Isolation and Identification of the Fungus *Colletotrichum cordylinicola* Causing Anthracnose Disease on *Cordyline fruticosa* in Florida

Kalpana Sharma

University of Florida, IFAS, Department of Plant Pathology, 1453 Fifield Hall, Gainesville, FL 32611

Erica Goss and Ariena H.C. van Bruggen¹

University of Florida, IFAS, Department of Plant Pathology, 1453 Fifield Hall, Gainesville, FL 32611; and the University of Florida, Emerging Pathogens Institute, 2055 Mowry Road, Gainesville, FL 32610

Additional index words. *Colletotrichum cordylinicola*, *Cordyline*, *Dracaena* spp., emerging plant pathogens, international trade

Abstract. Imported Hawaiian Ti *Cordyline* plants (*Cordyline fruticosa*) ‘Topsy Pink’ with anthracnose symptoms were found in Gainesville, FL, in 2013. A *Colletotrichum* spp. was isolated from symptomatic *Cordyline* plants and Koch’s postulates were fulfilled. The colony color on acidified potato dextrose agar (PDA) was orange with slight shades of pink and light gray aerial mycelium. Sclerotia and setae were absent. Conidia were one-celled, hyaline, guttulate, and cylindrical with round ends. The mean size of the conidia was $14.7 \times 5.0 \mu\text{m}$ and ranged from 12.5 to 17.5×3.8 to $7.5 \mu\text{m}$. Polymerase chain reaction (PCR) was performed on the internal transcribed space (ITS) and 28S rDNA regions of the isolate, and the sequences were compared with those of *Colletotrichum* spp. in GenBank. Sequence analysis indicated that the isolate belonged to *C. cordylinicola*. This is the first report of *C. cordylinicola* on *C. fruticosa* in Florida and the United States. Anthracnose symptoms developed on healthy-looking, latently infected Hawaiian Ti plants within 2 to 3 months, and 34% to 44% of the non-inoculated plants became diseased in 3 months. Reactions of several *Dracaena* and *Cordyline* species and varieties including Hawaiian Ti to *C. cordylinicola* were assessed. Several *Dracaena* and *Cordyline* species and varieties including Hawaiian Ti exhibited a differential response when inoculated with *C. cordylinicola*, but none of them was resistant. Hawaiian Ti was the most and lucky bamboo (*Dracaena sanderiana*) the least susceptible [area under the disease progress curve (AUDPC) = 71 vs. $10 \text{ cm}^2 \cdot \text{d}^{-1}$] to *C. cordylinicola*. The slope of the log-transformed disease progress regression line was steepest on Hawaiian Ti and *D. marginata* variety ‘Colorama’ plants, intermediate on varieties ‘Tarzan’ and ‘Magenta’, and least on lucky bamboo [slope = 0.046, 0.044, 0.036, and 0.034 vs. $0.020 \log(\text{cm}^2 + 1)/\text{d}$, respectively, with a mean SE of 0.0006].

The international trade in ornamental plants has increased significantly in recent years and represents an important pathway for the spread of exotic pests and pathogens from other countries to the United States (Rossman, 2009). There are several historical examples of pathogen and pest introductions through the ornamental trade, some of which have caused widespread and catastrophic epidemics not only on ornamentals, but also on agricultural and forest crops in the United

States (Liebhold et al., 2012; Parke and Grunwald, 2012).

In 2012, $\approx 71\%$ of 1.2 billion live ornamental plants that entered the United States came through the Miami Plant Inspection Station in Florida (U.S. Department of Agriculture–Agricultural Quarantine Activity System). It is estimated that a substantial percentage of exotic pests and pathogens arriving at U.S. ports of entry along with ornamental plant imports is missed because plants may be infected but may not express symptoms; fungicides may suppress disease temporarily; pots or potting media may be infested but go unnoticed; pathogens are particularly easy to miss when infecting roots; and symptoms may not be recognized by plant inspectors (Liebhold et al., 2012). As a consequence, new pests and diseases are frequently introduced into Florida (Buck and Ono, 2012; Momol, 2006) and become distributed throughout Florida and the United States through infected plant materials. Plant

pathogens such as *Puccinia hemerocallidis*, the causal agent of daylily rust, and *Ralstonia solanacearum* race 3, biovar 2, the causal agent of geranium bacterial wilt, entered the United States through Florida in 2000 (Buck and Ono, 2012) and 2003 (Momol, 2006), respectively.

