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Authors

Muramoto, Joji
Gliessman, Stephen R
Koike, Steven T
[et al.](#)

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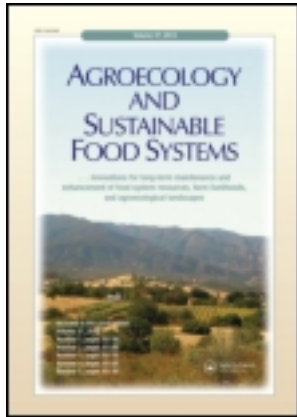
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Joji Muramoto^{ab}, Stephen R. Gliessman^{ab}, Steven T. Koike^c, Carol Shennan^{bd}, Carolee T. Bull^e, Karen Klonsky^f & Sean Swezey^d

^a Program in Community and Agroecology (PICA), University of California, Santa Cruz, California, USA

^b Department of Environmental Studies, University of California, Santa Cruz, California, USA

^c University of California Cooperative Extension, Monterey County, Salinas, California, USA

^d Center for Agroecology and Sustainable Food Systems (CASFS), University of California, Santa Cruz, California, USA

^e United States Department of Agriculture-Agricultural Research Services (USDA-ARS), Salinas, California, USA

^f Department of Agricultural and Resource Economics, University of California, Davis, California, USA

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Integrated Biological and Cultural Practices Can Reduce Crop Rotation Period of Organic Strawberries

JOJI MURAMOTO,^{1,2} STEPHEN R. GLIESSMAN,^{1,2}
STEVEN T. KOIKE,³ CAROL SHENNAN,^{2,4} CAROLEE T. BULL,⁵
KAREN KLONSKY,⁶ and SEAN SWEZEY⁴

¹*Program in Community and Agroecology (PICA), University of California, Santa Cruz, California, USA*

²*Department of Environmental Studies, University of California, Santa Cruz, California, USA*

³*University of California Cooperative Extension, Monterey County, Salinas, California, USA*

⁴*Center for Agroecology and Sustainable Food Systems (CASFS), University of California, Santa Cruz, California, USA*

⁵*United States Department of Agriculture-Agricultural Research Services (USDA-ARS), Salinas, California, USA*

⁶*Department of Agricultural and Resource Economics, University of California, Davis, California, USA*

A team of researchers conducted a replicated on-farm experiment with the break period between strawberry crops (continuous strawberries with broccoli residue incorporation, one-year break, two-year break, three-year break, and seven-year break) as the main plot and cultivar as the split plot in Moss Landing, Central Coastal California. We hypothesized that the use of non-host rotation crops for Verticillium wilt plus bio-fumigation with broccoli, incorporation of mustard cover crop residues, use of relatively resistant strawberry cultivars, and compost application would suppress disease sufficiently to grow strawberries successfully in rotation every two or three years. Although a positive correlation between break period and marketable fruit yield existed, integrated use of biological and cultural practices allowed one to three-year breaks

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Address correspondence to Joji Muramoto, Department of Environmental Studies, University of California, 1156 High Street, Santa Cruz, CA 95064, USA. E-mail: joji@ucsc.edu

to have a statistically similar yield as seven-year break for this low Verticillium dahliae pressure field over a five-year period.

KEYWORDS *crop rotation, organic farming, strawberries (Fragaria × ananassa Duch.), Verticillium dahliae, broccoli (Brassica oleracea L. (italica group))*

INTRODUCTION

With its Mediterranean climate of moist, mild winters and dry moderate summers, a broad range of fruit and vegetable crops can be grown year round on the central coast of California. Monterey and Santa Cruz counties combined produced \$912 million gross value of strawberries (*Fragaria × ananassa* Duch.) and over \$2.7 billion worth of vegetables in 2011 (Monterey County Agricultural Commissioner 2012; Santa Cruz County Agricultural Commissioner 2012). As the interest in organic farming and the demand for organic produce has increased during the last decade, organic farming on the central coast has also greatly increased. There were over 9,300 certified organic hectares in Monterey and Santa Cruz Counties in 2011, five times the number recorded in 1998 (Monterey County Agricultural Commissioner 1999, 2012; Santa Cruz County Agricultural Commissioner 1999, 2012). The total farm gate revenue from organic farming in these counties was over \$197 million in 2011, representing a dramatic 12-fold increase in 13 years (Klonsky and Richter 2005; Monterey County Agricultural Commissioner 2012; Santa Cruz County Agricultural Commissioner 2012). This trend is also true for organic strawberry production. In 2000, 77 ha of organic strawberries were grown in central coastal California, but by 2012 this had increased to 509 ha, representing 8.3% of the total strawberry production in the area (California Strawberry Commission 2004, 2012).

Continued growth of organic strawberry production in this area, however, faces the challenge of managing soil-borne diseases without the use of synthetic fumigants and fungicides. Verticillium wilt is a soil-borne disease caused by *Verticillium dahliae* that can damage a wide range of important crops in California. Host crops include lettuce, tomatoes, potatoes, apples, cotton, artichokes, and strawberries (Bhat and Subbarao 1999). Due to its resilient overwintering structure (microsclerotium), this pathogen can survive many years in soil even without host plants (Koike et al. 1994). In the pre-methyl bromide era, Verticillium wilt was a major limiting factor to strawberry production in California (Wilhelm and Paulus 1980). Today, Verticillium wilt is one of several key soil-borne diseases facing California strawberry production (Koike 2008; Koike et al. 2009) and poses a long-term threat for organic strawberry production in the state.

To avoid *Verticillium* wilt and other soil-borne diseases, as well as meet the requirements of the USDA National Organic Program (2000), organic strawberry growers must implement crop rotation. Due to its high sensitivity to the disease, several years between strawberry plantings are necessary (Gordon et al. 1994). For specialized strawberry growers in California, establishing a crop rotation system implies a major change in the design and management of the farming system. Due to the high costs of production (Bolda et al. 2006; Bolda et al. 2010) and the high leasing fees of crop lands (\$5,000 to \$7,500 ha⁻¹ year⁻¹), specialized organic strawberry growers need to minimize the break time between strawberry crops as much as possible to stay in business.

The following biological and cultural approaches to soil-borne disease management in strawberries have been tested: host resistance (Shaw et al. 1997; Duniway et al. 2001; Bull et al. 2005); small cell transplants (“plug plants”) (Sances 2000; Walter et al. 2005); organic amendments such as compost (Sances 2000; Arancon et al. 2004; Millner et al. 2004); high nitrogen organic fertilizers (Duniway et al. 2001; Lazarovits 2004); broccoli (*Brassica oleracea* L. (italica group)) residues (Sances and Ingham 1997); mustard residues (*Brassica juncea*, *Sinapis alba*, etc.), Sudan grass (*Sorghum* × *drummondii*), and other cover crops (Gordon et al. 1994; LaMondia et al. 2002; Seigies and Pritts 2006); microbial amendments including vesicular arbuscular mycorrhizal fungi (VAM) (Werner et al. 1990; Bull et al. 2005); plant growth promoting rhizobacteria (PGPR) (Bull and Ajwa 1999; Eayre 2000); crop rotations with broccoli, lettuce (*Lactuca sativa* L.) or Brussels sprouts (*Brassica oleracea* L. (gemmifera group)) (Subbarao et al. 2007); mustard seed meal (Mazzola 2011); soil-less trough production (Thomas and Legard 2013); and anaerobic soil disinfestation (Shennan et al. 2012). Further, a minimum of a three-year rotation is recommended for strawberries that do not use chemical fumigants in Europe (Bevan et al. 2001, Steffek et al. 2004), the Northeast and Midwest United States, and in eastern Canada (Pritts and Handley 1998). However, no research has yet integrated multiple biological and cultural practices for different rotation periods of organic strawberries in California.

