

# Response of Canola Cultivars to *Sclerotinia sclerotiorum* in Controlled and Field Environments

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## ABSTRACT

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Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, can be a devastating disease of canola (*Brassica napus*) in the northern United States. No canola cultivars are marketed as having resistance to SSR. Field trials were established in Red Lake Falls, MN (2001, 2003, and 2004) and Carrington, ND (2001, 2002, 2003, and 2004) to evaluate canola cultivars for resistance to SSR. These cultivars also were evaluated for resistance to SSR under controlled conditions using the following methods: petiole inoculation technique (PIT), detached leaf assay (DLA), and oxalic acid assay (OAA). Significant ( $P \leq 0.05$ ) differences were detected among cultivars for SSR and yield in the field trials, with SSR levels varying from low to high among years and locations. Cultivars with consistent high levels and low levels of SSR in the field trials were identified. Significant ( $P \leq 0.05$ ) differences were detected among cultivars for SSR using the PIT and OAA methods, but not the DLA method. No significant ( $P \leq 0.05$ ) correlations between SSR levels in the controlled studies with SSR levels in the field trials were detected; however, significant negative correlations were detected between SSR area under the disease process curve values from the PIT method and yield from Carrington, ND in 2001 and 2002. Although the PIT and OAA methods differentiated cultivars, neither method was able to predict the reaction of cultivars to SSR in the field, indicating that field screening for SSR resistance is still critical for the development of resistant cultivars.

Additional keywords: oilseed rape

North Dakota and Minnesota have the largest hectareage of canola-quality (low levels of erucic acid and glucosinolates) oilseed *Brassica napus* in the United States. *Sclerotinia sclerotiorum* (Lib.) de Bary, the causal agent of Sclerotinia stem rot (SSR), can cause severe economic damage to canola grown in these states. Estimated economic losses to canola growers caused by SSR in Minnesota and North Dakota were 17.3, 20.8, and 16.8 million dollars in 1999, 2000, and 2001, respectively (15–17). The primary methods of managing SSR in canola are rotation with nonhost crops and foliar fungicides. Because of the persistent nature of sclerotia of *S. sclerotiorum* in the soil (11), crop rotation alone is not always effective in managing SSR. Foliar fungicides are an added input cost, and canola growers may not achieve an economic benefit with the use of fungicides unless disease pressure

and yield potential are high. If genetic resistance to SSR was available to canola growers, reliance on fungicides would lessen, and canola production in Minnesota and North Dakota would become more profitable.

Field evaluations of canola cultivars for resistance to SSR are important; however, problems can be associated with field evaluations. Disease pressure may not be uniform in a field situation, which may lead to erroneous interpretation of results. Canola cultivars may differ in their plant architecture and maturity, which could result in measuring disease escape rather than physiological resistance in field screening experiments (19,21).

An efficient, reliable, and inexpensive screening method that would allow large-scale evaluation of canola germ plasm and cultivars for SSR resistance is needed to accelerate the development of SSR-resistant canola cultivars. Methods that have been used to screen canola-quality and non-canola-quality oilseed *B. napus* lines for resistance to SSR include petiole inoculation, leaf inoculation, and stem inoculation with *S. sclerotiorum* (1,4,7,25), as well as screening against oxalic acid (4,7), which is a known pathogenicity factor for *S. sclerotiorum* (3,8,9). Several

researchers have compared methods to screen soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), and sunflower (*Helianthus annuus*) for resistance to *S. sclerotiorum* (5,12,14,18,23,24); however, few researchers have compared SSR screening methods in canola or have related indoor screening methods to field screening of canola lines or cultivars. Fang (7) compared an oxalic acid assay along with inoculations of flowers, stems, and leaves of 18 oilseed *B. napus* lines with *S. sclerotiorum* in a controlled-environment growth room and identified the leaf inoculation as a consistent method to screen for resistance to SSR; however, no comparisons with field screening were made. Evaluating 15 oilseed *B. napus* lines, Chaocai (4) conducted an oxalic acid assay, inoculated plants with macerated mycelia of *S. sclerotiorum* and *S. sclerotiorum*-infested rye grain, and compared the methods with a field screening trial. Results from this study indicated that all screening methods were comparable and correlated to the field screening trial. The macerated mycelia and rye grain methods reported by Chaocai (4) required inoculation of plants at bolting or flowering stage. A method that would allow for inoculation of plants prior to bolting or flowering would accelerate the screening process and allow for screening of both spring and winter types of *B. napus* without having to meet vernalization requirements.

