Forest Productivity And Site Evaluation

Edited by

Tao Kiang
Jeng-Chuan Yang
Yu-Ping Kao

Taiwan, ROC–Arkansas, USA
Joint Seminar on Forest Productivity and Site Evaluation
November 10–11, 1987
Taipei, Republic of China

Organized by Council of Agriculture, ROC

Sponsored by Taiwan Forestry Research Institute, ROC
University of Arkansas, USA

Published by

Councial of Agriculture
Taipei, 1987

ASSOCIATION OF NITROGEN-FIXING BACTERIA WITH MYCORRHIZAL FUNGI AND FECES OF FOREST-DWELLING MAMMALS IN RELATION TO FOREST PRODUCTIVITY

C. Y. LI

U. S. Department of Agriculture, Forest Service,
Pacific Northwest Research Station, Forestry Sciences Laboratory
3200 Jefferson way, corvallis, Oregen 97331, U.S.A.

ABSTRACT

Growth of forest trees is often limited by nitrogen deficiency. In the Douglas-fir region of the Pacific Northwestern United States, however, a nitrogen-fixing system of potentially great value has been discovered. The nitrogen-fixing bacteria are associated with the root-colonizing mycorrhizal fungi that trees need to absorb nutrients from the soil. The bacteria produce the nitrogen-fixing enzyme only in the presence of the fungus and often are inoperative if the fungus is absent. For their spore dispersal, mycorrhizal fungi must be eaten by forest-dwelling mammals. The spores pass unharmed through animal's digestive tract and are defecated as inoculum "packets" from which spores are washed by rain into the soil to the tree roots. The nitrogen-fixing bacteria also survive passage through the animal and are active along with the spores.

INTRODUCTION

Isolation of bacteria from fungal sporocarps has been often reported. Swartz (1929) isolated bacteria from within sporocarps of Lycoperdaceae with either broken or unbroken peridia, although the functional importance of the bacteria is unknown. Larsen *et al.* (1978) demonstrated nitrogenase activity, measured by acetylene reduction, in sporocarps of decay fungi growing on dead tree boles. The responsible organisms were not isolated, but bacteria in sporocarps were detected with a scanning electron microscope. Spano *et al.* (1982) reported isolation of nitrogen-fixing bacteria from within sporocarps of *Fomitopsis pinicola* (Fr.) Karst. growing on decaying wood. The isolates were not tested for nitrogenase activity, however. Li and castellano (1985, 1987) identified acetylene-reducing (nitrogen-fixing) bacteria isolated from within sporocarps of ectomycorrhizal fungi that are common mycorrhizal associates of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) France). Also Li *et al.* (1986a, 1986b) demonstrated the presence of nitrogen-fixing bacteria in feces of mammals collected from St. Lawrence Island, Alaska, to the North Carolina-Tennesee border.

The present report is designed to summerize the author's findings and the role the forest mammals may play in maintaining the health of forest ecosystems.

METHODS

Nitrogenase activity was determined by placing one to five pieces of interior tissue of fungal sporocarp, or animal fecal pellets in 20 ml of Dobereiner's or Burk's Nitrogen-free liquid medium (Zuberer, 1987) in a 60 ml capacity serum bottle. After 3 days at 30°C under microaerophilic conditions the media became turbid. Acetylene was then injected into each bottle to 10% of the total gas volume. After 18 hrs, a 0.1 ml gaseous sample from each bottle was removed and analyzed for ethylene and acetylene with a gas chromatograph fitted with a 2 m x 2.1 mm, 80-100 mesh, Porapack R column.

Bacteria were isolated and purified by streaking the cultures an Dobereiner's nitrogen-free agar medium supplemented with 0.002% yeast extract.

Yeast populations in animal feces were determined by dilution-plating on sodium albumenate agar (Waksman and Fred, 1922).

Fecal extacts were prepared by the methods of Li. *et al.* (1986a).

RESULTS AND DISCUSSION

The nitrogen-fixing bacteria isolated from within sporocarps of ectomycorrhizal fungi were mostly those of *Azospirillum* that form a distinctive white pellicle with acetylene reduction activity in a nitrogen-free semi-solid malate agar (Table 1). The bacteria showed a characteristic spiral movement when observed in water under a light microscope. Cells were straight to slightly curved, plump, slightly pointed rods with two flagella at one end of the cell.

