Guide to handling of tropical and subtropical forest seed

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PHYTOSANITARY PROBLEMS AND SEED TREATMENT

7

Contents

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PHYTOSANITARY PROBLEMS AND SEED TREATMENT

7

7.1 Introduction

Seeds, being concentrated packages of high nutritive material like starch, protein and fat (oil), are attractive food supplies for a number of organisms. Seed-based food is the principal nutrition for human beings, and several large animals and birds feed largely or entirely on seeds. In nature, consumption by large animals often plays an important role in dispersal and regeneration (cf. ingestive dispersal chapter 2.6.1). Ants and termites occasionally feed on seed appendices like arils, leaving the seed intact behind. However, since small seed consumers like insects are too small to transport seeds, they must consume them on the spot, and they are therefore pure seed predators. In seed handling the distinction between dispersal and predation may appear academic, since anything removed from the seed crop, being it dispersal or predation is a loss. Predation by insects often causes major loss of seed crops just before and shortly after shedding or harvest. Appropriate timing of seed collection is often the most effective preventive measure to avoid both foraging by animals and birds and infestation by insects.

Susceptibility to pests and diseases often changes during the lifetime of the seed, and often the type of infection varies with external and internal conditions. For example, different species of infective organisms attack young and mature seeds respectively, and because there is often a radical change of environment from the field to storage, there is usually a concurrent change in pests and pathogens associated with the seeds at the two places. Seeds may contain innate barriers against infection, such as a hard seedcoat or chemical compounds. Immature, aged or damaged seeds normally have less protection and are accordingly more susceptible to infection than seeds with high physiological quality.

The natural ageing process, which is caused by progressive deterioration of cell constituents and which ultimately leads to loss of viability, will be dealt with specifically in chapter 8. The subject of this chapter is the kind of deterioration caused by external organisms. However, in terms of micro-organisms it is often not possible to distinguish between the two types. Where micro-organisms are present and active, they interact with natural ageing in the way that makes aged seeds more susceptible to infection than fresh seeds, but infecting organisms may cause similar breakdown of cell constituents as occurs during natural ageing.

For orthodox seeds, storage conditions which promote general seed longevity (low temperature and moisture content, see chapter 8) fortunately also lower the activity of storage pests and pathogens. Recalcitrant seeds pose a problem because their seeds must be stored at a temperature and moisture content conducive to insect and fungal development. Infecting organisms are often the major cause of deterioration for seeds stored at high temperature and moisture content.

Some seed-borne pests and pathogens are host specific in the sense that they are closely associated with one or a few plant species. Others can infect a wide range of species and may even attack other plant parts. Eventually, some seed-borne fungi do not cause any damage to the seed itself but only to seedlings or larger plants. In this case, seeds are a vehicle for dispersal of the pathogen rather than a food source. Detection and assessment of infection or infestation (the two terms explained below) will be dealt with in chapter 11.

Pests and diseases must be controlled during seed handling, both to prevent the infected seed itself being destroyed, and to prevent the pest being spread to other seeds during handling. Also, in the case of seed transmitted diseases, to prevent them from spreading to plants in the nursery or further afield. The latter is especially important where seeds are shipped into an area in which the pathogen is not found and where possible introduction could cause major loss. This problem is further discussed in chapter 15.

The type and level of seed pest and pathogen control vary with infection rate, type of infecting organism and the likelihood that the organism may multiply and destroy seeds during storage. Preventive measures like early collection, swift processing, good hygiene and appropriate storage conditions are often sufficient to reduce the loss caused by both insect and fungal attack and to make chemical control redundant. In cases where pesticide treatment cannot be avoided, e.g. because of sub-optimal storage conditions, recalcitrant seeds or for phytosanitary reasons, the use should be limited to the strictly necessary. It should be applied with due consideration to possible impairment of seed viability, risk to labourers during handling, and danger to the environment when disposed. It should be noted that most chemicals are generic (target specific) i.e. insecticides generally have little, if any, effect on fungi and fungicides on insects.

7.2 Terminology

External organisms that cause seed deterioration can be classified as insect pests and pathogens. Insects may be predators or parasites. Both predation and parasitism imply that the seed (or part of it) is consumed by the invading organism, and the terms are sometimes used synonymously (Janzen 1971). Generally, however, predation refers to a complete destruction of the seed in a relatively short time, while parasitism is the destructive sort of symbiosis[1] (living together) in which the parasite (in this case the insect or pathogen)

[1] The term 'symbiosis' is frequently used as the condition where both parts of an association benefit. This is more precisely called 'mutualism'.

derives its food from the host (in this case the seed) over a prolonged period without necessarily destroying it.

Pathogens are disease-inducing organisms like bacteria, viruses and fungi. The latter are by far the most important in seeds although most fungi are non-pathogenic. Some pathogens are parasites in the above sense of deriving their food from the host during a prolonged period. Often, however, injury to the host occurs because the pathogen releases enzymes or toxins detrimental to the host organism. The deterioration caused by pathogens is often manifested as poor or unhealthy performance (reduced 'vigour', cf. chapter 8.) of the seed or seedling; therefore the term 'disease-inducing'. Insects and pathogens may be carried on, in, or with the seeds, all of which are referred to as seed-borne. Organisms which cause no harm to the seed itself but only use the seed as a vehicle of dispersal (in practice only pathogens) are called seed transmitted. The distinction between infection and infestation is somewhat blurred. In the strict sense infection refers to the situation where the foreign organism lives inside the host (endoparasitism) while infestation is the invasion by exterior organisms (ectoparasites), e.g. some insects which feed from the outside of the seed. In connection with seed pathology, infection refers to a condition in which there is a direct connection between the living tissue of both the host and the pathogen. Infestation is here any condition where such connection does not occur, e.g. pathogen-infested soil. In this chapter, infection is mainly used in connection with pathogens, infestation in connection with insects.

7.3 Susceptibility to Pests and Diseases

Plant resistance to pests and diseases may be interpreted as a series of evolutionary steps, typically of any predator - prey association, in which the plant develops defensive and escape strategies, and the attacking organism develops measures to overcome these strategies. Innate protection against pests and diseases is typically in the form of a hard seed-coat or chemical compounds in the seeds (Halloin 1986, Neergaard 1979). Seed insects and pathogens have, as a counter-adaptation, often developed resistance to plant toxins or methods to penetrate hard seed-coats. For example, the testa of some legume seeds contains compounds which are toxic to bruchid larvae. As a counter-measure many bruchid larvae do not consume the testa when entering into the seed embryo.

Some plants are believed to having developed a regenerational strategy at least partly influenced by seed predation. In several Latin American rain forest species it has been observed that insects destroy almost the entire seed crop during years with low or intermediate seed production. Mast fruiting at long intervals satiates seed predators and the surplus crop that escapes predation is sufficient to ensure regeneration (Janzen 1971). Whether the theory of predator satiation holds as the principal explanation for periodicity or not, it is a generally observed fact that a much smaller proportion of the seed crop is infested by insects during mast years than is the case in low production years. Accordingly, seed orchards managed for a stable annual seed production sometimes experience a build up of insect pest populations, where in natural populations most insect pests die out during interim years of low seed production (Hedlin *et al.* 1981).

Individual seed susceptibility or resistance to pests and diseases is influenced by their genotype, development stage and the environment, and the interaction between these factors.

Genetics

Genetic resistance to pests and diseases is well known among agricultural crops. Several cultivars of e.g. grain with resistance to specific seed pests have been developed by intensive breeding. Genetic resistance is, however, not well documented in tree crops. Variation in infestation rate by bruchids among genotypes has been reported in herbaceous *Vigna unguiculata* (Adjadi *et al.* 1985). Since the same type of insect attacks woody legumes, e.g. *Acacia*, *Prosopis* and *Albizia* spp., it is likely that genetic variation also exists in these species. Resistance, caused by differences in chemical compounds or seed-coat structure, is variable and subject to continuous change. In years with low rates of attack, relatively resistant genotypes may escape or be attacked very late; in years of heavy attack the whole population may be affected.

Development

Ovules and seed may be prone to predation and infection by pests and pathogens from the early stages of development onto full maturity. Early infection or infestation often causes abortion of the attacked ovules or the whole fruit (Janzen 1972). If the attack occurs later during fruit development, the fruit usually matures normally but then contains empty and damaged seed. Where cone or fruit tissue is attacked, it sometimes develops abnormalities or deformities (Hedlin *et al.* 1981). Some insects and pathogens attack the fruit or seed only during a relatively short stage of development, e.g. when the seeds have reached mature or near mature size but before they have developed a hard seed-coat. This is the case with a large group of bruchids that only attacks green immature seeds or pods; other bruchid species prefer to attack mature seeds (Johnson 1983, Southgate 1983, Bonner *et al.* 1994). Most true bugs (Hemiptera), in which the adults penetrate the seed-coat, are only able to infest the seed-coat while this is still young and soft (Hedlin *et al.* 1981). However, in *Sterculia apetala* and *Hymenaea courbaril* insect infestation occurs only during the later part of the maturation process, because resin accumulation in the green pods yield effective protection against the insects. Predation of *S. apetala* seeds by the bug *Dysdercus fasciatus* occurs only when the pods have begun to open (Janzen 1972). In this rain forest species the mature seed-coat does not prevent penetration by the bug.

