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Propagation of Pacific Yews from Seed

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ABSTRACT

This paper describes five years of personal experience collecting, cleaning, testing, storing, stratifying, and germinating Pacific yew seed from native populations. Cultivation of seedlings for the three years of growth is also discussed. Recommendations are made for each activity. Annotated references about propagation of yews from seed are appended.

KEYWORDS: Seed, Pacific yew, *Taxus brevifolia*, stratification, germination, cultivation.

INTRODUCTION

Seeds of Taxus (yews) are more difficult to process than seeds from most other conifers. Collection and cleaning are laborious, long-term freezer storage is problematic, and stratification requirements are variable and lengthy. In this paper, I summarize observations and experience gained from processing 50,000 seeds from native Pacific yews (*Taxus brevifolia* Nutt.). I also discuss the culture of 1,000 seedlings. The conclusions and interpretations are based on observations, not empirical studies. These observations may be useful for selecting further useful scientific investigations of the yew tree's sexual reproduction and seed biology. They may also contribute to the development of practical *Taxus* regeneration methods which preserve and enhance the genetic variability of native populations.

COLLECTING

During the seasons of mid-August through early October of 1990, 1991, and 1992, I collected more than 17 seed lots from individual trees (open-pollinated), and more than 16 seed lots that included seed from many parent trees within a given population. Each collection contained at least 100 seeds, often considerably more. The seed lots came from eleven locations on private, state, and federal lands in the Willamette Valley and Cascade Mountains of Oregon. Collections sites ranged in elevation from 150 to 1500 meters. Each site was separated from others by at least four kilometers, so I believe they represented distinct populations.

Seed abundance varied greatly from tree to tree, and among locations. I selected populations with sufficient numbers of male and female trees for effective cross-pollination. The heaviest seed producers invariably were vigorous trees with abundant available water even in late summer, and moderate to full sunlight. However, even in populations where environmental conditions were relatively uniform, there still seemed to be great variability in fecundity among individual females as indicated by large crops of ripe seeds each year on certain trees. Trees growing in dense shade bore very few seeds, often ten or less. Sun scalded trees or sprouted stumps in clearcuts produced an intermediate number of seeds, averaging several hundred. The greatest seed producing tree I found was in the sunny yard of a residence whose owners left a hose running at the base of the tree all summer long. I estimate this tree, which was 10 meters tall, full canopied, and approximately 25 cm in diameter at breast height, produced more than 10,000 seeds each year.

I determined that for commercial seed collection, it was much more cost effective to scout a population extensively for good seed producers than to try to collect every seed from nearby trees or to climb trees to reach seeds in the upper canopies. Scouting for good seed producers also contributed to genetic diversity in offspring derived from a seed lot, because the parents were more widely dispersed trees. Although more seeds were collected per tree, the seeds were less likely to be related through common male pollen parents, and female parent trees were less likely to be vegetative clones derived from an earlier tree that was disturbed (burned, knocked over) and subsequently sprouted several new trees. On steep slopes or in areas that were hard to traverse, seed collection was more efficiently concentrated on readily accessible trees. Most native yews have long, resilient branches which can be bent down at the end for seed collection. A six to eight foot pole with a hook on the end allows the picker to reach many branch tips from the ground. A heavy pole will hold some branches bent sufficiently to free both hands for picking. I held a plastic ziplock freezer bag in my teeth to free both hands and to shorten the distance I needed to transfer each picked seed. This also allowed me to watch if the seed actually went into the collection bag. The ripe aril of a Pacific yew seed is very viscous if the outer membrane is broken. The skins of ripe arils break easily, and I needed to rinse or wipe the pulp off my fingers frequently to prevent newly picked seed from clinging to my fingers, thereby interfering with its transfer to the collection bag.

I counted the seeds as I picked them. To compensate for inaccuracies, and subsequent losses, I put an extra seed in the bag for every ten seeds counted, and several extra for every 100 counted. This method gave me a fairly accurate estimate of picked seeds, because I occasionally lost track of my count, and arils sticking to my fingers would fall off outside the bag.

