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Biological control of red alder using stem treatments with the fungus *Chondrostereum purpureum*

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Young red alder (*Alnus rubra* Bong.) trees were frilled at breast height and the frills inoculated with cultures of *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar in April, June, October, and in April of the following year. Over the ensuing 4 years, healing of the frills was less and tree mortality greater in trees inoculated with *C. purpureum* than in the uninoculated controls in the June and October treatments. Projected mortality for trees inoculated in June and October exceeded 80%. Among inoculation treatments, there was a strong correlation ($r = 0.816$) between percent tree mortality and the percentage of trees on which *C. purpureum* basidiocarps were recorded. It was concluded that summer or autumn inoculation with *C. purpureum* can be more effective in controlling red alder than frilling alone, and widens the treatment window for manual control.

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On a pratiqué des incisions en encoches groupées à hauteur de poitrine sur de jeunes aulnes rouges (*Alnus rubra* Bong.) et ces encoches ont été inoculées de cultures de *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar en avril, en juin et en octobre ainsi qu'en avril de l'année suivante. Au cours des 4 années qui ont suivi, la cicatrisation des encoches a été plus lente et le taux de mortalité plus élevé chez les arbres inoculés de *C. purpureum* que chez les arbres témoins non inoculés lors des traitements de juin et d'octobre. Le taux de mortalité prévu des arbres inoculés en juin et en octobre dépassait les 80 %. On a constaté une forte corrélation ($r = 0,816$) entre le pourcentage de mortalité des arbres et le pourcentage d'arbres sur lesquels des basidiocarpes de *C. purpureum* ont été notés d'un traitement d'inoculation à l'autre. On en est venu à la conclusion qu'une inoculation estivale ou automnale de *C. purpureum* peut être plus efficace pour réprimer l'aulne rouge que de simples incisions en encoches, qu'elle prolonge l'intervalle entre les traitements et qu'elle permet l'élimination manuelle des sujets indésirables.

Introduction

Hardwoods such as red alder (*Alnus rubra* Bong.), birches (*Betula* spp.), maples (*Acer* spp.), and poplars (*Populus* spp.) are increasing in economic value but will continue to require control on forest lands dedicated to production of softwood products as well as on utility rights-of-way and in thinned hardwood or mixedwood stands. On the Pacific coast, red alder is a primary invader of cutover areas and rights-of-way and suppresses conifer seedling growth on a variety of sites (Chan and Walstad 1987; Pendl and D'Anjou 1990). Use of chemical herbicides is not always permitted on forest land, especially in lowland areas and near streams, where these hardwoods are most vigorous. Manual cutting of hardwoods is often followed by rapid regrowth from basal stump sprouts and denser cover than that originally removed (Harrington 1984; Pendl and D'Anjou 1990). Girdling often fails because of wound healing and regrowth from adventitious shoots. Controls are needed that can be selectively applied to unwanted hardwood trees with minimal damage to crop trees (hardwood or softwood) and other nontarget species.

The fungus, *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar, has several features that make it attractive as a biological control for competing hardwoods. It has a broad host range but can be selectively applied through wounds without apparent damage to unwounded neighboring or adjacent trees (de Jong et al. 1990; Wall 1991). It is indigenous to Canadian forests (Fritz 1954; Thomas and Podmore 1953) and therefore can be treated as a naturally occuring microbial. When cultures of this fungus are placed on cut stumps of hardwoods, the cambium is often killed and adventitious growth usually prevented (Scheepens and Hoogerbrugge 1990; Wall 1990). The fungus is disseminated by short-lived basidiospores that are unlikely to pose a risk to nontarget trees (e.g., pruned fruit orchards) more than 0.5 km from sources of inoculum (de Jong et al. 1990).

In previous inoculation experiments with *C. purpureum* through patch grafts or axe wounds, suppression of wound healing and elongate cankers resulted (Wall 1991). It was apparent from these experiments that application of the fungus to complete frills could prevent cambial recovery and increase the likelihood of mortality in treated trees. The present study was conducted to determine the potential usefulness of this approach in the control of red alder.

