Effects of Small-Scale Aerobic Composting on Survival of Some Fungal Plant Pathogens

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ABSTRACT

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Aerobic composting for 21 days eliminated Armillaria mellea, Rhizoctonia solani, and Verticillium dahliae from colonized plant residues and killed Sclerotium rolfsii sclerotia sealed in glass vials or mesh bags. For eradication of the three pathogens protected in plant material and the sclerotia sealed in glass vials, the material containing the pathogens had to be turned with the compost every 2 or 3 days to expose the pathogens to the high temperatures in the center of the compost pile. S. rolfsii sclerotia in mesh bags, however, were killed even when exposed to sublethal temperatures in the corners of the compost bin. Inactivation of these sclerotia required various lengths of exposure depending on location and time of burial during the composting process. Temperatures in the center of the 0.7-m³ bin reached as high as 70 C, with temperatures above 45 C lasting 10-14 days. Temperatures in the corners did not reach levels lethal to the pathogenic fungi.

Composting, the controlled decomposition of organic material, has been widely used as a technique to stabilize and reduce large amounts of organic material for return to the soil in a less voluminous form. Under anaerobic, ie, limited oxygen, conditions (3), composting can be a slow process, requiring months for completion. If, however, decomposition is aerobic, ie, in the presence of oxygen (3), composting can be completed in 2-3 wk. Aeration necessary for composting to proceed rapidly is provided by turning the composting materials at 1- to 3-day intervals. Because metabolic heat generated during decomposition accumulates, frequent turnings also prevent temperature levels from becoming so high that compost microflora are destroyed. Through research aimed at refining

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composting as a method for treating solid municipal wastes, other conditions necessary for effective aerobic composting have been found, including a carbon to nitrogen ratio of 30:1, a water content of 65%, a relatively small particle size (3), and a minimum volume of a cubic yard

Several researchers have reported composting to be an effective method for eradicating some plant pathogens in diseased material. Hoitink et al (4) found that Phytophthora cinnamomi, Pythium irregulare, Rhizoctonia solani, Botrytis cinerea, and Erwinia carotovora var. chrysanthemi were eliminated from various plant materials composted along with hardwood bark. Eradication of Verticillium albo-atrum from hops (15) and cotton wastes (16) and of Didymella lycopersici from tomato stems (12) by the composting of these crop residues has also been reported. In contrast, Xanthomonas malvacearum survived treatment in all locations within a compost pile of cotton gin trash (17). In the same study, V. dahliae and Alternaria sp. could not be eradicated from the outer regions of the compost pile.

Because of its rapidity, the aerobic composting method is gaining popularity with home gardeners. However, there is a lack of information on the efficacy in pathogen eradication of short-term, ie, 3 wk, composting using the smaller volumes of plant material generated in a home garden. The objective of this study was to test the effect of small-scale aerobic composting on the survival of certain plant pathogens. Preliminary results have been reported (18).

MATERIALS AND METHODS

Composting was done in wooden bins 0.9 m high, 0.9 m deep, and 0.9 m across. Two types of materials were composted. The first was a mixture of dried spice plant residues provided by Spice Island Farms in Vacaville, CA, and softwood (redwood, pine, or fir) sawdust. The three types of softwood showed no differences in composting or antimicrobial properties in preliminary tests. The spice fraction was dill, rosemary, and sage, with dill making up over 75%. Sawdust and spice plant residues were combined in proportions to give a total carbon to nitrogen ratio of 25:1-30:1. The carbon and nitrogen contents of the materials were determined by the Walkley-Black method for carbon analysis (6) and the Kieldahl method for analysis of total nitrogen (1). Water was added to the final mixture to give a moisture content of 65-75% by dry weight.

The second type of material consisted of garden refuse: prunings, grass clippings, dried weeds, and fallen leaves. The proportions varied with each experiment, and carbon to nitrogen ratios were not determined. An attempt was made, however, to start composting with equal amounts of fresh green

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material (grass clippings, green leaves) and naturally dried material (fallen leaves, woody prunings, straw). Such proportions were reported by Raabe (13) to give a carbon to nitrogen ratio of approximately 30:1. Enough water was added at the start of composting to wet the material thoroughly, and water was added during the process when the material started to dry.

Every 2 or 3 days, the compost was turned from one bin into an adjacent empty bin. With each turning, the materials on the outside of the pile were moved to the center. Temperature was taken daily with a thermometer or thermocouples and recorded continuously on a Honeywell strip chart recorder.

