

Etiologic and Cultural Studies of *Kabatina juniperi*

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ABSTRACT

Ostrofsky, A., and Peterson, G. W. 1981. Etiologic and cultural studies of *Kabatina juniperi*. Plant Disease 65:908-910.

Kabatina juniperi caused a branch tip dieback of *Juniperus virginiana* and *J. scopulorum* in eastern Nebraska. Infected branch tips became discolored in early spring. Acervuli were present but not yet erumpent in February. Erumpent acervuli were abundant at the base of the dieback in April and May and were present in decreasing numbers through October. Spores germinated on water agar at temperatures from 5 to 32 C; percentage of germination and germ tube lengths after 24 hr of incubation were greatest at 24 C. Spores germinated on water agar at pH values of 3 through 8; percentage of germination and germ tube lengths were greatest at pH 6. Growth in malt extract broth was greatest at 24 C and at pH 5. Light had no effect on spore germination, germ tube length, or growth. Wounding was necessary before inoculation with spores for infection of healthy foliage in the greenhouse. Seedlings became infected when incubated at 100% relative humidity and 16–28 C for 5 days after inoculation. Wounded inoculated seedlings incubated for 24 hr at 100% relative humidity and 24 C also became infected. Scanning electron micrographs showed that the fungus entered foliage through wounds.

Additional key words: dieback, eastern redcedar, Rocky Mountain juniper

Juniperus virginiana L. (eastern redcedar) and *J. scopulorum* Sarg. (Rocky Mountain juniper) are important components of windbreak, wildlife, and landscape plantings in the Great Plains. In recent years, branch tips of both *Juniperus* spp. have been damaged by an unknown agent. *Kabatina juniperi* Schneider and v. Arx was found at the base of dieback on twigs of *J. virginiana*; its occurrence was the first reported in the United States (6). *K. juniperi* and *K. thujae* Schneider and v. Arx were originally found and described in Europe and recognized as agents of dieback of members of the Cupressaceae (3–5,7,8). *K. thujae* was associated with dieback of *Chamaecyparis nootkatensis* (D. Don) Spach after a severe winter in British Columbia (2).

This study investigated tip dieback of *Juniperus* spp. in eastern Nebraska. We sought to determine whether the fungus

associated with dieback of *J. virginiana* was the causal agent of the dieback of that species and of *J. scopulorum*, the development of symptoms in nature, when acervuli are present in nature, conditions influencing spore germination and growth of *K. juniperi* in vitro, conditions necessary for infection of seedlings in the greenhouse, and mode of entry of the fungus into host tissue.

MATERIALS AND METHODS

Field observations. We conducted field observations near Plattsmouth, NE, at Horning State Farm of the University of Nebraska, unless otherwise noted. Trees in a *J. virginiana* plantation known to be infected with *K. juniperi* were observed monthly for the presence of acervuli and for symptom development. Twenty-five diseased twigs from 10 trees were collected in April 1977. We measured length of dieback, length of twig covered with acervuli, and twig diameter at the base of dieback.

When diseased material from other states was encountered, we examined it for the presence of *K. juniperi*. *J. scopulorum* from Nebraska was also examined for acervuli of the fungus.

Germination studies. Germination, growth, and inoculation studies were conducted with *K. juniperi* isolated from *J. virginiana* from Plattsmouth, unless otherwise indicated. This single acervulus isolate was assigned number 283 in the culture collection maintained at the Forestry Sciences Laboratory, Lincoln.

Spores were obtained from 6- to 10-day-old colonies grown on Difco malt agar or Difco potato-dextrose agar

incubated at 20 C. Spores at a concentration of about 1.0×10^3 spores per milliliter of sterile distilled water were dropped onto 2% water agar in plastic petri dishes. Germination was monitored by observing 100 spores per plate at $\times 200$. Spores were considered germinated if a germ tube equal to or greater than spore width was visible. Thirty germ tubes per plate were measured at $\times 500$ with the aid of an ocular micrometer. Percentage of germination and germ tube lengths were determined for spores incubated at various times and temperatures, in the light (280 lux) or dark, and at six hydrogen ion concentrations adjusted with NaOH or HCl and maintained with citrate-phosphate buffer.