Inoculation of *Aluns rubra* Bong. with *Frankia* plus *Azospirillum* resulted in significantly larger seedlings – stem height, root collar diameter, and total shoot and root weights – than

Table 1. Nitrogen-fixing bacteria isolated from within sporocarps of ectomycorrhizal fungi (Li, 1987)

Fungal sporocarp	Geographic location	Bacterium isolated
Barssia oregonensis	Apiary, OR	*Azospirillum*
Hymenogaster parksii	Blodgett, OR	*Pseudomonas*
Hysterangium setchellii	Mary's Peak, OR	*Azospirillum*
Hebeloma crustulinforme	Grants Pass, OR	*Azospirillum*
Laccaria laccata	Corvallis, OR	*Azospirillum*
Leccinum scabrum	Corvallis, OR	*Azospirillum*
Rhizopogon parksii	Lincoln County, OR	not determined
R. vinicolor	Benton County, OR	*Azospirillum*
Suillus ponderosus	Corvallis, OR	*Pseudomonas*
Tuber melanosporum	France	*Klebsiella pneumoniae*

Table 3. Nitrogenase activity and microorganisms in feces of 51 mammals of 19 species from Alaska to North Carolina-Tennessee (Li *et al.* 1986b).

Animal	Geographic location	Nitrogenase[1] activity	Yeasts[2]	Actinomycetes[2]
INSECTIVORA				
Soricidae (shrews)				
Sorex cinereus (1)[3]	Morocco, IN	+	not determined	not determined
LAGOMORPHA				
Leporidae (hares and rabbits)				
Lepus Californicus (1)	Summer Lake, OR	+	+	+
Sylvilagus floridanus (1)	Morocco, IN	+	0	0
RODENTIA				
Sciuridae (squirrels)				
Cynomys leucurus (7)	Meeteetse, WY	0	+	0
Cynomys leucurus (3)	Laramie, WY	0	+	0
Eutamias townsendi (1)	Umpqua, OR	+	+	+
Glaucomys volans (2)	Morocco, IN	+	+	0
Spermophilus parryi (1)	St. Lawrence Island, AK	0	+	0
S. tridecemlineatus	Cedar Falls, IA	+	+	+
Tamias striatus (1)	Cedar Falls, IA	+	+	+
Geomyidae (pocket gophers)				
Geomy busarius (1)	Morocco, IN	+	0	+
Cricetidae (native mice)				
Peromyscus leucopus (6)	Morocco, IN	+	+	0
P. maniculatus (4)	Morocco, IN	+	0	0
Arvicolidae (voles)				
Clethrionomys gapperi (10)	Roon Mountain NC-TN	+	+	+
Lagurus curtatus (1)	Silver Lake, OR	+	+	+
Microtus ochrogaster (2)	Morocco, IN	+	+	0
M. oeconomus (4)	St. Lawrence Island, AK	+	+	0
M. oregoni (1)	Marys Peak, OR	+	+	+
Muridae (Old would mice)				
Mus musculus (1)	Morocco, IN	+	+	+
ARTIODACTYLA				
Cervidae (deer)				
Cervus elaphus (1)	Post, OR	0	+	0

[1] Four replicates (all positive or all negative).

[2] Average of three replications of one individual from each species.

[3] Number of individuals tested for nitrogenase activity.

inoculation with *Frankia* alone (Table 2). The dual inoculation also produced bigger and greater quantities of root nodules. Seedlings without *Frankia* inoculation or with *Azospirillum* inoculation were stunted and eventually died. *Azospirillum* might have facilitated nodule formation by promoting *Frankia* infection (Li *et al.* 1987).

Table 2. Effect of *Azospirillum- Frankia* inoculation on growth of *Alnus rubra* Bong. (Li *et al.* 1987).

Inoculation	Seedling height (mm)	Stem Caliper (mm)	Dry weight of shoot (mg)	Dry weight of roots & nodule (mg)	No. nodule
Azospirillum-Frankia	88	1.8	167	43	46
Frankia only	47	1.0	67	22	28
Azosprillum only	9.0	0.5	5.8	7.9	0
No inoculation	9.0	0.5	5.8	7.7	0

Data are average of 14 replicates of each treatment.

Some small forest-dwelling mammals eat mostly fruiting bodies of mycorrhizal fungi and disperse fecal pellets containing fungal spores, which germinated and form mycorrhizae with roots of forest trees (Hunt and Maser, 1985; Maser *et al.* 1978, 1985; Ure and Maser 1982). The feces of these animals also contained nitrogen-fixing bacteria and yeast (Table 3). The nutrient in the feces is as effective as yeast extract in promoting bacterial growth and nitrogences activity (Li *et al.* 1986a). When these animals dig at the bases of tree, the organisms in their feces could inoculate rootlets with nitrogen-fixing bacteria, yeast, and spores of mycorrhizal fungi.

