Sociedad Colombiana de Ciencias Hortícolas

MEMORIAS

SEGUNDO CONGRESO COLOMBIANO DE HORTICULTURA

COLOMBIA HORTÍCOLA:

RETOS Y OPORTUNIDADES

Bogotá, D.C., 12 - 14 de Septiembre de 2007
Centro de Convenciones Alfonso López Pumarejo
Universidad Nacional de Colombia

Transmission of plant viruses by water

Transmisión de virus de plantas por el agua

M. Bandte[1], H.J. Echevarria Laza[1], U. Paschek[1], C. Ulrichs[2], W. Pestemer[1, 4], D. Schwarz[3], and C. Büttner[1]

Introduction

With references in the eighties it has been shown that plant pathogenic viruses are widely spread in environment and occur in soil (Büttner and Nienhaus 1989 a) and surface waters like ditches, rivers, streams and lakes (Koenig, 1986, Büttner and Nienhaus 1989 b). Plant viruses were also detected in sea water. Carnation mottle virus (CarMV) was identified in a water sample from the Baltic Sea (Kontzog *et al.*, 1988) and Petunia asteroid mosaic virus (PAMV) has been isolated from the North Sea (Fuchs *et al.*, 1996). And remarkable, Carnation Italian ringspot virus (CIRV) was isolated from a creek in a forested area in West Germany (Büttner *et al.*, 1987). However, the presence of plant pathogens in water has rarely been monitored (Horvath *et al.*, 1999; Gosalves *et al.*, 2003). Tomato mosaic virus (ToMV), a very stable plant virus with a wide host range, has even been found in ancient glacial ice (Castello *et al.*, 1999). Castello *et al.* (1995) postulate an atmospheric spread of infectious plant viruses without invertebrate vectors which represents a potentially long-distance transport mechanism for stable plant viruses like ToMV. The authors detected ToMV in more than half of the investigated cloud samples collected from the summit of a mountain in New York and in 13 out of 22 fog samples from two collection sites along the coast of Main. It is discussed that the virus becomes airborne due to ToMV-contaminated soil particles that serve as cloud condensation nuclei. Jacobi and Castello (1991) detected ToMV in eight out of 29 water samples from streams and lakes draining forest stands in central New York and in the Adirondack Mountains. Recently, seven out of nine water sources from different locations in Slovenia were tested positive for ToMV (Boben *et al.*, 2007).

Plant viruses detected in water share certain features: they are stable, except a few examples as there are Cucumber mosaic virus (CMV) and Tomato spotted wilt virus (TSWV), possess wide host ranges, and occur in high concentration in plant tissue; all of them can infect plants through their roots. CMV and TSWV are known to be unstable but still they were detected for instance in Italian and Turkish Rivers (Piazolla *et al.*, 1986,

[1] Humboldt University of Berlin, Department of Horticultural Sciences, Section Phytomedicine, D-14195 Berlin
[2] Humboldt University of Berlin, Department of Horticultural Sciences, Assistant Professor: Urban Horticulture, D-14195 Berlin
[3] Institute of Vegetable and Ornamental Crops, Plant Nutrition, D-14979 Großbeeren
[4] before March 2006: Federal Biological Research Centre for Agriculture and Forestry, Institute for Ecotoxicology and Eco chemistry in Plant Protection, D-14195 Berlin. e-mail: carmen.buettner@agrar.hu-berlin.de

Erdiller and Akbas, 1994) respectively in recirculating nutrient solution (Büttner *et al.*, 1995a,b). The permanent infectivity of CMV supposed to be based on a protective action of sediments on the virus particles and is caused by chemical cross-linking (Piazolla *et al.*, 1986).

It has been demonstrated for a number of viruses that they can be released from undisturbed roots into the soil respectively the drainage water and that these viruses remain infectious for long periods of time (Koenig and Lesemann, 1985). Infected plants growing in the vicinity of waters may thus likewise be a source of plant viruses in rivers and lakes. Furthermore, dump material from vegetables and ornamentals as well as compost may be other sources of viruses in surface waters (van Dorst, 1969). A close relationship between the incidence of Cucumber green mottle mosaic virus (CGMMV) in muskmelon and watermelon in river water and plant debris was figured out by Vani and Varma (1993).

Plant viruses were first observed in irrigation water in the last century. Studies by Roberts (1950) demonstrated that Tobacco mosaic virus (TMV), Tomato bushy stunt virus (TBSV) and Potato virus X (PVX) can infect hydroponically grown tomato plants when leaf sap of virus-infected plants is added to the nutrient solution. Tomlinson and Faithfull (1984) showed that tomato plants became infected when grown in soil watered with virus suspension. The relevance of these water transmissible plant viruses still increase as agriculture and horticulture increasingly depends on the use of recycled water (Hong and Moormann, 2005). Plants will be repeatedly inoculated with viruses contaminating the water independent whether the initial source of water harbours viruses or viruses enter the water along the path of distribution. Hence the use of hydroponic systems with recirculating nutrient solutions may facilitate virus transmission as several studies by Hasky et al. (1993), Büttner *et al.* (1995a), and Büttner and Bandte (1999b) confirmed. The investigations demonstrate experiments on the transmission of many different viruses which infect plants through roots in recirculating irrigation systems within 1 to 3 months. Resulting questions on the perspective to control the transmission of viruses initiated further test on disinfectants (Büttner and Bandte, 1999a) The authors summarize the main given opportunities to prevent the spread of the pathogens (Büttner and Bandte, 2000). Timmermann *et al.* (2001) published the control of mechanical viroid transmission by successful disinfection of tables and tools.

