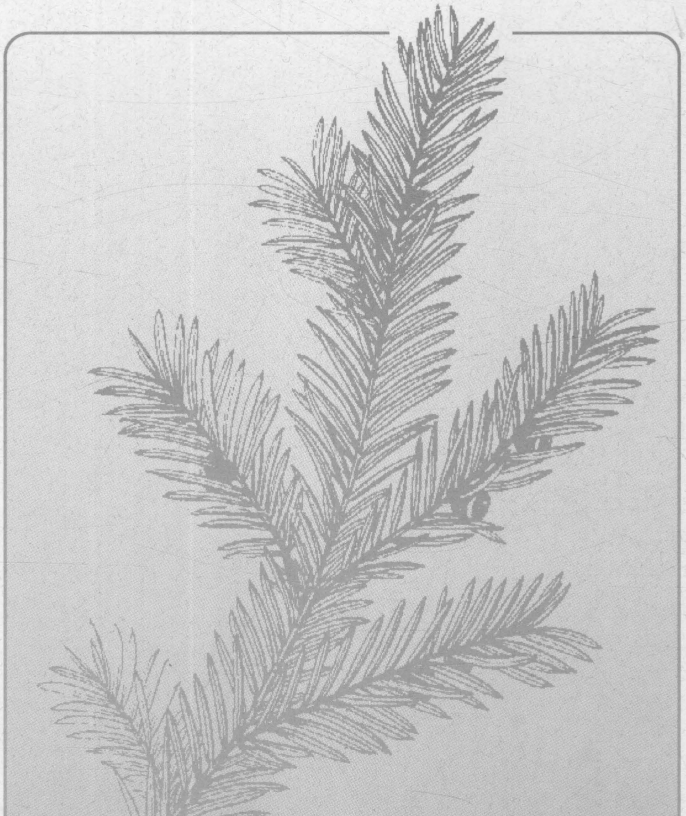
Taxus and Taxol

A Compilation of Research Findings



THE OHIO STATE UNIVERSITY

OARDC

December 1999
Special Circular 150
Ohio Agricultural Research and Development Center

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Taxus and Taxol

A Compilation of Research Findings

Edited By

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Contents

Preface

The development of the chemotherapeutic cancer drug, Taxol, was designated an "emergency priority" in the early 1990s by the National Cancer Institute (NCI). Taxol was identified as one of the most promising anti-cancer drugs to be discovered in 20 years.

According to the American Cancer Society, ovarian cancer afflicted an estimated 24,000 American women during 1992, and 13,600 died. A women's lifetime risk for ovarian cancer was one in 65. Breast cancer was diagnosed in approximately 182,000 women during 1994, and 46,000 died. A woman's lifetime risk was one in nine.

After more than three decades of research and development (see "History of the Development of Taxol as a Cancer-Fighting Drug" on page 11), the Food and Drug Administration (FDA) finally approved Taxol for treatment of ovarian cancer on December 29, 1992, and for treatment of breast cancer on April 15, 1994. Unfortunately, the drug was not found to be effective against most cancers that more typically afflict men.

NCI began the search for a chemotherapeutic cancer drug in 1958 with a general plant species screening program. Extracts from more than 35,000 species were collected for anti-cancer testing. The Pacific yew tree *(Taxus brevifolia* Nutt.) was the first plant species to demonstrate anti-cancer properties. These properties were isolated in very low concentrations from extracts found in the bark of the Pacific yew.

Taxus brevifolia is a species of the genus *Taxus* (commonly named yew). The common name for this species is the Pacific yew. These trees are often found to be 200 years old in their native habitat.

As Taxol showed more and more promise during its early development, research personnel recognized that harvesting trees for bark would not provide a renewable source of this natural product. Whole trees had to be sacrificed. On average, three to four 150- to 200-year-old trees would have to be harvested to supply the necessary raw material (bark) to treat one cancer patient. An adequate supply of the drug could not be assured.

The only place on the earth where the Pacific yew grew naturally was in forests in the northwestern United States, primarily in Oregon and Washington. Moreover, many citizens of the United States and other countries were opposed to the harvest of naturally occurring trees and shrubs because of perceived insults to a stable environment. In addition, this forested area was a natural home for the endangered, northern-spotted owl.

Other species of yews are commonly used in ornamental landscaping around our homes and public buildings. Major ones are *Taxus baccata* and *Taxus cuspidata*. A hybrid, *Taxus* x *media*, has also been designated an ornamental yew. While this hybrid cannot be verified, it is reported as *Taxus cuspidata* x *Taxus baccata*. More than 100 cultivars have been selected from these two species and this hybrid for landscape use. These yews have been used extensively in Eastern and Midwestern landscapes, especially since the turn of the century. Since they naturally mature to a medium- or large-sized shrub and are frequently used as small foundation plants in the landscape, they are regularly pruned to keep the planting in scale with the foundation area.

During the process of growing *Taxus* for landscape use, production nurseries annually prune *Taxus* plants to develop the small, dense, compact plant that is desired in the landscape marketplace. The clippings from these pruning operations fall to the ground and eventually break down and decompose. Dr. Edward M. Croom Jr., University of Mississippi, posed the following question: "Could these so-called waste clippings be collected after pruning and provide an annual renewable source of biomass for Taxol production?" Since only clippings were needed, whole plants would not have to be sacrificed.

For ornamental yews, the needles were found to be the primary source of Taxol, whereas the bark was the primary source from the Pacific yew. In addition, while the bark of the tree was found to have a Taxol content of approximately 0.01%, the needles of a popular ornamental hybrid yew, *Taxus* x *media* 'Hicksii,' were found to have more than 0.02%. Harvesting clippings offered the opportunity for a renewable Taxol source and associated economic benefits to nursery growers.

In the spring of 1991, the National Cancer Institute announced that a supply crisis for Taxol existed. There simply was not enough bark available to meet the demand for Taxol for clinical studies, let alone the anticipated demand if the drug were to be approved for general use. The question was raised: "Do we need to sacrifice a unique Pacific yew ecosystem in order to treat ovarian and breast cancer for our mothers, sisters, and daughters?"

Through funding from the NCI and the U.S. Department of Agriculture's Agricultural Research Service (USDA/ARS), a research team from the Ohio Agricultural Research and Development Center (OARDC) at The Ohio State University was asked to join the Research Institute of Pharmaceutical Sciences at the University of Mississippi and Zelenka Nursery, Inc., at Grand Haven, Michigan (the nation's largest producer of ornamental *Taxus* plants for landscapes), to form what was called the "Alliance for Taxol."

In response to the Taxol supply crisis, the Alliance for Taxol agreed to sign a contract to harvest, dry, store, and ultimately deliver 100,000 lbs. of *Taxus* clippings (wet-weight) to the National Cancer Institute by the summer of 1992. Approximately 25 research people from the three institutions contributed in one way or another to the work of the alliance. The contract was fulfilled.

A key to OARDC's participation in the alliance was the potential for inter-disciplinary research among departments at the research center and the years of *Taxus* research that had been conducted as a result of the *Taxus* collection at the Chadwick Living Herbarium of *Taxus* at OARDC's Secrest Arboretum. More than 100 types of *Taxus* plants were prophetically collected and planted, beginning in May 1942, by Dr. L. C. Chadwick and his graduate student, Raymond A. Keen. Fifty years later, as a part of the alliance's research efforts during 1991 and 1992, many samples of plant material were supplied to numerous research organizations worldwide that were involved in the search for the "super" *Taxus* cultivar that would ultimately yield maximum quantities of Taxol.

Fortunately, the Taxol supply crisis was at least temporarily eased by January 1994. Through a production process known as semi-synthesis, a precursor to Taxol, 10-deacetylbaccatin III, was successfully used to produce Taxol, which by then was generically identified as paclitaxel. Taxol was registered as the trade name for the formulated drug. The primary source of the taxane, 10-deacetylbaccatin III, was found from renewable biomass (twigs and needles) from the European yew *(Taxus baccata)* and the Himalayan yew *(Taxus wallichiana)*. Unfortunately, as the names suggest, this material grows in Europe and Asia. Therefore, the urgent need for a renewable source of *Taxus* biomass from Ohio nurseries and those in surrounding states did not materialize.

The papers contained in this publication resulted from the valiant efforts of the 25 research people who responded to the urgent call for delivery of *Taxus* biomass for clinical studies of Taxol treatments for ovarian and breast cancer patients during the spring of 1991. Three of the papers were published in refereed scientific journals; two were presented as papers at annual meetings of the American Society of Agricultural Engineers; and four were progress reports that outlined work that was initiated but never finished because research funding ceased as fast as it began. The final paper in this document was presented as a summary of the work of the Alliance for Taxol at the National Cancer Institute's Workshop on *Taxus*, Taxol, and Taxotere in September of 1992.

This special circular, representing a compilation of the reports resulting from the work of the Alliance for Taxol, benefited immensely from the editing skills of Joy Ann Fischer from the Section of Communications and Technology.

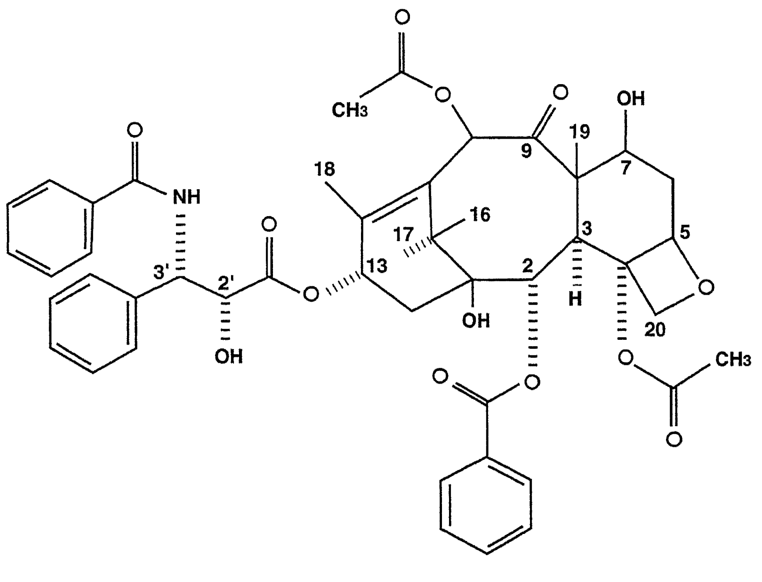
Robert C. Hansen
Editor

Taxol is used in this special circular to refer to the drug that now has the generic name paclitaxel and the registered trade name Taxol® (Bristol-Myers Squibb Company, New York, N. Y.).

Taxotere is used in this publication to refer to the drug that now has the generic name docetaxel and the registered trade name Taxotere® (Rhone-Poulenc-Rorer Pharmaceuticals, Inc., Collegeville, Pa.).

Dedicated to my sister,
Lois Jean Hansen Montoya,
who died from ovarian cancer,
December 28, 1998

History of the Development of Taxol As a Cancer-Fighting Drug



1963
The National Cancer Institute (NCI) found that yew samples showed activity against 9KB cancer-cell tissue culture. NCI sent a subsample to Monroe Wall, Ph.D., a medicinal chemist working under contract to NCI at Research Triangle Institute in North Carolina.

1964
Wall's group found that a crude extract of the yew bark was effective in both the cancer-cell tissue system and against a mouse leukemia. They worked to isolate the primary active principle of Taxol.

1966
Wall asked NCI to give the yew material special priority for research. He isolated the active principle and named it Taxol.

1969
NCI checked the activity of all parts of the Pacific yew. They now knew three things — the structure of Taxol, its success in cancer screens, and something about how it worked against cancer.

1971
Wall, with Mansukh Wani (at Research Triangle Institute) and Andrew McPhail (of Duke University), published the structure of the Taxol molecule, a complex diterpene with an unusual oxetane ring and an ester side chain.

1974
Taxol began to show results against a recently developed B16 mouse melanoma system. During the 1970s, cytotoxicity tests continued with tumor lines in new animal

screens, including human tumor xenografts (tissues grafted from one species to another.)

1977
Preclinical work on Taxol began. NCI contacted Susan Horowitz (professor of molecular pharmacology at Albert Einstein College of Medicine in the Bronx), working under an NCI Cancer Research Emphasis Grant, to ask her to investigate how Taxol worked on cancer cells. With graduate student Peter Schiff, she found that Taxol inhibited the replication of human tumor cells. Specifically, Taxol induces tubulin polymerization and inhibits disassembly of microtubules, an activity necessary to complete cell division.

1978
Taxol showed positive results in human cancer xenografts. Taxol showed activity in three systems, including a human breast cancer xenograft developed in the late 1970s.

1979
Horowitz and Schiff published their findings about Taxol's action of freezing microtubules and causing the cell to die.

1980
Toxicology studies began. Scientists looked for a suitable surfactant formulation for administering the insoluble drug.

1982
NCI approved Taxol for filing an Investigational New Drug Application (INDA) with the Federal Drug Administration.

1983
Phase I clinical trials began, testing patients who were not responding to other treatments, determining doses and toxicity, and generating data on dose limits of Taxol.

1987
NCI contracted for collection of 60,000 pounds of dry Pacific yew bark.

1988
Phase II clinical trials showed 30 percent improvement in patients with unresponsive cases of advanced ovarian cancer.

1989
Trials of Taxol progressed for other forms of cancers — breast, cervical, colon, gastric, non-small-cell lung, prostate, head and neck, small-cell lung, and renal. NCI contracted for an additional 60,000 pounds of dry bark.

1990
Phase II trials showed 48 percent tumor shrinkage with metastatic breast cancer patients who had at least one prior chemotherapy regime. (Metastatic refers to cancers which tend to spread from one body part to another.)

1991
The Ohio Agricultural Research and Development Center joined the Research Institute of Pharmaceutical Sciences, the School of Pharmacy of the University of Mississippi, and Zelenka Nursery, Inc., Grand Haven, Michigan, in a contract with the Cooperative State Research Service, USDA, to harvest and deliver critically needed *Taxus* clippings (100,000 lbs. fresh weight) to the National Cancer Institute by the summer of 1992.

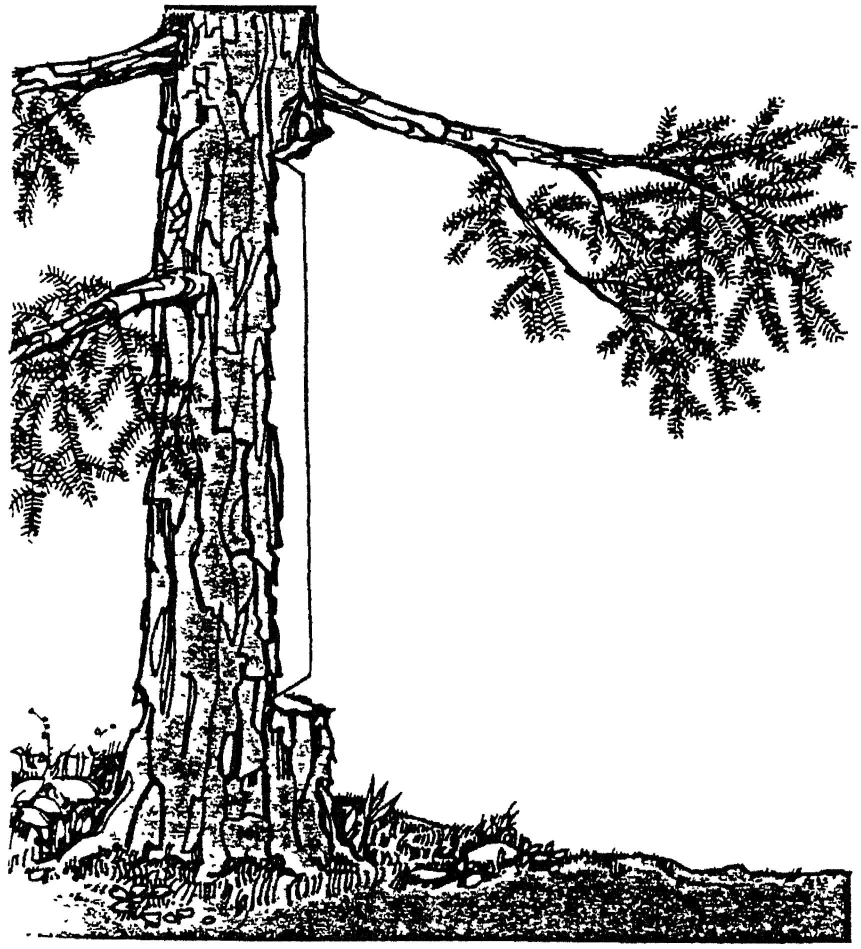
1992
Clinical trials were conducted at 20 centers on a number of different cancers, with some experimenting with combinations of therapies.

Based on the chronology presented in *Pacific Yew: Draft Environmental Impact Statement.* Appendices. U.S. Departments of Agriculture, Interior, and Health and Human Services. January 1993.

Traditional Uses of Yews

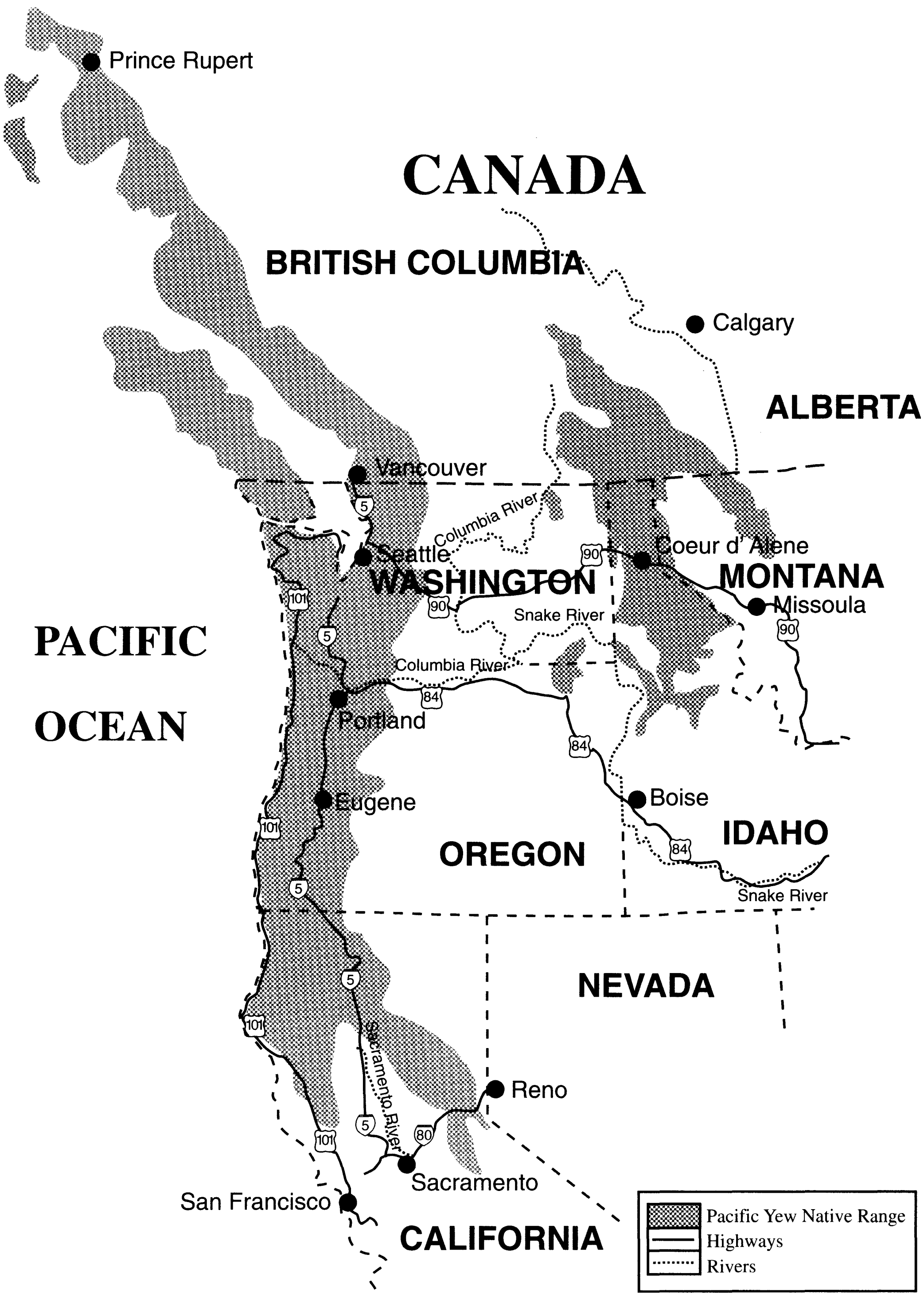
Native Americans have, historically, used the yew wood and needles in various aspects of their lives. Probably the highest use occurred along the coastal zones of Washington and British Columbia. It was here that uses were recorded for the manufacture of bows, arrows, harpoons, spear handles, paddles, war clubs, digging sticks, wedges, boxes, drums, spoons, dishes, cups, and bowls. Pacific yew is renowned for its value in making bows in native cultures from northern California to Canada and was formerly referred to as the "bow plant" by the Salish people. Bows made from Pacific yew tended to be broad, short, and flat.

