Journal of Integrative Agriculture 2015, 14(11): 2309–2318

Available online at www.sciencedirect.com

ScienceDirect

REIVEW

Anaerobic soil disinfestation: A chemical-independent approach to pre-plant control of plant pathogens

S L Strauss, D A Kluepfel

USDA-ARS Crops Pathology and Genetics Research Unit, University of California, Davis, CA 95616, USA

Abstract

Due to increasing regulations and restrictions, there is an urgent need to develop effective alternatives to chemical-dependent fumigation control of soilborne pests and pathogens. Anaerobic soil disinfestation (ASD) is one such alternative showing great promise for use in the control of soilborne pathogens and pests. This method involves the application of a carbon source, irrigation to field capacity, and covering the soil with a plastic tarp. While the mechanisms of ASD are not completely understood, they appear to be a combination of changes in the soil microbial community composition, production of volatile organic compounds, and the generation of lethal anaerobic conditions. The variety of materials and options for ASD application, including carbon sources, soil temperature, and plastic tarp type, influence the efficacy of pathogen suppression and disease control. Currently, both dry (e.g., rice bran) and liquid (e.g., ethanol) carbon sources are commonly used, but with different results depending on environmental conditions. While solarization is not an essential component of ASD, it can enhance efficacy. Understanding the mechanisms that mediate biological changes occurring in the soil during ASD will facilitate our ability to increase ASD efficacy while enhancing its commercial viability.

Keywords: anaerobic soil disinfestation, biological soil disinfestation, soilborne pathogens, fumigation

1. Introduction

Since the mid-20th century, fumigation of agricultural soils has been the primary pre-plant method for controlling soilborne plant pathogens, nematodes, and weeds. Pre-plant fumigation practices suppress a wide variety of plant pathogens, from *Verticillium* spp. which affect crops such as strawberries (Shennan *et al.* 2009) and eggplant (Momma *et al.* 2013), to *Agrobacterium tumefaciens* which affects such perennial woody crops as walnuts, almonds, and roses (Agrios 2005; Yakabe *et al.* 2014). Left unchecked, these diseases and many other soilborne pathogens can result in complete crop loss and potentially infect subsequent crops (Martin and Loper 1999). For the past 40 years, methyl bromide (MeBr) has been the dominant soil fumigant used to control soilborne pests for a wide range of high value crops from strawberries (Shennan *et al.* 2009) and tomatoes (Locascio *et al.* 1997), to fruit and nut trees (Ramos 1998). However, the 1993 *Montreal Protocol* required a complete phase-out of MeBr by 2005 in developed countries (http://www.epa.gov/Ozone/mbr), though certain crops and nurseries have been exempt. Since these exemptions will end soon, there is an increasing demand for alternatives (Browne *et al.* 2013; Hanson *et al.* 2013). In addition, current MeBr

Received 23 March, 2015 Accepted 25 June, 2015
S L Strauss, E-mail: sarah.strauss@ars.usda.gov;
Correspondence D A Kluepfel, Tel: +1-530-7521137,
Fax: +1-530-7547195, E-mail: daniel.kluepfel@ars.usda.gov

doi: 10.1016/S2095-3119(15)61118-2

alternatives, such as 1,3-dichloropropene and chloropicrin, are facing increased regulatory pressure as well. For example, there are 47 counties in California, USA, alone with binding township caps that limit application of chloropicrin (Carpenter *et al.* 2001). Barrier films and expanding buffer zones where fumigation is limited are also adding to the cost and difficulty of soil fumigation (Gao *et al.* 2011; Fennimore *et al.* 2013).

