

## Incidence of Wound-Associated Infection by *Cytospora* sp. in Mountain Alder, Red-Osier Dogwood, and Black Hawthorn in Oregon

### Abstract

Wildlife biologists are concerned that mortality of mountain alder (*Alnus incana*), a dominant riparian species in Oregon and Washington, would alter wildlife habitat. We chose to determine the cause of the mortality in alder and also examine the absence of dieback of associated species of red-osier dogwood (*Cornus stolonifera*) and black hawthorn (*Crataegus douglasii*). One hundred and fifty living stems each of alder, dogwood, and hawthorn were subjected to pathogenicity tests with the fungus *Cytospora* sp., the suspected pathogen. Trees of each species were wounded to the phloem or the xylem or left unwounded and then inoculated with *Cytospora* sp. After two growing seasons, incidence of infection was significantly higher in alder than in dogwood or hawthorn. Significantly more infection occurred in wounded alder stems than in unwounded stems. Cambial electrical resistance for alder was significantly higher (1) for inoculated than for uninoculated wounds and (2) for xylem wounds than for either phloem wounds or unwounded stems. We conclude that *Cytospora* sp. is associated with stem cankering and mortality of mountain alder in northeastern Oregon. This is the first report of mountain alder as a host for *Cytospora* sp. in Oregon. Because associated tree and shrub species are not affected, alder dieback should not seriously affect fish or wildlife habitat.

### Introduction

Mountain alder (*Alnus incana* (L.) Moench) is a dominant plant species along watercourses in Oregon and Washington, where it provides shade and helps to maintain cool water temperatures critical for anadromous and other valuable fish species. Alder also provides cover for wildlife dependent on riparian zones (Thomas *et al.* 1979). In recent years, much of the alder has been dying, whereas associated shrub species such as black hawthorn (*Crataegus douglasii* Lindl.) and red-osier dogwood (*Cornus stolonifera* Michx.) do not seem to be affected. Wildlife biologists are concerned that alder mortality will alter wildlife habitat.

Diseased trees exhibit multiple branch cankers and branch mortality. A fungus tentatively identified as *Cytospora* sp. was consistently isolated from canker margins (Filip *et al.* 1989). Shaw (1973) reported only one species of *Cytospora* on alder in the Pacific Northwest: *C. pulcherrima* Dearness & Hansbrough in British Columbia, where the fungus was found fruiting on fire-damaged stems of *A. tenuifolia* Nutt. (= *A. incana*) (Dearness and Hansbrough 1934). The objective of our study was to further characterize the relationship between fungus and host by testing two hypotheses: (1) alder is highly susceptible to infection by *Cytospora*

sp., but black hawthorn and red-osier dogwood are resistant, and (2) stem wounding is required for infection.

### Methods

The research site was a 4-ha area in the Grande Ronde River drainage, Wallowa-Whitman National Forest, Oregon. All selected trees were free of cankers or other injuries. Variability from multiple wounds, wound aspect, and wound height above ground was reduced by making only one wound per stem on the north side at 1.4 m above ground. Stem diameters at 1.4 m ranged from 3 to 5 cm. Stems were numbered at the groundline with a metal tag. All treatments were applied in spring (April 1986), the probable time of maximum natural spore release and infection.

One hundred and fifty living stems each of alder, hawthorn, and dogwood were subjected to six treatments (25 stems/treatment). These treatments, randomly assigned to each stem, were (1) wounded (xylem) and inoculated, (2) wounded (phloem) and inoculated, (3) unwounded and inoculated, (4) wounded (xylem) but uninoculated, (5) wounded (phloem) but uninoculated, and (6) unwounded and uninoculated. Only 444 of 450 total stems were actually sampled because six stems could not be relocated after two growing seasons.

Cultures of *Cytospora* sp. were obtained from cankers on infected alders near the study area.

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Bark was removed from cankers, and wood chips (5 x 5 x 15mm) were removed from necrotic wood and placed onto potato dextrose agar. The genus *Cytospora* was identified based on colony morphology and color (Spielman 1985). Only one isolate was used for all inoculations.

