Cytology and Histology

Progressive Stages of Discoloration and Decay Associated with the Canker-Rot Fungus, *Inonotus obliquus*, in Birch

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ABSTRACT

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The amount of discoloration and decay by the canker-rot fungus *Inonotus obliquus* (=*Poria obliqua*) in naturally infected *Betula papyrifera* was quantified throughout the columns of defect, and progressive stages of degradation were examined by scanning electron microscopy and histological techniques. Massive amounts of thick-walled mycelia penetrated the bark and phloem to incite cankers. The fungus was observed as a pioneer microorganism in the advancing front of the discolored column, colonizing and destroying vessel and parenchyma cell occlusions. Decay was always greatest immediately above and below the sterile conk. Multiple zones of discolored and decayed wood characteristic of *I. obliquus* decay apparently resulted from repeated attacts by the canker-rot fungus in the phloem and from host response to cambial death and infection. Although compartmentalization occurred, it did not successfully confine the fungus to wood formed prior to wounding. *I. obliquus* apparently evades and breaks down chemical and morphological barriers produced by trees in response to wounding and infection.

Inonotus obliquus (Fr.) Pilát [*Poria obliqua* (Pers.) Bres.] is a canker-rot fungus that causes extensive damage on various *Betula* species. A sterile conk is commonly produced at the site of infection (7,8,20,21,23). Shigo (13), investigating how cankers of *I. obliquus* are incited, reported that sterile conks push a wedge of mycelium into the outer bark and the resulting pressure splits the bark and kills the cambium. The progressive stages of decay and patterns of degradation attributed to canker rots have been difficult to understand (16).

Trees respond to infection by producing mechanical and chemical barriers that compartmentalize the affected tissues (18). A successional sequence of events can remove toxic or morphological obstructions making the wood suitable for colonization by decay fungi (2,12). Recent evidence suggests that some wood-destroying fungi occur alone in trees and can tolerate and destroy compounds produced in response to infection (22). The ability of canker-rot fungi to be primary invaders and break down barriers formed during compartmentalization has not been demonstrated.

This investigation was conducted to elucidate the discoloration and decay process in paper birch, *Betula papyrifera* Marsh., associated with *I. obliquus*, and to obtain a better understanding of how canker-rot fungi damage living trees.

MATERIALS AND METHODS

Twelve *B. papyrifera* trees naturally infected by *I. obliquus* and bearing one or more sterile conks, were cut into 30-cm bolts. Bolts, from the entire discolored and decayed columns, were split under

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aseptic conditions and sections from the exposed faces were used for isolations on agar media and for histological and ultrastructural studies. Small segments of wood were sequentially removed from the outer sapwood to inner regions of advanced decay and cultured on malt extract agar (15.0 g Difco malt extract, 15.0 g Difco bacto agar, 2.0 g yeast extract per liter), acidified malt extract agar (with 4 ml of 85% lactic acid added after autoclaving), and an agar medium selective for basidiomycetes (6).

Sections of wood were also prepared for scanning electron microscopy as previously described (3) and for light microscopic histological studies. Cubes of wood were cut with a razor blade and infiltrated with distilled water under low vacuum using a hand-operated vacuum pump. Radial, tangential, and transverse sections 15–20 μm thick were cut with a cryostat microtome at −20 C. Sections were stained with toluidine blue O (10) and safranin and fast green (11).

Wood disks 2.5 cm thick were cut from six additional naturally infected trees. Disks were removed at 30 cm intervals from the base of the tree through the discolored and decayed column until clear, unaltered wood was encountered. The amount of discolored and decayed wood was quantified for each cross-sectional area by using a video processor image analyzer (5).

To determine the effect of *I. obliquus* on xylem of birch, six healthy paper birch trees were inoculated. Drill wounds, 1.6 × 1.6 × 5 cm, were made on opposite sides of the tree. One wound served as a control while the other was inoculated with fungal material from inside an *I. obliquus* sterile conk. Wounds were covered with water-repellent tape. Trees were harvested and examined as previously outlined 18 wk after inoculation.

