

# MICROFLORA OF CONIFEROUS SEEDS IN TAIWAN<sup>(1)</sup>

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## Introduction

One of the most baffling problems in supplying coniferous seedling stock in Taiwan is the frequent recurrence of germination failures in nursery beds. The term "germination loss" has been employed for diminished emergence resulting from pre-emergence damping-off including either seed or radicle decay, or both. For control measures, both seed and soil treatments with Granosan have been recommended (Chen, 1961; Chen et al., 1963). However, controversies have raised among their results. Chen (1961) pointed out that the seed treatment was more effective than soil treatment, whereas Chen et al. (1963) suggested that seed treatment with Granosan and soil acidification with aluminum sulphate or ferrous sulphate were most effective for the disease control.

Hartley (1918) indicated that germination losses in coniferous seedbeds were attributed to the fungi, e. g. *Pythium*, *Rhizoctonia*, and *Fusarium*. Inhibitory effect of *Aspergillus*, *Mucor*, *Penicillium*, and *Rhizopus* on the germination of coniferous seeds was also reported by Vanino (1931). Rathbun-Gravatt (1931) showed that reduced emergence of coniferous seeds was brought about by the fungi, e. g. *Fusarium*, *Pythium*, and *Rhizoctonia*, inciting the radicle decay. Garbowski (1936) isolated 14 species of fungi from seeds of *Pinus sylvestris* and demonstrated that the poor germination of *P. sylvestris* was mainly due to *Pyronema ompholedes* and one species of *Botrytis*. From the inoculation experiments, Fisher (1941) found that the following organisms: *Botrytis* (*cinerea*?), *Fusarium*, spp., and undetermined phycomycetes, *Pythium debaryanum* Hesse, *P. ultimum* Trow, *Rhizoctonia*, *Sphaeropsis ellisii* Sacc., *Verticillium* and 4 undetermined fungi, caused decay of radicles just emerged from the seeds coats. Huss (1952) studying the effect of moulding on germinability of pine seeds exhibited that it was virtually without effect on seed of high viability but poor seed suffered a substantial reduction of germination. Nevertheless, Timonin (1964) recently reported that some saprophytes of the coniferous seed-coat

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could attack the seedling and produce symptoms of damping-off under certain condition.

From the review of literature available, it is not difficult to suspect that coniferous seeds associated with microorganisms carry causal organisms into the soil. An attempt to isolate the microorganisms associated with coniferous seeds in relation to damping-off disease seems to be plausible to understand disease incidence in order to establish effective control measure of the disease since the information of this nature has not been available from the coniferous seeds in Taiwan.

### Materials and Methods

Seeds of slash pine (*Pinus elliottii*), Luchu pine (*P. luchuensis*), Japanese black pine (*P. thunbergii*), Taiwan armand pine (*P. armandi* var. *mastersiana*), Taiwan red pine (*P. taiwanensis*), Taiwan white pine (*P. morrisanicola*), and horsetail pine (*P. massoniana*) were used for the present experiments. The seeds of slash pine, Taiwan armand pine, Taiwan red pine, Taiwan white pine, and horsetail pine were kindly supplied by the Taiwan Forestry Bureau, Luchu pine and Japanese black pine seeds were purchased from a forester. All the seeds used were collected on this island, except that a sample of slash pine imported from the States on March 26, 1962. They were stored in a cold room ranged at 0° to 5°C for a period of time prior to determination.

Coniferous seeds were respectively treated with 4 per cent sodium hypochlorite and 0.5 per cent mercuric chloride to distinguish the microflora of surface layers and internal layers of coniferous seeds as already mentioned in the previous experiment. The treated seeds were plated on potato dextrose agar and incubated at 28°C.

### Results

As shown in the previous experiments, seeds treated with 4.0 per cent sodium hypochlorite for one minute were partially sterilized, since most of the microorganisms associated with the surface layers of the coniferous seeds could appeared in a short term incubation at 28°C. The data obtained are shown in Table 1.

From 8 seed samples of 7 different species of pines, more than 17 genera of fungi were isolated. They were *Aspergillus*, *Cephalosporium*, *Chaetomium*, *Chaetomella*, *Curvularia*, *Diplodia*, *Fusarium*, *Graphium*, *Helminthosporium*, *Mucor*, *Penicillium*, *Pestalotia*, *Phoma*, *Rhizoctonia*, *Rhizopus*, *Sphaeropsis*, and *Verticillium*. The resultant data were almost similar to those obtained by other workers (Fisher, 1941; Garbowski, 1936; Sato, 1955; Timonin, 1964; Vanine et al., 1932).

