NOTES

Decline symptoms do not develop with grafting from dying yellow-cedar

P.E. Hennon and M.G. McWilliams

Abstract: Branchlets from dying yellow-cedar (*Chamaecyparis nootkatensis* (D. Don) Spach) trees were grafted on healthy saplings to determine if agents transmissible through grafts are associated with the widespread yellow-cedar decline in Alaska. A total of 216 branchlets were removed from 72 mature yellow-cedar trees that were apparently healthy, in early stages of dying, or nearly dead. These scions were grafted on 72 saplings and monitored for 5 years. The survival of grafted scions was reduced to 33% after 5 years. All surviving scions that were chlorotic when grafted became green. Chlorotic symptoms did not develop proximally to grafts in branches or generally in any of the saplings. Grafting treatment produced no detectable effect on height or diameter growth of the recipient saplings. This study produced no evidence that a graft-transmissible agent is associated with yellow-cedar decline.

Résumé : Des rameaux prélevés sur des chamaecyparis jaunes (*Chamaecyparis nootkatensis* (D. Don) Spach) mourants ont été greffés sur des gaules saines pour vérifier si des agents transmissibles par greffage sont associés au dépérissement du chamaecyparis jaune répandu en Alaska. Un total de 216 rameaux ont été prélevés sur 72 chamaecyparis jaunes matures qui étaient en apparence sains, au stade initial de la mort ou presque morts. Ces greffons ont été greffés sur 72 gaules et suivis pendant 5 ans. La survie des greffons a été réduite à 33% après 5 ans. Parmi les greffons qui ont survécu, tous ceux qui étaient chlorosés au moment du greffage sont devenus verts. Les symptômes de chlorose ne sont pas apparus autour des greffes dans les branches ni généralement chez les gaules. Le greffage n'a eu aucun effet apparent sur la croissance en hauteur ou en diamètre des gaules utilisées comme récepteurs. Cette étude n'a fourni aucun indice qu'un agent transmissible par greffage soit associé au dépérissement du chamaecyparis jaune.

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Introduction

Yellow-cedar (*Chamaecyparis nootkatensis* (D. Don) Spach) has suffered from a widespread and intensive mortality for more than 100 years in southeastern Alaska (Hennon and Shaw 1997). Dying trees have reduced radial growth and crowns with foliage that is yellow or light green and becomes sparse over time (Hennon et al. 1990*b*). The epidemiology and relation to site factors have been described (Hennon et al. 1990*a*). Results from studies on possible biotic causes suggest that fungi (Hennon 1990; Hennon et al. 1990*b*), insects (Shaw et al. 1985), and nematodes (Hennon et al. 1986) are not the primary agents that lead to tree death.

Viruses and phytoplasmas have been reported in conifers (Nienhaus and Castello 1989; Zhengmin and Zuoyi 1990), but little is known about their potential involvement in forest declines. In the virus–conifer pathosystem that has received the most attention to date, tomato mosaic virus infection caused significant reduction in the height, mass, and root volume but few foliar symptoms in red spruce (*Picea rubens* Sarg.) seedlings (Bachand et al. 1996). Infection is by atmospheric sources (Fillhart et al. 1997) such as fog and cloud water (Castello et al. 1995).

Many viruses and phytoplasmas are transmissible through grafts with the symptoms they cause developing in the recipient plants (Agrios 1997; Maramorosch 1979). Port-Orford-cedar (*Chamaecyparis lawsoniana* (A. Murr.) Parl.), a close relative of yellow-cedar, has been artificially infected with arabis mosaic nepovirus by using soil infected with dagger nematodes (Harrison 1964). This virus also is transmitted through grafts in other hosts. Rod-shaped bodies that causes chlorosis in Norway spruce (*Picea abies* (L.) Karst.) in Czechoslovakia can be transmitted by both aphids and grafting (Cech et al. 1961).

Zhengmin and Zuoyi (1990) reported the detection of a phytoplasma by Diene's stain and by transmission electron microscopy in the phloem tissues of symptomatic *Chamaecyparis obtusa* (Sieb. et Zucc.) Endl., another close relative of yellow-cedar. The chlorotic symptoms present in donor trees developed in recipient trees after grafting. The authors give the name "*Chamaecyparis* yellows" to the condition of chlorotic, symptomatic trees infected with this phytoplasma.

