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Transmission and Management of Plant Pathogens in Irrigation Water

Fungal and Viral Pathogens in Hydroponic Tomatoes in Greenhouses

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CHAPTER 1
General introduction

The water is essential to life and is fundamental to important prebiotic relationships. Its special properties allow it to fulfill important roles and functions in chemistry, biochemistry and biology (Westall and Brack, 2018). It has been calculated that the world contains approximately 1400 million km^3 of water and only 0.003 % (45000 km^3) correspond to fresh water that is used for human use, agriculture and industry. However, only 9000 to 14000 km^3 are economically accessible for human use and agriculture uses between 70 and 95 % of total water consumption (Anonymous, 2015; Anonymous, 2020).

Agricultural production is important to ensure food security and water is a key factor in this process. FAO has set a target of increasing world food production by 60 % by 2050. However, the intensive use of water by the agricultural sector can create great stress on water resources, due to its limitation by the deterioration of ecosystems (overpopulation and/or pollution) and climate change (Anonymous, 2021).

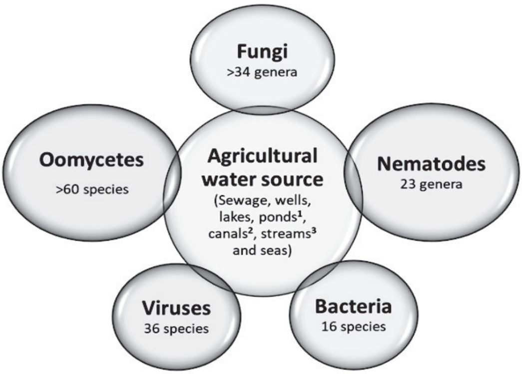
Water quality for agriculture

The quality of water used for crop irrigation is of great concern, as it directly affects food security and production costs (Anonymous, 2020). Thereby, mineral water quality and sanitary water quality and pollutants have to be considered. The mineral water quality largely determines the overall suitability of water for irrigation and nutrients solubility in the rhizosphere. In addition, specific elements, like iron, might disturb irrigation systems due to oxidation and thereby iron flocculation. The phytosanitary water quality is determined by the water source being used, by the design/maintenance of the irrigation system including the installed water treatment technologies as well as by plant and human pathogens (van Overbeek et al., 2014). In general surface water such as lakes, rivers, canals, drainage and recirculating irrigation water pose a much higher risk of harboring pathogens than ground water, municipal water and water collected from roofs and paved surfaces. Pathogens enter the surface water with plant debris, through soil erosion, wind, vectors, as a blind passenger in or on other organism or even due to human activities not related to agriculture (Moorman et al., 2014).

Detection of plant pathogen in irrigation water

Various detection techniques have been described to identify, quantify and assess plant pathogens in irrigation water in particular recirculating water and fertigation solution (Hong, 2014). Thus, filtration, centrifugation and ultracentrifugation have been used for isolation and concentration whereas electron microscopical, serological and molecular biological methods are applied for identification, monitoring and determination of biological and economic thresholds (Bandte and Pettitt, 2014; Büttner et. al., 2014; Von Bargen, 2014).

Some of the most important plant pathogen species that limit the production of crops of global economic interest have been detected in water sources for irrigation (Figure 1.). These include the oomycetes *Phytophthora* spp. and *Pythium* spp. (Hong and Moorman, 2005; Pottorff and Panter, 1997); the fungi *Fusarium* spp., *Rhizoctonia* spp. and *Verticillium* (Bewley and Buddin, 1921); the nematodes *Meloidogyne* spp., *Longidorus* spp., *Heterodera* spp, and *Radopholus similis* (Chabrier and Quénéhervé, 2008; Faulkner and Bolander, 1970; Hugo and Malan, 2010; van Reenen and Heyns, 1986); the plant viruses Pepino mosaic virus (PepMV), Carnation mottle virus (CarMV), Cucumber mosaic virus (CMV), Potato virus X (PVX), Tobacco mosaic virus (TMV), Tomato bushy stunt virus (TBSV) and Grapevine Algerian latent virus (GALV) (Büttner and Nienhaus, 1989; Cannizzaro et al., 1990; Erdiller and Akbas, 1994; Koenig and Lesemann, 1985; Plese et al., 1996; Schwarz et al., 2009; Tosic and Tosic, 1984), and finally the bacteria *Erwinia* spp., *Xanthomonas* spp., *Corynebacterium* spp., *Pseudomonas solanacearum* and *Ralstonia solanacearum* (Elphinstone et al., 1997; Harrison et al., 1987; Jenkins and Averre, 1983; Maddox and Harrison, 1988; Nelson, 1980).



