# Utilizing Electrolyzed Water in Conjunction with Electrostatic Spray and Biocatalytic Protein Adjuvant to Inhibit the Growth of "Powdery Mildew" the Fungal Pathogen *Erysiphe Necator*

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## Introduction:

Field application of electrolyzed water (EW) has been and is being tested in conjunction with electrostatic spray technologies with the objective of providing relatively environmentally safe systems for treatment of various agricultural detriments. Given EW's capability to treat various microbial pathogens, efficacy against the spreading of certain fungal species is of interest for testing and review. Application strategies provided by electrostatic spray systems in conjunction with a biocatalytic protein solution adjuvant have been proposed to further enhance the effective coverage of EW.

EW is available in both anolyte and catholyte forms. Activate is an enhanced biocatalytic protein surfactant that lowers interfacial and surface tension of fluid solutions at low dilutions (25ppm) which has been tested with EW in other white papers as anadjuvant. On-Target Spray Systems of Mount Angel, OR have engineered a versatileand effective electrostatic spray system with diverse capabilities. Coupling EW, an On-Target electrostatic sprayer and Activate is proposed as a plausible method for inhibiting the growth of *Erysiphe Necator* in various agricultural systems. A simple experiment to initiate testing into a synergistic system will be explored within this paper.

## Methods:

*Erysiphe necator* samples were taken from a private vineyard near Sunnyside, WA from infected vines and grapes. Samples were preserved with inside plastic sample bags and transported to Bozeman, MT in a cooler. Sterile swabs wetted with deionized water were used to inoculate tryptic soy agar plates with *Erysiphe necator* colonies sampled from the same, viable grape cluster. The inoculated plates were placed into a Quincy Laboratories analog incubator for 36 hours at 25°C. Colonies of *Erysiphe necator* were identified and isolated onto 12 plates.

EW water was tested for efficacy with a Hach® Pocket Pro ORP tester and Hach® Pocket Pro pH tester. On-Target Spray Systems provided a single unit application apparatus, which was fitted with a Husky 4-gallon air compressor and calibrated with a flow rate of 5 psi. Fungal colony treatments included: 1) Sterile deionized $H_2O$ (control); 2) Anolyte EW (AEW); 3) AEW + Activate added at 25 ppm; and 4) Catholyte then anolyte EW water + Activate at 25ppm. The EW was provided at strength ready to use formulation.

Three of the plates were randomly selected with a random number generator for each of the 4 treatments and randomly assigned a treatment with a random number generator. Plates were then sprayed with the assigned treatments for 15 seconds at 30 inches. One of the three plates was selected for each the four treatments by random selection to have batch cultures prepared from.

Batch cultures were prepared and grown overnight in tryptic soy broth (TSB) at 25°C. Culture optical density for *Erysiphe Necator* was adjusted to 0.5 at $Ab_{565}$. One ml of cell suspension was added to 9.0 ml of the four different treatments and briefly vortexed. The cell suspensions were serially diluted (10-fold) and plated onto TSB agar (in triplicate at each dilution). Viable cell counts were determined after 48 hours of incubation at 25 °C. Fungicidal efficacy was calculated by comparing viable plate counts derived from the control treatment against the counts obtained from the treated cells.

## Results:

All data are expressed in terms of number of observable colonies per ml. For all four treatments, EW, EW+*Activate* and Catholyte then Anolyte+Activate proved to have significant fungicidal effects; i.e. there were no viable counts at the lowest dilution of $10^{-3}$ derived from inoculating the TSB agar with 1 ml of $10^{-2}$ dilution. While claims of complete kill efficacy is not possible, the theoretical maximum viable counts could not exceed $9 \times 10^2$, and therefore viability decreased by approximately six orders of magnitude.

Combo nomenclature in table 1 indicates an initial application of catholyte water followed immediately by anolyte water.

Table 1. All counts on a per ml basis

| Treatment | Replicate 1 | Replicate 2 | Replicate 3 |
|---|---|---|---|
| AEW | $<1.0 \times 10^3$ | $<1.0 \times 10^3$ | $<1.0 \times 10^3$ |
| AEW + Activate | $<1.0 \times 10^3$ | $<1.0 \times 10^3$ | $<1.0 \times 10^3$ |
| Combo + Activate | $<1.0 \times 10^3$ | $<1.0 \times 10^3$ | $<1.0 \times 10^3$ |
| Control | $6.1 \pm 0.3 \times 10^8$ | $4.8 \pm 0.6 \times 10^8$ | $3.2 \pm 0.7 \times 10^8$ |

## Discussion:

Results of the experiment displayed in Table 1 show significant inhibitory capacity of all treatment groups against that of the control of distilled water. As stated in the results, complete kill efficacy is not possible given the technique utilized but the treatment groups were six orders of magnitude greater than that of the control. No viable cultures were observed at the $10^3$ magnitude. No detrimental effects were observed between the different treatment groups. As such, utilization of either the combo + Activate or AEW + Activate would likely give better coverage given past findings on the properties of Activate (Goldfeld et al., 2015, Sather and Goldstein, 2016). Note that AEW is effective by itself but would be enhanced in terms of coverage and total surface area treated.

During the experimental design process, it should be noted that On-Target's spray apparatus timing was calculated based upon speeds given by the private vineyards chief applicator's estimated travel time through a vineyard using a full sized sprayer system. The systems are designed to spray variable heights of crops with effective coverage. Difficulties making direct comparisons between the single unit

experiment apparatus and in-field applications should be noted. Observations not quantifiable during the experimental process at this time would suggest, given the amount of water displaced throughout the experimental chamber, that less time could be allowed for spraying to allow for effective coverage.

While literature regarding the use of electrolyzed water is somewhat limited in quantity, several other papers exist which have derived similar results. Abbasi and Lazarovits (2006), concluded that AEW significantly decreased the presence of fungal spores from *Botrytis* sp. and the expression of the subsequent root rot that occurs with a 2 minute exposure time. They also found greatly reduced incidences of diseased fruit. A journal article that only presented a brief summary of findings in English (the rest being in Japanese) tested AEW against an untranslatable species of powdery mildew. They concluded that the AEW treatment had a significant inhibitory effect upon the proliferation of powdery mildew on cucumber plants (Schorner et al., 1999).

While the effectiveness of AEW, Activate and electrostatic sprayers was significant in the laboratory situation, field conditions may vary. Timing of application within the seasonal operations of various crops should be explored. It can be concluded that AEW with the complimentary combinations of an electrostatic spray system and the Activate adjuvant can provide an effective, environmentally low impact solution to treating *Erysiphe Necator* varieties of powdery mildew.

## References


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