Vesicular-Arbuscular Mycorrhizae of Western Redcedar in Container Nurseries and on Field Sites After Slash Burning

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Container-grown western redcedar (Thuja plicata *Donn ex D. Don) was virtually nonmycorrhizal when lifted from both Koksilah and MacBean Nurseries on Vancouver Island, British Columbia. After one growing season in the field on a variety of sites, most plants were colonized by fine and/or coarse vesicular-arbuscular mycorrhizal (VAM) fungi. Increased intensity of slash-burn produced a trend toward increased colonization by fine VAM and decreased colonization by coarse VAM fungi.* Tree Planters' Notes 44(1):33-37; 1993.

Western redcedar *(Thuja plicata* Donn ex D. Don) forms mutually beneficial root associations with fungi known as vesicular-arbuscular mycorrhizae (VAM) in bareroot nurseries (Berch et al. 1992), in pots inoculated with forest soils or VAM fungi (Kough et al. 1985, Parke et al. 1983 a&b), and in nature (Carpenter and Trappe 1970). There is no published information on the mycorrhizal status of western redcedar in containerized nurseries of British Columbia, although Castellano and Molina (1989) predict that susceptible conifer hosts, including western redcedar, will not form mycorrhizae when grown in completely artificial media like peat-vermiculite.

Although western redcedar may not need its mycorrhizal fungi in nurseries where ample water and nutrients are supplied, the time immediately after outplanting is critical to the survival and vigor of seedlings. Thus, it is important that they form mycorrhizae quickly on sites where nutrients or water limit growth. Nothing has been published on the early mycorrhizal colonization of western redcedar on clearcuts, so it is possible that nonmycorrhizal container-grown seedlings planted into clearcuts may suffer from a lack of VAM inoculum, particularly if the soil has been disrupted by site preparation techniques such as slash burning.

Two studies are described here. The objectives of both were to determine the VAM status of western redcedar produced by container nurseries in south coastal British Columbia and the changes in VAM status occurring in these seedlings after outplanting.

Materials and Methods

Study sites and sampling. Study A was carried out in 1987-1988 using seedlot Cw4511 produced in the MacBean Nursery of MacMillan Bloedel Ltd., Nanaimo, British Columbia. Seeds had been planted in February to March 1987 in peat-vermiculite in containers, and seedlings were lifted February 1988 and cold-stored for 1 to 2 months. They were then outplanted onto a flat site east of Duncan, BC, in the coastal western hemlock biogeoclimatic zone, moist maritime subzone (CWHmm, Nuszdorfer et al. 1985) at 670 m elevation. The site had received a low-impact prescribed burn in the spring before planting. The soil was a well-drained to rapidly drained Humo-Ferric podzol with upper mineral soil at pH 4.6 (H_2O extractant). Dominant vegetation before burning consisted of salal *(Gaultheria shallon* Pursh), Oregon-grape *(Berberis nervosa* Pursh), blueberry species *(Vaccinium alaskaense* Howell and *V. parvifolium* Smith) and fireweed *(Epilobium angustifolium* L.). Spotted cats-ear (Hypo*chaeris radicata* L.), woodland groundsel *(Senecio sylvaticus* L.), and western hemlock *(Tsuga heterophylla* (Raf.) Sarg.) were also common. Twenty-five young cedars were randomly sampled from Styroblocks before outplanting in March (when the seedlings were 12 to 13 months old) for assessment of VA mycorrhiza development and size in the container nursery. Twenty-five other seedlings were randomly sampled after one growing season in September by excavating entire root systems for assessment of VA mycorrhizal colonization and growth.

Study B was carried out on sites burned in 1985 as part of a cooperative MacMillan Bloedel/ Forestry Canada study on the effects of prescribed fire on forest nutrition and productivity. The four sites were located in Sproat Lake Woodlands Division of MacMillan Bloedel Ltd., west of Port Alberni, BC, in the CWHmm biogeoclimatic subzone. All sites are south aspects between 450 and 600 m in elevation. Vegetation prior to planting was similar to that listed for study A, except that spotted cats-ear, woodland groundsel, and western hemlock were not common. The soils were Orthic HumoFerric podzols with upper mineral soil at pH 4.5 to 5.1.