Materials and methods

The study was established on southern Vancouver Island using local isolates of *C. purpureum* obtained from red alder during the previous 12 months. Isolate Cp46 was obtained from basidiocarps on a branch stub from a living tree near Sooke, and isolate Cp47 from basidiocarps on a dead alder stem near Port Renfrew, B.C. Both isolates were dikaryons obtained from germinated spores and cultured on malt agar Petri plates at 20°C and stored at 5°C. Inoculum of the fungus was prepared by growing the fungus on moist wheat bran in flasks incubated for 6 weeks at room temperature, and was applied as a thick slurry made by adding SAE 20 nondetergent motor oil immediately before treatment (Wall 1990).

The study site was located in the Sooke Highlands in the Leech River drainage system in southern Vancouver Island (48°31′N, 123°50′W, elevation 600 m) in the Coastal Western Hemlock biogeoclimatic zone. Soils are well-drained gravelly loams derived mainly from schist bedrock (Jungen 1985). The

TABLE 1. Percentage of trees with basidiocarps of *Chondrostereum purpureum*

Treatment	Time of treatment			
	April 1988	June 1988	October 1988	April 1989
Cpl	—	—	30	—
Cp46	—	85*	75*	—
Cp47	18	75*	70*	10
Control	10	16	10	10
Untreated check	9	—	5	0

NOTE: Based on observations made within a 4-year period after inoculation. Dashes indicate that the treatment was not done.
*Probability of a greater χ^2 less than 0.05 when compared with the control.

study area was a rectangular 0.4-ha block on a 5–10% slope located in a block that had been clear-cut and planted with Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Tree cover consisted entirely of Douglas-fir and red alder overtopping a ground cover consisting mainly of salal (*Gaultheria shallon* Pursh.) with scattered Oregon grape (*Berberis aquifolium* Pursh) and trailing blackberry (*Rubus ursinus* Cham. & Schlect). Both alder and Douglas-fir were about 10 years of age as determined by annual ring counts of increment cores collected from the base of five stems of each species. The alder trees occurred singly or in clumps and were associated with obviously suppressed Douglas-fir seedlings.

Within the study area, 265 healthy, codominant alder trees, 6–15 cm diameter at breast height (DBH), were numbered consecutively. Diameter at breast height was recorded for each tree and for 20–25 trees randomly selected for each treatment. Treatments (for a total of 13 experimental units) were as follows: one isolate of *C. purpureum* (Cp47) applied in late April (bud break), late June (full leaf), and early October (post leaf fall) of 1988, as well as early April (pre bud break) of 1989; a second isolate of *C. purpureum* (Cp46) applied in the June and October treatment periods; controls (oil slurries of sterile wheat bran) in all four treatment periods; and untreated checks (frilled only) in the April 1988, October 1988, and April 1989 periods (Table 1). At the time of treatment, trees were frilled at breast height throughout the full circumference with an alcohol sterilized hatchet, with the frills severing the cambium but not penetrating the xylem more than 0.5 cm. Inoculation or control treatments were applied to the frills with an alcohol-sterilized spatula immediately after frilling.

The study area was examined in the spring and autumn of each of the 4 years following treatment. Each tree was examined for the presence of basidiocarps of *C. purpureum* and evidence of mortality (crown deterioration, death of adventitious shoots, and discoloration of the cambium). The extent of healing of the frills was estimated as a percentage (0, no healing; 100, frills completely covered with callus growth). Percentages were transformed to arc sine percent values for analyses of variance, and inoculated and untreated-check treatments were compared with the controls using Dunnett's procedure (Zar 1984). The number of dead and dying trees, or the number of trees bearing basidiocarps of *C. purpureum* in a treatment were compared with the controls using χ^2-tests (Steel and Torrie 1980). The time of mortality, to the nearest 6 months, was also recorded, a tree being considered dead only when the cambium had become completely discolored, all adventious sprouts had died and there was no other evidence of aboveground living tissues. In the final tally (May 1993), healthy, living trees were given an arbitrary value of 10 years to mortality while dying trees were assigned a value of 6 years from inoculation to mortality. The Pearson correlation coefficient was used to determine the relationships among initial DBH, time of mortality, and wound healing as well as between percent mortality and percentage of trees on which *C. purpureum* basidiocarps had been recorded. Living trees were classified as healthy if they had full crowns and over 20% healing of the frills; or dying if crowns were broken, leaves discolored or reduced in size, or if frills were less than 20% healed over. To estimate time of 50% mortality due to a given treatment, percentages were converted to probits (Finney 1964) and plotted against time.

