Mycelial Compatibility Groups in Texas Peanut Field Populations of *Sclerotium rolfsii*

F. Ameena Nalim, James L. Starr, Kenneth E. Woodard, Suzanne Segner, and Nancy P. Keller

First, second, fourth, and fifth authors: Graduate research assistant, professor, research assistant, and assistant professor, respectively, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843-2132; third author: senior research associate, Texas Agricultural Experiment Station, Stephenville 76401.

This work was done in partial fulfillment of the masters requirement for F. A. Nalim.
We thank the Texas Peanut Producers Board for funding this research and T. A. Lee, Jr., for his help and encouragement with this project.
Accepted for publication 4 September 1995.

ABSTRACT

Nalim, F. A., Starr, J. L., Woodard, K. E., Segner, S., and Keller, N. P. 1995. Mycelial compatibility groups in Texas peanut field populations of *Sclerotium rolfsii*. Phytopathology 85:1507-1512.

Sclerotium rolfsii isolates (366) collected from 1985 to 1994 in Texas peanut fields were placed in 25 mycelial compatibility groups (MCG) based on the formation of an antagonism zone between incompatible mycelia of paired isolates. The same MCG were often detected in several geographically distant counties in Texas. Individual peanut fields contained one to five MCG, whereas individual plants were infected with isolates from one MCG. Two fields sampled extensively in 1992 maintained the same MCG in 1993: MCG 6 and 7 in Galen and MCG 1, 2, 3, 4, and 12 in Grissom. However, MCG 11, which consisted of a single fungicide-tolerant 1985 isolate of *S. rolfsii* from Galen, was not found among the 366 isolates collected nor was the trait of fungicide tolerance. DNA amplification patterns resulting from the use of the 18-base oligonucleotide primer NK2 and from restriction digests of the internal transcribed spacer (ITS) region of the rDNA were examined in a subset of 80 isolates from four fields to determine the genetic similarity of isolates within and between 12 MCG. Three NK2-amplified DNA patterns in genomic DNA and four *Mbo*I restriction digest patterns of the ITS region were found. All isolates within a MCG gave identical patterns for each marker, and some MCG shared the same ITS and NK2 patterns. MCG sharing the same DNA patterns were often from the same field.

Additional keywords: *Athelia rolfsii*, fungal genetics, southern blight, vegetative compatibility.

Sclerotium rolfsii Sacc., causal agent of southern blight of peanuts, is distributed worldwide and causes disease in over 500 plant species (3). In the United States, the disease is a problem in all peanut-producing states and is controlled primarily by the use of fungicides, particularly pentachloronitrobenzene (PCNB) (3,9,19). *S. rolfsii* does not produce asexual spores, and it overwinters in soil and on plant debris as sclerotia, the primary inocula (21,24). Although the basidial state, *Athelia rolfsii* (Curzi) Tu & Kimbrough, has been induced in the laboratory (22), the sexual stage is not thought to commonly occur in nature. Spatio-temporal studies on *S. rolfsii* have revealed that southern blight is a monocyclic disease (25,27).

Studies by Punja and Grogan (22,23) have shown that *S. rolfsii* can be placed in mycelial compatibility groups, (MCG), based on mycelial interactions similar to those described for vegetative compatibility groups (VCG) (14). Recent studies have suggested that the role of MCG and VCG is important in defining field populations and in facilitating genetic exchange in fungi in which the sexual stage has minimal impact on the disease cycle (11,14). Intraspecific grouping based on vegetative or mycelial compatibility has been shown in ascomycetes and deuteromycetes, including *Aspergillus flavus* (4), *Cryphonectria parasitica* (1), *Fusarium oxysporum* (10), *F. sambucinum* (20), and *Sclerotinia sclerotiorum* (11), and in the basidiomycete *Rhizoctonia solani* (2,28,29). Except for the intraspecific groups in *R. solani*, VCG in the above fungi are composed of isolates that are clones. Genetic exchange appears to be confined to members of the same MCG/VCG in *A. flavus* (4), *Sclerotinia sclerotiorum* (11), and *F. oxysporum* f. sp. *lycopersici* (10).

Corresponding author: N. P. Keller; E-mail address: npk3325@acs.tamu.edu

Although several MCG have been described for a limited worldwide collection of *S. rolfsii* isolates (8,22,23), no data has been gathered to establish the role of MCG in field populations. There are no data on the number or longevity of MCG found in a single plant, crop, or field nor on the amount of genetic variability within or between MCG. Aside from understanding the basic biology of *S. rolfsii* MCG, the putative role of MCG in maintaining genetic traits in a field population is important in light of the discovery of PCNB-tolerant strains of *S. rolfsii* isolated from a Texas peanut field in 1985 (19). Could this trait spread in a field population of *S. rolfsii*? If *S. rolfsii* MCG are clonal in nature, it is likely that genetic exchange would be limited to isolates within a single MCG and that the maintenance of such a MCG would be paramount to maintenance of a particular genetic trait. Our aims were to assess the occurrence of MCG of *S. rolfsii* and their distribution in peanut plants and fields in Texas and to establish a baseline on the level of variation within and between MCG.