The objective of this project was to demonstrate the effects of strawberry planting frequency in organic strawberry/vegetable rotations and combined biological and cultural practices on fruit yield and disease level. We hypothesized that the use of non-host rotation crops for *Verticillium* wilt plus bio-fumigation with broccoli, mustard cover crop residues, relatively resistant strawberry cultivars, and compost application would suppress disease sufficiently to grow strawberries in rotation every two or three years. To test the above hypothesis, in 2001, we initiated a five-year organic strawberry/vegetable rotation experiment in a commercial California field.

MATERIALS AND METHODS

Field Location and the Project History

The on-farm research site for this project was the Elkhorn Ranch in Moss Landing, Monterey County, CA, USA (36°49'50.94"N, 121°45'18.73"W). The ranch is adjacent to the Elkhorn Slough National Estuarine Research Reserve, one of the state's largest and the last remaining coastal wetlands and a habitat for hundreds of plant and animal species, including more than 135 aquatic bird species (Elkhorn Slough National Estuarine Research Reserve 2013). The field had a long history of conventional cultivation (strawberries, lettuce, Brussels sprouts, etc.) prior to the transition to organic production. The last conventional strawberry was grown in the 1997–1998 season and the last methyl bromide/chloropicrin fumigation was applied in the fall of 1997. Divided into three 8-ha parcels, in 1998 the land was placed into the mandatory three-year organic transition period, alternating winter cover crops with summer fallow. During the summer of 2001, romaine lettuce was grown on these fields by an organic vegetable grower and was harvested in early October 2001. Upon completion of the transition period and after discussions on experimental details with the grower, a replicated five-year rotation trial with 5 treatments was established in a 0.4 ha section of the ranch.

The soil type of the fields is Santa Ynez fine sandy loam (fine, montmorillonitic thermic, Ultic Palexerolls) with 2–9% slopes. Water infiltration is very slow due to a claypan at 45–150 cm deep. Organic matter content in the topsoil (0–15 cm) is as low as 10 g kg⁻¹ and the pH is 6.7–7.0 (Table 1). The baseline *V. dahliae* population in the topsoil was below the detection limit. With a typical Mediterranean climate, the majority of rainfall is concentrated in winter (from October to March). The average annual precipitation at the Elkhorn Slough National Estuarine Research Reserve was 498 mm and mean daily temperature ranged from 3.6 to 21.7°C (minimum to maximum) during the experiment.

Experimental Design

Our strategy for shortening the rotation period of organic strawberries was to apply biological and cultural practices in an integrated manner. The adopted biological practices were: using broccoli residues for bio-fumigation (Koike and Subbarao 2000; Subbarao et al. 2007); incorporating cover crops including mustard (Gordon et al. 1994; Seigies and Pritts 2006); and applying compost (Sances 2000; Duniway et al. 2001; Millner et al. 2004). These measures were combined with selecting strawberry cultivars less sensitive to pathogens (Bull et al. 2005; Shaw et al. 1996); and rotating with vegetables that do not host *V. dahliae* (Bhat and Subbarao 1999). We took a systems approach to examine the effects of interactions of all practices rather than to examine the effects of individual practices (Drinkwater 2002).

TABLE 1 Baseline soil characteristics of the rotation trial, Moss Landing, CA; soils were sampled in November 2001

Depth cm	pH	EC (1:1) dS m ⁻¹	O.M. ^a g kg ⁻¹	Total C g kg ⁻¹	Total N g kg ⁻¹	C/N	NH ₄ -N mg kg ⁻¹	NO ₃ -N mg kg ⁻¹	Olsen-P mg kg ⁻¹	Exchangeable cmol kg ⁻¹		
										Ca	Mg	Na
0-15	6.8	1.55	11.0	7.60	1.00	7.6	1.9	36.6	70.6	7.3	2.1	0.4
15-30	6.9	1.00	10.5	6.30	0.80	7.9	0.8	22.2	47.0	4.0	1.9	0.4
DTPA-soluble mg kg ⁻¹												
Depth cm	B (SP) mg kg ⁻¹			Cu	Zn	Mn	Fe	Sand %	Silt %	Clay %		
	Fe	Mn	Cu									
0-15	57.0	26.8	1.8	1.4	0.3	0.3	67.5	23.3	9.3			
15-30	56.4	21.4	1.5	1.3	0.2	0.2	67.5	22.5	10.0			

^aO.M.: organic matter.

The experimental design was a split plot arrangement of treatments in a randomized complete block with different break periods between strawberry plantings as the main plot (five treatments), and strawberry cultivar (two treatments) as subplots with four replications. The main treatments were: A) Continuous strawberries with pre-plant bio-fumigation using broccoli residues, B) one-year break before replanting strawberries, C) two-year break before replanting strawberries, D) three-year break before replanting strawberries, and E) no strawberries until the fifth year, which was a seven-year break because the last strawberries were grown in 1997–1998 in this field (Table 2). The subtreatments for the first year were cultivars “Aromas” and “Diamante.” From the second year onwards, Diamante was replaced with cv. “Seascope” because Seascope had performed better on other organic farms in the region (Bull et al. 2005). In addition to broccoli that was planted as a crop prior to strawberries for bio-fumigation, spinach (*Spinacia oleracea*) was chosen as a rotational crop since there were no reports at that time that it hosted *V. dahliae*. Each plot area was 91.3 m² (4.91 m wide × 18.6 m long) and consisted of 4 beds of strawberries (two plant rows per bed) or vegetables. We grew strawberries in all plots during the final (fifth) year (the 2005–2006 season).

Cultural Practices

Unless otherwise specified, common cultural practices for organic strawberry production on the central coast of California (Bolda et al. 2006) were used in the experiment. Dates of major cultural practices are provided in Table 3. All crops were managed by the collaborating grower. Cultural practices including application rates of compost, organic fertilizers, and broccoli residues were adjusted for each year based on discussion between the grower and the project coordinator reflecting N dynamics data from the previous year (Muramoto et al. 2007). Grape pomace/chicken manure-based compost was applied to all treatments in early October of each year. The application rates were 21.4, 17.8, 34.7, 5.6, and 10.1 Mg ha⁻¹ for years 1, 2, 3, 4, and 5,

TABLE 2 Rotation treatments based on a combination of broccoli residue incorporation, diverse winter cover crops including mustard, compost application, use of relatively resistant strawberry cultivars, and rotations with vegetables that do not host *Verticillium dahliae* at Moss Landing, CA, from November 2001 to October 2006

Treatment	Year 1 2001	Year 2 2002	Year 3 2003	Year 4 2004	Year 5 2005	Year 6 2006
A (0 yr* + br. res.)	st	st	st	st	st	st
B (1 year*)	st	cv-sp-br	st	cv-br	st	st
C (2 years*)	cv-sp-br	st	cv-sp-br	cv-br	st	st
D (3 years*)	st	cv-sp-br	cv-sp-br	cv-br	st	st
E (7 years*)	cv-sp-br	cv-sp-br	cv-sp-br	cv-br	st	st

*Period between strawberry plantings. br: broccoli. br. res.: applying broccoli residues before planting strawberries. cv: cover crop. sp: spinach. **st**: strawberries.

