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Integration of Steam with Allyl-isothiocyanate for Soil Disinfestation

Dong Sub Kim
Department of Plant Sciences, University of California–Davis, 1636 East Alisal Street, Salinas, CA 93905

Mark Hoffmann
Department of Horticultural Sciences, North Carolina State University, Raleigh, NC 27695

Steven Kim
Mathematics and Statistics Department, California State University, Monterey Bay, Seaside, CA 93955

Bertha A. Scholler and Steven A. Fennimore
Department of Plant Sciences, University of California–Davis, 1636 East Alisal Street, Salinas, CA 93905

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Abstract. **Steam has long been used to disinfest greenhouse soils. However, there is increasing interest in expanding the use of steam for in-field soil disinfestation as an alternative to chemical fumigants. Previous studies demonstrated that allyl-isothiocyanate (AITC) reduced viability of weed seeds and plant pathogen propagules, but AITC has a low vapor pressure and is relatively immobile in soil. Heat has been used in the past to enhance the mobility of soil fumigants such as methyl bromide (i.e., "hot gassing"). The effect of steam heat on the mobility of AITC is unknown. The objective of this study was to investigate the potential synergistic effect of steam plus ATIC against weed seeds and a plant pathogen. AITC alone did not reduce the viability of the four weed species and the number of *Verticillium dahliae* microsclerotia. The steam + AITC treatment reduced the viability of *V. dahliae* at 12.5 and 18 cm distances by 82% and 88%, respectively, and knotweed and nettle seeds at 70 cm from injection point by 75% and 86%, respectively, from the center of microplots compared with steam alone. The results suggest that AITC and steam have a complementary effect on soilborne pests because steam increases the mobility of AITC.**

The majority of U.S. field-grown specialty crop production depends on a few fumigants to control soilborne pests in the post-methyl bromide era: 1,3-dicloropropene (1,3-D), chloropicrin, methyl isothiocyanate and dimethyl disulfide (Porter and Mattner, 2002). Nonchemical tools such as biofumigants (e.g., mustard seed meal), physical treatments such as solarization, and flaming or biological control methods such as anaerobic soil disinfestation (ASD) have been proven to provide at least partial control of soilborne pests but have found only limited success in the U.S. specialty crop industry so far (Daugovish et al., 2016; Porter and Mattner, 2002; Samtani et al., 2011).

Soil-applied steam controls plant pathogens and weeds in strawberry fields (Fennimore et al., 2014; Hoffmann and Fennimore, 2017; Samtani et al., 2012). Although steam applications are cost-effective for soil disinfestation in greenhouse production (Baker, 1957; Knezevic et al., 2017; Kokalis-Burelle et al., 2016), the operating costs of open field steam applications are likely higher than cost of soil fumigation (Fennimore and Goodhue, 2016). Moreover, steam itself does not induce environmental pollution, but the longer the operating time, the fuel used, and more CO_2 released into the atmosphere do. The prototype steam machine used in Fennimore et al. (2014), Hoffmann and Fennimore (2017), and Samtani et al. (2012) is estimated to require ≈24 h to treat a 1-ha field. Fennimore et al. (2014) and Hoffmann and Fennimore (2017) suggest the use of steam in those areas that cannot be fumigated due to regulatory restrictions such as fumigant buffer zones. However, field-applied steam for soil disinfestation has so far had a small impact on U.S. agriculture, partly due to cost and lack of an efficient steam applicator.

Isothiocyanates (ITCs) are a group of phytochemicals with antimicrobial and herbicidal activities against soilborne pathogens and weeds (Lambrix et al., 2001). Among the ITCs, the antimicrobial and allelopathic effects of AITC are well documented. AITC has documented weed control activity (Bangarwa and Norsworthy 2014; Bangarwa et al., 2011, 2012; Bhowmik and Inderjit, 2003; Norsworthy and Meehan, 2005) and pathogen control activity (Fenwick et al., 1983; Kirkegaard et al., 1996; Mari et al., 1993; Sharma et al., 2008; Vaughn and Boydston, 1997).

However, questions remain as to whether the combination of steam with low vapor pressure pesticides will improve the efficacy of pest control under field conditions. We tested the hypothesis that steam efficacy on soilborne diseases and weeds could be improved by coapplication with AITC. Combinations of heat and pesticides have shown potential to increase pest control efficacy (Chamorro et al., 2014; Fennimore et al., 2014). Therefore, we investigated the weed and disease control of steam combined with AITC in repeated microplot experiments. The objective of this study was to determine whether steam increases the mobility of AITC in soil and efficacy against soil pests at a greater distance from the injection point than if applied at ambient temperatures.

Materials and Methods

Steam application. Four experiments were conducted: two tests on *V. dahliae* during March and June 2016 and two tests on weed seed viability during June 2018 and 2019 at Salinas, CA. The experimental units were 24 microplots (1 m^2) for all four studies. The Mar. and June 2016 and June 2018 experiments were located at the U.S. Department of Agriculture (USDA) main research station and the June 2019 study at the Spence research farm 10 km south of Salinas. The soil at the USDA research station and the Spence research farm was a sandy loam. Steam treatment time and sample distance from the center was determined based on soil temperatures after steam application from preliminary experiments because the soil temperature by the steam application can vary depending on soil humidity, air temperature, and soil compaction. Steam was injected into the exact center of the microplots at 50 kPa for 60 min in Mar. and June 2016 and June 2018 and at 50 kPa for 20 min in June 2019. Steam was applied through a single shank placed in the center of the microplots at a 15-cm depth with steam supplied by a SIOUX Steam-Flo SF-20 (Sioux Corp., Beresford, SD) with a boiler horsepower of 19.7. Dominus (99.8% AITC; Isagro USA Inc., Morrisville, NC) was applied at a rate of 18.7 $mL \cdot m^{-2}$ at the center of the microplots just before steam injection. A manifold with four outlets was used to apply steam on four microplots at once. The treatments are shown in Table 1. Soil temperature at 12.5 cm below surface was measured at 12.5 cm from the micro plot center for the Mar. and June 2016 experiments and at 10 cm