MATERIALS AND METHODS

Fungal collection. In 1992, isolates of *S. rolfsii* were collected from peanut plants with symptoms of southern blight from four fields (Galen, Eves, Grissom, and DeLeon) in the central Texas counties of Comanche, Eastland, and Erath (Table 1). The four fields were within 25 km of each other. One field, Galen, contained PCNB-tolerant isolates of *S. rolfsii* in 1985 (19). Both Galen and Grissom were resampled in 1993. Diseased plant tissue was collected from disease foci in the fields, and varying numbers of sclerotia were collected from each diseased plant. Additional *S. rolfsii* isolates collected from 1990 to 1994 were from arbitrarily sampled peanut fields in different counties in Texas (Table 1).

Sclerotia recovered from diseased plant tissue were identified by county, field, plant, and year (17). To establish permanent cultures of *S. rolfsii*, sclerotia were placed on 1.5% water agar at room temperature (23°C), and single hyphal tips were transferred from each germinating sclerotia to petri dishes of potato-dextrose agar (PDA) (Difco Laboratories, Detroit). Each culture was grown until numerous sclerotia formed. Sclerotia from dried cultures were collected and stored in sterile polypropylene tubes at room temperature and at –80°C.

MCG. *S. rolfsii* isolates were paired on 100 × 15-mm PDA dishes. All pairings were replicated twice. Controls were paired with themselves. Isolates were placed in different MCG based on the presence of an antagonism zone, which is the basis of incompatibility, between two paired fungal colonies (Fig. 1). MCG were defined within each field, and a representative tester isolate from each MCG was identified and given a special designation (17). Tester isolates for MCG from one field then were paired with tester isolates from MCG in other fields.

DNA extraction. *S. rolfsii* isolates were grown on PDA for 3 to 4 days at 29°C. Aerial mycelia, collected by scraping the agar surface with a sterile scalpel blade, were used to initiate fungal colonies in glass petri dishes containing 15 ml of a liquid complete medium (12). After 2 to 3 days of growth at 29°C, mycelial mats collected by filtering through Whatman No. 40 filter paper (Whatman, Clifton, NJ) were frozen at –80°C, lyophilized for at least 24 h, and stored at –80°C. Due to an undefined, viscous material coprecipitating with DNA, several DNA extraction procedures were tried before a modification of the Stratagene (La Jolla, CA) method was selected based on successful amplification by polymerase chain reaction (PCR) (17). The modified method was as follows: 4 ml of lysis buffer (50 mM Tris-HCl [pH 8.0], 20 mM EDTA [pH 8.0], 2.0% sodium dodecyl sulfate) was added to ~100 mg of lyophilized and crushed mycelial tissue and homogenized. Another 4 ml of lysis buffer was added, followed by addition of protease or proteinase K (final concentration 500 µg/ml). The mixture was incubated at 55°C for 2 h, placed on ice for 30 min, and 0.35 ml of 6 M NaCl was added per ml of lysis buffer. This mixture was centrifuged for 20 min at 7,000 rpm (JA-20 rotor) at 4°C. The pellet was discarded, and 2.5 volumes of cold 95% ethanol was added to the supernatant. The DNA was precipitated at 2,000 rpm for 5 min in a swinging bucket rotor at room temperature, washed with 70% ethanol, air-dried for 1.0 h, resuspended overnight in 300 µl of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), and stored at 4°C. RNaseA (final concentration 0.1 µg/µl) was added before the DNA was used for PCR.

DNA amplification using oligonucleotide primers. Genomic DNA of *S. rolfsii* isolates from each MCG was amplified with oligonucleotide primers using PCR. DNA amplification patterns were assessed for six 16- to 24-base primers. One 18-base primer, NK2 (5′ AAT TGG TTC CAC TTG GGC 3′) produced repeatable DNA amplification patterns useful in distinguishing certain MCG, whereas the other five primers did not give consistent results (this was partially attributed to difficulties in obtaining clean DNA). Amplifications were carried out in a final reaction volume of 30 µl that consisted of 1 µl (0.2 mM) of primer mix, 2.4 µl (0.8 mM) of total deoxyribonucleotide mix (dNTPs), 5 µl (4.1 mM) of $MgCl_2$, 0.1 µl (12 units/µl) of *Taq* Polymerase (Promega, Madison, WI), 3 µl (1×) of 10× PCR buffer (Promega), and 0.5 µl (approximately 100 ng/µl) of genomic DNA template overlaid with 30 µl of mineral oil. Temperature parameters for PCR reaction were (i) denaturation at 94°C for 1 min, (ii) primer annealing at 48°C for 2 min, and (iii) primer extension at 72°C for 2 min for 40 cycles using a Perkin-Elmer Cetus 480 thermal cycler (Norwalk, CT). PCR product (4 µl) was electrophoresed through a 1.5% agarose gel at 60 V for 1 h, stained with ethidium bromide for 15 min (30 µl of 10 mg of stock EtBr per ml per liter of ddH_2O), and visualized under UV light.