Some American Indian peoples traditionally associated Pacific yew with death and bereavement. The fragrant foliage was used as a deodorant and cleaning agent, and tonics made from Pacific yew were used medicinally by many peoples of the Pacific Northwest. Some Native American tribes in Washington used the yew boughs and needles for symbolic building of body strength. Several tribes also have dried the needles for smoking, either in combination with other products or later with tobacco. Although yew seeds are poisonous, the fleshy portions surrounding them were sometimes eaten.



The strength and flexibility of yew wood made it especially suited for making excellent bows. Native Americans removed staves from living trees to make bows, sometimes leaving the piece, trimmed at top and bottom and debarked, to cure, still attached to the tree. Trees showing scars resulting from this use are considered cultural artifacts.

From "The Yew and People: Cultural Resources" in *Pacific Yew: Draft Environmental Impact Statement*. U.S. Departments of Agriculture, Interior, and Health and Human Services. January 1993. Reprinted with permission.



The native range of the Pacific Yew.

From "The Yew and People: Cultural Resources" in *Pacific Yew: Draft Environmental Impact Statement.* U.S. Departments of Agriculture, Interior, and Health and Human Services. January 1993. Reprinted with permission.

A History of Yews in the United States

Kenneth D. Cochran

Introduction of the genus *Taxus* for ornamental horticulture in the United States appears to have begun with the seedling production work of Theophilus D. Hatfield, when he was the second head gardener at the Hunnewell Estate, Wellesley, Massachusetts (Shugert, 1985). Hatfield, born in Cottingham, Hull, Yorkshire, England, 1855, had a thorough training in gardening through noted horticulture establishments in Europe, including Kew Gardens (where *Taxus baccata* was growing), before he served as gardener to successive members of the well-known Hunnewell family (Craig, 1929).

Hatfield became well-acquainted with the species *Taxus cuspidata* and its Japanese variety *Taxus cuspidata* var. *nana,* as they were growing on the Hunnewell Estate. Dr. George R. Hall of Warren, Rhode Island, a missionary to Japan, imported these plants into America from Japan in 1862. Hall introduced the plants to the nursery trade through the Parsons Nursery at Flushing, Long Island, New York. Hatfield reported that the spreading varieties *Taxus cuspidata* var. *nana* and *Taxus cuspidata* var. *brevifolia* (later given the cultivar name 'Densa') were the first varieties to become popular in the nursery trade. They were followed by the upright-growing nursery form *Taxus cuspidata* var. *capitata* (Hatfield 1921, Hatfield 1929). These became quite plentiful and were eventually reproduced from cutting propagation.

Around 1904, Hatfield began a series of experiments raising yews from seed. In a propagation paper presented in 1929 entitled *Yews,* Hatfield indicated that the seedlings raised were probably a cross of the English (European) and Japanese types. No intentional crossing was reported. *Taxus baccata* had not been permanently cold hardy (following various winters) in upper Midwestern and northeastern U.S. landscapes (USDA Plant Hardiness Zone 5). Hatfield reported that when most *Taxus baccata* were planted, they would look good for a while, but after a few years of winter injury, they would look disgusting, especially due to browning of the foliage in winter. An exception was the procumbent form *Taxus baccata* 'Repandens,' which was often buried below the snow line.

Hatfield reported in 1929 that of the original English yews (*Taxus baccata)* that had been grown at the Hunnewell Estate, only one or two forms were in a presentable condition. While they grew well for a few years, an unusually severe winter would burn the south-facing side of the plant and otherwise disfigure them. Seedlings of *Taxus baccata* var. *fastigiata,* the Irish yew, took on the fastigiate form characteristic of the variety, and some proved perfectly hardy.

About the same time that Hatfield was growing seedlings at Wesley, nurseryman Henry Hicks of Westbury, Long Island, New York, was growing seedlings at his Long Island nursery. Henry

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was a crusader for yews for American gardens. Popularizing the Japanese yew was one of the achievements of the Hicks Nurseries, Inc. He thought that yews were fine plants as he had seen many beautiful specimens at the Dane Arboretum, Glen Cove, Long Island. In a 1924 Hicks Nursery catalog, Henry reported that a new selection, *Taxus* x *media* 'Hicksii,' obtained from a 1902 seed collection at the Dane Arboretum, was available for the first time.

The Hicks yew became a remarkable plant and has been propagated and distributed in great quantity all over the world. It made a definite hit with landscape architects. It became a substitute for the red cedar that was the standard for landscape hedges prior to 1920 (Cochran, 1991). For six decades, this selection has been the pick of the upright yews. 'Hicksii' is known as a female selection. Among Hicks' seedling plants, C. S. Sargent identified a male plant that L. C. Chadwick named *Taxus* x *media* 'Costich' (Chadwick and Keen, 1976). The author confirmed the original plant of 'Costich' as it was growing at the Hicks nursery in 1991, but the original 'Hicksii' no longer exists. Henry's daughter, Ester Emory, reported to this author in 1991 that "Father" would not plant the fruiting Hicks yew in landscapes where there were children, because he knew the seeds were poisonous. The Hicks nursery never used the name 'Costich;' they just called one yew a female 'Hicksii' and the other a male 'Hicksii' (Cochran, 1991).

Yews have been the most popular narrow-leaved evergreen landscape plants of the second half of the 20th Century in the Northeastern and the upper Midwestern United States. As Chadwick reported in an interview with this author prior to his death in 1993, "It is a good plant, dark green and hardy; there is no other plant like it" (Cochran, 1992). Its use in gardens for centuries has given it an unrivaled reputation. Chadwick and Keen's publication, *The Study of the Genus Taxus* (OARDC Research Bulletin 1086), their research work, the development of the Chadwick Living Herbarium of *Taxus* at the Secrest Arboretum on the campus of The Ohio State University/Ohio Agricultural Research and Development Center, Wooster, Ohio, and Chadwick's many lectures on the merits of *Taxus* have greatly furthered the marketing of *Taxus* through nurseries. Many landscape nurserymen think that yews are among the best of ornamental plants. They are serviceable the year around for the landscape as dark evergreen masses of foliage.

Low-profile plants for foundation, facing, low hedging, and groundcover uses will be in even greater demand for landscapes of the 21st Century. There are now several selections of *Taxus* available to fill that need. Large- and medium-sized *Taxus* cultivars, including 'Hicksii,' should continue to be popular. The value of the Hicks yew for hedging and screening should remain. Yews should continue to be the backbone for gardens of the Northeastern and upper Midwestern United States. Nurserymen are presently geared up for their production.

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Taxus Populations and Clippings Yields at Commercial Nurseries

Robert C. Hansen, Kenneth D. Cochran, Harold M. Keener, and Edward M. Croom Jr.

Summary

Commercial nurseries were surveyed during the summer and fall of 1991 as a basis for estimating populations of *Taxus* cultivars growing in the United States. Clippings of selected cultivars were sampled from nursery fields in Ohio and Michigan in order to estimate expected clippings yields as a function of cultivar and cultivar age.

More than 30 million *Taxus* plants were reported to be grown by the 19 major nurseries that responded to the survey. Approximately 88% of all *Taxus* plants reported in the survey were grown in the three-state area of Ohio, Michigan, and Pennsylvania. *Taxus* x *media* 'Densiformis,' 'Hicksii,' and 'Brownii' were found to be grown by nearly all nurseries in the survey. More than half grew *Taxus* x *media* 'Wardii' and *Taxus cuspidata* 'Capitata,' while other well-known cultivars seemed to have been specialties of one or two nurseries.

Annual clippings yields on a dry-weight basis (db) ranged from approximately 20 grams per plant to 140 grams per plant. Expected yields were found to be very dependent upon plant age and cultivar.

Taxus x *media* 'Hicksii' appeared to be the most ideal ornamental yew that could provide a renewable source of Taxol because of immediate availability and potential for mechanical harvesting of upright clippings. An estimated 3,000 to 4,000 ovarian cancer patients could have been treated annually with the Taxol available for extraction from *Taxus* x *media* 'Hicksii' clippings.

Introduction

A popular ornamental hybrid yew, *Taxus* x *media* 'Hicksii,' was evaluated extensively as a source of Taxol and taxanes (Croom *et al.*, 1991). New-growth shoots, which are typically pruned on an annual basis as a part of standard cultural practices in nurseries, and roots and needles from cull plants were the preferred sources of Taxol and other taxanes from ornamental yews.

Taxus x *media* 'Hicksii' had been targeted for priority evaluation for the following reasons:

- Taxol and taxane yields were comparable to or greater than those from other *Taxus* cultivars (Witherup *et al.*, 1990; ElSohly *et al.*, 1992; Mattina and Paiva, 1992).

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- Upright shoots produced clippings that facilitated mechanical harvesting.
- *Taxus x media* 'Hicksii' was among the more abundantly grown ornamental yews in the United States (see survey results later in this report).
- *Taxus* x *media* 'Hicksii' was grown as a nursery field crop and therefore offered an immediate renewable resource for annually pruned cuttings which were otherwise discarded.
- Clippings from ornamental yews offered the possibility of being less costly to harvest and process than bark from wild plants such as the Pacific yew tree (*Taxus brevifolia*).

While *Taxus* x *media* 'Hicksii' appeared to be a promising cultivar, many other *Taxus* cultivars were being collected and evaluated (Croom *et al.*, 1992a; ElSohly *et al.*, 1992; Mattina and Paiva, 1992). More than 100 cultivars are included in the Chadwick Living Herbarium of *Taxus* on the Wooster campus of The Ohio State University's Ohio Agricultural Research and Development Center.

An example of the concentrations of Taxol and 10-deacetylbaccatin III are shown in Table 1 for needles from six *Taxus* cultivars. While the highest concentration of Taxol came from *Taxus* x *media* 'Hicksii,' the highest concentration of the precursor came from *Taxus baccata* 'Repandens' Parsons and Sons.

In more recent research, Wheeler *et al.* (1992) reported that epigenetic factors (timing of harvest, plant tissue type, etc.) and environmental factors (soil type, climate, fertility, etc.) may be confounding efforts to establish expected Taxol and taxane yields for selected *Taxus* cultivars. They observed that higher Taxol yields were obtained from *Taxus brevifolia* that grew in cool, moist, shady environments than from trees that grew in warm, dry, sunny climates.

Protocols pertaining to harvesting, handling, drying, processing, and laboratory analyses needed to be defined. Each part of the system could have a significant effect on expected Taxol and/or taxane yields (Hansen *et al.*, 1992; Croom *et al.*, 1992b; Richheimer *et al.*, 1992; Hansen *et al.*, 1993; Ketchum and Gibson, 1993).

Along with identification of ideal cultivars and protocols for Taxol and/or taxane production on a renewable basis, an estimate of populations or acreage of each *Taxus* cultivar that was being grown in nurseries in the United States and other countries needed to be established. Other than for purposes of propagation, *Taxus* clippings had not been routinely harvested or stored; therefore, no estimates of potential

Table 1. Concentrations of Taxol and the Common Precursor, 10-deacetylbaccatin III, from Needles of Six *Taxus* Cultivars (Witherup *et al.*, 1990).

Taxus Cultivar	Concentration (Dry-Weight Basis) Taxol (mg/kg)	10-deacetylbaccatin III (mg/kg)
T. baccata 'Repandens'	30	200
T. brevifolia	60	100
T. canadensis	90	20
T. cuspitata 'Capitata'	80	20
T. x *media* 'Densiformis'	20	70
T. x *media* 'Hicksii'	100	90

yields for clippings and/or needles were available.

Objectives

The objectives of this research were:

- To survey nursery growers and account for the diversity and abundance of *Taxus* cultivars currently being propagated and cultivated for the ornamental market.
- To measure clippings yields as a function of *Taxus* cultivar and cultivar age.

Procedures

Survey forms, designed to determine types, ages, and quantities of ornamental *Taxus* grown by nurseries in the United States, were distributed to 68 nurseries in May 1991. The nurseries selected for the survey included all nurseries in the United States that were thought to be growing significant collections of one or more *Taxus* cultivars. The survey forms requested an accounting of propagation, field-grown, and container-grown *Taxus* plants. Results were returned from 18 nurseries during summer and autumn of 1991. One additional nursery returned a survey March 15, 1992. The data collected for field-grown plants for 17 of the 19 nurseries that responded to the survey were selected for the summaries and analyses included in this report. The two that were excluded reported that they did not grow *Taxus*.

During April and May 1992, the quantity of clippings to be expected during annual pruning of different ornamental *Taxus* plants at various ages was measured (see Table 2). Fifty plants were randomly selected from each field (selection was based on random number tables) independent of field size or number of plants in the field. In fields with plant populations greater than 3,000, four to six rows were randomly selected followed by random selection of 50 plants to be pruned from the identified rows. The degree of pruning and desired plant shape was determined according to the preferences of the grower. Soil conditions permitted retrieval of nearly all plant material pruned from the plants. Pruning was consistently done by one person skilled with the use of hand-pruning shears. All clippings, needles, and bits and pieces of plant material were retrieved from the soil surface below each plant and immediately placed in sealed plastic storage bags. The 50 bags were coded according to nursery, type of cultivar, and age (Table 2).

Upon returning to the laboratory, fresh weights of each of the 50 individual samples were recorded. Five of the 50 samples were randomly selected to be oven dried as a basis for calculating moisture content. Samples were dried at 60°C for 48 hours in standard, brown-paper grocery bags. Based on the average moisture content of the five dried samples, the average yields per plant in grams (dry basis) were calculated for each field along with the standard deviation.

Discussion of Results

Results for the 18 most numerous *Taxus* x *media* and the four most numerous *Taxus cuspidata* were tabulated in Table 3 based on responses from the 17 nurseries. *Taxus baccata* 'Repandens' is also shown. Rooted cuttings were counted as plants; cutting wood was not. The No. 1 cultivar in terms of total plants growing of all ages at the time of the survey was *Taxus* x *media* 'Densiformis' with a total population of 8.36 million plants. *Taxus* x *media* 'Hicksii' was second with 5.30 million plants while *Taxus cuspidata* 'Capitata' was third with 3.77 million. The fourth most numerous plant was *Taxus* x *media* 'Brownii' with 3.62 million plants followed by *Taxus* x *media* 'Dark Green Spreader' with 2.97 million.

Plant Population by Nursery and Cultivar

Table 3 is a summary of all cultivars that were grown in *significant* quantities. For the purpose of limiting the complexity of this analysis, a

Table 2. Summary of Field Data Obtained While Sampling Plants for Clippings Yields.

			Field Layout		Plant Population		Yield Per Plant*		Moisture Content	
							(grams, db)		(percent)	
Cultivar	Age (Yr)	Code	No. of Rows	Plants Per Row	Grower Esti- mate	Actual Count	Mean	Std. Dev.	Mean	Std. Dev.
[Taxus x *media]*										
'Hicksii'	7	SH7	2	200	500	400	66.1	21.9	57.4	0.8
'Densiformis'	3	SD3	12	325	3,100	3,900	23.6	9.8	53.4	1.3
'Densiformis'	7	SD7	30	150	4,500	4,500	70.0	35.7	57.0	1.4
'Runyan'	3	SR3	14	310	6,100	4,340	29.5	18.6	55.5	0.9
'Runyan'	7	SR7	—	—	3,750	3,750	65.5	29.5	55.9	1.2
'Hicksii'	3	BH3	—	—	—	2,750	18.8	8.1	51.3	0.9
'Densiformis'	4	BD4	—	—	450	190	190.2	103.9	51.4	2.9
'Densiformis'	5	BD5	—	—	1,725	1,624	135.7	62.9	49.8	1.7
'Hicksii'	5	RH5	66	80	—	5,280	26.8	16.2	51.5	1.0
'Hicksii'	7	RH7	14	787	—	11,018	50.5	21.7	53.7	0.5
'Densiformis'	6	LD6	27	236	10,000	6,372	142.4	51.3	54.8	0.5
'Densiformis'	7	LD7	36	149	6,260	5,364	60.8	26.7	55.7	0.5
'Densiformis'	7	ZD7	—	—	—	—	50.1	20.8	53.4	0.4

* Based on average moisture content of five plants (dry-weight basis) from each sample of 50 plants.

cultivar was designated *significant* if 100,000 plants (or more) of the cultivar were grown in at least one of the nurseries that responded to the survey. This number included rooted cuttings, all plants ranging from one to 10 years old, and plants greater than 10 years old. Cutting wood was excluded.

Some cultivars may have been left out because more than 40 nurseries did not respond to the census report. There may also be cultivars that were grown in small numbers (less than 100,000 plants) in a number of nurseries where combined production of that cultivar would exceed 100,000 plants. Although the designation of 100,000 plants was arbitrary, it was a conservative estimate of the minimum population of plants in any one nursery that would be required to produce enough clippings to economically justify collecting, handling, drying, and storing clippings for the Taxol/taxane market.

Review of Table 3 revealed that the three most numerous medias (hybrids) — *Taxus* x *media* 'Densiformis,' 'Hicksii,' and 'Brownii' — were grown by at least 15 of the 17 nurseries that responded to the survey. Eleven out of the 17 nurseries evaluated grew *Taxus* x *media* 'Wardii,' and 10 nurseries grew *Taxus cuspidata* 'Capitata.' Otherwise, most cultivars that were grown in significant quantities seemed to be specialties of one or two nurseries.

Plant Population by Age

A summary of plant populations of the more commonly grown cultivars as a function of age is provided in Table 4. A wide diversity in age of plantings was evident from the survey results. Grower estimates were subject to error; however, the survey results provided a good idea of what existing plant populations might have been.

Table 3. Field-Grown *Taxus* Plant Populations as a Function of Cultivar and Nursery.

