Host Specialization and Phylogenetic Diversity of *Corynespora cassiicola*

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ABSTRACT

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The fungus *Corynespora cassiicola* is primarily found in the tropics and subtropics, and is widely diverse in substrate utilization and host association. Isolate characterization within *C. cassiicola* was undertaken to investigate how genetic diversity correlates with host specificity, growth rate, and geographic distribution. *C. cassiicola* isolates were collected from 68 different plant species in American Samoa, Brazil, Malaysia, and Micronesia, and Florida, Mississippi, and Tennessee within the United States. Phylogenetic analyses using four loci were performed with 143 *Corynespora* spp. isolates, including outgroup taxa obtained from culture collections: *C. citricola*, *C. melongenae*, *C. olivacea*, *C. proliferata*, *C. sesamum*, and *C. smithii*. Phylogenetic trees were congruent from the ribosomal DNA internal transcribed spacer region, two

random hypervariable loci (*caa5* and *ga4*), and the actin-encoding locus *act1*, indicating a lack of recombination within the species and asexual propagation. Fifty isolates were tested for pathogenicity on eight known *C. cassiicola* crop hosts: basil, bean, cowpea, cucumber, papaya, soybean, sweet potato, and tomato. Pathogenicity profiles ranged from one to four hosts, with cucumber appearing in 14 of the 16 profiles. Bootstrap analyses and Bayesian posterior probability values identified six statistically significant phylogenetic lineages. The six phylogenetic lineages correlated with host of origin, pathogenicity, 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 genetic diversity within the species and provides evidence for host specialization on papaya.

Additional keywords: target spot.

Corynespora cassiicola (Berk. & M. A. Curtis) C. T. Wei, the type species of the genus, is one of >100 described *Corynespora* spp. delineated in part by host of isolation (13,59). The fungus has been found on plant leaves, stems, and roots; nematode cysts; and human skin (3,5,8,9,19,29,30,41). More than 530 plant species from 380 genera, including monocots, dicots, ferns, and one cycad, have been reported to support the growth of *C. cassiicola* (47,49). The majority of isolates reported are obtained from discrete lesions or are supported by Koch's postulates and, therefore, are classified as pathogens (2,3,7,9,17,21,22,24,31,32,41,48,51). Isolates obtained from dead organic material or that fail Koch's postulates are often reported and classified as saprotrophs on a given host (6,19,24,26,27,34). Isolates obtained from living, nonsymptomatic tissue are also reported and are classified as endotrophs (10,16,40,54). Some isolates show pathogenicity to a wide range of hosts, whereas others exhibit host specificity (12,24,33,34,36,38,43,50,51,53). Others are only pathogenic when associated with wounding (6,57).

The variability in host range within *C. cassiicola* is so great that grouping isolates based on host origin is problematic for predicting pathogenicity and genetic relatedness (45). Isolates with different pathogenicity profiles can be found on the same host (15). As many as eight different pathogenicity profiles were recognized among 28 isolates collected from *Glycine max* (soybean) in Mexico, *Cucumis sativus* (cucumber) in Florida, and

diverse hosts in Nigeria (34). An isolate collected from *Carica papaya* (papaya) debris was pathogenic on *Solanum lycopersicum* (tomato) and cucumber but not papaya, indicating that individual isolates can be both saprotrophic and pathogenic, depending on the host substrate (24). The host specificity and severity of the fungus on *Lantana camara* (lantana) in Brazil has led to the discovery that *Corynespora cassiicola* may be useful as a bioherbicide (36). Based on the vast number of weeds that serve as hosts and the host specificity of some isolates, there is potential for the discovery of additional isolates useful for biological control of weeds (51).

Past assessments of genetic diversity within *C. cassiicola* were largely limited to *Hevea brasiliensis* (rubber) isolates from Asia and were based on random amplified polymorphic DNA (RAPD) techniques, which are problematic with respect to repeatability and homology assessment (20). RAPD studies provide evidence for host specialization on individual rubber clones and papaya but do not indicate geographic associations between isolates (44,46). Restriction fragment length polymorphism (RFLP) of the ribosomal (r)DNA internal transcribed spacer (ITS) locus resulted in monomorphic patterns with diverse isolates (1,44,45). However, an isolate from rubber clone RRIM 2020 had a distinct RFLP pattern after digestion of the amplified rDNA ITS locus with *Hae*III (1).

C. cassiicola is a ubiquitous phytopathogen reported to cause major economic losses in >70 countries. The aim of this study was to expand our knowledge of the genetic variability among *C. cassiicola* isolates through the use of improved molecular methods and a large pool of diverse isolates. To accomplish these goals, we obtained 143 isolates from varied hosts and geographic locations. Isolates of target spot of *Ocimum basilicum* (basil), *Phaseolus vulgaris* (bean), *Vigna unguiculata* (cowpea), cucumber, papaya, soybean, *Ipomoea batatas* (sweet potato), tomato,

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and other crops were of particular focus because of the research implications for resistance breeding and disease management. Correlations among isolates' host, geographic locations, growth rate, pathogenicity profiles, and genotypes were investigated. This research will ideally improve disease management by identifying sources of inoculum and isolates for breeding and bioherbicide efforts.

MATERIALS AND METHODS

Collection of fungal isolates. *C. cassiicola* isolates were collected from diverse plant hosts during 5-day visits to locations in the Pacific—American Samoa (AS), Hawaii (HI), Palau (PW), Pohnpei (PH), Saipan (SN), and Yap (YP)—in summer 2005. More extensive surveys were conducted in Florida (FL) and Guam (GU) from 2004 to 2006. Host priority was given to basil, bean, cowpea, cucumber, papaya, soybean, sweet potato, and tomato plants as well as other susceptible crops, weeds, and naturalized or indigenous hosts of *C. cassiicola*. Leaves were collected and placed into individual plastic bags in the field. Priority was given to samples with discrete lesions, a common disease symptom of *Corynespora* leaf spot. In the laboratory, the leaves were placed abaxial side up in petri dishes lined with moistened paper towels and examined daily over the course of 4 days. Sporulating isolates arising from discrete lesions within 24 h were designated “lesioned” and those from lesion-free host tissue were designated “healthy” (Table 1). Sporulating isolates arising from decayed tissue with nondiscrete lesions or those that required 48 h or more to sporulate were designated “necrotic.” Conidia were captured at the end of a teasing needle and transferred to antibiotic V8 agar slants (340 ml of V8 juice, 660 ml of water, 3 g of CaCO₃, 17 g of agar, and ampicillin or kanamycin at 100 µg/ml), left at room temperature until colonies reached at least 5 cm in diameter, covered with autoclaved mineral oil, and stored at 5°C.

To obtain a more global sampling of *C. cassiicola*, isolates were requested from individual researchers (Table 1). Isolates of different *Corynespora* spp. were also obtained from culture collections to serve as potential outgroups. Cultures were single-spored after they were received (47). A complete list of the isolates used in this study, along with their hosts, geographic locations, and description of the host tissue from which they were isolated, can be found in Table 1.

Fungal cultures and extraction of genomic DNA. Genomic DNA from 143 isolates in long-term storage at 5°C was purified and amplified using Extract-N-Amp (Sigma-Aldrich, St. Louis) according to the manufacturer's specifications. To increase the likelihood that sequence data represent phylogenetic relationships, both variable (*caa5*, *ga4*, and *act1*) and conserved (rDNA ITS) loci were chosen for analysis. The following primers were used for polymerase chain reaction (PCR) amplification: ITS1 and ITS4 (58) for the ITS region, including the 5.8S rRNA coding region; ACT-512F and ACT-783R (4) for the single-copy nuclear actin locus *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 *ga4* locus; and 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 *caa5* locus (47). Primers for the *ga4* and *caa5* loci were designed from a *C. cassiicola* microsatellite library enriched for GA and CAA repeats. “Hot-start” PCR was performed with a MyCycler thermocycler (Bio-Rad Laboratories, Hercules, CA) with a program consisting of initial denaturation for 3 min at 94°C; followed by 30 cycles of 30 s at 94°C, 30 s at 58°C, and 45 s at 72°C; and a final cycle of 5 min at 72°C for the rDNA ITS, *ga4*, and *caa5* loci. For the *act1* locus, the program was identical except for an annealing temperature of 61°C. PCR products were purified using the QIAquick PCR purification Kit (QIAGEN Inc., Valencia, CA)

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 performed using the same primers that were used for amplification 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). Sequencing reactions were performed on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) following the manufacturer's protocols.

Phylogenetic analyses. Four loci (rDNA ITS, *caa5*, *ga4*, and *act1*) from 143 isolates were sequenced. Forward and reverse sequences from each PCR product were proofread and contiged in Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI) and samples with ambiguous bases were sent for resequencing or PCR reactions were repeated. Multiple alignments from each locus were executed separately with ClustalX 1.83.1 (56) and the alignments were inspected and adjusted manually using MacClade 4.08 OS X (28). A partition-homogeneity test (incongruence length-difference test [ILD]) was implemented to evaluate the homogeneity of different data partition subsets using PAUP* v4.0b10 (55). The test implemented 1,000 replicates (heuristic search; random simple sequence additions; tree-bisection reconnection (TBR); max-trees = 1,000). Comparisons were evaluated using a threshold of $P < 0.001$ and were made between all data partitions.

