Evaluation of Alternative Strategies for Management of Soilborne Plant Pathogens and Nematodes

Vijay K. Choppakatla, Sarah Budde-Rodriguez, and Ray Austin
SBrodriguez@BioSafeSystems.com

BioSafe Systems LLC, East Hartford, CT

Abstract:

Research into alternative methods to conventional soil fumigation has been increasing over the past decade due to growing concerns with fumigants' impact on human health, soil health, and the environment. One alternative strategy is the regenerative soils program being evaluated by BioSafe Systems, herewith referred to as BRSP. BRSP is an integrated approach aimed at reducing/controlling soil plant pathogen/nematode population and at improving the overall soil health by boosting beneficial soil microbiome and structure. The program sequentially applies biochemical (Peroxyacetic Acid; PAA) and *Bacillus*-based biological components immediately prior to planting and during crop growing season. Lab and greenhouse assays with these components against important soilborne pathogens/nematodes such as *Fusarium, Verticillium, Phytophthora,* and Root-knot nematode. Field research in pepper crops yielded promising results in reducing the pathogen/nematode populations in the soil, plant infection severity, and overall improvement in crop yields. The objective of this poster presentation is to discuss, in detail, the components of BRSP and the research associated with the program.

Introduction:

Soil fumigants are pesticides applied to the soil as a gas to control pests that can disrupt crop production and plant growth (EPA). Due to the increasing restrictions on fumigant use, researchers have been focused on developing non-fumigant alternatives to the traditional chemical fumigation. Peroxyacetic acid (PAA) is an equilibrium compound generated by the reaction of hydrogen peroxide (H_2O_2) with an organic acid (acetic acid). PAA is well known for its biocidal properties (Baldry, 1982) and found to be more effective as a bactericide, fungicide, and sporicide when compared to straight hydrogen peroxide by itself (Baldry, 1982; Alasri et al., 1992). PAA works primarily by oxidizing bacterial/fungal cells/spores by damaging the cellular macromolecules including lipids, proteins, and nucleic acids. Various studies have been conducted over the years looking into PAA's efficacy against various plant pathogens and nematodes. This poster reviews several studies conducted over the past few years exploring the use of PAA as a non-fumigant alternative for soil disinfestation.

Non-plant assays:

Three separate non-plant assays were conducted to evaluate the efficacy of PAA on the management of soilborne pathogens. All non-plant soil assays utilized autoclaved and artificially inoculated soil with the respective pathogens. A petri dish study using *Streptomyces scabies* inoculated soil (4.0 X 106 CFU/mL *S. scabies* added to 2.47 Kg of autoclaved soil) was conducted in 2019 in San Luis Valley, CO with soil treatments of 400, 500, 1,018, or 2,036 PPM of PAA and each treatment had three replicates. Each treatment in the study contained 50 g of autoclaved or *S. scabies* inoculated soil and 13 ml of the respective treatments were added to the petri plates and incubated for four hours. Soil (10 g) from each petri plate was sampled and suspended in thirty ml of sterile distilled water, shaken for 5 min on a vortex mixer. The suspension was used to start a 10-fold dilution series (10-1 to 10-5) and 100 µl of each dilution was plated on YME (Yeast Malt Extract) or TA (Tyrosine agar) medium and incubated for twelve days at 28°C. After incubation, *S. scabies* colonies were counted and expressed as CFU per gram of soil.

A non-plant soil assay was conducted in plastic cups with soil inoculated with *Meloidogyne incognita* juvenile 2 (J2) nematodes. The inoculated soil (200 J2/cup) was treated with distilled water, the standard (Fluopyram + Imidacloprid) at 0.32% v/v, or PAA concentrations of 50, 100, 500, 525, 1,018, 2,036, or 4,200 PPM and each treatment had four replications. After 24-hours, the soil from the cups was placed in pie-pan apparatus, which allow live J2 to move from the soil into water. After 48 hours, the water was removed from the pie-pan and sifted through a 500-mesh sieve. The living and mobile J2 nematodes were then counted for each sample. The percentage kill was determined by the number of recovered nematodes from the water control compared to the various treatments.

