Formation of Ponderosa Pine Ectomycorrhizae after Inoculation with Feces of Tassel-Eared Squirrels
Author(s): Martha M. Kotter and Robert C. Farentinos
Source: *Mycologia*, Vol. 76, No. 4 (Jul. - Aug., 1984), pp. 758-760
Published by: Taylor & Francis, Ltd.
Stable URL: https://www.jstor.org/stable/3793237
Accessed: 10-04-2024 16:20 +00:00

Mycologia, 76(4), 1984, pp. 758–760.

FORMATION OF PONDEROSA PINE ECTOMYCORRHIZAE AFTER INOCULATION WITH FECES OF TASSEL-EARED SQUIRRELS

MARTHA M. KOTTER[1]

Department of Zoology, The Ohio State University,
Columbus, Ohio 43210[1]

AND

ROBERT C. FARENTINOS

Department of Environmental, Population, and Organismic Biology,
University of Colorado, Boulder, Colorado 80303

Hypogeous mycorrhizal fungi appear to depend on small animals for spore dispersal. Sporocarp tissues are ingested by the animal, freeing spores which pass through the digestive tract and are defecated. Spores are ultimately washed into the soil, contact plant rootlets, germinate, and form mycorrhizae (Maser *et al.*, **1978**).

Recent investigations demonstrated spore germination and formation of vesicular-arbuscular (VA) mycorrhizae with grasses by spores of *Glomus* spp. after passage through rodent digestive tracts (Trappe and Maser, **1976**; Rothwell and Holt, **1978**; Ponder, **1980**). Unlike VA mycorrhizal fungal spores, ectomycorrhizal fungal spores do not germinate readily under laboratory conditions. An indirect test for viability of ectomycorrhizal spores is inoculating host tree seedlings with spores and observing ectomycorrhiza formation. Few investigations have demonstrated ectomycorrhiza formation after host inoculation with animal feces (Ure and Trappe, pers. comm.).

Our study examined feeding habits of tassel-eared squirrels (*Sciurus aberti* Gemlin). These squirrels inhabit certain ponderosa pine (*Pinus ponderosa* Dougl.) forests of the southern Rocky Mountains and depend heavily on hypogeous fungi for food during the summer months (Stephenson, **1975**; Kotter, **1981**). In Colorado, 12 species of fungi (10 hypogeous, 2 epigeous) were reported in the feces and stomach contents of a tassel-eared squirrel population. An estimated 200 million to 1 billion fungal spores per gram fecal material were disseminated by these squirrels (Kotter, **1981**). Our experiment was designed to determine: (1) if spores can remain viable after passage through squirrel digestive tracts, and (2) if tassel-eared squirrels can serve as spore vectors for hypogeous ectomycorrhizal fungi in ponderosa pine forests.

Ponderosa pine seeds were germinated and grown in a peat bed for 5 months. Afterwards they were stored at 5 C for 10 wk. Forty-five seedlings were treated as follows: (a) one-third were inoculated with one or two whole, dried fecal pellets from tassel-eared squirrels, (b) one-third were inoculated with dried, macerated hypogeous sporocarps, and (c) one-third were untreated. All squirrel feces used for inocula were obtained from live-trapped animals at Enchanted Mesa, Boulder, Colorado. All hypogeous sporocarps used were collected at the same location (Kotter, **1981**). Fungal spores in squirrel feces were examined by fragmenting fecal pellets in 1–2 drops of Melzer's solution on a microscope slide and observing them at 400–1000× with a phase-contrast microscope. Spores and sporocarps were identified using keys developed by Trappe (pers. comm.) and Smith and

---

[1] Present address: 2848 Neil Ave., Apt. 223-A, Columbus, OH 43202.

TABLE I

RESULTS OF ECTOMYCORRHIZAE INOCULATION EXPERIMENT ON PONDEROSA PINE SEEDLINGS WITH FECES OF TASSEL-EARED SQUIRRELS AND SPOROCARPS OF HYPOGEOUS FUNGI FROM ENCHANTED MESA, BOULDER COUNTY, COLORADO

Treatment	N	Mycorrhizal number	Seedlings per cent	Fungal taxa[a] present in inoculum each successful mycorrhizal seedling
Feces	15	5	33.3	Rhiz, Sede, Glom Rhiz, Sede, Glom Rhiz, Scle, Hyst, Sede, Elap Rhiz, Scle, Hyst, Sede, Tube, Glom Rhiz, Scle, Sede, Glom
Sporocarp	15	3	20	*Rhizopogon subcaerulescens* Smith *R. subcaerulescens* *Sclerogaster xerophilum* Fogel
Control	15	0	–	None

[a] Fungal taxa abbreviations are: Elap = *Elaphomyces* spp.; Glom = *Glomus* sp.; Hyst = *Hysterangium separabile* Zeller; Rhiz = *Rhizopogon* spp.; Scle = *Sclerogaster xerophilum*; Sede = *Sedecula pulvinata* Zeller; Tube = *Tuber* spp.

