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TAXOL CONTENT OF STORED FRESH AND DRIED *TAXUS* CLIPPINGS

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ABSTRACT.—The taxol content of dried *Taxus* biomass was monitored monthly for 15 months. Intact and finely ground biomass was stored at room temperature (22°–24°) as well as under refrigeration (2°–4°). In addition, intact fresh clippings stored under refrigeration in sealed plastic bags for up to 10 weeks were evaluated for changes in taxol content. Analysis indicates that properly dried *Taxus* clippings can be stored either intact or powdered at room temperature or under refrigeration with no apparent loss of taxol content. The taxol content in fresh intact clippings was also stable for at least 10 weeks when stored under refrigeration.

The importance of the anticancer drug taxol for the treatment of ovarian cancer (1) coupled with its low yield from *Taxus brevifolia* bark (2) has prompted the search for alternative sources (3–6). The needles of clippings of certain cultivars of ornamental *Taxus* have been found to be a possible economic and renewable source of biomass for the production of taxol and related compounds (7,8). Several studies related to the preservation of taxol content in post-harvest biomass were initiated by our group (9,10). Our efforts have shown that the clippings are best dried intact at 40° for a relatively short period of time (2–3 days) (10). These drying conditions were validated on large scale during the processing of a large batch of *Taxus* × *media* "Hicksii" clippings that was provided to the National Cancer Institute (approximately 18,000 kg) which resulted in a biomass of high taxol content (average 0.016 g%).

The present study was initiated to monitor the taxol content of intact fresh clippings of *T.* × *media* "Nigra" stored under refrigeration for 10 weeks, and the taxol concentration of dried clippings (intact and powdered) stored at room temperature and under refrigeration for 15 months.

The concentrations in ornamental *Taxus* clippings of taxol and of other related taxanes such as 10-deacetylbaccatin III, a valuable intermediate in the semi-synthesis of taxol, were found to be sufficiently high for this plant material to be considered as an economic source for these substances (8). Since the clipping of ornamental *Taxus* plants is a seasonal undertaking, potentially large amounts of biomass might become available for processing within a short period of time. To provide for the economic processing of biomass for taxol production, however, storage of plant material will be necessary. Therefore, this study was initiated to ascertain the stability of taxol in the biomass under various storage conditions and times of storage. Both intact and finely ground clippings were evaluated. In addition, the stability of taxol in fresh clippings stored under refrigeration was examined.

Table 1 shows the average taxol content of the fresh clippings of *T.* × *media* "Nigra" stored under refrigeration for 10 weeks. The data showed that no loss of taxol content was observed over the storage period.

Table 2 records the observed concentrations of taxol in dried clippings of *T.* × *media* "Nigra" stored both intact and as finely ground material under two temperature conditions, refrigeration (2°–

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TABLE 1. Taxol Content of Fresh Clippings of *T.* × *media* "Nigra" Stored Under Refrigeration (2°–4°).

Time (Week)	Average Taxol Content g%[a]±S.D. (c.v. n=5)
Start (Time)	0.0181±0.00235 (13%)
1	0.0190±0.00122 (6.4%)
2	0.0166±0.00065 (3.6%)
3	0.0159±0.00061 (3.8%)
4	0.0164±0.00065 (3.7%)
5	0.0193±0.00089 (4.2%)
6	0.0172±0.00210 (12.2%)
7	0.0210±0.00185 (8.8%)
10	0.0217±0.00068 (3.1%)

[a]Calculation based on fresh weight.

4°) and room temperature (22°–24°) for 15 months. It was observed that within each category there was no indication of any change in concentration ot taxol over the 15-month period. It is noteworthy that the observed taxol content of the powdered and refrigerated samples was very similar to that of both the powdered and intact samples stored at room temperature. However, the observed taxol content of the intact refrigerated samples was higher than levels observed for the other three categories. Whether this difference in taxol content is attributable to the samples themselves having a higher concentration to start with (i.e., sampling differences) is not known, since every effort was made to randomize the samples initially.

In conclusion, storage of fresh *Taxus* clippings under refrigeration for up to 10 weeks had no negative effect on their taxol content. Furthermore, dried clippings, either intact or powdered, did not show any reduction in taxol content when stored under refrigeration or at room temperature for up to 15 months. Therefore, it does not appear to be necessary for *Taxus* clippings to be processed immediately after harvesting and proper drying; they retain their taxol content and processing may be conducted over the entire calendar year.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Analytical instrumentation consisted of a Waters Model 590 solvent delivery system. The detector was an

TABLE 2. Taxol Content of Dried Clippings of *T.* × *media* "Nigra" Stored Under Refrigeration (2°–4°) and at Room Temperature (22°–24°).

