# Epidemiology and Control of a Blight of Juniperus virginiana Caused by Cercospora sequoiae var. juniperi

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ABSTRACT

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In eastern Nebraska, spores of *Cercospora sequoiae* var. *juniperi* were trapped as early as late April, but dispersal was not abundant until late May or early June and it extended into October. Spores were dispersed only during rainy periods. There was no evidence of long-distance dispersal; no spores were collected in volumetric traps 2 m from infected trees. Germination of spores began within 6 hr and after 24 hr was more than 90% over the range 16 to 28 C. Germ tube growth was optimum at 24-26 C. First infection of spur leaves of *Juniperus virginiana* occurred during the period 14-28 July in 1971 and 21 June - 5 July in 1972. Symptoms on spur leaves were first observed 8 August in 1971, 19 July in 1972, and 21 July in 1973. Only previous years' spur leaves and both current and previous years' juvenile leaves became infected. Whip leaves, the characteristic foliage of extremities of long shoots on secondary and tertiary branches, were not infected. Results provide a sound basis for determining when protective fungicides should be applied in eastern Nebraska.

*Additional key words:* eastern redcedar, Rocky Mountain juniper, *Juniperus scopulorum* Sarg.

*Cercospora sequoiae* Ell. & Ev. var. *juniperi* Ell. & Ev. causes a damaging leaf blight of eastern redcedar (*Juniperus virginiana* L.) and Rocky Mountain juniper (*J. scopulorum* Sarg.) in windbreaks and other plantings in the central Great Plains area of the USA. Successive seasons of heavy infection have killed 15- to 20-year-old trees. Our previous work (6) demonstrated that this fungus can be controlled by several applications of Bordeaux mixture, but the information needed for timing limited applications of fungicide has been lacking. Accordingly, investigations were made to determine: (i) when, and under what conditions, spores of the fungus are dispersed; (ii) when initial infection occurs; (iii) which tissues are susceptible; and (iv) when, and how many, applications of fungicide will adequately control this fungus.

## MATERIALS AND METHODS

Investigations were conducted at University of Nebraska's Horning State Farm near Plattsmouth in eastern Nebraska. The eastern redcedar trees studied were planted in 1962 and 1965 as filler trees or border rows in test plantings of conifers. All trees previously had been infected by the fungus.

**Spore trapping.**—Equipment for trapping spores consisted of petrolatum-coated slides suspended horizontally within the crown of infected junipers, and 7-day Burkard volumetric (10 liters per min) spore traps placed at measured distances from infected trees. A tipping-bucket rain gauge was used to measure rainfall, and temperature and relative humidity (RH) were recorded with a hygrothermograph. Wind velocity was continuously recorded and wind direction was recorded at 1-min intervals with a Science Associates, No. 470, all-purpose wind-recording system installed on-site.

**Germination of conidia.**—Spores used in these germination studies were produced in large numbers by isolates of *C. sequoiae* var. *juniperi* that originally had been isolated from eastern redcedar and then were cultured on carrot-leaf decoction agar (5). Fungal cultures were incubated for 7 days at 24 C under continuous fluorescent light (624 lux), then spores were washed from the cultures with water. Spores were placed on 2% water agar by dipping a glass rod (8-mm diameter) into a spore suspension and touching it to the agar surface. Thirty germ tubes were measured with an ocular micrometer, and germinated spores (number in 100) were counted at ×430 magnification in each of three replications. A spore was considered germinated when the length of the germ tube was equal to the width of the spore. Percent germination and germ tube lengths were determined for spores incubated (i) at different temperatures, (ii) at different time intervals at optimum germination temperature, and (iii) under either dark or light (fluorescent, approximately 1,076 lux) conditions.

The effect of desiccation of spores on germination was determined by (i) placing spores in distilled water for different time periods, (ii) then placing them on 1-cm squares of dialysis membranes (three replicates per treatment), (iii) drying them for various periods of time, and then (iv) incubating them on water agar at 24 C for 24 hr. In the first test, spores were hydrated for 0, 2, or 4 hr,

then desiccated for 0, 1, 2, 4, 6, 8, or 24 hr at 24 C and 20% RH. In the second test, spores were hydrated for 0, 2, 4, or 6 hr, then desiccated for 0 or 24 hr at 24 C, 30 C, or 35 C at 20% RH.

**Time of infection and control tests.**—The time of initial infection was determined by leaving foliage unprotected for different periods of time, then protecting it with Bordeaux mixture (8-8-100) applied with a hydraulic sprayer at 10.55 kg/cm$^2$ (150 psi) to run-off. In a test to determine the number and timing of fungicide sprays which would control this blight, branches were sprayed with Bordeaux mixture from one to six times at approximately 2-week intervals beginning 2 June. Infection was evaluated in mid-October by rating branches for the percentage of foliage that was necrotic according to the following rating scale: 1 = no necrosis; 2 = 1 to 20%; 3 = 21 to 40%; and 4 = over 40% necrosis. Treatments were assigned to individual branches and trees at random. Results were subjected to analysis of variance, and significant differences between means ($P = 0.05$) were determined by Duncan's multiple range test.

