CPS CENTER for PRODUCE SAFETY

**CPS 2013 RFP**
**FINAL PROJECT REPORT**

## Project Title
Remediation and recovery measures to expedite plant or replant of vegetables following soil contamination by *Salmonella enterica*

## Project Period
January 1, 2014 – December 31, 2015


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## Objectives
1. *Determine the optimal low-residue cover crop that will enhance die-off of Salmonella enterica in contrasting soils in Australia and California.*
2. *Determine which single or combined cover crop-solarization interval-flooding combination will facilitate die-off of Salmonella enterica in soil so that there is no re-contamination associated with the re-planting of leafy greens.*
3. *Assess the potential for increase of Listeria sp. and Listeria monocytogenes in cover crop amended soils in laboratory and research (Listeria innocua) and natural (L. monocytogenes) field conditions.*


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**FINAL REPORT**

**Abstract**
Contamination by soil amendments, flooding, contaminated irrigation water, or other sources has resulted in substantial losses of abandoned tender leafy greens for packaged salads. Replicated 1.5 x 3 m plots were inoculated with chicken manure/litter containing attenuated *Salmonella enterica* at $10^3$ or $10^4$ CFU $g^{-1}$, which was intentionally low to reflect a "real-world" natural contamination incident. Plots were treated using solarization or by growing cover crops. For solarization, plots were covered with clear polymer for 36 days. Cover crops (buckwheat, mustard and canola) were grown up to 50 days. Following incorporation, soil was sampled before plots were replanted with baby spinach. The aim of this study was to determine a practical strategy that will enhance remediation of *S. enterica*–contaminated soil and prevent subsequent product contamination following replant of leafy greens.

Trials in Davis silt loam soil revealed limited difference between the fallow controls and cover cropped plots. Unlike mesocosm trials in San Rafael in a high organic matter soil, *S. enterica* die-off in the Davis trials occurred within 30 days. This represented greater than 3-log reduction from the applied inoculum level. For solarization, *S. enterica* was not detected in any covered plot at 36 days, whereas 100% of the non-solarized plots were positive for the applied *S. enterica*. Temperatures at 6 cm under polyethylene row covers reached highs between 42 to 47.5°C during daily cycles while non-covered plots did not exceed 35.8°C. In plots where baby spinach was replanted, no contamination was detected at harvest.

**Background**
The U.S. is the world's largest poultry producer, with over 43 billion total pounds of meat produced annually (USDA, ERS 2013). Consequently, vast amounts of poultry farming by-products are generated every year. Poultry litter and chicken manure are widely utilized as the substrate in commercial leafy greens and leafy culinary herbs production as soil amendment to improve soil quality and for fertility management. Raw litter, stacked and aged manure, composted manure, and thermally-treated and pelletized manure are among the forms applied both pre-planting and as side-dressing during crop growth or following a harvest-regrowth cycle.

The use of animal waste increases the risk for soil contamination. Chicken manure-litter composting and aging prior to field application are of serious concern (Wilkinson et al., 2011; Singh et al., 2012). Reduction of *Salmonella* levels during composting relies on a combination of factors, including thermal pathogen inactivation (with temperatures 55 and 70°C), high moisture levels (60-65%), ammonia volatilization and periodical turning of the manure heap (Fremaux et al., 2008; Franz and Van Bruggen, 2008). However, variability in temperature, moisture and storage time can result in unpredictable compliance with pathogen reduction standards, in which survival and regrowth of enteric bacterial pathogens is possible (Wilkinson et al., 2011). *S. enterica* persistence in horticultural crop soils was demonstrated for extended periods under summer conditions in the Sacramento and San Joaquin Valleys (Danyluk et al., 2008; Lopez-Velasco et al., 2012). In earlier studies, Islam et al. (2004a, 2004b) found that both *Salmonella enterica* and *E. coli* O157:H7 persisted in soil amended with contaminated compost for more than 200 days in lettuce fields and more than 150 days in parsley fields.

There are no validated and economically practical remediation strategies, to date, that growers could implement to help reduce or eliminate human pathogens from soil. In this study we evaluated *S. enterica* survival in contaminated soil in relation to two potential remediation methods: cover crops and solarization.

For the cover crop trials, to anticipate the industry desire to minimize the remediation period and interference from non-crop plant residues during tender greens harvest with mechanized equipment, we tested three short growth-cycle (low-residue) cover crops—buckwheat (*Fagopyrum esculentum*), rapeseed (*Brassica napus*) and mustard (*Brassica juncea*)—selected for their known capabilities of secreting biochemical compounds, with antimicrobial characteristics against enteric pathogens (Moore et al., 1995 and 1998). Cover crop growth and subsequent incorporation could potentially introduce phytochemicals, such as glucosinolates and phenolic compounds, into the soil (Hertog et al., 1996; Rosa et al., 1997; Duthie et al., 2000; Mithen et al., 2003). Naturally occurring glucosinolates are found in the *Brassicaceae* family (Kushad et al., 1999; Cartea et al., 2008) and although biologically inactive, their degradation products are toxic for fungi and bacteria (Brader et al., 2006; Wilson et al., 2013). The *Brassicaceae* family has also been investigated for their phenolic content, constituted by a large group of secondary metabolites occurring more widespread in the plant kingdom (Llorach et al., 2003; Vallejo et al., 2004; Ferrerres, et al., 2005). Flavonoids and hydroxycinnamic acid are the most abundant phenolic compounds found in the plant kingdom, and are reported to have antifungal, antiviral or antibacterial properties (Cushnie and Lamb, 2005; Cartea et al., 2010).

Soil-borne diseases have been traditionally controlled with pesticides, although biological strategies have certainly been employed. However, due to an increased concern about their effects in human and animal health and the environment, solarization became a chemical-free alternative for soil disinfection. It can be a cost-effective and sustainable option when compared to biocidal chemical treatment, with an added advantage of weed suppression. In this study, soil inoculated with *S. enterica*–contaminated chicken manure was solarized by covering plots with clear low density polyethylene (LDPE) known to successfully reduce soil-borne pathogens (Barbour et al., 2002; D'Addabbo et al., 2010). Solarization effectiveness relies on many factors working in concert with the effects of daily trapping the heat from solar radiation. Moreover, adequate polyethylene cover application coupled with sufficient soil moisture content, to improve temperature conductivity, creates the necessary conditions for thermal and microbiological inactivation. Clear polymer covers allow solar radiation to pass into the soil, trapping heat and creating a greenhouse effect, thus increasing soil temperature. Soil color and texture play an important role in solarization (Katan, 1981; Stapleton et al., 1998, 2000 and 2007).

The objective of this study was to determine the practical strategy that will enhance remediation of *Salmonella*-contaminated soil and prevent subsequent product contamination following replant of leafy greens. Based on earlier field observations and preliminary studies, we hypothesized that a short duration of low-residue cover crops, solarization, or a combination of both will be a cost-effective and practical approach to eliminate of *S. enterica* from contaminated soil.

## Research Methods and Results

### Methods:

### 1.1. Inoculum preparation

In this study, stock cultures of avirulent *Salmonella enterica* sv Typhimurium (*att*PTVS 337; rifampicin-resistant, 80 mg $L^{-1}$, a derivative of *S. enterica* sv. Typhimurium $\chi$3895) were maintained at -80°C and aseptically streaked onto tryptic soy agar plates (TSA; Difco, Sparks, MD) amended with rifampicin (Fisher Scientific, Waltham, MA) (80 mg $L^{-1}$) and 1% (w/v) pyruvic acid sodium salt (Fisher, Bioreagents, Fair Lawn, NJ) (TSA-RP) and incubated for 24 h at 37°C. Following incubation, five well-isolated colonies were re-suspended in 1 mL of sterile

Butterfield's phosphate buffer (BPB; Whatman, Florham Park, NJ), 100 µL spread-plated on TSA-RP, and incubated at 37°C for 24 h to allow the formation of confluent colonies. Cell cultures were gently lifted from the agar surface by adding 2 mL of sterile BPB and using a sterile spatula to decant the cell suspension into a sterile beaker. The absorbance of the culture suspension was adjusted to 0.750 at 650 nm using a spectrophotometer (GeneQuant*pro* UV/Vis Spectrophotometer, Amersham Biosciences) to obtain a final inoculum concentration of approximately log 9.0 CFU $ml^{-1}$. Populations were confirmed by surface-plating serial dilutions on TSA-RP and incubating as described above.

### 1.2. Chicken manure and litter inoculation

Over twelve attempts to identify a naturally contaminated source of chicken manure from large commercial poultry operations and commercial compost yards were made to use as field inoculum. None were successful and, therefore, we resorted primarily to inoculating chicken manure pellets to have a standard inoculum. A total of 100 kg of chicken pellets (Nutri-Rich 4-3-2, Ca 7%, Organic Pellets; J & D Fertilize, Canby, OR), combined with collected non-treated chicken litter, were inoculated with *att*PTVS 337. For every 2 kg of dried chicken pellets, 600 mL of log 8 CFU $mL^{-1}$ of *att*PTVS 337 were added and mixed evenly for a subsequent organic matrix inoculation. After inoculation, pellets were left at room temperature for 3 days and manually broken up into smaller size particles. For field inoculation, 500 g of inoculated chicken pellets, and approximately 2 kg of chicken litter to increase organic content, were spread over plots (3 x 1.5 m) to obtain target concentrations of $10^3$ or $10^4$ CFU $g^{-1}$ of soil. Plots were then disked and mixed manure incorporated to a depth of 15-25 cm.

