Additional Data on the Thermal Sensitivity of Selected Fungi Associated with Agaricus bisporus

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ABSTRACT

Treatment of mineral soil with aerated steam at 54.4 C for 15 min eliminated *Dactylium dendroides, Geotrichum candidum, Mycogone perniciosa, Ostrachoderma* sp., *Trichoderma viride*, and *Verticillium malthousei* that had been experimentally established for 17 days in axenic soil culture. Repeated experiments with *T. viride* and *Ostrachoderma* sp. yielded inconsistent data when treatment time was 15 min, although they were consistently eliminated with 30 min treatment.

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Additional key words: soil treatment, aerated steam, mushroom pathogens.

The thermal death point for 14 plant pathogens propagated in sterilized greenhouse soil when treated with aerated steam was 30 min at 60 C (6). All but one of these pathogens studied by Bollen (6) survived

aerated steam treatment at 47.5 C for 30 min. *Mycogone perniciosa* and *Verticillium malthousei*, mushroom pathogens, plus *Geotrichum candidum* and *Trichoderma viride*, weed molds of the commercial mushroom, in addition to *Agaricus bisporus*, were eliminated from soil treated at 54.4 C for 30 min with aerated steam (8). This study considers other time X temperature effects of aerated steam on pathogens and weed molds of the commercial mushroom, *Agaricus bisporus* (Lange) Imbach. In addition, the treatment apparatus used by Wuest et al. (8) in earlier work on thermal sensitivity of mushroom pathogens was replaced with an alternate aerated steam treatment system (1).

MATERIALS AND METHODS.—Hagerstown silty clay loam, characteristics of which have been reported (8), was used in all experiments. Glass screw cap jars, 9 cm (diam) X 9 cm (height), were one-third filled with moist soil which had previously been passed through a 1.27-cm mesh sieve.

The jars of soil were autoclaved at 121 C for 60 min on each of 2 consecutive days. Soil crumbs from selected jars were plated onto potato-dextrose yeast-extract agar (PDYA) and incubated 5 days to assay for microbial growth. Microbial growth did not occur in any test plates. Soil moisture was adjusted to 19% (w/w) by the addition of an aqueous suspension of propagules (spores) of the test organisms.

Dactylium dendroides (Bull.) Fries, *Geotrichum candidum* Link, *Mycogone perniciosa* Magn., *Ostrachoderma* sp. Fries, *Trichoderma viride* Pers. and *Verticillium malthousei* Ware (isolate MS-PW-692) ex Fries were obtained from the culture bank of the Mushroom Laboratory of The Pennsylvania State University, and were used to seed sterile soil.

Mass transfers of the six fungi were grown for 24 days on PDYA at room temperature, 22 to 24 C. Aqueous spore suspensions of each test organism were prepared from a petri dish culture. Spore suspensions were standardized by means of a hemacytometer, and sterile soil in each jar was seeded with 2×10^6 spores. Moisture in control soil was adjusted by adding sterile distilled water. Jars of seeded soil were incubated in dark at 22 to 24 C for 17 days with their lids loosely sealed. Soil crumbs from each jar were plated onto PDYA to verify the presence of each fungus prior to treatment.

Soil was treated with aerated steam using equipment described by Aldrich & Nelson (1) with modifications previously reported (7). Flour sifters, containers for soil during treatment, and empty screw-cap glass jars used after soil treatment were heated overnight in an oven maintained at 121 C. Soil was treated for either 15 or 30 min at temperature intervals between 43.5 and 87.8 C. Treatment temperature was monitored using a multipoint potentiometric strip chart recorder, with a thermocouple buried in soil contained in each flour sifter; treatment temperatures varied ± 1 C. Warm-up time prior to treatment and cooling time after treatment were 7 and 6 min, respectively. Soil was dumped from each sifter into clean, unused glass jars after treatment, and the screw-caps were tightly affixed. Crumbs of soil were removed from each jar and plated onto PDYA within 12 hr of treatment. The plates were incubated 5 days at 22 to 24 C before microscopic identification of each fungus was attempted.

TABLE 1. Sensitivity of five fungi incubated for 17 days in soil to 30 min treatment with aerated steam at four temperatures

Fungus	Control[a]	Temperature, C			
		43.5	48.9	54.4	60.0
Dactylium dendroides	+[b]	+	+	–	–
Mycogone perniciosa	+	+	–	–	–
Ostrachoderma sp.	+	+	–	–	–
Trichoderma viride	+	+	+	–	–
Verticillium malthousei	+	+	–	–	–

[a] Denotes the presence of the fungi prior to treatment.
[b] + = growth; – = no growth on potato-dextrose yeast-extract agar.

TABLE 2. Sensitivity of six fungi incubated for 17 days in soil culture to treatment for 15 min with aerated steam at three temperatures

Fungus	Control[a]	Temperature, C		
		54.4	71.1	87.8
Dactylium dendroides	+[b]	–	–	–
Mycogone perniciosa	+	–	–	–
Ostrachoderma sp.	+	+	–	–
Trichoderma viride	+	+	–	–
Verticillium malthousei	+	–	–	–
Geotrichum candidum	+	–	–	–

[a] Denotes presence of respective fungi prior to treatment.
[b] + = growth; – = no growth on potato-dextrose yeast-extract agar.

The experiment in which treatment was for 30 min was repeated once for *D. dendroides*, *Ostrachoderma* sp., and *T. viride*, and conducted once for *M. perniciosa* and *V. malthousei*. Experiments wherein treatment time was 15 min were repeated once.

RESULTS.—Test fungi were eliminated from colonized soil by treatment with aerated steam at 54.4 C (130 F) for 30 min (Table 1). Treatment of colonized soil at 54.4 C for 15 min eliminated *D. dendroides*, *M. perniciosa*, *V. malthousei*, and *G. candidum* (Table 2). Elimination of *Ostrachoderma* sp. and *T. viride* at 51.5 C (125 F) with a 15-min treatment occurred in a third experiment. Average soil moisture before treatment was approximately 14.3% for data in Table 2, while soil moisture in the third experiment approximated 19.0%.

DISCUSSION.—The data suggest that aerated steam treatment at 54.4 C (130 F) will eliminate test fungi in as short a time as 15 min. There is a suggestion that soil moisture during incubation may influence the thermal sensitivity of some fungi to aerated steam treatment. Other authors have recognized the role of moisture in the heat sensitivity

of fungi (2, 3, 4, 5), but additional research should be directed to this question.

It has been documented that organisms of concern to growers of potted plants are eliminated by soil treatment at 60 C for 30 min with aerated steam under ideal conditions (5). Such treatment results in a flourish of microbial activity, and this minimizes the likelihood of subsequent colonization by pathogens. Colonization of mushroom casing (soil) by pathogens and competitors of the commercial mushroom is retarded by treatment with aerated steam treatments at 60 C for 30 min (7). Since lower temperatures and shorter treatment times eliminate selected pathogens and weed molds, one could speculate that such treatments should reduce the ability of a pathogen to colonize such soil when compared to soil treated at higher temperatures and/or for longer periods of time.

Additional studies which include other mushroom pathogens, pests, and competitors should be conducted to determine with certainty the minimum soil treatment with aerated steam required to provide *A. bisporus* the optimum eco-system for its growth.

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