The genus *Cordyline* consists of woody monocotyledonous flowering plants in the family Asparagaceae, subfamily Lomandroideae, and has ≈ 20 species, including *Cordyline fruticosa* L. A. Chev. (Hawaiian Ti) and *C. australis* G. Forst. Endl. (cabbage tree) (U.S. Department of Agriculture, Agricultural Research Service, 2011; 5 Mar. 2014 <<http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?401621>>). This genus is native to China, Taiwan, Myanmar, Indonesia, New Zealand, eastern Australia, and Hawaii. *Cordyline fruticosa* is the most frequently imported ornamental species into the United States from Guatemala (Agri-Starts, Inc., Apopka, FL, personal communication). It is a long-lived broadleaf evergreen plant that features thin lance-shaped leaves that emerge pinkish red but mature to deep green or variegated foliage. In the United States, *C. fruticosa* plants are grown outdoors in southern Florida, southwestern United States, and Hawaii but indoors in temperate climates.

Cordyline species are susceptible to several diseases in the United States and Florida. These include fungal diseases such as *Botrytis* blight, *Cercospora* leaf spot, fusarium leaf spot, fusarium stem and root rot, *Phyllosticta* leaf spot, *Phytophthora* leaf spot, *Sclerotium* southern blight as well as bacterial diseases such as *Erwinia* leaf spot and stem rot (Daughtrey and Chase, 1992; Moorman, 2014). However, new plant pathogens continue to be introduced. For example, we recently found that lucky bamboo (*Dracaena sanderiana* Sander ex Mast.) cuttings imported from China carried a latent infection of *Colletotrichum dracaenophilum*, a new species in Florida (Sharma et al., 2014). Similarly, other potential harmful exotic pests and pathogens currently not in the United States could potentially be imported with *Cordyline* plant materials.

In Apr. 2013, Hawaiian Ti ‘Topsy Pink’ plants were observed with anthracnose-like symptoms in retail stores in Gainesville, FL (Fig. 1). Plant stems had brown, sunken lesions surrounded by a dark brown border, which later became necrotic and spread to the entire stalk. Sticky masses of conidia were found in fruiting bodies (acervuli) on symptomatic tissue and appeared to belong to a *Colletotrichum* species. Over time, several leaves wilted and dropped off the plants, and the plants died. Phoulivong et al. (2010) reported a new species of *Colletotrichum*, *C. cordylinicola*, that was associated with an anthracnose on the leaves of *C. fruticosa* and *Eugenia javanica* in Thailand and Laos, respectively. To our knowledge, no *Colletotrichum* species has been associated with *C. fruticosa* in the United States.

Colletotrichum spp. are broad host range pathogens; many species can infect a given

Received for publication 11 Mar. 2014. Accepted for publication 27 May 2014.

This work was funded through a grant from the U.S. Department of Agriculture, Animal and Plant Health Inspection Service “Quality assessment of the U.S. Greenhouse Certification Program.”

We thank Agri-Starts, Inc., Apopka, FL, for donating the Hawaiian Ti (*Cordyline fruticosa*) plants.

¹To whom reprint requests should be addressed; e-mail ahcvanbruggen@ufl.edu.

Fig. 1. Hawaiian Ti *Cordyline* plants 'Topsy Pink' with typical anthracnose symptoms and numerous black fruiting bodies (acervuli, marked by arrow).

host, and a single species can infect diverse hosts, leading to serious cross-infection problems in ornamental nursery production (Freeman et al., 1998; Sanders and Korsten, 2003). With the introduction of new *Colletotrichum* spp., it is important to establish whether they are host-specific or have a wide host range. This will have important implications for disease management when susceptible ornamental plants are grown in the same greenhouse.

The objectives of this study were to isolate the suspect *Colletotrichum* spp. from Hawaiian Ti plants, perform Koch's postulates with the isolate, genetically identify the isolate and compare it with previously studied *Colletotrichum* strains, and determine the variation in aggressiveness of the isolate on different *Cordyline* and *Dracaena* species and cultivars.