**Table 1.** *Microflora of coniferous seeds treated with 4 per cent sodium hypochlorite\**

Species of conifer	<i>Pinus armandi</i> var. <i>mastersoniana</i>	<i>P. elliotii</i> *** A	<i>P. elliotii</i> *** B	<i>P. luchuensis</i>	<i>P. massoniana</i>	<i>P. morrissonicola</i>	<i>P. taiwanensis</i>	<i>P. thunbergii</i>
<i>Aspergillus</i>	2	12	4	9	3	5	1	21
<i>Cephalosporium</i>	—	1	—	—	—	—	—	—
<i>Chaetomium</i>	—	—	1	—	—	—	—	—
<i>Chaetomella</i>	1	—	—	—	—	—	—	—
<i>Curvularia</i>	1	—	—	—	—	—	—	—
<i>Diplodia</i>	7	208	99	20	11	185	1	21
<i>Fusarium</i>	9	8	3	—	25	79	2	28
<i>Graphium</i>	46	—	—	—	—	—	—	—
<i>Helminthosporium</i>	—	1	—	—	—	—	—	—
<i>Mucor</i>	1	—	—	—	—	—	—	—
<i>Penicillium</i>	—	—	11	—	—	—	—	—
<i>Pestalotia</i>	125	7	—	—	43	7	44	3
<i>Phoma</i>	1	—	—	—	—	—	—	—
<i>Rhizoctonia</i>	—	—	1	—	—	—	—	—
<i>Rhizopus</i>	—	—	—	—	11	—	8	7
<i>Sphaeropsis</i>	—	—	73	—	—	33	1	9
<i>Verticillium</i>	1	—	68	—	—	3	15	—
Undetermined**	1	1	1	1	—	—	1	42
Bacteria	946	927	699	785	954	926	902	991

\* Number of microorganisms from 1,000 grains of coniferous seeds incubated for 4 days in each case.

\*\* No spore formation was observed when the identification was made

\*\*\* Seeds imported from the States (A) and native to Taiwan (B).

It is worthy to note that one of the fungi, i.e. *Sphaeropsis* sp., is recently found to cause the bud blight of slash pine and Taiwan red pine. This disease may become a potential epidemic disease of slash pine in this area (unpublished). Nevertheless, this fungus was merely isolated from coniferous seeds native of Taiwan, e.g. slash pine, Taiwan white pine, Taiwan red pine, and Japanese black pine. The same was true in the case of *Verticillium* sp. A great number of seeds yielding *Diplodia*, *Fusarium*, and *Pestalotia* were determined to be very harmful to the germination of seeds and growth of coniferous seedlings in question.

Seeds completely surface-sterilized with 0.5 per cent mercuric chloride for 1 minute were ground into small pieces and mixed with melted potato dextrose

agar maintaining 43°C to determine the microflora of internal layers of the seeds. One hundred plates were made for each of the 8 seed samples comprised 7 species of pines aforementioned. The results are shown in Table 2.

**Table 2.** *Microflora of coniferous seeds treated with 0.5 per cent mercuric chloride\**

Species of conifer	<i>Pinus armandi</i> var. <i>masteriana</i>	<i>P. elliotii</i> *** A	<i>P. elliotii</i> *** B	<i>P. luchuensis</i>	<i>P. massoniana</i>	<i>P. merrisonicola</i>	<i>P. taiwanensis</i>	<i>P. thunbergii</i>
<i>Aspergillus</i>	1	—	—	—	—	—	—	—
<i>Chaetomium</i>	—	—	—	1	—	4	—	—
<i>Diplodia</i>	—	7	3	—	—	—	—	—
<i>Fusarium</i>	—	—	—	1	1	3	1	—
<i>Fusicoccum</i>	—	1	—	—	—	3	—	—
<i>Gloeosporium</i>	3	—	—	—	—	14	—	—
<i>Pestalotia</i>	2	—	—	—	4	—	—	—
<i>Phoma</i>	1	—	—	—	—	—	—	—
<i>Phomopsis</i>	—	—	—	—	1	—	—	—
<i>Sphaeropsis</i>	1	—	—	—	1	14	—	—
<i>Stemphylium</i>	—	—	—	—	1	—	—	—
<i>Verticillium</i>	—	—	—	—	—	4	—	—
Undetermined**	6	1	—	—	—	3	1	—
Bacteria	29	15	41	9	9	34	2	12

\* Number of microorganisms from 100 grains coniferous seeds incubated for 2 weeks in each case.

\*\* No spore formation was observed when the identification was made.

\*\*\* Seeds imported from the States (A) and native of Taiwan (B).

Eleven genera of fungi, e. g. *Aspergillus*, *Chaetomium*, *Diplodia*, *Fusarium*, *Fusicoccum*, *Gloeosporium*, *Pestalotia*, *Phoma*, *Phomopsis*, *Sphaeropsis*, *Stemphylium*, and *Verticillium*, were detected. *Fusicoccum*, *Gloeosporium*, *Phomopsis*, and *Stemphylium* were merely isolated from the seeds treated with the present method. Large number of seeds yielding bacteria was the characteristic of this series of experiments. Surprisingly, no fungi were isolated from one hundred seeds of Japanese black pine. Rare cases of *Diplodia*, *Phomopsis*, and *Verticillium* were also observed respectively from the seeds of slash pine, horsetail pine and Taiwan white pine.

