Methyl Bromide Alternatives for Strawberry Nurseries

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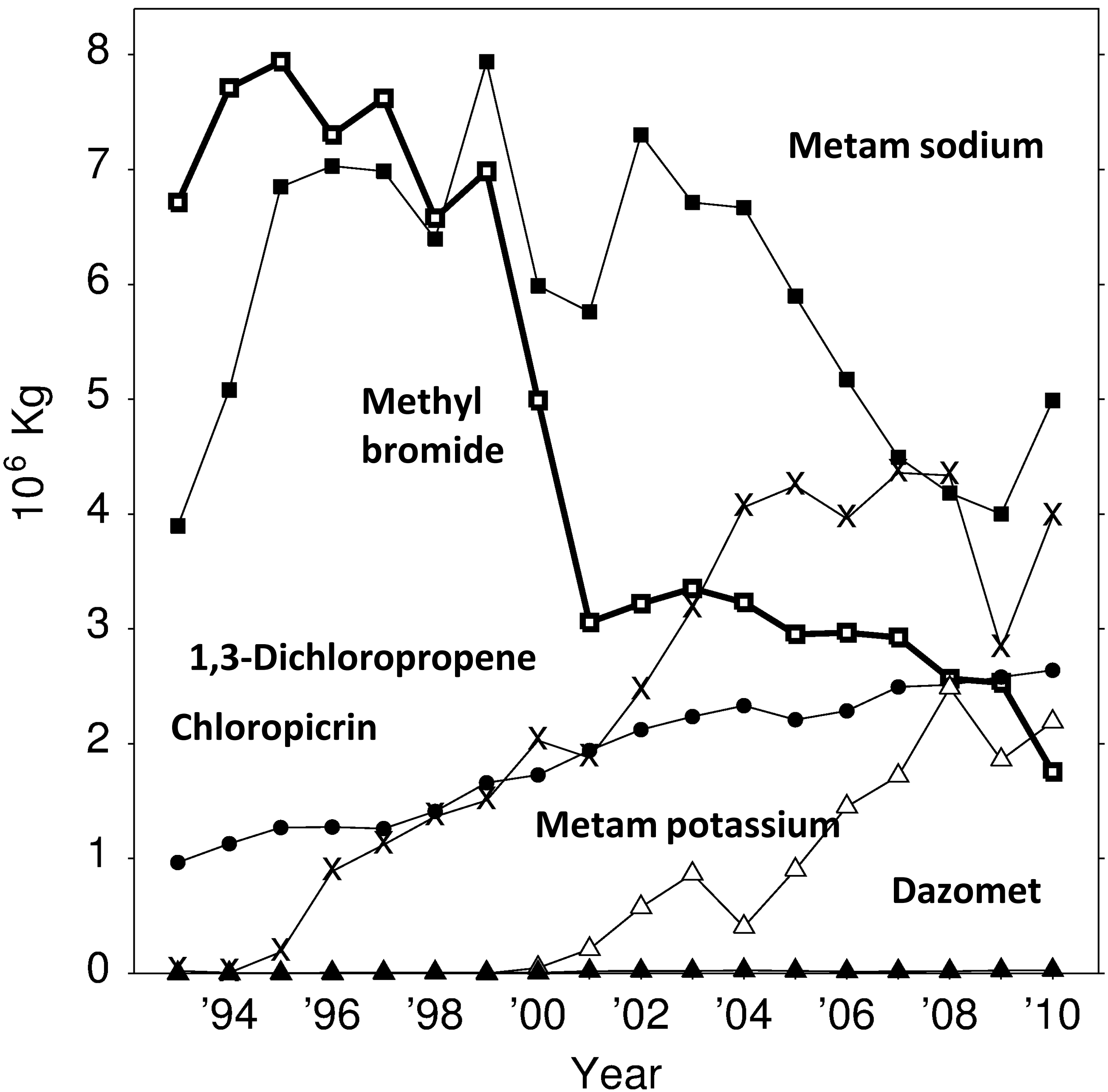
A fantastic cooperating nursery



PUR data of mass in millions of kg of agricultural fumigants used in California between 1993 and 2010

From Epstein & Zhang. 2014. The impact of IPM programs on pesticide use in California, USA. In: R. Peshin, D. Pimentel, *Integrated Pest Management: Experiences with Implementation, Vol. 4. Global Overview*, pp. 173-200. Springer, The Netherlands.

Trends in Fumigants of Regulatory Concern used in Agricultural Fields

Compound	2008 – 2010 annual average applications, kg	% change from 1993 - 1995	Risk groups[a]
1,3-Dichloropropene	**$3.7 X 10^6$**	**+5120**	**C, A**
Metam potassium	**$2.2 X 10^6$**	**(new)**	**A**
Metam sodium	**$4.4 X 10^6$**	-17	**R, C, A**
Methyl bromide	**$2.3 X 10^6$**	-69	**R, A**
Chloropicrin	**$2.6 X 10^6$**	**+130**	**(A)**

[a]A, listed as a DPR toxic air contaminant; (A) Newly listed as an air contaminant?
C, putative carcinogen
R, Proposition 65 reproductive toxin

From Epstein & Zhang. 2014. The impact of IPM programs on pesticide use in California, USA. In: R. Peshin, D. Pimentel, *Integrated Pest Management: Experiences with Implementation, Vol. 4. Global Overview*, pp. 173-200. Springer, The Netherlands.

