	D39
D42	D41
D44	D43
D46	D45
D48	D47
D50	D49
E2	E1
E4	E3
E6	E5
E8	E7
E10	E9
0	E11
F2	F1

Row 5

H15	H16
H13	H14
H11	H12
H9	H10
H7	H8
H5	H6
H3	H4
H1	H2
G7	0
G5	G6
G3	G4
G1	G2
F75	0
F73	F74
F71	F72
F69	F70
F67	F68
F65	F66
F63	F64
F61	F62
F59	F60
F57	F58
F55	F56
F53	F54
F51	F52
F49	F50
F47	F48
F45	F46
F43	F44
F41	F42
F39	F40
F37	F38
F35	F36
F33	F34
F31	F32
F29	F30
F27	F28
F25	F26
F23	F24
F21	F22
F19	F20
F17	F18
F15	F16
F13	F14
F11	F12
F9	F10
F7	F8
F5	F6
F3	F4

Row 6

H18	H17
H20	H19
0	I1
A2	A1
A4	A3
0	A5
B2	B1
B4	B3
B6	B5
B8	B7
B10	B9
B12	B11
B14	B13
B16	B15
B18	B17
B20	B19
B22	B21
B24	B23
B26	B25
B28	B27
B30	B29
B32	B31
B34	B33
B36	B35
B38	B37
B40	B39
B42	B41
B44	B43
B46	B45
B48	B47
B50	B49
B52	B51
B54	B53
B56	B55
B58	B57
B60	B59
B62	B61
B64	B63
B66	B65
B68	B67
B70	B69
B72	B71
B74	B73
0	B75
C2	C1
C4	C3
0	C5
D2	D1
D4	D3

Row 7

F37	F38
F35	F36
F33	F34
F31	F32
F29	F30
F27	F28
F25	F26
F23	F24
F21	F22
F19	F20
F17	F18
F15	F16
F13	F14
F11	F12
F9	F10
F7	F8
F5	F6
F3	F4
F1	F2
E11	0
E9	E10
E7	E6/8
E5	E6/8
E3	E4
E1	E2
D49	D50
D47	D48
D45	D46
D43	D44
D41	D42
D39	D40
D37	D38
D35	D36
D33	D34
D31	D32
D29	D30
D27	D28
D25	D26
D23	D24
D21	D22
D19	D20
D17	D18
D15	D16
D13	D14
D11	D12
D9	D10
D7	D8
D5	D6

Row 8

F40	F39
F42	F41
F44	F43
F46	F45
F48	F47
F50	F49
F52	F51
F54	F53
F56	F55
F58	F57
F60	F59
F62	F61
F64	F63
F66	F65
F68	F67
F70	F69
F72	F71
F74	F73
0	F75
G2	G1
G4	G3
G6	G5
0	G7
H2	H1
H4	H3
H6	H5
H8	H7
H10	H9
H12	H11
H14	H13
H16	H15
H18	H17
H20	H19
0	I1
0	W1
W2	W3
W4	W5
W6	W7
W8	W9
W10	W11
W12	W13
W14	W15
W16	W17
W18	W19
W20	W21
W22	W23
W24	W25

Row 9

W117	W116
W115	W114
W113	W112
W111	W110
W109	W108
W107	W106
W105	W104
W103	W102
W101	W100
W99	W98
W97	W96
W95	W94
W93	W92
W91	W90
W89	W88
W87	W86
W85	W84
W83	W82
W81	W80
W79	W78
W77	W76
W75	W74
W73	W72
W71	W70
W69	W68
W67	W66
W65	W64
W63	W62
W61	W60
W59	W58
W57	W56
W55	W54
W53	W52
W51	W50
W49	W48
W47	W46
W45	W44
W43	W42
W41	W40
W39	W38
W37	W36
W35	W34
W33	W32
W31	W30
W29	W28
W27	W26

Row 10

W118	W119
W120	W121
W122	W123
W124	W125
W126	W127
W128	W129
W130	W131
W132	W133
W134	W135
W136	W137
W138	W139
W140	W141
W142	W143
W144	W145
W146	W147
W148	W149
W150	W151
W152	W153

Spreader Bed

Spreader Bed

Spreader Bed

STEMPHYLIUM/ANTHRACNOSE/COMMERCIAL TRIAL LAYOUT 2022-23

Plant Date: 12/20/2022

Stemphyllium | Anthracnose | Commercial

Spreader Bed

Spreader Bed

58	59	60
55	56	57
52	53	54
49	50	51
46	47	48
43	44	45
40	41	42
37	38	39
34	35	36
31	32	33
28	29	30
25	26	27
22	23	24
19	20	21
16	17	18
13	14	15
10	11	12
7	8	9
4	5	6
1	2	3
61	62	63
58	59	60
55	56	57
52	53	54
49	50	51
46	47	48
43	44	45
40	41	42
37	38	39
34	35	36
31	32	33
28	29	30
25	26	27
22	23	24
19	20	21
16	17	18
13	14	15
10	11	12
7	8	9
4	5	6
1	2	3

63	62	61
3	2	1
6	5	4
9	8	7
12	11	10
15	14	13
18	17	16
21	20	19
24	23	22
27	26	25
30	29	28
33	32	31
36	35	34
39	38	37
42	41	40
45	44	43
48	47	46
51	50	49
54	53	52
57	56	55
60	59	58
63	62	61
3	2	1
6	5	4
9	8	7
12	11	10
15	14	13
18	17	16
21	20	19
24	23	22
27	26	25
30	29	28
33	32	31
36	35	34
39	38	37
42	41	40
45	44	43
48	47	46
51	50	49
54	53	52
57	56	55

Spreader Bed

Spreader Bed

Spreader Bed

34	35	36
31	32	33
28	29	30
25	26	27
22	23	24
19	20	21
16	17	18
13	14	15
10	11	12
7	8	9
4	5	6
1	2	3
61	62	63
61	62	63
58	59	60
55	56	57
52	53	54
49	50	51
46	47	48
43	44	45
40	41	42
37	38	39
34	35	36
31	32	33
28	29	30
25	26	27
22	23	24
19	20	21
16	17	18
13	14	15
7/10	8/11	9/12
4	5	6
1	2	3
61	62	63
58	59	60

39	38	37
42	41	40
45	44	43
48	47	46
51	50	49
54	53	52
57	56	55
60	59	58
63	62	61

Spreader Bed | Spreader Bed | Spreader Bed

25	26	27
22	23	24
19	20	21
16	17	18
13	14	15
10	11	12
7	8	9
4	5	6
1	2	3
25	26	27
22	23	24
19	20	21
16	17	18
13	14	15
10	11	12
7	8	9
4	5	6
1	2	3
25	26	27
22	23	24
19	20	21
16	17	18
13	14	15
10	11	12
7	8	9
4	5	6
1	2	3

