Longevity of Conidia of *Sirococcus clavigignenti-juglandacearum* in a Simulated Airborne State

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ABSTRACT

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Longevity of rain-dispersed conidia of *Sirococcus clavigignenti-juglandacearum* in a simulated airborne state was studied in the laboratory and forest by impacting conidia on fine spiderweb threads wound around rectangular frames. Frames bearing conidia were exposed to different controlled vapor pressure deficit (VPD) and temperature levels. After 32 hr of exposure to still air at 13 C, 81% of the conidia were viable at 9.7 mb VPD compared to 11% at < 0.8 mb VPD. At 25 C, 14% of the conidia survived for 32 hr at 20.6 mb VPD while less than 2% were viable at < 1.3 mb VPD. In field tests, spore longevity varied greatly with weather conditions. The highest percentage survival in any field experiment was 35% after 8 hr on a cool, rainy day with overcast skies.

Additional key words: butternut, *Juglans cinerea*.

Butternut canker, which is caused by *Sirococcus clavigignenti-juglandacearum* Nair, Kostichka, and Kuntz, was first observed in Wisconsin in 1967 (15). The disease now is found throughout the natural range of butternut (*Juglans cinerea* L.) in the midwestern and eastern United States (2,14) and threatens to eliminate this hardwood in some areas of the country (12). Symptoms of the disease include multiple cankers on branches, the main stem, and buttress roots. Coalescing perennial cankers eventually girdle and kill the tree. The fungus extrudes hyaline conidia in cirri from pycnidia embedded in a thin black stroma or in hyphal pegs formed beneath loose, necrotic, outer bark. The perfect state of the fungus has not been reported, nor have insect vectors been found (12).

Conidia are dispersed by raindrop impaction and have been trapped 40 m from a diseased tree (12,18), although cankers resulting from natural infection have been found over 100 m from the nearest cankered tree (18). Raindrop impaction disperses small droplets containing fungal spores into turbulent air where they can evaporate rapidly, leaving the spores suspended in air (9). Aerosols of bacteria (6,7,19) and fungal spores (3,4) have been produced by raindrop impaction. The generation of aerosols of conidia of *S. clavigignenti-juglandacearum* during rainfall may allow for long-distance dispersal of the pathogen.

Infection by conidia dispersed as aerosols would require that they remain viable in an aerial environment, yet relatively little is known about the longevity of fungal spores in the atmosphere. In the case of pigmented or thick-walled spores, a loss in viability by desiccation or the effects of ultraviolet radiation are presumably small. Kramer and Pady (10) found that 80% of the conidia of *Alternaria* sp. trapped in the air in Kansas were viable. However, the length of time the conidia had been airborne or the conditions to which they were exposed prior to trapping were not known. Pady and Kapica (13) trapped viable, dark-celled, fungal spores over the North Atlantic ocean. They concluded that the spores must have originated from land masses far to the southwest and remained viable in an airborne state for a considerable period of time. In contrast, hyaline, thin-walled, fungal spores and bacterial cells appear to have a relatively short lifespan in the air. Yarwood and Sylvester (20) calculated that 50% of the basidiospores of *Cronartium ribicola* would survive 5 hr in the air. Phytopathogenic bacteria have survived for 2 hr in the open air (8,16). The purpose of this study was to determine the longevity of conidia of *S. clavigignenti-juglandacearum* in a simulated airborne state and to examine the effects of temperature and vapor pressure deficit on their survival.

MATERIALS AND METHODS

A spider-silk (microthread) technique originally developed by May and Druett (11) for longevity studies of bacterial aerosols was used. Rectangular frames, used to support the spider silk (microthreads), consisted of two, 6-cm lengths of steel wire (1.2-mm diameter) spaced 2.0 cm apart and secured at each end by embedding the wire in 0.3 × 0.3 × 2.5-cm resin blocks. A removable wire handle (10 cm) was mounted at one end of the frame to facilitate the winding of the spider silk. Frames were sterilized in 70% ethanol before winding.

