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Resistance and Tolerance to Sclerotinia Stem Rot in Selected Short-Season Soybean Cultivars in Canada

Allen G. Xue[1*] • Sylvie Rioux[2] • Malcolm J. Morrison[1] • Yuanhong Chen[1] • Jinxiu Zhang[1] • Weikai Yan[1]

[1] Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, K.W. Neatby Building, 960 Carling Avenue, Ottawa, Ontario, K1A 0C6, Canada
[2] CEROM, 2700 Einstein, Quebec City, Quebec, G1P 3W8, Canada

Corresponding author: * **allen.xue@agr.gc.ca**

ABSTRACT

Fifteen short-season soybean cultivars released in Canada from 1934 to 2000 were evaluated under inoculated field conditions for resistance and tolerance to Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, in Saint-Bruno, Quebec in 2001, 2002 and 2003. Resistance to SSR was measured by the disease incidence, the severity, a disease severity index, the area under the disease progress curve, and tolerance by the reduction of yield and thousand seed weight (TSW) compared to a non-inoculated control. Significant differences in cultivar responses were found for all parameters. Averaged across the test years, 'AC Harmony', 'Maple Arrow', 'Maple Glen', 'Maple Ridge', and 'AC Orford', released after 1976, were among the most resistant cultivars based on their disease responses and were significantly better than 'Capital', 'Comet', 'Flambeau' and 'Mandarin', released before 1953, suggesting that newer cultivars are more resistant to the disease than older ones. The disease reduced yield by approximately 7-45% and TSW by < 4%. 'Altona', 'Crest', 'Mandarin', 'Maple Glen', 'Maple Ridge', 'Pagoda', and 'Portage' were among the most tolerant cultivars, with yield reduced by <18%, and were significantly better than 'AC Harmony', 'Capital', 'Comet', and 'Flambeau', with yields reduced by >33%. 'Maple Glen' and 'Maple Ridge' were the only cultivars with high levels of both resistance and tolerance. The four disease parameters were highly correlated ($r \geq 0.98$, $P < 0.01$), suggesting that a single measurement should be sufficient. All disease parameters were negatively correlated with yield and TSW under inoculation ($r \geq 0.36$, $P < 0.05$) but not correlated with yield and TSW reduction, suggesting that resistance and tolerance to SSR are two separately inherited traits in soybean.

Keywords: resistance, Sclerotinia stem rot, *Sclerotinia sclerotiorum*, soybean, tolerance
Abbreviation: AUDPC, area under the disease progress curve; **DSI**, disease severity index; **SSR**, Sclerotinia stem rot

INTRODUCTION

Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is an important disease of soybean (*Glycine max* (L.) Merr.) in the United States and Canada (Boland and Hall 1988; Doupnik 1993; Wrather 2001). The initial inoculum of the disease is soilborne sclerotia that form apothecia from which ascospores are released and germinate to colonize senescing flower parts as a nutrition base. Mycelia subsequently colonize leaf axils and stem nodes, then advance bilaterally on the main stems and lateral branches, resulting in white, bleached lesions on stems, leaves, and petioles in the lower part of the canopy (Cline and Jacobsen 1983; Boland and Hall 1988; Grau 1988). Under favorable environmental conditions, the appearance of lesions is followed by rapid wilting of the infected plants that are often scattered in irregular patches in the field. It is estimated that every 10% increase in SSR causes yield reductions of 83 to 330 kg/ha (Doupnik 1993; Hoffman *et al*. 1998; Yang *et al*. 1999; Danielson *et al*. 2004).

Current management strategies to reduce SSR include cultural practices, foliar application of fungicides, and the use of genetic resistance (Boland and Hall 1988; Grau *et al*. 1994; Mueller *et al*. 2002a; Dorrance and Mills 2008). Crop rotations to reduce initial inoculum and foliar fungicide applications to reduce disease severity have a limited effect and are often unsatisfactory (Oplinger and Philbrook 1992; Kurle *et al*. 2001; Mueller *et al*. 2002a, 2002b). In addition, fungicide applications present additional costs to producers and are generally not profitable (Dann *et al*. 1998; Mueller *et al*. 2002b; Rousseau *et al*. 2004; Dorrance and Mills 2008). Breeding for resistance remains the only viable option and is considered the most practical, economical, and environmentally safe measure against this disease (OMAFRA 2008).

