Cryptosphaeria Canker and Libertella Decay of Aspen

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ABSTRACT

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A recently discovered, widely distributed canker disease of aspen and other poplars throughout the Rocky Mountain region is described. Inoculations with ascospore and conidial isolates of *Cryptosphaeria populina* show the fungus is capable of causing branch, sprout, and sapling mortality, trunk cankers, and the discoloration and decay of aspen stems previously associated with its imperfect stage, *Libertella* sp. The greatest average canker elongation 50 mo after September inoculations was 33.5 cm with sapwood decay and discoloration extending to 401 cm. The fungus caused an average weight loss of 13.5% in bark, 27.0% in sapwood, and 19.1% in heartwood blocks. Incidence of decay in living trees in Colorado was 7.8%, increasing from the poorer sites to the better sites, and increasing with diameter class. Sapwood discoloration around and beyond the infection perimeter exhibits a yellowish fluorescence under ultraviolet light as does a water filtrate of the fungus grown in culture. Other aspen canker and decay fungi and their cultural filtrates exhibit similar fluorescence, which suggests that sapwood discoloration may be the result of materials produced by fungal pathogens rather than by the host response.

Additional key words: cottonwood, balsam poplar, Hypoxylon canker, sooty-bark canker, *Fomes igniarius*, heartrot, saprot.

This paper describes a canker of western aspen (*Populus tremuloides* Michx.), which recent research has linked with *Cryptosphaeria populina* (Pers.) Sacc., an ascomycete in the family Diatrypacea of the Sphaeriales (13–15,17,18). This disease, which here is called Cryptosphaeria canker, may rank in importance with Hypoxylon canker (caused by *Hypoxylon mammatum* (Wahl.) Mill.) and sooty-bark canker (caused by *Cenangium singulare* (Rehm) Davidson and Cash), since it causes not only tree mortality but also extensive trunk discoloration and decay, which is attributed to the imperfect stage, *Libertella* sp.

The research reported here was initiated to study its etiology, distribution, and potential pathogenic importance in aspen stands. The following section describes the disease and clarifies the confusion that exists in the literature concerning its etiology. Cryptosphaeria cankers, frequently associated with trunk wounds on aspen, are usually long and narrow (Fig. 1A). They may be only 5 to 10 cm wide and can extend up to 3 m in length, following the grain of the underlying wood. Annual extension of the canker margin may be only several millimeters laterally but several centimeters or more vertically. Small trees usually die several years after becoming infected and before the trunk is girdled. Large trees may have cankers that girdle branches and

enlarge onto the trunk. *Cytospora chrysosperma* (Pers.) Fr. frequently fruits at the canker perimeter and quickly colonizes the remaining bark after a tree dies.

The infected bark near the periphery of the canker usually becomes discolored light brown to orange, and annular callus formation inhibiting spread is obvious after 2 yr or more. The dead bark adheres tightly to the sapwood. Bark that has been dead for one or more years is black, stringy, and sootlike, similar to that affected by sooty-bark canker. However, the dead bark contains small (0.5–2.0 mm), scattered, lenticular, light-colored areas (Fig. 1B). Perithecia of the fungus develop separately within an effused pseudostroma, which may vary from 1–2 cm in width and up to 30 cm in length beneath the bark periderm that has been dead for at least 1 yr (Fig. 1C). Light-orange acervuli bearing filiform, nonseptate, curved conidia of the genus *Libertella* are occasionally found near the periphery of the canker.

The fungus colonizes the heartwood and sapwood, causing discoloration and decay. It then penetrates the cambium and bark causing the canker (Fig. 1D). The sapwood beneath the canker in stems less than about 5 cm in diameter is discolored gray. This discoloration usually extends up to 1 m or more above and below the canker. Various hues of gray, brown, yellow, orange, and even pink are associated with the brown mottled trunk decay from which the Libertella stage of the pathogen can be readily isolated in larger trees (Fig. 1E). Under ultraviolet light, the incipient and advanced decay exhibit various patterns of yellow fluorescence which is more intense in the discolored zone close to the infection perimeter (Fig. 1F).

The fungus was described by Persoon in 1801 (21) but was reclassified as *Cryptosphaeria* by Saccardo in 1882 (23) when he noted its occurrence on *P. tremula, P. nigra,* and *P. alba* in Europe. Although *C. populina* was collected from dead aspen in Colorado as early as 1897 (National Fungus Collections, USDA, Beltsville, MD, Bethel No. 1238), it was not associated with a canker in Colorado until 1969. In 1970, Krebill (18) found the canker on 2% of 4,016 trees in 100 sample plots on the Gros Ventre area, Teton National Forest, WY, during his study of aspen mortality caused by fungi and insects invading trees wounded by big game. Hinds (13) found the canker on 2% of the live and 8% of the dead aspens surrounding campsites in Colorado in 1973. He later noted the association of trunk decay with the canker (14), but proof that *C. populina* causes both cankers and decay had not been previously established.

In a 1977 survey of aspen cankers on 30 sites (2 0.4-ha circular subplots at each site) in nine national forests in Colorado, Juzwik et al (17) found the canker present on 83% of the sites and 1.1% of the 2,873 live trees examined. They attributed 26% of the tree mortality encountered to the canker. The canker was most frequent (10%) on trees in the smaller diameter classes and was found only on trees less than 28 cm in diameter. Juzwik et al (17) suggested that the canker may be important in natural thinning of younger stands in the 7- to 28-cm-diameter range because of its frequent association with tree mortality.

The canker is widespread throughout the range of aspen in the western United States where collections have been made. Cankers have been collected as far south as the Santa Catalina mountains of Arizona, in New Mexico, Colorado, Wyoming, and Utah and northward in the Rocky Mountains to British Columbia, the Yukon Territory, and north of Fairbanks, AK (15). Published host records of the fungus also include Oregon, Washington, Idaho, and

