



Guide to handling of tropical and subtropical forest seed

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Publication date:
2000

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Schmidt, L. H. (2000). Guide to handling of tropical and subtropical forest seed. Danida Forest Seed Centre.

DORMANCY AND PRETREATMENT

Contents	9.1 Introduction	1
	9.2 Ecological Significance of Dormancy	3
	9.3 Terminology and Classification of Dormancy	5
	9.4. Physiology of Seed Dormancy	7
	9.4.1 Location of dormancy in fruit and seed	7
	9.4.2 Variation in dormancy within species	8
	9.4.3 The legume seed	10
	9.5.1 Underdeveloped embryo	13
	9.5 Dormancy Types and Pretreatment Methods	13
	9.5.2 Mechanical dormancy	14
	9.5.3 Physical dormancy	16
	9.5.4 Chemical inhibitors	26
	9.5.5 Photo-dormancy	28
	9.5.6 Thermo - dormancy	30
	9.5.7 Combined dormancy	32
	9.5.8 Soaking in water	33
	9.5.9 Chemical or hormonal pretreatment	33
	9.6 Priming and Fluid Drilling	35
	9.7 Seed-coating and Pelleting	36
	REFERENCES	38

Other Chapters of the book Guide to Handling of Tropical and Sub-Tropical Forest Seed by Lars Schmidt soon available on www.dfsc.dk

- Chapter 1: Introduction
- Chapter 2: Seed Biology, Development and Ecology
- Chapter 3: Planning and Preparation of Seed Collections
- Chapter 4: Seed Collection
- Chapter 5: Fruit and Seed Handling between Collection and Processing
- Chapter 6: Seed Processing
- Chapter 7: Phytosanitary Problems and Seed Treatment
- Chapter 8: Seed Storage
- Chapter 10: Germination and Seedling Establishment
- Chapter 11: Seed Testing
- Chapter 12: Genetic Implications of Seed Handling
- Chapter 13: Microsymbiont Management
- Chapter 14: Seed Documentation
- Chapter 15: Trade and Transfer of Forest Seed

DORMANCY AND PRETREATMENT

9.1 Introduction

Seed dormancy refers to a state in which viable seeds fail to germinate when provided with conditions normally favourable to germination i.e. adequate moisture, appropriate temperature regime, a normal atmosphere and in some cases light. Dormancy has evolved as a strategy to avoid germination under conditions where seedling survival is likely to be low.

There are various degrees of dormancy varying from very slight to very strong (deep). Sometimes the development or degree of dormancy changes during the lifetime of the seed, usually as a response to external conditions. Hence, dormancy may be innate, develop, be broken and redevelop in seed.

Several types of dormancy exist, and sometimes more than one type of dormancy occurs in the same seed. In nature, dormancy is broken gradually or by a particular environmental event. The type of event that may break dormancy depends on dormancy type. Dormancy caused by a hard seed-coat may be overcome by a gradual or an instant abrasion, and darkness-induced dormancy by exposure to light. In seed handling the natural dormancy-breaking mechanism is applied or simulated during the process of pretreatment¹. In this chapter pretreatment is described in relation to different types of dormancy.

Dormancy in seeds may be advantageous or problematic during seed handling. The advantage is that it prevents seeds from germinating during storage and other handling procedures, and induction of dormancy, for example by drying and dark storage generally promotes storability. Indeed, seeds with no dormancy like recalcitrant seeds of rain forest species are very difficult to handle, e.g. because germination may begin already during transport and temporary storage. On the other hand, where dormancy is complex and seeds need a very specific pretreatment, failure to overcome these problems may result in very poor germination. A low germination rate of seeds which have proven to be sound and viable, in e.g. cutting or TTZ test (chapter 11), can often be ascribed to some type of dormancy. Seeds which have not been given an appropriate pretreatment to overcome dormancy may fail to germinate altogether, germination may be slow or germination of individual seeds in a seed lot may take place over a lengthy period.

¹ 'Pretreatment' is a term used for conditions or processes applied to break dormancy prior to germination, while 'treatment' is used for application of pesticides for control of pest and diseases.

The purpose of pretreatment is to ensure both that seeds will germinate, and that germination is fast and uniform. Pretreatment methods have been developed and described for many species. Yet, dormancy still causes problems of low germination rates for several tropical species, partly because of lack of general knowledge of their seed physiology, partly because of variation in dormancy rate. Pretreatment methods often have to be adjusted to individual species and seed lots based on experience and experiments. Knowledge of the biology and physiology of various types of dormancy and the occurrence in relation to regeneration biology may often suggest the nature of particular seed problems and possible pretreatment methods. Further, taxonomically related species often share similar types of dormancy, for example physical dormancy in legumes and thermo-dormancy in pines. Here the main variable is strength of dormancy, and elaboration of pretreatment methods can often be limited to increasing or shortening the duration of already known methods, rather than adopting new ones.

Pretreatment is normally undertaken shortly before sowing, i.e. after storage, both because dormancy normally promotes storability, because some types of dormancy may redevelop if pretreatment is undertaken earlier, and because pretreatment and germination conditions are often interlinked. In some cases dormancy is overcome by providing appropriate germination conditions, rather than a special pretreatment. An example of the latter is the light and fluctuating temperature conditions required to break physiological dormancy in some seeds. These conditions are conveniently provided during germination, although the germination process might take place under a wider range of conditions once dormancy has been broken (which actually proves that the phenomenon is dormancy). However, for most types of dormancy, conditions to overcome dormancy are quite different from those promoting germination.

Like any other seed handling operation pretreatment is subject to economic considerations. Where dormancy is weak, the gain of pretreatment may be marginal. Where several alternative methods exist, the technically most effective may not always be the most economical one.

Some pretreatment procedures are not directly related to seed dormancy, but are carried out in order to speed up the germination process or promote seedling establishment. Various hormones and nitrogenous compounds may help in breaking dormancy under certain conditions, and may simultaneously have a direct impact on germination. In priming, seeds are treated in a way to initiate germination without the process being carried as far as radicle protrusion. In pelleting, seeds are enclosed in a matrix to which may be added fertiliser, fungicide or microsymbiont inoculants. Both priming and pelleting are mainly used in connection with direct sowing (section 10.7).

9.2 Ecological Significance of Dormancy

Dormancy has evolved as a strategy to cope with situations in which seeds are likely to be exposed to conditions favourable to germination but where conditions for seedling survival and establishment may be poor or erratic. The type and degree of dormancy are largely reflections of these ecological conditions. Some examples will illustrate the connection of dormancy with environment.

1. Seeds of most temperate species, e.g. *Fagus*, *Quercus* and *Pinus*, mature during early or late autumn. Soil conditions (moisture and temperature) are generally favourable to germination at the time of shedding, but germination might be fatal for the young seedlings because of subsequent low winter temperatures. Seed dormancy, which is here overcome by a prolonged exposure to low temperature, prevents the seeds from germinating until the spring when the chances of survival of the offspring is much better.
2. Moist tropical rain forest floors provide favourable conditions for germination of most species. However, light demanding pioneers are not able to survive the shaded conditions under the canopy. Their regeneration is favoured by the formation of gaps in the canopy. Light and fluctuating diurnal temperatures are the two factors associated with gap formation, which break dormancy and trigger germination of pioneer seeds.
3. Seeds that happen to be buried under a thick layer of soil may be unable to reach the soil surface during germination. Such seeds may remain alive and dormant, and only germinate if they are uncovered. Light and temperature fluctuations are also in this case stimuli likely to trigger germination of the dormant seeds.
4. In dry areas, erratic light showers may be sufficient to cause seeds to imbibe and germinate, but not to provide adequate moisture for the seedlings to establish. By producing seeds with different degrees of dormancy, or dormancy which is gradually broken by environmental factors, e.g. gradually abrasion of hard seed-coats, the species saves part of the seed pool, so that some of the seeds are likely to germinate when conditions are favourable for seedling establishment (Mayer and Poljakoff-Mayber 1982).
5. In fire-prone areas seedling establishment is greatly improved after fire. Accordingly, many tree species from such areas e.g. some legumes, pines, eucalypts and *Banksia*, have developed dormancy which is broken only by exposure to high temperature.

Mangroves are the most striking example of an environment and a group of species in which dormancy is absent. In mangrove species, seed germination is often a more or less continuous process of seed maturation. Climax species of the humid tropical forests rarely have

post-dispersal seed dormancy; their seeds are adapted to rapid germination on the forest floor, where they often survive for long periods as dormant or suppressed seedlings, while awaiting improved light conditions for growth. Seed dormancy in climax forest species is usually related to dispersal.

Several types of dormancy relate to dispersal. For most species germination must be delayed until the seeds have reached a safe site, i.e. after dispersal. In fleshy fruits like berries and drupes, a juicy substance with high water content surrounds seeds, which is actually sufficient for imbibition. However, sugar content and frequently the presence of chemical inhibitors in these fruits prevent germination. As the fruit pulp is eaten by animals, decomposed by bacteria and fungi, or washed away by rain, the pulp and inhibitors are removed. A hard seed-coat (e.g. legumes) or endocarp (drupes) usually protects fruits or seeds adapted to dispersal by ingestion (section 2.6.2). These coverings also often restrict water uptake and thus imbibition, but ingestion usually partly abrades the seed-coats (Halevy 1974, Winer 1983). Seeds that are dispersed by a variety of animals, e.g. hard seeded acacias, tend to have very strong seed-coat dormancy, but also large variation in this type of dormancy (see section 2.6.2).

In some instances seeds only develop dormancy if exposed to unfavourable germination conditions. This phenomenon, known as secondary or induced dormancy, occurs for example in many species of Leguminosae where seeds germinate readily when young but develop strong seed-coat (physical) dormancy upon drying. Also light sensitivity often only develops in seeds that are exposed to darkness for a prolonged period (see discussion of innate and induced dormancy section 9.4).

Dormancy may be broken by an instant event like the above mentioned gap formation, ingestion or fire. In other cases dormancy is broken gradually by the influence of external factors, e.g. sand abrading hard seed-coats (Brown 1987), leaching of inhibitors by rainwater (Villiers 1972, Brown 1972), or natural decay of fleshy fruit substance (Mayer and Poljakoff-Mayber 1982).

Length of dormancy period varies with type and degree of dormancy and with strength and frequency of dormancy-breaking events. Seeds of berries may typically overcome dormancy once deposited by the dispersal agent. Temperate species typically stay dormant over one winter period. Many pioneer seeds on the rain forest floor perish because of soil living micro-organisms and predators unless conditions for germination occur relatively quickly. However, seeds with a strong innate protection (e.g. some Leguminosae, pines and eucalypts) may build up large soil seed banks of several years' accumulated seed production in areas with slow deterioration (e.g. cold or dry hot areas), where predation is small, and where the frequency of dormancy-breaking events, such as fire or rainfall, are rare.

9.3 Terminology and Classification of Dormancy

Dormancy has been classified in various ways, and there is no universally adopted system. Harper (1977) classified dormancy according to development into three types viz. 1) **innate dormancy** in which dormancy is present when the seeds are ready for dispersal, 2) **induced dormancy** in which dormancy develops as a response to external environmental factors, and 3) **enforced dormancy** in which germination is constrained because of external conditions. The latter group does not, however, comply with the usual definition of dormancy (see introduction) since germination, according to that definition, does not take place despite favourable germination conditions, not because of unfavourable conditions. The term is therefore avoided in this book; seeds which do not germinate because of external conditions are more correctly referred to as '**quiescent**' (Villiers 1972). Innate and induced dormancy are also referred to as primary and secondary dormancy respectively.

Another classification system uses the location of dormancy in different seed parts as criteria. Any cause of dormancy related to the embryo e.g. immature development or chemical inhibitors located in the embryo may collectively be referred to as **endogenous** or **embryo dormancy**. Analogously, mechanical resistance, physical impermeability, inhibitors or light sensitivity associated with the seed-coat are called **exogenous**- or **seed-coat dormancy** (see section 9.4.1). 'Seed-coat' is here used in the wide sense of any enclosing structure including e.g. endocarp or the entire pericarp.

In this book a simplified classification and terminology is used in which six main types of dormancy are described in relation to their physiological nature and the method of pretreatment used to overcome them. This system basically follows the one used by Hartmann *et al.* (1997) which in turn is based on Nikolaeva (1977) and Crocker (1916). A few modifications have been made: thermo-dormancy here encompasses all types of temperature related dormancies, whether high, low or fluctuating. Photo-dormancy encompasses all light related dormancy phenomena.