Every 1,000 seeds I would transfer to a cooler. 1,000 seeds took from one to four hours to collect, and the seeds and ripe arils fit conveniently into a pint Zip Lock freezer bag. I cleaned most of my seed within a week and kept it cool or refrigerated in the interim.

Pollen disbursal and fertilization may occur over a period of several months, because aril ripeness and presumably seed maturation occur over a three month period from August to October. Peaks of seed maturity at low elevations (150 meters in the Willamette Valley) seemed to be only one week earlier than at high elevations (1500 meters in the Cascade Mountains) during the years I collected. I judged seed maturation by how swollen, red, and soft the aril was. The greatest number ripened during the second half of September in most locations. Size and shape of the aril, as well as the degree to which it covered the seed, were quite variable and not obviously related to aril ripeness or seed maturity. The staggered nature of seed maturation meant that unless many seeds were bypassed because they were not quite ripe, seed collection on any given day typically included a variety of stages of aril ripeness.

In wild populations, birds and mammals are continuously harvesting ripened seeds, but I found no evidence of intensive activity. Every population from which I collected, had at least a few trees with an abundance of seeds. The seeds on any given tree seemed to have a uniform distribution of stages of maturity, which indicates to me that animals were not exerting heavy harvesting pressure on mature seeds. Many trees had mature seeds falling to the ground in October when frost caused the aril to rot. I found some seeds selectively removed, presumably by animals, from arils which were left on the tree. Rodents, at least, appear to prefer yew seeds to the arils. I made the following observation while helping a friend remodel an old farm house: when we tore out an interior wall, we found a mouse nest full of clean, neatly stored yew seeds from the old Taxus baccata plant on the north side of the house. I have not estimated the percentage of wild seeds that are harvested by forest denizens, but I do not feel that I was in significant competition with them. Perhaps they harvested from stouter branches in the higher canopy.

CLEANING

The viscous properties of the aril make it difficult to remove from the seed. Originally I thought some fermentation of the arils would ease separation from the seed, but it did not. (Fermented yew arils have a wonderfully pleasant, mild, fruity odor, and I would love to sample wine made from the pulp!) Likewise, 3 percent hydrogen peroxide did not reduce the viscosity of the pulp, or ease its separation from the seed. In 1990 and 1991, I mashed the arils in a metal kitchen colander under running water. Then I rinsed the pulp/seed mixture into an open three liter container and floated loose pulp off the top. This procedure needed to be repeated several times to completely clean the seeds. Metal screens abraded the outer (thin) seed coat, but did not damage the harder inner seed coat. I subsequently discovered that plastic screens worked as well or better for cleaning, and did not abrade the outer seed coat. Significant pressure must be applied to scrape the pulp off the seeds, so the screen needs to be reinforced, strong, or well supported. The screen matrix should be only slightly smaller than the seeds, so that the pulp flushes through freely. A seed lot of 1,000 seeds required approximately one half hour to clean. There are commercial seed cleaners (macerators) available which utilize rubber blades to remove fruit pulp from seeds. I do not have any experience with them, but most are designed for larger seed lots than I processed. I imagine few fruits are as viscous as that of the yew, but copious water flushing may make these machines practical. Dr. Nan Vance, with the USDA. Forest Service Pacific Northwest Research Station in Corvallis, indicated that a kitchen blender with rubber blades works well, but I have no personal experience with the method.

In 1992, I tried a procedure suggested by Pauline Montgomery-Borg of the Tiller Ranger District of the Umpqua National Forest. Dried ripe arils separate readily from seed; an effective method for cleaning small seed lots. The seed pops out very cleanly if the dried aril is squeezed from the base. Each seed must be individually handled again, so the process is rather laborious. It is a good opportunity for making an accurate count of the seeds.

