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Nicholas C. Wheeler, Keith Jech, Susan Masters, Susan W. Brobst, A. Belinda Alvarado, Andrew J. Hoover, and Kenneth M. Snader

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# EFFECTS OF GENETIC, EPIGENETIC, AND ENVIRONMENTAL FACTORS ON TAXOL CONTENT IN *TAXUS BREVIFOLIA* AND RELATED SPECIES

NICHOLAS C. WHEELER,* KEITH JECH, SUSAN MASTERS,

*Weyerhaeuser Research Center, 505 North Pearl St., Centralia, Washington 98531*

SUSAN W. BROBST, A. BELINDA ALVARADO,[1] ANDREW J. HOOVER,[1]

*Program Resources, Inc./DynCorp, NCI—Frederick Cancer Research and Development Center, P.O. Box B, Frederick, Maryland 21702*

and KENNETH M. SNADER

*Natural Products Branch, DTP, DCT, National Cancer Institute, Frederick, Maryland 21702*

ABSTRACT.—The demand for taxol, a promising cancer chemotherapeutic agent, far exceeds supply. Presently, taxol is derived from the bark of the Pacific yew, *Taxus brevifolia*, a small, slow-growing evergreen tree native to the northwestern United States. Knowledge of the distribution and magnitude of genetic and non-genetic sources of variation in taxol content in the genus *Taxus* is necessary if supply issues are to be met through plant harvesting. Analytical determinations of taxol, cephalomannine, and baccatin III in more than 200 trees representing several populations of *T. brevifolia* and other yew taxa indicate that (1) significant variation in taxane content exists among and within populations and species, (2) taxol levels exceeding those reported for *T. brevifolia* bark were found in shoots of individual trees from most taxa studied, and (3) the season in which samples are collected and handling procedures can influence taxane content.

The diterpene taxol was originally isolated at Research Triangle Laboratories (1) from a National Cancer Institute (NCI) collection of Pacific yew [*Taxus brevifolia* Nutt. (Taxaceae)] in Washington State. It recently emerged as a highly effective chemotherapeutic agent for the treatment of ovarian cancer (2) with a unique mechanism of action. It has been shown to promote tubulin polymerization and stabilize microtubules against depolymerization (3). The projected need for 20 to 25 kg of taxol per year to treat the number of ovarian cancer patients who would die each year (4) exemplifies the growing supply crisis.

The Pacific yew is a slow-growing understory tree found mainly in old-growth forests throughout the Pacific Northwest, and while it is not currently threatened or endangered (according to a report in response to the Environmental Defense Foundation from U.S. Fish and Wildlife, December 21, 1990), continued harvesting of the native plant for commercial preparation of taxol is untenable. Although recent reports indicate taxol may be found in tissues and species other than the bark of *T. brevifolia* (5,6), there remains much uncertainty regarding potential sources of variation in taxol content among populations, trees, plant tissue types, and season of collection of yew taxa. Understanding the nature and magnitude of these sources of variation can significantly influence recovery of taxol in existing plant populations.

The objectives of this study were to determine if patterns of genetic and/or environmental variation in taxol content exist in natural populations of the Pacific yew, evaluate the effect of season of collection on taxol content, evaluate the effect of plant tissue type on taxol content, and determine whether taxol and the related taxanes cephalomannine and baccatin III occur in *Taxus* species other than the Pacific yew.

[1]Chemical Synthesis and Analysis Laboratory.

Several analytical procedures have been reported (5–8) for the determination of taxol. For the purposes of this study an assay was needed that not only was amenable to handling large numbers of samples of various tissues, but also provided quantitation of the taxanes of interest. A 2D tlc system (7) capable of resolving compounds from crude *Taxus* extracts is rapid; however, it lacks the ability to quantitate the taxanes. Although a recent report (6) that describes an ELISA procedure using a polyclonal antibody provides a means of rapid detection of several taxanes, it does not discriminate between the various analogues.

A combination of tlc and hplc was applied to the examination of a number of tissues and three species of *Taxus* (5). The tlc-hplc method (5) is quite involved and does not lend itself to handling a large throughput of samples. Because the tlc mobile phase was developed for analyzing taxol and not for the other compounds of interest, baccatin III and cephalomannine do not form distinct bands. In addition, the hplc conditions (5) do not adequately resolve cephalomannine from taxol for the purpose of quantitation.

