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ORIGINAL PAPER

Plant viruses in irrigation water: reduced dispersal of viruses using sensor-based disinfection

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Abstract The increasing use of recirculating nutrient solutions and drainage water for irrigation purposes requires effective sanitation methods to minimise the dispersal of plant pathogens. Among these, plant viruses are of particular interest because they cannot be cured. A new disinfection system was tested in regard to its ability to inactivate plant viruses in nutrient solution in greenhouses. Potassium hypochlorite produced onsite by an electrolytic disinfector and injected once weekly into the nutrient solution by a sensor, prevented the dispersal of *Pepino mosaic virus* in the tomato crop. The management program assures that virus particles released from infected plants do not accumulate, forming an infectious virus reservoir which represents an inoculum potential in the hydroponic system. Both tested applications at 0.2 or 0.5 mg free chlorine/l nutrient solution for 60 or 30 min ensured virus inactivation and did not cause phytotoxicity. The yield of tomato plants grown in KCIO-treated nutrient solution was even significantly higher than that of control plants. PepMV-infected source plants solely bore unmarketable tomatoes showing discoloration. By inhibiting the dispersal of PepMV and the infection of test plants, the amount of unmarketable tomato fruits was reduced rigorously in treated variants.

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Introduction

Closed irrigation systems conserve resources and minimise production costs. However, there is a higher risk of infectious diseases due to improved conditions for the dispersal of waterborne plant pathogens in the recirculating nutrient solution. A considerable number of virulent pathogens which are difficult to manage are of significant concern in greenhouse crops and can cause severe economic losses (Stewart-Wade 2011). We focused on tomato which yielded an estimated 14.9 million tons of tomatoes in the EU in 2013 (Eurostat 2015). Although many different fungal, bacterial and viral pathogens affect tomato plants causing significant yield losses, we selected *Pepino mosaic virus* (PepMV), which has become a major threat to greenhouse tomato production around the world. It was transferred to the European and Mediterranean Plant Protection Organization (EPPO) A2 list in 2012. Pathogens and organisms listed there are present in the EPPO region but not widely distributed and are recommended for regulation as quarantine pests. The evidence that PepMV is causing commercial losses is largely based on anecdotal reports. PepMV was found to reduce the quality of tomato fruits significantly, but not the bulk yield (Spence et al. 2006). Losses correspond to the aggressiveness of the respective PepMV isolate (Peters et al. 2010). This aggressive isolate caused an overall yield loss of 4 % and taking into consideration only class I fruits as much as 14 % loss. In tomato crops PepMV can be transmitted from plant to plant by mechanical contact (Jones et al. 1980) through contaminated tools, hands, clothing and direct plant-to-plant contact as well as

by water and nutrient solutions (Schwarz et al. 2010). Seed transmission was first investigated by Bandte et al. (2003) and studies initiated thereafter summarised by Hanssen et al. (2010) have shown a low efficient seed transmissibility. Insects such as bumble bees (*Bombus* spp.) used as pollinators in tomato crops (Shipp et al. 2008), greenhouse whiteflies (*Trialeurodes vaporariorum*) (Noël et al. 2014) and the bug *Macrolophis caliginosus* (Klapwijk and Stijger 2000) have also been shown to transmit the virus.

Physical and chemical techniques such as filtration, pasteurisation, ultraviolet or ionising radiation, heating to elevated temperatures, water treatment by chlorination or ozonisation and surfactants have been described to decontaminate irrigation water (Hong et al. 2014). The main drawback of physical disinfection methods is the lack of a reservoir effect as they are only effective in the immediate surroundings of their operating devices (Kraft 2008). Chemical disinfection methods often demand transport, storage and use of hazardous substances. These substances require special handling, operator training and a high level of technical competence to run monitoring equipment for detection of active ingredients and residues. The efficacy of control depends mainly on the disease being targeted as well as the dose and contact time (Raudales et al. 2014). Furthermore pH value, temperature, concentration of suspended solids and the presence and amount of ions affect the efficacy of the treatment. With the exception of the cost-intensive thermodisinfection, none of the other treatments proved to be suitable for reliable inactivation of the multitude of relevant plant pathogens, in particular viruses, in hydroponic production systems.