Different insect species attack seeds of *Shorea* spp. during their development. Young seeds are attacked by *Nanophyes* spp. As the seeds enlarge, weevils of the genus *Alcidodes* attack them. Both of these weevil genera attack the seeds while they are still on the tree. As the seeds fall to the ground, they are attacked by scolytids (bark beetles) of the genus *Poecilips* (Singh 1976).
Stage of maturity at harvest influences seed susceptibility to especially

pathogen infection. Seeds that have not matured properly are often physiologically weaker, and failure of proper development of e.g. a protective seed-coat may give pests and pathogens easier access to the vulnerable interior of the seed. At the other end of the scale are seeds that have undergone progressive ageing. As will be discussed in connection with ageing in chapter 8, loss of viability is preceded by reduced vigour. Reduced vigour is e.g. reflected in susceptibility to infections of germinating seeds or their seedlings. For seeds stored with high moisture content like recalcitrant seeds, fungal infection in itself often contributes significantly to seed deterioration. Fungal infection is here a cause of deterioration rather than a symptom of ageing.

Environment

Specific climatic factors can promote or limit particular types of infection. A population of insects is influenced by such factors as reproductive success and predators (e.g. birds), which in turn may be influenced by weather conditions. Warm, sunny conditions may advance e.g. adult stages of infecting insects, which typically feed on flowers. Windy conditions may advance dispersal of fungal spores and sometimes also insects; humid conditions specifically benefit fungal development, with different species prevailing under warm and cold conditions respectively. The susceptibility of the individual seed may be influenced by its general physiological quality, the size of the seed crop (cf. the escape theory above) and rate of drying, which are all environmental impacts.

After harvest the most important impact that promotes infection is mechanical damage. Any crack or rupture in the seed-coat, occurring e.g. during mechanical processing, may serve as entry point for fungi. Temperature and moisture are of paramount importance during handling and storage. The lower the temperature and moisture content, the lower, generally, the rate of infection and infestation.

7.4 The Effect of Pests and Pathogens on Seed Quality

Infection or infestation may start in the fruit and affect mainly the fruit tissue. Insects and fungal infection of dry fruits like cones, capsules or dry multiple fruits may result in formation of web or fungal hyphae net (‘mycelium’) within and around the fruit or cone. Since the infection interferes with the natural dehiscent system (hygroscopic movement), the yield of seeds from such fruits or cones may become very low because of extraction problems, even where seeds themselves are not damaged.

In fleshy fruits, insect or micro-organism consumption and decomposition of the fruit flesh is generally considered beneficial for seed extraction. However, bulk decomposition often causes reduced viability of the seeds, either because of heat damage created by metabolism of the micro-organisms, or because fungi eventually infect the seeds (see chapter 6).

For the individual seed, the effect of infection depends on how much and which part of the seed is affected. Small damage to the endosperm or cotyledons may have little or no effect; and even where some part of the cotyledons has been eaten by larvae, the seedling may still survive and fully recover, albeit with some delay due to shortage of food reserve. Conversely, even minor damage to the vital part of the embryo like the radicle or hypocotyl rapidly causes the death of the seed (Lamprey *et al.* 1974).

Damage to the seed-coat only does not affect viability directly but may sometimes impair storability especially in cases where the pest or pathogen remains active during storage. On the other hand, in hard seeded legumes, insect damage to the seed-coat may help in overcoming physical dormancy. In cases where e.g. bruchid larvae have not damaged the delicate embryo axis, but only part of the cotyledons, the overall effect of infestation may be beneficial to germination (Lamprey *et al.* 1974, Halevy 1974). A similar positive effect has been observed after moderate fungal infection of *Pinus bungeana* seeds. The fungi were believed to break physical dormancy by degradation of pectin, cellulose and other compounds in the seed-coat (Li-feng *et al.* 1987).

Seed infecting or infesting organisms metabolise much more intensively than the seed itself at low moisture content. During their metabolism water and heat are released, both of which are conducive to micro-organism activity. Fungal development in particular may thus tend to be self accelerating. Fungi easily spread from one seed to another by their mycelium. For seed insects, spread from infested seeds to other seeds in the seed lot depends on the ability of the insects to reinfest seeds in storage, which in turn depends on the ability of the larvae to penetrate fully mature seed-coats when hatched, plus the ability of adult insects to complete their life cycle, i.e. survive and mate, within the seed lot where nutrients other than those in the seed are absent (see example of bruchids below).

Where conditions are favourable for the development and spread of pests or pathogens in storage, the whole seed lot may easily be lost.

7.5 Seed Insects

A wide range of insect species feeds on seed; most species belong to the groups beetles, bugs, moths, butterflies and wasps (section 7.5.1). Insects undergo a metamorphosis, i.e. a dramatic change of form from larva to adult, which for most groups occurs during a pupal stage (complete metamorphosis). In a few others like true bugs, a gradual (or incomplete) metamorphosis occurs with interim nymph stages. Where insect species undergo complete metamorphosis, seeds are most often exclusively fed upon by the larval stage, while adult insects typically feed on more easily digestible material such as flowers or nectar. Of species with gradual metamorphosis like the bug *Dysdercus* spp. both adults and nymphs feed on the seed by puncturing the seed-coat with their proboscis, injecting saliva and sucking out the mixture (Janzen 1972).

Seed insects can cause considerable lost seed production in the field and sometimes continue to do so during storage (Andersen 1988). Several generations of bruchid beetles in a seed lot of *Acacia* spp.

may completely destroy the crop (Doran *et al.* 1983). A weevil of the genus *Nanophyes* is reported to have destroyed up to 60% of the seed crop of *Terminalia ivorensis* in West Africa (Lamb and Ntima 1971). Other *Nanophyes* species cause heavy predation on dipterocarps in Malaysia. These and other insect pests may cause 70-95% loss of the seed in that area (Singh 1976).

The life cycle of seed insects is closely connected to the reproductive phenology of the host tree. This is especially crucial in species where host susceptibility is of short duration and where there is high host specificity on the part of the infesting insect. Survival conditions of the seed insect outside the infestive period are often decisive for the infestation rate. A precondition for a heavy infestation rate is a large population of adult individuals that can lay their eggs in/on the flowers, fruits or seeds. A prerequisite for a large population of adults is a successful previous infestation plus good survival of the juvenile stage.

7.5.1 Important seed insect orders, families and genera

The diversity of insect species is vast and far exceeds that of plant species. Yet the number of known seed-feeding insect species is small. Taxonomically related plant species often share the same type of insect pests, e.g. bruchids on legumes, *Megastigmus* on conifers, but many insect families and genera infest seeds of plants belonging to very different plant families. The majority of seed pests belong to the following five orders.

Coleoptera (Beetles)

Beetles have hard leathery fore wings (elytra) and membraneous hind wings used for flight and folded under the elytra when resting. Larvae have a distinct head capsule and usually thoracic legs. Both adult and larva have chewing mouth parts. The pupa resembles the adult. Important families are:

Bruchidae (bruchids) with 56 genera of which the most common are *Brunchus, Brunchidius, Tuberculobruchus, Conicobruchus, Caryedon, Callosobruchus, Acenthoscelides*. Bruchids are the prevalent pest in Leguminosae such as *Acacia, Prosopis, Albizia, Brachystegia, Dalbergia* spp., but are also found in e.g. *Cordia alliodora,* some *Eucalyptus* spp. and palms. Species of the subfamily Pachymerinae feed almost exclusively on palm seeds in America (Johnson *et al.* 1995). Bruchid species have been found in 32 plant families (Johnson 1983).
Curculionidae (weevils and snout beetles). An important genus is *Nanophyes,* which occurs throughout the tropics infesting e.g. *Terminalia ivorensis* in West Africa and dipterocarps in Malaysia (ref. see above). *Alcidodes dipterocarpi* attack *Shorea* spp. in Thailand (Eungwijarnpanya and Hedlin 1984), and *Apion* spp. feed on *Triplochiton scleroxylon*.
Scolytidae (bark beetles). The most common genus is *Conophteorus*, which causes vast destruction of North American pine cones (Hedlin *et al.* 1981).

Hemiptera (Plant bugs)

True bugs have leathery fore wings with membraneous tips; hind wings membraneous and shorter than fore wings. The wings lie flat over the abdomen. The mouthparts consist of a bundle of needle-like stylets within a segmented sheath which forms a beak or proboscis. During feeding the bug inserts its proboscis into the food material; salivary fluid dissolves the food which is then sucked up through the duct of the proboscis. Metamorphosis is gradual with the immature forms of nymphs resembling the adult in form and feeding habit.
Dysdercus spp. attack mainly species of the plant order Malvales (Sterculiaceae, Malvaceae, Bombacaceae, Tiliaceae). *Zulubius acaciaphagus* causes heavy attack on *Acacia cyclops* in S. Africa (Holmes and Rebelo 1988).

Lepidoptera (Moths and butterflies)

Adults with four membraneous wings almost wholly covered with minute overlapping scales. Mouthparts of most species consist of a long tube-like structure, coiled up when not in use. Adults usually feeding on flower nectar are important pollinators. Antennae are thread or feather like. Larvae (caterpillars) are usually cylindrical with three pairs of thoracic legs and up to five pairs of abdominal legs. Well developed heads with chewing mouthparts. Larvae produce silk cocoons. Pupae are compact with appendages fused to the body. Important families are:

Olethreutidae. This family of moths includes a large number of cone predators. Species of the genera *Barbara*, *Eucosma*, and *Laspeyresia* cause great damage to conifers in North and Central America (Hedlin *et al.* 1981). *Ofatulena* spp. feed on *Prosopis* flowers and fruits. *Cryptophlebia carpophagoides* is a South American species that attacks the pods and causes heavy loss in leguminous species like *Enterolobium contortisiliquum* in Argentina and *Prosopis tamarugo* and *P. juliflora* in Chile (Kock and Campos 1978, quoted in Johnson 1983). *Leptotes trigemmatus* feeds on *P. tamarugo* in Chile.

