Effect of acidic electrolyzed water on the viability of bacterial and fungal plant pathogens and on bacterial spot disease of tomato

P.A. Abbasi and G. Lazarovits

Abstract: Acidic electrolyzed water (AEW), known to have germicidal activity, was obtained after electrolysis of 0.045% aqueous solution of sodium chloride. Freshly prepared AEW (pH 2.3–2.6, oxidation–reduction potential 1007–1025 mV, and free active chlorine concentration 27–35 ppm) was tested in vitro and (or) on tomato foliage and seed surfaces for its effects on the viability of plant pathogen propagules that could be potential seed contaminants. Foliar sprays of AEW were tested against bacterial spot disease of tomato under greenhouse and field conditions. The viability of propagules of *Xanthomonas campestris* pv. *vesicatoria* (bacterial spot pathogen), *Streptomyces scabies* (potato scab pathogen), and *Fusarium oxysporum* f.sp. *lycopersici* (root rot pathogen) was significantly reduced 4–8 log units within 2 min of exposure to AEW. Immersion of tomato seed from infected fruit in AEW for 1 and 3 min significantly reduced the populations of *X. campestris* pv. *vesicatoria* from the surface of the seed without affecting seed germination. Foliar sprays of AEW reduced *X. campestris* pv. *vesicatoria* populations and leaf spot severity on tomato foliage in the greenhouse. In the field, multiple sprays of AEW consistently reduced bacterial spot severity on tomato foliage. Disease incidence and severity was also reduced on fruit, but only in 2003. Fruit yield was either enhanced or not affected by the AEW sprays. These results indicate a potential use of AEW as a seed surface disinfectant or contact bactericide.

Key words: electrolyzed oxidizing water, seed disinfectant, foliar sprays, bacterial spot control.

Résumé : L'eau d'électrolyse acide (EEA), connue pour ses activités germicides, a été obtenue par électrolyse d'une solution aqueuse de chlorure de sodium à 0,045 %. L'EEA fraîchement préparée (pH 2,3–2,6, potentiel d'oxydation–réduction 1007–1025 mV et concentration de chlore libre actif de 27–35 ppm) a été testée in vitro et (ou) sur le feuillage de tomate et sur la surface des graines, quant à son effet sur la viabilité de propagules de pathogènes de plantes qui pourraient potentiellement contaminer les graines. Une vaporisation foliaire de EEA a été testée contre la bactériose de la tomate en serre et sur le terrain. La viabilité de propagules de *Xanthomonas campestris* pv. *vesicatoria* (pathogène de la bactériose), *Streptomyces scabies (*pathogène de la gale commune de la pomme de terre) et *Fusarium oxysporum* f.sp. *lycopersici* (pathogène de la maladie de la pourriture des racines) a été significativement réduite de 4–8 unités logarithmiques suite à une exposition de 2 minutes à l'EEA. L'immersion de graines de tomates provenant de fruits infectés dans l'EEA pendant 1 et 3 minutes a significativement réduit la population de *X. campestris* pv. *vesicatoria* présente à la surface des graines, sans affecter la germination. La vaporisation foliaire de EEA a réduit la population de *X. campestris* pv. *vesicatoria* et la sévérité de la bactériose du feuillage des tomates en serre. Sur le terrain, des vaporisations multiples de EEA ont régulièrement réduit la sévérité de la bactériose du feuillage des tomates. L'incidence et la sévérité de la maladie a aussi été réduite sur le fruit, mais en 2003 seulement. La récolte de fruits a été soit augmentée, soit non affectée par la vaporisation de EEA. Ces résultats indiquent que l'EEA pourrait être potentiellement utilizée comme désinfectant de la surface des graines ou comme bactéricide de contact.

Mots clés : eau d'électrolyse acide, désinfectant des graines, vaporisation foliaire, lutte à la bactériose.

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Introduction

Acidic electrolyzed water (AEW) or electrolyzed oxidizing water has gained significant attention from the food, medical, and agricultural industries for use as a sanitizing agent (Izumi 1999; Kim et al. 2000*a*). AEW is prepared by the electrolysis of a dilute aqueous solution of sodium or potassium chloride in an electrolysis chamber of a water ionizer where anode and cathode electrodes are separated by a nonselective membrane (Kim et al. 2000*b*). The AEW is formed at the anode, whereas alkaline or electrolyzed reducing water is produced at the cathode. AEW has low pH, high oxidation–reduction potential (ORP), and contains hypochlorous acid, which is a weak acid but a very effective sanitizer. The electrolysis process also leads to the generation of reactive oxygen species and toxic radicals such as O^-, Cl^-, and OH^- in the AEW, which contribute to its bactericidal (Kim et al. 2000*a*;

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Kiura et al. 2002; Venkitanarayanan et al. 1999) and fungicidal (Buck et al. 2002) properties.