[1] including irrigation reservoirs, surface water retention basins, holding tanks (for ebb – and – flow and hydroponic systems in greenhouses). [2] including ditches. [3] including rivers and brooks.

Figure.1 Plant pathogens detected in different sources of irrigation water (Adapted from Hong, 2014).

In addition to running waters contaminated distribution systems have allowed the spread of pathogens across large geographic regions. Thus, the oomycetes *Phytophthora capsici* and *Pythium* *aphanidermatum*, which affect yellow pepper and pumpkin in Michigan, Georgia and Florida in the United States, and the bacteria *Ralstonia solanacearum* which spread throughout Europe and Florida were dispersed through contaminated irrigation water (Hong et al., 2014). The latter, *R. solanacearum*, is categorized as a quarantine pest in Europe and America (Chile, Mexico and Paraguay) and cause great losses mainly due to quarantine measures. These measures are carried out to eradicate the pathogen that means to prevent a further distribution. Severe economic losses would result from dispersal of *R. solanacearum* as the bacteria has i) a wide host range of at least 50 families including main crops such as tomato, potato, tobacco, and banana, and ii) a high aggressiveness (EFSA, 2019; EPPO, 2018; Hayward, 1994).

Hydroponics

Technologies for growing plants without soil have gained importance, as they are an alternative for producing food in those areas where there are contaminations with plant pathogens and heavy metals that limit the use of soil and water sources for agricultural use (Sambo et al., 2019; Savvas, 2003; Sharma et al., 2018). The challenge of optimizing water resources and minimizing the impact of plant pathogens to high quality horticultural crops for fresh consumption has allowed protected crop technology to expand in many countries of the world (Savvas, 2003; Sharma et al., 2018).

Hydroponics is a method that allows the growth of plants without using soil as a rooting medium, and where plant requirements are met by a mixture of inorganic salts and water through the irrigation system (Gericke, 1940; Gericke, 1945; Graves 1983; Hoagland and Arnon 1950; Jones Jr., 2005; Resh 2013; Santos et al., 2022; Savvas, 2003). Today, this technology is used to produce a wide variety of specialized crops, including ornamental and vegetable plants. Within this latter group lettuce, cucumber and tomato are the main vegetable crops cultivated for their high economic value (Brentlinger 1997; Jenner and Starkey, 1980; Jensen 1999; Song et al., 2004; Spensley et al., 1978).

In a hydroponic crop the nutritive solution that runs through the irrigation system can be administered using the open or closed method. When the open type is used the nutrient solution flows only once through the system and is drained wasting large amounts of water and nutrients (Jensen 1997; Nederhoff and Stanghellini 2010). For the closed–type method the nutrient solution recirculates through the irrigation system for an unspecified period of time, allowing reuse (Lykas et al., 2006). This closed recirculation technique is of great interest, as it is environmentally friendly and reduces production costs by reducing water and nutrient consumption by 20 – 40 % (Abd-Elmoniem et al., 2006; Nederhoff and Stanghellini, 2010; Vallejos, 2003).

Nutrient Film Technique

The nutrient film technique (NFT) is one of the most used within closed recirculation type (Jones Jr., 2005; Resh, 2013). NFT is characterized by a permanent nutrient flow, which flows around the roots in a thin "film" and thus supplies them. A pump conveys the nutrient solution to an inclined plane (tube), on which the plant roots lie, are washed around and thus continuously supplied. The constant flow prevents nutrient accumulation. Due to the special design of NFT systems, oxygen is added to the nutrient solution, usually through downpipes or vortex systems. Planting substrate is usually omitted, so that the roots have unhindered access to nutrients and oxygen and can grow quickly.

NFT was initially developed in 1966 by Allen Cooper in England (Cooper, 1975) and is currently based on containers (channels or tubes), which are supported on a support system with a slight slope of 0.3 to 2 percent (Cooper, 1975; Jones Jr., 2005; Van Os et al., 2008). The plants to be cultivated are suspended inside the containers to which the nutrient solution is supplied by the upper part of the canal, this flows superficially downhill passing in its path over the roots of the plants, at the end of the path it is collected and returned to the supply tank, where it is frequently monitored to maintain the composition of nutrients (Cooper 1975; Graves 1983; Jones Jr., 2005; Nederhoff and Stanghellini 2010; Resh 2013; Winsor et al., 1979). It is accepted that the appropriate film depth or surface flow is a few centimeters to a maximum of two inches (Jones Jr., 2005; Resh 2013). In crops such as chrysanthemum and lettuce slower flows of 3 to 8L m^{-2} h^{-1} are recommended (Benoit and Ceustermans, 1989; Ruijs et al., 1990a; Ruijs et al., 1990b), whereas for vegetables with fruits faster flows are recommended (Van Os et al., 2008). The main disadvantage of the recirculation of fertigation solutions in closed systems is the high risk of rapid spread of stable and viable plant pathogens. Thereby, initially sporadic infected plants form an inoculum that is quickly spread through the water and is responsible for many plants becoming diseased (Hong, 2014; Lévesque et al., 2022; Stewart-Wade, 2011).

