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Survival and Dissemination of Fern Anthracnose (*Colletotrichum acutatum*) Spores in Commercial Ferneries

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Fern anthracnose, *Colletotrichum acutatum*, is a highly contagious disease in commercial ferneries causing the immature fronds of leatherleaf fern to become unmarketable for floral use. This research identifies sources that could disseminate primary inoculum, examines pathways of spore spread in commercial ferneries and defines factors relating to spore longevity and survival. Spores of *C. acutatum* were found to survive up to 36 d on the surfaces of leatherleaf fronds and up to 49 d in leaf debris on the soil surface. Spores were able to survive up to 42 d on metal, rubber, or wood surfaces which could function as a major mechanism of spread. Significant differences in spore counts on the clothing of workers were found following harvesting activities under wet or dry foliage conditions. Temperature affected both spore survival and germination.

Introduction

Leatherleaf fern is a perennial crop generating sales of over $64 million per year in Florida. Fern anthracnose disease, incited by *Colletotrichum acutatum* Corda (fern isolate), causes immature leaflets of leatherleaf fern [*Rumohra adiantiformis* (Forst.) Ching] to become deformed or necrotic during development. At maturity infected fronds are unsuitable for floral use (Fig. 1) (Norman and Strandberg, 1997; Timmer et al., 1998). Fern anthracnose impacts the crop year-round, making disease management difficult (Timmer et al., 1998; Waller, 1992).

Fern anthracnose is highly contagious (Stamps et al., 1997, Strandberg et al., 1997). Infected fronds produce thousands of *C. acutatum* spores (Fig. 2) and due to efficient dissemination pathways of spores, control with fungicides is difficult (Strandberg et al., 1997). Spores do not infect mature fern fronds; however, if viable spores fall onto emerging fiddleheads, infection occurs (Strandberg et al., 1997). Spore persistence and survival in crop debris and in surrounding native soil has been reported for strawberry (Eastburn and Gubler, 1992; Freeman et al., 2002; Wilson et al., 1992) and pine (Nair et al., 1983) and transfer of spores via clothing has been reported for fern (Norman et al., 1997). The population of *C. acutatum* that infects leatherleaf fern is considered to be genetically distinct from those that infect other crops (MacKenzie et al., 2009; Schiller et al., 2006).

The purpose of this research was to quantify spore population, longevity and survival on mature fern fronds, spore viability duration in crop debris lying on the soil surface, and inoculum transfer via simulated equipment and clothing of workers. Protocols for disease management could then be determined in relation to spore survival, sources, and pathways.

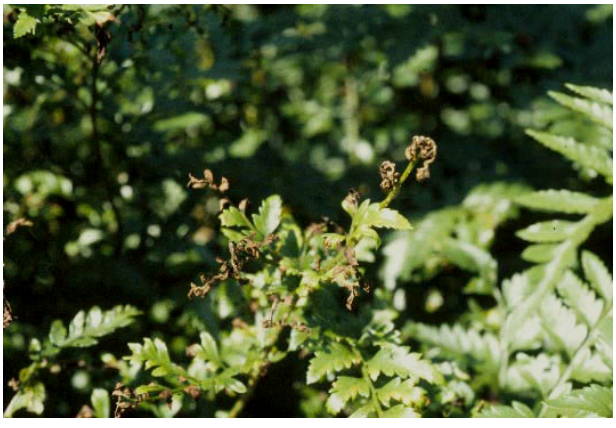

Fig. 1. Leatherleaf fern fronds infected with *Colletotrichum acutatum* become necrotic and/or deformed and unsaleable.

Fig. 2. Spores of *Colletotrichum acutatum* on infected leatherleaf fern.

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Materials and Methods

To isolate *Colletotrichum acutatum* for experimental purposes, diseased fern tissue was cut into 5-mm square sections, dipped briefly in 70% ethanol, immersed 1 to 2 min in 1% sodium hypochlorite, washed in sterile water, blotted dry on sterile paper towels, then placed on potato dextrose agar (PDA). After 3 to 4 d incubation at 24 °C, under 16-h cool-white fluorescent light, (150 $\mu mol \cdot s^{-1}) \cdot d^{-1}$ *Colletotrichum acutatum* colonies were identified by their unique salmon color and spore morphology. Spores for use in these experiments were produced on PDA plates under the same conditions.