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S.A.F. is the corresponding author. E-mail: safennimore@ucdavis.edu.

below surface at 30, 50, and 70 cm from the center for the June 2018 and June 2019 experiments. Soil temperatures were monitored with HOBO data loggers (Onset, Bourne, MA) for 3 d after steaming. The microplots were covered with plastic (0.15 mm, white) for 5 d after steam and AITC applications. The experiments were arranged in a randomized complete block design with three replications.

Bioassay. Bioassays of efficacy on *V. dahliae* were conducted on 20-g samples of infected soil, and 50 seeds each of common purslane (*Portulaca oleracea*), common knotweed (*Polygonum arenastrum*), burning nettle (*Urtica urens*), and pigweed (*Amaranthus retroflexus*) were used as bioassays. Life stage of the *V. dahliae* was microsclerotia in soil. The four weeds are common pests in California agricultural fields. The *V. dahliae*–infected soil and weed seeds were contained in nylon bags (polyethylene, 10 × 4 cm) attached to steel washers and colored ribbons. The sample bags were placed at the same locations where the temperatures were monitored. The *V. dahliae*–infected soil samples and weed seeds were buried 1 d before treatment and removed 1 week after the application. The *V. dahliae* samples were air-dried and pulverized in a mortar and pestle, and the number of microsclerotia per gram of soil were determined using the dry plating method on semiselective medium according to Kabir et al. (2004). Weed seeds at 50 per sample were tested for viability using the tetrazolium assay according to Cottrell (1947). A 0.1% (v/v) solution of 2,3,5-triphenyl-tetrazoilum chloride (TTC) (Sigma, St. Louis, MO) was used to stain the seeds from the recovered containers and probes. The seeds were plated on germination paper in petri dishes, cut in half, stained, and kept in the dark at 24 °C for 24 h. The viability of the individual seeds was evaluated under microscope, according to their staining intensity.

Statistical analyses. We assumed that the variation in a response variable is due to both fixed effect and random effect, so generalized linear mixed-effects models (GLMM) were considered. For the weed viability data (out of 25 per experimental unit), the two main effects (the treatments and the distance from the center of treatment injection) were tested using the logistic regression model which is specified as

$$\ln[\theta/(1-\theta)] = \beta_0 + \beta_1 \mathrm{D} + \beta_2 \mathrm{S} + \beta_3 \mathrm{SD} + \beta_4 d,$$

where $0 < \theta < 1$ denotes the proportion of viability, D = 1 denotes AITC, S = 1 denotes steam, SD = 1 denotes the combination of steam and AITC, and d denotes the distance (in centimeters) from the center of microplot (i.e., injection point).

To test whether the effect of SD is stronger than the effect of S at the maximally observed distance (18 cm) from the injection point, an interaction between treatment and distance was considered. Under the model, we tested for $\delta = \theta_{SD} - \theta_S$ where θ_{SD} and θ_S denote the proportion of viability for SD and D, respectively, at the maximum distance in this experiment.

To account for the mean–variance relationship in the count data of *V. dahliae*, the Poisson regression model was used with the log-link $\ln(\theta)$, where $\theta > 0$ denotes the expected count of microsclerotia. When the interaction terms were considered, the control group was removed because the distance was not measured in the control group.

The weed viability data were analyzed by species (purslane, knotweed, nettle, and pigweed), and the *V. dahliae* data were analyzed by month (March and June). All statistical analyses were performed in R version 3.6.1, and the lme4 package was used for the GLMM.

For the *V. dahliae* viability data, the four treatments were subjected to multiple comparisons with the least significant difference at the significance level of $\alpha = 0.05$, and the Bonferroni's correction was used to control type I error rate in the multiple comparisons. For the weed seed viability data, Duncan's multiple range test was performed at the significance level of $\alpha = 0.05$. All statistical analyses were performed in SPSS statistical software version 20 (SPSS Inc., Chicago, IL) and R version 3.6.1.

Results

V. dahliae viability test. In the Mar. 2016 experiment, the soil temperature 2 h after steam application and the time of above 60 °C at 12.5 cm from the injection point were 65.3 °C and 154 min, respectively (Table 2). The minimum and maximum soil temperatures were 20.8 and 25.6 °C in the control microplots, respectively. In the June 2016 experiment, the soil temperature 2 h after steam application and the time above 60 °C at 12.5 cm from the injection point were 71.1 °C and 199 min, respectively (Table 2). The minimum and maximum soil temperatures were 21 and 33.5 °C in the control microplots, respectively. The data from both experiments indicated that steam in the Mar. 2016 experiment cooled sooner than that in the June 2016 experiment because air temperature in the Mar. 2016 experiment (12.8 to 18.9 °C) was lower than that in the June 2016 experiment (12.8 to 23.9 °C).

