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Microbial Aerosols and Actinomycetes in Etiological Considerations of Mushroom Workers' Lungs

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Spent steamed compost, phase II compost, and dust emanating from spent compost during dumping of stationary-bed mushroom houses were examined bacteriologically. The total count for spent compost was 16×10^8 microorganisms per g. The total count for dust was 333 microorganisms per liter of air. Actinomycetes belonging to the genus *Streptomyces* often constituted 90% or more of isolates from dust, whereas mold spores constituted approximately 5%. Dust weight averaged 3.4 mg/liter of air and contained approximately 33% inanimate and 67% animate (microbial) particles. Spent compost and casing contained approximately 60% moisture; the average pH of compost was 6.93, and that of casing was 7.70. Ouchterlony precipitin results with antisera from workers afflicted with either farmer's ormushroom worker's lung were positive for *Bacillus licheniformis*, *Micropolyspora faeni*, *Thermoactinomyces vulgaris*, *Aspergillus fumigatus*, *Humicola grisea* var. *thermoidea*, spent compost, and phase II compost. Their usefulness in determining the etiology of this and related forms of allergic alveolitis is questioned and discussed. The relationship of dust particle size; microbial species, prevalence and antigenicity; and compost antigenicity to the etiology of mushroom worker's lung is discussed. The microbial ecology of mushroom compost and moldy hay associated with farmer's lung is compared.

Mushroom worker's lung, like farmer's lung, is a form of extrinsic allergic alveolitis. However, its etiology is poorly understood. It is known that farmer's lung is caused by inhalation of spores of the thermophilic actinomycetes, *Micropolyspora faeni* and *Thermoactinomyces vulgaris* (18), and it seems likely that actinomycetes are implicated in mushroom worker's lung (14). However, the species implicated have not been established beyond doubt.

Mushroom worker's lung and farmers' lung are clinically alike. Symptoms include malaise, sweating, chills, anorexia, breathlessness, and a sense of tightness in the chest. The accompanying cough is usually distressing and often nonproductive. Weight loss may occur. Chest X-rays may show diffuse micronodular densities, and pulmonary function may be impaired. In the microbiology laboratory, sputum specimens and lung biopsy are for the most part negative for microorganisms, whereas serum precipitin tests are sometimes positive for thermophilic actinomycetes (*M. faeni* and *T. vulgaris*) and fungus (*Aspergillus fumigatus*) (13). In farmer's lung, serum precipitins against *M. faeni* and *T. vulgaris* are usually present, but they have been found only occasionally in mushroom workers (19).

Previous studies suggest that mushroom worker's lung is most commonly associated with spawning operations (3, 4, 12, 19, 21, 22). However, in the United States, dust released when the spent compost is dumped may be important (2, 17). Mushrooms may be grown either in stationary beds or in portable trays. With the former, most processing is manual, with the worker close to the compost in a confined atmosphere in the mushroom house. By contrast, portable trays can be moved outside by forklift truck for processing. About 70% of the mushrooms in North America are grown in stationary beds (P. Greenlee, personal communication).

This study sought to establish the role of airborne microorganisms in mushroom worker's lung, to identify the species implicated (J. G. Kleyn and T. F. Wetzler, submitted for publication), and to study the nature of compost and dust produced by the dumping of spent compost.

MATERIALS AND METHODS

Collections of compost and dust samples. Samples of spent compost and dust were collected from six and seven stationary-bed mushroom houses, respectively, during the period of June through September 1977. There were four phase II compost samples col-

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lected during the period July through November 1978. Compost samples were collected from three locations in the mushroom house just before initiation of the dumping operation (Fig. 1): a front bottom shelf, a center top shelf, and a back bottom shelf. At each location, three 100-g samples were collected, one each from the top casing layer, the top layer of compost, and the bottom layer of compost. Each phase II compost sample was a composite of the casing, top, and bottom compost layers. Samples of compost dust were collected with a portable air sampling unit (MSA Model G portable air sampling unit; Mine Safety Appliance Co., Pittsburgh, Pa.) containing a 38-mm diameter, 0.45-μm membrane filter, in an area 4 to 8 feet (ca. 122 to 244 cm) from the workers who forked spent compost onto a conveyor belt. Samples for microbiological analysis were obtained at a flow rate of 2 liters per min with a 15-min collection period. Usually, two units were operated simultaneously, enabling collection of duplicate samples. For determining the amount of dust present in the air, a third sampler was used, and the collection period was extended to 1 h.

