Detection, Presence and Control of Seed-Borne Pests and Diseases of Trees with special reference to seeds of tropical and sub-tropical pines

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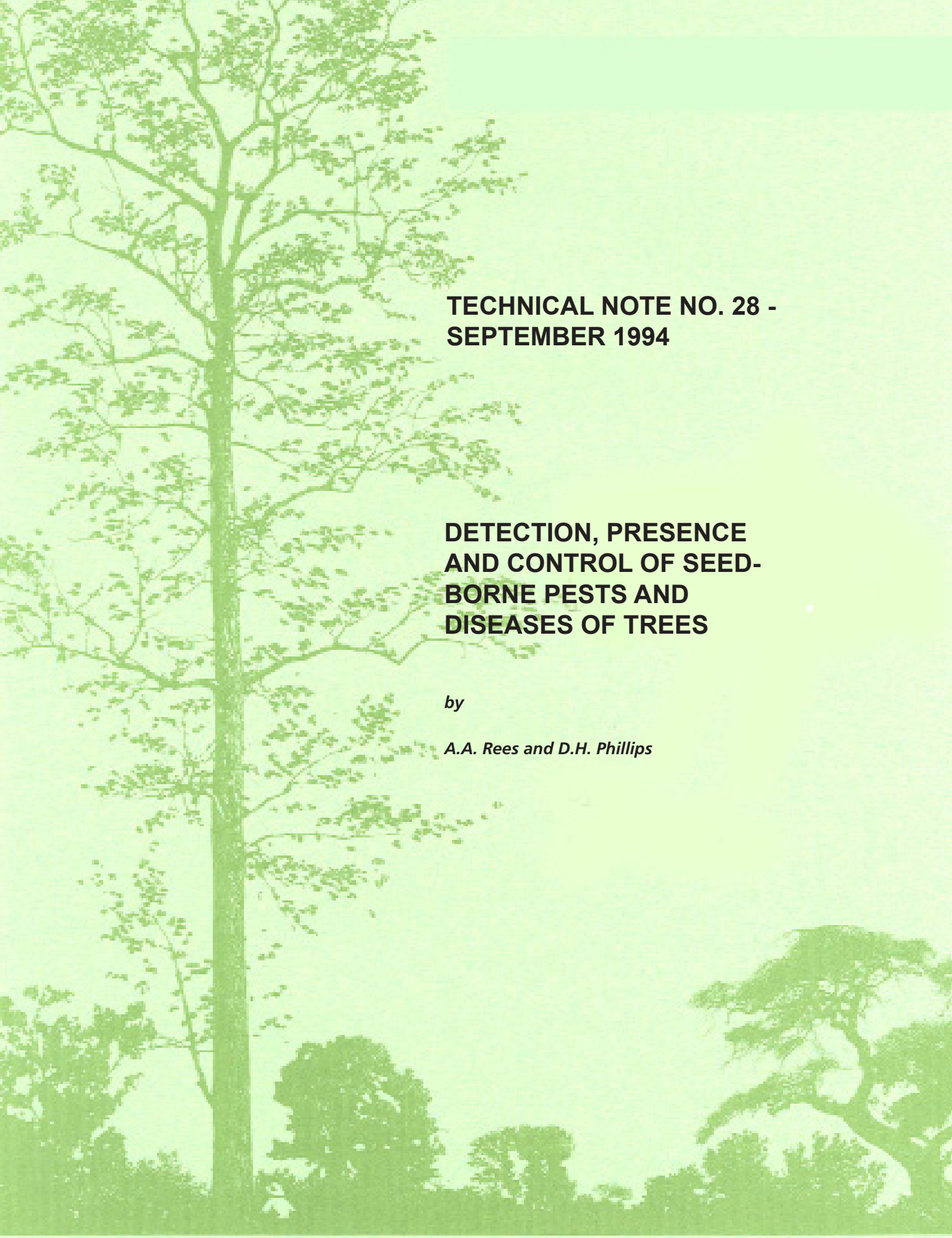
Publication date:
1986

Document version
Early version, also known as pre-print



TECHNICAL NOTE NO. 28 - SEPTEMBER 1994

DETECTION, PRESENCE AND CONTROL OF SEED-BORNE PESTS AND DISEASES OF TREES

by

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Titel
Detection, presence and control of seed-borne pests and diseases of trees

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Publisher
Danida Forest Seed Centre

Series - title and no.
Technical Note no. 28

ISSN:
0902-3224

DTP
Melita Jørgensen

Citation
A.A. Rees and D.H. Phillips**,** Detection, presence and control of seed-borne pests and diseases of trees, 1994. Technical Note nr. 28. Danida Forest Seed Centre, Humlebæk, Denmark

The report is available free of charge
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Danida Forest Seed Centre (DFSC) is a Danish non-profit institute which has been working with development and transfer of know-how in management of tree genetic resources since 1969. The development objective of DFSC is to contribute to improve the benefits ofgrowing treesfor the well-being ofpeople in developing countries. DFSC's programme isfinanced by Danish International Development Assistance (Danida).