Cultivar differences in resistance to *S. sclerotiorum* in soybean have been reported (Grau *et al*. 1982; Wegulo *et al*. 1998; Yang *et al*. 1999; Kim *et al*. 2000; Hoffman *et al*. 2002; Rousseau *et al*. 2004). To date, breeding for resistance has been only partly successful because of the lack of high levels of genetic resistance in soybean (Grau *et al*. 1982; Hoffman *et al*. 2002; Cober *et al*. 2003). In addition, the expression of resistance to SSR is complex and strongly influenced by disease pressure, environmental conditions, and other physiological and agronomic traits of the plants (Grau and Radke 1984; Pennypacker and Risius 1999; Kim and Diers 2000; Workneh and Yang 2000). Field conditions that enhance SSR development include extended plant surface wetness (Cline and Jacobsen 1983; Grau and Radke 1984; Boland and Hall 1988), cool and moist soil (Grau 1988) and cool canopy temperatures caused by closure (Chun *et al*. 1987; Boland and Hall 1988; Grau 1988; Nelson *et al*. 1991). Under conductive conditions, plants with upright plant architecture, open canopy, less lodging, short flowering period, and early-maturity reduce the possibility of SSR infection and severe disease development (Yang *et al*. 1999; Kim *et al*. 2000).

In Canada, soybean production has increased from approximately 4400 ha annually in 1940s to 1.2 M ha in 2008 (Dominion Bureau of Statistics 1959; Statistics Canada 2008) and yield increased 0.5 to 1.0% per year in the same period (Voldeng *et al*. 1997; Morrison *et al*. 1999, 2000, 2008). The expansion in production area and yield is largely due to the development of short-season cultivars (maturity

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Table 1 Name, year of release, maturity group, and pedigrees of 15 historical cultivars tested for resistance and tolerance to Sclerotinia stem rot in 2001, 2002 and 2003.

Cultivar	Year of release	Maturity group	Pedigree
Mandarin	1934	0	Machurian introduction
Pagoda	1939	00	Mandarin/Manitoba Brown
Capital	1944	0	171(Manchuria)/AK(Harrow)
Flambeau	1948	00	Selection from early Russian variety
Comet	1953	0	Pagoda/Mandarin
Crest	1957	00	Manitoba Brown/Mandarin/2/Mandarin
Portage	1964	00	Acme/Comet
Altona	1966	00	Flambeau/052-903
Maple Arrow	1976	00	Harosoy 63/840-7-3
McCall	1978	00	Acme/Chippewa/2/Hark
Maple Ridge	1984	00	Fiskeby III/Evans
Maple Glen	1987	00	BD22115-13/Premier
AC Bravor	1990	0	Maple Arrow/Wayne
AC Harmony	1992	00	Maple Presto/Williams/2/Weber
AC Orford	2000	0	OT80-18/X875-2-B-1

group 0 and earlier maturing) and the improved stress and disease tolerance of these early maturing cultivars (Voldeng *et al*. 1997; Morrison *et al*. 2000). There have been more than 100 short-season soybean cultivars registered for commercial production in Canada. Although the improvement of disease resistance, particularly SSR resistance, has been one of the major priorities of soybean breeding in Canada including the development of these short-season cultivars, no information is available on changes in SSR resistance among old and new short-season cultivars across a prolonged period of breeding and selection. Our objective was to examine the differences in resistance and tolerance to SSR within a group of 15 historical short-season cultivars representing 66 years of breeding and selection (1934-2000) in Canada.

MATERIALS AND METHODS

Growth of plants

Field experiments were conducted at the CEROM (Centre de Recherche sur les Grains Inc.), Saint-Bruno, Quebec, in 2001, 2002, and 2003. Fifteen soybean cultivars were chosen from maturity groups 0 and 00, which had been released in a 66-yr period and grown in eastern Canada (**Table 1**).

The field site from 2001 through 2003 was under conventional tillage in a 3-year rotation of soybean/corn/cereal (wheat, barley, or oat) and the soil type was a Farmington loam (Melanic Brunisol). Experiments were arranged in a split-split-plot design with four replications. Plants with or without the artificial inoculations were the main plots, two timings of inoculation were the sub-plots, and soybean cultivars were the sub-sub-plots. Plots were seeded at a rate of 60 seeds m^{-2} between May 10 and May 18 each year and consisted of five rows, 5.0-m long, with 0.20-m row spacing and 0.5-m between plots. Five rows of soybean as the buffer area were planted between the inoculated and non-inoculated main plots to reduce inter-plot influence. The fields were fertilized according to soil test recommendations and treated with pre-emergence application of Pursuit herbicide at a rate of 0.3 L/ha and post-emergence application of Basagran Forté at a rate of 1.7 L/ha for effective weed control each year. Plants of inoculated plots were hand harvested at maturity, air-dried, and threshed by a stationary-type, large-plot thresher (LPT; ALMACO, Nevada, Iowa). Non-inoculated plots were harvested using a small plot combine. Yield was adjusted to 13% moisture content and thousand seed weight (TSW) determined by weighing 200 seeds per plot.

