Temperature-Influenced Growth and Pathogenicity of *Cytospora abietis* on White Fir

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ABSTRACT

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Cytospora abietis grew in culture at constant temperatures that ranged from 5 to 30 C. The rate of linear growth of the fungus was most rapid at constant 25 and 30 C. No growth was observed at 35 C. Infection occurred and cankers developed on inoculated, greenhouse-grown white firs (*Abies concolor*) at constant temperatures of 17.1, 22.1, and 28.5 C. At 32.7 C, early symptoms of infection appeared but no cankers developed.

The fungus *Cytospora abietis* Sacc. causes a widespread and damaging disease of true firs (*Abies* spp.) in the western United States (6). Cankers, branch flagging, and top dieback are common types of damage. Although distribution of the disease and damage in the field are documented, little is known about the factors that influence fungus growth, infection, and disease development.

One factor known to predispose firs to infection by *C. abietis* is dwarf mistletoe (*Arceuthobium abietinum* Engelm. ex Munz.) (1,3). Portions of fir branches invaded by dwarf mistletoe are preferred sites for infection and development of *C. abietis*.

Another factor believed to predispose trees to infection by the fungus is moisture stress resulting from drought or poor growing site. Other factors that influence infection and disease development require further study before the epidemiology of the disease will be well understood. The range of temperature that regulates the survival, growth, and pathogenicity of *C. abietis* is currently unknown.

This paper reports the effect of temperature on growth of the fungus in culture, the temperatures at which the fungus will infect and develop in host tissue, and temperatures at which cankers develop and the host is damaged.

MATERIALS AND METHODS

Laboratory. A pure culture isolate of *C. abietis* was obtained from white fir, *Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr., in the Modoc National Forest, CA. The isolate was transferred to petri dishes containing potato-dextrose agar (Difco 40 g/L) (PDA), grown at room temperature (21–22 C) for 1 wk, and used for inoculation. For the growth study, the medium described was

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prepared, sterilized in an autoclave, and dispensed into 9-cm-diameter petri dishes, 25 ml per dish. Ten dishes were used at each of seven different temperatures—5, 10, 15, 20, 25, 30, and 35 C. Constant temperature was maintained to the nearest 0.5 C in the dark in laboratory incubators.

Cultures for inoculation were made by cutting a 5.5-mm disk from pure cultures and placing it in the center of a dish. Each dish was then inverted, and two perpendicular lines were drawn on the bottom to intersect the center of the disk. Linear growth of the fungus colony was measured in millimeters along each of these lines to obtain the mean diameter growth of each colony.

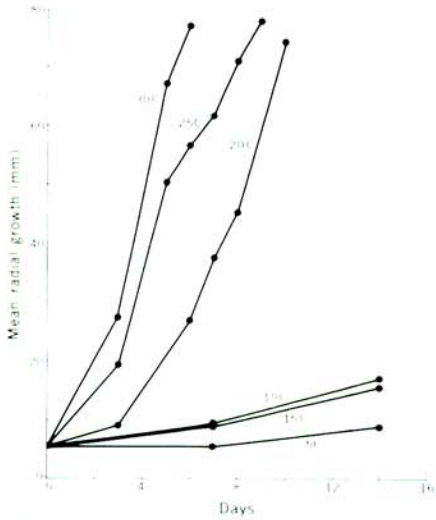

Fig. 1. Growth of *C. abietis* on PDA at different constant temperatures.

Immediately after inoculation, each group of 10 dishes was put into one of the incubators. Colonies were measured at periodic intervals until the dishes became overgrown or until the test was concluded. Colonies were measured under a dissecting microscope with a vernier caliper. Data were analyzed by Games and Howell's F modification for paired multiple comparisons with unequal variances (2) to determine differences in fungus growth at different temperatures.

Greenhouse. White firs were grown in nursery beds at the Forest Service Institute of Forest Genetics, Placerville, CA. Two-year-old root-pruned stock was lifted in February 1978, planted in 1-gal (3.8-L) pots, and held in a lathhouse until used for the tests in 1979.

In late June 1979, 32 trees were transported to Berkeley, where eight trees were placed in each of four temperature-controlled greenhouse cubicles. The trees were allowed to adapt to the temperatures in each cubicle for about 1 mo before they were inoculated. All trees were in an active stage of shoot elongation when placed in the cubicles. The test trees in each cubicle were checked daily and watered at the base as needed. No fertilizers or nutrients were used after the trees were placed in the cubicles.

Periodic temperature recordings during the test indicated that the mean cubicle temperatures were 17.1 ± 0.9 C, 22.1 ± 0.8 C, 28.5 ± 0.8 C, and 32.7 ± 0.6 C.

