CHARACTERIZATION OF *Sclerotinia minor* POPULATIONS IN TEXAS

A Thesis

by

MERRIBETH ANNETTE HENRY

Submitted to the Office of Graduate Studies
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2006

Major Subject: Plant Pathology

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Approved by:

Chair of Committee,	Charles M. Kenerley
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ABSTRACT

Characterization of *Sclerotinia minor* Populations in Texas. (August 2006)

Merribeth Annette Henry, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Charles Kenerley

Agriculture is a crucial component of the economy of Texas with millions of pounds of peanuts, cotton, wheat, and corn produced annually. However, Texas agricultural crops are not exempt from pathogens, especially *Sclerotinia minor* Jagger, which was introduced into Texas approximately 25 years ago. A dramatic increase in *S. minor* disease incidence in the High Plains of Texas during 2004 provided the basis for this study of the pathogen populations in Texas. To characterize the *S. minor* populations in Texas, aggressiveness and fungicide sensitivity assays were conducted to assess phenotypic characteristics as well as the use of five microsatellite markers to genotypically characterize the pathogen. A large diversity among the populations was found for the phenotypic characteristics; however, there was no evidence that a genotypically unique, highly aggressive, and fungicide resistant "super pathogen" had been introduced or evolved.

The populations of *S. minor* in Texas were moderately aggressive (26.15% of infected tissue), but there were also isolates found that have the inability to infect peanuts (less than 3% of infected tissue) as well as highly aggressive pathogens with the ability to infect more an 55% of the leaflet surface.

All fungicides tested were effective in limiting the growth of the pathogen; however, there were significant differences in the effectiveness of the fungicides. Thiophanate-methyl and dichloran were the least effective fungicides in inhibiting the growth of *S. minor* while boscalid, iprodione, and fluazinam were the best. Fluazinam exerted the most lasting suppressive effect on pathogen. A positive correlation between aggressiveness and fungicide sensitivity to fluazinam and boscalid was found; therefore, no ecological tradeoff was found when increasing these two phenotypic characteristics.

Whereas extensive genotypic diversity (50 unique genotypes) was found in Texas, the predominate pathogen was a clone. Genotype TX1 was a clone that accounted for more than 48% of genotypes in Texas populations, identified in all of the sampled counties. The index of association demonstrated that there was a lack of gene flow occurring in the *S. minor* populations, therefore confirming that the pathogen reproduced primarily through mycelogenic germination.

DEDICATION

I would like to dedicate this thesis to my family, Stephen and Pat Henry, Melissa Huffman, Michelle, Blake, and Britton Davis, and my best friend, Andy Gammons, for their unfailing support and love.

ACKNOWLEDGEMENTS

I would like to express my extreme gratitude to my committee chair, Dr. Charles Kenerley, for his tireless reading and rereading of this manuscript and my committee members: Dr. Heather Wilkinson, Dr. Terry Wheeler, and Dr. Patricia Klein. I would also like to thank my colleagues in the laboratory: Aric Wiest and Slavica Djonovic, for their help, advice, laughter, and friendship, and Charles Greenwald for his computer expertise. Andy Gammon's help on this thesis has been crucial as he willingly traipsed through fields looking for samples.

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INTRODUCTION

Agriculture is a crucial component of the economy of Texas with millions of pounds of peanuts, cotton, wheat, and corn harvested annually. Texas is the second largest producer of peanuts in the United States with 775.5 million pounds produced annually (Wickwire & Johnson 2005). However, Texas agricultural crops are not exempt from pathogen destruction. Each year thousands of pounds are lost due to the peanut pathogens: *Sclerotinia minor, Cercospora arachidicola, Cercosporidium personatum, Puccinia arachidis*, *Leptosphaerulina crassiasca, Didymella arachidicola,* and Tomato Spotted Wilt Virus (Lee *et al.* 1995). Several of these pathogens only cause minor damage to the plants; by contrast, *S. minor* effectively kills the plant and decreases yield.

Sclerotinia minor Jagger is a necrotrophic, sclerotial-forming fungus with a broad host range. At least 96 plant species belonging to 21 families and 66 genera are suitable hosts for this pathogen (Melzer *et al.* 1997; Hollowell & Shew 2001; Melouk *et al.* 1992; Serini 1944). The pathogen was first described in 1920 as a "drop" pathogen of lettuce that formed small sclerotia (Jagger 1920), but it is also known as Sclerotinia blight, white mold, cottony rot, and drop (Kohn 1979; Agrios 1997). The pathogen is a member of the family Sclerotiniaceae, which was erected and deliminated into species in 1945 by Whetzel through the use of ascospore color, presence of functional conidial state, and type of conidia (Whetzel 1945). The family Sclerotiniaceae contains

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This thesis follows the format of the journal of *Molecular Ecology*.

inoperculate discomycetes of the order Helotiales within the phylum Ascomycota. (Kohn 1979; Alexopolous *et al.* 1996).

The pathogen *S. minor* produces melanized sclerotia, which are the primary dissemination propagules and can survive in soil up to 20 years. The mycelia form sclerotia primarily on the roots, stems, and crown tissues of host plants, such as peanuts, unlike *S. sclerotiorum* where sclerotia form on the aerial foliar tissue (Hao *et al.* 2003). To initiate mycelogenic infection, sclerotia germinate to produce a cottony, white dense mat of mycelia that invades the lower branches of the host and cause the cells of the host to collapse (Maxwell & Lumsden 1970; Lumsden 1979). Mycelogenic germination is favored by temperatures between 18-21^{o}C and greater than 95 percent humidity (Maxwell & Lumsden 1970; Hao *et al.* 2003; Porter & Melouk 1997). The mycelia will girdle the stem and create water-soaked lesions that eventually become necrotic. The pathogen also produces pathogenicity factors, oxalic acid, pectinases, cellulases, and hemicellulases that aid in tissue injury and wilt (Maxwell & Lumsden 1970).

Sclerotia of *Sclerotinia* spp. can also germinate carpogenically to produce apothecia and ascospores. Although *S. minor* rarely reproduces carpogenically through the production of sexual ascospores on apothecia, the ascospores do have the ability to infect a host wherever they land. Apothecia of *S. minor* can be produced *in vitro* by incubating the sclerotia for 6-8 weeks under a diurnal temperature regime of 8 hours at 15^{o}C and 16 hours at 10^{o}C in the dark (Hawthorne 1973). When carpogenic germination does occur, the pale orange to white apothecia have concave or flat tops measuring 6 mm or more in diameter, and they produce hyaline, ellipsoidal ascospores (Hawthorne

1973; Backman 1997; Hao *et al.* 2003). Carpogenic germination is typically not epidemiologically important on peanut crops because it normally occurs during winter fallow in peanut production areas (Porter & Melouk 1997; Wadsworth 1979). No apothecia or ascospores have been reported in Texas.

The most economically important hosts of the *S. minor* include: peanuts (*Arachis hypogaea* L.) (Porter & Beute 1974), sunflower (*Helianthus annuus*) (Sedun & Brown 1989), and lettuce (*Lactuca sativa*) (Beach 1921; Sereni 1944). *S. minor* was first reported on peanuts in Virginia in 1971, North Carolina in 1972, and Oklahoma in 1972, but it was not reported as a pathogen of peanuts in Texas until the fall of 1981 (Woodard & Simpson 1993). The original infestation occurred in a peanut field in Mason County, which is located in central Texas (Woodard & Simpson 1993). Since its introduction into Texas, it has also been identified on Texas Bluebonnets (*Lupinus texensis*), the state flower of Texas (Woodard & Newman 1993). More than 70% of the total peanut production occurs in the western region, which includes the High Plains. The pathogen was not introduced into the High Plains until approximately 10 years ago, where disease incidence remained low until the 2004 growing season (Terry Wheeler personal communication). An unexpected increase in the incidence of *S. minor* in commercial peanut fields in Gaines County occurred during 2004. One field we defined as E, which was geographically isolated from the other fields, on a four year crop rotation, had no previously reported infections by *S. minor*, but had 2000 disease foci appear within a 30 acre area during 2004. These disease foci were less than three feet in diameter and did appear to coalesce. Another field, which we defined as W, had no

previously reported cases of *S. minor* but is located in close proximity to fields with a known history of *S. minor*, was found to have 120 out of 120 acres infested with the pathogen during 2004. Fungicide applications with a combination of fluazinam and thiophanate-methyl did not halt the spread of the disease in field W. These dramatic increases in *S. minor* and the lack of response to fungicide applications elicited major concern that the evolution or introduction of a more aggressive and/or fungicide insensitive as well as genetically unique isolate had occurred. For this study, we define a "super pathogen" as a fungicide insensitive, highly aggressive, and genotypically unique pathogen. To be fungicide insensitive, the pathogen must demonstrate an EC50 that is twice the mean EC50 for isolates assed for the tested fungicide. For a highly aggressive pathogen, it must have the ability to effectively colonize more than 55% of the leaflet tissue in a standard leaflet assay. More than one isolate can be defined as a "super pathogen" if they meet the defined criteria. The concern that a "super pathogen" had evolved or was newly introduced initiated the current research to characterize *S. minor* isolates from the newly infested regions as well as from regions historically known to harbor the pathogen.

Most research on *S. minor* has focused on developing resistant plants or effective fungicides (Chappell *et al.* 1995; Livingstone *et al.* 2005; Cruickshank *et al.* 2002). There are only three main cultivars that exhibit moderate resistance to *S. minor* in peanut production (Chappell *et al.* 1995). Aggressiveness, ability to infect and colonize the host, studies on *S. minor* were conducted to screen new peanut cultivars for resistance (Hollowell *et al.* 2003). *S. minor* isolates from peanut plants in North Carolina of

varying levels of aggressiveness were arbitrarily chosen to screen new peanut cultivars for resistance (Hollowell *et al.* 2003). There has been no assessment of aggressiveness for a large collection of isolates of *S. minor.* Considering the geographic isolation of Texas from the other major peanut producing regions of the US and the unexpected increase in disease incidence, we evaluated the levels of aggressiveness in populations of *S. minor* in Texas.

Fungicides have been used to protect plants against infection by *S. minor* since the pathogen was first identified. Some fungicides provide effective suppression of growth of *S. minor* while others, such as chlorothalonil, may induce sclerotial germination of *S. minor* (Beute & Rodriguez-Kabana 1979). The fungicides that have been most effective for controlling *S. minor* include: iprodione (Rovral, Bayer Crop Science), thiophanate-methyl (Topsin M, Cerexagri, Inc), dicloran (Botran 75W, Gowan Company), boscalid (Endura, BASF), and fluazinam (Omega 500F, Syngenta). They can be applied prior to or after disease symptoms appear. Each of the fungicides has a different mode of action that attributes to its efficacy. Thiophanate-methyl is classified as a benzimidazole fungicide which inhibits DNA synthesis (Cerexagri, Inc). Iprodione, an inhibitor of DNA and RNA synthesis, cell division, and cell metabolism, is classified as a dicarboximide fungicide (Bayer Crop Science). Field resistance to dicarboxamide fungicides was first reported in *S. minor* from peanut in Virginia in 1987 (Brenneman *et al.* 1987) yet the fungicide is still used for control of *S. minor*. Boscalid, a respiration inhibitor, is classified as a carboxamide fungicide (BASF). Dichloran is a chlorinated

nitroaniline fungicide, which inhibits protein synthesis. Fluazinam, a pyridinamine fungicide, inhibits fungal respiration (Syngenta).

There are many different reasons that fungicides are no longer effective against the pathogens they are listed to control. Possible explanations for failure of the fungicides to control disease are: (i) resistant isolates of *S. minor* have developed with continued fungicide use; (ii) fungicide applications are not being timed properly and plants are too large at the time of application for effective coverage of the soil surface; and (iii) continued use of the fungicides has increased the population of soil microflora that rapidly degrade the chemicals (Marcum *et al.* 1977; Slade & Fullerton 1992; Hubbard & Subbarao 1997).

Measures of fungicide sensitivity and aggressiveness for large populations of *S. minor* in Texas are limited. Characterizing populations of *S. minor* isolated from the peanut production regions of Texas for fungicide sensitivity and aggressiveness was the approach taken to provide information to assist in explaining the appearance of new disease outbreaks. Also, fungicide sensitivity assays for a large population may provide an indication of fungicide performance in field situations. These data may be very useful for the management of future disease occurrences.

While it is crucial to understand the phenotypic characteristics of a population, it also important to understand the genetic structure. Population genetics is the study of genetic variation within and among populations, focusing on the processes that affect genotypic and allele frequencies at one or a few gene loci (Hartl & Clark 1997). Populations of eukaryotes, such as fungi and many higher organisms, as well as some

prokaryotes have been studied genetically to determine if diversity exists. Populations of many pathogenic fungi, such as *S. sclerotiorum, Aspergillus flavus* and *Phytophthora ramorum*, have been characterized genetically and phenotypically (Kohli *et al.* 1995, Sexton & Howlett 2004, Atallah et al. 2004, Tran-Dinh & Carter 2000, Ivors et al. 2006). In contrast, there have been few studies that emphasized the genotypic characterization of *S. minor* populations. The genotypic studies have been conducted using DNA fingerprints generated by a probe for a multicopy transposon-like element in Southern analyses (Kohli *et al.* 1992). These DNA fingerprints demonstrated genotypic differences but are not sufficient to address index of equilibrium, or Hardy-Weinberg equilibrium. However, the use of microsatellite markers provides allele sizes and frequencies that can be used to examine these basic measures of diversity. Genotypic diversity is the probability that two individuals taken at random have different genotypes. Genotypic differentiation allows you to determine if genotypic distribution is identical or different across populations. Index of association (I_A) is the traditional measure of multilocus linkage disequilibrium to determine if individuals being the same at one locus makes them more likely to be the same at another locus (Brown *et al.* 1980; Maynard Smith *et al.* 1993; Haubold *et al.* 1998; Agapow & Burt 2001).

Twenty-five microsatellite primer pairs have been developed that amplify unambiguous regions of DNA of *S. sclerotiorum* (Sirjusingh & Kohn 2001). Fourteen of these established microsatellites amplify regions in the isolates of *S. minor* that were tested (Sirjusingh & Kohn 2001). Microsatellites are simple sequence repeats consisting of di-, tri-, tetra-, or pentanucleotides and found widely dispersed in eukaryotes but less

frequently in prokaryotes (Chamber & MacAvoy 2000). While there is controversy among scientists over the exact number of repeats that are considered microsatellites, 2-8 bp repeats (Goldstein & Pollock 1997) or 1-5 bp repeats (Schlötterer 1998), most agree that microsatellites have less than 1 kb of nucleotide repeats (Chamber & MacAvoy 2000). Microsatellites are found in regions of 'cryptic simplicity' (Tautz 1986). The predominate mechanism that produces polymorphic microsatellites is the 'slipped-strand mispairing,' which occurs during DNA synthesis and causes the gain or loss of one or more repeat units (Levinson & Gutman 1987; Eisen 1999). Microsatellite loci have high mutation rates and a multi-allelic nature, which permit the identification of differences among closely related isolates as well as inferring phylogenetic relations (Carbone & Kohn 2001). The major advantages of microsatellite markers include that they are locus specific, codominant (heterozygotes can be distinguished from homozygotes), and highly polymorphic.

The use of microsatellites has been crucial in resolving genotypic diversity and evolutionary differences in populations of many different eukaryotic organisms as well as phylogeography and migration patterns. Eight microsatellite markers applied to isolates of *S. sclerotiorum* identified that genetic differentiation exists among populations in canola-growing regions of south-eastern Australia and suggested that genetic recombination through outcrossing is occurring in addition to clonal reproduction (Sexton & Howlett 2004). Interestingly in another study, the population diversity of *S. sclerotiorum* in the Columbia Basin of Washington was not correlated with pathologically important phenotypes (Atallah *et al.* 2004). *Phytophthora ramorum*

microsatellite markers identified that genotypic diversity was higher in nurseries than in forests, and there were three distinct clades from different evolutionary lineages (Ivors *et al.* 2006). Microsatellite markers have also been used to identify migration patterns among global populations of *Mycosphaerella graminicola* (Banke & McDonald 2005). Microsatellite and mitochondrial variation identified that the slight difference between Arctic-nesting subspecies *Grus Canadensis candensis* and non Arctic-nesting subspecies *Grus Canadensis tabida* may have been caused by post-glacial secondary contact (Jones *et al.* 2005). Endangered Cape Fear Shiners *Notropis Mekistocholas* were found to be less genetically impoverished than thought because of their small numbers through the use of microsatellite markers (Gold *et al.* 2004).

Understanding the genotypic structure and mating system of the pathogen is important for devising disease management and resistance-screening strategies (Sexton & Howlett 2004). As there are no reports of the genetic difference of *S. minor* populations based upon microsatellites, aggressiveness, or fungicide sensitivity, these three measures were combined to examine the level of diversity among a collection of isolates of *S. minor* from Texas. The data that was collected can serve as a guide to identify if any new isolates that have been introduced in Texas as well as demonstrate population characteristics that need to be considered for disease management strategies.

MATERIALS AND METHODS

Sampling Methods and Population Isolation

Isolates of *S. minor* were obtained from symptomatic peanut plants recovered from six production fields in Gaines county (High Plains), four production fields in Erath county, one production field in Comanche county (Central Texas), and one production field in Atascosa county (South Texas) (Fig. 1). Fields from these counties represent the major peanut production areas of Texas.

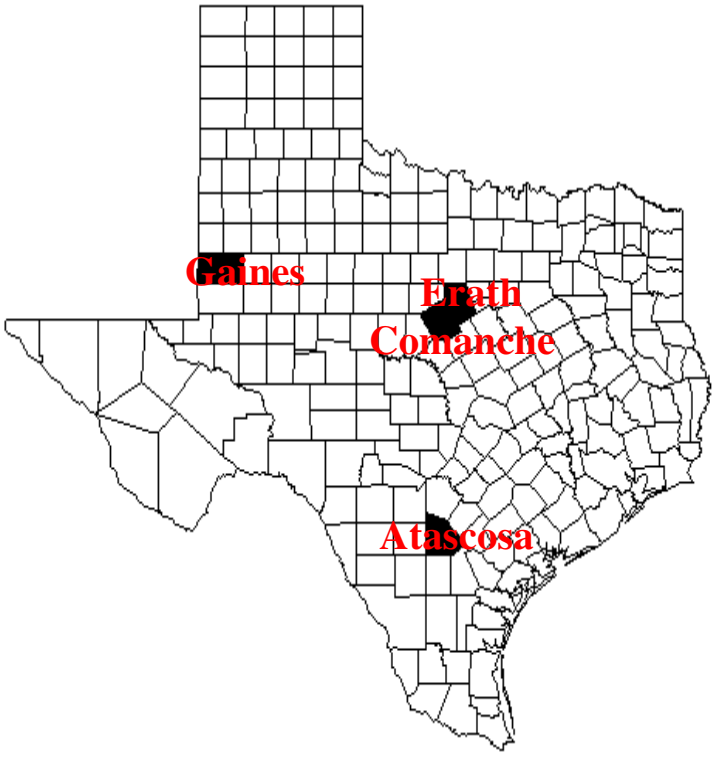

Fig. 1 Map of Texas counties illustrating the location of fields where peanut plants displaying symptoms of *Sclerotinia minor* were collected.

There are approximately 340 miles between Gaines and Erath county, 300 miles between Gaines and Comanche county, and 500 miles between Gaines and Atascosa county. Samples were arbitrarily collected from infested portions of peanut fields. Each sample included leaves and stem tissue from one infected plant; no roots were assayed for the pathogen. The samples were stored in plastic bags until assayed for the pathogen. Each plant sample was designated with a letter to identify the field and a number to indicate a unique sample. A total of 232 samples were assayed to obtain isolates of *S. minor* from the peanut production fields (Table 1).

Table 1 County, field designation, and number of isolates of *S. minor* assayed for aggressiveness and fungicide sensitivity.

Sampled Counties	Year Sample	Field Designation	Number of Isolates	Number of Isolates Selected For Fungicide Assay
Atascosa				
	2005	U	6	3
Comanche				
	2005	D	8	3
Erath				
	2004	F	6	2
	2004	L	5	2
	2004	P	6	2
	2004	T	9	6
Gaines				
	2005	A	10	3
	2005	B	20	3
	2004	E	84	24
	2005	H	18	3
	2004	J	12	6
	2004	W	48	9
Total Isolates			232	67

Plant stems from each sample were examined for as these propagules were easy to fiend in this tissue and yielded uncontaminated isolates in most cases. Sclerotia retrieved from each designated plant sample were surface sterilized in a 50:50 (vol:vol) mixture of 95% ethanol to bleach for 30 seconds and then rinsed in distilled water. The treated sclerotia were plated on potato dextrose agar (PDA) amended with streptomycin (100 mg/liter) and incubated at 25^{o}C. Unsuccessful attempts to isolate the pathogen due to contamination by bacteria or other fungi were addressed by treating another sclerotia from the same symptomatic plant sample with a longer sterilization period. Following sclerotial germination, mycelial plugs from the rapidly expanding colony were transferred with a number three cork borer to unamended PDA. After 5-9 days of incubation at 25^{o}C, newly-formed sclerotia were harvested, and stored in 15 ml conical tubes at 4^{o}C for future use.

Leaflet Aggressiveness Assay

A leaflet lesion assay modified from Hollowell *et al.* 2003 was conducted to assess the aggressiveness of the Texas isolates. Cultivar Tamrun96 peanut seeds, a *S. minor* susceptible variety, were planted 1 inch into moistened Metromix potting soil in pots (4 inch diameter) and incubated in a growth chamber with 50% humidity at 25^{o}C. The pots were covered with cellophane for 3 days to help maintain high humidity and increase seed germination. The plants were grown for three weeks, watered every two days with 300 mls of distilled water, and fertilized once a week with 100 mls of Peters Fertilizer (Scotts Fertilizer).

