

Recommended Procedures for Rooting Ground Hemlock (*Taxus canadensis*) Cuttings

by

L.D. Yeates, R.F. Smith, S.I. Cameron, and J. Letourneau



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Abstract

Methods for greenhouse propagation of rooted cuttings of Canada yew (*Taxus canadensis*) are described. Specific details on the collection and handling of cuttings, greenhouse culture, and post-rooting care of plants are provided. The equipment, facilities, and supplies required at all stages of production are listed. A brief description of ongoing research focusing on optimizing existing protocols for rooting dormant cuttings is also given.

Résumé

Des méthodes de multiplication par boutures de l'if du Canada (*Taxus canadensis*) en serre sont décrites, en donnant les détails de la collecte et de la manipulation des boutures, de la culture en serre et des soins apportés aux plants après leur enracinement. Les installations et le matériel requis à toutes les étapes de production sont énumérés. En outre, la recherche en cours visant à optimiser les protocoles pour l'enracinement des boutures dormantes est brièvement



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Introduction

Paclitaxel (Taxol®), initially found in the bark of the Pacific yew (*Taxus brevifolia*) is one member of a class of chemicals called terpenoids that are produced in species of the yew (*Taxus*) family. Over the past 10+ years, paclitaxel and some of the other “taxanes” have shown promise for use against more than 20 cancer and non-cancer diseases, and according to the U.S. National Institute of Health, paclitaxel is the most important anti-cancer drug to have been marketed in the last 15 years. In 1997, the Canadian Forest Service – Atlantic Forestry Centre (CFS-AFC) initiated a research program to “domesticate” ground hemlock (*T. canadensis*) for the production of taxanes. The domestication project is being used to convert ground hemlock into a nursery crop by a) selecting individuals from throughout the species’ range, b) testing and screening to select varieties with a high taxane content, and c) implementing a large-scale production program for these “elite” plants. Research is ongoing in five interdependent yet complementary areas: cultivar selection, nursery propagation with rooted cuttings, intensive nursery culture, tissue culture, and methods to increase taxane yields through culture. What follows is an overview of the progress made to date on nursery propagation using rooted cuttings.

Striking of Cuttings

When to take the cuttings

Cuttings can be rooted at almost any time of the year. However, costs and rooting success may vary significantly depending on the time of the year. Striking cuttings using elongating shoots (e.g., collected in May–July) can be problematic both because the actively growing new growth is susceptible to breakage, and because maintaining growing conditions within the desired temperature and humidity ranges in a greenhouse during the summer months can be difficult. The following recommendations are for dormant cuttings struck in the fall or early winter.

Size of cutting

Cuttings of almost any size can be rooted. The size you choose will depend on the size and type of container you use. Under our growing regime, there must be adequate space to allow for 1 year’s growth in the container. For *multipot 67* (Ropak Canada Inc., Springhill, N.S.) containers (65 cm³ volume), we recommend a cutting that is 7.5–10 cm in length (3–4 inches).

Where to make your cut

The best rooting occurs at growth nodes (Fig. 1). Making the cut at a node is more important than making a cutting of a predetermined length. The two most important considerations are to ensure that you make the cut at the correct spot, and that the cutting is of an appropriate size for the container.