PCR amplification of the internal transcribed spacer (ITS) region. The ITS region of *S. rolfsii* was amplified using "universal primers" 1 and 4 (constructed from conserved sequences of the 18s and 28s ribosomal genes [30]). These primers amplify ITS 1 and 2 and the 5.8s gene. Amplifications were carried out in a final reaction volume of 30 µl as described earlier, except the primers used were a 1-µl total of ITS primers 1 and 4 (6 mM each). Temperature parameters and number of cycles were as previously described. The unpurified, amplified products were digested using nine restriction enzymes. Of these, only *Mbo*I identified polymorphisms in the ITS region. *Mbo*I restriction digests of the ITS region of 1 to 20 representative isolates from each MCG were compared for polymorphisms. Digested sample (15 µl; ~1.0 µg) was electrophoresed on a 2.0% agarose gel and/or a 8.0% polyacrylamide gel (15) using a Mini-PROTEAN II electrophoresis cell (Bio-Rad Laboratories, Richmond, CA).

RESULTS

MCG. A total of 263 *S. rolfsii* isolates were obtained from Galen (148 isolates), Eves (30 isolates), Grissom (66 isolates) and DeLeon (19 isolates) in 1992. One to seven isolates of *S. rolfsii* were obtained from each diseased plant. Another 103 isolates

TABLE 1. Twenty-five *Sclerotium rolfsii* mycelial compatibility groups (MCG) found in 10 Texas counties from 1985 to 1994

Region / County	Isolates[a]	MCG[b] 1985	1990	1991	1992	1993	1994
West Texas							
Gaines	3		16	10	10		
Central Texas							
Bosque	1			16			
Brown	2		7				
Comanche	207	11[c]	4,5,7,21		1,5,6,7[c]	6,7[c],20,25	
Eastland	30				6,8,9		
Erath	90		10		1,2,3,4,6,12[d]	1,2,3,4,12[d]	
Southern Texas							
Atascosa	7		15		14		1
Frio	19			4,18,19			1,22,23,24
Southeast Texas							
Lavaca	2			13,17			
Waller	5			5			

[a] Total number of isolates collected from each county.
[b] *S. rolfsii* isolates collected from 1985 to 1994 belonged to 1 of 25 MCG.
[c] Underlined MCG indicate known isolates from Galen field.
[d] Underlined MCG indicate known isolates from Grissom field.

were collected from symptomatic plants from other Texas peanut fields for a total of 366 isolates (Table 1). All isolates were assigned to 25 MCG (Table 1). Ninety-six of the diseased peanut plants yielded two or more *S. rolfsii* isolates. Fungal isolates from the same diseased peanut plant belonged to the same MCG, except for one plant infected with MCG 4 and 12 isolates and one plant infected with MCG 9 and 8 isolates. Mycelia of isolates in the same MCG intermingled and formed a white ridge of mycelia (a compatible reaction), whereas mycelia of isolates from different MCG formed a cleared antagonism zone in the area of mycelial contact (incompatible reaction) (Fig. 1). Sclerotia formed on either side of this zone for most incompatible pairings. In general, isolates within the same MCG grew at similar rates and formed sclerotia in distinct patterns, e.g., many small sclerotia at the edge of the petri plate, a few large sclerotia in the center of the petri plate, etc. (17).

Of the four Texas fields intensively sampled in 1992, only DeLeon had a single MCG, MCG 6. Grissom contained MCG 1, 2, 3, 4, and 12; Galen contained MCG 5, 6, and 7; and Eves contained MCG 6, 8, and 9. Isolates of *S. rolfsii* obtained from Grissom in 1993 also belonged to MCG 1, 2, 3, 4, and 12, and isolates from Galen in 1993 belonged to MCG 6 and 7 (Table 1). No further isolates belonging to MCG 11 have been detected in Galen field since the original PCNB-tolerant strain was isolated in 1985. Additionally, no other PCNB-tolerant isolates have been identified (M.-Y. Shim and J. L. Starr, *unpublished data*). In fields containing more than one MCG, isolates from the same MCG were not grouped in any discernible pattern, although the single MCG 5 isolate from the 1992 sampling of Galen was collected in one corner of the field distant from other disease foci (data not shown).

Many MCG were present in more than one county in Texas. MCG 6 and 7 were clustered in bordering counties of central Texas, whereas MCG 1, 4, 5, 10, and 16 were found in distal regions of the state (Table 1).