Immediately after collection, membrane samples for microbiological analysis were transferred aseptically to 50-ml screw-cap test tubes containing 10 ml of 0.1% peptone broth for transport.

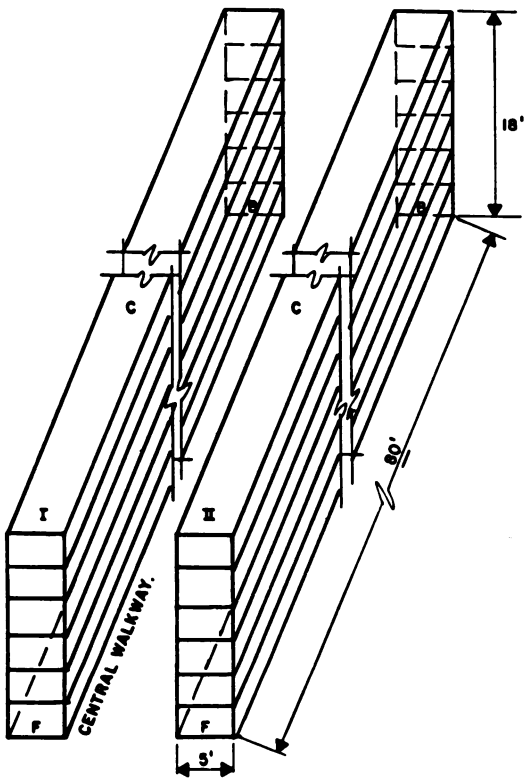

FIG. 1. *Diagram of bed and shelf arrangement inside stationary-bed mushroom house, showing compost sampling locations. I and II, Beds 1 and 2, respectively; F, C, and B, front, center, and back sampling locations, respectively.*

Microbiological assay of compost and dust samples. Dilution plating methods were used for isolating and quantifying the microorganisms present in each sample. Representative unknown microorganisms were submitted to experts for identification.

Direct microscopic examination of membrane filter samples. After determining the dry weight of dust on a membrane, central and outer sections of the membrane were placed on a clean slide. Immersion oil cleared the membranes. The ratio of spore to dust particles was determined after examination of 15 microscopic fields.

Ouchterlony precipitin serology. (i) Sera. The following sera were examined undiluted and at 1:0 dilutions: (a) antiserum (MM) from mushroom worker's lung; (b) antisera from farmer's lung patients (2424 and 2673) from D. W. R. Mackenzie, London School of Hygiene and Tropical Medicine, London, England; and (c) two serum controls (OX358 and PB320A) from George Kenny, University of Washington, Seattle.

(ii) Antigens. The following antigens were examined: (a) *T. vulgaris* (lot no. 0018101, Hollister Stier Laboratory, Spokane, Wash.); (b) *M. faeni* (lot no. 874001, Hollister Stier Laboratory, Spokane, Wash.); (c) *A. fumigatus, Bacillus licheniformis, Humicola grisea* var. *thermoidea, Streptomyces diastaticus,* and *Thermomonospora* spp.; (d) extract of mushroom compost from the end of phase II of composting; and (e) extract of steamed spent mushroom compost.

Fungi were incubated at 30°C on peptone glucose agar (8), and the bacteria and actinomycetes were incubated at 45°C on compost infusion agar (8) for 1 week. The growth from each plate was suspended in 1.5 ml of 0.2% Triton-X and 1.5 ml of 0.25% sodium deoxycholate. After placement in an ice bath, they were sonicated with a Bronwill Biosonik III for 5 min with 15-s bursts at 20 kc.

Mushroom compost extracts (d and e above) were prepared by first dehydrating 100 g of compost sample overnight in acetone; the sample was then extracted with 50 ml of Coca's solution (0.85% NaCl–0.5% phenol adjusted to pH 7.1 with 1 N NaOH). They were agitated for 1 week on a reciprocating shaker at room temperature. After shaking, the extracts were decanted and centrifuged at 10,000 rpm for clarification and then lyophilized. A 25-mg amount of the powdered supernatants was suspended in 1 ml of distilled water for the final extract.