### 1.3. Initial microcosm studies of *Salmonella* survival in crop residue

Two kilograms of Yolo silty clay loam (Table 1 – see Appendix 1) for each crop or cover crop residue treatment was sieved (U.S. Standard Sieve # 70; 0.21 mm) and 50 g of pulverized chicken manure pellet inoculum were blended in a polymer bag by repeated inversion. From the initial *Salmonella* population density of log 7 CFU $g^{-1}$, the final inoculum density in each soil matrix was determined to be approximately log 3.8 CFU $g^{-1}$. Three hundred grams of field grown and moderately chopped crop residue ('baby' arugula and spinach) or cover crop residue, approximately 4 $cm^2$, were incorporated into 1.5 kg of the inoculated soil and distributed among three plastic containers and randomized on a bench within a 20°C growth chamber. Three pots containing only 500 g of inoculated soil were included as No-residue Controls. Soil in all pots received a single irrigation at Day 0 to bring moisture content to approximately 70% of field capacity, as may occur in a field incorporation event, and was subsequently sampled after 1, 13, 20 and 34 days. At each sample time-point, three 10-g cores were removed from each replicated treatment. This study was repeated once.

### 1.4. Cover crop field trial

Following field inoculation using chicken manure pellets and chicken litter, plots were seeded using selected cover crops: buckwheat (*Fagopyrum esculentum*), rapeseed (*Brassica napus*) and mustard (*Brassica juncea*). Cover crops were grown for 30 (mesocosm trials in San Rafael, CA) and up to 50 (full field trial, UC Davis) days post emergence. The control treatment remained fallow during the entire trial duration, although weed emergence was significant in some plots/year due to inconsistent or lapsed contract farm management timing.

After mowing, cover crop residues were allowed to surface dry for 3 days, followed by incorporation into the soil. *S. enterica* populations in soil were monitored in all plots for 9 weeks. For each time point, 48 and 24 samples were collected in the Davis and San Rafael trials, respectively. To assess the potential for low levels of surviving populations of *S. enterica* in soil

to contaminate replanted crops, spinach and red chard were planted into the various treatment plots at 50 to 60 days post initial inoculation (DPI) and harvested when grown to 4-6 true leaves.

For Davis trials, two days prior to cover crop incorporation, Actigard 50WG (Syngenta; Basel, Switzerland), a selective non-pesticidal systemic compound, was applied at a labeled concentration of 0.84 Kg $ha^{-1}$ (0.75 lb $A^{-1}$) to half the plots per cover crops. Actigard 50WG, used for control of several fungal, bacterial, and viral plant diseases, has a unique mode of action that mimics the natural systemic activated resistance (SAR) response found in most plant species. We were interested to determine whether this treatment would induce elevated levels of antibacterial compounds in the cover crops, prior to incorporation.

### 1.4.1. Microcosm solarization

To preliminarily assess the effectiveness of soil temperature in *Salmonella* inactivation, prior to field testing, microcosm studies were conducted. Yolo silty clay loam soil for microcosm solarization was collected from the same location where field solarization was done. After collection, 180 g of soil was sieved, as described above, and transferred into clear polystyrene cups (9.5 x 9 cm; Hiplas, Mira Lomas, CA) followed by the addition of 20 g of inoculated chicken manure. After samples were homogenized, 20 mL of sterile Nanopure water was added, mixed once again, and total soil weight recorded. Soil weight was monitored every 3 days and water was added as needed to maintain soil water content to its starting weight value.

Cups were covered with polymer wrap with perforated holes (8 holes, 0.3 cm each) to allow ventilation. Covered soil cups were placed in plastic containers (30 x 43 x 20 cm) on moistened paper towels lining the container bottom to keep high relative humidity, as with field tarping. Soil cups in containers were held at 4 temperatures: 29, 37, 48, or 55°C. Lower temperature soil samples were assessed weekly for *S. enterica* survival, whereas higher temperature exposures were sampled more frequently within the first 24 h. Temperature and relative humidity in each master-container was monitored with a TempTale 4 data logger (Sensitech, Beverly, MA) whereas WatchDog B100 data loggers (Spectrum Technologies Inc., Aurora, IL) were used to record soil temperature.

## 1.5. Field solarization

During Year 1, the solarization trial was carried out at the seasonal summer peak for consistent solar radiation. After inoculation, plots were irrigated to soil field capacity and allowed to dry for 2 days. Each 3 × 1.5 m plot (n=6) was covered with sheets of either 4-mil (101.6 µm) or 6-mil (152.4 µm) thick clear polyethylene (Film-Gard, Minneapolis, MN). To prevent flapping and tearing, corners and edges were staked and covered with a layer of soil. Prior to plastic film application, WatchDog B100 data loggers were placed 6 cm below the surface in the center of each plot to monitor diurnal temperature fluctuations over the course of the experiment. Positive control plots remained fallow and uncovered during the entire trial. After solarization, soil samples were collected to assess *S. enterica* prevalence in soil.

In Year 2, the solarization trial was carried out in mid-fall to represent a more limited and variable condition for remediation. After inoculation, plots were irrigated to soil field capacity and allowed dry for 2 days. Each 3 × 1.5 m plot (n=6) was covered with sheets of 4-mil thick clear polyethylene (Film-Gard). Other conditions were identical to Year 1 trials described above.

## 1.6. Soil *Salmonella* recovery

### 1.6.1. Soil extraction

Following homogenization of composite soil samples, 100-g replicates were placed in Whirl-Pak bags (Nasco, Salida, CA) and 200 mL of 0.01 M sodium phosphate supplemented with 0.05% Tween 20 was added (Fisher, Fair Lawn, NJ) (NPT) to assist in detaching cells from the silt-clay fraction. The suspension was gently shaken and allowed to settle for 20 min (Gutierrez-

Rodriguez et al., 2011; Lopez-Velasco et al., 2012) and plated onto CHROMagar™-Salmonella Plus amended with rifampicin (80 mg.$L^{-1}$) and 1 g $L^{-1}$ sodium pyruvate (Fisher) (ChromSalP+RP) followed by incubation for 24 h at 37°C.

#### 1.6.2. Soil and plant enrichment

For direct soil enrichment, 100-g replicates of homogenized composite soil were placed in Whirl-Pak bags to which 200 mL of double strength (2X) buffered peptone water (BPW; Difco) amended with rifampicin (80 mg $L^{-1}$) (2X BPW-R) were added. Samples were gently massaged and incubated at 37°C for 12-14 h. Plants were harvested from the field by careful hand-cutting at the base, avoiding any soil contact. Once collected, plant material was diced, using a sterile stainless steel knife, into 5-cm squares, homogenized and weighed out into 150-g samples to later be placed in Whirl-Pak bags holding 400 ml $g^{-1}$ of BPW-R. Samples were massaged for 3 min and incubated for 24 h at 37°C

### 1.7. Spinach residue and cross contamination

#### 1.7.1. Spinach inoculation, incorporation and cross contamination

To complement cover crop studies, additional trials to assess options for management of contaminated crop residue were conducted. *S. enterica* inoculum preparation was performed as described above. Spinach (*Spinacia oleracea* L. cv Tigercat) was grown for 30 days in replicated plots and spray-inoculated with log 8 CFU $ml^{-1}$ *S. enterica*. Spray inoculation was performed by using a $CO_2$ powered handheld backpack sprayer set at 30 psi, with two nozzle spray boom and teejet 8005 tips. Three days post inoculation (DPI) plants were either (1) mowed and incorporated, (2) cut and left on top or (3) cut sprayed with Round up, followed by incorporation into the soil 6 days later. Soil was assessed for *Salmonella* soil presence after 2 and 3 weeks post spinach incorporation. After the last soil sampling, red chard (*Beta vulgaris* cv. *cicla*) was seeded to assess for *S. enterica* cross contamination. Red chard was harvested 21 days post emergence when grown to 4-6 true leaves.

### 1.8. *Salmonella* detection and culture confirmation

#### 1.8.1. Plant *Salmonella* quantification and enrichment

Plants were harvested from the field by cutting at the base, avoiding any soil contact. Once collected, samples were diced, using a sterile stainless steel knife, into 5-m squares, homogenized, and then 100-g samples were placed in Whirl-Pak bags holding 200 mL sterile potassium phosphate buffer (3.9 mMKH2PO4 and 6.1 mMK2HPO4) supplemented with 0.05 % Tween 20, and bags were massaged for 1 min to detach bacteria. For quantification, 100-µl and 1-mL aliquots were spread onto ChromSalP+RP and incubated for 24 h at 37°C. For qualitative analysis, an additional 200 mL of 2X BPW was added to sample bags, massaged for 2 min and incubated for 24 h at 37°C. After incubation, qualitative assessment was performed by spot plating triplicates of 33.3 µl onto ChromSalP+RP and incubating for 24 h at 37°C.

#### 1.8.2. Sample lysis for molecular detection

For DNA extraction, 1 mL of soil or plant enrichment was transferred to a 1.5-mL Eppendorf tube for pelleted centrifugation at 1500 ×g for 3 min. The pellet was re-suspended and washed three times in 1 mL BPB and finally re-suspended in 500 µL of BPB. To obtain a lysate for PCR screening, a 200-µL aliquot was transferred to another tube and placed in a heating block for 10 min at 95°C for 10 min.