Materials and Methods

Isolations from Hawaiian Ti 'Topsy Pink'. Isolations were made from symptomatic stalk tissue of Hawaiian Ti (*Cordyline fruticosa*) 'Topsy Pink' plants collected from an ornamental store in Gainesville, FL, in Apr. 2013. Diseased lesions were inspected using dissecting and compound microscopes for the occurrence of acervuli. Stem tissue containing lesions and acervuli was surface-disinfected with 10% Clorox regular bleach formulation (containing 5% sodium hypochlorite) and rinsed in sterile deionized water. Pure fungal cultures were obtained by transferring acervuli from disinfected tissue onto plates of acidified (pH 4.5) PDA (Difco Laboratories, Detroit,

MI) that were incubated at 23 °C in the dark. Fungal observations were made on 7-d-old cultures grown on acidified PDA plates. Mycelial growth, acervuli, and spores were inspected with a compound microscope (Olympus BX51; Olympus America Inc., Melville, NY). Spore sizes were measured on 50 spores. Single-spore cultures were prepared and maintained on acidified PDA using the procedure described by Cai et al. (2009).

Pathogenicity tests and reisolation. Healthy-looking rooted stem cuttings (≈30 cm long, 1.0 cm diameter, 2 months old) of Hawaiian Ti (*C. fruticosa*) 'Topsy Pink', imported from Guatemala, and donated by Agri-Starts, Inc., Apopka, FL, were used for pathogenicity tests. Single cuttings were planted in soilless Fafard® complete potting mix in 10-cm-diameter pots. The potting mix consisted of 45% Canadian sphagnum peat-moss, 30% processed pine bark, 15% vermiculite, 10% perlite, starter nutrients, a wetting agent, and dolomitic limestone (Conrad Fafard, Inc., Agawam, MA). Ten-d-old single-spore cultures of putative *C. cordylinicola* grown on acidified PDA were used to inoculate Hawaiian Ti plants by inserting a sporulating mycelial agar plug (5 mm diameter) into a cut on the upper half of the stalk with a sterile blade. The negative control consisted of a similar plug of sterile acidified PDA agar. The inoculated sites were then covered with parafilm strips (Bobev et al., 2008). Inoculated and control plants were kept in a greenhouse with a 12-h photoperiod at 25 to 30 °C. Plants were watered daily with deionized water and fertilized with 20N–20P₂O₅–20K₂O (J.R. Peters,

Inc., Allentown, PA) once every week. The experiments were laid out in a randomized complete block design with four replications (five plants per replication) per treatment. The experiment was repeated.

Plants were checked for symptom development on a daily basis. Disease severity was determined from the length and width of lesions with acervuli on individual plant, starting 6 d after inoculation and at 4-d intervals for 3 weeks. The diseased area per plant was calculated using the formula of an ellipse for each lesion. After the final assessments, stem tissues containing lesions and acervuli were disinfected with 10% Clorox regular bleach formulation and rinsed in sterile deionized water. Symptomatic tissue was aseptically removed and transferred to acidified PDA plates. Plates were incubated for 6 d at 24 °C and hyphal growth, shape, and size of conidia were examined using a compound microscope (Olympus BX51; Olympus America Inc.).

Latent infections. One hundred Hawaiian Ti 'Topsy Pink' cuttings imported from Guatemala, and donated by Agri-Starts, Inc., Apopka, FL, were planted individually in soilless Fafard® complete potting mix in 10-cm-diameter pots and kept in a separate greenhouse with a 12- to 13-h photoperiod at 25 to 30 °C to observe the latent presence of *Colletotrichum* spp. The greenhouse was completely disinfected with 10% Clorox regular bleach before the latent infection experiments were initiated. The plants were watered daily with deionized water and fertilized with 20N–20P₂O₅–20K₂O once every 3 weeks. Splash dispersal of the pathogen was prevented by watering individual plants with a beaker of water. The plants were assessed daily for symptom development for 3 months and then plants with anthracnose symptoms were counted. Plants were removed from the greenhouse as soon as acervuli or symptoms were observed. To confirm the identity of the pathogen, symptomatic tissue was aseptically removed and transferred to acidified PDA plates. Plates were incubated for 6 d at 24 °C and hyphal growth, shape, and size of conidia were examined. This study was conducted two times with plants of two different shipments from the same source.

