Latent Colonization and Pathogenicity of *Hypoxylon atropunctatum* on Oaks

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ABSTRACT

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Decay of phloem and sapwood by *Hypoxylon atropunctatum*, and stromata of this fungus that developed on inoculated and girdled oak trees were not associated with inoculation wounds. The fungus was recovered with equal frequency from discolored sapwood beneath inoculated and control wounds. *H. atropunctatum* was isolated from branches of 57% and trunks of 11% of healthy-appearing oaks. Isolation frequencies from black and white oaks were similar. Girdling of 196 uninoculated, healthy-appearing trees resulted in the development of *H. atropunctatum* on 77% of the black and 70% of the white oaks within 5 mo. Latent colonization probably accounts for the rapid increases in disease incidence following drought. The greater natural incidence of disease on black oaks than on white oaks may be related to host differences in drought sensitivity.

In oaks, *Hypoxylon atropunctatum* (Schw. ex Fr.) Cke. decays the phloem and sapwood, causing sloughing of the outer bark and forming extensive stromata. The disease is associated with tree decline and death (3,11,12,15,16). Incidence has risen following periods of drought (3,14,15), especially the drought in 1980 throughout the South (1,2,4,13). Incidence was greater on red oak species (subgenus *Erythrobalanus*) than on white oak species (*Leucobalanus*) (1,4).

This disease has been associated with drought stress, root injury, and other diseases (2,3,5,9,11,12,16,17), but little is known about the pathogenicity and ecological behavior of *H. atropunctatum*. Thompson (14,15) reported that *H. atropunctatum* could parasitize inoculated living oaks and cause tree death, but other workers have considered the fungus as only a weak parasite and a secondary invader of trees debilitated or killed by other diseases or abiotic factors (3,4,12,17).

These studies were undertaken to reexamine by inoculations the pathogenicity of *H. atropunctatum* to several oak species in relation to imposed stress and to locate the reservoir of inoculum that fosters rapid increases in incidence and severity.

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MATERIALS AND METHODS

Fungal isolates and culture techniques. Three polyascosporous isolates of *H. atropunctatum*, one each from a white oak (*Quercus alba* L.), a blackjack oak (*Q. marilandica* Muenchh.), and a black oak (*Q. velutina* Lam.), were obtained by attaching moistened pieces of perithecial stromata to the insides of petri dish covers. Ascospores were ejected onto agar media during 4–12 hr at room temperature. After spore germination, cultures were transferred to 2.5% malt agar slants and stored at 4 C. Identification of the fungus was based on stromal and ascospore characteristics (3).

Yeast-extract glucose agar (YEGA) containing 1.5 g yeast extract (Difco), 10 g D-glucose, and 20 g agar per liter of distilled water was used for isolation and culture studies. *H. atropunctatum* grew rapidly, produced distinctive white to gray mycelial strands, and consistently produced yellow to green pigments in this medium.

Inoculations. Inoculations were done to determine the pathogenicity of the three isolates of *H. atropunctatum* on four oak species. All inoculated trees were in a mixed oak and hickory stand on a Nixa cherty silt loam near Fayetteville, AR, and ranged from 8 to 25 cm in diameter at breast height (dbh). Each experimental group contained 20 trees: five black, five blackjack, five white, and five post oaks (*Q. stellata* Wangenh.).

In the first experiment, one group of trees was inoculated in June 1981 and girdled 1–3 cm into the sapwood in April 1982. A second group of trees was inoculated in August 1981 and left ungirdled. In a second experiment, two groups were inoculated in April 1982 and trees in one group were girdled in May 1982. In October 1982, all 80 trees were felled, dissected, and examined. Sapwood discoloration was measured and samples of the discolored wood were removed 3–8 cm from the inoculated and control wounds and plated on YEGA for recovery of *H. atropunctatum*.

Inoculations were made with mycelium in a manner similar to that used by Thompson (15) on oaks and by Shea (8) on aspen with *H. mammatum* (Wahl.) Mill. Holes 1.43 cm in diameter were drilled in the trunk to a depth of 2–3 mm into the xylem. Disks 1.4 cm in diameter from 4-day-old cultures on YEGA were inserted mycelium-first into the holes, which were then plugged with sterile cotton that was moistened with distilled water and covered with adhesive tape to retard moisture loss. For controls, the same procedure was followed, using disks of sterile YEGA. Inoculation instruments were dipped in alcohol and flamed between inoculations. Each tree received one control wound and three inoculated wounds, one for each host isolate of *H. atropunctatum*. Positions of the inoculation and control holes corresponded to cardinal compass points and were separated on the tree by vertical distances of 0.5 m.