The increasing use of recirculating nutrient solutions and drainage water for irrigation purposes requires effective sanitation methods to minimise the dispersal of plant pathogens. Among these, plant viruses are of particular interest because neither the virus in infected plants can be eradicated in the field nor can the plant be cured. The disinfection of the nutrient solution in hydroponics and of irrigation water would contribute significantly to integrated plant protection by preventing or inhibiting the dispersal of the virus and by the eradication of virus reservoirs.

With this premise a system of electrolytic water disinfection in greenhouses was developed. This system includes in situ production of disinfectants by anodic oxidation of a high concentrated salt solution and sensor-based injection into the nutrient solution tanks. So far electrochemical water disinfection is rarely seen in agriculture (Elmer et al. 2014) but is used for disinfection of various wastewaters, drinking water and water used in pre- and postharvest practices of fresh produce chain (Gil et al. 2015; van Haute et al. 2015; Särkkä et al. 2015). The technique is practical, as treatment does not require the addition of any chemical compounds to the water. Disinfecting agents such as ozone and chloride which are oxidised to free chlorine are produced electrochemically in the water by an electrical current and suitable electrodes (Kraft 2008). Sanitation is performed by the free chlorine produced. Free chlorine, the sum of hypochlorous acid and hypochlorite, is based on the release of atomic oxygen.

In the present study the efficacy and suitability of a sensor-based disinfection system to inactivate the viral pathogen *Pepino mosaic virus* and to reduce its dispersal in hydroponic systems in greenhouse production was evaluated. In vitro studies were carried out to ascertain dose relations of the disinfectant and in vivo studies to test the system under simulated field conditions.

Materials and methods

Plant material

Three herbaceous plants species were used for the propagation and diagnosis of PepMV: tomato (*Lycopersicon esculentum* Mill), *Chenopodium quinoa* Willd. and *Datura stramonium* L. Seeds were sown in propagation substrate (Gramoflor GmbH, Germany) and cultivated under greenhouse conditions (20 °C, 16-h photoperiod). *D. stramonium* plants were used in bioassays to confirm the phytosanitary effect of the water disinfection treatment.

In vivo trials were carried out with the small bush tomato cv. Hoffmanns Rentita in greenhouse cabins (22 °C, 16-h photoperiod). Seeds were sown in perlite, transferred to rockwool cubes (100 × 100 × 70 mm^3) 14 days after sowing (das) and inoculated mechanically at the two-leaf stage 28 das with PepMV isolate PV-0554 (DSMZ, Germany). Infection was verified 2 weeks later by DAS-ELISA (see section "Detection of Pepino mosaic virus by ELISA").

Electrolytic disinfection of nutrient solution

An electrolytic disinfector e-GW 30 system (newtec Umwelttechnik GmbH, Germany), specially developed for disinfection of irrigation water in greenhouses, was used. The system consists of an electrolysis reactor with titanium electrodes. A direct current of 10 A with a voltage of 13 V is applied to a solution containing potassium chlorite and fresh water leading to the formation of chlorine. The low concentrated potassium hypochlorite solution (0.4–0.6 %) generated was injected into the nutrient solution tanks using a dosing system. Thereto the target concentration of free chlorine was measured online with a special precious metal electrode, while the disinfecting solution was slowly added using a magnet membrane dosage pump with a maximum injection rate of 50 strokes per minute and a stroke volume of 0.09 ml per stroke. A control unit equipped with

a chlorine electrode regulated the supposed content of free chlorine. Two different dosage levels were tested. Weekly injection intervals provided 0.2 mg free chlorine/l nutrient solution for 60 min and 0.5 mg free chlorine/l for 30 min, respectively. To confirm the accuracy of the sensor-based measurement and injection, the content of free chlorine was checked manually using a hand-held apparatus (Pocket Colorimeter II, Hach Lange GmbH, Germany).

Detection of Pepino mosaic virus by ELISA

ELISA was performed using a commercially available assay (RT-1022) according to the suppliers' instructions (DSMZ, Germany). Each sample was tested with at least two replicates. The optical density (OD) of the samples at 405 nm was rated after 60 and 120 min substrate incubation. The cut-off value was defined as three times the mean value of three homogenates of different healthy (negative) samples. All samples with values above the cut-off were regarded as PepMV positive.

