Breaking of Tree Seed Dormancy at Controlled Moisture Content

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INTRODUCTION

In nature it is very common for seeds to have mechanisms that delay germination for shorter or longer periods. This delay or temporary inhibition of germination is often termed seed dormancy, and covers a range of different physical, chemical, and biological conditions in the seeds. More than 60% of Danish tree and shrub seeds have some kind of dormancy. In commercial production seed dormancy constitutes a major problem, causing losses of viable seed resources and reducing possibilities for optimizing the size of the production to the demand of the market.

In breaking physiological dormancy by a prechilling or cold stratification treatment, the seed has traditionally been fully hydrated and kept at a near maximum moisture content (MC), sometimes mixed with peat moss or sand, and prechilled at 2 to 5C for a time period required for the seed to initiate germination at the storage temperature. The duration of the prechilling treatment depends on the species and the seed lot involved and varies from a few weeks to many months.

The prechilling treatment is usually carried on until 5% to 10% of the viable seed has visible radicles and at this point the seed lot is either sown directly or stored for a short period at -2C to stop further germination of the lot.

A careful management of seeds prechilled by this method can provide good germination results.

WHAT IS THE PROBLEM?

The traditional method described above is based on a compromise and is quite sensitive to incorrect management. This means that the dormancy release of the seed lot cannot be fully optimized and even minor errors in management can produce major reductions in the potential of the seed lot to produce a good seedling stand.

Three factors in the behavior of tree seeds should be mentioned to understand this.

1) Many northern temperate tree and shrub seeds can germinate at temperatures close to 0C or even below this, and will germinate well at a prechill temperature of 3 to 5C.
2) Traditionally seeds are prechilled at full hydration, i.e. at a MC that allows germination of nondormant seeds.
3) The prechilling demand of individual seeds within a seed lot are not the same but can vary up to ± 4 to 6 weeks from the average prechilling demand.

The consequence of this is that both dormancy release and germination can occur simultaneously at the temperature and moisture content seeds are exposed to during the prechilling treatment. During the dormancy breaking treatment an increasing fraction of nondormant seeds will accumulate, which in turn will begin to germinate during the treatment. Germinated seeds with protruding radicles are very susceptible to damage during seed handling and sowing, with great risk of killing the seed or producing poor seedling stands of inferior plant quality.

Storage of fully hydrated seeds with protruding radicles is very difficult for even short periods, and accordingly any radicle protrusion during prechilling should be avoided.

If prechilling is stopped too early, the prechilling demand of seeds with the deepest dormancy might not be satisfied and consequently these seeds will not germinate after sowing. A compromise of prechilling long enough to release as many seeds as possible from dormancy but ended early enough so that only a few seeds will germinate during the treatment is the goal.

European scientists have suggested that up to 30% of viable tree and shrub seeds are lost in production due to nonoptimal methods of breaking seed dormancy. To eliminate the above compromise in the dormancy breaking method, it is necessary to allow dormancy release to occur but eliminate the ability of the nondormant seeds to germinate during prechilling. This would provide a possibility of prolonging the prechilling treatment until all seeds were released from dormancy without any seeds being lost due to early germination.

One method of inhibiting radicle growth is to lower the seed MC to a level which just inhibits the elongation of root cells. This critical MC will allow most physiological and biochemical processes in seeds, such as prechilling or priming, to go on although sometimes at a lower rate than at full hydration.

HISTORICAL DEVELOPMENT

The effect of reducing the MC in seeds during prechilling has been known for some years. In 1973 the English scientist Blundell described prechilling of *Rosa corymbifera* 'Laxa' seeds at a controlled MC that inhibited germination but allowed dormancy release (Blundell, 1973). In 1975 the Polish scientist Suszka described a method of prechilling at controlled MC in *Fagus sylvatica* (Suszka, 1975). With conifers the effect of holding seeds at different MC during cold storage was studied in the 1960s and 1970s, but was first adopted into a practical usable method in the beginning of the 1980s (Edwards, 1982; 1986).