Paralidae. The main genus attacking conifers is *Dioryctria* (coneworms), which infest cones and seeds as well as flowers and young shoots. It occurs both in the temperate and tropical region. *Dichocrocis punctiferalis* infests seed of *Tectona grandis* in Thailand (Eungwijarnpanya and Hedlin 1984). *Agathiphaga* is a moth that infests cones and seeds of *Agathis* spp. (Willan 1991).

Diptera (Flies)

Adults have a single pair of membraneous front wings, hind wings are reduced. Mouthparts are modified for piercing and sponging. The larva is legless and its head retracted into the thorax. Important families with members causing cone destruction in North American conifers are Cecidomyiidae, Anthomyiidae and Lonchaeidae (Hedlin *et al.* 1981).

Hymenoptera (Wasps, sawflies and seed chalcids)

Adults have four membraneous wings, the hind wings being distinctly smaller than the forewings. Wing venation is sparse. Mouthparts of the chewing type. Larvae differ: head capsules and legs are present in some families, absent in others. Pupa, frequently encased in a cocoon, resembles the adult. A single genus *Megastigmus* in the family Torymidae comprises a number of seed pests.
Megastigmus species are quite specialized with regard to host species, method of attack and feeding habit (Hedlin *et al.* 1981). They are common seed pests of conifers and also occur in e.g. *Eucalyptus* spp. (Eldridge *et al.* 1994) and *Sesbania grandiflora* (Eungwijarnpanya and Hedlin 1984). The female insect has a long ovipositor (egg laying organ) by which it penetrates the fruit and seed-coat to lay its eggs within the developing seed. The number of eggs varies from one to several per seed. The larvae feed inside the developing seed and pupation takes place within the seed. Infestation can thus not normally be recognized on the outside of the seed. The adult insect emerges via a round exit hole, which it chews through the seed-coat and sometimes through the fruit as well.

7.5.2 Life cycles and species specificity

Apart from the relatively few tree species with diffuse flowering and fruiting, most plant species only bear fruits during a short period of the year, and periodic species only at long intervals. Hence, insects must adjust to the reproductive cycle of their host plants, be able to survive periods where fruits and seeds are not available, and detect a reproductive event sufficiently early to take the full opportunity to infest the maturing fruits. Seed insects may survive the interim period between two seed crops in various ways e.g.:

1. A prolonged dormant pupal stage, inside or outside the seed.
2. An adult stage in which the insect is dormant or feeds on other food sources, e.g. flowers.
3. A prolonged infestative period where the insect feeds on seeds in soil seed banks, or in artificial seed stores.
4. A shift in predation to other species the seed of which are available at different times of the year.

Species with a narrow host range (pronounced host specificity) with a relatively short infestative period often have a long dormancy period (diapause) between the infestations. For example, many bruchid species spend a dormant stage as pupae, but some species that can infest mature seeds have a rapid generation turnover if seeds are abundant. The seed weevil *Sitophilus (Calandra) rugicollis*, which attacks seeds of *Shorea robusta*, *Syzygium cumini*, *Dipterocarpus allatus* and *Polyalthia longifolia* in India, survives as a dormant adult in the forest floor and emerges with the first monsoon rain, which coincides with the commencement of seed fall (Khatua and Chakrabarti 1990).

As an example of an insect life cycle, the cycle of a bruchid beetle is illustrated in fig. 7.1. Some variation occurs between different species.

For example, pupation within the seed is most frequent, but in some large species like *Caryedon*, the larva leaves the seed after completion of its development and pupates in the soil, or in the case of seed stores, outside and adhering to the seeds (Southgate 1983, El Atta 1993). The time of generation turnover and life cycles depends on species and the environment. Under favourable environmental conditions some species may complete their life cycle in little more than a month whilst others may take several months. Multivoltine species (those having several generations per year) emerge quickly after pupation while univoltine (one generation per year) stay dormant within the cocoon for several months, i.e. until the next seed season. Univoltine behaviour is accordingly typical for the group of species which only infests green pods or immature seeds, and must hence await a new green crop. In multivoltine species the insects mate and infest mature seeds shortly after their emergence. The adult stage is normally very short (< 2 weeks). Species of bruchids that infest only green pods may cause considerable loss of seeds in the field, but as their damage is limited to the seeds already infested at collection, the total loss is smaller and storage conditions are less critical for those seeds than for those where infestation may continue during storage. However, as the pupae may survive in the seeds, there is a risk that surviving insects may spread with the seed if exported into areas where the species is not originally present, cf. phytosanitary problems section 7.8. Because this group of species infests seeds before full maturity, infestation cannot generally be avoided by early collection (Southgate 1983).

Life cycles and species specificity are closely connected in the sense that the more host specific the insect, the more tightly it must be connected to the life cycle of the host. Some insects have adapted closely to one host. In some Latin American forests *Sterculia apetala* is attacked primarily by the bug *Dysdercus fasciatus* which in turn is not known from any other tree species (Janzen 1972). Similarly, *Conophthorus ponderosae* mainly attacks cones of *Pinus ponderosa* (Bohart and Koerber 1972). A high degree of host specificity is prevalent in bruchids attacking seeds of legumes. *Pseudopachymerina spinnipes* attacks almost exclusively *Acacia farnesiana* and *A. caven* (Southgate 1983). *Bruchidius albosparsus* is commonly associated with *Acacia tortilis* subsp. *raddiana*, and *Caryedon serratus* with *Acacia gerrardii* ssp. *negevensis* in Israel (Halevy 1974). From different regions in Latin America, Africa and India it was found that more than half of the bruchid species have only one host species and only few have many hosts (Labeyrie 1981, Janzen 1977, Singh and Bhandari 1988). Some bruchid species do, however, have a wider host range. *Caryedon gonagra* has been found in several species of *Acacia*, *Albizia*, *Prosopis* and *Cassia* plus *Tamarindus indica* (Singh and Bhandari 1988). Sometimes seed insects show preference for one species but may shift to other species if the number of main hosts is insufficient. In other cases host specificity is less pronounced in areas where several susceptible hosts occur. For example, the above mentioned *Caryedon serratus* has a wider host species range in India including e.g. *Acacia tortilis*, *A. nilotica* and *Albizia lebbeck* (El Atta 1993), and in Thailand the species feeds on both *Bauhinia* and *Cassia* species (Eungwijarnpanya and Hedlin 1984).

An illustration of host - insect specificity is given by Ernst *et al.* (1990): In Botswana *Bruchidius uberatus* is the only bruchid species affecting seeds of *Acacia nilotica* although several other species of bruchids occur in the area; in the Sudan *B. uberatus* is the prime infesting species but several other species also attack seeds of *A. nilotica*. In Botswana *A. nilotica* is the main host species of *B. uberatus*, but in other parts of Africa the bruchid attacks several other *Acacia* species. In a storage-infesting experiment in Botswana, *B. uberatus* showed a strong preference for *A. nilotica* seeds (75% infestation), but also attacked seeds of *A. tortilis*, *A. mellifera*, *A. burkei*, *A. erioloba*, *A. hebeclada* and *A. robusta* (all less than 25%). Some other species were not attacked at all. The example shows that host-insect specificity is not always absolute.

An account of host-insect specificity of the *Prosopis*-bruchid association in the Americas is given by Johnson (1983). Despite the above mentioned non-absolute nature of infestation patterns, and the inevitable problem that the full host range can rarely be proven, such host-predator specificity is useful in seed source management and seed handling. For example, seed insects with a wide host range are more likely to spread from one species to another during storage than host-specific species. Further, where one main seed pest prevails, it is easier to take preventive measures to avoid infestation in the field, e.g. treatment during a specific flying time of the insect.

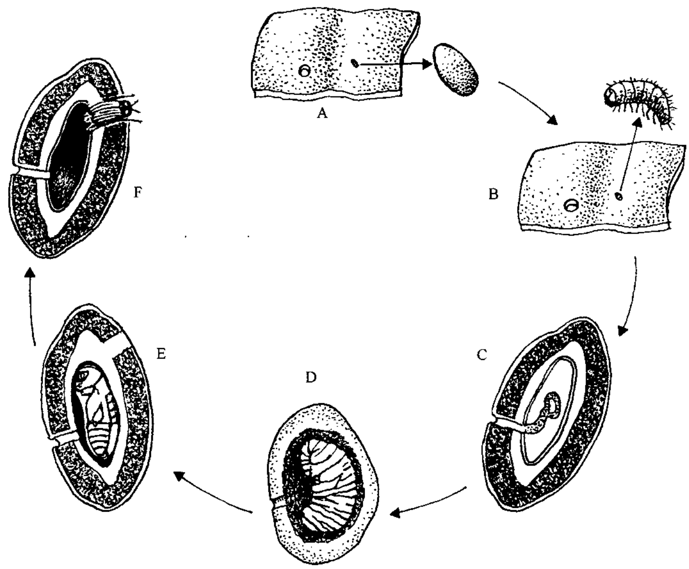

Figure 7.1.
Life cycle of bruchid beetle, here on *Prosopis* sp.
A. Eggs glued to pod surface or laid in cracks in pod or in emergence holes of an adult bruchid (round holes).
B. Entry holes of the first stage larvae that have burrowed through pod wall and first stage larva enlarged to show hairs, spines and legs which are modified for entering seeds.
C. Cross section of pod and seed showing the burrow made by the entering first stage larva.
D. Later stage larva inside cavity chewed in seed.
E. Pupa inside larval feeding chamber. The larva penetrates the testa except for a thin 'window' before it pupates.
F. Adult emerging through hole prepared by last stage larva.
(From Johnson 1983).

