Evaluation of a sclerotinia stem test protocol for canola co-op lines

Final report
Protocol, test coordination and data analysis provided by Lone Buchwaldt and Jennifer Adam. Report produced on behalf of the WCC/RRC Pathology Sub-committee. April 20, 2016.

Collaborators	Locations
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Background
Resistance to stem rot caused by *Sclerotinia sclerotiorum* is a quantitative trait conferred by several genes that work synergistically to reduced growth of the pathogen in the stem and prevent penetration into the vascular tissue. In 2011, the Western Canada Canola/Rapeseed Recommending Committee (WCC/RRC) requested that members of the Pathology Sub-committee develop a protocol for testing of co-op lines for sclerotinia resistance acceptable to canola breeders and other organisations in the industry. Members of the Sub-committee include canola breeders, plant pathologists, scientists and extension specialists in provincial, federal and private organizations.

Objective: To develop a standardized test for Sclerotinia resistance of canola entries in the co-operative system under the Western Canada Canola/Rapeseed Recommending Committee.

Testing of mycelium and ascospores as inoculum
Seed of lines expected to have some level of partial resistance (PR) to sclerotinia were provided by five canola breeding companies, Pioneer, Dupont, Bayer CropScience, Crop Protection Services (CPS, former Viterra) and Cargill. Line 45H29 and 45S52 (both from Pioneer) were selected as the susceptible (S) and partially resistant checks. Two screening methods were tested. One was inoculation of small field plots with ascospore suspension, and the other was inoculation of individual plants by attaching plugs of fungal mycelium to the stem. Useful data from ascospores inoculation were not obtained after three years of testing. In contrast, useful data from 32 stem tests were collected (page 3-11).

Experimental design of the stem test
Stem tests were conducted by plant pathologists and plant breeders in the private and public sectors listed above. The tests were carried out under field conditions at ten locations in Alberta, Saskatchewan and Manitoba over five growing seasons between 2011 and 2015. The test sites were subjected to various weather conditions except at Agriculture Canada in Saskatoon where high humidity was obtained using a semi-field nursery. Co-op lines were planted in rows with five plants per line in six replications using a completely randomized block design. *Sclerotinia sclerotiorum* isolate #321 was grown on PDA (potato dextrose agar) and mycelium was used for inoculation at all test locations. At full flower each plant was inoculated by attaching a 7 mm plug

of fungal mycelium to the main stem with Parafilm. Initially, disease rating consisted of measurements of stem lesion length 7, 14 and 21 days after inoculation (dai), but was later reduced to the 21 dai measurement for simplicity, and because there was a high correlation between the three time points. The depth of penetration into the stem was rated and used to calculate percent soft + collapse lesions (% s+c) for each line. The sclerotinia stem test protocol is available on page 15-18.

Results

There were a total of 33 test locations; however, one test (no 25) was not carried out because of hot, dry weather resulting in poor plant growth. The Pathology Sub-committee agreed to eliminate data from test locations with low infection e.g. when the average lesion length 21 days after inoculation was less than 20 mm on the susceptible control, 45H29 (tests no 5, 15 and 24).

Data from each location was analyzed statistically (GLM model, SAS program) and canola lines were separated by Fisher's test of least significant difference (LSD). The disease ratings on the different canola lines ranged from 0 to 260 mm stem lesion length and from 0 to 100% soft + collapsed stem lesions. Results from statistical analysis of data from locations combined by year are shown on page 3. Results from statistical analysis of data from individual locations are shown on page 4-11.

Conclusion

The stem test was able to differentiate between the susceptible check, 45H29, and most test lines expected to have some level of sclerotinia resistance. The PR check line, 45S52, was consistently better than the susceptible check in all five years based on stem lesion length measurements (page 3). Lines 45S54, 45S56, D3154S and VR9561G were better than the susceptible check in both years they were tested. Line 07H874 was better than the susceptible check based on three locations in one year, while 0CN0214 was better in two of three test years. Statistical analysis of percent soft + collapsed lesions was performed on data combined by year, which showed that none of the test lines were consistently better than the susceptible check in this aspect.

Criteria for labelling a canola co-op line partially resistant (PR)

Different criteria for labelling of canola lines partially resistant (PR) to sclerotinia stem rot was discusses by the Pathology Sub-committee. In December 2015, a group of 17 members with voting rights was created. The following motion was passed:

- **Motion: The stem inoculation testing protocol is recommended to WCC/RRC to be used to identify lines with significantly better sclerotinia stem resistance compared to the susceptible check 45H29.**

Subsequently, the WCC/RRC Board accept the sclerotinia stem test protocol and it was added to other disease protocols in December 2015.

Results from sclerotinia stem tests combined by year

S = susceptible
PR = expected partially resistant
Line with stem lesion length significantly better than the susceptible check 45H29
Line with percent soft and collapsed (% s + c) lesions significantly better than the susceptible check 45H29

2011 results from 3 locations combined.