The final non-plant soil assay was conducted in clay pots with V. dahliae inoculated soil treated with PAA concentrations of 471, 500, 942, 1,000, 1,889, or 2,000 PPM, and each treatment had four replications. All treatments for the non-plant soil assays were applied after the autoclaved soil was inoculated with the respective pathogens. After the *Verticillium* inoculum was applied to the soil, three soil samples were taken: prior to the soil treatment applications, twenty-four hours after treatment, and seven days after treatment application. *Verticillium* reduction was determined by evaluating the number of colony forming units per gram of soil (CFU/g of soil).

Greenhouse assay:

A greenhouse assay was conducted in Weslaco, TX to evaluate the efficacy of PAA on the management of *Phytophthora nicotianae* on sour orange seedlings. One-month -old sour orange seedlings with uniform height were selected for each treatment and were infested with 2.5 X 104 zoospores/ml of *P. nicotianae* for four to five hours then transferred to plastic pots filled with soil. The soil and seedlings were treated with soil drenches of either a PAA concentration of 900 PPM or to the standard (Mefenoxam at 2 qt/A) soil fumigant treatment. Two weeks post treatment applications, disease incidence was evaluated. Seedling heights were evaluated at 15-, 30-, 45-days post application, and after 45 days the roots were washed and scanned (data not shown). Pots were arranged in a completely randomized block design on a bench in the greenhouse. Each treatment had ten replicates and the experiment was performed twice.

Field trial:

A trial was conducted on field grown Crusader bell peppers in Arroyo Grande, CA to assess treatments on the management of Phytophthora capsisci that occurred naturally in the field. This field was designed as a randomized complete block design with four replications for the six treatments. The assessed treatments were comprised of an untreated check, a standard treatment (fluopicolide (4 fl. oz./A) + mandipropamid (8 fl. oz./A)), and PAA concentrations of 525, 1,018, 2,688, and 1,018 + 50 PPM. All PAA treatments were applied once on August 10, except for the 1,018 + 50 PPM PAA, which was applied on August 10 (1,018 PPM), August 18 (50 PPM), and August 25 (50 PPM). The two solutions that make up the standard treatment were applied at five points: mandipropamid (August 25, September 15, and October 13) and fluopicolide (August 11, and September 8). Peppers were evaluated for phytotoxicity six times during the growing season and phytophthora root severity wasevaluated using a scale of 0 to 10 by examining bisected roots from each plot on November 20.

Results:

Non-plant assays: In the petri dish study, *S. scabies* was undetectable in all negative controls except for one 10-3 dilution plate, indicating that the autoclaved soil had no detectable levels of *S. scabies.* The mean population of the *S. scabies* in the positive control was significantly higher than the other treatments except for the 400 PAA treatment **(Figure 1)**.

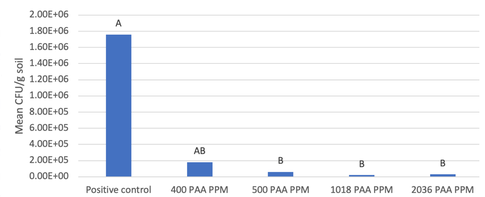

Figure 1. Mean colony forming units (CFU) per gram of soil detected for each of the Peroxyacetic Acid (PAA) soil treatments and the positive inoculated control for the management of *Streptomyces scabies*. Different letters above the bars indicate a statistical difference using Tukey's honest significant difference test.

For the root-knot nematode assay, seventy-seven J2 nematodes were recovered in the water check and were used to compare the percentage kill for each treatment. All PAA treatments greater than 100 PPM had significantly lower J2 nematodes in the soil compared to the water control **(Figure 2)**. All PAA treatments except for the 50 and 100 PPM, did not have significantly different nematode counts or percentage killed nematodes from the Fluopyram + Imidacloprid standard treatment.

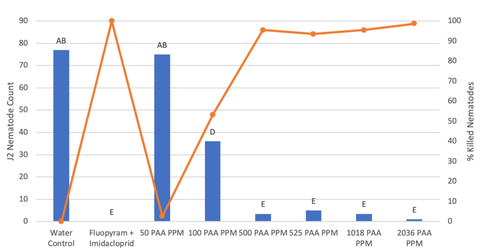

Figure 2. Mean Juvenile 2 root knot nematode (*Meloidogyne incognita)* count in 100 cc of soil (left vertical axis, blue bars) detected for each of the Peroxyacetic Acid (PAA) soil treatments, the Fluopyram + Imidacloprid (stanard), and the water control for the management of *Meloidogyne incognita*. Percentage killed nematodes calculated by percentage reduction of nematode count for each treatment compared to the water control (right vertical axis, orange line). Different letters above the bars indicate a statistical difference (*P<0.001*) between the treatments.