Verticillium in Chrysanthemum

costly diseose controlled by practice of culture• indexing and soil f11migation with chloropicrin

Stephen Wilhelm c,nd lllcltard H. Sclaronl

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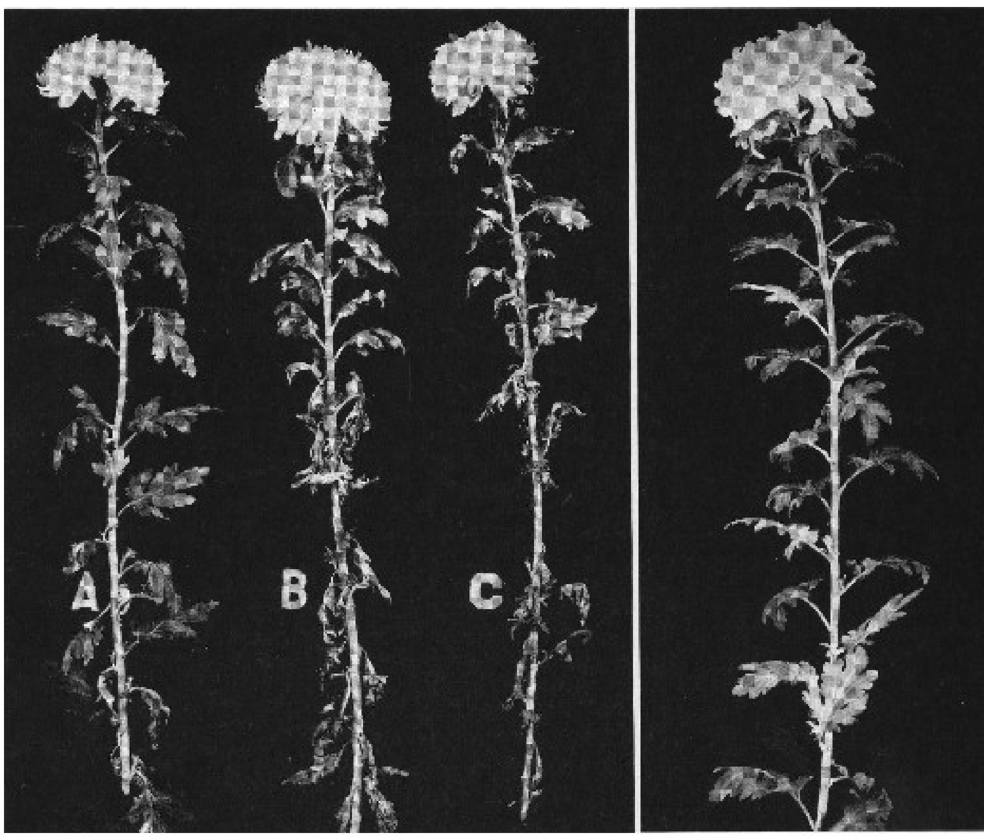
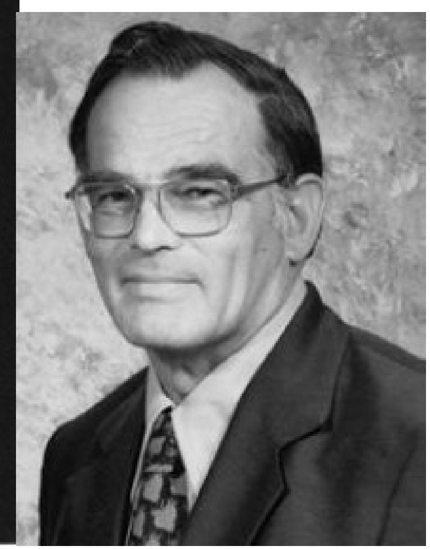




Soil Fumigation

FOUND ESSENTIAL FOR

Maximum Strawberry Yields

IN SOUTHERN CALIFORNIA

H. JOHNSON, JR. · A.H. HOLLAND · A. 0. PAULUS · S. WILHELM

Tests in two southern California counties resulted in similar data offering further proof of the value of soil fumigation for strawberry production. Yield increases as high as 85 per cent wei-e reported following soil treatment. Control of plant pathogens was excellent with full-coverage injections of a mixture of *2* parts of methyl bromide and 1 part chloropicrin applied at 225 pounds per acre.

FOLLOWING THE FIRST successful control of Verticillium wilt of strawberry by handgun application of chloropicrin in 1953, and the subsequent successful development of machine application, preplant soil fumigation became standard practice for many strawberry growers in California. One of the first areas fumigated by tractor-adapted equipment was in Los Angeles County in 1955, and other southern California growers were quick to note the advan. tages of soil fumigation . Where Verticilli t1111 wilt was a se rious proble m, 480 pounds per acre of chloropicrin applied lo moist soil gave excellent control. \\lhere Verticillium wilt was not a problPm, the application of lo wer rates of ch loropicrin, f rrq uently down to 150 pound per acre, resulted in improved plant growth and significantly increased yields.

More recently, other chemicals have been used in combination with chloropicrin such as the chlorinated C_3 hydro· carbons, particularly Telone, and methyl bromide. Methyl bromide is a very effec. tive herbicide. Extensive studies have demonstrated that mixtures of methyl bromide and chloropierin are more effective in controlling Verticillium wilt and weeds than is either component alone. The mixtureof two-thirds methyl bromide and one-third chloropicrin has been found to be particularly effective in soil preparation for annual replanting of strawberries.

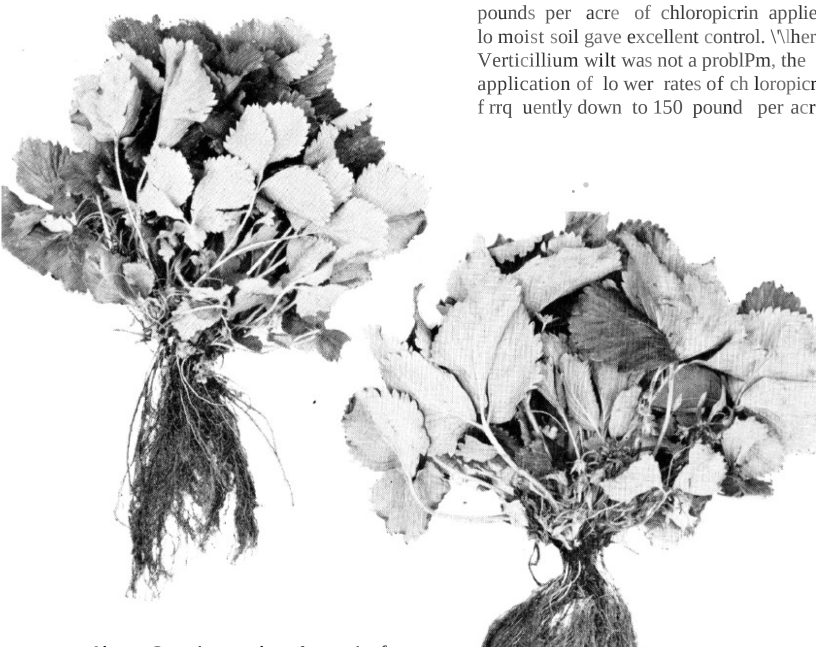
Loa Angeles trials

Dur ing 1961and 1962, trials were conducted in Los Angeles County to study the degree and nature of both yield and growth response in strawberries from soil fumigation. These trials also compared different fumigants and methods of application. The sites were fine sandy loam soils which had been fumigated and planted to strawberries the previous years.

