HOST RANGE, PHYLOGENETIC, AND PATHOGENIC DIVERSITY OF *Corynespora cassiicola* (Berk. & Curt.) Wei

By

LINLEY JOY SMITH

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To Peter, for making me laugh.

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Abstract of Dissertation Presented to the Graduate School
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By

Linley Joy Smith

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The fungus *Corynespora cassiicola* (Berk. & Curt.) Wei is a pathogen, endophyte, and saprophyte. It can be found growing on at least 530 plant species from 380 genera, primarily in the tropics. Isolates from diverse hosts were collected or solicited from locations in American Samoa, Brazil, Malaysia, Micronesia, and Florida, Mississippi, and Tennessee within the United States. Outgroup taxa including *C. citricola*, *C. melongenea*, *C. olivaceae*, *C. proliferata*, *C. sesamum*, and *C. smithii* were solicited from culture collections. A multilocus phylogenetic analysis using 143 isolates was performed to investigate how genetic diversity correlates with host-specificity, growth rate, and geographic distribution. Phylogenetic trees were congruent from the rDNA ITS region, two random hypervariable loci (*Cs caa5* and *Cs ga4*), and the actin encoding locus *CC act1*, indicating asexual propagation. Fifty isolates had different pathogenicity profiles when tested against eight known *C. cassiicola* hosts: basil, bean, cowpea, cucumber, papaya, soybean, sweet potato, and tomato. Phylogenetic lineage correlated with pathogenicity profiles, host originality, and growth rate, but not with geographic location. Common fungal genotypes were widely distributed geographically indicating long distance and global dispersal of clonal lineages. This research reveals an abundance of previously

unrecognized diversity within the species and provides evidence for redefining species distinctions within *Corynespora*, which will aid in future disease control strategies.

CHAPTER 1
INDEX OF PLANT HOSTS OF *CORYNESPORA CASSIICOLA*

Introduction

Corynespora cassiicola (Berk. & Curt.) Wei has been commonly reported as a plant pathogenic foliar fungus with a wide host range within tropical and subtropical areas (Holliday 1980; Farr et al. 1980; Romruensukharom et al. 2005). In addition to being a pathogen, on some hosts *C. cassiicola* is also reported to grow as an endophyte or saprophyte (Collado 1999; Gond et al. 2007; Promputtha et al. 2007; Suryanarayanan et al. 2002; Kingsland 1985; Hyde et al. 2001; Lee et al. 2004; Lumyong et al. 2003). Though the diseases attributed to *C. cassiicola* are mainly foliar, it may also cause fruit, stem, and root diseases (Jones et al. 1991). The generalization that individual *C. cassiicola* isolates have a wide host range is not supported by the literature because host specific isolates, isolates pathogenic to select hosts, and weak pathogens or secondary invaders of senescent tissue are known to exist (Onesirosan et al. 1974; Cutrim and Silva et al. 2003; Kingsland 1985; Pereira et al. 2003). Rarely reported outside the tropics and subtropics, there are occasional reports of the fungus from temperate regions, particularly on soybean (Boosalis and Hamilton 1957; Malvick 2004; Raffel et al. 1999; Seaman et al. 1965).

Disease symptoms attributed to *C. cassiicola* include necrosis, often with a surrounding yellow halo (Pernezny and Simone 1993) due to the production of a host specific protein toxin, cassiicolin (Barthe et al. 2007; Kurt 2004). With respect to foliage, young and mature leaves can be affected, although the pathogen is more commonly associated with older leaves (Pernezny et al. 2008). Substantial crop losses have been observed in many countries on numerous hosts: southern United States on ornamentals (Alfieri et al. 1984, 1994; Chase 1981,1982, 1984, 1986, 1987, 1993; El-Gholl and Schubert 1990; El-Gholl et al. 1997; Miller and Alfieri 1973;

McRitchie and Miller 1973; Simone 2000, 2000), cucumber (Abul-Hayja et al. 1978; Blazquez 1967; Strandburg 1971), and tomato (Bliss et al. 1973; Blazquez 1972; Jones and Jones 1984; Pernezny et al. 1996, 2002; Smith et al. 2006, Smith et al. 2008b); Midwestern United States on soybean (Boosalis and Hamilton 1957), cowpea (Olive and Bain 1945) and sesame (Stone and Jones 1960); India on ornamentals (Cheeran 1968; Mallaiah et al. 1981; Mehrotra 1987, 1997; Silva et al. 2000; Singh et al. 1982), *Hevea* rubber trees (Atan and Hamid 2003; Silva et al. 1998), cotton (Lakshmanan et al. 1990), and weeds (Philip et al. 1972); Brazil on ornamentals (Da Silva et al. 2005; Leite and Barreto 2000; Pohltronieri 2003), and weeds (Pereira et al. 2003); Philippines, Nigeria, and U.S. Virgin Islands on papaya (Quimio and Abilay 1979; Oluma and Amuta 1999; Bird et al. 1966); and Micronesia and Asia on ornamentals (Florence and Sharma 1987; Hasama et al. 1991), cucurbits (Yudin and Schlub 1998; Tsay and Kuo 1991), tomato (Schlub and Yudin 2002), and pepper (Kwon et al. 2001).

Most regions report *C. cassiicola* diseases on only a few host species, despite the broad host range of the fungus, prompting questions pertaining to isolate host specificity and distribution. Addressing such questions will have implications for disease control and quarantine. The host -specificity and severity of the fungus on *Lantana camara* in Brazil led to the discovery of a new forma specialis, *C. cassiicola* f. sp. *lantanae*, and the use of the isolate as a bioherbicide (Pereira et al. 2003). Based on the vast number of weeds that serve as hosts, and past demonstration of host-specificity in some isolates, there is great potential for the discovery of additional isolates useful for biological control. Further information on the fungal-host interaction and host range of individual isolates will be useful in the study of disease epidemics.

The objective of this study was to compile a list of *C. cassiicola* hosts into a single document, thereby aiding further research on the host range of individual isolates. Prior to this

study, the most complete host listing is in the fungal database of the ARS/USDA Systematic Mycology and Microbiology Laboratory, which included 257 plant host species (Farr et al. 2008). This study will provide a more complete index for use by those engaged in phylogenetic analysis of *Corynespora* spp. and in disease management. Awareness of the potential host range of the fungal species is vital to the determination of the host-specificity of individual isolates. The host range of individual isolates has direct implications for disease management, including the identification of potential inoculum sources, recommendations for intercropping and crop rotation, weed management, biological control candidacy, and isolate choice for resistance breeding.

In order to obtain an estimate of the completeness of the list of hosts known to harbor *C. cassiicola*, surveys were conducted to identify hosts in Guam and Florida. Guam is an ideal location to discover new hosts due to its tropical climate, wet and dry seasons, and lack heretofore of a *Corynespora* host survey (Schlub and Yudin 2002). Florida was included because outbreaks of target spot on tomato caused by *C. cassiicola* are common and it represents a subtropical environment located an ocean and a continent away from Guam.

Methods

Literature Survey and Host Index

An index of plant hosts of *C. cassiicola* was compiled from a search of world literature for any reference regarding its presence on plant tissue. All plant-fungus associations were included such as pathogenic, endophytic, and saprophytic. Resources included articles in refereed journals, graduate student theses, books, and web-based resources such as annual reports, production guides, and plant clinic lists. The final list of susceptible hosts of *C. cassiicola* was compiled from the literature and personal observation from surveys in Florida and Guam.

All plant species, genera, and families were named and classified according to the USDA Germplasm Resources Information Network (GRIN) taxonomy, which follows the APGII system. In some cases, the host name given in the original citation was changed to be consistent with GRIN taxonomy. In a few cases, neither the species cited nor a proper synonym was identified using GRIN taxonomy and the species name was kept as originally cited. Only one reference was provided per host, with emphasis on citing the first known report of that host. For some hosts, the only reference that could be found was a website, and in those cases the website is listed. The number of plant host species was conservatively determined by counting only unique species within each genus. Genera with unidentified species (e.g. *Crossandra* spp.) were counted only once when no other named species were present within that genus.

Guam and Florida Surveys

Surveys for the presence of *C. cassiicola* were conducted throughout Guam and Florida. The Guam survey was conducted for one year beginning in January of 2004 and the Florida survey was conducted for one year beginning in January of 2005. Survey areas focused on roadsides, nurseries, and farms. During the course of the survey, leaves from plants with characteristic *C. cassiicola* foliage disease symptoms were collected and placed in individual plastic bags. Known hosts of *C. cassiicola* were sampled more intensely through the additional collection of old and young asymptomatic leaves. An effort was made to sample from an equal number of individual plants and unique plant species in Florida and Guam.

To induce sporulation, leaf tissue was placed abaxial side up in the moisture chamber for 10 days. Moisture chambers were created on the lab bench by placing 10 ml of sterilized distilled water on a paper towel in a 150 mm petri plate. A plant species was identified as a host of *C. cassiicola* if characteristic structures of the fungus developed within 10 days. An isolate was labeled a pathogen if conidiophores arose from a necrotic spot and an endophyte if

conidiophores arose from healthy, green tissue. Petri plates were inspected under a dissecting microscope daily for spores and conidiophores of *C. cassiicola*. Structures were confirmed based on microscopic morphological features such as percurrent proliferation of the conidiophores and pseudoseptation. Single spore isolates were obtained for long-term storage by needle transfer of spores to antibiotic V8 agar agar slants (340 ml V8 juice, 660 ml water, 3 g $CaCO_3$, 17 g agar, 100 μg/ml ampicillin or kanamycin). Slants were left at room temperature until colonies reached at least 5 cm in diameter, covered with autoclaved mineral oil, and stored at 5^{o} C until further study.

Results

Over 900 individual plants were surveyed in both Guam and Florida from 320 unique plant species in Guam and 289 unique plant species in Florida. Compilation of *Corynespora cassiicola* hosts from the literature and surveys conducted in Guam and Florida resulted in an index of 530 plant species from 380 genera. The majority of index host species for *C. cassiicola* are herbaceous Eudicotyledonae, but 52 Monocotyledonae, eight Magnoliids, five Filicopsida (ferns), and one cycad are also represented. No hosts were found within the Anthocerotophyta (hornworts), Bryophyta (mosses), Equisetopsida (horsetails, sphenophytes), Lycopsida (lycophytes), or Marchantiomorpha (liverworts) (Table 1-1).

Hosts were found in two plant divisions: Filicopsida and Spermatopsida. The five hosts in the Filicopsida include *Arachniodes aristata* (*Davalliaceae*), *Athyrium niponicum* (*Dryopteridaceae*), *Adiantum cuneatum* (*Pteridaceae*), *Davallia repens* (*Davalliaceae*), and *Platycerium* spp. (*Pteridaceae*). The plant division Spermatophyta (*Cycadales, Magnoliidae, Monocotolydonae,* and *Tricolpates*) contains 99% of the host species (Table 1-1). There are eight species from the Magnoliidae. Three species are from the *Piperaceae* (*Piper betle, P.*

hispidinervum, and *Perperomia obtusifolia*). Three species are from the *Magnoliales* in the family *Annonaceae* (*Annona reticulata, A. squamosa* and *Asimina triloba*). Two species are from the *Laurales* in the *Hernandiaceae* (*Hernandia ovigera*) and the *Lauraceae* (*Ocotea leucoxylon*) (Table 1-2).

The 52 host species from the Monocotolydonae are from 16 families: *Araceae* (13 species), *Poaceae* (9 species), *Arecaceae* (7 species), *Dioscoreaceae* (5 species, all from the genus *Dioscorea*), *Orchidaceae* (4 species), *Agavaceae* (3 species), *Musaceae* (2 species), *Alismataceae* (1 species), *Asparagaceae* (1 species), *Bromeliaceae* (1 species), *Commelinaceae* (1 species), *Heliconiaceae* (1 species), *Hemerocallidaceae* (1 species), *Marantaceae* (1 species), *Restonaceae* (1 species), and *Strelitziaceae* (1 species), in decreasing order of host species numbers.

The remaining 464 host species are Eudicots. Families that contain the largest number of hosts include *Fabaceae* (70 species), *Lamiaceae* (33 species), *Malvaceae* (32 species), *Asteraceae* (26 species), *Apocynaceae* (21 species), *Acanthaceae* (20 species), *Euphorbiaceae* (20 species), *Verbenaceae* (17 species), *Convolvulaceae* (14 species), *Cucurbitaceae* (13 species), and *Solanaceae* (13 species), in decreasing order of host species numbers.

Between the two surveys, 91 new hosts species were identified, 87 of which were found in the survey conducted on Guam. New hosts were found in 32 families, of which three families had never been reported to harbor the fungus: *Hernandiaceae*, *Moringaceae*, and *Mutingiaceae*. Ten new host species were found to harbor the fungus in the survey conducted in Florida (*Cerinthe major, Corchorus aestuans, Fatshedera lizei, Hibiscus rosa-sinensis, Jatropha* spp., *Salvia farinacea, Salvia microphylla, Salcia officinalis, Sida spinosa,* and *Stachytarpheta*

jamaicensis). Six new hosts were found in both Guam and Florida (*Corchorus aestuans*, *Salvia farinacea*, *S. microphylla*, *S. officinalis*, *Sida spinosa*, and *Stachytarpheta jamaicensis*).

From the Guam and Florida surveys, *C. cassiicola* was more often identified as a pathogen than as an endophyte on 191 and 121 plant species, respectively. On 48 hosts, the fungus was identified as both a pathogen and an endophyte. Endophytic isolates of *C. cassiicola* were most likely recovered from young leaves and pathogenic isolates from older leaves.

Discussion

The index produced here contains 530 *C. cassiicola* host plant species. Four hundred thirty nine species were identified from the literature and 91 new species were identified from the field surveys conducted in Guam and Florida. The number of new hosts found to harbor the fungus in Guam was 87 and in Florida was 10, with six new hosts found in both Guam and Florida. This suggests that there are many additional host species remaining to be discovered.

Although most of the literature on *C. cassiicola* relates to the diseases it causes, in this study the fungus was often isolated from asymptomatic tissue, indicative of endophytic growth. There are likely many additional endophytic hosts that remain to be discovered considering only healthy leaves from previously reported hosts were sampled. The extent to which *C. cassiicola* was occurring as an endophyte was not appreciated prior to this survey. During the course of the Guam survey, *C. cassiicola* often sporulated from healthy tissue when placed in a moisture chamber instead of necrotic tissue. In these cases, *C. cassiicola* was likely not the cause of the necrosis because other fungi were often found to sporulate in those areas.

There seems to be no clear demarcation as to the presence of *C. cassiicola* on a particular host and its ability to grow endophytically or pathogenically. Publications on *C. cassiicola* are usually restricted to a description of symptoms on a particular host or as part of a list of fungi from an endophyte study. Koch's postulates are rarely completed, and when they are, often the

fungus is not pathogenic on the host it was isolated from without wounding (Kingsland 1985; Pernezny et al. 1996). This study recorded 48 cases from the Guam and Florida surveys where plants were found to harbor pathogenic isolates of *C. cassiicola* and in other locations harbor endophytic isolates. It may be that the fungus has the ability to delay symptoms by growing initially as an endophyte. Pathogenic isolates were often found on older leaves indicating that endophytic isolates may become pathogens as the host tissue ages or begins senescence. Despite the symptomless nature of an endophytic relationship with the host, it is likely that the potential exists for the fungus to switch to an opportunistic pathogen and/or a saprophyte on the same host because individual hosts were found to harbor both pathogenic and endophytic isolates.

The likelihood of finding the fungus as an endophyte or as a pathogen may depend on the plant family. In this study, plant families more likely found harboring the fungus growing as an endophyte were *Araceae, Bignoniaceae*, *Convolvulaceae*, *Crassulaceae, Elaeocarpaceae, Hernandaceae, Magnoliaceae*, *Meliaceae,* and *Moraceae*. *Magnolia liliifera* (*Magnoliaceae*) was recently reported as hosting a *Corynespora* spp. endophyte with ribosomal DNA (ITS1-5.8S-ITS2) sequence homology to *C. cassiicola* (Promputtha et al. 2007) and was therefore included in our list. In the Guam survey, *Hernandia* sp. (*Magnoliaceae*) was also found to support endophytic growth of *C. cassiicola*. Families that were likely to support pathogenic growth of the fungus in these surveys were *Acanthaceae*, *Amaranthaceae, Apocynaceae*, *Asteraceae*, *Begoniaceae*, *Boragniaceae*, *Gesnariaceae, Lamiaceae*, and *Verbenaceae*.

Throughout the survey, it was difficult to determine whether the *Corynespora* species observed were in fact *C. cassiicola*. At least one hundred and thirteen species of *Corynespora* are currently described, but a monograph is needed, including molecular analyses, in order to assess the validity of these species (Sivanesan 1996). Most species have been named according

to host identity, and only a few species have been described in culture. In addition, single isolates exhibit considerable morphological plasticity that depends on humidity, light, temperature, and substrate; therefore, morphological differences need to be compared with molecular differences. Although the hosts included in this index are restricted to those reported for *C. cassiicola*, some may actually be hosts of other *Corynespora* species due to misidentification. Likewise, there may be hosts reported to harbor other species of *Corynespora* that may, in fact, be harboring *C. cassiicola* because the morphological distinctions between species are based on overlapping, variable, morphological characters. Phylogenetic analyses of the isolates should help to clarify these issues.

Despite these complications, this is the first step taken to consolidate our knowledge of the potential host range of *C. cassiicola*, which is vital for further studies of the biology of individual isolates and ultimately in future studies of *Corynespora* species evolution. Although there is no teleomorphic stage currently known for *C. cassiicola*, the Ascomycete species *Corynesporasca caryote* and *Pleomassaria swidae* have unknown *Corynespora* species anamorphs (Sivanesan 1996; Tanaka et al. 2008). There is no evidence to suggest that *C. cassiicola* is reproducing other than by asexual spores. However, evidence for sexual recombination needs to be tested between isolates within and among host species. Insight into the evolutionary potential of the fungus will lead to a better understanding of how to control its diseases (McDonald 2004).

The literature search and surveys elucidated several characteristics of *C. cassiicola* that warrant further investigation: (1) the inability of some isolates recovered from symptomatic tissue to re-infect the original hosts; (2) the ability to be endophytic, pathogenic, and saprophytic on individual hosts; (3) the wide host range of the fungal species, yet restricted host ranges of individual isolates; (4) the ability to grow on some members of a plant taxonomic group and not

others; (5) a lack of understanding of the diversity within the fungal species and how it relates to host range; (6) the taxonomic validity of the 113 species of *Corynespora* considering the high morphological plasticity of individual isolates. Future research should attempt to address these issues and the organization of the plant hosts in a single publication will facilitate this.

Table 1-1. Taxonomic grouping of *Corynespora cassiicola* host species.

Plant Group	Number of Host Species in the Index	Number of Host Species Sampled in Guam	Number of Host Species Sampled in Florida
Anthocerotphyta (hornworts)	0	2	3
Bryophyta (mosses)	0	5	2
Filicopsida (ferns)	5	14	21
Spermatopsida (seed plants)	525	299	263
Conifers	0	3	6
Cycads	1	4	5
Gnetales	0	2	1
Angiosperms	524	290	251
Magnoliids	8	6	4
Monocotyledons	52	61	38
Eudicots	464	223	209

Table 1-2. Occurrence and fungal-host interaction of *Corynespora cassiicola* identified during 2004-2005 Guam and Florida surveys.

