Mycelial Compatibility Grouping and Aggressiveness of *Sclerotinia sclerotiorum*

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ABSTRACT

Kull, L. S., Pedersen, W. L., Palmquist, D., and Hartman, G. L. 2004. Mycelial compatibility grouping and aggressiveness of *Sclerotinia sclerotiorum*. Plant Dis. 88:325-332.

Population variability of *Sclerotinia sclerotiorum*, the causal organism of Sclerotinia stem rot of soybean, was determined by mycelial compatibility grouping (MCG) and isolate aggressiveness comparisons. MCG and aggressiveness of *S. sclerotiorum* isolates from diverse hosts and geographic locations (Diverse Set, 24 isolates), from a soybean field in Argentina (Argentine Set, 21 isolates), and from soybean fields in DeKalb and Watseka, Illinois (DeKalb Set, 124 isolates, and Watseka Set, 130 isolates) were assessed. Among 299 isolates tested, 42 MCGs were identified, and 61% were represented by single isolates observed at single locations. Within the Diverse Set, 17 MCGs were identified; 1 MCG consisted of six isolates, and 16 MCGs consisted of one isolate each. Nine MCGs were identified within the Argentine field with two MCGs composed of either five or six isolates, two MCGs composed of two isolates, and the remaining composed of one isolate each. Each Illinois field was a mosaic of MCGs, but MCG frequencies differed between the two fields. Common MCGs were identified among the Diverse, DeKalb, and Watseka Sets, but no MCGs within the Argentine Set were observed with other sets. MCG 8 was the most frequently sampled and widely dispersed MCG and occurred at a frequency of 29, 36, and 62% in the Diverse, DeKalb, and Watseka Sets, respectively. Variation in isolate aggressiveness was assessed using a limited-term, plug inoculation technique. Isolate aggressiveness varied ($P = 0.001$) within the Diverse, Argentine, DeKalb, and Watseka Sets. Within widely dispersed MCGs, isolate aggressiveness varied ($P \leq 0.10$); however, within locally observed MCGs detected only in single fields, isolate aggressiveness did not vary. Additionally, individual MCGs within the DeKalb and Watseka Sets differed in isolate aggressiveness. Using six soybean cultivars and six *S. sclerotiorum* isolates, no cultivar–isolate interaction was detected, but resistant and susceptible cultivars performed similarly when inoculated with either less or highly aggressive isolates. Pathogen population structure and variability in isolate aggressiveness may be important considerations in disease management systems.

A pernicious pathogen, *Sclerotinia sclerotiorum* (Lib.) de Bary has a wide geographic distribution and a diverse host range, including many agronomic crops (16). On soybean, *S. sclerotiorum* causes Sclerotinia stem rot (SSR), a disease recognized as an important yield-reducing disease in the United States (41) and a major disease on soybean in Illinois (15). Partial resistance associated with either escape mechanisms or physiological resistance to *S. sclerotiorum* has been reported in soybean (17). Extensive field and greenhouse evaluations to assess soybean resistance to *S. sclerotiorum* resulted in low correlations (18,32,39), and different experiments to assess cultivar response to *S. sclerotiorum* frequently were inconsistent (9,10). Problems associated with evaluating and breeding for resistance may be due in part to lack of consideration for pathogen population structure and variability in isolate aggressiveness.

S. sclerotiorum can spread by sexually and vegetatively produced propagules (16). Sclerotia, the over-wintering vegetative form of the fungus, reside in the soil and germinate during the cropping season to produce apothecia which release millions of sexually produced, airborne ascospores. Up to 90% of the ascospores, the primary inoculum source that infects soybean flowers, remain within 100 m of the dispersal site (3). Additionally, mycelia-infected seed can provide a soil inoculum source for continuance of the pathogen's life cycle (15,31). Although mycelia-infested seed can be dispersed widely throughout soybean-growing regions, no reports are available to suggest that the thin-walled ascospores are viable after long-distance wind dispersal. Mode and range of pathogen dispersal are important considerations potentially impacting population structure and disease control.

Naturally occurring *S. sclerotiorum* populations exist as a mosaic of clones, which tend to be genetically isolated from each other (2,11,25). Phylogenetic analysis of clones using DNA sequence data from four regions suggests a predominately clonal mode of evolution with no evidence of contemporary genetic exchange and recombination between individual genotypes (6). Clones of *S. sclerotiorum* can be identified by DNA fingerprinting using a dispersed, repetitive probe and/or mycelial compatibility groups (MCGs; 21,23,25). The *S. sclerotiorum* mycelial compatibility-incompatibility grouping system is a macroscopic assay of the self-nonself recognition system common in fungi and is determined using a side-by-side pairing system (23). Although the MCG system is similar to vegetative compatibility grouping systems, in which nuclei may be mobile within compatibility groups, self-self pairings may vary regarding movement of nuclei between hyphal filaments that appear compatible (27). Movement of nuclei (via sexual or vegetative means) is not known to occur between MCGs (27). Although MCG systems are propagated mitotically, they are not necessarily clonal, as indicated by MCGs associated with one or more DNA fingerprints (11,14) which has been attributed to (i) parallel gain or loss of fingerprint fragments resulting from transposable element activity or (ii) rare episodes of genetic exchange (6). Factors for MCG or clone designations were shown to be stable and unchanged through successive sexual generations and after serial culturing, and the correlation between an MCG and a DNA fingerprint or fingerprints supports the synonymous relationship between MCGs and clones of *S. sclerotiorum* (21). A system for identifying and naming *S. sclerotiorum* clones has been established (14,20,24) and currently utilized to report the global distribution of clonal genotypes. Both MCGs and clones

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Trade and manufacturers' names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

This work was supported by the Frerich's and Ingersoll Fellowships within the Crop Science Department of the University of Illinois, Illinois Soybean Checkoff Board, and the North Central Soybean Research program.

