# Taxane Contents of *Taxus* Cultivars Grown in American Nurseries[1]

**Hala N. ElSohly[2], Edward M. Croom Jr.[3], Wlodzimierz J. Kopycki[4], Alpana S. Joshi[5], Mahmoud A. ElSohly[6], and James D. McChesney[7]**
*National Center for the Development of Natural Products*
*Research Institute of Pharmaceutical Sciences, School of Pharmacy*
*The University of Mississippi, University, MS 38677*

## Abstract

The taxane content of the needles, stems and clippings of 92 cultivars obtained from ten nurseries in the United States is presented. The analysis of 10-desacetyl baccatin III (**1**), baccatin III (**2**), 10-desacetyl taxol (**3**) cephalomannie (**4**), 10-desacetyl-7-*epi*-taxol (**5**) and taxol (**6**) was carried out using a high performance liquid chromatographic method developed in our laboratory. The total 'useful' taxane content of the leaves, stems, and clippings in the studied cultivars ranged from 0.0135–0.1471%, 0.0055–0.0462% and 0.0121–0.1183%, respectively, with **1** and **6** being the most abundant. The survey identified 18 cultivars with a content of **1** ranging from 0.015% to 0.0622% and 24 cultivars with a content of **6** ranging from 0.012% to 0.062%; compound **2** was either undetectable or found in negligible amounts in all the cultivars studied. The contents of **3** varied between 0.0019–0.0264% and that of **4** between 0.0017–0.0386%, while that of **5** between 0–0.0107%.

**Index words:** taxol and related compounds, *Taxus*.

**Species used in this study:** *T. baccata* L. 'Repandens'; *T. cuspidata* Zieb. & Zucc.; *T. cuspidata* Zieb. & Zucc. 'Capitata'; *T. cuspidata* Zieb. & Zucc. 'Columnaris'; *T. cuspidata* Zieb. & Zucc. '*Fastigiata*'; *T. cuspidada* Zieb. & Zucc. 'Nana'; *T.* x *media* 'Rehd. Amherst'; *T.* x *media* Rehd. 'Angelica'; *T.* x *media* Rehd. 'Berryhill'; *T.* x *media* Rehd. 'Bobbink'; *T.* x *media* Rehd. 'Brevifolia'; T. x *media* Rehd. 'Brownii'; *T.* x *media* Rehd. 'Citation'; *T.* x *media* Rehd. 'Chadwick'; *T.* x *media* Rehd. 'Dark Green Spreader'; *T.* x *media* Rehd. 'Densiformis'; *T.* x *media* Rehd. 'Densiformis Henryi'; *T.* x *media* Rehd. 'Eldon'; *T.* x *media* Rehd. 'Everlow'; *T.* x *media* Rehd. 'Fairview'; *T.* x *media* Rehd. 'Greenwave'; *T.* x *media* Rehd. 'Halloran'; *T.* x *media* Rehd. 'Hatfieldii'; *T.* x *media* Rehd. 'Henryi'; *T.* x *media* Rehd. 'Hicksii'; *T.* x *media* Rehd. 'Hicks Verkade'; *T.* x *media* Rehd. 'Hiti'; *T. hunnewelliana* Rehd.; *T.* x *media* Rehd. 'Globet Hunnewelliana'; *T.* x *media* Rehd. 'Intermedia'; *T.* x *media* Rehd. 'Majestic'; *T.* x *media* Rehd. 'Nigra'; *T.* x *media* Rehd. 'Ohio Globe'; *T.* x *media* Rehd. 'Runyan'; *T.* x *media* Rehd. 'Sebian'; *T.* x *media* Rehd. 'Slavin'; *T.* x *media* Rehd. 'Stovekin'; *T.* x *media* Rehd. 'Sunburst'; *T.* x *media* Rehd. 'Super Densiformis'; *T.* x *media* Rehd. 'Tauntonii'; *T.* x *media* Rehd. 'Vermulen'; *T.* x *media* Rehd. 'Viridis'; *T.* x *media* Rehd. 'Thayerae'; *T.* x *media* Rehd. 'Tvurdy'; *T.* x *media* Rehd. 'TV'; and *T.* x *media* Rehd. 'Wardii'.

## Significance to the Nursery Industry

The need for an alternative source for taxol other than the bark of *T. brevifolia* is necessary to meet the expected increase in demand for the drug to treat different types of cancer. Commerical nurseries have more than 30 million *Taxus* plants in cultivation for use in landscape plantings. *Taxus* is grown for five to more than twelve years by nurseries before sale for landscape purposes. Annual shaping of the plants generates stem and needle clippings which are left in the field to rot or hauled away. In addition, entire plants that are not well formed are discarded as culls each year. The nursery industry has the potential to serve as the foundation of a renewable, sustainable, reliable and immediate source of *Taxus* biomass for production of taxol and related compounds. We have examined the variation in taxol and other taxanes in 46 *Taxus* cultivars represented by 92 accessions. The dried needles from *T.* x *media* 'Nigra', *T. cuspidata*, *T. cuspidata* 'Capitata', *T.* x *media* 'Hicksii', *T.* x *media* 'Tauntonii' were found to contain amounts of taxol three to six times higher than the typical levels reported in *T. brevifolia* bark (0.01–0.02%). In addition, the dried needles of *T. baccata* 'Repandens', *T. cuspidata*, *T.* x *media* 'Hicksii', *T.* x *media* 'Slavin' serve as a good source for 10-desacetyl baccatin III, a useful precursor for the synthesis of taxol.

## Introduction

*Taxus* (yew) is grown for 5 to more than 12 years by nurseries before being sold as a landscape shrub. A preliminary survey of nurseries found that approximately 30 million *Taxus* were in field production in 1991 (1). The nursery industry therefore has the potential to serve as the foundation of a sustainable, reliable and immediate source of *Taxus* biomass for the production of taxol and related taxanes (2–5). Previous work in our laboratory indicated that harvest and postharvest treatment are critical to the preservation of taxol in the clippings of *Taxus* plant material (6–9).

