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Impact of biosolarization with almond hull and shell amendments for the control of *Fusarium oxysporum* f. sp. *lactucae* in a lettuce/tomato cropping system

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ABSTRACT

Biosolarization is an alternative to chemical fumigation that couples solarization and organic amendments to control diseases by increasing temperature and microbial activity. A field study was conducted to evaluate the efficacy of biosolarization, using almond processing residues from two varieties, to control propagules of *Fusarium oxysporum* f. sp. *lactucae*, the causal agent of Fusarium wilt of lettuce. Peak soil temperatures of solarized and biosolarized soils were 6–15 °C higher than control soils, and biosolarized soils experienced 1 to 3 °C higher temperatures on average and 10 to 27°C-day higher cumulative temperatures than soil solarized without amendment. Residues were high in endogenous organic acids, between 144 and 298 mM combined acetic, lactic, succinic, and butyric acids. After eight days of treatment, solarized soils only reduced the population of *Fusarium oxysporum* f. sp. *lactucae* by 9% compared to the average non-treated non-amended control plots; whereas soils solarized and amended with residues from pollinator varieties of almonds showed a greater pathogen reduction of 63%. In this instance, Fusarium wilt had no effect on plant health due to low pressure and moderate innate plant resistance. Soils amended with nonpareil residues had similar lettuce biomass and health scores to untreated plots, but lower health scores than the solarized treatments. This may be due to residual organic acids detected in biosolarized plots 3–4 weeks after treatments (1–57 mM), which significantly correlated to poor lettuce health score. Roughly, one year after soil treatment, solarized plots continued to out-perform pollinator-residue amended plots. Germination assays indicate biosolarization outcomes may be improved with increased aerobic remediation time.

1. Introduction

Soilborne plant diseases limit crop production worldwide, resulting in yield losses of 50–75% for various crops such as wheat, cotton, maize, vegetables, fruit and ornamentals (Panth et al., 2020). These pathogens can survive in the soil for long periods, colonizing plant debris and organic matter or by forming resistant structures, making management difficult. Chemical fumigants have historically been applied to the soil to control these diseases. By the late 1950s, Wilhelm and Paulus (1980) began testing mixtures of chloropicrin with methyl bromide (MB) and showed that MB did not only eliminated soilborne pathogens but also provided excellent weed control. Due to health and environmental concerns, the use of MB and other related chemical products has become limited or prohibited (Lloyd et al., 2016). In response to the health concerns and regulations surrounding fumigants, alternative approaches are increasingly tested, including crop rotation, steam soil disinfection, soil solarization, resistant cultivars, and biological controls (Fennimore and Goodhue, 2016; Guerrero et al., 2019; Henry et al., 2019; Pastrana

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et al., 2016). These alternatives are compatible with organic production and for certain cropping systems remain the only feasible pest management strategy. However, none have been proven to completely eliminate pathogens from soils (Mihajlovic et al., 2017).

Biosolarization is a relatively novel alternative to chemical fumigation that couples solarization and microbial activity from organic amendments to reduce oxygen levels and accumulate biopesticides in form of organic acids (OAs) (Hestmark et al., 2019; Oka et al., 2007; Ros et al., 2008; Simmons et al., 2016). Soil biosolarization has been reported to successfully control weeds, nematodes, and fungal pathogens (Achmon et al., 2017; Basallote-Ureba et al., 2016; Jesús D. Fernández-Bayo et al., 2017; Fernández-Bayo et al., 2018). Efficacy depends on several factors, such as nature of the organic amendment used, application rate, soil type, agronomic practices, and other environmental factors (McSorley, 2011). Amendments used for biosolarization may be low-cost organic waste streams, may contain endogenous biopesticidal compounds, or can be subjected to fermentation by soil microbiota to form biopesticides (Fernández-Bayo et al., 2020; Oka, 2010).

Almond processing residues (hulls and shells) are agricultural byproducts from the isolation of edible almond kernels. Although their production is increasing worldwide, they are particularly abundant in California and can be obtained at low cost (Almond Board of California, 2020). Recent results validated almond byproducts as potential amendments for biosolarization, which were found to reduce the abundance of nematodes (*Pratylenchus vulnus*) and fungal (*Rosellinia necatrix*) pathogens when applied to soil (Fernández-Bayo et al., 2020; Vida et al., 2016). Particularly, extracts from soils containing at least 2.5% (dry weight) incorporated almond biomass showed an 84–100% mortality of *P. vulnus* nematode, correlated to organic acid concentrations above 2.0 g/L (Fernández-Bayo et al., 2020).

Fusarium oxysporum f. sp. *lactucae* (*F. o. lactucae*), the causal agent of Fusarium wilt of lettuce, is an important soilborne pathogen affecting lettuce production in California and worldwide. Once the pathogen is established in a field, it is difficult to eradicate due to chemical fumigation is economically prohibitive for the sector (Gordon and Koike, 2015). To advance the feasibility and benefit in cropping systems from soil biosolarization, studies should broaden the pathogens tested. One goal of this study was to evaluate the efficacy of biosolarization to reduce the number of propagules of *F. o. lactucae* under field conditions. Residual phytotoxicity from OAs and other bioactive compounds is a concern for amendments (Aslam and VanderGheynst, 2008). Organic acids from biosolarization may negatively affect subsequent crops with an insufficient equilibration period after biosolarization. Understanding the impact of soil biosolarization on crop yield and bioremediation practices to minimize potential phytotoxicity is necessary. Therefore, the second objective of this study was to assess the phytotoxicity of almond hull and shell amendments on lettuce and tomato yield post biosolarization as well as to determine how aerobic remediation affects post-treatment plant performance using in-lab phytotoxicity assays.

