Equipment Decontamination with a Mobile Power Washer Followed by Disinfectant Applications

Marissa L. Layman[1], Craig L. Ramsey[2] and Steve E. Newman[3,*]

[1]*Independent Research Associate, Thornton, CO 80229, USA*

[2]*Retired USDA-APHIS, Fort Collins, Colorado 80526, USA*

[3]*Colorado State University, Horticulture and Landscape Architecture, Fort Collins, CO, 80523, USA*

Abstract: Decontamination of field equipment has been used by farmers for many years in order to prevent the spread of plant and animal diseases. A greenhouse study evaluated the effects of an electrostatic sprayer, and several disinfectants on their efficacy to inactivate *Bacillus subtilis* spores. In addition, a field study was conducted with a two stage, decontamination system that evaluated a mobile power washer, five disinfectants, and repeated disinfectant applications on their efficacy to dislodge and inactivate *B. subtilis* spores. In the first study, EasyDecon@ DF 200 reduced viable spores by a log10 reduction of 1.42. In the second study, power washing effectively dislodged viable spores by nearly 3-fold compared to applying the disinfectants alone. EasyDecon@ DF 200 applied three times and Electro Biocide applied twice resulted in the greatest reduction of viable spores (4.51 log10) when applied after power washing. Two stage decontamination of agricultural equipment is effective for sanitizing most equipment that do not have exposed electronic instruments or sensors. Mobile power washers are economical for small scale sanitization of farm equipment daily.

Keywords: Equipment decontamination, Power washing, Disinfectants.

1. INTRODUCTION

Two-stage equipment decontamination involves power washing (first stage) followed by a disinfectant application (second stage). Several studies have shown the effectiveness of two stage decontamination for equipment or vehicles [1-4]. Decontamination systems can be mobile or stationary, depending on the capacity needs of the farmer or producer. Mobile systems provide the flexibility to conduct equipment cleaning on a “as needed” basis. Stationary systems can be automated with high equipment or truck processing rates, which are necessary at large processing facilities. Power washing with high water pressure (13,790 kPa) removes most of the bio-contaminates. Mobile power washers use large water volumes and, therefore, should be designed to recycle the wastewater in order to conserve water at sites where access to water may be limited. Mobile and stationary power washing systems should be further evaluated for their ability to decontaminate, prevent soil and water bio-contamination, and be cost effective.

Decontamination under harsh conditions and complex surface conditions, such as road grime on vehicles, biofilms on processing equipment, and dirt and oil on field equipment, generally require two stage decontamination using a combination of power washing and disinfectants [4, 5]. Such real-world conditions require that disinfectants effectively inactivate a wide range of pathogens on highly soiled surfaces [6]. Further research is needed to evaluate a range of microbial surrogates and tests that simulate real-world decontamination conditions. Disinfectant tests should also include repeat applications over time intervals that match the surface drying time of the disinfectant application in order to improve their effectiveness [7].

Electrostatic sprayers have the potential to improve disinfectant coverage on complex or semi-enclosed surfaces such as engines or processing equipment [8, 9]. Electrostatic sprayers typically generate small droplets (40 µm) with a negative charge that are attracted to positively charged surfaces [10]. Also, by generating smaller droplets with a uniform negative charge, the droplets repel each other and spread out covering a wider surface area.

In this study, *Bacillus subtilis* was used as the microbial surrogate to evaluate the effectiveness of each stage in the decontamination process. *B. subtilis* is a gram positive bacterium that is commonly found in the soil, air, and plant compost [11]. The endospore stage allows it to survive under the harshest environmental conditions, which makes it an excellent surrogate for field studies [12, 13].