Using a phenyl-bonded Si gel hplc column operated in a gradient mode, an enhanced detectability of the taxanes of interest was achieved (8). The authors noted (8) difficulties with excessive column backpressure after relatively few injections using this system. With hundreds of samples to analyze, such problems prohibited the use of this mobile phase. For the purposes of this study, a modification of the mobile phase proved applicable to large numbers of tissue samples and quantitated taxol, cephalomannine, and baccatin III in a single isocratic procedure.

## MATERIALS AND METHODS

SAMPLE COLLECTION, PROCESSING AND STORAGE OF *T. BREVIFOLIA*.—In early April 1989, 5 individual *T. brevifolia* trees were identified in each of 5 natural populations located in the Pacific Northwest (Table 1). Trees were permanently tagged, and major limbs from the lower crowns were removed at the tree bole. Trees exceeded 150 years of age and 6 inches in diameter in every case.

Branches were transported to Weyerhaeuser's Centralia Research Center within 6 to 48 h of collection. Needles and twigs were transported under refrigeration; branch wood was transported at ambient temperature. Upon receipt, shoots, consisting of small twigs and needles, and branch bark were removed from the branch wood, placed in screen bags, and dried in forced-air ovens at 50°–60° for 24 to 48 h (until brittle). All samples were processed within 48 h of receipt. Thus, the longest period of time between collection and processing was 48 h. All materials were stored at 2° between time of receipt and time of processing. Dried samples were ground in a Wiley Mill to pass through 1-mm pore sieves and stored in heat-sealed poly bags at 2° until shipped for analysis.

Between August 30 and September 7, the process described above was repeated for the same 25 trees. In addition, 5–7 trees from each of 2 other populations were included for evaluation. Samples were submitted for analysis within 40 days of collection. April collections were analyzed for taxol by Stanford Research Institute (SRI), and September collections were analyzed by Program Resources, Inc. (PRI) by the method described in this paper. Because of time and resource constraints, incomplete data sets exist for both collection dates. April and most of the September collections were assayed for taxol only. Three populations were assayed for taxol, cephalomannine, and baccatin III following the September collection. Because of the incompleteness of the data set, remnant samples of all tissues from all trees were re-extracted and analyzed again in August 1990. The samples had been stored at room temperature following the original assay.

In May 1991, the 5 trees from the Cowlitz, Washington, population were resampled; collections of trunk bark, branch bark, and shoot tissue were kept separate, treated as noted above and analyzed for taxanes within 1 week of collection. The purpose of this collection was to determine if the results of the earlier collection were biased by use of branch bark rather than trunk bark.

SAMPLE COLLECTION, PROCESSING AND STORAGE OF OTHER TAXA.—Between early September and late November of 1989, 180 additional shoot (foliage) collections representing *Taxus baccata* L., *Taxus canadensis* Marsh., *Taxus cuspidata* S.&Z., *Taxus floridana* Chapm., *Taxus chinensis* (Pilg.) Rehd., and *Taxus* x *media* Rehd. hybrids were obtained from arboreta or natural populations, prepared as noted above for *T. brevifolia*, and analyzed for taxol, cephalomannine, and baccatin III. A number of these samples were also re-extracted and analyzed in August 1990 to confirm earlier results.

GENERAL EXPERIMENTAL PROCEDURES.—Instrumentation consisted of a Waters 600E Multisolvent Delivery System, Waters WISP 700 Autosampler, and Waters 900+ Photodiode Array Detector

TABLE 1. Taxol Content (% dry wt) of *Taxus brevifolia* Individual Tree Samples.[a]