Stemphylium/Anthracnose Tolerance Trial

Plant Date: 12/20/2022
Plant Population: 2 mil

#	Seed Company	Variety	Germ	Seedcount	Treated	Treatment
1	Enza	E03D 1096	79	~72,000	no	
2	Seminis/BAYER	Mykonos	86	70,620	yes	Thiram
3	Nunhems/BASF	Nembus		55,542	no	
4	Nunhems/BASF	Volans		54,259	no	
5	Nunhems/BASF	Nun 7553 Aries		53,990	no	
6	Rijk Zwaan	51-IN539		52,817	yes	Maxim 480FS
7	Pop Vriend	Colusa	94	51,546	no	
8	Rijk Zwaan	51-or200		51,149	no	
9	Nunhems/BASF	Scorpius		49,669	no	
10	Pop Vriend	PV1611	96	49,348	no	
11	Pop Vriend	Laredo	97	49,304	no	
12	Pop Vriend	PV1664	96	46,327	no	
13	Pop Vriend	Cocopah	94	45,400	no	
14	Pop Vriend	Nevada	99	45,269	no	
15	Enza	E03D 1084	96	45,156	no	
16	Pop Vriend	Skarne PV1656	98	44,384	no	
17	Enza	Traverse	94	43,666	no	
18	Pop Vriend	PV1526	94	43,654	no	
19	Pop Vriend	PV1719	94	43,614	no	
20	Enza	Longhorn	99	43,369	no	
21	Rijk Zwaan	Baboon (51-529)	93	43,043	yes	Thiram 480 Apron XL
22	Pop Vriend	Dallas	94	42,430	no	
23	Pop Vriend	Denton PV1617	98	41,615	no	
24	Pop Vriend	PV1569	95	41,273	no	
25	Pop Vriend	Harmonica	95	41,086	no	
26	Pinnacle	E6T		40,000	no	
27	Rijk Zwaan	Tarsier (51-728)	93	39,831	no	
28	Rijk Zwaan	51-se730		39,501	no	
29	Pop Vriend	Bandera	94	38,803	no	
30	Pop Vriend	Onyx PV1713	92	38,803	no	
31	Rijk Zwaan	Boxfish (51-370)	87	38,117	no	
32	Pop Vriend	Quartz PV1720	95	37,706	no	
33	Nunhems/BASF	Callisto		34,149	no	
34	Rijk Zwaan	Aardvark (51-376)		33,419	no	
35	Nunhems/BASF	Nun 07557		30,650	no	
36	Nunhems/BASF	Minkar		30,550	no	
37	Nunhems/BASF	Formax		30,478	no	
38	Rijk Zwaan	Bonnethead (51-722)	95	30,385	no	
39	Nunhems/BASF	Alcor		30,128	no	
40	Nunhems/BASF	Tabit		29,999	no	
41	Bejo	Patton	92	29,248	no	
42	Pinnacle	2051		29,112	no	
43	Pop Vriend	PV1716	96	29,077	no	
44	Nunhems/BASF	Crater		28,936	no	
45	Pop Vriend	PV1610	97	28,553	no	
46	Rijk Zwaan	51-se734		28,201	yes	Apron XL: Metalaxyl M
47	Bejo	Pershing	94	28,189	no	
48	Enza	Crosstrek	91	26,858	no	
49	Pop Vriend	Opal PV1718	98	26,706	no	
50	Enza	Frontier	94	26,471	no	
51	Seminis/BAYER	Kona	96	25,954	yes	F300
52	Seminis/BAYER	Motutapu	87	25,669	yes	Thiram
53	Enza	E03D 1078	94	25,120	no	
54	Nunhems/BASF	Dracus		24,928	no	
55	Pop Vriend	Kiowa	96	24,814	no	
56	Seminis/BAYER	Jolo	95	24,642	yes	Thiram
57	Nunhems/BASF	Canopus		23,401	no	
58	Vilmorin	Fulla		~23,000	no	
59	Vilmorin	Odin		~23,000	no	
60	Nunhems/BASF	Corvus		22,682	no	
61	Seminis/BAYER	Rangitoto	95	22,192	yes	Thiram
62	Nunhems/BASF	Regor		21,572	no	
63	Vilmorin	Zisa		~18,000	no	

Commercial Showcase

Plant Date: 12/20/2022

Plant Population: 2 mil

#	Seed Company	Variety	Germ	Seedcount	Treated	Treatment
1	Seminis/BAYER	Mykonos	86	70,620	yes	Thiram
2	Nunhems/BASF	Tabit	96	56,408	no	
3	Rijk Zwaan	51-737		54,102	no	
4	Nunhems/BASF	Aries Nun 07553	97	53,990	no	
5	Rijk Zwaan	Yakalo (51-729)	95	49,443	no	
6	Pop Vriend	PV1664	96	46,327	no	
7	Pop Vriend	Cocopah	94	45,400	no	
8	Rijk Zwaan	51-539	92	50,000	no	
9	Pop Vriend	Skarne PV1656	98	44,384	no	
10	Enza	Traverse	94	43,666	no	
11	Pop Vriend	PV1569	94	43,654	no	
12	Enza	Longhorn	99	43,369	no	
13	Enza	Frontier	94	42,361	no	
14	Rijk Zwaan	Hammerhead	90	42,034	yes	
15	Nunhems/BASF	Nembus	93	33,460	no	
16	Nunhems/BASF	Regor	94	32,286	no	
17	Rijk Zwaan	Bonnethead (51-722)	95	30,385	no	
18	Bejo	Patton	92	28,189	no	
19	Pinnacle	2051		29,211	no	
20	Rijk Zwaan	51-734		28,201	yes	
21	Bejo	Pershing (3592)	94	29,248	no	
22	Enza	Crosstrek	91	26,858	no	
23	Seminis/BAYER	Kona	96	25,954	yes	F300
24	Seminis/BAYER	Motutapu	87	25,669	yes	Thiram
25	Pop Vriend	Kiowa	96	24,814	no	
26	Seminis/BAYER	Jolo	95	24,642	yes	Thiram
27	Seminis/BAYER	Rangitoto	95	22,192	yes	Thiram

Dr. Carlos Avila's Seed Breeding Project

Plant Date: 12/20/2022
Population: ~ 2 mil

plot-North	El Prado	Raccoon	El Giga	Corvus	Tabit	Minkar	Dracus	7553-Aries	Octans	road-South
	CA 1	CA 2	CA 3	CA 4	CA 5	CA 6	CA 7	CA 8	CA 9	

Stemphylium Leaf Spot of Spinach:

Susceptibility of Cultivars to *Stemphylium vesicarium*, Resistance of the Pathogens to Strobilurin (FRAC group 11) Fungicides, and Population Genetics of the Pathogen

Kayla Spawton & Lindsey du Toit, February 2023
Washington State University Northwestern Washington Research & Extension Center