Two decontamination studies were conducted in 2016-17 to evaluate multiple disinfectants and power washing disinfectant systems. The objective of the first

*Address correspondence to this author at the Colorado State University, Horticulture and Landscape Architecture, Fort Collins, CO, 80523, USA;
Tel: +1 (970) 491-7118; E-mail: Steven.Newman@colostate.edu

E-ISSN: 2409-9813/20

study was to determine the efficacy of four disinfectants applied with an electrostatic sprayer on the inactivation of *B. subtilis* spores on porous and non-porous surfaces. The objectives of the second study, a two stage decontamination strategy, were: 1) Determine the efficacy of power washing on *B. subtilis* spore removal, 2) determine the efficacy of five disinfectants on *B. subtilis* spore inactivation, and 3) determine the appropriate number of disinfectant spray applications to inactivate *B. subtilis* spores.

2. MATERIALS AND METHODS

2.1. Disinfectant and Sample Surface Study

The first study was conducted at the USDA-ARS Crops Research Laboratory greenhouse in Fort Collins, CO USA in January 2017. The experimental design was a factorial study, which evaluated the efficacy of four disinfectants for inactivating *B. subtilis* spores on porous (wool/nylon fabric) and non-porous (steel washers) inoculated samples with an air-assisted, electrostatic sprayer. The decontamination methods used in these studies generally followed the protocols from two earlier field studies [3,4].

A private lab (Microchem Laboratory, 1304 W. Industrial Blvd, Round Rock, TX USA) prepared the *B. subtilis* spore samples and assayed the samples after treatments for viable spore counts. They inoculated steel washers (5 cm) and nylon/wool samples (2.5 x 7 cm) with 100 µl of *B. subtilis* spores. The wool/nylon fabric samples were 2 mm thick to simulate a vehicle seat cover. Control washer and fabric samples, not treated with a disinfectant, were inoculated and used to determine the percent spore recovery. All samples were shipped in insulated boxes with ice packs and stored at 4°C upon arrival until the day of the experiment. The control and treated samples were returned in the same manner to the private lab to assay all samples for viable spore counts.

The disinfectants were: Clorox Concentrate (Clorox, Oakland, CA USA), EasyDECON® DF200 (Intelagard, Broomfield, CO USA), ElectroBiocide ((EB), Strategic Resource Optimization LLC, Baily, CO USA), and Virkon™-S (LANXESS Corp. Suffolk, UK). Clorox Concentrate (8.5%) was diluted and applied at 7,860 ppm by mixing 100 ml of bleach with 900 ml of water resulting in a 10% bleach solution. EasyDECON® DF 200 was diluted and applied at 54,782 ppm by mixing 39,102 ppm (3.9%) hydrogen peroxide with 15,680 ppm (1.57%) quaternary ammonium. Virkon™-S [pentapotassium bis(peroxymonosulphate) bis (sulphate)] was applied at 10,000 pm (1%) by dissolving 2 tablets in 946 ml of water. EB was a ready-to-use mix applied at 200 ppm. Disinfectants were applied at the recommended label application rates. Also, disinfectant spray application times were chosen to fully wet each sample. The diluted concentration, pH, and oxidation reduction potential (ORP) for the four disinfectants are listed in Table **1**.

The air-assisted, electrostatic sprayer (Model SC-EB, Electrostatic Spraying Systems Inc. (ESS), Watkinsville, GA) was modified in order to spray a positively charged spray onto the samples rather than the normal negatively charged spray. This was done so that the spray droplets would not interfere with the positive electrochemical charge of the disinfectants. Flow rate for the air assisted sprayer was approximately 3.8 L/hr, with an average droplet size of 50 microns. The liquid pressure was 103 kPa and the air pressure ranged from 206 to 275 kPa.

The spraying sequence for the ESS sprayer was 10 s/sample, followed by two minutes of drying time, followed by repeating the disinfectant applications for a total of one, two, or three applications per sample, for each disinfectant. A previous study evaluated the effects of low humidity conditions on disinfectant drying times which revealed that the average surface drying time was much less than 10 min. [4]. Therefore, this disinfectant application protocol was designed with repeated applications spaced at time intervals to maintain a wet sample surface over the 10 min. contact time that is required on most EPA-registered

Table 1: Average pH and Oxidation Reduction Potential (ORP) for the Disinfectants used in the Disinfectant and Sample Surface Study. The Final Diluted Concentration is Listed for Each Disinfectant

Disinfectant	Diluted concentration (ppm)	pH	ORP (mV)
Clorox Conc.	7,860	10.2	684
EasyDecon DF 200	54,782	-	89
ElectroBiocide	200	7.0	728
Virkon-S	10,000	2.9	417

disinfectants. In between each disinfectant, the ESS sprayer was purged completely in order to avoid any possible cross contamination. Finally, there was a five-minute time interval between third spray and the application of neutralizer.