2. Materials and methods

2.1. Biosolarization experiment

The experimental field was located in a research farm of the Department of Plant Pathology at University of California, Davis. The field soil was sandy clay loam (47%, 27%, and 26% of sand, silt, and clay, respectively), the organic matter (OM) content was 2.64% and the field capacity was 21.90% (wet basis) (J.D. J.D. Fernández-Bayo et al., 2017). The field was artificially infested with *F. o. lactucae* race 1 in 2002. Infestation with *F. o. lactucae* was achieved by incorporating sand inoculum into soil down to 25 cm (J. Scott et al., 2010a). During two cycles, the infested field was planted with a mix of lettuce cultivars susceptible to Fusarium wilt, and mature lettuce plants were incorporated into the soil (Scott et al., 2010a). High inoculum densities of the pathogen have been maintained since by regularly planting and incorporating susceptible lettuces (Paugh and Gordon, 2019). Prior to the experiment, the field was prepared by incorporating weeds present in the ground via rototiller. An area of approx. 645 m^2 with six 1.8 m-wide non-raised planting beds was divided into thirty-six equally sized plots (1.8 × 4.9 m). Buffer zones between plots within the same bed were 1 m. Almond residues for soil amendments included hulls and shells from two varieties, nonpareil (NP) and a pollinator variety mix (PM), were received whole (North State hulling Coop, Chico CA); the neutral detergent fiber, starch, and sugar content was 258, 3.9, and 267.4 g kg^{-1} dry matter for NP variety, and 345, 5.2, and 178 g kg^{-1} dry matter for the PM (Fernández-Bayo et al., 2020; Palma et al., 2020). The particle size of the biomass was <1.27 cm. Soil preparation for treated plots has been previously described (Shea et al., 2021a); one of six treatments was randomly assigned to each plot, with six replicates each: control (non-solarized which received no amendment and no cover), solarization (non-amended), biosolarization using NP at the amendment rate of 1.75% or 2.5%, and biosolarization using PM at the amendment rate of 1.75% or 2.5%. Biosolarized plots were amended and tilled down to 15 cm to achieve desired amendment rates of 1.75% or 2.5% by dry weight (equivalent to approximately 9 or 13 tons/ha, respectively). Temperature loggers (Omega, Norwalk, CT) were embedded 7.6 cm deep to monitor temperature every 30 min. Drip tape (30.5 cm emitter spacing) was set at the surface and plots were covered and sealed with total impermeable film (TIF, TriCal, Hollister, CA). Irrigation was initiated to reach field capacity in the soil at least down to approximately 1 m, at which point temperature logger data collection began. All plots, excluding the controls, underwent solarization under a 0.7 mm-clear totally impermeable film for 8 days (from August 7, 2018 through August 15, 2018), after which plastic tarps were removed and temperature loggers were retrieved. Plots were left for aeration until lettuce crop was set. Soil samples from the top 15 cm were taken for analyses after adding the amendment but before tarping, after biosolarization, and at the time of lettuce planting.

2.2. Soil organic acids and pH analyses

Organic acids (OAs) were analyzed as previously reported (Fernández-Bayo et al., 2020). Briefly, soil OAs were extracted from each soil sample by combining soil with water (1:1) in duplicate, and shaking via shaker for 30 min. Samples were centrifuged for 10 min at 8800 *g* (Eppendorf Centrifuge 5810R 15 Amp, Hamburg, Germany). pH values were obtained for supernatants. The supernatant was filtered via 0.2 μm syringe filter (Thermo Fisher Scientific Inc., San Diego, CA) into 2 mL HPLC (high performance liquid chromatography) vials (Agilent, Santa Clara, CA). Succinic, lactic, formic, acetic, propionic, isobutyric, and butyric acid contents in soil extracts were analyzed by HPLC (model UFLC-10Ai, Shimadzu, Columbia, MD; Aminex HPX-87H column; 300 × 7.8 mm, Bio-Rad, Hercules, CA) using previous methods (Fernández-Bayo et al., 2020). Measured OA concentrations in soil were normalized according to the moisture content of each extracted sample to yield concentration per volume soil water. Concentrations for individual OAs in each sample were summed to determine total OA concentration.

2.3. Population of Fusarium oxysporum f. sp. lactucae

The soil density of *F. o. lactucae* was estimated at each plot before and after biosolarization (initial and final timepoint samples, respectively). Soil samples were immediately air dried at room temperature, passed through a 4-mm sieve, and kept in a cold room at 4 °C for not longer than four weeks. For each soil sample, 10 g were suspended in 200 mL of 1% sodium hexametaphosphate and stirred for 5 min. Thereafter, a 10 ml of the suspension was diluted in 0.1% water agar (1:10) and agitated for another 5 min. Then, 400 μL was spread onto each of twelve Komada's plates (Komada, 1975). Plates were incubated at room temperature under continuous fluorescent light for ten days. Colonies were identified based on morphology on Komada's media, pink pigmentation on the underside of the colony and white aerial mycelium that was organized into variously sized tufts, as described and documented by Scott et al. (2010b).

2.4. Lettuce crop experiment

Two lettuce cultivars were selected: Steamboat (crisphead type) and Fossey (red leaf type). Cultivar Steamboat, moderately susceptible to Fusarium wilt, was chosen to evaluate the incidence of the disease after biosolarization. Cultivar Fossey, a resistant cultivar to Fusarium wilt, was selected to assess beneficial or negative effect of the treatments on crop production (Paugh and Gordon, 2019).