Host	Fungal-Host Interaction	Location	Reference
Acanthaceae Juss. (dicot)			
Acanthus ilicifolius L.	endophytic	GU	Sadaba et al. 1995
Aphelandra squarrosa Nees	pathogenic	FL, GU	Chase 1982
Asystasia spp. Blume			Alfieri et al. 1984
Asystasia gangetica (L.) T. Anders.	pathogenic	GU	Alfieri et al. 1984
Crossandra spp. Salisb.	pathogenic	FL	Alfieri et al. 1994
Eranthemum pulchellum Andrews	pathogenic	FL	Alfieri et al. 1994
Fittonia spp. Coem.	pathogenic	FL	Chase 1982
Fittonia albivenis (Lindl. ex hort. Veitch) Brummitt	endophytic, pathogenic	FL, GU	Chase 1982
Hygrophila spp. R. Br.		FL	Alfieri et al. 1994
Justicia spp. L.			Ellis 1957
Justicia brandegeeana Wasshausen & L.B. Sm.	pathogenic	FL, GU	Alfieri et al. 1994
Justicia carnea Lindl.	pathogenic	GU	Ellis 1957
Justicia ventricosa Wall. ex Hook.			Zhuang 2001
Meisosperma oppositifolium	endophytic	GU	Smith et al. 2007
Pachystachys coccinea (Aubl.) Nees			Urtiaga 1986
Pachystachys lutea Nees	pathogenic	FL, GU	Alfieri et al. 1994
Peristrophe spp. Nees			Alfieri et al. 1994
Pseuderanthemum spp. Radlk.			El-Gholl et al. 1997
Pseuderanthemum carruthersii (Seem.) Guillaumin	pathogenic	GU	El-Gholl et al. 1997
Ruellia humboldtiana (Nees) Lindau	endophytic, pathogenic	FL	Urtiaga 2004
Strobilanthes dyerianus M.T. Mast.	pathogenic	GU	Coile and Dixon 1994
Thunbergia fragrans Roxb.			Zhuang 2001
Warpuria clandestina Stapf.	pathogenic	GU	Ellis 1957
Actinidiaceae Gilg & Werderm. (dicot)			
Actinidia chinensis Planch.			Peregrine and Ahmad 1982
Adoxaceae E. Mey. (dicot)			
Viburnum spp. L.			Alfieri et al. 1994
Viburnum odoratissimum Ker Gawl.	endophytic	FL, GU	Alfieri et al. 1994

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Agavaceae Dumort. (monocot)			
Agave sisalana Perrine			Ellis 1957
Cordyline fruticosa (L.) Chev.	endophytic	GU	Situmorang and Budimen 1984
Dracaena spp. Vand. ex L.			Alfieri et al. 1984
Dracaena reflexa Lam.	endophytic, pathogenic	FL	Alfieri et al. 1994
Alismataceae Vent. (monocot)			
Echinodorus spp. Rich. ex Engelm.			Alfieri et al. 1994
Amaranthaceae Juss. (dicot)			
Achyranthes aspera L.			CABI, Herb. IMI 191361
Alternanthera ficoidea (L.) P. Beauv.	pathogenic	GU	first report
Amaranthus spp. L.			Alfieri et al. 1994
Amaranthus spinosus L.	pathogenic	FL, GU	Alfieri et al. 1994
Amaranthus tricolor L.			Peregrine and Ahmad 1982
Celosia argentea L. var. cristata (L.) Kuntze	pathogenic	GU	first report
Digera muricata (L.) Mart.			Sarma and Nayudu 1970
Anacardiaceae R. Br. (dicot)			
Lannea coromandelica (Houtt.) Merr.			CABI, Herb. IMI 266196
Mangifera indica L.			Rajak and Pandey 1985
Schinus spp. L.	endophytic, pathogenic	FL	Alfieri et al. 1984
Spondias purpurea L.	pathogenic	FL	Freire 2005
Vernicia montana Lour.	endophytic	FL	Ellis 1957
Annonaceae Juss. (dicot)			
Annona reticulata L.			Peregrine and Ahmad 1982
Annona squamosa L.	endophytic	GU	first report
Asimina triloba (L.) Dunal			CABI, Herb. IMI 364250
Apiaceae Lindl. (dicot)			
Foeniculum vulgare Mill.			Peregrine and Ahmad 1982

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Apocynaceae Juss. (dicot)			
Adenium obesum (Forssk.) Roem. & Schult.			El-Gholl 1997
Allamanda spp. L.	endophytic	FL	Alfieri et al. 1984
Allamanda cathartica L.	pathogenic	GU	Alfieri et al. 1994
Alstonia scholaris (L.) R. Br.	endophytic, pathogenic	FL	Suryanarayanan et al. 2002
Calotropis procera (Aiton) W. T. Aiton			CABI, Herb. IMI 173980
Carissa spp. L.	pathogenic	FL	Alfieri et al. 1994
Catharanthus roseus (L.) G. Don	pathogenic	FL, GU	McGovern 1994
Conopharyngia longiflora (Benth.) Stapf			Kranz 1963
Cryptolepis buchananii Schult.			CABI, Herb. IMI 221003
Funastrum clausum (Jacq.) Schltr.			Urtiaga 2004
Hoya spp. R. Br.	pathogenic	FL	Alfieri et al. 1994
Mandevilla spp. Lindl.			Alfieri et al. 1984
Mandevilla splendens (Hook. f.) Woodson	pathogenic	FL, GU	Alfieri et al. 1994
Nerium oleander L.	pathogenic	FL	Alfieri et al. 1994
Plumeria rubra L. *forma acutifolia* (Poir.) Woodson	endophytic, pathogenic	GU	Ellis 1957
Rauvolfia serpentina (L.) Benth. ex Kurz			CABI, Herb. IMI 122395
Tabernaemontana divaricata (L.) R. Br. ex Roem. & Schult.			CABI, Herb. IMI 209321
Tabernaemontana sananho Ruiz & Pav.			Urtiaga 2004
Tacazzea spp. Decne.			Ellis 1957
Telosma cordata (Burm. f.) Merr.	endophytic, pathogenic	GU	first report
Thevetia peruviana (Pers.) K. Schum.			CABI, Herb. IMI 231448
Trachelospermum jasminoides (Lindl.) Lem.	pathogenic	FL	Alfieri et al. 1984
Vinca spp. L.			Alfieri et al. 1994
Aquifoliaceae Bercht. & J. Presl (dicot)			
Ilex vomitoria Sol. ex Aiton	endophytic	FL	Alfieri et al. 1994
Araceae Juss. (monocot)			
Aglaonema spp. Schott	pathogenic	FL	Alfieri et al. 1994
Alocasia macrorrhizos (L.) G. Don	endophytic, pathogenic	GU	Mercado et al. 1997
Amorphophallus paeoniifolius (Dennst.) Nicolson			Puzari and Saikia 1981

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Anthurium spp. Schott	pathogenic		Alfieri et al. 1994
Anthurium andraeanum Linden ex André	pathogenic	GU	Alfieri et al. 1994
Anubias afzelii Schott			El-Gholl 1997
Caladium bicolor (Aiton) Vent.	endophytic, pathogenic	GU	first report
Colocasia esculenta (L.) Schott	endophytic	GU	Onesirosan et al. 1974
Dieffenbachia spp. Schott	endophytic	FL	Alfieri et al. 1994
Epipremnum pinnatum (L.) Engl.	pathogenic	FL	Alfieri et al. 1984
Philodendron bipinnatifidum Schott ex Endl.	endophytic	GU	first report
Syngonium podophyllum Schott	pathogenic	GU	Coile and Dixon 1994
Xanthosoma sagittifolium (L.) Schott	endophytic	GU	Ellis 1957
Zantedeschia spp. Spreng.			Raabe et al. 1981
Zantedeschia aethiopica (L.) Spreng.			Raabe et al. 1981
Araliaceae Juss. (dicot)			
Fatshedera spp. Guillaumin			Alfieri et al. 1984
Fatshedera lizei (hort. ex Cochet) Guillaumin	endophytic	FL	first report
Polyscias balfouriana L.H.Bailey			Alfieri et al. 1984
Polyscias fruticosa (L.) Harms	pathogenic	FL	Alfieri et al. 1994
Polyscias scutellaria (Burm. f.) Fosberg	pathogenic	GU	first report
Arecaceae Bercht. & J. Presl (monocot)			
Attalea butyracea (Mutis ex L. f.) Wess. Boer			Urtiaga 2004
Calyptronoma plumeriana (Mart.) Lourteig			Delgado-Rodriguez and Mena-Portales 2004
Cocos nucifera L.			CABI, Herb. IMI 317357
Dypsis lutescens (H. Wendl.) Beentje & J. Dransf.	endophytic, pathogenic	FL	Alfieri et al. 1994
Elaeis guineensis Jacq.			Ellis 1957
Licuala ramsayi (Mueler) Domin.			Shivas and Alcorn 1996
Rhopalostylis sapida H. Wendl and Drude			McKenzie et al. 2004
Asparagaceae Juss. (monocot)			
Asparagus officinalis L.			Urtiaga 2004
Asteraceae Bercht. & J. Presl (dicot)			
Ageratum conyzoides L.	pathogenic	GU	Smith and Schlub 2004

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Aspilia africana (Pers.) C. D. Adams	pathogenic		Onesirosan et al. 1974
Bidens spp. L.			Alfieri et al. 1984
Bidens alba (L.) DC.	pathogenic	FL, GU	Alfieri et al. 1984
Calyptocarpus vialis Less.	pathogenic	GU	Smith and Schlub 2004
Chromolaena odorata (L.) R. M. King & H. Rob.	pathogenic	GU	CABI, Herb. IMI 147913
Chrysanthemum spp. L.	endophytic	FL	Turner 1971
Chrysanthemum indicum L.			Peregrine and Ahmad 1982
Elephantopus mollis Kunth	endophytic	GU	first report
Elephantopus scaber L.			CABI, Herb. IMI 199985
Elephantopus tomentosus L.			Zhuang 2001
Emilia sonchifolia (L.) DC	pathogenic	GU	McKenzie 1990
Gaillardia aristata Pursh	pathogenic	GU	Ellis 1957
Lactuca sativa L.	pathogenic	GU	Ellis 1957
Liatris spp. Gaertn. ex Schreb.	endophytic, pathogenic	FL	Alfieri et al. 1994
Melanthera biflora (L.) Wild			Ellis 1957
Mikania micrantha Kunth	pathogenic	GU	Smith et al. 2007
Pseudelephantopus spicatus (B. Juss. ex Aubl.) C. F. Baker	endophytic	GU	first report
Pseudogynoxys chenopodioides (Kunth) Cabrera	endophytic, pathogenic	FL	Alfieri et al. 1994
Sphagneticola trilobata (L.) Pruski	endophytic	GU	Alfieri et al. 1994
Symphyotrichum novi-belgii (L.) G. L. Nesom			Dixon 1997
Synedrella nodiflora (L.) Gaertn.	pathogenic	GU	Onesirosan et al. 1974
Tithonia rotundifolia (Mill.) S. F. Blake			Wei 1950
Tridax procumbens L.	pathogenic	GU	first report
Verbesina turbacensis Kunth			Urtiaga 2004
Vernonia cinerea (L.) Less.	pathogenic	GU	Cutrim and Silva 2003
Zinnia violacea Cav.			Urtiaga 2004
Balsaminaceae A. Rich. (dicot)			
Impatiens balsamina L.	pathogenic	GU	Wei, 1950
Impatiens noli-tangere L.	pathogenic	FL	CABI, Herb. IMI 124564
Impatiens sultanii Hook. f.			Urtiaga 2004

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Impatiens walleriana Hook. f.			Alfieri et al. 1994
Begoniaceae C. Agardh (dicot)			
Begonia spp. L.			Chase 1982
Begonia coccinea Hook.	pathogenic	GU	Chase 1982
Begonia cucullata Willd.	pathogenic	GU	first report
Bignoniaceae Juss. (dicot)			
Bignonia spp. L.			Orieux and Felix 1968
Crescentia cujete L.	pathogenic	FL	Alfieri et al. 1994
Handroanthus serratifolius (Vahl) S. Grose			Mendes et al. 1998
Newbouldia laevis (P. Beauv.) Seem. ex Bureau	endophytic, pathogenic	GU	Ellis 1957
Radermachera sinica (Hance) Hemsl.	endophytic	FL	Alfieri et al. 1994
Radermachera xylocarpa (Roxb.) K. Schum.	endophytic	FL	Suryanarayanan et al. 2002
Stereospermum colais (Buch.-Ham. ex Dillwyn) Mabb.	endophytic	FL	Murali et al. 2007
Tabebuia spp. Gomes ex DC.			Mendes et al. 1998
Tabebuia aurea (Silva Manso) Benth. & Hook. f. ex S. Moore	pathogenic	FL	Alfieri et al. 1984
Tabebuia heterophylla (DC.) Britton	endophytic	GU	Alfieri et al. 1994
Tabebuia pallida (Lindl.) Miers	pathogenic	FL	Alfieri et al. 1994
Tabebuia odontodiscus (Bureau & K. Schum.) Toledo			Mendes et al. 1998
Tecoma capensis (Thunb.) Lindl.			Urtiaga 2004
Boraginaceae Juss. (dicot)			
Cerinthe major L.	pathogenic	FL	first report
Cordia collococca L.			Urtiaga 2004
Cordia curassavica (Jacq.) Roem. & Schult.			Urtiaga 2004
Cordia obliqua Willd.			Murali et al. 2007
Cordia wallichii G. Don.			Murali et al. 2007
Cordia subcordata Lam.	pathogenic	GU	first report
Tournefortia argentea L. f.	pathogenic	GU	first report
Brassicaceae Burnett (dicot)			
Brassica rapa L.			Peregrine and Ahmad 1982

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Bromeliaceae Juss. (monocot)			
Ananas comosus (L.) Merr.			Blazquez 1968
Burseraceae Kunth (dicot)			
Bursera simaruba (L.) Sarg.	endophytic, pathogenic	FL	Alfieri et al. 1994
Canarium album (Lour.) Raeusch.			Zhang and Ji 2005
Cannabaceae Martinov (dicot)			
Trema micrantha (L.) Blume			Arnold 1986
Trema orientalis (L.) Blume			CABI, Herb. IMI 256125
Capparaceae Juss**.** (dicot)			
Capparis spp. L.			CABI, Herb. IMI 259297
Caprifoliaceae Juss. (dicot)			
Lonicera japonica Thunb.	endophytic	FL	Alfieri et al. 1984
Lonicera sempervirens L.			Alfieri et al. 1994
Caricaceae Dumort. (dicot)			
Carica papaya L.	pathogenic	FL, GU	Beaver 1981
Vasconcellea cauliflora (Jacq.) A. DC.			Urtiaga 2004
Vasconcellea pubescens A. DC.			Johnston 1960
Celastraceae R. (dicot)			
Celastrus paniculatus Willd.			CABI, Herb. IMI 302698
Elaeodendron glaucum (Rottb.) Pers.			Murali et al. 2007
Euonymus spp. L.			Alfieri et al. 1994
Salacia senegalensis (Lam.) DC.			Ellis 1957
Combretaceae R. Br. (dicot)			
Anogeissus latifolia (Roxb. ex DC.) Wall. ex Guill. & Perr.			Suryanarayanan et al. 2002
Terminalia arjuna (Roxb. ex DC.) Wight & Arn.			CABI, Herb. IMI 302839
Terminalia catappa L.	endophytic	GU	first report
Terminalia crenulata Roth.			Murali et al. 2007
Terminalia elliptica Willd.			Suryanarayanan et al. 2002

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Commelinaceae Mirb. (monocot)			
Commelina benghalensis L.	pathogenic	GU	Cutrim and Silva 2003
Convolvulaceae Juss. (dicot)			
Evolvulus glomeratus Nees & Mart.	endophytic	GU	Alfieri et al.1994
Ipomoea alba L.	endophytic, pathogenic	GU	McKenzie 1990
Ipomoea aquatica Forssk.	endophytic	GU	McKenzie 1990
Ipomoea batatas (L.) Lam.	endophytic, pathogenic	FL, GU	Silva et al. 2003
Ipomoea indica (Burm.) Merr.	endophytic, pathogenic	GU	first report
Ipomoea littoralis (L.) Blume	endophytic, pathogenic	GU	first report
Ipomoea obscura (L.) Ker Gawl.	endophytic, pathogenic	GU	Smith and Schlub 2004
Ipomoea pes-caprae (L.) R. Br.	endophytic	GU	Hawaiian Ecosystems at Risk (HEAR) 2008
Ipomoea triloba L.	endophytic, pathogenic	GU	Smith and Schlub 2004
Lepistemon spp. Blume			Onesirosan et al. 1974
Merremia aegyptia (L.) Urb.	endophytic, pathogenic	GU	first report
Merremia peltata (L.) Merr.	endophytic, pathogenic	GU	first report
Operculina turpethum (L.) Silva Manso		GU	first report
Stictocardia tiliifolia (Desr.) Hallier f.	endophytic	GU	first report
Cornaceae Bercht. & J. Presl (dicot)			
Alangium chinense (Lour.) Harms			Guo 1992
Cornus florida L.			Alfieri et al. 1994
Crassulaceae J. St.-Hil. (dicot)			
Crassula ovata (Mill.) Druce			Alfieri et al. 1994
Kalanchoe spp. Adans.	endophytic	FL	Alfieri et al. 1994
Kalanchoe pinnata (Lam.) Pers.	endophytic	GU	first report
Kalanchoe thyrsiflora Harv.	endophytic, pathogenic	GU	first report
Sedum spp. L.			Chase 1982
Cucurbitaceae Juss. (dicot)			
Citrullus lanatus (Thunb.) Matsum. & Nakai	pathogenic	GU	Sobers 1966
Coccinia grandis (L.) Voigt	endophytic, pathogenic	GU	Philip et al. 1972
Cucumis anguria L.	endophytic	GU	Cutrim and Silva 2003

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Cucumis melo L.	endophytic, pathogenic	GU	Ellis and Holliday 1971
Cucumis sativus L.	pathogenic	FL, GU	Wei 1950
Cucurbita spp. L.			Grand 1985
Cucurbita maxima Duchesne			Williams and Liu 1976
Cucurbita moschata Duchesne			Minter et al. 2001
Cucurbita pepo L.	pathogenic	GU	Cutrim and Silva 2003
Lagenaria siceraria (Molina) Standl.	endophytic, pathogenic	GU	Ellis 1957
Luffa acutangula (L.) Roxb.	endophytic, pathogenic	GU	Onesirosan et al. 1974
Luffa aegyptiaca Mill.			Onesirosan et al. 1974
Momordica charantia L.	pathogenic	GU	Alfieri et al. 1994
Sechium edule (Jacq.) Sw.	endophytic, pathogenic	FL, GU	Alfieri et al. 1984
Davalliaceae M. R. Schomb. (dicot)			
Arachniodes aristata (G. Forst.) Tindale	endophytic, pathogenic	GU	Anderson and Dixon 2004
Davallia spp. Sm.			Alfieri et al. 1994
Davallia repens (L. f.) Kuhn	pathogenic	GU	Alfieri et al. 1994
Dioscoreaceae R. Br. (monocot)			
Dioscorea alata L.			CABI, IMI 229871
Dioscorea bulbifera L.	endophytic, pathogenic	GU	Onesirosan et al. 1974
Dioscorea cayenensis Lam.			CABI IMI 83832
Dioscorea esculenta (Lour.) Burkill	endophytic, pathogenic	GU	Onesirosan et al. 1974
Dioscorea pentaphylla L.			Peregrine and Ahmad 1982
Dryopteridaceae Herter (fern)			
Athyrium niponicum (Mett.) Hance	endophytic	GU	El-Gholl 1997
Ebenaceae Gürke (dicot)			
Diospyros montana Roxb.			Murali et al. 2007
Elaeocarpaceae Juss. ex DC. (dicot)			
Elaeocarpus joga Merr.	endophytic	GU	first report
Elaeocarpus tuberculatus Roxb.			Suryanarayanan et al. 2002
Muntingia calabura L.	endophytic	GU	first report

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Ericaceae Juss. (dicot)			
Oxydendrum arboreum (L.) DC.			Alfieri et al. 1994
Rhododendron spp. L.			Alfieri et al. 1984
Rhododendron canescens (Michx.) Sweet			
Rhododendron obtusum (Lindl.) Planch.	endophytic, pathogenic	FL	Ellis and Holliday 1971
Vaccinium corymbosum L.	pathogenic	FL	Hongn et al. 2007
Erythroxylaceae Kunth (dicot)			
Erythroxylum monogynum Roxb.			Murali et al. 2007
Euphorbiaceae Juss. (dicot)			
Acalypha macrostachya Jacq.			Urtiaga 2004
Bridelia ferruginea Benth.			Ellis 1957
Chamaesyce hirta (L.) Millsp.	pathogenic	GU	first report
Codiaeum variegatum (L.) A. Juss.	endophytic, pathogenic	GU	CABI, IMI 179212
Cnidoscolus aconitifolius (Mill.) I. M. Johnst.			Peregrine and Ahmad 1982
Croton bonplandianus Baill.	endophytic, pathogenic	FL, GU	Sarma and Nayudu 1970
Croton fragrans Kunth.			Urtiaga 2004
Drypetes alba Poit.			Mercado 1984
Euphorbia spp. L.			Ellis 1957
Euphorbia cyathophora Murray	endophytic, pathogenic	GU	Barreto and Evans 1998
Euphorbia pulcherrima Willd. ex Klotzsch	pathogenic	FL	Chase 1986
Euphorbia milii Des Moulins	pathogenic	GU	Smith et al. 2007
Givotia rottleriformis Griff.			Murali et al. 2007
Hevea brasiliensis (Willd. ex A. Juss.) Müll. Arg.			Silva et al. 1995
Hura crepitans L.			Urtiaga 1986
Jatropha spp. L.	pathogenic	FL	first report
Jatropha gossypiifolia L.	pathogenic	GU	Smith et al. 2007
Manihot spp. Mill.			Malvick 2004
Manihot carthagenensis (Jacq.) Müll. Arg.			Onesirosan et al. 1974
Manihot esculenta Crantz	endophytic, pathogenic	GU	Ellis 1957

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Phyllanthus amarus Schumach. & Thonn.	endophytic, pathogenic	GU	Mathiyazhagan et al. 2004
Phyllanthus emblica L.			Prakash and Garg 2007
Tragia spp. L.			Ellis 1957
Fabaceae Lindl. (dicot)			
Acacia spp. Mill.			Situmorang and Budimen 1984
Acacia auriculiformis A. Cunn. ex Benth.	endophytic, pathogenic	GU	first report
Afzelia africana Sm. ex Pers.			Dade 1940
Albizia lebbeck (L.) Benth.	endophytic	GU	first report
Albizia zygia (DC.) J. F. Macbr.			Ellis 1957
Alysicarpus vaginalis (L.) DC.	endophytic	GU	first report
Arachis hypogaea L.			Vyas et al. 1985
Bauhinia spp. L.			Alfieri et al. 1994
Bauhinia galpinii N. E. Br.	pathogenic	GU	Smith and Schlub 2004
Bauhinia purpurea L.	pathogenic	FL, GU	Ellis 1957
Bauhinia racemosa Lam.			Suryanarayanan et al. 2002
Butea monosperma (Lam.) Taub.			Murali et al. 2007
Caesalpinia granadillo Pittier			Urtiaga 2004
Cajanus cajan (L.) Millsp.			Lenné 1990
Calopogonium mucunoides Desv.	pathogenic	GU	Onesirosan et al. 1974
Cassia fistula L.	endophytic	GU	Suryanarayanan et al. 2002
Clitoria ternatea L.	pathogenic	GU	first report
Crotalaria goreensis Guill. & Perr.			Hyde and Alcorn 1993
Crotalaria juncea L.		GU	Wei 1950
Crotalaria micans Link			Shaw 1984
Crotalaria pallida Aiton			Turner 1971
Crotalaria retusa L.	endophytic, pathogenic	GU	first report
Crotalaria spectabilis Roth			Malvick 2004
Cyamopsis tetragonoloba (L.) Taub.			Spencer 1962
Dalbergia spp. L. f.			Ellis 1957

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Dalbergia latifolia Roxb.	endophytic		Suryanarayanan et al. 2002
Dalbergia lanceolaria L. f.			Murali et al. 2007
Delonix regia (Bojer ex Hook.) Raf.			CABI, Herb. IMI 314022
Desmodium spp. Desv.			Lenné, 1990
Desmodium incanum DC.	pathogenic	GU	Smith and Schlub 2004
Desmodium tortuosum (Sw.) DC.	pathogenic	GU	Smith and Schlub 2004
Desmodium triflorum (L.) DC.	pathogenic	GU	Smith and Schlub 2004
Erythrina spp. L.			Delgado-Rodriguez et al. 2002
Gliricidia sepium (Jacq.) Kunth ex Walp.			Boa and Lenné 1994
Glycine max (L.) Merr.	pathogenic	FL, GU	Olive et al. 1945
Glycine soja Siebold & Zucc.			Lenné 1990
Hymenaea courbaril L.			Urtiaga 2004
Lens culinaris Medik.			Khare 1991
Lupinus albus L.			Sobers 1966
Lupinus angustifolius L.			Sobers 1966
Lupinus luteus L.			Sobers 1966
Lupinus pilosus L.			Malvick 2004
Macrolobium spp. Schreb.			Kranz 1963
Macroptilium atropurpureum (Moc. & Sessé ex DC.) Urban	pathogenic	GU	first report
Macroptilium lathyroides (L.) Urban	pathogenic	GU	Smith and Schlub 2004
Mimosa diplotricha C. Wright			Silva 1995
Mimosa pudica L.	endophytic, pathogenic	GU	Smith and Schlub 2004
Mucuna pruriens (L.) DC.			Sobers 1966
Phaseolus lunatus L.			Malvick 2004
Phaseolus vulgaris L.	pathogenic	GU	Wei 1950
Pisum sativum L.	pathogenic	GU	first report
Pithecellobium dulce (Roxb.) Benth.	pathogenic	GU	first report
Psophocarpus tetragonolobus (L.) DC.			Ellis 1957
Pterocarpus indicus Willd.			Situmorang and Budimen 1984

