Intensive Cultivation of *Taxus* Species for the Production of Taxol®

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Taxol®, a novel anti-cancer drug registered in the U.S. in 1992 for the treatment of ovarian cancer, is derived from plant tissues of the genus *Taxus*. While the bark of Pacific yew (*T. brevifolia* Nutt.) is the only current approved source of Taxol, several alternative sources are under development. One promising alternative is the intensive cultivation of yew plants in tree nurseries, as is being developed by Weyerhaeuser Company. Since 1987 the company has been active in *Taxus* research and has embarked on a large scale production program to supply yew biomass for the production of Taxol. We believe this approach can best provide a dependable, long-term and economical supply of paclitaxel, the active compound on which Taxol is based. The program is supported by Bristol-Myers Squibb, the pharmaceutical company developing the drug in the U.S., and by the National Cancer Institute.

INTRODUCTION

Taxol® (paclitaxel) is considered by the U.S. National Cancer Institute (NCI) as one of the most important anti-cancer drugs to be developed in the past decade. The molecule paclitaxel is a diterpene, belonging to a group of closely related chemicals called taxanes, extracted from the bark and other plant tissues of yew trees (*Taxus* species). Taxol, the drug formulation of paclitaxel, has been approved by the U.S. Food and Drug Administration (FDA) for treatment of patients with ovarian cancer whose first-line or subsequent chemotherapy has failed. Taxol is being evaluated for the treatment of many other cancer types and appears promising for several, including breast, lung, head, and neck cancers.

The story of Taxol and its development began over 30 years ago. In 1958 the NCI initiated a long-term program, ultimately screening about 35,000 plant species for anti-cancer activity. As part of this program, samples of Pacific yew (*T. brevifolia*) bark, needles, and roots were collected in 1962 and sent to the NCI for evaluation. In 1964, extracts from these samples showed anti-cancer activity in *in vitro* cancer cell line screening trials.

The active ingredient of the yew bark extract (paclitaxel) was first identified and reported by Drs. M.C.Wani, Monroe Wall, and others of the Research Triangle Institute, North Carolina, in 1971 (Wani et al.). Due in part to the difficulty of obtaining the raw resource, and extracting and purifying the compound, further development of paclitaxel was delayed for several years. In 1979, the unique mechanism of action of paclitaxel was discovered by Dr. Susan Horwitz's laboratory at the Albert Einstein College of Medicine in New York (Schiff et al., 1979).

They found that paclitaxel inhibited the depolymerization of microtubules during mitosis, thus inhibiting the rapid growth of cancer by interrupting cell division. Virtually all other cell division inhibitors affect microtubule assembly. This uniqueness suggested that paclitaxel could be used in conjunction with other therapies with mechanisms of action that were different and in fact, the drug is currently being used in combination therapies in several clinical trials.

Human clinical trials were begun by the NCI in 1983. Initial results with ovarian cancers were very promising; those with melanomas less so. Early problems were experienced with formulations and delivery. Limited Phase II and III trials began in 1985 and 1990, respectively. Again, the limited availability of Taxol affected the speed at which clinical trials could proceed. Paclitaxel derived from Pacific yew bark was the only approved source for clinical trials, and the harvest from that resource lagged behind expectations and needs during the early years of clinical evaluations.

In 1991, following a competitive bidding process, Bristol-Myers Squibb (B-MS) was awarded a Cooperative Research and Development Agreement (CRADA) from the NCI to further develop and bring Taxol to market. In July of 1992, B-MS filed for a New Drug Application for the use of Taxol for ovarian cancer and in late December 1992, Taxol was approved by the FDA for treatment of patients with ovarian cancer whose first-line or subsequent chemotherapy had failed. In 1992, B-MS and their contractor, Hauser Chemical, harvested over 1.6 million pounds of bark and increased Taxol production from 5000 to 50,000 vials per month (Stull, 1992; DeFuria, 1992). This increase in available drug has permitted a dramatic increase in the number of clinical protocols, from a total of 30 between 1983 and 1990, to over 60 in 1992 alone (Arbuck, 1992). These trials included over 1700 patients. Today the numbers are even greater and virtually anyone in need of the drug can obtain it.