AEW has been shown to be effective for disinfecting food and agricultural products and sanitizing food preparation surfaces and hospital equipment (Kim et al. 2000*a*; Koseki et al. 2004; Venkitanarayanan et al. 1999; Wang et al. 2004). The use of AEW as a disease control agent in the agriculture and horticulture industries needs further testing, especially in the field and as a wash for stored fruit, vegetables, and cuttings. AEW has been shown to control powdery mildew on Gerbera daisy in the greenhouse (Mueller et al. 2003) and to reduce lesion development by *Botrytis cinerea* on geranium leaf disks (Buck et al. 2002). Foliar sprays of AEW were generally safe to foliage of a wide variety of bedding plants grown under greenhouse conditions (Buck et al. 2003). Fungicidal effectiveness of AEW was also demonstrated against post-harvest brown rot of peach, where it delayed the development of symptoms on the fruit (Al-Haq et al. 2001). Treatment with AEW of soil and seed containing *Tilletia indica*, the causal agent of Karnal bunt of wheat, improved detection of this pathogen by stimulating germination of teliospores and by eliminating bacterial and fungal contaminants (Bonde et al. 1999, 2003). The bactericidal activity of AEW has also been tested on seeds and sprouts (Bari et al. 2003*a*; Kim et al. 2003) and tomatoes (Bari et al. 2003*b*). Based on its properties, AEW may be an attractive alternative to contact pesticides because of its reduced risk to workers and the environment. Thus, AEW may have potential for use as a contact bactericide on foliar plant parts and as a seed surface disinfectant.

Xanthomonas campestris pv. *vesicatoria*, causal agent of bacterial spot of tomato, causes necrotic lesions on leaves, stems, and fruit, and reduces yield and fruit quality, leading to serious economic losses to growers. Copper-based bactericides (Kousik and Ritchie 1996) against this disease in the greenhouse production phase and in the field are available to growers in Canada, but there is a growing concern regarding the effect of these pesticides on the environment, their phytotoxicity, and the potential development of bacterial resistance.

The objectives of the study were to determine (*i*) the viability of propagules of plant pathogenic organisms, such as *X. campestris* pv. *vesicatoria*, *Streptomyces scabies*, and *Fusarium oxysporum* f.sp. *lycopersici*, after exposure to AEW; (*ii*) the efficacy of AEW as a seed treatment to eliminate populations of *X. campestris* pv. *vesicatoria* from infested tomato seed surfaces; (*iii*) the efficacy of AEW as foliar sprays in reducing pathogen population and leaf spot severity from foliage of greenhouse-grown tomato plants; and (*iv*) the effects of multiple sprays of AEW on bacterial spot disease and fruit yield in the field-grown tomatoes.

Materials and methods

Plant material and microorganisms

Tomato (*Lycopersicon esculentum* Mill. 'Bonny Best') seeds were purchased from OSC Seeds (Waterloo, Ont., Canada). Seedlings were grown as described below and were used in all greenhouse tests. Tomato seedlings of the commercial cultivar 'H9478', grown in a commercial plant growth mix in plug trays and supplied by H.J. Heinz (Leamington, Ont., Canada), were used in two greenhouse experiments and in both years of field trials. For in vitro tests, seeds were extracted from fruit of the processing tomato variety 'H9478' showing bacterial spot symptoms. These fruit were harvested from the field-grown plants during the 2002 season. The seeds were then air-dried in a laminar flow hood for 48 h and stored in a paper envelope at 24 °C until further use.

The bacterial culture of *X. campestris* pv. *vesicatoria* (Doidge) Dye strain DC 93-1 was provided by Dr. Diane Cuppels, Agriculture and Agri-Food Canada, London, Ont. The strains of *S. scabies* (Thaxter) Lambert & Loria and *F. oxysporum* Schlecht f.sp. *lycopersici* (Sacc.) Synder & Hansen used in this study were from the culture collection of Dr. George Lazarovits.

Preparation of electrolyzed water

AEW and alkaline electrolyzed water (alkaline EW) were prepared with a counter-top water ionizer (model BTM 3000; BION-TECH Co. Ltd., Jongro-Ku, Seoul, Korea) by electrolysis of a diluted aqueous solution of sodium chloride (Sigma Chemical Co., St. Louis, Mo.). The 0.045% aqueous solution of sodium chloride (1.8 g in 4 L deionized water) was added to both the anode and cathode chambers (2 L in each chamber) of the ionizer. Electrolysis was performed for 25 min, and AEW and alkaline EW were collected from anode and cathode, respectively. The freshly prepared water was analyzed for pH with a pH meter (model SB 20; VWR, Mississauga, Ont.) and for ORP with a hand-held redox meter (Orion Research Inc., Beverly, Mass.). The concentration of free active chlorine in the AEW was measured by the DPD (*N,N*-diethyl-*p*-phenylenediamine) colorimetric method with a portable spectrophotometer (model DR/2400; Hach Company, Loveland, Col.). Each experiment was performed with freshly prepared electrolyzed water.