7.5.3 Storage insects

Insect larvae that have infested seeds in the field may continue their predation in storage. However, only species that are able to breed and re-infest seeds in storage can be considered true storage pests. Most insects are unable to re-infest seeds because the adult insect cannot survive and mate under storage conditions. Alternatively, the infesting insect stage (larvae or adult) may be unable to penetrate the seed-coat during infestation.

Despite the hard seed-coat of legumes, a small group of bruchids are able to re-infest seeds during storage and pass several generations until the whole seed lot is destroyed, provided temperature is conducive to their survival and continuous activity. The adults of these bruchids need no food intake for reproduction (Southgate 1983).

Some species are field pests as well as storage pest. An example is *Caryedon gonagra* which attacks young developing seeds on the growing plant as well as stored seeds (Singh and Bhandari 1988). *C. serratus* showed a relatively low field infestation rate on *Acacia nilotica* in the Sudan (ranging from 10% on standing trees to 17% on the forest floor), but the infestation increased to 90% after 3 months' storage. Under storage conditions the following life cycle was revealed (El Atta 1993):

1. Egg incubation period, i.e. from oviposition to hatching, was 7-16 days.
2. Larval feeding; 4 larval stages ('instars') with average durations of 12.4, 10.6, 11.5 and 7.2 days respectively were recognized, i.e. a total feeding period of approx. 42 days.
3. Metamorphosis and pupal stage, 10-15 days.
4. Emergence and mating, 1 day
5. Time from mating to new oviposition, 2-3 days.

The total life cycle for this species under the given conditions is thus 63-76 days. The average number of eggs per female in the investigation was 93 of which some 80% hatched. However, as 12-25 eggs were laid per seed by this bruchid species, each female may have infested on average only 5-6 seeds. Hence, with a low initial infestation rate, several generations may be necessary for a total destruction of the seed lot. Storage under ambient conditions for less than one generation turnover (approx. 2 months in the example) may be acceptable if the initial infestation rate is low.

The life cycle of 2-2½ months given in the example above relates to optimal conditions for insect activity. A much longer life cycle would be expected where temperature and moisture content are low. The activity of insects during low temperatures varies with their specific environmental adaptations. Many tropical species show little activity below 15°C, while subtropical and high-altitude species may be active at temperatures down to a few degrees above zero. Moisture content may be limiting for feeding, but in this respect there are also large variations. While a moisture content below 10% is limiting for many field-infesting insects, others such as several bruchid species remain active at lower moisture content. However, though the activity of some insects may not be stopped altogether under a given storage regime, any reduction of temperature and moisture content below the physiologically optimal will delay their development. For example, the duration of reproductive cycles may be doubled or tripled under conditions of reduced temperature. It has also been suggested that insects will lay more eggs in a dark and damp store than in a light and dry one (Singh and Bhandari 1988).

Figure 7.2. Bruchid infestation of acacia seeds.

7.5.4 Control of seed insect infestation

Several methods are available in order to minimize destruction by seed insects. Generally, the earlier the control measure is implemented, the smaller the destruction. However, as insects may attack at any time during seed handling, a low initial infestation rate does not guarantee that the seed lot remains free from insects. Control measures must thus be observed throughout the seed handling process.

Control of infestation in the field

Collecting seeds which are free of, or have only a small infestation, can save a lot of work. Usually heavy crops and early crops are attacked much less than small and late ones. In *Cordia alliodora*, infestation by the bruchid genus *Amblycerus* was reduced by collecting the fruits three weeks before normal seed fall (Tschinkel 1967 quoted in Willan 1991). Also in many legumes infestation by bruchids may be reduced by collecting green and immature pods, which are then after-ripened under ambient conditions before seed extraction (Doran *et al.* 1983) (see also chapter 3 and 6).

When seeds are shed from the trees, they are exposed to soil living pests and pathogens in addition to those which may be present on the parent tree. Seed predation beneath the parent tree is often severe; collection of naturally fallen fruits often implies a risk of heavy infection and infestation (cf. chapter 4).

Protection against seed insects by shielding of individual fruit-bearing branches with nets has been used in *Acacia* spp. but is very labour intensive and applicable only on a small scale, e.g. in seed orchards or for research (Southgate 1983). Various types of chemical protection are available. In India, field infestation has been controlled by spraying individual fruit-bearing branches or trees with 0.25% endosulfan or fenitrothion water emulsions, or 0.05% monocrotophos or dichlorvos water emulsion (Singh and Bhandari 1987).

Several problems and limitations are, however, connected to the use of chemical spraying:

1. Spraying is only applicable to accessible fruits, i.e. relatively low trees and shrubs, unless aerial spraying is pertinent (Singh 1976).
2. The effect of the chemical treatment cannot be limited to the target pest, but will frequently affect harmless or beneficial organisms, e.g. seed pest predators or pollinators. Birds feeding on seeds or insects may also be affected.
3. Spraying cannot be applied to seed sources where there is public access, or to species whose seeds or fruits are used for consumption. For example, spraying of *Acacia nilotica* pods was discouraged in areas where the pods were used for local medicine or fodder for domestic animals (El Atta 1993).
4. It is difficult to apply the chemical in a dosage where it is effective over a long period.
5. Chemicals may have detrimental side effects on the tree as well as the surrounding environment, cf. 2.

In North America, Douglas-fir (*Pseudotsuga menziesii*) is susceptible to attack by e.g. coneworm (*Dioryctria reniculelloides*). An indirect effect on seed production is exerted by the bud worm (*Choristoneura occidentalis*) through its destruction of tree foliage. Repeated defoliations deplete the nutrient reserves and render the trees unable to produce cones and seeds. A field treatment against the two pests consists of implanting capsules containing 0.875g acephate insecticide into the sapwood every 10 cm around the tree bole, 1 m above the ground (Koerber 1989).

Where chemical treatment is used in the field, the timing of application is crucial both to achieve the optimal effect of the treatment (and hence the best economical result) and to avoid harmful side effects. For insect pollinated species, insecticide application during flowering should be avoided, as it may kill pollinators (Johnson 1983). On the other hand, insect larvae that have already entered into the interior of seeds may not be affected by treatment of the outside of fruits. Appropriate timing is especially difficult in species with prolonged flowering and where insects attack at an early stage of development. Generally, chemical control is only applicable in managed seed sources.

Insect elimination during processing

Elimination or reduction of insect infested seeds is particularly pertinent in cases where the insects are able to continue destruction and re-infestation of seeds under storage conditions. Even if the seed lot cannot be totally free from seed insects, a reduction of their population will greatly limit the re-infestation rate. Seed insects may be eliminated either by removing infested seeds, or by killing the insects inside the seeds.

During extraction procedures where fruits are exposed to high temperatures e.g. kiln drying, any stage of insect, whether adult, pupa or larva, present inside the fruit or seed is normally killed. In other words, species normally extracted by this method e.g. serotinous cones (chapter 6), are normally insect free after processing. In tree species where seeds are tolerant to a brief high temperature exposure, the method may be used as a treatment. Low temperature may also be effective in killing seed insects inside infested seeds. In Argentina, a cold treatment of -18°C for 10 days effectively killed all stages of bruchids in infested seeds of *Prosopis chilensis* (Mazzuferi *et al.* 1991). Both high and low temperature treatment must be carried out with due consideration to the seed tolerance. It should be recalled from chapter 6, that the higher the moisture content, the more sensitive seeds generally are to temperature extremes, especially high temperature. Therefore, temperature treatment for insect elimination is applicable only to orthodox and possibly intermediate seeds. Tropical recalcitrant seeds are not tolerant to the high or low temperature levels which are likely to kill infesting insects. Effective killing temperature varies according to the environmental adaptation of the particular insect species and with their ontogenetic stage of development. A temperature of 5°C was able to delay the development of bruchids in *Prosopis chilensis* but did not kill the insects (Stoyanova 1984).

Infested seeds are preferably removed from the seed lot before storage both to increase purity and quality of the seed lot, and to prevent them harbouring storage fungi, which may spread in the seed lot. Insect-infested seeds may be separated from sound seeds during the seed cleaning process if their physical properties differ. For example, where seed insects have consumed most of the interior of the seeds, they are normally lighter than the sound seeds and may be removed by blowing or flotation. An initial assessment of the infestation rate is appropriate. Where insect larvae live inside the seeds, their presence may sometimes only be detectable by X-ray tests (see chapter 11).

Storage conditions

Where seeds are insect-free when entering into storage, it is important that they remain so by excluding any later infestation. Left over seeds from a previous seed lot or insects (typically adults or pupae) left in cracks, corners, old containers etc. may also carry a potential source of seed destruction for a new stored seed lot. Thorough cleaning and possible disinfection of storerooms, bags and containers are measures to prevent transmission from a previous seed lot (Sing and Bhandari 1988). Where seeds are stored at ambient temperature, mechanical barriers such as plastic sheets or insect nets may prevent the entrance of flying insects and hence infestation (Howe 1972). However, whilst e.g. polyethylene sheet may be effective in preventing insects entering into the bags, escaping insects may easily penetrate the sheet upon departure. Such exit holes may impair seed moisture conditions by permitting free gaseous exchange (see chapter 8).

