

Article

A Combined Cleaning and Disinfection Measure to Decontaminate Tire Treads from Tomato Brown Rugose Fruit Virus

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Abstract: Mechanically transmissible and stable viruses such as tobamoviruses, which include *Tobamovirus fructirugosum* (syn. tomato brown rugose fruit virus (ToBRFV)), will continue to pose major challenges for farmers. Consequently, holistic hygiene concepts are being implemented to prevent the introduction and spread of these viruses. The decontamination of tires and castors was previously a weak point in many industrial hygiene concepts. For this reason, the ProfilGate clean-off zone was tested in combination with the disinfectant MENNO Florades for the decontamination of ToBRFV-contaminated tires. In total, 478 tire segments were sampled to evaluate the contamination of ToBRFV and the following decontamination of the tires. This treatment reliably removed high ($4.5 \mu\text{g}/\text{cm}^2$), medium ($0.45 \mu\text{g}/\text{cm}^2$), and low concentrations ($0.045 \mu\text{g}/\text{cm}^2$) of ToBRFV from the tires, as shown by a bioassay. The reduction in necrotic local lesions on susceptible indicator plants *N. tabacum* cv. Xanthi NN was between 91.9 and 97.6%. The reduction in ToBRFV contamination largely depended on the length of the rollover distance, i.e., the number of tire rotations. For transport trolleys with polyamide and rubber tires, depletions of 97.4 and 97.6%, respectively, was determined after 16 rotations. For transport wagons with tires twice the size and polyurethane tread, the depletion was still at least 91% after eight wheel turns. Even in the case of gross soiling of the tires, the mean reduction from the different tread materials was 80.9 to 98.9%. Subsequent analysis of the clean-off zone revealed that ToBRFV did not accumulate, even when the contaminated tires were driven over several times, but was safely inactivated completely in the disinfectant solution. This provides growers with an effective tool for preventing the introduction and spread of ToBRFV.

Keywords: ProfilGate; MENNO Florades; disinfection; greenhouse; clean gate; wheel; machinery; hygiene; cleaning; tobamovirus



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1. Introduction

Horticultural hygiene management to prevent the introduction and spread of harmful phytopathogenic pathogens in greenhouses is still dominated by the occurrence of stable and easily mechanically transmissible tobamoviruses, such as the tomato brown rugose fruit virus (ToBRFV) or cucumber green mottle mosaic virus (CGMMV). ToBRFV has spread to the European [1–3], North American [4–6], Asian [7–9], and African continents [10] in recent years. Several research studies have revealed that ToBRFV can quickly infect entire tomato crops due to the easy mechanical transmission of the virus [11,12].

The virus can infect up to 100% of the plants in a crop, with the intensity of symptoms varying depending on the variety, cultivation method, climatic conditions, and time of infection. The infection usually leads to a significant reduction in the vigor of the plants and, thus, to a shortening of the production period in which the tomato fruits are harvested [13]. Occasionally, premature death of the tomato plant is also observed. As a result, high losses are recorded as the symptomatic fruits lose their marketability [1] and fruit yields are

significantly reduced [14]. In addition to these direct losses, there is the economic burden on producers due to the costs of applying recommended or legally prescribed hygiene measures. Due to the loss of the market for seeds and seedlings, some producers have already had to switch to the cultivation of other plant species, which may be less profitable.

Therefore, it is imperative to prevent ToBRFV from entering production areas and spreading between different farm locations [15]. An important aspect of integrated disease management involves preventive hygiene measures. These combine cleaning and disinfection activities to remove dirt from surfaces as well as the reliable inactivation of pathogens adhering underneath. To provide an effective countermeasure against the entry and in-house spread of pathogens, the production site has to be compartmented using hygiene sluices. Previous studies have focused on interrupting the transmission routes of ToBRFV caused by humans [15–17]. It was found that, alongside an effective disinfectant, the mechanical cleaning of shoe soles is required for the safe decontamination of footwear [17]. However, the mechanical cleaning of tires, e.g., of forklifts or harvesting carts, cannot be ensured with conventional disinfection mats as these do not include active cleaning brushes. Manual machine cleaning is usually not integrated into operational processes for time and organizational reasons. It should also be emphasized that the contact time between the pathogen and disinfectant is particularly crucial for the efficacy of a disinfectant and is often very short in practice [18]. A question, therefore, arises as to how the entry of ToBRFV through contaminated tires can be prevented as automatically as possible in order to ensure a high level of biosecurity in production and to impair established work processes as little as possible.

In horticultural practice, vehicles and equipment with tires and rolls made of different tread materials are used: polyamide (PA), polyurethane (PU), and rubber. These materials have different properties, which should be considered when cleaning and sanitizing surfaces [19]. PA is a strong and tough material that resists abrasion, fatigue, and impact. It offers excellent chemical resistance with negligible permeation rates when used with organic solvents. However, it has poor resistance to strong mineral acids, oxidizing agents, and certain salts. PU belongs to the class of thermosetting polymers and is used for elastomers. These are characterized by extremely good abrasion resistance and hardness combined with good elasticity and resistance to grease and solvents. Natural rubber is the second most commonly used elastomer, amorphous polymers with linear-chain molecules with a certain degree of cross-linking. These provide a high degree of elasticity.