In early August 1979, the trees in each cubicle were inoculated with *C. abietis*. The isolate of the fungus was obtained from noble fir (*A. procera* Rehd.) in central Oregon and had been used previously in pathogenicity tests (5). The fungus was grown on PDA in petri dishes at room temperature for about 1 wk before it was used as inoculum.

Four trees in each cubicle were inoculated and four were left as controls. Trees were inoculated by placing a small (2–3 mm^2) block of PDA bearing the fungus onto a circular, 0.5-cm-diameter wound made in the cortex on the main stem of each tree. Control trees were wounded and inoculated with PDA only. The wound on each tree was covered with a small, moistened styrofoam pad and grafting rubber to provide a favorable environment for infection. The pads were moistened with tap water about every 2 days and removed at the end of 10 days. The trees were examined periodically for about 4 mo for cankers.

RESULTS

C. abietis grew in culture over a wide range of temperatures (Fig. 1). All statistical comparisons were made after 6 days of growth. In general, growth was slow at 15 C and lower temperatures. A significant difference in growth occurred between 5 and 10 C but not between 10 and 15 C. The rate of linear growth increased markedly above 15 C and was significantly different at five-degree increments between 15 C and 30 C. The fungus did not grow in culture at 35 C, but this high temperature was not lethal. When the cultures were taken from the 35 C incubator 4 wk after the start of the test and placed at 25 C, the fungus grew. *C. abietis* remained viable at all temperatures

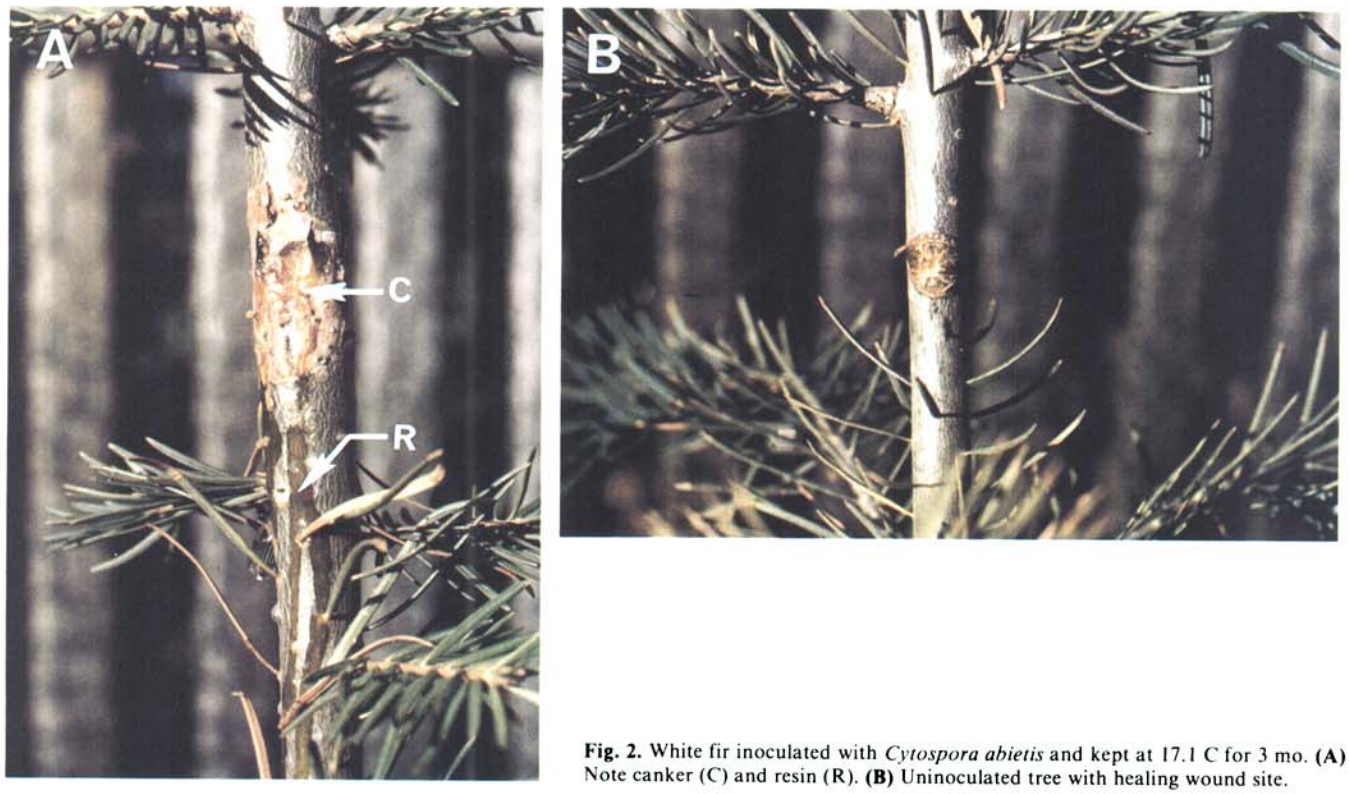

Fig. 2. White fir inoculated with *Cytospora abietis* and kept at 17.1 C for 3 mo. **(A)** Note canker (C) and resin (R). **(B)** Uninoculated tree with healing wound site.

