Final Report to the DPR: Grant Agreement 12-PML-R001

Title: Methyl bromide Alternatives to Strawberry Nurseries

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1. OVERVIEW

The project conducted a field trial at a commercial high elevation strawberry nursery to test the efficacy of alternatives to methyl bromide/chloropicrin (MB/C) fumigation with the following: 1) the most promising chemical based alternative, Pic-Clor 60 (1,3-dichloropropene/chloropicrin) under impermeable film; 2) the most practical non-chemical MB/C alternative, anaerobic soil disinfestation (ASD); and 3) an existing multi-year crop rotation that shows promise in potentially suppressing soil pathogens. The Pic-Clor 60 treatment can be used now to replace MB/C, and the ASD treatment could be used medium term as a non-fumigant alternative. In an organic system, crop rotation with wheat/peas plus compost for up to four years has already been observed to have promise as a long-term alternative to MB/C.

Treatment performance was evaluated by measuring nematode and weed control, yield and quality of nursery plants, and subsequent marketable and total fruit yield in Monterey. We used MiSeq DNA sequencing of ribosomal DNA to understand changes in the microbial community among the treatments and develop metrics for soil quality that are correlated with the production of high quality nursery transplants. In addition to gaining insight into how fumigants affect nutrient availability and the rhizosphere population, the correlation between the conditions in the nursery with the ultimate productivity of the plants in the fruiting field was examined. Consequently, both the efficacy of the best methyl bromide alternatives were evaluated and critical knowledge in the poorly understood growth promotion from MB/C were somewhat assessed.

2. SCOPE OF WORK

Goals

1) Develop efficacious and economical alternatives to methyl bromide/chloropicrin (MB/C) for the California strawberry nursery industry and for other agricultural users.
2) Develop longer-term IPM strategies, using MiSeq ribosomal DNA analysis as a culture-independent method to get insights into the growth promotion effects of MB/C.
3) Develop non-chemical strategies that will predictably and efficaciously promote plant growth and control soilborne pests and pathogens.

Objectives

Objective 1. Conduct general grant administration: meetings, progress reports, invoices, and final report as required. This objective was performed, except that the final report was filed after the 31 March 2016 due date so that we could have more time to finish data analysis. In addition to communication with strawberry nurseries, results were presented on-time in a verbal presentation at DPR on 11 February 2016.

Objective 2. To determine the effect of two fumigant treatments (MB/C, and Pic-Clor 60 under Impermeable Film, ASD and an untreated control on nematodes, weeds, pathogens, strawberry nursery yield, and marketable and total fruit yield of transplants.

This objective was performed in its entirety except that the results have not been published for multiple reasons: 1) we only had one year of the fully replicated methyl bromide alternatives trial, which is typically insufficient for publication in peer-reviewed journals; 2) there was an overall absence of pathogens in the nursery, which limits the inferences that can be made about the benefits and problems with the alternative treatments; and 3) the cooperating nursery does not want data on yield of their cultivars published.

Objective 3. To determine the effect of multiple and varied years of a wheat/pea and compost rotation in comparison to Pic-Clor 60 on the nematodes, weeds, pathogens, strawberry nursery yield, and marketable and total fruit yield of transplants.
This objective was performed in its entirety except that the results have not been published for multiple reasons: 1) the plots in the organic vs. conventional plots were pseudo-replicated, not independently replicated, which is typically insufficient for publication in peer-reviewed journals; 2) there was an overall absence of pathogens in the nursery, which limits the inferences that can be made about the benefits and problems with the alternative treatments; 3) the cooperating nursery insisted on changing cultivars after the first year and consequently we do not have two years of trial data for a single cultivar in the organic vs. conventional trial; and 4) the cooperating nursery does not want data on yield of their cultivars published.

Objective 4**.** To use nutritional analysis and MiSeq ribosomal DNA sequence and microbial community analysis to determine the microbial and nutritional factors that contribute to the methyl bromide growth promotion. The data collection was performed for this objective, but we did not observe a statistically recognizable methyl bromide/chloropicrin growth promotion. Consequently, we did collect potentially useful information on the strawberry rhizoplane biome, but it's not sufficient for publication at this time.

Major accomplishments and recommendations for outreach and further research.

