WOUND INOCULATIONS OF COLORADO BLUE SPRUCE WITH ISOLATES OF LEUCOSTOMA (CYTOSPORA) KUNZEI AND OTHER CYTOSPORA SPECIES

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Abstract. Thirty two isolates of *Cytospora* spp. recovered from various coniferous and deciduous hosts were used to wound inoculate intact and excised branch segments of mature Colorado blue spruce (*Picea pungens*) in two studies. All isolates of *C. kunzei* obtained from species of spruce consistently caused cankers on inoculated branches. Isolates of *C. kunzei* from other conifers varied in their ability to cause cankers. One of two isolates of *C. kunzei* from Douglas-fir and an isolate from eastern white pine were capable of inciting cankers while isolates from European larch and eastern hemlock failed to induce cankers. Isolates of other *Cytospora* species, collected from deciduous tree hosts, do not appear to play a significant role in the occurrence of Cytospora canker of Colorado blue spruce and should not be considered as a potential source of inoculum capable of infecting Colorado blue spruce.

Colorado blue spruce (*Picea pungens*) is widely planted in the midwestern, Great Lakes, and northeastern regions of the United States. This common and popular tree of the residential landscape is valued both for its color, which ranges from green to blue-green to silvery grey-blue on the new growth, and for its dense pyramidal crown (2). Unfortunately, mature Colorado blue spruce trees in these regions are generally disfigured by the fungal disease, Cytospora canker (5,7). This disease is characterized by diffuse resinous branch cankers, which first occur on the lower branches, and then affect limbs progressively higher on the tree (5,7). Cytospora canker rarely kills Colorado blue spruce trees. The discoloration, dieback, and loss of infected branches, however, dramatically reduces the aesthetic value of these large and prominent landscape specimens. The damage caused by this disease attracts the attention and concern of both homeowners and industry professionals.

Cytospora canker of blue spruce is caused by the fungus *Leucostoma kunzei* (1,3,4,5,7,9,11). The asexual stage of this fungus, *Cytospora kunzei*, is most frequently observed on the infected branches (4) and hence the name, Cytospora canker. While Colorado blue spruce is the most common host of *C. kunzei* in this area (9), it has also been isolated and reported from other spruce and conifer species (3,5,6,9,11).

Members of the genus *Cytospora* are associated with cankers and dieback on a wide range of trees and shrubs and can be found throughout our region (3,10). Hundreds of species of *Cytospora* have been described in the literature, based primarily on host associations (3,5,10). A relatively recent taxonomic review of the genus *Valsa* (sexual stage of many *Cytospora* species) has reduced the number of accepted species, some being identified from a wide variety of hosts (10). *Cytospora* species from conifers, however, were not included in that taxonomic revision. The variability of *Cytospora* fruiting bodies, even on a single host species, is high and species determinations are difficult (10,11). Differences in host tissues may affect the appearance of fruiting bodies. There have been few host range studies done with these fungi.

The spores of *C. kunzei* and other *Cytospora* species are disseminated primarily via rainsplash (5,8). As ornamental trees in this region, Colorado blue spruces occur in relatively isolated plantings in residential or municipal sites. Yet, despite their relative isolation from each other and other spruces, virtually every mature blue spruce tree in the region eventually becomes affected by Cytospora

canker. This poses the question, where is the inoculum coming from?

There are several possible explanations for the almost universal occurrence of Cytospora canker of blue spruce. One explanation may be that birds and/or insects are involved with long-distance spore dispersal. Another possible explanation is that trees are colonized by the fungus while still in the nursery (by spores from nearby spruce trees) but that cankers do not form until the trees mature and/or other disease requirements are met. A third possibility is that *Cytospora* spp. from other hosts may be capable of infecting spruce and that these fungi, when found on blue spruce, have been identified as *C. kunzei* merely because of the host association. This study addresses this latter possible explanation.