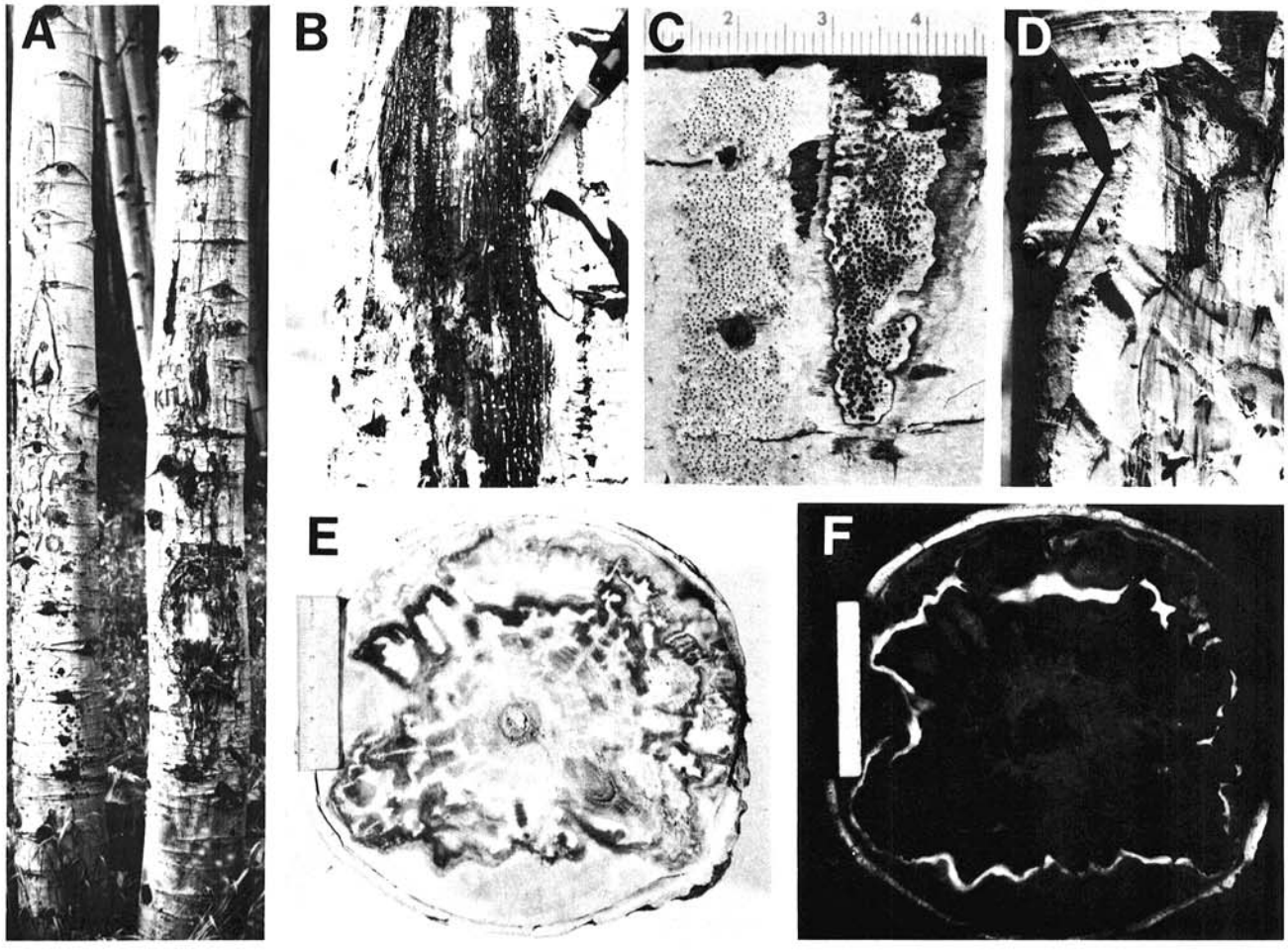

Fig. 1. Symptoms and signs of Cryptosphaeria canker in aspen. **A,** Cankers at 5-yr-old trunk wounds on 30-cm diameter aspens. **B,** Lenticular, light colored areas in the black, dead bark of canker. **C,** Formation of new perithecia in dead bark (left); epidermis removed (right) to show perithecia formed the previous year (scale bar is 1 cm). **D,** Sapwood discoloration with cambium and bark necrosis preceding canker formation. **E,** Cross section of discoloration and brown mottled trunk decay behind canker face on right side (scale bar is 1 cm). **F,** Fluorescence of cross section E under ultraviolet light.

Montana (26). Other poplar hosts include *P. deltoides* Marsh (5), *P. augustifolia* James (*unpublished*), *P. balsamifera* L., and *P. trichocarpa* Torr. and Gray (15).

The canker has been collected in the eastern United States on aspen near Lake George, MN, and the fungus reported near Ann Arbor, MI (28). Other hosts of *C. populina* include the Balm-of-Gilead poplar, *P. balsamifera* × *deltoides* var. *missouriensis* Henry, in New York (25), *Salix purpurea* L. in Pennsylvania (20) and limbs and bark of poplar in New Jersey and Illinois (8).

Wehmeyer (28) obtained the imperfect stage of *C. populina* within a stromatic tissue which he considered abnormal; however, Schreiner (25) later obtained pycnidia and conidia in culture and concluded that the imperfect stage belonged to the genus *Cytosporina*. During this study, diamalt agar cultures obtained from ascospores of *C. populina*, aspen canker tissue, discoloration or decay, and conidia from acervuli in infected bark produced pseudoacervuli with philoconidia typical of *Libertella* sp.

Libertella sp. has been consistently associated with stain, discoloration, and decay of aspen and other poplars; however, its status as a decay-producing organism has been questionable. Fungus X (probably a species of *Libertella*) was common in aspen and balsam poplar on Manitoulin Island in Ontario, Canada, and was found to cause an overall weight loss of 27.5% when tested on poplar heartwood (10). *Libertella* sp. was consistently isolated from wood with a red mottled stain which was frequently associated with knots, branches, and incipient decay by Basham (3) during a study of aspen decay in Ontario. The fungus, associated with the same stain, was almost uniformly frequent in all areas in a survey of 2,500 aspens sampled throughout the province (4). Thomas et al (27) also found a high incidence of heartwood stains in aspen and balsam poplar in Alberta; however, a limited test of *Libertella* sp. on wood blocks showed no weight loss after 3 mo.

Libertella sp. was associated with a white mottled top rot in a Colorado aspen decay study in 1959 (7) where it was found in 3.9% of the 976 trees sampled; however, its status as a decay-producing organism was uncertain. Ross (22) later isolated the fungus from advanced decay in aspen roots and found that it caused a 24.2% weight loss of sterile, dead root blocks by degrading mainly cell wall carbohydrates with no loss of lignin.

MATERIALS AND METHODS

Cultures. The following cultures used in this study were obtained from aspens at various locations and maintained on 2% diamalt agar media:

Cryptosphaeria populina

A-1. Ascospores, Roosevelt National Forest, CO, 1974.
A-5. Ascospores, Roosevelt National Forest, CO, 1976.
A-11. Ascospores, Cache National Forest, UT, 1976.
A-12. Ascospores, San Juan National Forest, CO, 1976.
C-1. Canker bark, Roosevelt National Forest, CO, 1974.
D-5. Canker saprot, Roosevelt National Forest, CO, 1974.
D-14. Canker saprot, Roosevelt National Forest, CO, 1976.

Libertella sp.