Accepted for publication 25 October 2003.

Publication no. D-2004-0120-02R

have been used to identify *S. sclerotiorum* genotypes over geographically long distances and disparate times and used to identify genetic variability in field populations (8,11,20,21,40). The extent of MCG diversity, pattern, and frequency, and variability in isolate and MCG aggressiveness associated with *S. sclerotiorum* populations infesting United States soybean fields has not been reported.

An important consideration that may impact cultivar resistance evaluations and long-term disease control is variability in MCG and isolate aggressiveness and the potential for interactions. "Pathogen aggressiveness" is defined as the relative ability to colonize the host and cause damage, and "virulence" as the relative capacity to produce disease (1,38). Greenhouse inoculation studies on canola suggest that some clones are more aggressive in forming lesions (13,20). Diverse isolates of both *S. sclerotiorum* and *S. trifoliorum* differed in virulence on alfalfa cultivars, and experiment–cultivar and experiment–isolate interactions were observed, but no isolate–cultivar interaction was observed (35). In recurrent phenotypic selection experiments to improve alfalfa resistance, isolates of *S. trifoliorum* that differed in virulence were used to measure the progress of recurrent selection and to test the durability of resistance in selected progeny after three cycles (34). To ensure correct field and greenhouse assessment of host resistance and to promote durable resistance, assessment of variability in isolate and MCG aggressiveness and information regarding possible interactions is important.

Various approaches have been used to understand population biology and variability of plant pathogens (5,25,28–30). Population biology studies of *S. sclerotiorum* MCGs or clones within Canadian canola and North Carolina and Louisiana cabbage fields indicated that (i) agricultural populations on canola consisted of a mosaic of genetically unique and reproductively conserved MCGs (25), (ii) some MCGs were associated with more than one DNA fingerprint, (iii) some MCGs or clones were distributed over long distances geographically (21), and (iv) no MCGs or clones were shared between a trans-Canadian and either North Carolina or Louisiana field strains (11). A fine-scale epidemiological study of 2,700 isolates in four Canadian canola fields indicated that a small number of clones (determined by DNA fingerprinting) represented the majority of the sample, and the spatial pattern of clones was random (20). MCGs and DNA fingerprints indicated that soybean in Ontario and Quebec is infected mainly by *S. sclerotiorum* genotypes residual from other crop or weed hosts and that the population structure was predominately clonal due to repeated recovery of MCGs, including clonal lineages 1 and 2 (14). DNA fingerprints indicated that soybean in Pennsylvania is infected by *S. sclerotiorum* clonal lineages 1 and 2 common to legumes and crucifers in New York and Canada (24). According to studies of *S. sclerotiorum* populations and the established system of identifying clonality groups, clones 1 and 2 are widely distributed geographically, are predominant genotypes on hosts in Canada and Pennsylvania (14,20,21,24), and were recovered initially on canola in 1989 (25).

The objectives of this study were to (i) identify MCGs among 299 *S. sclerotiorum* isolates, (ii) determine frequencies of MCGs within two Illinois soybean fields, (iii) assess variability in isolate and MCG aggressiveness, and (iv) determine if an isolate–cultivar interaction exists. To accomplish these objectives, *S. sclerotiorum* isolates from various hosts and locations and from two intensively sampled Illinois soybean fields were used. The discussion includes identification of MCGs in this study that are associated with previously characterized genotypes, clonal lineages 1 and 2 that dominate in *S. sclerotiorum* populations infecting Canadian soybean (14) and canola (25) and Pennsylvania soybean (24).

MATERIALS AND METHODS

***S. sclerotiorum* isolates.** Isolates are grouped in sets (Table 1) according to location, with the exception of the Diverse

Table 1. *Sclerotinia sclerotiorum* isolates grouped by set, isolate number, host, location, and source, including the curator code and clonal lineage

Set, isolate no.[a]	Host	Date collected	Location[b]	Source[c]
Diverse (24)				
D1	Soybean	1994	DeKalb, Illinois	H. W. Kirby
D2	Canola	1997	Canada	L. M. Kohn; LMK211, clone 2
D3	Soybean	1995	North Carolina	H. D. Shew; SS-M2-NC
D6	Soybean	1998	Tekamah, Nebraska	J. R. Steadman; I#265
D8	Dry bean	1998	Mitchell, Nebraska	J. R. Steadman; SSNeb152, clone 2
D12	Soybean	1996	Elkhorn, Wisconsin	L. S. Kull
D15	Soybean	1996	Clinton, Wisconsin	L. S. Kull; UIUC15, clone 1
D20	Soybean	1996	Iroquois, Illinois	L. S. Kull; UIUC20; clone 1
D30	Soybean	1996	Indiana	G. L. Hartman; UIUC30, clone 1
D33	Cabbage	1996	North Carolina	M. A. Cubeta; SS129
D36	Canola	1996	Canada	L. M. Kohn; Can-5, clone 1
D48	Soybean	1996	Iowa	G. V. Cook; 6675
D105	Soybean	1996	Story City, Iowa	G. V. Cook; 14082
D110	Pear	1997	Oregon	R. A. Spotts
D127	Soybean	1997	Switzerland	V. Michel; M7012, clone 1
D130	Rape	1997	Switzerland	V. Michel; #656
D133	Unknown	1997	Switzerland	V. Michel; #115
D144	Sunflower	1997	Switzerland	V. Michel; #111
D150	Soybean	1996	East Lansing, Michigan	L. P. Hart; 5.3, clone 1
D159	Soybean	1998	Ohio	A. E. Dorrance; 1548, clone 1
D160	Soybean	1998	Chacabuco, Argentina	Unknown
D457	Soybean	1997	Greeley, Colorado	J. R. Steadman; LMK77
D458	Sunflower	1995	Herts, Great Britain	J. R. Steadman
D459	Pinto	1996	O'Neil, North Carolina	J. R. Steadman
DeKalb (124)				
DK186-310	Soybean	1998	DeKalb, Illinois	L. S. Kull
Watseka (130)				
W311-443	Soybean	1998	Watseka, Illinois	L. S. Kull
Argentine (21)				
A167-185, A461, A462	Soybean	1998	San Pedro, Argentina	M. Scandiani

[a] Number in parentheses indicates number of isolates in the set.
[b] Country, state, city, and county from which isolates were obtained.
[c] The curator or collector of isolates. The curator code and clonal lineage, if available, is indicated.

