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I am submitting herewith a thesis written by Renae Erin DeVries entitled “Genetic diversity of *Sclerotinia homoeocarpa* delineated by AFLP and vegetative compatibility.” I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

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Genetic diversity of *Sclerotinia homoeocarpa* delineated by AFLP and vegetative compatibility

A Thesis Presented for the Master of Science Degree

The University of Tennessee, Knoxville Tennessee

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DEDICATION

This thesis is dedicated to my parents, Lavern and Regina DeVries, and all of my family and friends, for their constant support, advice, and encouragement.

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ABSTRACT

Sclerotinia homoeocarpa F.T. Bennett is the causal agent of dollar spot disease that infects turfgrasses of golf courses in Tennessee as well as around the world. Dollar spot taints the uniformity and aesthetic value of putting greens, therefore causing a serious problem on golf courses. Isolates were collected from across Tennessee and northern Mississippi and tested for fungicide resistance by Pamela Baird. Genetic diversity between the isolates was investigated by vegetative compatibility, conserved gene sequences, and AFLP (amplified fragment length polymorphism) to reveal associations with fungicide resistance. Vegetative compatibility tests were completed by pairing six tester strains with Tennessee and northern Mississippi isolates on potato dextrose agar. Additionally, isolates from all locations were paired against each other. After observations of the mycelial interaction zone, isolates were generally compatible within a location and incompatible between locations. Some isolates were delineated into vegetative compatibility groups (A, B, and D), and fungicide resistance could not be associated with a particular vegetative compatibility group. Genetic similarities of isolates at the vegetative compatibility level could be attributed to founder effects. Conserved gene sequences of the CPS region of CAD, EF1-α, β-tubulin, and ITS indicated 100% homology between isolates. The ITS region of *Sclerotinia* spp., *Rutstroemia* sp., and *Lanzia* sp. were compared using parsimony analysis to reveal *S. homoeocarpa* could be more genetically related to *Rutstroemia* spp. than *Sclerotinia* spp. AFLPs were detected by capillary gel electrophoresis, and calculated similarity indices

indicated 86-100% similarity between the 60 isolates. Pairwise differences ranged 0-14 implying some isolates were completely identical at all loci. Isolates did not cluster according to fungicide resistance during UPGMA analysis, but did appear to cluster according to vegetative compatibility group and location. Although associations could not be made between molecular markers and fungicide resistance, links between vegetative compatibility and AFLP markers provide a foundation from which other studies could be performed.

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CHAPTER ONE

Literature Review

Dollar Spot Disease:

Sclerotinia homoeocarpa F.T. Bennett is the causal agent of dollar spot disease that infects turfgrasses of golf courses in Tennessee as well as around the world. Dollar spot taints the uniformity and aesthetic value of putting greens, therefore causing a serious problem on golf courses (Burpee, 1997). Disease is most severe in areas with closely mowed grasses (Smiley, 1983) and low nitrogen (Pataky, 1999). To control the spread of this disease, turf managers implement fungicide programs, which can lead to survival of *S. homoeocarpa* isolates that are resistant to fungicides (Hsiang, 1998). As a result, dollar spot has become a persistent problem for golf course managers.

Epidemiology:

Sclerotinia homoeocarpa can be found in the spring through the fall, but is especially active during periods with warm temperatures, high humidity, and cool nights during the summer. These conditions provide a humid leaf canopy, which in addition to dry root systems create perfect environmental conditions for dollar spot to occur (Elliot, 2001). When temperatures are 10 °C to 32.2 °C (Chakraborty, 2003), dormant mycelium or stromata resume growth towards the humid air of the foliage canopy. However, if temperatures rise above 32.2 °C, fungal growth can be suppressed (Rimelspach, 1996). When growing mycelium reaches the surface of a moist leaf, infection can occur. Penetration of the leaf

occurs directly as the mycelium invades the tissue causing a lesion to form (Smiley, 1983).

Classification:

Conventionally, *S. homoeocarpa* is the preferred name for the pathogen that causes dollar spot (Bennett, 1937). Initially this disease was referred to as "small brown patch," since it was thought to be caused by a species of *Rhizoctonia* (Monteith and Dahl, 1937). Lack of sclerotia, fertile apothecia, and ascospores offers taxonomists with an immense challenge to classify this pathogen. Members of the *Sclerotinia* genus, such as *S. sclerotiorum* (Libert) de Bary, *S. minor* Jagger, *and S. trifoliorum* Eriks, are categorized by features that arise during sexual stages (Pratt, 1992). In general, *S. homoeocarpa* produces few morphological characteristics that would normally permit classification as a *Sclerotinia* species thus causing controversy as to whether it has been assigned properly to the correct genus.

Occasional formation of sterile apothecia has allowed classification of these fungi into either the *Lanzia, Moellerodiscus* (Kohn, 1979), or *Rutstroemia* genera (Whetzel, 1945). Whetzel (1945) was the first to suggest that the dollar spot pathogen was incorrectly classified when he observed that its apothecia did not arise from tuberoid sclerotia. *Sclerotinia homoeocarpa* ITS 1 (internal transcribed spacer) region sequences were compared with ITS 1 sequences of *Lanzia* sp., *Moellerodiscus* sp., and *Rutstroemia* sp. The dollar spot pathogen shared the largest amount of homology with *Rutstroemia* sp., which led the

researchers to believe that the pathogen was incorrectly included in the *Sclerotinia* genus (Powell and Vargas, 1999).

Hosts:

Dollar spot pathogen causes serious disease problems on a variety of turfgrasses. Its host range includes creeping bentgrass (*Agrostis stolonifera* L.), Kentucky bluegrass (*Poa pratensis* L.), annual blue grass (*Poa annua* L.), and fine-leaf fescues, perennial ryegrass, and tall fescue are often infected by this pathogen. Southern turfgrasses such as bermudagrass (*Cynodon dactylon* (L.) Persoon) and zoysiagrasses, tend to be more sensitive to this disease, and produce severe symptoms (Pataky, 1999).

Symptoms, Signs, and Life Cycle:

Symptoms of *S. homoeocarpa* infection are recognized as silver dollar-sized brown patches on turfgrass. A single patch usually does not grow to be larger than 5 cm on putting greens, but often, as the pathogen spreads several spots coalesce to create one large, brown patch. In residential lawns consisting of taller grasses, chlorotic areas can vary between 2 to 15 cm or more in diameter (Smiley, 1983).

Occasionally dollar spot disease is mistaken for brown patch symptoms caused by *Rhizoctonia solani* Kuhn since both diseases cause the development of straw-colored areas of dead grass. These two diseases can be differentiated by measuring the size of the diseased patch, since *Rhizoctonia solani* causes a larger brown patch (Elliot, 2001). *Rhizoctonia* also can be differentiated from *S. homoeocarpa* by its right-angled branching of hyphae and consistent hyphal

diameter. *Sclerotinia homoeocarpa* cytoplasm also appears granular in comparison to *R. solani* (Allen, 2005).

Leaf symptoms of dollar spot first appear as tiny yellow spots and expand outward to form hourglass-shaped lesions on infected grass blades (Rimelspach, 1996). These lesions turn light brown with dark brown borders and eventually the entire leaf becomes bleached (Vargas, 2005). A small, sunken, brown patch forms as the infection spreads to other leaves (Rimelspach, 1996).

On mornings with heavy dew, cottony mycelia are often visible (Elliot and Simone, 2001), and eventually disappear after the turf dries (Rimelspach, 1996). Mycelia or stromata spread by debris and grass clippings from infected turfgrass (Powell and Vargas, 2001). In subtropical and temperate regions of the world, *S. homoeocarpa* disease cycle is primarily maintained by mycelial growth from leaf blade to leaf blade. As the mycelium spreads, lesions form, more mycelium can grow, and the cycle repeats (Smiley, 1983).

Control:

Management of dollar spot has become very costly. Throughout the world, more money is spent to chemically control dollar spot than any other turfgrass disease (Vargas, 2005). Control of *S. homoeocarpa* is a high priority for golf course managers and several different measures can be taken to reduce damage from the disease. Cultural techniques such as proper use of fertilizers are often implemented for control (Pataky, 1999). Increased amounts and frequency of nitrogen applications can effectively promote maintenance of disease (Couch and Bloom, 1960). Disease can be prevented by avoiding

conditions of extremely low nitrogen. When the soil is nitrogen deficient, it is best to use a slow-release nitrogen fertilizer that can maintain the nitrogen balance. Light, frequent applications of foliar fertilizer may be applied to control dollar spot on putting greens. During the summer months, dollar spot can be controlled if one pound of nitrogen per 304.8 m^2 is applied each month. A balance of slow-release potassium fertilizer can be coupled with slow-release nitrogen fertilizer to suppress disease (Elliot and Simone, 2001).

Use of resistant cultivars (Bonos et al., 2004), proper watering, and mowing practices have also been implemented to control dollar spot disease. Several Kentucky bluegrass cultivars have demonstrated resistance (Pataky, 1999). Resistant clones of creeping bentgrass exhibited smaller lesion diameter and prevented the spread of the disease as well as coalescing of spots. Large trichomes produced by resistant clones appeared to aid in resistance to the disease in comparison to susceptible clones with smaller trichomes (Bonos et al., 2004). Early morning irrigation facilitates removal of exudates and dew that can promote fungal growth. This watering practice allows for leaves to dry during the day, thus impeding fungal growth. Use of sharp mower blades and mowing when the grass is dry can also decrease fungal spread (Allen, 2005). Light weight rolling three times a week soon after early morning mowing can also significantly reduce dollar spot by spreading accumulations of guttation water (Nikolai, 2002).

Fungicide Resistance:

Although cultural techniques are available, control of *S. homoeocarpa* is greatly dependent upon fungicide practices. Contact fungicides, such as anilazine, chlorothalonil, and quintozene commercial fungicides as well as systemic fungicides benomyl, thiophanate-methyl, iprodione, and propiconazole, have been applied to relieve symptoms (Serafinchon, 2001). Over dependence on fungicides has resulted in the selection of many isolates of *S. homoeocarpa* that are resistant to various fungicides.

Fungicide resistance has been a concern for some time for many researchers. For example, isolates collected from various areas across southern Ontario, Canada showned varying sensitivity to propiconazole, a demethylation inhibitor (DMI). Isolates that showed a reduction in sensitivity to propiconazole were less virulent and when propiconazole was not used isolates with less sensitivity demonstrated better survival (fitness) than those with greater sensitivity to the fungicide (Hsiang et al., 1998).

Molecular Markers:

Molecular markers are often used to quantify genetic diversity detected between organisms. DNA-based assays allow for the production of large numbers of molecular markers that can be more genetically revealing than morphological or biochemical traits. Polymorphisms, or differences between organisms, can be detected through DNA sequencing or DNA fingerprinting/profiling. These differences can be complied to estimate genetic similarities and differences between individuals and evolutionary relationships

within a population.

Arbitrarily primed PCR (polymerase chain reaction) and other PCR-based multi-locus profiling are popular techniques that use indiscriminate or semi-indiscriminate primers for amplification of DNA products. When using these techniques, prior knowledge of organism DNA sequence is not necessary. Sequencing of DNA can be costly and require a lot of skill (Karp and Edwards, 1997) thus, producing an arbitrary genetic profile or fingerprint of the organism provides a “short-cut” for discerning genetic diversity (O’Malley and Whetten, 1997).

Although arbitrary molecular markers are considered to be more efficient, little information is created about the alleles or genes. Conserved genes are characterized by a base sequence in a DNA molecule that has remained essentially unchanged throughout evolution. In essence, if the gene codes for an important protein, it would be expected to see little change in DNA sequence between individuals or populations. Some parts of genes show more variation than other such as introns, 5’-flanking sequences that may be regulatory, nontranscribed DNA after the 3’ end of a gene, and wobble (third positions) of a codon. Since changes in these areas do not typically cause an amino acid polymorphism, nucleotide differences can be tolerated and can be expected in these areas (Griffiths et al., 1999). Depending on the gene, sequence, or species 0.5 to 20 differences per kilobase of DNA sequence maybe seen when studying nucleotide diversity (O’Malley and Whetten, 1997). In essence, choosing the correct region of DNA for detection of genetic variation

depends on the amount of polymorphism that is needed to be produced for the study. Characterization of DNA by sequencing can resolve diversity at any taxonomic level (Kohn, 1992).

PCR (Polymerase Chain Reaction):

Development of the PCR technique set the ground-work for many other molecular techniques (Mullis and Faloona, 1987). DNA is first heated, which causes denaturation (approximately 95 °C), or separation of the double stranded DNA to single stranded templates (Figure 1.1). After denaturation primers, or short pieces of single stranded DNA, anneal to complementary sequences around the region of DNA of interest. The annealing temperature that is applied is dependent on the primer sequence, but typically occurs at/or around 58 °C. Once the primers attach, DNA polymerase extends the primers by adding nucleotides that are complementary to the opposite strand (approximately 72 °C). After a few cycles of these three steps, a fragment of DNA is produced from which a large number of copies can be created (Weaver, 2002).

DNA Sequencing:

A method to determine the exact sequence of a piece of DNA was developed by Sanger et al. (1977). The Sanger dideoxy method of DNA sequencing involves four different PCR reactions, each containing a dideoxyribonucleotide triphosphate (ddNTP) or a chain terminator. Each reaction contains all four normal deoxyribonucleotide thriphosphate (dNTPs) and one ddNTP (either C, T, A, or G). In addition to the ddNTP, one dNTP is also labeled with a radioisotope that can allow for fragment detection on exposed film after gel

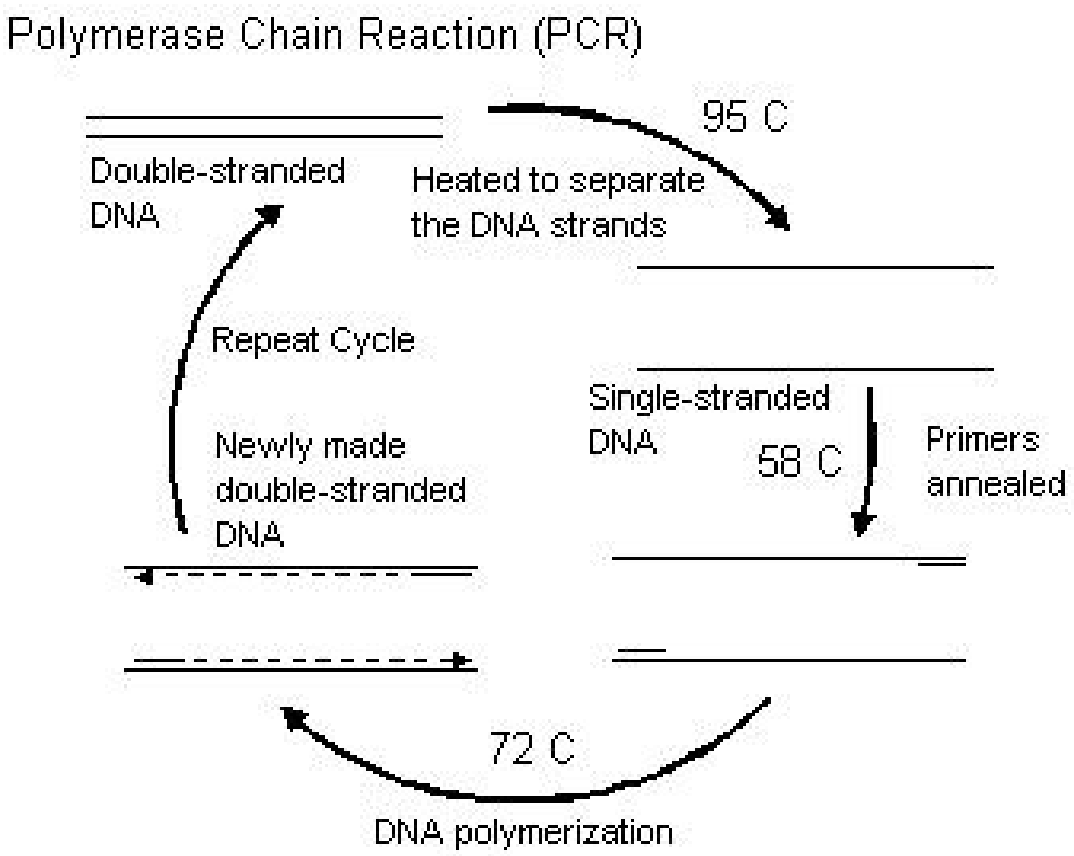

Figure 1.1: Polymerase chain reaction (PCR) (modified from Murdoch University, 2000). Once double-stranded DNA is denatured, primers can anneal, DNA polymerase then extends the primer by inserting nucleotides that are complementary to the opposite strand. After a few cycles, a fragment is produced that contains the DNA of interest.

electrophoresis. Deoxynucleotides already lack a hydroxyl group on the 2' carbon, but in contrast chain terminators lack a 3' hydroxyl group in addition to the 2'. Since DNA polymerase requires a 3' hydroxyl group in order to extend the DNA during replication, the incorporation of one ddNTP terminates replication of the rest of the chain. Termination of DNA replication at all possible positions results in as many fragments as there are bases in the piece of DNA. The products of each reaction are "electrophoresed" on a polyacrylamide gel, fragments appear and the DNA sequence can be manually read from the bottom of the gel to the top.

Although new technologies have developed since the Sanger sequencing method, the same basic concept is still applied. Conventionally, automated sequencers are used that have the ability to read sequences when a laser excites the fluorescently labeled ddNTPs (Figure 1.2). Each ddNTP is labeled with a different dye that fluoresces a different color, thus eliminating the need for more than one reaction. After fluorescence detection, the sequence is visualized on a computer as a chromatogram (Weaver, 2002).

Other Molecular Markers:

Sequencing and PCR techniques are very useful when studying diversity, but hybridization-based (non-PCR) techniques can also be used. For example, RFLP (restriction fragment length polymorphism) can be used when organisms are differentiated by analysis of patterns derived from enzymatic cleavage of DNA. If the distance between two restriction enzyme sites differs between two organisms, the length of the fragments produced will also vary after restriction

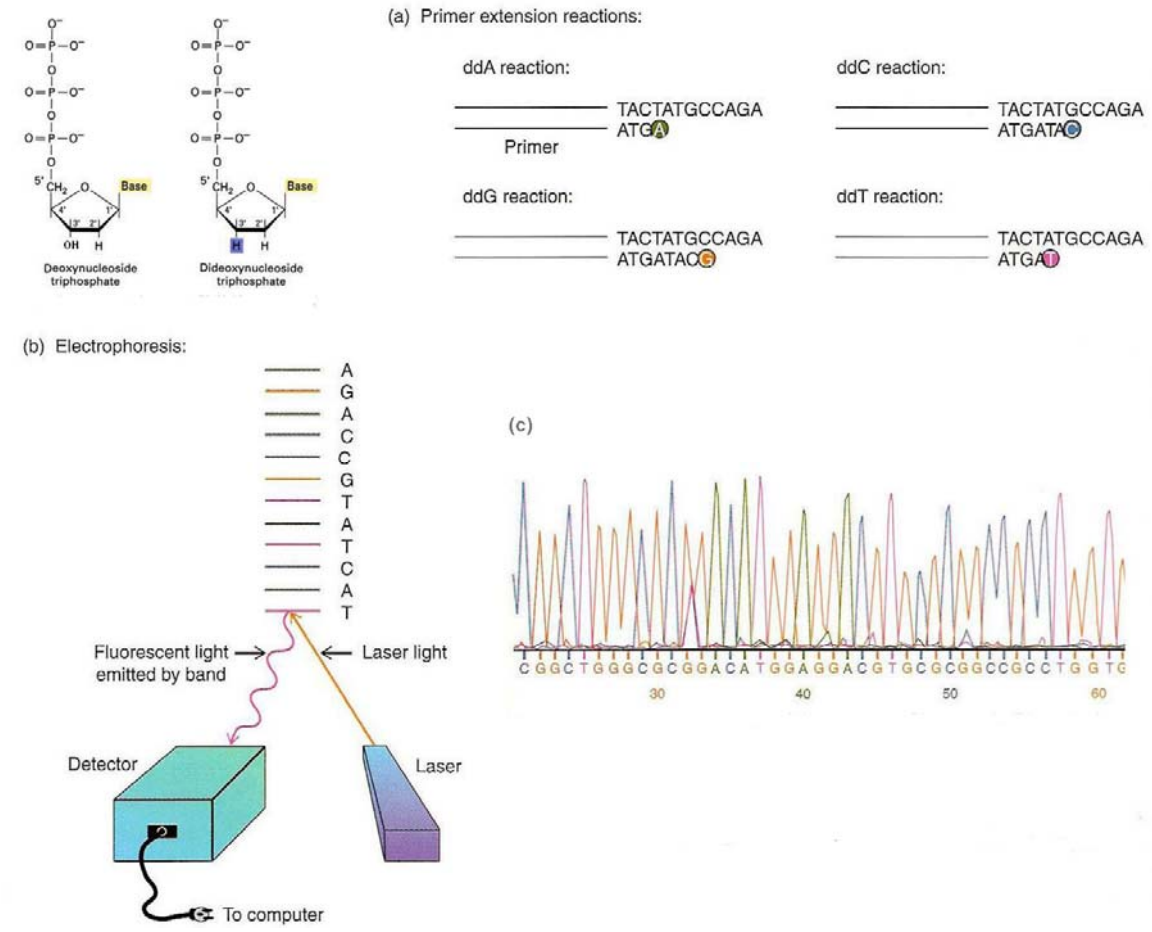

Figure 1.2: DNA sequences can be determined by using fluorescently labeled chain terminators or dideoxynucleotide triphosphates (ddNTPs) (modified from Weaver, 2002 and LaMunyon, 2001). a) If a ddNTPs is inserted during DNA replication, the reaction is terminated. b) One fragment for every base in the sequence is produced and is run on a gel. c) The fluorescence is detected and a chromatogram is produced depicting the DNA sequence.

enzyme digestion. The similarity of the patterns generated can be used to differentiate species or strains from one another. These polymorphisms can be visualized on an ethidium bromide stained agarose gel after electrophoresis and converted to binary data. Often a labeled probe that is complementary to an area of nucleotides in one or more of the restriction fragments is hybridized to digested DNA during Southern blot (Sealey and Southern, 1982) analysis. If the probe anneals successfully to a restriction fragment, restriction sites can be detected within the gene or region of interest (Griffiths et al., 1999).

Techniques vary in the length and sequence of the primers, stringency of PCR conditions, and type of fragment separation and detection. Some types of techniques that produce arbitrary molecular markers include RAPD (random amplified polymorphic DNA) (Williams et al., 1990), DAF (DNA amplification fingerprinting) (Caetano-Anollés et al., 1991), and AFLP (amplified fragment length polymorphism) (Vos et al., 1995). Each technique varies as to how many markers are produced and some can be more applicable for different taxonomical levels. For instance, production of RAPD markers is more applicable for detection of diversity at the intraspecific level whereas RFLPs can be useful at the intraspecific level and at the species level (Kohn, 1992).

AFLP (Amplified Fragment Length Polymorphsim) Analysis:

Amplified fragment length polymorphism (AFLP) is a technique that potentially allows for the recognition of genetic relationships between isolates. Even when little information is known about a genome, such as its genetic complexity or specific DNA sequences, the AFLP technique can be applied to

determine genetic variability (Vos et al., 1995). AFLP typically produces a large number of loci that can be assayed quickly, and be applied to most genetic variability studies since markers can be detected at almost any taxonomic level (Vos and Kuiper, 1997).

Although AFLP can be widely applied, it has limitations. If the organisms being studied share less than 90% homology, few bands may be found that are common to all samples indicating that even when similar sized bands are detected they may be attributed to chance (random). For example, lack of homology can decrease the usefulness of AFLP markers in bacterial studies since changes can occur very quickly in the genome. In contrast, if the genome being studied has little sequence variation, AFLP may also produce few polymorphic markers that are useful for genetic variation research (Vos and Kuiper, 1997).

AFLP includes the strategies of restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) in concert to detect selectively amplified fragments (Miyashita, 1999). This process includes the following three general steps: 1) digestion of the genomic DNA with restriction endonucleases and modification of the fragmented DNA by attachment of synthetic sequences, 2) selective amplification by polymerase chain reaction (PCR), and 3) analysis of fragments by gel electrophoresis (Figure 1.3). These steps are described in more detail later.