The 1960---61 trial was on a winter planting of the Lassen variety, established in November 1960. Preplant soil injections of 225 pounds per acre of a 2:1 mixture of methyl bromide-chloropicrin were made to compare three methods 0£ application. Treatments were: (I) full coverage application with chisels spaced 12 inches apart, (2) full coverage with chisels spaced 24 inches apart, and (3) application in the bed only, with two chisels 8 inches apart. AU treatments were injected 6 to 8 inches deep. Full coverage treatments were tarped within 20 minutes following application, using 2 mil polyethylene sheeting. The bed treatments were tarped with 11,4 mil polyethylene simultaneous with fumigation by a rear-mounted tractor attachment. In bed treatments, approximately only one-half of the area is fumigated,

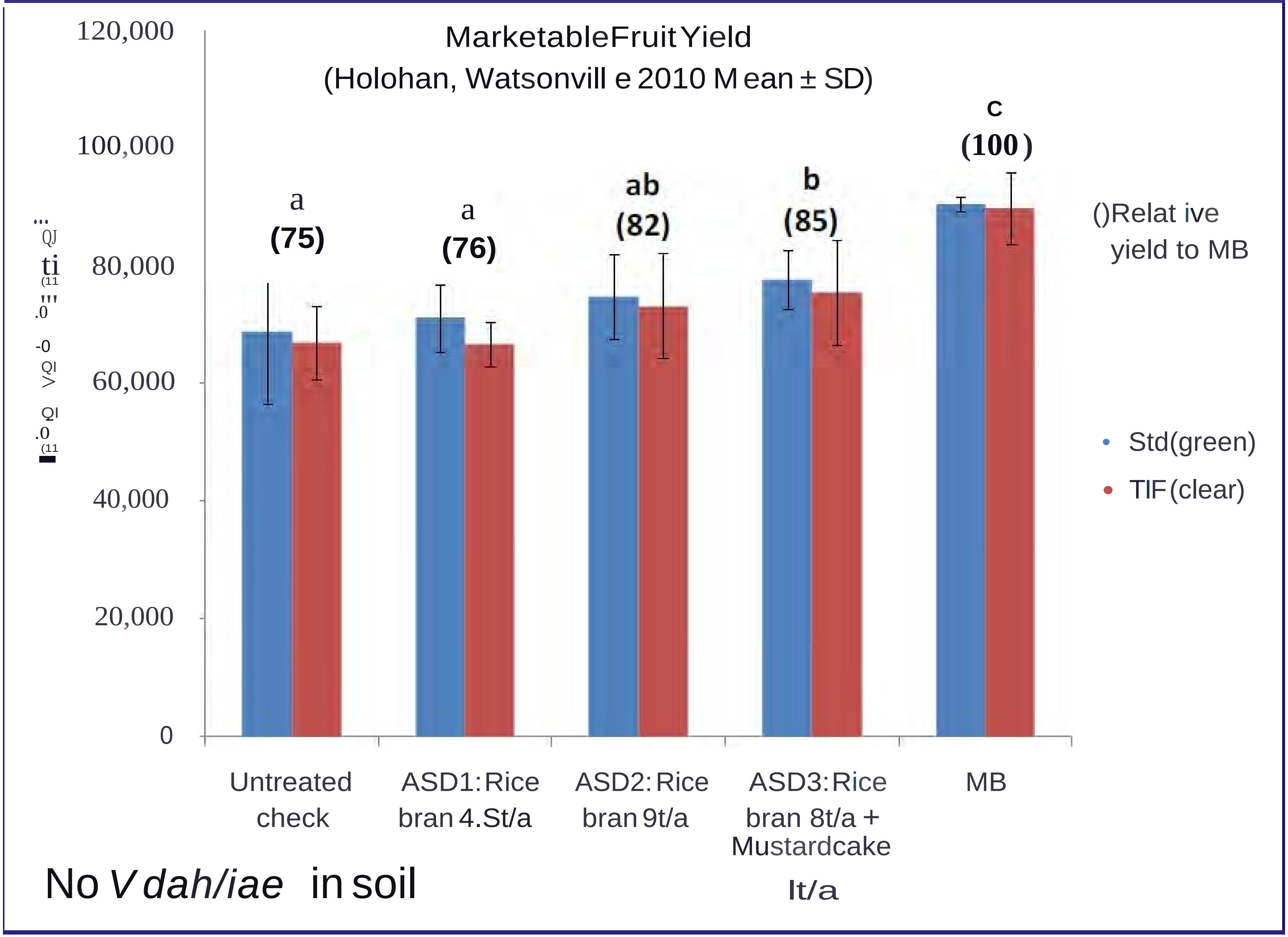
TABLE 1-EffECT Of METHOD OF APPLICATION OF 2,1 METHYL IROMIDE.CHLOROI'ICRIN MIXTURE AS A SOIL FUMIGANT ON TIIE YIELD OF IASSEN STAAW· BERRIES-WINTER PLANTING, LOS ANGELES COUNTY

	Number of 12.pint troya J*' acr• (No.1fruit}			
	To April 7	To May 5	To June ' HOIOn total	Goin* over check
Full coverog• 12• spocing..	308	799	1,456	535



Above: Strawberry olant from olot fu.

California Agriculture, 1962



From a presentation by Shennan, Muramoto, Bolda, Koike, Daugovish, Mochizuki, Klonsky, Rosskopf, Burelle, Butler, Fenimore & Samtani. Anaerobic soil disinfestation (ASD) for suppressing *Verticillium dahliae* in CA strawberries.

Goals

- To determine the efficacy of ASD/solar at a nursery in northern CA

- To identify the mechanism(s) of the “Pic Kick” (in the absence of known pathogens)
  - Nutritional?
  - Microbial?
    - Removal of “nibblers” and/or growth-inhibiting microbes?
    - Stimulation of growth-promoting microbes?

