

DEVELOPING IMPROVED NURSERY CULTURE FOR THE PRODUCTION OF ROOTED CUTTINGS OF CANADA YEW (*Taxus canadensis* Marsh.)

L. Webster^{1*}, R. F. Smith², S.I. Cameron² and M. Krasowski¹

ABSTRACT

Since the early 1990s, when the first anti-cancer drug containing paclitaxel was marketed, yew species (*Taxus* spp.) around the world have been threatened by unsustainable harvesting practices. Canada yew (*Taxus canadensis* Marsh.) a species native to eastern Canada and the north eastern United States is now facing similar pressures. In response to an increasing demand for biomass, in 1997, a domestication program was started. In 2003, a series of nursery trials was initiated to evaluate the potential of several plant growth regulators and cultural treatments for improving rooting of recalcitrant clones. This poster reports initial results from these trials.

INTRODUCTION

In 1958 the National Cancer Institute (NCI) initiated a search for chemotherapeutic cancer drugs. A plant screening program was developed to find natural compounds for the use in the fight against cancer. In 1972 the first species was found that contained the anti-cancer chemical paclitaxel (Taxol®): Pacific yew (*Taxus brevifolia* Nutt.), found in mountainous areas of the northwestern United States (Hansen 1999) and western Canada (Farrar 1995). Subsequently, paclitaxel has been found in the stem bark, roots and needles of several other yew species, including Canada yew (*Taxus canadensis* Marsh.) (Nandi, S. K. *et al* 1996, Senneville *et al* 2001). While all *Taxus* species contain paclitaxel, other specific ‘taxanes’ and related compounds and their concentrations vary greatly among species.

In the 1990’s the NCI designated Taxol as an “emergency priority”, since it was a very promising anti-cancer drug and already in short supply. The sustainability and survival of *Taxus* spp. around the world continues to be of great concern. The relatively high commercial value of taxane-yielding biomass has resulted in significant reductions in native populations of virtually all of the yew species throughout the world (Smith and Cameron 2002). Consequently, there is concern that similar overharvesting could threaten Canada yew populations (c.f. Senneville *et al* 2001). A sustainable harvest system is being developed; but insufficient research has been conducted to date to quantify the impact of different harvesting levels on the regrowth and abundance of the species (Smith and Cameron 2002).

As an alternative response to an increasing demand for biomass, in 1997 a domestication program was started, in which rooting cuttings are an integral component of field crop production. Canada yew shoots are moderately amenable to rooting, but cuttings are slow to root, and recalcitrant clones still cannot be rooted effectively. In 2003, a series of nursery trials was initiated to evaluate the potential of several plant growth regulators and cultural treatments for improving rooting of Canada yew cuttings.

¹ Faculty of Forestry and Environmental Management, University of New Brunswick, P.O. Box 4400, Fredericton, New Brunswick, Canada, E3B 5A3

² Natural Resources Canada, Canadian Forest Service, P.O. Box 4000, Fredericton, New Brunswick, Canada, E3B 5P7

Research objectives

The objectives of this research are: (i) to develop a method to increase rooting in recalcitrant clones of Canada yew, (ii) to evaluate methods to decrease time to root, cuttings and (iii) to test alternative rooting methods such as rooting directly into field crop beds. Improvement(s) in any of these areas would increase the efficacy, and decrease the cost, of producing rooted cuttings.

MATERIALS AND METHODS

Cuttings between 2 and 7 inches in length were collected in the fall of 2003. Shoots were cut just below the annual bud scar and comprised a maximum of two years of growth. All cuttings (with the exception of the no hormone treatment in the hormone trial) were dipped in a commercial rooting hormone mixture (Stim-Root No. 3, containing 0.8% indole-3-butyric acid). Excess rooting powder was tapped off. All cuttings were struck into 67 cell multi-pot trays with a standard mix of 2:1 peat moss: vermiculite. A 0.2" diameter hole with a depth of 0.75" was predrilled in each cell prior to striking the cutting. The soil was pinched around the cutting after sticking to hold it in place.

Three experiments were initiated: a terminal bud removal trial, a hormone trial and a cold storage trial. The number of cuttings struck per tray per clone varied from 30 to 67, based upon the total number of cuttings available within the specified size range from the parent plant. For each clone, the total number of cuttings was divided equally among treatments. The trays were arranged in the greenhouse by trial, clone, and treatment in a randomized block layout.

