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Effects of Nitrogen Fertilizer on Survival of Poria Weirii and Populations of Soil Fungi and Aerobic Actinomycetes

Fertilization of forest trees began in Europe in the early 1900's (Mayer-Krapoll, 1956). In the Pacific Northwest, nitrogen fertilizers substantially increased growth of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], especially on poor sites (Gessel *et al.*, 1965). Douglas-fir also benefits from soil enriched through biological nitrogen fixation by adjacent red alder (*Alnus rubra* Bong.) (Tarrant, 1961).

Effects of fertilization on root diseases merit special attention. In agriculture, nitrogen added to soil has affected disease severity, usually to the detriment of the host but sometimes to the detriment of the pathogen (Garret, 1956). Effects of nitrogen fertilization on forest tree diseases have not yet received close attention. A previous study in alder and conifer stands hinted that nitrogen in the soil might at least indirectly reduce survival of *Poria weirii* Murr. (Nelson, 1968). Supplementing forest soils with nitrate or ammonium salts might produce similar effects on this or other tree root pathogens without interplanting less desirable timber species such as red alder. To further examine this possibility, we tested the effects of fertilization with NH_4Cl and $NaNO_3$ (1) in the field, on survival of *P. weirii* in buried wood cubes and on numbers of soil fungi and actinomycetes, and (2) in the laboratory, on survival of *P. weirii* only.

Field Experiment

Methods

Eighteen 2-inch cubes, sawn from heartwood of Douglas-fir naturally infected with *P. weirii* and selected for uniformity of decay, were buried 8 inches deep at each point of a grid of 1-foot intervals on each of twenty-one 3- by 6-foot plots in the Coast Ranges of western Oregon. Soil, as determined from three pits distributed among the plots (Figure 2), was similar to the Slickrock series[1] as mapped in the nearby Alsea drainage and, at depth of cube burial, was a gravelly clay loam. After 2 months, three randomly selected cubes were removed from each plot and three 8-inch-long soil cores were taken near each cube.

The cubes were split and cultured onto malt agar from four points on the split face: center, one-half inch on either side of center, and one-half inch below center (opposite center from point of chisel penetration). *Poria weirii* was identified by cultural characteristics. Segments of soil cores from the 6- to 8-inch depth were combined for each plot, screened through a 2-mm soil sieve, and assayed for numbers of fungi and aerobic actinomycetes per gram and for concentration of NH_4^+ and NO_3^-. Numbers of fungi were determined by dilution plate counts (1:50,000) on peptone dextrose agar with rose bengal and streptomycin, incubated at room temperature (23° C) for 10 days. Numbers of aerobic actinomycetes were determined at the same dilution on sodium albuminate agar (Johnson *et al.*, 1959) at 30° C for 20 to 3

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[1] This series is provisional and not yet correlated.

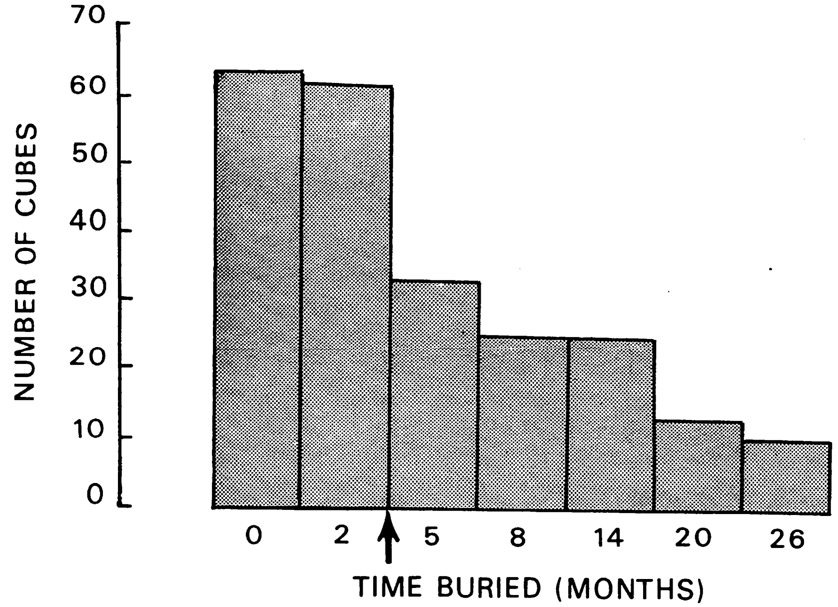

Figure 1. Number of wood cubes in which *Poria weirii* survived after burial. Each sample contained 63 cubes; ↑ indicates time fertilizer was added to soil.