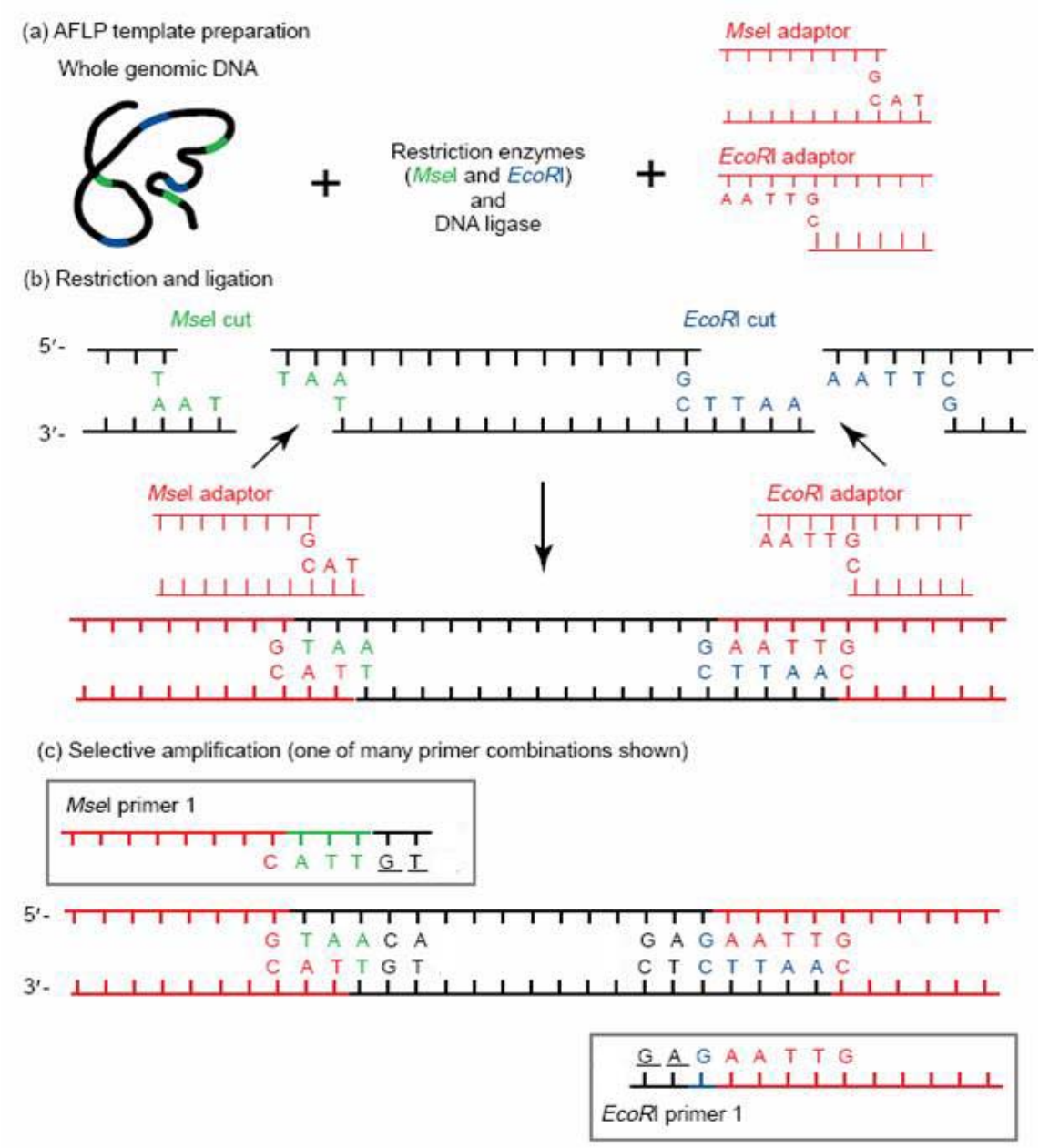

Figure 1.3: AFLP begins as the genomic DNA is digested by restriction enzymes. Adaptors can then be ligated to the over hangs created by the restriction enzymes (modified from Mueller et al., 1999). PCR can then be performed to select for fragments that have adaptors and have nucleotides next to the restriction site that match the selective primer.

Restriction/Ligation:

DNA is fragmented (restricted) using one restriction enzyme as a rare cutter, and one as a frequent cutter during the AFLP technique. For example, *Eco* RI recognizes a restriction site sequence that is 6 bp long whereas *Mse* I recognizes a restriction site sequence that is 4 bp long. Statistically speaking, since *Mse* I searches for a shorter DNA sequence it "cuts" the DNA more frequently than *Eco* RI. *Eco* RI and *Mse* I are conventionally used for most studies since other enzyme combinations do not produce high quality fingerprints. Due to the nature of the two endonucleases (*Eco* RI and *Mse* I), a sticky end is left after the palindromic restriction site sequence is identified. A sticky end refers to the single strand over-hang produced by the enzyme once it cuts at the restriction site. In this case *Eco* RI cuts less frequently than *Mse* I, therefore it is expected that more fragments will be produced with both ends being cut by *Mse* I. The only fragments that are amplified during this process are those that were cut by the rare cutter/frequent cutter endonucleases (Vos et al., 1995). Simultaneous with digestion, restriction fragments are modified by ligation of synthetic DNA adapters to the ends with DNA ligase.

Ligation can be accomplished by taking advantage of the sticky ends produced by the two restriction endonucleases. The base pairs of the complementary sequences of the adapters and the restriction site sequence overhang can anneal and the nick in the phosphodiester backbone can be sealed by the enzymatic activity of T4 DNA ligase and ATP. Taq polymerase then can complete the amplification of the fragment by the addition of deoxynucleotide

triphosphates (dNTPs) in the preselective reaction (Vos et al, 1995).

Preselective Reaction:

An unmanageable amount of fragments are usually produced from the restriction/ligation step, therefore it would be difficult to begin data analysis at this step. The preselective reaction helps decrease the number of fragments through amplification of only fragments with adaptors attached. When analyzing large genomes, primers in the preselective reaction select for one base within the sequence next to the restriction site. For instance, the primer (5' CTC GTA GAC TGC GTA CCA ATT CA 3') is annealed to *Eco* RI side and is complementary to the adapter and the *Eco* RI restriction site. In addition to the complementary sequence, it also has a one base extension, adenine, on the 3' end. The primer (5' GAC GAT GAG TCC TGA GTA AC 3') for the *Mse* I side of the fragment is complementary to the adapter and the *Mse* I restriction site and also has a one base extension, but uses cytosine (Vos et al., 1995).

The addition of one base extensions help decrease the number of fragments that are amplified, which in turn decreases the number of fragments visualized during capillary gel electrophoresis. When studying smaller genomes, such as fungi, primers are used during the preselective reaction that do not contain a one base extension, but are only complementary to the adaptor. The one base extension may be too discriminatory and enough useful fragments may not be produced for analysis (Vos and Kuiper, 1997).

Selective Reaction:

The selective reaction selects for fragments that contain specified bases next to the restriction site sequence. For example, only fragments that contain 5'ACC 3' on the *Eco* RI side of the fragment and 5'CCT 3' on the *Mse* I side of the fragment will be amplified. This step further reduces the number of fragments produced by PCR and visualized during capillary gel electrophoresis. Just as the preselective reaction, selection of three bases next to the restriction site causes too large of a reduction in fragments for analysis when studying smaller genomes. When studying microorganisms, bacteria or fungi, only a two base extension is used for selective amplification (Vos and Kuiper, 1997).

The preselective and the selective steps are performed separately to increase the selectivity of fragments by the primers. In studies by Vos et al. (1995), the amount of background smears were decreased in the fingerprint patterns, and bands were absent when the preselective step was not performed. These bands were otherwise present when both the preselective and the AFLP amplification were performed. Primers with a three base extension caused a decreased amount of base mismatching by the sequence adjacent to the restriction site during PCR in comparison to using four base extension primers (Vos et al., 1995).

Vegetative Compatibility:

A more superficial method to detect genetic variability is pairing of isolates for detection of vegetative compatibility or anastomosis. Vegetative compatibility is a classical genetic technique that allows for identification of clonal sub-

populations or individuals in a species. Although these pairings can indicate that high genetic diversity exists, often this information can be coupled with molecular markers (Kohn et al., 1992), such as AFLP (Vigi et al., 2004). Vegetative compatibility can be assessed by culturing two isolates on water agar (or other) medium and examining the contact region for either confluent hyphal growth (anastomosis).

Rhizoctonia solani has been studied extensively using vegetative compatibility and eleven anastomosis groups (AGs) have been recognized within this species (Correll, 1992). Although considered morphologically similar, the anastomosis groups are genetically isolated (Anderson, 1982). Genetic exchange occurs in the areas of anastomosis. When confluent hyphal growth (anastomosis) is observed, the two isolates are considered to be closely enough related that exchange of nuclei between hyphae is possible. However, if the isolates are vegetatively incompatible, an area of hyphal death or barrage zone is observed indicating there is no exchange of genetic material (Correll, 1992).

When individuals within the same species are physiologically different, a heterokaryon can be formed. If a stable heterokaryon, or a cell containing two or more genetically different nuclei, is established, the pair of isolates are said to be compatible and members of the same vegetative compatibility group (VCG). Vegetative incompatibility, or *vic*, loci are spread throughout the genome and control the phenotype of the VCG. VCGs are differentiated by either being different at all, some, or one *vic* loci. It is not possible to determine how many hetero-allelic *vic* loci are needed for an incompatible reaction to occur at the

phenotype level. Self-incompatibility can also exist when a mutation has occurred at the heterokaryon self-incompatibility loci (*his*) (Leslie, 1996).

The mechanism of this multilocus phenomenon of non-sexual genetic exchange has not been completely differentiated and at present it is unfeasible to know how many genes are actually involved. The following classes of mechanisms have been proposed for heterokaryosis: 1) formation and 2) maintenance. The formation mechanism is considered to be responsible for mechanical and structural events during heterokaryon formation. The maintenance mechanism is probably involved in killing machinery that results in cell death. Chemical agents that are produced during the killing mechanism could some day provide an ideal set of antifungal compounds (Leslie, 1996).

CHAPTER TWO

Evaluation of Vegetative Compatibility of *Sclerotinia homoeocarpa* Tennessee and Mississippi Golf Course Isolates

Introduction:

Evidence collected by Baird (2005) revealed varying resistance to fungicides by *S. homoecarpa* isolates from eight golf courses in Tennessee and northern Mississippi. The courses included in Tennessee were in western, central, eastern, and southeastern parts of the state. Isolates from Legends Golf Course in Franklin, TN contained the following three areas for collection: Legends (L), Little Course 1 (L1), and Little Course 2 (L2). Little Course 1 and 2 were adjoining courses where experimental fungicides were being tested. Samples were exposed to different concentrations of various fungicides. Resistance to iprodione, thiophanate-methyl, and propiconazole was detected. Some isolates displayed multi-resistance or sensitivity (Baird, 2005). Isolate locations, bentgrass cultivar hosts, and resistance have been summarized in Table 2.1. Varying resistance and sensitivity implies that genetic diversity is present between isolates locations and between individuals.

This portion of this thesis project will analyze vegetative compatibility between isolates and the ability to exchange genetic material asexually. Separation of isolates into vegetative compatibility groups will help understand possible clonal nature of these populations, especially if the differences in vegetative compatibility can be seen at a particular site. This could indicate that the pressure of more than one population or *de novo* mutations with in a

Table 2.1: Isolates of *Sclerotinia homoeocarpa*, golf courses, location, fungicide resistance and hosts.

Isolate	Golf Course	Location	Fungicide Resistance	Bentgrass Cultivar Host
BM4	Bays Mountain	Seymour, TN	none	Penncross, L 93, Crenshaw
BM7	Bays Mountain	Seymour, TN	none	Penncross, L 93, Crenshaw
BM8	Bays Mountain	Seymour, TN	none	Penncross, L 93, Crenshaw
BM9	Bays Mountain	Seymour, TN	none	Penncross, L 93, Crenshaw
BM10	Bays Mountain	Seymour, TN	Iprodione	Penncross, L 93, Crenshaw
CH1	Cherokee	Olive Branch, MS	Thiophanate-methyl	Crenshaw
CH2	Cherokee	Olive Branch, MS	Thiophanate-methyl	Crenshaw
CH3	Cherokee	Olive Branch, MS	Thiophanate-methyl	Crenshaw
CH4	Cherokee	Olive Branch, MS	Thiophanate-methyl	Crenshaw
CH5	Cherokee	Olive Branch, MS	Thiophanate-methyl	Crenshaw
T1	Cottonwoods	Robinsville, MS	Iprodione	Crenshaw
T2	Cottonwoods	Robinsville, MS	Iprodione	Crenshaw
T3	Cottonwoods	Robinsville, MS	Iprodione, Thiophanate-methyl	Crenshaw
T6	Cottonwoods	Robinsville, MS	Iprodione, Thiophanate-methyl	Crenshaw
T9	Cottonwoods	Robinsville, MS	Iprodione	Crenshaw
G2	Gettysvue	Knoxville, TN	Thiophanate-methyl	Crenshaw
G3	Gettysvue	Knoxville, TN	Thiophanate-methyl	Crenshaw
G4	Gettysvue	Knoxville, TN	Thiophanate-methyl	Crenshaw
G5	Gettysvue	Knoxville, TN	Thiophanate-methyl	Crenshaw
G8	Gettysvue	Knoxville, TN	Thiophanate-methyl	Crenshaw
L1	Legends	Franklin, TN	Iprodione	Pennlinks
L2	Legends	Franklin, TN	Iprodione	Pennlinks
L3	Legends	Franklin, TN	none	Pennlinks
L4	Legends	Franklin, TN	Iprodione	Pennlinks
L5	Legends	Franklin, TN	none	Pennlinks
L1-1	Little Course 1	Franklin, TN	Iprodione	18th Green
L1-2	Little Course 1	Franklin, TN	Iprodione	18th Green
L1-3	Little Course 1	Franklin, TN	Iprodione	18th Green
L1-4	Little Course 1	Franklin, TN	Iprodione	18th Green
L1-5	Little Course 1	Franklin, TN	Iprodione	18th Green

Table 2.1: Continued

Isolate	Golf Course	Location	Fungicide Resistance	Bentgrass Cultivar Host
L2-1	Little Course 2	Franklin, TN	none	SR 1020
L2-2	Little Course 2	Franklin, TN	Iprodione	SR 1020
L2-3	Little Course 2	Franklin, TN	none	SR 1020
L2-4	Little Course 2	Franklin, TN	Iprodione	SR 1020
L2-5	Little Course 2	Franklin, TN	Iprodione	SR 1020
W5	Willow Springs	Athens, TN	Thiophanate-methyl,Propiconazole, Iprodione	Crenshaw
W6	Willow Springs	Athens, TN	Thiophanate-methyl,Propiconazole, Iprodione	Crenshaw
W7	Willow Springs	Athens, TN	Thiophanate-methyl,Propiconazole, Iprodione	Crenshaw
W8	Willow Springs	Athens, TN	Thiophanate-methyl,Propiconazole, Iprodione	Crenshaw
W10	Willow Springs	Athens, TN	Thiophanate-methyl,Propiconazole, Iprodione	Crenshaw
WW1	Whispering Woods	Olive Branch, MS	Thiophanate-methyl	Crenshaw and L93
WW2	Whispering Woods	Olive Branch, MS	Thiophanate-methyl	Crenshaw and L93
WW3	Whispering Woods	Olive Branch, MS	Thiophanate-methyl	Crenshaw and L93
WW4	Whispering Woods	Olive Branch, MS	Thiophanate-methyl	Crenshaw and L93
WW5	Whispering Woods	Olive Branch, MS	Thiophanate-methyl	Crenshaw and L93
M1	Memphis National	Memphis, TN	none	Crenshaw
M2	Memphis National	Memphis, TN	none	Crenshaw
M3	Memphis National	Memphis, TN	none	Crenshaw
M4	Memphis National	Memphis, TN	none	Crenshaw
M5	Memphis National	Memphis, TN	none	Crenshaw
MID	NA	Michigan	Iprodione	unknown
MIB	NA	Michigan	Benzimidazole (Thiophanate-methyl)	unknown
MIDMI	NA	Michigan	Thiophanate-methyl, Propiconazole	unknown
MIW	NA	Michigan	none	unknown
VCG A	NA	Michigan	unknown	unknown
VCG B	NA	Michigan	unknown	unknown
VCG C	NA	Michigan	unknown	unknown
VCG D	NA	Michigan	unknown	unknown
VCG E	NA	Michigan	unknown	unknown
VCG F	NA	Michigan	unknown	unknown

a population could possibly be caused by fungicide selection. If groups are present, they could be associated with fungicide resistance.

Materials and Methods:

Vegetative Compatibility Between Isolates:

Fifty *S. homoeocarpa* isolates were selected from the original 90 provided by Pam Baird, University of Tennessee. Depending on resistance and susceptibility, five isolates from each of the ten collection sites were chosen. In addition to these fifty isolates, four isolates Baird received from Michigan State University (courtesy of J. Vargas) with known resistance to certain fungicides were also included (Table 2.1). Each isolate was incubated at 22 °C on autoclaved Difco™ PDA (potato dextrose agar) (starch 4.0 g/L, dextrose 20.0 g/L, 15.0 g/L agar) (Becton, Dickenson, and Co., Sparks, MD) in 60 X 15 mm petri dishes (Fisherbrand, Atlanta, GA) for one week (Figure 2.1). Mycelial plugs were cut with sterile plastic soda straws and placed approximately 1.5 cm apart on PDA dishes. Pairings were incubated at 26 °C for one week with 12 hour light and dark periods (Kohn and Anderson, 1990). All incubations took place in a Low Temperature Illuminated Incubator 818 (Precision Scientific, Chicago, IL). PDA was amended with 5 drops of red food coloring (Kroger Brand, Cincinnati, OH) during initial trials to aid in scoring of reactions due to dye accumulation in the contact zone in incompatible reactions (Kohn,1990). After pairing isolates on both regular PDA and amended PDA, no difference was found in the ability to score the reaction, thus PDA without food coloring was used for the rest of the pairings. Presence of an obvious dark barrage zone between PDA plugs was

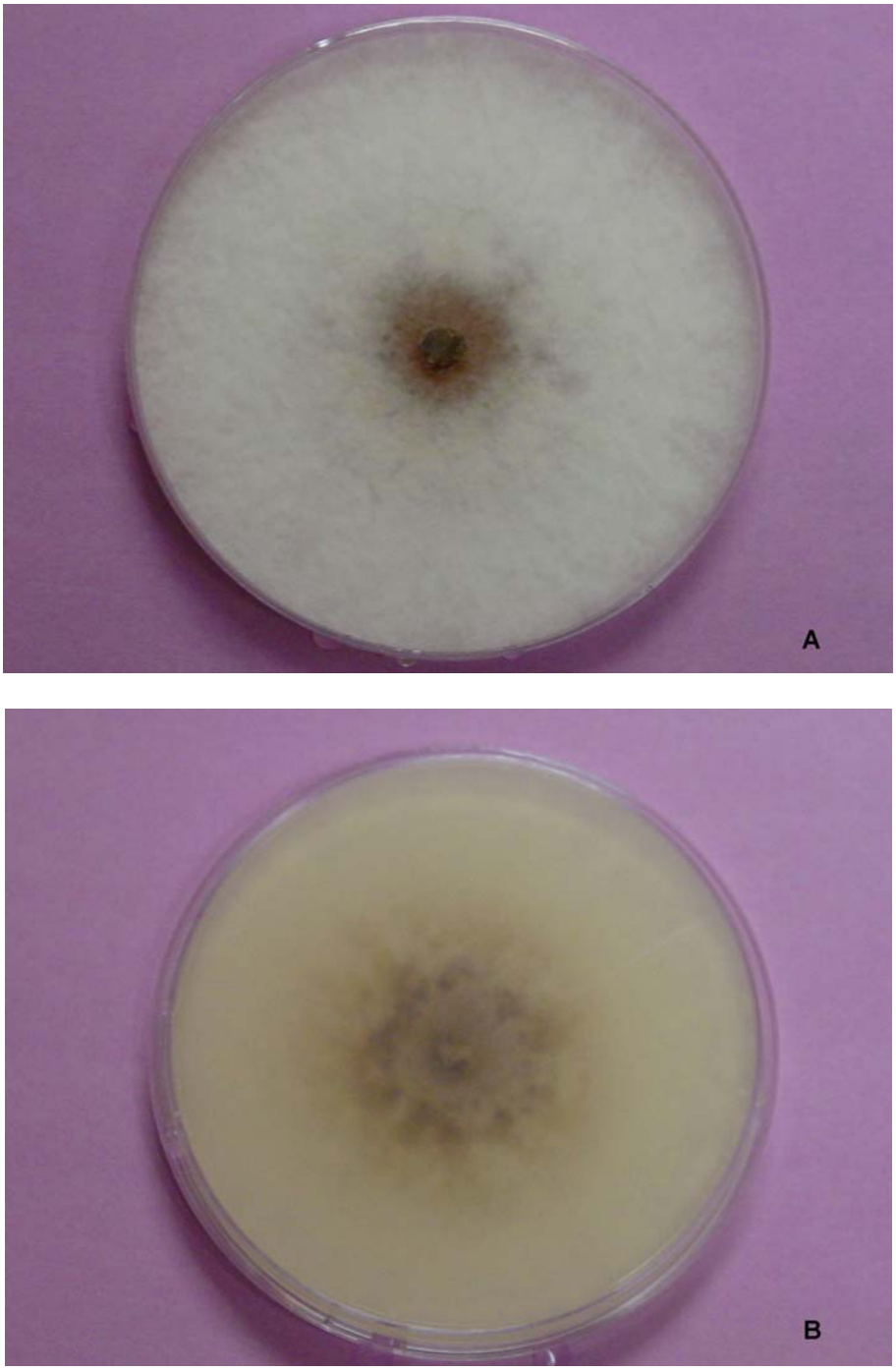

Figure 2.1: A) Top of *Sclerotinia homoeocarpa* culture on PDA.

B) Bottom of *Sclerotinia homoeocarpa* culture on PDA.

scored as incompatible. In contrast, confluent hyphal growth was scored as compatible. Compatible pairs were cultured twice for confirmation. Incompatible reactions between pairs were obvious and were not repeated. Reactions that could not be resolved were allowed to incubate for up to 3 weeks. If a reaction could not be characterized after that time, the pairing was repeated. All isolates were paired with itself and with all other isolates.

Vegetative Compatibility Between Isolates and Tester Strains:

Isolates were also paired with six different vegetative compatibility tester strains provided by Michigan State University (Powell and Vargas, 2001). All pairings were performed using the same method as described previously.

Results:

After incubation, isolates were observed for confluent hyphal growth or barrage zone (Figure 2.2). All isolates were self-compatible and isolates were generally compatible with most other isolates from the same location (Table 2.2), although there were some exceptions. For example, isolates G1, G2, G3, G4, and G5 were all exclusively compatible within location as were WW1, WW2, WW3, WW4, and WW5. Other isolates were also compatible with other isolates from the same location with a few exceptions. For instance, CH4 was only compatible with CH2 and CH3, but not CH1 or CH5 even though they were all collected from Olive Branch, MS. T1 was not compatible with any isolates from location T whereas T2, T3, T6, and T9 were all compatible. L1 was only compatible with L4 and isolates L1-1 and L1-4 were only compatible with each other and L1-5 suggesting that L1 and L4 are genetically different than the

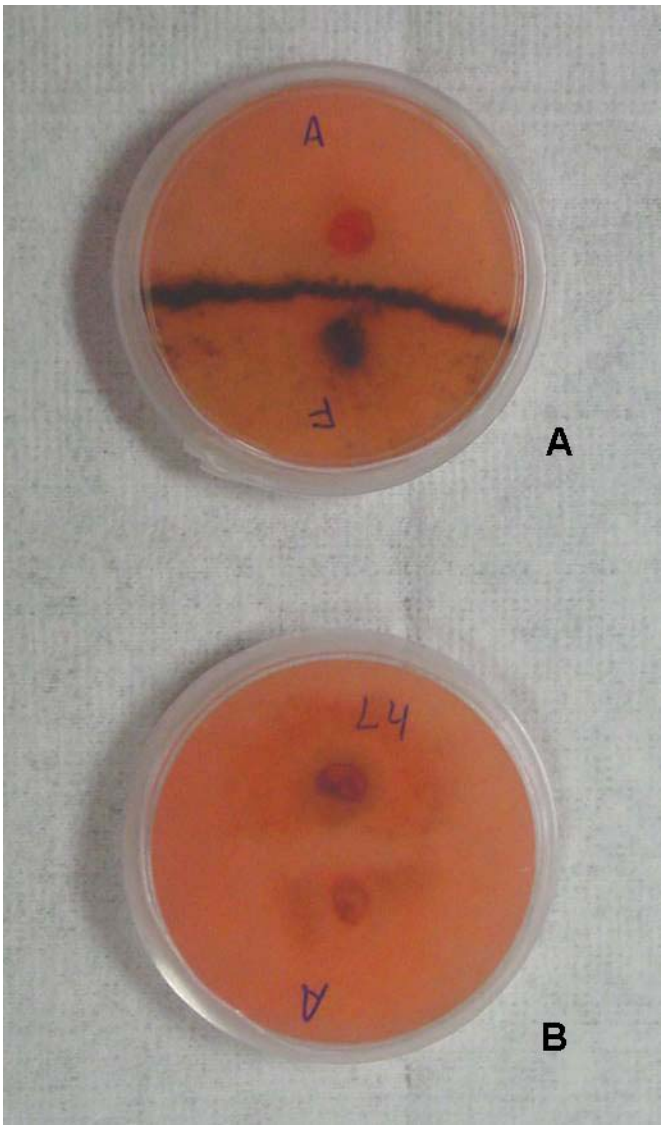

Figure 2.2: A) Typical barrage zone indicating vegetative incompatibility between two isolates of *Sclerotinia homoeocarpa*. B) Confluent hyphal growth indicating vegetative compatibility. Views are from the bottom of the petri dishes.

Table 2.2: Vegetative compatibility pairings of *Sclerotinia homoeocarpa* isolates.

