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Infection of Engelmann-Spruce Seed by *Geniculodendron pyriforme* in Western North America

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among isolates obtained from the same soil sample. Our data indicate that different genotypes were isolated including isolates from soil samples where mating types were predominantly or entirely (+) or (-).

Only 12% of the soil samples converted completely to the yeast phase in vitro, but this compares favorably with the 6% total conversion rate obtained by Kwon-Chung et al. (1974) with their soil isolates. In our study there was not a good correlation between complete convertibility and mating type, as can be seen in TABLE I. Of the 42 isolates (12%) which did convert well, 22 were (+) type, 20 were (-) type. It is also interesting to note that 52% of the isolates from gull nests converted completely to the yeast phase, well above the percentage obtained with isolates from the blackbird sites.

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INFECTION OF ENGELMANN-SPRUCE SEED BY *GENICULODENDRON PYRIFORME* IN WESTERN NORTH AMERICA

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Geniculodendron pyriforme Salt was identified and characterized by Salt (1974) as the fungus responsible for germination failure of Sitka

spruce [*Picea sitchensis* (Bong.) Carr.] seed in Canadian and British forest nurseries. It was confirmed that the fungus isolated by Epnors (1964) from seed of *Pinus resinosa* Ait., *P. sylvestris* L., *P. strobus* L., and *Picea glauca* (Moench.) Voss. in Canadian nurseries was the same (Salt, 1974). In addition, Salt (1970) demonstrated that the pathogen can infect seeds of western hemlock [*Tsuga heterophylla* (Raf.) Sarg.], lodgepole pine (*Pinus contorta* Dougl. ex Loud.), Japanese larch [*Larix leptolepis* (Sieb. and Zucc.) Gord.] and Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco].

In the western United States, natural regeneration of certain strands of Englemann spruce (*Picea engelmanni* Parry ex Engelm.) is hampered by a pathogenic agent limiting seed germination. *Geniculodendron pyriforme* has been identified by the authors as the pathogenic fungus infecting the embryo and endosperm of overwintering seeds of Englemann spruce. *Geniculodendron pyriforme* is known from Ontario, Canada (Epnors, 1964), and in western North America from British Columbia, Canada, and Washington, USA (Salt, 1970). The present paper is an account of *G. pyriforme* infection in western Englemann-spruce forests, where it invades not only Englemann-spruce seed but also seed of the associated conifer species, subalpine fir [*Abies lasiocarpa* (Hook.) Nutt.].

Englemann-spruce forest litter containing seed was collected from sites in Utah, Colorado, Arizona, Idaho and Wyoming. Infected seed often can be distinguished from normal viable seed by the fact that the former have varying amounts of blue discoloration of the seed coat and endosperm. However, reliable detection of seed infection and isolation of the pathogen can be accomplished by placing dissected portions of embryo and endosperm from surface-sterilized seed onto V-8-juice agar and incubating at 20 C. After 6 da at 20 C, the hyaline, aerial mycelium of the fungus can be seen growing from the infected portions. Further growth results in the formation of distinctive white, floccose colonies with conspicuous blue-colored areas. This blue coloration is intensified if other fungi or bacteria are growing in close proximity. Sporulation is evident after 4 wk of growth. The conidiogenous apparatus has a well-defined main axis and distinct dendritic branching. The conidia are produced in sympodial succession and form clusters at the apices of conidiogenous cells. *Geniculodendron pyriforme* infection could be demonstrated only in seeds sifted from litters collected in the Cache National Forest, Utah and Payette National Forest, Idaho (TABLE I).

In addition, healthy Englemann-spruce seeds, gathered in the fall from cones which had newly dropped, were stratified at 2 C in litters collected from the same sites throughout the intermountain area in an

TABLE I
Geniculodendron pyriforme IN SEEDS OR LITTER FROM VARIOUS SITES
 IN THE INTERMOUNTAIN AREA

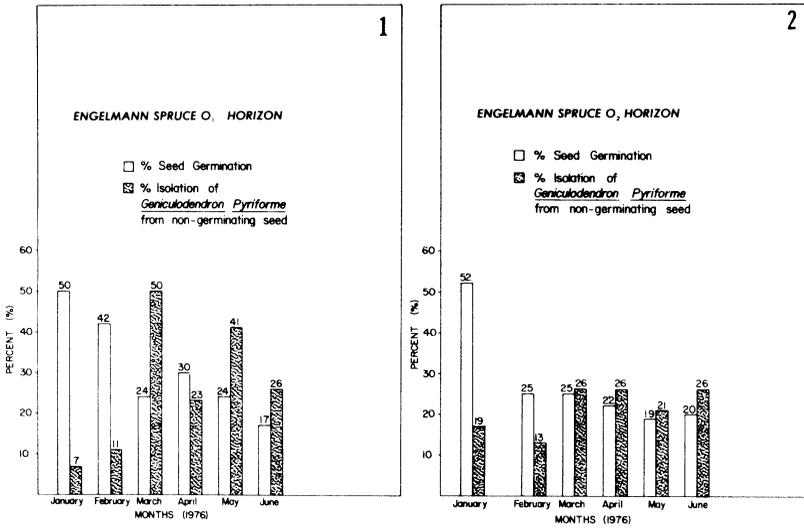
Location	In litter seeds	Infection in seeds stratified in Engelmann- spruce litter
Aquarius Plateau, Utah	—	+
Cedar Break, Utah	—	+
Mirror Lake, Utah	—	+
Barney Top, Utah	—	—
San Francisco Mts., Arizona	—	+
Mt. Graham, Arizona	—	+
Monarch Pass, Colorado	—	+
Buckskin Creek, Colorado	*	+
Fraser Expt. Stn., Colorado	—	+
Lizard Pass, Colorado	—	+
Cache National Forest, Utah	+	+
Payette National Forest, Idaho	+	+
Skalkaho Pass, Idaho	*	+
Togwotee Pass, Wyoming	—	—

