DISTRIBUTION AND OCCURRENCE OF *Stachybotrys chartarum* IN NORTH CENTRAL FLORIDA HABITATS

By

SARAH BRINTON CLARK SELKE

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To my parents, Newton and Patricia Clark, and my husband, Gregg Selke

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Abstract of Dissertation Presented to the Graduate School
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By

Sarah Brinton Clark Selke

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Stachybotrys chartarum is a mycotoxin-producing, cosmopolitan fungus that occurs on a variety of natural substrates as well as cellulose-based building materials such as drywall and ceiling tiles. This black mold has aroused public interest because it has been implicated in cases of sick building syndrome and pulmonary hemorrhage. Because it is likely that outdoor populations serve as the source of inoculum for mold colonies in water-damaged structures, it is critical to understand the types of environments that support natural populations of *S. chartarum*. The primary objective of this research was to identify outdoor habitats in north central Florida where *S. chartarum* is found and the times of year it is most abundant. Several semi-selective media were identified for this purpose; however, detection of *S. chartarum* from outdoor air was a rare event, suggesting that air sampling would not be appropriate for investigating the occurrence of *S. chartarum* in outdoor habitats. Instead, traps with pieces of wetted drywall were placed in four habitats in Gainesville, Florida: a pine grove, a citrus grove, a lakeside and a hardwood forest. Over the course of 24 months, *S. chartarum* was found growing at all four habitats but only rarely, occurring on only 0.02% of the pieces collected. Because the frequency of *S. chartarum* was so low, most differences in abundance between sites were not significant. *Stachybotrys chartarum* was recovered most frequently from the citrus grove, and at all sites, it

was found only during the summer months. There was a correlation between *S. chartarum* occurrence and temperature but not with rainfall. The morphological species *S. chartarum* contains two chemotypes, *S. chartarum* chemotype S and *S. chartarum* chemotype A, which produce different mycotoxins. The Florida field isolates were compared phylogenetically using the trichodiene synthase 5 and chitin synthase 1 gene fragments. Seventy percent of the outdoor isolates were identified as *S. chartarum* chemotype A and only 30% were identified as chemotype S. This may have a positive implication for public health in north central Florida since chemotype A does not produce highly toxic macrocyclic tricothecenes. As a diagnostic tool, neither locus correctly identified all isolates, and more accurate molecular markers should be identified.

CHAPTER 1
INTRODUCTION

Stachybotrys chartarum (Ehrenb.) Hughes (= *Stachybotrys atra* Corda) is a dermatiaceous hyphomycete of worldwide distribution. It has been isolated from soil (Barron 1968, Ellis 1971), from decaying plant material (Ellis 1971, Whitton et al. 2001), on animal fodder (Drobotko 1945), as a parasite of other fungi (Siqueira et al. 1984), and in association with living plants (El-Morsy 1999, Li et al. 2001). Some of the more unusual natural substrates include woodchuck dung (Jong and Davis 1976) and seaweed (Andersen et al. 2002). A strongly cellulolytic fungus, *S. chartarum* has also been isolated from a variety of cosmopolitan materials derived from plant fibers such as paper, cotton, and canvas (Bisby 1943, Jong and Davis 1976). Of current interest is the frequent isolation of *S. chartarum* from construction materials including drywall, ceiling tiles and wallpaper in buildings that have experienced water damage (Li and Yang 2005). The recovery of *S. chartarum* spores from indoor air samples and its known production of mycotoxins have led to increased public interest in this fungus in recent years (Nelson 2001, Money 2004).

Taxonomic History of *Stachybotrys chartarum* and Related Species

The genus *Stachybotrys* Corda was first described in 1837 to accommodate a black mold found growing on the wall of an apartment in Prague, Czech Republic. The type species is *S. atra* Corda, and the genus is characterized by septate and branched hyphae, conidiophores that terminate in a whirl of phialides, and two-celled pigmented conidia (Corda 1837). The etymology of *Stachybotrys* refers to its characteristic crown of phialides, with the Greek prefix stachy- referring to a “spike” and –botrys meaning a “bunch of grapes.” From 1837 to 1886, seven new species were described, one with two-celled spores, and the other six have one-celled

conidia. By 1943, there was a total of twenty *Stachybotrys* species, with the twelve new taxa all being unispored (Bisby 1943).

Bisby undertook the first major revision of the genus, reducing the number of *Stachybotrys* species to two, *S. atra* Corda and *S. subsimplex* Cooke. Based on studies of cultures and herbarium specimens, *S. alternans* Bonord., *S. asperula* Massee, *S. atrogrisea* Ellis & Everh., *S. cylindrospora* Jensen, *S. dakotense* Sacc., *S. dichroa* Grove, *S. elasticae* Koord., *S. gracilis* E.J. Marchal, *S. pulchra* Speg., *S. scabra* Cooke & Harkn., and *S. verrucosa* Cooke & Massee were reduced to synonymy with *S. atra* as were *Aspergillus alternatus* Berk., *Sporocybe lobulata* Berk., *Synsporium biguttatum* Preuss, and *Memnonium sphaerospermum* Fuckel. Bisby (1943) suggested that there was great morphological variation in *Stachybotrys atra* which accounted for the 15 synonyms and hypothesized that even an unnamed "pink *Stachybotrys*" could belong to this species. He attributed Corda's observation of two-celled conidia to the one, three or, most commonly, two guttulae which he observed, noting that under Corda's microscope such spores would seem definitely septate. Thus, the emended description of the genus *Stachybotrys* read as follows:

> Hyphae, phialophores, and phialides hyaline, brightly coloured, or dark; strands or ropes of hyphae may be produced. Conidia (slime-spores) one-celled, normally dark and accumulating into a cluster. The distinctive characteristic of the genus is the septate phialophore or simple conidiophore bearing a crown of phialides and generally becoming dark. A phialophore arises directly from a hypha, or, frequently, from another phialophore. Perfect stage unknown.

Stachybotrys atra was described as having phialides 10-16 x 5–7 µm and conidia 8-12 (14) x 4-9 (12) µm, elliptical to oval on younger growth of the fungus, often subglobose on older growth.

Stachybotrys subsimplex was described as having simple, rather than branched, conidiophores, and smaller phialides (6-12 x 4-6 µm) and conidia (4-10 x 3-5 µm) than *S. atra*.

The species was thought to be synonymous with *Gliobotrys alboviridis* Höhn. and *Memnoniella echinata* (Rivolta) Galloway (Bisby 1943). Bisby felt that the genus *Memnoniella* Höhn., which had as its only major difference from *Stachybotrys* the occurrence of its conidia in chains as opposed to a slimy head, could actually be *S. simplex* with slime production reduced sufficiently to allow the retention of spores in chains.

The binomial *Stachybotrys chartarum* was first used by Hughes in 1958 after reexamining the type material of *S. atra*. He identified three homotypic synonyms, *Stilbospora chartarum* Ehrenb. 1818, *Oidium chartarum* Ehrenb. ex Link 1824 and *Oospora chartarum* (Ehrenb. ex Link) Wallr. 1833. Hughes recombined the names as *Stachybotrys chartarum* (Ehrenb.) Hughes (= *S. atra* Corda). Although some authors continued to use *S. atra* for some time (Ellis 1971), *Stachybotrys chartarum* currently is the universally accepted name.

Jong and Davis (1976) recognized 11 species of *Stachybotrys* (S. *altipes* (Berk. & Broome) S.C. Jong & Davis, *S. bisbyi* (Sriniv.) G.L. Barron, *S. chartarum, S. cylindrospora, S. dichroa, S. kampalensis* Hansf., *S. microspora* B.L. Mathur & Sankhla, *S. nephrospora* Hansf., *S. oenathes* Ellis, *S. parvispora* Hughes, *S. theobromae* Hansf.) and two species of *Memnoniella*. *S. cylindrospora* and *S. dichroa* were taxa resurrected from Bisby's treaties, and two new combinations were proposed, *S. albipes* (Berk. & Br.) Jong & Davis and *S. microspora* (Mathur & Sankha) Jong & Davis. In addition, *S. saccharia* (Srinivasan) Barron was synonymized with *S. bisbyi* (Srinivasan) Barron, as was *S. reniformis* Tubaki. *Stachybotrys sinuatophora* Matsush. was considered the synonym of *S. nephrospora* Hansf. The most recently published key includes 52 species of *Stachybotrys*, and four species of *Memnoniella* (Pinruan et al. 2004). However, only a few of these fungi are reported frequently in literature (Andersen et al. 2003). *Stachybotrys sinuatophora* is not considered a synonym of *S. nephrospora* in this key.

Taxonomic uncertainty in the genus continues as recently as 2007 when the type species of *S. cylindrospora* was reclassified as *S. chartarum* and a new species, *S. eucylindrospora* Li was described (Li 2007)

Andersen et al. (2002, 2003) proposed two chemotypes of *S. chartarum* based on differing metabolite production. Chemotype S produces macrocyclic trichothecenes including satratoxins and roridins. Chemotype A produces atranones and dolabellanes.

Jong and Davis (1976) confirmed that *Stachybotrys* and *Memnoniella* represented two different genera based on morphological differences in the arrangement of the conidia, and reported work by Campbell (1972, 1974) that showed that in *Stachybotrys*, the new conidia arise after the previous ones are mature and have been released from the phialide neck. This is in contrast with *Memnoniella*, where the new conidia arise in basipetal succession before the previous ones are mature. Therefore, the conidial chains formed by *Memnoniella* are not a result of less slime production as suggested by Bisby (1943).

The status of the genus *Memnoniella* continues to be controversial (Li et al. 2003, Pinruan et al. 2004). Using sequence data from 18S, 28S, 5.8S rDNA genes and the ITS1 and ITS2 regions, Haughland et al. (2001) evaluated the evolutionary relationship between the two genera. They concluded that *Memnoniella* and *Stachybotrys* are paraphyletic and proposed renaming *M. echinata* and *M. subsimplex* as *S. echinata* and *S. subsimplex*, respectively. One strain in the study, identified as an isolate of *M. subsimplex*, showed morphological features typical of *M. subsimplex*, but fell into the *M. echinata* clade based upon a phylogenetic analysis. After further study, this culture was described as a new species, *Memnoniella longistipitata* D.W. Li, Chin S. Yang, Vesper & Haugland (Li et al. 2003) with the note that since *Memnoniella* was apparently still accepted, the new species was being placed in that genus. Other authors believe that

Memnoniella is not a valid genus and advocate transferring all *Memnoniella* species to *Stachybotrys* (Smith 1962, Carmichael et al. 1980, Pinruan et al. 2004).

Stachybotrys albipes is the only species for which a sexual state, *Melanopsamma pomiformis* (Pers. ex Fr.) Sacc., has been identified (Booth 1957, Castlebury et al. 2004). The genus *Melanopsamma* Niessel was placed in the Niessliaceae (Hypocreales) by Samuels and Barr (1997), but this classification has been questioned. Castlebury et al. (2004) investigated higher-level phylogenetic relationships of the genus *Stachybotrys* and found that it formed a previously unknown monophyletic lineage within the Hypocreales that also included species of *Myrothecium* Tode. Like *Stachybotrys*, *Myrothecium* produces macrocyclic trichothecenes (Jarvis et al. 1985), and the two genera share morphological features such as slimy dark black to green conidia that are produced from phialides. It has been suggested that these genera comprise a newly discovered sister lineage to the other families currently accepted in the Hypocreales (Castlebury et al. 2004).

Morphology of *Stachybotrys chartarum*

Bisby (1943) recognized the morphological variety that exists among isolates of *S. chartarum*, and subsequent authors have reaffirmed this observation (Andersen et al. 2002, Andersen et al. 2003, Li and Yang 2005). Jong and Davis (1976) described the species when grown on Corn Meal Agar as follows:

> . . . Condiophores determinate, macronematous, solitary or in groups, erect, straight or slightly curved, simple or irregularly branched, 2-4 septate, hyaline at the base, dark olivaceous toward the apex, length variable, up to 1000 µm long, 3-6 µm wide, the basal cell slightly inflated, sometimes minutely rough-walled at the upper parts, sometimes more or less smooth throughout the length, slightly enlarged at the apex which bears terminal phialides in a whorl of 3-9 around a central phialides.
>
> Phialides enteroblastic, determinate, discrete, unicellular, at first hyaline, later dark olivaceous, obovate or ellipsoid, smooth-walled, 9-14 x 4-6 µm, with conspicuous collarettes.

> Phialoconidia acrogenous, arising singly and successively as separate units, aggregated in slimy masses, at first hyaline, when mature dark olive gray, more or less opaque, smooth-walled or showing banded or ridged, ellipsoidal, unicellular, 7-12 x 4-6 µm. . . .

Jong and Davis (1976) described the distinguishing features of *S. chartarum* as the ridged or banded surface of the mature conidia as well as their size. Therefore, it is interesting that Ellis (1971) described the conidial dimensions as 8-11 x 5-10 µm and Bisby (1943) as 8-12 x 4-9 µm. The range of measurements in these various descriptions of *S. chartarum* led to some confusion regarding its species delineation (Andersen et al. 2002, 2003, Li and Yang 2005). The recognition of *S. chlorohalonata* B. Andersen & Thrane as a distinct species, and the use of metabolic profiles to identify chemotypes of *S. chartarum* may clarify the current situation (Andersen et al. 2003).

Stachybotrys chartarum, Mycotoxin Production and Human Health

Although some authors trace reports of mold-induced sick building syndrome back to books of Exodus and Leviticus in the Old Testament (Heller et al. 2003, Jarvis 2003), the widely-accepted first report of *S. chartarum* causing illness in humans or other animals was in the USSR in the 1930s when stachybotryotoxicosis epidemics occurred in horses in the Ukraine which had consumed *Stachybotrys*-infested hay (Drobotko 1945). Although stachybotryotoxicosis has occurred in animals in Eastern Europe throughout the 20th century, (Forgacs 1972), it has not been a problem in North America where a combination of favorable farming conditions and good agricultural practices have thwarted serious infestations of fodder with *S. chartarum* (Jarvis 2003).

Drobotko (1945) characterized the disease in horses as having three stages. Stage one is marked by irritation and ulcers of the mouth, throat, nose and lips. The second stage lasts several days to a month during which a low white blood cell count occurs. Only a few cases pass into the third stage where a fever of 40 – 41°C develops and remains until death. Disease progression

depends upon the amount of mold that is consumed and the period of time over which consumption occurs (Hintikka 1978b). An atypical form of the disease, characterized by neurological disorders including loss of reflex response and vision and noted by Forgacs et al. (1958), follows the ingestion of large quantities of contaminated feed. Afflicted animals usually die within 24 hours of showing symptoms.

Although it was suspected that stachybotryotoxicosis was caused by mycotoxins (Drobotko 1945), they were not characterized until Eppley and Bailey (1973) identified five toxic compounds from isolates of *S. chartarum*, including the macrocyclic trichothecenes roridin E and satratoxin G and H. Named after the fungus *Trichothecium roseum* (Pers.) Link from which the first trichothecene was isolated in 1948 (Desjardins et al. 1993), trichothecenes are an important class of sesquiterpenes that include the T-2 toxin produced by several species of *Fusarium* Link. A sub-class of this group is the macrocyclic tricothecenes which are the most potent inhibitors of eukaryotic protein synthesis; they are considered to be among the most important and acutely toxic of the mycotoxins (Jarvis 2003). Macrocyclic tricothecenes shown to be produced by *S. chartarum* include roridin E and L-2, satratoxins F, G and H, isosatratoxins F, G, and H, verrucarins B and J, and two types of trichoverroids, trichoverrols A and B and trichoverrins A and B (Nelson 2001).

Stachybotrys chartarum produces several other types of metabolites that are toxic to mammalian cells. Approximately 10-40 spirocyclic drimanes have been identified, and this class of metabolites has a broad range of activities including enzyme inhibition, cytotoxicity and neurotoxicity (Jarvis et al. 1995, Nielsen 2003). In addition, stachylysin causes leakage or rupturing of red blood cells (Vesper et al. 1999, 2001), and spirocyclic drimanes are potent immunosuppresants (Jarvis et al. 1995, Jarvis 2003). A novel class of compounds, the atranones,

was recently identified (Hinkley et al. 2000), although it is unknown if they have significant biological activity (Jarvis 2003).

In European regions that reported occurrences of equine stachybotryotoxicosis, humans who handled *Stachybotrys*-contaminated material developed symptoms similar to those found in horses (Forgacs 1972, Hintikka 1978a). Common symptoms included a rash, particularly in areas of perspiration such as underarms, dermatitis, pain, inflammation and/or a burning sensation in the mouth and nasal passages, tightness of the chest, cough, fever, headache and fatigue (Nelson 2001). The first report of stachybotryotoxicosis in North America was in a residence in Chicago (Croft et al. 1986). Over a five-year period, a family of five individuals exhibited symptoms including cold and flu-like illness, sore throats, diarrhea, headaches, fatigue, dermatitis, intermittent hair loss, and generalized malaise. *Stachybotrys chartarum* spores were isolated from an interior duct as well as from ceiling material. Extracts from spores collected from these areas were injected into mice, and within 24 hours of exposure, all of the animals died. Extracts from the ceiling fiber board were shown to contain the macrocyclic trichothecenes verrucarin J and satratoxin H as well as the precursors trichoverrins A and B.

This report was believed to be an isolated incident until the mid-1990s (Jarvis 2003, Money 2004). Between January 1993 and December 1994, ten infants were hospitalized with bleeding lungs at Rainbow Babies' and Childrens' Hospital in Cleveland, Ohio and diagnosed with acute pulmonary hemorrhage/ hemosiderosis (Anonymous 1994, 1997). These patients represented an unusually high number of cases, as only three infants had been diagnosed with acute pulmonary hemosiderosis from 1983-1993 (Anonymous 1994). All of the infants lived in homes that had been severely water damaged in the previous six-months, and *S. chartarum* was isolated from eight of nine homes that were tested (Anonymous 1997, Etzel et al. 1998, Dearborn

et al. 1999). R.A. Etzel, a pediatrician from the Centers for Disease Control (CDC, Atlanta, GA) and D.G. Dearborn, a pulmonary pediatrician from the Rainbow Babies' and Children's Hospital, both hypothesized that infant pulmonary hemorrhage might be caused by exposure to mycotoxins produced by *S. chartarum* (Anonymous 1997, Etzel et al. 1998, Dearborn et al. 1999). Although the CDC eventually retracted its earlier support of this association (Anonymous 2000), evidence continues to grow regarding the link between the two (Elidemir et al. 1999, Vesper and Vesper 2002).

Despite the significance of *S. chartarum* in issues regarding public health and the fact that it has been isolated from a wide variety of materials, a comprehensive ecological study that identifies different habitats that support *S. chartarum* populations in nature has never been undertaken. It is critical to understand the types of environments that support natural populations of *S. chartarum* because it is likely that the source of indoor contamination is air-borne spores that are deposited on the surface of building materials at their construction sites (Nelson 2001, Koster 2006). The development of a semi-selective growth medium that affords the usually slow-growing *S. chartarum* a competitive advantage when present with other common air-borne spores is the focus of chapter 2. Chapter 3 focuses on the distribution and occurrence of *Stachybotrys* spp. across four different habitats. The mycotoxin profile of *S. chartarum* can no longer be associated with particular morphological criteria (Andersen et al. 2003). A molecular characterization of the north central Florida isolates collected from these field studies was undertaken to distinguish between the two chemotypes and determine their prevalence (Chapter 4). In chapter 5, a summary of the information is provided and future suggestions for research are discussed.

CHAPTER 2
COMPARISON OF SEMI-SELECTIVE MEDIA FOR DETECTION OF *Stachybotrys chartarum* FROM AIR SAMPLES

Introduction

Stachybotrys chartarum is a mycotoxin-producing, cosmopolitan fungus that occurs on water-damaged, cellulose-based building materials such as drywall and ceiling tiles. This black mold has aroused public interest because it has been implicated in cases of sick building syndrome and pulmonary hemorrhage (Croft et al. 1986, Etzel et al. 1998, Dearborn et al. 1999). From a human health perspective, it is of paramount importance that *S. chartarum* be accurately and consistently isolated from environmental samples.

Common detection methods of suspected mold contamination are bioaerosol, swab, tape, or bulk sampling (Miller 2001). There is no ideal method for the sampling of fungal particles in indoor air, in part because no one medium exists for air sampling that is ideal for use in every environment (Samson et al. 1994). Recommended broad-spectrum media for use in indoor air sampling are Dichloran 18% Glycerol Agar (DG18), Malt Extract Agar (MEA) and Water Agar (Samson et al. 1994). However, *S. chartarum* has rarely been isolated from fungal air samples using these media (Andersen and Nissen 2000, Tiffany and Bader 2000). It is possible that *S. chartarum* spores do not become airborne easily because they are produced in wet slimy heads (Tucker et al. 2007). In addition, it has been reported that up to 90% of the spores that do become airborne may not be viable (Miller 1992).

An alternative hypothesis is that viable *S. chartarum* spores are airborne but that they are underreported in air sampling studies because 1) often they are found in conjunction with other common, fast-growing fungi such as species of *Penicillium, Aspergillus, Cladosporium*, and *Curvularia* that outcompete the slower-growing *S. chartarum* and 2) commonly-used media do not meet its growth requirements. For example, *S. chartarum* will not sporulate on DG18, which

is recommended for general detection of fungi indoors (Andersen and Nissen 2000). This is not surprising since DG18 is selective for xerophilic fungi; *Stachybotrys* requires a water activity higher than 0.9 for growth (Grant et al. 1989). *Stachybotrys chartarum* growth on MEA, another recommended medium, is very restricted, and some isolates will not sporulate on it (Andersen and Nissen 2000).