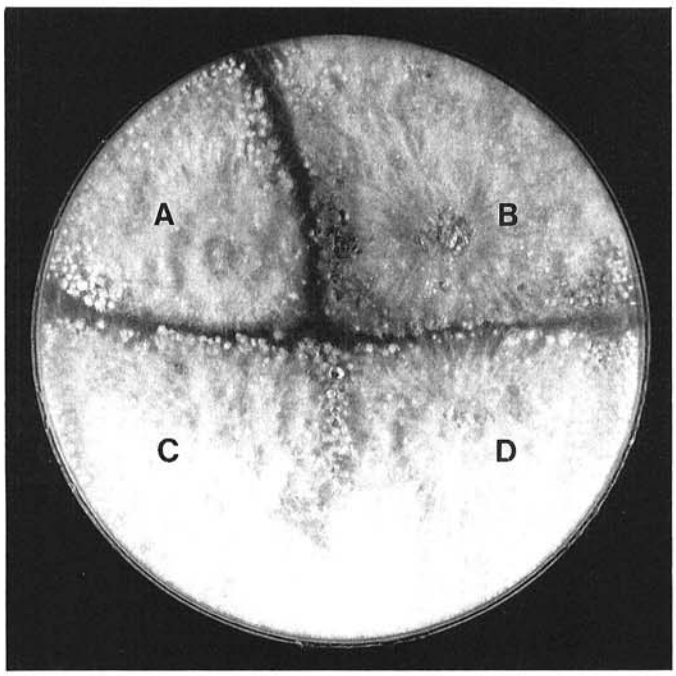

Fig. 1. Isolates of *Sclerotium rolfsii* form an antagonism zone when they are from different mycelial compatibility groups. *S. rolfsii* isolates were paired on petri plates of potato-dextrose agar to test for mycelial compatibility. For each pairing, mycelia either intermingled and formed a white ridge of mycelia (a compatible reaction) or formed a cleared antagonism zone in the area of contact between mycelia (incompatible reaction). **A and B** are incompatible and **C and D** are compatible.

NK2 amplification. Of six primers tested, only primer NK2 yielded easily discerned polymorphisms unique to the isolates tested. Three distinct NK2 amplification patterns, designated A, B, and C, were observed among the 80 isolates examined (Table 2). These patterns were based only on major DNA fragments that could be consistently amplified. The size of the major bands in pattern A were ~1,100 and 1,000 bp; in pattern B the major bands were at 1,500 and 900 bp; and in pattern C the major bands were at 1,500, 900, and 700 bp (17; Figs. 2 and 3A). There were other minor bands that were not always present and, therefore, not scored. All isolates that were tested within a MCG had the same NK2 amplification pattern (Fig. 2); this analysis was limited to the extent that some MCG were represented by only one member (Table 2). The MCG 5, 6, and 7 isolates were characterized by NK2 amplification pattern A; MCG 1, 3, 4, 10, 11, and 12 iso-

TABLE 2. DNA amplification patterns of 12 *Sclerotium rolfsii* mycelial compatibility groups (MCG)

Group MCG	Isolates[a]	ITS[b]	NK2[c]
Group 1[d]			
1	7	P1	B
4	12	P1	B
10	1	P1	B
11	1	P1	B
Group 2			
3	8	P1	C
Group 3			
6	15	P2	A
7	12	P2	A
Group 4			
8	4	P3	C
9	1	P3	C
12	7	P3	C
2	11	P3	C
Group 5			
5	1	P4	A

[a] Total number of isolates analyzed in each MCG.
[b] ITS = internal transcribed spacer region of rDNA. Patterns P1–P4 were obtained from *Mbo*I restriction digests of polymerase chain reaction (PCR)-amplified ITS DNA.
[c] NK2 = PCR amplification patterns A, B, and C formed using oligonucleotide primer NK2.
[d] MCG were placed in the same group if their NK2 and ITS DNA patterns were the same.

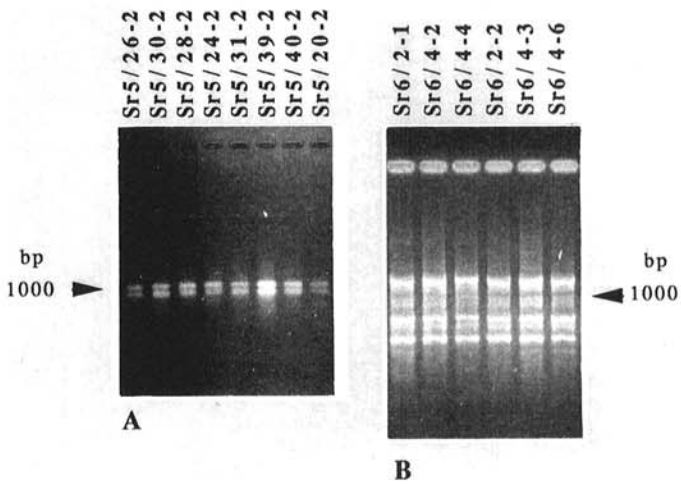

Fig. 2. Agarose gel electrophoresis of NK2 primer-amplified products of genomic DNA of *Sclerotium rolfsii* isolates. **A,** Isolates belonging to mycelial compatibility group (MCG) 6. **B,** Isolates belonging to MCG 2. The 1,000-bp marker is indicated on both agarose gels.

lates were characterized by NK2 amplification pattern B; and MCG 2, 8, and 9 isolates by were characterized NK2 amplification pattern C (Table 2; Fig. 3A).