Four pathogens were tested for survival through composting: R. solani, Armillaria mellea, V. dahliae, and Sclerotium rolfsii. The first three were introduced into both compost mixtures in colonized plant material; S. rolfsii was incorporated as sclerotia and was composted only in the spice-sawdust mixture. The sclerotia and colonized plant parts either were distributed evenly throughout the compost bins and turned along with the compost or were left continuously in the corners of the bins. Some material was kept in the laboratory as untreated checks.

 $R.\ solani$ was isolated from a stem rot on Senecio mikanioides Otto and allowed to colonize autoclaved millet seed. The seeds were bagged for composting in 2×3 cm polyester mesh $(34\times 38\ strands/cm)$ bags. After recovery following composting, the seeds were plated on water agar for 3 days at 25 C, and the plates were examined daily for growth of $R.\ solani$ hyphae.

A. mellea, isolated from pear from Watsonville, CA, was allowed to colonize cherry branches 2-4 cm in diameter and 6 cm long. Before composting, small sections were cut from each length, surface-sterilized in 0.5% NaClO for 5 min, and cultured on potato-dextrose agar slants containing 15 ppm of benomyl (14) and 100 ppm of streptomycin sulfate to assay the fungus in each piece. After composting, survival of the fungus in the wood was tested by the same procedure. All cultures were incubated for at least 21 days at room temperature before being examined for growth of the fungus.

Rose stems infected with V. dahliae and averaging 0.5 cm in diameter were cut into 6-cm lengths. The stems were cultured both before and after composting. Pieces of stem were surface-sterilized in 0.5% NaClO for 2 min and plated on cellophane agar (5). After incubation for 14 days at room temperature, the cultures were examined for formation of microsclerotia

S. rolfsii (isolate "Texas") was grown on autoclaved cornstalks for 1 mo at room temperature, then sclerotia were harvested, dried, and stored. When put

into the compost material, sclerotia were enclosed in bags of the same mesh material used to contain the *Rhizoctonia*-infested millet seed or in 2-ml glass vials. The vials were sealed with Parafilm before being closed with screw caps to ensure watertightness and airtightness. Twenty to 40 sclerotia were included in each container. After composting, 20 sclerotia from each container were plated on water agar, and the number germinating were counted after 5 days of incubation at 30 C.

RESULTS

Eradication of pathogens depended on their location within the compost pile. A. mellea, V. dahliae, and R. solani and S. rolfsii sclerotia in glass vials were completely inactivated after 21 days of treatment in either compost mixture when incorporated into the compost and moved throughout the bin by turning. When kept in the corners of the bin, these pathogens remained viable throughout the composting process. However, S. rolfsii sclerotia contained in mesh bags lost viability even when composted exclusively in the corners of the bin (Table 1).

S. rolfsii sclerotia were inactivated at different rates according to when they

were placed into the compost pile. Bags of sclerotia were placed in one corner of the bin, with one set of bags buried every 3 days. At 3-day intervals, five bags from each set were retrieved and the sclerotia tested for viability. Before treatment, the sclerotia germinated at a rate of 96%. Samples buried 2 and 5 days after initiation of the composting process required 9 days of treatment before germination was reduced to 10% or less. Samples buried day 8 through day 17 of the composting process reached that level of inactivation in 3-6 days of treatment. Temperatures measured in the corner during this experiment did not exceed 32 C (Table 2). In a similar assay of the viability of mesh-bagged sclerotia placed at 3-day intervals in the center of the bin, sclerotia were completely inactivated within 3 days after burial regardless of when they were placed in the compost.

Temperatures varied within the compost bin. In one bin, temperatures in the center rose rapidly the first 3 days, reaching as high as 70 C. Temperatures above 50 C were maintained for 10-14 days, then gradually dropped toward ambient. Temperatures measured in one corner of the bin remained close to ambient throughout the experiment without reaching 30 C (Fig. 1). One day

Table 1. Viability of Armillaria mellea, Verticillium dahliae, and Rhizoctonia solani in plant residues and Sclerotium rolfsii in mesh bags or glass vials after 21 days of treatment in compost piles made of spice-sawdust mixture or garden refuse

