Occurrence and Severity of Verticillium Disease of Mushrooms Produced on Casing (Soil) Treated with Aerated Steam

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ABSTRACT

Colonization of aerated, steam-treated soil by *Verticillium malthousei* was hindered more in soil treated at 60 and 82 C than at 98 C. Spore germination and germ tube length were reduced in soil treated at these two lower temperatures. Disease was most prevalent and yield of healthy mushrooms lowest on soil treated at 98 C and seeded 3 days before casing. Disease occurrence varied directly and yield (both weight and number of mushrooms) varied inversely with soil treatment temperatures of 60, 82, and 98 C. Similarly, disease occurrence varied directly and yield varied inversely with inoculum density.

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Additional key words: fungistasis, dry bubble, re-colonization.

Casing is a top-dressing placed on compost colonized by mycelium of the commercial mushroom, *Agaricus bisporus* (Lange) Imbach, to induce the formation of sporophores (12). Soil used for casing is pasteurized or fumigated to remove mushroom pests. The recommended pasteurization treatment of casing with steam is 82.2 C (180 F) for 30 min (13).

The use of low temperature aerated steam for treatment of soil employed in certain commercial greenhouse operations, suggested that the value of such treatment for mushroom growing should be measured (3, 4, 5, 9, 18). Wuest et al. (15) reported that *Geotrichum candidum, Mycogone perniciosa, Trichoderma viride, Verticillium malthousei*, and *Agaricus bisporus* were eliminated when infested soil was treated at 54.4 C for 30 min. Moore (9, 17) confirmed the work of Wuest, et al. for *G. candidum, M. perniciosa, T. viride*, and *V malthousei* and included two additional fungi: viz. *Dactylium dendroides* and *Ostrachoderma* sp. Fifteen minutes was adequate to eliminate all of these fungi in most instances (9, 17).

Determining the thermal sensitivity of these fungi to aerated steam treatment concerned the elimination of the pathogen from casing. The effect of such treatment on one mushroom pathogen's ability to incite disease is essential to establish a link between the ideas of Baker & Olsen (3) and Baker (4) with greenhouse potting soil to mushroom casing.

V. malthousei causes a disease known as Verticillium spot or dry bubble on commercial mushrooms. The disease has been described (8, 14). The ability of *V. malthousei* spores to germinate and