| Population/Accession | Lat. | Long. | Region[b] | Taxol Content | | | |
|---|---|---|---|---|---|---|---|
| | | | | Shoots | | Bark | |
| | | | | April[c] | September | April[c] | September |
| WASHINGTON | | | | | | | |
| Cowlitz | 47°35′ | 121°40′ | CM | | | | |
| 3334 | | | | 0.010 | 0.016 | 0.005 | 0.001 |
| 3332 | | | | — | 0.007 | 0.008 | 0.002 |
| 3335 | | | | — | 0.016 | 0.003 | 0.001 |
| 3336 | | | | — | 0.033 | 0.005 | 0.001 |
| 3337 | | | | 0.008 | — | 0.004 | — |
| Wind River | 44°15′ | 121°50′ | CM | | | | |
| 3328 | | | | 0.010 | 0.004 | 0.010 | 0.001 |
| 3329 | | | | — | 0.017 | 0.006 | 0.004 |
| 3325 | | | | — | 0.001 | 0.005 | 0.001 |
| 3326 | | | | 0.006 | 0.007 | 0.002 | 0.001 |
| 3327 | | | | — | — | 0.013 | — |
| OREGON | | | | | | | |
| McKensie Bridge | 44°15′ | 121°50′ | CM | | | | |
| 3351 | | | | 0.010 | 0.003 | 0.008 | 0.001 |
| 3352 | | | | 0.009 | 0.008 | 0.009 | 0.006 |
| 3353 | | | | — | 0.023 | 0.001 | 0.001 |
| 3354 | | | | — | 0.013 | 0.001 | 0.001 |
| 3355 | | | | — | — | 0.004 | — |
| Butte Falls | 42°30′ | 122°35′ | WD | | | | |
| 3360 | | | | 0.008 | 0.004 | 0.010 | 0.004 |
| 3339 | | | | — | 0.002 | 0.005 | 0.002 |
| 3362 | | | | — | 0.001 | 0.005 | 0.002 |
| 3338 | | | | 0.008 | 0.008 | 0.003 | 0.001 |
| 3361 | | | | — | — | 0.004 | — |

[a]Shoots and bark were collected in April and September of 1989 and assayed winter of 1989–1990. April collections from a fifth population in Idaho were not analyzed. Consequently, September results for Idaho are summarized in Table 3 only.

[b]CM = cool, moist; WD = warm, dry.

[c]April assays performed by SRI.

with an NEC APCIV computer (NEC Information Systems) for data processing. An IEC Model K Centrifuge was used for centrifugation. A Dynamax Microsorb 5 μm phenyl column (4.6 mm × 150 mm) with guard module (Rainin Instrument Company) was used for hplc analyses. Hplc grade MeCN, MeOH, $CH_2Cl_2$, $C_6H_{14}$, and $H_2O$ were purchased from Burdick and Jackson. Standards were weighed on a Cahn 21 automatic electrobalance.

EXTRACTION.—Because a reversed-phase hplc method was used for analysis of the extracts, it was necessary to remove non-polar substances which would interfere with the chromatography. This was especially true for the extraction of needles, which contain waxy, nonpolar components. Approximately 10 g of plant material was extracted in a cylindrical separatory funnel with 50 ml of hexane for 24 h at room temperature. The hexane extract was discarded, and the remaining plant material was extracted with $CH_2Cl_2$-MeOH (1:1) to 24 h at room temperature (9). The resulting organic extract was collected and evaporated to dryness at 35° under reduced pressure. The residue was dissolved in a minimal amount of MeOH and was partitioned between $CH_2Cl_2$ and $H_2O$ (1:1). In order to achieve better separation the mixture was centrifuged at 2700 rpm for 5 min. The aqueous layer was discarded, and the organic layer was evaporated to dryness at 35° under reduced pressure in a tared flask. The residue mass was determined and a 20 mg/ml solution in MeOH was prepared and filtered through a 0.2 μm filter.

STANDARDS.—Baccatin III and 10-deacetylbaccatin III were provided by Dr. David G.I. Kingston, Virginia Polytechnic Institute, Blacksburg, Virginia. Cephalomannine was isolated in our laboratory from

the bark of *T. brevifolia* and was identified based on spectral properties. A taxol reference standard was provided by the Developmental Therapeutics Program, National Cancer Institute. A 1 mg/ml solution of each standard in MeOH was prepared and filtered through a 0.2 μm filter prior to hplc analysis.

HPLC METHOD.—The hplc method is a modification of one previously reported (8). Through the replacement of $H_2O$ with an acidic buffer the aforementioned (8) backpressure problems were eliminated and the column lifetime was extended to enable hundreds of injections of extracts. The taxanes of interest were adequately resolved without the use of a gradient. A phenyl-bonded Si gel column was operated under isocratic conditions employing a mobile phase consisting of MeOH-MeCN-50 mM aqueous ammonium acetate buffer adjusted to pH 4.4 with HOAc (20:32:48). The flow rate was 1 ml/min, and the effluent was monitored at 228 nm. The injection volumes were 10 μl for the standards and 25 μl for the plant samples.