Growth, propagation, and treatments of bacteria and fungi

Xanthomonas campestris pv. *vesicatoria* DC 93-1 was grown overnight in 50 mL of autoclaved glucose-free nutrient broth yeast extract (NBY; Difco Laboratories, Detroit, Mich.) on an orbital shaker (150 r/min; 12 h). Bacterial cells were collected by centrifuging at 10 000 r/min (12 100*g*) for 15 min, resuspended in sterile saline solution (0.85% sodium chloride) to a concentration of about 10^8 colony-forming units (CFU) mL^{-1} (0.15 absorbance at 600 nm; model Cary 50 Spectrophotometer, Varian Instruments, Walnut Creek, Calif.). Spores of *S. scabies* were obtained from cultures grown on yeast – malt extract agar medium for 7–10 days by scraping the agar surface with a sterile wide loop. The spores were washed twice with 10 mL of sterile distilled water by centrifugation at 10 000 r/min (12 100*g*) for 10 min. *Fusarium oxysporum* f.sp. *lycopersici* spores were grown in 50 mL of potato dextrose (PD) broth (Difco) in a 250 mL Erlenmeyer flask. The PD broth flask was inoculated with 3–4 plugs of a 1-week-old culture of *F. oxysporum* f.sp. *lycopersici* grown on PD agar (Difco) medium and was placed in a shaker at 150 r/min for 4–5 days at 24 °C. Both of the spore suspensions were filtered through several layers of sterile cheesecloth and diluted to 10^8 spores mL^{-1} (10^6 spores mL^{-1} for *F. oxysporum* f.sp. *lycopersici*) with sterile water after counting with a haemocytometer. Bacterial cells or fungal spores were treated with AEW or sterile water for 0.5, 2, 5,

and 10 min by adding 1 mL of cell or spore suspension to 9 mL of the treatment solution in 15 mL sterile conical tubes (three replicate tubes per time period). The contents of the each tube were mixed individually by manually shaking and inverting the tubes. Tubes were kept on a rack on the laboratory bench at 24 °C until the treatment time. Immediately before the end of the treatment time, tubes were vortexed for 10–15 s, and serial dilutions of the treated cell or spore suspensions were made in sterile saline solution by transferring 1 mL of the treated solution to 9 mL of saline. Aliquots (100 μL) from serial dilutions (4–7 for the water controls and 1–4 for the AEW treatments) were plated on each of the three plates per dilution. Dilutions of *X. campestris* pv. *vesicatoria* were plated onto NBY agar medium (Vidaver 1967), *S. scabies* onto streptomyces semi-selective medium (Conn et al. 1998), and *F. oxysporum* f.sp. *lycopersici* onto PD agar medium. Plates were kept in the dark in an incubator set at 24 °C. Colonies of *X. campestris* pv. *vesicatoria* were counted after 3 days, *S. scabies* after 7 days, and *F. oxysporum* f.sp. *lycopersici* after 4 days of incubation. Experiments were repeated at least once.

Disinfestation of tomato seed with electrolyzed water

In preliminary experiments, the alkaline EW was not found to have any effect on the bacteria, and further tests were discontinued. Air-dried tomato seeds of uniform size and shape obtained from fruit infected with bacterial spot disease were treated by submerging 30 seeds per replicate (three replicates per treatment) in 5 mL of sterile water or AEW in 15 mL conical tubes. Seeds were manually shaken for 1, 3, 5, or 10 min and transferred to sterile Eppendorf tubes. Ten treated seeds were manually homogenized in 10 mL of sterile water in polyethylene stomacher bags (Seward Medical, London, UK) by applying pressure. Treatment solution and seed homogenate were diluted serially and plated (100 μL per plate and three plates per dilution) onto NBY agar and CKTM (Sijam et al. 1991; an agar medium for isolation and identification of *X. campestris* pv. *vesicatoria* from seed) media to determine external and internal *X. campestris* pv. *vesicatoria* CFUs, respectively. In a separate set of experiments, air-dried infested tomato seeds (30 seeds per replicate and three replicates per treatment) were treated with AEW or sterile water in Eppendorf tubes for 3 min or for 1 min three times (3 × 1 min). The 3 × 1 min treatments were exposed to the solutions for 1 min and immediately removed and placed in new solutions for a second and third time for 1 min each. Seeds were manually shaken, transferred to new Eppendorf tubes, and washed with 1 mL of sterile water by vortexing. External (treatment solution/wash water) and internal (seed homogenate) CFU of *X. campestris* pv. *vesicatoria* were determined as described above. In the case of treatment solution and wash water, the higher dilutions (5–7) of the water controls and the lower dilutions (1–3) of the AEW treatments, and in case of seed homogenate, the higher dilutions (5–7) of the water control and AEW treatments were plated (100 μL per plate and three plates per dilution). Germination of the treated seed was determined on water agar medium (10 g agar L^{-1} deionized water). Ten treated seeds were transferred to water agar medium and each seed was covered with 1–2 drops of sterile deionized water and incubated in the dark at 24 °C for 1 week to determine rates of germination. All the experiments were repeated once.

Foliar applications of AEW, inoculations of *X. campestris* pv. *vesicatoria*, and determination of leaf spot severity and the pathogen populations in the greenhouse-grown tomato plants