Growth studies. Spores were obtained from 6- to 10-day-old colonies grown on potato-dextrose agar at 20 C and added (0.15 ml; 1.0×10^6 spores per milliliter) to Erlenmeyer flasks each containing 75 ml of Difco malt extract broth. Flasks were not agitated during incubation. Fungal dry weight was determined by filtering flask contents through prewetted, dried, and weighed Whatman GF/A glass fiber filter paper in a Buchner funnel and drying specimens at 95 C. Dry weights were determined for fungi grown in malt extract broth at various temperatures, in the light (280 lux) or dark, and at various hydrogen ion concentrations adjusted with NaOH or HCl and maintained with citrate-phosphate buffer.

Data from growth and germination studies were evaluated with the two-sample *t*-test or by analysis of variance where appropriate. Duncan's multiple range test was used to compare means when analysis of variance revealed significant differences.

Inoculation studies. *J. virginiana*, *J. scopulorum*, and *J. deppeana* Steud. seedlings grown in the greenhouse from seed were used in infection studies. Spores for inoculations were obtained from 6- to 10-day-old colonies grown on Difco malt agar or Difco potato-dextrose agar at 20 C. Spores were diluted to about 1.0×10^6 spores per milliliter of sticker-spreader solution (2.5 g of gelatin and 0.5 g of sodium oleate per liter of sterile distilled water) and sprayed onto foliage with an atomizer. Before inoculation, foliage on branches was pierced 30 to 40 times with a dissecting needle. Inoculated seedlings were placed in ISCO growth chambers (Instrumentation Specialties Co., P.O. Box 5347, Lincoln, NE 68505)

Portion of a Ph.D. thesis by the senior author, University of Nebraska, Lincoln. Published with approval of the director as Paper 5965, Journal Series, Nebraska Agricultural Experiment Station.

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Accepted for publication 12 March 1981.

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at 100% relative humidity (RH) for 8 hr of dark and 16 hr of light (~ 2.2 klux). Wounded seedlings that were inoculated or uninoculated were incubated at 100% RH at various temperatures and times.

The infection of wounded *J. virginiana* foliage by *K. juniperi* was observed with a Cambridge SR-10 Stereoscan electron microscope. Several needles were each wounded once with a Kerr Style D Pulp Canal Reamer. Seedlings were sprayed with inoculum and incubated for 66 hr at 24 C, 100% RH, in 8 hr of dark and 16 hr of light. Needles were excised, fixed in 4% glutaraldehyde in citrate-phosphate buffer, and dehydrated in an acetone series to 100% acetone. They were dried in a Denton DCP-1 critical point dryer, mounted on stubs, and coated with gold-palladium in a Denton DV 515 thermal vacuum evaporator. The surfaces were then observed with the scanning electron microscope.

RESULTS

Field observations. *K. juniperi* acervuli were not found on *J. virginiana* in November, December, or January. Acervuli were present but not yet erumpent in February. The appearance of erumpent acervuli in March and April coincided with symptom development. Trees lost their winter coloration in spring, and the foliage turned green. Foliage of branches infected with *K. juniperi* turned yellow brown rather than green. Acervuli were usually present at the base of discolored foliage in a zone of sunken tissue. They were numerous in April and May and were present through the summer in declining numbers. In September and October, acervuli were rare and dead branch tips were often colonized by *Cytospora* sp. Unidentified insect larvae inhabited the foliage of some trees infected with *K. juniperi*.

The mean length of dieback of 25 diseased branches was 128 ± 50 mm. An average of 10 mm of this length was covered with acervuli. Mean twig diameter at the base of the dieback was 1.6 ± 0.4 mm. Wounds were often found near the base of the dieback.

Symptoms and signs of infection of *J. virginiana* by *K. juniperi* were less extensive in the spring of 1979 than of 1978. The average temperature for December, January, and February in 1977-1978 was -7.9 C; in 1978-1979, it was -8.5 C. The normal temperature for December, January, and February in the study area is -3.4 C (1).