Isolate	Compatible Isolates	VCG Group	Fungicide Resistance
BM4	BM4	- [a]	none
BM7	BM7	-	none
BM8	BM8	-	none
BM9	BM9, BM10	-	none
BM10	BM9, BM10	-	Iprodione
CH1	CH1, CH2, CH3, CH5	-	Thiophanate-methyl
CH2	CH1, CH2, CH4, CH5	-	Thiophanate-methyl
CH3	CH1, CH3, CH4, CH5	-	Thiophanate-methyl
CH4	CH2, CH3	-	Thiophanate-methyl
CH5	CH1, CH2, CH3, CH5	-	Thiophanate-methyl
T1	T1	-	Iprodione
T2	T2, T3 T6, T9, L3, W6, W7	-	Iprodione
T3	T2, T3, T6, T9	-	Iprodione, Thiophanate-methyl
T6	T2, T3, T6, T9, L2-1, W6, W7, W10	-	Iprodione, Thiophanate-methyl
T9	T2, T3, T6, T9	D	Iprodione
G2	G2, G3, G4, G5, G8	-	Thiophanate-methyl
G3	G2, G3, G4, G5, G8	-	Thiophanate-methyl
G4	G2, G3, G4, G5, G8	C	Thiophanate-methyl
G5	G2, G3, G4, G5, G8	-	Thiophanate-methyl
G8	G2, G3, G4, G5, G8	C	Thiophanate-methyl
L1	L1, L4	-	Iprodione
L2	L2, L3, L4, L5, L21, L2-2, L2-4, L2-5, W5, W6, W7	A,B,D	Iprodione
L3	T2, T6, L2, L3, L4, L5, W5, W6,W7. MID	A,B,D	none
L4	L1, L2, L3, L4, L5, L2-4, W5, W6, W7, MID	A,B,D	Iprodione
L5	L2, L3, L4, L5, L2-1, L2-2, L2-4, L2-5	B,D	none
L1-1	L1-1, L1-4, L1-5	-	Iprodione
L1-2	L1-2	-	Iprodione
L1-3	L1-3	-	Iprodione
L1-4	L1-1, L1-4, L1-5	-	Iprodione

[a]- Could not be determined using the tester strains in this study.

Table 2.2: Continued

Isolate	Compatible Isolates	VCG Group	Fungicide Resistance
L1-5	L1-1, L1-4, L1-5	-	Iprodione
L2-1	T6, L2, L3, L4, L5, L2-1, L2-2, L2-3, L2-4, L2-5, W5,W6, W7	A,B,D	none
L2-2	L2, L3, L4, L5, L2-1, L2-2, L2-3, L2-4, L2-5, W5,W6, W7	A,B,D	Iprodione
L2-3	L2-1, L2-2, L2-3, L2-5	-	none
L2-4	L2, L3, L4, L2-4, W8, W10	A,B,D	Iprodione
L2-5	L2, L3, L4, L2-1, L2-2, L2-3, L2-4, L2-5, W5,W6, W7	A,B,D	Iprodione
W5	L2, L3, L4, L2-1, L2-2, L2-5, W5 W6, W7, W8	A,B,D	Thiophanate-methyl,Propiconazole, Iprodione
W6	T2, T6, L2, L3, L4, L2-1, L2-2, L2-5, W5 W6, W7, W8, W10	A,B,D	Thiophanate-methyl,Propiconazole, Iprodione
W7	T2, T6, L2, L3, L4, L2-1, L2-2, L2-5, W5 W6, W7, W8, W10	A,B,D	Thiophanate-methyl,Propiconazole, Iprodione
W8	L2-4, W5, W6, W7, W8, W10	-	Thiophanate-methyl,Propiconazole, Iprodione
W10	L2-4, T6, W6, W7,W8, W10	-	Thiophanate-methyl,Propiconazole, Iprodione
WW1	WW1, WW2, WW3, WW4, WW5	-	Thiophanate-methyl
WW2	WW1, WW2, WW3, WW4, WW5	-	Thiophanate-methyl
WW3	WW1, WW2, WW3, WW4, WW5	-	Thiophanate-methyl
WW4	WW1, WW2, WW3, WW4, WW5	-	Thiophanate-methyl
WW5	WW1, WW2, WW3, WW4, WW5	-	Thiophanate-methyl
M1	M1	-	none
M2	M2, M4	-	none
M3	M3, M5	-	none
M4	M2,M4, M5	-	none
M5	M3, M4, M5	-	none
MID	L3, L4, MID	-	Iprodione
MIB	MIB	D,F	Benzimidazole (Thiophanate-methyl)
MIDMI	MIDMI	-	Thiophanate-methyl, Propiconazole
MIW	MIW	D	none
VCG A	L2, L3, L4, L2-1, L2-2, L2-4, L2-5, W5, W6, W7	A, B, D	unknown
VCG B	L2, L3, L4, L5, L2-1, L2-2, L2-4, L2-5, W5, W6, W7	A, B,D	unknown
VCG C	G4, G8	C	unknown
VCG D	T9, L2, L3, L4, L5, L2-1, L2-2, L2-5, L2-4, W5, W6, W7, MID	A,B,D	unknown
VCG E	-	E	unknown
VCG F	MIB	F	unknown

majority of isolates from Franklin, TN.

Isolates, such as BM4, BM7, BM8, T1, L1-2, L1-3, M1, MIB, MIDMI, and MIW, were incompatible with all isolates except themselves. Some isolates were compatible with isolates from different locations. For example, W isolates were compatible with several isolates from L, L1, and L2. W. isolates were collected in Athens, TN whereas L, L1, and L2 were from different courses in Franklin, TN

Sixteen Tennessee and Mississippi isolates were compatible with tester strains. Isolates from L, L1, L2, and W were compatible with tester strains A, B, and D. Two isolates (G4 and G8) were compatible with VCG tester strain C and only one isolate (MIB) was compatible with VCG tester strain F. Most isolates that were compatible with each other were also in the same VCG with a few exceptions. For example, isolate T6 was not compatible with any tester strains, but was compatible with sample L2-1 that was classified as being in VCGs A, B, and D. Another example is Michigan isolates MIW and MIB, which were in VCG D, but not compatible with any other isolate in that group. Isolates G4 and G8 were both found to be in VCG C and compatible with each other, yet no other isolate from that location was compatible with VCG C. Tester strains A, B, and D were also compatible with each other. Isolates that were compatible with these testers were common between all three strains.

Discussion:

Assessment of vegetative compatibility is a classical genetic technique that identifies related sub-populations within a species. Interactions of hyphae between some pairings were ambiguous and the reaction type was difficult to

categorize. Identification of vegetatively compatible or incompatible reactions was hindered by several physical features of the fungus. For instance, some isolates grew more vigorously than others and quickly colonized much of the surface of the medium before the other isolate could begin to grow. Another complication was excessive production of dark pigment by some isolates. Although most isolates grew as fluffy white mycelium on the surface of the medium, many isolates produced dark pigments that were incorporated into the agar and hindered visualization of the dead hyphae in the barrage zone. Production of black stroma also hindered the identification of the reaction. Pairings of isolates that could not be classified were incubated up to one to two weeks longer to allow additional mycelial growth. Black stromata were often produced before a decisive identification of the compatibility reaction could be made. Some pairings produced faint, indistinguishable barrage zones that did not always extend completely between the mycelial interaction zone. Areas where an obvious barrage was not present appeared to present confluent hyphal growth. Although these difficulties hindered quick identification, these reactions were differentiated after re-pairing. Varying types of barrage reactions were also observed by Deng et al. (2002). Barrages appeared as large gaps, thin gaps, or thick white lines of mycelium.

Samples within a location were typically compatible with each other, which supports the hypothesis that they are from the same source therefore genetically similar. Vegetative compatibility pairings and characterization into VCGs has also been performed using *Verticillium dahliae* Kleb isolates from around the

world (Korolev et al., 2000). Genetic homogeneity within a location and not between locations was described and appears to be similar to the diversity revealed between *S. homoeocarpa* isolates. Low diversity of both *V. dahliae* and *S. homoeocarpa* can be explained by the lack of sexual reproduction. Low genetic diversity may also be the result of the absence asexual spores. Dispersal of different genotypes via mycelium fragmentation is very limited and would not support combination of genetically dissimilar isolates. Diversity between isolates could be due to the parasexual cycle. The parasexual cycle offers an alternative to sexual recombination, but it does not provide the high diversity that is derived from sexual recombination. This cycle begins as hyphae from two haploid fungi fuse to form a heterokaryon. If nuclear fusion occurs, diploid heterozygous nuclei can form (at a frequency of approximately 10^{-6}). During mitosis, crossing over takes place between diploid nuclei (at a frequency of approximately 10^{-2}) and diploid nuclei can be restored to the haploid condition (at a frequency of 10^{-3}) (Pontecorvo et al., 1953).

Compatibility of isolates within a location could be explained by founder effects. Founder effects are an extreme example of genetic drift that is characterized by disproportional frequencies of alleles in a population resulting from a reduced number of gametes in the gene pool. Natural selection is not associated with genetic drift since adaptation is not involved and alleles are randomly chosen. If drift is the only evolutionary force, eventually one allele will drift to a frequency of 1 or become fixed. All other alleles will be lost, and

therefore a loss of heterozygocity. Drift especially impacts small, isolated populations and can produce rapid genetic divergence (Griffiths, et al., 1999).

When a natural barrier forms and divides the species into two separate groups, over succeeding generations, either through selection or random events, the populations become different. Statistically, even infinite populations will show homozygosity solely due to drift. Founder effects (or bottlenecks in population size) speed up this process by reducing a large population to a very small one that is not necessarily indicative of the allele frequencies in the large one. Physical movement of an asexual, immobile organism would almost instantaneously produce two divergent populations. In this current study, it is possible that one isolate of *S. homoeocarpa* was separated from a larger population and all isolates within the location could have been derived from the original progenitor. Multiple isolates with compatibility at the same location could be considered clones from an original isolate that spread due to poor sanitation and to high foot traffic (Harman et al., 2005). Purchasing of contaminated turfgrass seed or sod from the same source could explain this genetic similarity at the vegetative compatibility level between Tennessee and Mississippi locations. Since this pathogen depends on mycelium and stromata for dispersal, contaminated equipment used at many golf courses could easily spread disease (Deng, 2002).

Vegetative compatibility of *S. homoeocarpa* isolates has also been tested between isolates from Michigan, Illinois, and Wisconsin. Of these isolates, which were believed to be involved with seasonal infection of dollar spot, six VCGs

were delineated (Powell and Vargas, 2001). Most of the samples collected from these sites over the 3 year study were found primarily in VCGs A and B. Six tester strains were obtained from Vargas to characterize Tennessee and Mississippi isolates in the present study. Most isolates from L, L1, L2, and W were compatible with tester strains A, B, and D. In contrast, most isolates included in other studies were found to be in VCG A or VCG B, but not VCG D (Vigi et al., 2004; Powell and Vargas, 2001; Deng et al., 2002). Dominance of isolates in VCG B could be due an allele that permits survival over other genotypes. Selection pressures such as fungicides may support the dominance of one genotype over all others (Hartl, 2000). A correlation could not be made with fungicide resistance or location by VCG in the current study. Some isolates in groups A, B, and D lacked resistance or were resistant to multiple fungicides (Table 2.2). Therefore, these results do not support the hypothesis that fungicide resistance could be associated with VCGs.

Previous studies have indicated a correlation between pathogenicity VCGs (Vigi et al., 2004; Korolev et al., 2000; Leslie, 1996). Vigi et al. (2004) performed pathgenicity assays with isolates of *S. homoeocarpa* and attempted to correlate the results with VCGs. Generally, isolates that had similar pathogenicity were in the same VCG with a few exceptions. Isolates from Pennsylvania, New York, and Canada generally displayed weak to moderate pathogenicity and were in groups A, B, C, or D. Since the same tester strains were used in Vigi et al. (2004) and this study, it is possible that Tennessee and

Mississippi isolates also could be weak to moderately virulent, but this aspect of the biology was not investigated.

Most studies of vegetative compatibility with *S. homoeocarpa* do not indicate isolates are compatible with multiple tester strains. In this study, most isolates were compatible with multiple tester strains, especially A, B, and D. Interestingly, when the tester strains A, B, and D were paired against each other they were compatible. Isolates being compatible with more than one tester strain were also reported in a similar study. Pairing of isolates from the eastern United States and Canada indicated a single Canadian isolate was also in VCG C and D (Vigi et al., 2004). The same tester strains used in Vigi et al. (2004) were obtained from J. Vargas and used in this study. Isolates classified into more than one VCG implies that these tester strains are not as stringent as first thought, or that the tester strains have been genetically altered by storage and packaging.

In addition to multiple tester strain compatibility, some other isolates had unique pairing results. Isolates G4 and G8 were compatible with each other and with VCG C. G4 and G8 were also compatible with all other isolates from location G, but none of those isolates were compatible with VCG C. Perhaps these isolates are also compatible with VCG C, but compatibility was not obvious. Generally, physiological traits, such as reactions to temperature and pH, have been associated VCG in *Colletotrichum* systems (Nitzan and Tsror, 2003). However, all isolates were grown on the same medium and same temperature in this study and could not have been a factor in hyphal reactions. G4 and G8 could be incompatible with all other G isolates, but an obvious

barrage zone did not form. There are various types of phenotypic expression of barrage zones (Deng et al., 2002) that are controlled by several different molecular machineries that responsible for expression (Begueret et al., 1994). Possibly the combination of genes in between these G isolates produces a subtle incompatible reaction.

Summary:

Tennessee and northern Mississippi isolates with known fungicide resistance were paired on PDA for identification of sub-populations as well as paired with tester strains for delineation into VCG. Generally, isolates were compatible within location and incompatible between locations. Few isolates were delineated into vegetative compatibility groups, and fungicide resistance could not be associated with a particular vegetative compatibility group. Compatibility of isolates within a location could be due to founder effects. One isolate of *S. homoeocarpa* could have been separated from a larger population and all isolates within the location were perhaps derived from the original progenitor.

CHAPTER THREE

Evaluation of Conserved Gene Sequences of *Sclerotinia homoeocarpa* Tennessee and Mississippi Golf Course Isolates

Introduction:

In this study, portions of three conserved genes, elongation factor 1-α (EF1-α), β-tubulin, and carbomoylphospate synthase (CPS) domain of the CAD complex (CPS, aspartate transcarbamylase (ATC), and dihydroorotase (DHO), respectfully), were isolated by PCR and sequenced. In addition to gene sequences, the non-coding internal transcribed spacer (ITS) region between ribosomal subunit genes was sequenced. In preliminary studies, 20 samples were sequenced to assess or evaluate the level of diversity that could be detected at the nucleotide level. All of the samples contained the same sequence for all gene regions tested, and the AFLP technique was used to reveal molecular polymorphisms as a measure of genetic diversity (see Chapter 4). All gene sequences were submitted to GenBank.

EF1-α:

Ef1-α is a non-ribosomal protein (Voet et al., 2002) that plays a vital role in polypeptide chain elongation in eukaryotes. Along with the energy provided by GTP (guanosine 5'-triphosphate), it catalyzes the binding of aminoacyl t-RNAs to the A site of the ribosome. EF1-α has been characterized as having a molecular weight of 45,000-55,000 (Moldave, 1985). This conserved nuclear gene sequence has been amplified in several filamentous ascomycetes

(Carbone and Kohn, 1999). Comparison of Ef1-α sequences in conjunction with ITS sequences in anamorph isolates and teleomorph isolates have also been used to form phylogenetic linkages between the two forms of the fungus (Rehner and Buckley, 2005).

β-tubulin:

β-tubulin, in conjunction with α-tubulin, form heterodimers, which assemble to form strand-like structures called tubulin (Burns and Surridge, 1994). Dimers of tubulin join to form microtubules, which are involved in architecture, flagellar movement, and "rails" for movement of cellular components. Similarities in β-tubulin gene sequences have been used in phylogenetic studies, such as delineation of ancestry of some plant protists (Edgcomb, et al., 2001). β-tubulin has also been known as a target for benzimidazole in roundworms and fungi. Studies have examined associations between benzimidazole activity and β-tubulin sequence. Some residues of the β-tubulin sequence were correlated to benzimidazole vulnerability. Those particular residues were proposed to be involved in benzimidazole binding (Katiyar, et al., 1994). Another study involving comparison of field mutated and laboratory mutated strains of *Venturia inaequalis* Cook compared the sequences of β-tubulin. This study showed that changing of important codons provided benomyl resistance to the isolate (Koenraadt, et al., 1992).

CPS Domain of the CAD Complex:

CPS domain of the CAD complex is involved in the formation of carbamoylphosphate from CO_2, ATP, and ammonia or glutamine for pyrimidine biosynthesis, arginine biosynthesis, or the urea cycle (Liu et al., 1994). The CPS portions of CAD consist of nearly 4 kb of coding data. CPS sequencing has provided phyogenetic information that has been used to show divergence between Bacteria, Archaea, and Eukarya domains. Interestingly, Archaeal sequences show more homology to Eukarya than Bacteria (Lawson, et al., 1996). Primers were developed previously by Moulton and Wiegmann (2004), and were complementary to highly conserved regions, which could amplify the entire CPS domain.

ITS:

Often nuclear small-subunit rDNA sequences are used when studying phylogeny of organisms because like other conserved gene sequences, the sequences evolve slowly. In order to reveal more diversity, the internal transcribed region (ITS) can be used since it evolves at a quicker rate. ITS is a non-protein coding region, therefore base pair changes do not affect phenotype and is under little selection pressure even though it does appear in tandem along with genes for ribosomal proteins. Between small nuclear rDNA and 5.8S rDNA genes lies ITS region 1. Between 5.8S rDNA and nuclear large rDNA lies ITS region 2. Different primers can be used if both ITS regions need to be studied. ITS primers 1 and 4 amplify both ITS regions as well as the 5.8S rDNA. ITS primers 1 and 2 amplify only ITS 1 (White et al., 1990). The primer set that is

used is dependent on the amount of variability that is needed for delineation of organisms in a study (Kohn, 1992).

Materials and Methods:

DNA Extraction:

Isolates of *S. homoeocarpa* included in this study were the same of those used in the vegetative compatibility pairings. DNA was extracted using Qiagen DNeasy Plant DNA isolation kit #69106 (Qiagen, Valencia, CA). All 60 samples (Table 2.1) were cultured in autoclaved Difco™ Bacto® nutrient broth (Bacto beef extract 3.0 g/L, Bacto peptone 5.0 g/L dextrose) (Detroit, MI) for 7 days at 22 °C to grow isolates. The mycelial mass was separated from the media by centrifugation and frozen at -80 °C. Nutrient broth was used instead of potato dextrose broth because higher yields of DNA were obtained. Decreased yields were probably due to excessive carbohydrates in the mycelium. Samples were ground with liquid nitrogen in an autoclaved mortar and pestle. Instructions were followed as described by the Qiagen kit manual (Qiagen, Valencia, CA). RNase (4 µl) was added to each sample to digest any RNA that may have co-extracted with the genomic DNA. All DNA isolations were eluted in 10 mM Tris pH 8.0.

Electrophoresis of genomic DNA (5 µl) was performed on 1% agarose gel made with 1 X TAE buffer (Tris base, acetic acid, and EDTA) amended with 10 mg/ml ethidium bromide, and run at 100V for 60 min. All samples were visualized with UV light and quantified by comparing the relative intensity of the DNA in the gel to the intensity of a Low Mass DNA Ladder (Invitrogen, Carlsbad, CA).

Conserved Gene Isolation:

A standard protocol was used for all PCR reactions and separation of PCR products. PCR reactions contained the following components: 2 µl dNTPs (200 µM) (Eppendorf, Westbury, NY), 2 µl PCR buffer (10X), 2 µl forward primer (30 µM), 2 µl reverse primer (30 µM), 1.2 µl $MgCl_2$ (25 mM), 0.8 µl AmpliTaq Gold with GeneAmp® (5U/ µl), and 7 µl water. Buffer and $MgCl_2$ were provided with the AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA). All genomic DNA samples were diluted to 0.5 ng/µl and 2 ng were used in each 20 µl reaction. All primers were ordered from Integrated DNA Technologies (IDT), Coralville, IA. PCR products were electrophoresed under the same conditions as genomic DNA after extraction. A 100-2686 base pair (bp) eXACTGene Cloning DNA Ladder (Fisher, Atlanta, GA) or a 50-1000 bp BioMarker Low DNA Ladder (BioVentures, Murfreesboro, TN) were electrophoresed as a size standard for PCR products. All PCR reactions took place in an Eppendorf thermocycler in Fisherbrand thin-wall 0.2mL microcentrifuge tube strips with attached caps (Atlanta, GA).

Ef1-α:

A portion of Ef1-α was isolated using degenerate primers 526F (5'GTC GTY ATY GGH CAY GT3') and 1567R (5'AC HGT RCC RAT ACC ACC SAT CTT3') (Rehner, 2001). Other primer sets described by Rehner (2001) did not produce product after extensive modification to reaction conditions. This primer set amplifies about 2/3 of the gene or approximately 1000 bp. A modified touchdown PCR program was as follows: initial denaturation of 95 °C for 8 min;

10 cycles of 95 °C for 15 sec, 66 °C for 20 sec decreasing by 1 °C each cycle, 72 °C for 1 min 30 sec; 32 cycles of 95 °C for 15 sec, 56 °C for 2 min, 72 °C for 1 min 30 sec; final extension of 72 °C for 3 min, 4 °C hold (Rehner and Buckley, 2005).

β-tubulin:

Primers developed by Glass and Donaldson (1995) amplified only a small portion of the β-tubulin gene. Two primer sets to isolate two portions of the gene were described in this paper, but in preliminary trials, only one set generated products. The successful primer sequences were Bt2a: (5'GGT AAC CAA ATC GGT GCT GCT TTC3') and Bt2b: (5'ACC CTC AGT GTA GTG ACC CTT GGC3'). A modified thermal cycler program consisted of an initial denaturation of 94 °C for 8 min, 32 cycles of °94 C for 1 min, °58 C for 1 min, 72 °C for 1 min, final extension of °72 C for 5 min, and 4 °C hold (Glass and Donaldson, 1995).

CAD:

Primer sequences were obtained from Moulton and Wiegmann (2004). Two primer pairs were used to amplify approximately one half of the CPS domain, 54F/405R and 338F/680R. Degenerate primer sequences are as follows: 54F (5'GTN GTN TTY CAR CAN GGN ATG GT3'), 405R (5'GCN GTR TGY TCN GGR TGR AAY TG3'), 338F (5'ATG AAR TAY GGY AAT CGT GGH CAY AA3'), and 680R (5'AAN GCR TCN CGN ACM ACY TCR TAY TC3'). The following abbreviations for degenerate primer nucleotide were used: N-unknown, any nucleotide; Y- pyrimidine, either C or T; R- purine, either A or G; H- not G; M- amino, C or A. Modified thermal cycler program for the primer pair 54F/405R

consisted of initial denaturation of 94 °C for 5 min, 5 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min 30 sec, followed by 35 cycles of °94 C for °30 sec, 50 °C for 30 sec, 72 °C for 1 min 30 sec, final extension of 72 °C for 5 min, and 4 °C hold. Modified thermal cycler program for primer pair 338F/680R consisted of initial denaturation of 94 °C for 5 min, 5 cycles of 94 °C for 30 sec, 52 °C for 30 sec, 72 °C for 1 min 30 sec, followed by 35 cycles of 94 °C for 30 sec, 47 °C for 30 sec, 72 °C for 1 min 30 sec, final extension of 72 °C for 5 min, and 4 °C hold (Moulton and Wiegmann, 2004).

ITS:

Primers sequences were acquired from White, et al. (1990) and were as follows: ITS1 (5'TCC GTA GGT GAA CCT GCG G3') and ITS4 (5'TCC TCC GCT TAT TGA TAT GC3'). These primers cover the space between nuclear small rDNA and nuclear large rDNA. In the middle of this non-coding spacer region is the conserved gene sequence for 5.8s rDNA (White et al., 1990). The altered thermal cycler program was as follows: initial denaturation of 95 °C for 9 min, 35 cycles of 95 °C for 1 min, 56 °C for 1 min, 72 °C for 1 min, final extension at 72 °C for 7 min, and 4 °C hold.

PCR Clean-up:

In order to remove residual primers, dNTPs, and other components of the PCR reaction, the entire reaction was processed through a Qiaquick PCR Purification Kit #28106 (Qiagen, Valencia, CA). Protocol was followed as stated in the instruction manual and PCR fragments were eluted in water at or between

pH 7.5-8.0.

Fragment Excision:

For both primer combinations for the CPS portion of CAD, some nonspecific annealing of the primers occurred causing fragments other than the target sequences to be produced. Isolation of only the target fragment began by first separating the fragments in the entire reaction by electrophoresis on an agarose gel. The target band approximately 1000-1200 bp was then cut out with a No. 11 scalpel during visualization with UV light. Care was taken to ensure the smallest amount of agarose gel was excised as possible. The target fragment was then processed through a Qiaquick Gel Extraction Kit #28706 (Qiagen, Valencia, CA) following the manufacturers protocol. The purified DNA was eluted with water (pH 7.5-8.0).

Sequencing Reactions:

Standard master mixes were made for each gene that contained 3 µl of ABI buffer (5X), 1 µl of forward or reverse primer (3 pM/µl), and 1.5 µl Big Dye. All reagents except the primer were supplied with the Big Dye kit (Applied Biosystems, Foster City, CA). The remainder of the 20 µl reaction was filled by using 5-10 ng/100 bases of PCR fragment in water. For instance, if a 500 bp PCR fragment was being sequenced, 50 ng of purified product would be present in 14.5 µl of water. The following thermal cycler program was used: initial denaturation of 96 °C for 1 min, followed by 50 cycles of 96 °C for 20 sec, 49 °C for 30 sec, 62 °C for 4 min, and 4 °C hold. Forward and reverse sequencing reactions were performed for all samples using the PCR primers in order to

validate both strands of DNA.