Subsequent investigations focus on the transmissibility of selcted plant viruses Pepino mosaic virus (PepMV) and Cherry leaf roll virus (CLRV) through nutrient solutions to study virus dispersal in hydroponic systems. PepMV was initially described as causal agent of a viral disease of pepino (*Solanum muricatum* Ait.) in Peru 1974. Its host range is mainly limited to plant species within the family Solanaceae (Salomone and Roggero, 2002) and was detected in tomato plants (*Lycopersicon esculentum* Mill.) by Jones *et al.* in 1980 for the first time. In 1999 it was found in Europe as a virus disease of glasshouse tomatoes in the Netherlands (EPPO 2001, van der Vlught *et al.*, 2002). Since this first report in the European Union, PepMV was set on the EPPO Alert List and monitored during the next years. The virus was detected in the following years in several European tomato growing regions like Spain, France, Canary Islands, Belgium, and Germany, predominantly in

indoor cultivated tomato plants and were eradicated (Roggero *et al.*, 2001, EPPO, 2001). Because Pepino mosaic virus is easily transmitted by contact and propagation and tomato is a major crop in Europe, putative ways of transmission of PepMV as well as susceptible tomato cultivars have to be investigated in detail to evaluate the pathogen as invading pest and its potential of dispersal. The risk of PepMV transmission in glasshouse tomatoes grown in a recirculating hydroponic system was studied and is given below.

CLRV was first described in 1955 by Posnette and Cropley as causing a disease of sweet cherry (*Prunus avium* L.) in England. Since then it has been shown to exhibit a wide natural host range comprising 17 genera including a variety of herbaceous and woody plants. Some of the most common natural hosts of CLRV are common birch (Betula pendula Roth), black elderberry (*Sambucus nigra* L.), English walnut (*Juglans regia* L.) and sweet cherry. The virus is widely distributed and has been detected throughout Europe, the former USSR, North America, Chile, New Zealand, and Japan CLRV is naturally transmitted through seeds and pollen.

Material and methods

Cherry leaf roll virus-infected plants were obtained by mechanical inoculation of crude leaf homogenates prepared in 0.01 M sodium phosphate buffer (pH 7,0), using Celite as an abrasive onto suitable test plants (Tab. 1). The transmission of the virus to *C. quinoa respectively* L. esculentum was checked by applying electron microscopical, serological and molecular biological methods (Tab. 1).

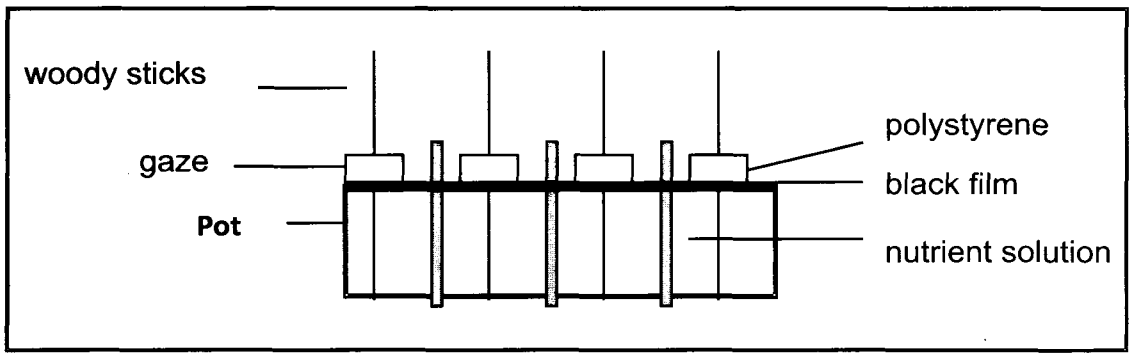

Table 1. Overview on test plants, virus isolates and methods applied to confirm the virus infection (x: applied, -: not applied, CLRV: cherry leaf roll virus, PepMV: Pepino mosaic virus EM: electron microscopy, ELISA: enzyme-linked-immunosorbent assay, IC-RT-PCR: immunocapture-reverse transcription-Polymerase chain reaction, RFLP: restriction fragment length polymorphism)

Electronmicroscopic methods were applied by negative staining to visualize the virus particles (Milne, 1993). Images were generated and evaluated with an EM 10 C electron microscope (Zeiss, Oberkochem, Germany).

The Double-antibody sandwich (DAS) ELISA was applied for PepMV detection following Clark *et al.*, (1976). PepMV-specific IgG was obtained from Plant Research International (Wageningen, Netherland) and IgG-AP by DSMZ (German Collection of Micro-

organism and Cell Cultures, Braunschweig, Germany). The optical densities of the sample fluid were measured with a microplate reader set at 405 nm. The samples were scored positive for the presence of the specific virus if the optical density value was at least twice that of the negative control.

