Clean Light for disinfection of trays

For UV Source b.v. and Van der Lugt,
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Concerning:
Effect on plantpathogene microorganisms of different methods of disinfection of trays with water or hypochloride in water and Clean Light technique.

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1. Trial setup en analysis methods

In accordance to out offer we did tests on methods of disinfections of trays by using water or hypochloride solutions, and Clean Light technique.
Before the treatments were carried out M. Hoekstra of UV Source b.v. checked the right setup and time of exposure to UV-c. He approved that the set-up was all right.

Materials and trial setup

UV Source b.v. supplied the following materials to use in the trials:

- Clean Light XL, we used only one of the tubes because the dosis of one lamp was high enough
- Helmed and glasses to protect face and eyes for radiation
- Gloves to protest hands
- Sensor to measure the right dosis of UV-c on the surface of the trays. In this way we could set the right time of exposure during the trials to get 50 and 5 mJ/cm^2 of dosis.
- Clean Light setup
    - Distance between treated tray (sensor) and UV-luminaire was 56cm.
    - One Clean Light XL was used to get the right dosis
    - The Clean Light XL is set on at least 5 minutes before use to warm
    - Dosis per second is 2,5 mJ/ cm^2.s
    - This means exposure time of 2 seconds for treatment of 5mJ/cm^2
    - This means exposure time of 20 seconds for treatment of 50mJ/ cm^2

Van der Lugt has supplied us with enough trays to do the tests

- We used several trays which we disinfected on forehand with water and high concentration of hypochloride. We let them dry for a day.
- We marked the area to be treated with black dots: 5 by 10 cm
- Just before starting the tests we again disinfected this area with alcohol

Pictures of trays

Furthermore we used ore own materials in the trials

- Hygene gloves to change for new ones before every new tray
- Quarantine area to prevent that other infections occur
- Severals materials to create a set up for the trial in de quarantine area
- Concentrated plantpathogenes
- Cotton pads to wipe the 5x10 area after disinfection
    - The whole 5x10cm area was wipes after treatments
    - Cotton pad is about 5 layers of cotton with 4cm diameter
    - Cottons pads where used to incubate the plants with 'virus'.
    - Cottons pads with (water)fungi and bacteria were treated with a small fixed solution of disinfected demiwater and put on pathogen specific agar in petri dishes in dilution series. Results for (water)fungi and bacteria are converted to c.f.u. (*) per cm2 tray.
- Pure demiwater or solution of demiwater with hypochlorite (150ppm)
  150 ppm is a high concentration, is a 0,1% solution, that means 1 liter of 15% concentrated sodium-hypochlorite on 1.000 liter water)
    - The area of 5x10cm is rinsed with 10cc by using a 25cc-syringe
      This is equal to 0,5 liter spraying over one tray of 40x60cm
    - When rinsing the trays was held in about 45 degreed angle.
    - The solution was spread over the 10cm edge of the 5x10cm
    - The volume is equal to 2mm

(*) c.f.u. means colony forming units (k.v.e. in Dutch). That means we count every colony that is grown from one fungi-spore or from one bacteria. By this mean we can quantify the amount on bacteria and fungi that is still infectious after the several different disinfection treatments.

For infection of the trays we used the following pathogens

- C-bacteria grown on agar: we used only the concentrated bacteria
  On an area of 5x10 we used a little pinch but a high dosis and spread that over 5x10cm. Despite the high dosis of infection after only one minute the suspension was dry and not visible anymore, but still infectious (as also proven later on).
- Phytophthora spp. grown in agar; we used the phytophthora plus some agar because this water-fungi grows in the agar. We used a little pinch and spread that over 5x10cm.
- Pythium spp. grown in agar; we used the pythium plus some agar because this water-fungi grows in the agar. We used a little pich and spread that over 5x10cm.
- Bacteria and fungi; non identified species grown on several agar media.
  We used a mix of high concentrated bacteria and fungi and spread a little pinch over 5x10cm. Despite the high dosis of infection after only one minute the suspension was dry and not visible anymore, but still infectious (as also proven later on).
- Cucumber virus: Fur cucumber virus (CGMMV): we used crunched leaves of infected plants. By this mean the area of 5x10 cm on the tray showed green after infection. Before further disinfection treatments the organic matter was more or less dry.
- Tomato virus: Pepino mosaic virus (PepMV); we used crunched leaves of infected plants. By this mean the area of 5x10 cm on the tray showed green after infection. Before further disinfection treatments the organic matter was more or less dry.