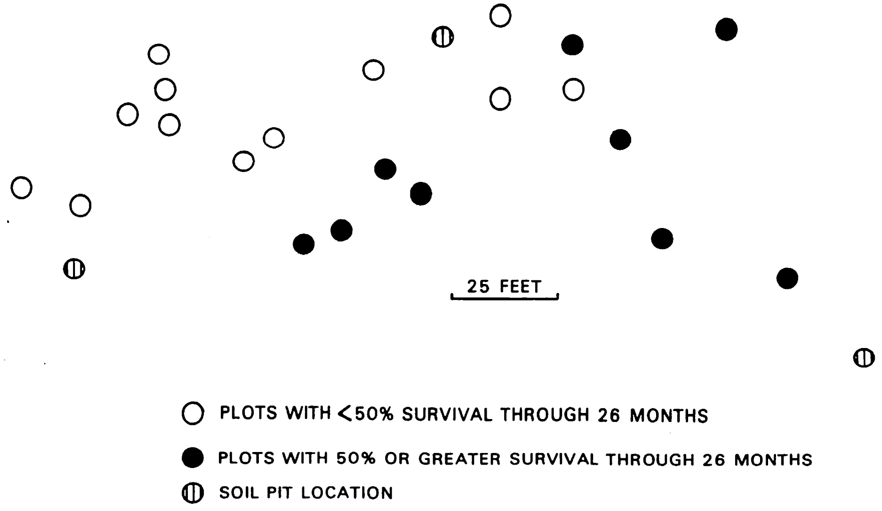

Figure 2. Effect of plot location on *Poria weirii* survival.

days. NH_4^+ and NO_3^- were determined by the methods of Shrinkhande (1941) and of Johnson and Ulrich (1950), respectively.

After the first samples were taken, sodium nitrate or ammonium chloride, at concentrations equivalent to 150, 300, and 600 pounds of N per acre, was applied in a gallon of water to each plot and the surrounding 1-foot buffer zone. Check plots similarly received 1 gallon of water alone. Three months later, cubes and soil cores were removed from each plot and treated as described earlier for determination of survival of *P. weirii*, number of fungi and actinomycetes, and concentrations of NH_4^+ and NO_3^-. Thereafter, cubes and soil cores were taken 6, 12, 18, and 24 months following fertilization, and were tested for survival of *P. weirii* and numbers of fungi and actinomycetes, respectively. Interrelationships among numbers of fungi and aerobic actinomycetes, kind and level of nitrogen applied, measured values of NO_3^- and NH_4^+ at the 6- to 8-inch depth, survival of *P. weirii*, and sampling date were tested by analysis of variance.

Results and Discussion

The NH_4^+ and NO_3^- salts, applied to the surface of the soil, were not detected at the 6- to 8-inch depth 3 months after application. Although combined NH_4^+ and NO_3^- nitrogen for all plots was somewhat greater after 3 months, changes in concentration from time of application were erratic and inconsistent with treatment levels on individual plots.

Fungus and aerobic actinomycete populations were highly correlated with time of sampling, most likely from seasonal influences. Populations of aerobic actinomycetes on control plots were not significantly different from fertilized plots, but a separate analysis excluding the highly variable controls showed significant differences between treatments (NO_3^- and NH_4^+) and interaction of levels and treatments. Populations were generally higher with each increment of NO_3^- but lower with each increment of NH_4^+ added (Table I).

Survival of *P. weirii* was not significantly correlated with kind or level of nitrogen applied or measured at 6- to 8-inch depth at the time of fertilization or at 3 months after, nor was it significantly correlated with population levels of fungi or aerobic actinomycetes. Overall, after 2 months' burial before fertilization, *P. weirii* survived in 61 of 63 cubes; 3 months after fertilization, in 32; 6 and 12 months after, in 24: 18 months after, in 12; and 24 months after, in 9 of 63 cubes (Figure 1). As i previous studies (Nelson, 1967; 1968), survival was closely associated with presenc of zone lines.

TABLE I. Aerobic actinomycete populations in soil after application of fertilizer.

(In tens of thousands per gram of soil)

Treatment	Level of N per acre	Months following application					
		3	6	12	18	24	Average
	Pounds	*Number*					
NH_4^+	150	239[1]	195	280	181	326	244
	300	287	210	256	144	281	236
	600	238	162	264	110	209	197
Check	0	310	225	281	165	269	250
NO_3^-	150	245	164	183	105	232	186
	300	364	318	420	208	271	316
	600	339	258	383	327	310	323

[1] Average of 3 plots.

The correlation of numbers of aerobic actinomycetes with applied NO_3^- and NH fertilizers is particularly interesting, since many of these organisms produce antagonist reactions toward *P. weirii in vitro* (Li, 1969). Further study is needed to interpr the wide variation in populations of aerobic actinomycetes on control plots compar with fertilized plots. We can now only guess that application of fertilizer reduc differences in soil factors affecting actinomycete populations. The main effect fertilization on *P. weirii* survival would very likely be indirect: i.e., changes in con position or populations of microflora induced by fertilization might encourage invasi of *Poria*-colonized wood substrates and replacement of the pathogen within the by certain soil-inhabiting microbes.

The inconclusive results in this study of survival of *P. weirii* may be part explained. First, plot location effects on survival may not have been overco through randomization (Figure 2); and second, treatments seemed to have little effe on N levels at the depth of cube burial 3 months after application. Timing fertilizer application may have accounted for the latter since most of the nitrog could have been held in the upper few inches of litter and soil following the relative dry period after application.