The purpose of this research was to determine whether isolates of *Cytospora* from a variety of deciduous and conifer hosts were capable of causing cankers in inoculated mature Colorado blue spruce trees. In addition to providing evidence as to whether *C. kunzei* is a distinct species, the goal was to determine if trees, other than spruces, might be serving as sources of inoculum for this common and important disease of Colorado blue spruce. This information could alter management and control strategies.

Materials and Methods

This paper combines the results of two separate studies. Study 1 was done at Michigan State University in 1984 and 1985 as part of the primary author's dissertation research. It involved both excised branch inoculations and inoculations in the field. Study 2 was done in 1992 at the Kellogg Forest Research Station of Michigan State University in Kalamazoo, Michigan. In Study 2, only field inoculations were performed.

Isolates. In Study 1, sixteen isolates of *Cytospora* were tested: four from spruce hosts, and twelve from other tree species (Table 1). In Study 2, sixteen new isolates were tested: again four from spruce hosts, and twelve from other tree species (Tables 1 and 2). Some of the isolates were obtained from infected host cambial/cortical tissues by aseptically removing pieces of affected tissues and placing them in petri plates containing potato dextrose agar (PDA). Other isolates were started from conidia or ascospores. Isolates were grown on 2% malt extract agar (MEA) at 26°C for 5-6 days to produce mycelial inoculum.

Excised branch inoculations. In Study 1, healthy, uncankered branches from mature blue spruce trees were collected and brought into the laboratory. The branches were collected from the same trees inoculated in the field portion of Study 1. The smaller branchlets and needles were removed. Branches at least 2 cm in diameter were cut into 30-40 cm segments. The cut branch ends and branchlet stubs were sealed with molten paraffin to reduce drying. Inoculation sites were surface disinfested by dabbing with 95% ethanol and flaming. Flap wounds (7 X 7 mm) were aseptically cut in the bark and mycelial inoculum was inserted under the flap. Three or four inoculations were spaced along and around each branch segment. Sterile 2% MEA was used for control inoculations. The flaps were closed and wrapped with Parafilm MR. The inoculated branches were placed into loosely closed plastic bags to incubate 7-10 days at room temperature. A total of 403 inoculations (20-30 reps per isolate and control) were made on excised twigs.

Intact branch inoculations. In Study 1, healthy, uncankered lower branches of three mature blue spruce trees were inoculated in July and August. A few naturally occurring cankers on these trees were pruned out prior to the inoculations. Flap wounds (7 X 7 mm) were cut in the bark and mycelial inoculum or sterile 2% MEA was inserted under the flap. The inoculated wound flaps were closed and wrapped with brightly colored tape to facilitate locating the inoculation sites later. Inoculations were spaced along the length of the branches from near the trunk to 3-yr-old branch tissue near the distal end. The inoculations were examined after 3-5 weeks. A total of 289 inoculations (17 inoculations per isolate and control) were made on intact branches.

In Study 2, one hundred 22-yr-old blue spruce trees that were part of a half-sib progeny test at the Kellogg Forest in Kalamazoo, Michigan, were inoculated. This study utilized trees representing a much broader genetic base than Study 1. While no Cytospora canker was noted in the planting at

the time of inoculation, the lower canopy of this stand was beginning to close and some of lowest branches were declining and dying due to shading. None of the resinosis associated with Cytospora canker was observed among the test trees. Inoculations were done in July as described above and examined after 4-5 weeks. There were 8-9 inoculations per tree (all different isolates), with a total of 800 inoculations (50 with each isolate and control, except for two isolates which had only 25 inoculations each).

Evaluation. The results of the inoculations were evaluated by cutting away the bark tissues surrounding the inoculation site and looking for canker development in the underlying cambial/cortical tissues. Healthy, uninfected tissues were white; cankered tissues were brown.