tested but grew fastest at temperatures between 20 and 30 C.

Infection, symptom development, and canker formation also varied among some of the inoculated firs at different temperatures, but the differences were not immediately apparent. Resin accumulation, the first symptom of infection, was observed at the inoculation site on 10 of the 16 trees 3 wk after inoculation and on 14 of the 16 trees after 1 mo. These early symptoms were also noted in a previous study (5). None of the control trees had appreciable accumulation of resin at the wound site.

Cankers first began to develop on trees 6 to 8 wk after inoculation in the 17.1, 22.1, and 28.5 C cubicles. Nine weeks after inoculation, three trees at 28.5 C and one tree at 17.1 C had stem cankers that were up to 2.5 cm long and 1.5 cm wide. Other inoculated trees had smaller but recognizable cankers. All cankers had reached their maximum dimensions within 3 mo after inoculation (Fig. 2A, Table 1). No further occurrence or enlargement of cankers were observed. The cankers varied in size but, in general, they usually extended somewhat farther along the vertical axis of the stem than they did around the circumference. Cankers ranged from 0.7–2.5 cm long to 0.7–1.5 cm wide. Uninoculated trees continued to heal at the wound site (Fig. 2B).

In the inoculated trees at 32.7 C, the highest temperature used, cankers did not develop even though resin had accumulated. Also, at this temperature, uninoculated trees had not started to heal at the wound site. All eight firs at 32.7 C grew poorly, lost foliage, and apparently were poorly adapted to the constant high temperature.

Pycnidia did not develop on any of the infected portions of the test trees during the study, and none of the cankers girdled trees or killed tops. At the end of the test, one inoculated and one uninoculated tree were taken from each cubicle, and wood chips were cultured for the presence of *C. abietis*. *C. abietis* was isolated from all the inoculated trees but not from the uninoculated trees.

Table 1. Occurrence and size of cankers caused by *Cytospora abietis* on white firs at different constant temperatures

Temp. (C)	No. with cankers[a]	Canker length		Canker width	
		Mean (cm)	Range (cm)	Mean (cm)	Range (cm)
17.1	3	1.8	1.0-2.1	0.9	0.8-1.0
22.1	4	1.4	0.7-1.7	0.9	0.7-1.0
28.5	4	1.7	0.8-2.5	1.1	0.8-1.5
32.7	0	...	...	...	...

[a]Four trees per treatment.

DISCUSSION

C. abietis grew in culture at temperatures ranging from 5 to 30 C. Infection and canker development occurred on firs at 17.1 to 28.5 C. The fungus survived at 32.7 C but did not produce cankers. High temperature levels that would affect fungus survival and low temperature limits for infection and canker development, however, were not established. It is possible that *C. abietis* can infect and damage trees at temperatures lower than those used in this study.

In nature, host and pathogen are exposed to fluctuating rather than constant temperatures. The ranges and patterns of fluctuating temperatures that influence fungus growth and infection in the field are not known. Because *C. abietis* can infect hosts over a wide range of constant temperatures, it may also be able to infect hosts over a wide range of fluctuating field temperatures.

The fungus did not produce pycnidia on any of the infected trees in the greenhouse cubicles, but when samples from these trees were cultured in petri dishes, pycnidia developed on the small bark chips from each of the infected test trees. In an earlier study, none of several test trees infected with *C. abietis* developed pycnidia in a lathhouse (5). The factors that regulate fruiting of *C. abietis* on true firs need further investigation.

Another observation in this study that was also noted earlier (4) is that cankers stopped developing about 3 mo after inoculation. It is not known why actively growing cankers suddenly stopped enlarging. In the field, *C. abietis* often continues to grow and kill branches, particularly those infected by dwarf mistletoe. In this study, however, no trees were girdled and killed by *C. abietis*. Further studies are needed to explain why some cankers caused by *C. abietis* girdle and kill branches and tops and others cease to grow and develop, thereby allowing the infected portions to heal.

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