The most efficient way of reducing insect damage during storage is by appropriate drying and storing at low temperature. Although storage insects are able to feed and infest seed at low moisture content, the activity of most species is reduced in very dry seed. Any reduction in temperature is also likely to reduce insect activity. For most tropical species there are little activity under 5-7°C, but even a smaller temperature reduction may lower both general activity and development. However, whenever the insects metabolize, they produce heat and water. They may thereby improve the micro-environment in their immediate vicinity, which in turn may promote their activity (Singh and Bhandari 1988).

7.5.5 Seed treatment

Where seed insects cannot be eliminated during processing, and where there is a risk that insect predation may continue or accelerate during storage, application of toxic or insect repellent treatment is applicable.

Fumigation

Fumigation denotes application of a metabolic inhibitory or toxic alloy in gaseous form. The method is used in other connections of plant propagation, e.g. sterilization of nursery soil. The advantages and disadvantages of fumigation, as compared to other seed treatment methods, like insecticide powders, relate to their volatile nature:

1. The effect is usually quick since the gas will always reach the target organism, unless it is deeply hidden within the seed.
2. Insects absorb gases through the 'skin'. The more metabolic activity, the larger and quicker the effect. Accordingly, fumigation has greater effect on adult stages and feeding larvae than on eggs and pupae; and the effect increases with temperature within the physiological limits.
3. The gases normally have no effect after they have escaped from the seeds.
4. Since the gases do not adhere to the seeds, no cleaning or preventive measures need to be taken after treatment, e.g. for export or during sowing.
5. The possible toxicity to humans is unfortunately easily overlooked because the gases are often invisible and without smell.
6. A prerequisite for application is that facilities and material impermeable to gases are available.

Several fumigants with proven effect on insect control are available. The most common ones are ethylene bromide, hydrocyanic gas, a mixture of carbon disulphide and carbon tetra-chloride, phosphine and pirimiphs (Willan 1991). For bruchid beetles in *Acacia tortilis*, fumigation with carbon disulphide, aluminium phosphide or chlorosal (a mixture of three part ethylene chloride and one part carbon tetra-chloride) has been used in India (Singh and Bhandari 1987). The above fumigants are all toxic to humans and should be handled with utmost care, and only by authorized staff using safety protection. Further, most of the fumigants are phytotoxic, so prolonged exposure of the seed should be avoided (Singh and Bhandari 1988).

One non-toxic gas, CO_2 has been successfully used for seed treatment of many species of orthodox seeds and is described in detail here.

Because CO_2 is harmless to dry seeds, the seeds can be stored with the gas for prolonged periods (Sary *et al.* 1993). CO_2 is a product of aerobic respiration. In a normal atmosphere it makes up some 0.03%, while in comparison O_2 makes up some 20%. CO_2 is non-toxic to the insects, but when present in large concentrations relative to oxygen, it blocks some physiological pathways of the respiration system. Since dry seeds are not metabolicly active at low moisture content, CO_2 is harmless to dry seeds whereas living insects are killed in an atmosphere of high CO_2 and low O_2 content. Adult and larval stages have the highest rate of respiration and are easier to kill. Pupae have active metabolism during their metamorphosis, but can also be dormant and therefore more resistant. Seeds with high moisture content and hence metabolism, e.g. recalcitrant seeds, do not tolerate prolonged exposure to CO_2 fumigation. In Australia a maximum of 10 days' fumigation with CO_2 is recommended for recalcitrant seeds (ATSC 1995).

Some technical details of CO_2 application equipment are given in appendix A7.1. The method of application is summarized here according to Sary et al. (1993):

The dry seeds are put into a plastic bag made of CO_2 impermeable and heat-sealable plastic sheet (see details A7.1). The bag is kept upright with its opening as flat as possible, yet allowing the air to escape as CO_2 is introduced. CO_2 is introduced through a tube into the bottom of the bag (see fig. 7.3). Because CO_2 is heavier than atmospheric air, it will flush out the air while filling the bag. It is recommended to flush the bags with twice the volume of the empty bag, e.g. 8 litres of CO_2 flushed into a bag of 4 litres. Flowing rate at a given pressure can be read directly on a flowmeter. Where a pressure reduction meter is used, filling an empty bag of known volume initially checks the flowing rate per minute. For larger bags it is advisable to seal the major part of the opening and only leave space for inserting the filling tube. Immediately after filling the bag is sealed with a heat sealer. A vacuum will develop as the seed absorbs CO_2. The sealed bag must initially be stored at room temperature for about 8 weeks. This is to assure a sufficiently high metabolic rate of the insects, which might otherwise be inhibited by low temperature.

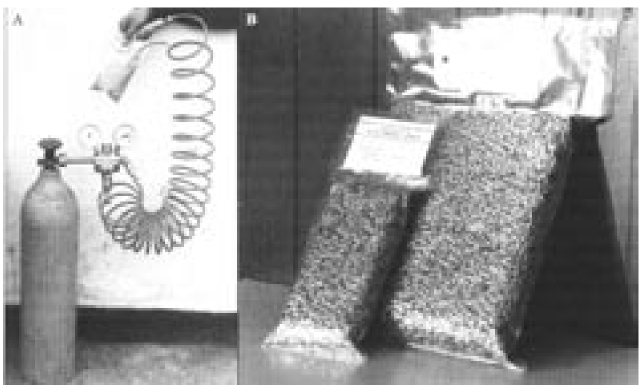

Figure 7.3.
Fumigation with CO_2.
A. Application of CO_2 to bag with dry seeds. CO_2 is heavier than atmospheric air, and when the gas is placed at the bottom of the bag, it will replace normal air and fill the inter-seed air space of the bag.
B. Bags of seeds after fumigation; because the seeds absorb CO_2, a vacuum is created. In these bags seeds were only filled to about 3/4 of the capacity so that space was left above the seeds to insert a seed label.

Insecticides

Insecticides may be used as an alternative to fumigation, or where a long-term effect is desired e.g. if hidden and dormant stages may escape a short treatment and appear later during storage. Several insecticides are available. Most of them have been developed and mainly used for agricultural seeds, e.g. so-called grain protectants. Application is normally in the form of dust where the seeds are mixed with the dry powdered chemical. Some insecticides have been banned or restricted, particularly in western countries, for environmental reasons. This pertains in particular to the group of chemicals known as chlorinated hydrocarbons which contains e.g. Lindane. DDT, Aldrin, Endrin and Chlordane. Most of these products are no longer produced and available in Europe and the United States but may still be found in a number of tropical countries. Their use should generally be discouraged and an environmentally less dangerous product used where necessary.

Organo-phosphate insecticides are environmentally safer. They contain a wide toxicity range; some are extremely poisonous to humans while others are relatively harmless. Among the moderate toxic ones is Phenitrothion, a relatively common seed insecticide known under various trade names such as Cytel and Folithion. In India, dusting of gunny bags with 5 % folithion dust for short term storage and 10 % for long term storage was recommended for control of bruchid beetles in *Acacia tortilis* seed in storage (Singh and Bhandari 1987). Phorate and malathion are other organo-phosphate seed insecticides with relatively wide use (Singh and Bhandari 1988, Creemer 1990). Among the environmentally safest insecticides is pyrethrum; originally it was extracted from flower heads of the herb *Chrysanthemum cinerariaefolium*, now a synthetic equivalent to the flower extract is manufactured. Pyrethrum insecticides such as pyrethin dust mixed with seeds are used in e.g. India (Singh and Bhandari 1988) and Australia (Cremer 1990).

Biological methods

Some plant species contain alleged insect repellent compounds, which have been traditionally used for seed protection in storage (table 7.1). Several of these species release a strong odour which is apparently avoided by insects. The plants or extracts often do not kill the insects or affect the activity of larvae already present within the seeds. In most cases the repellent effect is merely due to the odour. The efficacy of most traditional plants still needs to be proven. Golop and Webley (1980, quoted in Johnson 1983) also list a number of minerals with alleged insect repellent or insecticidal effects. These include diatomite, termite mound soil, kaolin and lime. Apart from pyrethrum mentioned above one of the most effective plants with insecticidal effect is neem (*Azadirachta indica*). Neem seeds have a particularly high concentration of the active compound, azadiractin, and crushed seeds or oil are especially effective. However, also other parts of the plants have proved effective (Soon and Bottrell 1994).

Another biological insect control method is through trapping, where the insects are attracted by pheromones (a group of female sex hormones which attracts male insects). Practical application of the method has not been documented for seed insects. Also the use of seed-insect predators or parasitoids contains a largely unused biological potential (Southgate 1983).

The main advantages of the biological methods are that they are likely to be non-toxic and hence safe for both labourers and the environment plus, for plants/ plant extracts, that they are often locally available.

Table 7.1.
Plant and plant parts used for insect control in storage, particularly for bruchids (Golob and Webley 1980, quoted in Johnson 1983).