The efficacy of AEW as foliar sprays to suppress populations of bacterial spot pathogen and leaf spot severity in tomato foliage was determined in a greenhouse. The first set of experiments was carried out with 'Bonny Best' tomato seedlings and the second set with commercially produced 'H9478' transplants. Seeds of 'Bonny Best' were germinated in 288-cell-plug flats filled with a commercial peat-based mix (Pro-Mix BX®; Premier Horticulture Ltd.; Rivière-du-Loup, Que., Canada). Two-week-old seedlings of 'Bonny Best' and commercially produced 'H9478' seedlings were transplanted in 10 cm plastic pots (one seedling per pot; five replicate pots per treatment) in the same mix and were fertilized with Nutricote® controlled slow-release fertilizer 14:14:14 (N–P–K) (Plant Products Co. Ltd., Brampton, Ont.) at the rate of 5–7 granules per pot. Pots were maintained in a greenhouse at 22–24 °C under a combination of sunlight and supplemental lighting (225 μE m^{-2} s^{-1} for 12 h day^{-1}) for 2 additional weeks in a completely randomized design. Plants were saturated with freshly prepared AEW (pH 2.6) by spraying with a hand-held sprayer 48 h before or after inoculations. Control plants were sprayed with tap water. Plants received one (weekly) or two (biweekly) sprays of AEW per week. In the second set of experiments with 'H9478' transplants, a single foliar spray of acibenzolar-*S*-methyl (Actigard 50WG; 30 mg a.i. L^{-1}; Syngenta, Greensboro, N.C.), an inducer of systemic acquired resistance (Lawton et al. 1996), was included as a positive control comparing it with once- or twice-a-week sprays of AEW. Plants were sprayed to runoff with an aqueous solution of Actigard 48 h prior to inoculations. For inoculations, *X. campestris* pv. *vesicatoria* DC 93-1 was grown in autoclaved glucose-free NBY broth (100 mL) on an orbital shaker (150 r/min; 24 h). Bacterial cells were collected by centrifuging at 10 000 r/min (12 100*g*) for 15 min, resuspended in tap water, and concentration adjusted to 10^8 CFU mL^{-1} with a spectrophotometer, as described above. Plants were inoculated with the bacterial suspension 48 h before or after initial applications of AEW to the foliar tissue. A noninoculated control group was also included and kept at a corner of the greenhouse bench. Total bacterial counts from the foliage of the noninoculated group averaged 4.58 log CFU (g tissue)$^{-1}$. Two weeks after initial inoculation, plants were rated visually for severity of disease development on a scale of 1–5 (Aldahmani et al. 2005): 1 = symptomless, 2 = one to five lesions per leaf or leaflet, 3 = many lesions and some coalesced lesions, 4 = coalesced lesions and some necrotic leaves or leaflets, and 5 = dead leaves or leaflets. The populations of *X. campestris* pv. *vesicatoria* were determined from each of five replicate plants by removing foliage, excluding the two lower leaves, and were collected into polyethylene stomacher bags (Seward Medical; London, UK). Fresh mass of the harvested foliage was determined and an equivalent volume of sterile saline was added. Pressure was applied manually to homogenize the tissue. Serial dilutions were plated onto CKTM

and NBY media and incubated in the dark at 24 °C for 4 days. Colonies resembling *X. campestris* pv. *vesicatoria* were counted and expressed as log CFU per gram of foliar tissue. All the experiments were repeated.

Foliar applications of AEW, inoculations of *X. campestris* pv. *vesicatoria*, and determination of bacterial spot disease and fruit yield in the field-grown tomatoes

Field experiments were conducted in 2003 and 2004 in London, Ont., at the Agriculture and Agri-Food Canada research farm on an alkaline loam soil (pH 8.0) with a moderate level of organic carbon. Tomato 'H9478' seedlings, grown in a commercial plant growth mix in plug trays, were supplied by H.J. Heinz. Standard cultivation practices were carried out (OMAF 2002). Six-week-old tomato seedlings were transplanted 0.45 m apart in single rows on 1.2 m centres with a planter. Plants were fertilized with the plant starter Plant Prod® (10–52–10; Plant Products Co. Ltd., Brampton, Ont.) during planting; each plant received approximately 150–180 mL of 0.5% starter fertilizer. Experimental plots (one 4.5 m row or replicate and 15 plants per row) were established in a randomized complete block design with four replicates per treatment. Treated rows were separated by a border row. Foliar sprays of aqueous solutions of Actigard (30 mg a.i. L^{-1}) were used as a positive control. Freshly made AEW or Actigard was sprayed onto tomato foliage once or twice a week throughout the growing season, starting 2 weeks after transplanting. Treatments were applied using a hand-held compressed-air sprayer (RL Flo-Master®; capacity 7.6 L) (Root–Lowell Manufacturing Co., Lowell, Mich.) at 30 ± 5 psi (1 pound-force per square inch (psi) = 6.895 kPa) with an adjustable cone nozzle. A total of 6 (7 in 2004) weekly and 12 (14 in 2004) biweekly sprays were applied. At each spray, approximately 15–25 mL of solution was sprayed onto each plant, depending on the plant age. Control plots were sprayed with tap water. The inoculum of *X. campestris* pv. *vesicatoria* DC 93-1 for inoculation was produced as described above. In 2003, tomato plots were inoculated twice. First inoculation was made onto the border rows 3 days after the first spray treatment, and bacterial spot was allowed to spread naturally to the treated plants. The 2003 growing season was dry during June and July and as a result, disease pressure was low on tomatoes. The tomato plots were reinoculated, with the second inoculation made directly onto the treated rows. Plots were also irrigated by overhead sprinklers for 4 h, 2 days after the second inoculation. Planting in the 2004 season was delayed almost 2 weeks because of heavy rains, and inoculations were made directly onto the treated plots. In both years, all plants in a plot were rated six times for foliar bacterial spot severity (2003 — 24 and 03 July; 07, 14, 18, and 22 August; 2004 — 30 July; 06, 10, 13, 20, and 27 August), using a modified Horsfall and Barrett (1945) disease rating scale (1 = no disease to 12 = 100% disease). The bacterial spot disease severity (% affected tissue) data for each treatment were converted to area under the disease progressive curve using the midpoints of each rating (Campbell and Madden 1990; Shanner and Finney 1977). All fruits from 10 plants in both years, in the middle of each plot (four replicate plots per treatment), were harvested when approximately 80% of the tomato fruit were ripe (light red to red). In both years, tomatoes were harvested on 5 September. Fruits were analyzed for the incidence of bacterial spot, and a fruit with one or more bacterial spot lesions was considered diseased. Incidence of bacterial spot disease was expressed as a percentage of total harvested fruit on a mass basis. Tomatoes free of disease symptoms were sorted as healthy fruit and weighed. Total yields were determined as well. Individual fruit was analyzed for bacterial spot severity by counting the number of lesions on each fruit.