Only steam + AITC suppressed *V. dahliae* at all distances in both Mar. and June 2016 experiments, whereas AITC alone did not control *V. dahliae* beyond the injection point. The control efficacy of steam + AITC was higher than that of steam alone at 12.5 cm from the injection point in the June 2016 experiment (Table 3). The statistical analysis

Table 1. Steam duration and AITC concentration applied in the four experiments.

	Mar. and June 2016		June 2018		June 2019	
Treatment	Steam (min, kPa)	AITC ($\mathrm{mL{\cdot}m^{-2}}$)	Steam (min, kPa)	AITC ($\mathrm{mL{\cdot}m^{-2}}$)	Steam (min, kPa)	AITC ($\mathrm{mL{\cdot}m^{-2}}$)
Steam alone	60, 50	—	60, 50	—	20, 50	—
AITC alone	—	18.7	—	18.7	—	18.7
Steam + AITC	60, 50	18.7	60, 50	18.7	20, 50	18.7

Table 2. The soil temperature 2 h after steam application and time above 60 °C in microplots.

Expt. date	Distance (cm)	Soil temp (°C)	Time above 60 °C (min)
Mar. 2016	12.5	65.3	154
June 2016	12.5	71.1	199
June 2018	30	74.9 b	553 b
	50	73.9 b	515 b
	70	65.3 a	373 a
June 2019	30	68.3 c	176 c
	50	61.4 b	128 b
	70	60.7 a	124 a

The letters indicate group separation by Duncan's multiple range test at the significance level of 0.05.

Table 3. The average count of *Verticillium dahliae* microsclerotia in each treatment and at four distances from the injection point.

		overall	2.5 cm	7.5 cm	12.5 cm	18 cm
Time	Treatment	Microsclerotia/g soil				
Mar. 2016	Control	4.1 ab	NA	NA	NA	NA
	AITC alone	5.5 a	6.7 a	4.5 a	4.9 a	5.7 a
	Steam alone	2.3 b	0.1 b	1.2 b	3.6 a	4.1 ab
	Steam + AITC	0.3 c	0.1 b	0.0 b	0.5 b	0.5 b
June 2016	Control	91.4 b	NA	NA	NA	NA
	AITC alone	115.8 a	90.2 a	100.1 a	83.5 a	92.0 a
	Steam alone	3.6 c	0.0 b	0.1 b	8.1 b	7.6 b
	Steam + AITC	0.0 c	0.0 b	0.1 b	0.0 b	0.0 b

The letters indicate group separation by the least significant difference test at the significance level of 0.05. NA = not analyzed.

by GLMM also supports the results (Table 4). The effect of AITC alone against *V. dahliae* was insignificant compared with the control group in both the Mar. and June 2016 experiments, but the effects of steam alone and steam + AITC were significantly better than the control. Moreover, both steam alone and steam + AITC controlled disease near the injection point, but the efficacy of steam + AITC was higher than that of steam alone at the maximally observed distance in both the Mar. and June 2016 experiments (Fig. 1). The marginal and conditional R^2 values were 0.742 and 0.969 for the March data, respectively, and the respective R^2 values were 0.917 and 0.969 for the June data.

Table 4. Estimated parameters, standard errors, and *P* values from the GLMM (Poisson model for the count of *V. dahliae*).

Month	Parameter	Estimate	SE estimate	*P* value
March	β_0	1.1131	0.4405	0.0058
	β_1	–0.7898	0.6593	0.1155
	β_2	–2.2208	0.7301	0.0012
	β_3	–4.5363	0.8072	<0.0001
	β_4	0.1061	0.0377	0.0025
	δ	–2.5535	0.8379	0.0012
June	β_0	4.7245	0.4892	<0.0001
	β_1	–1.0757	0.7450	0.0744
	β_2	–6.9941	0.8707	<0.0001
	β_3	–10.6679	1.3317	<0.0001
	β_4	0.0794	0.0452	0.0394
	δ	–6.5475	2.5229	0.0047

The generalized linear mixed-effects models (GLMM) assumes $\ln(\theta) = \beta_0 + \beta_1 D + \beta_2 S + \beta_3 SD + \beta_4 d$ for the fixed effect, where θ denotes the expected count of *V. dahliae*, D = 1 denotes AITC, S = 1 denotes steam, SD = 1 denotes the combination of steam and AITC, and *d* denotes the distance (cm) from the center of microplot (injection point). To test if the effects of S and SD are different at the maximum experimental distance (18 cm), interaction terms were considered in the model, and δ denotes the difference in the effects of S and SD at the maximum experimental distance (18 cm).