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% soft + collapsed	
45H29	Pioneer – S check	110	AB	100	72	AB
VT 500	Viterra - S	109	AB	99	65	ABC
5440	Bayer - S	101	B	92	60	ABC
73-75	Monsanto - S	117	A	106	70	AB
73-45	Monsanto - PR	96	B	88	75	A
07H874	Cargill - PR	79	C	72	52	BCD
Line	AAFC - PR	71	C	64	46	CD
0CN0214	Bayer - PR	47	D	43	35	D
9557S	Viterra - PR	72	C	66	57	ABC
45S52	Pioneer – PR check	70	C	63	52	BCD
LSD =		15			21	

2012 results from 3 locations combined.

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% soft + collapsed	
45H29	Pioneer – S check	128	AB	100	69	AB
VT 500	Viterra - S	128	A	101	79	A
5440	Bayer - S	122	ABC	95	72	AB
Topas	AAFC - S	110	CD	86	67	AB
Line	AAFC - PR	113	BCD	89	70	AB
0CN0214	Bayer - PR	86	E	67	45	C
9557S	Viterra - PR	100	DE	78	60	B
45S52	Pioneer – PR check	104	D	81	60	B
LSD =		15			13	

2013 results from 7 locations combined.

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% soft + collapsed	
45H29	Pioneer – S check	54	B	100	52	A
VT 500	Viterra - S	65	A	120	59	A
5440	Bayer - S	63	A	117	60	A
0CN0214	Bayer - PR	55	B	102	42	BC
9557S	Viterra - PR	45	C	83	41	BC
45S52	Pioneer – PR check	36	D	67	28	C
LSD =		6			9	

2014 results from 7 locations combined.

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% soft + collapsed	
45H29	Pioneer – S check	69	B	100	32	AB
Invigor 5770	Bayer - S (late)	81	A	117	36	A
L160S	Bayer - PR (late)	58	C	84	29	AB
D3154S	DuPont - PR	58	C	84	28	AB
VR9561G	CPS - PR	55	CD	80	27	B
45S56	Pioneer - PR	57	CD	83	27	B
45S54	Pioneer - PR	50	D	72	26	B
45S52	Pioneer – PR check	53	CD	77	26	B
LSD =		8			8	

2015 results from 8 locations combined.

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% soft + collapsed	
45H29	Pioneer – S check	67	BC	100	35	BC
Invigor 5770	Bayer - S (late)	94	A	141	50	A
L160S	Bayer - PR (late)	73	B	109	42	AB
D3154S	DuPont - PR	59	D	89	31	BC
VR9561G	CPS - PR	60	CD	90	33	BC
12H9054	Cargill - PR	59	D	88	30	CD
45S56	Pioneer - PR	51	E	76	22	D
45S54	Pioneer - PR	54	DE	81	29	CD
45S52	Pioneer – PR check	50	E	74	27	D
LSD =		7			10	

Results from sclerotinia stem tests at individual locations

Test 1. 2011 Alberta Innovates, Vegreville, AB

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	84	A	100	44
VT 500	Viterra - S	76	ABC	90	38
5440	Bayer - S	80	AB	95	38
73-75	Monsanto - S	81	AB	96	40
73-45	Monsanto - PR	62	ABCD	73	50
07H874	Cargill	57	BCD	68	27
Line	AAFC - PR	69	ABCD	82	38
0CN0214	Bayer - PR	47	DE	55	21
9557S	Viterra - PR	52	CDE	62	31
45S52	Pioneer – PR check	29	E	34	21
LSD =		27			na

Extra data

AUDPC		% of 45H29
40	A	100
36	AB	89
39	A	97
37	AB	92
33	AB	82
30	ABC	74
32	ABC	79
26	BC	64
27	BC	66
20	C	50
13		

Test 2. 2011 Monsanto, Carman, MB

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	43	B	100	
VT 500	Viterra - S	37	BC	86	
5440	Bayer - S	31	CD	72	
73-75	Monsanto - S	54	A	125	na
73-45	Monsanto - PR	35	BC	81	
07H874	Cargill - PR	30	CD	69	
Line	AAFC - PR	23	DE	53	
0CN0214	Bayer - PR	15	E	34	
9557S	Viterra - PR	22	DE	52	
45S52	Pioneer – PR check	39	BC	90	
LSD =		10			

Extra data

AUDPC		% of 45H29
21	B	100
19	BC	89
15	CDE	72
27	A	129
18	BCD	86
16	CDE	76
14	DE	67
9	F	44
12	EF	59
15	CDE	72
4		

Test 3. 2011 AAFC, Saskatoon, SK

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	176	AB	100	100
VT 500	Viterra - S	179	AB	102	92
5440	Bayer - S	163	BC	92	82
73-75	Monsanto -S	186	A	106	100
73-45	Monsanto - PR	167	AB	95	100
07H874	Cargill - PR	135	C	77	78
Line	AAFC - PR	100	D	57	54
0CN0214	Bayer - PR	72	E	41	50
9557S	Viterra - PR	124	C	70	83
45S52	Pioneer – PR check	136	C	77	83
LSD =		21			na