Given the limitations and regulations of chemical fumigation, there is a need to develop a biologically-based, integrated management strategy for soilborne diseases that facilitates profitable and sustainable production without the use of chemical fumigants. One such method showing great promise is anaerobic soil disinfestation (ASD), also termed biological soil disinfestation (Blok *et al.* 2000; Butler *et al.* 2012a). In this method, anaerobic conditions are generated after soil is amended with a carbon source (C-source), irrigated to field capacity, and covered with an impermeable plastic tarp (Blok *et al.* 2000; Momma *et al.* 2006). Independently developed in Japan (Momma *et al.* 2006) and the Netherlands (Blok *et al.* 2000), this method is currently being used on a commercial basis in California for strawberries (Muramoto *et al.* 2014), Japan for tomatoes, melons, and cut flowers (Momma *et al.* 2013), and the Netherlands for strawberries, asparagus, and tree nurseries (Shennan *et al.* 2014).

While the basic technique of ASD treatment is straight forward, the efficacy against a given target pathogen varies based on three key parameters: C-source used, tarp type, and soil temperature. As the use of ASD increases, it is critical that growers are provided with options and methods that are most effective for their target pathogens, crop, environmental conditions, and available carbon sources. Here, we provide an overview of the different options available when applying ASD as a pre-plant strategy to control soilborne pests and pathogens. In addition, we summarize the current understanding of the mechanisms that mediate ASD disease suppression.

2. Modes of action

The mode of action of ASD is not completely understood. In preliminary trials, others and we have documented ASD induced changes in the soilborne microbial community composition, production of volatile organic compounds, and the generation of anaerobic conditions that have all been shown to contribute to the suppression of phytopathogenic agents. Each of these factors will be discussed here.

ASD dramatically induced changes in the soil microbial community (Messiha *et al.* 2007; Mowlick *et al.* 2013, 2014), which is hypothesized to antagonize resident phytopathogenic microbial agents (Messiha *et al.* 2007). A next-generation sequence analysis was performed for bacterial 16S rRNA genes amplified from total DNA extracted from soil exposed to ASD conditions for 7 weeks. In these trials using 20.2 t ha^{-1} rice bran, we observed a significantly different bacterial community composition compared to adjacent non-treated soils (Fig. 1). Some of the most dramatic changes we observed in species composition included a significant increase in the abundance of *Clostridiales*, *Acidobacteria*, and *Burkholderia* (Fig. 2). Increases in *Clostridia*, a strict anaerobe, also were found in greenhouse ASD trials using wheat bran, *Brassica juncea*, or *Avena satvia* plants as a C-source (Mowlick *et al.* 2013, 2014).

Another contributing factor to ASD-mediated pathogen suppression is the production of volatile organic compounds (VOCs) such as isothiocyanates, alcohols, organic acids, organic sulfides, and esters (Hewavitharana *et al.* 2014), all of which were reported to contribute to the suppression of *Pythium ultimum*, *Fusarium oxysporum* and *Rhizoctonia solani* AG-5 in greenhouse trials (Hewavitharana *et al.* 2014). The production of acetic, propionic, and butyric acid in ASD treatments using a commercial organic amendment also were correlated with suppression of potato cyst nematode *Globodera pallida* (Runia *et al.* 2014). While similar compounds were generated in ASD trials using different C-sources, the proportions of these compounds differed as

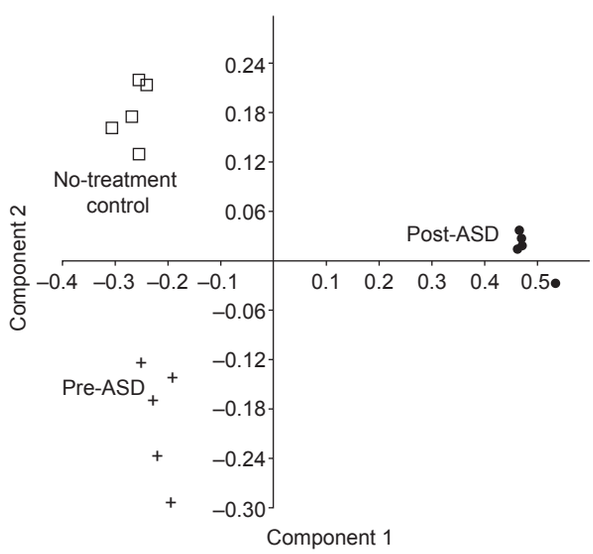

Fig. 1 PCA (variance-covariance) analysis of bacterial community composition data based on unweighted distance measurements (Unifrac) between samples of soils pre-ASD (+), post-ASD (•), and a no-treatment control (□). Post-ASD and no-treatment control samples were collected immediately following 7 weeks of ASD. No-treatment control plots were adjacent to ASD plots. ASD began in August 2013 at the University of California Kearney Agricultural Research and Extension Center in Parlier, CA. Soil was classified as Hanford sandy loam. Each treatment had *n*=5.