	Nursery Code																	Total
Cultivar	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	Plants
[Taxus x media]								*Number of plants (1,000s), all ages*										
'Densiformis'	211	153	12	535	87	60	—	552	310	874	11	84	154	910	333	—	4,069	8,355
'Hicksii'	113	9	2	155	39	3	69	469	34	557	10	99	288	453	108	—	2,892	5,300
'Brownii"	10	19	2	95	17	1	31	415	45	391	1	28	91	148	92	38	2,200	3,624
'Dark-green'	—	20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2,949	2,969
'Runyan'	—	11	—	—	36	—	—	—	—	—	11	192	490	—	—	—	887	1,627
'Wardii'	24	—	2	—	—	—	—	128	31	98	5	11	113	13	—	35	912	1,372
'Tautoni'	—	—	—	—	—	—	—	—	24	3	—	8	—	—	87	—	1,249	1,371
'Henryi'	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	630	630
'Fairview'	—	—	—	—	—	—	—	564	—	—	—	—	—	—	—	—	—	564
'Hatfieldii'	136	—	—	—	—	—	—	—	8	—	—	—	—	5	123	—	—	272
'Nigra'	—	—	—	—	—	—	—	—	10	—	—	—	—	4	201	—	—	215
'Angelica'	192	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	192
'Green-wave'	64	—	—	—	—	—	—	—	—	—	17	—	—	107	—	—	—	188
'Everlow'	—	—	—	—	11	—	—	—	—	3	—	23	—	—	—	143	—	180
'Vermuelen'	149	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	149
'Bobbink'	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	146	146
'Chadwick'	—	—	—	—	—	—	—	—	—	—	—	13	119	—	—	—	—	132
'Berryhill'	—	—	—	—	91	—	—	—	—	—	—	—	24	—	—	—	—	115
Subtotals	899	212	18	785	281	64	100	2,128	462	1,926	55	458	1,279	1,640	944	216	15,934	27,401
[T. cuspidata]																		
'Capitata'	40	—	1	—	—	—	—	849	—	42	1	24	—	179	86	10	2,538	3,770
(no cultivar)	—	—	—	—	—	—	—	56	—	—	—	—	31	6	23	—	161	277
'Nana'	112	—	—	—	—	—	—	—	—	—	—	—	—	—	63	—	—	175
'Thayerae'	—	—	—	—	43	—	—	—	—	—	—	—	119	—	—	—	—	162
Subtotals	152	0	1	0	43	0	0	905	0	42	1	24	150	185	172	10	2,699	4,384
[T. baccata]																		
'Repandens'	—	—	—	—	—	—	—	123	—	45	—	—	—	—	43	10	—	221
Totals	1,051	212	19	785	324	64	100	3,156	462	2,013	56	482	1,429	1,825	1,159	236	18,633	32,006

Table 4. Populations of More Commonly Grown *Taxus* Cultivars by Age and Total Plants Grown.

Cultivar	Cutting Wood	Rooted Cuttings	Age of Plants (Years) 1	2	3	4	5	6	7	8	9	10	>10	Total Plants
		(1,000s)												
[Taxus x *media]*														
'Densiformis'	370.0	1,378.5	1,318.8	1,508.0	1,492.7	866.8	952.8	400.2	260.5	105.5	67.8	2.3	—	8,353.9
'Hicksii'	368.0	990.5	903.1	888.0	632.6	538.4	516.3	216.1	227.8	124.0	130.5	91.8	40.0	5,299.1
'Brownii'	161.0	608.8	620.5	697.0	554.8	290.6	238.9	267.2	140.7	182.8	18.5	2.8	1.0	3,623.3
'Dark Green Spreader'	—	117.0	943.0	538.0	376.0	385.0	269.0	189.0	72.0	53.0	27.0	—	—	2,969.0
'Runyan'	278.4	456.4	457.0	334.0	97.8	160.5	56.1	30.5	26.8	5.8	1.3	0.8	—	1,626.9
'Wardii'	92.1	227.2	250.0	221.0	172.5	173.3	171.0	44.4	51.5	40.5	15.5	4.5	—	1,371.4
[Taxus x *cuspidata]*														
'Capitata'	9.0	67.2	290.4	1,039.4	235.5	314.3	606.6	87.8	177.3	449.5	88.2	150.1	264.0	3,770.3

Clippings Yields

A tabulation of measured and estimated clippings yields for three- to seven-year-old plants from five nurseries is shown in Table 5 for *Taxus* x *media* 'Hicksii' and 'Densiformis' as a function of age. Table 2 provides the resource data for Table 5. Data available from Table 2 for *Taxus* x *media* 'Runyan' were not included in Table 5. The yields per plant listed in Table 2 were samples of growth that occurred during 1991. The 1991 growing season was limited by low rainfall in Ohio and surrounding states. Average rainfall in Ohio is 100 cm; in 1991 the state average was 61 cm. These yields were therefore, at best, rough estimates of expected clippings yields. The large standard deviations were evidence of the wide variation in yields among plants. The moisture contents listed in Table 2 were used to determine clippings yields on a dry-weight basis.

Referring to the codes in column 3 of Table 2, BH3 and RH5 were the only results for expected clippings yields for three-year-old and five-year-old *Taxus* x *media* 'Hicksii' plants. The results given for BH3 and RH5 in column 8 — 18.8 and 26.8 — were rounded off to 19 and 27 g per plant (db) and recorded in Table 5. The result for seven-year-old *Taxus* x *media* 'Hicksii' plants, 58 g per plant, is the average of SH7 and RH7. Expected yields for four- and six-year-old plants were estimated by interpolation.

Table 5. Measured and Estimated Clippings Yields Per Plant Per Year for *Taxus* x *media* 'Hicksii' and 'Densiformis' as a Function of Age.

Cultivar	Age of Plants (Years) 3	4	5	6	7
[Taxus x *media]*	*g(db)/ plant/yr*				
'Hicksii'	19	23*	27	43*	58
'Densiformis'	24	80*	136	142	60

* Yields estimated by interpolation.

For three-, five- and six-year-old *Taxus* x *media* 'Densiformis' plants, results from only one field each (SD3, BD5, and LD6) were available for estimating expected yields in Table 2. Again, the raw data 23.6, 135.7, and 142.4 were rounded off and entered in Table 5. The result for four-year-old *Taxus* x *media* 'Densiformis' (BD4) was 190 g per plant. This yield was very high relative to all other results; therefore, it was excluded from the analysis. The field could have been unusually fertile or the age was improperly recorded. The yield for four-year-old plants was therefore interpolated from the results for three- and five-year-old plants. The result for seven-year-old *Taxus* x *media* 'Densiformis' plants, 60 g per plant, was the average of three fields — SD7, LD7, and ZD7. Recognizing that this data was a rough estimate or "first look" at expected yields, the results showed clippings from *Taxus* x *media* 'Densiformis' generally were more than double the expected yield of *Taxus* x *media* 'Hicksii.'

No information of this type is known to exist in nursery crop literature. Plants are pruned every year to develop compactness, i.e., a high density of limbs and branches. Clippings have generally been left on the ground. If these same clippings could be harvested for Taxol or taxane production, then what was heretofore waste has the potential to become a second crop.

Table 6 is a tabulation of expected clippings yields for three- to seven-year-old *Taxus* x *media* 'Densiformis' plants as a function of age and nursery. Plants that are three- to seven-years-old would have the greatest potential as a renewable source of *Taxus* clippings since they are in the productive cycle and have to be clipped each year. The results were calculated by multiplying expected yields (Table 5) times plant populations. These results indicated that an estimated 307,208 kg (db) of clippings could have been obtained from the 15 nurseries reporting that they grew *Taxus* x *media* 'Densiformis.'

Table 7 reveals expected yields for *Taxus* x *media* 'Hicksii' that were developed by following the same procedure described earlier for Table 6. Comparison of the total yields for all nurseries for ages three to seven showed expected yields for *Taxus* x *media* 'Hicksii' plants were only 20

Table 6. Expected Clippings Yields Per Year for Three- to Seven-Year-Old *Taxus* x *media* 'Densiformis' Plants by Nursery and Age of Plants.

Nursery Code	Age of Plants (Years)					Totals
	3	4	5	6	7	
A	1,080	2,320	—	2,130	—	5,530
B	672	480	680	—	—	1,832
C	288	—	—	—	—	288
D	4,200	4,000	12,240	2,840	600	23,880
E	456	1,040	272	639	360	2,767
F	—	—	—	—	—	0
G	—	—	—	—	—	0
H	2,328	5,600	9,656	5,254	6,300	29,138
I	1,200	4,000	5,440	—	—	10,640
J	4,128	—	4,624	3,834	240	12,826
K	—	—	—	—	—	0
L	432	1,000	—	28	—	1,460
M	569	2,184	1,333	639	270	4,995
N	600	—	30,872	—	—	31,472
O	1,152	3,840	4,080	4,260	1,800	15,132
P	—	—	—	—	—	0
Q	18,720	44,880	60,384	37,204	6,060	167,248
Totals	35,825	69,344	129,581	56,828	15,630	307,208

percent of expected yields for *Taxus* x *media* 'Densiformis.' The lower total plant population (Table 3), combined with much lower yields per plant (Table 5), led to the lower total expected clippings yield.

Traditionally, most field crops are evaluated in terms of yields per acre. When plant populations per acre are known, the yield data in Table 5 can then be used to estimate expected yield per acre. The section entitled *Land Area Required* (later in this report) can be used (or customized according to plant populations grown by individual nurseries) to facilitate the estimation of clippings yields per acre.

Based on plant population and taxane yield potential from needles only (see Table 1), *Taxus* x *media* 'Densiformis' might appear to have promise as a viable source of 10-deacetylbaccatin III. However, existing isolation technologies cannot produce taxanes cost effectively from such low concentrations (Croom *et al.*, 1991). Either more efficient chemical processing technologies must be developed or additional compounds in high concentrations that can be efficiently converted to Taxol must be found in *Taxus* x *media* 'Densiformis' for this material to become a viable commercial source.

While *Taxus baccata* 'Repandens' is apparently the best source of 10-deacetylbaccatin III, the results (based on the nurseries that responded to the survey) showed that only an estimated 221,000 plants were being grown (see Tables 1 and 3). While other ornamental *Taxus* plants may eventually be identified as promising sources of Taxol or Taxol precursors, *Taxus* x *media* 'Hicksii' prevails at present as the most ideal ornamental yew that could provide a re-

Table 7. Expected Clippings Yields Per Year for Three- to Seven-Year-Old *Taxus* x *media* 'Hicksii' Plants by Nursery and Age of Plants.

Nursery Code	Age of Plants (Years) 3	4	5	6	7	Totals
			(Kilograms, db)			
A	456	506	324	—	—	1,286
B	—	—	81	—	—	81
C	38	—	—	—	—	38
D	570	460	1,080	430	290	2,830
E	144	90	135	142	99	610
F	—	—	—	—	—	0
G	228	276	—	—	—	504
H	1,273	1,150	1,296	1,978	2,494	8,191
I	114	138	162	—	—	414
J	1,254	—	594	645	696	3,189
K	—	—	—	—	—	0
L	266	368	—	—	644	1,278
M	589	587	656	980	1,740	4,552
N	—	—	2,700	—	—	2,700
O	266	322	324	516	696	2,124
P	—	—	—	—	—	0
Q	6,821	8,487	6,588	4,601	6,554	33,051
Totals	12,019	12,383	13,940	9,292	13,212	60,847

newable source of the drug because of immediate availability and potential for mechanically harvesting upright clippings.

In addition, based on a new process developed by Croom *et al.* (1991) (patent pending), Taxol and taxane yields for *Taxus* x *media* 'Hicksii' may be more than double those shown in Table 1. Even if isolated Taxol yields of 0.010 to 0.014% are obtainable from *Taxus* x *media* 'Hicksii,' Croom *et al.* (1991) estimated 14 to 20 kg (db) of *Taxus* x *media* 'Hicksii' clippings would supply enough Taxol (2 g) to provide one full course of treatments for one ovarian cancer patient. Using only the *Taxus* x *media* 'Hicksii' clippings identified in Table 7, some 3,000 to 4,000 patients could be treated with the Taxol isolated from these clippings. The isolation and conversion of other taxanes to Taxol from *Taxus* x *media* 'Hicksii' and other cultivars could increase Taxol supplies several fold beyond these estimates.

Land Area Required

Some estimate of land area required to grow various *Taxus* cultivars should be noted. As an example, when plants were transplanted to fields at about three years of age, Zelenka Nursery, Inc., Grand Haven, Michigan, typically planted two rows to a bed with plants spaced 18 inches apart in the row. The rows were spaced 44 inches apart in beds which were laid out 84 inches apart. These spacings led to a theoretical population of 8,297 plants per acre.

The trend is to plant three rows in each bed with all other specifications the same as outlined earlier. This practice leads to a theoretical population of 12,446 plants per acre. After adjusting these populations for turn rows, irrigation ditches, and so forth, reasonable estimates for two-row and three-row plantings in beds may be 8,000 and 12,000 plants per acre, respectively.

Sources of Error

- An attempt was made to survey all nurseries with known significant populations of *Taxus* cultivars. Some nurseries may have been overlooked; some may not have received the survey forms; some may have lost the forms; and some may have elected not to fill out the forms and return them. Also, no attempt was made to contact nurseries outside the United States. In any event, the 17 nurseries referred to previously may still represent 75 to 90% of all commercially grown *Taxus* in the United States and therefore the information provided in this report would be relevant and useful.
- There may be *Taxus* cultivars that are grown in *significant* quantities somewhere (100,000 plants or more) that are not among the plants grown by the reporting nurseries.
- Because of historical propagation practices, growers may not know which cultivar they are growing. Plant populations within a given field may be generally one cultivar, but extraneous, unexpected cultivars may be dispersed throughout a given field. Even experts have difficulty identifying and distinguishing some cultivars.
- Growers vary in their assessment of plant age. Some determine age based on the time when cutting wood is "stuck" and rooting is initiated. Others may refer to a three-year-old plant based on the time it was transplanted in the field. The survey forms were carefully laid out to minimize these sources of error, but general agreement on plant age does not seem to exist.
- Tracking plant inventory is difficult for many nurserymen. Inventory tracking requires unusual dedication because of plant mortality in the field and the standard practice of selective harvest. Some plants may be alive, but are sufficiently defective that they may not be a viable source of clippings or may not be marketable as an ornamental plant. Accurate inventory is complicated further because of problems associated with distinguishing cultivars.

Recommendations for Future Research

- Survey *Taxus* plant populations periodically (at least once every five years).
- Focus surveys on cultivars shown to be promising sources of Taxol and taxanes.
- Continue to sample clippings yields annually for plants older than three years.
- Study the potential for harvesting other plant organs and whole plants for Taxol along with clippings.

Questions to be answered include:

- What would be the optimum plant age to harvest?
- What time of year should harvesting be done?
- Should plants be dried, after which needles only are stored and marketed?
- How can agronomic practices be adjusted (row spacing, fertilization, irrigation, choice of climate, etc.) to maximize Taxol/taxane yields per acre?
- How does the dedicated "whole-plant-for-Taxol" system compare economically to harvesting clippings and cull plants as by-products of traditional production and marketing of ornamental yews?

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Genetic Resources for Ornamental *Taxus* in the United States

Kenneth D. Cochran

Introduction

A study was conducted to investigate the genetic origin of ornamental *Taxus* in the United States and further verify the nomenclature and origin of *Taxus* x *media* 'Hicksii.' Observations were made in the Chadwick Living Herbarium of *Taxus* in the Secrest Arboretum on the Wooster campus of The Ohio State University's Ohio Agricultural Research and Development Center and through visits to selected commercial nurseries in Ohio, Michigan, Pennsylvania, New York, and some New England states.

Interviews were held with people who selected various *Taxus* cultivars. Gardens and arboretums were visited to understand the origin of *Taxus*, and literature was reviewed. The study determined that nomenclatural errors do exist in reference to *Taxus*, but there are opportunities to implement corrections.

Background

Classifying the genus *Taxus* has been discussed long and hard since Linnaeus wrote *Species Plantarum* in 1753. Subdividing the genus into one, six, seven, or eight species has been based on leaf characters, eventual size of plant, habit of growth, and geographical distribution of the genus throughout the world (Bailey, 1933). Naturally occurring varieties and selected cultivars have been named based on plant morphology and patterns of growth (Bailey, 1933; Chadwick and Keen, 1976). Sometimes plantspeople have designated cultivar names of *Taxus* solely on how they felt about a particular plant or how they found that plant to appear different in a nursery field from all others (Cochran, 1991-92).

In 1990, Dr. Edward M. Croom, botanist, University of Mississippi, and Ken Cochran, Secrest Arboretum, The Ohio State University, set out to identify leaf and branch characters for the 100-plus cultivars of *Taxus* in the Chadwick Living Herbarium of *Taxus*. With leaf and branch characteristics varying on a single cultivar according to the way the plant was oriented to the north, south, east, or west and according to the upper portion vs. the lower portion of a plant, it soon became apparent that the task was impossible. As Croom stated, "The best that we can do for starters is go to the wild and see what we can find in the different populations throughout the range for each species of *Taxus*."

Liberty Hyde Bailey stated in 1933 that it is impossible to designate a species that will include the exceptions of the horticultural varieties of *Taxus* (Bailey, 1933).

Summary of Information

About 1843, H. H. Hunnewell began an Arboretum at Wellesley, Massachusetts, near Boston. Hunnewell took great interest in planting

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species of evergreens that had not previously been available in the United States.

About 1866, Hunnewell received plants of the species *Taxus cuspidata* and *Taxus cuspidata* var. *nana.* These plants were received from the Parsons Nursery of Flushing, Long Island, New York. Parsons had obtained the plants from Dr. George R. Hall of Warren, Rhode Island. Hall brought these species to America from Japan through a plant collection trip in 1860. The author and Ethan Johnson of the Holden Arboretum, Kirtland, Ohio, along with Jack Cowles, their head gardener at Hunnewell, viewed the Hall plants growing on the Hunnewell Estate on September 21, 1991.

In 1886, Theophilus D. Hatfield began working for the Hunnewell family and eventually became the head hardener of the estate. Hatfield began a series of experiments about 1904 growing *Taxus* from seed, and his work was considered to be the largest ever undertaken for *Taxus* at that time in America (Klehm, 1929). He started by taking seed from *Taxus baccata* var. *fastigiata,* the Irish yew, which was growing near specimens of the Japanese yew, *Taxus cuspidata,* and the seedlings were raised. In a paper presented July 16, 1929, American Plant Propagators Association, Statler Hotel, Boston, Massachusetts, Hatfield reported that plants raised from his seedlings were a hybrid, concluding that the parent plants were growing near enough to cross with each other (Hatfield, 1929). There was no reporting of intentional crossing of *Taxus* by Hatfield. Hatfield died October 7, 1929, as a result of a fall from a ladder while picking seeds of Carolina hemlock; his left leg was badly broken; he sustained serious internal injuries; and tetanus was the immediate cause of death.

From Hatfield's writings we understand that he selected and numbered several varied forms of *Taxus* from his seedling experiments and eventually introduced them to the nursery trade as cultivars of a hybrid (Hatfield, 1921; Hatfield, 1929). Hatfield also reported that there were few marks of distinction in the various types of *Taxus* and that he was really led to believe that all yews are a form of *Taxus baccata* even though he used the designations *Taxus baccata, Taxus cuspidata,* and *Taxus* x *media.* His experiments also led him to believe that many well-defined forms would come true from seed. He was sure that his hybrid *Taxus* x *hunnewelliana* would come true from seed. He reported that most of the seedlings from seed of *Taxus baccata* var. *fastigiata* produced the upright type even though he named the offspring hybrids.

Hatfield indicated that the dwarf spreading variety, *Taxus cuspidata* var. *nana* got the lead in the nursery trade, but later the upright selection *Taxus cuspidata* var. *capitata* was brought into cultivation. *Taxus cuspidata* var. *capitata* was given a variety name, but the variety had the branching characteristics of the type of *Taxus cuspidata* which formed a central leader with distinct horizontal side branches.

In the Secrest Arboretum, all of the naturally seeded *Taxus* have taken on the central leader form of the type (species). The seed source for these seedlings has primarily been from more than 100 selections that have pollinated and fruited from among the various plants of *Taxus cuspidata, Taxus baccata,* and *Taxus* x *media.* There has been no indication of hybridization in the naturally seeded plants in the Secrest Arboretum. All seedlings have the appearance of a central leader plant.

In 1929, Hatfield reported to the American Plant Propagators Association that of the original English yews, *Taxus baccata,* only a few plants remained at Hunnewell. He further indicated that the Japanese varieties outperformed the English yew because of unusually severe winters that burned the south side of the plants and otherwise disfigured them. One particular selection, *Taxus baccata* 'Repandens,' was noted as a presentable performer, and it had an especially good chance to survive because in most winters it was below the snow line.

About the same time as Hatfield was growing seedlings at Wellesley, nurseryman Henry Hicks of Westbury, Long Island, New York, was growing seedlings at his Long Island nursery in the center of Nassa County, 25 miles from New York City. Henry was noted as an applied botanist, plant ecologist, and nurseryman of the Hicks Nurseries, Inc., one of America's oldest and finest nurseries (Wing, 1943). The nursery was started in 1853 by Henry's grandfather, Issac. Henry enrolled at Cornell University in 1888, the same year Liberty Hyde Bailey went there to teach and from there he saw a direction of where to go in his career. Henry was a crusader of yews for American gardens, and popularizing the Japanese yew was one of the achievements of the Hicks Nursery. Originally, Henry had an interest in hemlock, but he gave up that interest for yews. Henry cared about preserving the environment long before people knew what that concept meant (Cochran, 1991).