The necessity of a selective medium for *S. chartarum* has been recognized by professionals for over a decade (Samson et al. 1994), and researchers have proposed several possibilities that favor the isolation of *S. chartarum* from an environmental sample containing many different organisms. This medium must meet its growth requirements while restricting the growth of competing fungi. Since *S. chartarum* is a cellulose-digesting saprobe, a cellulose-based agar medium amended with Rose Bengal to inhibit other fungi has been recommended (Petri 1983, Henry and Stetzenbach 2000). El-Kady (1988) suggests a similar medium, Cellulose-Czapek's Agar, amended with Rose Bengal and adjusted to a pH of 8. Tsai et al. (1999) included Czapek's Cellulose Agar and Rose Bengal Agar in their survey of selective media, but they recommended using unamended Cornmeal Agar (CMA) because they found interference from species of *Aspergillus, Chaetomium, Cladosporium,* and *Penicillium* on Czapek's celluose agar. Billups et al. (1999) did not get satisfactory results using cellulose agar when trying to isolate *Stachybotrys* from mixed cultures with *Aspergillus flavus, A. niger, Cladosporium brevitomosum* and *Penicillium chrysogenum*. Potato dextrose agar (PDA) amended with the antibiotic/antimycotic agent Miconazole was a better isolation medium (Billups et al. 1999). Other authors have recommended Oatmeal Agar, Hay Agar (Samson et al. 2002), a low nutrient medium such as Water Agar with a piece of sterile filter paper on the surface (Harrington 2003) or V8 Agar with antibiotics (Andersen and Nissen 2000).

In this study, air samples were collected from indoor and outdoor air in Florida, a state with temperate and subtropical climates that are conducive to fungal growth. Huang and Kimbrough (1997) conducted a survey of 41 Florida homes with children ages 4-14, some of whom suffered from mold allergies. Species of *Cladosporium, Penicillium, Curvularia, Epicoccum* and *Alternaria* accounted for 80% of the total fungi isolated. Bishop (2002) sampled outdoor air during summer months in north central Florida and did not report the occurrence of any *Stachybotrys* spp. Species of *Cladosporium, Geotrichum, Fusarium,* and *Penicillium* accounted for over 85% of the total fungi found outdoors. This study used low pH Mycological Agar which is not considered to be a good medium for recovery of *S. chartarum*.

The obective of the present study was to identify a medium or media that are semi-selective for *S. chartarum* spores in indoor and outdoor air. A medium that favors the recovery of *S. chartarum* from indoor air samples would be useful to industrial hygienists. In addition, it has been suggested that the source of *S. chartarum* building contamination is likely inoculum from outdoor air (Koster 2006). *Stachybotrys chartarum* is only rarely reported from outdoor air samples; a semi-selective medium could be used to provide a better understanding of the presence of *S. chartarum* in outdoor environments.

Materials and Methods

In this study, ten media were exposed in a residence with known *S. chartarum* contamination. The media with the best recovery of *S. chartarum* were then evaluated in outdoor settings in order to determine which was most effective when recovering *S. chartarum* at low concentrations.

Indoor Sampling

In July 2006, air sampling was undertaken at a private residence in Gainesville, FL, USA, that was known to be contaminated with *S. chartarum*. There was visible growth of *S.*

chartarum that had been present for several weeks on the master bedroom carpet, covering approximately 0.3 m^3. Sampling was done in the master bedroom and the living room (Figure 2-1). Previously, *S. chartarum* had been recovered from the living room; remediation occurred at that time, and the carpet was removed, the mold cleaned, and wood flooring was laid. At the time of sampling, the master bedroom was unoccupied.

Air samples were taken for 2 min in the master bedroom and the living room using an Andersen one-stage culture plate impactor (N6, Andersen Samplers, Inc., Atlanta, GA) with an Aerolite pump (Aerotech Instruments, Aerotech P & K, Allegro Industries) drawing 28.3 L of air per minute. Sampling occurred at a height of 0.6 m above the ground. Effort was made not to disturb the area of fungal growth in the bedroom so as to prevent the release of spores that would not normally be airborne. Ten media were used: BBL™ Corn Meal Agar (CMA, Becton, Dickson and Co., Sparks, MD), Difco™ Potato Dextrose Agar (PDA, Becton, Dickson and Co, Sparks, MD), Potato Dextrose Agar with 0.05 g Rose Bengal per liter (PDA RB), Potato Dextrose Agar with1 ml Tergitol per liter (PDA T), Potato Dextrose Agar with 0.05 g Rose Bengal and 1 ml Tergitol per liter (PDA RB/T), V8 Agar with 2 g of $CaCO_3$ per L (V8/2, Atlas 1993), V8 Agar with 4 g of $CaCO_3$ per L (V8/4), Water Agar with sterile filter paper (WAFP, Atlas 1993), Water Agar with sterile filter paper and 0.05 g Rose Bengal per liter (WAFP RB), and wetted 4 cm x 4 cm squares of 1.3 cm (½") thick sterile drywall. All agar plates contained 24 mL of medium. There were three replications for each medium, and a randomized complete block design was used. The Andersen N6 sampler was wiped down with 95% ethanol before sampling, between each sample, and at the end of sampling. Sampling was repeated the following day. Samples were incubated in the dark at 24°C for seven days.

Total number of fungal and bacterial colonies and number of *S. chartarum* colonies were counted and recorded as colony forming units per cubic meter of air (CFU/m^3). *Stachybotrys chartarum* was identified microscopically at 400x. Representative colonies from each room were grown on Czapek Yeast Autolysate Agar (CYA, Samson et al. 2002) and incubated in the dark for seven days at $24^{o}C$ at which point they were evaluated for pigment production. This was done to differentiate between *S. chartarum* and *S. chlorohalonata* which are similar morphologically. *Stachybotrys chlorohalonata* produces a dark green extracellular pigment on CYA. Percent recovery of *S. chartarum* from total colonies was calculated. Data were log-transformed, except for percent recovery of *S. chartarum*, and the data were analyzed as a two-way factorial. The analysis of variance showed an interaction between room and media, so the data were analyzed by room. P-values less than 0.10 were considered significant, and in these cases, means were separated by the Waller-Duncan k-ratio t test. Data were analyzed using SAS statistical software (Version 9.1, SAS Institute, Cary, NC).

Outdoor Sampling

Four media from the indoor air study (CMA, PDA RB, V8/2, WAFP RB) were used in this study. The V8 agar was amended with 0.1 g streptomycin sulfate and 0.05 g chlorotetracycline hydrochloride per liter. Sampling occurred at two sites on the University of Florida campus, Gainesville, FL, USA, a managed citrus grove (29°38’10”N, 82°21’53”W) and a grassy parking area (29°38’17”N, 82°21’46”W). Sampling occurred between 8 AM and 11 AM on ten dates from Apr – July 2007 at intervals of approximately ten days. Air samples were taken for 2 min using the Andersen N6 sampler with an Aerolite pump (Aerotech Instruments, Aerotech P & K, Allegro Industries) drawing 28.3 L of air per minute. Sampling occurred at a height of 0.6 m. There were three replications for each medium, and a randomized complete block design was used. The Andersen air sampler was wiped down with 95% ethanol before sampling, between

each sample, and at the end of sampling. Samples were incubated in the dark at 24°C for ten days. Total numbers of colonies of filamentous fungi were counted on day 3 and recorded as colony forming units per cubic meter of air (CFU/m^3), with the exception of the WAFP RB plates which were read on Day 10. On day 5, the plates were observed at 30x, and the number of colonies of *Stachybotrys, Alternaria, Curvularia, Cladosporium, Bipolaris/Dreschlera, Epicoccum, Penicillium*, and *Aspergillus* was recorded for all samples on PDA RB and V8/2 media. The presence or absence of these genera was noted for samples on CMA and WAFP RB. Putative *S. chartarum* colonies were isolated and grown on Czapek Yeast Autolysate Agar (CYA, Samson et al. 2002) and incubated in the dark for seven days at 24°C, at which point they were evaluated for pigment production.

Results

Indoor Study

Because there was a significant interaction between room and media for total colonies ($p<0.01$ Day 1, $p<0.001$ Day 2), *Stachybotrys* colonies ($p<0.001$ Day 1, $p<0.001$ Day 2) and *Stachybotrys* as a percent of total colonies ($p<0.001$ Day 1, $p<0.01$ Day 2), the data were analyzed by room.

The means of the total number of CFU/m^3 of air for each room are reported in Tables 2-1 and 2-2. The media detected significantly different ($p<0.001$) numbers of fungal colonies in each room on both days. The two V8-based media recovered high numbers of colonies in both rooms; however, it was noted that the plates were dominated by bacterial colonies. The actual number of filamentous fungi recovered by these media was much lower. Zero to few colonies grew on the pieces of wetted drywall. Each medium recovered a higher number of colonies from the master bedroom than from the living room.

The means of the number of *S. chartarum* (CFU/m^3 of air) for each room are reported in Tables 2-3-and 2-4. In the master bedroom, differences in recovery among media were significant on both days (p<0.001). Seven media, WAFP RB, PDA RB, V8/2, V8/4, CMA, PDA RB/T, and PDA T, recovered multiple colonies of *S. chartarum* from the master bedroom on both days. WAFP recovered an average of 135 CFU/m^3 of *S. chartarum* on day 1 but none on day 2. Overall, fewer *S. chartarum* colonies were noted on day 2 in the master bedroom.

Relatively few colonies of *S. chartarum* were recovered from the living room. Only the two V8 media were significantly different (p<0.01) from the media that failed to recover any *S. chartarum*. Only V8/2 was able to capture *S. chartarum* from the living room on both days of sampling. Representative isolates from both rooms did not produce green pigment on CYA.

Recoveries of *S. chartarum* as a percent of total colonies are presented in Tables 2-5 and 2-6. In the bedroom, WAFP RB recovered a significantly higher percent of *S. chartarum* than all other media on both days (p<0.001 on day 1, p<0.01 on day 2). Other media for which *S. chartarum* represented 16% or more of the total colonies included CMA, WAFP, both V8 agars and PDA RB. In the living room, no significant differences among media occurred on either day (p=0.2935 on day 1, p=0.5181 on day 2).

Outdoor Study

The total number of fungal colonies recovered from all 240 samples taken in this study was 12,545 colonies at the citrus site and 10,422 colonies at the grassy parking area. Of these 22,967 total colonies, the recovery of *S. chartarum* was a rare event, occurring on only two of ten sampling dates and totaling only seven colonies from six plates, or 0.025% of all plates. One colony of *S. chartarum* was observed on a PDA RB plate and one colony on a WAFP RB plate from those sampled on May 25 2007 at the grassy lot. The plates from the Jun 5 2007 sampling period recovered four colonies of *S. chartarum* from the citrus grove (one colony on a V8/2

plate, two colonies on one WAFP RB plate and one colony on a second WAFP RB plate) and one colony from the grassy lot site, on a PDA RB plate. No *S. chartarum* colonies were recovered on any of the CMA plates. None of the putative *S. chartarum* isolates produced green extracellular pigment when grown on CYA.

Cladosporium spp. were the most commonly recovered colonies from all media at both sites. This genus represented anywhere from 39.3% of total colonies (PDA, citrus grove) to 69.7% (V8, grassy lot). The second most common genus was *Penicillium* which ranged from 1.9% of total colonies (V8, grassy lot) to 4.1% (PDA, grassy lot). Species of *Alternaria, Curvularia, Bipolaris/Dreschlera, Epicoccum,* and *Aspergillus* all appeared but less regularly and/or abundantly. No notable difference in the frequency or abundance of these genera appears in the two sampling dates that recovered *S. chartarum*.

It should be noted that mites were discovered in the plates from the May 25 and Jun 5 sampling dates, including the ones that had *S. chartarum* colonies. The sampled plates were kept in an incubator that also housed pure cultures of *S. chartarum* that were found to have mites. No mites were present in plates from the other ten sampling dates.

Discussion

There has been ongoing discussion about the accuracy of air sampling for detection of *S. chartarum* in cases of suspected mold contamination, whether due to sampling method, spore dispersal, spore viability, or competitiveness on common media (Andersen and Nissen 2000, Kuhn et al. 2005). A recent study (Tucker et al. 2007) examined the biomechanics of conidial dispersal of *S. chartarum* and concluded that its conidia are poorly adapted for dispersal by airspeeds common in indoor environments. In addition, *Stachybotrys* growth most commonly occurs hidden in natural cracks and openings in structures, such as behind wallpaper or in a wall cavity, protected from air flow (Andersen and Nissen 2000). *Stachybotrys chartarum* spores are

present in indoor air in low concentrations, so it is important to use a growth medium that is semi-selective and affords the best opportunity for detection of the fungus. This study showed that when using an appropriate agar medium, it is possible for an air sample to pick up viable *S. chartarum* spores. There are, however, limitations to this method.

In the indoor air study, there was ample and consistent recovery of *S. chartarum* on many different media from bedroom air when the sampler was in close proximity (<1m) to visible fungal growth. The percentage of *S. chartarum* spores of all collected spores in the bedroom was 16% or greater for WAFP RB, CMA, WAFP, V8/2, V8/4 and PDA RB. These results demonstrate that *S. chartarum* spores do become airborne under conditions of normal household activity and that these spores are viable. Because large numbers of bacterial colonies grew on the V8 plates, this medium should always be amended with antibiotics as suggested by Andersen and Nissen (2000). If the bacterial colonies had been suppressed, the percent recovery of *S. chartarum* by these two media would have been much higher. This study also confirms the suggestions that WAFP (Harrington 2003), V8 agar with antibiotics (Andersen and Nissen 2000), PDA (Billups et al. 1999), and CMA (Tsai et al. l999) may be the best culture media, of those tested, for recovering viable *S. chartarum* from bioaerosol samples. Of these, WAFP amended with Rose Bengal also recovered fewer total numbers of colonies. Thus it seems that this medium, along with V8 agar amended with antibiotics, best meets the challenge of selectively recovering *S. chartarum* while reducing the total number of other colonies recovered.

More colonies of *S. chartarum* were recovered in the master bedroom than in the living room and on a greater number of media. At the time of sampling, *S. chartarum* was present on carpet in the master bedroom while there was no visible growth in the living room, which is located at the other end of the house (Figure 2-1). It is likely that the distance of the sampler

from the source of primary inoculum affects the accuracy of detection. These results highlight the difficulty in detecting *S. chartarum* from indoor air even when inoculum is visible and semi-selective media are used. Previous studies have shown that fewer *S. chartarum* colonies were detected on agar plates than by other sampling methods (Tiffany and Bader 2000, Spurgeon 2003, Kuhn et al. 2005). In this study, it was expected that better detection of *S. chartarum* could be obtained by using semi-selective media; however, it is possible that other methods such as impaction plates or cassettes may be better suited for accurate sampling of this particular fungus.

Of the two rooms sampled in the indoor air study, the living room best represented typical outdoor air, which would likely have a low concentration of *S. chartarum* spores. Therefore, the media that successfully recovered *S. chartarum* from the living room air samples were used in the outdoor air study. In addition WAFP RB was selected because it consistently recovered the most colonies of *S. chartarum* in the master bedroom. Bishop (2002) showed that, in north central Florida, an Andersen sampler collects more fungi from outdoor air in the morning than in the afternoon. In addition, this same study showed that the amount of *Cladosporium* as a percent of total fungi measured is less at 9 AM than it is at 3 PM and 9 PM (Bishop 2002). In an effort to sample from the largest number of spores while limiting the amount of *Cladosporium* present, samples were collected in the morning for the outdoor air study. Sampling occurred at a time of year when *S. chartarum* is present outdoors (Chapter 3).

The detection of *S. chartarum* from outdoor air was a rare event although 1) the media used had successfully recovered *S. chartarum* in the indoor air study and 2) the samples were taken in an area and at a time of year that *S. chartarum* had been recovered by other sampling techniques (Chapter 3). It is likely that *S. chartarum* is present in outdoor air at such a low

concentration that it falls below the detectable threshold of even semi-selective media. This study suggests that air sampling would not be an appropriate method for research investigating the occurrence of *S. chartarum* in outdoor habitats. Studies that have used slide impaction or cassette samplers, which allow detection on non-viable spores, have reported higher levels of *S. chartarum* in outdoor air than this study, from 3 – 9% of samples (Baxter et al. 2005, Kuhn et al. 2005).

The fact that *S. chartarum* was recovered on plates from two of the ten sampling dates and only from plates that were also infested with mites suggests the possibility that it was, in fact, not ever recovered from outdoor air and was instead introduced from mites that migrated in the laboratory from pure cultures of *S. chartarum*. Fungi are known to be dispersed by a wide variety of arthropods such as beetles (Paine et al. 1997) and collembola (Visser et al. 1987). Mites are known to vector spores from a range of fungal taxa that includes Basidiomycota, Ascomycota and Zygomycota (Renker et al. 2005, Greif and Currah 2007). In particular, species that produce sticky spores, such as *Ophiostoma ulmi*, the causal agent of Dutch Elm disease, are associated with arthropods. *Stachybotrys* is unusual among common indoor air fungi in that it produces spores in a mucilaginous mass, whereas species of *Aspergillus*, *Penicillium,* and *Cladosporium* produce dry spores that seem well-adapted to air dispersal (Tucker et al. 2007). It has been hypothesized that *S. chartarum* can be spread indoors by insect movement, although research has yet to confirm this (Money 2004, Koster 2006). The results of this study suggest that further research should investigate mites as possible vectors of *S. chartarum* spores.

A B

Figure 2-1. Private residence in Gainesville, FL with *Stachybotrys chartarum* infestation. A) Bedroom showing *S. chartarum* growing on carpet. B) Living room and dining room.

Table 2-1. Total number of fungal and bacterial colonies recovered on different media from master bedroom, for two different samplings.

Day	Media[a]	Mean[b]	
1	PDA RB/T	3842.7	a
1	PDA T	3168.0	ab
1	PDA RB	2364.3	ab
1	V8/4	1830.4	ab
1	V8/2	1783.5	ab
1	PDA	2147.2	abc
1	WAFP RB	1302.4	abc
1	CMA	1009.1	bc
1	WAFP	662.9	c
1	drywall	0.0	d
2	V8/4	2405.3	a
2	V8/2	2217.6	a
2	PDA RB	2323.2	a
2	PDA T	1672.0	ab
2	CMA	1519.5	ab
2	PDA	1355.2	ab
2	PDA RB/T	1243.7	ab
2	WAFP RB	1014.9	ab
2	WAFP	727.5	b
2	drywall	5.9	c

[a] Media are Corn Meal Agar (CMA), Potato Dextrose Agar (PDA), Potato Dextrose Agar with 0.05 g Rose Bengal per liter (PDA RB), Potato Dextrose Agar with1 ml Tergitol per liter (PDA T), Potato Dextrose Agar with 0.05 g Rose Bengal and 1 ml Tergitol per liter (PDA RB/T), V8 Agar with 2 g of $CaCO_3$ per L (V8/2), V8 Agar with 4 g of $CaCO_3$ per L (V8/4), Water Agar with sterile filter paper (WAFP), Water Agar with sterile filter paper and 0.05 g Rose Bengal per liter (WAFP RB), and wetted 4 cm x 4 cm squares of 1.3 cm (½") thick sterile drywall.
[b] Differences among media are significant at $p<0.0001$. Means reported are actual means; units are colony forming units per cubic meter of air (CFU/m^3). For each sampling date, means in column followed by the same letter are not different, based on log(x+1) transformed data.

Table 2-2. Total fungal and bacterial colonies recovered on different media from living room, for two different samplings.

Day	Media[a]	Mean[b]	
1	V8 4	516.3	a
1	V8 2	440.0	ab
1	PDA	404.8	abc
1	CMA	305.1	abc
1	PDA T	275.7	abc
1	PDA RB/T	328.5	abcd
1	PDA RB	164.3	bcd
1	WAFP RB	146.7	cd
1	WAFP	111.5	d
1	drywall	0.0	e
2	V8 4	598.4	a
2	V8 2	580.8	a
2	PDA	287.5	ab
2	PDA T	211.2	bc
2	PDA RB	181.9	bcd
2	CMA	170.1	bcd
2	PDA RB T	129.1	bcd
2	WAFP	93.9	cd
2	WAFP RB	88.0	d
2	drywall	5.9	e

[a] Media are Corn Meal Agar (CMA), Potato Dextrose Agar (PDA), Potato Dextrose Agar with 0.05 g Rose Bengal per liter (PDA RB), Potato Dextrose Agar with1 ml Tergitol per liter (PDA T), Potato Dextrose Agar with 0.05 g Rose Bengal and 1 ml Tergitol per liter (PDA RB/T), V8 Agar with 2 g of $CaCO_3$ per L (V8/2), V8 Agar with 4 g of $CaCO_3$ per L (V8/4), Water Agar with sterile filter paper (WAFP), Water Agar with sterile filter paper and 0.05 g Rose Bengal per liter (WAFP RB), and wetted 4 cm x 4 cm squares of 1.3 cm (½") thick sterile drywall.
[b] Differences among media are significant at $p<0.0001$. Means reported are actual means; units are colony forming units per cubic meter of air (CFU/m^3). For each sampling date, means in column followed by the same letter are not different, based on log(x+1) transformed data.

Table 2-3. Total *Stachybotrys chartarum* colonies recovered on different media from master bedroom, for two different samplings.