Extra data

AUDPC		% of 45H29
98	A	100
103	A	105
99	A	101
107	A	109
95	AB	97
84	BC	86
46	D	47
40	D	41
71	C	72
71	C	73
13		

Test 4. 2012 Alberta Innovates, Vegreville, AB

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	84	AB	100	56
VT 500	Viterra - S	86	AB	102	64
5440	Bayer - S	86	AB	102	66
Topas	AAFC - S	108	A	128	82
Line	AAFC - PR	98	A	116	73
0CN0214	Bayer - PR	40	C	47	33
9557S	Viterra - PR	70	B	82	48
45S52	Pioneer – PR check	95	A	113	63
LSD =		24			na

Test 5. 2012 Monsanto, Carman, MB

Line	Company - expected reaction	Lesion length 12 dai, mm	% of 45H29	% s+c
45H29	Pioneer – S check	12		
VT 500	Viterra - S	12		
5440	Bayer - S	12		
Topas	AAFC - S	13		
Line	AAFC - PR	13		
0CN0214	Bayer - PR	11		
9557S	Viterra - PR	11		
45S52	Pioneer – PR check	10		
LSD =		1		

Test 5 eliminated since lesion length on 45H29 was less than 20 mm

Test 6. 2012 AAFC, Saskatoon, SK

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	257	A	100	97
VT 500	Viterra - S	230	AB	89	100
5440	Bayer - S	233	AB	91	100
Topas	AAFC - S	240	AB	93	94
Line	AAFC - PR	208	BC	81	97
0CN0214	Bayer - PR	214	BC	83	92
9557S	Viterra - PR	211	BC	82	100
45S52	Pioneer – PR check	185	C	72	92
LSD =		35			na

Extra data

AUDPC		% of 45H29
111	ABC	100
114	ABC	103
119	AB	107
127	A	115
101	CD	102
109	BC	98
105	BC	95
86	D	78
17		

Test 7. 2012 Dow, Saskatoon, SK

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	63	BC	100	50
VT 500	Viterra - S	86	A	136	72
5440	Bayer - S	67	B	107	48
Topas	AAFC - S	36	E	57	14
Line	AAFC - PR	50	D	79	36
0CN0214	Bayer - PR	23	F	36	5
9557S	Viterra - PR	42	DE	67	26
45S52	Pioneer – PR check	53	CD	84	18
LSD =		11			na

Extra data

AUDPC		% of 45H29
32	BC	100
52	A	164
33	B	105
14	E	44
27	CD	85
9	E	29
24	D	71
32	BC	62
6		

Test 8. 2013 Alberta Innovates, Vegreville, AB

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	59	CD	100	50
VT 500	Viterra - S	87	A	147	69
5440	Bayer - S	74	ABC	125	60
0CN0214	Bayer - PR	63	BCD	80	52
9557S	Viterra - PR	76	AB	129	49
45S52	Pioneer – PR check	48	D	81	27
LSD =		17			na

Extra data

Lesion length 14 dai, mm		% of 45H29
48	BC	100
62	A	129
50	B	104
45	BC	94
52	B	108
38	C	79
10		

Test 9. 2013 Monsanto, Carman, MB

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	74	B	100	47
5440	Bayer - S	109	A	147	76
VT 500	Viterra - S	108	A	146	70
0CN0214	Bayer - PR	61	B	95	37
9557S	Viterra - PR	64	B	86	43
45S52	Pioneer – PR check	34	C	46	13
LSD =		22			na

Extra data

Lesion length 14 dai, mm		% of 45H29
53	B	100
66	A	125
70	A	132
45	B	85
47	B	89
31	C	69
12		

Test 10. 2013 AAFC, Saskatoon, SK

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	54	A	100	23
VT 500	Viterra - S	57	A	106	37
5440	Bayer - S	57	A	106	47
0CN0214	Bayer - PR	48	AB	89	34
9557S	Viterra - PR	35	BC	65	13
45S52	Pioneer – PR check	31	C	57	10
LSD =		17			na

Extra data

Lesion length 14 dai, mm		% of 45H29
43	A	100
44	A	102
40	AB	93
36	ABC	84
30	BC	70
28	C	65
10		

Test 11. 2013 Dow, Saskatoon, SK

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	104	C	100	100
VT 500	Viterra - S	123	B	118	100
5440	Bayer - S	104	C	100	100
0CN0214	Bayer - PR	139	A	134	100
9557S	Viterra - PR	79	D	76	90
45S52	Pioneer – PR check	77	D	74	83
LSD =		10			na

Extra data

Lesion length 14 dai, mm		% of 45H29
86	C	100
94	B	109
80	C	93
104	A	121
66	D	77
58	D	67
8		

Test 12. 2013 Cargill, Aberdeen, SK

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	39	B	100	57
5440	Bayer - S	49	A	126	87
VT 500	Viterra - S	40	B	103	83
0CN0214	Bayer - PR	49	A	126	97
9557S	Viterra - PR	36	BC	92	23
45S52	Pioneer – PR check	30	C	77	20
LSD =		8			na

Extra data

Lesion length 14 dai, mm		% of 45H29
27	B	100
32	A	119
26	B	96
34	A	126
22	BC	81
20	C	74
5		

Test 13. 2013 Bayer, Saskatoon, SK

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	59	A	100	87
VT 500	Viterra - S	52	AB	88	78
5440	Bayer - S	48	B	81	72
9557S	Viterra - PR	31	C	53	45
45S52	Pioneer - PR	20	D	34	22
0CN0214	Bayer – PR check	17	D	29	16
LSD =		9			na