Analysis of the ITS region. The size of the amplified ITS region, including the 5.8s ribosomal gene, was 686 bp, determined by sequencing this region in one MCG 1 isolate (F. A. Nalim, M.-Y. Shim, J. L. Starr, and N. P. Keller, *unpublished data*). All other isolates produced a similarly sized ITS fragment, determined by gel electrophoresis. An aliquot of the PCR-amplified region, electrophoresed on a 2.0% agarose or a 8.0% polyacrylamide gel, confirmed the presence, size, and quantity of the desired product. The ITS region was digested using several restriction enzymes, with only *Mbo*I identifying consistent length polymorphisms. The 2.0% agarose gel did not resolve differences of less than 50 bp; therefore, a 8.0% polyacrylamide gel was necessary to accurately differentiate the digestion patterns. There were four *Mbo*I restriction digest ITS patterns: P1, which was common to isolates of MCG 1, 3, 4, 10, and 11; P2, which was common to MCG 6 and 7; P3, which was common to MCG 2, 8, 9, and 12; and P4, which was found in the single isolate in MCG 5 (Table 2). All of the isolates in a MCG had the same *Mbo*I restriction digest pattern (17; Fig. 4). Pattern P3 was the simplest pattern and contained a subset of the *Mbo*I fragments present in P1, P2, and P4 (Fig. 3B). Several MCG shared the same NK2 and ITS DNA patterns and could be placed into five groups (Table 2).

DISCUSSION

Several approaches have been used to understand the genetic structure of fungal populations (5,14,16,26). We chose to look at distribution of *S. rolfsii* MCG in peanut fields in Texas. Our results indicated that a rather small number of MCG exist within a single peanut field, with typically one MCG found on a single infected plant. On the other hand, an indefinite number of MCG exist within peanut fields throughout Texas. These observations may reflect the mode of reproduction and spread of *S. rolfsii* (e.g., lack of spores, sclerotial formation, and mycelial growth). MCG and VCG are considered genetic units in fungal populations, and accruing evidence suggests that these groups are often clonal (4, 11,14).

We hypothesized that isolates within a MCG would be more genetically similar than isolates in different MCG. To test this hypothesis, we examined the differences in the DNA from a subset of *S. rolfsii* isolates in 12 MCG isolated from four intensively sampled fields. We looked at the DNA amplification patterns of a random area of the genome using the primer NK2 and the ITS region of the ribosomal DNA (18,30) based on previous studies that suggested that randomly amplified DNA and restriction digests of the ITS region would be useful for such a purpose (7,8, 18,31). We observed three distinct NK2 amplification patterns (Fig. 3A). Significantly, all isolates within a MCG had the same NK2 amplification pattern (Fig. 2). For additional evidence that isolates in an MCG were genetically similar, we looked at the ITS region. Again, all isolates in an MCG had the same restriction digest pattern in the ITS region (Fig. 4). However, because the molecular markers used in this study were shared by several MCG (Table 2), we cannot prove that *S. rolfsii* MCG are clonal. Nevertheless, the identical DNA amplification patterns of isolates within a MCG, the morphological similarity of these isolates, the nature of hyphal fusion in *S. rolfsii* isolates in the same MCG (22), and the fact that all but 2 of 96 diseased plants were infected with isolates of the same MCG all support the hypothesis that these MCG are clonal. Possibly, those MCG placed in the same DNA amplification groups (Table 2) share a more recent ancestry, and an extended search for appropriate molecular markers may distinguish between these MCG.

Since the sexual stage of *S. rolfsii* is rare in nature and its role in the life cycle of the fungus is unknown, we suggest that genetic exchange in *S. rolfsii* is largely limited to isolates in the same MCG. This exchange would be further limited as new MCG arise. Previous studies have shown that mycelia of *S. rolfsii* isolates from different MCG can anastomose but lyse shortly thereafter, which may prevent nuclear and cytoplasmic exchange (22). We found that the original PCNB-tolerant isolate of *S. rolfsii* collected in 1985 constitutes the sole member of MCG 11 and that no other isolate in our collection maintains the same degree of PCNB tolerance (M.-Y. Shim and J. L. Starr, *unpublished data*). This suggests that this isolate had a unique MCG that may no longer be present in the field and that PCNB tolerance probably has not spread from this isolate.

