

DISINFESTATION OF EXPANDED-POLYSTYRENE SEEDLING TRAYS IN THE AUSTRALIAN TOBACCO INDUSTRY

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Prior to the announcement of the methyl bromide (MB) phase out, the tobacco industry worldwide was heavily reliant on MB to disinfest soils in nurseries against key pathogens, weeds and pests. However, a move by the industry to produce nursery transplants in semi-hydroponics systems (the 'float' system) reduced their reliance on MB significantly. The float system utilizes expanded polystyrene (EPS) trays seeded with tobacco, and floated on a common pool of water (containing measured doses of fertilisers) within polyhouses. By 2004, almost 100% of Australian tobacco growers had adopted the float system for transplant production. Growers re-use their trays as many times as possible to minimize cost and waste disposal issues. As EPS-trays age and deteriorate, their potential to harbour propagules of key tobacco pathogens (eg *Rhizoctonia*, *Fusarium*, *Botrytis* spp.) increases. Furthermore, the micro-environment of the float system (ie high plant densities and humidity, plant roots in a common pool of water, etc) favours the development of some diseases, particularly those caused by pathogens with motile spores (eg *Pythium* spp.). To manage the risk of disease occurring in the float system, Australian growers fumigated their trays with MB prior to re-use, up until its phase-out in 2005. Our research investigated four approaches to provide growers with disinfestation options for EPS-trays without MB; (a) improved hygiene practices, (b) heat disinfestation, (c) biocidal dips, and (d) alternative fumigants (1).

Survey of pathogens on used EPS-trays.

Used EPS-trays of varying ages and backgrounds were sampled from 35% of tobacco growers in the Australian industry. Trays were primarily colonized by a diversity of saprophytic fungi, dominated by *Cladosporium*, *Penicillium*, and *Trichoderma* spp. However, the tobacco pathogens *Fusarium oxysporum* and *F. tabacinum* were also regularly isolated from used trays. Artificial inoculum (sclerotia) embedded in trays of *Rhizoctonia solani* and *Botrytis cinerea* remained viable for more than 1 month. These results show the potential for tobacco pathogens to survive in used trays, and that tray disinfestation may reduce the risk of disease occurring in the float system.

Benchmarking the efficacy of methyl bromide

The effectiveness of MB for disinfestation of EPS-trays is poorly defined in the scientific literature, but is important for providing a benchmark for alternative treatments. Guitarrez et al. (1997) showed that MB reduced natural populations of *R. solani* on used trays from 7 CFUs/tray to nil (2). In our trials, fumigation with MB (98%, 100 g/m³) reduced populations of total fungi on used trays to undetectable levels (Fig 1). However, it only reduced the viability of artificial inoculum of *R. solani*, *B. cinerea* and *F. oxysporum* embedded in the trays by between 20-80% (e.g. Fig 2). Sclerotia (survival structures) were more tolerant of fumigation with MB than bare mycelium. These results suggest that in the past, MB may not have always eradicated all pathogens contained on trays in the disinfestation process.

Hygiene practices

A questionnaire survey was conducted in the Australian tobacco industry (returned by 92% of growers) to gather information on hygiene practices used in the float system. Based on responses, growers were categorized as employing upper (8%), medium (52%), lower (32%) and poor (8%) hygiene standards. There was a strong association between improved hygiene practices by growers and reduced fungal populations on their used trays (Fig 3). REML analysis showed that tray handling and storage practices, and disinfection were the most significant factors ($p = 0.01$ and 0.03 , respectively) in influencing fungal populations on used trays. Furthermore, controlled trials demonstrated that pressure washing (nozzle pressure, 1600 psi) could reduce fungal populations on used-trays by up to 99%. Although improved hygiene practices alone cannot reduce fungal populations on trays to the same level as MB, they are an essential component of any integrated method to disinfest trays.

Heat disinfestation

In vitro trials showed that exposure to periods of high temperature ($\geq 50^{\circ}\text{C}$) could kill a range of tobacco pathogens, including *Pythium ultimum*, *B. cinerea*, *R. solani*, *Chalara elegans*, *F. oxysporum* and *F. tabacinum*. The exposure time required to kill pathogens decreased as temperature increased. Chlamydospores of *F. oxysporum* were the most tolerant of periods of high temperature (requiring 4 hrs exposure to 60°C to kill), while mycelium of *P. ultimum* and *C. elegans* were the most sensitive. Three methods of delivering heat for commercial tray disinfestation were trialed: dry heat in ovens, aerated steam, and solarization of trays under transparent plastic in the polyhouse. Aerated steam (1 hr, maximum temperature 80°C) and solarization (6 wks, maximum temperature 75°C) provided the most practical and reliable results, reducing fungal populations on trays to undetectable levels and the viability of artificial inoculum of *R. solani* embedded in trays (sclerotia and bare mycelium) to nil (3). The efficacy of aerated steam and solarization for controlling *R. solani* in trays was greater than that of MB (Fig 2). Solarization is an attractive method for growers, as it allows them to utilize their polyhouses during the off-season (summer), and is a cheap and energy efficient disinfestation solution. Neither steam disinfestation nor solarization showed any evidence of reducing the structural integrity of trays (as determined by physical dimensions), although trays continuously solarized for 10 months showed minor discoloration.