**Disease development.**—Foliage of eastern redcedar was examined at frequent intervals to determine the chronology of disease development on the host.

Juniper foliage has been grouped into three types by Hall (1): (i) whip leaves, characteristic of long shoot growth which occurs terminally on secondary and tertiary shoots; (ii) spur leaves, characteristic of short (spur) shoots; and (iii) juvenile leaves, the characteristic foliage of seedlings. Observations were made so that differences in disease development on the three classes of foliage could be distinguished. Most of the test trees had whip and spur foliage only. However, localized juvenile foliage was observed following either pruning or severe infection

TABLE 1. Numbers of *Cercospora sequoiae* var. *juniperi* spores trapped on slides exposed beneath shoots of eastern redcedar in 1971 and 1972

| 1971 | | | | 1972 | | | |
|---|---|---|---|---|---|---|---|
| Date slide exposure began | Exposure time (days) | Rainfall during exposure (mm) | Spores[a] trapped (no.) | Date slide exposure began | Exposure time (days) | Rainfall during exposure (mm) | Spores[a] trapped (no.) |
| 12 Apr | 7 | 2 | 0 | 14 Apr | 6 | . . . | 0 |
| 19 | 7 | 10 | 0 | 20 | 7 | . . . | 0 |
| 26 | 7 | 6 | 0 | 27 | 8 | 24 | 18 |
| 3 May | 7 | 34 | 0 | 4 May | 4 | 30 | 60 |
| 10 | 7 | 18 | 0 | 8 | 2 | 0 | 0 |
| 17 | 7 | 105 | 0 | 10 | 7 | 16 | 4 |
| 24 | 7 | 0 | 0 | 17 | 7 | 13 | 24 |
| 31 | 7 | T[b] | . . . | 24 | 7 | 11 | 535 |
| | | | | 31 | 7 | 6 | 152 |
| 7 June | 7 | 11 | 609 | 7 June | 7 | 17 | 270 |
| 16 | 9 | 2 | 459 | 14 | 7 | 6 | 38 |
| 23 | 7 | 32 | 799 | 21 | 7 | 0 | 0 |
| 30 | 7 | 9 | 252 | 28 | 7 | 2 | 113 |
| 7 July | 7 | 33 | 1,048 | 5 July | 1 | 13 | 74 |
| 14 | 7 | 2 | 226 | 6 | 6 | 18 | 124 |
| 21 | 7 | 1 | 318 | 12 | 5 | 3 | 0 |
| 28 | 7 | 12 | 1,325 | 17 | 2 | 9 | 147 |
| | | | | 19 | 7 | 38 | 233 |
| | | | | 26 | 6 | 4 | 0 |
| 4 Aug | 7 | <1 | 4 | 1 Aug | 7 | 8 | 127 |
| 11 | 7 | 2 | 15 | 8 | 7 | 0 | 0 |
| 18 | 7 | 32 | 276 | 15 | 7 | 7 | 0 |
| 25 | 7 | 0 | 0 | 22 | 7 | 32 | 18 |
| | | | | 29 | 7 | 12 | 31 |
| 1 Sept | 5 | <1 | 0 | 5 Sept | 7 | 49 | 6 |
| 6 | 7 | 0 | 0 | 12 | 7 | 15 | 0 |
| 13 | 6 | 1 | 0 | 19 | 7 | 13 | 9 |
| 19 | 5 | 1 | 6 | 26 | 7 | 7 | 4 |
| 24 | 6 | 10 | 76 | | | | |
| 30 | 6 | 0 | 0 | | | | |
| 6 Oct | 7 | 4 | 168 | 3 Oct | 7 | 4 | 0 |
| 13 | 8 | 18 | 202 | 10 | 7 | 1 | 0 |

[a]Each figure is the average number of spores observed on 84 mm$^2$ of each of two petrolatum-coated slides.
[b]T = trace.

by *C. sequoiae* var. *juniperi* in trees of the same age as the test trees.

## RESULTS

**Spore trapping.**—Spores of *C. sequoiae* var. *juniperi* were trapped from late April through October (Table 1). Dispersal was not abundant until late May in 1972 or early June in 1971. Spores were first evident on fruiting bodies on collections of foliage (17 May 1971) that had been infected the previous year (1970). Spores were not trapped during rainfree exposure periods. Small amounts of rain (2 mm or less) sometimes resulted in dispersal of considerable numbers of spores.