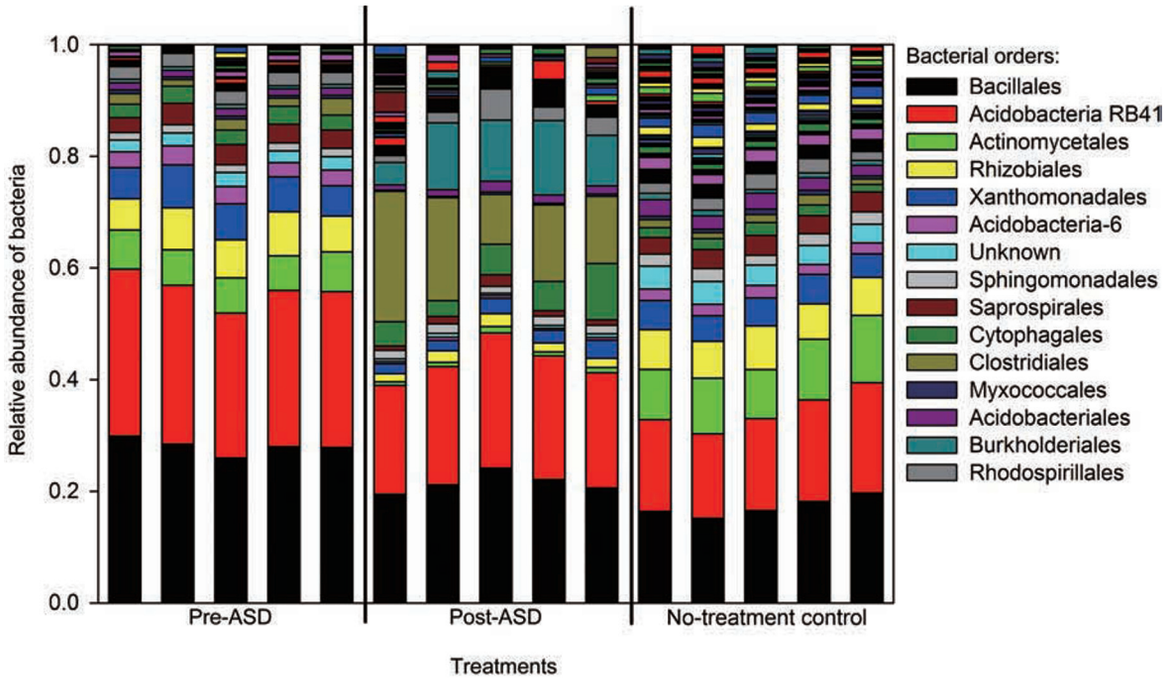

Fig. 2 Percent relative abundance of bacterial 16S rRNA gene taxonomic orders identified using the Ribosomal Database Project (RDP) classifier program (Wang 2007) within Quantitative Insights into Microbial Ecology (QIIME). DNA sequence data were derived from 16S rRNA genes amplified from the following soil treatments: pre-ASD, post-ASD, and a no-treatment control. ASD began in August 2013 at the University of California Kearney Agricultural Research and Extension Center in Parlier, CA, USA. Soil was classified as Hanford sandy loam. Each treatment had *n*=5.

a function of C-source (Hewavitharana *et al.* 2014), which in turn, may impact pathogen control. Carbon-sources typically used in ASD are discussed below. The production of other VOCs, particularly organic acids (e.g., acetic acid), also may have indirect effects on disease suppression *via* changes in soil pH and increases in manganese (Mn^{2+}) and iron (Fe^{2+}) ions (Momma *et al.* 2011). In ASD pot experiments with wheat bran, the decrease in soil pH was correlated with the accumulation of acetic and butyric acids (Momma *et al.* 2006; Momma 2008). These organic acids significantly reduced populations of *Ralstonia solanacearum* (Momma *et al.* 2006) and *Meloidogyne incognita* (Katase *et al.* 2009) when added to inoculated soils. Interestingly, *F. oxysporum* survival was significantly reduced when the pathogen was incubated in aqueous metal ion solutions (Momma *et al.* 2011).