L-2C, L-3C, L-4C. Conidia, Roosevelt National Forest, CO, 1976.
L-5D. Trunk rot, Roosevelt National Forest, CO, 1976.

Inoculations. A series of inoculations was made from 1974 to 1977 to test the pathogenicity of *C. populina* on aspens of different age and diameter classes. Trees used in these tests were either collected from or located in the Roosevelt National Forest and included 4-yr-old sprouts (transplanted and placed in an outside lathhouse the previous spring), saplings 2.5–4.0 cm d.b.h. (ranging from 15 to 26 yr of age) and trees in at least three clones (ranging from 30 to 140 yr of age and from 5.2 to 27.0 cm in diameter). Heights of inoculation points above ground level ranged from 25 to 50 cm on the main stem of sprouts and were at ~1.7 m on the trunks of saplings and larger trees. Inoculation points faced one of the four cardinal directions and also were made on the opposite side of the larger trees at 0.9 m. Inoculum consisted of cultures grown on autoclaved rye grains. Several infected grains were placed in 1-cm^2 wounds made into the sapwood. Autoclaved rye only was used as the control treatment. The wounds were covered with sterile cotton and wrapped with plastic tape.

Inoculations were made in 1977 to determine if wounds to the sapwood were necessary for infection and to test the ability of the imperfect Libertella stage isolates to cause infection. Trunk inoculations were made as before except half of the wounds penetrated only to inner bark. Live-branch inoculations were also made to test the ability of the fungus to enter the trunk from infected branches. Branch inoculations were similar to trunk inoculations except they were made alternately on top, bottom, and the two sides, 5–35 cm from the trunk on main branches throughout the tree crown and on some terminal growth of 13 trees. Branch diameters ranged from 0.7 to 3.0 cm and branch ages from 10 to 33 yr. Final observations and reisolations were made in September 1978.

Inoculation dates, number of inoculations made with the various isolates, and the host material used were as follows:

10 July 1974—20 A-1, 20 C-1, and 10 controls on 33 trees; 5 A-1, 5 C-1 on 10 saplings.
30 August 1974—15 A-1, 10 C-1, and 5 controls on 30 sprouts.
24 September 1974—25 A-1, 25 C-1, and 6 controls on 29 trees; 5 A-1, 5 C-1, on 10 saplings.
11 June 1975—25 A-1, 25 C-1, and 10 controls on 45 trees; 5 A-1, 5 C-1, on 10 saplings.
27 July 1977—50 A-11, 50 L-5D, and 10 controls on 55 trees.
28 July 1977—50 A-12, 50 L-3C, and 10 controls on 55 trees.
29 July 1977—25 A-12, 25 L-5D, and 10 controls on 53 branches, 7 terminals.

Decay test. Eight isolates from various sources were tested for ability to cause decay of freshly excised aspen bark, sapwood, and heartwood in 1977. Blocks (2.5 × 2.5 × 0.9 cm) were cut from a live 30.1-cm-diameter aspen, randomized, and incubated 24 wk with the isolates using the accelerated laboratory test of natural decay resistance of wood of the American Society for Testing and Materials (1). The blocks were sterilized with propylene oxide, placed directly in contact with moist soil in sterile bottles, and the fungus inoculum (approximately 1 cm square cut from a petri-dish culture) placed directly on top of the blocks. Six replicates of each isolate, and six controls were tested.

Decay in living trees. Decay of Colorado aspen in typical commercial aspen forests had been extensively sampled on 35 plots in 1954 to 1956 and represented 8.4% of the total gross cubic-foot volume (7). *Libertella* sp. was associated with 4.6% of the decay volume; however, its status as a decay-producing organism was uncertain at that time and its identification was not always complete during the early portion of the study. The data collected in 1956 on 14 plots in two national forests were reanalyzed to define the relationships between the incidence of infection and decay, and tree age, diameter, crown class, and site. Baker's (2) site quality criteria were used in this analysis, because not all stands sampled were even-aged.

Wood discoloration and fluorescence. Isolations from previous inoculations were made to determine the presence of the fungus in discolored wood which showed fluorescence under ultraviolet light. Fluorescent chips were taken adjacent to saprot, on the opposite side of the tree from an inoculation point, from 30 to 50 cm above and below inoculation points, from inoculated branches, and from discolored zones contiguous with heartwood and heartrot. The chips were placed on diamalt agar in petri dishes and observed for 1 mo.

The following preliminary experiments were carried out to determine whether the inoculated fungus was the cause of fluorescent discoloration of sapwood associated with infection or if the discoloration was caused by a host response. Distilled water was added to 20 petri dishes containing dried cultures of the fungus obtained from sapwood decay associated with the 1974 inoculations. After soaking for 24 hr the revived cultures were macerated in a blender and filtered through a Whatman No. 3 filter paper. The filtrate volume was reduced to one half by evaporation in a drying oven at 40 C. Half of the remaining filtrate was sterilzed by passage through a 0.2-μm Sartorius membrane filter and the other half was autoclaved for 15 min at 121 C. Distilled water was

also added to 10 petri dishes containing 1-mo-old cultures. After 24 hr, the cultures were macerated, the liquid was passed through a Whatman No. 3 qualitative filter paper, the filtrate volume was reduced to one half, and the remaining filtrate was left at room temperature. As a control, the same procedure was used on diamalt agar in petri dishes.

For comparison of culture filtrate and sapwood fluorescence material, sapwood beneath the canker of a freshly cut 1974 inoculation on a 17.5-cm-diameter aspen which exhibited yellowish fluorescence was macerated and soaked overnight in 1 L of distilled water. The aqueous extract was filtered and the filtrate volume was reduced to 250 ml in a drying oven. Half of the liquid was sterilized in an autoclave for 15 min and the remaining half was left at room temperature. Clear sapwood of the same tree received the same treatment.

RESULTS

Sprout inoculations. After the winter months of 1974–1975, 10 of the potted sprouts in the lathhouse (eight inoculated, two controls) were missing. Of the remaining 17 inoculated sprouts, two (one A-1, one C-1) did not become infected, six were girdled by the fungus, and nine had cankers ranging from 2 to 7 cm in length 9 mo after inoculation. One year after inoculation, two remained uninfected, nine were girdled, and cankers ranging from 3 to 19 cm in length had developed on six of the remaining live infected sprouts. The study was terminated after 24 mo; two sprouts remained uninfected, and the other 15 sprouts had been girdled and killed back either to within 5 cm of, or to the ground line. *Cytospora chrysosperma* readily colonized and fruited on the remaining bark once the stem was girdled. One of the three remaining controls became infected by *C. chrysosperma* and died 2 yr later, but the wounds at the inoculation points on the other two healed over without becoming infected.