Neighbor joining (NJ) and maximum parsimony (MP) analyses were conducted for each data set using PAUP* v4.0b10 (55). *C. smithii* IMI 5649b, *C. citricola* IMI 211585, *C. proliferata* CBS 112393, *C. citricola* CBS 169.77, and *C. olivacea* CBS 291.74 were selected as outgroups for rooting purposes. For the NJ analyses, default settings were used except ties were broken randomly by initial seed.

As a result of long computational time, MP analyses were conducted in the following manner. An initial heuristic search was conducted with one random addition replicate, TBR branch swapping, and the MulTrees option (saving all optimal trees) in effect. Using the above settings, a second heuristic search was conducted with 1,000 random addition replicates and saving no more than 10 trees with a score greater than or equal to the best tree score from 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 MP bootstrap (BS) replicates (14). 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 (18,42). An appropriate model of evolution (under the AIC criterion) was selected for each data partition using the program Modeltest v3.4 (39). All Bayesian analyses (individual loci and combined data) were conducted while retaining the appropriate model for each data partition. Two replicates of Markov Chain Monte Carlo were implemented with four heated chains and trees sampled every 1,000th generation for one million generations. To set the generation time, the number of generations required to achieve standard deviations of split frequencies of ≤ 0.01 was determined. A plot of likelihood scores at each sampling point was used to estimate burn-in. A 50% majority rule consensus tree was generated from the remaining trees, in which the percentage of nodes recovered represented their posterior probability (PoPr). Congruent nodes resulting from the NJ, MP, and BI analyses of the combined molecular data were used to assign isolates to a phylogenetic lineage (PL). Only isolates that fell within clades of high support (BS value > 70 and PoPr value > 95) were assigned to a PL.

TABLE 1. Origin and characteristics of *Corynespora* isolates used for phylogenetic analyses

Isolate ^a	Geographic location	Host ^b	PL ^c	Host tissue ^d	<i>Corynespora</i> spp. ^e
CABI 211585	New Zealand	<i>Poncirus trifoliatus</i>	O	Healthy	<i>C. citricola</i>
CBS 169.77	New Zealand	<i>Poncirus trifoliatus</i>	O	Healthy	<i>C. citricola</i>
NIAS 425231	Japan	<i>Ocimum basilicum</i>	1	Lesioned	<i>C. citricola</i>
NIAS 712045	Japan	<i>Solanum melongena</i>	–	Lesioned	<i>C. melongenae</i>
CBS 291.74	Netherlands	<i>Tilia</i> spp.	O	Necrotic	<i>C. olivacea</i>
CBS 112393	Italy	<i>Fagus sylvatica</i>	O	Healthy	<i>C. proliferata</i>
NIAS 305095	Japan	<i>Sesamum indicum</i>	1	Lesioned	<i>C. sesamum</i>
CABI 5649b	England	<i>Fagus sylvatica</i>	O	Necrotic	<i>C. smithii</i>
AS49	American Samoa	<i>Solanum lycopersicum</i>	3.1	Lesioned	<i>C. cassiicola</i>
AS50	American Samoa	<i>Solanum lycopersicum</i>	3	Lesioned	<i>C. cassiicola</i>
AS54	American Samoa	<i>Vigna unguiculata</i>	1	Necrotic	<i>C. cassiicola</i>
AS58	American Samoa	<i>Vigna unguiculata</i>	1.3	Necrotic	<i>C. cassiicola</i>
AS65	American Samoa	<i>Solanum melongena</i>	4.1	Necrotic	<i>C. cassiicola</i>
AS67	American Samoa	<i>Commelina benghalensis</i>	1	Lesioned	<i>C. cassiicola</i>
AS71	American Samoa	<i>Cucurbita pepo</i>	1	Necrotic	<i>C. cassiicola</i>
AS78	American Samoa	<i>Ocimum basilicum</i>	1	Lesioned	<i>C. cassiicola</i>
AS80	American Samoa	<i>Ocimum basilicum</i>	3.2	Lesioned	<i>C. cassiicola</i>
AS81	American Samoa	<i>Clerodendrum quadriloculare</i>	1	Lesioned	<i>C. cassiicola</i>
AS92	American Samoa	<i>Cucumis sativus</i>	4	Lesioned	<i>C. cassiicola</i>
AS98	American Samoa	<i>Cucumis sativus</i>	1	Lesioned	<i>C. cassiicola</i>
AS117	American Samoa	<i>Carica papaya</i> fruit	3.2	Necrotic	<i>C. cassiicola</i>
AS119	American Samoa	<i>Cucurbita pepo</i>	3.2	Necrotic	<i>C. cassiicola</i>
BZ DOA16b ^f	Brazil	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
BZ JMP216a ^f	Brazil	<i>Lantana camara</i>	4	Lesioned	<i>C. cassiicola</i>
BZ JMP217 ^f	Brazil	<i>Solanum lycopersicum</i>	4	Lesioned	<i>C. cassiicola</i>
BZ JMP218 ^f	Brazil	<i>Glycine max</i>	1	Lesioned	<i>C. cassiicola</i>
BZ RWB321 ^f	Brazil	<i>Plectranthus barbatus</i>	5	Lesioned	<i>C. cassiicola</i>
FL09	Florida, United States	<i>Lantana camara</i>	4	Lesioned	<i>C. cassiicola</i>
FL11	Florida, United States	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
FL12	Florida, United States	<i>Solanum lycopersicum</i>	4	Lesioned	<i>C. cassiicola</i>
FL15	Florida, United States	<i>Salvia farinacea</i>	4	Lesioned	<i>C. cassiicola</i>
FL21	Florida, United States	<i>Bauhinia galpinii</i>	4	Lesioned	<i>C. cassiicola</i>
FL34	Florida, United States	<i>Tabebuia pallida</i>	1	Lesioned	<i>C. cassiicola</i>
FL36	Florida, United States	<i>Catharanthus roseus</i>	2	Lesioned	<i>C. cassiicola</i>
FL37	Florida, United States	<i>Clerodendrum paniculatum</i>	1	Lesioned	<i>C. cassiicola</i>
FL50	Florida, United States	<i>Hydrangea macrophylla</i>	–	Lesioned	<i>C. cassiicola</i>
FL51	Florida, United States	<i>Vaccinium corymbosum</i>	–	Lesioned	<i>C. cassiicola</i>
FL62	Florida, United States	<i>Plectranthus barbatus</i>	1	Lesioned	<i>C. cassiicola</i>
FL757 ^g	Florida, United States	<i>Origanum vulgare</i>	1	Lesioned	<i>C. cassiicola</i>
FL2920 ^g	Florida, United States	<i>Solanum lycopersicum</i>	4	Lesioned	<i>C. cassiicola</i>
MS31 ^h	Mississippi, United States	<i>Solanum lycopersicum</i>	4	Lesioned	<i>C. cassiicola</i>
TN3-3 ⁱ	Tennessee, United States	<i>Saintpaulia ionantha</i>	6	Lesioned	<i>C. cassiicola</i>
GU01	Guam	<i>Cassia fistula</i>	4	Necrotic	<i>C. cassiicola</i>
GU06	Guam	<i>Hyptis suaveolens</i>	4	Healthy	<i>C. cassiicola</i>
GU08	Guam	<i>Lantana camara</i>	1	Lesioned	<i>C. cassiicola</i>
GU10	Guam	<i>Codiaeum variegatum</i>	1.3	Healthy	<i>C. cassiicola</i>
GU11	Guam	<i>Citrullus vulgaris</i>	1	Necrotic	<i>C. cassiicola</i>
GU12	Guam	<i>Calopogonium mucunoides</i>	4	Lesioned	<i>C. cassiicola</i>
GU14	Guam	<i>Calypotocarpus vialis</i>	4	Lesioned	<i>C. cassiicola</i>
GU16	Guam	<i>Asystasia gangetica</i>	3	Lesioned	<i>C. cassiicola</i>
GU21	Guam	<i>Buddleja asiatica</i>	1	Lesioned	<i>C. cassiicola</i>
GU23	Guam	<i>Ipomoea batatas</i>	4	Healthy	<i>C. cassiicola</i>
GU25	Guam	<i>Buchnera floridana</i>	1	Lesioned	<i>C. cassiicola</i>
GU28	Guam	<i>Solanum lycopersicum</i>	4	Lesioned	<i>C. cassiicola</i>
GU32	Guam	<i>Euphorbia heterophylla</i>	3	Healthy	<i>C. cassiicola</i>
GU38	Guam	<i>Allamanda cathartica</i>	2	Lesioned	<i>C. cassiicola</i>
GU41	Guam	<i>Eugenia uniflora</i>	4	Healthy	<i>C. cassiicola</i>
GU42	Guam	<i>Bidens alba</i>	1	Lesioned	<i>C. cassiicola</i>
GU44	Guam	<i>Jatropha curcas</i>	4.1	Healthy	<i>C. cassiicola</i>
GU49	Guam	<i>Syzygium jambos</i>	4	Healthy	<i>C. cassiicola</i>

(continued on next page)

^a Isolate designation.^b Original host from which the isolate was collected.^c Phylogenetic lineage (PL) designation within *Corynespora cassiicola* based on combined sequence analysis of ribosomal DNA internal transcribed spacer region, *caa5*, *ga4*, and *act*. Five isolates were defined as outgroups (O); – indicates that the isolate's clade was not statistically significant.^d Description of host tissue prior to incubation.^e *Corynespora* spp. designation. 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 and morphology. They are labeled herein according to the original culture collection designations, though they should be reclassified as *C. cassiicola*. The remaining isolates were collected as part of this study or obtained from other researchers and are listed according to geographic location.^f Received from Empresa Brasileira de Pesquisa Agropecuária-Embrapa.^g Received from John Scott, Gulf Coast Research and Education Center, University of Florida.^h Received from David Ingram, Central Mississippi Research and Extension Center.ⁱ Received from Justin S. Clark, University of Tennessee.^j Received from Safiah Atan, Rubber Research Board.