- **Background:** Historically, Stemphylium leaf spot of spinach was thought to be caused by the fungus *Stemphylium botryosum*. However, isolations completed at Washington State University (WSU) and the University of Arkansas (UA) over the last five years from spinach crops in Arizona, California, Florida, and Texas revealed at least two species of *Stemphylium* can cause this disease: 1) *S. vesicarium*, and 2) *S. beticola*. The isolates previously identified as *S. botryosum* are now known to be *S. beticola*, a species first described in 2017 from sugar beet crops in the Netherlands.
- ***Stemphylium vesicarium* is the predominant species causing Stemphylium leaf spot of spinach in the Wintergarden area of Texas:** Over the past five years, *S. vesicarium* was shown to be the cause of Stemphylium leaf spot for 26 of 27l spinach samples sent to WSU from the Wintergarden area of Texas (11 samples from the 2018-19 season, 11 from 2019-20, 2 from 2020-21, and 2 from 2021-22). Only one TX spinach sample was infected with *S. beticola*.
- **Resistance of *Stemphylium vesicarium* isolates to strobilurin (FRAC group 11) fungicides:** Isolates of *S. vesicarium* and *S. beticola* from spinach were tested for sensitivity to two FRAC group 11 fungicides commonly used on spinach: azoxystrobrin (e.g., Quadris) and pyraclostrobin (e.g., Cabrio). Most of the *S. vesicarium* isolates came from spinach crops in Texas and Florida, and all were far less sensitive to both fungicides than isolates of *S. beticola* (**Fig. 1**). The results were confirmed with greenhouse studies in which plants were sprayed with Quadris, Cabrio, or water and then inoculated with *S. vesicarium*. Neither of the fungicides was effective against the isolates of *S. vesicarium*. Additionally, all the isolates of *S. vesicarium* had a mutation in the *cytochrome b* gene (G143A) that is commonly associated with strobilurin fungicide resistance. In contrast, the isolates of *S. beticola* did not have this mutation. This explains why spinach growers in Texas have had difficulty controlling Stemphylium leaf spot with strobilurin fungicides.
- **Susceptibility of spinach cultivars to Stemphylium leaf spot and white rust:** Spinach cultivars were screened for resistance to Stemphylium leaf spot caused by *S. vesicarium* in a field trial near Crystal City, TX in each of three seasons. In 2020-21, 79 spinach cultivars were planted in three replicate plots per cultivar, at a baby leaf population density, and inoculated with *S. vesicarium*. White rust also developed in the trial from natural infection. Each plot was rated for severity of white rust eight weeks after planting, and severity of Stemphylium leaf spot a week later, on a 1 to 10 scale, with 1 = no symptoms and 10 = 100% of leaves symptomatic. There was a wide range in severity of symptoms among cultivars ($P < 0.001$) for both diseases (**Fig. 2**). Severity of Stemphylium leaf spot averaged 2.9 over the trial (ranged from 1.0 to 8.0 per plot). Of the 79 cultivars, 21 had no symptoms (mean severity of 1.0), 30 were partially resistant (1.1 to 3.0), 6 had moderate ratings (3.1 to 5.0), and 22 were susceptible (5.1 to 7.0) (**Fig. 2A**). White rust severity averaged 4.0 (range of 1.0 to 10.0 per plot). Five cultivars did not develop white rust (mean of 1.0), 24 were partially resistant (1.1 to 3.0), 29 had moderate ratings (3.1 to 5.0), 14 were partially susceptible (5.1 to 7.0), and 7 were highly susceptible (7.1 to 8.7) (**Fig. 2B**). No cultivar was completely resistant to both diseases. Salamander, PV 1569, Colusa, Sunangel, Spiros, Baboon, and Fantail had mean ratings ≤ 2.0 for both diseases. A similar trial was completed in 2021-22 with 81 spinach cultivars planted at a baby leaf population density, and 6 cultivars planted at a processing population density (**Fig. 3**). Of the 87 cultivars, 46 had been planted in the 2020-21 trial (**Fig. 4**). Severity of Stemphylium leaf spot in the 2021-22 trial ranged from 1.0 to 7.0, with an average of 2.1. Of the 87 cultivars, 10 had a mean rating of 1.0, 58 had a mean severity ranging from 1.1 to 3.0 (partially resistant), and 19 had a mean severity of 3.1 to 5.0 (moderate) (**Fig. 3A**). White rust severity ranged from 1.0 to 9.0, with an average of 5.2. None of

the cultivars was completely resistant to white rust (mean of 1.0), 11 were partially resistant (1.1 to 3.0), 32 were moderate (3.1 to 5.0), 33 were partially susceptible (5.1 to 7.0), and 11 were highly susceptible (7.1 to 8.3) (**Fig. 3B**). Of the 10 cultivars that exhibited no symptoms of Stemphylium leaf spot, one was partially resistant to white rust: San Juan. Of 11 cultivars partially resistant to white rust, nine were partially resistant to Stemphylium leaf spot: Cabezon, Kodiak, PV-1569, Bonnethead, Baboon, Sunangel, Waterbuck (51-727), Budgerigar, and Mandolin. The 2022-23 trial was planted in Dec. 2022 with 63 spinach cultivars.

- ***S. vesicarium* isolates from 2 Texas spinach crops with Stemphylium leaf spot had less genetic diversity than *S. vesicarium* isolates from the seed lots used to plant those crops:** Isolates of *S. vesicarium* were collected from two spinach crops in Texas in 2020, and were compared genetically to isolates obtained from the seed lots used to plant those crops. When the isolates were characterized and grouped genetically, those from Field 1 (*n* = 27 isolates) were placed into 4 genetic groups, while the isolates from the seed lot planted in that field (*n* = 33 isolates) were far more diverse, with 24 groups. Similarly, isolates from leaves in Field 2 (*n* = 34) were placed into 4 genetic groups, while isolates from the seed lot used to plant that crop (*n* = 26) were in 24 groups. A majority of isolates from the two fields were placed in the same genetic group. Six isolates from each of these four *S. vesicarium* populations were tested for pathogenicity on spinach. All 12 isolates from leaf samples were pathogenic but only 2 of the 12 seed isolates were pathogenic.
- **Seed isolates of *S. vesicarium* that were pathogenic on spinach were less diverse than seed isolates of *S. vesicarium* that were not pathogenic on spinach.** *S. vesicarium* isolates from 10 seed lots (two seed lots from each of Denmark, France, the Netherlands, New Zealand, and the Pacific Northwest USA) were tested for genetic diversity. For 5 of the 10 seed lots, a majority of the isolates tested were not pathogenic on spinach, and the number of genetic groups of the *S. vesicarium* isolates from these lots ranged from 20 to 33. For the other 5 seed lots, a majority of the isolates tested were pathogenic on spinach, and the number of genetic groups of the isolates from these lots ranged from 2 to 21. The results show that spinach seed can be colonized by pathogenic and non-pathogenic strains of *S. vesicarium*, and there tends to be much less genetic variability on seed lots infected primarily with isolates pathogenic on spinach than those infected primarily with non-pathogenic isolates.
- **Ongoing research:** The spinach cultivar trial planted near Crystal City, TX in Dec. 2022 will be rated for severity of Stemphylium leaf spot and white rust in Feb. 2023, and results compared with cultivar ratings from the previous two field trials. In addition, more isolates of *S. vesicarium* from the 12 spinach seed lots and 2 Texas field crops described above will be tested genetically to understand the population genetics of *S. vesicarium* isolates associated with spinach seed and spinach crops.

Acknowledgements

We thank the Texas Wintergarden Spinach Producers' Board, Puget Sound Seed Growers' Association, Washington State Commission on Pesticide Registration, ARCS Foundation, and Western SARE for funding this project; seed company personnel, seed growers, crop consultants, and other agricultural companies for in-kind support; and the Vegetable Seed Pathology team at Washington State University (Michael Derie, Marilen Nampijja, Tomasita Villaroel, and Babette Gundersen) for technical support. We also acknowledge the hard work of our collaborators in Texas, including Dr. Larry Stein, Ed and Paige Ritchie, Jimmy Crawford, Mike Phillips, and their respective teams who made the field trials possible.

For more details, contact:
Kayla Spawton (kayla.spawton@wsu.edu) or Lindsey du Toit (dutoit@wsu.edu).

