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For quality of life

Effectiveness of the cleaning process of Euro Pool System against pepino mosaic virus and bacteria (from the *Corynebacterium*-group) on rigid containers

Confidential Report

Martijn Schenk, Roel Hamelink & Ineke Stijger

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Wageningen UR Greenhouse Horticulture, Wageningen
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Summary

Viruses and harmful bacteria may have a large influence on greenhouse crops and affect yields and product quality. Pepino mosaic virus and Clavibacter are two examples of disease agents that currently cause problems in tomato crops. Pepino mosaic virus is transmitted mechanically and may, thus, be spread by containers in which fruits are carried. Clavibacter can also survive on containers in plant material or soil debris. Euro Pool System is a provider of reusable containers in the fresh food supply. Before the containers of Euro Pool System are recycled, they are washed in a washing installation.

This research examined whether the applied washing procedure is effective against pepino mosaic virus and a bacterium from the *Corynebacterium* group, to which Clavibacter also belongs. Two types of rigid containers were tested (M-size and T-size). The washing temperature and residence time of the containers was varied. One of the examined treatments was identical to the process that is currently used by Euro Pool System.

No infectious virus persists on the rigid containers in the process that is currently applied to wash the containers (washing at 70°C, addition of 0.13% of the cleansing agent Divoflow NTC, and a residence time of 60 seconds). The containers are also free of virus at a temperature of 60°C. However, pepino mosaic virus does persist on the M-sized containers at a temperature of 50°C. The T-sized containers are free of virus after all tested washing treatments. Residence time was varied between 45 and 60 seconds, which had no apparent effect on the effectively of the washing process. The difference in effectiveness of the washing process between M-sized and T-sized containers may be explained by the size of the small compartments underneath the upper edge of the containers. The bacteria from the *Corynebacterium* group did not survive on the containers in any of the treatments.

1 Introduction

Plant diseases caused by viruses and bacteria may have a large influence on cropping. Infections by these disease agents may lead to yield reductions or a reduced product quality. Part of the viruses is mechanically transmitted. This means that the transfer of the virus takes place through contact between plants and materials or surfaces or through plant-to-plant contact during routine crop management. For example, contaminated clothing, hands, tools and containers may spread these viruses. Bacteria can also stay alive on various surfaces and may subsequently spread to healthy crops.

Containers that are used in greenhouses and in which tomatoes are harvested and transported may become contaminated by sap of infected plants or fruits, or by infected plant remains. These containers enter the supply chain and are transported from growers to auctions and back. Thereby, these containers are a potential source of infection by viruses and bacteria. This can be overcome when the containers are thoroughly washed before they are recycled. Containers are washed in any case to remove plant debris, stickers and soil remains. In principle, the applied washing process might also be effective in removing the involved viruses and bacteria.

Pepino mosaic virus and Clavibacter are two organisms that are currently a major cause of disease in tomato cropping. Both organisms are only harmful for plants and pose no danger to human health at all. Because Clavibacter is a phytosanitary risk that is regulated by Quarantine measures, the organism itself can not be used for testing. However, a related bacterium that grows under comparable circumstance is available.

1.1 Pepino mosaic virus

Pepino mosaic virus is transmitted mechanically and causes major problems in tomato cropping. The virus has rapidly spread throughout Europe since its introduction in 1999. From 2005 onwards, problems have increased in severity. This is possibly related to the introduction of new virus variants. The virus is currently found throughout large parts of Europe and Northern-America.

Pepino mosaic virus may cause various symptoms on tomato, including mosaic, leaf deformation, nettle-like heads, yellow specks on the leaves and chlorosis (yellowing) between the veins (*Figure 1.1*). Some variants of the virus also cause necrosis (dying of tissue) on leaves or stems. These symptoms may be accompanied by yield losses. Fruit symptoms of the virus (uneven ripening, flamed fruits, marbling, and size reduction) cause most of the economic damage, because these symptoms affect the marketability of the fruits (*Figure 1.2*). Once a plant has been infected, it may recover to some extent, but will always stay infected.

Pepino mosaic virus is easily spread by mechanic transmission through contaminated hands, clothing, and tools. The virus survives on various surfaces and may subsequently infect new plants. Contact between healthy plants and a contaminated surface suffices to induce infection. Hygiene measures are therefore requited to limit the spread of pepino mosaic virus.