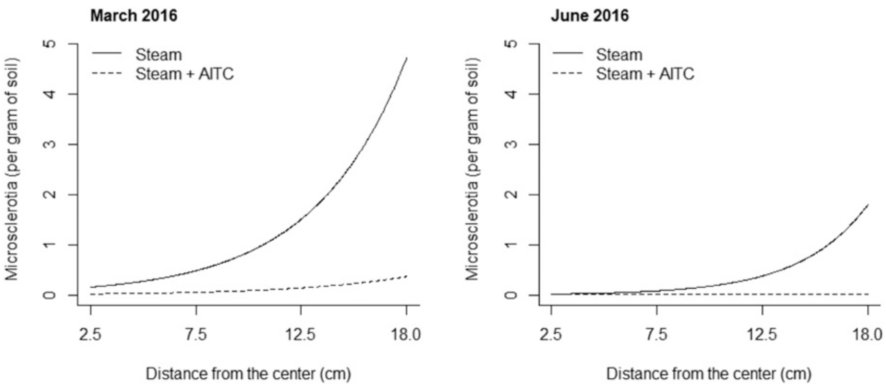

Fig. 1. The estimated expected count of *V. dahliae* microsclerotia per gram of soil with respect to distance (*d*) from the injection point for steam and steam + AITC (allyl-isothiocyanate). The data were collected Mar. (left) and June (right) 2016 (n = 18 replicated four times per experimental unit). Under the interaction model (which generates treatment-specific regression curves), the estimated regression equations are $e^{-2.44 + 0.22d}$ for steam (solid curve) and $e^{-4.41 + 0.19d}$ for steam + AITC (dotted curve) from the data collected in March (marginal $R^2 = 0.742$; conditional $R^2 = 0.969$; $P = 0.0012$ for the different effect at 180 mm). The respective estimated regression equations are $e^{-4.88 + 0.31d}$ for steam (solid curve) and $e^{-4.06-0.11d}$ for steam + AITC (dotted curve) from the data collected in June (marginal $R^2 = 0.917$; conditional $R^2 = 0.969$; $P = 0.0047$ for the different effect at 180 mm).

Weed seed viability. In the June 2018 experiment, the soil temperature 2 h after steam application was much lower at 70 cm than closer to the center. The time above 60 °C at 30, 50, and 70 cm from the injection point was 553, 515, and 373 min, respectively (Table 2). The minimum and maximum soil temperatures were 20.3 °C at 70 cm and 30.8 °C at 30 cm from the center in the control microplots. In the June 2019 experiment, the soil temperature 2 h after steam application decreased between 30 and 50 cm from the injection point and the time of above 60 °C at 30, 50, and 70 cm from the injection point were 176, 128, and 124 min (Table 2). The minimum and maximum soil temperatures were 17.9 °C at 70 cm and 31.5 °C at 30 cm from the center in the control microplots. The data from both the experiments indicated that the soil temperature in the June 2019 experiment cooled sooner than that in the June 2018 experiment due to the difference in the steam duration (Table 1).

Steam + AITC was more efficacious on weed seed control at a greater distance from the injection point than steam alone although both steam alone and steam + AITC reduced the weed seed viability more than AITC alone (Table 5). At 70 cm from the injection point, steam + AITC significantly reduced the viability of nettle seed in the June 2018 experiment and purslane seed in the June 2019 experiment compared with AITC alone and steam alone. In the June 2019 experiment, we also observed 0% weed seed viability at all distances in the steam + AITC treatment. The effect of AITC alone against the weed seed viability was no different from the control in the June 2018 and 2019 experiments, but the effects of steam alone and steam + AITC were significant in the GLMM analysis (Table 6). In addition, both steam alone and steam + AITC demonstrated greater efficacy on the four weeds near the injection point, but the control efficacy of steam + AITC was higher than that of steam alone at the maximally observed distance in both June 2018 and 2019 experiments (Fig. 2). The GLMM for each weed was useful to explain the experiment outcomes ($P < 0.0001$), and the *P* values for each model parameter are provided in Table 6. Using the estimated regression parameters reported in the table, the estimated viability percentage is graphically presented with respect to the distance

Table 5. The estimated percentage viability for each weed species (purslane, knotweed, nettle, and pigweed) at three distances from the injection point.

		Common purslane			Common knotweed			Burning nettle			Pigweed		
		30 cm	50 cm	70 cm	30 cm	50 cm	70 cm	30 cm	50 cm	70 cm	30 cm	50 cm	70 cm
Time	Treatment	Viability (%)											
June 2018	Control	84.0 a	86.7 a	72.0 a	94.7 a	84.0 a	97.3 a	74.0 b	97.3 a	89.3 a	74.7 a	97.3 a	72.0 a
	AITC alone	85.3 a	82.7 a	89.3 a	85.3 a	97.3 a	92.0 a	94.7 a	76.0 a	89.3 a	80.0 a	94.3 a	66.7 a
	Steam alone	0.0 b	6.7 b	5.3 b	5.3 b	17.3 b	10.7 b	0.0 c	16.0 c	56.0 a	1.7 b	9.3 b	48.0 a
	Steam + AITC	0.0 b	8.0 b	0.0 b	20.0 b	4.0 b	2.7 b	5.3 c	20.0 c	8.0 b	2.7 b	8.0 b	21.0 a
June 2019	Control	98.0 a	99.0 a	98.0 a	71.0 a	77.0 a	75.0 a	91.0 a	88.0 a	95.0 a	82.0 a	78.0 a	78.0 a
	AITC alone	66.0 a	99.0 a	99.0 a	55.0 b	60.0 b	67.0 a	83.0 a	84.0 a	96.0 a	65.0 b	79.0 a	66.0 a
	Steam alone	2.0 b	3.0 b	37.0 b	3.0 c	2.0 c	20.0 b	0.0 b	0.0 b	21.0 b	1.0 c	0.0 b	27.0 b
	Steam + AITC	0.0 b	0.0 b	0.0 c	0.0 c	0.0 c	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c	0.0 b	0.0 b

The letters indicate group separation by Duncan's multiple range test at the significance level of 0.05.

from the center in Fig. 2. The marginal R^2 values were 0.559, 0.434, 0.528, and 0.459 for purslane, knotweed, nettle, and pigweed, respectively. The conditional R^2 values were 0.597, 0.487, 0.595, and 0.514, respectively.