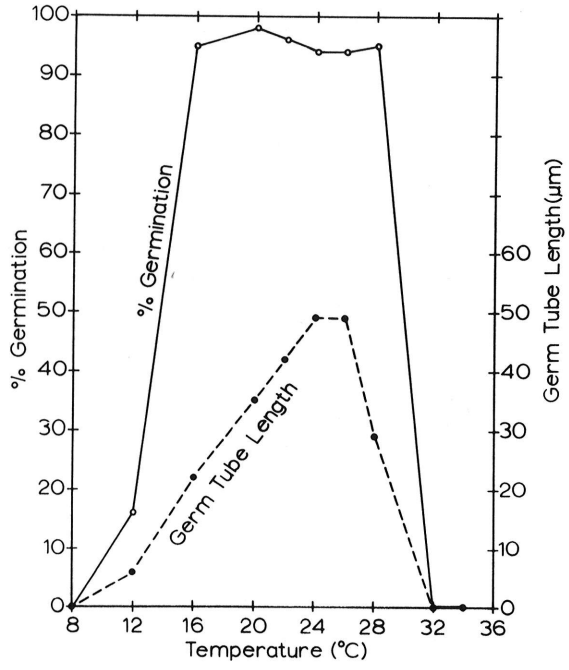


**Fig. 1.** Effect of temperature on germination of *Cercospora sequoiae* var. *juniperi* conidia incubated for 24 hr on water agar.

Spores were trapped in May 1972, but not in May 1971. The reason for this is not evident from weather data. There was only a slight difference in the average temperatures during May of both years and there was less rain in May 1972 than in May 1971.

During the 1971 growing season, no spores were collected in volumetric traps located 2 m from heavily infected trees; greased slides on these same trees trapped large numbers of spores.

**Spore germination.**—Over 90% of the spores germinated on water agar within 24 hr over the range 16 C to 28 C; no spores germinated at 8 C or 32 C (Fig. 1). Germ tube growth was optimum at 24-26 C (Fig. 1). Germination began within 6 hr at 24 C, and percent germination was near maximum within 16 hr. Percent germination and germ tube lengths were similar whether spores were incubated (24 C) in the dark or in the light.

None of the spore desiccation treatments prevented germination. Spores exposed to even the most severe desiccation treatment (6 hr of hydration and 24 hr of drying at 35 C) averaged 46% germination vs. 93% for controls.

The age of cultures from which spores were obtained strongly influenced spore germination and germ tube growth. After incubation for 24 hr at 24 C, germination and germ tube lengths for spores from 7-day-old cultures were 91% and 48 μm, from 10-day-old cultures 57% and 22 μm, and from 14-day-old cultures 16% and 10 μm, respectively.

**Disease development.**—The first infection in 1971 did not occur until the period 14-28 July (Table 2). There was very light infection of shoots sprayed on the four dates prior to 28 July, possibly because protection by the Bordeaux mixture was not adequate. It is unlikely that was due to infection prior to the first spray date, 2 June, since no spores were dispersed prior to 2 June (Table 1). Lack of extensive infections prior to late July could not be attributed to lack of spores, since high numbers of spores were dispersed in June and early July in 1971.

There is an indication that one or two applications of Bordeaux mixture applied in early June did not protect foliage for the entire growing season in 1971 (Table 3). A good level of protection was obtained by three or more applications when a 28 June application was included.

TABLE 2. Infection by *Cercospora sequoiae* var. *juniperi* of eastern redcedar shoots sprayed with Bordeaux mixture after various exposure periods in 1971 and 1972

| 1971 | | 1972 | |
|---|---|---|---|
| Initial spray date[a] | Infection rating[b,c,e] | Initial spray date[a] | Infection rating[b,d,e] |
| 2 June | 2.05 A | 10 May | 2.25 AB |
| 16 June | 2.05 A | 24 May | 2.00 A |
| 28 June | 2.15 A | 7 June | 2.00 A |
| 14 July | 2.10 A | 21 June | 2.25 AB |
| 28 July | 3.00 B | 5 July | 2.69 B |
| 11 August | 3.05 B | 19 July | 2.44 AB |
| Check (not sprayed) | 3.40 C | Check (not sprayed) | 3.50 C |

[a]Shoots were sprayed on all dates following initial application date.
[b]Rating: 1 = no necrosis, 2 = 1 to 20%, 3 = 21 to 40%, and 4 = over 40% necrotic foliage.
[c]Basis: Average from 20 branches (two branches on each of 10 trees) rated in mid-October.
[d]Basis: Average of four branches on four trees, rated in mid-October.
[e]Unlike letters denote difference, $P = 0.05$, according to Duncan's multiple range test.

In 1972, the first infection occurred during the period 21 June - 5 July, which was earlier than in 1971 (Table 2). The level of infection of untreated shoots was similar in 1971 and 1972 (Table 2). In 1973, Bordeaux mixture applied once (17 May) or twice (17 May, 14 June) provided complete control; however, the level of infection of untreated shoots was 2.6 compared to approximately 3.5 in 1971 and 1972.