Set, which we defined as a diverse sampling of isolates from various hosts and locations. The Diverse Set included 24 isolates; the Argentine Set, 21 isolates; the DeKalb Set, 124 isolates; and the Watseka Set, 130 isolates. Isolates composing the Diverse Set were from the *S. sclerotiorum* collection maintained at the National Soybean Research Center at the University of Illinois. Hyphal tip cultures were produced, and all mycelial cultures were maintained on potato dextrose agar (PDA) at 4°C. The Argentine isolates were gleaned from a single sclerotial-infested soybean seed lot produced in Chacabuco in 1999. All sclerotia from the Argentine seed lot were surface sterilized and cultured on PDA to produce mycelia. All soybean seed from the Argentine seed lot were placed on moistened KimPak (Butler Paper Company, Peoria, IL) in a seed germinator maintained at 25°C. After 7 days, sclerotia produced from *S. sclerotiorum*-infested seed were harvested and cultured to produce mycelial colonies. Isolates making up the DeKalb and Watseka Sets were from two severely infested Illinois soybean fields, 258 km apart, sampled in 1998. The DeKalb field was located in northern Illinois and the Watseka field in east-central Illinois. The sampling areas within each field were divided into five ranges, and each range was subdivided into plots. The sampling area within the Watseka field was 581 m^2 and divided into 135 plots, each 4.3 m^2; the DeKalb field was 813 m^2 and divided into 125 plots, each 6.5 m^2. To ensure that isolates used in this study were a result of the current year's infection cycle, a stem sclerotium or infested stem tissue was taken from each plot. Although each field location was severely infested with the fungus, several plots were not uniformly infested. If the fungus was not present in the designated plot location, the infested plant nearest to the designation point was sampled, and each isolate sample was nearly equidistant apart.

MCGs. Isolates were paired in all possible combinations on modified Patterson's medium (MPM) according to a previously published procedure (23). Mycelial reactions were recorded as incompatible when an apparent line of demarcation, a barrage zone, was observed between the confronting paired isolates. Barrage zone reactions were (i) a zone of sparse or no mycelium; (ii) a thin to wide band of uniform, aerial mycelium; (iii) a thin line of dark, pigmented mycelium; or (iv) a thin red line on the colony surface, reverse, or both. Pairings were evaluated at 7 and 14 days after transfer, and each pairing was performed twice. A universal MCG numbering system for *S. sclerotiorum* has not been established; thus, a numbering system was established for this research. Isolates that have been genotyped in other studies are noted (Table 1).

To facilitate MCG determination of all isolates listed in Table 1, a subset of 20 isolates from each field set were selected and paired in all possible combinations. MCGs were determined, and an isolate was selected to represent each MCG. These representative isolates were paired with all remaining untested isolates until all isolates were assigned to an MCG. If an incompatible reaction occurred with any MCG representative isolates, the newly tested isolate was established as a newly observed MCG and was used to represent the new MCG. All pairings were conducted at least twice.

Isolate aggressiveness within the four sets. Aggressiveness of isolates was determined using a limited-term, agar-plug inoculation technique (26). A partially resistant cultivar, NKS19-90 (17,18), was used for all aggressiveness tests. Soybean seed were grown in a soil and sand mix (1:1) in planting trays (27 by 54 by 8 cm) with four plants per row and 12 rows per tray. Plants were inoculated when the first trifoliolate was fully expanded. Isolate stock cultures maintained at 4°C were used to inoculate fresh PDA plates for each isolate and incubated at 20°C for 24 to 48 h to allow renewed mycelial growth. A mycelial plug from the growing colony edge of each isolate was transferred to a new PDA plate and incubated at 20°C for 24 h. To inoculate plants, 3-mm^2 mycelial plugs were removed from the advancing mycelial edge and singly placed mycelia-side down on one cotyledon adjacent to and touching the stem. Inoculated plants were hand misted with water, incubated in dew chambers (Percival, Boone, Iowa) maintained at 20°C ambient (13 and 34°C wall and water temperatures, respectively) for 24 h, transferred to a greenhouse bench, and maintained under two layers of 80% filtration shade cloth (Hummert International, Earth City, MO) in an air-conditioned greenhouse room at 20 ± 2°C. Approximately 24 h after trays were transferred from the dew chamber, the number of dead plants per row was recorded two times per day, and the area under disease progress curve (AUDPC; 37) was calculated from accumulated daily counts of number of dead plants per entry and treatment. Some of the isolates in the Diverse and Argentine Sets and subsets of 24 isolates each from DeKalb and Watseka were selected to conduct aggressiveness tests. Aggressiveness tests were conducted separately for each of the four sets. Experimental design for all isolate aggressiveness tests was a randomized, complete block with four replications and four plants per replication, and each experiment was conducted twice.

Isolate aggressiveness within MCGs. Aggressiveness within MCGs composed of isolates from multiple locations (MCGs 3, 8, and 14), which are described as widely dispersed MCGs, was compared with aggressiveness within MCGs composed of isolates from single-field locations (MCGs 13, 16, 17, 19, 21, 24, and 26), which are referred to as local MCGs. Isolate aggressiveness tests for each MCG were conducted separately; therefore, the AUDPC values are based on separate experiments and are not comparable. Aggressiveness tests were conducted as previously stated, and each experiment was conducted twice.