TABLE 1. (continued from preceding page)

Isolate ^a	Geographic location	Host ^b	PL ^c	Host tissue ^d	<i>Corynespora</i> spp. ^e
GU51	Guam	<i>Ochrosia oppositifolia</i>	1	Healthy	<i>C. cassiicola</i>
GU55	Guam	<i>Calopogonium mucunoides</i>	3.2	Lesioned	<i>C. cassiicola</i>
GU65	Guam	<i>Passiflora foetida</i>	3.3	Healthy	<i>C. cassiicola</i>
GU68	Guam	<i>Moringa oleifera</i>	3	Healthy	<i>C. cassiicola</i>
GU70	Guam	<i>Solanum melongena</i>	1.4	Healthy	<i>C. cassiicola</i>
GU79	Guam	<i>Acanthus ilicifolius</i>	3	Healthy	<i>C. cassiicola</i>
GU83	Guam	<i>Euphorbia heterophylla</i>	4.1	Healthy	<i>C. cassiicola</i>
GU90	Guam	<i>Stachytarpheta jamaicensis</i>	1	Lesioned	<i>C. cassiicola</i>
GU92	Guam	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
GU93	Guam	<i>Capsicum annum</i>	1.3	Healthy	<i>C. cassiicola</i>
GU98	Guam	<i>Spathodea campanulata</i>	4	Lesioned	<i>C. cassiicola</i>
GU99	Guam	<i>Saintpaulia ionantha</i>	6	Lesioned	<i>C. cassiicola</i>
GU101	Guam	<i>Euphorbia milii</i>	4	Necrotic	<i>C. cassiicola</i>
GU102	Guam	<i>Phaseolus vulgaris</i>	4	Necrotic	<i>C. cassiicola</i>
GU103	Guam	<i>Pilea nummulariifolia</i>	2	Healthy	<i>C. cassiicola</i>
GU104	Guam	<i>Macroptilium atropurpureum</i>	1.3	Lesioned	<i>C. cassiicola</i>
GU107	Guam	<i>Mikania micrantha</i>	4	Lesioned	<i>C. cassiicola</i>
GU109	Guam	<i>Bauhinia galpinii</i>	3	Lesioned	<i>C. cassiicola</i>
GU110	Guam	<i>Plectranthus ambionicus</i>	3	Lesioned	<i>C. cassiicola</i>
GU111	Guam	<i>Manihot esculenta</i>	4.1	Healthy	<i>C. cassiicola</i>
GU112	Guam	<i>Glycine max</i>	3.3	Healthy	<i>C. cassiicola</i>
GU114	Guam	<i>Teramnus labialis</i>	3.1	Healthy	<i>C. cassiicola</i>
GU115	Guam	<i>Vitex parviflora</i>	1	Lesioned	<i>C. cassiicola</i>
GU120	Guam	<i>Plectranthus barbatus</i>	4	Lesioned	<i>C. cassiicola</i>
GU128	Guam	<i>Solanum lycopersicum</i>	4	Lesioned	<i>C. cassiicola</i>
GU136	Guam	<i>Ficus benjamani</i>	4.2	Healthy	<i>C. cassiicola</i>
HI01	Oahu, Hawaii, United States	<i>Ocimum basilicum</i>	1	Lesioned	<i>C. cassiicola</i>
MY CBPP ^j	Malaysia	<i>Hevea brasiliensis</i> , clone unknown	1.2	Lesioned	<i>C. cassiicola</i>
MY CLN16 ^j	Malaysia	<i>Hevea brasiliensis</i> RRIM2020	1.2	Lesioned	<i>C. cassiicola</i>
MY CSB12 ^j	Malaysia	<i>Hevea brasiliensis</i> RRIM 725	2	Lesioned	<i>C. cassiicola</i>
PH01	Pohnpei	<i>Carica papaya</i>	1.1	Healthy	<i>C. cassiicola</i>
PW01	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW12	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW17	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW20	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW25	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW27	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW34	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW37	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW38	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW43	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW48	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW53	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW56	Palau	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
PW57	Palau	<i>Solanum lycopersicum</i>	4	Lesioned	<i>C. cassiicola</i>
PW63	Palau	<i>Solanum lycopersicum</i>	4	Lesioned	<i>C. cassiicola</i>
PW69	Palau	<i>Piper betle</i>	2	Healthy	<i>C. cassiicola</i>
PW79	Palau	<i>Pilea microphylla</i>	2	Lesioned	<i>C. cassiicola</i>
PW80	Palau	<i>Episcia reptans</i>	1	Lesioned	<i>C. cassiicola</i>
PW83	Palau	<i>Episcia reptans</i>	1	Lesioned	<i>C. cassiicola</i>
PW87	Palau	<i>Cucumis sativus</i>	1	Lesioned	<i>C. cassiicola</i>
PW89	Palau	<i>Chromolaena odorata</i>	4	Healthy	<i>C. cassiicola</i>
PW91	Palau	<i>Luffa acutangula</i>	1	Healthy	<i>C. cassiicola</i>
PW92	Palau	<i>Catharanthus roseus</i>	1.3	Lesioned	<i>C. cassiicola</i>
PW94	Palau	<i>Stachytarpheta jamaicensis</i>	1	Lesioned	<i>C. cassiicola</i>
PW99	Palau	<i>Momordica charantia</i>	3	Lesioned	<i>C. cassiicola</i>
PW101	Palau	<i>Vigna unguiculata</i>	5	Necrotic	<i>C. cassiicola</i>
SN03	Saipan	<i>Momordica charantia</i>	1	Lesioned	<i>C. cassiicola</i>
SN05	Saipan	<i>Ipomoea batatas</i>	1	Lesioned	<i>C. cassiicola</i>
SN06	Saipan	<i>Luffa acutangula</i>	3.2	Healthy	<i>C. cassiicola</i>
SN07	Saipan	<i>Carica papaya</i>	1.1	Healthy	<i>C. cassiicola</i>
SN18	Saipan	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
SN24	Saipan	<i>Solanum lycopersicum</i>	4	Lesioned	<i>C. cassiicola</i>
SN27	Saipan	<i>Solanum lycopersicum</i>	4	Lesioned	<i>C. cassiicola</i>
SN30	Saipan	<i>Solanum lycopersicum</i>	4	Lesioned	<i>C. cassiicola</i>
SN37	Saipan	<i>Vigna unguiculata</i>	4	Necrotic	<i>C. cassiicola</i>
SN40	Saipan	<i>Cucumis sativus</i>	4	Lesioned	<i>C. cassiicola</i>
SN43	Saipan	<i>Saintpaulia ionantha</i>	3	Lesioned	<i>C. cassiicola</i>
SN48	Saipan	<i>Coccinia grandis</i>	3.3	Healthy	<i>C. cassiicola</i>
SN53	Saipan	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
SN59	Saipan	<i>Lantana camara</i>	1.5	Lesioned	<i>C. cassiicola</i>
SN64	Saipan	<i>Asystasia gangetica</i>	5.1	Lesioned	<i>C. cassiicola</i>
SN69	Saipan	<i>Pachystachys lutea</i>	3.1	Lesioned	<i>C. cassiicola</i>

(continued on next page)

Pathogenicity analyses. Of the 143 *Corynespora* isolates used in the phylogenetic study, 50 were selected for pathogenicity profiling against eight known economically important susceptible crop plants commonly grown in the survey regions (Table 2). Isolates were chosen from hosts used in the inoculation studies and to represent diverse PLs. A factorial experiment was arranged in a completely randomized design with four replications and with isolates and crop plants as factors. Each isolate was spray inoculated onto four replicates of susceptible plants: 8-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. The experiment was repeated twice, and the data from the two experiments were pooled for analysis. Data were analyzed by analysis of variance (ANOVA) and treatment means compared by Fisher’s least significant difference (FLSD, $P \leq 0.05$) with SAS (SAS Institute, Inc., Cary, NC).