Extra data

Lesion length 14 dai, mm		% of 45H29
40	A	100
38	A	95
28	B	70
21	BC	53
15	CD	38
12	D	30
7		

Test 14. 2013 DL Seeds, Morden, MB

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	23	AB	100	20
VT 500	Viterra - S	39	A	170	18
5440	Bayer - S	30	AB	130	18
0CN0214	Bayer - PR	22	AB	96	20
45S52	Pioneer - PR	22	AB	96	28
9557S	Viterra – PR check	16	B	70	10
LSD =		19			na

Extra data

Lesion length 14 dai, mm		% of 45H29
12	AB	100
21	A	175
19	AB	158
14	AB	117
17	AB	142
9	B	75
11		

Test 15. 2014 Alberta Innovates, Vegreville, AB

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	8	B	100	0
Invigor 5770	Bayer - S (late)	23	A	283	10
L160S	Bayer - PR (late)	15	AB	178	3
D3154S	DuPont - PR	6	B	76	0
VR9561G	CPS - PR	12	B	143	3
45S56	Pioneer - PR	12	B	143	0
45S54	Pioneer - PR	12	B	141	0
45S52	Pioneer – PR check	14	AB	166	0
LSD =		10			

Test 15 eliminated since lesion length on 45H29 was less than 20 mm

Test 16. 2014 Monsanto, Carmen, MB

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	42	B	100	9
Invigor 5770	Bayer - S (late)	57	A	136	17
L160S	Bayer - PR (late)	26	C	64	6
D3154S	DuPont - PR	34	BC	83	6
VR9561G	CPS - PR	40	BC	95	8
45S56	Pioneer - PR	41	B	99	6
45S54	Pioneer - PR	29	BC	70	6
45S52	Pioneer – PR check	29	BC	70	0
LSD =		14			

Test 17. 2014 AAFC, Saskatoon, SK

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	80	AB	100	47
Invigor 5770	Bayer - S (late)	88	A	110	39
L160S	Bayer - PR (late)	66	BC	83	40
D3154S	DuPont - PR	48	CD	61	33
VR9561G	CPS - PR	48	CD	60	26
45S56	Pioneer - PR	41	D	52	23
45S54	Pioneer - PR	37	D	46	23
45S52	Pioneer – PR check	31	D	38	18
LSD =		20			

Test 18. 2014 Dow, Saskatoon SK

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer - S	129	A	100	83
Invigor 5770	Bayer - S (late)	136	A	105	90
L160S	Bayer - PR (late)	104	BC	80	83
D3154S	DuPont - PR	119	AB	92	90
VR9561G	CPS - PR	104	BC	80	80
45S56	Pioneer - PR	119	AB	92	97
45S54	Pioneer - PR	91	C	70	80
45S52	Pioneer - PR	119	AB	92	87
LSD =		30			

Test 19. 2014 Cargill, Aberdeen, SK

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	101	AB	100	70
Invigor 5770	Bayer - S (late)	106	A	104	70
L160S	Bayer - PR (late)	108	A	107	63
D3154S	DuPont - PR	90	BC	88	57
VR9561G	CPS - PR	91	BC	90	60
45S56	Pioneer - PR	89	BC	88	53
45S54	Pioneer - PR	83	C	82	55
45S52	Pioneer – PR check	77	C	76	50
LSD =		14			

Test 20. 2014 Bayer, Saskatoon, SK

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	%s+c
45H29	Pioneer – S check	36	B	100	3
Invigor 5770	Bayer - S (late)	52	A	145	13
L160S	Bayer - PR (late)	35	BC	98	0
D3154S	DuPont - PR	27	CD	76	0
VR9561G	CPS - PR	24	D	68	0
45S56	Pioneer - PR	23	D	65	0
45S54	Pioneer - PR	25	D	69	3
45S52	Pioneer – PR check	25	D	69	0
LSD =		8			

Test 21. 2014 DL Seed, Morden, MB

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	11	C	100	0
Invigor 5770	Bayer - S (late)	60	A	544	41
L160S	Bayer - PR (late)	23	B	207	6
D3154S	DuPont - PR	9	C	83	0
VR9561G	CPS - PR	12	C	105	7
45S56	Pioneer - PR	18	BC	162	3
45S54	Pioneer - PR	18	BC	162	6
45S52	Pioneer – PR check	22	B	198	0
LSD =		9			

Test 21 eliminated since lesion length on 45H29 was less than 20 mm

Test 22. 2014 Pioneer, Caledon, ON

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	50	BC	100	11
Invigor 5770	Bayer - S (late)	81	A	163	22
L160S	Bayer - PR (late)	31	C	62	3
D3154S	DuPont - PR	54	ABC	108	13
VR9561G	CPS - PR	44	BC	89	17
45S56	Pioneer - PR	38	BC	77	8
45S54	Pioneer - PR	57	ABC	114	13
45S52	Pioneer – PR check	61	AB	123	24
LSD =		29			

