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Propagation and Growth of Pacific Yew (*Taxus brevifolia* Nutt.) Cuttings

Abstract

Vegetative propagation techniques for Pacific yew (*Taxus brevifolia* Nutt.) are little known and could be useful for the management of this long-lived understory species whether for conservation, habitat restoration or for the production of Taxol® (paclitaxel), a promising anti-cancer agent. In this study, cuttings from natural stands of Pacific yew on Vancouver Island were used to test three components of a successful vegetative propagation program; nursery culture, rooting success, and shoot growth and orientation of rooted cuttings. Cultural studies showed that Pacific yew cuttings could be effectively propagated but success rates may not meet expectations derived from studies of other *Taxus* species. Rooting studies showed that cuttings from male and female Pacific yew could be rooted equally successfully. No significant differences were found in rooting percentages of parent trees from 6 Vancouver Island populations. Clonal variation in rooting was significant within those populations (87.5% maximum, 6.4% minimum). Shoot growth of rooted cuttings increased from 3.4 cm in the first year after transplanting to 15.7 cm in the second year. All the cuttings had branch-like orientation. Results showed that a Pacific yew conservation program for the Vancouver Island Region based on vegetative propagation of representative genotypes is feasible.

Introduction

Pacific yew (*Taxus brevifolia* Nutt.), having little commercial value in the past, is one species of which little is known about successful nursery propagation techniques. Such information would be useful in the management of this long-lived species which grows slowly and is scattered in the understory of Northwest forests (Busing et al. 1995). In addition, recently Pacific yew has received greater attention due to the clinical success of Taxol® (paclitaxel), a promising anti-cancer drug extracted from the bark. This has raised concern about the exploitation of natural stands by harvesting and the consequent loss of genetic resources (Scher and Schwarzschild 1989). Due to problems with the acquisition and germination of Pacific yew seed (Rudolf 1974), vegetative propagation could be a feasible way to achieve conservation and restoration goals as well as to meet the demand for paclitaxel (Heinstein and Chang 1994). Vegetative propagation has been successfully used in a program of conservation of genetic resources of some medicinal woody plants in Nigeria (Oni 1993).

The success of a Pacific yew vegetative propagation program depends on whether male and female trees from a variety of geographic locations can be effectively propagated, grown and outplanted. But little is known about rooting cuttings of Pacific yew (Mitchell 1992) and studies of rooting in other *Taxus* species have concentrated on ornamental varieties (Eccher 1988). As a result, rooting success of cuttings from natural stands of Pacific yew may not meet expectations. Cultural studies that test rooting methods established for other woody species would be an important first step in the development of a management program based on vegetative propagation (Oni 1993).

Pacific yew is a dioecious species (Bolsinger and Jaramillo 1990) and differences in rooting capacity between male and female trees may reduce the probability of success in a vegetative propagation program. In *Taxus cuspidata* (Japanese yew), a greater percentage of cuttings from female trees rooted than from male trees (Davidson and Olney 1964). In some dioecious species, differences in physiological and morphological traits have been found between males and females (Dawson and Ehleringer 1993). If rooting capacity is one of those traits, vegetative propagation of one sex or the other may be problematic.

Increasing parent tree age and low growth vigor are other factors known to reduce rooting success (Meier-Dinkel and Kleinschmidt 1990, Roberts and Moeller 1978, Libby and Conkle 1966) and cuttings from some individuals may not root at all, particularly if they are over 100 years old (Brix 1974). Pacific yew cuttings from natural stands may be difficult to root because parent trees can often be over 195 years-old and tend to be slow-growing (Busing et al. 1995). Therefore, old age and low vigor may combine to reduce rooting success in cuttings of Pacific yew from natural stands. This would tend to compromise the feasibility of a conservation program.

There are also indications that rooting may vary among parent trees from different geographic locations (Ticknor 1969). However, the extent to which environmental factors affect tree vigor and thus rooting is not well understood (Abedini and Marlats 1988, Libby and Conkle 1966). Pacific yew is widely scattered in its distribution (Bolsinger and Jaramillo 1990, Taylor and Taylor 1981). It is found at elevations up to 800 m and over the entire length and breadth of Vancouver Island (Krajina et al. 1982), an area of great climatic and ecological diversity (Green and Klinka 1994). Therefore, propagation of Pacific yew from stressful (e.g. dry or cold) sites may be problematic as a result of poor shoot growth vigor of the parent trees.