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[2]Research Professor. To whom correspondence should be addressed, Phone 601-232-7610.Fax 601-232-5118. E-mail: helsohly@sunset.backbone.olemiss.edu

[3]Research Associate Professor.

[4]Research Scientist.

[5]Research Associate.

[6]Research Professor.

[7]Barnard Distinguished Professor. Present address: NaPro BioTherapeutics, Inc. 6304-A Spine Road, Boulder, CO 80301.

In a previous publication (5), we reported on the taxane content of the needles of 57 cultivars grown in major nurseries in the United States using an HPLC method developed *in-house* (10). This work is directed towards the completion of this survey by evaluating the content of 10-desacetyl baccatin III (**1**), baccatin III (**2**), 10-desacetyl taxol (**3**), cephalomannine (**4**), 10-desacetyl-7-*epi*-taxol (**5**), and taxol (**6**) in 35 additional *Taxus* cultivars obtained from several nurseries in the United States.

## Materials and Methods

*General experimental procedures*. Instrumentation consisted of a Waters 600E Multisolvent Delivery System, Waters 712 W1SP Auto injector, and Waters 991 Photodiode Array (PDA) detector with a NEC Power Mate SX Plus Computer for controlling the analytical system and for data processing. The chromatograms were printed on a Waters 5200 Printer Plotter. A Curosil G HPLC column (250 × 4.6 mm i.d.; 6µm) and a precolumn (30 × 4.6 mm i.d.) with a proprietary bonded-phase packing material were purchased from Phenomenex (Torrance, CA). Samples were filtered prior to HPLC analysis using a Millex filtering unit (0.45 µm; Waters). HPLC grade MeCN, reagent ethanol (a mixture of 90.6% EtOH, 4.5% MeOH and 4.9% *iso*-PrOH), tetrahydrofuran, methyl-*t*-butyl ether and certified ethylene glycol monomethyl ether were purchased from Fisher Scientific Co. (Fair Lawn, NJ). The nanopure water (18MW) was obtained in-house using a Barnstead nanopure purificator. All solvents were filtered through a 0.45 µm nylon membrane (Alltech). A Roanoke Bulk Tobacco Curing Barn (Gregory Manufacturing Co., Inc., Lewistown-Woodville, NC) was used to dry the plant material. A New Brunswick Scientific G10 Gyrotory Shaker was used for the extraction of plant material. A Turbo Vap LV Evaporator (Zymark Corporation, Hopkinton, MA) was used for the evaporation of the extracts. Filtration was carried out using a Visiprep Vacuum Manifold purchased from Supelco (Bellefonte, PA).

*Plant material*. Fifty-seven accessions of yew (*Taxus*) cultivars (first shipment) were received during the Autumn of 1992 as potted plants (three to four years old) from five nurseries: Rhode Island Nurseries, Inc. (Newport, RI), Zelenka Nursery, Inc. (Grand Haven, MI), Fairview Evergreen Nurseries, Inc. (Fairview, PA) and the Cottage Gardens, Inc. (Lansing, MI). A second shipment consisting of thirty-five accessions was received during the spring of 1993 as potted plants (three to four years old) from five additional nurseries: Angelica Nurseries, Inc. (Kennedyville, MD,) , Clinton Nurseries (Clinton, CT), Ridge Manor Nursery (Madison, OH), Gardner's Nurseries, Inc. (Rocky Hill, CT), and Scarff's Nursery, Inc (New Carlisle, OH).

Plant cultivar names are as sold by the nursery. Species and cultivars used are listed in Table 1. Voucher specimens of each accession were deposited in the Herbarium, Biology Department, University of Mississippi. Before utilization, the plants were maintained in pots under shade cloth at the M.W. Quimby Medicinal Plant Gardens of the Research Institute of Pharmaceutical Sciences at the University of Mississippi. Clippings were taken from the previous season's growth from each of the individual plants (generally six plants/cultivar unless otherwise indicated in Table 1) received from the nurseries, except for Cottage Gardens Inc., where the clippings from several plants representing a cultivar were pooled together. Clippings were collected on November 30, 1992, for Cottage Gardens while those from the other nurseries described above were obtained between December 9–13, 1992. Cuttings from the 2nd shipment were made on December 14, 1993.

A shipment (100 lbs) of dry stem bark of *Taxus brevifolia* Nutt. was received from Patrick Connolly Yew Wood Industries Portland, OR, on May 1, 1989. The plant material was stored under refrigeration until November 2, 1993.

*Drying*. The cuttings were maintained intact and were dried in the drying barn at 40C (104F) for 72 hours. The moisture content of the clippings was approximately 60%.

*Extraction*. The dried clippings were separated into leaves and stems, and each organ ground in a coffee grinder (70 mesh size). The resulting finely powdered material was mixed well and a representative 1 g sample weighed and extracted with 95% EtOH by soaking with agitation (10 mL, 16 h × 2). The combined extract (25 mL) was evaporated to dryness, and the residue was partitioned three times between water (2 mL) and $CH_2Cl_2$ (4 mL). The organic fraction was evaporated to dryness. The residue was dissolved in EtOAc (5 mL) and MeOH (2 mL) and coated on 1 g of celite. The material coated on celite was packed into a small column which was then washed with 15 mL hexane until the eluate was colourless, followed by 15 mL $CH_2Cl_2$. The $CH_2Cl_2$ wash was evaporated to dryness and the residue reconstituted in reagent ethanol to produce a solution containing approximately 20 mg/mL, from which a 10 µL volume was submitted to HPLC analysis.