Test 23. 2014 Pioneer, Carman, MB

Line	Company - expected reaction	Lesion length 21 dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	37	BC	100	0
Invigor 5770	Bayer - S (late)	65	A	176	3
L160S	Bayer - PR (late)	27	C	72	8
D3154S	DuPont - PR	26	C	72	0
VR9561G	CPS - PR	31	BC	84	0
45S56	Pioneer - PR	43	B	117	0
45S52	Pioneer - PR	28	BC	77	0
45S54	Pioneer – PR check	28	C	75	0
LSD =		14			

Test 24. 2015 Alberta Innovates, Vegreville, AB

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	13	ABC	100	10
Invigor 5770	Bayer - S (late)	17	A	131	13
L160S	Bayer - PR (late)	10	BC	79	3
D3154S	DuPont - PR	12	ABC	90	7
VR 9561G	CPS - PR	7	C	55	0
12H9054	Cargill - PR	16	AB	122	17
45S56	Pioneer - PR	12	ABC	93	3
45S54	Pioneer - PR	8	C	57	3
45S52	Pioneer – PR check	13	ABC	97	3
LSD =		7			

Test 24 eliminated since lesion length on 45H29 was less than 20 mm

Test 25. 2015 Monsanto, Carman, MB. Not inoculated due to hot dry weather resulting in poor plant growth

Test 26. 2015 AAFC, Saskatoon, SK

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	140	BC	100	100
Invigor 5770	Bayer - S (late)	195	A	139	100
L160S	Bayer - PR (late)	160	B	114	100
D3154S	DuPont - PR	123	CD	88	100
VR 9561G	CPS - PR	120	CD	86	100
12H9054	Cargill - PR	102	DE	73	75
45S56	Pioneer - PR	86	E	61	70
45S54	Pioneer - PR	89	E	72	65
45S52	Pioneer – PR check	99	DE	71	80
LSD =		26			

Test 27. 2015 Dow, Saskatoon, SK

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	77	DE	100	52
Invigor 5770	Bayer - S (late)	116	AB	151	76
L160S	Bayer - PR (late)	120	A	156	96
D3154S	DuPont - PR	93	CD	121	76
VR 9561G	CPS - PR	97	C	126	76
12H9054	Cargill - PR	99	BC	129	75
45S56	Pioneer - PR	65	E	85	48
45S54	Pioneer - PR	94	CD	122	68
45S52	Pioneer – PR check	61	E	79	44
LSD =		18			

Test 28. 2015 Cargil, Aberdeen, SK

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	56	B	103	11
Invigor 5770	Bayer - S (late)	77	A	137	33
L160S	Bayer - PR (late)	60	B	106	34
D3154S	DuPont - PR	56	B	99	22
VR 9561G	CPS - PR	54	B	95	16
12H9054	Cargill - PR	55	B	98	19
45S56	Pioneer - PR	53	B	94	12
45S54	Pioneer - PR	39	C	70	6
45S52	Pioneer – PR check	29	C	52	0
LSD =		12			

Test 29. 2015 Bayer, Saskatoon, SK

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	35	B	58	10
Invigor 5770	Bayer - S (late)	60	A	171	37
L160S	Bayer - PR (late)	24	CD	116	0
D3154S	DuPont - PR	25	CD	71	0
VR 9561G	CPS - PR	30	BC	85	0
12H9054	Cargill - PR	32	BC	91	0
45S56	Pioneer - PR	27	BCD	76	0
45S54	Pioneer - PR	28	BCD	79	0
45S52	Pioneer – PR check	21	D	59	0
LSD =		9			

Test 30. 2015 DL Seeds, Morden, MB

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% s+c
45H29	Bayer – S check	53	A	100	51
Invigor 5770	Bayer - S (late)	43	AB	81	28
L160S	Bayer - PR (late)	50	A	94	41
D3154S	DuPont - PR	30	C	92	15
VR 9561G	CPS - PR	28	C	53	19
12H9054	Cargill - PR	33	BC	62	29
45S56	Pioneer - PR	36	BC	68	18
45S54	Pioneer - PR	26	C	48	11
45S52	Pioneer – PR check	32	BC	61	29
LSD =		12			

Test 31. 2015 Pioneer, Caldon, ON

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	95	BCD	100	19
Invigor 5770	Bayer - S (late)	129	A	137	43
L160S	Bayer - PR (late)	96	BCD	101	28
D3154S	DuPont - PR	92	CD	97	24
VR 9561G	CPS - PR	107	ABC	113	34
12H9054	Cargill - PR	72	D	76	16
45S56	Pioneer - PR	89	CD	94	19
45S54	Pioneer - PR	116	AB	123	42
45S52	Pioneer – PR check	93	BCD	99	37
LSD =		24			

Test 32. 2015 Pioneer, Carman, MB

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	44	BC	107	7
Invigor 5770	Bayer - S (late)	76	A	174	33
L160S	Bayer - PR (late)	58	B	132	10
D3154S	DuPont - PR	40	C	91	8
VR 9561G	CPS - PR	35	C	79	0
12H9054	Cargill - PR	43	C	97	3
45S56	Pioneer - PR	39	C	88	3
45S54	Pioneer - PR	37	C	84	3
45S52	Pioneer – PR check	41	C	93	7
LSD =		15			