TESTING

There are many tests of tree seed quality (Stein, Danielson, Shaw, Wolff, and Gerdis, 1986). I used a cut test to examine my seeds. This test is destructive, but it is cheap, easy, quick, and gives a good visual indication of seed quality. I sliced the seed in half longitudinally, so that the full length of the embryo was exposed. Freshly picked yew seeds have very small embryos which can be hard to find, but healthy endosperm tissue is firm and white. Embryos enlarge and become easier to observe as the seed is stratified. It was my experience that there were very few "empty" seeds. That is, unfertilized ovules or seeds probably abort. I do not know if most "filled" seed had a viable embryo, but my hunch is that most did. X-rays or ultrasound techniques would probably provide accurate non-destructive images of seed quality, but can be costly for small seedlots. During the lengthy stratification procedures outlined below, most seeds which were non-viable, or infected with pathogenic organisms become visibly rotted during the first several months and were simply removed. Yew seed stratification is an active biological process requiring 12 to 18 months or more, and brief germination tests are unlikely to be representative of final seed viability.

STORING

Yew seeds may or may not be amenable to long term freezer storage. All of the published reports on yew seed storage that I have found recommend storage at slightly above freezing temperatures, but they antedate the development of modern conifer seed storage methods. Most conifer seeds require drying to between six and ten percent moisture content before they can be frozen without intracellular ice crystal formation causing tissue damage. I know of no published literature where this has been tried with yew seeds. I do know that if immature seeds are dried, they are badly damaged. I destroyed some early collections in this manner. Whether mature yew seeds can be dried below ten percent moisture content without harm remains to be seen. I have air dried and re-hydrated mature seeds. Drying causes the endosperm to separate from the seed coat near the tip, and when I re-hydrated the seeds, this portion of the endosperm frequently became discolored.

Procedures for long term storage of yew seeds may be developed with close attention to physiological results. If yew seeds can not tolerate thorough drying without damage, my guess is that partially dried seeds could be frozen at 1 degree Celsius without intracellular ice crystals forming. Under these conditions, yew seeds could probably be stored for a decade or more without significantly diminishing their viability. This procedure would require precise and reliable control of freezer temperatures, and a separate freezer from other conifer seeds. If storage longer than 5 years is not important, it would be easier to simply refrigerate partially dried seeds.

STRATIFICATION

Few yew seeds germinate the first spring following maturation. Under nursery conditions, and presumably under natural conditions, peak germination occurs the second spring, and some germinate the third or subsequent springs. Three dormancy mechanisms may be involved (Hartmann, Kester, and Davies, 1990). The seed coat may have inhibitory compounds which are removed by passage through an animals gullet, or through the leaching action of rain or snow melt. Next, even in fully formed seeds with ripe arils, the embryo is very small. It grows in size slowly during the course of stratification, before it germinates. Finally, there is probably a genetically- or developmentally-predetermined physiological dormancy whereby either the embryo, or perhaps both the embryo and endosperm require a certain period of cold, wet conditions before the seed will germinate. Precocious germination of excised embryos reported by Flores and Sgrignoli (1991) and Chee (1994) indicates that physiological dormancy of the embryo may be related to seed maturity or embryo/endosperm interactions, because the excised embryos can be induced to grow in sterile culture. Precocious germination of excised embryos in petri plate media is a useful technique for studying seed biology or sterile inoculations of plantlets with selected organisms. Although these techniques can shorten seed germination times by 12 months, they are too expensive and labor intensive for use in large scale yew propagation efforts that seek to preserve genetic variability in native populations by propagation from seeds.

I originally thought that the most effective artificial stratification technique would be to mimic the conditions which yew seeds typically experience under natural circumstances; namely, cold and wet (1st winter), warm (summer), and then cold and wet (second winter). This technique worked, but I did not find it any more effective than merely keeping the seed continuously cold and wet. Warm wet conditions encourage fungus growth. What surprised me was how resistant yew seeds were to decay. Many seeds enlarged and cracked open during the warm period, and yet they did not germinate. Most of these "exposed" seeds remained uninfected and viable for the next eight to twelve months, and eventually germinated!

In every seed lot, some seeds decayed. I removed these and washed the remaining seeds with Ivory bar soap. Usually decayed seeds represented less than 5 percent of the seed lot. One year I tried a different collection technique with several trees that had numerous seeds. I spread plastic tarps under the trees, and picked the seeds quickly, merely letting them drop on the tarp. This procedure also resulted in a large amount of debris mixed with the seed. The seed was much harder to clean, requiring screening, hand cleaning, and frequent separations of debris with water floatation. Significantly, more than 50 percent of the seed in these lots decayed. The seeds were exposed to more contaminants during the cleaning process, but, as with all my seed lots, I washed the seed thoroughly with soap before putting them into stratification. When the seeds rotted, the fungus grew from the inside out. Cut tests originally showed infection spots on the endosperm. Later many examined seeds had deliquesced endosperms. Finally fungi grew out of individual seeds and spread in a hyphal mat between seeds. There were two macroscopically distinct fungi, one gray and one orange. When I discovered mats of fungi growing between seeds, I was tempted to discard the entire seed lots. Instead, I repeatedly removed the seeds from which the fungal hyphae had emerged and washed the remaining seeds. Eventually no more seeds decayed and I was left with sound seeds which germinated. Was this a seed lot that was contaminated by the collection and cleaning process, or was it already heavily infested with pathogenic fungi which invaded the seed before it fully developed?

I soaked portions of some seed lots in 3 percent hydrogen peroxide for a period of a week or two shortly after cleaning. I theorized that this procedure might improve leaching of inhibitory compounds from the seed coat, speed the maturation of the embryo, or hasten the fulfillment of physiological dormancy requirements. Concentrated (30 percent) hydrogen peroxide soaks are often used as a quick germination test with other conifer seeds (Stein, Danielson, Shaw, Wolff, and Gerdis, 1986). My procedure had no obviously discernable helpful or harmful effects, but I did not track germination quantitatively.

I currently use the following procedure for stratifying my yew seed lots. I clean large seed lots (greater than 1,000 seeds) with the screen abrasion, flushing, and flotation method. I dry smaller seed lots on screens in a cool, dry room and then separate the seeds from the arils by hand. Once cleaned, I soak the seed in tap water, at room temperate, for a week or more, changing the water daily, until leachates no longer discolor the water. I then dry the surface of the seeds to discourage fungal growth. I place the seeds in freezer Zip Lock bags, and use a needle to poke about ten holes in the bag for aeration. I then place the seeds in a refrigerator and check them monthly. If any seeds rot, I remove them. If external mold develops, I wash the seeds and dry them again before placing them in a clean bag. If the seeds appear to be drying, I wet them again, and put them in a bag with fewer holes. Ideally, a little moisture will condense on the inside of the bag from seed respiration, but not enough to wet the seed coats.

GERMINATION

I observed that germination rarely occurred with less than 12 months of cold stratification. Using the artificial stratification method described above, peak germination seemed to occur after 18 months. Some seeds start germinating earlier, even in the refrigerator, and some do not germinate after 18 months of cold stratification, even when placed in warmer temperatures. I conclude that stratification requirements vary greatly from seed to seed. Stratification requirements may be correlated with seedlots from individual parent trees, but if so, the difference was not great enough for me to note. Adequate stratification periods did seem to vary among populations, however, because seed lots from some collections sites germinated earlier than others collected the same year. The length of stratification required for germination may be related to seed maturity at time of collection, but I was unable to recognize a pattern. Since I use artificial stratification and container propagation, I discovered I needed a greenhouse to plant the seeds whenever they decided to germinate in the refrigerator. More seeds germinated in the spring when I warmed the seed lot, but there would always be seeds that would not respond to this encouragement, and germinated earlier or later while still refrigerated.

Natural stratification techniques seem to induce more uniform germination rates. The first year I collected seed, I planted several thousand seeds in styrofoam seedling trays after only six months of stratification. None of the seeds germinated that spring. I left the trays outside during that summer and the following winter. The second spring nearly all of the seed germinated. Seed beds are hard to maintain outside for two years, but protecting them for one winter is feasible. Yews could be artificially stratified the first winter, and naturally stratified the second. This approach should lead to very high, and relatively uniform germination rates.

CULTIVATION

Pacific yew germinants respond to nursery cultural practices in a manner similar to other conifers. They are somewhat vulnerable to "damping-off" fungi. Birds like to harvest the seed, and in the process, pull the germinant out of the soil. Slugs consume them as well. I lost some seedlings to *Fusarium* hypocotyl rot during the first summer and fall when I kept the soil too moist. Yew foliage continues to develop as long as temperature and moisture conditions are favorable, in contrast to the early bud set of most conifers with determinant growth. Nursery-grown seedlings therefore continue producing new foliage into the late autumn, and tender leaves may become discolored, turning reddish brown, during winter cold. I have observed the same phenomenon in mature native yew foliage, and also in the foliage of nursery-grown western red cedar seedlings, and presume it is a normal reaction of new foliage to cold temperatures.

Yew seedlings grow slowly compared to conifers propagated for timber production, but more rapidly than dwarf conifers cultivated for ornamental or landscape settings. I grow my conifers outdoors in the moderate climate of western Oregon. I use soilless potting media in tubes, shield the seedlings with a light (10-20%) shade cloth, and fertilize with the tomato formula of MiracleGro. Seedlings grown from seedlots collected in 1990 and germinated in 1992, are now 18" tall. Each growing season I root pruned and repotted the seedlings. I also pruned all the side branches, because the seedlings exhibited a strong preference for growing luxurious limbs, rather than upright leaders, and I want taller seedlings for out-planting.

USEFUL STUDIES OF YEW SEED PROPAGATION

There seems to be very little published literature on propagating yews from seed, and, until recently, none on Pacific yew seed. Few seed studies were conducted because ornamental varieties of yews are best propagated from cuttings to preserve their selected characteristics. Foresters have no reason to grow native yews in nurseries while timber production is their primary goal. Propagation of wildland plants to preserve genetic and biological diversity is a relatively new land management goal.

Preservation and restoration of biological diversity on our public lands will require a greater understanding of seed biology and development of specific practical methods for propagating a wide variety of native species from seed. Yews are a good starting point for study because they have an incredibly long association of usefulness to humanity (Hartzell, 1991), native populations throughout their natural range have been greatly diminished, their seeds are biologically interesting and complex, and because human society should restore the populations of yews harvested for taxol production.

The following studies would significantly contribute to our understanding of yew seed propagation:

1. Developing practical yew seed collection, cleaning, stratification, germination, and cultivation techniques would be useful for seedling nurseries.

2. Developing optimal long term yew seed storage techniques would enhance long term reforestation efforts and assist germplasm preservation of rare *Taxus* genomes.

3. Comparative results from whole seed versus excised embryo studies could be very useful for elucidating the mechanisms of physiological dormancy in conifer seeds and thereby improve stratification techniques in nurseries.

4. Studies of the mechanisms by which both mutualistic and pathogenic organisms (especially fungi) infect developing seeds would improve seed collection and cleaning methods, enhance nursery operations, and contribute to the fields of forest ecology and biotechnology.

CITED REFERENCES AND OTHER SOURCES OF INFORMATION

The following publications are the best that I have found for information on growing yews from seed. They are arranged in alphabetical order by the last name of the principal author, and a brief description follows each.

Chee, Paula. 1994. In Vitro Culture of Zygotic Embryos of Taxus Species. Hort. Science 29(6):695-697.

This article reports methods for precocious germination of embryos, and that embryos excised from mature seeds grew better.

DiFazio, Stephen P. 1995. The reproductive ecology of Pacific yew (*Taxus brevifolia* Nutt.) under a range of overstory conditions in western Oregon. Masters of Science Thesis. Oregon State University. Corvallis, Oregon. May 5, 1995.

Stephen, in conjunction with Dr. Nan Vance as a major professor, did a thorough study of native Pacific yew reproduction in conditions ranging from native forests to clearcut logging units. He quantified pollenization, seed development, seed attrition factors, and seed dispersal.

Devillez, F. 1976. After-ripening and dormancy breakdown in *Taxus baccata* L. seeds. In: Seed Problems. Proceedings of the Second International Symposium on Physiology of Seed Germination. International Union of Forest Research Organizations. Working Party S2. 01. 06. Fuji, Japan.

This article has a poor translation, but contains detailed information on stratification and germination temperature regimes.

Flores, Hector E. and Paula J. Sgrignoli. 1991. In vitro culture and precocious germination of Taxus embryos. In Vitro Cell. Biol. July 27:139-142

This article reports a higher germination rate for embryos excised from immature seeds, and germination of 50 percent of mature embryos which had been frozen for a week.

Hartmann, H.T., D.E. Kester and F.T. Davies, Jr. 1990. Plant Propagation: Principles and Practices. Chapter 6: Principles of propagation by seed. Fifth Ed. Prentice- Hall, Inc. Engelwood Cliffs, N.J. pp 104-136.

This chapter is a good, current review of the types and mechanisms of seed dormancy.

Hartzell, Jr., Hal. 1991. The Yew Tree, A thousand whispers. Hulogosi Press, Eugene, Oregon.

This is by far the most thorough reference on humanity's long association with yews.

Heit, C.E. 1969. Propagation from seed. Part 18: Testing

and growing seeds of popular *Taxus* forms. American Nurseryman 129(2):10-11, 118-128.

This article discusses *Taxus* stratification and germination regimes, but not those of *Taxus*

brevifolia.

Heit, C.E. 1967. Propagation from seed. Part 10: Storage methods for conifer seeds. American Nurseryman 126(8):632-34.

This article discusses storage of *Taxus baccata* seed.

Melzack, R.N. and D. Watts. 1982. Variations in seed weight, germination, and seedling vigor in the yew (*Taxus baccata*, L.) in England. Journal of Biogeography 9:55-63.

This article reports high variability in seed characteristics, but finds them unrelated to seed vigor.

Mitiska, L.J. 1954. The propagation of *Taxus* by seeds. Plant Propagators Society Proceedings 4:69-75.

This article discusses outdoor propagation of *Taxus* *baccata*.

Stein, W.I., R. Danielson, N. Shaw, S. Wolff, and D. Gerdis. 1986. Users Guide for Seeds of Western Trees and Shrubs. USDA. Forest Service, Pacific Northwest Range and Experiment Station. General Technical Report GTR-PNW-193.

This pamphlet is an excellent reference for seed testing procedures currently employed in seed commerce.

Suszka, B. 1978. The Yew *Taxus baccata*, L. (Cis pospolity *Taxus baccata*, L.) Warsaw, Poland. Translated from Polish. Published for the Department of Agriculture and the National Science Foundation by the Foreign Scientific Publications Department of the National

Center for Scientific, Technical, and Economic Information. Available from the U.S. Department of Commerce National Technical Information Service. Springfield, VA 22161. pp 87-92.

This is a thorough, but dated, reference on yew propagation. The translation is good and it is available on microfiche. Other sections discuss native yew stands in Poland, their history and ecology.

USDA. Forest Service. 1974. Seeds of woody plants in the United States. Agriculture Handbook No. 450.

This venerable tome is a seed bible, and has a good section on *Taxus*. James A. Young and Cheryl G. Young published a revised version through Dioscorides Press in 1992. Dr. Nan Vance has revised the section on *Taxus brevifolia* for yet another edition that is currently in press.