From the stem bark, a sample (500 g) was powdered in a coffee grinder (70 mesh size). The resulting finely powdered stem bark was mixed well and a representative 1 g sample was weighed, extracted, partitioned and purified as described. A validation of the celite clean-up procedure utilized was carried out by analyzing the level of 10-desacetyl baccatin III (**1**), baccatin III (**2**), 10-desacetyl taxol (**3**), cephalomannine (**4**), and taxol (**6**) in the leaf extract of *T.* x *media* 'Hicksii' before and after spiking. Spiking is the addition of a known amount of the reference compound(s) to the plant material during extraction so that the efficacy of the extraction procedure can be measured. The recovery of taxanes **1–4**, and **6** from the spiked samples (n = 3) was 89%, 92%, 94%, 97%, and 97%, respectively.

*Standards*. 10-Desacetyl baccatin III (**1**), baccatin III (**2**), 10-desacetyl taxol (**3**), cephalomannine (**4**), 10-desacetyl-7-*epi*-taxol (**5**), and taxol (**6**) were provided by Dr. Kenneth Snader of the National Cancer Institute. A mixture of standards **1–6** was prepared in reagent ethanol such that each standard was at a concentration of approximately 100 µg/mL from which 10 µL was submitted to HPLC analysis.

*Analytical HPLC method*. Analyses were performed on a Curosil G column (with a proprietary bonded phase packing material operating in the reversed-phase mode using an ethylene glycol monomethyl ether:water:MeCN mobile phase. The linear gradient elution profile started with 76:24 solvent B (ethylene glycol monomethyl ether:water, 8:92) and solvent C (100% MeCN) going to 70:30 and 55:45 (Solvent B:Solvent C) in 12 and 36 minutes respectively, and ending with a 50:50 mixture (Solvent B:Solvent C) for 46 minutes

Table 1. A summary of individual and total taxane content in the leaves of the different cultivars of *Taxus* by nursery.