ANALYSIS METHOD.—Peak areas were used to calculate the amount of taxane in the plant sample ascompared to the standard. The retention time of taxol was 14.9 min, cephalomannine 12.4 min, and baccatin III 4.5 min. The percent of taxanes in the plant material was obtained by dividing the amount of taxane found in the residue by the dry wt of plant material extracted and multiplying by 100.

VALIDATION.—Fourteen of the samples for which data are presented here were initially extracted and analyzed in duplicate with an average variation of ±0.001 in the % taxane content observed. A single sample each of bark and shoot tissue was subsequently analyzed independently 10 times. For taxol, mean and standard deviations for these observations were 0.0287 ± 0.001 and 0.0044 ± 0.0008, respectively. Given the apparent precision of the extraction/partition protocol, the large number of samples to assay, and the limited time and resources available, single extractions were performed on the remainder of the samples.

Reproducibility of injections expressed as the variation (%) of injection was 1%. Because an external standard method of calculating the amount of taxane represented by an area was used, linearity is important. Standards were injected at the beginning, middle, and end of each run. Standard curves showed a correlation of 0.99. Standard curves were developed for every set of analyses.

It is difficult to determine the absolute content of the compounds of interest in crude extracts and therefore difficult to measure with certainty completeness of extraction. Extraction trials with a number of solvents indicated $CH_2Cl_2$-MeOH (1:1) provided the highest percent extractables and best biological activity in the P-388 (in vitro) cell line assays (T.G. McCloud, personal communication, July 1988), and it was adopted as extraction solvent in this study.

Spiking studies with known concentrations of taxol, cephalomannine, and baccatin III evaluated with the experimental protocol cited here gave the following percent recoveries: 98%, 97%, and 97%, respectively.

## RESULTS

*T. BREVIFOLIA*.—This study demonstrated that (a) significant differences in taxol content exist among individuals within populations, among populations and between tissues and dates sampled, (b) cephalomannine and baccatin III occurred in relatively high concentrations in some trees, and (c) taxane content declined in processed tissue over time.

*Tissue*.—Taxol content was significantly greater in shoot tissue than in branch bark tissue at both sampling dates in 1989 (Tables 1 and 2). Differences were greatest in September when shoots possessed, on average, more than twice as much taxol as bark (0.008 vs. 0.0024% by weight). The exception to this trend was found in the trees from the Idaho population (Table 3), although for these samples taxol content was very low in both tissues.

Collections made in May 1991 exhibited the opposite pattern (Table 3). That is, taxol content was consistently greater in branch bark than in shoot tissue. Moverover, in every tree evaluated, trunk bark possessed the most taxol of the three tissues tested.

*Season*.—There was essentially no difference in taxol content between April and September collections for shoot tissue, but the differences were large and significant for bark tissue, April collections being higher (Tables 1 and 2).

*Tree, Population, Region*.—For collections made in 1989, taxol content varied dramatically among trees within populations (Tables 1 and 3); there was, however, rel-

TABLE 2. Selected Comparisons (paired *t*-tests) for Taxol Concentration, as Influenced by Tissue and Season of Collection for *Taxus brevifolia*.[a]

| Contrast | Mean Taxol % | N | T | Significance |
|---|---|---|---|---|
| *Shoot vs. Bark* | $x + SE$ | | | |
| April shoot[b] | 0.0086 ± 0.0005 | 8 | 2.39 | 0.048 |
| April bark | 0.0064 ± 0.0011 | | | |
| September shoot | 0.0080 ± 0.0019 | 21 | 2.82 | 0.010 |
| September bark | 0.0024 ± 0.0003 | | | |
| *April vs. September* | | | | |
| April shoot | 0.0087 ± 0.0006 | 7 | .92 | 0.390 |
| September shoot | 0.0072 ± 0.0027 | | | |
| April bark | 0.0053 ± 0.0013 | 16 | 5.18 | 0.0001 |
| September bark | 0.0019 ± 0.0003 | | | |

[a]Sample sizes vary because of incomplete assay information.
[b]April assays by SRI.

atively little variation among the 5 trees resampled in 1990 (Table 4). Using September 1989 shoot tissue as a standard reference, the mean taxol content (percent of dry wt) for the 38 trees evaluated was 0.0081, with a range of 0.0005 to 0.0330%. However, the median value was 0.003, and 28 of the 38 assays were at or below the average. Only 10 trees had taxol contents in shoot tissue that exceeded 0.01% in this study.