1. Using bagged citrus nematodes buried at 9 inches deep in the soil in a fully replicated trial, we demonstrated that ASD under an impermeable film at a high elevation nursery in California was as effective as either methyl bromide/chloropicrin or PicClor 60 in reduction of a "marker" nematode (Table 1). Currently, fumigant use in strawberry nurseries is largely driven by regulations from the California Department of Food and Agriculture (CDFA) for nematodes; if the nurseries use approved fumigants or other approved means of soil disinfection, the plants from the nurseries are pre-approved for certification, and consequently for out-of-county sale. (Note that essentially all of the strawberry plants from the high elevation nurseries are sold to out-of-county fruit producers.) Conversely, if growers do not use these methods, they are required to have sampling and testing at the end of the growing season; if the plants are not nematode free, out-of-county sale is prohibited. Consequently, even in the absence of pathogens, there is a regulatory-generated incentive to fumigate in the nursery, even though such an application is in conflict with the principals of Integrated Pest Management (IPM), which explicitly state that pesticides should not be used when pests are absent. To summarize, while sale of clean plants is an important part of an IPM program, we contend that CDFA regulations encourage unnecessary soil fumigation in uninfested soil in high elevation strawberry nurseries.

   But is ASD at high elevation nurseries a viable alternative for the currently approved fumigants and practices at high elevation nurseries? Although the CDFA regulations only pertain to nematodes, clearly nematodes were selected as an indicator of a broader disinfection of soil. In our trial conditions without significant "natural" pathogens or serious levels of weed seeds, we can't speculate. In addition, it is unclear whether CDFA would certify ASD as a pre-approved practice in the nursery; certainly CDFA would require monitoring citrus nematode bags at greater depth. However, the efficacy of the ASD is presumably dependent upon a number of factors including the soil temperature during the treatment period and the length of time the soil was tarped, and consequently predicting nematode kill at a level indistinguishable from that with approved materials would be difficult. Nonetheless, we demonstrated that ASD could be done on a commercial nursery scale.

2. <u>In the high elevation nursery environment without discernible pathogens and with excellent nutrition, we did not observe any growth benefit of methyl bromide/chloropicrin in either the nursery (Table 2) or in subsequent fruit production (Table 3).</u> Although we did not detect differences statistically in the incidence of weeds in the nursery plots (Table 4), I thought that I visually could see some differences, and, given that weed control is a major problem and that methyl bromide typically provides excellent weed control, we speculate that more intensive sampling would enable detection of differences in the mean incidence of weeds. We wanted to explore two hypotheses about the frequently observed methyl bromide/chloropicrin growth promotion: 1) treatments directly or indirectly resulted in differences in mineral nutrition; or 2) treatments directly or indirectly resulted in differences in microbial populations (particularly the increased incidence of minor pathogens). Perhaps not surprisingly in the absence of growth promotion, we did not observe what appeared to be any important differences in mineral nutrition in the treatments. However, we observed some differences in the bacterial populations on the strawberry root surface when we sampled the strawberry rhizoplanes on transplants. (Table 5). We note that the methyl bromide alternative treatments occurred during the summer of 2013, and then we waited to sample the rhizoplane populations from the high elevation nursery daughter plants at the time of their harvest in fall of 2014. The analysis in Table 5 is focused on specific bacterial species that are in relatively high incidence, because strawberry transplants could be infested with biocontrol agents, either when planted at the nursery, or when planted in the grower's fields. Interestingly, we discovered one species, *Arthrobacter psychrochitiniphilus* that we can presume accounted for 24% of the bacterial population on the rhizoplane in the methyl bromide/chloropicrin treatment and were less common on the lowest (14%) in the untreated. *Arthrobacter spp*. (in the phylum Actinobacteria) are common inhabitants in soil, and some have been reported to form growth promoting compounds; they have been isolated previously from strawberry plants (Scheublin & Leveau 2013). The type isolate of *A. psychrochitinphilus* was isolated from Antarctica (from Adele penquin guano), but has an optimal growth at 20 C, tolerates a range of temperatures (0 to 25 C) and has excellent chitin-utilizing ability (Wang et al. 2009), which has been frequently been associated with biocontrol activity for plant pathogens. Whether *A. psychrochitiniphilus* might be a potential biocontrol agent remains unknown, but it might be worth pursuing in a future grant. In addition to the characterizing the bacterial populations on the strawberry rhizoplanes, we also examined the fungal populations (Table 6).