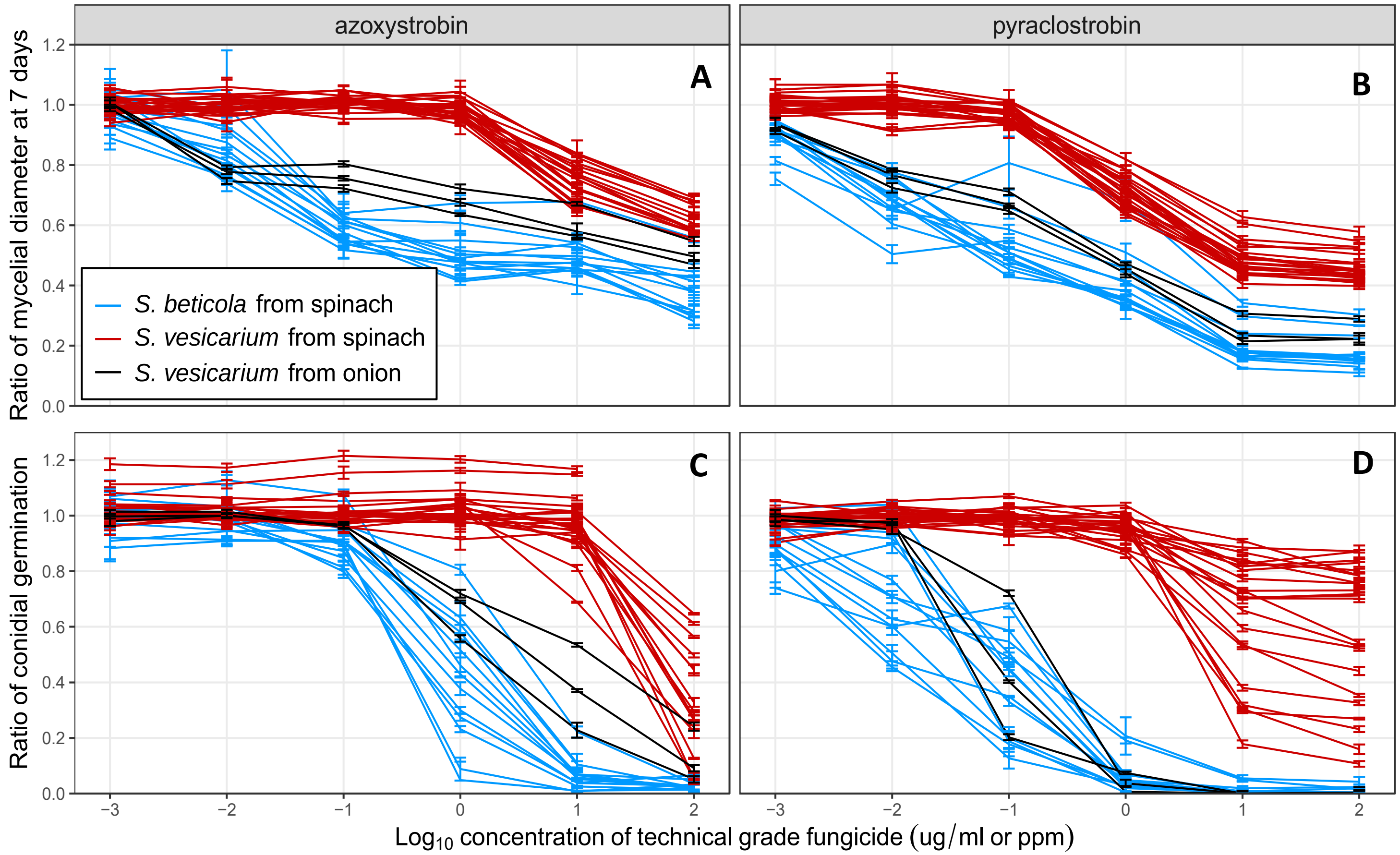


Fig. 1. Mycelial growth (**A** and **B**) and spore germination (**C** and **D**) of 23 isolates of *Stemphylium vesicarium* from spinach (red lines), 13 isolates of *S. beticola* from spinach (blue lines), and 3 isolates of *S. vesicarium* from onion (black lines) growing on agar medium amended with technical grade azoxystrobin (**A** and **C**) or pyraclostrobin (**B** and **D**) at 0.001, 0.01, 0,1, 1, 10, and 100 ppm. The ratios for mycelial growth and conidial germination were calculated as the colony diameter or percentage spore germination of each isolate on agar medium at each fungicide concentration divided by the colony diameter or percentage spore germination, respectively, of that isolate on agar medium not amended with fungicide.

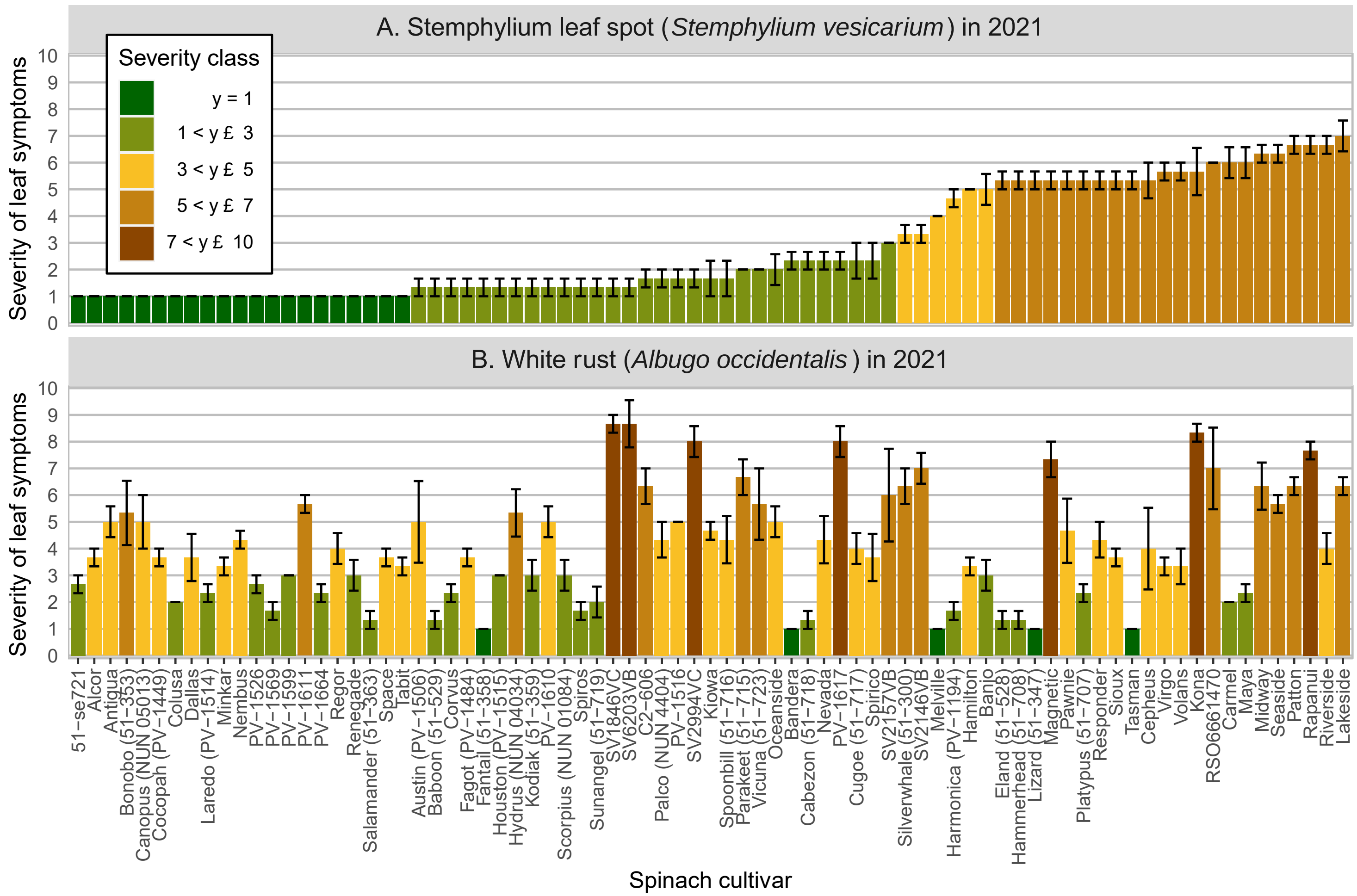


Fig. 2. Mean severity of Stemphylium leaf spot caused by *Stemphylium vesicarium* (**A**), and white rust caused by *Albugo occidentalis* (**B**), for each of 79 spinach cultivars in a field trial in Crystal City, TX in 2020-21. Disease ratings were completed 9 and 8 weeks after planting, respectively, on a scale of 1 to 10 (1 = no symptoms, and 10 = 90-100% of the canopy with symptoms). Each cultivar was planted in three replicate plots, with each plot 10 feet long x 1 bed wide. Plots were inoculated with a mix of three isolates of *S. vesicarium* from TX. White rust developed as a result of natural infection. Cultivars are arranged in the same order in **A** and **B** to highlight differences in susceptibility to the two diseases. Ratings are color-coded based on mean severity of symptoms for each disease.

