2015

CHARACTERIZATION OF *COLLETOTRICHUM* SPECIES CAUSING BITTER ROT OF APPLES IN KENTUCKY ORCHARDS

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CHARACTERIZATION OF *COLLETOTRICHUM* SPECIES CAUSING BITTER ROT
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THESIS

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A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food, and Environment
At the University of Kentucky

By

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Lexington, Kentucky

2015

ABSTRACT OF THESIS

CHARACTERIZATION OF *COLLETOTRICHUM* SPECIES CAUSING BITTER ROT
OF APPLES IN KENTUCKY ORCHARDS

Multiple species of *Colletotrichum* can cause bitter rot disease of apple, but the identities and relative representation of the species causing the disease in Kentucky are unknown. A total of 475 *Colletotrichum* isolates were collected from diseased apples in 25 counties and characterized both morphologically and by using various molecular approaches. Four morphotypes corresponded to reported descriptions of bitter rot species. Morphotype 1, distinguished by the production of a pink color on potato dextrose agar (PDA), orange conidial masses, and fusiform spores, was consistent with *C. acutatum.* Morphotype 2, which produced gray or white mycelial colonies with orange conidial masses and fusiform spores, was also similar to *C. acutatum.* Morphotype 3 had abundant gray mycelium and rounded spores and was identical to *C. gloeosporioides*. Morphotype 4 produced ascospores and resembled *Glomerella cingulata*. Species-specific polymerase chain reaction (PCR) indicated that both Morphotype 1 and Morphotype 2 belonged to the *C. acutatum* species complex, whereas Morphotype 3 and Morphotype 4 corresponded to the *C. gloeosporioides* complex. Multigene sequence analyses revealed that sample isolates belonged to several newly erected species within these species complexes. Morphotype 1 was identified as *C. fioriniae*, which resides within the *C. acutatum* species complex. Morphotype 2 was identified as *C. nymphaeae*, which is also a species within the *C. acutatum* species complex. Some isolates of Morphotype 3 were identified as *C. siamense* and some as *C. theobromicola;* both species are grouped within the *C. gloeosporioides* species complex. Morphotype 4 was identified as *C. fructicola*, which is also placed within the *C. gloeosporioides* species complex. *C. fioriniae* was the most common species causing bitter rot in Kentucky, comprising more than 70% of the isolates. Molecular fingerprinting using random amplified polymorphic DNA (RAPD) suggested that isolates within *C. fioriniae* belonged to a relatively homogeneous population, while isolates within *C. siamense, C. theobromicola* and *C. fructicola* were more diverse. Infectivity tests on detached fruit showed that *C. gloeosporioides* species-complex isolates were more aggressive than isolates in the *C. acutatum* species complex. However, isolates within the *C. acutatum* species complex produced more spores on lesions compared to isolates within

the *C. gloeosporioides* species complex. Aggressiveness varied among individual species within a species complex. *C. siamense* was the most aggressive species identified in this study. Within the *C. acutatum* species complex, *C. fioriniae* was more aggressive than *C. nymphaeae,* causing larger, deeper lesions. Apple cultivar did not have significant effect on lesion development. However, *Colletotrichum* species produced more spores on Red Stayman Winesap than on Golden Delicious. Fungicide sensitivity tests revealed that the *C. acutatum* species complex was more tolerant to thiophanate-methyl, myclobutanil, trifloxystrobin, and captan compared to the *C. gloeosporioides* species complex. The study also revealed that mycelial growth of *C. siamense* was more sensitive to tested fungicides compared to *C. fructicola* and *C. theobromicola*. These research findings emphasize the importance of accurate identification of *Colletotrichum* species within each species complex, since they exhibit differences in pathogenicity and fungicide sensitivity.

KEYWORDS: Bitter rot, apple, *Colletotrichum*, pathogenicity, fungicide sensitivity, Kentucky orchards

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June 26th 2015
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I dedicate this thesis to my dearly beloved and much missed son Hilal Mishbah, my beloved wife Camelia Misbakh, my parents, my family, my advisors Dr. Nicole Ward Gauthier and Dr. Lisa J. Vaillancourt, the apple growers of Kentucky, Fulbright Scholarship Program, the Institute of International Education (IIE), American Indonesian Exchange Foundation (AMINEF), Indonesian Rubber Research Institute (IRRI), Sembawa Research Center, and my friends and to those who have been tireless in supporting me.

ACKNOWLEDGEMENTS

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CHAPTER ONE: INTRODUCTION

The Economic Importance of Apples

Apples (*Malus domestica* Borkh.) are among the most widely cultivated fruit in the world and the third most traded fruit internationally, after bananas and grapes (48). Apples are grown in every state in the continental United States (U.S.) (72). The major apple-producing states are Washington, New York, Michigan, Pennsylvania, California, and Virginia. Approximately 7,500 U.S. apple growers operate orchards covering 379,000 acres (153,376 hectares). Most of these growers are smallholders who manage their own orchards ranging in size from less than 100 acres, up to 3,000 acres (40 to 1,214 hectares) (48; 72).

Apples have significant value both as an export and as a local agricultural commodity. In 2008, the export value of U.S. apples was $608 million, making the U.S. the fourth largest apple exporter in the world (48). By 2012, the export value of U.S. fresh apples had grown to a record-setting $1.2 billion positioning the U.S. as the world's second largest apple producer, behind China (72). As a local commodity, apples are the second most consumed fruit in the U.S. after oranges (33). In 2008, approximately 84% of the total U.S. apple production was domestically utilized, while only 16 % was exported (48).

In Kentucky, apples are an important local commodity. Apples and peaches are the principal tree fruits grown in the state (83). A high demand for locally-produced apples in Kentucky was observed mostly from local restaurants, farmer's markets, and local consumers (12). The 2015 Cooperative Agricultural Pest Survey (CAPS) (12) reported that there are approximately 25 U-pick apple orchards in Kentucky. In addition, Kentucky has

approximately 1000 acres of apple orchards (12). In 2008 total apple production in Kentucky was 6.9 million pounds, with a total value of $3.7 million (74).

Apple Production Processes and Practices in U.S. Orchards

Apples require a minimum of 6 to 8 hours of sun per day during the growing season, and 500 to 1,000 chilling hours (hours below 45 degrees F) during winter dormancy in order to produce a high quantity and quality of fruit (48). Apple trees can survive temperatures ranging from about -2.2°C to below -51.1°C (28°F to below –60°F), depending upon cultivar (51). However, freezing temperatures occurring during bloom can kill blossoms and thereby destroy entire crops (48). The lower the temperature, the greater the freezing damage (51).

Sunshine stimulates the development of fruit color and flavor, while cold temperatures promote winter dormancy and bloom during spring. Extremely high summer temperatures can cause sunburn on fruit, resulting in abnormal color development. In addition, dry summer conditions may limit tree and fruit growth in orchards. Well-distributed rainfall of 101 to 127 cm (40 to 50 inches) per year is also an important condition for optimum apple tree growth (48). Heavy rainfall can cause fruit damage such as cracking and can allow pathogens or insects to infect the fruits.

Most apple trees in the U.S. are grafted onto dwarf and semi-dwarf rootstocks in order to control plant size. The rootstock specifies hardiness and tree size at maturity; high density production requires a dwarfing rootstock (16). Apple industry standards recommend growers plant dwarf apple trees for specific purposes such as a shortened period of immaturity; increased productivity as a result of higher tree density; better fruit

quality and flavor due to increased sun exposure; and decreased production costs by minimizing labor for pruning, thinning and fruit harvesting (48).

In order to achieve high levels of production, apple trees also require pollination. Some growers cross-pollinate trees by growing different but compatible commercial varieties as pollen sources. Most commonly, honeybees are used as pollinators. Many apple growers depend upon commercial hives to pollinate orchards (48).

Another crucial factor influencing apple production is pruning and thinning. Pruning is performed during winter (late-dormancy) by removing extra wood and branches from trees. Dead, dying, and diseased wood is also removed during winter pruning. Unpruned trees will form many branches within canopies. These heavy canopies limit sunlight and provide favorable environments for insects and/or diseases, thereby lowering production. Thinning, which is carried out in the spring by removing young fruit shortly after bloom, is important for development of large fruit. Apple trees managed without thinning can become overloaded with many small and inconsistently-shaped fruit (48).

Apple yields depend upon varieties that are cultivated. Most apple growers produce multiple cultivars within orchards, while a smaller number focus primarily on a single cultivar (48). Approximately 100 apple cultivars are produced by commercial U.S. orchards. However, the most commonly grown include Cripps Pink, Empire, Fuji, Gala, Golden Delicious, Granny Smith, Honeycrisp, McIntosh, Red Delicious, and Rome (72).

If only one type of apple is cultivated, growers can optimize conditions within the orchard in order to maximize yields and quality for that particular cultivar. A disadvantage of a monoculture is that almost all fruit matures at the same time. Consequently, the fruit

must be sold quickly, flooding the market and reducing the price. Alternatively growers must store fruit in controlled-environment facilities at a high cost. Growing different apple cultivars (polyculture) allows growers to extend the harvest season and minimize the need for expensive storage. However, longer harvest periods result in higher labor costs. In spite of this, polycultures are more common in U.S. apple orchards since it enables more efficient cross-pollination and more variation in harvest times (48).

Challenges for Apple Production

U.S. apple growers are faced with several challenges and issues in terms of maintaining maximum levels of production. Apple trees and fruits are attacked by numerous destructive pathogens and insect pests. Poor pest management can result in significant crop losses and damage.

Insects

The insects that cause the most losses in Kentucky orchards are the codling moth and the plum curculio. Potato leafhoppers, wooly apple aphids, mites, and San Jose scales can also cause serious problems (16). Additional pest problems in Kentucky include apple maggots, brown marmorated stinkbugs, codling moths, dogwood borers, eastern tent caterpillars, European red mites, flatheaded apple tree borers, green June beetles, Japanese beetles, oriental fruit moths, plum curculio, ribbed cocoon makers, rosy apple aphids, San Jose scale, speckled green fruit worms, white apple leafhoppers, and wooly apple aphids (32). Most insect pests in Kentucky apple orchards are controlled by insecticides. A few, including codling moths and oriental fruit moths, are first monitored with pheromone traps and then controlled with insecticides (32).

Weeds

Weeds compete with apple trees for water and nutrients. Moreover, if left unchecked, weeds may harbor insects and pathogens and provide habitats for destructive wildlife (16). Common weeds in Kentucky orchards include honey vine milkweed, Johnsongrass, marestail/horseweed, and Palmer amaranth (32). Weeds in orchards are managed with both pre-emergence and post-planting herbicides (32).

Wildlife

Some types of wildlife such as deer, rodents, and rabbits can cause substantial damage in both new and established orchards (16). Wildlife problems in Kentucky orchards include deer, rabbits, voles, and yellow-bellied sapsuckers (32). Deer, rabbits and voles can cause injury to tree bark and roots, while yellow-bellied sapsuckers create holes that penetrate bark (32). Deer are commonly managed by installation of wire fences surrounding orchards. Rabbits and voles are commonly managed with commercial baits or deterred by clearing vegetation from beneath trees (32).

Horticultural Practices

Labor is the largest direct cost associated with apple production in the U.S., accounting for 60% of total production costs (48). Most horticultural practices associated with apple cultivation, including pruning, thinning, and harvesting, are performed manually. Thinning and harvesting by removing individual fruit is extremely labor intensive and is cost-prohibitive for some commercial orchards (16). This has become one of the justifications for some commercial orchards to provide u-pick operations for consumers. Unfortunately, u-pick operations can lead to other problems including plant damage and wasted produce. In addition, fruit left on trees or on the orchard floor can serve

as a source of pests and pathogens in the orchard, resulting in significant economic losses for the US apple industry (48).

Plant Diseases

Apple trees and fruits are susceptible to a wide range of diseases. Many of these are caused by endemic fungal and bacterial pathogens that also occur on wild crabapple and other members of the Rosaceae family. Monoculture contributes to buildup of primary inoculum of pathogens (67). This may be intensified in conditions of poor sanitation such as unpruned trees and unharvested fruit (32).

Plant diseases such as fruit rot, fire blight, frog eye leaf spot, rust, scab, sooty blotch/fly speck, and root/collar rot are commonly found in U.S. orchards (32; 68). Some diseases are particularly important in specific areas of the U.S. For example apple scab, cedar apple rust, and quince rust are important diseases in the eastern U.S. (68), while fruit rots are more significant in the southeastern U.S., including in Kentucky (32; 62). Bitter rot is the most important of these fruit rots (62). Bitter rot can cause significant economic losses during seasons with extended periods of warm and wet weather (62; 68). In temperate growing regions with high rainfall, bitter rot can cause crop losses as great as 50% (35).

Bitter Rot of Apple

Bitter rot is one of the most common fruit rot diseases of apple. Without proper disease management, bitter rot can destroy entire fruit crops in just a few weeks during periods of warm, wet weather (68). Bitter rot can be caused by any of several different *Colletotrichum* species (68).

Symptoms and Signs

Bitter rot appears initially as small circular lesions on fruit as they begin to mature. Lesions then enlarge, especially in warm and humid conditions. Within these lesions, fungal reproductive structures (acervuli, perithecia, or both) develop (62; 68). Under moist or rainy conditions, salmon-to-pink conidia appear in circular patterns (32), while in dry conditions, acervuli are visible in lesions (32). According to Sutton (68), bitter rot symptoms initiated by spores from a perithecial strain (which produces both ascospores and conidia) appear slightly different from those that are initiated by conidial strains (which produce only conidia). Lesions formed by conidial strains are described as circular, becoming sunken as they enlarge, while those initiated by perithecial strains are described as being darker brown in color and not sunken. Lesions produced by either type of strain appear as V-shaped in cross section. This V-shape is often used to differentiate bitter rot from white rot, which is characterized by a more cylindrical shape (68).

Disease Cycle

Sutton (68) reported that the bitter rot pathogens overwinter as perithecia and acervuli in mummified apples, and as mycelia in colonized dead wood, unpruned branches, cuttings that are left on the ground, and in cankers. In addition, unharvested fruits that fall to the orchard floor, as well as mummified fruits that remain attached to trees, can also be sources of inoculum. Conidia and ascospores are produced by the acervuli and perithecia respectively and are dispersed by rainfall throughout the growing season. Free water induces conidial and ascospore germination and production of appressoria. *Colletotrichum* spp. can penetrate plants directly and/or through wounds. It is commonly reported that infections occur mid- to late-season. However, infection can also occur before, during and

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just after bloom (68). Infected fruit consequently become a source of secondary inoculum throughout the growing season. Early infections combined with persistent wet weather can potentially result in severe disease epidemics (68).

Disease Management

Control of bitter rot is primarily focused on preventative actions such as sanitation and fungicide applications (68). Minimizing the source of primary inoculum by removing mummified fruit, cankers, and pruning remnants will significantly reduce epidemics. As a result, significant economic loss can be avoided (32).

Biggs and S.S. Miller in 2001 (6) classified several apple cultivars into four relative susceptibility groups to *C. acutatum,* the prevalent bitter rot pathogen in West Virginia and northern Virginia. The most susceptible cultivars were Ginger Gold, Honeycrisp and Pristin; highly susceptible cultivars included Arlet, Enterprise, Sansa, and Yataka; moderately susceptible cultivars were Creston, Golden Delicious, and GoldRush; and the least susceptible cultivar was Fuji. Shi and C.R. Rom in 1995 (63) reported that Jonafree, Jonagold, Melrose, Oregon Spur II, Red Cort, Red Delicious, Spartan, and VPI-9 showed good relative resistance to bitter rot pathogens.

Most existing apples cultivars are not sufficiently resistant to bitter rot pathogens for the disease to be managed by sanitation alone (68). Therefore, a protectant fungicide spray program is one of the most important disease control measures. In Kentucky orchards, growers use several fungicides representing different chemical groups and/or different modes of action to control bitter rot, as well as other diseases (36). During different stages of fruit development, different fungicides might be applied. Protective fungicides such as sulfur, captan, and ziram are usually used during the dormant and apple green tip stages,

while myclobutanil, trifloxystrobin, and thiophanate-methyl are applied during later stages of development including apple tight cluster, apple pink, or apple bloom (36).

Colletotrichum Species Associated with Bitter Rot of Apple

Colletotrichum is a cosmopolitan fungal genus that includes nine major clades (aka. "species complexes") comprised of 118 species (18; 31; 79). Some *Colletotrichum* species have wide host ranges, while others can infect only a single host species (28; 29; 49).

There are three *Colletotrichum* species that are most commonly reported as causal agents of bitter rot of apple. These are *G. cingulata*, *C. gloeosporioides* (usually considered to be an anamorph of *G. cingulata*), and *C. acutatum* (34; 35; 62; 68).

Several studies have been conducted to examine inter and intra-orchard variation of the various *Colletotrichum* spp. that are associated with bitter rot of apple (34; 35; 62). Shi et al (62) found that *C. acutatum* was the prevalent species associated with bitter rot in Arkansas, North Carolina, and Virginia. Gonzalez and Sutton (34) reported that *G. cingulata* was more abundant in some orchards in North Carolina.

Colletotrichum Taxonomy and Diagnosis

The taxonomy of *Colletotrichum* has changed significantly over the years. In the late 19th and early 20th centuries, species were described primarily on the basis of the host from which they were isolated. In the early 1900s, some of these species were combined based on morphology of spores and other structures, including sexual structures produced in culture. This process was taken to its extreme in 1957, when Von Arx reduced several hundred described species of *Colletotrichum* to just 11 morphological species (11). Over the next three decades, the numbers of described species slowly increased again, based on

subtle differences in conidial shape and size; appressorial shape and size; presence or absence of setae; and colony appearance and growth rate (11; 34; 35).

One of the challenges for identifying species within the *Colletotrichum* genus is morphological similarity among species. For example, *C. acutatum*, *C. fragariae*, and *C. gloeosporioides* (teleomorph, *G. cingulata*) appear similar in culture (39). In addition, it has been reported that spore shape and size of *Colletotrichum* isolates is dependent upon the growth medium used (1; 39). Cultural characteristics such as growth rate and colony pigmentation are also influenced by growth substrate and temperature (1; 35; 39). Furthermore, in the pathogen-host interaction, some of these *Colletotrichum* species produce very similar disease symptoms and infect the same hosts (4).

The morphological resemblance among *Colletotrichum* species, as well as the disease symptoms that they cause, can potentially raise another issue in disease management, since different *Colletotrichum* species can have different sensitivities to fungicides (4; 39; 44; 47; 49; 58). Differential sensitivity among Kentucky isolates of *C. acutatum* and *C. gloeosporioides* to certain fungicides has been observed (23). Thus, accurate pathogen identification is vital for development of effective disease management recommendations.

Molecular approaches began to be widely employed in the late 1990s to address issues related to characterization of *Colletotrichum* species (29; 39; 64; 66). More recently combined approaches that use both traditional (39) and molecular (35) methods, including species-specific polymerase chain reaction (PCR) amplification (7; 52; 65), RAPD fingerprinting (29; 45; 66), and sequence analyses of the internal transcribed spacer (ITS) of ribosomal DNA (rDNA) (7; 52; 65), and simultaneous sequencing of multiple genes

(17; 18) have been used to accomplish a more reliable identification of species. The result of these studies has been an increase in the number of named *Colletotrichum* species and a realization that the complexes traditionally known as *C. gloeosporioides* and *C. acutatum* actually consist of a large number of individual species.

The Role of Molecular Taxonomy/Diagnosis in Management of Bitter Rot

Molecular identification of fungi has provided in-depth knowledge and new insights into the diversity and ecology of many different groups of fungi (3). The ITS region of the nuclear ribosomal repeat unit has become the most popular sequence for fungal taxonomy and species identification. The advantages of the ITS include the ability to amplify this variable region using PCR primers that have been designed based on highly conserved flanking sequences. Amplification is facilitated by the fact that the region consists of many copies (3). Some species that cannot be reliably identified based on morphological traits can be clearly differentiated based on variation in the ITS sequence. Specific primer pairs based on the ITS region can be applied to rapidly identify and distinguish among some species (52; 65). Since the ITS region has been the most commonly used for fungal identification, it has been chosen for the DNA barcoding initiative, the goal of which is to provide a reliable database for fungal species identification (61).

Comparison of ITS sequences has commonly been used to identify species and species complexes within the *Colletotrichum* genus. Specific primer pairs have been designed based on the ITS region to differentiate morphologically similar *Colletotrichum* species and species complexes. For example, the specific primer pair, CaInt2-ITS4 was used initially by Sreenivasaprasad et al (65) for identification of *C. acutatum* isolated from strawberry. The primer pair CgInt-ITS4 was utilized initially by Mills et al (52) for

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detection and differentiation of *C. gloeosporioides* derived from several plants. Since then, these primer pairs have been widely used for identification of *C. acutatum* and *C. gloeosporioides* from different crops such as citrus (7), anemone (29) and fruits including apples (28). However, ITS based-sequence analysis has some problems as a tool for taxonomy and diagnosis of *Colletotrichum*. One hindrance is that it has insufficient resolution to separate species within *Colletotrichum* species complexes, including some that are morphologically and/or pathologically distinct (10; 11; 18; 24; 79). Another major obstacle with ITS species identification is that the species names associated with ITS sequences available in the public databases (eg Genbank) are often inaccurate (10; 17). Crouch et al (17) revealed a high rate of misidentification (86%) based on ITS sequence similarity comparison within the *C. graminicola* species complex. Cai et al. (10) also reported that ITS sequence data were frequently associated with an incorrect name. Utilization of sequence data from multiple genes provides better resolution of the species within complexes, and during the last decade, this has become the preferred method for *Colletotrichum* taxonomy (10; 11; 18; 24; 79). The number of new species defined using this approach has increased dramatically (11; 18; 79), and the challenge for plant pathologists now is to evaluate the potential significance of these sequence-based species definitions for disease diagnosis and management.

Problems Addressed in This Study

Bitter rot has emerged as one of the most important disease threats to the apple industry in Kentucky (32). As in other regions of the U.S., *C. acutatum*, *C. gloeosporioides*, and *G. cingulata* have all been reported to cause bitter rot in Kentucky (34; 35; 62; 68). The particular bitter rot-causing species within these complexes have not been identified

in Kentucky, although Kou et al. (44) recently reported *C. fiorinae*, a species within the *C. acutatum* complex, as the cause of fruit decay on Nittany apple. Multiple *Colletotrichum* species often occur within the same orchard or area, but the particular species that dominates in each location seems to vary (34; 62). The relative prevalence of the species causing bitter rot in Kentucky is unknown. Therefore, the first question addressed in this study was the identity and relative representation of the *Colletotrichum* species causing bitter rot in Kentucky. Molecular fingerprinting was used to investigate genetic diversity within the species in the state.

Symptomatic fruit were collected from commercial and some private orchards throughout the state. A total of 475 isolates were recovered and stored in a permanent collection. Twenty-six *Colletotrichum* isolates from several orchards representing all regions of the state were selected for more detailed analysis. A combination of morphological and molecular methods was used to identify these *Colletotrichum* isolates. A second important objective was to determine which identification method or methods would be most successful and reliable for diagnostic purposes.

Fungicides are commonly recommended for management of bitter rot, but sometimes they are not effective in controlling epidemics. Thus, this study also addressed whether the *Colletotrichum* species causing bitter rot in Kentucky differ in sensitivity to common fungicides used in orchards. Several other characteristics were also evaluated, including growth rate *in vitro*, sporulation *in vitro* and *in planta*, and pathogenicity to apple fruits. Pathogenicity tests were performed for each strain using detached apples. Fungicide sensitivity of selected strains representing each species was analyzed using common fungicides *in vitro*. Differential sensitivity of *Colletotrichum* species to benomyl has been

reported (1; 4; 23; 29; 57; 58; 76; 77). However, benomyl is no longer available on the U.S. market. Usually growers in the U.S. spray a combination of different fungicides representing different chemical groups and different modes of action, often with combinations of fungicides for each stage of fruit development (36). This study tested the sensitivity of representative *Colletotrichum* strains to thiophanate-methyl, myclobutanil, trifloxystrobin, and captan, since these fungicides are commonly used in Kentucky orchards (36).

The overall goal for this study was to provide data that could be used to design more effective disease management strategies in the field. Combining better diagnosis of specific species causing bitter rot with data about their relative pathogenicity and fungicide sensitivity can help growers to make the best management decisions.

CHAPTER TWO: CHARACTERIZATION OF *COLLETOTRICHUM* SPECIES

CAUSING BITTER ROT OF APPLES IN KENTUCKY ORCHARDS

Introduction

Bitter rot is one of the most common and economically important summer fruit rot diseases of apple in Kentucky (5). It can cause significant economic losses during long periods of warm, wet weather (62; 68). Failure to manage new infections by a combination of spraying preventative fungicides, removing dead and diseased wood, and removing diseased fruit, can exacerbate losses as disease intensity increases.

Bitter rot of apple can be caused by several species within the ascomycete fungal genus *Colletotrichum*. The most commonly reported causal species include *C. acutatum*, *C. gloeosporioides*, and *G. cingulata* (teleomorph of *C. gloeosporioides*) (34; 35; 62; 68). Multiple species are often present within the same orchard or location, but prevalence of specific *Colletotrichum* species seems to differ from one region to another (34; 62). Thus, location-specific species identification is important for effective disease management.

It can be challenging to identify species within the genus *Colletotrichum* because many of them are morphologically similar, with morphology sometimes varying in response to environmental factors (1; 35; 39). Furthermore, some *Colletotrichum* species produce similar disease symptoms and infect the same hosts (4). Molecular approaches have been widely employed since the 1990s to more effectively define and identify species of *Colletotrichum* (29; 39; 64; 66). Combinations of morphological-based identification and molecular approaches have been demonstrated in some studies to accomplish reliable results for identification of *Colletotrichum* species (7; 29; 34; 39; 45; 52; 65; 66).

Sequencing of the internal transcribed spacer (ITS) quickly became the dominant method for fungal taxonomy and species identification beginning in the 1990s, due to its practicality and convenience. ITS consists of large number of copies per cell, so the region can be sequenced even when the quantity of DNA is low (3). Universal ITS primers that correspond to highly conserved flanking regions have been developed for fungi (82). In addition, species-specific primers can be designed based on the ITS region; these can be used to rapidly identify and distinguish among species *in vitro* or *in planta* (52; 65). ITS also has been commonly used to evaluate species diversity in *Colletotrichum* (7; 28; 29; 35; 45; 52; 65; 66). However, the ITS method also has some recognized limitations for this purpose (10; 11; 17; 18; 79). Most importantly, ITS has insufficient resolution to separate some morphologically and/or pathologically distinct species within the *Colletotrichum* species complexes (10; 11; 18; 24; 79). Thus, multigene sequence-based identification has recently emerged as the preferred method for taxonomy and species identification in *Colletotrichum* (10; 11; 18; 24; 79).

With the application of multigene-based identification, the number of new *Colletotrichum* species has increased dramatically over the past decade. It is important to relate these sequence-based *Colletotrichum* species to characteristics such as pathogenicity and fungicide sensitivity. For example, in their characterization of *Colletotrichum* species associated with bitter rot of apple in Brazil and Uruguay, Velho et al (77) reported that some species within the *C. gloeosporioides* complex were more sensitive to benomyl fungicide than other species within *C. acutatum* complex. An ability to relate taxonomic data (also including the development of accurate and rapid diagnostic tools) to pathogenicity and fungicide sensitivity data will assist in management of bitter rot of apple.

The objectives of this study were to identify *Colletotrichum* species associated with bitter rot of apple in Kentucky and to determine which species were prevalent across the state; to determine the phylogenetic relatedness among the species and genetic diversity within the species; and to characterize and compare the pathogenicity and fungicide sensitivity of the species.

Materials and Methods

Isolation of *Colletotrichum* species from symptomatic apple fruit

In 2013, 475 *Colletotrichum* isolates were recovered from apples with symptoms of bitter rot collected from 25 counties across Kentucky. Fruits were sampled from commercial orchards and private residences; these included apples treated with regular fungicide regimes as well as non-sprayed trees. The fruits were stored for up to 7 d at 4°C until they were processed. Fruits were disinfested with 70% ethanol for 30 s and then air-dried in a laminar hood. Three small pieces of fruit skin and pulp were taken from the outer margin of each lesion and placed onto potato dextrose agar (PDA, Difco). Cultures were incubated at 25°C with constant light for 7 to 20 d. Monoconidial isolation was performed according to Du et al. (24), and all isolates were stored in a permanent collection as conidial suspensions on silica at -80°C (71). Two previously characterized monoconidial strains that had been isolated earlier from Kentucky apples with bitter rot symptoms were used as reference isolates for *C. gloeosporioides* and *C. acutatum* (24).

Morphological characterization

Morphological traits that were observed included colony color, conidial shape and size, and the ability to produce perithecia in culture. Colony color was determined after 7

to 15 d on PDA at 23^0C in constant light (39). Conidia were harvested from 14-day-old cultures by flooding each culture with 10 ml sterile water, scraping the upper surface of the culture with a sterile pestle, filtering the conidial suspension through a layer of cheesecloth, and then resuspending conidia in 45 ml of sterile water (24). To determine conidial shape, conidia were mounted in water and observed at 400x by using an Axioscop equipped with an Axio Cam HRc (Carl Zeiss Microscopy). Lengths and widths of 50 randomly chosen conidia were measured at 400x with phase contrast with the measurement module of the Zeiss AxioVision Rel. 48 computer program (24). The data were analyzed statistically with the univariate analysis and the Duncan's Multiple Range Test available through the SPSS statistical analysis package (IBM[©], SPSS[©], Statistic Version 21).

Growth rate assay

Mycelial growth rate of *Colletotrichum* isolates was measured by using a race tube assay developed by White and Woodward (81) and modified by another student in the laboratory (9). Linear mycelial growth was recorded at 3, 5, and 7 d after inoculation for cultures grown on PDA in 25 ml pipet tubes (USA Scientific) in the dark at 18, 21, 23, 25, or 30^0C. Three replications of each isolate at each temperature were evaluated. Growth rate was calculated as the 7-day average of mean daily growth rate (millimeters per day) for each culture and temperature. Growth rate data were analyzed statistically with multivariate analysis and the Duncan's Multiple Range Test available through the SPSS statistical analysis package (IBM[©], SPSS[©], Statistic Version 21).

Isolation of fungal DNA

Conidia from single-spore cultures were loosened by scraping gently with a pestle and were inoculated into 10 ml potato dextrose broth (PDB) in 9 x 50 mm Petri dishes.

Cultures were incubated for 7 to 10 d at 23^0C. Mycelia were harvested with a spatula. Excess media was removed by blotting the mycelia with sterile paper towels. Mycelia were lyophilized in 15 ml Eppendorf tubes with perforated lids for 24 h. Genomic DNA was extracted as described previously by Panaccione et al. (56) with changes in extraction buffer according to Porebski et al (59). DNA was dissolved in 0.5 ml 1 x TE buffer (10mMTris-HCl, 1 mM EDTA; pH 8.0) to an approximate concentration of 200 to 500 µg/ml and diluted to a final concentration of 10 ng/µl for PCR.

Molecular characterization

Species-specific PCR

Species specific PCR was performed by modifying existing protocols of Jelev et al (45) and Du et al (24) using primers *Ca*Int2 (GGGGAAGCCTCTCGCGG) specific for *C. acutatum* (65) and CgInt (GGCCTCCCGCCTCCGGGCGG) specific for *C. gloeosporioides* (7), each in combination with the conserved primer ITS4 (82). The previously characterized isolates APPR1 and APPY3, both collected earlier from symptomatic apples in Kentucky, were used as reference isolates for *C. acutatum* and *C. gloeosporioides* respectively (24). Reactions included 200 ng of genomic DNA, 1.5 mM MgCl$_2$, 1 X PCR buffer, 0.2 mM dNTP, 2.5 u of *Taq* DNA polymerase (Invitrogen) and 0.4 µM of each primer (45). The amplification cycle consisted of denaturation at 95^0C for 5 m followed by 40 cycles consisting of 30 s at 95^0C, 30 s at 50^0C and 1.5 m at 72^0C (45). PCR products were separated in agarose gels (2% w/v Methapor Agarose; 15 x 10 cm, W XL) in Tris/Borate/EDTA buffer electrophoresed at 100 V for 1 h. Each gel was stained with ethidium bromide and viewed on a UV transilluminator.

