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Short communication

A mechanized two-step cleaning and disinfection process strongly minimizes pathogen contamination on wooden potato storage boxes

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ABSTRACT

For storage of fruit and vegetables wooden boxes are often used. The reuse of these boxes provides a mean of pathogen transmission and food spoilage and therefore poses a risk for the consumers' health. The efficacy of a newly developed fully automatized machine for cleaning and disinfection of wooden potato storage boxes against various plant pathogens was tested. The moveable machine with two separate treatment chambers is fixed on a trailer. The technique is optimized for the disinfectant MENNO-Florades (90 g/L benzoic acid). In the first chamber the disinfectant is applied as a solution and in the second chamber as foam. The pathogens tested, *Pectobacterium carotovorum* subsp. *carotovorum*, *Xanthomonas campestris* pv. *campestris*, *Colletotrichum coccodes* and *Fusarium solani* were eliminated to 100, 96.9, 96.9 and 90.6% respectively. Additionally the effects against two quarantine pathogens (*Clavibacter michiganensis* subsp. *sepedonicus*, *Ralstonia solanacearum* race 3 biovar 2) were tested by simulating the process inside the machine in a quarantine greenhouse chamber. Both pathogens were eliminated completely. This shows that a combination of liquid and foam application of a disinfectant can successfully eliminate storage pathogens and in that way help to prevent pathogen spread and ensure crop health during storage.

Wooden boxes are still widely used in the agricultural industry. Compared to plastic boxes they have a low price and can easily be repaired on site (Schuur, 1988). Reuse of wooden boxes for storage or transport of potatoes and various other vegetables (e.g. carrots) involves a high risk of transmission of pathogens, pests and weed seeds (Kora et al., 2005). Therefore cleaning and disinfection of used storage boxes is an essential part of 'good plant protection practice'. According to the European Plant Protection Organization (EPPO) cleaning and disinfection procedures are even mandatory as soon as quarantine diseases such as ring rot and brown rot of potatoes are found (OEPP/EPPO, 2006). This is not only based on their high disease potential but also on the persistence of the microorganisms: ring rot bacteria can remain viable for over two years (OEPP/EPPO, 2006). In the European Union efficacy testing of disinfectants for the use in plant production is regulated by the EPPO-guideline PP 1/261 (OEPP/EPPO, 2008). The guideline provides several detailed procedures and protocols to determine disinfection efficacy under a broad range of conditions and for diverse different surface materials. Unfortunately, it does not contain any specific suggestions for testing disinfectants on wooden surfaces. Wood surely provides specific challenges regarding surface disinfection based on its irregular surface and material properties. Spotts and Cervantes (1994) have shown that several disinfectants perform better on plastic material than on wood regarding removal of a range of fungi that play a role during food storage. Therefore, the development of an optimized disinfection procedure for wood surfaces and specific testing methods is necessary. MENNO-Florades (90 g/L benzoic acid) is the only disinfectant with an official registration in plant production in Germany (BVL, 2016). Therefore the experiments were conducted with this specific disinfectant only.

We have shown previously that spray application of a disinfectant on artificially contaminated wooden boards immediately after cleaning (e.g. by a high pressure washer with water) on wet wood did not lead to a sufficient reduction of pathogens. Contrary to that, pathogens were completely eliminated when the disinfectant was applied on dry wood (data not shown). The disinfectant very likely diffuses too slowly into the water filled cracks and pores of the wood to achieve the necessary concentration before drying on the wood surface.

Based on these preliminary results the aim of the experiments

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Table 1
Amount of working solution of MENNO- Florades (3%) adhering to the wood surface in upright position. Measurements were taken on upright placed planks immediately after application and after 10 min. Mean values of n = 5 +/- standard deviation are shown.

Expansion ratio (foam)	Adhering solution, fluid application (g/m^2) after 0 min	Adhering solution, foam application (g/m^2) after 0 min	Adhering solution, foam application (g/m^2) after 10 min
7.0	85.7 ± 4.1	491.6 ± 57.2	208.5 ± 33.7
8.1	90.0 ± 7.2	474.0 ± 21.7	154.0 ± 15.4
8.2	68.9 ± 6.3	504.1 ± 62.8	202.0 ± 35.6

was the development of a system that ensures successful pathogen elimination by 1) extending the incubation time of the disinfectant on both the wood surface and inside the wood and 2) spatiotemporal separation of the high pressure wet cleaning followed by a two-step disinfection process.