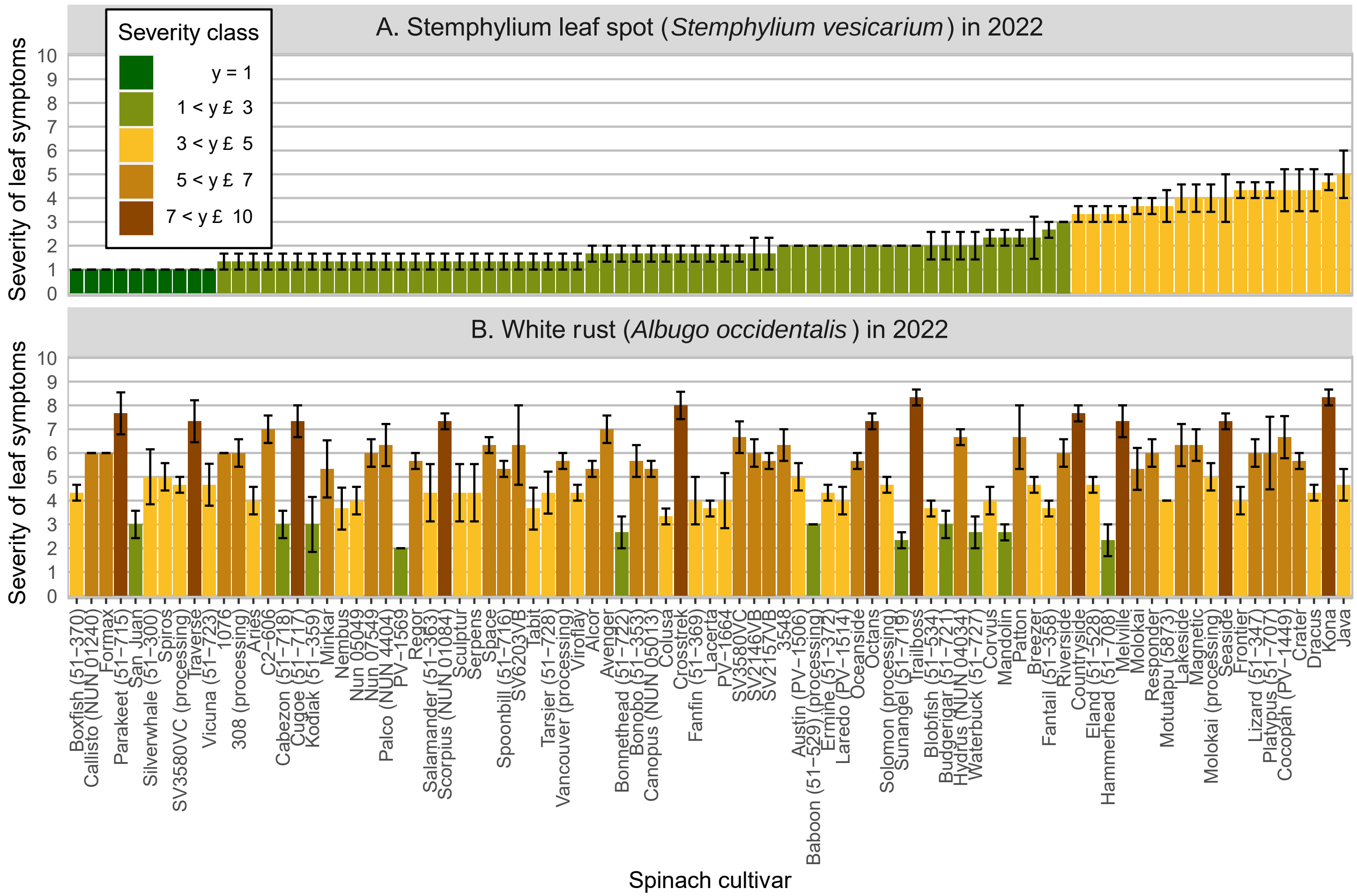


Fig. 3. Mean severity of Stemphylium leaf spot caused by *Stemphylium vesicarium* (**A**), and white rust caused by *Albugo occidentalis* (**B**), for each of 87 spinach cultivars planted in a field trial in Crystal City, TX in 2021-22. Disease ratings were completed on 15 Feb. 2022, 9 weeks after planting, on a scale of 1 to 10 (1 = no symptoms, and 10 = 90-100% of the canopy with symptoms). Each cultivar was planted in three replicate plots at 2.5 million seed/acre (except for 6 processing cultivars planted at 0.5 million seed/acre: 308, Vancouver, Baboon, Molokai, SV3580VC, and Solomon), with each plot ~3.05 m long x 1 bed wide. Plots were inoculated with a mix of three TX isolates of *S. vesicarium*. White rust developed as a result of natural infection. Cultivars are arranged in the same order in **A** and **B** to highlight differences in susceptibility to the two diseases. Ratings are color-coded based on mean severity of symptoms for each disease.

