Sterilization Method Effects on Germination of Wood Decay Fungus Spores Observed by the Contact Agar Method

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ABSTRACT

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Sterilization method significantly influenced spore germination of four wood decay fungi on spruce and aspen sapwood test blocks. Spore germination response to attempted sterilization methods was assessed on agar disks in diffusion contact with wetted wood samples. Of the five methods examined, brief immersion of blocks in vigorously boiling water most effectively minimized variation in germination percentages among replicates and allowed high germination levels for all wood/fungus combinations. Sensitivity of spore germination to even slight chemical modifications of wood, which may occur in attempted sterilization, must be considered in basidiospore testing programs for wood and forest products.

Studies of germination of spores of wood decay fungi on wood show that method of wood sterilization has little significant effect on wood decay fungus spore germination (5,6). These studies included steaming, autoclaving, and propylene oxide treatment of 60-μm thick wood sections to support basidiospore germination. Certain sterilization methods with heat or gaseous fumigation may influence the mycelial activity of decay fungi on both untreated and fungicide-treated wood or wood products (1,2,10) and it is reasonable to hypothesize that such modification of wood by sterilization also would affect spore germination. This study expands the numbers of test fungi and the number of sterilization methods employed to determine the influence of these methods on spore germination response of decay fungi.

MATERIALS AND METHODS

Wood blocks (1 cm^3) of aspen (*Populus tremuloides* Michx.) and white spruce (*Picea glauca* [Moench] Voss) cut from clear, air-dried sapwood were subjected to the following sterilization techniques:

(i) Autoclaving 15 min at 1.05 kg/cm^2 (15 psi).
(ii) Steaming 20 min at 100 C (at atmospheric pressure).
(iii) Ethylene oxide gas for 6 hr.
(iv) Reciprocal Tyndallization (5 days of alternate freezing and thawing (7).
(v) Boiling water immersion (blocks held for 2 sec in boiling water).

Control blocks were not treated. All blocks were wetted under vacuum (100 torr) in a volume of water equal to the sample weight just prior to sterilization with the exception of gas-treated blocks which were wetted after ethylene oxide exposure. Water agar disks (.6 cm in diameter × .3 cm thick) were fused to the radial surfaces of the wetted, sterilized, and control blocks with two drops of molten agar. These blocks then were incubated at 25 C for 24 hr in deep petri dishes (100 × 20 mm) stacked within a polystyrene box containing water to assure high humidity. Basidiospores, cast within the preceding 24 hr from inverted cultures grown following prescribed procedures (5,6,11), were suspended in sterile, deionized water and diluted until turbidity had just vanished. Suspensions were immediately seeded by micropipet onto agar disks, incubated for 24 hr, and counted for germination level (×400). At least 100 spores were counted from a random field observation of each of three replicate agar disks for each wood-fungus-sterilization method combination. Details of the adaptation of this contact agar method to the study spore germination of wood decay fungi have been published (8). Spores of the white rot fungi *Trametes hispida* Bagl. (ATCC 36206) and *Poria tenuis* (Schw.) Cooke (ATCC 36207) as well as two isolates (local MN50, and Madison 617) of the brown rot fungus *Gloeophyllum trabeum* (Pers.) Murr. were observed for germination response on agar disks. Spores also were seeded on 2% water agar plates and counted for germination after 24 hr.

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RESULTS AND DISCUSSION

Spore germination response to the various methods of attempted sterilization is shown in Fig. 1. For each decay fungus examined, two or more methods of attempted sterilization gave erratic germination response or inhibited spore germination on the contact agar blocks. Control (no sterilization) blocks and samples subjected to 'reciprocal Tyndallization' showed the greatest range of germination response. The effect presumably was due to bacterial and fungal contaminants which were seen either on the wood or agar disks at spore counting. Stimulation of spore germination of wood-decomposing Hymenomycetes by CO_2 is known (3), and microbial contamination may well induce variation in germination on this basis. Five freezing-thawing cycles were not effective in eliminating microflora from the two wood species tested.

Germination response among replicates within other wood-fungus-sterilization combinations showed acceptably low levels of variation (ie, less than ± 10%).

Inhibition of spore germination on agar disks fused to wood samples was dependent on fungus and wood species involved. Autoclaved blocks of aspen supported germination only of *T. hispida* spores, but all fungi germinated well on autoclaved spruce. It is known that autoclaving wood increases soluble sugars which may alter decay susceptibility (4). Likewise, the ethylene oxide treatment of aspen prevented spore germination of white rot fungi but not of brown rot fungi; no inhibition was noted on spruce. Steaming inhibited only *P. tenuis* on aspen. The chemical bases for these observed inhibitions remain largely speculative and are undoubtedly multiple.

Of the various commonly recommended methods of wood sterilization or disinfestation, a brief immersion of blocks in vigorously boiling water proved superior when such blocks are to be used in basidiospore germination tests. That is, germination of spores as counted on the fused agar disks showed limited variation and desirably high percentage for all fungi tested on either aspen or spruce. Due to lack of facilities, radiation sterilization of wood was not tested for influence on spore germination. This method has been reported to minimally alter the wood substrate, and should, therefore, be useful for reducing variability in biological testing procedures (2).