Day	Media[a]	Mean[b]	
1	WAFP RB	686.4	a
1	V8 4	733.3	ab
1	CMA	316.8	ab
1	V8 2	305.1	ab
1	PDA RB	258.1	ab
1	WAFP	134.9	b
1	PDA RB/T	105.6	b
1	PDA T	41.1	c
1	PDA	0.0	d
1	drywall	0.0	d
2	WAFP RB	164.3	a
2	PDA RB	146.7	a
2	V8 4	93.9	ab
2	CMA	70.4	ab
2	V8 2	58.7	ab
2	PDA RB/T	58.7	ab
2	PDA T	58.7	b
2	drywall	0.0	c
2	PDA	0.0	c
2	WAFP	0.0	c

[a] Media are Corn Meal Agar (CMA), Potato Dextrose Agar (PDA), Potato Dextrose Agar with 0.05 g Rose Bengal per liter (PDA RB), Potato Dextrose Agar with1 ml Tergitol per liter (PDA T), Potato Dextrose Agar with 0.05 g Rose Bengal and 1 ml Tergitol per liter (PDA RB/T), V8 Agar with 2 g of $CaCO_3$ per L (V8/2), V8 Agar with 4 g of $CaCO_3$ per L (V8/4), Water Agar with sterile filter paper (WAFP), Water Agar with sterile filter paper and 0.05 g Rose Bengal per liter (WAFP RB), and wetted 4 cm x 4 cm squares of 1.3 cm (½”) thick sterile drywall.
[b] Differences among media are significant at $p<0.0001$. Means reported are actual means; units are colony forming units per cubic meter of air (CFU/m^3). For each sampling date, means in column followed by the same letter are not different, based on log(x+1) transformed data.

Table 2-4. Total *Stachybotrys chartarum* colonies recovered on different media from living room, for two different samplings.

Day	Media[a]	Mean[b]	
1	V8 2	17.6	a
1	V8 4	11.7	ab
1	CMA	5.9	bc
1	WAFP	5.9	bc
1	PDA RB/T	0.0	c
1	PDA T	0.0	c
1	PDA	0.0	c
1	PDA RB	0.0	c
1	WAFP RB	0.0	c
1	drywall	0.0	c
2	V8 2	5.9	
2	PDA RB	5.9	
2	CMA	0.0	
2	drywall	0.0	
2	PDA RB/T	0.0	
2	PDA T	0.0	
2	PDA	0.0	
2	V8 4	0.0	
2	WAFP RB	0.0	
2	WAFP	0.0	

[a] Media are Corn Meal Agar (CMA), Potato Dextrose Agar (PDA), Potato Dextrose Agar with 0.05 g Rose Bengal per liter (PDA RB), Potato Dextrose Agar with1 ml Tergitol per liter (PDA T), Potato Dextrose Agar with 0.05 g Rose Bengal and 1 ml Tergitol per liter (PDA RB/T), V8 Agar with 2 g of $CaCO_3$ per L (V8/2), V8 Agar with 4 g of $CaCO_3$ per L (V8/4), Water Agar with sterile filter paper (WAFP), Water Agar with sterile filter paper and 0.05 g Rose Bengal per liter (WAFP RB), and wetted 4 cm x 4 cm squares of 1.3 cm (½") thick sterile drywall.
[b] Differences among media are significant at $p<0.01$. Means reported are actual means; units are colony forming units per cubic meter of air (CFU/m^3). For each sampling date, means in column followed by the same letter are not different, based on log(x+1) transformed data.

Table 2-5. *Stachybotrys chartarum* colonies as percent of total colonies recovered on different media from master bedroom, for two different samplings.

Day	Media[a]	Mean[b]	
1	WAFP RB	50.4	a
1	CMA	33.1	ab
1	V8 4	30.5	b
1	WAFP	18.8	bc
1	V8 2	18.1	bcd
1	PDA RB	16.5	bcd
1	PDA RB/T	5.1	cd
1	PDA T	3.1	cd
1	PDA	0.0	d
1	drywall	0.0	d
2	WAFP RB	13.6	a
2	PDA RB	5.4	b
2	CMA	4.8	b
2	PDA RB/T	4.3	b
2	V8 4	3.6	b
2	V8 2	2.8	b
2	PDA T	2.3	b
2	drywall	0.0	b
2	PDA	0.0	b
2	WAFP	0.0	b

[a] Media are Corn Meal Agar (CMA), Potato Dextrose Agar (PDA), Potato Dextrose Agar with 0.05 g Rose Bengal per liter (PDA RB), Potato Dextrose Agar with1 ml Tergitol per liter (PDA T), Potato Dextrose Agar with 0.05 g Rose Bengal and 1 ml Tergitol per liter (PDA RB/T), V8 Agar with 2 g of $CaCO_3$ per L (V8/2), V8 Agar with 4 g of $CaCO_3$ per L (V8/4), Water Agar with sterile filter paper (WAFP), Water Agar with sterile filter paper and 0.05 g Rose Bengal per liter (WAFP RB), and wetted 4 cm x 4 cm squares of 1.3 cm (½") thick sterile drywall.
[b] Differences among media are significant at p=0.0002 for Day 1 and p=0.0017 for Day 2. Means reported are actual means; units are colony forming units per cubic meter of air (CFU/m^3). For each sampling date, means in column followed by the same letter are not different, based on log(x+1) transformed data.

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Table 2-6. *Stachybotrys chartarum* colonies as percent of total colonies recovered on different media from living room, for two different samplings.

Day	Media[a]	Mean[b]
1	WAFP	5.6
1	V8 2	5.3
1	V8 4	2.7
1	CMA	1.4
1	PDA RB/T	0.0
1	PDA T	0.0
1	PDA	0.0
1	PDA RB	0.0
1	WAFP RB	0.0
1	drywall	0.0
2	PDA RB	3.3
2	V8 2	1.1
2	CMA	0.0
2	drywall	0.0
2	PDA RB/T	0.0
2	PDA T	0.0
2	PDA	0.0
2	V8 4	0.0
2	WAFP RB	0.0
2	WAFP	0.0

[a] Media are Corn Meal Agar (CMA), Potato Dextrose Agar (PDA), Potato Dextrose Agar with 0.05 g Rose Bengal per liter (PDA RB), Potato Dextrose Agar with1 ml Tergitol per liter (PDA T), Potato Dextrose Agar with 0.05 g Rose Bengal and 1 ml Tergitol per liter (PDA RB/T), V8 Agar with 2 g of $CaCO_3$ per L (V8/2), V8 Agar with 4 g of $CaCO_3$ per L (V8/4), Water Agar with sterile filter paper (WAFP), Water Agar with sterile filter paper and 0.05 g Rose Bengal per liter (WAFP RB), and wetted 4 cm x 4 cm squares of 1.3 cm (½") thick sterile drywall.
[b] Differences among media are not significant at $p \leq 0.1$, based on log(x+1) transformed data. Means reported are actual means; units are colony forming units per cubic meter of air (CFU/m^3).

CHAPTER 3
FREQUENCY AND ABUNDANCE OF *Stachybotrys chartarum* AND OTHER COMMON FUNGAL GENERA IN OUTDOOR HABITATS IN NORTH CENTRAL FLORIDA

Introduction

Stachybotrys chartarum (Ehrenb.) Hughes (= *Stachybotrys atra* Corda) is a dermatiaceous hyphomycete of worldwide distribution that has been isolated from a variety of substrates in both indoor and outdoor environments. In natural environments, it has been most commonly isolated from soil (Barron 1968, Ellis 1971, El-Morsy 1999); however, it has also been isolated from decaying plant material (Ellis 1971, Whitton et al. 2001), on animal fodder (Drobotko 1945), as a parasite of other fungi (Siqueira et al. 1984), and in association with living plants (El-Morsy 1999, Li et al. 2001). Some of the more unusual natural substrates include woodchuck dung (Jong and Davis 1976) and seaweed (Andersen et al. 2002). A strongly cellulolytic fungus, *S. chartarum* has also been isolated from a variety of human-made materials derived from plant fibers such as paper, cotton, and canvas (Bisby 1943, Jong and Davis 1976). Of current interest is the frequent isolation of *S. chartarum* from construction materials including drywall, ceiling tiles and wallpaper in buildings that have experienced water damage (Li and Yang 2005).

Stachybotrys chartarum, which produces macrocyclic trichothecenes that are among the most potent mycotoxins known to man (Jarvis 2003), has been implicated in cases of sick building syndrome and pulmonary hemorrhage (Croft et al. 1986, Etzel et al. 1998, Dearborn et al. 1999). A recent study suggests that outdoor inoculum is the most likely source for indoor *S. chartarum* contamination (Koster 2006), possibly from air-borne spores that are deposited on the surface of building materials at the construction site (Nelson 2001). Despite the significance of *S. chartarum* regarding public health, no comprehensive ecological study that identifies different habitats that support *S. chartarum* populations has been done.

Previous studies of outdoor air have reported none to very low levels of *S. chartarum* spores when sampling using a Samplair particle sampler (Li and Kendrick 1995, Baxter et al. 2005), an Air-O-Cell slit bioaerosal cassette (Baxter et al. 2005), or an Andersen N6 sampler (Chapter 2, Bishop 2002, Shelton et al. 2002). However, drywall is the most commonly found human-associated substrate for *S. chartarum*, and numerous studies have shown it supports extensive fungal growth when wet (Andersson et al. 1997, Nikulin et al. 1994, Karunasena et al. 2000, Hyvärinen et al. 2002). For this reason, drywall was the medium of choice for this study. The primary ingredient of drywall is the naturally occurring mineral gypsum or calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$), sandwiched between two sheets of paper. Both the cellulose in the liner and starch, which is used as an adhesive, have been shown to be important nutrient sources for *S. chartarum* (Murtoniemi et al. 2003).

The objective of this project was to identify outdoor habitats in north central Florida where *S. chartarum* is found and the times of year when it is most abundant. It is critical to understand the types of environments that support natural populations of *S. chartarum* because it is likely that these populations play an important role in the establishment of mold colonies in water-damaged structures.

Materials and Methods

Study Sites

Four study sites representing diverse habitats in Gainesville, Florida, USA, were selected (Figure 3-1). Three sites, the pine grove, the citrus grove, and the lakeshore, are located on the University of Florida campus. The fourth site, a hardwood forest, is on private property approximately 11 km from the other three sites.

The citrus and pine groves are managed agricultural sites, and the dominant plant species are species of *Citrus* and *Pinus elliottii* (Slash pine), respectively. The lakeshore and hardwood

forest sites are unmanaged and relatively ecologically diverse. Common tree species at the lakeshore site include *Acer negundo* (Ashleaved maple), *A. rubrum* (Red maple), *Taxodium distichum* (Bald cypress), *Quercus virginiana* (Live oak), *Sabal palmetto* (Cabbage palm), and *Salix nigra* (Black willow). Common shrubs and herbaceous plants include *Adiantum pedatum* (Maidenhair fern), *Ampelopsis arborea* (Peppervine), *Andropogon virginicus* (Broomgrass), *Baccharis halimifolia*, *Bidens alba* (syn. *B. pilosa*, Hairy beggarticks, Spanish needles), *Capsella bursa-pastoris* (Shepherd's purse), *Celtis occidentalis* (Common hackberry), *Cuscuta* sp. (Dodder), *Eupatorium capillifolium* (Dog fennel), *Laurocerasus caroliniana* (Carolina laurelcherry), *Menispermum canadense* (Moonseed), *Myrica cerifera* (Southern wax myrtle), *Parthenocissus quinquefolia* (Virgina creeper), *Rubus* sp. (Wild blackberry), *Sambucus* sp. (Elderberry), *Smilax bona-nox* (Saw greenbrier), *Typha* sp. (Cattail), and *Vitis rotundifolia* (Wild grape). Common tree species at the hardwood forest site include *Acer barbatum* (Southern sugar maple), *Betula lutea* (Yellow birch), *Carpinus caroliniana* (American hornbeam/Bluebeech), *Liquidambar styraciflua* (Sweetgum), *Magnolia grandiflora* (Southern magnolia), *Pinus taeda* (Loblolly pine), *P. glabra* (Spruce pine), *Quercus michauxii* (Swamp chestnut oak), *Q. nigra* (Water oak), *Q. phellos* (Willow oak), *Q. virginiana*, and *Tilia americana* (American basswood). Common understory trees, shrubs, and herbaceous plants include *Adiantum* sp., *Bidens alba*, *Callicarpa americana* (American beautyberry), *Celtis occidentalis*, *Fraxinus americana* (White ash), *Hedera* sp. (Ivy), *Koelreuteria paniculata* (Golden rain tree), *Laurocerasus caroliniana*, *Ligustrum vulgare* (Privet), *Mitchella repens* (Partridgeberry), *Morus rubra* (Red mulberry), *Phytolacca americana* (American pokeweed), *Serenoa repens* (Saw palmetto), *Smilax bona-nox*, *Toxicodendron radicans* (Poison ivy), and *Viola* sp. (Wild violet).

Description of Traps

In this experiment, traps were designed to hold eight pieces of drywall with dimensions 20 cm x 4 cm (Figure 3-2). Standard drywall sheets, 1.3 cm x 121.9 cm x 243.8 cm (½ in x 4 ft x 8 ft sheets) and manufactured in Palatka, Florida (Lafarge), were purchased at a national chain home-improvement store and cut into strips. The exception was October 2006 when Tough Rock brand drywall (George Pacific, Atlanta, GA) was purchased. A 12-cm x 4-cm grid consisting of 12 2-cm x 2-cm cells was drawn on the front of each strip. The strips were soaked overnight in tap water before being placed upright in 20 mm Nalgene Unwire™ test tube racks (250 x 102 x 83 mm) that were modified by removing some partitions. In each rack, the drywall pieces were oriented so that two pieces were placed along each side. Prior to being placed in the field, the wet drywall pieces and racks were sterilized for 5 minutes at full power in a 120V microwave (Frigidaire, Martinez, GA).

In the field, the sterile drywall strips and test tube racks were placed in 4-quart clear plastic storage boxes (34.3 cm x 20.3 cm x 10.2 cm, Sterilite, Townsend, MA) with drainage holes drilled 3.2 cm from the base. At each site, five traps were placed along a transect line, 10 m apart. GPS location data is reported in Appendix A (Table A-1). The drywall strips in each trap were oriented so that they faced due North, South, East, and West (Figure 3-2). One liter of sterile tap water was added to each trap when placed in the field. Traps were placed in the field on the following dates: June 05, Aug 05, Oct 05, Dec 05, Feb 06, May 06, Jun 06, Aug 06, Nov 06, Jan 07, and Apr 07.

Sampling

Each trap was sampled at four and eight weeks, with the exception of the May 06 traps which were only sampled once. Collection dates are summarized in Appendix A (Table A-2). A sample from one trap consisted of four drywall pieces, one from each direction. A total of 80

drywall pieces were collected each month. Samples were collected 21 times. In the lab, each strip of drywall was cut into three pieces using sterile instruments and placed in 100-mm x 20-mm disposable Petri dishes (Figure 3-3).

Each 2-cm x 2-cm grid was observed at 30x magnification under a dissecting microscope, and the presence of species of *Cladosporium, Alternaria, Epicoccum, Curvularia, Bipolaris, Dreschlera,* and *Stachybotrys* was recorded. Putative *Stachybotrys* colonies were isolated, grown on BBL™ Corn Meal Agar (Becton, Dickson and Co., Sparks, MD) in the dark for 14 days at 24°C, and identified at 400x and 1000x based on morphological features as described by Jong and Davis (1976), with the exception of *S. bisbyi* which was identified by tape mounts (Miller 2001) made directly from the drywall. In order to distinguish between *S. chartarum* and *S. chlorohalonata*, these isolates were inoculated on Czapek yeast autolysate agar (CYA, Samson et al. 2002), incubated in the dark for 7 days at 24°C, and then they were evaluated for pigment production. *Stachybotrys chlorohalonata* receives its name from the dark green pigment it produces on this medium.

Data Analysis

Data from each month were analyzed separately. Abundance was defined as the number of 2-cm x 2-cm squares on a 12-cm x 4-cm grid that contained *S. chartarum* colonies and was analyzed as a two-way factorial analysis of variance (ANOVA) to determine effects of location, direction, and location x direction interaction. When direction and the interaction were not significant, data were reanalyzed by one-way ANOVA to determine effects of location. P-values less than 0.10 were considered significant, and in these cases, means were separated by the Waller-Duncan k-ratio t test. Data were analyzed using SAS statistical software (Version 9.1, SAS Institute, Cary, NC). Occurrences of species of *Cladosporium, Alternaria, Epicoccum, Dreschlera*, *Bipolaris*, and *Curvularia* were analyzed in the same fashion, with the exception that

all data for species of *Dreschlera*, *Bipolaris*, and *Curvularia* were combined for statistical analysis. Approximate colony size was estimated by averaging the number of cells with *S. chartarum* growth at each site on each date. Weather information was gathered from the Florida Automated Weather Network for the Gainesville, FL, weather site which is located approximately 24 km from the campus sites and 18 km from the hardwood forest site (FAWN 2007).

Results

Stachybotrys chartarum

Over the course of 24 months, 1680 pieces of drywall were placed in the field. Fifteen pieces were damaged or unable to be recovered and were not included in the statistical analysis. *Stachybotrys chartarum* was found on 33 pieces of drywall or 0.02% of the pieces collected. Four pieces of drywall showed two colonies each of *S. chartarum* with distinct boundaries, so a total of 37 colonies were isolated from the field. *Stachybotrys chartarum* was found during 9 of the 24 months that traps were in the field: June, July, August, and September 2005, and May, June, August, September, and December 2006 (Tables 3-1 and 3-2). No *S. chartarum* was found on the drywall placed in the field January 17 – June 1, 2007. In 2005, *S. chartarum* occurred in the citrus grove, the pine grove, and the weedy lakeshore. In 2006, *S. chartarum* occurred in the citrus grove, the pine grove, and the hardwood forest. *Stachybotrys chartarum* was isolated most frequently from the citrus grove where it occurred during eight different months. Although it was not recovered from the hardwood forest in 2005, *S. chartarum* was recovered from that site in July, August, September, and December 2006. *Stachybotrys chartarum* was only recovered twice from the weedy lakeshore and three times from the pine grove during the course of this two-year study.

Abundance was defined as the number of cells per grid with *S. chartarum* growing on them; the maximum value was twelve. Abundance was not significantly affected by the direction that the drywall faced (N, S, E, W), except for December 2006 (p=0.0558); therefore, direction was pooled for each month. There was no significant difference in the abundance of *S. chartarum* among sites for each month except for September-October 2006 (p=0.0381, Table 3-4) and December 2006 (p=0.0558, Table 3-4) when the hardwood forest site had significantly more *S. chartarum* growing on the drywall pieces than did most other sites. Approximate colony size is reported in Table 3-5.

Putative *S. chartarum* colonies that were identified at 30x, but which could not be isolated in order to confirm their identification, occurred on four pieces. In each case, attempts to isolate the sample failed because only a few condiophores were found on the piece of drywall. These minute colonies occurred in July 2005 at the lakeside and pine sites, and in July and August 2006 at the lakeside. These colonies were not included in the statistical analysis because they could have been *S. chlorohalonata*.

Additionally, five pieces of drywall had large *S. chartarum* colonies growing off the grid (i.e. growing on the ungridded, bottom-third of the strip) that were visible to the naked eye. These colonies were isolated, grown on CMA and identified as *S. chartarum*. These isolates occurred in July 2005 at the citrus and pine sites, September 2005 at the hardwood and citrus sites, and May and September/October 2006 at the citrus site. These isolates were not used in the statistical analysis. All of these colonies occurred at months when on-grid colonies were recovered at the same sites with the exception of the September 2005 recovery of off-grid *S. chartarum* from the hardwood site. This off-grid colony was the only *S. chartarum* recovered from the hardwood site in 2005.

Average temperature, total rainfall and average relative humidity for each sampling period are presented in Table 3-6. The majority of *S. chartarum* isolates were recovered during months when average daily temperature ranged from 23.6 to 26.9°C. Average daily low temperatures during these months ranged from 16.2 °C to 21.6°C; average daily high temperatures during these months ranged from 31.4 °C to 34.1°C. The exception to this observation was December 2006 when average daily temperature was 15.3°C and *S. chartarum* was recovered from the hardwood forest site. All months that *S. chartarum* was not present on the drywall traps had average temperatures that fell below these ranges.

Other *Stachybotrys* species

Stachybotrys chlorohalonata colonies grew on the drywall grid four times during the 2-year study: citrus in Nov-Dec 2005, lakeside in July 2006, hardwood in Sept-Oct 2006 and citrus in May 2007 (Table 3-7). This species was never recovered from the pine site. Two colonies of *S. chlorohalonata* were visible with the naked eye but did not appear on the grid. These additional colonies occurred at the citrus site in September 2005 and the hardwood site in August 2006.

Five other species of *Stachybotrys* were recovered during the 2-year study. Of these, the two most commonly-occurring species were *S. kampalensis* and *S. bisbyi*. Both have unique morphological characteristics that make them easy to identify. *Stachybotrys bisbyi* produces hyaline conidiophores, phialides and conidia, and *S. kampalensis* produces hyaline phialides but very dark ellipsoidal spores that usually contain two oil drops (Jong and Davis 1976).

Stachybotrys bisbyi was the second most commonly found *Stachybotrys* species and was recovered 31 times (Table 3-8). It was found consecutively from June to September 2005 and June to December 2006. It was not recovered between January and June 1, 2007. It was found once at the citrus site (June 2005), twice at the hardwood site (July 2006 twice), and three times

at the pine site (July 2005 twice and August 2005 once). All of the other 25 occurrences were from the lakeside site. Significantly more *S. bisbyi* was found at the lakeshore site in September 2005 ($p=0.0537$), June 2006 ($p<0.01$), July 2006 ($p=0.0668$) and August 2006 ($p<0.01$) than at the other three sites at those times (Table 3-9).