Sheet-web spiders (*Frontinella* sp.), collected from the

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University of Wisconsin Arboretum, were used as a source of the spider silk. The spiders (body length 0.3–0.4 cm) are found commonly on small shrubs. Spiders were kept in 1-L glass jars containing wet paper towels and twigs. They were artificially reared for up to 6 mo by feeding on fruit flies (*Drosophila melanogaster*).

To begin the winding process, a spider was placed gently with forceps on the wire edge of a frame. The frame was tapped lightly to dislodge the spider and, as the spider dropped, a drag line of silk remained attached to the wire. The frame was rotated quickly for ~20–30 turns between the fingers as soon as the spider landed on a solid surface. The silk was pulled from the spinnerette of the spider. This method prevented the spider from re-attaching the drag line to another surface and kept tension on the silk as it was wound around the frame. Roughly parallel and equally spaced threads could be positioned by rotating the frame at a slight angle. A majority of the threads measured 1.5 μm or less in diameter, but occasionally two threads merged, resulting in a diameter of 2–3 μm.

The culture of *S. clavigignenti-juglandacearum* used in the experiments was a mass isolate obtained from a heavily cankered tree located in the University of Wisconsin Arboretum. Spore suspensions were prepared from 10- to 20-day-old cultures grown on 2% potato-dextrose agar (PDA) slants. Sterile, distilled water was added to the slants, which were then vibrated in a Vortex mixer (Scientific Products, Evanston, IL) to dislodge the conidia. Spore concentrations were measured with a hemacytometer and adjusted to 10^6 conidia per milliliter of water. Viability of the conidia in suspension always was greater than 90% as determined by germination on 2% water agar (WA). Aerosols of the spore suspension were generated by a nebulizer (Universal, West Germany) and were directed through the frames for 1 min. Conidia were impacted onto the spider silk. Conidia were not easily removed from the threads by wind although some loss was detected after outdoor exposure for 8 hr. Conidia always aligned such that the long axis of the conidium was parallel to the microthread. Usually, conidia were singly spaced on the microthread and not in contact with one another, but occasionally groups of two or more conidia were deposited. These groups were not counted in the germination studies.

Longevity of conidia in a small enclosure under controlled temperature and mb VPD levels was studied by placing the loaded frames inside a 2-L glass desiccator. A constant relative humidity (RH) of 35 or 65% was obtained by placing glycerine solutions (1) in the bottom 6 cm of the desiccator. Distilled water was used to obtain a RH greater than 95%. Desiccators were placed in constant-temperature incubators at 13 or 25 C in the dark. The RH within the desiccators at each temperature was monitored with a polystyrene hygrometer (Phys-Chemical Res. Corp., New York, NY 10011) and in all experiments was within ±3% of the desired value. VPD levels then were determined for each combination of temperature and RH. Microthread frames were mounted on the raised, perforated floor of the desiccators approximately 3 cm above the glycerine solution. The frames were taped perpendicular to the floor so that a maximum exposure of the threads to ambient conditions could be obtained. A total of 18 frames were placed in each desiccator at a given temperature and VPD level. Three frames were removed from the desiccator after 0, 4, 8, 16, 24 and 32 hr. The frames were placed immediately into petri dishes containing 2% WA and firmly pressed onto the agar surface. Microthreads then were cut from the frame with a razor blade. Frames were removed leaving behind the microthreads and conidia on the agar surface. Petri dishes were incubated for 48 hr at 25 C in the dark. Conidial viability was determined by examining spores on the WA with a light microscope at ×430 (Fig. 1). Mean percentage germination was based on counts of 200 conidia per frame and three frames per time interval. Spores were considered to have germinated if the germ tube equaled the length of the conidium. Experiments at a given temperature and VPD were repeated twice; similar results were obtained.

