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Mycelial Compatibility Group and Pathogenicity Variation of *Sclerotinia sclerotiorum* Populations in Sunflower From China, Canada and England

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Abstract: This study ascertained mycelial compatibility groups (MCGs) and pathogenicity variations of *Sclerotinia sclerotiorum* isolates sampled in sunflower from China, Canada and England. For this purpose, 205 isolates (5 from England, 33 from Canada and 167 from China) were grouped in 39 MCGs. Out of 39 MCGs, 64% were represented each by a single isolate. Sclerotinia isolates from the three countries' were incompatible between each other. Differences among MCGs were observed by comparing their differences in radial growth, sclerotial yield on PDA and detached leaves, aggressiveness and production of oxalic acid and total acids. Significant differences were found in radial growth, sclerotial yield, aggressiveness, production of oxalic acid and total acids within and among MCGs ($p<0.001$) regardless of their geographic origins. Correlation analysis indicated that *S. sclerotiorum* aggressiveness was positively related to oxalic acid production ($r = 0.739$, $p<0.01$) and negatively related to pH ($r = -0.436$, $p<0.01$), but not to the radial growth and the yields of sclerotia on PDA and detached sunflower leaves. Significantly, sclerotial yield on PDA was positively correlated to the sclerotia weight produced on detached sunflower leaf. There was a highly negative relationship between pH values (negatively indicated total acids secretion) and oxalic acid production ($r = -0.669$, $p<0.01$), suggesting that oxalic acid contributes most to the total production of acids released by *S. sclerotiorum*.

Key words: Oxalic acid, radial growth, aggressiveness, sclerotia, sunflower

INTRODUCTION

Sclerotinia sclerotiorum is capable of causing disease on at least 408 described species of plants from 278 genera in 75 families. The majority of these hosts are dicotyledonous, although a number of agriculturally significant monocotyledonous plants are also hosts (Boland and Hall, 1994).

Mycelial Compatibility Groups (MCGs) testing is a phenotypic, macroscopic assay of the self or non-self recognition system controlled by multiple loci common in fungi (Carbone *et al*., 1999). Mycelial incompatibility is a failure of different strains to fuse and form one cohesive colony and is characterized by the formation of dead cells and reduced growth between two incompatible colonies (Kohn *et al*., 1991). One of the criterions for detecting clonality is the mycelial compatibility grouping. When paired in culture, all members of the same MCGs can anastomose and form one confluent colony with no reaction line. These members also share a unique complex DNA fingerprint (Kohn *et al*., 1991; Carbone *et al*., 1999). MCGs or Microsatellite markers have not been associated with specific virulent characteristics or ecological adaptations of this pathogen. Indeed, a lack of variation in virulence among isolates from defined geographical areas has been reported in a number of studies on agricultural populations (Atallah *et al*., 2004; Auclair *et al*., 2004; Kull *et al*., 2004; Sexton and Hall, 2004). Differences in virulence may be detected only when isolates from widely separate geographical regions are compared. There has been no conclusive evidence to suggest host specialization among isolates of *S. sclerotiorum* (Kull *et al*., 2004).

Approximately 90% of its life cycle is spent in soil as sclerotia and their high persistence makes *S. sclerotiorum* a very successful pathogen (Adams and Ayers, 1979). Sclerotia are the asexual resting propagules that germinate to produce either hyphae or apothecia. Most diseases caused by this pathogen are initiated by ascospores (Abawi and Grogan, 1979). Disease initiated from myceliogenic germination occurs in only a few crops such as sunflowers and some vegetables where mycelia can directly infect susceptible root tissues. In sunflower, infection commonly occurs through the roots and

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progresses up into the stem. Since, sclerotia are the primary inoculum in the development of sclerotial wilt of sunflower, soil inoculum density is directly related to the amount of the disease (Holley and Nelson, 1986).

Oxalic Acid (OA) has been suggested to be an essential pathogenicity determinant in *S. sclerotiorum* (Dickman and Mitra, 1992). Oxalic acid production by this pathogen and the mechanisms of action have been proposed to explain its involvement in pathogenesis: (1) lowering infected tissues pH, which enhances the activity of extracellular enzymes produced by the pathogen (Bateman and Beer, 1965), (2) chelating cell wall Ca^{2+} by the oxalate anion, which softens plant cell wall and compromises the function of Ca^{2+}-dependent defense responses (Bateman and Beer, 1965), (3) directing toxicity to host plants, which weakens the plant and facilitates invasion (Noyes and Hancock, 1981) and (4) suppressing the host plant oxidative burst (Cessna *et al*., 2000). Therefore, the determination of oxalic acid in *S. sclerotiorum* is important for its pathogenesis research.

