

A Canker Disease of the Cupressaceae in Kansas and Texas Caused by *Seiridium unicorne*

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ABSTRACT

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Seiridium unicorne caused cankers on Oriental arborvitae (*Thuja orientalis*) and eastern red cedar (*Juniperus virginiana*) in Kansas and Texas and on Italian cypress (*Cupressus sempervirens*), Arizona cypress (*C. arizonica*), and Leyland cypress (*Cupressocyparis leylandii*) in Texas. The fungus also was associated with small, annual cankers on bald-cypress (*Taxodium distichum*) in Kansas landscape plantings. Cankers on Rocky Mountain (*J. scopulorum*) and Chinese (*J. chinensis*) junipers and on northern white-cedar (*T. occidentalis*) developed 1 mo after inoculation in greenhouse or field studies. No evidence of host specificity in fungal isolates was found.

Tree species in the cypress family (Cupressaceae) are planted extensively in the Great Plains of the United States because of their tolerance to a wide range of site conditions, low annual rainfall, and, in some cases, rapid and extreme temperature fluctuations. Eastern red cedar (*Juniperus virginiana* L.) and Rocky Mountain juniper (*J. scopulorum* Sarg.), which are native to certain areas of the Great Plains, and the introduced Oriental arborvitae (*Thuja orientalis* L.) are important components of windbreak and/or ornamental plantings throughout Kansas and central and western Texas. Other nonnative species, including Arizona cypress (*Cupressus arizonica* Greene), Italian cypress (*C. sempervirens* L.), and Leyland cypress (*Cupressocyparis leylandii* (Dallimore & Jackson)

Dallimore), are also used for ornamental purposes in the Texas panhandle.

A decline and dieback of these tree species have been noted in Kansas and Texas for many years. In 1988, a previously undescribed canker disease of Rocky Mountain juniper and eastern red cedar, caused by *Botryosphaeria stevensii* Shoemaker, was found in Kansas (12). Subsequent surveys in the state indicated that this pathogen was causing widespread damage to Rocky Mountain juniper plantings. During the surveys, a second canker disease, with symptoms similar to those of *Botryosphaeria* canker, was discovered on Oriental arborvitae, eastern red cedar, and bald-cypress (*Taxodium distichum* (L.) Rich., family Taxodiaceae). Similarly, in Texas, cankers were noted on Oriental arborvitae, eastern red cedar, and Arizona, Italian, and Leyland cypresses. In both states, elongate, resinous cankers, sometimes longer than 90 cm, were found on twigs, branches, and, occasionally, main stems of trees. Wood beneath the flattened area of the canker was stained dark brown (Fig. 1). Girdling cankers caused a rapid death of foliage beyond the canker margin on several of the species. The following research was

conducted to determine the cause of this disease. A preliminary report has been published (11).

MATERIALS AND METHODS

Isolation procedures. Branch cankers on two eastern red cedar, eight Oriental arborvitae, and two bald-cypress trees were collected from various locations in Kansas. Small sections of bark and wood were cut from the canker margins, soaked in a 0.5% sodium hypochlorite solution for 1-3 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. Single spore isolations from several samples were taken from conidiomata embedded in original bark samples or from conidiomata that formed in culture on PDA. Isolates were maintained at 25 C in the dark or under cool-white fluorescent lights (12 hr light/12 hr dark). In Texas, isolations were made from canker margins or, in some cases, fungal identity was determined by microscopic examination of morphological features of conidia and conidiomata removed from cankers on diseased trees.

