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Evaluation of a Steam Application by a Mobile Applicator for Soil Disinfestation in Strawberry Nurseries

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Abstract. Soil disinfestation with steam has been evaluated in strawberry fruiting fields as a nonchemical method of soil disinfestation; however, little is known about the use of steam for field production of strawberry daughter plants. The objective of this study was to compare daughter plant production in soils previously treated with steam compared to those treated with standard methyl bromide (MB) and chloropic-rin (Pic) treatments. A prototype field steam applicator and a self-propelled dieselfueled steam generator and applicator were tested at two high-elevation nurseries near Macdoel, CA, in Sept. 2018 and Aug. 2020, respectively. The steam application heated the soil above $60 \,^\circ C$ for ≈ 60 minutes to a depth of 25 cm at both nurseries. The pest control efficacy of the steam applications against weeds, *Verticillium* spp., *Tylenchulus semipenetrans*, and *Pythium ultimum* were similar to that of MB:Pic. The stolons and daughter plants densities in fields with steam treatment were similar to those in fields with MB:Pic treatment. Therefore, we suggest that soil disinfestation with steam may be a viable method of producing healthy strawberry plants. However, more research is needed to verify plant sanitation and quality.

Strawberry daughter plant production is a multi-location and multi-year process that begins in virus-free plant propagation facilities. Strawberry plants are vegetatively propagated in favorable warm climatic conditions at low-elevation nurseries (LENs) in California for one or two 8-month seasons (Voth, 1989). Strawberry daughter plants harvested from the LENs in winter are either moved to high-elevation nurseries (HENs) in northeastern California and southcentral Oregon for propagation of daughter plants for fruit production or planted as mother plants at LENs for a second year of propagation. Short photoperiods and cold night temperatures at the HENs increase subsequent plant productivity and vigor (Larson, 1994; Larson and Shaw, 2000; Voth and Bringhurst, 1990). The daughter plants are harvested at the HENs during September to November in preparation for planting in strawberry fruiting fields.

California strawberries are affected by soil-borne pests such as common chickweed (Stellaria media), common knotweed (Polygonum arenastrum Jord. ex Boreau), common purslane (Portulaca oleracea), little mallow (Malva parviflora), California burclover (Medicago polymorpha), Verticillium dahliae, Fusarium spp., Pythium ultimum, and

nematodes, which are primarily managed by soil fumigation (Fennimore et al., 2008a, 2008b, 2013; Samtani et al., 2011; Wilhelm, 1955; Wing et al., 1994). The California strawberry nursery industry is dependent on soil fumigants to control soil pests and enhance productivity (Kabir et al., 2005). The primary soil fumigants for soil disinfestation in California strawberry fruit production fields from the 1960s through the 2000s were MB and Pic and 1,3 dichloropropene; subsequently, MB was phased out (Ajwa et al., 2003; Wilhelm and Paulus, 1980). However, MB use continues to be allowed for strawberry nurseries under the quarantine and preshipment exemption of the Montreal Protocol because much of the daughter plant production is exported and subject to high phytosanitary standards (https://www.epa.gov/ods-phaseout/ methyl-bromide). Currently, some strawberry plants supplied to organic fruit producers come from fields fumigated with MB:Pic, and some producers are looking for methods to produce healthy strawberry plants without fumigation. Physical treatments such as steaming and solarization, nonchemical methods such as biofumigants (e.g., mustard seed meal), or biological control tools such as anaerobic soil disinfestation are a part of this approach for other crops (Daugovish et al., 2016; Kim et al., 2020; Porter and Mattner, 2002; Samtani et al., 2011). Steam is one of the more common methods of soil disinfestation for greenhouse soils. Steam does not leave harmful residues in the soil, and it is effective against a variety of soil pests. Samtani et al. (2012) found that steam disinfestation resulted in soil-borne pest control and fruit yield comparable to that of MB:Pic in strawberry fruiting fields. Although the effectiveness of steam for soil disinfestation in strawberry fruiting fields has been documented, steam effectiveness relative to MB has not been evaluated in strawberry nurseries. Therefore, the objective of this study was to evaluate daughter plant productivity and pest control after soil disinfestation with steam in strawberry daughter plant nurseries.