The entire sapwood of the infected sprouts was stained dark brown to black. Perithecia of *C. populina* developed on six of the cankered sprouts and acervuli of the Libertella imperfect stage formed near the base of two dead sprouts. Five girdled sprouts were cut during this study and the fungus was reisolated from necrotic bark tissue and black stained sapwood in each case.

Sapling inoculations. Three of the July 1974 inoculations were negative. Five saplings were girdled and dead within 23 mo, whereas the other two had cankers averaging 2.5 by 7.3 cm in size 50 mo later.

All of the September 1974 inoculations resulted in sapling mortality within 1 yr. Bark necrosis extended to 62 cm in length before stem girdling in one case, and sapwood discoloration of the past 10 yr growth extended from the ground line to the treetop.

Nine of 10 saplings inoculated in June 1975 became infected. Five of the inoculation points appeared to be callusing over in 1978; however, the sapwood behind the inoculation wounds was discolored and decayed. Four saplings had active cankers ranging from 3.0 to 4.5 by 5.0 to 18.0 cm in size (Fig. 2A) 39 mo later.

The fungus was reisolated from the sapwood of three infected saplings in each inoculation date. Sapwood discoloration and decay exhibiting fluorescence under ultraviolet light commonly extended from 0 to 2.1 m in the 4-m-tall saplings (Fig. 2 B and C). Perithecia of *Cryptosphaeria* were formed only on bark which had been dead at least 1 yr on three of the saplings. Once the saplings were girdled by the fungus, *Cytospora* rapidly invaded the remaining bark and soon fruited copiously.

Trunk inoculations. Trunk infection and perennial canker formation varied with time of inoculation and isolates used. In general, these differences were greater than those caused by variations in tree size, crown class, and clones. The ascospore isolate A-1 usually caused an annual increase of bark necrosis during the winter months with a prominent callus formation around its perimeter during the spring and summer. After 1–2 yr, the cankered area appeared as a flat grayish depression in the bark with necrosis often extending beyond the border of callus tissue at any time during the year. The canker isolate C-1 also caused bark necrosis, but often without ridges of callus tissue. These necrotic areas enlarged for 2 yr or more before any prominent callus formed and the infection resembled a typical canker. Unless a definite callus was formed, the necrotic areas were considered trunk lesions.

A summary of the 1974 and 1975 trunk inoculations is given in Table 1. Infection occurred at all inoculation points with the exception of two in which isolate C-1 was used in June. The C-1 isolate did not produce as many prominent cankers or cankers as large as those of isolate A-1, nor did it cause as extensive sapwood discoloration and decay. September inoculations produced larger cankers than inoculations made in June and July. The A-1 isolate resulted in an average annual canker enlargement of 1.8 by 8.4 cm whereas the same isolate used in June resulted in only 1.4 by 4.5 cm enlargement.

Canker size is probably of secondary importance to sapwood infection. Sapwood discoloration and decay exhibiting fluorescence (Fig. 2) was associated with the cankers. Based on time after inoculation, the average annual extension of discoloration and decay amounted to 113 cm with isolate A-1 used in September compared to 82 when used in June (Table 1).

In July 1977, 15 cankers were dissected, and the fungus reisolated from necrotic bark of five of the cankers, from sapwood discoloration and decay beneath the inoculation point of 14, and from the discolored heartwood of five. *Cytospora* was likewise isolated from necrotic bark of four cankers, two of which also yielded *C. populina*. In 1978, 20 cankers were dissected and the fungus recovered from all infections.

Mature perithecia of *C. populina* did not form in the cankers until 21 mo following inoculation and then only sporadically in bark tissue that had been dead for one year. Only 6% of the June, 10% of the July, and 40% of the September infections had produced perithecia 48 mo after inoculation. Immature perithecia formed in the spring matured during the summer and fall. The Libertella stage was not observed on the trunk cankers.

Three of the control inoculation points were infected by *Cytospora* but were healed over by 1978 and considered inactive. None of the remaining controls showed any external sign of infection; two were dissected and found to be uninfected.

All 1977 trunk inoculations with the four isolates in which the wound was made to the xylem became infected (Fig. 3A). Bark wound infections (Fig. 3B), on the other hand, varied as shown in Table 2. The percentage of successful infections and the average length of lesions and underlying sapwood discoloration was greater when xylem was wounded than when only the bark was wounded.

Forty-eight inoculations and eight controls were dissected in 1978 for reisolation and sapwood discoloration measurements. The fungus was reisolated from sapwood beneath 24 xylem and 12 bark inoculations. Twelve bark inoculations and the controls were found to be uninfected by *C. populina*. One control bark inoculation was infected by *Cytospora*.

Branch inoculations. Both isolates used for branch inoculations caused infection and branch mortality. In June 1978, 11 mo after inoculation, the 12-A isolate had girdled 11 branches with bark necrosis extending to the trunk (Fig. 3C and D); 10 other branches were infected. Three terminals were infected, and one girdled. Isolate L-5D caused five branches to die back to the trunk, 14 branches and two terminals were girdled, leaving three live branches and one infected terminal. After 14 mo, isolate 12-A had killed 18 branches to the trunk and had girdled three terminals. Three branches and one terminal remained alive but were one-half girdled. During this period isolate L-5D had caused the death of 15 branches and two terminals. Six branches and one terminal were girdled, leaving one live branch half girdled by the fungus. Bark infection in the spring had extended to 17 cm in the terminals when they were half girdled but proceeded to enlarge to 19 cm below the inoculation point by fall, 1978. Branch infections had spread from 6 to 17 cm before girdling occurred. Bark infection by *Cytospora* was common on the distal portion of branches girdled by *C. populina*.

Two trees with seven dead branches and two terminal inoculations were dissected in September, 14 mo after inoculation, to determine if branch infection had spread to the trunk sapwood. Only one branch had bark necrosis which extended onto the trunk. Branch sapwood discoloration with fluorescence extended 35.0 cm

TABLE 1. Summary data describing infection incidence and symptom development resulting from sequential 1974–1975 aspen trunk inoculations with *Cryptosphaeria populina* after 50, 48, and 39 mo, respectively

Kinds of data Avg. annual increase	Inoculation Date					
	10 July 1974		24 September 1974		11 June 1975	
Isolates identification	A-1	C-1	A-1	C-1	A-1	C-1
Inoculations (no.)	20	20	25	25	25	25
Infections (no.)	20	20	25	25	25	23
Trees girdled (no.)	0	1	3	1	2	0
Cankers (no.)	20	18	22	24	18	2
Canker size (avg cm)	5.4 × 19.5	5.0 × 11.9	7.1 × 33.5	6.1 × 24.1	4.5 × 14.8	3.4 × 9.5
Annual increase	1.3 × 4.7	1.2 × 2.9	1.8 × 8.4	1.5 × 6.0	1.4 × 4.5	1.0 × 2.4
Lesions (no.)	...	1	...	...	5	21
Lesion size (avg cm)	...	4.0 × 5.5	...	...	4.7 × 5.3	3.5 × 5.9
Annual increase	...	1.0 × 1.3	...	...	1.4 × 1.9	1.1 × 1.8
Sapwood discoloration (avg cm)	305(5)[a]	284(3)	401(12)	331(7)	251(4)	152(4)
Annual increase[b]	85	79	113	99	82	48

[a] Numbers in parentheses are the number of cankers dissected for reisolation purposes and length of discoloration and decay measurements.
[b] Based on length of time prior to dissection.