Results and discussion

The general sequence of changes in trees that died following treatment was as follows: bark discoloration and canker development above and below the frills, growth of adventitious shoots on living parts of the lower stem, fructification of *C. purpureum* on canker faces, increase in canker width until complete girdling occurred, yellowing of foliage, reduction in leaf size, reduction in crown density, death of adventitious shoots, discoloration of the bark and cambium throughout the tree, breakage of the main stem, and development of fructifications of secondary fungi (e.g., *Schizophyllum commune* Fr., *Coriolus versicolor* (L.:Fr.) Quel.) on dead stems. Complete cambial discoloration occurred very slowly in some trees, with patches of living bark and cambium often being found a few centimetres away from dead bark bearing basidiocarps of *C. purpureum*.

On trees that survived treatment, ridges of callus developed over the frills and the crowns remained intact. Adventitious sprouts developed below the frills on most trees and no differences in initial sprouting behaviour were evident among treatments or seasons.

Basidiocarps of *C. purpureum* appeared during prolonged rainy periods, mainly during winter. They occurred, for the most part, on inoculated trees (Table 1), but their occurrence on a few control and check trees indicated a certain level of natural infection. Relatively few were found on trees treated in April of either 1988 or 1989, suggesting poor fungus establishment in these two sets of treatments (Table 1). They were first observed less than a year after treatment in the trees inoculated in June of 1988, 1 year after treatment in the October inoculations, after 1.5 years in tree inoculated in April of either year, and 2 years later in the controls and checks. Basidiocarps were found almost entirely on dead and dying trees. There was a strong positive correlation (r = 0.815) between percent mortality after 4 years and the percentage of trees on which basidiocarps had been observed over the 4-year period. There was an even stronger correlation (r = 0.916) between percentage of trees that were dead and dying at the final tally and basidiocarp sightings. Of the trees on which *C. purpureum* basidiocarps had been observed before termination of the experiment, 95% were dead or dying in the final tally.

TABLE 2. Percent healing of frills on alder in relation to inoculation with *Chondrostereum purpureum*

Treatment	Time of treatment			
	April 1988	June 1988	October 1988	April 1989
Cpl	—	—	14.7	—
Cp46	—	0.7	2.7*	—
Cp47	2.6	1.4	2.6*	26.5
Control	1.1	3.6	33.1	22.7
Untreated check	12.4*	—	46.4	70.5*

NOTE: Data collected 4 years after treatment for each treatment date. Dashes indicate that the treatment was not done.
*Significantly different from the control at $p = 0.05$ using data transformed to arc sine percent values prior to analysis.

TABLE 3. Decline and mortality of red alder after frill inoculations with *Chondrostereum purpureum* cultures

Treatment	Cumulative percent mortality[a]				Percent dead and dying, final tally[b]
	1 year	2 years	3 years	4 years	
April 1988					
Cp47	5	23	36	46	73
Control	0	33	57	62	86
Untreated check	0	13*	13*	17*	52*
June 1988					
Cp46	20*	55*	60*	65*	85
Cp47	20*	55*	65*	75*	95
Control	0	21	32	42	74
October 1988					
Cp46	5	35*	45*	50*	80*
Cp47	5	45*	50*	65*	80*
Control	5	15	20	25	30
Untreated check	5	20	20	20	25
April 1989					
Cp47	0	10	15	25	45
Control	0	5	5	15	55
Untreated check	0	0	5	5	10*

[a]Mortality values are given for 1–4 years after inoculation.
[b]Final tally was taken in May 1993.
*Probability of a greater χ^2 less than 0.05 when compared with the control.