To date, the bark of the Pacific yew, a forest tree species native to the U.S. Pacific Northwest and British Columbia, remains the only approved source of Taxol for clinical trials or cancer therapy. Because of its limited occurrence and relatively slow growth rate, much concern was expressed over the environmental impact associated with the harvest of Pacific yew. The long-term supply of this resource seemed dubious. Consequently, one of the requirements of B-MS as stipulated in the CRADA was to identify and develop alternative sources of paclitaxel.

Several possible alternative sources have been identified and are receiving considerable research (and media) attention. They include foliage from wild populations of several species of *Taxus*, cell culture, total synthesis, semi-synthesis, and biomass from cultivated sources. Of these, semi-synthesis and biomass from cultivated sources appear most imminent as probable cost-effective alternative sources. Semi-synthesis requires taxane precursors derived from *Taxus* plants, whether from cultivated or wild sources. A combination of the above sources is probable in the future.

WEYERHAEUSER'S TAXOL PROGRAM—GOAL AND EARLY DEVELOPMENT

Of the above supply alternatives, Weyerhaeuser has focused its efforts on nursery cultivation, one of the Company's core businesses and an area of considerable expertise. The goal of Weyerhaeuser's Taxol Program is to provide, through intensive cultivation of *Taxus* biomass, a renewable, reliable and economic supply of paclitaxel and other desired taxane precursors for semi-synthetic production of Taxol.

Weyerhaeuser's Taxol Program began in 1987 when we were contacted by NCI's Natural Products Branch as a prospective contractor to supply Pacific yew bark. Weyerhaeuser is a major owner of timberland in the Pacific Northwest, managing nearly 3 million acres of private forest lands in the states of Washington and Oregon. However, only a relatively small portion of this land base contains native Pacific yew. We recognized and proposed the potential for intensive cultivation as a supply alternative. This was based not only on our knowledge of the relative scarcity of yew in the region, but also on the belief that cultivation would provide for a more reliable, long-term and environmentally friendly solution to the supply issue.

The initial steps toward establishing a cultivation program were directed at developing an information base, since virtually nothing was known about the genetics, physiology, or chemistry of the Pacific yew and related species. Our first research efforts included establishment of a large population/genetics study (Wheeler et al., 1992) and a survey of the taxane levels in literally hundreds of yew accessions. This was followed by establishment of a comprehensive research and production program, based on early research results of the genetics study and on extensive experience within the company in tree improvement, plant physiology and propagation research, as well as large-scale forest tree nursery operations. This program received support in 1991 with the signing of research and production agreements with Bristol-Myers Squibb and the awarding of a competitive research grant from the National Cancer Institute (NCI). NCI also supported earlier work through the provision of analytical support.

KEY ELEMENTS FOR A SUCCESSFUL CULTIVATION PROGRAM FOR TAXOL PRODUCTION

For a cultivation program to be successful—both technically and economically—several criteria must be met. Those key elements particularly necessary for a Taxol (paclitaxel) program are:

- An understanding of genetic and environmental factors contributing to taxane yields and plant growth. This understanding leads to the ability to select and use genetic material with the desired traits, and the ability to focus control on environmental variables affecting these traits. This includes identification of cultivation protocols that insure acceptable chemical profiles.
- Production systems capable of reliably and cost-effectively producing large amounts of high taxane-yielding biomass. This applies to all phases including propagation, growing, harvesting and processing.
- Crops dedicated to the specific purpose of Taxol/taxane production. This allows for the optimization of taxane content and biomass production, without the need to compromise for other product values, i.e. ornamental, form, etc.
- Strong research, technical, and analytical support. These are critical components for program start-up, optimization, and continuous improvement.

The cultivation approach is characterized by three discreet steps or processes : selection, propagation and grow-out.

With *Taxus*, three sources are available for genetic selection; (1) native or wild populations of several species, (2) unique genotypes found in arboreta and gardens, and (3) varieties or cultivars produced for the ornamental trade. The first contains the greatest amount of genetic variation and holds the most promise for continuous genetic improvement. The last has a more limited genetic base and thus a limited potential for improvement, but is available in numbers large enough to develop a large scale production program quickly.

