

Sensitivity of Selected Mushroom Pathogens to Aerated Steam

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ABSTRACT

Mushroom casing, the layer of soil applied atop colonized compost, is subject to undesirable effects when treated at 100 C. Thermal death times of four mushroom pathogens cultured in soil prior to treatment when subjected to aerated steam were determined over a range of temp between 43.5 and

82.2 C (110-180 F) for 30 min. *Agaricus bisporus*, *Geotrichum candidum*, *Mycogone perniciosa*, *Verticillium malthousei*, and *Trichoderma viride* could not be isolated from soil treated at 54.4 C, or higher. *Phytopathology* 60:1274-1275.

Casing is soil spread over compost that has been colonized by the mycelium of *Agaricus bisporus* (Lange) Sing. to a depth of 20 to 40 cm, and is the substrate on which the sporocarps of the mushroom are formed. Steam has been used for at least 33 years to eliminate weed molds and pathogens from casing (7). Usually, steam is introduced into the soil through a pipe grid located on the floor of a dump truck. The grid is uniformly covered with soil prior to treatment. Steam is introduced and treatment continues until the coolest spot in the soil mass is exposed to 82.2 C (180 F) for 30 min (11). Baker & Roistacher (6) found that most steam-treated greenhouse soils reached 100 C, which exceeded the desired temp. Such high temp may increase ammonia, soluble salts, or available magnesium to levels deleterious to *A. bisporus*, the mushroom of commerce. Physical changes may also occur when soil is treated at too high a temp, and the possibility of contamination is enhanced because of the biological vacuum (5).

Two pieces of equipment have been described which force aerated steam through the soil (1, 3, 4). Since some mushroom growers have indicated an interest in treating mushroom casing with aerated steam, the thermal death times of four soil-borne fungal pathogens of *A. bisporus* were ascertained using both pieces of treatment equipment.

MATERIALS AND METHODS.—A Hagerstown clay loam, used for casing at the Mushroom Research Center of The Pennsylvania State University, was used as the substrate for the test fungi. The soil had the following properties: pH 7.5, pH buffer 7.0; cation exchange capacity 8.3; organic matter 2%; electrical conductance 4×10^{-4} mhos; meq of potassium, magnesium, and calcium per 100 g soil: 0.42, 0.35, 7.5, respectively.

Screw-cap vials 5.8×2.2 cm were filled with soil to about 70% of their capacity. Soil moisture prior to treatment was approximately 16%; field capacity for this soil is about 24%. A number of vials was exposed to 5 megarads of gamma radiation from ^{60}Co for soil sterilization at the Brookhaven National Laboratory, Upton, N.Y. Plating crumbs of treated soil on various

media revealed that in some a slow growing bacterium was viable.

Another batch of screw-cap vials was partially filled with a similar soil, the caps were loosely affixed, and the vials were autoclaved at 121 C at 16 psi for 15 min on 2 consecutive days. Soil crumbs from each vial were plated on potato-dextrose agar (PDA) plates, some of which had been acidified. None of the plates showed either fungus or bacterial growth after incubation for 7 days at 21 C.

Vials of both irradiated and autoclaved soil were individually seeded with one of the five test organisms. The *A. bisporus* isolate was PSU strain 310; *Geotrichum candidum* Lk. ex Pers. emend Carmichael was isolated from mushroom casing; *Mycogone perniciosa* Magn. and *Verticillium malthousei* Ware were isolated from infected mushrooms; and *Trichoderma viride* Pers. was isolated from mushroom stumps. The soil was seeded with *A. bisporus* by placing small strips of basidiospore-laden filter paper into the soil, or by adding fragmented mycelial mats. *Agaricus bisporus* was recovered from soil seeded by both techniques after 18 days' incubation at 18 C. Aqueous spore suspensions of *G. candidum*, *M. perniciosa*, *T. viride*, and *V. malthousei*, or small pieces of agar on which the fungi were growing, were added to the soil, and the vials incubated at 18 C. Seven days after seeding, the organism that had been seeded was recovered from soil crumbs on PDA plates, except *G. candidum*, which was recovered 18 days after seeding.

