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EFFECT OF UREA ON *PORIA WEIRII* AND SOIL MICROBES IN AN ARTIFICIAL SYSTEM

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Summary—*Poria weirii* (Murr.) Murr. (an important root-infecting fungus of conifers in western North America) grown in alder stem sections and buried in soil at 15°C survived better when urea was not added to the soil. Survival over a 32 week period was inversely related to rate of urea applied. Fungus populations were greatest at 4 weeks after which numbers of *Trichoderma* began to increase. This corresponded with decreased survival of *P. weirii*; *Trichoderma* was the fungus isolated almost exclusively from alder sections when *P. weirii* was not. Increasing soil populations of actinomycetes with time may have had some effect on survival as well.

INTRODUCTION

The ability of *Poria weirii* (Murr.) Murr. to survive for many years in decaying root systems makes this destructive root pathogen extremely difficult to control in Pacific Northwest coniferous forests. Competing soil microorganisms replace this root-infecting fungus in decaying root systems but generally not before new disease centers develop in the regenerating forest. Centers thus formed enlarge with root to root spread as a stand develops. Opportunities for control of this disease appear best at final harvest when heavy equipment is present, mobility on the site is least restricted, harvest and reforestation options are available, and soil treatments such as organic amendments or chemicals can be applied.

Application of N fertilizer seems to be a promising control measure. Nelson (1975) found application of urea at the rate of 674 kg N ha^{-1} resulted in a dramatic reduction in survival of *P. weirii* in buried wood which he attributed to indirect effects of antagonists of *P. weirii*. This study relates rate of application of urea to microbial activity and consequent suppression of *P. weirii* in buried wood.

METHODS AND MATERIALS

Survival of *P. weirii* was tested in autoclaved alder stem sections of about 5 cm length × 2·5 cm dia in which the fungus had been established 3 months beforehand. Ten sections were buried in each of 18 plastic containers in 2·2 kg soil (dry wt) collected 10 days earlier from 10–30 cm depth beneath a stand of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), dried to 28·5% moisture content and screened through 6 mm mesh. Urea was mixed with soil at rates of 0, 147, and 294 g N/m^3 before the alder stems were buried.

Moisture content of soil at field capacity was estimated at 55% by the Buchner Funnel Method (Veihmeyer and Hendrickson, 1949). Soil in each container was adjusted to 80% of field capacity by sprinkling the soil surface with 350 ml distilled water. Moisture content in the loosely covered containers was maintained by adding water to bring each container to established gross weight at weekly intervals. Incubation was at 15°C in the dark.

At the beginning of the experiment, viability of *P. weirii* in alder sections was determined by splitting 10 randomly sampled sections and transferring two small chips of wood from each freshly split face onto malt agar slants. At 1, 2, 4, 8, 16 and 32 weeks after burial, one container of each treatment was removed and all sections split and treated as above. *P. weirii* is readily identified after 10 days at room temperature by macroscopic and microscopic characteristics, primarily by its distinctive setal hyphae. Soil from the emptied containers was thoroughly mixed and sampled to determine total N (¶2.045 in methods of analysis, Association of Official Agricultural Chemists, 1965), NO_3^- (Johnson and Ulrich, 1950), and NH_4^+ (Nichols and Foote, 1931). Populations of fungi were estimated from 5 replicate plates using a 10^{-6} soil dilution in peptone dextrose agar with streptomycin and Rose Bengal incubated at room temperature (23°C) for 1 week. Populations of bacteria and actinomycetes from 5 replicate plates were similarly estimated after 2 weeks' incubation on sodium albuminate agar (Johnson *et al.*, 1959).

Relationship between survival of *P. weirii* and time was tested by analysis of variance. Relationships among *Trichoderma* and *P. weirii* survival; bacteria, actinomycetes, fungi, NO_3^- and NH_4^+ with time; and NO_3^- and NH_4^+ with bacteria, actinomycetes and fungi were tested by analysis of covariance.

RESULTS

P. weirii was viable in all 10 alder sections sampled at the beginning of the experiment and remained viable in all sections through 4 weeks (Table 1). Growth of mycelium from some alder sections to the soil surface further attests to the initial vigor of the fungus (Fig. 1). After 4 weeks, survival declined with time and added N until the end of the experiment (32 weeks), when the fungus survived only in the control treatment. Analysis of variance indicated survival among treatments differed significantly ($P < 0{\cdot}021$) after a 4-week period of 100% survival.

Table 1. Survival of *P. weirii* in buried alder stem sections, nitrogen content and microbial populations in urea amended soil

Weeks after fertilization	Treatment gN/m^3 soil	*P. weirii* survival (%)	Soil nitrogen Total (%)	NH_4^+ parts/10^6	NO_3^- parts/10^6	Soil microbial populations (10^6/g dry soil) All fungi	Trichoderma	Bacteria	Actinomycetes
1	Control	100	0.28	20	20	47.2	0	25.6	6.4
	147	100	0.30	110	80	59.4	0	16.2	24.4
	294	100	0.33	290	60	271.2	0.2	12.8	25.4
2	Control	100	0.29	40	320	35.6	0	30.0	7.2
	147	100	0.31	110	420	28.4	0	13.8	27.2
	294	100	0.31	320	460	46.4	0	8.4	27.8
4	Control	100	0.38	40	480	363.8	0	17.2	7.4
	147	100	0.32	130	610	304.6	0	9.8	19.6
	294	100	0.33	330	640	305.0	0	11.6	35.8
8	Control	100	0.29	30	110	0.8	0.4	13.2	9.2
	147	80	0.30	40	140	2.6	1.2	18.4	28.0
	294	30	0.33	140	120	10.6	9.4	9.0	53.8
16	Control	90	n.d.	n.d.	n.d.	5.6	2.4	n.d.	n.d.
	147	70	n.d.	n.d.	n.d.	8.6	6.6	n.d.	n.d.
	294	20	n.d.	n.d.	n.d.	26.2	20.8	n.d.	n.d.
32	Control	80	0.30	30	10	n.d.	n.d.	n.d.	n.d.
	147	0	0.31	40	50	n.d.	n.d.	n.d.	n.d.
	294	0	0.31	60	130	n.d.	n.d.	n.d.	n.d.

