

# PATHOGENICITY OF CYTOSPORA FUNGI ON SIX HARDWOOD SPECIES

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**Abstract.** *Cytospora* canker is a serious fungal disease of many shade, fruit, and ornamental tree species in the urban forest, orchards, and nurseries. Because *Cytospora* species are difficult to identify and their host ranges are poorly understood, it is not known if disease occurrence on one host poses a threat to other host species. *Cytospora* isolates were collected from aspen (*Populus tremuloides*) (*Cytospora chrysosperma*), green ash (*Fraxinus pennsylvanica*) (*Cytospora pruinosa*), Siberian elm (*Ulmus pumila*) (*Cytospora sacculus*), alder (*Alnus* spp.) (*Cytospora umbrina*), cottonwood (*Populus* spp.) (*Cytospora chrysosperma*), and multi- and single-stemmed willow (*Salix* spp.) (*Cytospora fugax*). These isolates were inoculated into drought-stressed aspen, green ash, Siberian elm, thinleaf alder (*A. tenuifolia*), eastern cottonwood (*P. deltoides*), and single-stemmed willow. Ash, alder, and elm isolates were host specific. Aspen and cottonwood isolates were pathogenic only on aspen and cottonwood trees. Isolates from multi-stemmed willows caused cankers on aspen only and not single-stemmed willows. However, *Cytospora* spp. isolates collected from a single-stemmed willow were pathogenic on trees cloned from that willow. Water potential, as a covariate, did not explain variation in canker size among trees. Thus, *Cytospora* fungi that are host specific may not be a threat to other nearby tree species.

**Key Words.** *Cytospora* canker; pathogenicity; host specificity; drought stress; water potential; disease incidence; disease distribution.

*Cytospora* canker, caused by fungi in the genus *Cytospora* Ehrenb.:Fr., is a worldwide problem and affects more than 70 species of woody shrubs and trees (Agrios 1997). *Cytospora* refers to the anamorphic (asexual) stage of the causal fungi commonly found forming cankers. Numerous species of *Cytospora* are listed as causing *Cytospora* canker. However, species identification is difficult, even for the professional plant pathologist because *Cytospora* fruiting and vegetative structures, as well as spore size, vary greatly. A poor understanding of the host range of each species also contributes to the difficulty of identification (Waterman 1955; Spielman 1985). Thus, it is not known if *Cytospora* isolates found on diverse woody hosts in a local-

ized region are the same, are different strains of a single species, or are different species. Consequently, in the urban forest or nursery, it is not known if disease occurrence on one woody plant species poses a threat to other tree species.

The objective of this study was to determine if *Cytospora* isolates collected from six common urban and riparian tree species in northern Colorado, United States, would show single- or multiple-host specificity. A fungal isolate was considered to be either pathogenic or nonpathogenic on a particular host based on whether or not it caused tissue disruption as evidenced by a canker larger than the noninoculated control.

## MATERIALS AND METHODS

Four experiments were conducted during 1997 and 1998 to test the pathogenicity of *Cytospora* isolates collected from six hardwood tree species. In three experiments, inoculations were conducted on drought-stressed trees. One final experiment 1) addressed the pathogenicity of fungal isolates from multi- versus single-stemmed willows, and 2) attempted to determine if the infection of cottonwoods by elm isolates that occurred in one out of three experiments could be repeated.

### Plant Material

All experimental trees were grown in 19-L (5-gal) black plastic pots containing a potting mix composed of 25% sand, 20% peat, 5% composted manure and sawdust, and 50% clay loam. Green ash (*Fraxinus pennsylvanica* cv 'Patmore' Marsh.), eastern cottonwood (*P. deltoides* cv 'Siouxland', Bartr.), and multi-stemmed willow (*Salix* spp.) were clonal plant material, while thinleaf alder (*Alnus tenuifolia* Nutt.), aspen (*Populus tremuloides* Michx), and Siberian elm (*Ulmus pumila* L.) were seedlings (Table 1). Willows were obtained as cuttings taken from a mature single-stemmed willow in Fort Collins, Colorado. Cuttings were rooted in Fafard No. 4-P Growing Mix (Conrad Fafard, Inc., Agawam, MA) in the greenhouse. The

five other tree species were purchased from various nurseries. All trees were maintained outside under partial shade until fungal pathogenicity studies began. At study initiation, all trees were two to four years old and had an average height of 1.7 m (5.6 ft) and an average diameter of 1.6 cm (0.6 in.) measured 15 cm (6 in.) above the soil line.