Lettuce seeds cv. Fossey (uncoated seeds) were sown in a transplant tray filled with moistened potting soil (Sunshine Mix No. 1, Sun Gro Horticultural, MA) on July 26th, 2018. Trays were then covered with aluminum foil for 24 h and maintained in a growth chamber with day/night temperatures of 23/18 °C and 60% humidity. After six days, trays were moved to a greenhouse wherein temperatures ranged from 15 to 26 °C and a relative humidity between 10 and 75%. Five weeks later, or 20 days after biosolarization, seedlings were transplanted to half of the plots in the experimental field (three per treatment, n = 18). Lettuce seeds cv. Steamboat (coated seeds) were pre-germinated in a petri plate within two layers of wet filter paper and kept in darkness for 24–48 h. Pre-germinated seeds were placed in a transplant tray and maintained as explained above until they were ready to be transplanted to the field. Five weeks later, or 28 days after biosolarization, Steamboat seedlings were transplanted to the remaining half of the experimental plots (three per treatment, n = 18). Each plot represented 20 plants spaced approx. 30 cm apart and distributed in two parallel lines. Lettuces were watered as needed through drip irrigation. At the time of planting, 6 composite core soil samples were taken from the top 7 cm of soil for each plot to determine OA concentration in the lettuce root zone at the time of planting. Lettuce plants were rated for severity of Fusarium wilt based on an ordinal scale of 1–5 at four, six, eight, and ten weeks after transplanting. Ratings were assigned as follows: 1 = healthy, 2 = mild stunting, 3 = stunting and chlorosis/necrosis, and 4 = severe stunting and chlorosis/necrosis, 5 = dead, with intermediate ratings of 0.5 applied when appropriate (Paugh and Gordon, 2019). At the end of the experiment, plants were harvested, and their taproots were screened for discoloration. To recover fungi from discolored taproots, cross sections were rinsed with 0.1% Tween 20 (Sigma-Aldrich, St. Louis, MO), submersed in 70% ethanol for 20 s followed by 60 s in 1% NaOCl, and then placed on Komada's selective medium (Komada, 1975). After seven to 10 day at room temperature under continuous fluorescent light, colonies were identified as *F. o. lactucae* by morphology (Scott et al., 2010b). The above-ground portion of each plant was separated from the taproot and oven dried at 50 °C for ten days. Dry weight was recorded for each plant. Plants assumed to die independently of the disease (bad transplanting, damaged by insect or herbivore, or blown) were excluded from the dataset (Table S1). Any dead plants that tested positive for *F. o. lactucae* at the end of the experiment were also excluded from the biomass and health score analyzed data due to low prevalence (n = 1).

2.5. Tomato crop experiment

Tomato seeds cv. New Girl were sown in a transplant tray filled with moistened potting soil (Sunshine Mix No. 1, Sun Gro Horticultural, MA). Trays were maintained in a growth chamber with day/night temperatures of 28/20 °C until planted. The biosolarized plots after lettuce harvest were left undisturbed during the fall and winter 2019. The following spring, weeds were mowed, and plots were rototilled three times. Then, beds were pulled, and drip lines were installed. Twelve tomato plants were planted in each plot (April 25, 2019). Plants were watered as needed. When tomatoes reached maturity (105 days after planting), they were manually harvested four times (approx. every two weeks). For each harvest time, the wet weight of the fresh and rotten tomatoes was recorded. Total yield was calculated as the sum of all weights per plot.

2.6. Laboratory phytotoxicity assay

To assess phytotoxicity from the almond byproduct, biosolarization was simulated in the lab under conditions similar to the field. Soil was obtained from Parlier, CA (Achmon et al., 2016), air dried, and sieved through a 0.6 mm mesh to remove any plant residues. The same NP and PM almond hulls and shells were ground in a laboratory blender and sieved through 0.5 mm mesh to remove large particles. Soil was mixed with one of the two residues to achieve amendment rates of 2.5% on a dry weight basis. Nonamended soil was included as a control. Deionized water was added to achieve 80% water holding capacity. Bioreactors were prepared as previously described (Fernández-Bayo et al., 2020), where 240 g (wet weight) soil stock was aliquoted into 250 mL airtight glass jars (anaerobic reactors). Reactors were sealed and incubated for 8 days at 12 h cycles of 30 and 50 °C to simulate biosolarization. After fermentation, 180 g of biosolarized soil from each anaerobic reactor were added to 250 mL plastic flasks with air inlet at the bottom (aerobic reactors). For aerobic soil remediation, humidified air was passed through the biosolarized soil at 20 mL/min for 0, 2, or 4 weeks at 25 °C.

After remediation, phytotoxicity was assessed by measuring germination index (GI), the product of relative germination, and relative root length, as it is considered a more sensitive metric than relative germination rates alone (Tiquia et al., 1996). Radish seeds are commonly selected to calculate GI due to the sensitivity of roots to various phytotoxins (Aslam and VanderGheynst, 2008; Fernández-Bayo et al., 2020; Mitelut and Popa, 2011). Forty-five mL treated soil were applied to petri dishes (Falcon, NY, USA), and sterilized filter paper coated with 5 mL of deionized water was prepared as a negative control according to Aslam and VanderGheynst (2008). Seed incubations were performed according to previous studies (Ko et al., 2008; Mitelut and Popa, 2011): 10 radish seeds (var. *Sparkler*, *Raphanus sativus*, Ferry-Morse, KY, USA) were plated in triplicate and incubated in the dark at 24 °C for 72 h. After incubation, the total number of seeds to germinate (N) was counted and the germinated root lengths of each seed were measured and averaged (L_{avg}) for each plate. Germination was considered positive when root lengths were greater than 5 mm (Ko et al., 2008). The mean number of seeds to germinate (N_0) as well as the mean root length (L_0) was calculated for the non-amended control. For each soil plate, the germination index (GI) was calculated according to equation (1). Soils with a mean GI below 80% were considered phytotoxic (Tiquia et al., 1996).

$$GI = 100\% \left(\frac{L_{avg} N}{L_0 N_0} \right) \quad (1)$$

2.7. Data analysis

Analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) post-hoc tests were used to compare means for variables with normal distributions. Normality of data and residuals was analyzed using the Shapiro-Wilks test ($P > 0.05$), and parameters were transformed when necessary (see below). To assess differences in soil temperature, OA concentration, and pH between different amendment compositions, treatment levels included the untreated control soil (Con), nonamended solarized soil (Sol), soil biosolarized with either Nonpareil or pollinator residues at 1.75% or 2.5% (NP1.75, NP2.5, PM1.75, or PM2.5). Cumulative soil heating (degree-days) was calculated using the trapezoidal method. Temperature daily average, maximum, and ranges were calculated by averaging the 8 daily measurements. Individual organic acid concentrations were summed, and total OA concentration underwent log +1 transformations to ensure homoscedasticity and normality of residuals. To assess differences in the population of *F. o. lactucae* and crop performance, treatment levels included the untreated control soil (Con), nonamended solarized soil (Sol), and soil biosolarized with nonpareil or pollinator residues (NP or PM). Analysis of variance (ANOVA) of lettuce health and biomass was blocked by cultivar. Colony forming units of *F. o. lactucae* underwent square root transformations to ensure homoscedasticity and normality of residuals. Regression analysis was used to determine correlations between lettuce growth and log-transformed organic acid concentration. Calculated germination indices were corrected to non-amended controls, such that the mean GI for the controls of each batch was set at 100. All data analysis was performed using JMP (version 14).