Immunocapture was carried out according to Werner *et al.* (1997) using a concentration of 5.5 µg/mL of a polyclonal CLRV antiserum produced against an elderberry isolate of CLRV. First strand cDNA synthesis was performed directly in the immunocapture tubes in a total reaction volume of 10 □L using 5 units/□L M-MLV-Reverse transcriptase (Fermentas), 10 mM dNTP mix, 50 µM antisense-primer RW1 (5'-GTCGGAAAGATTACGTAAAAGG-3'). PCR amplification was done in a total volume of 50 □L using 10 □L of reverse transcription product, 2 mM MgCl2, 0.2 mM dNTP mix, 10 units Taq-DNA polymerase (Fermentas), 0.2 □M antisense-primer RW1 and 0.5 □M sense-primer RW2 (5'-TGGCGACCGTGTAACGGCA-3') in a Robocycler PCR machine (Stratagene). For both RT and PCR steps, the reaction buffers were those recommended by the supplier. The cycling scheme used was 2 minutes of denaturation at 94°C followed by 30 cycles at 94°C denaturation for 1 minute, 55°C annealing for 45 seconds, 72°C extension for 1 minute with final extension of 5 minutes at 72°C. Amplification products were analyzed on 1% (w/v) agarose gels and stained with ethidium bromide.

Restriction fragment length polymorphism (RFLP) analysis was carried out through treating PCR amplification products with three restriction endonucleases: EcoR I, Alu I, and Bsp 143 I to cleave 416 bp fragments generated by the PCR. The enzymatic reaction was carried out in a total volume of 15 µL including 10µL of the PCR amplification product and 5 µl of the specific restriction solution containing 6 U EcoRI (Promega), 5 U AluI (Fermentas) or 5 U Bsp143I (Fermentas) respectively; reaction buffers were applied as recommended by the supplier. The mix was incubated for 2 h at 37°C. The restriction products were analyzed on 2% (w/v) agarose gel and detected by ethidium bromide staining.

Transmissibility of CLRV – experimental design

All test plants were raised in soil (Einheitserde, Gramoflor) under greenhouse conditions with an average temperature of 22°C with a maximum of 28°C and a minimum of 16°C. The relative humidity (HR) ranged between 28-69%. Plants were watered daily with tap water. After four weeks plants were removed from the soil, rinsed thoroughly with tap water, and transferred into hydroculture with nutrient solution containing: 0,236 $mg \cdot L^{-1}$ of $CaNO_3$, 0,100 $mg \cdot L^{-1}$ of KNO_3, and 0,012 $mg \cdot L^{-1}$ of Fe chelat; HNO_3 was used to adjust the pH to 6.0. Each hydroponic unit covered 4 plants. Any kind of leaf- and root contact between plants could be excluded by using polystyrene and gaze (Fig. 1). Additionally black plastic film was placed on the surface of each unit to prevent algae development and the deposition of plant debris. Leaf samples were taken weekly to check CLRV-infection of the individual plants.

Transmissibility of PepMV – experimental design

Tomato seedlings cv. Hildares respectively Peto were pre-cultivated in rockwool cubes (100x100x70 mm^3) in a greenhouse and set up later on in separate experiments. Daily climate data averaged: temperature 25.0/23.4°C, relative humidity 83.7/65.1% and global radiation 12.5/14.1 MJ $m^{-2}s^{-1}$. The composition of the nutrient solution followed De Kreij *et al.* (1997). Forty eight plants of each variety were mechanically inoculated with PepMV. When first flowers started to bloom tomato plants were transferred in gullies (each 8 x 0.2 x 0.1 m). In each gully 18 plants were cultivated. To figure out the transmissibility of PepMV through nutrient solution the first eight plants of every second row were PepMV-infected (fig. 2). The experiment was designed with three replications; the distance of at least one meter between the gullies ensured that there was no leaf contact between the tomato plants. Moreover, a fleece (mesh aperture 50 µm) separated roots of the PepMV-