Inoculation techniques. Two- to three-year-old bare-rooted Rocky Mountain juniper (South Dakota seed source), eastern red cedar (South Dakota and Oklahoma seed sources), Oriental arborvitae (Oklahoma seed source), and northern white-cedar seedlings (*Thuja occidentalis* L.) (Wisconsin seed source) were planted in 15-cm-diameter pots in a steamed soil:sand:peat mixture (1:1:1, v/v). Oriental arborvitae and northern white-cedar seedlings were grown for 2 mo, and Rocky Mountain juniper and eastern red cedar seedlings for 1 yr, in

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the greenhouse before inoculation. Then, a 6-mm-diameter piece of bark, extending to the wood, was removed with a sterile cork borer from 12 seedlings of each species. Diameters of stems at points of wounding ranged from 7 to 15 mm. Wounds on four seedlings of each species were inoculated with a 6-mm plug of PDA containing mycelium of one of three fungal isolates, originally isolated from cankers on Oriental arborvitae (*Thuja*), eastern red cedar (*Juniperus*), and bald-cypress (*Taxodium*) in Kansas. After inoculation, bark plugs were reinserted and wounds were covered with small pieces of wet, sterile cotton and wrapped with Parafilm. The cotton and Parafilm were removed after 1 mo. Sterile PDA was inserted into wounds on four additional trees of each species in a similar manner. All plants were incubated in the greenhouse for 6 mo in a completely randomized, 4 × 4 factorial design with tree species and fungal isolates (including sterile PDA) as main treatments. Temperatures in the greenhouse ranged from 18 to 33 C, and trees were irrigated three times each week.

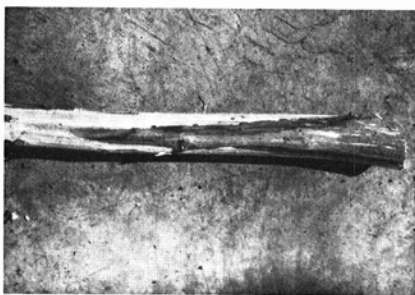


Fig. 1. Fusiform branch canker on *Thuja orientalis* caused by *Seiridium unicorne*. The bark is removed to show brown staining of wood.

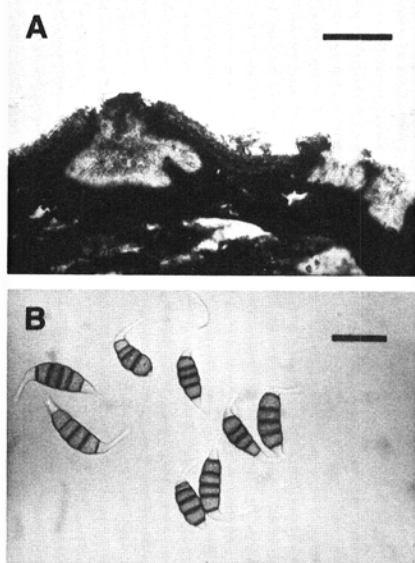


Fig. 2. *Seiridium unicorne*: (A) Acervuli embedded in bark of *Thuja orientalis*. Scale bar = 200 μ m. (B) Six-celled conidia with apical and basal appendages. Scale bar = 20 μ m.

Canker lengths along the vertical axis of the main stem were measured monthly. A fungus was isolated from canker margins on one tree from each treatment after 6 mo using the methods described above.

Canker development. Canker development on more mature, established trees was studied in juniper and Oriental arborvitae plantings at two Kansas State University experiment farms near Manhattan. A branch (5–15 mm diameter) on each of three 8-yr-old Rocky Mountain juniper cv. Welchii, eastern red cedar cvs. Oxford and Manhattan Blue, and Chinese juniper (*J. chinensis* L.) cv. Blue Point trees and 10 7-yr-old Oriental arborvitae trees was inoculated in April 1989 with the suspect pathogen (*Thuja* isolate) as described previously. Sterile PDA was inserted into a separate branch wound on each tree and served as a control. Canker lengths, as evidenced by discoloration of bark, were measured monthly.

Comparisons of isolates. Growth of the *Thuja*, *Juniperus*, and *Taxodium* isolates was compared by placing pieces of PDA containing mycelium of each isolate in the center of three replicate PDA plates and incubating the plates 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 10-day period to determine mean growth per day. The experiment was repeated once.

