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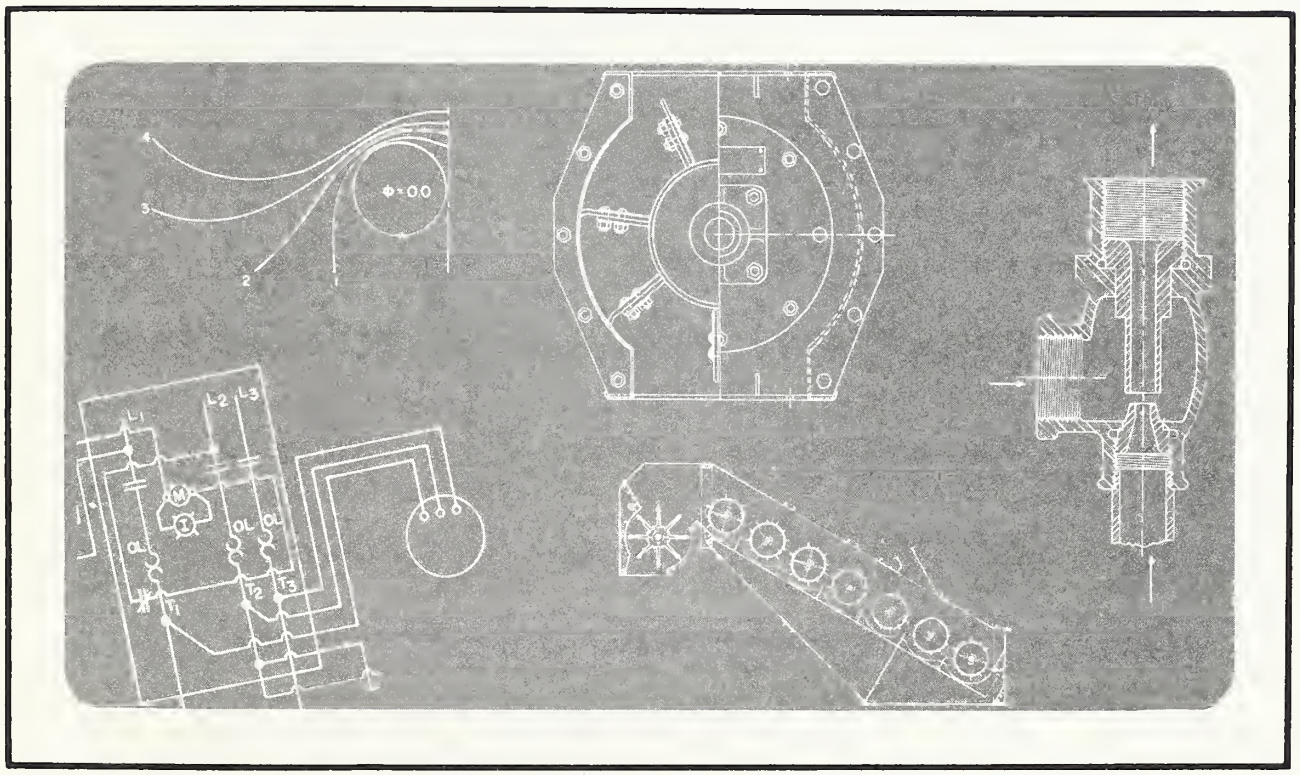
Control of *Cylindrocladium* Decay in Leatherleaf Fern Shipped From Florida to Europe



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Control of *Cylindrocladium* Decay in Leatherleaf Fern Shipped From Florida to Europe

By F. J. Marousky,[1] L. A. Risse,[2] and A. Dow[3]

