Comparing Biomass and Taxane Concentrations to Maximize Yield in Rooted Cuttings of Pacific Yew (*Taxus Brevifolia* Nutt)

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Abstract

Optimizing yields of important taxane compounds in yews under cultivation is a function of the genetic variation among individual genotypes in taxane concentration and vegetative growth response to hedging. This study examined yew clones grown from cuttings, their foliar biomass and regrowth after harvest in relation to their foliar taxane concentrations, and compared the performance of Pacific yew (*Taxus brevifolia* Nutt.) to an ornamental yew hybrid (*Taxus* x *media* Rehd.). To investigate the variation in these components, branch tips for cuttings were collected from an ornamental yew shrub, and nine Pacific yew trees. Cuttings were rooted in an outdoor rooting house then transplanted to raised beds. After three growing seasons in the raised beds, the clones were hedged for foliar biomass at one- and two-year intervals. Total taxanes, taxol, 10-deacetylbaccatin III and baccatin III concentrations for each clone were determined when biomass was first harvested. The highest yielding clones generally ranked in the top half in total taxane and taxol amount per clone over all years but not necessarily in growth of biomass. Taxane concentration appeared to be more important than biomass accumulation for optimizing yields. This study suggests that *T. brevifolia* could be managed through selection, propagation, and cultivation of high taxane-yielding genotypes as a sustainable source of these important compounds.

Keywords

yew propagation, yew cultivation, taxol

Introduction

Pacific yew (*Taxus brevifolia* Nutt.) is a long-lived gymnosperm native to the Pacific Northwest forests ranging from northern California to coastal Alaska and as far interior as the northern Rockies. In the early 1990s this species was harvested for its bark, the primary source of Taxol® (paclitaxel) a diterpene found in higher quantities

in the bark than in the foliage. Because of the positive clinical results, taxol was approved by the FDA for use in the treatment of breast and ovarian cancer (Croom 1995). Between 1991 and 1993 several million pounds of bark were collected from wild trees harvested on public and private lands.

A process was developed in the early 1990s that could sythesize taxol through esterification of taxol congeners, baccatin III and 10-deacetyl baccatin III (Holton et al. 1995). These terpenes were more plentiful in the foliage; thus, foliage, a renewable resource, became a viable source of taxol. In 1993 the foliage of English yew (*T. baccata* L.) and Himalayan yew (*T. wallichiana* Zucc.) trees harvested in central Europe and India became an important alternative source for taxol (Defuria and Horovitz 1993). Unfortunately, increased habitat disturbance, illegal trade and excessive collection of bark and foliage of *T. wallichiana*, resulted in acceptance of an Indian proposal to have it listed in Appendix II by the Convention on International Trade in Threatened and Endangered Species (CITES). Under this listing, treaty nations monitor and control species potentially threatened by trade (Robbins 1997). Ornamental yew shrubs native to Europe and Asia, primarily English yew, Japanese yew (*T. cuspidata* S. and Z.), and a number of the hybrid x media cultivars have been cultivated for centuries and were selected in part for being able to respond well to hedging and pruning. The ornamental yews have provided what may be a sustainable source of foliar material for the semi-synthesis of taxol (Croom 1995). Biomass production of cultivated yews has the added benefit of reducing harvest pressure on wild trees.

Several studies have demonstrated that Pacific yew can be cultivated from seed or cuttings in a nursery setting (Wheeler et al. 1995, Mitchell 1997); however no study has documented if Pacific yew could be economically cultivated and grown in plantations for foliar biomass production through repeated hedging or pruning. In a common-garden study, Wheeler et al. (1995) reported the results of isozyme, taxane and growth analysis on Pacific yew seedlings grown from seed sampled throughout most of its geographic range. Wheeler et al. (1995) found that variation in growth and bud flush traits occurred within populations spread over relatively short distances and that taxane concentrations are widely variable. Wheeler et al. (1995) concluded that this species offers potential for genetic selection and mass propagation to develop improved taxane and biomass production. However, the study did not examine the species' response to hedging and regrowth. We previously found considerable variation in concentrations of taxanes among Pacific yew trees growing under cultivation or in the wild (Kelsey and Vance 1992, Vance et al. 1994). To investigate how hedging affects biomass production and taxane yields, this study compared foliar biomass and foliar taxane concentrations and yields among hedged clones grown from rooted cuttings that were collected from nine Pacific yew trees and one yew hybrid *T.* x *media* Rehd. (*T. baccata* x *T. cuspidata*), an ornamental shrub.