3. In pseudo-replicated trials in 2013 and 2014, we compared the nursery organic production system to an adjacent field that was treated with the fumigant PicClor 60. Typically, plants in an organic system do not have the yield as those in a conventional system. Consequently, to increase the yield in the organic system, the nursery starts the organic system under plastic tunnels, in order to encourage early-season plant reproduction. While this represents current production practice, a comparison of organic vs. conventional is not a classical treatment effect in that there is much more than a difference in fumigation, as there was in the methyl bromide alternatives trial. Perhaps interestingly, in 2013, the yield of nursery plants was significantly greater in the conventional plants, but the organic plants were significantly larger (Table 7). In 2014, the differences were not as pronounced, but the organic plants tended to be larger (Table 8), consistent with the hypothesis that the organic plants reproduced more slowly than the fumigated plants. Although we did not perform an extensive time series analysis of mineral nutrition, at the time points that we examined, we were unable to attribute differences in growth

to differences in mineral nutrition in the soil, or in the plants. Because we did not want size to be a difference between those plants that were produced in the nursery under organic vs. conventional, we selected a standardized size of transplants for the fruiting trial; in the fruiting trial, all transplants were planted in fumigated soil. Regardless of whether the transplants came from an organic vs. a fumigated nursery production system, the transplants produced an indistinguishable quantity and quality of fruit in both years (Table 9). This result is consistent with our observations that organic production in this nursery during our study period was "clean," obviously in the absence of fumigation in the nursery, supporting our position in point #1, that regulations that encourage yearly fumigation in the nursery are unnecessary for optimal plant health and production. Rhizoplane populations on the organic vs. fumigated treatments were distinguishable, as determined by both a "Phylochip" (Table 10) and by MiSeq ribosomal DNA analysis (Table 11). However, not unexpectedly, rhizoplane populations are affected by the soil that they're planted in (high elevation nursery vs. fruit production field and strawberry cultivar, and to a lesser extent by organic vs. fumigated in the nursery) Phyla-level analysis is shown in Table 12. We note that *Arthrobacter psychrochitiphilus* was still detected on plants harvested from the fruiting fields, albeit at lower levels than observed in the high elevation nursery. Nonetheless we note the complexity of the bacterial communities on the strawberry rhizoplanes in both the organic vs. the fumigated soils; overall, we detected 2,814 bacterial species in our strawberry rhizoplanes, and consequently, finding a biocontrol agent that will be able to colonize and displace the soil flora could be extremely difficult. Regardless, *A. psychrochitiphilus* remains a possibility.

4. We had an excellent partnership with a cooperating nursery that assured that our data were collected in "real-world" high elevation nursery, and we're grateful for their cooperation in this research.

Table 1.

ASD results: citrus nematodes buried at 9 inches

Treatment	Mean Log_{10} (Number of nematodes per 50 cc +1)	Detransformed No. nematodes/50 cc
0 time Lab, 4 °C	2.8 a	684 a
0 time, Field & return	2.8 a	614 a
63 day Lab, 4 °C	2.6 a	413 a
Untreated Soil	2.5 a	332 a
PicClor 60	0.3 b	1.1 b
Methyl Bromide/chloropicrin	0.1 b	0.4 b
ASD	0.0 b	0.0 b

Means followed by the same letters are not significantly different by Tukey's HSD at α=0.05.

Table 2.

The effect of soil treatments in 2013 on the marketable yield of strawberry runners produced in 2014.

Treatment	No. market-able plants/m²	SEM	Wt per market-able plant, g	SEM	Crown diam, mm	SEM	No. roots/ daugh-ter	SEM
Untreated	103.4	10.9	9.6	0.3	10.8	0.4	17.0	0.5
MBr/Chlor	107.8	15.5	9.4	0.3	11.4	0.3	16.0	0.7
PicClor 60	105.2	10.4	9.0	0.4	11.2	0.4	15.7	0.6
ASD	91.9	10.6	9.5	0.6	11.6	0.4	17.1	0.7

[x]There were no significant differences between treatments in either the number of marketable plants (P=0.86), weight/marketable plant (P=0.81), crown diameter (P=0.58) , or the number of roots per daughter (P=0.35). No significant block effects (P=0.19, 0.42, 0.44, and 0.51), respectively.

[y]This trial was in a randomized complete block design with four replicated blocks.

Table 3.

Cumulative marketable strawberry fruit yield in Watsonville in 2015 from transplants that were produced in the nursery with the indicated treatments (and then planted into a fumigated production field)

Treatment	Marketable lbs/A ± SEM	Grams/ marketable berry± SEM	% Culls ± SEM
Untreated Control	74,621 ± 2,358	19.8 ± 0.10	0.22 ± 0.005
MBr/Chloropicrin	72,143 ± 2,662	20.1 ± 0.24	0.22 ± 0.01
PicClor 60	69,710 ± 2,274	19.9 ± 0.09	0.22 ± 0.001
ASD	73,309 ± 4,013	20.0 ± 0.27	0.22 ± 0.01
(*P*-value)	0.77	0.88	0.97

There were no significant treatment or block effects.