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Multigene amplification

PCR amplifications were performed for variable regions of two nuclear genes: glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*); and β-tubulin 2 (*TUB2*). These two regions have previously been used to differentiate species within the *C. gloeosporioides* and *C. acutatum* complexes (18; 79) and were reported to be the most effective for separating species within these complexes (Ulrike Damm, personal communication). PCR primer pairs used for the experiment included GDF1 (GCCGTCAACGACCCCTTCATTGA) and GDR2 (GGGTGGAGTCGTACTTG-AGCATGT), for *GAPDH* amplification (70); and T1 (AACATGCGTGAG-ATTGTAAGT) and T2 (TAGTGACCCTTGGCCCAGTTG), for *TUB2* gene amplification (55). PCR was performed according to Weir et al (79). PCR conditions for GAPDH amplification were 4 m at 95^0C, and then 35 cycles of 95^0C for 30 s, 60^0C for 30 s, 72^0C for 45 s, and then 7 m at 72^0C. The conditions for amplification of TUB2 were similar except that the annealing temperature was 55^0C (79).

Sequencing of PCR products

PCR products were purified using Qiagen's miniprep method (Qiagen Inc., Valencia California). Sequencing reactions were performed using the Sanger dideoxy chain termination method and the BigDye system (PE Applied Biosystems, Foster City CA) (24). Sequencing was performed by the Advanced Genetic Technologies Center, University of Kentucky, USA on an ABI 310 genetic analyzer (PE Applied Biosystem). Forward and reverse sequences were aligned and edited with Geneious Pro 4.5.7 software (Biomatter Ltd.). Homology searches to confirm species identity for the *GAPDH* and *TUB2* sequences were performed by using megablast against the GenBank nr database. Gene sequences

were submitted to the Q-bank fungal database that is designed to facilitate diagnosis of species within selected *Colletotrichum* complexes, including *C. gloeosporioides* and *C. acutatum* (www.q-bank.eu) (18; 79).

Phylogenetic analyses

Phylogenetic analyses were performed using MUSCLE for multiple alignment, Gblocks for automatic alignment curation, PhyML for tree building based on approximate Likelihood Ratio Test (aLRT), and TreeDyn for tree rendering, within the 'One Click' mode on the website www.phylogeny.fr (2; 15; 21; 22; 25; 38). Published sequences of 30 U.S. *Colletotrichum* isolates obtained from GenBank were included in the phylogenetic analyses as references.

RAPD fingerprinting analysis

RAPD (Random Amplified Polymorphic DNA) fingerprinting analyses were conducted with three different primers: OPA 13 (CAGCACCCAC); OPA 18 (AGGTGACCGT); and UBC 356 (GCGGCCCTCT). All reactions were incubated in a BIO-RAD C1000™Thermal Cycler in a total volume of 26.03 µl. PCR reactions contained 10.83 µl sterile water, 2.5 µl of 50 mM $MgCl_2$, 2.5 µl of 1 X PCR buffer, 4 µl dNTPs (each 0.2mM), 0.22µl of 500 U of *Taq* DNA polymerase (Invitrogen), 3 µl of 5 µM of primer, and 3 µl of 10 ng/µl genomic DNA. Amplification consisted of 3 cycles of 60 s at 95^0C, 60 s at 35^0C, 1.5 m at 72^0C, followed by 40 cycles consisting of 10 s at 95^0C, 20 s at 40^0C and 1.5 m at 72^0C. PCR products were separated in agarose gels (1% w/v Agarose GPG/LE (American Bioanalytical); 15 x 10 cm, W X L) in Tris/acetate/EDTA buffer electrophoresed at 90 V for 1 h. Each gel was stained with ethidium bromide and viewed on a UV transilluminator.

Pathogenicity tests

Pathogenicity tests were performed in the laboratory with two apple cultivars: Golden Delicious, reported to be susceptible to bitter rot (6) and Red Stayman Winesap, reported to be moderately resistant (60). Apples used in this study had not been sprayed with fungicides and were obtained from the Horticulture Research Farm, Department of Horticulture, University of Kentucky, located in Lexington, KY. Apples were harvested in August 2014 and stored in the dark at 4°C for up to 2 months before use. Detached fruits were surface-disinfested in 10% bleach for 5 m, washed in sterile distilled water, and then air-dried on sterile paper towels at room temperature. Fruits were wounded with a dissecting needle that had been modified to produce a puncture of 0.5 mm depth. The wounded fruits were then inoculated by placing 6 µl of conidial suspension ($1x10^5$ spores/ml) on the surface of the wound. The conidia used to make suspensions were harvested from 10-day-old cultures grown on PDA. Inoculated fruits were secured in plastic containers lined with wet paper towels and incubated for 14 d at 21°C in constant light. Containers were opened once a day for measurements of developing lesions. Lesion diameters were measured using a digital caliper. At 14 days post inoculation (dpi) fruits were cut through the centers of lesions for measurements of lesion depth. The entire surface area of each lesion was then removed with a scalpel and submerged in 10 ml of sterile water with one drop of Tween 80 (Fisher Scientific, Fair Lawn, N.J) in a 50 ml tube (USA Scientific). Spores were dislodged from the tissue samples by using a vortex for 10 s, and then conidia were filtered through a layer of cheesecloth. Spores were counted with a hemocytometer. The experiment was performed twice. The factorial experiment for experiment, replication, cultivar, species complex, species, isolate was evaluated using an

analysis of variance and means were separated using Least Square Means with Bonferroni correction provided in the statistical algorithms of SAS version 9.3 (SAS Institute, Cary, NC).

Fungicide sensitivity assays

Effect of fungicides on mycelial growth

Fungicide sensitivity of isolates was tested on PDA amended with fungicides in 25 ml race tubes (USA Scientific) as described above. Fungicides included thiophanate-methyl (Topsin M WSB 70 WP, Nippon Soda Company Ltd.; FRAC 1), myclobutanil (Rally 40 WSP, Dow AgroSciences LLC; FRAC 3), trifloxystrobin (Flint, Bayer CropScience; FRAC 11), and captan (Captan 80 WDG, Arysta LifeScience North America Co.; FRAC M). Race tube assays were performed according to Buiate (9), as discussed previously. Fungicide concentrations in the amended media were 0.01; 0.1; 1; 10; and 100 µg/ml for Topsin, Rally and Flint, and 0.1; 1; 10; 100; and 1,000 µg ai/ml for Captan. The linear growth of each isolate was measured at 10 d after inoculation. Percent inhibition was calculated by [(growth of the control − growth of the experimental)/growth of the control]*100. Relative growth was calculated by [100 − percent inhibition]. Half maximal effective concentration (EC50) was determined based on the relative growth data. Each treatment was replicated three times within the experiment, and the experiment was repeated once. The factorial experiment for experiment, replication, fungicide, species complex, species, isolate was evaluated using an analysis of variance and means of EC50 were separated using Least Square Means with Bonferroni correction provided in the statistical algorithms of SAS version 9.3 (SAS Institute, Cary, NC).

Effect of fungicides on conidial germination

Trifloxystrobin (a systemic fungicide) and captan (a protectant fungicide) were tested for their ability to inhibit spore germination. Fungicide solutions and fresh conidial suspensions were mixed with sterile water to a total of 15 ml. The final concentrations of fungicide were 0.001; 0.01; 0.1; 1; and 10 µg/ml, and the final concentration of conidia was 1 x 10^4 conidia per ml. Three 100-µl droplets of each suspension was placed into an empty 100-mm polystyrene Petri dish and then placed in humidity chambers and incubated for 20 to 24 h. After incubation, each droplet was covered with a cover slip and conidia were scored at 100x as either non-germinated or germinated according to Chaky et al (14) and Vincelli and E. Dixon (78).

Results

Morphological characterization

The 475 *Colletotrichum* bitter-rot isolates that were collected from 25 counties in Kentucky were divided into four morphological types (morphotypes) based on colony color and spore shape. These morphotypes corresponded to the descriptions of the three *Colletotrichum* species most commonly associated with bitter rot of apple: *C. acutatum*; *C. gloeosporioides*; and *G. cingulata* (Table 2.1; Figure 2.1; Appendix 1).

Isolates belonging to Morphotype 1 were characterized by the production of a pink to red coloration in the media (Figure 2.1). Orange conidial masses were submerged in the mycelia. Conidia were predominantly fusiform with pointed ends (Figure 2.1). Perithecia were not observed. Based on these morphological characteristics, all of the isolates in Morphotype 1 corresponded to *C. acutatum* (35; 62).

Morphotype 2 isolates did not produce pigment in the growth medium. Isolates formed white-to-gray mycelia, occasionally with a light yellow tint, and an abundance of orange conidial masses (Figure 2.1). Conidia of the isolates in Morphotype 2 were mostly fusiform, with ends that were somewhat more rounded than spores of Morphotype 1 (Figure 2.1). No perithecia were observed in cultures of this morphotype. The conidial shape of the isolates in Morphotype 2 corresponded to the description of *C. acutatum* (35; 62).

Isolates of Morphotype 3 were differentiated from other morphotypes by the presence of gray mycelia, with no coloration in the growth medium (Figure 2.1). Some isolates produced abundant aerial mycelia that were white-to-gray. Sectors were observed in the colony mycelia of some isolates of Morphotype 3 such as KY254; KY301; KY152; and KY153 (Appendix 1). Conidia of all isolates were cylindrical with rounded ends (Figure 2.1). Conidia were produced in small orange masses over the entire colony in most isolates. A few isolates produced larger orange scattered masses of conidia. No perithecia were observed in culture. The characteristics of the isolates in Morphotype 3 were consistent with descriptions of *C. gloeosporioides* (35; 62).

The mycelia of the isolates in Morphotype 4 were dark gray, with abundant mycelia (Figure 2.1). Isolates of Morphotype 4 produced no pigmentation in the media. Conidia were cylindrical with rounded ends, similar to those produced by isolates in Morphotype 3. Perithecia were produced in culture, and asci and ascospores were observed under the microscope (Figure 2.1). Based on these morphological characteristics, the isolates in Morphotype 4 belonged to *G. cingulata* (35).

Among the total of 475 isolates, 335 (70.53%) belonged to Morphotype 1; 15 (3.16%) belonged to Morphotype 2; 106 (22.32%) belonged to Morphotype 3; and 19 isolates (4%) belonged to Morphotype 4 (Table 2.2; Figure 2.2). Twenty-six of the 475 isolates were selected for further examination; the test isolates included representatives of all four morphotypes and were collected from northern, eastern, central, and western Kentucky (Table 2.3).

Sizes of conidia produced by each of the 26 selected isolates and two reference isolates were measured and compared (Figure 2.3; Figure 2.4; and Figure 2.5; Appendix 3). Average conidial length and width were significantly different among the four morphotypes (P < 0.05). Isolates of Morphotype 3 had the longest conidia, averaging 17.89 µm, while isolates of Morphotype 1 had the shortest, averaging 12.43 µm. Conidia of Morphotype 4 were the widest, with an average of 6.39 µm, while conidia of Morphotype 1 isolates were the narrowest, averaging 4.61 µm. Length-width ratios of isolates of Morphotype 1 and Morphotype 4 were not significantly different from one another ($P = 0.375$). Likewise, conidial length-width ratios of Morphotype 2 and Morphotype 3 did not differ from one another ($P = 0.323$). (Figure 2.3; Figure 2.4; and Figure 2.5).

Mycelial growth rate

The independent variables morphotype and temperature both had a significant effect on mycelial growth rate ($P < 0.05$). The growth rate of each morphotype was significantly different from the others ($P < 0.05$). The maximum growth rate occurred at 25°C for all isolates (Table 2.4; Appendix 3). Isolates of Morphotype 4 grew the fastest (average growth 4.99 mm/day), and isolates of Morphotype 2 were the slowest (average growth 3.43 mm/day) (Table 2.5; Appendix 3).

Molecular identification and phylogenetic analysis

Species-specific primer analyses

DNA amplification with the species-specific primers for *C. acutatum* (CaInt2-ITS4) yielded a single, relatively intense 490-bp product for isolates of Morphotype 1 and Morphotype 2, as well as from the *C. acutatum* reference isolate, APPY3. Multiple amplification products of lower intensity, including one of 490-bp, were generated with this primer for most of the isolates of Morphotype 3 and Morphotype 4, but not for the APPR1 *C. gloeosporioides* reference isolate (Figure 2.6).

Primers specific for *C. gloeosporioides* (CgInt-ITS4) yielded an intense 450-bp DNA product from all isolates in Morphotype 3 and Morphotype 4, as well as from the reference isolate of *C. gloeosporioides*, APPR1. No amplification products were produced with CgInt-ITS4 for isolates of Morphotype 1 and Morphotype 2 (Figure 2.6).

Multigene analyses

GAPDH and *TUB2* DNA fragments were successfully amplified and sequenced from all test isolates. The PCR products from the *GAPDH* and *TUB2* amplification were approximately 250 and 700 bp, respectively (Appendix 4). Homology searches with each individual sequence suggested that all tested isolates corresponded to the genus *Colletotrichum*.

Based on the sequence comparison of 26 tested isolates with published sequences in GenBank, all isolates in Morphotype 1 and Morphotype 2 belonged to the *C. acutatum* species complex. All isolates in Morphotype 1 (12 isolates) matched *C. fioriniae*, and all isolates in Morphotype 2 (3 isolates) could be identified as *C. nymphaeae*. Isolates within

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Morphotype 3 and Morphotype 4 belonged to the *C. gloeosporioides* species complex. Seven isolates in Morphotype 3 matched *C. siamense*, and 2 isolates were identified as *C. theobromicola.* All isolates in Morphotype 4 corresponded to *C. fructicola.* Homology searches to confirm species identity for the *GAPDH* and *TUB2* sequences against the Q-bank fungal data base was consistent with the homology searches by using megablast against the GenBank nr database.

Molecular phylogenetic analyses based on *GAPDH* and *TUB2* regions in a separate analysis by PhyML grouped the *Colletotrichum* isolates into two major clades, corresponding to the *C. acutatum* species complex and *C. gloeosporioides* species complex, with bootstrap support of 100% (Figure 2.7 and Figure 2.8). The *C. acutatum* clade consisted of two separate sub-clades, which corresponded to *C. fioriniae* and *C. nymphaeae*. The *C. gloeosporioides* clade consisted of three sub-clades, corresponding to *C. siamense*, *C. theobromicola,* and *C. fructicola* (Figure 2.7 and Figure 2.8).

RAPD fingerprinting analyses

Amplification products were obtained from all 26 test isolates with the random primers OPA 13, OPA 18, and UBC 356. Similar banding patterns were observed among isolates of *C. fioriniae* (Morphotype 1), and also among isolates of *C. nymphaeae* (Morphotype 2), suggesting that these populations are relatively homogeneous. Banding patterns within *C. siamense*, *C. fructicola,* and *C. theobromicola* isolates appeared to be more variable, indicating a higher level of diversity within those populations (Figure 2.9; Figure 2.10; and Figure 2.11).

Pathogenicity tests

All test isolates of *Colletotrichum* produced typical bitter rot symptoms on Golden Delicious and Red Stayman Winesap fruits. These symptoms included sunken lesions, and conical zones of necrotic tissue extending beneath the lesions (Appendix 6). Apple cultivar had no significant effect on lesion diameter or on lesion depth ($P > 0.05$). A significant difference was observed between the two replicate experiments that were performed for each cultivar ($P < 0.05$). The variable of species complex had a significant effect on lesion development ($P < 0.0001$). Isolates belonging to the *C. gloeosporioides* species complex, on average, produced larger and deeper lesions (34.22 mm and 22.03 mm, respectively) compared with the *C. acutatum* species complex isolates (20.11 mm and 12.12 mm, respectively) (Figure 2.12). The interaction of species complex and apple cultivar had no effect on lesion diameter or on lesion depth ($P > 0.05$) (Figure 2.12 and Figure 2.13).

The variable of species ($P < 0.0001$), as well as the interaction of species and apple cultivar ($P = 0.0135$), had significant effects on lesion development. Isolates of *C. siamense* produced the largest and deepest lesions (average of 40.18 mm in diameter and 25.17 mm deep) (Figure 2.12, Figure 2.13). Even though *C. fioriniae* and *C. nymphaeae* belong to the same species complex, *C. fioriniae* caused significantly larger lesions (average diameter 21.15 mm) than *C. nymphaeae* (average diameter 15.93 mm) (Figure 2.12). Isolates of *C. fioriniae* also produced significantly deeper lesions than *C. nymphaeae* ($P = 0.0002$) (Figure 2.13). However, the depth of lesions caused by *C. fioriniae* and by *C. theobromicola* was not significantly different ($P > 0.05$) (Figure 2.13).

Apple cultivar had a significant effect on the sporulation of *Colletotrichum* spp. ($P = 0.0097$). The average number of spores produced by *Colletotrichum* lesions on Red

Stayman Winesap apples was 0.56 x 10^5 spores/mm^2 while the mean number of spores produced by lesions on Golden Delicious was 0.37 x 10^5 spores/mm^2. The variable of species complex also had significant effect on sporulation ($P < 0.0001$). The mean number of spores produced by lesions caused by members of the *C. acutatum* complex was 0.88 x 10^5 spores/mm^2, while the average produced by lesions caused by isolates of the *C. gloeosporioides* complex was 0.06 x 10^5 spores/mm^2. Lesions caused by *C. fioriniae* produced more spores (average concentration 1.01 x 10^5 spores/mm^2) than any of the other species. The number of spores produced by *C. nymphaeae*, *C. siamense*, *C. theobromicola*, and *C. fructicola* was not significantly different ($P > 0.05$) (Figure 2.14).

Sensitivity of *Colletotrichum* isolates to fungicides

The independent variables fungicides (thiophanate-methyl, myclobutanil, trifloxystrobin, and captan), species complex, species, interaction of fungicide and species complex, and interaction of fungicide and species all had significant effects on the EC50s of selected fungicides against mycelial growth of *Colletotrichum* ($P < 0.0001$) (Figure 2.15). No differences were observed between the two replicate experiments ($P > 0.05$) (Figure 2.15).

The EC50 of captan against the mycelial growth of *Colletotrichum* spp. was higher than that of any of the other fungicides in the study (mean EC50 = 299.21 mg/L). The EC50s of thiophanate-methyl, myclobutanil, and trifloxystrobin were not significantly different from one another ($P > 0.05$) (Figure 2.15).

Based on mycelial growth, isolates belonging to the *C. gloeosporioides* complex were more sensitive, on average, to the four fungicides than isolates in the *C. acutatum* species complex. Isolates of *C. fioriniae, C. nymphaeae* and *C. theobromicola* were

significantly less sensitive to the four fungicides than isolates of *C. siamense* and *C. fructicola* (P < 0.05). The sensitivities of *C. fioriniae, C. nymphaeae,* and *C. theobromicola* were not significantly different from one other (*P* > 0.05). Similarly, *C. siamense* and *C. fructicola* did not differ in fungicide sensitivity (*P* > 0.05) (Figure 2.15).

The independent variables fungicide (trifloxystrobin and captan), species complex, species, fungicide-species complex interaction, and fungicide-species interaction all had significant effects on the EC50s of selected fungicides to the spore germination of each *Colletotrichum* species evaluated (*P* < 0.05) (Figure 2.16). The EC50 of trifloxystrobin (mean EC50 = 0.02 mg/L) for conidial germination of *Colletotrichum* spp. was significantly lower than the EC50 of captan (mean EC50 = 0.33 mg/L). The fungicide sensitivity of spores among members of the *C. gloeosporioides* species complex was, on average, significantly higher than the sensitivity of spores of isolates within the *C. acutatum* species complex. The sensitivity of spores of *C. theobromicola* to the two selected fungicides was significantly higher than the sensitivity of spores of the other species (*P* <0.05). (Figure 2.16).

Discussion

The major finding of this work suggested that *Colletotrichum* isolates causing bitter rot of apple in Kentucky orchards belong to five different species: *C. fioriniae*; *C. nymphaeae*; *C. siamense*; *C. theobromicola*; and *C. fructicola*. Among these, only *C. fioriniae* has been reported previously to cause bitter rot of apple in the U.S. (46). However, all five species have been found associated with bitter rot in other countries including Croatia (43), Brazil, and Uruguay (70). Furthermore, the publications that established and named these five new species included several isolates of *C. fioriniae*, *C. siamense*, and *C.*

fructicola that had been recovered from apple in the U.S. (18; 79). To my knowledge, this is the first study that has compared fungicide sensitivity and pathogenicity of these species on apple.

Diagnosis of species associated with bitter rot has traditionally been based on morphological features, particularly spore shape and type. This has allowed the recognition of three species of *Colletotrichum* as causal agents: *C. acutatum*, *C. gloeosporioides*, and *G. cingulata*. The 475 isolates recovered from diseased apples in Kentucky could be divided into two groups on the basis of spore shape, one with the spindle-shaped spores of *C. acutatum*, and the other with the ovoid spores of *C. gloeosporioides* (34). Among the latter group, a small percentage formed perithecia containing asci and ascospores in culture. These were grouped as Morphotype 4, differentiating them from the non-perithecial members of *C. gloeosporioides* which were named Morphotype 3. Isolates of *C. gloeosporioides* that form perithecia have traditionally been referred to as *G. cingulata*. However, there has recently been a move to eliminate the binary naming system for ascomycete fungi, and thus the teleomorph name *G. cingulata* has been rejected and both the sexual and asexual forms should now be referred to as *C. gloeosporioides* (42).

The assumption has generally been made that perithecial and non-perithecial isolates of *C. gloeosporioides* causing bitter rot represent the same strains undergoing different phases of development. However, this work clearly showed that isolates of *C. gloeosporioides* that formed perithecia were not the same as those that did not develop perithecia; perithecial isolates belonged to a different species within the *C. gloeosporioides* complex, *C. fructicola*. Isolates of *C. gloeosporioides* that did not form perithecia included

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two other species within the complex, *C. siamense* and *C. theobromicola*, which could not be distinguished by morphological features alone. Some of the Morphotype 4 isolates that had initially formed perithecia lost this ability in subsequent cultures. The loss of fertility in culture is not uncommon in *Colletotrichum* (75). Thus, presence of perithecia in culture may be a useful trait for diagnosis of *C. fructicola*, but their absence is not informative for diagnosis of bitter rot isolates within the *C. gloeosporioides* complex.

Isolates with the spindle-shaped conidia of *C. acutatum* occurred as two different color morphs in culture. A pink to red pigmentation was observed in some isolates (categorized as Morphotype 1). Other isolates with *C. acutatum*-type spores did not produce pigment and were categorized as Morphotype 2. Gonzalez et al (35) also reported the presence of two color morphs in the *C. acutatum* species. Isolates that produced red pigment in culture were identified as chromogenic *C. acutatum* and isolates that did not produce red pigmentation were identified as non-chromogenic *C. acutatum.* A similar observation was made in Du et al. (24) including some isolates from apple. Isolates of Morphotype 1 corresponded to chromogenic *C. acutatum*, and this work showed that all isolates of Morphotype 1 belonged to the species *C. fioriniae.* All the non-chromogenic isolates with *C. acutatum*-type spores belonged to *C. nymphaeae*. Thus, a combination of spore shape and color is useful for diagnosing species causing bitter rot within the *C. acutatum* complex in Kentucky.

Colletotrichum fioriniae was the most common causal agent of the bitter rot disease throughout the state. The dominance of *C. fioriniae* is consistent with previously published research by Shi et al. (62), who determined that most of the *Colletotrichum* isolates derived from apple bitter rot in the Southeastern U.S. were chromogenic *C. acutatum*. Although

the authors did not perform molecular characterization of their isolates, results of my study suggest that those isolates were probably *C. fioriniae*.

RAPD fingerprinting has been widely used for characterization of diversity within *Colletotrichum* populations (20; 26; 30; 45; 73). RAPD fingerprinting suggested relatively low levels of genetic diversity within the *C. fioriniae* population in Kentucky, and likewise among *C. nymphaeae* isolates. Results indicated a somewhat higher level of diversity within the *C. gloeosporioides* complex species *C. siamense* and *C. fructicola*. Greater relative diversity among isolates of *C. gloeosporioides* compared with *C. acutatum* has been frequently reported (8; 13; 41; 73). Reasons for this difference are unknown, but it has been suggested that it may be related to the presence of a sexual phase (*G. cingulata*) in *C. gloeosporioides*. The teleomorph of *C. acutatum* (*G. acutata*) has been observed only in the laboratory in the U.S. (37), although it has been reported in the field associated with some fruit rots in Asia (42). A sexual phase would be expected to increase population diversity due to marker recombination in the absence of selection. However, measuring genetic diversity of a population by using RAPD has several limitations. RAPD fingerprinting only determines amplification or lack of amplification (two alleles) for each amplicon locus; it does not provide measures of genetic diversity affected by the number of alleles at a locus (50). In addition, some technical limitations such as the size and specificity of RAPD primers, sensitivity to reaction condition, and the possibility of co-migration, may cause non-reproducible amplification products (40).

Spore shape was generally useful for differentiating *C. gloeosporioides* and *C. acutatum.* However, spores of the non-chromogenic *C. nymphaeae* were more rounded than the spindle-shaped spores of the chromogenic *C. fioriniae*, and thus they were more

difficult to classify as fusiform with complete confidence. Species-specific primers developed to identify *C. acutatum* and *C. gloeosporioides* gave consistent and reliable results in this study and may be a useful additional diagnostic technique in cases where spore shape is ambiguous. Random PCR products were often generated with *C. acutatum*-specific primers when they were used with *C. gloeosporioides*, and some of these amplicons matched the expected size for the specific *C. acutatum* product. However, the bands produced with *C. gloeosporioides* isolates were less intense. Additionally, if both primer pairs were used, results were unambiguous. These species-specific primers were designed from the ITS 1 region of the ribosomal DNA (rDNA) (52; 65). ITS sequences have been widely used for *Colletotrichum* species identification, but they do not allow separation of species within species complexes (19; 79). *GAPDH* and *TUB2* sequences have been used more recently to identify and differentiate *Colletotrichum* species within the *C. acutatum* and *C. gloeosporioides* complexes (19; 77). This study used these sequences to reveal the presence of five species associated with bitter rot of apple in Kentucky. In the future, these sequences could be used to design new species-specific primers that will allow identification and detection of each of these five species.

The presence of five different species causing bitter rot disease raised the possibility that they may differ in traits relevant to disease epidemiology and management. To my knowledge, my study is the first to thoroughly test this hypothesis.

Isolates within the *C. acutatum* species complex had a slower average growth rate than isolates in the *C. gloeosporioides* species complex. This result agrees with a previously published report (80). All test isolates had an optimal growth rate at 25°C, and that growth of all isolates was significantly inhibited at 30°C, in agreement with a previous

report by Velho et al. (77). In this study, both species within the *C. acutatum* complex (*C. nymphaeae* and *C. fioriniae*) grew at a similar rate. For isolates within the *C. gloeosporioides* complex, *C. theobromicola* grew more slowly than the other two species, and its growth rate was not significantly different from the two *C. acutatum* isolates. These results are in contrast with Velho et al. (77), who reported that *C. nymphaeae* grew more slowly than *C. theobromicola.*

All tested isolates from all five *Colletotrichum* species were pathogenic to wounded apple fruits. On average, isolates within the *C. acutatum* species complex produced smaller lesions than isolates in the *C. gloeosporioides* species complex. However, this study found significant differences in aggressiveness among the species within each complex. Thus, *C. siamense* in the *C. gloeosporioides* complex was the most aggressive species overall and was significantly more aggressive than the other two species within that complex. Similarly, within the *C. acutatum* species complex, *C. fioriniae* was more aggressive than *C. nymphaeae*. Isolates of *C. theobromicola* and *C. fructicola* within the *C. gloeosporioides* complex were not significantly different in aggressiveness from the two *C. acutatum* species. Thus, a diagnosis to species complex alone would be insufficient for a prediction of relative aggressiveness.

Lesion sizes did not differ on the two tested apple cultivars, Golden Delicious and Red Stayman Winesap. Red Stayman Winesap is reported to have partial resistance to bitter rot, whereas Golden Delicious is reported to be susceptible to the disease. This partial resistance may relate to factors other than resistance of the pulp to colonization once the skin has been broken. Most existing apples cultivars are not sufficiently resistant to manage the disease effectively in the absence of chemical treatments (68).

Isolates of *C. fioriniae* produced significantly more spores per unit lesion area than the other four species. Perhaps its relative fecundity is related to its dominance across the state of Kentucky. According to Shi et al (62) one of the variables influencing the frequency of a fruit rot pathogen is the abundance of initial inoculum.

On average, significantly more spores were produced on the Red Stayman Winesap apples than on the Golden Delicious apples. One possible explanation for this result could be differences in nutrient content, e.g. sugars or amino acids, between the two cultivars. Nour et al. (54) reported considerable variation in biochemical characteristics and mineral content between various apple cultivars, and nutritional factors are known to influence the sporulation of fungi *in vitro* (69). Differences due to variable storage periods between Golden Delicious and Red Stayman Winesap apple cannot be discounted as a contributing factor to this observation, as well as the lack of variation in susceptibility of the two cultivars. This study used the Golden Delicious apples in the first and second experiments, and during that time, the Red Stayman Winesap apples were kept in cold storage for 4 weeks. There was a significant difference in lesion size between the first and second experiments with each cultivar, with apples exhibiting greater susceptibility in the second experiment in each case. Apples used in the second experiments had been stored for about two weeks longer than apples used in the first experiments in each case. However, there was no difference in sporulation between the first and second experiments with each cultivar, so storage effects do not explain that difference.

U.S. growers typically manage bitter rot and other fungal diseases on apples with timed applications of fungicide mixes that include different modes of action. This study tested the sensitivity of the *Colletotrichum* bitter rot species to some common fungicides

used in Kentucky orchards. On average, species within the *C. acutatum* complex were more tolerant to thiophanate-methyl, myclobutanil, trifloxystrobin, and captan, than members of the *C. gloeosporioides* species complex. Similar results have been reported previously (1; 4; 23; 29; 57; 58; 76; 77). However, this study suggests that the story is more complex than this. The *C. acutatum* species *C. fioriniae* and *C. nymphaeae* were both more tolerant to fungicides used in this study compared with the *C. gloeosporioides* species *C. siamense* and *C. fructicola*. However, *C. theobromicola,* also within the *C. gloeosporioides* complex, did not differ significantly from the two *C. acutatum* isolates in its sensitivity to fungicides; it was significantly more tolerant than the other two *C. gloeosporioides* species. Thus, a diagnosis to species complex may not be sufficient to predict fungicide sensitivity among Kentucky bitter rot isolates.

Mycelial growth of *Colletotrichum* species was significantly less inhibited by captan than thiophanate-methyl, myclobutanil, or trifloxystrobin. Captan is a protectant fungicide with a multi-site mode of action; it is normally applied in higher doses compared with single-site mode of action fungicides (36). As a protectant fungicide, captan functions as a barrier to prevent infection from occurring by preventing spore germination (53). The mean $EC50$ of captan on conidial germination of *Colletotrichum* spp. is higher than the mean $EC50$ of trifloxystrobin. It confirms that *Colletotrichum* species in general are more sensitive to trifloxystrobin than to captan. Trifloxystrobin is a single-site fungicide from the group of quinone outside inhibitors (QoI) that inhibit respiration by targeting cytochrome bc1 at the Qo site (cyt b gene), while captan is a multi-site fungicide (27). On average, spores of members of the *C. acutatum* species complex were more tolerant to captan and trifloxystrobin than members of the *C. gloeosporioides* species complex.

However, when individual species were considered, only *C. fioriniae* and *C. theobromicola* differed significantly from one another, with *C. fioriniae* being more tolerant.

In conclusion, these findings demonstrate the importance of accurate pathogen identification for bitter rot management. It also shows the necessity to identify species beyond the species complex, because species within the species complex sometimes differed significantly in traits relevant to disease management, e.g. pathogenicity and fungicide sensitivity. Further study on comparing biological behavior of species within the species complex is necessary, especially for applied purposes such as disease management.