Aggressiveness of MCGs within the DeKalb and Watseka Sets. Aggressiveness of MCGs 8, 14, 16, and 17 within the Watseka Set and MCGs 3, 8, 13, and 14 within the DeKalb Set was calculated using AUDPC values from the isolate aggressiveness tests previously described.

Cultivar–isolate interaction tests. Six cultivars ranging in reaction from partially resistant to susceptible to *S. sclerotiorum* based on field evaluations were selected. Cvs. NKS19-90 and A2506 exhibited partial resistance; cvs. Bell and Elgin, intermediate reactions; and cvs. A2242 and Williams82, susceptible reactions (18). Six isolates (D3, D8, D105, D110, D160, and D458) that varied in aggressiveness (26) and host (including pear, pinto bean, soybean, and sunflower) were utilized to test for cultivar–isolate interactions. Inoculation was conducted as previously stated, and AUDPC values were calculated. The experiment was conducted twice.

Data analysis. Frequency of occurrence of MCGs within each of the DeKalb and Watseka fields was determined by χ^2 analysis. MCGs with less than five observed occurrences in each respective field were omitted from the analysis. For MCGs that were common between the DeKalb and Watseka fields (MCGs 8 and 14), χ^2 analysis at $P = 0.01$ was used to determine and compare MCG frequency between the two fields.

To determine if isolates varied in aggressiveness for the four sets (Argentine, DeKalb, Diverse, and Watseka), AUDPC data were analyzed using analysis of variance (PROC ANOVA; SAS Institute, Inc., Cary, NC), and means were compared by least significant differences (LSD) at $P = 0.05$. To compare the AUDPC values between repeated experiments, a general linear model (PROC GLM) and a nested model were used to test for homogeneity of variances and isolate by experiment interactions. Cultivar–isolate interaction was tested using PROC GLM within the SAS system. Means were compared by LSD at $P = 0.05$. To determine aggressiveness of MCGs within the DeKalb and Watseka Sets, contrast statements were analyzed using PROC GLM.

RESULTS

MCG diversity and distribution. Mycelial compatibility groups were determined for Diverse, Argentine, DeKalb, and Watseka Sets. Among 299 *S. sclerotiorum*

isolates, 42 MCGs were identified (Table 2). Most (61%) of the MCGs were unique, which describes an MCG represented by a single isolate observed at a single location. Three compatibility groups, MCGs 3, 8, and 14, were sampled at high frequency from multiple locations. MCG 8 was the most commonly sampled MCG and included isolates from both canola and soybean. MCG 8 was detected in Indiana, Michigan, Ohio, Wisconsin, Canada, and Switzerland and was observed at a high frequency in both Illinois soybean fields. MCG 8 was identified as clonal lineage 1 by DNA fingerprinting (L. M. Kohn, *personal communication*). MCG 14 included isolates from canola, dry bean, and soybean and was detected in Michigan, Nebraska, Canada, and both Illinois soybean fields. MCG 14 was identified as clonal lineage 2 by DNA fingerprinting (L. M. Kohn, *personal communication*). MCG 3 was collected from soybean in Wisconsin and also detected in the DeKalb field.

The observed MCGs and MCG frequencies differed within the four sets (Table 3). The Diverse Set consisted of 17 MCGs, whereas 14 and 6 MCGs were detected within the DeKalb and Watseka Sets, respectively. Both unique and common MCGs were observed within the Diverse, DeKalb, and Watseka Sets, but none of the MCGs identified in the Argentine Set were common with the other sets. Eight MCGs were identified from the Argentine Set, with four MCGs represented only once. MCGs 19 and 26 were sampled five times each; MCGs 21 and 24 were sampled two times each; and, MCGs 20, 22, 23, and 25 each were observed once. MCG 8 was the most frequently sampled MCG in the DeKalb and Watseka fields and represented 36 and 62% of the isolates, respectively. A similar trend was observed for MCG 14, which represented 14 and 4% of the isolates from the DeKalb and Watseka Sets, respectively. A χ^2 analysis confirmed that the DeKalb and Watseka fields did not have equal frequencies of occurrence of MCGs 8 and 14, and MCG 8 (clonal lineage 1) showed the highest frequency of occurrence in both fields (Fig. 1). MCG 3 was represented by 19% of the isolates from the DeKalb Set but was not observed in the Watseka Set. Five and two unique MCGs were observed in the DeKalb and Watseka Sets, respectively.

Table 3. Number of isolates and mycelial compatibility groups (MCGs) observed in each *Sclerotinia sclerotiorum* set

Set	No. of isolates	No. of MCGs	MCGs identified
Diverse	24	17	1–12, 14, 27, 40–42
DeKalb	124	14	3, 8, 13, 14, 15, 30, 31–38
Watseka	130	6	8, 14, 16–18, 39
Argentina	21	10	19–26, 28, 29

Table 2. Mycelial compatibility group (MCG) designation for 299 *Sclerotinia sclerotiorum* isolates

MCG[a]	Isolate code[b]
1	D1
2	D3
3	D12, 25 isolates from the DeKalb Set (Fig. 1)
4	D33
5	D48
6	D105
7	D110
8 (clone 1)	D15, D20, D30, D36, D127, D159, 44 isolates from the DeKalb Set (Fig. 1), and 82 isolates from the Watseka Set (Fig. 1)
9	D130
10	D133
11	D144
12	D160
13	DK219, DK222, DK257, DK273, DK280, and DK282
14 (clone 2)	D2, D8, D150, 18 isolates from the DeKalb Set (Fig. 1), and 5 isolates from the Watseka Set (Fig. 1)
15	DK292, DK300
16	16 isolates from the Watseka Set (Fig. 1)
17	25 isolates from the Watseka Set (Fig. 1)
18	W420
19	A167, A169, A173, A178, A181, and A184
20	A168
21	A170, A171
22	A172
23	A174
24	A176, A183
25	A177
26	A175, A179, A180, A182, and A185
27	D6
28	A461
29	A462
30	DK203, DK253, DK254, DK256, DK259, DK260, DK262, DK265, DK271, DK285, DK306, and DK309
31	DK240
32	DK221
33	DK270, DK293
34	DK264
35	DK286
36	DK267, DK269, DK276, DK305, and DK308
37	DK217, DK220, DK244, DK297, and DK304
38	DK198
39	W383
40	D457
41	D458
42	D459

[a] MCG is assigned for this research. Clonal lineage is shown if available.
[b] The isolate collection number is preceded by a letter to indicate set: A = Argentina, D = Diverse, DK = DeKalb, and W = Watseka.