In Europe significant research on prechilling of tree seeds at a controlled moisture content (CMC) occurred in the 1990s (Jones and Gosling, 1994; Suszka et al., 1994). An EU (European Union) research project running from 1993 to 1996 investigated and developed this method on a number of tree species. The method is now used commercially on a large scale with a few species in a number of European countries.

PRECHILLING AT CONTROLLED MOISTURE CONTENT IN NEW SPECIES

To establish this method in a new species it is critical to obtain knowledge about the critical MC for germination — or rather radicle protrusion — in the species. This can be obtained easiest by testing the germination ability of prechilled, non-dormant seeds kept at different constant levels of MC and at the prechilling temperature for different periods. At the higher MC the seeds will germinate and the critical MC will be at the highest MC where germination is completely restricted.

The critical MC for germination often lies between 3% and 8% below full hydration and generally only varies a little between seed lots. The critical MC for germination describes the upper limit of MC where you can safely prechill your seeds without having premature germination.

It is then necessary to investigate at what MC you will have the optimal rate and most efficient release of dormancy given the above moisture limit. The MC interval just below the critical MC for germination is relevant to investigate. It is further important to compare the rate of dormancy release at full hydration to the method at reduced MC to be able to transfer the new method into application, with regard to the duration of the prechilling.

The optimal MC for prechilling by CMC is found by cold stratifying seeds at different MCs and durations. Samples are withdrawn at intervals, imbibed to full hydration, and tested for germination capacity and speed of germination at a temperature that clearly shows if the seeds are dormant or not. Depending on the species both high, intermediate, or low temperatures could be used in these tests. After having calculated germination capacity (GC) and mean germination time (MGT) or another parameter describing the speed of germination, the efficacy of the treatments can be evaluated as an improvement in GC or a reduction in MGT. The optimal CMC for prechilling can then be defined as the MC giving the fastest improvement in GC and MGT. The optimal CMC for prechilling often lies just below the critical MC for germination.

The critical MC for prechilling describes the lowest MC where you can obtain a positive effect of a prechilling treatment. This lower MC limit and the critical MC for germination delimits the "treatment window" for a CMC prechill. The theoretical effect of MC on prechilling and germination in tree seeds based on experience with a number of species is shown in Fig. 1. The shown moisture scale is arbitrary and actual values would depend on the species. It cannot, however, be ruled out that some species can show other types of reaction patterns with regard to the effect of MC during prechilling.