| Cultivar | Nursery | Average taxane[z] concentration[y] (%W/W) ± S.D. 1 | 2 | 3 | 4 | 5 | 6 | Total taxanes (g %) |
|---|---|---|---|---|---|---|---|---|
| *T. baccata* 'Repandens' | Rhode Island | 395 ± 36 | —[x] | 37 ± 5 | 55 ± 9 | — | 59 ± 7 | 546 |
| | Fairview | 334 ± 43 | 20 ± 4 | 38 ± 8 | 36 ± 10 | — | 38 ± 15 | 466 |
| | Cottage Gardens | 662 ± 3 | — | 20 ± 0 | 28 ± 0 | — | Trace | 710 |
| | Ridge Manor[u] | 307 ± 91 | — | 19 ± 4 | 17 ± 12 | 14 ± 4 | 11 ± 3 | 368 |
| *T. cuspidata* | Zelenka | 45 ± 19 | 18 ± 2 | 142 ± 30 | 52 ± 15 | 32 ± 8 | 169 ± 36 | 458 |
| | Studebaker | 503 ± 148 | — | 74 ± 12 | 189 ± 39 | 15 ± 3 | 190 ± 38 | 971 |
| | Rhode Island | 103 ± 12 | 23 ± 3 | 164 ± 15 | 118 ± 16 | 17 ± 4 | 363 ± 52 | 788 |
| | Gardner's[u] | 142 ± 29 | 53 ± 15 | 431 ± 41 | 171 ± 25 | 107 ± 10 | 567 ± 64 | 1471 |
| *T. cuspidata* 'Capitata' | Rhode Island[v] | 173 ± 84 | — | 167 ± 74 | 243 ± 91 | 30 ± 11 | 518 ± 311 | 1131 |
| | Zelenka[v] | 273 ± 136 | 34 ± 23 | 149 ± 29 | 272 ± 63 | 16 ± 7 | 365 ± 88 | 1109 |
| | Fairview | 102 ± 98 | — | 130 ± 61 | 189 ± 130 | 29 ± 10 | 424 ± 186 | 874 |
| | Ridge Manor[u] | 34 ± 5 | — | 129 ± 11 | 71 ± 3 | 61 ± 10 | 57 ± 2 | 352 |
| | Gardner's[u] | 75 ± 38 | 14 ± 7 | 264 ± 71 | 386 ± 76 | 91 ± 17 | 509 ± 101 | 1338 |
| *T. cuspidata* 'Columnaris' | Studebaker | 169 ± 35 | 29 ± 7 | 196 ± 24 | 98 ± 18 | 17 ± 3 | 256 ± 18 | 765 |
| *T.cuspidata* 'Fastigiata' | Studebaker | 314 ± 130 | — | 96 ± 75 | 80 ± 19 | 16 ± 7 | 167 ± 78 | 673 |
| *T. cuspidata* 'Nana' | Fairview | Trace | — | 61 ± 31 | 70 ± 28 | — | 101 ± 28 | 232 |
| *T.* x *media* 'Amherst' | Studebaker[w] | 193 ± 34 | — | 72 ± 8 | 96 ± 12 | 24 ± 3 | 97 ± 15 | 482 |
| *T.* x *media* 'Angelica' | Angelica[u] | 37 ± 4 | — | 49 ± 7 | 19 ± 2 | 46 ± 2 | 32 ± 6 | 188 |
| *T.* x *media* 'Berryhill' | Studebaker[u] | 46 ± 13 | — | 23 ± 3 | 28 ± 3 | 22 ± 1 | 16 ± 5 | 135 |
| *T.* x *media* 'Bobbink' | Zelenka[u] | 192 ± 38 | 24 ± 8 | 90 ± 16 | 172 ± 47 | 16 ± 5 | 337 ± 36 | 831 |
| *T.* x *media* 'Brevifolia' | Rhode Island | 62 ± 25 | — | 101 ± 14 | 161 ± 48 | 14 ± 3 | 249 ± 48 | 587 |
| | Angelica[u] | 60 ± 8 | — | 160 ± 30 | 78 ± 6 | 75 ± 21 | 76 ± 18 | 449 |
| *T.* x *media* 'Brownii' | Rhode Island | 193 ± 38 | — | 87 ± 13 | 97 ± 10 | 13 ± 3 | 214 ± 31 | 604 |
| | Fairview | 117 ± 26 | — | 52 ± 8 | 71 ± 15 | 10 ± 1 | 119 ± 26 | 369 |
| | Studebaker | 304 ± 8 | 13 ± 1 | 63 ± 11 | 111 ± 5 | — | 102 ± 19 | 593 |
| | Cottage Gardens | 195 ± 8 | — | 66 ± 23 | 62 ± 26 | 18 ± 6 | 53 ± 31 | 394 |
| | Ridge Manor[u] | 110 ± 18 | — | 75 ± 3 | 60 ± 4 | 58 ± 6 | 53 ± 4 | 356 |
| | Gardner's[u] | 107 ± 4 | — | 64 ± 7 | 48 ± 1 | 35 ± 9 | 54 ± 5 | 308 |
| *T.* x *media* 'Citation' | Fairview | 156 ± 29 | 18 ± 6 | 67 ± 25 | 79 ± 32 | 13 ± 3 | 77 ± 9 | 410 |
| *T.* x *media* 'Chadwick' | Studebaker | 46 ± 11 | — | 50 ± 4 | 96 ± 12 | 28 ± 2 | 166 ± 15 | 386 |
| | Scarff's[u] | 55 ± 2 | — | 101 ± 10 | 48 ± 6 | 33 ± 12 | 146 ± 18 | 383 |
| *T.* x *media* 'Dark Green Spreader' | Zelenka[w] | 58 ± 19 | — | 103 ± 19 | 162 ± 32 | 21 ± 4 | 208 ± 39 | 552 |
| | Studebaker | 67 ± 15 | — | 91 ± 14 | 146 ± 20 | 65 ± 13 | 121 ± 3 | 490 |
| *T.* x *media* 'Densiformis' | Rhode Island | 180 ± 46 | —[x] | 209 ± 42 | 99 ± 66 | 10 ± 1 | 176 ± 35 | 674 |
| | Zelenka | 127 ± 28 | — | 88 ± 7 | 56 ± 8 | 14 ± 4 | 57 ± 9 | 342 |
| | Fairview | 79 ± 68 | — | 154 ± 34 | 65 ± 29 | 29 ± 32 | 89 ± 38 | 416 |
| | Studebaker | 229 ± 44 | — | 127 ± 25 | 96 ± 17 | 12 ± 2 | 81 ± 13 | 545 |
| | Cottage Gardens | 102 ± 7 | — | 106 ± 9 | 86 ± 6 | 15 ± 1 | 45 ± 5 | 454 |
| | Angelica[u] | 59 ± 16 | — | 92 ± 6 | 71 ± 3 | 43 ± 6 | 23 ± 3 | 288 |
| | Clinton[u] | 82 ± 21 | — | 122 ± 21 | 114 ± 23 | 84 ± 13 | 29 ± 9 | 431 |
| | Ridge Manor[u] | 39 ± 18 | — | 52 ± 36 | 36 ± 22 | 24 ± 18 | 26 ± 8 | 177 |
| | Gardner's[u] | 94 ± 19 | — | 142 ± 28 | 92 ± 38 | 26 ± 11 | 44 ± 5 | 398 |
| *T.* x *media* 'Densiformis Henryi[1] | Studebaker[u] | 33 ± 12 | —[x] | 39 ± 15 | 32 ± 3 | 29 ± 5 | 13 ± 4 | 146 |
| *T.* x *media* 'Eldon' | Studebaker[u] | 57 ± 7 | — | 90 ± 1 | 79 ± 16 | 46 ± 7 | 18 ± 4 | 290 |
| *T.* x *media* 'Everlow' | Studebaker[w] | 41 ± 13 | — | 75 ± 4 | 202 ± 16 | 31 ± 6 | 224 ± 14 | 573 |
| *T.* x *media* 'Fairview' | Fairview | 25 ± 12 | — | 119 ± 97 | 37 ± 31 | 9 ± 1 | 58 ± 4 | 248 |
| *T.* x *media* 'Greenwave' | Angelica[u] | 175 ± 65 | 34 ± 16 | 127 ± 31 | 106 ± 19 | 34 ± 8 | 222 ± 51 | 698 |
| *T.* x *media* 'Halloran' | Rhode Island | 79 ± 40 | — | 88 ± 11 | 66 ± 7 | 85 ± 10 | 227 ± 29 | 468 |
| *T.* x *media* 'Hatfieldii' | Rhode Island | 172 ± 18 | — | 195 ± 35 | 123 ± 18 | 9 ± 2 | 351 ± 40 | 850 |
| | Angelica[u] | 35 ± 7 | — | 181 ± 23 | 75 ± 5 | 63 ± 2 | 72 ± 6 | 426 |
| *T.* x *media* 'Henryi' | Zelenka | 114 ± 12 | — | 128 ± 14 | 104 ± 13 | 17 ± 2 | 76 ± 7 | 439 |
| *T.* x *media* 'Hicksii' | Rhode Island | 238 ± 37 | — | 124 ± 11 | 176 ± 24 | 10 ± 1 | 566 ± 30 | 1114 |
| | Fairview | 162 ± 29 | — | 86 ± 12 | 155 ± 26 | 14 ± 5 | 356 ± 53 | 773 |
| | Studebaker[w] | 277 ± 47 | — | 88 ± 9 | 155 ± 21 | 11 ± 3 | 241 ± 22 | 772 |
| | Cottage Gardens | 393 ± 16 | 23 ± 1 | 166 ± 5 | 143 ± 4 | 58 ± 1 | 285 ± 1 | 1068 |
| | Angelica[u] | 88 ± 23 | — | 136 ± 4 | 120 ± 3 | 70 ± 0 | 195 ± 15 | 609 |
| | Clinton[u] | 195 ± 28 | — | 209 ± 33 | 180 ± 25 | 135 ± 9 | 280 ± 33 | 999 |
| | Scarffs[u] | 276 ± 62 | — | 191 ± 39 | 148 ± 41 | 62 ± 5 | 318 ± 96 | 995 |
| | Ridge Manor[u] | 96 ± 33 | — | 144 ± 41 | 75 ± 4 | 59 ± 20 | 165 ± 14 | 539 |
| | Gardner's[u] | 214 ± 50 | 12.5 ± 3 | 232 ± 41 | 226 ± 92 | 74 ± 5 | 423 ± 22 | 1182 |
| *T.* x *media* 'Hicks Verkade' | Studebaker[u] | 31 ± 8 | — | 130 ± 24 | 46 ± 9 | 107 ± 12 | 88 ± 18 | 402 |
| *T.* x *media* 'Hiti' | Studebaker | 277 ± 47 | —[x] | 88 ± 9 | 155 ± 21 | 11 ± 3 | 241 ± 22 | 772 |
| *T. hunnewelliana* Rehd. | Zelenka[u] | 250 ± 16 | — | 61 ± 12 | 42 ± 4 | 19 ± 4 | 59 ± 7 | 431 |
| *T.* x *media* 'Globet Hunnewelliana' | Zelenka[u] | 81 ± 8 | — | 113 ± 37 | 73 ± 9 | 65 ± 18 | 34 ± 11 | 366 |
| *T.* x *media* 'Intermedia' | Cottage Gardens | 134 ± 13 | 28 ± 3 | 51 ± 6 | 57 ± 10 | 23 ± 11 | 35 ± 4 | 278 |
| *T.* x *media* 'Majestic' | Fairview | 16 ± 13 | — | 73 ± 13 | 37 ± 4 | 24 ± 2 | 93 ± 50 | 243 |
| *T.* x *media* 'Nigra' | Rhode Island | 56 ± 12 | 21 ± 12 | 194 ± 19 | 162 ± 26 | 15 ± 2 | 621 ± 74 | 1069 |
| | Fairview | 47 ± 39 | — | 188 ± 24 | 174 ± 52 | 11 ± 1 | 608 ± 112 | 1028 |
| | Cottage Gardens | 57 ± 3 | 23 ± 2 | 223 ± 19 | 135 ± 14 | 32 ± 4 | 393 ± 32 | 863 |
| *T.* x *media* 'Ohio Globe' | Studebaker[u] | 155 ± 24 | — | 168 ± 35 | 78 ± 46 | 71 ± 5 | 51 ± 13 | 523 |