Good shoot growth and upright shoot growth orientation of outplanted rooted cuttings will be important if they are to survive in natural stands, particularly on sites where competing vegetation is a problem for seedling establishment. Such sites are common in the Pacific Northwest (Herring and Etheridge 1976) and on Vancouver Island (Koppenaal and Mitchell 1992). Poor shoot growth is characteristic of Pacific yew in natural stands (Busing et al. 1995) and outplanting may not be successful if rooted cuttings also grow slowly. Branch-like form is frequently found in cuttings rooted from woody plants, particularly if the parent trees are old (Meier-Dinkel and Kleinschmidt 1990, Copes 1987, Rolund 1979, Black 1972). Because Pacific yew parent trees in natural stands can be over 195-years-old (Busing et al. 1995), branch-like form of rooted cuttings could be problematic for plantation establishment. In addition, poor shoot growth and branch-like form may lengthen the time needed to produce Pacific yew cuttings suitable for outplanting or hedging and reduce the feasibility of a vegetative propagation program (Thorpe and Harry 1990).

In this study, three elements of a successful program of Pacific yew propagation were investigated: (1) Cultural studies, (2) Rooting studies and (3) Shoot growth and orientation studies. The objectives were to establish basic cultural procedures for propagating Pacific yew and use them to determine whether cuttings from either male or female trees or from different aspects, elevations or latitudes would be difficult to propagate and grow successfully.

Materials and Methods

The following methods were used for both cultural and rooting studies. Branches were collected from the mid to lower crown of mature (bearing pollen or seed) Pacific yew (*Taxus brevifolia* Nutt.) found in natural stands on Vancouver Island. They were bagged in plastic and returned to the laboratory where they were stored at 2°C. All cuttings were made within 10 days of collection. Cuttings were set in a mixture of equal parts of sand, peat and perlite and placed in outdoor propagation boxes (Brix 1974) with bottom heat (20°C) to root. During rooting, high humidity was maintained and the medium was watered regularly.

Cultural Studies

Four basic cultural experiments were conducted. Because of a scarcity of suitable material for cuttings, each used different parent trees (=ortet). In any given experiment, parent trees were from the same geographic location. Equal numbers of cuttings (=ramet) were set in each treatment in each experiment. Rooting was assessed after 5 months by counting the number of cuttings with at least one root and expressing rooting as a percent of the total number of cuttings in that treatment. No statistical tests were conducted on the data. (1) Rooting was compared between cuttings treated with a basal application of Stimroot 3 (Plant Products, Bramalea, Ontario), that contained 0.8% IBA (indolebutyric acid) and untreated cuttings (two parent trees, 48 cuttings from each tree in each treatment). (2) Rooting of short (3 to 5 cm) and long (25 to 40 cm) cuttings were compared to determine the effects of cutting size (two parent trees, 40 cuttings in each treatment). (3) Effects of tree age were investigated by comparing rooting of reproductively immature (four trees) and mature (four trees) Pacific yew (48 cuttings from each tree in each treatment), and (4) Species variation in rooting was compared between cuttings from Pacific yew and English yew (*Taxus baccata* L.) (two parent trees of each species, 48 cuttings from each parent tree).

Rooting Studies

To assess variation in rooting between male and female trees, three sites were selected on which there were large trees of both sexes. Branches from one of the sites in the montane (800 m) on Vancouver Island were collected in October 1993. Cuttings were set in early November and assessed for rooting 6 months later. Experimental design consisted of four replicates of 150 cuttings (600 cuttings total) from each of three male and three female trees. Branches from the other two sites on southern Vancouver Island were collected in January 1994. Cuttings were set in early February 1994 and assessed for rooting 6 months later. Experimental design consisted of four replicates of 20 cuttings (80 cuttings total) from each of three male and three female trees from each location (6 trees). Rooting percentages (percent of the total number of cuttings in a replicate with at least one root) were transformed (Arcsin) for normality (Zar 1984). Comparisons were based on nested analysis of variance (Zar 1984) and used Tree(sex) as the error term. Planned contrasts (Mize and Schultz 1985) were made between male and female trees (SAS Institute, V6.0, Cary, NC). Means were separated using Tukey's Studentized Range test (HSD; $p<0.05$).