The objectives of this research were to (i) characterize the level of resistance to SSR in a selection of commercial canola cultivars using field and controlled environment screening methods and (ii) identify an efficient lab or greenhouse screening method that will predict the reaction of a canola cultivar to SSR in the field.

## MATERIALS AND METHODS

**Oxalic acid assay.** Because oxalic acid is a pathogenicity factor for *S. sclerotiorum* (3,8,9), measuring it has been used to screen plants for resistance to *S. sclerotiorum* (4,9,13,24). The oxalic acid assay (OAA) methods used in our trial were adapted from Wegulo et al. (24), in which the development of a soluble, pink-colored pigment in soybean stems in response to oxalic acid was measured with a spectrophotometer. In a preliminary trial, canola stems of some cultivars exposed to

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oxalic acid developed a pink-colored pigment which dissolved in the oxalic acid.

Trials were conducted on 19 canola cultivars grown in a greenhouse at  $24 \pm 3^\circ\text{C}$  with supplemental artificial light (12 h/day). Seed were planted in 266-ml plastic drinking cups with holes in the bottom for water drainage. All containers were filled with a potting mix (Sunshine Mix no. 1; SunGro Horticulture Canada, Ltd., Seba Beach, AB, Canada) and five seed of each cultivar were planted in them. After emergence, seedlings were thinned to one plant per cup. When canola reached the three- to four-leaf stage, plants were severed at the bottom of the stem with a razor blade and placed immediately in 8-ml test tubes containing 5 ml of 40 mM oxalic acid. Test tubes containing excised canola plants were arranged vertically in test tube racks in a randomized complete block (RCB) design with four replications. After 48 h, 3 ml of the oxalic acid in each test tube were transferred to 10-ml cuvettes and absorbance was determined by a spectrophotometer (Spectronic 21D; Spectronic Analytical Instruments, Leeds, United Kingdom) at 518 nm. The OAA was repeated once over time.

**Petiole inoculation technique.** An isolate of *S. sclerotiorum* which was recovered from canola in Benson County, ND was used. Canola seedlings, grown in 4-by-4-cm plastic pots (two plants per pot, three pots per replication) filled with potting mix, were inoculated when they reached the four- to five-leaf stage. The third fully expanded leaves of six seedlings per cultivar and replication were severed 2.5 cm from the main stem using a razor blade, and the petiole attached to the seedling was inoculated with *S. sclerotiorum* using a technique first described by del Rio et al. (6) for soybean and used on oilseed *B. napus* by Zhao et al. (25). Plants were evaluated for a 6-day period and mortality for each day was recorded. Mortality of a plant was recorded on the day that a plant exhibited an irreversible wilt (25) or exhibited a girdling lesion so severe that the stem portion above the lesion toppled over. An area under disease progress curve (AUDPC) was calculated by using the percent plants dead for each day as the dependent variable and the six dates as the independent variable (22). The pots were arranged in an RCB design with four replications per cultivar. The petiole inoculation technique (PIT) trial was conducted first in the greenhouse (same growing conditions as for the OAA trials) and again in a growth chamber set at  $21^\circ\text{C}$  with a 16-h photoperiod.

**Detached leaf assay.** The leaves excised for the PIT trials (described above) were used for these assays. Paper towels moistened with sterile distilled water were placed at the bottom of plastic containers (28 by 36 cm), a plastic mesh screen was placed over the paper towels, and the ex-

cised leaves were placed on the mesh screens. A plug (5 mm in diameter) taken from the margin of a 3-day-old culture of *S. sclerotiorum* grown on potato dextrose agar (PDA; same isolate used for PIT assay) was placed on the middle of each leaf. After inoculation, the containers were sealed and incubated on a laboratory bench for 24 h at  $21^\circ\text{C}$ . Lesion diameter was measured on each leaf 24 h after inoculation. The experimental design and the number of replications were the same as in the PIT trials.