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Our study was initiated to determine if an agent that is transmissible through branch grafts is associated with yellow-cedar decline. Either reduced sapling growth or the development of thinning or chlorotic foliage in the grafted branch or elsewhere in the sapling, symptoms typical of declining donor trees, would be taken as evidence of possible transmission of a pathogen.

Materials and methods

Scion material (i.e., the donor tissue used in grafting) was collected using a long pole pruner or shotgun from the branches of 72 small, mature yellow-cedar trees during March 1991. These donor trees (i.e., ortets) were growing in an unmanaged forest with a high concentration of dead and dying yellow-cedar trees at Waterfall Cove on Chichagof Island in southeastern Alaska. Donor trees averaged 24 cm in diameter at 1.37 m above ground. Although we did not determine their ages, results from another study conducted in the same area suggest that these yellow-cedar trees were 100–300 years old (Hennon et al. 1992). Of the 72 sample trees, 24 were classified as apparently "healthy" (crowns were judged to be more than 85% full and over 85% of foliage was green), 24 were in early stages of dying, "declining" (crowns 40–84% full or 40–84% of foliage was green), and 24 were "nearly dead" (crown less than 40% full or foliage color was less than 40% green).

All collected scion material was maintained at 1°C in an incubator until grafting in April, a time of active root growth but prior to shoot growth for yellow-cedar. Three scions approximately 10 cm long and 4 mm diameter from each mature donor tree were grafted on three lateral branches of randomly selected yellow-cedar saplings. Thus, the three scions taken from each mature tree were all grafted on the same sapling. These saplings had been planted 5 years earlier as 2-year-old seedlings on a harvested site on Etolin Island in southeastern Alaska. Saplings received no fertilization or shading at any time during or after planting. Recipient saplings had diameters of 28 ± 8 mm (mean ± SD) and heights of 142 ± 32 cm at the time of grafting. Scions were attached about midway along the branches' lengths using a side veneer graft technique (Hallett et al. 1981). Grafts were wrapped with grafting bands and the bands were covered with grafting wax.

The color and survival of each scion, the color of branch foliage (both distal and proximal to the grafts), and the overall foliage color of each recipient sapling were monitored seven times until 1996. These observations were made over 5 years because viruses may have a long latent period before expression of symptoms in forest trees (Nienhaus and Castello 1989). The percent scion survival was calculated for each sapling and for all scions in a treatment. Foliage color classes for scions, branches with grafts, and entire saplings were green, light green, yellow, and brown (dead). The color of approximately one half of the scions taken from declining and nearly dead trees was green, even though the tree crowns were sparsely foliated. Chi-square (Snedecor and Cochran 1980) was used to test ($P < 0.05$) for a difference in the proportion of recipient saplings with at least one surviving scion by treatment at the conclusion of the study.

The basal diameter (2 cm above ground) and height of each sapling were measured to the nearest 1 mm and 1 cm, respectively, at the time of grafting and in 1996 to determine if grafting treatments affected sapling growth. Differences in height and diameter growth and percent height and diameter growth among the three treatments were tested at $P < 0.05$ with one-way analysis of variance (Snedecor and Cochran 1980). Least-square means of these growth values were calculated and tested with analysis of covariance to account for variation in the diameter and height of saplings at the time of grafting (SAS Institute Inc. 1990).

Results and discussion

All scions survived during the few months until the first examination, but scion mortality, as expressed by a brown color, was high after 1 year with survival dropping to 47 of 72 for healthy, 35 of 72 for declining, and 38 of 72 for nearly dead treatments (Fig. 1). Thereafter, scion mortality increased slowly for each treatment and final counts of surviving scions 5 years after grafting were 36 of 72, 18 of 72, and 19 of 72 for three treatments, respectively. Survival of individual scions was independent of original scion color for the two dying treatments (i.e., green scions did not fare any better). Of surviving scions, scion color remained green or changed from light green or yellow to green in all but one scion. That particular scion, which was taken from a healthy donor tree, was green when grafted but became light green during the first remeasurement and remained light green during the study. Thus, all but one scion in the study (*i*) became brown and died, (*ii*) was off-color initially but changed to green, or (*iii*) was green initially and remained that color. At the conclusion of the study, 92, 46, and 65% (not significantly different) of the recipient saplings had at least one of their three grafts alive for healthy, declining, and nearly dead donor treatments.