The second leaflets of 3-week-old plants were excised and placed in sterile glass petri dishes with moistened filter paper (Fisherbrand Filter Paper P8). Four leaflets of uniform size were added to each petri dish, were uniformly misted with 1.5 ml of sterile distilled water from a spray gun (Preval), and inoculated with a 1 mm mycelial plug from the advancing edge of 3-day-old PDA cultures. Each leaflet within a petri dish was inoculated with a mycelial plug from a different isolate, which was placed facedown on each leaflet in the petri dish. Inoculation by each isolate was replicated on ten leaflets. The petri dishes were randomized, stored in sealed containers with moistened paper towels and beakers of water to maintain 100% humidity, and incubated at 21^{o}C in the dark for 48 hours. An example of a typical experiment unit is illustrated in Fig. 2. Following incubation, each infected leaflet was imaged using a scanner (Visioneer) and saved as a tiff file.

The tiff file of the infected leaflet was used to assess the area of each leaflet and any lesion using ImageJ (Abramoff *et al.* 2004). The measurements of the area of the leaflet and lesion were compiled into an Excel file, which was used to perform statistical analysis.

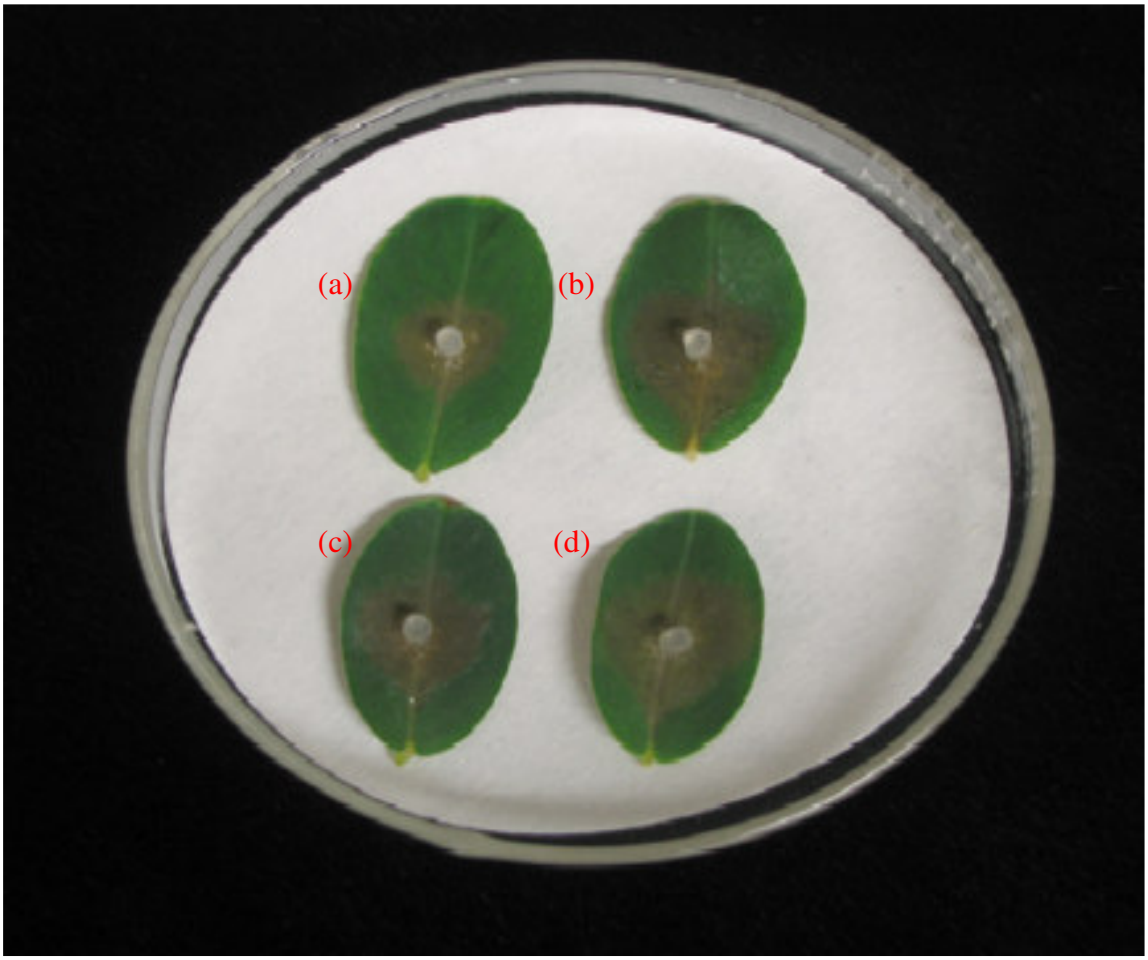

Fig. 2 Peanut leaflet assay used to demonstrate varying levels of aggressiveness. The leaflets are inoculated with isolates (a) E880 (b) L3 (c) J16 (d) E1850.

Fungicide Sensitivity Assay

The 67 isolates chosen for the fungicide sensitivity were a subset of the 232 isolates tested for aggressiveness. There were at least two isolates from each field sampled included in this assay. The isolates were chosen randomly through a computer generated data set. The fungicide sensitivity of the 67 isolates to iprodione (Rovral, Bayer Crop Science), thiophanate-methyl (Topsin M, Cerexagri, Inc), dichloran (Botran 75W, Gowan Company), boscalid (Endura, BASF), and fluazinam (Omega 500F, Syngenta) was determined by a modification of the spiral gradient dilution method (Förster *et al.* 2003). Each fungicide was tested at concentrations 0.01, 0.1, 1.0, 5, and 10 µg/ml of active ingredient (a.i.) in agar plates (Matheron & Porchas 2004) with isolates E880, J4, L7, and T1-3 in order to determine the most appropriate range for the spiral gradient application. The formula weight of each fungicide and the optimal range of a.i.were entered into the Spiral Gradient Endpoint software (Advanced Instruments), which determined the concentration of active ingredient required for fungicidal activity (Table 2).

Table 2 Each fungicide was tested at distinct concentration of a.i.

Fungicides	Concentration of a.i. (µg/ml)
Thiophanate-methyl	0.10-10.00
Dichloran	0.07-9.00
Iprodione	0.01-1.800
Fluazinam	0.01-1.50
Boscalid	0.01-3.00

The fungicides were measured, suspended in 10 ml of sterile distilled water, and vortexed to create uniform solutions. The fungicide solutions were applied in an Archimedes' spiral (Fig. 3) onto plates of PDA (50 ml) using the Autoplate 4000 (Advanced Instruments) and allowed to diffuse for 4 hours prior to applying the inoculum. The highest concentration of fungicide was applied at the center of the plate with a diminishing gradient towards the outside edge.

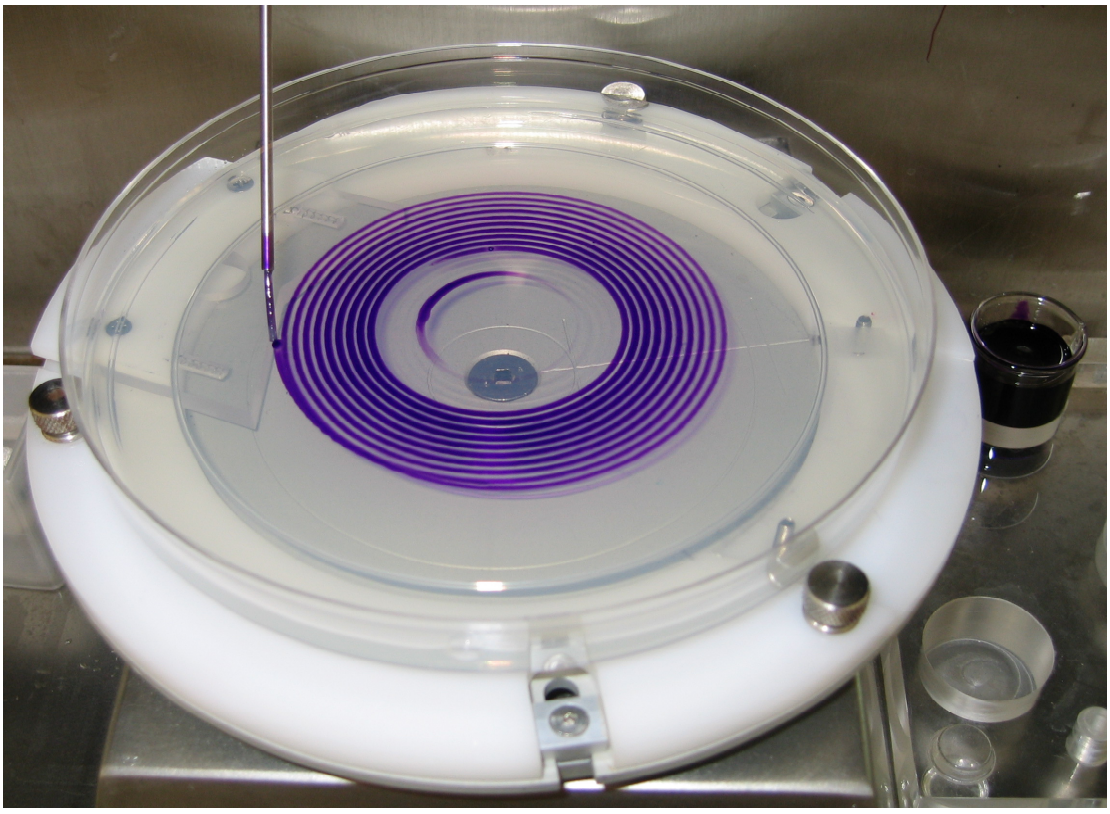

Fig. 3 Application of fungicides to 15 mm PDA plates in a concentration gradient as an Archimedes spiral as seen with crystal violet.

To produce uniform inoculum, six sterile filter paper strips (3-4 mm) (Fisherbrand Filter Paper P8) were placed concurrently with a mycelial plug of each isolate onto PDA and incubated for 2-3 days at 25°C. Four uniformly colonized strips of the different isolates were applied to each fungicide spiral plate (Fig. 4). There were five repetitions of each isolate for each fungicide.

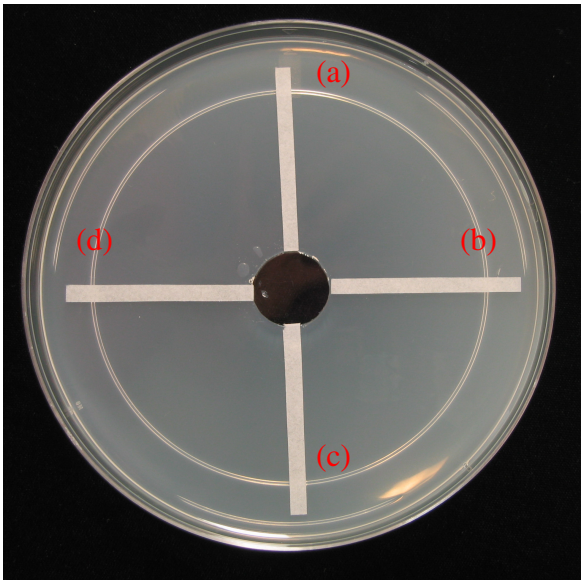

Fig. 4 Typical fungicide amended plate to which four different isolates of *S. minor* have been applied as colonized filter paper strips. The inoculated isolates are (a) E769 (b) W15 (c) H22 (d) U5.

Control plates for each isolate (five repetitions per isolate) were grown on 10 cm plates of unamended PDA. All plates were randomized and incubated in sealed containers at 21°C for 48 hours. The unamended plates were not intermixed with the fungicide plates in the storage containers to avoid fungicide contamination. The unique

effective concentration 50 (EC50) for each fungicide and isolate combination was determined first by measuring the radial growth of each isolate on the control plate (Fig. 5a, orange arrow). Digital calipers (Mitutoyo) were used all measurements. The location corresponding on the fungicide plate to half of the radial growth on the control plate was determined and named 50% inhibition (Fig 5b, green arrow). The radial distance between center of the fungicide plate and the 50% inhibition location were then measured (Fig. 5b, purple arrow) and entered into the Spiral Gradient Endpoint software as the ending radius (ER). The software uses the ending radius and information of the fungicide to calculate EC50 for each isolate.

After the measurements were completed, the fungicide plates were returned to the incubator for an additional 15 days to assess further growth and sclerotial production.

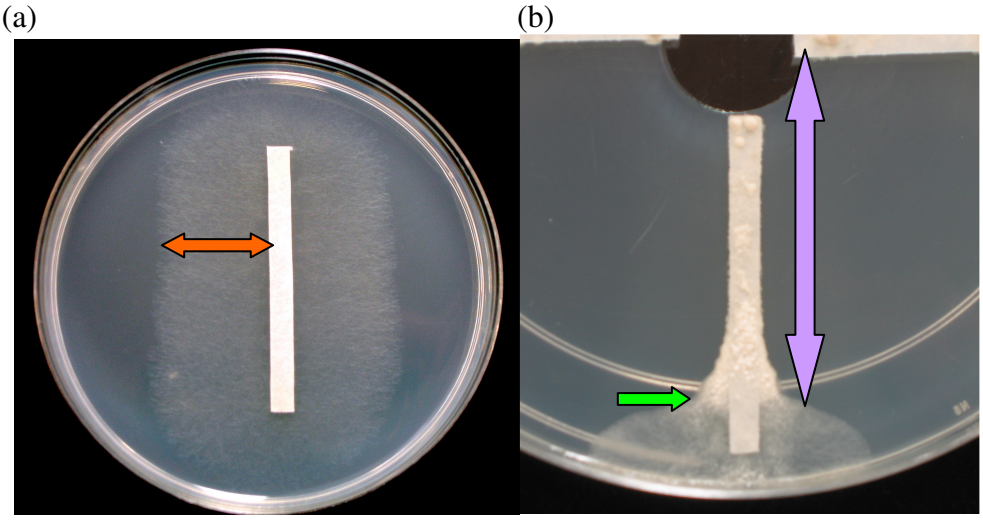

Fig. 5 Measurement of the growth of isolates to determine EC50. (a) The red arrow indicates that the radial growth on a *S. minor* isolate on an unamended PDA plate. (b) The green arrow represents where 50% inhibition occurs on the fungicicide plate. The purple arrow represents the distance between the 50% inhibition and the center of the fungicide plate, which was entered into SGE to calculate the EC50 of the fungicide.

Genotypic Characterization of S. minor *Populations in Texas*

Each fungal isolate obtained from an individual sclerotium was grown on a PDA plate for three to four days. Then a mycelial plug was transferred into a 50 ml liquid culture of glucose, yeast extract, and casein hydroxylate and shaken for 4-6 days at 145 rpm (Xu *et al.* 1996). The fungal tissue was then filtered, washed, and frozen in liquid nitrogen before being lyophilized. A small-scale phenol-chloroform extraction protocol (Grzegorski 2001) was used to extract DNA from the lyophilized tissue.

Genotypic differences among the *S. minor* isolates were assessed by using microsatellites. Fourteen polymorphic microsatellite primer pairs (Operon) developed by Sirjusingh and Kohn 2001 (Table 2) were tested to determine if they amplified microsatellite regions within four isolates of *S. minor*,E880, J4, L7, and T1-3, from diverse locations within Texas. Polymerase Chain Reaction (PCR) amplification mixtures (25 µL) contained 10 ng of DNA, 200 µM of each dNTP, 0.2 µM of each primer, 2.5 µL of 10x buffer (NEB) and 0.1 unit of Taq polymerase (NEB). Amplifications were performed in a Perkin-Elmer GeneAmp system 9700 Thermocycler (Applied Biosystems) programmed for initial denaturation at 95^{o}C for 4 min followed by 30 cycles of denaturation at 95^{o}C for 45 sec, primer annealing at 48 to 55^{o}C for 1:30 sec; and extension at 72^{o}C for 2:45 sec, with a final 5 min extension after the last cycle. The products were run on a 1.5% agarose gels stained with ethidium bromide and visualized with an Alpha Imager to determine if amplification occurred.

The forward primers from the five primer pairs that consistently produced amplification products were subsequently ordered with HEX and FAM fluorescent tags

(Table 3). These fluorescent primer pairs were used with the same PCR amplification cycles but with an essential final extension time of 30 minutes. The products were run on a 1.5% agarose gels stained with ethidium bromide and visualized with an Alpha Imager to determine if amplification occurred. The amplification product was diluted 1 µl of product to 50 µl of water. One µl of diluted amplification product was combined with 11 µl of size standard solution [10 µl of formamide to 1 µl of 500 Rox size standard (Applied Biosystems)], analyzed on an ABI PRISM 3130 Sequencer (Applied Biosystems), and allele sizes were estimated using the GeneMapper software (Applied Biosystems).

After all allele sizes were assessed to each locus, genotypes were assigned for each unique allele combination. A tab deliminated ASCI file was created that scored each isolate for the presence or absence of an allele, 1 or 0. This file was used to calculate linkage disequilibrium using MultiLocus (Agapow & Burt 2001); the physical linkage of the tested markers is unknown. The multilocus index of association (I_A) was tested by comparing the observed IA with the expected IA under a random mating hypothesis. The null distribution was calculated using 1000 randomizations of the individual alleles with the 13 (12 populations in Texas and one outgroup population from Brazil) defined populations as well as without the populations defined. The proportion of compatible pairs of loci was compared with the proportions in 1000 randomizations of the samples and a one-tailed P-value was generated. Loci were defined as compatible if all observed genotypes were created by mutations rather than recombination (Agapow & Burt 2001).

Table 3 Sequence of primer pairs tested for amplification of polymorphic loci of *Sclerotinia minor.*

Locus/GenBank Accession	Repeat Motif +	Primer Sequence (5` to 3`)*
5-2	$(GT)_8$	F: [HEX] GTAACACCGAAATGACGGC
AF377900		R: GATCACATGTTTATCCCTGGC
20-3	$(GT)_7GG(GT)_5$	F: [FAM]
AF377912		GACGCCTTGAAGTTCTCTTCC
		R: CGAACAAGTATCCTCGTACCG
36-4	$CA_6(CGCA)_2CAT_2$	F: [HEX] GAATCTCTGTCCCACCATCG
AF377914		R: AGCCCATGTTTGGTTGTACG
114-4	$(AGAT)_{14}(AAGC)_4$	F: [HEX] GCTCCTGTATACCATGTCTTG
AF377923		R: GGACTTTCGGACATGATGAT
117-4	$(TAC)_6C(TAC)_3$	F: [HEX] TCAAGTACAGCATTTGC
AF77924		R: TTCCAGTCATTACCTACTAC
6-2	$(TTTTTC)_2$ $(TTTTTG)_2$	F: GGGGGAAAGGGATAAAGAAAAG
AF377901	(TTTTTC)	R: CAGACAGGATTATAAGCTTGGTCAC
11-2	$(GA)_6GG(GA)_6(GGGA)_2$	F: CTTTCCTTTCGTTTGAGGG
AF377905		R: GGCAGGTAATGTTGCTTGG
17-3	$(TTA)_9$	F: TCATAGTGAGTGCATGATGCC
AF377911		R: CAGGGATGACTTTGGAATGG
23-4	$(TG)_{10}$	F: CTTCTAGAGGACTTGGTTTTGG
AF377913		R: CGGAGGTCATTGGGAGTACG
42-4	GA_9	F: GGTCTCATACAGTCTACACACA
AF377916		R: CTCTAGAGGATCTGCTGACA
55-4	$TACA_{10}$	F: GTTTTCGGTTGTGTGCTGG
AF377918		R: GCTCGTTCAAGCTCAGCAAG
99-4	$(GTAA)_2(GCAA)(GTAA)_3$	F: CTCATTTCATCCCATCTCTCC
AF377926		R: AATTCAAGCCTTCCTCAGCC
106-4	$(CATA)_{25}$	F: TGCATCTCGATGCTTGAATC
AF377921		R: CCTGCAGGGAGAAACATCAC
110-4	$(TATG)_9$	F: ATCCCTAACATCCCTAACGC
AF377922		R: GGAGAATTGAAGAATTGAATGC

+ Repeat motif and the number of times repeated in the genome of *S. sclerotiorum*.
* Fluorophores (HEX or FAM) used for labeling forward primer are specified with the primer sequence (The first five primer pairs in the table).

To calculate genotypic diversity and genotypic differentiation, a file that included the allele sizes and genotypes was input into GENEPOP 3.3 (Raymond & Rousset 1995). Genotypic diversity, probability that two individuals taken at random have different genotypes, was calculated using MultiLocus 1.2 (Agapow & Burt 2001) and GENEPOP 3.3 (Raymond & Rousset 1995). To test for differentiation within genotypes, an unbiased estimate of the P-value of a log-likelihood (G) based exact test was performed (Goudet *et al.* 1996).

A maximum parsimony tree representing the relationships among *S. minor* microsatellite genotypes in this study was inferred using PAUP 4.0b (Swofford 2002), set to parsimony criteria and using the heuristic search option with 1000 replicates of random step-wise addition.

Statistical Analysis

An analysis of variance (ANOVA) was performed to determine the significant differences ($P \geq 0.01$) among isolates for aggressiveness and fungicide sensitivity, differences in mean aggressiveness of the isolates among the counties, and differences among and within fungicides for EC50. Tukey's mean separation test was conducted among treatments for significant ANOVAs. Bartlett's test for homogeneity was performed to test for equal variances among the mean aggressiveness values for fields and counties. As the mean aggressiveness values for fields were found to be nonhomogenous, the Kruskal-Wallis rank test for significance was performed with these data. Linear regression was used to determine the relationship between fungicide sensitivity and aggressiveness for the 67 matched isolates.