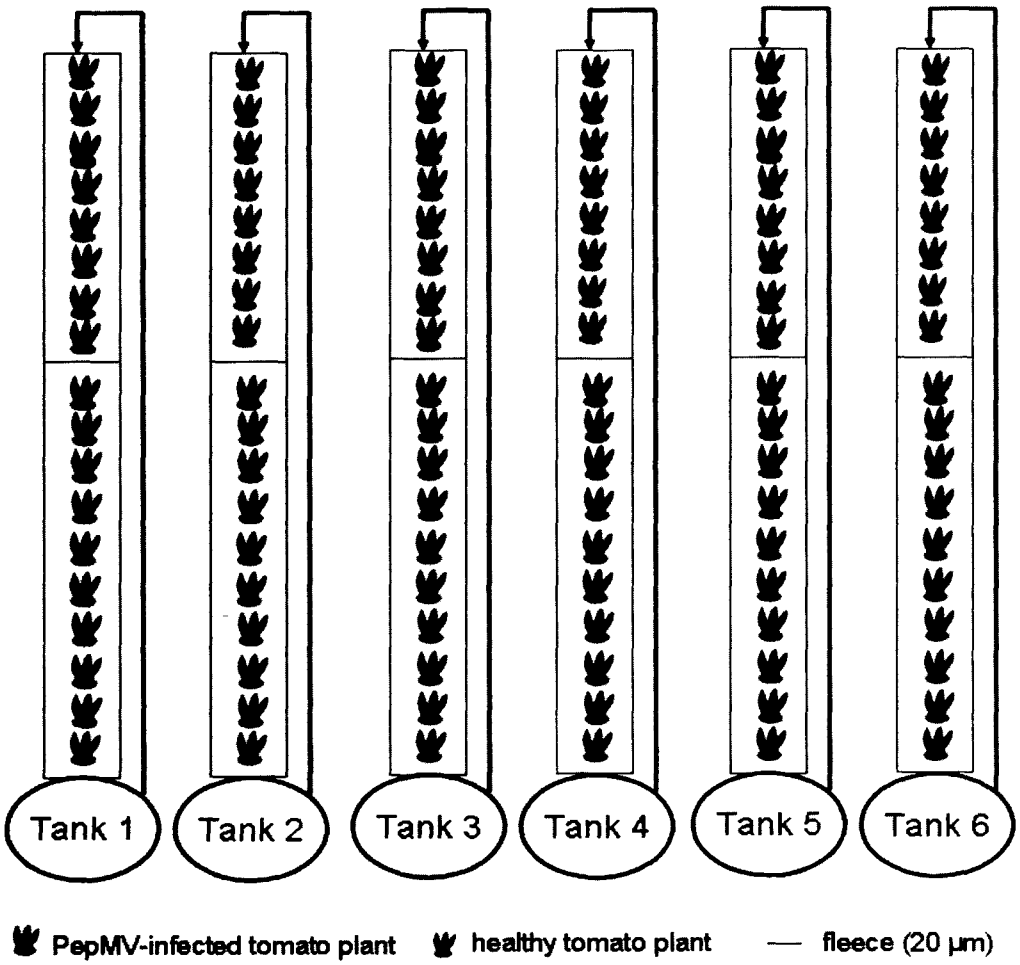

Figure 2. Schematic design the ebb-flow system used for investigations on the transmissibility of PepMV through nutrient solution. The arrows indicate the flow direction of the nutrient solution.

infected from the other plants. About 100 L of nutrient solution adjusted to pH 5.6 were recovered individually per gully and recirculated continuously at a flow rate of about 2 $L \cdot min^{-1}$. Leaf and root samples were taken weekly to check PepMV infection of individual plants as well as three fruits per healthy plant. Additionally fruit yield of cv. Hildares was ascertained every week by means of fresh weight.

Results

Transmission of PepMV

PepMV infected tomato seedlings of both varieties serving as inoculum in the gullies showed crimped and asymmetrically grown leaves after formation of the fifth leaf, often plants also displayed epinasty (Fig. 3). The infection was confirmed by electron microscopic visualization of flexible particles (Fig. 4) and ELISA.

In both experiments roots of formerly healthy tomato plants got infected by PepMV via nutrient solution. Finally half (cv. Peto) respectively all of the (cv. Hildares) tomato plants were PepMV-infected (Fig. 5 a, b). The viral pathogen was first detectable in roots of the plants, two weeks after setting up the experiment in cv. Hildares and after six weeks in cv. Peto. Within the survey period of 13 weeks 50% of the tomato plants cv. Peto got infected by PepMV through the nutrient solution. The infection rate of 50% was reached

Figure 3. Tomato plants grown in rockwool cubes left: PepMV-infected plant developing epinasty of leaves one week after being infected by mechanical inoculation right: healthy plant.

in cv. Hildares after 3 weeks, after 10 weeks all plants were proved to be infected with PepMV. Earliest one week after detecting the PepMV-infection in roots it could be confirmed in young leaves. The virus was also detectable in mature fruits of formerly healthy tomato seedlings.

PepMV-infected plants used as inoculum plants showed a decrease in fruit weight compared to healthy PepMV-free tomato plants. The reduction in yield constituted 41% after 7 weeks resulting in a decrease of about 20% that is an average of 1.3 kg fruits/ PepMV-infected and 1.6 kg fruits/ healthy plant at the end of the experiment.

Figure 4. Negative stained particle of PepMV; flexuous particle with a length of approx. 500 nm.

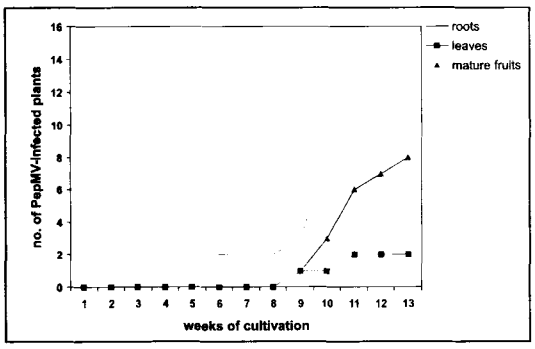

Figure 5a. *Detection of PepMV in roots, leaves and mature fruits of formerly PepMV-free tomato plants cv. Peto grown together with PepMV-infected tomato plants of the same variety in gullies using recirculating nutrient solution (n=30).*

