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Evaluation of electrolyzed oxidizing water for phytotoxic effects and pre-harvest management of gray mold disease on strawberry plants

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ABSTRACT

Near neutral (pH = 6.3–6.5) electrolyzed oxidizing water (EO water) has been demonstrated to inactivate fungi in pure culture and to mitigate infection on fruit surfaces. One possible alternative or supplement to traditional pre-harvest crop management practices that currently rely on the use of large quantities of fungicides is near neutral EO water. In the present work, treatment of *Botrytis cinerea* or *Monilinia fructicola* with near neutral EO water (50 or 100 ppm total residual chlorine (TRC)) in pure culture resulted in a 10^6 reduction and 100% inactivation as evidenced by negative broth enrichment. When applied in concert with 50 or 100 ppm EO water, treatments of Captan 50WP (captan), Rovral (iprodione), Iprodione 4LAG (iprodione), or Switch 62.5 WDG (cyprodinil and fludioxonil) effectively inhibited fungal growth of *B. cinerea* as evidenced by a 10^6 reduction on the direct plate and negative broth enrichment. Treatments of Captan 50WG (captan), Rovral (iprodione), Iprodione 4LAG (iprodione), Switch 62.5 WDG (cyprodinil and fludioxonil), Captan 80 WDG (captan), or Captevate (captan and fenhexamide) when applied in concert with 50 or 100 ppm EO resulted in a 10^6 reduction of *M. Fructicola* and 100% inactivation as evidenced by negative broth enrichment. Strawberry plants sprayed with EO water (pH = 6.3–6.5) at concentrations of 50 and 100 ppm TRC once per week, did not result in significant ($P > 0.05$) phytotoxicity relative to a water (0 ppm TRC) treatment. In this study, the application of 100 ppm EO water (pH = 6.3–6.5) twice per week to strawberry plants infected with *B. cinerea* was more effective ($P \leq 0.05$) than a once per week Captan application and as effective as a once per week captan/once per week EO treatment. The once per week captan/once per week EO treatment was significantly more effective ($P \leq 0.05$) than the captan once per week treatment. Dip treatments of strawberries in near neutral EO solutions (50 and 100 ppm TRC; pH = 6.3–6.5) did not leave a chlorine residue on the fruit relative to a water dip. The results from this study suggest that near neutral EO solutions could be used to manage infection of *B. cinerea* on strawberry plants in the field and also as a disinfection solution for harvesting equipment, greenhouses, packing houses and in commercial facilities to prevent or manage infections of *B. cinerea* and *M. fructicola*.

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1. Introduction

Pre and post-harvest fungal infections can result in significant economic losses to growers and retailers. Crop losses due to all plant pathogens within the U.S. are estimated to be $33 billion per year and growers spend approximately $720 million per year on fungicides (Pimentel et al., 2000). The fungus *Botrytis cinerea* Pers. 1794 is an important pathogen of grapes, strawberries and stone fruits (peaches, plums, cherries and nectarines). Fungal infestation by *Monilinia fructicola* (G. Winter) Honey 1928, which is responsible for brown rot in peaches, apples and other stone fruits, can result in significant pre and post-harvest losses (Emery, 2000; Elad et al., 2004). Infection by *B. cinerea* or *M. fructicola* can occur pre-harvest causing rotting on the plant and blossom blight and post-harvest as a latent infection during verasion which may lead to significant economic losses due to post-harvest decay during storage.

Traditional management practices for the control of fungal infestation rely mainly on the use of large quantities of fungicides, which can result in the development of resistant fungal strains. The occurrence of resistant fungal strains makes it difficult to develop an efficient management strategy for fungi and can lead to significant economic losses. Resistance to fungicides that affect a variety

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of crops, including grapes, strawberries, tomatoes and peaches, has been reported for populations of *B. cinerea and M. fructicola* (Elad et al., 2004; Schnabel et al., 2004). Concerns regarding disease resistance in plants, occupational exposure during fungicide application, negative environmental impacts and consumer demand for less chemical treatment of fruits and vegetables have resulted in the development of alternative methods for disease control. One possible alternative or supplement to traditional treatment methods is the use of electrochemical activation technology to produce solutions that effectively control fungal growth without the negative side effects caused by conventional practices. Near neutral electrolyzed oxidizing water (EO water) could be applied directly to the plants during the growing season via irrigation or spraying and also be used to treat harvested fruit by dipping or as a mist. Previous research indicates that near neutral EO water effectively mitigated post-harvest surface infections of *B. Cinerea* and *M. fructicola* on grapes and peaches, respectively (Guentzel et al., 2010).

Near neutral electrochemically activated solutions or "EO water" are produced via the passage of a dilute salt solution (~1% NaCl) through an electrolytic cell that contains a separate anode (oxidizing) and cathode (reducing) chamber. In the oxidizing chamber, chloride ions and water molecules are converted to chlorine oxidants (Cl_2, HOCl/ClO^-). Near neutral solutions of EO water (pH = 6.5; ORP 800–900 mV) contain primarily hypochlorous acid (HOCl) (≈95%), the hypochlorite ion (ClO^-) (≈5%), and trace amounts of chlorine (Cl_2) (Guentzel et al., 2008). The biocidal properties of HOCl are enhanced by the high oxidation-reduction potential (ORP) of solution. The high ORP of the EO water affects the outer membrane of the fungi which facilitates the transfer of HOCl across the cell membrane, thus resulting in further oxidation of intracellular reactions and respiratory pathways (Liao et al., 2007).