Table 4.

Weeds in the methyl bromide alternative plots on June 16, 2014.

Nursery soil treatment	No. weeds/m^2	SEM
Anaerobic soil disinfestation	6.0	0.5
Methyl bromide & chloropicrin	3.6	1.0
Pic-Clor 60	5.0	1.5
Untreated	8.9	2.4

The study is in a completely randomized block design with four blocks. There were no significant nursery treatment (P=0.24) or block effects (P=0.26) on the number of weeds.

Table 5.

Based on MiSeq rDNA 16S, the percentage of the six most frequently isolated bacterial species from the strawberry rhizoplanes in 2014

Treatment	*Arthro-bacter psychro-chitiniphilus*		*Nocard-ioides islandensis*		*Strepto-myces rose-ogilvus*	*Strepto-myces kaga-waensis*		*Sphingo-monas oligo-phenolica*	*Kaisto-bacter terrae*	
	Percentage of normalized counts[b]									
ASD	17.35	bc	3.53	b	0.76	0.95	ab	1.40	4.26	a
Methyl Bromide/CP	24.30	a	4.06	ab	0.48	0.38	b	1.71	1.06	b
PicClor60	21.28	ab	3.80	b	1.68	1.09	ab	1.60	2.46	ab
Untreated	13.85	c	4.70	a	2.23	1.65	a	1.53	1.55	b
Soil *P*	0.002		0.003		0.21	0.037		0.17	0.006	
Block *P*	0.12		0.85		0.24	0.79		0.43	0.28	

Ca. 1200 OTU/sample

Table 6.

Based on MiSeq rDNA ITS, the percentage of the 10 most frequently isolated fungal genera from the strawberry rhizoplanes in 2014: Average 348 OTU/sample

Nursery treatment	*David-iella*	*Phyllo-sticta*	*Coniella*	*Filo-basidium*[a]	*Alter-naria*	*Crypto-coccus*[a]	*Botry-tis*	*Mono-chaetia*	*Rhizo-phagus*[b]	*Sporidi-obolus*
ASD	18	8.9	1.4b	8.7	3.9	4.5	2.8b	5.1	4.1	2.7
Methyl Bromide/CP	18	8.6	2.1ab	8.4	5.4	4.4	4.5b	0.58	2.1	2.5
PicClor60	19	8.5	1.6b	8.1	5.2	6.6	8.1a	1.36	2.0	3.0
Untreated	16	7.8	5.2a	6.8	4.2	4.6	3.8b	1.01	2.9	2.6
P-value	0.97	0.99	0.012	0.86	0.18	0.4	0.0001	0.08	0.39	0.81

[a]Filobasidium & Cryptococcus are probably the same genus

[b]Presumably an arbuscular mycorrhizal fungus

Table 7.

Marketable yield of strawberry runners produced at high elevation in an organic versus and an adjacent "conventional" field in 2013

Production system	No. marketable plants/m² ± SEM	Wt per marketable plant, g ± SEM	Crown diam, mm ± SEM	No. main roots/ daughter ± SEM
Pre-plant fumigation	**68 ± 14***	9.2 ± 0.9	10.6 ± 0.3	12.5 ± 1.1
Organic	40 ± 11	**14.0 ± 0.7*****	**12.4 ± 0.5****	**20.4 ± 1.0****

Table 8.

Marketable yield of strawberry runners produced at high elevation in an organic versus and an adjacent "conventional" field in 2014[x]

Treatment	No. market-able plants/m² ± SEM	Wt per market-able plant, g ± SEM	Crown diam, mm ± SEM	No. roots/ Daughter ± SEM
Pre-plant fumigation	79.8 ± 7.4	7.0 ± 0.3	10.1 ± 0.4	16.5 ± 0.4
Organic	59.6 ± 7.4	**10.3** ± 0.7**	10.5 ± 0.2	**20.4** ± 0.7**

[x]There were highly significant differences in weight per marketable plant (***P*=0.0046**) and number of main roots per daughter (***P*=0.0039**) but no significant differences in either number of marketable plants (*P*=0.10) or crown diameter at the widest point (*P*=0.35).

Table 9.

Cumulative marketable strawberry fruit yield in Watsonville in the 2014 and 2015 seasons of runners that were produced either organically or conventionally in the nursery (and then planted into a fumigated production field)

	2014	2015
Nursery production	**Marketable Lbs/Acre ± SEM**	
Fumigated	**55,108 ± 2,743**	**74,132 ± 2,477**
Organic	**51,537 ± 2,165**	**73,360 ± 2,477**

There was no significant nursery treatment (P=0.52 and 0.89) or fruit row block effects (P=0.99 and 0.76) on fruit yield in either 2014 or 2015, respectively. Similarly, there were no significant differences in % culled.