TABLE 3 Major cultural practices in the rotation experiment in Moss Landing, CA, from October 2001 to September 2006

DATE	D.A.P.*	Strawberries	Cover crops and vegetables
Year 1			
10/02/01	-48	Compost application/incorporation	
10/13/01	-37	Broccoli residue application/incorporation (all treatments)	
10/22/01	-28	Listing beds and applying organic fertilizers	
11/19/01	0	Strawberry planting	Cover crop planting
1/08/02	50	Plastic mulch application	
3/01/02	102		Cover crop incorporation
5/02/02	164	Strawberry fruit harvest began	
6/19/02	212		Spinach planting
7/25/02	248		Spinach harvest
8/02/02	256		Broccoli transplanting
10/01/02	316	Strawberry fruit harvest ended	
10/05/02	320	Strawberry incorporation	Broccoli harvest/roto-tilling
Year 2			
10/05/02	-52	Broccoli residue application/incorporation (treatment A only)	
10/06/02	-51	Compost application/incorporation	
11/25/02	-1	Listing beds	
11/25/02	-1	Applying organic fertilizer and plastic mulch	
11/26/02	0	Strawberry planting	
12/07/02	11		Cover crop planting
4/08/03	133		Cover crop incorporation
5/01/03	156	Strawberry fruit harvest began	
5/21/03	176		Spinach planting
7/01/03	217		Spinach harvest
8/02/03	249		Broccoli transplanting
9/27/03	305	Strawberry fruit harvest ended	
10/01/03	309	Strawberry incorporation	Weed/broccoli roto-tilling
Year 3			
10/08/03	-37	Compost application/incorporation	
10/09/03	-36	Broccoli residue application/incorporation (treatment A and B)	

(Continued)

TABLE 3 (Continued)

DATE	D.A.P.*	Strawberries	Cover crops and vegetables
10/10/03	-35	Listing beds and applying organic fertilizers	
11/05/03	-9		Cover crop planting
11/13/03	-1	Plastic mulch application	
11/14/03	0	Strawberry planting	
4/05/04	143	Strawberry fruit harvest began	Cover crop incorporation
4/10/04	148		
4/29/04	167		Spinach planting
6/17/04	216		Weed/spinach roto-tilling
7/07/04	236		Broccoli transplanting
9/30/04	321	Strawberry fruit harvest ended	
10/05/04	326	Strawberry incorporation	Broccoli harvest/roto-tilling
Year 4			
10/05/04	-49	Broccoli residue application/incorporation (treatment A only)	
10/16/04	-38	Compost application/incorporation	
10/18/04	-36	Listing beds and applying organic fertilizers	
10/18/04	-36	Plastic mulch application	
11/23/04	0	Strawberry planting	
11/26/04	3		Cover crop planting
4/13/04	141	Strawberry fruit harvest began	
4/25/05	153		Cover crop incorporation
6/27/05	216		Broccoli transplanting
9/15/05	296	Strawberry fruit harvest ended	
9/20/05	301	Strawberry incorporation	Broccoli harvest/roto-tilling
Year 5			
9/20/05	-64	Broccoli residue application/incorporation (treatment A only)	
9/21/05	-63	Compost application/incorporation	
9/24/05	-60	Listing beds and applying organic fertilizers and plastic mulch	
11/23/05	0	Strawberry planting	
5/03/06	161	Strawberry fruit harvest began	
9/13/06	294	Strawberry fruit harvest ended	

*Days after strawberry planting in each year.

respectively. Organically certified fertilizers were either broadcast (sulfate of potash) or band applied in beds (pelleted organic fertilizers) to strawberry planted plots as pre-plant applications providing 82:34:267, 43:23:40, 67:25:23, 52:6:51, and 54:8:40 of N:P:K kg ha⁻¹ for years 1, 2, 3, 4, and 5, respectively. Fresh aboveground broccoli residues from an organic field in Gilroy, CA, were applied to all the plots before the first crop in year 1, and to treatment A from years 2 to 5 before planting strawberries. The application rates for years 1, 2, 3, 4, and 5 were 27.0, 28.1, 65.7, 27.9, and 43.2 Mg ha⁻¹, respectively. Application rates for years 2, 4, and 5 were determined so as to match the amount of broccoli biomass produced within the trial each year. Table 4 shows the amount of N inputs of organic materials and residues applied for all treatments.

Strawberry transplants were planted with two rows per bed (122 cm center-to-center width) in designated plots in mid- to late-November each year. Plant populations ranged from 47,340 to 52,340 ha⁻¹. A black plastic mulch was applied in January 2002 in year 1, whereas it was applied pre-plant from the second to the fifth year to slow down the rate of nitrate loss through leaching during the rainy season (Muramoto et al. 2004). A supplemental liquid organic fertilizer was applied to all plots via the drip tape system throughout the growing season of strawberries and vegetables. Application rates of liquid organic fertilizers for strawberries ranged from 6 to 75 kg-N ha⁻¹ during the study (Table 4).

Cover crops and vegetables were planted in designated plots each year according to the rotation design (Table 2). Prior to cover crop planting, organically certified fertilizers were applied in the same manner to strawberry-planted plots in years 1, 3, and 4 but no fertilizers were applied in year 2. A mixed cover crop of oats (*Avena sativa*, 10 kg ha⁻¹), bell beans (*Vicia faba*, 37 kg ha⁻¹), winter peas (*Pisum* sp., 26 kg ha⁻¹), common vetch (*Vicia purpurea*, 31 kg ha⁻¹), and mustard (*Brassica campestris* in year 1 and *Brassica juncea* in year 2, 5.6 kg ha⁻¹) was planted in mid-November of years 1 and 2, while only mustard (*Brassica juncea*, 39 kg ha⁻¹) was planted in years 3 and 4. However, mustard also dominated within the mixed cover crops in years 1 and 2 (80–90% of dry biomass). Cover crops were flail shredded and roto-tilled into the soil to a depth of 15 cm in March to April of the following year. Dry biomass of cover crops for years 1, 2, 3, and 4 averaged 2.8, 8.9, 10.8, and 3.7 Mg ha⁻¹, respectively. Spinach was then direct seeded at the rate of 7 kg ha⁻¹ in May to June in years 1 through 3. Because spinach was planted immediately after cover crop incorporation, no pre-plant fertilizer was used. Spinach was drip irrigated and 7 and 23 kg ha⁻¹ of supplemental N were applied through fertigation in years 2 and 3, respectively. Spinach was harvested in July of each year except for year 3 when harvestable plants were lacking due to poor germination. Spinach yields averaged 13.2 Mg ha⁻¹ in year 1 and 7.5 Mg ha⁻¹ in year 2. No spinach was planted in year 4 since

TABLE 4 Input and recycled nitrogen (kg ha^{-1}) to each treatment from organic fertilizers, broccoli residues brought from off-farm, cover crops, and crop residues recycled in the plot, 2001–2005