Kabatina juniperi has been found in six counties in Nebraska and in Indiana, Wisconsin, and New Hampshire. Cultural characteristics of isolates varied. The fungus was also found on branches of *J. scopulorum* exhibiting dieback in Lincoln in June 1979. This is the first report of *K. juniperi* on Rocky Mountain juniper.

Germination studies. Most *K. juniperi*

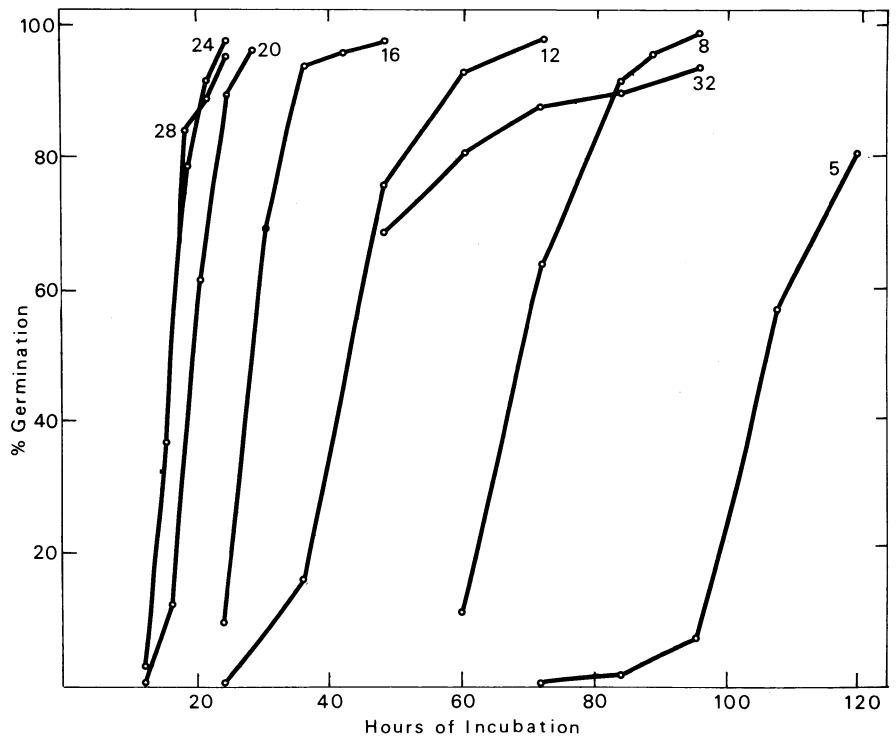


Fig. 1. Germination of *Kabatina juniperi* spores incubated on water agar at 5, 8, 12, 16, 20, 24, 28, and 32 C for various time periods.

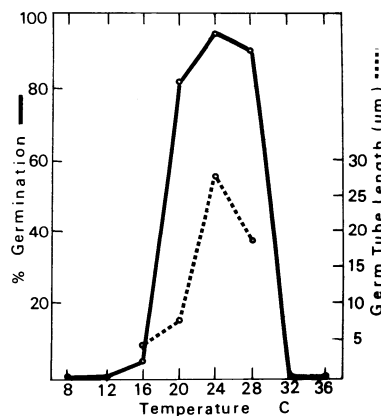


Fig. 2. Spore germination and germ tube lengths of *Kabatina juniperi* incubated on water agar for 24 hr at various temperatures.

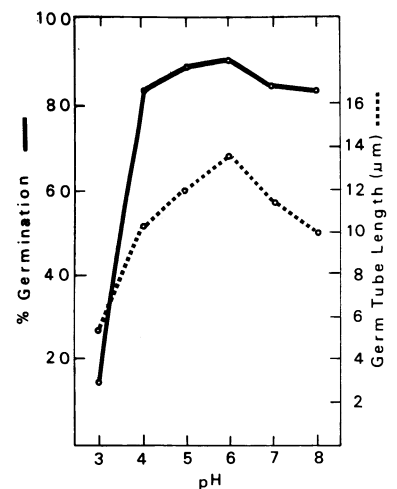


Fig. 3. Spore germination and germ tube lengths of *Kabatina juniperi* incubated for 24 hr on water agar at 24 C at six hydrogen ion concentrations (pH).

spores germinated on 2% water agar (six replicate plates) when incubated at 5, 8, 12, 16, 20, 24, 28, and 32 C (Fig. 1). Germination at temperatures less than 16 C and greater than 28 C often required several days of incubation. Spores incubated at 36 C did not germinate; they were no longer viable and did not germinate when transferred to a temperature favorable for germination.