### Discussion

From 8,000 coniferous seeds comprised 7 species of pines collected from different areas, more than 17 genera of fungi were isolated when seeds were

partially sterilized with 4 per cent sodium hypochlorite. They were *Aspergillus*, *Cephalosporium*, *Chaetomium*, *Chaetomella*, *Curvularia*, *Diplodia*, *Fusarium*, *Graphium*, *Helminthosporium*, *Mucor*, *Penicillium*, *Pestalotia*, *Phoma*, *Rhizoctonia*, *Rhizopus*, *Sphaeropsis*, and *Verticillium*. While *Fusicoccum*, *Gloeosporium*, *Phomopsis*, and *Stemphylium* were only present in the inner layers of seed structure. Most of them were formerly found to be in different localities to influence the germination of seeds as well as seedling stands by many workers (Carrera, 1951; Cockerill et al, 1951; Cox, 1953; Hartley, 1918; Holtzmann, 1955; Ito, 1962; Rathbun, 1922, 1923; Sato et al, 1955; Spaulding, 1914; Ten Houten, 1939; Timonin, 1964; Vaartaza, 1952, 1953a, 1953b, 1956; Verona, 1950). They frequently associated with diseased seedlings (Anonymous, 1952, 1953; Padgett, 1958; Timonin, 1964). And *Diplodia*, *Fusarium*, *Helminthosporium*, *Pestalotia*, *Rhizoctonia*, and *Verticillium* were reported to be pathogenic to seedlings of conifers in Taiwan (Chen, 1962; Chen et al, 1963).

The microflora of coniferous seeds varied qualitatively and quantitatively with respect of the seed samples collected from different localities and species of conifers. Relatively large number of bacteria were also observed. *Diplodia*, *Fusarium*, *Pestalotia*, *Sphaeropsis*, and *Verticillium* appeared frequently from both surface layers and inner parts of seeds tested, whereas the classical damping-off pathogen of *Rhizoctonia* was only secured from the seed-coat. One of the fungi isolated, *Sphaeropsis* sp. was recently found to cause bud blight of slash pine and Taiwan red pine of about 5 years old plantation. This disease may become a potential epidemic disease of slash pine in this island (unpublished).

### Summary

Microflora of coniferous seeds were studied by using 7 species of pines, e. g. slash pine (*Pinus elliottii*), Luchu pine (*P. luchuensis*), Japanese black pine (*P. thunbergii*), Taiwan armand pine (*P. armandi* var. *mastersiana*), Taiwan red pine (*P. taiwanensis*), Taiwan white pine (*P. morrisanicola*), and horsetail pine (*P. massoniana*). All the seed samples used were collected from different localities on this island, except a sample of slash pine imported from the States.

From 8,800 seeds comprised different pines mentioned above, more than 21 genera of fungi were isolated from the surface and inner layers of seed structure. They were *Aspergillus*, *Cephalosporium*, *Chaetomium*, *Chaetomella*, *Curvularia*, *Diplodia*, *Fusarium*, *Fusicoccum*, *Gloeosporium*, *Graphium*, *Helminthosporium*, *Mucor*, *Penicillium*, *Pestalotia*, *Phoma*, *Phomopsis*, *Rhizoctonia*, *Rhizopus*, *Sphaeropsis*, *Stemphylium*, *Verticillium*. Most of them were formerly found to be in different localities to cause damping-off disease in nursery beds. Relatively large number of bacteria were also observed.

# 臺灣松類的種子微生物

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本文係對有關七種松類的種子微生物羣之研究。材料取自臺灣各地，包括濕地松 (*Pinus elliottii*)，琉球松 (*P. luchuensis*)，日本黑松 (*P. thunbergii*)，臺灣華山松 (*P. armandi* var. *mastersiana*)，臺灣二葉松 (*P. taiwanensis*)，臺灣五葉松 (*P. morris-anicola*)及馬尾松 (*P. massoniana*) 等七種，另有美國進口濕地松種子以供實驗。

從 8,800 個採樣種子之表面及內面分離出 21 屬的真菌，它們包括 *Aspergillus*, *Cephalosporium*, *Chaetomium*, *Chaetomella*, *Curvularia*, *Diplodia*, *Fusarium*, *Fusioaccum*, *Gloeosporium*, *Graphium*, *Helminthosporium*, *Mucor*, *Penicillium*, *Pestalotia*, *Phoma*, *Phomopsis*, *Rhizoctonia*, *Rhizopus*, *Sphaeropsis*, *Stemphylium*, *Verticillium* 等，這些菌類大部份曾經被發現是各地苗床之倒伏病病原菌。另外尚有一些未被檢定之細菌亦被分離出來。

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