Discussion

van Loenen et al. (2003) reported that the minimum lethal temperature of the steam application for *V. dahliae* was 60 °C for 180 s and Melander and Jørgensen (2005) showed that the steam application of 60 °C for 70 s reduced seedling emergence of weeds by at least 90%. In the experiments reported here, soil temperatures were above 60 °C for more than 100 min (Table 2). The observed soil temperature was therefore probably enough to reduce *V. dahliae* and weed seed viability.

Table 6. Estimated parameters, standard errors, and *P* values from the GLMM (binomial model for the count of weed viability).

Weed	Parameter	Estimate	SE Estimate	*P* value
Purslane	β_0	0.8177	0.2549	0.0013
	β_1	–0.2865	0.3104	0.3560
	β_2	–4.1247	0.3111	<0.0001
	β_3	–5.0415	0.3123	<0.0001
	β_4	2.0954	0.0783	<0.0001
	δ	–1.7550	0.3279	<0.0001
Knotweed	β_0	0.9905	0.3900	0.0111
	β_1	–0.3413	0.2226	0.1252
	β_2	–3.3870	0.2233	<0.0001
	β_3	–4.0704	0.2247	<0.0001
	β_4	0.7506	0.0671	<0.0001
	δ	–1.7806	0.2346	<0.0001
Nettle	β_0	0.4612	0.3141	0.1420
	β_1	–0.1387	0.3603	0.7004
	β_2	–3.8174	0.3612	<0.0001
	β_3	–4.4346	0.3616	<0.0001
	β_4	2.4999	0.0738	<0.0001
	δ	–1.7346	0.4180	<0.0001
Pigweed	β_0	0.5037	0.3048	0.0985
	β_1	–0.2508	0.2860	0.3804
	β_2	–3.1086	0.2868	<0.0001
	β_3	–3.8697	0.2877	<0.0001
	β_4	1.3342	0.0646	<0.0001
	δ	–1.4032	0.3289	<0.0001

The generalized linear mixed-effects models (GLMM) assumes $\ln[\theta / (1 - \theta)] = \beta_0 + \beta_1 D + \beta_2 S + \beta_3 SD + \beta_4 d$ for the fixed effect, where θ denotes the probability of weed viability, D = 1 denotes AITC, S = 1 denotes steam, SD = 1 denotes the combination of steam and AITC, and *d* denotes the distance (m) from the center of microplot (injection point). To test if the effects of S and SD are different at the maximum experimental distance (0.7 m), interaction terms were considered in the model, and δ denotes the difference in the effects of S and SD at the maximum experimental distance (0.7 m).

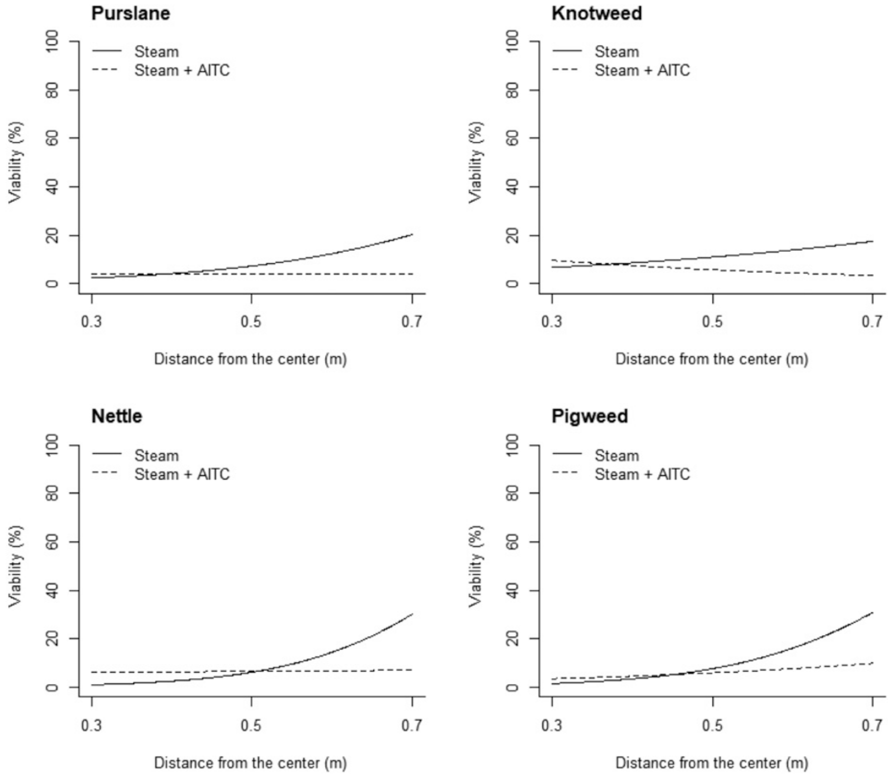

Fig. 2. The estimated expected weed viability percentage with respect to distance from the injection point for steam and steam + AITC (n = 25 replicated four times per experimental unit).

Numerous studies have been reported on weed and pathogen control by steam. Norberg et al. (2001) found that one steam application controlled *Calluna vulgaris* for 5 years. Fennimore et al. (2014) showed that steam reduced incidence of *Macrophomina phaseolina* in strawberry by 77% compared with the nontreated control. Steam reduced *V. dahliae* and weed densities and produced strawberry yields similar to methyl bromide plus chloropicrin (Samtani et al., 2012). Our results also indicated that steam alone reduced the viability of *V. dahliae* microsclerotia in soil, as well as that of seed of four weed species (Tables 3 and 4). However, the use of steam for soil disinfestation is limited by technology and economic factors (Hoffmann and Fennimore, 2017; Katan 2000; Samtani et al., 2012).