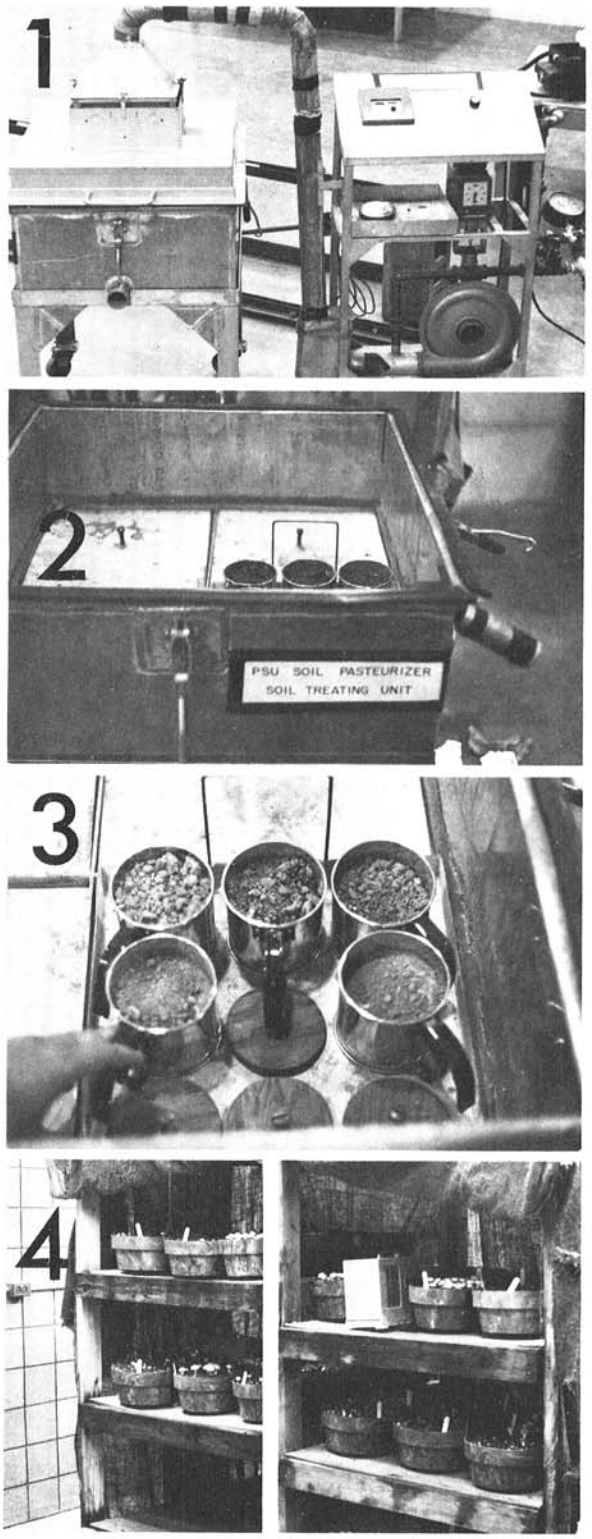

Fig. 1-4. 1) Aerated steam equipment used in treating soil; note aluminum box containing an Astrocel Hepa Filter above and affixed to plenum of soil treatment chamber. **2)** Interior arrangement of soil treatment chamber. **3)** A quandrant within soil treatment chamber showing circular one-cup chrome sifters containing soil plus circular wooden plug used to close-off 7.6-cm treatment section. **4)** Growth chamber used for yield studies with 25-cm plastic bulb pots in place.

grow, plus the ability of the pathogen to cause disease when introduced into casing treated with aerated steam, is reported in this study.

MATERIALS AND METHODS.—*Phialospore germination and growth. Soil treatment.*—Soil used in all experiments was a previously described Hagerstown silty clay loam (15).

Soil was sifted through a 0.64-, 1.27-, or 0.20-cm wire mesh sieve. Tap water was sprayed onto the soil after sieving to adjust the moisture content to approximately 19% moisture (w/w). Moisture content of soil was determined on the day of treatment.

Treatment with aerated steam was accomplished using a modified version (9) of equipment (Fig. 1, 2, 3) described by Aldrich & Nelson (1). Flour sifters heated overnight at 121 C were three-fourths filled with moist soil, and the soil treated at 60.0, 82.2, or 98 C for 30 min with aerated steam. Treatment temperature was monitored using a multi-point potentiometric strip chart recorder, with one copper-constantan thermocouple placed centrally in soil contained in each treatment chamber. After treatment, soil was placed into new polyethylene bags and used within 8 hr.

Slide preparation.—Water agar, 1.5%, was autoclaved and cooled to 42 to 44 C. The warm agar was poured into a sterile beaker and a phialospore suspension pipetted therein. Spore suspensions were prepared from 7-day-old *V. malthousei* cultures identified as C-PW-707 available at the Mushroom Laboratory of The Pennsylvania State University. A concentration of 2.0 to 2.5 $\times$ 10^6 spores/ml of water agar was prepared. Sterile microscope slides were dipped and swirled in the agar-spore suspension, removed and the agar coating allowed to solidify. Four or five coated slides were placed vertically in a glass jar and soil treated at one temperature poured into the jar to cover the agar surface ca. 6.5-cm deep. Jars containing the slides coated with the spore, water-agar combination were placed in an incubator to ascertain the ability of phialospores to germinate at 27 C in the dark. Slides were incubated for 24 or 72 hr, removed, and cleaned of soil particles by rinsing with water. Lacto-phenol solution (25%) was applied and a cover slip placed on the agar surface.