RESULTS

Sampling Methods and Population Isolation

S. minor isolates were obtained from all of the 12 peanut fields sampled (Table 1). The Gaines county fields were designated: A, B, H, E, J, and W. The Erath county fields were designated: F, L, P, and T. The Atascosa county field was designated U. The Comanche county field includes D. Fields E, J, W, F, L, P, and T were sampled during the 2004 production season, and Fields A, B, H, U, and D were sampled during the 2005 production season.

Leaflet Aggressiveness Assay

The analysis of variance indicated highly significant differences ($P \leq 0.01$) in aggressiveness among the 232 *S. minor* isolates (Table A1). In general, the distribution of aggressiveness of all of the isolates tested was bell-shaped but skewed towards the tail with smaller categories (Fig. 6). The mean percent of infected tissue was 26.15%. The least aggressive pathogens were J12 and E1095, only infecting 0.79% [±0.06 standard error (S.E.)] and 0.84% (±0.07) of the leaflet surface, respectively and, the most aggressive pathogens were E1410, W125, and E1750 infecting 58.44% (±0.75 S.E.), 60.23% (±0.76 S.E.), and 61.64% (±1.71 S.E.) of the leaflet surface, respectively (Table B1).

Each field population of *S. minor* was found to differ significantly ($P \leq 0.01$) in aggressiveness (Tables A2-A13). When plotted as frequency categories, several distinct patterns were found among fields (Fig. 7). Very few isolates had the ability to infect more than 45% of the leaflet surface; therefore, creating distribution graphs that were skewed right. Field B, D, and J exhibited bell-shaped distributions with the distribution was skewed right, below 25% of infected tissue. The bell-shaped distribution of fields E and W was also skewed to the right with more isolates having aggressiveness below 35% of infected tissue. The field H had a clumping of isolates with aggressiveness between 25-45% of infected tissue. Field L and P had uniform distributions of isolates with aggressiveness between 25-45% and 20-40% of infected tissue. Fields F, U, and T did not exhibit a distinct pattern in their distributions, more samples were needed to

explain the distribution in these fields. Field A showed a distribution that was skewed right with the majority of the isolates below 20% of infected tissue.

As there were unequal numbers of isolates obtained from each field, Bartlett's test for homogeneity of variances was conducted prior to performing an ANOVA. Bartlett's test for homogeniety (Table 4) indicated significant differences in variances among the populations from fields but not significant differences in variances among counties (Table 5). ANOVA (Table A14) indicated significant differences among the county populations with mean aggressiveness of each county unique (Fig. 8). Isolates from Atascosa county were the most aggressive pathogens with a mean percent of infected tissue of 38.34%. The Erath county population had the mean aggressiveness of 30.813%, and the Gaines county population had the mean aggressiveness of 25.44%. The least aggressive pathogens were found in Comanche county, only having the ability to infect 18.92%. The Kruskal-Wallace rank test indicated that there were significant differences among the field populations for aggressiveness and a visual representation of the mean aggressiveness for each field can be seen in Fig. 9.

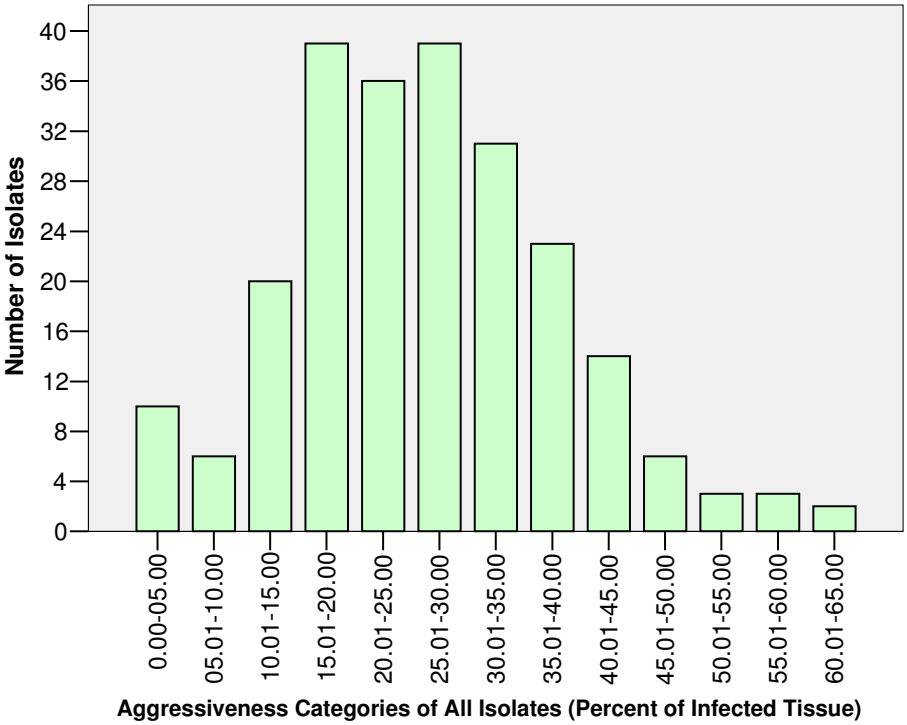

Fig. 6 Frequency distribution of aggressiveness for isolates of *S. minor* in Texas.

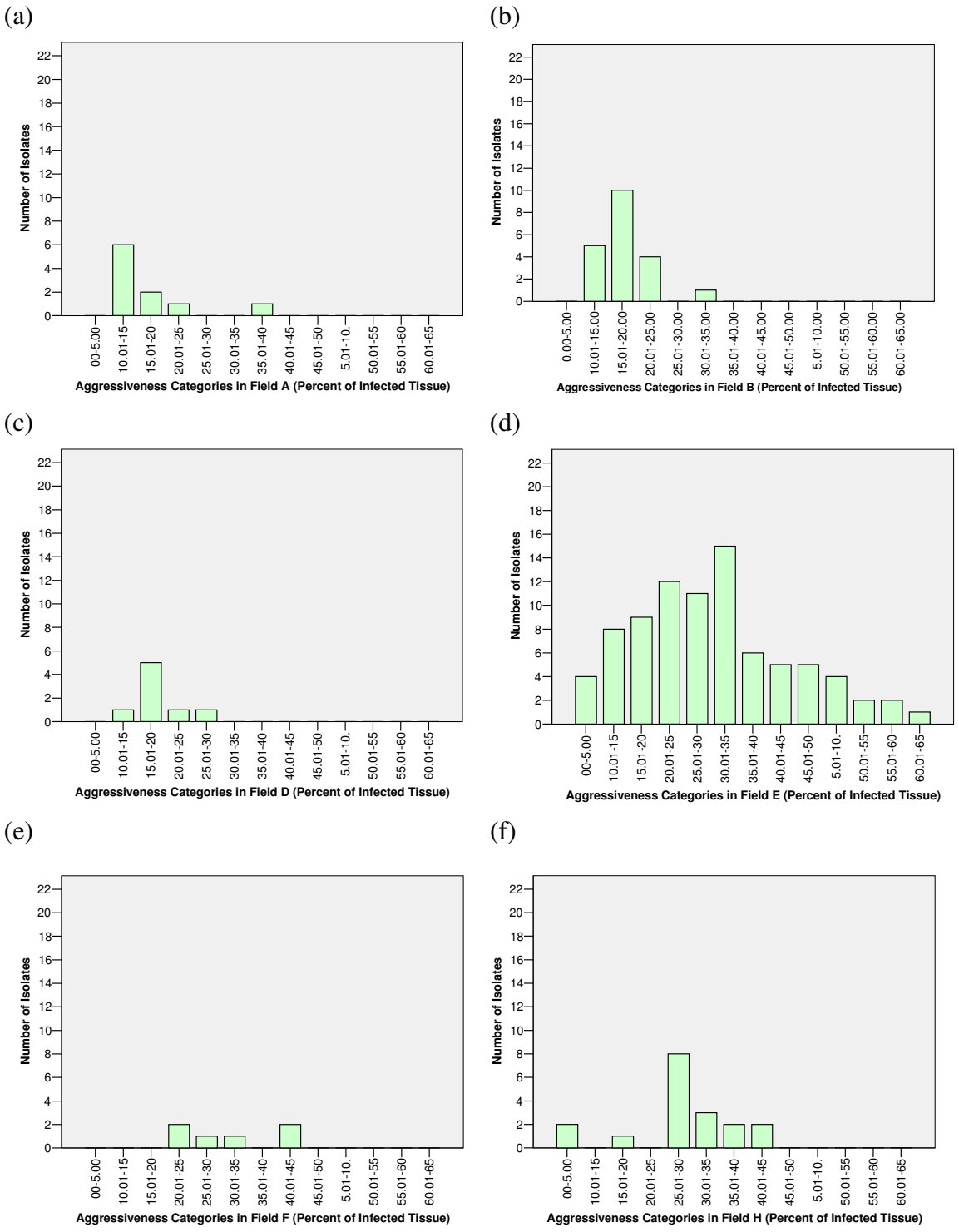

Fig. 7 Frequency distribution of aggressiveness for *S. minor* isolates within each field. Graphs represented are (a) Field A (b) Field B (c) Field D (d) Field E (e) Field F (f) Field H.

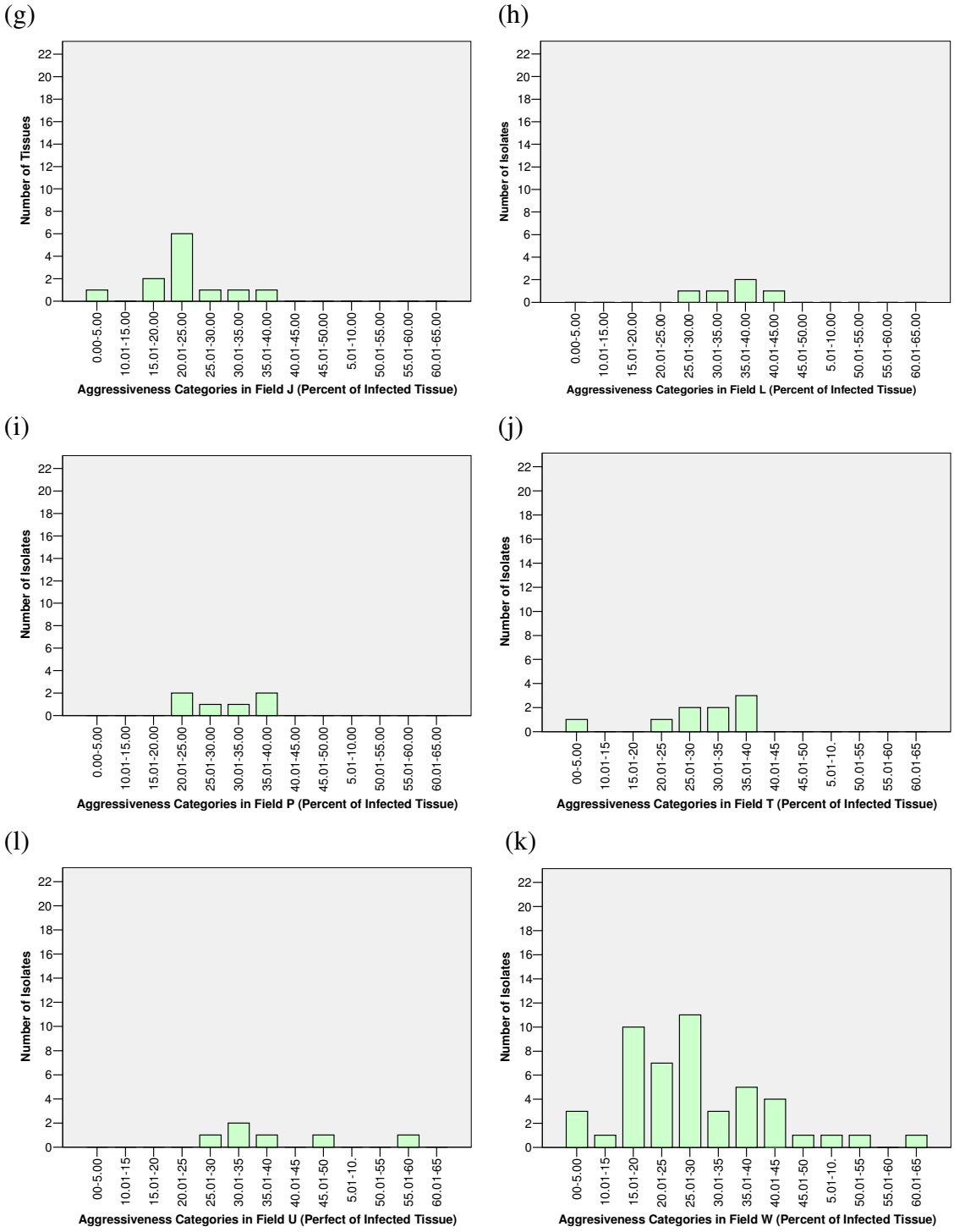

Fig. 7 continued The graphs represented are (g) Field J (h) Field L (i) Field P (j) Field T (k) Field U (l) Field W.

Table 4 Bartlett's test for homogeneity among fields.

Field	No. of Isolates	Sum of Squares ($f_i s_i^2$)	Degrees of Freedom (f_i)	Mean Square (s_i^2)	$f_i \log s_i^2$	$f_i \log s_i^2$	$1/f_i$
A	10	0.599	9	0.067	-1.174	-10.565	0.111
B	20	0.410	19	0.022	-1.658	-31.494	0.053
D	8	0.165	7	0.024	-1.620	-11.339	0.143
E	84	14.798	83	0.178	-0.750	-62.215	0.012
F	6	0.412	5	0.082	-1.086	-5.431	0.200
H	18	1.784	17	0.105	-0.979	-16.640	0.059
J	12	0.870	11	0.079	-1.102	-12.126	0.090
L	5	0.160	4	0.04	-1.398	-8.388	0.167
P	6	0.201	5	0.04	-1.398	-6.990	0.200
T	9	0.930	8	0.116	-0.936	-7.484	0.125
U	6	0.541	5	0.108	-0.967	-4.833	0.200
W	48	7.022	47	0.153	-0.815	-38.320	0.021
Total	232	27.892	220	1.014		-215.824	1.381
$\check{s}_i^2$	0.127						
$\sum \log \check{s}_i^2$	-197.327						
M*	36.1519						
C^	1.044						
X^2	34.620	d.f=11					

M* represents $2.3026f(\log \alpha \log \check{s}_i^2 - \sum f_i \log s_i^2)$
C^ represents $1 + (\alpha + 1)/3 \alpha f$
X^2 represents Chi-Square

Table 5 Bartlett's test for homogeneity among counties.

Field	No. of Isolates	Sum of Squares $f_i s_i^2$	Degrees of Freedom f_i	Mean Square s_i^2	$f_i \log s_i^2$	$f_i \log s_i^2$	$1/f_i$
Atascosa	6	540.921	5	108.184	2.034	10.171	0.200
Comanche	8	165.021	7	23.574	1.372	9.607	0.143
Erath	26	1933.682	25	77.347	1.888	47.211	0.040
Gaines	192	27980.459	191	146.495	2.166	413.672	0.005
Total	232	30620.083	228	355.600	7.461	480.661	0.388
$\check{s}_i^2$	134.298						
$\sum \log \check{s}_i^2$	485.200						
M*	10.452						
C^	1.043						
X^2	10.025						

M* = $2.3026f(\log \alpha \log \check{s}_i^2 - \sum f_i \log s_i^2)$
C^ = $1 + (\alpha + 1)/3 \alpha f$
X^2 represents Chi-Square

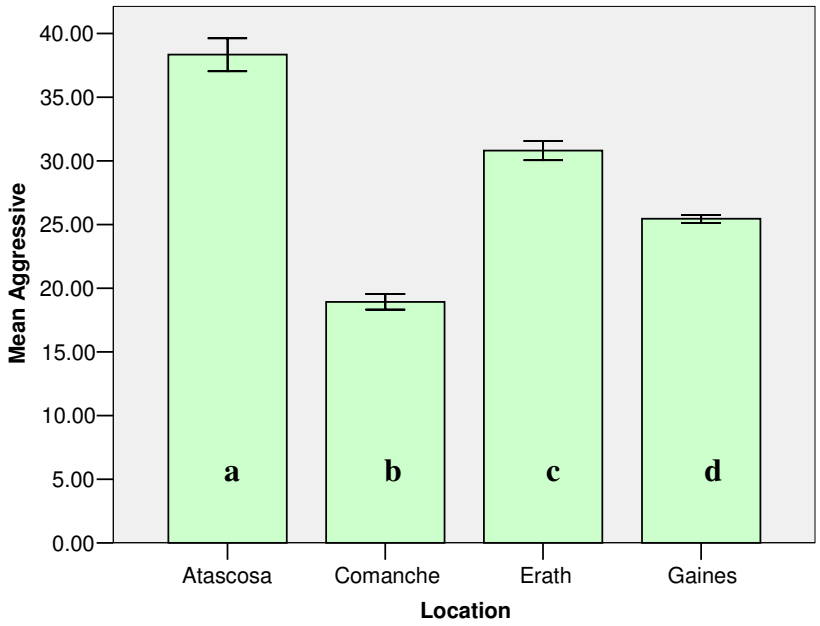

Fig. 8 Mean aggressiveness for each county sampled. Columns with a letter in common did not differ significantly according to Tukey's mean separation tests at a significance level of 0.01%; the error bars are ± one standard error.

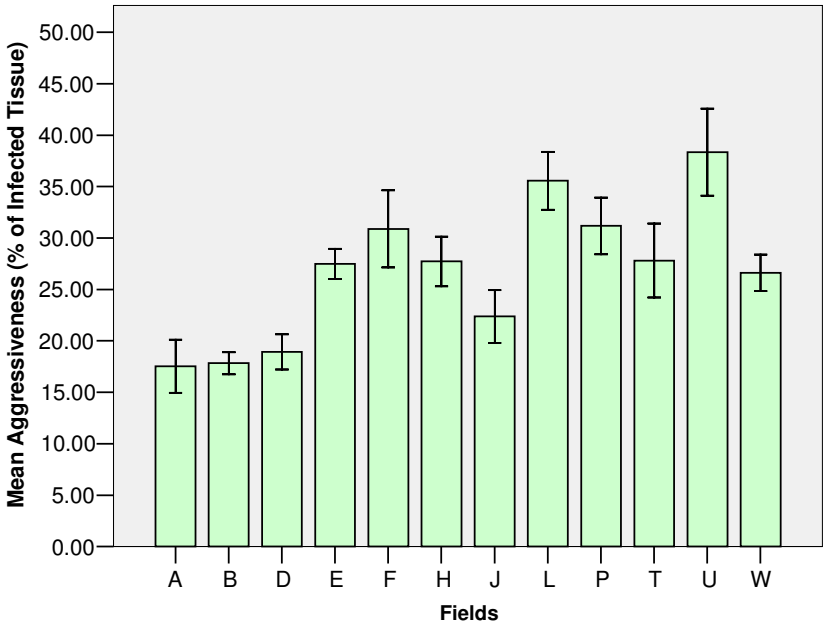

Fig. 9 Mean aggressiveness for each field sampled. The error bars are ± one standard error.

Fungicide Sensitivity Assay

The fungicides were all effective in reducing the growth of the pathogen over the levels tested after 48 hours of incubation. The isolates produced characteristic growth patterns on each fungicide gradient plate at the concentrations tested (Fig. 10). The plates amended with fluazinam and boscalid caused the inoculated isolates to produce gradient or gradual growth patterns. Plates amended with dichloran, thiophanate-methyl, and iprodione resulted in threshold response pattern where the fungal growth abruptly stopped where the EC50 occurred.

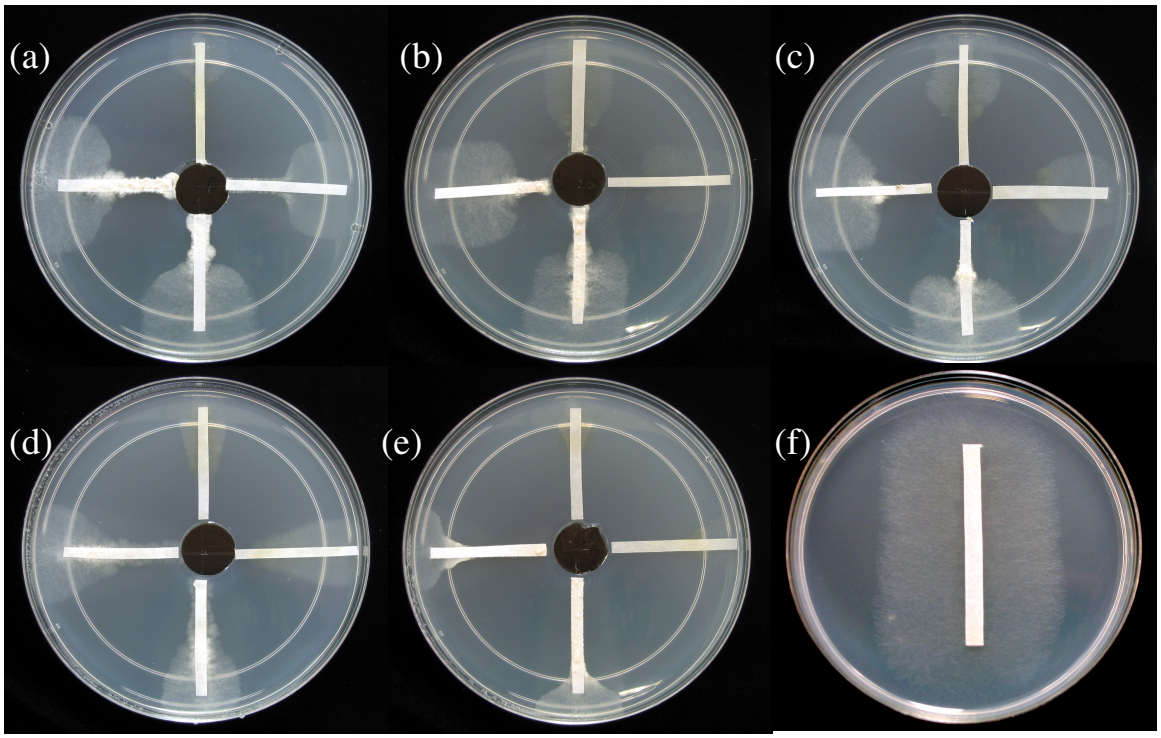

Fig. 10 Characteristic growth patterns of *S. minor* on PDA amended with a concentration gradient of a fungicide after 48 hours incubation. The fungicide plates are amended with only one fungicide (a) thiophanate-methyl (b) dichloran (c) iprodione (d) boscalid (e) fluazinam. Each fungicide plate was inoculated with isolates E769, W15, H22, and U5 (starting at the top and moving clockwise). Plate (f) is the unamended control, which was inoculated with H22.