Stachybotrys kampalensis was identified from colonies on 16 pieces of drywall (Table 3-10). It was discovered twice at the hardwood site (June and July 2006) and 14 times at the lakeside site. *Stachybotrys kampalensis* was not recovered from the pine or citrus sites. Significantly more *S. kampalensis* was found at the lakeside site in September 2005 ($p<0.01$) and August 2006 ($p=0.0393$) than was found at the other three sites at those times (Table 3-11).

Additional species of *Stachybotrys* that were observed include *S. albipes* (July 2006, hardwood site), *S. nephrospora* (July 2006, pine site), and *S. parvispora* (August 2006, hardwood site).

Other Genera

The abundance of species of *Cladosporium, Alternaria*, *Epicoccum*, *Dreschlera*, *Bipolaris*, and *Curvularia* is reported in Tables 3-12 to 3-15. All genera showed a significant difference in abundance among the four sites at different times over 21 months of sampling. The abundance of *Cladosporium* spp. and the *Dreschlera/Bipolaris/Curvularia* spp. group differed significantly among sites during 15 different months. *Epicoccum* spp. and *Alternaria* spp. differed during 13 and 11 months, respectively. There is no apparent seasonal pattern at any site in any of the four genera groups.

Discussion

Over the course of 24 months, *S. chartarum* was found growing in all four habitats. It was predicted that *S. chartarum* would be the dominant fungus on the drywall, occurring frequently, in multiple and large colonies. Instead, it was found rarely, on only 0.02% of the pieces

collected. Even if one considers the colonies that grew off-grid, the frequency of *S. chartarum* was unexpectedly low. In addition, it was thought that *S. chartarum* would be more abundant than observed. Abundance was measured as the number of cells with *S. chartarum* growth and reflected both the number of colonies present and the relative size of the colonies. On 4 of 33 pieces of drywall, two colonies were observed; all other pieces had only one colony. With the exception of the hardwood forest site in September-October 2006, the mean number of cells on the grid with *S. chartarum* was less than 1 cell. In part, this is due to the low number of pieces with *S. chartarum* growth, but it was also observed that the colony size was relatively small. It is, therefore, more appropriate to examine the mean number of cells with *S. chartarum* growth as an indicator of colony size. Two-thirds of samples (11 of 17), the average colony covered less than half of the 12-cm x 4-cm grid. It is unclear why the colonies did not dominate the drywall surface.

Because the frequency of *S. chartarum* was low, most differences in abundance between sites were not significant. The only two times that differences in abundance were significant, more *S. chartarum* was recovered at the hardwood site than at the other sites. It is unknown whether a large reservoir of inoculum was present, or if environmental conditions in Sept-Oct 2006 and December 2006 were particularly conducive to rapid growth at that site. The largest colony size also occurred during the Sept-Oct 2006 sampling period, with the average colony covering just over 75% of the total grid surface.

It is important to note, however, that *S. chartarum* was recovered more than once from each habitat. This is not surprising since 1) *S. chartarum* has been recovered from a variety of natural substrates in the past, and 2) its preferred substrate is cellulose which is common at all four sites. At all sites, *S. chartarum* was only found during the summer months, which was

anticipated since it is a hydrophilic fungus, and north central Florida typically receives abundant rainfall during this time. There was no trend in frequency of occurrence at the sites between years with the exception of the citrus grove where *S. chartarum* occurred both years from July to September. Originally, it was thought that the weedy lakeshore might harbor the highest populations of *S. chartarum* because the abundance of herbaceous annual plants in this habitat would supply a ready source of cellulose and the nearby water would keep relative humidity high. Instead, *S. chartarum* was recovered most frequently from the citrus grove where plant diversity was low. This site was included in the study because *S. chartarum* had been recovered previously in the area from citrus leaves (J.W. Kimbrough personal communication).

Several recent studies have suggested that *S. chartarum* spores are not naturally wind dispersed (Koster 2006, Tucker et al. 2007); thus it is unlikely that the inoculum was transported over long distances. Most likely the source of the drywall colonies is local; therefore, one can conclude that *S. chartarum* is found in a variety of outdoor habitats in north central Florida. If it is true that these outdoor populations serve as the inoculum for indoor contamination (Koster 2006), then there appear to be multiple reservoirs for mold contamination in natural habitats in north central Florida.

It is possible that *S. chartarum* was found less often than expected because unfavorable environmental conditions at the micro-level prevented viable spores that landed on the traps from sporulating (i.e. low moisture). If this were true, the traps would have underestimated the abundance of *S. chartarum* in each habitat. Water activity (a_w) is a measure of the available water in a substrate (Flannigan and Miller 2001). *Stachybotrys chartarum* requires a water activity between 0.91-0.93 for growth and sporulation (Grant et al. 1989) and is characterized as hydrophilic (Flannigan and Miller 2001). Nielsen et al. (2004) found *S. chartarum* growing on

drywall only at 95% relative humidity. However, *S. chartarum* requires a lower minimum water activity when grown at higher temperatures (Flannigan and Miller 2001). Although each trap was filled with 1 L sterile water when placed in the field, it was noted that the traps dried out during periods of low rainfall, so it is possible that moisture was a limiting factor. This idea is supported by the observation that most *S. chartarum* isolates were recovered from the citrus grove which is a managed agricultural site. The traps at this site were near irrigation heads and received more moisture than the traps at the other three sites. The citrus grove is irrigated year-round, so the citrus traps also received extra moisture during months when *S. chartarum* failed to be recovered at that site. In fact, of the four sampling periods that received the highest total rainfall, July 2005 (32.7 cm), Nov – Dec 2005 (26.6 cm), Feb 2006 (35.1 cm), and April 2006 (24.1 cm), *S. chartarum* was only recovered during the July 2005 period. Thus while it is possible that there were isolated incidents where a viable spore was unable to germinate due to limited moisture, it is unlikely that this was a consistent problem over the 24 months of sampling.

A positive correlation was seen between *S. chartarum* occurrence and daily temperature. With the exception of December 2006, every *S. chartarum* recovery was during a month with an average daily temperature above 23°C. Average daily low temperatures during these months did not fall below 16.2^{o}C. Average daily high temperatures during these months ranged from 31.4 ^{o}C to 34.1^{o}C. The recovery of *S. chartarum* from the December 2006 sampling shows that lower temperatures do not prevent it from sporulating on the drywall. Instead it is likely that the data accurately reflect that there are more airborne spores of *S. chartarum* outdoors during warmer months. A related finding was reported by Billups et al. (1999) who found that *S. chartarum* was preferentially recovered from air samples when the plates were incubated at 35°C as opposed to

25°C. However, it should be noted that the weather data in the present study are general data for the Gainesville, FL area, and they do not represent conditions that were present at the micro-level at each trap.

Stachybotrys chlorohalonata was found even more rarely than *S. chartarum*. This species was only recently recognized (Andersen et al. 2003) and has been found indoors in conjunction with *S. chartarum*. Initial studies have shown that *S. chlorohalonata* is found less commonly than *S. chartarum* indoors (Cruse et al. 2002, Koster et al. 2003, Andersen et al. 2003). This study is the first to compare population levels of these two species in nature, and it is interesting that the same trend has been observed. *Stachybotrys bisbyi* and *S. kampalensis* were the other two species most commonly found, and in both cases, significantly more fungal growth was found at the weedy lakeshore than at the other three sites.

Although this study found less *S. chartarum* than predicted, the use of drywall traps for measuring occurrence and frequency of common airborne fungi is a valid technique (Flannigan and Miller 2001). All of the other selected genera of fungi grew on the drywall traps, and differences in frequency were seen for all of the other genera measured in this study. Species of *Cladosporium* were the dominant drywall colonizers in all 21 months. This is not surprising, since this genus was also most commonly recovered from other outdoor air samples (Li and Kendrick 1995, Li et al. 1995, Bishop 2002, Shelton et al. 2002, Baxter et al. 2005).

Ultimately, the results of this study suggest that although *S. chartarum* is less common among the outdoor air spora of north-central Florida than had been predicted, it occurs in a variety of natural and managed habitats, primarily during summer months. Having identified these habitats, it would be interesting to expand sampling to other habitats to increase our knowledge about the geographic range of this fungus. It would also be useful to sample a variety

of substrates from these environments to determine if this species prefers a particular substrate. Caution should be taken during summer months to limit drywall exposure at building sites to prevent the deposition of *S. chartarum* spores that could germinate if there is a future water intrusion in the completed structure.

Figure 3-1. Study sites in Gainesville, Florida. A) Hardwood forest. B) Citrus grove. C) Lakeside. D) Pine grove.

Figure 3-2. Trap design.

A

B

Figure 3-3. Trap placement in the field. A) Citrus grove. B) Lakeside.

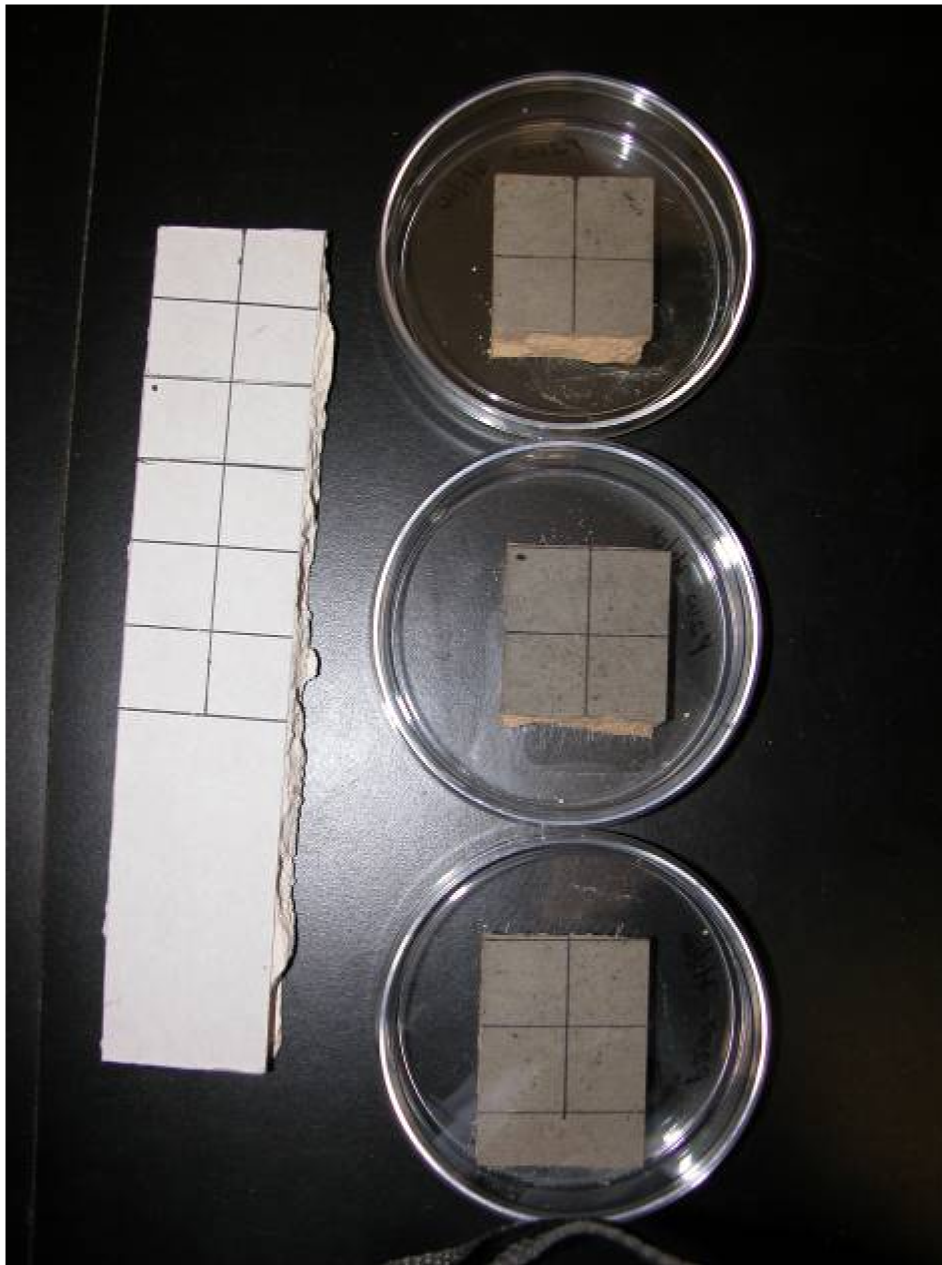

Figure 3-4. Sampling technique.

Table 3-1. Frequency of recovery of *Stachybotrys chartarum* at four sites, 2005.

Month[a]	Hardwood	Citrus	Lakeside	Pine
June	-[b]	+	-	+
July	-	+	+	+
August	-	+	-	-
September	-[c]	+	+	-

[a] Traps in field June 1, 2005 – December 17, 2005. No *S. chartarum* was recovered during months not shown in table.
[b] + = present; - = absent.
[c] *Stachybotrys chartarum* occurred in the Hardwood forest on one piece in September; however, the colony was located below the grid.

Table 3-2. Frequency of recovery of *Stachybotrys chartarum* at four sites, 2006.

Month[a]	Hardwood	Citrus	Lakeside	Pine
May	-[b]	+	-	-
July	+	+	-	+
August	+	+	-	-
Sept - Oct	+	+	-	-
December	+	-	-	-

[a] Traps in field December 20, 2005 – January 6, 2007. No *S. chartarum* was recovered during months not shown in table.
[b] + = present; - = absent.

Table 3-3. Abundance of *Stachybotrys chartarum* at four sites, 2005.

Month	Hardwood	Citrus	Lakeside	Pine
June	0.00[a]	0.13	0.00	0.05
July	0.00	0.25	0.85	0.70
August	0.00	0.45	0.00	0.00
September	0.00	0.90	0.45	0.00

[a] Data are means of 20 observations (5 replicates pooled across 4 directions). Data are mean numbers of cells in sampling grid. Max value = 12 cells per sample with fungus present. There were no significant differences ($p \leq 0.10$) among locations in any month.

Table 3-4. Abundance of *Stachybotrys chartarum* at four sites, 2006.

Month	Hardwood	Citrus	Lakeside	Pine
May	0.00[a]	0.10	0.00	0.00
June	0.00	0.00	0.00	0.00
July	0.30	0.13	0.00	0.05
August	0.40	0.40	0.00	0.00
Sept-Oct	2.06a	0.90ab	0.00b	0.00b
December	0.10a	0.00b	0.00b	0.00b

[a] Data are means of 20 observations (5 replicates pooled across 4 directions). Data are mean numbers of cells in sampling grid. Max value = 12 cells per sample with fungus present. Means in rows followed by the same letter do not differ ($p \leq 0.10$) according to Waller-Duncan k-ratio t-test. No letters in row indicate no differences at $p \leq 0.10$.

Table 3-5. Colony size determined as average number of sampled drywall cells with *Stachybotrys chartarum* growth.

Date	Location	Number of cells[a]
June 2005	Citrus	1.0
June 2005	Pine	1.0
July 2005	Citrus	2.0
July 2005	Lakeside	5.7
July 2005	Pine	7.0
August 2005	Citrus	9.0
September 2005	Citrus	6.0
September 2005	Lakeside	4.5
May 2006	Citrus	2.0
July 2006	Hardwood	6.0
July 2006	Citrus	2.0
July 2006	Pine	1.0
August 2006	Hardwood	8.0
August 2006	Citrus	4.0
Sept Oct 2006	Hardwood	9.3
Sept Oct 2006	Citrus	4.3
December 2006	Hardwood	1.0

[a] Means represent the total number of cells with *S. chartarum* growth divided by the total number of infested drywall pieces at each site on each sampling date.

Table 3-6. Weather data June 2005 to May 2007.

Date	Average daily temp	Low temp	High temp	Rainfall	% Relative humidity
June 2005	25.2	21.0	31.4	15.8	80.7
July 2005	26.2	21.6	32.4	32.7	79.9
August 2005	26.5	22.2	33.7	11.7	82.1
September 2005	26.0	21.3	33.0	11.7	79.5
Oct Nov 2005	17.3	10.3	25.7	3.3	76.7
Nov Dec 2005	15.4	8.4	23.4	26.6	76.7
January 2006	12.8	5.2	21.0	9.6	74.2
February 2006	12.0	4.2	20.2	35.1	72.4
March 2006	16.6	8.1	25.1	19.8	67.7
April 2006	17.8	9.8	26.1	24.1	69.3
May 2006	23.6	16.2	31.6	5.2	68.6
June 2006	25.2	18.5	32.7	14.5	73.3
July 2006	25.8	19.3	33.5	20.2	73.8
August 2006	26.9	21.2	34.1	7.4	75.3
Sept Oct 2006	24.7	18.2	32.3	14.2	74.1
November 2006	14.7	7.9	22.7	2.3	74.9
December 2006	15.3	8.8	22.8	16.5	77.1
Jan Feb 2007	10.9	3.5	18.6	6.5	67.0
March 2007	12.9	4.9	21.1	10.5	66.0
April 2007	18.4	9.4	27.1	3.2	60.6
May 2007	20.1	11.5	28.6	8.9	63.0

Note: Weather data were generated at the Alachua, Florida weather station (http://fawn.ifas.ufl.edu/).

Table 3-7. Frequency of recovery of *Stachybotrys chlorohalonata*, June 2005 to May 2007, at four sites.

Date[a]	Hardwood	Citrus	Lakeside	Pine
September 2005	-[b]	-[c]	-	-
Nov-Dec 2005	-	+	-	-
July 2006	-	-	+	-
August 2006	-[c]	-	-	-
Sept-Oct 2006	+	-	-	-
May 2007	-	+	-	-

[a] Traps in field June 1, 2005 – June 1, 2007. No *S. chlorohalonata* was recovered from the grid during months not shown in table.
[b] + = present; - = absent.
[c] *S. chlorohalonata* occurred twice off-grid, September 2005 at the citrus site and August 2006 at the hardwood site.

Table 3-8. Frequency of recovery of *Stachybotrys bisbyi*, June 2005 to May 2007, at four sites.

Date[a]	Hardwood	Citrus	Lakeside	Pine
June 2005	-[b]	+	-	-
July 2005	-	-	+	+
August 2005	-	-	+	+
September 2005	-	-	+	-
June 2006	-	-	+	-
July 2006	+	-	+	-
August 2006	-	-	+	-
Sept-Oct 2006	-	-	+	-
November 2006	-	-	+	-
December 2006	-	-	+	-

[a] Traps in field June 1, 2005 – June 1, 2007. No *S. bisbyi* was recovered during months not shown in table.
[b] + = present; - = absent.

Table 3-9. Abundance of *Stachybotrys bisbyi*, June 2005 to May 2007, at four sites.

Date	Hardwood	Citrus	Lakeside	Pine
June 2005	0.00[a]	0.10	0.00	0.00
July 2005	0.00	0.00	0.30	0.45
August 2005	0.00	0.00	0.60	0.15
September 2005	0.00b	0.00b	0.40a	0.00b
June 2006	0.00b	0.00b	0.40a	0.00b
July 2006	0.30ab	0.00b	0.55a	0.00b
August 2006	0.00b	0.00b	1.00a	0.00b
Sept-Oct 2006	0.00	0.00	0.15	0.00
November 2006	0.00	0.00	0.40	0.00
December 2006	0.00	0.00	0.20	0.00

[a] Data are means of 20 observations (5 replicates pooled across 4 directions). Data are mean numbers of cells in sampling grid. Max value = 12 cells per sample with fungus present. Means in rows followed by the different letters differ significantly ($p \leq 0.10$) according to Waller-Duncan k-ratio t-test. No letters in row indicate no differences at $p \leq 0.10$.

Table 3-10. Frequency of recovery of *Stachybotrys kampalensis*, June 2005 to May 2007, at four sites.

Date[a]	Hardwood	Citrus	Lakeside	Pine
September 2005	-[b]	-	+	-
June 2006	+	-	-	-
July 2006	+	-	+	-
August 2006	-	-	+	-
Sept-Oct 2006	-	-	+	-
December 2006	-	-	+	-

[a] Traps in field June 1, 2005 – June 1, 2007. No *S. kampalensis* was recovered during months not shown in table.
[b] + = present; - = absent

Table 3-11. Abundance of *Stachybotrys kampalensis*, June 2005 to May 2007, at four sites.

Date	Hardwood	Citrus	Lakeside	Pine
September 2005	0.00b[a]	0.00b	0.75a	0.00b
June 2006	0.25	0.00	0.00	0.00
July 2006	0.15	0.00	0.35	0.00
August 2006	0.00b	0.00b	0.20a	0.00b
Sept-Oct 2006	0.00	0.00	0.10	0.00
December 2006	0.00	0.00	0.10	0.00

[a] Data are means of 20 observations (5 replicates pooled across 4 directions). Data are mean numbers of cells in sampling grid. Max value = 12 cells per sample with fungus present. Means in rows followed by the different letters differ significantly ($p \leq 0.05$) according to Waller-Duncan k-ratio t-test. No letters in row indicate no differences at $p \leq 0.10$.

Table 3-12. Abundance of *Cladosporium* spp., June 2005 to May 2007, at four sites.