Results

The isolates of *Cytospora* varied in their ability to cause cankers on inoculated branches of blue spruce. Where cankers formed, an area of discolored cambial/cortical tissue could be measured extending beyond the margins of the inoculation site. In control inoculations, or where cankers did not form, only the tissues of the wound flap itself were discolored. Cankers which formed on inoculated intact branches (branches still attached to the tree) were always associated with heavy resin exudation at the inoculation site. Control inoculations, and inoculations with isolates which failed to cause cankers did not induce resinosis. Resinosis did not occur on the inoculated excised branch segments even when cankers formed.

Study 1. The only isolates capable on inciting cankers on inoculated intact branches were isolates of *C. kunzei* (Table 3) obtained from spruce and eastern white pine. These same isolates also induced cankers on inoculated excised branches (Table 3). Isolates of *C. kunzei* from Douglas-fir and eastern hemlock did not incite canker formation in this study. In contrast to the results observed on intact branches still attached to the trees, an isolate of *C. leucostoma* from peach did colonize and discolor cambial/cortical tissues in 10% of the inoculations on excised branch segments.

Table 1. Identification and origin of isolates of *Cytospora* used to inoculate Colorado blue spruce in Study 1.

Host	Origin of isolate	Identification
	Coniferous hosts	
Picea pungens	Colorado blue spruce	*C. kunzei*
P. pungens	Colorado blue spruce	*C. kunzei*
P. abies	Norway spruce	*C. kunzei*[a]
P. glauca	white spruce	*C. kunzei*
Pinus strobus	eastern white pine	*C. kunzei*
Pseudotsuga menziesii	Douglas-Fir	*C. kunzei*
Tsuga canadensis	eastern hemlock	*C. kunzei*
	Deciduous hosts	
Acer platanoides	Norway maple	*C. annulata*
Alnus rugosa	speckled alder	*C. leucosperma*
Fagus sylvatica	European beech	*C. leucosperma*
Malus pumila	apple	*C. leucosperma*
Populus grandidentata	big tooth aspen	*C. chrysosperma*
Populus sp.	hybrid poplar	*C. chrysosperma*
Prunus persica	peach	*C. leucostoma*
Quercus robur	English oak	*C. leucosperma*
Rhus typhina	staghorn sumac	*C. leucosperma*

[a] Isolate started from conidia, all other isolates obtained from infected host tissue.

Table 2. Identification and origin of isolates of *Cytospora* used to inoculate Colorado blue spruce in Study 2.

Host	Origin of Isolate	Identification
	Coniferous hosts	
Picea pungens	Colorado blue spruce	*C. kunzei*[a]
P. pungens	Colorado blue spruce	*C. kunzei*
P. pungens	Colorado blue spruce	*C. kunzei*
P. glauca	white spruce	*C. kunzei*
Larix decidua	European Larch	*C. kunzei*[a]
Pseudotsuga menziesii	Douglas-fir	*C. kunzei*[b]
	Deciduous hosts	
Acer palmatum	Japanese maple	*C. leucosperma*[a]
Acer platanoides	Norway maple	*C. annulata*
Cydonia oblonga	common quince	*C. cincta*[b]
Malus pumila	apple	*C. schulzeri*[a]
Populus X 'Robusta'	Robusta poplar	*C. nivea*[b]
Prunus persica	peach	*C. leucostoma*[a]
Quercus alba	white oak	*C. leucosperma*
Rhus typhina	Staghorn sumac	*C. leucosperma*
Salix babylonica	weeping willow	*C. chrysosperma*[a]
Salix matsudana	corkscrew willow	*C. leucosperma*[a]

[a] Started from conidia.
[b] Started from ascospores.

Study 2. All of isolates of *C. kunzei* from spruce and one from Douglas Fir consistently incited canker formation and resinosis on the inoculated branches (Table 4). An isolate of *C. kunzei* from larch did not produce cankers. In 17 of 500 inoculations with other isolates of *Cytospora* and the sterile control, resinosis and cambial/cortical discoloration were observed (Table 4).