These methods of infection are representative for what also happens with trays in practice. Trays from customers sometimes only come back after a few days after deliveries. Or they are held on stock before disinfection takes place. In that case organic matter sticks on the trays are dry green matter in which viruses can easy survive. For bacteria and fungi this means that before disinfection treatment the infection pressure can grow up to high levels.

All 6 pathogens are handled separately in separate trials.

Picture of trial setup

Treatments

Treatment 0
- control treatment
- add pathogens on tray
- without disinfection treatments
- wipe area with cotton pad
- use cotton pad for analysis or incubation of plants

Treatment 1
- disinfection with alcohol
- add pathogens on tray
- rinse area with water
- treat area with 50 mJ/cm^2 UV-c
- wipe area with cotton pad
- use cotton pad for analysis or incubation of plants

Treatment 2
- disinfection with alcohol
- add pathogens on tray
- rinse area with hypochlorite solution
- treat area with 50 mJ/cm^2 UV-c
- wipe area with cotton pad
- use cotton pad for analysis or incubation of plants

Treatment 3
- disinfection with alcohol
- add pathogens on tray
- rinse area with hypochlorite solution
- wait for 10 minutes
- wipe area with cotton pad
- use cotton pad for analysis or incubation of plants

Treatment 4
- disinfection with alcohol
- add pathogens on tray
- rinse area with hypochlorite solution
- treat area with **5** mJ/cm^2 UV-c
- wipe area with cotton pad
- use cotton pad for analysis or incubation of plants

Direct infection

To check the so called bio-test
- 2 plants are also treated directly with crunched infected leaves.
- 2 plants are not treated to check that no virus was in the plant before trial.

Analyse methods

C-bacteria
Add sample to specific C-bacteria-agar-medium to grow. That proves the bacteria are alive and thus still infectious. Identifying of the specific bacteria is done by Bio-PRC a DNA detection technique. The outcome is semi-quantitative, that means big differences must be seen as different. Small differences are seen as equal.

Phytophthora spp.
Add sample to specific Phytophthora-agar-medium to grow. That proves the waterfungi is alive and thus still infectious. Identifying of the specific waterfungi is done by r.t-PRC, a DNA detection technique. The outcome is + or -, as being still infectious (+) after the disinfection treatments or not (-).

Pythium spp.
Add sample to specific Pythium-agar-medium to grow. That proves the waterfungi is alive and thus still infectious. Identifying of the specific waterfungi is done by r.t-PRC, a DNA detection technique. The outcome is + or -, as being still infectious (+) after the disinfection treatments or not (-).

Bacteria and fungi
Add sample to a general agar-medium to grow. After several days we count for the amount of bacteria and fungi in so called colony formed units (c.f.u.). This gives an impression of a quantitative effect of the treatments.

Cucumber virus: Fur cucumber virus (CGMMV)
Incubate the sample to 2 cucumber plants. In case of infectious virus the plants mostly shows symptoms after about 3 weeks. Also without symptoms it is possible that the virus grows in the plant. The positive-control-plants show when symptoms are visible in time. Pieces of top-leaves are tested in specific Elisa test.
The outcome is + or -, as being still infectious (+) after the disinfection treatments or not (-).

Tomato virus: Pepino mosaic virus (PepMV)
Incubate the sample to 2 cucumber plants. In case of infectious virus the plants mostly shows symptoms after about 3 weeks. Also without symptoms it is possible that the virus grows in the plant. The positive-control-plants show when symptoms are visible in time. Pieces of top-leaves are tested in specific Elisa test.
The outcome is + or -, as being still infectious (+) after the disinfection treatments or not (-).