Date	Hardwood	Citrus	Lakeside	Pine	p-value
June 2005	7.45b[a]	9.69a	6.05b	9.95a	<0.0001
July 2005	4.90b	8.88a	4.60b	8.65a	<0.0001
August 2005	0.65b	1.50b	1.75b	6.65a	<0.0001
September 2005	0.45c	1.90b	1.30bc	4.70a	<0.0001
Oct-Nov 2005	7.55ab	8.95a	8.50a	6.25b	0.03
Nov-Dec 2005	8.90b	6.45c	9.75b	11.95a	<0.0001
January 2006	9.75a	9.80a	10.15a	1.95b	<0.0001
February 2006	10.95a	9.40b	11.10a	11.80a	<0.0001
March 2006	10.85a	9.95a	11.40a	10.55a	0.25
April 2006	11.58a	11.50a	12.00a	11.70a	0.13
May 2006	7.80b	9.90a	11.20a	10.15a	0.0008
June 2006	11.40a	11.70a	11.55a	11.60a	0.88
July 2006	10.40b	12.00a	11.85a	12.00a	<0.0001
August 2006	8.65b	6.90c	8.15bc	11.15a	0.0002
Sept-Oct 2006	5.94c	6.50bc	7.85b	10.30a	<0.0001
November 2006	12.00a	12.00a	11.95a	12.00a	0.40
December 2006	11.70a	11.90a	11.80a	11.60a	0.32
Jan-Feb 2007	10.00a	9.85a	10.00a	4.55b	<0.0001
March 2007	10.75a	10.60a	10.90a	10.10a	0.11
April 2007	9.80b	11.40a	5.65c	0.40d	<0.0001
May 2007	10.85b	11.15b	11.80a	12.00a	<0.0001

[a] Data are means of 20 observations (5 replicates pooled across 4 directions). Data are mean numbers of cells in sampling grid. Max value = 12 cells per sample with fungus present. Means in rows followed by the different letters differ significantly ($p \leq 0.10$) according to Waller-Duncan k-ratio t-test.

Table 3-13. Abundance of *Alternaria* spp., June 2005 to May 2007, at four sites.

Date	Hardwood	Citrus	Lakeside	Pine	p-value
June 2005	4.60a[a]	5.94a	4.30a	5.70a	0.58
July 2005	4.35a	4.50a	3.90a	4.40a	0.96
August 2005	0.05b	0.90ab	0.35b	1.60a	0.0048
September 2005	0.40a	0.65a	0.15a	0.20a	0.39
Oct-Nov 2005	0.05a	0.05a	0.00a	0.00a	0.59
Nov-Dec 2005	0.15b	0.00b	0.00b	0.75a	0.020
January 2006	0.00b	0.50a	0.05b	0.00b	0.0013
February 2006	0.10b	1.40a	0.00b	0.00b	<0.0001
March 2006	0.45b	0.55b	0.60b	2.05a	0.030
April 2006	0.74a	0.65ab	1.10a	0.05b	0.016
May 2006	0.05a	0.85a	0.55a	0.55a	0.33
June 2006	3.65a	2.10b	2.50b	0.20c	<0.0001
July 2006	4.45a	0.19c	0.40bc	1.10b	<0.0001
August 2006	1.05a	0.70a	0.90a	0.25a	0.21
Sept-Oct 2006	2.33a	0.00b	0.20b	0.00b	<0.0001
November 2006	7.40ab	8.85a	5.65b	9.00a	0.058
December 2006	6.65a	6.00a	5.60a	5.40a	0.85
Jan-Feb 2007	0.10a	0.00a	0.15a	0.00a	0.24
March 2007	0.25a	0.45a	0.60a	0.00a	0.14
April 2007	0.00b	0.80a	0.05b	0.00b	<0.0001
May 2007	0.75a	0.60a	0.50a	0.35a	0.80

[a] Data are means of 20 observations (5 replicates pooled across 4 directions). Data are mean numbers of cells in sampling grid. Max value = 12 cells per sample with fungus present. Means in rows followed by the different letters differ significantly ($p \leq 0.10$) according to Waller-Duncan k-ratio t-test.

Table 3-14. Abundance of *Epicoccum* spp., June 2005 to May 2007, at four sites.

Date	Hardwood	Citrus	Lakeside	Pine	p-value
June 2005	0.60b[a]	3.63a	0.85b	3.75a	<0.001
July 2005	0.05b	1.88a	1.05a	1.20a	0.0011
August 2005	0.00a	0.00a	0.050a	0.00a	0.40
September 2005	0.00	0.00	0.00	0.00	N/A
Oct-Nov 2005	0.00a	0.05a	0.00a	0.00a	0.40
Nov-Dec 2005	0.55a	0.050b	0.00b	0.50a	0.012
January 2006	0.050b	1.60a	0.050b	0.00b	<0.0001
February 2006	1.80a	0.45b	0.85b	0.85b	0.017
March 2006	0.15b	1.35b	1.40b	2.95a	0.0013
April 2006	1.58ab	1.75a	0.70bc	0.00c	0.0006
May 2006	0.10	0.75	0.80	0.00	0.089
June 2006	1.40ab	2.60a	0.95bc	0.05c	0.0024
July 2006	1.60a	1.38a	0.65a	0.70a	0.13
August 2006	0.00	0.00	0.00	0.00	N/A
Sept-Oct 2006	0.00	0.00	0.00	0.00	N/A
November 2006	3.00a	3.60a	3.40a	2.80a	0.73
December 2006	2.95b	1.80c	1.80c	4.75a	<0.0001
Jan-Feb 2007	0.15a	0.00a	0.50a	0.00a	0.1031
March 2007	1.05a	1.05a	0.55ab	0.00b	0.0040
April 2007	0.00b	3.20a	0.050b	0.00b	<0.0001
May 2007	3.50a	1.05c	2.15b	0.05c	<0.0001

[a] Data are means of 20 observations (5 replicates pooled across 4 directions). Data are mean numbers of cells in sampling grid. Max value = 12 cells per sample with fungus present. Means in rows followed by the different letters differ significantly ($p \leq 0.10$) according to Waller-Duncan k-ratio t-test.

Table 3-15. Abundance of *Bipolaris*, *Drechslera, Curvularia* spp.complex, June 2005 to May 2007, at four sites.

Date	Hardwood	Citrus	Lakeside	Pine	p-value
June 2005	2.20b[a]	4.69a	4.25a	5.05a	0.014
July 2005	1.30a	0.50a	1.30a	1.15a	0.40
August 2005	2.50b	6.65a	3.25b	8.00a	<0.0001
September 2005	2.15b	3.35a	1.60b	1.20b	0.0032
Oct-Nov 2005	0.20b	0.85a	0.00b	0.00b	0.0006
Nov-Dec 2005	1.85b	0.75c	1.15bc	3.35a	<0.0001
January 2006	0.00b	0.55a	0.00b	0.00b	0.0014
February 2006	0.00b	1.25a	0.00b	0.00b	<0.0001
March 2006	0.25a	0.35a	0.50a	0.65a	0.63
April 2006	0.95a	0.40bc	0.45b	0.00c	0.006
May 2006	0.00a	0.050a	0.25a	0.00a	0.29
June 2006	3.85a	1.85b	3.20a	0.00c	<0.0001
July 2006	2.30a	0.13b	0.85b	2.45a	<0.0001
August 2006	6.30ab	5.30b	6.80a	2.00c	<0.0001
Sept-Oct 2006	5.28a	1.80b	4.30a	0.50c	<0.0001
November 2006	6.00a	5.80a	6.75a	5.05a	0.31
December 2006	7.60b	8.25b	7.75b	9.70a	0.0063
Jan-Feb 2007	0.15a	0.00a	0.00a	0.00a	0.15
March 2007	0.20b	0.70a	0.10b	0.00b	0.0064
April 2007	0.00b	0.25a	0.00b	0.00b	0.0096
May 2007	0.20a	0.15a	0.30a	0.00a	0.29

[a] Data are means of 20 observations (5 replicates pooled across 4 directions). Data are mean numbers of cells in sampling grid. Max value = 12 cells per sample with fungus present. Means in rows followed by the different letters differ significantly ($p \leq 0.10$) according to Waller-Duncan k-ratio t-test.

CHAPTER 4
IDENTIFICATION OF PUTATIVE *Stachybotrys chartarum* ISOLATES FROM NORTH-CENTRAL FLORIDA HABITATS

Introduction

In the 1930s, Ukrainian horses faced a microscopic nemesis that threatened their usefulness in the Soviet cavalry. They were fed contaminated hay and fell ill; some eventually died. Russian scientists identified the causal agent as a black mold, *Stachybotrys chartarum* (Ehrenberg ex Link) Hughes, and they named the disease "stachybotryotoxicosis" (Hintikka 1978b). Sixty years later, doctors in Cleveland, Ohio questioned if an unexplained surge in infant deaths caused by bleeding of the lungs was due to the inhalation of toxic spores of the same fungus (Dearborn et al. 1999, Etzel et al. 1998). Although a connection was not conclusively proven, the health consequences of exposure to *S. chartarum* remain of great interest to physicians and the public alike.

An efficient cellulolytic degrader, this fungus is found indoors on building materials such as drywall, a major component of modern structures. Requiring high humidity for growth and sporulation, *S. chartarum* is particularly problematic in water-damaged structures. With the current public interest in indoor air pollution and "toxic mold", combined with the active 2004 and 2005 hurricane seasons in the United States, *S. chartarum* has remained at the forefront of mycological research (Kuhn et al. 2005, Li and Yang 2005, Koster 2006, Tucker et al. 2007).

Stachybotrys chartarum is known to produce several classes of mycotoxins, including trichothecenes, spirocyclic drimanes, hemolysin, and atranones (Jarvis et al. 1995, Jarvis 2003, Vesper et al. 2001). Trichothecenes are a class of sesquiterpenes that inhibit protein synthesis and include the well-known T-2 toxin produced by *Fusarium* spp. As a group, they are considered to be among the most important and acutely toxic of known mycotoxins (Jarvis 2003). In addition to many simple trichothecenes, *S. chartarum* produces macrocyclic

trichothecenes, including satratoxin G which Yang et al. (2000) reports to be more toxic to mammalian cells than T-2 toxin. In contrast, atranones have not been shown to possess significant biological activity to humans (Jarvis 2003).

As researchers worked to identify and characterize these *S. chartarum* metabolites, they repeatedly found variation in levels of toxin production among *S. chartarum* isolates (Jarvis et al. 1998, Ruotsalainen et al. 1998, Vesper et al. 1999, Elanskii et al. 2004). Randomly amplified polymorphic DNA (RAPD) analysis also showed variation among isolates (Vesper et al. 1999, Peltola et al. 2002) as did phylogenetic analysis of protein-coding genes (Andersen et al. 2002, Cruse et al. 2002, Andersen et al. 2003, Koster et al. 2003). Ultimately, the morphological species *S. chartarum* was recognized as three phylogenetic taxa (*Stachybotrys chlorohalonata* Andersen & Thrane, *S. chartarum* chemotype S and *S. chartarum* chemotype A) based on a combination of morphological, chemical, and molecular characteristics (Andersen et al. 2003).

Stachybotrys chlorohalonata is similar to *S. chartarum* morphologically. Conidiophores and phialids are similar in appearance, but *S. chlorohalonta* conidiophores and phialids are shorter, and *S. chlorhalonata* produces smooth spores while *S. chartarum* spores are rough (Andersen et al. 2003). In addition, *S. chlorohalonata* is named for the unique, green extracellular pigment it produces on Czapek Yeast Agar. *Stachybotrys chlorohalonata* isolates produce atranones and dolabellanes; they do not produce macrocyclic tricothecenes such as satratoxins or roridins (Andersen et al. 2002, 2003).

Stachybotrys chartarum chemotypes S and A are identical morphologically, but each produces a different set of toxins (Andersen et al. 2002, 2003). *Stachybotrys chartarum* chemotype S produces macrocyclic trichothecenes but no atranones, while *S. chartarum* chemotype A produces atranones but no macrocyclic trichothecenes (as does *S. chlorohalonata*).

These fungi must be identified based on differences in their toxological and/or molecular characteristics. It is crucial to differentiate between the two chemotypes because only *S. chartarum* chemotype S produces the highly-toxic macrocyclic trichothecenes. Genes that are currently known to distinguish between the two chemotypes are chitin synthase 1 and trichodiene synthase 5 (Cruse et al. 2002, Andersen et al. 2003). To date, neither *S. chartarum* chemotype A nor *S. chartarum* chemotype S has been conclusively identified as *S. chartarum sensu stricto*, nor has a new species been erected to accommodate the second chemotype.

The majority of the isolates (~90%) used in past phylogenetic studies were collected indoors (Andersen et al. 2002, 2003, Cruse et al. 2002, Koster et al. 2003). All of these studies included geographically diverse populations of *S. chartarum* chemotype A, *S. chartarum* chemotype S, and *S. chlorohalonata*. No study appears to have included multiple isolates from one location collected over a period of time. Andersen et al. (2002, 2003) and Koster et al. (2003) did not include any Florida isolates of *S. chartarum sensu lato* in their studies. Cruse et al. (2002) included a single isolate from Florida among the 30 isolates in his study, and this isolate was determined to belong to the new species, *S. chlorohalonata*.

This is the first study to use outdoor samples exclusively and the first to include multiple isolates from Florida, a state with environmental conditions that are conducive to mold growth. Most importantly, since the two chemotypes have different biochemical profiles, this study will also provide important toxicological data regarding the outdoor populations of *S. chartarum* in north central Florida. It is important to know which chemotype of *S. chartarum* is most prevalent in nature for reasons that range from public health to planning of building projects.

Materials and Methods

Preparation of Fungal Isolates

Forty-three putative environmental samples of *S. chartarum* were isolated from drywall that had been placed outdoors in Gainesville, Florida, USA (Chapter 3). Drywall traps were placed in four habitats, a managed citrus grove, a pine grove, a weedy lakeshore and a mature hardwood forest, for a period of one to two months. One sample, isolate # 52, was isolated from drywall from a Gainesville, Florida apartment with a history of water leaks, for a total of 44 Florida isolates (Table 4-1). Six isolates used in previous studies were obtained from the IBT culture collection at BioCentrum-DTU, Denmark: *S. chlorohalonata* IBT 9825 and IBT 40292, *S. chartarum* chemtotype A IBT 9290 and IBT 14915, and *S. chartarum* chemotype S IBT 7711 and IBT 40293 (Andersen et al. 2003).

Florida samples were tentatively identified as *S. chartarum* at 400x and 1000x based on morphological features as described by Jong and Davis (1976). Pure cultures were single-spored and maintained on Difco^{TM} Potato Dextrose Agar plates (Becton, Dickinson and Co., Sparks, MD). Mycelium for DNA extraction was grown at 22°C in 50 mL of yeast extract broth (20 g glucose, 10 g yeast extract, 2 g peptone per 1 liter water) as described by Cruse et al. (2002). After three days, mycelium was collected by vacuum filtration, frozen in liquid nitrogen and lyophilized. In addition, all Florida isolates were inoculated on Czapek Yeast Autolysate Agar (CYA, Samson et al. 2002) and incubated in the dark for seven days at 24°C at which point they were evaluated for pigment production. Proxy isolates were deposited at the University of Florida Mycological Herbarium.

DNA Extraction, PCR Amplification, and Sequencing

Lyophilized mycelium was manually disrupted using a metal spatula. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, California) and stored at -20°C.

Primer sequences for the trichodiene synthase 5 (*tri5*) and chitin synthase 1 (*chs1*) gene fragments were obtained from Cruse et al. (2002). The *tri5* 5' primer was 5'-CATCAATCCAACAGTTTCAC-3'. The *tri5* 3' primer was 5'-GCAACCTTCAAAGACTATTG-3'. The *chs1* 5' primer was 5'-ATCTCACCACAAGCACCGCCACACA-3'. The *chs1* 3' primer was 5'-GGAAGAAGATCGTTGTGTGCGTGGT-3'. The loci were amplified in 50 µL polymerase chain reactions (PCR) in a PTC-200 Peltier Thermal cycler (Bio-Rad Laboratories, Inc., Hercules, California) using Invitrogen reagents (Carlsbad, CA, USA). Each reaction contained 5 µL of 10x Taq buffer, 1.5 µL of 50 mM $MgCl_2$, 1 µL of 10 mM dNTPs, 1 µL each of 10 mM forward and reverse primers, 0.25 µL of Taq polymerase and 2 µL of template DNA. PCR conditions for *tri5* amplification were as follows: 94°C for 4 min, 35 cycles at 94°C for 1 min, 51°C for 1 min and 72°C for 1 min, with a final extension of 72°C for 4 min (Koster et al. 2003). PCR conditions for *chs1* amplification were identical with the exception of an annealing temperature of 61°C. PCR products were visualized on a 1% agarose gel before being submitted for sequencing.

Sequencing of the DNA sample was done at the University of Florida DNA Sequencing Core Laboratory using ABI Prism BigDye Terminator cycle sequencing protocols (part number 4303153) developed by Applied Biosystems (Perkin-Elmer Corp., Foster City, CA). The excess dye-labeled terminators were removed using MultoScreen® 96-well filtration system (Millipore, Bedford, MA). The purified extension products were dried in SpeedVac® (ThermoSavant, Holbrook, NY) and then suspended in Hi-di formamide. Sequencing reactions were performed using POP-7 sieving matrix on 50-cm capillaries in an ABI Prism® 3130 Genetic Analyzer

(Applied Biosystems, Foster City, CA) and were analyzed by ABI Sequencing Analysis software v. 5.2 and KB Basecaller.

Phylogenetic Analysis

Forward and reverse sequences obtained from the *tri5* and *chs1* primers were combined using Sequencher version 4.5 (Gene Codes Corporation, Ann Arbor, MI, USA), and only bases sequenced in both directions were included. The exceptions were isolate 27 for which only the *chs1* forward sequence was obtained and isolate 20 for which only a *chs1* reverse sequence was obtained. Two sequences from GenBank were included, *S. charatarum* AF468154 and *S. chartarum* AF 468155 (Cruse et al. 2002). Sequences were aligned in ClustalX version 1.83.1 using the default settings before being manually corrected in MacClade 4. The aligned sequences were exported as NEXUS files and analyzed using PAUP version 4.0b10. Phylogenetic trees for each locus were constructed with neighbor-joining (Saitou and Nei 1987) using default settings. Ties, if encountered, were broken randomly. Distance bootstrap values were determined using neighbor-joining with 100,000 replications.

Results

Of the 44 Florida isolates, six produced a dark green extracellular pigment on CYA, and eight other isolates produced an orange pigment. The remaining 30 isolates produced no pigment. (Figure 4-1, Table 4-2). Eight of the 11 isolates (73%) that were identified through sequencing as *S. chartarum* chemotype S produced orange pigment; three produced no pigment. None of the *S. chartarum* chemotype A isolates produced extracellular pigment on CYA.

The *tri 5* sequence is 485 base pairs with 29 informative characters, and the *chs 1* sequence is 297 base pairs with 12 informative characters. Sequence data have been submitted to Genbank (Table 4-3, Table 4-4); aligments are reported in Appendix B. Both the *tri5* and the *chs1* neighbor-joining trees separate the majority of the isolates into three clades that correspond with

the taxa/chemotypes *S. chlorohalonata*, *S. chartarum* chemotype A, and *S. chartarum* chemotype S (Figures 4-2 and 4-3). At both loci, there are multiple base pair differences between the *S. chlorohalonata* and the *S. chartarum* clades resulting in strong bootstrap support. In the case of the two *S. chartarum* clades, there is only a one nucleotide difference between the sequences at each locus that resulted in weaker bootstrap support. The difference in the *tri 5* sequence is at base pair 278 where *S. chartarum* chemotype A has a thymine and *S. chartarum* chemotype S has a cytosine. The difference in the *chs 1* sequence is at base pair 18 where *S. chartarum* chemotype A has a thymine and *S. chartarum* chemotype S has a guanine.

The two trees have similar topologies with the exception of isolate #1, which groups with the *S. chartarum* chemotype S clade in the *chs 1* tree and the *S. chartarum* chemotype A clade in the *tri 5* tree. This incongruence is due to a reversal of the nucleotide substitution at these loci. In addition the placement of isolate #41 is unresolved in the *chs 1* tree because it was not determined during sequencing if base pair 18 was a guanine or a thymine. In the *tri 5* tree, this isolate groups with the chemotype A clade. Two Florida isolates, isolates #12 and 46, as well as isolates ITB7711 and ITB40293 from Andersen et al. (2003), are unresolved in the *tri 5* tree and group as a sister clade to the two *S. chartarum* chemotypes. However, when the sequence data are examined, it is apparent that they should group with *S. chartarum* chemotype S clade because they all have a cytosine base at position 278. Their placement is unresolved because these isolates have a cytosine base at position 290 in common with the *S. chartarum* chemotype A clade, while the other *S. chartarum* chemotype S isolates have a thymine base at this position. The *tri 5* tree also shows the *S. chartarum* chemotype A clade and the *S. chlorohalonata* clade as each containing sister clades. These additional clades are also due to a one nucleotide substitution, at base pair position 480 and 116, respectively.

The one indoor isolate was identified as *S. chartarum* chemotype A. Of the 43 outdoor isolates, 6 were *S. chlorohalonata* (14%), 11 were *S. chartarum* chemotype S (26%), and 26 were *S. chartarum* chemotype A (60%). If only the *S. chartarum* isolates were considered, 70% were chemotype A (26 of 37 isolates) and 30% were chemotype S (11 of 37 isolates). The chemotype A isolates were recovered from all four habitats over the course of two years. However, 8 of the 11 chemotype S isolates were recovered from the hardwood site, and all were recovered in either September or October 2006 from the same replicate (Table 4-1).

