Mold Fungi and Their Relation to Forest Tree Seed

By Keith R. Shea

Immediate rehabilitation of harvested forest lands by planting or direct seeding has become a major endeavor of the Weyerhaeuser Company. Such an objective is dependent on an adequate supply of desirable tree seed. To meet this need, cone collection and seed processing have become an integral part of our forest practices. Along with foresters, specialists in the fields of soils, physiology, regeneration, silviculture, entomology, wildlife biology, and pathology, are engaged in co-ordinated research on aspects of seed procurement and regeneration. The purpose of the present paper is to discuss one aspect of the pathological problems involved, viz, the fungi (commonly called molds) on forest tree seed, especially Douglas-fir seed.

Examination of cones in sack storage revealed numerous fungi not only on the cone surfaces but between the cone scales and on the wings and seed coat of the seed. By the fall of 1957, sufficient evidence had been found to suggest that the molds might be the cause of unexplained losses in seed viability during cone storage prior to processing. Consequently, research was initiated with the initial objective of developing, methods for assay of the molds and for determining losses.

Review of Literature

A *review* of pertinent literature showed that little attention has been given to fungi associated with forest tree seed prior to sowing. Few seed-borne diseases of trees are known and many of these are not well substantiated or have received little attention. In 1955, Shea suggested that the indiscriminate introduction of seed and pollen from other countries was accompanied by the danger of introducing tree pathogens.

The molds of Douglas-fir seed were studied by Salisbury who found no clear correlation between high mold count and low viability of extracted seed. The most common molds found were species of Penicillium, Mucor, Aspergillus and Pullularia. However, most literature reviewed dealt with the molds associated with stored grains. It is these reports which provided background for investigations on molds associated with forest tree seed.

Deterioration of stored grains is manifested by decreased germination decreased processing quality, "sick" or germ damaged grain, heating, and mustiness. Over fifty fungi which appear to be involved may be divided into two groups, viz, field fungi and storage fungi. Harmful fungi increased greatly between harvest and arrival of grain at terminals. Invasion of seed by storage fungi impaired storability, whereas, field fungi appeared to be of little consequence.

No one medium or techniques suitable for determining **all** organisms that may be present in a given lot of seed. To determine fungi associated with grain,whole or macerated seed are used. Whole seed may be surface disinfected or the seed may be washed in a jet stream of water before plating. When it is desirable to determine the number-of viable fungus spores present, a given weight of seed is comminuted in a blender and dilutions are made until the desired dilution is obtained. -One ml. aliquots of the dilution are placed in petri dishes, the selected medium added, swirled, allowed' to harden and the dishes incubated. Counts of the mold colonies are made and the results expressed in colonies per gram of seed. Fungi selected for isolation and study usually are grown on other media..

The invasion of stored grain by fungi is influenced by a complex of conditions which in practice operate together. Moisture content, temperature, amount of previous infection, time, and the activities of grain-inhabiting insects and mites are the major ones. It appears that seed moisture contents below twelve percent are relatively safe provided no portion of the seed in storage is much above this level. Most fungi which invade stored grain grow best at about 85° F. A *few* will grow. at 130° F. and others as low as. 40° F. Time is associated intimately with moisture content-and temperature. Thus, within the limits of growth **of** the fungi, the higher the moisture content and temperature the shorter. the time seed may be stored without adverse effects. Apparently insect invasion of stored grain results in increased moisture content favoring mold development.

Control of molds of stored grain involve three general methods. They include treatment with fungicides, storage under toxic or inert gases, and drying the grain to reduce moisture: content to a safe level. Fungicides are most effective only so long as free water is available, a point which limits their use in grain storage, but may not be critical with moist, green forest tree cones. Storage under gases has practical limitations which limit their usefulness. Effective longtime storage of grain, may best be effected by obtaining a moisture content sufficiently low to inhibit invasion by storage fungi. It has been noted, however, that wheat seed stored outside in bags but protected from direct rain and snow picked up sufficient moisture from the air to raise the-moisture. content to fifteen percent at which level storage fungi flourish.