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Pueraria montana (Lour.) Merr.			Peregrine and Ahmad 1982
Ricinus communis L.			Spencer and Walters 1968
Saraca indica L.			CABI, Herb. IMI 210811
Senna alata (L.) Roxb.			Wei 1950
Senna occidentalis (L.) Link	pathogenic	GU	first report
Senna surattensis (Burm. f.) H. S. Irwin & Barneby	pathogenic	GU	first report
Senna tora (L.) Roxb.			Situmorang and Budimen 1984
Sesamum indicum L.	endophytic	GU	Wei 1950
Spathodea campanulata P. Beauv.	pathogenic	GU	Smith et al. 2007
Teramnus labialis (L. f.) Spreng.	pathogenic	GU	Smith et al. 2007
Trifolium repens L.			Cho and Shin 2004
Trigonella foenum-graecum L.			Komaraiah and Reddy 1986
Tylosema esculentum (Burch.) A. Schreib.			Alfieri et al. 1994
Vicia spp. L.			Alfieri et al. 1984
Vigna mungo (L.) Hepper			Gowda et al. 2001
Vigna radiata (L.) R. Wilczek			Malvick 2004
Vigna unguiculata (L.) Walp. subsp. *sesquipedalis* (L.) Verdc.	pathogenic	GU	Seaman et al. 1965
Vigna umbellata (Thunb.) Ohwi & H. Ohashi			Peregrine and Ahmad 1982
Wisteria sinensis (Sims) DC.	endophytic	FL	Alfieri et al. 1984
Fagaceae Dumort. (dicot)			
Quercus ilex L.			Collado et al. 1999
Gesneriaceae Rich. & Juss. (dicot)			
Aeschynanthus longicaulis Wall. ex R. Br.			Chase 1982
Aeschynanthus radicans Jack	pathogenic	GU	Chase 1982
Columnea spp. L.			Chase 1982
Episcia cupreata (Hook.) Hanst.	pathogenic	FL	Alfieri et al. 1994
Gloxinia perennis (L.) Fritsch			Brooks 2002
Nematanthus spp. Schrad.			Chase 1982
Saintpaulia ionantha H. Wendl.	pathogenic	GU	Smith et al. 2007

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Sinningia speciosa (Lodd. et al.) Hiern	pathogenic	FL	Alfieri et al. 1994
Streptocarpus spp. Lindl.			Alfieri et al. 1994
Streptocarpus rexii (Bowie ex Hook.) Lindl.	pathogenic	FL, GU	Alfieri et al. 1994
Heliconiaceae Nakai (monocot)			
Heliconia caribaea Lam.			Urtiaga 2004
Hemerocallidaceae R. Br. (monocot)			
Hemerocallis spp. L.			Peregrine and Ahmad 1982
Hernandiaceae Blume (dicot)			
Hernandia spp. L.	endophytic	GU	first report
Hernandia ovigera L.	endophytic	GU	first report
Hydrangeaceae Dumort. (dicot)			
Hydrangea spp. L.			Alfieri et al. 1984
Hydrangea macrophylla (Thunb.) Ser.	pathogenic	FL	Sobers 1966
Lamiaceae Martinov (dicot)			
Ajuga spp. L.			Alfieri et al. 1984
Ajuga reptans L.	pathogenic	FL	Alfieri et al. 1984
Anisochilus carnosus (L. f.) Wall. ex Benth.			CABI, Herb. IMI 151008
Coleus barbatus (Andrews) Benth.	pathogenic	FL, GU	Fernandes and Barreto 2003
Congea tomentosa Roxb.			Peregrine and Ahmad 1982
Clerodendrum inerme (L.) Gaertn.			Ahmad 1969
Clerodendrum infortunatum L.			CABI, Herb. IMI 112265
Clerodendrum speciosissimum Van Geert ex C. Morren			Urtiaga 1986
Hyptis suaveolens (L.) Poit.	endophytic	GU	Smith et al. 2007
Leucas aspera (Willd.) Link			Sarma and Nayudu 1970
Mentha arvensis L.	endophytic, pathogenic	GU	Cheeran 1968
Mentha ×piperita L.			Williams and Liu 1976
Moluccella spp. L.			Alfieri et al. 1984
Moluccella laevis L.			Alfieri et al. 1984
Monarda punctata L.			Alfieri et al. 1994

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Ocimum basilicum L.	endophytic, pathogenic	GU	Taba et al. 2002
Ocimum tenuiflorum L.			Sarma and Nayudu 1970
Origanum vulgare L.	pathogenic	FL, GU	
Perilla frutescens (L.) Britton	pathogenic	GU	Hasama et al. 1991
Plectranthus amboinicus (Lour.) Spreng.	pathogenic	GU	Miller 1991
Plectranthus barbatus Andrews			Smith et al. 2007
Plectranthus parviflorus Willd.	pathogenic	FL	Alfieri et al. 1994
Premna serratifolia L.	pathogenic	GU	first report
Premna tomentosa Willd.			Murali et al. 2007
Rosmarinus officinalis L.			Alfieri et al. 1994
Salvia spp. L.			Peregrine and Ahmad 1982
Salvia farinacea Benth.	pathogenic	FL, GU	first report
Salvia leucantha Cav.	pathogenic	FL	Riley 1960
Salvia microphylla Kunth	pathogenic	FL, GU	first report
Salvia officinalis L.	pathogenic	FL, GU	first report
Salvia splendens Sellow ex Schult.	pathogenic	FL	Chase 1982
Solenostemon scutellarioides (L.) Codd	pathogenic	FL, GU	Alfieri et al. 1994
Stachys floridana Shuttlew. ex Benth.			Alfieri et al. 1994
Thymus vulgaris L.	pathogenic	FL	Silva 1995
Tectona grandis L. f.			Murali et al. 2007
Teucrium canadense L.			El-Gholl 1997
Lauraceae Juss. (dicot)			
Ocotea leucoxylon (Sw.) Laness.			Delgado-Rodriguez et al. 2002
Lecythidaceae A. Rich. (dicot)			
Careya arborea Roxb.			Murali et al. 2007
Lecythis ollaria Loefl.			Urtiaga 2004
Loganiaceae R. Br. ex Mart. (dicot)			
Buddleja asiatica Lour.	pathogenic	GU	Smith and Schlub 2004
Strychnos potatorum L. f.			Murali et al. 2007

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Lythraceae J. St.-Hil. (dicot)			
Lagerstroemia indica L.	pathogenic	FL	Alfieri et al. 1994
Lagerstroemia microcarpa Wight			Murali et al. 2007
Lagerstroemia parviflora Roxb.			Murali et al. 2007
Pemphis acidula Forst. & Forst.	endophytic	GU	first report
Magnoliaceae Juss. (dicot)			
Magnolia champaca (L.) Baill. ex Pierre			CABI, Herb. IMI 254407
Magnolia liliifera (L.) Baill.	endophytic	FL	Promputtha et al. 2007
Malpighiaceae Juss. (dicot)			
Malpighia glabra L.			Poltronieri et al. 2003
Malvaceae Juss. (dicot)			
Abelmoschus esculentus (L.) Moench	pathogenic	GU	Wei 1950
Abutilon theophrasti Medik.	endophytic, pathogenic	GU	Spencer and Walters 1969
Ceiba pentandra (L.) Gaertn.	endophytic	GU	Mehrotra 1989
Ceiba speciosa (A. St.-Hil.) Ravenna			Ferreira 1989
Corchorus aestuans L.	pathogenic	FL, GU	Smith and Schlub 2004
Corchorus capsularis L.	pathogenic	GU	Wei 1950
Corchorus olitorius L.	endophytic, pathogenic	GU	Ellis 1957
Desplatsia spp. Bocq.			Ellis 1957
Durio zibethinus L.			Williams and Liu 1976
Gossypium barbadense L.	endophytic, pathogenic	GU	Jones 1961
Gossypium hirsutum L.			Jones 1961
Grewia tiliifolia Vahl			Suryanarayanan et al. 2002
Helicteres isora L.			Murali et al. 2007
Hibiscus spp. L.			Urtiaga 2004
Hibiscus cannabinus L.			Shaw 1984
Hibiscus mutabilis L.			Kwon and Park 2003
Hibiscus rosa-sinensis L.	endophytic	FL	first report
Hibiscus sabdariffa L.	endophytic	GU	Wei 1950

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Kydia calycina Roxb.			CABI, Herb. IMI 264454
Pavonia spp. Cav.			Urtiaga 2004
Pseudobombax septenatum (Jacq.) Dugand			Urtiaga 2004
Sida acuta Burm. f.	pathogenic	GU	Smith and Schlub 2004
Sida glomerata Cav.			Urtiaga 2004
Sida rhombifolia L.	pathogenic	GU	CABI, Herb. IMI 180198
Sida spinosa L.	pathogenic	FL, GU	first report
Sida urens L.			Ellis 1957
Sterculia apetala (Jacq.) H. Karst.			Urtiaga 2004
Talipariti tiliaceum (L.) Fryxell	endophytic	GU	first report
Theobroma cacao L.			Duarte et al. 1978
Thespesia populnea (L.) Soland. ex Correa	endophytic pathogenic	GU	first report
Triumfetta rhomboidea Jacq.	endophytic	GU	Onesirosan et al. 1974
Waltheria indica L.	endophytic	GU	CABI, Herb. IMI 123575
Urena lobata L.	pathogenic	GU	first report
Marantaceae R. Br. (monocot)			
Maranta leuconeura E. Morren	pathogenic	FL	Alfieri et al. 1994
Marcgraviaceae Bercht. & J. Presl (dicot)			
Norantea guianensis Aubl.	endophytic	GU	Wei 1950
Meliaceae Juss. (dicot)			
Chukrasia velutina M. Roem.	endophytic	GU	first report
Guarea guidonia (L.) Sleumer			Urtiaga 2004
Melia azedarach L.	endophytic	GU	first report
Moraceae Gaudich. (dicot)			
Artocarpus altilis (Parkinson) Fosberg			CABI, Herb IMI 351978
Broussonetia spp. L'Hér. ex Vent.			Pollack and Stevenson 1973
Broussonetia papyrifera (L.) L'Hér. ex Vent.	endophytic	GU	Alfieri et al. 1994
Ficus spp. L.			Ellis 1957
Ficus benjamina L.	endophytic	GU	Chase 1984

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Ficus elastica Roxb. ex Hornem.	endophytic	GU	Chase 1987
Ficus exasperata Vahl			Onesirosan et al. 1974
Ficus hispida L. f.			CABI, Herb IMI 311137
Ficus lyrata Warb.	endophytic	FL	Alfieri et al. 1994
Ficus racemosa L.			Gilson 2002
Ficus religiosa L.			CABI, Herb. IMI 217075
Moringaceae Martinov (dicot)			
Moringa oleifera Lam.	endophytic	GU	Smith et al. 2007
Muntingiaceae C. Bayer et al. (dicot)			
Muntingia calabura L.	pathogenic	GU	first report
Musaceae Juss. (monocot)			
Musa ×*sapientum* L.			Blazquez 1968
Musa acuminata Colla			Lumyong et al. 2003
Myrsinaceae R. Br. (dicot)			
Ardisia foetida Willd.			Urtiaga 2004
Myrtaceae Juss. (dicot)			
Eucalyptus spp. L'Hér.			
Eucalyptus grandis W. Hill ex Maiden			C.M.I. No. 303
Eucalyptus tereticornis Sm.			Vittal and Dorai 1994
Eugenia uniflora L.	pathogenic	GU	CABI, Herb IMI 99533
Psidium guajava L.			Alfieri et al. 1984
Syzygium aromaticum (L.) Merr. & L. M. Perry			Saikia and Sarbhoy 1981
Syzygium cumini (L.) Skeels	pathogenic	GU	Sarbhoy et al. 1971
Syzygium jambos (L.) Alston	pathogenic	GU	Smith et al. 2007
Nyctaginaceae Juss. (dicot)			
Bougainvillea spectabilis Willd.	endophytic	GU	first report
Mirabilis jalapa L.			CABI, Herb IMI 259283
Nymphaeaceae Salisb. (dicot)			
Nymphaea ampla (Salisb.) DC.			Urtiaga 2004

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Nyssaceae Juss. ex Dumort. (dicot)			
Nyssa spp. L.			Alfieri et al. 1994
Oleaceae Hoffmanns. & Link (dicot)			
Chionanthus retusus Lindl. & Paxton			Alfieri et al. 1994
Jasminum spp. L.			Alfieri et al. 1984
Jasminum laurifolium Roxb. forma *nitidum* (Skan) P. S. Green			Alfieri et al. 1994
Jasminum multiflorum (Burm. f.) Andrews			Alfieri et al. 1994
Jasminum sambac (L.) Aiton			CABI Herb. IMI 111858
Jasminum simplicifolium G. Forst.	pathogenic	FL	Alfieri et al. 1994
Ligustrum lucidum W. T. Aiton			Alfieri et al. 1994
Ligustrum japonicum Thunb.			Alfieri et al. 1994
Ligustrum sinense Lour.	endophytic	GU	Alfieri et al. 1994
Orchidaceae Juss. (monocot)			
Cattleya spp. Lindl.			Simone 2000
Dendrobium spp. Sw.			Alfieri et al. 1994
Phalaenopsis spp. Blume			Alfieri et al. 1994
Vanilla planifolia Andrews			Urtiaga 2004
Passifloraceae Juss. ex Roussel (dicot)			
Passiflora spp. L.			Pernezny and Simone 1993
Passiflora edulis Sims	endophytic	FL	Alfieri et al. 1994
Passiflora foetida L.	pathogenic	GU	Smith et al. 2007
Passiflora suberosa L.	endophytic	GU	first report
Pedaliaceae R. Br. (dicot)			
Josephinia imperatricis Vent.			Hyde and Alcorn 1993
Martynia annua L.			CABI, Herb IMI 264260
Sesamum indicum L.			Riley 1960
Piperaceae Giseke (dicot)			
Piper betle L.	endophytic, pathogenic	GU	Acharya et al. 2003

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Piper hispidinervum C. DC.			Poltronieri et al. 2003
Peperomia obtusifolia (L.) A. Dietr.	pathogenic	FL	Chase 1982
Poaceae Barnhart (monocot)			
Arundinaria pygmaea (Miq.) Asch. & Graebn.			LSU Ag Center 2008
Bambusa vulgaris Schrad. ex J. C. Wendl.	endophytic	GU	first report
Dendrocalamus spp. Nees			Lu et al. 2000
Oryza sativa L.			CABI, Herb IMI 280017
Ottochloa nodosa (Kunth) Dandy			Situmorang and Budimen 1984
Panicum repens L.			Situmorang and Budimen 1984
Pennisetum glaucum (L.) R. Br.			Lenné 1990
Megathyrsus maximus (Jacq.) B. K. Simon & S. W. L. Jacobs	endophytic	GU	Smith and Schlub 2004
Sorghum bicolor (L.) Moench			Mendes et al. 1998
Polypodiaceae Bercht. & J. Presl (fern)			
Platycerium spp. Desv.	pathogenic	FL	Alfieri et al. 1994
Polygonaceae Juss. (dicot)			
Coccoloba fallax Lindau			Urtiaga 2004
Pteridaceae E. D. M. Kirchn. (fern)			
Adiantum spp. L.			Situmorang and Budimen 1984
Adiantum tenerum Sw.	pathogenic	FL	Alfieri et al. 1984
Restionaceae R. Br. (monocot)			
Ischyrolepis subverticillata Steud.			Lee et al. 2004
Rhamnaceae Juss. (dicot)			
Colubrina retusa (Pittier) Cowan			Urtiaga 2004
Ziziphus cyclocardia S.F. Blake	pathogenic	FL	Urtiaga 2004
Ziziphus mauritiana Lam.	pathogenic	GU	first report
Ziziphus xylopyrus (Retz.) Willd.			Murali et al. 2007
Rosaceae Juss. (dicot)			
Malus pumila Mill.			CABI, Herb IMI 284207
Pyrus communis L.			Alfieri et al. 1984

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Rubiaceae Juss. (dicot)			
Guettarda speciosa L.	endophytic	GU	first report
Ixora coccinea L.			CABI, Herb. IMI 129296
Ixora nigricans R. Br. ex Wt. & Am.			Murali et al. 2007
Morinda citrifolia L.	endophytic	GU	first report
Nauclea diderrichii (De Wild.) Merr.			CABI, Herb. IMI 126192
Pentas lanceolata (Forssk.) Deflers	pathogenic	GU	first report
Spermacoce spp. L.			Situmorang and Budimen 1984
Rutaceae Juss. (dicot)			
Aegle marmelos			Gond et al. 2007
Naringi crenulata (Roxb.) Nicolson			Murali et al. 2007
Salicaceae Mirb. (dicot)			
Casearia decandra Jacq.			Urtiaga 2004
Sapindaceae Juss. (dicot)			
Acer negundo L.			El-Gholl 1997
Acer rubrum L.			Alfieri et al. 1994
Cupaniopsis anacardioides (A. Rich.) Radlk.			Alfieri et al. 1994
Dodonaea viscosa Jacq.			Singh et al. 1982
Litchi chinensis Sonn.			
Matayba scrobiculata (Kunth) Radlk.			Urtiaga 2004
Saxifragaceae Juss. (dicot)			
Saxifraga stolonifera Curtis			El-Gholl 1997
Tolmiea spp. Torr. & A. Gray			Alfieri et al. 1984
Tolmiea menziesii (Pursh) Torr. & Gray	pathogenic	FL	Alfieri et al. 1984
Scrophulariaceae Juss. (dicot)			
Alectra sessiliflora (Vahl) Kuntze			Urtiaga 2004
Antirrhinum majus L.			Alfieri et al. 1994
Buchnera americana L.	pathogenic	GU	Smith and Schlub 2004
Digitalis spp. L.			Alfieri et al. 1994

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Paulownia spp. Siebold & Zucc.			Mehrotra 1997
Paulownia tomentosa (Thunb.) Steud.	endophytic	GU	Mehrotra 1997
Russelia equisetiformis Schltdl. & Cham.	endophytic, pathogenic	FL, GU	Alfieri et al. 1984
Simaroubaceae DC. (dicot)			
Ailanthus excelsa Roxb.			CABI, Herb IMI 337615
Solanaceae Juss. (dicot)			
Capsicum annuum L.	endophytic	GU	Kwon et al. 2001
Capsicum frutescens L.			Pernezny and Simone 1993
Nicotiana glutinosa L.			Tsay and Kuo 1991
Nicotiana tabacum L.	pathogenic	GU	Fajola and Alasoadura 1973
Petunia ×hybrida hort. ex E. Vilm.	pathogenic	GU	Alfieri et al. 1994
Petunia integrifolia (Hook.) Schinz & Thell.			Peregrine and Ahmad 1982
Solanum erianthum D. Don			Shaw 1984
Solanum lycopersicum L.	pathogenic	FL, GU	Wei 1950
Solanum melongena L.	endophytic	GU	Onesirosan et al. 1974
Solanum nigrum L.	endophytic	FL, GU	Sarma and Nayudu 1971
Solanum torvum Sw.	endophytic	GU	Onesirosan et al. 1974
Solanum tuberosum L.			Peregrine and Ahmad 1982
Solanum viarum Dunal			Casady 1994
Strelitziaceae Hutch. (monocot)			
Strelitzia spp. Aiton			Alfieri et al. 1994
Strelitzia reginae Aiton	pathogenic	FL, GU	Alfieri et al. 1994
Theaceae Mirb. (dicot)			
Camellia sinensis (L.) Kuntze	endophytic	GU	El-Gholl et al. 1997
Turneraceae Kunth ex DC. (dicot)			
Turnera ulmifolia L.			Urtiaga 2004
Urticaceae Juss. (dicot)			
Boehmeria nivea (L.) Gaudich.			
Cecropia peltata L.			Minter et al. 2001

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Cecropia schreberiana Miq.			Minter et al. 2001
Laportea aestuans (L.) Chew			Alfieri et al. 1994
Pilea spp. Lindl.			Chase 1982
Pilea cadierei Gagnep. & Guillaumin	pathogenic	GU	Alfieri et al. 1994
Pilea microphylla (L.) Liebm.	pathogenic	GU	Smith and Schlub 2004
Pilea nummulariifolia (Sw.) Weddell	pathogenic	FL, GU	Alfieri et al. 1994
Verbenaceae J. St.-Hil. (dicot)			
Callicarpa americana L.			Alfieri et al. 1994
Citharexylum spinosum L.			El-Gholl 1997
Clerodendrum buchananii (Roxb.) Walp.	pathogenic	GU	first report
Clerodendrum paniculatum L.	pathogenic	FL	Ellis 1957
Clerodendrum quadriloculare (Blanco) Merr.	pathogenic	GU	first report
Clerodendrum thomsoniae Balf.	pathogenic	FL	Daughtrey 2000
Gmelina arborea Roxb.	endophytic	GU	Florence and Sharma 1987
Lantana camara L.	pathogenic	FL, GU	Pereira et al. 2003
Petrea spp. L.			Ellis 1957
Stachytarpheta angustifolia (Mill.) Vahl.	pathogenic	GU	Ellis 1957
Stachytarpheta cayennensis (Rich.) Vahl	pathogenic	GU	McKenzi 1990
Stachytarpheta jamaicensis (L.) Vahl	pathogenic	FL, GU	Smith and Schlub 2004
Vitex agnus-castus L.			Alfieri et al. 1994
Vitex negundo L.			CABI, Herb. IMI 244917
Vitex parviflora Juss.	pathogenic	GU	Smith and Schlub 2004
Vitex pinnata L.			Ellis 1957
Vitex trifolia L.	pathogenic	GU	McKenzie 1996
Vitaceae Juss. (dicot)			
Cissus spp. L.			Alfieri et al. 1994
Cissus alata Jacq.			Alfieri et al. 1994
Tetrastigma voinierianum (Baltet) Pierre ex Gagnep.			Alfieri et al. 1994
Vitis spp. L.			Alfieri et al. 1994

Table 1-2. Continued

Host	Fungal-Host Interaction	Location	Reference
Zamiaceae Horan. (gymnosperm)			
Encephalartos spp. Lehm.			Alfieri et al. 1994

Host plants are listed alphabetically by family (in bold). Each species is followed by the first known reported reference. Fungal-host interaction refers to the endophytic or pathogenic nature of the fungus and was only reported for hosts that were collected during the Guam (GU) and Florida (FL) surveys. Location refers to whether the plant species was found as a host of *C. cassiicola* in FL or GU. Forty of the hosts were found on the CABI online database website (http://194.203.77.76/herbIMI/DisplayResults.asp?strName=Corynespora+cassiicola CABI Databases: Herb. IMI records for Fungus: *Corynespora cassiicola*).