#### 1.8.3. Probe-based quantitative real time PCR

Soil enrichments were screened to detect the primary *Salmonella* pathogenicity marker using Taqman® probe-based quantitative real-time PCR (qrt-PCR). Each reaction was composed of 10 µL of a 2× Taqman Gene expression master mix (Applied Biosystems Inc., Foster City, CA), 0.5 µM of forward and reverse primers, 2.5 pmol of probe targeting *invA* gene (probe, FAM-CAATGGTCAGCATGGTATA-MGBNFQ; forward, TGGGCGACAAGACCATCA and reverse

TTGTCCTCCGCCCTGTCTAC) (Ziemer et al., 2003) and 2 µL of washed enrichment (BPW) for a final volume of 20 µL. Each reaction, including amplification of selected genes, was obtained by thermocycler (7300 Real Time PCR System, Applied Biosystems Inc.) protocol consisting of one cycle of 50°C for 5 min, one cycle of denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 30 s and annealing at 60°C for 1 min.

#### 1.8.4. Inoculum recovery and isolation and culture confirmation

*S. enterica* culture isolation was obtained by plating Salmonella immunomagnetic beads (Applied Biosystems, Life Technologies AS, Oslo) onto ChromSalP+RP followed by incubation for 24 h at 37°C. Mauve colonies on ChromSalP+RP were considered as presumptive positives. Isolated colonies were purified on the same media, and cell lysis was performed as described above. Confirmation was done with mPCR targeting the *inv*A gene (Ziemer et al., 2003).

### 1.9. Statistical analysis

Bacterial populations were converted to log scale and subjected to ANOVA using JMP 12.0 software (SAS Institute, Cary, NC). The Tukey-Kramer method was used to identify significant differences in populations of bacteria, with a *P* value of $< 0.05$ considered significant.

## Outcomes and Accomplishments

### Initial microcosm studies of *Salmonella* survival in crop residue

Survival of the inoculated *Salmonella* in the chicken manure pellet matrix was variable among the control and arugula, spinach, buckwheat, canola, and black mustard residues between initial incorporation and 34 days of incubation (Table 2 – see Appendix 1). From Day 1 to Day 20, *Salmonella* survival declined approximately 1.5 log in the soil-only controls and approximately 2.4 to 3.4 log in the cover crop residues. Crop residues, arugula and spinach, were observed to have a similar range of decline to quantitative recovery. At Day 34, *Salmonella* populations were near or at the established quantitative limit of detection (log 0.43 CFU $g^{-1}$), but 100% of control soils were positive by enrichment as compared with more variable survival among crop residue treatments, ranging from 33 to 100% among replicates.

### Mesocosm cover crop trials

*S. enterica*, at an initial concentration of log 3 CFU $g^{-1}$ of soil, declined slightly (1 log) in all treatments one month later, prior to cover crop incorporation (Table 3). Moreover, 11 days post incorporation (DPC) populations continued to decline to the quantitative limit of detection (log 0.43 CFU $g^{-1}$ of soil). Almost a month after incorporation, *S. enterica* was still quantifiable as in the previous time point. Qualitative detection with qPCR showed a decline in marked-*Salmonella* presence among canola and buckwheat cover-cropped plots, with 33.3 and 16% positive plots, respectively, whereas fallow control plots remained 100% positive.

Statistical analysis comparing *S. enterica* soil populations among treatments was not significant ($P > 0.05$) across all time points due to high variability. Regardless of the treatment, by 80 and 94 DPI, all plots (including control) were qualitatively negative for *S. enterica*. Spinach and red chard harvested 25 and 39 days after plant emergence (80 and 94 DPI, respectively) did not result in positive detection of marked *S. enterica* on the harvested leaves (Table 3).

In Year 2 trials, *S. enterica* soil concentration in all treatments declined by 5 logs in 5 weeks prior to cover crop incorporation and remained stable the rest of the trial at the quantitative limit of detection (log 0.43 CFU $g^{-1}$ of soil). At 4 weeks after incorporation, qualitative detection of *S. enterica* with qPCR showed a general decline in the number of positive plots, with canola and mustard cropped plots having 66.7% positives and buckwheat and control plots only 33.3% positives. In all treatments at any given time point post crop

incorporation, there was no significant difference ($P > 0.05$) when comparing *S. enterica* soil populations (Table 4 – see Appendix 2).

**Cross contamination study**

After spinach inoculation and incorporation, *S. enterica* soil populations ranged between log 2 and 3.4 CFU $g^{-1}$ of soil 14 days post incorporation (DSI). Populations remained almost unchanged 22 DSI. However, seeding of red chard into the treated plots resulted in no positive detection of *S. enterica* at 50 DPI or 43 DSI (Table 5).

**UC Davis cover crop field trials**

*S. enterica* initial populations declined in soil by approximately 1 log across all treatments in the full field trial conducted during the summer months. Within 30 days, populations dropped to below the standard limit of quantitative detection, representing greater than a 3-log reduction from applied inoculum levels. Soil sampling events at 50 and 62 DPI resulted in no detectable survivors by enrichment and qPCR (Table 3).

Due to the absence of *S. enterica* in soil before and after cover crop incorporation, no soil analysis was done comparing Actigard-treated and non-treated plots after incorporation (Table 3). Seeding of spinach and red chard into the treated plots 62 DPI and 12 DPC, did not result in positive detection of marked-*Salmonella* on the subsequent crop at 125 g of leaf tissue per plot (n = 48).

In Year 2 Davis field trials, *S. enterica* soil assessment prior to cover crop incorporation (50 DPI) resulted in no detectable survivors. The same results were obtained 12 days later. In contrast, during the mesocosm trial, conducted in a cooler climate and higher soil organic matter content, *S. enterica* survived well before cover crop incorporation (30 DPI) and remained present in low populations for the following two assessments (41 and 55 DPI), with canola and buckwheat treatments having the least detectable positives. Soil assessment outcomes in UC Davis silty clay loam taken before and after crop incorporation, revealed a rapid decline in recoverable *Salmonella* and limited difference between the fallow controls and replicated plots planted to Brassica rapeseed, black mustard, or buckwheat cover crops. Both trials took place in different locations within Northern California. The San Rafael mesocosm trial was conducted during late spring (May and August), while the Davis trial was took place in late summer (August-September). San Rafael's moderate climate, with maximum daily temperatures between 16.1 to 34°C, contrast to Davis Central Valley's dry and hot climate, with maximum daily temperatures between 21.6 and 40.6°C. Consequently, this difference in trial temperatures was reflected *S. enterica* soil survival/die-off prior to crop incorporation (Figure 1 and 2). Based on these outcomes, selected cover crops grown as a remediation for *S. enterica*–contaminated soils are inconclusive as die-off was uniform across all treatments.

*S. enterica* soil survival timelines are also determined by initial numbers of organism. In this project, inoculum levels were selected intentionally low to reflect real-world conditions seen in previous projects of naturally-contaminated-soil incidents. The initial low inoculum dose, combined with *S. enterica* environmental fitness and the potential for antagonistic indigenous soil microbiota, could also be responsible for its rapid decline and die-off before crop incorporation (Gerba and Bitton 1984; Santamaria et al., 2003; Jacobsen and Bech, 2012).

Additional cover crop trials were conducted in Year 2 with new varieties added as short-duration treatments. Similarly to previous trials, *S. enterica* soil populations declined to the limit of detection (log 0.43 CFU $g^{-1}$ of soil) with a ~6 log reduction in 4 weeks prior to cover crop incorporation. Following incorporation (5 and 20 DPC), *S. enterica* soil populations remained unchanged. During Year 2 Davis cover crop trial 2, *S. enterica* initial decline was followed by a prolonged survival. In this trial, however, all controls plots remained positive until the last sampling 48 DPI (Table 4). From the seven cover crops grown, broccoli A (cv. Greenbelt)

cropped plots had the least amount of *S. enterica* positives after incorporation and at the last time point (48 DPI). Although these results might indicate that broccoli could be a good candidate as a cover crop for remediation, more trials need to be conducted to affirm the results obtained. Unlike the 2014 trials, *S. enterica* soil quantification did not greatly differ between trials, surviving quantitatively and qualitatively before and after crop incorporation. Common Vetch populations 20 DPC were significantly higher ($P < 0.05$) than broccoli B, triticale, Sudangrass, barley, and control plots. Qualitative soil assessment of *S. enterica* survival resulted in all plots positive at any given time point for barley and common Vetch cropped plots. Conversely, the number of positives in broccoli B and triticale plots declined to 66.6% whereas broccoli A cropped plots had only 1 positive. These qualitative results were repeated 20 DPC and, together with Sudangrass, were the only treatments showing a decline in *S. enterica* positive cropped plots. Unlike in previous trials, all control plots stayed quantitatively and qualitatively positive for the entire trial (Table 4).

Subsequent replanting and growth of baby spinach and red chard resulted in no evidence of cross contamination from soil with qualitatively positive survival prior to the 60-day mark of no-crop in accordance to the replanting period by the Best Practice Guidance.