DNA isolation and sequencing. Genomic DNA was extracted from actively growing mycelium on acidified PDA plates using a quick DNA extraction protocol (Smith et al., 2011) and used for PCR, sequencing, and phylogenetic analyses. Both the ITS and the 28S subunit of ribosomal DNA (rDNA) regions was amplified with PCR using primer pairs ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Gardes and Bruns, 1993; White et al., 1990) and LROR (5'-ACCCGCTGAAGCTTAAGC-3') and LR5 (5'-TCCT GAGGGAAACTTCG-3') (Vilgalys and Hester, 1990), respectively. The PCR master mix consisted of 10× buffer, 10 mM dNTPs, 25 mM MgCl₂, DNA polymerase, sterile distilled water, and 10 μM of each primer in a total volume of 20 μL. Initial denaturation was at 94 °C for 2 min

with 39 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C (for primer pair ITS1-F and ITS4) or 50 °C for (primer pair LROR and LR5) for 30 s, and elongation at 72 °C for 1 min with a final elongation at 72 °C for 10 min (MyCycler Thermal Cycler; BioRad, Hercules, CA). PCR products were cleaned using the Wizard SV Gel and PCR Clean-up System (Promega, Madison, WI). Purified PCR products were Sanger-sequenced in both directions by the University of Florida Interdisciplinary Center for Biotechnology Research (Gainesville, FL). Base calls were checked manually and reads assembled in Geneious 6.16 (Biomatters Ltd.).

Isolate sequences were compared with ITS sequences from type cultures of *Colletotrichum* as described in Cannon et al. (2012). The sequence alignment used by Cannon and colleagues was downloaded from TreeBASE study S13170 and trimmed to include the ITS locus only. Species identification was obtained by aligning our ITS sequence with the trimmed Cannon alignment and constructing a maximum likelihood tree using PhyML (Guindon et al., 2010; Guindon and Gascuel, 2003) as implemented in Geneious 6.16. The GTR+I+G substitution model was used with both subtree pruning and regrafting and nearest neighbor interchange (NNI) topology searches. The size of the final alignment was 502 bp. Based on the phylogenetic relationships in Cannon et al. (2012), *C. dracaenophilum* was included as an outgroup to the clade containing *C. cordylinicola* to root the tree. Branch support was evaluated using 500 bootstrap samples under NNI tree searching.

The 28S sequence was subjected to BLAST analysis against the National Center for Biotechnology Information nucleotide database. The 28S gene was not included in the Cannon et al. (2012) analysis; thus, the sequence from the Florida isolate was compared with those from vouchered isolates of closely related species in the *C. gloeosporioides* species complex.

Host range experiment. One variety of Hawaiian Ti ‘Topsy Pink’ obtained from Agri-Starts, Inc., Apopka, FL; three varieties of *Dracaena marginata* var. ‘Tarzan’, ‘Magenta’, and ‘Colorama’ and one variety of lucky bamboo (*D. sanderiana*) obtained from Delray Plants Co., Venus, FL; and one isolate of *C. cordylinicola* were used for the host range experiment. Agar plug inoculation was performed as described previously for the pathogenicity test. Control plants were inoculated with sterile acidified PDA agar plugs. Inoculated cuttings were planted individually in 10-cm-diameter pots containing Fafard® complete soilless potting mix (Conrad Fafard, Inc.) and maintained in a greenhouse with a 12-h photoperiod at 25 to 30 °C. The plants were assessed daily for symptom development, and diseased area per plant was calculated as described for the pathogenicity test. AUDPC (Campbell and Madden, 1990) value per replication was calculated for the disease severity measurements. The experiment was laid out in a randomized complete block

design with four replications (one plant per treatment per replication). The experiment was repeated.

Statistical analysis. AUDPC values were compared with a generalized model analysis of variance (ANOVA) using SAS statistical software (PROC GLM, Version 9.2; SAS Institute Inc., Cary, NC). Data of each experiment were tested for normality using the Shapiro-Wilk test of residuals (Royston, 1992) and checked for outliers using Lund’s test of standardized residuals (Lund, 1975). No outliers were found in any data set. There was no effect of repetition or repetition–treatment interaction; therefore, the data of the repeated experiments were analyzed together. Data from the host range experiment were log-transformed and regressed on time without an intercept to quantify the relationship between diseased area and days after inoculation for plant cultivars and blocks separately. Estimates for the slope were compared with a generalized model ANOVA and means were separated using Tukey’s test at $P \leq 0.05$ using SAS statistical software.

