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Germination of Fern Spores in Natural Soils

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ABSTRACT.—In the presence of light, the germination rates of spores of *Nephrolepis exaltata*, *Phlebodium aureum* and *Cibotium glaucum* on three different soils were similar to those on water or water agar. All the soils tested promoted elongation of rhizoids of *N. exaltata* and stimulated growth of protonemata of *C. glaucum*. Spores of the fungus *Botryodiplodia theobromae* germinated completely on water or water agar under light or in darkness but failed to germinate on soils under the same conditions. The results suggest that spores of ferns are not sensitive to microbiostasis of soil. Contrary to microorganisms, insensitivity of spores to soil microbiostasis could be beneficial to ferns because it would be advantageous to their successful colonization of suitable habitats.

Natural soils contain fungal spores in great numbers (Warcup, 1955), as most fungi are sensitive to soil fungistasis which can be overcome by addition of organic nutrients to soil (Ko and Lockwood, 1967; Lockwood, 1977). Microbial quiescence in natural soils was subsequently extended to include actinomycetes and bacteria, and the term soil microbiostasis has been introduced to described the antagonistic phenomenon of soil against fungi, actinomycetes and bacteria collectively (Ko and Ho, 1984). Microbiostasis in natural soils is considered to be caused by nutrient deprivation resulting from microbial activity (Ho and Ko, 1986).

Natural soils also contain a great number of fern spores commonly referred to as the spore bank (Hamilton, 1988). When a small amount of soil was placed on a nutrient agar medium and exposed to light, fern spores in the soil readily germinated (Hamilton, 1988). Most fern spores are between 25 and 50 μm in diameter (Page, 1979; Devi, 1981), about the same size as many fungal spores (Walker, 1952). Because germination of fern spores in soil has not been quantitatively compared with that in non-soil medium, it is not known if fern spores are sensitive to soil microbiostasis. To address this question, spore germination of three different fern species on soils collected from three different locations was compared with that on water and water agar. Fungal spores *Botryodiplodia theobromae* Pat. were used as a control because they are sensitive to soil microbiostasis and like fern spores their germination does not require exogenous nutrients.

MATERIALS AND METHODS

Fertile fronds of the Hawaiian tree fern *Cibotium glaucum* (J.E. Smith) Hook. & Arn. (Dicksoniaceae), hare's foot fern *Phlebodium aureum* (L.) J. Smith (Polypodiaceae) and sword fern *Nephrolepis exaltata* (L.) Schott.

(Nephrolepidaceae) were collected from nature in the Hilo area. Fronds from each species were placed in an uncovered plastic box (13 × 24 × 35 cm) kept on the laboratory bench for air drying to discharge spores. A small quantity of spores was transferred to 5 ml sterile distilled water in a test tube with a pair of forceps. The concentrations of fern spores used ranged from 1.3×10^3 to 1.8×10^3 spores/ml as determined by a Pipetman (West Coast Scientific, Oakland, CA) microliter pipet (Ko et al., 1973). Fungal spores for comparison were obtained by growing *B. theobromae* on 10% V-8 agar (10% V-8 juice, 0.02% $CaCO_3$ and 2% agar) at 24°C under cool white fluorescent light (2,000 lx) for 9 days. Mature pycnidia were transferred to 5 ml sterile distilled water in a test tube, and crushed with a sterile spatula to release pycnidiospores. Spores were separated from crushed pycnidia by sedimentation before use. The concentrations of pycnidiospores used, ranged from 38×10^3 to 75×10^3 spores/ml.

Soil samples were collected from farm lands at Hilo (silty clay loam; pH 6.8), Volcano (silt loam, pH 6.8) and Mealani (silt loam, pH 5.3) on the island of Hawaii. Soils were taken from a depth of 0 to 15 cm after surface litter was cleared, sieved through a 2-mm screen and moistened to about 65% field capacity. These soils were stored in polyethylene bags for at least one month to allow microorganisms to exhaust nutrients which might have become available due to soil disturbance (Chuang and Ko, 1988).