Highly significant differences ($P \leq 0.01$) were demonstrated among the isolates to the fungicides tested and among the individual fungicides (Table A15-A19). These different patterns of response to the individual fungicides by the isolates can be seen in Figs.11 and 12. Average EC50 values were similar for boscalid, fluazinam, and iprodione but were significantly lower than thiophanate-methyl and dichloran (Fig. 13, Table A20). The mean EC50 for thiophanate methyl was 2.52 ±0.03 µg/ml, dichloran was 1.03±0.01 µg/ml, iprodione was .114 ±0.001 µg/ml, fluazinam was 0.091±0.001 µg/ml, and boscalid was .065 ±0.000 µg/ml.

When the mean EC50 values were determined among the isolates from each county and field, the greatest range was within thiophanate-methyl and dichloran (Fig. 14, 15). Iprodione, boscalid, and fluazinam were the most effective in inhibiting growth and the average response of the isolates to these fungicides appeared to have the smallest range of EC50 values (Figs. 14 and 15).

Linear regression identified that aggressiveness and the log EC50 of boscalid and fluazinam were positively correlated (Fig. 16a, b) at $P \leq 0.01$; therefore, an increase in aggressiveness was seen with an increase in EC50. Thiophanate-methyl, iprodione, and dichloran were not found to be correlated with aggressiveness (Fig. 16c, d,e).

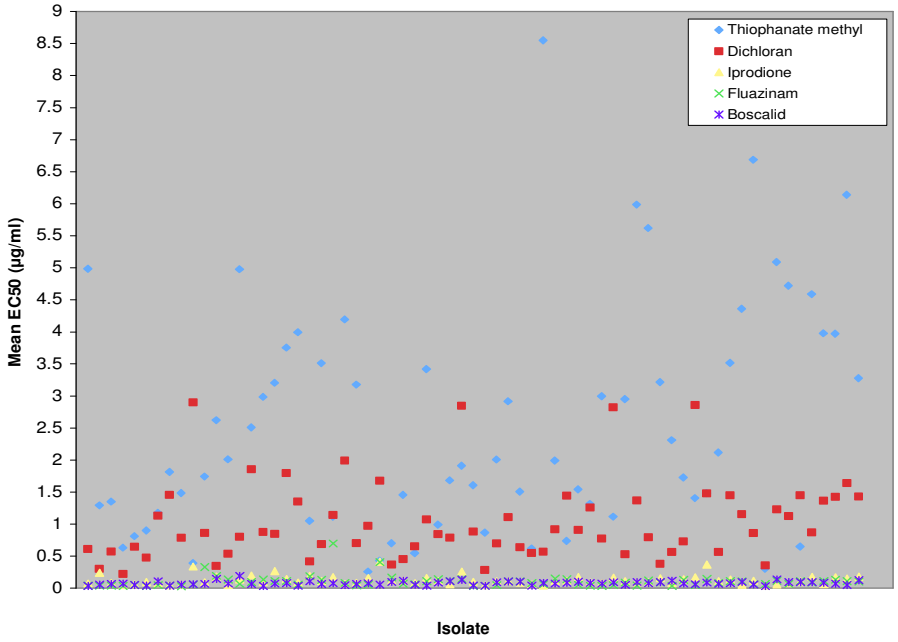

Fig. 11 Sensitivity among isolates of *S. minor* to five commercial fungicides.

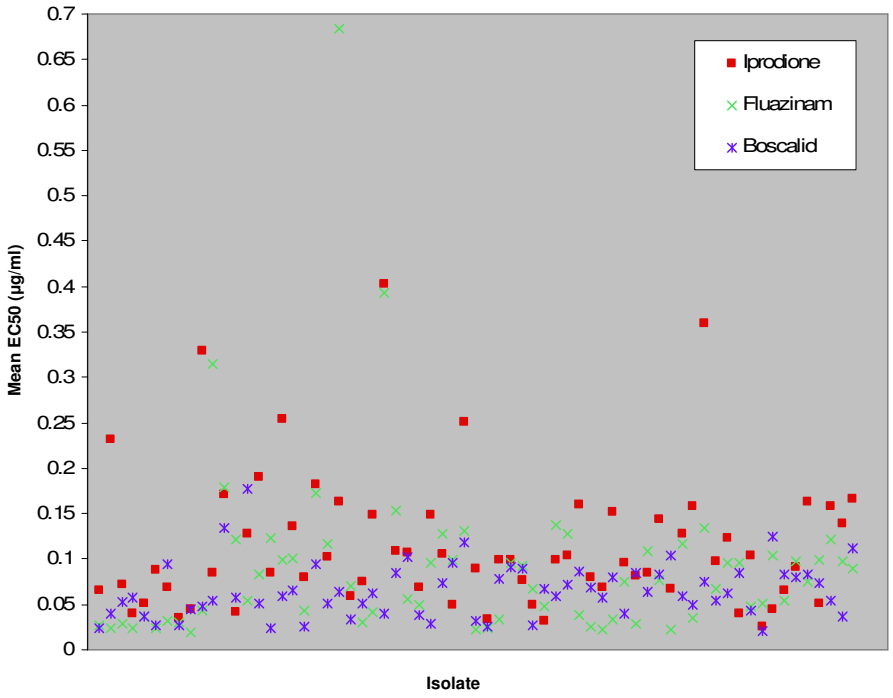

Fig. 12 Sensitivity among isolates of *S. minor* to iprodione, fluazinam, and boscalid with a range of 0.00 to 0.70 µg/ml of a.i.

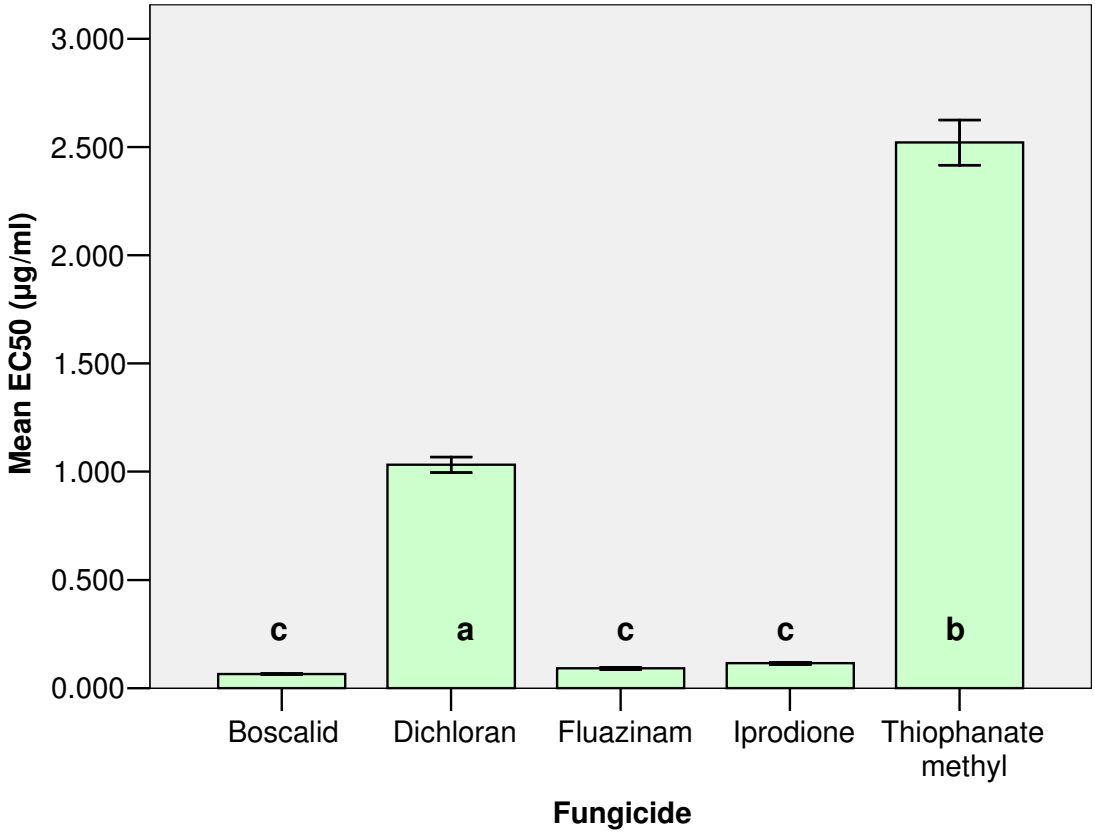

Fig. 13 The mean EC50 of sixty-seven isolates of *S. minor* for five commercial fungicides. Bars represent the 95% confidence interval and columns with a letter in common did not differ significantly according to Tukey's mean separation test at a significance level of 0.01%.

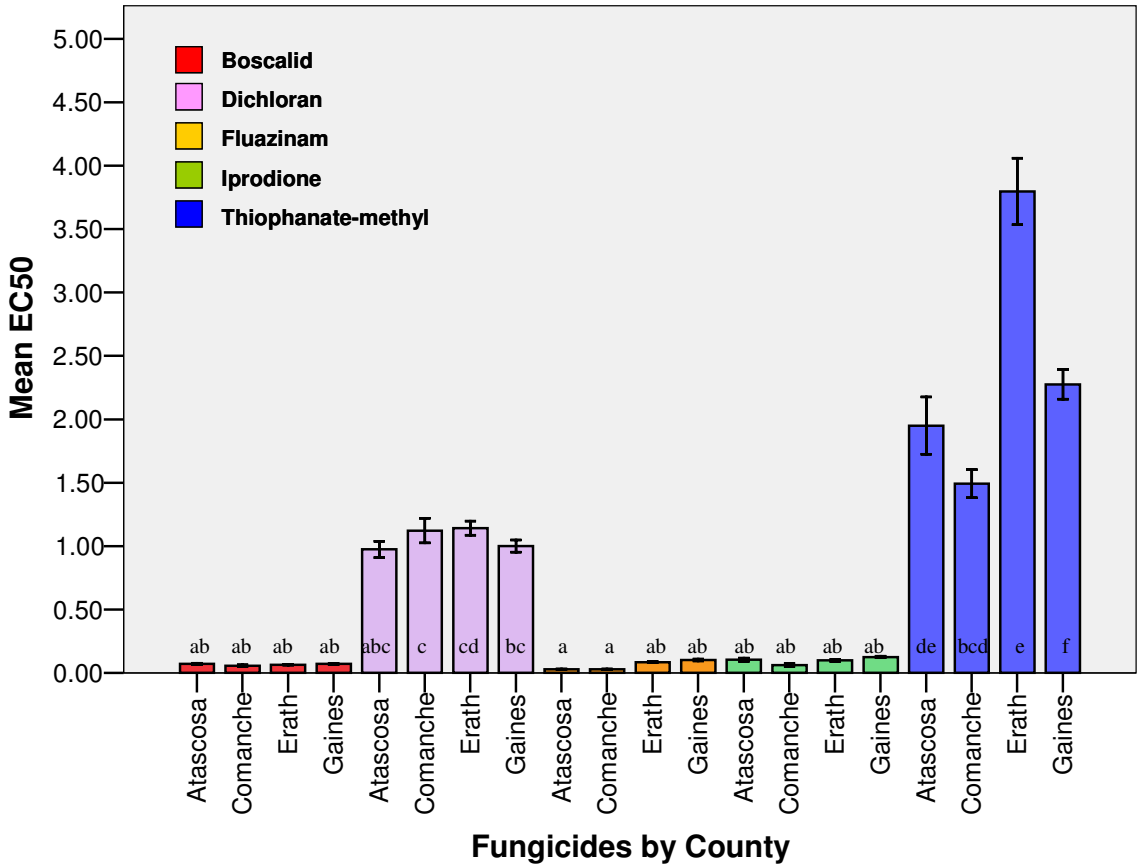

Fig. 14 The mean EC50 for five commercial fungicides among *S. minor* populations from four counties in Texas. Bars represent ± one standard error and counties within a fungicide with a letter in common did not differ significantly according to Tukey's mean separation test at a significance level of 0.01%.

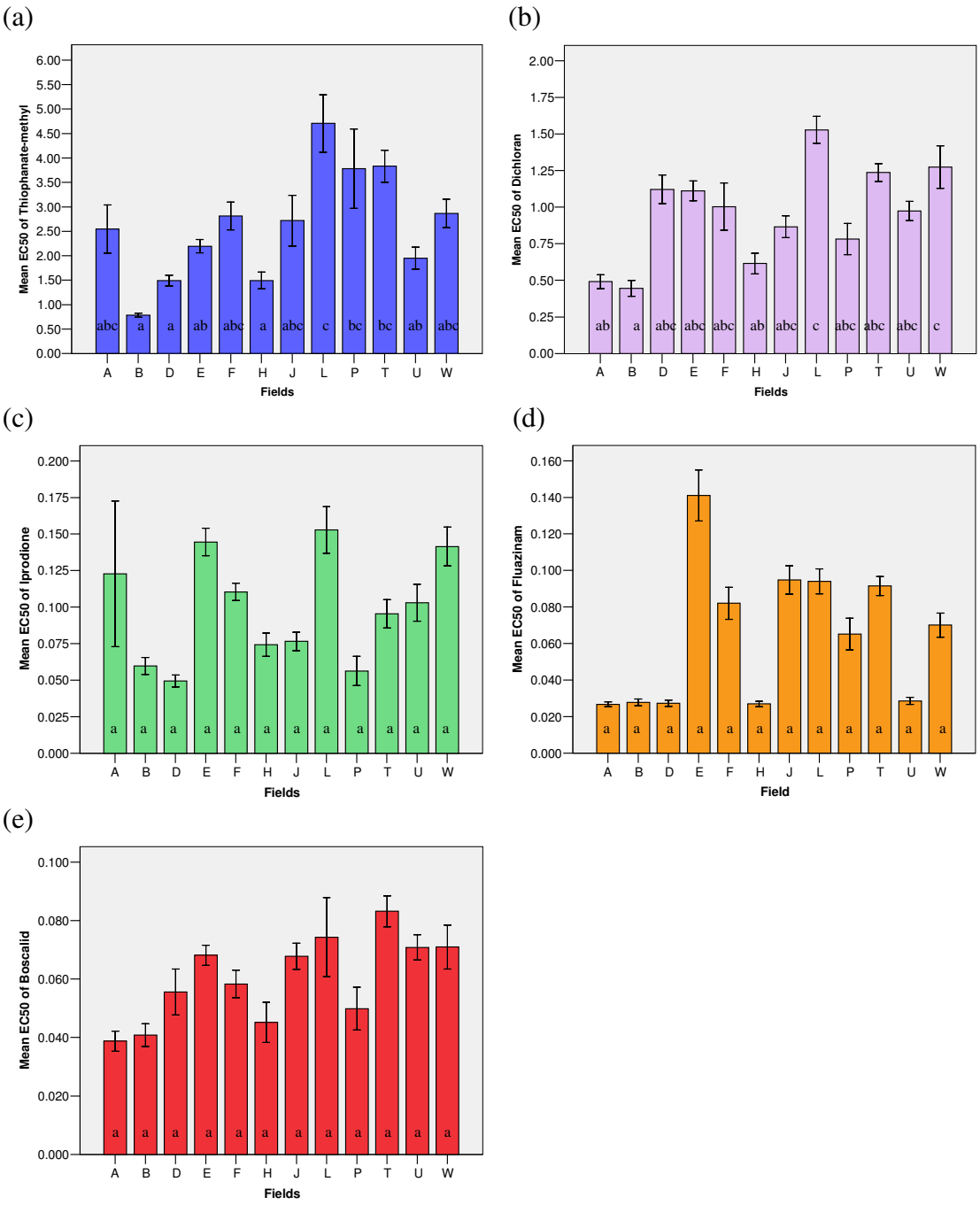

Fig. 15 The mean EC50 for five commercial fungicides among *S. minor* populations from 12 fields in Texas. Bars represent ± one standard error and columns with a letter in common did not differ significantly according to Tukey's mean separation test at a significance level of 0.01%.

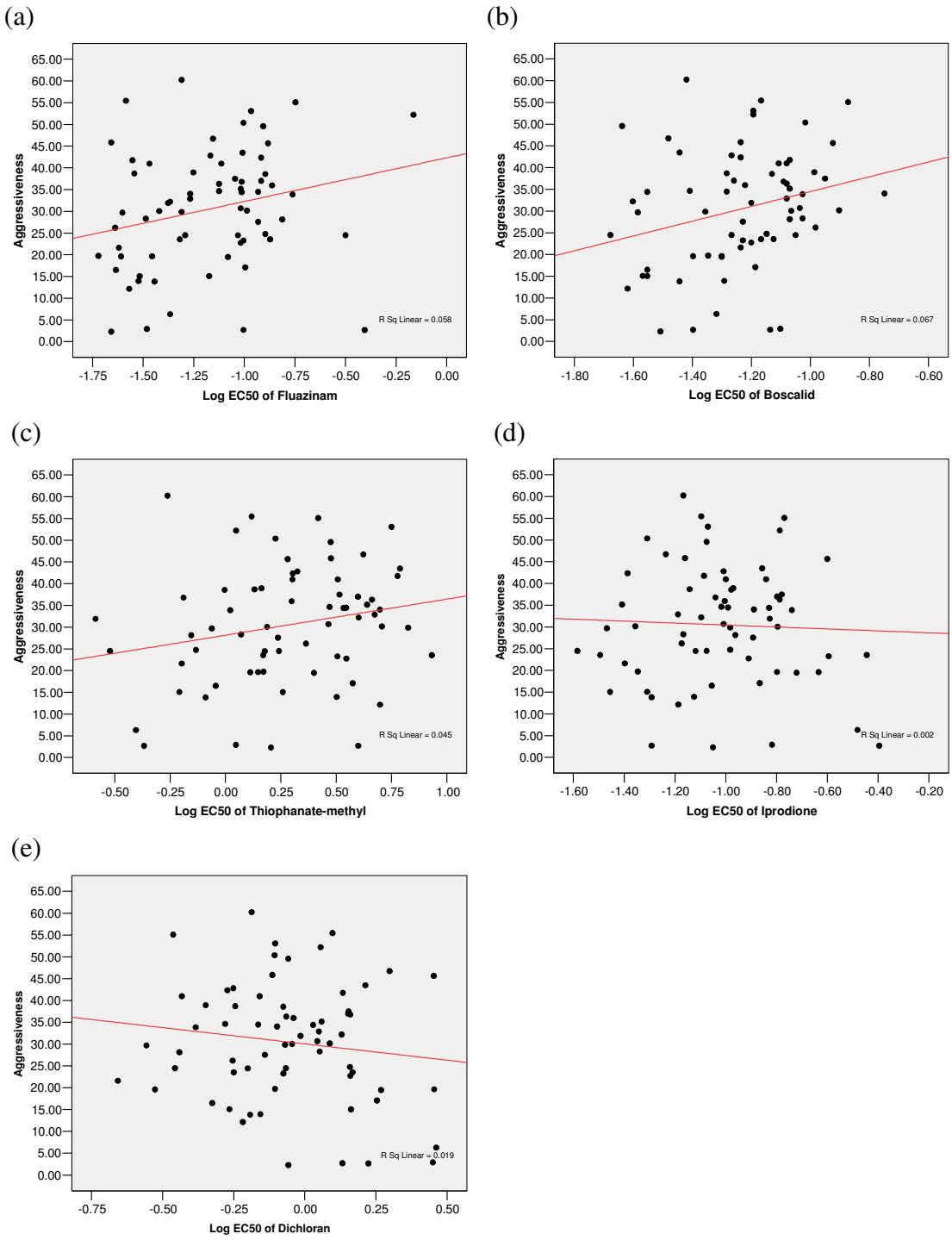

Fig. 16 Linear regression analysis of aggressiveness and the log EC50 of each fungicide.

After two weeks of growth on the fungicide amended plates, all strains produced sclerotia (Fig. 17). The fungicide amended plates containing fluazinam produced the smallest number of sclerotia as a consequence of the most limited growth of *S. minor*. Boscalid, thiophanate-methyl, iprodione and dichloran produced large numbers of sclerotia with the pathogen colonizing the highest portion of the plate.