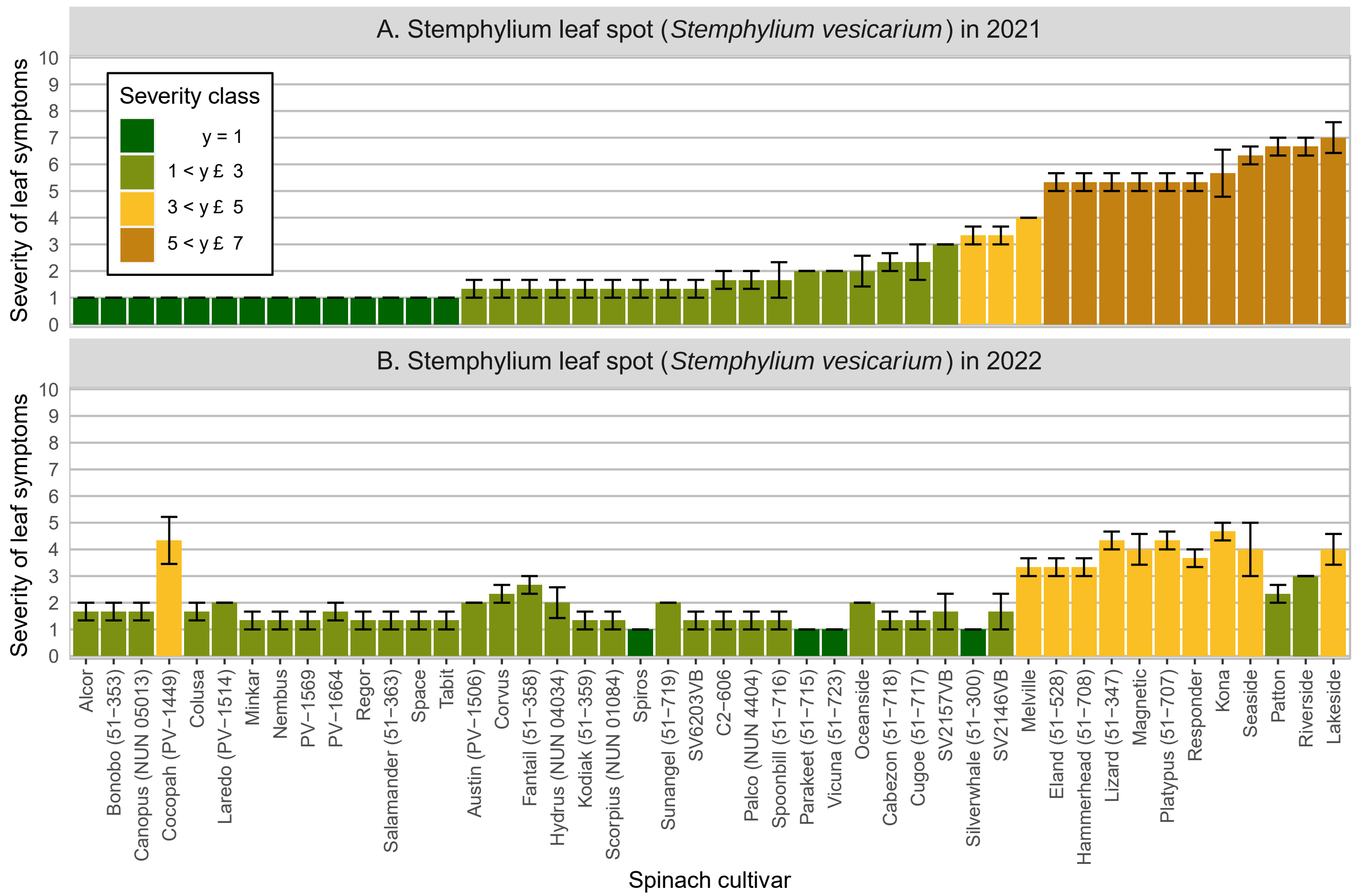


Fig. 4. Mean severity of Stemphylium leaf spot caused by *Stemphylium vesicarium* for each of the 46 cultivars planted in both the 2020-21 (**A**) and 2021-22 (**B**) field trials in Crystal City, TX. Disease ratings were completed 9 weeks after planting, on a scale of 1 to 10 (1 = no symptoms, and 10 = 90-100% of the canopy with symptoms). Each cultivar was planted in three replicate plots, with each plot 10 feet long x 1 bed wide. Plots were inoculated with a mix of three isolates of *S. vesicarium* from TX. Cultivars are arranged in the same order in **A** and **B** to highlight similarities in susceptibility of the cultivars between the two trials. Ratings are color-coded based on mean severity of symptoms.

BEYOND SALAD: HARVESTING SPINACH SEED FOR GRAIN CONSUMPTION

Carlos A. Avila[1,2,3], Samuel Zapata[4,5], Larry Stein[2,6]
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In response to consumer and producer demands, Texas A&M AgriLife in collaboration with the Wintergarden Spinach Producers Board have been working on developing solutions to increase the spinach industry competitiveness. Texas is one of the leading spinach-producing states in the US for both, the fresh and canning markets. Unfortunately, the higher-value fresh market production where producers make most of their profit, is constantly challenged by endemic diseases that significantly reduce producers' income. Even when resistant cultivars and cultural practices are used by growers, mild disease damage can happen, negatively affecting spinach quality and therefore reducing its commercial value for fresh market. In contrast, under those conditions, spinach could still produce significantly high yields of seed for grain with valuable nutritional content that can fetch premium prices for the gluten-free niche markets. Intriguingly, little or no deliberate efforts have been made to evaluate spinach seed yield and nutritional potential as food. This project funded by the Specialty Crop Block Grant program from the Texas Department of Agriculture evaluated spinach grain quality as a potential source of income at the end of the crop cycle when producers have finished leaf harvesting as an alternative source of income to the farmer.

1) Nutritional content of Spinach seed for its potential use as food additive

We performed measurements on grain nutritional content on USDA accessions at the Texas A&M AgriLife Research and Extension Center – Weslaco, TX. Seeds including the seed coat, were prepared for evaluation by grinding and performing solvent extractions as per standard protocol.

1.1 Amino acid content

Results indicate that there is a high diversity in seed amino acid content in spinach accessions (table 1). For all 19 amino acids evaluated, a wide range in content was observed. For example, aspartic acid population mean was 106.5 nmol/g with a minimum of 36.2 nmol/g and a maximum of 353.9 nmol/g (table 1). Similar results were observed in the rest of amino acid measured. This diversity can be used to improve nutritional content seed for grain (e.g. human feeding). In addition to it nutritional value, high amino acid content in seeds has been correlated to improved vigor and germination. Those traits are of high interest to the industry and producers to have uniform plant density in the field.

Table 1. Summary of Amino acid content in Spinach USDA collection (nmol/ mg). Grains from 242 USDA accessions were grinded and measured by UHPLC.

nmol/mg	Aspartic Acid	Glutamic Acid	Asparagine	Serine	Glutamine	Histidine	Threonine	Glycine	Arginine	Alanine
Mean	106.5	214.2	23.8	70.9	16.1	49.4	50.4	158.0	106.7	68.9
Std Dev	56.2	103.6	15.7	26.3	11.2	18.8	23.4	65.7	40.7	27.8
Min	36.2	84.2	5.0	20.2	0.0	9.9	13.5	61.3	34.9	22.9
Max	353.9	662.8	56.3	158.8	68.3	129.0	150.8	444.6	264.5	156.4
Range	317.7	578.6	51.4	138.7	68.3	119.1	137.3	383.3	229.5	133.5

nmol/mg	Tyrosine	Cystine	Valine	Methionine	Phenylalanine	Leucine	Tryptophan	Isoleucine	Lysine
Mean	16.5	28.7	61.3	4.4	49.8	43.0	27.5	67.4	39.4
Std Dev	7.5	19.7	32.8	2.0	32.4	21.1	59.8	26.2	15.1
Min	4.1	1.7	18.4	1.3	11.4	12.5	1.5	25.0	8.0
Max	47.0	93.2	196.7	9.5	187.5	145.8	259.2	151.3	81.5
Range	42.9	91.5	178.4	8.2	176.1	133.3	257.7	126.4	73.5

1.2 Mineral Content

Results indicate that there is a high diversity in seed mineral content in spinach accessions (table 2). For all 8 minerals evaluated, a wide range in content was observed. For example, K population mean was 9998.1 mg/kg with a minimum of 3227 mg/kg and a maximum of 24770 mg/Kg (table 1). Similar results were observed for Na, Ca, Mg, P, and S but not much range was observed in Mn and Cu. This diversity can be used to improve nutritional content seed for grain (e.g. human feeding). In addition to it nutritional value, mineral content in seeds has been correlated to improved vigor and germination. Those traits are of high interest to the industry and producers to have uniform plant density in the field.

Table 2. Summary of Mineral content in Spinach USDA collection (mg/ kg). Grains from 242 USDA accessions were grinded and measured by ICP-OES.

	Na (mg/Kg)	Ca (mg/Kg)	Mg (mg/Kg)	P (mg/Kg)	K (mg/Kg)	S (mg/Kg)	Mn (mg/Kg)	Cu (mg/Kg)
Mean	2233.0	5613.2	3424.0	4045.6	9988.1	3632.7	72.6	5.2
Std	1154.8	1705.3	675.8	708.4	3328.9	1571.1	43.1	2.3
Min	187	1570	1792	1580	3227	1579	10	1.7
Max	8289	10200	5370	5932	24770	8580	278	18.1
Range	8102	8630	3578	4352	21543	7001	268	16.4

1.3 Protein digestibility

Protein digestibility in spinach grain was evaluated using standard Megazyme Protein Digestibility Assay Kit (Medallion Labs, MN),no human or animal test was performed. Seedcoat was removed from spinach seeds (Fig 1) and seeds from relative crop species commercially grown for grain Amaranth and Quinoa were included as control for comparison.