The early symptoms consisted of bronzed tips of a few leaves on spur shoots. Subsequently, these leaves became entirely bronzed, then necrotic. Commonly, all leaves of a branchlet were affected. Usually, general necrosis of foliage on branchlets was not extensive until late September. By mid-October symptom development was most extensive; thus, final infection evaluations were made then. Affected branchlets usually were cast in October and November, and this resulted in the typical appearance of diseased trees–extremities of branches bearing healthy green foliage and the inner crown devoid of foliage.

Symptoms on spur leaves first were observed 8 August in 1971, 19 July in 1972, and 21 July in 1973. The incubation period (time between initial infection and first appearance of symptoms) was between 2 and 3 weeks in 1971 and 1972, since first infection occurred between 14-28 July in 1971 and 21 June - 5 July in 1972.

Long shoots remained free of infection for an average distance from their tips of 36 cm and 46 cm in 1971 and 1972, respectively. Most of the foliage on the infection-free shoot tips consisted of whip leaves, but there also were some spur leaves; no infection of whip leaves was observed.

Infection of spur leaves in 1972 was measured from the limit of infection in 1971 indicated by plastic bands that were placed on secondary and tertiary branches in the spring. The 1972 infection extended for an average distance of 29 cm. The new growth on the extremities of secondary and tertiary shoots averaged 10 cm. During the 3 years of tests, only spur foliage developed in previous years became infected; no infection of current-year spur foliage was observed.

Local necrosis on juvenile leaves first was evident 19 July in 1972. By 26 September most of the infected juvenile leaves were necrotic, and by 3 October necrosis was extreme. Thus, the disease developed more rapidly in juvenile leaves than in spur leaves. Furthermore, both current-year and previous years' juvenile leaves became infected, whereas only the previous years' spur leaves became infected.

Fruiting bodies (sporodochia) resulting from current-seasons' infection were observed in spur foliage 26 September 1972. They sporulated readily when incubated at 24 C and 100% relative humidity for 18 hr. In 1973, sporodochia first were found on spur foliage collected 9 September. Sporodochia first were found 5 September 1972 on juvenile leaves infected in 1972.

TABLE 3. Infection by *Cerspora sequoiae* var. *juniperi* of eastern redcedar sprayed from one to six times with Bordeaux mixture in 1971

| Spray dates | Infection rating[a,b] |
|---|---|
| 2, 16, 28 June; 14, 28 July; 11 August | 2.00 A |
| 2, 16, 28 June; 14, 28 July | 2.05 A |
| 2, 16, 28 June; 14 July | 2.20 A |
| 2, 16, 28 June | 2.30 A |
| 2, 16 June | 2.90 B |
| 2 June | 3.35 C |
| Check (not sprayed) | 3.80 D |

[a]Basis: Average from 20 branches (two branches on each of 10 trees) rated in mid-October. Rating: 1 = no necrosis; 2 = 1 to 20%; 3 = 21 to 40%; and 4 = over 40% necrotic foliage.

[b]Unlike letters denote difference, $P = 0.05$, according to Duncan's multiple range test.

## DISCUSSION

The results of this investigation provide a basis for control of Cercospora blight on *Juniperus virginiana* in Nebraska. Conidia were not dispersed in large numbers until late May. Eastern redcedar trees with whip and spur foliage were not initially infected before late June. Then only previous years' spur foliage became infected; there was no infection of whip foliage. Accordingly, a highly persistent fungicide applied before late June theoretically could protect trees with spur and whip foliage for the entire season. Because of weathering of fungicide, an additional application might be required.

Based on the results of this investigation, park managers in eastern Nebraska have used control procedures successfully against Cercospora blight on *Juniperus scopulorum* as well as on *J. virginiana* since 1973.

The control of this blight on eastern redcedar trees containing juvenile foliage would require more than a single application of fungicide, since both current-year and previous years' juvenile foliage becomes infected. Thus, additional fungicide applications would be needed to protect newly developing juvenile leaves.

The diseases caused by *C. sequoiae* var. *juniperi* and the related *C. sequoiae* Ell. & Ev. have been reported to be similar (2); however, cross inoculations have not been made to determine host response to these two fungi. Morphologically, *C. sequoiae* var. *juniperi* has shorter conidiophores, narrower conidia, larger stroma, and different colony characteristics than *C. sequoiae* (2, 3). *Cercospora sequoiae* causes a devastating disease of *Cryptomeria japonica* D. Don in Japan. Infection of 2-yr-old *C. japonica* seedlings by *C. sequoiae* in Japan was more effectively controlled by maneb than by Bordeaux mixture, the fungicide used in this study (4).

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