Wounds (314 mm<sup>2</sup>) were made with a 2-cm-diameter cork borer sterilized each time with 50 percent ethanol. Stems were surface-sterilized with ethanol before wounding. Phloem wounds penetrated only the inner bark; xylem wounds penetrated the cambium into the xylem. Bark disks were removed and replaced with a 2-cm-diameter agar plug (mycelium side toward pith) from 30-day-old cultures on potato dextrose agar. The agar disk was held in place with a wrap of Parafilm™. Wounded but uninoculated stems received a sterile agar plug. Unwounded but inoculated stems were marked in pen, and agar containing mycelium was placed on the marked but uninjured bark and held in place with a Parafilm™ wrap. Unwounded and uninoculated stems received a sterile agar plug.

In May 1987, cambial electrical resistance (CER) was measured (nearest K ohm) with a Shigometer Model OZ-67 (Osmose Wood Preserving Co., Buffalo, NY). The needle probes, sterilized with 50 percent ethanol, were pushed into the wood 1 cm above and below each treatment site.

In July 1987, after two growing seasons, the condition of the foliage on treated stems was observed for symptoms of advanced infection (chlorosis, stunting, death). The stem diameter at 1.4 m above ground was recorded again. A 1-m section of stem containing the treatment site was removed, placed in a plastic bag with the metal tag, returned to the laboratory, refrigerated, dissected, and cultured within 48 hours.

In the laboratory, the current wound area was calculated by measuring two diameters (nearest mm) at the longest and widest points between new callus tissue or living bark tissue. These diameters were multiplied together and then multiplied by 0.75 to approximate the area of an ellipse. The maximum length and width of discolored wood was measured (nearest mm) after the bark was aseptically removed. The discolored area was calculated the same way as the wound area.

Isolations were made from each stem. Ten wood chips (5 x 5 x 15 mm) were removed aseptically with a wood gouge at 2-cm intervals vertically

and horizontally from each treatment site. Tissue type (healthy or discolored) was recorded for each wood chip. Five chips were sequentially plated onto potato dextrose agar. Cultures were maintained for 4 weeks at room temperature. The genus *Cytospora* was identified on the basis of colony morphology and color (Spielman 1985). No attempts were made to identify other fungal species.

Significant ( $P \leq 0.05$ ) differences in isolation frequency among treatments were tested with chi-square analysis through the CATMOD program in the SAS/STAT Package Version 6 (SAS Institute Inc. 1987). Continuous response variables were analyzed by using the Advanced Statistics SPSS/PC+ (Norusis 1986). Discolored area, wound area, cambial electrical resistance, and percentage of change in stem diameter [(final diam.-initial diam.)/initial diam.] x 100 were subjected to a 2 x 3 x 3 factorial analysis of variance. Percentages were analyzed with arc sine transformation. When significant differences were found, means were subjected to Fisher's least significant difference test.

## Results

### Isolation Frequency

*Cytospora* sp. was consistently isolated from within discolored wood surrounding the original wound and from the margins of discolored and clear tissue in alder (Figure 1). A significantly higher isolation frequency of *Cytospora* sp. was obtained from alder than from dogwood or hawthorn (Table 1). Both inoculated and uninoculated stems of alder became infected, whereas only inoculated stems of dogwood and hawthorn became infected—but at very low frequencies. No infection occurred in unwounded alder treatments. There was no significant difference in the amount of infection between inoculated and uninoculated stems: some natural infection of alder occurred in wounded but uninoculated treatments. Many of the Parafilm™ wraps around each wound deteriorated by the end of the first growing season, and most were gone by the end of the second growing season. Except for two stems of dogwood, no unwounded stems became infected. There was no significant difference in isolation frequency between xylem wounds and phloem wounds.

After two growing seasons, none of the inoculated trees displayed foliage symptoms (chlorosis, stunting, death). Only 5 of 444 sample trees died



Figure 1. Among artificially wounded and inoculated alder stems, those affected have sunken areas (left) with discolored tissue (arrow) beneath the bark (right).

TABLE 1. Frequency of stem infection by *Cytospora* sp. in 444 artificially inoculated and uninoculated stems after two growing seasons.