To increase colony sporulation for inoculum preparation, aerial mycelium from 10-day-old V8 agar plates (100 mm) were gently scraped with a glass cover slip to flatten and then placed under constant cool-white fluorescent light (≈ 25 lux) (35). Three days later, the surface of the agar was scraped with a glass cover slip and the resulting mycelia were blended in 200 ml of sterile distilled water for 2 s and filtered through three layers of cheesecloth. Spore concentration was determined using a hemacytometer and adjusted to 20,000 spores/ml using sterile distilled water. One drop of Tween 20 per 100 ml was added to the inoculum. Plants preconditioned on a moisture bench under constant moisture conditions were sprayed with the spore suspension until runoff (≈ 500 ml), making sure that both leaf surfaces were fully covered. Plants were kept on a mist bench and rated for disease 7 days after inoculation using the disease rating system developed by Onesirosan et al. (33): 0 = symptomless: no lesions on leaves or stems; 1 = weakly virulent or hypersensitive response: a few to many nonexpanding pinpoint lesions; 2 = moderately virulent: many expanding lesions, some coalescing, but not resulting in blight; and 3 = highly virulent: lesions spreading to form large areas of dead tissue resulting in a blighting effect. Disease incidence (I) was recorded as the number of plants within a treatment with symptoms (a disease rating of ≥ 1). Disease severity (S) was calculated by averaging the disease rating of symptomatic plants within a treatment. For comparative purposes, a disease severity rating for each host was determined by averaging the severity ratings of all isolates that exhibited symptoms on that host.

Each isolate was assigned a pathogenicity profile (PP) based on the number of susceptible host species. A host species was considered susceptible if at least one of the replicates from the two experiments (total of eight plants) received a disease rating of 2 or 3. PPs were converted to a binary character matrix so that each isolate received either a zero or a one for each host. A zero was assigned to isolates that were nonpathogenic or weakly pathogenic on a given host (all replications with disease ratings of 0 or 1). A one was assigned to isolates that were pathogenic on a given

host (at least one replication with a disease rating of 2 or 3). Unweighted pair group method with arithmetic mean (UPGMA) trees were constructed from the binary matrix, and internal support for nodes were estimated using bootstrap analyses with 100,000 reps and an UPGMA algorithm (Fig. 1). The tree topology was visually compared with the PL designation of each isolate tested (Fig. 1). PPs were also visually mapped on the four-locus combined BI phylogenetic tree (Fig. 2).

Growth rate analyses. Growth rates of 77 isolates (selected to represent diverse hosts and geographic locations) were tested at 23 and 33°C, which are known extremes for the fungus (37). A factorial experiment was arranged in a completely randomized design with three replications. The factors studied were isolates of *C. cassiicola* and temperature. Small pieces of aerial mycelium were extracted from monospore cultures in long-term storage with tweezers and placed onto V8 agar plates. After 5 days, when the colonies had grown beyond their mineral oil, six 4-mm agar plugs were cut from the actively growing edge of each colony. Single plugs were transferred to the center of six V8 agar plates. Three replicate plates of each isolate were immediately placed in growth chambers at 23 and 33°C under 12 h of alternating fluorescent light (≈ 25 lux) and darkness. For each plate, the average of two colony diameters at 90° from each other was recorded at 48, 72, 96, 120, 144, and 166 h. Increase in colony diameter (mm) was plotted against time (h) and a line of best fit was generated for each replicate. Using SAS Statistical Software (version 8), the slope of the line of best fit ($R^2 > 0.98$) was determined and used to compare variation within replications with variation between isolates. The experiment was repeated with five randomly selected isolates to assess repeatability. Data were analyzed by ANOVA and treatment means compared by FLSD ($P \leq 0.05$) with SAS. For comparison purposes, treatment means were pooled according to host, geographic location, and PL and their means and standard errors were calculated and plotted (Fig. 3).

RESULTS

Phylogenetic analyses. Sequence are available in GenBank (accession numbers rDNA ITS: FJ852574 to FJ852716; GA4: FJ852717 to FJ852859; CAA5: FJ852860 to FJ853002; and FJ853003 to FJ853141). The final combined data set used for analysis contained 2,136 aligned characters (Table 3). ILD tests of the combined matrix showed no significant difference in the phylogenetic signal of the four regions (rDNA ITS versus *caa5* versus *ga4* versus *act*, $P = 0.983$). The general time reversible (GTR) model incorporating a proportion of invariable sites and a distribution of rates at variable sites modeled on a discrete gamma distribution (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 data set was partitioned. The first 25% of the total number of generations was discarded as burn-in. Tree topologies resulting from NJ, MP, and BI analyses recovered essentially the same well-supported nodes. The analyses revealed six PLs with high statistical support (BS value > 70 and PoPr value > 95) (Fig. 2).

TABLE 1. (continued from preceding page)

Isolate ^a	Geographic location	Host ^b	PL ^c	Host tissue ^d	<i>Corynespora</i> spp. ^e
YP01	Yap	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
YP08	Yap	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
YP17	Yap	<i>Carica papaya</i>	1.1	Lesioned	<i>C. cassiicola</i>
YP26	Yap	<i>Cucumis sativus</i>	1	Lesioned	<i>C. cassiicola</i>
YP27	Yap	<i>Cucumis sativus</i>	2	Lesioned	<i>C. cassiicola</i>
YP29	Yap	<i>Cucumis sativus</i>	1	Lesioned	<i>C. cassiicola</i>
YP41	Yap	<i>Saintpaulia ionantha</i>	2	Lesioned	<i>C. cassiicola</i>
YP42	Yap	<i>Solanum lycopersicum</i>	3	Lesioned	<i>C. cassiicola</i>
YP51	Yap	<i>Vigna unguiculata</i>	1	Necrotic	<i>C. cassiicola</i>
YP59	Yap	<i>Ipomoea batatas</i>	2	Healthy	<i>C. cassiicola</i>

The rDNA ITS alignment was composed of 1,013 characters, 400 of which are an insertion in the outgroup taxon *C. smithii*. Of the 612 remaining characters, 141 were variable and 107 were informative. The taxa from the NIAS culture collection, *C. sesamum* 305095, *C. citricola* 425231, and *C. melongenae* 712045, appear to be misidentified and are, in fact, *C. cassiicola*, based on 100% identity in rDNA ITS sequences and similar morphology. When the outgroup taxa *C. citricola*, *C. olivacea*, *C. proliferata*, and *C. smithii* were removed from the analyses, the rDNA ITS sequences of the *C. cassiicola* isolates contained only 3 informative characters out of 584 bases. Two of these characters

(base pairs 158 and 497) separate the isolates into three distinct haplotypes—CA, CG, and TG (no haplotype TA)—that are congruent with the PLs in the four-locus combined analysis. All isolates with haplotype CA are in PL4; all isolates with haplotype TG are in PL1; and isolates with haplotype CG are in PLs 2, 3, 5, and 6. CG is likely the ancestral haplotype, present in all outgroups except for *C. proliferata* (which is haplotype CA). The third informative character in the rDNA ITS sequence alignment 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 (base pair 123) that caused the