The cause of scion death was not always known; some died because grafts failed to form a good union and the scion actually broke away. Other scions appeared to be attached, but turned brown and died. In fruit trees, viruses can be agents of graft union rejection and virus transmission often occurs even when scions die soon after grafting (U.S. Department of Agriculture 1951). For *Chamaecyparis*, higher survival can be achieved when grafting with scion material from younger donor plants (M.G. McWilliams, unpublished data) than the material used here from mature trees, some of which were dying.

Typically, the foliage on the branches of the recipient sapling did not change appearance as a result of grafting. Of the 216 grafted branches, however, the distal portion on 11 branches died as a result of breakage at the graft. These were nearly equally distributed among the treatments, four each on the healthy and dying treatments, and three on the nearly dead treatment. Breakage at the graft union likely resulted when cuts made at the time of grafting were too deep and compromised the strength of the branch, causing failure during snow loading or strong wind. In addition, three branches were attacked by *Apostrasseria* sp., a fungal pathogen known to occur on yellow-cedar regeneration in the area (Hennon 1992). None of the 72 recipient saplings showed any evidence of general foliar discoloration or thinning throughout their crowns as might be expected if they had received a transmissible and systemic agent.

Grafting treatment had no detectable influence on diameter or height growth of recipient saplings (Table 1). Comparing the least-square means in analysis of covariance or excluding from analysis the saplings that had all three scions fail did not produce significant differences among treatments.

In conclusion, grafting scions from dying yellow-cedar trees to the branches of recipient saplings produced no visible symptoms of yellow-cedar decline and had no detectable effect on sapling growth. These results do not support the

Fig. 1. Frequency of scion color at the time of grafting (spring 1991) and at each measurement interval during spring (SP) and late summer (SU). Results are from 72 scions grafted onto 24 saplings for each of three treatments: scions taken from crowns of mature yellow-cedar trees that had no apparent symptoms of decline (A; healthy), were in early stages of dying (B; declining), or were in late stages of dying (C; nearly dead).