Material from the commercial sources are routinely propagated through the use of rooted cuttings. Tissue culture would be highly attractive for bulking-up desirable, but rare or unique genotypes; the status of this technology is currently under review. Seed orchards could provide seedling stock, but the loss of genetic integrity, due to gene segregation, would likely reduce genetic gain opportunities.

The current emphasis in Weyerhaeuser's program is on intensive nursery production. This approach provides the most rapid production scale-up of acceptable biomass in the shortest possible time, as well as the opportunity to incorporate improved material into the production program as quickly as it becomes available. Hedging could be a viable option once the desired trait is well understood (i.e., which taxane(s) are desirable), acceptable levels of it are found in the shearable portions of the plant, and ample time is available to reach the biomass production stage.

CHALLENGES FACED IN CULTIVATION OF *TAXUS* FOR TAXOL PRODUCTION

The cultivation of yew specifically for Taxol production has presented numerous technical and production challenges. Although yew is grown on a grand scale for ornamental use in the Upper Midwest and Northeast, when we began growing yew virtually nothing was known about optimizing plant growth for taxane yields.

Technical challenges of note include: (1) defining the sources and magnitude of variation in taxane content as a function of genetic, epigenetic, and environmental factors, (2) defining heritabilities and genetic correlations for traits of interest, (3) understanding postharvest stability issues, (4) evaluating growth regulator effects on biomass and chemistry of plants, (5) developing reliable and accurate assay protocols, (6) optimizing propagation and cultivation protocols, etc. to name but a few.

Production challenges are perhaps of greater interest to this group. Our production hurdles were defined primarily by the urgency and scale of start-up. We needed to develop reliable processes to integrate rapidly accumulating technical data into propagation and cultivation regimes that could accommodate literally millions of units within a period of 2 to 3 years. We did this by using the combined wisdom of experienced ornamental growers, like Zelenka Nurseries, and our own greenhouse and nursery staffs, who produce over 250 million conifer seedlings a year. In addition, we were able to benefit from a tremendous pool of expertise in our forestry research organization.

Our most notable progress came in the area of propagation. We jumped from a few hundred cuttings a year, in 1990, to over 12 million in 1993 (Fig. 1). Our initial efforts focused on sand and perlite rooting beds, standards of the ornamental industry. However, problems with control of pathogens and efficient handling lead us to develop a containerized system with rolling benches. Cuttings are stuck in assembly-line fashion on a moving belt (in a head-house), placed on aluminum-frame tables, and taken by trailer to the greenhouse where they are rolled into bays. At harvest the reverse process is used. Water and fertilizer are provided by an overhead gantry boom. We have taken a pro-active approach to disease control, using a two-tiered fungicidal attack: a sanitation dip prior to sticking , followed by preventative drenches. We are still experimenting to determine which fungicidal products are optimal in our system. Except for the use of containers, our rooting conditions are rather traditional, with bottom heat, provided by hot water fins, and high humidity provided by overhead foggers. Although environmental conditions are critical to health and rooting success, the single most important factor in rooting appears to be the cutting wood itself. Rooted cutting harvest is performed with both manual and mechanical extractors, the cuttings boxed and transported to one of our 4 western nurseries, and machine-transplanted, preferably within 24 to 48 h of harvest.

A great deal of research is underway to determine optimal protocols for the cultivation of yew for taxane production. We are becoming increasingly aware of a number of environmental factors that influence taxane yields, including fertility levels, soil types, etc. Coupled with genetic and epigenetic (time of harvest, tissue effects) sources of variation in taxane yields, these environmental factors result in a rather complex management protocol that we are still working to optimize. With