Figure 1-1. *Corynespora cassiicola* isolate from *Cucumis sativus* A) sporulating on naturally infected leaf tissue after 24 hours in the moisture chamber, B) germinating spore on water agar, and C) growing on V8 agar after single spore isolation (images are not shown to scale).

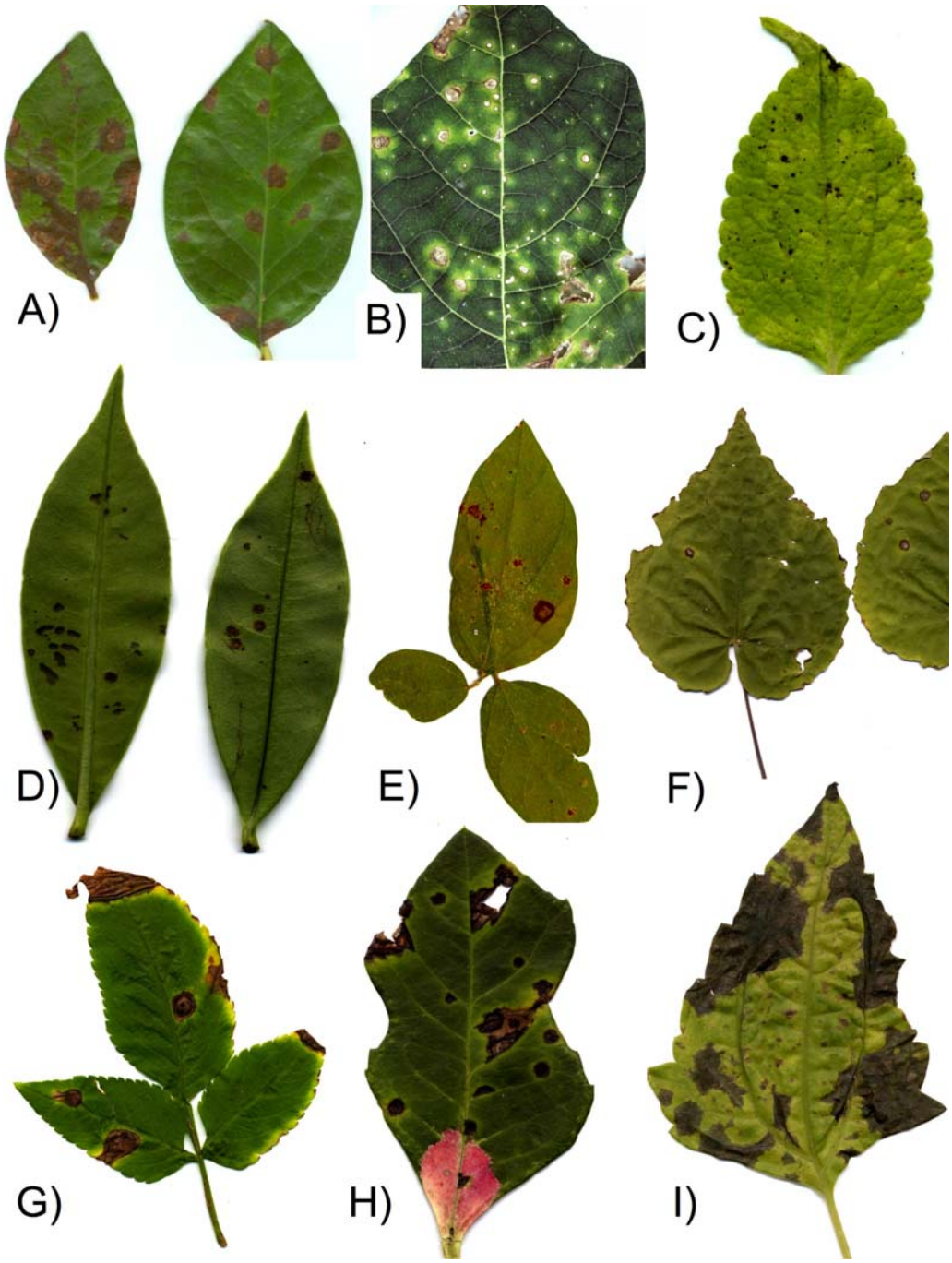

Figure 1-2. Various symptoms caused by *Corynespora cassiicola* on naturally infected leaves of A) *Vaccinium corymbosum*, B) *Carica papaya*, C) *Ageratum conyzoides*, D) *Allamanda* spp., E) *Macroptilium lathyroides*, F) *Abutilon theophrasti*, G) *Bidens alba*, H) *Euphorbia cyathophora*, I) *Chromolaena odorata* Continued. J) *Corchorus aestuans*, K) *Passiflora foetida*, L) *Ipomoea pes-caprae*, M) *Ipomoea obscura*, N) *Lantana camara*, O) *Merremia peltata*, P) *Bauhinia galpinii*, Q) *Catharanthus roseus*, R) *Phyllanthus amarus*, S) *Hydrangea macrophylla*, and T) *Salvia farinacea*. Images are not to scale.

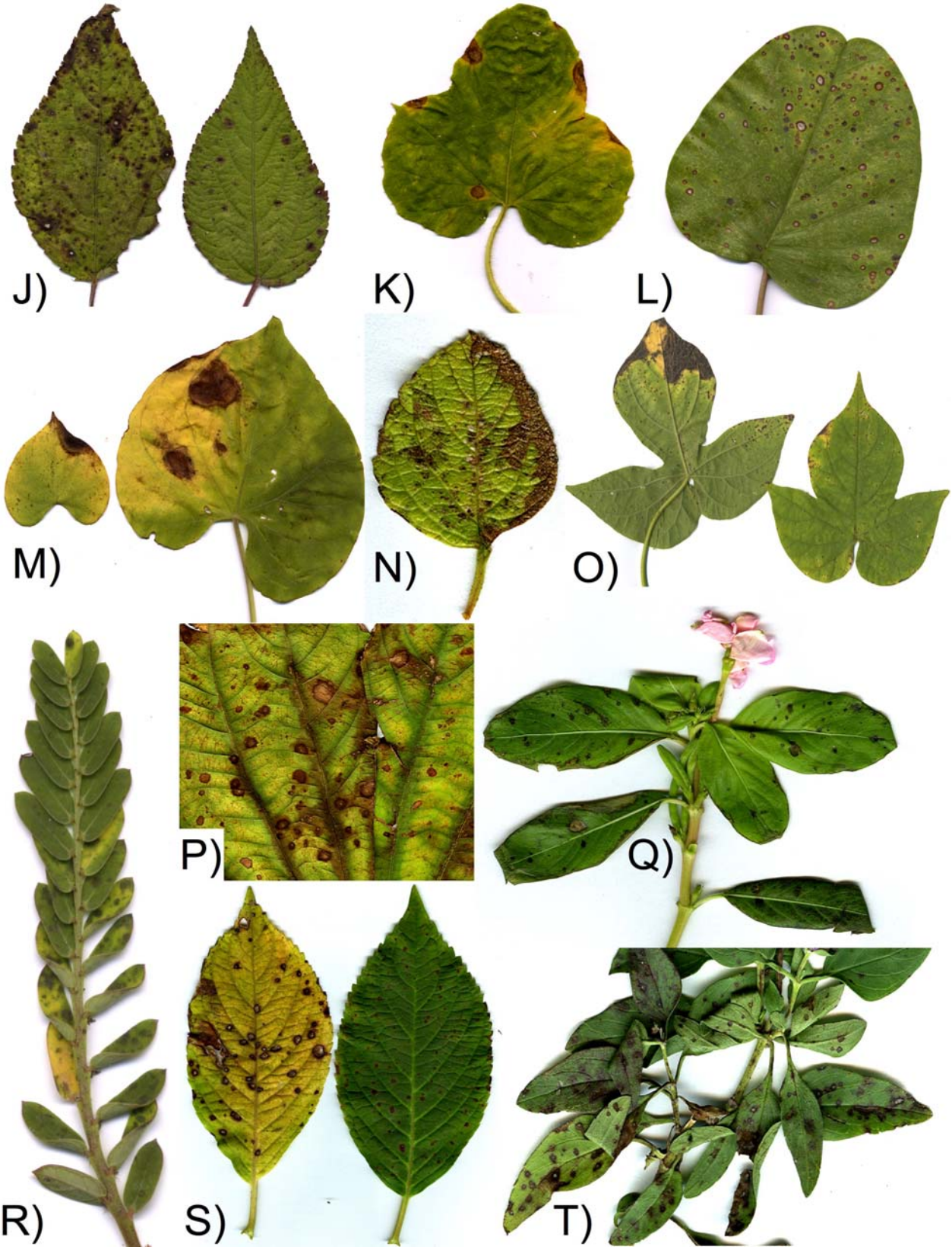

Figure 1-2. Continued.

CHAPTER 2
GENETIC AND PATHOGENIC DIVERSITY OF *CORYNESPORA CASSIICOLA*

Introduction

Target spot, caused by the fungal pathogen *Corynespora cassiicola* (Berk. & Curt.) Wei, is common in the tropics, subtropics, and greenhouses (Chase 1987). *C. cassiicola* is reported to infect 530 plant species from 380 genera, including monocots, dicots, ferns, and one cycad (Chapter 1, this dissertation). Isolate characterization is needed to determine which hosts might serve as sources of inoculum for target spot of tomato species and other hosts since there is much variability concerning the host range of individual isolates. Some isolates show pathogenicity to a wide range of hosts, whereas others exhibit host specificity, and some are only pathogenic when associated with wounding (Chase 1982; Cutrim and Silva 2003; Kingsland 1985; Onesirosan et al. 1973, 1974; Pereira et al. 2003; Poltronieri et al. 2003; Seaman et al. 1965; Smith and Schlub 2004; Smith and Schlub 2005; Spencer and Walters 1969; Volin and Pohronezny 1989). At least two races of the fungus have been distinguished based on their differential pathogenicity response on soybean and cowpea (Olive and Bain 1945; Spencer and Walters 1969). However, isolates from soybean, sesame, cowpea and cotton in Mississippi were alike in pathogenicity (Jones 1961). A more extensive study found eight different pathogenicity profiles among 28 isolates from soybean in Mexico, cucumber in Florida, and diverse hosts in Nigeria (Onesirosan et al. 1974). Furukawa et al. (2008) found that an isolate from *Salvia splendens* was not pathogenic to cucumber, green pepper or hydrangea; however isolates from these hosts were pathogenic to *Salvia splendens*. Furukawa et al. (2008), therefore, demonstrated that isolates with different pathogenicity profiles can be found on the same host.

Since the 1960's, a leaf and fruit spot disease of tomato caused by *C. cassiicola* has become increasingly serious in tropical countries worldwide (Jones and Jones 1984). It was first

reported in Florida in 1972 and has since become one of state's most damaging foliage and fruit diseases (Blazquez 1972; Pernezny et al. 1993, 1996, 2000, 2002). Under warm, humid, conditions the disease leads to heavy defoliation and significant losses in yield (Volin and Pohronezny 1989). Currently, there are no resistant tomato cultivars available, although resistance found in PI 120265 (*Lycopersicon esculentum*) and PI 11215 (*L. pimpinellifolium*) and was controlled by a single recessive gene (Bliss et al. 1973). Understanding the genetic and pathogenic diversity of the pathogen and its distribution is vital to isolate selection for resistance screening.

Kingsland (1985) compared three isolates from tomato, cucumber and papaya debris and found that tomato and cucumber were susceptible to all isolates, but the isolate from papaya debris was not pathogenic on papaya, indicating that it was possibly growing as a saprophyte. In many studies, isolates were found to be non-pathogenic on the hosts from which they were isolated, further indicating that *C. cassiicola* can grow as a saprophyte (Chase 1982; Kingsland 1985; Onesirosan et al. 1974; Hyde et al. 2001; Lee et al. 2004). Other studies show that isolates are only secondary invaders, or invaders of senescent tissue. Isolates from the ornamental hosts *Aeschynanthus pulcher* (lipstick vine), *Aphelandra squarrosa* (zebra plant), azalea and hydrangea were pathogenic on all hosts in cross-pathogenicity trials when wounded; however, only *A. pulcher* was susceptible without wounding (Chase 1982).

Silva et al. (1998) compared pathogenicity of 16 isolates from rubber trees in Sri Lanka and five isolates from diverse hosts in Australia. Papaya isolates from Australia were pathogenic to tomato and rubber, but not cowpea and eggplant. Mimosa and thyme isolates from Australia were pathogenic to eggplant, rubber, and tomato, but not cowpea. Isolates from Sri Lanka

collected from different rubber clones were either pathogenic to all hosts (cowpea, eggplant, rubber, and tomato), or pathogenic to all hosts but eggplant.

The host specificity and severity of the fungus on *Lantana camara* in Brazil has led to the discovery that *C. cassiicola* may be useful as a bioherbicide (Pereira et al. 2003). Based on the vast number of weeds that serve as hosts of the fungus, there is great potential for the discovery of several more isolates useful for biological control of weeds. Considering the wide variation in isolate pathogenicity that has been previously reported, additional studies are needed to further understand the host range of individual isolates from different hosts and locations.

Prior research on the genetic characterization of *C. cassiicola* is limited to restriction fragment length polymorphism (RFLP) of ITS rDNA and random amplified polymorphic DNA (RAPD) studies. No variation between five isolates of *C. cassiicola* collected from mimosa, papaya, and thyme in Australia was found based on RFLP of ITS (Silva et al. 1995). Silva et al. (1995) concluded that RFLP of the ITS regions of rDNA can be used to distinguish between *Corynespora* and the morphologically similar genus *Helminthosporium*, but not different isolates of *C. cassiicola*. However, the three isolates from papaya had identical RAPD patterns, growth rate, isolate color, and pathogenicity profiles, which were different from the isolates from mimosa and thyme, indicating an ongoing process of host specialization on papaya (Silva et al. 1995).

RAPD analyses from 27 isolates collected from *Hevea brasiliensis*, in Sri Lanka revealed correlations between host location, host genotype, isolate morphology, and isolate pathogenicity (Silva et al. 1998). Silva et al. (1998) concluded that a progenitor strain may have been spread in India by distribution of live plant material. Prior outbreaks of the disease on the susceptible

rubber clone RRIC 103 in other countries, and the sudden appearance and severity of target spot on the same clone in Sri Lanka in 1985, is evidence for such dissemination.

Silva et al. (2003) characterized 42 isolates from bitter gourd, cocoa, manihot, papaya, rubber, sweet potato, tomato, and wing-bean from various regions in India based on RAPD analyses. RAPD groups did not correlate with geographic origin, but isolates obtained from rubber clone RRIC 103 grouped together. This strain might be responsible for several recent outbreaks on this clone. In addition, all but one of the isolates from rubber clone RRIC 110 clustered in 2 RAPD groups, which may identify the strain that caused the outbreak on this clone in 1995. Silva et al. (2003) concluded that correlation of RAPD groups with pathogenicity was needed to help develop resistant clones against all pathogenic isolates.

Atan and Hamid (2003) characterized nine *C. cassiicola* isolates from *Hevea brasiliensis* in Malaysia using RAPD of genomic DNA and RFLP of amplified ITS regions. RFLP analyses with three restriction enzymes yielded monomorphic patterns. However, isolate OPEN 1 from clone RRIM 2020 had a distinct RFLP pattern from the other eight isolates after digestion with *Hae*III. RAPD results indicated the presence of at least two genetically distinct races that infect rubber. Seven isolates pathogenic to clones RRIM 600, RRIM 2009, and two unidentified rubber clones were molecularly similar and identified as Race 1. The remaining two isolates, both pathogenic on clone RRIM 2020, had identical banding patterns and were considered Race 2.

Unfortunately, the majority of the diversity assessments are limited to rubber isolates from Malaysia and Sri Lanka and are based on RAPD techniques, which is problematic with respect to repeatability and homology assessment (Isabel et al. 1999). In addition, all the RFLP studies used the ITS rDNA region which has minimal variation among isolates (Silva et al. 1995, 1998).

Investigations into the genetic variation among *C. cassiicola* isolates using more reliable molecular methods and more diverse isolates are needed.

In this study, we collected and solicited 143 isolates from diverse hosts and locations. To test whether *C. cassiicola* is panmictic throughout its range, allelic genealogies were constructed from four loci including the rDNA ITS region, two random hypervariable loci, *Cc caa5* and *Cc ga4*, and the single copy actin-encoding nuclear gene, *Cc act1*. Fifty of these isolates were spray inoculated on seedlings of eight crop plants to test pathogenicity profiles. Correlations among an isolate's pathogenicity profile, its host of origin, and genotype were investigated. The purpose of this research is to gain knowledge of the diversity within the species *C. cassiicola* because of its implications for resistance breeding and disease management of target spot of basil, bean, cowpea, cucumber, papaya, soybean, sweet potato, tomato, and potentially other crops.

Methods

Collection and Solicitation of Fungal Isolates

C. cassiicola isolates were collected from diverse plant hosts during 5-day collecting trips to locations in the Pacific: American Samoa (AS), Hawaii (HI), Palau (PW), Pohnepei (PH), Saipan (SN), and Yap (YP) in the summer of 2005. More extensive surveys were conducted to collect the fungus in Florida (FL) and Guam (GU) between 2004-2006 (see Chapter 1).

Farms, nurseries, and roadsides were surveyed for plants with target spot symptoms. First, second, and third priority was given to crops, weeds, and naturalized or indigenous hosts of *C. cassiicola*, respectively. Symptomatic leaves were put into individual plastic bags in the field and later placed abaxial side up in petri dishes with moistened paper towels in a laboratory. After 24 hours in the moisture chamber, petri plates were placed under the dissecting microscope and suspected spores and conidiophores of *C. cassiicola* were confirmed microscopically.

Single spores were captured at the end of a teasing needle and transferred to antibiotic V8 agar (340 ml V8 juice, 660 ml water, 3g $CaCO_3$, 17g agar, 100 μg/ml Ampicillin or Kanamycin) slants, left at room temperature until the colony reached at least 5 cm in diameter, whereby it was covered with autoclaved mineral oil, and stored at 5^{o} C until further study. Sporulation from non-symptomatic leaf material was noted, possibly indicating non-pathogenic growth.

To obtain globally diverse isolates, individual researchers in Brazil (BZ), Malaysia (MY), Mississippi (MS), and Tennessee (TN) were solicited for additional *C. cassiicola* cultures. Isolates from BZ on lantana (JMP216), papaya (DOA16b), soybean (RWB321) and tomato (JMP217) came from Alvaro Almeida, EMBRAPA. Isolates CBPP, CLN 16 and CSB1 2 were received from MY off of rubber from Dr. Safiah Atan, Malaysian Rubber Board. Isolate TN13-3 was received from Nashville, TN on greenhouse African violet from Justin S. Clark, University of Tennessee. Isolate MS01 was received from MS on greenhouse tomato leaves from David Ingram, Central MS Research and Extension Center.

Isolates of different species were also solicited from culture collections to serve as outgroups. Cultures from Commonwealth Agricultural Bureaux International (CABI) in the United Kingdom included *C. smithii* IMI 5649b and *C. citricola* IMI 211585. Cultures from National Institute of Agrobiological Sciences (NIAS) in Japan included *C. citricola* MAFF No. 425231, *C. melongenea* MAFF No. 712045, and *C. sesamum* MAFF No. 305095. Cultures from Centraalbureau voor Schimmelcultures (CBS) in the Netherlands included *C. proliferata* CBS 112393, *C. citricola* CBS 169.77, and *C. olivaceae* CBS 291.74. Cultures were single-spored after they were received. A complete list of isolates used in these studies, along with the plant host, geographic location and the type of association with the host plant (endophytic or pathogenic growth) can be found in Table 2-1.

Primer Development for Random Hypervariable Loci

Three *C. cassiicola* isolates from long-term storage (FL31, GU112, and PW56) were chosen based on unique host and location. A small piece of mycelium from the monosporic cultures was extracted with tweezers and placed onto a V8 agar plate. The isolates were grown under constant fluorescent light for 7 days. Aerial mycelium was scraped from the agar surface, placed in 1.5 ml microcentrifuge tubes, lyophilized overnight, and then frozen in liquid nitrogen. Genomic DNA was purified using the DNeasy plant Mini Kit (Quiagen, Inc.) according to the manufacturer's specifications.

Genomic DNA combined from all three isolates was digested with the *Sau* 3AI restriction enzyme (7.2 µl of DNA from each of the three isolates; 2.5 µl 10X buffer; 1.0 µl 10 U/µl *Sau* 3A I enzyme; incubated at 37^{o}C for 2 hours). The digested genomic DNA was fractionated to remove fragments less than 400 bp using a Chroma Spin® column (Chroma Spin + TE – 400, Clonetech Laboratories, Inc.) according to the manufacturer's specifications. The digested fractionated DNA was quantified and ligated to *Sau* 3AI linkers and incubated at 16^{o}C overnight. Excess linkers were removed using the same Chroma® Spin column as above. The linker-ligated fragments were PCR amplified using SauL-A primers and a program consisting of initial denaturation for 3 min at 94^{o}C, followed by 25 cycles of 94^{o}C for 1 min, 68^{o}C for 1 min, and 72^{o}C for 2 min, and a final amplification at 72^{o}C for 10 min.

The amplified genomic PCR library (composed of 400-1500 bp fragments) was enriched for fragments containing two different microsatellite repeats, $(CAA)_n$ and $(GA)_n$. The denatured genomic PCR library was hybridized to the following biotinylated oligoprobes: [5'$(CAA)_{15}$TATAAGATA-Biotin] and [5'$(GA)_{15}$TATAAGATA-Biotin] (Tepnel Lifecodes Corporation) and incubated at 48^{o}C overnight. The PCR fragments that hybridized to the repeat

probes were captured and eluted using two VECTREX® Avidin D matrix columns (cat. No. A-2020, Vector Laboratories, Burlingame, CA) according to the manufacturers specifications. The two mixtures containing genomic fragments enriched for the $(CAA)_n$ tri-repeat and the $(GA)_n$ di-repeat were PCR amplified using SauL-A primers following the same PCR conditions as above.

