Dose Curves of Disinfestants Applied to Plant Production Surfaces to Control *Botrytis cinerea*

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ABSTRACT

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Lethal dose curves were calculated using probit analysis for six disinfestants (chlorazene hydrosol, hydrogen dioxide, hydrogen peroxide, iodine, quaternary ammonium chloride, sodium hypochlorite) when applied on seven substrates (galvanized metal, stainless steel, polyethylene ground fabric, polyethylene pot plastic, pressure-treated pine, exterior latex-painted pine, raw pine) that had been inoculated with *Botrytis cinerea* conidia. Mortality was determined by percentage of ungerminated versus germinated conidia that had been rubbed off of a substrate onto half-strength potato dextrose agar (hPDA) 16 to 24 h previously. Based on overlapping confidence limits (95% CL) of the lethal doses resulting in 90 and 50% mortality (LD_{90} and LD_{50}, respectively) and significance of slopes, differences occurred between substrates with all six disinfestants. LD_{90} values ranged from 0.21 to 4.54 g a.i./liter for chlorazene hydrosol, 4.99 to 40.3 g a.i./liter for hydrogen dioxide, 63.0 to 233.1 g a.i./liter for hydrogen peroxide, 0.42 to 2.45 g a.i./liter for iodine, 0.64 to 6.46 g a.i./liter for quaternary ammonium chloride, and 0.87 to 6.84 g a.i./liter for sodium hypochlorite. For hydrogen dioxide, quaternary ammonium chloride, and sodium hypochlorite, a binomial lethal dose (LD_b) was calculated by plating the inverted inoculated substrates on hPDA, then recording the presence or absence of *B. cinerea* mycelial growth over 7 days. Lethal doses resulting in the absence of mycelial growth (LD_{b100}) were equal to or greater than the LD_{90} values for most disinfestants and substrates. Results demonstrate for the six disinfestants that dose should be selected based on the substrate being disinfested of *B. cinerea* conidia.

Additional keywords: biocidal, disinfectant, gray mold, sterilizing agent

Sanitation is a proven component of limiting the spread of pathogens in plant production systems, as well as in food preparation and medical facilities (9). Disinfestants are primary agents for killing viable propagules of pathogens present on stationary inert surfaces (e.g., bench top, floor, media mixing pad), transportable inert surfaces (e.g., pruning shears, shovel, cart, tractor tire), and biological organisms (e.g., human hands, plant propagation selections).

Much of the basic research on disinfestants has involved studies with human and animal pathogens (mainly bacteria and viruses) on surfaces typical of medical and food hygiene facilities (e.g., stainless steel, Formica, and ceramic tile). Within the agricultural sciences, more literature can be found related to animal production than to plant production systems. Most of the work in plant production has involved rates and selection of disinfestants used in cooling tanks, dump tanks, and spray systems of processing and packaging lines, and to a lesser degree for disinfesting pots and trays.

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In plant production systems, disinfestants are used on most stationary inert surfaces at specific rates, even though different pathogens (fungi, bacteria, and viruses), surfaces (metal, wood, and polyethylene surfaces), and cleanliness states (clean, and presence of algae, plant debris, and/or soil) are encountered. However, a single rate may not be equally effective on all surfaces (1,2,6,7). Nichols and Jodon (7) showed that 10% bleach and other disinfestants were not equally effective against a range of plant pathogens on clay and plastic pots, even with 30 min of submersion. Koponen et al. (6) found that iodine, quaternary ammonium, and sodium hypochlorite at a prescribed rate controlled a greater number of genera of plant pathogens on metal than on plastic surfaces, and provided poor control on raw pine with 60 min of submersion. Copes and Hendrix (1) found that quaternary ammonium and bromide sprayed on substrates at label rates provided limited control of *Thielaviopsis basicola* on ground fabric, pressure-treated wood, and galvanized metal surfaces. A 10% bleach solution fully controlled *T. basicola* on galvanized metal, but 20% bleach was required to obtain equal control on ground fabric and on pressure-treated wood (1).

Many disinfestants exist, but only a few are labeled for use in plant production areas. Multiple chlorine products are available, such as chlorine gas and hypochlorites. Chlorazene hydrosol is not used in ornamental plant production systems, but it effectively disinfested soiled concrete floors and wooden walls of *Actinobacillus pleuropneumoniae*, a gram-negative coccobacillus, in pig stables (4). Chlorazene hydrosol is a chlorine disinfestant reported to release hypochlorous acid more slowly than hypochlorites, be less irritating to humans, and be less affected by soiled surface contamination than hypochlorites (4). Hydrogen dioxide is labeled for use in plant production systems. A similar chemical compound is hydrogen peroxide, which is used to disinfest human wounds. Iodine is a common disinfestant that is available in dry and liquid formulations, has a low demand influence from nitrogenous compounds and organic matter, and is labeled as a medicinal product for humans (3).

The objective of this research was to establish a lethal dose curve response, with an emphasis on the dose resulting in 90% mortality (LD_{90}) of *Botrytis cinerea* conidia, for six disinfestants when applied on metal, plastic, and wood surfaces commonly present in plant production systems. This information will provide a baseline for these disinfestants under clean conditions. Since an upper dose range of the probit-predicted dose curves could behave as an asymptotic maximum, a binomial lethal dose will be established to compare a dose that caused 100% mortality with the mathematically predicted LD values. This would provide additional information for proposing dose recommendations.

MATERIALS AND METHODS

Inoculum production. *B. cinerea* (isolate Bc01 from coleus) was grown on PDA (potato dextrose agar; Difco Laboratories, Detroit, MI) for 18 to 26 days, flooded with autoclaved, distilled water, and conidia dispersed by rubbing a rubber policeman across the agar surface. The suspension was swirled and poured through four layers of cheesecloth. The plate was flooded a second time and the process repeated to maximize spore recovery. Spore number was determined with a hemacytometer.