Where two or more dormancy types occur in the same seeds it is called '**double dormancy**' or '**combined dormancy**'. Double or combined dormancy is for example found in fleshy fruits with chemical inhibitors combined with e.g. a hard endocarp (**physical dormancy**), or immature embryos combined with other dormancy types. A summary of dormancy types according to the classification used in this book is shown in table 9.1.

Table 9.1.
Classification and
characteristics of
seed dormancy.

Dormancy type	Characteristics	Examples of occurrence	Dormancy breaking stimulus	Seed handling
a. Immature embryo	Seeds are physiologically immature for germination	<i>Fraxinus excelsior</i> , <i>Ginkgo biloba</i>	Natural Post-dispersal development	After-ripening
b. Mechanical dormancy	Embryo development physi-cally restricted due to hard seed/fruit coat	<i>Pterocarpus</i> , some <i>Terminalia</i> spp., <i>Melia volkensii</i>	Gradual decomposition of hard structures, e.g. by termites	Mechanical cracking of restricting structure
c. Physical dormancy	Imbibition impeded because of impermeable seed-coat or fruit	Mainly hard seed Leguminosae, plus some Myrtaceae and others	Abrasion by sand, high tempera-tures, temperature fluctuations, ingestion by animals, or other mechanical or chemical impact	Mechanical scarification (e.g. abrasion or burning), boiling water or acid pretreatment
d. Chemical dormancy	Fruit and seed contain chemical inhibitory compounds that prevent germination	Fleshy fruit such as berries, drupes and pomes, plus some dry seeds	Ingestion by frugivores, leaching by rain, gradual decomposition of fruit pulp	Removal of fruit pulp plus leaching with water
c. Photo dormancy	Seeds fail to germinate unless exposed to appropriate light conditions/regime. Is operated by a biochemical phytocrome mechanism	Many temperate species, e.g. <i>Betula</i> Humid tropical pioneer species e.g. <i>Spathodea</i> and some eucalypts	Exposure to light conditions likely to promote seedling survival viz. white light or light relative rich in red light	Exposure to light, normally during germination, sometimes a distinct light-dark cycle of variable duration
f. Thermo dormancy	Germination low without pretreatment with appropriate temperatures	Most temperate species, e.g. <i>Fagus</i> , <i>Quercus</i> , <i>Pinus</i> Dry zone tropical - subtropical pio-neers e.g. <i>Hekeia</i> , <i>Pinus</i> , <i>Eucalyptus</i> , <i>Banksia</i> Humid tropical pioneers	Exposure to low winter tempera-ture Exposure to grass, bush or forest fires Diurnal fluctuating temperature in gaps	Stratification or chilling High temperature, e.g. kiln or light burning Fluctuating temperature

9.4. Physiology of Seed Dormancy

9.4.1 Location of dormancy in fruit and seed

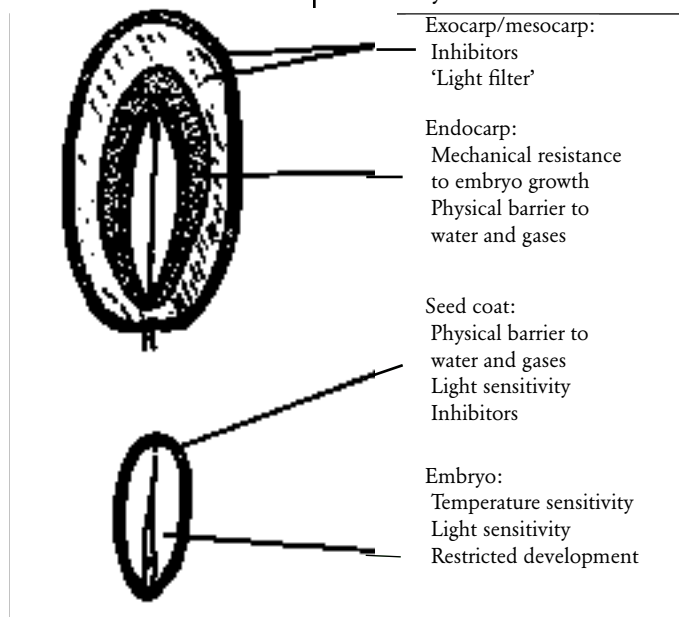
Any part of the fruit or seed, both live and dead tissue, may be involved in innate or induced dormancy. The location and type of dormancy may be revealed experimentally by removing or treating various parts of the fruit or seed separately. For example, if dormant seeds germinate after removal of their seed-coat, it may be concluded that the site of dormancy is the seed-coat. If excised embryos do not germinate when treated with extract from the fruit, it may indicate that the fruit contains inhibitory substances (Thapliyal and Naithani 1996).

The pericarp may impose dormancy in several ways. It may (1) form a mechanical barrier to the protrusion of the radicle or swelling of the embryo (mechanical dormancy), (2) be a physical barrier to water uptake and/or gaseous exchange (physical dormancy), (3) modify the light reaching the embryo (photo-dormancy), (4) contain inhibitory substances or (5) prevent escape of inhibitors from the embryo (Bewley and Black 1982, 1994, Ellis *et al.* 1985).

Several dormancy types can be associated with the seed-coat, e.g. mechanical resistance, physical barrier to moisture absorption or gaseous exchange, temperature or chemical inhibition, and light sensitivity. Where seeds remain enclosed inside a hard fruit enclosure like a samara or an endocarp, the seed-coat is usually thin and contributes little to dormancy. In many other seeds the seed-coat plays a major role in dormancy and often a more active one than the pericarp. Light sensitivity and moisture regulation are often associated with specialised structures in the seed-coat.

The endosperm rarely plays an important role in dormancy, although occasionally inhibitory substances in the endosperm impede germination. Several types of dormancy may be ascribed to conditions of the embryo itself; such types are (together with possible endosperm influence) collectively called endogenous dormancy as opposed to exogenous dormancy which is located in the outer coverings. Dormancy caused by immature or underdeveloped embryos is obviously a pure embryo character, and thermo-dormancy can probably also be restricted to the embryo itself. Although both chemical inhibitors and light ultimately are sensed by the embryo, both inhibitors and photo-dormancy are normally associated with the outer coverings, which can be verified by the observation that these dormancy types are normally overcome by removal of the pericarp or seed-coat.

Figure 9.1. Location of dormancy in various parts of the fruit and seed.



9.4.2 Variation in dormancy within species

Like other sectors of plant physiology, dormancy is influenced by developmental, genetic and environmental factors and interaction between the three. Within any one species, dormancy may vary from very shallow to very deep dormancy between different seed lots and between individual seeds in the lot. For example, young seeds of *Albizia gummifera* from the Kakamega provenance in Kenya germinate readily in the moist forest (fig. 9.2). Some degree of physical dormancy develops if the seeds dry out in the pods before they fall or are artificially dried after collection. Dry provenances of the same species naturally have more dormant seeds, with a dormancy that develops even more strongly upon artificial drying (pers. obs. 1987). For moist zone provenances, pretreatment to overcome dormancy may not only be redundant, but will most likely damage the seed; for dry zone provenances pretreatment may be a necessary precondition for rapid and uniform germination (see also section 9.5.3). Several additional examples are given below. Knowledge and awareness of the way in which individual factors influence and interact with dormancy are often important in handling dormant seed.

Developmental

Dormancy frequently changes during development. In the temperate species *Corylus avellana* dormancy is caused by chemical inhibitors. In freshly harvested seeds the inhibitors are located in the seed-coat (testa), and germination is stimulated by removal of the seed-coat. However, stored dry seeds are not stimulated by this pretreatment because the inhibitors have moved from the seed-coat into the embryo, thereby inducing embryo dormancy (Jarvis 1975 quoted in Richards and Beardsell 1987). The same phenomenon is observed after depulping of many fleshy fruits. Seeds depulped immediately after collection have a shallower dormancy than seeds where depulping is undertaken later (Schaefer 1989, see further section 9.5.4)

Since physical, and sometimes also mechanical, dormancy is related to seed moisture content, maturity stage and concurrent desiccation influence these types of dormancies in such a way that young seeds are likely to exhibit less dormancy than old ones (cf. example of *Albizia gummifera* above). Complete impermeability in Leguminosae develops around a moisture content of 12-14%. However, dormancy continues to develop at even lower moisture content. In *Leucaena leucocephala*, Duguma *et al.* (1988) investigated dormancy development in four classes of development, viz. green, light brown, dark brown (all fresh seeds) and dry seed. All freshly collected seeds germinated readily although germination percentage was low (70%) for green seeds. Moisture contents were 53% and 40% for light brown and dark brown seeds respectively. Dry seeds with a moisture content of 4% had pronounced physical dormancy (>80% impermeable).

Genetic

There is very little documentation of genetic variation in seed dormancy for tropical trees. Seeds of high altitude provenances of *Eucalyptus pauciflora* and *E. glaucescens* are reported to need longer stratification than



Figure 9.2.
Germination of seeds of *Albizia gummifera* from fresh pods.
The seeds do not dry out and hence do not develop physical dormancy under moist forest conditions; Kenya

seeds collected from seed sources of lowland provenances (Richards and Beardsell 1987). Rungu (1996) and Masamba (1994) investigated provenance variations of various African woody legumes; although some provenance variation occurred, variation was modest. In *Pinus monticola*, a large family variation was found as regards to required stratification period (Hoff 1987).

Environmental

Environmental factors are the cause of induced or secondary dormancy. Desiccation influence on the development and degree of physical dormancy has already been mentioned. Light or temperature sensitive seeds may typically change their degree of dormancy according to environment after dispersal. For example, in an experiment with seeds of the pioneer tree *Spathodea nilotica*, it was found that fresh seeds germinated readily and indiscriminately at any temperature within the range 15-25°C (both constant and fluctuating), and under both dark and light conditions. After 1½ month's storage, almost no germination took place under dark conditions, and germination rate at both constant 15°C and 20°C was low. Light greatly improved germination of those seeds, especially when combined with fluctuating temperature (pers. obs.). Another example of environmentally induced dormancy is thermo-dormancy² developing after exposure to high but non-lethal temperatures (Hartmann *et al.* 1997).

Most species of the family Leguminosae exhibit physical dormancy, which in this family is caused by advanced morphological structures of the seed-coat. Because of the importance of leguminous trees in tropical forestry and the widespread propagation constraints caused by this type of dormancy, the legume seed is described in detail in

² Note, Hartmann *et al.* restricted the use of the term 'thermo-dormancy' to this type of dormancy induced by high temperatures. In this book the term is used as any temperature related dormancy.

9.4.3 The legume seed

this section. Further discussion of physical dormancy in Leguminosae appears in section 9.5.3.

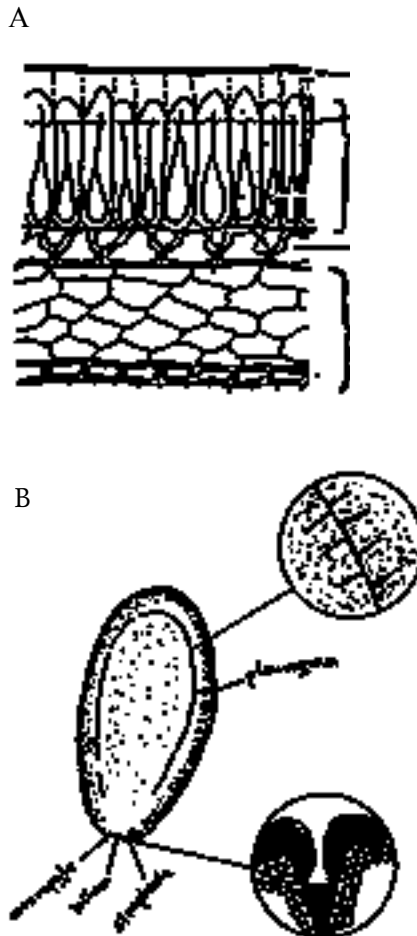
The impermeability of the legume seed-coat is relative in the sense that various species, various stages of maturity and various individuals within an apparently homogeneous seed lot exhibit different degrees of resistance to imbibition. The structure of the seed-coat is illustrated in fig. 9.3. It consists of four distinct layers: 1) the cuticle is the outermost layer which has a waxy and water-repellent character; 2) macrosclereids or palisade layer which consists of long, narrow, tightly packed, vertical cells; 3) osteosclereids which is a layer of more loosely packed cells; and 3) parenchyma layer which is made up of a layer of little differentiated cells. Impermeability can be ascribed to the two outer layers, since it has been shown that once these layers have been penetrated, the seed readily absorbs water. The thickness of the total seed-coat as well as the relative thickness of the individual layers vary with species. Several *Cassia* species have relatively thick cuticles, while the palisade layer is relatively thin. The relationship between morphological structure and pretreatment is, however, not well documented. For the seed-coat to become permeable, the palisade layer must be penetrated at least to the depth of the light line (fig. 9.3A).