The suppression of soilborne pathogens under anaerobic conditions generated during flooding is a well-known phenomenon (Stover 1955; Menzies 1963; Pullman *et al.* 1981; Ebihara and Uematsu 2010). Rotating paddy rice with cotton (Pullman *et al.* 1981), eggplant (Ebihara *et al.* 2010), or strawberries (Ebihara *et al.* 2010) significantly reduced *Verticillium dahliae* populations, with greater reduction occurring after multiple years of rice cultivation. Flooding soils without the cultivation of rice also decreased populations of *F. oxysporum* (Stover 1955). In all of these studies, anaerobic conditions were implicated as the primary mechanism responsible for pathogen suppression. Indeed, when exposed to anaerobic conditions, *V. dahliae* failed to grow, while *Phytophthora cactorum* and *F. oxysporum* survived, but exhibited significantly less growth when cultured anaerobically (Ebihara *et al.* 2014). However, flooding must occur for at least 17 weeks (Pullman *et al.* 1981) and incubation under anaerobic conditions for at least 90 days is required (Ebihara *et al.* 2014) for pathogen suppression. As these are significantly longer than the standard ASD treatment of 4–8 weeks, it is again likely that ASD pathogen suppression occurs through a combination of mechanisms rather than anaerobicity alone.

3. Carbon sources

Solid organic materials used in ASD, including rice bran, composted broiler litter, green manure, and cover crops, are often tilled into the soil prior to irrigation and covering with an impermeable tarp. Liquid amendments, such as molasses or ethanol, are sometimes introduced through existing irrigation lines under the plastic tarps. While all of the C-sources discussed here have been used for effective disease suppression, they have not all been tested against

the same pathogens or in the same soil conditions. Carbon sources currently reported in ASD treatments have C:N that range from 11 to 57.9 (Table 1). However, the C:N may be of less importance in the generation of anaerobic conditions than the type of carbon supplied (Blok *et al.* 2000; Butler *et al.* 2012b). In addition to C-type, the rate of application, and resulting soil conditions such as pH (Momma *et al.* 2006; Katase *et al.* 2009; Butler *et al.* 2012b) and temperature under the plastic tarp, may determine ASD effectiveness. For example, ASD trials using grass residues or ethanol exhibited similar levels of reduction in infection and infestation by *Pythium* spp. and *Pratylenchus penetrans* and produced similar levels of VOC (Hewavitharana *et al.* 2014). These are important observations, and imply growers may be able to use locally available C-sources (Butler *et al.* 2012b), which will likely reduce the cost of ASD without impacting its performance.

Table 1 Comparison of different anaerobic soil disinfestation (ASD) treatment components used in both field and pot studies