Inoculated samples were placed on a horizontal surface at a spacing of 0.38 m between samples. Samples were neutralized five minutes after the final application. EasyDecon® DF 200 and Clorox Concentrate were neutralized with Dey and Engley (D/E) Neutralizing Broth. The D/E neutralizing broth was mixed by dissolving 34 g D/E broth, 5 ml Polysorbate 80, and 1 L H_2O. ElectroBiocide and Virkon™-S were neutralized with sodium thiosulfate, which was mixed at 25 g/1,000 ml H_2O. Liquid neutralizers were applied with hand spray bottle at rate of approximately 7 ml/sample.

2.2. Two Stage Decontamination Study

The two-stage decontamination field study was conducted at the Colorado State University Agricultural Research Development and Education Center (ARDEC) near Fort Collins, CO from July – August 2016. The study was designed as factorial study with three study factors. There were 59 replicate runs due to hidden replication by limiting the data analysis to only two-way interactions. The three factors were: 1) with or without power washing, 2) five disinfectants, and 3) repeated disinfectant applications (1, 2, or 3 applications). Inoculated steel washers, as previously described, were attached with magnets to farming implement chisel bar to simulate disinfection of agricultural equipment.

The disinfectants were: EasyDECON® DF200, ElectroBiocide, and Virkon™-S as previously described. Two additional disinfectants were: Accel, (Virox, Oakville, ON, Canada), and Z-series (ICA Trinova, Macon, GA USA). Accel is a proprietary formulation based on hydrogen peroxide (H_2O_2), and the Z-series granules generate a liquid chlorine dioxide (ClO_2) disinfectant solution when mixed with water. The diluted concentration, pH, and oxidation reduction potential (ORP) for the five disinfectants are listed in Table **2**.

Inoculated samples, steel washers as previously described, were attached to the frame of a chisel plow to simulate disinfection of a farm implement. Each washer was spaced 0.38 m apart. A mobile power washer (S-K Environmental LLC, Okanogan, WA) was used to wash half of the washers in order to evaluate the efficacy of power washing on dislodging *B. subtilis* spores. The power washer had a pressure of 13.8 mPa, and a main tank volume of 1,892 L. The power washing nozzle was positioned approximately10 cm from the sample surfaces. Once the washers appeared to be dry, disinfectants were applied with a hand spray bottle (Double Mist Trigger Sprayer, Kwazar, UK). The approximate disinfectant spray volume was 4 ml/sample.

The power washing and disinfectant sequences included power washing for 10 s, sun dried for two minutes, application of the next disinfectant treatment, and then repeated. This was done either one, two or three times. The samples with only one spray application were neutralized after a ten-minute drying period. Most EPA-registered disinfectants have a label contact time of 10 minutes. Therefore, our disinfectant protocol followed the general label requirements for contact time. The samples with two spray applications, had a two-minute drying period between sprays, followed by an eight-minute drying period before neutralizing. Finally, the samples with three spray applications had a two-minute drying period between the sprays that was followed by a six-minute drying period until neutralization of the sample. The total disinfectant exposure time (approximately 10 min) and disinfectant treatment combination, for each sample, was the same for each spray in order to minimize any

Table 2: Average pH and Oxidation Reduction Potential (ORP) for the Disinfectants used in Two Stage Decontamination Study. The Final Diluted Concentration is also Listed for each Disinfectant

Disinfectant	Diluted concentration (ppm)	pH	ORP (mV)
Accel	2,600	1.9	530
EasyDecon DF 200	54,782	-	89
ElectroBiocide	200	7.0	728
Virkon-S	10,000	2.9	877
Z- series	250	6.8	771

effects due to extended exposure times for the second and third repeat applications. EasyDecon® DF 200 was neutralized with the D/E Neutralizing Broth as previously described. Accel, ElectroBiocide, Virkon™-S, and Z-series treated samples were neutralized with sodium thiosulfate, as previously described.

