A Canker Disease of Rocky Mountain Juniper Caused by *Botryosphaeria stevensii*

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ABSTRACT

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Botryosphaeria stevensii (anamorph *Diplodia mutila*) caused cankering of Rocky Mountain juniper (*Juniperus scopulorum*) in windbreak and ornamental plantings. Multiple, coalescing cankers resulted in branch dieback and sometimes tree mortality. The fungus also was pathogenic to and caused canker formation on eastern redcedar (*J. virginiana*) and Chinese juniper (*J. chinensis*) in greenhouse and field inoculation studies. Apple and juniper isolates were host-specific.

Eastern redcedar (*Juniperus virginiana* L.) and Rocky Mountain juniper (*J. scopulorum* Sarg.) are planted extensively in windbreaks and ornamental landscapes in the Great Plains of the United States. They are important components of most windbreaks in Kansas because they are well adapted to a wide range of site conditions. Most of the older juniper windbreaks in the state are composed of *J. virginiana*. In recent years, however, some landowners have favored the planting of *J. scopulorum* because of its more upright, compact form and dark blue foliage. Unlike *J. virginiana, J. scopulorum* does not reproduce well from seed in Kansas and therefore is less likely to encroach into nearby pastureland.

In 1986, a canker disease was found associated with branch dieback in several *J. scopulorum* plantings. Multiple cankers were noted on twigs, in branch crotches, and on main trunks of affected trees. Cankers were elliptical, flattened, and often resinous (Fig. 1). Branch cankers frequently were difficult to detect unless the outer bark was removed. Some cankers quickly girdled small branches, causing rapid death of foliage beyond the canker margin. Dull red, desiccated foliage was a good indicator of the presence of a girdling canker at the base of individual branches. Small black pycnidia were consistently observed in cankers and on dead branches. Coalescing cankers occasionally girdled main stems, causing death of the top 1–2 m of the crowns; some trees were killed. The following research was done to determine the cause of the canker disease of *J. scopulorum*. A preliminary report on this disease has been published (15).

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MATERIALS AND METHODS

Branch cankers on *J. scopulorum* were collected from 20 different locations in 18 Kansas counties. Small sections of bark and wood were cut from the canker margins, soaked in a 0.5% sodium hypochlorite solution for 3 min, blotted dry on a clean paper towel, and aseptically transferred to petri dishes containing acidified 2% (w/v) Difco potato-dextrose agar (PDA) adjusted to pH 4.5. The same fungus was isolated from cankers at all locations. Single-spore isolations from several samples were taken from pycnidia or pseudothecia embedded in the original bark sample or from pycnidia that formed on PDA. Isolates were maintained on PDA slants at 25 C under cool-white fluorescent lights (12 hr light/12 hr dark).

Two-year-old bare-rooted *J. scopulorum* (South Dakota seed source) and *J. virginiana* (South Dakota and Oklahoma seed sources) trees were planted in 15-cm-diameter pots in a steamed soil:sand:peat mixture (1:1:1, v/v) and placed in the greenhouse for a minimum of 1 mo before inoculation. Then, stems on nine *J. scopulorum* and five *J. virginiana* trees were each cut once with a sterile scalpel to leave wounds approximately 3–5 mm long and 2–3 mm deep and extending into the wood. Diameters of stems at points of wounding ranged from 5 to 10 mm. Wounds were inoculated with PDA containing mycelium of a 10-day-old single-spore culture of the fungus, covered with small pieces of wet, sterile cotton, and wrapped with Parafilm. The cotton and Parafilm were removed after 1 wk. Sterile PDA was inserted into wounds on nine additional *J. scopulorum* and five *J. virginiana* trees in a similar manner. All plants were incubated in a greenhouse for 1–6 mo, depending on canker development. Temperatures in the greenhouse ranged from 20 to 29 C, and trees were irrigated by hand three times a week. The fungus was isolated from canker margins using the methods described above.

Canker development after inoculation in the field was studied in a juniper planting at the Kansas State University Experimental Farm near Manhattan. A branch (5–15 mm diameter) on each of three 6-year-old *J. scopulorum* 'Wichita Blue,' *J. virginiana* 'Manhattan Blue,' and *J. chinensis* L. 'Blue Point' trees was inoculated on 19 August 1987 with the suspect pathogen in a manner similar to that described previously. Sterile PDA was inserted into a branch wound on each tree and served as a control.