In order to determine how long spores might persist on the surfaces of mature leaves and be available to infect the newly emerging fronds, a spore suspension (2.5×10^6 spores per mL) was applied to mature fern leaves of 34 plants growing in 1-L plastic pots in a warm, dry greenhouse at 21.1 °C and 32.7 °C, typical night and day temperature respectively. Spore concentrations were confirmed on day zero by dilution plating onto modified PDA (mPDA) containing 50 µg a.i. benomyl mL^{-1} (Benlate 50 WP), 50 µg a.i. triadimefon mL^{-1} (Bayleton 50 WP), 25 µg/mL of butylated hydroxy anisole (BHA, Sigma B 1253), and chlortetracycline–HCl 50 µg/mL (Sigma C 4881). Natural resistance to certain fungicides has been useful in semiselective isolation of *C. acutatum* (Norman and Strandberg, 1997). Plants were hand watered without wetting leaf surfaces in order to prevent spore germination. Each day, five leaves were collected at random, cut into approximately 4-cm^2 pieces (large veins and midrib discarded), placed in 100 mL sterile distilled water (SDW) and stirred with a magnetic stir bar. After 5 min, three 1-mL samples were plated onto mPDA plates. Leaf pieces were recovered, dried briefly, and the exact leaf area determined. After 5 d, colonies were counted and reported as average number of spores recovered per cm^2 of leaf surface. Experiment was repeated twice and a healthy fern leaf control was handled in the same manner as diseased samples.

In most ferneries a thick thatch of debris composed of old unharvested fern fronds develops underneath the actively growing fronds. Old fronds are continually added to this layer of organic mater. In order to assess spore population and spore survival within this debris, young fresh fern leaves were collected and cut into 1-cm^2 pieces. Sub-samples (10 g fresh weight) were packaged into 10×15 cm coarse, rayon fabric mesh packets and placed onto the soil surface of the experimental 1-L pots containing leatherleaf fern. Pots were kept in shade houses at 80% shade that are typical of those used in the industry. Once per week, three packets were recovered, and the leaf pieces were assayed for viable spores. Recovered leaf tissue was stirred using a magnetic stir plate and stir bar for 10 min in 200 mL of water then four 1-mL aliquots were removed with sterile pipettes, dispensed onto mPDA, then incubated and colonies counted. A healthy fern leaf control was also handled in the same manner as diseased samples. Experiments were repeated twice; once during June to July and again during July to August.

To investigate *Colletotrichum* spore survival on inert surfaces and the potential for spores spreading on surfaces of tools, vehicles and equipment moving in a commercial fernery, squares of unpainted wood (9.4 cm^2), rubber (0.6 cm^2) and aluminum (12 cm^2) were affixed to wooden garden stakes ($1.7 \times 0.3 \times 30$ cm). These sampling units on stakes were vigorously rubbed through wet, diseased, fern foliage. The sampling units were maintained outdoors from May through August under a roofed shelter without sidewalls (typical of commercial fernery equipment storage areas) that protected the sampling units against direct rain and sunlight. Each week, four sampling units per treatment were retrieved, returned to the laboratory and placed in 200 mL of SDW and stirred for 10 min. Four, 1-mL aliquots were plated on mPDA and incubated at 22 °C, under 16-h cool-white fluorescent light (150 $\mu mol \cdot s^{-1}) \cdot d^{-1}$. *C. acutatum* colonies were counted after 5 to 6 d.

To determine spore survival on denim fabric on workers, denim fabric patches (100 cm^2) were attached to boots, pants, and gloves of fern harvesters then collected after 6 to 7 h of harvesting activity in wet foliage (following 3 to 5.5 h of rainfall) or in dry foliage. Two wet and two dry sampling days were used with four or five workers on each day. From each air-dried patch, three 1-cm^2 pieces were cut, placed in 10 mL SDW, soaked for 1 h, stirred briefly by hand, then 1-mL portions were plated onto mPDA. Plates were incubated for 5 d, colonies counted, and populations expressed as numbers of spores recovered per cm^2 of fabric.