In the *Verticillium* non-plant assay, prior to the treatment drenches, there was no significant difference in the *Verticillium* population (CFU/g of soil) between the treatments **(Figure 3)**. However, 24-hours and 7-days after the soil was drenched with the respective treatments, there was a significant drop in the *Verticillium* population for all PAA treatments compared to the untreated control.

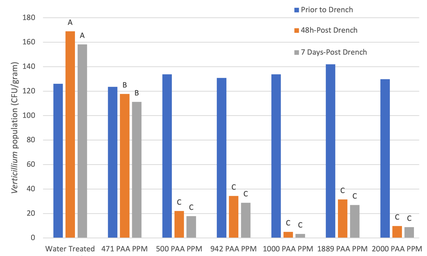

Figure 3. Mean *Verticillium dahliae* colony forming units (CFU) per gram of soil detected for each of the Peroxyacetic Acid (PAA) parts per million (PPM) soil treatments and the water treated control. With respect to each time point, columns with the same letter are not significantly different according to Fisher's protected least significant. difference test (α < 0.05).

Greenhouse assay:

All treatments showed more positive activity than the untreated inoculated control to reduce Phytophthora disease **(Figure4)**. The disease incidence percentage was statistically lower among all treatments compared to the untreated inoculated control.

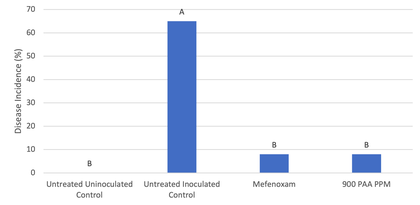

Figure 4. Mean disease incidence of *Phytophthora nicotianae* on citrus seedlings for each of the Peroxyacetic Acid (PAA) parts per million soil treatments, uninoculated and inoculated controls, and the Mefenoxam standard soil treatment. Different letters above the bars indicate a statistical difference (*P<0.05*) according to indipendent T-test.

Field trial:

Phytotoxicity was evaluated as a percentage where 0% indicates no phytotoxic damage, and 100% indicates plant death due to the chemical application. No phytotoxicity was observed on the six rating dates for any of the applied treatments. There was no significant difference between the treatments for the management of the Phytophthora root rot severity. However, the 1,018 and 2,688 PPM PAA treated peppers had numerically lower root rot severity compared to the Fluopicolide + Manipropamid standard treated peppers **(Figure 5)**. In addition, the 1,018 + 525 PPM PAA treated peppers had numerically lower root rot severity compared to the standard.

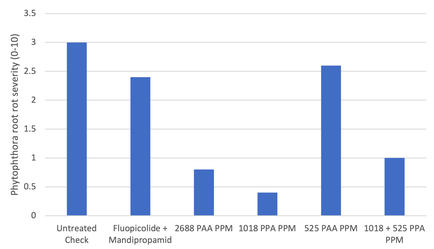

Figure 5. Mean disease severity of *Phytophthora capsici* on bell pepper roots for each of the Peroxyacetic Acid (PAA) parts per million treatments, untreated check, and the Fluopicolide + Mandipropamid standard treatment.

Conclusions:

The need for an alternative to traditional soil fumigants has increased the development and assessment of non-fumigant alternatives. In these various studies, the PAA treatments have been shown to be an effective alternative to the traditional fumigants on a lab and a greenhouse scale. The limited field data shows that the efficacy of PAA as an alternative for traditional fumigants is promising, however, more data should be collected.

References: Alasri, A., Roques, C., Michel, G., Cabassud, C., and Aptel, P. 1992. Bactericidal properties of peracetic acid and hydrogen peroxide, alone and in combination, and chlorine and formaldehyde against bacterial water stains. Canadian Journal of Microbiology. 38:635-642.

Baldry, M. 1983. The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid. J. Appl. Microbiol. 54:417-423. Environmental Protection Agency. (n.d.). EPA. Retrieved January 20, 2022, from https://www.epa.gov/soil-fumigants/what-are-soil-fumigants

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