First of all we determined the amount of disinfection solution adhering onto wood surfaces comparing fluidal to foam application of MENNO- Florades. Experiments were carried out in the greenhouse. Wooden planks were placed upright and treated with fluidal and foam solution with five replicates per treatment. Foam with different expansion ratios was produced and applied using a skumix®-foamer (MENNO Chemie- Vertrieb GmbH, Norderstedt, Germany). Immediately after application and after 10 min the weight of the adhering disinfectant was determined. Mean values are shown in Table 1. Our data shows that foam application mediates the adherence of a higher amount of disinfection solution to the wood surface compared to fluidal spray application. Even after 10 min the adhering amount of foam solution was still higher compared to fluidal application immediately after application. Foaming delays the drying process and therefore the reaction time of the disinfectant is extended. Finally, foaming allows the visual inspection of the disinfection coverage, disinfection gaps can be identified and thus excluded. Taken together, foaming increases the probability of disinfection success.

To test the practical applicability and efficacy of foam application for disinfection purposes a movable machine with two separate chambers, fixed on a trailer was constructed (Gaugele GmbH, Iffendorf, Germany; Fig. 1). The pre-cleaned boxes are washed in the first chamber (Fig. 1 a) with a recirculating disinfection solution, ensuring that the pores and cracks of the wood are filled with the disinfectant. While the boxes move into the second chamber (Fig. 1 b) most of the surface solution is blown off by a high pressure air stream. This prevents the entrance of the dirty wash solution into the tank of the second chamber. In the second chamber the disinfectant is applied as foam onto the boxes. The disinfectant solution in the second chamber is strictly separated from that of the first chamber and recirculated as well. Recirculation of the solutions prevents an unintentional contamination of the environment.

The efficacy of the described fully automated cleaning and disinfection machine, provided by the W&J Agrarhygiene GmbH & Co (Saerbeck, Germany), was tested with wooden potato boxes artificially contaminated with various pathogens. Test organisms were *Pectobacterium carotovorum Xanthomonas campestris* pv. *campestris*, *Colletotrichum coccodes*, *Fusarium solani* (Mart.) Sacc. *Clavibacter michiganensis* subsp. *sepedonicus* and *Ralstonia solanacearum* race 3 biovar 2.

Fungal pathogens were grown on potato dextrose agar (PDA) at 24 °C for 21 days, bacterial organisms on yeast-dextrose- chloramphenicol agar (YDC) at 27 °C for 2 days before artificial contamination of the boxes.

New wooden potato storage boxes (1.2 × 0.8 × 0.9 m) were used for the trials with the disinfection machine. The second plank from the top of a box was removed and 8 inoculation sites (1 cm × 2 cm each) were marked on the plank (Fig. 2a). One inoculation loop (diameter 5 mm) of bacterial slime or one 5 mm fully grown agar plug (fungal pathogens) was carefully spread with the help of a spatula onto the 2 cm^2 area of each inoculation spot. After letting dry for 24 h the planks were re-screwed onto the boxes (contaminated spots directed to the inner side of the box) and the disinfection tests were carried out (Fig. 2c). Per pathogen four individually disinfected storage boxes, representing 4 replicates were treated in the machine. In total 32 contamination sites per pathogen were analyzed.

The automatic disinfection process with the movable machine was conducted with the boxes that were previously contaminated with the pathogens without quarantine status, i.e. *X. campestris* pv. *campestris*, *P. carotovorum*, *C. coccodes*, and *F. solani*. In the first chamber 3% MENNO- Florades spray solution was applied, followed by a 2% MENNO- Florades foam application in the second chamber. Boxes were left to dry for 24 h in a storage hall to ensure homogeneous drying conditions and to avoid irregularly quick drying by excessive sunshine or disinfectant removal by rain.