**Solarization**

***Salmonella survival in soil***

**In-vitro solarization:** *S. enterica* soil survival during in-vitro solarization depended on soil temperature. The initial population of log 5 $CFU^{-1}g$ of soil was reduced 4 to 5 logs within 4 days for soil stored at 37 and 29°C, and within 18 and 2 h for soil treated at 48 and 55°C, respectively (Table 6). Elimination of *S. enterica* from soil needed 4, 13 and 21 days for soils stored at 48, 37 and 29°C, respectively, whereas 4 h was needed at 55°C. During solarization, it took 2.5, 7, 8.5 and 12.5 hours for soils at 29, 37, 48 and 55°C, respectively, to reach the desired temperature.

**Solarization field trials:** Initial *S. enterica* soil populations of log 5 CFU $g^{-1}$ declined 1 log prior to plastic application. During solarization, populations continued to decline by 2 to 3 logs prior to plastic removal; however, there was no statistical difference ($P > 0.05$) among treatments. Soil assessment after 20 days plastic removal showed that populations of *S. enterica* in solarized plots impacted by birds were greater than in fallow control plots ($P < 0.05$) (Table 7). Quantitative comparison between initial counts and final time point (DPI) indicated a 4.8, 4.1 and 3 log reduction for control, solarized, bird impacted plots respectively (Table 7). Qualitative analysis at any given time and for all treated and untreated plots resulted in 100% positive for *S. enterica* presence.

***Daily and hourly soil and air temperature Year 1***

Data loggers buried in soil under the polymer row covers highest recorded temperature reached between 35.5 to 37°C during daily cycles. On average, the maximum daily temperature recorded in solarized plots ranged between 31.5 to 14.5°C, and the 30°C mark was surpassed only in the first 6 days corresponding to 17% of the solarization timeline. Consequently, for 43% of the time the soil daily maximum temperature only ranged between 20 and 25°C. Control and solarized plots showed a greater temperature difference ($P < 0.05$) when solarized soil surpassed 30°C, and as the daily temperature declined this gap became smaller (Figure 3, 4).

Hourly soil average temperature reached its highest values of 24 and 19°C for solarized and control plots, respectively, for only 2-2.5 h (between 13:00 and 15:30). Similarly, average hourly air temperature reached 17.7°C between 15:00 and 16:00 (Figure 4).

Maximum hourly temperature readings of 35.5 to 37°C were reached between 12:30 and 15:00, 4.5 h before sunset (17:00), while the lowest temperatures were recorded at sunrise (6:30). Fallow control plots' maximum hourly temperature only exceeded 25°C by 12:00 and lasted for 5 hours. Hourly maximum soil temperature comparisons between solarized plots and

controls indicated a greater difference, above 9 °C between 12:00 and 13:00, overlapping the highest recorded reading of 37°C (Figure 4).

Initial *S. enterica* soil populations of log 4 CFU $g^{-1}$ were reduced by 2 logs prior to plastic application 4 days later. After 11 days of solarization treatment, *S. enterica* counts declined to below the standard limit of detection corresponding to a greater than 4-log reduction.

After a month of solarization with polyethylene tarps, both treatments of 4-mil and 6-mil resulted in no detectable post-enrichment recovery of *S. enterica*, while 100% of the non-solarized plots were positive for the applied *att*PTVS 337. Similar results were observed 8 days later at 47 DPI (Table 3). In plots where baby spinach was replanted into treated and fallow plots, no contamination by applied marked-*Salmonella* was detected at harvest.

***Daily and hourly soil and air temperature Year 2***

Data loggers buried in soil under the polymer row covers recorded temperature highs between 40.5 to 47.5°C during daily cycles. On average, solarized plots with 6-mil and 4-mil polymers showed a cumulative 5.5 and 6 h per day above the “kill zone” of 40°C between 14:30 and19:30 and 14:00 and19:30, respectively. Non-covered control plot temperatures did not exceed 35.8°C, and reached their highs also in the afternoon between 13:30 and 20:00 (Figure 3, 4).

Soil hourly temperature readings in solarized plots surpassed the 40°C “kill zone” only in the afternoon between 14:00 and 19:30. The cumulative hours between 40 to 45°C for 6-mil and 4-mil represented 23 and 25% of the day, respectively, equivalent to a total of 198-216 h for the entire solarization time. The control plots stayed 29% of the day at temperatures 10 °C lower (Figure 6, 7, 9).

Maximum hourly temperature readings were reached 2.5 h before sunset (19:30), while minimum temperatures were recorded 2 h after sunrise (8:00). The average highest hourly recorded temperatures were 43.3±2.0, 43.7±1.9 and 33±1.7 °C for 6-mil, 4-mil and control, respectively. Average lowest hourly recorded temperatures for 6-mil, 4-mil and control were 28.1±1.6, 27.8±1.7, 21.8±2.0 °C, respectively (Figure 4).

Fallow control plot hourly temperatures only exceeded 30°C by 13:30, and although lasting for 7 h, never surpassed 36°C. Hourly soil temperature comparison between solarized plots with fallow controls indicated a greater difference, above 10 °C between 15:00 and 19:30, coinciding with the highest reading above 40°C of the solarized plots.

Air temperatures were lowest (13.8±1.6 °C) before sunrise, and reached 30°C and higher by 14:00 and then remained between 30 to 33°C for 5 hours before starting to decline one hour before sunset (Figure 4).

**Soil analytical assessments**

Due to the lack of significant differences among treatments in the UC Davis field trials in two years, the compositional analysis of antimicrobial compounds from cover crops and impacts on the soil microbiota were deferred. In the absence of treatment effects, these were not likely to be productive to explore mechanisms for optimization. However, all retained frozen soil samples have been extracted for analysis and, although delayed by lack of technical staff, these objectives are in progress and will be added as a Supplemental report to CPS and to journal manuscripts.

Similarly, the final stage of detection, post-enrichment, of *Listeria* in soil following cover cropping was deferred because of limited priority due to the primary outcomes.

## Summary of Findings and Recommendations

The outcome of the primary objective, cover cropped experimental field trials to assess the effectiveness of treatments on the rate of applied *S. enterica* die-off, was greatly limited by the accelerated loss of viability in fallow controls, very similar to plots with cover crop residues. Achieving the Expected Measurable Outcomes in relation to developing recommendations for benefits from limited duration crop growth periods for cover crops as a remediation treatment was therefore largely unresolved.

In contrast, solarization trial outcomes were more generally satisfactory in that the goals proposed in this project were realized by successfully eliminating *S. enterica* from contaminated soils prior to the industry standard 60-day no-crop period recommended by Best Practices guidance. However, repetition of solarization trials should be conducted under different field conditions and soil types to develop accurate recommendations for remediation treatments.

There are many factors contributing to *S. enterica* elimination in solarized soil. Thermal inactivation of pathogens is the most important physical mechanism during solarization, in which soil is heated to lethal temperatures for bacteria, plant, soil pathogens, pests, etc. It has been suggested that solarized soil lethal temperatures should be higher than 39–40°C (Stapleton and DeVay, 1995; Wu et al., 2009; D'Addabbo et al., 2010). Wu et al. (2009) successfully inactivated *E. coli* from soil after 4 weeks of solarization with 40°C or higher temperatures.

In-vitro solarization results from the present study are in accord with suggested lethal temperature above 40°C. When soil cups were incubated in temperatures below threshold, *S. enterica* survival was prolonged for up to 13 and 21 days for 29 and 37°C, respectively, whereas elimination at 55 and 48°C only needed 4 and 96 h, respectively. Based on 2014 solarization results, solarized soil temperature during the summer will be maintained at temperatures above 40°C for 6 h per day. Consequently, 94 consecutive hours at 48°C of in-vitro solarization would represent 16 days of field solarization in above 37°C weather.

Polyethylene covers increase soil temperature by trapping solar radiation that passes through the cover and converting it to longer wavelength infrared energy, thus producing a "greenhouse" effect. The highest temperatures in soil profiles are found in the upper 15 cm and decrease with depth to sub-lethal temperatures (Stapleton 2000, D'Addabbo et al., 2010). During solarization trials, temperatures dropped on two occasions, both of them after rainstorm events with cumulative precipitation levels of 21.7 and 11.8 mm, respectively. Thermal soil effects, together with moisture retention and an increase of soluble mineral nutrient availability, have strong implications in soil microflora population shifts. While mesophilic populations rapidly decrease, thermotolerant and thermophilic bacteria thrive and re-colonize the soil (Katan, 1987; Stapleton and DeVay, 1995).

A limitation in the execution of this objective was the presence of high populations of American crow birds, which were attracted to feed on the chicken manure pellets and thereby damaged the plastic tarp, letting heat escape. This problem was especially acute in Year 2. Evidence of severe bird damage in half of the solarized plots could explain the increase in population counts at the last time point. Although there was no significant difference ($P > 0.05$) in soil temperature between damaged and intact plots, heat penetration might not be deep enough to inactivate *S. enterica* in the soil top layer.

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## APPENDICES

### Publications and Presentations

No publications have been submitted but one for cover crop trials and one for solarization trials are being prepared.

Six presentations of the work in progress have been made during the project period, including at the 2014 and 2015 CPS Annual Research Symposium and at meetings organized by the CA Leafy Greens Research Board and the University of Sydney (by Co-PI McConchie).

### Budget Summary

The budget for this project was adjusted to reflect the inability to hire an appropriate Postdoctoral Researcher to play a senior role in its implementation. Full reconciliation of the budget line items to reflect full expenditure of allocated funds is in progress.