Isolate aggressiveness within the four sets. Isolate aggressiveness varied ($P \leq 0.01$) within the four sets (Tables 4 and 5). Mean AUDPC values between repeated experimental runs were significantly correlated ($P \leq 0.05$) with correlation coefficients of $r = 0.79$, 0.79, 0.48, and 0.48 for Diverse, Argentine, DeKalb, and Watseka Sets, respectively. The AUDPC values for each set did not exhibit replication–experiment interactions, but an isolate–experiment interaction was observed within the DeKalb and Watseka Sets. The Diverse Set had mean AUDPC values ranging from 22 to 287, and the LSD was 63 (Table 4). The least aggressive isolates (D110 and D133) were isolated from pear and an unknown host, respectively, and the most aggressive isolate (D160) was from soybean in Argentina. The Argentine set had mean AUDPC values ranging from 41 to 165, and the LSD was 43 (Table 4). The DeKalb Set had mean AUDPC values ranging from 0 to 250 (LSD = 88) and 0 to 188 (LSD = 78) for two experiments that showed isolate–experiment interactions (Table 5). Isolate 209, responsible for the AUDPC value of 0, grew on PDA but did not infect soybean in either of the two experimental runs. The Watseka set had mean AUDPC values ranging from 44 to 188 (LSD = 76), and 7 to 250 (LSD = 63) for two experiments that showed isolate–experiment interactions (Table 5).

Isolate aggressiveness within widely dispersed MCGs. Aggressiveness values for isolates within MCGs composed of members from multiple locations (MCGs 3, 8, and 14) were highly significant ($P \leq 0.001$). Isolate aggressiveness within locally observed MCGs composed of members from a single location (MCGs 13, 16, 17, 19, 21, 24, and 26) did not differ ($P = 0.05$). Selected isolates from MCG 8, the most geographically diverse and frequently sampled MCG, had a range of AUDPC values from 105 to 416. These two extreme values were exhibited by isolates collected from the DeKalb field. MCG 3, composed of isolates from two different locations (Wisconsin and Illinois) ranged in AUDPC values from 23 to 105. MCG 14, composed of isolates from five locations (Michigan, Nebraska, Canada, and the DeKalb and Watseka fields) ranged in AUDPC values from 0 to 223, with the least aggressive isolate from Michigan and the most aggressive from the DeKalb Set.

MCG aggressiveness within the DeKalb and Watseka Sets. AUDPC values for MCGs 3, 8, 13, and 14 from the DeKalb Set and for MCGs 8, 14, 16, and 17 from the Watseka Set were compared. Within the DeKalb Set, AUDPC values for MCGs 3 and 8 differed at $P \leq 0.1$, and for MCGs 3 and 13 at $P \leq 0.01$. Within the Watseka Set, AUDPC values for MCGs 8 and 17 differed at $P \leq 0.001$, for MCGs 14 and 17 at $P \leq 0.001$, and for MCGs 16 and 17 at $P \leq 0.05$. AUDPC values did not differ significantly for MCGs 8 and 14 within either the DeKalb or Watseka Sets.

Cultivar–isolate interaction tests. A cultivar–isolate interaction was not detected ($P = 0.05$), but AUDPC values varied by isolate (Table 6). No differences in mean AUDPC values were observed ($P = 0.05$) between susceptible cv. Williams 82 and resistant cvs. NKS19-90 and A2506 when inoculated with isolate 160. When inoculated with isolates 8 and 105, no differences ($P = 0.05$) in mean AUDPC values were observed for resistant cv. A2506 and susceptible cvs. A2242 and Williams82. When inoculated with isolate 458, mean AUDPC values did not differ ($P = 0.05$) for all six cultivars. Resistant cv. NKS19-90 consistently resulted is the lowest AUDPC values, and A2242 and Williams82 resulted in the highest AUDPC values for all isolates except 458.

DISCUSSION

Populations of *S. sclerotiorum* from two Illinois soybean fields were a heterogeneous mix of MCGs. This corroborates reports of *S. sclerotiorum* MCG population structure on canola in Canada (21), Norwegian vegetable crops (8), sunflower in Manitoba (20), cabbage in North Carolina (11), and soybean in Argentina (12) and Canada (14). In our study, each soybean field contained two or three MCGs observed at high frequency, and a larger proportion of MCGs sampled at low frequencies. This frequency trend was observed previously in Canadian studies of *S. sclerotiorum* on canola (20) and on soybean in Argentina (12) and Canada (14). The population structure of *S. sclerotiorum*, based on MCGs, appears similar irrespective of host crop and field location. The genetic and evolutionary mechanisms that contribute to the maintenance of such a mosaic pattern have not been determined experimentally, but comparisons of *S. sclerotiorum* populations on cultivated canola