Deterioration of Forest Tree Seed

Deterioration of tree seed by fungi involves problems of similar nature but differing in many respects from those' of grain. For example,' forest tree seed are exposed to many ' conditions which permit the development' of mold fungi. The cones are collected, placed in burlap sacks, and stored at collecting points or proce'ssin plants for indeterminate periods prior to kiln drying and seed extraction. After extraction, the seed are dried and stored for varied periods until used in field or nursery seedings.

Investigations of the molds of forest tree seed involve the development of methods and techniques unique to forest tree seed procurement. It is essential to determine the kinds of fungi involved and the manner in which they develop. Before control methods can be justified, the losses attributable to molds must be assessed. Initial studies at the Forestry Research Center have shown that methods developed by other workers are suitable for most purposes.

Over forty isolates of fungi have been obtained from Douglas-fir seed in our laboratories. The most common fungi include species of Penicillium and Aspergillus. Other fungi include species of Rhizopus, Thamnidium, Pullularia, Trichoderma, and Trichothecium, to name a few. Additional fungi are now being identified.

Development of molds in cones and on seed apparently can begin on the tree. Examination of fresh cones commonly reveals no mycelium on the cone surfaces, although fungus spores may be present. Norman Johnson, ou.r entomologist, who has studied the insects of Douglas•fir cones has noted the development of fungi in the galleries of cone and seed insects. Often, the areas adjacent to the gallery are dead and invaded by fungi. Whether these are saprophytic fungi attacking tissues killed by insect activity or parasitic is open to question. Currently, we do, not know the effect the fungi associated with insect damage may have on seed. However, it appears that additional damage to the cone and seed is the direct result of fungus attack arising from infection courts provided by insect activity.

Whether there are two distinct groups of organisms, the field and storage fungi, as with grain is open to question. There are, however, increased numbers of Aspergillus and Penicillium species in relation to other fungi associated with seed as cone storage time increases. Numerous other fungi also appear to be involved with the various stages during cone procurement and processing.

Douglas-fir cones collected and stored in burlap sacks may become covered with mold fungi. Coordinated experiments on after ripening of Douglas-fir cones with Dr. Rediske, our physiologist, have shown that the fungi are associated with decreased viability of seed from cones stored for varied periods. As viability decreased, mold counts increased. Cones dipped in fungicides before storage yielded seed of greater viability over longer periods than did seed from untreated check cones. Later in the season when seed were mature and cones more ripe, losses were less and the causes more poorly defined. Additional studies are being continued with the most promising fungicides in pilot scale tests to determine their effectiveness for control of molds. Initial operations of this size were not too effective probably because of adverse storage and deterioration of seed before treatment.

Mold growth and yield of viable seed are affected markedly by the conditions under which cones are stored. Apparently three factors--moisture, temperature, and time of storage, are involved. Cones stored under adverse environmental conditions are subject to heating as a result of biological activity. Examination of seed from cones under Very adverse storage condtions has shown that molds decreased potential yields markedly. Seed in all stages of deterioration were found: In extreme instances, fungi had invaded the seed coat and were observed fruiting on the deteriorating endosperm. To date, all evidence suggests that the fungi are a major cause of reduced seed viability in stored cones. Research is underway to determine practical methods for preventing such losses attributable to fungi.

Extracted forest tree seed may be stored for prolonged periods before utilization in the field or nursery. Douglas-fir seed have been shown to retain full viability for three years when stored **at.** 65 F. and 5.8 or 13.6 percent moisture content. If seed were stored at similar moisture contents and at 40° F., marked deterioration resulted after twelve months at 13.6 moisture content: Certain storage molds of grain are capable of attacking seed at this temperature and moisture content.

Perhaps, part of the loss in viability of stored Douglas-fir seed could be attributed to fungi. In most instances, however,. extracted forest tree seed are stored at temperatures and moisture:contents which- prevent mold growth.. Consequently, any reduction in .:viability of extracted seed must occur before seed is stored or-after storage when environmental conditions are favorable for fungus development. Our experiments have shown that molds on tree seed retain viability for at least twelve months when seed are stored at 10^0 *F.* and approximately 8 percent moisture content. During this time no reduction in germination was noted. However, seed remain well inoculated and need only favorable conditions for continued mold development on and in seed.