**Carrington, ND field trials.** Field trials were conducted at Carrington, ND during the 2001 to 2004 growing seasons. In, all 16 cultivars were evaluated in 2001, 18 cultivars were evaluated in 2002, and 6 cultivars were evaluated in 2003 and 2004. Plots were planted at approximately 1.7 million viable seed/ha on 10, 16, 15, and 18 May in 2001, 2002, 2003, and 2004, respectively, and were 6 m long and seven rows wide (18-cm row spacing). The field site, which had been used as a Sclerotinia disease nursery for several years to evaluate canola, common bean, and sunflower, had a history of Sclerotinia diseases. Ascospores of *S. sclerotiorum* (supplied by Dr. Mike Boosalis, University of Nebraska, Lincoln) were applied to canola plants at approximately 10 to 40% bloom and at the 50 to 80% bloom stage to help ensure adequate disease pressure. The ascospore suspension ( $1 \times 10^3$  ascospores/ml) was applied to plots using a  $\text{CO}_2$ -pressurized hand sprayer at 207 kPa and 131 liters/ha. A mist-irrigation system that had 1.2-m risers spaced 4.6 m apart misted plots for 3 min every 30 min. The plots were misted beginning just prior to ascospore inoculation through just prior to swathing (approximately 5 to 6 weeks). Fifty adjacent plants in the middle of each plot were evaluated for SSR incidence; a plant was considered infected if the main stem or a branch was bleached in color or shredded, with sclerotia present (20). Disease data were recorded on 9, 12, 21, and 26 August in 2001, 2002, 2003, and 2004, respectively. Plots were cut with a swather on 10, 13, and 21 August in 2001, 2002, and 2003, respectively, and on 15 September 2004 and left to dry in a windrow. A small plot combine was used to harvest the plots on 17, 21, and 26 August in 2001, 2002, and 2003, respectively, and on 20 September 2004. Harvested seed were weighed and yields per hectare were calculated.

**Red Lake Falls, MN field trials.** Field trials were conducted at Red Lake Falls, MN during the 2001, 2003, and 2004 growing seasons. The same six cultivars were evaluated each year. Plots were planted at approximately 1.5 million viable seed/ha on 10 and 16 May in 2001 and 2003, respectively, and 28 April 2004, and were 6 m long and 10 rows wide (15-cm row spacing). The field had a known his-

tory of Sclerotinia disease, and ascospores were applied when canola plants were at 10 to 40% bloom and at 50 to 80% bloom using the same methods as at the Carrington trials. A mist-irrigation system that had 1.5-m risers spaced 3.7 m apart misted plots for 10 min every hour. The plots were misted beginning just prior to ascospore inoculation through just prior to swathing (approximately 5 to 6 weeks). Disease data were recorded on 31 July, 5 August, and 10 August in 2001, 2003, and 2004, respectively, using the same methods as in Carrington. Plots were cut with a swather on 1, 6, and 11 August in 2001, 2003, and 2004, respectively, and left to dry in a windrow. A small plot combine was used to harvest the plots on 13, 18, and 31 August in 2001, 2003, and 2004, respectively. Harvested seed were weighed and yields per hectare were calculated.

**Statistical analyses.** Data from the OAA, PIT, and detached leaf assay (DLA) trials were analyzed using the general linear model procedure (PROC GLM) with SAS statistical analysis software (SAS Institute, Inc., Cary, NC), and means were compared using Fisher's least significant difference (FLSD) test where  $\alpha = 0.05$ . Data from the field trials at Carrington in 2001 and 2002 were analyzed separately using PROC GLM due to the number of different cultivars evaluated, and means were compared using FLSD test.

Data from the six common cultivars (Pioneer 44A89, Pioneer 46A76, Hyola 357, Hyola 401, Invigor 2663, and LG 3455) included in each field trial were analyzed together using PROC GLM, with year considered as a random effect. Least-square means were compared using the PDIF option, where  $P = 0.05$ .

Correlations among the variables OAA, PIT, DLA, SSR incidence, and yield were tested using the Pearson correlation procedure (PROC CORR) in SAS within each year and location and across years and locations combined.