Management of pathogens in recycled water

Preventing (i) contact of water with plant material and soil infected/infestested by pathogens, (ii) transport of pathogens, and (iii) propagation of pathogens in water are the objectives of sanitizing irrigation water in agriculture and fertigation solution in horticulture, respectively (Armitage 1993; Hong and Moorman 2005). Cultural measures such as storing water at specific temperatures prior to use in production areas, use of buried pipes, management of the type, volume and frequency of irrigation, or having the captation tube high with respect to the base of the tank pursue the goal of avoiding sediment, reducing the transport and viability of pathogens, and even eliminating infectious propagules of particular pathogens (Hong and Moorman, 2005; Nielsen et al., 2004; Rattink, 1990; Van Kuik, 1992).

Different methods for managing plant pathogens in recycled irrigation water are available (Lévesque et al., 2022). They can be categorized as cultural, biological, physical and chemical measures (table .1). The last two groups are the most used in water disinfestation treatments. The selection of the treatment to be used is influenced by factors such as volume of water collected, flow of water to be disinfected, number of irrigations to be carried out and irrigation time, among others (Van Os, 2000). It is also important to consider biological and detection thresholds in order to define the level of economic damage and to justify the action of the use of disinfection treatment (Ehret et al., 2001; Hong and Moorman, 2005; Steinberg et al., 1994; Stewart-Wade, 2011).

Table. 1. Relevant aspects of the main technologies for the control of microorganisms in irrigation water and nutrient solutions (Adapted from Dandie et al., 2020; Pettitt, 2015 and Haute et al., 2015).

Water treatment	Technology	Strength	Weakness
Physical	Heat treatment	Efficient control: Kills all pathogenic groups (virus, bacteria, fungi)	High energy consumption, high installation and maintenance costs, inactivates all organism
	Particle and membrane filters	Retention of very small organism	High maintenance costs
	Ultraviolet radiation	Destroys nucleic acid, e.g. bacteria and fungi	High maintenance costs
Ecological	Slow sand filtration	Environmentally friendly	Requires space and cause high cost
	Biosurfactants		
	Constructed wetland		
Chemical	Chlorine	Efficient and moderate installation and maintenance costs	Transport and storage of hazardous chemicals, phytotoxicity, unwanted by-products, difficult to dose according to requirements
	Chlorine dioxide		
	Copper, silver, bromine		
	Ozone		Difficult to dose according to requirements
	Hydrogen peroxide and activated peroxygen		

Thus, **filtration**, **UV-light** and **heating** are frequently applied procedures which are all based physical mode of action. In the former procedure, mats, meshes or thin films are used to exclude the organisms and thereby prevent the spread of propagules. For example, irrigation mats at the bottom of containers, trays channels and basins reduce or even prevent the spread of infectious propagules with irrigation and fertigation water in greenhouses. Van der Gaag et al. (2001) reported on a significantly reduced spread of *Phytophthora* spp. in *Gerbera* and *Spathiphyllum* by apparently limiting the flow of the inoculum between the dams and the irrigation system.

However, **filtration** can also be performed with porous materials that retain solid particles and allow liquids to pass through its structure. For example, slow filtration uses materials such as sand and gravel of different sizes, which are arranged in layers within a containment structure that makes it possible to drain and control the flow of water within it (Werres and Wohanka, 2014). Different trials have reported the partial or total elimination of plant pathogens such as Tobaco mosaic virus (TMV), *Phytophthora* spp., *Pythium* spp., and *Fusarium oxysporum* in irrigation water and fertigation solutions, using layers of materials such as rook wool, volcanic granules and coral particles within the filters (Belbahri et al., 2007; Fitzell and Peak., 1990; Oki et al., 2017; Wohanka, 1989; Wohanka, 1995). Another filtration system used is membrane filtration, which is subdivided into Microfiltration (1 μm), Ultrafiltration (100 nm), Nanofiltration (10 nm) and Reverse Osmosis (1 nm), depending on the size of the pores of the material that makes up the membrane. The membrane is mostly ceramic, polymers or cellulose acetate. Additionally, membrane filtration technique is applied in two different variants: i) dead-end and ii) cross-flow. This distinction refers to the position of the feed in relation to the membrane surface. This can be perpendicular or parallel. A parallel course of the feed to the membrane surface enables continuous recirculation (recycling) of the retained current. Such a membrane filtration system allows the elimination of plant pathogens in irrigation water and fertigation solutions efficiently.