#### Fungal Isolates

A total of 18 *Cytospora* isolates were used in the four experiments (Table 1). For the first three experiments, 12 isolates of *Cytospora* were tested. Two isolates from each of six host tree species were obtained during 1995 and 1996 from infected bark tissue. Isolates from aspen, green ash, and Siberian elm were identified as *Cytospora chrysosperma* (Pers.:Fr.) Fr., *Cytospora pruinosa* Fr.:Fr., and *Cytospora sacculus* (Schw.) Gvrit., respectively. These isolates from urban trees species were collected from landscapes in Fort Collins, Colorado. The isolates from alder, cottonwood, and multi-stemmed willow were identified as *Cytospora umbrina* (Bonord.) Sacc, *Cytospora chrysosperma* (Pers.:Fr.) Fr., and *Cytospora fugax* (Bull.:Fr.) Fr., respectively. These isolates from riparian tree species were collected in the Poudre Canyon northwest of Fort Collins. In the final experiment, six willow isolates of *C. fugax* were obtained from a single-stemmed willow in Fort Collins. This tree was the parent plant from which all the willows were cloned. *Cytospora* isolates from multi-stemmed willow did not produce significantly larger cankers than controls on the single-stemmed willow trees in the first three experiments. For this reason, new *Cytospora* isolates were collected to determine if these isolates were pathogenic on the cloned single-stemmed willow material.

Potato dextrose agar (PDA) and a defined glucose medium (Kastirr 1985) served as media for the initial isolations. All isolates were transferred to petri dishes containing PDA and grown in the laboratory under

light at 23°C ± 2°C (73.4°F) for one week prior to inoculation. Isolates were maintained on PDA slants at 5°C (41°F). Asexual and sexual fruiting bodies on the original plant material from which the isolates were obtained were used to identify *Cytospora* species using Spielman's (1984) monograph on *Valsa* and *Cytospora* species. The identification of the willow species was from asexual and sexual fruiting bodies on multi-stemmed willow, but only asexual fruiting bodies were found on the single-stemmed willow.

#### Induction of Drought Stress

All trees in the first three experiments and half of the trees in the final experiment were drought stressed for two weeks prior to and for four weeks following inoculation. Drought stress was achieved by withholding moisture from trees until leaves reached predawn water potentials of -1.5 to -2.5 MPa. Predawn water potential (the measure of water stress in the tree) was measured daily on one leaf from each tree by means of a pressure chamber (Spomer 1985). Supplemental water (approximately 250 to 500 mL [8.5 to 17 oz]) was added as needed to maintain trees at approximately the same level of drought stress. Half the trees in the final experiment were well-watered prior to and for four weeks following inoculation. Predawn water potential was measured weekly on one leaf from each tree by means of a pressure chamber.

#### Inoculations

Stems were surface disinfested with 95% ethanol and allowed to dry before wounding. Trees were wounded by removing a bark disk to the xylem with an 8-mm-diameter (0.3-in.) flame-sterilized leather punch. Each tree received four wounds. Each wound was located 10 to 15 cm (4 to 6 in.) above and 90° clockwise around the stem from the one below. A 6-mm-diameter (0.2 in.) plug of mycelium from an actively growing colony of a *Cytospora* isolate was placed into each of the

Table 1. Trees and fungi used in *Cytospora* pathogenicity studies.

