Sustainable Development of Natural Sources of the Emerging Anti-cancer Agent, Taxol

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Introduction

Promising clinical trials of taxol, the anti-cancer agent isolated from the bark of Pacific yew (*Taxus brevifolia* Nutt.), have resulted in an increasing demand for the raw material and raised concerns about the unsustainable exploitation of natural stands in the Pacific Northwest. Massive research and development programs aimed at increasing taxol supply have been undertaken in the United States, Canada and France. These include studies of the full and partial chemical synthesis of taxol, and intensive culture of *Taxus* species and cultivars for taxol production. However, expectations are that it will take from 2 to 10 years before alternatives to taxol extracted from bark are certified for clinical use and production is scaled up to meet the demand.

The following investigations are being conducted to foster the sustainable utilization of Pacific yew for taxol: 1) tolerance of yew to environmental stresses associated with disturbance; 2) vegetative propagation of representative genotypes; 3) impacts of pathogens on propagated and wild trees; and 4) environmental effects on taxol production.

Methods

Tolerance of yew to environmental stresses associated with disturbance

Foliar physiology and morphology were studied in seedlings and excised shoots taken from trees growing in different light environments. Measurements of photosynthesis were made under different light intensities (0–850 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD, photosynthetic photon flux density), 20°C, and 1.5 kPa VPD (vapour pressure difference), using an open gas exchange system. Branch and leaf structure of trees growing in sun and shade were characterized with respect to foliage age classes, specific leaf areas ($cm^2 \cdot g$), and the distribution of stomata.

Vegetative propagation of representative genotypes

Pacific yew branches representative of different sites and tree ages and of male and female trees were collected and cuttings treated with 0.8% IBA were set in flats containing sand:peat:perlite under cool air and warm soil (20°C) conditions. Cuttings from English yew (*Taxus baccata* L.) were similarly treated. After 8 months, rooting percentages were determined.

Results and Discussion

Little is known about native North American yews. Of the over 300 articles surveyed (Mitchell 1992a), only 11 were on Pacific yew and 11 on Eastern yew (*Taxus canadensis* Marsh). The majority of references pertain to the English yew, its cultivars, and hybrids.

Tolerance of yew to environmental stresses associated with disturbance

Shade acclimation of Pacific yew was characterized by unusually large shifts in photosynthetic light responses (Mitchell 1992b). At low light intensity (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD), photosynthetic rates were 30% of maximum (100%; 4.4 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) in sun grown leaves and 70% of maximum (100%; 3.1 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) in shade grown leaves. Photosynthetic rates remained within 10% of maximum over a broad range of light intensities (250–450 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD) in both sun and shade grown leaves. At high light

intensity (850 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD), rates declined to 40% of maximum in shade leaves and 85% of maximum in sun leaves. Within a single tree, a mosaic of sun and shade leaves were found, each acclimated to the light environment in which they grew.

Foliar morphology and branch structure of Pacific yew were also affected by exposure (Mitchell 1992b). Shade grown leaves had higher specific leaf areas ($cm^2 \cdot g$) than sun grown leaves. On a single leaf basis, sun and shade grown leaves did not differ in the number of stomata. Branches from exposed trees tended to have fewer age classes of leaves (7) and more current shoots (69% of total) than branches from shade grown trees (11 age classes and 31% of total). Shade grown trees tended to invest in leaf maintenance, while exposed trees tended to invest in leaf production.

Vegetative propagation of representative genotypes

In general, cuttings from *T. baccata* rooted significantly faster and with a higher success rate (near 100%) than those from *T. brevifolia* (Mitchell 1992c). Cuttings from young *T. brevifolia* rooted better, 70.8%, than those from old trees, 48.2%, and variability was high among individuals, 14.5–87.5%. Collection site also appeared to affect rooting success, but variability among individuals from a given location masked significant differences. Of the seven locations sampled, the highest rooting percentage was 66, and the lowest was 25. Comparisons were also made between cuttings collected from sunny and shady sites, and rooting percentages were similar, 41.5% and 50.7%, respectively. There was no significant difference in rooting between male and female trees, 53 and 47.2% respectively.

Conclusions

- There are inter-specific differences between English and Pacific yew biology; not all information can be directly transferred.
- Male and female trees did not differ in foliar physiology or morphology, regardless of the light environment in which they grew.
- Sun and shade grown trees differ with respect to both carbon acquisition (photosynthetic light optima) and allocation (shoot maintenance or production).
- Propagation of high taxol-yielding individuals for intensive culture may be difficult if the parent trees are old.

Future Research Priorities

1. Drought tolerance of yew and its implications for harvesting and stand management.
2. Pests and diseases associated with yew and their implications for replanting and intensive cultivation.
3. Assessment of *in situ* and *ex situ* management practices for taxol yield.

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