Substrates. Seven substrates were used in a clean state. Substrates were galvanized metal, stainless steel, polyethylene pot plastic, polyethylene ground fabric (tightly woven, 5-oz. fabric, needle punched and UV stabilized, 98.7% opaque), pressure-treated pine, exterior latex-painted pine, and raw pine. Galvanized metal and stainless steel washers were used that had a diameter, center hole diameter, and thickness of 18, 8, and 1 mm, and 22, 5, and 1 mm, respectively. Pine substrates and ground fabric were cut to form 196 mm^2 squares. Raw pine and painted pine substrates were 3 mm thick. The painted pine was raw pine pieces painted on all sides with an exterior-latex paint. Pressure-treated pine was 4 mm thick. Polyethylene pot plastic was a 19-mm-diameter disk that was a by-product from punching holes in the bottom of 3.8-liter and larger plastic pots. Many of the pot plastic disks had folds with approximately 15° angled bends, which corresponded to the bend at the bottom of the pot. Disks were flattened by placing them between two sheets of heavy-gauge aluminum foil and ironing them at a wool temperature setting for 30 s. All substrate pieces were stored at room conditions (21°C) and never exposed to environmental degradation.

Disinfestants. Disinfestant treatments were (i) chlorazene hydrosol (Chloramin-T, 99.99% a.i., WPC Brands, Inc., Wilmington, OH), (ii) hydrogen dioxide (ZeroTol, 30% a.i., Biosafe Systems, Glastonbury, CT), (iii) hydrogen peroxide (ACS grade, 27% a.i., Fisher Scientific, Atlanta, GA), (iv) iodine (iodine Lugol, 5% a.i., Fisher Scientific), (v) quaternary ammonium chloride (10% *n*-Alkyl [60% C14, 30% C16, 5% C12, 5% C18] dimethyl benzyl ammonium chloride and 10% *n*-Alkyl [68% C12, 32% C14] dimethyl ethylbenzyl ammonium chloride) (Green Shield, 20% a.i., Whitmire Micro-Gen Research Laboratories, St. Louis, MO), and (vi) sodium hypochlorite (Ultra Clorox, 6% a.i., The Clorox Co., Oakland, CA). Disinfestants were mixed in sterile deionized water to a volume of 30 ml and applied with a Kontes reagent sprayer (500 ml) using pressurized air at 6 psi. Aluminum foil trays containing substrate pieces were set on a wooden block that had an upper surface at a 15° angle. The sprayer was held parallel to and at a distance of about 14 cm from the substrates, and six to eight sweeps were made to achieve thorough coverage.

Lethal dose curve response. Each of the six disinfestants was tested in six separate experiments that were repeated for a total of 12 experiments. Experimental design was a randomized complete block with four replications. All substrates being treated per dose per replication were placed in a 9 × 13.5 cm heavy gauge aluminum tray with 2 cm side walls. To maximize mathematical accuracy, each substrate was treated with a different selection of 7 to 10 doses, based on preliminary trials done to bracket a dose range for each substrate and disinfestant so at least one dose resulted in 0 and 100% mortality each and multiple doses resulted in a range of mortalities greater than 0 and less than 100%. Doses were 0.00, 0.04, 0.08, 0.12, 0.16, 0.24, 0.41, 0.82, 1.63, 2.45, 4.08, and 6.13 g a.i. of chlorazene hydrosol per liter of water; 0.0, 0.9, 1.8, 2.7, 3.6, 4.5, 5.4, 7.2, 9.0, 12.6, 14.4, and 18.0 g a.i. of hydrogen dioxide per liter of water; 0.0, 5.4, 10.8, 16.2, 22.5, 29.7, 45.0, 55.8, 60.3, 67.5, 78.3, 90.0, 112.5, 119.7, 135.0, 150.3, 157.5, 180.0, and 225.0 g a.i. of hydrogen peroxide per liter of water; 0.00, 0.08, 0.17, 0.25, 0.33, 0.42, 0.50, 0.67, 0.83, 1.00, 1.17, 1.33, 1.50, 1.67, 1.83, 2.00, 2.17, and 2.50 g a.i. of iodine per liter of water; 0.00, 0.07, 0.17, 0.20, 0.33, 0.47, 0.50, 0.67, 0.87, 1.13, 1.53, 2.00, 2.67, 3.33, 4.00, 5.33, 6.67, 8.33, 9.33, 10.00, 12.00, and 14.67 g a.i. of quaternary ammonium chloride per liter of water; and 0.00, 0.06, 0.14, 0.20, 0.40, 0.60, 0.70, 0.87, 0.98, 0.99, 1.00, 1.40, 1.75, 1.80, 2.40, 2.62, 3.00, 3.50, 4.00, 4.38, 5.00, 5.25, 6.00, 7.88, 9.00, 10.50, 12.00, and 18.00 g a.i. of sodium hypochlorite per liter of water.

Approximately 0.01 ml of a water suspension of 2 × 10^6 conidia per ml was applied with a nongraduated sterile glass pipette to the upper surface of the substrates. Infested substrates were maintained at room conditions (21°C, 10 to 11 h light per day from fluorescent ceiling lamps) but protected from direct sunlight. Sixteen to 20 h later, on day two, substrates were sprayed with the disinfestant doses. Three to 5 h later, which allowed complete drying of disinfestants from the surfaces, substrates were inverted and gently rubbed against the surface of half-strength PDA (hPDA) to dislodge hundreds to over a thousand conidia. Plates were maintained at room conditions. On day three, greater than one hundred conidia per treatment and replication were counted as germinated or not germinated.