The extent of healing of the frills through formation of callus tissues is shown in Table 2. In the October treatments, there was significantly less healing in the inoculated trees than in the controls or checks. In addition, the April treatments (both years) showed an interesting pattern, with significantly more healing in the untreated checks than in the controls or inoculated trees. Evidently, a component of the oil–bran slurry applied to frills had some impact on wound healing.

Mortality attributable to *C. purpureum* inoculation could be seen in the June and October inoculations (75% and 65%, respectively, of the trees inoculated with isolate Cp47), but not in the April inoculations (Table 3). The October inoculations showed the greatest differences from the controls and checks. In the April inoculations (both years), fewer trees were dead or dying in the untreated checks than in the controls or inoculated trees, suggesting that the oil–bran mixture, rather than infection by *C. purpureum*, was the main cause of mortality (Table 3). In the June inoculations, considerable mortality associated with *C. purpureum* occurred in the second year (i.e., 13–24 months) after treatment, but in subsequent years many of the controls also died (Table 3). There were no significant correlations between original tree diameter and time of mortality ($r = 0.297$) or percent wound healing ($r = 0.139$) in the June and October inoculations or in any of the other inoculation, control or check treatments.

Times required to reach 50% mortality were estimated for further comparison of treatments. Inoculation with *C. purpureum* in June resulted in 50% mortality in less than 2 years as opposed to 4–5 years in the controls. In the October set of treatments, 50% mortality occurred in 2 years in inoculated trees in contrast to a projected time lapse of over 10 years in the controls and untreated checks.

The results show that inoculation of frilled red alder with *C. purpureum* during the summer and autumn can prevent wound healing and hasten mortality, thereby broadening the normally accepted June–August treatment window (Harrington 1984; Pendl and D'Anjou 1990) for manual control of this species. Treatment effectiveness could likely be further improved by additional seasonal testing. Because the fungus has been shown to vary in virulence (Ekramoddoullah et al. 1993), effectiveness as a mycoherbicide could also be improved by deliberate selection of virulent isolates. Optimization of culture conditions, culture media, adjuvants used with the inoculum, and application technology would further improve the practicality and effectiveness of using this fungus as a bioherbicide. In this study, the best treatments provided more than 80% control if dying trees noted at the end of the experiment were taken into account. This could translate into a sufficient reduction in alder cover to release suppressed conifers.

The slow death of inoculated trees and resultant gradual reduction in alder cover observed in these experiments might not appear satisfactory to some land managers. However, there may be silvicultural advantages to a slow release in terms of replacement of the alder cover by other competing species as often occurs after chemical control or felling.

The occurrence of *C. purpureum* basidiocarps on a few of the controls and untreated checks indicates that frilling alone might occasionally result in sufficient natural infection to achieve control. *Chondrostereum purpureum* is a common forest fungus and naturally occurring inoculum sources are widespread (Williams and Cameron 1956; Setliff and Wade 1973; Bishop 1979). However, sporulation of the fungus occurs only during prolonged wet weather when it may be difficult to perform silvicultural operations.

Failure of the early spring inoculations to cause substantial fungus establishment and to account for much of the mortality is not well understood. In earlier work, spring inoculations of yellow birch (*Betula alleghaniensis* Britton) and beech (*Fagus grandifolia* Ehrh.) resulted in less penetration and smaller cankers than summer inoculations (Wall 1991). In the April inoculations performed in the present study, there was similar evidence of poor fungus establishment. However, greater mortality in the inoculated and control treatments than in the untreated checks suggested that components of the inoculum, possibly oil, also had some bearing on the results.

The results of this and past studies indicate that inoculation with *C. purpureum* provides a useful improvement to manual or mechanical control of unwanted hardwoods. When a consistently effective product has been developed with this fungus, large-scale field tests on a variety of species and sites plus measurements of crop response are required as further measures of effectiveness.

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