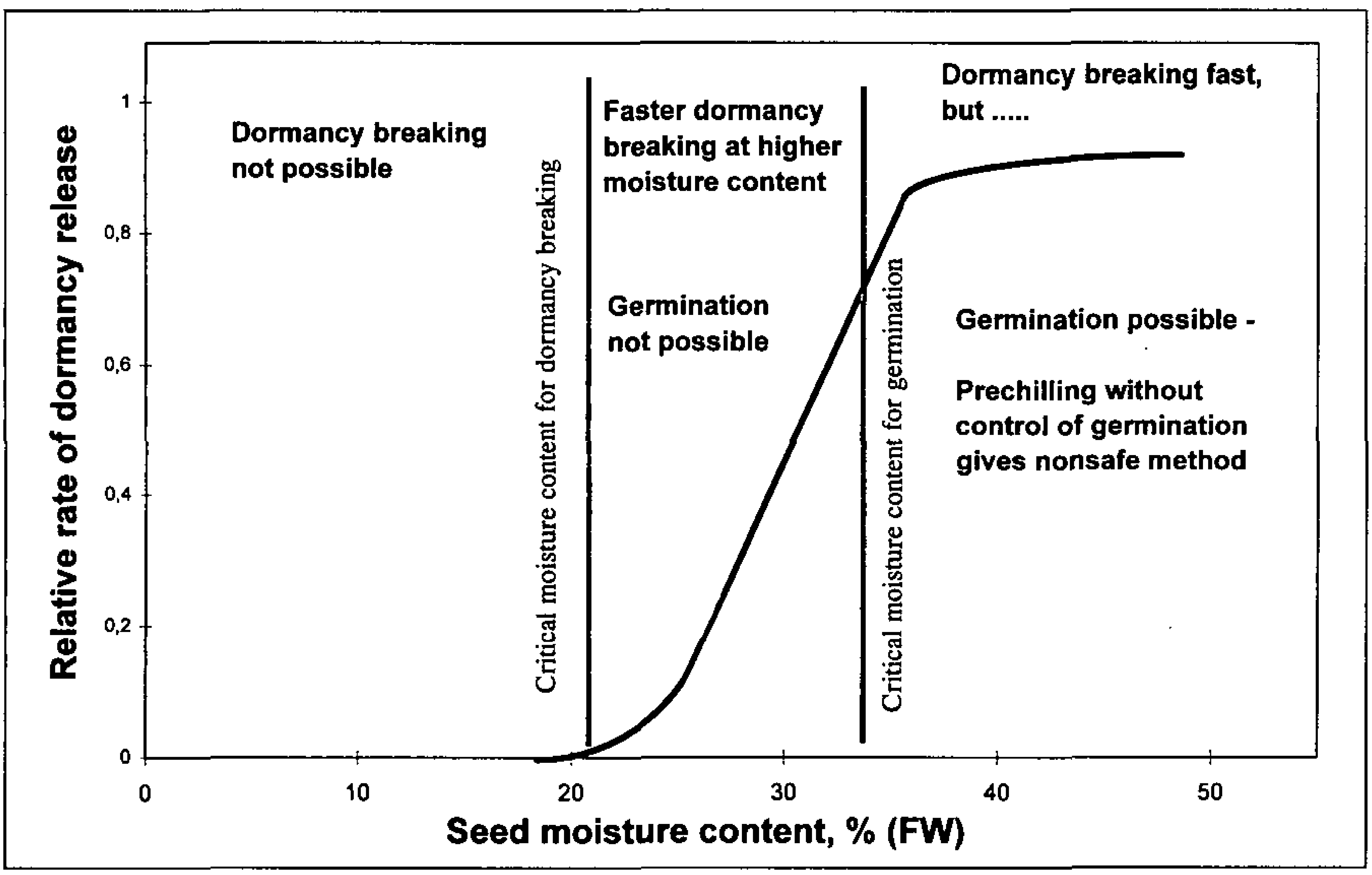

Figure 1. Theoretical effect of different controlled moisture content values during prechilling of seeds. The moisture interval between the two critical m.c. levels is the "treatment window", where dormancy can be released without having premature germination during the prechill. Critical moisture content levels vary between species.

HOW LONG CAN THE TREATMENT DURATION BE PROLONGED WITHOUT HARMING THE SEEDS?

Studies with a number of conifer species suggest that the CMC prechill can be prolonged significantly without reducing the quality of the seed, i.e. with no reduction in GC or MGT at optimal conditions (Derkx and Joustra, 1996; Jensen 1996; Jones and Gosling, 1994). Experiments with *F. sylvatica* and *Abies nordmanniana* showed that the duration of the CMC prechill should be almost doubled to obtain maximal benefit of the treatment. Other experiments show that prolonging the CMC prechill significantly longer than traditional treatment durations will reduce both GC and MGT (Suszka, 1994). The prechill period should therefore only be continued until no further improvements in GC or MGT is seen. At this point the seeds should either be sown or kept at -2C for short-term storage. If it has been shown that seeds of a species can be dried back and stored after being prechilled at CMC, this could be a third option.

The optimal duration of the prechill treatment is related partly to the level of dormancy in the seeds, which varies according to the seed lot, year of harvest, and processing method, and partly to the rate of dormancy release which is affected mainly by the prechilling temperature and seed moisture content. A low quality seed lot usually has a shorter optimal duration of the prechill period than a high quality seed lot. Therefore, a general treatment duration can not be listed for individual species. The theoretical effect of the duration of the prechill period on the speed of germination (MGT) is shown in Fig. 2. The average optimal prechill duration at optimal MC will often be a few weeks longer than the duration of a traditional prechill of fully hydrated seeds. It is recommended that a small sample of the seed lot be tested for the optimal duration of the treatment before scaling up. Individual