Year	Input/Recycled	Source	Treat. A	Treat. B	Treat. C	Treat. D	Treat. E	Average	
Year 1 (2001-02)	Input	Broccoli residues from off farm	147	147	147	147	147	147	
		Compost	86	86	86	86	86	86	
		Pelleted organic fertilizers*	82	82	82	82	82	82	
	Recycled	Liquid organic fertilizers	36	36	15	36	15	15	343
		Total	351	351	330	351	330	330	343
		Cover crops	0	0	82	0	63	63	29
Year 2 (2002-03)	Input	Broccoli residues from off farm	136	0	0	0	0	0	0
		Compost	117	117	117	117	117	117	117
		Pelleted organic fertilizers	43	0	43	0	0	0	0
	Recycled	Liquid organic fertilizers	52	22	52	22	22	22	195
		Total	348	139	212	139	139	139	195
		Strawberry or broccoli residues	50s**	54s	133b**	56s	131b	131b	131b
Year 3 (2003-04)	Input	Cover crops	0	168	0	175	188	188	191
		Total	50	222	133	231	319	319	191
		Broccoli residues from off farm	266	229	0	0	0	0	0
	Recycled	Compost	198	198	198	198	198	198	198
		Pelleted organic fertilizers	67	67	164	164	164	164	164
		Liquid organic fertilizers	79	79	51	51	51	51	51
Year 4 (2004-05)	Input	Total	610	573	413	413	413	413	484
		Strawberry residues	57s	0	74s	0	0	0	0
		Cover crops	0	0	157	144	151	151	151
	Recycled	Total	57	0	231	144	151	151	117
		Broccoli residues from off farm	126	0	0	0	0	0	0
		Compost	50	50	50	50	50	50	50
Recycled	Pelleted organic fertilizers	52	190	190	190	190	190	190	
	Liquid organic fertilizers	73	40	40	40	40	40	40	
	Total	301	280	280	280	280	280	280	

Year 5 (2005-06)	Recycled	Strawberry or broccoli residues	37s	45b	61s	118b	89b
		Cover crops	0	49	49	49	49
		Total	37	94	110	167	138
Year 5 (2005-06)	Input	Broccoli residues from off farm	95	0	0	0	0
		Compost	163	163	163	163	163
		Pelleted organic fertilizers	54	54	54	54	54
		Liquid organic fertilizers	80	80	80	80	80
		Total	392	297	297	297	297
Recycled	Strawberry or broccoli residues	33s	97b	131b	105b	124b	98
	Total	33	97	131	105	124	98
Average (per year)	Input	Broccoli residues from off farm	154	75	29	29	29
		Compost	123	123	123	123	123
		Pelleted organic fertilizers	60	79	107	98	98
		Liquid organic fertilizers	64	51	48	46	42
		Total	400	328	306	296	292
Recycled	Strawberry or broccoli residues	35	39	80	56	69	69
	Cover crops	0	43	58	74	90	90
	Total	35	83	137	129	159	109

*Pelleted organic fertilizers were applied for strawberries (year 2 and 5), strawberries and cover crop (year 1), or strawberries, cover crop, and broccoli (year 3 and 4).

**s: strawberry crop residues; b: broccoli crop residues.

the extended rainy season prohibited planting in the spring. Following spinach, 4- to 6-week old transplants of broccoli were planted in mid-July to early August for years 1 through 4. Broccoli was grown as a summer cover crop for suppressing *V. dahliae* in the soil and planted at a density of $\sim 50,000 \text{ ha}^{-1}$ throughout the study. A limited amount of supplemental N (15 kg-N ha^{-1}) was applied in years 1 and 2 but the N rate was increased to 114 and 178 kg ha^{-1} in years 3 and 4, respectively, to increase broccoli biomass (Table 4). The entire broccoli crop was lost due to damage by ground squirrels in year 2. To compensate for the loss, above-ground broccoli residues (66.5 Mg ha^{-1} fresh weight) were collected from an organic broccoli field in Gilroy, CA, and applied to treatments A and B (to be followed with strawberries in year 3); these broccoli residues were taken from the same size area as the experimental plot (0.08 ha) but had greater biomass due to the higher plant density of the Gilroy site. Fresh biomass of broccoli residues (florets, leaves, and stems) grown in years 1, 3, and 4 averaged 27.9, 28.1, and 43.2 Mg ha^{-1} , respectively. In September to October each year these residues were mowed and rototilled into the plots where these were grown (Table 3).

Yield, Mortality, Biomass Survey, and C/N analysis

Fresh strawberry fruit yield was measured for each cultivar once or twice per week from 40 designated plants during the harvest period. Marketable and cull fruit yields were weighed separately by experienced harvesters. Mortality of strawberry plants was examined from May to the end of the season (September to October) by counting living and dead plants in the middle two beds of each plot. During year 5, 20 transplants were sampled on November 23, 2005, and 4 whole plants (shoots, roots, and immature fruits) of strawberry were dug out from the middle two beds of each plot on January 8, March 13, June 2, and October 5 in 2006. After washing out soil from roots with running water, samples were dried at $60 \text{ }^\circ\text{C}$ in a convection oven to constant weight. The dried samples were weighed for dry biomass, ground to pass through a 0.5-mm sieve with a Wiley mill, and analyzed for total C and total N content (AOAC 1997) using a Vario Max CNS analyzer (Elementar America, Mount Laurel, NJ, USA). Twenty mature fruits from each plot were sampled on June 20, 2006, frozen at -20°C , and lyophilized by a freeze dryer. Freeze dried samples were passed through a 0.5-mm sieve and analyzed for total C and N content using the fore-mentioned method. Fresh moisture content of fruit was calculated from the difference between pre- and post-lyophilized weight of fruits. Cumulative total fruit yield, total N, and fresh moisture content of mature fruits were then used to estimate N uptake by fruit on June 2 and October 5, 2006, which was combined with whole plant biomass-N to calculate plant biomass N plus harvested fruit N on these dates. Since the focus was on strawberry disease management, broccoli was

managed as a cover crop for disease control in this experiment and broccoli floret yield was not recorded. About 1 kg of fresh broccoli and cover crop samples from each plot was brought to the lab and biomass and total C and N content were determined by the above-mentioned methods. Approximately 1 kg of composts and organic fertilizers used in the trial were sampled, oven dried, ground, and analyzed for total C and total N in the same manner. Also ~100 g subsamples were taken to gravimetrically determine moisture content by heating at 105°C for 48 hours in a convection oven.

Leaf Tissue Test

To evaluate the nutrient status of the strawberry plants in year 5, twenty leaf samples (petioles and leaf blades) were taken from the middle two beds of each plot in mid (July 11, 2006) and late (October 5, 2006) harvest, dried and analyzed for total N, P, K, Ca, Mg, S, and B content (leaf blade) and NO₃-N (petiole) content by the UC Davis Analytical Lab, Davis, CA, USA.