Discussion

Wound inoculations of both excised and intact branches confirmed that Cytospora canker of blue spruce is caused by *Cytospora kunzei* (sexual stage: *Leucostoma kunzei*). All isolates of *C. kunzei* obtained from spruce species common in the residential landscape (*P. pungens, P. glauca, P. abies*, and *P. pungens* var. *glauca*) were capable of causing cankers in inoculated Colorado blue spruce trees. Earlier vegetative-compatibility studies with *C. kunzei* revealed a similar ability for this fungus to occur on more than one spruce species (9). All spruce trees (regardless of species) with Cytospora canker should be considered as a source of inoculum capable of infecting Colorado blue spruce.

Isolates of *C. kunzei* from conifers other than spruce varied in their ability to incite cankers in these inoculation studies. While these other coniferous hosts of *C. kunzei* need to be considered as potential sources of inoculum, their impact and importance is probably limited under most conditions. One limiting factor is that *C. kunzei* has not been frequently reported from hosts other than spruce (3). Based on personal experience (primary author) attempts to obtain isolates of *C. kunzei*, as part of the vegetative-compatibility study (9) or while performing isolations as part of disease diagnostic tests, failed to produce many isolates of *C. kunzei* (only those few isolates used in these tests). Additionally, isolates of *C. kunzei* from non-spruce hosts did not consistently produce cankers as did all spruce isolates of *C. kunzei*. In a previ-

Table 3. The percent of canker initiation on blue spruce branches wound inoculated with isolates of different Cytospora species: Study 1.

Isolate origin	Percent canker initiation[a] Excised branches[b]	Intact branches[c]
Coniferous hosts		
Picea pungens var. glauca	80	78
P. pungens	85	87
P. abies	80	93
P. glauca	96	90
Pinus strobus	95	88
Pseudotsuga menziesii	0	0
Tsuga canadensis	0	0
Deciduous hosts		
Acer platanoides	0	0
Alnus rugosa	0	0
Fagus sylvatica	0	0
Malus pumila	0	0
Populus grandidentata	0	0
Populus sp.	0	0
Prunus persica	10	0
Quercus robur	0	0
Rhus typhina	0	0
Control	0	0

[a] Basis: The number inoculations causing canker development / number of total inoculations.
[b] 20-30 inoculations per isolate.
[c] 17 inoculations per isolate.

ous study by Waterman (11), isolates *C. kunzei* from non-spruce hosts also failed to cause cankers in inoculated nursery-aged Colorado spruce trees. The isolate of *C. kunzei* from eastern white pine used in Study 1 appears to be very atypical. It was recovered from a dead twig on a tree near naturally infected Colorado Spruce trees and the isolate was of the same vegetative- compatibility group as those spruce isolates (9) indicating they were clonal in nature. As part of that vegetative compatibility study, many dead or dying branches of eastern white pine in other locations were sampled and in no other case was *C. kunzei* isolated. Eastern white pine probably is not an important source of inoculum for this fungus. White pine and Bhutan pine (*Pinus wallichiana)* are the only pine species reported to be a host of *C. kunzei* in the United States (3,11). Of the two isolates of *C. kunzei* from Douglas fir tested, one

Table 4. The percent of canker initiation on blue spruce branches wound inoculated with isolates of different Cytospora species: Study 2.

Isolate origin	Percent canker initiation[a]
Coniferous hosts	
Picea pungens	100
P. pungens	92
P. pungens var. glauca	100
P. glauca	100
Larix decidua	0
Pseudotsuga menziesii	74
Deciduous hosts	
Acer palmatum	4
Acer platanoides	4
Cydonia oblonga	4
Malus pumila	6
Populus X 'Robusta'	0
Prunus persica	4
Quercus alba[b]	4
Rhus typhina	4
Salix babylonica	0
Salix matsudana[b]	8
Control	2

[a] Basis: The number of inoculations resulting in cankers / total number inoculations
[b] 25 inoculations per isolate, all others with 50 per isolate

was capable of inciting cankers. Douglas fir is not widely used as an ornamental tree in this region and its impact as a source of inoculum is also probably limited.