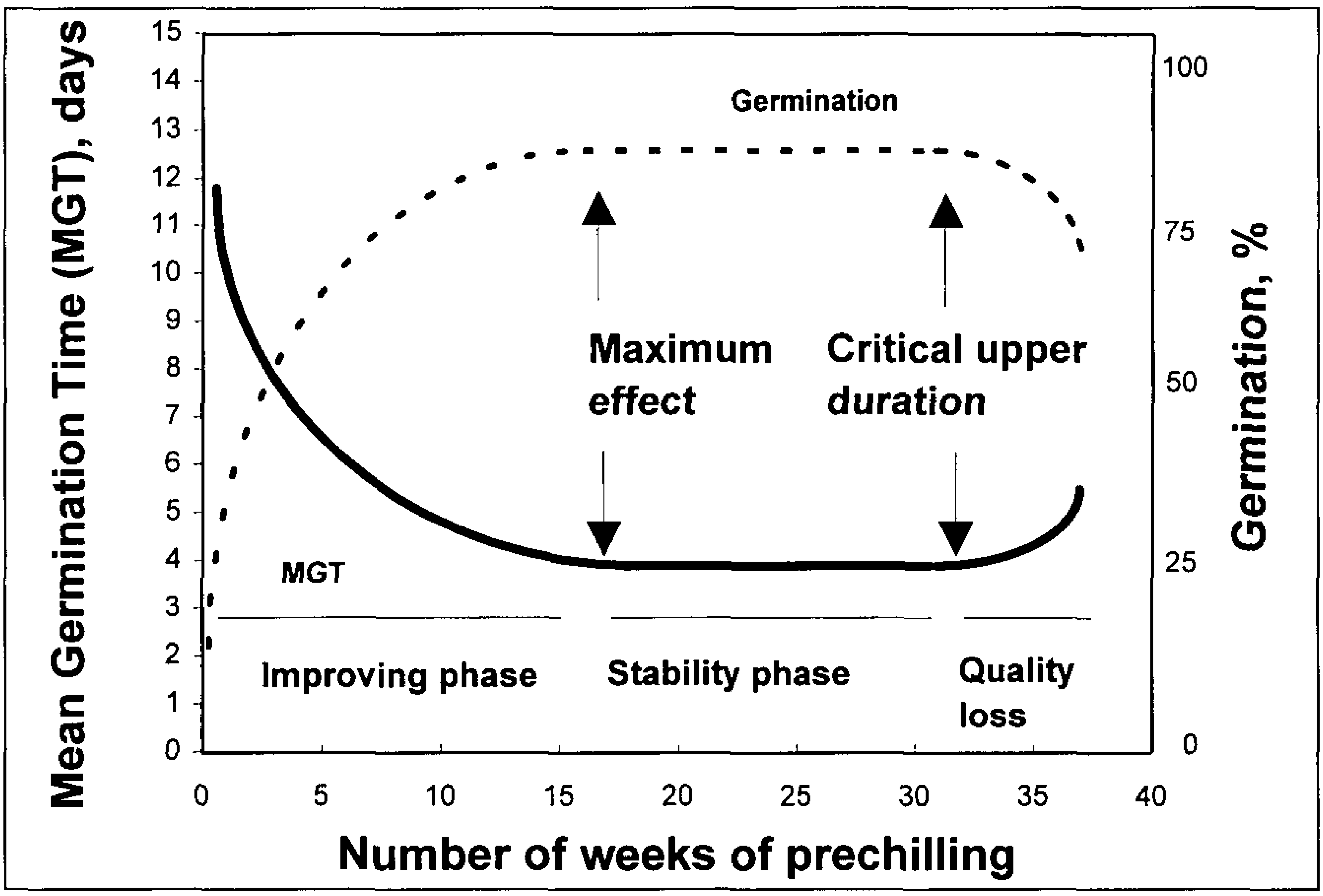

Figure 2. Theoretical effect of a prolonged prechill at controlled moisture content on the germination capacity and mean germination time. Absolute levels of GC, MGT, and prechilling duration varies between species.

growers should also test this method on the specific seed lots they use in order to develop a standard procedure adjusted to their seed provenances and nursery practice.

