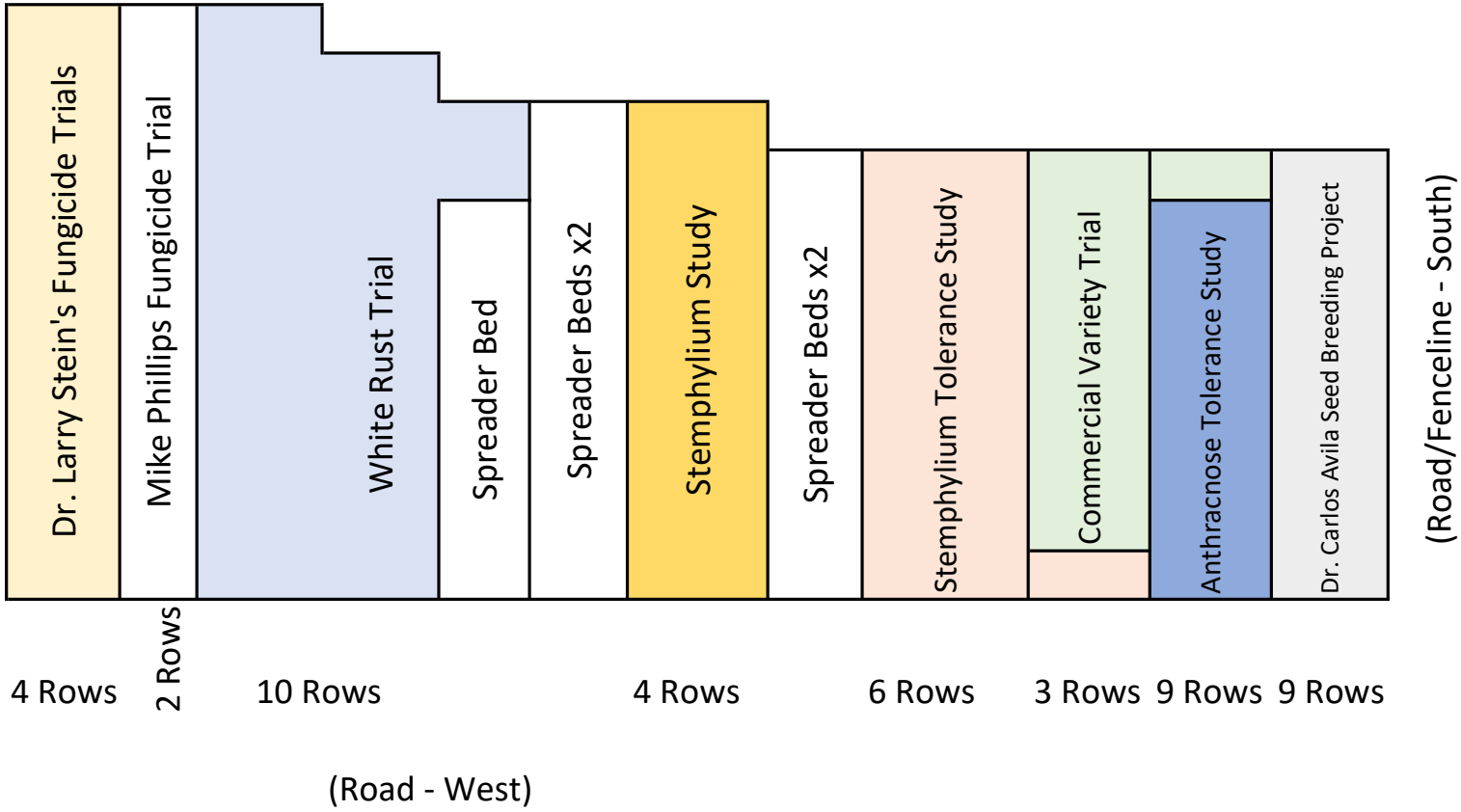
Research Plot Layout 2021-22



WHITE RUST TRIAL 2021-22

Plant Date: 11/17/2021

Row 1

E113	E114
E111	E112
E109	E110
E107	E108
E105	E106
E103	E104
E101	E102
D175	0
D173	D174
D171	D172
D169	D170
D167	D168
D165	D166
D163	D164
D161	D162
D159	D160
D157	D158
D155	D156
D153	D154
D151	D152
D149	D150
D147	D148
D145	D146
D143	D144
D141	D142
D139	D140
D137	D138
D135	D136
D133	D134
D131	D132
D129	D130
D127	D128
D125	D126
D123	D124
D121	D122
D119	D120
D117	D118
D115	D116
D113	D114
D111	D112
D109	D110
D107	D108
D105	D106
D103	D104
D101	D102
C5	0
C3	C4
C1	C2
B5	0
B3	B4
B1	B2
A5	0
A3	A4
A1	A2

Row 2

E116	E115
E118	E117
E120	E119
E122	E121
E124	E123
E126	E125
E128	E127
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	F29
F32	F31
F34	F33
F36	F35
F38	F37
F40	F39
G2	G1
G4	G3
G6	G5
G8	G7
G10	G9
G12	G11
G14	G13
G16	G15
G18	G17
G20	G19
G22	G21
G24	G23
G26	G25
G28	G27
G30	G29
G32	G31
G34	G33
G36	G35
G38	G37
G40	G39
G42	G41
G44	G43
G46	G45
G48	G47
G50	G49
G52	G51
H2	H1

Row 3

D113	D114
D111	D112
D109	D110
D107	D108
D105	D106
D103	D104
D101	D102
C5	0
C3	C4
C1	C2
B5	0
B3	B4
B1	B2
A5	0
A3	A4
A1	A2
I75	0
I73	I74
I71	I72
I69	I70
I67	I68
I65	I66
I63	I64
I61	I62
I59	I60
I57	I58
I55	I56
I53	I54
I51	I52
I49	I50
I47	I48
I45	I46
I43	I44
I41	I42
I39	I40
I37	I38
I35	I36
I33	I34
I31	I32
I29	I30
I27	I28
I25	I26
I23	I24
I21	I22
I19	I20
I17	I18
I15	I16
I13	I14
I11	I12
I9	I10
I7	I8
I5	I6
I3	I4
I1	I2
H3	H4

Row 4

D116	D115
D118	D117
D120	D119
D122	D121
D124	D123
D126	D125
D128	D127
D130	D129
D132	D131
D134	D133
D136	D135
D138	D137
D140	D139
D142	D141
D144	D143
D146	D145
D148	D147
D150	D149
D152	D151
D154	D153
D156	D155
D158	D157
D160	D159
D162	D161
D164	D163
D166	D165
D168	D167
D170	D169
D172	D171
D174	D173
0	D175
E102	E101
E104	E103
E106	E105
E108	E107
E110	E109
E112	E111
E114	E113
E116	E115
E118	E117
E120	E119
E122	E121
E124	E123
E126	E125
E128	E127
F42	F41
F44	F43
F46	F45
F48	F47
F50	F49
F52	F51