Longevity of conidia in the forest was studied on six occasions in 1981 in the University of Wisconsin Arboretum. Conidia were nebulized onto microthreads under ambient conditions in the forest. Frames were suspended from a string 2 m above the forest floor in a small clearing such that the frames were exposed to direct sunlight during clear days. At various time intervals, three frames were removed and placed in petri dishes containing WA. Temperature, RH, rainfall, and wind speed were recorded during most sampling periods. During rainfall, a small plastic tarp was placed 10 cm above the microthread holders to prevent raindrops from impacting on the microthreads.

RESULTS

The application of conidia onto microthreads did not reduce spore viability. Germination percentage of conidia (0 hr) directly after impaction onto microthreads was >90% in all studies and was similar to the germination percentage of conidia in the original spore suspension.

Conidial longevity on microthreads exposed to 25 C in the laboratory varied with differences in VPD (Fig. 2). Spore viability at <1.3 mb VPD (>95% RH) decreased rapidly; <30% of the conidia survived for 4 hr. In contrast, spore viability at 20.6 mb VPD (35% RH) was 94% after 4 hr and decreased gradually to 14% at 32 hr. Longevity of conidia at 11.1 mb VPD (65% RH) was intermediate; however, few conidia survived for 32 hr. Similar effects of VPD levels were found at 13 C (Fig. 3). Longevity was shorter at <0.8 mb VPD (>95% RH) than at 9.7 mb VPD (35% RH).

Temperature also influenced spore longevity. Viability consistently was higher at 13 C than at 25 C for similar time intervals and VPD levels. Maximum longevity (81% after 32 hr) was obtained at 13 C and 9.7 mb VPD (35% RH).

Conidial longevity always was shorter in the forest (Table 1) than

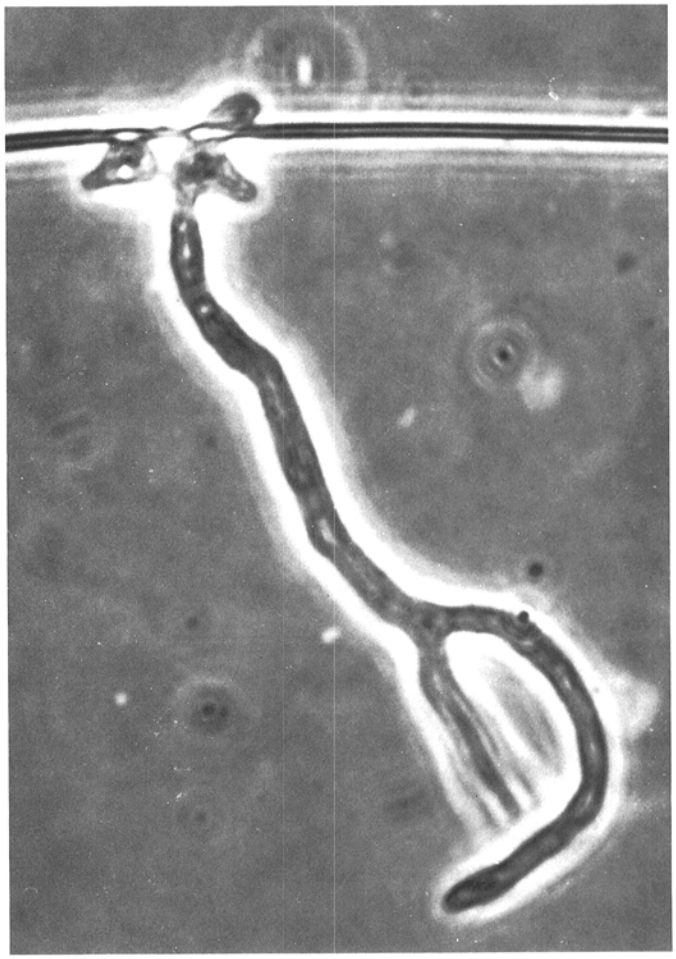

Fig. 1. Germinating conidium of *Sirococcus clavigignenti-juglandacearum* attached to microthread after exposure to the air and incubation on WA for 48 hr at 25 C (×1,500).