Inoculum preparation and inoculation

The *S. sclerotiorum* strain NB5, isolated from a soybean stem collected in a field from the Huntingdon region of Quebec, was used for producing the inoculum of sclerotia each year. The NB5 strain was chosen because it is known to be aggressive (Cober *et al*. 2003). Sclerotia were produced on a potato substrate in plastic autoclavable bags, each containing 250 g of potato fries (frozen fried, McCain), autoclaved for 25 min, inoculated with pieces of agar and mycelium of *S. sclerotiorum* grown on potato dextrose agar (PDA), and then incubated at 22°C in darkness for 4 weeks. Sclerotia were extracted from potato fries. To condition sclerotia for carpogenic germination, they were washed and placed in Erlenmeyer flasks half-filled with tap water, which were aerated with an aquarium air pump. The flasks were incubated at 4°C in darkness for 11 weeks and the water was changed weekly. The vernalization process was modified from that of Rousseau *et al*. (2004) and the sclerotia were air-dried and stored at 4°C until field inoculation.

Plants were inoculated early (V4, Fehr *et al*. 1971) or late (V7) each year. The dates of two timings of inoculation were July 5 and July 16 in 2001, June 25 and July 12 in 2002, and June 25 and July 9 in 2003, for early and late inoculation treatments, respectively. Within the inoculated main-plots, an across-section of 1-m long was selected for each of the two timings of inoculation. The early and late inoculation treatments were separated by plastic strings laid out on the ground before the inoculation. At each inoculation, 20 randomly selected sclerotia were buried 2-cm deep evenly in the 4 inter-rows for each sub-sub plot. The inoculated plots were irrigated with pierced hosepipes on the soil as needed, to keep the soil surface wet from the day of early inoculation until the beginning of seed filling.

Disease and yield assessment

The development of SSR was monitored by visually estimating disease severity on all plants (approximately 60) in each inoculated plot on a scale of 0 to 3, where 0 = no symptoms, 1 = lesions on lateral branches only, 2 = lesions on main stem but little effect on pod fill, and 3 = lesions on main stem resulting in plant death and poor pod fill. Assessments were carried out three times during each growing season at growth stages R4, R5, and R6, respectively. The dates of these assessments were August 29, September 7, and September 19 in 2001, August 30, September 5, and September 16 in 2002, and August 30, September 5, and September 16 in 2003. Severity of SSR over time was calculated as area under the disease progress curve (AUDPC) for each plot using the formula described by Wilcoxson *et al*. (1975). In addition, final disease incidence and severity for each plot were rated at the beginning of leaf senescence (R6, a few days before the appearance of a pod with mature color). The disease incidence was calculated based on percentage of infected plants and disease severity by the average of individual plant ratings (0-3 scale). The disease severity index (DSI) was calculated using the formula (Grau *et al*. 1982; Kim and Diers 2000; Cober *et al*. 2003):

$$DSI = \frac{\text{Sum (severity class} \times \text{no. of plants in class)}}{3 \times \text{total number of plants rated}} \times 100$$

Data analysis

Residuals for each parameter for each experiment were examined for normality and homogeneity of variances. An angular transformation of disease incidence and DSI and logarithmic transformation of yield were used in the analysis of variance to stabilize variances (Snedecor and Cochran 1980). Data analysis was conducted for each year separately and across years in a combined analysis using the GLM procedure of SAS (SAS Institute 2004). The assumption of normality based on Shapiro Wilk's test was examined using the Univariate procedure of SAS and random and homogeneous distribution of residuals was examined using the Plot procedure of SAS. Means of untransformed data were presented and separated by Fisher's least significant difference (LSD) tests at a probability level $P \leq 0.05$, based on the analysis of transformed data. Pearson correlation analysis was used to examine the relationships among the disease and yield parameters. Contrasts were used to compare the yield and TSW reductions of cultivars under the inoculated condition. To observe cultivar changes across time, the cultivar means of the disease parameters and reductions in yield and TSW were plotted against the year of cultivar release. A straight line was fitted through the points using simple linear regression.

GGE biplot analysis (Yan and Kang 2003) was done to visualize and verify the relationships among various measurements and to identify resistant and tolerant soybean cultivars. The GGE biplot was based on singular value decomposition of trait-standardized data (Yan and Rajcan 2002). In such a biplot, the correlation between two traits is approximated by the cosine of the angle between them; the relative value of a cultivar for a trait is approximated by the product of the distance of the cultivar from the biplot origin, the distance of the trait from the biplot origin, and the cosine of the angle between the cultivar and the trait.