Growth characteristics in culture and pathogenicity of the suspect pathogen on *J. scopulorum* and *J. virginiana* were compared with a morphologically similar isolate of *Botryosphaeria stevensii* Shoemaker (ATCC 6180), which was originally isolated as the cause of black rot of apple (*Malus pumila* Mill.) in New Zealand. Because preliminary experiments determined that all juniper isolates had similar morphologies and growth rates on PDA, one ascospore isolate of the suspect pathogen was compared with ATCC 6180. Small pieces of PDA containing mycelium of each isolate were placed in the center of three replicate

Fig. 1. Elliptical branch canker on *Juniperus scopulorum* caused by *Botryosphaeria stevensii*. The bark is removed to show brown staining of the wood.

PDA plates and incubated in darkness at 5-C intervals from 5 to 35 C. Colony diameters were measured daily in two directions on each plate and averaged over a 3- to 7-day period to determine mean growth per day. For pathogenicity tests in the greenhouse, sterile PDA or mycelium of the *J. scopulorum* isolate or the apple isolate was inserted into wounds on three trees of each juniper species in the manner described previously. The pathogenicity of each fungal isolate on apple was determined by fruit inoculation. Recently harvested Golden Delicious apples were washed and surface-sterilized with a 70% ethanol solution. Three equally spaced plugs (6 mm diameter and 3 mm deep) on each of three apples were removed with a sterile cork borer. Small pieces of PDA or PDA containing mycelium of the apple or juniper isolate were inserted into the wounds. The plugs of apple tissue were reinserted into the wounds, and the fruit were incubated at 25 C in plastic bags for 2 wk.

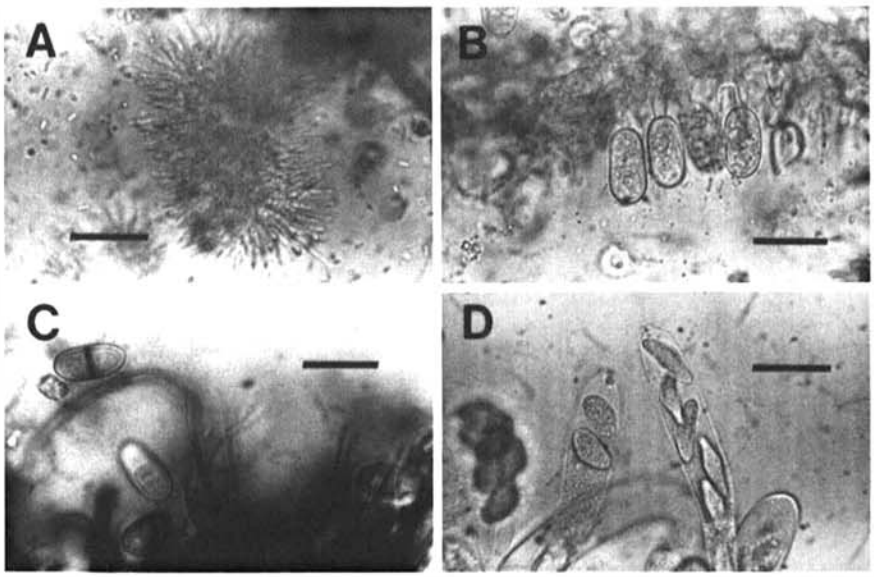

Fig. 2. *Botryosphaeria stevensii:* **(A)** Microconidia. Scale bar = 40 μm. **(B)** Hyaline macroconidia and **(C)** two-celled, dark macroconidia of the anamorph *Diplodia mutila*. Both microconidia and macroconidia were occasionally produced within the same conidioma. Scale bars = 28 μm. **(D)** Bitunicate asci and ascospores. Scale bar = 40 μm.