RESULTS

Seiridium unicorne (Cooke & Ellis) Sutton (= *Monochaetia unicornis* (Cooke & Ellis) Sacc.), identified by A. Y. Rossman and T. R. Nag Raj, was consistently isolated from and observed fruiting in cankers on eastern red cedar, Oriental arborvitae, and bald-cypress in

Kansas and on Oriental arborvitae, eastern red cedar, and Arizona, Italian, and Leyland cypresses in Texas. Acervular conidiomata were abundant in cankers (Fig. 2A). Conidia were six-celled and 22–30 μ m long × 8–10 μ m wide; the four inner cells were dark brown, and the hyaline apical and basal cells had thin, unbranched appendages 5–10 and 1–4 μ m long, respectively (Fig. 2B). Guba (5) and Sutton (9) give more complete descriptions of the fungus. The teleomorph, *Lepteutypa cupressi* (Natrass, Booth, & Sutton) Swart (10), was not found.

Cultures of *S. unicorne* on PDA initially were off-white with irregular, appressed margins, but the mycelium later turned pale green, beige, or light orange. Acervuli were common in recently isolated cultures grown on PDA under lights but not in cultures grown in the dark. The ability of isolates to form acervuli decreased with serial transfers or storage on PDA for longer than 3–4 mo. Growth rates for all isolates were optimal at 25 and 30 C. No growth was observed at 5 and 35 C (Fig. 3). The *Juniperus* isolate had a slightly faster growth rate at 25 C but otherwise was indistinguishable from the *Taxodium* and *Thuja* isolates.

All species of trees, except one northern white-cedar and one eastern red cedar, developed sunken, resinous cankers 1 mo after inoculation with *S. unicorne* in the greenhouse (Table 1). Significant differences in canker lengths among isolates or fungal isolate × tree species interactions were not observed after 6 mo. However, there were differences among species when canker lengths were averaged for all isolates. Cankers on northern white-cedar, which were shorter ($P < 0.05$) than those on Rocky Mountain juniper, expanded for 3 mo

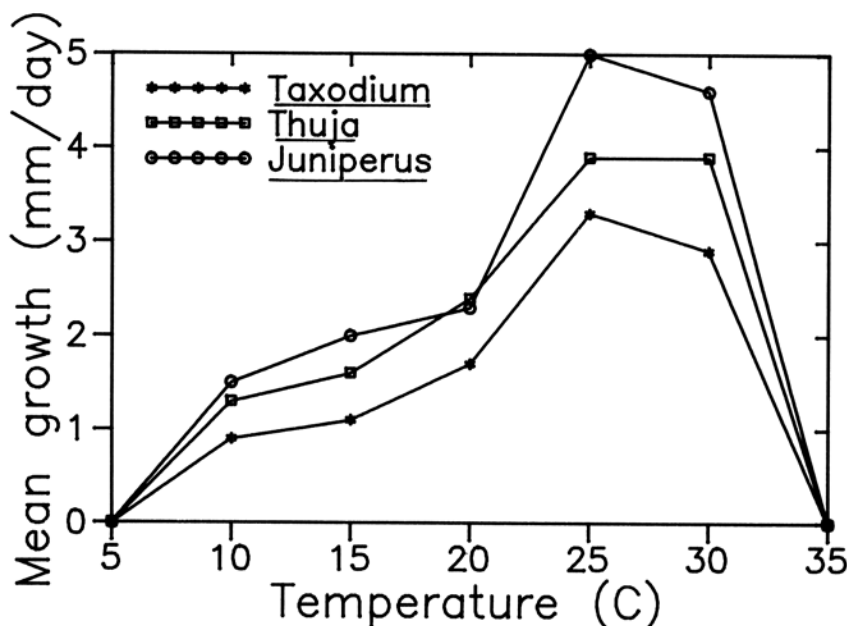


Fig. 3. Mean growth rates of isolates of *Seiridium unicorne* on potato-dextrose agar at different temperatures. Mean growth rates are averages of three replicate plates at each temperature.