Materials and Methods

Steam application equipment. Steam was applied to a fallow field before planting strawberry plants at two nurseries near Macdoel. CA. Two types of steamer applicators were used in this study (Fig. 1). The prototype fieldscale steam applicator (Southern Turf Nurseries, Elberta, AL) used in 2018 was equipped with a 300-hp diesel-fueled Cleaver Brooks steam generator mounted on a trailer towed by a tractor (Fig. 1A). Steam was injected using a 3-m-wide reverse tiller that was set to till 30 to 40 cm deep (Northwest Tillers, Yakima, WA). The self-propelled diesel-fueled steam generator and applicator called the Steamy (JSE, Daegu, South Korea) used in 2020 was equipped with 20 straight shanks that could treat 2 m per pass (Fig. 1B). Steam was injected to a depth of 30 cm through a tube on the back of the shanks. HOBO (Onset Computer, Bourne, MA) loggers were placed immediately behind the steam applicator and left for 24 h to

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Fig. 1. The mobile applicator for soil disinfestation used in 2018 (A) and 2020 (B).

measure soil temperatures at depths of 10, 20, 25, and 35 cm below the soil surface.

Field trials. Four studies were initiated in the Butte Valley of Northern California, two each at Sierra Cascade Nursery (SCN) and Lassen Canyon Nursery (LCN), in both 2018 and 2020. The trials in the first (2018–19) and second (2020-21) seasons were conducted at different sites of the same nursery. Soils at both sites were sandy loam soils with less than 1% organic matter. At SCN, the studies included three treatments with four replicates arranged in a randomized complete block design. Treatments included MB:Pic 57:43 covered with high-density polyethylene (HDPE), MB:Pic 45:55 covered with totally impermeable film (TIF), and steam. Steam was applied using the Southern Turf Nurseries applicator on 6 Sept. 2018, and MB:Pic 57:43 with HDPE at 40 $g \cdot m^{-2}$ and MB:Pic 45:55 with TIF were applied at 50 $g \cdot m^{-2}$ on 22 Oct. 2018. Steam was applied using the Steamy on 27 Aug. 2020, and MB:Pic 57:43 with HDPE was applied at the rate of 40 $g \cdot m^{-2}$ on 31 Aug. 2020. Plots were 6.7 m wide \times 30.5 m long in 2018, and 6.1 m wide \times 12.2 m long in 2020. Strawberry plants (Fragaria ×ananassa cv. 'Maverick' 2019 and 'EW017' 2021) were transplanted on 11 Apr. 2019 and 11 May 2021 (Table 1). At LCN, the treatments included steam and MB:Pic replicated four times and arranged in a randomized complete block design. Steam was applied using the Southern Turf Nurseries applicator on 5 Sept. 2018, and MB:Pic was applied at a rate of 40 g·m⁻² on 6 Sept. 2018. Steam was applied using the Steamy on 31 Aug. to 2 Sept. 2020, and MB:Pic was applied at a rate of 40 $g \cdot m^{-2}$ on 12 Sept. 2020. Plots were 5.5 m wide \times 91.4 m long in 2018, and 6.7 m wide \times 30.5 m long in 2020. The field was fallowed over the winter months. Strawberry plants (Fragaria ×ananassa cv. 'Merced' 2019 and 'Monterey' 2021) were transplanted 25 Apr. 2019 and 20 Apr. 2021 (Table 1).

Data collection. Weed densities were measured over the entire length of the four beds per replicate per treatment. Both stolon and daughter plant counts were collected in 0.25-m² sample areas of each bed. Three sub-samples per plot were collected at both the SCN and LCN sites. Weed densities and numbers of stolons and daughter plants were assessed at monthly intervals from May to Sept. 2019 and from June to Oct. 2021 (Table 1). The plots were weeded and cultivated between weed counts; therefore, these

data represent sequential weed flushes that have been summed to provide cumulative seasonal weed emergence densities. Verticillium dahliae samples of infected soil were contained in nylon mesh bags in 2018. An isolate of Verticillium longisporum was used as a surrogate for V. dahliae to avoid the potential risk of accidental release of a strawberry pathogen into the nurseries in 2020. Ten pieces of filter paper with viable microsclerotia were put into sachets and buried 15 mm deep in the field before the steam and MB:Pic treatments. After the sachets were recovered, the filter pieces were plated onto NP10 media. If any growth of Verticillium spp. was observed, then that piece of filter paper was counted as having viable Verticillium spp. Sachets that contained 50 mL of soil inoculum containing citrus nematode (Tylenchulus semipenetrans) obtained from TriCal Inc. were buried at the nurseries. Sachets were gently placed into Baermann funnels lined with a mesh screen and Kimwipe filter, saturated with water, and allowed to incubate at room temperature for 3 d. The nematodes were incubated for 3 d under saturated conditions (water-saturated