One to three *S. rolfsii* MCG were found in the central Texas peanut fields. In studies of MCG in *Sclerotinia sclerotiorum*, Kohn et al. (11) found that one field had 26 MCG from 30 isolates, and another field had a total of 6 MCG from 33 isolates, with 2 MCG predominant. In our study, five MCG were identified from 66 isolates from one field, three MCG were identified from 148 isolates in Galen, three MCG were identified from 30 isolates in Eves, and one MCG was identified from 19 isolates in DeLeon, with

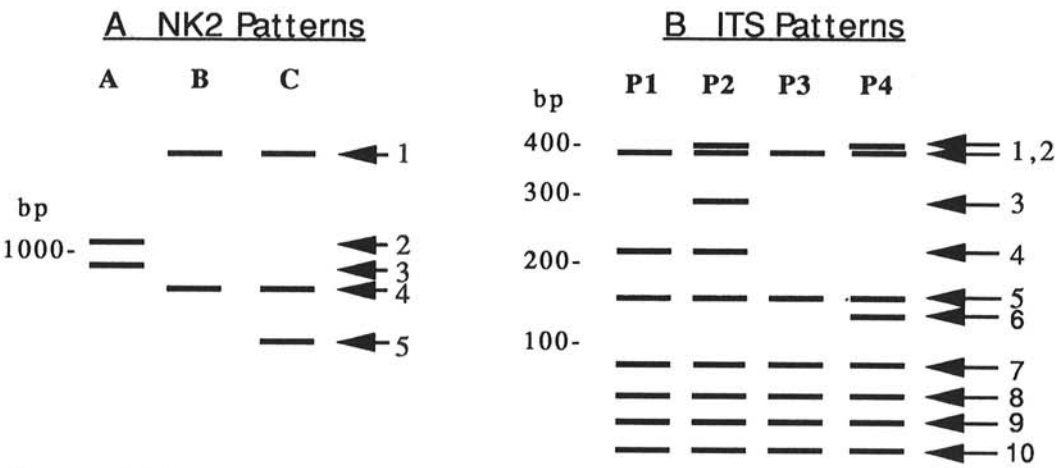

Fig. 3. Diagram of the scored DNA fragments of the three NK2 patterns and four internal transcribed spacer (ITS) region *Mbo*I digest patterns. **A,** polymerase chain reaction amplification patterns of *Sclerotium rolfsii* genomic DNA using oligonucleotide primer NK2. The scored DNA fragments are shown on the right: pattern A contains fragments 2 and 3, ~1,100 and 1,000 bp, respectively; pattern B contains fragments 1 and 4, ~1,500 and 900 bp, respectively; and pattern C contains fragments 1, 4, and 5, ~1,500, 900, and 700 bp, respectively. **B,** *Mbo*I restriction digests of the ITS region of *S. rolfsii*. The scored DNA fragments are shown on the right: pattern P1 contains fragments 2, 4, 5, 7, 8, 9, and 10; pattern P2 contains fragments 1, 2, 3, 4, 5, 7, 8, 9, and 10; pattern P3 contains fragments 2, 5, 7, 8, 9, and 10; and pattern P4 contains fragments 1, 2, 5, 6, 7, 8, 9, and 10. Fragments smaller than fragment 7 were shared by all isolates but were not always clearly visible due to their small size (Fig. 4).

MCG 6 predominant in three of these fields. Perhaps the selection for specific MCG is greater in the *S. rolfsii* population than that of *Sclerotinia sclerotiorum*, thus accounting for the smaller number of *S. rolfsii* MCG per field. Within the fields, the MCG appeared scattered within distinct disease foci, and several adjacent plants in the same focus were frequently infected with *S. rolfsii* isolates of different MCG. However, with the exception of 2 of 96 plants, all plants sampled had isolates that belonged to a single MCG, which suggests that the isolates found on a single plant may be clones.