## RESULTS

**OAA.** A pink-colored pigment appeared in the stems of some cultivars after exposure to the oxalic acid; however, no plants exhibited wilting or lesions from the oxalic acid exposure. In an attempt to measure this pink pigment, the oxalic acid solution from each test tube was analyzed with a spectrophotometer. Significant ( $P = 0.0001$ ) variation among cultivars occurred with the spectrophotometer readings (Table 1). Means of absorbance values ranged from 0.002 to 0.033.

**PIT.** Significant ( $P = 0.0001$ ) variation among cultivars occurred with the PIT screening method (Table 1). Cv. Hyola 357 had a significantly lower AUDPC value than all other cultivars except Hyclas 601.

**DLA.** No significant variation among cultivars occurred with the DLA screening method (Table 1).

**Carrington field trials, 2001–02.** Significant differences in SSR incidence were detected among cultivars at Carrington in 2001 and 2002 (Table 2). Differences among cultivars were somewhat inconsistent among the years. For example, Invigor 2663 had one of the lowest SSR incidence values in 2002 but one of the highest SSR incidence values in 2001. Cvs. Skyhawk and Pioneer 44A89 responded similarly each year, in which Skyhawk consistently had a low SSR incidence and Pioneer 44A89 consistently had a high SSR incidence.

Significant differences in yield also were detected among cultivars at Carrington in 2001 and 2002 (Table 2). Yields of cultivars tended to be more stable among years than SSR incidence. Cv. Crusher consistently had low yield in both years and had the significantly lowest yield of all cultivars in 2001. Cvs. Invigor 2573 and Invigor 2663 had consistently high yields in both years.

**Field trials with six common cultivars.** Six common cultivars (Pioneer 44A89, Pioneer 46A76, Hyola 357, Hyola 401, Invigor 2663, and LG 3455) were evaluated at Carrington, ND and Red Lake Falls, MN (Table 3). Due to significant

year–cultivar, location–year, and location–cultivar interactions for SSR incidence and significant location–year–cultivar and year–location interactions for yield, results are presented by location and year (Table 4). Cv. Pioneer 44A89 consistently had high SSR incidence, because it had the significantly greatest SSR incidence among all of the cultivars in four of the seven trials. The remaining five cultivars (Pioneer 46A76, Hyola 357, Hyola 401, Invigor 2663, and LG 3455) differed significantly among each other in only two of the seven trials. Within these five cultivars, Hyola 357 and Invigor 2663 differed from each other at Red Lake Falls in 2001, and Invigor 2663 and LG 3455 differed from Hyola 401 at Red Lake Falls in 2003.

Significant yield differences among cultivars occurred at all locations and years. Cv. Pioneer 44A89 was consistently one of the lowest yielding cultivars and had the significantly lowest yield of the six cultivars at Carrington in 2002 and 2003. Even under low disease pressure at Carrington in 2004, Pioneer 44A89 had significantly

lower yield than three other cultivars (Hyola 357, Invigor 2663, and LG 3455).

**Correlations.** Few significant correlations were detected among the variables tested. Significant correlations between yield and PIT AUDPC values were detected for the 19 cultivars evaluated at Carrington in 2001 ( $P = 0.0177$ ;  $R = -0.58$ ) and 2002 ( $P = 0.0380$ ;  $R = -0.48$ ). A significant correlation between SSR incidence and yield was detected for Red Lake Falls in 2003 ( $P = 0.0394$ ;  $R = -0.83$ ). No other correlations were significant for each location and year, and no significant correlations were detected when data were combined across years and locations.

## DISCUSSION

From our studies, the PIT appeared to be a good method to compare the level of resistance to SSR among canola cultivars under controlled conditions. Zhao et al. (25) also demonstrated that the PIT could be used effectively to differentiate oilseed *B. napus* accessions for resistance to SSR. The PIT had the lowest coefficient of variation (CV) of all three methods evalu-

**Table 1.** Reactions of 19 canola cultivars to the oxalic acid assay (OAA), petiole inoculation technique (PIT), and detached leaf assay (DLA), which are potential screening methods for resistance to *Sclerotinia sclerotiorum*