To assess geographic variation in rooting of Pacific yew, branches were collected from six natural stands on Vancouver Island chosen to represent wet and dry (west and east), cool and warm (north and south), and coastal and montane (low and high elevation) sites. Cuttings were set in late November and assessed 6 months later. Experimental design consisted of four replicates of 12 cuttings (48 cuttings total) from each parent tree at each location. Rooting percentages (percent of the total number of cuttings in a replicate with at least one root) were transformed (Arcsin) for normality (Zar 1984). Comparisons were based on analysis of variance (Zar 1984) in which locations were blocked and trees were nested within blocks. Tree(sex*block) was used as the error term. Planned contrasts (Mize and Schultz 1985) were made between north and south, east and west, and low and high elevation sites (SAS Institute, V6.0, Cary, NC). Means were separated using Tukey's Studentized Range test (HSD; $p<0.05$).

Shoot Growth and Orientation Studies

In March of 1994, rooted cuttings from 3 male (n=101) and 3 female trees (n=80) from a single Vancouver Island location (Iron River) were transplanted into styroblocks (PSB 615A, 45cc) in equal parts of peat, sand and vermiculite and placed in a shaded greenhouse. They were fed once weekly with 100 ppm 20-20-20 (Plant Products, Bramalea, Ontario) and watered as required. Height growth was measured in October of 1994 and 1995. Comparisons were based on nested analysis of variance (Zar 1984) and used Tree(sex) as the error term. Planned contrasts (Mize and Schultz 1985) were made between male and female trees (SAS Institute, V6.0, Cary, NC). Means were separated using Tukey's Studentized Range test (HSD; $p<0.05$).

Shoot growth orientation was assessed in October 1995 on the same two-year-old cuttings as were measured for height growth. A protractor was used to measure the shoot growth orientation (90° = vertical). Male and female trees were compared (Table 4) using nested analysis of variance (Zar 1984) with Tree(sex) as the error term (SAS Institute, V6.0, Cary, NC). Means were separated using Tukey's Studentized Range test (HSD; $p<0.05$).

Results and Discussion

Cultural Studies

Rooting hormone (IBA) increased rooting success from 30.6% to 50.0% (Table 1). Similar results have been found using IBA with a number of other yew cultivars (Eccher 1988, Van Hoff 1978, Von Kornya 1976, Wells 1961). The positive rooting response of Pacific yew cuttings to IBA treatment indicated that hormone treatment was important for a successful propagation program. It may even be essential to successfully

TABLE 1. Summary of rooting percentages (± s.e.) in cultural studies. Hormone treatment was 0.8% IBA. Juvenile trees were less than 1 m tall and did not have reproductive structures. Mature trees were more than 5 cm in diameter and bearing pollen or seed.

Experiment	Treatment	% Rooted
Hormone	+ IBA	50.0 (6.3)
	- IBA	30.6 (9.8)
Size	3-5 cm	55.7 (6.5)
	25-40 cm	50.6 (6.8)
Age	Juvenile	70.8 (6.2)
	Mature	48.6 (8.3)
Species	Pacific yew	45.8 (5.3)
	English yew	95.4 (4.2)

root cuttings from some parent trees (Loach 1988), as was found in Douglas-fir (Brix 1974). Long woody cuttings (37 to 42 cm) rooted successfully (50.6%) and at comparable percentages to short (3 to 5 cm) cuttings (55.7%). Successful propagation of long cuttings has also been found in hardwood shrubs (Wainwright and Hawkes 1988) and in *Taxus* (Bell 1975, Wells 1961). Therefore, long cuttings may be used to accelerate a propagation and outplanting program by producing large trees suitable for outplanting in one less year than if short cuttings were used (Bell 1975). This would also be of benefit in the establishment of hedges for paclitaxel production in nurseries.

Cuttings from juvenile trees rooted 70.8% while those from sexually mature trees rooted 48.6% (Table 1). This has been found for a number of other tree species (Meier-Dinkel and Kleinschmidt 1990, Morgenstern et al. 1984, Brix 1974). Rooting cuttings from juvenile trees may therefore provide a more efficient way to produce large numbers of trees for outplanting than if cuttings from mature trees were used (Thorpe and Harry 1990). In addition, field performance may be better in rooted cuttings from juvenile than mature trees (Ritchie 1991). The use of juvenile parent trees would therefore improve the feasibility of both restoration and paclitaxel production programs.

English yew cuttings rooted with higher success rates (95.4%) than cuttings from Pacific yew (45.8%)(Table 1). Interspecific differences have previously been found in rooting of various *Taxus* species and cultivars (Von Kornya 1976, Wells 1961). Therefore, published procedures for vegetative propagation of English yew (Mitchell 1992) may not work as well when they are applied to rooting Pacific yew cuttings. Nursery programs for paclitaxel production may also be more successful if yew species and cultivars other than Pacific yew are used. Many nursery trials have been done to improve rooting percentages of other woody species (Loach 1988) and great gains have been made through such trials (Mendel 1992). Similar experiments will be needed on Pacific yew cuttings if rooting is to meet expectations derived from previous studies of *Taxus*.