Concentration of Pepino mosaic virus in nutrient solution

Detection of PepMV in nutrient solution requires concentration of the virus prior to testing by ELISA (Büttner et al. 2014). Samples of 10 l each were concentrated by tangential flow filtration (TFF) using the Pellicon 2 cassette with the prefilter Opticap XL capsule (10 µm) and the Biomax hydrophilic polyethersulfone membrane (10 kDa) (Merck Millipore, Germany). The pressure was adjusted according to the manufacturer's instructions. Retentates were filtered continuously until the desired volume of 60 ml was achieved. The filter was washed twice with tap water and sanitised with 1 N NaOH to remove any residual plant viruses. The cassette was stored at 4 °C in 0.1 N NaOH between tests. The retentate from TFF was further concentrated using ultracentrifugation. It was centrifuged at 28.000 rpm for 90 min in a Beckman Coulter Optima L-70K ultracentrifuge, with a type 70Ti rotor (Beckmann Coulter, Germany). The pellets were re-suspended and pooled in 300 µl high-purity water and tested by ELISA (Bandte and Pettitt 2014).

In vitro tests to evaluate treatment efficacy

Inactivation of *Pepino mosaic virus* by the disinfectant was tested in vitro according to the standard "Disinfection in plant production" (Anonymous 2008). Testing covered a range of different concentrations (0, 1, 2, 6, 12 and 18 mg free chlorine/l displayed by the electrolytic processed potassium hypochlorite) and incubation times (10, 30 and 60 min). Water and buffer, respectively, were used as untreated controls. Neutralisation was conducted with 0.01 M sodium thiosulfate pentahydrate. The test suspension was mixed with an abrasive (Celite), three leaves of *D. stramonium* inoculated, and screened for characteristic systemic mosaic symptoms of PepMV. Sanitation was achieved when none of the inoculated leaves exhibited characteristic symptoms. Assessments for leaf symptoms were carried out daily for a time span of 14 days. The trial comprised four plants per variant and two replications. All inoculated plants were tested subsequently by ELISA to confirm sanitation success.

In vivo tests to evaluate treatment efficacy

The effect of the disinfection procedure on PepMV dispersal and fruit yield was evaluated in tomato plants cultivated in NFT (Nutrient Film Technique). Thirteen plants were positioned in two channels which were supplied with nutrient solution via a 400 l tank (Fig. 1). The channels were flushed with this nutrient solution applied at a flow rate of 600 l h^{-1} for 24 h a day. One storage tank was subjected to the disinfection procedure, and the other tank with only nutrient solution acted as the non-treated control. A root contact between healthy and infected plants was prevented by a root barrier. Plant handling was carried out using disposable rubber gloves that were changed after each PepMV-infected plant in order to prevent mechanical

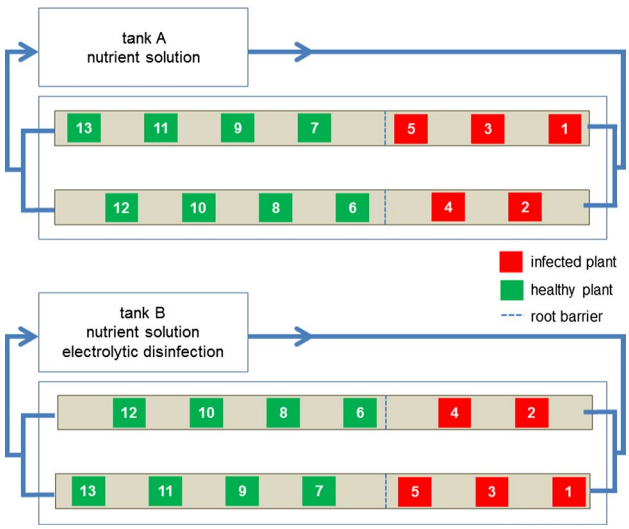

Fig. 1 Schematic view of experimental set-up. Nutrient solution was supplied continuously with recirculation (pump power 600 l/h at 50 Hz). Tank A and B provided the solution to the 13 plants, well positioned in two rows. A root barrier (mesh size of 252/in^2) hampered root contact between PepMV-infected donator plants and healthy plants. The bushy habitus of the selected tomato variety 'Hoffmanns Rentita' and spacing prevented a PepMV transmission from plant to plant. The experiment was performed with two repetitions

Table 1 Number of PepMV-infected plants after mechanical inoculation of the indicator plant *Datura stramonium* with a PepMV-infected leaf homogenate treated with a KClO solution taking into account different concentrations and contact times ($n = 8$)

Contact time (min)	Concentration of the disinfectant free chlorine/ml water (mg)					
	0	1	2	6	12	18
5	8	8	8	8	8	8
10	8	8	8	8	8	8
30	8	8	8	8	8	7
60	8	8	8	8	8	0