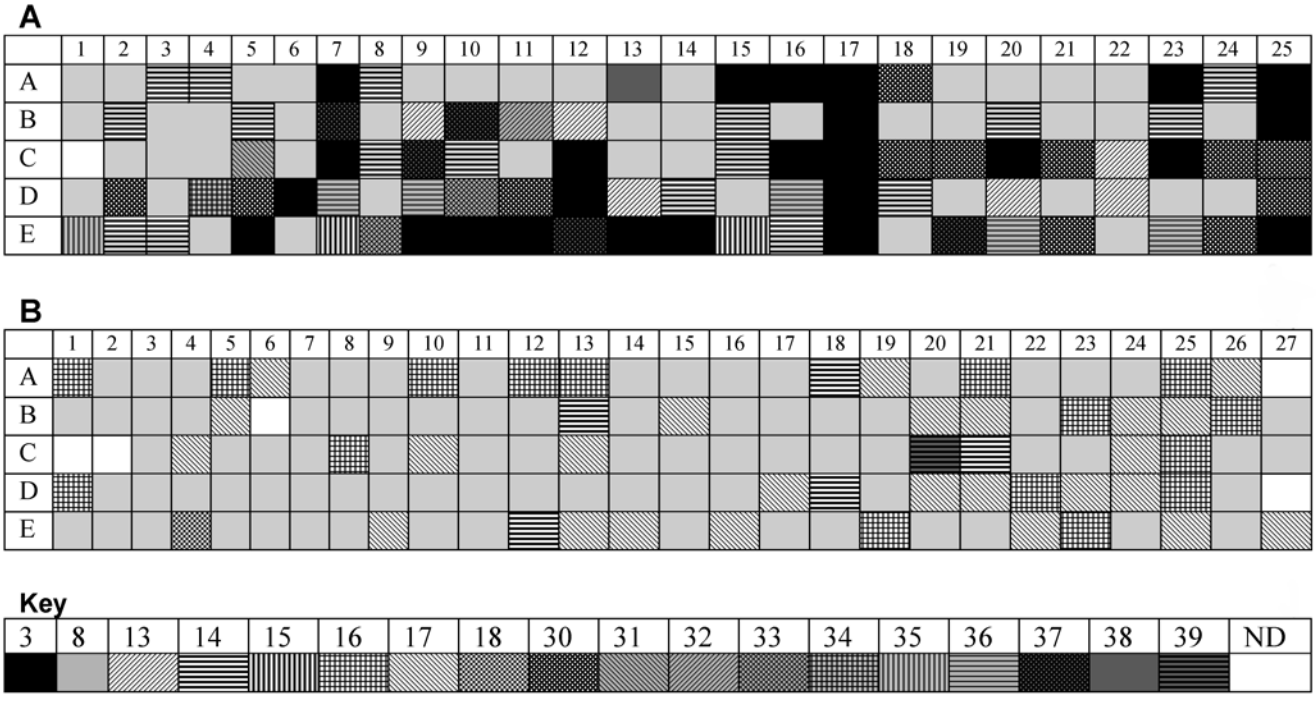

Fig. 1. Schematic distribution of mycelial compatibility groups (MCGs) of *Sclerotinia sclerotiorum* within two Illinois soybean fields. The sampled area at **A,** DeKalb was 813 m^2 and divided into 125 subplots. The sampled area at **B,** Watseka was 581 m^2 and divided into 135 subplots. Not determined (ND) indicates that the MCG could not be determined.

and on an isolated wild perennial host, *Ranunculus ficaria*, indicated four major differences between agricultural and wild populations (22). DNA fingerprint diversity was high in agricultural populations and low in wild populations; agricultural populations showed no evidence of out crossing, but out crossing occurred in wild populations; and MCGs were dispersed randomly in agricultural populations, but strong spatial substructuring was indicated in isolated wild populations. Comparison of DNA fingerprint patterns from *S. sclerotiorum* populations in New Zealand indicated substantial local movement of isolates but provided little evidence for long-range dispersal (8). MCG structure of *S. sclerotiorum* on cultivated hosts appears to be more complex, indicating that agricultural practices may influence MCG frequencies and patterns.

Within agricultural populations, MCG frequencies and patterns vary. *S. sclerotiorum* MCGs on soybean may be unique and exist locally at low frequencies, as indicated by 65% of the MCGs sampled being represented by one isolate. Localization of unique MCGs was observed in vegetable-growing regions in New Zealand (8) and in winter canola in Harrison, Ontario (25). Explanations for unique, low-frequency MCGs in a sampling area may be recent MCG introduction events or random mutation events. The emergence of new genotypes localized in single fields may be an indication of MCGs or clones adapted to specific field microclimates or hosts, and is less likely the result of genetic exchange and recombination (3,14). MCGs may exist locally at high frequency, as observed with MCGs 16 and 17 in the Watseka field and MCG 13 in the DeKalb field. Growers who utilize a portion of each crop for seed for the next growing season or continually plant the same soybean cultivar may tend to localize and increase the frequency of specific MCGs.

MCGs may exist at high frequencies with wide geographic distribution. A trans-Canadian study of *S. sclerotiorum* isolated from canola revealed that one widely distributed genotype was sampled in Ontario, Manitoba, and Saskatchewan provinces (21). A single *S. sclerotiorum* MCG, clonal lineage 1, represented 46% of the samples from Ontario and Quebec soybean (14). In this study, MCG 8 was detected in soybean fields in several states, on soybean in Switzerland, and on canola in Canada. MCG 8 was observed in 62 and 36% of the isolates sampled within the Watseka and DeKalb Sets, respectively. The clonal lineage represented in this study by MCG 8 initially was sampled on canola in 1989 (25) and is clonal lineage 1 (L. M. Kohn, *personal communication*). MCG 14 was detected in three states, in Canada, and on three different hosts (soybean, canola, and dry bean). In the Watseka and DeKalb Sets, MCG 14 composed 4 and 14% of the isolates, respectively. The clonal lineage represented in this study by MCG 14 also was sampled initially on canola in 1989 (25) and is clonal lineage 2 (L. M. Kohn, *personal communication*). Since 1989, both clonal lineages 1 and 2 have been sampled at high frequency over large geographical areas (14). Pervasive MCGs may be due to movement of sclerotia or infested seed through established distribution channels. Additionally, isolate fecundity factors may influence proliferation of certain genotypes. Isolate differences in the number of apothecia produced per sclerotium have been observed (4). Sclerotia from isolate D150 (Table 1) produced a relatively larger number of apothecia (L. S. Kull, *unpublished data*). Isolate D150 is associated with MCG 14 (clonal lineage 2), which is a high-frequency genotype sampled over large geographical areas. The association between clonal lineage and fecundity is testable and may help to explain differing