ABSTRACT

In air and sea shipments to Rotterdam, the Netherlands, postharvest decay caused by *Cylindrocladium pteridis* and *C. heptaseptatum* was controlled by proper use of a fungicidal dip and maintenance of the proper temperature. Fern inoculated with *C. pteridis* and held at low temperature during the handling and transit period did not decay, but inoculated fern held at 21° C for 24 to 48 hours after harvest and subsequently transported at 2.8° C arrived in Rotterdam severely decayed. Fern inoculated with *C. pteridis* and dipped in a mixture of benomyl and chlorothalonil 24 hours later became severely decayed; the fungicide treatment was effective only when applied within 4 hours of harvest. Fern inoculated with *C. heptaseptatum* and *C. pteridis* and shipped airfreight arrived in Rotterdam severely decayed, whether precooled or not; temperatures during the 46-hour transit were recorded at 15°–21° C. *C. heptaseptatum* produced more decay than *C. pteridis*, but it was not determined if the difference was due to virulence or inoculum levels. Index terms: airfreight, benomyl, chlorothalonil, *Cylindrocladium heptaseptatum* Sobers, Alfieri, et Knauss, *Cylindrocladium pteridis* Wolf, export handling (of produce), fungicide treatments (for produce), leatherleaf fern, postharvest handling, *Rumohra adiantiformis* (G. Forst.) Ching, refrigerated van containers, shipping methods, shipping temperatures.

INTRODUCTION

Leatherleaf fern, *Rumohra adiantiformis* (G. Forst.) Ching, is an important cut-foliage crop in Florida. During the 1980–81 season, wholesale shipments were valued at over $50 million, and about 27% of this fern was transported to western Europe by ship in refrigerated van containers, while a small portion was shipped unrefrigerated by air, according to the Florida Department of Agriculture and Consumer Services (1982).

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The transit period to Europe can vary from a few days by air to 3 weeks by sea (Miller et al. 1979). During this interim, the fern can be subjected to adverse environmental conditions, pathogens, and physical damage. European receivers have increasingly complained of decay, and so efforts have been made to identify the pathogens. Schickedanz (1974) found *Cylindrocladium macrosporum* (that is, *C. pteridis* Wolf, 1926) in decaying Florida leatherleaf fern shipped into Germany. Marousky and De Wildt (1982) isolated a *Rhizoctonia* sp., *Cylindrocladium pteridis*, and *C. heptaseptatum* Sobers, Alfieri, et Knauss (1975), from decaying leatherleaf fern. All three fungi were pathogenic, but the *Cylindrocladium* spp. were the most virulent. The fungi were easily controlled by dipping freshly harvested fern in benomyl[4] and chlorothalonil.[5] Similarly, Knauss (1971) controlled a *Rhizontonia* sp. on potted fern with benomyl, thiabendazole[6], or chlorothalonil. Marousky et al. (1981) showed that decay caused by *C. heptaseptatum* in commercial shipments could easily be controlled by dipping the fern in fungicide and controlling the pretransit and transit temperatures.

No information is available on decay caused by *Cylindrocladium* when ferns are shipped by unrefrigerated airfreight. In this paper we report the influence of pretransit temperatures, postharvest fungicidal use, and airfreight and refrigerated shipping containers on decay of Florida leatherleaf fern inoculated with *C. pteridis* or *C. heptaseptatum*.

METHODS AND MATERIALS

Fern. — The leatherleaf fern used in these experiments was obtained from commercial nurseries around Zellwood and DeLand, Fla. Freshly harvested fronds were bunched (25 stems per bunch) in the field in the morning and transported to our laboratory in Orlando for treatment that afternoon.

Inocula. — *Cylindrocladium pteridis* and *C. heptaseptatum* were grown on petri plates containing potato dextrose agar (Difco). The plates were incubated at 23°–24° C, and sporulation occurred in about 10 days. To make the inocula, 10–15 sporulating cultures of each species were scraped lightly with a sterile needle, each plate was rinsed with 500 ml of sterile water, and the conidial effluents of each fungus were combined. The fungi were originally isolated from decayed ferns as described by Marousky and De Wildt (1982).

Inoculation. — Entire bunches of fern were dipped momentarily in tapwater (the controls) or in one of the conidial suspensions. After dipping, the bunches were drained for approximately 30–40 seconds, placed in a controlled temperature (21° C) and humidity (88%–92%) room, covered with polyethylene sheets, and held for 8–48 hours, depending on the test.

Fungicide treatments. — The fern bunches were dipped in a mixture of benomyl (0.35 g/l of active ingredient, supplied as Benlate 50 WP) and chlorothalonil (1.0 g/l of active ingredient, supplied as Daconil 75 WP) or in tapwater (the controls) for 30 seconds and then drained. All ferns were treated except where temperature was to be the experimental variable.