Table 2.1. Description of morphological type of isolate based on colony color, conidial shape, presence and absence of perithecia in culture and distribution of conidia or perithecia in culture

Morphotype	Colony	Conidial shape	Perithecia	Conidia or Perithecia distribution	Species[a]
Morphotype 1	Red pigment in culture	Fusiform with pointed ends	Absence	Orange conidial masses within mycelium	*C. acutatum*
Morphotype 2	Gray/white mycelium with light orange due to conidial masses, no pigment	Fusiform with pointed ends	Absence	Orange conidial masses over entire colony	*C. acutatum*
Morphotype 3	Gray/white abundant mycelium, no pigment	Cylindrical with rounded end	Absence	Dark acervuli, small or large orange masses scattered or over entire colony	*C. gloeosporioides*
Morphotype 4	Gray/dark abundant mycelium, no pigment	Cylindrical with rounded end	Presence	Scattered small group or clump	*G. cingulata*

[a] Species designation was assigned after morphological observation

Table 2.2. Sample of *Colletotrichum* spp. isolate from symptomatic fruit in Kentucky orchards

Origin (county)	Year of isolation	Number of isolates[a]				Total isolates
		M-1	M-2	M-3	M-4	
Clay	2013	1	-	-	-	1
Whitley	2013	6	4	-	-	10
Harlan	2013	4	1	1	-	6
Bourbon	2013	150	10	33	18	211
Jessamine	2013	2	-	-	-	2
Martin	2013	19	-	1	-	20
Woodford	2013	27	-	3	-	30
Owen	2013	-	-	-	1	1
Breathitt	2013	14	-	4	-	18
Fayette	2013	34	-	21	-	55
Cumberland	2013	4	-	3	-	7
Madison	2013	12	-	-	-	12
Clinton	2013	-	-	3	-	3
Knott	2013	3	-	-	-	3
Perry	2013	-	-	3	-	3
Nelson	2013	20	-	-	-	20
Bell	2013	11	-	1	-	12
Hancock	2013	4	-	-	-	4
Magoffin	2013	11	-	-	-	11
Lyon	2013	-	-	12	-	12
Marshall	2013	7	-	3	-	10
Montgomery	2013	-	-	17	-	17
Estill	2013	4	-	-	-	4
Allen	2013	2	-	-	-	2
Caldwell	2013	-	-	1	-	1

[a] M-1 indicates Morphotype 1; M-2 indicates Morphotype 2; M-3 indicates Morphotype 3; M-4 indicates Morphotype 4.

Table 2.3. Test isolates of *Colletotrichum* spp. isolates from Kentucky orchards

Isolates[a]	Host (cultivar)	Origin (county)	Region of Kentucky	Morphotype
HC 296	Honeycrisp	Bourbon	North	1
KY 228	Unknown	Fayette	North	1
KY 95	Jona Gold	Madison	North	1
KY 123	Jonathan	Bourbon	North	1
HC 557	Honeycrisp	Bourbon	North	1
KY 28	Gibson Golden	Martin	East	1
KY 6	Empire	Harlan	East	1
KY 105A	Unknown	Whitley	East	1
KY 320	Unknown	Allen	Central	1
KY 162	Unknown	Nelson	Central	1
KY 291	Granny Smith	Marshall	West	1
KY 191	Unknown	Hancock	West	1
KY 9	Empire	Harlan	East	2
HC 646	Honeycrisp	Bourbon	North	2
HC 647	Honeycrisp	Bourbon	North	2
KY 263	Pink Lady	Montgomery	North	3
KY 254	Unknown	Lyon	West	3
KY 301	Granny Smith	Marshall	West	3
KY 146	Unknown	Clinton	Central	3
KY 305	Unknown	Cumberland	Central	3
KY 152	Unknown	Perry	East	3
KY 153	Unknown	Perry	East	3
KY 128	Gold Rush	Bourbon	North	3
HC 540	Honeycrisp	Bourbon	North	4
KY 40	Unknown	Owen	North	4
KY 8	Unknown	Harlan	East	3

[a] Isolates were selected as representative of each morphotype and region from where the isolates were collected

Table 2.4. Average growth rate of *Colletotrichum* isolates at five selected temperatures

Temperature (°C)	Mean growth rate (mm/day)[x]
18	2.67 a
21	3.25 b
23	4.69 c
25	6.12 d
30	3.90 e

[x] Values followed by the same letter were not significantly different based on Duncan's multiple range test ($P < 0.05$).

Table 2.5. Average growth rate of each morphotype at five selected temperatures

Morphotype	Average growth rate (mm/day)					Mean (mm/day)[x]
	18	21	23	25	30	
Morphotype-1	2.59	2.94	4.43	5.69	2.76	3.68 a
Morphotype-2	2.70	3.08	3.32	5.05	3.01	3.43 b
Morphotype-3	2.74	3.83	5.15	6.75	5.30	4.75 c
Morphotype-4	2.79	2.70	6.25	7.42	5.78	4.99 d

[x] Values followed by the same letter were not significantly different based on Duncan's multiple range test ($P < 0.05$).
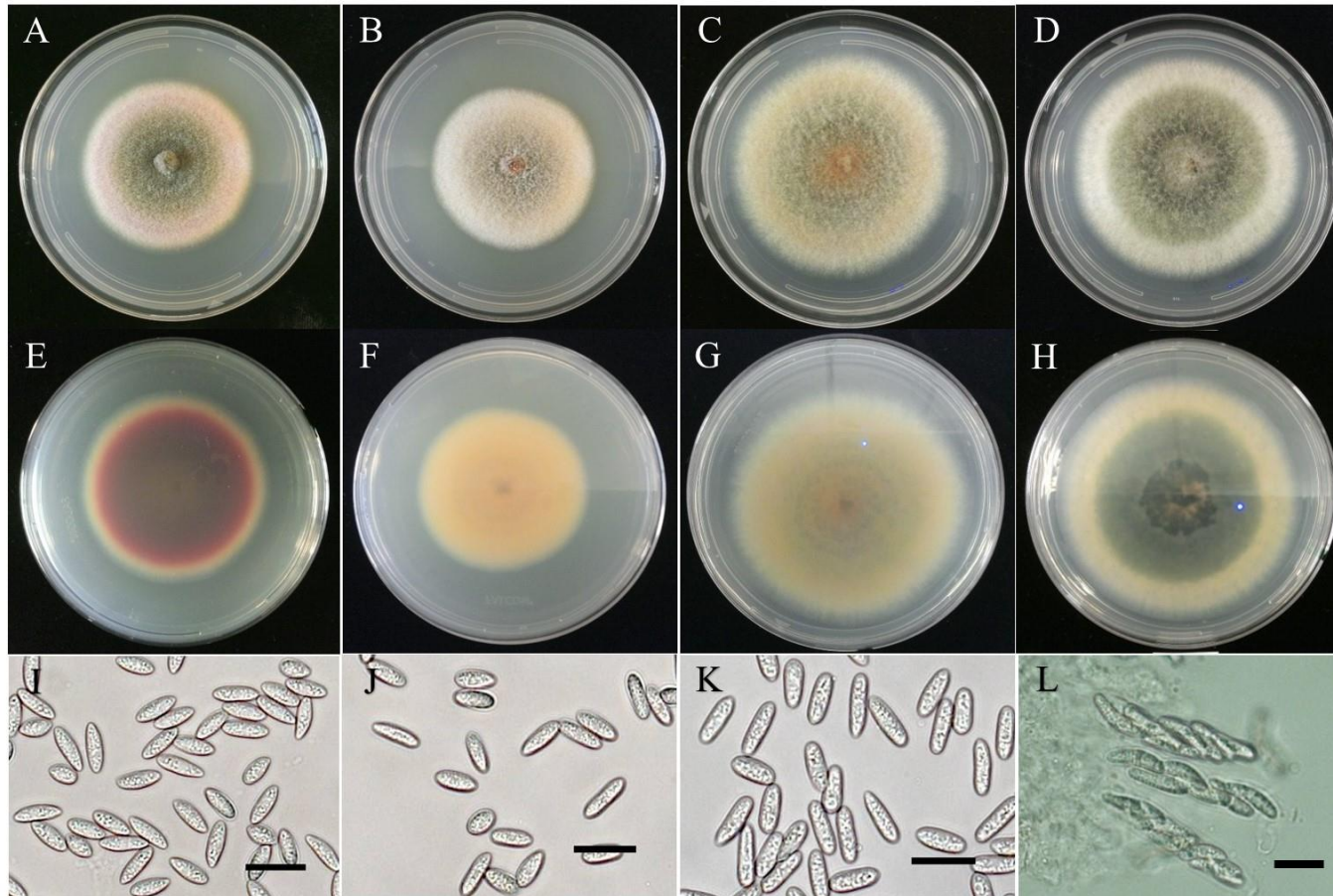
Figure 2. 1: Morphological types found among isolates of *Colletotrichum* spp. isolated from symptomatic fruit collected in several orchards in Kentucky. Characterization was based on colony color, conidial shape, and the ability to produce sexual spores. Colony color of (A, E) Morphotype 1; (B, F) Morphotype 2; (C, G) Morphotype 3; and (D, H) Morphotype 4. Colony color was observed from (A, B, C, D) the top; and (E, F, G, H) the bottom. Conidial shape of (I) Morphotype 1; (J) Morphotype 2; (K) Morphotype 3; and asci of (L) Morphotype 4. Colonies were 7 days old. Bars = 20 μm.

Figure 2.2. The number and the proportion of isolates within each morphotype from a total of 475 isolates collected from Kentucky orchards.
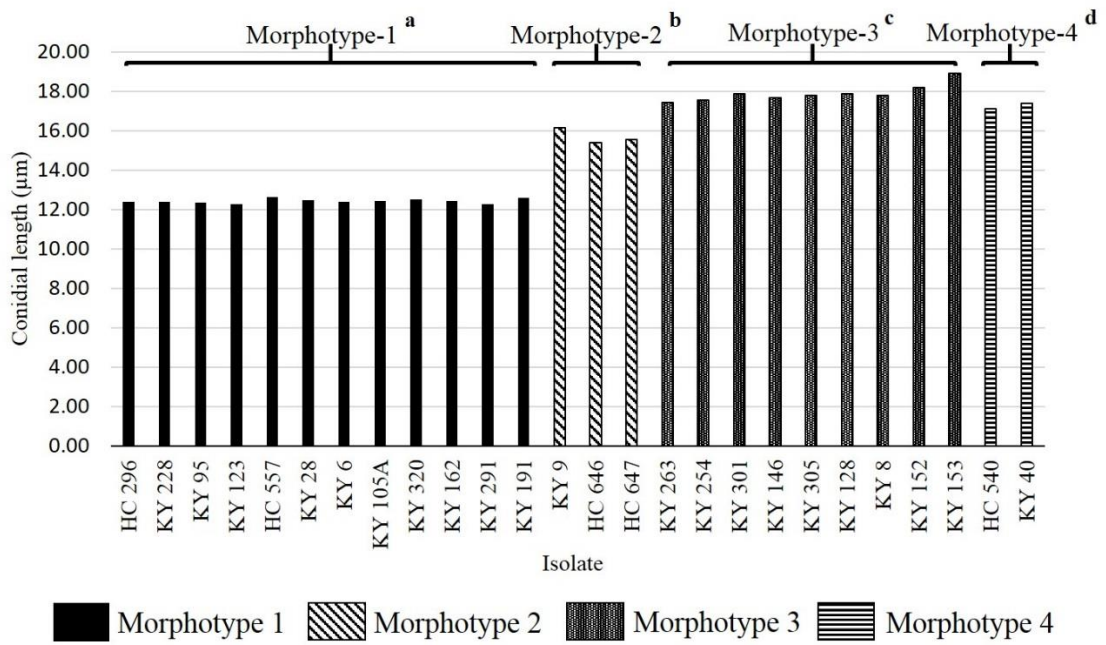
Figure 2.3. Conidial length among morphotypes. Morphotype name followed by the same superscript letter were not significantly different using Duncan's multiple range test (P < 0.05).
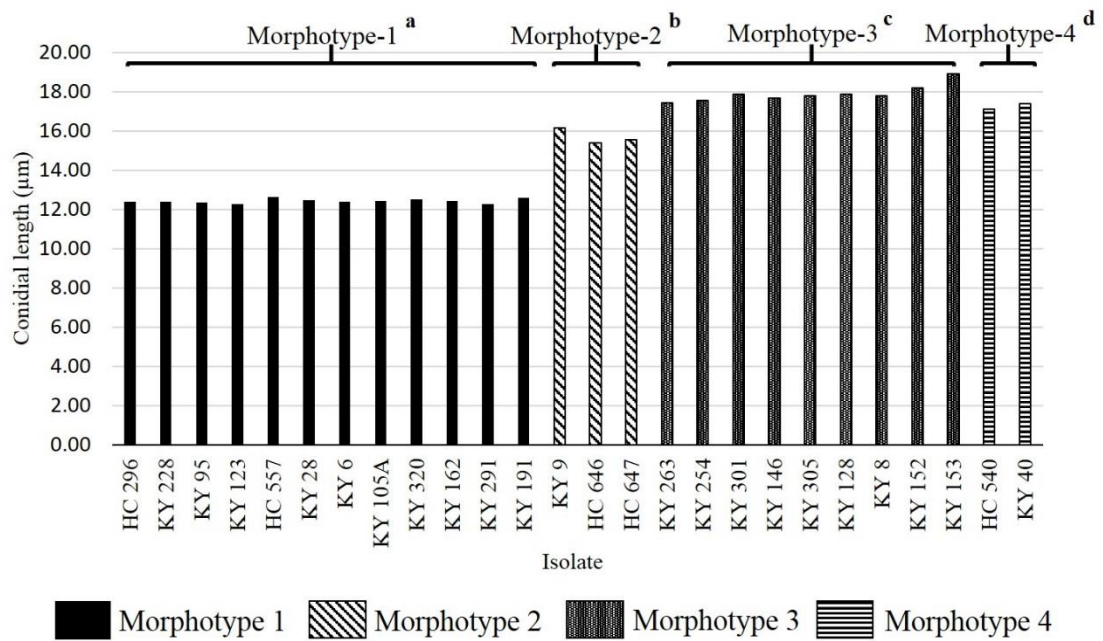
Figure 2.4. Conidial width among morphotypes. Morphotype name followed by the same superscript letter were not significantly different using Duncan's multiple range test (P < 0.05).
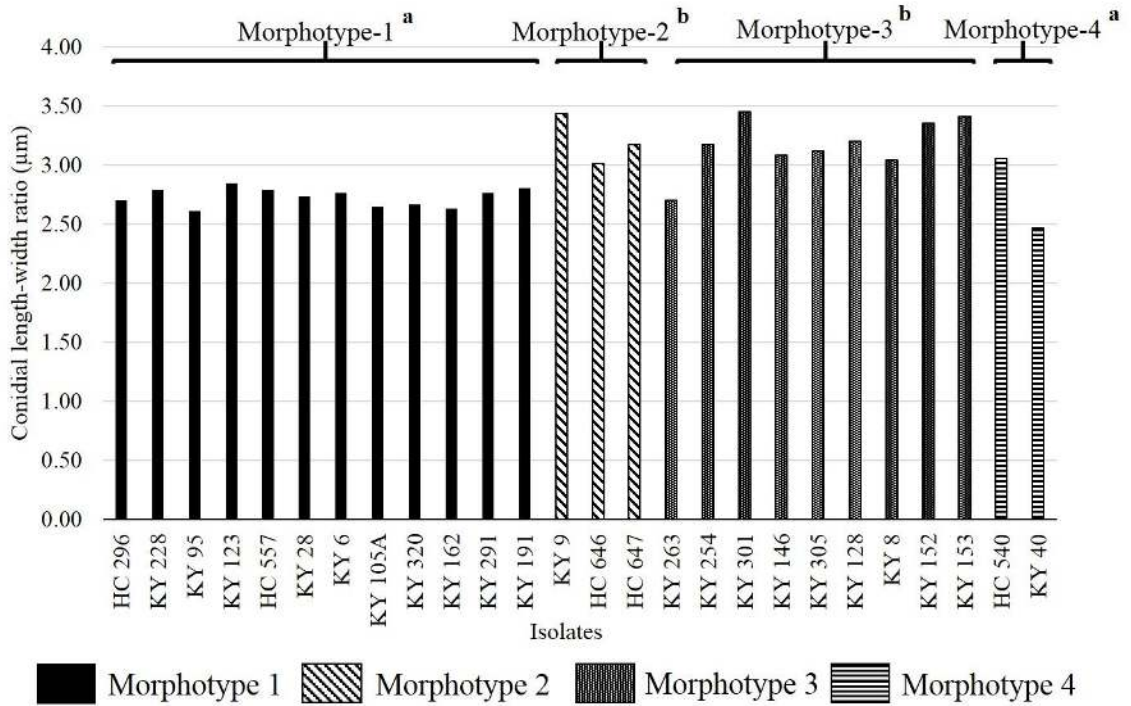
Figure 2.5. Conidial length-width ratio among morphotypes. Morphotype name followed by the same superscript letter were not significantly different using Duncan's multiple range test (P < 0.05).
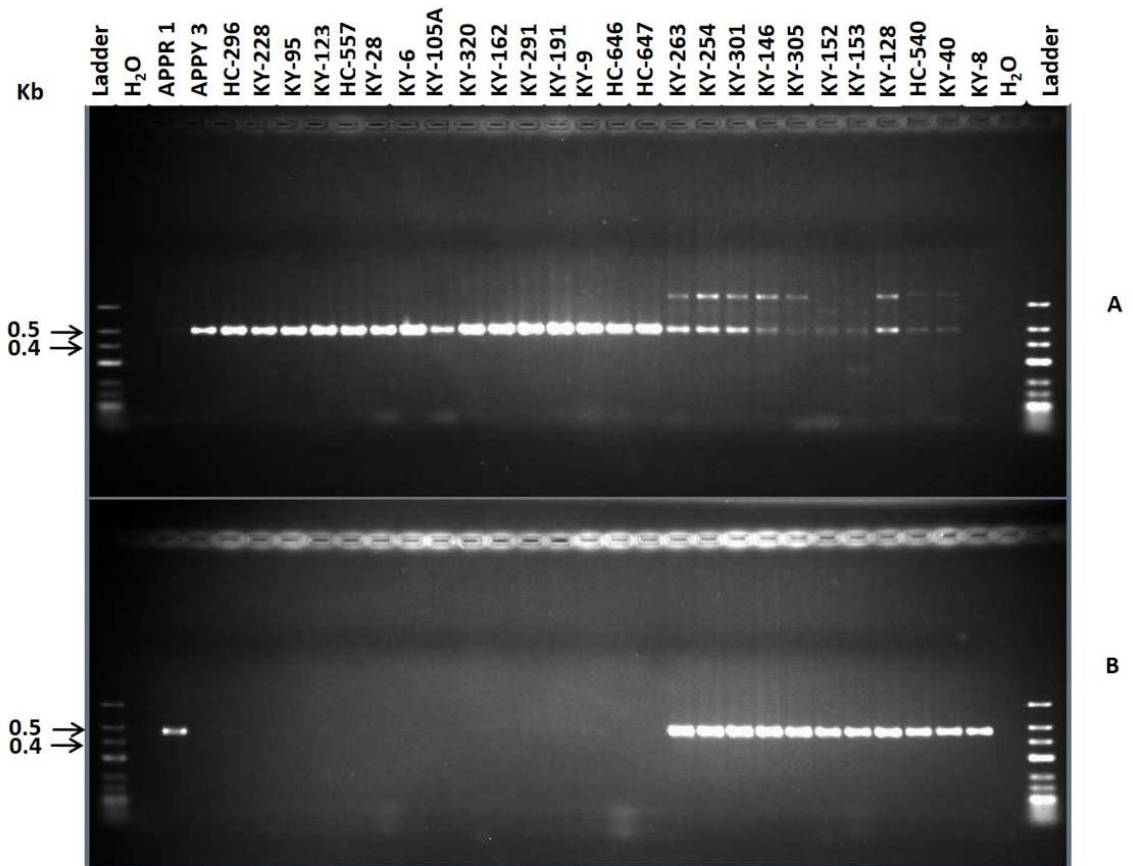
Figure 2.6. Species identification by using species-specific primers of *C. acutatum* (A – primer CaInt2 in combination with primer ITS4) and *C. gloeosporioides* (B – primer CgInt in combination with primer ITS4). Ladder: O'Gene Ruler
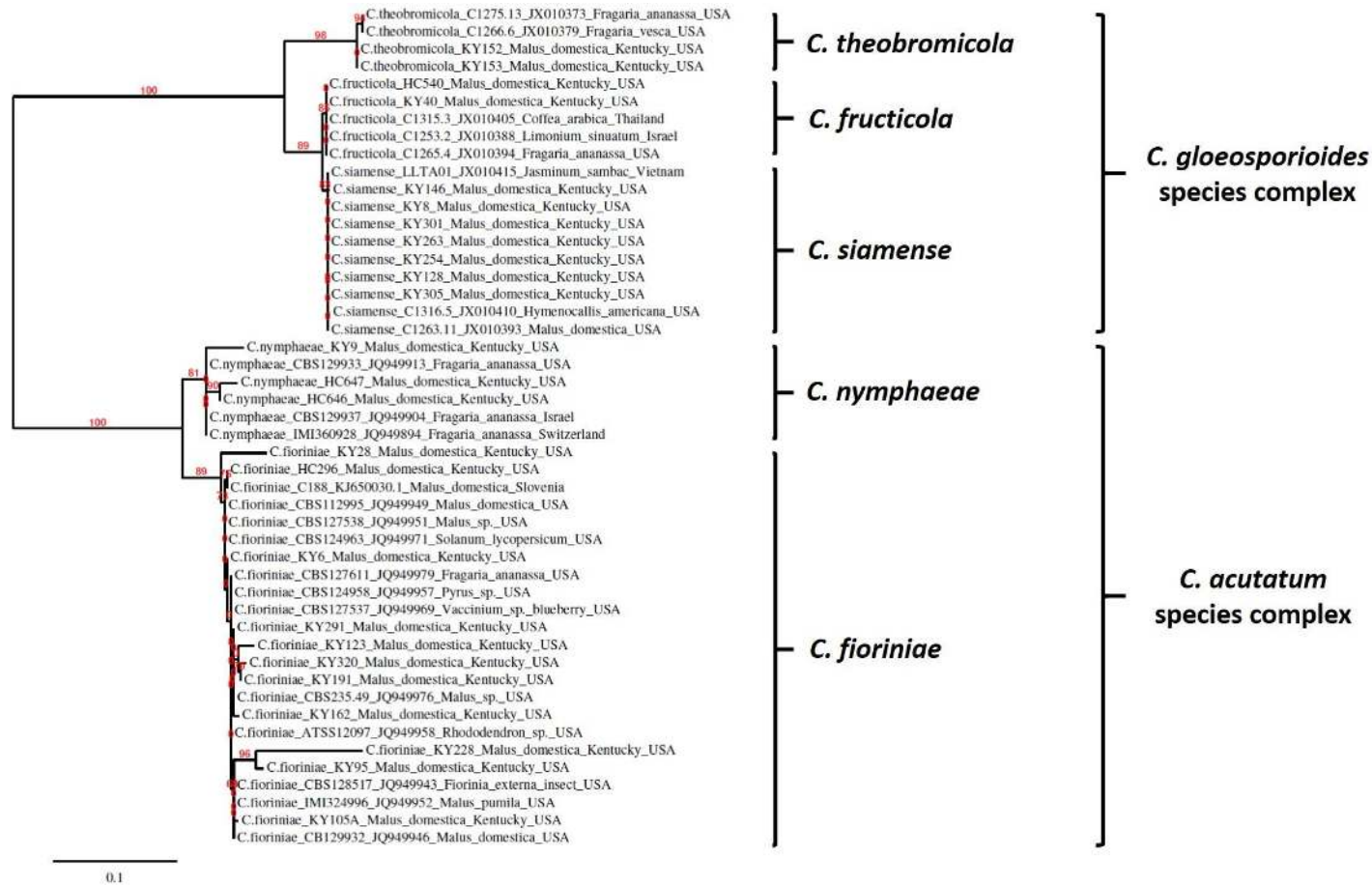
Figure 2.7. Un-rooted *TUB2*-based phylogenetic tree. The tree was produced using PhyML based on approximate Likelihood Ratio Test (aLRT) and TreeDyn for tree rendering. Numbers at each branch indicate percentage of branch support values based on maximum likelihood.
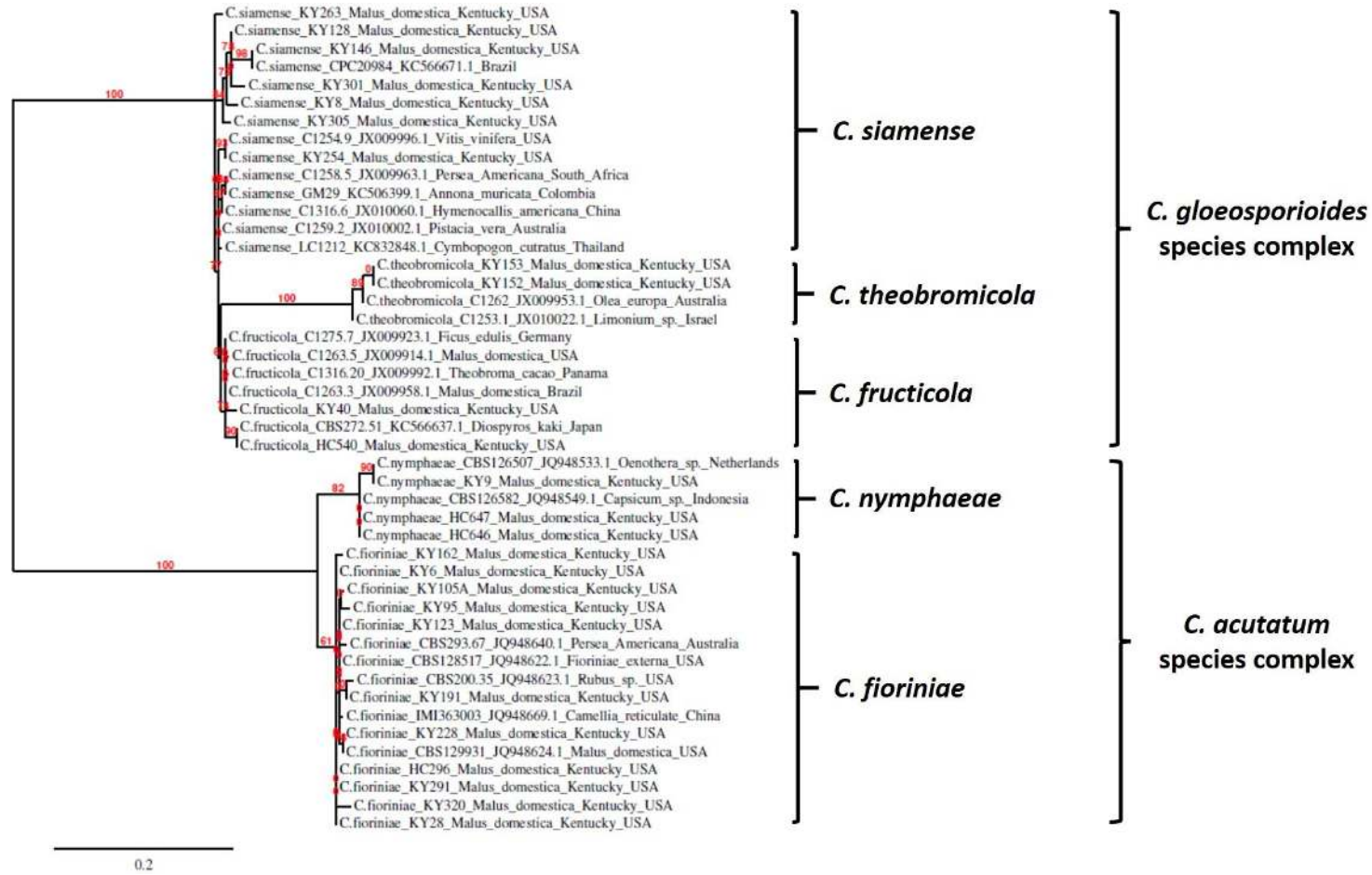
Figure 2.8. Un-rooted *GAPDH*-based phylogenetic tree. The tree was produced using PhyML based on approximate Likelihood Ratio Test (aLRT) and TreeDyn for tree rendering. Numbers at each branch indicate percentage of branch support values based on maximum likelihood.
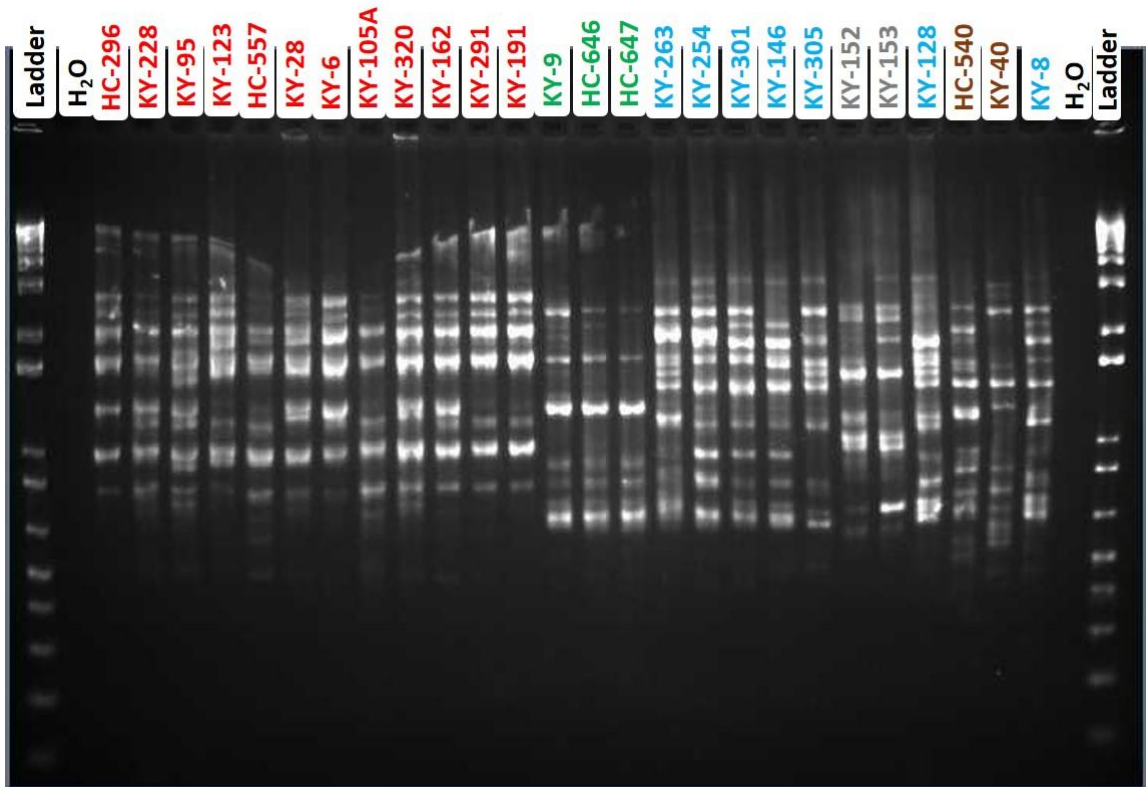
Figure 2.9. Banding patterns of random amplified polymorphic DNA (RAPD) of *Colletotrichum* spp. using primers OPA13 Ladder: 1 kb Plus DNA ladder. Colors show species according to multilocus based identification (red = *C. fioriniae*; green = *C. nymphaeae*; blue = *C. siamense*; grey = *C. theobromicola* and brown = *C. fructicola*).
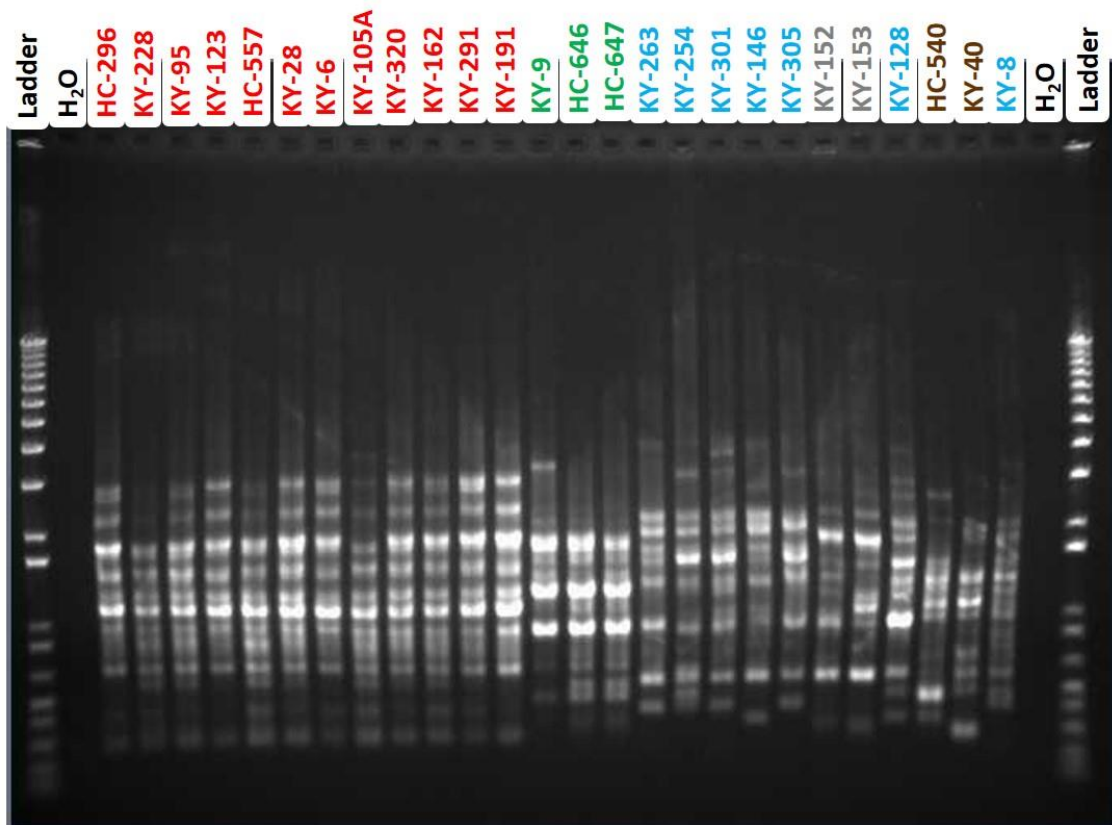
Figure 2.10. Banding patterns of random amplified polymorphic DNA (RAPD) of *Colletotrichum* spp. using primers OPA18 Ladder: 1 kb Plus DNA ladder. Colors show species according to multilocus based identification (red = *C. fioriniae*; green = *C. nymphaeae*; blue = *C. siamense*; grey = *C. theobromicola* and brown = *C. fructicola*).
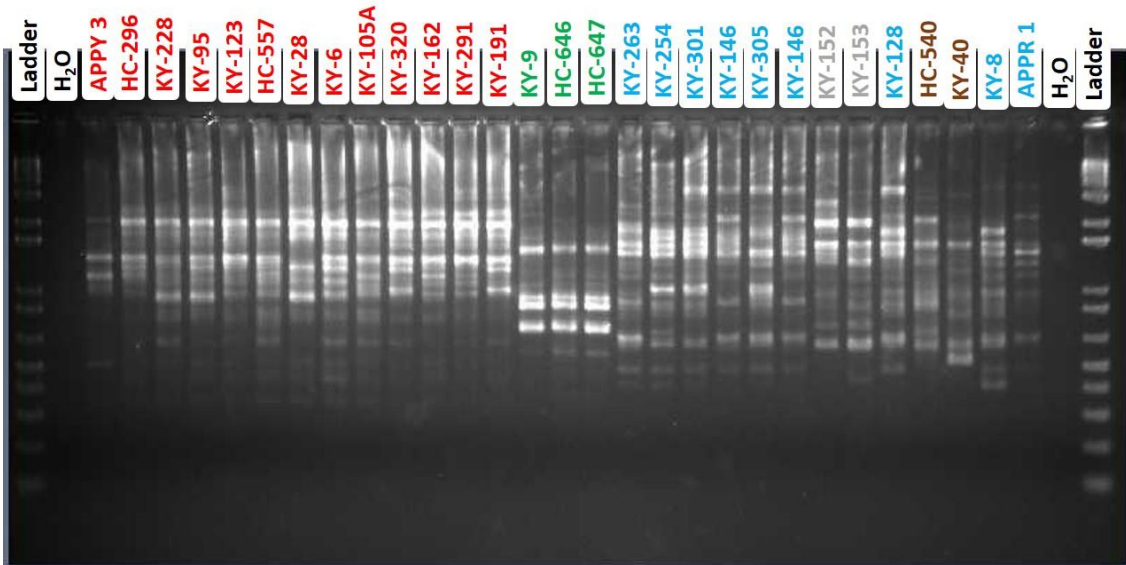
Figure 2.11. Banding patterns of random amplified polymorphic DNA (RAPD) of *Colletotrichum* spp. using primers UBC 356 Ladder: 1 kb Plus DNA ladder. Colors show species according to multilocus based identification (red = *C. fioriniae*; green = *C. nymphaeae*; blue = *C. siamense*; grey = *C. theobromicola* and brown = *C. fructicola*).
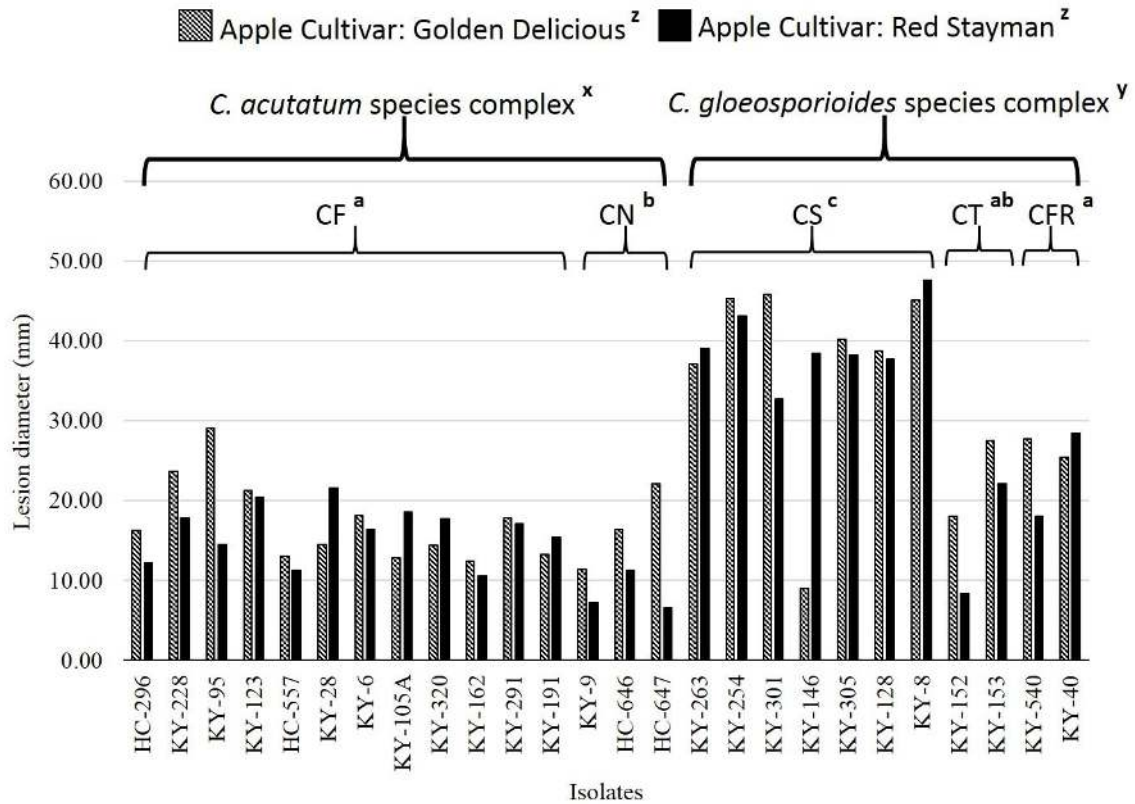
Figure 2.12. Lesion diameter caused by *Colletotrichum* spp. Species name indicated with CF = *C. fioriniae*; CN = *C. nymphaeae*; CS = *C. siamense*; CT = *C. theobromicola*; CFR = *C. fructicola*. Species name, species complex, and apple cultivar followed by the same superscript letter were not significantly different based on Least Square Means, Adjustment for Multiple Comparison: Bonferroni.
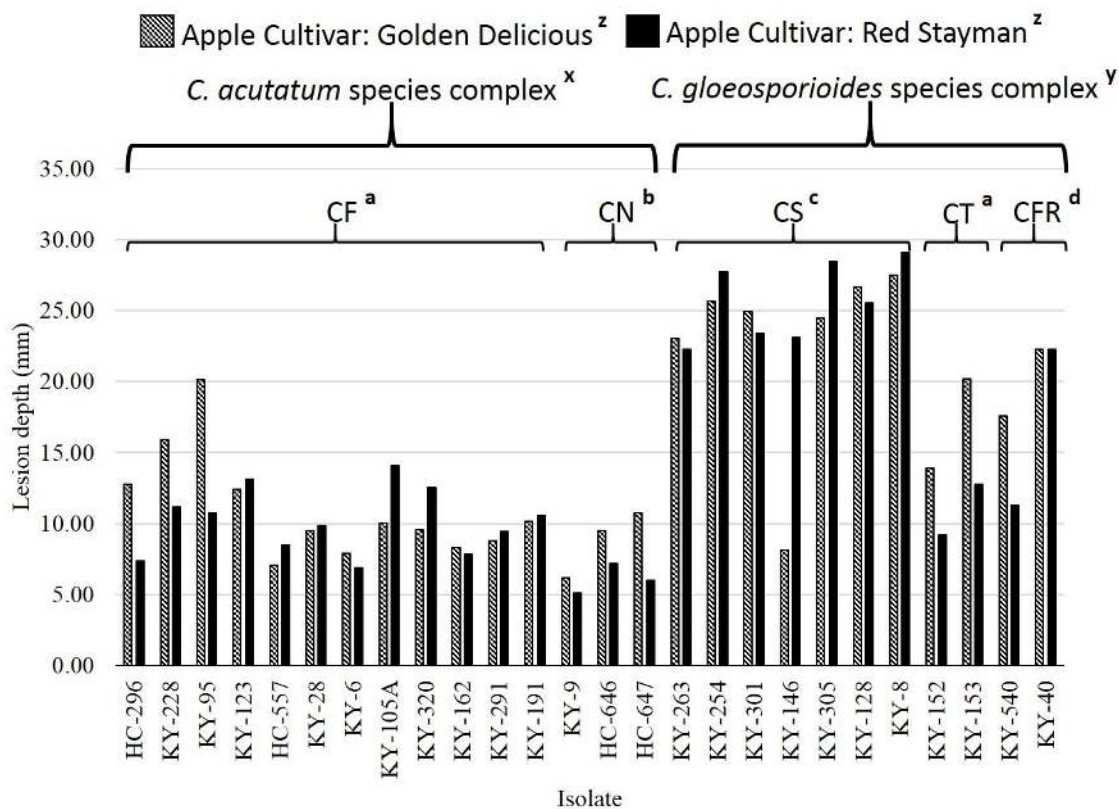
Figure 2.13. Lesion depth caused by *Colletotrichum* spp. Species name indicated with CF = *C. fioriniae*; CN = *C. nymphaeae*; CS = *C. siamense*; CT = *C. theobromicola*; CFR = *C. fructicola*. Species name, species complex, and apple cultivar followed by the same superscript letter were not significantly different based on Least Square Means, Adjustment for Multiple Comparison: Bonferroni.
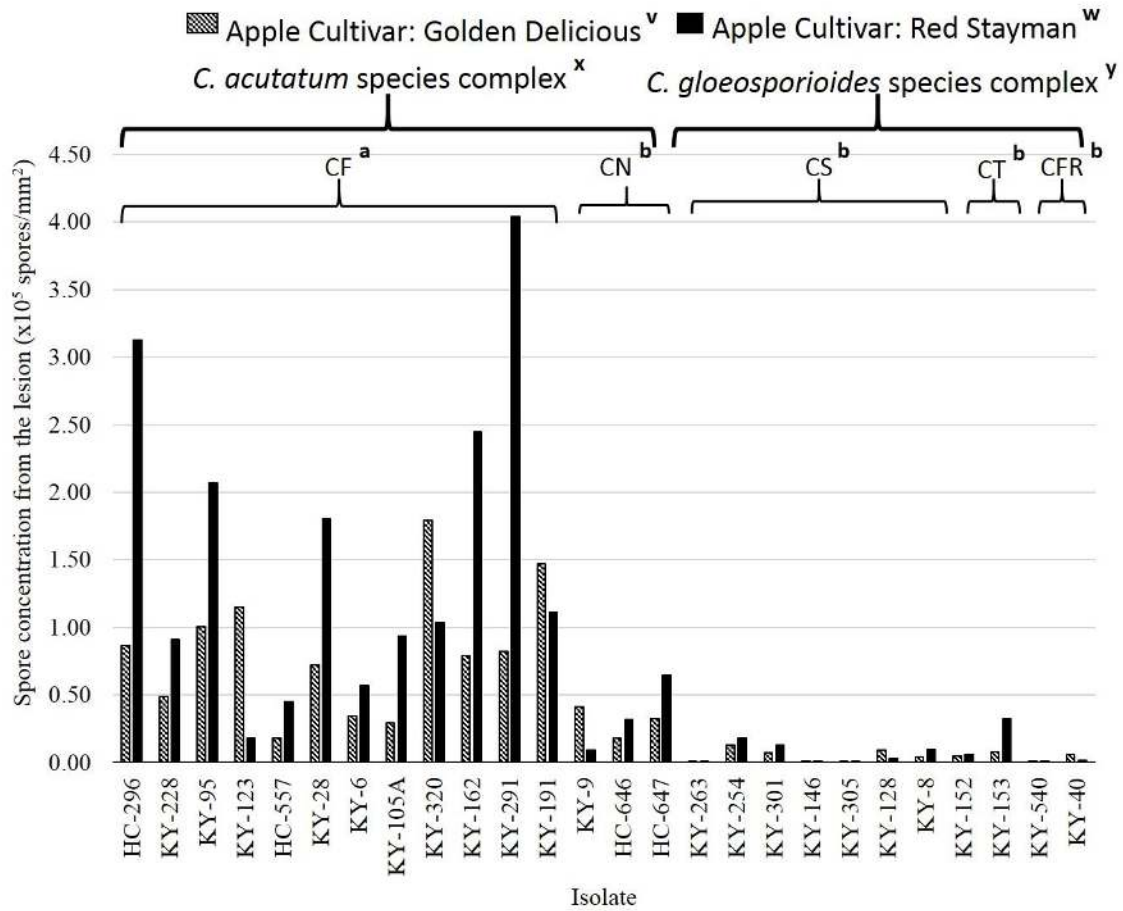
Figure 2.14. Spore concentration from the lesion on detached apple infected with *Colletotrichum* spp. Species name indicated with CF = *C. fioriniae*; CN = *C. nymphaeae*; CS = *C. siamense*; CT = *C. theobromicola*; CFR = *C. fructicola*. Species name, species complex, and apple cultivar followed by the same superscript letter were not significantly different based on Least Square Means, Adjustment for Multiple Comparison: Bonferroni.
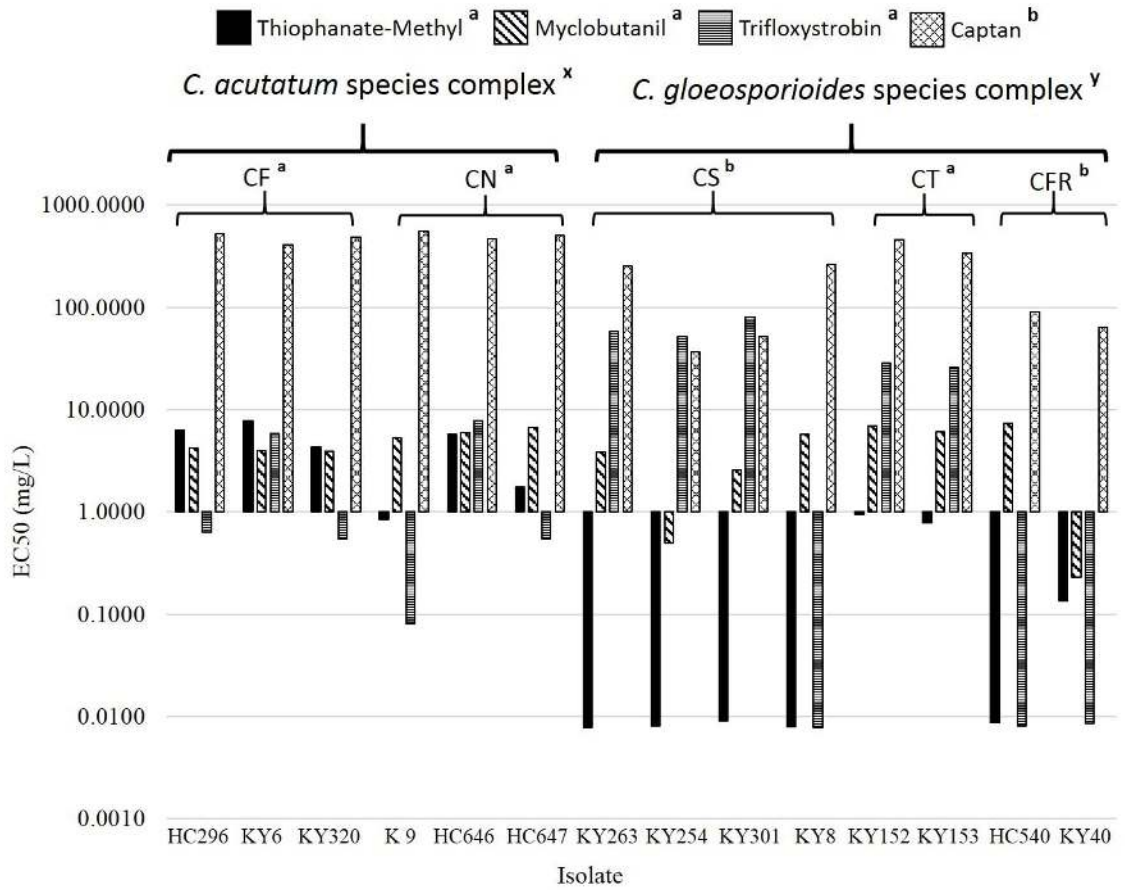
Figure 2.15. EC50 of four selected fungicides on mycelial growth of *Colletotrichum* spp. Species name indicated with CF = *C. fioriniae*; CN = *C. nymphaeae*; CS = *C. siamense*; CT = *C. theobromicola*; CFR = *C. fructicola*. Species name, Species complex, and fungicides followed by the same superscript letter were not significantly different based on Least Square Means, Adjustment for Multiple Comparison: Bonferroni.
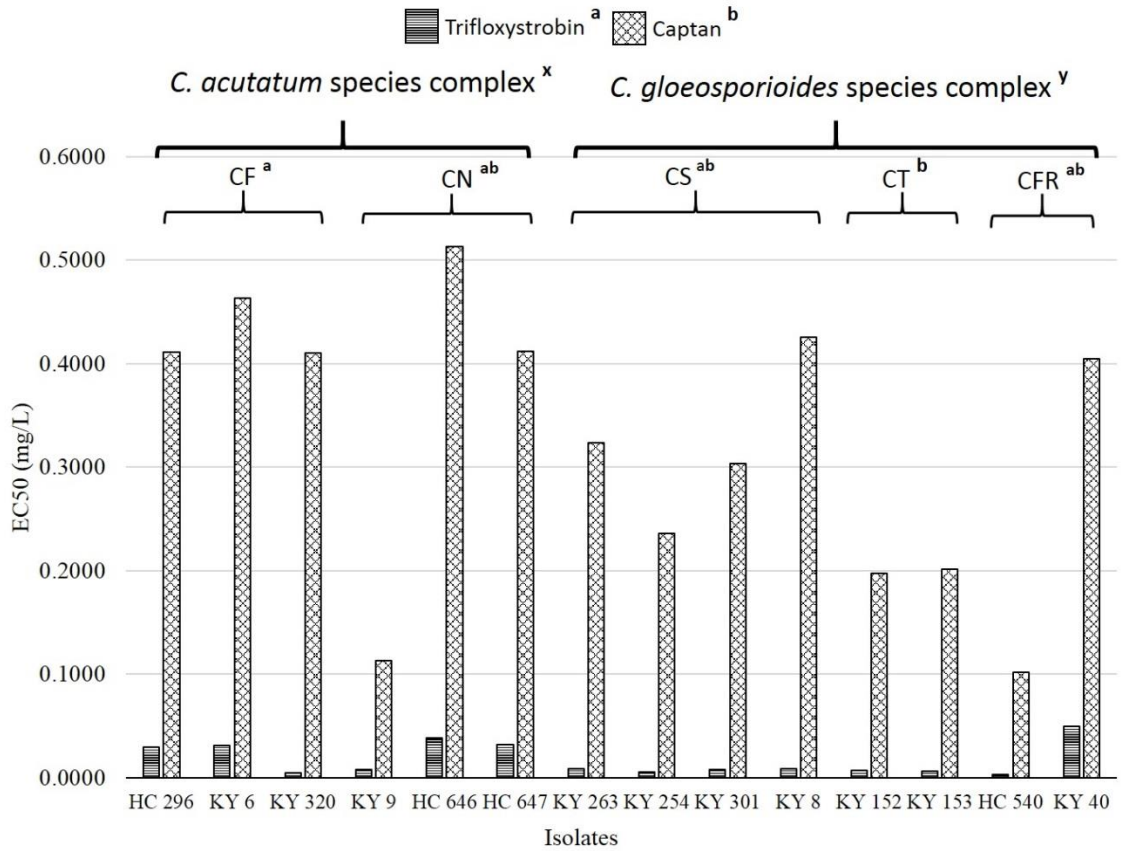
Figure 2.16. EC50 of two selected fungicides on spore germination of *Colletotrichum* spp. Species name indicated with CF = *C. fioriniae*; CN = *C. nymphaeae*; CS = *C. siamense*; CT = *C. theobromicola*; CFR = *C. fructicola*. Species name, Species complex, and fungicides followed by the same superscript letter were not significantly different based on Least Square Means, Adjustment for Multiple Comparison: Bonferroni