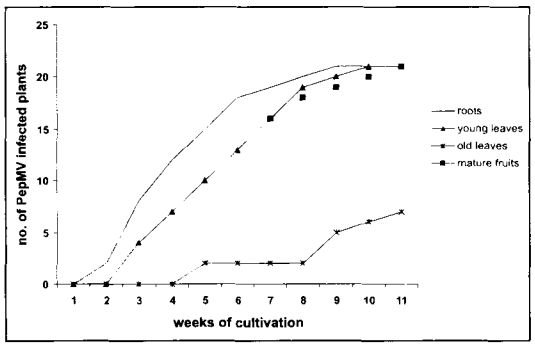

Figure 5b. *Detection of PepMV in roots, leaves and mature fruits of formerly PepMV-free tomato plants cv. Hildares grown together with PepMV-infected tomato plants of the same variety in gullies using recirculating nutrient solution (n=21).*

Transmission of CLRV

C. quinoa plants cultivated in hydroculture developed four to five days post mechanical leaf inoculation with CLRV (Fig. 6) characteristic chlorotic local lesions. The infection was confirmed by electron microscopic visualization of the particles (Fig. 7) and IC-RT-PCR. The inoculum used to infect the CLRV donator plants by mechanical inoculation was checked prior application by IC-RT-PCR and the virus isolate was confirmed by RFLP analysis.

Discussion

Our investigation carried out in hydroculture demonstrates that CLRV is transmissible

Figure 6. Leaf of C. quinoa with characteristic chlorotic lesions induced by CLRV.

Figure 7. Negative stained particles of CLRV isometric particles, approx. 28 nm in diameter.

through nutrient solution although its longevity in-vitro is only four to sixteen days (Brunt *et al.*, 1996). The wide distribution and host range covering woody as well as herbaceous plants could not be explained singly by transmission through seed and pollen. Therefore, virus distribution through water/nutrient solution may serve as an alternative transmission mode. This has to be taken into consideration for CLRV dispersal under natural conditions and has already been shown for other viruses exhibiting a wide host range and found in surface waters like TMV and ToMV (König, 1986, Boben et al., 2007). The viruses can be released from infected plants into drainage water and then spread to other plants.

The use of CLRV-contaminated irrigation water possesses a potential risk to nurseries and fruit orchards cultivating CLRV host plants. Therefore strict control and determination

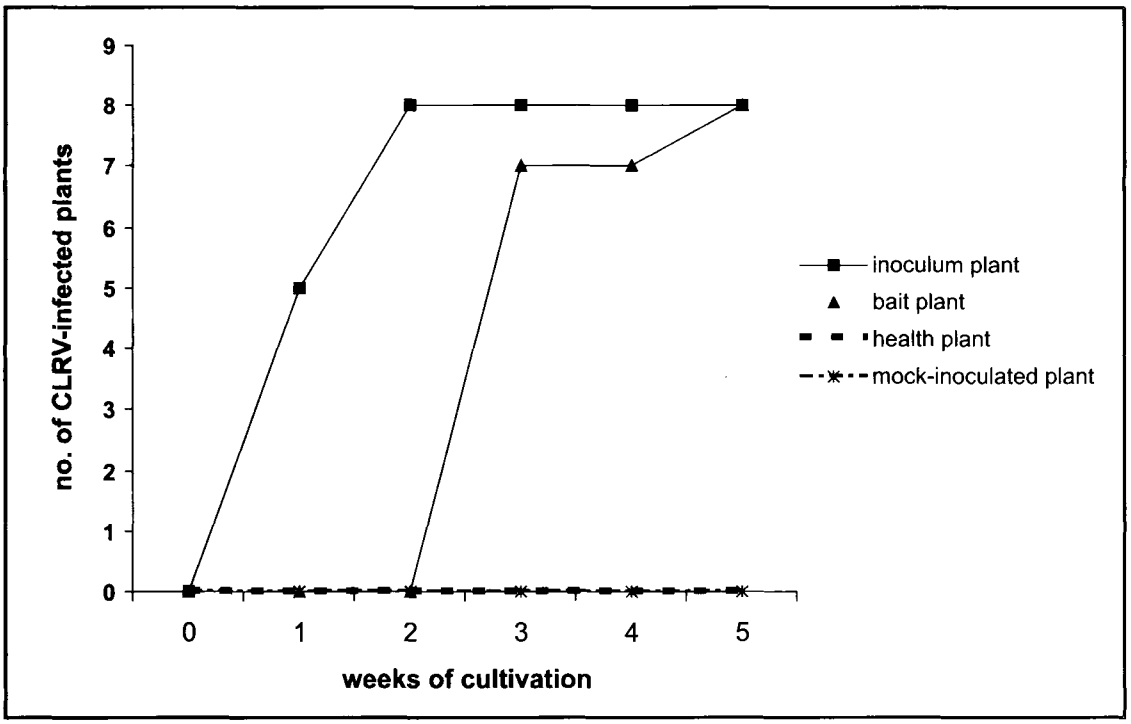

Figure 8. Detection of CLRV in C. quinoa cultivated in hydroculture. (inoculum plant: CLRV infected by mechanical inoculation at the beginning of the experiment, n=8; bait plant: healthy plant serving as indicator plant, n=10 healthy plants and mock-inoculated plants, each n=4).

of the sanitary state of the water resources is indispensable to ensure sustainable high yields in quantity and quality. Reused water or such water designed to be released from greenhouses into the environment has to be tested categorical in regard to plant pathogenic viruses. So far this is no accepted practice. Hence it is not astonishing that Cucumber leaf spot virus (CLSV) was found in large quantities in drainage water collected for example from cucumber greenhouses in Israel (Rosner *et al.*, 2006).

