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Effects of Soil Treatments on the Survival of Soil Microorganisms[1]

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Abstract: In biological control studies of plant-parasitic nematodes in field soil improved methods are needed for reducing or eliminating specific soil inhabiting microorganisms. Microwave heating of soil decreases soilborne fungi and bacteria, but not *Pasteuria* spp. Bacterial and fungal colony forming units were reduced to nondetectable levels in microwaved heated field soil (650 watts) at 5.2% moisture when treated for 6 minutes and 4 minutes, respectively.

Many physical and chemical methods have been developed to eliminate or reduce populations of soilborne plant pathogens for phytopathological studies (8). Treating soil with fungicides or irradiation have been used in the evaluation of nematode suppressive soil (2,4,5–7,9). Microwave heating of soil may be useful in eliminating soilborne fungi with limited reduction of prokaryotic organisms (3). Subjecting soil to autoclaving or steam heating is useful for killing both soilborne fungi and prokaryotic organisms (8). Our objectives were to evaluate the effects of microwave heating, formalin, autoclaving, and storage at room temperature on reducing soil bacteria and fungi in soil used for evaluating the effects of fungal antagonists of plant-parasitic nematodes.

MATERIAL AND METHODS

Microwave heating: Soil was taken from six plots with a bucket auger (10-cm-d) on 30 May 1992 in a soybean field infested with *Heterodera glycines* at the University of Florida Agronomy Farm, Green Acres, Alachua County, Florida. The soil texture was 90.5% sand, 5.3% silt, and 4.2% clay; the soil contained 1.6% organic matter at pH 5.7. The soil was passed through a 6-mm-aperture sieve and mixed, and soil moisture was measured before treatment. The soil was either treated with microwave heating (3) for 2, 3, 4, 5, or 6 minutes, or left untreated. For the microwave-heated treatment, 1-kg lots of soil were placed in even layers (2 cm deep) in open plastic bags and heated individually in a microwave oven (650 watts). The densities of prokaryotes and the densities of fungi in the soil samples were determined by plating serial dilutions (1:300, 1:3,000, and 1:30,000) of soil suspensions in sterilized water on potato dextrose agar (PDA) and PDA containing 100 mg of streptomycin, 50 mg of chlortetracycline, and 1.0 ml of tergitol/liter of medium (10). Each dilution was replicated three times.

Microwave heating and other methods: Microwave heating of soil for 4 or 6 minutes as described above, autoclaving, formalin, storage treatments, and a control (untreated), were compared. Soil was taken on 17 June 1992 from the same field as described above. The soil was sieved and mixed, and the moisture was determined. The soil was autoclaved once at 120 C at 1.4 kg/cm^3 for 20 minutes. For the formalin treatment, 40% formaldehyde was applied at a rate of 3.8 ml/liter of soil, after which the soil was sealed in a plastic tray with plastic wrap and maintained for 3 days in a greenhouse (23–26 June). Following treatment, the soil was placed in 15-cm-d pots in the greenhouse and drenched with water daily for 10 days (26 June–6 July). For the storage treatment, soil was

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TABLE 1. Effects of soil treatments on survival of soil microorganisms.

Soil treatment		Soil moisture (%)	Bacteria (10^6 cfu/g of soil)[a]	Fungi (10^4 cfu/g of soil)[b]
			Microwave heated	
Untreated		3.3	1.42	2.91
Microwave heated (minutes)	2	3.3	1.48	0.30
	3	3.3	0.38	0.09
	4	3.3	0.33	0.012
	5	3.3	0.021	0
	6	3.3	0.021	0
			Microwave heated and other methods	
Untreated		5.2	1.68 b	1.93 c
Microwave heated (minutes)	4	5.2	0.022 c	0 d
	6	5.2	0 d	0 d
Autoclaved		5.2	0 d	0 d
Formalin-treated		5.2	41.67 a	6.82 a
Stored at 23–24 C for 14 days		5.2	0.91 b	2.56 b

Data are means of three replicates. The data were transformed to $\log_{10}(x + 1)$ values before statistical analyses. In microwave-heated soil, the densities of bacteria or fungi are correlated negatively with time of exposure to microwave heating according to linear regression ($r = -0.90$ for bacteria, $r = -0.96$ for fungi; $P \leq 0.05$). The same letters in columns indicate no significant differences at $P \leq 0.05$ according to Duncan's multiple-range test.

[a] Population in colony-forming units (cfu)/g soil determined after 2 days of incubation on potato dextrose agar (PDA).

[b] Population in cfu/g soil determined after 7 days of incubation on PDA containing 100 mg of streptomycin, 50 mg of chlortetracycline, and 1 ml of tergitol/liter of medium.

stored at room temperature (23–24 C) for 14 days. The densities of prokaryotes and fungi were determined according to the procedures described above.

The data were transformed to $\log_{10}(x + 1)$ values before statistical analyses. The densities of bacteria or fungi were regressed on time of exposure of the soil to microwave heating. The densities of bacteria and densities of fungi in soil among treatments were compared with Duncan's multiple-range test.

RESULTS AND DISCUSSION

Populations of the bacteria and fungi in soil decreased with the time of exposure to microwave heating ($P \leq 0.05$, Table 1). No bacterial colony forming units were detected in soil treated for 6 minutes when the soil moisture was 5.2%. No fungi were detected in soil treated with microwave heating for 5 minutes at a soil moisture of 3.3%, or for 4 minutes at 5.2%. No bacterial or fungi were recovered from autoclaved soil. The number of bacterial colonies increased 24-fold and the fungal colonies increased 3.5-fold in soil following formalin treatment compared with untreated soil ($P \leq 0.05$). The dominant fungal species encountered in the soil treated with formalin were *Trichoderma* spp. Storage of soil at 23–24 C for 14 days had little effect on total bacterial and fungal densities (Table 1).

These results indicate that microwave heating is probably the best among the treatments tested for reducing population densities of fungi in soil when needed for evaluating the effects of fungal antagonists on nematode population densities in soil. Also, microwave heating may be useful for separating the roles of fungal antagonists and *Pasteuria* spp. in soil suppressive to plant-parasitic nematodes. The time required for effective treatment with microwave heating varies with soil moisture, and output of the microwave oven (3). The microwave heating treatment of 4 minutes (soil moisture 5.2%) was successfully used in our studies on the effects of mycoflora on the population development of *H. glycines* (1).

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