PCR products from the amplification of the enriched microsatellite library were ligated into a plasmid vector (pCR 2.1-TOPO® vector; Invitrogen, Inc.) and transformed into *E. coli* (One Shot™ TOP 10 Cells, Invitrogen, Inc.) using the TOPO TA Cloning Kit® (Invitrogen, Inc.) according to the manufacturers instructions. Transformed colonies were lifted and crosslinked onto nylon membranes in an UV chamber (GS Gene Linker™, Bio-Rad Laboratories, Inc., Hercules, CA) using the "optimal crosslink" program.

Nylon membranes were hybridized with alkaline phosphatase-labeled repeat probes ($(CAA)_n$ and $(GA)_n$) and the Quick-Light™ hybridization Kit (Tepnel Lifecodes Corp.) according to the manufacturers recommendation. Colonies containing plasmids that tested positive for inserts with repeats were sequenced in one direction. Primers were designed to amplify 300-500 base pair fragments flanking low repeat number (<10) sequences using Primer3 (v. 0.4.0). Sequences with repeats of less than 10 were likely to be non-variable microsatellite loci, but may contain polymorphic flanking sequences. Sequences were screened for polymorphisms using five isolates from different hosts and locations. Primers that amplified the *Cc ga4* and *Cc caa5* loci were chosen for further study because they amplified sequences with relatively high levels of polymorphism (>5%).

Fungal Cultures and Extraction of Genomic DNA

Genomic DNA from 143 isolates (Table 2-2) in long-term storage was purified and amplified using Extract-N-Amp™ (Sigma-Aldrich) according to the manufacturer's specifications.

The following primers were used for PCR amplification: ITS1 and ITS4 (White et al. 1990) for the internal transcribed spacer region, including the 5.8 rRNA coding region; ACT-512F and ACT-783R (Carbone and Kohn 1999) for the single copy nuclear actin locus *Cc act1*; GA4-F (5'-CCT GCT CCG ACT TTG TTG AG-3') and GA4-R (5'-GTC TGG GAG CAG CAA AGA CT-3') for the random hypervariable *Cc ga4* locus; CAA5-F (5'-GTC CAC AAG TGG AAC CTC GT-3') and CAA5-R (5'-CCT CGT CTG CCA GTT CTT CT-3') for the random hypervariable *Cc caa5* locus. "Hot-start" PCR was performed with a MyCycler™ thermocycler (BioRad) with a program consisting of initial denaturation for 3 min at 94°C, followed by 30 cycles of 30 sec at 94°C, 30 sec at 58°C, and 30 sec at 72°C, and a final cycle of 5 min at 72°C for the ITS, *Cc ga4*, and *Cc caa5* loci. For the *Cc act1* locus, the program was identical except for an annealing temperature of 61°C. PCR products were purified using the QIA quick PCR purification Kit (QIAGEN Inc.) according to the manufacturer's instructions. The purified products were then quantified on 1% ethidium bromide-stained agarose gels. Sequencing of the DNA samples was done at the University of Florida DNA Sequencing Core Laboratory using ABI Prism BigDye Terminator cycle sequencing protocols (part number 4303153) developed by Applied Biosystems (Perkin-Elmer Corp., Foster City, CA). The excess dye-labeled terminators were removed using MultiScreen® 96-well filtration system (Millipore, Bedford, MA, USA). The purified extension products were dried in SpeedVac® (ThermoSavant, Holbrook, NY, USA) and then suspended in Hi-di formamide. Sequencing reactions were performed using POP-7 sieving matrix on 50-cm capillaries in an ABI Prism® 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and were analyzed by ABI Sequencing Analysis software v. 5.2 and KB Basecaller.

Phylogenetic Analyses

Four loci (rDNA ITS, *Cc caa5*, *Cc ga4*, and *Cc act1*) from 143 isolates were sequenced. Forward and reverse sequences from each PCR product were concatenated in Sequencher™ 4.8 and trimmed to include only bases sequenced in both directions. Samples with ambiguities were sent for re-sequencing. Multiple alignments from each locus were executed separately with Clustal X (1.83.1) and the alignments were inspected and adjusted manually using MacClade 4.08 OS X (Maddison and Maddison 2005). Data from ITS rDNA, *Cc ga4*, *Cc caa5*, and *Cc act1* loci were partitioned to facilitate different permutations of combined analysis. A partition-homogeneity test (incongruence length-difference test or ILD) was implemented to evaluate the homogeneity of different data partition subsets using PAUP* v4.0b10 (Swafford 2002). The test implemented 1,000 replicates (heuristic search; random simple sequence additions; TBR; max-trees = 1,000). Comparisons were evaluated using a threshold of $p < 0.001$ and were made between all data partitions.

With the ILD test indicating the combinability of all molecular data, neighbor joining (NJ) and maximum parsimony (MP) analyses were conducted for each data partition and the combined data set using PAUP* (Swafford 2002). *C. smithii* IMI 5649b, *C. citricola* IMI 211585, *C. proliferata* CBS 112393, *C. citricola* CBS 169.77, and *C. olivaceae* CBS 291.74 were defined as outgroups. Cultures from National Institute of Agrobiological Sciences (NIAS) in Japan (*C. citricola* MAFF No. 425231, *C. melongenea* MAFF No. 712045, and *C. sesamum* MAFF No. 305095) were not included as outgroups because they grouped with *C. cassiicola* isolates in phylogenetic analyses (see Results below). For the NJ analyses, default settings were used except ties were broken randomly by initial seed.

Due to long computational time, MP analyses were conducted in the following manner. An initial heuristic search was conducted with one random addition replicate, TBR (tree-

bisection-reconnection) branch swapping, and the MulTrees option (saving all optimal trees) in effect. A second heuristic search was conducted using 1000 random addition replicates with the above settings and saving no more than 10 trees with a score greater than or equal to the best tree score from the first replicate in the previous analysis. In all analyses, gaps were treated as missing data. Strict consensus trees were generated from analyses with multiple equally parsimonious trees. For all MP analyses, statistical support for nodes was estimated using maximum parsimony bootstrap (BS) replicates (Felsenstein 1985). For the combined data set, BS estimates were obtained using 1,000 replicates, each with 100 random taxon addition replicates and saving no more than 1,500 trees per bootstrap replicate, TBR branch swapping and the MulTrees option in effect.

All data were also analyzed by Bayesian inference (BI) methods with MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). An appropriate model of evolution (under the AIC criterion) was selected for each data partition using the program Modeltest v3.4 (Posada and Crandall 1998). All Bayesian analyses (individual loci and combined data) were conducted while retaining the appropriate model for each data partition. Markov Chain Monte Carlo was implemented with four heated chains and trees were sampled every 1,000th generation for one million generations. The first 25 percent of the total number of generations was discarded as burn-in. A 50 percent majority rule consensus tree was generated from the remaining trees, in which the percentage of nodes recovered represented their posterior probability (PP).

Congruent nodes resulting from the NJ, MP, and BI analyses of the combined molecular data was used to assign isolates to a phylogenetic lineage (PL). Only isolates that fell within clades of high support (BS value >70 and PP value > 95) were assigned to a PL.

Pathogenicity Analyses

Fifty out of the 143 *Corynespora* isolates were used for pathogenicity profiling (Table 2-3) on eight crop plants. Isolates originally isolated from crop plants and from all phylogenetic lineages were chosen. Each isolate was spray-inoculated onto four replicate plants of eight-week-old: basil 'Italian Large Leaf' (Ba), bean 'Bush Kentucky Wonder' (Be), cowpea 'California black-eye' (Co), cucumber 'Straight 8' (Cu), soybean 'AG00901' (So), and tomato 'Rutgers' (To) seedlings; 8-week-old sweet potato 'Beauregard' (Sw) cuttings; and 12-week-old papaya 'HI Sunrise' (Pa) seedlings. Cultivars were chosen based on their known susceptibility in the survey regions.

To increase colony sporulation for inoculum preparation, aerial mycelium from 10-day-old V8 agar plates was gently scraped with a glass cover slip to flatten mycelium and then placed under constant cool-white fluorescent light (Onesirosan et al. 1975). Three days later, the surface of the agar was scraped with a glass cover slip and the resulting mycelia was blended in 200 ml sterile distilled water for two seconds and filtered through three layers of cheesecloth. Spores were counted under a hemacytometer and the concentration was adjusted to 20,000 spores/ml. One drop of Tween® 20 per 100 ml was added to the inoculum. Plants were sprayed with the spore suspension until leaf run off (about 500 ml), making sure that both leaf surfaces were fully covered. Plants were kept on a mist bench to maintain constant leaf moisture 3 days prior to inoculation and for the remainder of the experiment. Plants were rated 7 days after inoculation using the rating system developed by Onesirosan et al. (1973): (0) symptomless, no lesions on leaves or stems; (1) non pathogenic hypersensitive response, a few to many non-expanding pinpoint lesions; (2) moderately virulent, many expanding lesions, some coalescing, but not resulting in blight; (3) highly virulent, lesions spreading to form large areas of dead tissue resulting in a blighting effect. Incidence (I), defined as the number of plants showing symptoms

(with ratings of 1, 2, or 3), and severity (S), defined as the average rating for all symptomatic plants, were recorded. The experiment was repeated.

Each isolate was assigned a pathogenicity profile (PP), which is a list of susceptible hosts. Hosts were considered susceptible if at least one of the replicates from the two experiments (total of eight plants) received a rating of 2 or 3. PPs were converted to a binary character matrix so that each isolate received a zero (non-pathogenic, all reps with ratings of 0 or 1) or a one (pathogenic, at least one rep with a rating of 2 or 3) for each host. Unweighted pair group method with arithmetic mean (UPGMA) trees were constructed from the binary matrix and internal support for nodes was estimated using bootstrap analyses with 1,000 reps and a UPGMA algorithm. The tree topology was visually compared to the PL designation of each isolate tested (Figure 2-6). PPs were also visually mapped on the four-locus combined BI phylogenetic tree (Figure 2-1).

Growth Rate Analyses

Seventy-seven isolates were tested for growth rate at two temperatures (23 C and 33 C). A small piece of aerial mycelium was extracted from the monosporic cultures in long-term storage with tweezers and placed onto a V8 agar plate. After 5 days, the 77 colonies had grown beyond the mineral oil and six 4 mm agar plugs were cut from actively growing mycelium at the colony edge. A single plug was placed in the center of six V8 agar plates. Three replicate plates of each isolate were immediately placed in growth chambers at 23 C and 33 C under 12 hours of alternating fluorescent light (ca. 25 lux) and dark.

The average of two colony diameters at 90 degrees from each other was recorded at 48, 72, 96, 120, 144, and 166 hours. Average colony diameter was plotted against time and a line of best fit was generated for each replicate. The slope of the line of best fit (R^2>0.98) was used to compare variation within reps to variation between isolates in SAS® Statistical Software

(Version 8, 1999). The experiment was repeated with five isolates with no statistically significant variation (data not shown). A correlation between isolate growth rate and phylogenetic lineage was tested using SAS statistical software.

Results

Phylogenetic Analyses

The General Time Reversible model (GTR + I + Γ) was selected by Modeltest for each of the four gene partitions. The corresponding model for each locus was applied to all BI analyses and the combined dataset was partitioned. The final combined dataset contains 2,136 aligned characters used for analyses. Tree topologies resulting from NJ, MP, and BI analyses recovered essentially the same well-supported nodes. The analyses reveal four major phylogenetic lineages (PL) with high statistical support (BS value >70 and PP value > 95) (Figure 2-1).

All major PLs contain isolates from diverse locations, indicating their global dispersal. PL1 contains a distinct clade with high statistical support (designated PL1.1) containing only isolates collected from papaya from around the world indicating specialization on this host. PL1.2 contains two isolates from *Stachytarpheta jamaicensis,* collected from Guam and Palau, indicating potential specialization on this host. This supports pathogenicity studies showing isolate specificity to this host (Smith and Schlub 2005). Isolates from diverse hosts are present in PL1 including crops (basil, bitter melon, eggplant, cowpea, cucumber, oregano, pumpkin, rubber, soybean, sweet potato, watermelon), ornamentals (*Buddleja*, *Catharanthus, Codiaeum*, *Coleus*, *Episcia*, and *Tabebouia*), and weeds (*Bidens*, *Buchnera*, *Clerodendrum, Commelina*, *Lantana*, *Macroptilium*, *Meisosperma*, *Vitex*). Tomato isolates are missing from PL1, indicating that isolates in this lineage may not be pathogenic to tomato.

Isolates in PL2 are also globally distributed and include crops (cucumber, rubber, sweet potato), ornamentals (African violet, *Allamanda*, *Catharanthus, Pilea*), and weeds (*Piper, Pilea*).

There is also a lack of tomato isolates in PL2 indicating that isolates from this lineage may be nonpathogenic on this host. Though PL2 was highly supported (BS and PP values of 100), its sister relationship to PL1, PL3, and PL4 remains unresolved.

Globally distributed isolates from PL3 include crops (basil, bitter melon, cucumber, pumpkin, soybean, tomato), ornamentals (*Bauhinia*, *Moringa*, *Pachystachys*, *Plectranthus*, *Saintpaulia*), and weeds (*Acanthus*, *Asystasia*, *Calopogonium*, *Coccinia*, *Euphorbia*, *Luffa*, *Passiflora*, *Teramnus*). These are hosts that may harbor isolates pathogenic to tomato. PL5 and PL6 group with PL3 with low support (MPBS value of 60). PL5 contain *C. cassiicola* isolates from African violet in Guam and Tennessee that are very similar in sequence, especially at the *Cc-caa5* locus, indicating specialization on this host. African violet isolates from Saipan and Yap are found in PL3. PL6 is highly supported and contains isolates from Brazil on *Coleus*, Palau on cowpea, and Saipan on *Asystasia*.

The majority of tomato isolates group in PL4 from diverse locations including American Samoa, Brazil, Florida, Guam, Mississippi, Palau, and Saipan. These twelve tomato isolates also group with isolates from crops (bean, cassava, cucumber, sweet potato), ornamentals (*Bauhinia*, *Cassia*, *Coleus*, *Eugenia*, *Ficus*, *Jatropha*, *Salvia*, *Syzygium*), and common weeds (*Calopogonium*, *Calyptocarpus*, *Chromolaena*, *Euphorbia*, *Hyptus*, *Lantana*, *Mikania*, *Spathodea*), which are likely inoculum sources for the initiation of disease on tomato.

The rDNA ITS region (Figure 2-2) is composed of 1,013 characters, 400 of which are an insertion in the outgroup taxa *C. smithii*. Of the 612 remaining characters, 141 are variable and 107 are informative. The rDNA ITS sequences reveal the misidentification of three outgroup taxa from the NIAS culture collection. *C. sesamum* 305095, *C. citricola* 425231, and *C. melongenea* 712045 should be reclassified as *C. cassiicola* based on rDNA ITS sequences.

When the outgroup taxa *C. citricola, C. olivaceae, C. proliferata,* and *C. smithii* are removed from the analyses, the rDNA ITS sequences of *C. cassiicola* contain only three informative characters out of 584 bases. Two of these characters separate the isolates into three distinct phylogenetic lineages that correlate with the PLs in the combined analysis. These two characters are base pair 158 (C or T), and base pair 497 (A or G) of the *C. cassiicola* rDNA ITS alignment. Three haplotypes are represented by these two characters: CA, CG, and TG (no haplotype TA); all isolates with haplotype CA group in PL4, isolates with haplotype TG group in PL1, isolates with haplotype CG group in PL2, PL3, PL5 and PL6. CG is also the ancestral haplotype, present in all outgroups except for *C. proliferata* (haplotype CA). The third informative character in the rDNA ITS sequences of *C. cassiicola* is base pair 123, which is a T in the majority of isolates, but a C in isolates PW101 (PL5), RWB321 (PL5), SN64 (PL5.1), and TN13-3 (PL6). It is this character (bp 123) that caused the polymorphic band pattern observed by Atan and Hamid (2003) in their RFLP analysis of the rDNA ITS region of rubber isolates using *HaeIII* (recognition sequence GGCC).

The sister relationships between the phylogenetic lineages remain unresolved in the analyses of the individual loci and in the combined analyses. In addition, the ITS rDNA region was the only locus that showed good support for *C. citricola, C. olivaceae, C. proliferata,* and *C. smithii* as sister taxa to the ingroup of *C. cassiicola* isolates. The phylogenetic placement of the outgroup taxa was not well supported in the combined analyses, or the GA4 locus. The CAA5 locus showed support for *C. olivaceae, C. proliferata,* and *C. smithii* as basal to PL1, PL2, PL3, PL5, and PL6, but PL4 and *C. citricola* fell basal to that group. The apparent paraphyly of *C. cassiicola* at the *Cc-caa5* locus may be a result of character variation that occurred at this locus

before the species evolved. Additional loci containing characters that reveal the sister relationships of the different PLs are needed in future analyses.

The *Cc-caa5* locus (Figure 2-3) reveals similar tree topologies to the combined analyses with high support for the four major PLs. Differences at this locus in PL1 include the lack of PL1.1 that distinguishes papaya isolates from other isolates in PL1 in the combined analyses. In addition, PL1.2, which includes two rubber isolates from Malaysia, has low support. GU70 and SN59, isolates basal to PL1 in the combined analyses, groups with other isolates in PL1 at this locus. The *Cc-caa5* locus shows strong support for PL2 with the same nine isolates as in the combined analysis. The *Cc-caa5* locus does not resolve PL3.1 or PL3.3 as distinct from PL3, although the five isolates in PL3.2 group together with strong support. This locus does not distinguish isolates FL2920, GU120, GU136 as distinct from other isolates in PL4. PL5 and PL5.1 isolates are group basal to PL3, but with low support. PL6, which includes African violet isolates from Guam and Tennessee, group with isolate NIAS 712045 with high support. Isolates FL50 (*Hydrangea macrophylla*) and FL51 (*Vaccinium corymbosum*) are unresolved at this locus as well as in combined analyses.

The *Cc-ga4* locus (Figure 2-4) highly supports PL1, PL2, PL4, and PL6, although the sister relationships between the PLs are unresolved. Isolates in PL3 form a clade with low support. The *Cc-ga4* locus did reveal a shared haplotype between papaya isolates with a point mutation from an A to a G at base 74. *C. cassiicola* isolates are not monophyletic at this locus because the outgroups *C. proliferata*, *C. olivaceae*, and *C. smithii* fall basal to PL1, PL2, and PL6 with low support. This may be a result of character variation before speciation, a high incidence of homoplasious characters, or the convergent evolution of specific adaptations.

The *Cc-act1* locus could not be amplified in the outgroup taxa *C. citricola*, *C. proliferata*, and *C. smithii*, perhaps due to mutations in the primer annealing site. The locus did amplify in *C. olivaceae* and shows high variation from *C. cassiicola* isolates (Figure 2-5). There is good support for PL3, PL2, and PL4 at this locus, although only marginal support for PL1 (BS value of 68). Again, the sister relationships between the PLs are unresolved. The *Cc-act1* locus also reveals a shared haplotype between papaya isolates with a point mutation from an A to a G at base 229.

Pathogenicity Analyses

As a result of screening fifty isolates for pathogenicity on eight index hosts, 16 unique pathogenicity profiles (PP) were developed (Table 2-3). The most common PP was CuTo, followed by Pa and CuSwTo. Cucumber was the most susceptible host, with all isolates producing symptoms and an average severity rating of 2.3. Tomato was also highly susceptible with 49 out of 50 isolates showing symptoms with an average severity rating of 1.8. Even though only eight isolates were pathogenic on papaya, the average severity rating was 2.1 indicating that pathogenic isolates were highly virulent. Isolates pathogenic to basil, bean, cowpea, soybean and sweet potato were less virulent on these hosts with average severity ratings less than 1.5.

There was a strong correlation between PP and PL (Figure 2-6). Seven out of ten isolates with PP CuTo were from PL4 and all isolates with PP CuSwTo and BeCuSwTo were from PL4. In PL4, all isolates but SN37 were highly virulent on tomato (average severity ratings ranging from 2.5 to 3) and all isolates but GU28 were pathogenic to cucumber (average severity ratings ranging from 1.3 to 3). In addition, the only isolates pathogenic to bean were from PL4, although these five isolates were weakly virulent (average severity ratings ranging from 1.3 to 1.9).

In PL3, all isolates were strongly pathogenic to cucumber (average severity ratings ranging from 2.3 to 2.8) and six out of seven isolates were strongly pathogenic to tomato (average severity ratings ranging from 2.5 to 3). Four out of the six isolates also were pathogenic to basil and all isolates with PP BaCuTo were from PL3.

Pathogenicity profile CuSw was unique to isolates from PL2 and all isolates tested from PL2 had this profile. In addition, isolates collected in the field from papaya in PL1.1 were specific to papaya in pathogenicity studies, although all isolates were weakly virulent on cucumber with average severity ratings of 1.3 or less. All isolates from PL1 were pathogenic to cucumber with average severity ratings ranging from 1.3 to 3. Nine out of the 13 isolates from PL1 were pathogenic to cowpea and seven were pathogenic to basil. The only other host susceptible to isolates from PL1 was soybean, which was only weakly susceptible when inoculated with isolate PW87.

Growth Rate Analyses

The null hypotheses of no growth rate differences among isolates, phylogenetic lineage, and temperatures were rejected ($P<0.0001$), while the null hypotheses of no growth rate differences among repetitions was accepted with a probability of 0.7546. The 77 isolates tested all grew faster at 23 C than 33 C. At 23 C, average isolate growth rate (average of three repetitions) was between 0.1479 and 0.474 with an overall mean of 0.3855 (Table 2-4). At 33 C, average isolate growth rate was between 0.1382 and 0.4153, with an overall mean of 0.2958 (Table 2-5). At 23 C, there were 29 significantly different growth rates and at 33 C there were 39 significantly different growth rates. Among the fastest growing isolates at both temperatures were FL37, GU90, GU99, AS67, and HI01. The slowest growing isolates were very different at the two temperatures. Slow growing isolates at 23 C were PH01, JMP216a, GU120, FL15, and

DOA16b. Slow growing isolates at 33 C were AS119, AS117, JMP217, AS49, GU120, and GU112.