CONTENTS

1. INTRODUCTION

Interest in the raising of fast-growing pines is increasing in many tropical and subtropical areas. This has led to the planting of many exotic species, and therefore to the movement of seed from country to country and often from one continent to another. Seed may carry harmful organisms, so its passage across international boundaries and often over natural barriers multiplies the risk of the transmission of diseases and pests. To some degree this can be guarded against, partly by precautions when collecting, extracting and handling the seed in its country of origin, and partly by quarantine measures imposed by importing countries. It is unfortunate that in many instances tree and shrub seed regulations are promulgated ahead of scientific ability, as there is still so much work to be done in developing the techniques necessary to detect the pests and pathogens in the quarantine measures with any level of confidence. Indeed, information on the disease organisms carried by seeds of tropical pines is very incomplete, and that on their corresponding pests is even more scanty. This makes it difficult to devise rational steps against them.

When seed (especially that of softwood trees) is examined during routine seed tests, e.g. for germination and purity, it may appear to be healthy even when badly infected by some microbial pathogens, and the presence of a resident fauna may be inferred only if bore holes made by emerging insects are found in the testae. Also, harmful organisms may occur on or in so few seeds that they may remain undetected if the size of the sample examined is inadequate. It is difficult to assess the importance of this in individual cases because the presence of a harmful organism within a seed collection is not necessarily indicative of its effect in the field. Some organisms (especially many moulds) are in the nature of laboratory weeds, which are a nuisance in the seed laboratory because they interfere with routine seed tests. Others are seed destroyers and so affect the quality of seed samples. The remainder, with which we are primarily concerned in this note, are pathogens or pests which may persist and be transmitted to cause damage in the crops raised from the affected seed.

New plantings of introduced trees are in special need of protection against pests and diseases. In their country of origin, trees form part of a more or less stable ecosystem in which their pests and diseases are to a considerable degree held in check by competing organisms and by parasites and predators. When these trees are planted elsewhere, the associated organisms which act to some extent as natural controls are absent, so pests and diseases have increased opportunities to spread through the crop. These opportunities are further increased because exotic forests are planted as monocultures, often covering large uniform areas over which pests and diseases can spread quickly and easily. In these forests, it is usually difficult and expensive to use chemical controls (even when they are available). Instead, the forester can often only thin out diseased trees, gradually replace affected crops with other, less vulnerable, species and perhaps change some of the methods of management. All these measures take time to have any effect and usually lead to financial loss. Herbaceous crops can usually be harvested after one season or less, but forest crops, even of relatively fast-growing species, take longer to mature. Introduced organisms therefore have a long time in which to become established and to spread, and in a short time they may wipe out the investment of many years. Hence it is important for the forester that all practicable steps should be taken to prevent the entry of exotic pests and diseases.

If such steps are to be taken to good effect, they must be based on good information on the diseases and pests occurring in the countries of origin of the exotic trees concerned and on the organisms carried with their seeds and fruits (and on other planting material, though this is not the special subject of this note). The information required can be obtained in part by a study of available literature and by contacts through regional plant health organizations such as SEAPCC (the Plant Protection Committee for the South East Asia and Pacific Region) and CIPA (Comite Interamericano de Protección Agricola) (Smith, 1979). In part, however, it must come from continuing research, including that coordinated by bodies such as ISTA (the International Seed Testing Association) and IUFRO (the International Union of Forest Research Organizations).

The present note provides some information on methods available for the examination of seed for pest and disease organisms, on harmful organisms already known to occur on seeds of tropical pines, and on methods for their control, including some details of quarantine measures to prevent the movement of infested seed. Although we are concerned especially with seeds of tropical pines, much of the material discussed is inevitably of wider interest.

2. CARRIAGE OF PESTS AND DISEASES WITH SEEDS AND THEIR FURTHER TRANSMISSION

Neergaard (1979), developing the ideas of Leach (1940), pointed out that all the organisms contaminating the surfaces of seeds or infecting their tissues can be described as **seed-borne**. Only those seed-borne organisms which establish themselves on and cause infection in the plants raised from the seed can be described as **seed-transmitted**. Whether the organisms mentioned below as found on or in seed are seed-transmitted or only seed-borne is not certainly known. Indeed, information of this nature is rarely available in the case of seeds of trees, though it is known, for example, that *Megastigmus spermotropus*, a seed wasp affecting seeds of Douglas fir *(Pseudotsuga menziesii*) entered Britain on seeds imported from North America, and became established there (Phillips &Bevan, 1966). That so few pests and diseases of forest trees are known to be seed-transmitted may reflect the true position, but it may be because too little research has been done to reveal this. It is certainly true to say that in the case of other crops, which have been the subject of more research, many examples of seed-transmission are known, and a great many have been listed by Neergaard (1979). All countries attempting to establish new forests based on exotic trees would therefore be well advised to take seriously the possibility of seed-transmission of alien pests and diseases. Organisms which are seed-borne but not seed-transmitted in the above sense are also of importance (though they are not the primary subject of this note), as they affect the quality of the seed, and also interfere with seed testing in the laboratory.