Table 1. A summary of individual and total taxane content in the leaves of the different cultivars of *Taxus* by nursery, continued.

| Cultivar | Nursery | Average taxane[z] concentration[y] (%W/W) ± S.D. | | | | | | Total taxanes (g %) |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| *T.* x *media* 'Runyan' | Zelenka | 86 ± 28 | — | 59 ± 21 | 44 ± 21 | 17 ± 2 | 53 ± 25 | 259 |
| | Studebaker | 113 ± 32 | — | 93 ± 10 | 56 ± 5 | 12 ± 2 | 174 ± 17 | 448 |
| | Scarff's[u] | 65 ± 7 | — | 103 ± 44 | 87 ± 27 | 37 ± 11 | 111 ± 30 | 403 |
| *T.* x *media* 'Sebian' | Cottage Gardens | 64 ± 4 | — | 51 ± 1 | 36 ± 1 | 13 ± 1 | 206 ± 16 | 370 |
| *T.* x *media* 'Slavin' | Studebaker | 537 ± 13 | 19 ± 6 | 91 ± 9 | 121 ± 13 | 10 ± 2 | 169 ± 9 | 947 |
| *T.* x *media* 'Stovekin' | Studebaker | 34 ± 13 | 22 ± 6 | 76 ± 8 | 53 ± 3 | 93 ± 3 | 97 ± 5 | 375 |
| *T.* x *media* 'Sunburst' | Fairview | 132 ± 20 | — | 25 ± 3 | 83 ± 12 | — | 37 ± 11 | 277 |
| *T.* x *media* 'Super Densiformis' | Cottage Gardens | 118 ± 4 | — | 107 ± 1 | 89 ± 2 | 18 ± 1 | 65 ± 4 | 397 |
| *T.* x *media* 'Tauntonii' | Rhode Island | 35 ± 17 | — | 88 ± 5 | 109 ± 8 | 12 ± 1 | 535 ± 44 | 779 |
| | Zelenka | 37 ± 18 | — | 85 ± 22 | 73 ± 21 | 18 ± 2 | 298 ± 81 | 511 |
| | Studebaker | 39 ± 7 | — | 88 ± 14 | 99 ± 18 | 23 ± 8 | 391 ± 50 | 640 |
| | Gardner's[u] | 35 ± 4 | — | 80 ± 6 | 67 ± 6 | 40 ± 2 | 329 ± 37 | 511 |
| *T.* x *media* 'Vermeulen' | Angelica[u] | 31 ± 3 | — | 78 ± 12 | 85 ± 10 | 44 ± 3 | 61 ± 10 | 299 |
| *T.* x *media* 'Viridis' | Angelica[u] | 46 ± 15 | — | 45 ± 8 | 57 ± 3 | 34 ± 4 | 52 ± 11 | 234 |
| *T.* x *media* 'Thayerae' | Studebaker | 327 ± 71 | — | 129 ± 10 | 144 ± 22 | 12 ± 3 | 172 ± 30 | 784 |
| *T.* x *media* 'Tvurdy' | Studebaker[u] | 36 ± 16 | — | 143 ± 9 | 61 ± 7 | 101 ± 16 | 133 ± 17 | 474 |
| *T.* x *media* 'TV' | Scarff's[u] | 123 ± 23 | — | 56 ± 12 | 59 ± 3 | 23 ± 3 | 44 ± 10 | 305 |
| *T.* x *media* 'Wardii' | Zelenka | 27 ± 11 | —[x] | 79 ± 32 | 47 ± 34 | 18 ± 5 | 67 ± 22 | 238 |
| | Fairview | 47 ± 4 | — | 98 ± 9 | 39 ± 8 | 14 ± 1 | 87 ± 8 | 285 |
| | Studebaker[w] | 60 ± 15 | — | 92 ± 8 | 32 ± 3 | 11 ± 2 | 75 ± 8 | 270 |
| | Cottage Gardens | 57 ± 3 | — | 108 ± 6 | 59 ± 37 | 47 ± 3 | 51 ± 3 | 263 |
| | Ridge Manor[u] | 34 ± 5 | — | 111 ± 21 | 116 ± 70 | 106 ± 40 | 127 ± 68 | 494 |

[z]Taxanes Code: **1** = 10-Desacetyl Baccatin III, **2** = Baccatin III, **3** = 10-Desacetyl Taxol, **4** = Cephalomannine, **5** = 10-Desacetyl-7-*epi*-Taxol and **6** = Taxol.