Soil infested with *A. bisporus*, *G. candidum*, *M. perniciosa*, *T. viride*, or *V. malthousei* was treated with aerated steam at temp of 43.5, 48.9, 54.4, 60.0, 65.6, 71.1, 76.7, and 82.2 C, 14 to 43 days after seeding. Infested soil was transferred directly from the incubation vial into the treatment tube (4) to a depth of 1 inch. After adjusting the steam:air mixture to the desired temp, the treatment tube was attached to the aerated steam source. Timing of the 30-min period commenced when the soil heated to the treatment temp. Following treatment, the steam was turned off and the soil air-cooled to 32-38 C. Sensitivity of the seeded fungi to each treatment was determined by fungus

TABLE 1. The sensitivity of four mushroom pathogens and *Agaricus bisporus* following treatment of infested soil with aerated steam for 30 min

Fungi	Check	Temp, C							
		43.5	48.9	54.4	60.0	65.6	71.1	76.7	82.2
<i>Agaricus bisporus</i>	+ ^a	—	—	—	—	—	—	—	—
<i>Geotrichum candidum</i>	+	+	+	—	—	—	—	—	—
<i>Mycogone perniciosa</i>	+	+	+	—	—	—	—	—	—
<i>Trichoderma viride</i>	+	+	+	—	—	—	—	—	—
<i>Verticillium dahliae</i>	+	+	+	—	—	—	—	—	—

^a + = recovery on PDA; — = nonrecovery.

recovery following the plating of treated soil crumbs on PDA plates. The plates were incubated at 21 C for 3 to 14 days prior to data collection. Treatments of *A. bisporus* and *G. candidum*-infested soil were repeated once; treatment of other test soils was repeated twice.

In a second study, the Hagerstown clay loam was steamed to 82.2 C for 30 min, then placed in trays with 2-ft³ capacity. An aqueous spore suspension of *M. perniciosa* and *T. viride* or a combination of the two fungi was added to each tray as it was being filled with steam-treated soil. The soil was incubated at 20 C for 21 days. The presence of the fungi was ascertained by recovering each fungus on PDA plates seeded with soil crumbs. This infested soil was then treated with aerated steam (1) at 60 C (140 F) for 30 min and immediately applied as casing to compost which was thoroughly colonized with *A. bisporus* mycelium.

RESULTS.—Aerated steam forced through the soil at a temp of 54.4 C (130 F) and above for 30 min eliminated all of the test fungi from the infested soil. *Agaricus bisporus* was killed in the 43.5-C (110 F) treatment, but the other fungi survived treatment at 48.9 C (120 F) for 30 min. The thermal sensitivity of the five fungi is shown in Table 1. There was no difference between results when fungi were seeded in irradiated or double-autoclaved soil.

Mushrooms harvested from the treated casing of the second study were disease-free, and there was no evidence of *T. viride* on the soil. On the other hand, mushrooms infected with *M. perniciosa* were harvested from untreated casing, and *T. viride* was observed growing on untreated soil 10 days after casing.

DISCUSSION.—Most early studies on thermal death times of mushroom pathogens were conducted by exposing test tube cultures to dry heat for extended periods. Lambert (9) showed that *M. perniciosa* in agar was killed after 60 min at 50 C or in 6 hr at 42 C, while 12 hr at 45 C were required when the fungus had been grown in soil. Lambert & Ayers (10) determined the thermal death times for nine pathogens with 2- to 16-hr treatments at 50 to 65 C in a humidified incubator. Six were killed in 2 hr at 60 C; this group included *M. perniciosa* and *V. psalliotae*. *Trichoderma koningi* was killed in 4 hr at 60 C, while *Geotrichum* sp. required 6 hr.

Anderson (2) subjected dry and moist spores of 12 mushroom pathogens and competitors to heat treat-

ment in an incubator for 30 min. When spores were mixed with moist soil, *M. perniciosa* could not withstand treatment at 50 C. *T. viride* was not recovered after treatment at 70 C, while *V. malthousei* was eliminated at 80 C. Bollen (7) found that most isolates of *Trichoderma* were eliminated from greenhouse soil treated with unsaturated aerated steam for 30 min at 50 C.

Our results clearly indicate that forcing steam at 54.4 C (130 F) through the soil for 30 min eliminated the fungi considered. These findings are in general agreement with previously reported thermal death times (2, 9, 10), although the fungi reported on in these studies had not been allowed to colonize soil prior to treatment. Since the fungi were in their natural habitat prior to treatment, our results can be applied to immediate practical use.

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