For testing germination of spores on soil surfaces, approximately 25 g of soil adjusted to about 75% field capacity was placed in a Petri plate (100 mm diam.). It was compressed to form a disk (ca. 60 mm diam.) and the surface was smoothed with a bent spatula. Three drops (ca. 0.15 ml) of spore suspension were added to a sterile polycarbonate membrane (8 μm, 47 mm diam.; Nuclepore Co., Pleasanton, CA) laid on each soil disk in the Petri plate. Inoculated plates were incubated at 24°C under cool white fluorescent light (2,000 lx) or in darkness for 5–9 days for fern spores and 12 hr for fungal spores. After incubation, each polycarbonate membrane was transferred from the soil disk to a moistened paper towel to wipe off soil particles on the lower surface of the membrane. The membrane was then placed on the cover of the Petri plate, and germination of spores was observed under a 40× objective. To determine if exogenous nutrients were required for germination, spore germination was similarly tested on polycarbonate membranes floating on the surface of sterile distilled water in Petri plates or directly on 2% water agar. Percentage germination was determined by counting 100 spores in each treatment. For each treatment, two of the longest rhizoids were measured and the average length was recorded. Two replicates were used and all experiments were done at least twice.

Results

Nephrolepis exaltata.—In the presence of light, spores of *N. exaltata* germinated by producing a protonemal cell and an elongating rhizoid. The average percent germination in 5 days on the three different soils separated by polycarbonate membranes was 50%, which was similar to that on distilled

TABLE 1. Germination of fern spores of *Nephrolepis exaltata* on natural soils under light and in darkness after incubation at 24°C for 5 days. Standard deviations are given in parentheses.

	Light		Dark	
Medium	Germination (%)	Rhizoid length (μm)	Germination (%)	Rhizoid length (μm)
Hilo soil	42.5 (0.5)	377.5 (9.5)	0	0
Volcano soil	52.5 (0.5)	304.5 (14.5)	0	0
Mealani soil	53.5 (1.5)	285.5 (4.5)	7.5 (0.5)	0
Water	42.0 (1.0)	155.0 (10.0)	0	0
Water agar	42.5 (2.5)	193.5 (9.5)	0	0

water separated by polycarbonate membrane or on water agar directly (Table 1). The mean length of rhizoids from spores germinated on the soils was 323 μm, about 108% and 67% longer than those on water and water agar, respectively. Without light, all or nearly all the spores of *N. exaltata* failed to germinate on soils, water or water agar (Table 1). On Mealani soil, 7.5% of spores examined produced a green protonemal cell but no rhizoids after 5 days in darkness.

Phlebodium aureum.—The germination pattern of *P. aureum* spores on soils was similar to that of *N. exaltata* spores. Under light, *P. aureum* also geminated by producing a protonemal cell and an elongating rhizoid, and the average germination rate of 57% after 6 days on the three soils was similar to that on distilled water or water agar (Table 2). The average length of rhizoids from spores germinated on the soils was 290 μm which was about the same as that on water and 97% longer than that on water agar. In darkness, all or nearly all the spores of *P. aureum* failed to germinate on soils, water or water agar (Table 2). On Mealani soil, 8.5% of spores tested produced a green protonemal cell, without rhizoids after 6-day incubation without light.

Cibotium glaucum.—In the presence of light, spores of *C. glaucum* germinated by producing an expanding protonema and an elongating rhizoid. The average germination rate on the three soils was 58% after 9-day incubation, similar to that on water or water agar (Table 3). All the soils tested stimulated growth of protonemata. The protonemata on soils consisted of 3 to 5 cells each,

TABLE 2. Germination of fern spores of *Phlebodium aureum* on natural soils under light and in darkness after incubation at 24°C for 6 days. Standard deviations are given in parentheses.

	Light		Dark	
Medium	Germination (%)	Rhizoid length (μm)	Germination (%)	Rhizoid length (μm)
Hilo soil	55.5 (1.5)	285.5 (4.5)	0	0
Volcano soil	51.0 (1.0)	265.5 (5.5)	0	0
Mealani soil	64.0 (2.0)	319.5 (9.5)	8.5 (0.5)	0
Water	51.5 (2.5)	249.5 (7.5)	0	0
Water agar	43.5 (0.5)	145.5 (9.5)	0	0

TABLE 3. Germination of fern spores of *Cibotium glaucum* on natural soils under light and in darkness after incubation at 24°C for 9 days. Standard deviations are given in parentheses.

	Light		Dark	
Medium	Germination (%)	Rhizoid length (μm)	Germination (%)	Rhizoid length (μm)
Hilo soil	52.0 (2.0)	244.5 (21.5)	0	0
Volcano soil	63.5 (4.5)	314.5 (58.5)	0	0
Mealani soil	57.5 (2.5)	300.0 (5.0)	0	0
Water	60.0 (3.0)	321.5 (65.5)	0	0
Water agar	42.0 (2.0)	225.0 (27.0)	0	0

whereas those on water and water agar contained only 1 or 2 cells each. The average length of rhizoids from spores germinated on soils was 286 μm, similar to those on water or water agar. In darkness, none of the *C. glaucum* spores examined germinated on soils, water, or water agar (Table 3).