Sanitation by **ultraviolet radiation (UV)** takes place through specific lamps that emit UV-C radiation at 254 nm (Newman, 2004; Newman, 2014). This radiation induces effects on the DNA and RNA of cells and cellular organisms which are mutagenic or lethal (Newman, 2014). To achieve a germicidal effect on pathogenic fungi, a dose of 100 mJ/cm^2 and 250 mJ/cm^2 is recommended to inhibit all organisms including viruses (Ehret et al., 2001; Runia, 1994b; Runia, 1995; Van Os and Stanghellini, 2002). Several authors reported on the reduction of populations of important pathogens such as *Alternaria*, *Colletotrichum*, *Fusarium*, *Pythium*, *Phytophthora*, nematodes, bacteria and viruses such as TMV and ToMV in irrigation water (Amsing and Runia 1995; Banihashemi et al., 1992; Ehret et al., 2001; Fitzell and Peak 1990; Mebalds et al., 1996; Rey et al., 2001). In the last two decades, this technique has been improved by the additional use of semiconductors such as Zinc oxide (ZnO), Iron oxide (FeO), Cadmium sulphide (CdS), Zinc sulphide (ZnS) and Titanium oxide (TiO_2). The latter is considered the best photocatalyst for water disinfection treatments. Heterogeneous photocatalytic oxidation with TiO_2 increases the reaction and causes complete oxidation of substances with CO_2 and other inorganic species (Malato et al., 2009).

Finally, **heating** is one of the most used procedures for sanitation of fertigation solutions and irrigation water, due to its high reliability to inactivate plant pathogens. Currently, this system is used especially in the Netherlands and the United Kingdom in nurseries (Hao et al., 2014). The technology allows the solutions to be heated by means of specialized equipment, controlling exposure time and temperature. It has been confirmed to effectively control different plant pathogens of economic importance like *Phytophthora infestans, Verticillium dahliae, Fusarium oxysporum* f.sp. *melongenae, Pythium ultimátum, Thielavidopsis basicola, Agrobacterium tumefaciens,* and plant viruses such as TMV among others (Bollen, 1985; Hao et al., 2014). Different protocols have been evaluated varying contact time and temperature. In regard to effectiveness, the most recommended treatment applies 203°F (95°C) for a period of 30 seconds to sanitize waters (McPherson et al., 1995; Runia et al., 1988). According to the authors it allows the inactivation of infectious propagules and prevent the spread of the corresponding diseases to new plants.

Chemical measures are based on components such as **chlorination**, **surfactants** and **ozone**. Already for 100 years, Chlorine is the most widely used in drinking water disinfection because of its germicidal properties and low cost (LeChevallier and Kwok-Keung, 2004; Stewart-Wade, 2011). The most common **chlorine** sources used in the irrigation water disinfection process are chlorine gas (Cl_2), calcium hypochlorite (solid), electrochemical hypochlorination and the most popular sodium hypochlorite (liquid) (Fisher et al., 2014; Lévesque et al., 2022; Lévesque et al., 2023). Three types of residual chlorine are generated during the chlorination process, the first being total chlorine, which is formed by free chlorine plus combined chlorine. The second is combined chlorine, which corresponds to chloroderivative compounds generated in water with different elements such as ammonium (NH_3) and organic nitrogen. And finally, free chlorine which is composed of dissolved chlorinated gas (Cl_2), hypochlorite (OCl) ions and hypochlorous acid (HOCl) (White, 2010; Hong et al., 2003). The HOCl molecule is a strong oxidant and is responsible for the greatest microbicidal effect during chlorination (De Hayr et al., 1994; Fisher et al., 2014); as it destroys pathogens through cell wall penetration (because of its small size and non-polar characteristic), causing damage to membranes, metabolic processes and proteins. This acid in turn can be dissociated into hydrogen ions (H^+) and hypochlorite ions (OCL^-), a separation that is affected by the pH value (Fisher et al., 2014). The inactivation of many microbial pathogens is at doses of 1-3 ppm of free residual chlorine. Although, depending on the water source higher levels of about 5-10 mg/L are required to compensate chlorine demand due to organic matter and ammonium (Clark and Smajstrla, 1992; Daughtry, 1984; De Hayr et al., 1994; Ewart and Chrimes, 1980). There are vegetables sensitive to chlorine, for example lettuce expresses toxicity at doses of 0.5 mg/l of chlorine (McGehee and Raduales, 2023). However, there are plants that are more sensitive even to concentrations of 0.05 mg/l, but in general doses higher than 5 mg/l can cause phytotoxic problems (Lykogianni et al., 2023). In post-harvest the use of chlorine is also relevant; For the disinfection of tomato fruits, doses of 80 ppm can reduce decomposition and improve their shelf life by up to 10 days (Saikumar et al., 2023).