3. PROTECTIVE MEASURES AGAINST THE LONG-DISTANCE MOVEMENT OF HARMFUL ORGANISMS WITH SEED

Measures to prevent the movement of harmful organisms with seed need to be taken by both the exporting and importing countries concerned. Liaison between them is important so that each can understand the nature of the problems of the other, and so that importing countries can make their requirements clear.

3.1 Measures to be taken by the exporting countries

3.1.1 Production of clean seed for export

It is easier and more effective to take preventive measures than to cure the results of contamination. Precautions should begin with the collection of cones (and the seed of broadleaved trees) straight from the trees instead of taking them from squirrel caches or picking them off the forest floor, for the leaf litter harbours many microorganisms. If possible, the cones should come from specially selected trees, from seed stands made up of trees of high quality, or from seed orchards of genetically desirable material. Once collected, cones should not be left in damp, warm conditions, which stimulate microbial development prior to processing. It is desirable to extract the seeds quickly, as seed contamination greatly increases when the cones open, though care must be taken during extraction to prevent mechanical damage to the testas. The debris often associated with a collection should be removed, as it adulterates the seed sample, and itself carries harmful organisms.

Sub-optimal storage conditions reduce seed viability, thereby rendering the seed susceptible to attack by the microorganisms present on the testa. Before sealing in the polythene bags used for storage, seed of pines should be dried to bring the moisture content down to 10 %. It is then advisable to store the seed in sealed containers at a temperature between 0 and 2°C.

When testing seeds in the laboratory, seed analysts should monitor germination tests for seedling health, making notes and isolating any suspicious microorganisms for identification if the viability of the seed falls below the expected percentage for the species. In those cases in which the examination shows a high proportion of the seeds in a sample harbour a pathogen, the collection concerned should not be exported; it should be destroyed, or (if appropriate) retained for use in its country of origin. This is particularly important if the seed is found to show internal infection.

Data from the examination of many samples in the seed laboratory can be accumulated. It may then show that certain sites or areas habitually produce seed infested by one or more dangerous pests or disease organisms. It may then be possible to take control measures in the places concerned, or it may be necessary to desist from seed collection in them.

In the past it has been common practice for seed exporters to dress seeds of trees and shrubs with chemical fungicides and insecticides in the hope that this will destroy any pests or disease organisms present. This has often been done in response to the seed

legislation of importing countries, which rarely specify the organisms against which treatment is to be given or the precise treatment required. Often exporters do not indicate in their export documents the treatments they have applied. This may lead to problems, partly because the importers may themselves in ignorance then apply a treatment to already treated seed. This may do harm, as many of the chemicals used reduce seed viability, and additional treatments have cumulative ill effects. Further, some chemicals used to dress seed are harmful to workers (including seed analysts) who handle the seed.

Though the situation may change in future as a result of further research, it is at present best to avoid the chemical treatment of exported seed. It is better to take the steps briefly mentioned above to produce seed which is as clean as possible, and to leave further treatment to be done in the importing country. This involves agreement between the countries concerned.

3.1.2 Certification schemes

Once sufficient information has accumulated, it may become possible to set up certification schemes, to indicate for example that certain named pests and diseases do not occur in the country of origin of the seed, or in the part of the country from which the seed has been collected. Certification schemes are discussed in some detail by Neergaard (1979) and by Hewett (1979). Here it may be briefly suggested that if these schemes are to have a sound basis, information is needed on the pests and diseases known to be transmitted on the seed concerned and on their distribution in the exporting country. Their likely significance in the country proposing to import the seed also needs to be considered. This involves assessment, which is often difficult. Some of the problems in this field in the case of organisms attacking trees have been discussed by Phillips (1979, 1980).

3.2 Measures available to importing countries

3.2.1 Quarantine measures

As now understood, quarantine covers all legislative and associated organisational measures taken to prevent the entry of alien pests and diseases. Here we are concerned with seeds of pines, and at their most extreme, importing countries may prohibit the importation of all pine seeds because they may (or may not) introduce unwanted organisms. As these seeds may be required to set up new exotic forests or to introduce additional species or provenances, such an extreme approach may be self-defeating. Some less absolute form of control must usually be sought. This implies at least some degree of risk. It is therefore best if the controls to be introduced are not decided upon by individual countries, but are agreed upon by international discussion between representatives of all the countries concerned in a committee set up by their regional plant health organization.

Various approaches can then be considered. The first may be to allow the entry of the seed concerned, but demand that it is covered by certificates declaring that it is free of all pests and diseases. This approach is still unrealistic, however, as some pest and disease organisms will already be established in the importing area, and others will be of only trivial significance. It is therefore necessary to consider the matter more carefully, assessing the harmful organisms likely to be brought in on seed from abroad, and listing for prohibition only those (a) not established in the importing region and (b) likely to become established and to cause significant loss if they were imported.

Once legislation has been produced, exporting countries must be notified, so that they can undertake the necessary inspections and seed tests and produce the certificates needed to cover their seed exports.