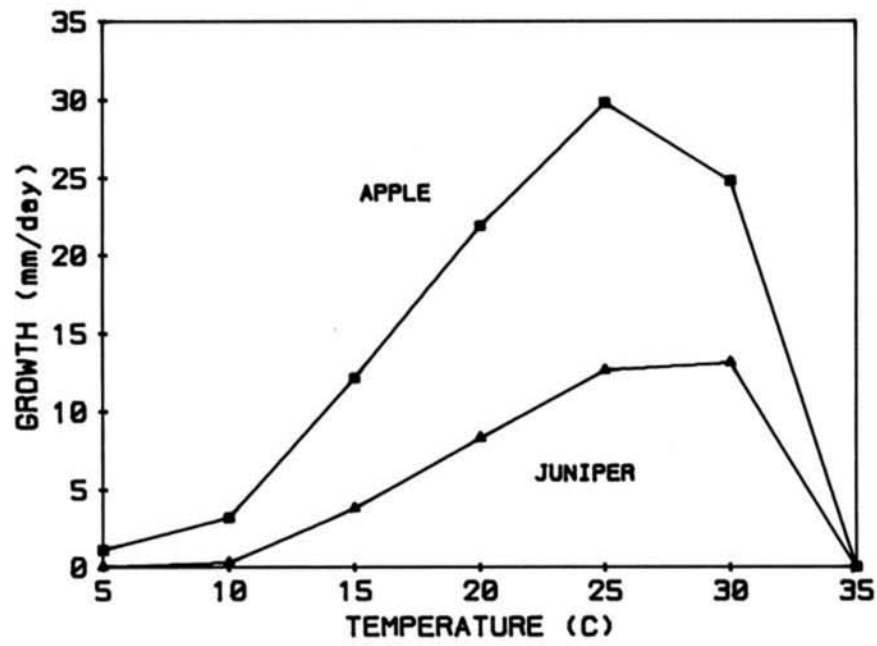

Fig. 3. Mean growth rates at different temperatures of apple and juniper isolates of *Botryosphaeria stevensii* on potato-dextrose agar. Coefficients of variation for each datum point varied from 0 to 0.86.

RESULTS

A fungus identified as *B. stevensii* (= *Physalospora mutila* (Fr.) Stevens) was consistently isolated from and observed fruiting in cankers on *J. scopulorum*. Conidiomata were pycnidial, immersed, unilocular, and 5–6 mm in diameter. Both microconidia and macroconidia were produced in conidiomata, in some cases within the same locule. Microconidia (Fig. 2A) were hyaline, thin walled, and unicellular and measured 3.0–6.0 (5.0) × 0.75–1.5 (1.0) μm. Microconidia were common in pycnidia in young cultures grown on PDA and were occasionally found in pycnidia on naturally infected branches. The production of microconidia in association with the anamorph has been reported previously (16). Attempts to germinate the microconidia on various media were unsuccessful. Macroconidia were hyaline at first (Fig. 2B) but eventually turned brown and developed single (Fig. 2C) or, rarely, two median septations. Mature conidia were thick walled and measured 23–32 (28) × 12–15 (14) μm. Although rare, the teleomorph was observed in dead branches at several locations. Pseudothecia were immersed with asci 120 × 16 μm. Ascospores (Fig. 2D) were hyaline, smooth, thick walled, elliptical to ovate, and 32–40 (37) × 12–16 (14) μm. Ascospores have been reported to eventually turn pale brown and develop septations (10,11), although this was not observed in our studies. Sivanesan (11) and Sutton (14) give a more complete description of the fungus. Single-ascospore and macroconidia isolates were morphologically similar in culture. A single-ascospore isolate has been deposited with the ATCC (number 64483).

Colony morphology of the juniper and apple isolates of *B. stevensii* on PDA were distinctly different. Most juniper isolates first developed a white, feathery margin and eventually turned light to dark green. Several of the isolates produced a water-soluble red pigment. Pycnidia were produced sparingly in culture after 20 days of incubation. Addition of sterile *J. scopulorum* twigs to the agar aided in the development of pycnidia. Microconidia were commonly found in pycnidia in all juniper isolates. The formation of red pigment and the production of microconidia were not observed in the apple isolate. Both isolates had similar optimal temperatures for growth on PDA (Fig. 3), but the apple isolate grew at a faster rate at all temperatures.