Figure 9.3.

Legume seed.

A. Cross section of the legume seed-coat. Seeds become permeable when the cuticle and the outer part of the palisade cells are penetrated.

B. Entire seed with demarcation of 'weak sites', sites most likely to become permeable during pretreatment. Inserts: i) cracks along the pleurogram after hot water pretreatment; ii) cross section of the seed-coat in the strophiolar region.



The seed-coat structure is fairly uniform except for a few sites, with different cell arrangements. One site is the hilar region, which is the attachment site of the funicle and which contains the micropyle, hilum and strophiole (see fig. 2.12 and 9.3). The cells of this region have a corky structure and no cuticle. The seed-coat along the pleurogram, a horseshoe formed usually green line on e.g. *Acacia* and *Albizia* seeds is also slightly different (fig. 9.3B). The hilar region and to a smaller degree the pleurogram are relatively weak sites of the seed-coat and most likely to become permeable during pretreatment. Hot water pretreatment is believed to influence permeability especially of the strophiole (Dell 1980). However, any part of the seed-coat may be turned into the weaker site where water will ultimately penetrate (Werker 1980).



Figure 9.4. Increase in size during imbibition of *Acacia mellifera* seeds. Left: not imbibed, right: imbibed.

As the seed loses water during maturation, the palisade cells of the seed-coat become more tightly packed and the seed-coat more impermeable. It is generally observed that fresh legume seeds need no or less pretreatment than dry stored seeds. A large difference in physical dormancy between different species of legumes is encountered even within the same area. Generally, seeds dispersed by ingestion have a much harder seed-coat, and hence require a much more radical pretreatment, than wind-dispersed seeds. Physical dormancy also differs from one seed to another in a seed lot. Usually a few seeds in any seed lot will imbibe when submerged into water. Another part of the seed lot may remain impermeable even after one or more severe pretreatments.

Variation in physical dormancy between species, stages of maturity and individual seeds in a seed lot can be illustrated by a simple imbibition experiment. Imbibition is easily recognised in legumes since the seed swells 2-3 times its dry size in a couple of hours once dormancy is broken (fig. 9.4). In the two experiments illustrated in fig 9.5A and 9.5B), the effect of several repeated standard pretreatment was measured on different seed lots. In each experiment the seeds (5X100) were submerged in water day '0'. The number of imbibed seeds were counted and removed after 1 day; the remaining seeds were pre-treated with boiling water, left to cool and imbibe in the water, and the number of imbibed seeds was counted and removed after 24 hours. This pretreatment and imbibition procedure was repeated for 14 days. The cumulated average daily imbibition rate was plotted against time. The shape of the curve is a measure of seed-coat hardness.

Figure 9.5.

Relative seed-coat hardness expressed by cumulative imbibition after soaking in cold water day '0', and subsequent pretreatment with boiling water and soaking day 1-13. A. Three maturity stages of *Acacia mellifera*. B. Seven *Acacia* spp. from East Kenya (seeds sun-dried to 6-8% m.c.) (Schmidt 1988).

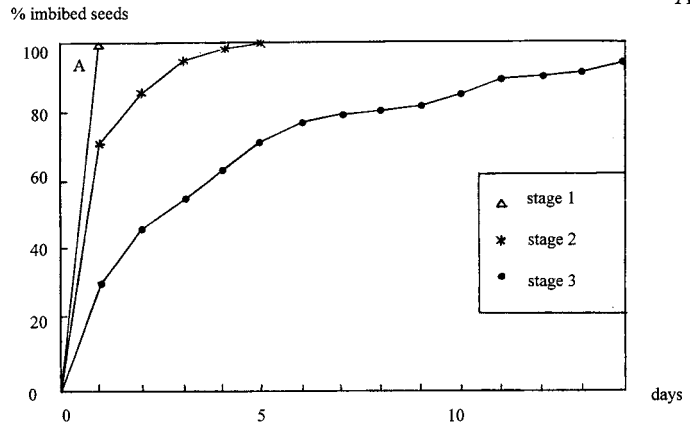
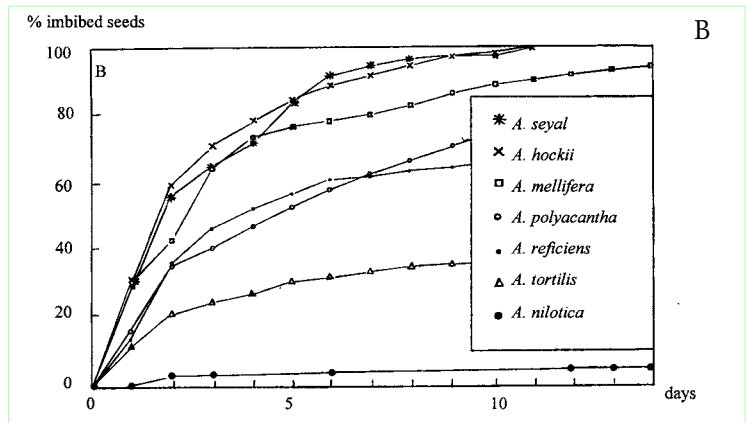


Fig. 9.5A and B show that in seed lots with relatively weak dormancy (e.g. young seeds or mature seeds of *Acacia seyal* and *A. hockii*) many seeds imbibe readily in cold water or after only one or few treatments. In species with strong dormancy, there is little imbibition even after several pretreatments with boiling water. It should be noticed that *A. seyal*, *A. hockii*, *A. mellifera*, *A. polyacantha* and *A. reficiens* are all wind dispersed, while *A. nilotica* and *A. tortilis* are dispersed mainly by ingestion by large herbivores.



Individual seed lot variation can be illustrated by the fact that 15% of *Acacia reficiens* seeds imbibed readily in cold water, while about 35% remained dormant even after 13 pretreatments with boiling water.

It should be noticed that imbibition is a purely physical process and therefore independent of whether the seed is alive or dead. It is therefore a more direct measure of physical dormancy than germination, but on the other hand not an expression of viability. Seeds with heat sensitive embryos may be killed by boiling water treatment and yet imbibe perfectly, apparently indicating that dormancy has been overcome.

Bebawi and Mohamed (1985) used average imbibition after soaking in cold water for 3, 6, 12, 24, 48 and 192 hours as a measure of physical dormancy. *Acacia nilotica*, *A. albida* (now *Faidherbia albida*) and *A. nubica* (all ingestively dispersed species) showed also here a very strong dormancy, while the two wind-dispersed species *A. spirocarpa* and *A. ebrenbergiana* had the weakest dormancy; *A. seyal* in this experiment was intermediate.

9.5 Dormancy Types and Pretreatment Methods

Pretreatment is a 'pre-sowing-treatment' carried out in order to enhance rapid and uniform germination of seed sown in the nursery, field or for testing. In some cases pretreatment is a mere acceleration of the natural processes of dormancy release, in others it is a simulation of these processes. Dormancy is often an advantage during storage because it prevents germination under storage conditions, and in some species and under some conditions dormant seeds store better than non-dormant. Therefore, pretreatment is often deliberately delayed until after storage, i.e. immediately before sowing. In some instances processing acts as a pretreatment, e.g. leaching of inhibitors during depulping, abrasion of seed-coats during tumbling, after-ripening of seeds with immature embryos, or when mechanically dormant seeds are extracted from their fruits before storage e.g. to reduce bulk. Hot water treatment against insect or seed-borne pathogens may also occasionally serve as a pretreatment.

Where pretreatment can be undertaken without interfering with storability, it is sometimes conveniently done before storage because seeds can then be taken directly from storage to nursery. This is typically the case with certain pretreatment procedures for mechanical and physical dormancy. Generally, however, any prolonged wet treatment should be avoided before storage because it implies a risk of imbibition and germination. In some instances storage in itself acts as a pretreatment, viz. where dormancy is broken by prolonged exposure to low temperature (chilling), typical of many temperate and high altitude species. On the other hand, storage may also induce dormancy, e.g. photo-dormancy in light sensitive seeds, and pretreatment is here obviously not applicable before storage.

It should be noted that any dormancy type involving physiological processes within the seed must be broken under moist conditions, which means that in practice only physical and mechanical dormancy can be overcome when seeds are dry. In some cases there is no practical distinction between pretreatment and germination conditions. Light and fluctuating temperature demand are strictly speaking dormancy phenomena, but in practice such dormancy is overcome by providing germination conditions suitable to overcome dormancy, rather than giving a special pretreatment.

Usually, particular pretreatment methods are designed to overcome particular dormancy problems. Some methods may, however, be effective for more than one type. Cold moist stratification may thus be effective on thermo-dormancy as well as on softening of the seed-coat, and both physical and chemical dormancy may be overcome by soaking.

In many species specific knowledge of seed dormancy is scarce. However, adoption of methods known to work for related species, or duplication or simulation of natural conditions believed to influence dormancy are often effective (Hartmann *et al.* 1997).

9.5.1 Underdeveloped embryo

Seeds with underdeveloped embryos at the time of dispersal are unable to germinate under normal germination conditions and thus comply with the term 'dormancy'. The phenomenon is sometimes classified

9.5.2 Mechanical dormancy

morphological dormancy, referring to the immature morphological stage of the embryo. This should not be confused with the condition of immature embryos in early collected seeds, although the distinction is not always clear and pretreatment method is similar. Seeds with immature embryos occur in e.g. *Arecaceae* (palms), *Ginkgo biloba* and several *Fraxinus* species. The stage of embryo development at the time of dispersal differs between species that show this type of dormancy. In *Ginkgo biloba* even fertilization may take place after dispersal; in *Ilex opaca* and some palms the embryo consists of a core of undifferentiated cells, while in *Fraxinus* the embryo is fully differentiated but small. *Pinus* spp. from northern latitudes and high elevations are also reported to have morphological dormancy (Bonner *et al.* 1994).

For germination to proceed the embryo must grow to full size, which is promoted by a period of warm moist treatment; it is in practice an after-ripening similar to that used for early collected seeds (cf. section 6.3). Dormancy caused by immature embryos is often combined with other dormancy types, e.g. thermo-dormancy in *Fraxinus* spp.

Mechanical dormancy refers to the condition in which the embryo development is physically restricted due to a hard enclosing structure. Imbibition may take place but the radicle is unable to split or penetrate its enclosure, which is often the fruit or part of the fruit. The term mechanical dormancy does not include impermeability to water and gases, which forms a special dormancy type, 'physical dormancy' (see below), but in practice most mechanically dormant seeds have some restriction to water uptake. Where impermeability is complete, it is correctly referred to as combined mechanical and physical dormancy. Mechanical dormancy here typically restricts germination after physical dormancy has been overcome. It should be emphasized that the connection is more rare the other way around: only a minority of physically dormant seed also exhibits mechanical dormancy.

Mechanical dormancy is quite common in several tropical and subtropical genera, e.g. *Pterocarpus* (*P. indicus*, *P. angolensis* and others), *Terminalia* (*T. brownii*, *T. mollis*, *T. tomentosa*, *T. superba*) and *Melia* (*Melia volkensii*). Mechanical dormancy has also been suggested in seeds of *Eucalyptus delegatensis* and *E. pauciflora* (Bachelard 1967, quoted in Turnbull and Doran 1987). Some mechanical resistance to embryo enlargement is probably present in most seed, but the resistance must have a certain strength and thus a delaying effect on germination to qualify for the term 'dormancy'.

Mechanical restriction to embryo development may be overcome in one of two ways: 1) by gradual softening of the enclosing seed-coat or pericarp to allow embryo expansion, or 2) by extracting the seeds from a mechanically restricting pericarp.

Softening of the pericarp or seed-coat occurs during any moist pretreatment, and seeds pretreated by moist stratification to overcome e.g. thermo-dormancy (*Eucalyptus delegatensis*, *E. pauciflora*), usually also overcome possible mechanical dormancy (Boland *et al.* 1980).

Duration of stratification depends on temperature, species and degree of dormancy, but typically ranges between three and five weeks. Fruits of *Pterocarpus* spp. are often abraded during the normal processing procedure (chapter 6), and softening of the fruit by stratification is hence shorter than for intact fruits. There are several problems and limitations to overcoming mechanical dormancy by stratification:

1. The procedure is generally slow, and duration of stratification period cannot be predicted
2. It can only be undertaken shortly before sowing
3. Seeds will germinate once dormancy is broken, and because of variation in dormancy, germination will not be uniform.