Besides these aspects that can affect the effectiveness of disinfection, the virus concentration on the ground and, thus, the potential level of contamination on the tires is also a decisive factor. The ToBRFV concentration can vary between plant parts [20]. If leaves, fruits, or other plant parts fall to the ground and are rolled over, the virus concentration on the tires will presumably also differ. Therefore, different virus concentrations need to be included in tests in order to obtain generalizable results.

Thus, we investigated (i) whether tires in tomato production pose a risk of introducing and spreading ToBRFV and (ii) whether the decontamination of tires in a ProfilGate clean-off zone filled with the disinfectant MENNO Florades is effective for different virus concentrations, tire materials, roll lengths, and in the presence of organic soiling.

2. Materials and Methods

2.1. Plant Materials and Virus Preparation

The indicator plant *Nicotiana tabacum* L. cv. Xanthi NN, which is characterized by the development of necrotic local lesions after infection with ToBRFV, was used for all investigations. The plants were grown at 20 °C/16 °C day/night and 16 h/8 h light/dark in a greenhouse and supplied with water and conventional universal fertilizer as required. The virus source was homogenized ToBRFV-infected leaf material of *Nicotiana clelandii* A. Gray. The ToBRFV isolate originated from the first ToBRFV outbreak in Germany in 2018 [1] and was purchased from the DSMZ (German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany). In addition, another virus source was used.

In order to simulate tire contamination as close to reality as possible, in terms of both the virus concentration and type of contamination, infected tomato fruits obtained from a farm with a ToBRFV outbreak were used.

2.2. Inoculum Preparation

To contaminate the wheels of the trolleys, ToBRFV-infected *N. clevelandii* plant material was ground 1:5 (*w/v*) with deionized water to produce an inoculum containing 2 mg/mL of ToBRFV. Quantification was performed according to the recently presented method in [21,22]. In this method, the number of local lesions obtained from inoculation of serially diluted virus suspensions was correlated with defined virus concentrations to obtain standard curves based on the different models such as the Kleczkowski model or the growth curve model. Besides the plant homogenate, an inoculum of purified ToBRFV particles was also used. The particles were mixed with uninfected *Nicotiana tabacum* cv. Xanthi NN plant sap and also adjusted to 2 mg/mL of ToBRFV.

In addition, plant material from a commercial tomato production plant with a confirmed ToBRFV outbreak was used. Plant debris (stems, leaves, fruit stalks, and unripe fruits), tomato fruits with characteristic ToBRFV-associated color changes, and other non-marketable tomato fruits were available at the end of the vegetation. This material was used (i) uncrushed or as (ii) macerate or (iii) tomato fruit halves for the experiments.

2.3. Transport Trolleys and Wagons

Four-wheeled trolleys and wagons with standard tires that are commonly used in horticultural practice were used. Accordingly, treads made of polyamide (PA), polyurethane (PU), and rubber were included in the tests. For testing, four-wheeled trolleys and wagons with standard tires were selected. The trolleys had either rubber tires or polyamide tires with a diameter of 10 cm, or tires with a diameter of 20 cm and polyurethane tread. The tread surfaces were separated into individual segments using duct tape and resulted in four segments (approx. 22 cm² each) for the small tires and six segments (approx. 45 cm² each) for the larger ones (Figure 1).

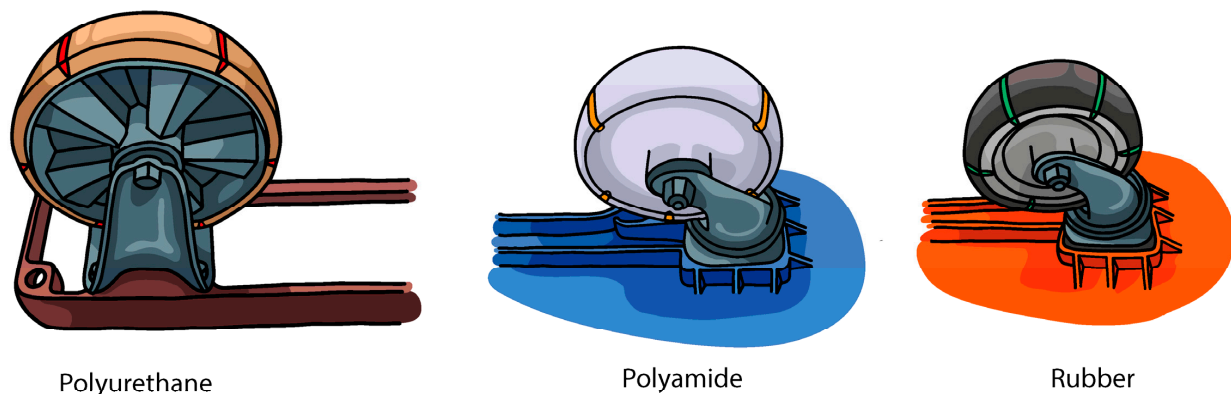


Figure 1. Tires of transport wagons (left) and trolleys (middle and right) with different tread surfaces. These were marked and divided with duct tape.

2.4. Set-Up of the Clean-Off Zone

The stainless-steel tray (approx. L × W 2 × 1 m) was first placed horizontally, and the gratings with integrated brush strips were inserted. Approx. 45 L of a disinfectant solution were then added. MENNO Florades (a.i. benzoic acid) was mixed with deionized water and tested with a 4% concentration (Figure 2). The pH of the solution was 1.