RESULTS

Plants in the non-inoculated plots remained healthy throughout the experiments whereas those inoculated with the pathogen developed SSR symptoms. Significant differences ($P < 0.05$) were observed among cultivars within each of the three years for all parameters examined under both the inoculated and non-inoculated conditions (**Table 2**). The timing of inoculation significantly affected the disease incidence, DSI, AUDPC, yield, and TSW in 2001 and yield in 2003, but had no effect on any disease parameters in 2002 and 2003. In the combined analysis, significant differences were observed among the test years, for cultivars within a year, and there was a significant year × cultivar interaction for all parameters examined under the inoculated conditions. The effect of inoculation timing was significant for disease parameters and yield, but not for TSW, and year × inoculation timing was significant for yield only. The effect of inoculation timing × cultivar interaction and the year × inoculation timing × cultivar interaction was not significant for all parameters. Under the non-inoculated conditions, crop yield was not affected by the year, but there were significant differences among cultivars as well as a year × cultivar interaction. There were significant differences in TSW as affected by year, cultivar and year × cultivar interaction. Yield and TSW reductions were significantly affected by year and cultivar, but not by inoculation timing.

The early inoculation resulted in significantly more severe SSR disease and lower yield than the late inoculation, over the average of 15 soybean cultivars and three test years (**Table 3**). However, the two inoculation timings were not significantly different in TSW and reductions in yield and TSW.

There were significant differences among cultivars for all SSR resistance and tolerance parameters measured

Table 2 Mean squares from analysis of variance for the effects of inoculation timing (IT) and cultivar, and their interactions on Sclerotinia stem rot incidence, severity, disease severity index (DSI), area under the disease progress curve (AUDPC), yield, thousand seed weight (TSW), yield reduction, and TSW reduction of soybean from 2001 to 2003.

	DF	Incidence	Severity	DSI	AUDPC	Yield			TSW		
						Inoculation	Non-inoculated	Reduction	Inoculation	Non-inoculated	Reduction
2001											
Replicate	3	727.0 *	0.97 *	536.7 **	3.1 **	0.01	0.03 **	135.7	309.1 **	153.8 **	262.6**
IT	1	636.6 *	0.27	307.5 *	0.9 *	0.59 **		3066.6 *	151.9 *		34.6*
Cultivar	14	1242.4 **	1.28 **	819.9 **	5.0 **	0.29 **	0.21 **	986.8 **	4434.1 **	3000.0 **	150.0**
IT × cultivar	14	159.4	0.16	103.7	0.7	0.09		467.6	62.0		18.0
Error	84	131.7	0.15	85.9	0.6	0.05	0.01	362.8	70.7	76.4	45.7
2002											
Replicate	3	276.7	0.04	21.3	0.1	0.03	0.05 **	436.4	114.5	145.4 **	90.3
IT	1	30.6	0.01	10.9	0.2	0.46		3617.5	350.2		124.2
Cultivar	14	1054.4 **	0.44 **	268.2 **	2.1 **	0.22 **	0.24 **	1517.2 **	3498.9 **	3292.1 **	72.5
IT × cultivar	14	66.1	0.02	10.7	0.2	0.07		750.0	92.2		32.9
Error	84	116.2	0.04	21.0	0.1	0.09	0.01	649.2	82.1	65.2	59.9
2003											
Replicate	3	5572.3 **	7.28 **	4443.0 **	30.4 **	0.46 *	1.13 **	1439.3	865.4	203.2 **	183.5
IT	1	100.4	0.08	68.3	0.5	1.03 **		4794.8 *	114.1		10.0
Cultivar	14	2051.7 **	2.72 **	1793.2 **	12.4 **	0.89 **	0.20 **	2113.6 **	5847.5 **	4807.7 **	147.9
IT × cultivar	14	136.8	0.18	101.8	0.9	0.20		498.0	158.4		54.5
Error	84	165.5	0.25	157.2	1.2	0.13	0.03	410.2	193.9	145.2	129.2
Combined analysis											
Replicate	3	1152.2	1.64	989.4	6.6	0.25	0.60	675.8	707.7	122.1	442.2*
Year	2	33284.3 **	28.74 *	16534.2 *	94.1 *	2.41 **	0.91	28236.7 **	9680.4 **	30234.4 **	2204.4**
Error A	6	2711.9	3.33	2005.8	13.5	0.13	0.30	667.9	290.7	190.1	47.1
IT	1	554.5 **	0.27 *	282.3 **	1.5 **	0.41 **		1385.7	6.1		1.5
Year × IT	2	106.6	0.04	52.2	0.1	0.84 **		5046.6 **	305.0		83.7
Error B	9	46.4	0.04	20.4	0.1	0.03		294.7	141.7		51.6
Cultivar	14	2760.6 **	2.95 **	1903.2 **	12.1 **	0.74 **	0.37 **	2759.3 **	11582.3 **	9683.1 **	184.0**
Year × cultivar	28	794.0 **	0.75 **	489.1 **	3.7 **	0.32 **	0.14 **	929.2 **	1099.1 **	708.3 **	93.2
IT × cultivar	14	121.8	0.15	83.6	0.7	0.08		408.0	101.6		39.1
Year × IT × cultivar	28	120.2	0.10	66.3	0.5	0.14 *		653.8	105.5		33.2
Error C	252	137.8	0.15	88.1	0.7	0.09	0.02	474.0	115.6	95.6	78.3

* Significant at P < 0.05; ** Significant at P < 0.01. DF, degree of freedom.