CHAPTER THREE: SUMMARY AND SIGNIFICANCE OF THE STUDY

Morphology-based identification, as well as species-specific primer analyses, revealed that the *Colletotrichum* isolates sampled from bitter rot lesions in symptomatic apple from Kentucky orchards belong to two species complexes: the *C. acutatum* species complex and the *C. gloeosporioides* species complex. Multi-locus gene sequence-based identification using the TUB2 and GAPDH genes identified species within the *C. acutatum* species complex as *C. fioriniae* and *C. nymphaeae*, and species within the *C. gloeosporioides* species complex as *C. siamense, C. theobromicola,* and *C. fructicola*. *Colletotrichum fioriniae* is distinguished from the other species by the production of a red pigment in culture, and this enabled *C. fioriniae* to be recognized as the most abundant bitter rot species in Kentucky (approximately 70% of the total number of isolates). Pathogenicity to apple fruit differs between the two species complexes, as well as among the species within each species complex. The *C. gloeosporioides* species complex was more aggressive than the *C. acutatum* species complex, on average. *Colletotrichum siamense* was the most aggressive species among the five, causing larger and deeper lesions. The sensitivity of *Colletotrichum* species to thiophanate-methyl, myclobutanil, trifloxystrobin and captan was different, on average, between the two species complexes, as well as among each species within each species complex. The *C. acutatum* species complex was more tolerant to tested fungicides compared to the *C. gloeosporioides* species complex. Among all five species, *C. fioriniae* was the most tolerant to the fungicides used in this study. Fingerprinting using RAPD analyses suggested that *C. fioriniae* represented a relatively homogenous population. Relatively diverse RAPD banding patterns were

observed within species in the *C. gloeosporioides* species complex, indicating a potentially higher level of diversity.

The overall differences observed between the two species complexes causing bitter rot were consistent with previous reports in the literature. However, this study is the first, to my knowledge, to thoroughly examine differences among species within species complexes in the U.S. and one of the first worldwide. It revealed that there are differences in pathogenicity and fungicide sensitivity among these species that could have significant implications for disease management. A diagnosis to species complex is not sufficient for prediction of behavior for these traits.

Accurate pathogen identification is the first step in the process of developing an effective disease management program. *Colletotrichum* species identification can be challenging due to morphological similarity among species and morphological plasticity in culture. In addition, the wide range of hosts and the ability of some *Colletotrichum* species to infect the same host make species boundaries ambiguous and confusing. This study included cases in which several *Colletotrichum* species were found infecting the same tree in the same orchard. Moreover, two different species were sometimes found in the same lesion. Different *Colletotrichum* species cause very similar bitter rot symptoms, which makes disease diagnosis in the field even more challenging. In the meantime, errors in disease diagnoses may result in ineffective disease control.

Each pathogen identification method used to address the complexity of *Colletotrichum* species identification has advantages and disadvantages. The traditional identification technique, which is based only on pathogen morphology, seems to be straightforward. However, it is time-consuming and this study clearly exhibited the

inadequacy of morphologically-based identification to differentiate *Colletotrichum* species. Identification by sequencing or species-specific amplification provided rapid and reliable identification to species complex by using PCR. However, the disadvantages of this method include the possibility for DNA contamination or inaccurate identifications of standards in Genbank, as well as insufficient resolution to differentiate species within species complexes of *Colletotrichum*. Multi-locus sequence based identification is currently the preferred method for discrimination of species within the species complexes, and it was applied successfully for this study. However, this technique required more advanced methods including sequencing technology and cost more compared to other techniques.

My research findings demonstrated the importance of identifying and studying the behavior of species within the species complexes of *Colletotrichum*. Findings suggest that species within each species complex differed in pathogenicity and in fungicide sensitivities. Thus it is important to be able to apply multi-locus sequence-based identification. Future work should include development of specific primer pairs based on the variable multi-locus sequences.

For the apple industry in Kentucky, these research findings provide some important information about the causal agent of bitter rot. Most importantly, *C. fioriniae* appears to be the most common species causing bitter rot in Kentucky orchards; it also appears to be relatively tolerant to common fungicides that are used in Kentucky orchards. However, the EC50 of some common fungicides to *C. fioriniae* showed in this study are still far below the lowest label rates; thus thiophanate-methyl, myclobutanil, trifloxystrobin and captan should still provide control for *C. fioriniae* in Kentucky orchards, provided optimal

conditions and coverages with fungicides are achieved. However, optimal condition and optimal coverage of fungicide in fields is often difficult to achieve, and this may lead to variation in efficacy. Applications of protectant fungicides and/or other fungicides based on local recommendations is critical. Growers should also incorporate cultural practices such as maintaining orchard sanitation and monitoring disease incidence and disease spread.

Preliminary fingerprinting analyses using the RAPD technique suggested that the dominant *C. fioriniae* population within the state was relatively homogenous. However, further studies on the genetic diversity of isolates using more stringent fingerprinting techniques such as RFLP are necessary to evaluate the genetic diversity of species. Genetic diversity data will be useful to describe how the population disperses in nature. A genetic diversity study might also be expanded to include these same species that infect different crops surrounding orchards, such as blueberry and strawberry, in order to study cross-infection potential, diseases cycle and potential cultural practices to control diseases caused by these pathogens. Cross infection potential of species could also be evaluated by conducting infectivity tests (Koch's postulates) to different fruits.

Differences in sporulation of *Colletotrichum* spp. on two different apple cultivars suggest that planting more resistant or tolerant cultivars might be able to reduce the rate of disease spread in Kentucky orchards. However, field research would be needed to test this hypothesis, since my work was done in the laboratory with a limited numbers of isolates. Field studies on the pathogenicity of species-within-species complex is needed to illustrate the ability of each species in causing disease in orchards and to show whether or not some species are experiencing fitness penalty in field.

Appendix One: Morphological Types of Tested Isolates

Table A1.1. Morphological types of 26 tested isolates

Isolates	Morphological type	Colony	Conidial shape	Perithecia	Conidia or Perithecia distribution	Identification
HC 296	Morphotype 1	Red pigment in culture	Fusiform with pointed ends	absence	Orange conidial masses within mycelium	*C. acutatum*
KY 228	Morphotype 1	Red pigment in culture	Fusiform with pointed ends	absence	Orange conidial masses within mycelium	*C. acutatum*
KY 95	Morphotype 1	Red pigment in culture	Fusiform with pointed ends	absence	Orange conidial masses within mycelium	*C. acutatum*
KY 123	Morphotype 1	Red pigment in culture	Fusiform with pointed ends	absence	Orange conidial masses within mycelium	*C. acutatum*
HC 557	Morphotype 1	Red pigment in culture	Fusiform with pointed ends	absence	Orange conidial masses within mycelium	*C. acutatum*
KY 28	Morphotype 1	Red pigment in culture	Fusiform with pointed ends	absence	Orange conidial masses within mycelium	*C. acutatum*
KY 6	Morphotype 1	Red pigment in culture	Fusiform with pointed ends	absence	Orange conidial masses within mycelium	*C. acutatum*
KY 105A	Morphotype 1	Red slightly yellow pigment in culture	Fusiform with pointed ends	absence	Orange conidial masses within mycelium	*C. acutatum*
KY 320	Morphotype 1	Red pigment in culture	Fusiform with pointed ends	absence	Orange conidial masses within mycelium	*C. acutatum*
KY 162	Morphotype 1	Red pigment in culture	Fusiform with pointed ends	absence	Orange conidial masses within mycelium	*C. acutatum*
KY 291	Morphotype 1	Red pigment in culture	Fusiform with pointed ends	absence	Orange conidial masses within mycelium	*C. acutatum*
KY 191	Morphotype 1	Red pigment in culture	Fusiform with pointed ends	absence	Orange conidial masses within mycelium	*C. acutatum*
KY 9	Morphotype 2	Gray mycelium, no pigment	Fusiform but with more rounded ends	absence	Orange conidial masses over entire colony	*C. acutatum*
HC 646	Morphotype 2	Gray mycelium, no pigment	Fusiform but with more rounded ends	absence	Orange conidial masses over entire colony	*C. acutatum*
HC 647	Morphotype 2	Gray mycelium, no pigment	Fusiform but with more rounded ends	absence	Orange conidial masses over entire colony	*C. acutatum*
KY 263	Morphotype 3	Gray abundant, no pigment	Cylindrical with rounded end	absence	Large orange scattered masses	*C. gloeosporioides*
KY 254	Morphotype 3	Gray abundant mycelium, no pigment	Cylindrical with rounded end	absence	Small orange masses over entire colony	*C. gloeosporioides*
KY 301	Morphotype 3	Gray abundant mycelium, no pigment	Cylindrical with rounded end	absence	Small orange masses over entire colony	*C. gloeosporioides*
KY 146	Morphotype 3	Gray abundant mycelium, no pigment	Cylindrical with rounded end	absence	Dark acervulli, conidia mostly within mycelium	*C. gloeosporioides*
KY 305	Morphotype 3	Gray abundant mycelium, no pigment	Cylindrical with rounded end	absence	Dark acervulli, conidia mostly within mycelium	*C. gloeosporioides*
KY 152	Morphotype 3	Gray abundant mycelium, no pigment	Cylindrical with rounded end	absence	Dark acervulli, conidia mostly within mycelium	*C. gloeosporioides*
KY 153	Morphotype 3	Gray abundant mycelium, no pigment	Cylindrical with rounded end	absence	Dark acervulli, conidia mostly within mycelium	*C. gloeosporioides*
KY 128	Morphotype 3	Gray abundant mycelium, no pigment	Cylindrical with rounded end	absence	Dark acervulli, conidia mostly within mycelium	*C. gloeosporioides*

Table A.1. Continued

Isolates	Morphological type	Colony	Conidial shape	Perithecia	Conidia or Perithecia distribution	Identification
HC 540	Morphotype 4	Dark gray abundant mycelium	Cylindrical with rounded end	presence	Scattered small group or clump	*G. cingulata*
KY 40	Morphotype 4	Dark gray abundant mycelium	Cylindrical with rounded end	presence	Scattered small group or clump	*G. cingulata*
KY 8	Morphotype 3	Gray abundant mycelium, no pigment	Cylindrical with rounded end	absence	Dark acervulli, sometime large orange scattered masses appear	*C. gloeosporioides*