Measurements.—Spore germination at 500 X was ascertained from ten random fields, five each on the upper and lower half of the agar-slide surfaces. The same focal plane, ca. 0.15 units of the fine focus below the surface, was needed to count spores after which percent germination was calculated. A spore was considered germinated if a germ tube was observed.

Germ tube lengths were measured with the aid of an ocular micrometer. Whenever possible, five random germ tubes were counted per field assayed

TABLE 1. Percent germination of *Verticillium malthousei* phialospores incubated in soil previously treated with aerated steam at 60, 82, and 98 C

Incubation time	Soil treatment temperature (C)			
	Control	98	82	60
24 hr	64.5[a]	57.9	23.1	11.3
72 hr	61.2[b]	21.2	12.1	7.8

[a] Data is mean of three experiments; $LSD_{.01}$ = 39.3.
[b] Data is mean of two experiments; $LSD_{.01}$ = 18.8.

for germination. The 24-hr incubation experiment was repeated three times, whereas the 72 hr incubation experiment was repeated twice.

Mushroom yield as related to disease occurrence.—*A. bisporus* (P.S.U. 310) was used as the suscept for all experiments. Cultural procedures included those previously described (15) with the exception that plastic pots, 24-cm diam, were used as experimental units. The pots were sanitized in a dilute solution of calcium hypochlorite prior to filling with 1,400 g of compost and 20 g of rye grain spawn. When spawn had colonized the compost (15), usually a duration of 21 to 26 days, the pots were placed into the growth chamber (Fig. 4). The chamber was maintained at 98 to 100% relative humidity and 17 to 19 C throughout the experiment as monitored by a hygrothermograph. The pots were cased with treated soil to a depth of 2 to 3 cm and the soil raised to field capacity (19% w/w) within 2 days after casing by light and frequent sprays of tap water.

Treatment of casing with aerated steam was accomplished using equipment already described. Soil was treated at 60, 82, or 98 C for 30 min 3 days prior to casing. Approximate warmup times prior to treatment and cooling time following treatment were 10 and 20 min, respectively, for all three treatments. After cooling, soil was placed into new, clean polyethylene bags and stored.

Inoculum density varied from 0 to 5 X 10^5 phialospores per pot. Spore preparation was identical with that previously described. Soil was seeded at three different times after treatment: 3 days before casing; 2 days after casing; 12 days after casing. Spores were atomized onto soil 3 days before casing, and the soil then mixed. This procedure was followed for aliquots of soil treated at 60, 82, or 98 C. Soil seeding after casing occurred by atomizing a spore suspension onto the soil surface.

Data were taken on the number of infected and healthy mushrooms, disease occurrence throughout the harvest period, and the weight of mushrooms harvested in each disease category. Harvesting was limited to ca. 14 days during which two breaks (flushes) of mushrooms developed. Mushrooms were classified as: (i) healthy; (ii) diseased, i.e. spotted and/or deformed. Three or four replicate pots were used per treatment and the experiment repeated four times.

RESULTS.—*Phialospore germination and growth.*—Lower soil treatment temperatures resulted in reduced germination of *V. malthousei* phialospores irrespective of incubation time; viz. 24 or 72 hr. Percentage germination of phialospores in contact with soil for 24 hr was significantly less, 0.01 level, in