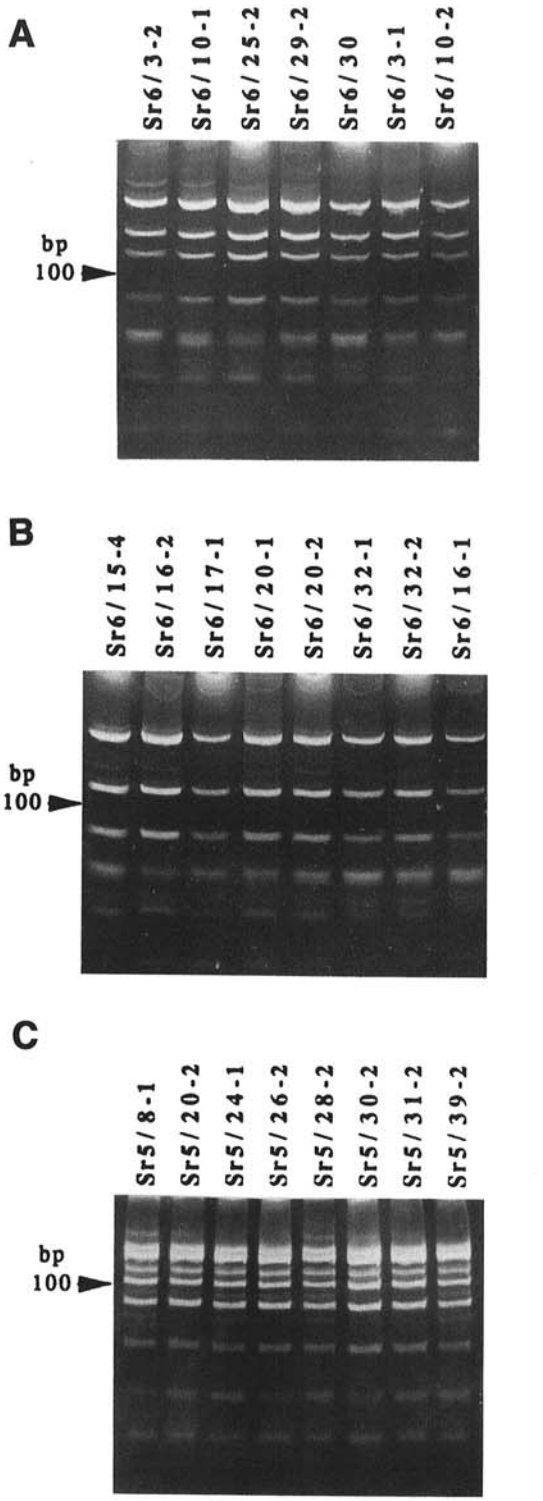

Fig. 4. Polyacrylamide gel electrophoresis of *Mbo*I restriction digests of polymerase chain reaction-amplified internal transcribed spacer (ITS) regions of *Sclerotium rolfsii* isolates. **A,** Isolates belonging to mycelial compatibility group (MCG) 1. **B,** Isolates belonging to MCG 12. **C,** Isolates belonging to MCG 6. The 100-bp marker is indicated on each polyacrylamide gel.

In a study designed to observe the change in VCG over time, Bayman and Cotty (4) found that *A. flavus* VCG changed considerably over a 3-year period in one cotton field. Although our sample size was not sufficient to thoroughly study the distribution or change in *S. rolfsii* MCG over time and space, we did find that 1993 isolates from Galen and Grissom belonged to the same MCG as the previous year even though the 1985 MCG 11 was no longer present. This suggests that MCG are fairly stable on a year-to-year basis. We also observed that some MCG were widely distributed in Texas. Because *S. rolfsii* produces no spores, the fungus most likely moves as mycelia and sclerotia from field to field via infected pods or equipment. Should *S. rolfsii* MCG prove to be a stable trait, it is possible that MCG may present an easily scored and novel means to track the role of man as a vector in transmitting this fungus.

The ITS region of *S. rolfsii* contained several different *Mbo*I restriction patterns (Table 2, Fig. 4); this observation also has been noted by Harlton et al. (8). In many organisms, the ITS region within a species is generally conserved (5,11,13), but recent reports have shown that the ITS region has diverged within some species (6,13,20). For example, sequence variation within the ITS 1 and 2 and the 5.8s ribosomal DNA gene among 86 isolates of *F. sambucinum* from diverse geographic locations was reported (20). Since *F. sambucinum* also has a predominantly asexual life history, it was hypothesized that the three ITS patterns corresponded to isolates of three asexual populations, among which sexual reproduction rarely occurred (20), although the relationship between these ITS patterns and VCG was not established. In our and Harlton's (8) studies, results showed that the *S. rolfsii* ITS *Mbo*I fragment sizes of certain MCG added up to more than the entire ITS region of ~680 bp. Harlton et al. (8) partially resolved this curiosity by generating basidiospore progeny from some of these *S. rolfsii* isolates. The basidiospore strains showed segregation patterns in relationship to their parental *Mbo*I restriction patterns, which suggested that the parental isolates were heterokaryons (8). Our preliminary DNA sequencing results (F. A. Nalim, M.-Y. Shim J. L. Starr, and N. P. Keller, *unpublished data*) suggest that ITS differences in the Texas isolates may reflect the presence of both homo- and heterokaryons in the field and even ITS heterogeneity within an isolate. Resolution of this issue will contribute to understanding the biology of *S. rolfsii* populations in peanut fields.