Methods

Preparation and Rooting of Cuttings

Material for rooted cuttings was obtained from three T. brevifolia trees growing in the central Cascades on the Willamette National Forest between 1200 and 1300 m (CAS1-3), and from six trees in the McDonald-Dunn Research Forest in the foothills of the Coast Range near Corvallis, Oregon (CST1-6). Additional cuttings were obtained from ramets of an ornamental yew hybrid, T. x media (TXM) growing on the grounds of the Forest Service Forestry Sciences Laboratory in Corvallis. Vigorous and healthy branches were collected in late autumn 1990 and cold-stored at about 4°C. Cuttings were prepared by clipping the branchlets 8 to 10 cm from the tip, usually below the first branching junction. The bare stem base of the cuttings was immediately dipped in a saturated Captan drench, then into a 10,000 ppm IBA solution (10 g IBA to 500 ml of 95% ETOH brought to 1 liter with H_2O) and promptly stuck in an outdoor rooting bed. A block of 10 randomized rows was replicated 8 times with each row in each block composed of a clone of 13 cuttings. The rooting bed consisted of a 2:1 (v:v) sphagnum peat- fine sand mix. Stems of cutting were inserted into dibbled holes about 4 cm deep. The rooting house, was equipped with overhead misters for maintaining moist cuttings and media, and underground heat set at about 20-

22°C (Copes 1983).

Harvested Biomass Measurements

The cuttings were stuck in December 1990. Onset of rooting varied among clones but most clones had begun to root by February 1991. In May 1991 the rooted cuttings were tallied, lifted and transplanted in raised outdoor beds near the laboratory at a spacing of 16 x 20 cm in a randomized design. In November 1993, after three growing seasons in the raised bed, foliage of each of the transplanted clones, which were the size of small shrubs, was harvested by clipping all stems and branches 15 cm from the base of the plant. The harvested stems and branches of each individual clone were clipped into small pieces, placed in a paper bag, dried at 65°F and weighed to the nearest 0.01g. Needles and stems were weighed as a single sample. After one full growing season (1994), a subset of the clones (approximately half the clones) were systematically selected so as not to introduce bias, their foliage (needles and stems) harvested and weighed as described above. These same individuals were re-harvested following a successive growing season (1995). The remaining clones constituted a second subset that was allowed to grow in the bed over two consecutive growing seasons (1994 and1995) before harvesting.

Taxane Analysis

Ten individual clones replicated twice (two ramets per clone) were selected for taxane analysis (20 individuals total). The analysis was performed as described in Kelsey and Vance (1992) and Vance et al. (1993) except stems were included with needles. Separation, identification and quantification of the taxanes were by HPLC with a UV detection system using taxane standards. Concentrations were calculated and reported as percent dry weight.

Statistics

Statistical analysis of log transformed data was by one-way ANOVA. Means were compared by Fischer's protected LSD. All analyses and tests including tests of variance were performed using Statgraphics software.

Results

Cuttings from all 10 yew clones rooted. The Pacific yew cuttings varied greatly in rootability (Table 1). The percentage of live cuttings that rooted ranged from 12.8 to 81.0%. Although there was no apparent difference in rootability between the Cascade and Coast Range yews, the ornamental yew was the earliest to root (personal observation), had the greatest number of live cuttings, and the highest rooting percentage (81%), thus, produced the greatest amount of rooted cuttings. The rooting percent significantly correlated in a linear model (r^2=0.42, p=0.042) with the accumulated biomass harvested three growing seasons after yews were transplanted into the outdoor growing bed (Cut1, Table 2.).

The clonal differences in mean foliar biomass from each of the harvests (Cut 1, Recut 1A, Recut 1B, and Recut 2) were significant. Mean foliar biomass accumulated by the first harvest (Cut 1) ranged from 11.81 g (CAS1) to 41.36 g (CST3). Three coastal yews (CST3, CST2, CST5) and the ornamental yew (TXM) had the highest amount of biomass from the first hedging. A significant difference was detected in mean biomass harvested (Cut 1) and the biomass that accumulated one year later (Recut 1A). The mean foliar biomass collected one growing season later decreased for all Pacific yew clones, but not for TXM. The mean biomass for all clones (Recut 1A) was not significantly different from that of Recut 1B, the succeeding year's harvest. The combined mean biomass of the two annual harvests (Recut1A+1B) was significantly less than the mean biomass harvested after two growing seasons (Recut 2). TXM was not significantly higher in biomass at the first harvest (Cut 1) but under successive harvests (Recut1A, Recut

Table 1. Rooting percent of cuttings from nine *Taxus brevifolia* trees (clones) and one hybrid yew, *T.* x *media* stuck in Fall 1990 and tallied in early Spring 1991. n = number of live cuttings.