Identifying an association between MCGs and OA, one of the main determinants of *S. sclerotiorum* pathogenicity, is important for developing strategies to combat *S. sclerotiorum* spread among agronomically important crops, particularly because some MCGs have been shown to persist from year to year especially as sclerotia in soil, covering a very wide geographical area being some of them responsible for a large proportion of infections (Hambleton *et al*., 2002; Durman *et al*., 2003).

The objectives of this study were to determine MCGs among 205 isolates from China, Canada and England, to comprehensively compare the pathogenic differences among MCGs with regards to aggressiveness, radial growth and sclerotial production on medium and detached sunflower leaves, as well as production of oxalic acid and total acids in PDB and to analyze the correlation among those factors related to pathogenicity.

MATERIALS AND METHODS

Isolates: A total of 205 isolates of *S. sclerotiorum* sampled in 2006 from sunflower fields were grouped in three sets according to the country of origin (Table 1). The China set included 167 isolates; the Canada set, 33 isolates and England set, 5 isolates. For the Chinese set, 167 isolates sampled from 12 counties crossing West, Middle and East ecological regions in Inner Mongolia (more than 3000 km away from the West to the East), which is main sunflower growing province in China; for the Canada set, 33 isolates sampled from 5 sunflower fields in southern Manitoba; for England set, 5 isolates sampled from 5 sunflower fields in the North and Central England.

Sclerotia were surface sterilized using 10% commercial bleach (0.5% NaHCl) for 3 min, washed with sterile water and then incubated on Potato Dextrose Agar (PDA) for 3 days. Mycelial tips were cut and transplanted at least three times to obtain the genetically identical cultures.

Determination of MCGs: For the MCGs test, three isolates were co-cultured triangularly on a PDA plate amended with bromophenol blue (BPB) 50 (mg L^{-1}), which was added to enhance the visibility of the incompatible reaction. Each isolate was paired with itself as a control for compatibility. Different isolates were incubated in the dark for 1 week at room temperature (23-25°C). Isolates from the same set were confronted and then representatives of each MCG were paired with representatives of each of other MCGs. Mycelial pairings were scored as incompatible when a yellow barrier between colonies of paired isolates and/or when a yellow to green line between colonies was observed. Compatibility occurred when no reaction line occurred and two colonies grew together. The assay was repeated three times.

Radial growth and sclerotial yield on PDA: Plates containing 20 mL of PDA amended with 50 mg L^{-1} of BPB were prepared. A 7 mm PDA plug removed from the margin of an actively growing colony of the individual isolate was centered on PDA with four replicates for each isolate. Plates were sealed with parafilm and incubated at room temperature (23-25°C) in the dark. After of 48 h, the diameter of colony was measured. All plates were cultured continually in the same conditions for 14 days and dry sclerotial yields were measured. The sclerotial yield was expressed as grams per plate.

Aggressiveness assessment and sclerotial yield on detached sunflower leaf: The same size true and second leaves from 30 day old seedlings of sunflower cultivar IS8048 (Canadian uniform confection sunflower hybrid) were used to assess aggressiveness and sclerotial yield of each isolate in a 9 cm Petri dish. A leaf was put on a layer of filter paper (Waterman, No. 4) moistened with 2 mL of sterile distilled water. A 7 mm PDA disc removed from the growing edge of the colony for each isolate was centered on the detached leaf. Three replicates were made for each isolate. Inoculated leaves were incubated at room temperature (23-25°C) in a mist container and illuminated with 12 h ambient light. The diameters of necrotic lesions were measured 48 h after inoculation. The dry weight of sclerotia per leaf was measured 14 days after incubation at the same conditions and, expressed as sclerotial grams per leaf.

Table 1: The origin and mycelial compatibility group of *Sclerotinia sclerotiorum* isolates