Morphotype-1 isolates

(Left to right = upper colony surface-lower colony surface-conidia; bars = 20μm)
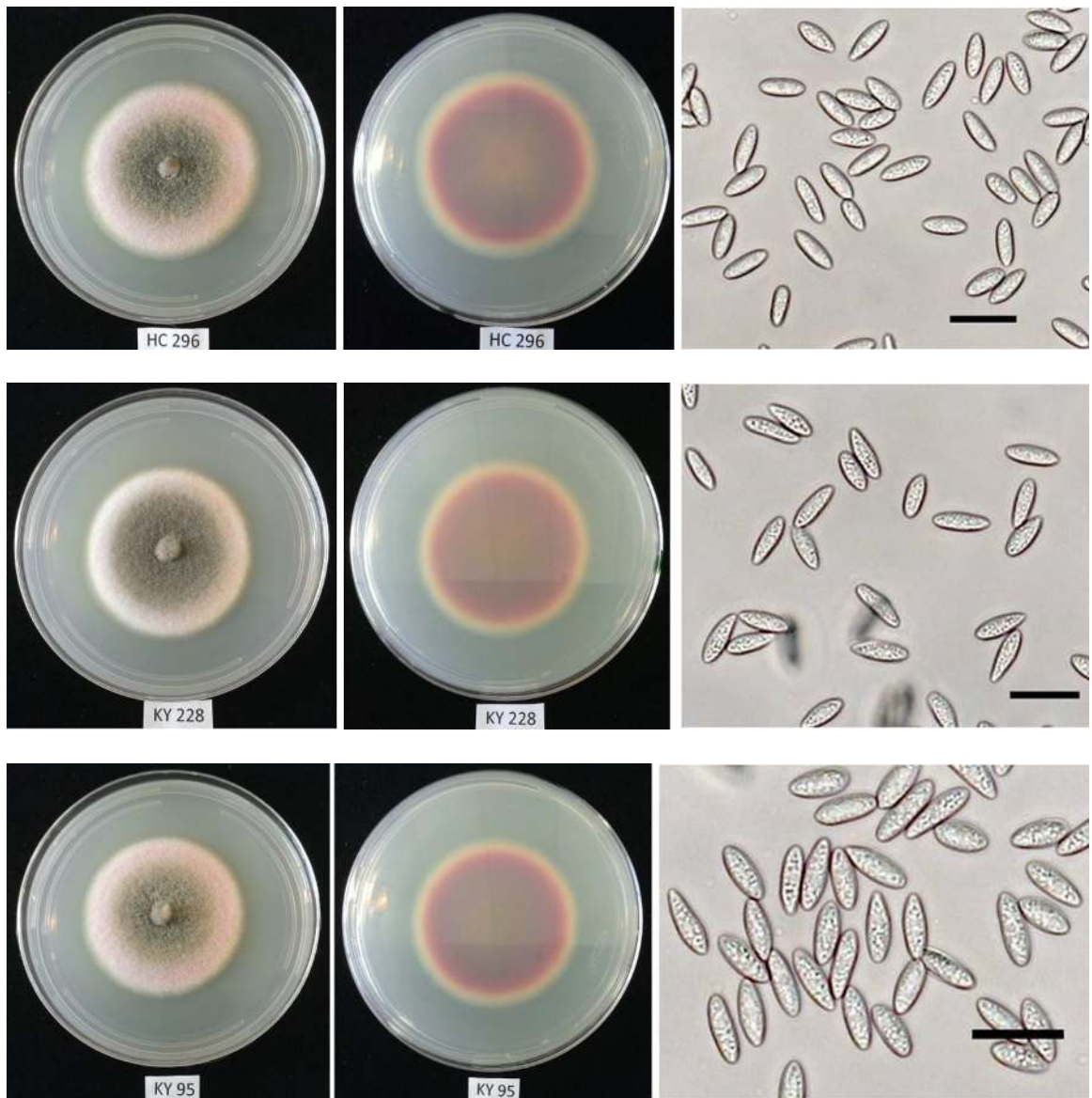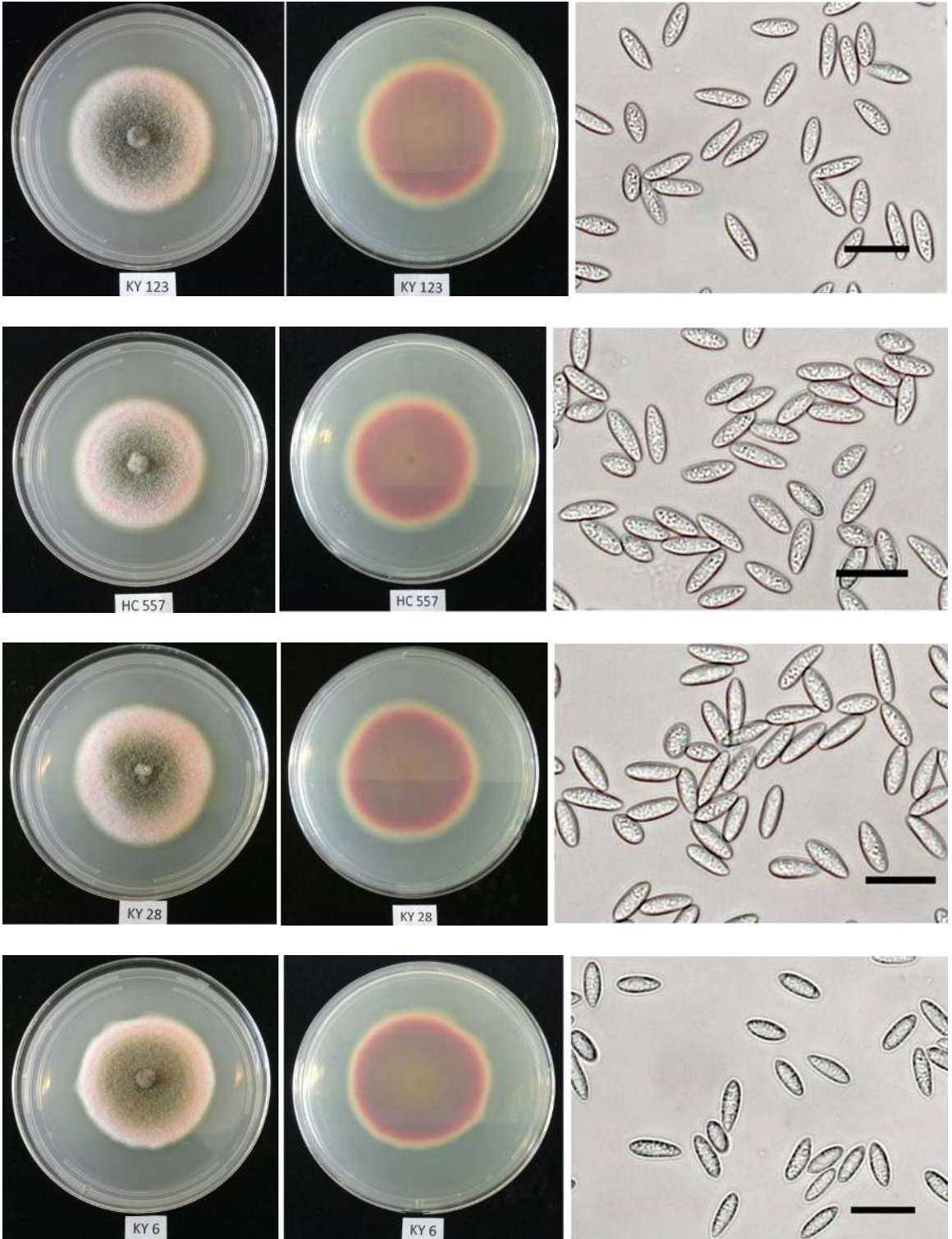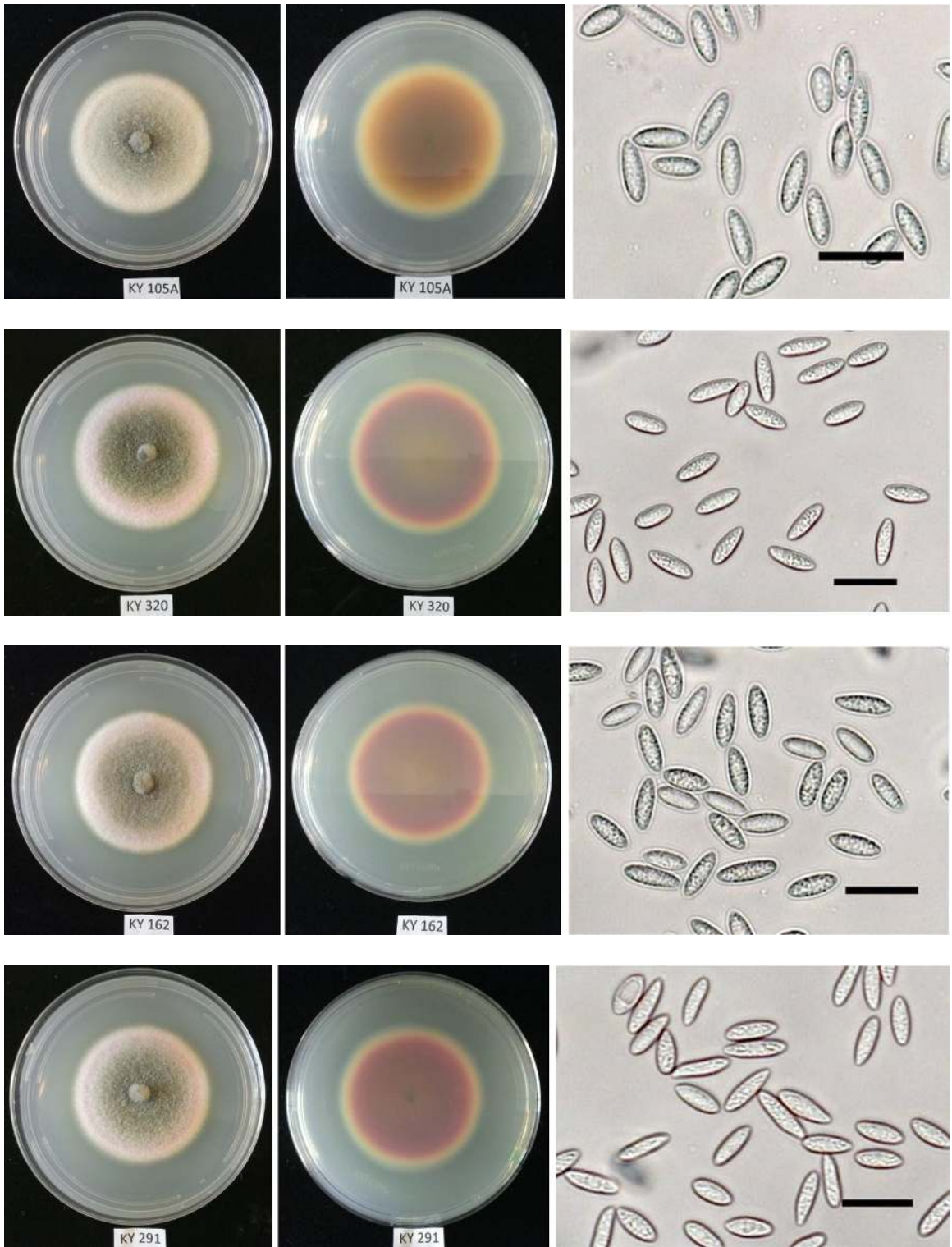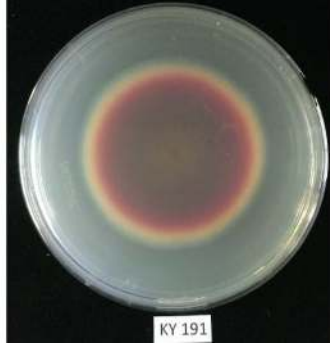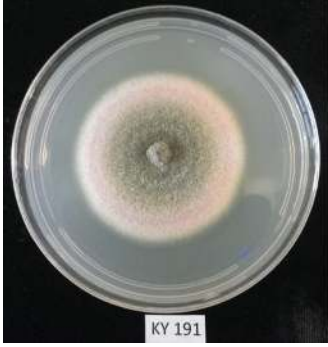
Morphotype-2 isolates

(Left to right = upper colony surface-lower colony surface-conidia; bars = 20μm)
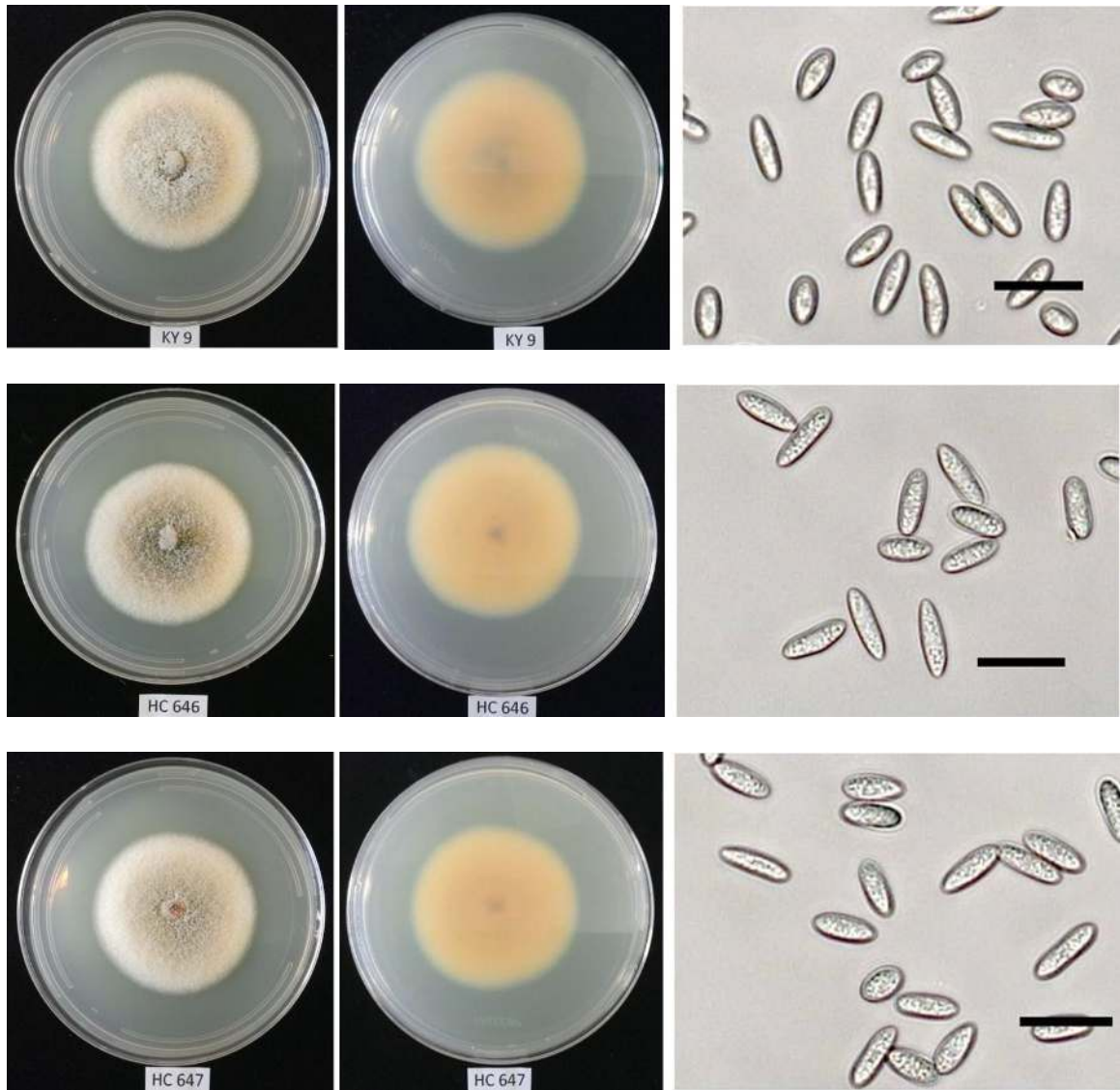
Morphotype-3 isolates

(Left to right = upper colony surface-lower colony surface-conidia; bars = 20µm)
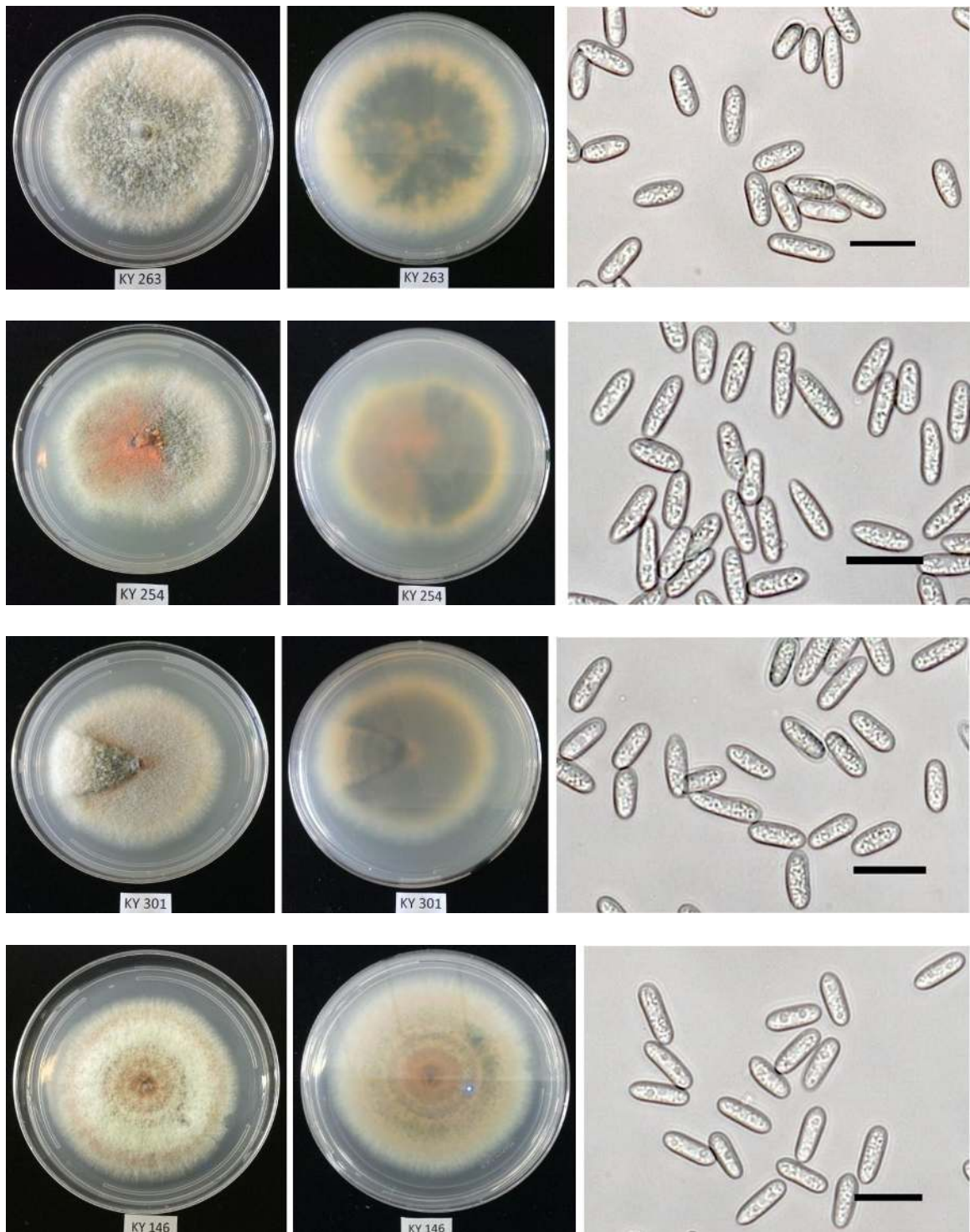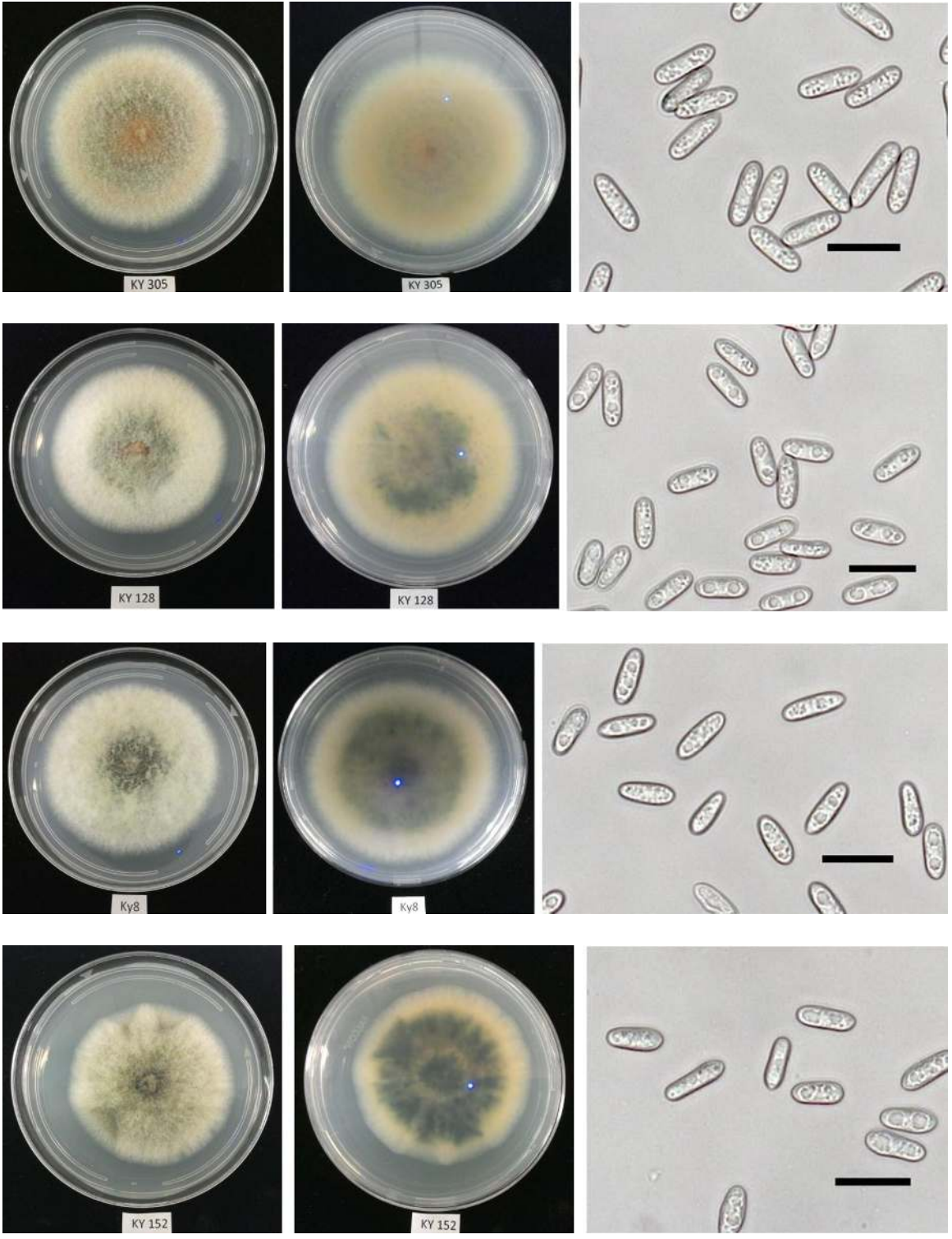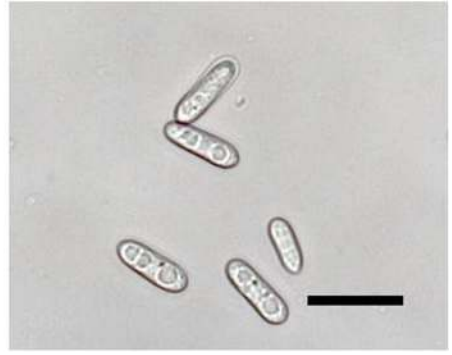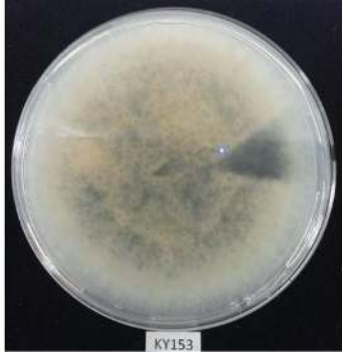
Morphotype-4 isolates

(Left to right = upper colony surface-lower colony surface-conidia; bars = 20μm)
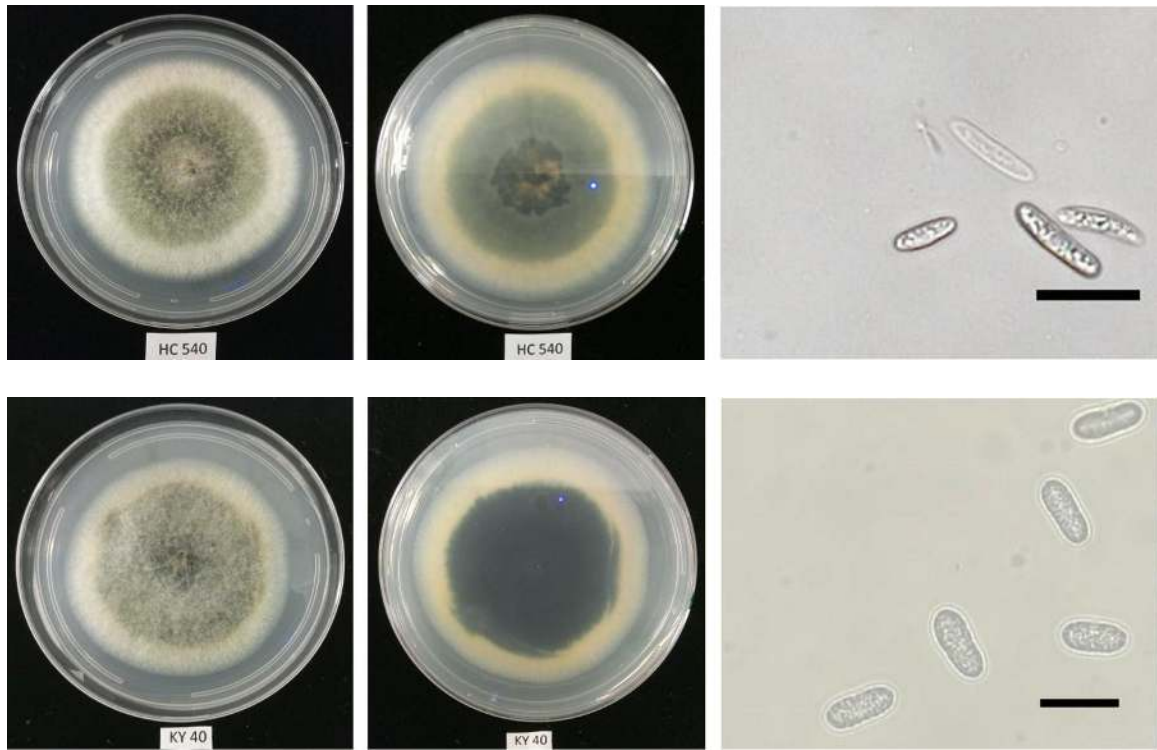
Perithecia and ascospores of morphotype-4 isolates

(Left to right = perithecia, ascospores)
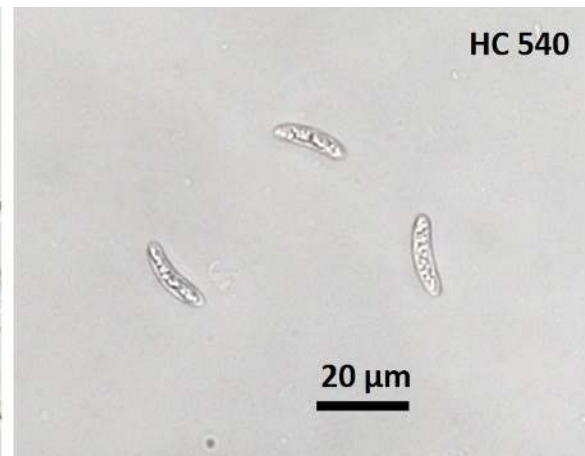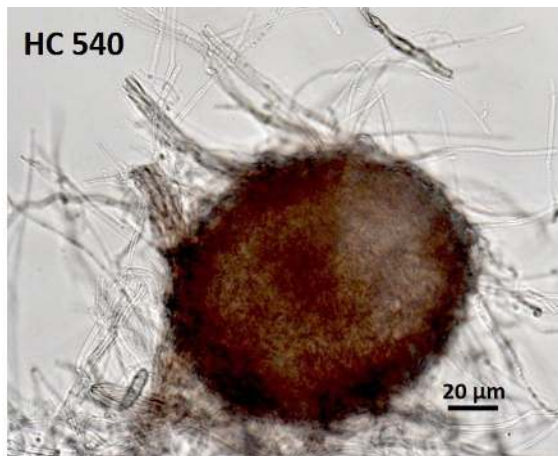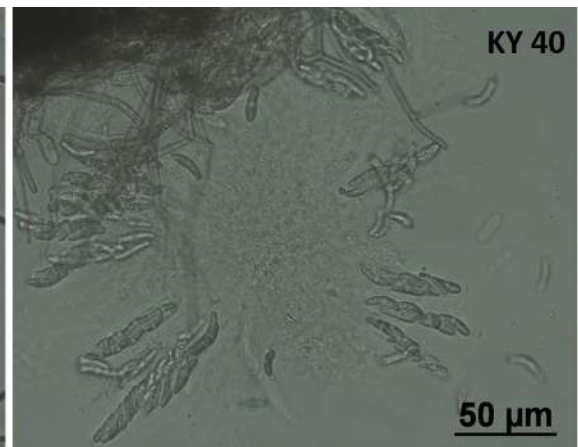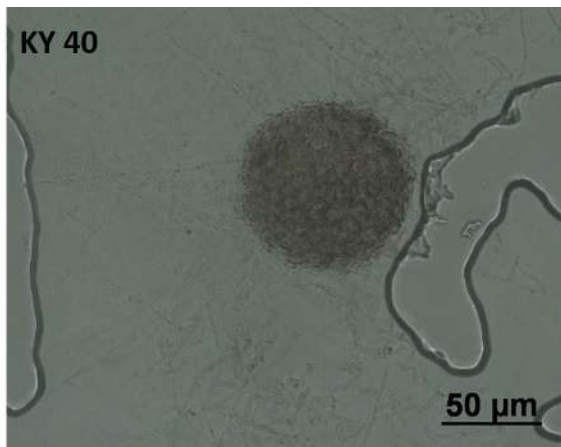
Appendix Two: Conidial Size of Tested Isolates

Table A2.1. Comparison of conidial size of 28 isolates of *Colletotrichum* spp. grown on potato dextrose agar (PDA)

Isolates	Morphotype	Length (µm)	Width (µm)	Length - wide ratio (µm)
HC 296	1	12.40 ± 0.40	4.61 ± 0.30	2.70 ± 0.20
KY 228	1	12.41 ± 0.46	4.48 ± 0.41	2.79 ± 0.26
KY 95	1	12.37 ± 0.50	4.78 ± 0.51	2.61 ± 0.27
KY 123	1	12.26 ± 0.45	4.42 ± 0.71	2.85 ± 0.48
HC 557	1	12.64 ± 0.52	4.56 ± 0.36	2.79 ± 0.22
KY 28	1	12.47 ± 0.40	4.61 ± 0.41	2.73 ± 0.31
KY 6	1	12.41 ± 0.44	4.52 ± 0.35	2.76 ± 0.24
KY 105A	1	12.44 ± 0.51	4.74 ± 0.39	2.65 ± 0.26
KY 320	1	12.51 ± 0.58	4.79 ± 0.64	2.66 ± 0.40
KY 162	1	12.42 ± 0.65	4.77 ± 0.40	2.63 ± 0.31
KY 291	1	12.29 ± 0.45	4.47 ± 0.31	2.76 ± 0.21
KY 191	1	12.59 ± 0.73	4.55 ± 0.51	2.80 ± 0.38
KY 9	2	16.14 ± 0.62	4.76 ± 0.52	3.43 ± 0.40
HC 646	2	15.41 ± 0.90	4.94 ± 0.59	3.01 ± 0.42
HC 647	2	15.57 ± 0.71	4.91 ± 0.37	3.17 ± 0.30
KY 263	3	17.41 ± 0.84	6.50 ± 0.60	2.70 ± 0.28
KY 254	3	17.55 ± 0.87	5.60 ± 0.63	3.17 ± 0.38
KY 301	3	17.86 ± 0.78	5.23 ± 0.54	3.45 ± 0.41
KY 146	3	17.66 ± 0.54	5.78 ± 0.50	3.08 ± 0.28
KY 305	3	17.78 ± 0.85	5.76 ± 0.60	3.12 ± 0.34
KY 152	3	18.21 ± 0.65	5.47 ± 0.48	3.35 ± 0.35
KY 153	3	18.90 ± 1.04	5.57 ± 0.43	3.41 ± 0.30
KY 128	3	17.87 ± 0.93	5.63 ± 0.53	3.20 ± 0.34
HC 540	4	17.10 ± 0.91	5.69 ± 0.76	3.05 ± 0.38
KY 40	4	17.41 ± 0.39	7.10 ± 0.55	2.47 ± 0.19
KY 8	3	17.81 ± 0.97	5.90 ± 0.45	3.04 ± 0.28
APPR1	3	17.82 ± 0.72	5.93 ± 0.57	3.03 ± 0.26
APPY 3	1	12.78 ± 0.61	5.04 ± 0.41	2.55 ± 0.23

Conidial length among species
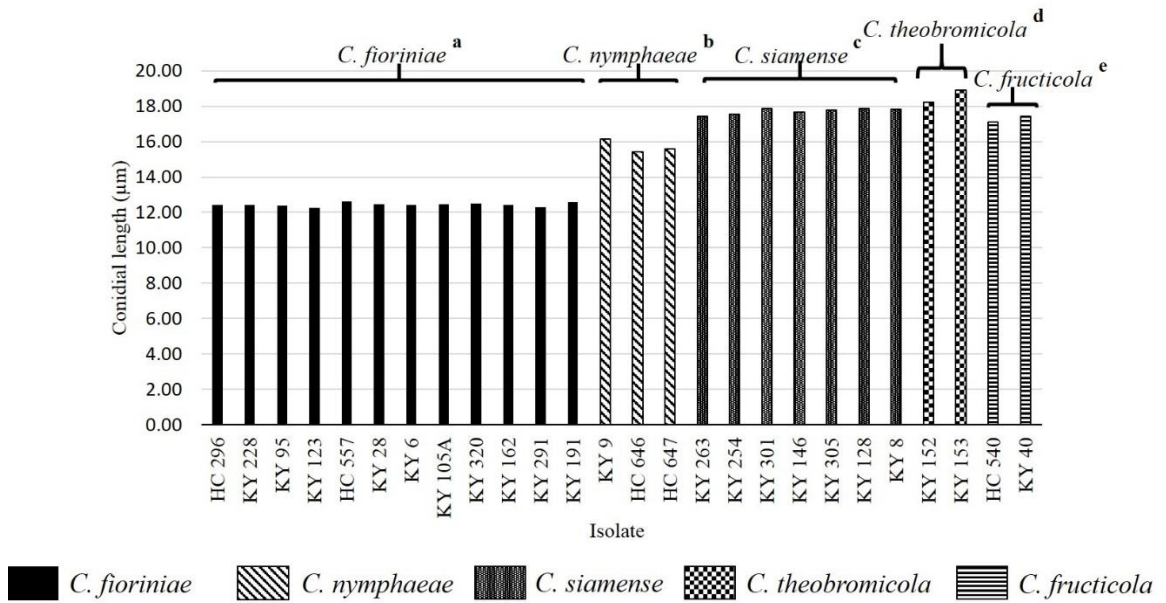
Figure A2.1. Conidial length among species. Species name followed by the same superscript letter were not significantly different using Duncan's multiple range test (P < 0.05).