Fig. 2 Characteristic mosaic on the leaf induced by PepMV on the indicator plant *Datura stramonium* (12 dpi). *Left* control inoculation without disinfectant, *right* treatment with the disinfectant KClO at 18 mg free chlorine/l and contact time of 60 min prior inoculation

spread of the virus. Cultivation lasted for 16 weeks following commercial practices: 22 °C, 16-h photoperiod, relative humidity 30–60 %. The nutrient solution consisted of tap water and a stock solution of macro- and micronutrients (Gohler and Molitor 2002). The composition of the nutrient solution was measured weekly in the laboratory and corrected when necessary. The pH of the nutrient solution was maintained at a value of 6.0 using phosphoric acid or caustic soda. All experiments were performed twice.

Data collection and analysis

Plants were assessed individually weekly for PepMV infection by ELISA. Fruits were harvested every week starting 42 days after set-up. Number and fresh weight of tomato fruits were determined per plant. Fruits with a diameter <40 mm, severe discoloration or growth cracks were defined unmarketable.

Data on tomato fruits were subjected to two-way analysis of variance. Means were compared by Fisher's F test followed by Tukey's t test at significance level $\alpha = 0.05$. Significant differences are represented by different letters.

Results

Efficacy of the treatment in vitro

Six concentrations each with four different contact times were tested. As plant viruses are obligate pathogens and not cultivable on nutrient media, tests had to be performed on suitable indicator plants. In total 183 out of 192 plants were infected with PepMV. Except for the dosage of 18 mg free chlorine/l and a contact time of 60 min, none of the treatments succeeded to inactivate the viral pathogen PepMV completely (Table 1). Furthermore treatment of test suspensions for 30 min at 18 mg free chlorine/l inhibited infection in one out of eight plants. Infected plants developed characteristic leaf mosaic (Fig. 2). These symptoms became visible 2 weeks after mechanical inoculation. The infection of the indicator plants was confirmed in all cases by ELISA.

Effect of treatment on virus dispersal

The nutrient solution of both tanks was contaminated continuously and naturally by the infected control plants. Treatment with the disinfectant hampered the dispersal of PepMV in all experimental series. None of the tomato plants supplied with nutrient solution treated weekly with 0.2 mg free chlorine/l for 60 min were infected with PepMV. In contrast six out of eight (A07 to A11 and A13) in the first test and eight out of eight (A6 to A13) tomato plants in the second test became infected with PepMV by the untreated nutrient solution (Fig. 3). First infected plants were detected 11 and 10 weeks after set-up, respectively. The infection of these plants was by chance and not by their position. For example plant A06 remained PepMV-free in the first survey although it was closest to an infected plant. A concentration of 0.5 mg free chlorine/l in conjunction with a contact time of 30 min prevented infection of tomato plants, whereas half of the control plants irrigated with untreated nutrient solution were infected (data not