Table 4. Aggressiveness of 18 *Sclerotinia sclerotiorum* isolates from various hosts and locations (Diverse Set) and 20 *S. sclerotiorum* isolates from San Pedro, Argentina[a]

Isolate[b]	MCG[c]	AUDPC[d]
Diverse		
D160	12	287
D105	6	285
D20	8	282
D30	8	268
D6	27	268
D127	8	263
D36	8	256
D1	1	256
D15	8	253
D12	3	216
D48	5	190
D33	4	179
D130	9	156
D144	11	147
D3	2	146
D159	8	138
D133	10	56
D110	7	22
LSD	…	63
Argentine		
A172	22	165
A178	19	161
A177	25	160
A160	12	157
A180	26	152
A170	21	143
A184	19	139
A182	26	137
A171	21	136
A169	19	129
A183	24	124
A179	26	121
A173	19	118
A181	19	116
A185	26	112
A176	24	97
A175	26	87
A174	23	85
A167	19	79
A168	20	41
LSD	…	43

[a] Aggressiveness on soybean seedlings was based on area under disease progress curve (AUDPC) values averaged over two greenhouse experiments.
[b] LSD = least significant difference at $P = 0.05$.
[c] Mycelial compatibility group (MCG) assigned for this research. No standard system of naming MCGs has been established for *S. sclerotiorum*.
[d] Based on accumulation of disease recorded over time (37). AUDPC values for the Diverse and Argentine Sets are based on separate experiments and are not comparable.

Table 5. Aggressiveness of 48 *Sclerotinia sclerotiorum* isolates from two Illinois soybean fields, DeKalb and Watseka[a]

		AUDPC	
Isolate[b]	MCG[c]	Run 1	Run 2
DeKalb			
DK198	38	250	175
DK202	3	250	188
DK233	14	238	88
DK228	8	225	100
DK245	14	225	163
DK237	8	225	119
DK268	8	225	175
DK309	30	225	125
DK193	14	219	113
DK230	14	207	150
DK221	32	207	163
DK261	na	200	69
DK280	13	200	31
DK278	14	200	56
DK251	3	188	138
DK303	8	163	131
DK187	8	163	50
DK292	15	157	150
DK219	13	150	81
DK257	13	138	31
DK287	14	113	100
DK269	3	75	81
DK277	3	38	106
DK209	14	0	0
LSD	…	88	78
Watseka			
W328	14	188	163
W367	17	188	250
W441	17	171	225
W342	na	163	200
W407	17	150	238
W391	16	150	188
W435	16	138	175
W428	14	138	76
W336	17	138	163
W362	16	134	200
W213	8	125	150
W315	16	125	151
W357	17	125	200
W423	8	123	7
W324	8	113	38
W378	8	113	113
W388	16	113	213
W416	8	107	225
W365	na	88	138
W349	14	88	50
W384	14	82	132
W337	8	69	38
W408	14	57	44
W420	18	44	57
LSD	…	76	63

[a] Aggressiveness in two experiment runs on soybean seedlings inoculated in the greenhouse was based on area under disease progress curve (AUDPC) values, based on accumulation of disease over time (37).
[b] LSD = least significant difference.
[c] Mycelial compatibility group (MCG) assigned for this research; na = not determined.

MCG frequencies among field populations and over large geographic distances.

Widely dispersed MCGs showed within-genotype variability in aggressiveness that was not observed in local MCGs. Isolates within local MCGs, defined in this study as MCGs detected in one location or field, did not vary in aggressiveness, whereas MCGs composed of members from more than one location or field varied in aggressiveness. Uniformity within local MCGs could be due to a founder effect, limited local proliferation, and continual adaptation for a particular set of field conditions. Individual MCGs within each field varied in aggressiveness, indicating that MCGs may maintain fitness phenotypes. Within the Watseka field, MCG 17 was the most aggressive, and MCGs 8 and 14 (clonal lineages 1 and 2, respectively), the two most pervasive genotypes in this study, were the least aggressive. This genetic association of low aggressiveness and high pervasiveness supports the popular idea held by evolutionary biologists that pathogens can evolve to become harmless, more deadly, or anything in between depending on the forces guiding natural selection; such forces can pull the pathogen in opposite directions at the same time, creating an evolutionary tradeoff between fecundity and infectivity factors (19).

Unique MCGs, defined as one MCG composed of a single isolate member, were observed in each set. Five and two unique MCGs were detected in the DeKalb and Watseka Sets, respectively. Three of these unique MCGs were included in aggressiveness tests. Watseka MCG 18, isolate 420 and DeKalb MCG 38, isolate 198 (Table 5) resulted in the lowest and highest AUDPC values, respectively, of the 24 isolates compared in aggressiveness tests for each set. Interestingly, MCG 18 isolate 420, being the most aggressive, was not associated with the most pervasive MCG within the Watseka Set. Unique MCGs ranging in aggressiveness from high to low may be indicative of genetic tradeoffs between aggressiveness genes allowing rapid, successful infection and fecundity genes allowing pervasive proliferation (19). These genetic scenarios may be challenging to test experimentally but may be considerations in successful management of this ubiquitous pathogen.