Binomial lethal dose. Hydrogen dioxide, quaternary ammonium chloride, and sodium hypochlorite were tested in three separate experiments that were repeated. Experimental design was a completely randomized design with five replications. All seven substrates were placed in a tray (described previously) per dose and replication. A 0.01-ml volume of a water suspension of 5 × 10^5 conidia per ml was applied with an adjustable pipette (Eppendorf Series 2100, 10 to 100 µl) to the upper surface of the substrates. Timing sequence of tasks and spraying of disinfestants were done as previously described. Based on the probit-predicted lethal dose curves generated previously, a zero dose and six to seven doses ranging from approximately the LD_{50} to greater than the LD_{99} were applied to all substrates to obtain some germination at lower doses and zero germination at higher doses. Doses were 0.0, 5.2, 10.5, 15.8, 21.0, 26.8, and 31.5 g a.i. of hydrogen dioxide per liter of water; 0.0, 3.5, 7.0, 10.5, 14.0, 17.5, and 21.0 g a.i. of quaternary ammonium chloride per liter of water; and 0.0, 0.6, 1.2, 2.6, 3.8, 5.0, 7.5, and 10.1 g a.i. of sodium hypochlorite per liter of water. Different from the previously described methods, substrate pieces were inverted and plated on hPDA, with three to four substrate pieces per 100-mm plastic petri dish. Substrate pieces were checked daily over a 7-day period for the appearance of mycelium. The measured binomial response was "nongermination of the approximately 5,000 spores" or "one or more spores germinated". When mycelium was macroscopically detected beyond the edge of the substrate, plates were moved to a laminar air flow hood. A solid agar piece that comprised agar from a minimum of 0.5 cm outside the radius of the mycelium and the substrate was excised with a flame-sterilized scalpel and moved to an empty sterile petri dish. A maximum of three agar pieces and substrates were placed in one petri dish, with 1.5 to 2 cm of space between the agar pieces. Plates were kept at room conditions (21°C, 10 to 11 h light from fluorescent ceiling lamps) for approximately 1 week until cultures sporulated and *B. cinerea* could be confirmed.

A second set of binomial lethal dose experiments was done, with separate experiments for quaternary ammonium chloride and sodium hypochlorite that were repeated. Each replication of a dose consisted of one raw pine substrate per tray. Doses were 0, 50, 100, 150, and 200 g a.i. of quaternary ammonium chloride per liter of water and 0, 30, 45, and 60 g a.i. of sodium hypochlorite per liter of water. Experiment protocol was followed exactly as described in the preceding paragraph.

Statistical analysis. The experimental design of the lethal dose curve response was a randomized complete block of four blocks with a nested treatment structure of seven substrates and 7 to 10 rates per substrate. Block was equivalent to replication and was done on separate days. Analysis of variance was performed on "spore mortality" at zero rate using PROC MIXED, followed by a mean comparison between substrates (SAS Institute, Cary, NC).

Probit analysis was performed to estimate a lethal dose curve response for each substrate. First, spore mortality was adjusted for mortality due to substrate by the formula

$$Madj = (Mi - Mctrl)/(1 - Mctrl)$$

where Mi is the mortality at rate i for that substrate, Mctrl is the mortality of the control (zero rate) for that substrate and replication, and Madj is the adjusted mortality. The adjusted mortality formula resets the mortality at zero rate to zero and proportionally scales all values between 0 and 1, except for values of 1. Resulting negative values were treated as zero.

Probit regressions were estimated for log 10 transformed adjusted mortality (Madj) values as a function of rate. Based on theses equations, estimates of the values of the dose (lethal dose) that yields a desired mortality were computed using PROC PROBIT (SAS Institute). Confidence limits about these estimated lethal doses are inverse confidence limits because they are computed for the value of the independent variable (dose) that yields a specified response (mortality). The estimated lethal doses that yield a 50 and 90% mortality are referred to as the LD_{50} and LD_{90}, respectively.

Comparisons of substrates were made using the LD_{50} and slope of the probit regression. The LD_{50} measures the average (μ) dose required to prevent germination of a conidium, and the slope (1/σ) provides a measure of variance of the dose around the LD_{50} (8). Additionally, the LD_{90} values (theoretical dose for control) were compared.

Pairwise comparisons of slopes between experiments and between substrates within an experiment were performed using a χ^2 test ($P \leq 0.05$) (SAS OnlineDoc Version Eight, The PROBIT Procedure, example 54.2: Multilevel Response, SAS Institute). A significantly larger slope would indicate there is a greater change in mortality per unit change in concentration from the LD_{50} than with a smaller slope. With a smaller slope, more units of a disinfestant would be required to reach a higher LD value than with a larger slope.

The 95% inverse confidence limits were calculated on the log scale and back transformed to the original scale for LD_{50} and LD_{90} values (5). The confidence limits were used to compare the LD values between substrates within experiments. LD values were declared significantly different if their corresponding 95% inverse confidence limits did not overlap.

For the binomial lethal dose, the experimental design was completely randomized with a factorial treatment structure of seven substrates, seven doses per substrate, and five replications. Analysis of variance (PROC MIXED) was used to compare experiments and to compare substrates based on the number of pieces from which *B. cinerea* grew for all doses. The error term was the random effects of replication, experiment, and their interaction. Differences among substrates were determined by pairwise comparison of *t* tests for the statistical significance of the difference between least square means (LSMEANS). However, the main objective of the experiment was to obtain a mean minimum dose at which no conidia germinated from the approximately 5,000 conidia per substrate for five replications. The mean minimum dose at which no growth occurred was compared directly to the lethal doses calculated in the probit analysis.