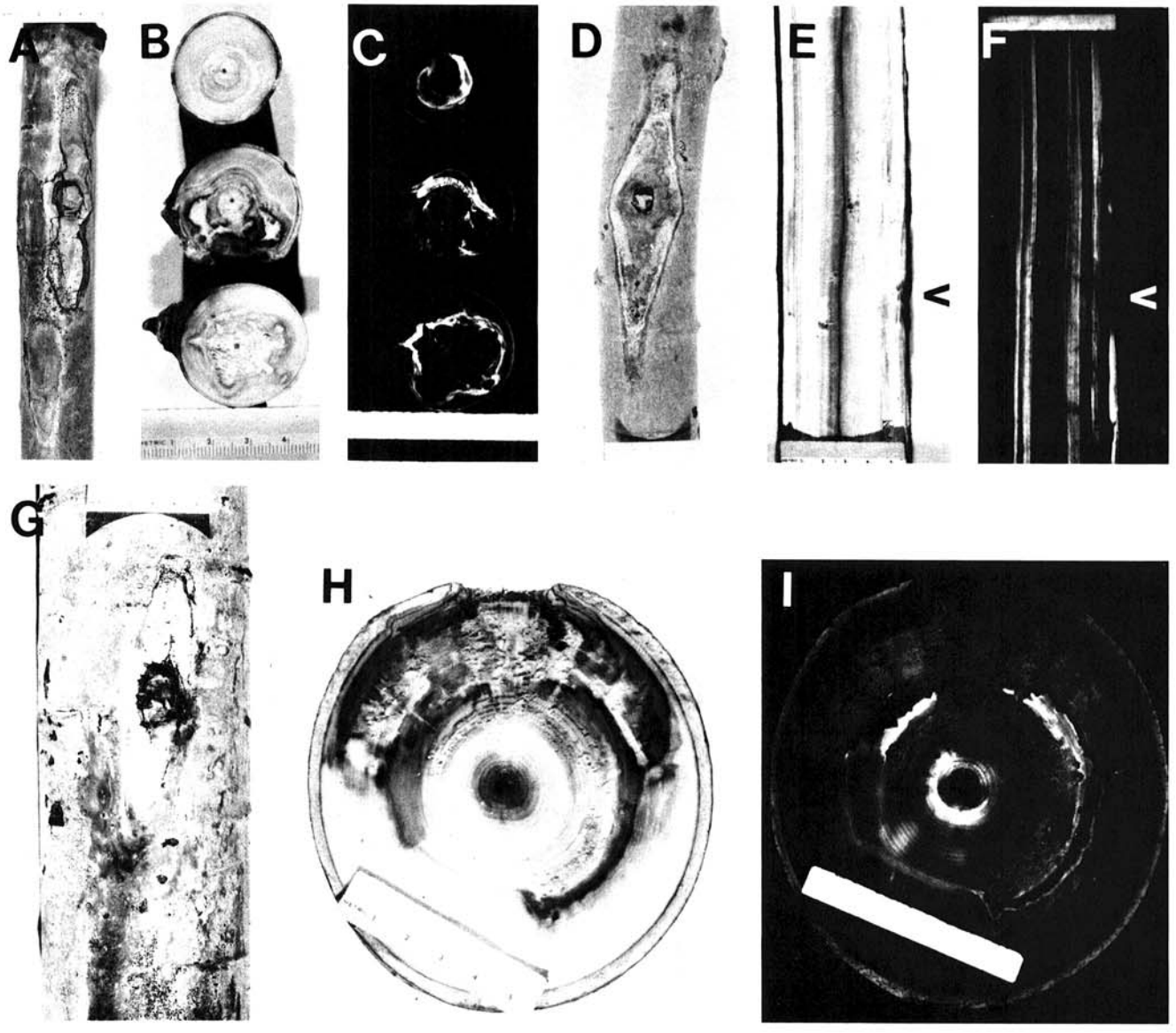

Fig. 2. *Cryptosphaeria populina* inoculations on aspen. **A,** Sapling canker 39 mo after inoculation with isolate A-1 (scale bar is 1 cm). **B,** Discoloration and decay in cross sections of canker in A at 0.6 m (bottom), inoculation point, and 2.0 m. **C,** Fluorescence of sections in B under ultraviolet light. **D,** Canker 6.0 by 19.5 cm on a 5.6-cm-diameter tree, 34 mo after inoculation. **E,** Sapwood discoloration in longitudinal section behind canker in D (arrow indicates inoculation point). **F,** Fluorescence associated with discoloration in E. **G,** Canker 4.0 by 15.0 cm on a 10.4-cm-diameter tree, 37 mo after June inoculation with isolate A-1. **H,** Cross section through inoculation point of canker in G; decay extended 1.4 m, discoloration 2.4 m in trunk. **I,** Fluorescence associated with discoloration and decay in section H.

in length and 1.2 cm deep in the trunk behind the dead branch. Sapwood discoloration with fluorescence had penetrated the trunk for a distance of 9.0 cm behind four other dead branches. While the terminal growth of both trees had been girdled, sapwood discoloration extended approximately 10 cm below the necrotic bark limit. Although the fungus was reisolated from discolored sapwood of the branches and terminals, it was only isolated from the trunk of three of the five infections that entered the trunk. Immature perithecia of *C. populina* were observed on three branches and on both girdled terminals.

TABLE 2. Infection incidence and symptom development in aspen trunks 13 mo after bark or sapwood inoculation with *Cryptosphaeria populina* isolates

Kinds of data Type of inoculation	Isolate number			
	A-11	A-12	L-5D	L-3C
Inoculations (no.)				
Xylem	25	25	25	25
Bark	25	25	25	25
Infection (%)				
Xylem	100	100	100	100
Bark	84	52	76	36
Average lesion size (cm)				
Xylem	4.1 × 11.4	3.3 × 7.4	3.9 × 9.2	3.5 × 8.0
Bark	4.7 × 5.9	3.8 × 4.9	4.9 × 8.1	3.6 × 5.3
Average length of sapwood discoloration (cm)				
Xylem	88.4(5)[a]	74.9(7)	48.0(6)	45.7(6)
Bark	33.0(3)	25.9(4)	27.9(4)	17.8(1)

[a] Numbers in parenthesis are the numbers of lesions dissected.