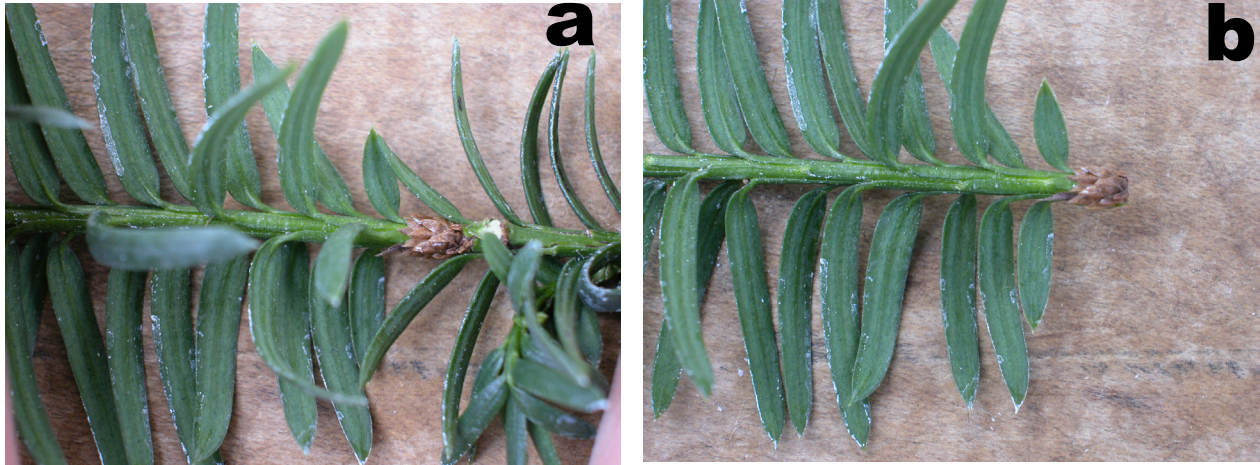


Figure 1. a) A 2-year-old shoot with a node, and b) a close-up of the cut end. (Photographs: J. Letourneau.)

When to strike

It will normally take a minimum of 12–16 weeks for cuttings to callus and start to produce roots. The greenhouse temperature should be maintained at 22–25°C day and 18°C night during this initial rooting period. The later in the fall you strike the cuttings, the higher your heating costs will be. Therefore, we recommend you strike your cuttings before October 1.

Media

A variety of media are available. The following recommendation is what we routinely use at CFS-AFC.

We use a 2:1 peat:vermiculite mix composed of a medium-to-coarse horticulture vermiculite and a coarse peat. At this time, we do not incorporate any fertilizer into our potting mix. NOTE: A 3:1 mixture may lack adequate aeration (Doug Beaton, Nova Scotia Department of Natural Resources, *personal communication*).

If you make your own mix, first break the peat moss up in a mixer, shredding it for the least amount of time possible, as excessive shredding may destroy the fibers, and result in reduced air and water drainage in the final media mix. Add the vermiculite to the shredded peat. Water should be added to the mixer when mixing the peat and vermiculite.

When filling the containers, it is important to ensure that the cells have a uniform density. We use a vibrating belt system, which has the added advantage of eliminating air spaces within





Figure 2. The end of the cutting is dipped into a rooting compound and the excess shaken or tapped off. (Photograph: J. Letourneau.)

the cells. After filling, place the trays in the greenhouse and water thoroughly. Media should be moist but not saturated when the cuttings are struck. Saturated conditions inhibit rooting and root development, and can increase the chances of basal rot.

How to strike

We routinely dip the cuttings in a rooting hormone such as Seradex #3 (3-Indolybutyric acid 0.8% manufactured by May & Baker (Canada) Ltd., Montreal, QC). Take a small amount of the rooting hormone and place it on tinfoil or in a small dish. Dip the end of the cutting into the hormone (Fig. 2), give the cuttings a light tap with your finger to shake off excess powder,¹ and then insert the cutting into the cavity.

There are a number of commercially available rooting compounds suitable for use on yew. In general, callus formation and early root formation are enhanced by using a rooting compound, but we have not found a significant improvement in rooting success for either easy- or difficult-to-root clones (unpublished data).

¹ NOTE: Do not return unused rooting compound to its original container because you may contaminate the rooting compound or introduce moisture into the powder.