Results

Isolations from Hawaiian Ti ‘Topsy Pink’. One *Colletotrichum* isolate was isolated from symptomatic stalk tissue on Hawaiian Ti obtained from an ornamental store in Gainesville, FL, in Apr. 2013. Lesions were beige to

light brown surrounded by a dark brown border (Fig. 1). Acervuli on the surface of the lesions were black. Waxy, subepidermal acervuli with salmon-colored spores produced on conidiophores were observed in lesion centers. The colony color on acidified PDA was orange with slight shades of pink and light gray aerial mycelium (Fig. 2A). Sclerotia and setae were absent. Colonies on acidified PDA produced abundant conidia that were one-celled, hyaline, guttulate, and cylindrical with round ends (Fig. 2A). The mean size of the conidia was $14.7 \times 5.0 \mu\text{m}$ and ranged from 12.5 to 17.5×3.8 to $7.5 \mu\text{m}$ (Fig. 2B) as measured with a compound microscope at 125× magnification. The fungus was putatively considered to be *C. cordylinicola* based on the colony color and morphology, conidial dimensions, and the host from which it was isolated.

The pathogenicity test and reisolation. Typical anthracnose symptoms were observed on all Hawaiian Ti plants inoculated with the isolated *Colletotrichum* spp., but no symptoms were found on control plants during the assessment period (22 d). Disease symptoms consisted of light brown lesions surrounded by a dark brown border that spread from the point of inoculation. Dark brown acervuli developed in the symptomatic tissue 6 d after inoculation, and disease symptoms continued to develop (Fig. 3). Inoculated plants had a 1-cm² diseased area on Day 6 that increased

Fig. 2. (A) Colony morphology of *Colletotrichum cordylinicola* on an acidified potato dextrose agar plate and (B) *Colletotrichum cordylinicola* conidia spores seen under the compound microscope.

Fig. 3. Disease progression of a single spore isolate of *Colletotrichum cordylinicola* that was isolated from a Hawaiian Ti Cordyline plant ‘Topsy Pink’ on plants of the same cultivar. Data are the means ± SD of two tests, each with four replications (five plants per replication per treatment).

to 2.1 cm² on Day 10 and continued to increase to 6.1 and 10 cm² on Days 18 and 22, respectively.

Colonies of the reisolates looked like the original *Colletotrichum* isolate, and shape and size of conidia appeared the same as those of the original isolate.

Latent infections. The levels of latent plant infection varied between the two batches from the same source. In the first shipment, 18 of 100 Hawaiian Ti plants developed typical anthracnose symptoms with the dark brown acervuli after 2 months. After 3 months, 34% of the plants had anthracnose symptoms. For the second shipment of cuttings, 12% of the plants had anthracnose symptoms after 2 months and increased to 44% in 3 months. A *Colletotrichum* spp. was isolated from latent stem infections that resembled the one from pathogenicity test.

DNA sequencing. The ITS of the *Colletotrichum* isolate in this study exhibited 100% sequence identity to the ITS sequence of the ex-holotype *C. cordylinicola* ICMP 18579 based on comparison with GenBank accession JX010226 (Fig. 4; Weir et al., 2012). The *C. cordylinicola* ITS sequence contains three nucleotides that distinguish it from close relatives *C. psidii* and *C. kahawae* (99.4%

sequence identity). Sequence identity to other species in the *C. gloeosporioides* clade shown in Figure 4 ranged from 98.0% to 99.2%. Although 28S sequences were not available for *C. cordylinicola*, the sequence we obtained differed by only 1 bp from the 28S sequences from bar-coded *C. asianum* isolates and *C. gloeosporioides* isolate CBS 122687 (GenBank accessions JN940407-10 and EU552111, respectively), confirming that the Florida isolate falls within the *Gloeosporioides* clade (Cannon et al., 2012). The ITS and 28S sequences have been deposited to GenBank under accessions KJ186112 and KJ186113, respectively.