Henry thought that yews were fine plants as he had seen many beautiful specimens at the Dane Arboretum, Glen Cove, Long Island. In a 1924 Hicks catalog, Henry reported a new variety, *Taxus* x *media* 'Hicksii' from a 1902 collection at the Dane Arboretum. From among 1,000 seedlings of an old yew plant at the Arboretum, the Hicks yew was selected — "an interrogative point form, fruiting, dark green and handsome." Hicks called this a hybrid, but there was no intentional crossing indicated. From Hicks' seedling population, another upright form was selected. Eventually Chadwick gave the second plant the name *Taxus* x *media* 'Costich' as it was a male selection. Hicks Nursery did not use the name 'Costich;' they just designated them as male or female Hicks yew (Cochran, 1991).

Results and Conclusion

It is apparent that the origin and production of Taxus in the United States originated from the collections of Hall, seedling work of T. D. Hatfield, the Parsons Nursery, and nurserymen like Henry Hicks. It also seems likely that the Japanese types were hardy in New England, but the Japanese types along with the English types were hardy in Long Island. If there is only one species of *Taxus* as some have proposed, *Taxus baccata*, then at least it seems that provenance does indeed make a difference with regard to hardiness. It would seem convenient to the nursery trade to keep the two species designations *Taxus baccata* and *Taxus cuspidata*, with hardiness being a deciding factor between the two, the latter being the hardier type.

With *Taxus* cultivars, pressed specimens are of little value. Croom and Cochran would not have been able to observe the varied leaf and branch characteristics on a single cultivar without constant recourse to the living plants in the Secrest Arboretum. Pressed specimens cannot show the plant forms that are so critical to the ornamental character of the cultivars; therefore, a living collection is essential for identification.

Taxonomists have suggested the name *Taxus* x *media* to be the hybrid designation for most of the cultivars that have been introduced since the work of Hatfield and Hicks. The author's research has not shown any intentional hybridization of *Taxus;* therefore, the genetic basis for *Taxus* x *media* needs to be verified by more genetic analysis.

Ever since modern *Taxus* cultivars were first named, nurserymen and plantspeople have made cultivar selections from seedling populations and from designated differences within a nursery field of known *Taxus.* In seed propagation, segregation of the genes is to be expected, and variability is the outcome. Why not indicate that seed propagation resulted in a complex of seedlings of the species rather than the outcome of presumed hybridization? The probability of the progeny originating from a species seems at least as acceptable as would be the probability of hybrid origin.

Some cultivars have been designated from vegetatively propagated *Taxus.* Unlike most plants, *Taxus* cuttings continue the habit of growth determined by the position the cutting had on the parent plant. It seems reasonable that within a collection of cuttings there could be a potential of phenotypic variation just because of where the cuttings were taken, and thus a nursery field could show variable plants, but certainly not because of hybrid variation.

And who is to say that some cuttings or plants of designated cultivars may have been mixed up in propagation or planting and thus show a variant or two in a nursery field? Still another possibility is a branch mutation that was collected in cutting propagation. Certainly, all of these factors could be traced back to genetic parentage of already existing cultivars vs. a hybrid possibility.

Some nurserymen reported endless variation from seed propagation; others said there was much similarity in plants propagated from seed. From my understanding of hybridization, I have a hard time substantiating a hybrid if a plant comes true from seed propagation as Hatfield speculated of *Taxus* x *hunnewelliana.*

In addition to explaining the variability of progeny due to gene segregation of seed-propagated plants, branch mutation, or branch orientation of vegetatively propagated plants, there is also the possibility of variation due to soil-fertility levels, soil types, or just plain poor stand of seedling or "runt of the litter."

Commercial nurseries have in the past and, to a large extent, still today propagate *Taxus cuspidata* var. *capitata* from seed imported from Japan. It is the conclusion of this author that this selection should be designated as a botanial variety rather than the cultivar designation that is indicated in the nursery trade. The late nurseryman Laddie J. Mitiska grew his stock of *Taxus cuspidata* 'Capitata' from a single isolated plant of *Taxus cuspidata* and produced very uniform plants (Mitiska, 1954). Since *Taxus* is dioecious, the pollen had to come from some other plant, no matter how isolated it was. *Taxus* is wind-pollinated, and pollen can carry a great distance. The variety has the same plant form that is described for the species. It seems that it would be more significant to the nursery trade if the variety designation was used rather than the species. The Zelenka Nursery Company of Grand Haven, Michigan, still produces the var. *capitata* in great numbers from seed the company receives from Japan.

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Root Rot of *Taxus* spp. in Ohio Caused by *Phytophthora cinnamomi*

Michael A. Ellis, Sally A. Miller, A. F. Schmitthenner, and Kenneth D. Cochran

Phytophthora cinnamomi Rands has been reported to cause a serious die-back and root rot disease of *Taxus* spp. in Indiana (4). The disease occurred on cuttings in rooting benches and rooted cuttings and older plants in lath houses and in the field. Symptoms on infected plants included a gradual loss of normal green color (chlorosis) and die-back of the aerial portion of affected plants. The root system of affected plants showed extensive discoloration (reddish-brown to black) and a dry root rot. The disease was most severe in plantings on poorly drained sites or under conditions of high soil moisture. Growers frequently attributed losses to poor soil drainage, unfavorable weather, or poor quality stock (4).

During the summer of 1991, several accessions in the *Taxus* collection at the Secrest Arboretum on the Wooster campus of The Ohio State University's Ohio Agricultural Research and Development Center (OARDC) developed symptoms of foliar chlorosis, reduced growth, and eventual dieback of the aerial portions of affected plants. The purpose of this paper is to report the results of studies to determine the cause of these symptoms.

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Materials and Methods

Isolation of the Pathogen

In October 1991, the species and cultivars of *Taxus* listed here were dug and the roots examined — *T. cuspidata* 'TV spreading,' *T.* x *media* 'F&F compacta,' *T.* x *media* 'Mitiska Upright,' and *T.* x *media* 'Wilsonii.' All plants showed above-ground symptoms similar to those described earlier. Plants were dug to include major lateral roots and the crown. Root samples were taken from each plant, placed in individual plastic bags, and stored at 4°C until they were assayed for the presence of *Phytophthora* spp. No sample was stored more than 24 hours prior to conducting isolations.

Root samples were washed in running tap water then examined for lesions. A lesion was considered to be an area of discoloration (reddish brown to black) with a sharp line of demarcation between healthy (white) and diseased tissue. The epidermis was removed by scraping with a scalpel, and discolored tissue samples were taken from near the edge (line of demarcation) of the lesion. A minimum of 25 tissue pieces (five pieces per plate) were placed on penta-chloronitrobenzene-benomyl-neomycin-chloramphenicol (PBNC) medium (3), a selective medium for *Phytophthora* spp. Plates were incubated at 24°C for five to seven days. The recovery of fungi was recorded from day five through seven. Representative isolates of the different fungi recovered were made for identification purposes.

Pathogenicity Testing

One species of *Phytophthora* was consistently recovered from lesions on affected roots. Pathogenicity tests using this fungus were conducted by inoculating two-year-old transplants of *T.* x *media* 'Densiformis.' Bare-rooted plants were planted in a peat-sand-soil mix (1-1-2) containing infested oat kernels. Oat kernels were autoclaved, allowed to cool, then inoculated with plugs of the test fungus taken from the margin of five-day-old cultures on PBNC medium. Kernels were incubated at room temperature for eight weeks. Fifteen grams (approximately 150 kernels) of infected oat kernels were thoroughly mixed with each liter of potting mix. Plants were planted in two liters of potting mix in 2.5 liter ceramic pots. Plants planted in potting mix containing noninfected oat kernels served as controls. Pots were saturated with water each day, but were not maintained under flooded conditions. The test consisted of 10 inoculated and 10 noninoculated plants in individual pots. After 12 weeks, all plants were removed from pots and examined for disease symptoms. Isolations were conducted to recover the test fungus from diseased roots.

Results and Discussion

The *Phytophthora* species that was consistently isolated from lesions on affected plants was *P. cinnamomi.* Identification of the fungus was confirmed by A. F. Schmitthenner, OARDC. This is the first report of this pathogen on *Taxus* in Ohio. No other *Phytophthora* spp. or other fungi were recovered during the course of this study. After 12 weeks, all inoculated plants were dead, and *Phytophthora cinnamomi* was recovered from lesions on roots of all inoculated plants. Plants in noninfested soil developed no above-ground symptoms, and their roots appeared normal.

The results demonstrated that *P. cinnamomi* does cause a root rot of *Taxus* spp. in Ohio. Another species of *Phytophthora (P. citrophthora)* has also been reported to cause a root rot of *Taxus* in Ohio (2). *Phytophthora* root rot is usually associated with poorly drained areas or other situations that result in periods of high and sustained soil moisture (1). The years 1990 and 1992 have seen some of the wettest growing seasons in recorded history. Conditions such as this could result in an increased incidence of this disease in many regions of Ohio.

The extent to which this disease occurs in Ohio is not known. Several other disorders can cause above-ground symptoms similar to those observed with this disease. Crown and root feeding by the black vine weevil, *Otiorhynchos sulcatos* F., results in above-ground symptoms on *Taxus* that are almost identical to those caused by *Phytophthora* root rot; however, the two disorders are easily distinguished upon visual observation of exposed crowns and roots. Physical damage (tunnels) caused by feeding of the black vine weevil larvae was not observed on any of the plants used in this study. Further research is needed to determine the extent to which this disease is distributed in Ohio.

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Growth Rate of Container-Grown *Taxus* x *media* 'Hicksii' and 'Densiformis' Compared at Two Levels of Nutrition and Irrigation

Robert C. Hansen, Kenneth D. Cochran, and R. Peter Fynn

Introduction

The effect of fertilization on the growth of *Taxus* cultivars and other woody ornamental species has been studied previously. Flint (1962) defined the upper limit of soil nitrogen for *Taxus* x *media* 'densiformis' along with four other woody ornamentals, i.e., *Forsythia intermedia* 'Lynwood Gold,' *Viburnum plicatum* var. *tomentosum*, *Rhododendron catawbiense* 'grandiflorum,' and *Pieris japonica*. The rooted cuttings and *Pieris japonica* seedlings were planted in 15 cm containers using standard potting medium. N was supplied by injecting soluble concentrates in the irrigation water. N levels of 100, 200, 300, and 400 ppm were compared while the concentration of K in the irrigation water was held constant at 200 ppm. This resulted in mean levels of 10, 19, 31, and 41 ppm N, respectively, and 19 ppm K in the soil extract. Growth was measured by oven drying detached plant tops 100 to 110 days after planting. The upper limit of N for optimum growth was between 10 and 19 ppm for *Viburnum* and *Rhododendron* and between 19 and 31 ppm for *Pieris*. Meanwhile, the upper limit of N for optimum growth was between 31 and 41 ppm in soil extract for *Taxus* x *media* 'densiformis' and *Forsythia*.

Gouin and Link (1966) studied the effects of three levels each (27 different combinations) of N, P, and K on total growth and nutrient levels in the foliage of *Taxus* x *media* 'Hatfieldii.' Rooted cuttings were planted in 25 cm pots in a standard medium and grown for 19 months after which plant tops were pruned to the top of the pots and dried. Maximum total growth occurred when levels of N, P, and K were 224, 75, and 135 ppm, respectively. According to Gouin and Link (1996): "Any variation from these levels not only resulted in significant decrease in total growth, but also caused drastic changes in the nutrient content of the foliage." They concluded that *Taxus* growth was strongly affected by any deviation from a nutritional balance of 3-1-2 for N-P-K, respectively.

Meyer and Tukey (1967) showed that the timing of nutrient applications and root temperature have important effects on root growth of *Taxus* x *media* 'Hicksii' and *Forsythia intermedia* 'Spring

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Glory.' Roots of both species grew appreciably over winter, while the above-ground parts were dormant. The average root growth of plants receiving nutrient applications was much greater than that of plants receiving none. In addition, increase in nutrient reserves during the dormant season increased the amount of shoot growth during the following spring.

While research has been targeted to identify nutritional and cultural practices that maximize the production of *Taxus* biomass, no recommendations have been reported for sustaining and maximizing annual production of clippings while the plant continues to grow year after year. Also, which nutritional and cultural practices lead to optimum concentration of Taxol in clippings? The research reported here was a first attempt to explore these issues.

Objective

The objective of the experiment was to measure the effect of *Taxus* cultivar, nutrition level, and irrigation frequency on the total mass of *Taxus* clippings produced and on the Taxol concentration in the clippings.

Materials and Methods

Experiments were conducted June 1, 1992, to September 15, 1993, in a double-polyethylene greenhouse located at the Ohio Agricultural Research and Development Center (OARDC), Wooster, Ohio (41° 30′ N Latitude). Greenhouse air temperatures ranged from 20 to 40°C. The specific greenhouse compartment used in this experiment was 5 m wide x 30 m long with an evaporative pad and fan ventilation system across the short dimension.

The plants were potted June 15, 1992, in 22 cm (8.5 in./#2) containers using 60% pine bark, 30% Sphagnum peat moss, and 10% Haydite®. The mix also included 3 kg/m^3 (5 lbs/yd^3) of lime. The plants were placed on a dirt floor covered with a weed mat made of black fabric.

Supplemental lighting was supplied with HID lights December 14, 1992, through April 5, 1993, in an attempt to avoid plant dormancy. The lights were set to come on at 7 a.m. and were shut off at 10 p.m.

The experiment was designed to compare two cultivars — *Taxus* x *media* 'Hicksii' and *Taxus* x *media* 'Densiformis.' Two hundred plants were obtained from Zelenka Nursery, Inc., Grand Haven, Michigan — 100 'Hicksii' as two-year liners and 100 'Densiformis' as three-year liners. Half of the plants in each cultivar were irrigated two times per week [Monday and Friday (M-F)] while the other half were irrigated four times per week [Monday, Wednesday, Friday, and Sunday (M-W-F-Sun)].

Finally, the plants were supplied with nitrogen, phosphorus, and potassium from soluble concentrates (21-7-7) in the irrigation water. Approximately 100 ppm N, 33 ppm P, and 33 ppm K were delivered to half of the plants as the low level of nutrition compared to 200 ppm N, 66 ppm P, and 66 ppm K as the high level for the other half. Appropriate levels of soluble micronutrient concentrates were included in each solution.

Tables 1 and 2 summarize the factors and levels used in the full factorial, three-factor, two-level experimental design. The design resulted in eight treatments where each treatment was rep-

Table 1. A Summary of Factors and Associated Levels to Be Evaluated in the Experiment.

Controllable Factors	Levels	
	1	2
A. Cultivar	'Hicksii'	'Densiformis'
B. Irrigation Frequency	M-F	M-W-F-Sun
C. Nutrition Level	100 ppm N	200 ppm N

Table 2. **The Experimental Design Matrix Showing All Eight Combinations of Three Factors at Two Levels With Their Designated Treatment Number.**

Treatment No.	A Cultivar	B Irrigation Frequency	C Nutrition Level
1	'Hicksii'	M-F	100 ppm N
2	'Hicksii'	M-F	200 ppm N
3	'Hicksii'	M-W-F-Sun	100 ppm N
4	'Hicksii'	M-W-F-Sun	200 ppm N
5	'Densiformis'	M-F	100 ppm N
6	'Densiformis'	M-F	200 ppm N
7	'Densiformis'	M-W-F-Sun	100 ppm N
8	'Densiformis'	M-W-F-Sun	200 ppm N

licated four times using five plants per replication. Therefore, 80 'Hicksii' and 80 'Densiformis' were included in the experiment. An additional 52 plants were used as border plants.

Figure 1 is a schematic drawing showing the randomized location of each treatment within four replications. The drawing also illustrates the layout for four irrigation lines that were required to deliver high and low levels of nutrition using the two irrigation schedules referred to earlier. Water and nutrients were delivered through 16 mm polyethylene tubing used as a main line. Water was delivered to each individual plant through 3 mm spaghetti tubing using 0.03 L/min (0.5 gph) woodpecker drippers. Each irrigation event required 39 minutes on average before water dripped through the bottom of the pots. During this time period, approximately 760 ml of water along with designated nutrient levels were delivered to each pot. Automatic timers were used to set the days of the week and the duration for each irrigation.

A Q-COM (Q-COM Corporation, Irvine, CA 92614) moisture tension measurement system was used to monitor tension in one container per treatment. Each tensiometer was placed half way down into the growing medium of each of the four containers. These tensiometers used a "high flow" ceramic cup for the tip and had a relatively fast reaction time to changes in moisture tension. The tension readings typically ranged between 2 kPa and 10 kPa throughout the study. This particular regimen seemed to maintain good soil moisture conditions while avoiding water drainage from the bottom of the containers.

Growth rate was measured based on plant volume increase and weight of clippings collected after a typical pruning event. Plant volume was represented with a pseudo cylinder by averaging two diametrical measurements of each plant, calculating a projected area, and then multiplying by plant height. Measured diameters were approximately at right angles to each other. Height was defined by the distance from a plane defined by the top of the pot to the furthermost extent of branching in the vertical direction. Each diameter and height dimension was defined with a caliper. The quantity of clippings harvested and weighed was determined by subjectively simulating what a grower would typically remove during an annual pruning event to bring about compactness and an aesthetically pleasing shape.

Measurements of plant volume were done on one randomly selected plant per treatment per replication, i.e., eight treatments times four rep-

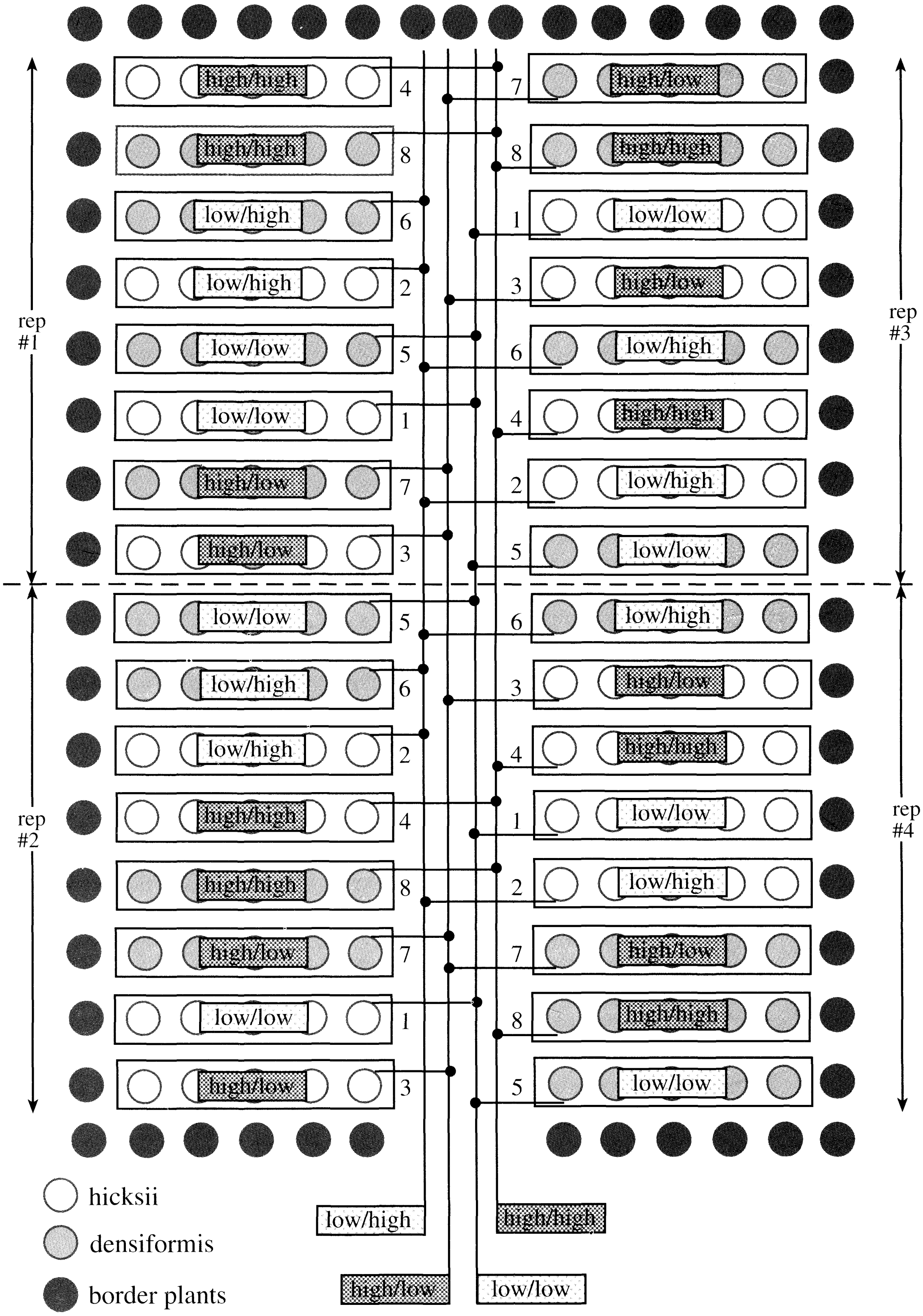

Figure 1. Top view of the experimental layout showing random placement of eight treatments and associated irrigation lines within four replications.

lications equals 32 plants. Plant volumes were first measured 8/4/92. Thereafter, measurements were made 10/4/92, 11/4/92, 1/12/93, 2/23/93, and 5/4/93 and subtracted from 8/4/92 results to determine volume increase. All 80 'Densiformis' plants were pruned 7/8/93, while all 80 'Hicksii' plants were pruned 9/8/93. The clippings were collected, immediately placed in one-gallon plastic zip-lock bags, and weighed as harvested without drying.