- To determine if DNA-based methods for quantification of microbial rhizoplane populations will be useful for:
  - Identifying microbes that are associated with high-yielding vs. low-yielding plants
  - Selection of potential biocontrol/plant growth promoting agents that would actually survive and protect plants

DPR grant field trials

Fumigation alternatives:

Summer 2013 (replicated):

1) ASD/solar
2) Untreated
3) Methyl bromide/chloropricrin
4) PicClor 60

↓

2014: Nursery production

↓

2015: Fruit production

Fumigated vs. organic nursery production (pseudo-replicated):

Nursery production (2013 & 2014)

↓

Fruit production (in 2014 & 2015)

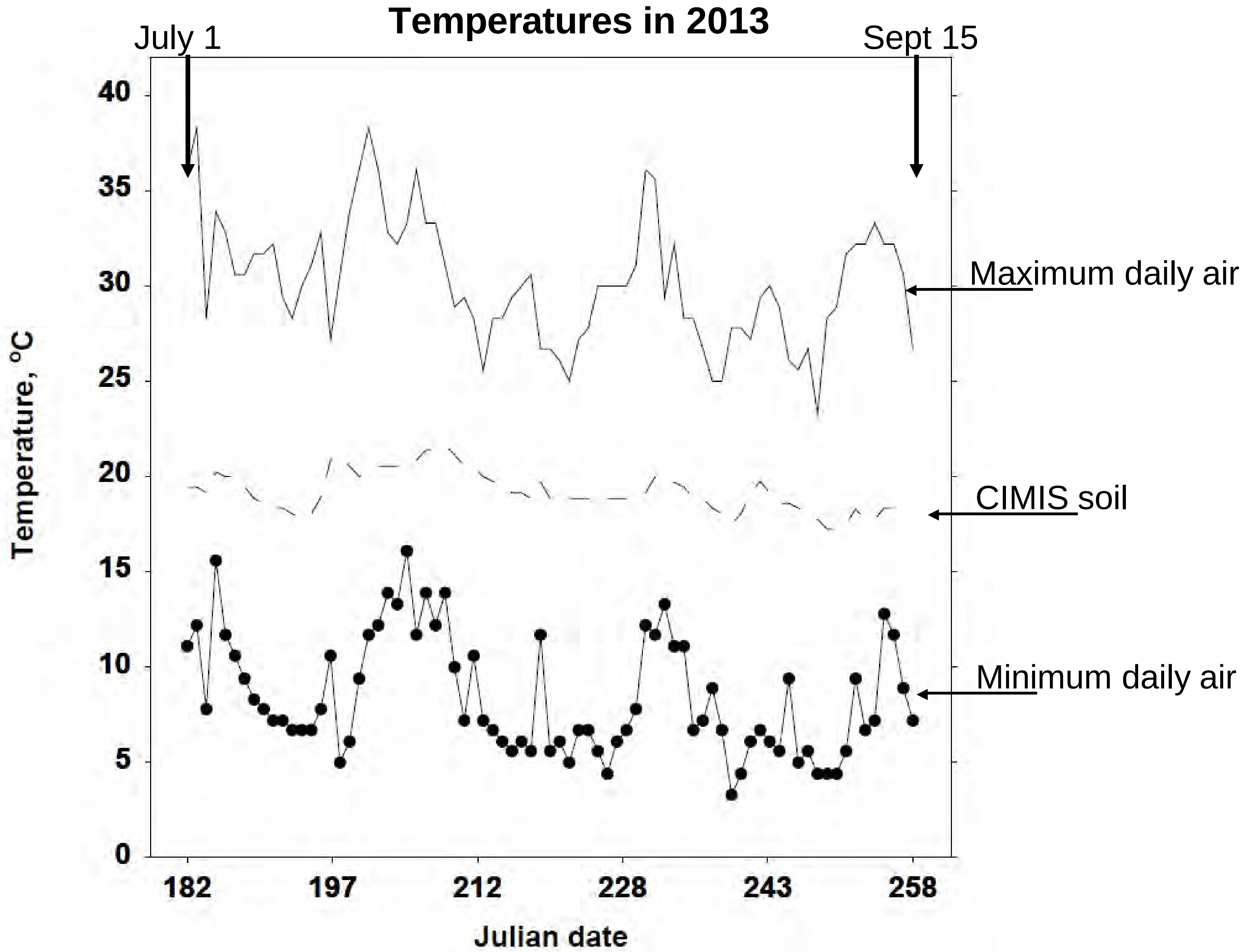
ASD/solarization Schedule

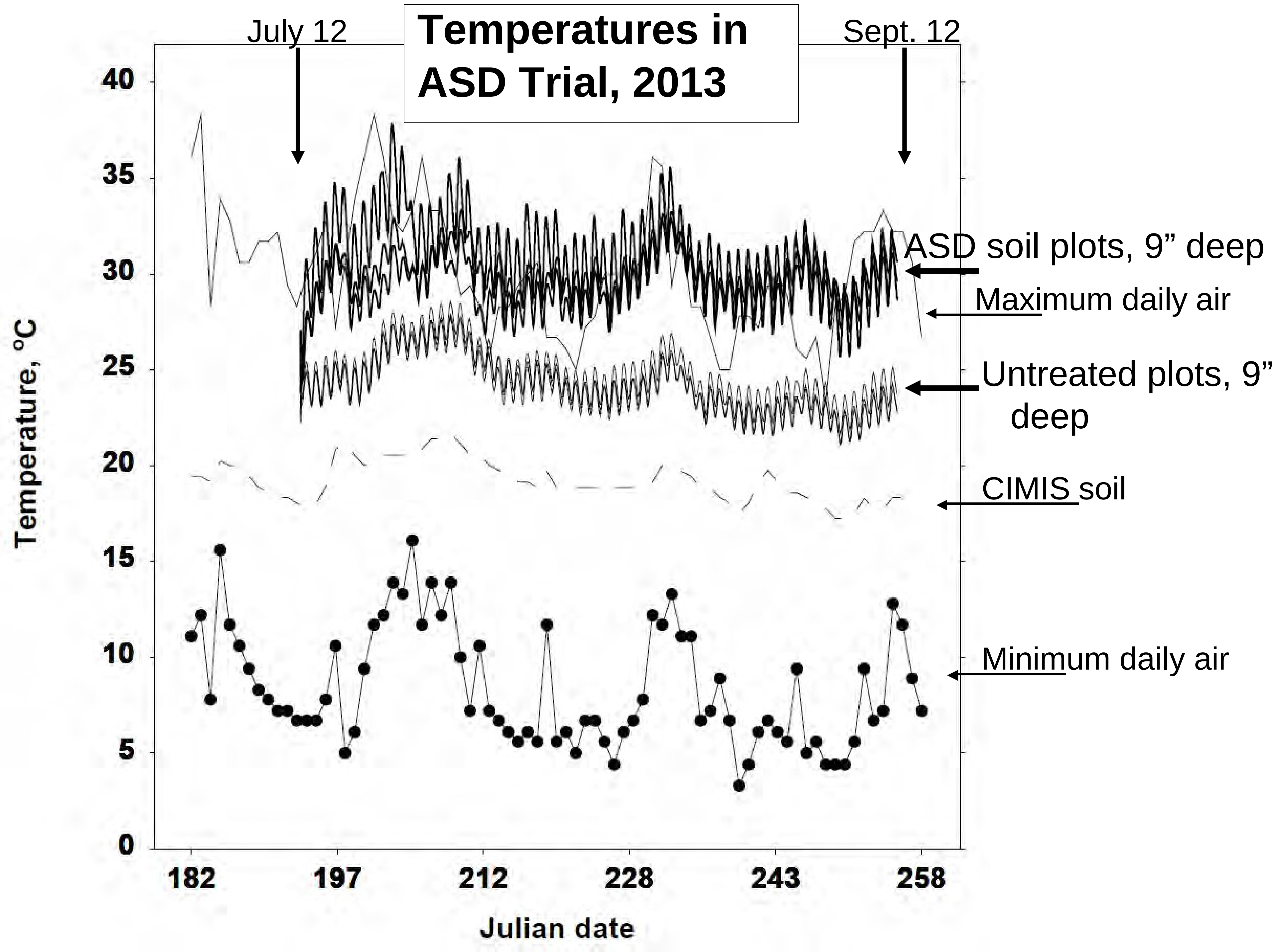
- July 11: Incorporated 9 tons of rice bran/acre