Host range trial. None of the tested plant species were resistant to the Florida isolate of *C. cordylinicola*. Overall, mean diseased area was the highest for Hawaiian Ti ‘Topsy Pink’ and least for lucky bamboo, whereas *Dracaena marginata* variety ‘Tarzan’, ‘Magenta’, and ‘Colorama’ showed an intermediate response throughout the entire assessment period (Fig. 5). Among three *Dracaena marginata* varieties, ‘Colorama’ had larger disease areas than ‘Tarzan’ and ‘Magenta’, and the response pattern of those two varieties was the same ($P = 0.001$). The increase in log (diseased area +1) was linear over time. The slope

of the regression line was significantly higher ($P = 0.0001$) on Hawaiian Ti and ‘Colorama’ plants, intermediate on ‘Tarzan’ and ‘Magenta’, and least on lucky bamboo (slope = 0.046, 0.044, 0.036, and 0.034 vs. 0.020 cm²·d⁻¹, respectively, with a mean SE of 0.0006). The same was true for AUDPC values, which were significantly different ($P = 0.0001$) for Hawaiian Ti and ‘Colorama’ plants vs. ‘Tarzan’ and ‘Magenta’ vs. lucky bamboo. The mean AUDPCs in decreasing order were 71 cm²·d⁻¹ on Hawaiian Ti, 34 cm²·d⁻¹ on ‘Colorama’, 23 cm²·d⁻¹ on ‘Tarzan’, 19 cm²·d⁻¹ on ‘Magenta’, and 10 cm²·d⁻¹ on lucky bamboo with a mean SE of 0.94 cm²·d⁻¹.

Discussion

The most important finding of this study was the isolation and identification of *Colletotrichum cordylinicola* causing stem anthracnose on *Cordyline fruticosa* in Florida. The causal agent was consistently reisolated from inoculated *C. fruticosa* plants, thus fulfilling Koch’s postulates. To our knowledge this is the first report of *C. cordylinicola* on *C. fruticosa* in the United States. *Colletotrichum cordylinicola* was first isolated and described

Fig. 4. Maximum likelihood phylogenetic tree for the *Colletotrichum gloeosporioides* clade rooted with *C. dracaenophilum*. The Florida isolate from a Hawaiian Ti Cordyline plant ‘Topsy Pink’ is identical in internal transcribed space sequence to the *C. cordylinicola* ex-holotype. Branch labels show bootstrap support. Branch lengths are substitutions per site.

Fig. 5. Reaction of several *Dracaena* species and varieties including Hawaiian Ti ‘Topsy Pink’ to *Colletotrichum cordylinicola* isolated from Hawaiian Ti. Before regression analysis, diseased area data were log-transformed and regressed, but nontransformed means are presented in the figure for uniformity of presentations. Data are the means $\pm$ SD of two tests, each with four replications (one plant per replication per treatment).

from leaves of *C. fruticosa* and *Eugenia javanica* in Thailand and Laos by Phoulivong et al. (2010).

Colletotrichum cordylinicola is morphologically similar to several species in the *C. gloeosporioides* species complex and also to *C. dracaenophilum* (Bobev et al., 2008; Cannon et al., 2012; Sharma et al., 2014). Species of *Colletotrichum* are difficult to differentiate based solely on morphology. Morphological and cultural characteristics of *C. cordylinicola* described in this study match the description of *C. cordylinicola* by Phoulivong et al. (2010), although conidial dimensions differed slightly. Conidial dimensions for the Asian isolate ranged from 11 to 20 $\times$ 4 to 5 μm , whereas the Florida isolate ranged from 12.5 to 17.5 $\times$ 3.8 to 7.5 μm . The length range of the Florida isolate was smaller than that of the Asian isolate and the width range of the Florida isolate was greater. Moreover, both setae and sclerotia were absent on infected plants and in acidified PDA culture plates of the Florida isolate. Nevertheless, the pathogen’s identity as *C. cordylinicola* was further confirmed by sequencing and had a 100% identical ITS sequence to ex-holotype *C. cordylinicola* ICMP 18579 based on comparison with GenBank accession JX010226 (Weir et al., 2012). Multilocus sequencing of all members of the *C. gloeosporioides* complex and closely related *Colletotrichum* species would be needed to more clearly separate members of this complex and related species.