MCG	Isolate	Origin
1	ZQ1-1, ZQ1-2, ZQ1-4, ZQ1-5, ZQ1-6, ZQ1-7, ZQ1-8, ZQ1-9, ZQ1-10, ZQ2-1B, ZQ2-2B, ZQ2-7, ZQ2-10, ZQ3-8, ZQ3-9, ZQ3-10, ZQ4-1, ZQ4-2, ZQ4-3, ZQ4-6, ZQ4-7, ZQ4-9, ZQ4-10, ZQ5-1, ZQ5-4, ZQ6-1, ZQ6-2, ZQ6-3, ZQ6-4, ZQ6-5, ZQ6-6, ZQ6-7, ZQ6-8, ZQ7-2, ZQ7-5, ZQ7-6, ZQ7-10, ZQ8-8, ZQ8-9, ZQ9-1, ZQ9-5, ZQ10-3, ZQ10-6, ZQ11-2, ZQ11-5, ZQ11-7, ZQ11-9, ZQ12-2, ZQ12-8, ZQ12-9, ZQ12-11, ZQ12-12, ZQ13-4, ZQ13-7, ZQ13-8, ZQ14-2, ZQ14-4, ZQ14-5, ZQ14-6, ZQ14-8, ZQ14-10, ZQ15-1, ZQ15-4, ZQ16-1, ZQ16-2, ZQ16-7, ZQ16-8, ZQ16-10, ZQ22-3, ZQ22-4, ZQ22-5, ZQ22-10, ZQ23-1, ZQ23-4, ZQ17-2, ZQ17-3, ZQ17-4, ZQ17-6, ZQ17-7, ZQ17-8, ZQ17-9, ZQ18-1, ZQ18-3, ZQ18-6, ZQ18-7, ZQ18-8, ZQ18-9, ZQ18-10, ZQ19-1, ZQ20-3, ZQ21-7, ZQ21-8, ZQ21-9, ZQ24-2, ZQ24-3, ZQ24-5, ZQ24-9, ZQ24-10, ZQ25-3, ZQ25-4, ZQ25-6, ZQ25-8, ZQ25-9, ZQ25-10, ZQ37-1, ZQ37-4, ZQ37-6, ZQ37-7, ZQ37-8, ZQ34-1, ZQ34-2, ZQ34-3, ZQ34-4, ZQ34-5, ZQ34-6, ZQ35-6, ZQ36-5, ZQ36-9, ZQ38-2; ZQ38-3, ZQ38-5, ZQ39-1, ZQ39-2, ZQ28, ZQ40-1.	China
2	ZQ16-3, ZQ16-5	China
3	ZQ23-3	China
4	ZQ22-9	China
5	ZQ24-1, ZQ25-1, ZQ25-2, ZQ25-5	China
6	ZQ34-7, ZQ34-8, ZQ34-9, ZQ35-3, ZQ35-8, ZQ37-2	China
7	ZQ34-10	China
8	ZQ35-7	China
9	ZQ35-9	China
10	ZQ35-10	China
11	ZQ36-2	China
12	ZQ36-7, ZQ36-8	China
13	ZQ37-10	China
14	ZQ38-6, ZQ38-7, ZQ38-9, ZQ38-10	China
15	ZQ39-5	China
16	ZQ38-1, ZQ38-8	China
17	ZQ39-3, ZQ39-4, ZQ39-6	China
18	ZQ41-2, ZQ41-5	China
19	ZQ41-4, ZQ41-6	China
20	ZQ33-1 ZQ33-4-1, ZQ33-4-4, ZQ33-4-5, ZQ33-5-1, ZQ33-5-2, ZQ33-5-4, ZQ33-5-5, ZQ33-5-6, ZQ33-5-7, ZQ33-6-3, ZQ33-6-4, ZQ33-6-7, ZQ33-6-8, ZQ33-8-2, ZQ33-9-4 ZQ33-10-4	Canada
21	ZQ33-6-2, ZQ33-6-5, ZQ33-10-3, ZQ33-10-5	Canada
22	ZQ33-2, ZQ33-9-3	Canada
23	ZQ33-5-9	Canada
24	ZQ33-3-1	Canada
25	ZQ33-3-2	Canada
26	ZQ33-3-3	Canada
27	ZQ33-4-2	Canada
28	ZQ33-4-3	Canada
29	ZQ33-4-7	Canada
30	ZQ33-5-8	Canada
31	ZQ33-9-1	Canada
32	ZQ33-10-2	Canada
33	ZQ26-1	China
34	ZQ26-2	China
35	ZQ26-3	China
36	ZQ26-4	China
37	ZQ29	China
38	ZQ31	China
39	ZQ32-Py1, ZQ32-M17, ZQ32-M23, ZQ32-S3-2, ZQ32-GH	UK

MCG: Mycelial Compatibility Group

Estimation of OA and the pH: For the OA determination, two 7 mm PDA discs removed from the actively growing edge of the colony for representative isolates were grown in 50 mL centrifuge tube containing 30 mL of potato dextrose broth (PDB, 2% glucose and 0.4% fresh potato extract in distill water). Tubes were statically incubated for 3 days at room temperature. Cultures were vacuum filtered, the mycelia was dried 12 h in Freeze Dryer (LABCONCO) at -100°C, 0-5 microns Hg, then weighed and the supernatant was used as an oxalic acid and pH test sample. Oxalic acid was determined following Durman *et al.* (2005). Reaction mixture contained 0.2 mL of sample (or standard oxalic acid solution), 0.11 mL of BPB (1 mM), 0.198 mL of sulfuric acid (1 M), 0.176 mL of potassium dichromate (100 mM) and 4.8 mL of distilled water. The mixture was placed in a water bath at 60°C and quenched by adding 0.5 mL sodium hydroxide solution (0.75 M) after 10 min. The absorbance was measured at 600 nm by means of a spectrophotometer (GeneQuant Pro, Amersham Pharmacia Biotech) and PDB was used as the blank control. Oxalic acid concentration was calculated comparing with a standard curve and was expressed as μg oxalic acid mg^{-1} dry mycelia. Total acid production was estimated by testing pH value of supernatant using a pH meter. The assay was conducted in triplicate and repeated once.