Figure 1.1. Several symptoms that are characteristic of pepino mosaic virus. Leaves can be affected by chlorosis (top left), leaf bubbling (top right), and mosaic (bottom left). Plant heads may be affected by nettle-like leaves (bottom right).

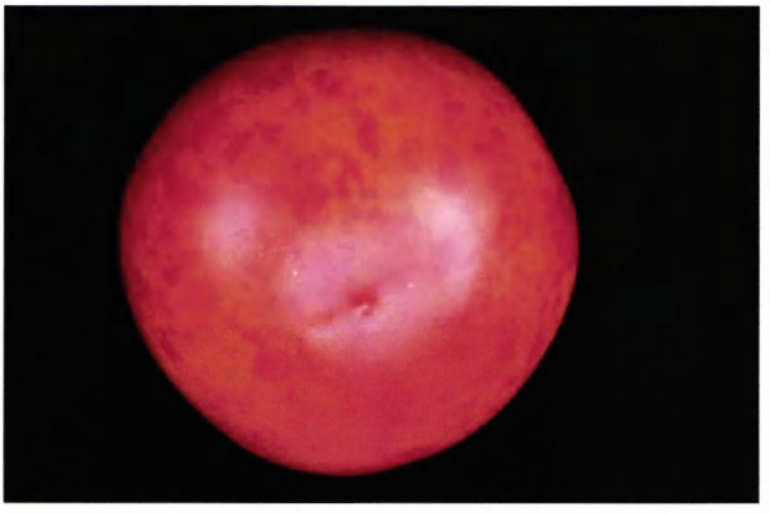

Figure 1.2. Tomato fruit of a plant that is infected by pepino mosaic virus.

1.2 Clavibacter

Problems caused by Clavibacter have reoccurred in the Netherlands since 2007. The bacterium causes the so called 'bacterieverwelkingsziekte' that may cause serious damage to tomato plants. Clavibacter is transmitted through seeds, by young plants, fruits, water and during routine crop handling. In addition, the bacterium survives in the soil or in substrate. Infected leaf material or soil debris that stays behind in containers is therefore a potential source of infection of this bacterium. Because Clavibacter is a Quarantine organism according to EU regulations, one is obliged to prevent the spread of this organism.

Plants that have become infected by Clavibacter are affected by various symptoms on leaves and stems. First of all, glassy patches appear between the veins of a leaf. These leaves become light brow or start yellowing, while leaf tops wilt or dry out. This is succeeded by wilting of the entire plant. Once a plant has become infected, it will not recover and the disease will lead to yield reduction and quality losses (*Figure 1.3*).

Figure 1.3. Fruits of a plant that is infected by Clavibacter.

1.3 Research aims

In research that was carried out in 2000, we examined the washing process that was applied by Euro Pool System at that time. The effectiveness of this process against Pepino mosaic virus was evaluated for T-size and L-size rigid containers. In 2000, the washing processes involved a 2.5 section washing installation in which 0.35% of the cleansing agent Sekwea was added at a washing temperature of 70°C.

Currently, the washing process has been improved. Future adaptations may take place that consider energy savings and the speed of the washing procedure. Therefore, new research was desirable and carried out. Wageningen UR Glastuinbouw has determined whether the washing process that is currently applied by Euro Pool System is effective against Pepino mosaic virus and a bacterium from the *Corynebacterium* group. During these tests, temperature of the water tanks and residence time of the containers has been varied to determine whether future adaptations are possible without affecting the risks for contamination by plant viruses and bacteria.

2 Methods

2.1 Production of infectious virus and bacteria

Tomato plants were grown at the greenhouse facilities of Wageningen UR Glastuinbouw (Bleiswijk, The Netherlands). These plants were infected by pepino mosaic virus. Infected plants were maintained at 20-22°C, and under a constant light regime. Infected leaves were harvested and subsequently ground after the addition of demineralized water. The leaf suspension was not filtered through cheese cloth, so that small leaf remains were still present in the suspension.

Because Clavibacter is a phytosanitary risk that is regulated by Quarantine measures, the organism itself can not be used for testing. Therefore, a closely related bacterium *Corynebacterium* group was selected for testing. This bacterium has its optimal growth under comparable circumstance as Clavibacter. Fresh material of this bacterium was cultured on nutrient agar one week before the tests took place. Bacterium cultures were scraped form the surface of the agar and suspended in a saline solution (0.9% NaCl).