### Tables and Figures

See Attachment for Appendix 1 and 2:

Appendix 1. Tables and Figures 2014 (includes Tables 1–3, Figures 1–5)
Appendix 2. Tables and Figures 2015 (includes Tables 4–7, Figures 6–9)

## Appendix 1. Tables and Figures 2014

**TABLES**

**Table 1.** Soil analysis for microcosm and field trials 2014-15; Yolo silty clay loam.

| Depth Range (cm) | Horizon Designation | Percent Silt | Percent Clay | Percent Sand | Percent Organic Matter | pH by water Extraction |
|---|---|---|---|---|---|---|
| 0 - 36 | H1 | 66 | 21 | 11.3 | 2 | 7 |
| 36 - 152 | H2 | 68 | 22.5 | 9.5 | 0.75 | 7.5 |

**Table 2.** Initial microcosm trial of *Salmonella enterica* sv. Typhimurium (attenuated) in Yolo clay loam.

| Crop Residue or Cover Crop Trial 1 | 1 DPI | 13 DPI | | | 20 DPI | | | 34 DPI | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Log CFU/g soil ± st dev (% enrichment positives) | | | | | | | | |
| **Arugula** | 3.84 ± 0.05 | 2.48 | ± 0.46 | (100) | 1.24 | ± 0.68 | (100) | 0.43 | ± 0.00 | (66.6) |
| **Spinach** | | 2.56 | ± 0.71 | (100) | 0.58 | ± 0.17 | (100) | 0.43 | ± 0.00 | (100) |
| **Buckwheat** | | 2.80 | ± 0.54 | (100) | 1.13 | ± 0.16 | (100) | 0.45 | ± 0.03 | (66.6) |
| **Canola** | | 2.53 | ± 0.18 | (100) | 0.46 | ± 0.03 | (100) | 0.43 | ± 0.00 | (100) |
| **Mustard** | | 2.31 | ± 0.35 | (100) | 0.80 | ± 0.37 | (100) | 0.43 | ± 0.00 | (33.3) |
| **Control** | | 3.09 | ± 0.95 | (100) | 2.43 | ± 0.59 | (100) | 0.61 | ± 0.30 | (100) |

| Crop Residue or Cover Crop Trial 2 | 1 DPI | 13 DPI | | | 20 DPI | | | 34 DPI | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Log CFU/g soil ± st dev (% enrichment positives) | | | | | | | | |
| **Arugula** | 3.84 ± 0.05 | 2.74 | ± 0.05 | (100) | 0.71 | ± 0.48 | (66.6) | 0.43 | ± 0.00 | (66.6) |
| **Spinach** | | 2.67 | ± 0.23 | (100) | 0.99 | ± 0.69 | (100) | 0.61 | ± 0.30 | (66.6) |
| **Buckwheat** | | 2.47 | ± 0.63 | (100) | 1.31 | ± 0.56 | (100) | 0.43 | ± 0.00 | (66.6) |
| **Canola** | | 2.59 | ± 0.88 | (100) | 0.80 | ± 0.37 | (100) | 0.43 | ± 0.00 | (33.3) |
| **Mustard** | | 2.27 | ± 0.55 | (100) | 0.48 | ± 0.00 | (100) | 0.43 | ± 0.00 | (66.6) |
| **Control** | | 3.09 | ± 0.95 | (100) | 2.43 | ± 0.59 | (100) | 0.61 | ± 0.30 | (100) |

**Table 3.** *Salmonella enterica* soil recovery during cover crop and solarization remediation treatments.

| | | | **Log CFU g$^{-1}$ soil ± st dev** *(% enrichment positives)* | | | |
|---|---|---|---|---|---|---|
| **San Rafael Mesocosm Trial** | | | | | | |
| **Cover Crop** | **n** | **3 DPI*** | **30 DPI€ £** | **41 DPI (11 DPC)** | **55 DPI (25 DPC)** | **80 (25 DPSe) &94 DPI** |
| **Canola** | 6 | 3.33 ± 0.42 | 2.01 ± 0.93 A[a] (100) | 0.43 ± 0.00 A (83.3) | 0.43 ± 0.00 A (33.3) | 0.43 ± 0.00 A (0) |
| **Mustard** | 6 | | 2.32 ± 0.72 A (100) | 0.43 ± 0.00 A (66.7) | 0.57 ± 0.34 A (83.3) | 0.43 ± 0.00 A (0) |
| **Buckwheat** | 6 | | 1.78 ± 0.68 A (100) | 0.61 ± 0.43 A (50) | 0.61 ± 0.43 A (16.7) | 0.43 ± 0.00 A (0) |
| **Control** | 6 | | 2.29 ± 0.49 A (100) | 0.43 ± 0.00 A (83.3) | 0.89 ± 0.43 A (100) | 0.43 ± 0.00 A (0) |
| **Cover crop Davis Field Trial** | | | | | | |
| **Cover Crop** | **n** | **0 DPI** | **1 DPI** | **21 DPI** | **29 DPI** | **50 DPI€ £ & 62 DPI** |
| **East Side *** **Canola** | 6 | 3.20 ± 0.05 | 1.99 ± 0.21 | 0.85 ± 0.43 A (100) | nd | 0.43 ± 0.00 A (0) |
| **Mustard** | 6 | | 2.24 ± 0.36 | 0.72 ± 0.34 (100) | nd | 0.43 ± 0.00 A (0) |
| **Buckwheat** | 6 | | 1.97 ± 0.45 | 0.72 ± 0.34 (100) | nd | 0.43 ± 0.00 A (0) |
| **Control** | 6 | | 2.26 ± 0.49 | 0.91 ± 0.54 (100) | 0.43 ± 0.00 (16.7) | 0.43 ± 0.00 A (0) |
| **West Side** **Canola** | 6 | 3.32 ± 0.14 | 1.93 ± 0.32 | 0.94 ± 0.43 (100) | nd | 0.43 ± 0.00 A (0) |
| **Mustard** | 6 | | 2.22 ± 0.45 | 0.75 ± 0.38 (100) | nd | 0.43 ± 0.00 A (0) |
| **Buckwheat** | 6 | | 1.93 ± 0.24 | 0.72 ± 0.31 (100) | nd | 0.43 ± 0.00 A (0) |
| **Control** | 6 | | 2.16 ± 0.29 | 0.93 ± 0.41 (100) | 0.43 ± 0.00 (0) | 0.43 ± 0.00 A (0) |
| **Soil Solarization** | | | | | | |
| **Treatments** | **n** | **0 DPI** | **4 DPI & 1 DPS** | **14 DPI & 11 DPS** | **39 DPI & 36 DPS** | **47 DPI & 8 DPR** |
| **Polyethylene 4 mil** | 3 | 4.20 ± 0.33 | 2.06 ± 0.57 A[a] (100) | 0.43 ± 0.00 A (33.3) | 0.43 ± 0.00 A (0) | 0.43 ± 0.00 A (0) |
| **Polyethylene 6 mil** | 3 | | 2.44 ± 0.59 A (100) | 0.43 ± 0.00 A (100) | 0.43 ± 0.00 A (0) | 0.43 ± 0.00 A (0) |
| **Positive Control** | 2 | | 2.12 ± 0.90 A (100) | 0.43 ± 0.00 A (100) | 0.43 ± 0.00 A (100) | 0.43 ± 0.00 A (100) |

[a] Means with different letters represent populations that differ in significance ($P < 0.05$) between wash water treatments.
Cover crop trials abbreviations: Days post inoculation (DPI), Days post cover crop incorporation (DPC), Days post seeding (DPSe)
€ Cover crop incorporation.
£ Soil samples were collected prior to cover crop incorporation.
*Actigard 50W sprayed on east side of the field, 2 days before cover crop incorporation (48 DPI)
Solarization trial abbreviations: Days post solarization/plastic application (DPS) and Days post plastic removal (DPR)
nd: Not done.
Limit of detection: log 0.43 CFU g$^{-1}$ of soil