Table 1. Critical trial events and dates.

Event	Date
Steam application	6 Sept. 2018, 27 Aug. 2020
MB:Pic application	22 Oct. 2018, 31 Aug. 2020
Transplanting	11 Apr. 2019, 11 May 2021
Steam application	5 Sept. 2018, 31 Aug. to 2 Sept. 2020
MB:Pic application	6 Sept. 2018, 12 Sept. 2020
Transplanting	25 Apr. 2019, 20 Apr. 2021
Weed counts	6 May, 13 June, 10 July, 15 Aug. 2019 4 June 2021
Stolon counts	13 June, 10 July 2019, 15 July 2021
Daughter plant counts	15 Aug., 11 Sept. 2019, 26 Aug., 12 Oct. 2021
	MB:Pic application Transplanting Steam application MB:Pic application Transplanting Weed counts Stolon counts

Table 2. Duration of the soil temperature at four depths for 1 h after steam application. Different steam applicators were used in 2018 and 2020.

		Time (min)					
		2018–19			2020–21		
Location	Depth (cm)	60 to 65 °C	65 to 70 $^\circ \mathrm{C}$	>70°C	60 to 65 °C	65 to 70 $^\circ \mathrm{C}$	>70°C
Sierra Cascade	10	2	56	0	0	0	0
Nursery	20	1.5	57.5	0	0	0	0
	25	0.5	44	15	0	0	0
	35	0	0	0	0	0	0
Lassen Canyon	10	0	0.5	59	0	0.5	59.5
Nursery	20	0	1	58.5	0	0	60
-	25	0.5	0.5	59	27.5	31.5	1
	35	1	0	0	0	0	0

soil in funnel) because up to 3 d were required for nematodes to swim to the bottom of the funnel and tubing. With flooded conditions, nematodes will swim downward. In real-world scenarios, this mimics rain on the soil surface; therefore, nematodes swim down to less saturated soil zones (more oxygen in the soil moisture). Nematodes collected in solution were then transferred to a test tube and a 1-mL aliquot was used to enumerate total live nematodes per funnel-extracted nematode solution when extrapolated to total nematode solution arising from the extraction process. Recovered nematodes were counted using a microscope. The quantity of propagules of Pythium ultimum from the soil at LCN was assayed before and after treatment using the method described by Klose et al. (2008). Recovered nematodes were counted using a microscope. The quantity of propagules of Pythium ultimum from the soil at LCN was assayed before and after treatment using the method described by Klose et al. (2008).

Statistical analyses. Duncan's multiple range test and the independent samples t-test were performed using SPSS statistical software version 20 (SPSS Inc, Chicago, IL). All statistical hypotheses tests were performed at a significance level of $\alpha = 0.05$.

Results

When the soil temperature was measured for 1 h after steam treatment, the soil temperatures at depths of 10 to 25 cm remained above 60 °C for 58 to 60 min at both SCN and LCN in 2018 (Table 2). The steam applicator used in 2020 did reach critical temperatures (>60 °C) to a depth of 25 cm at LCN in 2020, but it did not increase soil temperatures to 60° at any depth at SCN. Neither steam applicator was able to increase soil temperatures to critical temperatures to a depth of 35 cm.