Biocidal dips and alternative fumigants

Biocidal dips such as electronically activated water (25-100% anolyte solution, dipped for 2 min) and sodium hypochlorite (10% solution, dipped for 1 min) reduced fungal populations on used EPS-trays by 90-99%, but were not as effective as MB. Fumigation with the alternative, methyl iodide (100%, 100 g/m^3), also reduced fungal populations on used trays (Fig 1), but further work is required to optimise rates for this fumigant to obtain equivalent efficacy to MB. An important drawback to use of biocides and alternative fumigants for tray disinfestation is their potential to cause phytotoxicity in tobacco transplants. For example, residues of methyl iodide were detected in trays for 7 days following their removal from fumigation chambers.

Conclusions

In late 2006, the Australian tobacco industry accepted a buy-out to cease production. Prior to closure, the industry was preparing the infrastructure required to adopt steam and solarization systems for disinfesting used trays. Together with the previous

adoption of the float system, this move would have eliminated the use of MB in the industry. The identification of disinfestation options for used EPS-tray in Australia may assist other tobacco-producing nations in phasing out of MB.

References

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3. Mattner, S.W. et al. (2007) Integrated disinfestation of expanded-polystyrene seedling trays using washing practices and solarisation. MBAO Conference, San Diego.

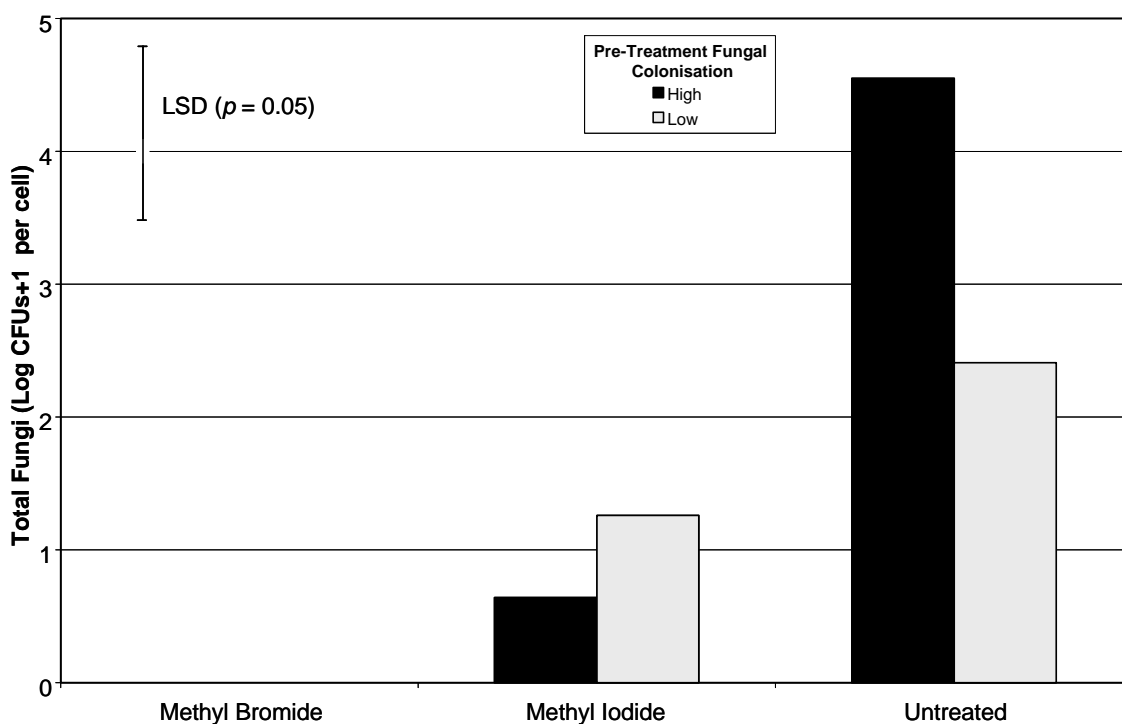


Figure 1. Effect of disinfestation with methyl bromide and methyl iodide on total fungi on used EPS-trays.