2.2 Contamination of the containers

Euro Pool System delivered 22 rigid containers of the T-size and 22 rigid containers of the M-size to the facilities of Wageningen UR Glastuinbouw in Bleiswijk. Bacterium suspension was spread on these containers at three locations (*Figure 2.1*) on October 13, 2008. These locations were marked by permanent markers beforehand. After the suspension was added, containers were shortly kept at room temperature to allow the suspension to dry up slightly. Containers were packaged separately to avoid infection of the negative controls by contaminated containers. The containers were transported to the washing installation at Geldermalsen (The Netherlands) on October 14. Two containers stayed behind in Bleiswijk to serve as (unwashed) controls.

The same containers were contaminated on three locations (*Figure 2.1*) by sap of plants that were infected by pepino mosaic virus on November 6, 2008. These locations were marked by permanent markers beforehand. After the suspension was added, containers were shortly kept at room temperature to allow the suspension to dry up slightly. Containers were packaged separately to avoid infection of the negative controls by contaminated containers. The containers were transported to the washing installation at Geldermalsen (The Netherlands) on November 7. Two containers stayed behind in Bleiswijk to serve as (unwashed) controls.

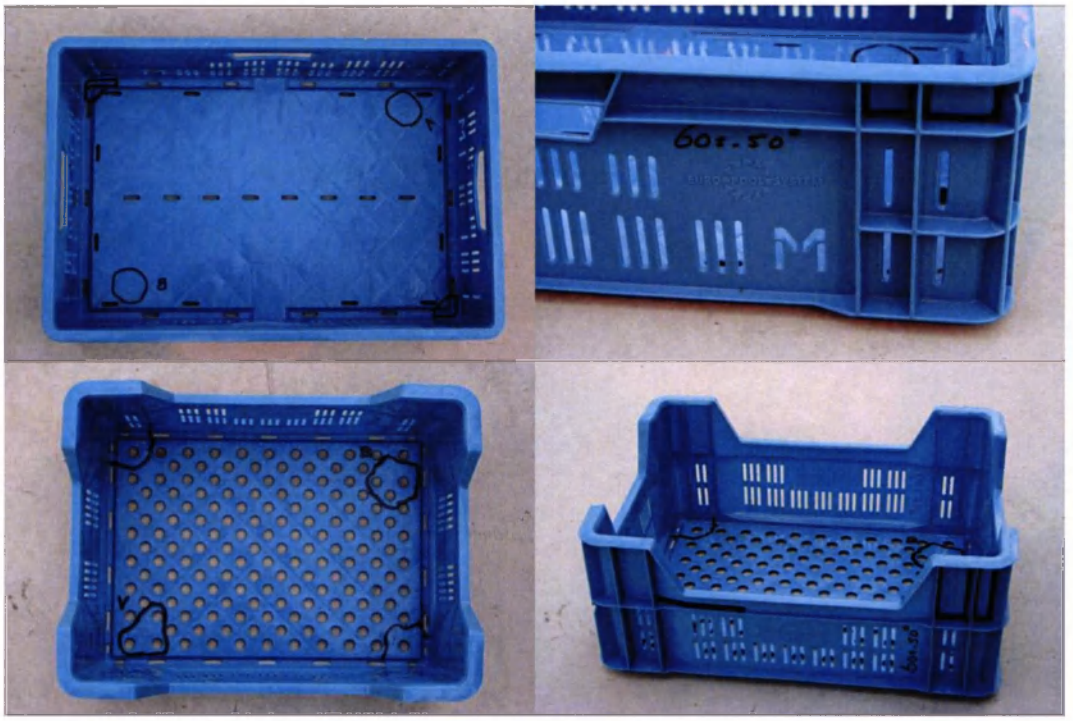

Figure 2.1. The locations at which the virus and bacterium suspensions were spread on M-sized (left) and T-sized rigid containers (right). The virus suspension was spread on the side of the container marked with a V, while the bacterium suspension was spread on the side marked with a B.

2.3 Treatments

In total, we tested eleven different treatments (*Table 2.1*) for both the virus and bacterium tests. Besides two control treatments, three different water temperatures (50°, 60°, and 70°C) and three different residence times (45, 50, and 60 seconds) were examined.

Table 2.1. The order in which the treatments were tested during the tests at Euro Pool System in Geldermalsen. Two containers of both the T-size and M-size were tested in each treatment.