Test 33. 2015 Pioneer, Saskatoon, SK

Line	Company - expected reaction	Lesion length 21dai, mm		% of 45H29	% s+c
45H29	Pioneer – S check	68	BC	100	53
Invigor 5770	Bayer - S (late)	115	A	171	73
L160S	Bayer - PR (late)	73	B	108	57
D3154S	DuPont - PR	52	BCDE	77	37
VR 9561G	CPS - PR	50	CDE	74	45
12H9054	Cargill - PR	61	BCD	90	43
45S56	Pioneer - PR	34	E	51	27
45S54	Pioneer - PR	49	CDE	72	56
45S52	Pioneer – PR check	42	DE	62	37
LSD =		22			

Sclerotinia stem test protocols by year

	2011	2012	2013	2014	2015
Sclerotinia isolate	#321	#321	#321	#321	#321
Growth media	Sclerotia plate on V8 agar then onto glucose media	Sclerotia plated directly onto PDA	Sclerotia plated directly onto PDA	Sclerotia plated directly onto PDA	Sclerotia plated directly onto PDA
Inoculum	Mycelium plug + petal	Mycelium plug + petal	Mycelium plug	Mycelium plug	Mycelium plug
Inoculation sites per stem	2	1	1	1	1
Plants per line	6	5-6	5	5	5
Replications	4	5-6	6	6	6
Lesions per line	48	25-36	30	30	30
Stem lesion length, mm	21 dai	21 dai	21 dai	21 dai	21 dai
Lesion appreance	% soft + collapsed	% soft + collapsed	% soft + collapsed	% soft + collapsed	% soft + collapsed
Extra data collected	7 and 14 dai, AUDPC	14 dai	14 dai		
Susceptible check	45H29	45H29	45H29	45H29	45H29
Partially resistant check	45S52	45S52	45S52	45S52	45S52
Susceptible (S) lines	5440, VT500, 73-75	5540, VT500, Topas	5540, VT500	Invigor 5770 (late flowering)	Invigor 5770 (late flowering)
Expected partially resistant (PR) lines	0CN0214, 9557S, Line, 07H874, 73-45	0CN0214, 9557S, Line	0CN0214, 9557S	45S54, VR9561G, 45S56, D3154S, L160S (late)	45S54, VR9561G, 45S56, D3154S, L160S (late), 12H9054

Blue text indicate factors that were changed

Numbering of test locations

Collaborators			2011	2012	2013	2014	2015
Ralph Lange	Vegreville	Alberta Innovates, AB	1	4	8	15	24
Jed Christianson	Carman	Monsanto, MB	2	5	9	16	25
Lone Buchwaldt	Saskatoon	AAFC, SK	3	6	10	17	26
Jianwei Zhao, Sherry Gore	Saskatoon	Dow, SK		7	11	18	27
Yu Chen	Aberdeen	Cargill, SK			12	19	28
Godfrey Chongo	Saskatoon	Bayer, SK			13	20	29
Evan Gillis, Marc Vincent	Morden	DL Seeds, MB			14	21	30
Igor Falak	Caledon	Pioneer, ON				22	31
Navratan Bahia	Carman	Pioneer, MB				23	32
Julian Heath	Saskatoon	Pioneer, SK					33

Tests eliminated since stem lesion length was less than 20 mm on the susceptible check 45H29.

Test not inoculated due to hot dry weather resulting in poor plant growth.

Planting and inoculation dates

Location	2011			2012			2013			2014			2015		
	Planting date	Inocula-tion date	Days from first to last inoculation	Planting date	Inocula-tion date	Days from first to last inoculation	Planting date	Inocula-tion date	Days from first to last inoculation	Planting date	Inocula-tion date	Days from first to last inoculation	Planting date	Inocula-tion date	Days from first to last inoculation
Alb. Inn., Vegreville, AB	24-May	25-Jul	**62**	31-May	16-Jul	**46**	28-May	19-Jul	**62**	03-Jun	23-Jul	**50**	May 29	July 20	**52**
Monsanto, Carmen, MB	20-May	18-Jul	**42**	30-Apr	16-Jun	**58**	27-May	22-Jul	**56**	27-May	18-Jul	**52**		not inoculated	
AAFC, Saskatoon, SK	19-May	July 4-16	**48-58 (10)**	19-May	July 9-17	**50-58 (8)**	21-May	July 8-15	**48-55 (7)**	30-May	July 14-23	**45-54 (9)**	May 20	July 6 - 14	**47-55 (8)**
Dow, Saskatoon, SK				01-Jun	July 13-19	**43-49 (6)**	30-May	12-Jul	**43**	02-Jun	15-Jul	**43**	June 2	July 20-22	**48-50 (2)**
Cargill, Arberdeen, SK							28-May	July 25-Aug 1	**58-65 (7)**	28-May	29-Jul	**61**	May 21	July 17	**57**
Bayer, Saskatoon, SK							31-May	July 18-24	**48-54 (6)**	03-Jun	27-Jul	**54**	June 2	July 20	**48**
DL Seeds, Morden, MB							17-May	08-Jul	**52**	22-May	July 8-14	**47-53 (6)**	May 27	July 10-13	**44-47 (3)**
Pioneer, Caldon, ON										24-May	16-Jul	**53**	May 8	July 9	**62**
Pioneer, Carman, MB										13-May	21-Jul	**69**	May 12	July 23	**72**
Pioneer, Saskatoon, SK													May 19	July 6 - 20	**48-62 (14)***

Later inoculation than the average

Earlier inoculation than the average

*Some dark mycelium developed on some plugs; re-inoculation was necessary resulting in 14 days from first to last inoculation.