One-way analysis of variance showed that differences in taxol content among populations was also significant (at $P = 0.05$ level; Figure 1). Ecological grouping of populations indicated that trees from cool, moist sites, such as found on the west slopes of the

TABLE 3. Taxane Content (% dry wt) in *Taxus brevifolia* Individual Tree Samples.[a]

| Population | Lat. | Long. | Region | % Taxol | % Cephalomannine | % Baccatin III |
|---|---|---|---|---|---|---|
| IDAHO | 41°50′ | 115°27′ | WD[b] | | | |
| 3363 Needle | | | | 0.003 | 0.008 | 0.013 |
| Bark | | | | 0.003 | 0.002 | 0.001 |
| 3364 Needles | | | | 0.001 | 0.002 | 0.016 |
| Bark | | | | 0.005 | 0.027 | 0.050 |
| 3365 Needles | | | | 0.001 | 0.004 | 0.023 |
| Bark | | | | 0.004 | 0.021 | 0.010 |
| 3366 Needles | | | | 0.002 | 0.007 | 0.023 |
| Bark | | | | 0.005 | 0.015 | 0.006 |
| 3379 Needles | | | | 0.002 | 0.006 | 0.030 |
| Bark | | | | 0.003 | 0.019 | 0.024 |
| BLUE MOUNTAINS, OR | 46°10′ | 117°27′ | WD | | | |
| 3342 | | | | 0.005 | 0.024 | 0.063 |
| 3343 | | | | 0.001 | 0.002 | 0.002 |
| 3344 | | | | 0.001 | 0.002 | 0.020 |
| 3345 | | | | 0.002 | 0.008 | 0.001 |
| 3346 | | | | 0.001 | 0.004 | 0.000 |
| PLACERVILLE, CA | 38°41′ | 120°26′ | Transition | | | |
| 3315 | | | | 0.003 | 0.002 | 0.045 |
| 3316 | | | | 0.014 | 0.008 | 0.062 |
| 3317 | | | | 0.024 | 0.031 | 0.084 |
| 3318 | | | | 0.011 | 0.020 | 0.021 |
| 3319 | | | | 0.007 | 0.011 | 0.055 |
| 3320 | | | | 0.013 | 0.018 | 0.206 |
| 3321 | | | | 0.005 | 0.001 | 0.038 |

[a]Based on September shoot and bark collections (where noted) from three populations.
[b]WD = warm, dry.

TABLE 4. Taxane Content (% dry wt) in Plant Parts of Five *Taxus brevifolia* Trees in the Cowlitz, WA Population.[a]

| Accession | Taxol | | | Cephalomannine | | | Baccatin III | | |
|---|---|---|---|---|---|---|---|---|---|
| | S[b] | BB[b] | TB[b] | S | BB | TB | S | BB | TB |
| 3332 | — | 0.008 | 0.019 | —[•] | 0.012 | 0.020 | — | 0.009 | 0.020 |
| 3334 | 0.006 | 0.008 | 0.011 | 0.004 | 0.012 | 0.013 | 0.003 | 0.008 | 0.012 |
| 3335 | 0.005 | 0.009 | 0.020 | 0.003 | 0.012 | 0.022 | 0.002 | 0.009 | 0.020 |
| 3336 | 0.004 | 0.010 | 0.015 | 0.002 | 0.011 | 0.015 | 0.002 | 0.010 | 0.015 |
| 3337 | 0.006 | 0.009 | 0.018 | 0.003 | 0.013 | 0.018 | 0.003 | 0.009 | 0.018 |
| MEANS | 0.005 | 0.009 | 0.017 | 0.003 | 0.012 | 0.018 | 0.003 | 0.009 | 0.017 |

[a]Samples were collected in May, 1991.
[b]S = shoots, BB = branch bark, and TB = trunk bark.

Cascade Mountains, had more taxol, on average, than trees from warm, dry sites like Idaho, the Blue Mountains, and southern Oregon (single degree of freedom contrast was significant at $P = 0.05$ level).

*Other Taxanes.*—As with taxol, large differences in concentrations of cephalomannine and baccatin III were observed among trees within populations (Table 3). Both compounds were found in greater abundance, on average, than taxol in collections made in 1989. In particular, baccatin III occurred at very high concentrations in some trees and populations; values above 0.05% were not uncommon, and the California population averaged 0.073%. However, it should be noted that a number of trees appeared to have no cephalomannine or baccatin III. For the single population in which both shoots and bark were analyzed for all taxanes, shoots possessed substantially more baccatin III and bark possessed substantially more cephalomannine. For trees collected in 1990, cephalommanine and baccatin III content reflected the pattern observed for taxol; concentrations were greatest in trunk bark and least in shoot tissue (Table 4). All taxanes were found in approximately equivalent amounts at this sampling date.