Table 3 Sclerotinia stem rot incidence, severity, disease severity index (DSI), area under the disease progress curve (AUDPC), yield, thousand seed weight (TSW), yield reduction, and TSW reduction by *Sclerotinia sclerotiorum* on 15 short-season soybean cultivars grown at Saint-Bruno, Quebec from 2001 to 2003[†].

	Incidence (%)	Severity (0-3)	DSI (%)	AUDPC	Yield (kg/ha)			% reduction	TSW (g)			% reduction
					Inoculated	Non-inoculated	Difference		Inoculated	Non-inoculated	Difference	
Cultivar												
Mandarin	49.8 bc	1.0 c	24.4 c	1.9 c	3152.5 a	3884.6a	732.1**	16.0abc	213.0a	212.5a	-0.5	-0.4abcd
Pagoda	32.3 efg	0.6 ef	13.1 de	1.2 de	2136.7 f	2481.7h	345.0**	12.6ab	162.8f	159.0i	-3.8	-3.5abc
Capital	73.3 a	1.7 a	44.8 a	3.3 a	1848.2 cd	3270.4efg	1422.1**	44.6f	144.3g	144.3j	0.0	-0.1bcd
Flambeau	53.3 b	1.3 b	32.6 b	2.4 b	2309.3 bc	3459.4cde	1150.1**	32.3def	173.5e	179.0fg	5.5	2.8d
Comet	49.8 b	1.1 bc	25.7 bc	2.2 bc	2368.1 a	3897.4a	1529.3**	35.8ef	172.6e	175.0g	2.5	0.4cd
Crest	31.7 efg	0.6 ef	12.4 de	1.0 ef	2632.4 de	3104.2fg	471.8**	14.3ab	207.3ab	200.8bc	-6.5*	-4.0abc
Portage	32.0 de	0.6 ef	9.6 de	1.2 de	2845.6 cd	3324.2def	478.6**	12.9ab	186.2d	181.5ef	-4.7	-3.0abc
Altona	33.9 efg	0.7 d	15.6 d	1.4 d	2659.6 e	2954.0g	294.4	6.5a	181.7d	182.4ef	0.7	-0.3abcd
Maple Arrow	22.8 h	0.4 f	6.4 e	0.8 ef	2876.3 a	3724.3abc	847.9**	21.3bcd	198.6c	190.4d	-8.2**	-4.7ab
McCall	39.3 cd	0.7 d	11.4 d	1.3 d	2812.6 a	3755.4ab	942.8**	21.6bcd	161.2f	167.7g	6.6*	3.3d
Maple Ridge	25.8 gh	0.5 ef	7.0 e	0.9 ef	2725.5 de	3188.1efg	462.6*	10.2ab	174.4e	166.5u	-7.9**	-5.2a
Maple Glen	22.7 h	0.4 f	6.1 e	0.7 f	3047.0 ab	3721.0abc	674.1**	14.6abc	204.0bc	197.0c	-6.9	-3.9abc
AC Bravor	34.7 def	0.7 d	13.6 d	1.3 de	2712.4 a	3858.2a	1145.9**	27.0cde	184.8d	186.1de	1.3	0.1bcd
AC Harmony	26.0 gh	0.5 ef	8.2 e	1.0 ef	2216.3 c	3492.2bcd	1275.9**	31.2de	145.4g	149.2j	3.8	2.2d
AC Orford	27.5 fgh	0.6 ef	9.3 de	1.0 ef	2979.9 a	3809.2a	829.3**	21.0bcd	206.8ab	206.3b	-0.5	-0.6abcd
Timing of inoculation												
Early	38.7 a	0.8 a	17.1 a	1.5 a	2559.5 b		902.1	23.4a	181.0a		-1.1	-1.2a
Late	35.3 b	0.7 b	14.9 b	1.4 b	2683.5 a		778.1	19.5a	181.2a		-1.4	-1.1a

[†]Data were means of three years (2001-2003). Values followed by the same letter in a column, among cultivars or between early and late inoculation timings are not significantly different at P = 0.05 (LSD).
* Significant at P < 0.05; ** Significant at P < 0.01.