The Ouchterlony technique employed was a slide modification which used minimal amounts of reactants and demonstrated high sensitivity to the antigens (6).

RESULTS

Quantitative analysis of microorganisms present in compost and dust. The composite average total count for spent steamed compost was 16×10^8 microorganisms per g (dry weight) and for the casing layer, 41×10^8 microorganisms per g (dry weight) (Table 1). The composite average total count for dust emanating from spent compost during dumping was 353 microorganisms per liter of air (Table 2). Of this

amount, approximately 94% were procaryotes and 6% were eucaryotes. Upon observing compost infusion agar plates, it was not uncommon to find actinomycete colonies comprising 90% or more of the total microbial population. The highest count obtained for individual samples was 6,000 microorganisms per liter of air.

Predominant isolates from phase II and spent compost. This subject is detailed in a related manuscript (Kleyn and Wetzler, submitted for publication). Predominant isolates from phase II compost included *B. licheniformis*, *T. vulgaris*, and *H. grisea* var. *thermoidea*. Predominant isolates from spent compost and dust included *B. licheniformis*, *S. diastaticus* (syn. *S. griseoflavus*), and *A. fumigatus*. *M. faeni* was not isolated.

A direct microscopic examination of the membrane showed that approximately 33% of the dust was composed of inanimate particles and 67% was composed of microbial spores (see Fig. 2a and b). It was difficult, due to their small size, to distinguish accurately bacterial spores, but of the mold and actinomycetal spores, approximately 3% were molds and 97% were actinomycetes. The size of most of the inanimate particles was 5 μm or greater. The average size of spores was as follows: *A. fumigatus*, 2.38 μm; *T. vulgaris*, 0.85 μm; and *S. diastaticus*, 0.8 × 1.1 μm. The average dust weight was 3.5 mg/liter of air sampled.

TABLE 1. *Total counts of spent casing and compost samples plated on compost infusion agar and peptone glucose agar*

Plating medium	Avg total count (× 10^8/g [dry wt])[a]		
	Casing	Top compost	Bottom compost
Compost infusion agar	60.4	20.1	14.6
Peptone glucose agar	22.2	12.7	16.1
Composite avg	41.3	16.4	15.3

[a] Each result represents an average of six samples obtained from six stationary-bed mushroom houses.

Moisture and pH of compost and casing samples. Results for the six mushroom houses examined showed that the composite average moisture content of spent compost was 62%, and that of the casing layer was 59% (Table 3). Moisture levels for individual samples ranged from 43 to 85%. The average pH of casing was 7.70, and that of compost was 6.93. The pH of the casing layer ranged from 7.54 to 7.80, and that of the compost layer ranged from 6.42 to 8.60.

Antigenic analyses of mushroom compost and predominant microbial isolates. Negative Ouchterlony reactions were obtained for all five antisera when *S. diastaticus* was the test antigen (Table 4 and Fig. 3). *Thermomonospora* spp., another actinomycete commonly found in mushroom compost, was also negative (see Fig. 3, 3a).

Positive Ouchterlony reactions were obtained for *B. licheniformis*, *T. vulgaris*, and *M. faeni* when mushroom worker's lung antiserum was used (Table 4 and Fig. 3, 1c, 1d, and 3c). However, two of the organisms, *T. vulgaris* and *M. faeni*, also evoked positive reactions with negative control antisera (OX358 and PB320A). The most intense reactions were obtained when *M. faeni* was tested against antisera of workers diagnosed as having farmer's lung (2424 and 2673) (Fig. 3, 4b).

Weak postive Ouchterlony reactions were obtained when *A. fumigatus* and *H. grisea* var. *thermoidea* antigens were tested against mushroom worker's lung antiserum (Table 4). One of the negative control antisera (PB320A) was also positive with *A. fumigatus*.

A strong positive Ouchterlony reaction occurred when phase II compost was tested against mushroom worker's lung antiserum (Table 4 and

TABLE 2. *Frequency of procaryotic (actinomycetes and bacteria) compared with eucaryotic (molds) cells in dust*

House no.	Sampling date (1977)	Microorganisms per liter of air			
		Avg no.[a]		%	
		Procaryotes	Eucaryotes	Procaryotes	Eucaryotes
13	10 June	50	0.08	99.8	0.2
19	6 July	118	8	93.7	6.3
22	16 July	308	2.5	99.2	0.8
8	29 July	75	29	72.1	27.9
13	7 September	1,696	13	99.2	0.8
20	3 October	115	2.9	97.5	2.5
7	26 October	53	1.7	96.9	3.1
Composite avg		345	8	94.1	5.9

[a] Results are a composite of 49 dust samples collected from seven houses.