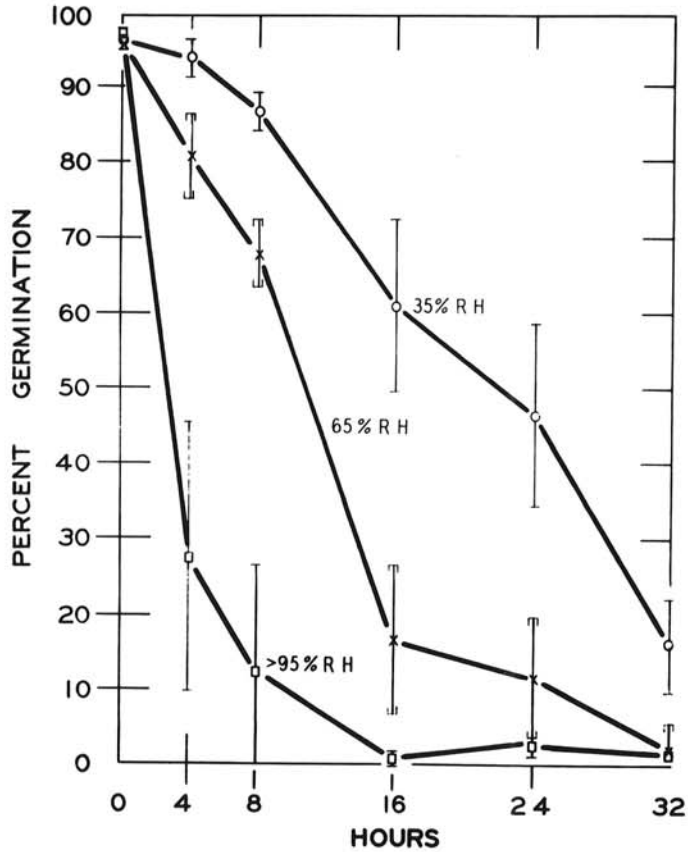

Fig. 2. Percentage germination of conidia of *Sirococcus clavigignenti-juglandacearum* after exposure to air at 20.6, 11.1, and < 1.3 mb VPD (35, 65, and >95% RH) and 25 C. Bars represent 90% confidence intervals.

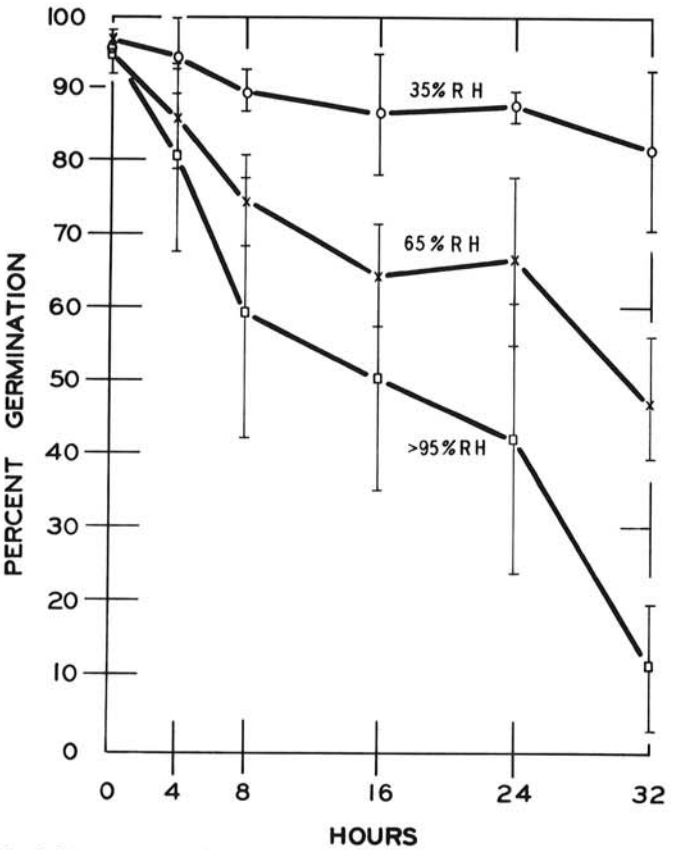

Fig. 3. Percentage germination of *Sirococcus clavigignenti-juglandacearum* conidia after exposure to air at 9.7, 5.2, and <0.8 mb VPD (35, 65, and >95% RH) and 13 C. Bars represent 90% confidence intervals.