Effect of temperature and age on spore germination. *Colletotrichum* spore suspensions were washed from 3-d-old mPDA plates, diluted to 2.5×10^3 spores per ml and 1-ml samples spread on each of five water agar plates then incubated in the dark at 5, 10, 15, 20, 25, and 30 °C. At 0, 3, 5, and 7 h after plating, spores within a 4-cm^2 area of each plate were examined and percent germination determined. The experiment was repeated twice. Spores germinated poorly (<5%) below 15 °C (results not presented). Above 15 °C, some spores (5% to 10%) germinated by 3 h, but most required 5 h or more. By 7 h, almost all spores (95%) had germinated at 15 to 30 °C. There were no large differences in temperature effects between 15 and 30 °C. Because of observations made above, spores from PDA plates collected at 2 d and 9 d were compared for germination responses at 16, 20, 24, and 28 °C.

Results and Discussion

Fern anthracnose spores applied to the surfaces of mature fronds survived and were detected in decreasingly smaller numbers for up to 36 d after placement (Fig. 3). Numbers of viable spores for each sampling date were statistically compared using a linear regression analysis (Fig. 3). Data closely followed a linear model for each test with data from the first test ($R^2 = 0.96$). Evaluation of the survival of spores in crop debris on the soil surface showed

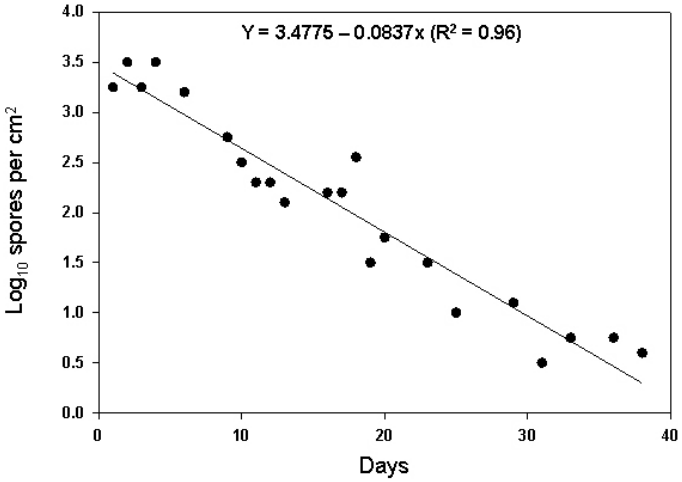

Fig. 3. Survival of *Colletotrichum acutatu*m spores on mature leatherleaf fern leaves maintained under greenhouse conditions without overhead irrigation.

that numbers of spores recovered remained high in the first experiment (3.2×10^4 spores/g) for 21 d after leaf debris placement, then numbers declined steadily as leaves turned brown and decayed (Fig. 4). After 14 d we observed a steady decline in viable spores in both experiments. At 41 d the spore concentration was 6.4×10^3 spores/g. A more rapid decline in spore numbers was observed in the second experiment; this was likely due to consistent warm temperatures of July and August along with more frequent rain. Even with these warm temperatures a spore count greater than 6.3×10^2 viable spores/g was detected on day 49. No viable spores were detected in the healthy fern leaf controls in either experiment. Numbers of viable spores for each sampling date were statistically compared using a linear regression analysis. Data closely followed a linear model ($R^2 = 0.95$).

Fern anthracnose spores survived in small numbers (1 to 10 spores/cm^2) on wood, rubber and aluminum surfaces for up to 42 d (Fig. 5). Initial spore populations were 948, 1388, and 1713 spores per cm^2 for wood, metal, and rubber, respectively, but numbers decreased greatly after 7 d, and were barely detectable after 14 d except for occasional samples where small numbers were recovered for up to 42 d. Small pieces of leaf debris adhered to surfaces of all three materials, increasing the likelihood that spores would survive longer than on a clean, inert surface. Combined data from three replicate experiments are displayed in Figure 5.