Row 5

I17	I18
I15	I16
I13	I14
I11	I12
I9	I10
I7	I8
I5	I6
I3	I4
I1	I2
H3	H4
H1	H2
G51	G52
G49	G50
G47	G48
G45	G46
G43	G44
G41	G42
G39	G40
G37	G38
G35	G36
G33	G34
G31	G32
G29	G30
G27	G28
G25	G26
G23	G24
G21	G22
G19	G20
G17	G18
G15	G16
G13	G14
G11	G12
G9	G10
G7	G8
G5	G6
G3	G4
G1	G2
F79	F80
F77	F78
F75	F76
F73	F74
F71	F72
F69	F70
F67	F68
F65	F66
F63	F64
F61	F62
F59	F60
F57	F58
F55	F56
F53	F54

Row 6

I120	I119
I122	I121
I124	I123
I126	I125
I128	I127
I130	I129
I132	I131
I134	I133
I136	I135
I138	I137
I140	I139
I142	I141
I144	I143
I146	I145
I148	I147
I150	I149
I152	I151
I154	I153
I156	I155
I158	I157
I160	I159
I162	I161
I164	I163
I166	I165
I168	I167
I170	I169
I172	I171
I174	I173
0	I175
A2	A1
A4	A3
0	A5
B2	B1
B4	B3
0	B5
C2	C1
C4	C3
0	C5
D102	D101
D104	D103
D106	D105
D108	D107
D110	D109
D112	D111
D114	D113
D116	D115
D118	D117
D120	D119
D122	D121

Row 7

F95	F96
F93	F94
F91	F92
F89	F90
F87	F88
F85	F86
F83	F84
F81	F82
E127	E128
E125	E126
E123	E124
E121	E122
E119	E120
E117	E118
E115	E116
E113	E114
E111	E112
E109	E110
E107	E108
E105	E106
E103	E104
E101	E102
D175	0
D173	D174
D171	D172
D169	D170
D167	D168
D165	D166
D163	D164
D161	D162
D159	D160
D157	D158
D155	D156
D153	D154
D151	D152
D149	D150
D147	D148
D145	D146
D143	D144
D141	D142
D139	D140
D137	D138
D135	D136
D133	D134
D131	D132
D129	D130
D127	D128
D125	D126
D123	D124

Row 8

F98	F97
F100	F99
F102	F101
F104	F103
F106	F105
F108	F107
F110	F109
F112	F111
F114	F113
F116	F115
F118	F117
F120	F119
G2	G1
G4	G3
G6	G5
G8	G7
G10	G9
G12	G11
G14	G13
G16	G15
G18	G17
G20	G19
G22	G21
G24	G23
G26	G25
G28	G27
G30	G29
G32	G31
G34	G33
G36	G35
G38	G37
G40	G39
G42	G41
G44	G43
G46	G45
G48	G47
G50	G49
G52	G51
H2	H1
H4	H3
I2	I1
I4	I3
I6	I5
I8	I7
I10	I9
I12	I11
I14	I13
I16	I15

Row 9

W35	W36
W33	W34
W31	W32
W29	W30
W27	W28
W25	W26
W23	W24
W21	W22
W19	W20
W17	W18
W15	W16
W13	W14
W11	W12
W9	W10
W7	W8
W5	W6
W3	W4
W1	W2
I75	0
I73	I74
I71	I72
I69	I70
I67	I68
I65	I66
I63	I64
I61	I62
I59	I60
I57	I58
I55	I56
I53	I54
I51	I52
I49	I50
I47	I48
I45	I46
I43	I44
I41	I42
I39	I40
I37	I38
I35	I36
I33	I34
I31	I32
I29	I30
I27	I28
I25	I26
I23	I24
I21	I22
I19	I20
I17	I18

Row 10

W38	W37
W40	W39
W42	W41
W44	W43
W46	W45
0	W47

Spreader Bed

Spreader Bed

Spreader Bed

* Planter was plugged

STEMPHYLIUM PI TRIAL 2021-22

Plant Date: 11/17/2021

Spreader Bed

Spreader Bed

Row 1

89	90
87	88
85	86
83	84
81	82
79	80
77	78
75	76
73	74
71	72
69	70
67	68
65	66
63	64
61	62
59	60
57	58
55	56
53	54
51	52
49	50
47	48
45	46
43	44
41	42
39	40
37	38
35	36
33	34
31	32
29	30
27	28
25	26
23	24
21	22
19	20
17	18
15	16
13	14
11	12
9	10
7	8
5	6
3	4
1	2

Row 2

92	91
94	93
96	95
98	97
100	99
102	101
104	103
106	105
108	107
110	109
112	111
114	113
116	115
118	117
120	119
122	121
124	123
126	125
128	127
130	129
132	131
134	133
136	135
138	137
140	139
142	141
144	143
146	145
148	147
150	149
152	151
154	153
156	155
158	157
160	159
162	161
164	163
166	165
168	167
170	169
172	171
174	173
176	175
178	177

Row 3

263	264
261	262
259	260
257	258
255	256
253	254
251	252
249	250
247	248
245	246
243	244
241	242
239	240
237	238
235	236
233	234
231	232
229	230
227	228
225	226
223	224
221	222
219	220
217	218
215	216
213	214
211	212
209	210
207	208
205	206
203	204
201	202
199	200
197	198
195	196
193	194
191	192
189	190
187	188
185	186
183	184
181	182
179	180

Row 4

266	265
268	267
270	269
272	271
274	273
276	275
278	277
280	279
282	281
284	283
286	285
288	287
290	289
292	291
294	293
296	295
298	297
300	299
302	301
304	303
306	305
308	307
310	309
312	311
314	313
316	315
318	317
320	319
322	321
324	323
326	325
328	327
330	329
332	331
0	333

Spreader Bed

Spreader Bed

Spreader Bed

STEMPHYLIUM/COMMERCIAL/ANTHRACNOSE TRIAL LAYOUT 2021-22

Plant Date: 12/15/2021

Spreader Bed

Spreader Bed

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
85	86	87
82	83	84
79	80	81
76	77	78
73	74	75
70	71	72
67	68	69
64	65	66
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

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
66	65	64
69	68	67
72	71	70
75	74	73
78	77	76
81	80	79
84	83	82
87	86	85
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
66	65	64
69	68	67
72	71	70
75	74	73
78	77	76
81	80	79
84	83	82
87	86	85

Stemphyllium

13	14	15
10	11	12
7	8	9
4	5	6
1	2	3
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
85	86	87
82	83	84
79	80	81
76	77	78
73	74	75
70	71	72
67	68	69
64	65	66
61	62	63