Except in the control soil at 4 weeks, total N was nearly constant by treatment throughout the experiment. The high concentration of N at 4 weeks, especially NO_3^-, corresponds with the unusually high populations of fungi at that time. The declining survival of *P. weirii* at 8 weeks and beyond is timed with decline in total fungus populations and increased numbers of *Trichoderma* spp. The correlation between *Trichoderma* populations and *P. weirii* survival was significant in all treatments ($P < 0{\cdot}05$); and in nearly all cases, *Trichoderma* spp. were isolated from the alder sections when *P. weirii* was not.

Because of the small number of observations, it was difficult to show significant relationships between forms of N, microbial populations, and time by analysis of covariance. Relationships were apparent in some treatments but not in others. It is probably best that the reader judge for himself the validity of these interrelationships from data in Table 1.

DISCUSSION

The adverse effects of urea on *P. weirii* survival demonstrated in the laboratory (Nelson, 1970) and in modified field experiments (Nelson, 1975) appear to some extent to be rate dependent; that is, 294 g N/m^3 had a greater effect than 147 g N/m^3. The inverse relationship between *Trichoderma* populations and *P. weirii* survival observed here complements earlier works where the principal fungus invading wood colonized by *P. weirii* was *Trichoderma* (Nelson, 1964).

There are numerous reports of effects of N on longevity of soil-borne plant pathogens in colonized host tissues. Many of these refer to cereal crop pathogens in buried straw where the N applied may cause direct and indirect effects on saprophytic survival. Garrett (1970), reviewing work of several investigators, cites a generally favorable effect of N on survival of fungi inhabiting straw in soil. The N applied shifts the ratio of C to N in favor of continued growth and, consequently, survival of the fungus. Butler (1953) proposed an indirect effect of N on survival. Antagonism of soil microflora to cereal root rot fungi was enhanced by added N, and their replacement was hastened.

The size, composition and, under normal circumstances, protective covering of woody materials in soil leads one to suspect an indirect effect of N on survival of the primary colonizing fungus. Bliss (1951) reported "biological" control of *Armillaria mellea* in citrus orchards by treating soil with sub-lethal concentrations of CS_2. The fumigant failed to kill *A. mellea* directly but allowed its replacement in its wood

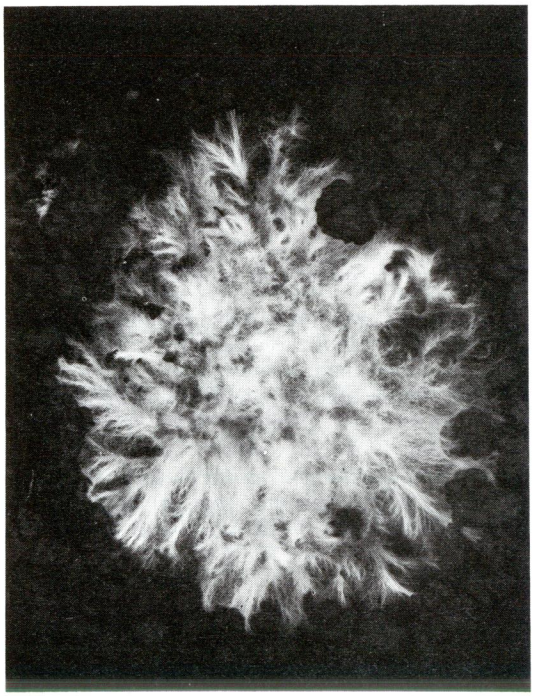

Fig. 1. Colony of *P. weirii* growing on surface of soil. This colony had grown from an alder section, 9 mm beneath the soil surface.

substrate by *Trichoderma*. Darley and Wilbur (1954) concluded that more than soil microbial changes due to fumigation were necessary for replacement of *A. mellea* in buried wood—the pathogen, itself, must be weakened. In their experiment, *Trichoderma* replaced *A. mellea* in 30 days. Varghese and Chew (1975) reported similar findings for a species of *Ganoderma* in tea plantations fumigated with methyl bromide. Addition of mulch containing NP and K further stimulated soil microflora after fumigation.

My results show that urea can stimulate development of *Trichoderma* in soil within 8 weeks and the magnitude depends directly on rate of application. No attempt was made to measure a direct effect on *P. weirii* and it is unlikely that one of significance exists. In this case, rise in *Trichoderma* populations followed a rapid decline in measured soil fungus populations. The cause–effect relationship of these two events, if one exists, is unknown. The increased numbers of *Trichoderma* in soil or on dilution plates may have suppressed development of some other fungi and may account for some of the reduction in total fungus populations. Competition from increasing numbers of actinomycetes during the period may have been partly responsible for reduced fungus populations.

In nature, *P. weirii* often survives in very large, woody residues, sometimes covered by thick bark and nearly isolated by bands of resin. Whether the fungus in this habitat can be affected by N applied to the soil surface remains to be seen. Field plots have been established to determine this possibility, but the answer will not be apparent for years. Other field trials are planned combining N with mechanical disruption of rotted root systems to make the fungus more vulnerable to attack. It seems likely that natural control of *P. weirii* by its replacement in wood by competing soil microflora can be practical and effective if encouraged by proper stand and soil manipulations.

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