Treatment	Infected	Temperature	Residence time
1	Yes	50 °C	60 sec
2	Yes	50 °C	50 sec
3	Yes	50 °C	45 sec *1
4	Yes	60 °C	60 sec
5	Yes	60 °C	50 sec
6	Yes	60 °C	45 sec *1
7	Yes	70 °C	60 sec
8	Yes	70 °C	50 sec
9	Yes	70 °C	45 sec *1
10	No	70 °C	45 sec
11	Yes	Unwashed	Unwashed

*1 *In the original experimental set-up, a residence time of 40 seconds was scheduled. However, this was not feasible as the maximum speed of the installation was reached at 45 seconds.*

The treatment with the uninfected containers served as a control treatment that was carried out to determine whether pepino mosaic virus or test bacteria stayed behind in the washing installation itself.

2.4 Washing procedure

The test with the bacterium was carried out in Geldermalsen on October 15, while the test with pepino mosaic virus was carried out at this location on November 10. The washing installation in Geldermalsen is considered to be representative for the other installations used by Euro Pool System. The installation consists of six water tanks of 3 m^3 each. The first water tank is non-heated and is used for the first washing step. The water tanks two to five are heated and the cleansing agent Divoflow NTC is added to a concentration of 0.13%. The sixth water tank is used to rinse off any cleansing agent (*Figure 2.2*). The temperature of the water tanks two to five was varied from 50°C to 70°C during the tests. Euro Pool System currently uses a temperature of 70°C in these tanks.

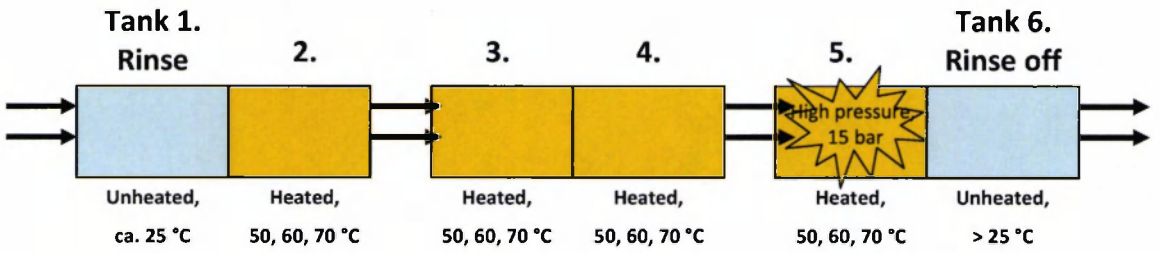

Figure 2.2. Schematic overview of the washing installation of Euro Pool System in Geldermalsen.

The containers pass through the installation on a double-tracked chain driven conveyor. Containers are washed both on the inside and outside by nozzles during the transmission. In water tank 5, this takes place under high pressure (20 bar according to the specifications, but approximately 15 bar is reached in practice). The pressure attained by the nozzles in the other tanks is approximately 3 bar. During the tests, the residence time of the containers on the conveyor was varied from 45 to 60 seconds. Euro Pool System currently uses a residence time of 60 seconds at the installation in Geldermalsen. Under these circumstances, the capacity of the washing installation is approximately 4,250 containers per hour for the T-size containers and 3,000 containers per hour for the M-size containers. *Figure 2.3* shows the washing installation in operation.

Figure 2.3. The washing installation of Euro Pool System in Geldermalsen.

2.5 Detection of virus and bacteria

The effectiveness of each treatments against pepino mosaic virus was determined using test plants. These tests started at November 12 and indicated whether infectious virus persisted on the containers after washing. A wad of cotton was soaked in inoculation buffer and subsequently rubbed over the location on the container that was initially contaminated. Then, the wad of cotton was rubbed over a leaf of a healthy plant. Leaves were dusted with carborundum powder beforehand to encourage a possible infection. Two test plants were used per contaminated location on the container. The plant sap that was used to contaminate the containers was also tested for its infectivity on two test plants.

Because the symptoms of the applied virus isolate of pepino mosaic virus are relatively mild, it is not possible to establish the presence or absence of infections visually. Therefore, leaf material of the test plants was picked and tested for the presence of a viral infection by using a DAS-ELISA (serological test) 14 days after the tests started. This testing procedure does not allow quantification of the effectiveness of a treatment and will only yield a yes/no answer to whether a treatment is effective.