RESULTS

Large columns of discolored and decayed wood were associated with sterile conks of *I. obliquus* (Table 1). Each sterile conk was associated with various types of stem wounds. The greatest cross-sectional area of the stem was at the site of infection (0.3 m above or below the sterile conk) (Table 1). This swelling was characteristic of infection by *I. obliquus* and has been referred to as a "bowling pin effect" (17). The amount of decayed wood was always greatest near the sterile conk. At all levels within the column, there was usually a larger percentage of discolored wood than decayed wood (Table 1).

I. obliquus was easily isolated from discolored and decayed wood throughout the columns of defect. The fungus was the only microorganism isolated from the advancing front of discolored wood at the top of the column. The pattern of discolored and decayed wood from representative locations where samples were removed for histological study and scanning electron microscopy is illustrated in Fig. 1.

Transverse sections from Zone A (Fig. 1), the lightly discolored wood surrounding the dark discolored region (Zone B), contained very few occlusions (Fig. 2a). In Zone B (Fig. 1), vessels, parenchyma, and fibers were often profusely plugged while some xylary tissues were free of occlusions (Fig. 2b). Hyphae of *I. obliquus*, as confirmed by isolation, were frequently found colonizing occluded vessels (Fig. 2c) of Zones B and D. The multiple columns of discolored and decayed wood (Zones C and D in Fig. 1) are regions of densely plugged cells and severely degraded wood (Fig. 2d). Hyphae were present in both zones, but significant cell wall deterioration occurred only in cells free of plugs.

Since *Betula* species do not produce vessel occlusions in response

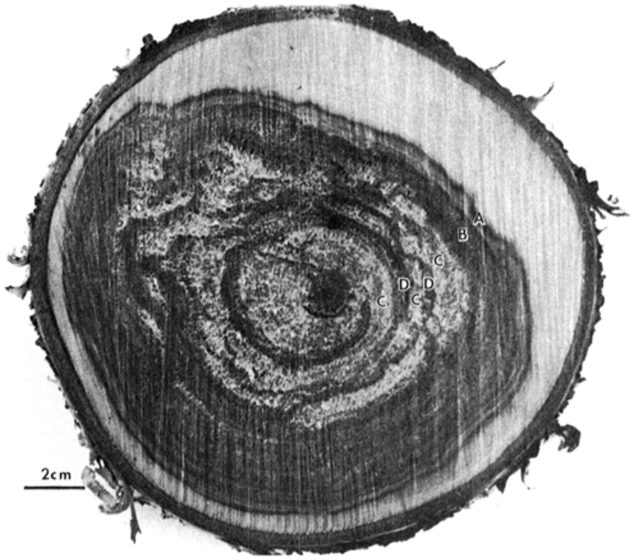

Fig. 1. Cross section of birch trunk being decayed by *Inonotus obliquus* 1 m above the sterile conk, demonstrating lightly discolored wood (A) and multiple zones of discolored (B = outer zone, D = inner zones) and decayed wood (C).

TABLE 1. Area (square centimeters) of total disk, discolored, and decayed wood associated with *Inonotus obliquus* in birch (*Betula papyrifera*)