This research was conducted to determine whether we can increase the efficacy and mobility of AITC by coapplication with steam and also reduce the cost of steam by coapplication with AITC. Faster steam application may allow reductions in fuel, machine, and labor costs. Multitactic treatments, using steam in combination with other soil disinfestation methods, were previously reported to be successful. Daugovish et al. (2013) combined mustard seed meal and solarization with steam application in strawberries. The combinations increased marketable strawberry fruit yield. Samtani et al. (2011) showed that the combination of steam and a liquid organic fertilizer application (AgroThrive, 2.5–2.5–1.5 NPK) reduced the weed density similar to steam alone and resulted in more marketable strawberry fruit than steam alone. The combinations, however, did not show greater efficacy on weed and disease control than steam alone.

The antimicrobial and allelopathic activities of ITCs are induced by disruption of the mitochondrial membrane potential (Calmes et al., 2015) and by inhibition of redox-based defenses and subsequent inhibition of sulfhydryl enzyme activities (Jacob and Anwar, 2008; Kolm et al., 1995; Tang and Tang, 1976). Olivier et al. (1999) also found that mustard genotypes with higher AITC content showed higher antimicrobial activity against plant pathogens. AITC as a soil fumigant has been reported to be effective against soilborne pathogens (Dhingra et al., 2004; Harvey et al., 2002; Sharma et al., 2008), weeds (Brown and Morra, 1996; Stiehl and Bible, 1989), nematodes (Zasada and Ferris, 2003), and insects (Noble et al., 2002; Wu et al., 2009). Mayton et al. (1996) reported that AITC completely suppressed radial growth of *V. dahliae* in vitro test. Vaughn and Boydston (1997) showed that AITC strongly suppressed germination of many plant species. Ren et al. (2018) reported that AITC is registered in 30 U.S. states as a preplant soil biofumigant. Our results, however, found that AITC alone did not reduce the viability of the four weed species tested here and the number of *V. dahliae* microsclerotia because the viability and the number of the microsclerotia did not differ from the control beyond the injection point (Tables 3 and 5). The data indicated that AITC did not disperse well in soil. Dhingra et al. (2009) found that the vapor pressure of AITC is 5 mm Hg at 25 °C, whereas in comparison, the vapor pressure of methyl bromide is 1600 mm Hg.

The steam + AITC application had significant higher weed and disease control efficacy than steam alone at greater distances from the injection point. For instance, at 12.5 and 18 cm from the injection point in the Mar. 2016 experiment and at 70 cm from the injection point in June 2018 and June 2019 experiments, the steam + AITC controlled *V. dahliae* and burning nettle and common purslane seeds, whereas steam alone did not (Tables 3 and 4). The differences between the steam + AITC and steam-alone treatments against the pathogen and weed seeds indicate that AITC was more mobile when coapplied with steam than when applied alone (Ojaghian et al., 2012). Shin et al. (2010) reported that AITC vapor was more effective against *Salmonella typhimurium* and *Listeria monocytogenes* than liquid AITC. Suhr and Nielsen (2003) showed that the minimum inhibitory concentration of the gaseous AITC was 250 times lower than that of the liquid AITC. Solarization, in which a transparent plastic cover is used to increase temperature, can also induce vaporization of liquid AITC (Kokalis-Burelle et al., 2016).

If fuel for operation of the steamer can be reduced by addition of AITC, the cost of the steam application will be reduced. Schweigkofler et al. (2014) reported that diesel used for running the same model as our steamer was 11.5 $L \cdot h^{-1}$. On the basis of their report, if diesel price is \$1/L, the fuel cost is \$0.05/m^2 when four manifold was used. The AITC cost in this experiment was \$0.09/m^2. The calculation indicated that reducing the steamer operating time above 2 min in the steam + AITC application can reduce the cost for soil disinfestation. Moreover, the fuel and AITC costs are lower than the cost of 1,3-dichloropropene (Pic-Clor 60) fumigant (\$0.47/m^2) (Fennimore and Goodhue, 2016). This economic analysis can be used as information for farmers to choose the steam + AITC application.

Conclusion

Our results indicate that the steam + AITC application increased the pest control efficacy in soil compared with AITC alone and steam alone. Therefore, AITC can complement the effect of steam application for soil disinfestation. To evaluate the fuel cost savings by addition of AITC, comparison of pest control in the steam alone and steam + AITC treatments according to steam duration should be considered in future studies. Also, to enhance the pesticidal activity of AITC, combination of solarization and AITC should be evaluated in future research. Additionally, the logistics of coapplying steam and AITC at field-scale were not addressed here. Likely steam and AITC would need to be coinjected and immediately tarped to trap the volatiles. It is also possible that the rate of AITC coapplied with steam could be reduced from the currently recommended label rate of AITC applied at ambient temperatures.

Literature Cited

Baker, K.F. (ed.). 1957. The U. C. system for producing healthy container-grown plants through the use of clean soil, clean stock, and sanitation. Univ. CA Div. Agricultural Sci. Agricultural Expt. Sta. Ext. Serv.

Bangarwa, S.K. and J.K. Norsworthy. 2014. Purple nutsedge control with allyl isothiocyanate under virtually impermeable film mulch. Weed Technol. 28:200–205.