Plant species	Plant part or extract used
Azadirachta indica	neem kernels, seed oil, powdered leaves or bark
Chrysanthemum cinerariaefolium (pyrethrum)	whole plants or flower heads
Capsicum	pepper chillies
Cactus spp.	stem powder
Anona reticulata	custard apple seed powder
Mundulia sericca	stem bark powder
Piper nigrum	black pepper powder, extract
Madhura latifolia	stem bark powder
Acorus calamus	rhizome powder, oil
Thevetia nerifolia	powder of drupes
Adhatoda vasica	leaf powder
Ipomea cornea	leaf powder
Derris elliptica	oil
Pogostemon heyneanus	Pachouli oil
Nigella sativa	black cumin oil
Phaseolus vulgaris	bean oil
Allium cipa and *A. sativum*	oil

7.6 Fungal Infections

Fungal infection may occur on any part of the plant. A large group of fungi, the saprophytes, infect dead plant material and are hence a natural part of the decomposition of dead tissue. Conversely, pathogenic or parasitic fungi infect living tissue causing fungal disease which may ultimate kill the plant or the infected part. There is no clear distinction, however, between saprophytes and parasites since many fungi can live on both living and dead tissue, and all parasites are able to live some part of their life cycle on dead tissue. Fungi may be grouped into four classes according to their preferred growth habit: **obligate parasites** have parasitism as a necessary part of their life cycles; **facultative saprophytes** live mainly as parasites, i.e. on living plant material, but are able to complete their life cycle as saprophytes, typically when their infection has caused the death of the plant or tissue; **facultative parasites** live mainly as saprophytes, but they may occasionally infect living tissue, hence being opportunistic parasites (Halloin 1986). The last group, **obligate saprophytes** is of less importance in seed handling because they do not cause infection of living seeds.

Fungi multiply by spores, sometimes of different types. Spores are produced in vast numbers; they are tiny, long-lived and usually dispersed by wind, which can carry them over long distances. Once the spores are deposited on a suitable substrate, provided temperature and humidity are appropriate, the spores may germinate and form minute thread-like filaments 'hyphae' that penetrate into the plant tissue. Hyphae and their aggregate network 'mycelium' make up the vegetative stage of the fungus. Whereas the fungal spores are relatively resistant to adverse environments (e.g. drought), the hyphae normally grow only under high moisture conditions and warm temperatures (see section 7.6.4). The hyphae penetrate between and within cells while absorbing organic material. However, for most pathogenic fungi the damage to the host plant is not so much caused by the depletion of nutrients as by damage to the cell caused by the release of enzymes and toxic metabolites by the infecting fungus (Halloin 1986, Vijayan and Rehill 1990). Some of the fungal exudates cause damage to the cell membranes, others inhibit vital life processes of the germinating seeds. A moderate infection may reduce germination energy and affect embryo development during germination, e.g. causing malformation or discolouration (inhibition of the chlorophyll synthesis) of the seedling (Christensen 1973, Halloin 1986). Infection of the radicle of germinating *Pinus* spp. by the fungi *Alternaria alternata*, *Aspergillus*, *Penicillium* and *Trichoderma* spp. either killed or caused temporary setback of the seedling. However, some seedlings were able to fully recover from the attack (Rees and Phillips 1986).

Fungi may spread over short distances, e.g. from one seed to another in a seed lot, by the mycelium (cf inoculation with mychorrhiza, chapter 11). As the fungus grows and completes its life cycle, it forms new spores which may infect new plant parts on the spot or be dispersed into other areas. The time to complete the life cycle depends on the species and the conditions surrounding it. Many fungi multiply primarily vegetatively when conditions of moisture and temperature are optimal for growth, while spore formation becomes important under stressed conditions, e.g. when food supply is depleted or under suboptimal temperature and moisture conditions. Many fungi are able to survive long periods as spores. The severity of fungal attack is proportional with the initial infection rate (inoculation) plus the condition during growth and development.

7.6.1 Fungus types and species specificity

Fungi may be classified as seed-borne or seed-transmitted according to the general terminology of pathogens: seed-borne fungi include all fungal types contaminating the surface of the seed or infecting its tissue. Seed-transmitted fungi cause no infection of the seed itself but only infect seedlings in the nursery or in the field (Neergaard 1979). Because fungal spores are almost omnipresent and have a high resistance to adverse environments, probably most fungi are seed transmitted to some degree, but in most cases they are harmless because of innate resistance of the plant. Many fungal species are primarily dispersed by wind or nursery soil and are only occasionally seed borne. The risk of seed-borne pathogens infecting living plants also depends on mode of infection. For example, fungal types that primarily attack the reproductive structures, e.g. some seed rust and smut fungi are easily transmitted with fruits and seeds, but their likelihood of being dispersed from the seed back to a susceptible flower or fruit is small. On the other hand, pathogens which primarily infect young plants ('nursery pathogens') are less likely to be introduced into the seed lot, unless seeds are collected from the ground, but once introduced their dispersal and infection can be very fast. In cases where seeds carry pathogens capable of infecting newly germinated plants, the route from seed transmission to infection is short.

Most fungi infecting fruits and seeds in the field require relatively high humidity for germination and growth. Typical field fungi genera are *Fusarium*, *Botrytis*, *Ciboria*, *Sclerotina*, *Phomopsis*, *Valsa* and *Gloesporium* (Bonner *et al.* 1994). Most storage fungi attacking orthodox seeds belong to the genera *Aspergillus* and *Penicillium* (see below). In the Philippines, Dayan (1986) found 17 fungal species associated with orthodox seeds. Mohanan and Sharma (1991) have listed seed-borne micro-organisms (including fungi) for a number of indigenous and exotic trees in India, and an extensive worldwide list of micro-organisms associated with tree seeds is summarised by Mittal *et al.* (1990).

An example of a seed-transmitted disease is 'damping off', a common nursery disease caused by i.a. the fungal genera *Fusarium*, *Phytophthora*, *Rhizoctonia* and *Phytium*. The symptoms are described in chapter 10. The disease usually originates from nursery soil but is occasionally introduced via infected seeds (Gardner 1980, Ivory and Spreight 1993). In three *Araucaria* species in Australia seed-borne *Rhizoctonia solani* proved to be the principal cause of damping off (Kamara *et al.* 1981).

Rust and smut diseases are seed and fruit infections common in herbs and agricultural crops, but relatively rare in trees. In Central America, *Pinus oocarpa* is frequently infected by pine cone rust, and in West Africa a smut disease in *Triplochiton scleroxylon* can seriously impair seed production (Ivory and Spreight 1993). Rust and smut fungi produce vast quantities of spores which are likely to be mixed with the whole seed lot during processing and hence be transmitted with the seed. As stated above, however, their chances of infecting new cones or fruits are probably small.

As among the seed insects there are fungal specialists and generalists as regards to host plant. Examples of host-specific fungi are *Phyllachora balansae* on *Cedrela* spp. in Central and S. America and *Dothiorella mahagoni* on *Swietenia macrophylla* in the West Indies. Both species cause stem disease of nursery seedlings (Ivory and Spreight 1993). Another host-specific stem disease in seedlings and young trees in Central America is the eastern gall rust of pines. This fungus has a complex life cycle with two host species viz. *Quercus* and *Pinus*. The pine seedlings are infected by airborne spores from infected leaves of nearby *Quercus* spp.; the infected seedlings or young trees form large galls which produce another type of spore which infects *Quercus* spp. (ibid). Least host specific are storage fungi like *Aspergillus niger* and *A. flavus* which attack seeds of a wide range of species (Mittal *et al.* 1990).

7.6.2 Conditions and mode of infection

Fungal infection of fruit and seed may occur at any time of development but is often more abundant during the later stage of maturation, especially in areas where that stage of development coincides with the season of high atmospheric humidity (rainy season). Most fungi are dispersed by spores and are therefore also dependent on windy conditions. Hence, weather conditions during seed maturation have a strong impact on the severity of fungal attack. Obligate or facultative saprophytic field fungi often infect decaying floral or fruit tissue. From there they may spread to the seed via direct connections of the vascular tissue (Halloin 1986). Often, however, they do not infect the seed but are borne on the surface, especially where small cracks or rough surface ease their attachment. The vast majority of seed-borne fungi are harmless saprophytes; only a small number are seed transmitted or seed pathogens.

Fungi are normally unable to penetrate intact seed-coats, unless the coats are very thin, but use cracks or damage to the seed-coat as entry points (Halloin 1986). Also natural 'weak sites' of the seed-coat may occasionally be attacked. A natural 'weak' site is the chalazal region consisting of easily penetrable parenchyma tissue, through which fungi may invade (Christensen 1973). Germination of fungal spores requires high humidity. Since spores germinate on the surface of the seed, humidity rather than seed moisture is the critical factor.

Sound healthy seeds are less likely to be infected by fungi than aged and damaged seeds. An intact seed-coat forms a physical and chemical barrier to infection (Halloin 1986). Hence, a major reason why hardcoated seeds remain viable both in storage and soil seed banks is their resistance to fungal infection.

7.6.3 Control of seed infection in the field

Because fungal spores are tiny and air-borne, long-lived and produced in an enormous quantity, they are virtually ubiquitous; unless one works under sterile laboratory conditions, there is almost always a pool of potentially infective spores on and within a seed lot (Halloin 1986). Although most seed-borne fungi are harmless saprophytes, precautions during collection should be undertaken to reduce the general contamination with pathogens, and minimize the risk of introducing specific ones. By collecting seeds as early as possible after maturation, the period during which seeds are prone to infection can be greatly reduced (Mohanan and Sharma 1991). For example, seed collected from the ground are likely to be contaminated with soil-borne fungi, which may easily become seed-borne and potentially disease transmitting. In temperate *Picea sitchensis* it was found that seeds collected from the trees directly were free from contamination with *Caloscypha fulgens*, a fungus causing germination failure of several conifer seeds, whereas seeds of cones collected from the ground were heavily infected (Southerland 1981).