**FIGURES**

**Figure 1 and 2.** Daily air temperature during cover crop and solarization trials

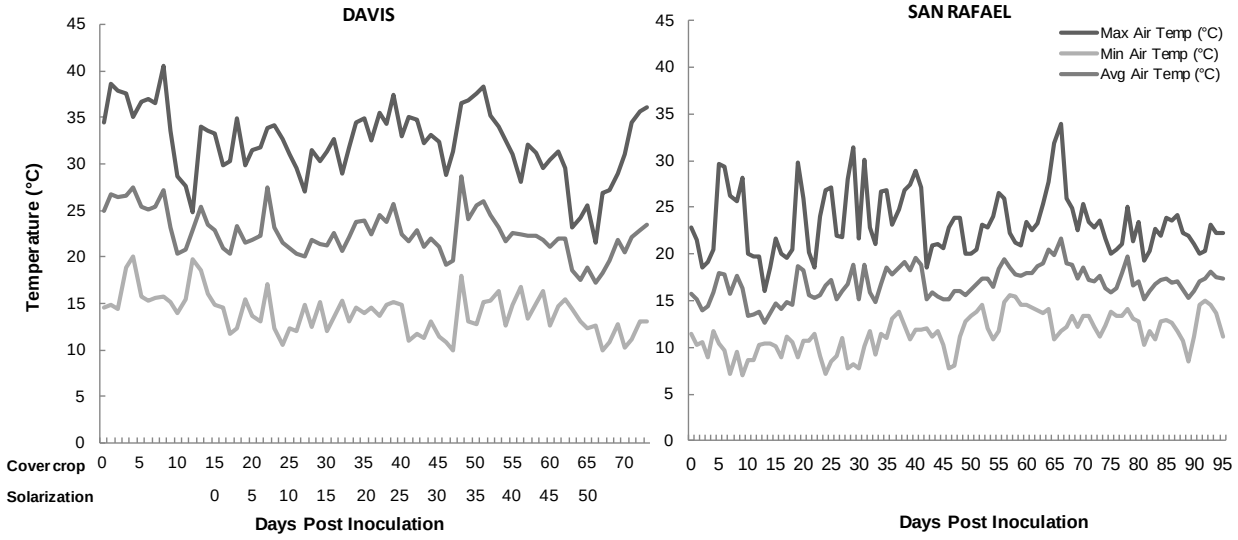



**Figure 3.** Daily maximum soil temperature during bed solarization

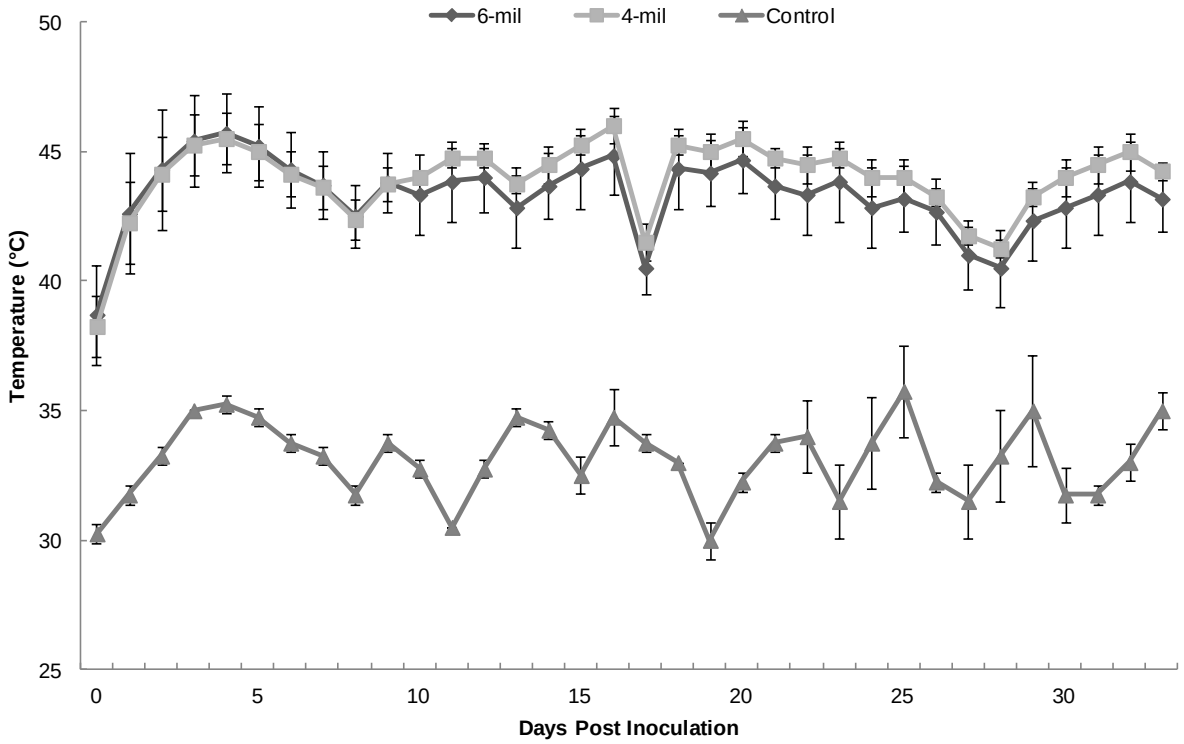


**Figure 4.** Hourly soil and air temperature during bed solarization

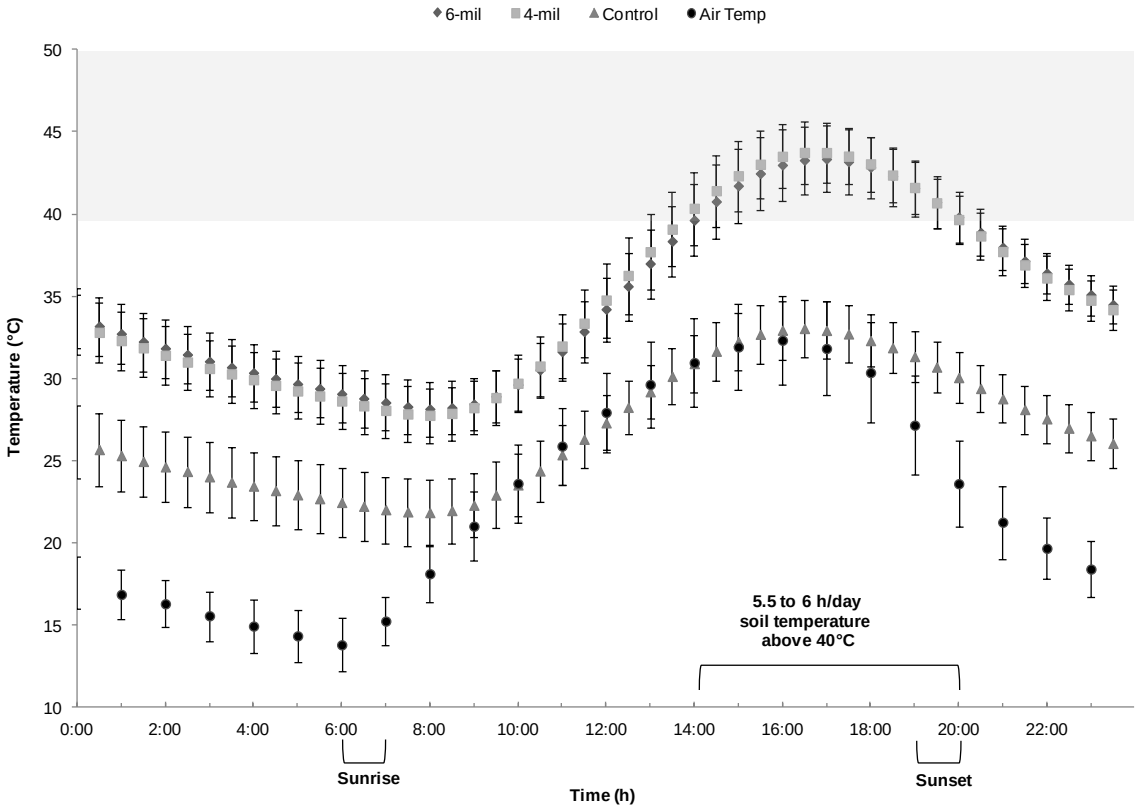
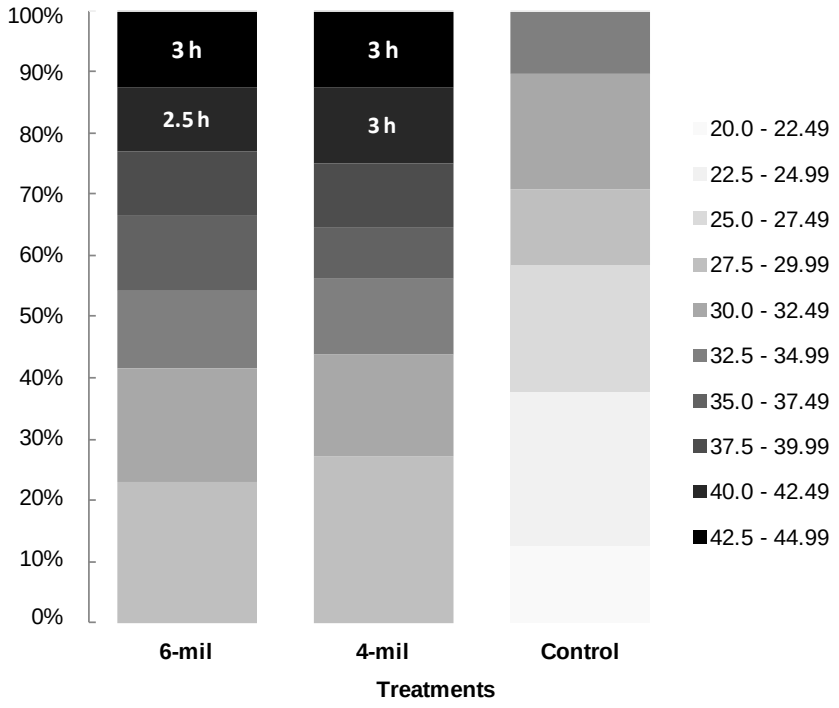



