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

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Abstract. Imported Hawaiian Ti Cordyline plants (Cordyline fruticosa) ‘Tipsy 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 acidic potato dextrose agar (PDA) was orange with slight shades of pink and light yellow 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 × 5.0 μm and ranged from 12.5 to 17.5 × 3.8 to 7.5 μm. 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 cm²-d⁻¹] to C. cordylinicola. The slope of the log-transformed disease progression 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(cm² + 1)/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 (Liebold et al., 2012; Parke and Grunwald, 2012).

In 2012, ≈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 (Liebold 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, Ceratocystis leaf spot, fusarium leaf spot, fusarium stem and root rot, Phylosticta 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 ‘Tipsy 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 leaves which later became necrotic and spread to the entire stalk. Sticky masses of conidia were found in fruiting bodies (acervuli) on symptomatic leaves which later became necrotic and spread to the entire stalk.
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 ‘Tipsy Pink’*. Isolations were made from symptomatic stalk tissue of Hawaiian Ti *(Cordyline fruticosa)* ‘Tipsy 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*) ‘Tipsy 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 plates 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 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’-CTTGGTCAATTTAGAGGAATTAA-3’) and ITS4 (5’-TCCCTCCGTTATTGATATGC-3’) (Gardes and Bruns, 1993; White et al., 1990) and LROR (5’-ACCCGCTTAACCTAAAG-3’) and LBS (5’-TTCCTACGGGAGGCAGCAGC-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.
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, 1995) 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 ‘Tipsy 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 fungus was putatively identified as *C. cordylinicola* based on the colony color and morphology, conidial dimensions, and the host from which it was isolated.

**Host range experiment.** One variety of Hawaiian Ti ‘Tipsy 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 from 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).
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 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 ‘Tipsy 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 ‘Tipsy 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.
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 × 4 to 5 μm, whereas the Florida isolate ranged from 12.5 to 17.5 × 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 noninoculated 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**