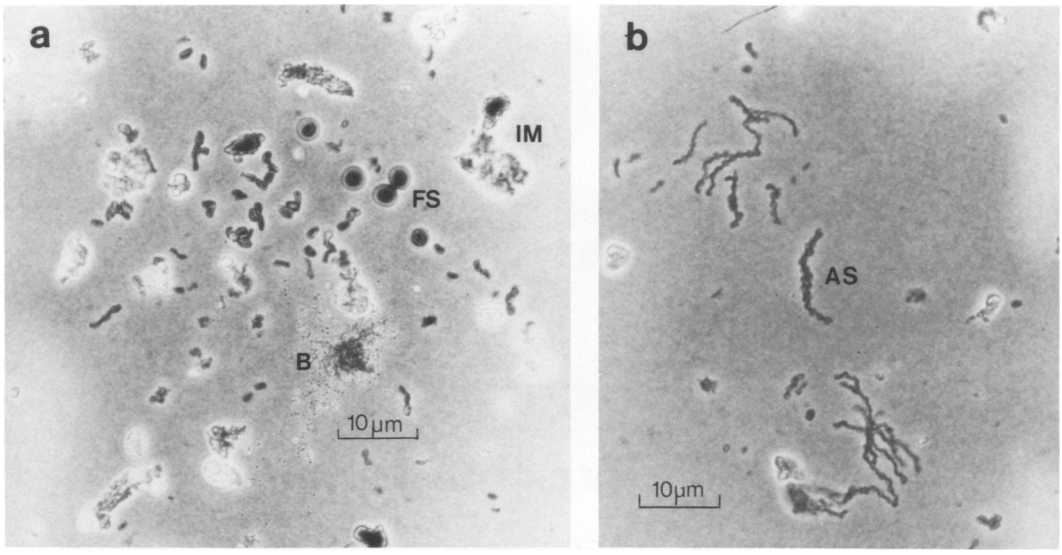

FIG. 2. *Direct microscopic examination of dust on the surface of a membrane filter. Zeiss ultraphotomicroscope with neofluor 40× phase objective (×400). AS, Chain of actinomycete spores; B, bacteria; FS, fungal spores; IM, inanimate matter. Note chains of actinomycete spores in (b) and large size of particles of inanimate matter in (a). Also note low frequency of fungal spores as compared with actinomycete spores.*

TABLE 3. *Moisture content of casing and compost samples collected from stationary-bed mushroom houses*

Stationary-bed house no.	Sampling date (1977)	Moisture (%)[a]											
		Casing				Top compost				Bottom compost			
		F	C	B	Avg	F	C	B	Avg	F	C	B	Avg
13	13 June	83				70				53			
19	1 July	43	65	57	55	61	67	65	64	62	65	57	61
22	15 July	65	69	55	63	67	71	62	67	72	71	61	68
8	29 July	44	71	55	57	51	65	56	57	63	60	51	61
13	1 September	44				46				50			
20	27 September	61	69	50	60	63	67	50	60	62	60	56	59
Avg		57	69	54		60	68	58		60	64	58	
Composite avg					59				62				62

[a] F, Front bottom shelf; C, center top shelf; B, back bottom shelf.

TABLE 4. *Antigenicity of mushroom compost and its predominant microbial isolates*

Antigen	Ouchterlony reaction intensity with antiserum				
	MM[a]	OX358[b]	PB320A[b]	2424[c]	2673[c]
B. licheniformis	+	−	−	++	−
S. diastaticus	−	−	−	−	−
T. vulgaris	++	++	++	+	−
M. faeni[d]	+	+	+	++++++	+++++
A. fumigatus	+	−	+	+	−
H. grisea var. *thermoidea*	+	−	−	−	−
Phase II compost	+++	++	+	++	++
Spent compost	++	+	−	+	+

[a] Antiserum from mushroom worker's lung patient.
[b] Negative control antisera.
[c] Antisera from farmer's lung patients.
[d] Included since etiologically important in farmer's lung.