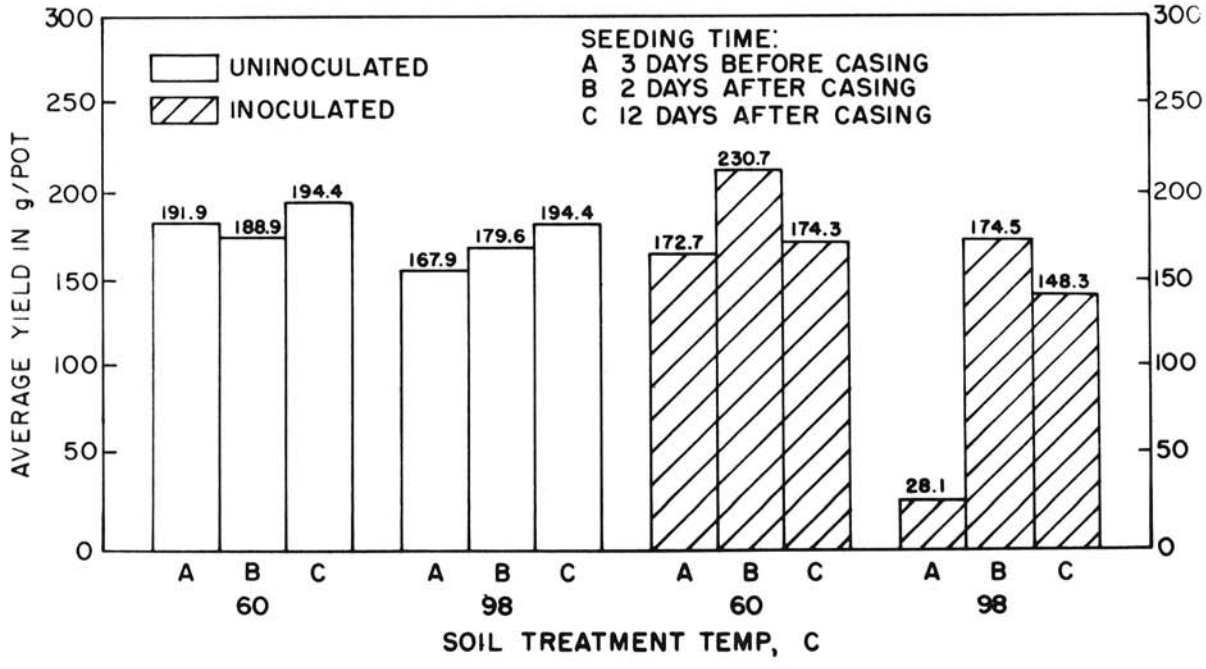

Fig. 5. The effect of aerated steam treatment of casing followed by seeding with *Verticillium malthousei* phialospores on the yield in grams of healthy mushrooms; the seeding rate was 5,000 phialospores per replicate.