3. Results

3.1. Temperature

Treatment significantly affected mean, minimum, maximum, and cumulative temperature (P < 0.001), but not temperature range (P = 0.252; Fig. 1, Table 1). Regardless of residue type and rate, biosolarized plots had statistically equal mean, minimum, maximum and cumulative temperature. Biosolarized soils had average temperatures 1–3 °C significantly higher than non-amended solarized soils (P < 0.001) and 10–12 °C significantly higher than control soils (P < 0.001). Similarly, biosolarized soils had minimum temperatures 2–5 °C significantly higher than non-amended solarized soils (P < 0.001) and 9–12 °C significantly higher than control soils (P < 0.001). Maximum daily temperatures were similar between biosolarized and solarized soil (P = 0.904) and were 6–15 °C higher than controls (P < 0.001). Taken together, biosolarized soils had the highest cumulative heating, 10–27 °C•days significantly higher than solarized (P < 0.001) and 79–96 °C•days significantly higher than control soils (P < 0.001).

3.2. Soil organic acids and pH levels in the soil

Before solarization, the presence of amendment effected OA concentration (P = 0.001, Fig. 2A). Due to high variation, soils amended with 1.75% PM or NP amendment were not statistically different from the non-amended soils, despite over 10-fold higher mean concentrations at 88 and 144 mM, respectively. However, soils amended with 2.5% PM or NP amendment did have significantly higher total OAs than non-amended soil, with a near 30-fold increase, at 263 and 298 mM, respectively. After 8 days of solarization treatment, organic acids were present in the treated soils at lower total quantities than initial values (Fig. 2B). At this timepoint, NP-amended soil had still greater total OAs than non-amended soil, with 69- and 91-mM total OA at 1.75 and 2.5% amendment rates, respectively compared to the control and solarized treatments, which were below 10 mM. PM-amended soils had greater total OAs than non-amended soils at 2.5% amendment rates with 86 mM, but no significant difference was observed 1.75% amendment rate (45 mM). In amended soil before and after biosolarization, acetic acid was the major product, making up 31–46% of the total acids, followed by lactic (19–42%), succinic (11–15%), and butyric acid (3–10%). Formic and isobutyric acids were detected in trace amounts (<3%). In general; rate, residue type, and the time sampled had no effect on the fraction of OA species, with the exception of propionic acid which significantly increased in amended soils from <1% before biosolarization to 11–20% after the 8-day treatment (P = 0.008). By the time of Fossey lettuce planting 20 days after treatment, OAs were still detected (2–57 mM) but were not significantly higher than non-amended soil; when Steamboat lettuce was planted 28 days after treatment, OAs were still even lower (<21 mM).

Before solarization, the treatments significantly affected pH (Table 2), with soil amended with 2.5% PM having lower pH than the nonamended samples. After treatment, only 2.5% NP had significantly lower pH than the non-amended samples (P < 0.05). No significant pH

Table 1
Temperature responses at 7.5 cm depth.

Treatment[a]	Mean Temp (°C)[b]	Min Temp (°C)[b]	Max Temp (°C)[b]	Cumulative Temp (°C × day)[c]
Control	27.0 ± 0.1 C	19.7 ± 1.2 C	35.3 ± 2.1 B	207 ± 1 C
Solarization	35.3 ± 0.6 B	26.3 ± 0.6 B	46.3 ± 1.2 A	276 ± 1 B
Biosolarization NP1.75	37.7 ± 0.6 A	29.7 ± 1.2 A	47.7 ± 1.2 A	295 ± 6 A
Biosolarization NP 2.5	37.7 ± 0.6 A	30.3 ± 0.6 A	46.3 ± 0.6 A	293 ± 4 A
Biosolarization PM1.75	37.3 ± 0.6 A	30.3 ± 0.6 A	45.3 ± 2.3 A	290 ± 6 A
Biosolarization PM 2.5	38.3 ± 0.6 A	29.7 ± 1.2 A	48.3 ± 3.5 A	299 ± 7 A
P-Value	<0.0001	<0.0001	<0.0001	<0.0001

Within each column, values that do not share a letter are significantly different according to Tukey's HSD and a significance threshold of P = 0.05.

[a] Indicate treatment at which plots were exposed for 8 days: control (non-solarized which received no amendment and no TIF), solarization (non-amended), biosolarization using nonpareil residues at the amendment rate of 1.75% (NP1.75) or 2.5% (NP2.5), and biosolarization using pollinator residues at the amendment rate of 1.75% (PM1.75) or 2.5% (PM2.5).

[b] Mean daily parameter indicates the average value over 8 days of treatment ± standard deviation (n = 3 plots): mean daily average (mean), mean daily minimum temperature (min), and mean daily maximum temperature (max).

[c] Values indicates the cumulative soil heating (degree-days) over 8 days of treatment ± standard deviation (n = 3), calculated for each plot using the trapezoidal method.

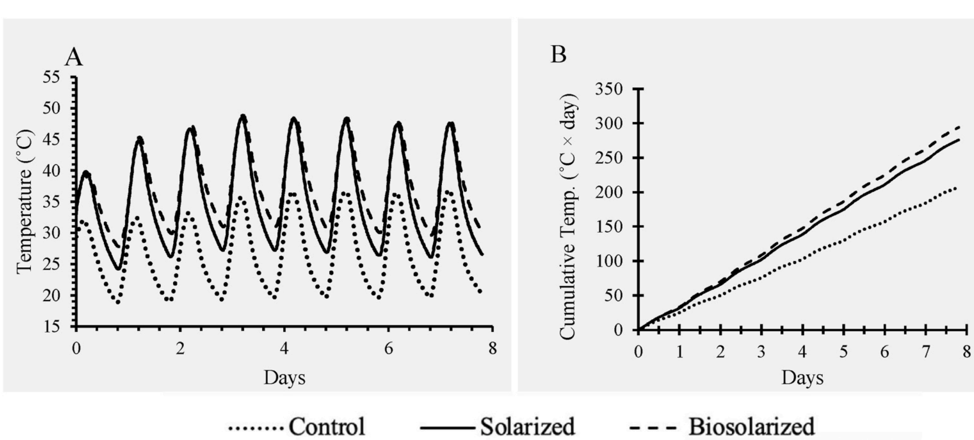

Fig. 1. Temperature during 8-day biosolarization treatment. Soil temperature was measured in 15-min intervals at a depth of 7.5 cm for control (n = 3), solarized (n = 3), as well as biosolarized plots (n = 12). Fig. 1A represents raw mean temperature, and Fig. 1B represents the integral of Fig. 1B, the cumulative temperature.