The same seed inspections and tests necessary to confirm the absence of specified restricted organisms can be carried out, if desired, by the importing country to corroborate in information on the export certificates. The certificates themselves should be internationally agreed (normally those of the Food and Agriculture Organization of the United Nations).

Seedlings and plants raised from imported seed should be watched more carefully for possible diseases than similar plants of indigenous origin.

3.2.2 Seed treatments

In some circumstances, after testing imported non-tree seed, importing countries may decide they can safely treat some infected lots to rid them of harmful organisms, usually by dusting them with or steeping them in chemical preparations or by placing them in a hot water bath. The hot water and chemical methods were combined in the thiram soak method of Maude (1968). The methods and chemicals available have been reviewed by Neergaard (1979).

Little work has so far been done on treatments for the seeds of trees. Most chemical dressings have been developed for use on agricultural and horticultural crops and are either systemic or non-systemic in nature (Jeffs, 1978; Suryanarayana 1978). Many are designed to control a specific pathogen. The dressings used on tree seed are usually the non-specific chemicals applied to crops in the field. The seeds may be dusted with a powder, or, if sowing is imminent, a drench may be preferred. It is important to label clearly any seed lot treated with a chemical so that this can be taken into account in subsequent handling.

At the Official Seed Testing Station, Alice Holt Lodge, Great Britain, when in the past importing countries have required an unspecified chemical treatment of tree seed, thiram has been used as a general fungicide, and malathion as a general insecticide. Thiram or benomyl have been advocated by Delatour & Morelet (1979) to control *Ciboria batschiana* in acorns. In the case of conifers, Epners (1964) suggested pelleting with captan if seed was infected with *Geniculodendron pyriforme* (the anamorph of *Caloscypha fulgens*). In work on the microflora of *Pinus resinosa*, Vaartaja (1976) investigated the efficiency of pyrrolnitrin, stendomycin, kalafungin, haloprognin, sodium selenate, benomyl and demon as fungicides and bacitracin as a bactericide, and it was found that they had potentially useful selective effects against seed contaminants. Kelley &Williams (1985) found that they could protect seedlings of *Pinus teada* from seed-borne *Cronartium fusiforme* by dressing seed with triadimefon, which is compatible with thiram. When Motta (1984) treated seed of *Cupressus* spp. with benomyl or thiophanate-methyl, he was able to eliminate or much reduce infection by *Seiridium cardinale*.

It must be borne in mind that the seed and its microflora form a delicately balanced ecosystem. If one section of the microbial population is removed, other groups in the community tend to increase, often to the detriment of the host. Further, seed dressings are to some degree phytotoxic, and the effect on seed germination of any proposed treatment should be considered before its general introduction. Different formulations of the same chemical may differ in their effects on seeds. As manufacturers may alter the formulation of their products from time to time, this also may have to be taken into account.

4. METHODS USED TO DETECT HARMFUL ORGANISMS ON AND IN SEEDS

4.1 Detection of disease organisms

The causes of transmitted plant diseases include the viruses, mycoplasma-like organisms (MLO), rickettsia-like organisms (RLO), the bacteria and the fungi. Special techniques are needed to show the presence of viruses, and MLOs and RLOs in seeds. Information is given on these in specialist literature on viruses and virus-like diseases (see e.g. Matthews, 1970; Nienhaus, 1985).

Many methods have been used to isolate bacteria and fungi on or in seeds. Some are suitable for use in routine seed testing, while others, at least at present, are more suited to research. Some give a general impression of seed infestation, others attempt to differentiate between surface contamination and the presence of organisms within the seed tissues.

It has been calculated that there may be as many as 10,000,000 viable spores on every gram of conifer seed. They may be of many species, and the method chosen to detect an individual organism can bring success or failure, depending mainly on how closely linked the organism may be with its host, and in particular whether the infesting organism is at one extreme a saprophyte or at the other an obligate parasite.

The methods available can be divided into the general and the special.

4.1.1 General methods

These are all basically variations on techniques in which seeds are incubated either on agar or on filter paper (the blotter method).

The agar plate method: When using this technique in its basic form, seeds are placed initially on the surface of a solid natural or artificial nutrient medium. Microorganisms grow out from the seeds on to the surrounding agar. They can then be identified. In the case of seeds of the size of *Pinus caribaea*, ten seeds can conveniently be examined in a 9 cm plastic petri dish filled with 17 ml of agar. Bigger sterile containers may be used for larger seeds or fruits.

Various media are available, including the artificial and semi-artificial Czapek Dox, nutrient and malt agars, and others using the natural ingredients of wheat straw, oats, potato and carrot. Alternatively, use can be made of sterilized material of the host species, such as wood shavings and crushed leaves or seeds, provided they do not contain components toxic to microorganisms. If desired, selective media may be used, to favour the growth of particular microbial species or groups. For the best results, the seeds must be induced to germinate, so that organisms within the seeds as well as on their surfaces can be isolated. For this reason the chosen medium should not be too concentrated, for the lowered water availability of such a substrate reduces seed germination. In general microbial surveys of tropical pine seeds, a 2 % malt and 2 % technical agar mixture has been found suitable.