in laboratory tests. Nevertheless, on all days except 5 August, spore viability was greater than 84% for the first 2 hr of exposure to air. Field results supported laboratory findings, indicating that cool temperatures increase spore longevity. On 5 August, less than 6% of the conidia were viable after a 2.5-hr exposure at temperatures ranging from 22.4 to 24.5 C. Longevity also was short on 24 September during a 5-hr interval in which temperatures increased from 11.8 to 20.0 C. In contrast, on 16 September, 89% of the conidia were viable after 2 hr and 30% were viable after 5 hr; temperatures ranged from 11.2 to 14.4 C. Highest spore survival

TABLE 1. Longevity of conidia of *Sirococcus clavigignenti-juglanacearum* exposed on spider web microthreads to ambient conditions in the forest

Exposure[a] (hr)	Germ.[b] (%)	Temp.[c] (C)	RH[d] (%)	VPD[e] (mb)	Max. WS[f] (m/sec.)	Cld. Cv.[g] (%)
5 August						
0	91.5 ± 2.4	22.4	91	2.4	0.5	0
0.5	85.5 ± 0.8	22.7	91	2.4	0.5	0
1.0	60.0 ± 8.8	23.3	90	2.8	0.5	0
1.5	32.5 ± 5.1	23.7	89	3.2	0.5	25
2.0	12.5 ± 6.6	24.3	89	3.3	0.5	25
2.5	5.5 ± 2.4	24.5	88	3.7	1.0	25
14 August						
0	98.2 ± 0.5	20.1	91	2.1	0.9	100
0.5	94.0 ± 1.7	20.1	91	2.1	···	100
1.0	95.3 ± 1.0	19.9	91	2.1	0.9	100
2.0	91.7 ± 4.2	20.2	91	2.1	0.8	100
3.0	63.7 ± 1.9	20.9	91	2.2	0.5	100
4.0	23.0 ± 8.4	21.4	91	2.2	1.0	100
9 September						
0	98.3 ± 0.5	18.0	69	6.4	0.5	0
0.5	98.5 ± 0.8	19.2	65	7.7	···	0
1.0	98.8 ± 1.3	20.1	54	10.8	0.6	0
2.0	84.7 ± 3.1	21.7	52	12.7	···	0
3.0	32.5 ± 3.7	22.0	68	8.4	0.7	0
3.5	30.2 ± 6.4	21.9	51	13.0	0.8	0
16 September						
0	96.7 ± 2.6	11.2	81	2.5	0.9	25
1.0	94.8 ± 1.8	12.5	72	4.0	1.1	25
2.0	89.1 ± 4.0	13.4	62	5.7	1.0	50
3.0	84.2 ± 3.0	14.4	62	6.0	0.6	50
4.0	77.2 ± 2.7	14.4	59	6.4	0.7	50
5.0	30.0 ± 8.5	13.2	65	5.2	0.7	50
24 September						
0	97.8 ± 0.5	11.8	93	1.0	0.5	100
1.0	96.8 ± 1.0	12.6	93	1.0	0.5	75
2.0	88.7 ± 1.3	14.2	93	1.1	0.5	25
3.0	10.2 ± 15.1	16.9	84	3.0	0.5	0
4.0	1.3 ± 1.2	18.9	79	4.4	0.5	0
5.0	0.7 ± 1.3	20.0	69	7.2	0.5	0
14 October						
0	93.3 ± 2.6	13.9	>95	<0.8	0.9	100
1.0	90.5 ± 1.2	13.9	>95	<0.8	0.8	100
2.0	92.7 ± 4.9	13.6	>95	<0.8	···	100
4.0	67.0 ± 4.8	13.7	>95	<0.8	0.5	100
6.0	53.3 ± 8.4	13.7	>95	<0.8	0.7	100
8.0	34.7 ± 7.8	13.8	>95	<0.8	0.5	100

[a] Exposure of conidia on microthreads to ambient conditions in the forest on 5 August, start time 0820 hours, no rain; 14 August, start time 1100 hours, trace of rain from 1330–1430 hours; 9 September, start time 1035 hours, no rain; 16 September, start time 1000 hours, no rain; 24 September, start time 0930 hours, 0.25 cm rain from 0900–0915 hours; and 14 October, start time 0830 hours, 0.76 cm rain from 0930–1100 hours.