Cordyline fruticosa can be easily confused with *Dracaena marginata*, and anthracnose symptoms are very similar on both species. Moreover, *C. cordylinicola* cultures look very similar to those of *C. dracaenophilum*. Therefore, these *Colletotrichum* species were cross-inoculated on varieties of both plant genera. The results with *C. dracaenophilum* have been reported (Sharma et al., 2014). Similar to the results with *C. dracaenophilum*, all of the tested plant species and varieties

in the current study were susceptible to *C. cordylinicola* isolated from Hawaiian Ti. *Colletotrichum cordylinicola* caused more severe disease on Hawaiian Ti than on *Dracaena* spp. and varieties. This study provides further evidence that *Colletotrichum* spp. are not absolutely host-specific. This is consistent with the findings of Phoulivong et al. (2012) who showed that most *Colletotrichum* species can infect many hosts, whereas the same host can be infected by many *Colletotrichum* species. Nevertheless, a significant differential interaction was observed in this study and our previous study with *C. dracaenophilum* (Sharma et al., 2014). It is therefore important to identify *Colletotrichum* isolates at the species levels using molecular techniques. Identification at the species level would be helpful in developing a systems approach to disease management in ornamental nurseries (Parke and Grunwald, 2012; van Bruggen et al., 2014).

Approximately 34% to 44% of the non-inoculated plants in the current study showed anthracnose symptoms over a 3-month period. It is therefore likely that Hawaiian Ti plants imported from Guatemala carried *C. cordylinicola* in latent infections. It is important that nursery staff members in Guatemala and the United States are able to recognize the symptoms of anthracnose infections. In addition, the development of an efficient and sensitive detection technique to estimate latent infections of *C. cordylinicola* in imported plants is critical so that infected plant material could be intercepted at the border and information on disease risk and management can be provided in advance to reduce the development of epidemics. Future research should be done to investigate methods for the rapid detection of latent infections of *C. cordylinicola* in asymptomatic plants.

Much of the *Colletotrichum* research in recent years has been targeted at controlling the pathogen. Considering the latent presence of *C. cordylinicola* on Hawaiian Ti, control

methods need to be developed to eliminate latent infections. Recently, Sharma et al. (2014) found that the systemic fungicide azoxystrobin not only prevented and controlled new infections by *C. dracaenophilum* on lucky bamboo, but also cured latent anthracnose infections. Several other fungicides are effective at managing *Colletotrichum* spp. on a variety of hosts (Daugovish et al., 2009). Azoxystrobin and other systemic fungicides could also be tested for managing latent infections of *C. cordylinicola* on Hawaiian Ti. This could have important implications for disease management when different susceptible ornamental plants are grown in the same greenhouse.

In conclusion, Hawaiian Ti imported from Guatemala carried latent infections of the fungal pathogen *C. cordylinicola*. The system approach should be applied to identify key points of vulnerability in the nursery operation where steps can be taken to minimize *C. cordylinicola* risks and to maintain access to national and international markets. Proper disease control tactics cannot be implemented unless the correct disease problems are identified. It is therefore very important that nursery staff members at the source and destination of a production chain are well trained to recognize and manage *C. cordylinicola* as well as other pathogen species in the ornamental industry. Similarly, careful inspection at the ports of entry is very important. Each and every individual shipment needs to be inspected carefully for signs, symptoms, and pathogens, and shipments with contaminated plant materials should be destroyed by autoclaving.

Literature Cited

- Bobev, S.G., L.A. Castlebury, and A.Y. Rossman. 2008. First report of *Colletotrichum dracaenophilum* on *Dracaena sanderiana* in Bulgaria. *Plant Dis.* 92:173.
- Buck, J.W. and Y. Ono. 2012. Daylily rust. The plant health instructor. 2 July 2014. DOI: 10.1094/PHI-I-2012-0516-01.
- Cai, L., K.D. Hyde, P.W.J. Taylor, B.S. Weir, J. Waller, M.M. Abang, J.Z. Zhang, Y.L. Yang, S. Phoulivong, Z.Y. Liu, H. Prihastuti, R.G. Shivas, E.H.C. McKenzie, and P.R. Johnston. 2009. A polyphasic approach for studying *Colletotrichum*. *Fungal Divers.* 39:183–204.
- Campbell, C.L. and L.V. Madden. 1990. Introduction to plant disease epidemiology. Wiley-Interscience, New York, NY.
- Cannon, P.F., U. Damm, P.R. Johnston, and B.S. Weir. 2012. *Colletotrichum*—Current status and future directions. *Stud. Mycol.* 73:181–213.
- Daughtrey, M.L. and A.R. Chase. 1992. Ball field guide to diseases of greenhouse ornamentals. Ball Publishing, Batavia, IL.
- Daugovish, O., H. Su, and W.D. Gubler. 2009. Preplant fungicide dips of strawberry transplants to control anthracnose caused by *Colletotrichum acutatum* in California. *HortTechnology* 19: 317–323.
- Freeman, S., T. Katan, and E. Shabi. 1998. Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. *Plant Dis.* 82:596–605.
- Gardes, M. and T.D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes—Application to the identification of mycorrhizae and rusts. *Mol. Ecol.* 2:113–118.