The use of seed scrapes: It was suggested above that whole seeds could be placed on agar plates. If during visual inspection of a seed collection, tissue colour or texture appears abnormal, small pieces of material may be scraped off with a sterile needle and

deposited on the agar. This technique is usually employed when large seeds, such as chestnuts or acorns, are cut open to estimate viability.

Dilution-plating: When this method is used, the seed itself is not placed on the agar. Instead the contaminating microorganisms are removed from the seed surface by shaking a given quantity of seed in sterile distilled water. The water is then diluted, and various dilutions are spread over the surface of an agar medium. The resulting colonies give an indication of the number and kind of viable propagules contaminating the seed. If desired, a selective medium can be used to favour the growth of a particular microbial species. The figure obtained by this method is an underestimate, because experience has shown that shaking the seed never removes all the microorganisms from the testa. Nevertheless, this technique has the advantage that after use the seeds can be dried and used for sowing in the nursery.

The ultrasound technique: This is a variation of the dilution plate method, but the seed is placed either in lactophenol or in sterile distilled water, and treated in an ultrasound water-bath. If distilled water is used, the seeds can be dried and later sown as previously described. The water can also be diluted and spread on to agar to estimate viable propagule types and numbers. It can also be centrifuged to concentrate its contents, which can then be examined with a light microscope. When treating tropical pine seeds, it has been found that a ten minute 19.67 KHz treatment is sufficient, when a few drops of the surfactant 'Tween 80' are added to the seed water.

If the seed is placed in lactophenol, it can be removed after treatment, and the lactophenol centrifuged. Samples of the centrifugate can be stained with lactophenol cotton blue, and examined under the microscope. A haemocytometer slide can be used to calculate spore numbers.

Spores are only identifiable under the microscope if they have a characteristic shape, colour and size, but the method is useful because in suitable cases large seed samples can be examined quickly at a relatively low cost, and with relatively little expertise.

The method gives faster results than simply shaking the seed, but if the treatment time is too long, the spores begin to rupture, and if it is too short, many spores remain on the seeds.

The blotter method: When this technique is used, seeds are placed individually on moist, sterile paper in a petri dish or some other suitable container depending on seed size. On incubation in the damp chamber thus formed, organisms present on and in the seed develop on the seed surface and on the filter paper. They can then be identified microscopically.

The great advantage of the blotter method is that it is simple, and it is the most economical when used for the examination of pine seeds because it uses materials similar to those already used in germination tests. However, this technique tends to favour the fungal seed microflora at the expense of the bacteria and actinomycetes. Also, because of the level of skill required to carry out the difficult task of identifying the many seed-borne microbial species, it may be preferable to employ the agar technique using tap water, as this medium encourages a wider spread from the seed, and so facilitates isolation.

4.1.2 Special methods

These methods include the use of the scanning electron microscope (which allows a

direct examination of organisms on the seed coat) and of enzyme electrophoresis, and techniques which go at least some way towards differentiating between organisms contaminating the surface of the seeds and those within their tissues.

Scanning electron microscopy is the only method which permits the direct observation of microorganisms on the seed coat. It has five main drawbacks, however:

(1) test seed material must be dried below a 10 % moisture content;
(2) the equipment is expensive, and the operator needs experience;
(3) very few seeds can be examined, so that the probability of discovering rare spores is reduced;
(4) to be identifiable, the propagules must have a characteristic shape and size; and
(5) it is impossible to differentiate living propagules from dead ones.

Seeds may be prepared for the SE microscope by sticking them down with doublesided sticky tape and coating them with gold. Better SE micrographs can be obtained, however, by glueing the specimen to an aluminium stub with a conductive carbon cement, before painting the slides with a conductive silver paint and finally spatter-coating with gold. Further details of SE-microscopy techniques should be sought in makers' manuals issued with the equipment to be used, as well as in specialist literature.

Isoenzyme electrophoresis: Using electrophoresis on polyacrylamide gels, it has been found that seeds of *Picea sitchensis* infected by *Caloscypha fulgens* (Pers.) Boud. show different isoenzyme patterns from those of uninfected seeds. Sutherland, Rink *et al.* (1981) have further discovered that the high alkaline phosphatase activity displayed by infected seeds can be used as an indicator. This technique can be employed only when searching for a known microorganism in the seed of a particular host species.

Seed tissue excision: This is really a variation of the agar plate technique, designed to sample the microflora within the seed. To reveal the microorganisms it is necessary to remove as much as possible of the surface microflora by chemical means before planting the seeds or (more effectively) dissected parts from their inner tissues on to agar.

Many workers have described their own variations on this technique in the study of pines and other conifers. Salisbury (1953) treated seeds of *Pseudotsuga menziesii* with 0.1 % mercuric chloride solution. Epners (1964) used the same chemical on seeds of *Pinus resinosa*, *P. sylvestris*, *P. strobus* and *Picea glauca*. He subsequently rinsed the seed in sterile distilled water, prior to plating out naked endosperms. Miller & Bramlett (1978) soaked seed of *Pinus elliottii* var. *elliottii* in 95 % ethanol for ten minutes, cut the seeds in half longitudinally, removed the testas, and exposed one half of each seed for ten minutes in a 0.5 % sodium hypochlorite solution before plating on agar.

