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Mycoflora of recalcitrant tree seeds and it's effects on germination and storability

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Reduction in seed viability during storage of recalcitrant seeds has been a major constraint in success of several plantation programmes. Unlike orthodox seeds which can tolerate desiccation to low (4-12%) moisture content (m.c.), recalcitrant seeds are sensitive to desiccation and store best with relatively higher m.c., for example *Quercus robur* at about 45% (Holmes and Buszevicz, 1958), and *Acer saccharinum* at 36% (Jones, 1920). Recalcitrant seeds of temperate tree species may be stored at or slightly below freezing temperature whereas of tropical species can not survive the same low temperatures (Bonner, 1990). However, some recalcitrant seeds may exhibit different storage patterns also. For example, *Shorea parvifolia* seeds with m.c. above 40% have storage characteristics similar to recalcitrant seeds while those with m.c. less than 40% store like orthodox seeds (Yap, 1988). Farrant *et al.* (1988) reported continuum of recalcitrance and that the seeds might be grouped as showing a minimum, moderate or high degree of recalcitrance. Seeds with minimum recalcitrance will tolerate a fair amount of water loss and relatively low temperatures. The concerned species are mainly indigenous to temperate and sub-tropical regions. Highly recalcitrant seeds produced by the tree species of moist tropical forests, will tolerate only a little water loss and are generally markedly sensitive to low temperature. Seeds with moderate recalcitrance are tropical in distribution and characterized by intermediate degree of dehydration and ability to tolerate mid range temperatures.

Tropical recalcitrant seeds are usually large in size and are mainly found in (a) tree species of moist forests belonging to the families of Dipterocarpaceae and Araucariaceae, (b) important tropical plantation crops such as rubber, cocoa, and coconut, and (c) tropical fruit crops such as mango, mangosteen, durian, rumbutan, and jack fruit. Seeds of this group are desiccation-sensitive and chilling-sensitive, with many intolerant to freezing temperatures (e.g. *Hevea brasiliensis* and *Nepheliom lappaceum).* Some can not tolerate temperatures of 4 *Shorea roxburghii (=talura)* of even 15 *(Theobroma cacao).*

The recalcitrant tropical tree seeds usually have a short viability period of a few weeks to a few months (King and Roberts, 1979). They have to be stored at a critical (lowest safe) m.c. which is usually a little lower than the seed m. c. at harvest, and at a relatively high and often ambient temperature. They loose viability with decrease in their m.c. Studies by Lin (1996) of the seed storage behaviour of three genara of the Lauraceae family, viz., *Neolitsea parvigemma, Lindera megaphylla* and *Cinnamomum subavenium*, revealed that the safest seed m.c. for storage behaviour of three genera of the Lauraceae family, viz., *Neolitsea parvigemma, Lindera megaphylla* and *Cinnamomum subavenium,* revealed that the safest seed m.c. for storage is that of the mature seeds at the time of harvest, thus supporting the observations by Tompsett (1982) on *Araucaria hunsteinii* and by Hong and Ellis (1990) on *Acer pseudoplatanus.* However, Lin (1996) observed that the *Lindera megaphylla* seeds with 30.7% m.c., had 7.5% m.c. and still 97% germination after 18 days storage at 23°C and 82% relative humidity. These seeds with 7.5% m.c. when further stored at 4°C and 82% relative humidity, were still having 7% m.c. but the germinability was reduced to 93% after two months and was only 10% after four months. Therefore, he expected that the seed viability could not be determined according to a critical m.c., and the initial sensitivity to desiccation and the final longevity might not be strongly associated. Also, the recalcitrant seeds generally show variation in m.c. at shedding within any particular harvest of a single species. Results of a survey of 21 recalcitrant species, have shown that without exception there was a marked variation in the m.c. on a seed-to-seed basis for both embryonic axis and storage tissues (Grabe 1989) which might make it difficult to obtain a critical safe moisture content level for seeds.

Besides other factors, viz., improper time and place of harvest, and improper method of seed collection and handling, the storage of recalcitrant tropical tree seeds with their high m.c. and at a relatively high and ambient temperatures offer fortune to the micro-organisms expecially fungi, to infect such seeds. But the characteristics of these fungi i.e. which are the species associated with different tree species, when, where and how their infections take place, what damage they cause to seeds and seedlings, and what can be done to prevent the damage, have not been throughly investigated.