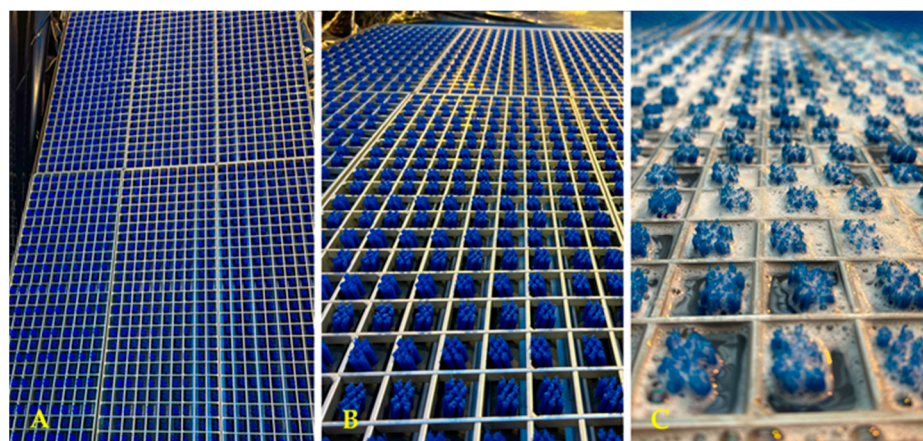


Figure 2. Set-up of the clean-off zone. (A) ProfilGate consists of a stainless-steel tray in which gratings with integrated brush strips are placed. (B) The brush strips generate a vibration due to their inclined position and pre-tensioning, which mechanically cleans the tires. (C) The disinfectant MENNO Florades was added to the stainless-steel tray.

2.5. Detection of ToBRFV and Evaluation

The numbers of necrotic local lesions on the indicator plants were scored 6–7 days after the mechanical inoculation to evaluate the decontamination success of the wheels. This method enables the determination of infectious ToBRFV and has proven to be particularly suitable for determining the quantitative depletion of infectious ToBRFV after disinfectant application [21]. Mixed samples of leaves with necrotic local lesions were then analyzed for ToBRFV with a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) (DAS-ELISA) using commercially available antibodies (RT-1236; DSMZ, Braunschweig, Germany). The assay was carried out according to the supplier's protocol [17].

2.6. Experimental Design and Contamination of Tires under Semi-Practical Conditions

The series of tests were carried out in order to test the contamination of the wheels as closely as possible to real-life conditions and, thus, to demonstrate the risk of this transmission pathway for tomato cultivation. For this, the transport trolleys, weighed down with a weight of 40 kg, were driven through a mixture of infected plant material and contaminated soil residues; alternatively, ToBRFV-infected tomato fruit halves were rubbed onto the tread surfaces of the individual tires (Table 1). In each case, the inoculum was then left to dry on the wheels. Subsequently, the respective tread surface segments were wiped with a water-moistened viscose swab into a tube ($L \times \varnothing 101 \times 16.5$ mm; Sarstedt AG & Co. KG, Nümbrecht, Germany). To sample the tread surface, one swab was used per tire segment. Each swab sample was inoculated onto 3 leaf halves of a *Nicotiana tabacum* cv. Xanthi NN plant, which had been injured in advance with abrasive non-washed diatomaceous earth [CAS 61790-53-2].

Table 1. Overview of the experimental series on the contamination of tires with ToBRFV. R: rubber, PA: polyamide, PU: polyurethane, * R and PA (2), PU (4), and RMs: running meters.

Experiment	Inoculum	Contamination, Procedure	Contamination, Unit	Tread Material
1	Plant debris	Run over, 8.8 RM	8.8 RM	R and PA
2	Plant homogenate	Run over, 8.8 RM	8.8 RM	R and PA
3	Tomato fruit halves	Mechanical inoculation	2 resp.4/tire *	R, PA, and PU

2.7. Experimental Design of the Decontamination Trials

The decontamination studies were carried out according to a standardized procedure (Figure 3). First, the wheels of the transport trolleys were contaminated with ToBRFV,

control samples were taken, the decontamination treatment was carried out, the comparison segments were sampled, and, finally, all swab samples were inoculated on the indicator plants to detect infectious ToBRFV.