Commercial

18	17	16
21	20	19
24	23	22
27	26	25
30	29	28
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
66	65	64
69	68	67
72	71	70
75	74	73
78	77	76
81	80	79
84	83	82
87	86	85

Anthracnose

85	86	87
82	83	84
79	80	81
76	77	78
73	74	75
70	71	72
67	68	69
64	65	66
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

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
66	65	64
69	68	67
72	71	70
75	74	73
78	77	76
81	80	79
84	83	82
87	86	85

Commercial Trial

Plant Date: 12/15/2021

Plant Population: 1.5 mil

	Seed Company	Variety	Germ	Seedcount	Treated
1	Enza Zaden	1076		60,000	No
2	Rijk Zwaan	51-370	90	55,027	No
3	Sakata	Countryside	87	52,911	No
4	Sakata	SSP9621	97	51,718	Yes
5	Nunhems BASF	Tabit		46,408	No
6	Pop Vriend	PV-1712	99	46,327	
7	Nunhems BASF	Corvus		46,239	No
8	Bejo	Patton	92	45,352	No
9	Bejo	Space	92	43,130	No
10	Enza Zaden	Traverse		42,696	No
11	Enza Zaden	Frontier		42,361	No
12	Bejo	Spiros	92	41,314	No
13	Pop Vriend	PV-1724	93	40,177	
14	Rijk Zwaan	Tarsier (51-728)	93	39,831	No
15	Pop Vriend	PV-1723	92	39,478	
16	Pop Vriend	PV-1713	97	38,803	
17	Pop Vriend	PV-1720	99	37,521	
18	Sakata	Seaside	98	36,943	No
19	Rijk Zwaan	51-727	86	35,809	No
20	Sakata	C2-606	97	35,118	Yes
21	Rijk Zwaan	51-733		33,677	No
22	Nunhems BASF	7553		32,000	No
23	Nunhems BASF	Octans		31,188	No
24	Rijk Zwaan	Bonnethead (51-722)	95	30,385	No
25	Bejo	3548	90	30,059	No
26	Enza Zaden	Crosstrek		29,550	No
27	Bejo	Responder	90	29,510	No
28	Nunhems BASF	Crater		28,958	No
29	Sakata	Riverside	89	28,544	No
30	Enza Zaden	Trailboss		28,306	No

Stemphylium/Anthracnose Tolerance Trial

Plant Date: 12/15/2021

Plant Population: 1.5 mil

	Seed Company	Variety	Germ	Seedcount	Treated
1	Nunhems BASF	Tabit		20,624	No
2	Nunhems BASF	Serpens		20,915	No
3	Nunhems BASF	Sculptur		21,248	No
4	Nunhems BASF	Alcor		21,319	No
5	Rijk Zwaan	51-369	91	23,969	No
6	Pop Vriend	Mandolin	94	24,674	
7	Seminis/Bayer	SV6203VB	90	24,706	No
8	Nunhems BASF	Regor		24,756	No
9	Seminis/Bayer	5873-Motutapu	87	25,669	Yes
10	Rijk Zwaan	Silverwhale (51-300)	89	27,234	Yes
11	Pinnacle	San Juan	95	28,138	No
12	Pop Vriend	Austin	95	28,199	
13	Enza Zaden	Trailboss		28,306	No
14	Sakata	C2-606	93	28,410	No
15	Sakata	Riverside	89	28,544	No
16	Rijk Zwaan	Hammerhead (51-708)	90	28,781	No
17	Seminis/Bayer	Melville	93	29,120	No
18	Bejo	Responder	90	29,510	No
19	Enza Zaden	Crosstrek		29,550	No
20	Sakata	Oceanside	89	29,891	No
21	Bejo	3548	90	30,059	No
22	Rijk Zwaan	Cugoe (51-717)	94	30,119	No
23	Rijk Zwaan	Bonnethead (51-722)	95	30,385	No
24	Nunhems BASF	Formax		30,478	No
25	Nunhems BASF	Minkar		30,550	No
26	Nunhems BASF	Aries		30,773	No
27	Nunhems BASF	Octans		31,253	No
28	Nunhems BASF	NUN 4404 Palco		31,552	No
29	Rijk Zwaan	Eland (51-528)	90	31,829	No
30	Nunhems BASF	Lacerta		31,842	No
31	Nunhems BASF	Nun 05049		32,407	No
32	Nunhems BASF	Nun 07549		31,253	No
33	Rijk Zwaan	Vicuna (51-723)		35,426	No
34	Rijk Zwaan	Lizard (51-347)	95	35,865	No
35	Seminis/Bayer	Magnetic	95	36,000	No
36	Rijk Zwaan	51-372	95	36,586	No
37	Pop Vriend	Viroflay	95	36,911	
38	Sakata	Seaside	98	36,943	No
39	Seminis/Bayer	Kona		38,000	No
40	Rijk Zwaan	Sunangel (51-719)	92	38,024	No
41	Seminis/Bayer	Java	91	38,166	No
42	Seminis/Bayer	SV3580VC	94	38,852	No
43	Rijk Zwaan	Budgerigar (51-721)		38,901	No
44	Seminis/Bayer	SV2146VB	98	39,347	No

45	Rijk Zwaan	Tarsier (51-728)	93	39,831	No
46	Rijk Zwaan	Bonobo (51-353)	89	40,052	No
47	Seminis/Bayer	SV2157VB	95	40,148	No
48	Seminis/Bayer	Avenger	92	40,212	No
49	Rijk Zwaan	Parakeet (51-715)	93	40,982	No
50	Bejo	Spiros	92	41,314	No
51	Seminis/Bayer	Molokai	91	41,353	No
52	Rijk Zwaan	Salamander (51-363)	95	41,844	No
53	Enza Zaden	Frontier		42,361	No
54	Rijk Zwaan	Fantail (51-358)	92	42,487	Yes
55	Enza Zaden	Traverse		42,696	No
56	Rijk Zwaan	Blobfish (51-534)		43,056	No
57	Bejo	Space	92	43,130	No
58	Rijk Zwaan	Kodiak (51-359)	95	43,944	No
59	Rijk Zwaan	Platypus (51-707)	96	44,422	No
60	Pop Vriend	Laredo	93	44,510	
61	Pop Vriend	PV1664	87	44,950	
62	Bejo	Patton	92	45,352	No
63	Pop Vriend	Cocopah	94	45,400	
64	Pop Vriend	Colusa	95	45,957	Yes
65	Sakata	Lakeside	98	46,089	No
66	Nunhems BASF	Corvus		46,239	No
67	Pop Vriend	PV1569	97	46,327	
68	Mission	Breezer	93	46,387	No
69	Nunhems BASF	Crater		47,405	No
70	Rijk Zwaan	Spoonbill (51-716)		47,761	No
71	Rijk Zwaan	51-727	80	48,450	No
72	Nunhems BASF	NUN 04034 Hydrus		49,060	No
73	Nunhems BASF	NUN 01084 Scorpius		49,773	No
74	Enza Zaden	1076		50,000	No
75	Rijk Zwaan	Cabezon (51-718)	92	50,466	No
76	Nunhems BASF	Nembus		50,569	No
77	Nunhems BASF	NUN 01240 Callisto		50,834	No
78	Nunhems BASF	NUN 05013 Canopus		51,725	No
79	Sakata	Countryside	87	52,911	No
80	Rijk Zwaan	51-370	90	55,027	No
81	Nunhems BASF	Dracus		57,843	No