Data analyses

All the experiments were repeated at least once. Because the repeat data in laboratory and greenhouse experiments showed similar trends and had homogeneous variances, they were pooled for statistical analyses. All disease severity data were analyzed according to the Kruskal–Wallis nonparametric test statistics. Analysis of variance was performed and means were separated according to the Student–Newman–Keuls test. Disease severity data on two or more factors and field data were subjected to analysis of variance, using the general linear model procedure of MINITAB, and means were separated according to Tukey's procedure. Greenhouse data on bacterial populations in tomato foliage were transformed to the logarithmic scale and then subjected to analysis of variance with MINITAB statistical software (Version 13, Minitab Inc., State College, Penn.), and means were separated according to Fisher's protected least significant difference (LSD) test.

Results

Properties of AEW

The pH, ORP, and free active chlorine concentration of the AEW at 24 ± 1 °C prepared from aqueous solution of 0.045% sodium chloride ranged from 2.3–2.6, 1007–1025 mV, and 27–35 ppm, respectively. The pH and ORP of AEW did not change, whereas free active chlorine concentration reduced to 9.5–11 ppm within 4 h.

Reduction in viability of propagules of plant pathogens after exposure to AEW

Treatment with AEW significantly reduced or eliminated viability of propagules of plant pathogenic organisms, such as *X. campestris* pv. *vesicatoria*, *S. scabies*, and *F. oxysporum* f.sp. *lycopersici* (Table 1). Bacterial cells of *X. campestris* pv. *vesicatoria* and spores of *S. scabies* and *F. oxysporum* f.sp. *lycopersici* were dead after a 2 min exposure in AEW (Table 1). The cells of *X. campestris* pv. *vesicatoria* and spores of *S. scabies* and *F. oxysporum* f.sp. *lycopersici* were also killed after just a 30 s exposure in AEW (data not shown).

Disinfestation of tomato seed with electrolyzed water

Treating tomato seed from fruit infected with bacterial spot pathogen by soaking in AEW for 1, 3, 5, and 10 min killed the bacteria from the seed surface after 1 and 3 min of exposure (Table 2). However, the internal bacterial CFU from the seed homogenate were not affected by the AEW treatments (Table 2). The rate of germination of the treated seed on water agar was not affected by any treatment (Table 2). All treatments averaged 86% germination or higher.

In subsequent experiments, treatment of the infected tomato seed with AEW for 3 min or three times for 1 min each

Table 1. Viability of *Xanthomonas campestris* pv. *vesicatoria*, *Streptomyces scabies*, and *Fusarium oxysporum* f.sp. *lycopersici* propagules after exposure to acidic electrolyzed water (AEW) for various times.

		Pathogen propagules (CFU mL^{-1})		
Treatment	Time (min)	*X. campestris* pv. *vesicatoria*	*S. scabies*	*F. oxysporum* f.sp. *lycopersici*
Water	2	17±2a	25±3a	38±3a
AEW	2	0±0b	0±0b	0±0b
Water	5	10±0.6a	21±3a	44±3a
AEW	5	0±0b	0±0b	0±0b
Water	10	11±0.2a	29±2a	37±2a
AEW	10	0±0b	0±0b	0±0b

Note: Means ± standard error are the average of two experiments (n=6). Means followed by the same letter do not differ significantly according to Fisher's protected least significant difference test at $P \leq 0.05$. *Xanthomonas campestris* colonies were counted 4 days after plating onto nutrient broth yeast extract agar medium, *S. scabies* colonies were counted 1 week after plating onto streptomyces semi-selective medium, and *F. oxysporum* colonies were counted 3 days after plating onto potato dextrose agar medium. The CFU mL^{-1} for water treatments are $\times 10^8$ for *X. campestris*, $\times 10^6$ for *S. scabies*, and $\times 10^4$ for *F. oxysporum*. No colonies were detected for AEW treatments with any of the lower dilutions (1–4) plated.

Table 2. Effects of seed treatment with acidic electrolyzed water (AEW) on external and internal populations of *Xanthomonas campestris* pv. *vesicatoria* in the infected tomato 'H9478' seeds and on seed germination.

		X. campestris pv. *vesicatoria* populations (10^6 CFU mL^{-1})*		
Seed treatment	Time (min)	External†	Internal	Seed germination (%)‡
Water	1	208±61a	71±13a	100±0a
AEW	1	0±0b	49±10a	94±6a
Water	3	338±77a	51±9a	94±6a
AEW	3	0±0b	39±10a	100±0a
Water	5	—	38±4a	92±8a
AEW	5	—	65±13a	100±0a
Water	10	—	24±4a	90±6a
AEW	10	—	14±4a	86±6a

Note: Values are the means ± standard error and are the average of two experiments (n=6). Means followed by the same letter do not differ significantly, according to Fisher's protected least significant difference test at $P \leq 0.05$.