Results and Discussion

Since the plants were potted in June, the greatest flush of growth for *Taxus* cultivars was expected during late summer, between July and October. A summary of growth in terms of volume increase for each treatment is shown in Table 3 for the two-month period (8/4/92 to 10/4/92). Each measurement is the average of four replications of randomly selected plants. The average growth for the 'Densiformis' samples was nearly five times as great as the growth measured for 'Hicksii' (2,109 vs. 425 cm^3/month). Treatment No. 7 consisting of 'Densiformis' irrigated four times per week (M-W-F-Sun) using 100-33-33 ppm N-P-K resulted in the greatest growth of all treatments with 2,534 cm^3/month on average while 'Hicksii' irrigated two days per week (M-F) using 100-33-33 ppm N-P-K resulted in the least volume increase with 219 cm^3/month. The effect of growing 'Densiformis' using the low nutrition level of 100-33-33 ppm N-P-K (Treatment No. 5 and 7) averaged 2,471 cm^3/month while the high level of nutrition 200-66-66 ppm N-P-K (Treatment No. 6 and 8) averaged only 1,747 cm^3/month (Table 3). Finally, there was little difference in volume increase for 'Densiformis' when irrigating two times per week (2,029 cm^3/month) vs. four times per week (2,189 cm^3/month). Based on these very preliminary results using data from the two-month period (8/4/92 to 10/4/92), the best combination of factors and levels for maximum growth was 'Densiformis' irrigated two times per week using the low nutrition level 100-33-33 ppm N-P-K.

Table 4 shows example diameter and height measurements along with calculated volume for one of the largest 'Densiformis' plants and one of the largest 'Hicksii' plants, 8/4/92 through 5/4/93. Even though HID lighting was used over winter, the results show that little growth occurred and that a period of dormancy was not avoided. Decreases in volume (e.g., 'Densiformis,' Nov. 4 to Jan. 12; 'Hicksii,' Oct. 4 to Jan. 12) would imply plants diminished in size somehow. Since no pruning was done, the results most probably indicate variation in the

Table 3. A Comparison of Growth as a Function of Treatment Number as Measured by Volume Increase, 8/4/92 to 10/4/92. Each Measured Response Is the Average of Four Replications.

Treatment No.	A Cultivar	B Irrigation Frequency (days)	C Nutrition Level (ppm N)	Volume Increase (cm^3/month)
1	'Hicksii'	M-F	100	219
2	'Hicksii'	M-F	200	345
3	'Hicksii'	M-W-F-Sun	100	667
4	'Hicksii'	M-W-F-Sun	200	524
5	'Densiformis'	M-F	100	2,408
6	'Densiformis'	M-F	200	1,650
7	'Densiformis'	M-W-F-Sun	100	2,534
8	'Densiformis'	M-W-F-Sun	200	1,844

Table 4. Growth as Measured by Volume Increase for Two Selected Plants, One *Taxus* x *media* 'Densiformis' and One *Taxus* x *media* 'Hicksii', 8/4/92 to 5/4/93.

Date	Diameter One (mm)	Diameter Two (mm)	Height (mm)	Volume (cm^3)
		'Densiformis'		
Aug. 4	286	181	152	6,509
Oct. 4	285	210	190	9,141
Nov. 4	285	225	200	10,214
Jan. 12	255	195	240	9,543
Feb. 23	265	205	290	12,578
May 4	280	260	290	16,604
		'Hicksii'		
Aug. 4	123	116	166	1,862
Oct. 4	110	120	200	2,077
Nov. 4	105	120	195	1,938
Jan. 12	115	105	200	1,901
Feb. 23	130	120	200	2,454
May 4	150	195	215	5,025

technician's ability to subjectively identify representative diameters month to month. Note that the 'Densiformis' plant increased in volume from 6,509 to 16,604 cm^3/month (larger by a factor of 2.55, 8/4/92 through 5/4/93) while the 'Hicksii' increased from 1,862 to 5,025 cm^3/month (larger by a factor of 2.70, 8/4/92 through 5/4/93). Because of the preliminary nature of this research, the impact of dormancy and undesirable environmental conditions in the greenhouse throughout the winter, results obtained for volume increase after 10/4/92 through 5/4/93 for the rest of the plants did not merit further analysis.

A preliminary analysis of expected clippings mass that might be harvested from *Taxus* x *media* 'Hicksii' and *Taxus* x *media* 'Densiformis' is shown in Table 5. No clippings were harvested until near the end of the experiment. 'Densiformis' was pruned 7/8/93 and 'Hicksii' was pruned two months later, 9/8/93, i.e., the 'Hicksii' grew for 15 months after potting and the 'Densiformis' grew for only 13 months before the clippings were harvested. Based on the results for increase in volume (Table 3), the clippings mass for 'Densiformis' was expected to be much greater than for 'Hicksii.' Instead, the average yield for the 80 'Hicksii' plants was 28 grams per plant compared to 26 grams per plant on average for the 80 'Densiformis' plants. The plant volume represented by the pseudo cylinder clearly did not correlate with mass of clippings harvested. 'Densiformis' clippings did appear to be more delicate (smaller stem diameter than 'Hicksii') and more open and widely dispersed in the pseudo cylinder than 'Hicksii.' 'Hicksii' was typically more compact. This observation might partially explain the unexpected results. Based on an appropriate statistical analysis of variance, no significant difference was discerned between the eight treatments for the clippings mass response variable. This was due in part to the large standard deviation found within the treatment results.

As a comparison, Hansen *et al.* (1994) reported clippings yields for three-year 'Densiformis' were 23.6 grams per plant (dry basis) while yields for three-year 'Hicksii' were 18.8 grams per plant (dry basis). These measurements were obtained from field-grown plants at commercial nursery operations. Typical moisture contents for clippings were 51 to 57% (wb).

Conclusions

- Growth in terms of volume increase was measured for the two-month period (8/4/92 to 10/4/92). The average growth for the 'Densiformis' samples was nearly five times as great as the growth measured for 'Hicksii' (2,109 vs. 425 cm^3/month).

- Treatment No. 7, consisting of 'Densiformis' irrigated four times per week (M-W-F-Sun) using 100-33-33 ppm N-P-K, resulted in the

Table 5. A Comparison of Growth as a Function of Harvested Clippings Mass (Undried) for All Eight Treatments. Each Measured Response Is the Average Yield for 20 Plants.

Treatment No.	A Cultivar	B Irrigation Frequency (days)	C Nutrition Level (ppm N)	Clippings Mass (g)
1	'Hicksii'	M-F	100	30
2	'Hicksii'	M-F	200	24
3	'Hicksii'	M-W-F-Sun	100	31
4	'Hicksii'	M-W-F-Sun	200	27
5	'Densiformis'	M-F	100	14
6	'Densiformis'	M-F	200	32
7	'Densiformis'	M-W-F-Sun	100	28
8	'Densiformis'	M-W-F-Sun	200	29

greatest growth of all treatments with 2,534 cm^3/month on average while 'Hicksii' irrigated two days per week (M-F) using 100-33-33 ppm N-P-K resulted in the least volume increase with 219 cm^3/month.

- The effect of growing 'Densiformis' using the low nutrition level of 100-33-33 ppm N-P-K (Treatment No. 5 and 7) averaged 2,471 cm^3/month while the high level of nutrition 200-66-66 ppm N-P-K (Treatment No. 6 and 8) averaged only 1,747 cm^3/month.

- One of the best 'Densiformis' plants increased in volume from 6,509 to 16,604 cm^3/month (larger by a factor of 2.55, 8/4/92 through 5/4/93) while the best 'Hicksii' increased from 1,862 to 5,025 cm^3/month (larger by a factor of 2.70, 8/4/92 through 5/4/93).

- Based on harvested clippings mass (wb), the average yield for 80 'Hicksii' plants was 28 grams per plant compared to 26 grams per plant on average for 80 'Densiformis' plants.

Sources of Error

Taxus cultivars are typically grown outdoors in soil rather than in a greenhouse in potting medium. The effect of growing these plants in an artificial environment without full spectrum lighting is not known. Also, the attempt to provide HID lighting during the winter months to avoid dormancy is questionable. Since dormancy occurred anyway and growth was minimal, the plants in treatments set for irrigation four times per week (M-W-F-Sun) were no doubt watered in excess. In addition, the continual addition of nutrients during dormancy may have led to a build up of salts in the potting media, leading to detrimental results. A final difficulty was encountered when attempting to monitor growth by measuring plant diameter and height. It was difficult to determine actual dimensions to be measured. Because the choice was very subjective, measurements varied significantly between technicians and from month to month with the same technician.

Some of the plants developed rust-colored leaves/needles by May of 1993. The needles would fall off when the plant was mildly shaken. A plant pathologist thought the symptom might be due to salt build up in the potting medium. He did not recognize any disease symptoms. Since new growth was evident, the pathologist was convinced the plants were not dying.

Original objectives for the experiment included measurement of the Taxol content of harvested clippings. Because of the experimental difficulties and associated sources of error noted in the two earlier paragraphs and because of the cost of conducting laboratory analyses for Taxol, these measurements did not seem to be justified and therefore were not done.

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Taxus Clipping Harvester

Robert G. Holmes and Don Wuertz

Introduction

Identification of ornamental yews as a potential renewable source of Taxol and related taxanes has led to the need for efficient, effective methods for growing, harvesting, drying, storing, and handling *Taxus* clippings and needles. Communication with Bristol-Myers Squibb suggested that the demand (during 1993, 1994, 1995) for *Taxus* biomass would be met with bark from Pacific yew trees that were already under contract to be harvested and from naturally occurring sources in Nepal and India. However, many citizens of the United States and other countries are opposed to the harvest of naturally occurring trees and shrubs because of perceived insults to a stable environment. For example, on average, three to four Pacific yew trees, each 150 to 200 years old, would have to be sacrificed to obtain enough Taxol to treat one ovarian cancer patient.

A sensible alternative would be to harvest clippings from cultivated ornamental yews commonly grown in nurseries. For selected cultivars, clippings could be captured and processed as a by-product of annual pruning activities. Since the entire plant is not sacrificed, the potential for providing a renewable source of *Taxus* biomass is evident. The nursery grower would be in a position to grow dual purpose plants — clippings for the pharmaceutical marketplace and shrubs for the ornamental marketplace. Clipping is normally done with hand shears or by using a powered hedge trimmer. Both are labor-intensive operations.

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Harvester Design and Operation

The machine shown in Figure 1 was designed to mechanically cut and collect the clippings from the top of upright growing plants such as *Taxus* x *media* 'Hicksii.' The machine was powered by a medium-sized tractor (30 to 40 hp) and was constructed by modifying the header from a small grain combine.

The standard cutter bar and guards were replaced by 1-1/2-inch-wide "quick cut" knives and guards. In addition, the conventional knife hold-down plates were replaced by special 1/4-inch-thick hold-down plates on top of each guard to give a cleaner cut and prevent the small clippings from falling through the knife and being recut and lost on the ground (see Figure 2).

The standard reel was modified by reducing its diameter to 38 inches, by installing 12 batts in place of the normal six and by fastening 1/8-inch thick flexible rubber belting along the edge of each reel batt to "sweep" the cuttings off the cutter bar and back onto the canvas conveyor.

Text continues on page 44

Figure 1. View of *Taxus* clipping harvester during field operation.

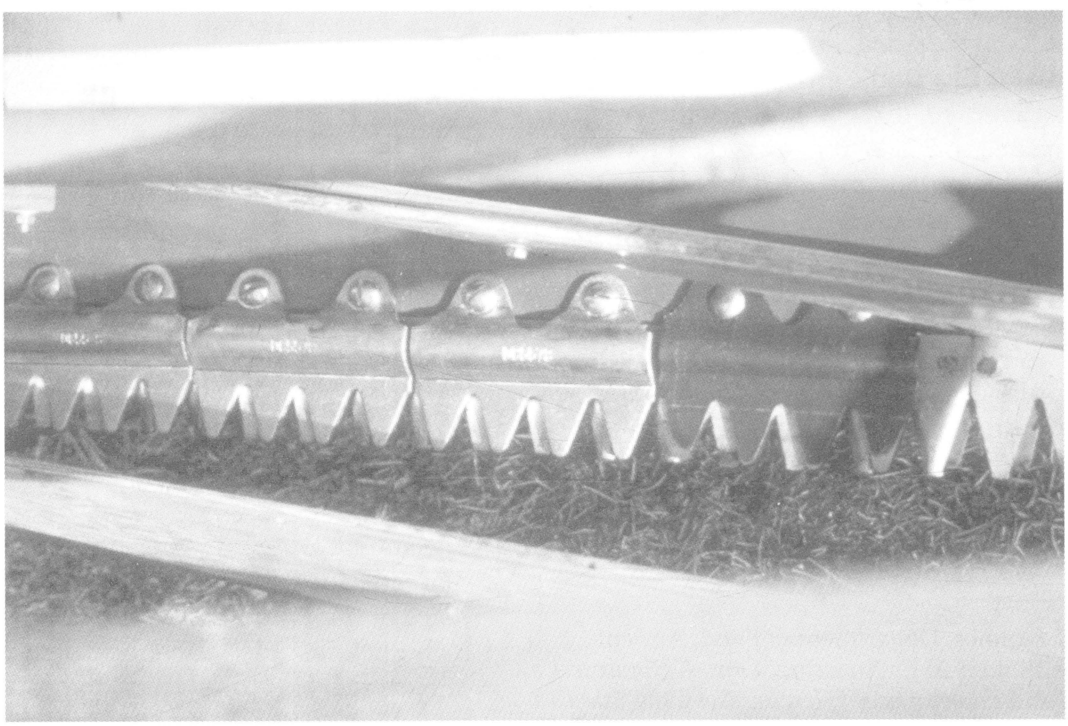

Figure 2. Close-up view of "quick cut" knives and guards.

Figure 3. Plywood collector box showing wire-mesh bottom.

Figure 4. View of two-pallet collection system.

With this system, a clean cut was achieved, and almost no cuttings were lost on the ground.

After being cut, the clippings were conveyed up the canvas conveyor and deposited into a 60-inch x 30-inch x 18-inch plywood collector box shown at the rear of the harvester (Figure 3). The collector box was constructed with a wire-mesh bottom to retain the clippings and needles but allow free airflow up through the plant material for drying.

The cutter bar, reel, and conveyor were all driven by hydraulic motors powered from the tractor hydraulic system. In addition, the entire machine and the header can be hydraulically positioned (independently) to allow trimming of plants from four inches to 30 inches in height (Short *et al.,* 1999).

When full, the front pallet box was slid to the rear, and the empty box was moved to the front (see Figure 4). A full box containing approximately 225 lbs. of clippings was off-loaded from the harvester to a truck or trailer and hauled to a dryer.

Harvesting Results

During 1992 approximately 1,000 acres of various *Taxus* cultivars were clipped with this machine. Ground speeds up to 1-1/2 mph were used. The rate of harvest varied considerably, depending on the plant spacing, age, cultivar, and growth conditions. Both five-foot-wide beds and three rows of individual plants were harvested. Maximum harvest rates of 300 lbs./hr. were attained while harvesting *Taxus* x *media* 'Hicksii' while rates up to 1,350 lbs./hr. were attained while harvesting *Taxus* x *media* 'Densiformis.'

Future Research

Future plans are to:

- Evaluate alternative cutting mechanisms to allow faster ground speeds while maintaining low clipping losses.
- Investigate mechanisms to lift side branches to allow trimming plants with a spreading growth pattern.
- Develop a system to trim the plant sides.
- Improve further the mechanical handling efficiency of the harvested material.

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Short, T. H., R. E. Young, and R. C. Hansen. 1999. *Mechanization of Landscaping and Outdoor Nursery Crop Production: Planting, Harvesting, and Handling.* Video, 12 minutes. The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, Ohio.

Thin-Layer Drying of Cultivated *Taxus* Clippings

Robert C. Hansen, Harold M. Keener, Hala N. ElSohly

Abstract

The ornamental yew, *Taxus* x *media* 'Hicksii,' has been identified as a renewable source of Taxol. However, clippings from the plant must be properly and efficiently harvested, dried, and stored. Thin-layer drying studies of *Taxus* clippings, needles, and stems were conducted. Parameters for the thin-layer drying equation were successfully determined for drying temperatures of 30°, 40°, 50°, and 60°C. The results showed that drying rates increased 28-, 15-, and 3-fold as drying temperatures increased from 30°, 40°, and 50°C to 60°C, respectively. Also, stems dried at a faster rate than needles, and needles dried at a faster rate than whole clippings. Taxol yields (g/100 g db) from stems were nearly constant for the four temperatures tested. However, yields from needles increased linearly as drying temperatures increased from 30° to 60°C. The highest Taxol yields were obtained from clippings. Nearly constant yields were obtained (~ 0.014 g/100 g db) for drying temperatures of 40°, 50°, and 60°C. The lowest yields for all three plant components occurred when the drying temperature was set at 30°C. The results suggested 60°C was the best temperature set point for drying *Taxus* x *media* 'Hicksii,' but temperatures higher than 60°C should be evaluated.

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Introduction

Taxol has been obtained from the bark of the Pacific yew tree, *Taxus brevifolia*, which grows in forests primarily located in Oregon and Washington. Because of low concentrations of Taxol in the bark (less than 0.01%) and a limited supply of trees, research was directed to finding additional sources for the drug. The ornamental yew, *Taxus* x *media* 'Hicksii,' was found to have concentrations of Taxol on the order of 0.02% in clippings (Croom, 1991). Croom (1991) stated Taxol yields from *Taxus* clippings are very sensitive to drying temperatures and procedures. Ornamental yews are typically pruned on an annual basis as a part of standard nursery practices. For commercial Taxol production, the clippings must be properly and efficiently harvested, dried, and stored. However, no published data exist on the effect of drying temperature on rate of drying and on extractable Taxol yield.

Objectives

The objectives of this research were to:

- Measure the rate of drying for *Taxus* clippings, needles, and stems as a function of temperature.