Tree	Tree species	<i>Cytospora</i> species	Isolate numbers	Fungal source location
Aspen	<i>Populus tremuloides</i> Michx.	<i>C. chrysosperma</i>	52,67	Fort Collins, CO
Cottonwood	<i>Populus deltoides</i> Bartr.	<i>C. chrysosperma</i>	54,59	Poudre Canyon, CO
Green ash	<i>Fraxinus pennsylvanica</i> Marsh.	<i>C. pruinosa</i> (Fr.:Fr.)	11,15	Fort Collins, CO
Siberian elm	<i>Ulmus pumila</i> L.	<i>C. sacculus</i> (Schw.) Gvrit.	28,31	Fort Collins, CO
Alder	<i>Alnus</i> spp.	<i>C. umbrina</i> (Bonord.) Sacc.	45,65	Poudre Canyon, CO
Multi-stemmed willow	<i>Salix</i> spp.	<i>C. fugax</i> (Bull.:Fr.)Fr.	35,36	Poudre Canyon, CO
Single-stemmed willow	<i>Salix</i> spp.	<i>C. fugax</i> (Bull.:Fr.)Fr.	89,90,91,92,93,94	Fort Collins, CO

three wounds with the mycelium facing the wound. The fourth wound received a 6-mm-diameter plug of sterile PDA as a control. All wounds were wrapped with wax film for two weeks following inoculation, after which the film was removed.

Canker size measurements were taken four weeks after inoculation. Canker size was recorded as the sum of horizontal and vertical dimensions of discolored bark measured through the center of each wound. Isolations were attempted from 20% of the inoculation sites to confirm presence of the fungus. Surface-disinfested chips of bark and xylem tissue were placed in petri dishes containing PDA amended with streptomycin sulfate at 100 mg/L.

### Experimental Design

All experiments were conducted in a glass house with shade cloth in which temperatures were maintained at 22°C to 30°C (71.6°F to 86°F). The first three experiments used a randomized block split-plot design and consisted of two replications per experiment. Each block (replication) consisted of six plots, and each plot contained four trees, for a total of 24 trees per replication. Each plot within a replication contained one tree species. Each of the four trees within a plot received four wounds. Three wounds were inoculated with a different *Cytospora* isolate, while the fourth received sterile agar and served as a control. The isolates and controls were randomly chosen and placed into the wounds on the four trees in each plot. Using this protocol, all six tree hosts were inoculated with 12 fungal isolates. To adjust for unequal variances and skewed data, canker measurements were  $\log^{10}$  transformed. To determine whether there were differences among isolates or host tree species, data were analyzed by analysis of covariance using SAS software (PROC MIXED, SAS Institute 1996). Water potential varied among trees within a species and was included as a covariate. When host tree species by isolate interactions were significant, paired comparisons using Student's *t*-tests were performed to detect differences among isolates for each host tree species. Because canker size did not significantly differ between isolates obtained from the same host tree species, the two isolates were pooled in the statistical analysis. One isolate each from green ash, alder, and cottonwood was not used after the first experiment due to inconsistent canker development. These isolates were replaced with other isolates for the remaining experiments.

In the final experiment, twelve single-stemmed willows and six cottonwood trees were used in a split-plot design with three replications. A plot for willow consisted of two willow trees inoculated with six single-stemmed willow isolates as either drought-stressed or well-watered treatments (Table 1). For cottonwood, a plot consisted of a single cottonwood tree inoculated with the two elm fungal isolates, as either drought-stressed or well-watered treatments. This resulted in six plots each (two treatments by three replications) for both willow and cottonwood trees. Each of the two willow trees within a plot received four wounds. Three of the wounds were inoculated with different *Cytospora* isolates, while the fourth received sterile agar and served as a control. Each cottonwood tree in a plot received five wounds. Four wounds were inoculated, two wounds each with one of the two different *Cytospora* isolates, while the fifth received sterile agar and served as a control. The isolates and controls were randomly chosen and placed into the wounds on tree(s) in each plot. Data analysis was handled as previously described.