Clone	n	Rooting %
CAS1	78	12.8
CAS3	91	15.4
CST1	86	32.6
CST6	101	33.5
CST5	81	34.6
CST4	84	36.5
CST2	97	41.2
CST3	91	49.4
CAS2	54	75.9
TXM	104	81.0
Mean		41.3

Table 2. Foliar biomass in g dry wt. of yew clones clipped 15cm from base of plant. Different letters in each column signify significant differences among clones. Cut1 is first biomass harvest 2 seasons after planting; Recut 1A is reclipped biomass of all clones following 1 growing season; Recut 1B is biomass of same subset of clones clipped the following year; Recut 1A+1B is the sum of the mean biomass of 1A and 1B; Recut 2 is the mean biomass of clones clipped after 2 years' growth.

Clone	Cut 1	Recut 1A	Recut 1B	Recut 1A+1B	Recut 2
CAS1	11.81 a	8.09 a	11.01 ab	19.10	47.32 abcd
CAS3	13.11 ab	10.11 ab	10.20 a	20.31	30.64 a
CST4	18.10 abc	9.78 a	16.82 bcd	26.60	34.87 ab
CST1	20.17 bcde	13.01 abcd	17.18 abcd	30.19	70.89 cd
CST6	25.95 cde	16.68 bc	19.35 cd	36.03	50.88 bcd
CAS2	26.05 bcd	25.20 cd	24.52 d	49.72	61.36 cd
CST5	32.45 de	19.70 cd	14.69 abc	34.39	62.40 cd
CST2	38.75 de	22.67 cd	27.77 de	50.44	55.79 abc
TXM	39.14 e	39.81 e	44.23 e	84.04	170.96 e
CST3	41.36 e	24.74 d	22.83 cd	47.57	81.94 d
Mean	26.69[1]	18.98[2]	20.86	39.84[3]	66.71

[1]Paired t test of unequal means between Cut 1 and Recut 1A; p=0.003.
[2]Paired t test of unequal means between Recut 1A and Recut 1B; n.s. p=0.141.
[3]Paired t test of unequal means between Recut 2 and Recut 1A+1B; p=0.007.

1B, and Recut 2), TXM with the exception of CST2 (Recut 1B), was significantly higher than the Pacific yew clones in amount of biomass accumulated. Clones of TXM accumulated a biomass of 170.96 g, which was the greatest increase in biomass after two years without hedging (Recut 2). The mean biomass of TXM ranged from 5.6 times that of CAS3 (30.64 g) to 2.1 times that of CST3 (81.94 g), the second highest producer of biomass.

Differences among clones in concentration of commercially important taxanes 10-deacetylbaccatin III, baccatin III and taxol were significant (Table 3.). The concentration of 10-BAD ranged from 0.0023 to 0.0089. The clones TXM and CST2 were among the highest in concentration of 10-DAB. Taxane yield (amount of taxane per clone) equals the concentration of taxanes in percent dry weight multiplied by the mean biomass (values taken from Cut1). The CST2 and TXM clones were the highest in yield of 10-DAB, and also among the highest in biomass. The hybrid TXM was significantly lower in BAC concentration than all nine of the Pacific yew clones; TXM was also the lowest in yield. Only CST4, which had the highest taxol concentration, was significantly different from CST6 which was lowest in taxol concentration. The mean biomass of CST6 was 25.95, about midpoint among the clones, but CST4 was not significantly different than the lowest ranked clone in mean biomass (Table 2). Taxol yield was lowest for CST6 and highest for CST4. The total taxane concentrations ranged from 0.0344 to 0.1511% dr wt. The difference among clones in total concentration was significant. The hybrid TXM was significantly lower that the Pacific yew clones in total taxane concentration, but CAS1 was the lowest ranked clone in yield.

Discussion

The hybrid yew clones demonstrated traits that are important to propagators maintaining pure lines of cultivars in commercial nurseries, as they rooted quickly and produced the greatest number and percent of rooted cuttings. However, the particular yew shrub from which rooting material had been taken, had been periodically hedged for several decades which promotes rootability through rejuvenation and reinvigoration of tissue (Copes 1983). This study would not be able to ascertain between the cultural or genetic contribution to these traits. The foliar biomass of three Pacific yew clones (CST5, CST2 and CST3) were comparable to the TXM in 1993 when biomass was collected for the first time (Cut 1). The harvested biomass of CST3 was almost 3.5 times that of CAS1 in 1993 and 2.7 time that of CAS3 in 1995 indicating a range of variation that can provide potential for selecting desirable genotypes. In 1993, the mean foliar biomass of several Pacific yew clones was equivalent to that of TXM, but by the end of the second growing season in 1995 (Recut

Table 3. In ascending order, mean concentrations in % dry wt of 10-Deacetylbaccatin III (10-DAB), Baccatin III (BAC), Taxol and total Taxanes; and yield shown as taxane amount per clone (mg/Cl) based on the product of mean taxane concentration (% drwt) and biomass (g) of first harvest (Table 2 Cut1).