Table 1. Mean canker lengths on four tree species 6 mo after inoculation with one of three isolates of *Seiridium unicorne* in greenhouse experiments

Treatment	Canker length ^x (mm)
Isolate ^y	
Sterile PDA	6.3 a
<i>Taxodium</i>	24.6 b
<i>Thuja</i>	26.1 b
<i>Juniperus</i>	37.3 b
Tree species ^z	
<i>Thuja occidentalis</i>	11.7 a
<i>T. orientalis</i>	22.3 ab
<i>Juniperus virginiana</i>	23.0 ab
<i>J. scopulorum</i>	37.2 b

^x Bark wounds on plants were inoculated with a 6-mm plug of PDA containing mycelium of each isolate, and canker lengths were measured along the vertical axis of the inoculated stem. Means for each treatment main effect followed by the same letter are not significantly different ($P > 0.05$) by FLSD test. There was no isolate \times tree species interaction ($P > 0.05$).

^y Fungal isolates *Thuja*, *Taxodium*, and *Juniperus* were originally isolated from *T. orientalis*, *T. distichum*, and *J. virginiana*, respectively, in Kansas. Isolate values represent mean canker lengths for all tree species tested.

^z Mean canker lengths for all isolates on each tree species.

but were surrounded by callus after 5 mo and appeared inactive. *S. unicorne* was reisolated from canker margins on all treatment combinations except control plants.

All 10 Oriental arborvitae trees developed resinous cankers, 3.5–11.5 cm long, 6 mo after inoculation in the field. None of the cankers had girdled the stem, but three inoculated branches had light green to yellow, desiccated foliage beyond the canker margin and were in a state of decline. Similarly, mean canker lengths on branches of Rocky Mountain juniper cv. Welchii, eastern red cedar cvs. Manhattan Blue and Oxford, and Chinese juniper cv. Blue Point were 2.5, 2.3, 2.9, and 3.1 cm, respectively, after 6 mo; *S. unicorne* was reisolated from canker margins. No cankers developed on control branches of any tree species.

DISCUSSION

S. unicorne is a pathogen of many tree species of the Cupressaceae in Africa (7), New Zealand (1,2), and Japan (8). It also causes cankers on Arizona cypress in the southeastern United States (3,4). The closely related fungus *S. cardinale* (Wag-

ner) Sutton & Gibson is a well-known pathogen of the Cupressaceae in many areas of the world (1,2,9,13).

Jones (7) previously reported canker development on Oriental arborvitae after inoculation with *S. unicorne* in greenhouse tests but did not observe natural infection of trees in Kenya. However, cankers caused by *S. unicorne* are common and widespread on Oriental arborvitae in Kansas and also occur on this species in Texas. In a tree improvement planting near Manhattan, Kansas, 15.7% (20/127) of the Oriental arborvitae trees had one or more branch or main stem cankers.

Cankers caused by *S. unicorne* also have been observed on eastern red cedar and bald-cypress in Kansas and on Leyland cypress in Texas. *S. unicorne* has previously been shown to cause cankers on Leyland cypress in New Zealand (1) and on eastern red cedar after inoculation in greenhouse experiments (4). Our report, however, is the first of natural infection of these species in the United States. The pathogenicity of *S. unicorne* on bald-cypress remains uncertain because Koch's postulates have not been completed. Rocky Mountain and Chinese juniper and, to some extent, northern white-cedar also appear to be susceptible to *S. unicorne*, although we have not observed natural infection of these species. *S. unicorne* is widely distributed in Kansas and Texas and is a potential pathogen to many species of the Cupressaceae grown in the Great Plains.

The severity of *Seiridium* canker on Oriental arborvitae and cypress species in Kansas and Texas seems to increase during spring and summer months after severe winters characterized by prolonged subfreezing temperatures. Cambial or bark injury caused by low temperatures may predispose trees to infection or colonization by the pathogen.

We found no evidence of host specificity among isolates of *S. unicorne* collected from different tree species. Jones (6) described three morphologically distinct strains of *S. unicorne* in Kenya. Two strains, each composed of many isolates, were pathogenic to *Cupressus* spp. and *J. procera* L. but differed slightly in virulence on each host. A third strain was pathogenic only to *J. procera*. Our failure to detect host specificity or differences in virulence may have resulted from the low number of isolates tested and/or the short duration of the experiments. Differences in canker

lengths also may not accurately reflect relative susceptibility of genera or species in the Cupressaceae. Further information on host specificity, differences in virulence among *S. unicorne* isolates, and distribution of the disease in North America is needed.

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