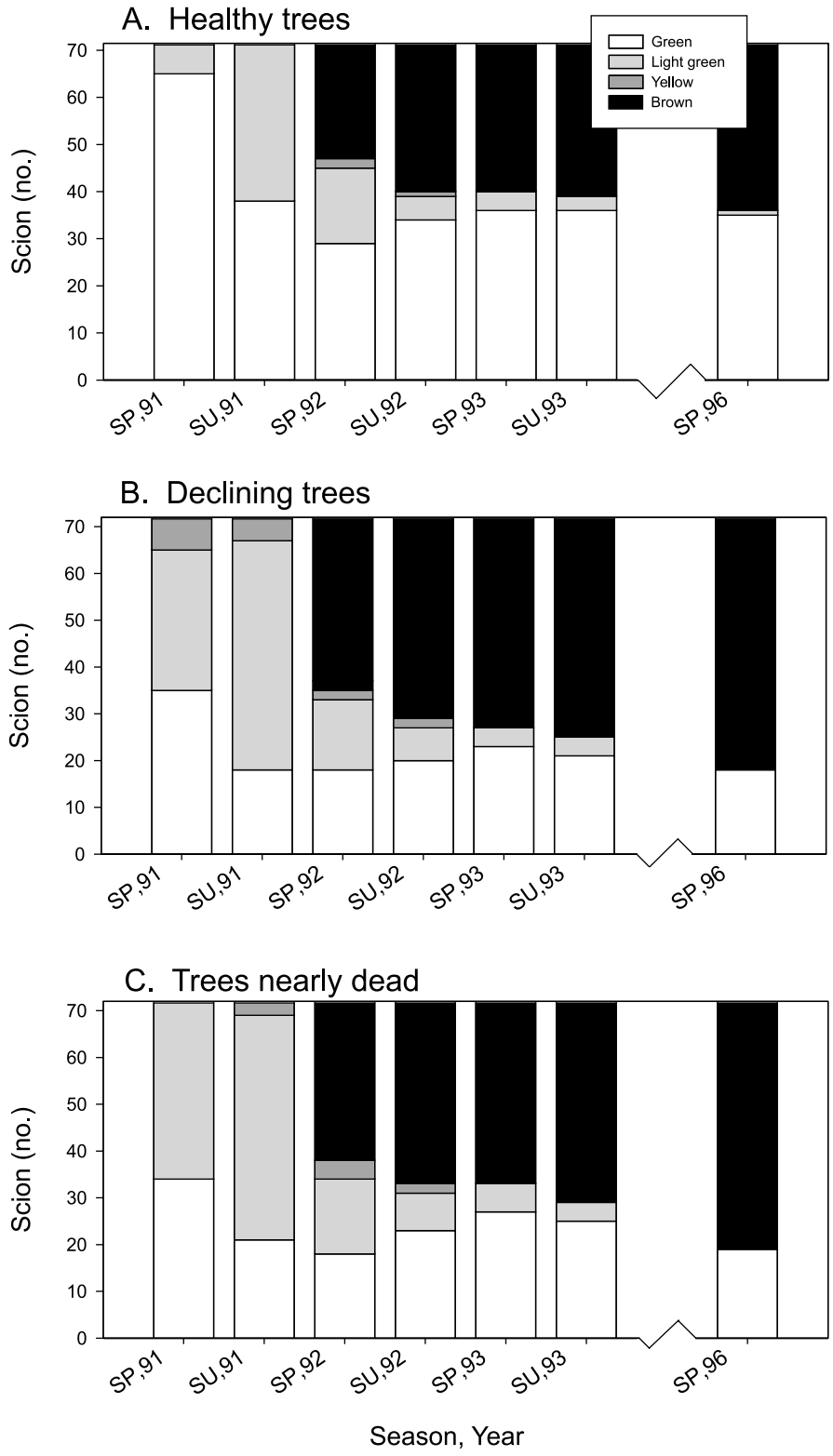

Table 1. Growth of yellow-cedar saplings in 5 years after receiving grafted scions from healthy, declining, and nearly dead mature, donor yellow-cedar trees.

		Diameter growth		Height growth	
Treatment	*N*	mm	%*	cm	%*
Healthy	24	46.0±1.9	267.2±10.2	176.7±7.3	228.0±6.4
Declining	24	44.5±3.3	267.0±10.4	179.1±12.6	236.7±18.1
Nearly dead	24	44.3±1.6	265.2±10.9	172.3±8.9	224.0±8.5

Note: Values for diameter and height growth are means ± 1SE. *N*, number of recipient saplings.
*Percent growth determined by subtracting final values from initial values and multiplying by 100.

hypothesis that a graft-transmissible agent is the primary cause of yellow-cedar decline.

Viruses may be more common or more concentrated in the roots of conifer trees than in other tissues (Nienhaus and Castello 1989; Jacobi and Castello 1992; Jacobi et al. 1992). Thus, attempted recovery and observation of viruslike particles in the roots of dying yellow-cedar trees (i.e., transmission electron microscopy) would be the next logical steps in a search for these types of pathogens. Detection methods such as baiting with *Chenopodium* plants (Fillhart et al. 1998), ELISA, or methods with greater specificity (Jacobi et al. 1998) could be attempted, because some virus pathogens do not incite obvious disease symptoms. Interestingly, recent work on viruses (Bachand et al. 1996) and phytoplasmas (Zhengmin and Zuoyi 1990) in conifers indicates that these agents alter susceptibility to freezing damage, a factor that we have hypothesized to be the primary causal factor in yellow-cedar decline (Hennon and Shaw 1997). More study is needed to conclusively evaluate whether or not a virus, phytoplasma, or some other biotic agent is responsible for the widespread forest decline in Alaska.

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