Pretreatment with acid or hot water, agents effectively used for physically dormant seed (see below), is generally not applicable to species with purely mechanically dormant seed. Since the seed-coat of such seed is permeable to the liquid, the embryo is prone to be damaged by any toxic agent. However, Khasa (1992) found that pretreatment of *Terminalia superba* seeds with concentrated sulphuric acid (95-98% V/V) for 15-60 min greatly improved germination, while any exposure to boiling water killed the seeds. It was suggested that the acid, having a higher viscosity than water, did not penetrate slits in the pericarp and hence did not come into physical contact with the embryo. Sodium hypochlorite (5.25% V/V) also greatly improved germination of this species, presumably because it penetrates into the slits of the pericarp and softens it from inside, yet is harmless to the embryo. Acid pretreatment is frequently used where mechanical dormancy is combined with an impermeable seed-coat (double dormancy). For example, it has been used successfully to improve germination of *Pterocarpus angolensis* (Groome *et al.* 1957, quoted in Willan 1985), and *Terminalia bellirica* (Bhardwaj and Chakraborty 1994). In the latter case both total germination and germination speed were greatly improved by an optimal 12 minutes' soaking in concentrated sulphuric acid as compared to the control.

Acid pretreatment may in some instances be applied prior to (warm) moist stratification. The seeds are initially treated with acid which scarifies the coat, but the treatment is stopped well before acid has penetrated the pericarp or seed-coat. After careful washing, the seeds are exposed to moist stratification until dormancy has been overcome. This procedure reduces the time required for moist stratification and reduces the risk of damage by prolonged acid treatment (Gordon and Rowe 1982).

Extraction of seeds from hard fruits is sometimes undertaken before storage; a discussion of aspects in relation to timing of extraction appears in section 6.4.1. The advantage of extraction as compared to stratification is that the seeds germinate quickly and uniformly, and sowing is easier. Because the seeds do not germinate during the procedure, sowing may also be undertaken at any convenient time, limited though by shortened longevity, especially where seeds are likely to be damaged during extraction. The two main problems and limitations are: 1) that it may be very labour intensive as there is no effective mechanical method of extraction, and 2) that seeds are highly exposed to damage during the process.

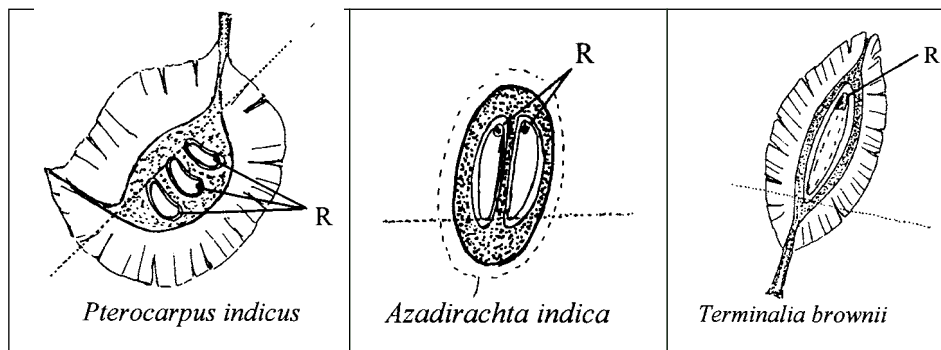


Figure 9.6. Orientation of the embryo in some fruits with hard pericarp enclosure. 'R' indicate radicle and dotted line the cutting line for extraction or mechanical pretreatment.

Because seed protection is performed entirely by the hard fruit cover, hard pericarps are always associated with fragile seeds with very thin seed-coats. Although inevitably some seeds will be damaged during extraction, the damage can be limited by initially familiarizing oneself with the seed to identify the location of the radicle. This is the most sensitive part of the embryo and should be avoided or approached with great care. The orientation of the embryo in relation to the outer morphology of some fruits is illustrated in fig. 9.6. Where fruits have been partly processed, e.g. by depulping or dewinging, it can be very difficult to locate the embryo end. For example, fruits of *Pterocarpus* spp., which are modified pods, always have a pointed apex and the pedicel at one side of the samara, which is the side to which the radicles of the seeds point. These points of orientation are obviously lost where fruits have been dewinged and there is an increased risk of damaging the seeds if they are manually pretreated or extracted. A common way of extracting seeds from these fruits is to clip approx. 0.5 centimetre inside the wing around the samara with secateurs; inserting a strong knife in the suture and twisting carefully can then separate the two halves. Cracking hard seed and fruit coats with a hammer will almost inevitably cause damage and should be avoided.

In *Melia volkensii* the seeds are enclosed in a stony endocarp from which they must be extracted. Milimo (1986, quoted in Kamondo and Kalanganire 1996) recommended the following procedure:

1. Place the dry stony endocarp horizontally on a large cut tree stump.
2. Place a sharp pocket knife midway across the endocarp and gently apply several hammer blows until the crack develops. The knife blade must not penetrate deeper than the stony endocarp wall, otherwise the seeds will be damaged.
3. Carefully penetrate and open the crack with the sharp edge of the knife blade by pushing and twisting. The aim is to separate the two stony endocarp halves.
4. Pull out the seeds from their locules in the stony endocarp.

9.5.3

Physical dormancy

Physical dormancy is caused by a hard and impermeable seed-coat or fruit enclosure which prevents imbibition and sometimes also gaseous exchange. The phenomenon is often referred to as 'hard seed', although this term is usually reserved for impermeable seeds of Leguminosae.

In addition to Leguminosae, physical seed-coat dormancy also occurs in some members of the families Myrtaceae (*Eucalyptus* and *Melaleuca*), Cupressaceae (*Juniperus procera*) and Pinaceae (*Pinus* spp.). Physical dormancy caused by the pericarp or part of the pericarp occurs in Rhamnaceae (*Ziziphus* spp.), Verbenaceae (*Tectona grandis*), Combretaceae (*Terminalia* spp.), Santalaceae (*Santalum* spp.), Ulmaceae (*Trema* spp.) and several others. Because most legume trees exhibit some degree of physical dormancy, this dormancy type is by far the most common in tropical environments, particularly in arid zones. Physical dormancy because of fruit structures, e.g. impermeable endocarps, are functionally analogous, and the same range of pretreatment methods applies. However, because of the anatomical differences between seed-coat and pericarp, the character of pretreatment often differs.

A wide range of methods have been developed to overcome this type of dormancy. All methods are derivations of the same principle: to pierce the seed-coat to an extent that will render it permeable to water so that imbibition can take place. Unless physical dormancy is combined with mechanical dormancy, penetration at one point is sufficient to ensure permeability. Because the impermeability in legumes is exerted by the outer layer of the coat and the palisade cells absorb water, a relatively superficial treatment may overcome dormancy in these seeds. A more homogeneous structure of the seed cover and a deeper impermeable layer of e.g. some endocarps may require a more drastic scarification.

Since the degree of physical dormancy differs between species, stage of maturity and degree of desiccation (section 9.4), pretreatment must be adjusted accordingly. As the individual seeds in a seed lot vary in dormancy, adjustment of pretreatment can be difficult. Manual pretreatment of individual seeds e.g. by abrasion or burning is quite efficient in overcoming dormancy without damaging the seeds but is labour intensive. Bulk pretreatment faces the problem that, when aiming at overcoming dormancy in the most resistant individuals in the seed lot, the seeds with relatively thin seed-coats may be damaged by the pretreatment. We call this type of damage 'over-treatment'. Where dormancy is related to genotype, an average treatment may have genetic implications cf. chapter 12. Examination of initial imbibition must be done before treatment of thin coated or freshly harvested seed. Such seed may need no pretreatment at all, and any applied treatment could be lethal.

In *Trichilia emetica*, a non-legume with physical dormancy, it has been found that the aril has a strong influence on dormancy. Removal of the aril was sufficient to break dormancy in the majority of seeds, while the remaining seeds needed an additional scarification (Masanga and Maghembe 1993). The importance of the aril in imposing physical dormancy is also known in e.g. *Azelia xylocarpa* and *Sindora siamensis* (Pukittayacamee 1990).

Mechanical scarification

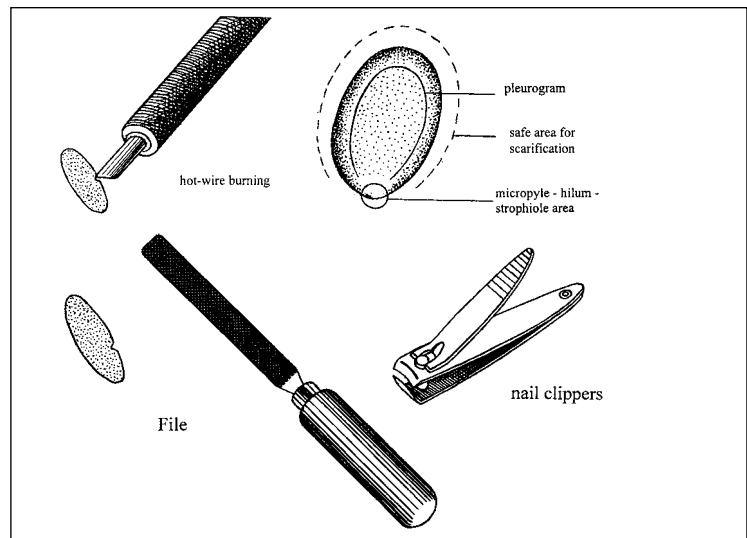
Manual scarification of the seed-coat by piercing, nicking, chipping, filing or burning with the aid of a knife, needle, file, hot wire burner,

abrasion paper or the like is usually considered the most effective way of overcoming physical dormancy. Since each seed is handled manually, it can be given individual treatment according to the thickness of the seed-coat. It is often used as a reference method to which the effectiveness of other methods is compared. Virtually all seed can be made permeable, and the risk of over-treatment (damage) is small, provided that the radicle region is avoided.

Any site of the seed-coat can be turned into a weak site where imbibition will start (cf. section 9.4.2). In legume seeds, the cells of the palisade layer of the seed-coat take up water, and the softening process spreads from the initial site of imbibition into the whole seed-coat within few hours when submerged in water. Simultaneously the embryo imbibes (fig. 9.4). The abrasion should penetrate at least through the cuticle and half way through the palisade layer (fig. 9.3). Manual scarification is effective at any site of the seed-coat, but the micropylar region should be avoided as it is the most sensitive site of the seed where the radicle is located (cf. discussion of mechanically dormant seed). Accidental damage to this region may damage the seed, while minor damage to the cotyledons is unlikely to affect germination (Cremer 1990). In a comparative study of hot wire scarification and conventional pretreatment methods of 10 hard seeded species, hot wire scarification was shown to be the most effective for all species (Sandiford 1988).

Figure 9.7.

Simple instruments for mechanical scarification of hard seeded legumes. Note that any point of the larger part of the seed surface may be scarified, while the micropylar region must be avoided.

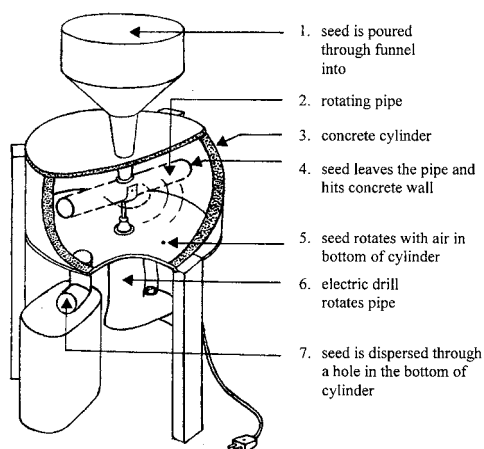


The main problem with manual scarification is its labour intensiveness. However, with the manual thread burner one person may pretreat more than 100 seeds per minute (Sandiford 1988), which at least for smaller seed lots may easily compete with bulk methods. Small seeds such as Australian acacias can be stuck to sticky tape to hold them still during hot wire pretreatment. It is, however, difficult for very small seeds.

Bulk scarification may be carried out by tumbling the seed in a cement mixer together with sand, gravel or any other sharp abrading material. Smaller seed lots may be scarified by gently stirring them in a mortar

with sharp sand. Abrading material should obviously have a size that makes it easy to separate it from the seed again. Duration of treatment depends on seed type and should be determined by experience. In fast imbibing seeds such as Leguminosae the effectivity in overcoming physical dormancy can easily be determined in an imbibition test: if the majority of a sample of seeds imbibe within a couple of hours, the pretreatment is sufficient; if only few imbibe, prolonged pretreatment is necessary. Mechanical bulk scarification may also be carried out with a so-called seed gun, the technical details of which are described in fig. 9.8. During operation the seeds are filled into a central funnel with an outlet in a fast rotating horizontal pipe. The seeds are slung against the wall of the enclosing concrete pipe by which treatment their coats crack (Poulsen and Stubsgaard 1995). The device has proven efficient for a number of species, but the number of damaged seed can be fairly high.