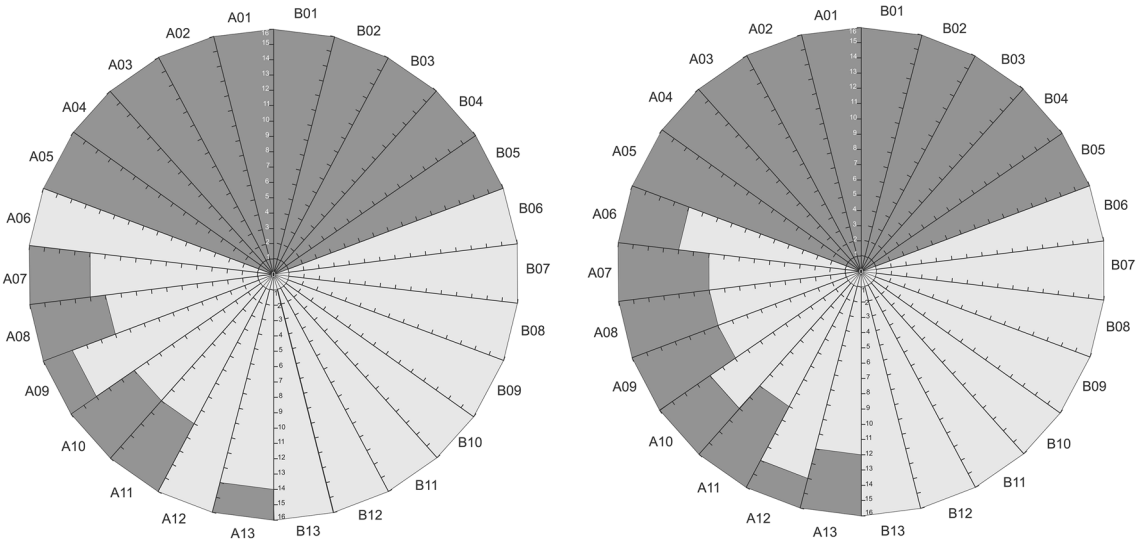

Fig. 3 Dispersal of PepMV by nutrient solution in a NFT system and infection of tomato plants within a 16 week survey dependant on a treatment sanitising the nutrient solution. Initially PepMV-infected (plant A01–A05 and B01–B05) and non PepMV-infected (plant A06–A13 and B06–B13) tomato plants are cultivated in a NFT system using recirculating nutrient solution. Plants indexed by B are supplied with treated nutrient solution (0.2 mg free chlorine/l for 1 h a week) whereas plants indexed by A are provided with untreated nutrient solution. Plants were tested weekly due to an infection with PepMV. The first time PepMV was detected in the individual plant is marked by a dark line. *Dark grey fields* PepMV-infected, *light grey fields* not PepMV-infected. *Left* survey 1, September–December 2014, *right* survey 2, January–May 2015

Fig. 4 Rating of tomato fruits. *Left* unmarketable tomato fruits showing discoloration, *right* marketable tomato fruits without any discoloration and cracks

shown). Here the first infected tomato test plant was also detected 10 weeks after experimental set-up. In all experimental runs PepMV was detected solely in the untreated nutrient solution and not at any time in those treated with the disinfectant.

Effect of treatment on plant growth and fruit yield

None of the plants supplied with treated nutrient solution showed phytotoxic foliar injury and/or growth differences. Furthermore, none of the PepMV-infected source or test plants developed leaf symptoms when cultivated in treated or untreated nutrient solution. A number of fruits had to be graded unmarketable (Fig. 4) as they exhibited a diameter <40 mm, severe discoloration or growth cracks. Interestingly fruits of PepMV-infected source plants were never marketable.

Source as well as test plants cultivated in untreated nutrient solution exhibited the lowest total fruit weight (Table 2). Control test plants produced only two-thirds of fruit weight compared to the treated ones. In both experimental runs the treatment with potassium chloride significantly increased the number of fruits/plant of infected control as well as test plants (Table 3). However, a high percentage of unmarketable fruits (48 %) emerged in control test plants. The corresponding plants supplied with treated nutrient solution yielded only about 5 % of unmarketable tomatoes. Those fruits had cracks or were too small, but they never showed discoloration.

Taking into consideration the groups of tomato plants (infected control plants and test plants) irrigated with the same recirculating nutrient solution, the mean total fruit yield of individual tomato plants was significantly higher in treated nutrient solution compared to those plants irrigated with untreated nutrient solution (Fig. 5). The two different concentration and contact time treatments did not differ significantly from another. The results for both, tomato quality and quantity, were similar in the second experimental runs as shown for the treatment concentration of 0.2 mg free chlorine/l and a contact time of 60 min (Fig. 6).

Table 2 Tomato fruit yield during 10 harvest weeks dependent on the sensor-based injection of an electrolytic produced disinfectant

	Tomato plants	Total yield (kg)		Yield/plant (kg)	
		Control	Sanitation	Control	Sanitation
First run	PepMV-infected control plants	2.64	3.32	0.53 ± 0.24 a	0.66 ± 0.11 a
	Test plants	6.03	9.47	0.75 ± 0.18 a	1.18 ± 0.28 b
Second run	PepMV-infected control plants	2.34	3.99	0.47 ± 0.18 a	0.80 ± 0.17 a
	Test plants	6.32	10.16	0.79 ± 0.14 a	1.27 ± 0.26 b

The data represents mean values (infected control plants $n = 5$, test plants $n = 8$). First run (September–December 2014) and second run (January–May 2015). Control = no sanitation, sanitation = 0.2 mg free chlorine/l for 60 min, weekly. Comparisons were calculated using Tukey's test. Values followed by different letters differ significantly from each other ($p < 0.05$). Values with the prefix ± represent the standard deviation

