Postharvest Decay in Florida Leatherleaf Fern

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ABSTRACT

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A *Rhizoctonia* sp., *Cylindrocladium pteridis*, and *C. heptaseptatum* were isolated from leatherleaf fern (*Rumohra adiantiformis*) arriving in Europe from Florida. In laboratory tests, the three organisms were pathogenic. Inoculation of fronds with *Rhizoctonia* sp. resulted in symptoms at 24 C, but decay of the fern increased when injured. Leaflets inoculated with *Rhizoctonia* sp. and held at 10 C showed little decay. *C. pteridis* and *C. heptaseptatum* were highly infectious and more virulent than *Rhizoctonia* sp. Leaflets inoculated with *C. heptaseptatum* or *C. pteridis* became severely infected when held at 24 C but were not infected when held at 4.5 C. Inoculated leaflets held at 24 C for 2 days and subsequently held at 4.5 C were severely infected. Leaflets inoculated with either *Cylindrocladium* species developed lesions that coalesced and produced a bronze-brown decay over 20–100% of the leaflet surface. Benomyl effectively controlled postharvest decay caused by *Rhizoctonia* sp. and *C. pteridis*. A delay in benomyl treatment after inoculation increased the incidence of decay in leaflets.

Additional key words: fungicide, simulated shipping temperature

In 1979, the wholesale value of Florida leatherleaf fern was estimated at $30 million, with about 31% of the crop exported to western Europe (2). The transit period for fern exported to Europe varies from 10 to 23 days (5). During postharvest handling and transit, fern can be subjected to a variety of environmental conditions that may make it vulnerable to deterioration and potential decay (4,5). About 62% of the fern is transported to western Europe in temperature-controlled van containers, whereas the remaining portion is shipped by air (2). Most products shipped via air are subjected to higher ambient temperatures than those shipped in refrigerated containers.

A decay was observed on Florida cut leatherleaf fern (*Rumohra adiantiformis* (G. Forst.) Ching.) arriving in the western European market. The principal symptom was a bronze-brown decay occurring at the margins and apexes of the fronds. In severe instances, entire fronds were decayed. Fronds located in the centers of bunches typically had more decay than those located on the exterior. A species of *Rhizoctonia, Cylindrocladium pteridis* Wolf (9), and *C. heptaseptatum* Sobers, Alfieri, and Knauss (8) were isolated from various diseased lots arriving from Florida. Symptoms caused by these organisms have been described on growing ferns, but few reports are available on postharvest decay (3,7,8). The decay symptoms observed on cut fern were more severe than those reported for intact ferns (8). However, various species of *Cylindrocladium* can cause a multiplicity of symptoms on different ornamental crops (7). In Germany, Schickedanz (6) found *C. macrosporum* (*C. pteridis*) on fronds of leatherleaf fern imported from the United States.

Fungicidal dip of cut leatherleaf fronds is a standard postharvest practice. However, little research has been published on the use of fungicides in cut leatherleaf fern. De Wildt (*unpublished data*) found *Rhizoctonia* sp. in decaying leatherleaf fern arriving in Europe and demonstrated effective control with benomyl. Knauss (3) showed that *Rhizoctonia* sp. is pathogenic to fronds of Florida Ruffle fern during warm periods and is particularly active when plants are crowded and remain wet for long periods. The fungus was controlled by benomyl, thiabendazole, and chlorothalonil.

This paper reports the pathogenicity and symptoms of decay caused by *Rhizoctonia* sp., *C. pteridis,* and *C. heptaseptatum* and the method for their control on cut leatherleaf fern during the postharvest period.

MATERIALS AND METHODS

Detached leaflets (7–8 cm long) were surface-sterilized in sodium hypochlorite solution (5,000 ppm) for 1–1.5 min and rinsed in running tap water for 5 min. A single leaflet was placed in a sterile 9-cm petri plate containing clean, moistened filter paper. Inoculated and control leaflets were held in the dark in an incubator at 24 C, except where temperature was an experimental variable. All organisms were grown on Difco potato-dextrose agar (PDA) and were initially isolated from decayed tissue. *C. pteridis* and *C. heptaseptatum* conidial suspensions were prepared by lightly scraping 10-day-old sporulating PDA cultures with a sterile loop and rinsing the mycelial mat with sterile water. *C. pteridis* and *C. heptaseptatum* inocula typically contained about 2,000 and 2,800 ml of conidia per milliliter, respectively. Distilled or deionized water was used for making conidial suspensions and moistening filter paper. The fungi were reisolated from test leaflets to confirm pathogenesis. All experiments were repeated at least once, and six leaflets were used per treatment.

In experiments with *Rhizoctonia* sp., PDA cultures 1 wk to 10 days old were used to inoculate leaflets. Blocks of PDA (0.25 cm^2) containing the mycelium were placed on injured and uninjured leaflets. Sterile PDA blocks were used on all control (uninoculated) leaflets. Leaflets were held at 4.5, 10, 21, and 25.5 C

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for 10 days. Number of infected leaflets was noted after 6, 8, and 10 days.