- July 12: Nematode bags and probes buried
- July 16: Tri Cal shank buried (1-2”) the drip tape, emitters every 8” lines every 18“ covered by clear standard plastic and 1 mil TIF & plot watered
- Sept 12: ASD terminated

Applying rice bran

Modified Tri Cal applicator

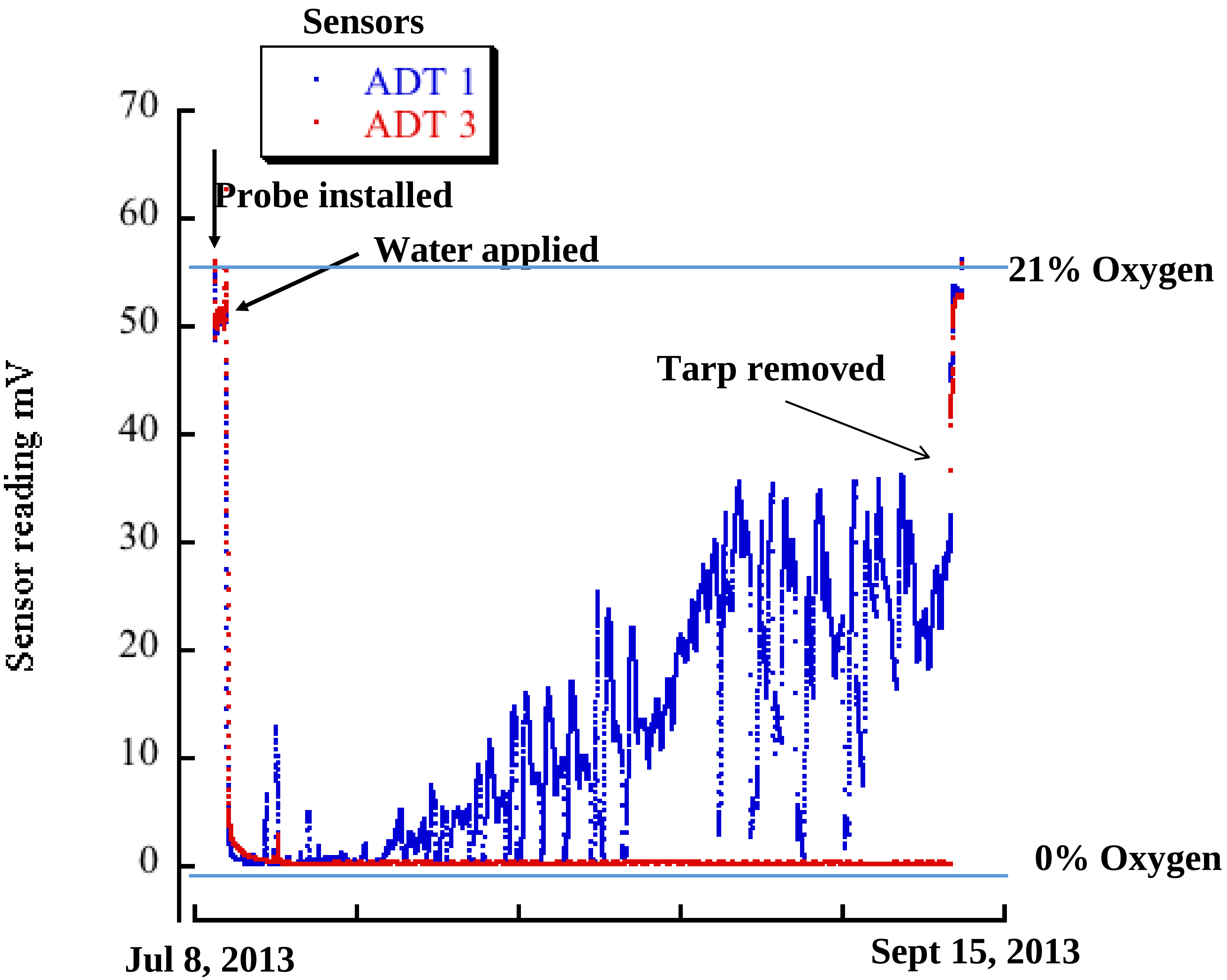
Plots 44 ft wide, 75 long


Temperatures in 2013
July 1
Sept 15
Temperature, °C
40
35
30
25
20
15
10
5
0
182
197
212
228
243
258
Julian date
Maximum daily air
CIMIS soil
Minimum daily air



Temperatures in ASD Trial, 2013
July 12
Sept. 12
ASD soil plots, 9" deep
Maximum daily air
Untreated plots, 9" deep
CIMIS soil
Minimum daily air
Temperature, °C
40
35
30
25
20
15
10
5
0
182
197
212
228
243
258
Julian date

Oxygen Sensor Data in Two ASD Replicates



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.