Dominant weed species were clover (Trifolium repens L.), California burclover (Medicago polymorpha L.) and filaree (Erodium cicutarium L.) at SCN, whereas pigweed (Amaranthus retroflexus L.) and Russian thistle (Salsola tragus L.) were most common at LCN. The cumulative total weed densities between the steam and MB:Pic treatments in 2018-19 and 2020-21 were similar at both nurseries, suggesting that steam was as effective as MB:Pic at weed suppression (Table 3). The MB:Pic and steam applications reduced the number of active Tylenchulus semipenetrans and survival rates of Verticillium dahliae and V. longisporum in 2018-19 and 2020-21, regardless of nursery (Table 4). Especially at SCN, the MB:Pic 57:43 with HDPE and steam applications completely suppressed V. dahliae in 2018 to 2019. The steam application in 2020-21 did not control V. longisporum at SCN; however, it controlled T. semipenetrans and V. longisporum similar to MB:Pic at LCN (Table 4). The MB:Pic application completely eliminated Pythium ultimum in the soil in 2018-19 and 2020-21, whereas the steam application reduced P. ultimum densities by 100% in 2018-19 and by 83% in 2020-21 at LCN (Table 5).

Plant production and health during the season resulting from steam disinfestation was not different from that of MB:Pic. The stolon and daughter plant production and crown diameter with steam and MB:Pic treatments were not different at either nursery regardless of the season (Tables 6 and 7, Supplemental Table 1). Moreover, this trend was found during all trials. The growth of crown diameters with the MB:Pic treatment at SCN in the 2020–21 trial was slower than that with the steam treatment at the beginning; however, later in the season, it was similar (Supplemental Table 1).

Discussion

Melander and Jørgensen (2005) reported that the steam application of more than 60 °C for 70 s and 70 °C for 90 s reduced weed emergence by 90% and 99%, respectively. van Loenen et al. (2003) found that the minimum lethal temperature of the steam application for Pythium ultimum and Verticillium dahliae was 60°C for 180 s. Tylenchulus semipenetrans was controlled by maintaining 45 °C for 25 min (Baines et al., 1949) or 50 °C for 10 to 20 min (Silva et al., 1987). During our study, the temperature was more than 60 °C for \approx 60 min at the depths of 10 to 25 cm at both nurseries (Table 2). Benvenuti et al. (2001) reported that the emergence depth limit of weed species was considerably less than 10 cm; therefore, the observed soil temperature in this trial was probably sufficient to reduce weed seedling emergence.

Weeds compete with strawberry plants for sunlight, water, and nutrients, and they serve as alternate hosts of strawberry pathogens (Johnson and Fennimore, 2005). The final Table 3. The cumulative season-long weed densities and hand weeding times for the steam and MB:Pic treatments.

		Weed	Weed density	
		2018-19	2020-21	2020-21
Location	Treatment	No	./m ²	$s \cdot m^{-2}$
Sierra Cascade	MB:Pic 57:43 w/HDPE	$1051 \pm 87 \ a^{z}$	56.0 ± 14.0 a	$290 \pm 60.0 \text{ a}$
Nursery	MB:Pic 45:55 w/TIF	351 ± 49 a	-	_
•	Steam	$655 \pm 61 a$	$30.0 \pm 6.9 \text{ a}$	$215 \pm 6.5 a$
Lassen Canyon	MB:Pic	$124 \pm 37 a^{y}$	$7.3 \pm 4.1 a$	$3.4 \pm 0.3 a$
Nursery	Steam	237 ± 98 a	7.8 ± 0.8 a	$3.7\pm0.5~a$

^zMean separation by Duncan's multiple range test.

^yMean (±sE) separation by independent samples t-test.

Means followed by the same letter within columns do not differ significantly at the 5% level.

product of strawberry nurseries, daughter plants, can be infected from the soil, and soil pathogens can be transferred from mother plants grown in the infested soil to daughter plants (Gordon et al., 2002). Infection of daughter plants at any step in the process can cause the crop to be discarded. Therefore, soil disinfestation is important to produce pathogen-free daughter plants. Many studies have demonstrated that MB:Pic provides excellent weed control at strawberry nurseries (Fennimore et al., 2008a, 2008b;

Table 4. The number of active nematodes of *Tylenchulus semipenetrans* and survival rates of *Verticil-lium dahliae* and *V. longisporum* at a depth of 25 cm.