Numbers of spores recovered from denim fabric patches attached to pants and gloves of fern workers, but not to their boots, were significantly different (*t*-test) following harvesting activity in wet or dry foliage. Average numbers of spores per cm^2 from harvesting in wet and dry foliage were recovered from patches on boots were: 24.3 and 1.23 ($t = 1.075$, $P = 0.477$); from pants: 76.8 and 4.0 ($t = 0.2769$, $P = 0.024$); and from gloves: 75.2 and 2.3 ($t = 3.28$, $P = 0.011$) respectively. No spores were recovered from unexposed (control) fabric.

Effect of temperature and age on spore germination. Slower spore germination was observed with the 9-d-old spores (Fig. 6). For example, at 20 to 28 °C, moderate to large numbers of 2-d-old spores germinated within 2 h (5% to 55%), whereas 9-d-old spores required at least 5 to 6 h to germinate equivalent numbers of spores.

Our results demonstrated that spores can survive on mature fern leaves for several days under conditions unfavorable for germination and infection. Spores survived on fern leaves in large to moderate numbers for 5 d, and small numbers could be isolated up to 36 d when the experiment was terminated. Survival capabilities of pathogenic Colletotrichum species in soil and crop debris has been frequently studied (Eastburn and Gubler, 1990; Freeman et al., 2001; Lipps, 1983; MacKenzie et al., 2009; Nair, et al., 1983; Strandberg, 2003; Timmer et al., 1998; Urena-Padilla et al., 2001; Vizvary and Warren, 1982). A thick thatch of leaf litter is normally present in fern production beds, so pathogen survival in diseased leaves is of primary importance. Leaf-debris tests in this study demonstrated that spores can survive in fresh or decaying leaf debris on the soil surface for at least 3.5 weeks. These data are very similar to and corroborate earlier research on spore survival in debris incorporated into the soil (Norman and Strandberg, 1997). Increases in spore numbers recovered during the first 14 to 20 d indicate *C. acutatum* can produce additional new spores on detached diseased leaves. A similar response was observed on diseased leatherleaf fern debris incorporated into soil under both laboratory and outdoor conditions (Norman and Strandberg, 1997) and was also observed during survival studies on C. gloeosporioides (Urena-Padilla et al., 2001).

Germination of 2-d-old spores required 4 to 5 h and temperatures greater than 15 °C. Overall, 2-d -old spores germinated much faster than 9-d-old spores. Our results suggest that spore age and loss of germination capabilities are also important factors in determining the efficacy of surviving spores, but additional studies are needed.

Experimental conditions simulated those likely to be encountered by spores of *C. acutatum* found on tools, vehicles, equipment and clothing. Fern workers often work or harvest under contract and can visit several farms and fields each day. Cultural work and harvesting is routinely done when foliage is wet from rainfall or dew. Foliage wet from rain or dew may not dry until mid-day. Spores found on small pieces of leaf debris survived up to 42 d giving ample opportunities for the pathogen to be picked up on surfaces and spread between farms. Large numbers of spores were recovered from fabric patches attached to clothing of work-

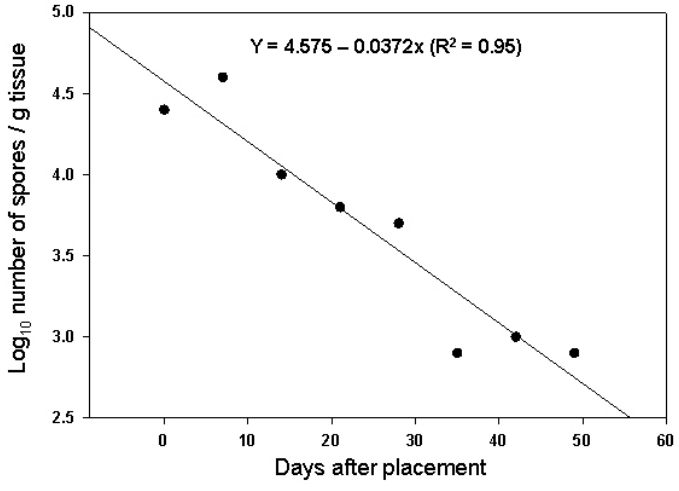

Fig. 4. Recovery of viable *Colletotrichum acutatum* spores from leaf debris samples placed on soil surface of leatherleaf planting. Values are averages for four replicates.