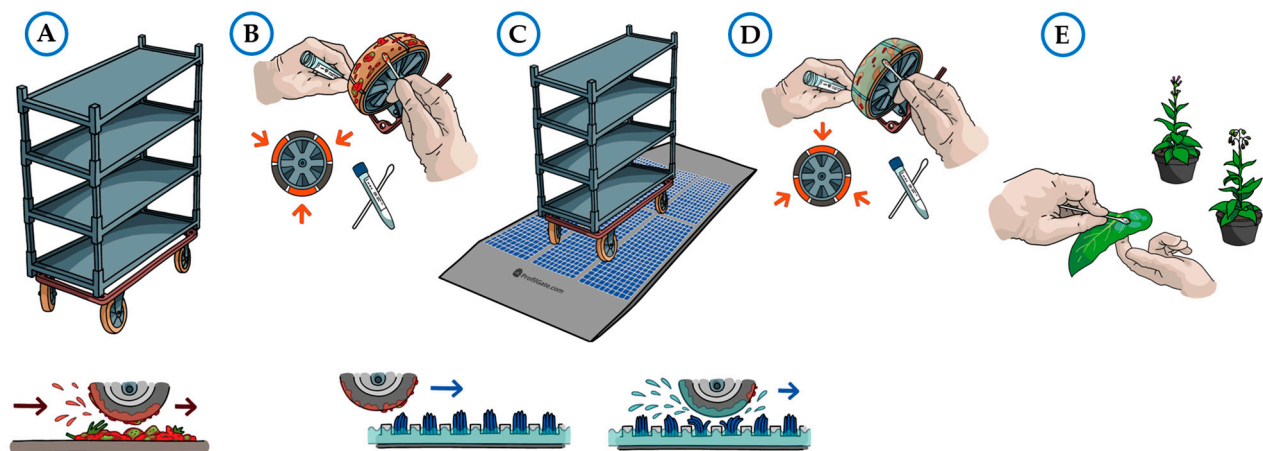


Figure 3. Approach of the decontamination trials. (A) ToBRFV-contaminated of tires; (B) swab samples of tires segments for positive control; (C) treatment of the contaminated tires on ProfilGate filled with MENNO Florades (4%); (D) swab samples of the decontaminated tire segments; (E) inoculation of all swab samples on the indicator plant *Nicotiana tabacum* L cv. Xanthi NN.

To evaluate the decontamination effect of the ProfilGate system filled with MENNO Florades, the trolleys were placed on the brushes and loaded with a 40 kilo weight to simulate the cleaning process as closely as possible to practical conditions. The trolleys were moved over the clean-off zone corresponding to a distance of 5 m. Due to the characteristics of the brushes, there is an active movement of the brushes when driving over them, which is intended to ensure successful cleaning. The remaining tire segments were then wiped with the swabs and immediately inoculated onto the indicator plants. Each swab sample was inoculated onto 3 leaf halves of a *Nicotiana tabacum* cv. Xanthi NN plant, which had been injured in advance with abrasive non-washed diatomaceous earth [CAS 61790-53-2]. Samples of the brushes, the grid, and the disinfectant solution were also taken at intervals of one week after the tests were carried out to confirm that ToBRFV did not accumulate in the clean-off zone. Different inocula, viral loads, and tread materials were included in the individual test series (Table 2).

Table 2. Overview of the decontamination tests on tomato brown rugose fruit virus-contaminated tires using a ProfilGate clean-off zone and MENNO Florades.

Experiment	Objective	Viral Load	Soiling	No. of Tires
4	Different virus concentrations	0.45 to 4.5 $\mu\text{g}/\text{cm}^2$	No	8
5	Different tire materials	2 $\mu\text{g}/\text{cm}^2$	No	8
6	Different tomato debris	Unknown	Yes	8

3. Results

3.1. Contamination of Tires under Semi-Practical Conditions

3.1.1. Contamination by Plant Debris

Even rolling over plant residues of ToBRFV-infected plants that were not further shredded led to contamination of the tire treads. Swab samples taken from both polyamide and rubber tires led to ToBRFV infection of sensitive indicator plants (Figure 4). The average number of necrotic local lesions for the rubber tires was 29.8, while the number for the polyamide treads was lower, at 12.2. Two rubber tires showed an extremely high contamination with ToBRFV, at 54 and 119 nLLs, respectively.

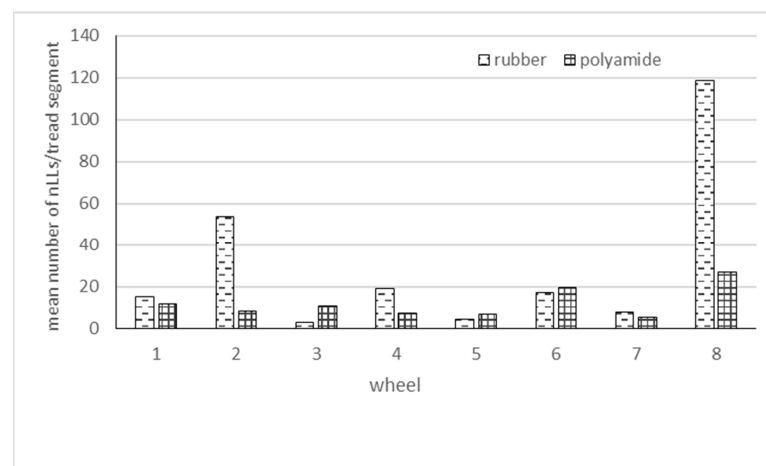


Figure 4. Mean number of ToBRFV-induced necrotic local lesions (nLLs) on the susceptible indicator plant *N. tabacum* cv. Xanthi NN after mechanical inoculation of swab samples taken from four tread surface segments (approx. 22 cm² each) of rubber and polyamide tires after rolling 8.8 m (approx. 28 wheel turns) over a mixture of tomato plant debris from a ToBRFV-infested commercial tomato production site. N = 64.

3.1.2. Contamination by Plant Homogenates

The rolling over of homogenized tomato plant residues from a ToBRFV-affected tomato production site led to contamination regardless of the tire tread. After a roll-over distance of 8.8 m, all tires were contaminated with infectious ToBRFV (Figure 5). Swab samples of the individually sampled tread segments of the rubber and polyamide tires resulted in an average of 29.6 and 34.4 nLLs on the indicator plants.