Sutherlands & Woods (1978) treated *Picea sitchensis* seed with a 1 % sodium hypochlorite solution for five minutes, followed by a rinse. In a later investigation, Sutherland, Lock & Farris (1981) treated seeds of *Picea sitchensis*, *P. glauca* and *P. engelmannii* with a 30 % hydrogen peroxide solution for 30 minutes followed by a rinse. Wicklow-Howard & Skujins (1980) surface sterilized seeds of *Picea engelmannii* with 0.1 % silver nitrate and then dissected out portions of the endosperm and embryo on to agar.

During researches by one of us (A.A.R.) on seed of *Pinus oocarpa*, *P. caribaea* and *P. pseudostrobus*, using the tissue excision technique with a number of seed pretreatments, it was possible not only to locate an internal microflora, but also to indicate the

efficiency of the various surface sterilants. As a first step, full and empty seeds in each sample were differentiated with the aid of an X-ray machine and photographic paper. Five pretreatments, involving four chemicals, were tested:

5 % glutaraldehyde for ten minutes
0.1 % mercuric chloride for ten minutes
6 % available chlorine in sodium hypochlorite for five minutes
30 % w/v hydrogen peroxide for five minutes
30 % w/v hydrogen peroxide for fifteen minutes

A few drops of the surfactant 'Tween 80' were added to each sterilant.

After treatment, the seeds were rinsed in sterile distilled water and left to soak overnight to allow imbibition. The three main seed components, namely the testa, endosperm and embryo, were placed in numbered positions on 2 % malt or Czapek Dox agars on three separate plates. The resultant microbial growth from the seed coats indicated that the best surface sterilization was achieved by the mercuric chloride and the 15-minute peroxide soaks, while the least effective results were obtained by the glutaraldehyde and the 5-minute peroxide treatments.

Using this method it was found that species of *Aspergillus*, *Chaetomium* and *Penicillium*, a *Cytospora sp.* and a *Trochoderma sp.*, and *Botryodiplodia theobromae*, *Pestalotiopsis palmarum* and *Schizophyllum commune* were isolated from the internal tissues. It was noticed that many of the seeds harbouring *B. theobromae* had dead or decaying embryos.

We recommend that when using this technique, the seed should be surface sterilized with 0.1 % mercuric chloride for ten minutes, and then rinsed and soaked. If it is preferred to avoid the use of the highly toxic mercuric chloride, treatment with 30 % hydrogen peroxide for 15 minutes can be used instead. The testa should then be removed, the naked endosperm longitudinally halved slightly off-centre, and the half containing the embryo then plated out. By observing the location of microbial growth with a dissecting microscope it is usually possible to determine whether a microorganism is genuinely located within the tissue or present only as a result of damage to the seed coat.

Seed sectioning: By this means, permanent stained microscopical preparations can be made. Sutherland, Lock & Farris (1981) made such preparations of seeds of *Picea spp.*, first cutting the seeds in half and fixing in formalin acetic acid before sectioning with a cryostat.

While working with tropical pines, however, it was found (Rees, 1983) that if seeds were cut before fixing, the contents tended to fall out or be squeezed out of position; further, if the seed was decayed, the contents spilt out, possibly removing evidence of an internal infection.

It was therefore decided to devise a pretreatment which did not involve seed mutilation prior to fixation. Using this method, full seeds were placed in perforated containers and left for two days at 100 % humidity. After one day in 4 % phenol, which softened the testas, they were left for one month in the fixative formal saline, followed by a further month in 4 % phenol. The samples were then dehydrated over a period of 93 days in a methanol, absolute ethanol and chloroform series. Finally the chloroform was replaced by paraffin wax prior to block casting the samples. Sections were then cut with a rotary

microtome and stuck to slides smeared with Meyers albumen. Wrinkled sections were treated with 1 % potassium dichromate and heated gently. Finally the sections were stained with Magdala Red and Fast Green FCF, and made permanent. By this means, fragmented and septate hyphae of *Botryodiplodia theobromae* were observed inside seeds of *Pinus caribae*a.

Seed preparation time may be shortened by carefully puncturing the seed coat after surface sterilization, to facilitate penetration of the chemicals.

4.2 Detection of pests

Pest organisms may be revealed during seed tests, when exit holes on seeds indicate the partial or complete departure of an infesting organism. Infested seeds may also be unusually large, and further examination of enlarged seeds may reveal the presence of a pest organism. X-ray photography may be used to examine general seed samples or samples of enlarged seeds or seeds from samples showing exit holes. In the case of most softwood seed, using Hewlett Packard 43805N machine and Ilfo-speed 31M glossy photographic paper, three minutes at 30 kvp and 2.5mA is a sufficient exposure to obtain a clear picture of the interior of the seed. It was by this means that one of us (A.A.R.) discovered larvae of a species of *Megastigmus* in unnaturally large seeds of *Pinus oocarpa*.