Most seed-borne pathogens may remain at low level and harmless if their conditions for growth and development are controlled. This implies primarily that processing should take place as soon as possible after collection, and that seeds should subsequently be stored under conditions unfavourable to further development, i.e. low temperature and low moisture content.

7.6.4 Storage fungi

While most field-infecting fungi become inactive at a moisture content below approx. 25%, another group of fungi may remain active, and become increasingly dominant, at moisture content down to less than 15%. Though often present in the field, these fungi normally play a minor role in disease development under field conditions. It should be noted that recalcitrant seeds, being sensitive to desiccation and therefore stored with high moisture content, may continue to support a microflora of fungi usually only active under field conditions. For example, Mycock and Berjak (1990) found for four recalcitrant species that *Fusarium* sp., a typical field fungus, became dominant during storage. Storage fungi, commonly called moulds, are facultative saprophytes living on most dead organic materials. There is, accordingly, little host specificity among storage fungi. Most species belong to the genera *Aspergillus, Penicillium, Rhizopus*, *Chaetomium* and *Mucor. Aspergillus* is by far the most common in seed store infections; *Penicillium* is more common in the temperate than in the tropical regions (Agarwal and Sinclair 1997), although Hong (1981) found several *Penicillium* species on stored dipterocarp species in Malaysia. Storage fungi are frequent where seed moisture cannot be brought below a safe level, generally around 10%. In India, *Aspergillus niger* is a frequent storage fungus attacking seeds of *Shorea robusta*. The fungus may attack at a moisture content of 12% (equivalent to a RH of approx. 75%) and the attack becomes increasingly worse at higher humidity and seed moisture content (Singh *et al.* 1979). 12% m.c. is probably the lowest safe moisture content for this 'intermediate' seed to avoid desiccation damage. Seeds collected during the rainy season may contain more than 50% moisture which is difficult to bring down to safe level during conditions of high air humidity (see chapter 6). Such seed will, accordingly, often be extremely prone to fungal attack.

The activity and damage exerted by storage fungi depend on the infection rate and their growth conditions.

1. **Initial infection**. Fungi that are already present in the seed when entering into storage may continue to develop during storage if conditions are favourable. Also seed-borne spores may form a potential infective source, becoming activated under conditions favourable to germination.

2. **Debris**. Most debris like fruit parts, soil particles, leaves have a higher moisture absorption and retention ability than the seeds. Consequently, insufficiently cleaned seeds are more susceptible to fungal infection than clean seeds (Christensen 1973). Further, the debris itself is likely to contain pathogens. What in the cleaning process appears as 'dust' may to a large extent consist of fungal spores plus infective debris.

3. **Seed condition**. Any damage to the seed-coat or unhealthy condition of the seed is likely to accelerate deterioration by fungi. Damaged seeds may harbour a fungal flora which can easily spread to other seeds in the seed lot, cf. 2.

4. **Insects and mites.** Insects or mites infesting seeds generate

heat and moisture by respiration which in turn promotes fungal activity (ibid). Damage to seed-coats, e.g. entry or exit holes, may also serve as entry points for fungal infection.

5. **Moisture content and humidity**. Storage fungi grow at moisture content in equilibrium with a relative humidity of 65-75 to 85-95%, depending on species. Some *Aspergillus* spp. are active at very low humidity while most *Penicillium* spp. have a minimum requirement of 85-95% (ibid). Since fungi start growing on the surface of the seed, they are more dependent on air humidity than on the moisture content of the seed itself. Hence, seeds which have been dried to low moisture content and do not easily re-absorb water (e.g. legumes) may be infected with storage fungi if RH increases.

6. **Temperature**. Storage fungi may be active at a temperature range from 0 to 55°C, some even as low as minus 5°C (ibid). However, below 10°C the activity of most fungi is extremely low. Temperature reduction is the safest way of reducing fungal infection in seed stored with a relatively high moisture content (recalcitrant seed).

7.6.5 Fungal treatment

In most cases preventive measures like ensuring appropriate time and method of collection, and appropriate processing and storage make chemical treatment redundant. However, where seeds are heavily infected with seed-borne pathogenic fungi and these are likely to cause damage during storage or germination, treatment may be indispensable. Further, where seeds are to be exported, treatment may be necessary for phytosanitary reasons (see below). A number of chemicals are available, some of which are listed below. The basic requirements to a seed treatment chemical are, according to Agarwal and Sinclair (1997):

1. Effective under different agro-climatic conditions.
2. Harmless to the seed and seedling, i.e. non-phytotoxic.
3. Safe to operators during handling and sowing, and to wildlife.
4. Not leaving harmful residues in plants or in the soil.
5. Compatible with other seed treatment chemicals.
6. Low in price.

It may in practice be impossible to find chemicals which fulfil all these requirements. For example, most seed treatment chemicals are phytotoxic even when used in safe prescribed doses, but may still be economically beneficial by outweighing the detrimental effect of the pathogens. Potentially harmful effects to humans may in most cases be overcome by safety precautions during handling (see below).

Harmful environmental effects are subject to increasing concern, and in many countries a number of chemicals have been banned for environmental reasons. Mercury (Hg) chemicals were formerly common seed fungicides, now being replaced by more rapidly decomposable and less harmful products (see list table 7.2). Mercury-based fungicides can also be harmful to some seed species, e.g. certain *Pinus* spp.

(Willan 1991). When seed for export is treated with pesticides, the rules and legislation of the importing countries should be consulted. Failure to comply with such rules may cause import problems (cf. chapter 15).

Most seed treatment fungicides are targeted to a wide range of fungi and are likely to affect the total microflora and fauna on the seeds, including beneficial organisms such as mychorrhiza, rhizobia and *Frankia*. As a consequence it is generally not possible to apply fungicides together with e.g. microsymbiont inoculants e.g. by pelleting, and during any inoculant application seeds must be cleaned for possible adhering fungicides (see chapter 13). The problem may in some instances be overcome by using an instant treatment like heat or surface sterilization rather than pesticides with a long-term effect.

Fungicides applied before storage are normally targeted only at seed-borne and potential storage fungi. Another fungicide treatment is sometimes applied just before sowing, targeted at the seed-borne and soil-borne fungi that may attack the germinating seed or seedlings in the nursery.

Surface sterilization

Fungi adhering to the surface of the seeds may be exterminated by an instant exposure to a sterilizing agent. Various types are available; the following are listed by Bonner *et al.* (1994):

1. Hydrogen peroxide (H_2O_2) (e.g. 30% for 20 min).
2. Sodium hypochlorite (NaHCl) (10% solution of commercial bleach)
3. 75% ethanol[2] (C_2H_5OH)

Under laboratory conditions seed surfaces may be sterilized by 0.1% solution of mercuric chloride ($HgCl_2$); because of its content of the heavy metal 'Hg', the agent should be handled and disposed of especially carefully and safely.

Prolonged exposure of seed to all the above agents is harmful. Exposure time and concentration should be adjusted to the individual species to achieve the highest efficacy whilst avoiding phytotoxic side effects. After exposure the seed should be rinsed in water to remove possible residues of the chemical. Sterilizing agents are effective for pathogens adhering to the seed surface and those present in superficial seed-coat crevices, while deep infecting fungi will normally escape the treatment. Surface sterilization is widely used in experimental work on small seed lots but is impracticable on a larger scale.

Heat treatment

Brief exposure to high temperature applied by dry air or submersion in hot water is applicable in cases where the fungus is heat sensitive and the seed heat tolerant (Agarwal and Sinclair 1997). In temperate oak (*Quercus* spp.) a 2-2½ hours' submersion of seed in water at 40-45°C is used to control fungal infection of *Ciborea*. Such prolonged

[2] Should be pure alcohol and not denaturated alcohol as the latter may damage the seed.

exposure must be carefully adjusted in time and temperature since too long exposure is likely to be harmful to the seed. Further, the heat treatment may leave the seed-coat more vulnerable to invasion of other pathogenic fungi. Therefore a fungicide treatment may still be necessary (Knudsen 1997).

Fumigation

Fumigation with methyl bromide is effective to control certain fungal pathogens. Other less widely used fumigants are HCN, carbon disulphide and aluminium sulphide.

Fungicides

Some of the most common fungicides are listed in table 7.2. It should be noticed that chemicals based on the same active compound are sometimes sold under different trade names by different manufacturers in different countries. Because of the larger market and use of agricultural seeds, most chemicals are accompanied with instruction and dosage for application for agricultural seeds only. Seed size and structure of the seed-coat should be considered when determining the dose.

Some fungicides are only effective if they are in direct contact with the fungi. Hence, fungi already present deep within the seed are likely to escape treatment (Christensen 1973, Gardner 1980). Systemic pesticides like triadimethol, ethirimol and metalaxyl are effective against deep seated seed-borne fungal organisms (Mohanan and Sharma 1991). In Tasmania, Australia, 2 calico bags each containing 50g of paradichlorbenzine are added to each tin (approx. 12 litres) of seed, one at 2/3 depth and another at the top of the seed for fungal protection (For. Com. 1994).