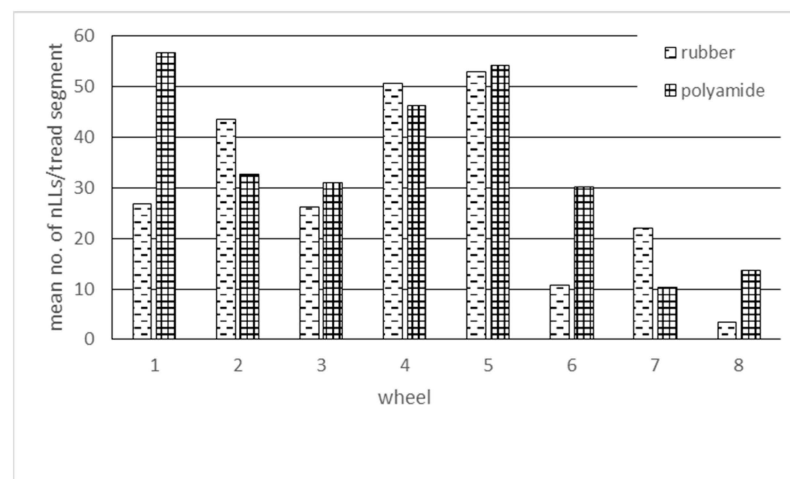


Figure 5. Mean number of ToBRFV-induced necrotic local lesions (nLLs) on the susceptible indicator plant *N. tabacum* cv. Xanthi NN after mechanical inoculation of swab samples taken from four tread surface segments (approx. 22 cm² each) of rubber and polyamide tires after rolling 8.8 m (approx. 28 wheel turns) over a mixture of homogenized tomato plant debris from a ToBRFV-infested commercial tomato production site. N = 64.

3.1.3. Contamination by Tomato Fruits

All wheels were contaminated with ToBRFV from the tomato fruits, regardless of the tread material (Figure 6). It is striking that the polyurethane tires had a significantly lower viral load. For the small polyamide and rubber tires, the average contamination, measured by the number of nLLs induced on the indicator plants, was 363 and 339, respectively, while for the PU tires, it was only 41.

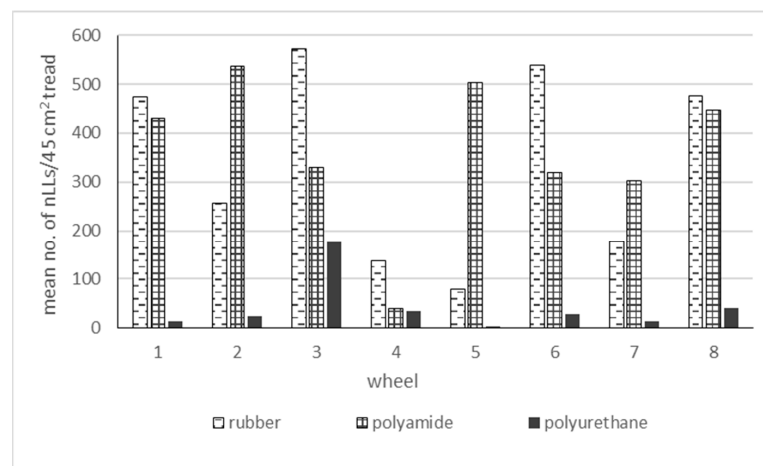


Figure 6. Mean number of ToBRFV-induced necrotic local lesions (nLLs) on the susceptible indicator plant *N. tabacum* cv. Xanthi NN after mechanical inoculation of swab samples taken from two (rubber and polyamide) and three (polyurethane) tread surface segments, which were rubbed with tomato fruit halves from a ToBRFV-infested commercial tomato production site. In order to compare the viral load per cm², the respective number of nLLs of the two segments of the smaller polyamide and rubber wheels were added together, and an arithmetic mean of the three segments of the polyurethane wheels was calculated. N = 46.

3.2. Efficacy of ProfilGate System in Cleaning and Decontamination of Tire Tread Surfaces

3.2.1. Effects of Different Virus Concentrations on Decontamination Success

The results indicate that a ProfilGate clean-off zone containing 4% MENNO Florades is a very effective measure for the decontamination of ToBRFV, regardless of the viral load. In the PU tires, treatment at a high viral concentration (4.5 µg/cm²) led to a reduction in necrotic local lesions of approx. 94% compared with the non-treated control. At a medium viral load (0.45 µg/cm²), the reduction was 97%, and at a low viral load (0.045 µg/cm²), the reduction was 91% (Figure 7).