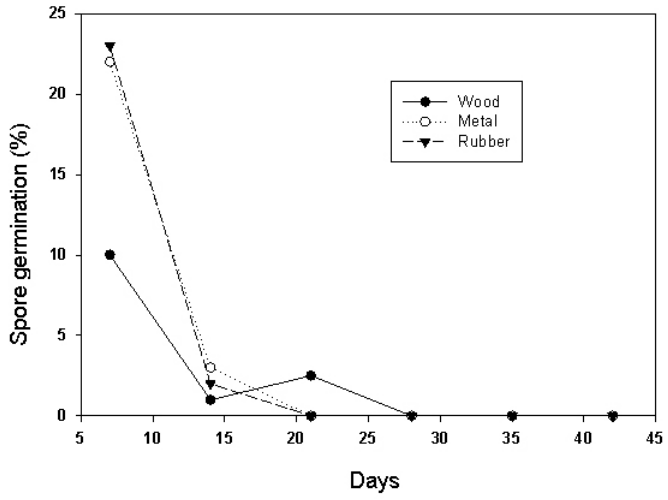

Fig. 5. Percent germination of *Colletotrichum acutatum* spores compared to the initial mean germination on day zero compared with day zero values recovered from inert substrates following exposure to infected fern leaves. Sampling units were kept outdoors, but protected from rain and direct sunlight.

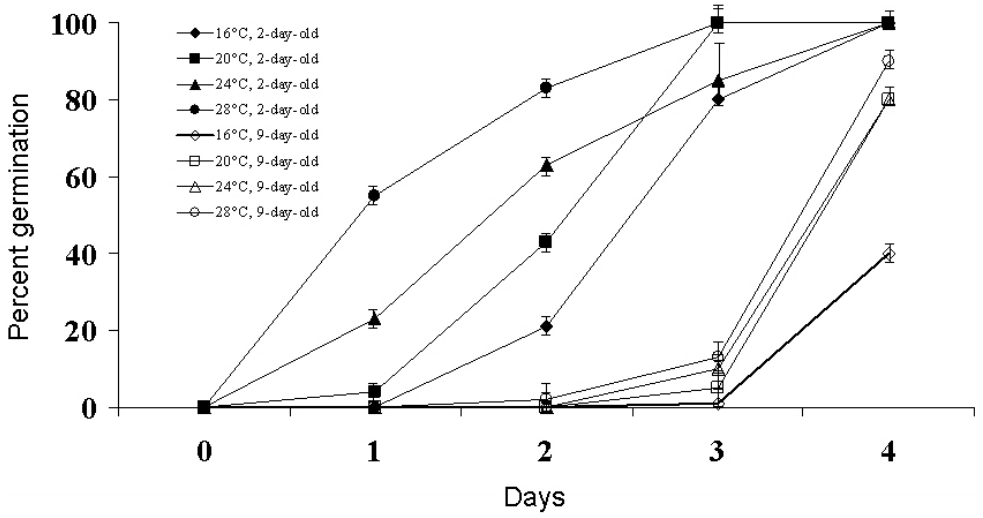

Fig. 6. Effect of temperature (16 to 28 °C) on the germination of 2- and 9-d-old spores of *Colletotrichum acutatum*.

ers engaged in harvesting activities, and significant differences existed between wet and dry harvesting conditions. A previous study showed that spores could survive on dry cotton fabric at 25 °C for up to 6 weeks in numbers sufficient to infect fern but could be killed by laundering (Norman and Strandberg, 1997; Urena-Padilla et al., 2001). Contamination of worker's clothing and equipment coupled with prolonged survival capabilities, and subsequent dissemination, demonstrate likely methods by which fern anthracnose is rapidly spread between and within ferneries (Norman and Strandberg, 1997; Urena-Padilla et al., 2001). Rubber sampling units in this study used to simulate worker rubber boots contact and carry numerous spores, but spores are easily dislodged or washed-off by continued contact with wet foliage and soil, whereas, spores adhering to clothing remain there. Spores on inert surfaces such as tools or equipment could be eradicated by several methods including chemicals (Norman and Strandberg, 1997; Urena-Padilla et al., 2001).