All *J. scopulorum* and *J. virginiana* trees inoculated with the juniper isolate of *B. stevensii* in greenhouse experiments developed sunken lesions within 1 mo. The flattening of the stem and accumulation of resin at the canker were identical to symptoms observed in the field. After 3 mo, lesions had girdled stems on all nine *J. scopulorum* and three

of the five *J. virginiana* trees, resulting in death of foliage beyond the canker. After 5 mo, cankers had girdled stems on the two remaining *J. virginiana* trees. Pycnidia were abundant in all cankers after 1 mo. *B. stevensii* was reisolated from all cankers. None of the control trees developed lesions or were killed after 6 mo.

In the experiment to compare pathogenicity of the juniper isolate of *B. stevensii* with that of an apple isolate (ATCC 6180), girdling cankers were formed on all *J. scopulorum* and *J. virginiana* trees within 3 wk of inoculation with the juniper isolate and all portions of the trees beyond the canker were dead after 1 mo. Pycnidia were abundant on all dead stems. *J. scopulorum* trees inoculated with the apple isolate developed small lesions around the wounds, but callus tissue formed around the necrotic area within 2 wk and no further canker development was observed. No lesions developed on *J. virginiana* trees inoculated with the apple isolate or on any of the control trees.

Golden Delicious apples inoculated with the apple isolate of *B. stevensii* developed large lesions and extensive rotting of tissue within 2 wk. Lesions did not develop on apples inoculated with the juniper isolate or in wounds in which sterile PDA was inserted.

All three *J. scopulorum* 'Wichita Blue,' *J. virginiana* 'Manhattan Blue,' and *J. chinensis* 'Blue Point' trees developed girdling branch cankers within 2 mo after inoculation in the field. Branch dieback was identical to symptoms that occurred on naturally infected trees. *B. stevensii* was recovered from all canker margins. No cankers developed on the control branches.

DISCUSSION

B. stevensii is saprophytic on a number of woody plants (11,14) and is known to cause black rot of apple (7,13,16), a rot of kiwifruit (*Actinidia chinensis* Planch.) (6), and twig dieback of *Pyrus, Quercus, Vitis*, and *Populus* (16). Luttrell (8) reported a twig blight of Arizona cypress (*Cupressus arizonica* Greene) caused by an unidentified *Botryosphaeria* that may be *B. stevensii*. Other species of *Botryosphaeria* resembling *B. stevensii* cause canker diseases of conifers in western North American and have been described by Funk (1–4). These species differ significantly from *B. stevensii* in having larger ascospores and/or lacking an anamorph.

Neither *B. stevensii* nor the anamorph *Diplodia mutila* (Fries) Mont. have previously been reported to cause a canker disease of juniper in the United States. However, two specimens of this species were found in the U.S. National Fungus Collections, one from Indianapolis, IN, collected on field-grown *Juniperus* sp. imported from Holland and identified as *D. juniperi* West f. *foliicola*, and one from Germany on branches of *J. sabina* L. identified as *D. juniperi*. Hall (5) also found a dieback of *J. sabina* 'Blue Danube' in Ontario, Canada, caused by an unidentified *Botryosphaeria* that was morphologically similar to *B. stevensii*.

Botryosphaeria canker has been confirmed in *J. scopulorum* windbreaks throughout central and eastern Kansas and has been found in western Missouri. Disease incidence in 14 windbreak plantings of *J. scopulorum* in Kansas ranged from 2 to 22%. The disease is now considered to be a serious problem and could limit further plantings of *J. scopulorum* in windbreaks or shelterbelts. Botryosphaeria canker also is causing significant branch dieback and mortality on *J. scopulorum* 'Welchii,' 'Wichita Blue,' and 'Wren' in urban landscape plantings.

The relative susceptibility of other juniper species to Botryosphaeria canker is unclear. Our studies indicate that canker development and branch dieback will occur on *J. virginiana* and *J. chinensis* after artificial inoculations. The fungus also has been isolated from dead branches and canker margins on two naturally infected *J. virginiana* trees. This disease, however, does not appear to be widespread in *J. virginiana* windbreaks in Kansas.

We found evidence of host specialization between the apple and juniper isolates of *B. stevensii*. The juniper isolate was pathogenic on vigorously growing junipers in the greenhouse, whereas the apple isolate was not. Conversely, the juniper isolate failed to cause extensive rotting of apple. This specialization is similar to that found for *B. corticis* (Demaree & Wilcox) Arx & Müller (9) but contrasts with what is known about *B. dothidea* Ces. & de Not. (12,17).

ACKNOWLEDGMENT

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