Spores incubated at 16, 20, 24, and 28 C on water agar (10 replicate plates) germinated within 24 hr, with the largest percentage of germinated spores and the longest germ tubes at 24 C ($P=0.05$) (Fig. 2).

Germination was 98% on 2% water agar (10 replicate plates) incubated for 24 hr at 24 C in the light or in the dark. Germ tube lengths—19.6 µm when produced in the light, 18.7 µm in the dark—were not

significantly different ($P=0.05$).

Spores incubated for 24 hr at 24 C on water agar germinated at pH values of 3, 4, 5, 6, 7, and 8 (five replicate plates) (Fig. 3). Spore germination and germ tube lengths were greatest at pH 6. Germination at pH 5 and pH 6 were not significantly different from one another, but were greater than germination at other hydrogen ion concentrations ($P=0.05$). Germ tubes grown at pH 6 were significantly longer than those grown at other hydrogen ion concentrations.

Growth studies. Dry weights of *K. juniperi* grown in malt extract broth (five replicate flasks) held at room temperature (23 C) for 24 hr and then at 8, 12, 16, 20, 24, 28, and 32 C for 7 days were compared

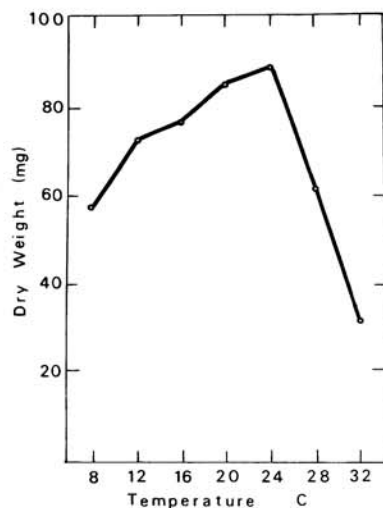


Fig. 4. Growth of *Kabatina juniperi* in malt extract broth held at 23 C for 1 day and at various temperatures for 7 more days.

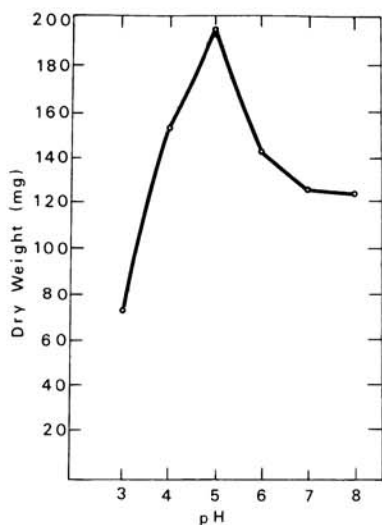


Fig. 5. Growth of *Kabatina juniperi* in malt extract broth buffered at six hydrogen ion concentrations and incubated at 24 C for 2 wk.

(Fig. 4). Growth was maximum at 24 C, although not significantly different from growth at 20 C in this experiment. When growth at 16, 20, 24, and 28 was compared using six replicates, growth at 24 C was significantly greater than growth at other temperatures ($P=0.05$).

Dry weights of *K. juniperi* grown in malt extract broth (eight replicate flasks) at 24 C for 14 days in the light and in the dark were not significantly different ($P=0.05$). The mean dry weight per flask of fungi grown in the light was 131 mg and in the dark was 125 mg.

Dry weights of *K. juniperi* grown in malt extract broth (five replicate flasks) at 24 C for 2 wk at pH 3, 4, 5, 6, 7, and 8 were compared (Fig. 5). Growth was greatest at pH 5, although not significantly different from growth at pH 4 ($P=0.05$). Growth at pH 3 was significantly less than growth at other hydrogen ion concentrations.