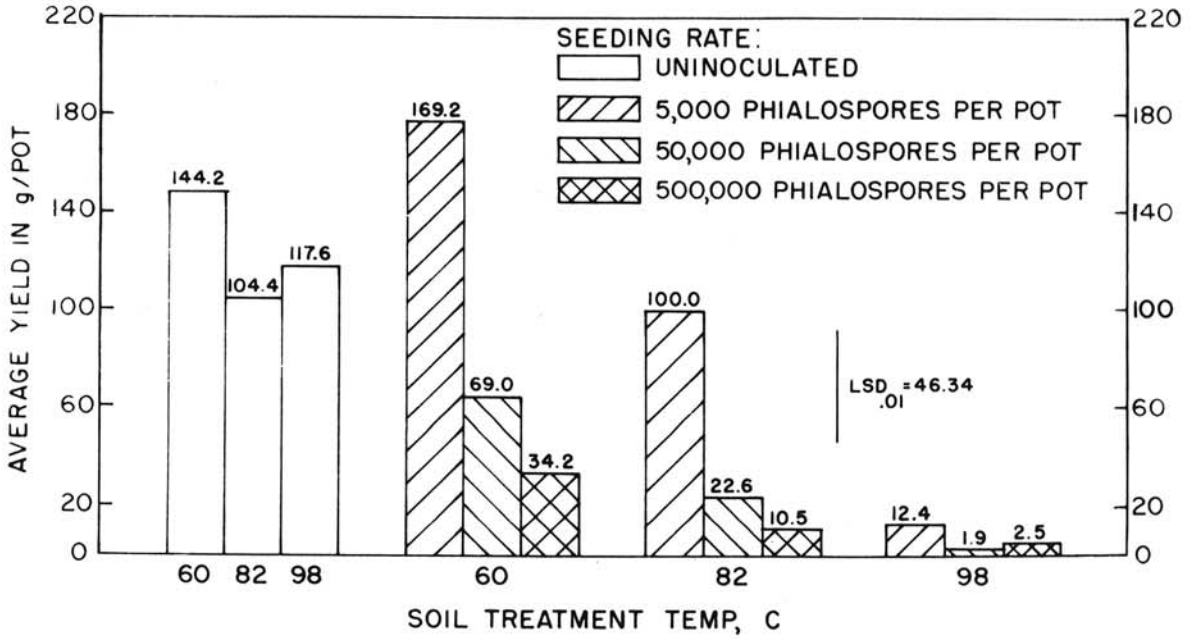

Fig. 6. The effect of aerated steam treatment of casing followed by seeding with *Verticillium malthousei* phialospores on the yield in grams of healthy mushrooms; seeding time was 3 days before casing.

soil treated at 60 C versus 98 C or with germination in moist chambers (Table 1).

Average length of germ tubes from phialospores placed in contact with soil previously treated at 60 or 82 C were equivalent irrespective of incubation time. Phialospores in contact with soil previously treated at 98 C and incubated for 24 or 72 hr produced germ tubes three and nine times longer, respectively, than germ tubes from phialospores in contact with soil previously treated at 60 or 82 C.

Mushroom yield as related to disease occurrence.—Disease was most severe on mushrooms growing on soil seeded before casing and treated at 98 C (Fig. 5, 6, 7a, b, c, vs. 7d, e, f) irrespective of inoculum density.

TABLE 2. Disease occurrence as influenced by seeding time with *Verticillium malthousei* phialospores following soil treatment at 60 and 98 C for 30 min

Soil treatment temperature (C)	Seeding time days[a]	Diseased mushrooms[b] (%) I	II	III
60	−3	40-50	0-3	5-12
	+2	5-12	1-7	0-4
	+12	10-33	0-10	0
98	−3	85-100	45-85	90-91
	+2	30-40	0-12	0
	+12	60-75	8-35	3-13

[a] Casing = day zero (0).

[b] Inoculum density: I = 5×10^5; II = 5×10^3; III = 5×10^3 phialospores per replicate

In assessing the influence of inoculation time versus soil treatment temperature, the highest percentage of diseased mushrooms, 91%, and lowest mean weight, 28.1 g, developed on soil treated at 98 C and seeded before casing (Table 2, Fig. 5). The next highest disease occurrence was on soil treated at 98 C and seeded 12 days after casing. There was a statistical difference in yield at the 0.05 level between means for treatment temperature and time of seeding, but no statistical difference for the interaction of

TABLE 3. Percentage of diseased mushrooms present on casing treated at 60, 82, or 98 C for 30 min and seeded with three different inoculum densities of *Verticillium malthousei* phialospores 3 days prior to casing

Soil treatment temperature, C	Inoculum density[a]	Diseased mushrooms[b] (%)
60	0	4
	5×10^3	17
	5×10^4	65
	5×10^5	81
82	0	5
	5×10^3	44
	5×10^4	92
	5×10^5	96
98	0	2
	5×10^3	92
	5×10^4	100
	5×10^5	98

[a] Number of spores added to soil prior to casing.

[b] Average of two breaks (flushes of sporophore formation).