Figure 9.8. Seed gun for bulk mechanical scarification of hard seeds. The speed of the revolving central pipe can be regulated and determines the centrifugal force and hence the treatment. For very hard seeds, the speed is increased; for relatively soft-coated seeds the speed is decreased. Poulsen and Stubsgaard (1995).



Hot water

Hot water overcomes physical dormancy in Leguminosae by creating tension which consequently causes cracking of the macrosclerid layer (Brant *et al.* 1971), or by affecting the strophilar plug (Dell 1980). The method is most effective when seeds are submerged into the hot water, not heated together with the water. A quick dip is also better to avoid heat damage to the embryo. Most thick-coated *Acacia* species tolerate a brief (e.g. <1 min.) submersion into boiling water. Longer exposure has rarely any additional effect; the quick temperature change rather than the high temperature cause tension; during longer exposure, heat may be transmitted into the embryo, which may then be damaged. However, for several hard coated Australian species 2 minutes' boiling was found superior to 1 minute, and some species are pretreated by boiling for up to 5 minutes (ATSC 1995). A common procedure is to pour the seeds into boiling water and then leave them to cool and imbibe in the water for 12-24 hours. The temperature of the water decreases quickly enough not to cause damage to most species with relatively thick seed-coats and resistant embryos.

However, high temperatures may damage seeds with relatively thin seed-coats. In an experiment on *Cassia siamea* in Thailand, 1-2 min. soaking in 85°C warm water, or submersion at 85°C with subsequent cooling in the water for 12-36 hours gave a germination percentage of 82-89. Longer soaking at 85°C slightly decreased germination percentage. Soaking from 1 to 3 min. in water at 95°C caused rapid reduction of viability; it was 71% after 1 min, 47% after 2 min. and 40% after 3 minutes. Hence, for this species a brief exposure to high temperature or prolonged exposure to 85°C apparently caused heat damage (Kobmoo and Hellum 1984).

Heat damage was also observed for *Cassia sieberiana* (Todd-Bockarie *et al.* 1993) although the seeds of this species still maintained high viability (75%) after 2 minutes' boiling; longer boiling and any dry heat treatment rapidly reduced viability. In *Cassia fistula* a quick dip in boiling water killed 50% of the seeds, and 68% were killed after 5 minutes' boiling (Babeley and Kandya 1988). Boiling water was lethal to 5 out of 20 tested species in Ethiopia viz. *Acacia seyal*, *A. tortilis*, *A. senegal*, *Cassia decapetala*, and *C. spinosa* (Teketay 1996b); all other species showed somewhat improved germination after a brief submersion in boiling water. However, for *Entada abyssinica* germination after 15 seconds of boiling was only 1/3 of that after 5 seconds, and longer exposure was completely lethal. It should be noted that heat damage to *Acacia tortilis* seeds already at 5 seconds as observed in the referred experiment is unusual; brief boiling is a common pretreatment method for that species elsewhere. Heat damage at 100°C was also detrimental to *Paraserianthes falcataria* and *Albizia procera*, while 60-80°C greatly improved germination (Sajeevukumar *et al.* 1995). However, in a study by Kannan *et al.* (1996), no reduced germination capacity was observed by boiling water pretreatment.

In an experiment on *Prosopis alba* and *P. flexuosa* damage in the form of dead seeds and abnormal seedlings occurred after pretreatment in 90°C water, and only 20-30% of the seeds produced normal seedlings after pretreatment in 98°C water (Catalan and Macchiavelli 1991). Both figures refer to hulled seeds; seeds enclosed in the endocarp have a higher temperature tolerance because of the shielding effect of the endocarp. On the other hand germination is poorer because the endocarp also makes pretreatment less efficient. However, in an experiment by Lopez and Aviles (1988), where seeds were submerged into boiling water and left to cool in the water, heat damage was neither observed in these two *Prosopis* species nor in *P. chilensis* and *P. tamarugo*. All the species tested in their experiment had a high germination after treatment with boiling water.

The conclusion of these experiments is that temperature sensitivity varies both between and within species. Also, some Australian acacias are preferably treated below boiling point. 90°C water for 1 minute is preferred for e.g. *A. coriaceae*, *A. pachycarpa* and *A. pendula* (ATSC 1995). Usually, mature dry seeds of relatively thick-coated species tolerate at least a brief exposure to boiling. However, where temperature sensitivity is not known, the effect of high temperature pretreatments should be related to germination and not only to imbibition (which is an entirely physical process).

Species with very hard-coated seeds, e.g. several African acacias, show little response to boiling water pretreatment. In the Sudan the method was found inferior to both manual scarification and acid pretreatment of especially the very hard coated species *Acacia nilotica*, *A. nubica* and *Faidherbia albida* (Bebawi and Mohamed 1985). Boiling water pretreatment for 30-60 seconds with the seeds being left to cool in the water was the most effective method for non-leguminous *Juniperus procera* (Laurent and Chamshama 1987).

Heating or burning

Dry heat has a similar effect on seed-coat of dry fruits as boiling water: tension in the outer cells causes the formation of cracks through which gas and water can penetrate. The effectiveness of dry heat and burning is normally enhanced by rapid temperature change e.g. by rapidly pouring the seeds into cold water after heat pretreatment. This also reduces the risk of heat damage to the embryo. Dry heat is often less effective than boiling water in overcoming physical dormancy at least in legumes, but seeds may be easier to store after pretreatment provided they are cooled quickly without being left in water to imbibe. The above reference to temperature sensitivity also holds for dry heating: some species are very sensitive and consequently easily damaged. Both temperature level and duration of exposure are crucial for effect and possible damage.

Kiln drying for extraction of e.g. *Acacia mangium* may serve as an incidental pretreatment. Oven drying at 100°C for 10 minutes followed by cold water immersion was found an effective pretreatment for *A. mangium* in Sabah (Bowen and Eusebio 1981, quoted in Adjers and Srivastava 1993). 83% of the seeds germinated after this treatment as compared to 3% for untreated and 92% for 30 sec. boiling water pretreatment. 5 min. oven-drying was apparently too short (67% germination, 80% imbibition), while 15 min. or longer at 100°C apparently damaged the seeds (more than 95% imbibed but germination rate fell from 80 to 50% after over-heating for 15 and 60 min. respectively).

Dry heat provided by grass burning has been used for several species. In the Philippines, seeds of *Aleurites moluccana* are pretreated by burning grass in one of two ways: 1. Seeds are spread evenly on the ground, covered with a 3 cm thick layer of imperata grass (*Imperata cylindrica*; alang-alang) which is set on fire. As soon as the grass is burned, the seeds are poured into cold water. 2. Alternatively, seeds are sown in the seedbed at correct spacing but only half covered with soil. A layer of imperata grass is spread over the seedbed and set on fire. After burning, the seedbed is immediately sprinkled with water and the seeds pushed 2 cm into the soil and watered thoroughly (Seeber and Agpaoa 1976). Scorching by burning a cover of *Pennisetum* grass also enhanced germination of *Enterolobium cyclocarpum* and *Hemenaeca courbaril* in India (Brahmam 1996). However, the method was inferior to other pretreatments (hot water and acid scarification) and some seeds were damaged by high intensity burning (thick layer of grass). Similar results were obtained after fire scorching of *Juniperus procera* seeds in Tanzania; although scorching improved germination from 0 to 50-60% (depending on fire intensity), the results were also for this

species poorer than with hot water and acid pretreatment (Laurent and Chamshama 1987). Dry heat caused complete failure of germination in *Albizia procera* and *Paraserianthes* (former *Albizia*) *falcataria* in India (Sajeevukumar *et al.* 1995). The method probably often gives a poorer result than many other pretreatments, because the fire intensity and consequently the temperature and duration of exposure are difficult to control.

Acid pretreatment

Acid used for seed pretreatment is almost exclusively concentrated sulphuric acid (H_2SO_4). The acid causes some kind of wet combustion of the seed-coat and works equally well in legumes and non-legumes. However, the method is not applicable to seeds that easily become permeable because the acid then penetrates and damages the embryo. The practical application of acid pretreatment is as follows:

A container type that is not corroded by the acid should be used, e.g. a glass beaker for small lots under laboratory conditions (testing) or thick plastic bucket or bowl for large quantities. Seeds removed from cold storage rooms should be left in the closed containers until they have come to air temperature to avoid moisture condensing on the seed surface reacting with the acid (Willan 1985). Soaking in acid should be at ambient temperature (15-25°C). Duration of treatment varies according to the following factors:

- a. Seed-coat thickness (depending on species, maturity, age etc.)
- b. Temperature (longer treatment is required at lower temperature)
- c. Strength of the acid (new acid is stronger than re-used acid)
- d. Stirring (stirring during treatment reduces duration of treatment)
- e. Relative volume of the acid (a relatively large volume of acid as related to volume of seed is likely to reduce time required for pretreatment).

To avoid over-treatment by excessive soaking in acid, the duration of treatment must be adjusted. The imbibition test mentioned under Mechanical scarification is feasible for initial testing of a small sample. Conditions b, c and d should obviously be as close as possible to final treatment conditions.

After soaking, the seed is removed from the acid and rinsed under running water for at least 10 minutes. The seeds can then be sown (possibly after soaking in water to enhance imbibition) or re-dried and stored for a period, possibly up to 1-2 months.

Acid may be re-used several times, although its strength will gradually decline, and the treatment time must consequently be extended.

Duration of acid pretreatment should aim at reaching a balance in which the seed-coat (or pericarp) is sufficiently ruptured to permit the seed to imbibe, but without the acid itself reaching the embryo. In *Cassia siamea*, Kobmoo and Hellum (1984) found 15-45 minutes'

soaking in concentrated sulphuric acid highly effective, resulting in about 98% germination, while germination was lower for both shorter and longer exposure. 1-10 min. soaking was apparently too short to fully overcome dormancy, while 60 min. and more reduced germination rate probably because of damage. The seed lot in this experiment was apparently quite uniform, since pretreatment of 15-45 min. soaking rendered almost all seeds germinable without destroying a significant number of seeds (germination after mechanical treatment was 99.75%).

Similar results were found in experiments of variation in duration of acid treatment for several Ethiopian species (Teketay 1996b). Acid pretreatment improved germination in all tested species but prolonged soaking reduced germination capacity. However, the tolerance range was very different. In *Albizia lebbeck*, 40 min. was effective while both 20 min. and 60 min. gave poorer germination; in *Caesalpinia spinosa* any duration of soaking within the tested pretreatment time from 1-4 hours gave almost 100% germination. Eventually, in an acid pretreatment of *Hemenaea courbaril* and *Enterolobium cyclocarpum* 15 min. soaking was found suitable for both species. Longer duration of soaking (20-25 min.) gave slightly poorer results (Brahman 1996). Table 9.2 lists the recommended duration for sulphuric acid treatment of some additional species.

Acid pretreatment is commonly used for African acacias and other legumes (Doran *et al.* 1983, Bebawi and Mohamed 1985). It must be considered one of the most effective pretreatments for hard seed, especially those with very hard coats like *A. nilotica* and *Faidherbia albida*. For thin-coated species there is a risk of damaging seeds by over-treatment and less severe methods are normally preferred. In Australia the method is only used for species with very thick seed-coats such as *Acacia bidwillii* and *A. stenophylla* (ATSC 1995). Acid treatment also greatly improved germination of non-leguminous *Juniperus procera* (from 0 to >70%) (Laurent and Chamshama 1987).

Where physical dormancy is caused by a thick pericarp, a long soaking treatment in sulphuric acid is often necessary. Vasista and Soni (1988) investigated the effect of up to 60 min soaking of drupes of *Trema politoria* and found that germination increased proportionally with duration of soaking. However, for *Terminalia bellirica*, Bhardwaj and Chakraborty (1994) found that 10-12 minutes' dipping in concentrated sulphuric acid was the most suitable pretreatment, which almost doubled the percentage of germination as compared to untreated control.

Table 9.2. Duration of soaking in concentrated sulphuric acid to overcome seed-coat dormancy in some legume seed. In all the experiments the seeds were carefully washed after pretreatment, allowed to imbibe in water and sown under optimal germination conditions. The figures in parenthesis indicate germination percentage after pretreatment.