Data analysis: To determine the variability of isolates in aggressiveness, radial growth, sclerotial yield, pH and oxalic acid production among the 39 MCGs, data were analyzed using Analysis of Variance (ANOVA) for unequal sample sizes and means were separated by Least Significant Differences (LSD) at $p \leq 0.05$. To analyze the correlations among aggressiveness, mycelial growth, pH and oxalic acid of MCGs, the means of these factors were also used for estimation of the correlation coefficient (r) for their different combinations. All statistical analyses were performed by SPSS 12.0 for Windows (SPSS Inc., Chicago, USA).

RESULTS

Determination of MCGs: In the 205 isolates tested, 39 MCGs were identified (Table 1). Of the 39 MCGs, 25 groups (64%) were unique, in which each MCG is represented only by one isolate. The China set, in which 167 isolates were grouped in 25 MCGs, contained 15 MCGs each only consisting of a single isolate. There were 13 and 1 MCGs identified from Canada and England, respectively. The largest population was MCG1 which included 125 China isolates, only 4 isolates from the East Inner Mongolia, 121 isolates from West and Middle Inner Mongolia. Isolates from different locations with long geographic distances, such as isolates among sets of China, Canada and England, were incompatible.

Radial growth and aggressiveness: On PDA amended with BPB, all isolates of *S. sclerotiorum* grew rapidly. Due to the secretion of OA and other organic acids by mycelia, the media's color quickly changed from blue to yellow. After 48 h incubation, colonies attained diameters ranging from 2.75 cm (isolate ZQ26-2) to 6.23 cm (isolate ZQ 35-10). Significant differences ($p<0.05$) were observed on the radial growth of isolates regardless of their geographic origins and MCGs (data not shown). When isolates were grouped by mycelial compatibility, the average growth rates in 48 h varied from 2.75 cm (MCG 34) to 6.23 cm (MCG 10) (Table 2). Significant variability was found in radial growth among MCGs ($p<0.001$) (Table 3, Fig. 1).

Table 2: Radial growth, sclerotial production, lesion size oxalic acid production and pH of different mycelial compatibility groups of *Sclerotinia scleritiorum**

MCG	Radial growth (cm/48 h±SE)	Sclerotia weight on PDA (g/plate±SE)	Lesion diameter on leaf (cm/48 h±SE)	Sclerotia weight on leaf (g/leaf±SE)	pH	Oxalic acid (μg/mg±SE)
1	4.15±0.066	0.150±0.003	14.87±0.57	0.030±0.001	3.69±0.015	17.98±0.48
2	4.11±0.163	0.140±0.077	19.67±1.84	0.040±0.005	3.53±0.031	22.53±5.5
3	3.63±0.366	0.100±0.00	15.00±2.89	0.050±0.003	3.97±0.645	16.10±0.27
4	4.58±0.545	0.200±0.00	4.67±0.88	0.040±0.006	3.49±0.055	11.28±0.4
5	4.42±0.246	0.180±0.03	14.00±3.76	0.030±0.003	4.12±0.177	13.44±3.4
6	4.41±0.242	0.210±0.026	7.17±2.25	0.030±0.004	3.99±0.081	10.61±1.4
7	3.30±0.507	0.250±0.029	26.67±1.86	0.040±0.003	3.71±0.01	29.15±6.95
8	3.93±0.411	0.040±0.031	2.00±1.53	0.010±0.006	3.99±0.11	17.52±1.65
9	3.95±0.67	0.060±0.029	2.00±1.53	0.010±0.007	4.42±0.785	10.77±0.11
10	6.23±0.278	0.180±0.023	4.00±0.58	0.020±0.003	3.20±0.00	14.15±1.76
11	5.28±0.111	0.130±0.033	27.33±1.45	0.030±0.003	2.49±0.14	33.36±6.12
12	5.11±0.196	0.110±0.006	16.67±1.38	0.040±0.006	3.34±0.046	20.22±1.09
13	5.33±0.32	0.180±0.017	16.67±1.67	0.040±0.006	3.34±0.125	20.47±1.33
14	5.64±0.269	0.160±0.012	15.17±1.76	0.020±0.004	3.65±0.067	14.83±4.87
15	4.30±0.443	0.010±0.01	13.33±1.45	0.003±0.0	3.68±0.005	16.75±5.28
16	3.86±0.334	0.140±0.019	20.67±3.89	0.030±0.006	3.75±0.107	28.95±7.3
17	3.63±0.733	0.170±0.012	16.11±3.5	0.040±0.006	3.42±0.059	29.11±7.16
18	3.45±0.401	0.200±0.014	20.00±3.474	0.040±0.005	3.23±0.068	30.57±3.09
19	3.09±0.297	0.130±0.021	10.17±4.73	0.010±0.005	3.64±0.236	18.99±5.32
20	4.44±0.14	0.180±0.006	15.12±1.53	0.030±0.002	4.07±0.083	9.67±1.39
21	4.18±0.234	0.190±0.019	18.08±2.59	0.030±0.004	4.12±0.145	13.81±4.86
22	4.28±0.333	0.170±0.021	14.17±0.83	0.030±0.007	3.78±0.157	11.76±2.39
23	5.08±0.149	0.187±0.13	6.00±1.00	0.050±0.009	3.64±0.04	15.23±4.81
24	4.15±0.301	0.200±0.00	24.67±1.45	0.030±0.006	3.32±0.115	22.69±6.69
25	4.10±0.268	0.170±0.033	2.00±1.00	0.040±0.006	3.41±0.045	11.80±1.79
26	4.63±0.063	0.200±0.00	2.00±1.00	0.020±0.006	4.64±0.095	1.65±0.32
27	4.75±0.104	0.170±0.033	0.67±0.33	0.040±0.006	4.56±0.055	3.25±0.19
28	4.60±0.258	0.200±0.00	3.67±1.76	0.040±0.006	3.68±0.08	11.30±2.58
29	4.73±0.16	0.200±0.00	3.00±1.00	0.030±0.007	3.74±0.02	8.20±1.86
30	4.53±0.17	0.170±0.033	16.00±2.08	0.030±0.006	3.87±0.06	10.49±3.53
31	4.05±0.357	0.100±0.00	9.00±1.00	0.030±0.003	3.81±0.02	9.78±1.19
32	2.93±1.055	0.130±0.033	13.00±2.08	0.050±0.009	4.05±0.525	15.59±6.69
33	4.83±0.717	0.070±0.033	0.33±0.33	0.003±0.00	4.42±0.295	9.34±0.38
34	2.75±0.777	0.100±0.00	2.00±0.58	0.030±0.003	3.76±0.095	18.49±1.55
35	5.45±0.32	0.130±0.033	7.33±0.33	0.030±0.003	3.73±0.025	19.62±4.48
36	6.05±0.272	0.100±0.00	19.33±4.98	0.020±0.006	3.65±0.04	22.46±4.25
37	4.63±0.384	0.133±0.033	1.33±0.88	0.020±0.007	3.89±0.07	9.66±0.22
38	5.13±0.757	0.070±0.033	2.33±0.88	0.010±0.009	3.14±0.1	14.64±3.52
39	4.15±0.267	0.150±0.019	16.67±1.82	0.020±0.005	3.61±0.231	22.90±4.89