2.3. Statistical Analysis

All study designs were created with the SAS-JMP Design of Experiment (DOE) program in order to reduce the number of samples and cost for each study. Analysis of each study was limited to two-way interactions for all model terms. Statistical significance was set at α=0.05. The average *B. subtilis* viable spore count for the storage and transit control samples was 5.75 log /sample. The storage and transit control, and treated *B. subtilis* viable spore counts from each sample were transformed into log10 reduction data. The log10 reduction formula was as follows: A was the number of viable spores recovered from the control treatment surfaces, and B was the number of actual sores recovered from the treated surfaces:

$$Log10\ Reduction\ of\ Viable\ B.subtilis\ spores = log(A/B)$$

The SAS-JMP Least Squares Fit model was used to analyze the log10 reduction data for each study.

3. RESULTS

3.1. Disinfectant and Sample Surface Study

Of the two study factors, disinfectants and sample surfaces, only the differing disinfectants applied to the different surfaces resulted in differences in *B. subtilis* spore inactivation (p-value = <0.0001). There were no differences between the porous and non-porous surfaces in viable spores after application of the disinfectants. There were no interactions between the two study factors; therefore, only the log10 reduction estimates in viable spore reduction are reported (Table **3**). The disinfectant with the greatest level of viable spore inactivation was EasyDecon® DF 200 with a log10 reduction of 1.42. Clorox Concentrate and Virkon™-S had a log10 reduction of 1.3 and 1.1, respectively.

3.2. Two-Stage Decontamination Study

Two, two-way interactions were included in the final model used to estimate the log10 reduction values for this study (Table **4**). The Least Squares model, based on the three factors listed in Table **4**, was used to estimate the log10 viable spore reduction values for each of the study treatments (Table **5**).

Table 3: *Bacillus subtilis* log10 Spore Reduction Estimates, Based on the Least Squares Fit Model, after Spray Application of Disinfectants on Porous and Non-Porous Surfaces (Data Combined)

Disinfectant	Log10 Reduction of *B. subtilis* spores
Clorox Conc.	1.29
EasyDecon DF 200	1.42
ElectroBiocide	0.68
Vikron-S	1.14

Table 4: Least Squares Fit Model Results with *p*-Values for the Power Washing and Disinfectant Spray Application Two-Stage Decontamination Study

Source	Prob > F
Disinfectant	<.0001
Power wash	<.0001
Number of disinfectant applications	0.5173
Disinfectant *Power wash	<.0001
Disinfectant*Number of disinfectant applications	0.0068

Power washing nearly tripled the levels of log10 inactivation of *B. subtilis* spores regardless of which disinfectant was applied (Table **5**). Three applications of EasyDecon® DF200 provided the greatest level of viable *B. subtilis* spore inactivation when the surfaces were not power washed (Table **5**). Power washing followed by disinfectant applications more effectively inactivated *B. subtilis* spores compared to no power washing followed by spray applications. Multiple applications of disinfectants increased spore inactivation slightly. The greatest reduction in spore inactivation with power washing was 4.51 log10 reduction, including: 1) EasyDecon® DF-200 applied three times and 2) ElectroBiocide applied twice (Table **5**).