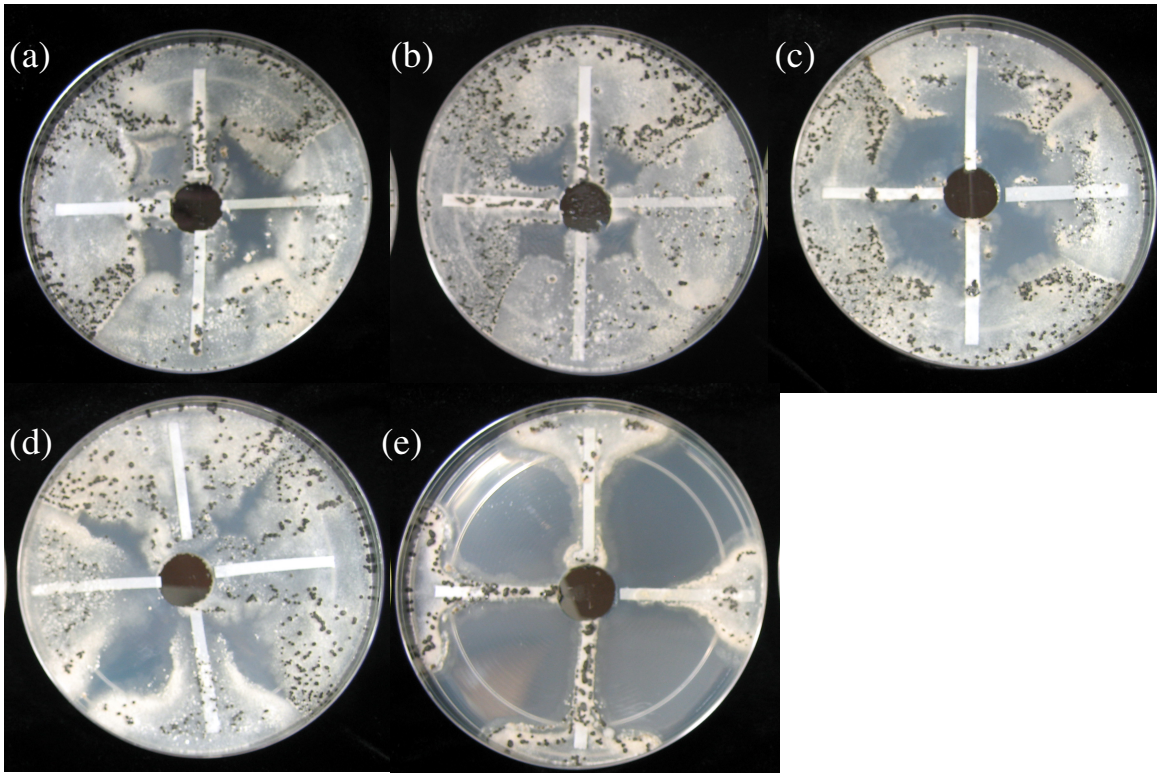

Fig. 17 Characteristic growth patterns and sclerotial production of *S. minor* on PDA amended with a concentration gradient of a fungicide after two weeks incubation. The plates are amended with one fungicide (a) thiophanate-methyl (b) dichloran (c) iprodione (d) boscalid (e) fluazinam. Each fungicide plate was inoculated with isolates E769, W15, H22, and U5 (starting at the top, rotating clockwise).

Genotypic Characterization of S. minor *Populations in Texas*

Of the 14 fourteen polymorphic microsatellite primer pairs tested, five consistently produced an aplicon with our samples. These five polymorphic loci, 5-2, 20-3, 36-4, 114-4 and 117-4 (Sirjusingh & Kohn 2001) were then selected for genotyping the collection of *S. minor* isolates. Microsatellite alleles were determined for 232 isolates as well as 4 isolates from Brazil at the 5 loci using primers labeled with FAM or HEX (Table 2).

Microsatellite allele sizes from all isolates were scored by visualizing chromatograms in GeneMapper (Table C1). The number of alleles observed at each locus ranged from four to seven, with the largest number of alleles identified at loci 117-4. The calculated allelic frequency for each allele ranged from 0.0038 to 0.9662 (Table 6). Each unique genotype was designated with a name and resulting in the identification of 50 genotypes from Texas and one genotype from Brazil (Table 7). The predominate genotype, TX1, consisting of 108 isolates, was designated a clone, defined in this study as five or more isolates with the same genotype. TX1 was found in every population in Texas except in fields L and P; both of these fields are located in Erath county. The five isolates from Brazil were a single unique genotype, being unique at all loci except loci 20-3 and 114-4.

Table 6 Allelic frequency at each microsatellite locus of *S. minor*.

Loci	Alleles	Frequency
5-2		
	302	.9325
	304	.0380
	298	.0506
	300	.0127
	301	.0253
	305	.0084
20-3		
	298	.6667
	297	.2574
	302	.1266
	300	.0464
	284	.0211
	286	.0253
36-4		
	429	.9451
	408	.0127
	407	.0169
	350	.0042
	420	.0211
	428	.0211
144-4		
	350	.9662
	351	.0169
	336	.0038
	349	.0127
117-4		
	377	.0211
	387	.9536
	388	.0084
	386	.0169
	368	.0042
	372	.0042
	370	.0042

The allele sizes were visualized in chromatograms and the allelic frequency was calculated from number of allele divided by the total number of alleles.

Table 7 Genotype differences among populations of *S. minor* in Texas.

Genotype *	Isolate	Locus 5-2	Locus 20-3	Locus 36-4	Locus 144-4	Loci 117-4
TX1 (45.76%)	Clone (108 TX isolates)	302/-	298/-	429/-	350/-	387/-
TX2 (14.41%)	Clone (34 TX isolates)	302/-	297/-	429/-	350/-	387/-
TX3 (3.81%)	Clone (9 TX isolates)	302/-	298/302	429/-	350/-	387/-
TX4 (1.27%)	A3 A15 A20	302/-	298/-	429/-	350/-	388/-
TX5 (1.69%)	B1 E1730 H7 F10	302/-	302/-	429/-	350/-	387/-
TX6 (0.42%)	B8	304/-	297/-	429/-	350/-	386/-
TX7 (0.42%)	B16	302/-	297/298/302	429/-	351/-	387/-
TX8 (0.42%)	B22	302/-	297/-	429/408/407	350/-	387/-
TX9 (0.42%)	B25	302/-	297/-	408/-	350/-	387/-
TX10 (0.42%)	E865	302/-	298/300	429/-	350/-	387/-
TX11 (2.11%)	E880 E935 E955 E1360 E1660	302/-	298/-	429/-	350/336	387/-
TX12 (0.84%)	E975 E985	302/-	298/-	429/407	350/-	387/-
TX13 (0.42%)	E1145	302/-	297/-	429/-	351/-	387/-
TX14 (0.42%)	E1190	302/-	298/-	429/408	350/-	387/-
TX15 (0.42%)	E1220	302/-	284/297/302	429/-	350/-	387/-
TX16 (0.42%)	E1250	302/-	284/297	428/-	350/-	387/-
TX17 (0.42%)	E1310	304/-	298/-	429/-	350/336	387/-
TX18 (0.42%)	E1420	302/-	298/-	429/-	350/336	387/368
TX19 (0.42%)	E1440	302/298	298/302	429/-	350/-	387/-
TX20 (1.69%)	P9 E1290 E1300 E1460	302/-	286/300	429/-	350/-	387/-
TX21 (1.27 %)	E1540 E1760 H23	302/-	300/-	429/-	350/-	387/-
TX22 (0.42%)	E1620	302/-	302/-	429/-	350/336	387/-
TX23 (0.42%)	E1780	304/298	298/-	429/-	350/-	387/-
TX24 (0.42%)	E1800	302/-	284/298	429/-	350/-	387/-
TX25 (0.42%)	E1810	298/-	298/-	429/-	350/-	387/-
TX 26 (4.24%)	Clone (10 isolates)	302/-	297/302	429/-	350/-	387/-
TX27 (0.42%)	F2	302/-	-/-	429/-	350/-	387/-
TX28 (0.42%)	H1	302/-	298/-	429/-	350/-	387/372
TX29 (1.69%)	H15 L10 W140 W180	302/298	297/-	429/-	350/-	387/-
TX30 (2.12%)	H16 H18 H19 H21 W245	302/298	298/-	429/-	350/-	387/-
TX31 (0.42%)	H22	301/304/305	298/-	429/-	350/-	386/-
TX32 (0.42%)	H25	305/300	300/-	429/-	350/-	387/-
TX33 (0.42%)	J13	302/-	300/298	429/-	351/-	387/-

* The frequency of the genotype in the population of Texas.

Table 7 continued

Genotype	Isolate	Locus 5-2	Locus 20-3	Locus 36-4	Locus 144-4	Loci 117-4
TX34 (0.42%)	J14	302/-	284/298/302	429/-	350/-	387/-
TX35 (0.42%)	T2-2	302/-	284/-	429/-	350/-	387/-
TX36 (0.42%)	T2-3	302/-	284/-	429/-	350/-	387/370
TX37 (0.42%)	W80	300/304	298/-	429/-	350/-	387/-
TX38 (0.42%)	W87	300/304	297/-	429/350	350/-	387/-
TX39 (0.42%)	W100	302/-	302/286	429/-	350/-	387/-
TX40 (0.84%)	W125	302/-	298/-	429/-	349/-	387/-
	B14					
TX41 (0.42%)	U1	304/-	286/-	429/-	350/-	387/-
TX42 (0.42%)	U5	302/-	298/-	429/-	351/-	387/-
TX43 (0.42%)	U7	304/-	298/-	429/-	350/-	387/-
TX44 (0.84%)	F8	302/-	298/302	428/-	350/-	387/-
	B13	302/-	298/302	428/-	350/-	387/-
TX45 (.049%)	T1-4	302/-	298/-	428/-	349/-	387/-
TX46 (0.42%)	E830	304/-	300/-	429/-	350/-	387/-
TX47 (0.42%)	E1170	302/-	297/-	429/407	350/-	387/-
TX48 (0.42%)	E810	302/-	298/-	428/-	350/-	387/-
TX49 (0.84%)	W285	302/-	297/-	429/-	350/-	386/-
	B2					
TX50 (0.49%)	W195	302/-	296/-	429/-	350/-	387/-
BR1 (2.12%)	Clone (5 isolates)	301/-	298/-	420/-	350/-	377/-

Genotypes TX7, TX8, TX15, TX 31, and TX 34 were excluded from further analysis due to the presence of three alleles at different loci, which could not be managed in GENEPOP. Genotypic diversity of the loci was 0.766. Genotypic differentiation indicated significant differences in genotypes based on location ($P \leq 0.01$).

Data sets of each multilocus genotype observed were analyzed to determine the role of sexual recombination in shaping the genotypic structure of the isolates sampled. The index of association, I_A, has an expected value of zero if there is no association of alleles at unlinked loci as assumed in randomly mating populations. Therefore, high within-population levels of linkage disequilibria are expected in largely clonal species (Ivors *et al.* 2006). The observed I_A value (I_A =1.18) for *S. minor*

populations was significantly higher ($P \leq 0.001$) than the I_A calculated from 1000 artificially recombined data sets; therefore, the population is clonal.

A maximum parsimony tree was constructed from clonally corrected genotypes with the data scored as 0 or 1 for presence or absence of an allele. Based on the number of alleles at each of the five microsatellite, there were loci 31 characters total. In general, the presence of a band is more likely to be a homologous than absence of the band; thereby the character type was set to dollo.up. Since parsimony based analyses of datasets with such large numbers of taxa are computationally intensive with characters that are equally weighted (and since the allele frequencies at these tested loci were quite inconsistent), the reweighting characters feature based on the rescaled consistency index (RC) criteria (Quicke *et al.* 2001) was employed.

The maximum parsimony tree (Fig. 18) demonstrated that there were 13 distinct clades and many unique individuals. Clade 1 included only TX1, which was the only genotype that was found in every field sampled. Clade 2 included: TX2, TX36, TX38, TX6, TX49, TX29, TX13, and TX16. Clade 3 encompassed the largest number of genotypes: TX7, TX8, TX9, TX47, TX12, TX14, TX15, and TX26. Clade 4 included: TX24, TX27, TX45, TX35, TX34, TX40, TX50, TX48, and TX44. Clade 5 included: TX10. TX20, TX41, TX46, TX32, TX21, TX31, and TX33. Clade 7 included TX3, TX5, TX11, TX18, TX17, and TX22. Clade 8 included TX23, TX25, TX43, and TX37. Clades that did not represent more than one genotype were clade 6-TX39, clade 9-TX30, clade 10-TX28, clade11-TX42, and clade 12-TX4. Clade 13 was genotype BR1, which were the five isolates from Brazil, which were incorporated as the outgroup in the tree.

The maximum parsimony tree demonstrates that the clades that contain more than one genotype were not isolated by county while clades with only one genotype were isolated county (Fig. 18). The maximum parsimony tree also demonstrated that many of the genotypes are isolated by field; TX1, TX2, TX3 (clones) are the only genotypes that occur in more than four fields (Fig. 19).

While each genotype had a unique distribution of aggressiveness (Fig. 20), no significant differences in aggressiveness were found at $P \leq 0.01$ (Table A21). The lack of significant differences among genotypes can be attributed to the wide array of aggressiveness within each genotype as seen in TX1 (Fig. 21).

After assessing the data for each phenotypic and genotypic characteristic tested, no “super pathogen” was found that was fungicide insensitive, highly aggressive, and genotypically unique. Five isolates, E790, U7, E1410, E1750, and W125, were found that exhibit at least two of the characteristics (Table 8).

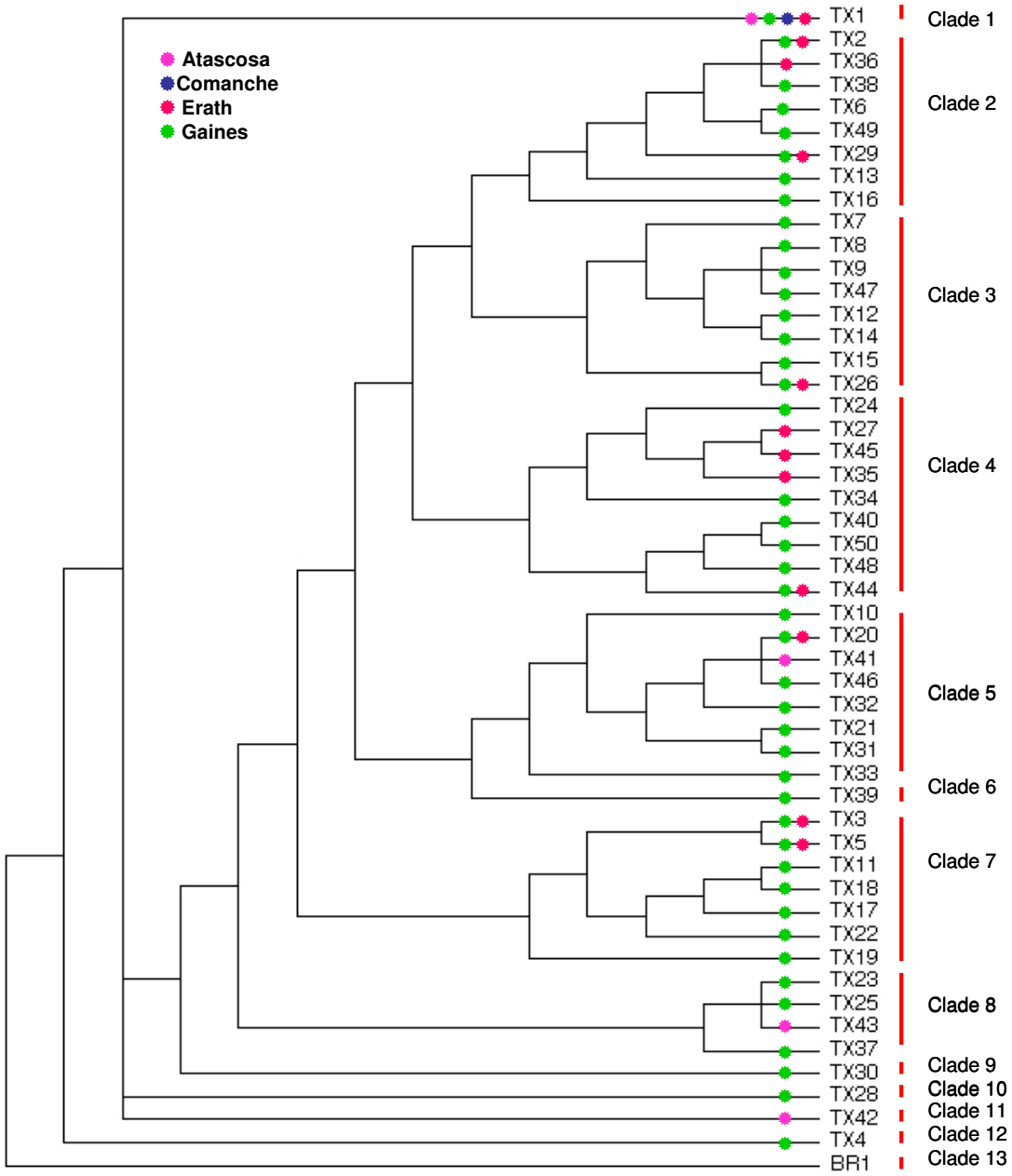

Fig. 18 Maximum parsimony tree of unique genotypes of *S. minor* in Texas indicates the genotypes within each county and in which clade the genotypes reside.

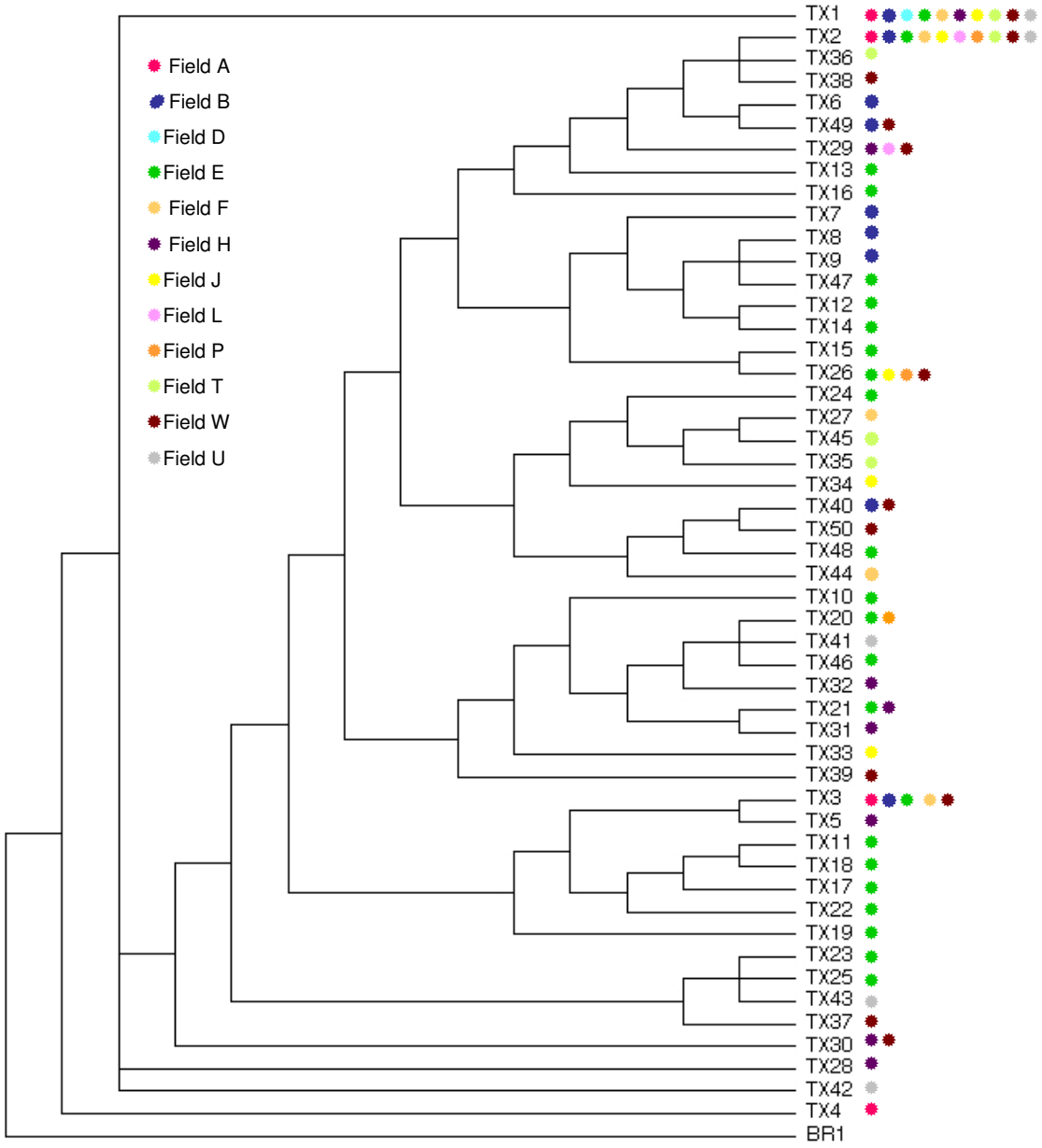

Fig. 19 Maximum parsimony tree that demonstrates the location of genotypes in the sampled fields.