Sequencing Reaction Clean-up:

To ensure proper sequence detection by the MJ BaseStation DNA Sequencer (BioRad, Waltham, MA) each reaction was purified by running it through sephadex gel matrix columns. Sephadex columns were hydrated with 800 µl deionized water, shaken, and allowed to incubate at room temperature on the desktop. After 2 hours, the bottom cap was removed and the water was allowed to drain for about 30 min or until no water was visible on the top of the sephadex plug. Columns were then dried by centrifugation at 1000 x g for 2 min in a 2 ml collection tube. An entire sequencing reaction was applied to a column with special care not to touch the sides of the column or sephadex gel matrix. Samples were centrifuged at 500 x g for 90 sec and collected in a 1.5 ml collection tube. The eluted purified dye-labelled fragments were dried in a speed vacuum for one hour at 40 °C. Samples were resuspended in 6 µl of blue formamide loading dye formula (trace of crystal violets added to the formamide) and incubated at 75 °C for 10 min. After vortexing for approximately 1-3 sec, each sample was loaded into a MJ BaseStation-compatible 96 well plate and stored at 4 °C until analysis.

Sequencing:

A horizontal polyacrylamide gel electrophoresis system was used to separate the sequencing fragments. Approximately 15 ml of a 5% solution of BasePack™ acrylamide stock and KBB (Tris/TAPS) buffer was cast between two 30 cm MJBaseStation compatible glass plates. After polymerization using a UV

light box, the sequencing gel was placed in the sequencing machine as well as the 96 well sample plate. Running buffer (1X KBB) was added to the anode chamber and 120 mls of 18.2 mOhm water was added to the cathode chamber. Samples were injected into the wells of the gel by the sequencers robot loader and electrophoresed using the following conditions: 200V pre-run voltage for 1 min; 5000V injection voltage for 60 sec; 2200V run voltage for 20,000 scans. A preview of the gel and fluorescence of the nucleotides could be visualized on the computer screen as the samples separated. The accompanying Cartographer sequence analysis software was used to track and score lanes, obtain Phred values, and export the sequences in a format compatible with Sequencher (Gene Codes Corporation, Ann Arbor, Michigan).

Sequence Analysis:

Forward and reverse sequences were aligned using Sequencher™ Version 4.2 (Gene Codes Corporation, Ann Arbor, Michigan) sequence editing software. Introns were annotated and edited sequences were submitted to GenBank on the National Center Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/). Introns are region of non-protein coding DNA that interrupt the transcribed part of a gene. These regions are transcribed, but are removed by slicing, or the process of connecting two RNA exons, once the intron is removed. Introns were identified by searching for stop codons (TAA, TAG, and TGA) contained in DNA consensus sequence. A stop codon in the middle of the gene sequence implies that the gene is a pseudogene or an intron is present (Weaver, 2002). If the stop codon is bracketed by potential splice sites, generally

GT before the stop codon and AG following, it can be assumed and intron is present if removal of the fragment reestablishes the reading frame (Ogura et al., 1997). The 3' end of the fragment of the 54F/405R and the 5' end of the 338F/680R overlap slightly thus the two fragments were aligned and combined to form one complete sequence. Nucleotide-nucleotide BLAST (basic local alignment search tool) (blastn) searches were performed on the NCBI website to confirm the identity of sequences to known genes sequences.

Comparison to Similar Species:

According to Powell and Vargas (1999), *S. homoeocarpa* grouped with members of the genus *Rutstroemia* rather than members of *Lanzia* and *Moellerodiscus* when ITS1 sequences subjected to phylogenetic analysis using parsimony criteria. Since it was not indicated whether ITS 1 sequences from members of the *Sclerotinia* genera were included in the study, sequences were compared to the dollar spot pathogen in addition to the *Lanzia* genera and *Rutstroemia* genera. Another study by Carbone and Kohn (1993) comparing the ITS 1 region between *S. sclerotiorum*, *Piceomphale bulgariodes* Rabenh, *R. petiolorum* Rob, *R. sydowiana* Rehm, *R. firma* Pers,, *R. henningsiana* Plottn, *Lambertella subrenispora* Korf and Zhuang, and *S. homoeocarpa* also indicated genetic similarities between *S. homoeocarpa* and *Rutstroemia* spp. These results implied that *S. homoeocarpa* could be placed in the *Rutstroemia* genus. The present study further investigated this concept by comparing ITS 1 and 2 sequences for *S. sclerotiorum* (Accession ID DQ329538), S. *minor* (DQ287337), *S. trifoliorum* (DQ314296), *R. firma* (Z80893), and *L. allantospora* (AY755334)

from GenBank on the NCBI website. *Moellerodiscus* was excluded from the study because the ITS sequence was not available on the NCBI website. Alignment and phylogenetic analysis of the ITS 1 and 2 sequences could provide additional information as to the proper taxonomy of *S. homoeocarpa*. All ITS sequences were aligned in Sequencher and analyzed using PAUP* version 4.0 to create a most parsimonious phylogenetic tree (Swofford, 2001). Nonparametric bootstrap sampling was conducted to evaluate node support. Bootstrapping tests the reliablility of a dataset by resampling. Bootstrap scores below 50 were excluded from the tree.

Results:

EF1-α PCR products were approximately 1100 bp (Figure 3.1) whereas the CAD 338F/680R and 54F/ 405R PCR products were approximately 1000 and 1200 bp, respectfully (Figure 3.2, 3.3). β-tubulin primers produced a 500 bp PCR product (Figure 3.4) and ITS primers produced a 550 bp PCR product (Figure 3.5). An example of CAD 338F/680R 1100 bp PCR product after agarose gel extraction is depicted in Figure 3.6. After sequencing, the slight overlap of CAD 338F/680R and 54F/ 405R PCR products were aligned to form a contiguous 1820 bp sequence.

Introns were annotated and sequences of all genes were submitted to Genbank with the following Accession IDs: (Table 3.1). One intron was detected in the β-tubulin fragment as well as the CAD fragment. Estimated location of introns and primer annealing sites for each gene are illustrated on Figure 3.7.

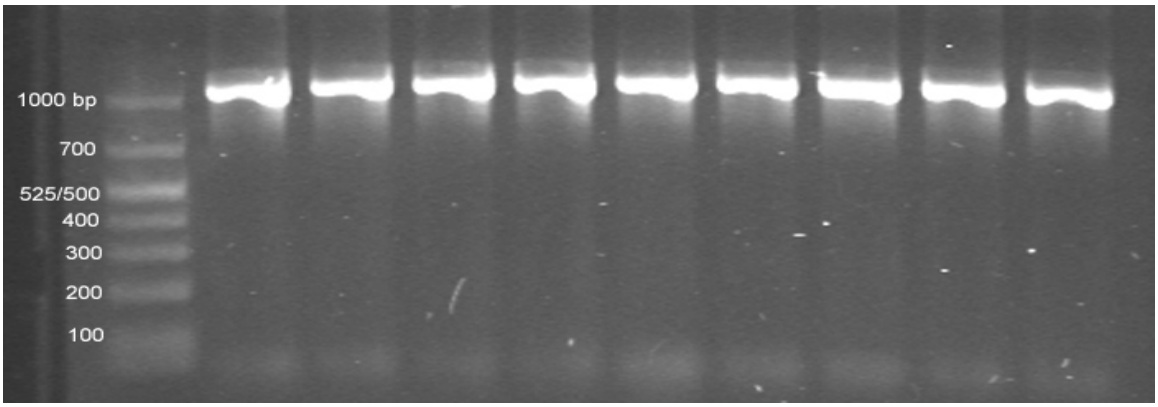

Figure 3.1: *Sclerotinia homoeocarpa* PCR products (3 µl) generated by EF1-α 526F and 1567R. Fragments were approximately 1000 bp when compared to 3 µl Biomarker Low DNA Ladder (BioVentures, Murfreesboro, TN).

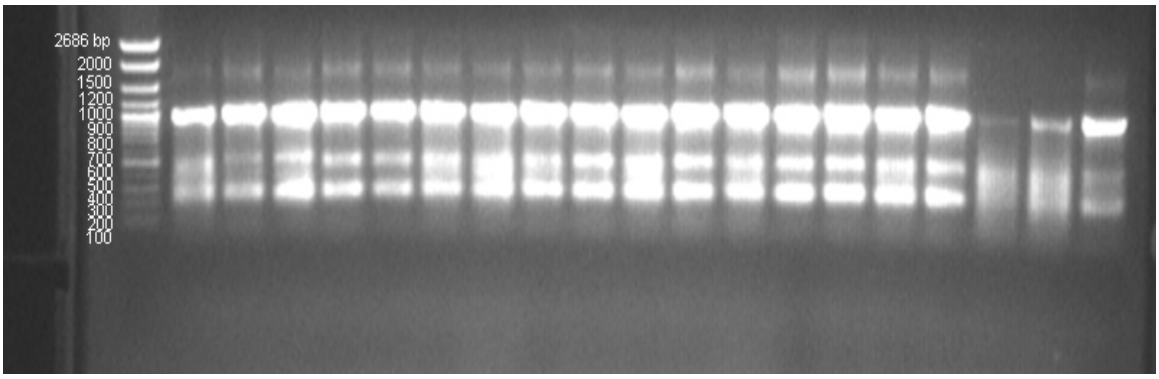

Figure 3.2: *Sclerotinia homoeocarpa* PCR products (3 µl) generated by CAD primers 338F/680R. Fragments were approximately 1100 bp when compared to 5 µl eXACTGene Cloning DNA Ladder (Fisher, Atlanta, GA).

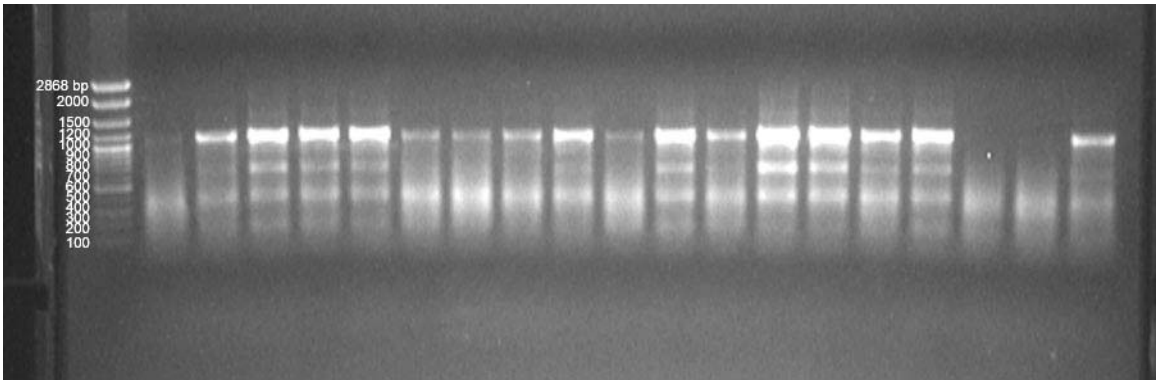

Figure 3.3: *Sclerotinia homoeocarpa* PCR products (3 µl) generated by CAD primers 54F/405R. Fragments were approximately 1200 bp when compared to 5 µl eXACTGene Cloning DNA Ladder (Fisher, Atlanta, GA).

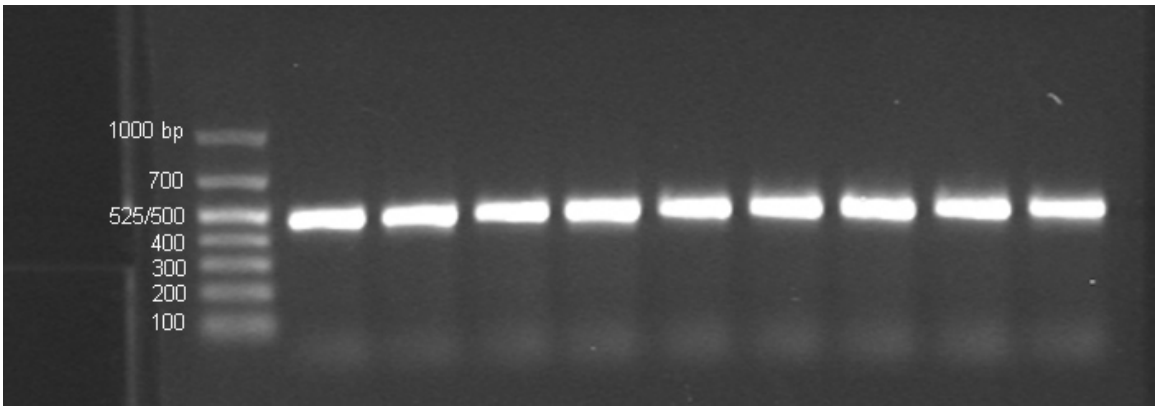

Figure 3.4: *Sclerotinia homoeocarpa* PCR products (3 µl) generated by β-tubulin primers Bt2a and Bt2b. Fragments were approximately 500 bp when compared to 3 µl Biomarker Low DNA Ladder (BioVentures, Murfreesboro, TN).

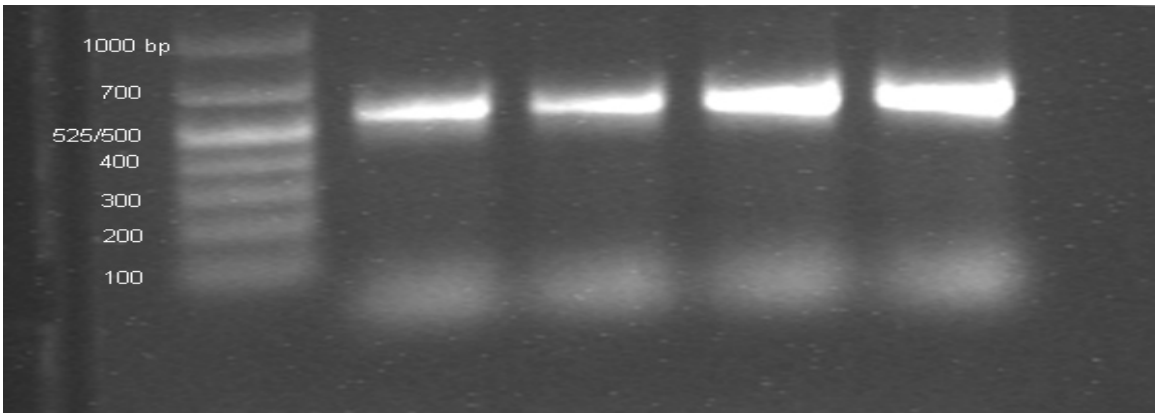

Figure 3.5: *Sclerotinia homoeocarpa* PCR products (3 µl) generated by ITS primers ITS1 and ITS4. Fragments were approximately 550 bp when compared to 3 µl Biomarker Low DNA Ladder (BioVentures, Murfreesboro, TN).

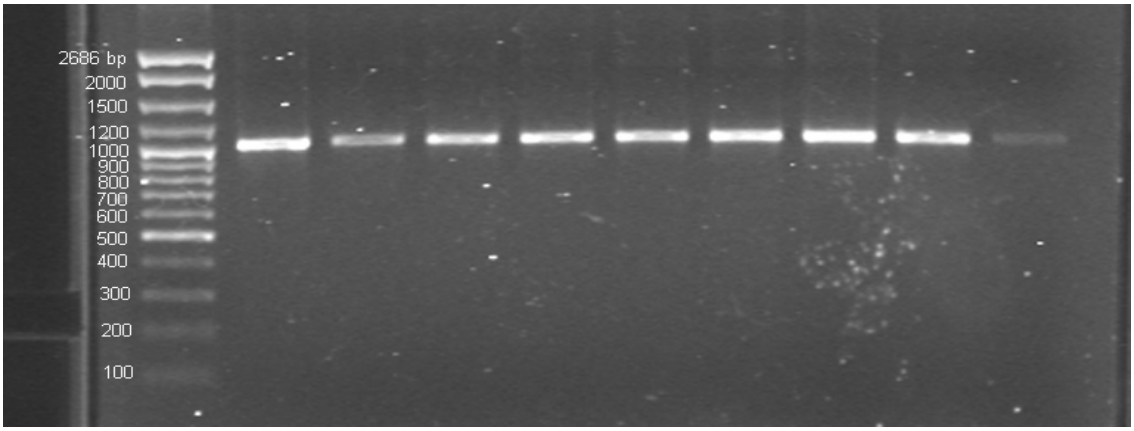

Figure 3.6: An example of clean gel extractions of CAD 54F/405R PCR product (3 µl) from *Sclerotinia homoeocarpa*. Fragments were approximately 1100 bp when compared to 5 µl eXACTGene Cloning DNA Ladder (Fisher, Atlanta, GA).

Table 3.1: Length, exon and intron position, and GenBank accession numbers for *S. homoeocarpa.*

Gene	Length (bp)	Exon Positions (bp)	Intron Position (bp)	Accession Number
EF1-α	974	64-122, 317-974	1-63, 123-316	DQ448301
β-tubulin	424	1-106,199-424	107-198	DQ448299
CPS	1817	1-454,476-1817	455-475	DQ448300
ITS 1, 5.8S, ITS 2	542	N/A	N/A	DQ448302

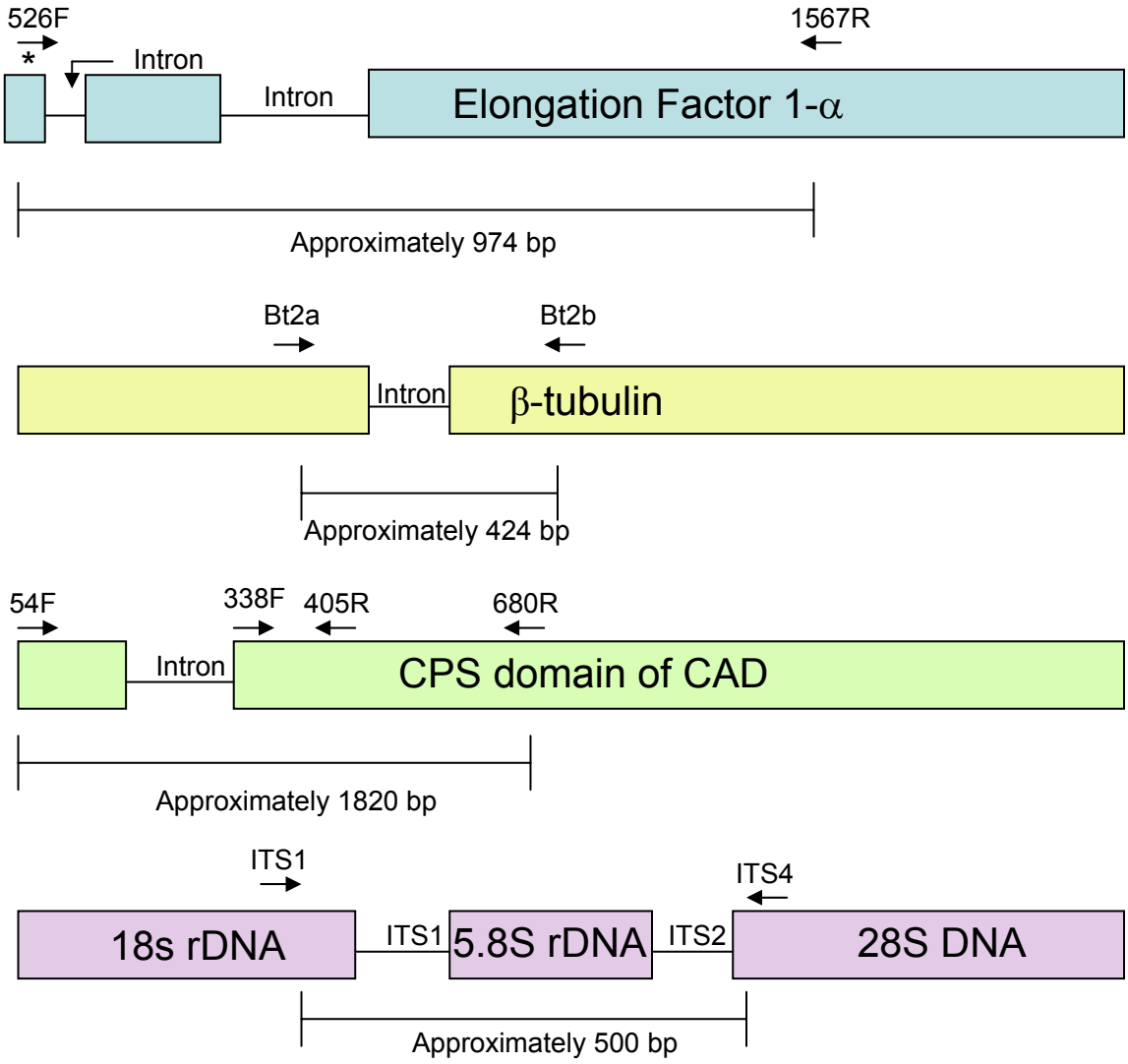

Figure 3.7: Estimated intron and primer annealing sites on *S. homoeocarpa* conserved genes. Elongation factor 1-α (modified from Rehner and Buckley, 2005), β-tubulin (modified from Glass and Donaldson, 1995), CPS domain of CAD (modified from Moulten and Wiegmann, 2004), ITS (modified from White et al., 1990). ***** Indicates area where an exon matched the degenerate primer site. These sequences along with other irresolvable nucleotides were removed during sequence editing and were not included in the final base pair count.

All edited gene and spacer sequences are described in Appendix I. During nucleotide-nucleotide BLAST searches, all genes matched the appropriate sequences except the CAD fragment. The CAD sequence was confirmed by alignment with the known CAD sequence of a *Discula* species.

Alignment of ITS regions of *S. homoeocarpa*, *S. sclerotiorum*, S. *minor*, *S. trifoliorum*, *R. firma*, and *L. allantospora* indicated homology between all genera and species (Figure 3.8). The phylogram produced by parsimony analysis contained two major clades; one grouping *S. homoeocarpa* and *R. firma* and one grouping *S. sclerotiorum*, S. *minor*, and *S. trifoliorum* (Figure3.9). The node containing *S. homoeocarpa* and *R. firma* had a bootstrap value of 52 whereas the node containing *S. sclerotiorum*, S. *minor*, and *S. trifoliorum* had a boostrap value of 100.

Discussion:

Sequencing of β-tubulin yielded a fragment approximately 424 bp with one intron. In contrast, Glass and Donaldson (1995) found 3 introns in the 495 bp fragment using the same primers with *Neurospora crassa*. The portion of EF1-α amplified by the same primers used in this study was found to be about the same length as *Beauveria* isolates (1200bp) in Rehner and Buckley (2005). Four introns were detected in this portion of EF1-α, but only 2 were found in the current study. An exon exists before the first intron, but it was removed since it was a degenerate primer annealing site. About half of the CPS domain of CAD was isolated in this experiment and contained one intron. Originally the primers for amplification of CAD were designed for insect studies

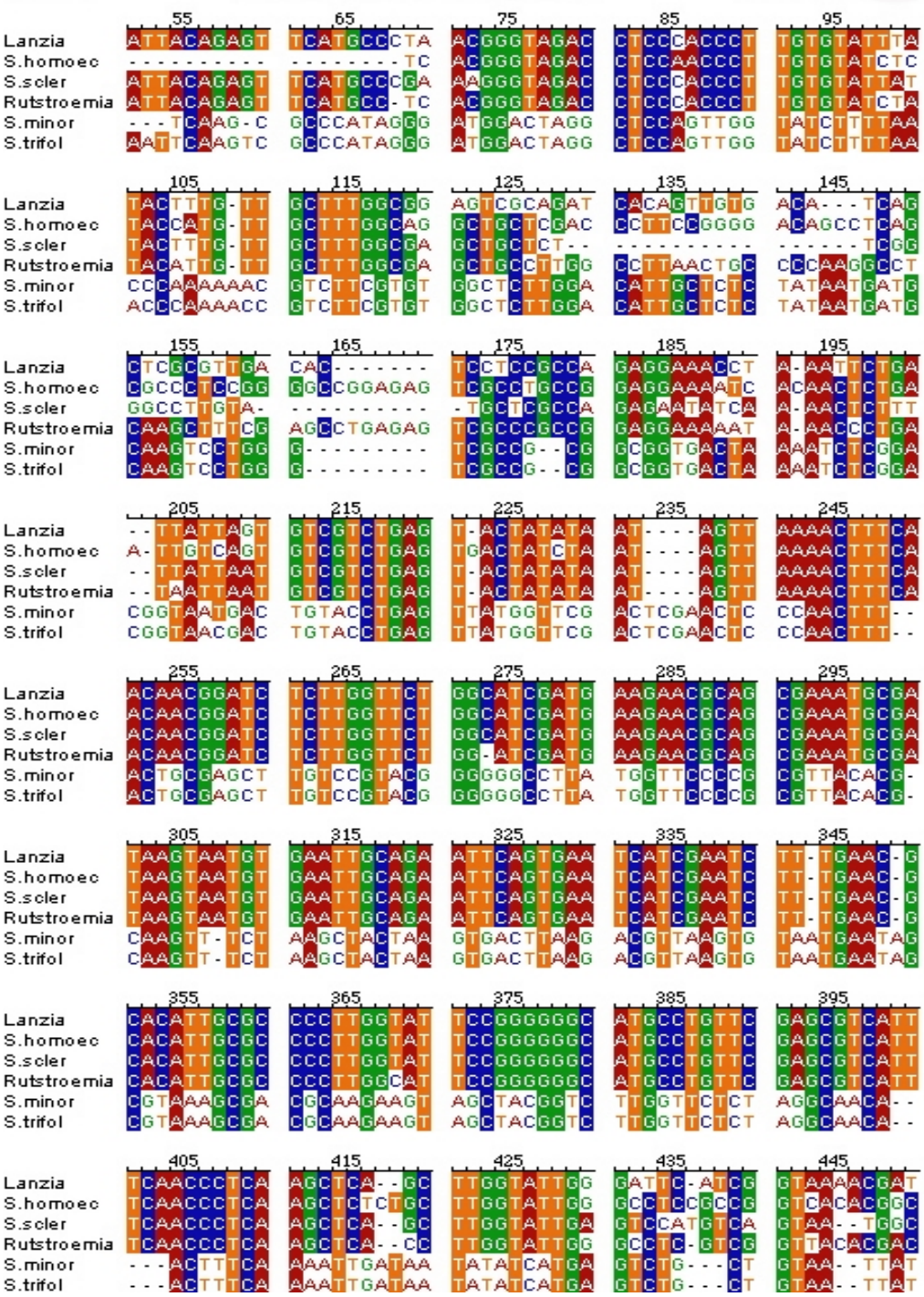

Figure 3.8: Alignment of *Sclerotinia homoeocarpa*, *S. sclerotiorum, S. minor*, *S. trifoliorum*, *Rutstroemia firma*, and *Lanzia allantospora* ITS sequences.