Carbon source	C:N	Application rate (t DW ha^{-1})	Pathogens suppressed	Weed control	Mean soil temperature (°C)	Treatment time	Crop	Location	Reference
Wheat bran	NR	10	*Fusarium oxysporum* MTC 6	NA	32.4	14 d	NA	Japan	Momma *et al*. (2010)
	NR	10	*F. oxysporum*	NA	46	20 d	Carnation	Argentina	Yossen *et al*. (2008)
	NR	2	*Meloidogyne incognita*	NA	35	24 d	Tomato	Japan	Katase *et al*. (2009)[2)]
Rice bran (RB)	NR	20.2	*V. dahliae*	Yes	16.6-21.1	3 wk	Strawberry	CA, USA	Shennan *et al*. (2010)
	NR	20.2	*V. dahliae*	Yes	16.6-21.1	6 wk	Strawberry	CA, USA	Shennan *et al*. (2010)
	NR	10.8	*V. dahliae*	Yes	21.1-26.6	4 wk	Strawberry	CA, USA	Shennan *et al*. (2010)
	19	11.1	*Pratylenchus penetrans*, *Rhizoctonia solani* AG-5, *Pythium ultimum*, *F. oxysporum*	NA	NR	2 wk	Apple	WA, USA	Hewavitharana *et al*. (2014)[2)]
	NR	15.7	*Agrobacterium tumefaciens*, *Pythium* spp.	Dicots	32	7 wk	Walnut	CA, USA	Strauss *et al*. (2015)
	NR	20.2	*A. tumefaciens*, *Pythium* spp.	Dicots	32	7 wk	Walnut	CA, USA	Strauss *et al*. (2015)
RB+*S. alba* seed meal (SM)	NR	17.9 RB+ 2.2 SM	*V. dahliae*	Yes	30	3 wk	Strawberry	CA, USA	Shennan *et al*. (2010)
RB+SM	NR	17.9 RB+ 2.2 SM	*V. dahliae*	Yes	30	6 wk	Strawberry	CA, USA	Shennan *et al*. (2010)
B. juncea cv. Pacific Gold SM	16	4.9	*Pythium* spp., *P. penetrans*	NA	NR	2 wk	Apple	WA, USA	Hewavitharana *et al*. (2014)[2)]
Orchard grass (*Dactylis glomerata* L.)	19	20–40	*Pythium* spp., *P. penetrans*	NA	NR	2 wk	Apple	WA, USA	Hewavitharana *et al*. (2014)[2)]
Composted steer manure	11	11.1	*Pythium* spp., *P. penetrans*	NA	NR	2 wk	Apple	WA, USA	Hewavitharana *et al*. (2014)[2)]
Ryegrass	NR	8	*F. oxysporum*, *V. dahliae*, *R. solani*	NA	NR	15 wk	NA	Netherlands	Blok *et al*. (2000)
	NR	4.6	*V. dahliae*, *Pratylenchus* spp., *Trichodorus* spp.	NA	NR	10–13 wk	Maple and catalpa	Netherlands	Goud *et al*. (2004)
	NR	50	*P. penetrans*, *V. dahliae*	NA	NR	12 wk	Potato	Netherlands	Korthals *et al*. (2014)
Cowpea	16.8	0.08	*M. incognita*, *F. oxysporum*, *Sclerotium rolfsii*	NA	25	3 wk	Tomato	FL, USA	Butler *et al*. (2012b)[2)]

(Continued on next page)

Table 1 (Continued from preceding page)