TABLE 2. Pathogenicity profiles for 50 isolates of *Corynespora cassiicola*^a

Path Pro ^b	PL ^c	Isolate ^d	H ^e	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	1.0	2	1.0
BaCoCu	1.3	AS58	Co	6	2.3	0	—	3	2.3	6	3.0	0	—	0	—	0	—	7	1.0
BaCoCu	1	YP29	Cu	7	2.1	0	—	3	1.7	8	3.0	0	—	0	—	0	—	0	—
BaCoCu	1	AS71	Pu	8	2.1	0	—	2	2.0	7	1.3	0	—	1	1.0	0	—	2	1.0
BaCoCuSo	1	PW87	Cu	5	2.2	0	—	5	1.6	8	2.5	0	—	2	1.5	0	—	7	1.0
BaCu	1	HI01	Ba	4	2.5	0	—	0	—	7	3.0	0	—	0	—	1	1.0	8	1.0
BaCu	1	SN05	Sw	7	1.9	1	1.0	0	—	6	2.7	0	—	0	—	0	—	2	1.0
BaCuTo	3	AS50	To	5	2.2	0	—	2	1.0	6	2.7	0	—	1	1.0	2	1.0	8	3.0
BaCuTo	3	YP42	To	6	2.3	0	—	0	—	7	2.6	0	—	0	—	0	—	8	2.6
BaCuTo	3.2	AS80	Ba	5	1.8	0	—	0	—	8	2.5	0	—	1	1.0	0	—	8	2.8
BaCuTo	3.2	AS117	Pa	7	1.9	0	—	2	1.0	8	2.8	0	—	2	1.0	8	1.0	7	2.9
BeCoCuSw	4	SN37	Co	7	1.0	5	1.6	5	1.4	8	2.8	0	—	2	1.0	3	1.3	8	1.0
BeCuSwTo	4	JMP217	To	5	1.0	7	1.9	0	—	8	2.9	0	—	0	—	1	2.0	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	2.0	8	2.6
BeCuTo	4	PW57	To	0	—	6	1.7	0	—	8	2.1	0	—	2	1.0	0	—	8	3.0
CoCu	1	AS98	Cu	0	—	5	1.0	6	1.8	8	2.6	0	—	0	—	0	—	7	1.0
CoCu	1	YP26	Cu	0	—	7	1.0	4	1.5	7	2.4	0	—	0	—	8	1.0	8	1.0
CoCu	1	JMP218	So	0	—	0	—	7	2.1	8	3.0	0	—	0	—	7	1.0	7	1.0
CoCu	1	GU08	La	0	—	0	—	3	1.7	7	3.0	0	—	0	—	0	—	8	1.0
Cu	1	AS54	Co	0	—	1	1.0	0	—	8	3.0	0	—	1	1.0	8	1.0	1	1.0
Cu	1	YP51	Co	0	—	0	—	0	—	8	3.0	0	—	0	—	7	1.0	1	1.0
CuPa	1.1	DOA16b	Pa	7	1.0	0	—	0	—	8	1.1	8	2.3	0	—	8	1.0	1	1.0
CuPa	1.1	FL11	Pa	0	—	5	1.0	0	—	8	1.3	7	2.6	0	—	0	—	8	1.0
CuPa	1.1	PH01	Pa	1	1.0	1	1.0	0	—	7	1.1	6	1.8	0	—	0	—	8	1.0
CuSo	3.3	GU112	So	0	—	0	—	5	1.0	8	2.8	0	—	1	2.0	1	1.0	2	1.0
CuSw	2	YP27	Cu	1	1.0	0	—	2	1.0	6	2.8	0	—	0	—	5	1.4	2	1.0
CuSw	2	YP59	Sw	2	1.0	0	—	1	1.0	7	2.6	0	—	0	—	6	1.4	7	1.0
CuSw	1.5	SN59	La	1	1.0	5	1.0	0	—	8	2.8	0	—	0	—	7	1.3	7	1.0
CuSwTo	4	PW63	To	4	1.0	0	—	7	1.0	8	2.6	0	—	0	—	1	2.0	8	3.0
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	—	0	—	8	3.0	0	—	0	—	5	1.2	8	2.9
CuSwTo	4	GU23	Sw	0	—	0	—	7	1.0	8	2.4	0	—	0	—	5	1.8	8	2.9
CuSwTo	4	FL09	La	0	—	1	1.0	0	—	8	1.4	0	—	2	1.0	3	1.3	8	2.8
CuTo	3	AS49	To	0	—	0	—	0	—	4	2.8	0	—	0	—	1	1.0	7	2.6
CuTo	3.1	AS119	Pu	0	—	0	—	0	—	8	2.3	0	—	1	1.0	7	1.0	8	2.5
CuTo	4	FL12	To	0	—	5	1.0	5	1.0	8	3.0	0	—	2	1.0	1	1.0	8	3.0
CuTo	4	FL2920	To	4	1.0	0	—	0	—	8	2.0	0	—	0	—	0	—	8	2.6
CuTo	4	MS31	To	0	—	7	1.0	8	1.0	8	2.8	0	—	0	—	1	1.0	8	2.3
CuTo	4	GU128	To	0	—	1	1.0	0	—	8	2.9	0	—	2	1.0	1	1.0	8	2.5
CuTo	4	SN30	To	0	—	0	—	0	—	8	3.0	0	—	2	1.0	2	1.0	8	3.0
CuTo	4	AS92	Cu	2	1.0	1	1.0	0	—	8	2.8	0	—	0	—	1	1.0	8	2.9
CuTo	4	JMP216a	La	7	1.0	0	—	0	—	7	1.3	0	—	0	—	2	1.0	8	3.0
CuTo	5	PW101	Co	0	—	0	—	4	1.0	7	2.9	0	—	0	—	0	—	7	1.3
Pa	1.1	GU92	Pa	2	1.0	0	—	0	—	1	1.0	7	2.1	0	—	1	1.0	7	1.0
Pa	1.1	PW01	Pa	0	—	0	—	0	—	8	1.0	8	2.4	0	—	0	—	8	1.0
Pa	1.1	PW12	Pa	0	—	0	—	1	1.0	1	1.0	4	1.4	2	1.0	0	—	1	1.0
Pa	1.1	SN07	Pa	1	1.0	0	—	0	—	8	1.0	6	2.3	0	—	1	1.0	1	1.0
Pa	1.1	YP01	Pa	0	—	7	1.0	0	—	1	1.0	8	1.9	0	—	0	—	8	1.0
To	4	GU28	To	0	—	0	—	0	—	8	1.0	0	—	0	—	1	1.0	8	3.0

^a Incidence (I): number of plants within a treatment of eight plants that showed symptoms 7 days after inoculation with 20,000 *C. cassiicola* spores per milliliter and severity (S): average disease rating of all the symptomatic plants. Disease rating: 0 = symptomless; 1 = nonpathogenic or weakly virulent, a few to many nonexpanding 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; and — indicates no symptomatic plants out of eight replicates.

^b Pathogenicity profile (Path Pro): list of susceptible hosts (all plants with a disease rating >1).

^c Phylogenetic lineage (PL) designation based on combined sequence analysis of ribosomal DNA internal transcribed spacer region, *caa5*, *ga4*, and *act*.

^d *C. cassiicola* isolate designation.

^e Original host (H) from which the isolate was collected: Ba (basil), Be (bean), Co (cowpea), Cu (cucumber), La (lantana), Pa (papaya), Pu (pumpkin), Sw (sweet potato), and To (tomato).

polymorphic band pattern observed by Atan and Hamid (1) in their RFLP analysis of the rDNA ITS region of rubber isolates using *Hae*III (recognition sequence GGCC).

All PLs contain isolates from diverse geographic locations, indicating wide dispersal. Within PL1 (Fig. 2), a distinct clade with high statistical support (designated PL1.1) contains 23 isolates from papaya from diverse geographic locations. Pathogenicity studies with eight of these isolates support specificity to papaya. PL1.2 contains two isolates from *Stachytarpheta jamaicensis* collected from Guam and Palau. The genetic similarity of these isolates is supported by pathogenicity studies indicating host specificity to *S. jamaicensis* (51). Isolates from diverse hosts are present in PL1, including crops (basil, *Momordica charantia* [bitter melon], eggplant, cowpea, cucumber, oregano, papaya, *Cucurbita pepo* [pumpkin], rubber, soybean, sweet potato, and *Citrullus vulgaris* [watermelon]), ornamentals (*Buddleja asiatica*, *Catharanthus roseus*, *Codiaeum variegatum*, *Plectranthus barbatus*, and *Tabebuia pallida*), and weeds (*Bidens alba*, *Buchnera floridana*, *Clerodendrum paniculatum*, *Commelina benghalensis*, lantana, *Macroptilium atropurpureum*, *Ochrosia oppositifolia*, and *Vitex parviflora*). Missing from PL1 are isolates obtained from or that are pathogenic to tomato (Fig. 2; Table 2).

The nine isolates in PL2 are also globally distributed and include crops (cucumber, rubber, and sweet potato), ornamentals (African violet, *Allamanda cathartica*, *Catharanthus roseus*, and *Pilea nummulariifolia*), and weeds (*Piper betle* and *Pilea microphylla*). As in the case of PL1, there is a lack of isolates obtained from or pathogenic to tomato. In addition, only PLs 1 and 2 contain isolates from rubber.

Twenty-one globally distributed isolates are in PL3 and were collected from crops (basil, cucumber, pumpkin, bitter melon, soybean, and tomato), ornamentals (African violet, *Bauhinia galpinii*, *Moringa oleifera*, *Pachystachys lutea*, and *Plectranthus ambionicus*), and weeds (*Acanthus ilicifolius*, *Asystasia gangetica*, *Calopogonium mucunoides*, *Coccinia grandis*, *Euphorbia heterophylla*, *Luffa acutangula*, *Passiflora foetida*, and *Teramnus labialis*). These are hosts that may harbor isolates pathogenic to tomato. PL5 and PL6 group with PL3 with low support (MP BS value of 60). PL5 is highly supported and contains isolates collected from *Plectranthus barbatus* (Brazil), cowpea (Palau), and *Asystasia gangetica* (Saipan). PL6 contains *Corynespora cassiicola* isolates from African violet in Guam and Tennessee that are very similar in sequence, especially at the *caa5* locus. Given the low level of sampling on African violet, it is impossible to differentiate between specialization on this host and plants that may have originated from the same horticultural source. African violet isolates from Saipan and Yap are found in PL3.