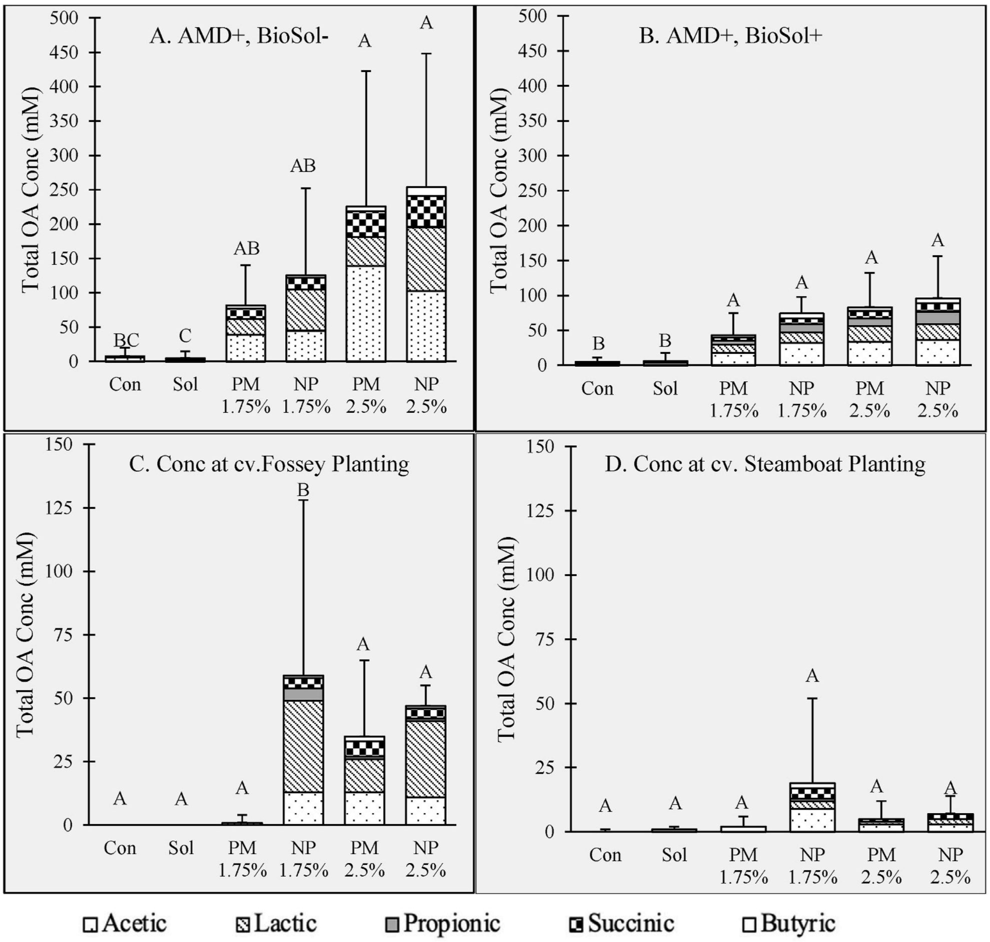

Fig. 2. Organic acids detected in treated soil. Acetic, lactic, propionic, succinic, butyric, formic, and isobutyric acids were measured in control soil, solarized soil, and soil biosolarized with 1.75% and 2.5% Nonpareil residues (NP), and biosolarized with 1.75 and 2.5% pollinator residues (PM). Soil was analyzed at four time points, (A) after amendment but before biosolarization (AMD+, BioSol-); (B) after 8 days of biosolarization (AMD+, BioSol+); (C) at the time of cv. Fossey lettuce planting, 20-days post-treatment; and (D) at the time of Steamboat lettuce planting, 28-days post-treatment. The bars indicate the mean concentration of each OA and the stacked bar indicates the sum of all detected acids in a given treatment (total OAs). Different capital letters represent significant differences for each treatment according to Tukey's HSD after log +1 transformations, and error bars represent the standard deviation of total OAs, where n = 6 for the first two time points and n = 3 for the last two time points.

Table 2
Soil pH before and after treatment. Six treatments are shown, including control, solarized, biosolarized with 1.75% and 2.5% NP, and biosolarized with 1.75 and 2.5% PM, both before and after the treatment. Values indicate mean daily parameter ± standard deviation (n = 3 plots). P-value indicates the result of one-way ANOVA performed for each time point. Different lowercase letters represent significant differences for each parameter according to Tukey's HSD for each time point (column).

Treatment[a]	pH initial[b]	pH final[c]
Control	7.4 ± 0.6 ab	7.6 ± 0.3 a
Solarization	7.7 ± 0.2 a	7.3 ± 0.4 ab
Biosolarization NP1.75	7.1 ± 0.4 ab	6.7 ± 0.5 bc
Biosolarization NP 2.5	6.9 ± 0.3 bc	6.6 ± 0.9 c
Biosolarization PM1.75	7.0 ± 0.4 bc	7.3 ± 0.5 ab
Biosolarization PM 2.5	6.5 ± 0.7 c	7.0 ± 0.6 abc
P-Value	*P = 0.007*	*P = 0.038*

[a] Indicate treatment at which plots were exposed: control (non-solarized which received no amendment and no TIF), solarization (non-amended), biosolarization using nonpareil residues at the amendment rate of 1.75% (NP1.75) or 2.5% (NP2.5), and biosolarization using pollinator residues at the amendment rate of 1.75% (PM1.75) or 2.5% (PM2.5).

[b] Mean pH of soil immediately before treatment ± standard deviation (n = 6 plots).

[c] Mean pH of soil immediately after 8 days of treatment ± standard deviation (n = 6 plots).

depression was observed for any treatment before and after treatment (all pairwise t-tests > 0.05). Organic acid concentration was inversely correlated to pH ($P < 0.001$).