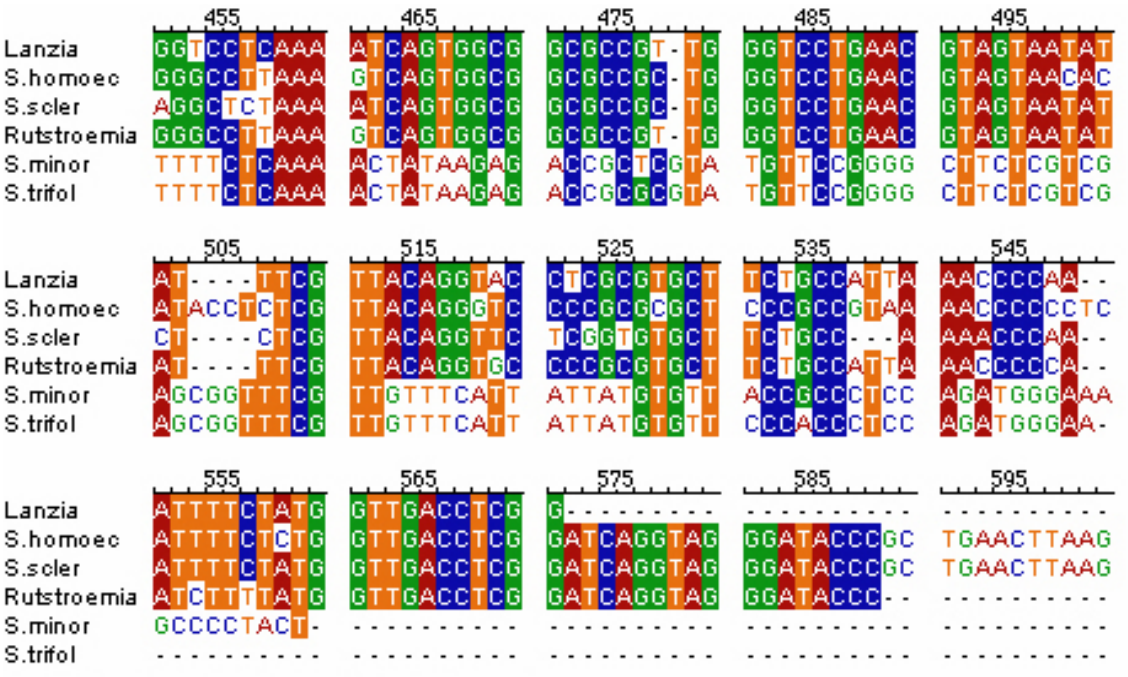

Figure 3.8: Continued.

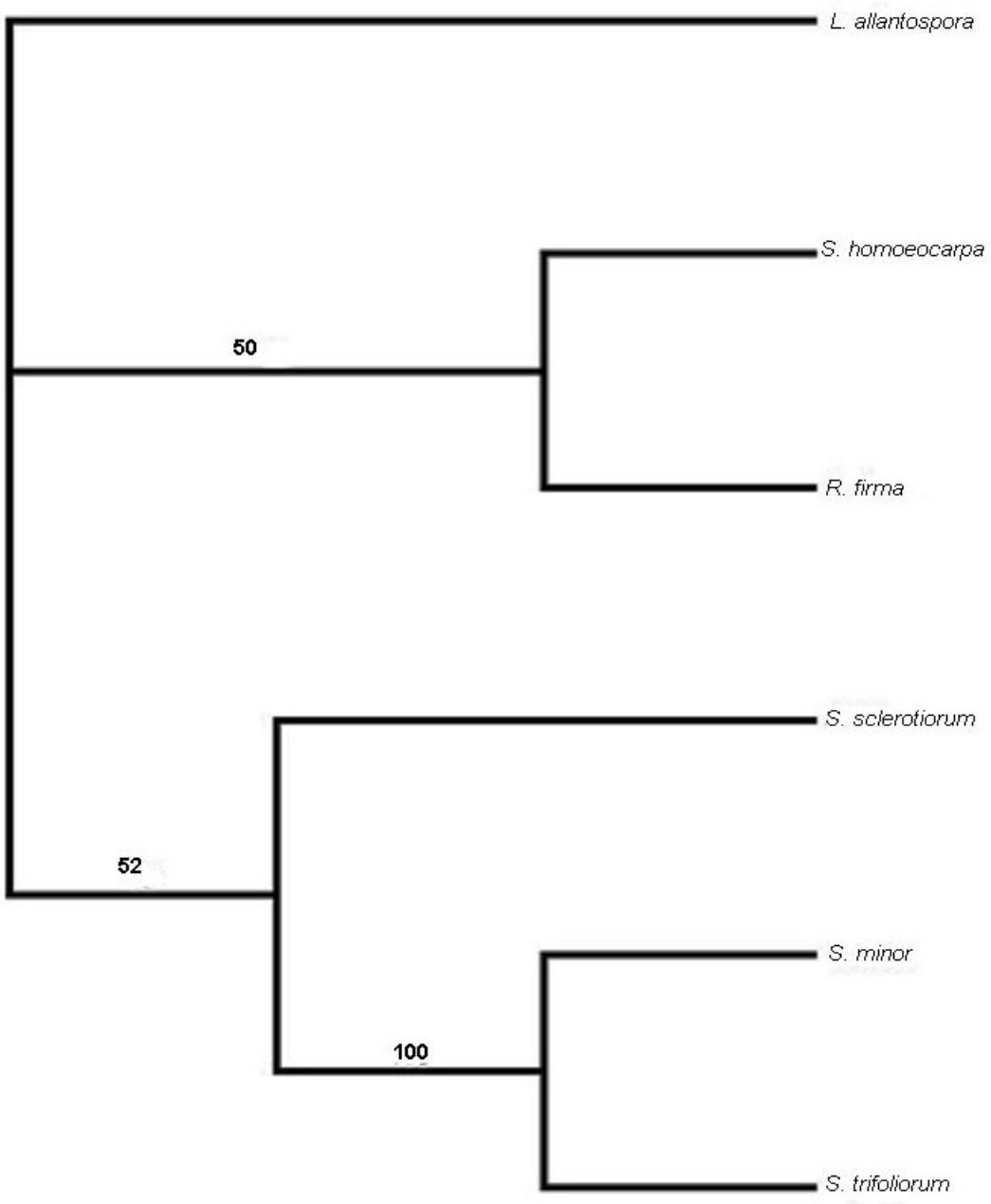

Figure 3.9: Phylogentic tree produced by parsimony analysis using ITS 1 and 2 sequences. *Sclerotinia homoeocarpa grouped with R. firma,* whereas all other *Sclerotinia* species grouped separately. Bootstrap value of 100 strongly groups *Sclerotinia minor* and *trifoliorum* in the same clade.

(Moulton and Wiegmann, 2004), and their successfulness in this study implies high conservation of the regions where primers anneal. The combined length of ITS 1, 5.8S rDNA and ITS 2 were comparable to White et al., (1990).

Lack of variation of between conserved gene sequences and ITS regions implies little diversity between isolates. Powell and Vargas (2001) found that comparison of ITS 1 regions of *S. homoeocarpa* isolates indicated no diversity between isolates from different locations. ITS sequences are not under selection pressure because they do not code for proteins, thus a large amount of diversity would be expected in this region when studying various organisms. All other genes studied are essential to cell function and it was expected to see little variation between isolates. Lack of variation at all loci further supports the hypothesis that *S. homoeocarpa* reproduction is highly dependent on dissemination by mycelium, not sexual or asexual reproduction.

Although alignment of the ITS 1 and 2 region and bootstrap values indicate that *S. homoeocarpa* could be placed in the *Rutstromia* genus, only one region of DNA was tested (Carbone and Kohn, 1993; Powell and Vargas, 1999). Other regions of DNA such as β–tubulin, EF1-α, and CPS domain of CAD were not available for other species in GenBank, thus only one region of DNA could be tested for diversity. Carbone and Kohn (1993) aligned ITS 1 sequences to determine if *Sclerotinia* spp. (tuberlike sclerotium) and *Rutstroemia* spp. (platelike stroma) with similar stromata type clustered during parsimony analysis. Clustering indicated that *S. homoeocarpa* isolates were significantly more similar to *Rutstroemia* species than other *Sclerotinia* species. Although strong cluster

support for these clusters was revealed, it was concluded that ITS 1 sequences were not enough to differentiate diverging stromata type lineages of *Sclerotinia* and *Rutstroemia* genera. In this study, sequence comparison of ITS 1 and 2 did not indicate variation from the Carbone and Kohn (1993) study; *S. homoeocarpa* grouped with *Rutstroemia firma*. Significant differences could possibly be seen at different loci that could provide evidence *S. homoeocarpa* belongs to the *Sclerotinia* genus. Further study of different genes and more species from the various genera will need to be performed to confirm the appropriate classification of *S. homoeocarpa.*

Summary:

Three conserved genes, EF1-α, β-tubulin, and CPS domain of the CAD as well as ITS 1 and 2 were isolated by PCR and sequenced. In preliminary studies, 20 samples were sequenced to assess or evaluate the level of diversity that could be detected at the nucleotide level. All of the samples contained the same sequence for all gene regions tested. Gene sequences were annotated for introns and submitted to GenBank. Alignment of ITS 1 and parsimony analysis in previous studies indicated *S. homoeocarpa* and *Rutstroemia* spp. shared more sequence homology than with *Sclerotinia* spp. *Sclerotinia homoeocarpa*, *S. sclerotiorum*, S. *minor*, *S. trifoliorum*, *R. firma*, and *L. allantospora* ITS 1 and 2 sequences also were aligned and compared in parsimony analysis. Results were congruent with previous studies indicating that *S. homoeocarpa* could potentially be re-classified as a *Rutstroemia* species. Several other markers need to be analyzed before a final decision could be made as to its classification.

CHAPTER FOUR

AFLPs Used to Evaluate the Genetic Diversity of *Sclerotinia homoeocarpa* Isolates from Tennessee and Mississippi Golf Courses

Introduction:

AFLP markers have been used to resolve genetic relationships between organisms and to discover unique molecular markers. AFLP and vegetative compatibility pairings in concert can be even more informative if correlations can be identified (Kohn, 1992). Genetic analysis of geographically diverse resistant and non-resistant isolates of *S. homoeocarpa* may determine if any molecular markers are specific to VCG members or isolates resistant to the same fungicide formulation. Cluster analysis could also elucidate groups with similar resistance or sensitivities. Discovery of molecular markers would increase the knowledge of this pathogen, and help with the efforts to decrease fungicide resistance. If markers can be correlated with a certain type of resistance, educated decisions could be made as to what fungicide to use against a particular isolate by testing for the diagnostic allele. By not using a "trial and error" type fungicide system, money could be saved by using the appropriate fungicide the first time.

Materials and Methods:

The AFLP protocol (Vos et al.,1995) used in this study labeled fragments during the selective reaction. Utilizing fluorescently labeled primers for the various selective reactions becomes very costly. To remedy this issue, an AFLP protocol developed by Habera et al. (2004) was used that includes a separate and simple PCR reaction to label fragments. This reaction uses a single Eco+0

primer that can be used for all fluorescent labeling reactions. Before a separate labeled Eco side primer would be needed for each possible selective primer combination, which increased costs tremendously (Habera et al., 2004).

DNA Extraction:

DNA was extracted, quantified, and qualified using the same methods used for conserved gene study previously described. Most AFLP reactions require approximately 500 ng of high quality DNA (low fragmention, high purity, etc.) though this amount varies depending on the genome of interest (smaller genomes require more DNA) (Figure 4.1). DNA was speed vacuumed and then resuspended in 5 µl of 10 mM Tris pH 8 to reach a concentration of 100 ng/µl.

Restriction/Ligation:

Adaptors for the restriction/ligation reaction are obtained as single stranded oligonucleotides that must be annealed together for ligation to the restriction site. Four different oligos were ordered including 2 *Eco* RI adapators and 2 *Mse* I adaptors. The following adaptor sequences were used: *Eco* RI adapter 1- (5' CTC GTA GAC TGC GTA CC 3'), *Eco* RI adapter 2- (5' AAT TGG TAC GCA GTC TAC 3'), *Mse* I adapter 1- (5' GAC GAT GAG TCC TGA G 3'), and *Mse* I adapter 2- (5' TAC TCA GGA CTC AT 3'). Upon receiving lyophilized adaptors, 10 mM Tris pH 8.0 was used to resuspend to a concentration of 200 µM. In a thin-walled Eppendorf PCR tube, 5 µl of each adaptor pair was mixed and heated at 95 °C for 5 min in a themocycler. At this time the adaptor pairs became annealed and were slowly cooled to room temperature overnight to ensure complete annealing.

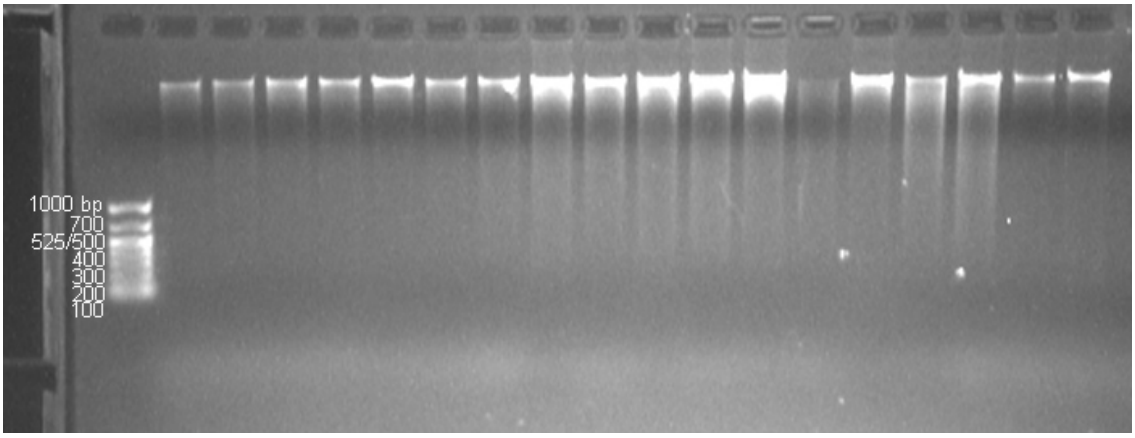

Figure 4.1: Example of high quality genomic DNA extraction from *Sclerotinia homoeocarpa*. Bands are clear and bright with minimal streaking indicating little fragmentation of DNA occurred during extraction. BioVenture Biomarker Low DNA ladder (3 µl) (Murfreesboro, TN) was electrophoretically separated along with 3 µl of genomic DNA.

Genomic DNA was digested using *Eco* RI and *Mse* I simultaneously then adaptors were ligated to fragments by T4 ligase (New England Biolabs, Beverly, MA) in Fisherbrand 96 well flexible plates (Atlanta, GA). The reaction mixture contained 1.1 µl of ligase buffer (10X) (included in T4 ligase kit), 1.1 µl of 0.5 M NaCl, 0.5 µl of bovine serum albumin (BSA) (1 mg/ml), 1 µl *Mse* I adaptor (50 µM), 1 µl *Eco* RI adaptor (5 µM), 0.5 µl *Eco* RI (10 U/ µl) (Invitrogen, Carlsbad, CA), 0.25 µl *Mse* I (5 U/ µl) (Invitrogen, Carlsbad, CA), and 0.33 µl T4 ligase (3 U/ µl). The master mix was added to 5 µl of 100 ng/µl genomic DNA, and incubated on the benchtop over night at room temperature (~20 °C). In the morning, the entire reaction was diluted with 189 µl of 10 mM Tris pH 8. This dilution deactivated enzymes that could interfere with subsequent reactions. Plates were stored at -20 °C until pre-selective reactions were performed. All of the following reactions were also completed using Fisherbrand 96 well flexible plate (Atlanta, GA) and stored at -20 °C until subsequent reactions were performed.

Preselective:

Digestion of the genomic DNA produces a large amount of fragments that are very difficult to differentiate from each other. The preselective reaction reduces the number of fragments that can be amplified by PCR to those that only have adaptors ligated to restriction sites. When using AFLP to study relatively small genomes such as fungi, no additional nucleotides are selected for next to the restriction site in the preselective step. In larger genomes, such as plants, additional nucleotides adjacent to the restriction site are used to selectively

reduce the complexity of fragments amplified in the preselective reaction. In this study, primer sequences were complementary to the adaptor only. Primers (Integrated DNA Technologies, Coralville, IA) were designated as Eco+0 (5' GAC TGC GTA CCA ATT C 3') and Mse+0 (5' GAT GAG TCC TGA GTA A 3'), "0" indicated no base extensions other than the sequence that was complementary to the adaptor. The following reaction constituents were added to 5 μl of diluted restriction/ligation reaction: 6.8 μl water, 2 μl 10X Taq buffer, 2 μl dNTPs (Eppendorf, Westbury, NY) (2 μM), 2 μl Eco+0 primer (2.75 μM), 2 μl Mse+0 (2.75 μM), and 0.2 μl of Eppendorf Master Taq DNA polymerase (5 U/μl). Eppendorf Master Taq DNA polyermase kit provided the 10 X Taq buffer, which contained 25 mM $MgCl_2$. The following thermal cycler program was used: initial incubation at 72 °C for 2 min followed by 20 cycles of 94 °C for 20 sec, 56 °C for 30 sec, 72 °C for 2 min, final extension at 72 °C for 2 min, final incubation at 60 °C for 30 min and 4 °C hold. After amplification, 5 μl of each PCR product was separated by electrophoresis at 100 V for 1 hour in a 1% agarose gel containing ethidium bromide dye. If the restriction/ligation and preselective reactions were successful, a smear was expected in the lanes of the gel. Preselective products were then diluted with 135 ml of 10 mM Tris pH 8.0 and the dilutions served as template for several subsequent selective reactions.

Selective:

Selective reactions decreased the amount of restriction fragments for analysis by selecting for nucleotides next to the restriction site. Vos and Kuiper (1997) suggest using 2 base extensions on both primers for selective reactions in

fungal DNA studies. Preliminary testing for useful primers was completed with eight isolates from different locations. Eco+AC/Mse+CA selective reaction was separated by electrophoresis on a 1% agarose gel containing ethidium bromide at 100 V for 60 min to ensure PCR was successful before proceeding to the fluorescent reaction. Agarose gel electrophresis of fragments provided incomplete resolution, but possible polymorphisms were observed between 1000-2000bp (Figure 4.2). Capillary gel electrophoresis of fluorescently labeled Eco+AC/Mse+CA fragments yielded monomorphic products. Therefore the *Mse* I side primer was shorted by one base (Mse+C) as described by Vigi et al. (2004). Thus, a combination of primers from Vos and Kuiper (1997) and Vigi et al. (2004) were used and revealed polymorphic DNA. Several other primer combinations were tested (Eco+AC/Mse+C; Eco+AT/Mse+C; Eco+TT/Mse+C; Eco+TA/Mse+C; Eco+TG/Mse+C) and two primer combinations (Eco+AA/Mse+C and Eco+TG/Mse+C) that yielded the most polymorphisms were used for all samples. Primers were exactly the same in the preselective reactions, except for the addition of base extensions.

Selective reactions contained 8 μl of water, 2 μl 10X Taq Buffer, 0.8 μl dNTPs (2 mM), 2 μl Eco+(AA or TG) primer (2.75 μM), 2 μl Mse+C primer (2.75 μM), and 0.2 μl of Taq polymerase (5 U/μl). The following touchdown thermal cycler program was used: initial DNA denaturation temperature of 94 °C for 2 min, then 94 °C for 20 sec, 66 °C for 30 sec, 72 °C for 2 min, followed by 9 cycles of 94 °C for 20 sec, decrease by 1 °C/cycle from 66 °C, 72 °C, 20 cycles of 94 °C

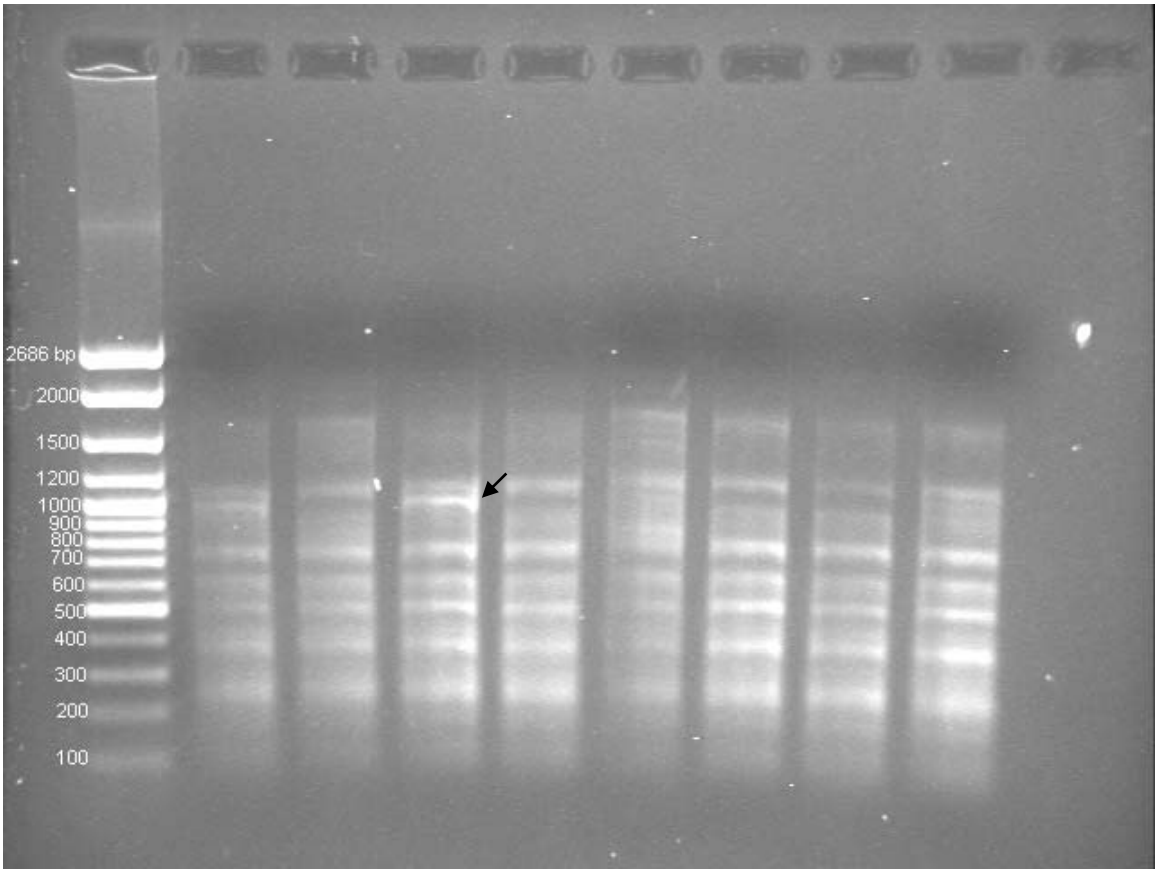

Figure 4.2: Gel electrophoresis of Eco+AC/Mse+CA *Sclerotinia homoeocarpa* selective reactions on 1% agarose gel offered incomplete resolution of bands. Some possible *Sclerotinia homoeocarpa* polymorphisms are visible around 1000 bp (indicated by arrow). Selective reaction products (5 µl) were quantified using 5 µl eXACTGene Cloning DNA Ladder (Fisher, Atlanta, GA).

for 20 sec, 56 °C for 30 sec, 72 °C for 2 min, final incubation at 60 °C for 30 min, and 4 °C hold. After PCR, samples were diluted 1:500 with 10 mM Tris pH 8.0. Plates were stored at -20 °C until fluorescent labeling reactions.

Fluorescent Labeling:

Selective reaction products were labeled with WellRED (Sigma-Aldrich, St. Louis, MO) phosporamidite dye using a labeled Eco+00 primer composed of the same sequence as preselective reaction, and Mse+C primer from the selective reaction (Habera et al., 2004). Due to the light sensitivity of the fluorescent dyes, the reactants were maintained on ice and in diminished (overhead lights in lab turned off). Labeled primer was resuspended with 10X TE buffer to 1.38 μM, and 50 μl aliquots were stored wrapped in foil at -80 °C until use. Each reaction mixture contained 4.8 μl of water, 2 μl 10X Taq buffer, 2 μl dNTPs (2 mM), 4 μl Eco+0 primer (1.38 μM), 2 μl Mse+C primer (2.75 μM), and 0.2 μl Taq polymerase (5 U/μl). Thermal cycler program was the same as the selective reaction.