The investigations confirmed that the viral pathogen Pepino mosaic virus can infect healthy tomato plants of two cultivars in closed hydroponic systems via recirculating nutrient solution. The later infection of cv. Peto compared to cv. Hildares is probably due to the different genetic characteristics of the cultivars as nutrient supply was the same and climate conditions similar. Earliest two weeks after setting up the experiment, PepMV was detectable in roots of formerly healthy plants. For this reason the infection occurs even faster than described by Krczal *et al.* (1995). The authors found Pelargonium flower break virus (PFBV) in previously uninfected plants six weeks after setting up the plants after the pathogen released from PFBV-infected Pelargonium plants was detected already in the nutrient solution two weeks after starting the culture. Comparable to our own results on PepMV all plants were infected with PFBV within fourteen weeks. Previous studies on the transmissibility of Arabis mosaic virus (ArMV), CGMMV, Pelargonium leaf curl virus (PLCV), TMV, Tobacco necrosis virus (TNV), ToMV and TSWV through water demonstrated that a

detectable virus contamination of the nutrient solution takes between three to five weeks while the virus concentration of the originally healthy plants cultivated in an ebb-flow system increases continuously (Büttner *et al.*, 1995 a).

Significant yield losses due to a PepMV-infection occurred only in infected plants, after mechanical inoculation in the second leaf stage. This early infection is assumed to be the reason for the observed losses. Production losses were estimated at 15 to 80% in tomatos grown in polyethylene greenhouses in different regions of Spain (Jorda et al., 2001). In contrast Spence *et al.* (2006) found a significant reduction in the quality of PepMV-infected tomato fruits but no decrease in bulk yields. The loss of quality was mainly a result of blotchy ripening, gold marbling, gold spot and symptoms directly attributed to PepMV infection. Currently, there is no source of resistance available in the tomato cultivars. Recently, Ling and Scott (2007) found three *Solanum habrochaites* plants possessing a resistance to the U.S. as well as European type isolates of PepMV which might provide a durable resistance that can be introgressed into tomato cultivars.

The findings on the transmissibility of PepMV through water should lead farmers to consider strategies to prevent spread of the disease as it seems possible that already a few infected plants could be a source for an epidemic in soilless grown tomatoes using recirculating nutrient solution.

Detection at early stages of viral infection, subsequent elimination of the infected plant material and prophylactic measures while establishing a new culture constitute the main approach to combat viral disorders. Moreover, the longevity of the infectivity of sap - >90 days in the case of PepMV and four to sixteen days for CLRV - emphasis the importance of inactivating any viral focus. Methods introduced to reduce or eliminate plant pathogens from water cover slow sand filtration, ultraviolet light, chlorination, ozonation, heat, pressure, surfactants, sedimentation, antimicrobial compounds, suppressive potting mixes and biological control agents (Hong and Moorman, 2005). None of these methods has been proved to be suitable for sole application to eliminate or inactivate plant pathogenic viruses in general.

Disinfectants can be used for decontamination of pots, tables, sand filters (Runia *et al.*, 1988), and other tools. Thereby the required incubation time and concentration of the disinfectant depend on the specific plant pathogenic virus to be inactivated. In Germany only one disinfectant called Menno-Florades (Menno-Chemie Vertriebs mbH, Norderstedt, Germany) is permitted as pesticide. In order to inactivate PepMV it has to be applied on tables, gullies and tanks in a concentration of 2% for 16 hours. The worldwide easily available commercial bleach at 7% (v:v) or NaOH at 0.5% (w:v) was found to inactivate six carnation viruses after a 60 seconds treatment. The investigations were carried out in a systemic Saponaria vaccaria bioassay allowing the RNA-viruses Carnation mottle virus (CarMV), Carnation vein mottle virus (CVMV), Carnation ringspot virus (CRSV), Carnation Italian ringspot virus (CIRV) and Carnation latent virus (CLV) as well as the DNA-virus Carnation etched ring virus (CERV) (Sanchez-Navarro *et al.*, 2007).