Discussion

Extracellular pigment production and molecular analyses of suspected isolates of *S. chartarum sensu lato* are necessary to differentiate among *S. chlorohalonata*, *S. chartarum* chemotype S, and *S. chartarum* chemotype A. This study confirms that *S. chlorohalonata* can be identified accurately by production of a dark green extracellular pigment on CYA (Andersen et al. 2003). All of the isolates that produced this pigment also fell into the *S. chlorohalonata* clade in the phylogenetic analysis. Researchers have remarked on the difficulty of distinguishing between *S. chlorohalonata* and *S. chartarum* isolates based on morphological characteristics alone (Li and Yang 2005, Koster 2006). In the present study, it was not possible to consistently identify isolates of *S. chlorohalonata* using oil immersion (1000x magnification), and pigment production and DNA sequencing were used for identification. Given the time and expense of preparing a sample for DNA sequencing and the reliability of the pigment test on CYA, it is recommended that all putative *S. chartarum* isolates be screened on CYA.

Seventy-three percent of the *S. chartarum* chemotype S isolates produced a yellow-orange pigment on CYA. This is a much higher percentage than observed by Andersen et al. (2003), who found only three of nine *S. chartarum* chemotype S isolates produced a yellow-orange pigment. However, these results still suggest that extracellular pigment production cannot be

used alone as a method to differentiate between *S. chartarum* chemotypes S and A. While an isolate that produces orange pigment on CYA can be identified confidently as *S. chartarum* chemotype S, lack of pigment would require further testing for identification purposes. It should also be noted that one pigment-producing isolate, isolate #1, was incongruent between the two loci in the phylogenetic analysis. Andersen et al. (2003) reported a similar situation from a non-pigment producing isolate and used metabolite production to confirm its identification. In this case, pigment production was able to confirm that isolate #1 was *S. chartarum* chemotype S. However, these examples illustrate the challenge of using sequence data with only a single nucleotide substitution, as do the cases of isolates #12, 41 and 46, all of which were unable to be placed into either *S. chartarum* clade in one of the two trees.

Generally speaking, sequence analysis of both gene fragments used in this study successfully differentiated between the two chemotypes. However, as a diagnostic tool, neither locus was 100% accurate, and final identification relied on information from both loci plus pigment production. Additional loci, hopefully with more than one nucleotide substitution between the two chemotypes, should be identified if sequencing is to be widely used. Other species-specific molecular primers for use in PCR assays have been designed for the purpose of identifying *S. chartarum* from indoor samples; however, none of these authors differentiated between *S. chlorohalonata* and the two *S. chartarum* chemotypes (Haugland and Heckman 1998, Haugland et al. 1999, Cruz-Perez et al. 2001, Dean et al. 2005). An alternative molecular technique is amplified fragment length polymorphism (AFLP) fingerprinting, which Koster (2006) successfully used to study intraspecific genetic variation among *S. chartarum* isolates. This technique could be developed into a diagnostic tool, as could measurement of metabolite production.

Previous studies that have used DNA sequencing to identify *S. chlorohalonata* and chemotypes of *S. chartarum* have looked primarily at indoor isolates from diverse geographic origins. Andersen et al. (2002) examined isolates of which the majority were collected from buildings in Europe and the United States; approximately 20% of the 122 isolates were identified as *S. chlorohalonata*, 49% as *S. chartarum* chemotype A, and 31% as *S. chartarum* chemotype S. Cruse et al. (2002) examined 30 isolates collected primarily in northern California; approximately 23% were *S. chlorohalonata*, 47% were *S. chartarum* chemotype S and 30% were *S. chartarum* chemotype A. While the exact distribution of isolates among the three taxa varied between the two studies, these studies as well as another (Koster 2006) found no correlation between genetic isolation and geographic distance.

In this study, the same four habitats were repeatedly sampled in order to accumulate a collection that accurately reflected the population distribution of the two chemotypes in natural settings in north central Florida. A previous study of isolates sampled within a restricted geographical region found genome-wide genetic variation (Koster 2006). Fourteen percent of the outdoor isolates in the present study were identified as *S. chlorohalonata*, 60% as *S. chartarum* chemotype A and 26% as *S. chartarum* chemotype S. It should be noted that 8 of the 11 *S. chartarum* chemotype S isolates were recovered from the hardwood site, and all were recovered in either Sept or Oct 2006 from the same trap. It is likely that a single source of inoculum was responsible for all of these isolates, in which case, the occurrence of *S. chartarum* chemotype S would be even less than suggested by the results of this study. Thus, while all three taxa are present in north central Florida habitats, this study recovered fewer macrocyclic trichothecene-producing isolates (*S. chartarum* chemotype S) than non-macrocyclic

trichothecene producers. It is unknown why *S. chartarum* chemotype A was the clade most commonly recovered.

Koster (2006) hypothesizes that outdoor sources of *S. chartarum* serve as reservoirs of inoculum for indoor contamination. If this is true, then the present study suggests that *S. chartarum* chemotype A would also be found more commonly indoors than *S. chartarum* chemotype S in north central Florida. This may have a positive implication for public health since *S. chartarum* chemotype A does not produce macrocyclic tricothecenes. Further research should compare the distribution of *S. chlorohalonata* and *S. chartarum* chemotypes in outdoor and indoor environments of close proximity.

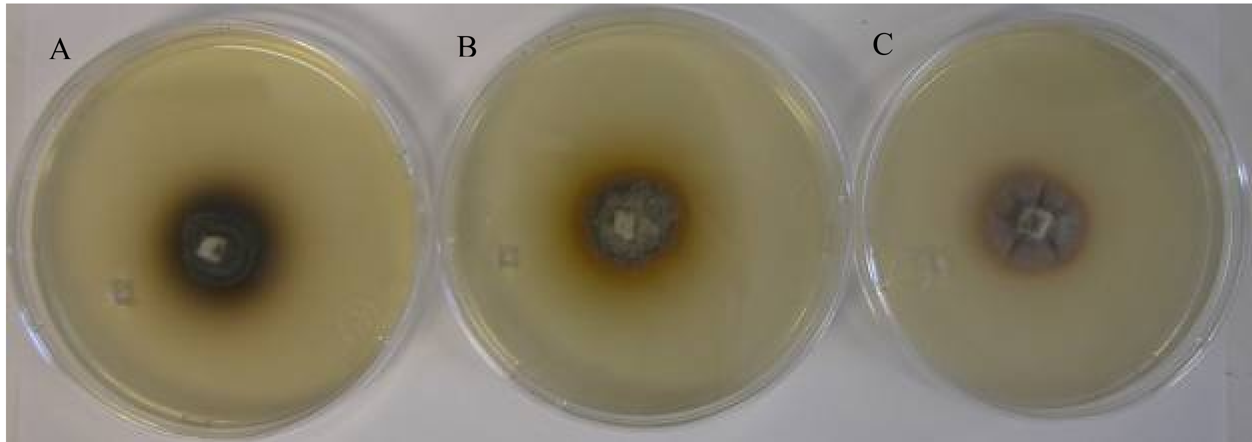

Figure 4-1. Extracellular pigmentation on Czapek yeast autolysate agar (CYA). A) *S. chlorohalonata* with green pigmentation. B) *S. chartarum* chemtoype S with orange pigmentation. C) *S. chartarum* chemotype A with no pigmentation.

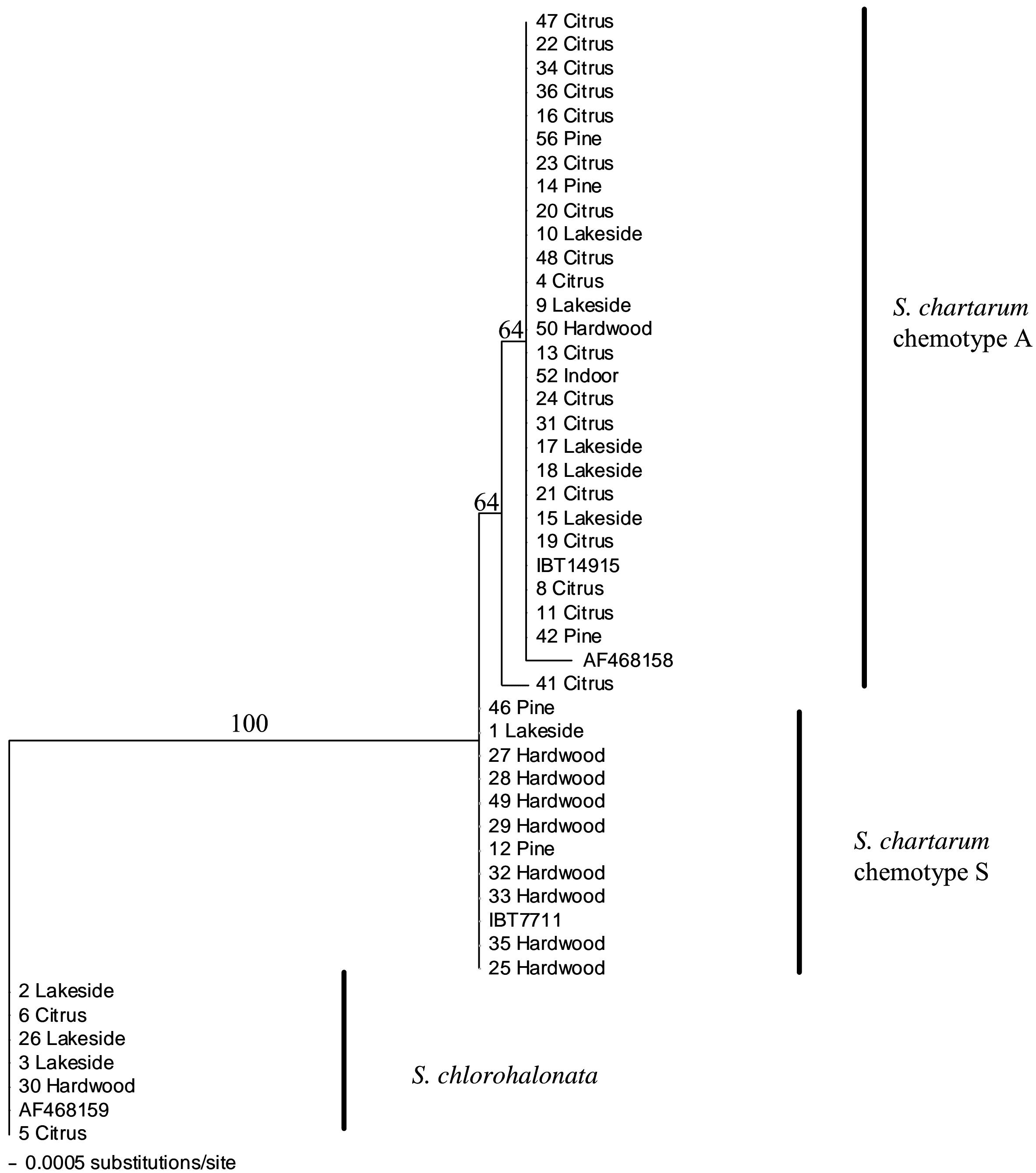

Figure 4-2. Neighbor-joining tree for *chs 1* gene for *Stachybotrys chartarum* and *S. chlorohalonata* outdoor isolates. Numbers above branches are bootstrap percentages. AF 468158 and AF 468159 are Genbank sequences published by Cruse et al. (2002). IBT 14915 and IBT 7711 are isolates from Andersen et al. (2003).

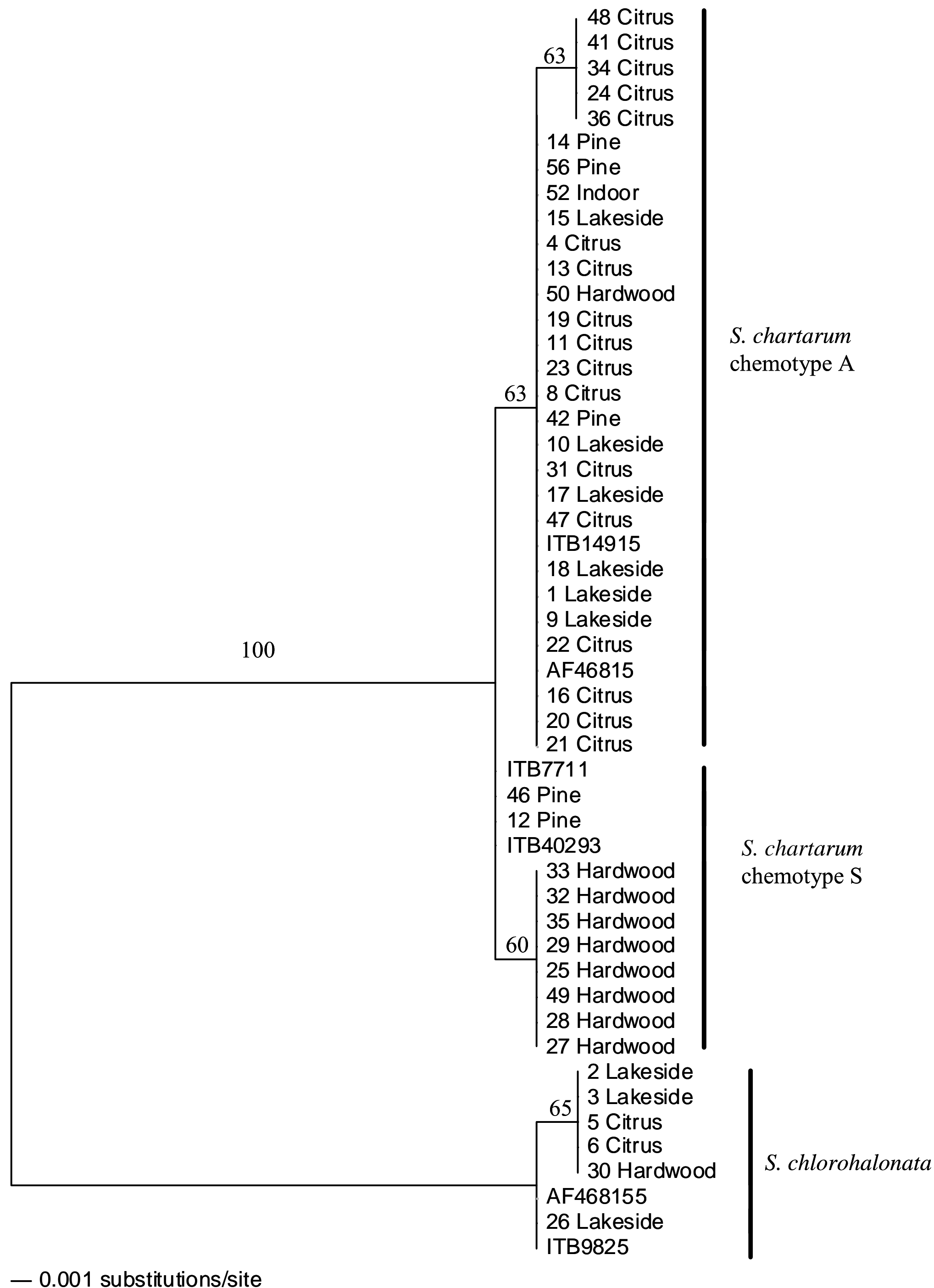

Figure 4-3. Neighbor-joining tree for *tri 5* gene for *Stachybotrys chartarum* and *S. chlorohalonata* outdoor isolates. Numbers above branches are bootstrap percentages. AF 468158 and AF 468159 are Genbank sequences published by Cruse et al. (2002). IBT 14915, IBT 40293, IBT 7711, and IBT 9825 are isolates from Andersen et al. (2003).

Table 4-1. Isolate number, species, origin and collection date of 44 *Stachybotrys* isolates from outdoor habitats in north central Florida.

Isolate	Species	Site	Collection	Isolate	Species	Site	Collection
1	S[a]	lakeshore	Jul 04	24	A	citrus	Jun 06
2	Cl	lakeshore	Jun 05	25	S	hardwood	Aug 06
3	Cl	lakeshore	Jun 05	26	Cl	lakeshore	Aug 06
4	A	citrus	Aug 05	27	S	hardwood	Oct 06
5	Cl	citrus	Oct 05	28	S	hardwood	Oct 06
6	Cl	citrus	Dec 05	29	S	hardwood	Oct 06
8	A	citrus	Jul 05	30	Cl	hardwood	Oct 06
9	A	lakeshore	Aug 05	31	A	citrus	Sep 06
10	A	lakeshore	Oct 05	32	S	hardwood	Sep 06
11	A	citrus	Aug 05	33	S	hardwood	Sep 06
12	S	pine	Jul 05	34	A	citrus	Sep 06
13	A	citrus	Oct 05	35	S	hardwood	Oct 06
14	A	pine	Aug 05	36	A	citrus	Oct 06
15	A	lakeshore	Aug 05	41	A	citrus	Aug 06
16	A	citrus	Oct 05	42	A	pine	Aug 05
17	A	lakeshore	Oct 05	46	S	pine	Jul 04
18	A	lakeshore	Oct 05	47	A	citrus	Oct 06
19	A	citrus	Oct 05	48	A	citrus	Oct 06
20	A	citrus	Aug 05	49	S	hardwood	Oct 06
21	A	citrus	Sep 05	50	A	hardwood	Oct 05
22	A	citrus	Sep 05	52	A	indoor	2004
23	A	citrus	Oct 05	56	A	pine	Aug 06

[a] Cl = *S. chlorohalonata*, A = *S. chartarum* chemotype A, S = *S. chartarum* chemotype S.

Table 4-2. Comparison of pigmentation and *tri5* and *chs1* sequence data for *Stachybotrys chartarum* and *S. chlorohalonata* isolates from outdoor habitats in north central Florida.

Isolate	Pigment	Clade	Isolate	Pigment	Clade
1	orange	S[a]	24	none	A
2	green	Cl	25	orange	S
3	green	Cl	26	green	Cl
4	none	A	27	orange	S
5	green	Cl	28	orange	S
6	green	Cl	29	orange	S
8	none	A	30	green	Cl
9	none	A	31	none	A
10	none	A	32	orange	S
11	none	A	33	orange	S
12	none	S	34	none	A
13	none	A	35	orange	S
14	none	A	36	none	A
15	none	A	41	none	A
16	none	A	42	none	A
17	none	A	46	none	S
18	none	A	47	none	A
19	none	A	48	none	A
20	none	A	49	none	S
21	none	A	50	none	A
22	none	A	52	none	A
23	none	A	56	none	A

[a] Cl = *S. chlorohalonata*, A = *S. chartarum* chemotype A, S = *S. chartarum* chemotype S.

Table 4-3. Genbank accession numbers for *chs1* and *tri5* nucleotide sequences for *Stachybotrys chartarum*.

Isolate	*chs1*	*tri5*
1	EU288762	EU288803
4	EU288766	EU288813
8	EU288772	EU288815
9	EU288778	EU288817
10	EU288788	EU288822
11	EU288770	EU288805
12	EU288783	EU288834
13	EU288784	EU288799
14	EU288791	EU288806
15	EU288789	EU288814
16	EU288790	EU288819
17	EU288792	EU288801
18	EU288773	EU288816
19	EU288774	EU288804
20	EU288771	EU288820
21	EU288785	EU288807
22	EU288776	EU288800
23	EU288782	EU288802
24	EU288786	EU288808
25	EU288779	EU288826
27	EU288761	EU288828
28	EU288764	EU288830
29	EU288796	EU288833
31	EU288780	EU288823
32	EU288793	EU288831
33	EU288798	EU288829
34	EU288794	EU288811
35	EU288765	EU288832
36	EU288781	EU288809
41	EU288777	EU288812
42	EU288769	EU288818
46	EU288763	EU288836
47	EU288768	EU288825
48	EU288795	EU288810
49	EU288797	EU288827
50	EU288775	EU288824
52	EU288787	EU288835
56	EU288767	EU288821

Table 4-4. Genbank accession numbers for *chs1* and *tri5* nucleotide sequences for *Stachybotrys chlorohalonata*.

Isolate	*chs1*	*tri5*
2	EU288837	EU288843
3	EU288838	EU288844
5	EU288839	EU288846
6	EU288842	EU288847
26	EU288840	EU288848
30	EU288841	EU288845

CHAPTER 5
DISCUSSION

Stachybotrys chartarum is a mycotoxin-producing, cosmopolian fungus that occurs on a variety of natural substrates as well as cellulose-based building materials such as drywall and ceiling tiles. This black mold has aroused public interest because it has been implicated in cases of sick building syndrome and pulmonary hemorrhage (Croft et al. 1986, Etzel et al. 1998, Dearborn et al. 1999). Because it is likely that outdoor populations serve as the source of inoculum for mold colonies in water-damaged structures (Koster 2006), it is critical to understand the types of environments that support natural populations of *S. chartarum*. The primary objective of this project was to identify outdoor habitats in north central Florida where *S. chartarum* is found and the times of year it is most abundant.

Initially, it was thought that air sampling would be an appropriate method despite the fact that *S. chartarum* is only rarely reported from outdoor air samples (Li and Kendrick 1995, Tiffany and Bader 2000, Bishop 2002). The development of a semi-selective medium was intended to overcome the difficulties that broad-spectrum media have isolating *S. chartarum* due to its slow growth in relation to common airborne fungi. However, the detection of *S. chartarum* from outdoor air in this study was a rare event if it was even isolated at all. It is quite possible that the infrequent occurrence of *S. chartarum* on plates in this study was actually due to mite contamination. Thus, it is likely that if *S. chartarum* is present in outdoor air, it is at such a low concentration that it falls below the detectable threshold of even semi-selective media. This study suggests that air sampling would not be an appropriate method for research investigating the occurrence of *S. chartarum* in outdoor habitats.

