EFFECT OF ERADICANTS ON THE MICROBIOLOGICAL PROPERTIES OF NURSERY SOILS[1]

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Current nursery practice employs a large number of toxic compounds, or eradicants, for the control of destructive insects, parasitic fungi, and noxious weeds. The application of these chemicals is not without adverse influence upon the beneficial soil organisms, state of soil fertility, and the growth of nursery stock. This study aimed to detect the effects of commonly used eradicants upon microbiological characteristics of soils which serve as indicators of unimpaired soil productive capacity.

The trials were conducted in greenhouse cultures with outwash siliceous sand possessing a reaction of about pH 5.0, exchange capacity of 1.9 m.e. per 100 g., and 0.7 per cent of organic matter. The biocides studied included chlordane, benzene hexachloride, calomel, thiosan, aluminum sulfate, formaldehyde, allyl alcohol, and Stoddard solvent. These were applied at the rates slightly exceeding those used in current nursery practice. Such treatments were justified in view of the local concentration of chemicals resulting from their uneven distribution under conditions of actual soil management. The following microbiological characteristics were investigated: the relative density of micropopulation, rate of cellulose and protein decomposition, nitrification capacity, rate of carbon dioxide evolution, growth of excised roots under the influence of volatile substances emitted by the soil, growth responses of *Aspergillus niger,* and the development of mycorrhizal short roots. Monterey pine, *Pinus radiata,* was used as a test plant and a carrier of symbiotic organisms. The extreme poverty of the soil, the use of enclosed containers, and fluctuating content of soil moisture, unavoidable in watering by hand, all undoubtedly contributed to the adverse effects of biocides.

The number of microorganisms present in untreated and biocide-treated soils was determined on the basis of colonies developed on the molecular membrane filters (Clark et al., 1951).

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The results, presented in Table 1, indicate that all biocides used appreciably decreased the soil micro-population. The most drastic reduction of microorganisms was caused by calomel and benzene hexachloride. The least harmful effect was exerted by chlordane.

The activity of cellulose- and protein-decomposing microorganisms was appraised on the basis of a modified method of Richard (1945). The soils were incubated for two weeks at 24° C in a saturated environment and then transferred into petri dishes together with the standard cellulose and protein cords. Cultures with cellulose cords were incubated for one week; cultures with protein cords were incubated for two weeks. The tensile strength of the cords was recorded by means of a wire tensilometer, and the results were expressed in percent of the tensile strength of sterile cords. The averages of quadruplicate determinations, given in Table 1, indicate that the processes of organic matter decomposition are retarded to a marked degree by application of all eradicants. The reaction of cellulose-decomposers and protein-decomposers suggests that these two groups of microorganisms vary considerably in their tolerance of different chemical compounds.

The rate of nitrification was determined by the standard phenoldisulfonic method using an Evelyn colorimeter. The samples were enriched in ammonium sulfate, applied at the rate equivalent to 400 lbs. per acre, and incubated for 3 weeks at 28° C. The results, reported in Table 1, show a depressing effect of all eradicants on the activity of nitrifying bacteria, especially sharply pronounced in treatments with calomel, Stoddard solvent, and chlordane.

For the determination of the rate of respiration of treated soils, 250 g. of air-dry samples were placed in 500 ml Erlenmeyer flasks, moistened, and incubated for 48 hours at 28° C. Before the analyses, 0.5 g. of dextrose was added to each culture. The determination of carbon dioxide evolution was made by the procedure of Heck (1929), using 48 hour aeration periods. The results, presented in Table 1, provide a clear-cut picture of the adverse influence of the biocides.

The effect of volatile substances, emitted by soils, was studied using Cholodny's biotest (Cholodny, 1951; Persidsky and Wilde, 1954). The results of these trials (Table 1) show that the growth of excised roots of blue lupine is depressed in part by the direct toxicity of applied chemicals, and in part by the reduction of the microbiological activity responsible for the release of growth-promoting volatile substances. As indicated by the average growth and longevity of roots, calomel and formaldehyde exert

TABLE 1

EFFECT OF ERADICANTS ON MICROBIOLOGICAL CHARACTERISTICS OF A COARSE SANDY SOIL (AVERAGE RESULTS)