RESULTS

Lethal dose curve response. Germination data were pooled for 0 g a.i./liter from 12 disinfestant studies to test the effect of substrate. A significant difference ($P < 0.05$) in mortality resulted from the substrate (Table 1). As a result, mortality due to substrate was rescaled per replication.

For all six disinfestants, some substrates had significantly different probit regression trends between experiments; therefore, data are shown individually for repeated experiments (Table 2). Based on comparison of regression trends and confidence limits of LD_{50} values, substrates were significantly different (Table 2).

For chlorazene hydrosol, LD_{90} values ranged from 0.21 to 4.54 g a.i./liter (Table 2). Relative order of substrates from the lowest to highest LD_{90} value was similar between experiments. The relative value and differences of LD_{90} values closely reflected patterns of the LD_{50} values and not differences in slope. Differences in slopes existed but were relatively small among most substrates. The steepest slope occurred with the highest LD_{50} value (raw pine). The LD_{90} value of six substrates in experiment one and five substrates in experiment two were less than the median value of 2.38 and 1.75 g a.i./liter per experiment, respectively.

For hydrogen dioxide, LD_{90} values ranged from 4.99 to 19.95 g/liter (Table 2). The relative order of substrates from the lowest to highest LD_{90} value was not similar between experiments, although polyethylene ground fabric and raw pine were consistently in the low and high part of the range, respectively. The number of substrates with overlapping confidence limits and the relative order of substrates was greater between LD_{50} values than between LD_{90} values. Relative order of substrates from the lowest to steepest slope was more similar than LD_{50} values, but the wide range in slopes accentuated differences between LD_{50} values and was reflected in the pattern of LD_{90} values (Table 2). Low LD_{90} values for ground fabric in both experiments resulted from intermediate LD_{50} values and steep slopes. Most other substrates had a higher LD_{90} value than ground fabric regardless of the relationship between LD_{50} values because the other substrates had lower slopes. Raw pine in both experiments had high LD_{90} values despite having steep slopes because they also had high LD_{50} values. The LD_{90} values of three substrates per experiment were less than the median value of 14.27 and 9.42 g a.i./liter for experiments one and two, respectively.

For hydrogen peroxide, LD_{90} values ranged from 63.0 to 233.1 g/liter (Table 2). Relative orders of substrates from the lowest to highest LD_{90} and LD_{50} values were not similar between experiments, except for polyethylene ground fabric, which had low values, and raw pine and galvanized metal, which had high values. Raw pine and galvanized metal had high LD_{90} values despite having steep slopes because they also had high LD_{50} values. The LD_{90} values of five substrates in experiment one and of three substrates in experiment two were less than the median values of 130.6 and 148.1 g a.i./liter per experiment, respectively.

For iodine, LD_{90} values ranged from 0.42 to 2.45 g/liter (Table 2). The relative order of substrates from lowest to highest LD_{90} value, LD_{50} value, and slope was similar between experiments. Within experiments, confidence limits of multiple LD_{90} values overlapped. Painted pine had a low LD_{90} value in both experiments despite having the lowest slope because of a low LD_{50} value. Pot plastic had a low LD_{90} value in both experiments because of an intermediate slope and relative low LD_{50} value. The relative order of LD_{90} values within an experiment differed for stainless steel mainly due to differences in slope rather than to LD_{50} values. Raw pine had high LD_{90} values despite having steep slopes because they also had high LD_{50} values. The LD_{90} values of five substrates in experiment 1 and six substrates in experiment 2 were less than the median values of 0.80 and 1.62 g a.i./liter per experiment, respectively.

For quaternary ammonium chloride, LD_{90} values ranged from 0.64 to 8.62 g/liter (Table 2). The relative orders of substrates from the lowest to highest LD_{90} values were similar between experiments based on stainless steel and galvanized metal having the two lowest LD_{90} values, painted pine, pressure-treated pine and ground fabric having intermediate LD_{90} values, and raw pine having the highest LD_{90} value. The relative orders of substrates from the lowest to highest LD_{50} values were also similar between experiments, but the order of slopes varied more.

Table 1. Mean mortality of *Botrytis cinerea* conidia after 18 to 22 h contact with materials found in plant production systems[y]

Substrate	Mean[z]
Pine (raw)	0.07 a
Pressure-treated pine	0.21 b
Polyethylene ground fabric	0.35 c
Galvanized metal	0.36 c
Polyethylene pot plastic	0.50 d
Exterior latex-painted pine	0.59 e
Stainless steel	0.60 e
Standard error	0.041

[y] Data were the pooled zero dose treatments (n = 48 per substrate) from 12 disinfestant experiments.

[z] Substrate was significantly different at $P < 0.0001$ based on PROC MIXED model (SAS Institute). Means with the same letter are not significantly different at $P = 0.05$ based on analysis of least square means.

The LD_{90} values of five substrates per experiment were less than the median values of 4.69 and 3.55 g a.i./liter for experiments 1 and 2, respectively.