As the bacterial pathogens *C. michiganensis* subsp. *sepedonicus* and *R. solanacearum* are quarantine organisms, their reaction to the disinfection process was tested in a quarantine cabinet of an experimental greenhouse. This was done to ensure compliance of the appropriate safety standards to avoid pathogen introduction into the environment. Artificially contaminated boxes as described

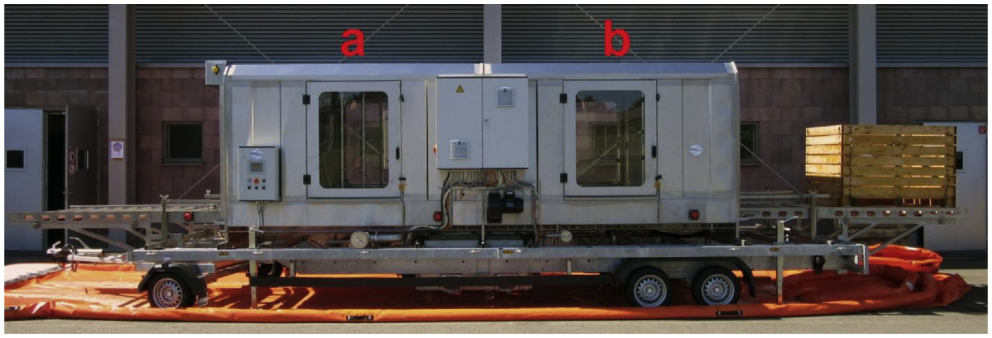

Fig. 1. Cleaning and disinfecting machine for wooden potato storage boxes. Boxes are loaded onto the machine on the left side, followed by liquid cleaning (a) and foam disinfection (b). Storage boxes leave the machine on the right side.

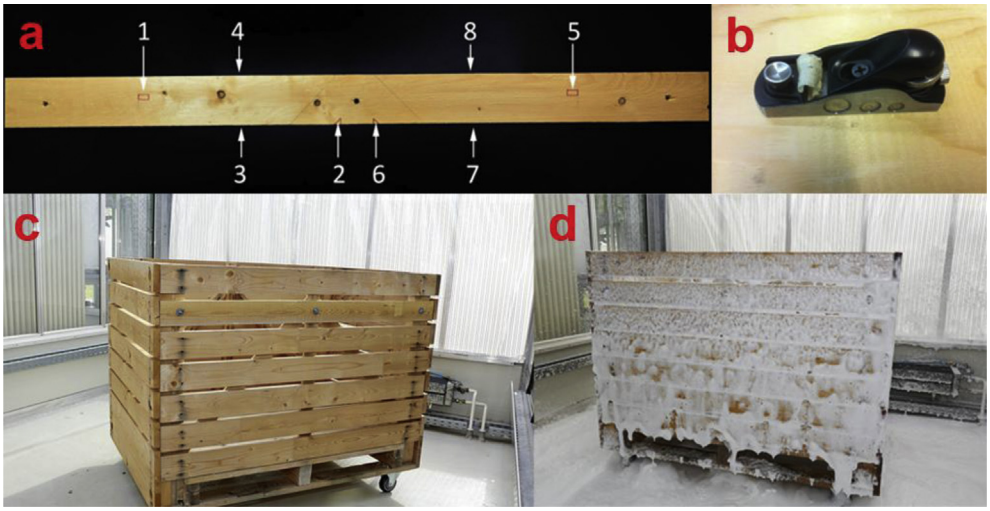

Fig. 2. Disinfectant efficacy testing with artificially contaminated potato boxes. a) 8 sites per plank were infected with one pathogen; b) sampling of thin wooden slides with a plane after the treatment; c) The second plank from the top was removed, inoculated as shown in a) and re-screwed in the storage box before treatment with the inoculation sites facing to the inside of the box; d) 4 storage boxes were treated individually per pathogen, here shown for one box with a quarantine organism in the greenhouse.

above were treated with 3% MENNO-Florades solution with a hand operated sprayer. After removal of solution adhering to the surface with pressurized air for 15 s 2% MENNO-Florades foam was applied using the skumix®-foamer (Fig. 2d). Both pathogens were tested in 4 individual replicates, i.e. 4 individually treated boxes.