	Seed Company	**Variety**	**Germ**	**Seedcount**	**Treated**
82	Pinnacle	308		19,976	No
83	Seminis/Bayer	Vancouver	94	25,540	Yes
84	Rijk Zwaan	Baboon (51-529)	86	28,184	Yes
85	Seminis/Bayer	Molokai	97	28,710	Yes
86	Seminis/Bayer	SV3580VC	90	39,789	Yes
87	Seminis/Bayer	Solomon	81	40,284	Yes

(82-87 Planted at 500,000 plant population for processing)

Stemphylium Leaf Spot of Spinach:
Susceptibility of Cultivars to *Stemphylium vesicarium*, Resistance of the Pathogens to Strobilurin (FRAC group 11) Fungicides, and the Potential for Onion to Serve as a Host Crop

Kayla Spawton & Lindsey du Toit, February 2022
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 in du Toit's lab at Washington State University (WSU) and Correll's lab at the University of Arkansas (UA) over the last five years from spinach crops in Arizona, California, Florida, and Texas revealed there are at least two species of *Stemphylium* that can 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 are of Texas:** Over the past four years, *S. vesicarium* was the cause of Stemphylium leaf spot symptoms on almost all of the spinach samples received at Washington State University from the Wintergarden area of Texas (including 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* from spinach and onion, as well as isolates of *S. beticola* from spinach were tested in the lab for sensitivity to two FRAC group 11 fungicides commonly used in spinach crops: azoxystrobrin (e.g., Quadris) and pyraclostrobin (e.g., Cabrio). Most *S. vesicarium* isolates were from spinach crops in Texas and Florida, and all were far less sensitive to both fungicides than the 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*. 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:** In 2020-21, 79 spinach cultivars were planted in a field trial in La Pryor, TX (three replicate plots per cultivar) and inoculated with *S. vesicarium* to identify cultivars with resistance. 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 the 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. 1A**). 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. 1B**). 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.
- **Onion is a symptomatic host of *Stemphylium* isolates from spinach:** Isolates of *S. beticola* and *S. vesicarium* from spinach fields in California, Texas, and Washington, and an isolate of *S. vesicarium* from an onion crop in Washington, all caused leaf spots and leaf dieback on the onion cv. Hamilton in a greenhouse trial. The most severe symptoms were caused by the Washington onion isolate of *S.*

vesicarium. The results suggest onion may be a host for isolates of *S. vesicarium* in areas where spinach and onion are grown in proximity.

- **Ongoing research:** Two field trials were planted in the Texas Wintergarden area in late 2021 to evaluate the reactions of: 1) 333 Plant Introduction (PI) lines from the USDA spinach germplasm collection, and 2) 87 spinach cultivars for relative susceptibility to *S. vesicarium*. The cultivar trial is a repeat of the 2020-21 trial (**Fig. 2**) with additional cultivars. Furthermore, we are examining the diversity of *Stemphylium* species associated with spinach seed, and the genetic diversity of *S. vesicarium* isolates from spinach seed grown in all the main regions of the world where seed is produced. We also are comparing populations of *S. vesicarium* from symptomatic spinach crops in Texas to the seed lots used to plant those crops. This research will help us understand the potential distribution of isolates on seed and the potential relative significance of seedborne inoculum in outbreaks of the disease.

Acknowledgements

We thank the Texas Wintergarden Spinach Producers' Board, Puget Sound Seed Growers' Association, Washington State Commission on Pesticide Registration, and ARCS Foundation 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, Sanjaya Gyawali, Paul Morgan, Eliza Mae Andrews, Alex Batson, Ryan Solemslie, 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, and their respective teams who made the field trials possible.

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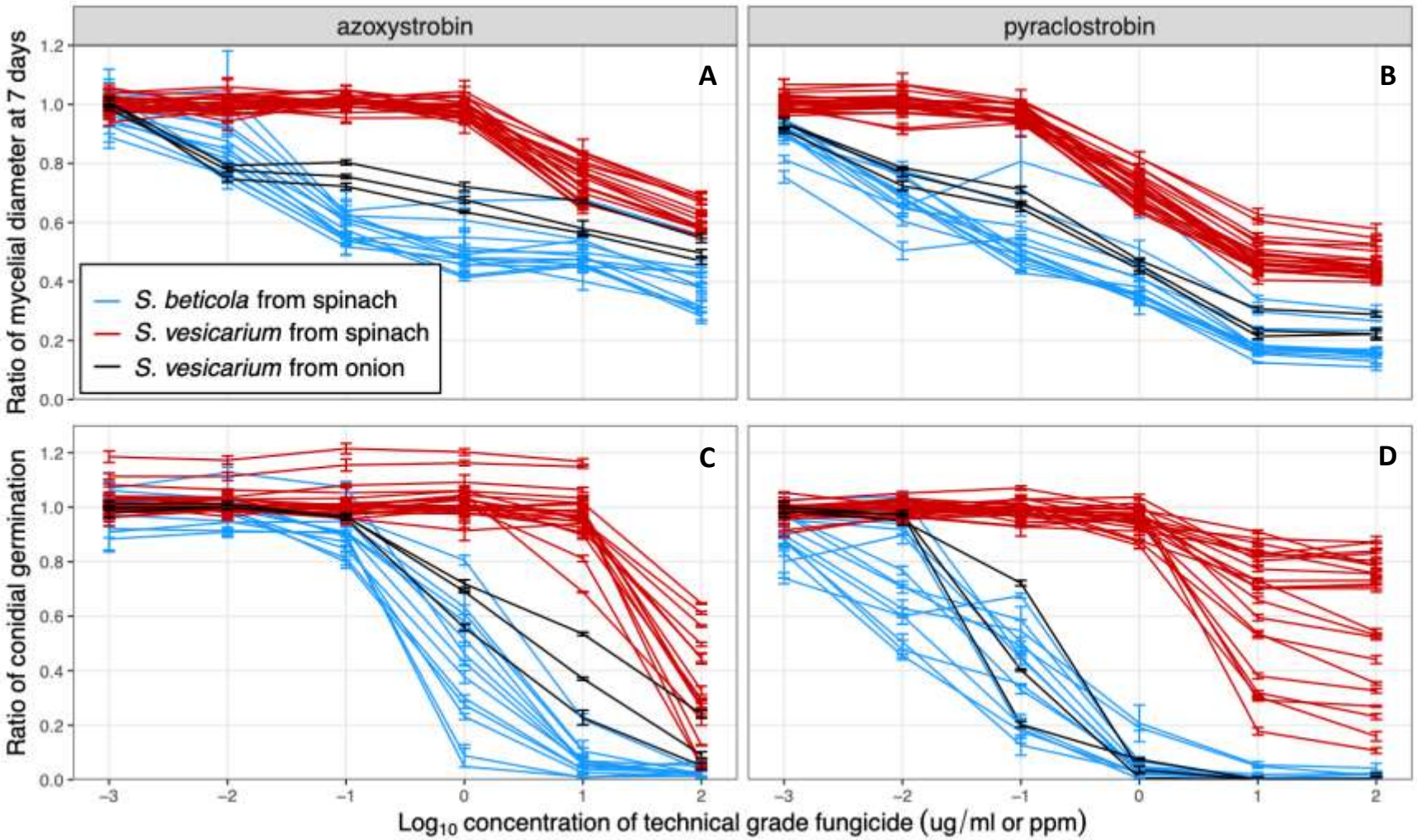