For sodium hypochlorite, LD_{90} values ranged from 0.64 to 5.63 g/liter (Table 2). The relative orders of LD_{90} values were similar between experiments based on stainless steel and galvanized metal consistently having low LD_{90} values; ground fabric, painted pine, and pot plastic having fluctuating but intermediate LD_{90} values;

Table 2. Probit prediction of disinfestant concentration (g a.i./liter) when sprayed on materials of various production surfaces (galvanized metal, stainless steel, polyethylene ground fabric, polyethylene pot plastic, pressure-treated pine, exterior latex-painted pine, raw pine) contaminated with *Botrytis cinerea* conidia[v]

Disinfestant – Exp.[w] Substrate	LD_{90} (95% CL)[x] (g/liter)		LD_{50} (95% CL)[y] (g/liter)		Slope (SE)[x]		n[z]
Chlorazene hydrosol - Exp. 1							
Latex painted	0.21	(0.19-0.24)	0.04	(0.03-0.05)	1.82	(0.089)	24
Stainless	0.49	(0.44-0.56)	0.11	(0.10-0.11)	1.93	(0.081)	25
Polyethylene	0.57	(0.52-0.63)	0.15	(0.14-0.16)	2.19	(0.075)	27
Pressure-treated	0.62	(0.57-0.70)	0.15	(0.14-0.16)	2.02	(0.066)	27
Galvanized	0.40	(0.37-0.44)	0.12	(0.11-0.12)	2.36	(0.081)	27
Polyethylene	1.52	(1.37-1.71)	0.27	(0.25-0.29)	1.70	(0.051)	29
Raw pine	4.54	(4.03-5.18)	0.99	(0.93-1.06)	1.94	(0.060)	29
Chlorazene hydrosol - Exp. 2							
Latex painted	0.51	(0.47-0.56)	0.13	(0.12-0.14)	2.16	(0.070)	28
Stainless	0.63	(0.57-0.71)	0.09	(0.08-0.10)	1.55	(0.058)	28
Polyethylene	0.68	(0.61-0.77)	0.10	(0.09-0.11)	1.54	(0.057)	28
Pressure-treated	0.70	(0.63-0.79)	0.13	(0.12-0.14)	1.75	(0.058)	28
Galvanized	1.71	(1.46-2.07)	0.16	(0.15-0.18)	1.26	(0.050)	28
Polyethylene	2.02	(1.84-2.24)	0.40	(0.37-0.43)	1.81	(0.056)	28
Raw pine	2.99	(2.75-3.29)	0.73	(0.69-0.78)	2.10	(0.058)	28
Hydrogen dioxide - Exp. 1							
Polyethylene	8.58	(8.19-9.02)	3.91	(3.76-4.06)	3.76	(0.116) e	28
Stainless	18.56	(17.18-20.25)	6.15	(5.90-6.41)	2.67	(0.095) c	28
Polyethylene	15.08	(13.94-16.47)	4.34	(4.12-4.55)	2.37	(0.087) b	28
Pressure-treated	12.78	(11.87-13.88)	3.82	(3.61-4.02)	2.44	(0.090) bc	28
Latex painted	9.39	(8.59-10.36)	2.16	(2.02-2.30)	2.01	(0.072) a	28
Galvanized	14.35	(13.24-15.70)	4.01	(3.79-4.22)	2.31	(0.087) b	28
Raw pine	19.95	(18.68-21.46)	7.66	(7.40-7.94)	3.08	(0.096) d	31
Hydrogen dioxide - Exp. 2							
Polyethylene	6.02	(5.79-6.28)	3.27	(3.17-3.37)	4.83	(0.151) de	29
Stainless	4.99	(4.75-5.27)	2.18	(2.08-2.28)	3.56	(0.120) c	29
Polyethylene	6.07	(5.82-6.34)	3.13	(3.03-3.23)	4.46	(0.139) d	32
Pressure-treated	9.90	(9.22-10.72)	3.24	(3.10-3.38)	2.91	(0.092) b	28
Latex painted	13.84	12.95-14.90)	5.03	(4.84-5.23)	2.64	(0.078) a	31
Galvanized	9.79	(9.33-10.31)	3.99	(3.83-4.15)	3.29	(0.101) c	31
Raw pine	11.78	(11.36-12.24)	6.41	(6.25-6.57)	4.85	(0.132) e	32
Hydrogen peroxide - Exp. 1							
Polyethylene	74.4	(70.7-78.6)	32.5	(30.9-34.1)	3.56	(0.124) d	24
Pressure-treated	86.2	(81.2-92.1)	31.9	(29.7-34.0)	2.97	(0.126) c	24
Stainless	104.2	(97.5-112.1)	33.7	(31.6-35.8)	2.62	(0.096) b	24
Latex painted	70.6	(63.6-79.6)	13.6	(12.1-15.0)	1.79	(0.008) a	24
Polyethylene	96.0	(92.2-100.2)	51.0	(49.4-52.6)	4.67	(0.151) e	24
Raw pine	167.2	(159.5-176.3)	91.2	(88.7-93.7)	4.87	(0.176) e	24
Galvanized	190.6	(173.6-212.4)	55.8	(53.0-58.5)	2.40	(0.104) b	24
Hydrogen peroxide - Exp. 2							
Polyethylene	63.0	(59.1-67.5)	19.6	(18.4-20.7)	2.52	(0.084) c	31
Pressure-treated	133.4	(127.8-139.6)	66.5	(64.6-68.4)	4.24	(0.116) e	32
Stainless	121.0	(109.9-134.9)	23.2	(21.5-24.9)	1.79	(0.070) b	31
Latex painted	190.0	(164.6-224.1)	22.6	(20.9-24.4)	1.39	(0.056) a	33
Polyethylene	225.5	(195.6-266.2)	28.4	(26.1-30.7)	1.42	(0.062) a	31
Raw pine	233.1	(219.3-249.5)	93.0	(90.0-96.0)	3.21	(0.102) d	33
Galvanized	202.1	(193.9-211.4)	109.2	(106.7-111.8)	4.80	(0.144) f	33
Iodine - Exp. 1							
Polyethylene	0.52	(0.49-0.55)	0.21	(0.20-0.22)	3.28	(0.116) c	31
Latex painted	0.55	(0.52-0.59)	0.16	(0.15-0.17)	2.38	(0.084) a	34
Polyethylene	0.42	(0.40-0.44)	0.20	(0.18-0.21)	3.86	(0.180) d	28
Stainless	0.44	(0.42-0.46)	0.20	(0.19-0.21)	3.80	(0.136) d	24
Galvanized	0.74	(0.69-0.80)	0.27	(0.25-0.28)	2.88	(0.107) b	23
Pressure-treated	1.09	(1.04-1.14)	0.48	(0.46-0.50)	3.62	(0.114) d	33
Raw pine	1.18	(1.14-1.22)	0.72	(0.70-0.73)	5.92	(0.158) e	35

(*continued on next page*)

[v] Dose curve response is characterized by the lethal doses resulting in 90 and 50% mortalities (LD_{90} and LD_{50}), and the slope.