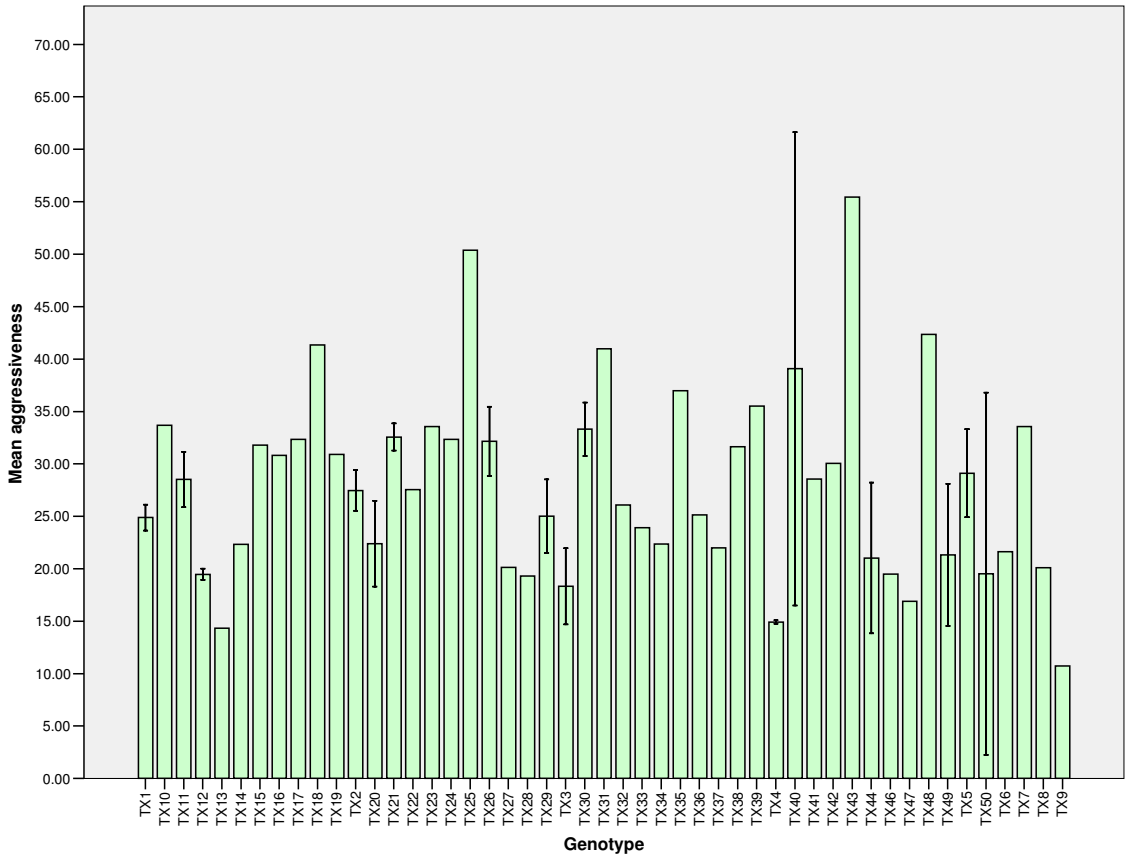

Fig. 20 Aggressiveness distribution of the genotypes. Error bars represent +/- one standard error.

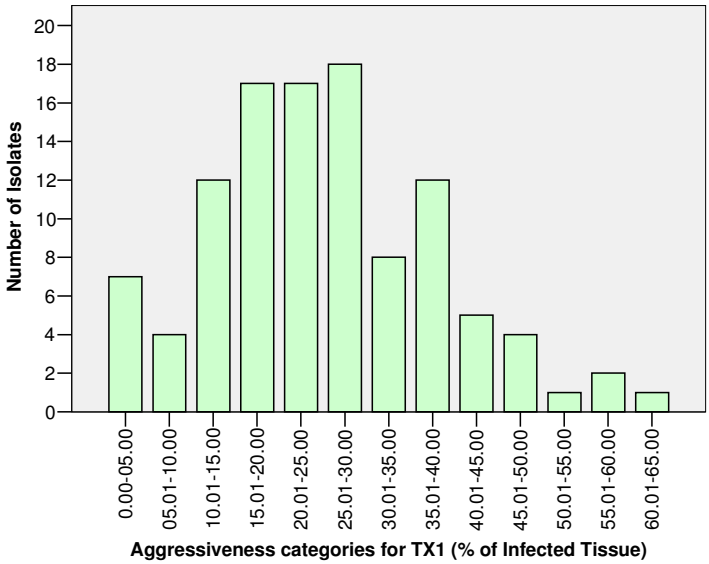

Fig. 21 Frequency distribution of aggressiveness of TX1.

Table 8 Assessing most aggressive pathogens in genotype and fungicide insensitivity.

Isolate	Aggressiveness		Fungicide Insensitivity		Genotype
E790	√	55.07	*X*	√	TX1
U7	√	55.44	*X*	√	TX43
E1410	√	58.44	No Data	√	TX1
E1750	√	60.23	*X*	√	TX1
W125	√	61.64	No Data	√	TX40

√ The isolate meets the criteria for the characteristic.
X The isolate did not meet the criteria for the characteristic.

DISCUSSION

The isolates of *S. minor* characterized were significantly different in aggressiveness, genotypically unique, and responded differently to the tested fungicides. The hypothesis that a "super pathogen" had evolved or was introduced into Gaines county and was the predominate pathogen was rejected. No isolate was found to be highly aggressive, genotypically unique or fungicide insensitive. Only five of the 232 tested isolates were found to have at least two of the three defined characteristics, genotypically unique and highly aggressive. Four of these highly aggressive and genotypically isolate, E790, E1410, E1750, and W125 were found in Gaines county; therefore, continued heavy application of fungicide and continual cropping of peanuts could create a "super pathogen" that was fungicide insensitive and was capable of rapidly decimating its host.

An alternative hypothesis to explain the sudden increase in disease incidence in Gaines county is that the pathogen has always resided in the area; however, the rotation of crops and fungicide application may have kept the population density low if not detectable. During 2004 in the High Plains, there was more moisture, the temperatures were considerably lower than previous years (noaa.org), and a host of *S. minor* was present. These three conditions may have been so conducive to disease development compared to previous year that a dramatic increase in incidence of *S. minor* occurred. No historical data are available that indicates the presence of the pathogen prior to 1996 in Gaines county (Terry Wheeler personal communication).

Another hypothesis is that the pathogen was introduced multiple times through cultivar or equipment contamination. Due to many different genotypes present in each field and lack of evidence of sexual reproduction as indicated by the index of association, the pathogen diversity seems unlikely to be attributed to an ascospore shower during fallow periods.

The aggressiveness of the pathogen throughout Texas was significantly different. The Gaines county population's mean aggressiveness was among the least across the entire state, but the most aggressive isolates (capable of generating lesions on more than 55% of peanut tissue) were found within this area. The most aggressive isolates accounted for less than 3% of the total isolates of *S. minor*; this is incredibly interesting as a positive correlation between aggressiveness and fungicide insensitivity was found for fluazinam and boscalid. An ecological tradeoff, an increase in one characteristic requires a decrease in another characteristic, was not found between aggressiveness and fungicide insensitivity; therefore, another phenotypic characteristic such as sclerotial hardiness, must be decreased thereby preventing the highly aggressive pathogens from being the predominate isolates in the population. Additional phenotypic characteristics needed to be assessed to hypothesize what is preventing these highly aggressive pathogens from becoming the predominate isolates within the field. Since the highly aggressive pathogens can rapidly infect under favorable conditions, spread plant-to-plant by mycelial networks, and propagate and survive as sclerotia, there is the potential for a population shift. The shift in population structures has been observed in populations of *Phytophthora infestans*, where endemic populations have been replaced by more

aggressive introduced genotypes (Goodwin *et al.* 1994; Spielman *et al.* 1991). Perhaps an annual evaluation of a limited sample size is advisable to monitor for increases in percent of highly aggressive isolates.

While there are isolates of *S. minor* that are highly aggressive on peanuts, there are also isolates that do not rapidly infect peanuts. These isolates may be still present in the population because their primary host is not peanuts. Agricultural fields in Texas have wild populations of *Helianthus annuus* L., *Cucumis melo*, *Lupinus texensis* (Siders & Henry 2002; Woodard & Simpson 1993), which are all known hosts of *S. minor* (Melzer *et al.* 1997). Therefore, these non-aggressive isolates (unable to infect more than 1% of the peanut tissue) may survive in the population because of the presence of an alternate host in these production fields. Alternate hosts that provide a suitable habitat for the pathogen insure production of sclerotia. These sclerotia of less aggressive isolates could germinate and infect weakened or aging peanut plants.

While the fungicide sensitivity assay demonstrated that less sensitive isolates exist, no "super pathogen" was found that was highly resistant (requiring more than two times the mean EC50) to the tested fungicides at the concentrations tested. No region of the state was found to have isolates that were distinctively resistant to any of the fungicides tested. Thiophanate-methyl and dichloran were found to be inhibitory, but they required much higher concentrations of active ingredient than fluazinam, ipodione, or boscalid.

Thiophanate-methyl was the least effective fungicide in inhibiting growth of *S. minor* on the spiral fungicide plates. The mean EC50 of thiophanate-methyl for the

isolates of *S. minor* was 25 times the concentration mean EC50 for boscalid, fluzinam, and iprodione and 2.5 times the EC50 of dichloran as seen in Fig. 13. This suggests that this fungicide would not be cost-effective nor highly efficacious in treating outbreaks of *S. minor*. Interestingly, the mean EC50 of thiophanate-methyl required to inhibit *S. minor* is only 0.05 times the EC50 for *S. sclerotiorum* (Mueller *et al.* 2002); this phenomenon may occur because the species are so closely related and the fungicide's mode of action effectively inhibits the both pathogens.

Dichloran is not typically used to control *S. minor* blights on peanuts because it requires higher concentrations of active ingredient in comparison to fluazinam, boscalid, or iprodione. However, it is used to effectively control *Botrytis cinerea* (Jim Starr personal communication). Therefore, an outbreak of both pathogens might be controlled with this fungicide.

Iprodione was an effective fungicide on populations of *Sclerotinia minor* from Texas, and its effective concentration is comparable to levels that inhibit the growth of *S. minor* isolates from California (Hubbard & Subbaro 1997). However, development of resistance towards this fungicide has been found in populations of *S. minor* throughout the major agricultural production areas in the United States and does not provide effective control in the field (Hubbard & Subbaro 1997).

The average EC50 for boscalid was comparable between Texas and the Arizona isolates (Matheron *et al.* 2004). While the EC50 for fluazinam was one of the lowest among the fungicides tested, the isolates from Texas required higher concentrations of fluazinam to inhibit growth than recorded EC50 levels for isolates from lettuce in

Arizona. The average EC50 for fluazinam for our isolates of *S. minor* is nine times greater than the fluazinam concentration that effectively suppresses 82 to 84% of the mycelial growth of *S. minor* from Arizona (Matheron *et al.* 2004). This is interesting since *S. minor* in Arizona has been present since 1925 (Brown & Butler 1936) and only found in Texas in 1981 (Woodard & Simpson 1993). Therefore, it would be logical for isolates within Arizona to require higher concentrations of active ingredient of the fungicide since these areas have been treating for the pathogen for a longer period of time. Yet, we have found the opposite effect. One possible hypothesis for the difference in the required a.i. is that the fungicides were not tested in the same manner; our fungicides were spirally applied to the surface of the agar at 25^{o}C while there fungicides were applied into agar at 50^{o}C.

Large differences in growth and sclerotial production among the isolates of *S. minor* were observed on the different fungicide amended plates. Fluazinam provided the most effective growth inhibition of the pathogen after 14 days while boscalid, thiophanate-methyl, iprodione, or dichloran PDA plates were no longer effective in inhibiting growth or sclerotial production. The effectiveness of these fungicides in the laboratory may be comparable to their field application as their recommended application schedules may indicate differing levels of residual activity. The recommended application schedule for fluazinam is every 21-28 days while boscalid, thiophanate-methyl, and dichloran application schedules are every 7-14 days and iprodione is 14-21 days. Therefore, fluazinam seems to provide the most effective cover with prolonged residual activity.

Isolates of *S. minor* from 12 fields in Texas and one field in Brazil (incorporated to act as an outgroup) were genotypically characterized. Analyses of genetic variation using allelic frequencies at 5 microsatellite loci indicated significant variation among populations between continents and within Texas. There were 49 unique genotypes from Texas while the Brazil isolates were found to be a clone, which was unique at loci 5-2, 20-3, 36-4, and 117-4. Loci 5-2, 20-3, 36-4, and 117-4 were polymorphic and the most informative loci. Locus 114-4 was the most conservative locus tested; it was only found to be unique in 10 isolates and polymorphic in five isolates from Texas.

There were 50 unique genotypes described in this study, six were clones (five isolated in Texas and one clone from Brazil) contributing to more than 74.55% of the population structure. Two studies have examined populations of *S. sclerotiorum* using microsatellite markers. Attalah *et al.* 2004 using 11 microsatellite markers found 148 genotypes from 167 isolates in Washington. A study of Australian isolates found 82 unique genotypes out of 154 isolates of *S. sclerotiorum* using eight microsatellite markers (Sexton & Howlett 2004). Compared to these previous studies, the results reported here suggest that less diversity is present within the populations of *S. minor* in Texas. Less diversity naturally occurs in *S. minor* populations or additional markers are required to further separate some of our populations.

Genotypic diversity was found within all fields tested. No field was found to be genotypically uniform, consisting of a single clone; however TX1 was found to be the predominate pathogen throughout the state. Field E (Gaines county) had the most diverse populations of *S. minor*; however, the number of samples was at least twice the number

of isolates from any other field sampled. This strongly suggests that increasing the number of pathogen samples would yield further diversity in the other fields. Even though field E was highly diverse, the predominate pathogens were the clones, TX1, TX2, TX3, and TX26. The diversity of the pathogen population within a field may be attributed to several independent introductions of unique pathogens to the area or by previous infections by sexual progeny, ascospores. However, there are no reports of apothecia or ascospores occurring in Texas and our data does not support the occurence that ascospores as the primary mechanism of *S. minor* dissemination.

There were significant differences in genotypic differentiation among the populations tested. No population was found to have the exact percentage of genotypes as another tested population. The populations in Gaines county exhibited the most differences between populations in spite of being geographically isolated from the other peanut producing regions in Texas and being in close proximity (less than 50 miles between each tested field).

Genotype TX1, monomorphic at the loci tested, accounted for almost 50% of the total pathogen population and was isolated in all three main peanut producing areas in Texas. It was found in every field tested except two fields in Erath county. Most likely a migration of the genotype has occurred, which may have been natural through the dissimination of propagules in air, water, or animals or inadvertently by the action of man spreading contaminated equipment or cultivars. The maximum parsimony tree constructed showed that this genotype was a distinct clade, but a frequency distribution indicated that this clade had large diversity among the aggressiveness.

The maximum parsimony tree, in addition to illustrating that genotype TX1 was a unique clade, indicated that there were 12 more unique clades among the populations. The clades that encompassed more than one genotype were not found to be geographically isolated, genotypes were found in more than one county. Only clades 9, 10, and 12 were geographically isolated to Gaines county and contained only one genotype. The most probable explanation for the lack of geographic isolation among the genotypes is that the pathogen has migration has occurred from the regions of historical cases of *S. minor* (Atascosa, Mason, Comanche, and Erath county) to Gaines county.

The clonal genotypes were found within their own unique clades with other genotypes that were not clones except clones TX3 and TX11 were combined into Clade 7. These clonal genotypes were created by separate, unique mutations of the organism's genotype; thereby, creating the unique clades seen in Fig. 18. These clonal genotypes were also found in more than one geographic location except clone TX11, which was only found within field E. TX11 may have been caused by a later mutation that is isolated to this field or region. The geographic diversity of the clonal genotypes indicates that multiple introductions of the pathogen or migration may have occurred to create the diversity.

Linkage disequilibrium, I_A=1.18, indicated that there was a lack of gene flow occurring in the *S. minor* populations of Texas. The populations of *S. minor* in Texas were clonal as indicated by the index of association and the large number of clones isolated in our study. The populations of *Phytophthora ramorum* were also found to be clonal throughout the United States and Europe, and this was confirmed through an

I_A=17.1 (Ivors *et al.* 2006). In the studies of populations of *S. sclerotiorum* from Washington state and Australia, linkage disequilibrium, 0.086 and 0.18, respectively, indicated that gene flow was occurring (Attalah *et al.* 2004). Since apothecia were found in the Washington populations, they are the source most likely generating the gene flow on these two continents (Attalah *et al.* 2004). No apothecia were found during the survey of fields to collect the isolates of *S. minor* used in this study. Additionally there are no reports of apothecia from fields in Texas. Therefore, future pathogen sampling of *S. minor* should be focused on determining if apothecia are occurring in Texas to verify if outcrossing can occur even though there was a lack of gene flow occurring in our populations.

SUMMARY

We have demonstrated that populations of *Sclerotinia minor* in Texas are diverse phenotypically as well as genotypically. No "super" pathogen, which was phenotypically and genotypically unique, was discovered that had been introduced or evolved in Gaines County.

There were significant differences among the isolates in aggressiveness as well as unique distributions of aggressiveness within each field population. No highly aggressive pathogen was found to be the dominant phenotype. However, population structures have the ability to shift and a pathogen that was initially found to be in small numbers could rapidly become the dominant pathogen.

While differences in aggressiveness were seen throughout the populations of *S. minor*, there were also differences in the effective concentrations of the fungicides tested. Thiophanate-methyl and dichloran were found to be inhibitory, but they required much higher concentrations of active ingredient than fluazinam, ipodione, or boscalid. We found that fluazinam provided the most lasting growth inhibition of the pathogen.

In addition to phenotypic diversity, genotypic diversity of *S. minor* was found throughout Texas. There were distinct populations of clones found throughout the state; however, evidence (index of association) demonstrated that a lack of gene flow was occurring. Therefore, populations within Texas while genotypically diverse did not arise from sexual reproduction.

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APPENDIX A

Table A1 Analysis of variance (ANOVA) table on the effect of different isolates on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates*	32.477	230	0.141	32.205	0.000
Error	9.102	2076	0.004		
Total	199.376	2307			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A2 Analysis of variance (ANOVA) table on the effect Field A on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates in field A*	0.599	9	0.067	94.855	0.000
Error	0.063	90	0.001		
Total	3.738	100			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A3 Analysis of variance (ANOVA) table on the effect Field B on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates in field B*	0.410	19	0.022	30.659	0.000
Error	0.126	179	0.001		
Total	6.807	199			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A4 Analysis of variance (ANOVA) table on the effect Field D on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates in field D*	0.165	7	0.024	23.504	0.000
Error	0.072	72	0.001		
Total	3.102	80			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A5 Analysis of variance (ANOVA) table on the effect Field E on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates in field E*	14.798	83	0.178	26.915	0.000
Error	5.015	757	0.007		
Total	83.473	841			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A6 Analysis of variance (ANOVA) table on the effect Field F on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates in field F*	0.412	5	0.082	14.093	0.000
Error	0.310	53	0.006		
Total	6.292	59			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A7 Analysis of variance (ANOVA) table on the effect Field H on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates in field H*	1.784	17	0.105	92.563	0.000
Error	0.184	162	0.001		
Total	15.792	180			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A8 Analysis of variance (ANOVA) table on the effect Field J on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates in field J*	0.870	11	0.079	15.166	0.000
Error	0.553	106	0.005		
Total	7.352	118			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A9 Analysis of variance (ANOVA) table on the effect Field L on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates in field L*	0.160	4	0.040	3.829	0.009
Error	0.471	45	0.010		
Total	6.920	50			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A10 Analysis of variance (ANOVA) table on the effect of Field P on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates in field P*	0.201	5	0.040	4.572	0.002
Error	0.476	54	0.009		
Total	6.648	60			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A11 Analysis of variance (ANOVA) table on the effect of Field T on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates in field T*	0.930	8	0.116	15.052	0.000
Error	0.626	81	0.008		
Total	8.510	90			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A12 Analysis of variance (ANOVA) table on the effect of Field U on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates in field U*	0.541	5	0.108	113.52	0.000
Error	0.051	54	0.001		
Total	9.411	60			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A13 Analysis of variance (ANOVA) table on the effect of Field W on aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates in field W*	7.036	47	0.150	55.963	0.000
Error	1.156	432	0.003		
Total	41.844	480			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A14 Analysis of variance (ANOVA) table on the effect of counties on Aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Counties	19715.421	3	6571.807	38.368	0.000
Error	396182.608	2313	171.285		
Total	1999342.423	2317			

*Dependent variable: aggressiveness (percent of infected tissue)

Table A15 Analysis of variance (ANOVA) of fungicide sensitivity to thiophanate-methyl by isolates.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolate*	1048.324	66	15.884	23.518	0.000
Error	181.003	268	0.675		
Total	3357.124	335			

*Dependent variable: EC50 of thiophanate-methyl

Table A16 Analysis of variance (ANOVA) of fungicide sensitivity to dichloran by isolates.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates*	128.373	66	1.945	27.577	0.000
Error	18.902	268	0.071		
Total	503.340	335			

*Dependent variable: EC50 of dichloran

Table A17 Analysis of variance (ANOVA) of fungicide sensitivity to iprodione by isolates.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates*	1.878	66	0.028	8.829	0.000
Error	0.864	268	0.003		
Total	7.161	335			

*Dependent variable: EC50 of iprodione

Table A18 Analysis of variance (ANOVA) of fungicide sensitivity to fluazinam by isolates.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates*	3.129	66	0.047	26.587	0.000
Error	0.478	268	0.002		
Total	6.377	335			

*Dependent variable: EC50 of fluazinam

Table A19 Analysis of variance (ANOVA) of fungicide sensitivity to boscalid by isolates.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Isolates*	0.299	66	0.005	8.978	0.000
Error	0.135	268	0.001		
Total	1.844	335			

*Dependent variable: EC50 of boscalid

Table A20 Analysis of variance (ANOVA) of EC50 among fungicides.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Fungicide	1513.822	4	378.455	456.865	0.000
Error	1383.385	1670	0.828		
Total	3875.845	1675			

*Dependent variable: EC50 of all of the tested fungicides

Table A21 Analysis of variance (ANOVA) on the effect of genotype on Aggressiveness.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significant
Genotype*	6075.954	48	126.582	0.874	0.703
Error	26507.146	183	144.848		
Total	191239.386	232			

*Dependent variable: aggressiveness

APPENDIX B

Table B1 The genotype, county, field, aggressiveness ± one standard error (S.E.), and fungicide insensitivity ± S.E or no data (n.d.) for each isolate.