Isolates of *Cytospora* from deciduous hosts failed to cause cankers in inoculated intact trees in Study 1. An isolate of *C. leucostoma* did colonize the cambial/cortical tissues in 10% of the inoculations on excised branch segments. *Cytospora leucostoma* is a common and widespread pathogen of peach and other *Prunus* species in the region (3). The cultural characteristics of *C. leucostoma* and *C. kunzei* are distinctly different and *C. leucostoma* was not isolated in the expanded survey of the vegetative-compatibility study (9) or has it been recovered during many subsequent isolations from spruce. It seems very unlikely that *C. leucostoma* is a contributor in Cytospora canker of spruce.

In the much larger field inoculations of Study 2, scattered cankers were observed among the

control inoculations and inoculations using isolates of *Cytospora* from deciduous hosts (17 of 500 inoculations). Whether these cankers were due to limited colonization by these other fungi or due to natural infections of the inoculation sites was not determined. Given that one control inoculation yielded a canker, natural infection of at least that inoculation site is indicated. If infrequent colonization by these other species did occur, the canopy closure noted at the study site may have played a role. The vigor of the branches inoculated undoubtedly varied and if low may have allowed a response as seen on the excised branch segments with *C. leucostoma*). The infrequent nature of the canker initiation caused by these other isolates of *Cytospora* suggests that they are not playing an important role in Cytospora canker of spruce. Supporting this statement, the cultural characteristics of isolates of *C. kunzei* are different from these other *Cytospora* species and past experience with a large number of isolations from spruce indicate that other *Cytospora* species are not associated with Cytospora canker of spruce. Deciduous trees do not appear to serve as a significant source of inoculum capable of causing the common disease, Cytospora canker of Colorado blue spruce.

In terms of disease management, the results of this study indicate that all spruces, regardless of species, should be considered as potential inoculum sources of *C. kunzei*. Planting Colorado blue spruces near other spruces with Cytospora canker should be avoided. Douglas-fir should also be considered as a potential inoculum source. Pines, other conifers, and deciduous trees appear to pose a minimal risk in terms of producing inoculum capable of causing Cytospora canker of blue spruce.

One of the potential explanations for the almost universal occurrence of this disease in our region has now been eliminated. Cytospora canker of spruce is caused by *C. kunzei* and not by a variety of *Cytospora* species. This still leaves the question unanswered, where is the inoculum coming from? Given that the spores of *C. kunzei* are disseminated primarily via rainsplash, and given that spruces are distributed discontinuously across the region (no native spruces occur through most of this region), why is this disease so ubiquitous? It would seem than some trees would escape infection, a phenomenon rarely observed. Further studies dealing with long distance dispersal (via birds and/or insects) and infection of nursery stock (with delayed disease development) will be required to address this interesting and important question.

Inoculation studies should also be used to explore the existing forest genetic plantings of Colorado blue spruce to try and identify breeding lines which may offer some resistance to this common pathogen. Colorado blue spruce remains popular with the public so this disease will continue to cause concern within the industry for many years to come.

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Résumé. Trente-deux souches de *Cytospora* spp., récoltées de différentes espèces de conifères et de feuillus, ont été utilisées pour inoculer des segments de branches intactes ou coup'es d'épinette bleue du Colorado (*Picea pungens*) à maturité. Toutes les souches de *C. kunzei* obtenues à partir d'épinettes causèrent naturellement des chancres sur les branches inoculées. Les souches de *C. kunzei* provenant d'autres conifères avaient une capacité variable à causer la formation d'un chancre. Les souches d'autres espèces de *Cytospora*, recueillies d'espèces-hôte feuillues, ne semblaient pas devoir jouer un rôle significatif dans l'occurence du chancre cytosporéen sur l'épinette bleue du Colorado.