Small mammals have often been seen as detrimental to timber management (Crouch and Radwan, 1975; Hooven, 1975; Sullivan, 1979, 1980), and poisons and habitat manipulation have been used against them. But the more forests are altered by human actions, the more evident becomes the need to understand the interactions of all organisms in the ecosystam. These small mammals are the first to occupy clearings after logging or fire and disperse these microorganisms that are essential in maintaining ecosystem health.

REFERENCES

1. Crouch, G. L. and M. A. Radwan. 1975. Coating and impregnation with Endrin Protects field-sown Douglas-fir seeds. Pestic. Sci. 6: 337-345.
2. Hooven, E. 1975. Baiting to reduce losses of conifer seeds to small forest mammals. Res. Note 55, For. Res. Lab., Oregen State University, Corvallis, Oregen. 3 pp.

3. Hunt, G. A. and Z. Maser. 1985. Consumption of hypogeous fungi by the deer mouse (*Peromyscus maniculatus*). p. 272 *In* Proceedings of the 6*th* North American Conference on Mycorrhizae (ed. R. Molina), Corvallis, Oregen State University Forestry Research Laboratary.
4. Larsen, M. J., M. F. Jurgensen, A. E. Harvey, and J. C. Ward 1978. Dinitrogen fixation associated with sporophores of *Fomitopsis pinicola*, *Fomes fomentarius* and *Echinodontium tinctorium*, Mycologia 70: 1217-1221.
5. Li, C. Y. and M. A. Castellano. 1985. Nitrogen-fixing bacteria isolated from within sporocarps of three ectomycorrhizal fungi. *In* Proceedings of the 6th North American Conference on Mycorrhizae (ed. R. Molina), p. 264. Corvallis, Oregen State University Forestry Research Laboratary.
6. Li. C. Y., C. Maser, Z. Maser, and B. A. Caldwell. 1986a. Role of three rodents in forest nitrogen fixation in western Oregon: Another aspect of mammal-mycorrhizal fungus-tree mutualism. Great Basin Naturalist 46: 411-414.
7. Li. C. Y., C. Maser and H. Fay. 1986b. An initial survey of acetylene reduction and selected microorganisms in the feces of nineteen species of mammals. Great Basin Noturalist 46: 646-650.
8. Li. C. Y. and M. A. Castellano 1987. *Azospirillum* isolated from within sporocorps of the mycorrhizal fungi *Hebeloma Crustuliniforme*, *Laccaria laccata* and *Rhizopogon vinicolor*. Trans. Br. Mycol. Soc. 88: 563-565.
9. Li. C. Y., K. V. B. R. Tilak and R. Molina 1987. Growth enhancement of red alder by inoculation with *Frankia-Azospirillum* combination. p. 247 (Abstract), 1987. American Society of mirobiology Annual meeting.
10. Li. C. Y. 1987. Diazotrophic bacteria in sporocarps of ectomycorrhizal fungi *Barssia oregonensis*, *Hysterangium setchellii*, *Leccinum scabrum*, and *Rhizopogon parksii*. *In* Procceedings of the 7*th* North American Conference on Mycorrhizae. (In Press).
11. Maser, C., J. M. Trappe and R. A. Nussbaum. 1978. Fungal-small mammals interrelationships with emphasis on oregen coniferous forest. Ecology 59: 799-809.
12. Maser, Z., C. Maser and J. M. Trappe. 1985a. For habits of the northern flying squirrel (*Glaucomys sabrinus*) in Oregen. Can. J. Zool. 63: 1084-1088.
13. Spano, S. D., M. F. Jurgensen, M. J. Larsen and A. E. Harvey. 1982. Nitrogen-fixing bacteria in Dougles-fir residue decayed by *Fomitopsis pinicola*. Plant and Soil 68: 117-123.
14. Sullivan, T. P. 1979. Demography of populations of deer mice in coastal forest and clear-cut (logged) habitats. Can. J. Zool. 57: 1636-1648.
15. Sullivan, T. P. 1980. Comparative demography of *Peromyscus maniculatus and Microtus Oregoni*: Populations after logging and burning of coastal forest habitats. Can. J. Zool. 58: 2252-2259.
16. Swartz, D. 1929. Bacteria in puffballs. Michigan Academy of Science, Arts and Letters 11: 285-296.
17. Ure, D. C. and C. Maser. 1982. Mycophagy of red-backed voles in Oregen and Washington. Can. J. Zoology 60: 3307-3315.

18. Waksman, S. A. and E. B. Fred 1922. A tentative outline of the plate method for determining the number of microorganisms in the Soil. Soil Sci. 14: 27-28.

19. Zuberer, D. A. 1987. Collection, isolation, cultivation, and maintenance of associated N_2-fixing bacteria. *In* Symbiotic Nitrogen fixation technology (ed. G. H. Elkan), pp. 95-125. Marcell Dekker, Inc. New York.