3.3. Population of *Fusarium oxysporum f. sp. lactucae*

Initially, plots prepared for different treatments showed average levels of FOL between 83 and 271 CFU/g. After amendment, means were between 50 and 149 CFU/g and showed no statistically significant differences ($P = 0.412$) regardless of amendment. Even before treatment, *F. o. lactucae* population were 66% lower in PM-amended soil than in control plots, but differences were not significant. After eight days under the tarp, the pathogen population (CFU/g soil) didn't change in the solarized (Sol) plots compared to the control (Con) plots. However, it decreased by 50% in the NP biosolarized soils ($P = 0.207$, not significant), and was significantly reduced by 63% in the PM biosolarized soil ($P = 0.024$).

3.4. Lettuce performance after biosolarization

At the end of the experiment, just one taproot showed discoloration and was infested by the pathogen, meaning that inoculum densities of *F. o. lactucae* after biosolarization were not high enough to cause Fusarium wilt symptoms in lettuce plants cv. Fossey or Steamboat. Therefore, results from both cultivars were pooled together as significant differences between them were not observed. Treatment significantly affected lettuce biomass ($P = 0.002$) and lettuce health score ($P = 0.001$). Lettuce grown in solarized soil, as well as soil biosolarized with PM amendment, had significantly greater biomass than lettuce grown in soil biosolarized with NP ($P = 0.005, 0.022$). However, lettuce grown in solarized soil had lower health scores than biosolarized soil, regardless of NP or PM amendments ($P = 0.001, 0.035$). Regression analysis found lettuce health score correlated to log total organic acid concentrations at the time of planting ($P < 0.0001$, Fig. 2C, figure S1), but biomass was not affected by OA concentration ($P = 0.364$). Lettuce biomass and health score from biosolarized soil was not statistically different to the control plots (Fig. 4).

3.5. Tomato yield

Tomato was planted eight months after biosolarization in the same undisturbed soils plots to understand longer-term impacts of the treatment on crop yields. Amendment treatment from the previous summer affected tomato yield ($P = 0.047$), in which PM-amended soil had significantly lower yield than solarized soil (Fig. 5). However, NP-amended soils had statistically equal yield to solarized and control soil.

3.6. Laboratory phytotoxicity assay

The GI of radish was affected by remediation time ($P < 0.001$) but not residue type ($P = 0.994$), where remediation time generally decreased phytotoxicity. For both residues, 2.5% amended soils resulted in high initial toxicity (0% germination). After two weeks of aerobic remediation, NP-amended soil remained severely phytotoxic, with a GI of 36%, where PM-amended soil was moderately phytotoxic, with a GI at 68%. After 4 weeks of remediation, phytotoxicity was removed from the NP-amended soils indicated by a GI of 96%, where PM-amended soils did not see significant increase in GI after 4 weeks (Fig. 6).

4. Discussion

Several studies have shown the physical thermal killing of soilborne pathogens when the soil upper layers are submitted to solarization (Katan, 1976; Martyn, 1986). Often, a beneficial microbial shift is also observed in the less heated soil layers, which eventually could contribute to pathogen control (Culman et al., 2006; Katan, 2017; Ozyilmaz et al., 2016). *F. o. lactucae* has not been an exception, and a disease reduction of 42–91% was observed in a four-year study when comparing lettuces growing in solarized soils with non-solarized plots (Matheron and Porchas, 2010). Conversely, our results show no reduction in the population of *F. o. lactucae* in solarized plots, and presumptively, it has to do with the number of days the soil was heated: eight days. The length of the technique is one of the main limitations of solarization, and even when 14 days could be enough to kill some organisms, four to six weeks of treatment in full sun are recommended (Elmore et al., 1997). One of the ways to improve its effectiveness in a short period of time is the combination with the generation of organic acids, as occurs during biosolarization. Our study showed that NP residues reduced *F. o. lactucae* population by 50% when compared to the control. However, differences were not statistically significant. On the other hand, results confirmed that the addition of PM residues combined with soil heating was able to significantly reduce the population of *F. o. lactucae* in soil by more than 60% compared to control treatment (Fig. 3). It must be noted that the addition of PM residues was also able to reduce the population of the pathogen before the solarization treatment, although this difference was not significant when compared to the control. This decline could be attributed to the natural higher levels of OA found in the NP-amended soils (Fig. 2A). However, no correlation in this case was found between the accumulation of organic acids or changes in the pH and the pathogen population. The effect on the population of *F. o. lactucae* has also been reported by Fernández-Bayo et al. (2018), when a partially stabilized liquid digestate amendment was used at room temperature (Fernández-Bayo et al., 2018). Furthermore, similar studies using PM and NP residues at the same 2.5% rate also showed high initial levels of OA that resulted in the inactivation of the parasitic nematode *Pratylenchus vulnus* (Fernández-Bayo et al., 2020). OA are not the only mechanisms to control fungal pathogens. Other parameters and/or the combination of several factors are also involved in the reduction of this disease, like changes in soil microbial communities and metabolome, the production of $Mn2+$ and $Fe2+$, the formation of toxic byproducts and/or anaerobic conditions, as reported for similar techniques (Hewavitharana et al., 2019; Hewavitharana and Mazzola, 2016; Momma et al., 2006, 2011). Furthermore, previous studies have observed that, while microbial metabolites controlled most fungal pathogens, *Fusarium* species were uniquely resistant (Vespermann et al., 2007).