MCG: Mycelial Compatibility Groups; SE: Standard Error

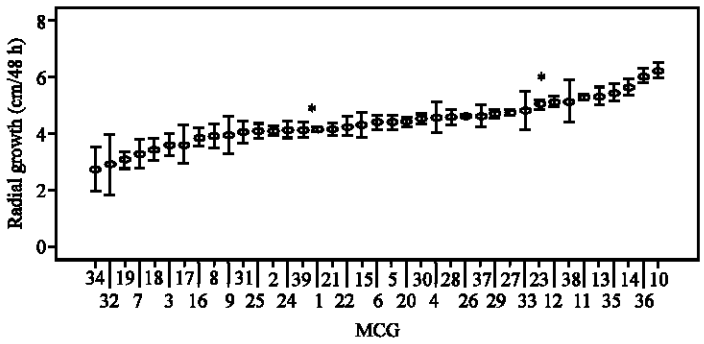

Fig. 1: Radial growth on PDA by the *Sclerotinia sclerotiorum* isolates from the different Mycelial Compatibility Groups (MCGs). Each point is the mean of all the isolates from the same MCG. Vertical bars represent the standard error of the mean. *significantly different at $p \leq 0.05$

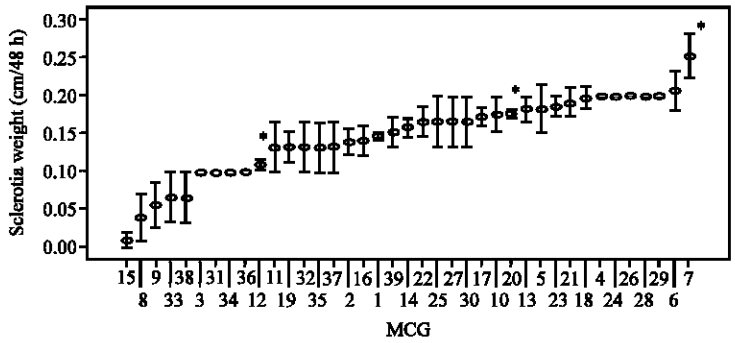

Fig. 2: Sclerotia weight on PDA by the *Sclerotinia sclerotiorum* isolates from the different Mycelial Compatibility Groups (MCGs). Each point is the mean of all the isolates from the same MCG. Vertical bars represent the standard error of the mean. * significantly different at $p \leq 0.05$