The effectiveness of each treatment against the test bacterium was determined in a detection test. A wad of cotton was soaked in a sterilized saline solution (0.9% NaCl) and rubbed over the location on the container that was initially contaminated. Then, the wad of cotton was soaked in 10 ml of saline solution for two hours and subsequently spread on nutrient agar in three different dilutions. This testing procedure allows the quantification of the number of bacteria and thereby the quantification of the effectiveness of each treatment. Petri dishes were incubated at 28°C and visually inspected for the presence of the applied *Corynebacterium* after 7 days. The inspection was repeated after 10 and 14 days.

3 Results

3.1 Pepino mosaic virus

The infectivity of the plant sap that was used to contaminate the rigid containers with pepino mosaic virus was tested on two test plants. Both test plants gave a positive reaction in the DAS-ELISA, indicating that the plant sap was infectious. The results of the tests on pepino mosaic virus are shown in *Table 3.1*. This table indicates whether infectious virus was still present on the containers in each of the 11 treatments after the washing procedure. In one treatment, the containers were not washed in the washing installation. These containers tested positive for the presence of pepino mosaic virus at all three locations. This indicates that pepino mosaic virus is able to survive on containers and stay infectious in plant sap for several days.

Infectious virus was detected after the washing process in two treatments. Washing took place at 50°C in both 'positive' treatments, while using a transit time of 50 and 60 seconds, respectively. No infectious virus was recovered at 50°C when a transit time of 45 seconds was applied. The contaminations were detected at the outside of the containers on both occasions.

The containers in the 'uninfected control treatment' were free of virus after the washing process. This indicates that pepino mosaic virus which ends up in the water tanks is not able to infect clean containers afterwards.

Table 3.1. The effectiveness of the various treatments against pepino mosaic virus. Treatments were carried out at the washing installation in Geldermalsen. In each treatment, four rigid containers (two M-size and two T-size) were contaminated with pepino mosaic virus at three locations (bottom, corner, outside). Two test plants were evaluated per container. The table displays the number of plants that tested positive for the presence of pepino mosaic virus (0, 1 or 2).

Treatment	Location*[1]	M-size, container 1	M-size, container 2	T-size, container 1	T-size, container 2
1.	Bottom	0	0	0	0
(50 °C, 60 sec)	Corner	0	0	0	0
	Outside	2	2	0	0
2.	Bottom	0	0	0	0
(50 °C, 50 sec)	Corner	0	0	0	0
	Outside	2	0	0	0
3.	Bottom	0	0*[2]	0	0
(50 °C, 45 sec)	Corner	0	0*[2]	0	0
	Outside	0	0*[2]	0	0
4.	Bottom	0	0	0	0
(60 °C, 60 sec)	Corner	0	0	0	0
	Outside	0	0	0	0
5.	Bottom	0	0	0	0
(60 °C, 50 sec)	Corner	0	0	0	0
	Outside	0	0	0	0
6.	Bottom	0	0	0	0
(60 °C, 45 sec)	Corner	0	0	0	0
	Outside	0	0	0	0
7.	Bottom	0	0	0	0
(70 °C, 60 sec)	Corner	0	0	0	0
	Outside	0	0	0	0
8.	Bottom	0	0	0	0
(70 °C, 50 sec)	Corner	0	0	0	0
	Outside	0	0	0	0
9.	Bottom	0	0	0	0
(70 °C, 45 sec)	Corner	0	0	0	0
	Outside	0	0	0	0
10.	Bottom	0	0	0	0
(uninfected	Corner	0	0	0	0
containers)	Outside	0	0	0	0
11.	Bottom	2	2	2	2
(unwashed)	Corner	2	2	2	2
	Outside	2	2	2	2

*[1] The **bottom** location is the bottom on the inside of the container, the **corner** location is the corner on the inside where the bottom meets the side of the container, **outside** locations are the compartments on the outside of the container beneath the upper edge (see also Figure 2.1).*

[2] This container was stuck in the installation during the procedure, and its transit time was 70 seconds rather than 45 seconds.

Table 3.2. The effectiveness of the various treatments against Corynebacterium. Treatments were carried out at the washing installation in Geldermalsen. In each treatment, four rigid containers (two M-size and two T-size) were contaminated with Corynebacterium at three locations (bottom, corner, outside). The presence of bacteria after washing was determined in three dilutions (10^0, 10^{-1} and 10^{-2}). The table indicated whether infectious bacteria were detected (yes, no).