Capillary Gel Electrophoresis:

Samples were prepared for capillary gel electrophoresis by Beckman Coulter CEQ™ 8000 Genetic Analysis System by first aliquoting 5 μl of each undiluted fluorescently labeled product into a CEQ™ 8000 Genetic Analysis System compatible 96-well plate in the dark. To each sample 30 μl of CEQ™ SLS (sample loading solution) and 0.5 μl of fluorescently labeled CEQ™ DNA Size Standard (600 bp ladder) were added. A drop of mineral oil was added to

the top of each sample and covered with PCR amplification tape, and then wrapped in foil to protect it from the light. A 96-well flat-bottom plate (diameter-6.4 mm; Corning Glass Company, Corning, NY) was also filled with CEQ™ Separation Buffer for gel electrophoresis. After the gel cartridge, sample plate, and buffer plate were in the machine the capillary was purged with 0.5 ml of gel before each run and the optics aligned. The CEQ program began with 2 min of capillary preparation at 50 °C, followed by denaturation at 90 °C for 120 sec, injection at 2 kV for 30 sec, and separation at 4.8 kV for 75 min with no pause between samples.

Fragment Analysis:

Electorpherograms produced by the CEQ™ 8000 were analyzed by comparing fragments to a 600 bp CEQ™ DNA Size Standard using a quartic model. Any samples that did not pass the analysis or had too low of signal were repeated through troubleshooting reactions. All samples were manually edited to ensure the software correctly called the peaks and for low background noise. The Y threshold for signal strength was set conservatively at 5000 RFU and bin at 1.5 nucleotides. Through the AFLP analysis option in the CEQ™ 8000 software, binary data was produced. The binary data, or “1” representing presence of a fragment or “0” representing absence of a fragment, was exported into an Excel file. The CEQ™ software made false calls therefore it was necessary to compare the binary data to the electropherograms after AFLP analysis and manually edit the data.

Data Analysis:

Once all binary outputs were completely edited, all data was combined from the two primer sets. Similarity indices were calculated by NTSYSpc version 2.20b (Exeter Software, 2005) using Jaccard association coefficient (Jaccard, 1908). The equation is as follows: **GS(ij) = 2a/(2a + b + c)**, where **GS (ij)** is the measure of genetic similarity between individuals **i** and **j**, **a** is the number of polymorphic bands that are shared by **i** and **j**, **b** is the number of bands present in **i** and absent in **j**, and **c** is the number of bands present in **j** and absent in **i**. In addition, pairwise absolute distances were determined using PAUP (Swofford, 2001) and combined on a table with similarity indices. From the similarity indices cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method, which produced a tree dendrogram. In addition, Principle Coordinates Analysis (PCoA) was performed using the similarity indices. Bootstrap values were calculated for cluster support using WinBoot (Yap, 1991). Bootstrapping tests the reliability of nodes on a tree by resampling. Higher bootstrap values give confidence that a particular node of a tree appears the same after each time the dataset is resampled (Opperdoes, 1997). Values below 50 were not labeled on the tree as in Trigiano et al. (2004).

Results:

After the restriction and ligation, the preselective reaction yielded a smear containing various fragment lengths on a 1% agarose gel containing ethidium bromide (Figure 4.3). Fragment sizes ranged between 100 bp-2000 bp. Although samples appeared to lack DNA, fragments were still detected during

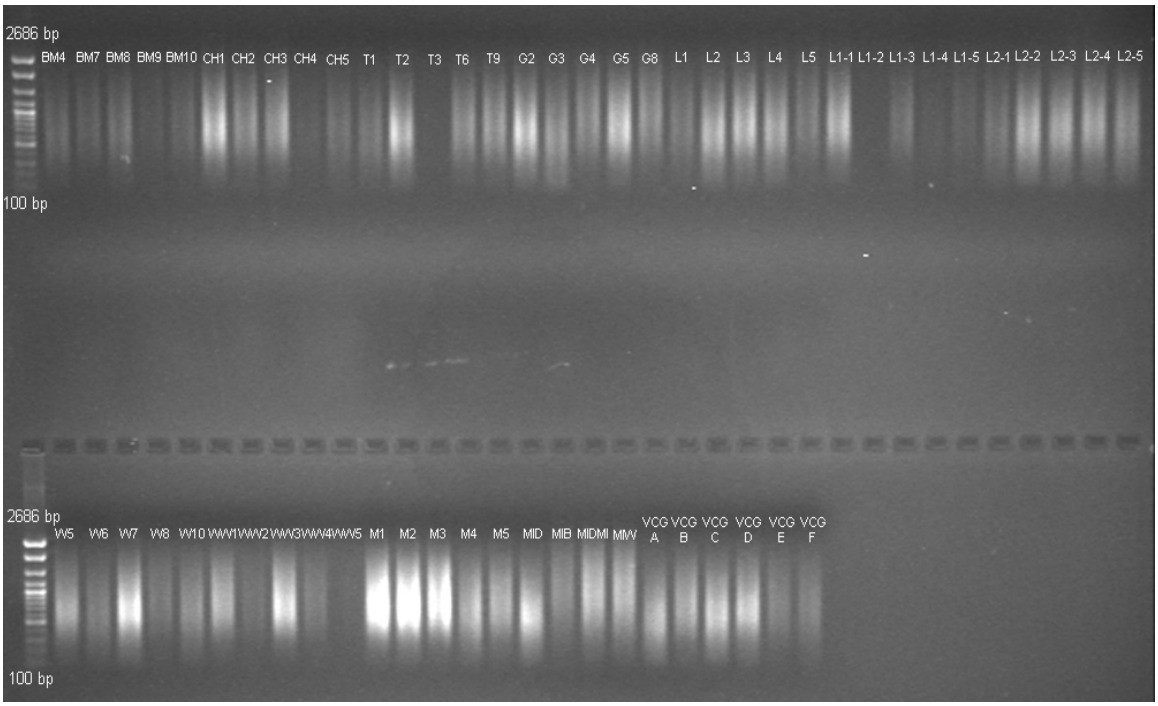

Figure 4.3: Preselective reaction of *Sclerotinia homoeocarpa* DNA fragments electrophoresed on agarose gel. The smear indicates the reaction was successful and an enormous amount of fragments were produced by restriction digestion and amplification of restriction fragments. Preselective reaction products (5 µl) were quantified using 5 µl eXACTGene Cloning DNA Ladder (Fisher, Atlanta, GA) to determine the range of fragment sizes. Labels above the lanes are abbreviations for isolate location and identification (see Table 2.1).

capillary gel electrophoresis. Once the electropherogram was created, each sample was observed for irregularities. If a sample was irregular in comparison to several other samples, another fluorescent reaction was completed. After repeating the reaction, usually electropherograms appeared similar to other samples. If the sample failed again, troubleshooting reactions were performed to "back track" which reaction failed (selective, preselective, etc). Preselective reactions were rarely repeated and the restriction/ligation was never repeated.

Eco+TG/Mse+C and Eco+AA/Mse+C primer combinations yielded fragments that ranged between 51-711 and 55-620 bp, respectively, during capillary gel electrophoresis. Fragments above 600 bp were excluded from the study due to low fluorescence. Eco+TG/Mse+C primer combination produced 44 polymorphic fragments while Eco+AA/Mse+C produced 62 polymorphic fragments. Setting the Y-threshold to 5000 allowed peaks with lower signal to be included in the AFLP study. Peaks with lower signal were only considered if several other samples had the same peak with higher signal. Although the Beckman Coulter CEQ™ 8000 Genetic Analysis Systems was very convenient and required little input from the operator, during data analysis several qualifying peaks were miss-called. Manual confirmation of peaks was necessary before conversion of the electropherogram into binary data. After the binary data were processed by the CEQ software, binary data was compared to the electropherogram for errors. Corrected binary data appears in Appendix II.

Similarity indices were calculated from the binary data, which ranged between 0.86-1.00. The most diverse isolates were L1-2/ L2-2 at 0.86. Similarity

indices within locations ranged as follows: BM 0.94-1.00; CH 0.94-1.00; T 0.99-1.00; G 0.95-0.99; L, L1, and L2 0.86-1.00; W 0.96-1.00; WW 0.96-1.00; M 0.97-1.00; MID, MIB, MIDMI, and MIW 0.92-0.97; VCG A, B, C, D, E, and F 0.91-0.99. Location abbreviations are listed on Table 2.1. Pairwise absolute character differences ranged from 0-14 fragments, indicating some isolates were the same at all loci (Table 4.1).

Similarity indices were used for cluster analysis to produce a dendrogram using UPGMA method. Two major groups (labeled as 1 and 2) were produced. Four smaller subgroups were also visible and labeled with letters A, B, C, and D. Fungicide resistance and VCG was overlaid on the AFLP tree (Figure 4.4 and Figure 4.5, respectfully). Isolates from the same location clustered together; some examples were WW isolates in subgroup A and L, L1, and L2 isolates in subgroup C. Some isolates from the same location did not group together. For instance, isolates BM4 and BM10 were in group 1 whereas BM9 was in group 2. Most bootstrap values calculated by WinBoot were less than 50, which indicated little support for almost all clusters in the dendrogram and were omitted, with a few exceptions. Sufficient bootstrap values ranged between 52 and 99. PCoA 3-D plot analysis also presented similar results as cluster analysis. No major groupings were identified and most samples plotted on top of each other (Figure 4.6). Isolate L2-2 appears to be grouping by itself, but this is due to absence of data because the Eco+AA/Mse+C primer set reaction failed.

Table 4.1: Calulcated similarity indices of *Sclerotinia homoeocarpa* below diagonal, pair wise differences above.

	Bm4	BM7	BM8	BM9	BM10	CH1	CH2	CH3	CH4	CH5	T1	T2	T3	T6	T9	G2	G3	G4	G5	G8	L1
Bm4	-	3	3	6	0	5	5	6	9	5	5	5	5	5	6	5	4	6	4	7	4
BM7	0.97	-	0	9	3	2	2	3	8	2	4	4	4	4	5	2	1	5	3	6	3
BM8	0.97	1.00	-	9	3	2	2	3	8	2	4	4	4	4	5	2	1	5	3	6	3
BM9	0.94	0.91	0.91	-	6	11	11	12	7	11	11	11	11	11	10	11	10	8	10	7	8
BM10	1.00	0.97	0.97	0.94	-	5	5	6	9	5	5	5	5	5	6	5	4	6	4	7	4
CH1	0.95	0.98	0.98	0.89	0.95	-	0	1	6	0	6	6	6	6	7	2	1	5	3	6	5
CH2	0.95	0.98	0.98	0.89	0.95	1.00	-	1	6	0	6	6	6	6	7	2	1	5	3	6	5
CH3	0.94	0.97	0.97	0.88	0.94	0.99	0.99	-	5	1	7	7	7	7	8	3	2	6	4	7	6
CH4	0.91	0.92	0.92	0.93	0.91	0.94	0.94	0.95	-	6	12	12	12	12	11	8	7	5	7	4	11
CH5	0.95	0.98	0.98	0.89	0.95	1.00	1.00	0.99	0.94	-	6	6	6	6	7	2	1	5	3	6	5
T1	0.95	0.96	0.96	0.90	0.95	0.94	0.94	0.93	0.88	0.94	-	0	0	0	1	4	5	7	5	8	3
T2	0.95	0.96	0.96	0.90	0.95	0.94	0.94	0.93	0.88	0.94	1.00	-	0	0	1	4	5	7	5	8	3
T3	0.95	0.96	0.96	0.90	0.95	0.94	0.94	0.93	0.88	0.94	1.00	1.00	-	0	1	4	5	7	5	8	3
T6	0.95	0.96	0.96	0.90	0.95	0.94	0.94	0.93	0.88	0.94	1.00	1.00	1.00	-	1	4	5	7	5	8	3
T9	0.94	0.95	0.95	0.90	0.94	0.93	0.93	0.92	0.89	0.93	0.99	0.99	0.99	0.99	-	5	6	6	6	7	4
G2	0.95	0.98	0.98	0.89	0.95	0.98	0.98	0.97	0.92	0.98	0.96	0.96	0.96	0.96	0.95	-	1	3	1	4	5
G3	0.96	0.99	0.99	0.90	0.96	0.99	0.99	0.98	0.93	0.99	0.95	0.95	0.95	0.95	0.94	0.99	-	4	2	5	4
G4	0.94	0.95	0.95	0.92	0.94	0.95	0.95	0.94	0.95	0.95	0.93	0.93	0.93	0.93	0.94	0.97	0.96	-	2	1	8
G5	0.96	0.97	0.97	0.90	0.96	0.97	0.97	0.96	0.93	0.97	0.95	0.95	0.95	0.95	0.94	0.99	0.98	0.98	-	3	6
G8	0.93	0.94	0.94	0.93	0.93	0.94	0.94	0.93	0.96	0.94	0.92	0.92	0.92	0.92	0.93	0.96	0.95	0.99	0.97	-	9
L1	0.96	0.97	0.97	0.92	0.96	0.95	0.95	0.94	0.89	0.95	0.97	0.97	0.97	0.97	0.96	0.95	0.96	0.92	0.94	0.91	-
L2	0.95	0.96	0.96	0.89	0.95	0.98	0.98	0.97	0.92	0.98	0.96	0.96	0.96	0.96	0.95	0.96	0.97	0.93	0.95	0.92	0.97
L3	0.95	0.96	0.96	0.89	0.95	0.98	0.98	0.97	0.92	0.98	0.96	0.96	0.96	0.96	0.95	0.96	0.97	0.93	0.95	0.92	0.97
L4	0.94	0.93	0.93	0.92	0.94	0.95	0.95	0.94	0.95	0.95	0.93	0.93	0.93	0.93	0.94	0.93	0.94	0.96	0.94	0.95	0.94
L5	0.94	0.93	0.93	0.92	0.94	0.95	0.95	0.94	0.95	0.95	0.93	0.93	0.93	0.93	0.94	0.93	0.94	0.96	0.94	0.95	0.94
L1-1	0.94	0.97	0.97	0.89	0.94	0.97	0.97	0.96	0.91	0.97	0.97	0.97	0.97	0.97	0.96	0.99	0.98	0.96	0.98	0.95	0.94
L1-2	0.93	0.94	0.94	0.90	0.93	0.94	0.94	0.93	0.92	0.94	0.94	0.94	0.94	0.94	0.93	0.96	0.95	0.97	0.97	0.96	0.91
L1-3	0.94	0.97	0.97	0.89	0.94	0.97	0.97	0.96	0.91	0.97	0.97	0.97	0.97	0.97	0.96	0.99	0.98	0.96	0.98	0.95	0.94
L1-4	0.95	0.98	0.98	0.89	0.95	0.98	0.98	0.97	0.92	0.98	0.96	0.96	0.96	0.96	0.95	0.98	0.99	0.95	0.97	0.94	0.95
L1-5	0.93	0.94	0.94	0.93	0.93	0.94	0.94	0.93	0.96	0.94	0.92	0.92	0.92	0.92	0.93	0.94	0.95	0.97	0.95	0.96	0.91
L2-1	0.94	0.95	0.95	0.88	0.94	0.97	0.97	0.96	0.91	0.97	0.95	0.95	0.95	0.95	0.94	0.95	0.96	0.92	0.94	0.91	0.96
L2-2	0.93	0.95	0.95	0.91	0.93	0.95	0.95	0.95	0.91	0.95	0.98	0.98	0.98	0.98	0.98	0.95	0.95	0.91	0.93	0.91	1.00

Table 4.1: Continued

	Bm4	BM7	BM8	BM9	BM10	CH1	CH2	CH3	CH4	CH5	T1	T2	T3	T6	T9	G2	G3	G4	G5	G8	L1
L2-3	0.95	0.96	0.96	0.89	0.95	0.98	0.98	0.97	0.92	0.98	0.96	0.96	0.96	0.96	0.95	0.96	0.97	0.93	0.95	0.92	0.97
L2-4	0.95	0.96	0.96	0.89	0.95	0.98	0.98	0.97	0.92	0.98	0.96	0.96	0.96	0.96	0.95	0.96	0.97	0.93	0.95	0.92	0.97
L2-5	0.95	0.96	0.96	0.89	0.95	0.98	0.98	0.97	0.92	0.98	0.96	0.96	0.96	0.96	0.95	0.96	0.97	0.93	0.95	0.92	0.97
W5	0.95	0.96	0.96	0.89	0.95	0.98	0.98	0.97	0.92	0.98	0.96	0.96	0.96	0.96	0.95	0.96	0.97	0.93	0.95	0.92	0.97
W6	0.95	0.96	0.96	0.89	0.95	0.96	0.96	0.95	0.9	0.96	0.98	0.98	0.98	0.98	0.97	0.96	0.97	0.93	0.95	0.92	0.97
W7	0.94	0.95	0.95	0.89	0.94	0.95	0.95	0.94	0.89	0.95	0.99	0.99	0.99	0.99	0.98	0.97	0.96	0.94	0.96	0.93	0.96
W8	0.95	0.94	0.94	0.9	0.95	0.94	0.94	0.93	0.9	0.94	0.98	0.98	0.98	0.98	0.97	0.96	0.95	0.95	0.97	0.94	0.95
W10	0.94	0.95	0.95	0.89	0.94	0.95	0.95	0.94	0.89	0.95	0.99	0.99	0.99	0.99	0.98	0.97	0.96	0.94	0.96	0.93	0.96
WW1	0.96	0.95	0.95	0.9	0.96	0.95	0.95	0.94	0.91	0.95	0.95	0.95	0.95	0.95	0.94	0.97	0.96	0.96	0.98	0.95	0.92
WW2	0.97	0.96	0.96	0.91	0.97	0.96	0.96	0.95	0.92	0.96	0.94	0.94	0.94	0.94	0.93	0.96	0.97	0.95	0.97	0.94	0.93
WW3	0.96	0.95	0.95	0.9	0.96	0.95	0.95	0.94	0.91	0.95	0.95	0.95	0.95	0.95	0.94	0.97	0.96	0.96	0.98	0.95	0.92
WW4	0.96	0.95	0.95	0.9	0.96	0.95	0.95	0.94	0.91	0.95	0.95	0.95	0.95	0.95	0.94	0.97	0.96	0.96	0.98	0.95	0.92
WW5	0.94	0.95	0.95	0.9	0.94	0.95	0.95	0.96	0.91	0.95	0.93	0.93	0.93	0.93	0.92	0.95	0.96	0.92	0.94	0.91	0.94
M1	0.95	0.98	0.98	0.89	0.95	1.00	1.00	0.99	0.94	1.00	0.94	0.94	0.94	0.94	0.93	0.98	0.99	0.95	0.97	0.94	0.95
M2	0.95	0.98	0.98	0.89	0.95	0.98	0.98	0.97	0.92	0.98	0.96	0.96	0.96	0.96	0.95	0.98	0.99	0.95	0.97	0.94	0.97
M3	0.93	0.96	0.96	0.87	0.93	0.98	0.98	0.97	0.92	0.98	0.94	0.94	0.94	0.94	0.93	0.96	0.97	0.93	0.95	0.92	0.95
M4	0.94	0.97	0.97	0.90	0.94	0.97	0.97	0.96	0.91	0.97	0.95	0.95	0.95	0.95	0.94	0.97	0.98	0.94	0.96	0.93	0.98
M5	0.94	0.97	0.97	0.90	0.94	0.97	0.97	0.96	0.91	0.97	0.95	0.95	0.95	0.95	0.94	0.97	0.98	0.94	0.96	0.93	0.98
MID	0.96	0.95	0.95	0.90	0.96	0.97	0.97	0.96	0.93	0.97	0.95	0.95	0.95	0.95	0.94	0.95	0.96	0.94	0.96	0.93	0.96
MIB	0.95	0.92	0.92	0.95	0.95	0.94	0.94	0.93	0.96	0.94	0.90	0.90	0.90	0.90	0.89	0.92	0.93	0.93	0.93	0.94	0.91
MIDMI	0.92	0.93	0.93	0.94	0.92	0.93	0.93	0.92	0.97	0.93	0.91	0.91	0.91	0.91	0.92	0.93	0.94	0.96	0.94	0.97	0.90
MIW	0.92	0.91	0.91	0.94	0.92	0.93	0.93	0.92	0.97	0.93	0.91	0.91	0.91	0.91	0.92	0.91	0.92	0.94	0.92	0.95	0.92
VCGA	0.95	0.96	0.96	0.89	0.95	0.96	0.96	0.95	0.90	0.96	0.98	0.98	0.98	0.98	0.97	0.96	0.97	0.93	0.95	0.92	0.97
VCGB	0.94	0.95	0.95	0.89	0.94	0.95	0.95	0.94	0.89	0.95	0.99	0.99	0.99	0.99	0.98	0.97	0.96	0.94	0.96	0.93	0.96
VCGC	0.94	0.97	0.97	0.88	0.94	0.97	0.97	0.96	0.91	0.97	0.95	0.95	0.95	0.95	0.94	0.97	0.98	0.94	0.96	0.93	0.94
VCGD	0.94	0.95	0.95	0.89	0.94	0.95	0.95	0.94	0.89	0.95	0.99	0.99	0.99	0.99	0.98	0.97	0.96	0.94	0.96	0.93	0.96
VCGE	0.94	0.93	0.93	0.90	0.94	0.91	0.91	0.92	0.89	0.91	0.93	0.93	0.93	0.93	0.92	0.93	0.92	0.92	0.94	0.91	0.90
VCGF	0.97	0.94	0.94	0.91	0.97	0.94	0.94	0.93	0.90	0.94	0.96	0.96	0.96	0.96	0.95	0.96	0.95	0.95	0.97	0.94	0.93

Table 4.1: Continued

	L2	L3	L4	L5	L1-1	L1-2	L1-3	L1-4	L1-5	L2-1	L2-2	L2-3	L2-4	L2-5	W5	W6	W7	W8	W10	WW1
Bm4	5	5	6	6	6	7	6	5	7	6	3	5	5	5	5	5	6	5	6	4
BM7	4	4	7	7	3	6	3	2	6	5	2	4	4	4	4	4	5	6	5	5
BM8	4	4	7	7	3	6	3	2	6	5	2	4	4	4	4	4	5	6	5	5
BM9	11	11	8	8	12	11	12	11	7	12	4	11	11	11	11	11	12	11	12	10
BM10	5	5	6	6	6	7	6	5	7	6	3	5	5	5	5	5	6	5	6	4
CH1	2	2	5	5	3	6	3	2	6	3	2	2	2	2	2	4	5	6	5	5
CH2	2	2	5	5	3	6	3	2	6	3	2	2	2	2	2	4	5	6	5	5
CH3	3	3	6	6	4	7	4	3	7	4	2	3	3	3	3	5	6	7	6	6
CH4	8	8	5	5	9	8	9	8	4	9	4	8	8	8	8	10	11	10	11	9
CH5	2	2	5	5	3	6	3	2	6	3	2	2	2	2	2	4	5	6	5	5
T1	4	4	7	7	3	6	3	4	8	5	1	4	4	4	4	2	1	2	1	5
T2	4	4	7	7	3	6	3	4	8	5	1	4	4	4	4	2	1	2	1	5
T3	4	4	7	7	3	6	3	4	8	5	1	4	4	4	4	2	1	2	1	5
T6	4	4	7	7	3	6	3	4	8	5	1	4	4	4	4	2	1	2	1	5
T9	5	5	6	6	4	7	4	5	7	6	1	5	5	5	5	3	2	3	2	6
G2	4	4	7	7	1	4	1	2	6	5	2	4	4	4	4	4	3	4	3	3
G3	3	3	6	6	2	5	2	1	5	4	2	3	3	3	3	3	4	5	4	4
G4	7	7	4	4	4	3	4	5	3	8	4	7	7	7	7	7	6	5	6	4
G5	5	5	6	6	2	3	2	3	5	6	3	5	5	5	5	5	4	3	4	2
G8	8	8	5	5	5	4	5	6	4	9	4	8	8	8	8	8	7	6	7	5
L1	3	3	6	6	6	9	6	5	9	4	0	3	3	3	3	3	4	5	4	8
L2	-	0	3	3	5	8	5	4	8	1	0	0	0	0	0	2	3	4	3	7
L3	1.00	-	3	3	5	8	5	4	8	1	0	0	0	0	0	2	3	4	3	7
L4	0.97	0.97	-	0	8	7	8	7	5	4	2	3	3	3	3	5	6	5	6	8
L5	0.97	0.97	1.00	-	8	7	8	7	5	4	2	3	3	3	3	5	6	5	6	8
L1-1	0.95	0.95	0.92	0.92	-	3	0	1	5	6	3	5	5	5	5	3	2	3	2	2
L1-2	0.92	0.92	0.93	0.93	0.97	-	3	4	4	9	6	8	8	8	8	6	5	4	5	3
L1-3	0.95	0.95	0.92	0.92	1.00	0.97	-	1	5	6	3	5	5	5	5	3	2	3	2	2
L1-4	0.96	0.96	0.93	0.93	0.99	0.96	0.99	-	4	5	3	4	4	4	4	2	3	4	3	3
L1-5	0.92	0.92	0.95	0.95	0.95	0.96	0.95	0.96	-	9	5	8	8	8	8	6	7	6	7	5
L2-1	0.99	0.99	0.96	0.96	0.94	0.91	0.94	0.95	0.91	-	0	1	1	1	1	3	4	5	4	8
L2-2	1.00	1.00	0.95	0.95	0.93	0.86	0.93	0.93	0.89	1.00	-	0	0	0	0	1	1	2	1	5