The majority of tomato isolates group in PL4 and are from diverse geographic locations, including American Samoa, Brazil, Florida, Guam, Mississippi, Palau, and Saipan. These 11 tomato isolates group with 25 isolates from crops (bean, *Manihot esculenta* [cassava], cucumber, and sweet potato), ornamentals (*Bauhinia galpinii*, *Cassia fistula*, *Plectranthus barbatus*, *Eugenia uniflora*, *Ficus benjamini*, *Jatropha curcas*, *Salvia farinacea*, and *Syzygium jambos*), and common weeds (*Calopogonium mucunoides*, *Calypocarpus vialis*, *Chromolaena odorata*, *Euphorbia milii*, *E. heterophylla*, *Hyptis suaveolens*, lantana, *Mikania micrantha*, and *Spathodea campanulata*), which are likely inoculum sources for the initiation of disease on tomato.

The sister relationships between the PLs remain unsupported in the individual and combined locus analyses. In addition, the rDNA ITS region was the only locus that showed good support for *Corynespora citricola*, *C. olivacea*, *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. olivacea*, *C. proliferata*, and *C. smithii* as basal to PLs 1, 2, 3, 5, and 6, but PL4 and *C. citricola*

fell basal to that group. The apparent paraphyly of *C. cassiicola* at the *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 PLs are needed in future phylogenetic analyses.

Pathogenicity analyses. As a result of screening 50 isolates for pathogenicity on eight index hosts, 16 unique PPs were found (Table 2). There were no statistically significant variations in pathogenicity when the experiment was repeated ($P = 0.963$); therefore, the data were combined. The null hypotheses of no pathogenicity differences among isolates, PL, and their interactions were rejected ($P < 0.001$), while the null hypothesis of no pathogenicity differences among replicates was accepted with a probability of 0.154. The most common PP was CuTo, followed by Pa and CuSwTo (Table 2). Cucumber was the most susceptible host, with all isolates producing symptoms and a disease severity rating of 2.3. Tomato was also highly susceptible, with 49 of 50 isolates showing symptoms with a disease severity rating of 1.8. All isolates pathogenic on papaya were originally isolated from papaya and were highly virulent to papaya (disease severity rating = 2.1). Isolates pathogenic to basil, bean, cowpea, soybean, and sweet potato were less virulent on these hosts, with disease severity ratings < 1.5 .

There was a strong correlation between PP and PL ($P < 0.001$), (Figs. 1 and 2). Of 10 CuTo isolates, 7 were from PL4 and this lineage contained all CuSwTo and BeCuSwTo isolates. In PL4, all isolates but SN37 were highly virulent on tomato (disease severity ratings of 2.5 to 3) and all isolates but GU28 were pathogenic to cucumber (disease severity ratings of 1.3 to 3). In

Fig. 1. Unweighted pair group method with arithmetic mean (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), and 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 100,000 UPGMA bootstrap repetitions is indicated.

(Continued on next page)

Fig. 2. Majority rule (50%) consensus tree phylogram from Bayesian inference analysis of combined data from ribosomal DNA internal transcribed spacer (rDNA ITS), *ga4*, *caa5*, and *act1* sequences. Numbers above branches indicate maximum parsimony bootstrap > 70% and numbers below branches indicate posterior probability values > 0.90. Phylogenetic lineage (PL) indicates clades of high support. The branch length for outgroups *Corynespora olivacea* and *C. proliferata* was shortened in half to increase readability. Of the total 143 isolates, 50 were spray inoculated on eight crop plants to determine pathogenicity profiles. rDNA ITS haplotype designation is based on two mutations at base pairs 158 and 278 in a 584 base pair rDNA ITS alignment with only *C. cassiicola* taxa represented (no outgroups).

Four of the six isolates also were pathogenic to basil and all BaCuTo isolates were from PL3 (Fig. 1).

All isolates from PL2 had pathogenicity profile CuSw and this profile was unique to isolates from PLs 2 and 1.5 (Fig. 1). In addition, isolates collected in the field from papaya in PL1.1 were specific to papaya in pathogenicity studies, although these isolates

were weakly virulent on cucumber, with disease severity ratings of ≤ 1.3 (Table 2). Excluding PL1.1, all isolates from PL1 were pathogenic to cucumber (disease severity ratings of 1.3 to 3), 9 of 13 were pathogenic to cowpea, and 7 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. The only isolate tested from PL5 (PW101) was weakly pathogenic on the host from which it was isolated (cowpea) but was virulent to cucumber and tomato.

Growth rate analyses. The null hypotheses of no growth rate differences among isolates, PL, temperatures, and their interactions were rejected ($P < 0.001$), while the null hypothesis of no growth rate differences among repetitions was accepted with a probability of 0.755 (Fig. 3). There were no statistically significant variations in growth rate for the five randomly selected isolates in which the growth analyses were repeated ($P < 0.001$). Apart from isolate PH01, all isolates grew faster at 23 than at 33°C. At 23°C, isolate growth rate ranged from 0.148 to 0.474, with an overall mean of 0.387 ± 0.048 . At 33°C, isolate growth rate ranged from 0.138 to 0.415, with an overall mean of 0.296 ± 0.07 . At 23°C, there were 29 significantly different growth rates and, at 33°C there were 39 significantly different growth rates, and it appears that there is a continuum in growth rates within the species. Among the fastest growing isolates at both temperatures were FL37, GU99, GU90, AS71, AS67, HI01, SN03, and SN37 and the slowest growing were GU120, JMP216a, JMP217, and AS117. Of note was the fact that PH01, the slowest growing

isolate at 23°C, was ranked 36th at 33°C, and AS119, the slowest growing isolate at 33°C, was ranked 13th at 23°C.

Growth rate was correlated with temperature, isolate, geographic location, PL, and host and all interactions were significant ($P < 0.001$). Isolates from the host genera *Clerodendrum*, *Stachytarpheta*, and *Macroptilium* were among the fastest growing at both temperatures (Fig. 3A). In addition, isolates from the host genera *Salvia*, *Eugenia*, *Plectranthus*, and *Allamanda* were among the slowest growing at both temperatures. Isolates from the host genera *Cucurbita*, *Pilea*, *Spathodea*, *Phaseolus*, and *Carica* grew proportionally faster at 33°C when compared with isolates from other genera, indicating a greater tolerance to warmer temperatures. Isolates from the host genera *Plectranthus*, *Allamanda*, *Solanum*, *Bauhinia*, and *Tabebuia* grew proportionally slower at 33°C compared with isolates from other genera, indicating a lesser tolerance to warmer temperatures. Isolates collected from American Samoa and Hawaii grew fastest at 23°C (Fig. 3B). Isolates from Pohnpei, Brazil, Florida, and Malaysia had slower growth rates at both temperatures. Isolates collected from American Samoa, Hawaii, and Malaysia grew proportionally much faster at 23 than at 33°C, which may be a result of collecting at higher elevations where temperatures are cooler. Isolates from PLs 6 and 1 grew fastest at both temperatures (Fig. 3C). Isolates collected from papaya in PL1.1 grew proportionally faster at 33 compared with 23°C, indicating a higher tolerance to warmer temperatures. Isolates from PLs 2 and 4 grew slowest at 23°C and isolates from PLs 3 and 5 grew slowest at 33°C.

Fig. 3. Growth rate of *Corynespora cassiicola* isolates at 23 and 33°C from different **A**, host genera; **B**, geographic locations; and **C**, phylogenetic lineages (PLs). Bars represent $\pm$ one standard deviation.

Interestingly, isolates from PLs 3 and 5 group together with low bootstrap support (MP BS value of 67) and have similar growth rates at both temperatures.

DISCUSSION

The current study presents the first robust phylogeny using globally distributed *C. cassiicola* isolates. Based on sequence data from four loci, there is evidence for high genetic diversity within the species. This genetic diversity likely arose from accumulated mutations in clonal lineages based on the congruence of the phylogenetic trees from all four loci. All loci distinguish six clonal lineages within *C. cassiicola* and identical haplotypes are widely distributed geographically. The low level of sequence variation at the rDNA ITS region within the species relative to the outgroup *Corynespora* spp. suggests that these lineages are clonal populations rather than taxonomically distinct species (25).

The lack of correlation between phylogenetic data and geographic location provides evidence for recent global movement of isolates from all six PLs. In addition, geographically diverse isolates from the same host plant often share identical haplotypes, potentially indicating a degree of host specialization. For example, isolates collected from tomato in Brazil, Florida, Guam, Mississippi, Palau, and Saipan have identical haplotypes at all four loci. Although unlikely, the possibility remains that the fungus was distributed on tomato seed from the same source. Isolates collected from lantana in Florida and Brazil are also identical at all four loci, possibly indicating host specialization. Isolates collected from African violet in Guam and Tennessee are unique from all other isolates and nearly identical to each other at the loci sequenced. Isolates from different *Pilea* spp. in Guam and Palau are both in PL2. Given additional sequencing at loci with more resolution, isolates from the same host in different geographic locations may prove to be unique, indicating host specialization or recent global movement of plant material from a similar source. The most compelling evidence for host specialization is the host specificity of papaya isolates in the inoculation studies and the identical nucleotide sequences of geographically diverse papaya isolates (Fig. 2) (11). In addition, only isolates collected from papaya were found to be pathogenic on papaya.