Acidic EO water solutions also show promise as a fungal decontamination agent for control of foliar diseases of plants and as a post-harvest dip treatment for peaches and pears (Al-Haq et al., 2001, 2002; Buck et al., 2002, 2003; Mueller et al., 2003). Strongly acidic EO water (pH<3) has biocidal properties, but rapidly loses Cl_2 through the evolution of chlorine gas thus reducing the biocidal effectiveness of the solutions over time (Len et al., 2000) and causing a potential human health and safety issue through the formation of chlorine gas (Guentzel et al., 2008). The high acidity of the solution causes corrosion of equipment and surfaces and is slightly phytotoxic to some species of bedding plants (Buck et al., 2003). The application of EO water at a near neutral pH reduces the negative health and safety effects caused by chlorine off-gassing, minimizes corrosion of surfaces and may limit phytotoxic side effects while maximizing the application of the hypochlorous acid species (Guentzel et al., 2008). The objectives of this study were to evaluate the effectiveness of using near neutral EO water to inactivate *B. cinerea* or *M. fructicola* when concomitantly applied with chemical fungicides in pure culture, its possible phytotoxicity to strawberry plants and its potential for use as a pre-harvest treatment of strawberry plants during the growing season.

2. Methods and materials

2.1. Preparation of near neutral electrolyzed oxidizing water

Electrolyzed oxidizing water (EO water) was produced using room temperature dilute salt water (~1%NaCl in tap water) and a patented EcaFlo® system (Model C101; Integrated Environmental Technologies, Little River, SC). The system is equipped with a C-100 electrolytic cell constructed from titanium, ceramic and plastic and operates at 20 V, 50 amps and 79.5 L per hour. The C-100 cells contain a ceramic diaphragm that separates the anode and cathode, which allows for the separate production of oxidizing anolyte solutions (EO water) and reducing catholyte solutions. The EcaFlo® system produces near neutral (pH = 6.5–6.7) EO water while maintaining a high oxidation/reduction potential (ORP = 800–900 mV). Hypochlorous acid (HOCl) is the dominant chlorine species (96%) at this pH range (6.5–6.7) (Faust and Aly, 1998). The pH and ORP were measured using an Oakton 110 series meter equipped with pH and ORP electrodes (model #s WD-35615-24 and 35805-15 Oakton Instruments, Vernon Hills, IL). The concentration of total residual chlorine species (TRC) was determined using iodometric titration (Method 8209; detection limit = 0.1 mg/l, HACH Co., Loveland, CO). The EO water used for the experiments in this study was generated daily and used within 8 h of production.

2.2. Preparation of pure cultures

Individual cultures of *B. cinerea* and *M. fructicola* were grown separately on BD™ BBL™ Potato Dextrose Agar at 25 °C for 10 days. Fungi were harvested by dislodging the spores from the plate surface using a sterilized angled glass rod and a small amount of sterile water. The spores were filtered through 3 layers of sterile cheesecloth and diluted with sterile water to a concentration of 10^8 spores/ml. The concentrations of conidia in solution were counted using a hemocytometer.

2.3. Procedure for treatment of pure cultures with EO water and or fungicides

Fungicides were obtained from the manufacturers. The low and high application rates were determined according to the manufacturer's instructions and were normalized to active ingredient per milliliter (ai/ml) (Table 1). A volume of 100 μl of each stock culture (10^8 spores/ml) was combined with 9.9 ml of sterile water, EO water, each specific fungicide, or a mixture of EO water and each specific fungicide (Table 2) for a final cell count of 10^6 spores/ml per treatment. The treatments were vortexed and incubated at 25 °C for 10 min. Following a 10 min time period, the treatments were vortexed and diluted 1/100 with sterile water to stop or minimize

Table 1
Application rate of fungicide for compatability testing with EO water.

Fungicide treatment	Active ingredient	Application rate	
		Low	High
Captan 50WP	Captan	1000 μg ai/ml	2000 μg ai/ml
Captan 80 WDG	Captan	900 μg ai/ml	1800 μg ai/ml
Elevate	Fenhexamid	300 μg ai/ml	450 μg ai/ml
Captevate 68 WDG	Captan and Fenhexamid	1450 μg ai/ml	2176 μg ai/ml
Rovral	Iprodione	450 μg ai/ml	600 μg ai/ml
Iprodione 4L AG	Iprodione	600 μg ai/ml	1500 μg ai/ml
Vangard 75WG	Cyprodinil	562 μg ai/ml	1124 μg ai/ml
Switch 62.5 WDG	Cyprodinil and Fludioxonil	3433 μg ai/ml	4370 μg ai/ml
Topsin-M	Thiophanate methyl	210 μg ai/ml	840 μg ai/ml

ai/ml = active ingredient per milliliter. Captan (N-Trichloromethylthio-4-cyclohexene-1, 2-dicraboxamidie); Fenhexamid (N-(2,3-dichloro-4-hydroxyphenyl)-1-methyl-cyclohexane carboxamide); Iprodione (3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidnecarboxamide); Cyprodinil (4-cyclopropyl-6-methyl-N-phenyl-pyrimidinamine); Fludioxonil (4-(2,2-difluro-1,3-bensodioxol-4-yl)-1H-pyrrole-3-carbonitrile) Thiophanate methyl (dimethyl [(1,2-phenylene)-bis(iminocarbonothioyl)]bis[carbamate]). (Captan Pro 50WP, Captan 80 WDG, Elevate, Captevate 68 WDG; Arysta Lifescience, 100 First Street, Suite 1700, San Francisco, CA 94105, USA) (Rovral; Bayer Crop Science, Crop Protection Division, 2 T.W. Alexander Drive, RTP, North Carolina 27709, USA) (Iprodione 4L AG, Micro Flo Company, LLC, P.O. Box 772099, Memphis, TN 38117, USA) (Vangard 75WG; Switch 62.5 WDG; Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419, USA) (Topsin-M 70 WDG; Cerezagri-Nisso LLC 630 Freedom Business Center Suite 402, King of Prussia, PA 19406, USA).