Fig. 1. Mycelial growth (**A** and **B**) and spore germination (**C** and **D**) of 23 isolates of *Stemphylium vesicarium* from spinach (red lines), 13 isolates of *S. beticola* from spinach (blue lines), and 3 isolates of *S. vesicarium* from onion (black lines) growing on agar medium amended with 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.

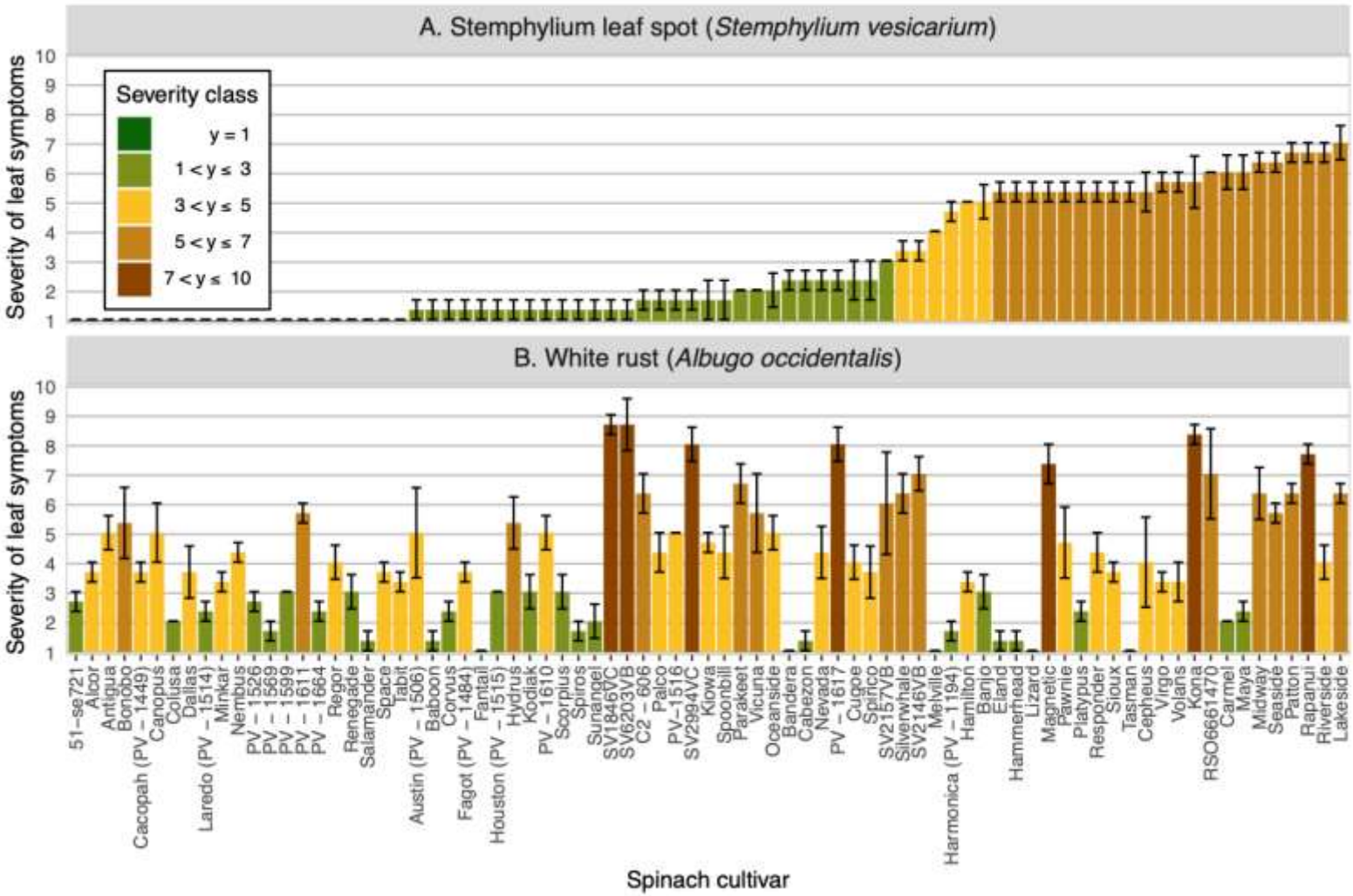

Fig. 2. Mean severity of Stemphylium leaf spot caused by *Stemphylium vesicarium* (**A**) and white rust caused by *Albugo occidentalis* (**B**) for each of 79 spinach cultivars in a field trial in La Pryor, TX in 2020-21. Disease ratings were completed 9 and 8 weeks after planting, respectively, on 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.