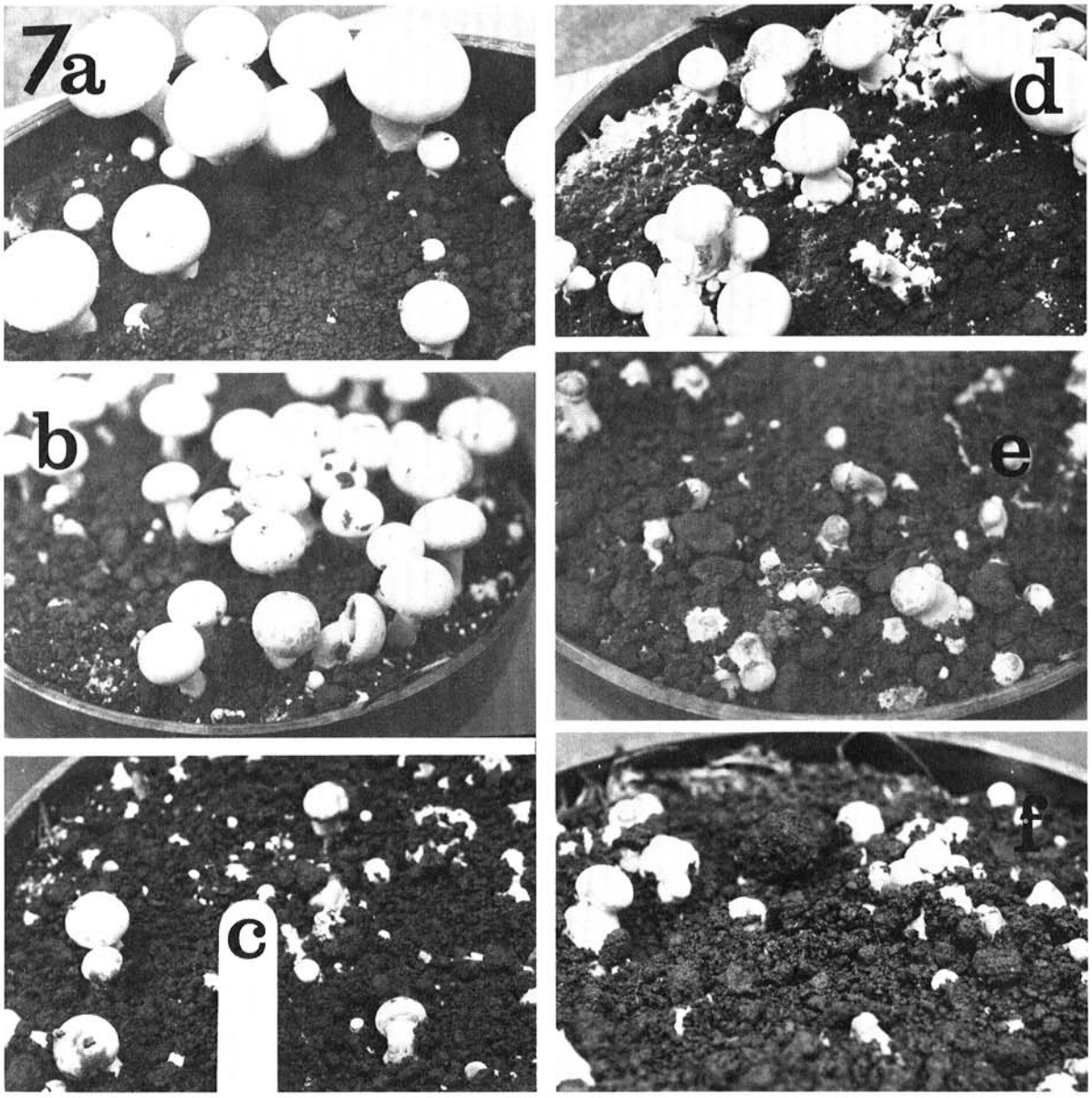

Fig. 7. First break mushrooms which developed on casing treated at 60 C (a, b, c) or 90 C (d, e, f), with 5,000 (a, d), 50,000 (b, e), or 500,000 (c, f) phialospores of *Verticillium malthousei* seeded after treatment but 3 days before application (casing) of horse manure compost colonized by *Agaricus bisporus*.

temperature X time (Fig. 5). Disease occurrence seems to be directly related to inoculum density.

In assessing inoculum density versus soil treatment temperature, disease occurrence varied directly and yield (number and weight) varied inversely with temperature of soil treatment (Table 3, Fig. 6). Percentage of diseased mushrooms increased concurrently with yield decreases as inoculum density increased (Table 3, Fig. 6). In soil treated at 98 C and seeded before casing, the crops produced few healthy mushrooms (Fig. 5, 6, and 7). Inoculum density had little effect on the percent diseased mushrooms when casing was treated at 98 C. These data are in contrast with that for soil treated at 60 C, where disease occurrence was as low as 27% with the lowest inoculum density (Table 3), and yield varied significantly with varying inoculum density (Fig. 6). There was a significant difference between means of temperature X rate at the 0.01 level (Fig. 6).