For *C. pteridis,* leaflets were inoculated by being dipped in an aqueous conidial suspension. Leaflets were dipped in benomyl immediately, 6 hr, or 24 hr after inoculation. Control (uninoculated) leaflets were dipped in water or benomyl. Lesions on each leaflet were counted after 3 days, and the percentage of the leaflet decayed was estimated after 15 days.

For *C. heptaseptatum,* leaflets were dipped in a conidial aqueous suspension or in water and placed in incubators in the dark at 4.5, 10, 21, and 26.5 C for 10 days. Number of lesions per leaflet and percentage of leaflet decayed after 3, 5, and 10 days, respectively, were noted.

Leaflets were inoculated with *C. pteridis* or *C. heptaseptatum.* Control and inoculated leaflets were placed at 4.5 C or placed at 24 C for 1 or 2 days and subsequently placed at 4.5 C. A group of inoculated and control leaflets was held continuously at 24 C. The percentage of decay caused by *C. heptaseptatum* and *C. pteridis* was estimated after 12 and 18 days, respectively.

Leaflets were inoculated with *C. pteridis* and *Rhizoctonia* sp. and compared. The organisms were grown on PDA as outlined above and in Blakeslee's malt extract medium (1). Fungi from both media were used for inoculating leaflets. Ten to 15 ml of the medium was added to cover the bottom of a 500-ml flask. The mycelial mat from 10-day-old cultures was washed with sterile water, broken up (chopped) with a sterile glass rod, and suspended in 100 ml of sterile water. Leaflets were inoculated by being dipped in one of the two fungal suspensions. Sections (0.25 cm^2) of mycelial mat from PDA cultures were placed on other leaflets. Leaflets were also dipped in an aqueous conidial suspension of *C. pteridis.* Control (uninoculated) fronds were dipped in sterile water. Disease severity was rated after 3 and 8 days on a 0–3 scale: 0 = no infection; 1 = incipient infection, 1–2% of tissue decayed; 2 = moderate infection, 3–20% of tissue decayed; 3 = severe infection, more than 20% tissue decayed.

RESULTS AND DISCUSSION

Rhizoctonia sp. caused postharvest decay in leatherleaf fern, although leaflets had to be injured for early disease development (*data not shown*). After 5 days, injured and inoculated leaflets had incipient decay that after 10 days turned bronze-brown. Leaflets inoculated but not injured showed symptoms at 10 days; however, the decay was not as severe as that in injured and inoculated leaflets. Control (uninoculated, injured and uninjured) leaflets were free of decay symptoms.

Injured and inoculated leaflets held at 4.5 C did not decay. As temperature increased, the incidence of decay increased. Leaflets held at 21 and 25.5 C were infected after 6 days and turned bronze-brown after 10 days. Two of the inoculated leaflets held at 10 C had incipient decay but did not turn bronze-brown.

Leaflets inoculated with *C. pteridis* and held at 24 C developed lesions and decayed (Table 1). Initial infections by *C. pteridis* were small, irregular, circular, brown lesions. Lesions enlarged and coalesced; the affected decay area became bronze-brown and water-soaked. Benomyl reduced the number of lesions and the incidence of decay, but the longer the benomyl dip was delayed after inoculation, the greater the incidence of decay.

C. heptaseptatum caused serious decay (Table 2). Initial disease symptoms were similar to those previously described (6). Lesions on inoculated fronds continued to enlarge, eventually coalescing. Leaflets held at 21 and 26.5 C had severe decay after 5 days. The late stage of the decay was a bronze-brown rot, similar to that caused by *C. pteridis.*

Leaflets inoculated with *C. pteridis* and held at 4.5 C for 18 days had no visible symptoms of infection (Table 3). Inoculated leaflets held for 2 days at 24 C and subsequently held at 4.5 C for 16 days (18 days from inoculation) had 40% of the leaflet surface decayed. Inoculated leaflets held continuously at 24 C were severely decayed. Inoculated leaflets held at 24 C for 1 day and moved to 4.5 C for 17 days were not infected.

Leaflets inoculated with *C. heptaseptatum* and held at 4.5 C for 12 days had few lesions (Table 3). Inoculated leaflets held at 24 C for 2 days had lesions that were beginning to coalesce and turn the leaf bronze-brown. Leaflets held at 24 C had 83% decay after 3 days (*data not shown*) and 99% decay after 12 days.