Cargile Consulting
Trial ID: SPINACH 2022 Location: Trial Year: 2021
Protocol ID: Investigator (Creator): Mike Phillips, Cargile Consulting

No.	Name Type	Rate Unit	1	2	3	4
1	UNTREATED CHECK		101	203	309	409
2.	LUNA SENSATION FL	7 FL OZ/A	102	210	307	409
3.	MERIVON FL	7 FL OZ/A	103	206	301	402
4.	REASON FL	7 FL OZ/A	104	201	308	407
5.	MIRAVIS PRIME FL	13.4 FL OZ/A	105	211	305	412
6.	MIRAVIS PRIME FL +ACTIGARD WG	13.4 FL OZ/A 0.38 OZ WT/A	106	204	311	403
7.	MIRAVIS PRIME FL +PROPHYTE FL	13.4 FL OZ/A 48 FL OZ/A	107	208	306	401
8.	MIRAVIS PRIME FL +LIFEGARD WG	13.4 FL OZ/A 1 FL OZ/A	108	202	303	406
9.	CABRIO WG	16 OZ WT/A	109	207	302	408
10.	INSPIRE EC	7 FL OZ/A	110	212	304	410
11.	INSPIRE SUPER FL	20 OZ/A	111	209	310	405
12.	VELTYMA BAS 751 FL	10 FL OZ/A	112	205	312	411

2021 - 2022 Spinach Fungicide Trial for White Rust Control

Larry A. Stein, Texas A&M AgriLife Extension Service

	TREATMENT
1.	Untreated check
2.	Life Gard 2 oz alt. Merivon 7 oz alt. Miravis Prime 13.4 alt. Merivon
3.	Life Gard 2 oz alt. Presidio 3 oz alt. Merivon 7 oz alt. Presidio
4.	Life Gard 2 oz alt. Merivon 7 oz alt. Reason 7 oz alt. Merivon
5.	BAS 751 (Veltyman) 10 oz/A
6.	Life Gard 2 oz alt. Prophyt 1.5 qts/A alt. Oso 8.5 oz alt. Prohyt
7.	Miravis Prime 13.4 oz/A (.25% NIS) alt. Switch 14 oz/A alt. Orondis Ultra 8 oz (.25% NIS) alt. Miravis Prime 13.4 oz/A (.25% NIS) alt. Switch 14 oz/A
8.	Miravis Prime 13.4 oz/A (NIS) + Actigard .38 oz/A alt. Switch 14 oz/A +Actigard .38 oz/A alt. Orondis Ultra 8 oz/A + Actigard .38 oz/A alt. Miravis Prime + Actigard (as before) alt. Switch + Actigard (as before)

Colletotrichum spinaciae Infested Oat Inoculum Protocol

Kimberly Cochran, Ph.D.

Plant Pathology Extension Specialist and Assistant Professor

Texas A&M University, AgriLife Extension

Field trial inoculation is an important aspect of conducting product efficacy trials and variety trials so that even disease pressure can be achieved for comparison across treatments and varieties. In my early spinach pathology efforts, I found an older protocol[1] that used glass flasks to create *Colletotrichum* sp. inoculum, which made breaking up clumps of fungal growth and getting inoculum out of flasks very difficult. Additionally, limited lab space was an issue that made large scale inoculum production with flasks a challenge in my lab. Consequently, I utilized components of the older protocol with my knowledge of creating large amounts of inoculum in past research with the goal of creating inoculum that was more easily scaled up or down for different project needs with evenly colonized consistent quality inoculum, all while being affordable, user friendly, and easily reproduceable.

Supplies:

- Single spore isolate *Colletotrichum spinaciae* culture- several plates (1/3 plate/bag of inoculum)
- Scalpels
- Autoclave
- Autoclave indicator strips
- Impulse sealer
- Metal center twist ties
- Aluminum foil
- Permanent marker
- Distilled or reverse osmosis water (500 ml/ 1190 g (42 oz, one large container) of oats)
- Mushroom spawn bags, vented (Fig 1) Use 0.2 micron size vent only, available online.
- Old fashioned oats, sufficient for project plus 10% extra. Must be old fashioned oats*, NOT instant oats.

*Raw oats (ex: animal feed) or steel cut oats are also suitable. However, I find the old-fashioned oats crumble well for hand spreading inoculum. Animal feed grade oats may need sifting prior to use.

Instructions:

1. Place 1190 g (a single large 42 oz container) of oats in a 8" x 5" x 19" large size 0.2 micron vented spawn bag, LOOSELY close and secure with a twist tie. Loosely cover openings with foil, place on an autoclave tray, and autoclave twice on a 60 min dry cycle, 24 hr apart. Place a fresh autoclave indicator strip in the autoclave for each cycle. Cool completely. Keep sterile until use.
    a. It is important to use a vented bag and not seal it tightly before autoclaving. Failing to do so can result in the bag popping or compromising the vent filter or seams.
    b. While these bags can hold more, I find they perform better if you leave ample space for mixing/shaking the bags later.
    c. Be mindful to not overload your autoclave with spawn bags. Only place bags in a single layer, preferably with a bit of space between, the quantity varies by autoclave model obviously.
2. Autoclave distilled water (500ml per 1190g (42 oz) container of oats used), twice 24 hr apart and cool completely. Keep sterile until use.
3. In a laminar flow hood or other sterile work space, add sterile water so oats are moist but not saturated using sterile technique. Close the bag and shake vigorously for approx. 30 sec so that water coats the oats and doesn't settle in the bottom.
4. Using aseptic technique, cut 1/3 of a 100mm petri dish culture into small (3-4mm squares) pieces and place into the spawn bag with the oats and water.
    a. More or less culture material can be used, which will affect the time it takes for oats to become fully colonized.
5. Promptly seal with the impulse sealer and shake vigorously to evenly distribute moisture and fungal culture pieces.
    a. I prefer to double seal bags for more durability during later mixing steps.
6. Label each bag with date and fungal isolate information and lay flat on a clean surface.
7. Incubate infested oat inoculum at or slightly above room temperature. Shake inoculum bags and break up clumps of colonized oats every other day. Inoculum should be ready in about 14-20 days, depending on incubation temperature (optimal approx. 77-80°F; 24-26.5°C). Bags should look like Fig. 2 during this process.
8. Infested oat inoculum is ready for use when you see dark fungal growth evenly throughout the oats. If I am using more than one bag I bulk the inoculum in a large container to ensure uniformity for the experiment, breaking up any clumps larger than a pea as I go. Rubbing inoculum over a mesh screen in a frame may be helpful in this step.
9. Spread infested oat inoculum evenly through the desired plot(s) with a gloved hand.
    a. I typically use approx. ½ cup per 10 x 2 ft. plot section, but this may vary by project goals. I use a scoop so that each plot gets the same quantity.
    b. Prior to use I weigh the infested oat inoculum in the scoop, then dry this and weigh it again for my records.

Tips to ensure success:

- Empty and combine bags of inoculum no earlier than the night before inoculation for optimal quality.
- If facing cooler than ideal temperatures for the fungus, inoculate plants with adequate growth so that the canopy is fairly closed to protect the inoculum from the elements.
- Irrigate after inoculation with overhead sprinkler irrigation lightly every day (ideally afternoon) for 5 days for best results. This fungus thrives with moist conditions.