The four sites had received different site preparation before planting:

1. Unburned control
2. Low impact burn in spring 1985
3. Moderate impact burn in fall 1985
4. Severe impact burn in fall 1985

Sites 1 and 2 were adjacent; site 3 and 4 were at separate locations. The light bum consumed 25% of the forest floor by weight and exposed 4% of the mineral soil. The moderate burn consumed 47% of the forest floor by weight and exposed 29% of the mineral soil. The severe bum consumed 67% of the forest floor by weight and exposed 74% of the mineral soil. Further details of burn impacts are available (Beese 1992).

A total of 150 western redcedar seedlings (seedlot Cw7303, Koksilah Nursery, Duncan, British Columbia) were examined: 25 before cold storage (at the end of January 1986, 11 to 12 months after seeding); 25 after storage (mid-March 1986); and 100 at the end of the first growing season (November 1986, with 25 from each treatment: control, light impact, moderate impact, severe impact). Only 20 seedlings per treatment were examined for mycorrhizal colonization, as 5 seedlings per treatment were sacrificed for root weight measurements. All 25 seedlings from each treatment site were assessed for height and caliper and shoot dry weight.

Mycorrhizal colonization. For study A at all times, the entire root system was examined for mycorrhizal colonization, with the plug roots being assessed separately from the new roots. For study B at all times, the extent of VAM colonization was determined on approximately 2 g (wet weight) per seedling of cedar roots less than 1 mm diameter. For both studies, percentage root colonization was determined using the gridline intersect method (Giovannetti and Mosse 1980) after clearing in KOH and alkaline H_20_2 then staining in trypan blue (Kormanik and McGraw 1982). Based on presence or absence of VAM, the percentage of seedlings that were mycorrhizal was also determined.

Also for both studies, five to ten 1-cm-long colonized root segments per seedling were mounted on microscope slides and examined at 500 x to distinguish between coarse and fine VAM endophytes and other nonmycorrhizal root-inhabiting fungi. Coarse VAM endophytes have hyphae approximately 5 µm diameter, vesicles approximately 50 to 100 µm long, and spores generally 50 to 1000 µm long that form in the soil or in roots. This contrasts with fine VAM endophytes, which have hyphae that are 2 to 3 µm diameter, vesicles 5 to 10 µm long, and relatively small spores (10 to 15 µm long). Presence or absence of fine and coarse endophytes was recorded but the endophytes were not evaluated separately for extent of roots colonized.

Only in study B were some of the data analyzed statistically. Because data were not normally distributed for the adjacent unburned and low-impact burn sites, the difference in percentage colonization was analyzed using the Mann-Whitney U test (Siegel 1956). Data from the moderate-impact and high-impact burned sites were not included in the analysis because appropriate on-site controls had not been included in the experimental design.

Plant growth. Plant growth was assessed to ensure that plants were growing reasonably well. When outplanted seedlings were harvested, shoot height, caliper, and dry weight were measured. Root dry weight was determined by converting from fresh weight. For 5 seedlings per plot per treatment site, roots were weighed fresh, dried at 100 /C for 24 h, and then reweighed dry. From these data, a conversion factor was determined so that root fresh weight of the other seedlings could be converted to dry weight. Data from the control and low-impact burn sites were compared using the student t test (Snedecor and Cochran 1967).

Results and Discussion

In study A, post-storage redcedars were virtually nonmycorrhizal (table 1). In study B, only 10% of the pre-storage seedlings and 5% of the poststorage seedlings were mycorrhizal, and of these, the extent of mycorrhizal colonization in each seedling was low, with less than 1% of the roots colo-

Table 1 -*Summary of growth and mycorrhizal colonization for western redcedar before and after outplanting*