Soil Sampling and Analysis

Ten to twenty soil cores at 0–15 cm (topsoil) were sampled by soil probes (2 cm diameter) at the middle two beds of each plot to make a composite sample. Samplings were conducted 2–3 times per year for the entire trial period. About 100 g of composite sample were air dried in the lab for six weeks and then ground and plated onto *V. dahliae* semi-selective medium (NP-10) using the Anderson Sampler dry sieve technique (Koike et al. 1994) to test the number of viable microsclerotia of *V. dahliae*. NP-10 plates were incubated in the dark for three weeks, then examined for typical microsclerotia formation by using a dissecting microscope. During year 5 (October 2005 to October 2006), topsoil (0–15 cm) and subsoil (15–30 cm) were sampled monthly in the same manner. After mixing well in a bucket, about 5 g of soil were taken from the composite sample and transferred on site into a pre-weighed screw top plastic tube containing 25 ml of 2M KCl. Each tube was tightly sealed and kept on ice in an ice chest and transferred to the lab. In the lab, samples were reweighed, shaken for one hour with a reciprocal shaker, filtered to obtain clear supernatant, and kept at –20 °C until analysis. NH₄-N and NO₃-N concentrations in the KCl extracts were determined by flow injection analysis (Lachat Instruments 1993a, 1993b) and the sum of NO₃-N and NH₄-N was expressed as inorganic N in the 0–30 cm soil layer. Separately, a ~100 g subsample was taken from a composite soil sample to determine gravimetric soil moisture content. The remainder of soils sampled at the end of the strawberry harvest season in October 2006 were air dried, passed through a 2-mm sieve, and analyzed for pH and electrical conductivity (soil: water =1: 1). Ten gram subsamples were further ground to pass through a 0.5 mm sieve and analyzed for total C and N content using

the above-mentioned methods. Soil bulk density was also determined at the end of the harvest season in October 2006 using the soil core method (core size: 5 cm inner diameter \times 15 cm long) for 0–15 cm and 15–30 cm depths of the strawberry beds. Four cores were sampled per depth and bulked to form composite samples.

Disease Diagnosis

During years 1 and 4, plants were regularly observed for disease symptoms by the grower and researchers and tested as needed. At the end of year 5, plants showing dieback, stunting, or collapsing symptoms were collected from all plots on October 5, 2006. For some of the Seascape plots, plants in general showed few signs of distress. Plants were taken to the lab and isolations made on roots and crowns. Plant material was first washed free of dirt and debris. Crowns and roots were then surface sanitized by soaking in a 0.1% bleach solution for 3 min and then thoroughly rinsing in sterile distilled water. Using aseptic technique, crowns and roots were dissected and symptomatic pieces of tissue were placed into separate Petri dishes containing acidified corn meal agar (for general pathogens), PARP semi-selective medium (for *Phytophthora*), or NP-10 (for *Verticillium*). Isolations were evaluated on October 16, 2006.

Statistical Analysis

Prior to analysis of variance (ANOVA), data were log transformed to fulfill ANOVA's assumptions when needed. Split plot two-way ANOVA (for strawberry plots, with rotation patterns (main plot) and cultivars (split plot) as variables) was applied to test statistical differences among treatments. Repeated measure analysis was conducted to examine treatment effects on *V. dahliae* population in soils. Tukey's HSD post-hoc test at $P = 0.05$ was used for mean separation. Regression analysis was conducted for examining relationships between marketable fruit yield of strawberries in year 5 and the length of break period between strawberries. Statistical analysis package Statistix (Analytical Software 2008) was used for these analysis.

RESULTS AND DISCUSSION

Strawberries

GROWTH AND YIELD DURING YEARS 1 THROUGH 4

During the first four years of the study, organic strawberries grew well and no major disease and pest problems were observed in any plots. Mortality of strawberry among all plots averaged 1.1% and the average marketable yield of main treatments ranged from 675 to 841 g plant⁻¹ during the period. These

yields are equivalent to 34.0 to 42.3 Mg ha⁻¹, which is higher than the typical organic strawberry yield (22 to 36 Mg ha⁻¹) for the central coast (Bolda et al. 2006) and is approximately 60% of the average strawberry yield of Monterey and Santa Cruz Counties during the same period of which about 97% are conventionally produced (Monterey County Farm Bureau 2003–2006; Santa Cruz Farm Bureau 2003–2006). Significant differences in marketable yields were not found between any rotation treatments (Table 5). Notably, yield in continuous strawberry treatment A was relatively consistent. Yields dropped 10% from year 1 to year 2, but increased in years 3 and 4 back to year 1 levels. On the other hand, fruit yields varied between cultivars: Aromas had higher yields than Diamante (year 1) and Seascape (years 2 to 4)(Table 5).

GROWTH AND YIELD IN YEAR 5

Cultivars Aromas and Seascape were planted in all plots in year 5. Strawberries in all plots grew well without any major pest problems and disease symptoms. The average plant mortality was 1.2%, a level similar to the first four years, and no significant difference in mortality was found between any treatments.

In the early harvest stage, rotation treatment did not affect fruit yield whereas, between cultivars, cv. Seascape had a higher yield compared to cv. Aromas (Table 5). This is a common pattern in years 2 through 4 (data not shown). In the mid-harvest season, however, yield was highest in treatment E (seven-year break between strawberries) followed by D (three-year break between strawberries), C (two-year break between strawberries), B (one-year break between strawberries), and A (continuous strawberries with broccoli residue incorporation) (Table 5). A significant difference was found between treatments E and A. In the late season, no significant differences were found between any treatments. Overall cumulative marketable yield in year 5 ranged from 650 to 890 grams per plant, a comparable range with the first four years. Seascape had a significantly higher yield than Aromas (Table 5), which was the opposite trend for years 2 through 4; yields for Seascape exceeded those for Aromas in mid- to late season in the previous three years but not for year 5. Among rotation treatments, though a significant difference was found only between treatments A and E, the longer the years between strawberries, the greater the marketable fruit yield. Since the interaction was not significant ($P = 0.222$. Table 5), cumulative marketable fruit yield of both cultivars were pooled and relative yields to the average of the seven- year rotation yield for both cultivars calculated. Then the correlation between the relative yields and the length of break periods were analyzed. The result showed a strong positive linear correlation between the two factors ($P = 0.0003$; Figure 1). Numerically, marketable yield in treatment A was approximately 20% lower than treatment E. Total fruit yield showed a similar trend with marketable yield ranging from 891 g per plant (treatment A) to 1093 g per plant (treatment E) (Table 5).

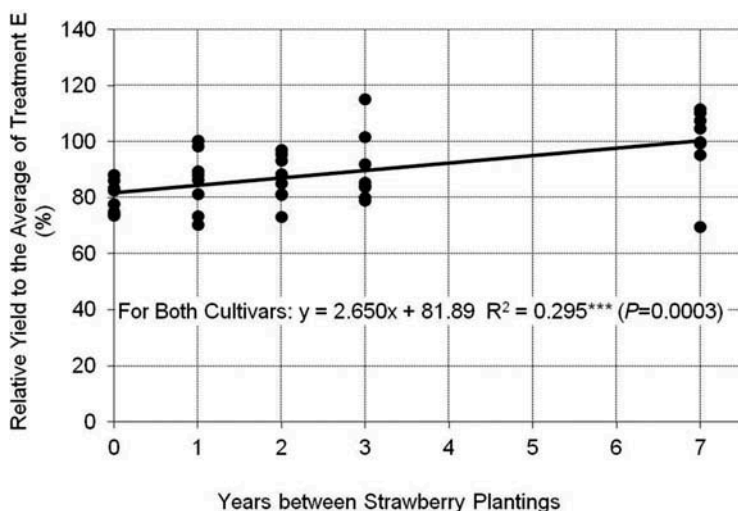


FIGURE 1 Regression between break periods between strawberry plantings (years) and relative marketable fruit yield (%) based on the average yield of treatment E (seven-year break treatment) in year 5 in organic strawberry/vegetable rotation experiment in Moss Landing, CA. Data for both cvs. Aromas and Seascape are pooled; $n = 40$.