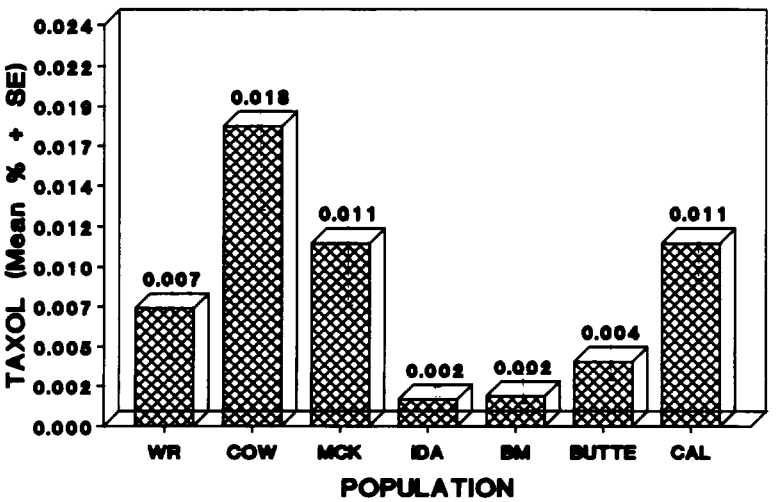


FIGURE 1. Population mean and standard error for taxol content (% dry wt) in *Taxus brevifolia*. Values are based on assays performed on fall-collected shoot tissue of 4–7 trees per population. Population abbreviations are: WR = Wind River, Cow = Cowlitz, McK = McKensie Bridge, Ida = Idaho, BM = Blue Mountains, Butte = Butte Falls, Cal = Placerville.

*Effects of Storage.*—Dried and ground shoot and bark tissue samples, reassayed after being maintained at room temperature for 12–17 months, had significantly less taxanes than when they were originally surveyed. Taxol content declined to about 40% of the original estimates; the other taxanes declined to about 20%.

OTHER TAXA.—All taxa evaluated in this study possessed taxol, cephalomannine, and baccatin III (Table 5). Estimates of the quantity of compound found in each taxa were highly variable, as noted with *T. brevifolia*, and were generally commensurate with numbers published previously (9) in a study of limited scope. Because of the likelihood of sampling error (small sample size) giving misleading results, we have chosen to present summary data only on those taxa well represented in our survey.

TABLE 5. Mean, Standard Error, and Range of Taxane Concentration (% dry wt) for *Taxus* spp. Based on Fall Shoot Collections.

| Species | Taxol | Cephalomannine | Baccatin III |
|---|---|---|---|
| | X ± SE (Range) | X ± SE (Range) | X ± SE (Range) |
| *Taxus baccata* | 0.0088 ± 0.0023 | 0.0246 ± 0.0049 | 0.0285 ± 0.0045 |
| (*n* = 27) | (0.0008–0.049) | (0.0033–0.1237) | (0.0005–0.0899) |
| *Taxus brevifolia* | 0.0081 ± 0.0014 | 0.0093 ± 0.0021 | 0.0466 ± 0.0122 |
| (*n* = 17–33) | (0.0005–0.0333) | (0.0008–0.0305) | (0.0004–0.2063) |
| *Taxus cuspidata* | 0.0077 ± 0.0015 | 0.0237 ± 0.0038 | 0.0322 ± 0.0050 |
| (*n* = 40) | (0.0003–0.0390) | (0.0013–0.1146) | (0.0001–0.1418) |
| *Taxus media*[a] | 0.0056 ± 0.0008 | 0.0242 ± 0.0036 | 0.0243 ± 0.0026 |
| (*n* = 90) | (0.0000–0.0513) | (0.0007–0.2225) | (0.0002–0.0963) |

[a]*Taxus baccata* by *Taxus cuspidata* hybrids.

Mean species values for taxol were comparable across all taxa. On average, *T. brevifolia* possessed more baccatin III and less cephalomannine than other species. When added together, all taxa evaluated appeared to possess essentially equivalent amounts of total taxanes (Table 5).