	Areas of cross-sectional disks[a] from trunks of:																	
	Tree 1			Tree 2			Tree 3			Tree 4			Tree 5			Tree 6		
Height (m)	Total	Dis-colored	Decayed	Total	Dis-colored	Decayed	Total	Dis-colored	Decayed	Total	Dis-colored	Decayed	Total	Dis-colored	Decayed	Total	Dis-colored	Decayed
0.3	254.9	36.0	4.6	527.1	126.5	36.5	211.2	16.9	0.4	271.4	29.8	36.3	74.8*	43.8	13.6	143.1	10.4	0
0.6	212.9	26.1	21.2	526.0	134.1	76.3	192.0	23.5	2.4	248.9*	45.3	33.2	64.0	34.6	12.3	145.6	12.3	0
0.9	439.7	26.9	12.7	434.8*	120.9	58.5	186.2	28.1	7.2	196.2	19.1	36.5	57.8	26.6	7.4	144.0	12.4	0
1.2	238.2*	63.9	16.6	438.1	85.7	69.4	184.5	44.6	9.9	179.5	16.6	25.1	54.6	25.8	1.9	143.7	16.2	0
1.5	339.2	102.5	14.6	405.0	73.9	58.8	203.1	58.2	10.1	167.9	23.7	9.4	53.2	22.3	0.7	169.0	22.2	1.7
1.8	215.7	54.5	19.0	311.6	64.2	37.7	239.6*	48.7	9.9	161.0	23.8	6.3	50.0	16.0	0.5	122.3	10.6	1.7
2.1	212.9	60.9	13.2	357.2	64.9	21.8	168.1	32.0	13.0	164.3	20.5	4.4	48.4	9.8	0	137.1	15.6	0
2.4	199.2	41.5	22.8	398.1	75.3	7.3	151.4	22.3	6.4	149.5	18.5	1.6	43.9	6.5	0	119.7	12.2	2.5
2.7	175.4	26.2	9.3	350.4	64.7	2.1	153.0	12.9	3.2	144.1	17.2	2.5	43.9	4.5	0	127.8	14.9	11.1
3.0	175.7	24.3	5.3	330.5	59.1	5.5	146.4	7.9	1.4	132.4	13.0	0.6	42.9	1.6	0	134.6	27.5	10.8
3.3	175.0	17.5	2.8	220.7	49.5	2.9	128.0	7.2	0.2	142.0	10.5	0	40.3	0.6	0	148.2	39.6	4.6
3.6	158.5	17.1	0.6	298.7	42.4	2.4	124.4	70	0	103.0	3.5	0				191.5*	58.4	9.0
3.9	147.3	10.8	0.9	287.6	38.8	0	135.4	5.8	0	57.9	0.3	0				113.6	20.1	7.6
4.2	145.1	7.2	0.1	282.6	27.3	2.8	118.4	4.2	0							105.8	12.1	4.4
4.5	148.7	4.6	0	274.4	36.1	0										97.0	9.5	2.4
4.8	153.1	5.7	0	263.9	16.0	4.7										95.1	10.0	0.5
5.1	129.1	0.1	0	258.8	15.0	1.6										95.7	3.2	0
5.4	···	···	···	251.8	7.9	0.9										86.0	1.8	0
5.7	···	···	···	250.1	3.3	0												
6.0	···	···	···	236.1	0.9	0												

[a] Disks 2.5 cm thick taken at 30-cm intervals from ground level to healthy wood.