Survey and isolations. A total of 300 black/red (mostly black) oaks and white oaks growing at three locations in the Ozark National Forest that showed no symptoms of disease or injury were sampled for *H. atropunctatum*. They ranged from 10 to 50 cm dbh and included all crown classes, from suppressed to dominant. Two branch sections about 1 cm in diameter were removed from each tree with a pole pruner, and two trunk pieces were removed with a hand ax. Samples were stored in individual plastic bags at 4 C for up to 3 days. The outer bark was removed from the trunk samples and these were trimmed to make three to five subsamples (about 1 cm^3), each containing phloem, cambium, and sapwood. Three to five bark-covered cross sections (about 0.5 cm^3) were cut from each branch sample, dipped in 95% ethanol, flamed, and plated on YEGA. Plates were incubated at 30 C and examined after five or more days. Identification of *H. atropunctatum* growing from the samples was based on comparisons of culture characteristics with those of stock cultures grown at the same time.

Girdling experiment. Girdling was used as a standard treatment to compare development of *H. atropunctatum* on severely stressed black and white oaks. A total of 196 black oaks and white oaks of

different crown classes and with no signs of *H. atropunctatum*, injury, wilt, or dieback were selected from among the trees growing in a mixed oak stand on a Nixa cherty silt loam soil. In May 1982, the trees were girdled at the base with a chain saw to depths varying from 1.5 to 3 cm, depending on dbh (10–25 cm) and bark thickness. Girdled trees were observed during the next 4–5 mo and signs of *H. atropunctatum* recorded. A survey was done to determine the incidence of *H. atropunctatum* on black oaks and white oaks in the vicinity of the girdled trees.

Table 1. Vertical extent of sapwood discoloration in oaks inoculated with three isolates of *Hypoxylon atropunctatum*[x]

Oak species	Length of discoloration (cm) by isolate[y]				
	Control	Black	Blackjack	White	Average
Inoculated June 1981, girdled April 1982					
Black	8.5[z]	23.2	13.4	18.2	15.8 a
Blackjack	3.9	11.9	12.2	11.4	9.9 b
White	5.6	15.0	11.7	12.5	11.2 b
Post	4.9	6.9	6.4	7.1	6.4 c
Average	5.7 b	14.3 a	11.0 a	12.3 a	···
Inoculated August 1981, not girdled					
Black	12.4	19.0	17.6	15.7	16.2 a
Blackjack	7.2	10.0	11.3	13.4	11.0 b
White	8.6	10.5	15.3	12.0	11.6 b
Post	3.6	7.3	10.3	9.6	7.7 b
Average	7.9 b	11.7 ab	13.6 a	13.2 a	···

[x] All trees were dissected and measurements made in October 1982.
[y] Isolates are designated by the oak species from which they were obtained.
[z] Average of five control (uninoculated) or inoculated wounds. Within both the nongirdled and girdled groups, an analysis of variance (*F*-test, $P = 0.05$) indicated significant effects of oak species and of isolates but no significant interaction among oak species and isolates. Average values followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