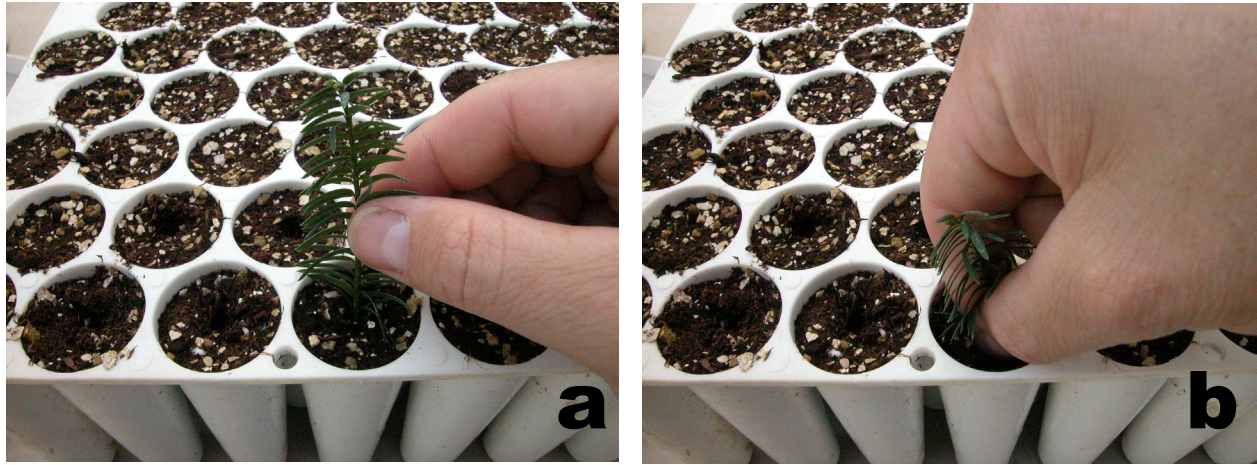


Figure 3. The cutting is a) placed into pre-drilled holes in the cavity, and b) the medium is pinched around the base to ensure good contact between the cutting and the medium. (Photographs: J. Letourneau.)

We use two methods for striking the cuttings: with and without pre-drilling a hole before striking the cutting.

Without pre-drilled hole. Take cutting and insert it directly into the medium to a depth of 2.4–4 cm (1–1.5 inches).

With pre-drilled hole. The depth of the hole should be slightly less than the amount of the cutting to be inserted. The diameter of the hole should be approximately the same as that of the cutting. A simple method is to use a sharpened pencil to “drill” the holes.

It is not necessary to remove the needles from the cutting. Insert cutting to a depth slightly greater than the depth of the hole, thereby ensuring good contact between the end of the cutting and the rooting medium. Pinch the medium around the cutting (Fig. 3).

Regardless of the method chosen (with or without pre-drilled holes), ensure that the end of the cutting makes good contact with the rooting medium.

The medium will usually continue to settle after the cuttings have been struck. Therefore, the cuttings must be inserted AT LEAST the minimum recommended depth (3.0–4.0 cm). If this is not done, after the medium has settled, the end of the cutting may not be embedded sufficiently to support the cutting. When this happens, rooting success will decrease. Although we have not examined this in detail, planting the cuttings deep (5 cm or more) does not appear to have a negative effect on rooting success.





Figure 4. Grit is applied to help retain moisture, limit algae growth, and reduce the establishment of weed seeds. (Photograph: J. Letourneau.)

After cuttings are struck, cover the cells with a layer of light grit (Fig. 4). This helps retain moisture, limits algae growth, and reduces the establishment of weed seeds. We routinely use calcitic grit because it releases some calcium. This is important for us: given our particular water source and medium, plants in our greenhouse require the supplemental calcium. Our objective is to ensure that the medium is at a pH between 5.5–6.



General Greenhouse Culture: Post-Striking Care of the Cuttings

Misting

Maintain the relative humidity (RH) in the greenhouse at or above 70%. We use a Micro-cool™ (Nortec Industries, Inc., Palm Springs, CA) fogging system to accomplish this. Although misting also helps maintain RH in the greenhouse, its main purpose is to reduce plant desiccation by maintaining a fine layer of moisture on the needle surfaces. We try not to maintain them so wet that water routinely runs off the needles. This is best accomplished by using a fine nozzle, such as a Tee Jet 800067 (John Brooks Co. Ltd., Mississauga, ON). In our system, these nozzles are put on a watering cart that passes (double pass) over the crop every 2 h. This misting allows fine water droplets to form on the foliage, thus preventing desiccation, but not wetting the medium. The frequency at which misting is required will be determined by your greenhouse conditions, and will be greatly influenced by outdoor weather conditions. During cool, cloudy weather, less frequent misting is required, whereas on hot and sunny days, five or six mistings a day may be needed to maintain the RH at desired levels. In our greenhouse system, we mist every 2 h through the day and 4 h through the night.

Before root formation, the main source of water loss from the medium occurs through evaporation. Good callus formation and subsequent root formation and growth will occur when the medium is at, or slightly below, field capacity. Therefore, be very careful not to overwater during the rooting phase. When irrigating (watering), always apply sufficient water to drip, i.e., water drips out of the bottom of the trays.