- Quantify parameters for the thin-layer drying equation.
- Measure the effect of drying temperature on Taxol yield.

Literature Review

Other than for purposes of propagation, *Taxus* clippings have not been routinely harvested or stored. Efforts to measure drying rates of any form of *Taxus* biomass have not been reported in research literature. However, drying of cereal grains and forage crops has been studied extensively (Hukill, 1947; Henderson and Perry, 1966; Brooker *et al.*, 1974; Hall, 1980; Parker, 1991).

Conceptualization of drying involves the single particle alone and the particle en masse. Many drying models have been developed for both cases. When drying a single particle (or thin-layer drying), the empirically fitted curves as well as theoretical curves based on heat and mass transfer for rate of moisture loss take the general form:

$$MR = \frac{(M - M_e)}{(M_o - M_e)} = \sum_{i=1}^{j} a_i e^{-k_i t} \qquad (1)$$

where j takes values between 1 and 4.

(See nomenclature on page 52.)

Studies have shown the parameter values to be dependent upon material, type, temperature, moisture level and its distribution within the kernel, and airflow. For the case of j = 1, k is known as the drying constant and is evaluated from thin-layer drying curves by plotting on semilog paper (Hall, 1980).

For corn, Henderson and Henderson (1968), Thompson *et al.* (1968), and Barre *et al.* (1971) have expressed k as a function of the saturation vapor pressure and air velocity:

$$k = bp_s^m v_a^n \qquad (2)$$

Using low-temperature (10°C) thin-layer drying data for corn, Sabbah *et al.* (1979) determined the constants b and m as 0.0722 and 0.722, respectively. The value for m derived by Henderson's and Thompson's data for shelled corn was 0.83.

According to Henderson and Pabis (1961) and Henderson and Henderson (1968), the effect of air velocity on the drying constant is trivial and, hence, the value of n becomes zero. However, when applying the drying constant to deep-bed drying, results of Barre *et al.* (1971) and Sabbah *et al.* (1979) give the value of n as 0.8 for corn.

Keener (1991) summarized values derived for k for grains based on published research. Most k values are reported as a function of temperature only. Other forms of thin-layer dryer equations have been proposed and are discussed by Brooker *et al.* (1974). For this study on thin-layer drying, velocity was fixed and no evaluation of n was attempted.

Materials and Methods

Clippings were collected from the cultivar *Taxus* x *media* 'Hicksii' on a research farm near Wooster, Ohio. The plants had been growing on the site for 13 years. Cuttings were obtained December 17, 1991, and were representative of one full season of growth. Within one hour after cutting, the material was packaged in one-gallon sealable plastic storage bags after which the samples were stored and refrigerated at 3.5°C.

The laboratory dryer consisted of a blower that directed air past three electrical resistance heating coils with one connected to a variable voltage controller. The resistance heating unit was adjusted and calibrated to operate at four temperatures — 30°, 40°, 50°, and 60°C.

The drying unit contained nine stacked trays (see Figure 1). Thermocouples located above each tray were used to monitor air temperatures at each tray. An orifice plate was used to monitor airflow rate. Temperature of the air at the orifice plate, barometric pressure, and ambient dew point temperatures were also recorded. Temperature readings from the thermocouples were recorded every 15 minutes with a Digi III Kaye Datalogger and a MFE tape recorder.

Previous research results showed Taxol yields were diminished if *Taxus* needles were separated from stems before drying (Croom, 1991). Therefore, trays 1 to 5 were dedicated to drying clippings. Trays 6 and 7 were used to dry needles only, and trays 8 and 9 were used to dry stems only. This arrangement permitted a rough comparison of drying rates for clippings, needles, and stems while at the same time permitting trays 1 and 2 to provide data for evaluating thin-layer drying rates. In addition, Taxol yields obtained by separating needles and stems prior to drying could be compared to earlier findings reported by Croom (1991), and trays 3, 4, and 5 could provide a first look at the variability of Taxol yield within a drying zone.

One batch of clippings, needles, and stems was dried at each of the four temperatures noted previously. Each tray was 26.7 cm x 26.7 cm x 3.5 cm deep. The weight of each sample of standard clippings before drying was approximately 180 g. After drying, the final weight was about 75 g. Subsamples of needles were stripped from clippings and separated from stems. The needles that were dried in trays 6 and 7 were similar in weight to the whole clippings dried in trays 1 to 5. The stems, dried in trays 8 and 9, weighed approximately 100 g before drying and about 50 g after drying. Average cross-sectional airflow velocity was 0.039 m/s. The trays were 6.4 cm apart (Figure 1).

The *Taxus* samples were weighed at various times throughout each of the four tests. For example, for the test where the temperature was set at 60°C, samples were weighed at 0, 2, 4, 16, 19, 22, 28, and 46.5 h. At 40°C, weights were

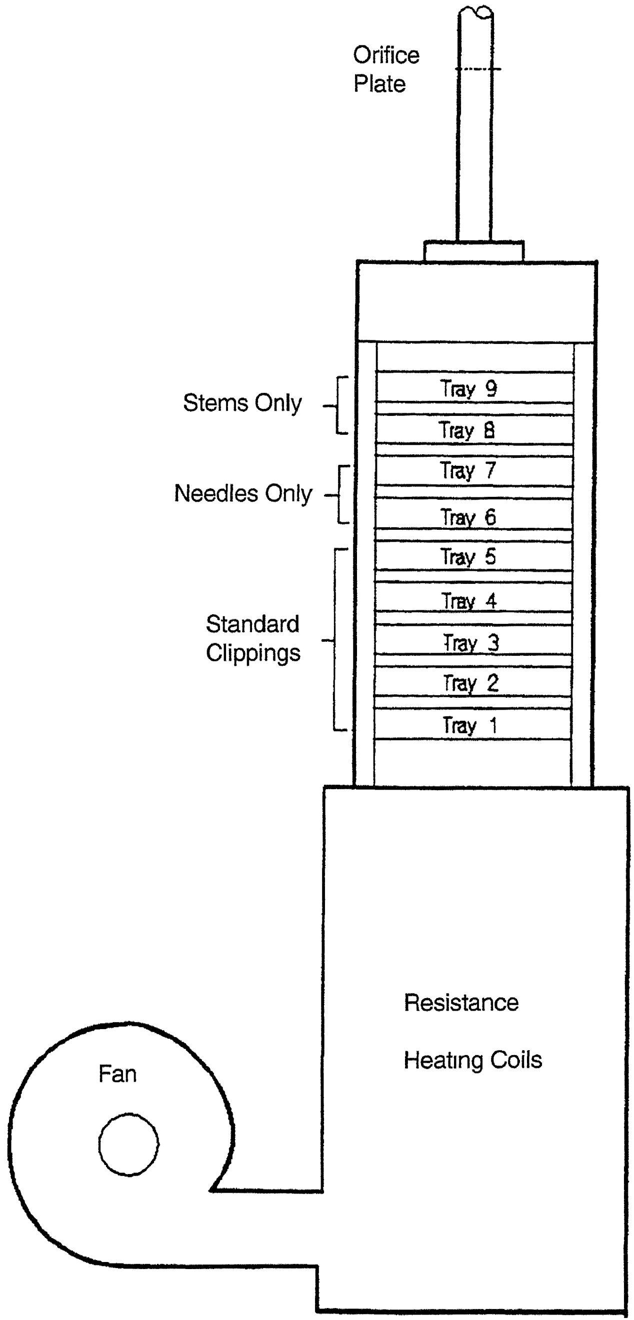

Figure 1. Schematic illustration of the thin-layer dryer showing the relative location of the drying trays.

recorded at 0, 2, 4, 8, 12, 23.25, 27.25, 31.25, 38, 65, 77, 82.5, 86, and 101 h. The dryer door was opened and remained open during the time period required to remove each tray, weigh it, record it, and return it to the appropriate location in the dryer. The fan and the heater continued to operate during this time. The time period required to weigh all nine trays was about two minutes. When a constant weight was obtained for three consecutive readings, it was concluded that all moisture was volatilized, and the test was terminated. Relative humidity levels ranged from 21.8% for the 30°C test down to 0.3% for the 60°C test.

For calculations of moisture ratios in the results presented in this article, the equilibrium moisture content (M_e in Equation 1) was set equal to zero. Setting M_e equal to zero was appropriate since the 180 g samples dried at 30°, 40°, and 50°C reached equivalent final weights and were equal to the final weight for plant samples dried at 60°C. If the equilibrium moisture contents were significantly different than zero, final weights would have been different for one or more of the four drying conditions. Initial moisture content of fresh *Taxus* clippings ranged from 55 to 60% wet basis.

Taxol yields for the tests were determined by the Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi. After a drying run was completed, the contents of each tray were sealed in plastic bags in preparation for shipment. All samples were stored at room temperature for about six weeks prior to shipment to Mississippi.

Results and Discussion

In general, the lowest trays dried at a faster rate than the higher ones, indicating that location was a factor and that the drying air picked up sufficient moisture to reduce rates of drying in the upper trays. However, temperature variations within trays 1 to 5 were less than 1°C after 8–12 hours for all tests.

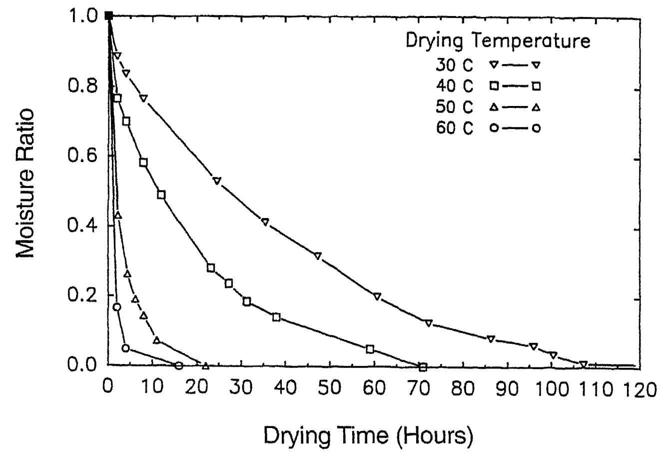

Figure 2. Drying curves for *Taxus* x *media* 'Hicksii' clippings as a function of drying temperature.

In order to compare thin-layer drying rates for the four drying temperatures studied, moisture ratios as a function of time were averaged for trays 1 and 2 and plotted in Figure 2. The results showed that drying time decreased dramatically as drying temperatures increased. For example, total drying time for 40°C was nearly three days while 50°C required about one day; i.e., total drying time was reduced to one-third with only a 10°C increase in temperature. These conclusions are, of course, based on data for

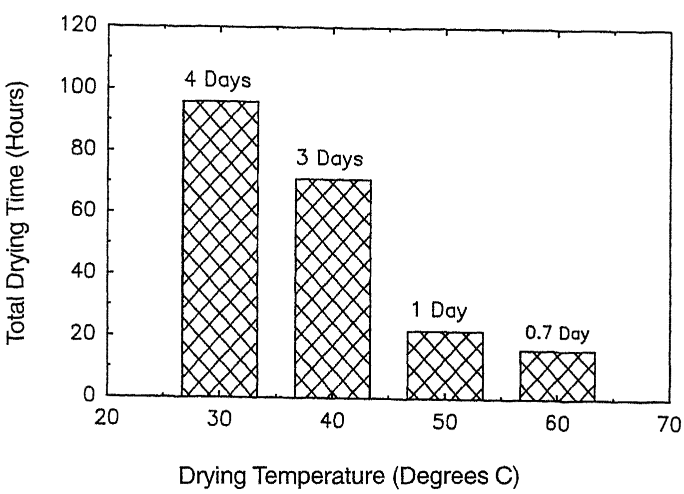

Figure 3. Total drying time for *Taxus* x *media* 'Hicksii' clippings as a function of drying temperature.

thin-layer drying and do not account for the effects of deep beds. Figure 3 illustrates the effect of temperature on total drying time.

Hall (1980) described a procedure for determining the drying constant (k) by drawing a semilogarithmic plot of moisture ratio (MR) vs. time (t). Since Equation 1 is an exponential relationship, the data should plot as a straight line for j = 1, where the slope is equal to k. Figure 4 is a semilogarithmic plot of the same results that are plotted in Figure 2, with the exception that the curves were truncated for MR < 0.1. The data come very close to fitting straight-line relationships.

Results of a regression analysis are shown in Table 1 where parameters are tabulated for the thin-layer drying equation (Equation 1) for each of the four drying temperatures tested. A regression analysis of k vs. p_s for drying temperatures tested gave:

$$k = 1.62 \times 10^{-10} \, p_s^{2.233} \quad (r^2 = 0.967) \qquad (3)$$

A comparison of estimated drying rates for clippings, needles, and stems is shown in Figure 5 for a drying temperature of 40°C. To minimize the effect of bed depth, the results were obtained by averaging the drying rates for trays 4 and 5 for clippings, trays 6 and 7 for needles, and trays 8 and 9 for stems. Even though the stems were in the top two trays, the drying rate was much higher than it was for needles or clippings.

Taxol yields (g/100 g db) are compared for clippings, needles, and stems as a function of drying temperature in Table 2 and Figure 6. The

Table 1. Evaluation of Thin-Layer Drying Constants for *Taxus* Clippings at 30° to 60°C, $MR = Ae^{-k\theta}$.

Temperature Dry Bulb (°C)	Temperature Dew Point (°C)	Relative Humidity (%)	No. of Data Pts.	A	k (h^{-1})	r^2
30	5.8	21.8	18	0.981	0.027	0.986
40	1.5	9.2	18	0.888	0.049	0.978
50	5.3	7.2	10	0.839	0.234	0.930
60	-0.5	0.3	6	0.912	0.762	0.983

Evaluation of A and k are based on linear regression using y = 1n MR and x = θ.

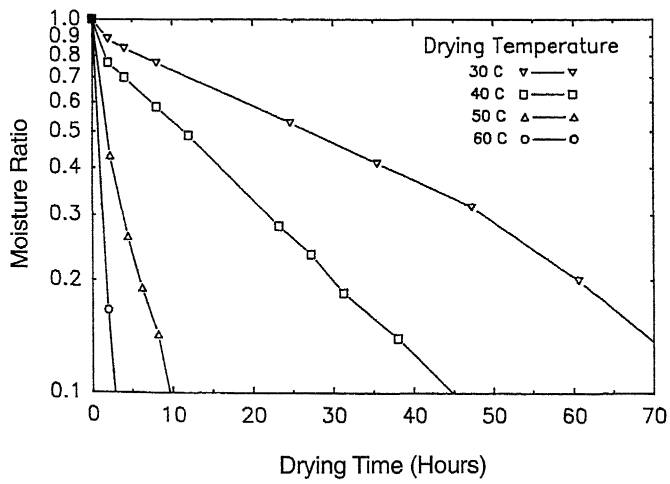

Figure 4. Semilogarithmic plot of drying curves for *Taxus* x *media* 'Hicksii' clippings as a function of drying temperature.

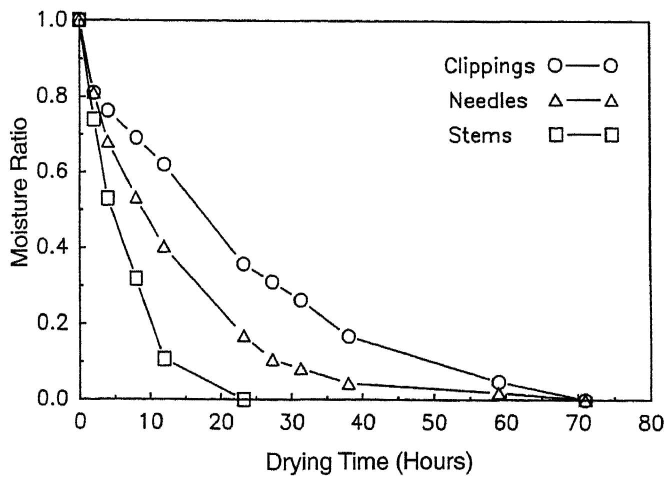

Figure 5. Drying curves for *Taxus* x *media* 'Hicksii' clippings as a function of plant parts. Temperature set point for dryer was 40°C.

Table 2. Listing of Average Taxol Yields Along with Sample Standard Deviations for Identified Components of *Taxus* x *media* 'Hicksii' as a Function of Drying Temperature.

	Clippings			Needles			Stems		
Drying Temp. (°C)	Sample Size (n)	Taxol Yield (g/100g) (avg.)	(s.d.)	Sample Size (n)	Taxol Yield (g/100g) (avg.)	(s.d.)	Sample Size (n)	Taxol Yield (g/100g) (avg.)	(s.d.)
30	5	0.0077	0.0037	2	0.0051	0.0037	2	0.0033	0.0004
40	5	0.0138	0.0016	2	0.0072	0.0011	2	0.0042	0.0007
50	4*	0.0135	0.0004	2	0.0092	0.0014	2	0.0034	0.0005
60	5	0.0137	0.0009	2	0.0120	0.0006	2	0.0039	0.0004

* Due to experimental error, one sample was excluded from the analysis.

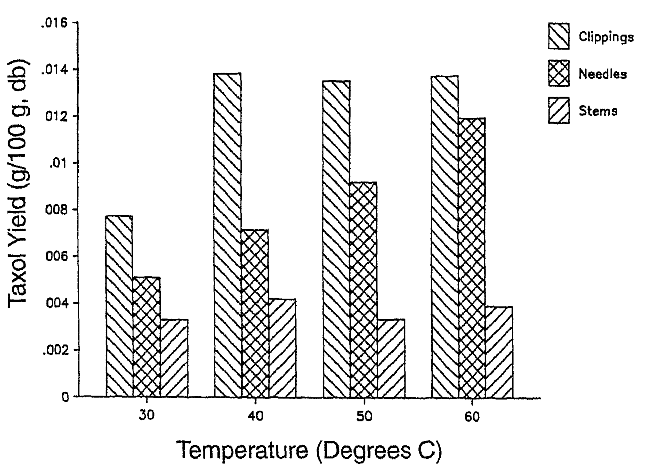

Figure 6. Taxol yields from *Taxus x media* 'Hicksii' clippings, needles, and stems as a function of drying temperature.

results indicated that yields from stems were unaffected by drying temperature; the yield from needles increased linearly as the temperature increased from 30° to 60°C; and the yield for clippings was nearly constant at 0.014% for 40°, 50°, and 60°C while being much lower at 0.008% for 30°C.

When comparing 30°, 40°, 50°, and 60°C drying temperatures, Figures 3 and 6 clearly showed that *Taxus* x *media* 'Hicksii' clippings should be dried at 60°C. Although the effects of depth of the drying bed have not been evaluated here, the results indicated that drying clippings at 60°C could be accomplished under ideal conditions in less than 16 hours with Taxol yields equal to or better than drying at the lower temperatures tested. A drying temperature of 30°C may bring about enzymatic activity which destroys Taxol. This effect, along with the extra time required, would suggest that drying should be done at 60°C or higher.

Although preliminary results indicated that needles should not be separated from stems prior to drying because Taxol would be lost (Croom, 1991), the results in Table 2 and Figure 6 showed increasing yields from separated needles as temperatures increased from 30° to 60°C. These results suggested that drying temperatures greater than 60°C should be evaluated for both clippings and needles.

Conclusions

Thin-layer drying studies of clippings, needles, and stems were conducted in a laboratory dryer. The thin-layer drying equation was found to be applicable for the prediction of drying rates for the *Taxus* materials tested at 30°, 40°, 50°, and 60°C drying temperatures. Parameters for the drying equations were successfully determined. The results showed that the drying constant at 60°C was 3.2 times greater than k at 50°C and 15.5 times greater than k at 40°C. Also, stems were found to dry at a faster rate than needles, and needles dried at a faster rate than whole clippings.

Comparison of Taxol yields for four drying temperatures from 30° to 60°C suggests that 60°C is the most ideal temperature for drying clippings and separated needles and stems. Drying at 30°C resulted in low yields. Future testing should be directed at drying temperatures greater than 60°C. Perhaps *Taxus* biomass should be dried a few hours at a high temperature to stop enzymatic activity followed by a step down to a lower temperature to finish the process.