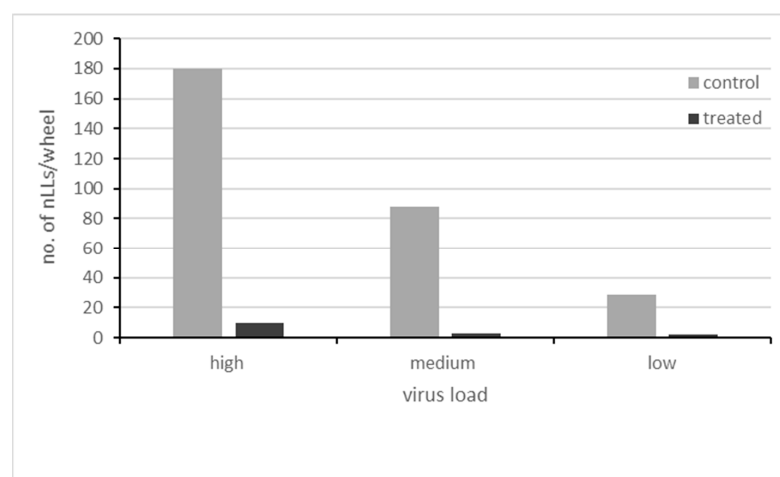


Figure 7. Depletion of infectious ToBRFV from tires with polyurethane tread surfaces by rolling over a ProfilGate clean running zone with a distance of 500 cm, corresponding to 8 wheel turns, depending on the viral load. The tires were contaminated by applying a standardized ToBRFV suspension (high: 4.5 µg/cm², medium: 0.45 µg/cm², and low: 0.045 µg/cm²) to two segments of each of the eight wheels. One of these segments was sampled before and the other after rolling over the ProfilGate zone using swabs. These samples were immediately inoculated onto ToBRFV-sensitive test plants (*N. tabacum* cv. Xanthi NN). The depletion was determined on the basis of the mean value of the number of necrotic local lesions (nLLs) on the indicator plants. N = 48.

3.2.2. Effects of Different Tread Materials

Due to the promising results of the tire decontamination with a high ToBRFV concentration of $4.5 \mu\text{g}/\text{cm}^2$, we adjusted a high virus concentration in the inocula to investigate the influence of different tire materials on the clean-off treatment. The depletion results of ToBRFV from the wheels were found to be independent of which type of inoculum was selected and which type of tire was investigated. The results were very homogeneous between the inoculum type and the rubber and polyamide tires, with a reduction in necrotic local lesions of 97.4–98.4% compared with the non-treated control (Figure 8). Thereby, the depletion of infectious ToBRFV was determined on the basis of the mean value of the number of necrotic local lesions (nLLs) on the indicator plants.

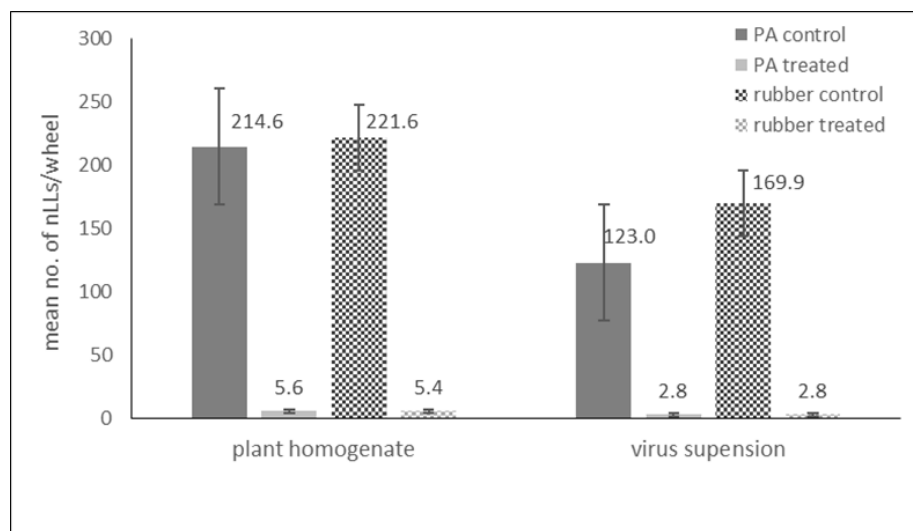


Figure 8. Inactivation of ToBRFV from tires with polyamide (PA) or rubber tread surfaces by rolling over a ProfilGate clean running zone with a distance of 500 cm, corresponding to 16 wheel turns. The tires were contaminated by applying a standardized ToBRFV suspension ($2 \mu\text{g}/\text{cm}^2$) and a ToBRFV-infected plant suspension ($2 \mu\text{g}/\text{cm}^2$) to two segments of each of the eight wheels. One of these segments was sampled before and the other after rolling over the ProfilGate zone using swabs. These samples were immediately inoculated onto ToBRFV-sensitive test plants (*N. tabacum* cv. Xanthi NN), and ToBRFV-induced necrotic local lesions (nLLs) were counted 5 days post-inoculation (dpi). N = 128.

3.2.3. Effect of Heavy Soiling on the Decontamination Success

Coarse contamination of the rubber and polyamide tires of transport trolleys with homogenized ToBRFV-infected plant residues could be largely cleaned by rolling over the ProfilGate system (Figure 9 and Table 3).

Table 3. Inactivation of ToBRFV from tires with polyamide or rubber tread surfaces by rolling over a ProfilGate clean running zone with a distance of 500 cm, corresponding to 16 wheel turns. The tires were contaminated with infected tomato fruits. The mean numbers of necrotic local lesions on the susceptible indicator plant *N. tabacum* cv. Xanthi NN after the mechanical inoculation of the respective swab samples are shown, which were taken from two tread sections per wheel. N = 64.