SPECIES	DURATION OF ACID PRETREATMENT	REFERENCE
<i>Acacia nilotica</i>	> 15 min	Rungu 1996
<i>Acacia tortilis</i>	30 min - 2h (100%)	Teketay 1996b
<i>Albizia lebeck</i>	40 min (85%)	Teketay 1996b
<i>Caesalpinia spinosa</i>	1-4 hrs (100%)	Teketay 1996b
<i>Cassia sieberiana</i>	45 min. (90-95%)	Todd-Bockarie <i>et al.</i> 1993
<i>Cassia fistula</i>	45 min (75%) - 90 min (84%)	Babeley and Kandya 1988
<i>Ceratonia siliqua</i>	20 min (89%)	Martins-Loucau <i>et al.</i> 1996
<i>Delonix regia</i>	3-6 hrs	Sandiford 1988 and Teketay 1996b
<i>Erythrina abyssinica</i>	5-20 min	Laurent and Chamshama 1987
<i>Leucaena leucocephala</i>	30 min (95%)	Duguma <i>et al.</i> 1988
<i>Prosopis alba</i>	6-24 min (100%)	Lopez and Aviles 1988
<i>P. flexuosa</i>	6.24 min (100%)	
<i>P. chilensis</i>	6-24 min (95%)	
<i>P. tamarugo</i>	6-24 min (95%)	
<i>P. juliflora</i>	15-60 min (95-100%)	Teketay 1996b
<i>Senna bicaparis</i>	60 min (95-100%)	Teketay 1996a
<i>S. didymobotrya</i>	60 min (95-100%)	
<i>S. multiglandulosa</i>	60 min (95-100%)	
<i>S. occidentalis</i>	60 min (95-100%)	
<i>S. septemtrionalis</i>	60 min (95-100%)	

Acid pretreatment has several advantages in overcoming physical dormancy:

1. It is applicable to many species, not only leguminous
2. The duration of treatment is short as compared to other scarification methods
3. It is the most effective method of bulk treatment for very hard coated seeds
4. It requires no special equipment
5. Seeds can be stored for a period after treatment

The method also has some major problems:

1. It implies a serious safety hazard to workers
2. Seeds are in risk of being damaged by over-treatment
3. It can be difficult to safely dispose of waste acid
4. It can be expensive in some places

While over-treatment may easily be avoided by appropriate adjustment to the method, safety hazards remain the major obstacle to the method. The method should be carried out with utmost care, since the chemical can cause serious injuries. A number of safety rules are summarised below. It should also be noted that acid causes corrosion of a lot of materials such as fabric and metal, while glass and most plastics are resistant.

Sulphuric acid, H_2SO_4 belongs to the group of strong acids and used in concentrated form (95%, 36N) it implies high safety hazards during handling.

Strict **safety rules** should therefore be observed:

1. Use acid only in a well ventilated place as evaporated gases can cause serious irritation when inhaled. Avoid inhaling the gas when opening bottles.
2. Always use safety glasses, protective gloves (good quality rubber gloves without perforations), and protective clothing (e.g. laboratory coat or apron).
3. Never pour water into undiluted acid; if the acid is to be diluted, carefully and slowly pour the acid into water.
4. Beware that even diluted acid can corrode skin, eyes and clothes. Protective clothing should be used throughout the operation, i.e. also during rinsing after pretreatment.
5. Store the acid locked up in a safe place when not in use. Make sure that the container type used will not be corroded by the acid, that containers are not leaking, and that they are distinctly marked 'STRONG ACID'. This also holds for containers containing used acid.
6. Dispose used and ineffective acid safely, i.e. heavily diluted with water.
7. Always have plenty of water, preferably a water tap within easy reach during any handling of acid.
8. In case acid is spilled on cloth or skin, rinse with plenty of water. If acid comes into contact with the eyes, rinse with plenty of water and contact a doctor immediately.

Other chemicals

A number of alternative chemicals have been tested on breaking physical dormancy, none of which, however, have given results comparable to conventional pretreatment methods. In a comparative study of 66 different pretreatment methods by Todd-Bockarie *et al.* (1993) the only chemical, apart from sulphuric acid, which gave some promising result was ethanol (95% conc. 9 hours' soaking). The improvement in germination was, however, only about 40% at its maximum and usually significantly less. Besides being less effective than several other pretreatments, the main problem with ethanol is its detrimental effect on viability; for most species few seeds survived more than 40 min. exposure to the alcohol. Kerosene appeared quite harmless to *Cassia sieberiana*, but on the other hand it was not very effective.

Hydrogen peroxide (H_2O_2) has long been known to improve germination in several species, though the mechanism is not fully understood. Ching-Te Chien and Tsan-Piao Lin (1994) found that the observed improvement of germination of *Cinnamomum camphorum* from 0-11% before treatment to 51-58% after treatment with 15% H_2O_2 could probably be ascribed entirely to the chemical helping to release physical dormancy (see further section 9.5.9).

9.5.4

Chemical inhibitors

Biological methods

Biological methods such as ingestion by large animals or the effect of insects or microbes are rarely used as a managed pretreatment method, but incidents of such action frequently result in improved permeability. Seeds of *Acacia* species extracted from goat faeces are often less dormant than non-ingested dry seed (Ahmed 1986). The effect depends, however, on species, since in some of the relatively soft coated species a large number of seeds are digested. Although many of the seeds which have passed through the digestive system of an animal may have been made permeable an equal amount may have been digested (pers. obs.). Ingestion of *Cassia sieberiana* by sheep gave a poor result in the comparative study of Todd-Bockarie *et al.* (1993). Ingestion may, in some places, be a very efficient procedure for handling legume seeds. Feeding pods to goats or other livestock and collecting the seeds from the faeces (see chapter 6.) has the following advantages:

1. Seeds are extracted and pre-treated during the same process
2. Seeds are efficiently cleaned from infestation by infecting organisms, e.g. bruchids and seed-borne pathogens
3. Adhering dung serves as fertiliser for the seedling
4. The pods serve as food for the animal eating them

Also non-legumes are often rendered more permeable after ingestion. Seeds from ingested drupes of *Melia volkensii* reportedly have improved germination (Kamondo and Kalanganire 1996).

Selection of pretreatment method

Several comparative studies have been carried out on the relative effectiveness of a range of pretreatment methods on one or several species (Teketay 1996a and b, Masamba 1994, Bebawi and Mohamed 1985, Khasa 1992, and others). Apart from scarification of each individual seed, which universally seems to be the most effective method, no single pretreatment method is equally effective for all species. Since relative dormancy also varies within species, preliminary trials are often necessary to find the best method. In most instances time factor, safety risk (primarily acid treatment), available equipment and relative cost are important factors to be balanced against the physiological advantage. A less effective pretreatment may be most efficient in total accounting if seeds are relatively abundant.

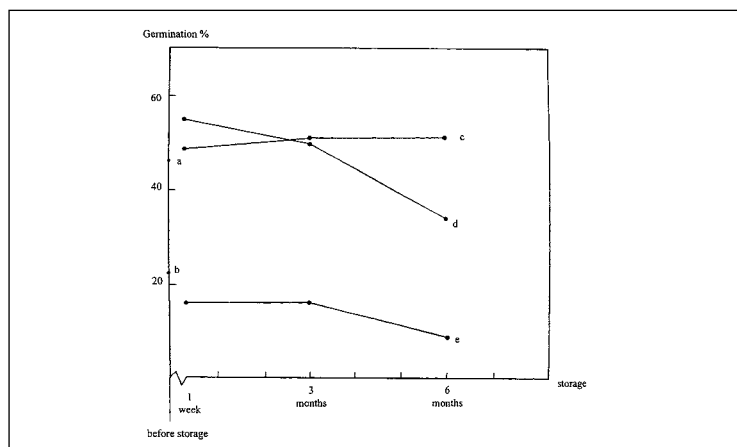
A number of species contain chemical inhibitors in fruit or seed which prevent germination from proceeding, e.g. by blocking the metabolic processes necessary for germination. Sugars and other substances in fleshy fruits prevent germination because they exert osmotic pressure, which impedes imbibition. Osmotic inhibition is not strictly a dormancy phenomenon since seeds will germinate readily when removed from the high osmotic pressure, e.g. by placing them in water. In addition to sugars many fleshy fruits contain inhibitory compounds for example coumarin in the pulp, which prevent germination. In order to overcome such dormancy the inhibitors must be removed. Under natural conditions dispersal, decomposition and/or rainwater

leaching gradually relieve dormancy, but the natural process may be slow and result in very uneven germination.

Germination inhibitors may be located in several places in the fruit or seed. The most frequent inhibitors are those occurring in fleshy fruit pulp. Even where seeds are sown immediately after harvest, such seeds usually need extraction and washing to remove inhibitors. *Dobera glabra* fruits consist of two distinct layers: an outer green coreaceous exocarp and an inner red soft mesocarp. Both layers contain inhibitors: removal of the exocarp increased germination from 8 to 57%, removal of the mesocarp further increased germination to 70% (Schaefer 1989). Seeds of *Gmelina arborea* completely failed to germinate without extraction. Extraction followed by thorough washing in running water to leach out inhibitors enhanced germination to 50-90% depending on the preceding fermentation/softening procedure. Fermentation in running water or piled up in the soil was recommended for this species (Ogunnica and Kadeba 1993).

In *Prunus africana* very low germination was achieved when whole fruits were sown, while 75-90 % of the seed germinated after extraction/depulping (Schaefer 1989). In this species it was also shown that depulping should be done before storage; when depulping was delayed for 20 days, germination fell to 50%, possibly because inhibitors enter deeper into the seed parts which are not removed by depulping. *Vitex* spp. have a sticky pulp which is difficult to remove when the fruit is fresh. Depulping is normally done after drying and sometimes after storage. In the experiments of Schaefer (1989) (fig 9.9) all seeds of *Vitex keniensis* sown with pulp showed a markedly reduced germination as compared to depulped seeds. However, fresh seeds sown with pulp had 23% germination capacity as compared to 46% for depulped. Germination rapidly declined with a longer time lapse between collection and depulping. After 6 months' storage, germination capacity for seeds depulped immediately after storage remained at approximately 50%. Similar observations were made on seeds of *Ziziphus mauritiana*, *Z. mucronata* and *Maesopsis eminii* in Kenya (pers. obs). The observations seem to indicate that germination inhibitors are more difficult to remove after a period of storage. It must therefore be concluded that prompt depulping of fleshy fruits helps to avoid development of this type of dormancy.

Figure 9.9.
Effect of time of depulping on germination of *Vitex keniensis*.
a. Fresh seeds without fruit pulp.
b. Fresh seeds with pulp.
c. Dried 1 week with pulp, then depulped.
d. Dried 3 weeks with pulp, then depulped.
e. Dried 1 week with pulp, stored with pulp.
(Graph drawn from data in Schaefer 1989).



9.5.5

Photo-dormancy

Since depulping before storage is normally necessary to maintain viability, inhibitors that may be present in the pulp are thus removed by routine processing. Where seeds are stored with the dry pulp, removal of inhibitors by washing and leaching is necessary before sowing. For both fresh fleshy fruits and those stored with their pulp, pretreatment is essentially a pre-sowing extraction, in practice similar to that taking place during normal processing (chapter 6.).

While inhibitors present in fruit structures are readily removed by extraction, those located in non-removable structures e.g. remaining pericarps, seed-coats, endosperms or embryos must either be removed or inactivated by special seed treatments. Water soluble inhibitors are often effectively removed by leaching. Seeds are either subjected to running water or soaked in several changes of water.

The treatment may work both by physically removing the inhibitors with the discharged soaking water, and by a gradual decomposition. Once the concentration of inhibitors has been adequately diluted, the seeds are capable of germinating. In teak (*Tectona grandis*) several alternate cycles of soaking and drying seem to gradually reduce chemical dormancy simultaneously with breaking physical dormancy (see below). Also stratification, primarily designed to overcome thermo-dormancy may reduce inhibitors.

Dormancy in legumes has generally been ascribed only to the impermeable seed-coat, but Sajeevukumar *et al.* (1995) also found indications of the presence of water soluble inhibitors in the seed-coat of *Albizia procera* and *Paraserianthes falcataria* (former *Albizia falcataria*). The presence of an inhibitor has also been shown in seed-coats of *A. odoratissima* (Kannan *et al.* 1996). 24 hours' soaking in running water after scarification is therefore recommended as a standard pretreatment for these species.