LITERATURE CITED

1. Anagnostakis, S. L. 1977. Vegetative incompatibility in *Endothia parasitica*. Exp. Mycol. 1:306-316.
2. Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. Annu. Rev. Phytopathol. 20:329-347.
3. Aycock, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii* or the status of Rolf's fungus after 70 years. N.C. Agric. Exp. Stn. Tech. Bull. 174.
4. Bayman, P., and Cotty, P. J. 1991. Vegetative incompatibility and genetic diversity in the *Aspergillus flavus* population of a single field. Can. J. Bot. 69:1707-1710.
5. Bruns, T. D., White, T. J., and Taylor, J. W. 1991. Fungal molecular systematics. Annu. Rev. Ecol. Syst. 22:525-564.
6. Buchko, J., and Klassen, G. R. 1990. Detection of length heterogeneity in the ribosomal DNA of *Pythium ultimum* by PCR amplification of the intergenic region. Curr. Genet. 18:203-206.
7. Duncan, S., Barton, J. E., and O'Brian, P. A. 1993. Analysis of variation in isolates of *Rhizoctonia solani* by random amplified polymorphic DNA assay. Mycol. Res. 97:1075-1082.
8. Harlton, C., Levesque, C. A., and Punja, Z. K. 1994. Genetic diversity and variation within *Sclerotium (Athelia) rolfsii* and related species. (Abstr.) Phytopathology 84:1149-1150.
9. Harrison, A. L. 1961. Control of *Sclerotium rolfsii* with chemicals. Phytopathology 51:124-128.
10. Karol, E. S., Lichtman-Pleban, D. Z. T., and Katan, T. 1993. Population structure of *Fusarium oxysporum* f. sp. *lycopersici*: RFLPs provide genetic evidence that VCG is an indicator of evolutionary origin. Mol. Plant-Microbe Interact. 6:565-572.

11. Kohn, L. M., Stavoski, E., Carbone, I., Royer, J., and Anderson, J. B. 1991. Mycelial incompatibility and molecular markers identify genetic variability in field populations of *Sclerotinia sclerotiorum*. Phytopathology 81:480-485.
12. Leach, J., Lang, B. R., and Yoder, O. C. 1982. Methods for selection of mutants and in vitro culture of *Cochliobolus heterostrophus*. J. Gen. Microbiol. 128:1719-1729.
13. Lee, S. B., and Taylor, J. W. 1992. Phylogeny of five fungus-like protoctistan *Phytophthora* species, inferred from the internal transcribed spacers of ribosomal DNA. Mol. Biol. Evol. 9:636-653.
14. Leslie, J. F. 1993. Fungal vegetative compatibility. Annu. Rev. Phytopathol. 31:127-150.
15. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
16. Michelmore, R. W., and Hulbert, S. H. 1987. Molecular markers for genetic analysis of phytopathogenic fungi. Annu. Rev. Phytopathol. 25: 383-404.
17. Nalim, F. A. 1995. A genetic study of mycelial compatibility groups of *Sclerotium rolfsii*. M.S. thesis. Texas A&M University, College Station.
18. Nazar, R. N., Hu, X., Scmidt, J., Culham, D., and Robb, J. 1991. Potential use of PCR-amplified ribosomal intergenic sequences in the detection and differentiation of Verticillium wilt pathogens. Physiol. Mol. Plant Pathol. 30:1-11.
19. Nelin, M. 1992. White mold in peanuts. Peanut Grower June:18-20.
20. O'Donell, K. 1992. Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum*. Curr. Genet. 22:213-220.
21. Punja, Z. K. 1988. *Sclerotium (Athelia) rolfsii*, a pathogen of many plant species. Pages 523-534 in: Advances in Plant Pathology. Vol. 6, Genetics of Plant Pathogenic Fungi. G. S. Sidhu, ed. Academic Press, London.
22. Punja, Z. K., and Grogan, R. G. 1983. Basidiocarp induction, nuclear condition, variability, and heterokaryon incompatibility in *Athelia (Sclerotium) rolfsii*. Phytopathology 73:1273-1278.
23. Punja, Z. K., and Grogan, R. G. 1983. Hyphal interactions and antagonism among field isolates and single-basidiospore strains of *Athelia (Sclerotium) rolfsii*. Phytopathology 73:1279-1284.
24. Punja, Z. K., and Rahe, J. E. 1992. *Sclerotium*. Pages 166-170 in: Methods for Research on Soilborne Phytopathogenic Fungi. L. L. Singleton, J. D. Mihail, and C. M. Rush, eds. The American Phytopathological Society, St. Paul, MN.
25. Punja, Z. K., Smith, V. L., Campbell, C. L., and Jenkins, S. F. 1985. Sampling and extraction procedures to estimate numbers, spatial pattern, and temporal distribution of sclerotia of *Sclerotium rolfsii* in soil. Plant Dis. 69:469-474.
26. Rosewich, U. L., and McDonald, B. A. 1994. DNA fingerprinting in fungi. Methods Mol. Cell. Biol. 5:41-48.
27. Shew, B. B., Beute, M. K., and Cambell, C. L. 1984. Spatial pattern of southern stem rot caused by *Sclerotium rolfsii* in six North Carolina peanut fields. Phytopathology 74:730-735.
28. Vilgalys, R. 1988. Genetic relatedness among anastomosis groups in *Rhizoctonia* as measured by DNA/DNA hybridization. Phytopathology 78:698-702.
29. Vilgalys, R., and Gonzalez, D. 1990. Ribosomal DNA restriction fragment length polymorphisms in *Rhizoctonia solani*. Phytopathology 80:151-158.
30. White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal DNA for phylogenetics. Pages 315-322 in: PCR Protocols: A Guide to Methods and Applications. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, San Diego, CA.
31. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingy, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6532-6535.