Terminal Bud trial

The terminal bud trial consisted of twenty-one clones. There were two trays per clone for a total of 42 trays. One tray per clone had the terminal bud removed and the other tray the terminal bud was left intact.

Hormone trial

The hormone trial consisted of eight clones. There were two trays per clone for a total of 16 trays. One tray per clone had IBA (indole-3-butyric acid) hormone applied, and the other tray cuttings did not have hormone applied.

Storage trial

The storage trial consisted of twenty-eight clones. There were two trays per clone for a total of 56 trays. Material for one tray was struck on the day of collection and the material for the second tray was stored in a cooler at 4°C. Ten clones were stored for one week, nine clones were stored for four weeks and nine clones were stored for six weeks.

Cuttings were grown in a greenhouse at the Canadian Forest Service in Fredericton, New Brunswick. Post-striking, trays containing the cuttings were placed in the greenhouse with misting to maintain the relative humidity at 70%. The day/night temperature was 22/18 °C. Ambient light levels ranged between 37 and 146 micromole/sec/m² for the rooting period. Once callus and (or) roots formed, the temperature, frequency of watering and light in the greenhouse were reduced to provide a cold period of eight weeks where the greenhouse temperature was at maintained at 5 °C. Greenhouse growing resumed in the spring with 22/18 °C day/night temperatures, relative humidity of 60% and a 16-18 hour photo period extended through

supplementary lighting (high pressure sodium). Cuttings were maintained at these conditions throughout the summer and harvested during the fall of 2004.

Measurements

Cuttings were harvested individually, placed into plastic bags, and put into frozen storage until processed. Cuttings that did not have roots were discarded. At the time of processing, roots were washed and the following measurements taken:

Terminal bud trial: The length of the initial cutting, length of new growth, root collar diameter, and terminal shoot diameter were measured and then the samples dried at 65°C for 48-72 hours. Dry weights were measured separately for roots, the original cutting needles and stem, terminal shoot (current-year) needles and stem, and lateral shoot(s) (current-year) needles and stem(s).

Hormone trial and storage trial: The length of the cuttings with new growth was measured. Dry weights were measured separately for roots, cutting, and current year growth.

RESULTS AND DISCUSSION

Terminal Bud trial

The production and basipetal translocation of auxins from terminal buds is important in stimulating adventitious rooting (Cooper 1936). Removing the terminal bud reduced sugar:starch concentration ratios in the basal stem of *Pinus banksiana* cuttings, which may have caused a reduction in rooting success (Haissig 1989). The effect of terminal bud removal has not been studied on *Taxus* spp. so it was unknown how cuttings would be affected.

Operationally, there would be an advantage if removal of the terminal bud did not decrease rooting success, since an increased number of cuttings can be taken from each parent plant. However the removal of Canada yew terminal buds caused a decrease in mean survival for all clones from 39% to 22%. Survival rate was typically higher for all clones with terminal bud attached. With the exception of two clones (designated B and R) which had a minimal increase in survival with terminal bud removed (Fig 1). Therefore, there may be auxins or other phytohormones within the terminal bud that are translocated at time of rooting to the basal stem (Haissig 1989). For cuttings that rooted, the amount of new growth produced by cutting was positively correlated with the initial cutting length ($P < 0.0001$). There was no significant difference in growth between cuttings with terminal bud removed and without terminal bud removed ($p=0.59$).

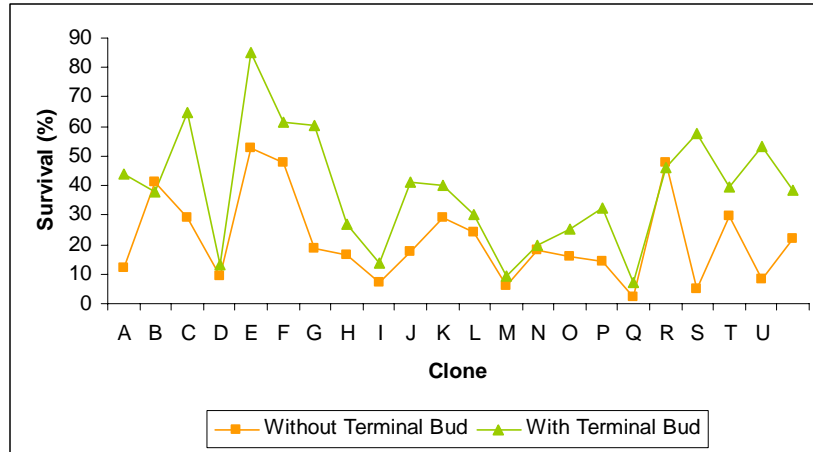


Figure 1. Survival between clones with and without a terminal bud.