Smith (**1973**), and by comparing spores with fresh and dried material in a voucher collection from the Oregon State University Herbarium. Identifications were confirmed by J. M. Trappe and R. Fogel. The prepared inoculum was placed on rootlets of seedlings which were then planted in clear plastic boxes (12.7 × 6.4 × 6.4 cm) in a sterile soil mixture of 1:1 peat to vermiculite. The boxes were covered with aluminum foil to exclude light. They were kept in the Department of Botany Greenhouse at The Ohio State University. The seedlings were watered every 2–4 da or when the surface soil dried. After 6 months, rootlets were examined with a dissecting microscope for the presence of ectomycorrhizae.

Results indicate the spores remained viable after passage through digestive tracts of squirrels (TABLE I). Tassel-eared squirrels can serve as vectors of spore dissemination for hypogeous ectomycorrhizal fungi. Squirrels appear to be the link in perpetuating the symbiotic relationship between the host, ponderosa pine, and mycorrhizal fungi. Success of ectomycorrhiza formation in the fecal treatment (33% success) and the sporocarp treatment (20% success) did not differ significantly ($\chi^2 < 3.84$, 1 df, $p < 0.05$). Although not apparent in our study, it is possible some fungal species require the physical and chemical scarification from a rodent digestive tract to induce germination (Fogel and Trappe, **1978**). The results of this study may apply to other forest ecosystems where hypogeous ectomycorrhizal fungi and mammalian spore vectors are present.

This study was supported in part by National Science Foundation Grants (BNS-76-05069 and DEB-76-80423) to R. C. Farentinos, Department of Zoology, The Ohio State University, and P. J. Capretta; by a Sigma Xi Grant-in-Aid of Research, and a Osburn Memorial Fund Fellowship to M. M. Kotter. We thank D. Ure, J. M. Trappe, C. P. P. Reid, L. Rhodes for their suggestions of spore inoculation technique. J. M. Trappe and R. Fogel were invaluable for identification of fungi and reviewing the manuscript.

Key Words: animal spore vectors, ectomycorrhizae, hypogeous fungi, tassel-eared squirrels.

LITERATURE CITED

Fogel, R., and J. M. Trappe. 1978. Fungus consumption (mycophagy) by small animals. *Northwest Sci.* **52**: 1–31.

Kotter, M. M. 1981. Interrelationships of tassel-eared squirrels, ponderosa pine, and hypogeous mycorrhizal fungi. M.S. Thesis, The Ohio State Univ., Columbus. 92 p.

Maser, C., J. M. Trappe, and D. C. Ure. 1978. Implications of small mammal mycophagy to the management of western coniferous forests. *Trans. N. Amer. Wildlife Nat. Resources Conf.* **43**: 78–88.

Ponder, F. 1980. Rabbits and grasshoppers: vectors of endomycorrhizal fungi on new coal mine spoils. *U.S.D.A. For. Serv. N. Central For. Exp. Sta. Res. Note NC-250.* 2 p.

Rothwell, R. M., and C. Holt. 1978. Vesicular-arbuscular mycorrhizae established with *Glomus fasciculatus* spores isolated from the feces of cricetine mice. *U.S.D.A. For. Serv. Northeast For. Exp. Res. Note NE-259.* 4 p.

Smith, H. V., and A. H. Smith. 1973. *How to know the non-gilled fleshy fungi.* Brown, Dubuque, Iowa. 402 p.

Stephenson, R. L. 1975. Reproductive biology and food habits of Abert's squirrels in central Arizona. M.S. Thesis, Arizona State Univ., Tempe. 66 p.

Trappe, J. M., and C. Maser. 1976. Germination of spores of *Glomus macrocarpus* (Endogonaceae) after passage through a rodent digestive tract. *Mycologia* **68**: 433–436.

Mycologia, 76(4), 1984, pp. 760–763.

NUCLEAR CONTROL OF MONOKARYOTIC FRUITING IN *SCHIZOPHYLLUM COMMUNE*[1]

JOHN F. LESLIE[2]

Department of Genetics, University of Wisconsin,
Madison, Wisconsin 53706

AND

THOMAS J. LEONARD

Departments of Botany and Genetics, University of Wisconsin,
Madison, Wisconsin 53706

Monokaryotic fruiting in *Schizophyllum commune* Fr. was first reported by Wakefield (**1909**). Recent studies of the initiation of monokaryotic fruiting (Leslie and Leonard, **1979a**, **b**; **1980**; Esser *et al.*, **1979**) revealed a complex genetic basis of at least 8–10 loci for this trait. The data supporting this formal genetic model are segregation ratios of fruiting progeny to non-fruiting progeny from selected crosses. Recently, Eger-Hummel (**1983**) reported that monokaryotic fruiting in *Pleurotus* was under partial cytoplasmic control. We differentiated between the nuclear inheritance and the cytoplasmic inheritance of monokaryotic fruiting in *S. commune* by forming known dikaryons and then separating the dikaryon's nuclei (dedikaryotization) by growth on a cholic acid-containing medium.

Monokaryotic fruiting by *S. commune* in response to mechanical injury was first noted by Leonard and Dick (**1973**). At least four loci (*hap-1, hap-2, hap-3,* and *hap-4*) control the ability of a strain to respond to this stimulus (Leslie and Leonard, **1979b**). The presence of any *inj* allele at the *hap-1* locus, or of an *inj* allele at each of the *hap-2, hap-3,* and *hap-4* loci enables a strain to respond to

---

[1] Paper No. 2708 from the Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin.

[2] Present address: Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506.