Species	Treatment	Number of stems*		
		Unwounded	Wounded	
			To phloem	To xylem
Alder	Inoculated	0(0)a	16(64)b	12(50)b
	Uninoculated	0(0)a	5(22)b	6(26)b
Dogwood	Inoculated	2(7)a	2(8)a	3(12)a
	Uninoculated	0(0)a	0(0)a	0(0)a
Hawthorn	Inoculated	0(0)a	1(4)a	0(0)a
	Uninoculated	0(0)a	0(0)a	0(0)a

\*Number in parentheses is percentage of total stems with *Cytospora* sp. Within a column or row, frequencies followed by the same letter are not significantly ( $P \leq 0.05$ ) different according to chi-square analysis by the CATMOD procedure.

after two growing seasons. Two of these were dogwoods: one wounded and inoculated and the other unwounded and inoculated. Two were hawthorns: one wounded and inoculated and the other wounded and uninoculated. The one dead alder was unwounded and inoculated. *Cytospora* sp. could not be isolated from the treatment area of any of the dead trees. In all five cases, the trees apparently died from causes other than *Cytospora* sp.

## Wound Size

After two growing seasons, phloem wounds were significantly larger than xylem wounds for all species (Table 2). Wounds inoculated with *Cytospora* sp. were not statistically different in size than uninoculated wounds. Except for dogwood xylem wounds, all inoculated wounds were larger after two growing seasons than they were originally. Xylem wounds that were uninoculated were all smaller after 2 years than they were originally, but phloem wounds that were uninoculated were the same size as or larger than the original wound.

TABLE 2. Wound size after two growing seasons in 294 stems artificially wounded and either uninoculated or inoculated with *Cytospora* sp. Wounds were originally 314 mm<sup>2</sup>.

Species	Treatment	Wound area (mm <sup>2</sup> )*	
		Wound depth	
		Phloem	Xylem
Alder	Inoculated	1232a	496b
	Uninoculated	313a	134b
Dogwood	Inoculated	343a	77b
	Uninoculated	458a	82b
Hawthorn	Inoculated	696a	339b
	Uninoculated	440a	95b

\*Within a column or row, means followed by the same letter are not significantly ( $P \leq 0.05$ ) different according to Fisher's least significant difference test.

## Wound-Associated Discoloration

As stated above, *Cytospora* sp. was consistently isolated from discolored tissue surrounding wounded alder stems. None of the wounds had discoloration that completely girdled the stem. There were no statistically significant differences in amount of discoloration between xylem wounds and phloem wounds and between inoculated wounds and uninoculated wounds (Table 3). It is obvious from isolation results (Table 1) that the wound-associated discoloration in dogwood and hawthorn, both inoculated and uninoculated, was caused by microorganisms other than *Cytospora* sp.

## Stem Diameter Growth

Within each tree species, diameter growth after two growing seasons was not significantly different (1) between stems with inoculated or uninoculated wounds or (2) between wounded or unwounded

TABLE 3. Wound-associated discoloration after two growing seasons in 294 stems artificially wounded and either uninoculated or inoculated with *Cytospora* sp.

Species	Treatment	Discolored area (mm <sup>2</sup> )*	
		Wound depth	
		Phloem	Xylem
Alder	Inoculated	1447a	1538a
	Uninoculated	334a	214a
Dogwood	Inoculated	478a	1737a
	Uninoculated	603a	1320a
Hawthorn	Inoculated	922a	1338a
	Uninoculated	676a	1350a

\*Within a column or row, means followed by the same letter are not significantly ( $P \leq 0.05$ ) different according to Fisher's least significant difference test.

TABLE 4. Increase in diameter of 444 stems either uninoculated or artificially inoculated with *Cytospora* sp., after two growing seasons.

Species	Treatment	Diameter increase (%)*		
		Unwounded	Wounded	
			To phloem	To xylem
Alder	Inoculated	18c	28d	22d
	Uninoculated	20c	29d	27d
Dogwood	Inoculated	10a	6a	11a
	Uninoculated	9a	7a	9a
Hawthorn	Inoculated	14ab	11b	11ab
	Uninoculated	8ab	16b	13ab

\*Within a column or row, means followed by the same letter are not significantly ( $P \leq 0.05$ ) different according to Fisher's least significant difference test.