Conidial width among species
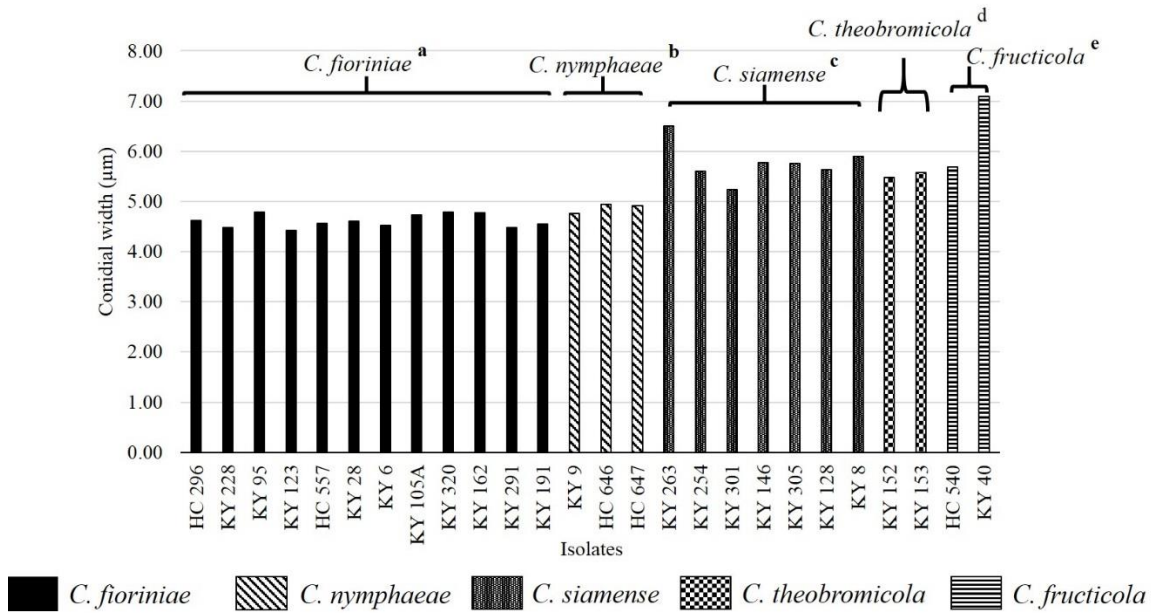
Figure A2.2. Conidial width among species. Species name followed by the same superscript letter were not significantly different using Duncan's multiple range test (P < 0.05).

Conidial length-width ratio among species
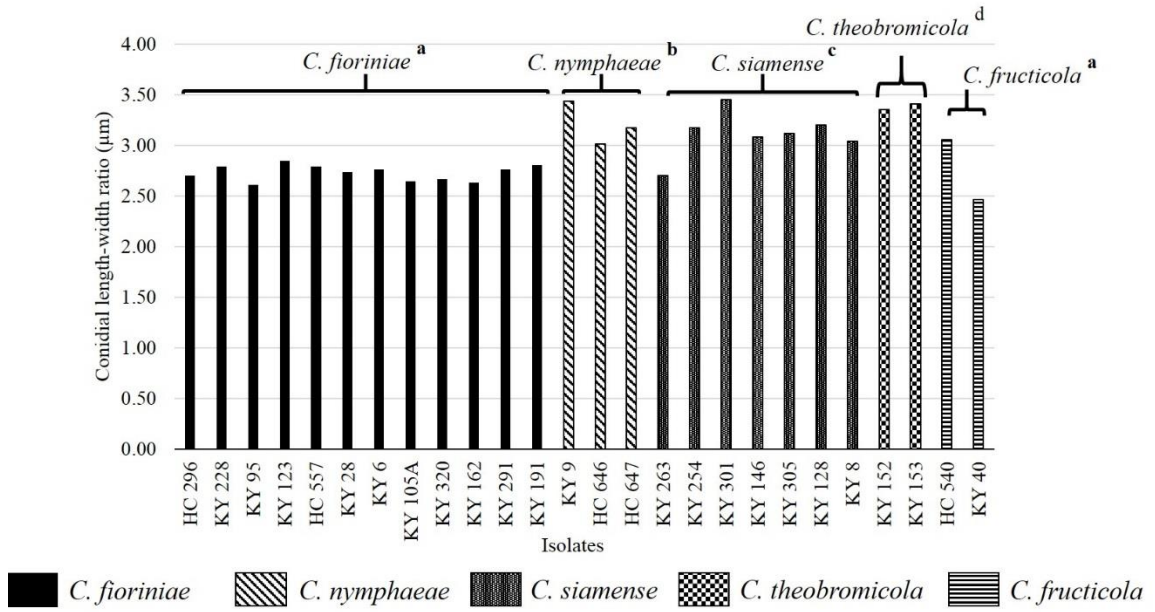
Figure A2.3. Conidial length-width ratio among species. Species name followed by the same superscript letter were not significantly different using Duncan's multiple range test ($P < 0.05$).

Appendix Three: Growth Rate of Tested Isolates

Table A3.1. Growth rate of *Colletotrichum* isolates at five selected temperatures

| Isolate	Morphotype	Growth rate (mm/day)				
		18	21	23	25	30
HC 296	1	2.40	2.61	4.18	5.24	2.64
KY 228	1	2.37	3.17	4.53	5.81	3.11
KY 95	1	2.52	2.76	3.32	5.66	3.02
KY 123	1	2.76	2.90	4.39	5.60	2.84
HC 557	1	2.67	3.20	4.86	5.69	2.87
KY 28	1	2.84	3.38	5.07	6.13	2.67
KY 6	1	2.52	2.79	3.59	5.57	2.58
KY 105A	1	2.67	3.11	4.59	5.96	2.40
KY 320	1	2.31	2.49	4.71	5.96	3.41
KY 162	1	2.67	2.87	4.36	5.16	2.40
KY 291	1	2.76	3.08	4.68	5.75	3.11
KY 191	1	2.55	2.93	4.89	5.78	2.01
KY 9	2	2.34	2.64	3.29	4.27	2.79
HC 646	2	3.35	4.00	3.56	5.45	3.20
HC 647	2	2.40	2.61	3.11	5.42	3.05
KY 263	3	2.49	3.56	5.81	6.16	3.67
KY 254	3	2.67	3.56	5.24	7.64	6.22
KY 301	3	2.76	3.67	5.19	6.37	5.42
KY 146	3	3.02	3.76	5.16	7.23	5.69
KY 305	3	2.84	3.73	3.61	7.02	4.80
KY 152	3	1.60	3.02	4.47	4.00	4.21
KY 153	3	3.23	4.18	5.13	6.76	5.78
KY 128	3	3.11	4.86	6.19	7.17	5.16
HC 540	4	2.31	2.04	4.98	6.90	5.69
KY 40	4	3.26	3.35	7.53	7.94	5.87
KY 8	3	2.93	4.12	5.51	8.36	6.73
APPR1	3	2.76	3.23	4.89	7.73	6.81
APPY3	1	2.61	2.37	2.58	5.42	3.82

Statistical analysis on growth rate among species

Table A3.2. Average growth rate of *Colletotrichum* spp. at five selected temperatures

Species	Average growth rate (mm/day)					Average [x]
	18	21	23	25	30	
C. fioriniae	2.59	2.94	4.43	5.69	2.76	3.68 a
C. nymphaeae	2.70	3.08	3.32	5.05	3.01	3.43 a
C. siamense	2.83	3.89	5.24	7.14	5.38	4.90 b
C. theobromicola	2.41	3.60	4.80	5.38	4.99	4.24 a
C. fructicola	2.79	2.70	6.25	7.42	5.78	4.99 b

[x] Values followed by the same letter were not significantly different based on Duncan's multiple range test ($P < 0.05$).

Table A3.3. Growth rate of *Colletotrichum* spp. at five selected temperatures

Temperature (°C)	Average growth rate (mm/day)
18	2.67 a
21	3.25 b
23	4.69 c
25	6.12 d
30	3.90 e

[x] Values followed by the same letter were not significantly different based on Duncan's multiple range test ($P < 0.05$).

Appendix Four: Amplification of *GAPDH* And *TUB2* Genes
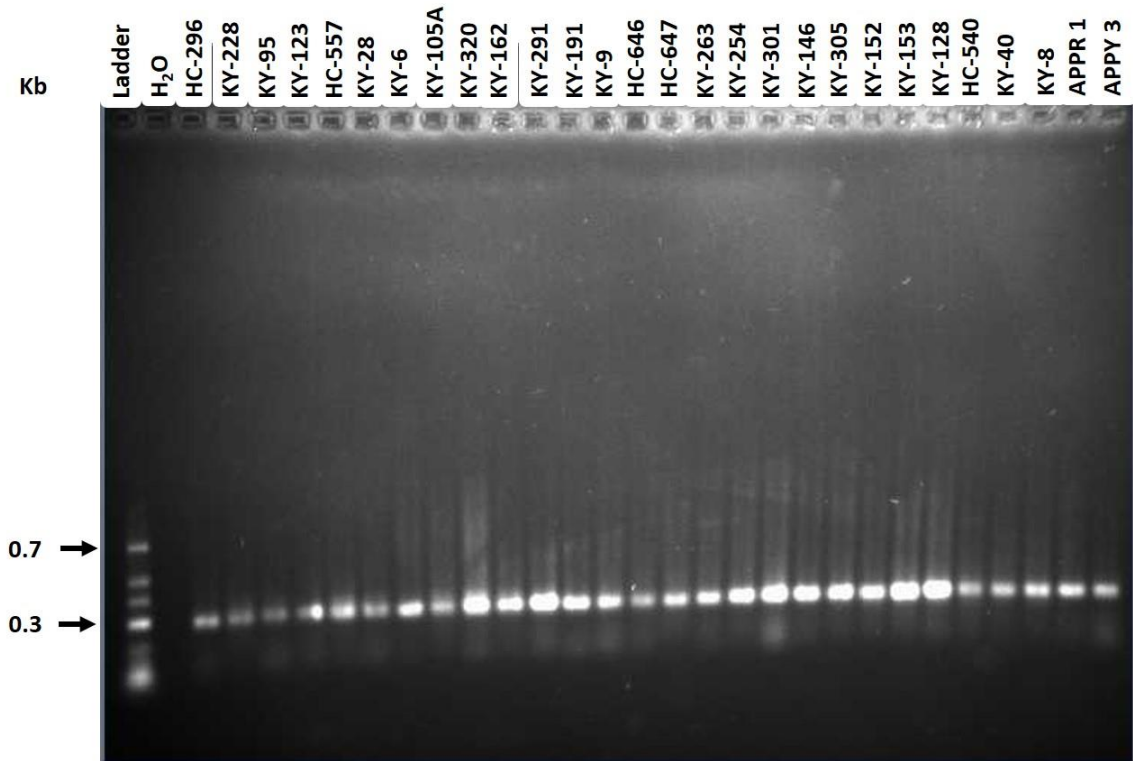
Figure A4.1. Amplification of *GAPDH* genes by using primers GDF1 and GDR2 (70). Ladder: O'Gene Ruler.
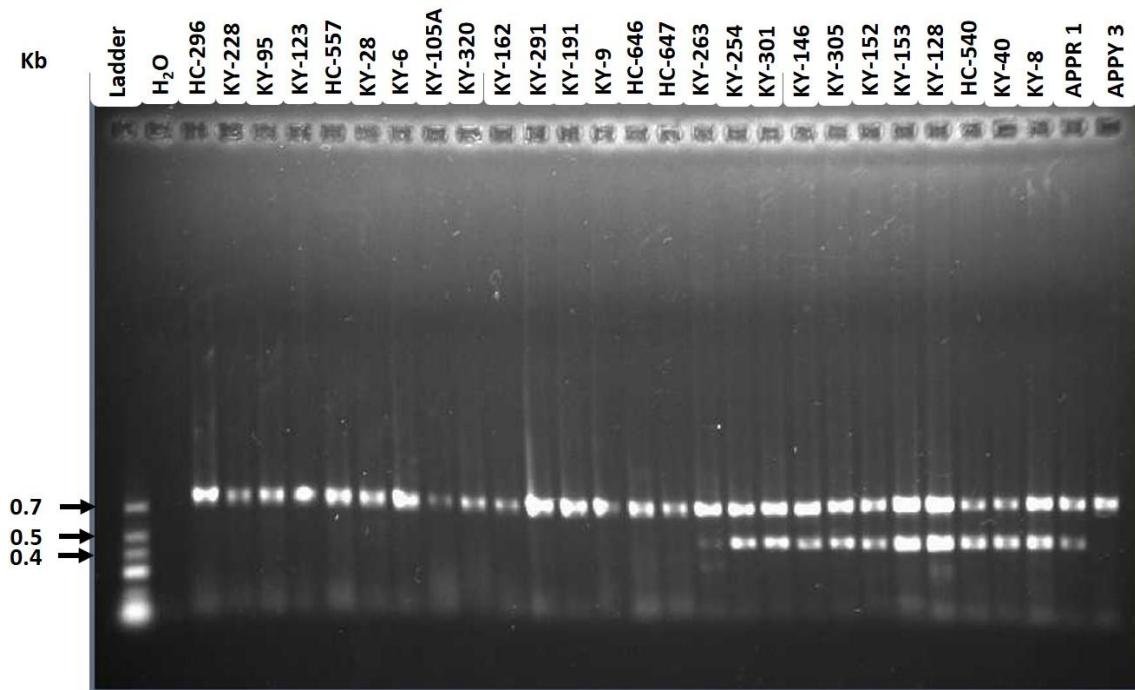
Figure A4.2. Amplification of *TUB2* genes by using primers T1 and T2 (55). Ladder: O'Gene Ruler.

Appendix Five: Alignments Used To Create The Un-Rooted Phylogenetic Trees in

Chapter 2

PART 1: *GAPDH* SEQUENCES

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C.nymphaeae_CBS126507        -------------CCTTCATTGAGACCAAGTACGCTGTGAGTATCA-TCCCACTTTACCC
C.nymphaeae_KY9              ---------GGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-TCCCACTTTACCC
C.nymphaeae_CBS126582        -------------CCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCACTTTACCC
C.nymphaeae_HC647            GCCGTCAA-AGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCACTTTACCC
C.nymphaeae_HC646            -------------------TTGAGACCAAGTACGCTGTGAGTATCA-CCCCACTTTACCC
C.fioriniae_KY320            GCCGTCAA-CTCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_CBS200.35        -------------CCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_CBS293.67        -------------CCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_KY228            GCCGTCAA-CGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_CBS129931        -------------CCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_KY162            GCCGTCAACCGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_KY105A           GCCGTCAA-CGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_IMI363003        -------------CCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_KY191            GCCGTCAA-CGACCCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCAYTTTACCC
C.fioriniae_KY95             GCCGTCAA-CGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_KY6              GCCGTCAA-CGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_KY291            -------A-CGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_HC296            GCCGTCAA-CGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_CBS128517        -------------CCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_KY123            -------------------TTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.fioriniae_KY28             GCCGTCAA-CGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCACCCCCCACTTTACCC
C.theobromicola_C1253.1      GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-TTACCC
C.theobromicola_C1262        GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-TTACCC
C.theobromicola_KY153        --CGTCAA-GCACCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-TTACCC
C.theobromicola_KY152        -------------------TTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-TTACCC
C.siamense_KY305             --------AGGCCCCTTCATTGAGACCARGTACGCTGTGAGTATCA-NNCCAC-CTACCC
C.fructicola_KY40            GCCGTCAACC-CCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTTCCC
C.siamense_KY146             ----TCAAGTGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.siamense_CPC20984          -------------------TTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.siamense_KY301             ---GTCAA-CGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.siamense_KY8               -CCGTCAA-CGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.fructicola_CBS272.51       -------------------TTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.fructicola_HC540           -------------------TTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.fructicola_C1263.5         GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.fructicola_C1316.20        GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.fructicola_C1275.7         GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.fructicola_C1263.3         GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.siamense_KY263             --CGTCAACGGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.siamense_C1254.9           GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.siamense_KY254             ----------------------------------------AGTATCA-CCCCAC-CTACCC
C.siamense_C1258.5           GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.siamense_GM29              GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.siamense_C1316.6           GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.siamense_LC1212            GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.siamense_KY128             GCCGTCAA-CGCCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
C.siamense_C1259.2           GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCA-CCCCAC-CTACCC
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C.nymphaeae_CBS126507      CTCCATC------ATGATATCACGT-CTGCCACGATAACACCAGCTTCGTCGATATCCA-
C.nymphaeae_KY9            CTCCATC------ATGATATCACGT-CTGCCACGATAACACCAGCTTCGTCGATATCCA-
C.nymphaeae_CBS126582      CTCCATC------ATGATATCACGT-CTGCCACGATAACACCAGCTTCGTCGATATCCA-
C.nymphaeae_HC647          CTCCATC------ATGATATCACGT-CTGCCACGATAACACCAGCTTCGTCGATATCCA-
C.nymphaeae_HC646          CTCCATC------ATGATATCACGT-CTGCCACGATAACACCAGCTTCGTCGATATCCA-
C.fioriniae_KY320          CTCCNTA------ATGATATCACGTACTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_CBS200.35      CTCCATG------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_CBS293.67      CTCCATA------ATGATATCACGT-CTGCTACAATAACACTAGCTTCATCGGTAACCA-
C.fioriniae_KY228          CTCCATA------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_CBS129931      CTCCATA------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_KY162          CTCCATA------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_KY105A         CTCCATA------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_IMI363003      CTCCATA------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_KY191          CTCCATA------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_KY95           CTCCATA------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_KY6            CTCCATA------ATGATATCACGTNCTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_KY291          CTCCATA------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_HC296          CTCCATA------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_CBS128517      CTCCATA------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_KY123          CTCCATA------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.fioriniae_KY28           CTCCATA------ATGATATCACGT-CTGCTACAATAACACCAGCTTCATCGGTAACCA-
C.theobromicola_C1253.1    CTCCAAACTCGCCACTACTTCACACC------CGCCACCGCCGCTGCTGTC-GTTCACA-
C.theobromicola_C1262      CTCCAAACTCGCCACTACTTCACACC------CGCCACGGCTGCTGCTGTC-GTTCACA-
C.theobromicola_KY153      CTCCAAACTCGCCACTACTTCACACC------CGCCACGGCTGCTGCTGTC-GTTCACA-
C.theobromicola_KY152      CTCCAAACTCGCCACTACTTCACACC------CGCCACGGCTGCTGCTGTC-GTTCACA-
C.siamense_KY305           CTCCNARMTCGCCATGAMTTYANATCCAT---CACCACCNCCACCGCTGTCNATMTA---
C.fructicola_KY40          CTCCAAACTCGCCATGACTTCACATCACCACCACCACCACCACCGYTGWM-ATMWACAG
C.siamense_KY146           CTCCAAACTCG-CATGACTTCACATYCAT---CACCACCACCACCGCTGTC--WYTACA-
C.siamense_CPC20984        CTCCAAACTCGCCATGACTTCACATCCAT---CACCACCACCACCGCTGTC-ATCTACA-
C.siamense_KY301           CTCCAAACTCGCCATGACTTCACATCCAT---CACCACCACCACCGCTGTC-ATCTATA-
C.siamense_KY8             CTCCANACTCGCCATGACTTCTCATCCAT---CACCACCACCACCGCTGTC-ATCTACA-
C.fructicola_CBS272.51     CTCCAAACTCGCCATGACTTCGCATCCATCCCCACCACCACCACCGCTGTC-ATCTACA-
C.fructicola_HC540         CTCCNAACTCGCCATGACTTCGCATCCATCCCCACCACCACCACCGCTGTC-ATCTACA-
C.fructicola_C1263.5       CTCCAAACTCGGCATGACTTCACATCCATCACCACCACCACCACCGCTGTC-ATCTACA-
C.fructicola_C1316.20      CTCCAAACTCGCCATGACTTCACATCCATCACCACCACCACCACCGCTGTC-ATCTACA-
C.fructicola_C1275.7       CTCCAAACTCGCCATGACTTCACATCCATCACCACCACCACCACCGCTGTC-ATCTACA-
C.fructicola_C1263.3       CTCCAAACTCGCCATGACTTCACATCCATCACCACCACCACCACCGCTGTC-ATCTACA-
C.siamense_KY263           CTCCAANCTCGCCATGACTTCACATC------CATCACCACCACCGCTGTC-ATCTACA-
C.siamense_C1254.9         CTCCAAACTCGCCATGACTTCACATGCAT---CACCACCACCACCGCTGTC-ATCTACA-
C.siamense_KY254           CTCCAAACTCGCCATGACTTCACATGCAT---CACCACCACCACCGCTGTC-ATCTACA-
C.siamense_C1258.5         CTCCAAACTCGCCATGACTTCACATCCAT---CACCACCACCACCGCTGTC-ATCTATA-
C.siamense_GM29            CTCCAAACTCGCCATGACTTCACATCCAT---CACCACCACCACCGCTGTC-ATCTATA-
C.siamense_C1316.6         CTCCAAACTCGCCATGACTTCACATCCAT---CACCACCACCACCGCTGTC-ATCTACA-
C.siamense_LC1212          CTCCAAACTCGCCATGACTTCACATCCAT---CACCACCACCATCGCTGTC-ATCTACA-
C.siamense_KY128           CTCCAAACTCGCCATGACTTCACATCCAT---CACCACCACCACCGCTGTC-ATCTACA-
C.siamense_C1259.2         CTCCAAACTCGCCATGACTTCACATCCAT---CACCACCACCACCGCTGTC-ATCTACA-
                           ****         *  *  *                  *   *   *
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C.nymphaeae_CBS126507      ----------------CGGGAAAAGAGTCGGAGCTAGCACTCTCAACTCTTTTGCCCCAA
C.nymphaeae_KY9            ----------------CGGGAAAAGAGTCGGAGCTAGCACTCTCAACTCTTTTGCCCCAA
C.nymphaeae_CBS126582      ----------------CGGGAAAAGAGTCGGAGCTAGCACTCTCGACTCTTTTGTCCCAA
C.nymphaeae_HC647          ----------------CGGGAAAAGAGTCGGAGCTAGCACTCTCGACTCTTTTGTCCCAA
C.nymphaeae_HC646          ----------------CGGGAAAAGAGTCGGAGCTAGCACTCTCGACTCTTTTGTCCCAA
C.fioriniae_KY320          ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_CBS200.35      ----------------CGGGAAAAGAGCCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_CBS293.67      ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_KY228          ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_CBS129931      ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_KY162          ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_KY105A         ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_IMI363003      ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_KY191          ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_KY95           ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_KY6            ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_KY291          ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_HC296          ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_CBS128517      ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_KY123          ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.fioriniae_KY28           ----------------CGGGAAAAGAGTCAGAGCTAGTACTCTCGACTCTTTGGACCCAA
C.theobromicola_C1253.1    CCTTCCCGCCTGTATTTGGCAGACTAG--AAGGCCA----ACATGAATT-----------
C.theobromicola_C1262      CCTTCCCGCCTGTATTTGGCAGACTAG--AAGGCCA----ACATGAATT-----------

C.theobromicola_KY153      CCTTCCCGCCTGTATTTGGCAGACTAG--AAGGCCA----ACATGAATT-----------
C.theobromicola_KY152      CCTTCCCGCCTGTATTTGGCAGACTAG--AAGGCCA----ACATGAATT-----------
C.siamense_KY305           TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAANG-----------
C.fructicola_KY40          TATNNCCNNCCGCGTTTGGTAANCAAG--AAGGCCG----TC-TGAATG-----------
C.siamense_KY146           TCTCGCCACCTGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.siamense_CPC20984        TCTCGCCACCTGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.siamense_KY301           TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.siamense_KY8             TCTTGCCATCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.fructicola_CBS272.51     TCTCGCCACCCGCGTTTGGTAAATAAG--AAGGCCG----TCATGAATG-----------
C.fructicola_HC540         TCTCGCCACCCGCGTTTGGTAAATAAG--AAGGCCG----TCATGAATG-----------
C.fructicola_C1263.5       TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.fructicola_C1316.20      TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.fructicola_C1275.7       TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.fructicola_C1263.3       TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.siamense_KY263           TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.siamense_C1254.9         TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.siamense_KY254           TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.siamense_C1258.5         TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.siamense_GM29            TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.siamense_C1316.6         TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.siamense_LC1212          TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.siamense_KY128           TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
C.siamense_C1259.2         TCTCGCCACCCGCGTTTGGTAAACAAG--AAGGCCG----TCATGAATG-----------
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C.nymphaeae_CBS126507        -GGTTTCGATT-GGGC----TTGTTGTAACGACACGACGTGACACA----ATCATGCAGAA
C.nymphaeae_KY9              -GGTTTCGATT-GGGC----TTGTTGTAACGACACGACGTGACACA----ATCATGCAGAA
C.nymphaeae_CBS126582        -GGTTTCGATT-GGGC----TTGTTGTAACGACACGACGTGACACA----ATCATGCAGAA
C.nymphaeae_HC647            -GGTTTCGATT-GGGC----TTGTTGTAACGACACGACGTGACACA----ATCATGCAGAA
C.nymphaeae_HC646            -GGTTTCGATT-GGGC----TTGTTGTAACGACACGACGTGACACA----ATCATGCAGAA
C.fioriniae_KY320            -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAAAA
C.fioriniae_CBS200.35        -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAGAA
C.fioriniae_CBS293.67        -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAGAA
C.fioriniae_KY228            -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACAACGTGACACA----ATCATGCAGAA
C.fioriniae_CBS129931        -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACAACGTGACACA----ATCATGCAGAA
C.fioriniae_KY162            NGGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAAAA
C.fioriniae_KY105A           -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAAAA
C.fioriniae_IMI363003        -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAGAA
C.fioriniae_KY191            -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAGAA
C.fioriniae_KY95             -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAGAA
C.fioriniae_KY6              -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAGAA
C.fioriniae_KY291            -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAGAA
C.fioriniae_HC296            -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAGAA
C.fioriniae_CBS128517        -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAGAA
C.fioriniae_KY123            -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAGAA
C.fioriniae_KY28             -GGTTTCGATT-GGGCTCGTTGTTGTAATGATACGACGTGACACA----ATCATGCAGAA
C.theobromicola_C1253.1      -GATGCCAATT-GATACCATGGCT----CGGCACGGCCGGACACA-GCTATCACT-CATC
C.theobromicola_C1262        -GACGCCAATT-GATACCATGGCT----CGGCACGGCCGGACACA-GCTATCACT-CATC
C.theobromicola_KY153        -GACGCCAATT-GATACCATGGCT----CGGCACGGCCGGACACA-GCTATCACT-CATC
C.theobromicola_KY152        -GACGCCAATT-GATACCATGGCT----CGGCACGGCCGGACACA-GCTATCACT-CATC
C.siamense_KY305             -GAGGCCAATT-GARACCATGGSC----CGGGACGGCCGGACACACATGCTATCACT-CATA
C.fructicola_KY40            -GAGGCCAATT-GAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
C.siamense_KY146             -GAGGCCAATTGGAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-TTTW
C.siamense_CPC20984          -GAGGCCAATTGGAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-TTTA
C.siamense_KY301             -GAGGCCAATT-GAAACCATGGGC----CGGGACGGCCGGACACATGCTATCACTNCATA
C.siamense_KY8               -GAGGCCAATT-GAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
C.fructicola_CBS272.51       -GAGGCCAATT-GAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
C.fructicola_HC540           -GAGGCCAATT-GAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
C.fructicola_C1263.5         -GAGGCCAATT-GAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
C.fructicola_C1316.20        -GAGGCCAATT-GAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CACA
C.fructicola_C1275.7         -GAGGCCAATT-GAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
C.fructicola_C1263.3         -GAGGCCAATT-GAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
C.siamense_KY263             -GAGGCCAATT-GAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
C.siamense_C1254.9           -GAGGCCAATT-GAAATCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
C.siamense_KY254             -GAGGCCAATT-GAAATCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
C.siamense_C1258.5           -GAGGCCAATT-GAAACCATGGGC----CGGGACGGCCGGACACATGCTATCACT-CATA
C.siamense_GM29              -GAGGCCAATT-GAAACCATGGGC----CGGGACGGCCGGACACATGCTATCACT-CATA
C.siamense_C1316.6           -GAGGCCAATT-GAAACCATGGGC----CGGGACGGCCGGACACATGCTATCACT-CATA
C.siamense_LC1212            -GAGGCCAATT-GAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
C.siamense_KY128             -GAGGCCAATT-GAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
C.siamense_C1259.2           -GAGGCCAATT-GAAACCATGGGT----CGGGACGGCCGGACACATGCTATCACT-CATA
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C.nymphaeae_CBS126507    ACAGCCGAGACAA----AACTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.nymphaeae_KY9          ACAGCCGAGACAA----AACTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.nymphaeae_CBS126582    ACAGCCGAGACAA----AACTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.nymphaeae_HC647        ACAGCCGAGACAA----AACTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGT-
C.nymphaeae_HC646        ACAGCCGAGACAA----AACTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGT-
C.fioriniae_KY320        ACAGCCNNNACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAMGTA
C.fioriniae_CBS200.35    ACAGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.fioriniae_CBS293.67    ACGGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.fioriniae_KY228        ACAGCCCAA---------------------------------------------------
C.fioriniae_CBS129931    ACAGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.fioriniae_KY162        ACAGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.fioriniae_KY105A       ACAGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATG--------
C.fioriniae_IMI363003    ACAGCCCAAACAA----ACTTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.fioriniae_KY191        ACAGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.fioriniae_KY95         ACAGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTC
C.fioriniae_KY6          ACAGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.fioriniae_KY291        ACAGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.fioriniae_HC296        ACAGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.fioriniae_CBS128517    ACAGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.fioriniae_KY123        ACAGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGT-
C.fioriniae_KY28         ACAGCCCAAACAA----AATTTGCTGACAGACAATCATCACAGGCCTACATGCTCAAGTA
C.theobromicola_C1253.1  TCAGCCCCATCTGTCA-CATGTACTGAC--TCGCACTTCACAGGCCTACATGCTCAAGTA
C.theobromicola_C1262    TCAGCCCCATCTGTCA-CATGTACTGAC--TCGCACTTCACAGGCCTACATGCTCAAGTA
C.theobromicola_KY153    TCAGCCCCATCTGTCA-CATGTACTGAC--TCGCACTTCACAGGCCTACATGCTCAAGT-
C.theobromicola_KY152    TCAGCCCCATCTGTCA-CATGTACTGAC--TCGCACTTCACAGGCCTACATGCTCAAGT-
C.siamense_KY305         TCAGCCCTATCTGTCA-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.fructicola_KY40        TCAGCCCCATCTGTCG-CN-NYACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.siamense_KY146         TCAGCCCCATCTGTCA-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTC
C.siamense_CPC20984      TCAGCCCCATCTGTCA-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGT-
C.siamense_KY301         TCAGCCCTATYYGTCA-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTC
C.siamense_KY8           TCAGCCCTATCTGTCA-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.fructicola_CBS272.51   TCAGCCCCATCTGTCG-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGT-
C.fructicola_HC540       TCAGCCCCATCTGTCG-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGT-
C.fructicola_C1263.5     TCAGCCCCATCTGTCG-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.fructicola_C1316.20    TCAGCCCCATCTGTCG-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.fructicola_C1275.7     TCAGCCCCATCTGTCG-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.fructicola_C1263.3     TCAGCCCCATCTGTCG-CA-TTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.siamense_KY263         TCAGCCCTATCTGTCA-CATTTACTGAC--TAGCTCTTCACAGGCCTACATGCTCAAGTA
C.siamense_C1254.9       TCAGCCCTATCTGTCA-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.siamense_KY254         TCAGCCCTATCTGTCA-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.siamense_C1258.5       TCAGCCCTATCTGTCA-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.siamense_GM29          TCAGCCCTATCTGTCACCATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.siamense_C1316.6       TCAGCCCTATCTGTCA-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.siamense_LC1212        TCAGCCCTATCTGTCA-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
C.siamense_KY128         TCAGCCCTATCTGTCA-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTC
C.siamense_C1259.2       TCAGCCCTATCTGTCA-CATTTACTGAC--TCGCTCTTCACAGGCCTACATGCTCAAGTA
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C.nymphaeae_CBS126507      C-------------
C.nymphaeae_KY9            CG-ACTCCACCC--
C.nymphaeae_CBS126582      C-------------
C.nymphaeae_HC647          CG-ACTCCACCC--