**Figure 5.** Cumulative hours of soil temperature distribution per day



## Appendix 2. Tables and Figures 2015

**TABLES**

**Table 4.** Cover crop trials.

| | | | **Log CFU $g^{-1}$ soil ± st dev** *(% enrichment positives)* | | | |
|---|---|---|---|---|---|---|
| **San Rafael Mesocosm Trial** | | | | | | |
| **Cover crops** | **n** | **O DPI** | **33 DPI** | **39 DPI & 0 DPC€,£** | **60 DPI & 21 DPC** | **67 DPI & 28 DPC** |
| **Canola** | 3 | 5.62 ± 0.14 | 2.01 ± 0.23 AB[a] *(100)* | 0.66 ± 0.36 A *(100)* | 0.43 ± 0.00 A *(100)* | 0.75 ± 0.55 A *(66.7)* |
| **Mustard** | 3 | | 2.00 ± 0.33 AB *(100)* | 0.78 ± 0.52 A *(100)* | 0.45 ± 0.03 A *(100)* | 0.65 ± 0.37 A *(66.7)* |
| **Buckwheat** | 3 | | 1.56 ± 0.28 B *(100)* | 0.43 ± 0.00 A *(100)* | 0.43 ± 0.00 A *(100)* | 0.68 ± 0.43 A *(33.4)* |
| **Control** | 3 | | 2.67 ± 0.26 A *(100)* | 0.45 ± 0.03 A *(100)* | 0.95 ± 0.87 A *(100)* | 1.06 ± 1.10 A *(33.4)* |
| **Cover crop Davis Field Trial 1** | | | | | | |
| **Cover crops** | **n** | **0.5 DPI** | **24 DPI & 0 DPC€,£** | **44 DPI & 20 DPC** | **51 DPI & 27 DPC** | **73 DPI & 49 DPC** |
| **East side*** **Canola** | 6 | 4.11 ± 0.37 A *(100)* | 1.49 ± 0.14 A[a] *(50)* | 1.43 ± 0.00 A *(100)* | 1.44 ± 0.02 A *(50)* | 1.43 ± 0.00 A *(16.7)* |
| **Mustard** | 6 | 3.59 ± 0.36 AB*(100)* | 1.63 ± 0.49 A *(66.7)* | 1.43 ± 0.00 A *(83.3)* | 1.43 ± 0.00 A *(33.3)* | 1.43 ± 0.00 A *(16.7)* |
| **Buckwheat** | 6 | 3.46 ± 0.37 AB *(100)* | 1.43 ± 0.00 A *(16.7)* | 1.56 ± 0.30 A *(83.3)* | 1.43 ± 0.00 A *(33.3)* | 1.43 ± 0.00 A *(33.3)* |
| **Control** | 6 | 3.96 ± 0.38 AB *(100)* | 1.43 ± 0.00 A *(66.7*) | 1.43 ± 0.00 A *(83.3)* | 1.43 ± 0.00 A *(33.3)* | 1.43 ± 0.00 A *(0)* |
| **West side** **Canola** | 6 | 3.72 ± 0.41 AB *(100)* | 1.45 ± 0.02 A *(100)* | 1.43 ± 0.00 A *(66.7)* | 1.54 ± 0.26 A *(33.3)* | 1.43 ± 0.00 A *(16.7)* |
| **Mustard** | 6 | 3.69 ± 0.28 AB *(100)* | 1.63 ± 0.33 A *(66.7)* | 1.43 ± 0.00 A *(83.3)* | 1.43 ± 0.00 A *(83.3)* | 1.43 ± 0.00 A *(33.3)* |
| **Buckwheat** | 6 | 3.31 ± 0.39 A *(100)* | 1.45 ± 0.02 A *(50)* | 1.49 ± 0.14 A *(100)* | 1.43 ± 0.00 A *(33.3)* | 1.43 ± 0.00 A *(16.7)* |
| **Control** | 6 | 3.35 ± 0.66 AB *(100)* | 1.71 ± 0.68 A *(83.3)* | 1.43 ± 0.00 A *(100)* | 1.49 ± 0.14 A *(50)* | 1.43 ± 0.00 A *(16.7)* |
| **Cover crop Davis Field Trial 2** | | | | | | |
| **Cover crops** | **n** | **0 DPI** | **22 DPI** | **28 DPI & 0 DPC€,£** | **33 DPI & 5 DPC** | **48 DPI & 20 DPC** |
| **Broccoli-A (Greenbelt)** | 3 | 6.22 ± 0.09 | 3.41 ± 0.41 *(100)* | 0.43 ± 0.00 A[a] *(100)* | 0.45 ± 0.03 A *(33.3)* | 0.46 ± 0.03 AB *(33.3)* |
| **Broccoli-B (Di Ciccio)** | 3 | | | 0.56 ± 0.19 A *(100)* | 0.61 ± 0.30 A *(66.7)* | 0.61 ± 0.30 B *(66.7)* |
| **Triticale** | 3 | | | 0.56 ± 0.19 A *(100)* | 0.43 ± 0.00 A *(66.7)* | 0.43 ± 0.00 B *(66.7)* |
| **Sudangrass** | 3 | | | 0.46 ± 0.03 A *(100)* | 0.45 ± 0.03 A *(100)* | 0.43 ± 0.00 B *(66.7)* |
| **Barley** | 3 | | | 0.78 ± 0.30 A *(100)* | 0.99 ± 0.45 A *(100)* | 0.45 ± 0.03 B *(100)* |
| **Purple Vetch** | 3 | | | 0.45 ± 0.03 A *(100)* | 0.82 ± 0.34 A *(66.7)* | 0.48 ± 0.00 AB *(100)* |
| **Common Vetch** | 3 | | | 0.45 ± 0.03 A *(100)* | 0.76 ± 0.49 A *(100)* | 1.07 ± 0.59 A *(100)* |
| **Control** | 3 | | | 1.26 ± 0.54 A *(100)* | 0.96 ± 0.44 A *(100)* | 0.43 ± 0.00 B *(100)* |

[a] Means with different letters represent populations that differ in significance ($P < 0.05$) between treatments.
Cover crop trials abbreviations: Days post inoculation (DPI) and Days post cover crop incorporation (DPC).
€ Cover crop incorporation.
£ Soil samples were collected prior to cover crop incorporation.
Limit of detection: log 1.43 CFU $g^{-1}$ of soil

**Table 5.** *Salmonella* soil survival followed contaminated spinach incorporation and subsequent red chard cross contamination.

| Treatments | n | Log CFU $g^{-1}$ soil ± st dev *(% enrichment positives)* | | |
|---|---|---|---|---|
| | | Soil | | Red Chard |
| | | 23 DPI & 16 DSI | 29 DPI & 22 DSI | 50 DPI & 43 DSI (21 DPSe) |
| **(1) Mowed and incorporated**[£] | 3 | 3.14 ± 0.15 A[a] *(100)* | 2.69 ± 0.16 A *(100)* | 1.43 ± 0.00 A *(0)* |
| **(2) Cut and left on top**[€] | 4 | 2.01 ± 0.32 B *(100)* | 2.61 ± 0.31 A *(100)* | 1.43 ± 0.00 A *(0)* |
| **(3) Round up applied**[€] | 4 | 3.21 ± 0.45 A *(100)* | 3.20 ± 0.45 A *(100)* | 1.43 ± 0.00 A *(0)* |
| **Negative Control** | 2 | 0.43 ± 0.00 C *(0)* | 0.43 ± 0.00 B *(0)* | 1.43 ± 0.00 A *(0)* |

[a] Means with different letters represent populations that differ in significance ($P < 0.05$) between treatments.
Days post inoculation (DPI), Days post inoculated spinach incorporation (DSI), Days post seeding (DPSe)
[£] Spinach incorporated immediately after mowed
[€] Spinach incorporated after 6 days
Limit of detection: log 1.43 and 0.43 CFU $g^{-1}$ of soil and plant respectively

**Table 6.** In-vitro solarization (hourly and weekly).

| Time | | log CFU/g of soil ± stdev | | | |
|---|---|---|---|---|---|
| | | Temperature (°C) | | | |
| | | 55 °C | 48 °C | 37 °C | 29 °C |
| **Hours** | **0 h** | 5.07 ± 0.27 | | | |
| | **2 h** | 1.35 ± 0.50 | nd (100) | nd (100) | nd (100) |
| | **4 h** | 0.43 ± 0.00 (0) A[a] | | | |
| | **18 h** | 0.43 ± 0.00 (0) A | 0.72 ± 0.27 (100) A | | |
| | **24 h** | 0.43 ± 0.00 (0) B | 0.88 ± 0.17 (100) A | | |
| | **48 h** | 0.43 ± 0.00 (0) B | 0.43 ± 0.00 (66.7) A | | |
| **Days** | **4 D** | 0.43 ± 0.00 (0) B | 0.43 ± 0.00 (0) B | 1.08 ± 0.84 (100) A | 1.44 ± 1.01 (100) A |
| | **13 D** | 0.43 ± 0.00 (0) B | 0.43 ± 0.00 (0) B | 0.43 ± 0.00 (0) B | 0.43 ± 0.00 (100) A |
| | **21 D** | 0.43 ± 0.00 (0) A | 0.43 ± 0.00 (0) A | 0.43 ± 0.00 (0) A | 0.43 ± 0.00 (0) A |
| **Time needed to deactivate *Salmonella*** | | **4 h** | **96 h** | **312 h** | **504 h** |

[a] Means with different letters represent populations that differ in significance ($P < 0.05$) between treatments.
Limit of detection: log 0.43 CFU $g^{-1}$ of soil