4. DISCUSSION

In the first study, there were no differences in *B. subtilis* spore survival between the porous and non-porous surfaces when sprayed with the disinfectants. One explanation is that the air-assisted electrostatic sprayer provided complete coverage of the wool/nylon

Table 5: *Bacillus subtilis* log10 Spore Reduction, Based on the Least Squares Fit Model, for the Two-Stage Decontamination Study with Power Washing followed by Spray Application of Disinfectants

Disinfectant	Power wash (yes or no)	One disinfectant application	Two disinfectant applications	Three disinfectant applications
		Log10 reduction		
Accel	No	1.06	1.07	1.39
DF 200	No	1.88	1.61	2.03
ElectroBiocide	No	0.97	1.24	1.11
Virkon-S	No	0.84	0.82	1.05
Z-series	No	1.56	1.80	0.92
Accel	Yes	2.91	2.92	3.23
DF 200	Yes	4.36	4.10	4.51
ElectroBiocide	Yes	4.24	4.51	4.39
Virkon-S	Yes	3.62	3.60	3.83
Z-series	Yes	4.05	4.29	3.41

fabric including all the enclosed or “shielded” fibers [14,15]. The second, and more plausible, explanation is that all the samples received three spray applications, which tripled the disinfectant applied and exposure time for each sample. DeQueiroz and Day [16] reported similar results when comparing disinfectants applied to porous and non-porous surfaces for *B. subtilis* spore inactivation.

A similar, two-stage decontamination study conducted in 2015 had an optimal treatment of 10 seconds of power washing followed with a spray of EasyDecon® DF-200 which resulted in a 4.03 log10 reduction of *B. subtilis* spores [3]. The 2015 study only used wool fabric samples and a nozzle distance of 20 cm. In this study, EasyDecon® DF 200 was evaluated again, which resulted in a 4.36 log10 reduction of *B. subtilis* spores, after power washing and one spray application to steel washer samples (Table **5**).

In this study, viable spore reduction was a function of both dislodging the *B. subtilis* spores from the sample surface via power washing and spore inactivation by the disinfectants [3, 4]. Power washing greatly increases the effectiveness of equipment decontamination; however, the wastewater from power washing should also be treated to inactivate all pests and pathogens before the wastewater can be disposed or recycled [1,2,5]. Wastewater should be filtered then treated with disinfectants, ozone, heat, and/or ultraviolet light. This will add to the cost of two-stage equipment decontamination. However, the additional cost of treating the wastewater could be offset by the savings through recycling the water for additional decontamination of equipment or risking the spread of pests or pathogens.

Comparison of the log10 reduction viable spore counts between the first study and the second study shows a 30 and 39% increase in spore inactivation for EasyDecon® DF-200 and ElectroBiocide, respectively, with three applications of disinfectant with no power washing. In contrast, Virkon was 9% less effective when comparing the second study results with the first study. However, comparing the two studies is not necessarily appropriate because two different disinfectant sprayers were used (electrostatic sprayer vs hand bottle) along with two differing disinfectant exposure times (6 vs 10 min).

5. CONCLUSION

Two-stage power washing with disinfectant application is essential for decontamination of farm equipment, transport vehicles, processing and storage facilities. Automated equipment washing systems are being commercialized for large processing stations to prevent the spread of pests and diseases from site to site. There is still a need to find economical methods that effectively sanitize large numbers of equipment, under a range of conditions from freezing to extreme heat, while cleaning semi-hidden surfaces and sensitive instruments and electronics.

ACKNOWLEDGEMENTS

We would like to thank Sheila and Jeff Kennedy, and Alan Rubert from S-K Environmental for the use of

their mobile pressure washer. Also, we thank John Breedlove and Mike Peters from Strategic Resource Optimization (SRO) for their advice and expertise on the chlorine dioxide formulations. Thank you to Karl Whitman from Colorado State University-ARDEC Farm, for the use of farm facilities and farm equipment. Thank you as well to Tracey Ayer and Ben Hollis of the USDA-APHIS-PPQ-CPHST for their assistance and photographs from this experiment, and Ned Jones for his help and assistance in designing and completing the results and statistics for this study.