Further a comparison of the disinfecting capacity of different agents was included. This was done in the greenhouse as well. As no other foaming agent was available, we used Hydrosan 1% as fluid application (active agent hydrogen peroxide), applied as described for MENNO- Florades with a hand operated sprayer twice with a treatment with pressurized air in between both applications. We compared it with MENNO- Florades foam application regarding its efficacy against *F. solani* and *X. campestris* pv. *campestris*.

In all cases, with the movable machine as well as for the greenhouse experiments 24 h after treatment samples for the determination of pathogen survival were taken. For this purpose a thin (ca. 0.2 mm) layer of wood was cut from the contaminated spots with a small plane (Fig. 2b). Pathogen material was extracted from the wood tissue after 5 min of sonication. Determination of pathogen growth was done on selective media for 7 days at 24 °C (fungi) or for 3 days at 27 °C (bacteria). A control treatment with contaminated, but further non-treated wood planks was carried out in parallel for each organism. Pathogen recovery was determined qualitatively as a yes-or-no result, even the survival of one

Table 2
Disinfection efficacy of the combined liquid (3%) and foam (2%) treatments of MENNO-Florades. A plank with 8 contamination sites was disinfected individually. 4 replicates (planks) per pathogen were tested, i.e. 32 contamination spots in total were analyzed. The number and percentage of sites where pathogen elimination was successful +/- standard deviation are shown. The quarantine organisms *R. solanacearum* and *C. michiganensis* were tested in the greenhouse, the other pathogens directly on boxes treated in the disinfection machine.

Organism	Expansion ratio	Pathogen recovery (no. of contamination sites)		Reduction (%)
		yes	no	
F. solani	7.2	3	29	91 ± 6.25
C. coccodes	7.2	1	31	97 ± 6.25
X. campestris pv. *campestris*	6.1	1	31	97 ± 6.25
P. carotovorum	6.1	0	32	100
R. solanacearum	7.2	0	32	100
C. michiganensis subsp. *sepedonicus*	7.2	0	32	100
Control (all organisms)		32	0	0

Table 3
Disinfection efficacy of the combined liquid and foam treatments of MENNO-Florades and combined liquid-liquid treatments of Hydrosan. A plank with 8 contamination sites was disinfected individually. 4 replicates (planks) per pathogen were tested, i.e. 32 contamination spots in total were analyzed. The number and percentage of sites where pathogen elimination was successful +/- standard deviation are shown. All experiments were carried out in the greenhouse under standard conditions.

Organism	Disinfecting agent	Expansion ratio	Pathogen recovery (no. of contamination sites)		Reduction (%)
			yes	no	
F. solani	MENNO- Florades	6.7	0	32	100
X. campestris pv. *campestris*	MENNO- Florades	8.0	1	31	97 ± 6.25
F. solani	Hydrosan	liquid	21	11	34 ± 15.7
X. campestris pv. *campestris*	Hydrosan	liquid	23	9	28 ± 21.3
Control (all organisms)			32	0	0

single colony was recorded as no disinfection success. This was set in relation to the total number of contamination sites (Tables 2 and 3).

Using MENNO- Florades pathogen elimination was very high (Table 2), between 91 and 100% of the pathogens were not detectable anymore after the combined liquid and foam treatment. The untreated control treatments were fully grown. Interestingly, the disinfecting potential was lower for Hydrosan, where only 34% (*F. solani*) and 28% (*X. campestris pv. campestris*) pathogen reduction was achieved (Table 3). Whether this is caused by the different active ingredients or the longer adherence of the foam cannot be distinguished here. But the results clearly show, that clear differences between the products are observable.

Our data show that a combination of liquid and foam application of a disinfecting agent provides a suitable method to highly reduce fungal and bacterial pathogens on wooden surface material. In case of *P. carotovorum*, *R. solanacearum* and *C. michiganensis* subsp. *sepedonicus* even complete pathogen elimination was possible. One reason most likely is the use of foam, that mediates the adherence of higher amounts of disinfecting agent and therefore longer disinfection time. An important factor for wood treatment is the use of dry material to mediate uptake of the disinfecting agent into the wood.

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