To prevent diseases in horticultural and agricultural crops the understanding of three interacting factors is of capital importance: host plant susceptibility, pathogen and favourable environment. As all three factors are necessary for an infection with fungi, bacteria or even viruses altering any one of these factors has to be evaluated in regard to an effective management. In some cases the host plant can be changed by growing disease-resistant varieties or species that are relatively disease free. The possibilities to influence the environment so that it is less favourable for disease exceed in greenhouse crops compared to field crops. The package of measures includes the spacing and pruning of plants to promote airflow and reduce humidity, avoiding overhead watering that increases leaf wetness and watering in the morning rather than evening so leaves have time to dry out. Using growing practices that maintain good plant vigour will also make plants less susceptible to disease. Also the application of pesticides or plant growth enhancers contributes to an optimal healthy status of the plants. Therewith best conditions will be created to produce reasonable yields even with virus-infected plants which can not be cured by application of pesticides. On all accounts prophylactic measures causing a reduction of the infection pressure as the removing of debris, weeds and infected plants should be conducted.

Literature cited

Boben, J., Kramberger, P., Petrovic, N., Cankar, K., Peterka, M., Strancar, A., and Ravnikar, M. 2007. Detection and quantification of Tomato mosaic virus in irrigation waters. Eur. J. Plant Pathol. 118, 59-71.

Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L., and Zurcher, E.J. (eds.). 1996 onwards. Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996.' URL. In: http://biology.anu.edu.au/Groups/MES/vide/

Büttner, C., Jacobi, V., and Koenig R. 1987. Isolation of Carnation Italian ringspot virus from a creek in a forested area south west of Bonn. J. Phytopatol. 118, 131-134.

Büttner, C. and Nienhaus, F. 1989 a. Virus contamination of soils in forest ecosystems of the Federal Republic of Germany. Eur. J. For. Pathol. 19, 47-53.

Büttner, C. and Nienhaus, F. 1989 b. Virus contamination of waters in two districts of the Rhineland area (FRG). Eur. J. For. Pathol. 19, 206-211.

Büttner, C., Marquardt, K. and Führling, M. 1995a. Studies on transmission of plant viruses by recirculating nutrient solution such as ebb-flow. Acta Hort. 396, 265-272.

Büttner, C., Marquardt, K., and Schickedanz, F. 1995b. Studien zur Virusübertragbarkeit von Cucumber mosaic virus (CMV) und Cucumber green mottle mosaic virus (CGMMV) über die Nährlösung in einer Ebbe-Flut-Anlage. Gartenbauwiss. 60, 104-109.

Büttner, C. and Bandte, M. 1999. Überprüfung der viruziden Wirksamkeit von Desinfektionsmitteln am Beispiel von Menno-Florades. Gartenbauwiss. 64, 214-219.

Büttner, C., and Bandte, M. 1999. Orchids: the mechanical transmission of viruses by water, tools, and tables and perspective to control. Hort. Industry 4, 36-37.

Büttner, C., and Bandte, M. 2000. Elimination of plant viruses by horticultural disinfectant. Med. Fac. Landbouww. Univ. Gent, 65/2b, 703-708.

Castello, J.D., Lakshman, D.K., Tavantzis, S.M., Rogers, S.O., Bachand, G.D., Jagels, R., Charlisle, J., and Liu, Y. 1995. Detection of infectious Tomato mosaic tobamovirus in fog and clouds. Phytopathol. 85, 1409-1412.

Castello, J.D., Rogers, S.O., Starmer, W.T., Catranis, C.M., Ma, L., Bachand, G.D., Zhao,

Y., and Smith, J.E. 1999. Detection of tomato mosaic tobamovirus RNA in ancient glacial ice. Polar Biol. 22, 207-212.
Clark, M.F., Adams, A.N., and Barbara, D.J. 1976. The detection of plant viruses by enzyme-linked immunosorbent assay (ELISA). Acta Hort. 67, 43-49.
De Kreij, C., Voogt, W., Van den Bos, A.L., Baas, A.L., and Baas, R. 1997. Voedingsoplossingen voor de teelt van tomaat in gesloten teeltsystemen. Proefstation voor Bloemisterij en Glasgroente, Naaldwijk, Niederlade, First Ed. 22 p.
EPPO. 2001. EU survey on Pepino mosaic potexvirus. In: http://www.maff.gov.uk/planth/pestnote/pepino.htm
EPPO. 2006. EPPO Alert List-Viruses. Pepino mosaic potexvirus – a new virus of tomato introduced into Europe. In: http://www.eppo.org/QUARANTINE/Alert_List/viruses/PEPMV0.htm
Erdiller, G., and Akbas, B. 1994. Plant viruses in Ankara rivers and lakes. J. Turkish Phytopathol. 23, 119-126.
Fuchs, E., Schlufter, C., Kegler, H. 1996. Occurrence of a plant virus in the northern sea. Arch. Phytopath. Pflanz. 30, 365-366.
Gosalves, B., Navarro, J.A., Lorca, A., Botella, F., Sánchez-Pina, M. A., and Pallas, V. 2003. Detection of Melon necrotic spot virus in water samples and melon plants by molecular methods. J. Virological Methods, 113, 87–93.
Hasky, K., Büttner, C., Schickedanz, F., and Sadowska-Rybak, M. 1993. Untersuchungen zur Virusausbreitung über die Nährlösung in geschlossenen Bewässerungssystemen gartenbaulicher Kulturen. Gartenbauwiss. 58, 233-238.
Hong, C.X. and Moormann, G.W. 2005. Plant pathogens in irrigation water: challenges and opportunities. Critical Rev. Plant Sci. 24, 189-208.
Jacobi, V., Castello, J.D., 1991. Isolation of Tomato Mosaic Virus from waters draining forest stands in New York State. Phytopathol. 81, 1112-1117.
Jones, R.A.C., Koenig, R., and Lesemann, D.E., 1980. Pepino mosaic virus, a new potexvirus from pepino (*Solanum muricatum*). Ann. Appl. Biol. 94, 61-68.
Jorda, C., Lázaro Pérez, A., Martínez-Culebras, P., Abad, P., Lacasa, A., and Guerrero, M.M. 2001. First Report of Pepino mosaic virus on Tomato in Spain. Plant Dis. 85, 1292.
Koenig, R. 1986. Plant viruses in rivers and lakes. Adv. Virus Res. 31, 321-333.
Koenig, R. and Lesemann, D.E. 1985. Plant viruses in German rivers and lakes. J. Phytopathol. 112, 105-116.
Kontzog, H.G., Kleinhempel, H., and Keller, H. 1988. Nachweis pflanzenpathogener Viren in Gewässern. Arch. Phytopathol. Pflanzenschutz 24, 171-172.
Krczal, G., Albouy, J., Damy, I., Kusiak, C., Deogratias, J.M., Moreau, J.P., Berkelmann, B., and Wohanka, W. 1995. Transmission of Pelargonium flower break virus (PFBV) in irrigation systems and by thrips. Plant Dis. 79, 163-166.
Ling, K.-S. and Scott, J.W. 2007. Sources of resistance to pepino mosaic virus in tomato accessions. Plant Dis. 91, 749.
Milne, R.G. 1993. Electron microscopy of in-vitro preparations. In: Matthews, R.E.F. (ed.). Diagnosis of plant virus diseases. CRC Press, Boca Raton. pp. 215-251.
Piazolla, D.J., Castellano, M.A., and DeStradis, A. 1986. Presence of plant viruses in some rivers of Southern Italy. J. Phytopathol. 116, 244-246.