- Guindon, S., J.F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst. Biol.* 59:307–321.
- Guindon, S. and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52:696–704.
- Liebhold, A.M., E.G. Brockerhoff, L.J. Garrett, J.L. Parkeand, and O.K. Britton. 2012. Live plant imports: The major pathway for forest insect and pathogen invasions of the U.S. *Front. Ecol. Environ.* 10:135–143.
- Lund, R.E. 1975. Tables for an approximate test for outliers in linear models. *Techometrics* 17: 473–476.
- Momol, T.M. 2006. Recovery plan for *Ralstonia solanacearum* Race 3 biovar 2, causing brown rot of potato and southern wilt of geranium. National Plant Disease Recovery System (NPDRS), Dept. of Homeland Security, Washington, DC.
- Moorman, G.W. 2014. Cordyline (Ti plant) diseases. Penn State Extension. 7 July 2014. <<http://extension.psu.edu/pests/plant-diseases/all-fact-sheets/cordyline-ti-plant-diseases>>.
- Parke, J.L. and N.J. Grunwald. 2012. A system approach for management of pests and pathogens of nursery crops. *Plant Dis.* 96:1236–1244.
- Phoulivong, S., L. Cai, N. Parinn, H. Chen, K.A. Abd-Elsalam, E. Chukeatirote, and K.D. Hyde. 2010. A new species of *Colletotrichum* from *Cordyline fruticosa* and *Eugenia javanica* causing anthracnose disease. *Mycotaxon* 114:247–257.
- Phoulivong, S., E.H.C. McKenzie, and K.D. Hyde. 2012. Cross infection of *Colletotrichum* species; a case study with tropical fruits. *Curr. Res. Environ. Appl. Mycol.* 2:99–111.
- Rossman, A.Y. 2009. The impact of invasive fungi on agricultural ecosystems in the United States. *Biol. Invasions* 11:97–107.
- Royston, P. 1992. Approximating the Shapiro-Wilk W-test for non-normality. *Stat. Comput.* 20: 11–19.
- Sanders, G.M. and L. Korsten. 2003. A comparative morphology of South African avocado and mango isolates of *Colletotrichum gloeosporioides*. *Botany* 81:877–885.
- Sharma, K., J.L. Merritt, A. Palmateer, E. Goss, M. Smith, T. Schubert, R.S. Johnson, and A.H.C. van Bruggen. 2014. Isolation, characterization and management of *Colletotrichum* spp. causing anthracnose on lucky bamboo (*Dracaena sanderiana*). *HortScience* 49:1–7.
- Smith, M.E., T.W. Henkel, M.C. Aime, A.K. Fremier, and R. Vilgalys. 2011. Ectomycorrhizal fungal diversity and community structure on three co-occurring leguminous canopy tree species in a Neotropical rainforest. *New Phytol.* 192:699–712.
- U.S. Department of Agriculture, Agricultural Research Service. 2011. *Cordyline fruticosa* (L.) A. Chev. Germplasm Resources Information Network (GRIN) online database. National Germplasm Resources Laboratory, Beltsville, MD. 6 Jan. 2014. <<http://ars-grin.gov/cgi-bin/npgs/html/taxon.pl?401621>>.
- van Bruggen, A.H.C., K. Sharma, J.L. Merritt, G.S. Ali, E.R. Dickstein, and C.L. Harmon. 2014. Pests and diseases in ornamental greenhouse crops: International trade, diagnosis and management, and audit-based certification systems. StorterChilds, A Consolidated Graphics Company, Gainesville, FL.
- Vilgalys, R. and M. Hester. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* 172:4238–4246.
- Weir, B.S., P.R. Johnston, and U. Damm. 2012. The *Colletotrichum gloeosporioides* species complex. *Stud. Mycol.* 73:115–180.
- White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315–322. In: Innis, M.A., D.H. Gelfand, J.J. Sninsky, and T.J. White (eds.). *PCR protocols: A guide to methods and applications*. Academic Press, San Diego, CA.