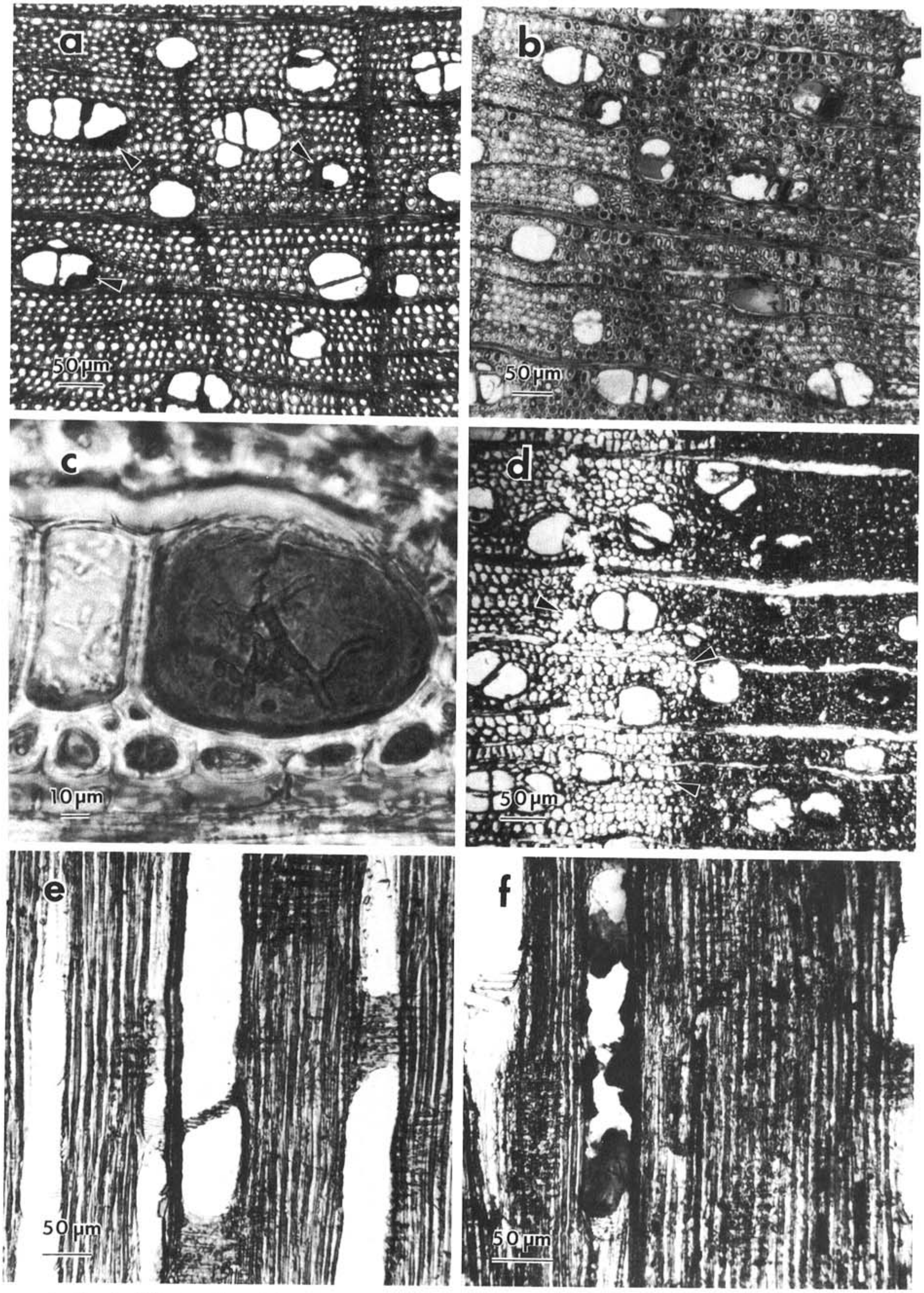

Fig. 2. Transverse (**a–d**) and radial (**e,f**) sections of birch wood stained with toluidine blue O (**a,d–f**) and safranin and fast green (**b,c**). **a,** Light discolored wood (Zone A in Fig. 1) with few vessel occlusions (arrowheads). **b,** Extensive occlusions in vessels and other xylary cells of discolored wood (Zone B in Fig. 1). **c,** Hyphae of *Inonotus obliquus* colonizing a vessel occlusion. **d,** Occluded discolored xylem and nonoccluded decayed xylem (arrowheads) from border of Zone C and D in Fig. 1. **e,** Xylem above uninoculated drill wound demonstrating the lack of vessel occlusions in response to wounding. **f,** Vessel occlusions in xylem above drill wounds inoculated with sterile conk tissue.