Treatment	Location*[1]	M-size 10^0	M-size 10^{-1}	M-size 10^{-2}	T-size 10^0	T-size 10^{-1}	T-size 10^{-2}
1.	Bottom	no	no	no	no	no	no
(50 °C, 60 sec)	Corner	no	no	no	no	no	no
	Outside	no	no	no	no	no	no
2.	Bottom	no	no	no	no	no	no
(50 °C, 50 sec)	Corner	no	no	no	no	no	no
	Outside	no	no	no	no	no	no
3.	Bottom	no	no	no	no	no	no
(50 °C, 45 sec)	Corner	no	no	no	no	no	no
	Outside	no	no	no	no	no	no
4.	Bottom	no	no	no	no	no	no
(60 °C, 60 sec)	Corner	no	no	no	no	no	no
	Outside	no	no	no	no	no	no
5.	Bottom	no	no	no	no	no	no
(60 °C, 50 sec)	Corner	no	no	no	no	no	no
	Outside	no	no	no	no	no	no
6.	Bottom	no	no	no	no	no	no
(60 °C, 45 sec)	Corner	no	no	no	no	no	no
	Outside	no	no	no	no	no	no
7.	Bottom	no	no	no	no	no	no
(70 °C, 60 sec)	Corner	no	no	no	no	no	no
	Outside	no	no	no	no	no	no
8.	Bottom	no	no	no	no	no	no
(70 °C, 50 sec)	Corner	no	no	no	no	no	no
	Outside	no	no	no	no	no	no
9.	Bottom	no	no	no	no	no	no
(70 °C, 45 sec)	Corner	no	no	no	no	no	no
	Outside	no	no	no	no	no	no
10.	Bottom	no	no	no	no	no	no
(uninfected	Corner	no	no	no	no	no	no
containers)	Outside	no	no	no	no	no	no
11.	Bottom	no	no	no	no	no	no
(unwashed)	Corner	no	no	no	no	no	no
	Outside	no	no	no	no	no	no

*[1] The **bottom** location is the bottom on the inside of the container, the **corner** location is the corner on the inside where the bottom meets the side of the container, **outside** locations are the compartments on the outside of the container beneath the upper edge (see also Figure 2.1).*

3.2 Corynebacterium

The suspension that was used to contaminate the rigid containers with *Corynebacterium*, was tested for its infectivity by culturing the suspension on nutrient agar at three different dilutions (*Figure 3.1*). The number of bacteria in the suspension can be deduced by counting the number of bacterial colonies. This test indicated that the suspension contained 3.7 x 10^9 colony-forming units per ml suspension.

In one treatment, the containers were not washed in the washing installation. All four containers in this treatment tested negative for the presence of *Corynebacterium* at all three locations. This indicates that *Corynebacterium* is not able to survive on containers under the test conditions for more than three days. Obviouosly, no test bacteria were recovered in any of the treatments after the washing procedure (*Table 3.2)*. Other than the absence of the test bacteria, large numbers of bacteria and fungi were present on the Petri dishes after the washing procedure.

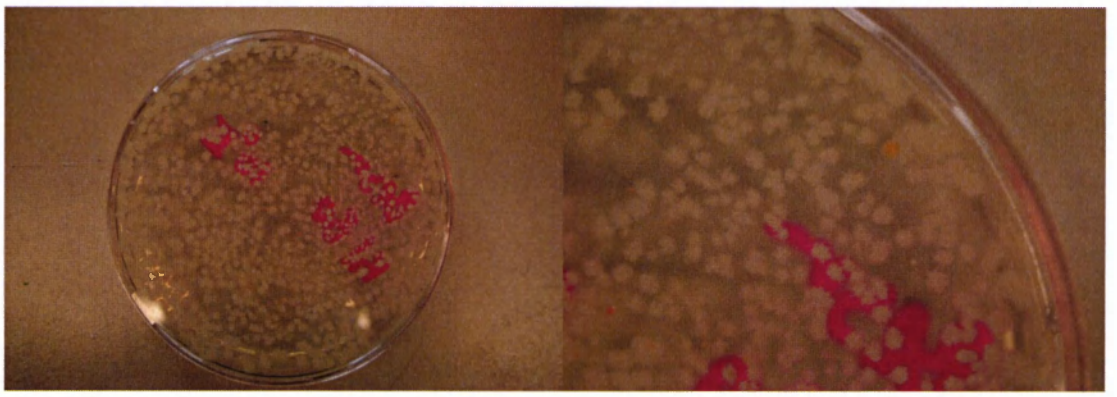

Figure 3.1. One of the Petri dishes on which the diluted bacteria suspension was spread (left). A section on which a colony of the test bacterium (Corynebacterium) is present (bright orange).