Table 10.

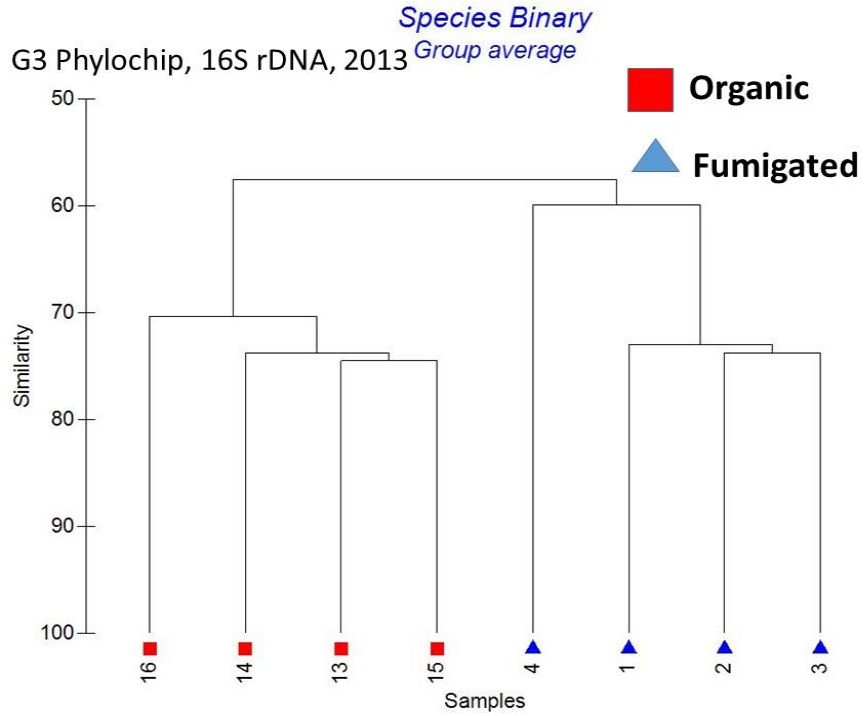

Table 11.

Based on MiSeq rDNA, the percentage of the six most frequently isolated bacterial species from the strawberry rhizoplanes in 2014

Treatment	*Arthro-bacter psychro-chitiniphilus*		*Nocard-ioides islandensis*		*Strepto-myces rose-ogilvus*		*Strepto-myces kaga-waensis*		*Sphingo-monas oligo-phenolica*	*Kaisto-bacter terrae*	
	Percentage of normalized counts										
Nurs-Con13, Fruit14	3.8	b	1.0	c	6.8	a	5.4	ab	2.8	0.35	b
Nurs-Org13,Fruit14	1.2	b	0.9	c	9.5	a	7.4	a	2.8	0.20	b
Nurs-Conv14	11.4	a	4.1	a	1.1	b	0.9	c	1.7	0.25	b
Nurs-Org14	10.5	a	3.3	b	0.6	b	3.3	bc	1.7	1.61	a
P-value	<0.0001		<0.0001		<0.0001		0		0.02	<0.0001	

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Table 12.

Bacterial Phyla on the organic vs conventional nursery rhizoplanes (Del Rey), and on San Juan after fruit production: most Proteobacteria, next Actinobacter

Treatment	Proteo-bacteria		Actino-bacteria		Firmi-cutes		Bactero-idetes		Verruco-microbia		Chloro-flexi	Planctomycetes	Teneri-cutes	Acido-bacteria		Gemmati-monadetes	Thermi	Syner-gistetes	
	Percentage of normalized counts																		
13Con14Fruit	49.8	b	33.7	a	7.6	ab	3.5	a	1.9	a	1.4	0.52	0.54	0.16	b	0.17	0.14	0.03	b
13Org14Fruit	54.2	b	32.2	a	6.4	b	2.3	b	1.7	a	1.2	0.50	0.49	0.12	b	0.08	0.10	0.03	b
Conventional14	62.0	a	21.8	b	8.5	ab	3.3	a	1.0	b	1.5	0.45	0.50	0.12	b	0.17	0.15	0.09	a
Organic14	51.6	b	31.4	a	9.0	a	3.2	ab	0.9	b	1.5	0.56	0.56	0.38	a	0.20	0.17	0.04	b
Soil P	0.0004		0.0017		0.040		0.0096		0.0006		0.46	0.11	0.72	0.0002		0.051	0.055	<.0001	