### RESULTS

Canker size in the first three experiments was dependent upon the host tree species–fungal isolate combination. Aspen and cottonwood isolates each produced significantly ( $P < 0.05$ ) larger cankers than controls on aspen and cottonwood trees in all three experiments (Figures 1 and 2). Only on their respective hosts did the ash and alder isolates produce significantly ( $P < 0.05$ ) larger cankers than controls (Figures 1 and 2). This occurred in all three experiments with ash isolates. However, significantly larger cankers than controls occurred only in the first experiment for alder isolates. Nevertheless, mean canker size for alder isolates was always greater than control canker size. Elm isolates produced significantly ( $P < 0.05$ ) larger cankers than controls on elm trees in the first and third experiments as well as on cottonwood trees (Figure 1). Although isolates from multi-stemmed willows failed to produce significantly larger cankers than the controls on single-stemmed willow trees in any of the three experiments, they did, produce significantly ( $P < 0.05$ ) larger cankers on aspen trees (Figure 2). Water potential, as a covariate, did not explain variation in canker size among trees in any experiment.

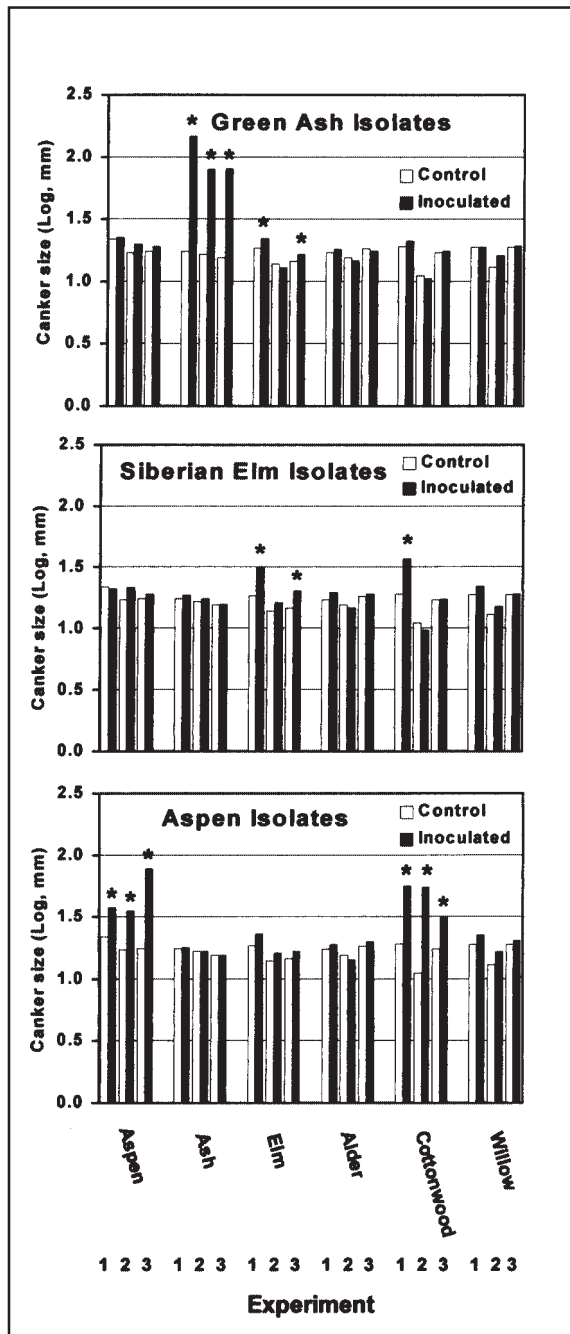


Figure 1. Canker size on three urban hardwood species four weeks post-inoculated with two *Cytospora* isolates from six tree species. Measurements were log<sup>10</sup> transformed, n = 4; isolate data were pooled. Asterisks indicate means, which are significantly different than that of controls (P - 0.05).