Clone	10-DAB[1] % drwt	Clone	10-DAB mg/Cl	Clone	BAC % drwt	Clone	BAC mg/Cl
CAS3	0.0023 a	CAS3	0.3015	TXM	0.0011 a	TXM	0.4110
CST4	0.0043 ab	CAS1	0.6968	CAS3	0.0298 b	CAS1	3.8910
CST3	0.0053 bc	CST4	0.7873	CST4	0.0325 b	CAS3	3.9002
CAS1	0.0059 bcd	CST1	1.7044	CAS1	0.0330 b	CST4	5.8825
CST5	0.0072 bcde	CST6	2.0110	CAS2	0.0340 b	CST1	8.3605
CST6	0.0077 cde	CAS2	2.1101	CST2	0.0355 bc	CAS2	8.8700
CST2	0.0081 cde	CST3	2.1714	CST5	0.0369 bc	CST6	11.8330
CAS2	0.0081 cde	CST5	2.3360	CST1	0.0415 bc	CST5	11.9578
CST1	0.0084 de	CST2	3.1390	CST3	0.0415 bc	CST2	13.7756
TXM	0.0089 e	TXM	3.4640	CST6	0.0456 c	CST3	17.1644
Clone	Taxol % drwt	Clone	Taxol mg/Cl	Clone	Total % drwt	Clone	Total mg/Cl
CST6	0.0021 a	CST6	0.3110	TXM	0.0344 a	CAS1	11.3910
TXM	0.0024	CAS1	0.6440	CAS1	0.0965 b	TXM	13.4450
CAS1	0.0054	TXM	0.9200	CST2	0.1003 b	CAS3	16.0400
CST3	0.0057	CAS3	1.0490	CAS3	0.1224 bc	CST1	26.4830
CST2	0.0062	CST1	1.8350	CST3	0.1246 bc	CST4	27.3400
CAS2	0.0075	CAS2	1.9540	CST5	0.1257 bc	CAS2	37.4470
CST5	0.0080	CST3	2.3780	CST1	0.1313 bc	CST6	37.6660
CAS3	0.0080	CST2	2.3830	CAS2	0.1438 bc	CST2	38.8660
CST1	0.0091	CST5	2.5800	CST6	0.1452 bc	CST5	40.7730
CST4	0.0154 b	CST4	2.7870	CST4	0.1511 c	CST3	51.5140

[1] Means in column followed by a letter indicate significant difference ($p<0.05$).

2), the data indicate that TXM had accumulated significantly more harvestable biomass. By contrast, within the *T. brevifolia* group, the ratio in mean biomass between the highest and lowest clone declined from 3.5 to 2.7.

Foliar biomass collected from two annual hedgings clearly yields less than that collected from a single, bi-annual hedging. Interestingly, the effect was just as strong on the lowest biomass producer (CAS1) as it was on the higher producers (TXM and CST3). It may be that the narrow spacing of the rooting bed led to competitive effects among the clones. Other spacing and growing environments might improve the response to hedging. Pre-hedging trees for several years before collecting cuttings for propagation and cultivation could also be effective for enhancing rooting (Copes 1983).

The yield per clone is a function of the percent taxane concentration on a dry weight basis (g/g), and mean biomass (g) per clone. This may shift the order in which the productivity of clones is rated. Even though TXM ranked among the highest in biomass, it ranked among the lowest in BAC yield. The TXM and CST1 clones, among the highest producers of biomass in 1993 and also the highest in 10-DAB concentrations, yielded the

greatest quantity of this taxane. The hybrid TXM clones ranked among the lowest in concentrations of BAC, taxol and total taxanes. The difference was sufficient to place TXM among the lowest ranked clones in yield of those compounds, despite its high accumulation of foliar biomass. The clone CST3 was a good performer, placing in the top half in yield for 10-DAB and taxol and at the top for BAC and total taxanes. CST3, had the third highest rooting percentage, the highest biomass at the time of the first harvest, and the second highest biomass after two years. This suggests that variation in taxane concentration may have more weight than the differences in accumulated foliar biomass, but that selection for high performers in taxane concentration and biomass is possible. Potential to improve yield is possible through genetic selection by selecting the genotypes that produce the highest quantities of foliar biomass among the clones that produce the highest concentrations of targeted taxanes.

Conclusions

The study demonstrated that in the growing environment of a narrowly spaced planting bed, annual hedging for biomass is not as likely to produce a sustainable harvest as a two-year hedging cycle. *T. brevifolia* clones appeared to respond more poorly to the annual hedging treatment than did the ornamental yew clones. *T. brevifolia* clones varied widely in foliar taxane concentrations even among the small number selected over a limited geographic area for this study. Variation in rootability and foliar biomass accumulation and recovery, and in the concentration of important derivable taxanes can be used to select for high yielding genotypes, and could have potential for sustainable taxane production while conserving the *T. brevifolia* species.

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