Vitagreen

3 Results and conclusions

In general

Overall you could say that doing the maximum to inactivate the plantpathogens is the best. In this trial that means first flush the trays with water and hypochlorite, soaking the trays for about 10 minutes and than treat them with 50mJ/cm^2 Clean Light. But for every specific pathogen more or less treatments on trays are needed to inactivate that specific pathogen.

Bacteria and fungi in general and C-bacteria specific

Treatment	*c.f.u. bacteria /cm2*	*% b*	*c.f.u. fungi /cm2*	*% f*	*C-bacteria*	*% C-b*
0: Control from tray (100%)	2.500.000	100%	78.000	100%	100.000.000	100%
1: H2O -> 50 mJ/cm^2	220.000	8,8%	20.000	0,8%	200	0,01%
2: Hypochlorite -> 50 mJ/cm^2	140.000	5,6%	11.000	0,4%	79	0,00%
3: Hypochlorite -> 10 minutes	180.000	7,2%	17.000	0,7%	18	0,00%
4: Hypochlorite -> 5 mJ/cm^2	1.300.000	52,0%	22.000	0,9%	100.000	4,00%

Conclusions on bacteria and fungi and C-bacteria

- Treatments 1, 2 and 3 are all the same in result compared to the reference (0).
- An UV-c treatment of 5 mJ/cm^2 is too little for a good result on dirty trays
- Conclusion is that first washing the trays with water or hypochlorite gives the same result when a 50 mJ/cm^2 UV-c treatment follows. So washing trays first with water only is enough to flush most infection away, when 50 mJ/cm^2 follows

Phytophthora and Pythium

These water-fungi produce hyphae, spores and (so called) survival spores. The last two ones are meant for surviving severe circumstances.

Treatment	*Phytophthora*	*Pythium*
0: Control from tray (100%)	+	+
1: H2O -> 50 mJ/cm^2	+	+
2: Hypochlorite -> 50 mJ/cm^2	+	-
3: Hypochlorite -> 10 minutes	+	-
4: Hypochlorite -> 5 mJ/cm^2	+	-

Conclusions on Phytophthora and Pythium

- Treatments 2, 3 and 4 are all the same in result compared to the reference (0). That could mean that only the hypochloride is enough to inactivate Pythium, but not for Phythophthora.
- Washing the trays with water only does not inactivate Pythium on trays.
- 50 mJ/cm^2 UV-c gives no better result on these water-fungi than 5 mJ/cm^2.
- Discussion: probably we incubated the tray with hyphae with a lot of spores of Phytophthora in agar-medium. It is known that UV-c inactivates hyphae quit easy, but spore are harder to inactivate.
- Conclusion: Of all 4 treatments I would recommend treatment 2

Fur cucumber virus (CGMMV) and Pepino mosaic virus (PepMV)

Of CGMMV it is known that for this virus in water it needs 240-250 mJ/cm^2 of UV-radiation to inactivate this virus. For PepMV120 mJ/cm^2 is enough according to the same literature.

Treatment	*CGMMV*	*PepMV*
0: Control from tray (100%)	+	+
1: H2O -> 50 mJ/cm^2	-	+
2: Hypochlorite -> 50 mJ/cm^2	-	+/-
3: Hypochlorite -> 10 minutes	+	+
4: Hypochlorite -> 5 mJ/cm^2	+	+
Control direct	+	+

Conclusions on Fur cucumber virus (CGMMV) and Pepino mosaic virus (PepMV)

- In non of the treatments PepMV is inactivated.
- The 2 treatments with 50 mJ/cm^2 inactivated the CGMMV in cucumber.
- Discussion: to incubate the trays a mix of leaves is used. In this way al lot of organic (green)matter clung to the tray, that is hard to remove. By only flushing the tray with little solution, without soaking it to solve the organic matter, it does not flush away with the solution.
- Conclusion: when seeing this results treatment 2 is post preferable.