*Colonies resembling *X. campestris* on nutrient broth yeast extract agar or CKTM agar medium from external (treatment solution) and internal (seed homogenate) seed surfaces.

†No *X. campestris* colonies were detected for AEW treatments with any of the lower dilutions (1–3) plated. —, not determined.

‡Determined on water agar.

Table 3. Effect of seed treatment with acidic electrolyzed water (AEW) on populations of *Xanthomonas campestris* pv. *vesicatoria* of infected tomato 'H9478' seeds.

		Population of *X. campestris* pv. *vesicatoria* (10^6 CFU mL^{-1})*		
Seed treatment†	Time (min)	Treatment solution‡	Wash water‡	Seed homogenate
Water	3	48±5a	65±6a	64±28a
Water	1 (3 times)	21±2a	44±3a	68±28a
AEW	3	0±0b	0±0b	2±2b
AEW	1 (3 times)	0±0b	0±0b	0.1±0.1b

Note: Values are the means ± standard error and are the average of two experiments (n=6). Means followed by the same letter do not differ significantly according to Fisher's protected least significant difference test at $P \leq 0.05$.

*Colonies resembling *X. campestris* on nutrient broth yeast extract or CKTM agar medium from external (treatment solution and wash water) and internal (seed homogenate) seed surfaces.

†Seeds were immersed in AEW or water for 3 min or three times for 1 min each.

‡No *X. campestris* colonies were detected for AEW treatments with any of the lower dilutions (1–3) plated.

effectively reduced bacteria from the seed surface to below the detection level (Table 3). The number of bacteria from seed homogenate (internal tissue) was also reduced to just detectable levels by both AEW treatments (Table 3).

Effect of foliar sprays of AEW on *X. campestris* pv. *vesicatoria* populations and leaf spot severity in the greenhouse-grown tomato plants

Although the number of plants per replication was low, the leaf spot lesions were evenly distributed over the entire plant and variation was minimal among the replicates. A single spray application of AEW 48 h after inoculation with bacterial spot pathogen reduced the populations of *X. campestris* pv. *vesicatoria* in tomato foliage compared with that of the controls (Table 4). A second spray application 4 days after the first spray did not further reduce the bacterial population on the AEW-treated foliage. Bacterial leaf spot severity was reduced in tomato plants receiving one or two spray applications of AEW compared with those receiving water sprays (Table 4). In the second set of experiments, the effects from weekly and biweekly sprays of AEW on tomato plants 48 h before inoculation were compared with those from a single spray of Actigard. Compared with the nontreated control, the populations of *X. campestris* pv. *vesicatoria* were reduced and the severity of bacterial spot moderately suppressed, following either weekly or biweekly sprays of AEW (Table 5). A single foliar application of the plant activator Actigard at 30 mg a.i. L^{-1} water was comparable to AEW in

Table 4. Effect of foliar sprays of acidic electrolyzed water (AEW) on the populations of *Xanthomonas campestris* pv. *vesicatoria* and on leaf spot severity on tomato 'Bonny Best' foliage.

Spray treatment*	Total no. sprays	*X. campestris* pv. *vesicatoria* population (log CFU (g tissue)$^{-1}$)†	Leaf spot severity‡
Water	1	9.0±0.1a	2.9±0.2a
Water	2	9.1±0.1a	3.0±0.2a
AEW	1	8.3±0.2b	2.2±0.2b
AEW	2	8.3±0.2b	2.2±0.1b

Note: Values are the means ± standard error and are the average of two experiments (n=10). Means followed by the same letter do not differ significantly according to Fisher's protected least significant difference test at $P \leq 0.05$.

*Plants were sprayed 48 h after *X. campestris* inoculations.

†In all leaves from five replicate plants per treatment per experiment.

‡Plants were rated 2 weeks after inoculation based on a 1–5 rating scale in which 1 = healthy and 5 = dead leaves or leaflets.

Table 5. Effect of weekly and biweekly foliar sprays of acidic electrolyzed water (AEW) on the populations of *Xanthomonas campestris* pv. *vesicatoria* and on leaf spot severity on tomato 'H9478' foliage.

Spray treatment*	Sprays per week	*X. campestris* pv. *vesicatoria* population (log CFU (g tissue)$^{-1}$)†	Leaf spot severity‡
Control	—	7.9±0.1a	2.6±0.3a
AEW	1	7.3±0.2b	2.2±0.2b
AEW	2	7.1±0.2b	2.2±0.2b
Actigard	1	7.1±0.1b	1.6±0.2c

Note: Values are the means ± standard error and are the average of two experiments (n = 10). Means followed by the same letter do not differ significantly according to Fisher's protected least significant difference test at $P \leq 0.05$.

*Plants were sprayed with AEW once or twice a week or with Actigard (acibenzolar-*S*-methyl) at 30 mg a.i. L^{-1} 48 h before *X. campestris* inoculations.

†In all leaves from five replicate plants per treatment per experiment.

