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Toxicity of mixed-oxidant electrolyzed oxidizing water to in vitro and leaf surface populations of vegetable bacterial pathogens and control of bacterial diseases in the greenhouse

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Abstract

Mixed-oxidant (MO) electrolyzed oxidizing (EO) water was generated by electrolysis of a 1.7% KCl (aq. w/v) brine solution. The MO EO water was a powerful bactericide in vitro at a dosage of mixed oxidants equivalent to 50 mg L^{-1} free available chlorine. Populations of *Xanthomonas campestris* pv. *vitians*, *Pseudomonas syringae* pv. *coriandricola*, and *Erwinia carotovora* subsp. *carotovora* were reduced from log 9 to log 10 CFU mL^{-1} to undetectable levels after 1 min exposure. Only *E. carotovora* subsp. *carotovora* was sensitive to a 5 mg L^{-1} dose of MO EO water. In greenhouse disease control experiments, 50 or 100 mg L^{-1} MO EO water failed to control bacterial leaf spot of lettuce, bacterial spot of tomato and pepper, or bacterial leaf spot of radish. A spray application of a copper hydroxide/mancozeb suspension was effective for control of bacterial leaf spot of lettuce and bacterial spot of tomato and pepper, reducing foliar disease levels up to 45%. Some phytotoxicity was observed at the 100 mg L^{-1} MO EO water dose. The lack of disease control on greenhouse plants with MO EO water may in large part be due to low mortality of the pathogen on leaf surfaces. In five of six experiments, no significant reductions in leaf surface populations were found for 50 mg L^{-1} MO EO water. In contrast, copper/mancozeb treatments reduced pathogen leaf surface populations by up to 5 log units when copper-sensitive strains were involved.

Keywords: Electrolyzed oxidizing water; *Xanthomonas*; *Pseudomonas*; *Erwinia*; Lettuce; Tomato; Pepper

1. Introduction

Bacterial diseases represent some of the most recalcitrant and difficult-to-manage pest problems affecting commercial vegetable production in Florida. The humid, warm conditions, coupled with frequent rain events, are ideal for development of bacterial disease epidemics. Losses from bacterial diseases in Florida can be substantial. For example, losses of 50% in extra-large and large tomato fruit were recorded in plots where epidemics were initiated before first fruit set (Pohronezny and Volin, 1983). In the mid-1990s, millions of dollars in revenue were denied southern Florida lettuce growers because of outbreaks of bacterial leaf spot (Pernezny et al., 1995). Copper resistance and the frequent occurrence of new races of *Xanthomonas campestris* pv. *vesicatoria* have been implicated in a seemingly neverending battle to control bacterial spot of pepper (Ritchie and Dittapongpitch, 1991; Pernezny et al., 1999).

Electrolyzed oxidizing (EO) water represents an exciting technology for disinfection and management of infectious disease agents. The process was initially developed in Japan (Shimizu and Hurusawa, 1992). Briefly, the technology is based on the passage of an electric current through a chloride salt solution (typically 2 M NaCl or KCl). In many systems, a membrane separates solution output at the anode and cathode terminals. The anode water achieves effective

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microbiocidal properties through development of high available chlorine levels and high oxidation–reduction (REDOX) potential (+1100 mV) (Kim et al., 2000). EO water has provided noteworthy antimicrobial activity in the food industry (Kim et al., 2000; Venkitanaroyanan et al., 1999a), surgical theaters (Ohno et al., 2000), and potable and waste water treatment systems (McPherson, 1993).

Recently, Mueller et al. (2003) showed that EO water can be used to provide control of a fungal disease, powdery mildew of gerbera daisy, in the greenhouse. While the exposure of crop plants to chlorine and the high-REDOX potential solutions might well raise concerns of phytotoxicity, Buck et al. (2003) did not find EO water to cause appreciable damage to floricultural crops.