4 Discussion

A summary of the test results per treatment is shown in *Table 4.1.* The test bacterium from the *Corynebacterium*-group is not able to survive the treatment as a whole (drying out, washing, and again drying out). The fact that this bacterium does not survive the positive control treatment as well, suggests that the test bacterium does not withstand drying out of the suspension.

In contrast, pepino mosaic virus is very well capable to survive on containers for a long period of time. The virus is also able to withstand and survive part of the examined washing procedures. Infectious virus is present on the M-size containers after washing these containers at a temperature of 50°C. We found no apparent effect of residence time on the effect of the washing process, but the varied parameters (45, 50, and 60 seconds) are perhaps too much alike. The amount of virus that was used to contaminate the containers with pepino mosaic virus can be considered as high. In this respect, the tests represent a *worst case* scenario and one may expect that less virus is present when an infection takes place in practice. In contrast, the containers that were used in these tests were new and did not have any cracks or rough surfaces. In practice, such cracks or surfaces will be more difficult to wash and may contain virus particles or bacteria.

Table 4.1. Effectiveness of the various washing procedures against pepino mosaic virus as tested in the washing installation in Geldermalsen. Plus signs in the table indicate that pepino mosaic virus or Corynebacterium was discovered on the rigid containers after the washing procedure.

Treatment	M-size virus	M-size bacterium	T-size virus	T-size bacterium
1. (50 °C, 60 sec)	-	-	-	-
2. (50 °C, 50 sec)	+	-	-	-
3. (50 °C, 45 sec)	+	-	-	-
4. (60 °C, 60 sec)	-	-	-	-
5. (60 °C, 50 sec)	-	-	-	-
6. (60 °C, 45 sec)	-	-	-	-
7. (70 °C, 60 sec)	-	-	-	-
8. (70 °C, 50 sec)	-	-	-	-
9. (70 °C, 45 sec)	-	-	-	-
10. (neg. control)	-	-	-	-
11. (unwashed)	+	-	+	-

The T-size containers were free of virus after all tested washing procedures, while the M-size containers were not. The difference in effectiveness of the washing process between the M-size and T-size containers may be explained by the size of the small compartments underneath the upper edge of the containers. These compartments are difficult to wash due to their shape and size. The outside of the containers has a smaller chance of becoming contaminated with infected plant sap than the inside. Contact between infected fruits and the containers will take place on the inside when the containers are used while harvesting tomatoes. However, since the outside of the containers is touched by hands on which plant sap is present, it is likely that containers become contaminated by virus on the outside. Plant sap will also get on the outside when containers are used during crop management practices.

Several differences were found when comparing the present results to the tests that were carried out in 2000. In the former tests, T-size and L-size rigid containers were tested, while the washing processes involved a 2.5 section washing installation to which 0.35% of the cleansing agent Sekwea was added. In 2000, the presence of infectious pepino mosaic virus was established at washing temperatures of 60°C or 65°C. Both examined types of containers were free of virus at 60°C in the current tests. However, several testing parameters have been altered since 2000, namely (I.) the installation now consists of four heated water tanks rather than two, (II.) the cleaning agent that is now applied is Divoflow NTC rather than Sekwea, and (III.) high pressure is now applied in one of the water tanks. Additionally, one type of rigid container that tested positive in 2000 (L-size) was not tested in the current research. One of the abovementioned variables or a combination of multiple variables may account for the difference in effectiveness at 60°C.

After the containers are washed, they are generally stored before they actually enter circulation again. The duration of the storage will also affect the survival of viruses and bacteria. Most viruses that are mechanically transmitted are able to stay infectious on contaminated surfaces for several days, weeks or perhaps even months. Infectivity will, however, decrease in the course of time. By storing the containers for a longer period of time, it is theoretically possible to wash at a temperature of 50°C without running the risk of infection. How long this storage time will have to be in order to reduce the risks to acceptable levels is unknown, and therefore no specification can be given.

5 Conclusion

The washing procedure that is currently applied by Euro Pool System on the type of installation as present in Geldermalsen, has been examined for its effectiveness against pepino mosaic virus and a bacterium from the *Corynebacterium* group.