MYCOFLORA OF TREE SEEDS

Forest tree seeds harbour micro-organisms which include bacteria, virus and predominantly, the fungi (Mittal *et al.*, 1990). Fungi associated with tree seeds in general, have been categorized differently by different workers. Urosevic (1961) divided the acorn mycoflora into two groups : (i) parasites and semiparasites such as *Ciboria batschiana*, *Ophiostoma* sp., *Gloeosporium quercinum*, *Phomopsis quercella*, *Cytospora intermedia*, *Botrytis cinerea* and *Pestalotia* sp., and (ii) saprophytes including species of *Alterna, Aspergillus, Fusarium, Penicillium, Trichoderma* and others. Christensen and Kaufmann (1974) classified the fungi found associated with seeds into field fungi which gained access to the seed prior to or immediately after harvest when seed moisture content is still relatively high, and the storage fungi like species of *Aspergillus* and *Penicillium*, which might initially be inconspicuous prior to, or at harvest, but come to dominate the interior of (orthodox) seeds once they are dried down to m.c. suitable for storage.

Depending upon their location, the seed-borne saprophytic and pathogenic fungi can in general, be classified under two gropus, viz., externally seed-borne and internally seed-borne. Fungi of the first group are usually not host specific and particularly, the internally seed-borne fungi may cause deterioration of seed quality and pre-and post-emergence mortality of seedlings (Singh and Mathur, 1993). Sutherland (1995) classified the seed-borne fungi of conifers on the basis of their pathogenicity, into (i) saprophytes or weak pathogens, (ii) pathogens, such as the seed or cold fungus *Caloscypha fulgens*, which consistently kill seeds, (iii) pathogens mainly important as seedling pathogens, e.g. *Sirococcus conigenus*, and (iv) fungi, e.g. *Fusarium* spp., whose pathogenicity depends upon factors including fungus species and pathogenic strain, and host and host stress.

Thus, the fungi may be found on the surface (externally seed-borne) or within (internally seed-borne) the seeds. They may not be causing any disease (saprophytes) or may do so (pathogens). Amongst the saprophytes, certain fungi though harmless but may cause serious damage if optimum environment for their activity prevails or may facilitate infection by pthogens. Infected seeds may act as carriers of disease into new areas also. It is therefore, important to study the characteristics of all the fungi associated with the seeds.

Seed-borne nature of fungi like species of *Phytophthora, Pythium* and *Fusarium* which caused pre and post-emergence damping-off of seedlings ; *Ciboria batschiana, Sclerotinia sclerotiorum, Phomopsis quercella, Gloeosporium quercinum* on oak acorns ; *Endothea parasitica* on chest nuts ; *Fusarium oxysporum* in larch ; *Trichothecium roseum* on Scotch pine, Norway spruce, *Rubinia pseudoacacia*, ash, birch, Euonymus, beech and oak, and of *Botrytis cinerea* on 48 species of 32 tree genera, etc. has earlier been reported (Neergaard, 1969). In addition, several fungi, viz., species of *Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium, Mucor, Rhizopus, Trichoderma, Trichothecium* and many others have now been reported on the seeds of tropical trees (Mittal *et al.*, 1990). However, very few attempts have been made to identify the fungal species associated with the recalcitrant tree seeds in particular, though their importance in seed deterioration has been expected for quite some time. It is often difficult to categorize a tree species as having recalcitrant seeds without specific knowledge about it's seeds, since the species within same genus behave differently. Some work on the mycoflora of *Quercus* acorns is available, sometimes without mention of particular species. Specific reference to *Quercus robur* which is a temperate species with recalcitrant seeds is also occasionally available. The work on oak acorns in general, is discussed here for reference. *Phomopsis quercella, Fusarium oxyporum, Ceratocystis* sp. and *Pestalotia* sp. have been recorded both on acorns and as casual organisms of seedling diseases. *Gloeosporium quericinum,* which causes the leaf spots, has also been recorded infecting acorns (Potlaichuk, 1953). Fungi of recalcitrant tropical tree seeds have recently been studied by Mycock and Berjak (1990), Gomide *et al.* (1994), Berjak (1996) and Kumi *et al.* (1996). Gomide *et al.* (1994) identified *Alternaria, Aspergillus, Cladosporium, Curvularia, Helminthosporium, Mucor, Nigrospora, Penicillium* and *Rhizopus* from the infected seeds of *Eugenia dysenterica. Phytophthora* spp. has been isolated from the cacao seeds and has been studied to cause blackening of pods and leaf spots, wilting and stunting of seedling (Kumi *et al.*, 1996). In *Tachigalia versicolor*, a neotropical leguminous tree, pathogens ranked very high in importance in seed destruction and caused nearly 25% of full sized seeds to become nonviable (Kitajina and Augupurger, 1989).

The time, place and method of collection of seeds and subsequent handiing during transport, extraction and processing affect the development and spread of mycoflora on seeds. Seeds collected from the ground or squirrel catches, or late in the season, or damaged by insects or mechanically during extraction and processing, or stored and transported in poorly ventilated humid conditions, are known to suffer more from fungal infections. Cones and seeds in temperate climates escape many conditions which favour fungal development and spread, however encountered very frequently by the tropical tree seeds, leading to much higher losses of them.