[y]Numbers shown are multiplied by $10^4$.

[x]Denotes no detectable level.

[w]7 plants used.

[v]10 plants used.

[u]3 plants used.

The system was then purged for 3 minutes using a 50:50 mixture of Solvent A (reagent ethanol: tetrahydrofuran:methyl-*t*-butyl ether, 50:30:20) and Solvent C, followed by 10 minutes equilibration with the initial solvent composition. The total analysis time was 1 hour per sample. The flow rate was 1.1 mL/minute. The spectral data were collected over the 210–300 nm range of the absorption spectrum and all chromatograms were plotted at 227 nm, the absorption maximum of taxol. The external standard method of quantitation was used.

The $R_t$ values for 10-desacetyl baccatin III (**1**), baccatin III (**2**), 10-desacetyl taxol (**3**), cephalomannine (**4**), 10-desacetyl-7-*epi*-taxol (**5**), and taxol (**6**) were 10.77, 18.76, 35.91, 39.64, 41.34 and 42.07, respectively.

The linearity of the detector response was determined for taxanes **1** to **6** by injection of a series of standard solutions ranging in concentration from 10–240 µg/mL. A linear relationship was observed between the peak areas and the concentration of taxanes **1** to **6** ($r$ = 0.995, 0.999, 0.999, 0.999, 0.999 and 0.999, respectively) at the concentration range studied. The day-to-day variation in the peak area of taxanes **1** to **6** was minimum (CV 7.8%, 7.4%, 7.7%, 5.8%, 9.1% and 9.8% respectively; $n$ =12). Variation within a day in the peak area of taxanes **1** to **6** was even less (CV = 1.5%, 1.4%, 1.4%, 1.4%, 1.3% and 1.5%, respectively; $n$ =4). The detection limit of the different taxanes was determined to be below 100 ng for 10-desacetyl baccatin III (**1**) and baccatin III (**2**), and below 50 ng for 10-desacetyl taxol (**3**), cephalomannine (**4**), 10-desacetyl-7-*epi*-taxol (**5**) and taxol (**6**).

In summary, plants representing ninety-two accessions of yew (*Taxus* ) cultivars were acquired for this study and were received in two shipments. The first shipment arrived during the Autumn of 1992 and the 2nd during the Spring of 1993. Plants were sampled from November 15 to December 13, 1992 (1st shipment), and December 14, 1993 (2nd shipment). All plants were dormant at that time. The clippings were dried at 40C for 72 hours and the dried clippings separated into leaves and stems. Each organ was powdered (70 mesh) and extracted with 95% EtOH followed by partitioning of the extract between water and $CH_2Cl_2$. The latter fraction was cleaned-up prior to analysis using a celite solid phase extraction procedure and the purified extracts were analyzed by HPLC using a Curosil G column. The elution system was selected so that polar and relatively non-polar taxanes could be separated from other plant constituents. Replicates (3–10, mostly 6) were generated from samples of individual plants within each of the cultivars received from Rhode Island, Zelenka, Fairview Evergreen, Studebaker, Scarff's, Angelica, Clinton, Ridge Manor and Gardner's nurseries. Samples from Cottage Gardens, however, were pooled and analyzed in triplicates.

## Results and Discussion

In an effort to ascertain the viability of *Taxus* as a source for the anticancer drug taxol a study was initiated to survey as many of the available cultivars as possible for their content of taxol and other related taxanes. Clippings representing 92 accessions of *Taxus* collected from 10 nurseries were dried intact then separated into leaves and stems. Although each organ was analyzed and a calculated value was obtained for the respective clipping, we report here the data related to the leaves only. The taxane contents of the stem, for the six compounds studied, showed the same trend in concentration as that of the leaves but in lesser amounts (20–40% the values for the leaves). The average dry clipping weight was 12.4 ± 5.5 (n = 83) and the average ratio of leaf to stem was 3.1 ± 1.3 (n = 83). The total taxane content of the leaves, stems and clippings in the studied cultivars ranged from 0.0135–0.147%, 0.0055–0.046% and 0.0121–0.118%, respectively. Table 1 summarizes the results of the average concentrations of compounds **1–6** and the percent of total taxanes in the leaves of the 92 accessions studied. This survey resulted in the identification of 24 cultivars with needles rich in taxol with concentrations exceeding 0.01% and as high as 0.062%. These are (by cultivar): *T. cuspidata*, *T. cuspidata* 'Capitata' (4 out of 5 nurseries), *T. cuspidata* 'Columnaris', *T. cuspidata* 'Fastigiata', *T.* x *media* 'Bobbink', *T.* x *media*, 'Brevifolia', *T.* x *media* 'Brownii' (3 out of 6 nurseries), *T.* x *media* 'Chadwick', *T.* x *media* 'Dark Green Spreader', *T.* x *media* 'Densiformis', (1 out of 9 nurseries), *T.* x *media* 'Everlow', *T.* x *media* 'Greenwave', *T.* x *media* 'Halloran', *T.* x *media* 'Hatfieldii', *T.* x *media*, 'Hicksii', *T.* x *media* 'Hiti', *T.* x media 'Nigra', *T.* x *media* 'Runyan', *T.* x *media* 'Sebian', *T.* x *media* 'Slavin', *T.* x *media* 'Tauntonii', *T.* x *media* 'Thayerae', *T.* x *media* 'Tvurdy', and *T.* x *media* 'Wardii' (1 out of 5 nurseries).