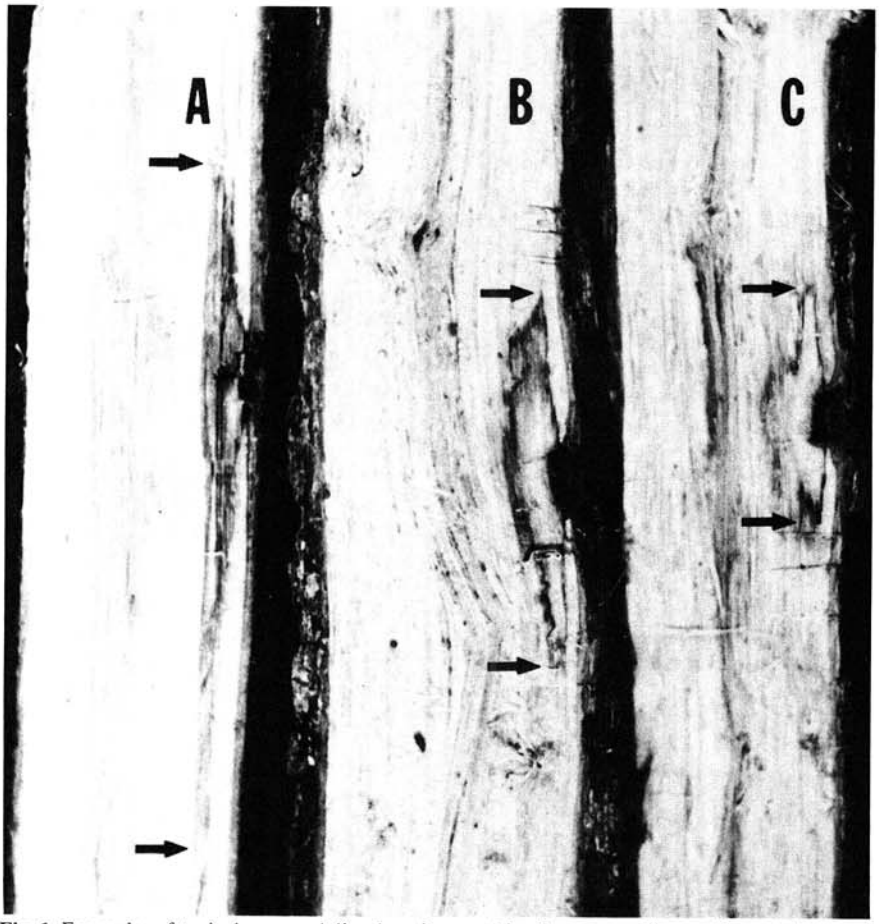

Fig. 1. Examples of typical sapwood discoloration resulting from wounding and inoculating oaks with *Hypoxylon atropunctatum*. Extent of discoloration (arrows) shown in **(A)** an inoculated black oak, **(B)** an inoculated post oak, and **(C)** an uninoculated control post oak.

RESULTS

Inoculations. In a preliminary test, *H. atropunctatum* grew readily on YEGA from inoculum disks removed from black oaks and white oaks up to 10 days after inoculation (the longest time tested). Also, diffusates from pieces of nonsterile living sapwood that were placed adjacent to inoculum disks on YEGA plates did not inhibit growth of the fungus.

Only one of the 40 inoculated, nongirdled trees in the two experiments (a blackjack oak) developed phloem and sapwood decay and a stroma of *H. atropunctatum*, while 18 of the 40 inoculated, girdled trees developed similar decay and stromata (five black, eight blackjack, three white, and two post oaks). In no case was phloem and sapwood decay centered on an inoculation wound and no stromata were associated with inoculation wounds.

Sapwood discoloration was associated with all inoculation and control wounds. The discolored wood was generally wet, fetid, and brown to black, especially near the margins of the discolored zones (Fig. 1). There was no evidence of decay or distinct black zone lines characteristic of *H. atropunctatum* in either the sapwood or phloem.

Discoloration was greatest in the vertical dimension (Table 1). On the average, discolored sapwood extended 1 cm radially and 2 cm tangentially from the inoculated and control wounds and did not differ among species. Because results from both experiments were similar, only data from the first experiment are presented in Table 1. No significant ($P = 0.05$) interactions were found among the oak species and fungal isolates in either group of trees (Table 1). The average length of the discolored sapwood in black oaks was significantly greater than in the other species. There were no differences among the ungirdled blackjack, white, and post oaks, but girdled post oaks had significantly less discoloration than girdled blackjack and white oaks. Longer discolored zones were associated with inoculated than with control wounds, but there were no significant differences among the three fungal isolates. Girdling of inoculated trees did not increase the extent of sapwood discoloration.

From girdled trees, *H. atropunctatum* was recovered in YEGA culture from samples of discolored sapwood associated with 17 of 20 inoculation wounds and 16 of 20 control wounds. From nongirdled trees, *H. atropunctatum* was recovered from only three of 30 samples.