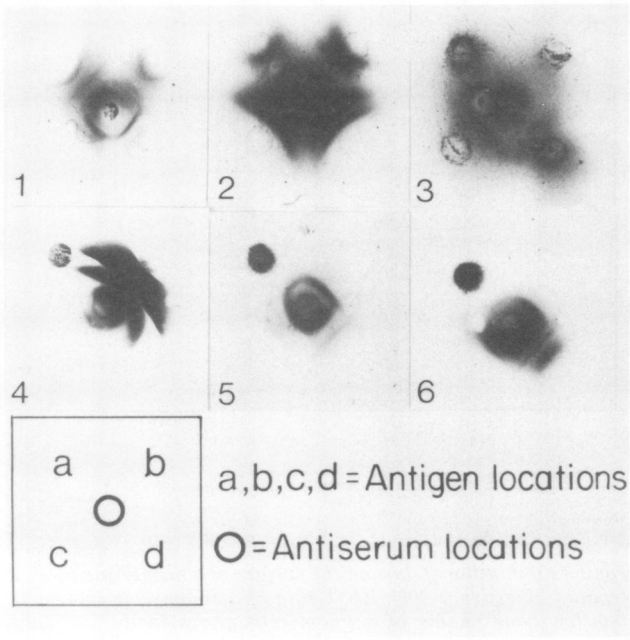

FIG. 3. *Ouchterlony precipitin reactions with mushroom worker's lung serum (MM), farmer's lung serum (2424), and control sera (PB320A and OX358). (1) MM with (a) phase II compost, (b) spent compost, (c) M. faeni, and (d) T. vulgaris. (2) MM with (a and b) spent compost and (c and d) phase II compost. (3) MM with (a) Thermomonospora spp., (b) S. diastaticus, (c) B. licheniformis, and (d) H. grisea var. thermoidea. (4) 2424 with (a) A. fumigatus, (b) M. faeni, (c) phase II compost, and (d) T. vulgaris. (5) PB320A with (a) A. fumigatus, (b) M. faeni, (c) T. vulgaris, and (d) phase II compost. (6) OX358 with (a) A. fumigatus, (b) M. faeni, (c) T. vulgaris, and (d) phase II compost.*

Fig. 3, 2c). Spent mushroom compost also evoked a strong Ouchterlony reaction, although somewhat weaker than that of phase II compost (Table 4 and Fig. 3, 2a).

DISCUSSION

Comparison of spore counts to those in moldy hay. Gregory and Lacey (11) reported that the outstanding characteristic of dust from farmer's lung hay was the large number of actinomycete spores, i.e., more than 6×10^7/g (dry weight). Lacey and Lacey (15) estimated that moldy hay from cow sheds contained as many as 1.6×10^9 spores per m^3 and that 98% of these spores were actinomycetes. The highest spore count of dust from spent mushroom compost was somewhat less than that described above (approximately 1.8×10^6 spores per g or 6×10^6 spores per m^3). Qualitatively, our results compare with theirs inasmuch as actinomycete spores in excess of 90% often occurred on dilution plates.

Gregory (10) estimated that the lung is normally exposed to 12,000 to 15,000 fungal spores per m^3 of inspired air. Our calculations show that this amount is only 0.2% of the concentration to which mushroom workers are exposed.

Retention of spores in the lung. During a spent compost dumping operation (approximately 2.5 h), a worker could inhale as many as 6.4×10^7 spores. Qualitatively, our results compare with theirs inasmuch as actinomycete spores in excess of 90% often occurred on dilution plates.

Altman and Dittmer (1) state that retention of particles within the lung is dependent on particle size. Lacey et al. (16) state that spores smaller than 10 μm penetrate to the lungs and that spores smaller than 5 μm penetrate to the alveoli. One would therefore expect *S. diastaticus*, *T. vulgaris*, and *A. fumigatus* spores to penetrate to the alveolar region of the lungs since their average spore sizes ranged from approximately 1 to 2.5 μm. One would expect many of the inanimate particles to be trapped in either the nasal passages or upper regions of the lung since most were 5 μm or greater in diameter.