In summary, the basic knowledge necessary to develop a controlled moisture content prechill for a new species requires the following three tasks:

1) Establishment of the critical MC for germination (radicle protrusion) by investigating the effect of different levels of MC on the germination ability of seeds at 2 to 5C.
2) Establishment of the optimal MC for CMC prechilling by investigating the effect of different MC levels just below the critical MC for germination on the rate of and final level of improvement in GC and MGT.
3) Establishment of the optimal duration of the CMC prechill by investigating the effect of different prechill durations at the optimal MC on the level of improvement in GC and MGT.

The primary advantage of using CMC prechill is that seeds will not be able to initiate radicle protrusion during the prechill treatment. This means that the duration is not as critical as in the traditional method, thereby reducing the need for intensive monitoring during the last weeks of prechill and reducing or eliminating the risk of loosing seeds due to premature germination. The method provides an opportunity to obtain optimal germination capacity by prolonging the prechill treatment until all seeds have been released from dormancy.

A prolonged prechill treatment also enhances the speed of germination and the synchronization of germination of individual seeds in a seed lot which provides a much better starting point in production. Seeds which are sensitive to induction into secondary dormancy by high temperatures, e.g. *Prunus* spp., become less sensitive to high temperatures when the prechilling treatment is extended. The CMC prechill