Strawberry biomass showed a similar trend with the marketable fruit yield; at the end of early harvest stage (June 2, 2006), shoot biomass of three-year and seven-year break plots were greater than continuous plots ($P = 0.0012$) and the cultivar Aromas had greater immature fruit yield than Seascape ($P = 0.037$). However, no such differences were found in the late harvest stage (data not shown). A similar trend was also observed for biomass N in strawberries with a significant difference between treatments only at the early harvest stage (Figure 2).

DISEASE DIAGNOSIS OF STRAWBERRY PLANTS IN YEAR 5

No major strawberry pathogens (*Phytophthora*, *Verticillium*, *Colletotrichum*) were recovered from any of plants sampled at the end of the season. Various Aromas plants showed apparent stress or senescence symptoms in some of the crowns (purple discoloration). Seascape plants generally did not show this symptom. Minor pathogens such as *Cylindrocarpum* and *Pythium*, as well as saprophytic *Fusarium* species, were recovered only from Aromas, which may have contributed to lower yields of this cultivar compared to Seascape in year 5.

TISSUE TEST OF STRAWBERRY PLANTS IN YEAR 5

To determine if any differences in nutritional status existed between any treatments, strawberry foliage was sampled at mid- and late harvest seasons

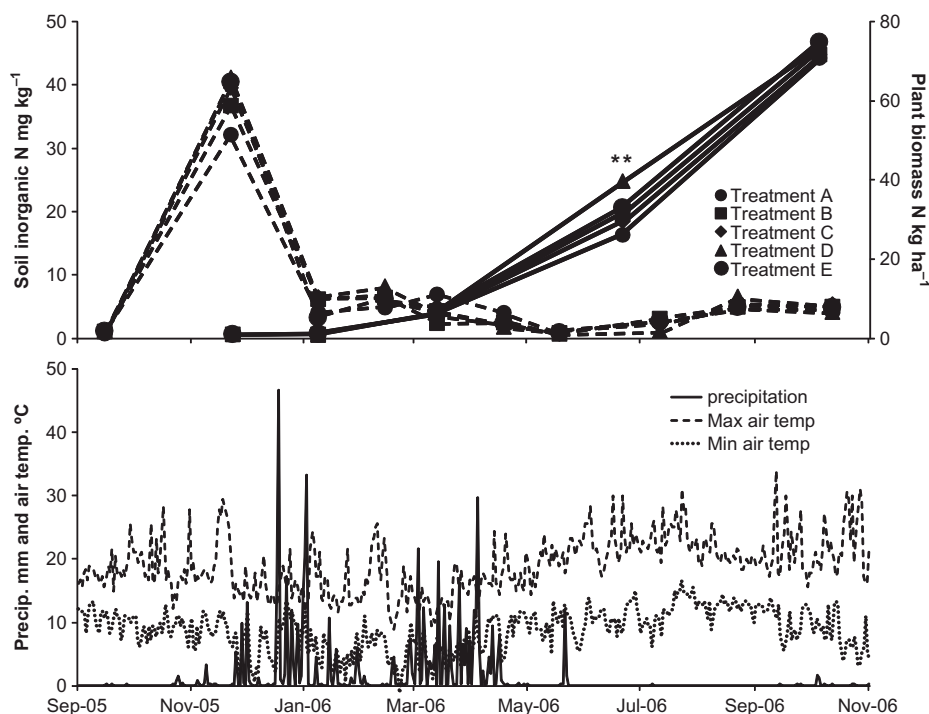


FIGURE 2 Changes in inorganic N concentration in 0–30 cm depth soil (broken line) and strawberry plant biomass N including cumulative harvested strawberry fruit N at cv. Seascape subplot (solid line) (above), and precipitation, maximum air temperature, and minimum air temperature at the Elkhorn Slough National Estuarine Research Reserve, Moss Landing, CA, (below) during the 2005–2006 season (year 5). **indicates significant at $P = 0.01$. No significant differences at $P = 0.05$ were found between any treatments in soil inorganic N and plant biomass N at any other dates. See [Table 2](#) for treatments.

in year 5 and analyzed for N, P, K, Ca, Mg, S, and B (leaf blades) and $\text{NO}_3\text{-N}$ (petioles). Results were compared with the optimum range of each element for California strawberries (Bottoms et al. 2013). A significant difference was found only for Mg in mid-season and $\text{NO}_3\text{-N}$ in late season ([Table 6](#)). Both showed the same general trend with the fruit yield; the longer the rotation, the higher the concentration. None of nutrients, including Mg and $\text{NO}_3\text{-N}$, were below the optimum range except for B in mid-season for continuous plots and in late season for all plots, and $\text{NO}_3\text{-N}$ in late season continuous plots. This indicates a slight B deficiency trend for all plots especially in the late season. For N, although petiole $\text{NO}_3\text{-N}$ was below the optimum range in late season continuous plots, total N in leaf blade in the late season at the same plots was in the optimum range.

TABLE 6 Nutrient content in leaf tissues of organic strawberries from the rotation experiment in Moss Landing, CA, at mid- and late season samplings in year 5

Sampling date/Treatment	Petiole									
	Blade N g kg ⁻¹	NO ₃ -N mg kg ⁻¹	Blade P g kg ⁻¹	Blade K g kg ⁻¹	Blade Ca g kg ⁻¹	Blade Mg g kg ⁻¹	Blade S mg kg ⁻¹	Blade B mg kg ⁻¹		
7/11/2006										
Main plot										
A (Continuous)	24.3 a ^a	624 a	3.03 a	15.6 a	10.6 a	3.05 a	1669 a	41.7 a		
B (1-year break)	24.6 a	651 a	3.05 a	15.0 a	10.3 a	3.19 ab	1712 a	43.1 a		
C (2-year break)	24.1 a	634 a	2.89 a	15.3 a	11.3 a	3.28 b	1703 a	44.9 a		
D (3-year break)	24.3 a	740 a	3.04 a	15.2 a	10.6 a	3.22 ab	1792 a	44.6 a		
E (7-year break)	24.8 a	669 a	2.94 a	15.0 a	11.4 a	3.36 b	1762 a	46.6 a		
ANOVA (P)	0.695	0.323	0.325	0.134	0.241	0.008**	0.150	0.105		
Split plot										
Aromas	24.5 a	626 a	2.97 a	15.4 a	11.0 a	3.24 a	1722 a	44.9 a		
Seascape	24.4 a	702 a	3.01 a	15.0 a	10.6 a	3.20 a	1733 a	43.4 a		
ANOVA (P)	0.713	0.632	0.459	0.556	0.216	0.740	0.739	0.220		
Interaction ANOVA (P)	0.705	0.950	0.933	0.996	0.925	0.998	0.984	0.929		
10/5/2006										
Main plot										
A (Continuous)	24.6 a	111 a	4.45 a	19.5 a	10.1 a	3.20 a	2232 b	35.6 a		
B (1-year break)	25.4 a	378 ab	4.30 a	18.1 a	9.75 a	3.61 a	1958 a	36.7 a		
C (2-year break)	24.7 a	256 ab	4.40 a	18.1 a	9.66 a	3.44 a	2047 ab	35.8 a		
D (3-year break)	25.6 a	623 b	4.40 a	18.0 a	9.28 a	3.56 a	1941 a	36.2 a		
E (7-year break)	25.6 a	573 ab	4.36 a	18.0 a	9.33 a	3.56 a	1958 a	35.9 a		
ANOVA (P)	0.730	0.037*	0.793	0.061	0.342	0.121	0.004**	0.976		
Split plot										
Aromas	25.2 a	373 a	4.38 a	18.2 a	9.38 a	3.46 a	2006 a	35.6 a		
Seascape	25.1 a	404 a	4.39 a	18.5 a	9.85 b	3.49 a	2048 a	36.5 a		
ANOVA (P)	0.890	0.740	0.915	0.528	0.038*	0.779	0.554	0.244		
Interaction ANOVA (P)	0.380	0.522	0.992	0.798	0.214	0.948	0.728	0.586		
Optimum range ^b	24–30	250–1900	3–4	13–17	9–23	3.1–4.1	1600–2000	43–77		

^aValues within a given treatment followed by the same letter are not significantly different according to Tukey's HSD test at the $P = 0.05$.