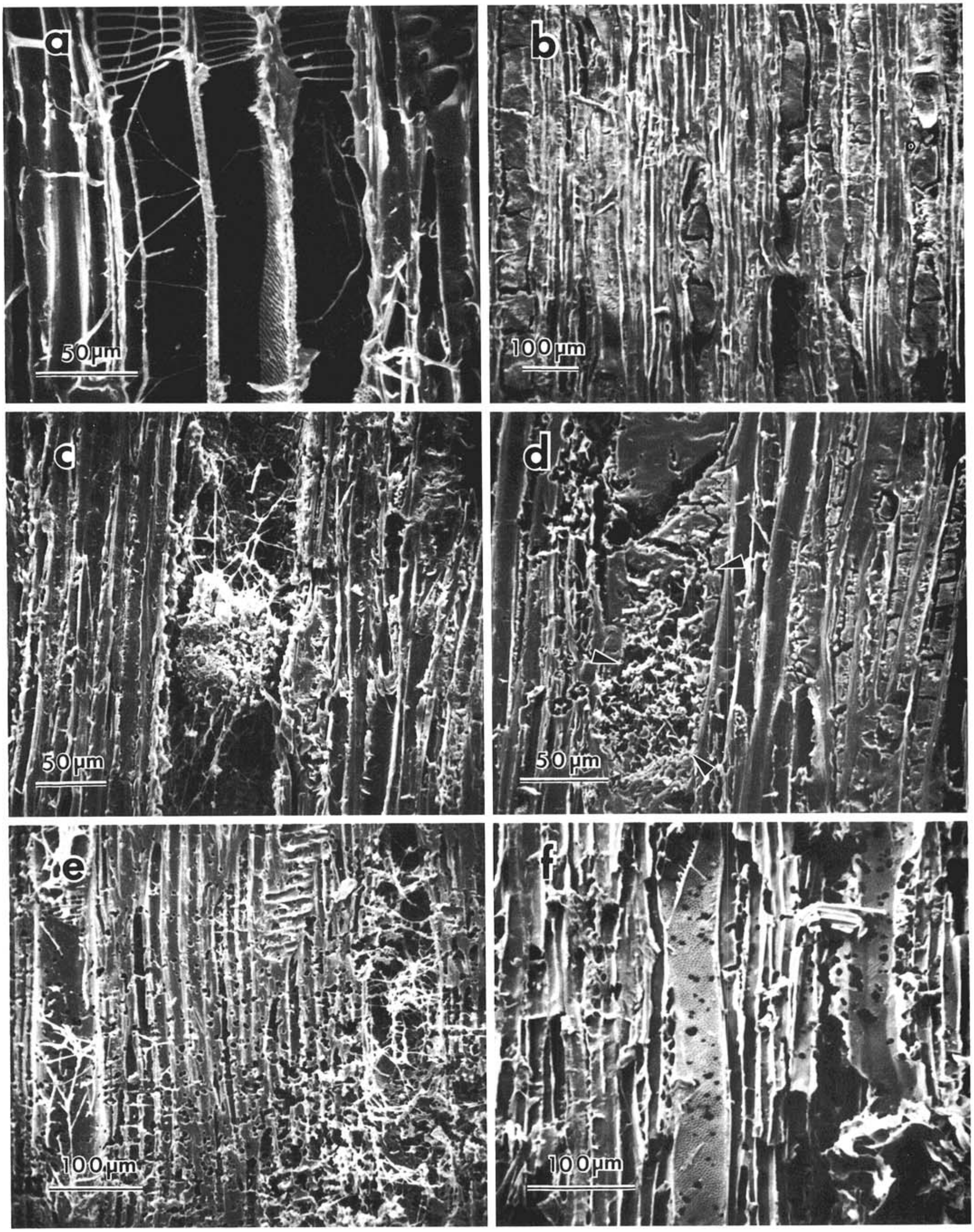

Fig. 3. Scanning electron micrographs of radial sections from discolored and decayed birch wood. In the early stages of discoloration the wood contained **a,** xylem free of vessel occlusions and **b,** regions with copious amounts of vascular plugging. *Inonotus obliquus* was observed colonizing the regions without plugs (a). In older discolored wood, *I. obliquus* **c,** colonized vessel occlusions and **d,** apparently destroyed them (arrowheads). Wood with advanced decay was free of occlusions and the decay appeared to be **e,** of the white rot type with **f,** lignin and cellulose being removed in localized areas.

to wounding (1), the host response to drill wounds filled with material from a sterile conk of *I. obliquus* was tested. Uninoculated wounds reacted as indicated by Bauch et al (1); extensive plugging of xylary elements did not occur (Fig. 2e). However, wood above and below wounds containing sterile conk tissue had vessel occlusions and greater amounts of plugs in other xylem cells (Fig. 2f).

Scanning electron microscopy showed that xylary cells from Zone B (Fig. 1) around the circumference of the decay column contained copius amounts of plugs as well as areas free of occlusions. Hyphae of *I. obliquus* were observed in vessels without occlusions (Fig. 3a), but not in occluded xylem of the outermost discolored wood (Zone B in Fig. 1) (Fig. 3b). Discolored wood towards the center of the column (Zone D in Fig. 1) contained vessels and other cells with hyphae of *I. obliquus* colonizing occlusions and apparently destroying them (Figs. 3c and d). Regions of decay, Zone C, were free of vessel plugs, and the decay appeared to be characteristic of the white rot type (Figs. 3e and f) (4). Discolored wood between the decay was filled with occlusions in various stages of degradation.