Although some soybean seed produced in the United States routinely is increased in Argentina for subsequent distribution in the United States, no common MCGs were observed between Argentina and any other location. Lack of common MCGs may indicate little or no movement of propagules or indicate selection for specific MCG genotypes influenced by environmental conditions and competition. In a 1992 study, highly significant differences in clone frequencies in lesions verses petals suggested processes of positive selection may have occurred (20). A previous report of *S. sclerotiorum* isolates on cabbage in North Carolina indicated that MCGs or clones were shared between fields 75 km apart, but no common MCGs or clones were detected between North Carolina and Canada or between North Carolina and Louisiana (11). The evolutionary history of population haplotypes indicated that isolates may be grouped into subtropical, temperate, wild, and two relatively recently evolved temperate-subtropical populations (7). This geographic clustering infers that ecological conditions may contribute to adaptations associated with growth temperature range, light intensity, or sclerotial vernalization requirements. The Argentine isolate set is a small representative sample, and lack of common MCGs with other locations may be a result of a small sample size. Additional sampling of Argentine fields may result in detection of common MCGs; alternatively, phylogenetic analysis comparing isolates from geographically and environmentally distinct regions may provide evidence that geographically similar genotypes cluster (L. S. Kull, *unpublished data*).

Few reports regarding variation in *S. sclerotiorum* aggressiveness on soybean are available. A 1975 report (36) comparing pathogenicity of 14 *S. sclerotiorum* isolates on 11 different hosts (soybean was not included) showed a variation in degree of pathogenicity dependent on host species. The current study revealed a notable range in isolate AUDPC values for both a diverse sampling and local field samplings of the fungus. An isolate–experiment interaction was observed between the two experimental runs for the DeKalb and Watseka Sets. Although mean AUDPC values were correlated significantly for each of the DeKalb and Watseka experiments, correlation coefficients were low (r = 0.48). The results do not indicate evidence for specific causes of interactions, but similar interactions previously have been reported (35) and support reasons for further experimentation. For the current study, environmental sensitivity to light and temperature may contribute to the unpredictability of disease development and progress observed in the *Sclerotinia* pathosystem (33). Prior to inoculation, plants for separate experimental runs were grown in the greenhouse at various times throughout the year. Ambient conditions of light intensity, day length, and temperature may fluctuate in controlled-environment facilities and result in subtle differences in growth rates and degree of stem etiolation of seedlings to be inoculated. A consistent observation evident in all experimental runs was quantifiable differences in levels of variability in isolate AUDPC values.

When selecting an isolate for varietal resistance evaluations, variation in isolate aggressiveness should be considered. Partially resistant and susceptible cultivars were compared by inoculation with highly and weakly aggressive isolates, and disease occurred on both cultivars, but partially resistant and susceptible cultivars were not effectively identified. Although no isolate–cultivar interaction was detected, effective separation of resistant and susceptible cultivars was dependent on isolate selection. A recent report indicated that successful identification of resistant and susceptible cultivars and detection of interactions was influenced by isolate, inoculation technique, and type of statistical analysis (26).

The differences in MCG frequency between fields and variation in aggressiveness of *S. sclerotiorum* within naturally infested disease-screening nurseries may help to explain the mixed results across field locations used for resistance screening trials (18) and the lack of correlation between field and laboratory evaluations (17,32,39). For example, the DeKalb and Watseka fields were a mosaic of MCGs (Fig. 1). MCG frequencies differed between the two fields, and MCGs and individual isolates varied in aggressiveness. Although greenhouse resistance screening programs may reduce interactions due to pathogen variability across experimental field plots, greenhouse resistance screening protocols typically are conducted with one

Table 6. Comparison of partially resistant (NKS19-90 and A2506), intermediate (Bell and Elgin), and susceptible (A2242 and Williams82) soybean cultivars inoculated with six *Sclerotinia sclerotiorum* isolates differing in level of aggressiveness[a]

	Isolate[b]					
Cultivar[c]	**D160**	**D105**	**D3**	**D110**	**D8**	**D458**
NKS19-90	232	217	113	66	74	3
A2506	300	282	238	179	238	53
Bell	269	282	151	166	100	35
Elgin	282	257	153	110	119	16
Williams82	301	294	190	221	206	60
A2242	351	319	238	257	238	44
LSD (0.05)	75	66	76	96	105	65

[a] Relative aggressiveness of isolates is D160 and D105 > D3 and D110 > D8 and D458. Cultivar performance was based on area under disease progress curve (AUDPC), and lower values represent less disease.

[b] Isolates were taken from different hosts and differ in aggressiveness; data is AUDPC (37).

[c] LSD = least significant difference at P = 0.05.

isolate that may not represent the range of pathogen variability present on the host. Lack of consideration for pathogen variability could result in low correlation between greenhouse and field screens, and also result in mixed results across locations. Designing a field experiment to eliminate or compensate for the variability in MCG and isolate aggressiveness may not be possible. Nelson et al. (32) attributed the lack of correlation between field and laboratory evaluations to an ineffective greenhouse screening method, but information about field escapes, spatial uniformity of field infection, cropping history, or pathogen population structure were not reported. To enhance disease management efforts, selecting several isolates to evaluate resistance or to develop disease resistance markers may be more effective than evaluations conducted with one isolate. Assessment of the range in aggressiveness of *S. sclerotiorum* populations on cultivated hosts and monitoring of year-to-year changes in population structure within cropping regions may contribute to efficient management strategies for controlling diseases caused by *S. sclerotiorum*.

ACKNOWLEDGMENTS

We thank R. Warsaw for helping with field isolate collection and greenhouse screening; M. Raymond, K. Roemer, K. Smith, and K. Urish for greenhouse and laboratory assistance; and various individuals for donation of *S. sclerotiorum* isolates.

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