Sclerotinia stem test protocol for canola

Developed by Lone Buchwaldt and modified by Pathology Sub-committee members to suit WCC-RRC's need for sclerotinia testing of canola lines in the co-op system.
December, 2015.

Experimental design

Sclerotinia stem-tests are carried out under field conditions. Establishment of a healthy and uniform plant stand is required. Irrigation and misting is generally not needed for symptom development unless weather conditions are hot and dry during flowering. The negative effect of wind out can be mitigated by planting the test by planting guard rows around the test site or by planting in a sheltered or low-lying and area. Fertilizer application, weed and insect control should be carried out as for a commercial canola crop. However, all fungicide treatments should be avoided. The seed can either be planted directly in the field or in a greenhouse followed by transplanting of seedlings at the 3-4 true leaf stage.

Test locations: Tests can be located in canola growing areas of Alberta, Saskatchewan and Manitoba.

Plot lay-out*:* All lines are planted in 6 replications in a complete randomized block design. Data is needed from 5 plants per line per replication. Planting of extra seed is recommended to ensure that enough plants are at the full flower stage at the time of inoculation.

Susceptible and resistant checks: The susceptible is 45H29 and partially resistant check is 45S52 both from Pioneer. In cases when a late flowering co-op line is included, a suitable late flowering susceptible check will be identified for comparison.

Inoculum

Fungal isolate: Sclerotinia sclerotiorum isolate #321 is recommended for inoculation to allow comparison between test locations. The isolate was collected in a commercial canola field in Olds, Alberta in 1992 (Kohli and Kohn, 1995 Mol Ecol. 4: 69-77). Sclerotia of this isolates can be obtained from Lone Buchwaldt (email: Lone.Buchwaldt@agr.gc.ca).

Inoculum production: Sclerotia are divided into batches matching the number of expected inoculation dates. They are surface sterilize in 10% Javex (10 ml Javex + 90 ml water) and rinsed 2-3 times in sterile water. Sclerotia are cut in half and each half placed on PDA in the centre of a 9 cm Petri plate. The plates are sealed with Parafilm and incubated up-side-down (to avoid condensation) at 20-22^{o}C in a 16/8 hour light/dark cycle (an incubator or lab bench is equally good).

Age of mycelium cultures: The cultures are ready for inoculation when the mycelium is 1-2 cm from the edge of the Petri plate which normally takes 4 to 6 days. Cultures that have reached the edge of the plate are too old for inoculation. If needed, the growth of cultures can be slowed down by placing them at 5-8^{o}C (in a fridge); however, this should only be for a maximum of 24 hours. A cooler can be used for transport of the cultures to the field, but direct contact with ice should be avoided.

Mycelium plugs: Plugs should be cut with a 7 mm diameter cork borer approximately 5 mm behind the growing margin under clean but not necessarily sterile conditions. Ensure that each plug contains a similar amount of mycelium by holding the plates up against a light source while

cutting thereby avoiding areas with sparse hyphal growth. For consistency, only one or two circles of plugs should be cut from each culture. Between 15 and 30 plugs can be obtained per Petri plate depending on the size of the culture.

Inoculation

Time of inoculation: Each plant is inoculated when it is at full flower. Full flower is defined as the stage when petals senesce at the same rate as new flowers open. This corresponds to 65 on the BBCH canola growth scale; see http://en.wikipedia.org/wiki/BBCH-scale_(canola). There is usually a 7 day window for inoculation at individual test locations. However, at some locations it may be necessary to inoculate on 2 or 3 different days due to variable flower time between lines or even between plants of the same line.

Preparation of Parafilm: First cut several 2 meter long 3 cm wide ribbons of Parafilm, remove the paper, combine 6-8 layers of ribbon and cut them into 3 x 4 cm pieces. Stretch each piece to 4 x 7 cm and place them on a tray. Place one plug with the mycelium facing up in the middle of each piece of Parafilm. The inoculum should be used within one hour of preparation.

Site of inoculation: The middle internode of the main stem is inoculated. This indernode is identified as half way between the soil surface and the lowest side branch, about 30-50 cm above the soil surface. The internode is inoculated by attaching a 7 mm plug to the stem with the mycelium facing the stem surface. The two ends of the Parafilm are twisted around one another on the opposite side of the stem while ensuring that the plug keeps its shape.

Weather considerations: Inoculation is best carried out at temperatures between 18^{o} and 23^{o}C. If the weather is hot and dry then inoculation should be postpone to the evening or to a cooler day.

Verification of inoculation: Evidence of successful inoculation can be verified after 5-7 days by looking for small lesions under the mycelium plugs. If uniform infection is not achieved, it is possible to re-inoculate the stems using the internode above the previously inoculated internode given that the plants are still flowering.

