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EFFECTS OF DROUGHT-STRESS AND WOUNDING ON CYTOSPORA CANKER DEVELOPMENT ON COLORADO BLUE SPRUCE[1]

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Abstract. Trees subjected to drought-stress in the greenhouse following inoculation with inoculum produced from monoascospore cultures developed significantly ($p=0.001$) more cankered branches than did inoculated, non-drought-stressed trees. Inoculation of drought-stressed and non-drought-stressed trees with inoculum produced from monoconidium cultures did not cause infection. No infection with either inoculum occurred in the absence of wounding.

Colorado blue spruce (*Picea pungens*) is one of the most commonly planted and highly valued ornamental, shelterbelt, and Christmas trees in north temperate zones of the U.S. (8). Cytospora canker caused by *Valsa kunzei* (imperfect stage is *Cytospora kunzei*) is the most destructive and easily recognized disease of blue spruce in Michigan. Blue spruce trees are rarely killed by the disease, but the continuous removal of cankered branches destroys the symmetry and subsequently the aesthetic value of the tree.

Drought is believed to be a predisposing factor in the development of Cytospora canker of white and Norway spruce (9). Numerous workers have found wounds to be necessary for the development of Cytospora canker in artificial inoculation studies of spruce trees (6, 7, 9, 11).

Several aspects of the etiology of the disease are still in question, i.e., the major source of inoculum and environmental conditions favoring natural infections. Ascospores, conidia and mycelium derived from these spores are assumed to be infectious (6, 9, 11). However, greenhouse inoculation of healthy Colorado blue spruce trees with mycelium derived from monoconidium cultures consistently failed to produce cankers, yet Cytospora canker is very common. The purposes of the present investigation were: 1) to study sites of *Cytospora* infection, 2) to determine the effect of drought-stress on Cytospora canker development, and 3) to determine the infective capacity of conidia and mycelium derived from ascospores and conidia of *V. kunzei.*

Materials and Methods

The effects of drought-stress and wounding on Cytospora canker development were studied in the greenhouse during the summers of 1978 and 1979. Drought-stressed trees received 1000 ml of water once a week while non-drought-stressed trees received 350 ml of water per day for a period of 10-12 weeks. Six five-year old potted Colorado blue spruce were drought-stressed in 1978. Two trees were wound-inoculated with discs of mycelium from monoascospore culture of *V. kunzei.* Two trees were wound-inoculated with discs of mycelium from monoconidium culture of *C. kunzei* and the remaining two trees served as wounded-non-inoculated controls. Four non-drought-stressed trees plus two controls were

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treated as above. These experiments were repeated with new trees in 1979.

In 1978, each of the inoculated trees in both the drought and non-drought-stressed experiments consisted of four wound-inoculated branches plus one wounded but non-inoculated control branch. In addition, four wounded but non-inoculated branches on each of two control trees in each of drought and non-drought-stressed experiment provided additional controls. In 1979, each of the inoculated trees in the drought and non-drought-stressed experiments had six wound-inoculated branches plus one wounded but not inoculated control branch. Four wounded but non-inoculated branches on each of two control trees in each of drought and non-drought-stressed experiments provided additional controls.

Inoculation zones were swabbed with 2.62% sodium hypochlorite solution, rinsed with sterile distilled water and wounded through the bark with a sterile scalpel. Inoculum in the form of mycelial blocks or mycelium growing on sterilized blue spruce twigs on agar medium was placed onto the wounded zones, wrapped with sterile cheesecloth and covered with parafilm. Control branches were treated in the same manner but received water agar blocks. The wrappings were removed after 2 weeks, and final data on resin production, needle drop and fungus fructification were recorded after 12 weeks.

Wounded and inoculated branches in the non-drought-stressed tree experiment described above served as the wounded and inoculated experimental branches. In 1978, 4 branches on each of 4 non-drought-stressed trees and on 2 non-drought-stressed trees in 1979 treated in the same manner except for wounding served as non-wounded treatments. Evaluation of disease was made 10-12 weeks after inoculation for resin production, needle drop and for the formation of fruiting bodies.

Infection of blue spruce via needle inoculation was also studied. In 1978, 12 potted Colorado blue spruce seedlings in the greenhouse received a 48-hr pre-inoculation misting period in a mist chamber. Thirty needles on 3 seedlings were individually inoculated with either 0.01 ml of a suspension containing 3.8×10^7 freshly harvested conidia per ml or 2.0×10^6 freshly harvested ascospores per ml of water. An additional 60 needles on 6 seedlings were inoculated with sterile water. Inoculated seedlings were covered with a plastic bag and left in the mist chamber for another 48-hr period. The seedlings were placed on a bench in the greenhouse and the plastic bags removed after 4 days. The plants were checked daily for symptoms of infection. These experiments were repeated in 1979.

Results

All drought-stressed trees inoculated with inoculum produced from monoascospore culture exhibited needle drop on all 20 wound-inoculated branches (Table 1), followed by death of inoculated branches. The first symptoms of infection appeared 23 days after inoculation. None of the non-inoculated, drought-stressed trees suffered death or drop of needles from branches. Twelve of 20 inoculated branches on non-drought-stressed trees inoculated with monoascospore culture developed cankers in 63 days after inoculation. Thus, the period between inoculation and canker development was longer in the non-drought-stressed trees than in the drought-stressed trees. None of the branches on control trees developed cankers. Typical resinosis on infected branches was evident. Re-isolation from infected branches yielded cultures identical to the cultures used in the inoculation.