Bangarwa, S.K., J.K. Norsworthy, and E.E. Gbur. 2012. Allyl isothiocyanate as a methyl bromide alternative for weed management in polyethylene-mulched tomato. Weed Technol. 26:449–454.

Bangarwa, S.K., J.K. Norsworthy, E.E. Gbur, J. Zhang, and T. Habtom. 2011. Allyl isothiocyanate: A methyl bromide replacement in polyethylene-mulched bell pepper. Weed Technol. 25:90–96.

Bhowmik, P.C. and Inderjit. 2003. Challenges and opportunities in implementing allelopathy for natural weed management. Crop Prot. 22:661–671.

Brown, P.D. and M.J. Morra. 1996. Hydrolysis products of glucosinolates in *Brassica napus* tissues as inhibitors of seed germination. Plant Soil 181:307–316.

Calmes, B., G. N'Guyen, J. Dumur, C.A. Brisach, C. Campion, B. Iacomi, S. Pigné, E. Dias, D. Macherel, T. Guillemette, and P. Simoneau. 2015. Glucosinolate-derived isothiocyanates impact mitochondrial function in fungal cells and elicit an oxidative stress response necessary for growth recovery. Front. Plant Sci. 6:414.

Chamorro, M., L. Miranda, P. Domínguez, J. Medina, C. Soria, F. Romero, J.M. Lopez Aranda, and B. de los Santos. 2014. Evaluation of biosolarization for the control of charcoal rot disease (*Macrophomina phaseolina*) in strawberry. Crop Prot. 67:279–286.

Cottrell, H.J. 1947. Tetrazolium salt as a seed germination indicator. Nature 159:748.

Daugovish, O., A. Howell, S. Fennimore, S. Koike, T. Gordon, and K. Subbarao. 2016. Non-fumigant treatments and their combinations affect soil pathogens and strawberry performance in Southern California. J. Fruit Sci. 16:37–46.

Daugovish, O., S. Fennimore, T. Gordon, S. Koike, J. Muramoto, C. Shannon, and K. Subbarao. 2013. Non-fumigant treatments for management of *Fusarium oxysporum* and *Macrophomina phaseolina* on strawberry cultivar San Andreas. Proc. Annu. Intl. Conf. Methyl Bromide Altern. Emissions Reductions, San Diego, CA, 4–6 Nov. <http://mbao.org/>.

Dhingra, O.D., C. Mln, and S. Gjjr. 2004. Potential of allyl isothiocyanate to control *Rhizoctonia solani* seedling damping off and seedling blight in transplant production. J. Phytopathol. 152: 352–357.

Dhingra, O.D., G.N. Jham, F.A. Rodrigues, G.J. Silva, and M.L.N. Costa. 2009. Fumigation with essential oil of mustard retards fungal growth and accumulation of ergosterol and free fatty acid in stored shelled groundnuts. J. Stored Prod. Res. 45:24–31.

Fennimore, S.A. and R.E. Goodhue. 2016. Soil disinfestation with steam: A review of economics, engineering, and soil pest control in California strawberry. Intl. J. Fruit Sci. 16:1–13.

Fennimore, S.A., F.N. Martin, T.C. Miller, J.C. Broome, N. Dorn, and I. Greene. 2014. Evaluation of a mobile steam applicator for soil disinfestation in California strawberry. HortScience 49:1542–1549.

Fenwick, G.R., R.K. Heaney, W.J. Mullin, and C.H. VanEtten. 1983. Glucosinolates and their breakdown products in food and food plants. CRC Crit. Rev. Food Sci. Nutr. 18:123–201.

Harvey, S.G., H.N. Hannahan, and C.E. Sams. 2002. Indian mustard and allyl isothiocyanate inhibit *Sclerotium rolfsii*. J. Amer. Soc. Hort. Sci. 127:27–31.

Hoffmann, M. and S.A. Fennimore. 2017. A soil probe system to evaluate weed seed survival in soil disinfestation trials. Weed Technol. 31:752–760.

Jacob, C. and A. Anwar. 2008. The chemistry behind redox regulation with a focus on sulphur redox systems. Physiol. Plant. 133:469–480.

Kabir, Z., R.G. Bhat, and K.V. Subbarao. 2004. Comparison of media for recovery of *Verticillium dahliae* from soil. Plant Dis. 88:49–55.

Katan, J. 2000. Physical and cultural methods for the management of soil-borne pathogens. Crop Prot. 19:725–731.

Kirkegaard, J.A., P.T.W. Wong, and J.M. Desmarchelier. 1996. *In vitro* suppression of fungal root pathogens of cereals by Brassica tissues. Plant Pathol. 45:593–603.

Knezevic, S., S. Fennimore, and A. Datta. 2017. Thermal weed control, p. 463–468. In: B. Thomas, B.G. Murray, and D.J. Murphy (eds.). Encyclopedia of applied plant sciences, Vol. 3. Academic Press, Amsterdam.

Kokalis-Burelle, N.K., E.N. Rosskopf, D.M. Butler, S.A. Fennimore, and J. Holzinger. 2016. Evaluation of steam and soil solarization for *Meloidogyne arenaria* control in Florida floriculture crops. J. Nematol. 48:183–192.

Kolm, R.H., U.H. Danielson, Y. Zhang, P. Talalay, and B. Mannervik. 1995. Isothiocyanates as substrates for human glutathione transferases: Structure-activity studies. Biochem. J. 311: 453–459.