Table 3 Number and unmarketability of tomato fruits during 10 harvest weeks dependent on the sensor-based injection of an electrolytic produced disinfectant

	Tomato plants	Fruit/plant (No.)		Unmarketable fruits (%)	
		Control	Sanitation	Control	Sanitation
First run	PepMV-infected control plants	10.40 ± 4.22 b	14.60 ± 5.42 ab	100	100
	Test plants	16.12 ± 4.12 ab	23.87 ± 4.09 a	48.4	5.0
Second run	PepMV-infected control plants	11.40 ± 6.02 b	17.00 ± 6.36 ab	100	100
	Test plants	17.75 ± 5.84 ab	22.62 ± 5.42 a	47.6	6.3

The data represents mean values (infected control plants $n = 5$, test plants $n = 8$). First run (September–December 2014) and second run (January–May 2015). Control = no sanitation, sanitation = 0.2 mg free chlorine/l for 60 min, weekly. Unmarketable fruit: diameter <40 mm, discoloration or cracks. Comparisons were calculated using Tukey's test. Values followed by different letters differ significantly from each other ($p < 0.05$). Values with the prefix ± represent the standard deviation

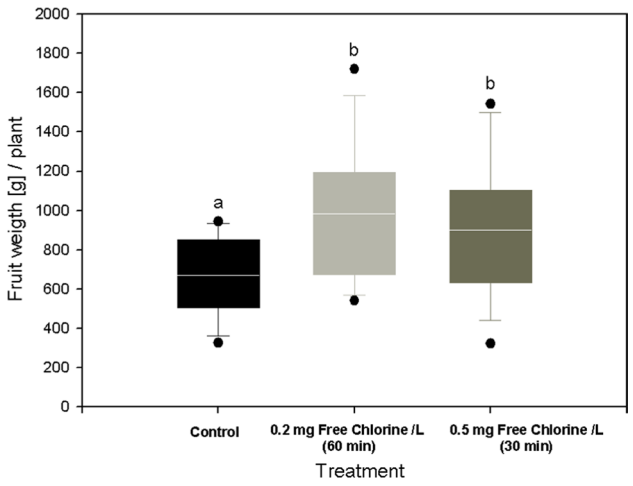

Fig. 5 *Box* and *whisker* plot showing the maximum and minimum values (*whiskers*), the upper and lower quartiles (*boxes*) and the median (*middle horizontal line*) of the mean total yield of individual tomato plants during 10 harvest weeks dependent on a sanitising treatment of the nutrient solution. *Circles* denote outliers beyond the whiskers. Significant differences are represented by *different letters*. $n = 13$

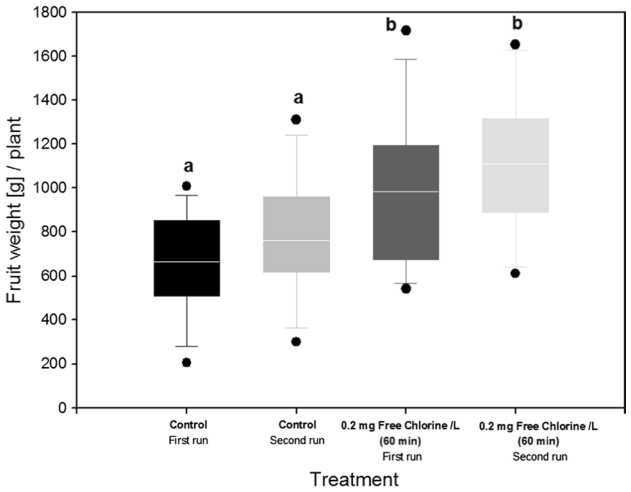

Fig. 6 *Box* and *whisker* plot showing the maximum and minimum values (*whiskers*), the upper and lower quartiles (*boxes*) and the median (*middle horizontal line*) of the mean total yield of individual tomato plants during 10 harvest weeks dependent on a sanitising treatment of the nutrient solution. *Circles* denote outliers beyond the whiskers. Significant differences are represented by *different letters*. $n = 13$

Although the mean total yield was generally slightly higher in the second run, the differences were not significant.