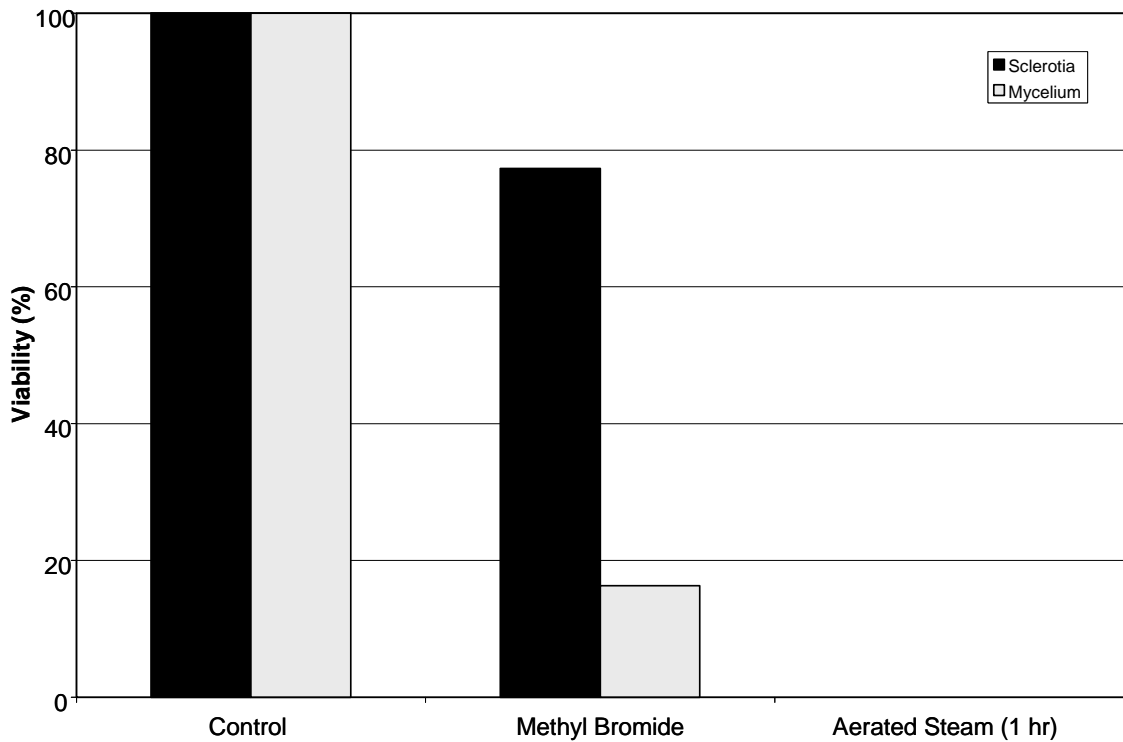


Figure 2. Comparison of the viability of *Rhizoctonia solani* inoculated into EPS-trays as sclerotia or bare mycelium and exposed to aerated steam for 1 hour or methyl bromide.

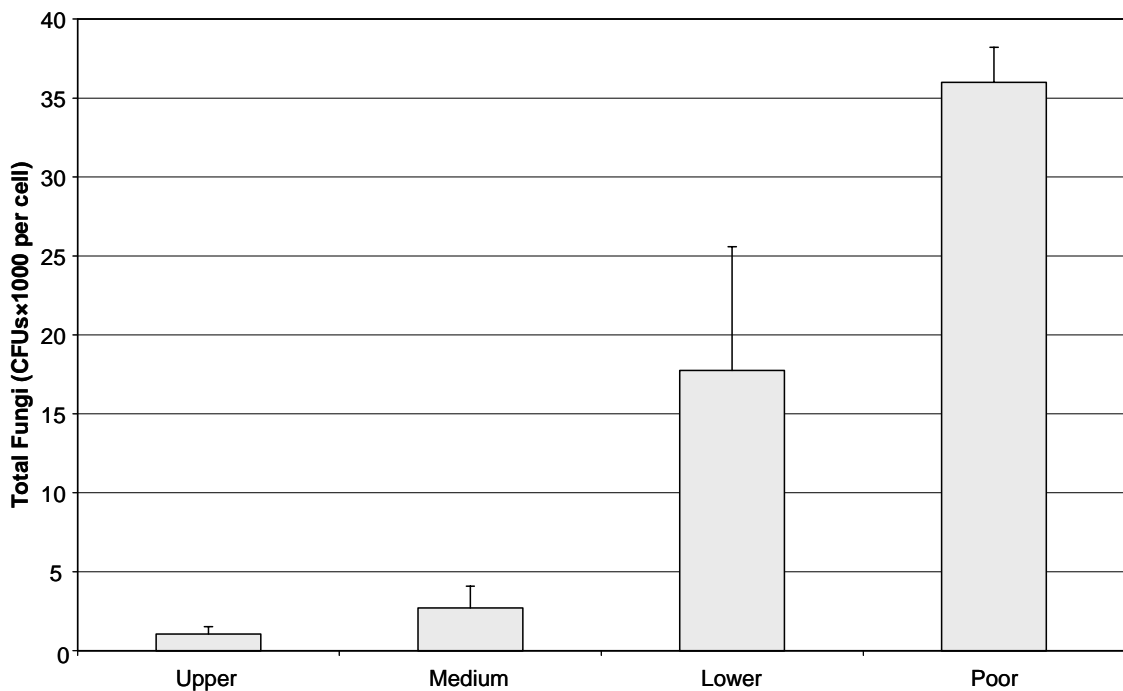


Figure 3. Populations of fungi recovered from used EPS-trays from Australian tobacco growers employing high, medium, lower or poor hygiene practices. Bars are standard errors, where P = 0.05.