Fungal infection in acorns was found facilitated by the weevil damage ; 50% of those infected by weevil were infected as against 3% which were not damaged by the weevil (Potlaichuk, 1953). Similar observations on fungal infestation of oak acorns facilitated by insects, principally by weevil and a totricid, have made by Jones (1959) and Hepting (1971).

The later the acorns were collected from the fields, the more infected they were with fungi (Potlaichuk, 1953). *Penicillium, Fusarium, Alternaria* and *Trichothecium roseum* were found to be the most common fungi during acorn development. Development of *Cladosporium herbarum*, which was frequently encountered on the acorns in the first and last samples during their development, depended upon the amount of rainfall, the highest occurrence during the period of heavy rainfall.

Several fungi have been found in natural infections as well as in artificial inoculations, to reduce the quantity and quality of seed germination in tree species. The damage usually depended upon the quality of seeds used and the testing environment. During the initial germination testing of *Prunus africana* in sterilized sand, growth of a white coloured fungus was observed which was more prevalent in the green seeds with a moisture content of 25% than the purple or purplish green seeds or the seeds with 8 and 15% m.c. (Weres, 1997).

STORAGE OF RECALCITRANT TREE SEEDS AND THE FUNGAL DAMAGES

The recalcitrant seeds are fast perishable in contrast to orthodox seeds which can tolerate low m.c. and low storage temperature and therefore, can be stored successfully for often longer periods. Longevity of recalcitrant seeds which generally require wet storage varies from one to the other species. On the basis of the work done on unrelated recalcitrant seeds of *Avicennia marina, Landolphia kirki, Castenospermum australe, Scadoxus puniceus, Podocarpus henkelii, Coffea canephora* var. *robusta* and *Camellia sinensis,* Boyce (1989) summarized that those species which have a rapid rate of germination, have a more curtailed storage life span too. Maximum longevity of recalcitrant tree seeds has been reported more in wet than in the dry storage. It is also interesting to note that the slowly dried seeds survived longer in storage than the rapidly dried seeds, but they lost their viability at higher m.c. than the rapidly dried seeds.

Several theories have been proposed as regards the seed deterioration during storage. These include changes in protein structure (the protein coagulation caused loss of viability) ; depletion of food reserves for embryos ; development of fat acidity (reduced seed germination accompanied by an increase in fat acidity) ; enzymatic activity (for example catalase, phenolase) ; chromosomal changes ; membrane damages (free radical damage to membranes and enzyme systems) and increased respiration (increased respiration with increased seed m.c.). However, none explains satisfactorily how seeds deteriorate although membrane damage is definitely assocaited with seed deterioration (Justice and Bass, 1979).

In an examination of some of the biochemical events leasing to the loss of viability under various storage treatments in seeds of *Guilfoylia monostylis,* Nkang (1988) did not find any correlation between the decline in viability and pentose phosphate pathway dehydrogense or peroxidase activities, however reported that the status of certain hydrolytic enzymes and stored reserves might be important to seed viability.

King and Roberts (1979) pointed out that one of the factors contributing to the short storage life of recalcitrant seeds is microbial contamination. Potlaichuk (1953) reported that acorns could loose upto 70% of their germinability due to fungus infection in storage. *Sclerotinia pseudotuberosa, Phomopsis quercella, Gloeosporium quercinum* and *Cytospora intermedia* were found most harmful as they penetrated deep into cotyledons and embryo and destroyed them by planting time beside spreading infection during storage. In the oak acorns during storage, the blackening and deterioration by *Ciboria batschiana,* dry rot by *Phomopsis* spp., decay by *Fusarium oxysporum,* and wilt and dieback by *Coniothyrium quercinum,* have been reported (Murray, 1974). Similar detrimental effects of associated fungi to *Quercus robur* acorns were demonstrated by Hangyal-Balul (1986), Werres *et al.* (1992) and Siwecki (1994). However, Andersson (1992) reported that the fungal infested acorns germinated more than the unifested ones.

The desiccation sensitive, wet shed, recalcitrant seeds have been found to have mycoflora dominated by *Fusarium* species rather than the *Aspergillus* and *Penicillium* species of air dried, desiccation tolerant, orthodox seeds. According to Mycock and Berjak (1990), a fungal succession occurred during storage of homoiodryous seeds as in the orthodox seeds, and they found a narrowing in the range of fungal species during wet storage. After only six days of wet storage, *Fusarium* spp. had apparently outcompeted the other fungi on the pericarp as well as on the surfaces of the cotyledons and embryonic axis in *Avcennia marina* seeds. Although bacteria initially constituted the major class of initial contaminant, it had declined markedly after six days of wet storage and no bacteria could be isolated after 13 days when *Fusarium* spp. emerged as the sole contaminant of all the *A. marima* seeds.