Rooting Studies

There were no significant differences in rooting percentages between male and female Pacific yew trees from the three sites selected to test for sex-specific effects (Table 2). In contrast, cuttings from female Japanese yew (*Taxus cuspidata*) rooted with higher success rates than those from male trees but differences were confounded by clonal effects (Davidson and Olney 1964). Significant clonal variation was also seen in this experiment and rooting percentages of individual parent trees ranged from a high of 49.1% to a low of 1.3% (Table 2). Since both male and female trees rooted with equal success, vegetative propagation could provide a viable means for the conservation of Pacific yew from natural stands.

Rooting percentages were not significantly different over the six Vancouver Island sites selected to test for effects of geographic location (Table 3). Rooting percentages varied among the sites from a low of 30.8% to a high of 66.0%.

TABLE 2. Rooting percentages of cuttings from male (M) and female (F) Pacific yews from three Vancouver Island sites.[1] Row Means (± standard error, se) followed by the same letter are not significantly different (Tukey's Studentized Range; HSD, $p \leq 0.05$). Maximum and minimum rooting percentages were selected from means of 6 parent trees at each site.

Site[1]	Sex	% Rooted (se)	Sex	% Rooted (se)	% Rooted Maximum	% Rooted Minimum
RR	M	15.8 (6.3) a	F	38.3 (4.8) a	46.3	1.3
RP	M	17.5 (3.9) a	F	19.6 (5.7) a	36.3	1.3
IR	M	34.5 (5.4) a	F	19.8 (3.3) a	49.1	6.9

Planned Contrasts	df	MS	F	Pr>F
Male vs Female (RR & RP)	1	0.3921	1.51	0.2536
Male vs Female (IR)[2]	1	0.2276	0.61	0.4801

[1]Abbreviations:RR, Royal Roads; RP, Rocky point; IR, Iron River.

[2]Iron River is tested separately because it is geographically different from the other twoVictoria area sites and because cuttings were set 3 months earlier than the others.

TABLE 3. Rooting percentages of Pacific yew cuttings from six Vancouver Island sites. Number of parent trees (N) from which maximum (Max) and minimum (Min) rooting percentages were selected. Means (± standard error, se) followed by the same letter are not significantly different (Tukey's Studentized Range; HSD, $p \leq 0.05$).

Site	Lat.	Elev.	N	% Rooted (se)	Max.	Min.
Harmac (HA)	49°08'	50m	3	66.0 (8.5)a	87.5	31.2
Haslam Creek (HC)	49°03'	50m	3	59.0 (7.6)a	87.5	6.4
San Juan Creek (SJ)	48°40'	600m	9	59.7 (3.4)a	75.0	25.0
Rooney Lake (RL)	50°21'	350m	2	42.7 (8.7)a	60.4	25.0
Jordan River (JR)	48°32'	150m	3	34.9 (7.5)a	65.0	14.6
Tsitika (TS)	50°14'	400m	6	30.8 (3.6)a	43.8	14.2

Planned Contrasts	df	MS	F	Pr>F
[1]North vs South (cool vs warm)	1	0.5940	2.76	0.1122
[2]High vs Low (montane vs coast)	1	0.0096	0.04	0.8438
[3]East vs West (dry vs wet)	1	0.1829	0.85	0.3674

[1]RL,TS vs JR,HC,HA; [2]SJ vs JR,HC,HA; [3]SJ,JR vs HA,HC,TS

Planned contrasts between wet and dry sites, cool and warm sites, and coastal and montane sites showed that there was no significant effect of geographic location or elevation on rooting despite potential climatic affects on shoot growth and vigour of parent trees. In Eucalyptus, different ecotypes also had rooting percentages that were not significantly different (Abedini and Marlats 1988). Individual Pacific yew parent trees had rooting percentages as low as 6.4% and as high as 87.5%. This could be the result of parent trees being different ages. Increasing age of parent trees has been found to decrease rooting success and increase clonal variability (Meier-Dinkel and Kleinschmidt 1990, Thorpe and Harry 1990, Black 1972) perhaps through its effects on tree vigour (Roberts and Moeller 1978). High variation in rooting among clones has also been observed in *Larix laricina* (Morgenstern et al. 1984) and in Douglas-fir (Brix 1974). As a result, large numbers of cuttings from parent trees across a broad range of sites may be required for a successful conservation program, as suggested by Wheeler et al. (1995). This may limit the number of rooted cuttings that can be produced in a reasonable time (Thorpe and Harry 1990).