Most seeds with photo-dormancy germinate only under light conditions. Such seeds are therefore also called light sensitive. Photo-dormancy is common among pioneer forest trees. It is operated by a biochemical phytochrome mechanism, which shall be briefly explained here:

Phytochrome appears in two forms, P_r and P_{fr} (subscripts meaning 'red' and 'far-red') which can be reversibly converted to either form by radiation at different wavelengths (fig. 9.10). Germination is determined by the amount of P_{fr} relative to the total amount of phytochrome (Mayer and Poljakoff-Mayber 1982). Phytochrome in the P_r form inhibits germination, whereas P_{fr} allows germination to proceed. Dormant seeds have a large quantity of P_r , in non-dormant seed the phytochrome is mainly in the P_{fr} form. Dormancy in a photo-dormant seed may be broken by exposure to light with a high red/far-red ratio, e.g. white light. Conversely, non-dormant seed may turn dormant (induced or secondary dormancy) if exposed to illumination with light relatively rich in the far-red wavelength. The latter occurs e.g. where light is filtered through a dense canopy (Mayer and Poljakoff-Mayber 1982, Richards and Beardsell 1987) or

where seeds are enclosed in a chlorophyll-rich (green) fruit or seed-coat (Cresswell and Grime 1981). Eventually, seeds exposed to dark conditions (e.g. buried or dark storage) gradually develop dormancy because P_{fr} is converted to P_r .

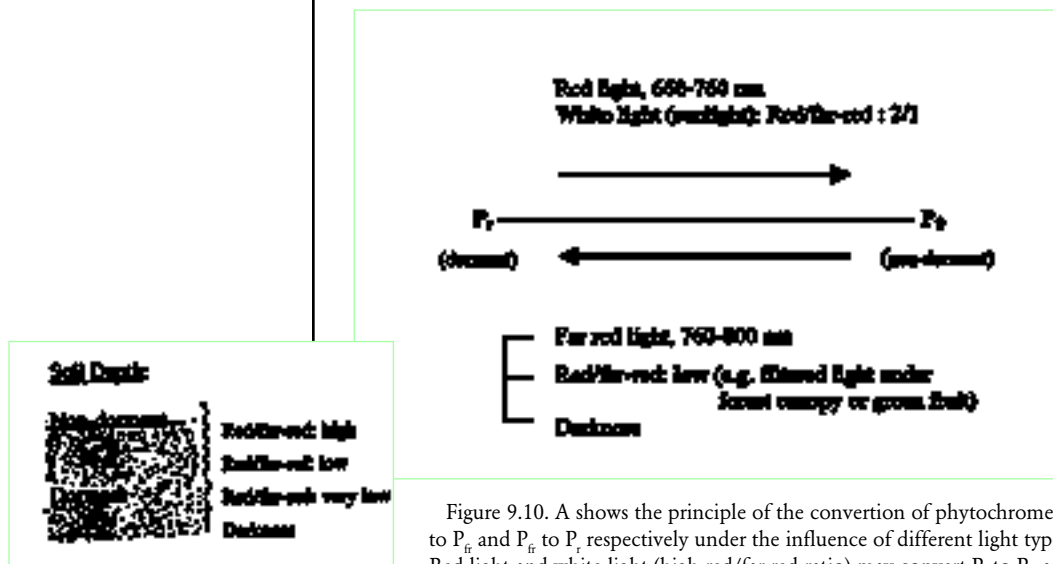


Figure 9.10. A shows the principle of the conversion of phytochrome P_r to P_{fr} and P_{fr} to P_r respectively under the influence of different light types. Red light and white light (high red/far-red ratio) may convert P_r to P_{fr} and thus break dormancy (indicated by top arrow). Far red light or light with a low red/far-red ratio (e.g. filtered light) will convert P_{fr} to P_r and thus induce dormancy in seed with a phytochrome dormancy system. In complete darkness, P_{fr} may revert to P_r and the seed consequently become dormant. The three conditions are indicated by the lower arrow, going from P_{fr} to P_r . Notice that 'r' and 'fr' refer both to a stage of the phytochrome and to the wavelength of the light that transforms the phytochrome. B shows an example of the conversion of phytochrome at different soil depth. Since red light has a lower penetration into the soil than far-red light, the relative amount of light of the two wavelengths changes. At the upper soil levels the light will be rich in red light and there will be no dormancy. At some depth in the soil, there will be very little red light penetrating, and dormancy may be induced; the same will happen at greater depth, where no light penetrates.

The phytochrome dormancy mechanism seems to some degree influenced by temperature. High or fluctuating temperatures appear to overcome photo-dormancy in some instances. In nature, light and temperature are obviously interrelated.

Although photo-dormancy has been most frequently documented from herbal species, it also occurs among some tree pioneers. The phytochrome dormancy system has been documented for e.g. *Cecropia obtusifolia* and four Latin American *Ficus* species (Vasquez-Yanes *et al.* 1996). *C. obtusifolia* showed the strongest dependence and had very low germination under dark or far red light. None of the *Ficus* species germinated in the dark, but there was a great difference with regard to far red (which simulates a forest canopy); two of the species had largely the same germination rate as in white or red light, only *F. insipida* showed significantly reduced germination under far-red conditions. Also, germination of many *Eucalyptus* spp. are believed to be determined by light (Boland *et al.* 1980).

9.5.6

Thermo - dormancy

The condition of light requirement in pioneers is the simplest sort of photo-dormancy. In some species seeds require specific duration of light-dark cycles for germination to proceed. Under tropical conditions a cycle of 12h light - 12 hour dark is prevalent. Under temperate conditions, longer exposure to light is sometimes required which corresponds to the longer day light hours during the temperate spring and summer.

Photo dormant seeds normally require only a brief illumination after imbibition to break dormancy. In practice photo-dormancy is not overcome by pretreatment, but by germinating seeds under appropriate light conditions that will break the dormancy (see chapter 10).

The term thermo-dormancy is here used in its widest sense to cover all types of dormancy in which temperature plays a role in the development or release from dormancy (see footnote to section 9.4.2). Seeds with thermo-dormancy require exposure to a temperature regime which is often different from that required for the actual germination process. Dormant seeds of eucalypts, pines, acacias and others, though benefiting from fires during germination and regeneration, are not considered thermo-dormant since the high temperature in itself is not necessary for breaking dormancy, rather one of several methods of breaking physical dormancy. The distinction is, however, not always clear.

Low temperature thermo-dormancy is experienced in most temperate species, e.g. *Fagus*, *Quercus*, *Pinus*, *Abies* and some highland tropical species of pines and eucalypts. Such seed needs exposure to cold, moist pretreatment for a period to break dormancy. Any cold and moist condition is called chilling. Pre-chilling applies specifically to the conditions when applied for breaking dormancy. Pre-chilling was previously undertaken in practice by placing the seeds in alternate layers with a moist medium in a cold environment, e.g. an outdoor pit exposed to ambient low winter temperatures. The common term 'stratification' or 'cold stratification' originates from this practice of layering. Warm stratification is analogously used for any type of warm, moist pretreatment (Bonner *et al.* 1994). Warm stratification is used in connection with after-ripening, for overcoming dormancy caused by underdeveloped embryo and for softening hard pericarps or seed-coats (mechanical dormancy).

High altitude (alpine) eucalypts e.g. *E. delegatensis*, *E. pauciflora* and *E. glaucescens* require cold moist pretreatment to overcome dormancy. Stratification at 3-5°C for 4-8 weeks is recommended pretreatment for these species (Boland *et al.* 1980, Turnbull and Doran 1987). In other eucalypt species e.g. *E. camaldulensis*, *E. tereticornis* and *E. nitens*, it has been shown that stratification may substitute for light requirement, another example of linkage of photo- and thermo-dormancy. For *Terminalia chebula*, a highland Indian species, Bhardwaj and Chakraborty (1994) found improved germination after cold moist stratification in cowdung. 5 weeks' stratification was considered optimum and less than 3 weeks was insufficient to overcome dormancy.

In order to break thermo-dormancy by cold moist treatment, seeds must be imbibed. Hence, general cold storage of dry seed does not substitute for stratification since the seeds only respond when moist. Since imbibed seeds respire (albeit at a low rate at low temperature) good aeration must be provided during pretreatment. The necessary period of pretreatment varies, but as long as temperature is kept too low for germination to proceed, there is little risk of damage by over-treatment. However, most seed requiring cold moist treatment also germinates at fairly low temperature, so in practice it is difficult to avoid initial germination once dormancy has been overcome. In temperate regions, where thermo-dormancy is very common, stratification takes place during the late winter months up to normal sowing time in early spring. Because temperature increases during that period, seeds may germinate once dormancy is broken. The onset of splitting of the seed-coat and radicle protrusion is an indication of terminated dormancy. Seeds may be transferred directly from stratification to the seedbed before the radicles have elongated. Immediate sowing is necessary to avoid mechanical damage to the radicle during handling (Aldhous 1972). In comparison with other dormancy types, thermo-dormancy requires a fairly long pretreatment period. Therefore, appropriate scheduling according to time of sowing is important. A practical method of stratification used in the temperate region but also applicable to tropical highlands is described by Alhous (1972), here slightly modified:

1. A stratification pit should be located where the temperature is relatively low, e.g. a shaded site on a north facing slope in the northern hemisphere, south facing in the southern hemisphere, with good drainage.
2. A depth of 60-80 cm is convenient. The form and volume should be adapted to site and seed volume. A 60-80 cm wide trench of variable length or several circular pits are applicable. The side of the pit may be provided with a frame to protect the sides from falling in. As a protection against rodents, sides and bottom may be lined with wire mesh.
3. The bottom of the pit or trench is covered with a layer of sand or gravel for drainage.
4. The seeds to be stratified should be mixed with moist sand 4 times their weight, or filled into the pit in alternate layers of seeds and sand in the above proportion. The pit is filled to 15 cm from the top. The top 15 cm is filled with pure sand.
5. The pits are covered with a wire mesh cover to protect against rodents.

Where cool rooms are available, cold stratification is preferably carried out indoors at 1 - 5°C. The seeds are soaked in water for 24-48 hours, then mixed with a moisture retaining medium e.g. moist sand, vermiculite, peat or a mixture. Occasionally seeds are pre-chilled 'naked' i.e. without mixing with a moisture retaining medium, but that procedure makes control of moisture and temperature within the seedlot more difficult during treatment. Willan (1985) recommends use of a medium for long term pre-chilling and any warm moist pretreatment, while 'naked' pre-chilling is suitable for species needing only a few weeks' cold pretreatment.

Indoor pre-chilling may take place in various types of containers. The main requirements are sufficient drainage and ventilation during the process. Boxes, cans, drums, trays, or woven bags all make up suitable containers, although bags are obviously less applicable where seeds are mixed with sand. Polythene bags (100 micron) are suitable since they retain moisture, yet allow some ventilation. Where polythene bags are used, they should be only loosely closed, be opened regularly and the seed stirred to avoid heating and assure ventilation. Prechilling of naked seeds in trays in cool rooms is now the most common method for several temperate species (fig. 9.11). Seeds are regularly moistened during the period. Moisture content during prechilling is crucial. Too low moisture content slows down or stops the dormancy-breaking process; too high a moisture content may cause deterioration. During the latter part of the prechilling period too high a moisture content may induce germination. Measuring moisture content on samples during the treatment period helps in adjusting m.c. and hence in controlling germination during the pretreatment process.

Thermo-dormancy can in some instances be partly or fully overcome by chemical pretreatment (see section 9.5.9).

Figure 9.11.
Naked prechilling of beech nuts (*Fagus sylvatica*) in a cool room. Danish Tree Improvement Station.



9.5.7 Combined dormancy

Where two or more types of dormancy are present in the same species, dormancy must be broken either by successive methods that work on different dormancy types, or by methods with multiple effects. The latter is usually applied in the combination of mechanical and physical dormancy. Where the two types occur together, any method aiming at breaking physical dormancy will also work on mechanical dormancy. In practice it is often difficult to distinguish between the two. Since some pretreatment methods work on different types of dormancy, the nature of dual or combined dormancy is not always evident. For example, *Terminalia tomentosa* evidently has a pronounced physical dormancy with almost no germination unless mechanically scarified (Negi and Todaria 1995). However, since seeds extracted completely from the fruit showed greatly improved germination as compared to those that were only scarified, it is likely that there is a second dormancy type, which could be mechanical or caused by inhibitors in the fruit.