Tomato isolates from diverse geographic locations are found only in PL3 and PL4 and only isolates from these lineages were pathogenic to tomato. Though there were no isolates collected from tomato 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 isolates may have lost the ability to cause disease on tomato either by mutation in the pathogen or acquired resistance in the host. Isolates from other hosts in PL3 and PL4 were also pathogenic to tomato in inoculation studies and, therefore, may serve as source hosts for target spot of tomato in the field. These hosts include common crops (basil, bean, bitter melon, cassava, cucumber, papaya, pumpkin, soybean, and sweet potato), weeds (*Acanthus ilicifolius*, *Calopogonium mucunoides*, *Calyptocarpus vialis*, *Chromolaena odorata*, *Coccinia grandis*, *Euphorbia heterophylla*, lantana, *Macroptilium atropurpureum*, *Mikania micrantha*, *Momordica charantia*, *Passiflora foetida*, and *Teramnus labialis*), and ornamentals (African violet, *Asystasia gangetica*, *Bauhinia galpinii*, *Cassia fistula*, *Plectranthus barbatus*, *Eugenia uniflora*, *Euphorbia milii*, *Ficus benjamina*, *Hyptis suaveolens*, *Jatropha curcas*, lantana, *Luffa acutangula*, *Moringa oleifera*, *Pachystachys lutea*, *Plectranthus ambonicus*, *Salvia farinacea*, *Spathodea campanulata*, and *Syzygium jambos*). Based strictly on these data, control of target spot on tomato should involve isolation of tomato fields from these plant species when possible. Our inoculation methods were chosen to maximize consistency of results; however, disease incidence and severity values may be inflated compared with those that occur under natural field conditions.

There are at least 16 unique pathogenicity profiles within *Corynespora cassiicola* on the eight crop plants that were tested, and isolates from the same lineages show similar but not identical profiles (Figs. 1 and 2). Hosts susceptible to isolates from only two of the six lineages included basil, cowpea, sweet potato, and tomato. Isolates pathogenic to bean are only in PL4. Interestingly, the majority of isolates, excluding the isolates collected from papaya, were pathogenic on cucumber. These pathogenicity data are similar to those obtained by Onesirosan et al. (33) using 18 *C. cassiicola* isolates from Nigeria, the southern United States, and Mexico in that both studies found isolates specific to papaya and cucumber. Likewise, both studies found that isolates pathogenic to tomato were likely to be pathogenic to several other hosts. The number of isolates screened compared with the number of unique pathogenicity profiles in both studies indicates that gains and losses of pathogenicity are common.

Though our phylogenetic data do not correlate with geography, growth rate experiments of ours and others provide evidence for adaptability of isolates to tropical and temperate environments. Using an isolate collected from tomato in Florida, Pernezny et al. (37) found the best colony growth occurred at 32°C, whereas Sobers (52) reported an optimum growth rate at 24°C for Florida isolates collected from hydrangea and azalea. Jones and Jones (23) report higher disease severity on tomato inoculated and maintained at temperatures of 20 to 23°C. In this study, two temperature extremes (23 and 33°C) were chosen in an attempt to discern between isolates adapted to temperate and tropical climates. Though the majority of isolates were collected from tropical climates, all isolates but one (PH01) grew faster at 23 than at 33°C. Though only two temperatures were tested, the ideal temperature for colony growth is likely to be very near 23°C based on the fact that the majority of isolates grew better at 23 than at 33°C, and the standard error of all isolates at 23°C was ± 0.048 compared with ± 0.07 for 33°C. Growth rate did, however, correlate with PL ($P < 0.001$). Results indicate that isolates from PL1.1, PL4, and PL2 may be more adapted to warmer temperatures and isolates from PL5 and PL3 may be more adapted to cooler temperatures. Physiological traits that correlate with phylogenetic data, such as growth rate and pathogenicity profile, may be useful for isolate classification when further characterized.

The description of the original host tissue from which isolates were obtained in the field was useful in further understanding the diversity of trophic capabilities within *C. cassiicola*. Isolates collected from lesioned tissue were pathogenic to their original host 29 of 30 times. Interestingly, all isolates collected from healthy tissue were pathogenic to the original host of isolation, pointing toward the occurrence of latent infections in nature. AS54 (cowpea), AS58 (cowpea), AS117 (papaya fruit), and YP51

TABLE 3. Summational sequence data from four loci used to confirm the phylogenetic lineage of *Corynespora cassiicola* isolates

Locus	Total	Variable	Informative	Tree score	No. of trees ^a
Combined ^b	2,136	248	174	330	7,430
rDNA ITS	1,013	135	100	158	9,990
rDNA ITS ^c	584	4	3	4	1
ga4	414	31	25	40	9,530
ga4 ^c	414	28	25	36	9,560
caa5	366	38	34	52	40
caa5 ^c	366	37	32	46	12
act1	343	44	15	49	11
act1 ^c	343	16	15	17	4

^a Number of maximum parsimony trees.

^b Combined loci: ribosomal DNA internal transcribed spacer region (rDNA ITS), *ga4*, *caa5*, and *act1*.

^c Locus analyzed with only *Corynespora cassiicola* taxa represented (no outgroups).

(cowpea), which were all collected from necrotic tissue, were nonpathogenic when inoculated on their original hosts, indicating saprophytic growth in the field. Based on these data, it is likely that individual isolates have the ability to be pathogens, endotrophs, and saprotrophs on different hosts. In addition, a given host likely harbors pathogenic, endotrophic, and saprotrophic isolates of *C. cassiicola*.

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 based on two mutations at base pairs 158 and 278: PL1 (haplotype TG); PL4 (haplotype CA); and PLs 2, 3, 5, and 6 (haplotype CG). These haplotypes correlate with phylogenetic data from the combined four-locus data set (Fig. 2). 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 PLs 3 and 4 (rDNA ITS haplotypes CG and CA) should be used to screen for resistance to target spot in tomato. In addition, genotyping by restriction digest of the amplified rDNA 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 identified isolates with the same unique genotype found in Atan and Hamid's (1) RFLP analysis of the rDNA ITS region using *HaeIII*. However, only 4 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.

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 associated diseases. Despite evidence for host specificity on papaya, 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. Although informative, it fails to explain why *C. cassiicola* is rare on all cultivated crops in Hawaii except basil or why, on Guam, outbreaks of target on cucumber are sporadic. Finally, further investigations are needed to determine the role played by the fungus's endotrophic and saprotrophic capabilities within its associated disease cycles.