Strawberry nursery harvest, 2014

Untreated MBr/Chlor PicClor 60 ASD

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

Treatment	No. marketable plants/m²	SEM	Wt per marketable plant, g	SEM	Crown diam, mm	SEM	No. roots/ daughter	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.

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.

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.

The (non-)effect of soil treatment in 2013 on soil microbial content pre-harvest in Oct. 2014.

	Soil microbial content mg/kg soil	
	Soil Depth, cm	
Treatment	0-15	15-30
Untreated Control	98	35
MBr/Chloropicrin	74	38
PicClor 60	66	34
ASD	74	29
(*P*-value)	(0.19)	(0.70)

The effect of soil treatments in 2013 on soil nitrate and ammonium pre-harvest in Oct. 2014.

	Soil Depth, cm			
	0-15	15-30	0-15	15-30
Treatment	Microgram nitrate/g soil		Microgram ammonium/g soil	
Untreated Control	0.1	3.3	2.38	0.09 b
MBr/Chloropicrin	0.2	9.8	0.03	0.15 b
PicClor 60	1.6	12.1	0.09	1.00 a
ASD	1.0	8.4	0.15	0.06 b
(*P*-value)	(0.28)	(0.06)	(0.44)	**(0.0012)**

[x]Within a column, variables with a significant (P<0.05) F test are shown with a bolded P-value. For those variables with a significant F test, mean comparisons with a Tukey's HSD multiple comparison procedure are shown; means followed by the same letter were not significantly different at α=0.05.

[y]This trial was in a randomized complete block design with four replicated blocks. There were no significant block effects for any of the variables

Mineral content of whole plants and strawberry runners from the nursery in 2014

	Transplants (Harvested 19 Oct. 2014, trimmed and marketable)[a]										
	P, %	K, %	S, ppm	B, ppm	Ca, %	Mg, %	Zn, ppm	Mn, ppm	Fe ppm[b]	Cu ppm	Mo, ppm
ASD	0.16	0.94	1340	29	0.66	0.36ab	28	344a	24060	23	1.5
MeBr/Chlor	0.17	0.95	1385	30	0.75	0.38a	33	224ab	29300	20	1.3
PicClor60	0.17	0.96	1182	29	0.70	0.33b	28	184b	20210	18	1.1
Untreated	0.17	0.96	1202	29	0.71	0.34b	26	180b	22930	21	1.9
Tmt P value	0.86	0.98	**0.043**	0.41	0.14	0.01	0.096	**0.002**	0.53	0.64	0.76

	Whole plants (16 Oct. 2014)[a]										
	P, %	K, %	S, ppm	B, ppm	Ca, %	Mg, %	Zn, ppm	Mn, ppm	Fe ppm	Cu ppm	Mo, ppm
ASD	0.22	1.4	830	54 b	2.0	0.53	23	776a	366	9.8	0.31
MeBr/Chlor	0.22	1.3	875	66 ab	2.2	0.52	21	479ab	319	5.2	0.18
PicClor60	0.24	1.4	930	63 ab	2.1	0.48	20	309b	309	6.2	0.23
Untreated	0.22	1.3	863	71 a	2.3	0.47	17	224b	312	5.8	0.31
Tmt P value	0.56	0.10	0.32	**0.016**	0.31	0.11	0.29	**0.0097**	0.42	0.42	0.68

Summary of frequency of confirmed pathogens from a total 1600 marketable and 1600 unmarketable strawberry runners harvested in Oct. 2014 from a high elevation nursery.

	No. plants						
Plant category	Total # with pathological symptoms	*Coniella fragariae* (CF) only	*Macro-phomina phaseolina* (MP) only	*Botrytis cinerea* & *CF*	Both *Cf* & *Mp*	*Rhizoc-tonia spp.*	No pathogen isolated
Market-able Plants	22	17	2	0	1	1	1
Unmar-ketable plants	10	8	0	1	1	0	1

In both years, pathogen levels in the nursery were too low for study!! Fantastic news for the nursery, but not such great news for the study!