Treatment	Location*	Height (cm)	Caliper (mm)	Dry weight (g) Shoot	Root	Shootroot ratio	% of seedlings with VAM	% of live roots with VAM
Study A								
Post-storage	-	27.8	2.6	1.3	0.6	2.2	0	0
Low-impact fire	7c	32.5	3.9	2.9	1.7	1.7	60	8 (plug) 15 (new)
Study B								
Pre-storage	-	-	-	-	0.5 (25) a	-	10	<1 a
Post-storage	-	-	-	-	0.4 (28) a	-	5	<1 a
Unburned	C	38.3 (±4.4) a	3.6(± 0.5) a	2.7(± 0.7) a	1.1(± 0.4) b	2.8(± 1.1) a	95	22.3(± 13.4) b
Low-impact fire	C	40.5(± 4.9) a	4.2(± 0.5) b	4.1(± 1.5) b	1.2(± 0.4) b	3.7(± 0.8) b	95	31.5(± 18.4) b
Medium-impact fire	K	35.5	4.1	3.4	1.2	3.1	90	28.1
High-impact fire	M	35.3	4.4	4.0	1.5	2.9	85	17.4

Values are mean and (standard deviation). Means within columns not followed by a common letter differ at $P< 0.05$ using Bonferroni significance levels.
- = data not available.
*Location: 7c =Duncan, C = Cous, K = Kanyon, M = Mactush.

nized. These findings are in agreement with what Castellano and Molina (1988) had predicted of VAM-forming conifer seedlings grown in peatvermiculite potting mix in container nurseries. In contrast, in a bareroot nursery bed in southwestern British Columbia, western redcedar fine roots can be over 50% VA mycorrhizal (Berch et al. 1992). Most VAM fungi, notably those known as coarse endophytes, are not readily disseminated in air or water because their propagules consist of large spores formed in the soil or in roots, colonized root fragments, or hyphae attached to colonized roots. This contrasts with the situation for fine VAM endophytes, which have relatively small spores that may be capable of easier dispersal through air and water. Similarly, pines, firs, spruce, and most other North American conifers of commercial importance associate with ectomycorrhizal fungi, many of which are wind or water disseminated.

For study A, 60% of seedlings and for study B, 85% or more of seedlings were colonized after one growing season in the field (table 1). In study A, virtually all nonmycorrhizal seedlings from the MacBean Nursery had been colonized 6 months after outplanting and the mean percentage colonization was about 8% for roots in the original plug and 15% for roots that had grown out of the plug. These levels were slightly lower than those found in study B, which averaged from about 17 to 32%. In study A, plants were harvested 6 months after outplanting, while in study B, plants were harvested after 8 months. During the fall, root growth may slow, permitting the mycorrhizal fungi to colonize a greater proportion of the fine roots. The vegetation on site for both studies before burning included Oregon-grape, which is commonly VA mycorrhizal and the roots of which could serve as a source of inoculum for cedars planted after a relatively low-impact slash burn.

Since the unburned site was not adjacent to the moderate and severely burned sites, it cannot serve as a control for these two treatments and we cannot compare the sites directly. However, on the adjacent low-impact burn and unburned sites, 95% of the seedlings on both sites had formed VAM (table 1). Percentage colonization of roots was not significantly greater after the low-impact burn (32%) than on the adjacent unburned control area (22%) even though shoot caliper, shoot dry weight, and shoot-root ratios were significantly higher than those of the control. This suggests that the light burn had little or no effect upon VAM colonization but promoted plant growth (except root weight), perhaps through weed suppression or release of available nutrients. In study A, the cedars were planted on another site pretreated with a lowimpact burn. Although the extent of colonization was lower on this site (15% for roots outside of plugs) than the low-impact burn site in study B, this may have been due to the shorter time in the field.

Despite the lack of statistical analysis for the comparison of unburned and low-impact slashburned sites with the moderate-impact and highimpact slash-burned sites, some general trends were evident. Percentage of seedlings mycorrhizal showed a decreasing trend with increasing intensity of burn (table 1) in study B, assuming equal preburn levels of inoculum among sites. We also observed a trend toward a greater proportion of the mycorrhizal seedlings on the unburned site having only coarse endophyte VAM than on any of the burned sites (figure 1). The number of seedlings with only coarse endophyte VAM seemed to decrease as burn intensity increased. The high-impact burn had a greater proportion of seedlings with only fine endophyte VAM than any of the other sites, with a decreasing trend as burn intensity decreased. This suggests that burning may have an adverse impact on coarse endophyte VAM and a favourable impact on fine endophyte VAM, but this hypothesis would have to be tested.