Results of this study help to explain the rapid spread of fern anthracnose. Spores are long lived in and survive on crop foliage, crop debris and on inert surfaces. Growers who are knowledgeable concerning sources and survival capabilities of *C. acutatum* inoculum can integrate appropriate sanitation and cultural procedures with effective fungicide programs to successfully manage fern anthracnose.

Literature Cited

Eastburn, D.M. and W.D. Gubler. 1990. Strawberry anthracnose: Detection and survival of *Colletotrichum acutatum* in soil. Plant Dis. 74:161–163.

Eastburn, D.M. and W.D. Gubler. 1992. Effects of soil moisture and temperature on the survival of *Colletotrichum acutatum*. Plant Dis. 76:841–842.

Freeman, S., S. Horowitz, and A. Sharon. 2001. Pathogenic and nonpathogenic lifestyles in *Colletotrichum acutatum* from strawberry and other plants. Phytopathology 91:986–992.

Freeman, S., Z. Shalev, and J. Katan. 2002. Survival in soil of *Colletotrichum acutatum* and *C. gloeosporioides* pathogenic on strawberry. Plant Dis. 86:965–970.

Lipps, P.E. 1983. Survival of *Colletotrichum graminicola* in infested corn residues in Ohio. Plant Dis. 67:102–104.

MacKenzie, S.J., N.A. Peres, M.P. Barquero, L.F. Arauz, and L.W. Timmer. 2009. Host range and genetic relatedness of *Colletotrichum acutatum* isolates from fruit crops and leatherleaf fern in Florida. Phytopathology 99:620–631.

Nair, J., F.J. Newhook, and J.B. Corbin. 1983. Survival of *Colletotrichum acutatum* f. sp. *pinea* in soil and pine debris. Trans. Br. Mycol. Soc. 81:53–63.

Norman, D.J. and J.O. Strandberg. 1997. Survival of *Colletotrichum acutatum* in soil and plant debris of leatherleaf fern (*Rumohra adiantiformis*). Plant Dis. 81:1177–1180.

Schiller, M., M. Lübeck, T. Sundelin, L.F.C. Meléndez, S. Danielsen, D.F. Jensen, and K.M. Ordeñana. 2006. Two subpopulations of *Colletotrichum acutatum* are responsible for anthracnose in strawberry and leatherleaf fern in Costa Rica. Eur. J. Plant Pathol. 116:107–118.

Stamps, R.H., D.J. Norman, and J.O. Strandberg. 1997. Incidence, severity, and control of anthracnose of leatherleaf fern. Proc. Fla. State Hort. Soc. 110:115–118.

Strandberg, J.O., R.H. Stamps, and D.J. Norman. 1997. Fern anthracnose; A guide for effective disease management. Fla. Agr. Expt. Sta. Tech. Bul. 900. Gainesville, FL.

Strandberg, J.O. 2003. Seasonal variations in production and development of leatherleaf fern leaves. Ann. Appl. Biol.143:235–243.

Timmer, L.W., G.E. Brown, and S.E. Zitko. 1998. The role of *Colletotrichum* spp. in postharvest anthracnose of citrus and survival of *C. acutatum* on fruit. Plant Dis. 82:415–418.

Urena-Padilla, A.R., D.J. Mitchell, and D.E. Legard. 2001. Over summer survival of inoculum for *Colletotrichum* crown rot in buried strawberry crown rot tissue. Plant Dis. 85:750–754.

Vizvary, M.A. and H.L. Warren. 1982. Survival of *Colletotrichum graminicola* in soil. Phytopathology 72:522–525.

Waller, J.M. 1992. Colletotrichum diseases of perennial and other cash crops, p. 167–185. In: J.A. Bailey and M.J. Jeger (eds.). *Colletotrichum*: Biology, pathology and control. CAB Intl., Wallingford, Oxon, UK.

Wilson, L.L., L.V. Madden, and M.A. Ellis. 1992. Overwinter survival of *Colletotrichum acutatum* in infected strawberry fruit in Ohio. Plant Dis. 76:948–950.