Packaging and shipping. — After inoculation and handling, the bunches of fern were usually placed in large polyethylene bags and packed in standard commercial waxed fiberboard shipping cartons 30 inches long by 14 inches wide by 9 inches high (75 by 35 by 23 cm). Ryan temperature recorders were placed in the center of each carton. For each test, duplicate groups of fern were packaged. One group of cartons was taken to a commercial fern exporter for shipment in a refrigerated van container set at 2.8° C or by airfreight to Rotterdam, the Netherlands. The other group was held in Orlando under refrigeration at 1°–2° C to simulate transit conditions.

Decay rating. — The ferns in Orlando were removed from simulated transit conditions at about the time the corresponding ferns arrived in Rotterdam. At both places each frond in each bunch was evaluated for decay and an average rating determined. Each frond was again evaluated after being held for 1 or 2 weeks. In Rotterdam, ferns were held at 21° C, except those in test 1 (table 1), which were held at 4.5° C. In Orlando, the stem ends were trimmed, and the ferns were held in a storage room maintained at 21° C and 88%–92% relative humidity. In Rotterdam, the fungi

[4]Methyl 1 - (butylcarbamoyl) - 2 - benzimidazolecarbamate.

[5]Tetrachloroisophthalonitrile.

[6]2-(4-Thiazolyl) benzimidazole.

Table 1. — Influence of *Cylindrocladium* inoculation and treatment with benomyl-chlorothalonil mixture on decay of fern shipped in a refrigerated van container

Inoculation	Fungicide-treated?	Average decay rating[1] at —			
		Rotterdam —		Orlando[2] —	
		On arrival	After 2 weeks at 4.5° C	On arrival	After 1 week at 21° C
None	No	1.0c	1.1c	1.1c	1.3c
	Yes	1.2c	1.2c	1.1c	1.1c
C. pteridis	No	2.2b	2.5b	2.2b	2.7b
	Yes	2.0b	2.2b	1.7bc	2.1b
C. heptaseptatum	No	4.0a	4.3a	4.0a	4.5a
	Yes	4.0a	4.7a	3.6a	4.0a

[1]Ratings are based on the percentage of frond surface area that decayed: 1, no decay; 2, little decay, 1%–2%; 3, severe decay, 3%–15%; 4, 16%–50%; 5, 51% and over. Means in a column followed by different letters are significantly different at the 1% level by Duncan's new multiple-range test.

[2]Simulated shipment; see text.

Table 2. — Influence of *Cylindrocladium pteridis* inoculation and time of treatment with benomyl-chlorothalonil mixture on decay of fern shipped in a refrigerated van container

Fungicide treatment —	Average decay rating[1] at —		
	Rotterdam on arrival	Orlando[2] —	
		On arrival	After 1 week at 21° C
For uninoculated fern:			
None	1.0b	1.1b	1.5b
24 hours after inoculation	1.0b	1.1b	1.3b
For inoculated fern:			
None	2.5a	1.8a	3.9a
Immediately after inoculation	1.0b	1.0b	1.1b
4 hours after inoculation	1.3b	1.0b	1.3b
24 hours after inoculation	2.3a	1.7a	3.0a

[1]Ratings are based on the percentage of frond surface area that decayed: 1, no decay; 2, little decay, 1%–2%; 3, severe decay, 3%–15%; 4, 16%–50%; 5, 51% and over. Means in a column followed by different letters are significantly different at the 1% level by Duncan's new multiple-range test.

[2]Simulated shipment; see text.

Table 3. — Decay of fern as influenced by *Cylindrocladium pteridis* inoculation and time held at 21° C before shipment in a refrigerated van container

Time at 21° C before shipping[1] —	Average decay rating[2] at —			
	Rotterdam —		Orlando[3] —	
	On arrival[4]	After 1 week at 21° C[5]	On arrival[6]	After 1 week at 21° C[5]
Uninoculated fern:				
None	1.0	1.0	1.1	1.5
8 hours	1.0	1.0	1.1	1.2
24 hours	1.0	1.0	1.1	1.3
48 hours	1.0	1.0	1.4	1.7
Inoculated fern:				
None	1.0	1.2	1.1	2.1
8 hours	1.0	1.5	1.2	2.0
24 hours	1.0	2.0	1.2	2.4
48 hours	1.5	2.0	1.7	2.9

[1]When not at 21° C, ferns were held at 1°–1.5° C.