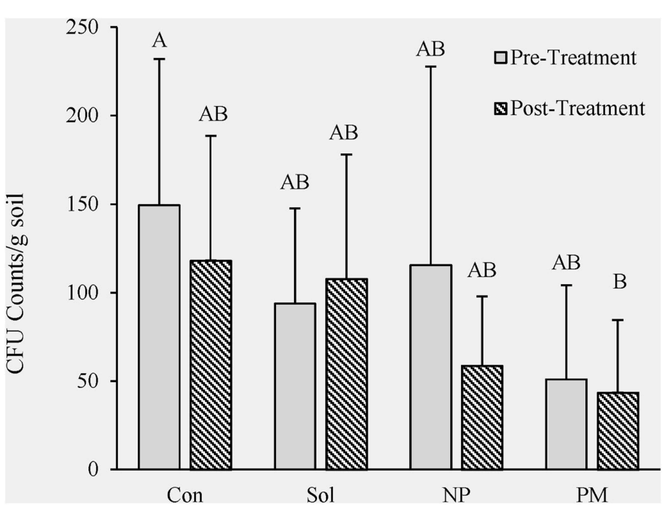

Fig. 3. Fusarium oxysporum f. sp. lactucae population (CFU/g soil) in soil before and after treatment. Colony forming units were measured in amended soil before (initial timepoint, T0) and after 8 days of biosolarization with NP (1.75% and 2.5%), biosolarized with PM (1.75 and 2.5%), and solarization compared to controls (final timepoint, T8). Bars represent the raw counts and error bars indicate standard deviation (n = 12 for NP and PM; n = 6 for control and solarized treatments). Letters represent significant differences in pathogen population according to one-way ANOVA with square root transformation, followed by Tukey's HSD.

The addition of amendments also helped to elevate minimum, average, and cumulative temperatures, which were significantly higher in the eight days-biosolarized plots than in the solarized. Laboratory mesocosm experiments found that the addition of unstable biomass increased soil temperatures over non-amended controls, which may increase the efficacy of thermal pest inactivation (Achmon et al., 2016; Simmons et al., 2013). In this study, these increments were not translated in increasing pest control efficacy over solarization, which may indicate *F. o. lactucae* control in this case relied on OA accumulation and other control mechanisms, rather than thermal heating.

Lettuce plants cv. Fossey which are resistant to Fusarium wilt were chosen to assess additional beneficial or negative impact of the treatments on crop production. On the other hand, lettuce plants cv. Steamboat, only moderate susceptible, were grown to analyze the incidence of the disease after biosolarization. However, the population of *F. o. lactucae* in the soil was not high enough (avg. = 44–118 CFU/g) to cause disease symptoms on lettuces cv. Steamboat planted in mid-September. Recently, Paugh and Gordon (2019), reported little or no impact of Fusarium wilt on lettuce cultivars of intermediate susceptibility in warm planting windows (August) when grown in soil with inoculum levels under 125 CFUs per gram (Paugh and Gordon, 2019). Moreover, symptoms were typically absent in cooler planting windows (October). Lettuce taproot analysis showed that only one plant was affected by the soilborne pathogen in our trial, which was not representative for the disease and it was then discarded from the analysis. Then, the health score measurement was associated to phytotoxicity instead to Fusarium wilt. No significant differences between cultivars were either found when analyzing plant biomass or health score. These reasons supported the combination of data from both cultivars for statistical analysis.

Increased growth response of lettuces and tomatoes growing in

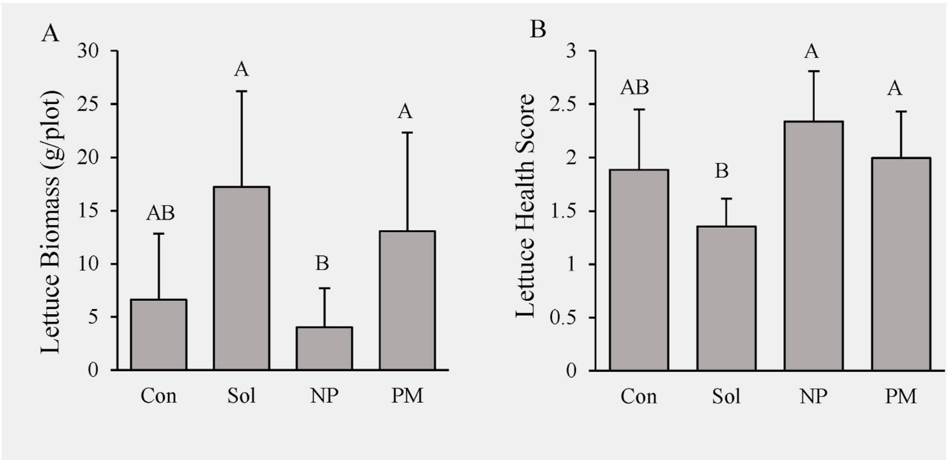

Fig. 4. Lettuce biomass (4A) and health score (4B). Treatments included control, solarized, biosolarized with NP (1.75% and 2.5%), and biosolarized with PM (1.75 and 2.5%). Biomass indicates total lettuce dry mass (g/plot). Health ratings were assigned as follows: 1 = healthy, 2 = mild stunting, 3 = stunting and chlorosis/necrosis, and 4 = severe stunting and chlorosis/necrosis, 5 = dead, with intermediate ratings of 0.5 applied when appropriate. Error bars indicated standard deviation and different capital letters represent significant differences for each parameter according to Tukey's HSD.

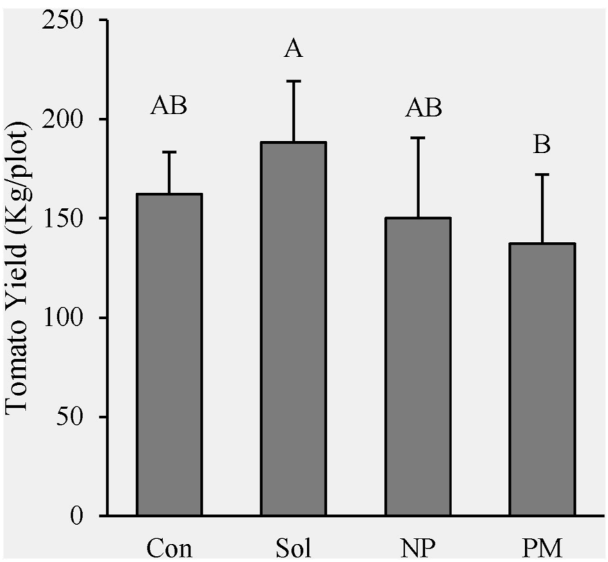

Fig. 5. Total tomato yield (Kg/plot) in treated soil 194 days after the treatment ended. Treatments included control, solarized, biosolarized with NP (1.75% and 2.5%), and biosolarized with PM (1.75 and 2.5%). Error bars indicated standard deviation and different capital letters represent significant differences for each parameter according to Tukey's HSD.