Table 4.1: Continued

	L2	L3	L4	L5	L1-1	L1-2	L1-3	L1-4	L1-5	L2-1	L2-2	L2-3	L2-4	L2-5	W5	W6	W7	W8	W10	WW1
L2-3	1.00	1.00	0.97	0.97	0.95	0.92	0.95	0.96	0.92	0.99	1.00	-	0	0	0	2	3	4	3	7
L2-4	1.00	1.00	0.97	0.97	0.95	0.92	0.95	0.96	0.92	0.99	1.00	1.00	-	0	0	2	3	4	3	7
L2-5	1.00	1.00	0.97	0.97	0.95	0.92	0.95	0.96	0.92	0.99	1.00	1.00	1.00	-	0	2	3	4	3	7
W5	1.00	1.00	0.97	0.97	0.95	0.92	0.95	0.96	0.92	0.99	1.00	1.00	1.00	1.00	-	2	3	4	3	7
W6	0.98	0.98	0.95	0.95	0.97	0.94	0.97	0.98	0.94	0.97	0.98	0.98	0.98	0.98	0.98	-	1	2	1	5
W7	0.97	0.97	0.94	0.94	0.98	0.95	0.98	0.97	0.93	0.96	0.98	0.97	0.97	0.97	0.97	0.99	-	1	0	4
W8	0.96	0.96	0.95	0.95	0.97	0.96	0.97	0.96	0.94	0.95	0.95	0.96	0.96	0.96	0.96	0.98	0.99	-	1	3
W10	0.97	0.97	0.94	0.94	0.98	0.95	0.98	0.97	0.93	0.96	0.98	0.97	0.97	0.97	0.97	0.99	1.00	0.99	-	4
WW1	0.93	0.93	0.92	0.92	0.98	0.97	0.98	0.97	0.95	0.92	0.88	0.93	0.93	0.93	0.93	0.95	0.96	0.97	0.96	-
WW2	0.94	0.94	0.93	0.93	0.97	0.96	0.97	0.98	0.96	0.93	0.88	0.94	0.94	0.94	0.94	0.96	0.95	0.96	0.95	0.99
WW3	0.93	0.93	0.92	0.92	0.98	0.97	0.98	0.97	0.95	0.92	0.88	0.93	0.93	0.93	0.93	0.95	0.96	0.97	0.96	1.00
WW4	0.93	0.93	0.92	0.92	0.98	0.97	0.98	0.97	0.95	0.92	0.88	0.93	0.93	0.93	0.93	0.95	0.96	0.97	0.96	1.00
WW5	0.93	0.93	0.90	0.90	0.96	0.93	0.96	0.97	0.93	0.92	0.90	0.93	0.93	0.93	0.93	0.95	0.94	0.93	0.94	0.96
M1	0.98	0.98	0.95	0.95	0.97	0.94	0.97	0.98	0.94	0.97	0.95	0.98	0.98	0.98	0.98	0.96	0.95	0.94	0.95	0.95
M2	0.98	0.98	0.95	0.95	0.97	0.94	0.97	0.98	0.94	0.97	0.97	0.98	0.98	0.98	0.98	0.98	0.97	0.96	0.97	0.95
M3	0.98	0.98	0.95	0.95	0.95	0.92	0.95	0.96	0.92	0.97	0.97	0.98	0.98	0.98	0.98	0.96	0.95	0.94	0.95	0.93
M4	0.97	0.97	0.94	0.94	0.96	0.93	0.96	0.97	0.93	0.96	0.97	0.97	0.97	0.97	0.97	0.97	0.96	0.95	0.96	0.94
M5	0.97	0.97	0.94	0.94	0.96	0.93	0.96	0.97	0.93	0.96	0.97	0.97	0.97	0.97	0.97	0.97	0.96	0.95	0.96	0.94
MID	0.99	0.99	0.98	0.98	0.94	0.93	0.94	0.95	0.93	0.98	0.98	0.99	0.99	0.99	0.99	0.97	0.96	0.97	0.96	0.94
MIB	0.94	0.94	0.95	0.95	0.91	0.92	0.91	0.92	0.94	0.93	0.91	0.94	0.94	0.94	0.94	0.92	0.91	0.92	0.91	0.93
MIDMI	0.91	0.91	0.94	0.94	0.94	0.95	0.94	0.95	0.99	0.90	0.89	0.91	0.91	0.91	0.91	0.93	0.92	0.93	0.92	0.94
MIW	0.95	0.95	0.98	0.98	0.90	0.91	0.90	0.91	0.95	0.94	0.95	0.95	0.95	0.95	0.95	0.93	0.92	0.93	0.92	0.90
VCGA	0.98	0.98	0.95	0.95	0.97	0.94	0.97	0.98	0.94	0.97	0.98	0.98	0.98	0.98	0.98	1.00	0.99	0.98	0.99	0.95
VCGB	0.97	0.97	0.94	0.94	0.98	0.95	0.98	0.97	0.93	0.96	0.98	0.97	0.97	0.97	0.97	0.99	1.00	0.99	1.00	0.96
VCGC	0.95	0.95	0.92	0.92	0.98	0.95	0.98	0.99	0.95	0.94	0.93	0.95	0.95	0.95	0.95	0.97	0.96	0.95	0.96	0.96
VCGD	0.97	0.97	0.94	0.94	0.98	0.95	0.98	0.97	0.93	0.96	0.98	0.97	0.97	0.97	0.97	0.99	1.00	0.99	1.00	0.96
VCGE	0.89	0.89	0.88	0.88	0.94	0.93	0.94	0.93	0.91	0.88	0.88	0.89	0.89	0.89	0.89	0.91	0.92	0.93	0.92	0.96
VCGF	0.94	0.94	0.93	0.93	0.97	0.96	0.97	0.96	0.94	0.93	0.91	0.94	0.94	0.94	0.94	0.96	0.97	0.98	0.97	0.99

Table 4.1: Continued

	WW2	WW3	WW4	WW5	M1	M2	M3	M4	M5	MID	MIB	MIDMI	MIW	VCGA	VCGB	VCGC	VCGD	VCGE	VCGF
Bm4	3	4	4	6	5	5	7	6	6	4	5	8	8	5	6	6	6	6	3
BM7	4	5	5	5	2	2	4	3	3	5	8	7	9	4	5	3	5	7	6
BM8	4	5	5	5	2	2	4	3	3	5	8	7	9	4	5	3	5	7	6
BM9	9	10	10	10	11	11	13	10	10	10	5	6	6	11	12	12	12	10	9
BM10	3	4	4	6	5	5	7	6	6	4	5	8	8	5	6	6	6	6	3
CH1	4	5	5	5	0	2	2	3	3	3	6	7	7	4	5	3	5	9	6
CH2	4	5	5	5	0	2	2	3	3	3	6	7	7	4	5	3	5	9	6
CH3	5	6	6	4	1	3	3	4	4	4	7	8	8	5	6	4	6	8	7
CH4	8	9	9	9	6	8	8	9	9	7	4	3	3	10	11	9	11	11	10
CH5	4	5	5	5	0	2	2	3	3	3	6	7	7	4	5	3	5	9	6
T1	6	5	5	7	6	4	6	5	5	5	10	9	9	2	1	5	1	7	4
T2	6	5	5	7	6	4	6	5	5	5	10	9	9	2	1	5	1	7	4
T3	6	5	5	7	6	4	6	5	5	5	10	9	9	2	1	5	1	7	4
T6	6	5	5	7	6	4	6	5	5	5	10	9	9	2	1	5	1	7	4
T9	7	6	6	8	7	5	7	6	6	6	11	8	8	3	2	6	2	8	5
G2	4	3	3	5	2	2	4	3	3	5	8	7	9	4	3	3	3	7	4
G3	3	4	4	4	1	1	3	2	2	4	7	6	8	3	4	2	4	8	5
G4	5	4	4	8	5	5	7	6	6	6	7	4	6	7	6	6	6	8	5
G5	3	2	2	6	3	3	5	4	4	4	7	6	8	5	4	4	4	6	3
G8	6	5	5	9	6	6	8	7	7	7	6	3	5	8	7	7	7	9	6
L1	7	8	8	6	5	3	5	2	2	4	9	10	8	3	4	6	4	10	7
L2	6	7	7	7	2	2	2	3	3	1	6	9	5	2	3	5	3	11	6
L3	6	7	7	7	2	2	2	3	3	1	6	9	5	2	3	5	3	11	6
L4	7	8	8	10	5	5	5	6	6	2	5	6	2	5	6	8	6	12	7
L5	7	8	8	10	5	5	5	6	6	2	5	6	2	5	6	8	6	12	7
L1-1	3	2	2	4	3	3	5	4	4	6	9	6	10	3	2	2	2	6	3
L1-2	4	3	3	7	6	6	8	7	7	7	8	5	9	6	5	5	5	7	4
L1-3	3	2	2	4	3	3	5	4	4	6	9	6	10	3	2	2	2	6	3
L1-4	2	3	3	3	2	2	4	3	3	5	8	5	9	2	3	1	3	7	4
L1-5	4	5	5	7	6	6	8	7	7	7	6	1	5	6	7	5	7	9	6
L2-1	7	8	8	8	3	3	3	4	4	2	7	10	6	3	4	6	4	12	7
L2-2	5	5	5	4	2	1	1	1	1	1	4	5	2	1	1	3	1	5	4

Table 4.1: Continued

	WW2	WW3	WW4	WW5	M1	M2	M3	M4	M5	MID	MIB	MIDMI	MIW	VCGA	VCGB	VCGC	VCGD	VCGE	VCGF
L2-3	6	7	7	7	2	2	2	3	3	1	6	9	5	2	3	5	3	11	6
L2-4	6	7	7	7	2	2	2	3	3	1	6	9	5	2	3	5	3	11	6
L2-5	6	7	7	7	2	2	2	3	3	1	6	9	5	2	3	5	3	11	6
W5	6	7	7	7	2	2	2	3	3	1	6	9	5	2	3	5	3	11	6
W6	4	5	5	5	4	2	4	3	3	3	8	7	7	0	1	3	1	9	4
W7	5	4	4	6	5	3	5	4	4	4	9	8	8	1	0	4	0	8	3
W8	4	3	3	7	6	4	6	5	5	3	8	7	7	2	1	5	1	7	2
W10	5	4	4	6	5	3	5	4	4	4	9	8	8	1	0	4	0	8	3
WW1	1	0	0	4	5	5	7	6	6	6	7	6	10	5	4	4	4	4	1
WW2	-	1	1	3	4	4	6	5	5	5	6	5	9	4	5	3	5	5	2
WW3	0.99	-	0	4	5	5	7	6	6	6	7	6	10	5	4	4	4	4	1
WW4	0.99	1.00	-	4	5	5	7	6	6	6	7	6	10	5	4	4	4	4	1
WW5	0.97	0.96	0.96	-	5	5	7	4	4	8	9	8	12	5	6	4	6	6	5
M1	0.96	0.95	0.95	0.95	-	2	2	3	3	3	6	7	7	4	5	3	5	9	6
M2	0.96	0.95	0.95	0.95	0.98	-	2	1	1	3	8	7	7	2	3	3	3	9	6
M3	0.94	0.93	0.93	0.93	0.98	0.98	-	3	3	3	8	9	7	4	5	5	5	11	8
M4	0.95	0.94	0.94	0.96	0.97	0.99	0.97	-	0	4	9	8	8	3	4	4	4	10	7
M5	0.95	0.94	0.94	0.96	0.97	0.99	0.97	1.00	-	4	9	8	8	3	4	4	4	10	7
MID	0.95	0.94	0.94	0.92	0.97	0.97	0.97	0.96	0.96	-	5	8	4	3	4	6	4	10	5
MIB	0.94	0.93	0.93	0.91	0.94	0.92	0.92	0.91	0.91	0.95	-	5	3	8	9	9	9	11	6
MIDMI	0.95	0.94	0.94	0.92	0.93	0.93	0.91	0.92	0.92	0.92	0.95	-	4	7	8	6	8	10	7
MIW	0.91	0.90	0.90	0.88	0.93	0.93	0.93	0.92	0.92	0.96	0.97	0.96	-	7	8	10	8	14	9
VCGA	0.96	0.95	0.95	0.95	0.96	0.98	0.96	0.97	0.97	0.97	0.92	0.93	0.93	-	1	3	1	9	4
VCGB	0.95	0.96	0.96	0.94	0.95	0.97	0.95	0.96	0.96	0.96	0.91	0.92	0.92	0.99	-	4	0	8	3
VCGC	0.97	0.96	0.96	0.96	0.97	0.97	0.95	0.96	0.96	0.94	0.91	0.94	0.9	0.97	0.96	-	4	6	5
VCGD	0.95	0.96	0.96	0.94	0.95	0.97	0.95	0.96	0.96	0.96	0.91	0.92	0.92	0.99	1.00	0.96	-	8	3
VCGE	0.95	0.96	0.96	0.94	0.91	0.91	0.89	0.90	0.90	0.90	0.89	0.90	0.87	0.91	0.92	0.94	0.92	-	5
VCGF	0.98	0.99	0.99	0.95	0.94	0.94	0.92	0.93	0.93	0.95	0.94	0.93	0.91	0.96	0.97	0.95	0.97	0.95	-

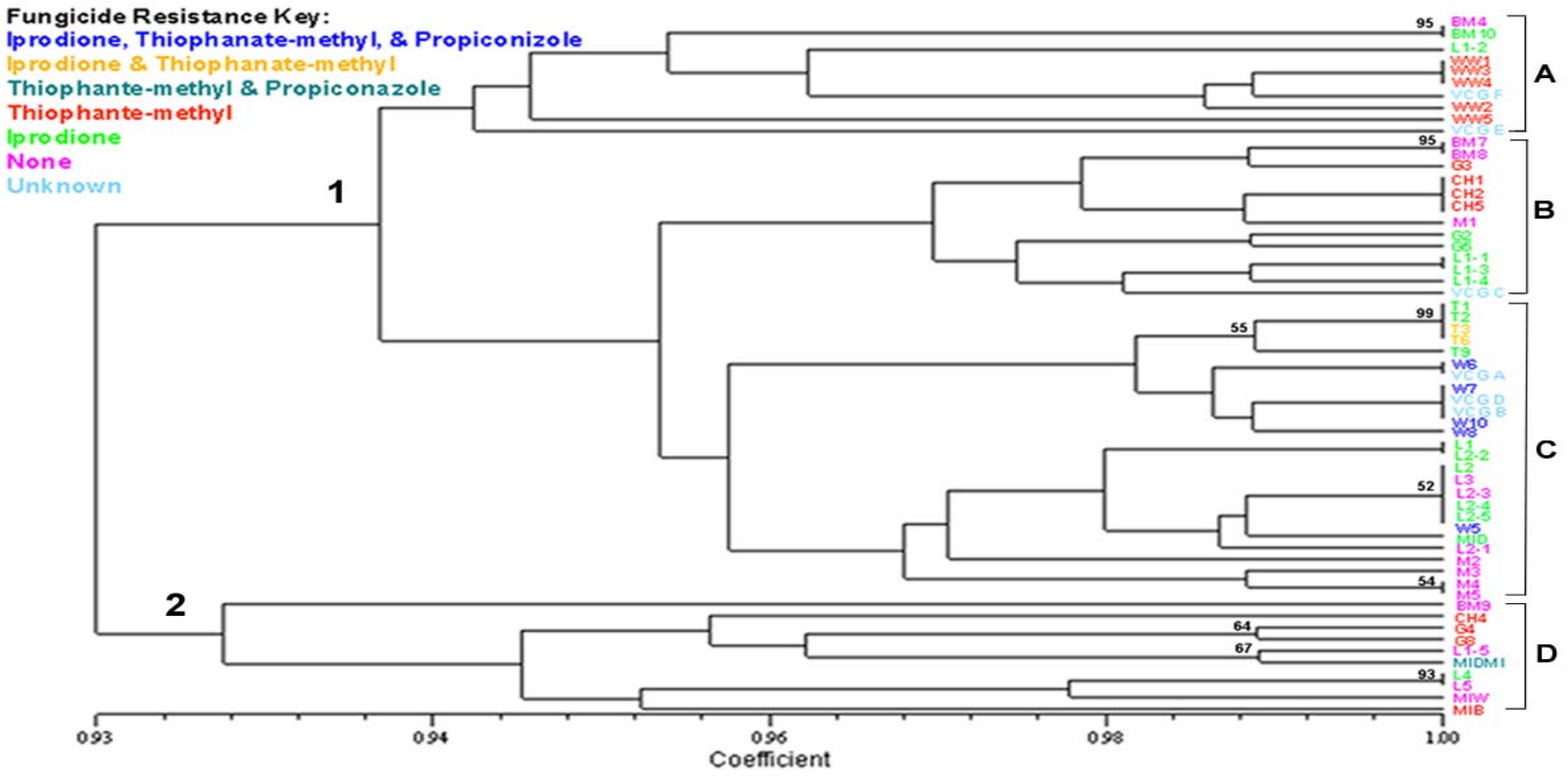

Figure 4.4: Dendrogram produced from calculated similarity indices of *Sclerotinia homoeocarpa* isolates and overlaid with fungicide resistance in color. Abbreviations for isolate name and can be found on Table 2.1.

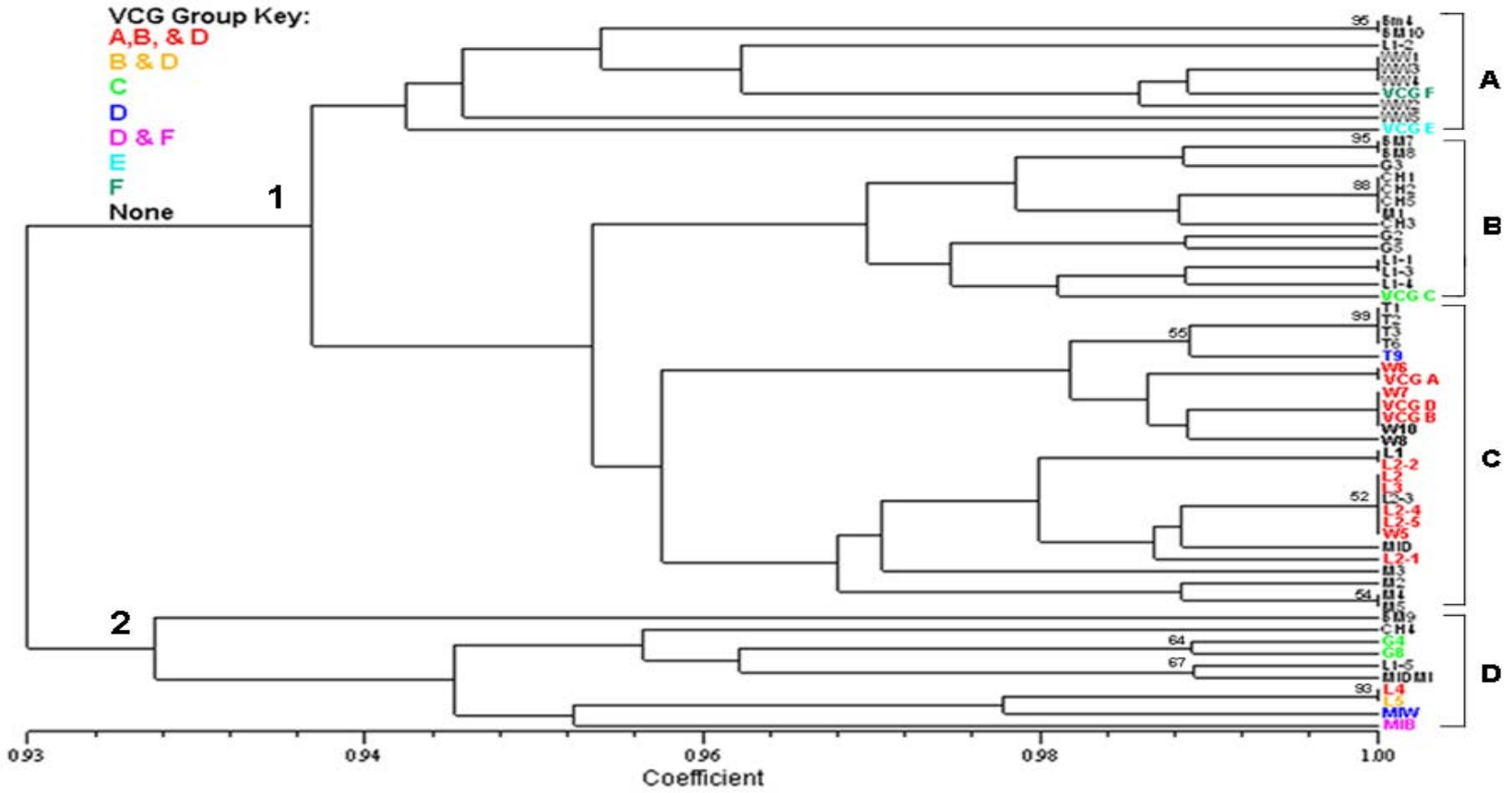

Figure 4.5: Dendrogram produced from calculated similarity indices of *Sclerotinia homoeocarpa* isolates and overlaid with VCG data in color. Abbreviations for isolate name and can be found on Table 2.1.

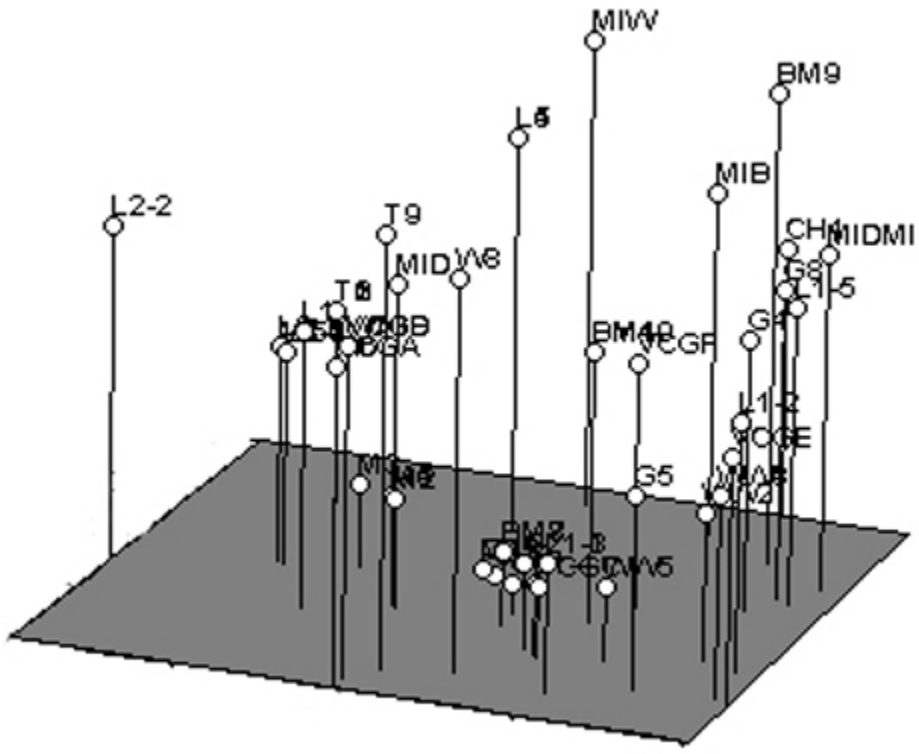

Figure 4.6: PCoA 3-D plot for AFLP data from *Sclerotinia homoeocarpa* isolates. Abbreviations for isolates name and location can be found on Table 2.1.

Discussion:

AFLP has proven to be a useful technique for assessing genetic diversity in *S. homoeocarpa*. One of the goals of this study was to determine if it was possible to expose genetic similarity between isolates with the same fungicide resistance. During UPGMA, isolates did not appear to cluster according to fungicide resistance. In subgroups A, B, C, and D, a mixture of fungicide resistance was illustrated. For instance, in subgroup C, many of the isolates had iprodione resistance in addition to thiophanate-methyl, propiconazole, or no resistance. Bootstrap values were calculated and were insufficient to support branches, therefore there is little confidence in the apparent clustering of isolates. Clusters with sufficient bootstrap support were not consistently resistant to a single fungicide. For instance, the branch with BM4/BM10 and L4/L5 had bootstrap values above 90, which indicated strong support to the grouping, but there were two different types of fungicide resistance. A similar study using UPGMA and principle component analysis to relate RAPDs to host specificity also was not able to clearly expose a relationship between genotype and phenotype. Some clustering occurred of isolates with similar host specificity, but the significance was difficult to interpret (Hsiang et al., 2000).

In contrast to the fungicide resistance, isolates within a similar VCG appeared to cluster together in subgroups. For example, in subgroup C, isolates that were compatible with VCG A, B, and D clustered together as well as tester strains A, B, and D. Interestingly, B and D tester strains have a similarity index of 0.99, which suggests that relatively little variation was found between these

strains. Since these tester strains were so similar it is feasible to think they really are not dependable for delineation of isolates for vegetative compatibility. Another study has shown similar results in the respect that isolates in the same VCG grouped on a dendrogram. The same VCG tester strains from Michigan State University were used by Vigi et al. (2004) and isolates successfully separated into their known VCGs. In the current study, isolates G4 and G8 assembled together in subgroup D, but did not cluster with tester strain C in subgroup B. Vigi et al. (2004) also found that isolates in VCG C separated into two different subgroups. Only one primer combination was used (Eco+AG/Mse+C) while 2 different pairs were used in this study. Interestingly, during preliminary primer trials in this study no polymorphisms were identified using the Eco+AG primer set. Regions of DNA associated with the genes for compatibility appeared to be selected for using these primer combinations.