Carbon source	C:N	Application rate (t DW ha^{-1})	Pathogens suppressed	Weed control	Mean soil temperature (°C)	Treatment time	Crop	Location	Reference
Sunn hemp	19.1	0.03	*M. incognita*, *F. oxysporum*, *S. rolfsii*	No	25	3 wk	Tomato	FL, USA	Butler *et al*. (2012b)[2]
Pearl millet	35.1	0.03	*M. incognita*, *F. oxysporum*, *S. rolfsii*	No	25	3 wk	Tomato	FL, USA	Butler *et al*. (2012b)[2]
Sorghum-sudangrass	57.9	0.03	*M. incognita*, *F. oxysporum*, *S. rolfsii*	No	25	3 wk	Tomato	FL, USA	Butler *et al*. (2012b)[2]
Broccoli	NR	5	*F. oxysporum*, *V. dahliae*, *R. solani*	NA	39	15 wk	NA	Netherlands	Blok *et al*. (2000)
Potato	NR	30	*R. solanacearum*	NA	NR	6 wk	NA	Netherlands	Messiha *et al*. (2007)[3]
Avena sativa	NR	4.4	*F. oxysporum*	NA	35.5	3 wk	Spinach	Japan	Mowlick *et al*. (2013)
B. juncea var. *cernua*	NR	11	*F. oxysporum*	NA	35.5	3 wk	Spinach	Japan	Mowlick *et al*. (2013)
B. juncea var. *cernua*+ *B. juncea* var. *crispifolia*	NR	96	*F. oxysporum*	NA	33.1	3 wk	Spinach	Japan	Mowlick *et al*. (2014)
Radish	NR	200	*F. oxysporum*	NA	33.1	3 wk	Spinach	Japan	Mowlick *et al*. (2014)
Herbie 22[1]	NR	NR	*Globodera pallida*	NA	NR	4 wk	NA	Netherlands	Runia *et al*. (2014)[2]
		NR	*R. solanaceraum*, *G. pallida*	NA	15.6–16.5	21 wk	Tomato	Netherlands	van Overbeek *et al*. (2014)
Dried molasses	35.9	NR	*R. solani*	Monocots	20.8	4 wk	Tomato, red bell pepper	TN, USA	McCarty *et al*. (2014)[4]
Cereal rye	18.5	0.134	*R. solani*	Monocots	20.8	4 wk	Tomato, red bell pepper	TN, USA	McCarty *et al*. (2014)[4]
Mustard +arugula +dried molasses	11.2	0.004 mustard+ 0.004 arugula+NR molasses	*R. solani*	Monocots	20.8	4 wk	Tomato, red bell pepper	TN, USA	McCarty *et al*. (2014)[4]
Mustards+ arugula	9.3	0.004 mustard+ 0.004 arugula	*R. solani*	Monocots	20.8	4 wk	Tomato, red bell pepper	TN, USA	McCarty *et al*. (2014)[4]
Cereal rye+dried molasses	12.6	0.134 rye+NR molasses	*R. solani*	Monocots	20.8	4 wk	Tomato, red bell pepper	TN, USA	McCarty *et al*. (2014)[4]
Molasses	17.5	8.2	*P. capsici*, *F. oxysporum*, *M. incognita*	Yellow nutsedge	40	3 wk	Eggplant, bell pepper	FL, USA	Butler *et al*. (2012a)
	NR	1% v/v	*F. oxysporum* MTC 6	NA	33	2 wk	NA	Japan	Momma *et al*. (2010)
Ethanol	NR	2% v/v	*F. oxysporum*	NA	30	12 d	NA	Japan	Momma *et al*. (2008)
	NR	10% v/v	*Pythium* spp., *P. penetrans*	NA	NR	2 wk	Apple	WA, USA	Hewavitharana *et al*. (2014)[2]

[1] Commercial organic matter mixture developed by Thatchtec (www.thatchtec.com), see Runia *et al*. (2014).
[2] Greenhouse/Pot experiment.
[3] Both pot and greenhouse experiments performed in study, but only field experiment results reported in Table 1.
[4] Two experiments performed, only first year reported in Table 1.
Only papers reporting positive pathogen suppression are represented in Table 1. NR, not reported; NA, not applicable.

4. Solid organic materials

ASD using rice and wheat brans are being used commercially in California strawberry (Shennan *et al.* 2014) and Japanese tomato and melon crops (Momma *et al.* 2013), respectively. In field trials, both wheat and rice bran applied at a rate of 10 t ha^{-1} were successful at suppressing *F. oxysporum* (Momma *et al.* 2010) and *V. dahliae* populations (Shennan *et al.* 2009), respectively. In our preliminary field trial examining ASD for nut-tree nurseries, rice bran application at both 15.7 and 20.2 t ha^{-1} significantly suppressed *A. tumefaciens* (Fig. 3-A) and *Pythium* spp. (Fig. 3-B). While anaerobic conditions using 15.7 t ha^{-1} rice bran were lost earlier than with 20.2 t ha^{-1} (Fig. 4), pathogen populations were still significantly suppressed at the lower carbon application rate. These data suggest, depending on soil type and environmental conditions, it may be possible to use C-sources at rates lower than 20.2 t ha^{-1} to generate lethal conditions during ASD.