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LITERATURE CITED

- Atan, S., and Hamid, N. H. 2003. Differentiating races of *Corynespora cassiicola* using RAPD and internal transcribed spacer markers. *J. Rubber Res.* 6:58-64.
- Blazquez, C. H. 1972. Target spot of tomato. *Plant Dis. Rep.* 56:243-245.
- Boosalis, M. G., and Hamilton, R. I. 1957. Root and stem rot of soybean caused by *Corynespora cassiicola*. *Plant Dis. Rep.* 41:696-698.
- Carbone, I., and Kohn, L. M. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91:553-556.
- Carris, L. M., and Glawe, D. A. 1986. Isolation of the soybean pathogens *Corynespora cassiicola* and *Phialophora gregata* from cysts of *Heterodera glycines* in Illinois. *Mycologia* 78:503-506.
- Chase, A. R. 1982. *Corynespora* leaf spot of *Aeschynanthus pulcher* and related plants. *Plant Dis.* 66:739-740.
- Chase, A. R. 1986. *Corynespora* bract spot of *Euphorbia pulcherrima* in Florida. *Plant Dis.* 70:1074.
- Chase, A. R. 1987. Pages 22-23 in: *Compendium of Ornamental Foliage Plant Diseases*. American Phytopathological Society, St. Paul, MN.
- Chase, A. R. 1993. *Corynespora* leaf spot and stem rot of *Salvias*. *CFREC-Apopka Res. Rep.* RH-93-12.
- Collado, J., Platas, G., Gonzalez, I., and Pelaez, F. 1999. Geographical and seasonal influences on the distribution of fungal endotrophs in *Quercus ilex*. *New Phytol.* 144:525-532.
- Couch, B. C., and Kohn, L. M. 2002. A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia* 94:683-693.
- Cutrim, F. A., and Silva, G. S. 2003. Pathogenicity of *Corynespora cassiicola* to different plant species. *Fitopatol. Brasil.* 28:193-194.
- Ellis, M. B., and Holiday, P. 1971. *Corynespora cassiicola* (Berk. & Curt.) Wei. Commonwealth Mycological Institute Descriptions of Fungi and Bacteria No. 31, Sheet 303.
- Felsenstein, J. 1985. Confidence limit on phylogenies: an approach using the bootstrap. *Int. J. Org. Evol.* 39:783-791.
- Furukawa, T., Ushiyama, K., and Kishi, K. 2008. *Corynespora* leaf spot of scarlet sage caused by *Corynespora cassiicola*. *J. Gen. Plant Pathol.* 74:117-119.
- Gond, S. K., Verma, V. C., Kumar, A., Kumar, V., and Kharwar, R. N. 2007. Study of endotrophic fungal community from different parts of *Aegle marmelos* Corraeae (*Rutaceae*) from Varanasi (India). *World J. Microbiol. Biotechnol.* 23:1371-1375.
- Hongn, S., Ramallo, A., Bairo, O., and Ramallo, J. C. 2007. First report of target spot of *Vaccinium corymbosum* caused by *Corynespora cassiicola*. *Plant Dis.* 91:771.
- Huelsenbeck, J. P., and Ronquist, F. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17:754-755.
- Hyde, K. D., McKenzie, E. H. C., and Dalisay, T. U. 2001. Saprobic fungi on bamboo culms. *Fungal Divers.* 7:35-48.
- Isabel, N., Beaulieu, J., Theriault, P., and Bousquet, J. 1999. Direct evidence for biased gene diversity estimates from dominant random amplified polymorphic DNA (RAPD) fingerprints. *Mol. Ecol.* 8:477-483.
- Jayasuriya, K. E., and Thennakoon, B. I. 2007. First Report of *Corynespora cassiicola* on *Codiaeum variegatum* (Croton) in Sri Lanka. *Ceylon J. Sci. Biol. Sci.* 36:138-141.
- Jones, J. P. 1961. A leaf spot of cotton caused by *Corynespora cassiicola*. *Phytopathology* 51:305-308.
- Jones, J. P., and Jones, J. B. 1984. Target spot of tomato: epidemiology, and control. *Proc. Fla. State Hort. Soc.* 97:216-218.
- Kingsland, G. C. 1985. Pathogenicity and epidemiology of *Corynespora cassiicola* in the Republic of the Seychelles. *Acta. Hort.* (ISHS) 153:229-230.
- Kohn, L. M. 2005. Mechanisms of fungal speciation. *Annu. Rev. Phytopathol.* 43:279-308.
- Lee, S., Melnik, V., Taylor, J. E., and Crous, P. W. 2004. Diversity of saprobic hyphomycetes on *Proteaceae* and *Restionaceae* from South Africa. *Fungal Divers.* 17:91-114.
- Lumyong, P., Photita, W., McKenzie, E. H. C., Hyde, K. D., and Lumyong, S. 2003. Saprobic fungi on dead wild banana. *Mycotaxon* 85:345-346.
- Maddison, D. R., and Maddison, W. P. 2005. MacClade 4, version 4.08. Sinauer Associates, Sunderland, MA.
- Mahgoub, E. S. 1969. *Corynespora cassiicola*, new agent of maduro-mycetoma. *J. Trop. Med. Hyg.* 1069:217-212.
- Mathiyazhagan, S., Kavitha, K., Nakkeeran, S., Chandrasekar, G., Manian, K., Renukadevi, P., Krishnamoorthy, A. S., and Fernando, W. G. D. 2004. PGPR mediated management of stem blight of *Phyllanthus amarus* (Schum and Thonn) caused by *Corynespora cassiicola* (Berk and Curt) Wei. *Arch. Phytopathol. Plant Prot.* 37:183-199.
- McGovern, R. J. 1994. Target spot of *Catharanthus roseus* caused by *Corynespora cassiicola*. *Plant Dis.* 78:830.
- Olive, L. S., and Bain, D. C. 1945. A leafspot of cowpea and soybean caused by an undescribed species of *Helminthosporium*. *Phytopathology* 35:822-831.
- Onesirosan, P. T., Army, D. C., and Durbin, R. D. 1973. Target spot of tomato incited by *Corynespora cassiicola* (Berk. & Curt.) Wei. Ph.D. dissertation, University of Wisconsin, Madison.
- Onesirosan, P. T., Army, D. C., and Durbin, R. D. 1974. Host specificity of Nigerian and North American isolates of *Corynespora cassiicola*. *Phytopathology* 64:1364-1367.
- Onesirosan, P. T., Army, D. C., and Durbin, R. D. 1975. Increasing sporulation of *Corynespora cassiicola*. *Mycopathologia* 55:121-123.
- Pereira, J. M., Barreto, R. W., Ellison, C. A., and Maffia, L. A. 2003. *Corynespora cassiicola* f. sp. *lantanae*: a potential biocontrol agent from Brazil for *Lantana camara*. *Biol. Control* 26:21-31.
- Pernezny, K., Datnoff, L. E., Rutherford, B., and Carroll, A. 2000. Relationship of temperature to growth, sporulation, and infection of tomato by the target spot fungus. Pages 16-19 in: *Florida Tomato Committee Tomato Research Report for 2000*.

38. Poltronieri, L. S., Duarte, M. L. R., Alfenas, A. C., Trindade, D. R., and Albuquerque, F. C. 2003. Three new pathogens infecting Antilles cherry in the state of Para. *Fitopatol. Brasil.* 28:424-426.
39. Posada, D., and Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817-818.
40. Promputtha, I., Lumyong, A., Dhanasekaran, V. McKenzie, E. H. C., Hyde, K. D., and Jeewon, R. 2007. A phylogenetic evaluation of whether endotrophs become saprotrophs at host senescence. *Microbiol. Ecol.* 53:579-590.
41. Raffel, S. J., Kazmar, E. R., Winberg, R., Oplinger, E. S., Handelsman, J., Goodman, R. M., and Grau, C. R. 1999. First report of root rot of soybeans caused by *Corynespora cassiicola* in Wisconsin. *Plant Dis.* 83:696.
42. Ronquist, F., and Huelsenbeck, J. P. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572-1574.
43. Seaman, W. L., Shoemaker, R. A., and Peterson, E. A. 1965. Pathogenicity of *Corynespora cassiicola* on soybean. *Can. J. Bot.* 43:1461-1469.
44. Silva, W. P. K., Deverall, B. J., and Lyon, B. R. 1995. RFLP and RAPD analyses in the identification and differentiation of isolates of the leaf spot fungus *Corynespora cassiicola*. *Aust. J. Bot.* 43:609-618.
45. Silva, W. P. K., Deverall, B. J., and Lyon, B. R. 1998. Molecular, physiological and pathological characterization of *Corynespora* leaf spot from rubber plantations in Sri Lanka. *Plant Pathol.* 47:267-277.
46. Silva, W. P. K., Karunanayake, E. H., Wijesundera, R. L. C., and Priyanka, U. M. S. 2003. Genetic variation in *Corynespora cassiicola*: A possible relationship between host origin and virulence. *Mycol. Res.* 107:567-571.
47. Smith, L. J. Host range, phylogenetic and pathogenic diversity of *Corynespora cassiicola* (Berk. & Curt.) Wei. Ph.D. dissertation, University of Florida, Gainesville.
48. Smith, L. J., Datnoff, L. E., Pernezny, K. L., Roberts, P. D., Rollins, J. A., Schlub, R. L., and Scott, J. W. 2006. Characterization and host-range of the tomato target spot fungus, *Corynespora cassiicola* and resistance of tomato cultivars. Pages 14-20 in: Florida Tomato Committee, Tomato Research Report for 2004-2005.
49. Smith, L. J., Datnoff, L. E., Pernezny, K. L., Rollins, J. A., and Schlub, R. L. 2008. Phylogenetic analyses of diverse *Corynespora cassiicola* isolates indicate an evolutionary correlation with host not geography. In: Meeting Abstracts, 9th Eur. Conf. Fungal Genet. Edinburgh.
50. Smith, L. J., and Schlub, R. L. 2004. Host range of *Corynespora cassiicola* and its occurrence on weeds, ornamentals and crops of Guam. (Abstr.) *Phytopathology* 94:S97.
51. Smith, L. J., and Schlub, R. L. 2005. Foliar fungi on weeds of Guam and the potential for *Corynespora cassiicola* as a bioherbicide for *Stachytarpheta jamaicensis*. (Abstr.) *Phytopathology* 95:S93.
52. Sobers, E. K. 1966. A leaf spot disease of azalea and hydrangea caused by *Corynespora cassiicola*. *Phytopathology* 59:455-457.
53. Spencer, J. A., and Walters, H. J. 1969. Variations in certain isolates of *Corynespora cassiicola*. *Phytopathology* 59:58-60.
54. Suryanarayanan, T. S., Murali, T. S., and Venkatesan, G. 2002. Occurrence and distribution of fungal endotrophs in tropical forests across a rainfall gradient. *Can. J. Bot.* 80:818-826.
55. Swofford, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4. Sinauer Associates, Sunderland, MA.
56. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25:4876-4882.
57. Volin, R. B., and Pohronezny, K. 1989. Severe spotting of fresh market tomato fruit incited by *Corynespora cassiicola* after storm-related injury. *Plant Dis.* 73:1018-1019.
58. White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: PCR Protocols: A Guide to Methods and Applications. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, Inc., New York.
59. Zhang, G. M., and Zhang X. G. 2007. Two new species of *Corynespora* from Guangdong, China. *Mycotaxon* 99:347-351.