5. MICROFLORA AND FAUNA OF SEEDS

5.1 The microflora

Some seed micro-organisms are solely or mainly saprophytic, while others are parasites. Some occur chiefly on the outsides of the seeds, but the use of techniques outlined above has shown that some occur in the internal tissue. Information on the microflora of seeds of tropical pines is still very imperfect. Most relates to the fungi, a little to the actinomycetes and bacteria. In the notes below, some recent information is summarised, and brief reference made also to a few well-documented cases of infestations of seeds of other conifers and some broadleaved trees.

5.1.1 The external microflora: saprophytes

Saprophytes form by far the largest group of micro-organisms on seeds, and in a recent study of seeds of tropical pines, 183 out of 210 microbial species fell into this category (Rees, 1983). The most common genera found in this study were bacteria of the genus *Bacillus*, some actinomycetes from the *Streptomyces* group and species of *Trichoderma*, *Chaetomium*, *Aspergillus* and *Penicillium* from among the fungi.

In certain circumstances, particularly when a seed collection is heavily contaminated, a few members of the saprophytic microflora become opportunistic parasites. As the testa opens or ' chits' prior to germination proper, *Streptomyces spp.* and the fungus *Trichothecium roseum* can penetrate the internal tissues, causing a loss of viability in seeds of *Pinus oocarpa*, *P. caribaea* and *P. pseudostrobus*. During standard germination tests on these pines, severe damage to the apex of emergent radicles has sometimes been observed, caused by *Alternaria alternata* and species of *Penicillium*, *Aspergillus*,

Chaetomium and *Trichoderma* among the fungi, and by the bacterium *Bacillus cereus* var. *mycoides*. This damage has sometimes proved fatal, but it has usually caused only a temporary setback as the affected seedlings, after an initial stunting of growth, overcome the effects by apical regrowth or lateral root production.

5.1.2 The external microflora: parasites

Pathogens which attack pines: The external microflora also includes some species which are harmful to nursery plants and established trees. On seeds of pine collected from tropical and sub-tropical areas by the Commonwealth Forestry Institute, Oxford, England, this group included the fungi *Fusarium equiseti*, *F. oxysporum*, *F. semitectum*, *F. solani*, *F. merismoides* var. *acetilereum*, *Botryosphaeria ribis*, *Phialophora parasitica*, *Phacidiopycnispseudotsugae*, *Sphaeropsis sapinea* and *Botrytis cinerea*. These fungi were often isolated, and not a single seedlot was heavily contaminated. For this and other reasons it was doubtful whether these fungi would cause more than sporadic losses in the nursery. In the seed laboratory, however, where seeds are placed for testing on moist filter paper, the fungi can interfere with germination tests. These may then be spectacularly poor, as one infected seed can affect the remainder.

Pathogens of other hosts: The members of this category were very infrequently isolated from seeds of tropical pines (Rees, 1983). These species identified included the crop pathogens *Nigrospora oryzae*, *Curvularia lunata* and *Hendersonula toruloidea*, the soft rot fungi *Mucor racemosus* and *Rhizopus stolonifer* and the palm parasite *Pestalotiopsis palmarum*. Also found were *Absidia corymbifera* and *Rhizopus microsporus*, which cause phycomycosis in man, *Pithomyces chartarum*, a pathogen of rice and sorghum which also causes facial eczema in sheep, and the bacterium *Xanthomonas campestris* p.v. *campestris*, a well documented pathogen of crucifers.

5.1.3 Internal seed pathogens

This category is potentially the most important of all the seed microflora because these organisms can cause pre-germination death of the seeds, and many of them are also pathogenic to established host trees; because of their location within the tissues, they are difficult to control.

Very few species have been well documented, and this may indicate either that the incidence of pathogens within tree seed is low, or that our information is insufficient because research in this field is in its infancy.

Botryodiplodia theobromae (*Lasiodiplodia theobromae*) and *Schizophyllum commune* were found in seed of *Pinus caribaea* by Rees (1983), while Miller & Bramlett (1978) found *Fusarium moniliforme* var. *subglutinans* and *Diplodia gossypina* in seed of *Pinus elliottii* var. *elliottii*. Agmata (1979) found many seeds of *Pinus merkusii* infected by a *Cephalosporium sp*.

Other fungi have been recorded from time to time in seeds of other coniferous and broadleaved trees. They include *Calyscypha fulgens* (anamorph *Geniculodendron pyriforme*) in *Picea sitchensis* (Sutherland & Woods, 1978) and *P. engelmannii* (Wicklow-Howard &Skujins, 1980), *Sirococcus strobilinus* in *Picea sitchensis*, *P. glauca* and *P. engelmannii* (Sutherland, Lock & Farris, 1981), *Fusarium oxysporum* in *Pseudotsuga menziesii* (Graham & Linderman, 1983), and (in seeds of broadleaved trees) *Ciboria batschiana* (*Stromatinia pseudotuberosa*) in *Quercus spp.* (Delatour &Morelet,1979; Ellis & Ellis, 1985) and a species of *Coniothyrium* in *Betula alleghaniensis* (Shigo &Yelenosky, 1963).