DISCUSSION.—Cross & Jacobs (6) state that *V. malthousei* spores germinate rapidly to infect mushroom pins when mushroom mycelium is present in mineral soil or peat. These authors speculate that nutrients required by spores to germinate are released

from mushroom mycelium. The suggestion of Cross & Jacobs is not clarified by current data, since disease occurred when soil was seeded prior to contacting mushroom mycelium. Disease occurred when phialospores were sprayed onto soil 12 days after casing, which suggests mycelium and rhizomorphs did not deter disease development. Perhaps the most significant finding was the amount of disease when soil was seeded 2 days after casing. In these instances a significant fungistatic mechanism was apparently operating when mushroom mycelium began to colonize casing since disease severity was not as great as that associated with seeding at other times. This fungistatic factor was generally greater when soil was treated at 60 vs. 98 C irrespective of time of seeding with the pathogen.

Dobbs & Hinson (7) have suggested that such a phenomenon may occur when liberated nutrients have been absorbed by microorganisms. The return of fungistasis may depend on survival of endemic antagonistic microorganisms to steam treatment.

Wuest & Forer (16) reported that rhizomorph exudates and volatiles produced by colonized compost, mycelium-impregnated casing or casing supporting mushrooms had no significant effect on *V. malthousei* spore germination. Thus, it seems the effect of the mushroom mycelium on *V. malthousei* remains unclear although the presence of a fungistatic factor is quite probable.

Results from current studies substantiate the hypothesis of Baker & Olsen (3, 10) who propose that a pathogen may luxuriate after soil is treated with steam. Baker (2) found the retardant effect of resident microorganism on *Rhizoctonia solani* was less at 100 C and 71.1 C than at 60 C. Olsen & Baker (10) found *Bacillus subtilis* survived in soil treated with aerated steam and inhibited *R. solani* introduced into the soil. These reports are closely aligned with less disease on soil treated at 60 vs. 98 C.

Seeding soil treated at 60 C at the rate of 5×10^3 spores per pot resulted in less disease (Table 3) and greater yields (Fig. 6) when compared with higher inoculum densities. This disease decrease suggests an interruption during pathogenesis which may have been caused by: (i) inhibition of spore germination; (ii) lysis of germ tubes; (iii) lack of nutrients; (iv) presence of antagonists to mention but a few of the possibilities. Inhibition of spore germination is widespread in nonsterilized soils and is termed "soil fungistasis" (7). Fungistasis can be overcome by the addition of utilizable substrates such as natural products or by the sterilization of soil. Recently, Romine & Baker (11) have reported on a volatile fungistatic factor (VFF), but they did not consider the effect of soil treatment on VFF.

Data further suggested that inoculum density can override the factor(s) which are responsible for less disease at lower temperatures. One can therefore conclude that this factor is operative within specific limits and is therefore an exhaustible commodity. Such a phenomenon is not unique in biology for the law of limiting factors has long been known. Since a limiting factor exists, it should be possible to identify and measure the factor or factors responsible for less disease at 60 C soil treatment.

The fact that soil seeded with propagules of *V. malthousei* after casing resulted in few symptoms on developing mushrooms is contrary to a report by Holmes (8), who found highest disease incidence occurred when casing was seeded with spores 14 days after casing. His work did not include the use of soil seeded with the pathogen prior to its use as casing. A higher disease percentage occurred on mushrooms from soil seeded after casing versus noninoculated controls (Table 3), but disease occurrence was considerably less than that associated with soil seeded 3 days before casing. Contamination of soil by a pathogen between treatment and use is possible; thus, contaminated casing must be considered a likely reservoir of inoculum.

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