Though there were not enough replicates to test for interactions among effects, growth rate alone correlated with location, phylogenetic lineage, and with host. Isolates from Oahu and Palau grew the fastest at both temperatures. Isolates from Brazil, Florida and Malaysa tended to have slower growth rates at both temperatures. Surprisingly, American Samoan isolates grew proportionately much faster at 23 C than 33 C, despite its tropical climate. Isolates from PL6 and PL1 grew the fastest at both temperatures. Isolates from PL2 and PL4 grew the slowest at 23 C and isolates from PL5 and PL3 grew the slowest at 33 C. All isolates from *Clerodendrum*, *Commelina*, *Ficus*, *Macroptilium*, pumpkin, and *Stachytarpheta* were fast growing at both temperatures. In addition, isolates from *Allamanda*, *Coleus*, eggplant, *Lantana*, and tomato isolates had slower growth rates at both temperatures.

Discussion

The current study presents the first robust, global phylogeny of the species *Corynespora cassiicola*. Based on sequence data from four unique loci, there is evidence for high genetic diversity within the species. The highly clonal nature of *C. cassiicola* is demonstrated in the congruence of the phylogenetic trees from distinct loci. All loci distinguish four major clonal lineages within *C. cassiicola*. The low level of sequence variation at the rDNA ITS region within the species relative to other *Corynespora* species suggests that these lineages are in fact clonal populations, rather than taxonomically distinct species.

As reported previously, the pattern of distribution of the diversity within the species correlates with the host (Smith et al. 2008a). Identical haplotypes are widely distributed geographically. The lack of correlation between phylogenetic data and location provides evidence for the recent global dispersal of isolates from all four phylogenetic lineages. In

addition, geographically diverse isolates from the same host plant shared identical haplotypes, potentially indicating host specialization. For example, isolates collected from tomato in Brazil, Florida, Guam, Mississippi, Palau, and Saipan had identical haplotypes at all four loci. Isolates collected from *Lantana* in Florida and Brazil were also identical at all four loci. Isolates collected from African violet in Guam and Tennessee were unique from all other isolates and nearly identical to each other. Perhaps the most compelling evidence for host specialization is the shared identical sequences of all isolates collected from papaya from very diverse locations. Tomato isolates from diverse locations including North and South America and the Pacific Islands are found in only two of the five major phylogenetic lineages (PL3 and PL4). Isolates from other hosts that fall into these same PLs are likely pathogenic to tomato and may serve as source hosts or alternative hosts for target spot of tomato. Tomato isolates are genetically similar to isolates from common crops (basil, bean, bitter melon, cassava, cucumber, papaya, pumpkin, soybean, sweet potato), weeds (*Acanthus*, *Calopogonium*, *Calyptocarpus*, *Chromolaena*, *Coccinia*, *Euphorbia*, *Lantana*, *Macroptilium*, *Mikania*, *Momordica*, *Passiflora*, and *Teramnus*), and ornamentals (*Asystasia*, *Bauhinia*, *Cassia*, *Coleus*, *Eugenia*, *Euphorbia*, *Ficus*, *Hyptus*, *Jatropha*, *Luffa*, *Moringa*, *Pachystachys*, *Plectranthus*, *Saintpaulia*, *Salvia*, *Spathodea*, and *Syzygium*). Based strictly on these data, control of target spot should involve isolation of tomato fields from these plant species, when possible.

Pathogenicity testing, in addition to phylogenetics, should be used to determine which hosts might serve as sources of inoculum for the initiation of target spot of tomato. There are at least sixteen unique pathogenicity profiles within *C. cassiicola* on the eight crop plants that were tested. Isolates from the same lineages show similar but not identical profiles (Figure 2-1 and Figure 2-6). For example, all but two isolates in PL3 and PL4 are pathogenic to tomato, and

isolates from all other lineages are nonpathogenic to tomato. All isolates pathogenic to basil are from PL1 and PL3, but not all isolates in these clades are pathogenic to basil. Interestingly, the majority of isolates, excluding the isolates collected from papaya, were pathogenic on cucumber. Though there were no isolates collected from tomato that grouped in PL1 and PL2, isolates from these lineages produced a hypersensitive response on tomato, showing pinpoint lesions that were given a disease rating of one.

These data are similar to pathogenicity tests using 18 *C. cassiicola* isolates from Nigeria, the Southern U.S., and Mexico (Onesirosan et al. 1973) in that both studies found isolates specific to papaya and cucumber. Likewise, both studies found that isolates pathogenic to tomato also were likely to be pathogenic on several other hosts. The number of isolates screened compared to the number of unique pathogenicity profiles in both studies indicates that gains and losses of pathogenicity are common.

Growth rate at different temperatures has provided evidence for isolates adapted to tropical and temperate environments. Using an isolate collected from tomato in Florida, Pernezny et al. (2000) found the best colony growth occurred at 32C, whereas Sobers (1966) reported an optimum growth rate at 24C for Florida isolates collected from hydrangea and azalea. Jones and Jones (1984) report higher disease severity on tomato inoculated and maintained at temperatures between 20-23 C. In this study, two temperature extremes (23 C and 33 C) were chosen in attempt to discern between isolates adapted to temperate and tropical climates. Though the majority of isolates were collected from tropical climates, all isolates grew faster at 23 C than 33 C. Growth rate also strongly correlated with phylogenetic lineage. Isolates from PL2 and PL4 may be more adapted to warmer temperatures, and isolates from PL5 and PL3 might be more adapted to cooler temperatures. Such physiological traits, including growth rate and

pathogenicity profile, correlate with phylogenetic data and may be useful for isolate classification.

These studies have shown that the rDNA ITS sequence will be useful for the initial screening of isolates and for isolate selection for resistance breeding. The rDNA ITS region was useful for the grouping of isolates into three groups (PL1 (haplotype TG), PL4 (haplotype CA), and PLs 2, 3, 5, and 6 (haplotype CG)) that correlate with phylogenetic data from the combined four locus data set. For example, isolates from PL1 (rDNA ITS haplotype TG) should be used to screen for resistance to target spot in papaya. In contrast, isolates from PL2 and PL4 (rDNA ITS haplotypes CA and CG) should be used to screen for resistance to target spot in tomato. In addition, genotyping by restriction digest of the amplified ITS region is possible now that specific polymorphisms have been identified and mapped. For example, use of the enzyme *HpyCH4V* (recognition sequence TGCA) will cut in two positions in haplotypes CA and CG, but only one position in haplotype TG. Additionally, this research found isolates with the same unique genotype found in Atan and Hamid's (2003) RFLP analysis of the rDNA ITS region using *HaeIII*. However, only four of the 143 isolates we sequenced shared this polymorphism at base pair 123, rendering RFLP analysis of the rDNA ITS region using *HaeIII* ineffective for distinguishing among the majority of isolates.

Despite evidence for host specificity (on African violet, *Lantana*, papaya, and *Stachytarpheta*, for example), the combined pathogenicity and phylogenetic data indicate that there are many hosts with the potential to harbor *C. cassiicola* isolates pathogenic to susceptible crops such as basil, cucumber, and tomato. Studies that incorporate many isolates from the same host across diverse locations, the sequencing of additional loci, and subsequent pathogenicity screening, will no doubt reveal additional genetic diversity and host specificities.

It is hoped that this research will aid others in unraveling the many complexities that remain to be discovered with respect to *C. cassiicola* and its disease development in the field. For example, more studies are needed to explain why *C. cassiicola* is rare in Hawaii on all cultivated crops except basil, if there are isolates adapted to tropical and temperate climates, and how isolate genotype and pathogenicity profiles are correlated using more diverse isolates and hosts.

Table 2-1. Isolate designations, geographic location of isolation, host of isolation, phylogenetic lineage (PL), type of growth on associated host, and species of *Corynespora* used in the phylogenetic analyses.

Isolate ID	Location	Host	PL	Growth	Species
CABI 211585	New Zealand	*Poncirus trifoliatus*	O	endophytic	*C. citricola*
CBS 169.77	New Zealand	*Poncirus trifoliatus*	O	endophytic	*C. citricola*
NIAS 425231	Japan	*Ocimum basilicum*	1	pathogen	*C. citricola*
NIAS 712045	Japan	*Solanum melongenea*	?	pathogenic	*C. melongenae*
CBS 291.74	Netherlands	*Tilia* spp.	O	saprophyte	*C. olivacea*
CBS 112393	Italy	*Fagus sylvatica*	O	endophytic	*C. proliferata*
NIAS 305095	Japan	*Sesamum indicum*	1	pathogenic	*C. sesamum*
CABI 5649b	England	*Fagus sylvatica*	O	saprophyte	*C. smithii*
AS49	Amer. Samoa	*Solanum lycopersicum*	3	pathogenic	*C. cassiicola*
AS50	Amer. Samoa	*Solanum lycopersicum*	3	pathogenic	*C. cassiicola*
AS54	Amer. Samoa	*Vigna unguiculata*	1	saprophyte	*C. cassiicola*
AS58	Amer. Samoa	*Vigna unguiculata*	1	saprophyte	*C. cassiicola*
AS65	Amer. Samoa	*Solanum melongenea*	6	saprophyte	*C. cassiicola*
AS67	Amer. Samoa	*Commelina benghalensis*	1	pathogenic	*C. cassiicola*
AS71	Amer. Samoa	*Cucurbita pepo*	1	saprophyte	*C. cassiicola*
AS78	Amer. Samoa	*Ocimum basilicum*	1	pathogenic	*C. cassiicola*
AS80	Amer. Samoa	*Ocimum basilicum*	3.1	pathogenic	*C. cassiicola*
AS81	Amer. Samoa	*Clerodendrum quadriloculare*	1	pathogenic	*C. cassiicola*
AS92	Amer. Samoa	*Cucumis sativus*	6	pathogenic	*C. cassiicola*
AS98	Amer. Samoa	*Cucumis sativus*	1	pathogenic	*C. cassiicola*
AS117	Amer. Samoa	*Carica papaya fruit*	3.1	saprophyte	*C. cassiicola*
AS119	Amer. Samoa	*Cucurbita pepo*	3.1	saprophyte	*C. cassiicola*
DOA16b	Brazil	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
JMP216a	Brazil	*Lantana camara*	6	pathogenic	*C. cassiicola*
JMP217	Brazil	*Solanum lycopersicum*	6	pathogenic	*C. cassiicola*
JMP218	Brazil	*Glycine max*	1	pathogenic	*C. cassiicola*
RWB321	Brazil	*Coleus barbatus*	4	pathogenic	*C. cassiicola*
FL09	FL, USA	*Lantana camara*	6	pathogenic	*C. cassiicola*
FL11	FL, USA	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
FL12	FL, USA	*Solanum lycopersicum*	6	pathogenic	*C. cassiicola*
FL15	FL, USA	*Salvia farinacea*	6	pathogenic	*C. cassiicola*
FL21	FL, USA	*Bauhinia galpinii*	6	pathogenic	*C. cassiicola*
FL34	FL, USA	*Tabebouia pallida*	1	pathogenic	*C. cassiicola*
FL36	FL, USA	*Catharanthus roseus*	2	pathogenic	*C. cassiicola*
FL37	FL, USA	*Clerodendrum paniculatum*	1	pathogenic	*C. cassiicola*
FL50	FL, USA	*Hydrangea macrophylla*	?	pathogenic	*C. cassiicola*
FL51	FL, USA	*Vaccinium corymbosum*	?	pathogenic	*C. cassiicola*
FL62	FL, USA	*Coleus barbatus*	1	pathogenic	*C. cassiicola*
FL757	FL, USA	*Origanum vulgare*	1	pathogenic	*C. cassiicola*
FL2920	FL, USA	*Solanum lycopersicum*	6	pathogenic	*C. cassiicola*
MS31	MS, USA	*Solanum lycopersicum*	6	pathogenic	*C. cassiicola*
TN3-3	TN, USA	*Saintpaulia ionantha*	5	pathogenic	*C. cassiicola*
GU01	Guam	*Cassia fistula*	6	saprophyte	*C. cassiicola*

Table 2-1. Continued.

Isolate ID	Location	Host	PL	Growth	Species
GU06	Guam	*Hyptus suarelens*	6	endophytic	*C. cassiicola*
GU08	Guam	*Lantana camara*	1	pathogenic	*C. cassiicola*
GU10	Guam	*Codiaeum variegatum*	1	endophytic	*C. cassiicola*
GU11	Guam	*Citrullus vulgaris*	1	saprophyte	*C. cassiicola*
GU12	Guam	*Calopogonium mucunoides*	6	pathogenic	*C. cassiicola*
GU14	Guam	*Calyptocarpus vialis*	6	pathogenic	*C. cassiicola*
GU16	Guam	*Asystasia gangetica*	3	pathogenic	*C. cassiicola*
GU21	Guam	*Buddleja asiatica*	1	pathogenic	*C. cassiicola*
GU23	Guam	*Ipomoea batatas*	6	endophytic	*C. cassiicola*
GU25	Guam	*Buchnera floridana*	1	pathogenic	*C. cassiicola*
GU28	Guam	*Solanum lycopersicum*	6	pathogenic	*C. cassiicola*
GU32	Guam	*Euphorbia heterophylla*	3	endophytic	*C. cassiicola*
GU38	Guam	*Allamanda cathartica*	2	pathogenic	*C. cassiicola*
GU41	Guam	*Eugenia uniflora*	6	endophytic	*C. cassiicola*
GU42	Guam	*Bidens alba*	1	pathogenic	*C. cassiicola*
GU44	Guam	*Jatropha curcas*	6	endophytic	*C. cassiicola*
GU49	Guam	*Syzygium jambos*	6	endophytic	*C. cassiicola*
GU51	Guam	*Meisosperma oppositifolium*	1	endophytic	*C. cassiicola*
GU55	Guam	*Calopogonium mucunoides*	3.1	pathogenic	*C. cassiicola*
GU65	Guam	*Passiflora foetida*	3	endophytic	*C. cassiicola*
GU68	Guam	*Moringa oleifera*	3	endophytic	*C. cassiicola*
GU70	Guam	*Solanum melongenea*	1.3	endophytic	*C. cassiicola*
GU79	Guam	*Acanthus ilicifolius*	3	endophytic	*C. cassiicola*
GU83	Guam	*Euphorbia heterophylla*	6	endophytic	*C. cassiicola*
GU90	Guam	*Stachytarpheta jamaicensis*	1	pathogenic	*C. cassiicola*
GU92	Guam	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
GU93	Guam	*Capsicum annum*	1	endophytic	*C. cassiicola*
GU98	Guam	*Spathodea campanulata*	6	pathogenic	*C. cassiicola*
GU99	Guam	*Saintpaulia ionantha*	5	pathogenic	*C. cassiicola*
GU101	Guam	*Euphorbia milii*	6	saprophyte	*C. cassiicola*
GU102	Guam	*Phaseolus vulgaris*	6	saprophyte	*C. cassiicola*
GU103	Guam	*Pilea nummulariifolia*	2	endophytic	*C. cassiicola*
GU104	Guam	*Macroptilium atropurpureum*	1	pathogenic	*C. cassiicola*
GU107	Guam	*Mikania micrantha*	6	pathogenic	*C. cassiicola*
GU109	Guam	*Bauhinia galpinii*	3	pathogenic	*C. cassiicola*
GU110	Guam	*Plectranthus ambionicus*	3	pathogenic	*C. cassiicola*
GU111	Guam	*Manihot esculenta*	6	endophytic	*C. cassiicola*
GU112	Guam	*Glycine max*	3	endophytic	*C. cassiicola*
GU114	Guam	*Teramnus labialis*	3	endophytic	*C. cassiicola*
GU115	Guam	*Vitex parviflora*	1	pathogenic	*C. cassiicola*
GU120	Guam	*Coleus barbatus*	6	pathogenic	*C. cassiicola*
GU128	Guam	*Solanum lycopersicum*	6	pathogenic	*C. cassiicola*
GU136	Guam	*Ficus benjamani*	6.1	endophytic	*C. cassiicola*
HI01	Oahu, Hawaii	*Ocimum basilicum*	1	pathogenic	*C. cassiicola*
CBPP	Malaysia	*Hevea brasiliensis clone unk.*	1.2	pathogenic	*C. cassiicola*
CLN16	Malaysia	*Hevea brasiliensis RRIM 2020*	1.2	pathogenic	*C. cassiicola*

Table 2-1. Continued.

Isolate ID	Location	Host	PL	Growth	Species
CSB12	Malaysia	*Hevea brasiliensis RRIM 725*	2	pathogenic	*C. cassiicola*
GU136	Guam	*Ficus benjamani*	6.1	endophytic	*C. cassiicola*
HI01	Oahu, Hawaii	*Ocimum basilicum*	1	pathogenic	*C. cassiicola*
CBPP	Malaysia	*Hevea brasiliensis clone unk.*	1.2	pathogenic	*C. cassiicola*
CLN16	Malaysia	*Hevea brasiliensis RRIM 2020*	1.2	pathogenic	*C. cassiicola*
CSB12	Malaysia	*Hevea brasiliensis RRIM 725*	2	pathogenic	*C. cassiicola*
PH01	Pohnpei	*Carica papaya*	1.1	endophytic	*C. cassiicola*
PW01	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW12	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW17	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW20	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW25	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW27	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW34	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW37	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW38	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW43	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW48	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW53	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW56	Palau	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
PW57	Palau	*Solanum lycopersicum*	6	pathogenic	*C. cassiicola*
PW63	Palau	*Solanum lycopersicum*	6	pathogenic	*C. cassiicola*
PW69	Palau	*Piper betle*	2	endophytic	*C. cassiicola*
PW79	Palau	*Pilea microphylla*	2	pathogenic	*C. cassiicola*
PW80	Palau	*Saintpaulia ionantha*	1	pathogenic	*C. cassiicola*
PW83	Palau	*Saintpaulia ionantha*	1	pathogenic	*C. cassiicola*
PW87	Palau	*Cucumis sativus*	1	pathogenic	*C. cassiicola*
PW89	Palau	*Chromolaena odorata*	6	endophytic	*C. cassiicola*
PW91	Palau	*Luffa acutangula*	1	endophytic	*C. cassiicola*
PW92	Palau	*Catharanthus roseus*	1	pathogenic	*C. cassiicola*
PW94	Palau	*Stachytarpheta jamaicensis*	1	pathogenic	*C. cassiicola*
PW99	Palau	*Momordica charantia*	3	pathogenic	*C. cassiicola*
PW101	Palau	*Vigna unguiculata*	4	saprophyte	*C. cassiicola*
SN03	Saipan	*Momordica charantia*	1	pathogenic	*C. cassiicola*
SN05	Saipan	*Ipomoea batatas*	1	pathogenic	*C. cassiicola*
SN06	Saipan	*Luffa acutangula*	3.1	endophytic	*C. cassiicola*
SN07	Saipan	*Carica papaya*	1.1	endophytic	*C. cassiicola*
SN18	Saipan	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
SN24	Saipan	*Solanum lycopersicum*	6	pathogenic	*C. cassiicola*
SN27	Saipan	*Solanum lycopersicum*	6	pathogenic	*C. cassiicola*
SN30	Saipan	*Solanum lycopersicum*	6	pathogenic	*C. cassiicola*
SN37	Saipan	*Vigna unguiculata*	6	saprophyte	*C. cassiicola*
SN40	Saipan	*Cucumis sativus*	6	pathogenic	*C. cassiicola*
SN43	Saipan	*Saintpaulia ionantha*	3	pathogenic	*C. cassiicola*
SN48	Saipan	*Coccinia grandis*	3	endophytic	*C. cassiicola*
SN53	Saipan	*Carica papaya*	1.1	pathogenic	*C. cassiicola*

Table 2-1. Continued.

Isolate ID	Location	Host	PL	Growth	Species
SN59	Saipan	*Lantana camara*	1.4	pathogenic	*C. cassiicola*
SN64	Saipan	*Asystasia gangetica*	4.1	pathogenic	*C. cassiicola*
SN69	Saipan	*Pachystachys lutea*	3	pathogenic	*C. cassiicola*
YP01	Yap	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
YP08	Yap	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
YP17	Yap	*Carica papaya*	1.1	pathogenic	*C. cassiicola*
YP26	Yap	*Cucumis sativus*	1	pathogenic	*C. cassiicola*
YP27	Yap	*Cucumis sativus*	2	pathogenic	*C. cassiicola*
YP29	Yap	*Cucumis sativus*	1	pathogenic	*C. cassiicola*
YP41	Yap	*Saintpaulia ionantha*	2	pathogenic	*C. cassiicola*
YP42	Yap	*Solanum lycopersicum*	3	pathogenic	*C. cassiicola*
YP51	Yap	*Vigna unguiculata*	1	saprophyte	*C. cassiicola*
YP59	Yap	*Ipomoea batatas*	2	endophytic	*C. cassiicola*

Information on *Corynespora cassiicola* isolates used in this study including location, original host, phylogenetic lineage (PL), type of growth in association with the host (endophytic or pathogenic), and the *Corynespora* species. The first eight isolates were solicited from culture collections as outgroups (O). Three isolates from the NIAS culture collection (305095, 425231, and 712045) are likely misidentified because they grouped with *C. cassiicola* isolates according to sequence data. They are labeled here according to the original culture collection designations, though they should be re-classified as *C. cassiicola*. The remaining isolates were collected as part of this study or solicited from other researchers and are listed according to geographic location.

Table 2-2. Summary of sequence data from four loci used to confirm the phylogenetic lineage of *Corynespora cassiicola* isolates.

Locus	Total	Variable	Informative	Tree Score	No. MP Trees
Combined[a]	2136	248	174	330	7430
rDNA ITS	1013	135	100	158	9990
rDNA ITS[b]	584	4	3	4	1
Cc-ga4	414	31	25	40	9530
Cc-ga4[b]	414	28	25	36	9560
Cc-caa5	366	38	34	52	40
Cc-caa5[b]	366	37	32	46	12
Cc-act1	343	44	15	49	11
Cc-act1[b]	343	16	15	17	4

[a] Combined loci: rDNA ITS, *Cc-ga4*, *Cc-caa5*, and *Cc-act1*.
[b] Locus analyzed with only *C. cassiicola* taxa represented (no outgroups).

Table 2-3. Pathogenicity profiles for 50 *Corynespora cassiicola* isolates.