10-Desacetyl baccatin III (**1**), a useful precursor currently used in the semisynthesis of taxol (11) was found in concentrations exceeding 0.01% and as much as 0.066% in 18 cultivars. These are (by cultivar): *T. baccata* 'Repandens', *T. cuspidata* (2 out of 4 nurseries), *T. cuspidata* 'Capitata' (2 out of 5 cultivars), *T. cuspidata* 'Columnaris', *T. cuspidata* 'Fastigiata', *T.* x *media* 'Amherst', *T.* x *media* 'Bobbink', *T.* x *media* 'Brownii', *T.* x *media* 'Citation', *T.* x *media* 'Densiformis' (4 out of 9 nurseries), *T.* x *media* 'Greenwave', *T.* x *media* 'Hatfieldii' (1 out of 2 nurseries), *T.* x *media* 'Hicksii' (7 out of 9 nurseries), *T.* x *media* 'Hiti', *T. hunnewelliana*, *T.* x *media* 'Ohio Globe', *T.* x *media* 'Slavin', and *T.* x *media* 'Thayerae'.

Cephalomannine (**4**), a taxane with antileukemic properties of its own (12) was found to be present in amounts ranging from one fifth to four times those of taxol among all cultivars. Seventeen cultivars were identified with cephalomannine content exceeding 0.01% and as high as 0.0386%. These are (by cultivar): *T. cuspidata* (3 out of 4 nurseries), *T. cuspidata* 'Capitata' (4 out of 5 nurseries), *T.* x *media* 'Bobbink', *T.* x *media* 'Brevifolia' (1 out of 2 nurseries), *T.* x *media* 'Brownii' (1 out of 6 nurseries), *T.* x *media* 'Dark Green Spreader', *T.* x *media* 'Densiformis' (1 out of 9 nurseries), *T.* x *media* 'Greenwave', *T.* x *media* 'Hatfieldii' (1 out of 2 nurseries), *T.* x *media* 'Henryi', *T.* x *media* 'Hicksii' (8 out of 9 nurseries), *T.* x *media* 'Hiti', *T.* x *media* 'Nigra', *T.* x *media* 'Slavin', *T.* x *media* 'Tauntonii' (1 out of 4 nurseries), *T.* x *media* 'Thayerae', and *T.* x *media* 'Wardii' (1 out of 5 nurseries).

10-Desacetyl taxol (**3**) is another major taxane which could easily be converted to taxol. Twenty cultivars were found to contain significant concentrations of **3** (0.01% and up to 0.026%). These are (by cultivar): *T. cuspidata* (2 out of 3 nurseries), *T. cuspidata* 'Capitata', *T. cuspidata* 'Columnaris', *T.* x *media* 'Brevifolia', *T.* x *media* 'Chadwick' (1 out of 2 nurseries), *T.* x *media* 'Dark Green Spreader' (1 out of 2 nurseries), *T.* x *media* 'Densiformis' (6 out of 9 nurseries), *T.* x *media* 'Fairview', *T.* x *media* 'Greenwave', *T.* x *media* 'Hatfieldii', *T.* x *media* 'Henryii', *T.* x *media* 'Hicksii' (7 out of 9 nurseries), *T.* x *media* 'Hicks Verkade', *T.* x *media* 'Globet Hunnewelliana', *T.* x *media* 'Nigra', *T.* x *media* 'Runyan' (1 out of 3 nurseries), *T.* x *media* 'Super Densiformis', *T.* x *media* 'Thayerae', *T.* x *media* 'Tvurdy', and *T.* x *media* ' Wardi' (2 out of 5 nurseries)

Baccatin III (**2**) and 10-desacctyl-7-*epi*-taxol (**5**) are two minor taxanes in the cultivars studied. Although **5** exists in almost all cultivars, concentrations at or above 0.01% are rare. The monitoring of 10-desacetyl 7-*epi*-taxol is not as much for its economical value as it is for the difficulty of its separation from taxol during the isolation of the latter. On the other hand, although baccatin III is a taxane with beneficial utility as a precursor for taxol, it was only found in a few cultivars and at negligible concentration (Table 1).

It is evident from the data shown in Table 1 that there is a wide variation in the taxanes content for some cultivars grown in different nurseries. For example the total taxanes content varied from 0.0177 to 0.0674% among the nine nurseries producing *T.* x *media* 'Densiformis'; even more variation existed for the individual taxanes e.g. the taxol content ranged from 0.0026% to 0.0176%. The variation among nurseries for the total taxanes and the individual taxanes is less drastic for *T.* x *media* 'Hicksii' (9 nurseries), where the range for total taxanes was 0.061–0.118% and for taxol was 0.016–0.057%. Even less variation was observed with *T.* x *media* 'Nigra'(3 nurseries) where the total taxanes ranged from 0.086–0.107% and the taxol content ranged from 0.039% to 0.062%. The variation among nurseries could be due to different cultivation practices, soils and climatic conditions.

It is therefore demonstrated from this study that clippings of several cultivars of *Taxus* are rich in taxanes and surpass the bark of *T. brevifolia* (contents of **1–6** of 0.0249, 0.0013, 0.0059, 0.0029, 0.00054 and 0.0157, respectively) as a source for taxol and other taxanes. The dried needles, from *T.* x *media* 'Nigra', *T. cuspidata*, *T. cuspidata* 'Capitata', *T.* x *media* 'Hicksii', and *T.* x *media* 'Tauntonii' were found to contain amounts of taxol three to six times higher than the typical levels reported in *T. brevifolia* bark (0.01–0.02%). Further, the dried needles of *T. baccata* 'Repandens', *T. cuspidata*, *T.* x *media* 'Hicksii', and *T.* x *media* 'Slavin' serve as a good source for 10-desacetyl baccatin III, a useful precursor for the synthesis of taxol. Plant selections of taxane rich chemovars should increase the concentration of taxanes to even higher yields.