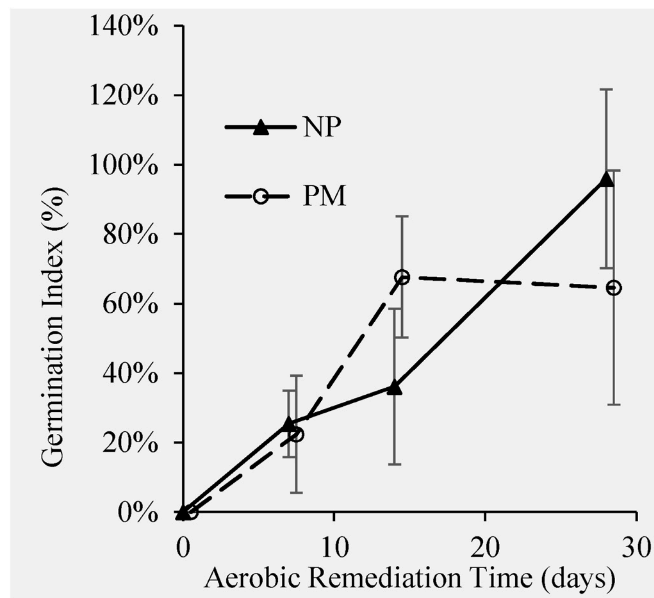

Fig. 6. Mean germination index of radish planted in biosolarized soil after 0, 7, 14, and 28 days of aerobic remediation. Soil was mixed in mesocosms and fermented under thermophilic conditions after 2.5% amendment with NP or PM. Error bars indicated standard deviation (n = 3).

solarized non-amended soils is noticed when compared with untreated soils, although differences were not significant. This response is well-known and has been associated with microbial, chemical, and physical changes in the soil after solarization (Gamliel and Stapleton, 1993; Stapleton and De Vey, 1984). In this study, however, a significantly lower lettuce and tomato yield was observed when comparing NP and PM-amended with solarized soils. Lettuce health was also affected by both organic amendments, as biosolarized plots were significantly less healthy than solarized. Although plants growing in solarized plots performed the best in all cases, no significant differences were observed in plant performance between biosolarized and untreated plots.

The incorporation of crop residues into soil can sometimes decrease productivity of subsequent crops (Bonanomi et al., 2007). One major factor for this is the accumulation of toxic organic acids from the continuous degradation of cellulosic biomass under insufficient aeration (Lynch, 1977). Previous studies using almond hulls and shells as growth media for peppers found significantly lower growth compared to controls despite the higher nutrient contents from the amendments (Valverde et al., 2013), and this was attributed to poor aeration, which may have caused OA accumulation during residue degradation. Previous studies suggested aerobic remediation time of at least 2 weeks can eliminate phytotoxins from biosolarized soil (Achmon et al., 2016), but in this case residual OAs remained in the soil 20–28 days after treatment, and may have contributed to lower lettuce health score. Therefore, the laboratory germination assay confirmed 2 weeks was not sufficient for remediating the soil showing phytotoxicity for radish with GI values under 80%, even with finely-ground amendments. Four weeks of remediation was sufficient to remediate the nonpareil-amended soils, but pollinator-amended soils remained phytotoxic with a GI below 70%. This may be due to the different compositions of the biomass: compositional analysis found nonpareil hulls have on average 41% greater sugar content than pollinator varieties, where pollinator hulls have 36% more fiber and 54% more lignin than nonpareil varieties (Palma et al., 2020). Due to the high soluble sugar content, the NP residues may have a more rapid degradation than the PM, leading them to both become phytotoxic more quickly and remediate quickly. Although laboratory assays cannot be directly compared to field trial results due to differences in plant type, growth stage, scale, and amendment particle size, they are useful in determining trends in phytotoxin remediation. Higher degradation rates from almond hulls may have also contributed to greater phytotoxicity of this amendment compared to the lignin-rich shells (Valverde et al., 2013).

Two hundred and fifty-four days after field biosolarization, tomato plants growing in pollinator-amended plots had a significantly lower yield than in solarized soils, but they were not statistically different to the untreated soils. Presumably, this duration would be sufficient for remediating phytotoxins. One potential reason for the difference in performance of lettuce and tomato plants after PM-amendment may be the different nutritional requirements of the plants. Previous studies correlate extended degradation of high C:N material with lower yield, as decomposition immobilizes nitrogen, leading to deficiencies in plants (Benito et al., 2005). Then, it was one of the reasons the experiment was not able to be repeated in a timely manner, as the waiting time for the almond biomass to completely decompose in the soil to avoid crossed effect or potential phytotoxicity was unknown.

To increase the adoption of the technique, biosolarization must be optimized to ensure disinfestation without diminishing yields of subsequent crops and without extended remediation time. One metadata analysis found phytotoxicity was common where studies amended with over 1.9% crop residues, on the order of those used in this study (Bonanomi et al., 2007). Other studies noted that degradation rate of cellulosic biomass can be increased by grinding material and decreasing particle size (Angers and Recous, 1997; Tarafdar et al., 2001; White and Webber, 2018). Authors have observed that decreasing the particle size of the biomass to 2 mm (smaller than the one applied in this study) will significantly decrease the remediation time (Shea et al., 2021b). Finally, further studies are needed to specify all the mechanisms of almond residue driven phytotoxicity. Besides organic acid toxicity and nitrogen immobilization, plant productivity can be effected by microbial activity, which can decrease oxygen availability (Epstein, 2017; Tiquia, 2010). This study shows how biosolarization could potentially have the same, or greater, benefit than solarization since temperatures were significantly higher. However, it also emphasizes the importance to know the characteristics of the organic amendments and the application procedures and timing that optimize the beneficial effect of disinfesting the soil, while limiting their phytotoxic effect to the following crops. This has applications as an alternative to chemical fumigation in US lettuce cultivation, as lettuces are mainly grown in areas with hot seasonal windows ideal for biosolarization, such as the San Joaquin Valley in California and Yuma County in Arizona. In addition, this sector often cannot afford the cost of chemical applications to disinfect soils, so there is particular need for lower-cost alternatives.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2021.105856.

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