Cultivar	OAA <sup>1</sup>	PIT <sup>2</sup>	DLA <sup>3</sup>
Pioneer 44A89	0.007	248	2.9
Pioneer 46A76	0.005 <sup>w</sup>	227	2.5
Hyola 357	0.002 <sup>w</sup>	31	1.9
Hyola 401	0.009	89	2.9
Invigor 2663	0.025	219	3.3
LG 3455	0.003 <sup>w</sup>	128	4.7
Crusher	0.012	242	3.8
Gladiator	0.024	231	3.3
Hudson	0.022	195	2.2
Hyclass 601	0.011	85	2.1
IMC 204	0.014	274	6.2
Invigor 2573	0.014	134	3.4
Invigor 2733	0.033	126	4.1
Minot RR	0.020	211	3.6
Q2	0.010	128	4.2
Rider	0.014	215	2.9
Roughrider	0.013	265	3.1
Skyhawk	0.014	235	4.6
SWP 9828000	0.022	148	4.1
LSD <sub>0.05</sub> <sup>x</sup>	0.012	55	NS <sup>y</sup>
CV (%) <sup>z</sup>	86	31	80

<sup>1</sup> Combined data from two OAA trials. Cultivar means reported are absorbance values (518 nm) of soluble pigments dissolved in 5 ml of 40 mM oxalic acid.

<sup>2</sup> Combined data from two PIT trials. Cultivar means reported are area under disease progress curve units.

<sup>3</sup> Combined data from two DLA trials. Cultivar means reported are lesion diameters (mm).

<sup>w</sup> No visible pink pigment appeared in the stem of the cultivar after exposure to oxalic acid.

<sup>x</sup> Fisher's least significant difference (LSD), where  $\alpha = 0.05$ .

<sup>y</sup> *F* test was not significant (NS) at  $P \leq 0.05$ .

<sup>z</sup> Coefficient of variation (CV).

**Table 2.** Sclerotinia stem rot incidence, disease severity index values, and yield of canola cultivars inoculated with *Sclerotinia sclerotiorum* ascospores and grown under mist irrigation at Carrington, ND from 2001 to 2002

Cultivar	2001		2002	
	Incidence (%)	Yield (kg/ha)	Incidence (%)	Yield (kg/ha)
Pioneer 44A89	36	2,257	18	1,262
Pioneer 46A76	23	2,495	7	1,664
Hyola 357	34	2,709	9	2,116
Hyola 401	21	2,992	14	1,928
Invigor 2663	34	2,737	4	2,188
LG 3455	41	2,284	9	1,735
Crusher	33	1,395	11	9,14
Hudson	25	1,860	17	1,121
IMC 204	32	2,078	12	1,100
Invigor 2573	43	2,859	16	2,298
Minot RR	32	2,304	5	1,701
Q2	31	2,394	23	1,655
Rider	27	2,453	11	2,182
Roughrider	25	1,942	5	1,462
Skyhawk	17	1,969	5	1,316
SWP 9828000	15	2,093	8	2,129
Gladiator	...	...	16	1,904
Hyclass 601	...	...	14	1,661
Invigor 2733	...	...	15	1,458
LSD <sub>0.05</sub> <sup>z</sup>	16	372	10	390

<sup>y</sup> ... Indicates cultivar not planted in this trial.

<sup>z</sup> Fisher's least significant difference (LSD), where  $\alpha = 0.05$ .

**Table 3.** Combined analysis of variance for Sclerotinia stem rot (SSR) incidence and yield of six canola cultivars planted between 2001 and 2004 at Carrington, ND and Red Lake Falls, MN

	SSR incidence			Yield		
	MS error	<i>F</i> value	<i>P</i> > <i>F</i>	MS error	<i>F</i> value	<i>P</i> > <i>F</i>
Year	17,372	187.69	0.0001	9,169,690	112.02	0.0001
Block (location × year)	215	2.33	0.0033	243,626	2.98	0.0002
Location (loc)	737	7.96	0.0058	6,436,116	78.63	0.0001
Cultivar (cv)	2,730	29.50	0.0001	1,869,750	22.84	0.0001
Year × cv	285	3.08	0.0004	120,611	1.47	0.1305
Loc × year × cv	110	1.19	0.3051	158,085	1.93	0.0498
Loc × year	6,014	64.98	0.0001	4,039,796	49.35	0.0001
Loc × cv	591	6.38	0.0001	81,113	0.99	0.4274
Error	93	...	...	81,858	...	...