C.nymphaeae_HC646          -------------
C.fioriniae_KY320          GC-ACTCCACCC--
C.fioriniae_CBS200.35      C-------------
C.fioriniae_CBS293.67      C-------------
C.fioriniae_KY228          -------------
C.fioriniae_CBS129931      C-------------
C.fioriniae_KY162          CG-ACTCCACCA--
C.fioriniae_KY105A         -------------
C.fioriniae_IMI363003      C-------------
C.fioriniae_KY191          CGAACTCCACAA--
C.fioriniae_KY95           GA-ACTCCACCA--
C.fioriniae_KY6            CG-ACTCCACCC--
C.fioriniae_KY291          -G-ACTCCACCC--
C.fioriniae_HC296          -G-ACTCCACCC--
C.fioriniae_CBS128517      C-------------
C.fioriniae_KY123          -------------
C.fioriniae_KY28           CG-ACTCCACCC--
C.theobromicola_C1253.1    CG-ACTCCACCC--
C.theobromicola_C1262      CG-ACTCCACCC--
C.theobromicola_KY153      CA-ACTCCACCC--
C.theobromicola_KY152      -------------
C.siamense_KY305           GT-ACTCCACCC--
C.fructicola_KY40          -G-ACTCCACCC--
C.siamense_KY146           GA-ACTCCACCC--
C.siamense_CPC20984        -------------
C.siamense_KY301           C--ACTCCACCA--
C.siamense_KY8             G--ACTCCACCCCA
C.fructicola_CBS272.51     -------------
C.fructicola_HC540         -------------
C.fructicola_C1263.5       CG-ACTCCACCC--
C.fructicola_C1316.20      CG-ACTCCACCC--
C.fructicola_C1275.7       CG-ACTCCACCC--
C.fructicola_C1263.3       CG-ACTCCACCC--
C.siamense_KY263           CG-ACTCCACCC--
C.siamense_C1254.9         CG-ACTCCACCC--
C.siamense_KY254           CG-ACTCCA-----
C.siamense_C1258.5         CG-ACTCCACCC--
C.siamense_GM29            CG-ACTCCACCC--
C.siamense_C1316.6         CG-ACTCCACCC--
C.siamense_LC1212          CG-ACTCCACCC--
C.siamense_KY128           G--ACTCCACCC--
C.siamense_C1259.2         CG-ACTCCACCC--
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Multiple alignments were produced using CLUSTAL multiple sequence alignment by MUSCLE 3.8.

PART 2: *TUB2* SEQUENCES

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C.fructicola_HC540         ---------TGCAGTCCATCACCAC-AATCACAACAACGCT--------------------
C.fructicola_KY40          ---------TGCAGTCCATCACCAC-AATCACAACAACGCT--------------------
C.fructicola_C1253.2       ---------------CCATCACCAC-AATCACAACAACGCT--------------------
C.fructicola_C1315.3       ---------------CCATCACCAC-AATCACAACAACGCT--------------------
C.fructicola_C1265.4       ---------------CCATCACCAC-AATCACAACAACGCT--------------------
C.siamense_C1316.5         ---------------CCATCACCAC-AATCACAACAACGCT--------------------
C.siamense_KY8             ---------------CCATCACCAC-AATCACAACAACGCT--------------------
C.siamense_KY146           ---------TGCAGTCCATCACCAC-AATCACAACAACGCT--------------------
C.siamense_KY128           ---------TGCAGTCCATCACCAC-AATCACAACAACGCT--------------------
C.siamense_LLTA01          ------------------------------------------------------------
C.siamense_C1263.11        ---------------CCATCACCAC-AATCACAACAACGCT--------------------
C.siamense_KY254           ---------TGCAGTCCATCACCAC-AATCACAACAACGCT--------------------
C.siamense_KY263           ---------TGCAGTCCATCACCAC-AATCACAACAACGCT--------------------
C.siamense_KY301           ---------TGCAGTCCATCACCAC-AATCACAACAACGCT--------------------
C.siamense_KY305           ---------TGCAGTCCATCACCAC-AATCACAACAACGCT--------------------
C.theobromicola_C1275.13   ---------------CCATCACCAC-AATCACAACACCACT--------------------
C.theobromicola_C1266.6    ---------------CCATCACCACAAATCACAACACCACT--------------------
C.theobromicola_KY152      ---------CGATGTCCATCACCAC-AATCACAACACCACT--------------------
C.theobromicola_KY153      ---------CGATGTCSRTCACCAC-AATCACAACACCACT--------------------
C.fioriniae_KY228          ----------------CGCTCTCTCC-CATCACAACAAATTT--------------------
C.fioriniae_KY28           ----------------CGCTCTCTCC-CATCACAACAATTTT--GGNNGTAAACGNNTGGSG
C.fioriniae_KY95           ----------------CGCTCTCTCC-CATCACAACAAATYT--------------------
C.fioriniae_KY320          ----------------------------GCAMTA--------------------------
C.fioriniae_KY123          ----------------CGCTCTCTCC-CATCACAACAATTCT--------------------
C.fioriniae_KY162          ----------------CGCTCTCTCC-CATCACAACAATTCT--------------------
C.fioriniae_KY191          ----------------CGCTCTCTCC-CATCACAACAATTTT--------------------
C.fioriniae_KY291          ----------------CGCTCTCTCC-CATCACAACAATTTT--------------------
C.fioriniae_KY105A         ----------------CGCTCTCTCC-CATCACAACAATTCT--------------------
C.fioriniae_HC296          ----------------CGCTCTCTCC-CATCACAACAATTCT--------------------
C.fioriniae_KY6            ----------------CGCTCTCTCC-CATCACAACAATTTT--------------------
C.fioriniae_CB129932       ------------------------------------------------------------
C.fioriniae_CBS128517      ------------------------------------------------------------
C.fioriniae_CBS112995      ------------------------------------------------------------
C.fioriniae_CBS127538      ------------------------------------------------------------
C.fioriniae_IMI324996      ------------------------------------------------------------
C.fioriniae_CBS124958      ------------------------------------------------------------
C.fioriniae_ATSS12097      ------------------------------------------------------------
C.fioriniae_CBS127537      ------------------------------------------------------------
C.fioriniae_CBS124963      ------------------------------------------------------------
C.fioriniae_CBS235.49      ------------------------------------------------------------
C.fioriniae_CBS127611      ------------------------------------------------------------
C.fioriniae_C188           ----------------CGCTCTCTCC-CATCACAACAATTCT--------------------
C.nymphaeae_HC647          GCGAGATTGTAAGTCGCTCTCTCCCATCRCCAAMAATY----------------------
C.nymphaeae_KY9            ----------------CGCTCTCTCC-CATCACCAACAATTTTGCTAAGGGGTNNNNTGGNN
C.nymphaeae_HC646          ----GATTGTAAGTCGCTCTCTCCCATCACCAACAATY----------------------
C.nymphaeae_IMI360928      ------------------------------------------------------------
C.nymphaeae_CBS129937      ------------------------------------------------------------
C.nymphaeae_CBS129933      ------------------------------------------------------------
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C.fructicola_HC540         -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.fructicola_KY40          -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.fructicola_C1253.2       -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.fructicola_C1315.3       -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.fructicola_C1265.4       -TGCGACGCGTT----------------TATCCGCCTTG-CCCTGAGCGTACCCCGCCGA
C.siamense_C1316.5         -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.siamense_KY8             -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.siamense_KY146           -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.siamense_KY128           -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.siamense_LLTA01          ------------------------------------------------------------
C.siamense_C1263.11        -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.siamense_KY254           -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.siamense_KY263           -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.siamense_KY301           -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.siamense_KY305           -TGCGACGCGTT----------------TATCCGCCTTGCCCCTGAGCGTACCCCGCCGA
C.theobromicola_C1275.13   -TGCGACGCGTT----------------TATCCGCTTTGCCCCTGAGCGTACCCCGCCGA
C.theobromicola_C1266.6    -TGCGACGCGTT----------------TATCCGCTTTGCCCCTGAGCGTACCCCGCCGA
C.theobromicola_KY152      -TGCGACGCGTT----------------TATCCGCTTTGCCCCTGAGCGTACCCCGCCGA
C.theobromicola_KY153      -TGCGACGCGTT----------------TATCCGCTTTGCCCCTGAGCGTACCCCGCCGA
C.fioriniae_KY228          --GCGTAGCGTCTGGSATGGYYAMWATTTCNGCGRNAGRTGTCTGARCGTACCCCGACGA
C.fioriniae_KY28           GRAGGANNANTA----------------TTTSGGGATAGAMCNTGAGCGTACMCMGMCGA
C.fioriniae_KY95           --GCGTAACGTYGGGTGGCACTA-----TTTCSGGATAGYCCCTGAGCGTACCCCGYCGA
C.fioriniae_KY320          ---------------------------TTTCGGGATAGCCCCTGAGCGTACCCCGCCGA
C.fioriniae_KY123          --GCGTRGCGTNCGGGRAAGGSCACNTATTTCGGGATAGCCCCTGAGCGTACCCCGCCGA
C.fioriniae_KY162          --NCGAYSMGTT---GGGGAGTNCCAMTATTTCGGGATAGSCCCTGAGCGTAYCCCGCCGA
C.fioriniae_KY191          --GCGTAGCGTTNGGGGAANGGCCACTATTTCGGGATAGCCCCTGAGCGTACCCCGCCGA
C.fioriniae_KY291          --GCGWCGCGTTTGRG----GGCCACTATTTCGGGATAGCCCCTGAGCGTACCCCGCCGA
C.fioriniae_KY105A         --GSGTCGCGTC---GGGAAGGGCACTATTTCGGGATAGCCCTTGAGCGTACCCCGCCGA
C.fioriniae_HC296          --GCGACGCGTC---GGNATGGCCACTATTTCGGGATAGCCCCTGAGCGTACCCCGCCGA
C.fioriniae_KY6            --GCGWMGCGTC-TGNGGATGGCCACTATTTCGGGATAGCCCCTGAGCGTACCCCGCCGA
C.fioriniae_CB129932       ------------------------------------------------------------
C.fioriniae_CBS128517      ------------------------------------------------------------
C.fioriniae_CBS112995      ------------------------------------------------------------
C.fioriniae_CBS127538      ------------------------------------------------------------
C.fioriniae_IMI324996      ------------------------------------------------------------
C.fioriniae_CBS124958      ------------------------------------------------------------
C.fioriniae_ATSS12097      ------------------------------------------------------------
C.fioriniae_CBS127537      ------------------------------------------------------------
C.fioriniae_CBS124963      ------------------------------------------------------------
C.fioriniae_CBS235.49      ------------------------------------------------------------
C.fioriniae_CBS127611      ------------------------------------------------------------
C.fioriniae_C188           --GCGACGCGTC---GGAATGGCCACTATTTCGGGATAGCCCCTGAGCGTACCCCGCCGA
C.nymphaeae_HC647          -TGCGACGCGTC--GGNAATGGCCACTATTTCGGGGTAGCCCCTGAGCGTACCCCGCCGA
C.nymphaeae_KY9            GAATGGCCAATA----------------TTTCGGGGTAGCCCCTGAGCGTACCCCGCCGA
C.nymphaeae_HC646          -TGCGACGCGTC---GGAATGGCCACTATTTCGGGGTAGCCCCTGAGCGTACCCCGCCGA
C.nymphaeae_IMI360928      ------------------------------------------------------------
C.nymphaeae_CBS129937      ------------------------------------------------------------
C.nymphaeae_CBS129933      ------------------------------------------------------------
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C.fructicola_HC540         CATTTTTACCCGACTTCTATGCACAACAAACCCGCGACGCCTGTCAAT--CATCGACGCC
C.fructicola_KY40          CATTTTTACCCGACTTCTATGCACAACAAACCCGCGACGCCTGTCAAT--CATCGACGCC
C.fructicola_C1253.2       CATTTTTACCCGACTTCTATGCACAACAAACCCGCGACGCCTGTCAAT--CATCGACGCC
C.fructicola_C1315.3       CATTTTTACCCGACTTCTATGCACAACAAACCCGCGACGCCTGTCAAT--CATCGACGCC
C.fructicola_C1265.4       CATTTTTACCCGACTTCTATGCACAACAAACCCGCGACGCCTGTCAAT--CATCGACGCC
C.siamense_C1316.5         CATTTTTACCCGACTTCTATGCTCAACAAACCCGCGACGCCTGTCAAT--CATCGACGTC
C.siamense_KY8             CATTTTTACCCGACTTCTATGCTCAACAAACCCGCGACGCCTGTCAAT--CATCGACGTC
C.siamense_KY146           CATTTTTACCCGACTTCTATGCTCAACAAACCCGCGACGCCTGTCAAT--CATCGACGTC
C.siamense_KY128           CATTTTTACCCGACTTCTATGCTCAACAAACCCGCGACGCCTGTCAAT--CATCGACGTC
C.siamense_LLTA01          ------------------------------------------------------------
C.siamense_C1263.11        CATTTTTACCCGACTTCTATGCTCAACAAACCCGCGACGCCTGTCAAT--CATCGACGTC
C.siamense_KY254           CATTTTTACCCGACTTCTATGCTCAACAAACCCGCGACGCCTGTCAAT--CATCGACGTC
C.siamense_KY263           CATTTTTACCCGACTTCTATGCTCAACAAACCCGCGACGCCTGTCAAT--CATCGACGTC
C.siamense_KY301           CATTTTTACCCGACTTCTATGCTCAACAAACCCGCGACGCCTGTCAAT--CATCGACGTC
C.siamense_KY305           CATTTTTACCCGACTTCTATGCTCAACAAACCCGCGACGCCTGTCAAT--CATCGACGTC
C.theobromicola_C1275.13   CACTTTCATTCGGAGTTCCATGTTCACCAAACCCGCGACGCCCGTTAAT----CCGACCCC
C.theobromicola_C1266.6    CACTTTCATTCGAGTTCCATGTTCACCAAACCCGCGACGCCCGTTAAT----CCGACCCC
C.theobromicola_KY152      CACTTTCACCCGAGTTCCATGTTCACCAAACCCGCGACGCCCGTCAAT----CCGACCCC
C.theobromicola_KY153      CACTTTCACCCGAGTTCCATGTTCACCAAACCCGCGACGCCCGTCAAT----CCGACCCC

C.fioriniae_KY228          TRCCCCCAYSGMRAAWGACWWWTNASCAAWNG------GCCCMGANGTGGCKTTGSYCAC
C.fioriniae_KY28           TANNNCNTCCAAAARGACA-ATGASYNAAACARGKCCMGACTAGCATCTCCAACGMAWCK
C.fioriniae_KY95           TATTMCCACSCAAA-AMGANRTTTACCAAACATGGCCCGACTWGNATCTCCARCGATTCT
C.fioriniae_KY320          TATTCCCACCCAAA-ACGACNTTTACCAAACATGGCCCGACTTGCATCTCCNACGCTTCT
C.fioriniae_KY123          TATTCCCACCCAAA-ACGACATTTACCAAACATGGCCCGACTTGCATCTCCAACGCTTCT
C.fioriniae_KY162          TAYTCCCACCCAAA-ACGACATTTACCAAACATGGCCCGACTTGCATCTCCANCGCTTCT
C.fioriniae_KY191          TATTCCCACCCAAA-ACGACATTTACCAAACATGGCCCGACTTGCATCTCCAACGCTTCT
C.fioriniae_KY291          TATTCCCACCCAAA-ACGACATTTACCAAACATGGCCCGACTTGCATCTCCAACGCTTCT
C.fioriniae_KY105A         TATTCCCACCCAAA-ACGACNTTTACCAAACATGGCCCGACTTGCATCTCCAACGCTTCT
C.fioriniae_HC296          TATTCCCACCCAAA-ACGACATTTACCAAACATGGCCCGACTTGCATCTCCAACGCTTCT
C.fioriniae_KY6            TATTCCCACCCAAA-ACGACATTTACCAAACATGGCCCGACTTGCATCTCCAACGCTTCT
C.fioriniae_CB129932       ------------------------------------------------------------
C.fioriniae_CBS128517      ------------------------------------------------------------
C.fioriniae_CBS112995      ------------------------------------------------------------
C.fioriniae_CBS127538      ------------------------------------------------------------
C.fioriniae_IMI324996      ------------------------------------------------------------
C.fioriniae_CBS124958      ------------------------------------------------------------
C.fioriniae_ATSS12097      ------------------------------------------------------------
C.fioriniae_CBS127537      ------------------------------------------------------------
C.fioriniae_CBS124963      ------------------------------------------------------------
C.fioriniae_CBS235.49      ------------------------------------------------------------
C.fioriniae_CBS127611      ------------------------------------------------------------
C.fioriniae_C188           TATTCCCACCCAAA-ACGACATTTACCAAACATGGCCCGACTTGCATCTCCAACGCTTCT
C.nymphaeae_HC647          TATTCCCACCCAAG--CCATGCTCGCCAAACATGGCCCGACTTGCATCTCCAACGCTCCT
C.nymphaeae_KY9            TATTCCCA-CCCAA-GCCATGCTCGCCAAACATGGCCCGACTTGCATCTCCAACGCTCCT
C.nymphaeae_HC646          TATTCCCACCCCAA-GCCATGCTCGCCAAACATGGCCCGACTTGCATCTCCAACGCTCCT
C.nymphaeae_IMI360928      ------------------------------------------------------------
C.nymphaeae_CBS129937      ------------------------------------------------------------
C.nymphaeae_CBS129933      ------------------------------------------------------------
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C.fructicola_HC540          CAACT-----CTGGATTGTTTTGCTGACTGCTGCT--TTTTTTCTCTACAGGTTCACCTC
C.fructicola_KY40           CAACT-----CTGGATTGTTTTGCTGACTGCTGCT--TTTTTTCTCTACAGGTTCACCTC
C.fructicola_C1253.2        CAACT-----CTGGATTGTTTTGCTGACTGCTGCT--TTTTTTCTCTACAGGTTCACCTC
C.fructicola_C1315.3        CAACT----CTGGATTGTTTTGCTGACTGCTGCT--TTTTTTCTCTACAGGTTCACCTC
C.fructicola_C1265.4        CAACT-----CTGGATTGTTTTGCTGACTGCTGCT--TTTTTTCTCTACAGGTTCACCTC
C.siamense_C1316.5          CAACT-----CTGGAATGTTTTGCTGACTGCTGCTT-TTTTTTGTCTACAGGTTCACCTC
C.siamense_KY8              CAACT-----CTGGAATGTTTTGCTGACTGCTGCTT-TTTTTTGTCTACAGGTTCACCTC
C.siamense_KY146            CAACT-----CTGGAATGTTTTGCTGACTGCTGCTT-TTTTTTGTCTACAGGTTCACCTC
C.siamense_KY128            CAACT-----CTGGAATGTTTTGCTGACTGCTGCTT-TTTTTTGTCTACAGGTTCACCTC
C.siamense_LLTA01           ------------------------------------------------------------
C.siamense_C1263.11         CAACT-----CTGGAATGTTTTGCTGACTGCTGCTTTTTTTTTGTCTACAGGTTCACCTC
C.siamense_KY254            CAACT-----CTGGAATGTTTTGCTGACTGCTGCTT-TTTTTTGTCTACAGGTTCACCTC
C.siamense_KY263            CAACT-----CTGGAATGTTTTGCTGACTGCTGCTT-TTTTTTGTCTACAGGTTCACCTC
C.siamense_KY301            CAACT----CTGGAATGTTTTGCTGACTGCTGCTT-TTTTTTGTCTACAGGTTCACCTC
C.siamense_KY305            CAACT-----CTGGAATGTTTTGCTGACTGCTGCTTTTTTTTTGTCTACAGGTTCACCTC
C.theobromicola_C1275.13    CTACT-----GCGGGATGCTTTGCTGACTGCCGC---TTTTCTCTACAGGTTCACCTC
C.theobromicola_C1266.6     CTACT-----GCGGGATGCTTTGCTGACTGCCGC---TTTTCTCTACAGGTTCACCTC
C.theobromicola_KY152       CTACT-----GCGGGATGCTTTGCTGACTGCCGC---TTTTCTCTCTACAGGTTCACCTC
C.theobromicola_KY153       CTACT-----GCGGGATGCTTTGCTGACTGCCGC---TTTTCTCTCTACAGGTTCACCTC
C.fioriniae_KY228           KAACTAGCAYGGGAAACATTYCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCACCRT
C.fioriniae_KY28            AGCAW-----GGGAAACATTYCSCNSACCATTGATTGTTTTCTCGCAATAGGTTCACCKT
C.fioriniae_KY95            AGCAT-----GGGAAACATTCCGCTGACCMTTGATTGTTTTCTCGCAATAGGTTCACCTT
C.fioriniae_KY320           AGCAT-----GGGAAACATTCCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCACCTT
C.fioriniae_KY123           AGCAT-----GGGAAACATTCCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCACCTT
C.fioriniae_KY162           AGCAT-----GGGAAACATTCCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCACCTT
C.fioriniae_KY191           AGCAT-----GGGAAACATTCCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCACCTT
C.fioriniae_KY291           AGCAT-----GGGAAACATTCCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCACCTT
C.fioriniae_KY105A          AGCAT-----GGGAAACATTCCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCACCTT
C.fioriniae_HC296           AGCAT-----GGGAAACATTCCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCACCTT
C.fioriniae_KY6             AGCAT-----GGGAAACATTCCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCACCTT
C.fioriniae_CB129932        ------------------------------------------------------------
C.fioriniae_CBS128517       ------------------------------------------------------------
C.fioriniae_CBS112995       ------------------------------------------------------------
C.fioriniae_CBS127538       ------------------------------------------------------------
C.fioriniae_IMI324996       ------------------------------------------------------------
C.fioriniae_CBS124958       ------------------------------------------------------------
C.fioriniae_ATSS12097       ------------------------------------------------------------
C.fioriniae_CBS127537       ------------------------------------------------------------
C.fioriniae_CBS124963       ------------------------------------------------------------
C.fioriniae_CBS235.49       ------------------------------------------------------------
C.fioriniae_CBS127611       ------------------------------------------------------------
C.fioriniae_C188            AGCAT-----GGGAAACATTCCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCACCTT
C.nymphaeae_HC647           GTCGT-----GGGAAACATTCCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCMCCTT
C.nymphaeae_KY9             GTCGT-----GGGAAACATTCCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCACCTT
C.nymphaeae_HC646           GTCGT-----GGGAAACATTCCGCTGACCATTGATTGTTTTCTCGCAATAGGTTCACCTT
C.nymphaeae_IMI360928       ------------------------------------------------------------
C.nymphaeae_CBS129937       ------------------------------------------------------------
C.nymphaeae_CBS129933       ------------------------------------------------------------
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C.fructicola_HC540        CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.fructicola_KY40         CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.fructicola_C1253.2      CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.fructicola_C1315.3      CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.fructicola_C1265.4      CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.siamense_C1316.5        CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.siamense_KY8            CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.siamense_KY146          CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACTG--CCTGATTGC-----
C.siamense_KY128          CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.siamense_LLTA01         --------------CGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.siamense_C1263.11       CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.siamense_KY254          CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.siamense_KY263          CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.siamense_KY301          CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.siamense_KY305          CAGACCGGCCAGTGCGTAAGTCT-TCCCAAGCCAAATCCAACCG--CCTGATTGC-----
C.theobromicola_C1275.13  CAGACCGGCCAGTGCGTAAGTAG-TCCCAAAGCAAATTCAATCG--CCTGATTGCAGGGC
C.theobromicola_C1266.6   CAGACCGGCCAGTGCGTAAGTAG-TCCCAAAGCAAATTCAATCG--CCTGATTGCAGGGC
C.theobromicola_KY152     CAGACCGGCCAGTGCGTAAGTAG-TCCCAAAGCAAATTCAATCG--CCTGATTGCAGGGC
C.theobromicola_KY153     CAGACCGGCCAGTGCGTAAGTAG-TCCCAAAGCAAATTCAATCG--CCTGATTGCAGGGC
C.fioriniae_KY228         CARACCGGCCAGTGCGTAWSTATCTCCTGATCTCAACTCARCGAG-CCGGAATGG-----
C.fioriniae_KY28          CAGACCGGCCAGTGCGTAWSTATCTCCTGATCTCAACTCAACGAG-CCGGARTGS-----
C.fioriniae_KY95          CAGACCGGCCAGTGCGTAAGTATCTCCTGATCTCAACACAACGAG-CCGGARTGS-----
C.fioriniae_KY320         CAGACCGGCCAGTGCGTAAGTATCTCCTGATCTCAACACAACGAG-CCGGARTGC-----
C.fioriniae_KY123         CAGACCGGCCAGTGCGTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_KY162         CAGACCGGCCAGTGCGTAAGTATCTCCTGATCTCAACACAACGAG-CCGGARTGC-----
C.fioriniae_KY191         CAGACCGGCCAGTGCGTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_KY291         CAGACCGGCCAGTGCGTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_KY105A        CAGACCGGCCAGTGCGTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_HC296         CAGACCGGCCAGTGCGTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_KY6           CAGACCGGCCAGTGCGTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_CB129932      ---------------GTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_CBS128517     ---------------GTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_CBS112995     ---------------GTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_CBS127538     ---------------GTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_IMI324996     ---------------GTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_CBS124958     ---------------GTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_ATSS12097     ---------------GTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_CBS127537     ---------------GTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_CBS124963     ---------------GTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_CBS235.49     ---------------GTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_CBS127611     ---------------GTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.fioriniae_C188          CAGACCGGCCAGTGCGTAAGTATCTCCTGATCTCAACACAACGAG-CCGGAGTGC-----
C.nymphaeae_HC647         CAGACCGGCCAGTGCGTAAGTATCTCCTGATCTCAACCCAACAAGNCCAGAGTGS-----
C.nymphaeae_KY9           CAGACCGGCCAGTGCGTAAGTATCTCSTGATCTCAAYCCAACAAG-CCAGAGTGC-----

C.nymphaeae_HC646         CAGACCGGCCAGTGCGTAAGTATCTCCTGATCTCAACCCAACAAG-CCAGAGTGG-----
C.nymphaeae_IMI360928     ---------------GTAAGTATCTCCTGATCTCAACCCAACAAG-CCAGAGTGC-----
C.nymphaeae_CBS129937     ---------------GTAAGTATCTCCTGATCTCAACCCAACAAG-CCAGAGTGC-----
C.nymphaeae_CBS129933     ---------------GTAAGTATCTCCTGATCTCAACCCAACAAG-CCAGAGTGC-----
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C.fructicola_HC540        --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.fructicola_KY40         --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.fructicola_C1253.2      --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.fructicola_C1315.3      --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.fructicola_C1265.4      --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.siamense_C1316.5        --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.siamense_KY8            --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.siamense_KY146          --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.siamense_KY128          --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.siamense_LLTA01         --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.siamense_C1263.11       --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.siamense_KY254          --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.siamense_KY263          --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.siamense_KY301          --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.siamense_KY305          --GGGGCTAACCTCCTTGTACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.theobromicola_C1275.13  TAAGGGCTAATCTCTCGCAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.theobromicola_C1266.6   TAAGGGCTAATCTCTCGCAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.theobromicola_KY152     TAAGGGCTAATCTCTCGCAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.theobromicola_KY153     TAAGGGCTAATCTCTCGCAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTACGTGACGA
C.fioriniae_KY228         --GGGGCTRAYTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_KY28          --GGGGCTAACTTCWWTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_KY95          --GGGGCTRACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCR
C.fioriniae_KY320         --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_KY123         --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_KY162         --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_KY191         --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_KY291         --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_KY105A        --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_HC296         --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_KY6           --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_CB129932      --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_CBS128517     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_CBS112995     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_CBS127538     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_IMI324996     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_CBS124958     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_ATSS12097     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_CBS127537     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_CBS124963     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_CBS235.49     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_CBS127611     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.fioriniae_C188          --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.nymphaeae_HC647         --GGGGCTAACTTCTTTGAMCAGGGTAACCAGATTGGTGCTSCCTTCTGGTGSGTASCCA
C.nymphaeae_KY9           --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.nymphaeae_HC646         --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTSCCTTCTGGTGCGTAGCCA
C.nymphaeae_IMI360928     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.nymphaeae_CBS129937     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA
C.nymphaeae_CBS129933     --GGGGCTAACTTCTTTGAACAGGGTAACCAGATTGGTGCTGCCTTCTGGTGCGTAGCCA