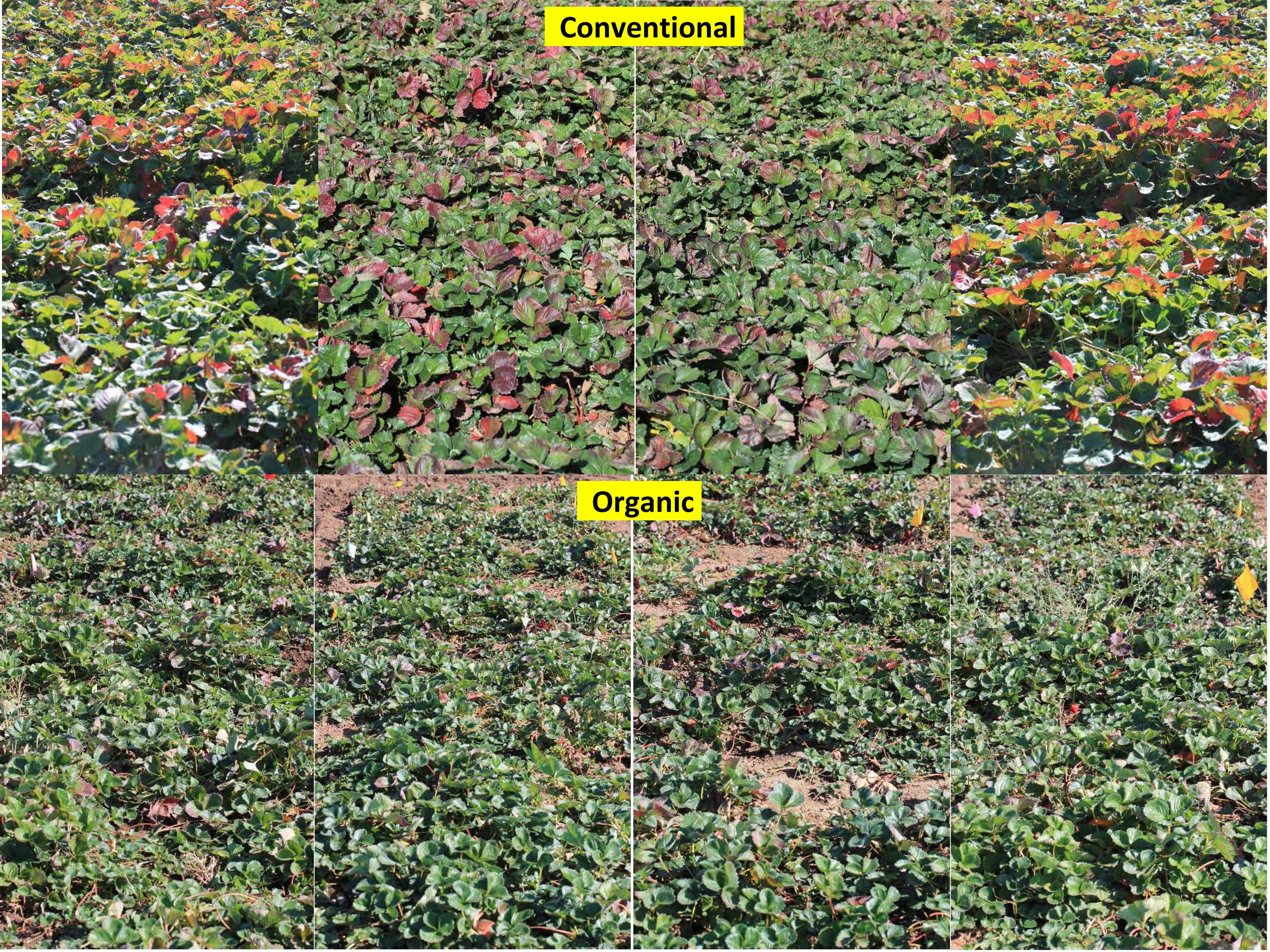
Some comments & conclusions:

Based on buried citrus nematode bags, anaerobic soil disingestion (ASD) was as effective in controlling nematodes as methyl bromide/chloropicrin and PicClor 60.

Based on standard IPM guidelines ("Pesticides are used only after monitoring indicates they are needed*…"), no pre-plant pest or pathogen treatment was necessary in any of our trials in the nursery.

Are the CDFA (nematode-based) requirements for strawberry nurseries encouraging unnecessary fumigant use?

*http://www.ipm.ucdavis.edu/GENERAL/ipmdefinition.html



Conventional
Organic

---Fumigated---- ---------Organic---------

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/m^2 ± 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** **

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).

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.

2013, nursery, mid-season %N

	% N		
	Mother roots	Mother, above ground	Runners and daughters (above-ground)
Pre-plant fumigation	1.03	1.66	1.95
Organic	0.99	2.22	2.59

Mineral content of strawberry transplants produced in an organic vs. a conventional production system in 2013 in McArthur, CA

Nursery	C	N	P	Fe	Mg	K	S	B	Mn	Co	Ni	Cu	Mo	Zn
Fumi-gated	43	1.1	0.17	0.24	0.20	0.50	0.07	31	103	2.1	15	20	0.74	27
Organic	44	1.1	0.18	0.23	0.24	0.58	0.08	33	146	2.7	13	22	0.82	32

There were no significant treatment effects by ANOVA, α=0.05.

Soil nitrate and ammonium in a strawberry nursery produced at high elevation in an organic versus and an adjacent “conventional” field at harvest

	Year					
	2013		2014			
	Soil Depth, cm					
	0-15	15-30	0-15	15-30	0-15	15-30
Treatment	µg nitrate N/g soil				µg ammonium/g soil	
Pre-plant fumigation	0.02	0.25	0.08	0.03	-0.02	0.26
Organic	0.47	0.75	0.65	0.20	0.32**	0.31
(*P*-value)	**<0.05**		**(0.051)**	(0.31)	**(0.005)**	(0.78)

Mineral content of transplants and whole plants produced at high elevation

	Runners (Harvested 19 Oct. 2014, trimmed and marketable)										
	P, %	K, %	S, ppm	B, ppm	Ca, %	Mg, %	Zn, ppm	Mn, ppm	Fe ppm[a]	Cu ppm	Mo, ppm
Conv	0.16	1.09	1068	30	0.84	0.34	28	354	2877	18	0.71
Org	0.17	1.12	1232	30	0.78	0.33	25	317	3447	18	1.17
***P*-value**	0.32	0.77	0.26	0.51	0.41	0.15	0.35	0.48	0.13	0.87	**0.043**

	Whole plants (16 Oct. 2014)										
	P, %	K, %	S, ppm	B, ppm	Ca, %	Mg, %	Zn, ppm	Mn, ppm	Fe ppm	Cu ppm	Mo, ppm
Conv	0.20	1.6	675	67	2.3	0.46	16	607	598	4.8	0.20
Org	0.26	1.7	822	59	2.0	0.50	18	322	755	4.9	0.52
***P*-value**	**0.0023**	0.21	**0.020**	**0.040**	0.10	0.34	0.58	**0.003**	0.16	0.80	0.07

Note the Bonferroni correction for multiple comparisons would “reset” an α=0.05 to α=0.0023

Soil microbial content (MBC) pre-harvest in a strawberry nursery produced at high elevation in an organic versus an adjacent “conventional” field. Different plots were sampled in 2013 and 2014

	Year			
	2013		2014	
	Soil Depth, cm			
	0-15	15-30	0-15	15-30
Treatment	**Microbial biomass, mg/kg soil**			
Pre-plant fumigation	132	84	47.2	33.8
Organic	162	118	70.2**	40.8
(*P*-value)	NS	NS	*(0.006)*	*(0.06)*

Runner harvest from fumigated vs. organic rotation, 2013

Treatment	Marketable runners only				
	Wet weight, g/m^2	No. plants per m^2	Wet wt, g per plant	Crown diam, cm	No. Roots per plant
Fumigated	**627 a**	**68 a**	**9.2 b**	**1.06 b**	**12.5 b**
Organic-normal rotation	**568 ab**	**40 b**	**14.0 a**	**1.24 a**	**20.4 a**
Organic-year 2 rotation	280 b	21 b	13.9 a	1.23 ab	18.5 a
Organic-year 1 rotation	335 ab	28 b	12.0 ab	1.17 ab	19.6 a

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

A conclusion:

In the organic plots in the nursery, there tended to be fewer but larger strawberry runners compared to their fumigated controls. However, fruit yields from the organically-produced nursery transplants were indistinguishable from their fumigated controls in both years.