Results indicate that protein in spinach provides ~50% of all amino acids required in the diet as compared with Amaranth and Quinoa protein in grain that provides ~20% of all amino acid required. **Therefore, spinach grain has a higher nutritional content as compared with highly demanded Amaranth and Quinoa grains.**

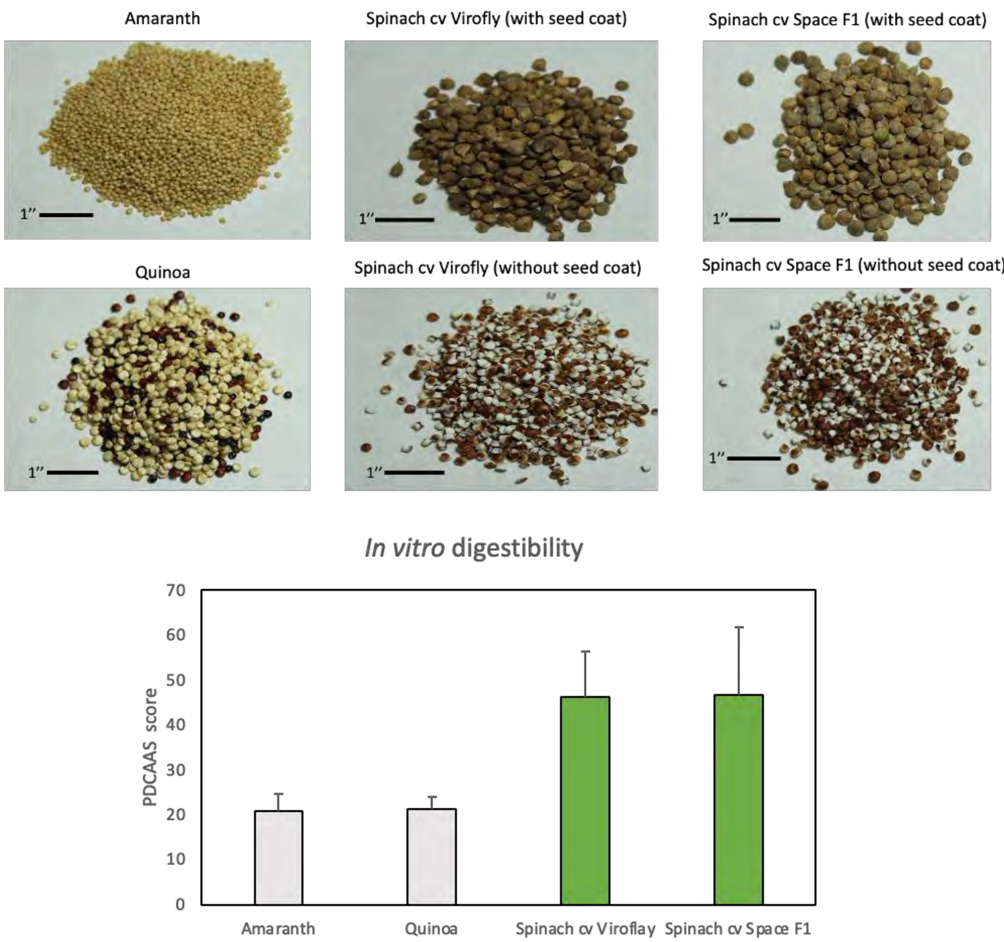

Figure 1. Spinach, Amaranth and Quinoa grain in vitro protein digestibility. Protein Digestibility Corrected Amino Acid Score (PDCAAS) indicate that protein will provide estimated % of all the amino acids required in the diet (higher PDCAAS score indicates better protein quality).

2) Seed for grain yield potential

Trials were established to evaluate a diversity panel of 320 spinach accessions from the USDA national germplasm system and three commercial cultivars to determine seed yield potential in both wild and commercial germplasm using ~2.5 ft^2 plots. Out the 320 accessions evaluated, only 232 were able to bolt and produce seed with an average of 28.38 g/plot, with a minimum of 2.599 g/plot and maximum of 166.5 g/plot , indicating a great potential for grain production (Figure 2). Commercial cultivars Viroflay, Freja and Banjo yielded 4.05, 4.13, and 2.60 g/plot; respectively. Low yield in commercial cultivars may be the reflect of breeding efforts to improve bolt resistance in spinach. In summary, results indicate there is a great potential to increase seed production not just for their utilization as grain but also to improve commercial seed yields that can potentially result in reduction of seed cost to producers.

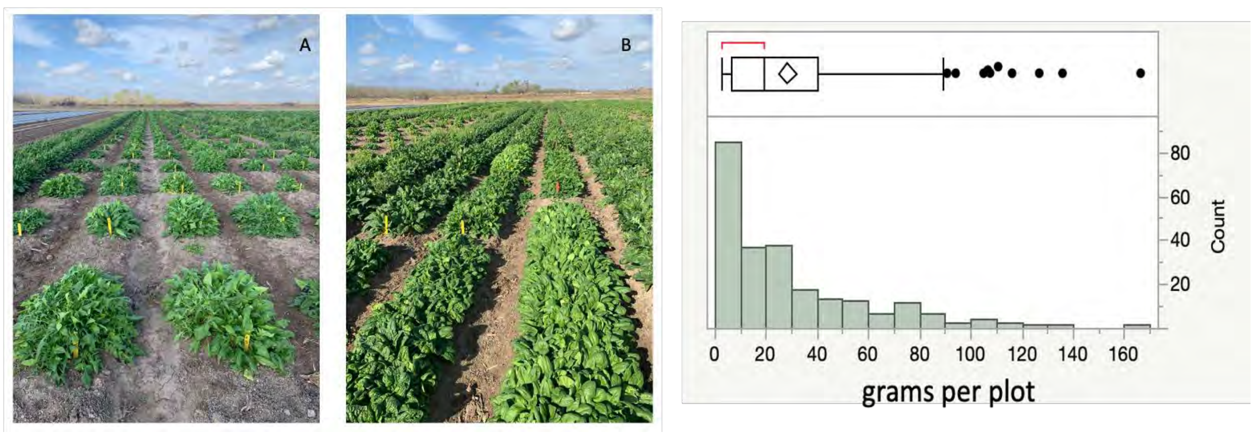

Figure 2. Evaluation of spinach USDA diversity panel (A) and commercial cultivars (B) for seed grain yield

3) Development of breeding tools to improve seed grain nutritional content

3.1 Molecular tools for Amino Acid selection in spinach grain

A genome-wide association studies (GWAS) was conducted to identify genomic regions responsible for 16 essential amino acids content in spinach grain by using single-locus (three models) and multi-locus (six models) methods. Amino acid content was measured by HPLC on a diverse panel of 223 USDA spinach accessions (see above). A total of 94 significant SNPs detected by both methods. The highest and lowest number of significant SNPs were identified for Threonine (26 SNPs) and Glycine (3 SNPs). 24 SNPs were found in more than one amino acid, in which, 16 SNPs were identified in more than three amino acids, indicating pleiotropic genetic control for 16 amino acids in spinach grain. The results of this study could guide future experimental validation, helping to understand the genetic mechanisms of amino acids content that eventually, could accelerate the genetic improvement of amino acids content in spinach grain.

3.2 Molecular tools for Mineral Content selection in spinach grain

The aim of this study was to identify the genetic basis of eight mineral elements (Ca, Cu, K, Mg, Mn, Na, P, and S) in spinach grain by using single-locus (three models) and multi-locus (six models) methods of genome-wide association studies (GWAS), using 223 USDA spinach accessions. Grain mineral content was measured by ICP-OES. A total of 55 SNPs were detected by both methods, in which, 8 and 43 SNPs were identified by single and multi-locus methods, respectively, and 2 were common between them. The highest number of significant SNPs were found for S (13 SNPs), followed by Na (12), K (8), and Ca (7) and the remaining each of them had 4 SNPs. Only two SNPs were common between Ca and Mg, indicating the possibility of pleiotropic genetic control. Identified quantitative trait loci (QTL) are valuable resources for future genetic studies, gene functional characterization, helping to understand the complex molecular mechanisms of mineral uptake, transport in spinach.