Table 3: Analysis of variance for radial growth, sclerotial production, lesion size, pH and oxalic acid production among mycelial compatibility groups of *Sclerotinia sclerotiorum*

Parameters	Sum of squares	df	Mean square	F	p-level
Radial growth (cm)	144.095	38	3.792	2.065	<0.001
Sclerotia wt. on PDA (g)	0.473	38	0.012	2.853	<0.001
Leaf lesion (cm)	9609.536	38	252.883	2.456	<0.001
Sclerotia wt. on leaf (g)	0.023	38	0.001	2.580	<0.001
pH	20.859	38	0.549	5.813	<0.001
Oxalic acid ($\mu g\ mg^{-1}$ dry mycelia)	8320.698	38	218.966	3.169	<0.001

Significant differences were found among the MCGs not only from different countries such as MCG10 from China, MCG32 from Canada and MCG39 from England, but also from the same country. For example, in the China set, MCG10 showed significantly rapid growth compared to MCG8 and MCG9 (Fig. 1).

Visible symptoms occurred on leaves at 12 h after inoculation. Isolates were significantly different in their ability to infect and spread on sunflower leaves ($p < 0.05$), resulting in different lesion sizes in 48 h ranging from 0.33 cm (ZQ26-1 from China) to 27.33 cm (ZQ36-2 also from China). When the same analyses were performed for MCGs, significant differences of pathogenic variability were observed among MCGs ($p<0.001$) (Table 3, Fig. 3), where the lesion size varied from 0.33 cm (MCG33 from China) to 27.33 cm (MCG11 from China) in 48 h (Table 2, Fig. 3). These pathogenic differences of isolates and MCGs were not found to relate to their geographic origin.

Sclerotia yield: Sclerotia formed on the leaves and PDA was dry weighed after 14 days. Isolates showed variability in sclerotial shape, size, weight and number. While dry weight of sclerotia produced on PDA ranged from 0.01 (ZQ39-5) to 0.25 g (ZQ34-10) per plate, on leaves it ranged from 0.003 g per leaf (ZQ39-5 from China) to 0.05 g per leaf (ZQ33-5-9 from Canada). Significant differences occurred in sclerotia production of MCGs on both leaf and PDA ($p<0.001$) (Table 2, 3 and Fig. 2, 4).

Estimation of OA and the pH: When production was compared among MCGs, the mean production of oxalic

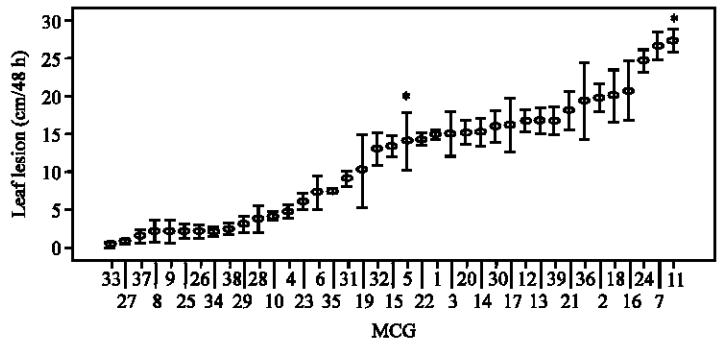

Fig. 3: Diameter of lesion on detached leaf by the *Sclerotinia. sclerotiorum* isolates from the different Mycelial Compatibility Groups (MCGs). Each point is the mean of all the isolates from the same MCG. Vertical bars represent the standard error of the mean. *significantly different at $p \leq 0.05$

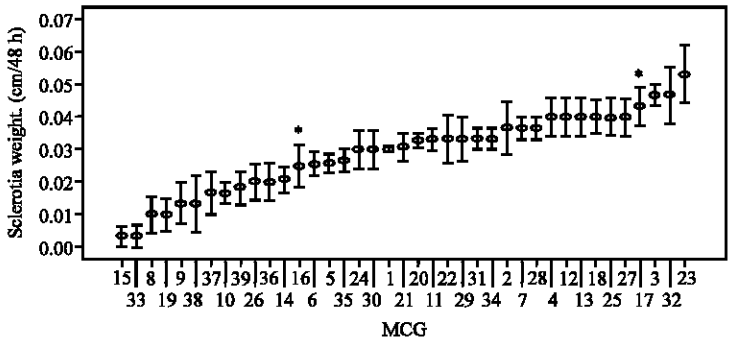

Fig. 4: Sclerotia weight on detached leaf by the *Sclerotinia sclerotiorum* isolates from the different Mycelial Compatibility Groups (MCGs). Each point is the mean of all the isolates from the same MCG. Vertical bars represent the standard error of the mean. *significantly different at $p \leq 0.05$