In addition to isolates clustering into VCGs on the dendrogram, isolates appear to be grouping due to location, though insufficient bootstrap values do not support this hypothesis. Some isolates did not group with other members from the same location, such as BM9. Most of the isolates from location L, L1, and L2 clustered together in subgroup C as well as isolates from WW, T, CH, and M. RFLP of the IGS (intergenic spacer) region and RAPD analysis have also indicated that isolates from Ontario, Canada clustered according to population origin with a few exceptions (Hsiang and Mahuku, 1999). In addition, the similarity calculations among Tennessee and Mississippi isolates ranged between 0.86-1.00, which is also similar to the results found between Ontario

isolates by Hsiang and Mahuku (1999) and Vigi et al. (2004). This lack of variability is thought to be due to the lack sexual recombination, which therefore indicates these populations are clonal in nature (Hsiang and Mahuku, 1999; Vigi et al., 2004).

Grouping of isolates during cluster analysis from one location could also be explained by the founder effect. Founder effects are a type of genetic drift that is characterized by a change in the frequencies of alleles in a population resulting in sampling error in drawing gametes from the gene pool. If drift is the only evolutionary force, eventually one allele will drift to a frequency of 1 or become fixed. All other alleles will be lost, and therefore a loss of heterozygocity. Drift especially impacts small, isolated populations and can produce rapid genetic divergence. When a barrier forms and divides the species into two separate groups, over succeeding generations, either through selection or random events, the populations become different. Natural selection is not associated with genetic drift since adaptation is not involved and alleles are chosen randomly (Griffiths et al., 1999). In this current study, it is possible that one isolate of *S. homoeocarpa* was separated from a larger population and all isolates within the location could have been derived from the original progenitor.

One of the drawbacks of using a 600 base pair size standard and an automated capillary gel electorphoresis system is that it may not allow complete recognition of all polymorphisms. After electrophresis of the selective reaction, possible polymorphisms were detected on a 1% agarose gel between 1000-2000bp using primers Eco+AC/Mse+CA. Since there is little variation in this

species, perhaps if polymorphisms could be detected at all fragment lengths, additional variation could be revealed.

Arbitrarily primed PCR profiling, such as AFLP, have been popular techniques that use indiscriminate or semi- indiscriminate primers for amplification of DNA products (O'Malley and Whetten, 1997). Although arbitrary molecular markers are considered to be more efficient than sequencing, little information is created about the alleles. Theoretically, AFLPs have two alleles per loci, one that was amplified and one that was not, and genetic difference is not known (McDonald, 1997). Additionally, arbitrarily primed PCR techniques do not give information about what region of genomic DNA was amplified. For example, members of the same VCG clustered together in the dendrogram indicating that AFLP primers could be amplifying regions of DNA associated with the genes for vegetative compatibility. Similarity indices indicated low diversity between Tennessee and Mississippi isolates, therefore it could be assumed that bands that comigrate are the same (Trigiano et al., 1995).

Summary:

Tennessee and Mississippi *S. homoeocarpa* isolates restriction fragments were selectively amplified and fluorescently labeled by PCR during AFLP analysis to determine if any molecular markers are common to VCG members or isolates resistant to the same fungicide formulation. Similarity indices and pairwise differences calculated indicate large amounts of homology between isolates (0.86-1.00 and 0-14, respectively). Grouping of isolates with similar fungicide resistance was not revealed by cluster analysis, although grouping of

isolates from the same VCG and location occurred. Unfortunately, clusters were not supported by sufficient bootstrap values. Clustering of members of the same VCG suggests that AFLP primers could be amplifying regions of DNA associated with genes for vegetative compatibility. Clustering of isolates from the same location could be due to founder effects. Arbitrarily primed PCR techniques, such as AFLP, do not give information about what region of genomic DNA was amplified, thus more work will need to be done to determine the origin of these polymorphisms.

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APPENDICES

APPENDIX I: CONSERVED GENE SEQUENCES

Partial DNA sequence of β-tubulin of *Sclerotinia homoeocarpa*. The intron is indicated in red.

1 CCTTCCTTCCACGAAATAAATTTCTGCACATGCGCGACGATGGAGACACAGTACTTACGA
61 CGATACAGGCAAACAATCTCTGGCGAGCACGGCTTGGATGGCTCTGGTGTGTAAGTGACC
121 TCTACCAGTCTCTATGTGGTGTGCACAAAACTGATTTTTATTTACAGCTACAATGGCACT
181 TCCGATCTTCAACTTGAGCGCATGAACGTCTACTTCAACGAGGTAAATACCGACCGTCCA
241 TAATACGAGCTGCGGATAATAGAATTCTTGCTAATGCCCATAAAGGCTTCAGGAAACAAG
301 TATGTTCCTCGTGCCGTTCTCGTCGATTTGGAACCAGGAACCATGGACGCTGTCCGTGCT
361 GGTCCTTTCGGTCAACTTTTCCGCCCCGACAACTTCGTTTTCGGACAATCTGGTGCTGGT
421 ACAT

Partial DNA sequence of 18S ribosomal RNA gene of *Sclerotinia homoeocarpa*. Complete DNA sequence of internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2. Partial sequence of 28S ribosomal RNA gene.

1 TCACGGGTAGACCTCCAACCCTTGTGTATCTCTACCATGTTGCTTTGGCAGGCTGCTCGA
61 CCCTTCCGGGGACAGCCTCAGCGCCCTCCGGGGCCGGAGAGTCGCCTGCCGGAGGAAAAT
121 CACAACTCTGAATTGTCAGTGTCGTCTGAGTGACTATCTAATAGTTAAAACTTTCAACAA
181 CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAAT
241 TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGG
301 GGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTCTGCTTGGTATTGGGCCTCC
361 GCCGGTCACACGGCGGGCCTTAAAGTCAGTGGCGGCGCCGCTGGGTCCTGAACGTAGTAA
421 CACATACCTCTCGTTACAGGGTCCCCGCGCGCTCCCGCCGTAAAACCCCCCTCATTTTCT
481 CTGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAG
541 GA

Partial DNA sequence of EF1-α of *Sclerotinia homoeocarpa*. Introns are indicated in red.

1 ACTACCGGTAAGCAGAACCCTCGACTTCTGCTGATCTACACATGGTTCTTACATTATATT
61 TAGGTCACTTGATCTACAAGTGCGGTGGAATTGACAAGCGTACTATTGAAAAGTTCGAGA
121 CGGTATGACTTCTCCACCTTTCTCTTGCTATCTTTTCCCGTCCTTCTCATCGAGATCAGT
181 GTCTGCGATCTTGGTGCTGATGGATTTATCGGGTTGCGTTTTCTCTCATGCGCGGAGCAT
241 ACATCCGAATTCTCAACCCTTTGAACATTACCACATTGCCTTTCCAGAATCCCTTTGCTA
301 ACCCGTTAATAGGAAGCCAAGGAGATGGGAAAGGGTTCCTTCAAGTACGCATGGGTTTTG
361 GACAAGTTGAAGGCTGAGCGTGAGCGTGGTATCACCATCGACATTGCCCTCTGGAAGTTC
421 GAGACACCTAAGTACAATGTTACTGTCATTGGTATGTGTACGAATTCTTTATGCCAACTG
481 AAGTATATTAACCCATTCGCAGATGCCCCCGGTCATCGTGATTTCATCAAGAACATGATC
541 ACTGGTACCTCCCAAGCTGATTGTGCCATTCTTATCATCGCTGCCGGTGTTGGTGAGTTC
601 GAGGCTGGTATCTCCAAGGATGGTCAGACCCGTGAGCACGCTCTTCTTGCGTACACTCTT
661 GGTGTTAAGCAACTTATCGTTGCCATCAACAAGATGGACACCACCAAGTGGTCCAAGGAT
721 CGTTTCGAGGAAATCATCAAGGAGACAACCAACTTCATCAAGAAGGTTGGCTACAACGCC
781 AAGACTGTTCCCTTCGTGCCGATCTCTGGATTCGAGGGTGATAACATGATTGAGCCCTCA
841 ACTAACTGCCCATGGTACAAGGGCTGGGAGAGAGAGTCCAAGGAGTCTGGCAAACACACC
901 GGCAAGACCCTTCTTGAGGCCATCGACAGCATGGACCTGCCTGAGCGTCCTATCAAGAAG
961 CCTCTCCGTCTCCT

Partial DNA sequence of CPS Domain of the CAD complex *Sclerotinia homoeocarpa*.
The intron is indicated in red.

1 TCCATAACTGACCCTCATATCGCGGGTCAAATCTTGGTCATCACGTTCCCTTTAGTGGGC
61 AACTATGGTGTGCCATCAAGAGA ACTATGGACGAACTCCTTAAGGATCTGCCAGCACAT
121 TTCGAGGCTTCTGA ATCCATATTGCAGGCTTAAT ACCGCCTCCTATGCCGGAGAAGAC
181 TTCTCCCATTTCCTCGCTACTTCTTCACTCGGCACATGGTTGAAGGAGCAGGGTGTCCCG
241 GCCATATATGGTGTTGACACCAGAGCCTTGACCCAGAGGATACGAGAGGAAGGTAGTATG
301 CTAGGTCGCATGCTACTGCAAAAGCAAGATCTAACCAATGGACATGCCGATGGATCCAAT
361 GCCAATAAGTCAGCTCTTTCATCACAGGACTGGAGGTCATACTTTGAATTGATTGAATGG
421 GATGATCCTAACAAGAAGAATCTTGTGGCAGATGGTGAGTTTCGATTGAGTTCTAGCCGG
481 AATGAGAGGCTAATACCTTGGCTTAGTATCCATTCGCGAGCCAAAATTATACTCACCCGA
541 TCCTTCAAATGCTCTCAAGAATCCTTCCGGTAGACCTATACGTGTTTTGTGTTTGGATGT
601 GGGTTTGAAGTACAATCAATTACGCTGCCTTGTGAAGAGGGGCGTAGAAGTTCTGGTCTG
661 TCCATGGGATTACGATTTCCCTGCACTGGCTGGAAAGGATTATGATGGATTGTTCATCTC
721 AAACGGTCCTGGTGATCCAGCCATGATGGAAAAGACTGTCAAGCACATCCAATCCGCCTT
781 GGCAGAGAATAGAACACCYATCTTTGGTATCTGTCTTGGACATCAACTTCTTGCYAGAGC
841 TGCTGGTGCCGAGACTGAGAAGATGAAGTTTGGTAATCGTGGACACAATATCCCATGTAC
901 GAATCTGTTGACTGGAAAATGTCACATCACTTCGCAAAACCACGGATATGCTGTCAAGAC
961 TAGCAGTTTACCATCCGAATGGACCGAACTTTTCGTCAATGCTAATGATGGAAGCAACGA
1021 AGGTATCCGCCACGTAGGCAGACCGTACTTCAGTGTTCAATTCCATCCTGAAAGCACTCC
1081 AGGGCCTCGAGATACGGAGTTCCTCTTTGATGTGTTCATAGACACAATCATGAAAACAAT
1141 TGTCAACAAAGATCTTCTCTCTGCCCCTGTTGCCTTCCCAGGTGGTGAAGCAGCCGAGAA
1201 CGAGAGACTGCACCCGAAAGTTGAAGTCAAGAAAGTACTCGTGTTGGGCAGTGGTGGCCT
1261 GAGTATTGGTCAAGCCGGAGAGTTCGATTACTCCGGAAGTCAAGCCATCAAGGCACTCAA
1321 GGAAGAGGGTATCTATACTATTCTGATCAACCCCAACATTGCCACTATTCAAACTTCCAA
1381 GGGCTTAGCGGACAAGGTCTATTTCCTACCTGTCACTGCTGAATTTGTACGAAAAGTTTT
1441 GGTGTACGAGAGGCCTGACGCAATTTACGTCACATTTGGTGGACAGACAGCTTTGCAAGT
1501 CGGTATTCAGTTGAAGGATGAGTTTGAGGAACTTGGTGTCAAAGTTCTTGGAACCCCAAT
1561 CGATACAATCATCACCACTGAAGATCGTGAGCTCTTTGCCCGAAGCATGGAGTCCATTGG
1621 TGAAAAATGTGCCAAATCTGCTTCTGCAAGCACCGTTGATGAGGCCATGCGCGTTGTCAA
1681 GGGTATCGGATTCCCAGTCATTGTCCGAGCTGCTTATGCGCTAGGTGGTCTTGGAAGTGG
1741 ATTTGCAGACAACGAACAAGAATTGCTAGATCTCTGCAACAAAGCCTTCGCTGCCCGTCC
1801 CCCGGTTTTGATTGAGCCAA

APPENDIX II: CORRECTED BINARY DATA FROM AFLP ANALYSIS

Primer set Eco+AA/Mse+C binary data for *Sclerotinia homoeocarpa*.

Bin	55	57	59	64	66	69	72	74	75	89	91	97	99	100	103
Bm4	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
BM7	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
BM8	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
BM9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BM10	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
CH1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
CH2	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
Ch3	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
CH4	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
CH5	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
T1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
T2	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
T3	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
T6	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
T9	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
G2	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
G3	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
G4	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
G5	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
G8	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L3	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L4	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L5	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L1-1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L1-2	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L1-3	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L1-4	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L1-5	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L2-1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L2-2	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
L2-3	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L2-4	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
L2-5	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
W5	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
W6	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
W7	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
W8	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
W10	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
WW1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
WW2	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
WW3	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
WW4	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
WW5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
M2	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
M3	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
M4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MID	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1

Primer set Eco+AA/Mse+C binary data for *Sclerotinia homoeocarpa*.

Bin	55	57	59	64	66	69	72	74	75	89	91	97	99	100	103
MIB	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
MIDMI	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
MIW	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
VCG A	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
VCGB	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
VCG C	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
VCGD	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
VCG E	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
VCG F	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1

Bin	105	114	132	133	139	140	152	154	158	165	169	170	183	184	188
MIW	1	1	0	1	0	1	1	1	1	1	1	0	1	0	1
VCG A	1	0	0	1	0	1	1	1	1	1	1	0	1	1	1
VCGB	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
VCG C	1	0	0	1	0	1	1	1	1	1	0	0	1	1	1
VCGD	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
VCG E	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1
VCG F	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1

Bin	193	198	201	204	206	210	215	217	229	237	251	255	259	268	269
MIW	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VCG A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VCGB	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VCG C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VCGD	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VCG E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VCG F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Bin	279	325	336	340	343	359	369	378	387	393	435	452	456	499	589
MIW	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
VCG A	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
VCGB	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
VCG C	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
VCGD	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
VCG E	1	1	1	1	1	0	0	1	1	1	1	0	1	1	1
VCG F	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1

Primer set Eco+AA/Mse+C binary data for *Sclerotinia homoeocarpa*.

Bin	105	114	132	133	139	140	152	154	158	165	169	170	183	184	188
Bm4	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1
BM7	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1
BM8	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1
BM9	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1
BM10	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1
CH1	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
CH2	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
Ch3	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
CH4	1	1	0	1	0	1	1	1	1	1	1	0	1	0	1
CH5	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
T1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1
T2	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1
T3	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1
T6	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1
T9	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
G2	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
G3	1	0	0	1	0	1	1	1	1	1	1	0	1	1	1
G4	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1
G5	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
G8	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1
L1	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1
L2	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
L3	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
L4	1	1	0	1	0	1	1	1	1	1	1	0	1	0	1
L5	1	1	0	1	0	1	1	1	1	1	1	0	1	0	1
L1-1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
L1-2	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
L1-3	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
L1-4	1	0	0	1	0	1	1	1	1	1	1	0	1	1	1
L1-5	1	1	0	1	0	1	1	1	1	1	1	0	1	1	1
L2-1	1	0	1	1	0	1	1	1	1	1	1	0	1	0	1
L2-2	9 [a]	9	9	9	9	9	9	9	9	9	9	9	9	9	9
L2-3	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
L2-4	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
L2-5	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
W5	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
W6	1	0	0	1	0	1	1	1	1	1	1	0	1	1	1
W7	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
W8	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
W10	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
WW1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
WW2	1	0	0	1	0	1	1	1	1	1	1	0	1	1	1
WW3	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
WW4	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1
WW5	1	0	0	1	0	1	1	1	1	1	1	0	1	1	1
M1	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
M2	1	0	0	1	0	1	1	1	1	1	1	0	1	1	1
M3	1	0	0	1	0	1	1	1	1	1	1	0	0	0	1
M4	1	0	0	1	0	1	1	1	1	1	1	0	1	1	1
M5	1	0	0	1	0	1	1	1	1	1	1	0	1	1	1
MID	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
MIB	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1
MIDMI	1	1	0	1	0	1	1	1	1	1	1	0	1	1	1

[a]-Indicates missing data.

Primer set Eco+AA/Mse+C binary data for *Sclerotinia homoeocarpa*.

Bin	193	198	201	204	206	210	215	217	229	237	251	255	259	268	269
Bm4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BM7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BM8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BM9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BM10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CH1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CH2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ch3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CH4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CH5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
T1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
T2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
T3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
T6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
T9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L1-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L1-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L1-3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L1-4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L1-5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2-2	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
L2-3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2-4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2-5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
W5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
W6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
W7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
W8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
W10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
WW1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
WW2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
WW3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
WW4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
WW5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MID	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MIB	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MIDMI	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Primer set Eco+AA/Mse+C binary data for *Sclerotinia homoeocarpa*.

Bin	279	325	336	340	343	359	369	378	387	393	435	452	456	499	589
Bm4	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
BM7	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
BM8	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
BM9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BM10	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
CH1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
CH2	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
Ch3	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1
CH4	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1
CH5	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
T1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
T2	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
T3	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
T6	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
T9	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
G2	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
G3	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
G4	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
G5	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
G8	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1
L1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
L2	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
L3	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
L4	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
L5	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
L1-1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
L1-2	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
L1-3	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
L1-4	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
L1-5	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1
L2-1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
L2-2	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
L2-3	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
L2-4	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
L2-5	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
W5	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
W6	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
W7	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
W8	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
W10	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
WW1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
WW2	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
WW3	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
WW4	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
WW5	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1
M1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
M2	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
M3	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
M4	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
M5	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
MID	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1
MIB	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
MIDMI	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1

Primer set Eco+AA/Mse+C binary data for *Sclerotinia homoeocarpa*.

Bin	607	620
Bm4	1	1
BM7	1	1
BM8	1	1
BM9	1	1
BM10	1	1
CH1	1	1
CH2	1	1
Ch3	1	1
CH4	1	1
CH5	1	1
T1	1	1
T2	1	1
T3	1	1
T6	1	1
T9	1	1
G2	1	1
G3	1	1
G4	1	1
G5	1	1
G8	1	1
L1	1	1
L2	1	1
L3	1	1
L4	1	1
L5	1	1
L1-1	1	1
L1-2	1	1
L1-3	1	1
L1-4	1	1
L1-5	1	1
L2-1	1	1
L2-2	9	9
L2-3	1	1
L2-4	1	1
L2-5	1	1
W5	1	1
W6	1	1
W7	1	1
W8	1	1
W10	1	1
WW1	1	1
WW2	1	1
WW3	1	1
WW4	1	1
WW5	1	1
M1	1	1
M2	1	1
M3	1	1
M4	1	1
M5	1	1
MID	1	1
MIB	1	1
MIDMI	1	1

Bin	607	620
MIW	1	1
VCG A	1	1
VCGB	1	1
VCG C	1	1
VCGD	1	1
VCG E	1	1
VCG F	1	1

Primer set Eco+TG/Mse+C binary data for *Sclerotinia homoeocarpa*.

Bin	58	61	63	66	67	70	72	76	79	82	85	91	92	98	102
Bm4	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
BM7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BM8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BM9	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
BM10	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
CH1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CH2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CH3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CH4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CH5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
T1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
T2	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
T3	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
T6	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
T9	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
G2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
L2	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
L3	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
L4	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
L5	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
L1-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L1-2	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
L1-3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L1-4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L1-5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2-1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
L2-2	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
L2-3	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
L2-4	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
L2-5	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
W5	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
W6	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
W7	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
W8	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
W10	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
WW1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
WW2	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
WW3	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
WW4	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
WW5	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
M1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M2	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
M3	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
M4	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
M5	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1

Primer set Eco+TG/Mse+C binary data for *Sclerotinia homoeocarpa*.

Bin	58	61	63	66	67	70	72	76	79	82	85	91	92	98	102
MID	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
MIB	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
MIDMI	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MIW	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
VCG A	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
VCG B	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
VCG C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VCG D	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
VCG E	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
VCG F	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1

Bin	107	113	118	120	124	133	135	139	144	146	160	166	183	186	190
MID	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1
MIB	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
MIDMI	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MIW	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
VCG A	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
VCG B	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
VCG C	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
VCG D	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
VCG E	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
VCG F	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1

Bin	192	194	204	208	212	218	231	289	297	311	392	457	505	511
MID	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MIB	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MIDMI	1	1	1	1	1	1	1	1	1	1	0	1	1	1
MIW	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VCG A	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VCG B	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VCG C	1	1	1	1	1	1	1	1	1	1	0	1	1	1
VCG D	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VCG E	1	1	1	1	1	1	1	1	1	1	0	1	1	1
VCG F	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Primer set Eco+TG/Mse+C binary data for *Sclerotinia homoeocarpa*.

Bin	107	113	118	120	124	133	135	139	144	146	160	166	183	186	190
Bm4	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1
BM7	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
BM8	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
BM9	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
BM10	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1
CH1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
CH2	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
CH3	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
CH4	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
CH5	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
T1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
T2	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
T3	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
T6	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
T9	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
G2	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
G3	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
G4	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
G5	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1
G8	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
L1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
L2	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
L3	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
L4	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
L5	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
L1-1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
L1-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L1-3	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
L1-4	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
L1-5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2-1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
L2-2	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
L2-3	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
L2-4	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
L2-5	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
W5	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
W6	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
W7	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
W8	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
W10	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
WW1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
WW2	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
WW3	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
WW4	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
WW5	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
M1	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
M2	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
M3	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
M4	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
M5	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1

Primer set Eco+TG/Mse+C binary data for *Sclerotinia homoeocarpa*.

Bin	192	194	204	208	212	218	231	289	297	311	392	457	505	511
Bm4	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BM7	1	1	1	1	1	1	1	1	1	1	0	1	1	1
BM8	1	1	1	1	1	1	1	1	1	1	0	1	1	1
BM9	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BM10	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CH1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
CH2	1	1	1	1	1	1	1	1	1	1	0	1	1	1
CH3	1	1	1	1	1	1	1	1	1	1	0	1	1	1
CH4	1	1	1	1	1	1	1	1	1	1	0	1	1	1
CH5	1	1	1	1	1	1	1	1	1	1	0	1	1	1
T1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
T2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
T3	1	1	1	1	1	1	1	1	1	1	1	1	1	1
T6	1	1	1	1	1	1	1	1	1	1	1	1	1	1
T9	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G2	1	1	1	1	1	1	1	1	1	1	0	1	1	1
G3	1	1	1	1	1	1	1	1	1	1	0	1	1	1
G4	1	1	1	1	1	1	1	1	1	1	0	1	1	1
G5	1	1	1	1	1	1	1	1	1	1	0	1	1	1
G8	1	1	1	1	1	1	1	1	1	1	0	1	1	1
L1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L3	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L4	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L1-1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
L1-2	1	1	1	1	1	1	1	1	1	1	0	1	1	1
L1-3	1	1	1	1	1	1	1	1	1	1	0	1	1	1
L1-4	1	1	1	1	1	1	1	1	1	1	0	1	1	1
L1-5	1	1	1	1	1	1	1	1	1	1	0	1	1	1
L2-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2-3	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2-4	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L2-5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
W5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
W6	1	1	1	1	1	1	1	1	1	1	1	1	1	1
W7	1	1	1	1	1	1	1	1	1	1	1	1	1	1
W8	1	1	1	1	1	1	1	1	1	1	1	1	1	1
W10	1	1	1	1	1	1	1	1	1	1	1	1	1	1
WW1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
WW2	1	1	1	1	1	1	1	1	1	1	0	1	1	1
WW3	1	1	1	1	1	1	1	1	1	1	0	1	1	1
WW4	1	1	1	1	1	1	1	1	1	1	0	1	1	1
WW5	1	1	1	1	1	1	1	1	1	1	0	1	1	1
M1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
M2	1	1	1	1	1	1	1	1	1	1	0	1	1	1
M3	1	1	1	1	1	1	1	1	1	1	0	1	1	1
M4	1	1	1	1	1	1	1	1	1	1	0	1	1	1
M5	1	1	1	1	1	1	1	1	1	1	0	1	1	1

VITA

Renae Erin DeVries was born in November of 1980 and raised in Allison, IA. Her father was the high school science teacher in the small rural town and her mother worked in the insurance department at a hospital in Waverly, IA. In May of 1999, Renae graduated high school with honors.

In August of 1999, Renae began her freshman year at the University of Northern Iowa in Cedar Falls, IA. During her undergraduate career, Renae was involved in resident hall government and was the secretary of the University of Northern Iowa Biotechnology Club. Before graduating with her Bachelor of Arts in Biology with an emphasis in honors biotechnology and a minor in chemistry, she completed her undergraduate honors thesis.

In August 2004, Renae moved to Knoxville, TN to attend graduate school at the University of Tennessee. After one year, she obtained a position as a Research Specialist II in her advisor's lab and worked while attending graduate classes. In May 2006, she completed a Master of Science degree in Plant Pathology. After graduation Renae plans to obtain a job in the plant pathology or molecular biology field.