Microflora of allergic alveolitis. Gregory and Lacey (11) reported that farmer's lung hay had many more spores of *Humicola lanuginosa* and *A. fumigatus* than did other hay. Large

numbers of fungi similar to those named above were present in mushroom compost. Fergus (8) reported finding *H. grisea* var. *thermoidea* more commonly than *H. lanuginosa*, whereas Deahl (K. Deahl, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, Q119, p. 213) supports Gregory and Lacey (11).

Gregory and Lacey (11) also stated that farmer's lung hays were specially characterized by many actinomycetes, and furthermore, thermophilic molds and actinomycetes predominated in farmer's lung hays. Dust from spent mushroom compost was characterized by a large actinomycete population which included many thermotolerant and thermophilic molds and actinomycetes.

Two microorganisms thought to be closely associated with farmer's lung are *M. faeni* (syn. *Thermopolyspora polyspora*) and *T. vulgaris* (syn. *Micromonospora vulgaris*). They evoked a positive precipitin test with mushroom worker's lung antiserum (Table 4). Whether or not *M. faeni* plays a prominent role in the etiology of mushroom worker's lung is questioned at this time since, at least in our studies, it was not isolated from mushroom compost. Other investigators (5, 8, 14) report finding it only on occasion. Deahl (personal communication) reports finding it in large numbers in his spent compost studies.

Moisture content and pH of farmer's lung hay and mushroom compost. Festenstein et al. (9) and Gregory and Lacey (11) showed that the antigenic activity of hay was dependent upon its moisture content. Hays with moisture contents of 29% or less contained very little farmer's lung hay antigen, whereas those with higher water contents (40 to 48%) contained more farmer's lung hay antigen. As the water content increased from 47 to 57%, the amount of farmer's lung hay antigens decreased, and at 68% water, only a very weak immunoelectrophoretic pattern was observed. These studies have shown that mushroom compost was antigenically active when tested against mushroom worker's lung and farmer's lung antisera. Spent mushroom compost as shown in these studies had a high average moisture content (61%). This finding suggests that spent mushroom compost should have a low farmer's lung hay antigen content. Our studies also have shown that a considerable range was experienced in the moisture content of spent mushroom compost so that it was often similar to that found for moldy hay with high farmer's lung hay antigen levels (Table 3).

Gregory and Lacey (11) found that the pH of farmer's lung hay was 7.0, whereas that of other hays was 5 to 6. We observed that the average mushroom compost pH was 6.94 and therefore approximated that of farmer's lung hay.

Clinical significance. Case reports have implicated both phase II and spent mushroom compost in the etiology of mushroom worker's lung (13). Our studies help to confirm the implications of etiology in selected allergic alveolitis. (i) Both spent and phase II compost evoked positive Ouchterlony precipitin reactions with serum from a worker afflicted with mushroom worker's lung. (ii) Support for phase II compost playing a more active role than spent compost in the etiology of this disease is as follows: (a) *T. vulgaris* was more predominant in phase II than in spent compost; (b) phase II compost evoked a stronger positive Ouchterlony precipitin reaction than spent compost with antiserum from a worker afflicted with mushroom worker's lung; and (c) *H. grisea* var. *thermoidea*, a new fungus found to evoke a positive Ouchterlony precipitin reaction with serum from a worker afflicted with mushroom worker's lung, was more predominant in phase II than in spent compost. (iii) We note that *B. licheniformis*, a bacterial species commonly found in compost, evoked a positive Ouchterlony precipitin reaction with a mushroom worker's lung serum. In this conjunction, enzymes of a closely related species, *Bacillus subtilis*, have been suggested as being responsible for allergic bronchial reactions (7).

The usefulness of precipitin reactions for determining the etiology of mushroom worker's lung as well as related forms of allergic alveolitis is questioned. Such reactions can be viewed as indirect evidence that workers have been exposed to airborne thermophilic molds or actinomycete spores in the work area. Whether or not the organisms studied here may be directly responsible for the development of clinical manifestations associated with the disease remains to be shown irrefutably.

The primary agent of mushroom worker's lung is unproven here. It could be due to *S. diastaticus*, the most common actinomycete found in dust, even though serologically negative in our study. It could conceivably be due to domestic mushroom viruses. It might be due to chemicals present in compost.

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