Genotype	Isolate	Field	County	Aggressiveness ± S.E.	Thiophanate-methyl ± S.E.	Iprodione ± S.E.	Dichloran ± SE	Fluazinam ± S.E.	Boscalid ± S.E.
TX1	A1	A	Gaines	12.15 ± 0.18	4.985 ± 0.131	0.065 ± 0.001	0.605 ± 0.021	0.027 ± 0.001	0.024 ± 0.001
TX3	A2	A	Gaines	19.61 ± 0.31	1.293 ± 0.185	0.232 ± 0.065	0.297 ± 0.037	0.025 ± 0.001	0.040 ± 0.001
TX4	A3	A	Gaines	15.2 ± 0.31	n.d.	n.d.	n.d.	n.d.	n.d..
TX1	A4	A	Gaines	13.79 ± 0.18	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	A11	A	Gaines	12.31 ± 0.14	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	A12	A	Gaines	11.77 ± 0.22	n.d.	n.d.	n.d.	n.d.	n.d.
TX4	A15	A	Gaines	14.91 ± 0.14	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	A17	A	Gaines	38.68 ± 0.48	1.352 ± 0.099	0.072 ± 0.002	0.569 ± 0.009	0.029 ± 0.001	0.052 ± 0.002
TX4	A20	A	Gaines	14.62 ± 0.29	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	A25	A	Gaines	22.33 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.
TX5	B1	B	Gaines	18.69 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.
TX49	B2	B	Gaines	14.53 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.
TX3	B3	B	Gaines	17.01 ± 0.29	n.d.	n.d.	n.d.	n.d.	n.d.
TX3	B5	B	Gaines	19.06 ± 0.33	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	B6	B	Gaines	16.44 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.
TX6	B8	B	Gaines	21.60 ± 0.28	0.634 ± 0.023	0.040 ± 0.001	0.220 ± 0.004	0.024± 0.001	0.058 ± 0.002
TX1	B10	B	Gaines	22.08 ± 0.27	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	B11	B	Gaines	15.30 ± 0.07	n.d.	n.d.	n.d.	n.d.	n.d.
TX44	B13	B	Gaines	13.82 ± 0.16	0.813 ± 0.031	0.051 ± 0.002	0.642 ± 0.023	0.036 ± 0.001	0.036 ± 0.002
TX40	B14	B	Gaines	16.49 ± 0.18	n.d.	n.d.	n.d	n.d.	n.d.
TX1	B15	B	Gaines	16.04 ± 0.31	0.903 ± 0.015	0.088 ± 0.007	0.472 ± 0.032	0.023 ± 0.000	0.028 ± 0.001
TX7	B16	B	Gaines	33.54 ± 0.64	n.d.	n.d.	n.d.	n.d.	n.d.
TX3	B18	B	Gaines	16.81 ± 0.25	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	B19	B	Gaines	15.95 ± 0.16	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	B20	B	Gaines	13.67 ± 0.24	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	B21	B	Gaines	22.41 ± 0.28	n.d.	n.d.	n.d.	n.d.	n.d.
TX8	B22	B	Gaines	20.08 ± 0.38	n.d.	n.d.	n.d.	n.d.	n.d.
TX3	B23	B	Gaines	18.26 ± 0.25	n.d.	n.d.	n.d.	n.d.	n.d.
TX9	B25	B	Gaines	10.72 ± 0.16	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	B26	B	Gaines	14.10 ± 0.08	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	D1	D	Comanche	17.53 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	D2	D	Comanche	15.82 ± 0.25	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	D4	D	Comanche	28.31 ± 0.52	1.176 ± 0.045	0.068 ± 0.002	1.127 ± 0.048	0.033 ± 0.001	0.094 ± 0.003
TX1	D5	D	Comanche	13.04 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	D6	D	Comanche	15.05 ± 0.22	1.815 ± 0.100	0.035 ± 0.001	1.452 ± 0.075	0.030 ± 0.001	0.028 ± 0.000
TX1	D7	D	Comanche	19.74 ± 0.26	1.485 ± 0.058	0.045 ± 0.002	0.785 ± 0.027	0.019 ± 0.000	0.045 ± 0.002
TX1	D8	D	Comanche	22.80 ± 0.37	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	D9	D	Comanche	19.09 ± 0.35	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	E740	E	Gaines	19.85 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E769	E	Gaines	6.31 ± 0.21	0.394 ± 0.013	0.330 ± 0.012	2.895 ± 0.11	0.043 ± 0.001	0.048 ± 0.002
TX1	E773	E	Gaines	24.49 ± 0.68	1.742 ± 0.087	0.084 ± 0.001	0.857 ± 0.016	0.316 ± 0.058	0.054 ± 0.003
TX1	E775	E	Gaines	13.47 ± 0.17	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E781	E	Gaines	25.20 ± 0.3	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E785	E	Gaines	26.87 ± 0.13	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E790	E	Gaines	55.07 ± 2.09	2.621 ± 0.131	0.170 ± 0.008	0.344 ± 0.013	0.179 ± 0.003	0.134 ± 0.002
TX1	E795	E	Gaines	16.89 ± 0.3	n.d.	n.d.	n.d.	n.d.	n.d.
TX48	E810	E	Gaines	42.33 ± 1.38	2.011 ± 0.222	0.041 ± 0.003	0.534 ± 0.031	0.121 ± 0.004	0.058 ± 0.001
TX1	E815	E	Gaines	34.03 ± 1.16	4.978 ± 0.134	0.129 ± 0.007	0.798 ± 0.028	0.054 ± 0.004	0.178 ± 0.003
TX1	E825	E	Gaines	20.87 ± 0.18	n.d.	n.d.	n.d.	n.d.	n.d.
TX46	E830	E	Gaines	19.47 ± 1.23	2.510 ± 0.097	0.190 ± 0.016	1.852 ± 0.055	0.083 ± 0.002	0.050 ± 0.000
TX1	E835	E	Gaines	49.56 ± 2.45	2.985 ± 0.046	0.084 ± 0.002	0.874 ± 0.053	0.124 ± 0.002	0.023 ± 0.000
TX1	E845	E	Gaines	7.955 ± 0.11	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E850	E	Gaines	15.43 ± 0.19	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E860	E	Gaines	1.412 ± 0.14	n.d.	n.d.	n.d.	n.d.	n.d.
TX10	E865	E	Gaines	33.68 ± 0.46	n.d.	n.d.	n.d.	n.d.	n.d.
TX11	E880	E	Gaines	23.26 ± 0.59	3.203 ± 0.118	0.254 ± 0.046	0.840 ± 0.025	0.099± 0.003	0.059 ± 0.002
TX1	E885	E	Gaines	20.46 ± 0.24	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E895	E	Gaines	17.08 ± 0.36	3.753 ± 0.376	0.136 ± 0.006	1.792 ± 0.157	0.101 ± 0.002	0.065 ± 0.001
TX2	E915	E	Gaines	32.20 ± 0.95	3.994 ± 0.171	0.080 ± 0.005	1.346 ± 0.071	0.043 ± 0.003	0.025 ± 0.001
TX11	E935	E	Gaines	33.87 ± 0.74	1.050 ± 0.081	0.182 ± 0.008	0.413 ± 0.018	0.173 ± 0.006	0.094 ± 0.006
TX1	E940	E	Gaines	34.47 ± 1.07	3.510 ± 0.133	0.102 ± 0.002	0.685 ± 0.019	0.117 ± 0.001	0.052 ± 0.001
TX1	E945	E	Gaines	52.19 ± 2.85	1.116 ± 0.063	0.163 ± 0.011	1.135 ± 0.019	0.685 ± 0.019	0.064 ± 0.001

Table B1 continued.

Genotype	Isolate	Field	County	Aggressiveness ± S.E.	Thiophanate-methyl ± S.E.	Iprodione ± S.E.	Dichloran ± SE	Fluazinam ± S.E.	Boscalid ± S.E.
TX11	E955	E	Gaines	21.14 ± 0.48	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E965	E	Gaines	46.72 ± 1.4	4.193 ± 0.201	0.058 ± 0.002	1.984 ± 0.061	0.070 ± 0.003	0.033 ± 0.001
TX12	E975	E	Gaines	18.93 ± 0.51	n.d.	n.d.	n.d.	n.d.	n.d.
TX12	E985	E	Gaines	19.99 ± 0.29	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E995	E	Gaines	9.09 ± 0.5	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1005	E	Gaines	20.61 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1025	E	Gaines	13.94 ± 0.21	3.177 ± 0.203	0.075 ± 0.001	0.697± 0.01	0.030 ± 0.001	0.051 ± 0.004
TX1	E1035	E	Gaines	26.24 ± 0.55	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1045	E	Gaines	22.57 ± 0.31	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1055	E	Gaines	21.94 ± 0.44	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1065	E	Gaines	20.01 ± 0.12	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1075	E	Gaines	35.77 ± 0.48	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1095	E	Gaines	0.847 ± 0.07	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1105	E	Gaines	21.94 ± 0.45	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1115	E	Gaines	22.77 ± 0.27	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	E1125	E	Gaines	5.544 ± 0.16	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	E1132	E	Gaines	19.50 ± 0.31	n.d.	n.d.	n.d.	n.d.	n.d.
TX13	E1145	E	Gaines	14.32 ± 0.22	n.d.	n.d.	n.d.	n.d.	n.d.
TX47	E1170	E	Gaines	16.89 ± 0.61	n.d.	n.d.	n.d.	n.d.	n.d.
TX14	E1190	E	Gaines	22.32 ± 0.32	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	E1200	E	Gaines	38.63 ± 0.65	n.d.	n.d.	n.d.	n.d.	n.d.
TX15	E1220	E	Gaines	31.79 ± 0.17	n.d.	n.d.	n.d.	n.d.	n.d.
TX16	E1250	E	Gaines	30.80 ± 0.33	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	E1270	E	Gaines	44.03 ± 0.38	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1280	E	Gaines	29.15 ± 0.14	n.d.	n.d.	n.d.	n.d.	n.d.
TX20	E1290	E	Gaines	29.25 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.
TX20	E1300	E	Gaines	10.52 ± 0.22	n.d.	n.d.	n.d.	n.d.	n.d.
TX17	E1310	E	Gaines	32.33 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.
TX11	E1360	E	Gaines	31.07 ± 0.31	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1380	E	Gaines	1.57 ± 0.11	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1390	E	Gaines	14.18 ± 0.46	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1400	E	Gaines	11.60 ± 0.22	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1410	E	Gaines	58.44 ± 0.75	n.d.	n.d.	n.d.	n.d.	n.d.
TX18	E1420	E	Gaines	41.34 ± 1.2	n.d.	n.d.	n.d.	n.d.	n.d.
TX19	E1440	E	Gaines	30.89 ± 0.27	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	E1450	E	Gaines	29.57 ± 0.26	n.d.	n.d.	n.d.	n.d.	n.d.
TX20	E1460	E	Gaines	25.26 ± 0.22	n.d.	n.d.	n.d.	n.d.	n.d.
TX3	E1490	E	Gaines	10.88 ± 0.16	n.d.	n.d.	n.d.	n.d.	n.d.
TX26	E1520	E	Gaines	41.55 ± 0.53	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1530	E	Gaines	36.59 ± 1.00	n.d.	n.d.	n.d.	n.d.	n.d.
TX21	E1540	E	Gaines	33.21 ± 0.22	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1560	E	Gaines	25.77 ± 0.39	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1600	E	Gaines	38.61 ± 0.52	n.d.	n.d.	n.d.	n.d.	n.d.
TX26	E1610	E	Gaines	28.66 ± 0.39	n.d.	n.d.	n.d.	n.d.	n.d.
TX22	E1620	E	Gaines	27.52 ± 0.15	n.d.	n.d.	n.d.	n.d.	n.d.
TX26	E1640	E	Gaines	26.82 ± 0.4	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1650	E	Gaines	44.59 ± 0.69	n.d.	n.d.	n.d.	n.d.	n.d.
TX11	E1660	E	Gaines	33.21 ± 0.24	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1680	E	Gaines	31.88 ± 1.19	0.259 ± 0.008	0.149 ± 0.008	0.965 ± 0.047	0.042 ± 0.003	0.063 ± 0.001
TX1	E1690	E	Gaines	2.68 ± 0.24	0.429 ± 0.015	0.402 ± 0.009	1.672 ± 0.008	0.393 ± 0.004	0.040 ± 0.001
TX1	E1720	E	Gaines	28.13 ± 0.57	0.700 ± 0.067	0.109 ± 0.008	0.362 ± 0.013	0.154 ± 0.007	0.085 ± 0.001
TX5	E1730	E	Gaines	38.92 ± 1.51	1.453 ± 0.000	0.107 ± 0.003	0.448 ± 0.018	0.056 ± 0.002	0.103 ± 0.003
TX26	E1740	E	Gaines	46.60 ± 1.27	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1750	E	Gaines	60.23 ± 1.71	0.547 ± 0.087	0.068 ± 0.001	0.650 ± 0.022	0.049 ± 0.002	0.038 ± 0.001
TX21	E1760	E	Gaines	34.39 ± 0.68	3.416 ± 0.159	0.148 ± 0.007	1.068 ± 0.035	0.097 ± 0.004	0.028 ± 0.001
TX23	E1780	E	Gaines	33.55 ± 0.40	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	E1790	E	Gaines	38.56 ± 1.57	0.991 ± 0.02	0.105 ± 0.01	0.839 ± 0.068	0.127 ± 0.004	0.074 ± 0.001
TX24	E1800	E	Gaines	32.33 ± 0.36	n.d.	n.d.	n.d.	n.d.	n.d.
TX25	E1810	E	Gaines	50.37 ± 2.02	1.681 ± 0.087	0.049 ± 0.003	0.782 ± 0.022	0.099 ± 0.008	0.096 ± 0.004
TX26	E1850	E	Gaines	45.64 ± 1.3	1.909 ± 0.038	0.251 ± 0.006	2.838 ± 0.056	0.131 ± 0.006	0.119 ± 0.005
TX27	F2	F	Erath	20.12 ± 0.32	n.d.	n.d.	n.d.	n.d.	n.d.
TX3	F3	F	Erath	42.79 ± 0.51	2.113 ± 0.115	0.098 ± 0.002	0.560 ± 0.046	0.068 ± 0.006	0.054 ± 0.001

Table B1 continued.

Genotype	Isolate	Field	County	Aggressiveness ± S.E.	Thiophanate-methyl ± S.E.	Iprodione ± S.E.	Dichloran ± SE	Fluazinam ± S.E.	Boscalid ± S.E.
TX1	F4	F	Erath	40.39 ± 0.71	n.d.	n.d.	n.d.	n.d.	n.d.
TX44	F8	F	Erath	28.22 ± 1.8	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	F9	F	Erath	22.74 ± 0.43	3.514 ± 0.101	0.123 ± 0.003	1.444 ± 0.043	0.096 ± 0.003	0.063± 0.004
TX5	F10	F	Erath	31.06 ± 0.25	n.d.	n.d.	n.d.	n.d.	n.d.
TX28	H1	H	Gaines	19.30 ± 0.36	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	H3	H	Gaines	26.36 ± 0.28	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	H5	H	Gaines	27.68 ± 0.5	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	H6	H	Gaines	30.86 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.
TX5	H7	H	Gaines	27.74 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	H8	H	Gaines	37.13 ± 0.45	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	H10	H	Gaines	2.29 ± 0.07	1.605 ± 0.095	0.089 ± 0.001	0.875 ± 0.018	0.022 ± 0.001	0.031 ± 0.002
TX1	H11	H	Gaines	5.75 ± 0.26	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	H12	H	Gaines	26.49 ± 0.39	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	H13	H	Gaines	28.60 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.
TX29	H15	H	Gaines	31.27 ± 0.5	n.d.	n.d.	n.d.	n.d.	n.d.
TX30	H16	H	Gaines	40.32 ± 0.33	n.d.	n.d.	n.d.	n.d.	n.d.
TX30	H18	H	Gaines	38.62 ± 0.57	n.d.	n.d.	n.d.	n.d.	n.d.
TX30	H19	H	Gaines	29.69 ± 0.17	0.867 ± 0.043	0.034 ± 0.001	0.277 ± 0.008	0.025 ± 0.001	0.026 ± 0.002
TX30	H21	H	Gaines	29.65 ± 0.35	n.d.	n.d.	n.d.	n.d.	n.d.
TX31	H22	H	Gaines	40.97 ± 0.16	2.006 ± 0.133	0.100 ± 0.002	0.693 ± 0.025	0.034 ± 0.001	0.078 ± 0.004
TX21	H23	H	Gaines	30.05 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.
TX32	H25	H	Gaines	26.06 ± 0.33	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	J1	J	Gaines	30.68 ± 1.50	2.916 ± 0.112	0.098 ± 0.005	1.105 ± 0.029	0.096 ± 0.007	0.092 ± 0.003
TX1	J2	J	Gaines	29.55 ± 0.43	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	J4	J	Gaines	24.45 ± 0.39	1.504 ± 0.018	0.076 ± 0.004	0.630 ± 0.049	0.093 ± 0.012	0.089 ± 0.003
TX26	J6	J	Gaines	15.08 ± 0.59	0.619 ± 0.012	0.049 ± 0.004	0.543 ± 0.011	0.067 ± 0.001	0.027 ± 0
TX1	J8	J	Gaines	23.53 ± 0.49	8.546 ± 0.248	0.032 ± 0.001	0.562 ± 0.056	0.048 ± 0.000	0.068 ± 0.001
TX1	J11	J	Gaines	21.07 ± 0.14	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	J12	J	Gaines	0.791 ± 0.06	n.d.	n.d.	n.d.	n.d.	n.d.
TX33	J13	J	Gaines	23.92 ± 0.43	n.d.	n.d.	n.d.	n.d.	n.d.
TX34	J14	J	Gaines	22.34 ± 0.32	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	J15	J	Gaines	16.32 ± 0.57	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	J16	J	Gaines	35.96 ± 0.90	1.990 ± 0.227	0.099± 0.007	0.912 ± 0.05	0.137 ± 0.005	0.060 ± 0.002
TX26	J17	J	Gaines	24.75 ± 1.29	0.735 ± 0.028	0.104± 0.003	1.440 ± 0.09	0.127 ± 0.002	0.071 ± 0.001
TX2	L1	L	Erath	27.73 ± 0.34	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	L2	L	Erath	38.37 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	L3	L	Erath	43.48 ± 2.1	6.136 ± 0.117	0.139 ± 0.005	1.631 ± 0.08	0.098 ± 0.005	0.036 ± 0.003
TX2	L7	L	Erath	37.49 ± 0.77	3.273 ± 0.304	0.166 ± 0.013	1.424 ± 0.009	0.090 ± 0.004	0.112 ± 0.003
TX29	L10	L	Erath	30.76 ± 0.26	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	P1	P	Erath	39.98 ± 0.28	n.d.	n.d.	n.d.	n.d.	n.d.
TX26	P2	P	Erath	22.61 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.
TX26	P3	P	Erath	35.16 ± 1.46	4.358 ± 0.452	0.039 ± 0.002	1.145 ± 0.068	0.096 ± 0.009	0.085 ± 0.002
TX2	P7	P	Erath	34.93 ± 0.54	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	P8	P	Erath	29.85 ± 1.31	6.681 ± 0.37	0.104 ± 0.005	0.852 ± 0.053	0.049 ± 0.002	0.044 ± 0.002
TX20	P9	P	Erath	24.49 ± 1.01	0.301 ± 0.028	0.026 ± 0.001	0.349 ± 0.005	0.051 ± 0.001	0.021 ± 0.002
TX1	T1-1	T	Erath	23.08 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	T1-2	T	Erath	32.89 ± 1.06	4.717 ± 0.215	0.065 ± 0.004	1.119 ± 0.075	0.054 ± 0.002	0.083 ± 0.005
TX1	T1-3	T	Erath	30.16 ± 1.05	5.085 ± 0.145	0.044 ± 0.003	1.223 ± 0.072	0.103 ± 0.003	0.125 ± 0.005
TX45	T1-4	T	Erath	36.78 ± 1.23	0.646 ± 0.017	0.091 ± 0.000	1.443 ± 0.046	0.097 ± 0.005	0.081 ± 0.003
TX1	T1-5	T	Erath	36.31 ± 1.53	4.585 ± 0.141	0.163 ± 0.008	0.860 ± 0.031	0.075 ± 0.001	0.083 ± 0.006
TX1	T1-8	T	Erath	2.71 ± 0.25	3.974 ± 0.338	0.051 ± 0.003	1.355 ± 0.026	0.099 ± 0.008	0.073 ± 0.003
TX35	T2-2	T	Erath	36.98 ± 0.74	3.965 ± 0.265	0.159 ± 0.003	1.419 ± 0.072	0.121 ± 0.001	0.055 ± 0.002
TX36	T2-3	T	Erath	25.12 ± 0.36	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	T2-5	T	Erath	26.13 ± 0.26	n.d.	n.d.	n.d.	n.d.	n.d.
TX41	U1	U	Atascosa	28.55 ± 0.34	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	U2	U	Atascosa	36.90 ± 0.16	n.d.	n.d.	n.d.	n.d.	n.d.
TX42	U5	U	Atascosa	30.04 ± 0.15	1.542 ± 0.05	0.16± 0.008	0.901 ± 0.02	0.038 ± 0.001	0.086 ± 0.001
TX43	U7	U	Atascosa	55.44 ± 0.36	1.313 ± 0.116	0.08± 0.001	1.250 ± 0.038	0.026 ± 0.001	0.068 ± 0.003
TX1	U8	U	Atascosa	45.83 ± 0.45	2.993 ± 0.087	0.069± 0.004	0.768 ± 0.031	0.022 ± 0.001	0.058 ± 0.003
TX1	U9	U	Atascosa	33.28 ± 0.29	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W6	W	Gaines	27.62 ± 1.29	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W10	W	Gaines	2.90 ± 0.23	1.115 ± 0.048	0.152 ± 0.009	2.816 ± 0.107	0.033 ± 0.000	0.079 ± 0.002

Table B1 continued.