Heat and light

In winter, cuttings root sooner in direct sun, but in summer, shade is required to reduce the light level (approximately 50% of light transmittance) (Phillion *et al.* 1982). There must be sufficient light to sustain some photosynthesis. Optimum range of light is between 300-ft candles to 1200-ft candles for rooting, whereas 1000-watt HID lamps are used for growth. When light levels are reduced by shading, temperatures tend to be lowered, which prevents damage and excessive evapotranspiration. Rooting of the cuttings does not appear to depend on light or the length of the day.

NOTE: We use below-bench heating. It has been well documented in the literature for other species that keeping the medium warm promotes root growth, and yew appears to be no different.



Sanitation

Maintaining high RH and relatively warm temperatures in the greenhouse provides an ideal environment for diseases. Inspect the cuttings regularly for basal rot and other pest and disease problems. Remove infected and dead cuttings so they do not provide a source of infection for other cuttings. Unless a very aggressive manual culling system is used, some form of fungicide schedule will likely be needed. Two of the successful regimes we have used are described below.

1) Weekly schedule of fungicide applications.

Fungicide was applied weekly, starting right after the cuttings were struck. This was continued until the beginning of the cold treatment, i.e., around the end of December (see section on hardening off). The five fungicides used in the rotation were:

Benlate™ 50WP (DuPont Canada, ON)
 Maestro™ 80DF (replaces Captan™) (Tomen Agro, Inc., San Francisco, CA, USA)
 Rovral™ (Rhône-Poulenc Canada Inc., ON)
 Thiram™ 80WP (Plant Products Co. Ltd., Brampton, ON)
 Daconil™ 2787 (Fermenta ASC, OH, USA)

The fungicides must be rotated to ensure that disease-resistant strains do not develop.

2) An alternate fungicide schedule, based on recommendations from Doug Beaton and Brian White (Nova Scotia Department of Natural Resources) has also been used successfully at CFS-AFC.

The biological fungicide Mycostop® (Biobest Canada Ltd., Leamington, ON) was applied every 3 weeks according to the label recommendations, with intervening applications of Maestro 80DF, e.g., Mycostop at 3 weeks, Maestro 80DF 1 week, Mycostop at 3 weeks. (Note: in accordance with the label directions, do not apply Mycostop within 10 days after a Maestro 80DF application.) The main advantage of using this second regime is that Mycostop reportedly lasts 6–9 weeks, whereas chemical fungicide treatments typically must be applied every 7–10 days. Furthermore, although not observed thus far with *Taxus canadensis* in our studies, high fungicide application rates, or repeated application of the fungicide Maestro 80DF, can result in abnormal root development, and may be a root growth inhibitor (White 1983).



When applying Mycostop, take off all filters and apply with a watering boom, but leave screens on the watering boom.

Overwintering the First Year

The temperature, watering, and light in the greenhouse can be reduced once callus and (or) roots are produced. Not all cuttings will have rooted, as there is considerable variation among genotypes in both the speed and percentage of rooting. Therefore, it will be necessary to examine the cuttings and then “decide” when a sufficient number of the cuttings have reached this stage before beginning to shut the greenhouse down for the first winter.

Gradually (over a 2- to 3-week period) reduce the greenhouse temperature. We typically provide a 7- to 10-week cold period during which the greenhouse is maintained at 5°C. During this period, no supplemental light is provided and the containers are only watered as required.

Spring and Summer of Year Two

Following the cold treatment, start up the greenhouse with the following conditions: 18°C night and 22°C day temperatures, while maintaining RH at 60% and a 16–18 h photoperiod. Begin applying a starter fertilizer (e.g., 11-41-8, Plant Products (Brampton, ON) Forestry Fertilizers with micro nutrients) at a rate of 50 ppm N.

Once most of the cuttings have produced some roots, you may increase the rate of starter to 100 ppm N. However, this decision should be based on the stage of development of YOUR plants. If good callus and root formation occurred in the fall and early spring, shift to a standard grower fertilizer (e.g., 20-8-20) at 100 ppm N rather than increasing the rate of starter fertilizer.