The ability of *I. obliquus* to incite cankers was due to penetration of bark by massive amounts of thick-walled fungal mycelia (Fig. 4a). Fig. 4b demonstrates how a wedge-shaped mass of mycelium penetrated the bark and phloem. The host responded to this infection by forming new callus tissue around the dead cambium. Both phloem and xylem reacted to the wound caused by the fungus. Callus tissue around the area of canker formation responded by extensive plug formation (Fig. 4c). This host response provided a barrier to fungal invasion, but new sites of entry occurred as the canker expands (Fig. 4d). New tissues formed after wounding were directly penetrated from the outer edge of the phloem thus circumventing the strongest barrier, the cells formed after wounding (18).

DISCUSSION

After trees are wounded, morphological and chemical barriers are formed that compartmentalize the defect (15–18). *I. obliquus* is unlike most wood-destroying fungi, because it can incite cankers and break down internal compartments formed by the tree. This

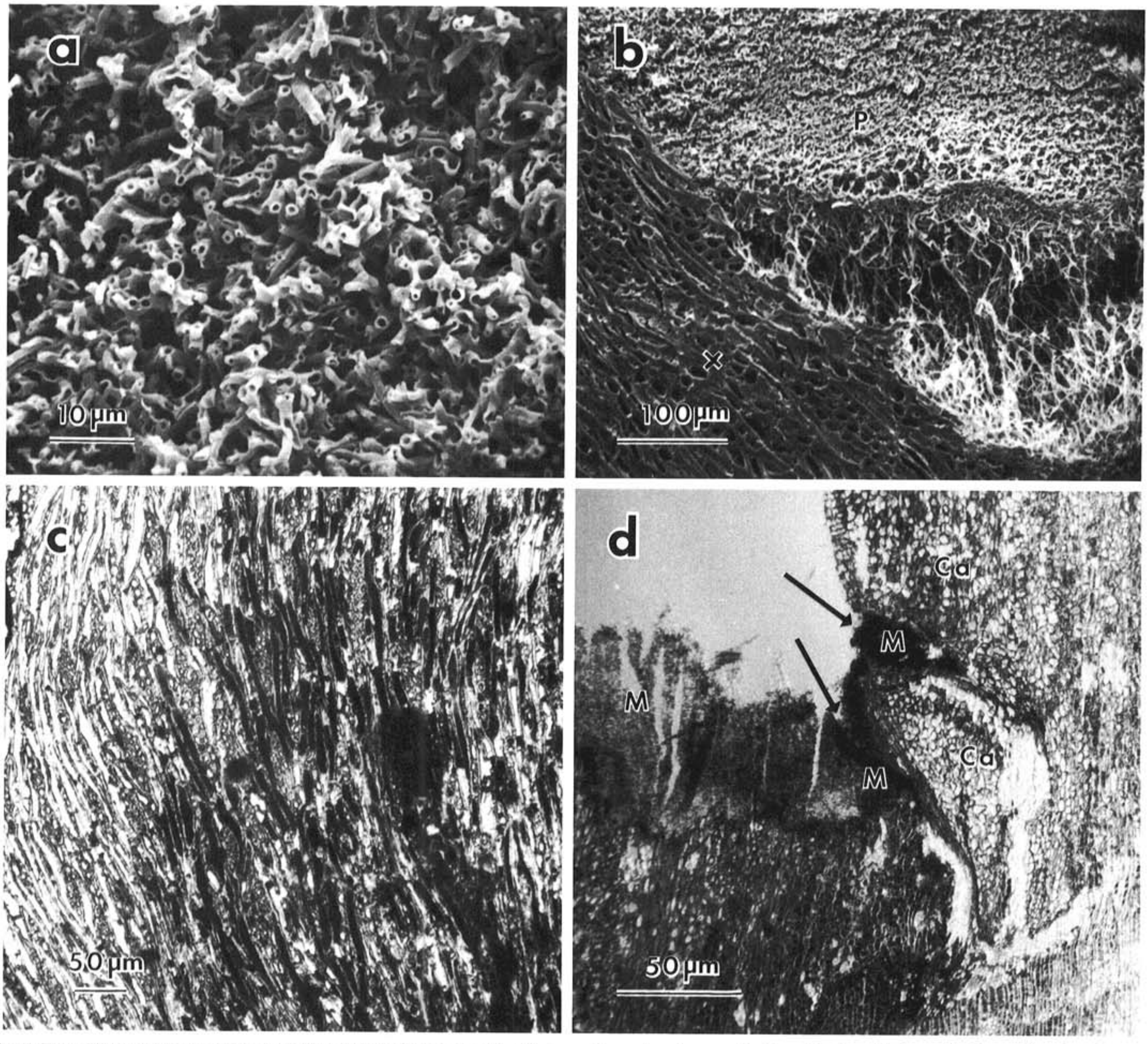

Fig. 4. Scanning electron micrographs (**a,b**) and light micrographs (**c,d**) from regions of cankers on birch. **a,** Thick-walled mycelia from edge of canker. **b,** Wedge-shaped mass of mycelia penetrating the phloem (P) and xylem (X). **c,** Callus tissue adjacent to the canker with a strong host response of xylarly occlusions. **d,** New cells formed after wounding are repeatedly attacked by the fungus (arrows); M = mycelium and Ca = Callus tissue.

allows the fungus to continually reinvade the tree and attack newly formed cells. Although the host responds each time cambial cells are killed, the strongest barrier, the cells formed after wounding (18), can be avoided. The fungus repeatedly moves around and beyond the morphological and chemical barriers. This compartment functions to restrict fungi moving out from the xylem, but apparently has little or no effect when the attack comes from the outer bark. Internal barriers (ie, vessel and parenchyma occlusions) can also be tolerated and destroyed by *I. obliquus*. In hardwood rots, usually a succession of microorganisms are required to alter and detoxify the discolored wood and eventually provide an environment and substrate suitable for colonization by decay fungi (2,12,14). *I. obliquus* does not need these successional changes to occur before colonizing the discolored wood. The fungus can be isolated from and observed in the advancing front of the discoloration column. *Phellinus tremulae* (Bond.) Bond. et Borisov may also have the ability to act as a primary invader of *Populus* (22).