[w] Disinfestants tested were chlorazene hydrosol (Chloramin-T, 99.99% a.i.), hydrogen dioxide (ZeroTol, 27% a.i.), hydrogen peroxide (ACS grade, 27% a.i.), quaternary ammonium chloride (Green Shield, 20% a.i.), iodine (Lugol iodine, 5% a.i.), and sodium hypochlorite (bleach, 6% a.i.). Each disinfestant was done as a separate experiment and each was repeated (Exp. 1 and 2).

[x] LD values with overlapping 95% confidence limits (CL) are not significantly different. All probit curves had a χ^2 probability of <0.0001.

[y] Significant differences of slopes were based on pairwise analysis of substrates using PROC PROBIT. Standard error of the slope (SE) is in parentheses. Slopes with the same letter are not significantly different.

[z] Number of doses × replications (n) per dose curve calculation. The number of doses varied per replication as a dose(s) sometimes was added or deleted in subsequent replications.

and pressure-treated pine and raw pine consistently having high LD_{90} values. The relative orders of substrates from the lowest to highest slopes were also similar between experiments, but the order of LD_{50} values varied more. Raw pine had high LD_{90} values despite having steep slopes because they also had high LD_{50} values. The LD_{90} values of five substrates in experiment 1 and six substrates in experiment 2 were less than the median values of 3.24 and 3.99 g a.i./liter per experiment, respectively.

Binomial lethal dose. Mean number of pieces over all doses with growth of *B. cinerea* were significantly different for substrates at $P = 0.10$ for hydrogen dioxide and $P = 0.05$ for quaternary ammonium and sodium hypochlorite (Table 3). Painted wood was not included in the analysis for all three disinfestants because all values were zero with zero variance. Raw pine was not included in the analysis for quaternary ammonium because all values were one with zero variance.

The LD_{b100} values for hydrogen dioxide corresponded to doses estimated by the probit regression from LD_{85} to outside of the confidence limits of the LD_{99} values (Table 4). The differences between the LD_{b100} values that were greater than the LD_{99} and the upper limit of the LD_{99} confidence intervals were from 9.8 to 20.9 g a.i./liter. The LD_{b100} values for quaternary ammonium corresponded to doses from LD_{90} to outside of the confidence limits of the LD_{99} values (Table 4). More than half of the LD_{b100} values were outside of the confidence limits of the LD_{99}. However, the differences between the LD_{b100} values that were greater than the LD_{99} and the upper limit of the LD_{99} confidence intervals were only from 0.1 to 10.6 g a.i./liter. The LD_{b100} values for sodium hypochlorite corresponded to doses from LD_{70} to LD_{99} (Table 4).

B. cinerea grew from all of the raw pine pieces treated with up to 21 g of quaternary ammonium chloride per liter. In a second experiment with raw pine as the only substrate, *B. cinerea* grew from every piece even with rates up to 200 g a.i. of quaternary ammonium chloride per liter (pure product). *B. cinerea* grew from all of the raw pine pieces treated with up to 10 g of sodium hypochlorite per liter. In a second experiment with raw pine as the only substrate, *B. cinerea* grew from every piece even with rates up to 60 g a.i. of sodium hypochlorite per liter (pure product). For hydrogen dioxide, quaternary ammonium, and sodium hypochlorite, *B. cinerea* did not grow from any of the latex-painted pine pieces even at 0 g/liter.

DISCUSSION

Several types of materials are used to construct production surfaces in greenhouses and nurseries, such as pressure-treated wood, painted wood, and galvanized fencing for bench tops, stainless steel for head-house benches, and ground fabric in greenhouses and production pads in nurseries. The fact that different doses of disinfestants are required in response to the type of material being disinfested brings to the industry's attention that these surfaces are neither uniform nor inert. Typically, disinfestants are simple chemicals, and their effectiveness is in part due to their highly reactive nature. Yet the reasons for dose differences may vary, and include (i) reactiveness of a disinfestant with the surface material (a high demand load that results in the need to proportionally increase dose), (ii) vapor potential of the active chemical moiety when a disin-

Table 2. (*continued from previous page*)