NOTE: At some time in the middle of the growing season, the tips of the cuttings will likely begin to turn yellow. If and when this occurs, increase the grower fertilizer rate to 150 ppm N and maintain this rate until it is time to begin to harden off the cuttings.

Beginning approximately in late July or early August, leach the cuttings for 2 weeks. After leaching, apply a finisher fertilizer (e.g., 8-20-30) at 35 ppm N for 3 more weeks.

Frozen Overwinter Storage of 1-Year-Old Cuttings

One of the techniques used operationally in several conifer nurseries is overwinter frozen storage. Under this regime, conifer seedlings are first “hardened off” using well-established nursery practices. Once the seedlings are shown to be hardy to -20°C, they are then put into plastic bags, boxed, and placed in frozen storage. The advantage of this method is that seedlings can be held dormant in storage considerably longer into the late spring/early summer than plants being held outdoors. Given that yew species are particularly prone to late spring frost damage



because of their tendency toward early spring flushes, refining this system for yew species would be of considerable benefit to nurseries by reducing early post-planting frost damage, and extending the planting season. We have now successfully used this system for 3 years with *T. canadensis*. Although *T. canadensis* cuttings respond well to extended greenhouse culture, they are slow to become dormant, e.g., “shut down.” Therefore, careful monitoring, sampling, and testing to confirm that they attain the desired frost hardiness IS A MUST. It will likely take longer for these plants to harden off than for many other conifers.

NOTE: *Taxus* foliage turns a purple to brown color in the fall, similar to young jack pine seedlings.

Summary of Ongoing Research

Most of the research on nursery propagation has focused on reducing the time required to produce a plantable rooted cutting. Initial findings indicate that it is possible to strike *T. canadensis* cuttings early in the fall, and thereby produce plantable cuttings for the following summer. These can then be planted directly into fields or hedges. In addition to reducing the time between when cuttings are struck and when they can be outplanted by 1 year, this system should also result in improved early post-planting performance.

Additional Nursery Trials

Weed control is a significant problem in the nursery, and to date has been accomplished in these studies using manual weeding only, which is both time consuming and expensive. We have now conducted two small-scale herbicide trials in order to provide a cost-effective alternative to manual weeding. *Taxus canadensis* is particularly sensitive to overspray with herbicides such as Vision® (Monsanto Canada, Inc., Mississauga, ON) (glyphosate). After having examined the list of herbicides currently being used on *Taxus* species in commercial nurseries in the United States, we opted to conduct a series of trials using three different herbicides from the current list of products that are registered both for use in Canada and for use on *Taxus* species. Results from these trials will be reported as they become available.

Acknowledgments

The results of numerous rooting trials conducted over the past 8 years have led to the recommendations in this manual. We would like to express our sincere thanks to the following people for technical support over these past years: Shawn Farrell, Dan Flemming, Terry Hay, Fiona McBain-Hogg, Jay Phillips, and Paula Stewart-Leblanc. This publication would not have been possible without their hard work. A special thanks to Joe Lewis, retired CFS-AFC greenhouse manager. More recently, from 2001-2003, we received financial support from Napro

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White, B. 1983. Production of highland black spruce from cuttings. Nova Scotia Department of Lands and Forests, Forest Technical Note #7

Fungicides

Benlate 50WP (Benomyl 50% CAS #17804-35-2) PCP #11062 (DuPont Canada, Mississauga, ON). Rate 2–3 kg/1000 L/ha.

Maestro 80DF (Captan 80% CAS #133-06-2) PCP #26408 (Tomen Agro Inc., San Francisco, CA, USA). Rate 1.25 kg/1000 L water and apply at a rate of 50–85 L/100 m².

Rovral 50W (Iprodione 500 g/kg CAS #36734-19-7) PCP #15213. Rhône-Poulenc Canada Inc., Mississauga, ON). Rate 1.5–2 kg/ha in 1100 L water/ha.

Thiram 80WP (Thiram 80% CAS #137-26-8) PCP #9871 (Plant Products Co. Ltd., Brampton, ON). Rate 250 g/100 m².

Daconil 2787 (Chlorothalonil 40.4% CAS #1897-45-6) PCP #15724 (Fermenta ASC, Mentor, OH, U.S.A.)