Eradicant Applied and Rate of Application per Acre	Number of Colonies per Membrane	Loss in Tensile Strength of Cellulose Cords	Loss in Tensile Strength of Protein Cords	Nitrification Capacity NO_3	Evolution CO_2	Behavior of Excised Roots of Blue Lupines	
						Longevity	Growth
		per cent	per cent	p.p.m.	mg/g	days	mm
Untreated soil	518	65.3	72.4	7.0	168.3	5.5	7.5
Chlordane 10 lbs.	334	45.4	42.8	1.1	141.9	4.5	5.1
Allyl alcohol 50 gal.	253	29.3	45.7	3.5	58.3	2.0	3.0
Stoddard oil 100 gal.	221	33.3	38.1	0.8	34.1	3.5	4.2
Thiosan 120 lbs.	138	28.1	42.8	2.9	52.8	3.5	2.0
Aluminum sulfate 500 lbs.	51	46.7	44.7	1.7	23.1	4.0	4.3
Formaldehyde 0.7%, 5,000 gal.	63	22.7	33.3	2.7	37.4	1.0	1.6
Calomel, 40 lbs.	48	37.3	38.1	0.6	72.6	1.0	1.5
Benzene hexachloride, g.i. 1 lb.	38	32.0	47.6	1.3	25.3	4.0	2.6

TABLE 2

GROWTH OF MYCELIA OF *Aspergillus niger* IN SOILS TREATED WITH VARIOUS BIOCIDES. RESULTS ON OVEN-DRY BASIS

ERADICANT APPLIED	RATE OF APPLICATION PER ACRE	AVERAGE WEIGHT OF MYCELIUM grams			
		Culture A	Culture B	Culture C	Ave.
Untreated soil	.....	1.273	1.398	1.487	1.386
Chlordane (actual compound)	10 lbs.	1.083	1.137	1.170	1.130
Allyl alcohol	50 gal.	0.563	0.706	0.819	0.696
Stoddard oil solvent	100 gal.	0.752	0.993	1.039	0.928
Thiosan	125 gal.	0.762	0.829	0.896	0.829
Aluminum sulfate	500 lbs.	0.754	0.838	0.901	0.831
Formaldehyde (0.7 per cent solution)	5200 gal.	0.810	0.996	1.019	0.942
Calomel	40 lbs.	0.638	0.718	0.759	0.705
Benzene hexachloride (gamma isomer)	1.0 lb.	0.735	0.813	0.858	0.802

TABLE 3

EFFECT OF ERADICANTS ON THE GROWTH AND MYCOTROPHIC FEATURES OF ONE YEAR OLD SEEDLINGS OF MONTEREY PINE, *Pinus radiata*, RAISED IN SAND CULTURES. OVEN-DRY WEIGHTS OF AVERAGE SEEDLINGS

ERADICANT APPLIED AND RATE OF APPLICATION PER ACRE	SEEDLING "IN TOTO" g	TOP g	ROOT g	TOP—ROOT RATIO	TYPE OF MYCORRHIZAL ORGANS OR "SHORT ROOTS"
Untreated soil	.177	.143	.034	4.2	Abundant normal bifurcate short roots
Chlordane, 10 lbs.	.172	.132	.040	3.3	Abundant nearly normal bifurcate elongated short roots
Allyl alcohol, 50 gal.	.191	.143	.048	2.9	Fairly abundant simple short roots
Stoddard oil, 100 gal.	.180	.108	.072	1.5	Abundant simple "pseudo-mycorrhizal" short roots
Thiosan, 120 lbs.	.146	.114	.032	3.6	Sparse pedunculate roots of "pseudomycorrhizal" type
Aluminum sulfate, 500 lbs.	.130	.087	.043	2.1	Fairly abundant short roots of a slingshot pattern
Formaldehyde, 0.7% 5,000 gal.	.140	.089	.056	1.5	Fairly abundant simple and bifurcate pedunculate short roots
Calomel, 40 lbs.	.107	.098	.009	10.8	Very sparse depressed sessile lobate short roots
Benzene hexachloride, g.i. 1 lb.	.176	.130	.046	2.8	Sparse depressed sessile clovate short roots

the most unfavorable influence. A strong depressing effect is also caused by thiosan and benzene hexachloride. Chlordane proved to be the least inhibiting; this behaviour may have a bearing upon observations of Voigt (1953) who recorded a high rate of oxygen uptake by root tips in the presence of chlordane suspensions.