Fraxinus spp. are commonly known to possess two types of dormancy viz. underdeveloped embryo and thermo-dormancy. The former is broken by warm moist stratification, the latter by subsequent cold moist stratification. Teak (*Tectona grandis*) is one of several species where physical dormancy is combined with chemical inhibitors in the fruit. In addition the fruits often need a period of after-ripening which must be carried out before the seeds respond to other pretreatment procedures (Bedell 1989). A recommended pretreatment of teak fruits is alternate soaking and drying, plus sometimes sun baking. The duration of each treatment and number of cycles vary; Keiding (1993) and Willan (1985) list variations of the procedure:

- a. 4 times soaking and 3 times drying, each of 30-35 min. for scarified seed.
- b. 5-10 cycles of 1 day soaking and 3-5 days' drying and sun-baking.
- c. Alternate 24 hours' soaking and 24 hours' drying for 2 weeks.

Prolonged soaking in running water for one to several days also serves both to leach inhibitors and soften fruit or seed-coat. This method is also applicable to teak (Keiding 1993). In India, Yadav (1992) found prolonged soaking a suitable alternative to the alternate treatment (see section 9.5.8).

Chemical inhibitors in combination with physical dormancy has also been suggested for two albizias (see section 9.5.4).

9.5.8 Soaking in water

Soaking in stagnant or running water has been mentioned above as a method of leaching out chemical inhibitors in fruits and seeds. Soaking in water for 6 days was found to be a suitable alternative to alternate soaking and drying in order to overcome physical dormancy in teak (*Tectona grandis*) in India (Yadav 1992). Although germination was delayed after prolonged soaking alone (as compared to alternate soaking and drying) total germination was equivalent, and the method is less laborious. Where physical dormancy is relatively weak e.g. fresh legume seeds, soaking is often sufficient to render the seed-coat permeable. The effect of prolonged soaking on hard seed varies with species. In some species seeds become gradually permeable, in other species there is little effect of continuous soaking. Generally, however, soaking alone is a very slow procedure to overcome physical dormancy, and there is a great risk that imbibed seeds will die if left in the water until the remaining seeds become permeable.

However, many species without dormancy or where dormancy has been broken, benefit from soaking in water, usually 12-24 hours before sowing. Soaking allows a quicker imbibition than is usually the case in a nursery bed or container (see chapter 10). Prolonged soaking (i.e. beyond one day) should, however, be avoided as it may cause anoxia. When longer submersion is necessary, it is advisable to renew the water regularly.

9.5.9 Chemical or hormonal pretreatment

Several chemical compounds promote seed germination. Some compounds interact with the physiological mechanisms of some dormancy types. Application may break or relieve dormancy, e.g. partly substitute for temperature or light pretreatment, or for leaching of germination

inhibitors. Some compounds stimulate individual metabolic processes during germination without being directly linked to dormancy. The same type of hormones is for example often involved in both dormancy release and germination processes. The effect of germination stimulants is often most evident under sub-optimal germination temperatures. A detailed discussion of the physiological mechanism behind the effect of germination promoters is beyond the scope of this book. Interested readers are referred to Hartmann *et al.* (1997), Bewley and Black (1982 and 1994), and Meyer and Poljakoff-Mayber (1982). Total germination percentage, germination speed and seedling vigour may be promoted by application of germination stimulants. The two main groups of stimulants are growth regulators (e.g. gibberellic acid (GA_3), benzyl adenine (BA)) and nitrogenous compounds (e.g. potassium nitrate (KNO_3), and thiourea). Comparative studies on the effect of different germination stimulants have been carried out on seeds of *Ziziphus mauritiana* (Murthy and Reddy 1989), *Casuarina equisetifolia* (Maideen *et al.* 1990), and *Acacia nilotica* (Palani *et al.* 1995). Neither hormones nor other germination compounds are much used in practical seed propagation, but widely used in seed research.

Growth regulators

Gibberellic acid or gibberellines (GA) is a group of naturally occurring plant hormones. The hormones play a central role in the early germination processes by activating enzyme production and mobilising storage reserves. In connection with dormancy GAs may overcome the inhibitory effect of e.g. coumarin or abscisic acid (ABA). Application of gibberellic acid (usually GA_3) has been shown to have an effect in overcoming thermo-dormancy (e.g. induced dormancy caused by high temperatures), photo-dormancy (e.g. inducing dark germination in light sensitive seeds) and dormancy caused by inhibitors (Bewley and Black 1982, Villiers 1972). Murthy and Reddy (1989) used a concentration of 200 ppm. for stimulating germination in *Ziziphus mauritiana*. These seeds were apparently not dormant, but GA_3 had a particularly positive effect on shoot development and vigour.

Benzyl adenine (BA) is a synthetic plant hormone of the cytokinin group. Cytokinins are essential for cell division. The interaction between cytokinin and another plant hormone, auxin, is well established in plant propagation: a high auxin/cytokinin ratio favours root development, a high cytokinin/auxin ratio favours shoot development. Application of cytokinins sometimes promotes germination but because of their specific effect on shoot development, both germination and seedling development may be abnormal; seeds treated with cytokinins sometimes germinate with the shoot before the radicle (Bewley and Black 1994). In connection with dormancy release, cytokinins interact with e.g. gibberellic acid but their role is not fully understood. Applied cytokinins (kinetin) have been reported to overcome high-temperature dormancy in lettuce seed (Smith *et al.* 1968, quoted in Hartmann *et al.* 1997). In *Ziziphus mauritiana* BA (100 ppm) stimulated both total germination and vigour of seeds, but the effect was generally lower than for the other compounds tested (Murthy and Reddy 1989).

Nitrogenous compounds

Potassium nitrate (KNO_3) is one of the frequently used germination stimulants. It is used both in connection with testing (ISTA 1996, cf. chapter 11), and in operational plant propagation. Its physiological role is not clear (Hartmann *et al.* 1997). KNO_3 had a strong effect on both germination percentage and vigour on acid pre-treated *Acacia nilotica* seeds (Palani *et al.* 1995). At 1% concentration, germination increased from 37% (control) to 79%, and at 2% conc. it increased to 85%. In *Casuarina equisetifolia* germination increased from 46% in the control to 65% after soaking in 1.5% KNO_3 for 36 hours. Both higher and lower concentration, and shorter duration of soaking showed a lower germination in that experiment (Maideen *et al.* 1990). In seed testing, 0.2% is the recommended concentration (ISTA 1996). In the study on *Ziziphus mauritiana*, KNO_3 was less effective than GA_3 , thiourea and BA in all germination parameters except root length (Murthy and Reddy 1989).

Thiourea has a stimulating effect on breaking dormancy possibly by deactivating the effect of inhibitors, e.g. ABA (Hartmann and Kester 1983). It has proved effective in overcoming photo-dormancy in a number of light sensitive seeds (Mayer and Poljakoff-Mayber 1982). In temperate *Quercus*, *Larix* and *Picea* species it has been used instead of stratification (Deubner 1932, Johnson 1946, quoted in Mayer and Poljakoff-Mayber 1982). Among several compounds studied, thiourea proved the most effective germination stimulant for *Ziziphus mauritiana*. 24 hrs. soaking in a 1% solution enhanced total germination percentage from 41% (control) to 78% at 30°C, which was considered the optimal germination temperature. In addition it alleviated the deleterious effects of sub-optimal temperatures, both in terms of total germination and vigour.

9.6 Priming and Fluid Drilling

Priming is a method to promote rapid and uniform germination of seeds, by controlling imbibition to an extent where germination is initiated, but insufficient to cause radicle emergence. The priming process carries germination further than pure imbibition, viz. as close as possible to phase three, the radicle expansion phase, in the germination process (see chapter 10). During priming the variation in initial imbibition rate is overcome: all seeds tend to reach a stage where they are ready to germinate once they are provided with optimum germination conditions (Maude 1996). Because germination process is in the second 'lag' phase without protrusion of the sensitive radicle, primed seeds can be stored and handled at least for some time without damage to the seeds. Priming is not a dormancy breaking treatment; possible dormancy must be broken by an appropriate pretreatment prior to priming. Under normal nursery practice for forest trees, priming is not much used, but the method is becoming increasingly important in connection with direct sowing in dry areas. Because the germination process has already started before sowing, germination and seedling establishment is fast and primed seeds thus have a competitive advantage under field conditions.

9.7 Seed-coating and Pelleting

The most common type of priming is osmo-priming in which the seeds are soaked in a priming fluid with high osmotic pressure. Usually polyethylene glycol (PEG) is used as priming fluid. Conditions and duration of priming varies with species. Hartmann *et al.* (1997) indicate range of condition of osmotic potential (i.e. PEG concentration) from -5 to -15 bars (= -0.5 to -1.5 Mpa), temperature from 10 to 25°C, and duration of treatment from 1-15 days. A common priming condition is 15°C for 5-10 days. Stirring or bubbling is essential during priming of large quantities in containers, both to assure uniform treatment and to assure proper aeration while the seeds are being soaked. Small quantities may be primed on filter paper irrigated with PEG (Maude 1996). Once priming is completed, the seedlot is washed, dried superficially and coated with a film, e.g. sodium alginate. The priming fluid may be re-used. Drying rate and coating depend mainly on time of priming in relation to sowing date. Seeds to be sown immediately are only slightly dried, seeds to be sown later may need slightly more drying, e.g. by warm air, and protection against fungi. Fertilizer, pesticide or inoculant may be added as an integrated part of the coating process (see below). Fungicides are also occasionally added to the priming fluid (Maude 1996).

In fluid drilling the germination process is allowed to proceed until radicle emergence. Germination takes place in aerated water, and once the radicle has emerged, the seed is mixed with a viscous gel to protect the radicle from mechanical injuries and desiccation.

Coating and pelleting are practices of covering seeds with an inert substance prior to sowing. In coating, seeds are covered with the substance with or without an adhesive applied to the seed-coat. The coating does not significantly increase seed size or weight. In pelleting the functional substrate is mixed with an adhesive before application. Pelleted seeds achieve in that way a larger, heavier and more uniform size which facilitates some types of handling, e.g. machine sowing. The coating material in both types of treatment yields in itself some protection to the seed. Special coating material may add particular protection, e.g. alginate as an anti-desiccant and lime at low pH. In addition, various substances which promote germination and early seedling development may be added to the coating or pelleting material. These include: 1. Fertilizers, 2. Growth regulators, 3. Fungicides or insecticides, 4. Rodent and bird repellent. 5. Microsymbionts (mycorrhiza, rhizobia, frankiae). The substances can rarely be applied all to the same seeds at the same time. Fertilizer and fungicides are for instance normally antagonistic to microsymbionts. As a major purpose of pelleting is to increase size, the major component of pelleting material is a filler, kaolin clay, vermiculite, gypsum, peat etc.

Coating and pelleting is rarely economically feasible when seeds are raised in nurseries where moisture and other planting conditions are easily controlled, and fertilizer, microsymbionts etc. are easily applied directly to the seed bed or nursery soil. Only where seeds are very small and machine sowing applicable can the advantage of an increased and more uniform size counterbalance the cost of pellet-

ing seeds sown under nursery conditions. The practice is, however, relevant for forest trees in connection with direct sowing (section 10.7) since this practice often requires some of the above protection and application, which is normally beyond control in the field. In addition, for very small seeds like eucalypts and *Enthocephalus spp.* direct sowing becomes practically difficult without artificially increasing the size of the seed.

Some methods of application are described below:

- Seeds are moistened and mixed with a dusty substrate which will adhere to the seed-coat. The method is applicable only if the seed-coat is relatively rough, and only a small amount of coating material needs to be applied. Seeds pre-treated with acid tend to get a rough surface which promotes adhesion. Inoculants and pesticides may be applied this way.
- Where seeds are smooth, adhesion may be promoted by covering the surface with an adhesive prior to application of the dry coating material. 40% (w/v) gum arabic, 1.5% (w/v) methyl cellulose or vegetable or paraffin oils are applicable as binders or stickers. The seeds are rolled in the sticker until evenly coated, then transferred to the dusty substrate. More substrate can be applied by this method than by the method above, but the risk of losing coating material such as inoculant during handling is high.
- Substrate may alternatively be mixed with the sticker (gum arabic or methyl cellulose) and applied by rolling the seeds in the mixture until evenly covered. As much substrate as required can be applied by extending the time of rolling. When sufficient substrate has been applied and the seed has reached a reasonable size, they are rolled in powdered rock phosphate, calcium carbonate or the like to avoid them agglutinating. The pelleting protects possible inoculants applied with the substrate and the seeds are easy to handle.

Coating and pelleting exclude any further pretreatment such as dormancy release, and is normally undertaken shortly before sowing. Pelleting may in some instances delay germination since the pelleting material needs to dissolve before imbibition, and the pellet may act as a physical barrier to water and oxygen absorption.

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