^bAccording to Bottoms et al. (2013).

*,** indicates significant at $P = 0.05, 0.01$, respectively.

Changes in the Soil

VERTICILLIUM DAHLIAE POPULATION IN THE SOIL

Pre-experiment soil samples (collected November 2001) indicated that the population of *V. dahliae* was below the detection limit. Although numbers varied, the Verticillium detection tests resulted in generally low counts during the five-year rotation experiment, that is, less than 5 microsclerotia per gram of soil (ms g^{-1}) (Figure 3). Repeated-measure analysis indicated that rotation treatments did not affect *V. dahliae* in soil throughout the five-year experiment ($P > 0.17$). In year 5, average *V. dahliae* counts in soil was 0.2 ms g^{-1} , below the detection limit, and 0.6 ms g^{-1} at planting, mid-season, and end of harvest, respectively, and no significant difference was found between any treatments ($P > 0.12$).

Interestingly numbers of *V. dahliae* microsclerotia in soils tended to drop after broccoli residue incorporation regardless of treatment (Figure 3). In treatments B and D, microsclerotia increased late in year 2 when broccoli was lost due to the damage caused by ground squirrels. This field was weedy at that time and the major weed, Shepherd's purse (*Capsella bursa-pastoris*), a known host of *V. dahliae* (Koike et al. 2007), could have contributed to this increase. Although spinach was not considered a host of *V. dahliae* when the experiment began in 2001, du Toit et al. (2005) later reported that spinach seed crops can become infected with this pathogen; the extent to which fresh market production spinach harbors *V. dahliae* has not been documented. In our experiment, when spinach was present in the field, *V. dahliae* population in the soil was not affected in year 1 but slightly increased in year 2 (Figure 3). In the fifth year, *V. dahliae* population in soils decreased to undetectable levels in all treatments due probably to broccoli residue incorporation in the fall 2005. Therefore, it is unlikely that the yield reduction found in shorter break-period rotations in the final year was attributed to *V. dahliae*.

SOIL CHEMICAL AND PHYSICAL PROPERTIES

During year 5, inorganic N content in 0–30 cm soil depth was $30\text{--}40 \text{ mg kg}^{-1}$ at planting in November 2005, but decreased to below 10 mg kg^{-1} in January 2006 after multiple storm events and remained at this level for the rest of the growth period (Figure 2 for Aromas subplot). There was no significant difference in inorganic N content in topsoil between any treatments during the strawberry growth period in year 5 ($P > 0.39$), indicating no difference in N supplies between any treatments. At the end of harvest in year 5, no significant differences between any treatments were found at 0–15 cm and 15–30 cm depth in bed soil for total C, total N, pH, electrical conductivity, and bulk density (data not shown). Although we cannot eliminate the possibility of combined effects from all these factors, these results indicate that poor

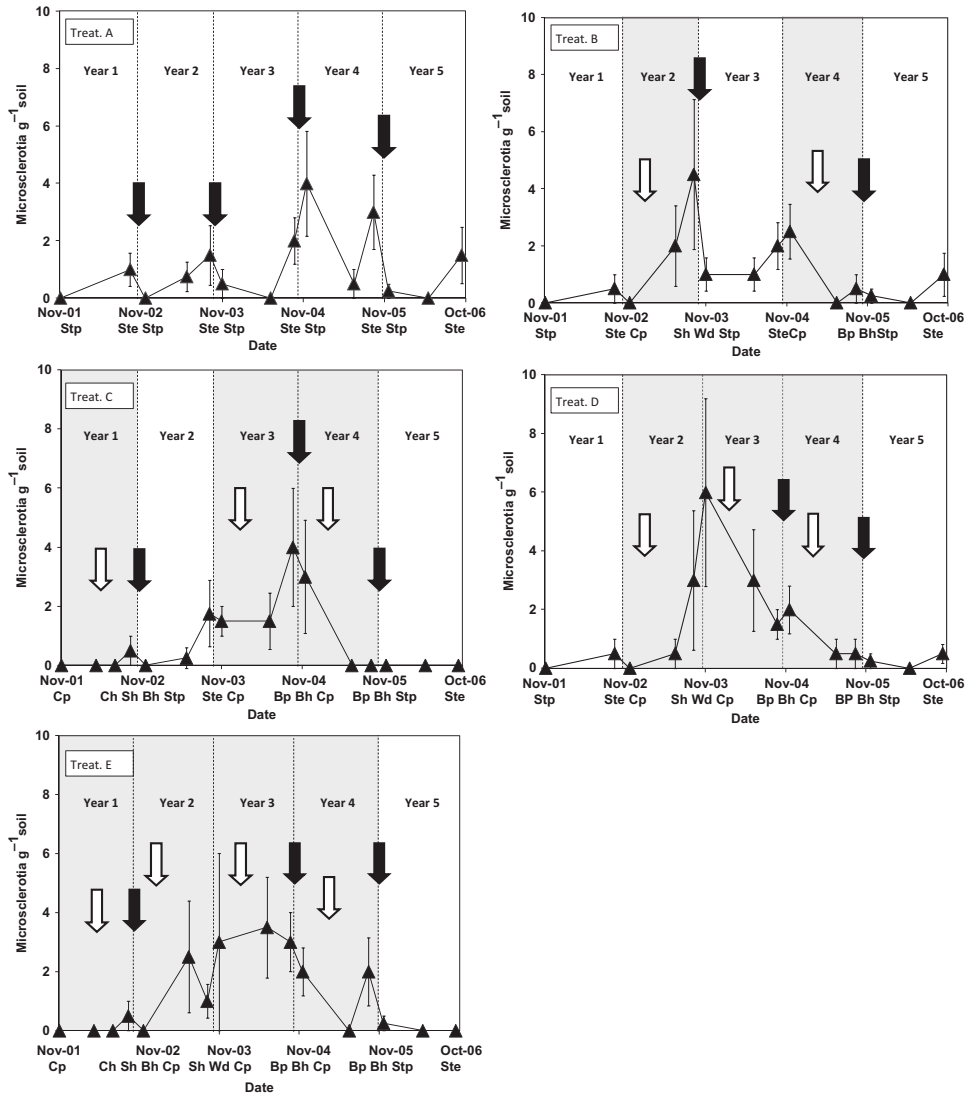


FIGURE 3 Microsclerotia of *Verticillium dahliae* in the soil of each treatment during the organic strawberry/vegetable rotation trial from 2001 to 2006. Year with darker background indicates cover crop and vegetable production period and blank background means strawberry production period. Stp = strawberry planting, Ste = end of strawberry harvest, Cp = cover crops planting, Ch = cover crop harvest, Sh = spinach harvest, Wd = weedy condition due to loss of broccoli, Bp = broccoli planting, Bh = broccoli harvest. White arrows and black arrows indicate date of cover crops and broccoli residues incorporation, respectively. Vertical bars represent the standard error of means. See Table 2 for treatments.

soil chemical and physical conditions such as soil compaction, acidification, or salinization (Huang et al. 2006) were not the causes of the yield decline in the present study.