Ongoing work

- Commercial cultivar multi-year and multi-location yield trials at Wintergarden and Rio Grande Valley
- Estimation of production costs

Acknowledgements

- We would like to than Mr. Ed Ritchie, III at Tiro Tres Farms, Crystal City and the Wintergarden Spinach Production board for supporting this project including on farm field trials.
- This project was funded by the Texas Department of Agriculture, Specialty Crop Block Grant #SC-2021-18

TEXAS A&M UNIVERSITY
Plant Pathology
& Microbiology

TEXAS A&M
AGRILIFE
EXTENSION

Kimberly Cochran, Ph.D.

Plant Pathology Extension Specialist

- Root knot nematode (RKN), species pending molecular ID
- North-northeast edge of disease nursery
- Patchy bare areas, stunting, yellowing, dead plants
- Possibly exacerbated by fungal pathogens
- Full impact is unknown
    - What population threshold is required for meaningful economic/plant health impact
    - Geographic distribution is unknown
- If you have seen something similar on your farm, please contact me!

830.988.6151

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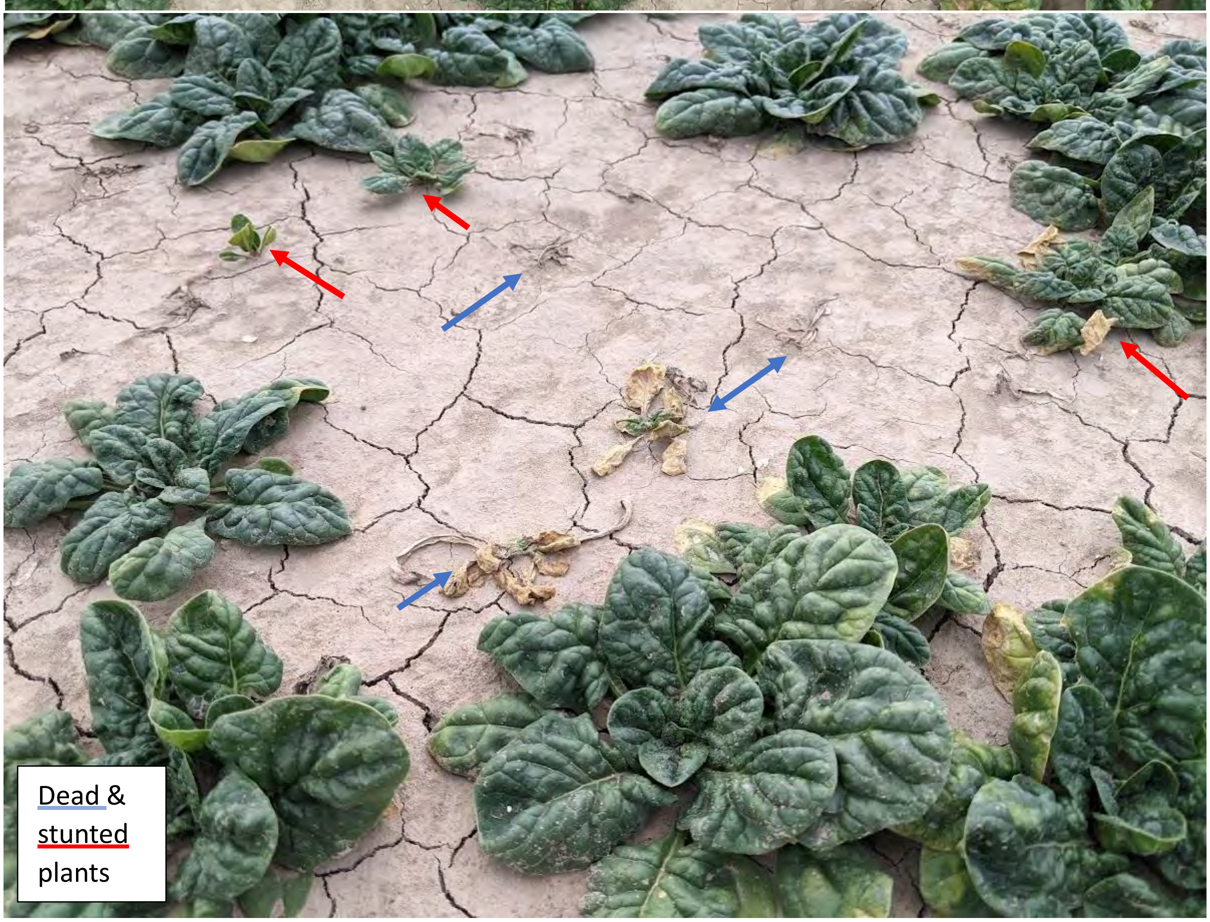
Yellowed & stunted plants,
symptomatic of RKN damage


Dead &
stunted
plants

Targeting *Stemphylium vesicarium* with Fungicide Sprays Trial ID: Spinach 2022 Mike Phillips, Cargile Consulting				
Trt No.	**Type**	**Treatment Name**	**Form Type**	**Rate Unit**
1	CHK			
2	FUNG	LUNA SENSATION	SC	7 FL OZ/A
3	FUNG	REASON	SC	7 FL OZ/A
4	FUNG	MERIVON	SC	7 FL OZ/A
5	FUNG	CABRIO	WG	16 OZ WT/A
6	FUNG	VELTYMA	SC	10 FL OZ/A
7	FUNG	MIRAVIS PRIME	SC	13.4 FL OZ/A
8	FUNG	INSPIRE	EC	7 FL OZ/A
9	FUNG	INSPIRE SUPER	SC	20 FL OZ/A
10	FUNG	GWN-10320 (ECOSWING)	SC	24 FL OZ/A
11	FUNG	GWN-9999	SC	24 FL OZ/A
12	FUNG	FUNGICIDE TO BE ADDED		

Replications: 4, Untreated treatments: 1, Design: Randomized Complete Block (RCB), Treatment units: US standard, Treated 'Plot' experimental unit size Width: 1 meters, Treated 'Plot' experimental unit size Length: 5.5 meters, Application amount: 20 GAL/AC, Mix size: .8 L, Format definitions: G-All7.def, G-All7.frm

2022 - 2023 Spinach Fungicide Trial for White Rust Control Larry A. Stein, Texas A&M AgriLife Extension Service Leslie Dominguez, Zavala CEA-AG/NR	
	TREATMENT
1	UTC
2	Water treated check
3	Double Nickel 1qt/A plus non-ionic surfactant
4	LifeGard 4.5 oz/100 gals alt. LifeGard 4.5 oz/100 gals plus Reason 8 fl.oz/A plus NIO
5	Double Nickel 1 qt/A plus NIO alternated with LifeGard 4.5 oz/100 gals plus Reason 8 fl.oz/A plus NIO
6	Double Nickel 1qt/A plus NIO alternated with Reason 8 fl.oz/A plus NIO
7	Reason 8 fl.oz/A alternated with Reavus 8 oz/A
8	Oso 6.5 oz/A plus NIO
9	LifeGard 4.5 oz/100 gals plus NIO
10	Double Nickel 1 qt/A plus NIO alternated with LifeGard 4.5 oz/100 gals plus Merivon 8 fl.oz/A plus NIO
11	Oso 6.5 oz/A plus NIO alternated with LifeGard 4.5 oz/100 gals plus Merivon 8 fl.oz/A plus NIO
12	LifeGard 4.5 oz/100 gals alternated with LifeGard 4.5 oz/100 gals plus Merivon 8 fl.oz/A plus NIO
13	Reason 8 fl.oz/A alternated with Merivon 8 fl.oz/A
14	Veltyma 10 oz/A
15	Timorex 35fl.oz/A
16	Check

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****

A tremendous THANK YOU to all who contributed to the preparation of this field day, from the sponsors listed in this program, to the farm laborers and the cooks. No doubt, without their help this event would not be possible.

****

***12/01/22; planting the white rust trial**

***12/20/22; planting the leaf spot and commercial trials**

***02/03/2023; Inoculating anthracnose**

Spinach Field Day

February 16, 2022

Tiro Tres Farms

Crystal City, TX