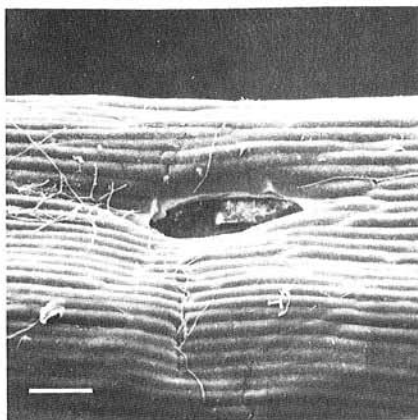


Fig. 6. Scanning electron micrograph of *Kabatina juniperi* growing on wounded needle of *Juniperus virginiana* 66 hr after inoculation. Calibration bar = 50 μ m.

Inoculation studies. Foliage of all wounded and inoculated branches of *J. virginiana* (three branches on each of four trees per temperature) became infected with *K. juniperi* when incubated at 16, 20, 24, and 28 C, and 100% RH for 5 days. Foliage of unwounded, inoculated branches on the same trees did not become infected, nor did foliage of wounded or unwounded branches of uninoculated seedlings incubated similarly.

Foliage of all wounded and inoculated branches of *J. scopulorum* (three branches on each of three trees) became infected with *K. juniperi* when incubated at 24 C and 100% RH for 5 days. Foliage of unwounded, inoculated branches on the same trees did not become infected.

Foliage of all wounded and inoculated branches of *J. depeana* (three branches of each of four trees) became infected with *K. juniperi* when incubated at 24 C and 100% RH for 5 days. Foliage of unwounded, inoculated branches on the same trees did not become infected, nor did foliage of wounded or unwounded branches of uninoculated seedlings incubated similarly.

When wounded, inoculated *J. virginiana* seedlings were incubated at 24 C and 100% RH for 72 hr after inoculation, eight of nine wounded branches on three trees became infected with *K. juniperi*. When wounded, inoculated seedlings were incubated at 24 C and 100% RH for 24 or 48 hr after inoculation, three of nine wounded branches on three trees became infected. Seedlings that were wounded, inoculated, and placed directly in the greenhouse did not become infected.

Observations by scanning electron microscope of *K. juniperi* on needle surfaces revealed that spores germinated on the surfaces of both wounded and unwounded foliage. Fungal growth was abundant around wounds, and sporulation was observed. Hyphae of *K. juniperi* entered the needle through wounds (Fig. 6). No hyphae of *K. juniperi* were seen to enter the needle through stomates.

DISCUSSION

The association of *K. juniperi* with juniper tip dieback in the field and the infection, disease development, and formation of acervuli on inoculated junipers indicate that *K. juniperi* was the causal agent of tip dieback of *Juniperus* spp. in Nebraska. The symptoms of infection of *J. virginiana* by *K. juniperi* in Nebraska were similar to those found on *Juniperus* species in Europe (3). Branch tips became discolored in early spring, and acervuli formed in necrotic regions at the base of discolored tissue. The symptoms occurred on foliage produced during the previous growing season and appeared before new growth was initiated. This, together with the absence of acervuli in the winter, suggests that infection occurred during the previous growing season.

The occurrence of *K. juniperi* in several states extends its known range in the United States and suggests that the fungus is probably more widespread than is now recorded.

In cultural studies, *K. juniperi* germinated and grew over a wide range of temperatures and pH and was not affected by light conditions. Effects of temperature on spore germination and growth agree with results reported for European isolates of the fungus (3,4). A lower pH optimum for growth was found, which may reflect differences in isolates or in techniques used (dry weight vs. colony diameter) (3). The fungus survived cool temperatures, but the influence of unusually cold weather on disease development is not known.

Hoffman and Fliege (3) suggested that *K. juniperi* is a wound parasite. Results of inoculation tests and observations by scanning electron microscope confirmed that *K. juniperi* entered foliage through wounds.

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