Molds associated with seed may have adverse effects on seed in the field. Gibson (1957) in Africa has reported that loss of Pinus patula seed associated with damage to the seed coat was due to invasion by soprophytic seedborne fungi. The destruction of the seed appeared to depend largely on growth rate of the seedling which became tore resistant as germination progressed. He suggested that fungal invasion of seed was facilitated by minute damage incurred during mechanical dewinging. Laboratory Studies with eighteen of the common fungi we have isolated have -shown that all were capable of destroying seedlings under Conditions favorable to mold development.

Thus, it seems likely that the molds prevalent on tree seed may contribute to seed decay and pre-emergence damping-off in the field. A' field study in 1959 on our Wynoochee Tree 'Farm in western Washington has shown that seed decay and damping-off were major causes of seed loss. Deterioration of the seed and damping-off 'may result from direct attack by seedborne fungi or from general depletion of vigor predisposing the seed to other fungi in the soil. This theory is borne out by the report of Noble, et **al,** (1958) who in their list of seedborne diseases have reported that Aspergillus niger and other Aspergillus spp. attack seed. Penicillium oxalicum and Penicillium spp. are known to cause seed rot of sorghum and corn. Likewise Rhizopus spp. cause loss of cotton seed and seedlings, sorghum, and corn. Koehler and Hotbert (1930) have shown that Aspergillus, Rhizopus, and Penicillium invade the scutellum and adjacent embryo of corn damaging the endosperm and weakening the endosperm. The similarities with fungal invasion of Douglas-fir seed are so striking that there seems little doubt but what comparable events can and do occur. Research now is being planned to explore more fully damping-off and seed decay in the field and the role of seedborne fungi in the damping-off complex.

Future Needs

It has been established that the molds of forest tree seed can cause reduction in yield and viability. Evidence suggests that the effects of molds may carry over into the field and contribute to seed decay and damping-off. The question arises as to what lines of approach future research should take.

The prevalence of a wide variety of molds and the classes into which they fall suggest three possible groupings. Certain molds may be wholly saprophytic and contribute little, if anything, to the damage of seed. Others may actually be beneficial in that they are antagonistic to the third class, the harmful fungi. With such possibilities, it seems that the causes of seed deterioration should be explored more fully and the causal agents isolated. If fungi antagonistic to damaging molds are found, it might be possible to encourage their growth at the expense of the harmful fungi.

Once the causal agents are well defined, it is essential to determine the epidemiology of seed deterioration. Studies on the effects of those factors in the environment which contribute to the development of mold fungi could provide basis devising control measures. Of special concern are temperature, moisture content, relative humidity, tine of exposure to these predisposing factors and the environmental conditions in the field. Such studies call for joint participation of pathologists, physiologists, soils specialists, and regeneration experts, as well as others.

Sound control measures should be based on thorough investigations of the etiology and epidemiology of seed deterioration. Control measures must be divided into two facets. The first involves control of damage to seed in the cone prior to processing. The second involves the seed in the soil in the field. Reducing the severity of contamination and infection in the cone and processing stages may alleviate damage in the field. Thus, it appears inevitable that the two phases of control be investigated jointly. Mold control in the cone may be attempted through chemicals, by creating conditions unfavorable for development of damaging fungi, or encouragement of fungi antagonistic to the harmful ones. In the field, the addition of fungicides to standardized seed treatments appears most promising. Any chemical additive should, however, be assayed for phytotoxicity and compatibility with rodenticides or other seed treatments.

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Following the papers, Dick Bingham presented a very interesting slide talk on the "Role of Genetics in Producing Blister Rust Resistant Seedlings." This work, started in the late forties i progressing much faster than originally expected.

The 2-1 progeny of selected blister rust resistant trees are showing such good resistance that it now appears that stock for field planting will be available in 1970.

A forty-acre seed orchard has been established at Moscow on land provided by. the University of Idaho.

The new genetics center, complete with laboratory and greenhouse facilities, is also located on the campus. A seventeen-acre seed orchard has been established at Sandpoint on the administrative site there.

All in all the future looks very bright in this: important development in white pine management.

The meeting adjourned at about 10 p.m. following a short summary statement and God-speed by Supervisor Krueger.

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