Surfactants are amphiphilic molecules that possess chemical properties of great interest, as they are composed of a hydrophobic and a hydrophilic part. They allow the formation of interfaces or micelles between phases, such as air and a liquid or liquids with different polarities (Tsuji and Tanaka, 1998). Surfactants are commonly used in various fields of application, both in private household as well as industrial processes. According to their polar charge surfactants are grouped into anionic (negative charge), cationic (positive charge), noionic (absence of charge) and amforetic (with both positive and negative charges). In addition to the large group of synthetic surfactants, there are so-called biosurfactants, which are formed by microorganisms. These biosurfactants are supposed to have no toxic effect on the environment compared to synthetic ones (Hultberg and Alsanius, 2014; Mulligan, 2005). The amphiphilic properties of surfactants, mainly those of the cationic group, are commonly used for the decontamination of irrigation water, as they prevent the spread of diseases by killing microorganisms quickly by breaking their membranes (Hultberg and Alsanius, 2014).

And finally, **Ozone** (O_3) is considered a gas have interesting properties to kill algae, fungi, bacteria, nematodes and viruses. Compared to chlorine ozone is 1.5 folds more oxidizing, easily allowing the rupture of cell membranes (Igura et al., 2004; Newman, 2004; Runia, 1995; Yiasoumi et al., 2005). Its use in irrigation water purification treatments began in the 1980s. However, its use is not very common in horticulture (Burlenson et al., 1975; Farooq et al., 1977; Runia, 1994a). This gas must be produced in generators for an *in situ* disinfection as it is unstable and easyly decomposable. Different studies reported the inactivation of pathogens such as *Verticillium dahliae*, *Botrytis cinérea*, *Phytophthora nicotianae*, *Pythium ultimum*, *Fusarium oxsporum*, Cucumber green mottle mosaic virus (CGMMV) and Tomato mosaic virus (ToMV) after sanitation applying ozone with different levels and exposure times (Elmer et al., 2014; Ruiz-Espin et al., 2023; Runia, 1994a; Spotts and Cervantes, 1992; Stewart-Wade, 2011; Vanachter et al., 1988).

Research aims and thesis outline

Currently, irrigation water is a limited resource, promoting gardeners, farmers and nurserymen to use recirculating nutrient solution systems to optimize their production. The presence of various plant pathogens and their easy spread by this technique is one of the main problems limiting its application; moreover, the risk of recontamination of the solution endangers the production system.

Electrolytic water disinfection is known from drinking water purification, where an antimicrobial hypochlorite, a salt of the hypochlorous acid HClO, is generated *in situ*. In the context of the present work it should be examined to what extent the procedure is suitable for the sanitation of horticultural nutrient solutions in intensive crop production in greenhouses such as tomatos.

In order to assess the suitability of the method for horticultural practice, the antimicrobial effective concentration and exposure time for individual pathogens or pathogen groups as well as the possible occurrence of plant damage and undesirable residues in the crop must first be determined. These studies were conducted and are presented in Chapters 2 through 4. Hypochlorite was produced on-site in a single chamber brine electrolysis plant which has since been further developed and commercialized.

- The ability of electrolytically-derived KCLO to inactivate viral particles and its inhibitory effect on the spread using the example of Pepino mosaic virus (chapter 2).

- Efficacy of the electrolytically-derived KCLO to inactivate plant pathogenic fungi such as *Fusarium oxysporum* and *Rhizoctonia solani* (chapter 3). Both *in vitro* and *in vivo* studies were carried out. But, focus was on interruption of pathogen dispersal via nutrient solution under semi commercial conditions.

- Decontamination of a nutrient solution naturally contaminated with two viral and two fungal pathogens in tomatos hidroponic under semi-commercial production conditions, each using a sensor-based electrolytic disinfection approach (chapter 4).