ated under controlled conditions. The CVs of the OAA and DLA trials possibly could have been lowered with the inclusion of additional plants per cultivar in the trials; however, the additional plants would require more space and would reduce efficiency. The PIT also has been used to identify soybean (10) and common bean (*L. E. del Río, unpublished*) cultivars and lines with improved resistance to *S. sclerotiorum*. In our trials, the PIT was the only method that had any significant correlation to field data. These significant negative correlations were between PIT

**Table 4.** Sclerotinia stem rot incidence and yield of canola cultivars inoculated with *Sclerotinia sclerotiorum* ascospores and grown under mist irrigation at Carrington, ND from 2001 to 2004 and Red Lake Falls, MN in 2001, 2003, and 2004

Location, year, cultivar	Incidence (%)	Yield (kg/ha)
Carrington, 2001		
Pioneer 44A89	36 a <sup>2</sup>	2,257 c
Pioneer 46A76	23 a	2,495 bc
Hyola 357	34 a	2,709 abc
Hyola 401	21 a	2,992 a
Invigor 2663	34 a	2,737 ab
LG 3455	41 a	2,284 bc
Carrington, 2002		
Pioneer 44A89	18 a	1,262 d
Pioneer 46A76	7 ab	1,664 c
Hyola 357	9 ab	2,116 ab
Hyola 401	14 ab	1,928 abc
Invigor 2663	4 b	2,188 a
LG 3455	9 ab	1,735 bc
Carrington, 2003		
Pioneer 44A89	90 a	903 c
Pioneer 46A76	51 b	1,538 b
Hyola 357	61 b	1,903 ab
Hyola 401	55 b	2,162 a
Invigor 2663	62 b	1,745 b
LG 3455	53 b	1,573 b
Carrington, 2004		
Pioneer 44A89	5 a	2,469 b
Pioneer 46A76	0 a	2,825 ab
Hyola 357	3 a	3,063 a
Hyola 401	1 a	2,435 b
Invigor 2663	2 a	2,990 a
LG 3455	1 a	3,026 a
Red Lake Falls, 2001		
Pioneer 44A89	77 a	1,224 bc
Pioneer 46A76	34 bc	1,199 c
Hyola 357	29 c	1,901 a
Hyola 401	34 bc	1,605 ab
Invigor 2663	45 b	1,549 abc
LG 3455	40 bc	1,251 bc
Red Lake Falls, 2003		
Pioneer 44A89	76 a	1,343 c
Pioneer 46A76	21 bc	1,682 abc
Hyola 357	18 bc	1,927 a
Hyola 401	11 c	1,957 a
Invigor 2663	30 b	1,826 ab
LG 3455	29 b	1,504 bc
Red Lake Falls, 2004		
Pioneer 44A89	25 a	2,124 c
Pioneer 46A76	1 b	2,170 c
Hyola 357	7 b	2,828 a
Hyola 401	7 b	2,747 ab
Invigor 2663	4 b	2,840 a
LG 3455	3 b	2,381 bc

<sup>2</sup> Means within a location and year followed by a common letter are not different according to least square means *t* tests ( $P \leq 0.05$ ).

AUDPC values and yield in the field trials. Although these correlations were statistically significant, they may not necessarily indicate that the PIT method would predict the response of a cultivar to SSR in the field. Because no significant relationships between PIT AUDPC values and SSR incidence in the field were observed, the PIT–yield relationship should be interpreted with caution because it could be just a matter of coincidence. Cultivars that differed significantly in the PIT trials, such as Pioneer 44A89 and Hyola 357, tended to have significant differences in yield regardless of disease pressure. For example, very low disease pressure was present at Carrington in 2004, yet Pioneer 44A89 and Hyola 357 still differed significantly in yield. Zhao et al. (25) rated accessions using a qualitative score on a daily basis (similar to our mortality rating) and a quantitative lesion phenotype score on the final day of rating in their PIT trials. We did not use a quantitative scoring system to rate canola cultivars in our PIT trials, and it is possible that a quantitative scoring system could have significantly correlated to field screening results. Both the qualitative and quantitative scoring systems should be used in future trials using the PIT method to screen for resistance to SSR in canola germ plasm.