Table 4 Correlation coefficients among Sclerotinia stem rot incidence, severity, disease severity index (DSI), area under the disease progress curve (AUDPC), yield, thousand seed weight (TSW), yield reduction, and TSW reduction by *Sclerotinia sclerotiorum* on 15 short-season soybean cultivars representing 66-yr of genetic improvement measured across 3-yr test period.

	Incidence	Severity	DSI	AUDPC	Yield	TSW	Yield reduction
Severity	0.987**						
DSI	0.978**	0.992**					
AUDPC	0.986**	0.994**	0.989**				
Yield	-0.526**	-0.560**	-0.579**	-0.589**			
TSW	-0.382*	-0.381*	-0.356*	-0.426**	0.824**		
Yield reduction	0.056	0.054	-0.001	0.034	0.488**	0.319*	
TSW reduction	-0.299	-0.298	-0.283	-0.248	0.824**	0.978**	0.417**

(**Table 3**). Averaged across the test years, 'AC Harmony', 'Maple Arrow', 'Maple Glen', 'Maple Ridge', and 'AC Orford', released after 1976, were among the most disease resistant cultivars and significantly better than 'Capital', 'Comet', 'Flambeau' and 'Mandarin', released before 1953, based on their disease incidence, severity, DSI, and AUDPC. Yield reduction ranged from less than 7% for 'Altona' to 45% for 'Captial'. The reduction in yield was significant for all cultivars except for 'Altona'. 'Crest', 'Mandarin', 'Maple Glen', 'Maple Ridge', 'Pagoda', and 'Portage' were also among the most tolerant cultivars, with a reduction in yield of < 18%, and were significantly better than 'AC Harmony', 'Capital', 'Comet', and 'Flambeau', that had yield reductions > 33%. 'Maple Glen' and 'Maple Ridge' were the only cultivars with high levels of both resistance and tolerance. The reduction in seed weight was observed in four of the 15 cultivars and by less than 4% only. Significant reduction in seed weight was observed only for 'McCall'. Three cultivars including 'Crest', 'Maple Arrow', and 'Maple Ridge' significantly increased seed weight under the inoculated conditions.

The four disease parameters were highly correlated ($r \geq 0.98$, $P < 0.01$) and these disease parameters were negatively correlated with yield under inoculation ($r \geq 0.36$, $P < 0.05$) but not correlated with yield and TSW reduction (**Table 4**). Yield was highly correlated with seed weight under both inoculated and non-inoculated conditions ($r \geq 0.82$, $P < 0.01$), which was in accordance with the results of GGE biplot analysis (**Fig. 1**).

When the mean of disease incidence, severity, DSI, AUDPC, yield, TSW, and reductions of yield and TSW from three test years of a cultivar was plotted against the year of cultivar release, the linear regression revealed that resistance to SSR increased significantly across the 66-yr breeding period by 56.7, 62.4, 84.3, and 66.3%, or 0.9, 1.0, 1.3, and 1.1% per year, for disease incidence, severity, DSI, and AUDPC, respectively (**Fig. 2A, 2B, 2C, 2D**). Yield increased by 14.5%, or 0.2% per year across the 66-yr breeding period (**Fig. 2E**); however, the yield increase with year of release was not statistically significant. Seed weight and SSR tolerance measured by the percentage of yield and TSW reductions remained unchanged across the 66-yr breeding period (**Fig. 2F, 2G, 2H**).

DISCUSSION

The 15 soybean cultivars tested in this study represent a wide range of differences in resistance to SSR that is currently available in the Canadian short-season soybean. Although breeding for SSR resistance has always been a major priority of soybean cultivar development programs in Canada, the magnitude of cultivar differences among the recently released and oldest landrace cultivars has not been examined. Our results indicated that the resistance to SSR increased from 66-yr of plant breeding and selection by more than 58%, at approximately 1% per year. The relatively higher levels of SSR resistance in these recently released short-season soybean cultivars have not been