TABLE 5. Cambial electrical resistance (CER) 1 cm above and below a wound or corresponding location in 444 stems artificially inoculated or uninoculated with *Cytospora* sp., after two growing seasons.

Species	Treatment	CER (K ohms)*		
		Unwounded	Wounded	
			To phloem	To xylem
Alder	Inoculated	28ab	34b	59c
	Uninoculated	27ab	26ab	28b
Dogwood	Inoculated	20a	23ab	20a
	Uninoculated	20a	20a	21ab
Hawthorn	Inoculated	29b	32b	34b
	Uninoculated	29b	30b	29b

\*Within a column or row, means followed by the same letter are not significantly ( $P \leq 0.05$ ) different according to Fisher's least significant difference test.

stems—except for alder, which grew more when wounded ( $P = 0.028$ , Table 4). Alder grew significantly ( $P < 0.01$ ) more in diameter than either dogwood or hawthorn.

### Cambial Electrical Resistance

Cambial electrical resistance above and below a wound was significantly ( $P < 0.01$ ) different among species (Table 5). Higher CER values usually indicate reduced host vigor. The highest CER occurred around xylem wounds of inoculated alder. Such wounds had significantly ( $P < 0.01$ ) higher CER than uninoculated wounds or unwounded stems. There was no apparent correlation between CER and either wound size or amount of wound-associated discoloration.

### Discussion

The fungus associated with the alder dieback has been identified as *Cytospora* sp., an anamorph of several species of *Valsa* (L. J. Spielman, pers. comm. 1990; Filip and Parks 1991). Species of *Valsa* are separated by disk color, ostiole arrangement, and ascospore size and therefore require the teleomorphic fruiting bodies that occur only on natural cankers in the field. We were not able to obtain these. Nevertheless, no species of *Valsa* has been reported on alder in Oregon (Spielman 1985). It is quite possible that the fungus in question is *C. chrysosperma* (Pers.: Fr.) Fr. (= *C. pulcherrima*), which is the anamorph of *V. sordida* (Spielman 1985) and was reported as *C. pulcherrima* on mountain alder in British Columbia (Dearness and Hansbrough 1934).

We suspect that *Cytospora* sp. may be causing the damage observed on alder in northeastern Oregon, although our study did not prove this—for two reasons: (1) canker development, stem girdling, and foliar symptom expression may take longer than the 2 years we allowed and (2) natural infection of uninoculated wounds made unclear the role of the fungus in canker development.

Empirical and experimental data indicate that stem wounding is required for fungal infection. Stem wounding in northeastern Oregon can occur in several ways: the most common is from damage by domestic cattle, deer, or elk that frequently use riparian zones containing alder (Thomas *et al.* 1979). Mass movement of river ice also causes periodic killing and wounding of trees and shrubs in riparian zones (Filip *et al.* 1989). Wounding does

not have to be so severe that the xylem is exposed: wounds to the inner bark are severe enough to result in infection. It is rare that an entire clump of alder is killed; only wounded stems are affected, and sprouting is common below killed branches. We could not determine the time between infection and stem dieback because in all cases infected stems were destroyed for sampling before the development of advanced symptoms.

Although we have observed alder dieback in other riparian zones in eastern Oregon besides the Grande Ronde River basin, the geographical extent of alder dieback in Oregon or other parts of western North America has not been determined. We did not determine whether other species of Pacific Northwest alder (*A. sinuata* (Regel.) Rydb., *A. rubra*

Nutt., and *A. rhombifolia* Nutt.) are affected by *Cytospora* sp. Wildlife managers need not be concerned that alder dieback will seriously affect fish or wildlife habitat. Associated tree and shrub species, such as dogwood and hawthorn, are not affected.

### Acknowledgments

We thank Bobbi Fuller and Judy Engle for assistance in the field; Kevin Hosman, Tim Max, and Janet Nelson for assistance in statistical analysis; Linda Spielman for fungal identifications; and Craig Schmitt, Bill Bloomberg, Bob Scharpf, Linda Spielman, and an anonymous reviewer for manuscript review. This is Paper 2761, Forest Research Laboratory, Oregon State University, Corvallis.

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Received 7 June 1991

Accepted for publication 22 October 1991