[2]Ratings are based on the percentage of frond surface area that decayed: 1, no decay; 2, little decay, 1%–2%; 3, severe decay, 3%–15%; 4, 16%–50%; 5, 51% and over.

[3]Simulated shipment; see text.

[4]Inoculation and time main effects and inoculation × time interaction are not significant.

[5]Inoculation and time main effects and inoculation × time interaction are significant at the 5% level.

[6]Time main effect is significant at the 1% level. Inoculation main effect and inoculation × time interaction are not significant.

were reisolated from test leaflets to confirm pathogenesis. No reisolations were made in Orlando.

TEST RESULTS

Inoculation and fungicide treatments. — Inoculated ferns and controls were held under plastic sheeting at 21° C for 24 hours, dipped in the fungicide mixture or tapwater, covered with plastic sheeting and held at 21° C for 48 hours, and finally packaged for transit in a refrigerated van container. Transit time to Rotterdam was 15 days. The inoculated ferns transported to Rotterdam and held in Orlando were similarly decayed (table 1). *C. heptaseptatum* produced more decay, but we could not determine if this result was due to greater virulence of this fungus or to the larger number of conidia that it produced. The fungicidal dip 24 hours after inoculation did not prevent infection or control decay. The temperature recorded inside the cartons during transit was maintained within ±1.5° C of the thermostat setting, 2.8° C.

Time of fungicide dip. — Thirty-two bunches of fern were inoculated with *C. pteridis* and 16 bunches were dipped in water (the controls), covered with plastic sheeting and held at 21° C for 48 hours, and during this interval, dipped in the fungicide mixture at various times (table 2). Seventy-two hours after inoculation, the ferns were packaged and shipped to Rotterdam in a refrigerated van container. The fern reached Rotterdam in 13 days; the fern in Orlando was evaluated after 15 days of simulated transit. The ferns dipped in benomyl-chlorothalonil immediately or 4 hours after inoculation decayed little, while those dipped 24 hours after inoculation decayed about the same as those not treated with the fungicide mixture (table 2). Results were similar in Orlando.

Pretransit temperature. — Thirty-two bunches of fern were inoculated with *C. pteridis* and 32 were dipped in water (the controls). Random

Table 4. — Influence of *Cylindrocladium pteridis* inoculation and temperature before shipment on decay of fern shipped airfreight and in a refrigerated van container

Treatment[1]	Average decay rating[2] at —			
	Rotterdam —		Orlando[3] —	
	On arrival	After 1 week at 21° C	On arrival	After 1 week at 21° C
	Shipped airfreight			
Held at 21° C before shipment:				
Uninoculated	1.0	1.0	1.0	1.1
Inoculated	2.5	3.8	1.2	2.8
Held at 1.5° C before shipment:				
Uninoculated	1.0	1.0	1.1	1.1
Inoculated	1.0	2.5	1.1	1.2
	Shipped in refrigerated container			
Held at 21° C before shipment:				
Uninoculated	1.0	1.3	1.2	1.5
Inoculated	1.3	2.3	1.5	2.1
Held at 1.5° C before shipment:				
Uninoculated	1.0	1.3	1.1	1.2
Inoculated	1.0	2.5	1.4	1.9

[1]Holding time 24 hours.

[2]Ratings are based on the percentage of frond surface area that decayed: 1, no decay; 2, little decay, 1%–2%; 3, severe decay, 3%–15%; 4, 16%–50%; 5, 51% and over. Inoculation, shipping method, and temperature main effects and all interactions for a given column are significant at the 1% level.

[3]Simulated shipment; see text.

bunches from each lot were placed in polyethylene bags and held at 21° C for 0, 8, 24, or 48 hours (the ferns were held at 1°–1.5° C when not at 21° C), after which the bunches were packed and shipped to Rotterdam in a refrigerated van container. Transit time to Rotterdam was 12 days; the fern held in Orlando was rated after 10 days. At both locations the bunches were held at 21° C for 1 week and reevaluated for decay. The uninoculated fern held at 21° C for up to 48 hours before being shipped arrived in Rotterdam free of decay, and even the infected fern had little decay on arrival (table 3). Holding at 21° C for 1 week increased decay. (Note that there was little decay in this test compared to the previous ones.) Results in Orlando were similar.