Fig 1: Mushroom spawn bag with 0.2 micron vent. I use 8" X 5" X 19" size.

Fig 2: Infested oat inoculum during the incubation process.

[1]Original Colletotrichum oat inoculum by John Damicone, Ph.D., modified by Kimberly Cochran, Ph.D.

WHITE RUST RATINGS

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
A1	4	3	7	5	4	4
A2	x	7	8	x	8	8
A3	4	4	7	3	4	6
A4	x	5	7	x	5	8
A5	6	7	6	5	5	7

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
B1	8	8	9	8	8	8
B2	x	7	8	x	8	8
B3	6	8	8	7	4	7
B4	x	8	7	x	6	6
B5	7	8	8	8	8	8

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
C1	6	7	7	5	5	6
C2	x	8	7	x	5	8
C3	8	7	7	5	4	7
C4	x	9	8	x	6	8
C5	8	8	8	7	8	8

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
D1	3	8	7	7	8	8
D2	x	7	6	x	7	7
D3	1	2	2	2	3	3
D4	x	3	8	x	6	7
D5	4	6	6	4	5	4
D6	x	7	8	x	6	7
D7	6	7	8	5	6	7
D8	x	8	8	x	6	7
D9	1	5	3	3	4	5
D10	x	5	6	x	5	7
D11	4	4	6	4	5	6

WHITE RUST RATINGS

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
D12	x	3	3	x	4	4
D13	3	4	6	5	4	5
D14	x	7	7	x	6	6
D15	4	8	6	7	8	8
D16	x	7	7	x	7	5
D17	6	8	8	8	7	8
D18	x	8	7	x	4	6
D19	4	6	3	5	5	5
D20	x	4	6	x	5	5
D21	6	8	8	5	4	6
D22	x	7	8	x	5	8
D23	2	5	6	3	5	4
D24	x	6	7	x	5	5
D25	2	4	7	4	3	4
D26	x	8	8	x	7	8
D27	3	4	3	3	3	4
D28	x	8	7	x	7	6
D29	5	8	7	6	8	7
D30	x	8	8	x	6	8
D31	7	8	9	6	5	7
D32	x	8	8	x	7	8
D33	3	6	8	5	6	7
D34	x	6	7	x	4	5
D35	2	5	4	2	6	5
D36	x	6	2	x	3	4
D37	3	7	7	4	4	6
D38	x	7	7	x	5	6
D39	4	8	8	6	7	8
D40	x	6	7	x	4	5
D41	7	8	8	6	6	7
D42	x	8	8	x	7	8
D43	6	8	8	7	8	8
D44	x	9	9	x	8	9

WHITE RUST RATINGS

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
D45	5	8	9	6	8	8
D46	x	6	8	x	7	7
D47	6	7	8	5	5	6
D48	x	8	7	x	4	5
D49	3	8	8	8	7	8
D50	4	8	8	6	6	7
D51	6	8	9	6	7	8
D52	5	8	8	5	5	8
D53	6	8	8	7	7	7
D54	6	8	8	8	7	7
D55	7	8	9	7	7	8
D56	2	2	4	3	3	4
D57	2	3	3	5	4	6
D58	3	8	6	5	5	8
D59	2	8	7	4	7	6
D60	4	8	6	6	7	7
D61	2	5	5	3	5	3
D62	3	7	5	4	5	4
D63	8	8	8	4	6	5
D64	4	6	4	3	4	3
D65	7	6	5	3	6	5
D66	8	9	8	8	8	8
D67	4	8	7	5	8	5
D68	4	8	7	5	7	7
D69	3	7	7	3	6	5
D70	3	4	4	6	4	5
D71	3	4	4	7	4	6
D72	4	7	5	8	5	6
D73	5	7	6	6	7	7
D74	4	7	6	6	4	5
D75	5	7	7	6	5	6

WHITE RUST RATINGS

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
E101	3	6	7	5	5	5
E102	5	7	5	6	5	5
E103	5	7	7	4	6	4
E104	5	8	6	5	6	5
E105	6	7	7	6	8	7
E106	6	8	6	4	7	6
E107	4	8	8	5	8	7
E108	4	9	7	6	7	6
E109	4	7	6	6	8	7
E110	3	2	2	4	3	4
E111	2	3	2	3	5	3
E112	4	7	4	4	4	5
E113	2	5	3	3	6	4
E114	4	7	6	5	6	6
E115	3	6	4	4	8	4
E116	4	6	6	4	6	5
E117	5	6	7	5	8	6
E118	5	7	7	5	8	6
E119	4	9	7	4	8	6
E120	x	8	7	x	8	5
E121	6	8	6	7	8	6
E122	x	7	6	x	7	6
E123	5	7	4	5	8	5
E124	x	8	5	x	7	5
E125	5	6	6	5	8	6
E126	x	8	6	x	7	6
E127	7	7	6	4	8	6
E128	x	8	6	x	7	6

WHITE RUST RATINGS

			Date: 1/13/2022			Date: 2/1/2022		
			Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
F1	F41	F81	5	9	6	7	8	7
F2	F42	F82	X	8	6	X	6	5
F3	F43	F83	7	8	8	6	8	7
F4	F44	F84	X	8	5	X	8	5
F5	F45	F85	4	8	4	6	8	6
F6	F46	F86	X	10	8	X	8	8
F7	F47	F87	8	10	8	7	8	8
F8	F48	F88	X	10	5	X	8	5
F9	F49	F89	8	9	8	6	9	7
F10	F50	F90	X	9	7	X	8	7
F11	F51	F91	8	10	7	8	9	7
F12	F52	F92	X	10	6	X	8	6
F13	F53	F93	9	9	7	8	8	7
F14	F54	F94	X	8	8	X	7	8
F15	F55	F95	8	9	7	8	8	8
F16	F56	F96	X	4	4	X	6	5
F17	F57	F97	9	8	5	6	8	5
F18	F58	F98	X	5	6	X	5	4
F19	F59	F99	4	7	6	5	8	4
F20	F60	F100	X	6	7	X	7	6
F21	F61	F101	6	8	6	6	8	7
F22	F62	F102	X	8	7	X	6	6
F23	F63	F103	4	7	3	4	8	4
F24	F64	F104	X	6	7	X	6	6
F25	F65	F105	5	7	4	5	8	6
F26	F66	F106	X	4	7	X	5	4
F27	F67	F107	3	4	5	3	7	6
F28	F68	F108	X	8	8	X	8	6
F29	F69	F109	5	8	7	6	8	7
F30	F70	F110	X	7	7	X	7	6
F31	F71	F111	8	8	8	7	8	8
F32	F72	F112	X	8	8	X	7	6
F33	F73	F113	7	7	7	6	8	7