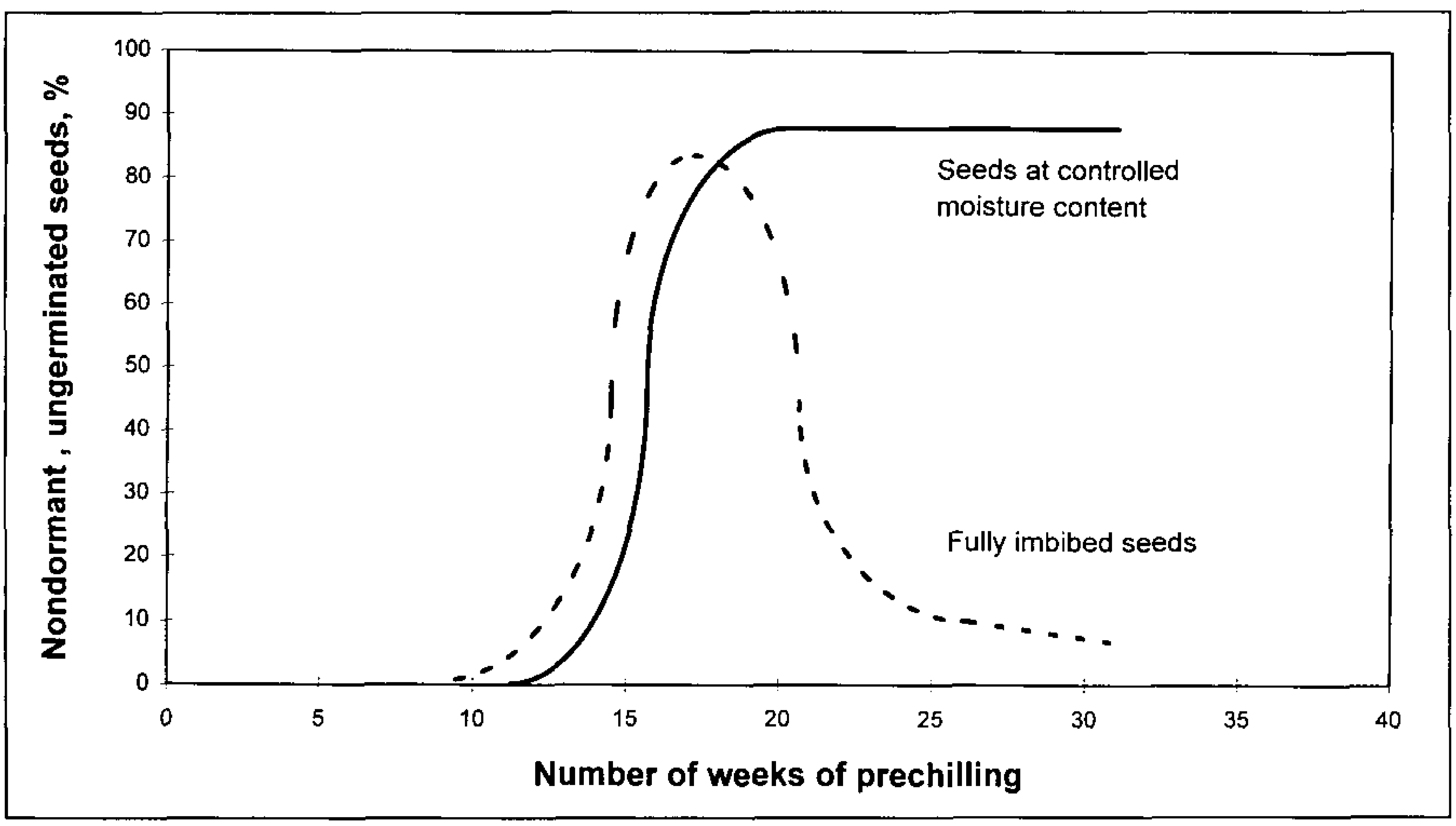

Figure 3. Theoretical changes in the number of nondormant but ungerminated seeds during a controlled moisture content prechill or a traditional prechill at full hydration. At full hydration seeds begin to germinate during the prechill. Germinated seeds are often damaged during sowing and retain little ability to produce normal seedlings.

method accordingly provides a unique possibility for reducing the risk of induction of seeds into secondary dormancy.

The method also adds flexibility to the sowing time as the CMC prechill can be extended without reducing germination potential of the seed lot. Seeds are generally surface dry and free-flowing during a CMC prechill. This means that the rate of growth of fungal hypha at least on the surface of the seeds is reduced significantly. The relatively high MC in the seeds may, however, provide opportunity for fungi to proliferate within the seeds.

The CMC method is superior to the traditional method mainly because it provides much better control of biological processes during the prechill treatment which in turn improves our ability to optimise the dormancy release process. The theoretical difference in dormancy release and premature germination during a prechill treatment of CMC prechilled seeds and traditionally prechilled seeds at full MC is shown in Fig. 3.

CONTROLLED MOISTURE CONTENT PRECHILL IN INDIVIDUAL SPECIES

As the critical MC for germination and the optimal CMC for prechilling varies with the species, it is necessary to investigate and develop the method for each new species.

Literature that describes the effect of seed MC on the biological activity of the seeds including the ability to germinate would provide a sound basis for choosing relevant moisture content levels for investigations on CMC prechill in new species. Table 1 provides a summary of MC levels or intervals reported in the literature to restrict germination but allow dormancy release to occur. The results are from investigations on either prechilling or priming/invigoration of tree and shrub seeds. As minor variations in optimal MC would be expected due to differences in provenances, etc., the data should only be used as a guideline when developing protocols for any particular seed lot.

WHAT WILL FUTURE RESEARCH AND DEVELOPMENT BRING?

Only a small number of species have been investigated so far and the method is only used commercially with a few species in Europe. However, in those species there are still a number of problems remaining that need to be studied in future research.

As the development of the CMC prechill method in a new species, using the above described scientific approach, is costly and time consuming it will be difficult to adapt the CMC prechill concept to all tree and shrub species propagated by seed. It is, therefore, of great interest to develop a fast, easy, and less expensive protocol for establishing the optimal CMC prechill in new species.

Potential similarities in reaction patterns and optimal moisture contents for prechilling of closely related species should be used as a basis for predicting CMC values in new species. In addition to this a general understanding of the relations between the biochemical composition of the seed, barriers to water transport, and MC or water potential of the seed would significantly improve the ability to predict optimal MC. With knowledge on the content and character of the main biochemical compounds (e.g. lipids, proteins, carbohydrates) and the distribution of these and water within the principal seed tissues controlling germination and dormancy release, it should be possible from moisture sorption/desorption references to predict more precisely the critical MC of interest.

Table 1. Seed moisture content or moisture intervals found to restrict germination but allow dormancy release or priming to occur.