The fine endophyte type is known to be most common on harsh sites, such as low-pH soils (Wang et al. 1985), high elevations (Crush 1973), and coal spoils (Daft et al. 1972). In a prefumigated nursery bed in southwestern British Columbia, bareroot western redcedar was colonized only by fine endophyte, whereas plants in the nonfumigated bed were colonized by fine and coarse VAM endophytes (Berch et al. 1992).

Recommendations

The lack of mycorrhizal colonization of western redcedar in peat-vermiculite mixes of containerized nurseries is of particular interest because it provides nonmycorrhizal controls that could easily be compared with seedlings inoculated with VAM fungi obtained from commercial sources or raised on host plants in pot culture. In contrast, ectomycorrhizal fungi spread easily in nurseries, and noninoculated plants often become mycorrhizal before the experiment is concluded, which confounds the interpretation of nursery-based experiments with ectomycorrhizal fungi. Thus, for studies of mycorrhizal fungi as agents of biocontrol of root diseases or as means of improving fertilizer efficiency under operational conditions, VA mycorrhizae would be a superior test system.

Observations from this work and the literature indicating that fine endophyte VAM may be more resistant to stresses, including severe burn, suggest some questions worthy of future study. Are propagules of the coarse endophytes more susceptible to burning, fumigation, and other stresses than those of fine endophytes? Are fine endophytes stimulated to sporulate by burning or other stresses? Are fine endophytes more rapidly dispersed to disturbed sites than coarse endophytes? Are fine and coarse VAM fungi equally capable of contributing to the growth of western redcedar?

The main conclusions of this study are that western redcedar remains virtually nonmycorrhizal in container nurseries in British Columbia but develops VAM on a variety of clearcut sites within one growing season of outplanting. The suggestion that fine VAM fungi may be found more commonly than coarse VAM fungi after high-impact slash burn would have to be examined more extensively. The fact that western redcedar seedlings produced in containerized nurseries are nonmycorrhizal when lifted would present a situation where inoculation with selected VAM fungi, whether fine or coarse, could be easily carried out with minimal risk of cross-contamination.

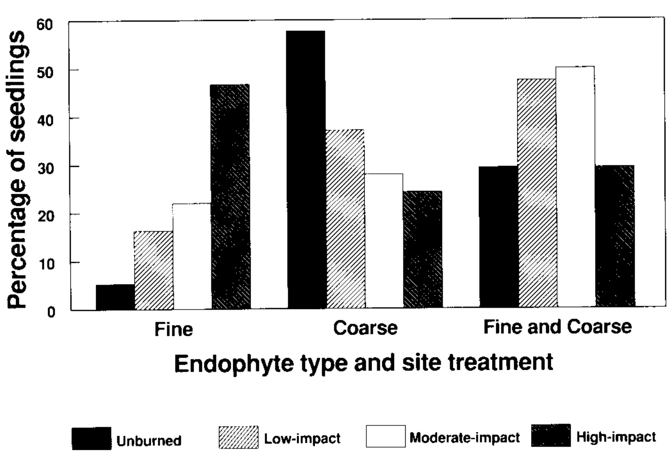

Figure 1—*Relative proportions of mycorrhizal western redcedar seedlings colonized by only fine, only coarse, or fine and coarse vesicular-arbuscular endophytes one season after outplanting on clearcuts having received different intensity slash burns.*

Acknowledgments

S. M. Berch was supported by an operating grant from the Natural Sciences and Engineering Research Council of Canada, A. Roth by Macmillan Bloedel, Ltd., and a G.R.E.A.T. Award from the Science Council of British Columbia, and W. J. Beese by Macmillan Bloedel Ltd., and the British Columbia Ministry of Forests, Vancouver Forest Region.

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