GENERAL DISCUSSION

The loss of soil productivity when crops are grown repeatedly on the same land resulting in poor plant growth and reduced yields is called “yield decline” (Bennett et al. 2012), “soil sickness” (Huang et al. 2006), or “replant problem” (Utkhede 2006). Such losses, called here “yield decline,” have been reported in many crops worldwide (see Bennett et al. 2012, for recent review) including strawberries (Wilhelm and Paulus 1980; Cao and Wang 2007; Huang et al. 2006). Biotic (pests, pathogens, nematodes, deleterious rhizosphere microorganisms, allelopathic autotoxicity, and weeds) and abiotic (soil physical and chemical properties) factors can cause yield decline. Further, although one factor may possibly be responsible for yield decline, it is more likely that a combination of factors interact to cause the effect (Bennett et al. 2012; Huang et al. 2006; Zucconi 1993). This study also demonstrated the challenges researchers face when using a participatory process where farmer involvement is a key part of the experiment. Farmers cannot wait until the end of a long-term experiment such as this before making adjustments in their farming practices. Changes occur as needed, and the experimental design needed to shift as well. This definitely added to our difficulty in pointing to single factors causing yield decline.

The present study showed that the integrated practices of broccoli residue incorporation, compost application, mustard cover crop incorporation, use of a relatively resistant cultivar, and rotation with non-host crops allowed the one- to three-year rotations to have a statistically similar marketable fruit yield to the seven-year rotation in organic strawberries grown in this region of coastal California. Although the trial was conducted in a field with a low baseline *V. dahliae* population and results may be different for fields with greater disease pressure, it should be noted that the *V. dahliae* population in the soil was kept low ($<5 \text{ ms g}^{-1}$) during the five-year study (Figure 3) and no *V. dahliae* or other major pathogens were detected from strawberry plants at the end of year 5. The low *V. dahliae* population in the soil during the trial may be due to broccoli residue incorporations (Subbarao et al. 2007) though the application rate of broccoli residues in this trial was lower than many farmers are using today. Nevertheless, a significant positive correlation of the break period between strawberries and the marketable fruit yield in year 5 existed (Figure 1), and marketable fruit yield in continuous strawberry plots (treatment A) was 20% lower than seven-year break plots (treatment E) (Table 5). Given these results, the cause of the yield reduction at the shorter rotations appears to be something other than major soil-borne pathogens (e.g., *V. dahliae* and *Phytophthora* spp.). It is known that other minor sublethal pathogens such as species of *Colletotrichum*, *Pythium*, *Rhizoctonia*, and *Cylindrocarpum* can reduce fruit yield of strawberries in California (Fort et al. 1996; Subbarao et al. 2012), which may have played a role in yield reduction of the shorter rotation treatments in

the trial. Further, Seigies and Pritts (2006) showed that three sowings of brown mustard (*Brassica juncea*) cover crops enhanced successive strawberry growth compared to continuous strawberries regardless of relatively high levels of fungal infection on the strawberry roots. Use of mustard cover crops in the longer rotation treatments of the present study may have had a similar positive effect.

Soil nutrient imbalance after continuous cropping can also cause yield decline. In the present study, fertility management changed from year to year in order to reduce N loss during the rainy winter (Muramoto et al. 2004, 2007) and by the grower's preference. To assure adequate nutrient status, we conducted tissue tests for strawberries and monitored soil inorganic N dynamics during the strawberries' growth period. Although strawberry leaf blade analysis in the mid- and late season of year 5 showed differences in Mg and NO₃-N among treatments, none of these appeared to be critically deficient. Further, even though plant tissues in shorter rotations showed lower petiole NO₃-N, it is difficult to interpret whether it was due to the lack of N supply, reduced N absorption by roots infested by non-lethal pathogens, or both (Copeland and Crookston 1992). In fact, no significant difference was found between any treatments in soil inorganic N content during year 5 (Figure 2), suggesting the lower total-N content in leaf blades may be attributed to the poor root health in shorter rotations rather than the lack of N supply.

Cao and Wang (2007) showed that root exudates from strawberries inhibited the growth of strawberries due to autotoxicity. In hydroponics, Kitazawa et al. (2005) identified the causal compound of strawberry autotoxicity as benzoic acid. Further, Asao et al. (2008) demonstrated that destroying benzoic acid by electrodegradation in hydroponic strawberries increased the plant growth and fruit yield. In soil culture, however, the accumulation and fate of autotoxic compounds in the rhizosphere is not well understood (Bennett et al. 2012). For example, a toxic level (<20 mg L⁻¹) of benzoic acid was absorbed to soil particles, which may explain the reason for the limited allelopathic effect of the compound at concentrations often recorded in natural soil (Inderjit and Bhowmik 2004).

Overall, the causes of yield decline are complex in general and the exact cause in this study is unknown. However, we demonstrated that the use of the integrated approach can reduce the amount of yield loss, the goal of the study. Future studies on the effect of interactions of minor pathogens on strawberry fruit yield are warranted. For example, although each pathogen alone did not cause significant damage, combination of *Cylindrocarpon* spp., and *Pythium* spp. caused significant root disease in apple seedlings (Braun 1995). More information is needed on how this cropping system would be influenced by rotations with different crops (including major vegetable crops such as lettuce, cauliflower, celery, and spinach) and by emerging pathogens such as *Fusarium oxysporum* and *Macrophomina phaseolina* (Koike et al. 2012).

Actual crop rotations of organic strawberries and vegetables in the central coast of California are complex. Specialized berry growers in California usually lease land and grow strawberries on different fields every year (Bolda et al. 2006), whereas small-scale organic growers tend to grow both strawberries and vegetables on the same fields. For a specialized organic strawberry grower to rotate fields with other organic vegetable growers, choosing which vegetable crops will be used in rotation as done in the present study may not be an option. Further, if a host crop susceptible to *V. dahliae* (e.g., lettuce) was planted during the break period between strawberries, the level of yield decline can be greater than we found in this study (Subbarao et al. 2007).

Finally, the present study demonstrated the positive effect of a longer rotation on strawberry production. In Europe (Bevan et al. 2001, Steffek et al. 2004), the Northeast and Midwest United States, and in eastern Canada (Pritts and Handley 1998), a minimum of a three-year rotation is recommended for strawberries that do not use chemical fumigants. Some diversified small-scale organic growers in central coastal California have been maintaining a five-year rotation for their strawberries. By diversifying their cropping rotations, they benefit both from improved strawberry yields and access to alternative markets such as farmers' markets or Community Supported Agriculture systems where greater diversity provides economic advantage (Gliessman and Muramoto 2010). The results of this study justify such practice when it is feasible.

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