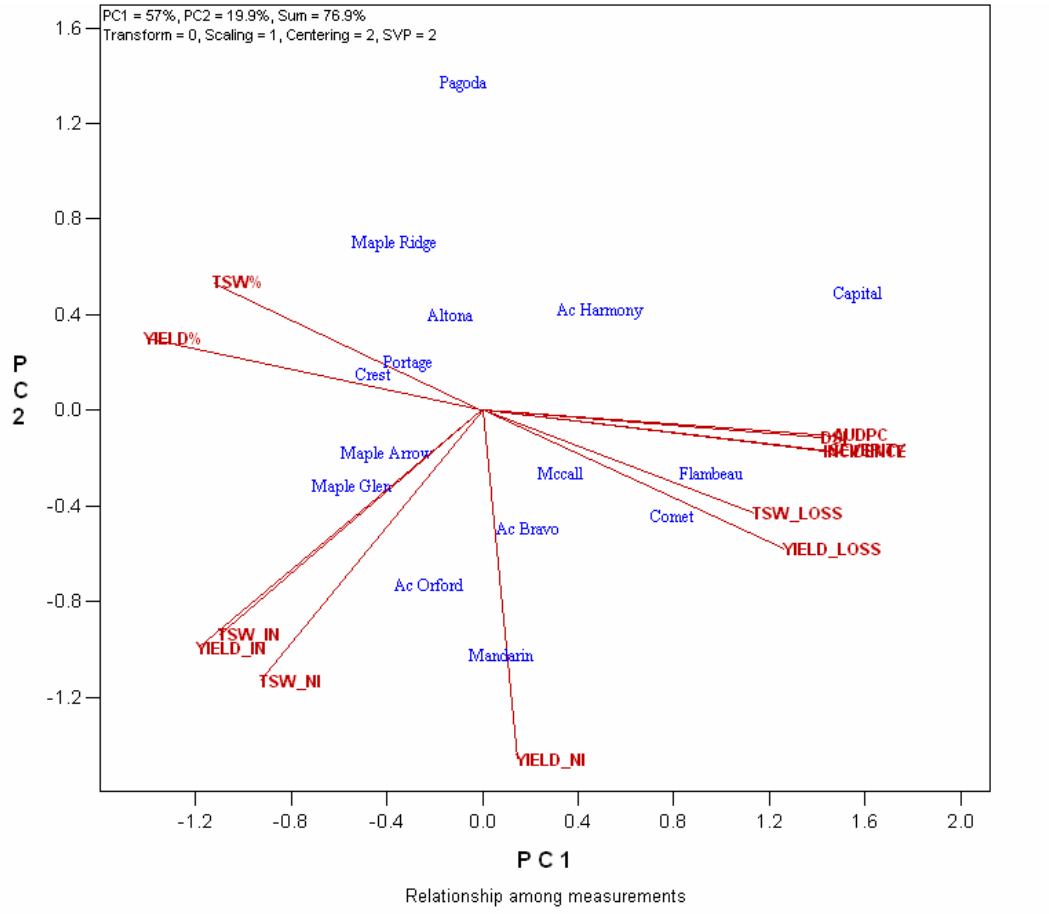

Fig. 1 GGE biplot for the genotype from data of yield under inoculation (IN) and yield non-inoculation (NI) in Table 3.

previously reported.

This study demonstrated that the timings of inoculation had no significant effect on cultivar resistance to SSR (**Table 2**). This result is in agreement with Vuong *et al*. (2004) who reported that variety resistance to *S. sclerotiorum* was not affected by plant age. However, severity of SSR was significantly influenced by timings of inoculation under field conditions. The early inoculation is preferable as it would result in higher disease pressures and allow for little chance for disease escape than the late inoculation. Although moderate SSR developed in all three test years, disease severity was relatively low in 2002 than in 2001 and 2003 (data not shown). The significant year × cultivar interactions for all disease resistance and tolerance parameters found in the present study were also in agreement with previous reports that SSR disease severity and cultivar resistance varied with environmental conditions (Cline and Jacobsen 1983; Chun *et al*. 1987; Boland and Hall 1988; Kim *et al*. 2000). These results suggest that evaluation of cultivar resistance to SSR shall be conducted under different environments and with multiple years. Correlations among the four disease parameters were high ($r \geq 0.98$, $P < 0.01$) (**Table 4, Fig. 1**), indicating that any single measure would suffice in cultivar evaluation for SSR resistance.

Our data indicated that the yield increased from 66-yr of plant breeding and selection by 0.22% per year. This rate of increase was lower than that of Morrison *et al*. (1999, 2000), who reported yield improvement of 0.45% per year, using the same set of cultivars but without AC Orford (released in 2000), grown in Ontario from 1993 to 1998. The variation in yield improvement per year among these studies was likely due to the different test locations and the possible confounding effect of the field environments. Seed weight remained unchanged over the time. This result was in agreement with Morrison *et al*. (1999, 2000), suggesting that breeders have increased yield by selecting the number of seeds per plant, not by increasing seed size. While there was considerable variation in seed size among soybean cultivars, it appears that the increase in numbers of seed per plant did not occur at the expense of seed size.

The present study also demonstrated significant differences among cultivars in level of tolerance to SSR, measured as percentage of yield and TSW reduction under the inoculated field conditions. Cultivar 'Altona', released in 1966, had no significant yield reduction (**Table 3, Fig. 1**). While 'Capital', released in 1944, reduced yield by 45%. However, regression coefficients obtained from regression of cultivar means of tolerance to SSR on year of release were not significantly different from zero (**Fig. 2F, 2H**). These results suggest that breeders have either not successfully increased the SSR tolerance over the time or not considered SSR tolerance as a desirable trait for new cultivars.