A pink pigment developed in some canola stems after exposure to oxalic acid that was similar to what was observed on soybean by Wegulo et al. (24); however, no wilting or stem lesions were observed. Wegulo et al. (24) suggested that the pink pigments observed in the soybean stems were related to resistance to SSR, and reported that results of this method were repeatable and correlated well to field reactions of soybean cultivars to SSR. In our studies, the OAA method did significantly differentiate cultivars, but did not have a significant correlation with any field trial data. Because no wilting or lesions were caused by oxalic acid in our trials, tolerance to oxalic acid was not truly measured. A higher percentage of oxalic acid in the solution may be required to cause wilting and lesions on canola (40 mM was used in our trials); however, Chaocai (4) and Fang (7), using 15 and 40 mM, respectively, were able to cause wilting and lesions on oilseed *B. napus*. Differences in methodology existed; however, because Chaocai (4) evaluated entire plants by submersing roots in oxalic acid and Fang (7) evaluated detached leaves in oxalic acid. The oxalic acid procedure conducted by Chaocai (4) correlated significantly with field results, but the oxalic acid procedure conducted by Fang (7) did not relate to resistance to *S. sclerotiorum*. Both Chaocai (4) and Fang (7) evaluated a mixture of canola-quality and non-canola-quality oilseed *B. napus*, whereas only canola-quality oilseed *B. napus* cultivars were evaluated in our trials. This could

have contributed to different reactions and results among the different oxalic acid trials, because Zhao et al. (25) found that non-canola-quality accessions tended to be more resistant to SSR than canola-quality accessions. It appears from our and others' results that more research is needed to design an OAA that can differentiate canola-quality oilseed *B. napus* cultivars effectively and relate to field reactions to *S. sclerotiorum*.

A DLA method was used successfully by Bailey (1) to differentiate oilseed rape accessions for resistance to *S. sclerotiorum*. The methods used in our DLA trials were very similar to that of Bailey (1); however, the DLA in our trials did not provide differentiation among the cultivars tested or have a significant correlation with any of the field data. Although ascospores were used to inoculate plants in the field and are the primary inocula under natural conditions, the DLA mimics what can happen in a natural epidemic in the field, because the *S. sclerotiorum*-infested PDA plug is similar to an infected flower petal that landed on a leaf. Leaf resistance to *S. sclerotiorum* in canola, although important, may not provide as much of a benefit as stem resistance, because destruction of the stem causes the largest damage to the canola plant.

Disease pressure in the field trials varied from year to year. Even though the field plots were misted and artificially inoculated with ascospores, not all environmental conditions needed for infection can be controlled in the field. Uncontrollable factors in the field, such as temperature and relative humidity, play a role in ascospore survival and infection (2) and may have contributed to variation in disease pressure across locations and years.

Six canola cultivars common to every field trial were Hyola 357, Hyola 401, Invigor 2663, LG 3455, Pioneer 44A89, and Pioneer 46A76. The ranking among these cultivars for SSR incidence was somewhat inconsistent among locations and years. Pioneer 44A89, however, consistently had a high SSR incidence and ranked sixth (lowest to highest) for SSR incidence in six of the seven trials. Results of our PIT trials and those conducted by Zhao et al. (25) indicated that Pioneer 44A89 had a lower level of resistance to SSR than many other cultivars and accessions. Although only a small percentage of canola cultivars available to growers were tested in our trials, it is apparent that there are differences in levels of resistance to SSR in the pool of commercial canola cultivars. Efforts in breeding for SSR-resistant canola cultivars and screening for new resistant sources should be continued to improve the levels of resistance in commercial canola cultivars.

Although a lab or greenhouse screening method that could accurately predict the reaction of a canola cultivar to SSR in the

field was not identified, screening methods that could differentiate canola cultivars were identified. From this study, we agree with Zhao et al. (25) that the PIT is a good method to be used to differentiate canola cultivars or *B. napus* accessions for their reaction to *S. sclerotiorum*. Any material identified as having a high level of resistance to *S. sclerotiorum* using the PIT should be evaluated for their reaction to SSR in the field as well.

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