If we were to eliminate, as far as possible, the effects of plot location and assu distribution of fertilizer around wood cubes colonized by *P. weirii*, might surviv then be affected by the added NH_4^+ or NO_3^- nitrogen? To answer this questio we conducted a small but better controlled laboratory study of a similar nature.

Laboratory Experiment

Methods

Two-inch wood cubes from Douglas-fir naturally infected with *P. weirii* were agai used as the test material. Soil from a Douglas-fir stand near the original fiel experiment was sifted through a 4-mesh screen, thoroughly mixed in a concre mixer, and divided into three treatment lots. The first was remixed in the sam mixer; the second was mixed with 46.5 g $NaNO_3$/cubic foot of soil (600 lb. N acre-ft.); the third was mixed with 29.2 g NH_4Cl/cubic foot of soil (600 lb. N/acr ft.).

For each soil treatment, 30 cubes were potted individually in plastic quart co tainers with bottoms perforated for drainage. Pots were lightly watered from abov at approximately weekly intervals. After incubation at 15° C for 6 months, all cub were removed and cultures taken from them as described in the field study.

Results and Discussion

Results of the experiment were striking. *P. weirii* survived for 6 months in only on of the 60 pots with either NH_4^+ or NO_3^- forms of nitrogen mixed with soil. Th fungus survived in 14 of the 30 pots of unfertilized soil. As was the case in th field, survival was closely linked with presence of zone lines in the cubes (Table II)

TABLE II. Survival of *Poria weirii* in potted wood cubes.

Treatment	Number of cubes Total	w/zone lines	w/*P. weirii*
NH_4Cl	30	0[1]	0
$NaNO_3$	30	1[2]	1
Check	30	15	14

[1] Five additional cubes had traces of zone lines.
[2] Two additional cubes had traces of zone lines.

Previous observations have led to the hypothesis that *P. weirii* survival is reduced in soil under red alder because of large amounts of NO_3^- in these soils. Nitrate is unavailable to *P. weirii,* a fungus lacking the enzyme nitrate reductase, but usable by many of its competitors in the soil-wood environment (Li *et al.,* 1967).

Apparently, high levels of nitrogen, either as NO_3- or NH_4^+, can decrease survival of *P. weirii.* The additional supply of nitrogen apparently stimulates development of soil micro-organisms which invade the colonized wood cubes and replace *P. weirii.* Another possible but less likely explanation is that the higher levels of nitrogen inhibited formation of zone lines by *P. weirii*—zone lines are suspected of resisting invasion of the *Poria*-colonized substrate. The problem in field application of nitrogen fertilizers might well be getting the nitrogen to the place in the soil where its effect is desired.

Modified field studies are planned to further examine effects on survival of *P. weirii* of nitrogen form and level as well as time and method of application.

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The Distribution of Ground-Dwelling Beetles in Relation to Vegetation, Season, and Topography in the Rattlesnake Hills, Southeastern Washington*

Introduction

The ecological distribution of insects in the semiarid shrub steppe region of sout eastern Washington has received little study. This paper reports the results of pitfall-trap survey of four common species of ground-dwelling beetles inhabiting rel tively undisturbed plant communities in the Rattlesnake Hills, Benton County, Was ington during 1966 and 1967.

The major land use of the northeasterly facing slopes of the Rattlesnake Hil prior to 1943 was livestock grazing. After 1943, this area was incorporated int the United States Atomic Energy Commission's Hanford Reservation and since the only occasional grazing has occurred mostly confined to the vicinity of a few widel scattered watering places.

The northeasterly facing slopes of the Rattlesnake Hills have not received dire applications of chemical insecticides or weedicides and probably represent the lea man-modified landscape now available for ecological study within the shrub step region of southeastern Washington.

Description of Study Sites

Eight study sites were established at different elevations ranging between 460 an 3,450 feet. The sites were spread over a straight line distance of about eight mile Site number one is 460 feet above mean sea level located on more or less lev topography. The soil is coarse sand with buried cobbles and boulders. The domina vegetation is big sagebrush (*Artemisia tridentata*) and bitterbrush (*Purshia tridentata* with a sparse herbaceous understory comprised mostly of Sandberg bluegrass (*P* *secunda*) and cheatgrass brome (*Bromus tectorum*). Site number two is at an elevatio of 530 feet. This site is floristically and topographically similar to site number on Site number three is located on a gentle slope at 630 feet in elevation. The vegetatio is dominated by big sagebrush with a few scattered clumps of spiny hopsage (*Gray* *spinosa*). The understory is comprised mostly of Sandberg bluegrass, cheatgra brome, and a few scattered plants of carey balsamroot (*Balsamorhiza careyana* The soil profile lacks stones and is less sandy than sites one and two. Site numb four is located on a gentle slope at 925 feet elevation. Big sagebrush is the onl shrub. The understory is dominated by large perennial bunchgrass, especially blu

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