Hormone trial

Mean survival for all clones with and without hormone treatments was 49% and 36% respectively. This is similar to previous results from IBA hormone application to Pacific Yew cuttings where rooting success increased from 30.6% to 50.0% (Mitchell 1997). Hormone application had no significant effect on survival within clones (Fig. 2). Cuttings receiving hormone produced significantly more top ($p < 0.005$) and root ($P < 0.009$) growth than did the untreated controls. Many clones rooted but produced no current shoot growth. Hormone treatment did not increase rooting success in recalcitrant clones.

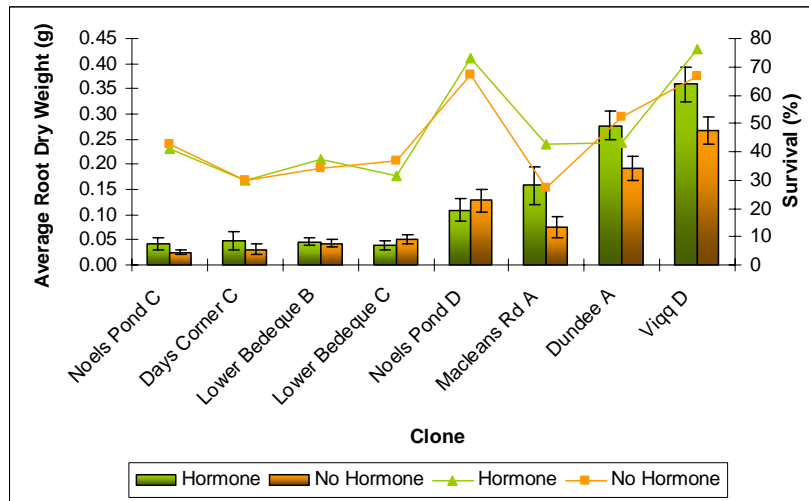


Figure 2. Survival (lines with points) and Average root weight (bars) between clones with and without hormone treatment.

Storage trial

Several studies have shown that cold storage of dormant conifer cuttings prior to striking is an effective treatment for improving rooting success (Behrens 1988). This was also true for Canada yew cuttings. The average survival of cuttings that had cold storage treatment was about 10% higher at any of the three storage times compared to cuttings that were immediately struck at the time of collection (Fig. 3). This implies that rooting success is not enhanced by increasing the

cold storage times. Operationally, however, cold storage does allow for material to be collected and stored for striking at a later time, while actually increasing rooting potential without degrading the plant material.

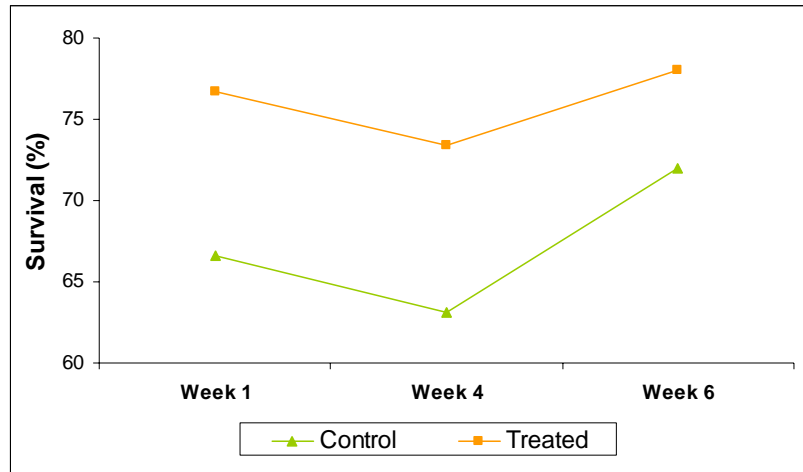


Figure 3. Average survival of cuttings struck at time of Collection versus those stored for one, four, or six weeks (n = 10, 9, 9 clones respectively).

CONCLUSION

Clonal differences were significant for all treatments and experiments. For the clones that had poor survival, the cuttings that did root typically did not exhibit bud flush and subsequent top growth. The standard practice of using a hormone dip to root yew cuttings should also be used for Canada yew, but it does not appear to enhance rooting in recalcitrant clones. Preliminary findings indicate that storing cuttings for as little as one week prior to striking can achieve modest gains in rooting efficiency.

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