[b] Mean viability of conidia for three replicates, 200 conidia per replicate ± 90% confidence intervals. Germination test was conducted after exposure of conidia to the air.

[c] Temperature at each sampling time.

[d] Relative humidity at each sampling time.

[e] Vapor pressure deficit for each sampling time.

[f] Maximum wind speed during sampling interval.

[g] Approximate percent cloud cover.

was found on 14 October when 34.7% of the conidia were viable at 8 hr. Temperature on this date remained near 14 C. Temperatures and longevity of conidia were similar after 2 hr on 14 August and 9 September, even though the VPD level on 14 August remained near 2.1 mb while VPD levels on 9 September increased from 6.4 to 12.7 mb.

DISCUSSION

The microthread technique proved useful in studying the longevity of conidia of *S. clavigignenti-juglandacearum* in a simulated airborne state. Conidia readily adhered to the microthreads and were not easily removed by low wind speeds. The frames were small and portable, facilitating studies in both the laboratory and field. In these experiments, the microthread technique was not compared to other methods of studying the viability of airborne spores, but May and Druett (11) found that the longevity of bacteria on spider silk was similar to that in aerosols suspended in a Goldberg rotating drum apparatus (5).

The diameter of the microthread (1.5 μm) was approximately the same size or smaller than the width of the conidium, allowing for maximum exposure of conidia to air. This presumably would give more precise data on conidial longevity in the air than would spore survival on glass microscope slides or plant surfaces. Large surface areas would increase boundary layer effects and could give different survival rates.

An assumption made in these studies was that the ability of the conidia to germinate on water agar after exposure to the aerial environment reflected the viability of the conidia. While we did not detect differences in germination on WA or PDA after 48 hr, exogenous nutrients on host tissue may result in higher germination rates. Therefore, the technique may underestimate the viability of the conidia. Another minor problem associated with the technique in forest experiments was the trapping of unwanted fungal spores on the fine threads. The contaminants germinated rapidly on WA and made counts of the slower germinating conidia of *S. clavigignenti-juglandacearum* difficult at exposure times >8 hr.

Conidia of *S. clavigignenti-juglandacearum* survived for appreciable lengths of time in an airborne state, but meteorological conditions strongly influenced their longevity. Survival under laboratory conditions was highest at a cool temperature (13 C) and a high VPD (9.7 mb). The fact that survival of the hyaline, thin-walled conidia increased at a high VPD suggests that dehydration extends the life of conidia in an airborne state. Dehydration has been shown to be effective in extending the viability of some fungal spores during storage (17).

Longevity of conidia in the forest was favored by cool temperatures, overcast skies, and stable VPD levels. Rapid changes in temperature or VPD, or a change from cloudy to clear skies resulted in a loss in viability under the conditions tested. Although the effects of ultraviolet light on spore longevity were not tested directly, results from 9 and 24 September (Table 1) suggest that such radiation plays a significant role in the reduction of spore viability.

The generation of conidial aerosols by raindrop impaction and subsequent longevity of the conidia once airborne may be important in the dispersal of the pathogen over long distances. Butternut trees tend to grow in small, isolated groves or pockets in mixed hardwood forests. Our results indicate that conidia would remain viable during dispersal from one pocket of butternut trees to another during or following rain provided that the spores were not washed from the air by raindrops or removed by impaction. Whether or not the concentration of conidia in the air at distances of 1 km or more would result in infection of healthy trees remains uncertain.

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