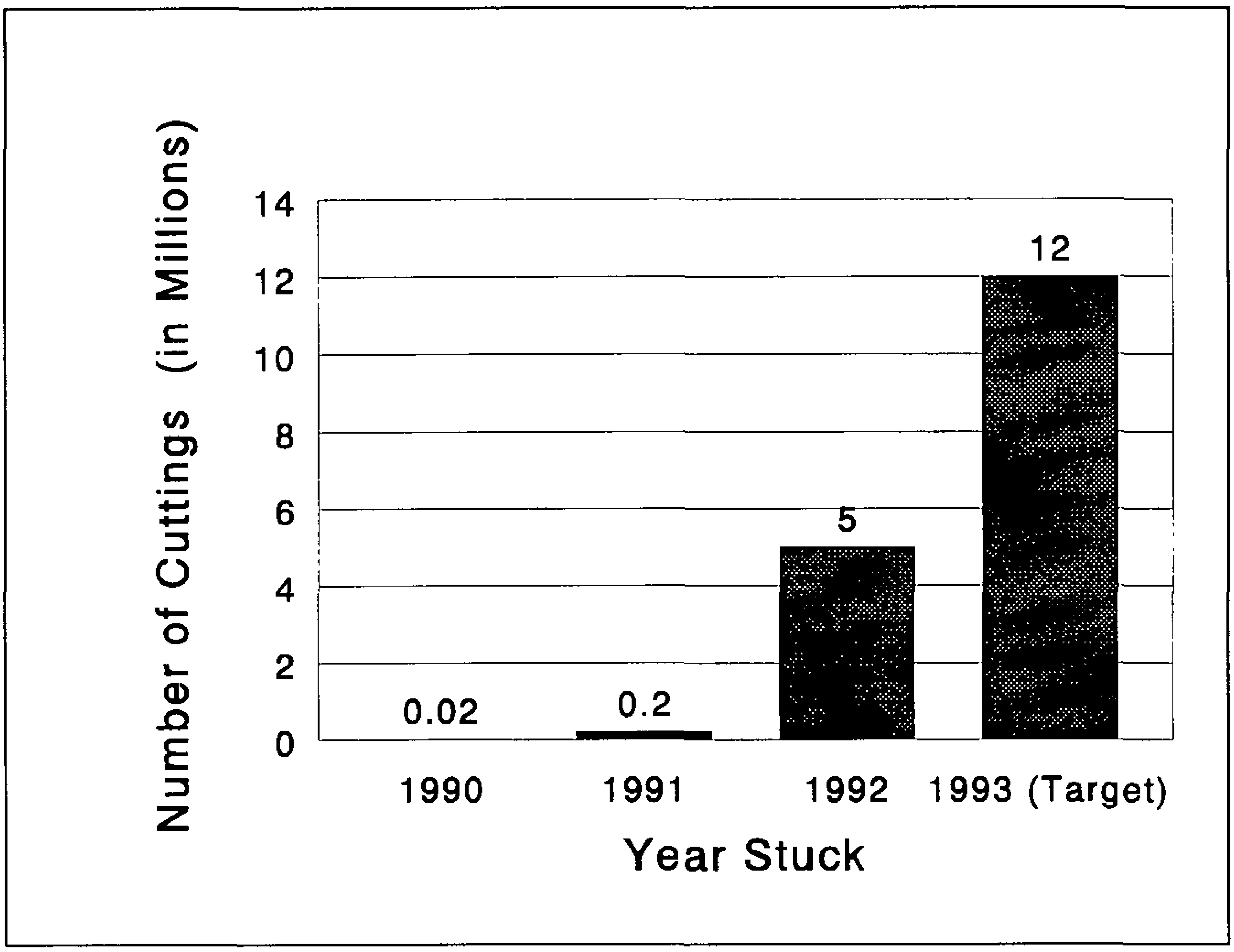

Figure 1. Number of yew cuttings stuck during the start-up of Weyerhaeuser's Taxol production program.

over 100,000 plants in some 70 research trials, and about 15,000,000 plants in production plantings, Weyerhaeuser is aggressively seeking to provide a renewable and cost-effective source of Taxol for years to come. Weyerhaeuser's yew cultivation approach will not only provide for a reliable and uniform source of material for Taxol, but will allow for an early exit from natural forests, thus insuring the maintenance of this small component of diversity in our forest ecosystems. The Taxol program is a welcome addition to an ever-expanding conifer nursery business that also insures we will never run out of trees.

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