Wounds in *Betula* species usually result in much larger columns of discolored and decayed wood than in other tree species (19). Bauch et al (1) recently postulated that gasses and microorganisms can easily penetrate longitudinally because *Betula* species do not form vessel occlusions in response to wounding. In the study presented here, extensive occlusions were found in vessels of *B. papyrifera* when *I. obliquus* was present. The living host cells respond differently to wounds than to colonization of the wood by *I. obliquus*. The response was observed in advance of the fungus. Cells a considerable distance from sites of cambial death produced extensive plugs (Fig. 4c). Trees that were wounded and inoculated with pieces of a sterile conk also contained vessel occlusions (Fig. 2f), but the fungus could not be microscopically observed in the immediate area of the plugs. The production of vessel occlusions in birch may be initiated by a host response to enzymes or other substances produced by *I. obliquus* (14,18,19), or the occlusions may be formed from materials produced by the fungus as described by Hinds (9).

The multiple zones of discolored and decayed wood (Fig. 1) appear to be due to repeated attacks by the canker-rot fungus. When the cambium is killed, the tree responds both to the wound and to the fungus. The host response does not result in uniformly occluded xylary tissues. Instead, some areas are occluded more extensively than others. When the fungus enlarges the canker and colonizes the wood, heavily plugged zones result. Zones of relatively few occlusions apparently are formed when the fungus is confined or successfully compartmentalized for a period of time. Although *I. obliquus* can colonize and destroy occlusions in vessel and parenchyma cells, degradation in heavily plugged areas is a slow process. In contrast, areas free of plugs are quickly colonized followed by subsequent degradation. As the canker enlarges, previously formed internal compartments on the canker side of the tree become less effective barriers and *I. obliquus* continually invades new xylem.

Canker-rot fungi are able to cause extensive damage to trees because of their ability to overcome the host response to wounding and infection. These characteristics make them an exception to the rules of compartmentalization (15) and indicate they have evolved a system to evade or destroy the trees' defense mechanisms.

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