Drought-stressed and non-drought-stressed seedlings inoculated with inoculum produced from monoconidium culture were not infected, and wounded branches healed with abundant callus formation. Growth resumed when drought-stressed seedlings were returned to a regular watering schedule as indicated by budbreak in late September.

None of the non-wounded branches inoculated with inoculum produced from monoconidium and single ascospore culture became infected. They remained healthy and appeared to be free of cankers. Periodic examination of inoculated needles indicated no infection 3 months after inoculation.

Table 1. Effect of Drought-Stress on Cytospora Canker Development. 1978-1979.

Stress treatment	*Type of inoculum*	*No. of wound-inoculated trees*	*No. of branches inoculated*	*No. of infected branches*
1000 ml water per week	Monoascospore mycelia	4	20	20/20[a]
	non-inoculated	2	0	0
	Monoconidium mycelia	4	20	0
	non-inoculated	2	0	0
350 ml water per day	Monoascospore mycelia	4	20	12/20[b]
	non-inoculated	2	0	0
	Monoconidium mycelia	4	20	0
	non-inoculated	2	0	0

a is significantly different from b at 0.001 level. p=0.001 (T-test).

Discussion

The literature on Cytospora disease of many hosts stresses the role of predisposition in disease development (1, 2, 3, 4, 5, 9). In the case of Colorado blue spruce in Michigan, the major predisposing factors appear to be drought and wounds.

The results provide indirect evidence that drought-stress in the host influences the development of Cytospora canker. Although direct water potential or relative bark moisture was not measured in the present study, indirect evidence for influence of drought-stress comes from the fact that growth resumed when drought-stressed trees were placed on a regular watering schedule. Even thought drought-stressed trees developed significantly more cankers than did non-drought-stressed trees, there is always inherent stress associated with potted seedlings. That the incidence of Cytospora canker of Colorado blue spruce in the field increases as bark moisture decreases should be investigated.

Cytospora species have been characterized as wound parasites that require wounds to gain entry to a host (6, 9, 10, 11). This study has found wounds to be necessary for the development of Cytospora canker on Colorado blue spruce trees artificially inoculated. In addition, there is no evidence to indicate that Cytospora infection occurs through needles. That insects are present and may play a role in infection must be examined in future studies. Insect wounds may serve as infection courts.

The *Valsa* stage (ascospore) appears to be the infective stage confirming the work of other investigators. Marsden (10) was able to show that cankers and death of Norway spruce branches occurred following inoculation with mycelium from single spore cultures of *Valsa*. He did not, however, test the pathogenicity of the species of *Cytospora* which causes the canker disease of spruce. He was unable to obtain sufficient fertile perithecia to determine the importance of the ascospores in the natural spread of Cytospora canker. Waterman (11) obtained infection on Douglas-fir, and blue, Norway and white spruce using mycelium from isolates of *Valsa kunzei* var. *piceae*. She did not report details of conidial inoculations.

Only two studies have reported infection of spruce with conidia of *C. kunzei* (6, 9). In 1936, Gilgut (6) succeeded in obtaining artificial infection of wounded Norway spruce in the field with freshly harvested spores or mycelium of *C. kunzei*. He did not state whether the origin of the culture was pycnidia or perithecia. His spore suspension was made by dissolving several freshly exuded spore tendrils in distilled water. His mycelial inoculum was obtained from a single spore culture from spore suspension. Jorgensen and Cafley (9) were able to induce infection on white and Norway spruce with mycelium, pycnidiospores, or ascospores of *V. kunzei*. The monoascospore cultures used in the inoculation experiments reported in the present study always produced pycnidia in culture and were infective.

Therefore, knowledge of the original culture and the type of fruiting structure(s) from which the inoculum was obtained is extremely important. Both types of fruiting bodies exude spores in indistinguishable tendrils on wetting. The possibility of mixed inoculum is conceivable.

The conflicting results regarding infection of spruce by mycelium and conidia of *C. kunzei* may result from differences in host susceptibility, isolate virulence, host reaction to wounding, host physiology and environmental conditions at the time of inoculation. Although it has not yet been established that the conidial stages found on these hosts are identical, and extensive cross-inoculation studies have not been attempted, there is little evidence to suggest host specificity among the isolates. For example, the ascospore cultures used in this study were obtained from Norway spruce and were pathogenic to blue spruce. Three *Cytospora* isolates from each of blue and Norway spruce were tested in separate experiments and found to be non-infective.

The question arises as to the role of conidia in the life cycle of *C. kunzei*. Although they germinate and produce mycelial colonies, strong corroborating evidence of infection either from conidia or from mycelial colonies derived from them is lacking and should be investigated. The results of this study suggest they probably are not involved in the infection process. Pycnidia arise in the same tissue as the perithecia. However, the two types of fruiting bodies have never been found in the same stroma (13). Possibly conidia serve as gametes in hybridization of compatible perithecial initials. With the exception of Wehmeyer (12), none of the other investigators (6, 9, 11) has been able to induce perithecial formation in culture. Investigations of Cytospora canker have been concerned with describing canker development. The details of perithecial and pycnidial development in *V. kunzei* have not been reported. Failure to obtain reproducible results calls for a study to elucidate the developmental morphologies of the two spore types.

The results of wound and needle inoculation support the hypothesis that wounds are important for Cytospora canker development. However, they do not exert a general predispositional influence on the tree. For disease development, the tree must also be weakened by some other factors, e.g., drought-stress.

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