Time (Month)	Average Taxol Content g%[a]±S.D. (c.v. n=5)			
	Refrigeration		Room Temperature	
	Intact	Powdered	Intact	Powdered
Start	0.0257±0.00106 (4.1%)	0.0224±0.00087 (3.9%)	0.0196±0.00145 (7.4%)	0.0217±0.00191 (8.8%)
1	0.0327±0.00192 (5.9%)	0.0234±0.00099 (4.2%)	0.0195±0.00057 (2.9%)	0.0216±0.00083 (3.8%)
2	0.0248±0.00100 (4.1%)	0.0204±0.0015 (7.5%)	0.0236±0.00090 (3.8%)	0.0177±0.00180 (10.3%)
3	0.0308±0.00138 (4.5%)	0.0220±0.00096 (4.4%)	0.0270±0.00162 (6%)	0.0212±0.00160 (7.6%)
4	0.0306±0.00184 (6%)	0.0206±0.00256 (12%)	0.0208±0.00159 (7.7%)	0.0197±0.00165 (8.4%)
5	0.0369±0.00311 (8.5%)	0.0195±0.00101 (5.2%)	0.0242±0.00147 (6%)	0.0182±0.00168 (9.2%)
6	0.0340±0.00337 (9.9%)	0.0161±0.00105 (6.5%)	0.0244±0.00164 (6.7%)	0.0200±0.00175 (8.7%)
7	0.0369±0.00315 (8.5%)	0.0196±0.00236 (12%)	0.0213±0.00142 (6.7%)	0.0211±0.00107 (5.1%)
8	0.0350±0.0020 (5.7%)	0.0227±0.00206 (9%)	0.0237±0.0020 (8.75%)	0.0217±0.00171 (7.8%)
9	0.0293±0.00240 (8.2%)	0.0204±0.00140 (5.6%)	0.0197±0.00082 (4.2%)	0.0196±0.00152 (7.8%)
10	0.0287±0.00280 (9.9%)	0.0186±0.00140 (7.7%)	0.0255±0.00115 (4.5%)	0.0210±0.00037 (1.7%)
12	0.0375±0.00396 (10.6%)	0.0233±0.00214 (9.2%)	0.0249±0.00072 (2.9%)	0.0244±0.00154 (6.8%)
15	0.0336±0.00132 (3.9%)	0.0233±0.00207 (8.9%)	0.0264±0.00218 (8.2%)	0.0225±0.00071 (3.2%)

[a]Calculation based on dry weight.

LDC Milton-Roy variable wavelength detector set at 227 nm with aufs of 0.1. A μBondapak 10-μm C-18 column (3.9 mm × 300 mm; Waters Associates, Milford, MA) was used together with a reversed-phase guard column (3.2 mm × 15 mm; Brownlee Laboratory, Inc., Santa Clara, CA). Hplc grade MeOH and H_2O were used. Samples were filtered through a Millex HV filtering unit (0.45 μm, Millipore Corporation, Bedford, MA) prior to hplc analysis.

PLANT MATERIAL.—The cultivar name reported is as sold by the nursery. The fresh clippings (ca. 8.8 kg) were from a previous season's growth of 3–6 year old *T.* × *media* "Nigra" plants and were obtained from Rhode Island Nursery (Newport, Rhode Island). These were cut on March 1, 1991 and received on March 5, 1991. A voucher specimen is deposited in the Herbarium, Biology Department, University of Mississippi (MISS 55972).

A representative sample of the fresh clippings as received (600 g) was taken and divided into nine parts (each approximately 65 g) which were stored in sealed plastic bags under refrigeration (2°–4°). The first sub-sample was extracted and analyzed using 5 replicates of 10 g each (time zero). The remaining eight sub-samples were each analyzed at 1, 2, 3, 4, 5, 6, 7, and 10 weeks.

The balance of the plant clippings (8.24 kg) was divided into two parts (4.12 kg each). The first part was dried at 40° in a Roanoke bulk curing barn (no light, continuous air flow by the barn's fan) for 3 days. The barn-dried clippings were powdered in a Wiley Mill (one-tenth inch screen size), the powder mixed well, and 50-g aliquots transferred into sealed plastic bags. A total of 28 packages (50 g each) were prepared, of which half were stored at room temperature (22°–24°) and half under refrigeration (2°–4°). The second batch of the fresh clippings (4.12 kg) was divided into 28 brown bags each containing ca. 165 g fresh clippings. Each bag was dried individually under barn conditions (so as to contain any dried fallen leaves). The bags were divided into two sets of 14 bags each. One set was stored at room temperature and the other set was placed in sealed plastic bags and stored under refrigeration.

EXTRACTION AND SAMPLE PREPARATION.—*Fresh clippings.*—Samples of 10 g each (× 5) were weighed and extracted with 95% EtOH (100 ml) in a blender. The blended material was transferred quantitatively into a 250-ml Erlenmeyer flask and allowed to macerate for 24 h, then filtered. The extraction was repeated three additional times and the combined filtrates and washings (500 ml) were evaporated to dryness *in vacuo* at 40°.

Dried powdered clippings.—Samples of 10 g each (× 5) were weighed and extracted with 95% EtOH (100 ml×4) by maceration for 24 h according to the procedure described above.

Dried intact clippings.—The plant material was powdered in a Wiley Mill (one-tenth inch screen size), and 10-g samples (× 5) were weighed and extracted as described for the powdered clippings.

Standard.—Authentic taxol was obtained from the National Cancer Institute. MeOH solutions containing approximately 100 μg/ml taxol were prepared and a 10-μl volume was injected.

Preparation of extracts for hplc analysis.—Approximately 100 mg-samples of the dried EtOH extracts were partitioned and purified by solid-phase extraction using a procedure described in detail elsewhere (7).

ANALYTICAL HPLC METHOD.—Analyses were performed on a column packed with a $C\text{-}_{18}$-bonded Si gel phase using a mobile phase consisting of MeOH-H_2O (65:35). The flow rate was 1.2 ml/min, and all chromatograms were plotted at 227 nm, the uv absorption maximum of taxol. Sample injection volumes were 10μl. Samples for injection were prepared by dissolving the solid phase purified samples in MeOH (1 ml). The external standard method of quantitation was employed. The hplc conditions were adjusted such that cephalomannine was at least 50% resolved from taxol and little or no cephalomannine was included in the taxol measurements.

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