The effectiveness of different fungicides to control different fungal species varies. In India, the relative efficacy of five commonly used fungicides viz. Dithane M-45, Bavistin, Fytolan, Caresan, and Thyride (all 0.1% concentration) was tested on eight common storage fungi on seeds of three different tree species (Purohit *et al.* 1996). One of the fungicides, Fytolan had no effect on *Aspergillus niger*. The effect of Thyride depended on tree species.

Table 7.2.
List of some common fungicides under their common names. The chemicals are sometimes sold under different trade names

Bavistin-SD
Thyride
Ceresan
Brassicol
Thiram
Panoctine 35%
Orthocid
Dithane M-45
Fytolan
Agrosan GN
Captan
RH-2161
Octave

Application of fungicides

Most fungicides are applied as dry powder mixed with the seeds. This method is mostly applicable to seeds with a relatively rough surface to which the powder will adhere. For larger quantities of seed the best method is to mix seed and powder by tumbling in a rotating drum. Where the seed surface is smooth, the fungicide may be applied by a dip or slurry method in which the seeds are dipped into an aqueous solution of the fungicide; sometimes a glue or binder may be added to improve the retention of the material. The dip and slurry method also assists in the absorption of the chemical (Agarwal and Sinclair 1997).

Fungicides can also be applied to pelleting material. During pelleting the seeds are tumbled with an adhesive material such as gum arabic, gelatin, methyl cellulose or the like, plus an inert filler such as gypsum talc, kaolin clay, limestone, peat or vermiculite. A fungicide powder may be mixed throughout the coating material or can be added in discrete layers or in the outermost part of the pellet (Mohanan and Sharma 1991, Agarwal and Sinclair 1997).

Biological methods

There is little experience in the use of biological agents to control fungal development in tropical forest seed. Schaefer (1990) reports that storing recalcitrant *Prunus africana* and *Podocarpus milanjianus* seeds in sawdust restricts fungal development, but it is not known whether the sawdust has any anti-fungal properties. In India a *Eucalyptus* hybrid oil was found effective in controlling mould development in *Shorea robusta* seeds at high humidity. A minimum of 3 cm^3 oil per 1000 cm^3 of storage container was effective (Singh *et al.* 1979).

Biological control measures on fungi in agricultural seeds include the application of fungi antagonistic to pathogenic fungi (Knudsen 1997).

7.7 Safety Precaution during Handling of Pesticides

Use of pesticides in seed handling should be limited to the absolutely necessary. Where pesticides are used, they must be handled with due respect, and the seed handler must comply with the safety precautions prescribed by the manufacturer. Any toxic chemical should be provided with a label from the manufacturer indicating toxicity, e.g. in classes A, B, C, etc. The rules vary from one country to another. Highly toxic pesticides should only be handled by authorised personnel under observation of strict safety rules throughout handling. Some general rules and precautions are listed here:

1. Read instructions from the manufacturer carefully and handle the remedy accordingly
2. Use the concentration prescribed by the manufacturer
3. Never experiment by mixing different chemicals
4. Prepare prescribed pesticide mixtures in a well-ventilated place
5. Always use gloves during preparation and application; for liquid remedies, waterproof rubber gloves should be used

6. Use masks and protective glasses when applying toxic fumigants and sprays
7. Check and repair any leak from containers and equipment. Replace worn gaskets in equipment used for fumigation and spraying
8. Do not leave pesticides unattended. Have a locked up room or cabinet especially for pesticides and application equipment
9. Dispose any left over remedy safely
10. Be prepared for accidents; the universal emergency agent is water, which should always be available within reach.

To these points should be added that only personnel having received appropriate instruction and training should be allowed to handle pesticides.

7.8 Phytosanitary Problems during Transfer of Planting Material

In natural ecosystems, seed pests and diseases normally take their toll of the seed production, but they are, in turn, normally controlled by natural enemies. The number of seed insects is, for example, controlled by birds and insect parasites. When plants are grown outside their natural environment, they are also often out of reach of their natural enemies. That will, in the first phase, normally benefit the plant. Eucalypts grown as exotics often perform better in their new growth area than they did in their original area, because they have escaped their natural predators (Eldridge *et al.* 1994). *Swietenia macrophylla* grown in Fiji is another example of an exotic which performs excellently as an exotic, while native plantations are heavily infested by a shoot borer, *Hypsipyle* spp. However, if an insect pest or pathogen happens to be spread into a new plantation area, it is likely that it will cause much more damage than in its native area, because its natural enemies are absent. The problem has frequently been encountered in agricultural crops, and often with disastrous consequences; the most serious probably being the Irish potato famine of last century. Where the exotic tree has some innate resistance to a seed-transmitted pathogen, introduction of such a pathogen may be harmless. However, if other susceptible host species occur, e.g. related endemic species without or with low resistance, the pathogen may spread into these populations (Ivory and Spreight 1993).

Generally, seed-borne insect pests may be easier to detect and treat than seed-borne pathogens; yet they impose a potential risk of being accidentally transferred e.g. as eggs adhering to the seed surface or as dormant pupae inside the seed. Bruchids are usually only visible by the pupal 'window' or emergence hole, chalcids only by the latter. Because of the unsuccessful effect of treatment by e.g. insecticides while the insect is 'safe' inside the seed, accidental escape is a risk. Seed-borne pathogens are more numerous, individual seeds often carrying several species. In *Eucalyptus* spp. FAO/IPGRI (Ciesla *et al.* 1996) list more than 30 seed-borne pathogenic fungi, and several seed-borne insects.

Because of the potential danger of introducing seed-borne pests and diseases together with planting material, most countries have strict regulations on import of e.g. seeds. The aim is to exclude exotic pathogens from areas where they do not already exist, and where they might cause serious problems if they are introduced. A phytosanitary or health certificate, which guarantees the absence of particular pathogens normally has to be provided with any seed lot that is imported. In addition the importing country normally wishes to verify for themselves that no pathogen accidentally escapes into their country. A laboratory may perform a screening of the phytosanitary conditions (see chapter 11). Meanwhile the seed lot is exposed to quarantine regulations. Some countries as a routine treat imported seeds with fungicides or expose them to high temperature in order to kill possible pests and pathogens. A major problem of these import treatments is partly the time taken by the screening procedure which, together with administrative procedures, may be critical in relation to seed longevity in transit, partly that seeds may be treated with large doses of phytotoxic remedies which may impair seed viability. The practical implications of phytosanitary rules on seed transfer are discussed in chapter 15.

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APPENDIX A7.1 Equipment for application of CO_2 to stored seeds

CO_2 is available in most parts of the world since it is extensively used for e.g. fire extinguishing and thin plate welding. It is available in re-fillable metal bottles from approx. 6 kg and larger. Stored under pressure of approx. 40 atmospheres (bars), CO_2 is liquid. The pressure in the bottle decreases as the CO_2 is used. An adjustable pressure reduction valve must be connected to the bottle to reduce the pressure of the outlet and allow a steady flow of the gas. The most convenient type is a flowmeter in which the pressure of the outlet can be adjusted quite exactly, which makes it easier to determine the time it takes to fill a bag with CO_2. A hose is fitted to the flowmeter/reduction valve at one end and a blowing pistol for pressurized air at the other. All connections should be provided with gaskets and closely fit to avoid leaking of the gas. When all joints are fitted, the pressure of the bottle is released by opening the main valve. Possible leaks may be checked by applying a thin layer of soap water or thin oil to fittings with a paint brush; escaping gas will be detected as bubbles blown in the liquid.

Seed to be fumigated with CO_2 is placed in heat-sealable plastic bags with low permeability to CO_2. Laminated plastic material consisting of an outer layer of approx. 0.03 mm thick polyamide (nylon, low CO_2 permeability) and an inner layer of approx. 0.07 mm low density polythene (ordinary plastic, heat-sealable) is suitable. An alternative laminate has aluminium foil in stead of the outer polyamide layer. Bags less than 4 litres are preferred as larger bags are more difficult to fill and tend to puncture easily.

Sealing of the bag after filling is done by the aid of an electric heat sealer. Special heat sealers make a broad tight seal and allow adjusting of temperature and sealing time to the particular material thickness. It is important that the temperature and time are adjusted so that the two sides melt together without melting holes in the material. During sealing the bag is kept upright and the sealing site kept clean (For further information on CO_2 fumigation, see Sary *et al.* 1993).

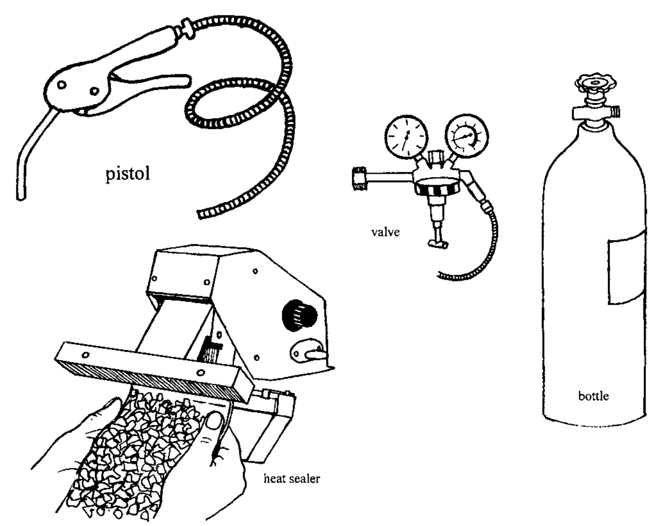

Figure A7.1. CO_2 fumigation equipment.