Acknowledgments

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Nomenclature

a,b,A coefficients

k drying constant (h^{-1})

m,n exponents

M grain moisture, decimal dry basis (dimensionless)

M_e equilibrium moisture content, decimal dry basis (dimensionless)

M_o initial moisture content, decimal dry basis (dimensionless)

MR moisture ratio at a location in bed, decimal (dimensionless)

p_s vapor pressure at saturation (Pa)

t time (h)

v_a superficial air velocity, based on empty containers (ms^{-1})

q time (h)

Evaluation of Bin Drying of *Taxus* Biomass

Robert C. Hansen, Ralph B. Shugert Jr., Hala N. ElSohly, Edward M. Croom Jr., Harold M. Keener

Summary

Standard grain-storage bins equipped with dryers were used to dry *Taxus* biomass. Drying temperatures were monitored and compared to specifications of 115 ± 15°F. Attempts to improve temperature control and to reduce variation were only marginally successful. The drying system was not capable of drying *Taxus* biomass to designated specifications.

Introduction

Clippings and needles from *Taxus* plants have been investigated as a source of Taxol, a promising drug for treatment of ovarian and breast cancers. Drying has been specified as a critical step in the preparation of *Taxus* biomass for storage. Drying is also generally recommended prior to the Taxol extraction process. The need for drying was established during preliminary laboratory research (Croom *et al.*, 1991); however, attempts to dry *Taxus* clippings and/or plants on a commercial scale have not been reported in the literature.

Objectives

The objectives of this research were to measure drying temperature distributions while drying *Taxus* biomass in a standard grain-drying bin and to determine the effect of drying temperatures on Taxol yields.

Literature Review

Research identifying specifications for drying *Taxus* clippings and needles was reported by Croom *et al.* (1992). Optimum Taxol yields were obtained when drying temperatures were maintained between a lower limit of 100°F and an upper limit of 130°F. In addition, percent Taxol yields from needles were found to be significantly higher when the needles were dried while remaining attached to stems rather than being separated from stems before drying.

Hansen *et al.* (1993) used a thin-layer laboratory dryer to measure drying rates as a function of temperature for clippings, needles, and stems from *Taxus* x *media* 'Hicksii.' Based on their re-

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sults, parameters were determined for a thin-layer drying equation for drying temperatures of 30°, 40°, 50°, and 60°C. The results showed that drying rates increased dramatically as drying temperatures increased. Also, stems were found to dry at a faster rate than needles, and needles dried at a faster rate than whole clippings.

Procedure

The drying study was conducted through the cooperation of Zelenka Nursery, Inc., on a farm about 10 miles south of Grand Haven, Michigan. A circular 27-ft.-diameter grain-storage bin, seven rings high, which was equipped with a perforated floor, was available. The floor provided space (18 inches high) for a plenum to which was attached a 10-hp, 3,450-rpm, 28-inch-diameter vane axial drying fan with a propane heating unit rated at 2 million Btu/hour. (See Figure 1.)

Results in this paper pertain to data collected while drying whole *Taxus* x *media* 'Hicksii'

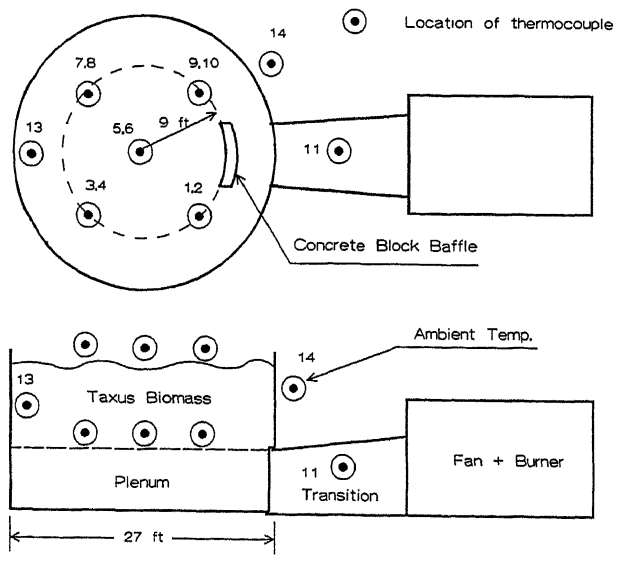

Figure 1. Schematic drawing of the drying system showing the location of temperature measurement points for Lot Nos. 105–109.

plants which averaged 36 inches in height. The plants had matured to the point where they were not marketable as ornamental plants and were therefore deemed to be culls. For most of the drying runs, roots were removed to reduce weight and contamination of the bin with soil.

Plants identified by nine lot numbers were dried in various quantities and drying conditions as summarized in Table 1. As indicated in Note 3, Table 1, the fan and heater were replaced with new units during the time when Lot No. 103 was being dried. The temperature control system on the old dryer appeared to be defective. Since retention of Taxol content was apparently dependent on being able to hold drying temperatures at 115 ± 15°F, purchase of a new unit seemed to be prudent. Also, problems with the old heating unit had caused shutdowns at night while drying previous material. Specifications on the new fan and heater were 7.5- to 10-hp, 3,450-rpm, 24-inch-diameter vane axial fan with a 1,035,000 Btu/hr heater. In order to double drying capacity, a second 27-ft.-diameter bin was equipped identical to the first. Both bins were used to dry Lot Nos. 107, 108, and 109.

A Leeds and Northrup strip recorder was set up to monitor drying temperatures with thermocouples. Temperatures were recorded one channel at a time every 1.875 minutes. While monitoring temperatures for one bin using 13 channels, a complete cycle of recordings required approximately 24 minutes and 20 seconds. When two bins were being monitored with 24 thermocouples, 45 minutes were required for a complete cycle. Chart speed was set at three inches per hour.

While drying Lot Nos. 101–104, 10 thermocouples were distributed to monitor temperatures in the bin on top of the perforated floor. In addition, one was placed in the transition channel between the burner and the bin, and one was placed in a shaded position outside to measure ambient temperatures. A schematic of the thermocouple layout is shown in Figure 2. After finishing the drying for Lot No. 104 and before

Table 1. **Summary of Drying Activity for Cull Plants, *Taxus* x *media* 'Hicksii,' at Zelenka Nursery, Inc., Lot Nos. 101–109, Nov. 22, 1991, to Jan. 16, 1992.**

Lot No.	Drying Schedule Start		Drying Schedule Stop		Drying Time (hrs)	No. of Plants	Undried Plants (lbs)	Dry Needles (lbs)	Yield (%)
101[1]	11/22	4:30 p.m.	11/26	8:00 a.m.	87.50	340	3,400	830	24.4
102[2]	11/26	5:00 p.m.	11/30	12:00 n	91.00	300	3,260	560	17.2
103[3]	12/02	1:30 p.m.	12/08	6:00 a.m.	136.50	285	2,700	520	19.3
104[4]	12/09	4:30 p.m.	12/13	9:30 a.m.	89.00	600[4](c)	5,820	1,120[4](c)	19.2
105[5]	12/13	4:30 p.m.	12/18	7:00 a.m.	100.50	600[4](c)	5,300	1,400[4](c)	26.4
106[6]	12/18	2:30 p.m.	12/24	3:00 p.m.	144.50	1,250	9,060	2,760	30.5
107B1[7]	12/26	4:15 p.m.	01/01	4:30 p.m.	144.25	900	9,780	2,000	20.4
107B2[7]	12/26	3:30 p.m.	01/01	4:30 p.m.	145.00	900	9,240	1,980	21.4
108B1[8]	01/02	4:00 p.m.	01/09	6:00 a.m.	170.00	1,000	13,500	2,420	17.9
108B2[8]	01/02	4:00 p.m.	01/09	6:00 a.m.	170.00	1,000	13,500	2,420	17.9
109B1	01/09	4:45 p.m.	01/16	7:00 a.m.	170.25	1,000	13,500	2,490	18.4
109B2	01/09	4:45 p.m.	01/16	7:00 a.m.	170.25	1,000	13,500	2,290	17.0

1. (a) The 50 plants standing upright were dry in 36 hours.
   (b) Nov. 25. Turned dryer off at 1:30 p.m. Out of LP gas. Turned on again at 2:30 p.m.

2. Nov. 29. Checked dryer at 7:00 a.m. Pilot light went off during the night. After service call, restarted dryer at 1:30 p.m.

3. (a) Dec. 3. Pilot light went out at 3:30 a.m. All temperatures dropped to 25°F until the dryer was restarted at 6:00 a.m. Pilot light went out again at 6:30 p.m. Restarted at 8:30 p.m. Went out again at 9:00 p.m. Dryer stayed off until it was restarted at 5:30 a.m. Dec. 4. All temps dropped to 25°F to 30°F during down time.
   (b) Dec. 4. Shut down dryer at 12:30 p.m. for the purpose of installing a new fan and burner. Restarted dryer with new fan and burner plus baffle about noon on Dec. 5.
   (c) Dec. 6. Natural gas odor detected around dryer.

4. (a) Dec. 9. Loaded entire floor of drying bin by standing plants upright with one or two layers of plants placed horizontally on top. A new ignitor was installed before starting dryer.
   (b) Dec. 11. Still detecting a gas odor. Applied foam insulation (11:00 a.m. to 1:00 p.m.) to outside bin wall (9-ft. high) and to transition.
   (c) Dec. 13. Sorted the 600 plants after shutting down the dryer at 9:30 a.m. Decided to retain 45 plants that were not dry enough; placed them along the bin wall and included them in Lot No. 105 for additional drying. Therefore, dry needles were only harvested from 555 plants for Lot No. 104. Lot No. 105 actually consisted of 645 plants.

5. No comment.
6. No comment.

7. The 900 plants in Bin No. 2 were composed of 500 plants without roots (as per usual) and 400 plants with roots.
8. Two hundred of the 1,000 plants were loaded into Bin No. 2 with roots. Assume 800 plants in Bin No. 2 and 1,000 plants in Bin No. 1 were without roots.

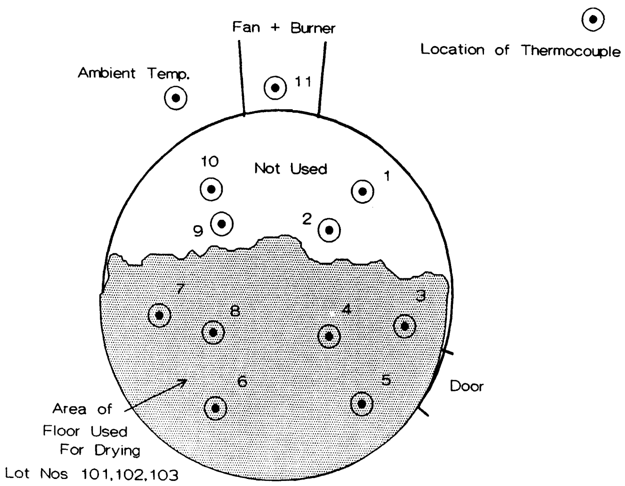

Figure 2. Map of temperature measurement points for Lot Nos. 101–104. The limited area of the floor used for drying Lot Nos. 101–103 is also identified.

starting the drying for Lot No. 105, the configuration for monitoring temperatures was changed so that measurements at the top of the plant material could be compared to measurements directly below at floor level. Also, temperatures a few inches away from the bin wall were recorded at one point in the bin. (See schematic in Figure 1.)

In an attempt to reduce drying time to avoid unnecessary heat loss at the bin wall and to improve energy use efficiency, foam insulation was applied on the outside of the wall of the bin and over the transition channel. The foam was applied two inches thick and about 20 inches high around the plenum and then narrowed to one inch of thickness to a total height of nine feet. The insulation was applied on Dec. 11 during the drying of Lot No. 104. Bin No. 2 was also insulated in the same manner before it was used for the first time on Dec. 26 for Lot No. 107.

Various configurations for placement of the plant material on the bin floor were tried. Without static pressure build-up in the plenum that is typical for grain drying, wide distributions of airflow and drying temperatures occurred. Higher airflow rates and higher temperatures were measured on the side of the bin opposite the fan and heating unit. Therefore, *Taxus* material located on the hotter areas of the plenum would dry more quickly than those in cooler areas.

One strategy, used for Lot Nos. 101, 102, and 103, was to cover the cooler area of the floor with plywood and only dry using the hotter areas. (See layout in Figure 2.) While this procedure generally led to satisfactory results, drying capacity in terms of plant biomass dried per batch was severely restricted. Before drying Lot No. 101, a three-inch layer of pea gravel was distributed on the perforated floor in Bin No. 1 in an attempt to build up static pressure in the plenum and to reduce temperature variation from one part of the floor to the other. This strategy did not bring about measurable improvement, and therefore pea gravel was not applied to the floor in Bin No. 2.

Another strategy for distributing heat energy more uniformly throughout the plenum was the placement of a concrete block baffle, four-feet long and two blocks high, in a circular arc about four ft. away from the bin wall in line radially with the transition. (See Figure 1.) This was installed on Dec. 5 along with the new fan and burner for Bin No. 1. A similar baffle was installed in Bin No. 2.

Starting with Lot No. 104, plants were placed in upright positions (roots removed) and distributed across the entire floor. One or two layers of plants were placed horizontally across the top of the upright plants. With the new fan, burner, temperature controller, and baffle in place, temperature variation was expected to be significantly reduced. In addition, insulation was applied to Bin No. 1 during the drying of Lot No. 104 from which improvements in drying efficiency were expected.

Taxus biomass is considered to be dry when the needles become brittle. Evidence of brittleness is identified when needles break or snap as they are stressed or pinched longitudinally between the thumb and forefinger of an observer's hand.

Biomass color also changes from green to light brown. The point at which brittleness occurs corresponds with a moisture content of 2.5 to 3.0 percent, wet basis.

Dried needles were successfully removed from the branches of shrubs by feeding them through an electrically powered, rotating drum to which rows of 0.5-inch-diameter by three-inch-long hard rubber fingers were attached. The machine is commonly known as a power-driven mechanical chicken plucker. Relatively small quantities of stems and bark were harvested with the needles. The harvested needles were successfully stored in an unheated outdoor shed after being wrapped in double layers of polypropylene, approximately 200 lbs. per pallet.

Discussion of Results

The drying schedule for Lot Nos. 101 to 109 (12 batches), showing starting and stopping times, is tabulated in Table 1. Increased drying times corresponded with efforts to increase batch size. As the number of plants per batch increased, the depth of the *Taxus* biomass in the bin increased from an average depth of 2.5 to 3.0 ft. to an average depth of 3.5 to 4.0 ft. Simultaneously, ambient temperatures were generally decreasing as starting dates progressed from late in November to early in January. Therefore, increasing drying times most likely resulted both from increasing biomass depth and colder days. Total drying time ranged from under four days to seven days. The last column in Table 1 shows that yields of dry needles as a percent of the weight of undried plants ranged from 17 to 30%. The moisture content of fresh, undried *Taxus* biomass studied to date ranged from 50 to 60 percent, wet basis.

Table 2 is a summary of drying statistics for the Lot Numbers identified in Table 1. Statistics for Lot Nos. 101 to 103 were based on thermocouple locations 3 to 8 since a significant portion of the drying floor was not used. (See Figure 2.) All locations were included in calculations for Lot No. 104 since the lot size was doubled and the entire floor was filled with plants. As indicated under the procedure section (see previously), thermocouple locations for Lot Nos. 105 to 109 were distributed as

Table 2. Summary of Temperature Means and Measures of Variation as Monitored at the Plenum Floor. Temperature Specifications: 115 ± 15°F.

Lot No.	Mean (°F)	Std. Dev. (°F)	Min. Temp. (°F)	Max. Temp. (°F)	Above Spec. (%)	Within Spec. (%)	Below Spec. (%)	Taxol Yield (%)
101	112.2	10.4	88	132	1.8	87.5	10.7	2.14
102	112.0	12.8	84	143	9.0	76.4	14.6	2.09
103	109.0	14.5	48	146	7.8	71.1	21.1	2.15
104	112.2	11.1	79	142	7.6	82.1	10.3	1.67
105	113.9	13.0	90	141	11.1	70.6	18.3	1.34
106	109.0	13.4	84	146	6.7	63.7	29.6	1.34
107B1	111.4	10.8	88	137	1.4	78.1	20.5	1.49
107B2	—	—	—	—	—	—	—	—
108B1	115.4	11.7	89	140	7.7	79.2	13.1	1.64
108B2	114.9	9.9	90	140	5.0	91.1	3.9	—
109B1	111.7	9.3	64	135	1.1	89.6	8.3	1.47
109B2	114.5	8.1	93	134	1.1	93.6	5.3	—

shown in Figure 1. Therefore, the results shown in Table 2 for Lot Nos. 105 to 109 were determined only from the five thermocouples located on the drying floor. (See Figure 1.)

As drying proceeded for each lot and as floor temperatures were monitored with the Leeds and Northrup recorder, the burner temperature was adjusted in an effort to meet drying specifications of 115 ± 15F°. Data from the strip charts were tabulated for each temperature reading approximately once every three hours as a basis for calculating mean and variation statistics summarized in Table 2.

Table 2 shows that mean temperatures for each lot generally were slightly below the target value of 115°F. Lot Nos. 108B1, 108B2, and 109B2 were nearly on target. Standard deviations ranged from a low for Lot No. 109B2 of 8°F to a high for Lot No. 103 of 14.5°F. As noted in Table 1, Lot No. 103 was subjected to numerous starts and stops caused by burner malfunctions. Additional measures of temperature variation at the plenum floor include minimum and maximum temperatures recorded and percentage of temperature recordings tabulated

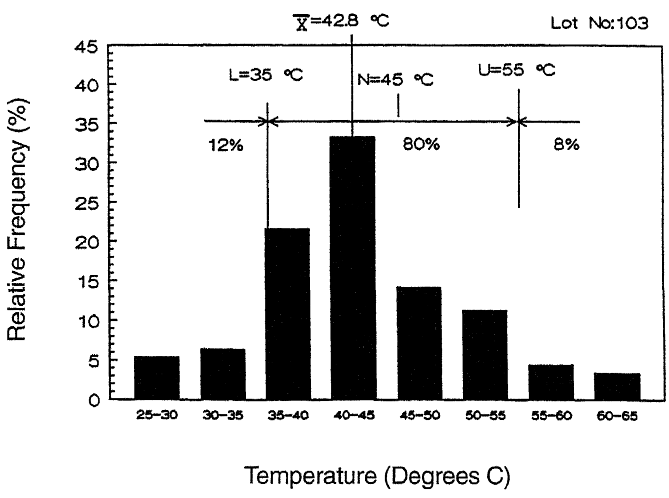

Figure 3. Histogram of temperatures at the drying bin floor for Lot No. 103.

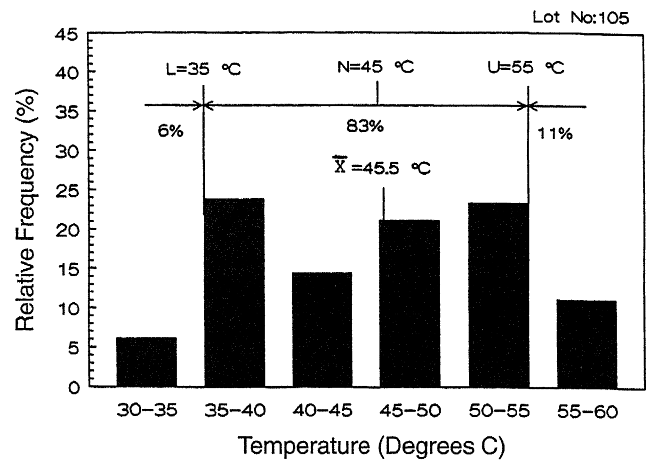

Figure 4. Histogram of temperatures at the drying bin floor for Lot No. 105.

that were above, within, and below specification limits (see Table 2.)