Fast deterioration of the recalcitrant seeds by the storage fungi has been expected to be due to the debilitation of seeds caused by internal moisture stress generated within the cells or tissues as a result primarily of the water requiring process of vacuolation (Berjak, 1996). This debilitation impaired phytoalexin synthesis by the seeds, facilitating proliferation of associated fungi or bacteria.

MANAGEMENT OF FUNGI DURING STORAGE

In view of the specific storage requirements of the recalcitrant seeds, a multidimensional approach including collection of healthy seeds form healthy sites and at appropriate time, management of seed moisture content and storage temperature at optimum during seed storage, will be required for management of fungal damages to them during storage. Prevention of fungal activity during storage can be more easily achieved by controlling the m.c. of the seeds which is easier also, than by controlling the storage temperature. If m.c. and relative humidity are high enough, fungal activity has been found possible between -8°C and +80°C (Roberts, 1972). Hence, it is important to find out optimum m.c. and storage requirements for individual reclcitrant tree seeds. With partial desiccation and surface dressing with fungicide, Chin (1995) observed prolonged seed viability from an initial few weeks to a few months to presently upto one year. Nkang (1988) recommended slow drying of seeds to a m.c. slightly below the m.c. (56%) of the freshly collected seeds of *Guilfoylia monostylis,* before their long term storage at 5°C in sealed polythene bags with suitable fungicides.

Treatment of seeds with hot water, fungicide or gel, had been found effective in controlling the mycoflora during storage. Delatour *et al.*, (1980) observed that the oak acorns which received heat treatment by soaking in water at 38-40°C for several hours before storage, had 80-90% germination as against 40% in untreated seeds. Hangyal-Balul (1986) found reduced fungal damage to oak acorns by seed dressing with Dithane M 45 + Fundazol 50 WP (Benomyl) at 1 g per kg of seed, however the treatment had little effect on survival after first growing season. In an effort to find out an effective method to control lethal fungi of *Penicillium*, *Aspergillus, Botryodiplodia* and less destructive fungi of *Rhizopus* and *Paecilomyces* in cocoa seed storage, Hor (1988) found that 80% germination was maintained for 12 to 18 weeks in the seeds treated with 0.2% w/w of an equal benlate-thiram mixture, whether applied as dust or by soaking the seeds in fungicide solution.

Gomide *et al.,* (1994) evaluated eight fungicides to control the fungi and their effects on seed germination in *Eugenia dysenterica*, before sowing in sand, and found that the treatment with 5% aqueous solution of Benomyl for 10 min had the lowest percentage of seeds with fungal growth, and the highest percentage emergence and speed of emergence when seeds tested after one day or 50 days storage in a humid cold chamber. Treament with sodium hypochlorite and carboxin + thiram were found phytotoxic.

Reduced fungal growth on seeds due to their treatment with fungicides has been successfully achieved in several other tree species including both conifers and hardwoods. However, the fungicides sometimes caused abmormal germination as reported by Cram and Vaartaja (1957), Vaartaja (1964) and Kozlowski (1986) in conifers, and therefore, have to be applied carefully after testing their phytoxicity.

According to Motete *et al.*, (1997). the storage duration of seeds of *Avicennia marina*, a highly recalcitrant species, was multiplied four times when coated with an alginate gel, and that the preliminary results indicated the antifungal property of the gel.

In cases where fungi are located on the seed surfaces or within the seed coat only, storing the seeds without them curtailing the fungal proliferation, could be attempted. Cryopreservation of such seeds, or the excised embryos is thus, a possibility for long term storage of recalcitrant tropical tree seeds. There may however, be a requirement of pre-storage treatment with surface cleaning agent like sodium hypochlorite, hydrogen peroxide, etc., or with fungicide or simply hot water or air. It is therefore, important to find out and manage the optimum seed m.c., storage conditions (temperature and relative humidity) and requirements of a safe treatment before storage.

EPILOGUE

Seeds being the main source of raising planting stocks of trees, seed health testing assumes great significance for the success of plantation programmes. An organized collaborative effort at international level is required for reviewing the existing knowledge and develop suitable techniques ans guidelines for study of micro-organisms on tree seeds, evalaution of their effects on seed health during storage and germination, identification of pathogens,risk analysis for ascertaining their quarantine significance, optimization of seed storage requirements, management of seed pathogens and formulation/revision of seed health and quarantine regulations for important tree species.

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