Rebenstorf, K., Candresse, T., Dulucq, M.J., Büttner, C., and Obermeier, C. 2006. Host species-sependent population structure of a pollen-borne plant virus, Cherry leaf roll virus. J. Virology 80, 2453-2462.

Roberts, F.M. 1950. The infection of plants by viruses through roots. Ann. Appl. Biol. 37, 385-396.

Roggero, P., Masenga, V., Lenzi, R., Coghe, F., Ena, S., and Winter, S. 2001. First report of Pepino mosaic virus in tomato in Italy. Plant Pathol. 50, 798.

Rosner, A., Lachmann, O., Pearlsman, M., Feigelson, L., Maslenin, L. and Antignus, Y. 2006. Characterization of cucumber leaf spot virus isolated from recycled irrigation water of soil-less cucumber cultures. Ann. Appl. Biol. 149, 313-316.

Runia, W.T., van Os, E.A., and Bollen, G.J. 1988. Disinfection of drainwater from soilless cultures by heat treatment. Neth. J. Agric. Sci. 36, 231-238.

Salomone, A., Roggero, P. 2002. Host range, seed Transmission and Detection by ELISA and Lateral Flow of an Italian Isolate of Pepino mosaic virus. J. Plant Pathol. 84, 65-68.

Sanchez-Navarro, J.A., Canizares M.C., Cano, E.A. and Pallas, V. 2007. Plant tissue distribution and chemical inactivation of six carnation viruses. Crop Protection 26, 1049-1054.

Spence, N.J., Basham, J., Mumford, R.A., Hayman, G., Edmondson, R., and Jones, D.R. 2006. Effect of Pepino mosaic virus on the yield and quality of glasshouse-grown tomatoes in the UK. Plant Pathol. 55, 595-606.

Timmermann, C., Bandte, M. Mühlbach, H.P., and Büttner, C. 2001. Control of mechanical viroid transmission by the disinfection of tables and tools. Med. Fac. Landbouww. Univ. 66/2a, 151-156.

Tomlinson, J.A. and Faithfull, E.M. 1984. Studies on the occurrence of tomato bushy stunt virus in English rivers. Ann. Appl. Biol. 104, 485-495.

Van der Vlugt, R.A.A., Cuperus, C., Vink, J., Stijger, I.C.M.M., Lesemann, D.E., Verhoeven, J.T.J., and Roenhorst, J.W. 2002. Identification and characterization of Pepino mosaic potexvirus in tomato. Bulletin OEPP/EPPO Bulletin 32(3), 503-508.

Van Dorst, H.J.M. 1969. Virus diseases of cucumbers. Ann. Rep. Glasshouse Crops Res. And Exp. Station. Naaldwijk, The Netherlands. 75 p.

Vani, S. and Varma, A. 1993. Properties of cucumber green mottle mosaic virus isolated from water of river Jamuna. Indian Phytopathol. 46, 118-122.

Werner, R., Mühlbach, H.-P., and Büttner, C. 1997. Detection of Cherry leaf roll nepovirus (CLRV) in birch, beech and petunia by immunocapture RT-PCR using a conserved primerpair. Eur. J. For. Pathol. 27, 309-318.