Based on the results of this part of the study, it was decided to investigate other means of measuring *S. chartarum* frequency and abundance outdoors. Since water-damaged drywall is a

common substrate for indoor *S. chartarum* contamination, traps were designed that incorporated this material and were placed in four habitats in Gainesville, Florida. Over the course of 24 months, *S. chartarum* was found growing at all four habitats. It was expected that *S. chartarum* would be the dominant fungus on the drywall, occurring frequently, in multiple and large colonies. Instead, it was found rarely, on only 0.02% of the pieces collected. In addition, it was expected that the abundance of *S. chartarum* would be more than what was observed. The frequency of *S. chartarum* was low and, therefore, most differences in abundance between sites were not significant. *Stachybotrys chartarum* was recovered more than once from each habitat, was recovered most frequently from the citrus grove, and was found only during the summer months at all sites. There was a correlation between *S. chartarum* occurrence and temperature but not with rainfall. A closely related species, *S. chlorohalonata,* was found even more rarely than *S. chartarum*. Ultimately, the results of this study suggest that although *S. chartarum* is less common among the outdoor air spora of north-central Florida than had been predicted, it occurs in a variety of natural and managed habitats, primarily during summer months.

The morphological species *S. chartarum* is recognized as three phylogenetically distinct taxa based on a combination of morphological, chemical and molecular characteristics: *Stachybotrys chlorohalonata* Andersen & Thrane, *S. chartarum* chemotype S and *S. chartarum* chemotype A (Andersen et al. 2003). This study was the first to use outdoor samples exclusively and the first to include multiple isolates from Florida, a state with environmental conditions that are conducive to mold growth. Most importantly, since the two *S. chartarum* chemotypes have different biochemical profiles, this study provides important toxicological data regarding the outdoor populations of *S. chartarum* in north central Florida. It is important to ascertain which chemotype of *S. chartarum* is most prevalent in nature for reasons that range from public health

to planning of building projects. Seventy percent of the outdoor isolates in this study were identified as *S. chartarum* chemotype A and 30% as chemotype S. It is hoped that the collection of Florida outdoor isolates compiled in this study will be useful to future researchers investigating the population genetics of *S. chartarum*.

Future work with *S. chartarum* should focus on the following two areas: 1) development of alternative sampling methods in order to expand our knowledge of the distribution and occurrence of *S. chartarum* in indoor and outdoor environments, and 2) further development of molecular markers for accurate identification of chemotypes A and S. Neither sampling method used here (selective media and drywall) recovered *S. chartarum* as often as previously was expected. Although the research presented here does elucidate some understanding of frequency and abundance of *S. chartarum*, it was difficult to discern differences between habitats because of the limited number of fungus recoveries. It is possible that bulk samples of soil and leaf litter plated out on the semi-selective media evaluated in Chapter 2 would be more appropriate than drywall traps which are, essentially, modified air samplers. In particular, this could determine if populations of *S. chartarum* truly decline in the winter months, or if that observation was an artifact due to lower moisture levels in the drywall traps.

The molecular techniques used in this study to distinguish between *S. chartarum* chemotype A and *S. charatrum* chemotype S were based on minor differences in the genomic DNA and were not infallible. If loci with more sequence differences are not found, an alternative method such as AFLP fingerprinting should be employed. A rapid and accurate diagnostic tool will be key to answering remaining questions about the distribution and occurrence of the two *S. chartarum* chemotypes.

APPENDIX A
LOCATION OF SITES AND SAMPLING DATES FOR FIELD STUDY

Table A-1. GPS location of study sites

Site	Latitude	Longitude
Citrus 1[a]	29°38’12” N	82°21’51” W
Citrus 2	29°38’12” N	82°21’51” W
Citrus 3	29°38’13” N	82°21’51” W
Citrus 4	29°38’13” N	82°21’51” W
Citrus 5	29°38’13” N	82°21’51” W
Hardwood 1	29°41’21” N	82°24’43” W
Hardwood 2	29°41’21” N	82°24’43” W
Hardwood 3	29°41’22” N	82°24’44” W
Hardwood 4	29°41’22” N	82°24’44” W
Hardwood 5	29°41’22” N	82°24’45” W
Lakeside 1	29°38’18” N	82°21’17” W
Lakeside 2	29°38’18” N	82°21’17” W
Lakeside 3	29°38’18” N	82°21’16” W
Lakeside 4	29°38’18” N	82°21’16” W
Lakeside 5	29°38’18” N	82°21’15” W
Pine 1	29°38’12” N	82°21’54” W
Pine 2	29°38’13” N	82°21’54” W
Pine 3	29°38’13” N	82°21’54” W
Pine 4	29°38’13” N	82°21’54” W
Pine 5	29°38’14” N	82°21’54” W

[a] Numbers 1 through 5 represent replicates.

Table A-2. Summary of sampling dates

Month	Date placed in field	Date Collected
June 2005	6/1/05	6/28/05
July 2005	6/1/05	7/27/05
August 2005	8/1/05	8/31 – 9/1/05
September 2005	8/1/05	9/26/05
October-November 2005	10/15/05	11/17-11/20/05
November-December 2005	10/15/05	12/14-12/17/05
January 2006	12/20/05	1/19-1/24/06
February 2006	12/20/05	2/14-2/17-06
March 2006	2/21/06	3/22-06, 4/8/06
April 2006	2/21/06	4/20-4/26/06
May 2006	5/5/06	6/2-6/5/06
June 2006	6/1/06	6/30-7/10/06
July 2006	6/1/06	8/5-8/606
August 2006	8/1/06	9/1-9/4/06
September-October 2006	8/1/06	10/18-10/19/06
November 2006	11/1/06	12/1-12/7/06
December 2006	11/1/06	1/3-1/6/07
January-February 2007	1/17/07	2/18-2/23/07
March 2007	1/17/07	3/21/07
April 2007	4/2/07	5/1-5/2/07
May 2007	4/2/07	6/1/07

APPENDIX B
ALIGNMENTS OF *TRI5* AND *CHS1* NUCLEOTIDE SEQUENCES

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13  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
22  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
17  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
23  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
1   TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
19  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
11  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
14  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
21  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
24  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
36  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
48  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
34  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
41  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
4   TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
15  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
8   TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
18  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
9   TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
42  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
16  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
20  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
56  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
10  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
31  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
50  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
47  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
25  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
49  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
27  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
33  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
28  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
32  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
35  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
29  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
12  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
52  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
46  TGGAGGCATTCCCGACCGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
2   TGGAGACATTCCCGACTGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
3   TGGAGACATTCCCGACTGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
30  TGGAGACATTCCCGACTGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
5   TGGAGACATTCCCGACTGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
6   TGGAGACATTCCCGACTGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
26  TGGAGACATTCCCGACTGAGTACTTCCTGGGCACCGCTGTGCGGCTGCTG  [50]
         *          *
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Figure B-1. Alignment of *tri5* nucleotide sequence. * = informative base pair differentiating *Stachybotrys chlorohalonata* and *S. chartarum.* + = informative base pair differentiating *S. chartarum* chemotypes A and S.

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13 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
22 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
17 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
23 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
1  GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
19 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
11 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
14 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
21 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
24 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
36 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
48 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
34 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
41 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
4  GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
15 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
8  GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
18 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
9  GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
42 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
16 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
20 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
56 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
10 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
31 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
50 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
47 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
25 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
49 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
27 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
33 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
28 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
32 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
35 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
29 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
12 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
52 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
46 GAGAATGTCAAGTACAGGGACAGTAACTACACCAGGGAGGAGCGTGTTGA [100]
2  GAGAACGTCAAGTACAGAGACAGCAACTACACGAGGGAGGAGCGCGTTGA [100]
3  GAGAACGTCAAGTACAGAGACAGCAACTACACGAGGGAGGAGCGCGTTGA [100]
30 GAGAACGTCAAGTACAGAGACAGCAACTACACGAGGGAGGAGCGCGTTGA [100]
5  GAGAACGTCAAGTACAGAGACAGCAACTACACGAGGGAGGAGCGCGTTGA [100]
6  GAGAACGTCAAGTACAGAGACAGCAACTACACGAGGGAGGAGCGCGTTGA [100]
26 GAGAACGTCAAGTACAGAGACAGCAACTACACGAGGGAGGAGCGCGTTGA [100]
        *           *     *        *           *
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Figure B-1. Continued

```
13  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
22  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
17  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
23  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
1   AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
19  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
11  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
14  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
21  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
24  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
36  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
48  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
34  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
41  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
4   AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
15  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
8   AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
18  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
9   AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
42  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
16  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
20  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
56  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
10  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
31  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
50  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
47  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
25  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
49  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
27  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
33  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
28  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
32  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
35  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
29  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
12  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
52  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
46  AAATCTCCAGTATGCCTACAACAAGGCTGCGGCCCACTTCGCACAAGAGC  [150]
2   AAATCTTCAGTATGCTTACAACAAGGCTGCGGCTCATTTCGCACAAGAAC  [150]
3   AAATCTTCAGTATGCTTACAACAAGGCTGCGGCTCATTTCGCACAAGAAC  [150]
30  AAATCTTCAGTATGCTTACAACAAGGCTGCGGCTCATTTCGCACAAGAAC  [150]
5   AAATCTTCAGTATGCTTACAACAAGGCTGCGGCTCATTTCGCACAAGAAC  [150]
6   AAATCTTCAGTATGCTTACAACAAGGCTGCGGCTCATTTCGCACAAGAAC  [150]
26  AAATCTTCAGTATGCCTACAACAAGGCTGCGGCTCATTTCGCACAAGAAC  [150]
          *        *                 *  *          *
```

Figure B-1. Continued

13 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
22 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
17 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
23 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
1 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
19 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
11 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
14 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
21 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
24 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
36 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
48 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
34 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
41 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
4 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
15 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
8 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
18 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
9 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
42 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
16 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
20 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
56 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
10 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
31 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
50 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
47 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
25 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
49 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
27 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
33 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
28 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
32 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
35 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
29 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
12 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
52 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
46 GGCAGCAACAGATTTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
2 GGCAGCAACAGATCTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
3 GGCAGCAACAGATCTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
30 GGCAGCAACAGATCTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
5 GGCAGCAACAGATCTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
6 GGCAGCAACAGATCTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
26 GGCAGCAACAGATCTTGAAGGTCAGCCCCAAGAGACTGGAGGCTTCCCTT [200]
*

Figure B-1. Continued

13 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
22 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
17 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
23 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
1 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
19 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
11 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
14 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
21 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
24 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
36 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
48 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
34 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
41 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
4 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
15 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
8 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
18 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
9 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
42 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
16 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
20 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
56 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
10 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
31 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
50 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
47 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
25 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
49 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
27 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
33 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
28 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
32 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
35 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
29 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
12 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
52 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
46 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
2 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
3 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
30 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
5 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
6 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]
26 CGAACCATTGTCGGCATGGTGGTTTACTCCTGGGCCAAGGTTTCCAAGGA [250]

Figure B-1. Continued

13 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
22 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
17 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
23 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
1 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
19 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
11 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
14 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
21 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
24 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
36 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
48 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
34 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
41 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
4 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
15 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
8 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
18 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
9 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
42 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
16 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
20 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
56 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
10 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
31 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
50 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
47 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
25 GCTCATGGCAGATCTCAGCATCCACTACACCTACACTCTTATCCTCGATG [300]
49 GCTCATGGCAGATCTCAGCATCCACTACACCTACACTCTTATCCTCGATG [300]
27 GCTCATGGCAGATCTCAGCATCCACTACACCTACACTCTTATCCTCGATG [300]
33 GCTCATGGCAGATCTCAGCATCCACTACACCTACACTCTTATCCTCGATG [300]
28 GCTCATGGCAGATCTCAGCATCCACTACACCTACACTCTTATCCTCGATG [300]
32 GCTCATGGCAGATCTCAGCATCCACTACACCTACACTCTTATCCTCGATG [300]
35 GCTCATGGCAGATCTCAGCATCCACTACACCTACACTCTTATCCTCGATG [300]
29 GCTCATGGCAGATCTCAGCATCCACTACACCTACACTCTTATCCTCGATG [300]
12 GCTCATGGCAGATCTCAGCATCCACTACACCTACACTCTCATCCTCGATG [300]
52 GCTCATGGCAGATCTCAGCATCCACTATACCTACACTCTCATCCTCGATG [300]
46 GCTCATGGCAGATCTCAGCATCCACTACACCTACACTCTCATCCTCGATG [300]
2 GCTCATGGCAGATCTCAGCATTCACTACACCTACACTCTCATCCTCGATG [300]
3 GCTCATGGCAGATCTCAGCATTCACTACACCTACACTCTCATCCTCGATG [300]
30 GCTCATGGCAGATCTCAGCATTCACTACACCTACACTCTCATCCTCGATG [300]
5 GCTCATGGCAGATCTCAGCATTCACTACACCTACACTCTCATCCTCGATG [300]
6 GCTCATGGCAGATCTCAGCATTCACTACACCTACACTCTCATCCTCGATG [300]
26 GCTCATGGCAGATCTCAGCATTCACTACACCTACACTCTCATCCTCGATG [300]
* + +

Figure B-1. Continued

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13   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
22   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
17   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
23   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
1    ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
19   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
11   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
14   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
21   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
24   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
36   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
48   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
34   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
41   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
4    ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
15   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
8    ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
18   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
9    ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
42   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
16   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
20   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
56   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
10   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
31   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
50   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
47   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
25   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
49   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
27   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
33   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
28   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
32   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
35   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
29   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
12   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
52   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
46   ATAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
2    ACAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
3    ACAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
30   ACAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
5    ACAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
6    ACAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
26   ACAGCGAGGACGACCCTCACCCTCAGATGTTGACATACTTTGATGATCTT   [350]
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Figure B-1. Continued

```
13  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
22  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
17  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
23  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
1   CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
19  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
11  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
14  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
21  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
24  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
36  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
48  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
34  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
41  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
4   CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
15  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
8   CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
18  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
9   CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
42  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
16  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
20  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
56  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
10  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
31  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
50  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
47  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
25  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
49  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
27  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
33  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
28  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
32  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
35  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
29  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
12  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
52  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
46  CAGAGTGGCAACCCGCAAAAGCATCCCTGGTGGATGCTGGTCAACGAGCA [400]
2   CAGAGTGGCAACCAGCAAAAGCATCCTTGGTGGATGCTGGTCAACGAGCA [400]
3   CAGAGTGGCAACCAGCAAAAGCATCCTTGGTGGATGCTGGTCAACGAGCA [400]
30  CAGAGTGGCAACCAGCAAAAGCATCCTTGGTGGATGCTGGTCAACGAGCA [400]
5   CAGAGTGGCAACCAGCAAAAGCATCCTTGGTGGATGCTGGTCAACGAGCA [400]
6   CAGAGTGGCAACCAGCAAAAGCATCCTTGGTGGATGCTGGTCAACGAGCA [400]
26  CAGAGTGGCAACCAGCAAAAGCATCCTTGGTGGATGCTGGTCAACGAGCA [400]
                 *             *
```

Figure B-1. Continued

13 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
22 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
17 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
23 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
1 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
19 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
11 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
14 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
21 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
24 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
36 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
48 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
34 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
41 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
4 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
15 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
8 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
18 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
9 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
42 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
16 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
20 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
56 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
10 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
31 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
50 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
47 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
25 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
49 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
27 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
33 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
28 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
32 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
35 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
29 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
12 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
52 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
46 CTTCCCTAATGTGCTGAGACACTTTGGCCCTTTCTGCTCGCTAAACCTCA [450]
2 CTTCCCTAATGTGCTCAGACACTTTGGCCCTTTCTGCTCCTTGAACCTCA [450]
3 CTTCCCTAATGTGCTCAGACACTTTGGCCCTTTCTGCTCCTTGAACCTCA [450]
30 CTTCCCTAATGTGCTCAGACACTTTGGCCCTTTCTGCTCCTTGAACCTCA [450]
5 CTTCCCTAATGTGCTCAGACACTTTGGCCCTTTCTGCTCCTTGAACCTCA [450]
6 CTTCCCTAATGTGCTCAGACACTTTGGCCCTTTCTGCTCCTTGAACCTCA [450]
26 CTTCCCTAATGTGCTCAGACACTTTGGCCCTTTCTGCTCCTTGAACCTCA [450]
* ** *

Figure B-1. Continued

```
13  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
22  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
17  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
23  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
1   TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
19  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
11  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
14  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
21  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
24  TTCGCAGCACACTGGACTGTAAGTCTATAGTCGAC  [485]
36  TTCGCAGCACACTGGACTGTAAGTCTATAGTCGAC  [485]
48  TTCGCAGCACACTGGACTGTAAGTCTATAGTCGAC  [485]
34  TTCGCAGCACACTGGACTGTAAGTCTATAGTCGAC  [485]
41  TTCGCAGCACACTGGACTGTAAGTCTATAGTCGAC  [485]
4   TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
15  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
8   TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
18  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
9   TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
42  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
16  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
20  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
56  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
10  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
31  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
50  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
47  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
25  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
49  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
27  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
33  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
28  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
32  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
35  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
29  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
12  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
52  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
46  TTCGCAGCACACTGGACTGTAAGTCTATACTCGAC  [485]
2   TTCGCAGCACACTGGACTGTAAGTCTGCACTGTAA  [485]
3   TTCGCAGCACACTGGACTGTAAGTCTGCACTGTAA  [485]
30  TTCGCAGCACACTGGACTGTAAGTCTGCACTGTAA  [485]
5   TTCGCAGCACACTGGACTGTAAGTCTGCACTGTAA  [485]
6   TTCGCAGCACACTGGACTGTAAGTCTGCACTGTAA  [485]
26  TTCGCAGCACACTGGACTGTAAGTCTGCACTGTAA  [485]
                              ** * ** *
```

Figure B-1. Continued

27 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
46 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
1 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
28 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
35 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
4 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
56 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
47 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
42 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
11 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
20 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
8 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
18 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
19 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
50 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
22 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
41 GGAGTTTCCTCCAGGTCKGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
9 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
25 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
31 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
36 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
23 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
12 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
13 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
21 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
24 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
52 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
10 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
15 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
16 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
14 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
17 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
32 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
34 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
48 GGAGTTTCCTCCAGGTCTGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
29 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
49 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
33 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACACAGATGTTGG [50]
2 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACGCAGATGTTGG [50]
3 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACGCAGATGTTGG [50]
5 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACGCAGATGTTGG [50]
26 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACGCAGATGTTGG [50]
30 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACGCAGATGTTGG [50]
6 GGAGTTTCCTCCAGGTCGGGTACCGGCATCGATGAGGACGCAGATGTTGG [50]
+ *

Figure B-2. Alignment of chs1 nucleotide sequence. + = informative base pair differentiating *S. chartarum* chemotypes A and S. * = informative base pair differentiating *Stachybotrys chlorohalonata* and *S. chartarum.*

```
27  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
46  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
1   GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
28  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
35  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
4   GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
56  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
47  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
42  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
11  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
20  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
8   GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
18  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
19  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
50  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
22  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
41  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
9   GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
25  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
31  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
36  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
23  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
12  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
13  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
21  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
24  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
52  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
10  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
15  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
16  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
14  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
17  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
32  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
34  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
48  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
29  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
49  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
33  GATCCAGGACACGACCAAATGCCTGGAAGAACCATCTGTGCGAATTGATC [100]
2   GATCCAGGACGCGACCAAAGGCCTGGAAGAACCATCTGTGCGAGTTGATC [100]
3   GATCCAGGACGCGACCAAAGGCCTGGAAGAACCATCTGTGCGAGTTGATC [100]
5   GATCCAGGACGCGACCAAAGGCCTGGAAGAACCATCTGTGCGAGTTGATC [100]
26  GATCCAGGACGCGACCAAAGGCCTGGAAGAACCATCTGTGCGAGTTGATC [100]
30  GATCCAGGACGCGACCAAAGGCCTGGAAGAACCATCTGTGCGAGTTGATC [100]
6   GATCCAGGACGCGACCAAAGGCCTGGAAGAACCATCTGTGCGAGTTGATC [100]
              *        *                       *
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Figure B-2. Continued

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27   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
46   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
1    TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
28   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
35   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
4    TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
56   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
47   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
42   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
11   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
20   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
8    TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
18   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
19   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
50   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
22   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
41   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
9    TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
25   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
31   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
36   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
23   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
12   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
13   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
21   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
24   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
52   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
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16   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
14   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
17   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
32   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
34   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
48   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
29   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
49   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
33   TTCTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGCTGGCG  [150]
2    TTTTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGTTGGCG  [150]
3    TTTTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGTTGGCG  [150]
5    TTTTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGTTGGCG  [150]
26   TTTTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGTTGGCG  [150]
30   TTTTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGTTGGCG  [150]
6    TTTTTCTGGTTCTTTTCCTTCAGGCAAAAGATCATCTGCACGGGTTGGCG  [150]
       *                                         *
```

Figure B-2. Continued

27 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
46 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
1 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
28 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
35 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
4 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
56 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
47 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
42 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
11 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
20 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
8 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
18 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
19 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
50 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
22 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
41 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
9 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
25 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
31 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
36 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
23 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
12 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
13 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
21 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
24 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
52 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
10 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
15 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
16 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
14 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
17 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
32 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
34 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
48 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
29 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
49 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
33 GCGGTGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
2 GCGATGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
3 GCGATGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
5 GCGATGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
26 GCGATGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
30 GCGATGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
6 GCGATGCACGAGCGAGACGACGTCGTTCTTGAGCTGCAGGTGAGTCTGCG [200]
*

Figure B-2. Continued

27	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
46	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
1	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
28	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
35	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
4	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
56	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
47	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
42	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
11	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
20	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
8	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
18	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
19	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
50	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
22	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
41	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
9	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
25	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
31	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
36	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
23	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
12	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
13	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
21	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
24	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
52	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
10	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
15	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
16	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
14	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
17	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
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48	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
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33	TGGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTAACCTGCTGC	[250]
2	TCGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTGACCTGCTGC	[250]
3	TCGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTGACCTGCTGC	[250]
5	TCGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTGACCTGCTGC	[250]
26	TCGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTGACCTGCTGC	[250]
30	TCGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTGACCTGCTGC	[250]
6	TCGTGTACTCGTAAATGTGCGCCGTTACGTCCTTGCCGTTGACCTGCTGC	[250]
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Figure B-2. Continued

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27      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
46      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
1       TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
28      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
35      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
4       TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
56      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
47      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
42      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
11      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
20      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
8       TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
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41      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
9       TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
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12      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
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24      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
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10      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
15      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
16      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
14      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
17      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
32      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
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48      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
29      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
49      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
33      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGCACGGCTTT   [297]
2       TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGTACGGCTTT   [297]
3       TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGTACGGCTTT   [297]
5       TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGTACGGCTTT   [297]
26      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGTACGGCTTT   [297]
30      TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGTACGGCTTT   [297]
6       TTTGCAATACCTTCCTGGTACACTCCCATACCAGAGAGTACGGCTTT   [297]
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Figure B-2. Continued

LIST OF REFERENCES

Andersen B, Nielsen KF, Jarvis BB. 2002. Characterization of *Stachybotrys* from water-damaged buildings based on morphology, growth and metabolite production. Mycologia 94:392–403.