Many of these fungi not only destroy the seeds, but are also pathogenic to nursery plants and plantation trees.

5.2 The microfauna

Information on pests carried on and in tree seeds is almost nonexistent. Most of the information on pests of seeds and cones relates to damage in seed orchards and seed stands rather than to transport with the seeds. Much of the information on conifer cone and seed pests has been summarised by Stadnitskii *et al.* (see Yates, 1979). Janzen (1980) has detailed the specificity of beetles attacking seed in Costa Rica. Rowen & DeBarr (1974) mention *Leptoglossus corculus* (a leaf-footed seed bug) and *Tetyra bipunctata* (a shield-back bug) as the cause of damage to seed of *Pinus elliottii* var. *elliottii*.

When many samples of seeds of tropical pines were examined by Rees (1983), a pest infestation was found in only one case, when larvae of an undetermined species of *Megastigmus* were found in seeds of *Pinus oocarpa*. In the same study, pests (mainly Bruchids) were also found in the seeds of other tropical and sub-tropical trees, particularly of the *Leguminosae* and of species of *Corda*.

In the past, pests of various coniferous trees of temperate areas have been intercepted in seed imported into Great Britain (Phillips & Bevan, 1966). Among them were *Megastigmus piceae*, in seed of spruce, and of another *Megastigmus sp.* in seed of Lodgepole pine (*Pinus contort*a).

6. DISCUSSION

Tree pathologists and seed analysts are often placed in difficulties when asked to certify that seed of tropical and subtropical pines (and indeed of many other tree seeds) conforms to the requirements of an importing country. This is partly because the requirements set out by importing countries may not be clear, or they may be unrealistic, or (in the case of chemical treatments) they may be harmful to the seed or to those who may handle it. These problems arise mainly because there is lack of sufficient information on the pest and disease organisms carried by tree seeds and on their significance, on specific methods of detection, and on suitable chemical and other controlling treatments.

In the long term, this situation can be remedied only by a coordinated and increased research programme on the various aspects of tree seed pathology. The necessary coordination could best be done by a body such as the Danida Forest Seed Centre, which has world-wide contacts and already handles seed of trees of non-temperate areas, and so understands the problems involved. The cooperation of other interested organizations such as IPPC, FAO, IUFRO and ISTA, and governmental bodies responsible for overseas aid could also be sought. By this coordination and cooperation, duplication of projects could be avoided, and the best use made of resources available. In due course, as information accumulated, collaborative projects could lead to the development of standard seed laboratory tests for known seed-transmitted pests and diseases, and eventually to the training of seed analysts in the procedures involved. The tests would be comparable to those used to examine seed germination, proposed by ISTA (1976). First of all, however, more information is needed on the presence of actinomycetes,

bacteria, invertebrate pests and viruses, as well as of fungi, occurring on tree seeds. The knowledge gleaned from this research could also lead to suggestions on suitable sample sizes, especially for the detection of pathogens of low incidence. At present, too, organisms are commonly scored only as 'present' or 'absent' in a seed sample. As our ability to isolate specific pathogens increases, it should be possible to provide a more detailed and accurate scoring system. However, as the microbial profile of each seed collection changes during storage, it will probably be necessary to carry out a series of tests over the storage period.

Further, the more general information needs to be augmented by other work to establish which of the organisms found are seed-transmitted.

A concerted effort is also needed to discover suitable methods for the control of the harmful organisms associated with seeds of trees and shrubs. So far, chemicals have been the main agents used to limit the dissemination of these pathogens and pests. New chemicals and formulations should be tested to find materials which penetrate the seed coat, are not phytotoxic at the time of application and do not later break down to leave harmful residues, and which are safe to handle. It is important to note the effect of any chemical on seed viability (which may vary with the tree concerned). It is pointless to examine proprietary materials if their active ingredients are not known; and accounts of work done on proprietary materials should provide the brand names as well as those of the active ingredients.

It may be productive also to examine possible alternatives to chemical control, such as the use of ultrasound techniques, which are relatively inexpensive and easy to use, and are not dependant on the whims and vagaries of chemical companies.

It would be desirable if the work done could lead to a simplification of the administrative paper work involved in the movement of seed. At present, seed samples may need to be accompanied by import permits, certificates of origin, papers certifying viability, purity, etc., phytosanitary certificates, and information on any treatments applied to the seed. Time could be saved if the seed could be accompanied by one standard certificate detailing all the required information. Much of this would be provided by the exporting country. As already noted, however, we consider that at present, chemical seed treatments should be applied in the importing country, and information on any chemicals used (with rates and methods of application) would then be added as the final entry on the certificate.

If the above suggestions could be implemented, we consider that our knowledge of tree seed pathology, which is still relatively scanty, could be improved and be made to equal that in the fields of agriculture and horticulture. The possibility of the seed-transmission of tree diseases and pests from continent to continent could thus be reduced.

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