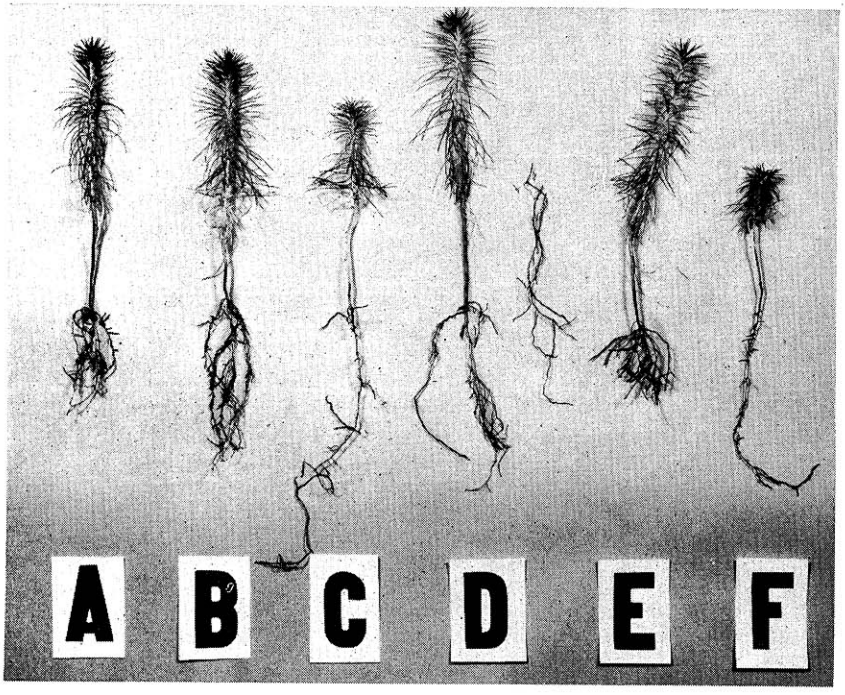

FIGURE 1. Growth of 1–0 *Pinus radiata* seedlings in a coarse sandy soil treated with different biocides at the indicated rate of application: A—Untreated soil; B—Chlordane, 10 lbs/A; C—Thiosan, 125 lbs/A; D—Allyl alcohol, 50 gal/A; E—Benzene hexachloride (gamma isomer), 1.0 lbs/A; F—Calomel, 40 lbs/A.

The direct influence of different biocides on the development of fungi was investigated by observing the growth of *Aspergillus niger* in a suspension prepared from 20 g. of soil and 30 ml of nutrient solution (Mehlich, Truog, and Fred, 1933). Inoculated soils were incubated for 5 days at 35° C. The weights of mycelia, given in Table 2, indicate that the most unfavorable influence, reducing the growth of mycelia about 50 per cent, is exerted by allyl alcohol and calomel. Chlordane proved to be the least toxic. The triplicate results give rather small deviations from the aver-

age values and suggest that the direct observations of the behavior of microorganisms may have considerable value in analyses of soils treated with biocides and commercial fertilizers (Wilde and Krumm, 1946).

The effect of eradicants on the growth of nursery stock and the development of symbiotic mycorrhizal fungi was studied in sand cultures, using *Pinus radiata* as the test plant. The results are presented in Figure 1 and Table 3.