‡Plants were rated 2 weeks after inoculation based on a 1–5 rating scale in which 1 = healthy and 5 = dead leaves or leaflets.

reducing the bacterial populations but better than AEW in reducing bacterial spot severity (Table 5).

Effect of foliar sprays of AEW on bacterial leaf spot disease and fruit yield of field-grown tomatoes

Bacterial spot was the predominant disease on tomatoes in both 2003 and 2004. The 2003 growing season was comparatively dry during June and July, and as a result, disease pressure was low earlier on the tomato plots. Disease spread quickly when the treated plots were reinoculated directly. Planting in the 2004 season was delayed almost 2 weeks because of heavy rains, and inoculations were made directly onto the treated plots. Foliar sprays of Actigard and AEW significantly reduced bacterial spot disease severity on tomato foliage compared with the water control in both years (Table 6). Sprays applied once or twice a week were equally effective. In 2003, both weekly and biweekly sprays of Actigard and AEW significantly reduced the incidence of bacterial spot on tomato fruit on an average by 65%–67% and the number of spots per fruit by 76%–79%. The effect of disease reduction on the fruit was not seen in 2004 by any treatment (Table 6).

In 2003, the relative proportion of healthy fruit significantly increased (47%–69% on average) by both weekly and biweekly sprays of Actigard and AEW compared with that of the water control (Table 7). In 2004, both treatments also appeared to increase healthy fruit but the effect was not statistically significant. Compared with the control, total fruit yield was significantly increased by both weekly and biweekly AEW sprays in 2003 and by only biweekly AEW sprays in 2004 (Table 7). Multiple foliar sprays of AEW did not cause any phytotoxic effects on the tomato foliage.

Discussion

The need for reduced-risk technologies for management of plant pathogens is increasing as concerns on the impact of pesticides on environment and human health become a concern to the public. The electro-chemically generated AEW could be an environmentally safer pathogen management tool as it has no known residual effects. It is also easy to make and relatively inexpensive. In this study, we further confirm the antimicrobial effects of AEW against bacteria and fungal spores. AEW prepared from a diluted aqueous solution (0.045%) of sodium chloride rapidly killed the bacteria *X. campestris* pv. *vesicatoria* and *S. scabies*. Hotta et al. (1994) also reported similar results on the loss of viability of *Streptomyces* spores after treatment with the AEW, although they used 0.1% sodium chloride solution to prepare acidic water in their study. Buck et al. (2002) reported that a 30 s or less exposure time in AEW prepared from 2 mol L^{-1} salt solution is enough to kill thin-walled spores of *Botrytis* sp. and *Monilinia* sp., whereas the germination of thicker-walled and pigmented fungal spores is significantly reduced after a 2 min or longer exposure time. However, in our study, the germination of propagules of a root rot pathogen of tomato (*F. oxysporum* f.sp. *lycopersici*) was reduced within 2 min of exposure to AEW prepared from only 8 mmol L^{-1} salt solution.

Wang (2004) reported that dipping scabby tubers in an acid fraction of electrolyzed water for 10 min reduces the population of *Streptomyces* spp. without affecting sprouting. Thus, AEW may be very useful for eliminating inoculum from tuber surfaces, as visually clean seed tubers have been found to carry pathogenic *Streptomyces* spp. (Wang 2004; Wang and Lazarovits 2004, 2005). Although treatment of seed tubers may not be enough to protect new daughter tubers from getting infected from the inoculum present in soil, cultivation of AEW-treated healthy tubers may be useful in soils not infested with pathogenic *Streptomyces* spp. Disinfesting tubers with AEW may also minimize losses during storage by reducing other sensitive pathogenic microorganisms from the tuber surface.

The presence of any nonselective reducing agents and organic material in the soil may react with the free radicals in AEW, making its germicidal effects ineffective (Oomori et al. 2000). It has been demonstrated that the presence of material containing proteins, amino acids, lipids, and minerals, etc., may interfere with the free available chlorine in the AEW, resulting in the reduced bactericidal activity of AEW (Oomori et al. 2000). Addition of 10% AEW (*v*/*m*) to a muck soil naturally infested with damping-off pathogens also did not

Table 6. Effect of weekly and biweekly foliar sprays of acidic electrolyzed water (AEW) on the bacterial spot severity on tomato foliage and incidence and severity on tomato fruit in the field.

		Foliar AUDPC[†]		Diseased fruit (%)[‡]		Spots per fruit[‡]	
Foliar sprays*	Sprays per week	2003	2004	2003	2004	2003	2004
Water	1	230±6a	371±67a	35±5a	31±2a	1.5±0.2a	1.1±0.2a
Water	2	188±9a	388±63a	37±5a	35±1a	1.9±0.2a	1.2±0.1a
Actigard	1	35±3b	160±50cd	11±3b	27±6a	0.3±0.1b	0.7±0.2a
Actigard	2	30±9b	122±11d	14±5b	22±1a	0.4±0.2b	0.6±0.0a
AEW	1	63±3b	235±43bc	11±1b	26±2a	0.4±0.2b	0.8±0.1a
AEW	2	87±8b	271±19b	13±3b	28±2a	0.4±0.1b	0.8±0.1a

Note: Values are the means ± standard error and are the average of four replicate plots per year. Means followed by the same letter in each column do not differ significantly, according to Fisher's protected least significant difference test at $P \leq 0.05$.