Disease rating

Lesion length: The length of each stem lesion is measured in millimeters (no decimals) 21 days after inoculation (dai). Measurements made one day before or after is acceptable i.e. 20 to 22 dai. The longest part of the lesion is measured including the dark margin that separates the necrotic and healthy stem tissue (Figure 1).

Wilted plants: It may be difficult to determine the upper edge of the infected area if the plant is wilting. In these cases it is often possible to measure from the site of inoculation to the lower edge of infection. This length is then multiplied by 2 and recorded as the total lesion length.

Lesion firmness: The appearance of each lesion is recorded as black (bl), firm (f), soft (s) or collapsed (c) (**Fig 2**). The firmness is assessed by applying a slight pressure to the lesion with two fingers (**Fig 3**). A cross section of a firm lesion shows infection is confined to the stem surface while soft lesion affects the vascular tissue and the stem pith (**Fig 4**). Generally, collapsed lesions girdle the stem, while soft lesions do not.

Many small, black flecks: The stem under the plug can sometimes show many small, black flecks indicating many attempts of infection. In such cases, the longest fleck is measured (**Fig 2**).

Escapes: If no symptoms can be seen at the site of inoculation it is most likely an escape due to problems with the inoculum or weather conditions, and should therefore not be attributed to resistance of the stem. These escapes are rare, but the missing values should be recorded with 'e' not zero (0).

Thin stems: Small plants with thin stems (< 6 mm diameter) should be avoided and not inoculated since they are more susceptible than normal-sized stems of the same line.

Other observations: In order to determine if a data set should be excluded it is essential to describe damages to the plant stand caused by blackleg or other diseases, insect pests, weeds, hail, excessive moisture, drought etc. It is also important to mention variability in the test caused by human error.

Guidelines for the WCC/RRC Pathology Sub-committee

The committee maintains a list of collaborators who have the capacity to conduct the stem test.

Number of co-op lines: Each test can accommodated up to eight co-op lines plus the susceptible and resistant check lines for a total of 300 plants (10 lines x 5 plants x 6 replications). If more co-op lines need evaluation, they will be divided into two or more groups with similar flowering dates and planted in separate tests.

Coded names: Seed of co-op lines should be sent to WCC/RRC co-op coordinator, Raymond Gadoua, CCC. Seed envelopes will be labelled with code names and distributed to collaborators. A key for converting to actual names will be available after statistical analysis and first discussion by the Pathology Sub-committee.

The disease rating data, planting and inoculation dates should be emailed to the stem test coordinator, Lone Buchwaldt, AAFC (Lone.Buchwaldt@agr.gc.ca). Any problems regarding plant growth, weather, human errors and other comments should be included in this email.

Statistical analysis: A GLM (general linear model) analysis of stem lesion length and % soft + collapsed lesions will be conducted for individual test location and for lines over locations combined. Lines will be separated using Fisher's LSD analysis.

Elimination of test locations: Data from a test location will be eliminated if the average stem lesion length is less than 20 mm on the susceptible check line 45H29. Other reasons for exclusion of data are poor or uneven plant growth, extreme weather conditions and human errors. Data from all other test locations will be included in the final evaluation.

Minimum number of test locations: Five is the minimum number of test locations for evaluation of each co-op line. Data can be obtained from field tests in one or more years. Data from indoor testing are not permitted.

Criteria for PR-labeling of a co-op line: A co-op line is considered partially resistant to sclerotinia when the average stem lesion length at 21 days is significantly less than the susceptible check 45H29 in the analysis of test locations combined.

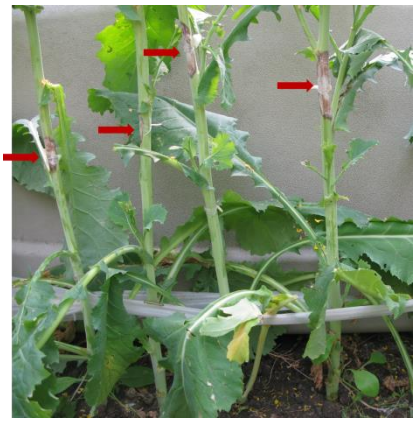

Figure 1. The middle internode of the main stem (red arrow) inoculated with a mycelium plug of *S. sclerotiorum* held in place with a piece of Parafilm.

Figure 2. The four different notations used to describe lesion appearance: black, firm, soft and collapsed.

Figure 3. A slight pressure with two fingers on the stem lesion is used to assess whether the lesion is firm, soft or collapsed.

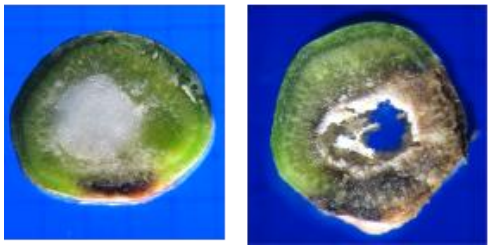

Figure 4. Cross sections of canola stems inoculated with a *S. sclerotiorum* showing a firm and a soft lesion, respectively.