Discussion

In hydroponic systems, plants like tomato are typically grown for almost a whole year. Therefore, the high stability of several plant pathogens, e.g., plant viruses in aqueous environments might allow them to accumulate in and on root systems (Büttner and König 2014). To date, the transmission of plant viruses through water and their inactivation has been paid little attention even though the sanitation of the nutrient solution would counteract the spread of the virus and reduce economic losses. Current disinfection treatments of irrigation water include filtration, ultraviolet irradiation, ozonation, the use of non-ionic surfactants and ionised copper, and chlorination (Stewart-Wade 2011). Thus far, none of these sanitation methods ensure a safe and reliable inactivation or elimination of the multitude of plant pathogenic organisms in hydroponic systems. The individual processes require different contact times and concentrations depending on the pathogen. For instance, the free chlorine threshold and the critical contact time at which there is no detection of plant pathogenic fungi differ between genera and species as seen for *Phytophthora infestans*, *P. cactorum*, *Pythium aphanidermatum*, *Fusarium oxysporum,* and *Rhizoctonia solani,* all common pathogens in nurseries (Cayanan et al. 2009).

Chlorination, originally developed to treat municipal water, is one of the most economical water decontamination methods. Higher concentrations of chlorine require a shorter contact time for sanitation and conversely the efficacy of a low chlorine dose is increased with a longer duration of exposure (Cayanan et al. 2009). But the excessive use of chlorinated irrigation water may lead to severe phytotoxic effects, reduce the marketability of the plants due to visible injury such as chlorosis, foliar necrosis, premature abscission of foliage, decrease in plant growth, leaf discoloration and deformation, and form undesirable by-products (Raudales et al. 2014; van Haute et al. 2015). The sensitivity of plant species to free chlorine concentrations of irrigation water varies. For instance, Cayanan et al. (2008) stated that irrigation water containing <2.5 mg of free chlorine l^{-1} for 6 weeks should not adversely affect the growth or appearance of ornamental woody shrubs. Chlorine reacts with various substances in water, including ferrous ions, ammonium ions and other inorganic and organic contaminants, making the chlorine unavailable for disinfestation (Stewart-Wade 2011). Therefore, it is essential to monitor the free residual chlorine concentration to ensure efficacy of the treatment, as considered in the disinfection system tested in the present study.

To date, about 20 studies have been conducted on the efficacy of chlorine to sanitise irrigation water (Raudales et al. 2014). Most of them were carried out in vitro focusing on pathogen mortality, whereas pathogen dispersal and disease development are most important to commercial crop production. In all cases the chlorine was applied in the form of NaOCl or Cl_2 and not as in our experiments with KClO. Potassium, a macronutrient, is required and absorbed by the plant in a much larger scale than sodium. Excess sodium may lead to phytotoxic reactions and crystallizes easily, being deposited in the channels, lines, and tubes. Our investigations reveal differences in results and implications gained by in vitro and in vivo experiments and confirmed the necessity to confirm data in vivo. In contrast to the high dose (18 mg free chlorine/l, 60 min) required in vitro to avoid virus infection of the indicator plants, only a fraction of the dose (0.2 mg free chlorine/l, 60 min weekly or 0.5 mg free chlorine, 30 min/weekly) inhibited virus transmission in a greenhouse situation. These differences in the dosage are related to the virus titre present in an artificial and natural inoculum. The virus titre is extremely high in infected tomato plant material compared to a very low one in naturally contaminated nutrient solution. Already Raudales et al. (2014) point to observed discrepancies between pathogen mortality and disease incidence reviewing measures to control waterborne microbes in irrigation. Among others they refer to the fact that the efficacy of disinfectants greatly differs depending on whether evaluations were conducted in pure water or in nutrient solution.

So far only few studies were conducted in vivo. Poncet et al. (2001) describe disinfecting with chlorine gas as an excellent preventive method in roses which neither cause phytotoxic effects nor yield losses. An amount of 4 mg NaOCl/l applied for a contact time of 30 min in in vitro studies was sufficient to obtain inactivation of *Agrobacterium tumefaciens*. Although chlorine was effective in reducing the incidence of the fungal pathogen *Plasmodiophora brassicae* causing clubroot of cabbage at 2 mg Cl/l and contact time of at least 5 min, field trails reveal that the treatment of infested irrigation water with 200 mg Cl/l only reduced disease incidence while leading to reduced plant height, fresh weight and stand count (Datnoff et al. 1987). However, in vivo studies are rare; Rosner et al. (2006) stated that virus dissemination in the greenhouse can be effectively controlled by treating the recycled water with 4 mg of hypochlorite/l for 30 min as shown with *Cucumber leaf spot virus* (CLSV). Our studies show that the electrolytically manufactured disinfectant at a dose of only 0.2 mg free chlorine/l and 60 min contact on a weekly base is sufficient to eradicate PepMV in nutrient solution. Although CLSV and PepMV differ in shape, both virions contain a monopartite, positive sense, single-stranded RNA genome and possess