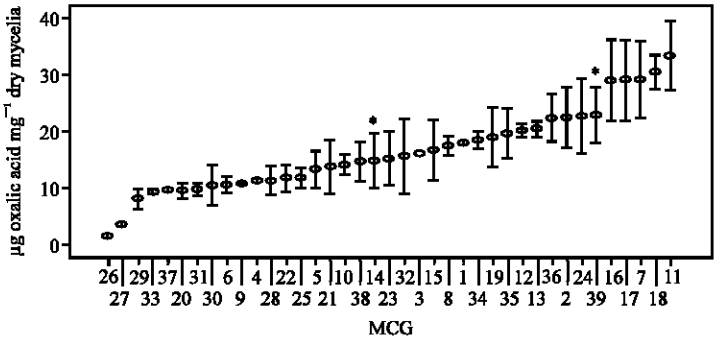

Fig. 5: Oxalic acid produced and released in PDB by the *Sclerotinia. sclerotiorum* isolates from the different Mycelial Compatibility Groups (MCGs). Each point is the mean of all the isolates from the same MCG. Vertical bars represent the standard error of the mean. *significantly different at $p \leq 0.05$

acid varied from 1.65 (MCG26) to 33.36 (MCG11) µg mg^{-1} dry weight. A high degree of variability in oxalic acid production occurred among the MCGs and significant differences among them could be detected ($p<0.001$) (Table 2, 3 and Fig. 5). These distinct differences were observed both among and within MCGs. For example, in the Canadian MCGs, MCG24 produced much higher concentration of oxalic acid than MCG26 and MCG27. All isolates of *S. sclerotiorum* actively secreted acids, which resulted in pH values of the culture medium decreasing from 5.24 of the original level (CK) to 2.49 of MCG11 (data not shown). When total acid production levels among MCGs were compared by the pH levels, significant differences in total acid production were found among

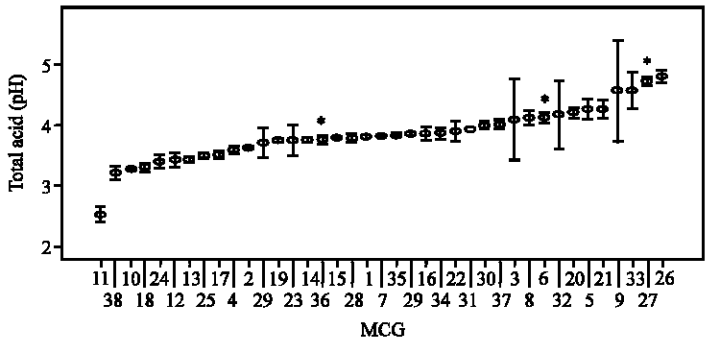

Fig. 6: Estimation of total acid produced and released in PDB by the *Sclerotinia sclerotiorum* isolates from the different Mycelial Compatibility Groups (MCGs). Each point is the mean of all the isolates from the same MCG. Vertical bars represent the standard error of the mean. *significantly different at $p \leq 0.05$

Table 4: Correlation analysis among radial growth, sclerotial production, lesion size and oxalic acid production in mycelial compatibility groups of *Sclerotinia sclerotiorum**

Parameters	Radial growth (cm)	S. wt. on PDA (g)	Leaf lesion (cm)	S. wt. on leaf (g)	pH	Oxalic acid (μg mg^{-1} dry mycelia)
Radial growth (cm)	1.000	0.015	-0.103	-0.183	-0.218	-0.180
Sclerotia wt. on PDA (g)	0.015	1.000	0.249	0.534**	-0.077	0.017
Leaf lesion (cm)	-0.103	0.249	1.000	0.260	-0.436**	0.739**
Sclerotia wt. on leaf (g)	-0.183	0.534**	0.260	1.000	-0.195	0.154
pH	-0.218	-0.077	-0.436**	-0.195	1.000	-0.669**
Oxalic acid (μg mg^{-1} dry mycelia)	-0.180	0.017	0.739**	0.154	-0.669**	1.000

*Correlation is significant at $p \leq 0.01$. Entry: Correlation coefficient (r); S: Sclerotinia sclerotiorum; wt: weight

MCGs ($p<0.001$) (Table 2, Fig. 6). MCG26 produced the lowest amount of acid (pH = 4.64), while MCG11 contributed the highest acid production (pH = 2.49) (Table 2, Fig. 6).

Correlation analysis of six factors related to the pathogenicity: Correlation analysis was conducted among the combinations of radial growth, aggressiveness, production of oxalic acid, pH and sclerotia yields (Table 4). Results indicated that *S. sclerotiorum* mycelial growth on PDA was not related to the other 5 factors related to pathogenicity. Its aggressiveness was positively related to oxalic acid production ($r = 0.739$, $p<0.01$) and negatively to the pH ($r = -0.436$, $p<0.01$), but not to the radial growth and the yields of sclerotia produced on both PDA and leaf. Significantly, sclerotial yield produced on PDA was positively related to the sclerotial weight produced on detached sunflower leaf ($r = 0.534$, $p<0.01$). There was a highly negative relationship ($r = -0.669$, $p<0.01$) between the pH value and oxalic acid production, it meant that total acid production had a positive relationship with oxalic acid released, suggesting that oxalic acid contributed mostly to the production of total acids released by *S. sclerotiorum*.

DISCUSSION

The MCG analysis of *S. sclerotiorum* populations in this study suggests that in a given location there is a heterogeneous mix of MCGs (In the 205 isolates tested, 39 MCGs were identified). This agrees with previous reports on *S. sclerotiorum* MCG population structures on different crops (Carpenter *et al*., 1999; Cubeta *et al*., 1997; Hambleton *et al*., 2002; Durman *et al*., 2003; Kull and Pedersen, 2004). A group of isolates sharing the same DNA fingerprint and MCG was interpreted as a clone (Hambleton *et al*., 2002). Population studies of *S. sclerotiorum* have suggested a predominantly clonal mode of reproduction (Cubeta *et al*., 1997) with some evidence of out crossing contributing to the population structure in a few regions (Atallah *et al*., 2004; Sexton and Howlett, 2004). In the current study, isolates from geographically separated countries were incompatible. This could be a result of geographic barriers and failure of sexual recombination. Of the 39 MCGs in this study, the largest population was MCG1 which included 125 China isolates, where only 4 isolates were from the East Inner Mongolia, 121 isolates from West and Middle Inner Mongolia. This suggested that both populations of West and Middle Inner Mongolia isolates essentially did not have significant differentiation since West IM and Middle IM are two major sunflower production areas in the Autonomous region of Inner Mongolia. Although both areas are approximately 300 km apart, sunflower fields are actually continuous between the two regions, providing a pathway of cross infection for pathogen isolates from the two areas, also frequent seed exchanges have been occurring and the same varieties have been planted in

both areas for many years, in addition, both production areas share the same growth season, similar weather conditions, soil structures and agriculture practices, suggesting that no barrage exits in ecological adaptation of the pathogen isolates in any one of these areas, both populations have the higher heterozygosis among the three China populations. All of these factors probably resulted in no differentiation between two pathogen populations. Unlike the West and Middle Inner Mongolia populations, the East Inner Mongolia is more than 2500 km away from the Middle Inner Mongolia. Meanwhile weather and the flowering period of sunflower in the East IM are different with that in West and Middle Inner Mongolia. All these may contribute to the independent genetic evolution in the western or eastern population and thus resulted in differentiation between the East Inner Mongolia population and the populations from the West and Middle part of the province.

Variations in aggressiveness of *S. sclerotiorum* have been investigated previously. On the basis of a detached celery petiole assay 50 MCGs identified from 160 Argentinean isolates (Durman *et al*., 2003), were found not to differ in aggressiveness among MCGs. By using a limited-term, plug inoculation technique, Kull *et al.* (2004) reported that aggressiveness varied between isolates and MCGs from different locations, but not in MCGs produced from isolates originating from infections in single fields. Present results indicate that significant differences in aggressiveness are present not only within a MCG, but also between MCGs, regardless of the isolate origins. To evaluate pathogenicity of *S. sclerotiorum* dispersed in a region, or to screen resistant cultivars, these results suggest that more representative isolates should be included.

Oxalic Acid (OA) has long been associated with pathogenicity of some *Sclerotinia* species. OA, as a major source of virulence of *S. sclerotiorum*, has drawn more and more attention. Marciano *et al.* (1989) found that highly and weakly aggressive isolates could equally utilize several components of the host cell wall as nutrients for mycelial growth, but differed in their ability to utilize them for oxalate production. The poor ability to produce OA by weakly aggressive isolate seems to be due to a lower efficiency in the synthetic pathway. OA as an essential determinant of *S. sclerotiorum* pathogenicity has been strongly confirmed by Godoy *et al.* (1990), who found the mutants of *S. sclerotiorum* that were deficient in the ability to synthesize oxalate were nonpathogenic, whereas reverting strains that regain their oxalate biosynthetic capacity exhibited normal virulence. It has been reported that there were significant differences among *S. sclerotiorum* MCGs in the release of both OA and organic acids (Durman *et al*., 2005). Our results confirm this conclusion and additionally indicate that these differences do not relate to the geographic origins of MCGs.

Numbers of sclerotia of *S. sclerotiorum*, as inoculum and the primary long-term survival structure in soils, relate directly to the extent of damage caused by this disease in fields. However, up to now, little attention has been given to the determination factors of sclerotia formation. Our test has shown a high variation for the strains in different MCGs to produce sclerotia on host tissue. Conclusively, there is high genetic diversity in *S. sclerotiorum* populations, as determined by MCG and high variation in six pathogenicity-related factors between isolates and MCGs, irrespective of their region of origin.

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