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C.fructicola_HC540        GACCGCCGACGACCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.fructicola_KY40         GACCGCCGACGACCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.fructicola_C1253.2      GACCGCCGACGACCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.fructicola_C1315.3      GACCGCCGACGACCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.fructicola_C1265.4      GACCGCCGACGACCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.siamense_C1316.5        GACCGCCGACGATCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.siamense_KY8            GACCGCCGACGATCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.siamense_KY146          GACCGCCGACGATCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.siamense_KY128          GACCGCCGACGATCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.siamense_LLTA01         GACCGCCGACGATCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.siamense_C1263.11       GACCGCCGACGATCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.siamense_KY254          GACCGCCGACGATCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.siamense_KY263          GACCGCCGACGATCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.siamense_KY301          GACCGCCGACGATCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.siamense_KY305          GACCGCCGACGATCCGGCAATATATAC-----TTGCGAGGACGGCAGATGTTGACGATAG
C.theobromicola_C1275.13  GACCGCCGACGAC-CGGCAACAAACACCACAATTGCGAGAACGAGAGATGTTGACGATGG
C.theobromicola_C1266.6   GACCGCCGACGAC-CGGCAACAAACACCACAATTGCGAGAACGAGAGATGTTGACGATGG
C.theobromicola_KY152     GACCGCCGACGAC-CGGCAACAAACACCACAATTGCGAGAACGAGAGATGTTGACGATGG
C.theobromicola_KY153     GACCGCCGACGAC-CGGCAACAAACACCACAATTGCGAGAACGAGAGATGTTGACGATGG
C.fioriniae_KY228         -ACCGMWCRYGACGCGGCGATTTCSACMTTTGATACGATTTCG-----TASTGACCTTGG
C.fioriniae_KY28          -ACCGMWCRYGACGCGGCGATTTCSACATTTGATACGATTTCG-----TASTGACSWTGG
C.fioriniae_KY95          -ACCGCTCRYGACGCGGCGATTTCSACMTTTGATACGATTTCG-----TASTGACCTTGG
C.fioriniae_KY320         -ACCGCTCACGACGCGGCGATTTCSACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_KY123         -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_KY162         -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_KY191         -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_KY291         -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_KY105A        -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_HC296         -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_KY6           -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_CB129932      -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_CBS128517     -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_CBS112995     -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_CBS127538     -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_IMI324996     -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_CBS124958     -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_ATSS12097     -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_CBS127537     -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_CBS124963     -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_CBS235.49     -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_CBS127611     -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.fioriniae_C188          -ACCGCTCACGACGCGGCGATTTCGACATTTGATACGATTTCG-----TACTGACCTTGG
C.nymphaeae_HC647         -ACCSCCAGCGACGCGGCGATCCCGACATTTGACACGATCTCG-----TACTGMCCTTGG
C.nymphaeae_KY9           -ACCGCCAGCGACGCGGCGATCCCGACATTTGACACGATCTCG-----TACTGACCTTGG
C.nymphaeae_HC646         -ACCSCCAGCGACGCGGCGATCCCGACATTTGACACGATCTCG-----TACTGACCTTGG
C.nymphaeae_IMI360928     -ACCGCCAGCGACGCGGCGATTCCGACATTTGACACGATCTCG-----TACTGACCTTGG
C.nymphaeae_CBS129937     -ACCGCCAGCGACGCGGCGATTCCGACATTTGACACGATCTCG-----TACTGACCTTGG
C.nymphaeae_CBS129933     -ACCGCCAGCGACGCGGCGATTCCGACATTTGACACGATCTCG-----TACTGACCTTGG
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C.fructicola_HC540          AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.fructicola_KY40           AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.fructicola_C1253.2        AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.fructicola_C1315.3        AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.fructicola_C1265.4        AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.siamense_C1316.5          AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.siamense_KY8              AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.siamense_KY146            AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.siamense_KY128            AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.siamense_LLTA01           AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.siamense_C1263.11         AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.siamense_KY254            AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.siamense_KY263            AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.siamense_KY301            AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.siamense_KY305            AGTAGGCAAAACATTTCTGGCGAGCACGGCCTCGACAGCAATGGAGTGTATGTCATGCCC
C.theobromicola_C1275.13    AACAGGCAAAACATTTCTGGCGAGCACGGCCTTGACAGCAATGGAGTGTATGTCATGCCC
C.theobromicola_C1266.6     AACAGGCAAAACATTTCTGGCGAGCACGGCCTTGACAGCAATGGAGTGTATGTCATGCCC
C.theobromicola_KY152       AACAGGCAAAACATTTCTGGCGAGCACGGCCTTGACAGCAATGGAGTGTATGTCATGCCC
C.theobromicola_KY153       AACAGGCAAAACATTTCTGGCGAGCACGGCCTTGACAGCAATGGAGTGTATGTCATGCCC

C.fioriniae_KY228           KACAGGCAAAACATYTYTGGCGAGCACGGTCTMGACAGCAAYGGMRTGTATGTCAC----
C.fioriniae_KY28            TACAGGCAAAACATYTYTGGCGAGCACGGTCTCGACAGCAATGGMATGTATGTCAC----
C.fioriniae_KY95            TACAGGCRAAACATCTYTGGCGAGCACGGTCTMGACAGCAATGGMGTGTATGTCAC----
C.fioriniae_KY320           TACAGGCAAAACATCTYTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_KY123           TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_KY162           TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_KY191           TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_KY291           TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_KY105A          TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_HC296           TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_KY6             TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_CB129932        TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_CBS128517       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_CBS112995       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_CBS127538       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_IMI324996       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_CBS124958       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_ATSS12097       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_CBS127537       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_CBS124963       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_CBS235.49       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_CBS127611       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.fioriniae_C188            TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.nymphaeae_HC647           TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.nymphaeae_KY9             TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.nymphaeae_HC646           TACAGGSAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.nymphaeae_IMI360928       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.nymphaeae_CBS129937       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
C.nymphaeae_CBS129933       TACAGGCAAAACATCTCTGGCGAGCACGGTCTCGACAGCAATGGCGTGTATGTCAC----
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C.fructicola_HC540          CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.fructicola_KY40           CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.fructicola_C1253.2        CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.fructicola_C1315.3        CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.fructicola_C1265.4        CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.siamense_C1316.5          CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAT
C.siamense_KY8              CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.siamense_KY146            CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.siamense_KY128            CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.siamense_LLTA01           CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.siamense_C1263.11         CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.siamense_KY254            CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.siamense_KY263            CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.siamense_KY301            CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.siamense_KY305            CTTATCTGGCCACATTGGTGGTTGACCGCTAAACTCGA--------------ACAGCTAC
C.theobromicola_C1275.13    GTTATCTGGCCACATTGATGGATGACCGCTAAACTCGA--------------ACAGCTAC
C.theobromicola_C1266.6     GTTATCTGGCCACATTGATGGATGACCGCTAAACTCGA--------------ACAGCTAC
C.theobromicola_KY152       GTTATCTGGCCACATTGATGGATGACCGCTAAACTCGA--------------ACAGCTAC
C.theobromicola_KY153       GTTATCTGGCCACATTGATGGATGACCGCTAAACTCGA--------------ACAGCTAC
C.fioriniae_KY228           ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAKCTAATTATACCASAGGTAC
C.fioriniae_KY28            ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAKCTAATTATACCACAGGTAC
C.fioriniae_KY95            ----TTTGTCCTCCASTGCGGCTGCCCCATGGACCTAGTAKCTAATTATACCACAGGTAC
C.fioriniae_KY320           ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_KY123           ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_KY162           ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_KY191           ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_KY291           ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_KY105A          ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_HC296           ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_KY6             ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_CB129932        ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_CBS128517       ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_CBS112995       ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_CBS127538       ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_IMI324996       ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_CBS124958       ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_ATSS12097       ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_CBS127537       ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_CBS124963       ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_CBS235.49       ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_CBS127611       ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.fioriniae_C188            ----TTTGTCCTCCAGTGCGGCTGCCCCATGGACCTAGTAGCTAATTATACCACAGGTAC
C.nymphaeae_HC647           -----TCGTCCTCCAGTGTGGCTTCCCCGTGGACCCAGCAGCTAATCATACCATAGGTAC
C.nymphaeae_KY9             -----TCGTCCTCCAGTGTGGCTTCCCCGTGGACCCAGCAGCTAATCATACCATAGGTAC
C.nymphaeae_HC646           -----TCGTCCTCCAGTGTGGCTTCCCCGTGGACCCAGCAGCTAATCATACCATAGGTAC
C.nymphaeae_IMI360928       -----TCGTCCTCCAGTGTGGCTTCCCCGTGGACCCAGCAGCTAATCATACCATAGGTAC
C.nymphaeae_CBS129937       -----TCGTCCTCCAGTGTGGCTTCCCCGTGGACCCAGCAGCTAATCATACCATAGGTAC
C.nymphaeae_CBS129933       -----TCGTCCTCCAGTGTGGCTTCCCCGTGGACCCAGCAGCTAATCATACCATAGGTAC
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C.fructicola_HC540          AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.fructicola_KY40           AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.fructicola_C1253.2        AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.fructicola_C1315.3        AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.fructicola_C1265.4        AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.siamense_C1316.5          AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.siamense_KY8              AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.siamense_KY146            AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.siamense_KY128            AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.siamense_LLTA01           AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.siamense_C1263.11         AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.siamense_KY254            AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.siamense_KY263            AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.siamense_KY301            AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.siamense_KY305            AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAC
C.theobromicola_C1275.13    AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTATTTCAACGAAGTTTGTTAC
C.theobromicola_C1266.6     AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTATTTCAACGAAGTTTGTTAC
C.theobromicola_KY152       AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTATTTCAACGAAGTTTGTTAC
C.theobromicola_KY153       AACGGCACCTCTGAGCTCCAGCTCGAGCGCATGAGCGTCTATTTCAACGAAGTTTGTTAC
C.fioriniae_KY228           RACGGCACTTCCGAGCTCCASCTCGAGYGTATKAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_KY28            AACGGCACTTCCGAGCTCCASCTCGAGYGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_KY95            AACGGCACTTCCGAGCTCCAGCTCGAGYGTATGAGCGTCTACWTYAACGAAKTTTGTTAT
C.fioriniae_KY320           AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_KY123           AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_KY162           AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_KY191           AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_KY291           AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_KY105A          AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_HC296           AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_KY6             AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_CB129932        AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_CBS128517       AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_CBS112995       AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_CBS127538       AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_IMI324996       AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_CBS124958       AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_ATSS12097       AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_CBS127537       AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_CBS124963       AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_CBS235.49       AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_CBS127611       AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.fioriniae_C188            AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.nymphaeae_HC647           AACGGCACTTCCGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.nymphaeae_KY9             AACGGCACTTCCGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAT

C.nymphaeae_HC646           AACGGSACTTCCGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.nymphaeae_IMI360928       AACGGCACTTCCGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.nymphaeae_CBS129937       AACGGCACTTCCGAGCTCCAGCTCGAGCGTATGAGCGTCTACTTCAACGAAGTTTGTTAT
C.nymphaeae_CBS129933       AACGGCACTTCCGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAACGAAGTTTGTTAT
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C.fructicola_HC540            CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.fructicola_KY40             CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.fructicola_C1253.2          CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.fructicola_C1315.3          CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.fructicola_C1265.4          CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.siamense_C1316.5            CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.siamense_KY8                CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.siamense_KY146              CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.siamense_KY128              CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.siamense_LLTA01             CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.siamense_C1263.11           CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.siamense_KY254              CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
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C.siamense_KY301              CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.siamense_KY305              CTTATAGCCCCCAGAGTGCAAGATAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.theobromicola_C1275.13      CTTATAGCCCCCAGAGTGCAAGACAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.theobromicola_C1266.6       CTTATAGCCCCCAGAGTGCAAGACAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.theobromicola_KY152         CTTATAGCCCCCAGAGTGCAAGACAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
C.theobromicola_KY153         CTTATAGCCCCCAGAGTGCAAGACAAACATATTGACGAGTACTGACCTTCGCTCCTACCC
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C.fioriniae_KY28              CCTA-RTCCCCCAGTGTGCASG-CAATCCTATTGACGAATGCTGACCTTMTCACCCAATC
C.fioriniae_KY95              CCTA-GTCCCCCAGTGTGCAGG-CAATCYTATTGACGAATGCTGACCTTMTCACCCAATC
C.fioriniae_KY320             CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAATC
C.fioriniae_KY123             CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAATC
C.fioriniae_KY162             CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAATC
C.fioriniae_KY191             CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAATC
C.fioriniae_KY291             CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAATC
C.fioriniae_KY105A            CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAATC
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C.fioriniae_CBS128517         CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAATC
C.fioriniae_CBS112995         CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAATC
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C.fioriniae_CBS124958         CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAATC
C.fioriniae_ATSS12097         CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAATC
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C.fioriniae_CBS127611         CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAATC
C.fioriniae_C188              CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAATC
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C.nymphaeae_KY9               CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAACC
C.nymphaeae_HC646             CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGSTGACCTTCTCACCCAACC
C.nymphaeae_IMI360928         CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAACC
C.nymphaeae_CBS129937         CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAACC
C.nymphaeae_CBS129933         CCTA-GTCCCCCAGTGTGCAGG-CAATCCTATTGACGAATGCTGACCTTCTCACCCAACC
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C.fructicola_KY40         AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.fructicola_C1253.2      AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.fructicola_C1315.3      AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.fructicola_C1265.4      AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.siamense_C1316.5        AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.siamense_KY8            AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.siamense_KY146          AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.siamense_KY128          AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.siamense_LLTA01         AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.siamense_C1263.11       AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.siamense_KY254          AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.siamense_KY263          AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.siamense_KY301          AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.siamense_KY305          AGGCTTCCGGCAACAAGTACGTG-CCCCGTGCCGTCCTCGTCGATTTGGAGCCCGGTACC
C.theobromicola_C1275.13  AGGCTTCCGGCAACAAGTATGTG-CCCCGTGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.theobromicola_C1266.6   AGGCTTCCGGCAACAAGTATGTG-CCCCGTGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.theobromicola_KY152     AGGCTTCCGGCAACAAGTATGTG-CCCCGTGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.theobromicola_KY153     AGGCTTCCGGCAACAAGTATGTG-CCCCGTGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_KY228         AGKNMTCCGGCAANAARTACTTT-CCCCGCGCCGTCCKCNTCAACTTGAARCCCGGARCC
C.fioriniae_KY28          AGGCCTCCGGCAACAAGTACNTTYCCCCGCGCCGTCCTCGTCRACTTGGAGCCCGGWACC
C.fioriniae_KY95          AGGCCTCCGGCAACAAGTACTTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_KY320         AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_KY123         AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_KY162         AGGCCTCCGGCAACAAGTACTTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_KY191         AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_KY291         AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_KY105A        AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_HC296         AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_KY6           AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_CB129932      AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_CBS128517     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_CBS112995     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_CBS127538     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_IMI324996     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_CBS124958     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_ATSS12097     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_CBS127537     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_CBS124963     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_CBS235.49     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_CBS127611     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.fioriniae_C188          AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.nymphaeae_HC647         AGGSCTCCGGCAACAAGTACGTT-CCCCGCSCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.nymphaeae_KY9           AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.nymphaeae_HC646         AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.nymphaeae_IMI360928     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.nymphaeae_CBS129937     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
C.nymphaeae_CBS129933     AGGCCTCCGGCAACAAGTACGTT-CCCCGCGCCGTCCTCGTCGACTTGGAGCCCGGTACC
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C.fructicola_HC540           ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCT-CCG-C-CCCGACA-ACTTCGT-
C.fructicola_KY40            ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCT-CCG-C-CCCGACA-ACTTCGT-
C.fructicola_C1253.2         ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTTCCG-C-CCCGACA-ACTTCGT-
C.fructicola_C1315.3         ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTTCCG-C-CCCGACA-ACTTCGT-
C.fructicola_C1265.4         ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTTCCG-C-CCCGACA-ACTTCGT-
C.siamense_C1316.5           ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTTCCG-C-CCCGACA-ACTTCGT-
C.siamense_KY8               ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCNTCCG-C-CCCGACA-ACTTCGT-
C.siamense_KY146             ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCT-CCG-C-CCCGACA-ACTTCGT-
C.siamense_KY128             ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTNCCG-C-CCCGACA-ACTTCNT-
C.siamense_LLTA01            ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTTCCG-C-CCCGACA-ACTTCGT-
C.siamense_C1263.11          ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTTCCG-C-CCCGACA-ACTTCGT-
C.siamense_KY254             ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCT-CCG-C-CCCGACA-ACTTCGT-
C.siamense_KY263             ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTNCCG-C-CCCGACA-ACTTCGT-
C.siamense_KY301             ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTNCCG-C-CCCGACA-ACTTCGT-
C.siamense_KY305             ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTNCCG-C-CCCGACA-ACTTCGT-
C.theobromicola_C1275.13     ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTTCCG-C-CCCGACA-ACTTCGT-
C.theobromicola_C1266.6      ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTTCCG-C-CCCGACA-ACTTCGT-
C.theobromicola_KY152        ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCT-CCG-C-CCCGACA-ACTTCGT-
C.theobromicola_KY153        ATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCTCTNCCG-C-CCCGACA-ACTTCNT-

C.fioriniae_KY228            -NGGACSCCTTCCGTGCCGGTCCCTTTGGCCAGCTGTTCCG-C-CRSGACM-AMATCGT-
C.fioriniae_KY28             ATGGACSCMTTCCGTGCCGGTCCCTTTGGCCAGNTTTTCCGCCMCCCTACAWATNACSTC
C.fioriniae_KY95             ATGGACGCCTTCCGTGCCGGTCCCTNTGGCCAGCTNNTCCG-C-CCCGACN-NAATCGT-
C.fioriniae_KY320            ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTGTTCCS-C-CCCRACR-WMWTCGT-
C.fioriniae_KY123            ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCGCCC-CCCAACCANATTCGT-
C.fioriniae_KY162            ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCMGCTTTTCCG-C-CCCGACR-ACTTCGT-
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C.fioriniae_KY6              ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGNTTTTCCG-C-CCCGACANACTTCGT-
C.fioriniae_CB129932         ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-AC------
C.fioriniae_CBS128517        ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-AC------
C.fioriniae_CBS112995        ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-AC------
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C.fioriniae_IMI324996        ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-AC------
C.fioriniae_CBS124958        ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-AC------
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C.fioriniae_CBS127537        ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-AC------
C.fioriniae_CBS124963        ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-AC------
C.fioriniae_CBS235.49        ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-AC------
C.fioriniae_CBS127611        ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-AC------
C.fioriniae_C188             ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-ACTTCGT-
C.nymphaeae_HC647            ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTNCCG-C-CCCGACA-ACTTCGT-
C.nymphaeae_KY9              ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGGTTKTCCG-C-CCGACCA-AMTACGT-
C.nymphaeae_HC646            ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTNTCCG-C-CCCGACA-ACTTCGT-
C.nymphaeae_IMI360928        ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-AC------
C.nymphaeae_CBS129937        ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-AC------
C.nymphaeae_CBS129933        ATGGACGCCGTCCGTGCCGGTCCCTTTGGCCAGCTTTTCCG-C-CCCGACA-AC------
                             **** *  ************ *  **** * *     *    * *
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C.fructicola_HC540          CTTCGGCCAGTCTG----------------------------------------
C.fructicola_KY40           CTTCGGCCAGTCTG----------------------------------------
C.fructicola_C1253.2        CTTCGGCCAGTCTG----------------------------------------
C.fructicola_C1315.3        CTTCGGCCAGTCTG----------------------------------------
C.fructicola_C1265.4        CTTCGGCCAGTCTG----------------------------------------
C.siamense_C1316.5          CTTCGGCCAGTCTG----------------------------------------
C.siamense_KY8              CTTCGGCCAGTCTG----------------------------------------
C.siamense_KY146            CTTCGGCCAGTCTG----------------------------------------
C.siamense_KY128            CTTCGGCCAGTCTG----------------------------------------
C.siamense_LLTA01           CTTCGGCCAGTCTG----------------------------------------
C.siamense_C1263.11         CTTCGGCCAGTCTG----------------------------------------
C.siamense_KY254            CTTCGGCCAGTCTG----------------------------------------
C.siamense_KY263            CTTCGGCCAGTCTG----------------------------------------
C.siamense_KY301            CTTCGGCCAGTCTG----------------------------------------
C.siamense_KY305            CTTCGGCCAGTCTG----------------------------------------
C.theobromicola_C1275.13    CTTCGGCCAGTCTG----------------------------------------
C.theobromicola_C1266.6     CTTCGGCCAGTCTG----------------------------------------
C.theobromicola_KY152       CTTCGGCCA---------------------------------------------
C.theobromicola_KY153       CTTCGGCCAGTCTG----------------------------------------
C.fioriniae_KY228           CTTTGGCCAGTCCG----------------------------------------
C.fioriniae_KY28            CTTTGGCCAGTCCG----------------------------------------
C.fioriniae_KY95            CTTTGGCCAGTCCG----------------------------------------
C.fioriniae_KY320           CTTTGGCCAGTCCG----------------------------------------
C.fioriniae_KY123           CTTTGGCCAGTCCG----------------------------------------
C.fioriniae_KY162           CTTTGGCCAGTC------------------------------------------
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C.fioriniae_CB129932        ------------------------------------------------------
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C.fioriniae_ATSS12097       ------------------------------------------------------
C.fioriniae_CBS127537       ------------------------------------------------------
C.fioriniae_CBS124963       ------------------------------------------------------
C.fioriniae_CBS235.49       ------------------------------------------------------
C.fioriniae_CBS127611       ------------------------------------------------------
C.fioriniae_C188            CTTTGGCCAGTCCG----------------------------------------
C.nymphaeae_HC647           CTTTGGCCAGTCCGGTGCCGGCAACAACTGGGCGGGGGGGGCGATAAA
C.nymphaeae_KY9             CTTTGGCCAGTCCG----------------------------------------
C.nymphaeae_HC646           CTTKGGCCMGTCCGGTGCCGGCAACAACTGGGCCAGGGGTCACT----
C.nymphaeae_IMI360928       ------------------------------------------------------
C.nymphaeae_CBS129937       ------------------------------------------------------
C.nymphaeae_CBS129933       ------------------------------------------------------
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Multiple alignments were produced using CLUSTAL multiple sequence alignment by MUSCLE 3.8.

Appendix Six: Lesion Characteristics

Lesion diameter and sporulation on lesions caused by each species of *Colletotrichum*.
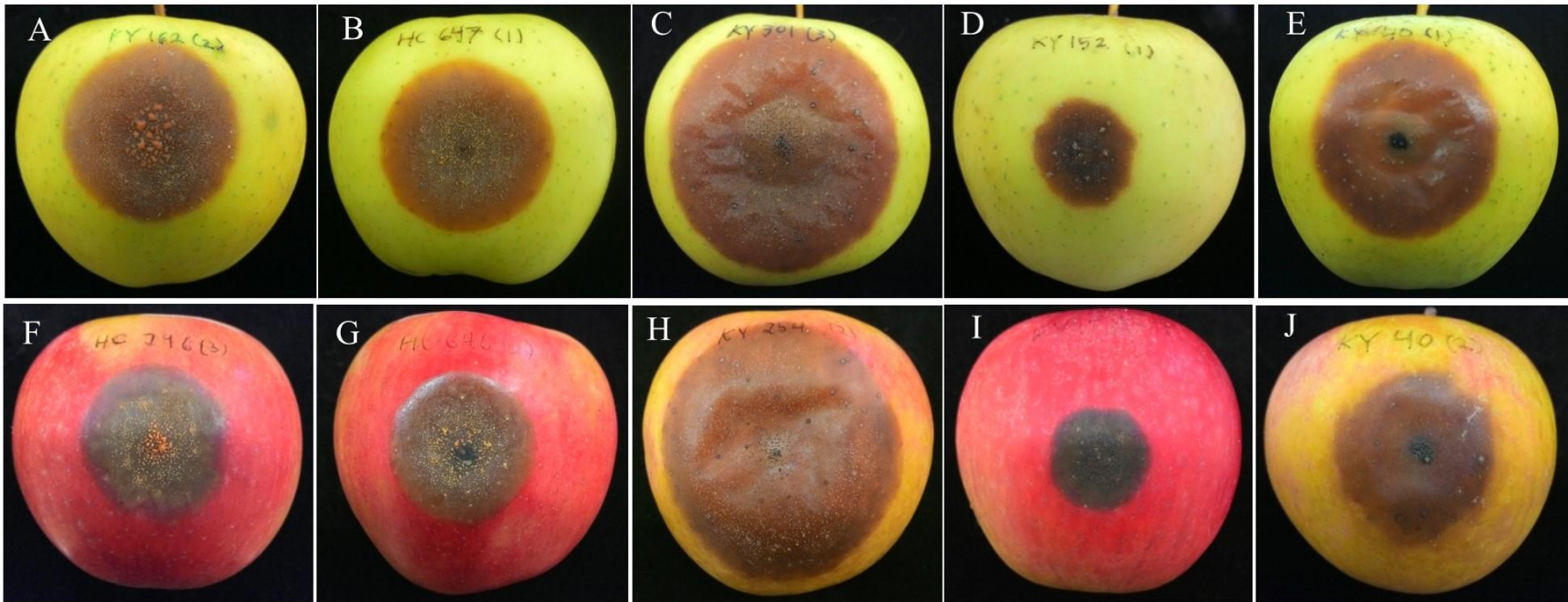

Figure A6.1. Artificial inoculation of (A and F) *C. fioriniae*; (B and G) *C. nymphaeae*; (C and H) *C. siamense*; (D and I) *C. theobromicola*; and (E and J) *C. fructicola* on (A,B,C,D,E) Golden Delicious and (F, G, H, I, J) Red Stayman Winesap. Lesions were 14 days post inoculation (dpi).

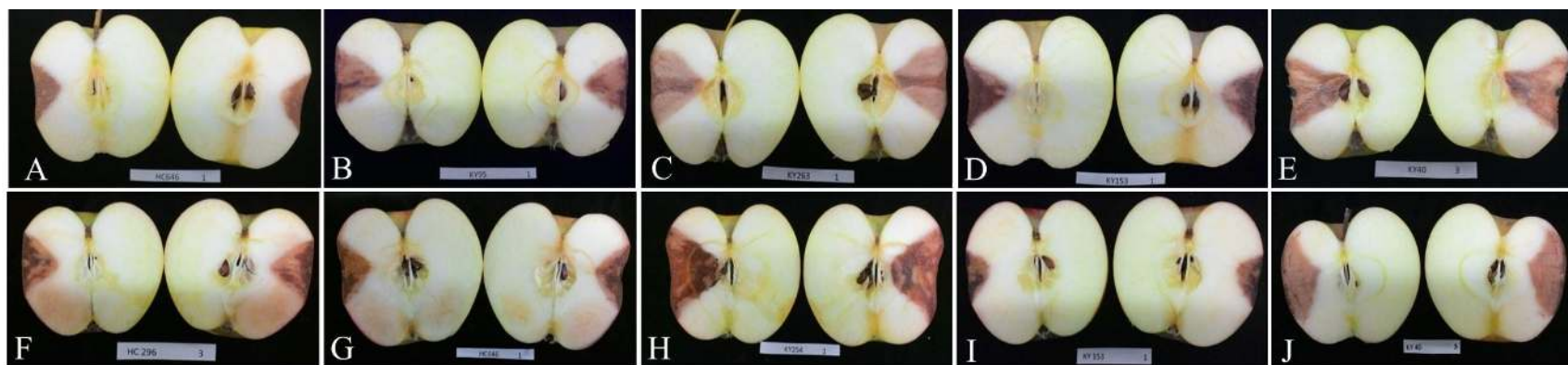**Appearance of affected flesh beneath lesions caused by each species of *Colletotrichum*.**

Figure A6.2. Conical necrotic tissue beneath lesions due to artificial inoculation of (A and F) *C. fioriniae*; (B and G) *C. nymphaeae*; (C and H) *C. siamense*; (D and I) *C. theobromicola*; and (E and J) *C. fructicola* on (A,B,C,D,E) Golden Delicious and (F, G, H, I, J) Red Stayman Winesap. Necrotic tissues were 14 days post inoculation (dpi).

Appendix Seven: Liner Growth of *Colletotrichum* Species on Media Amended with

Selected Fungicides

Linear growth of *Colletotrichum* species on the medium amended with thiophanate-methyl at 10 days post inoculation (dpi).
Fungicide concentration on each strain from top to bottom: 0; 0.01; 0.1; 1; and 100 mg/L.

C. fioriniae

C. nymphaeae

C. siamense

C. theobromicola

C. fructicola

Linear growth of *Colletotrichum* species on media amended with myclobutanil at 10 days post inoculation (dpi).

Fungicide concentration on each strain from top to bottom: 0; 0.01; 0.1; 1; and 100 mg/L.

C. fioriniae

C. nymphaeae

C. siamense

C. theobromicola

C. fructicola

Linear growth of *Colletotrichum* species on media amended with trifloxystrobin at 10 days post inoculation (dpi).

Fungicide concentration on each strain from top to bottom: 0; 0+SHAM; 0.01; 0.1; 1; and 100 mg/L.

C. fioriniae

C. nymphaeae

C. siamense

C. theobromicola

C. fructicola

Linear growth of *Colletotrichum* species on media amended with captan at 10 days post inoculation (dpi).

Fungicide concentration on each strain from top to bottom: 0; 0.1; 1; 10; 100; and 1000 mg/L.
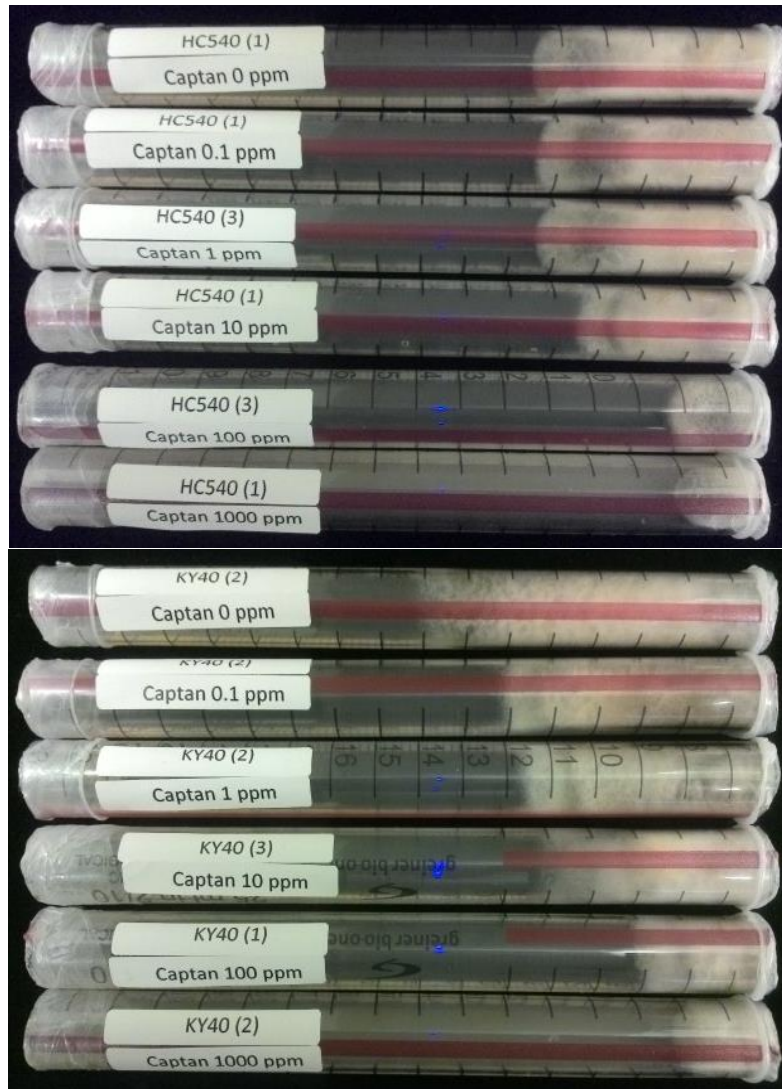

C. fioriniae

C. nymphaeae

KY263 (1) Captan 0 ppm	KY254 (1) Captan 0 ppm
KY263 (3) Captan 0.1 ppm	KY254 (2) Captan 0.1 ppm
KY263 (1) Captan 1 ppm	KY254 (2) Captan 1 ppm
KY263 (2) Captan 10 ppm	KY254 (1) Captan 10 ppm
KY263 (3) Captan 100 ppm	KY254 (2) Captan 100 ppm
KY263 (1) Captan 1000 ppm	KY254 (3) Captan 1000 ppm
KY301 (1) Captan 0 ppm	KY8 (3) Captan 0 ppm
KY301 (2) Captan 0.1 ppm	KY8 (1) Captan 0.1 ppm
KY301 (2) Captan 1 ppm	KY8 (3) Captan 1 ppm
KY301 (1) Captan 10 ppm	KY8 (3) Captan 10 ppm
KY301 (2) Captan 100 ppm	KY8 (2) Captan 100 ppm
KY301 (2) Captan 1000 ppm	KY8 (2) Captan 1000 ppm

C. siamense

C. theobromicola

C. fructicola

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2014   Departmental Seminar: "Can modern taxonomy help us manage anthracnose and other diseases caused by *Colletotrichum* spp.?"

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PROFESSIONAL PUBLICATIONS

- Munir, M. et al. 2008. Effects of *Ocimum sanctum* leaf extract on radial growth of *Corynespora cassiicola*, a causal agent of leaf fall disease on Hevea rubber. *Proceeding of Indonesian Entomologist and Phytopathologist Association*, Palembang, Indonesia. October 18th 2008.

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