Disinfestant – Exp.[w] Substrate	LD_{90} (95% CL)[x] (g/liter)		LD_{50} (95% CL)[y] (g/liter)		Slope (SE)[x]		n[z]
Iodine - Exp. 2							
Polyethylene	0.78	(0.74-0.84)	0.28	(0.27-0.30)	2.90	(0.080) c	33
Latex painted	0.80	(0.73-0.88)	0.16	(0.15-0.17)	1.84	(0.067) a	33
Polyethylene	0.91	(0.85-0.99)	0.27	(0.25-0.28)	2.40	(0.071) b	33
Stainless	1.12	(1.05-1.20)	0.35	(0.34-0.37)	2.54	(0.075) b	35
Galvanized	1.71	(1.61-1.82)	0.61	(0.59-0.64)	2.89	(0.073) c	36
Pressure-treated	1.44	(1.38-1.52)	0.58	(0.56-0.61)	3.26	(0.097) d	33
Raw pine	2.45	(2.31-2.62)	0.95	(0.92-0.98)	3.12	(0.092) cd	32
Quaternary ammonium chloride - Exp. 1							
Stainless	0.75	(0.69-0.83)	0.13	(0.11-0.15)	1.67	(0.097) d	29
Galvanized	1.05	(1.00-1.11)	0.45	(0.44-0.47)	3.52	(0.112) g	30
Polyethylene	2.98	(2.52-3.65)	0.15	(0.11-0.18)	0.98	(0.055) a	30
Latex painted	2.76	(2.35-3.34)	0.22	(0.18-0.25)	1.16	(0.063) b	32
Pressure-treated	5.82	(5.32-6.43)	1.46	(1.38-1.54)	2.14	(0.073) e	30
Polyethylene	4.04	(3.56-4.67)	0.47	(0.43-0.52)	1.38	(0.054) c	28
Raw pine	8.62	(8.01-9.35)	2.84	(2.71-2.98)	2.66	(0.089) f	30
Quaternary ammonium chloride - Exp. 2							
Stainless	0.75	(0.72-0.79)	0.32	(0.20-0.33)	3.41	(0.106) d	28
Galvanized	0.64	(0.60-0.70)	0.17	(0.15-0.18)	2.18	(0.078) b	28
Polyethylene	0.93	(0.87-1.01)	0.23	(0.21-0.24)	2.08	(0.069) b	33
Latex painted	5.63	(4.61-7.16)	0.27	(0.23-0.30)	0.97	(0.049) a	30
Pressure-treated	2.02	(1.87-2.20)	0.45	(0.37-0.53)	1.96	(0.123) b	27
Polyethylene	3.15	(2.68-3.79)	0.10	(0.05-0.17)	0.87	(0.084) a	27
Raw pine	6.46	(6.03-6.97)	2.12	(2.02-2.22)	2.65	(0.081) c	28
Sodium hypochlorite - Exp. 1							
Stainless	0.87	(0.71-1.08)	0.02	(0.01-0.03)	0.77	(0.068) a	24
Galvanized	1.07	(0.98-1.19)	0.26	(0.24-0.28)	2.06	(0.076) e	24
Polyethylene	1.41	(1.28-1.56)	0.30	(0.25-0.34)	1.90	(0.096) de	23
Latex painted	0.87	(0.77-0.99)	0.12	(0.10-0.14)	1.51	(0.077) c	22
Polyethylene	2.09	(1.94-2.26)	0.67	(0.62-0.72)	2.59	(0.098) f	23
Pressure-treated	5.61	(4.78-6.35)	0.33	(0.22-0.43)	1.04	(0.080) b	29
Raw pine	3.34	(3.06-3.70)	0.63	(0.53-0.73)	1.77	(0.100) d	29
Sodium hypochlorite - Exp. 2							
Stainless	1.13	(1.04-1.22)	0.33	(0.31-0.34)	2.39	(0.065) e	32
Galvanized	1.17	(1.09-1.27)	0.39	(0.37-0.41)	2.67	(0.074) f	32
Polyethylene	1.16	(1.06-1.28)	0.27	(0.25-0.29)	2.02	(0.059) d	32
Latex painted	3.49	(3.01-4.12)	0.35	(0.32-0.38)	1.28	(0.046) a	33
Polyethylene	1.59	(1.45-1.75)	0.32	(0.30-0.34)	1.84	(0.047) c	32
Pressure-treated	3.86	(3.47-4.37)	0.35	(0.28-0.41)	1.22	(0.057) a	39
Raw pine	6.84	(6.20-7.64)	1.02	(0.94-1.10)	1.55	(0.065) b	39

festant is dispersed thinly on a surface (increased volatilization with a large surface area per volume ratio), (iii) water tension properties of a disinfestant (poorly dispersed thus poor coverage), (iv) hydrophobicity properties of substrates (droplets that do not spread), and (v) irregular topography of a substrate's surface (escape sites for pathogen propagules and resulting air pockets that reduce coverage). None of these factors was isolated as a treatment in this study, so it was not known whether one or a combination of these reasons, or other reasons, caused the need for dose differences.

Substrate not only affected the effectiveness of a disinfestant but also affected conidial germination of *B. cinerea*, which indicates that conidial survival may be affected by the substrate, particularly ground fabric, exterior latex-painted pine, and stainless steel. The range of mortality due to substrate was unexpected and the cause not known. However, in the case of exterior paint, it is likely that it contained an antifungal compound. Other studies will be needed to determine the practical implications of spore survival due to substrate and the reason(s) for these results. In addition, high mortality due to substrate did not automatically result in low LD_{90} values.

The smallest range across LD_{90} values for all substrates tested was with chlorazene hydrosol and iodine. The lower reactivity of both products with surrounding compounds like organic matter was the reason they were included in this study. Both products have the disadvantage of relatively short shelf lives from the date of manufacturing, less than 3 years. Since neither was tested in an LD_b response, a comparison between the LD_{b100} and LD_{90} couldn't be made.

Hydrogen peroxide had LD_{90} values that were as much as 10 times greater than those of hydrogen dioxide. Some LD_{90} values approached the dose equivalent to pure product, 270 g a.i./liter. The chemical nature of the differences between the products containing hydrogen dioxide and hydrogen peroxide was beyond the scope of this project. The results indicate that hydrogen peroxide would be a poor substitute for hydrogen dioxide.