		T. semip	T. semipenetrans		V. longisporum
		2018-19	2020-21	2018-19	2020-21
Location	Treatment	No. of active 1	nematodes/50 g	Surv	rival %
	Nontreated control	790.3 ± 79.9 b	638.3 ± 43.0 c	$77.5 \pm 14.4 \text{ b}^{z}$	$1049.0 \pm 601 \text{ b}$
Sierra Cascade Nursery	MB:Pic 57:43 w/HDPE	$10.8\pm4.0~a$	$2.4\pm0.7~a$	0.0 a	33.0 ± 19.4 a
5	MB:Pic 45:55 w/TIF	2.0 ± 2.0 a	-	$2.5\pm2.5~a$	-
	Steam	$17.0 \pm 6.3 \text{ a}$	100.6 ± 44.9 b	0.0 a	$424.8 \pm 174 \text{ ab}$
Lassen Canyon	MB:Pic	_	$4.3 \pm 2.5 \ a^{y}$	_	59.0 ± 35.7 a
Nursery	Steam	_	14.5 ± 6.7 a	_	41.5 ± 19.9 a

^zMean (\pm se) separation by Duncan's multiple range test.

^yMean separation by independent samples T-test.

Means followed by the same letter within columns do not differ significantly at the 5% level.

Table 5. *Pythium ultimum* control before and after treatment with steam and fumigant at Lassen Canyon Nursery.

	2018–19				2020–21	
	Pretreatment	Posttreatment	_	Pretreatment	Posttreatment	_
Treatment	Propagules (n/g)		Reduction %	Propagules (n/g)		Reduction %
MB:Pic Steam	$\begin{array}{c} 9.7 \pm 2.8 \ a^z \\ 10.0 \pm 3.8 \ a \end{array}$	0.0 a 0.0 a	100 100	35.7 ± 7.9 a 47.7 ± 17.7 a	$0.0 \text{ a} \\ 8.0 \pm 4.7 \text{ a}$	100 83.2

^zMean (\pm se) separation by independent samples t-test.

Means followed by the same letter within columns do not differ significantly at the 5% level.

		2018–19		2020-21
		Stolon densities		
		6 June	10 July	15 July
Location	Treatment		No./m ²	
Sierra Cascade Nursery	MB:Pic 57:43 w/HDPE MB:Pic 45:55 w/TIF Steam	$1.0 \pm 0.3 \text{ a}^{z}$ $3.5 \pm 0.8 \text{ a}$ $3.0 \pm 0.9 \text{ a}$	18.0 ± 0.4 a 18.5 ± 0.8 a 21.5 ± 1.1 a	$12.2 \pm 1.7 \text{ a}$ - $8.8 \pm 1.3 \text{ a}$
Lassen Canyon Nursery	MB:Pic Steam	$6.3 \pm 1.0 a^{y}$ $9.7 \pm 1.5 a^{y}$	33.0 ± 2.2 a 32.3 ± 3.9 a	57.8 ± 8.0 a 66.4 ± 3.3 a

^zMean (±sE) separation by Duncan's multiple range test.

^yMean separation by independent samples T-test.

Means followed by the same letter within columns do not differ significantly at 5% level.

Table 7. The daughter plant densities for the steam and MB:Pic treatments.

		2018–19		2020–21		
		Daughter plant densities				
		15 Aug.	11 Sept.	26 Aug.	12 Oct.	
Location	Treatment	No./m ²				
Sierra Cascade Nursery	MB:Pic 57:43 w/HDPE	$21.8 \pm 2.2 \ a^{z}$	65.8 ± 14.2 a	33.9 ± 1.8 a	$44.9 \pm 2.2 \text{ a}$	
,, ,	MB:Pic 45:55 w/TIF	$22.8 \pm 1.5 a$	85.8 ± 17.0 a	-	_	
	Steam	24.5 ± 1.6 a	80.3 ± 19.1 a	$33.1 \pm 8.8 \text{ a}$	39.2 ± 1.1 a	
Lassen Canyon	MB:Pic	$41.5 \pm 8.2 \ a^{y}$	211.8 ± 25 a	84.4 ± 18.5 a	_	
Nursery	Steam	$53.0\pm5.9\;a$	$227.7\pm23~a$	$113.9 \pm 10.5 \text{ a}$	-	

^zMean (±sE) separation by Duncan's multiple range test.

^yMean separation by independent samples t-test.

Means followed by the same letter within columns do not differ significantly at the 5% level.