Path Pro	PL	Isolate	Host	Ba		Be		Co		Cu		Pa		So		Sw		To	
				I	S	I	S	I	S	I	S	I	S	I	S	I	S	I	S
BaCoCu	1	AS78	Ba	5	2.6	0	-	4	1.8	6	2.3	0	-	0	-	1	1	2	1
BaCoCu	1	AS58	Co	6	2.3	0	-	3	2.3	6	3	0	-	0	-	0	-	7	1
BaCoCu	1	YP29	Cu	7	2.1	0	-	3	1.7	8	3	0	-	0	-	0	-	0	-
BaCoCu	1	AS71	Pu	8	2.1	0	-	2	2	7	1.3	0	-	1	1	0	-	2	1
BaCoCuSo	1	PW87	Cu	5	2.2	0	-	5	1.6	8	2.5	0	-	2	1.5	0	-	7	1
BaCu	1	HI01	Ba	4	2.5	0	-	0	0	7	3	0	-	0	-	1	1	8	1
BaCu	1	SN05	Sw	7	1.9	1	1	0	0	6	2.7	0	-	0	-	0	-	2	1
BaCuTo	3	AS50	To	5	2.2	0	-	2	1	6	2.7	0	-	1	1	2	1	8	3
BaCuTo	3	YP42	To	6	2.3	0	-	0	-	7	2.6	0	-	0	-	0	-	8	2.6
BaCuTo	3.1	AS80	Ba	5	1.8	0	-	0	-	8	2.5	0	-	1	1	0	-	8	2.8
BaCuTo	3.1	AS117	Sap	7	1.9	0	-	2	1	8	2.8	0	-	2	1	8	1	7	2.9
BeCoCuSw	4	SN37	Co	7	1	5	1.6	5	1.4	8	2.8	0	-	2	1	3	1.3	8	1
BeCuSwTo	4	JMP217	To	5	1	7	1.9	0	-	8	2.9	0	-	0	-	1	2	8	2.5
BeCuSwTo	4	GU102	Be	0	-	7	1.3	0	-	8	2.6	0	-	0	-	2	1.5	8	2.5
BeCuSwTo	4	SN40	Cu	0	-	6	1.3	0	-	8	2.5	0	-	0	-	1	2	8	2.6
BeCuTo	4	PW57	To	0	-	6	1.7	0	-	8	2.1	0	-	2	1	0	-	8	3
CoCu	1	AS98	Cu	0	-	5	1	6	1.8	8	2.6	0	-	0	-	0	-	7	1
CoCu	1	YP26	Cu	0	-	7	1	4	1.5	7	2.4	0	-	0	-	8	1	8	1
CoCu	1	JMP218	So	0	-	0	-	7	2.1	8	3	0	-	0	-	7	1	7	1
CoCu	1	GU08	La	0	-	0	-	3	1.7	7	3	0	-	0	-	0	-	8	1
Cu	1	AS54	Co	0	-	1	1	0	-	8	3	0	-	1	1	8	1	1	1
Cu	1	YP51	Co	0	-	0	-	0	-	8	3	0	-	0	-	7	1	1	1
CuPa	1.1	DOA16b	Pa	7	1	0	-	0	-	8	1.1	8	2.3	0	-	8	1	1	1
CuPa	1.1	FL11	Pa	0	-	5	1	0	-	8	1.3	7	2.6	0	-	0	-	8	1
CuPa	1.1	PH01	Pa	1	1	1	1	0	-	7	1.1	6	1.8	0	-	0	-	8	1
CuSo	3	GU112	So	0	-	0	-	5	1	8	2.8	0	-	1	2	1	1	2	1
CuSw	2	YP27	Cu	1	1	0	-	2	1	6	2.8	0	-	0	-	5	1.4	2	1
CuSw	2	YP59	Sw	2	1	0	-	1	1	7	2.6	0	-	0	-	6	1.4	7	1
CuSw	2	SN59	La	1	1	5	1	0	-	8	2.8	0	-	0	-	7	1.3	7	1
CuSwTo	4	PW63	To	4	1	0	-	7	1	8	2.6	0	-	0	-	1	2	8	3
CuSwTo	4	SN24	To	0	-	0	-	0	-	8	2.5	0	-	0	-	2	1.5	8	2.6
CuSwTo	4	SN27	To	5	1	0	-	0	-	8	3	0	-	0	-	5	1.2	8	2.9

Path Pro	PL	Isolate	Host	Ba		Be		Co		Cu		Pa		So		Sw		To	
				I	S	I	S	I	S	I	S	I	S	I	S	I	S	I	S
CuSwTo	4	GU23	Sw	0	-	0	-	7	1	8	2.4	0	-	0	-	5	1.8	8	2.9
CuSwTo	4	FL09	La	0	-	1	1	0	-	8	1.4	0	-	2	1	3	1.3	8	2.8
CuTo	3	AS49	To	0	-	0	-	0	-	4	2.8	0	-	0	-	1	1	7	2.6
CuTo	3.1	AS119	Pu	0	-	0	-	0	-	8	2.3	0	-	1	1	7	1	8	2.5
CuTo	4	FL12	To	0	-	5	1	5	1	8	3	0	-	2	1	1	1	8	3
CuTo	4	FL2920	To	4	1	0	-	0	-	8	2	0	-	0	-	0	-	8	2.6
CuTo	4	MS31	To	0	-	7	1	8	1	8	2.8	0	-	0	-	1	1	8	2.3
CuTo	4	GU128	To	0	-	1	1	0	-	8	2.9	0	-	2	1	1	1	8	2.5
CuTo	4	SN30	To	0	-	0	-	0	-	8	3	0	-	2	1	2	1	8	3
CuTo	4	AS92	Cu	2	1	1	1	0	-	8	2.8	0	-	0	-	1	1	8	2.9
CuTo	4	JMP216a	La	7	1	0	-	0	-	7	1.3	0	-	0	-	2	1	8	3
CuTo	5	PW101	Co	0	-	0	-	4	1	7	2.9	0	-	0	-	0	-	7	1.3
Pa	1.1	GU92	Pa	2	1	0	-	0	-	1	1	7	2.1	0	-	1	1	7	1
Pa	1.1	PW01	Pa	0	-	0	-	0	-	8	1	8	2.4	0	-	0	-	8	1
Pa	1.1	PW12	Pa	0	-	0	-	1	1	1	1	4	1.4	2	1	0	-	1	1
Pa	1.1	SN03	Pa	1	1	0	-	0	-	8	1	6	2.3	0	-	1	1	1	1
Pa	1.1	YP01	Pa	0	-	7	1	0	-	1	1	8	1.9	0	-	0	-	8	1
To	4	GU28	To	0	-	0	-	0	-	8	1	0	-	0	-	1	1	8	3

Path Pro (Pathogenicity Profile): A list of susceptible hosts, or plants with an average disease rating greater than 1.
PL: Phylogenetic lineage designation based on combined sequence analysis of ITS rDNA, CAA5, GA4, and ACT.
Isolate: *Corynespora cassiicola* isolate code.
Host: Original host the isolate was collected from. Ba (*Ocimum basilicum*), Be (*Phaseolus vulgarus*), Co (*Vigna unquiculata*), Cu (*Cucumis sativus*), La (*Lantana camara*), Pa (*Carica papaya*), Pu (*Cucurbita pepo*), Sw (*Ipomoea batatas*), To (*Solanum lycopersicum*).
I (Incidence): Number of plants (out of 8 reps) that showed symptoms seven days after inoculation with 20,000 *C. cassiicola* spores per ml.
S (Severity): Average rating of symptomatic plants (these rated 1, 2, or 3). Plants were rated with the following scale: (0) symptomless; (1) non pathogenic hypersensitive response, a few to many non-expanding pinpoint lesions; (2) moderately virulent, many expanding lesions, some coalescing, but not resulting in blight; (3) highly virulent, lesions spreading to form large areas of dead tissue resulting in a blighting effect.

Table 2-4. Growth rate of *Corynespora cassiicola* isolates at 23°C.

Iso. ID	PL	Location	Host	Avg GR	LSD
GU99	6	Guam	Saintpaulia	0.4743	a
AS81	1	Samoa	Clerodendron	0.4639	ab
GU90	1	Guam	Stachytarpheta	0.4528	abc
HI01	1	Oahu	Basil	0.4521	abcd
PW94	1	Palau	Stachytarpheta	0.4521	abcd
FL37	1	Florida	Clerodendron	0.4514	abcd
GU104	1	Guam	Macroptilium	0.4507	abcde
AS67	1	Samoa	Commelina	0.4479	bcde
AS54	1	Samoa	Bean	0.4444	bcdef
GU08	1	Guam	Lantana	0.4438	bcdef
AS71	1	Samoa	Pumpkin	0.4410	bcdef
SN03	1	Saipan	Bitter melon	0.4389	cdef
YP26	1	Yap	Cucumber	0.4375	cdef
GU136	4	Guam	Ficus	0.4375	cdef
PW80	1	Palau	Saintpaulia	0.4375	cdef
SN05	1	Saipan	SwPotato	0.4375	cdef
AS58	1	Samoa	Bean	0.4368	cdef
YP29	1	Yap	Cucumber	0.4361	cdef
YP51	1	Yap	Bean	0.4326	cdef
SN37	4	Saipan	Bean	0.4313	cdef
GU115	1	Guam	Vitex	0.4278	defg
PW92	1	Palau	Catharanthus	0.4264	efg
AS78	1	Samoa	Basil	0.4229	fgh
GU21	1	Guam	Buddleja	0.4215	fgh
AS80	3	Samoa	Basil	0.4202	fgh
PW91	1	Palau	Luffa	0.4202	fgh
AS50	3	Samoa	Tomato	0.4063	ghi
FL34	1	Florida	Tabebouia	0.4055	ghi
YP08	1	Yap	Papaya	0.4000	hij
PW79	2	Palau	Pilea	0.3951	ijk
SN59	1	Saipan	Lantana	0.3951	ijk
RWB321	5	Brazil	Coleus	0.3945	ijk
JMP218	1	Brazil	Soybean	0.3924	ijkl
CSB12	2	Malaysia	Rubber	0.3917	ijklm
SN06	3	Saipan	Luffa	0.3903	ijklmn
PW37	1	Palau	Papaya	0.3896	ijklmn
FL2920	4	Florida	Tomato	0.3882	ijklmn
SN07	1	Saipan	Papaya	0.3868	ijklmno
PW101	5	Palau	Bean	0.3833	ijklmno
PW99	3	Palau	Bitter melon	0.3833	ijklmno
SN64	5	Saipan	Asystasia	0.3822	ijklmno
GU109	3	Guam	Bauhinia	0.3820	ijklmno
AS98	1	Samoa	Cucumber	0.3819	ijklmno
CLN16	1	Malaysia	Rubber	0.3778	jklmnop
PW89	4	Palau	Chromolaena	0.3778	jklmnop
YP59	2	Yap	SwPotato	0.3778	jklmnop

Table 2-4. Continued.

Iso. ID	PL	Location	Host	Avg GR	LSD
AS49	3	Samoa	Tomato	0.3771	jklmnopq
GU107	4	Guam	Mikania	0.3729	klmnopqr
CBPP	1	Malaysia	Rubber	0.3695	lmnopqrs
AS119	3	Samoa	Papaya	0.3687	lmnopqrst
YP42	3	Yap	Tomato	0.3674	mnopqrstu
YP01	1	Yap	Papaya	0.3667	nopqrstu
AS92	4	Samoa	Cucumber	0.3632	opqrstu
GU112	3	Guam	Bean	0.3632	opqrstu
FL12	4	Florida	Tomato	0.3556	pqrstuv
GU98	4	Guam	Spathodea	0.3549	pqrstuv
GU92	1	Guam	Papaya	0.3529	qrstuvw
FL09	4	Florida	Lantana	0.3521	rstuvw
YP17	1	Yap	Papaya	0.3521	rstuvw
GU102	4	Guam	Bean	0.3507	rstuvwx
GU38	2	Guam	Allamanda	0.3507	rstuvwx
PW57	4	Palau	Tomato	0.3500	rstuvwx
YP41	2	Yap	Saintpaulia	0.3480	stuvwxy
AS117	3	Samoa	Papaya	0.3458	stuvwxy
SN40	4	Saipan	Cucumber	0.3444	tuvwxy
AS65	4	Saipan	Eggplant	0.3437	uvwxy
PW01	1	Palau	Papaya	0.3368	vwxyz
GU41	4	Guam	Eugenia	0.3361	vwxyz
YP27	2	Yap	Cucumber	0.3340	vwxyz
FL36	2	Florida	Catharanthus	0.3312	vwxyz
JMP217	4	Brazil	Tomato	0.3299	wxyz
SN24	4	Saipan	Tomato	0.3264	xyz
DOA16b	1	Brazil	Papaya	0.3250	yz
FL15	4	Florida	Salvia	0.3146	z
GU120	4	Guam	Coleus	0.2792	A
JMP216a	4	Brazil	Lantana	0.2535	B
PH01	1	Pohnpei	Papaya	0.1479	C

PL: Phylogenetic Lineage based on sequence data from 4 loci.
Avg GR: Average slope (growth rate) of three replicate plates.
LSD: Average slope (growth rate) values followed by different letters are significantly different from one another according to least significant difference test ($P<0.05$).

Table 2-5. Growth rate of *Corynespora cassiicola* isolates at 33°C.

Iso. ID	PL	Location	Host	Avg GR	LSD
AS71	1	Samoa	Pumpkin	0.4153	a
FL37	1	Florida	Clerodendron	0.3972	b
AS78	1	Samoa	Basil	0.3965	b
SN37	4	Saipan	Bean	0.3917	bc
PW92	1	Palau	Catharanthus	0.3813	bcd
SN03	1	Saipan	Bitter melon	0.3799	bcd
GU90	1	Guam	Stachytarpheta	0.3778	cd
GU99	6	Guam	Saintpaulia	0.3771	cde
AS67	1	Samoa	Commelina	0.3764	cde
PW79	2	Palau	Pilea	0.3722	def
PW80	1	Palau	Saintpaulia	0.3715	defg
HI01	1	Oahu	Basil	0.3680	defgh
GU136	4	Guam	Ficus	0.3674	defghi
YP51	1	Yap	Bean	0.3653	defghi
SN05	1	Saipan	SwPotato	0.3597	efghij
GU115	1	Guam	Vitex	0.3577	fghij
GU21	1	Guam	Buddleja	0.3576	fghij
YP26	1	Yap	Cucumber	0.3569	fghij
YP29	1	Yap	Cucumber	0.3548	fghij
AS54	1	Samoa	Bean	0.3542	ghijk
AS58	1	Samoa	Bean	0.3542	ghijk
GU104	1	Guam	Macroptilium	0.3542	ghijk
PW91	1	Palau	Luffa	0.3514	hijkl
GU08	1	Guam	Lantana	0.3500	ijklm
AS98	1	Samoa	Cucumber	0.3451	jklmno
SN07	1	Saipan	Papaya	0.3368	klmno
YP08	1	Yap	Papaya	0.3354	lmno
AS92	4	Samoa	Cucumber	0.3327	mnop
PW94	1	Palau	Stachytarpheta	0.3292	nopq
JMP218	1	Brazil	Soybean	0.3285	nopq
GU98	4	Guam	Spathodea	0.3278	nopq
PW89	4	Palau	Chromolaena	0.3278	nopq
YP17	1	Yap	Papaya	0.3236	opqr
PW37	1	Palau	Papaya	0.3224	opqr
GU107	4	Guam	Mikania	0.3215	opqr
PH01	1	Pohnpei	Papaya	0.3174	pqrs
DOA16b	1	Brazil	Papaya	0.3132	qrst
GU102	4	Guam	Bean	0.3125	qrstu
PW01	1	Palau	Papaya	0.3063	rstuv
GU92	1	Guam	Papaya	0.3028	stuv
YP01	1	Yap	Papaya	0.3014	stuv
PW57	4	Palau	Tomato	0.2993	tuvw
GU41	4	Guam	Eugenia	0.2959	tuvwx
AS81	1	Samoa	Clerodendron	0.2952	uvwx
SN40	4	Saipan	Cucumber	0.2951	uvwx
PW101	5	Palau	Bean	0.2951	uvwx

Table 2-5. Continued.

Iso. ID	PL	Location	Host	Avg GR	LSD
YP41	2	Yap	Saintpaulia	0.2903	vwxy
FL2920	4	Florida	Tomato	0.2889	vwxy
SN64	5	Saipan	Asystasia	0.2829	wxyz
CLN16	1	Malaysia	Rubber	0.2792	xyz
CSB12	2	Malaysia	Rubber	0.2771	yz
CBPP	1	Malaysia	Rubber	0.2736	yz
YP59	2	Yap	SwPotato	0.2688	zA
FL15	4	Florida	Salvia	0.2686	zA
YP27	2	Yap	Cucumber	0.2667	zA
FL36	2	Florida	Catharanthus	0.2521	AB
PW99	3	Palau	Bitter melon	0.2486	BC
GU109	3	Guam	Bauhinia	0.2438	BC
SN24	4	Saipan	Tomato	0.2431	BC
FL12	4	Florida	Tomato	0.2326	CD
AS80	3	Samoa	Basil	0.2313	CD
GU38	2	Guam	Allamanda	0.2312	CD
SN06	3	Saipan	Luffa	0.2250	DE
RWB321	5	Brazil	Coleus	0.2188	DE
FL09	4	Florida	Lantana	0.2097	EF
AS65	4	Saipan	Eggplant	0.2076	EF
AS50	3	Samoa	Tomato	0.1993	FG
YP42	3	Yap	Tomato	0.1938	GFH
SN59	1	Saipan	Lantana	0.1875	GHI
FL34	1	Florida	Tabebouia	0.1763	HIJ
JMP216a	4	Brazil	Lantana	0.1750	IJ
GU112	3	Guam	Bean	0.1709	IJK
GU120	4	Guam	Coleus	0.1688	JKL
AS49	3	Samoa	Tomato	0.1660	JKL
JMP217	4	Brazil	Tomato	0.1549	KLM
AS117	3	Samoa	Papaya	0.1521	LM
AS119	3	Samoa	Papaya	0.1382	M

PL: Phylogenetic Lineage based on sequence data from 4 loci.
Avg GR: Average slope (growth rate) of three replicate plates.
LSD: Average slope (growth rate) values followed by different letters are significantly different from one another according to least significant difference test ($P<0.05$).

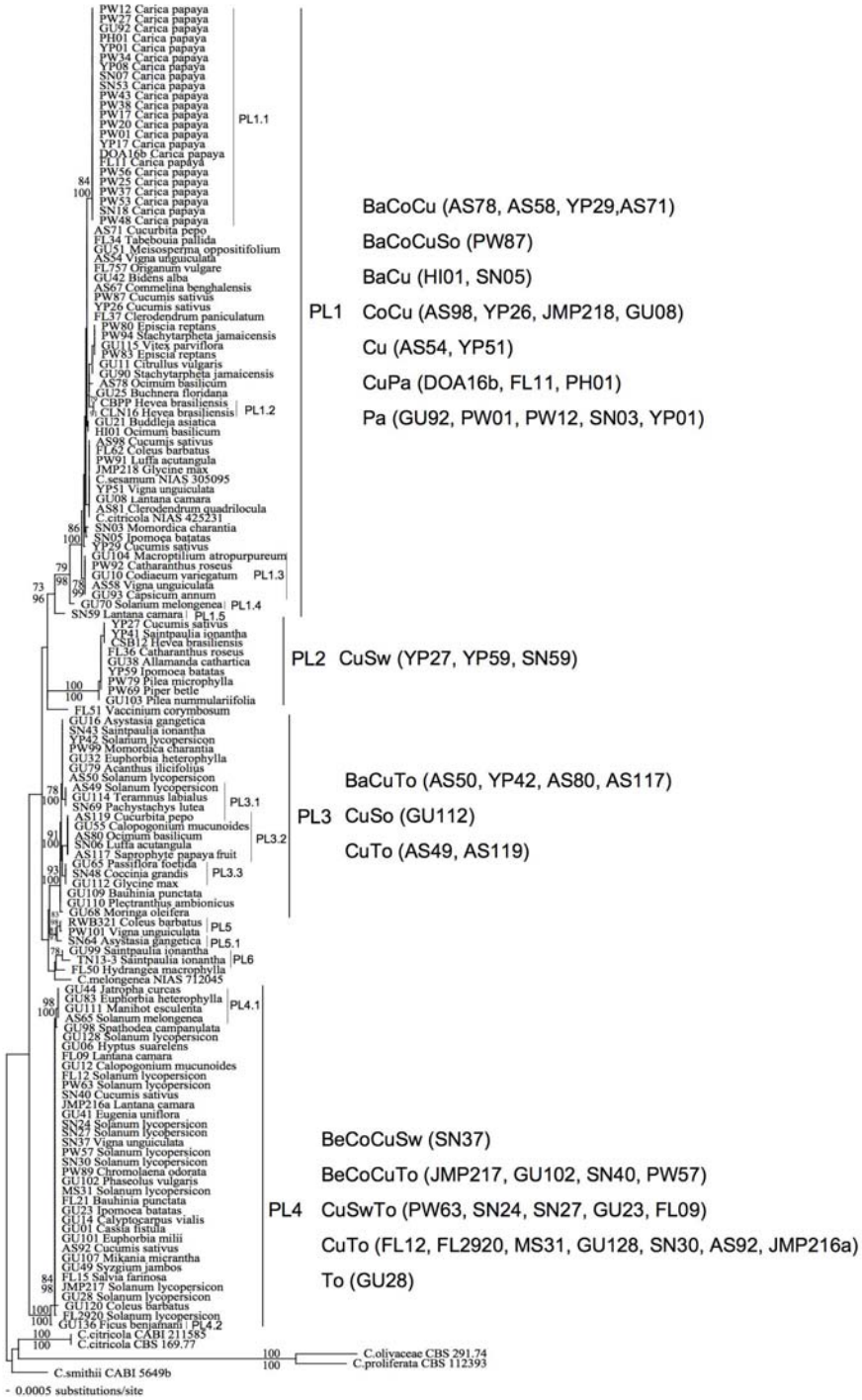

Figure 2-1. Fifty percent majority rule consensus tree-phylogram from Bayesian inference analysis of combined data from rDNA ITS, *Cc-ga4*, *Cc-caa5*, and *Cc-act1* sequences. Numbers above branches indicate maximum parsimony bootstrap > 70% and numbers below branches indicate posterior probability values > 0.90. Pathogenicity profiles on eight crop plants: basil (Ba), bean (Be), cowpea (Co), cucumber (Cu), papaya (Pa), soybean (So), sweet potato (Sw), and tomato (To), and phylogenetic lineage (PL) are indicated.