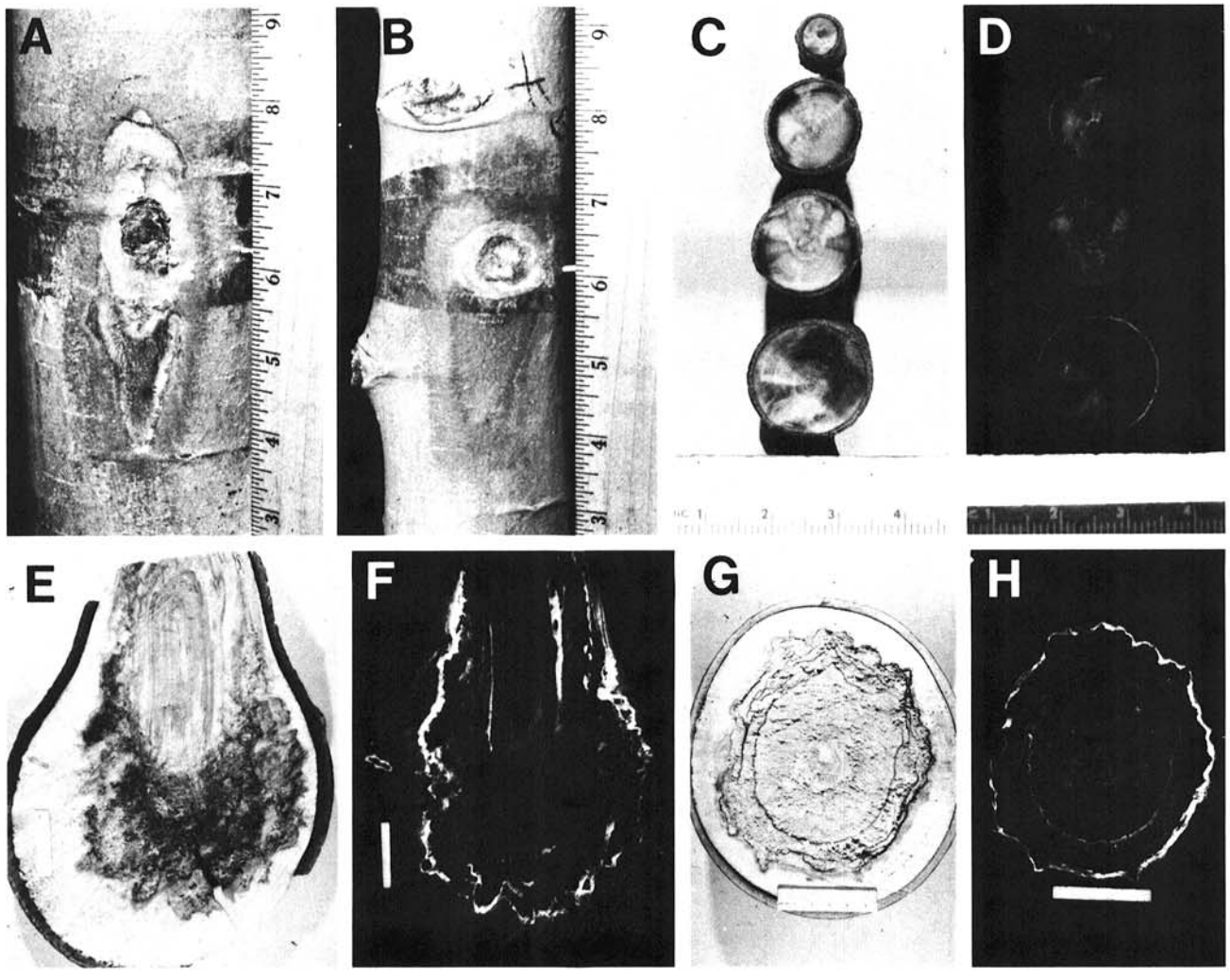

Fig. 3. Discoloration and decay in aspen trees inoculated with *Cryptosphaeria populina* or its *Libertella* conidial stage compared with decay caused by *Phellinus tremulae*. **A,** Xylem wound 13 mo after inoculation with Libertella isolate L-3C: bark necrosis 3.0 × 11.0 cm, sapwood discoloration 71 cm (scale is in.). **B,** Bark wound 13 mo after inoculation with *C. populina* isolate A-11: bark necrosis 3.5 × 7.0 cm, sapwood discoloration 33 cm. **C,** Sapwood discoloration in cross sections of 11-yr-old girdled branch at 0, 25, 40, and 70 cm from trunk, 11 mo after inoculation with *C. populina* isolate A-12 (scale is cm). **D,** Fluorescence of sections in C under ultraviolet light. **E,** *Libertella* sp. decay associated with large and dead branch stub in living aspen collected in 1956 (scale is cm). **F,** Fluorescence associated with decay in E. **G,** Cross section of *Phellinus tremulae* advanced decay and discoloration in living aspen (scale is cm). **H,** Fluorescence associated with decay in G.

Decay test. The isolates rapidly colonized the bark cortex, turning it black. The white mycelium grew abundantly over the entire surface but caused an average of only 13.5% weight loss. Decay of sapwood and heartwood blocks was similar, although average weight loss caused by all isolates was 27.0 and 19.1%, respectively (Table 3). Decay was soft, grayish tan, and more advanced in the lower portion of the block which was in contact with the moist soil. The area adjacent to the more advanced decay exhibited fluorescence under ultraviolet light.

Decay in living trees. Of 449 trees sampled in the 1956 portion of the Colorado aspen decay study (7), 7.8% were infected with *Libertella* sp. based on cultural diagnosis. Trunk decay columns with which *Libertella* was associated ranged from 0 to 17.1 m in height with an average length of 4.6 m. Most decay columns occurred in the mid and upper trunk areas. Sixty-one percent of the infections were associated with dead or broken tops, living and dead branches, and trunk wounds. The entrance of the other infections was undetermined. The amount of decay attributed to *Libertella* in individual trees varied from 0.1 to 14.5 ft^3 (0 to 43% of the tree gross volume); however, the decay amounted to only 0.8% of the total cubic foot volume.

Significant relationships were found between incidence of infection and tree diameter and site. Infection decreased from 12% on the best site to 8% on the intermediate site and to only 3% on the poorest site. The incidence of decay increased with increased tree diameter (Fig. 4).