## DISCUSSION

This study represents the first significant effort to define the magnitude and pattern of genetic and non-genetic sources of variation in taxane concentrations of natural populations of Pacific yew and other taxa. The data are encouraging in many respects, but must be interpreted with great care. Notable implications of these results are (a) opportunities for genetic selection among individuals and populations appear to exist, (b) epigenetic factors such as timing of collection and plant tissue type can significantly influence taxol content within plants, (c) taxol content in stored tissue samples may decline with time, and (d) taxol occurs in other yew taxa. Each of these points is discussed below.

GENETIC VARIATION.—The large differences observed among individuals and populations suggest that substantial opportunity exists to apply strong selection pressure in identifying superior individuals for taxol/taxane content. However, virtually nothing is yet known about the heritability of taxane content; much of the variability observed in this study may have been environmentally induced. The currently held view among forest geneticists is that a number of terpenes found in conifers are rather simply inherited (i.e., under the control of relatively few genes) (10–13). Still, our understanding of the biosynthetic pathways leading to the production of the relatively

simple monoterpenes, not to mention highly functionalized, complex diterpenes such as taxol, is not complete (14–16). It is probable that taxanes are the products of biosynthetic pathways involving a dozen or more enzymes (genes), all of which may be under the influence of one or more regulatory genes. To fully understand opportunities for obtaining genetic gains through selection, population and family-structured genetic tests in carefully controlled environments are required. Such tests are currently under way with *T. brevifolia* at Weyerhaeuser nurseries, but results will not be available for at least 2 years.

NON-GENETIC VARIATION.—For convenience, non-genetic sources of variation can be categorized as either epigenetic or environmental. Epigenetic variation is that which occurs due to variable expression of genetic potential, such as differences due to time of collection or plant tissue type. Such variation is of considerable practical importance.

This study represents the first compelling evidence that taxol content in shoot tissue of *T. brevifolia* may exceed or equal that in bark tissue of the same plant. Previous comparisons have shown bark to have the highest taxol concentrations of any plant part (5, NCI, unpublished data). Unfortunately, much of what was previously known about taxol content in plant tissues has been confounded by lack of experimental control over other genetic, epigenetic, and environmental factors. This study attempted to control these factors by sampling within individuals, across seasons, and with uniform post-harvest handling procedures. Also evident from the results presented here are the significant effects time of collection may have on taxol content, and the potential for large and confusing interaction effects among factors influencing taxane production. For instance, it is difficult to explain why taxol content for the Cowlitz population was consistently higher in shoots than branch bark on both collection dates in 1989, but lower in May 1990. Nor is it easy to explain the shift in relative performance of the individual trees with time. Clearly, these results imply that epigenetic sources of variation are large and relatively poorly understood, and a good deal of investigation will be required to insure efficient use of available resources. Alternatively, the results may suggest a need for greater standardization of sample handling, preparation, and assay.

Environmental variation is that which may be due to physical or chemical factors such as soil pH, climate, water relations, temperature, etc. As noted above, not all variation in concentration of taxanes observed among individuals, populations, or geographic regions is likely to be heritable. Indeed, for most traits in conifers controlled by more than one or a few genes, environmental effects are very large (17, 18). Determining precisely what environmental factors are important and how to control them will take considerable time and experimentation. The most notable environmental association detected in this study appears to be a tendency for trees with higher taxol contents to occur in cool, moist, and shady environments rather than warm, dry, sunny climates. Potential geoclimatic trends such as this should become more clear with the completion of a much more extensive population survey now being cooperatively undertaken by the U.S. Forest Service, the National Cancer Institute, and PRI.

STORAGE.—Based on the results of this and other unreported work, we now believe taxol content in shoot tissues, both processed and unprocessed, may be highly unstable or labile under normal room conditions. This could explain why most previous efforts to detect taxol in Pacific yew needles have been unspectacular. Clearly, if taxol content in foliar tissues is to ever be utilized, handling and processing protocols will need to be carefully defined. A number of studies are currently under way by various organizations to improve understanding of this potential source of post-harvest, environmental variability.

OTHER TAXA.—The presence of taxol and other taxanes in yew species and hybrids other than the Pacific yew is of considerable, practical importance. It greatly increases the genetic base from which future domestic population development can occur, and it could provide a previously ignored source of biomass for meeting current pharmaceutical needs for taxol. Extensive efforts to improve understanding of these opportunities are currently under way by a number of public and private organizations.

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