Andersen B, Nielsen KF, Thrane U, Szaro T, Taylor JW, Jarvis BB. 2003. Molecular and phenotypic descriptions of *Stachybotrys chlorohalonata* sp. nov. and two chemotypes of *Stachybotrys chartarum* found in water-damaged buildings. Mycologia 95:1227-1238.

Andersen B, Nissen AT. 2000. Evaluation of media for detection of *Stachybotrys* and *Chaetomium* species associated with water-damaged buildings. Int Biodeter Biodegr 46:111-116.

Andersson MA, Nikulin M, Köljalg U, Andersson MC, Rainey F, Reijula K, Hintikka EL, Salkinoja-Salonen M. 1997. Bacteria, molds, and toxins in water-damaged building materials. Appl Environ Microbiol 63:387-393.

Anonymous. 1994. Acute pulmonary hemorrhage/hemosiderosis among infants – Cleveland, January 1993-November 1994. MMWR 43:881-883.

Anonymous. 1997. Update: pulmonary hemorrhage/hemosiderosis among infants – Cleveland, Ohio, 1993-1996. MMWR 46:33-35.

Anonymous. 2000. Update: pulmonary hemorrhage/hemosiderosis among infants – Cleveland, Ohio, 1993-1996. MMWR 49:180-184.

Atlas RM. 1993. Handbook of microbiological media. Boca Raton: CRC Press. 1079 p.

Barron, GL. 1968. The genera of hyphomycetes from soil. Baltimore: Williams & Wilkins. 364 p.

Baxter DM, Perkins L, McGhee CR, Seltzer JM. 2005. A regional comparison of mold spore concentrations outdoors and inside "clean" and "mold contaminated" southern California buildings. J Occup Environ Hyg 2:8-18.

Billups RA, Tilton KS, Warden PS. 1999. Identification of *Stachybotrys chartarum* utilizing various media and two temperature settings [abstract]. In: 1st NSF international conference on indoor air health: impacts, issues and solutions; May 3-5 1999; Denver CO. Ann Arbor, MI: NSF Int. p 166-174.

Bisby GR. 1943. *Stachybotrys*. Trans Br Mycol Soc 26:133-143.

Bishop A. 2002. Diurnal response and periodicity of the common aerial fungal genera and species found in north central Florida [Masters thesis]. College of Agricultural and Life Sciences, University of Florida, Gainesville, FL. 39 p.

Booth C. 1957. Studies of pyrenomycetes: I. Four species of *Chaetosphaeria*, two with *Catenulaira* conidia. II. *Melanopsamma pomiformis* and its *Stachybotrys* conidia. Mycol Papers 68:1–27.

Campbell R. 1972. Ultrastructure of conidium ontogeny in the Deuteromycete fungus *Stachybotrys atra* Corda. New Phytol. 71:1143-1149.

Campbell R. 1974. The ultrastructure of the formation of chains of conidia in *Memnoniella* echinata. Mycologia. 67:760-769.

Carmichael JW, Kendrick WB, Conners IL, Sigler L. 1980. Genera of hyphomycetes. Edmonton: Univ Alberta Press. 386 p.

Castlebury LA, Rossman AY, Sung GH, Hyten AS, Spatafora JW. 2004. Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. Mycol Res 108:864-872.

Corda ACJ. 1837. Icones fungorum hucusque cognitorum. Vol I. Prague: Apud JG Calve. 86 p.

Croft WA, Jarvis BB, Yatawara CS. 1986. Airborne outbreak of trichothecene mycotoxicosis. Atmos Environ 20:549-552.

Cruse M, Telerant R, Gallagher T, Lee T, Taylor JW. 2002. Cryptic species in *Stachybotrys chartarum*. Mycologia 94:814-822.

Cruz-Perez P, Buttner MP, Stetzenbach LD. 2001. Specific detection of *Stachybotrys chartarum* in pure culture using quantitative polymerase chain reaction. Mol Cell Probes 15:129-138.

Dean TR, Roop B, Betancourt D, Menetrez MY. 2005. A simple multiplex polymerase chain reaction assay for the identification of four environmentally relevant fungal contaminants. J Microbiol Meth 61:9-16.

Dearborn DG, Yike I, Sorenson WG, Miller MJ, Etzel RA. 1999. Overview of investigations into pulmonary hemorrhage among infants in Cleveland, Ohio. Environ Health Persp 107:S495-S499.

Desjardins AE, Hohn TM, McCormick SP. 1993. Trichothecene biosynthesis in *Fusarium* species: chemistry, genetics, and significance. Microbiol Rev 57:595-604.

Drobotko VG. 1945. Stachybotryotoxicosis: a new disease of horses and humans. Am Rev Sov Med 2:238-242.

El-Kady IA. 1988. New technique for isolation of *Stachybotrys chartarum*. Naturalia Monspeliensia 52:53-56.

El-Morsy, ESM. 1999. Microfungi from the ectorhizosphere-rhizoplane zone of different halophytic plants from the Red Sea Coast of Egypt. Mycologia 91:228-236.

Elanskii SN, Petrunina YV, Lavrova OI, Likhachev AN. 2004. A comparative analysis of *Stachybotrys chartarum* strains isolated in Russia. Microbiology 73:60-65.

Elidemir O, Colasurdo GN, Rossmann SN, Fan LL. 1999. Isolation of *Stachybotrys* from the lung of a child with pulmonary hemosiderosis. Pediatrics 104:964-966.

Ellis MB. 1971. Dematiaceous Hyphomycetes. Kew, England: Commonwealth Mycological Institute. 608 p.

Eppley RM, Bailey WJ. 1973. 12-13-epoxy-Δ^9-trichothecenes as probable mycotoxins responsible for stachybotryotoxicosis. Science 181:750-758.

Etzel RA, Montana E, Sorenson WG, Kullman GJ, Allan TM, Dearborn DG. 1998. Acute pulmonary hemorrhage in infants associated with exposure to *Stachybotrys atra* and other fungi. Arch Pediatr Adol Med 152:757-762.

Flannigan B, Miller JD. 2001. Microbial growth in indoor environments. In: Flannigan B, Samson RA, Miller JD, eds. Microorganisms in home and indoor work environments: diversity, health impacts, investigation and control. London: Taylor & Francis. p 35-68.

FAWN (Florida Automated Weather Network). 2007. Published on the Internet at http://fawn.ifas.ufl.edu/.

Forgacs J. 1972. Stachybotryotoxicosis. In: Kadis S, Ciegler A, Ajl S, eds. Microbial toxins. New York: Academic Pr. Vol 8, Fungal Toxins; p 95-128.

Forgacs J, Carll WT, Herring AS, Hinshaw WR. 1958. Toxicity of *Stachybotrys atra* for animals. T New York Acad Sci 20:787-808.

Grant C, Hunter CA, Flannigan B, Bravery AF. 1989. Moisture requirements of moulds isolated from domestic dwellings. Intl Biodeter Biodegr 25:259-284.

Greif MD, Currah RS. 2007. Patterns in the occurrence of saprophytic fungi carried by arthropods caught in traps baited with rotted wood and dung. Mycologia 99:7-19.

Harrington B. 2003. The effect of medium used on the isolation of *Stachybotrys chartarum* from air samples [abstract]. In: 103rd general meeting of the American society for microbiology; May 18-22; Washington, DC. Herndon, VA: ASM Pr. p 212.

Haugland RA, Heckman JL. 1998. Identification of putative sequence specific PCR primers for detection of the toxigenic fungal species *Stachybotrys chartarum*. Mol Cell Probes 12:387-396.

Haugland RA, Vesper SJ, Harmon SM. 2001. Phylogenetic relationships of *Memnoniella* and *Stachybotrys* species and evaluation of morphological features for *Memnoniella* species identification. Mycologia. 93:54-65.

Haugland RA, Vesper SJ, Wyner LJ. 1999. Quantitative measurement of *Stachybotrys chartarum* conidia using real time detection of PCR products with the TaqMan^{TM} fluorogenic probe system. Mol Cell Probes 13:329-340.

Heller RM, Heller TW, Sasson JM. 2003 "Tsara'at," Leviticus, and the history of confusion. Perspect Biol Med. 46:588-591.

Henry JL, Stetzenbach LD. 2000. Isolation of *Stachybotrys chartarum* from environmental samples using a cellulose-based medium [abstract]. In: 100th general meeting of the American society for microbiology; May 21-25; Los Angeles, CA. Herndon, VA: ASM Pr. p 566.

Hinkley SF, Mazzola EP, Fettinger JC, Lam YKT, Jarvis BB. 2000. Atranones A-G, from the toxigenic mold *Stachybotrys chartarum*. Phytochemistry 55:663-673.

Hintikka EL. 1978a. Human stachybotryotoxicosis. In: Wyllie TD, Morehouse LG, ed. Mycotoxic fungi, mycotoxins, mycotoxicoses: an encyclopedic handbook. Vol 2. New York: Marcel Dekker. p 87-89.

Hintikka EL. 1978b. Stachybotryotoxicosis in horses. In: Wyllie TD, Morehouse LG, ed. Mycotoxic fungi, mycotoxins, mycotoxicoses: an encyclopedic handbook. Vol 3. New York: Marcel Dekker. p 181-185.

Huang S, Kimbrough JW. 1997. Mold allergy is a risk factor for persistent cold-like symptoms in children. Clin Pediatr 36:695-699.

Hughes SJ. 1958. Revisiones Hyphomycetum aliquot cum appendice de nominibus rejiciendis. Can J Bot. 36:727-836.

Hyvärinen A, Meklin T, Vepsäläinen A, Nevalainen A. 2002. Fungi and actinobacteria in moisture-damaged building materials – concentrations and diversity. Intl Biodeter Biodegr 49:27-37.

Jarvis BB. 2003. *Stachybotrys chartarum*: a fungus for our time. Phytochemistry 64:53-60.

Jarvis BB, Pavanasasivam GP, Bean,GA. 1985. Mycotoxin production from *Myrothecium* species. In: Lacey J. ed. Trichothecenes and other mycotoxins. New York: John Wiley. p 221-231.

Jarvis BB, Salemme J, Morais, A. 1995. *Stachybotrys* toxins 1. Nat Toxins 3:10-16.

Jarvis BB, Sorenson WG, Hintikka EL, Nikulin M, Zhou Y, Jiang J, Wang S, Hinkley S, Etzel RA, Dearborn DG. 1998. Study of toxin production by isolates of *Stachybotrys chartarum* and *Memnoniella echinata* isolated during a study of pulmonary hemosiderosis in infants. Appl Environ Microbiol 64:3620-3625.

Jong SC, Davis EE. 1976. Contribution to the knowledge of *Stachybotrys* and *Memnoniella* in culture. Mycotaxon 3:409-485.

Karunasena E, Markham N, Brasel T, Cooley JD, Straus DC. 2000. Evaluation of fungal growth on cellulose-containing and inorganic ceiling tile. Mycopathologia 150:91-95.

Koster B. 2006. Evolutionary relationships among members of the fungal form-genus *Stachybotrys*, with emphasis on indoor-occurring taxa and the phylogenetic distribution and evolution of the trichothecene biosynthetic gene, tri5 [Doctoral Dissertation]. University of Toronto, Ontario, Canada. 267 p.

Koster B, Scott J, Wong B, Malloch D, Straus N. 2003. A geographically diverse set of isolates indicates two phylogenetic lineages within *Stachybotrys chartarum*. Can J Bot 81:633-643.

Kuhn, RC, Trimble MW, Hofer V, Lee M, Nassof RS. 2005. Prevalence and airborne spore levels of *Stachybotrys* spp. in 200 houses with water incursions in Houston, Texas. Can J Microbiol 51:25-28.

Li CS, Hsu LY, Chou CC, Hsieh KH. 1995. Fungal allergens inside and outside the residences of atopic and control children. Arch Environ Health 50:38-43.

Li DW. 2007. *Stachybotrys eucylindrospora*, sp. nov. resulting from a re-examination of *Stachybotrys cylindrospora*. Mycologia 99:332-339.

Li DW, Kendrick B. 1995. A year-round comparison of fungal spores in indoor and outdoor air. Mycologia 87:190-195.

Li DW, Yang CS. 2005. Taxonomic history and current status of *Stachybotrys chartarum* and related species. Indoor Air 15:5-10.

Li DW, Yang CS, Haugland R, Vesper S. 2003. A new species of *Memnoniella*. Mycotaxon 85:253-257.

Li S, Hartman GL, Jarvis BB, Tak H. 2001. A *Stachybotrys chartarum* isolate from soybean. Mycopathologia 154:41-49.

Miller JD. 1992. Fungi as contaminants in indoor air. Atmos Environ 26A:2163-2172.

Miller JD. 2001. Mycological investigations of indoor environments. In: Flannigan B, Samson RA, Miller JD, eds. Microorganisms in home and indoor work environments: diversity, health impacts, investigation and control. London: Taylor & Francis. p 231-246.

Money NP. 2004. Carpet monsters and killer spores: a natural history of toxic mold. Oxford: Oxford Univ Press. 178 p.

Murtoniemi T, Nevalainen A, Hirvonen MR. 2003. Effect of plasterboard composition on *Stachybotrys chartarum* growth and biological activity of spores. Appl Environ Microbiol 69:3751-3757.

Nelson BD. 2001. *Stachybotrys chartarum*: the toxic indoor mold. ASPnet feature story. Published on the internet at http://www.apsnet.org/online/feature/stachybotrys/.

Nielsen KF. 2003. Mycotoxin production by indoor molds. Fungal Genet Biol 39: 103-117.

Nielsen KF, Holm G, Uttrup LP, Nielsen PA. 2004. Mould growth on building materials under low water activities, influence of humidity and temperature on fungal growth and secondary metabolism. Intl Biodeter Biodegr 54:325-336.

Nikulin M, Pasanen A-L, Berg S, Hinitikka E-L. 1994. *Stachybotrys atra* growth and toxin production in some building materials and fodder under different relative humidies. Appl Environ Microbiol 60:3421-3424.

Paine TD, Raffa KF, Harrington TC. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annu Rev Entomol 42:179-206.

Peltola J, Niessen L, Nielsen KF, Jarvis BB, Andersen B, Salkinoja-Salonen M, Moller EM. 2002. Toxigenic diversity of two different RAPD groups of *Stachybotrys chartarum* isolates analyzed by potential for trichothecene production and for boar sperm cell motility inhibition. Can J Microbiol 48:1017-1029.

Petri A. 1983. Selective medium for the isolation of *Stachybotrys* molds. Magy Allatorvosok 38:697-698.

Pinruan U, McKenzie EHC, Gareth Jones EB, Hyde KD. 2004. Two new species of *Stachybotrys*, and a key to the genus. Fungal Divers 17:145-157.

Renker C, Otto P, Zimdars B, Maraun M, Buscot F. 2005. Oribatid mites as potential vectors for soil microfungi: study of mite-associated fungal species. Microbiol Ecol 50:518-528.

Ruotsalainen M, Hirvonen MR, Hyvärinen A, Meklin T, Savolainen K, Nevalainen A. 1998. Cytotoxicity, production of reactive oxygen species and cytokines induced by different strains of *Stachybotrys* sp. from moldy buildings in RAW264.7 macrophages. Environ Toxicol Pharm 6:193-199.

Samson RA, Flannigan B, Flannigan ME, Verhoeff AP, Adan OCG, Hoekstra ES, eds. 1994. Health implications of fungi in indoor environments. Amsterdam: Elsevier. Chapter 7, Recommendations; 529-538.

Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O, eds. 2002. Introduction to food- and air borne fungi. 6th ed. Utrecht: Centraalbureau voor Schimmelcultures. 379 p.

Samuels GJ, Barr ME. 1997. Notes on and additions to the Niessliaceae (Hypocreales). Can J Bot 75:2165-2176.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425.

Shelton BG, Kirkland KH, Flanders WD, Morris GK. 2002. Profiles of airborne fungi in buildings and outdoor environments in the United States. Appl Environ Microbiol 68:1743-1753.

Siqueira, JO, Hubbell DH, Kimbrough JW, Schenck NC. 1984. *Stachybotrys chartarum* antagonistic to azygospores of *Gigaspora margarita* in vitro. Soil Biol Biochem 16:679-681.

Smith G. 1962. Some new and interesting species of micro-fungi. III. Trans Br Mycol Soc 45:387-394.

Spurgeon JC. 2003. A method for detecting fungal contaminants in wall cavities. Am Ind Hyg Assoc J 64:40-47.

Tiffany JA, Bader HA. 2000. Detection of *Stachybotrys chartarum*: the effectiveness of culturable-air sampling and other methods. J Environ Health 62:9-11.

Tsai SM, Yang CS, Heinsohn P. 1999. Comparative studies of fungal media for the recovery of *Stachybotrys chartarum* from environmental samples. In: Johanning E, ed. Bioaerosols, fungi and mycotoxins: health effects, assessment, prevention and control. Albany, NY: Boyd Printing. 330-334.

Tucker K, Stolze JL, Kennedy AH, Money NP. 2007. Biomechanics of conidial dispersal in the toxic mold *Stachybotrys charatarum*. Fungal Genet Biol 44:641-647.

Vesper SJ, Dearborn DG, Yike I, Sorenson WG, Haugland RA. 1999. Hemolysis, toxicity and randomly amplified polymorphic analysis of *Stachybotrys chartarum* strain. Appl Environ Microbiol 65:3175-3181.

Vesper SJ, Magnuson ML, Dearborn DG, Yike I, Haugland RA. 2001. Initial characterization of the hemolysin stachylysin from *Stachybotrys chartarum*. Infect Immun 69:912-916.

Vesper SJ, Vesper MJ. 2002. Stachylysin may be a cause of hemorrhaging in humans exposed to *Stachybotrys chartatrum*. Infect Immun 70:2065-2069.

Visser S, Parkinson D, Hassall M. 1987. Fungi associated with *Onychiurus subtenuis* (Collembola) in an aspen woodland. Can J Bot 65:635-642.

Whitton SR, McKenzie EHC, Hyde KD. 2001. Microfungi on the Pandanaceae: *Stachybotrys*, with three new species. New Zeal J Bot 139:489-499.

Yang G-H, Jarvis BB, Chung Y-J, Pestka JJ. 2000. Apoptosis induction by the satratoxins and other trichothecene mycotoxins: relationships to ERK, p38 MAPK, and SAPK/JNK activation. Toxicol Appl Pharm 164: 149-160.

BIOGRAPHICAL SKETCH

Sarah Brinton Clark Selke was born in West Hartford, Connecticut, the elder daughter of Newton and Patricia Clark. She graduated from Bates College, Lewiston, ME, in 1995 with a major in biology and a minor in French. Her first job was working for Bates College's Fall Semester in Nantes, France, in Fall 1995. Enjoying the traveler's life, Sarah pursued temporary employment for the next few years while traveling to Ecuador, New Zealand, Australia, Indonesia, Singapore, Thailand, Turkey, and France. In 1999, Sarah joined the Peace Corps and worked in the Kingdom of Tonga, South Pacific, where she taught biology and English at Mailefihi-Siu'ilikutapu high school on the island of Vava'u.

Sarah returned to the United States in 2001 and taught biology and algebra at Bloomfield High School in Bloomfield, Connecticut, while obtaining her grade 7-12 biology teaching certification. In 2002, she began her studies at the University of Florida and joined Dr. Jim Kimbrough's lab in early 2004. During her doctoral studies, Sarah was the lab instructor for Fundamentals of Plant Pathology and worked with Dr. Bill Zettler in his distance-education program. For her efforts, Sarah was awarded teaching awards from the National Association of Colleges and Teachers of Agriculture in 2006 and from the University of Florida Graduate School in 2007.

Sarah and her husband, Gregg, currently live in Rhode Island. Sarah looks forward to continuing her teaching career.