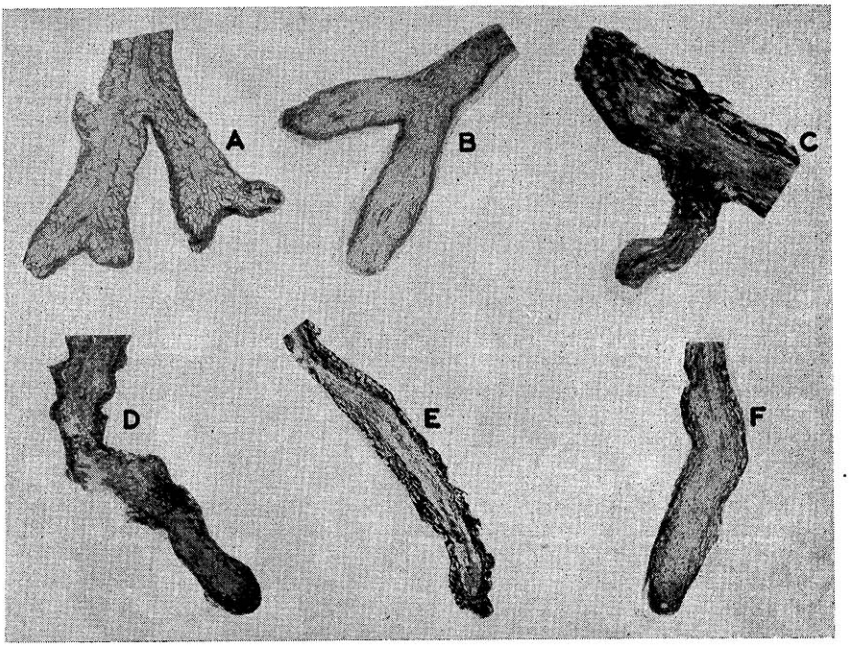

FIGURE 2. Effect of biocides on the development of root tips or "short roots" of *Pinus radiata* seedlings. A and B, normal bifurcate mycorrhizal short roots with a well developed Hartig net, produced in biocide-free soils or in the presence of mildly concentrated less toxic biocides; C to F, simple pedunculate short roots of a "pseudomycorrhizal" type, prevalent in soils or soil regions containing biocides in a high concentration.

Calomel is the only chemical which conspicuously inhibited the development of seedlings and drastically disrupted the normal top-root ratio. Other biocides at this rate of application did not decrease significantly the weights of total seedlings, their tops, or their roots. In some instances, the application of biocides stimulated the production of dry matter. As a rule, the presence of eradicants reduced the quantitative top-root ratios. However, as many previous studies have shown, the appraisal of nursery

stock on a weight basis may lead to grossly erroneous conclusions (Wilde and Voigt, 1954). Even a cursory examination of the seedlings' morphology (Figure 1) is sufficient to reveal some of the negative features which the planting material has acquired under the influence of eradicants. This is particularly true of the arrested downward growth of roots (benzene hexachloride), undue elongation of tap roots (allyl alcohol, thiosan), and reduction of laterals (thiosan, calomel).

A more elaborate analysis of the root systems disclosed further detrimental influences—the reduction of mycorrhizal short roots. This reduction was usually accompanied by alterations of the normal bifurcate mycorrhizae into simple pedunculate or clovate short roots of a "pseudomycorrhiza" type (Figure 2). In some cases, especially frequent in the presence of calomel and benzene hexachloride, eradicants caused a complete castration of mycotrophic organs or their deformation into sessile swellings of a lobate pattern (Table 3).

The reason why the unfavorable alterations of the morphology of root systems and mycotrophic organs have not always depressed the growth of seedlings is understood considering the special conditions of greenhouse cultures or nursery beds. The periodic watering of closed containers or treatment of nursery beds with liquid fertilizers supply root systems with nutrients in the form of solution and thus eliminate the need for the solid phase feeding. The intensity of the latter process under natural conditions is directly related to the size of the absorbing surface of roots and the participation of symbiotic fungi.

The study in its entirety suggests that the use of eradicants must be paralleled by a search for ameliorating buffering substances which would reduce the biocide-caused deterioration of plants, beneficial soil organisms, and soil fertility. This task is of a greater importance in the production of forest nursery stock than it is in the production of any other crop. Extermination by drastic means of the undesirable forms of soil life is only a part of nursery management program; another and the essential part of such a program is the production in the same soil of healthy and vigorous planting material destined to form the future forests.

Literature Cited

Cholodny, N. G. 1951. Soil atmosphere as a source of organic nutrient substances for plants. Pedology, 1:16–29.

Clark, H. F., et al. 1951. The membrane filter in sanitary bacteriology. Public Health Reports. 66:951–977.

HECK, A. F. 1929. A method for the determination of total carbon and also for the estimation of carbon dioxide evolved from soils. Soil Sci. 23: 225–232. Illus.

MEHLICH, A., E. TRUOG, and E. B. FRED. 1933. The *Aspergillus niger* method of measuring available potassium in soil. Soil Sci. 35:259–279.

PERSIDSKY, D. J. and S. A. WILDE. 1954. The effect of volatile substances released by soil, humus, and composts on the growth of excised roots. Plant Phys. 29:484–486.

RICHARD, F. 1945. Der biologische Abbau von Zellulose-und Eiweiss- Testschnüren im Boden von Wald- und Rasengesellschaften. Mitt. d. Schweiz. Anst. F. forst. Versuchswesen. 24:295–397.

VOIGT, G. K. 1953. The effects of fungicides, insecticides, herbicides, and fertilizer salts on the respiration of root tips of tree seedlings. Soil Sci. Proc. 17:150–152.

WILDE, S. A. and C. J. Krumm. 1946. Allowable concentration of commercial fertilizer salts in composts used in forest nurseries. Jour. For. 44:662–665.

WILDE, S. A. and G. K. VOIGT. 1949. Absorption-transpiration quotient of nursery stock. Jour. For. 47:643–645.