## Literature Cited


1. Hansen, R.C., K.D. Cochran, H.M. Keener, and E.M. Croom, Jr. 1994. *Taxus* populations and clippings yields at commercial nurseries. HortTechnology 4:372–377.

2. Croom, E.M. Jr. 1990. Ornamental *Taxus* cultivars as sustainable, abundant plant materials for clinical Taxol supplies. Workshop on Taxol and *Taxus*. Current and Future Perspectives. Session IIIa. Presentation #6.

3. Croom, E.M., Jr. 1995. *Taxus* for taxol and taxoids p. 37–70; *In*: Matthew Suffness, Ed., Taxol Science and Application, CRC Press, New York.

4. ElSohly, H.N., E.A. El-Kashoury, E.M. Croom, Jr., M.A. ElSohly, and J.D. McChesney. 1994. Taxol content of the fresh needles and other organs of various cultivars of ornamental *Taxus*. Zagazig J. Pharm. 3:104–110.

5. ElSohly, H.N., E.M. Croom, Jr., W.J. Kopycki, A.S. Joshi, and J.D. McChesney. 1995. Concentrations of Taxol and related taxanes in the needles of different Taxus cultivars. Phytochem. Anal. 6:149–156.

6. ElSohly, H.N., E.M. Croom, Jr., W.J. Kopycki, A.S. Joshi, and J.D. McChesney. 1997. Diurnal and seasonal effects on the taxane content of the clippings of certain *Taxus* cultivars. Phytochem. Anal. 8:124–129.

7. ElSohly, H.N., E.A. El-Kashoury, E.M. Croom, Jr., M.A. ElSohly, K.D. Cochran, and J.D. McChesney. 1995. Effect of drying *Taxus* needles on their taxol content: The impact of drying intact clippings. Planta Med. 61:290–291.

8. ElSohly, H.N., E.M. Croom, Jr., E.A. El-Kashoury, A.S. Joshi, W.J. Kopycki, and J.D. McChesney, 1997. Effect of drying conditions on the taxane content of the needles of ornamental *Taxus*. Planta Med. 63:83–84.

9. ElSohly, H.N., E.M. Croom, Jr., E.A. El-Kashoury, M.A. ElSohly, and J.D. McChesney. 1994. Taxol content of stored fresh and dried Taxus clippings. J. Nat. Prod. 7:1025–1028.

10. Kopycki, W.J., H.N. ElSohly, and J.D. McChesney. 1994. HPLC determination of taxol and related compounds in Taxus plant extracts. J. Liq. Chromatogr. 17:2569–2591.

11. Holton, R.A. 1991. Method for preparation of taxol using an oxazinone. U.S. Patent # 5,015,744.

12. Miller, R.W. and R.G. Powell. 1980. Cephalomannine and its use in treating leukemic tumors. U.S. Patent # 4,206,221.



# Effect of Split Fertilizer Application and Irrigation Volume on Nitrate-Nitrogen Concentration in Container Growing Area Soil[1]

**David J. Colangelo and Mark H. Brand**[2]
*Department of Plant Science, U-67*
*University of Connecticut, Storrs, CT 06269-4067*

## Abstract

Outdoor-grown, containerized, *Aronia melanocarpa* (Michx.) Ell. and *Rhododendron* 'Roseum Elegans' were grown atop soil-filled boxes that had been recessed into a grassed field in separate studies. *Aronia* were fertilized with either a single application of controlled-release fertilizer (CRF) or a split application of CRF separated by 36 days. *Rhododendron* were supplied a single application of CRF and either a standard or excessive irrigation volume on each irrigation day. Soil samples were taken in 30 cm (12 in) layers to a depth of 90 cm (36 in) beneath containers at 14-day intervals and soil $NO_3$-N concentrations were determined. Accumulation of $NO_3$-N was immediate in the 0–30 cm (0–12 in) layer for both species with accumulation of $NO_3$-N in the deeper soil layers occurring later. Split application of a CRF was somewhat effective at reducing $NO_3$-N accumulation at specific times and in specific soil layers, but reductions were not as substantial as studies on $NO_3$-N concentrations in leachate have indicated. The large irrigation volumes used in the irrigation volume study resulted in $NO_3$-N moving rapidly through the soil profile beneath containers.

**Index words:** *Aronia melanocarpa, Rhododendron* 'Roseum Elegans', controlled-release fertilizer, leaching, slow-release fertilizer, groundwater.

## Significance to the Nursery Industry

Nursery crop management practices that contribute to nitrate-N loading to soil beneath container crops are regarded as a threat to groundwater quality and are of concern to the nursery industry and the public. Use of controlled-release fertilizers (CRFs) is considered to be more environmentally sound than use of soluble fertilizers and split applications of CRFs are thought to cause less $NO_3$-N leaching than a single, large CRF application. In this study we showed that CRFs can contribute substantial amounts of $NO_3$-N to the soil profile beneath a container production area even when a split CRF application is used. Furthermore, the large and frequent irrigation volumes typically applied to container crops, combined with the intensive nature of container production, may cause $NO_3$-N to leach deep into the soil profile. These data suggest that growers must be careful to avoid excessive irrigation volumes which are the result of continuing irrigation beyond the time needed to supply an adequate amount of water to the crop. This study is applicable to growers having container production on gravel or porous fabric-covered areas, but not to production on plastic-covered areas.

## Introduction

Nursery crops are increasingly grown in containers due to the economic benefits of container production and consumer demand. This trend away from field production is likely to continue. Container crops are grown in porous, highly organic media that require frequent irrigation and large irrigation volumes to optimize crop growth (6). Large irrigation volumes supplied to containerized crops have been shown to increase the volume of leachate and quantity of $NO_3$-N exiting the container (10, 12). A recent survey of container nursery operations revealed that some samples of irrigation

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[2]Graduate Student and Associate Professor, respectively.