* No seed could be found in the litter.

attempt to discover presence of the pathogen in the litter. Following 2 mo of incubation the seeds were retrieved and germinated on water agar. Nongerminating seeds were then surface sterilized with 0.1% silver nitrate, dissected, and portions placed on V-8 agar to be incubated at 20 and 10 C. Engelmann-spruce litter from Utah, Arizona, Colorado and Idaho contained the pathogen (TABLE I). Control seeds placed in sterile Petri dishes at 2 C for several mo remained healthy with high germination rates.

The presence of *G. pyriforme* in materials normally present in Engelmann-spruce litter (needles, cone scales, seed wings, sterile seed) also was investigated. Methods used to isolate or observe the pathogen on these materials included: detergent wash, surface sterilization and epidermal impression. We were unable to isolate or show the presence of *G. pyriforme* in any of these materials. The organism can be found only in seeds which have been in contact with the litter either in the laboratory or in the field.

To demonstrate the percentage of seeds infected as well as the season when most infection occurs, in situ studies were undertaken using viable, noninfected seed taken from newly dropped cones and planted into the O₁ and O₂ layers of the Englemann-spruce stand in Cache National Forest, Utah. The seeds were planted in fall 1975 and harvested at regular weekly intervals from January through June 1976.



FIGS. 1, 2. Percent germination of Engelmann-spruce seed and occurrence of *Geniculodendron pyriforme* at mo intervals.

There was fair to good germination with a low incidence of *G. pyriforme* infection during the coldest mo of January and February. As temperatures warmed in March, April and May (to about 0 C or above), percent germination decreased while occurrence of *G. pyriforme* increased (FIGS. 1 and 2).

TABLE II
GROWTH OF *Geniculodendron pyriforme* AND OTHER FUNGAL ISOLATES FROM ENGELMANN-SPRUCE LITTERS AFTER 28 DA AT 10 C

Test organism	Average colony diam, mm
<i>Geniculodendron pyriforme</i>	39
<i>Mycelia sterilia</i>	32
<i>Chrysosporium</i> sp.	26
<i>Stephanosporium</i> sp.	14
<i>Penicillium</i> sp.	18
<i>Mortierella isabellina</i> Oudemans & Koning	31
<i>Cephalosporium</i> sp.	20
<i>Chaetomium</i> sp.	42
<i>Trichoderma</i> sp.	42
<i>Absidia</i> sp.	85
<i>Aureobasidium pullulans</i> (deBary) Arnaud	26
<i>Cylindrocarpon</i> sp.	30
<i>Acremonium</i> sp.	22

Growth of *G. pyriforme* in vitro is good at 10 C, in contrast to the very poor growth of most other fungal isolates from Engelmann-spruce litter. When tested with 12 other fungi for growth at 10 C, *G. pyriforme* exhibited an average colony diam that was greater than the average diam of nine of the other litter fungi after 28 da (TABLE II). Similar tests at 22 C indicated that most fungi were able to outgrow *G. pyriforme*.

In the ecologically competitive situation, *G. pyriforme* appears to exhibit its maximum pathogenicity in the spring upon snowmelt when temperatures are 2 to 12 C. Little is known about its life history or its mode of infection; however, it seems to be present in litter and to infect through contact with seed in that substrate.

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VEGETATIVE PROPAGATION OF SOME SAPROLEGNIACEAE UNDER SIMULATED-ESTUARINE-CULTURE CONDITIONS¹

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The vegetative phases of saprolegniaceous fungi are more tolerant of salinity stress than are the asexual and sexual reproductive structures (Te Strake, 1959; Harrison and Jones, 1974). In this connection, Harrison and Jones (1974) have stated that, “. . . the inhibitions imposed by salinity on reproduction . . . of these organisms confines them to fresh water or water of extremely low salinity.” This statement supports results of field studies such as that of Te Strake (1959), which suggest that water molds do not grow actively in estuarine waters where salinity exceeds about 2.8 parts per thousand (ppt).

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