Another electrolytically derived solution, generally referred to as mixed oxidants, has also been used to effectively disinfect microbially contaminated water sources. In this system, the anode and cathode waters from electrolysis of a brine solution are collected together in a receiving vessel (Robson, 1984). The resultant solution consists of a mixture of oxidants, including free chlorine, ozone, and hydrogen peroxide. Mixed-oxidant (MO) solutions have been very effective for inactivation of *Clostridium perfringens* spores that are normally resistant to standard drinking water chlorine treatments (Venczel et al., 1997). MO solutions are particularly attractive for plant disease management, because pH and REDOX potential values are not as extreme as those found in pure anode water. Therefore, one might expect fewer problems with phytotoxicity with the MO product at higher concentrations of oxidants.

The objectives of this study were to: (i) investigate the in vitro activity of MO EO water against a number of important bacterial pathogens of vegetables in Florida, (ii) determine the efficacy of MO EO water for control of bacterial diseases in the greenhouse, and (iii) measure mortality of leaf surface populations of bacteria after exposure to MO EO water sprayed on plants.

2. Materials and methods

2.1. Electrolyzed, MO water

All experiments used a MO solution of EO water. Stock solutions of EO water were prepared using a 12-V MIOX disinfectant apparatus, Brine Pump System version no.4 (MIOX Corp., Albuquerque, NM, USA), according to the manufacturer's user's guide (Robson, 1984). The brine solution was prepared as a 1.7% KCl (aq. w/v) solution. The unit was operated at 10 A for approximately 4 min and the EO water captured from the polypropylene discharge tube in a clean amber glass jar with a tightly fitting, screw cap lid. Generally, the MO EO water was used within 1 h of generation.

The free available chlorine was determined by the DPD ferrous titrimetric method using N, N-diethyl-p-phenylenediamine as the indicator (Palin, 1967). Typical output values of free available chlorine were 300 mg L^{-1}. Dilutions to desired chlorine concentrations were made in distilled water. The pH and REDOX potential of fresh MO EO water were 7.0 and −5.1 mV, respectively, as measured with a Denver Basic dual scale pH meter (Denver Instrument Co., Arvada, CO, USA).

2.2. In vitro mortality experiments

The effect of MO EO water on in vitro survival of three representative genera of plant-pathogenic bacteria was determined. *Xanthomonas campestris* pv. *vitians*, strain L7 from lettuce (*Lactuca sativa* L.) (Pernezny et al., 1995); *Erwinia carotovora* subsp. *carotovora*, strain ECC2, originally from a soft-rotted tomato (*Lycopersicon esculentum* Mill.) fruit; and *Pseudomonas syringae* pv. *coriandricola*, strain Cil1 from cilantro (coriander) (*Coriandrum sativum* L.) (Pernezny et al., 1997), were grown on nutrient agar amended with 0.5% (w/v) glucose (GNA) for 48–72 h. Plates were flooded with sterile phosphate-buffered saline (PBS) (Leben et al., 1968) and resultant suspensions adjusted turbidimetrically to approximately 1×10^8 CFU mL^{-1}. Suspensions of bacteria and diluted MO EO water were then mixed in sterile beakers in ratios to give final concentrations of either 50 or 5 mg L^{-1} free available chlorine. After 1 min exposure, suspensions were serially diluted in a decimal series and 100 μL of each dilution was plated in triplicate on GNA and spread with a sterile, bent glass rod. Bacterial suspensions mixed with sterile PBS served as controls. Plates were incubated at 28 °C for 48 h (*E. carotovora* subsp. *carotovora*) or 72 h and colonies enumerated. There were three replicate dilutions of each treatment. Data were expressed as $\log_{10}$ CFU mL^{-1}. In order to detect very low populations of these bacteria, three replicate tubes of 9 mL of sterile glucose-nutrient broth-liquid cultures were seeded with 1 mL of the test suspension, exposed to treatment for 1 min. Tubes were agitated on an orbital shaker at 150 rpm and 25 °C for 48 h. Populations were recorded as zero when no colonies were detected on spread plates and no liquid cultures showed any sign of turbidity after 48 h shaker incubation.

All permanent cultures were maintained as turbid suspensions in 15% aqueous glycerol solution at −70 °C (Sleesman and Leben, 1978). Working cultures were maintained on yeast extract-glucose-calcium carbonate slants at 4 °C (Shaad, 2001) for up to 3 weeks.

2.3. Greenhouse disease control

A series of four greenhouse control experiments were conducted to determine the efficacy of MO EO water for