Genotype	Isolate	Field	County	Aggressiveness ± S.E.	Thiophanate-methyl ± S.E.	Iprodione ± S.E.	Dichloran ± SE	Fluazinam ± S.E.	Boscalid ± S.E.
TX26	W15	W	Gaines	34.63 ± 0.83	2.949 ± 0.325	0.096 ± 0.009	0.524 ± 0.02	0.075 ± 0.002	0.039 ± 0.000
TX1	W20	W	Gaines	28.87 ± 0.39	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W25	W	Gaines	41.74 ± 0.3	5.982 ± 0.291	0.082 ± 0.003	1.360 ± 0.105	0.028 ± 0.001	0.085 ± 0.008
TX1	W30	W	Gaines	25.17 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W40	W	Gaines	36.14 ± 1.51	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	W45	W	Gaines	53.06 ± 0.81	5.616 ± 0.209	0.085 ± 0.002	0.786 ± 0.019	0.108 ± 0.011	0.064 ± 0.004
TX1	W65	W	Gaines	40.97 ± 0.8	3.213 ± 0.227	0.144 ± 0.007	0.369 ± 0.003	0.077 ± 0.003	0.083 ± 0.004
TX1	W70	W	Gaines	26.21 ± 0.23	2.308 ± 0.199	0.067 ± 0.001	0.557 ± 0.011	0.023 ± 0.000	0.104 ± 0.029
TX2	W75	W	Gaines	27.55 ± 0.56	1.725 ± 0.118	0.128 ± 0.009	0.723 ± 0.026	0.117 ± 0.002	0.059 ± 0.002
TX37	W80	W	Gaines	21.98 ± 0.18	n.d.	n.d.	n.d.	n.d.	n.d.
TX38	W87	W	Gaines	31.64 ± 0.76	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W95	W	Gaines	19.63 ± 0.28	1.404 ± 0.054	0.159 ± 0.003	2.849 ± 0.148	0.035 ± 0.001	0.050 ± 0.003
TX39	W100	W	Gaines	35.49 ± 0.22	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	W110	W	Gaines	28.77 ± 0.30	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	W115	W	Gaines	19.29 ± 0.25	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	W120	W	Gaines	9.77 ± 0.04	n.d.	n.d.	n.d.	n.d.	n.d.
TX40	W125	W	Gaines	61.64 ± 0.76	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W130	W	Gaines	26.14 ± 0.38	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W135	W	Gaines	35.13 ± 0.91	n.d.	n.d.	n.d.	n.d.	n.d.
TX29	W140	W	Gaines	17.83 ± 0.44	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W145	W	Gaines	18.57 ± 0.26	n.d.	n.d.	n.d.	n.d.	n.d.
TX3	W155	W	Gaines	1.29 ± 0.10	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W160	W	Gaines	17.06 ± 0.36	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W165	W	Gaines	22.86 ± 0.36	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W170	W	Gaines	35.65 ± 0.32	n.d.	n.d.	n.d.	n.d.	n.d.
TX3	W175	W	Gaines	19.08 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.
TX29	W180	W	Gaines	20.12 ± 0.40	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W185	W	Gaines	19.87 ± 0.31	n.d.	n.d.	n.d.	n.d.	n.d.
TX50	W195	W	Gaines	2.23 ± 0.06	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W200	W	Gaines	17.68 ± 0.19	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W210	W	Gaines	48.71 ± 0.63	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W215	W	Gaines	18.23 ± 0.29	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W220	W	Gaines	27.75 ± 0.30	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W225	W	Gaines	21.29 ± 0.34	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W230	W	Gaines	12.16 ± 0.20	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	W235	W	Gaines	18.44 ± 0.37	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	W240	W	Gaines	40.08 ± 0.74	n.d.	n.d.	n.d.	n.d.	n.d.
TX30	W245	W	Gaines	28.20 ± 0.44	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W250	W	Gaines	20.10 ± 0.17	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W260	W	Gaines	36.72 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	W265	W	Gaines	30.25 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.
TX1	W270	W	Gaines	23.54 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	W275	W	Gaines	44.66 ± 0.88	1.480 ± 0.126	0.359 ± 0.008	1.471 ± 0.071	0.134 ± 0.002	0.075 ± 0.005
TX2	W280	W	Gaines	24.04 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.
TX49	W285	W	Gaines	28.09 ± 0.14	n.d.	n.d.	n.d.	n.d.	n.d.
TX2	W290	W	Gaines	28.55 ± 0.43	n.d.	n.d.	n.d.	n.d.	n.d.
BR1	Bra1	n.d.	Brazil	n.d.		n.d.	n.d.	n.d.	n.d.
BR1	Bra2	n.d.	Brazil	n.d.		n.d.	n.d.	n.d.	n.d.
BR1	Bra3	n.d.	Brazil	n.d.		n.d.	n.d.	n.d.	n.d.
BR1	Bra6	n.d.	Brazil	n.d.		n.d.	n.d.	n.d.	n.d.
BR1	Bra5	n.d.	Brazil	n.d.		n.d.	n.d.	n.d.	n.d.

APPENDIX C

Table C1 Genotypic differences of isolates at microsatellite loci 5-2, 20-3, 36-4, 114-4, and 117-4.

Genotype	Isolate	Field	County	Locus 5-2	Locus 20-3	Locus 36-4	Locus 14-4	Locus 117-4
TX1	A1	A	Gaines	302/-	298/-	429/-	350/-	387/-
TX3	A2	A	Gaines	302/-	302/298	429/-	350/-	387/-
TX4	A3	A	Gaines	302/-	298/-	429/-	350/-	388/-
TX1	A4	A	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	A11	A	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	A12	A	Gaines	302/-	298/-	429/-	350/-	387/-
TX4	A15	A	Gaines	302/-	298/-	429/-	350/-	388/-
TX2	A17	A	Gaines	302/-	297/-	429/-	350/-	387/-
TX4	A20	A	Gaines	302/-	298/-	429/-	350/-	388/-
TX2	A25	A	Gaines	302/-	297/-	429/-	350/-	387/-
TX5	B1	B	Gaines	302/-	302/-	429/-	350/-	387/-
TX49	B2	B	Gaines	302/-	297/-	429/-	350/-	386/-
TX3	B3	B	Gaines	302/-	298/302	429/-	350/-	387/-
TX3	B5	B	Gaines	302/-	298/302	429/-	350/-	387/-
TX1	B6	B	Gaines	302/-	298/-	429/-	350/-	387/-
TX6	B8	B	Gaines	304/-	297/-	429/-	350/-	386/-
TX1	B10	B	Gaines	302/-	298/-	429/-	350/-	387/-
TX2	B11	B	Gaines	302/-	297/-	429/-	350/-	387/-
TX44	B13	B	Gaines	302/-	298/302	428/-	350/-	387/-
TX40	B14	B	Gaines	302/-	298/-	429/-	349/-	387/-
TX1	B15	B	Gaines	302/-	298/-	429/-	350/-	387/-
TX7	B16	B	Gaines	302/-	297/298/302	429/-	351/-	387/-
TX3	B18	B	Gaines	302/-	298/302	429/-	350/-	387/-
TX2	B19	B	Gaines	302/-	297/-	429/-	350/-	387/-
TX1	B20	B	Gaines	302/-	298/-	429/-	350/-	387/-
TX2	B21	B	Gaines	302/-	297/-	429/-	350/-	387/-
TX8	B22	B	Gaines	302/-	297/-	429/408/407	350/-	387/-
TX3	B23	B	Gaines	302/-	298/302	429/-	350/-	387/-
TX9	B25	B	Gaines	302/-	297/-	408/-	350/-	387/-
TX1	B26	B	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	D1	D	Comanche	302/-	298/-	429/-	350/-	387/-
TX1	D2	D	Comanche	302/-	298/-	429/-	350/-	387/-
TX1	D4	D	Comanche	302/-	298/-	429/-	350/-	387/-
TX1	D5	D	Comanche	302/-	298/-	429/-	350/-	387/-
TX1	D6	D	Comanche	302/-	298/-	429/-	350/-	387/-
TX1	D7	D	Comanche	302/-	298/-	429/-	350/-	387/-
TX1	D8	D	Comanche	302/-	298/-	429/-	350/-	387/-
TX1	D9	D	Comanche	302/-	298/-	429/-	350/-	387/-
TX2	E740	E	Gaines	302/-	297/-	429/-	350/-	387/-
TX1	E769	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E773	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E775	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E781	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E785	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E790	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E795	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX48	E810	E	Gaines	302/-	298/-	428/-	350/-	387/-
TX1	E815	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E825	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX46	E830	E	Gaines	304/-	-/300	429/-	350/-	387/-
TX1	E835	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E845	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E850	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E860	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX10	E865	E	Gaines	302/-	298/300	429/-	350/-	387/-
TX11	E880	E	Gaines	302/-	298/-	429/-	350/336	387/-
TX1	E885	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E895	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX2	E915	E	Gaines	302/-	297/-	429/-	350/-	387/-
TX11	E935	E	Gaines	302/-	298/-	429/-	350/336	387/-
TX1	E940	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E945	E	Gaines	302/-	298/-	429/-	350/-	387/-

Table C1 continued.

Genotype	Isolate	Field	County	Locus 5-2	Locus 20-3	Locus 36-4	Locus 14-4	Locus 117-4
TX11	E955	E	Gaines	302/-	298/-	429/-	350/336	387/-
TX1	E965	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX12	E975	E	Gaines	302/-	298/-	429/407	350/-	387/-
TX12	E985	E	Gaines	302/-	298/-	429/407	350/-	387/-
TX1	E995	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1005	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1025	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1035	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1045	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1055	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1065	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1075	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1095	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1105	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1115	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX2	E1125	E	Gaines	302/-	297/-	429/-	350/-	387/-
TX2	E1132	E	Gaines	302/-	297/-	429/-	350/-	387/-
TX13	E1145	E	Gaines	302/-	297/-	429/-	351/-	387/-
TX47	E1170	E	Gaines	302/-	297/-	429/407	350/-	387/-
TX14	E1190	E	Gaines	302/-	298/-	429/408	350/-	387/-
TX2	E1200	E	Gaines	302/-	297/-	429/-	350/-	387/-
TX15	E1220	E	Gaines	302/-	284/297/302	429/-	350/-	387/-
TX16	E1250	E	Gaines	302/-	284/297	428/-	350/-	387/-
TX2	E1270	E	Gaines	302/-	297/-	429/-	350/-	387/-
TX1	E1280	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX20	E1290	E	Gaines	304/-	286/300	429/-	350/-	387/-
TX20	E1300	E	Gaines	304/-	286/300	429/-	350/-	387/-
TX17	E1310	E	Gaines	304/-	298/-	429/-	350/336	387/-
TX11	E1360	E	Gaines	302/-	298/-	429/-	350/336	387/-
TX1	E1380	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1390	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1400	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1410	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX18	E1420	E	Gaines	302/-	298/-	429/-	350/336	387/368
TX19	E1440	E	Gaines	302/298	298/302	429/-	350/-	387/-
TX2	E1450	E	Gaines	302/-	297/-	429/-	350/-	387/-
TX20	E1460	E	Gaines	304/-	286/300	429/-	350/-	387/-
TX3	E1490	E	Gaines	302/-	298/302	429/-	350/-	387/-
TX26	E1520	E	Gaines	302/-	297/302	429/-	350/-	387/-
TX1	E1530	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX21	E1540	E	Gaines	302/-	300/-	429/-	350/-	387/-
TX1	E1560	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1600	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX26	E1610	E	Gaines	302/-	297/302	429/-	350/-	387/-
TX22	E1620	E	Gaines	302/-	302/-	429/-	350/336	387/-
TX26	E1640	E	Gaines	302/-	297/302	429/-	350/-	387/-
TX1	E1650	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX11	E1660	E	Gaines	302/-	298/-	429/-	350/336	387/-
TX1	E1680	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1690	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	E1720	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX5	E1730	E	Gaines	302/-	-/302	429/-	350/-	387/-
TX26	E1740	E	Gaines	302/-	297/302	429/-	350/-	387/-
TX1	E1750	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX21	E1760	E	Gaines	302/-	-/300	429/-	350/-	387/-
TX23	E1780	E	Gaines	304/298	298/-	429/-	350/-	387/-
TX1	E1790	E	Gaines	302/-	298/-	429/-	350/-	387/-
TX24	E1800	E	Gaines	302/-	284/298	429/-	350/-	387/-
TX25	E1810	E	Gaines	-/298	298/-	429/-	350/-	387/-
TX26	E1850	E	Gaines	302/-	297/302	429/-	350/-	387/-
TX27	F2	F	Erath	302/-	-/-	429/-	350/-	387/-
TX3	F3	F	Erath	302/-	298/302	429/-	350/-	387/-

Table C1 continued.

Genotype	Isolate	Field	County	Locus 5-2	Locus 20-3	Locus 36-4	Locus 14-4	Locus 117-4
TX1	F4	F	Erath	302/-	298/-	429/-	350/-	387/-
TX44	F8	F	Erath	302/-	298/302	428/-	350/-	387/-
TX2	F9	F	Erath	302/-	297/-	429/-	350/-	387/-
TX5	F10	F	Erath	302/-	302/-	429/-	350/-	387/-
TX28	H1	H	Gaines	302/-	298/-	429/-	350/-	387/372
TX1	H3	H	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	H5	H	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	H6	H	Gaines	302/-	298/-	429/-	350/-	387/-
TX5	H7	H	Gaines	302/-	302/-	429/-	350/-	387/-
TX1	H8	H	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	H10	H	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	H11	H	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	H12	H	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	H13	H	Gaines	302/-	298/-	429/-	350/-	387/-
TX29	H15	H	Gaines	302/298	297/-	429/-	350/-	387/-
TX30	H16	H	Gaines	302/298	298/-	429/-	350/-	387/-
TX30	H18	H	Gaines	302/298	298/-	429/-	350/-	387/-
TX30	H19	H	Gaines	302/298	298/-	429/-	350/-	387/-
TX30	H21	H	Gaines	302/298	298/-	429/-	350/-	387/-
TX31	H22	H	Gaines	301/304/305	298/-	429/-	350/-	386/-
TX21	H23	H	Gaines	302/-	300/-	429/-	350/-	387/-
TX32	H25	H	Gaines	300/305	300/-	429/-	350/-	387/-
TX1	J1	J	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	J2	J	Gaines	302/-	298/-	429/-	350/-	387/-
TX2	J4	J	Gaines	302/-	297/-	429/-	350/-	387/-
TX26	J6	J	Gaines	302/-	297/302	429/-	350/-	387/-
TX1	J8	J	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	J11	J	Gaines	302/-	298/-	429/-	350/-	387/-
TX2	J12	J	Gaines	302/-	297/-	429/-	350/-	387/-
TX33	J13	J	Gaines	302/-	298/300	429/-	351/-	387/-
TX34	J14	J	Gaines	302/-	284/298/302	429/-	350/-	387/-
TX1	J15	J	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	J16	J	Gaines	302/-	298/-	429/-	350/-	387/-
TX26	J17	J	Gaines	302/-	297/302	429/-	350/-	387/-
TX2	L1	L	Erath	302/-	297/-	429/-	350/-	387/-
TX2	L2	L	Erath	302/-	297/-	429/-	350/-	387/-
TX2	L3	L	Erath	302/-	297/-	429/-	350/-	387/-
TX2	L7	L	Erath	302/-	297/-	429/-	350/-	387/-
TX29	L10	L	Erath	302/298	297/-	429/-	350/-	387/-
TX2	P1	P	Erath	302/-	297/-	429/-	350/-	387/-
TX26	P2	P	Erath	302/-	297/302	429/-	350/-	387/-
TX26	P3	P	Erath	302/-	297/302	429/-	350/-	387/-
TX2	P7	P	Erath	302/-	297/-	429/-	350/-	387/-
TX2	P8	P	Erath	302/-	297/-	429/-	350/-	387/-
TX20	P9	P	Erath	304/-	286/300	429/-	350/-	387/-
TX1	T1-1	T	Erath	302/-	298/-	429/-	350/-	387/-
TX1	T1-2	T	Erath	302/-	298/-	429/-	350/-	387/-
TX1	T1-3	T	Erath	302/-	298/-	429/-	350/-	387/-
TX45	T1-4	T	Erath	302/-	298/-	428/-	349/-	387/-
TX1	T1-5	T	Erath	302/-	298/-	429/-	350/-	387/-
TX1	T1-8	T	Erath	302/-	298/-	429/-	350/-	387/-
TX35	T2-2	T	Erath	302/-	284/-	429/-	350/-	387/-
TX36	T2-3	T	Erath	302/-	297/-	429/-	350/-	387/370
TX2	T2-5	T	Erath	302/-	297/-	429/-	350/-	387/-
TX41	U1	U	Atascosa	304/-	286/-	429/-	350/-	387/-
TX1	U2	U	Atascosa	302/-	298/-	429/-	350/-	387/-
TX42	U5	U	Atascosa	302/-	298/-	429/-	351/-	387/-
TX43	U7	U	Atascosa	304/-	298/-	429/-	350/-	387/-
TX1	U8	U	Atascosa	302/-	298/-	429/-	350/-	387/-
TX1	U9	U	Atascosa	302/-	298/-	429/-	350/-	387/-
TX1	W6	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W10	W	Gaines	302/-	298/-	429/-	350/-	387/-

Table C1 continued.

Genotype	Isolate	Field	County	Locus 5-2	Locus 20-3	Locus 36-4	Locus 14-4	Locus 117-4
TX26	W15	W	Gaines	302/-	297/302	429/-	350/-	387/-
TX1	W20	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W25	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W30	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W40	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX2	W45	W	Gaines	302/-	297/-	429/-	350/-	387/-
TX1	W65	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W70	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX2	W75	W	Gaines	302/-	297/-	429/-	350/-	387/-
TX37	W80	W	Gaines	300/304	298/-	429/-	350/-	387/-
TX38	W87	W	Gaines	304/300	297/-	429/350	350/-	387/-
TX1	W95	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX39	W100	W	Gaines	302/-	286/302	429/-	350/-	387/-
TX2	W110	W	Gaines	302/-	297/-	429/-	350/-	387/-
TX2	W115	W	Gaines	302/-	297/-	429/-	350/-	387/-
TX2	W120	W	Gaines	302/-	297/-	429/-	350/-	387/-
TX40	W125	W	Gaines	302/-	298/-	429/-	349/-	387/-
TX1	W130	W	Gaines	302/-	297/-	429/-	350/-	387/-
TX1	W135	W	Gaines	302/-	297/-	429/-	350/-	387/-
TX29	W140	W	Gaines	302/298	297/-	429/-	350/-	387/-
TX1	W145	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX3	W155	W	Gaines	302/-	298/302	429/-	350/-	387/-
TX1	W160	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W165	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W170	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX3	W175	W	Gaines	302/-	298/302	429/-	350/-	387/-
TX29	W180	W	Gaines	302/298	297/-	429/-	350/-	387/-
TX1	W185	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX50	W195	W	Gaines	302/-	296/-	429/-	350/-	387/-
TX1	W200	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W210	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W215	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W220	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W225	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W230	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX2	W235	W	Gaines	302/-	297/-	429/-	350/-	387/-
TX2	W240	W	Gaines	302/-	297/-	429/-	350/-	387/-
TX30	W245	W	Gaines	302/298	298/-	429/-	350/-	387/-
TX1	W250	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX1	W260	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX2	W265	W	Gaines	302/-	297/-	429/-	350/-	387/-
TX1	W270	W	Gaines	302/-	298/-	429/-	350/-	387/-
TX2	W275	W	Gaines	302/-	297/-	429/-	350/-	387/-
TX2	W280	W	Gaines	302/-	297/-	429/-	350/-	387/-
TX49	W285	W	Gaines	302/-	297/-	429/-	350/-	386/-
TX2	W290	W	Gaines	302/-	297/-	429/-	350/-	387/-
BR1	Bra1	n.d	Brazil	301/-	298/-	420/-	350/-	377/-
BR1	Bra2	n.d.	Brazil	301/-	298/-	420/-	350/-	377/-
BR1	Bra3	n.d.	Brazil	301/-	298/-	420/-	350/-	377/-
BR1	Bra6	n.d.	Brazil	301/-	298/-	420/-	350/-	377/-
BR1	Bra5	n.d.	Brazil	301/-	298/-	420/-	350/-	377/-

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