Tread Surface	Treatment	No. of nLLs/Wheel								Mean
		1	2	3	4	5	6	7	8	
Rubber	Control	237.5	128.5	286.5	68.5	39.0	269.5	88.5	238.5	169.6
	Treated	16.5	4.5	10.5	24.0	73.5	7.0	20.5	102.5	32.4
Polyamide	Control	215.0	268.0	164.5	19.0	251.5	159.5	151.5	223.0	181.5
	Treated	1.0	0.5	1.0	0.0	2.5	2.5	0.5	10.0	2.3

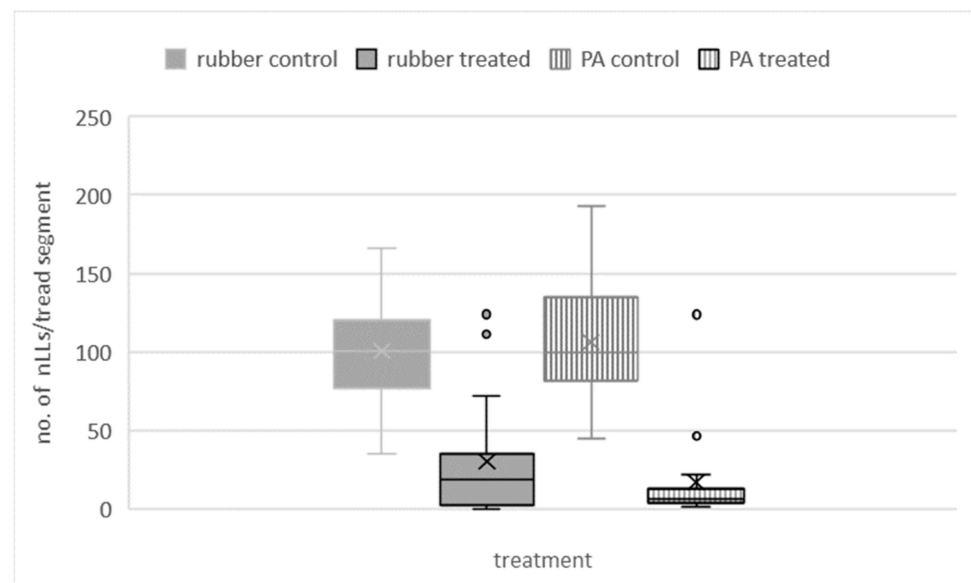


Figure 9. No. of necrotic local lesions (nLLs) on *N. tabacum* cv. Xanthi NN per tread segment after mechanical inoculation of swab samples. Samples were taken from tires with a rubber or polyamide tread initially contaminated by rolling over homogenated plant debris of an ToBRFV-infested commercial tomato production site (8.8 running meters; approx. 28 wheel turns) and, subsequently, after rolling over a ProfilGate clean running zone with a distance of 5 running meters, corresponding to 16 wheel turns. N = 64.

Especially before cleaning, the control segments contaminated with homogenized plant debris showed the scattering that is expected when using infected plant material that has not been further processed and adjusted to a definite viral load. The swab samples from the control segments yielded an average of around 100 nLLs on the indicator plants (Figure 9). After cleaning with the ProfilGate clean running zone, the counts were reduced to an average of 27 (rubber) and 17 (polyamide). Thus, the depletion under the selected test conditions amounted to 72.4% (rubber) and 83.7% (polyamide). The lower average ToBRFV depletion in the rubber tires was due to the greater dispersion of the 16 segments tested in each case. There were six almost completely decontaminated tire segments (<10 nLLs) but also three tire segments that were responsible for >50 nLLs.

Similar results were obtained with the use of ToBRFV-infected tomato fruits to contaminate the tires (Table 3). The removal of ToBRFV calculated by the formula $[100 - (\text{mean treatment} \times 100 / \text{mean control})]$ was, on average, 98.7% for the polyamide tires and 80.9% for the rubber tires. Here, too, the treatment of rubber tires led to different cleaning results. While four of the eight tires showed a removal of >90%, one tire was not freed from the viral load at all.

3.3. Inactivation of ToBRFV within the Clean-Off Zone

Swab samples from the clean-off zone showed that ToBRFV did not accumulate on the brush strips, grids, or in the disinfectant solution but was reliably inactivated. No necrotic local lesions were detected for the disinfectant solution, the swab samples of the brushes showed an average of 0.2 necrotic local lesions, and the swab samples of the grid resulted in 0.4 necrotic local lesions per indicator plant.

4. Discussion

As shown, tires of vehicles such as trolleys, tractors, or containers are contaminated with ToBRFV after driving over infected plant residues for only a few meters. Therefore, they contribute to the dispersion the virus on farms and beyond. For this reason alone, there is a need for a measure that leads to the safe decontamination of vehicle tires in horticulture.

As expected, the viral loads on the tires significantly differed in some cases depending on the inoculum used in the practical contamination. Thus, not only do the individual plant organs have different virus concentrations [20], but the degree of comminution of the plant residues and the (weight) proportion of ToBRFV-infected plant parts also contribute to a scattering of the viral load on the running surfaces naturally contaminated by rolling over the plant material. Therefore, we included an artificial contamination with a defined virus concentration to calculate a reliable degree of depletion considering different viral loads.