*All plants in a plot were sprayed with tap water, Actigard (acibenzolar-*S*-methyl, 30 mg a.i. L^{-1}), or AEW.

[†]Tomato plots were rated on a 1–12 rating scale in which 1 = no disease to 12 = 100% disease. AUDPC, area under the disease progressive curve.

[‡]Fruit was harvested from 10 plants from each of four replicate plots in each year.

Table 7. Effect of weekly and biweekly foliar sprays of acidic electrolyzed water (AEW) on the yield of healthy and total tomato fruit in the field.

		Healthy fruit (kg (10 plants)$^{-1}$)		Total fruit (kg (10 plants)$^{-1}$)	
Foliar sprays*	Sprays per week	2003	2004	2003	2004
Water	1	15.3±2.8b	12.4±1.5ab	24.9±0.7b	16.9±1.4ab
Water	2	15.6±1.3b	9.3±0.5b	24.1±2.4b	14.3±0.5b
Actigard	1	22.6±1.8a	11.6±2.3ab	26.0±2.4b	17.6±2.3ab
Actigard	2	23.0±1.0a	14.8±1.3a	27.6±0.9ab	18.9±1.4ab
AEW	1	27.9±0.8a	11.8±2.1ab	31.9±0.7a	16.5±2.5ab
AEW	2	24.3±4.0a	14.7±1.9a	30.7±2.5a	20.4±2.8a

Note: Values are the means ± standard error and are the average of four replicate plots for each year. Means followed by the same letter in each column do not differ significantly, according to Fisher's protected least significant difference test at $P \leq 0.05$.

*Tomato plots were sprayed with tap water, Actigard (acibenzolar-*S*-methyl, 30 mg a.i. L^{-1}), or AEW.

protect cucumber seedlings from damping-off (unpublished data). This may simply be due to inadequate concentration of AEW in the soil. In any case, soil application of AEW may not be practical. Thus, AEW will likely be useful for the reduction of relative simple organisms on the plant surfaces where there is not a lot of organic debris.

The populations of *X. campestris* pv. *vesicatoria* from the surface of the tomato seeds extracted from fruit infected with bacterial spot pathogen harvested from field-grown plants were effectively reduced by AEW to levels below the detection limit. The germination of the AEW-treated seeds and the untreated control seeds was similar. Internal bacterial CFU in the seed homogenate were not affected by the AEW, as there were no consistent significant differences. AEW must be in contact with bacteria in the seed for effectiveness. Seed treatment with AEW might have implications in the greenhouse industry in the production of pathogen-free and healthy transplants, particularly, for organic production systems where the use of conventional agro-chemicals is limited. Seeds harboring the pathogen represent the main source of primary inoculum for bacterial spot disease (Bashan et al. 1982). Although seed treatments can reduce the disease, total elimination of the pathogen from seed has not been accomplished so far (Bashan et al. 1982). Despite the use of pathogen-free transplants, bacterial spot still can spread in the field through airborne inoculum of the pathogen (McInnes et al. 1988; Pohronezny et al. 1990), therefore, additional preventive sprays may be required once the crop is in the field.

Foliar sprays of AEW reduced populations of *X. campestris* pv. *vesicatoria* and leaf spot severity on tomato foliage in the greenhouse-grown tomato plants without any symptoms of phytotoxicity. Multiple sprays were effective in reducing bacterial spot disease on tomato foliage and fruit (2003 only) in the field as well. None of the treatments, including the positive control (Actigard), reduced bacterial spot disease on fruit in 2004. Delayed planting because of heavy rains and high disease pressure because of direct inoculations of the treated plots may have been responsible for ineffectiveness of the treatments in 2004. It is also interesting to test foliar sprays of AEW prepared from higher concentrations of salt solutions. Since the activity of AEW is like a contact bactericide or fungicide, foliar sprays may work well if applied when the pathogen is on the surface or before establishment of infection. In a recent study, biweekly foliar sprays of AEW applied for 7 weeks on Gerbera daisy foliage in the greenhouse reduce powdery mildew (Mueller et al. 2003). AEW was also shown compatible with several fungi-

cides and insecticides in the same study. A couple of foliar sprays of AEW per week are generally safer towards a variety of bedding plants, however, three sprays per week cause a slight damage to some plants (Buck et al. 2003). We did not see any phytotoxic effects on the tomato foliage with one or two sprays of AEW per week, although AEW used in this study was prepared from 8 mmol L^{-1} NaCl solution compared with 2 mol L^{-1} salt solution used by Buck et al. (2003).

In summary, AEW reduced the viability of propagules of plant pathogenic organisms, such as *S. scabies, F. oxysporum* f.sp. *lycopersici*, and *X. campestris* pv. *vesicatoria*. AEW as a seed treatment reduced populations of *X. campestris* pv. *vesicatoria* from infested tomato seeds, and foliar sprays reduced *X. campestris* pv. *vesicatoria* population and moderately suppressed leaf spot severity on tomato foliage in the greenhouse and reduced bacterial spot disease on tomato foliage and fruit in the field. The field efficacy of AEW may be affected by high disease pressure and weather conditions, such as heavy rains. Thus, AEW may prove to be a reduced-risk, convenient, and economic way of disinfesting seeds, fruit, foliar plant parts, and plant cuttings.

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