Wood discoloration and fluorescence. Sapwood discoloration around the perimeter of decay infections associated with the inoculation points varied from light brown to brownish-orange and was often mottled in appearance. The discolored zone fluoresced under ultraviolet light. The discoloration often completely enveloped the sapwood of saplings and branches. It often encircled portions of the sapwood of larger trees, and extended considerable distances above and below the inoculation point (Table 1). In many instances the fluorescent zone associated with this discoloration extended into the heartwood.

Of 282 fluorescent sapwood chips, 43% yielded *C. populina*, 38% were sterile, 17% yielded other fungi, and 2% yielded bacteria. Of 84 heartwood isolations, 8% yielded the fungus, 67% were sterile, 13% yielded other fungi, and 12% bacteria. The other fungi commonly isolated were not wood-decaying fungi.

The first aqueous extract from dried and 1-mo-old cultures was light yellow and showed light yellow fluorescence. Upon concentration, the extract became light brown and the fluorescence intensified. The filtrate left unsterilized at room temperature soon showed signs of biological activity. Within 1 mo there were large, rod-shaped bacteria and aggregates of mycelial growth of the fungus within the liquid. The filtrate changed during this period from about pH 4.0 to 5.0. An aqueous extract from malt agar likewise showed a slight fluorescence; it was whitish in color and very faint.

The aqueous extract from discolored fluorescent sapwood was light brown in color and exhibited dark orange fluorescence. The healthy sapwood extract was clear but fluoresced only faintly, and then with a whitish glow.

TABLE 3. Average weight loss (percent) of aspen bark, sapwood, and heartwood blocks caused by various isolates of *Cryptosphaeria populina*[a]

Bark		Sapwood		Heartwood	
Isolate	Mean[b]	Isolate	Mean[b]	Isolate	Mean[b]
L-4C	10.6 a	A-5	19.8 a	L-5D	16.6 a
L-2C	11.0 ab	A-11	22.8 ab	L-2C	17.0 a
L-5D	11.2 ab	L-4C	24.0 ab	L-4C	17.3 a
A-5	12.2 bc	D-5	25.5 ab	A-11	17.3 a
A-11	16.2 bc	L-2C	27.4 b	D-5	18.5 a
D-14	16.3 bc	L-5D	29.3 b	A-5	20.1 a
D-5	16.8 c	D-14	40.3 c	D-14	26.5 b

[a] Percentage weight loss of all decayed samples was corrected by subtracting the small weight loss of the controls.

[b] Means with common letters within a column are not considered different at $\alpha = 0.5$ by Tukey's test.

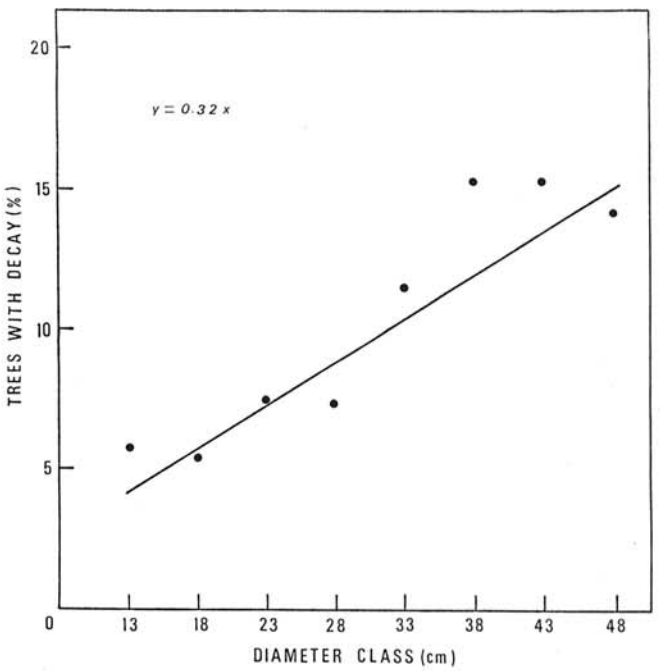

Fig. 4. Relationship between percent of trees with *Libertella* decay and diameter class in 449 live aspens in natural stands in Colorado.

DISCUSSION

Although the distribution of Cryptosphaeria canker is largely known from the western range of aspen and includes other poplar hosts, its known distribution will, no doubt, increase in the future as more intensive observations are made. The inoculation tests confirm that *C. populina* is the causal agent of this common canker and that tree mortality is often caused by sapwood parasitism rather than by canker girdling of the cambium and live bark.

Results of the sprout and sapling inoculations show that *C. populina* can cause death of smaller stems within 1 yr. Sprout mortality, although not yet recorded, may also be common in the field. Sapling mortality was evidently related to time of infection, for June inoculations did not result in stem mortality as did the July and September inoculations.

The general lack of perithecial formation on small trees and the common occurrence of Cytospora fruiting on the stems could lead to an incorrect diagnosis of cause of death when *C. populina* was present, unless isolations were made from sapwood tissue. *Cytospora chrysosperma* is usually considered a weak parasite of declining trees, and as suggested by Christensen (6), the fungus often is not responsible for the injury with which it is associated. Aspen mortality caused by *C. populina* has probably been misidentified in the past.

The elongated Cryptosphaeria cankers are caused by the rapid development of the fungus in the sapwood. The length-to-width ratio of bark necrosis in the 1977 inoculations was less than 3:1 (Table 2). While this ratio evidently increases with canker age, the development of the associated sapwood infection and decay is probably more important. Bark necrosis associated with the xylem inoculation extended to 11.4 cm with the A-11 infections in 13 mo but sapwood infection amounted to 88.4 cm, a ratio of 7.7:1. After 48 mo, the September-inoculated C-1 cankers had extended to 24.1 cm (Table 1) but by this time discoloration and decay had extended to 331 cm; a ratio of 13.7:1. The C-1 canker discoloration ratio amounted to 23.9:1 in 50 mo. With this large extension of sapwood infection behind the canker, eventual cambium and bark death would cause canker length to extend rather rapidly over a period of time.

The branch inoculations demonstrate that these infections, starting with bark lesions, can lead to trunk infections. Branch infections may represent a major source of trunk infection and may

be more significant in terms of trunk decay than other investigators had supposed. Etheridge (9) isolated *Libertella* sp. from dead aspen branches at their point of juncture with the stem but not from the stems of 34-yr-old aspens. He found *C. chrysosperma, Phoma* sp., *Libertella* sp., and several other fungi colonizing aspen branches 6 yr in advance of the wood-destroying basidiomycetes. The branch mortality with which *Libertella* sp. was associated may have been due to the primary action of the *Libertella* sp. infection rather than the mortality he attributed to suppression. Based on branch mortality caused by *C. populina* in this study, it appears that the organism can be considered a primary cause of branch mortality and that other organisms appear in later successional stages.