WHITE RUST RATINGS

			Date: 1/13/2022			Date: 2/1/2022		
			Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
F34	F74	F114	X	6	8	X	8	6
F35	F75	F115	6	7	7	5	8	4
F36	F76	F116	X	8	8	X	7	5
F37	F77	F117	7	7	6	4	7	6
F38	F78	F118	X	8	8	X	8	8
F39	F79	F119	7	8	8	5	8	7
F40	F80	F120	X	8	7	X	8	

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
G1	7	8	6	5	9	7
G2	x	8	7	x	6	7
G3	8	8	8	7	9	8
G4	x	8	8	x	9	8
G5	7	8	8	6	9	8
G6	x	8	8	x	8	7
G7	8	8	7	7	9	6
G8	x	8	6	x	6	4
G9	8	8	8	8	9	8
G10	x	7	8	x	6	8
G11	7	8	8	8	9	7
G12	x	7	7	x	9	8
G13	8	8	8	9	8	8
G14	x	8	8	x	6	6
G15	7	7	8	9	8	7
G16	x	8	8	x	9	8
G17	8	8	8	9	9	8
G18	x	7	8	x	6	5
G19	6	6	7	6	7	6
G20	x	6	7	x	6	5
G21	7	7	7	8	9	8
G22	x	8	8	x	8	7
G23	8	8	8	8	9	8

WHITE RUST RATINGS

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
G24	x	6	7	x	7	8
G25	8	8	9	9	9	8
G26	x	7	9	x	8	8
G27	8	7	8	7	8	8
G28	x	7	8	x	6	8
G29	7	8	7	8	6	8
G30	x	5	7	x	8	7
G31	7	7	7	7	6	6
G32	x	7	8	x	7	7
G33	8	8	8	8	8	8
G34	x	5	6	x	6	4
G35	8	7	8	9	8	8
G36	x	7	8	x	7	8
G37	7	7	7	6	5	6
G38	x	8	8	x	7	7
G39	6	6	7	7	6	6
G40	x	7	8	x	7	8
G41	8	8	8	9	8	7
G42	x	5	8	x	6	7
G43	8	7	5	8	6	8
G44	x	3	4	x	4	5
G45	6	7	6	7	7	6
G46	8	7	8	8	8	8
G47	8	8	8	9	8	8
G48	8	8	8	9	7	7
G49	8	8	8	9	8	8
G50	8	8	8	8	8	8
G51	7	8	8	8	7	8
G52	8	8	8	7	6	7

WHITE RUST RATINGS

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
H1	8	8	8	8	7	8
H2	6	6	7	6	6	7
H3	8	8	8	7	8	9
H4	8	8	8	8	7	8

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
I1	3	6	7	4	5	4
I2	4	5	6	5	5	3
I3	8	8	8	7	6	7
I4	8	8	8	7	5	6
I5	8	8	8	8	7	8
I6	8	7	8	8	7	8
I7	8	8	8	8	7	8
I8	7	7	8	8	6	7
I9	8	8	8	8	6	8
I10	7	7	7	4	5	6
I11	7	5	8	5	5	8
I12	6	4	6	4	3	6
I13	7	7	7	7	6	7
I14	8	7	8	8	6	8
I15	7	8	8	8	8	8
I16	9	8	8	8	8	8
I17	4	6	7	7	6	5
I18	5	7	6	7	5	5
I19	5	9	8	9	8	8
I20	7	8	8	8	6	8
I21	6	6	6	5	5	5
I22	5	6	6	6	5	7
I23	7	6	8	5	5	7
I24	6	7	8	7	6	8
I25	8	9	8	8	8	8
I26	5	8	7	7	6	7
I27	8	8	8	8	8	8

WHITE RUST RATINGS

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
I28	7	8	9	8	8	8
I29	7	8	9	7	8	8
I30	8	7	8	7	6	8
I31	8	8	8	8	8	8
I32	8	9	9	8	8	9
I33	7	8	9	9	8	8
I34	8	8	9	9	7	9
I35	5	8	8	8	7	9
I36	6	8	8	8	8	8
I37	8	8	8	8	8	8
I38	8	8	8	8	6	7
I39	5	7	7	7	7	7
I40	6	5	7	6	5	5
I41	5	6	5	4	5	5
I42	7	7	8	5	8	7
I43	5	7	7	5	5	5
I44	7	8	8	8	7	8
I45	8	8	8	7	7	8
I46	8	8	8	8	5	8
I47	7	6	6	7	6	7
I48	7	8	6	8	7	7
I49	7	8	7	7	8	7
I50	6	7	6	6	7	7
I51	4	7	7	7	6	6
I52	7	8	6	8	7	8
I53	7	8	8	8	8	8
I54	5	7	6	8	9	7
I55	6	8	8	8	8	8
I56	7	7	7	5	6	7
I57	7	8	7	5	7	7
I58	7	8	6	5	6	7
I59	7	8	7	5	6	6
I60	7	8	7	5	6	7

WHITE RUST RATINGS

	Date: 1/13/2022			Date: 2/1/2022		
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
I61	7	6	7	4	6	7
I62	8	8	6	5	6	7
I63	6	8	7	5	7	6
I64	8	7	7	4	7	7
I65	6	7	7	5	6	7
I66	8	8	6	7	8	7
I67	7	7	7	7	6	7
I68	7	7	8	6	6	8
I69	7	7	7	7	7	7
I70	7	7	8	5	7	8
I71	7	6	6	7	7	5
I72	7	7	7	7	7	7
I73	7	7	8	7	7	8
I74	8	8	7	7	7	7
I75	7	7	8	4	7	7

	Date: 1/13/2022	Date: 2/1/2022
	Rep 1	**Rep 1**
W1	5	5
W2	5	4
W3	5	5
W4	5	5
W5	5	4
W6	5	6
W7	4	5
W8	6	5
W9	5	5
W10	5	4
W11	5	6
W12	5	5
W13	5	5
W14	5	5
W15	6	4

WHITE RUST RATINGS

	Date: 1/13/2022	Date: 2/1/2022
	Rep 1	**Rep 1**
W16	6	6
W17	6	5
W18	7	5
W19	6	5
W20	6	4
W21	5	4
W22	5	5
W23	4	3
W24	6	4
W25	5	4
W26	5	4
W27	5	3
W28	6	6
W29	5	4
W30	4	6
W31	5	5
W32	5	5
W33	5	5
W34	5	5
W35	5	4
W36	5	5
W37	6	5
W38	6	5
W39	6	4
W40	6	4
W41	6	4
W42	6	5
W43	7	6
W44	7	5
W45	6	6
W46	7	5
W47	7	5

2021-2022 Spinach Trial
Tiro Tres Farms
Crystal City, TX

2022 Spinach Research Trial *Photo provided by: Julia Paige Ritchie, Tiro Tres Farms*