Leaflets inoculated with *C. pteridis* became infected earlier and had more tissue decayed than leaflets inoculated with *Rhizoctonia* sp. (Table 4). Leaflets infected with *Rhizoctonia* sp. did not develop any lesions, but after 8 days affected areas were bronze-brown, similar to the decay caused by *C. pteridis.* After 8 days, it was difficult to differentiate differences in symptoms of decay in leaflets infected with *Rhizoctonia* sp. or *C. pteridis.*

All fungi tested caused postharvest decay in leatherleaf fern. The initial symptoms caused by the two *Cylindrocladium* species were lesions that were similar to those previously described (6). In the late stage of infection, the symptoms caused by *C. pteridis, C. heptaseptatum,* and *Rhizoctonia* sp. were similar and could not be differentiated. Entire leaflets inoculated with the two *Cylindrocladium* species decayed. The symptoms we observed in these tests were similar to those observed in commercial fern shipments arriving in Europe. *C. heptaseptatum* has been found in fern imported into Florida from Honduras (6). However, this is the first time the organism has been reported in a terminal market.

Temperature had a profound effect on disease development, which is very significant from the commercial standpoint. In our tests, only fern subjected to elevated temperature (ie, 24 C) decayed. Fern shipped from Florida to western Europe is transported in refrigerated containers at 1.1–4.4 C (3). This suggests that commercial fern arriving with severe decay must have been naturally inoculated before or during the harvest and handling periods and subsequently exposed to warm temperatures at some time during the postharvest period. We observed that ferns sent via air had a greater incidence of decay than those shipped by refrigerated van containers. Horticultural products shipped via air usually have higher temperatures during transit than those shipped by refrigerated containers. We have no in-transit temperature records for air shipments, but fern shipped from Florida by air in late spring to autumn had more decay problems than fern shipped by containers.

Table 1. Influence of *Cylindrocladium pteridis* inoculation and benomyl dip on number of lesions and percentage of decay on cut leatherleaf fern leaflets held at 24 C

Treatment	Benomyl or water dip	Time of benomyl dip	Lesions after 3 days (no.)	Decay after 15 days (%)
Control (uninoculated)	Water	···	0.0	4.0
Control (uninoculated)	Benomyl	After inoculation	0.0	0.0
Inoculated	Water	···	57.3	100.0
Inoculated	Benomyl	After inoculation	1.0	19.0
Inoculated	Benomyl	6 hr after inoculation	2.7	43.0
Inoculated	Benomyl	24 hr after inoculation	35.5	98.0

Table 2. Influence of temperature and inoculation with *Cylindrocladium heptaseptatum* on number of lesions after 3 days and percentage of decay after 5 and 10 days on leatherleaf fern leaflets

Temperature (C)	Lesions/leaflet after 3 days[z]	Decay (%) after 5 days	Decay (%) after 10 days
4.5	0	1	1
10	2.5	6	18
21	9.0	39	99
26.5	23.5	65	94

[z]Control (uninoculated) leaflets held at all temperatures had no symptoms.

Table 3. Influence of *Cylindrocladium heptaseptatum* and *C. pteridis* inoculations on percentage of decay on cut leatherleaf fern leaflets exposed to 24 C for 1 or 2 days and subsequently held at 4.5 C

Treatment	Days held at 24 C	*C. heptaseptatum* Decay (%) after 12 days	*C. pteridis* Decay (%) after 18 days
Control	0[y]	0.0	0.0
Inoculated	0[y]	1.4[z]	0.0
Control	1	0.0	0.0
Inoculated	1	1.6[z]	0.0
Control	2	0.0	0.0
Inoculated	2	4.0[z]	40.0
Control	Continuous	0.0	0.0
Inoculated	Continuous	99.0	100.0

[y] Held continuously at 4.5 C.
[z] Indicates number of lesions per leaflet. Lesions were beginning to coalesce on leaflets exposed to 24 C for 2 days

Table 4. Influence of *Rhizoctonia* sp., *Cylindrocladium pteridis*, and method of inoculation on severity of infection in leatherleaf fern when held at 24 C for 8 days

Pathogen	Inoculation method	Disease severity[z] after 3 days	Disease severity[z] after 8 days
Control (no inoculation)	...	0.0	0.0
Rhizoctonia sp.	Mycelial section	0.3	0.3
Rhizoctonia sp.	Chopped mycelium	0.3	1.3
Cylindrocladium pteridis	Mycelial section	0.5	2.3
C. pteridis	Chopped mycelium	3.0	3.0
C. pteridis	Conidial suspension	3.0	3.0

[z] 0 = no decay; 1 = incipient infection, 1–2% of tissue decayed; 2 = moderate infection, 3–20% of tissue decayed; 3 = severe infection, more than 20% of tissue decayed.

Benomyl used as a postharvest fungicide on leatherleaf fern 6 hr after inoculation effectively controlled *C. pteridis* for 3 days (Table 1). Benomyl used as a dip 1 day after inoculation delayed but did not prevent decay as effectively as a dip applied immediately after inoculation. Control (uninoculated) leaflets remained free of decay and had no visible symptoms of deterioration after 15 days.

These data demonstrate that timely postharvest application of benomyl coupled with proper temperature control are critically important for delivery of decay-free leatherleaf fern to western Europe.

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