Results of the soil block tests substantiate the earlier reports that the fungus causes decay. The fungus may be a primary organism in the succession of decay, for it has often been reported in association with other decay fungi. *Libertella* sp. was one of three fungi frequently isolated by Basham (3) from heartwood stains above and below columns of advanced heart rot. He suggested that the heartwood of living aspen was invaded and colonized by one or more fungi which are not generally associated with advanced decay prior to the development of advanced heart rot. Fritz (10) also isolated the fungus from many specimens of rot associated with characteristic heartwood and sapwood decay of logs in association with other types of decay.

Libertella sp. has been found in advance of *Fomes igniarius* var. *populinus* (Neu.) Campb. decay. Good and Nelson (11) studied the fungi associated with *F. igniarius* in living poplar trees and their probable significance in decay. Sixty-four fungi, including *Libertella* sp., plus bacteria, were isolated from 25 trees containing typical *F. igniarius* decay. *Libertella* sp. was consistently isolated from most zones of stain and decay from 10 to 34 feet in height. The number of fungi associated with *F. igniarius* was so large that, if there is a regular succession of these fungi during the course of decay, the details of such a succession could not be made in their study; the wood into which *F. igniarius* progressively grew had, in most cases, already been colonized by other fungi. Based on discoloration, decay, and cultural descriptions, the species of *Libertella* involved in the previous studies of poplar and aspen is very likely the same, and its role in succession in the decay process should be investigated.

The incidence of Libertella infection in live trees may increase with tree size and decrease with site class because infection takes longer to cause tree mortality in larger trees on good sites than on trees of smaller size on poorer sites. Therefore, the increase in numbers of small, living, infected trees is limited by the short survival of these trees. The canker was found on 2% of the live trees and 36% of the dead trees under 15 cm d.b.h. in the 1977 survey (17). Although less than 1% of the trees over 15 cm d.b.h. had canker, the decay was found in nearly 8% of the live trees over 13 cm d.b.h. in the Colorado decay study. Consequently, the incidence of live tree infection is probably greater than the survey indicated. Observations indicate that the fungus is somewhat limited in penetrating thicker bark and expressing canker symptoms in older trees.

It was first thought that the yellowish fluorescence associated with the gray to brownish mottled saprot of *Libertella* sp. might be diagnostic (Fig. 3E and F). However, other aspen decays exhibit this yellowish fluorescence, particularly in the discolored zones (incipient decay) around the advanced decay. Other decay common in live Colorado aspen which show fluorescence include that caused by the following fungi: *Peniophora polygonia* (Pers. ex Fr.) Bourd. et Galz., *Phellinus tremulae* (Bond.) Bond. et Boris (Fig. 3G and H), *Inonotus rheades* (Pers.) Bond. et Sing., *Daldinia concentrica* (Fr.) Cs. and DeNot., *Armillariella mellea* (Vahl ex Fr.) Karst., *Flammulina velutipes* (Curt. ex Fr.) Sing., *Ganoderma applanatum* (Pers. ex Wallr.) Pat., *Pholiota squarrosa* (Fr.) Kumm. *Pleurotus ostreatus* Jacq. ex Fr., *Bjerkandera adusta* (Willd. ex Fr.) Karst., *Hirschiporus paragemenus* (Fr.) Bond. et Sing., *Sistotrema raduloides* (Karst.) Donk, and *Pleurotus elongatipes* Pk.

Zones of fluorescent material are common in aspen. They were noticed at the control inoculation points which healed over and also found in sapwood behind Ceratocystis, sooty-bark, and Hypoxylon cankers. Oberg et al (19) compared normal sapwood with chromotograms prepared from extracts of fluorescent material from Nectria canker, poplar borer infested wood, knots, and wetwood. Because all these samples were similar in composition, Oberg et al (19) suggested that the development of the zones of discoloration may result from a common stimulus. They also suggested that the nature of discoloration which develops in aspen wood is independent of the nature of the associated agent of injury, and that these agents (ie, Nectria canker, etc.) are merely a stimulus for the initiation of physiological changes in the cells of affected zones. These changes result in the eventual death of the cell. Hossfeld et al (16) later showed fluorescent discolored wood of knots, Nectria canker, and wetwood to be decay resistant. Wound- and Saperda-discolored wood was not decay resistant when tested against three decay fungi. Extracts from the zones of fluorescence were also shown to have a toxicity towards two decay fungi. They suggested that zones of fluorescent wood associated with discoloration contain extractive components, some of which are toxic toward wood decay fungi, and that zones of durable wood observed in untreated aspen fence posts coincided with the occurrence of these zones of fluorescent wood.

The fluorescent liquid extracted in water from discolored sapwood beneath an inoculation was sterilized and used for a preliminary toxicity test. Two concentrations, plus a control of sterile water, were added to diamalt agar media and tested in duplicate with seven fungi commonly found in aspen decay. Although there was slight differences in growth rate of the fungi upon the three media at the end of 10 days, there were no great differences at the end of 24 days.

As cultures of *C. populina* produce a fluorescent water extractive similar in color under ultraviolet light to the extractive from fluorescent discolored aspen associated with the decay, the following simple test was made to see whether other decay fungi produce fluorescent material or that it is a host response. The previously mentioned decay fungi were grown in petri dishes on a diamalt agar media for 2 mo and treated similar to the *C. populina* cultures. All of the decay fungi tested, in addition to cultures of canker fungi including *Ceratocystis fimbriata* Ell. & Halst., *Cenangium singulare, Nectria galligena* Bres., and *H. mammatum,* yielded a filtrate that was fluorescent under ultraviolet light.

In summation, it has been shown that *C. populina* is capable of causing an aspen canker and the discoloration and decay previously attributed to a *Libertella* sp. that apparently is the same as the Libertella imperfect stage of *C. populina.* Fluorescent materials produced by this canker fungus and other decay fungi in culture are similar to fluorescence associated with discoloration and decay in living trees. This suggests that this material may be toxic to living cells and a precursor to the eventual discoloration and decay caused by the fungi. Toxin production by other fungi that cause tree cankers has been shown by Schipper (24) in his study of Hypoxylon canker. This fluorescent material may be translocated well in advance of the fungal hyphae where the affected cells are then penetrated by bacteria and other fungi. It seems likely that this fluorescent material contains polyphenol oxidases which oxidize certain acids and phenols to more toxic quinones which are then polymerized to insoluble nontoxic melanins causing the discoloration around the perimeter of infection (12). Research is continuing along these lines.

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