Pretransit temperature and transit methods. — Thirty-two bunches of fern were inoculated with *C. pteridis* and 32 were dipped in water (the controls). Each bunch was placed in an individual plastic bag, and the bags were packed in cartons, which were held at 21° or at 1.5° C for 24 hours before being shipped. One-half of the cartons held at each temperature were transported to Rotterdam by airfreight; the other half were transported by ship in a refrigerated van container. The cartons transported by airfreight were at ambient temperature, while the cartons transported in the van container were at 2.8° C, as before. Shipping time was 74 hours by air and 17 days by sea. In Orlando, duplicate ferns were held at 4.5° C for 4 and 17 days to simulate transit by air and ship. The same experiment was done with *C. heptaseptatum*, but transport time was 69 hours by air and 23 days by sea.

Ferns inoculated with *C. pteridis* and held at 21° C for 24 hours prior to transit had decay on arrival in Rotterdam, and those transported by air had more decay than those transported in a temperature-controlled container (table 4). No

Table 5. — Influence of *Cylindrocladium heptaseptatum* inoculation and temperature before shipment on decay of fern shipped airfreight and in a refrigerated van container

Treatment[1]	Average decay rating[2] at —			
	Rotterdam —		Orlando[3] —	
	On arrival	After 1 week at 21° C	On arrival	After 1 week at 21° C
	Shipped airfreight			
Held at 21° C before shipment:				
Uninoculated	1.0	1.3	1.0	1.1
Inoculated	4.7	5.0	5.0	5.0
Held at 1.5° C before shipment:				
Uninoculated	1.0	1.0	1.0	1.0
Inoculated	1.4	5.0	1.0	1.2
	Shipped in refrigerated container			
Held at 21° C before shipment:				
Uninoculated	1.0	1.1	1.1	1.1
Inoculated	1.2	2.6	5.0	5.0
Held at 1.5° C before shipment:				
Uninoculated	1.0	1.0	1.0	1.0
Inoculated	1.1	1.7	1.0	1.2

[1]Holding time 24 hours.

[2]Ratings are based on the percentage of frond surface area that decayed: 1, no decay; 2, little decay, 1%–2%; 3, severe decay, 3%–15%; 4, 16%–50%; 5, 51% and over. Inoculation, shipping method, and temperature main effects and all interactions for a given column are significant at the 1% level.

[3]Simulated shipment; see text.

uninoculated fern decayed, irrespective of shipping method. Holding inoculated fern for an additional week at 21° C increased decay. Holding uninoculated air-shipped fern produced no decay, whereas holding uninoculated sea-shipped fern resulted in slightly increased levels. Fern held in simulated transit conditions in Orlando had lower levels of decay than those observed in Rotterdam.

Ferns inoculated with *C. heptaseptatum* and held at 21° C for 24 hours before transport by air to Rotterdam arrived severely decayed, while those held at 1.5° C were less decayed (table 5). Inoculated ferns transported in a temperature-controlled container arrived in Rotterdam with less decay than those transported by air. All inoculated fern had more decay after being held at 21° C for 1 week.

All fern transported by air was too hot (between 15°–20° C during the 46-hour trip), while fern transported in a refrigerated van container had good temperature control (a bit less than 1.5° C during the 23-day voyage).

DISCUSSION

The symptoms of postharvest decay in leatherleaf fern caused by both *Cylindrocladium* species were similar to those reported earlier, and postharvest dips in benomyl-chlorothalonil controlled *C. pteridis* as effectively as those reported for *C. heptaseptatum* (Marousky and De Wildt 1982). But timing was important: Freshly inoculated fern dipped in fungicide did not decay, but there was little decay control when fern was dipped in fungicide 1 day after inoculation. This suggests that chance infections during harvest would be controlled by fungicidal dips but that fern infected in the nursery would not. For postharvest

decay control, it is essential to control *Cylindrocladium* in the nursery. Next, it is important to control postharvest temperatures. Uninoculated fern held at 21° C for 1 day and exposed to warm transit conditions did not decay; only inoculated ferns held at high temperatures decayed, and the longer they were held at warm temperatures, the greater the decay was (tables 3 and 4). Fern transported airfreight had more decay because temperatures were not controlled and were in a range for growth of *Cylindrocladium* (Marousky and De Wildt 1982).

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