Shoot Growth and Orientation Studies

Pacific yew cuttings grew an average of 3.4 cm in the first year after propagation, but in the second year, height growth increased over four-fold to an average of 15.7 cm (Table 4). No significant differences in growth were found between rooted cuttings from male and female trees in either the first or second year after rooting. Clonal variation in growth was significant, particularly in the second year after rooting when the best clone grew 31.2 cm and the poorest grew 9.7 cm. This is in contrast to Pacific yews in natural stands where shoot growth tends to be poor (Busing et al. 1995). There are also indications that rooted

TABLE 4. Height growth (cm) of rooted cuttings from male and female trees from Iron River in the first (1994) and second (1995) years after rooting. Column means (± s.e.) followed by the same letter are not significantly different (Tukey's Studentized Range; HSD, $p \leq 0.05$).

Sex	Parent Tree	Height growth (cm) 1994	1995
Male	1	1.7 (0.2) a	9.7 (0.7) a
	11	4.5 (0.5) b	31.2 (2.6) b
	9	3.4 (0.4) b	11.8 (0.9) a
Female	2	2.5 (0.3) a	14.8 (1.4) a
	4	4.3 (0.4) b	10.2 (1.4) a
	7	4.1 (0.6) b	16.7 (1.4) a
Male (Average)		3.2 (0.4) A	17.5 (1.6) A
Female (Average)		3.6 (0.5) A	13.9 (1.4) A

TABLE 5. Shoot growth orientation (vertical=90°) of two-year-old Pacific yew rooted cuttings. Means (± s.e.) followed by the same letter are not significantly different (Tukey's Studentized Range: HSD, $p \leq 0.05$).

Site[1]	Sex	Age	Parent Trees	No. of Cuttings	Orientation
IR	Male	2	3	101	55.8° (2.4) a
	Female	2	3	80	52.4° (1.5) a

[1]Abbreviation:IR; Iron River

cuttings may not grow as well as seedlings (Ritchie and Long 1986). Planting trials will be needed to test whether good growth rates in the nursery will be carried to the field. All the rooted cuttings had branch-like form (Table 5). No significant differences were found between cuttings from male and female trees and the average angle of shoot growth was 54.1° from horizontal. Branch-like form is not uncommon in cuttings rooted from woody plants, particularly if the parent trees are old (Meier-Dinkel and Kleinschmidt 1990, Copes 1987, Black 1972). This condition would tend to compromise the survival of cuttings planted on sites where competing vegetation is a problem for seedling establishment. Such sites occur frequently in the Vancouver Island Region (Koppenaal and Mitchell 1992). An upright form may be regained in time (Thorpe and Harry 1990, Rolund 1979) and it may be possible to promote it by staking the trees.

Summary and Conclusions

Cultural studies

Pacific yew can be propagated using published techniques for other *Taxus* species but expectations of rooting success may not be met. Further experiments are needed to increase rooting percentages of Pacific yew so that sufficient numbers of rooted cuttings can be produced for a successful conservation program.

Rooting Studies

Cuttings from male and female trees rooted equally well and cuttings from all parent trees from 6 sites on Vancouver Island were successfully rooted. This is an essential element of a vegetative propagation program aimed at genetic conservation and habitat restoration. Significant clonal variation in rooting percentages indicated that some parent trees may be difficult to root and implied that large numbers of cuttings from each tree would be needed for a viable vegetative propagation program.

Shoot Growth and Orientation Studies

Rooted cuttings grew well in the nursery but two years were required to produce trees tall enough to plant. All the clones had branch-like shoot growth orientation and this may be problematic for survival and growth during the outplanting phase of a Pacific yew conservation program. Field trials of Pacific yew rooted cuttings are needed to test whether the good growth rates achieved in the nursery will continue and whether branch-like form is persistent.

The success of vegetative propagation and subsequent good growth rates of rooted cuttings from Pacific yew indicated that genetic resources of natural stands could be conserved using that approach. Studies are now under way to determine the tolerance of Pacific yew to exposure and drought and to measure the success of outplantings on disturbed sites. Those studies will provide further indications of whether a Pacific yew management program based on vegetative propagation is viable.

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