Since the emergence of the Tobacco mosaic virus, disinfection tests on tobamoviruses have a long history and are now the focus of the evaluation of hygiene measures in horticulture due to the worldwide re-emergence of tobamoviruses, such as the cucumber green mottle mosaic virus or the newly emerging tomato brown rugose fruit virus. These studies have focused on the disinfection of seeds [23–25], greenhouse surfaces [21,26,27], plant sap [28–30], personnel [16,17], and the soil [31]. Despite these various studies, the specific requirements and challenges in horticulture, such as organic and mineral pollution, porous surfaces, and short periods of crop cycles, still pose serious concerns when it comes to preventing the introduction and spread or eradication of tobamoviruses.

One of the vulnerabilities of many hygiene concepts within a company is the prevention of phytopathogens being introduced by the wheels of vehicles not belonging to the company and the carry-over between different greenhouse compartments by internal harvesting trolleys. A tire may have different loads of dirt adhesions, be made from different materials and have different tread depths. At the same time, due to the high weight of the vehicles, the demands on such a decontamination system are very high.

Using a clean-off zone filled with the disinfectant MENNO Florades, it was shown that tomato brown rugose fruit virus can be safely removed from tires when driving over this system. The depletion of ToBRFV was shown for different virus concentrations, for different tire materials, and in the presence of heavy soiling. When driving over a clean-off zone, the contaminated tread of a tire only comes into contact with the disinfectant solution for a few seconds, making the mechanical cleaning of infectious material from the tires very important. The importance of combining mechanical cleaning and chemical disinfection has already been highlighted for shoe sole decontamination [17]. The virus particles are mechanically cleaned from the treads together with the organic and mineral contaminants before falling into the disinfectant solution in the steel trays. The inactivation of ToBRFV then takes place over time in the trays. Accordingly, the contact time between the stable virus particles and the disinfectant is not just a few seconds of passing over the clean-off zone but is permanent in the working solution. This approach avoids the challenge of short contact times, which naturally reduce the effectiveness of a disinfectant. Wales and colleagues already highlighted that very short contact times were not yet covered in standard disinfection tests [18]. By combining the mechanical cleaning and chemical disinfection measures, these short contact times of <1 min have now been scientifically evaluated in a standardized way. Under clean, laboratory conditions without any pollution on the tires, the reduction in necrotic local lesions through the clean-off measure was between 91 and 98%. The relative depletion rates were nearly the same for the very high virus concentration as well as the medium and low concentrations. This suggests that it is irrelevant for the system how high the pathogen concentration is, but it is crucial not to have too much dirt on the running surfaces. If plant and fruit residues, i.e., gross organic soiling, had dried on the tires, the reduction was between 81 and 99%. It should be noted that a single tire showed no depletion of ToBRFV. Unfortunately, it was not possible to clarify whether this was due to technical or human error. However, these very promising results showed a relatively large variation. The results of decontamination trials are also supported by the results of the contamination of the tires by driving over a macerate (Figures 4 and 5), which led to highly variable virus concentrations on the tires.

The results of most of the decontamination tests were achieved with a tread of 5 m. Such a tread can be easily integrated into existing production facilities and, with the tire diameters used, allows the tires to be rolled over 8 or 16 times. The more often the

wheels can be rolled, the greater the decontamination effect. However, it goes without saying that a shorter length of the clean-off zone leads to less virus depletion on the contaminated wheels.

The effect of the tread material on the decontamination success was of minor importance in the investigations. In particular, the highly standardized test with a defined virus concentration and without any organic soiling (Figure 8) showed almost identical depletion rates.

The decontamination results show that this system can strongly enhance in-house hygiene concepts by separating black/white areas, i.e., areas with different hygiene requirements, and preventing adherent pathogens such as ToBRFV from further dispersal.

Alongside the depletion efficiency on the tires, a key requirement for the use of a clean-off zone is the phytosanitary safety of the brushes, grids, and all other components of the system. To ensure that ToBRFV and other pathogens do not accumulate in them, an effective and, above all, stable active ingredient is required that does not quickly degrade as a result of the inevitable presence of dirt. MENNO Florades has already been able to demonstrate these requirements for the decontamination of ToBRFV-contaminated shoe soles in a disinfection mat, and this has now also been confirmed in the ProfilGate clean-off zone [17]. The virus was reliably inactivated on brushes and grits and in the working solution despite several passes with some very high levels of ToBRFV contamination on the wheels ($4.5 \mu\text{g}/\text{cm}^2$). It must be continuously ensured that there is sufficient MENNO Florades solution in the trays so that both the top of the grid is wetted and the brushes are constantly in the solution to ensure that adhering ToBRFV virions can be reliably inactivated.

Despite the progress that has been made in breeding new ToBRFV-resistant varieties, the control of viral pathogens of plants will continue to require effective hygiene measures. Recent findings by Zisi et al. (2024) demonstrate that one mutation of the movement protein of a ToBRFV isolate has already been able to overcome new resistance under practical conditions. Furthermore, the authors concluded that viral pressure must be kept low through hygiene measures in order to ensure the resistance progresses in the long term [32]. Based on the present results, the ProfilGate clean-off zone containing the disinfectant MENNO Florades is an effective hygiene measure. It will help growers to eliminate another entry point for plant pathogens and minimize the spread of ToBRFV.

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