similar physical features related to their stability. Infectivity of CLSV in crude sap of *C. sativus* is lost after dilution between 10^{-6} and 10^{-7} or storing for 20 days at 22 °C (Weber et al. 1982), dilution endpoint of PepMV is determined at 10^{-4}–10^{-5} and infectivity was retained for at least 3 months at 20 °C (Jones et al. 1980). The comparability of virus stability enables a direct comparison of the two disinfection methods. The tested system enables the reduction of the disinfectant dosage to about a tenth compared to conventional treatment.

PepMV can survive and remain infectious for several weeks in plant debris, on contaminated surfaces and in water and remains infectious in water at 20 °C for up to 3 weeks (Mehle et al. 2014). The release of PepMV from roots into water has already been shown for different strains of the pathogen (Schwarz et al. 2010; Mehle et al. 2014). The transmission of PepMV by nutrient solution was first shown for the European strain PepMV-EU by Schwarz et al. (2010) and Mehle et al. (2014) confirmed the finding for the Chilean isolate PepMV-Ch2. Hydroponic experiments on water-mediated transmission of that isolate resulted in four out of six plants being infected within 4 months. The results of our two experiments confirmed these previous findings and showed transmission rates of at least four out of eight plants within 16 weeks.

PepMV symptomatology and host range have been extensively studied (Blystad et al. 2015). General symptoms in tomato included mosaic and yellowing of leaves, bubbling, necrosis and fruit discoloration. But diagnosis of PepMV based on disease symptoms is not reliable because not all PepMV-infected plants show symptoms. For qualitative detection and identification of PepMV, DAS-ELISA is recommended as the initial test of choice (Anonymous 2013). Whereas PepMV was not detectable in samples of nutrient solutions in practical greenhouse experiments carried out by Schwarz et al. (2010), Mehle et al. (2014) were able to detect PepMV directly in the nutrient solution of a small-scale hydroponic set-up. In our studies we detected PepMV directly in the nutrient solution after concentration by ultrafiltration and ultracentrifugation by applying ELISA. The experimental design served as proof of the infectivity of the virus as almost all tomato test plants were infected.

Already in the last decade Hong et al. (2003) suggested electrolysed water (EW) may have potential for disinfesting irrigation water. But there is still a lack of data on the efficacy of EW in commercial greenhouse production (Stewart-Wade 2011). Just recently electrochemical disinfection has been demonstrated to be effective in eliminating a wide spectrum of human pathogens in processed water of fresh-cut vegetable production (Gil et al. 2015). Now our investigations show the efficacy of electrolytically produced KClO injected into nutrient solution to inactivate PepMV and to hamper the dispersal of the devastating plant virus. Last but not least, there is strong evidence that the proposed treatment of the nutrient solution not only prevents the spread of PepMV in the crop but leads to an increase in crop yields. In all experiments fruit biomass and the amount of marketable fruits were significantly higher in plants cultivated in treated nutrient solution. Such an increase in yield should also increase the acceptance of this prophylactic measure and its integration into commercial production processes.

Conclusions

This is the first study that successfully applies a combination of techniques to sanitise drainage water in greenhouses, naturally contaminated with a plant pathogenic virus. The main findings are:

- The tested system combines onsite production of a disinfectant by anodic oxidation, custom-tailored sensor injection in recirculating nutrient solution and monitoring and control of the injection by an electrode suitable to measure the amount of free chlorine in salty solutions used in greenhouse production.
- For the first time a disinfection method is available which inhibits the dispersal of the economic important *Pepino mosaic virus* reliably, even though PepMV-infected plants have the continuing ability to release infectious virus particles to the drainage water.
- Most likely the system is also suitable to inactivate pathogenic root-invading fungi and bacteria which are a limiting factor in greenhouse production of vegetables and horticultural crops. The technology needs to be tested under commercial conditions and prove its efficiency and robustness. In those real life scenarios factors such as water source, flow rates, contaminants, temperature, required investment, and operating costs have to be considered.
- Only sanitation of drainage water and its reuse enables resource-conserving production without sacrificing quality and yield of agricultural production.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest and that neither animal nor human rights are violated.

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