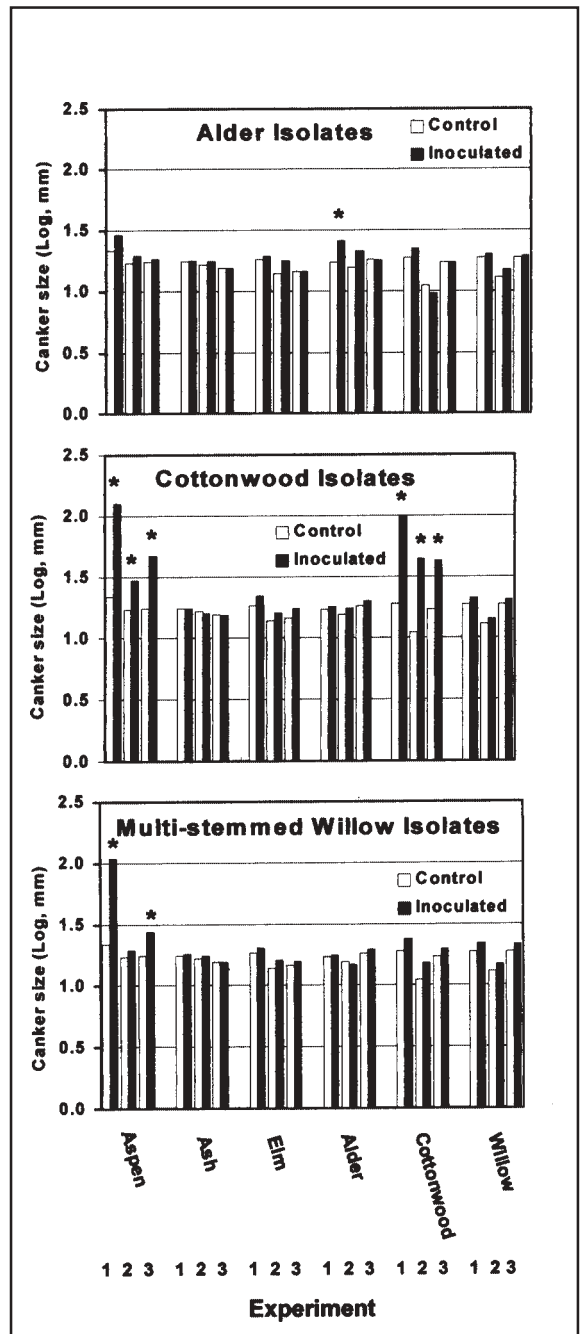


Figure 2. Canker size on three riparian hardwood species four weeks post-inoculated with two *Cytospora* isolates from six tree species. Measurements were log<sup>10</sup> transformed, n = 4; isolate data were pooled. Asterisks indicate means, which are significantly different than that of controls (P - 0.05).

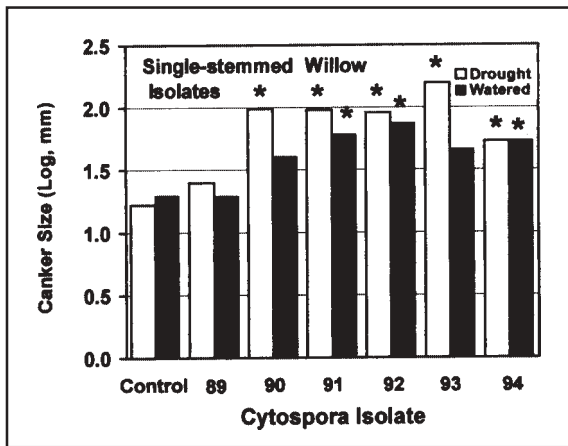


Figure 3. Canker size on single-stemmed *Salix* spp. four weeks post-inoculated with six willow isolates from single-stemmed willow and subjected to drought stress. Measurements were  $\log_{10}$  transformed,  $n = 3$ . Asterisks indicate means, which are significantly different than that of controls ( $P < 0.05$ ).

On the single-stemmed willow trees in the final experiment, five of the six *Cytospora* isolates from single-stemmed willows produced significantly ( $P < 0.05$ ) larger cankers than controls on drought-stressed trees (Figure 3). Three of these five *Cytospora* isolates produced significantly ( $P < 0.05$ ) larger cankers than controls on watered trees (Figure 3).

On the cottonwood trees in the final experiment, neither *Cytospora* isolate from elm produced cankers on cottonwood trees. There also was no significant difference in canker size between drought-stressed and well-watered treatments with elm isolates on cottonwood trees.

In all experiments, reisolation to confirm the presence of the fungus were 50% positive for drought-stressed trees and 73% positive for well-watered trees. All reisolation from nonexpanding wounds were negative. This finding indicates the fungi placed in the wounds were the cause of the canker expansion.

## DISCUSSION

*Cytospora* canker size was dependent upon the host tree species–fungal isolate combination. This result is consistent with previous reports indicating that many species of *Cytospora* parasitize diverse woody plant hosts (Helton and Konicek 1961), while other *Cytospora* species are host specific (Helton and Moisey

1955; Farr et al. 1989; Proffer and Hart 1994). There appears to be host specificity among the species of *Cytospora* examined in the current study, and future research is needed to determine the effect of these *Cytospora* isolates on tree species other than those tested.

The involvement of predisposing environmental stresses in the ability of *Cytospora* fungi to infect hosts is not well understood. *Cytospora* fungi are mostly opportunistic pathogens and attack hosts that are stressed and of poor vigor (Schreiner 1931; Hinds and Stewart 1965; Schoenweiss 1967; Hinds 1985; Sinclair et al. 1987; Biggs 1989; Guyon et al. 1996; McIntyre et al. 1996). Helton (1961b) and Dhanvantari (1978) reported that low-temperature injury could predispose stone fruit trees to infection by *Cytospora*.

Among drought-stressed trees, ash isolates were host specific, and although pathogenicity occurred in only one of three experiments, it appears that alder isolates were host specific as well. The lack of canker development in two of the three experiments with alder suggests that possibly a stress other than drought is involved in pathogenesis. Although elm isolates were pathogenic on cottonwood trees in the first experiment, results from the final experiment indicated they were not. One might assume that the canker development on elm trees was a result of variation in environmental stresses that we did not monitor. Based on preliminary tests that were positive, and the fact that elm trees were the only other host on which cankers developed, it seems reasonable to conclude that elm isolates are host specific.

The range of tree hosts of *Cytospora chrysosperma* is not completely defined from our study. This species is often reported as the pathogen associated with *Cytospora* canker on aspen, cottonwood, and willow trees (Christensen 1940; Hinds 1985; Walla and Conway 1986; Sinclair et al. 1987). Therefore, one might expect isolates of *Cytospora* from aspen, cottonwood, and willow to behave similarly on these hosts. Although aspen and cottonwood isolates each produced significantly larger cankers than controls on drought-stressed aspen and cottonwood trees, multi-stemmed willow isolates caused cankers on aspen trees only. Had willow isolates not infected aspen trees, the conclusion could be made that they were perhaps saprophytic, and therefore not capable of being pathogenic on trees other than multi-stemmed willow. The

results, however, are similar to those Helton (1961a) observed in a host range study in which a willow isolate (*Salix* spp.) did not cause cankers on golden willow (*Salix alba*). Additionally, Helton found five out of ten *Cytospora* isolates failed to induce cankers on trees of the same or closely related species from which they were isolated, and only two of the ten isolates produced their largest cankers on the host species or variety from which they were isolated. Clearly there are important factors other than host specificity that affect *Cytospora* canker development, and they appear to be related to the type and timing of stress.

The lack of canker development by multi-stemmed willow isolates on single-stemmed willow trees was explained by the final experiment. Data from the test indicated that single-stemmed willow isolates could cause cankers on single-stemmed willow trees. Thus, multi-stemmed willow isolates are host specific and will not infect single-stemmed willows.

Numerous species of *Cytospora* are listed in the literature as causing *Cytospora* canker, yet only a few have been tested in pathogenicity studies. These facts, combined with the broad host range of *Cytospora* spp., make questionable any conclusions regarding which species of *Cytospora* occur in a local region. Because species identification of *Cytospora* is difficult due to variation in fruiting and vegetative structures and spore size, examining differences among isolates from various hosts by means of host range and pathogenicity studies is important. The host specificity exhibited by ash isolates, as well as host preference of aspen and cottonwood isolates, indicates these fungi have specific host ranges. The possible host specificity of alder and elm isolates, and the host specificity displayed by willow isolates, lends further support to host specificity.

*Cytospora* species described on the six hosts we studied are numerous, but recent studies (Spielman 1985; Farr et al. 1989) have reduced the number of species by 80% to 90%. These reduced number of species are listed below; those in bold we found on our plant material. *Cytospora* species on alder are *C. sacculus*, *C. umbrina*, and *C. chrysosperma*; ash species are *C. fugax*, *C. leucosperma*, *C. minuta*, *C. pruinosa*, *C. sacculus*, and *C. annually*; poplar species are *C. chrysosperma*, *C. leucosperma*, *C. leucostoma*, *C. nivea*, and *C. sacculus*; willow species are *C. chrysosperma*, *C. fugax*, *C. germanica*, *C. leucosperma*, *C. nivea*, and *C. sacculus*; and

elm species are *C. carbonacea*, *C. chrysosperma*, *C. leucosperma*, and *C. sacculus*.

Techniques for characterizing races, varieties, and species of *Cytospora* are needed to further examine host–fungus species relationships. This study supports the contention that molecular techniques to examine differences among host-specific *Cytospora* spp. should be used in conjunction with host range and pathogenicity studies.

There appears to be both host-specific and host-nonspecific *Cytospora* species associated with *Cytospora* canker of hardwood trees. Knowledge of host specificity may aid nursery managers, landscape planners, and arborists in tree species selection. Planting trees that are attacked only by host-specific *Cytospora* near hosts susceptible to other *Cytospora* would be a good preventive practice in nurseries and landscapes. Knowing the specificity of a *Cytospora* fungus causing cankers on a tree would allow arborists to decide if the fungus is a threat to nearby trees and what actions are needed.

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Résumé. Le chancre cytosporéen est une maladie sérieuse chez plusieurs espèces d'arbres fruitiers et ornementaux au sein des forêts, des vergers et des pépinières. Parce que les espèces de *Cytospora* sont difficiles à identifier et les hôtes de chacune méconnus, il est difficile de dire si la présence de la maladie sur un hôte pose un risque pour d'autres espèces hôtes. Des souches de *Cytospora* ont été recueillies sur du peuplier faux-tremble (*Populus tremuloides* – *Cytospora chrysosperma*), du frêne de Pennsylvanie (*Fraxinus pennsylvanica* – *Cytospora pruinosa*), de l'orme de Sibérie (*Ulmus pumila* – *Cytospora sacculus*), de l'aulne (*Alnus* spp. – *Cytospora umbrina*), le peuplier (*Populus* spp. – *Cytospora chrysosperma*) et du saule à tige unique et multi-tiges (*Salix* spp. – *Cytospora fugax*). Ces souches ont été inoculées sur des peupliers faux-trembles, des frênes de Pennsylvanie, des ormes de Sibérie, des aulnes à petites feuilles (*A. tenuifolia*), des peupliers deltoïdes (*Populus deltoides*) et des saules à tige unique soumis à un stress hydrique. Les souches recueillies sur les frênes, les aulnes et les ormes sont spécifiques à leur hôte. Les souches des peupliers faux-trembles et deltoïdes sont pathogéniques seulement sur les espèces du genre *Populus*. Les souches provenant des saules multi-tiges causent des chancres seulement sur le peuplier faux-tremble mais pas sur le saule à tige unique. Cependant, les souches de *Cytospora* provenant de saules à tige unique étaient pathogéniques sur les arbres clonés à partir de ce saule. Le potentiel en eau, à titre de covariable, n'expliquait pas la variation dans la dimension des chancres parmi les arbres. De ce fait, les souches de *Cytospora* qui sont des hôtes spécifiques ne constitueraient donc pas un risque pour les arbres des autres espèces.

Zusammenfassung. Cytospora-Krebs ist eine ernste Pilzerkrankung bei vielen Schatten-, Frucht- und Zierbaumarten in öffentlichen Anlagen, Obstgärten und Baumschulen. Weil der *Cytospora*-Arten schwierig zu identifizieren sind und ihr Wirtsspektrum noch nicht genau verstanden ist, bleibt ungeklärt, ob beim Auftreten an einem Wirt ein Übergreif auf andere Arten zu erwarten ist. An folgenden Bäumen wurden *Cytospora*-Erreger isoliert: *Populus tremuloides*: *Cytospora chrysosperma*, *Fraxinus pennsylvanica*: *Cytospora pruinosa*, *Ulmus glabra*: *C. sacculus*,

*Alnus* spp.: *C. umbrina*, *Populus* spp.: *C. chrysosperma*, *Salix* spp.: *C. fugax*. Diese Isolate wurden in folgende trockenheitsgestresste Bäume infiziert: Espe, Esche, Ulme, Erle, Pappel und einstämmige Weide. Die Isolate von Espe, Erle und Ulme waren wirtsspezifisch. Die Isolate von Espen und Pappeln waren nur an Espen und Pappeln pathogen. Die Isolate der mehrstämmigen Weide verursachten Krebs nur an Espen und nicht an einstämmigen Weiden. Aber die Isolate von einstämmigen Weiden waren an allen Klonen dieser Weide. Das WasserPotential, als eine KoVariante, erklärte nicht die unterschiedliche Krebsgröße zwischen den Bäumen. Daher stellen wirtsspezifische *Cytospora*-Pilze keine direkte Bedrohung für andere Baumarten dar.

Resumen. El cancro *Cytospora* es una seria enfermedad fungosa de muchas especies de árboles frutales, de sombra y ornamentales en los huertos, bosques urbanos y viveros. Debido a que las especies de *Cytospora* son difíciles de identificar y su rango de hospederos no es bien conocido, no se sabe si la ocurrencia en un hospedero sea una amenaza para otras especies. Se colectaron aislamientos de *Cytospora* de álamo (*Populus tremuloides*) (*Cytospora chrysosperma*), fresno (*Fraxinus pennsylvanica*) (*Cytospora pruinosa*), olmo siberiano (*Ulmus pumila*) (*Cytospora sacculus*), aliso (*Alnus* spp.) (*Cytospora umbrina*), álamo (*Populus* spp.) (*Cytospora chrysosperma*), y sauce mono y multitronco (*Salix* spp.) (*Cytospora fugax*). Estos aislamientos fueron inoculados en álamo, fresno, olmo siberiano, aliso de hoja fina (*A. tenuifolia*), álamo del este (*P. deltoides*) y sauce de un tallo, todos estresados por la sequía. Los aislamientos de fresno, aliso y olmo fueron hospederos específicos. Los aislamientos de los álamos fueron patogénicos solamente sobre los árboles de estas especies. Los aislamientos de los sauces multitrancos causaron canchros solamente en álamo y sauces de más de un tronco. Sin embargo, los aislados de *Cytospora* sp. colectados de sauces de un tronco fueron patogénicos en árboles clonados de esos sauces. El potencial del agua, como una covariada, no explicó la variación en el tamaño del cancro entre especies. Por consiguiente, los hongos de *Cytospora* que son hospederos específicos pueden no ser un riesgo para otras especies de árboles vecinos.