REFERENCES

[1] Alphin R, Ciaverelli C, Hougentogler D, Johnson K, Rankin M, Benson E. Postoutbreak Disinfection of Mobile Equipment. Avian Diseases 2010; 54: 772-776. https://doi.org/10.1637/8763-033109-ResNote.1

[2] Guan J, Chan M, Brooks BW, Rohonczy E, Miller LP. Vehicle and Equipment Decontamination During Outbreaks of Notifiable Animal Diseases in Cold Weather. Applied Biosafety 2017; 3: 114-122. https://doi.org/10.1177/1535676017719846

[3] Layman M, Ramsey CL, Freebury P, Newman D, and Newman S. Two Stage Decontamination of Agricultural Equipment Using Power Washing Followed by Disinfectant Treatments. Glob J Agric Innov Res Dev 2018(a); 5: 38-45. https://doi.org/10.15377/2409-9813.2018.05.5

[4] Layman M, Ramsey CL, Freebury P, Newman D, Newman S. Decontamination using Chlorine Dioxide Disinfectant with Adjuvants Versus Hydrogen-Peroxide and Pentapotassium Disinfectants on Farm Equipment. Glob J Agric Innov Res Dev 2018(b); 5: 29-37. https://doi.org/10.15377/2409-9813.2018.05.4

[5] Dee S, Deen J, Burns D, Douthit G, Pijoan C. An Evaluation of Disinfectants For the Sanitation of Porcine Reproductive and Respiratory Syndrome Virus-Contaminated Transport Vehicles at Cold Temperatures. Canadian J Vet Res 2004; 68: 208-214

[6] Calfee M, Touati A, Pongur SG, McArthur T, Wyrzykowska-Ceradini B. Effectiveness of Spray-Based Decontamination Methods for Spores and Viruses on Heavily Soiled Surfaces. 2016. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&TIMSType=&count=10000&dirEntryId=329391&searchAll=&showCriteria=2&simpleSearch=0

[7] Block S. Disinfection, Sterilization, and Preservation. Lippincott Williams and Wilkins, Philadelphia PA 2000; p. 1162.

[8] Law SE, Cooper SC. Air-Assisted Electrostatic Sprays for Postharvest Control of Fruit and Vegetable Spoilage Microorganisms. IEEE Transactions on Industry Applications 2001; 37(6): 1597-1602. https://doi.org/10.1109/28.968166

[9] Lyons SM, Harrison MA, Law SE Electrostatic Application of Antimicrobial Sprays to Sanitize Food Handling and Processing Surfaces for Enhanced Food Safety. 2011; Pages 012014 *in* Journal of Physics: Conference Series. IOP Publishing. https://doi.org/10.1088/1742-6596/301/1/012014

[10] Zhao S, Castle G, Adamiak K. Factors Affecting Deposition in Electrostatic Pesticide Spraying. Journal of Electrostatics 2008; 66(11-12): 594-601. https://doi.org/10.1016/j.elstat.2008.06.009

[11] Earl AM, Losick R, Kolter R. Ecology and Genomics of *Bacillus subtilis*. Trends in Microbiology 2008; 16(6): 269-275. https://doi.org/10.1016/j.tim.2008.03.004

[12] Maillard JY. Innate Resistance to Sporicides and Potential Failure to Decontaminate. J Hosp Infect 2011; 77(3): 204-209. https://doi.org/10.1016/j.jhin.2010.06.028

[13] Russell A. Bacterial Resistance to Disinfectants: Present Knowledge and Future Problems. J Hosp Infect 1999; 43: S57-S68. https://doi.org/10.1016/S0195-6701(99)90066-X

[14] Coates W, Palumbo J. Deposition, Off-Target Movement, and Efficacy of Capture and Thioden Applied to Cantaloupes using Five Sprayers. Amer J Ag Eng 1997; 97: 181-188. https://doi.org/10.13031/2013.21595

[15] Matthews GA. Electrostatic spraying of pesticides: A review. Crop Prot 1989; 8: 3-15. https://doi.org/10.1016/0261-2194(89)90093-8

[16] DeQueiroz GA, Day DF. Disinfection of *Bacillus subtilis* spore-contaminated surface materials with a sodium hypochlorite and a hydrogen peroxide-based sanitizer. Letters in Applied Microbiology 2008; 46: 176-180. https://doi.org/10.1111/j.1472-765X.2007.02283.x

Received on 08-05-2020 Accepted on 12-06-2020 Published on 13-11-2020

DOI: https://doi.org/10.15377/2409-9813.2020.07.3