Species	Critical moisture content %FW	Reference
Abies amabilis	30	Leadem, 1986
A. amabilis, grandis, lasiocarpa	30-35	Edwards, 1982; 1986
A. lasiocarpa	30	Leadem, 1989
A. nordmanniana	33-34	Jensen, 1997
A. nordmanniana	28-35	Poulsen, 1996
A. procera	30-34	Poulsen, 1996
A. procera	25-30	Tanaka and Edwards, 1986
Picea abies	30	Bergsten, 1987; 1989; 1991
P. glauca	30	Downie et al., 1993
P. glauca	22-30	Edwards, 1986
P. mariana	30	Downie et al., 1993
P. sitchensis	24-27	Poulsen, 1996
P. sitchensis	31	Jones and Gosling, 1994
P. sitchensis	27-30	Jones et al., 1991
P. sitchensis	25-30	Gosling and Rigg, 1990
P. sitchensis	25	Edwards, 1986
Pinus banksiana	30	Downie et al., 1993
P. contorta	35	Jones and Gosling, 1994
P. contorta	25	Edwards, 1986
P. strobus	30	Downie and Bergsten, 1991
P. sylvestris	30	Bergsten, 1987; 1989; 1991
Pseudotsuga menziesii	35-37	Derkx, 1996a
P. menziesii	32-34	Muller, 1996 (pers. comm.)
P. menziesii	35-36	Poulsen, 1996
P. menziesii	36-37	Jones and Gosling, 1994
P. menziesii	35	Edwards, 1986
Acer palmatum (fruit)	36-40	Derkx, 1996 (pers. comm.)
A. platanoides (fruit)	36-38	Derkx, 1996a
A. platanoides (fruit)	35-40	Suszka et al., 1994
A. pseudoplatanus (fruit)	44-50	Knudsen, 1996
A. pseudoplatanus (fruit)	48	Derkx, 1996a
A. pseudoplatanus (fruit)	44-50	Suszka et al., 1994
Berberis thunbergii	40	Derkx, 1996b
Fagus sylvatica (before storage)	30-32	Muller, 1996 (pers. comm.)
F. sylvatica (after storage)	32-34	Muller, 1996 (pers. comm.)
F. sylvatica	30	Derkx and Joustra, 1996
F. sylvatica	30-33	Knudsen, 1996
F. sylvatica	30-32	Suszka et al., 1994
F. sylvatica (before storage)	28	Suszka, 1975
Fraxinus excelsior (fruit)	55-60	Muller, 1996 (pers. comm.)
F. excelsior (fruit)	45	Derkx, 1996a
F. excelsior (fruit)	55-60	Suszka et al., 1994
Prunus avium (incl. stone)	27-29	Muller, 1996 (pers. comm.)
P. avium (incl. stone)	26-28	Knudsen, 1996
P. avium (incl. stone)	28-30	Suszka et al., 1994
Syringa vulgaris	40-45	Derkx, 1996 (pers. comm.)
Tilia cordata	40	Derkx, 1996 (pers. comm.)

In order to be able to provide growers with pretreated, nondormant seed it is important to be able to redry and store prechilled seeds. Shipment of pretreated seeds over great distances is largely dependent on this ability, since hydrated seeds are very sensitive to conditions during transport.

In a number of species, mainly conifers, drying of pretreated seeds can be done without damaging the seeds. In other species drying has been shown to reduce speed of germination or even partly reinduce dormancy in pretreated seeds. Some species seem to have lost their desiccation tolerance and storage ability after a prechill and will not survive drying. The loss of tolerance to drying could be caused at least partly by the pretreatment conditions. A prolonged treatment and especially too high MC during prechilling will potentially allow the seed to initiate the early phase of radicle protrusion, which is often associated with a loss of desiccation tolerance and storage ability. Indeed, prechilling at slightly reduced CMC values has in some species been found to conserve the desiccation tolerance and make drying possible (Muller, pers. comm.). At the moment practical experience on the optimal duration of a CMC prechill or the use of a reference sample CMC prechill test are the only methods to assess the optimal treatment duration in a seed lot. A quick, easy and nonexpensive biochemical or immunological test for assessing the level of dormancy in a seed lot or its potential for fast and complete germination would be of great advantage in future optimization of prechilling. Even if significant progress in prechilling of tree seeds has been made many important questions still remain to be answered in future research.

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