García-Méndez et al., 2008). Weeds such as prostrate knotweed, common purslane, common chickweed, hairy nightshade, and pigweed were completely controlled by MB:Pic. Our results indicate that the soil-borne pest control was similar with the steam and MB:Pic treatments. Fennimore et al. (2014) showed that steam and steam plus mustard seed meal applications suppressed seed viability of seven weed species, cumulative weed biomass and density, and P. ultimum populations in strawberry fruiting fields. Samtani et al. (2012) also reported that steam application reduced common chickweed, common knotweed, common purslane, little mallow, yellow nutsedge, and V. dahliae similar to MB:Pic standard treatment in fruiting fields. The combined results suggested that steam is as effective as the MB:Pic standard applications in the strawberry nurseries.

García-Méndez et al. (2008) showed that weed control and daughter plant production by fumigation with MB:Pic, chloropicrin, 1,3dichloropropene:chloropicrin, metam sodium plus chloropicrin, dimethyl disulphide plus chloropicrin, and propylene oxide were positively correlated. Similar effects on pest control determined in this study could result in similar effects on stolon and daughter plant productivity. This may be because no significant differences were found in the cumulative weed densities, the survival rates of V. dahliae and V. longisporum, the number of active T. semipenetrans, and P. ultimum control before and after treatment with the steam and MB:Pic standard applications in the early season (Tables 3-7). Moreover, the weeds, stolons, and daughter plants were assessed 8 to 10 months after the applications of steam and MB:Pic, and the data indicate that the longterm effects of steam are similar to the longterm disinfestation effect of MB.

Chemical alternatives to MB for weed control and daughter plant production in strawberry nurseries have been evaluated by many researchers (Fennimore et al., 2008a, 2008b; García-Méndez et al., 2008; Kabir et al., 2005; Larson and Shaw, 1995, 2000; López-Medina et al., 2007); however, to the best of our knowledge, effective steam has not been previously tested in California strawberry nurseries. For instance, solarization was studied by Hartz et al. (1993), but it was not suitable as an alternative for most daughter plant producers because of the cool conditions at the highelevation nurseries in California. In that sense, steaming, one of the more reliable physical methods, may be an alternative for strawberry nurseries.

The combination of steam and organic fertilizers should be considered to improve stolon and daughter plant productivity in strawberry nurseries. Samtani et al. (2011) reported that the combination of steam and an organic fertilizer application (AgroThrive, 2.5-2.5-1.5 NPK) in California conventional strawberry production fields resulted in higher marketable fruit yields than steam alone, although weed and disease control data for both treatments were similar. Fennimore et al. (2014) showed that the combination of steam and mustard seed meal application increased NH_4^+ and NO_3^- concentrations in the soil in strawberry production fields. Furthermore, to improve the efficiency of steam treatment, the combination of steam and exothermic compound (KOH and CaO) was considered by Bàrberi et al. (2009) and Peruzzi et al. (2012). Therefore, the combinations might be helpful for daughter plant producers in strawberry nurseries.

Conclusion

Our data collected at two strawberry nurseries indicate that steam application by the mobile steam applicator was as effective as MB:Pic for weed and phytopathogen control and production of daughter plants. However, development of more effective steam applicators and verification of their efficacy will be necessary if steam application is to be considered a dependable soil disinfestation method for strawberry daughter plant nurseries, where phytosanitation is required at each cultural production step. To implement more widescale use of steam in strawberry nurseries, it will be necessary to 1) demonstrate that plants are high-quality and pathogen-free to meet strict phytosanitary standards and 2) develop an effective commercial-scale steam applicator or purchase existing steam applicator technology.

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Supplemental Table 1	. Crown diameters	for the steam and MB:Pi	c treatments during 2020-21.
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		Crown diameters		
		26 Aug.	12 Oct.	
Location	Treatment	mm		
Sierra Cascade Nursery	MB:Pic 57:43 w/HDPE	8.4 a ^z	11.6 a	
-	Steam	10.4 b	11.6 a	
Lassen Canyon Nursery	MB:Pic	12.0 a		
	Steam	12.2 a		

^zMean separation by independent samples t-test. Means followed by the same letter within columns do not differ significantly at the 5% level.