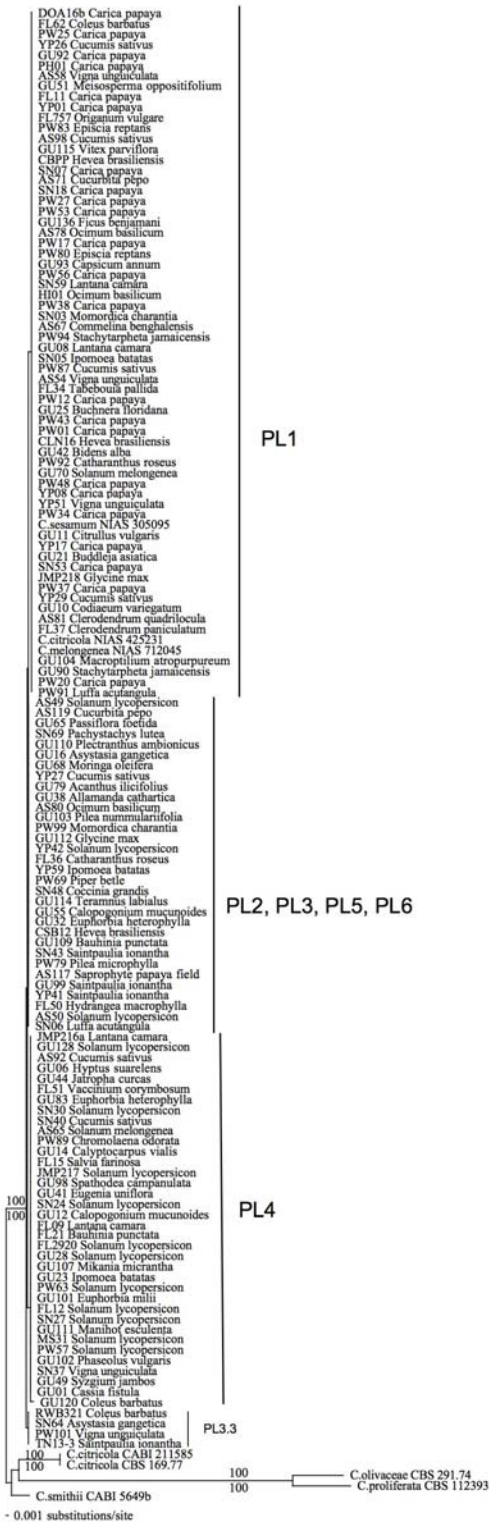

Figure 2-2. Fifty percent majority rule consensus tree-phylogram from Bayesian inference analysis of rDNA ITS locus. Numbers above branches indicate maximum parsimony bootstrap > 70% and numbers below branches indicate posterior probability values > 0.90. 100,000 maximum parsimony trees were a result of only 3 informative characters within *C. cassiicola*. Phylogenetic lineages (PL) are indicated.

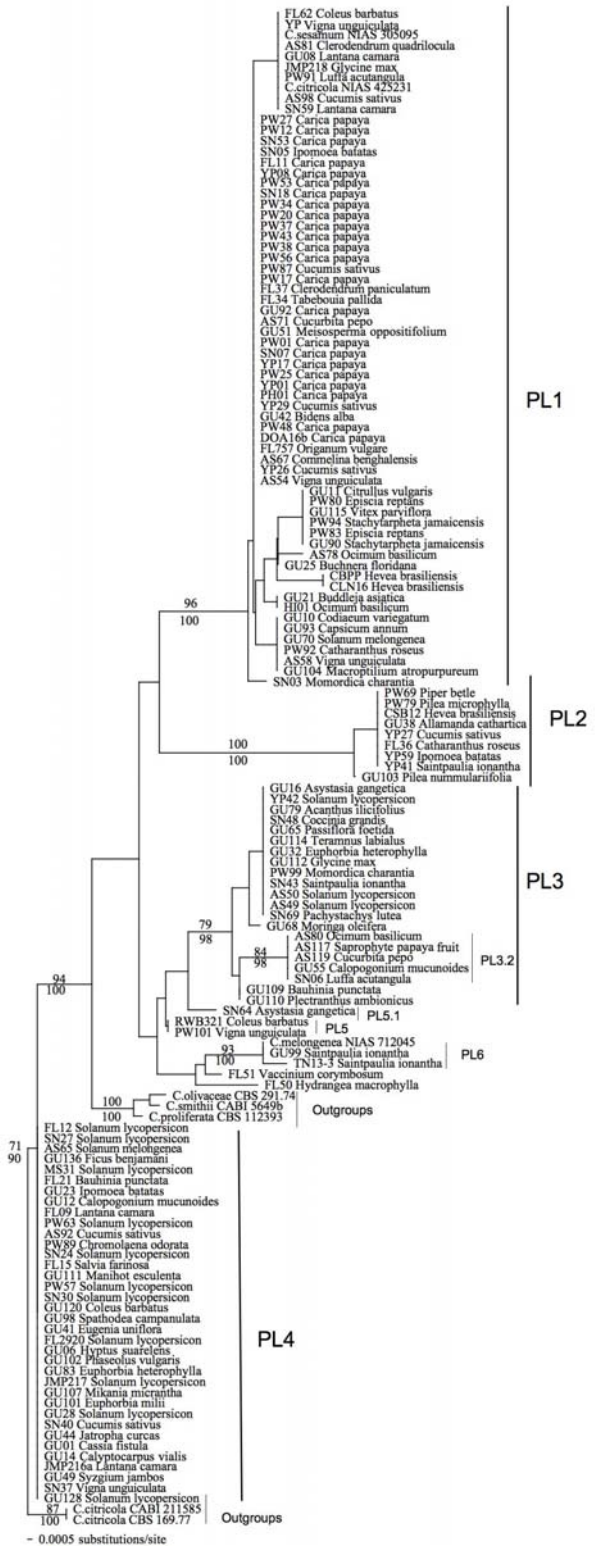

Figure 2-3. Fifty percent majority rule consensus tree-phylogram from Bayesian inference analysis of the *Cc-caa5* locus. Numbers above branches indicate maximum parsimony bootstrap > 70% and numbers below branches indicate posterior probability values > 0.90. Phylogenetic lineages (PL) are indicated.

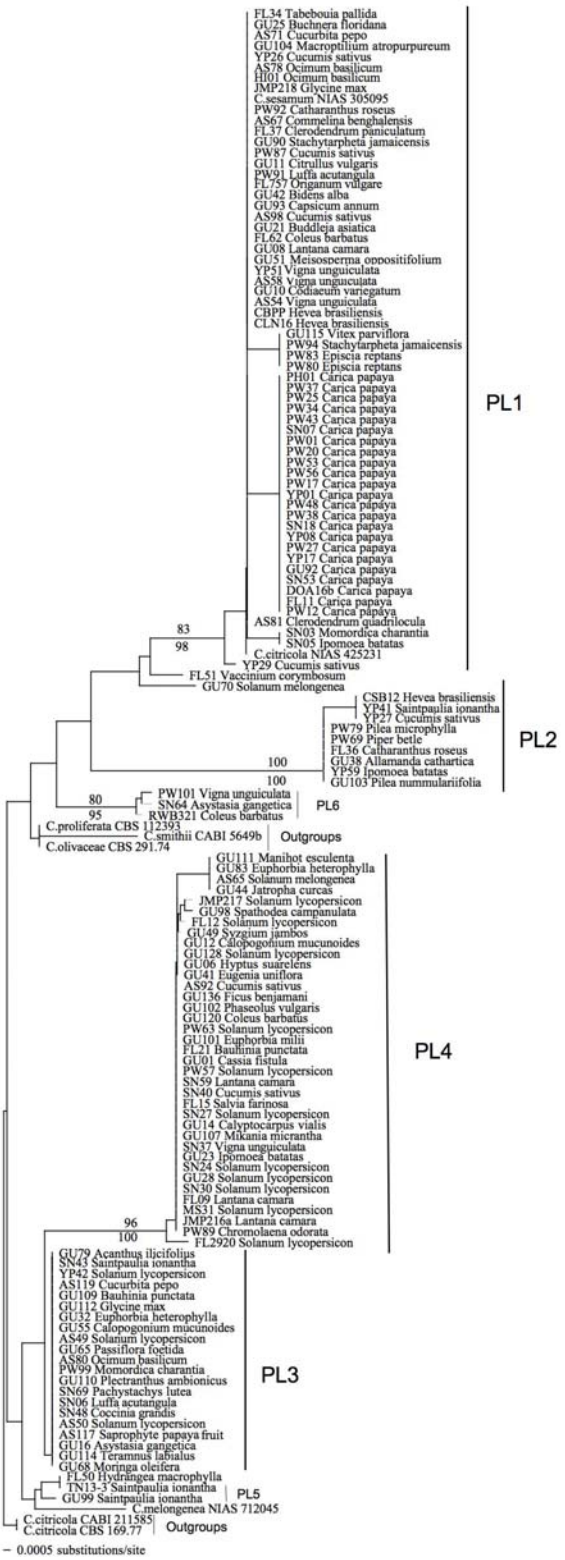

Figure 2-4. Fifty percent majority rule consensus tree-phylogram from Bayesian inference analysis of the *Cc-ga4* locus. Numbers above branches indicate maximum parsimony bootstrap > 70% and numbers below branches indicate posterior probability values > 0.90. Phylogenetic lineages (PL) are indicated.

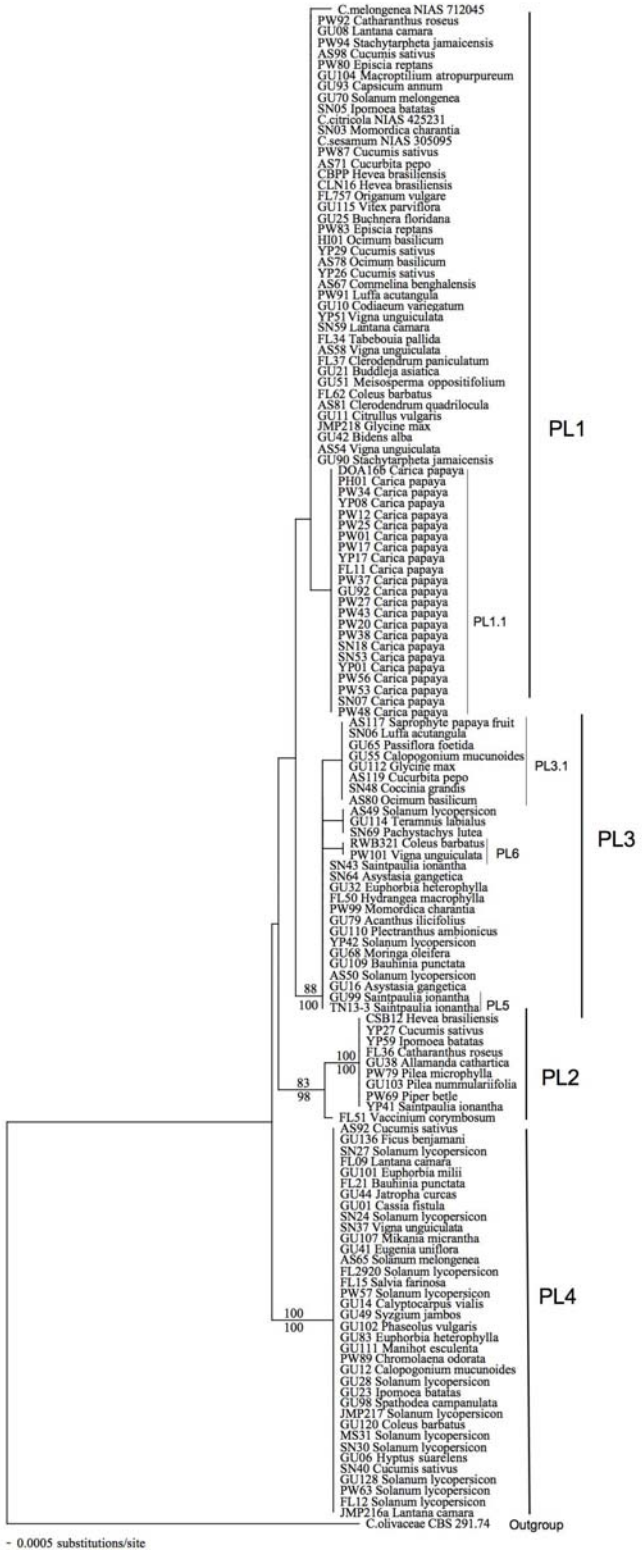

Figure 2-5. Fifty percent majority rule consensus tree-phylogram from Bayesian inference analysis of the *Cc-act1* locus. Numbers above branches indicate maximum parsimony bootstrap > 70% and numbers below branches indicate posterior probability values > 0.90. Phylogenetic lineages (PL) are indicated.

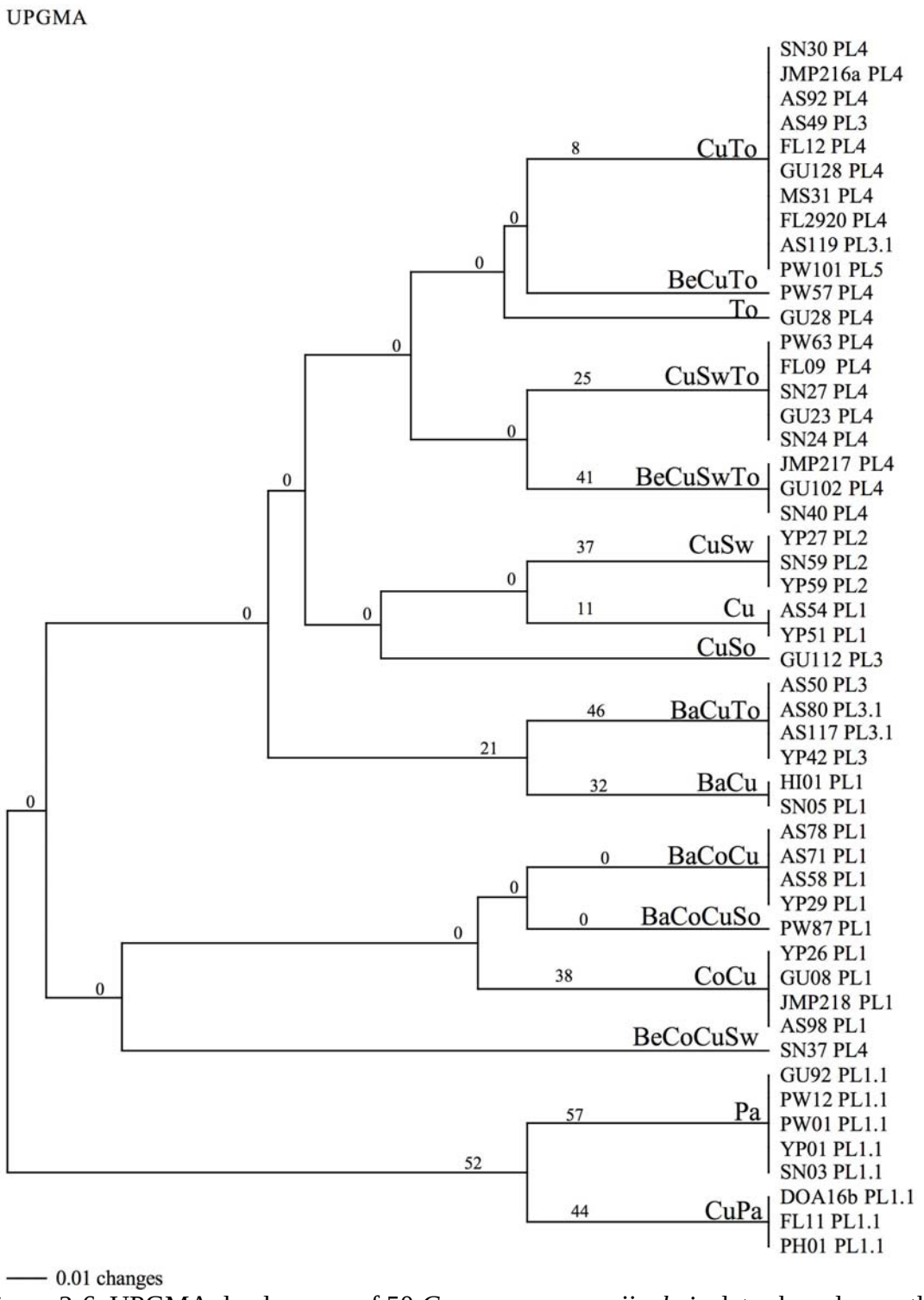

Figure 2-6. UPGMA dendrogram of 50 *Corynespora cassiicola* isolates based on pathogenicity profiles on eight crop plants: basil (Ba), bean (Be), cowpea (Co), cucumber (Cu), papaya (Pa), soybean (So), sweet potato (Sw), tomato (To). Isolates are labeled with their phylogenetic lineage (PL) designation to demonstrate that isolates from the same PL cluster together. Statistical support for nodes by 1,000 UPGMA Bootstrap repetitions is indicated.

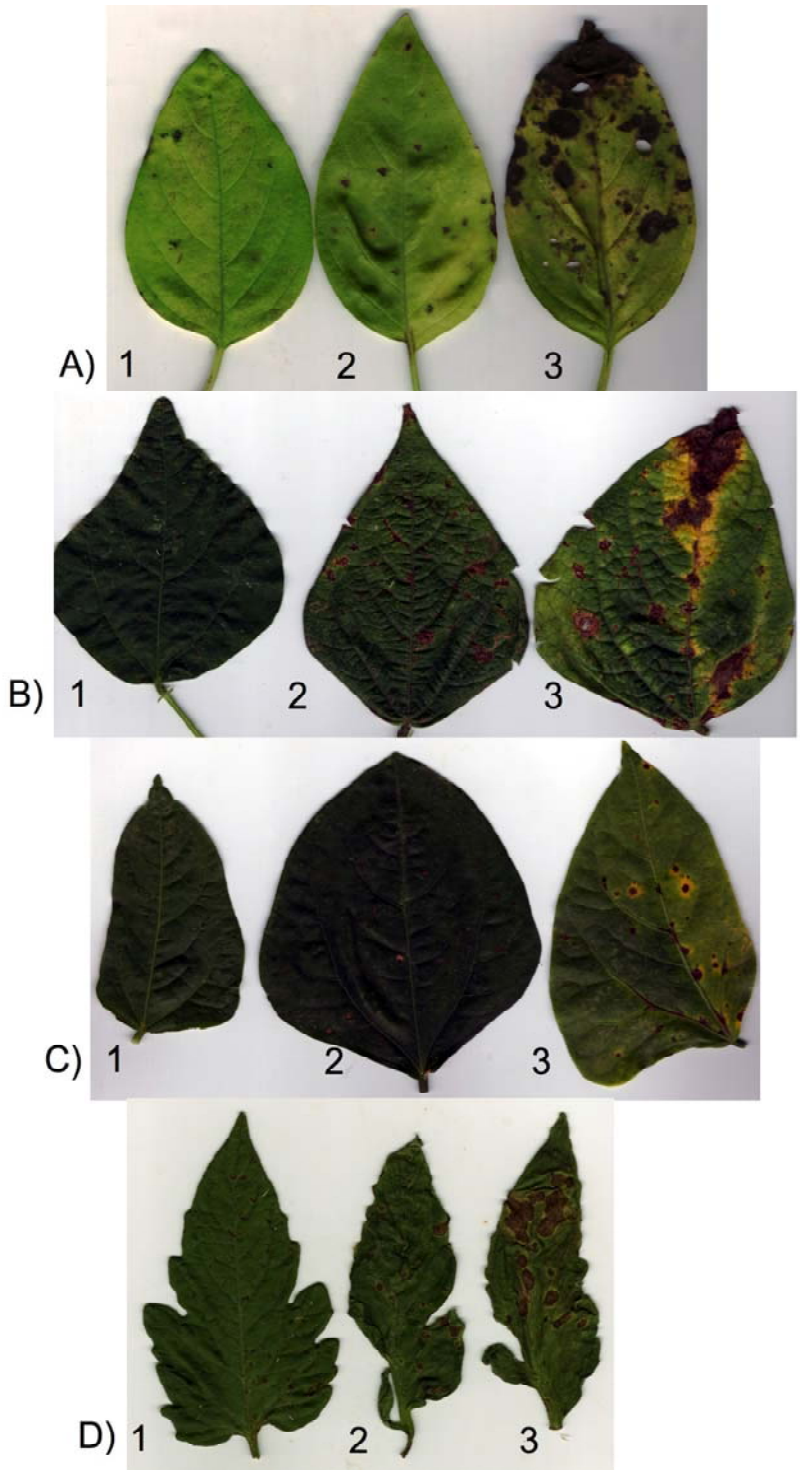

Figure 2-7. Demonstration of the *C. cassiicola* disease rating system. Symptoms on A) basil, B) bean, C) cowpea, and D) tomato plants seven days after inoculation with different isolates of *Corynespora cassiicola*. Plants were rated with the following scale: (0) symptomless; (1) non pathogenic hypersensitive response, a few to many non-expanding pinpoint lesions; (2) moderately virulent, many expanding lesions, some coalescing, but not resulting in blight; (3) highly virulent, lesions spreading to form large areas of dead tissue resulting in a blighting effect.

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BIOGRAPHICAL SKETCH

I was born in Pompano Beach, FL to Janis Therrell and Kenneth Wayne Smith on October 15, 1977. I have an older sister, Allison, and two younger brothers, Scott and Reid. Our family moved to Baltimore, MD when I was nine and I attended Baltimore Friends School, where my father was head of the Middle School. Though I have always loved biology and gardening, my interest in agriculture took off in high school when I attended Maine Coast Semester, a small school for students in their junior year located on a coastal farm in Wiscasset, Maine.

I received my B. A. at Colorado College in 2000 with a major in Biology, while fostering my interest in farming through summer jobs at nurseries, CSA's, and internships. My sophomore year in college, I traveled abroad to East Africa through The School for Field Studies where I learned the importance of economic value in conservation by focusing on wildlife ranching, national parks, and medicinal plant use as case studies.

In 2002, I received my Masters Degree from West Virginia University in Plant Pathology as part of the Organic Farm Project by studying the effect of intercropping on diseases caused by *Alternaria solani* and *Meloidogyne incognita*. I then spent two years in Micronesia on the island of Guam as a Research Assistant documenting pathogens of agronomically important weeds and working in the diagnostic clinic. It was in Guam where I first became aware of *Corynespora cassiicola* as an agent of disease. An opportunity presented itself to continue the work begun on this pathogen at the University of Florida in the Fall of 2004. At the University of Florida, I became well trained in Phylogenetics and this has become my specialty. I plan to continue studying fungal systematics beginning in September, 2008 as a post-doc with the USDA in Beltsville, MD.