**Table 7.** Solarization field trials.

| Treatment | n | Log CFU $g^{-1}$ soil ± st dev *(% enrichment positives)* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **0 DPI** | **4 DPI & 0 DPS** | **14 DPI & 10 DPS** | **22 DPI & 18 DPS** | **28 DPI & 24 DPS** | **32 DPI & 5 DPR** | **48 DPI & 24 DPR** |
| **Polyethylene Covers** | 3 | 5.41 ± 0.57 | 4.24 ± 0.63 | 2.37 ± 0.64 A[a] *(100)* | 1.92 ± 0.41 A *(100)* | 0.94 ± 0.89 A *(100)* | 1.51 ± 0.38 A *(100)* | 1.32 ± 0.77 AB *(100)* |
| **Bird impacted Covers** | 3 | | | 2.56 ± 0.44 A *(100)* | 2.71 ± 0.53 A *(100)* | 1.14 ± 0.31 A *(100)* | 1.89 ± 0.36 A *(100)* | 2.45 ± 0.34 A *(100)* |
| **Control** | 2 | | | 3.78 ± 0.52 A *(100)* | 2.26 ± 1.17 A *(100)* | 1.84 ± 0.58 A *(100)* | 1.45 ± 0.03 A *(100)* | 0.60 ± 0.25 B *(100)* |

[a] Means with different letters represent populations that differ in significance ($P < 0.05$) between treatments.
Limit of detection: log 0.43 CFU $g^{-1}$ of soil
DPI: Days Post Inoculation, DPS: Days Post Solarization, DPR: Days Post Plastic Removal

## FIGURES

**Figure 6.** Daily air temperature during cover crop and solarization trials

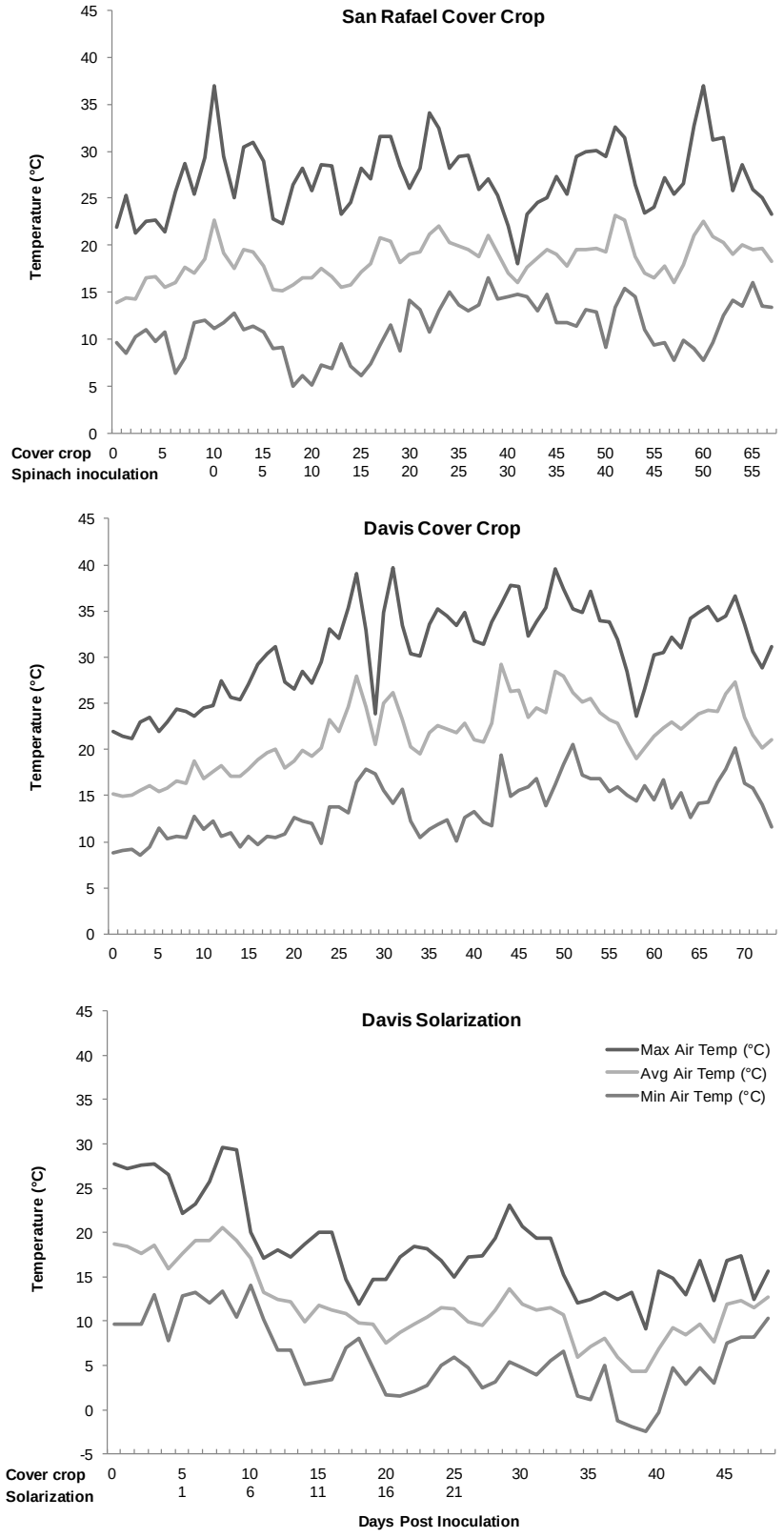


**Figure 7.** Qualitative *Salmonella* detection after crop incorporation in Davis field trial 1 (east and west side combined)

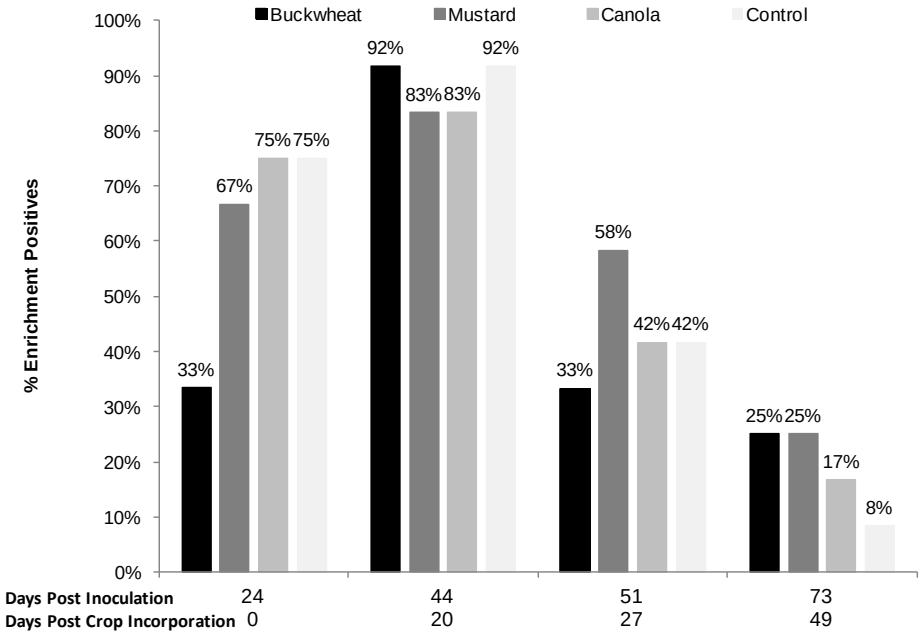



**Figure 8.** Daily maximum and minimum soil temperature during bed solarization

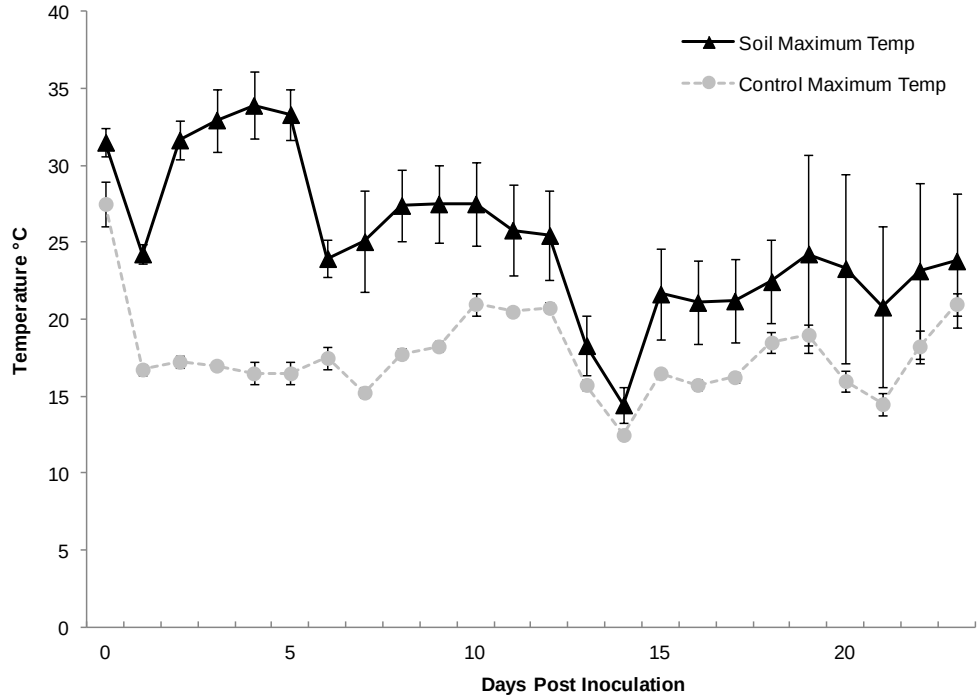


**Figure 9.** Hourly soil temperature during bed solarization