Botryodiplodia theobromae.—Light had no effect on the germination of fungal spores of *B. theobromae*, which germinated by producing an elongating germ tube. Nearly all the spores tested geminated on water or water agar after incubation for 12 hr under light or darkness (Table 4). However, under the same conditions spore germination was completely inhibited on the three different soils tested.

DISCUSSION

Spores of the fungus *B. theobromae* germinated completely on water or water agar with or without light, but remained inactive on soils under the same conditions. This shows that the three different soils used in this study are suppressive to microorganisms, as are most soils (Lockwood, 1977; Ko and Ho, 1984). However, in the presence of light, the germination rates of spores of all three fern species tested on soils were similar to that on water and water agar, indicating that fern spores are not sensitive to soil microbiostasis. The general phenomenon by which germinable spores of microorganisms are rendered static in soils (Bruehl, 1986), therefore, does not appear to apply to spores of ferns.

TABLE 4. Germination of fungal spores of *Botryodiplodia theobromae* on natural soils under light and in darkness after incubation at 24°C for 12 days. Standard deviations are given in parentheses.

	Germination (%)	
Medium	Light	Dark
Hilo soil	0	0
Volcano soil	0	0
Mealani soil	0	0
Water	99.5 (0.5)	99.5 (0.5)
Water agar	99.5 (0.5)	98.0 (1.0)

Spores of many fungi are nutritionally dependent and require exogenous nutrients for germination, but others are nutrient independent and are capable of germination in nutrient-free water (Ko and Lockwood, 1967). All the nutrient-dependent, and most of the nutrient-independent spores, are sensitive to soil microbiostasis. Only some of the nutrient-independent types can germinate freely on soil (Ko and Lockwood, 1967; Hwang and Ko, 1974). Fern spores appear to be similar to the latter group although a greater range of fern species awaits investigation. The germination rate of each fern species tested on water was similar to that on water agar. Because water agar contains sufficient nutrients for spore germination (Ho and Ko, 1980), the results suggest that fern spores are nutritionally independent. This is in accordance with previous findings of the ability of a number of fern species to germinate on water (Dyer, 1979).

Insensitivity of fungal spores to microbiostasis is detrimental to their survival in nature as germ mycelia from the germinating spores will be lysed due to unavailability of organic nutrients for their growth in soil (Ko and Lockwood, 1970). However, this is not the case with fern spores as inorganic nutrients needed for their growth are available in soil. Therefore, ability to germinate freely on soil is advantageous to ferns for their colonization of suitable habitats.

Most species of ferns depend on light for germination of spores (Weinberg and Voeller, 1969). When fern spores fall to the ground in scattered masses from sporophytes after maturation, a portion of them will percolate into the pore space of soil and remain quiescent due to the absence of light. This might be an important source of fern spores in the spore bank. A large number of those spores on the soil surface remain ungerminated as shown by the observation that about 50% of spores of all the three species of ferns tested remain dormant on soil even in the presence of light. These spores may be dispersed and buried in soil through the activity of earthworms (Hamilton, 1988; Hamilton and Lloyd, 1991) and become part of the spore bank. Light is inhibitory to spore germination of some fern species (Whittier, 1973; 1977; 1978). In this case, ungerminated spores on the soil surfaces would also become part of the spore bank.

All of the test soils appeared to promote elongation of rhizoids of *N. exaltata* but not *P. aureum* or *C. glaucum*. The activation of rhizoid elongation may be due to minerals present in soils. Elongation of rhizoids in the fern *Onoclea sensibilis* has been shown to be promoted by mental ions (Miller et al., 1983). Minerals in soils may also account for the growth promotion of protonemata of *C. glaucum* on soils. However, the actual cause of the stimulatory effects of soils on rhizoid elongation and protonemal growth remains to be investigated.

Approximately 8% of *N. exaltata* and *P. aureum* spores germinated by producing a green protonemal cell without any rhizoid on Mealani soil in darkness. It is not know what factor in the soil is responsible for such a phenomenon. The fate of these germinated spores on soil after an extended period of time also remains to be investigated.

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