- Under the circumstances that are currently applied by Euro Pool System in Geldermalsen (washing at 70°C, addition of 0.13% of the cleansing agent Divoflow and a residence time of 60 seconds) no infectious pepino mosaic virus or *Corynebacterium* was detected behind on rigid containers of the M-size and T-size. This applies to three locations on the containers, including a location on the outside that is difficult to access by the installation.
- After a reduction of the transit time of containers from 60 to 45 seconds, no infectious pepino mosaic virus or *Corynebacterium* was detected on rigid containers of the M-type and T-type under the circumstances that are currently applied by Euro Pool System in Geldermalsen (washing at 70°C, addition of 0.13% of the cleansing agent Divoflow).
- After a reduction of the washing temperature from 70°C to 60°C, no infectious pepino mosaic virus or *Corynebacterium* was detected on rigid containers of the M-size and T-size under the circumstances that are currently applied by Euro Pool System in Geldermalsen (addition of 0.13% of the cleansing agent Divoflow and a residence time of 60 seconds).
- A further reduction of the washing temperature to 50°C is not possible as infectious pepino mosaic virus is then able to stay behind on rigid containers of the M-size. This introduces the risk of spreading the virus by containers.

6 Statements

6.1 M-sized rigid containers

To whom it may concern
Euro Pool System International B.V., Rijswijk, the Netherlands has requested Wageningen UR Glastuinbouw (project number 32 42058400) to test whether their washing process effectively removes viruses and bacteria from their containers.
For this purpose, containers were provided, intentionally contaminated and washed as follows:

Sample description:	M-sized container.
Plant diseases:	Pepino mosaic virus and bacterium from the (*Corynebacterium*-group).
Washing installation:	Euro Pool System Geldermalsen, Netherlands.
Washing parameters:	50°C-60°C, 45 seconds, 0.13% cleaning agent Divoflow.
Test period:	October - November 2008.

Test and regulations
Virus and bacterium suspension were intentionally spread on the containers at the facilities of Wageningen UR in Bleiswijk, the Netherlands.
The containers were shortly kept at room temperature to allow the suspension to dry up. The containers were then transported and washed.

Results and conclusion
It was shown that rigid containers of the M-size were free of pepino mosaic virus after the washing procedure applied by Euro Pool System in Geldermalsen in which the following parameters were used: 0.13% of the cleansing agent Divoflow is added to the water tanks, a residence time of minimal 45 seconds is used, and the water temperature in the water tanks is at least 60°C.

It was shown that rigid containers of the M-size were free of *Corynebacterium* test bacteria after the washing procedure applied by Euro Pool System in Geldermalsen in which the following parameters were used: 0.13% of the cleansing agent Divoflow is added to the water tanks, a residence time of minimal 45 seconds is used, and the water temperature in the water tanks is at least 50°C.

The results are described in detail in the Report.

Approved by:

6.2 T-sized rigid containers

To whom it may concern
Euro Pool System International B.V., Rijswijk, the Netherlands has requested Wageningen UR Glastuinbouw (project number 32 42058400) to test whether their washing process effectively removes viruses and bacteria from their containers.
For this purpose, containers were provided, intentionally contaminated and washed as follows:

Sample description:	T-sized container.
Plant diseases:	Pepino mosaic virus and bacterium from the (*Corynebacterium*-group).
Washing installation:	Euro Pool System Geldermalsen, Netherlands.
Washing parameters:	50°C, 45 seconds, 0.13% cleaning agent Divoflow.
Test period:	October - November 2008.

Test and regulations
Virus and bacterium suspension were intentionally spread on the containers at the facilities of Wageningen UR in Bleiswijk, the Netherlands.
The containers were shortly kept at room temperature to allow the suspension to dry up. The containers were then transported and washed.

Results and conclusion
It was shown that rigid containers of the T-size were free of pepino mosaic virus after the washing procedure applied by Euro Pool System in Geldermalsen in which the following parameters were used: 0.13% of the cleansing agent Divoflow is added to the water tanks, a residence time of minimal 45 seconds is used, and the water temperature in the water tanks is at least 50°C.

It was shown that rigid containers of the T-size were free of *Corynebacterium* test bacteria after the washing procedure applied by Euro Pool System in Geldermalsen in which the following parameters were used: 0.13% of the cleansing agent Divoflow is added to the water tanks, a residence time of minimal 45 seconds is used, and the water temperature in the water tanks is at least 50°C.

The results are described in detail in the Report.

Approved by: