STRATIFICATION OF TREE SEED

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Stratification of seed that is otherwise difficult to germinate is a very old practice, probably pre-dating most of our agriculture and forestry texts and handbooks. It is an art developed from observation, experiment, and experience. To date it is not strongly science-based although its effects will undoubtedly be explained in due course in terms of removal of specific blocks to germination—the ultimate goal of the seed.

Stratification is one of a number of treatments commonly used to overcome seed dormancy. Such include the use of light or other radiation, the use of various chemicals such as thiourea, nitrates, ethylene gas, citric acid, and gibberellin, soaking in hot water and acids and many others. Some of these appear to be interchangeable. In particular, stratification can substitute for the others in many instances.

The suggestion has been made and generally accepted that the course of germination may follow one of several pathways, that blocks may be present in one or more, and these may respond to different kinds of treatment or be by-passed (E. H. Toole, 1961). Stratification—usually cold, sometimes warm, and sometimes a combination—results in a by-passing of other requirements and presumably of the related blocks.

It may be fortuitously, or more likely through long and rigorous selection, that many woody plants have acquired a system of alternative pathways from dormancy to germination. Whatever the cause, the process probably favors survival of the wild species. It takes care of some of the vagaries of seasons and environments to permit eventual germination and initial establishment under a variety of adverse or unusual conditions. Such conditions would probably often be unsuitable for seed provided with only one road to germination and one block that *had* to be broken down for germination to take place.

What is stratification?

Usually stratification implies a cold, moist treatment of seed for periods of a few days to as long as several months. The term is also used, however, to include a moist treatment at any temperature that will not normally cause germination. Thus, Mahlstede and Haber (1957) include both cold and warm treatments as well as the combination of cold and warmth used to overcome so-called "double dormancy". In the Woody Plant Seed Manual (U. S. Forest Service, 1948) also, the terms "cold stratification" and "warm stratification" are both used.

Variations in actual practice are several. What may be the original system of placing seeds between layers of moist sand or other medium in a pit in the ground, is still used, probably fairly commonly. Instead of being buried outdoors, the seed may be layered in a container placed in refrigerator, cold room, or root cellar. Often the seed is mixed with a moist medium, rather than layered, and placed in a rigid container or polyethylene bag and refrigerated.

Another modification is to pre-soak the seed, then dry its surface and place it in vials, jars, or plastic sacks. Mahlstede and Haber (1957) suggest that small lots of seed can be mixed with moist, shredded sphagnum moss and placed in a polyethylene bag at 41°F. Large lots of conifer seed can be stratified in cotton sacks separated by wet medium into layers, in barrels provided with good drainage (U. S. Forest Service, 1948). Any large-scale operation requires frequent checking of temperature and moisture conditions around the seed. Fine seeds have been placed between appropriate cloth material which is alternated with layers of stratification medium such as sand, peat, or vermiculite (Mahlstede and Haber, 1957).

What does stratification do?

Cold stratification is thought to break at least some forms of internal dormancy; that is, to remove or circumvent physiological blocks to necessary processes or events leading to germination. Little is known as to the nature of the blocks or even the processes, although some of the changes that take place have been determined (see, for example, Baldwin, 1942; Barton and Bray, 1967). Such changes must be preceded by activation of appropriate enzyme systems that direct metabolism along a successful pathway. Such changes may involve modification of substrates or removal or change of toxic or inhibitory materials, as well as activation of an enzyme system (E. H. Toole, 1961).

On the other hand, warm stratification, usually in combination with a cold period, is used to make the seed coat permeable for effective cold stratification to follow. Other treatments commonly used as alternatives to warm stratification include soaking in hot water or strong acid, and scarification of the seed coat. Stratification is often favored as the more natural method, probably less hazardous to both seed and user and adaptable to both small- and large-scale operations.

An interesting application of stratification by Hagner and Simak (1958) resulted in the further development of immature embryos of *Pinus cembra*. The best treatments involved the use of moist vermiculite at about 5°C. with the seed placed on its surface, embedded in it, or mixed with the medium. Over a 5-month period, some embryos grew from less than half to almost full seed length. Such a technique may have considerable value for far-north or high-elevation species or races whose seed often does not develop fully. Incidentally, Hagner and Simak used x-radiography to follow embryo development in individual seeds during treatment. They advocate use of the x-ray technique to diagnose immaturity of the kind reported.

Natural basis for stratification

H. I. Baldwin (1942), in his near-classic *Forest Tree Seed*, stated "Knowledge of the proper pretreatment to induce prompt and complete germination can best be gained by a study of the ecological factors affecting the seed in its natural habitat between the time of maturity and germination. With rare exceptions this has not been done. . . . . . many things can be learned from a careful study of the life history of a seed". Baldwin's statement still holds after 25 years even though we have added to the species and varieties we can successfully treat to make them germinate. Many seeds still pose problems; such include sweet fern, the euonymuses, some hollies and honeysuckles, yews, California nutmeg and others (Mahlstede and Haber, 1957; U. S. Forest Service, 1948).

Very often, observations of seed behavior in field or nursery suggest whether stratification is desirable or necessary. Black cottonwood seed, for example, matures and is disseminated in early summer and germinates almost immediately or perishes. Willows are similar and so is sugar maple. Seeds having these characteristics are not likely to benefit from stratification or other treatment, but should be sown as soon as collected.

Western broadleaf maple, on the other hand, matures and drops its seed in late autumn; germination occurs the next spring. Seed with such characteristics usually responds to stratification if sown in spring, or to fall sowing if not treated.

A fairly large number of species, particular trees and shrubs, produce seeds that germinate irregularly over a period of two, three, or even four years. If sown in the late fall they may germinate mainly in the second year; if sown in summer they may germinate well the next spring. If we know this from experience or observation, we can be fairly certain that such seed requires compound stratification, that is, warm followed by cold. Temperatures commonly used during the warm treatment are 68-86°F. alternating (16 and 8 hours, respectively). Some, but not all, of this group drop their mature seed in summer when it is exposed naturally to a period of "warm stratification", then to one of cold. Examples of species whose seeds generally respond to double or compound stratification include (U. S. Forest Service, 1948): basswood or linden (*Tilia americans*), the ashes (*Fraxinus excelsior, F. nigra,* and others), Eastern red cedar (*Juniperus virginiana*), hawthorns (*Crataegus* spp.), American mountain ash (*Sorbus americana*), dogwoods (*Cornus* spp. except *C. nuttalli*), cotoneasters. Western white pine is reported to respond to compound treatment: 30 days at 24°C. followed by 30 days at 2-5°C. was most effective (Anderson and Wilson, 1966).

Many kinds of seeds are disseminated in the fall or early winter, lie exposed for several months, and germinate more or less completely the following spring. Such seeds respond well to simple cold stratification if spring sown; if fall sown they rarely require treatment. Many north temperate conifers are in this group. Although deep dormancy causing one or more full years' delay in germination is not characteristic of them, spring sowing without pretreatment usually leads to prolonged germination over the first season and a consequent lack of uniformity and often a high cull factor in the case of nursery stock. For this reason, most forest tree nurserymen practice stratification for some, if not all, of their species.

It is interesting to note that of some 80 groups of trees, mostly genera representing several species, cited by Mahlstede and Haber (1957), over 50 require cold stratification if spring sown, but 39 of those require no treatment if fall sown. Nine require double stratification and two respond to warm stratification alone.

Ecological significance of a stratification requirement

A number of authors have noted that geographically different sources of a species tend to have different germination requirements. Thus, Fowler and Dwight (1964) and Mergen (1963) found that seeds of *Pinus strobus* from southern sources required longer stratification than did seeds from northern sources. A generally comparable behavior seems to be true also for Douglas fir (Allen, 1960, 1961).

Noted many times is the fact that outdoor overwintering ("natural stratification"), or cold stratification, lowers the temperature required for germination by as much as 20° C, perhaps more. Such a mechanism serves to prevent germination during mild weather in fall or winter and to favor germination in the spring as soon as soil temperatures rise somewhat above freezing. This kind of response protects effectively against premature germination at a time when killing temperatures can still occur. Although it has no direct value as a protection against a severe drought following hard on germination, it favors early germination and establishment while soil moisture is still adequate; thus it probably has indirect protective value against drought most years.

Because naturally "stratified" seed has a much lower temperature requirement for germination, ecological studies that use spring-sown seed, untreated after dry storage, are likely to produce results that are difficult or impossible to interpret and apply to situations that actually occur in nature.

Technique

Most of the following remarks are based on experience with seed of western conifers, particularly Douglas fir. They are probably applicable in principle to many seeds that respond to cold stratification.

Various media have been used, as well as no medium, that is, so-called "naked stratification" (Allen and Bientjes, 1954). The latter consists of soaking the seed in water for a suitable time, removal of surface moisture by towelling or air-drying, and storage in loosely closed vials, bottles, or polyethylene bags.

Use of a medium provides a reservoir of moisture and probably helps to prevent heating. The disadvantage is that the medium and seed will likely have to be separated prior to sowing by mechanical means. With no medium, the seed is usually surface-dry at the end of stratification; sowing is easier. With or without a medium, periodic examination is desirable to ensure that moisture and aeration are adequte. With Douglas fir, presoaking of the seed for 24-30 hours at room temperature brings its moisture content up to 60-70 per cent (o.d.w. basis). Over a period of two or three months this will drop to 30-40 per cent. Within this range, stratification has proven fully effective, but not at lower moisture contents. In a study of loblolly pine, MacKinney and McQuilkin (1938) found that peat or sand wetted to 25, 50, 75, and 100 per cent of their moisture-holding capacities, were all equally effective in hastening germination. Such suggests that moisture limits may be quite broad and non-critical, particularly when a medium is used.

Some other characteristics of stratified seeds

Duration of the cold stratification treatment used depends partly on the objective of treating the seed. For testing at an optimal temperature, a relatively short period may be quite satisfactory. But the longer the treatment, the lower will be the temperature at which a given fraction of the seed will germinate. Hence, for field or nursery use, longer treatments more closely simulating natural overwintering may be selected.

In the case of Douglas Fir and the true firs (*Abies* spp.), for example, after extended stratification, many of the seeds will germinate near the freezing point and the radicles will continue to grow at that temperature (0-2°C.). Such behavior has been reported for a number of plants including grasses. After natural overwintering just below the soil surface, Douglas fir seed germinates in February or early March in this climate (Vancouver, B. C.) when near-surface soil temperature is about 10°C. or less. But no shoots appear above ground until daytime soil temperature is about 15°C. —a relatively "safe" temperature. Hence, the early germination or establishment resulting from fall sowing (or stratification if seed is sown early enough) might go unnoticed or be underestimated. It is doubtful that the alternatives to cold stratification have such marked effect upon the temperature requirement for germination.

Experiments with Douglas fir suggest that some 80-120 days of cold stratification fairly closely simulates 5-7 months of overwintering on the ground; the latter, of course, involves fluctuating moisture and temperature whereas the former is usually accomplished at constant temperature and either a constant or a slowly decreasing moisture content.

Another feature is that the longer the period of stratification (up to some limit, possibly 80-120 days), the more uniformly will the seed behave. Whereas untreated seed of individual Douglas fir trees may have median germination times of 5 and 30 days at 25°C. in somewhat extreme cases, such times can be brought to a low and similar level by cold stratification, usually of 80 days or longer. Extreme differences of this kind do occur within single lots of seed derived from a number of parents. Uniformity, often a most desirable feature in large-scale operations, can be promoted by long, cold stratification.

Although stratification does not harm high quality seed of species known to benefit, it may lower production from poor seed. In such cases, the longer the treatment, the greater the drop. Seed that reacts in this way is suspect and should be used as soon as possible and stratified for a minimum period.

Special applications

Stratification has some advantages for seed testing. Response will indicate the degree of dormancy and the treatment likely to be effective; but differences between laboratory and field conditions for germination should be kept in mind in interpreting the laboratory results. In many cases, cold stratification makes unnecessary other treatments for dormant or slow-to-germinate seeds and, in many seed-testing laboratories, is therefore used as a matter of routine.

For research, stratification has specific values:

a) For studies of the ecological requirements for germination, it provides seed that is similar physiologically to seed that was shed in the fall and overwintered on the ground; the artificial treatment, however, is reproducible.
b) For studies of the physiology of dormancy, it provides seeds whose dormancy has been broken, for comparison with untreated, dormant seeds.
c) For seedling studies of such things as growth, photosynthetic efficiency, and nutrition, it provides a way of producing relatively uniform experimental material.

Reversing the stratification process

The most effective stratification treatment for a given set of conditions can be worked out by laboratory experiment. Unfortunately, the predicted and planned-for conditions may not come about and ability to modify plans is highly desirable. For example, we might have a perfectly good 60-day treatment based on careful studies, only to find the nursery flooded on the planned sowing date. The question is: what can we do with the seed if we can't sow? — and what if anything can we do with leftover treated seed? The following comments apply to Douglas fir seed, both Coast and Interior sources; they probably apply to seeds of a number of other conifers that react favorably to cold stratification.

Good seed is durable if carefully handled; care is essential when the seed is moist and ready to germinate. It can be continued in stratification for months with little or no loss and will retain its readiness to germinate. Some seed may germinate at the low temperature but will remain viable if handled with care. Instead of being continued in stratification the seed can be dried down to 10 per cent moisture content or less (at room temperature and moderate relative humidity) and returned to cold storage. Another method is to expose the seed to the atmosphere in cold storage and allow it to lose moisture slowly. As the seed dries by either method it slowly loses its readiness to germinate and will go back into full dormancy in time. Field observations of seed prevented from germinating by sudden cold or drought generally support the above conclusions derived from laboratory studies.

Before concluding, I should like to stress the fact of biological variation among the seed of wild species. Prescriptions for seed testing or treatment may differ because the experimenters used material that differed in some important way(s). The history of the fruits or cones, and seeds, may be a factor; thus, the parentage the season's weather, the seed's maturity, the way it was handled from collection through extraction and cleaning, to storage may all have their effects. The need for replication is obvious. To give just one example, I once had a Douglas fir tree that produced seeds that were very slow to germinate when untreated. Dormancy in this tree's seeds lay largely in the endosperm membrane. If the membrane was punctured or cut anywhere on its surface, that seed germinated very rapidly, as if stratified. I thought I had a real discovery but seed from other trees nearby showed very little response to membrane treatment. The interesting finding was that all of the seed referred to responded equally well to cold stratification.

A final example taken from some nursery studies made in the early 1940's illustrates again the uniformity-promoting feature of cold stratification for Douglas fir. With spring sowing, seedlings from stratified seed began to appear above ground 2-4 weeks earlier than those from untreated seed; the treated seed completed its germination two months earlier, producing much more uniform and larger stock.

Conclusion. I have discussed some aspects of the practice of stratification and very little of the scientific base or interpretation. The reason is simple. Too little is known about dormancy as such or the action of various agents that remove or destroy the blocks to germination to overcome dormancy. Knowledge about these two aspects will almost certainly increase at about the same rate since they are so closely interdependent. Stratification is probably the most universal of the treatments used and, as such, is likely to figure importantly in future studies designed to shed light on dormancy and the processes that overcome it.

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MODERATOR JACK: Thank you, Dr. Allen, for a most interesting discussion. Mr. Jack Doty of Viewcrest Nursery, Vancouver, Washington, (not British Columbia) will now talk to us on field production of tree seedlings in Washington. Mr. Jack Doty:

FIELD PRODUCTION OF TREE SEEDLINGS IN WASHINGTON

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In growing seedlings we try to duplicate the processes which would come about naturally. Seeds have an inherent ability to hold back germination in the fall to avoid winter-killing. By stratification we gain a quick germination at