Treatment	Proportion of residue samples containing viable pathogen ^a			Proportion of viable S. rolfsii sclerotia germinated ^b		
Compost material	A. mellea	V. dahliae	R. solani	Mesh bags	Glass vials	
Turned with compost						
Spice-sawdust	0/20	0/15	0/81	0.00	0.00	
Garden refuse	0/26	0/15	0/25	¢	•••	
Left in corner of pile			,			
Spice-sawdust	3/5	5/5	•••	0.00	0.78	
Garden refuse	5/7					
Untreated ^d	,					
Spice-sawdust	5/5	5/5	10/10	0.80	0.80	
Garden refuse	7/7	6/6	10/10	•••		

^a A. mellea in cherry wood, V. dahliae in rose stems, and R. solani in millet seed. All residue samples contained viable pathogens before treatment.

Table 2. Percent germination of *Sclerotium rolfsii* sclerotia buried in one corner of a compost pile on different days after initiation of composting and retrieved after varying lengths of time^a

No. of days in compost	No. of days after initiation of composting when sclerotia were buried									
	2	5	8	11	14	17	20			
-3	20	100	89	54	1	11	84			
6	34	88	9	0	0	1				
9	4	8	0	0	0	•••	•••			
12	0	0	0	0						
15	0	0	0	•••						
18	0	0								
21	0									
Temperature										
in corner (C)	32	24	19	20	19	21	21			

^a Sclerotia were in polyester mesh bags. One set of bags was buried every 3 days, and five bags from each set were retrieved from compost every 3 days. Germination of 20 sclerotia from each bag was measured by incubation on water agar for 5 days at 30 C. Germination rate before treatment was 96%.

^bValues are means of 8-10 replicates. Proportion of sclerotia germinating before experiment was 0.83.

[°]Not tested.

^dUntreated samples left in laboratory at room temperature during each composting experiment.

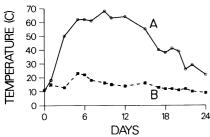


Fig. 1. Temperatures measured during aerobic composting in (A) the center and (B) one corner of a compost bin.

when heat levels were at their highest in this bin, temperature measurements were taken at 65 equally spaced points; only 55% of the total volume of the compost reached 50 C or higher. Such high temperatures were restricted to the center portion of the compost. Similar temperature trends were recorded in the other compost bins.

DISCUSSION

Eradication of the organisms in these experiments can be attributed mostly to the high temperatures generated by microbial metabolism. Temperatures measured in the center of the small, rapid compost piles were as high as those reported in other studies describing composting under larger scales. When left in the center of a rapid compost pile for only one day within the 10-14 days of peak heat generation, the pathogens were exposed to temperatures and durations far exceeding those necessary for thermal kill. In other studies, V. dahliae was killed by a 10-min exposure to 50 C heat (9), whereas A. mellea required 30 min (8) and R. solani 60 min at 50 C (10). In preliminary experiments, S. rolfsii sclerotia did not survive a hot water (50 C) treatment for 40 min.

Temperatures lethal to fungal pathogens are severely limited spatially in small compost piles. This is reflected in the survival of the pathogens through composting when plant materials containing mycelia or glass vials containing sclerotia were left undisturbed in the corners of the compost pile.

Eradication of the pathogens may also involve mechanisms other than heat, as demonstrated by the loss in viability of S. rolfsii sclerotia in mesh bags composted exclusively in the corners of the bin where no lethal temperatures were recorded. These mechanisms may include the production of toxic substances during organic matter decomposition and microbial antagonism. Microbial antagonism around exposed sclerotia may have been intensified by the leakage of nutrient materials from the sclerotia that occurs with exposure to sublethal heat (7) or with rewetting of dried sclerotia (2). Inactivation of sclerotia in mesh bags at sublethal temperatures did not reach maximum levels until composting had progressed for over a week. This lag period was perhaps required for microbial antagonism and production of toxic byproducts to reach lethal levels. Pathogen eradication by these processes may require that the pathogen be directly exposed. S. rolfsii in glass vials and A. mellea and V. dahliae in woody tissues were protected and thus survived composting at sublethal temperatures.

Rapid composting in which periodic turning of the materials exposes all parts of the pile to high temperatures during the composting process is effective in killing disease-producing organisms. Even with turning, the outer portion of the composting material does not reach temperatures lethal to disease-producing organisms. This suggests that in the slower method of composting in which turning is not used, plant refuse containing disease-producing organisms should not be used.

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