Lambrix, V., M. Reichelt, T. Mitchell-Olds, D.J. Kliebenstein, and J. Gershenzon. 2001. The Arabidopsis epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. Plant Cell 13:2793–2807.

Mari, M., R. Iori, O. Leoni, and A. Marchi. 1993. *In vitro* activity of glucosinolate-derived isothiocyanates against postharvest fruit pathogens. Ann. Appl. Biol. 123:155–164.

Mayton, H.S., C. Olivier, S.F. Vaughn, and R. Loria. 1996. Correlation of fungicidal activity of Brassica species with allyl isothiocyanate production in macerated leaf tissue. Phytopathology 86:267–271.

Melander, B. and M.H. Jørgensen. 2005. Soil steaming to reduce intrarow weed seedling emergence. Weed Res. 45:202–211.

Noble, R.R.P., S.G. Harvey, and C.E. Sams. 2002. Toxicity of Indian mustard and allyl isothiocyanate to masked chafer beetle larvae. Plant Health Prog. 3:1–7.

Norberg, G., A. Dolling, A. Jäderlund, M.C. Nilsson, and O. Zackrisson. 2001. Control of heather (*Calluna vulgaris* (L.) Hull) by steam treatment: Effects on establishment and early growth of Scots pine. New For. 21:187–198.

Norsworthy, J.K. and J.T. Meehan. 2005. Herbicidal activity of eight isothiocyanates on Texas panicum (*Panicum texanum*), large crabgrass (*Digitaria sanguinalis*), and sicklepod (*Senna obtusifolia*). Weed Sci. 53:515–520.

Ojaghian, M.R., H. Jiang, G.L. Xie, Z.Q. Cui, J. Zhang, and B. Li. 2012. In vitro biofumigation of Brassica tissues against potato stem rot caused by *Sclerotinia sclerotiorum*. Plant Pathol. J. 28:185–190.

Olivier, C., S.F. Vaughn, E.S.G. Mizubuti, and R. Loria. 1999. Variation in allyl isothiocyanate production within Brassica species and correlation with fungicidal activity. J. Chem. Ecol. 25:2687–2701.

Porter, I.J. and S.W. Mattner. 2002. Non-chemical alternatives to methyl bromide for soil treatment in strawberry production. Proceedings of international conference on alternatives to methyl bromide. 43–47.

Ren, Z., Y. Li, W. Fang, D. Yan, B. Huang, J. Zhu, X. Wang, X. Wang, Q. Wang, M. Guo, and A. Cao. 2018. Evaluation of allyl isothiocyanate as a soil fumigant against soil-borne diseases in commercial tomato (*Lycopersicon esculentum* Mill.) production in China. Pest Manag. Sci. 74:2146–2155.

Samtani, J.B., H.A. Ajwa, J.B. Weber, G.T. Browne, S. Klose, J. Hunzie, and S.A. Fennimore. 2011. Evaluation of non-fumigant alternatives to methyl bromide for weed control and crop yield in California strawberries (*Fragaria ananassa* L.). Crop Prot. 30:45–51.

Samtani, J.B., J.B. Weber, and S.A. Fennimore. 2012. Tolerance of strawberry cultivars to oxyfluorfen and flumioxazin herbicides. HortScience 47:848–851.

Schweigkofler, W., K. Kosta, V. Huffman, S. Sharma, K. Suslow, and S. Ghosh. 2014. Steaming inactivates Phytophthora ramorum, causal agent of sudden oak death and ramorum blight, from infested nursery soils in California. Plant Health Res. 15:43–47.

Sharma, H.K., A. Garg, S. Charanjiv, and B.C. Sarkar. 2008. Effect of pretreatment conditions on the allyl isothiocyanate from mustard meal. J. Food Sci. Technol. 45:420–422.

Shin, J., B. Harte, E. Ryser, and S. Selke. 2010. Active packaging of fresh chicken breast, with allyl isothiocyanate (AITC) in combination with modified atmosphere packaging (MAP) to control the growth of pathogens. J. Food Sci. 75:M65–M71.

Stiehl, B. and B.B. Bible. 1989. Reaction of crop species to thiocyanate ion toxicity. HortScience 24:99–101.

Suhr, K.I. and P.V. Nielsen. 2003. Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. J. Appl. Microbiol. 94:665–674.

Tang, C.S. and W.J. Tang. 1976. Inhibition of papain by isothiocyanates. Biochim. Biophys. Acta 452:510–520.

van Loenen, M.C.A., Y. Turbett, C.E. Mullins, N.E.H. Feilden, M.J. Wilson, C. Leifert, and W.E. Seel. 2003. Low temperature–short duration steaming of soil kills soil-borne pathogens, nematode pests and weeds. Eur. J. Plant Pathol. 109:993–1002.

Vaughn, S.F. and R.A. Boydston. 1997. Volatile allelochemicals released by crucifer green manures. J. Chem. Ecol. 23:2107–2116.

Wu, H., G.A. Zhang, S. Zeng, and K.C. Lin. 2009. Extraction of allyl isothiocyanate from horseradish (*Armoracia rusticana*) and its fumigant insecticidal activity on four stored-product pests of paddy. Pest Manag. Sci. 65:1003–1008.

Zasada, I.A. and H. Ferris. 2003. Sensitivity of *Meloidogyne javanica* and *Tylenchulus semipenetrans* to isothiocyanates in laboratory assays. Phytopathology 93:747–750.