Most of the LD_{b100} values were within the LD_{90} and LD_{99} values, which indicates that the LD response curves accurately calculated doses needed for control. LD_{b100} values were consistently higher than the LD_{99} values with hydrogen dioxide on polyethylene ground fabric and with quaternary ammonium chloride on galvanized metal and stainless steel. While the difference in dose between the LD_{b100} values and upper confidence limit of the LD_{99} values were greatest with hydrogen dioxide, it should be compared relative to the changes in dose along the lethal dose curve response. In this study, the range between the LD_1 and LD_{50} values was smaller than the range between the LD_{95} and LD_{99} values. Therefore, differences between the LD_{b100} and LD_{99} values maybe relatively small.

The LD values calculated by probit analysis provide quantitative data that provide a direct measure of all the intermediate dose responses. The results of this study demonstrate that an LD_b value validates many of the LD curves but also identified circumstances when additional replications or alternative approaches would have improved the probit prediction. Considerably less effort is required to demonstrate an effective dose with an LD_b response than with the LD curve response.

Two response deviations were identified with the LD_b response, those with raw pine and with exterior latex-painted pine. With both substrates, an LD curve was calculated, yet *B. cinerea* grew from all raw pine pieces treated with quaternary ammonium chloride and sodium hypochlorite, and no *B. cinerea* grew from any painted pine pieces. In the case of painted pine, a fungicidal component of the paint is likely responsible. In developing the LD response curve, conidia were in contact with the paint surface for nearly 24 h. During that time, a percent mortality occurred due to the substrate and the disinfestant dose, but viable conidia germinated once dislodged onto agar. In developing the LD_b response, conidia had a sustained contact of 7 days with the paint surface that completely inhibited fungal growth.

The response pattern with raw pine was specific to quaternary ammonium chloride and sodium hypochlorite. One possible explanation is that a few conidia escaped

Table 3. Proportion of substrate pieces, across all concentrations (g a.i./liter) of a disinfestant, from which *Botrytis cinerea* grew

Substrate	Hydrogen dioxide[z]	Quaternary ammonium	Sodium hypochlorite
Pine (raw)	0.43 ab	1.00	0.94 a
Polyethylene ground fabric	0.57 a	0.40 a	0.13 b
Polyethylene pot plastic	0.50 ab	0.24 b	0.15 b
Galvanized metal	0.39 b	0.14 b	0.12 b
Stainless steel	0.36 b	0.19 b	0.18 b
Pressure-treated pine	0.36 b	0.19 b	0.14 b
Exterior latex-painted pine	0	0	0
Standard deviation	0.166	0.140	0.080

[z] Data were analyzed using PROC MIXED (SAS Institute). Means with the same letter are not significantly different at $P = 0.05$ based on analysis of least square means. Means with no letter were excluded from the analysis of variance because of zero variance.

Table 4. The lowest binomial lethal dose (LD_{b100}, g a.i./liter) of a disinfestant at which it and all higher doses resulted in no growth from substrates infested with 5,000 *Botrytis cinerea* conidia per surface that had been placed on potato dextrose agar for 7 days[y]

		Hydrogen dioxide			Quaternary ammonium			Sodium hypochlorite		
Substrate	Exp.	LD_{b100}	LD (%) Exp. 1	LD (%) Exp. 2	LD_{b100}	LD (%) Exp. 1	LD (%) Exp. 2	LD_{b100}	LD (%) Exp. 1	LD (%) Exp. 2
Galvanized metal	1	21.0	94	99	3.5	>99	>99	2.6	98	98
	2	15.8	91	97	3.5	>99	>99	0.6	80	70
Stainless steel	1	21.0	91	99	10.5	>99	>99	3.8	96	99
	2	21.0	91	99	7.0	>99	>99	0.6	90	75
Polyethylene fabric	1	21.0	99	>99	14.0	98	96	2.6	96	97
	2	31.5	99	>99	10.5	96	95	0.6	70	75
Pot plastic	1	21.0	94	>99	14.0	97	>99	3.8	97	97
	2	31.5	98	>99	3.5	90	>99	2.6	93	95
Pressure-treated pine	1	10.5	85	85	10.5	96	>99	10.1	93	96
	2	26.2	98	98	10.5	96	>99	5.0	90	92
Pine (raw)	1	21.0	94	99	–[z]	–	–	–	–	–
	2	15.8	91	97	–	–	–	–	–	–

[y] Dose of the LD_{b100} is matched to the lowest percent spore mortality (e.g., LD_{94}) with an equivalent dose that was calculated in the lethal dose curve response using probit analysis. Values are compared for the two binomial lethal dose experiments and the two lethal dose curve response experiments because no relationship existed between the experiment labels "1" and "2" of the two types of experiments.

[z] An LD_{b100} value was not attained because no dose, even 100% product, resulted in zero growth of *B. cinerea* from the substrate pieces.

in untreated niches. The reason for a calculable mortality in the LD response curve was that the conidia in the niches were not dislodged onto the agar, and therefore the sample used to develop the LD test was biased. However, this may not be the case, because the same response pattern did not occur with hydrogen dioxide. An alternative explanation is that the raw pine provided an extremely high demand load because its surface is highly reactive even with an excessively high dose of the disinfestant. If this were true, it would be difficult to explain why an LD response curve was calculable. It is interesting that a steep slope was typically associated with raw pine regardless of the disinfestant, yet no relationship between that result and the results with quaternary ammonium chloride and sodium hypochlorite seem apparent.

Disinfestant rates developed in medical and food hygiene environments are done on relatively smooth and often expensive surfaces (ceramic tile, Formica, linoleum, and stainless steel) that have been cleaned to regulated standards. Horticultural production surfaces and sanitation practices are not regulated and vary among businesses based on economical consideration, management style, and past history of problems. The study shows that doses should be selected based on which clean substrate is being treated. Further work is needed to determine the dose needed to disinfest surfaces soiled with small amounts of algae, fines of peat and bark, and other components of potting media. Furthermore, dose may be dependent on the pathogen being targeted.

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