The lack of correlation between SSR tolerance and disease resistance parameters (**Table 4, Fig. 1**) suggests that SSR resistance and tolerance may be controlled by different genes and inherited independently. More research is needed to examine the genetics of SSR tolerance identified in the present study, in order to effectively use these tolerant cultivars in breeding programs.

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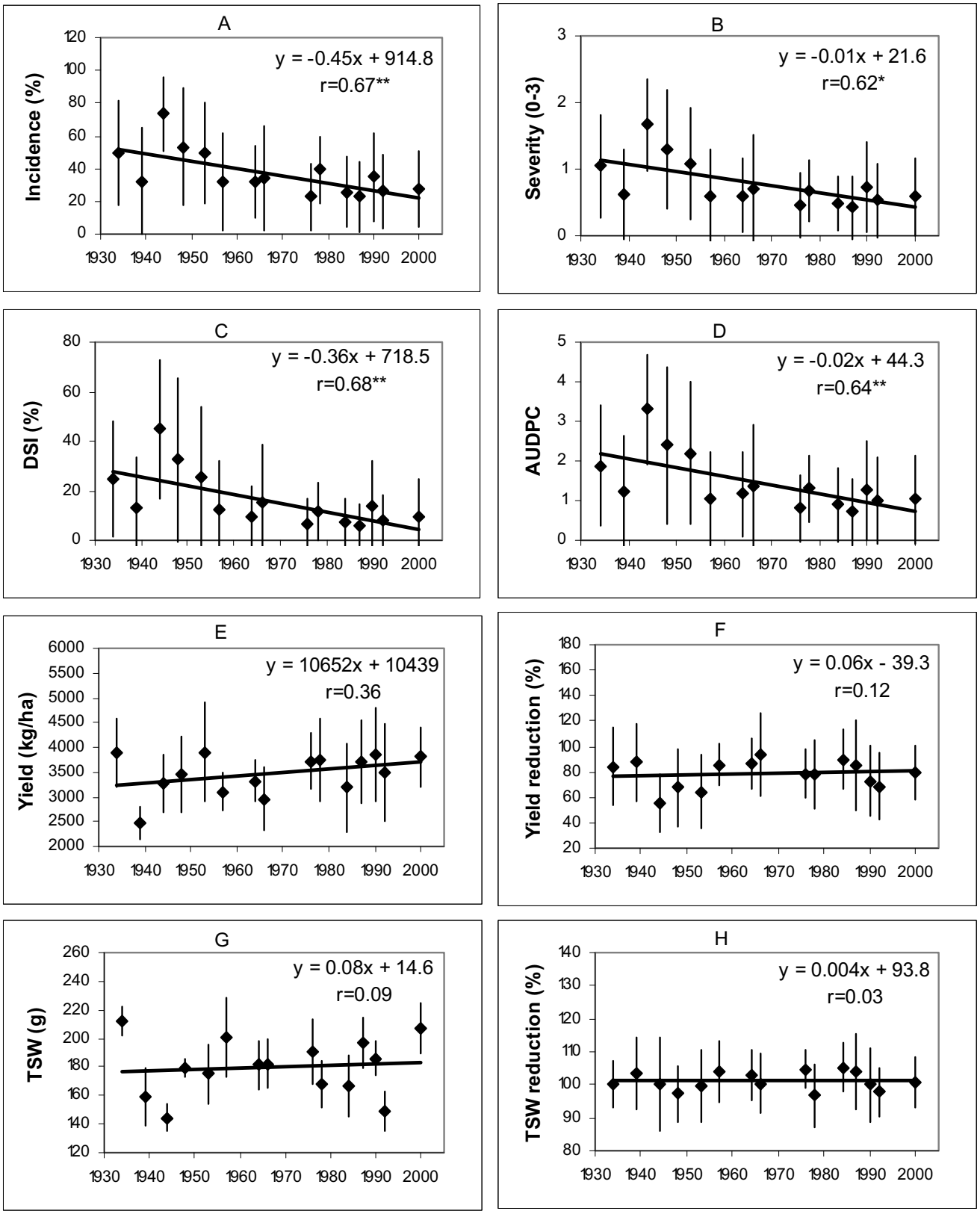

Fig. 2 The relationship between year of cultivar release and a 3-yr mean for: (**A**) Sclerotinia stem rot incidence; (**B**) severity; (**C**) disease severity index (DSI); (**D**) area under the disease progress curve (AUDPC); (**E**) yield; (**F**) yield reduction; (**G**) thousand seed weight (TSW); and (**H**) TSW reduction. The vertical bars represent the maximum and minimum range of the cultivars across the 3-yr test period.

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