Isolations from healthy trees. *H. atropunctatum* was readily isolated from the living tissues of healthy-appearing oaks (Table 2). The fungus was obtained

Table 2. Frequencies of isolation of *Hypoxylon atropunctatum* from healthy-appearing oaks at three locations in the Ozark National Forest, May–July 1982

Portion of tree sampled	*Hypoxylon*-positive trees/total sampled by location and species									
	Wedington		Devil's Den		White Rock		All locations			
	Black/red[a]	White	Black/red	White	Black/red	White	Black/red	White	Total	Percentage
Branches[b]	52/88	32/65	22/35	16/35	25/35	18/34	99/158	66/134[c]	165/292	57
Trunks[d]	11/91	10/69	4/35	5/35	2/36	2/34	17/162	17/138	34/300	11

[a] Mostly black oaks (*Quercus velutina*).
[b] Lateral branches (about 1 cm diam.); all tissues were plated.
[c] Ratio for white oak branches was significantly less than for black/red oak branches (chi-square, $P = 0.05$).
[d] Phloem and sapwood were plated; outer bark was removed.

from branches of 57% of the trees and from trunks of 11% of the trees. Within or among the three locations, the fungal isolation frequencies between black/red and white oaks were not significantly different for either branches or trunks. When data from all locations were combined, the isolation frequency from branches was significantly greater for black/red than for white oaks.

Girdling experiment. Girdling of trees in May 1982 resulted in death of 99% of the black oaks and 93% of the white oaks within 5 mo. Most of the trees—77% of 108 black oaks and 70% of 88 white oaks—developed stromata of *H. atropunctatum*. Stromata of *H. atropunctatum* were found on 5.5% of the black oaks and 0.2% of the white oaks in the vicinity of the girdled trees.

DISCUSSION

The most important finding of this study was the natural occurrence of *H. atropunctatum* within the branch (57%) and trunk (11%) tissues of healthy oaks. Because only a very small sample was cultured from each tree, the isolation frequencies probably underestimate the actual proportion of trees colonized by the fungus. Latent colonization of tissues of healthy oaks by *H. atropunctatum* prior to any known stress may explain how this fungus is capable of rapid increases following drought. Inoculum dispersal and initial infection of trees after they are stressed or killed would be unnecessary for epidemic development of *H. atropunctatum* on oaks as occurred after the 1980 drought. Whether the fungus infects living cells without causing observable symptoms, survives as a dormant structure(s), or subsists on nonliving cells within the phloem and sapwood has yet to be determined.

The sampling procedure did not indicate the specific tissue(s) colonized or the extent of colonization within individual trees. All tissues from the branch samples were plated, but the outer bark was removed from the trunk samples. The related species *H. punctulatum* (Berk. & Rev.) Cke. may colonize the sapwood of healthy oaks (5), but in contrast to our results, this species was isolated frequently from trunk samples but rarely from the crown.

Our experiments did not confirm Thompson's report (15) that *H. atropunctatum* causes decline, sapwood discoloration and decay, and death when inoculated into trees but did confirm that stress induced by girdling stimulates development of the fungus. The sapwood discoloration associated with both inoculated and control wounds was not similar to that observed in oaks naturally invaded by *H. atropunctatum*. No attempt was made to identify microorganisms other than *H. atropunctatum* in the discolored sapwood; however, its appearance and odor indicated that various pioneer wound invaders (10) were probably present. The high frequency of colonization of healthy oaks by *H. atropunctatum* and the high incidence of fungal development on uninoculated, girdled trees made it impossible to demonstrate pathogenicity by Koch's postulates. Decay of phloem and sapwood and presence of stromata on 18 of 40 inoculated and girdled trees probably developed from the latent fungus within the trees. This explains the lack of an association between the phloem and sapwood decay and stromata with the inoculation wounds and also explains why *H. atropunctatum* was recovered with equal frequency from the discolored sapwood associated with both inoculated and control wounds.

In a recent survey of living and dead trees, *H. atropunctatum* was found more than twice as frequently on black and blackjack than on white and post oaks (1). In these studies, which concentrated on black oak and white oak, isolation frequencies from healthy trees and the incidence of decay and stromatal development induced by girdling were similar for both species. This indicates that the natural differences in incidence of *H. atropunctatum* on the two species cannot be attributed to differences in the ability of the fungus to survive in and cause extensive disease on these hosts. The difference in natural incidence could be related to the greater ability of white oak to avoid and tolerate drought stress (7). Various stresses within host plants trigger the development of facultatively parasitic canker pathogens (6). Work is needed to determine how various types of host stresses trigger the development of *H. atropunctatum*.

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