Evidence that the rhizoplane microflora differs in the organic vs. conventional systems

Data sets of :

1) G3 phylochip of bacterial rDNA, organic vs. fumigated, 2013

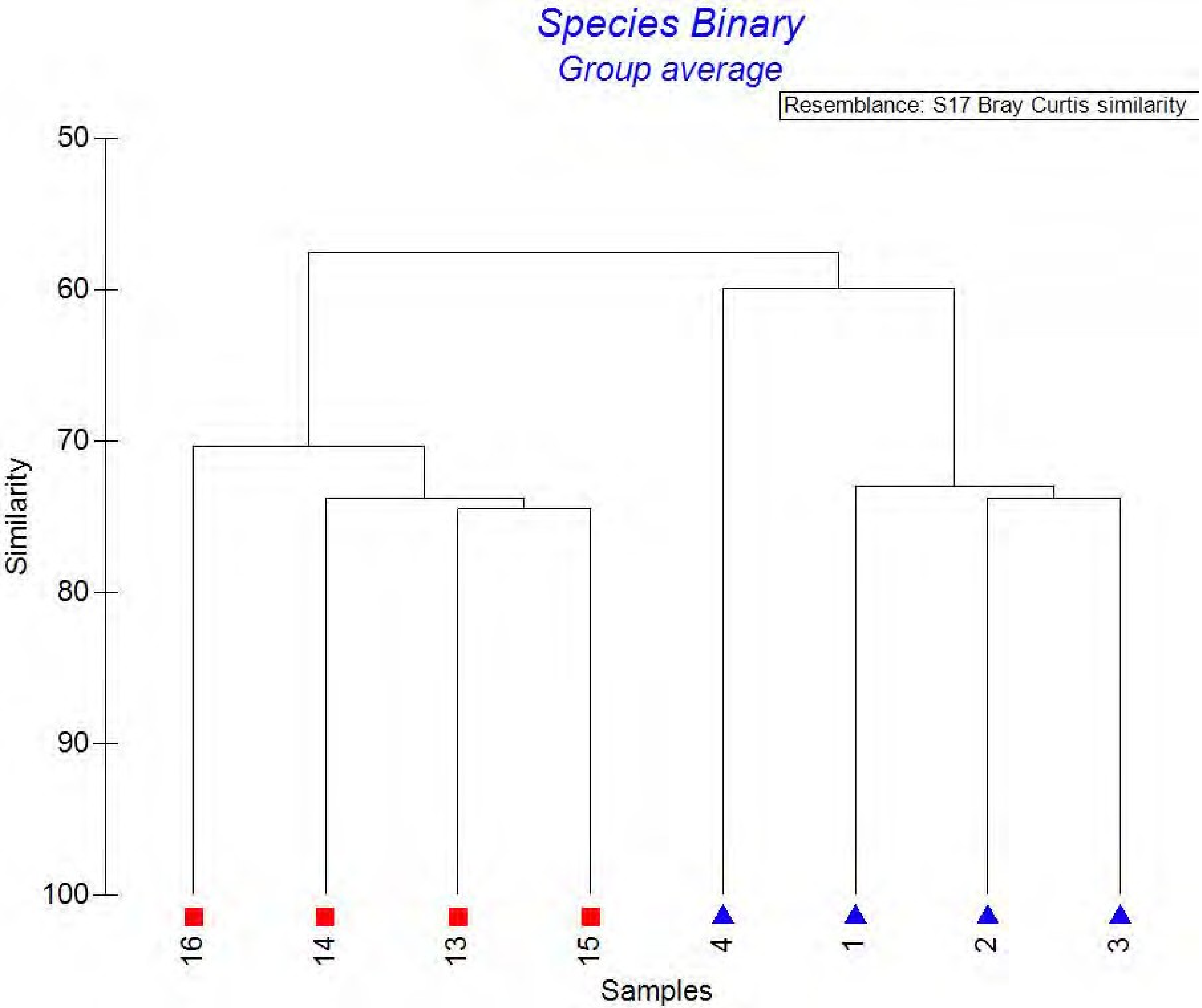
2) MiSeq DNA sequence

2013 - organic vs. fumigated bacterial rDNA MiSeq DNA sequence

2014 – all nursery plots and fruit production plots from 2013 nursery

Bacterial rDNA and fungal ITS MiSeq DNA sequence

Issues: 2,016 bacterial “species” and 1,341 fungal “species” detected, most at <u>very</u> low incidence.


Species Binary
Group average
Resemblance: S17 Bray Curtis similarity
50
60
70
80
90
100
Similarity
16
14
13
15
4
1
2
3
Samples

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

Treatment	*Arthrobacter psychrochitiniphilu*		*Nocardioides islandensis*		*Streptomyces roseogilvus*	*Streptomyces kagawaensis*		*Sphingomonas oligophenolica*	*Kaistobactert errae*	
	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	

	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	

Conclusions:

Rhizoplane populations differ in different locations (high elevation nursery vs. production field)

Rhizoplane populations differ between fumigated and organic fields

Rhizoplane populations are extremely diverse and complex.

Based on the bioinformatics (only), *Arthrobacter psychrochitiniphilus* strains may be the best candidate as bioinoculant for roots of strawberry transplants.

What are we doing now?

-Finishing rhizoplane analysis

-Trying to establish an assay system that will allow us to identify the mechanism of soil fungistasis

-Testing the *Allium* extract VEG`LYS as a potential product to kill *Fusarium oxysporum* in soil (looked promising in first trial but not in second)