Histograms of recorded temperatures graphically compare temperature distributions with the target temperature and specification limits (based on Celsius units) in Figure 3 for Lot No. 103 and in Figure 4 for Lot No. 105. A tolerance based on 45 ± 10°C is somewhat more relaxed than 115 ± 15°F. In both cases, drying temperatures averaged within 2.2°C of being right on the nominal temperature, and 80 percent or more of recorded temperatures at the floor were within upper and lower specification limits. The histogram for Lot No. 103 exemplifies temperature distributions before the new fan and heating unit were purchased and the baffle and insulation were in place. The histogram for Lot No. 105 is typical of results obtained after the improvements were installed. Although a significant reduction in temperature variation was not achieved as expected, the improvements did permit doubling and even tripling batch size without increasing temperature variation. There were some evidences of reductions in temperature variation in a few batches (see Table 2).

Figures 5 and 6 are plots of temperature histories for Lots No. 103 and 105, respectively, throughout the time periods that drying oc-

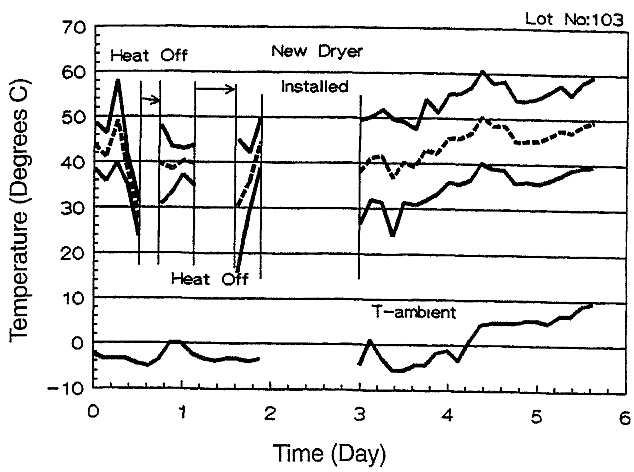

Figure 5. Temperature histories of maximum, mean, and minimum floor temperatures compared to a history of ambient temperature for Lot No. 103. Interruptions in the drying process are noted.

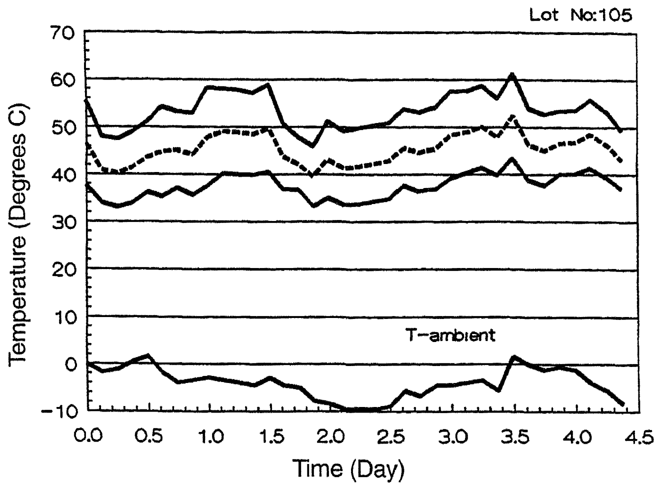

Figure 6. Temperature histories of maximum, mean, and minimum floor temperatures compared to a history of ambient temperatures for Lot No. 105.

curred for each batch. The problems that disrupted the drying of Lot No. 103 are noted in the graph in Figure 5. Even after the new drying unit was installed, note that maintenance of mean temperatures on a 45°C target was not attained. The dryer did not control temperatures to a prescribed set point. On the contrary, the temperatures seemed to parallel ambient temperatures.

Figure 6 exemplifies the same concerns for Lot No. 105, although, in general, control seems to be improved.

Summary

Twelve batches of *Taxus* x *media* 'Hicksii' whole plants were dried in two 27-ft.-diameter grain-storage bins that were equipped with perforated floors, vane axial drying fans, and propane burners. Temperatures were monitored at the top surface of the plenum floor with thermocouples and a strip-chart recorder.

After temperature variations ranging from 30° to 40°F were recorded, efforts were made to reduce variation. The plenum and bin walls were insulated to reduce heat loss, a concrete block baffle was installed to diffuse heated air more uniformly throughout the plenum, and new drying units were added to improve temperature control. While these changes permitted drying more *Taxus* biomass per batch, reduction in temperature variation was small. Temperature control to a prescribed set point was poor. The drying system was not capable of drying *Taxus* biomass to specifications of 115 ± 15°F.

Acknowledgments

The authors gratefully acknowledge the dedicated assistance of Charles Wolters, Craig Schwander, and Dean Souden of Zelenka Nursery, Inc., Grand Haven, Michigan.

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Hansen, R. C., H. M. Keener, and H. N. ElSohly. 1993. Thin-layer drying of cultivated *Taxus* clippings. *Transactions of the ASAE.* 36(6):1873–1877.

Selecting Bed Depth for Drying Cultivated *Taxus* Clippings

Robert C. Hansen and Harold M. Keener

Summary

The ornamental yew, *Taxus* x *media* 'Hicksii,' has been identified as a renewable source of Taxol. However, clippings from the plant must be properly and efficiently harvested, dried, and stored.

Analysis of deep bed drying systems for *Taxus* plant material was done using the logarithmic model of drying. Results show drying capacity, airflow, energy costs, and efficiency as a function of fan power and bed depth.

Introduction

Ornamental yews are pruned on an annual basis as a part of standard nursery practices. Pruning typically occurs either in April/May or September/October. For commercial Taxol production, the clippings must be properly and efficiently harvested, dried, and stored. Hansen *et al.* (1993) have published data on thin-layer drying of *Taxus* and showed 50° to 60°C gave high rates of drying with no loss in Taxol yield. However, no published data exist on the optimum airflows and depth that would be energy efficient and cost effective for drying *Taxus* material.

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Objective

The objective of this research was to evaluate and optimize airflow rate and bed depth as design parameters for minimizing cost of drying *Taxus* plant material.

Analysis

Logarithmic Model of Drying

Sabbah *et al.* (1979) reported that the analytic model described as a logarithmic model application to deep bed drying (Hukill, 1954; Barre *et al.*, 1971) could be used to predict average drying-time history and forecast the average moisture content of a deep bed of grain dried with ambient or solar-heated air. This model was used in this study instead of finite difference models of grain drying (Morey *et al.*, 1978; Bakker-Arkema *et al.*, 1978; Keener *et al.*, 1978), because of computational speed and flexibility in solving for optimum dryer designs.

The equations used to analyze for time to dry a batch (or bin) of product from initial moisture

M_o to final moisture $\overline{M}_f$ and to calculate the energy cost per ton of grain dried (see Nomenclature on page 65), were:

Average Moisture Ratio

$$\overline{MR} = \frac{\overline{M}_f - M_e}{M_o - M_o} \quad (1)$$

Drying Constant

$$k = 1.62 \times 10^{-10} p_s^{2\,233} \quad (2)$$

Depth Unit

$$D = \frac{\rho_g\, kL\,(M_o - M_e)Y}{3600\, q\rho_a\, C_a\,(T_o - T_e)} \quad (3)$$

Time to Dry

$$t_d = \frac{1.0}{k}\,\ln\left(\frac{e^D - 1.0}{e^{D\overline{MR}} - 1.0}\right) \quad (4)$$

Equilibrium Moisture

$$M_e = \left[\frac{\ln(1\text{-}RH)}{-0.0000753\; T_o}\right]^{\frac{1}{2.4}} /100 \quad (5)$$

Parameter Values

Analysis for efficiency of drying requires knowledge of parameter values and average weather conditions for Ohio. Keener *et al.* (1981) defined energy conversion values used in efficiency analysis. Hansen *et al.* (1993) collected thin-layer drying data. Evaluation of M_e for Taxol was based on relative humidity levels of three percent. Average weather conditions for Wooster, Ohio, are 15.6°C dry bulb and 8.8°C dew point in May and 11.7°C dry bulb and 6.4°C dew point in October.

Computer Program for Optimization

The computer program used was written in FORTRAN IV and run on an HP-3000 computer system. The program contains:

- An input section for fan size, bin diameter, product parameters, energy conversion coefficients, initial and final product moisture, and ambient air conditions along with desired temperature.
- A calculation section to determine product depth, fan heat, and air drying at conditions entering the drying bed as a function of airflow rate.
- A drying model for analysis of time to dry.
- An output section.

In the computer program, iterations are performed on airflow until a minimum energy to dry the product is found. Output is optimum airflow rate and product depth to minimize energy as a function of fan power and drying air temperature.

For *Taxus* material, maximum depth for the analysis was limited to 10 ft. because of practical considerations pertaining to loading clippings into and out of bins. As a result, optimization was not attained. However, useful

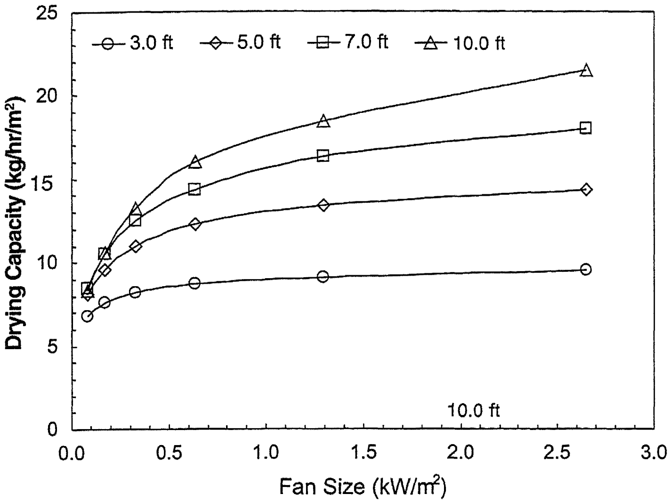

Figure 1. Drying capacity vs. fan size as a function of bed depth for drying *Taxus* clippings using a plenum temperature of 60°C and air conditions reflecting north-central Ohio in May (15.6°C dry bulb and 8.8°C dew point).

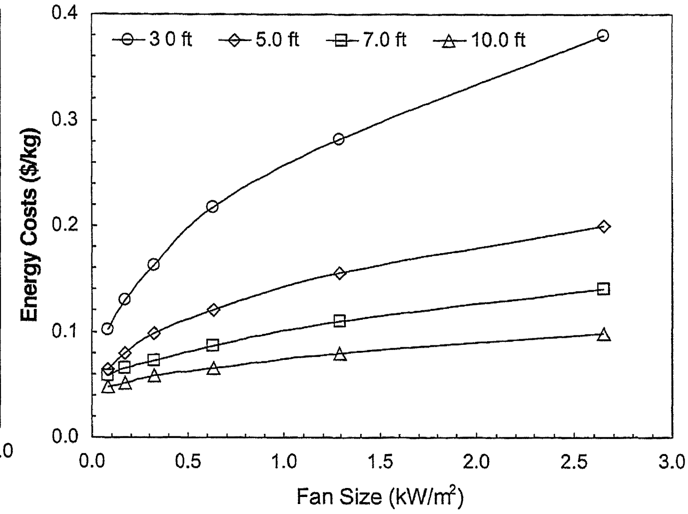

Figure 3. Energy cost vs. fan size as a function of bed depth for drying *Taxus* clippings using a plenum temperature of 60°C and air conditions reflecting north-central Ohio in May (15.6°C dry bulb and 8.8°C dew point).

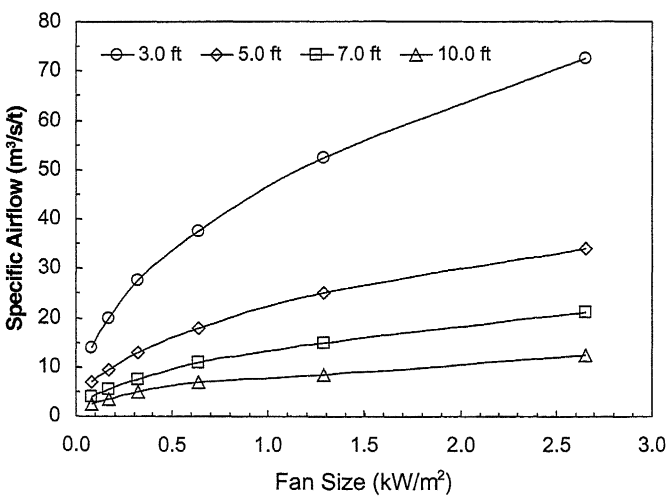

Figure 2. Specific airflow rate vs. fan size as a function of bed depth for drying *Taxus* clippings using a plenum temperature of 60°C and air conditions reflecting north-central Ohio in May (15.6°C dry bulb and 8.8°C dew point).

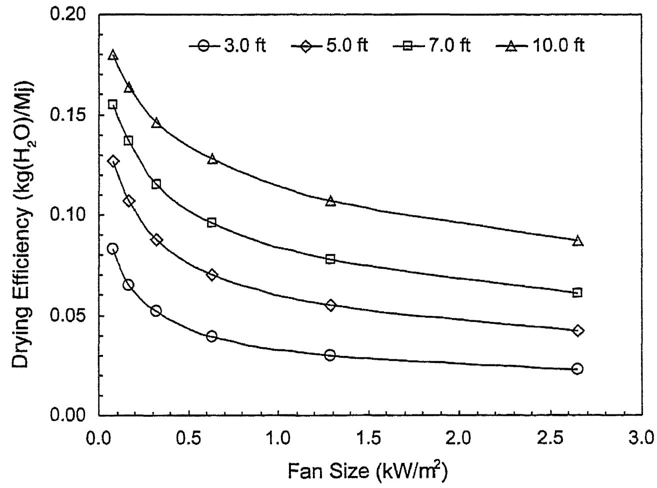

Figure 4. Drying efficiency vs. fan size as a function of bed depth for drying *Taxus* clippings using a plenum temperature of 60°C and air conditions reflecting north-central Ohio in May (15.6°C dry bulb and 8.8°C dew point).

information was provided by the program by simulating specific drying conditions.

In this report, drying (fossil fuel) efficiency is defined as the ratio of latent heat in the water evaporated to the fossil-fuel energy input. Because electrical power generation and delivery is approximately 33 percent efficient in terms of energy conversion, the electrical energy was converted to a fossil-fuel equivalent by multiplying by a factor of three. Similarly, LP gas was converted to a fossil-fuel equivalent by dividing by a factor of 0.90 for its energy conversion ratio.

Simulation Results

Based on a plenum temperature of 60°C and average weather conditions for north central Ohio in May, Figures 1, 2, 3, and 4 can be used to predict drying capacities, specific airflow rates, energy costs, and drying efficiencies for drying *Taxus* clippings as a function of fan size and bed depth. Use of these simulated results can be illustrated with an example.

Suppose rate of harvest and allowable storage time for fresh-cut *Taxus* clippings required a drying capacity of 267 kg/hr. Also, assume a standard 27-ft.-diameter grain bin with a 15-hp fan was available (floor area equals 16.2 m^2). By dividing 267 kg/hr. by 16.2 m^2, drying capacity requirements can be estimated at 16.5 kg/hr/m^2. A 15-hp fan is roughly equivalent to 1 kW/m^2. Figure 1 can now be used to select a bed depth of 8.5 ft.

Figure 2 can be used to estimate required airflow. Using 1 kW/m^2 and a depth of 8.5 ft., specific airflow rate is shown as 10 m^3/s/t. Similarly, based on these same criteria, Figure 3 shows cost of energy for drying *Taxus* clippings would be about $0.007/kg (dry basis), and Figure 4 shows drying efficiency would be 0.1 kg of water per MJ of energy.

The significance of the effect of bed depth on drying conditions is shown by decreasing depth from 8.5 ft. to 7 ft. Based on a specific airflow requirement of 10 m^3/s/t, Figure 2 shows the fan size is now cut in half to 0.5 kW/m^2. Similarly, Figure 1 shows drying capacity will be reduced to 13.5 kg/hr/m^2 (dry basis) while drying efficiency (Figure 4) drops to 0.085 kg of water per MJ of energy.

Summary

A logarithmic drying model developed for grain drying was adapted for use in predicting expected drying results for *Taxus* clippings. Results of the simulation were displayed graphically, showing drying capacity, airflow, energy costs, and drying efficiency as a function of fan size and bed depth. An example of a drying situation is described, showing how simulation results can be used to predict the effects of varying bed depth based on a plenum temperature of 60°C.

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Nomenclature

Log Model

C_a = specific heat of air, kJ $kg^{-1}°C^{-1}$)

D = depth unit of the bed, dimensionless

k = drying constant, h^{-1}

L = latent heat of moisture evaporation, kJ/kg

M_f = average moisture content of the bed, decimal dry basis

M_e = equilibrium moisture content, decimal dry basis

M_o = initial moisture content, decimal dry basis

MR = average moisture ratio of the bed, decimal dimensionless

p_s = vapor pressure at saturation, Pa

q = airflow, m^3/s (at STP)

ρ_a = air density, kg/m^3

ρ_g = material bulk density, kg/m^3

RH = relative humidity

T_g = temperature of material, °C

T_e = outlet air temperature at equilibrium with the grain in the downstream; for sufficiently deep bed, T_e is equivalent to the wet bulb temperature, °C

T_o = time-averaged drying temperature, °C

Y = bed depth, m

t_d = drying time, h

Progress Report and Summary: National Cancer Institute Workshop on *Taxus*, Taxol, and Taxotere

Robert C. Hansen, Robert G. Holmes, Ralph B. Shugert Jr., Edward M. Croom Jr., Hala N. ElSohly, Harold M. Keener, and Kenneth D. Cochran

Taxus x *media* 'Hicksii' has been identified as a potential renewable source of Taxol (Witherup *et al.*, 1990; Croom, 1991). 'Hicksii' is somewhat unique because new shoots generally grow vertically, thus facilitating mechanization of clipping and capture. Ornamental yews are pruned on an annual basis as a part of standard cultural practices in nurseries. Other than for purposes of propagation, *Taxus* clippings have not been routinely harvested, dried, or stored. *Taxus* x *media* 'Hicksii' clippings have been found to have concentrations of Taxol equal to or greater than 0.02 percent (Croom, 1991).

A mechanical harvester for clipping and collecting 'Hicksii' clippings was designed and developed. The height of the machine can be adjusted as plant height varies. A reel and canvas conveyor transports clippings to a 5 ft. x 2-1/2 ft. x 1-1/2 ft. pallet after cutting. When full, the pallets can be conveniently transferred to four-wheel trailers and towed from the field to any specified storage building. The harvester was successfully field tested during the spring 1992 pruning season.

A commercial 27-ft.-diameter bin dryer was used to dry *Taxus* x *media* 'Hicksii' clippings and whole plants in a shallow bed in preparation for storage (Hansen *et al.*, 1992). Variations in drying temperatures were recorded throughout the three- to five-day period during which drying occurred for 10 lots of biomass. Data were analyzed using statistical process control charts. Results suggested that commercial grain bin drying systems are not effective in meeting currently accepted tolerances for drying *Taxus* biomass and may require extensive modification.

To facilitate design of a more effective drying system, thin-layer drying studies of *Taxus* x

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media 'Hicksii' clippings, needles, and stems were conducted in a laboratory dryer (Hansen *et al.*, 1993). The thin-layer drying equation (Keener, 1991) was found to be applicable to drying rates for the *Taxus* materials tested at 30°, 40°, 50°, and 60°C drying temperatures. Parameters for the drying equations were successfully determined. The results showed that drying rates increased dramatically as drying temperatures increased. Also, stems were found to dry at a faster rate than needles, and needles dried at a faster rate than whole clippings.

More than 100,000 lbs. (wb) of *Taxus* clippings and/or needles were harvested, dried, and stored in preparation for Taxol extraction and purification. Each 200-lb. lot of the clippings and/or needles was randomly sampled and analyzed for Taxol content at each stage of the process preparatory to extraction, i.e., before drying, after drying, and after storage (two to four months), and after transporting to the extractors (Grand Haven, Michigan, to South Hackensack, New Jersey; Grand Haven, Michigan, to Long Mount, Colorado). Percent Taxol yield was maintained at an acceptable level throughout all stages of processing. However, Taxol yield was found to be most sensitive to drying protocol.

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