This experiment has shown the sensitivity of basidiospore germination response to chemical modifications of wood, presumably caused by method of sterilization, and the presence of

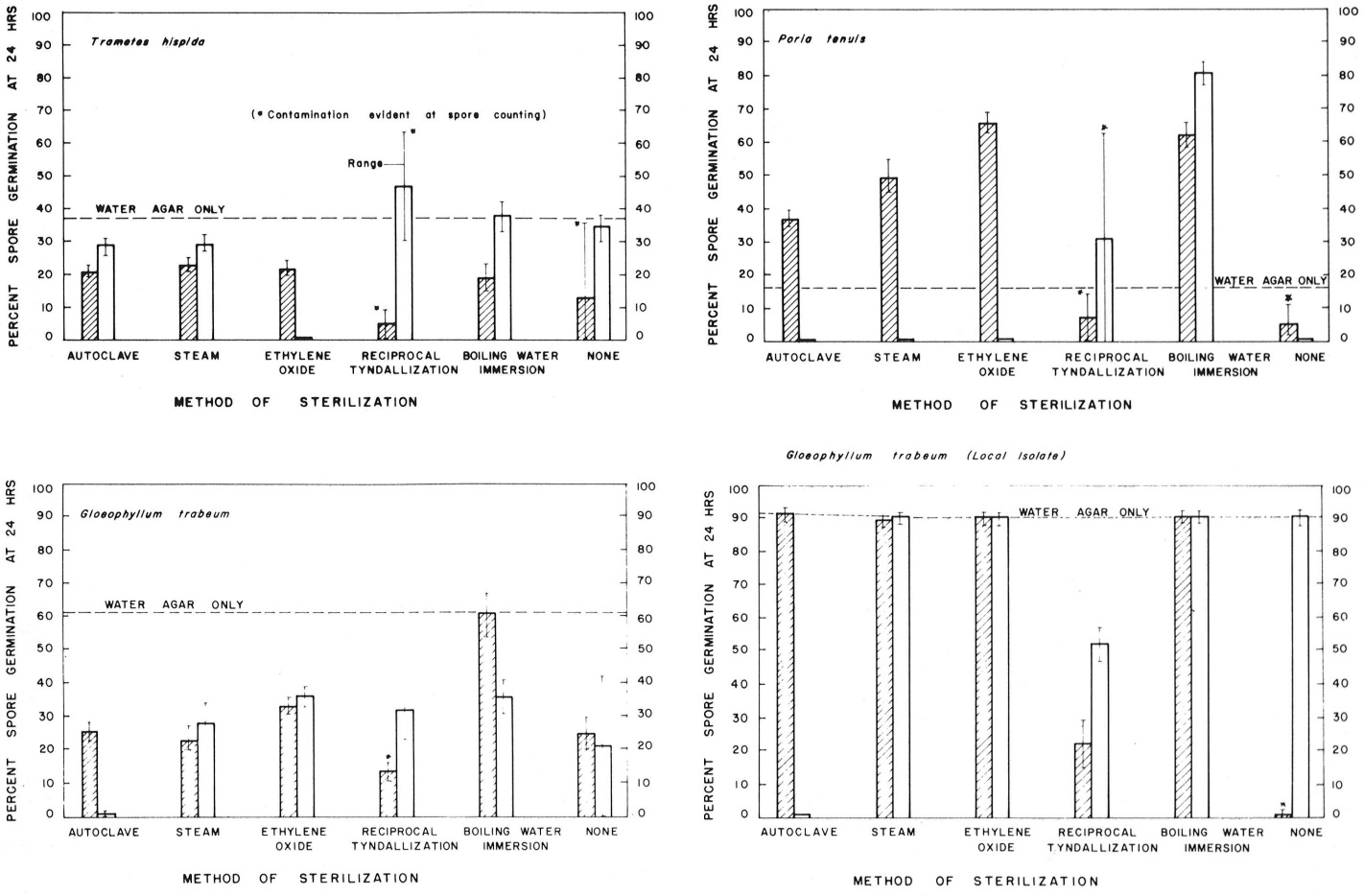

Fig. 1. Spore germination of wood decay fungi on contact agar disks fused to spruce (hatched) and aspen (open) blocks earlier subjected to various methods of sterilization. (Each value averaged from three replicates.)

other obvious microflora. The use of 1 cm^3 wood blocks rather than thin wood sections in tests of the influence of sterilization method on spore germination is preferred to better approximate the chemical nature of the liquid film required for spore germination on wood. The concentration of spore germination inhibitors in an agar disk fused to wetted wood apparently is related to the wood mass (9). This may partially explain the results of experiments in which only 20–60 μm sections of wood were used but there was no influence of substrate modification on spore germination (5,6,11).

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