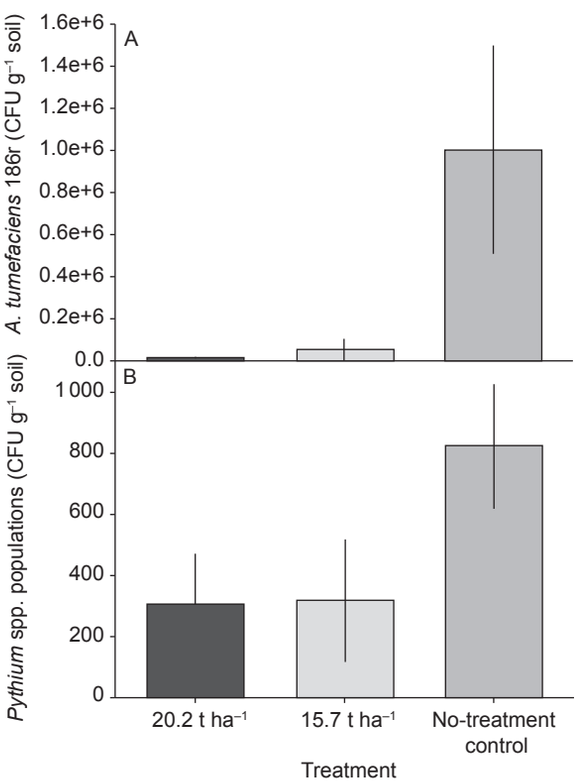

Fig. 3 Abundance of *A. tumefaciens* 186r (A) and *Pythium* spp. (B) at a depth of 46 cm after 7 wk of ASD with the following treatments: 20.2 t ha^{-1} rice bran, 15.7 t ha^{-1} rice bran, and a no-treatment control. ASD began in August 2013 at the University of California Kearney Agricultural Research and Extension Center in Parlier, CA, USA. Soil was classified as Hanford sandy loam. Values are means±95% confidence intervals (n=5). Means without overlapping intervals were considered significantly different (P<0.05).

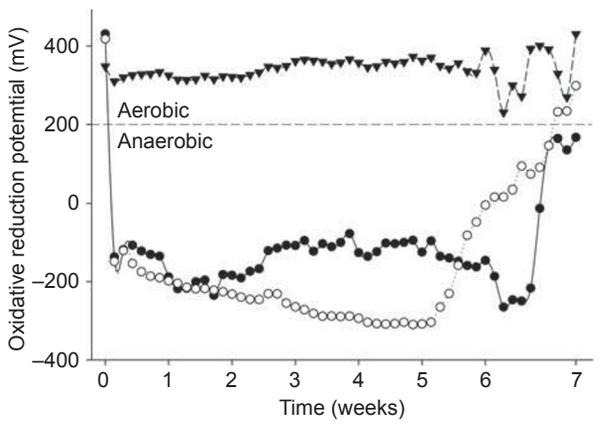

Fig. 4 Oxidative reduction potential (mV) during 7 weeks of ASD treatment with 20.2 t ha^{-1} rice bran (●), 15.7 t ha^{-1} rice bran (○), and a no-treatment control (▼). Oxidative reduction potential sensors were buried at 46 cm. ASD began in August 2013 at the University of California Kearney Agricultural Research and Extension Center in Parlier, CA, USA. Soil was classified as Hanford sandy loam. Values are the mean of n=4 for each treatment.

Brassicaceae seed meal applications have successfully controlled several soilborne pathogens, including *Rhizoctonia* spp. and *Pratylenchus* spp. (Mazzola 2007). The VOCs generated by these seed meals appear to contribute to pathogen suppression, but the use of seed meals as a C-source for ASD produced mixed results. ASD treatments using *Brassica juncea* as the C-source exhibited similar suppression of *Pythium* spp. and *P. penetrans* as observed with a non-*Brassica* cover crop and ethanol C-sources. However, seedling survival decreased likely due to phytotoxicity (Hewavitharana *et al.* 2014). Interestingly, *Sinapis alba* seed meal combined with 17.9 t ha^{-1} of rice bran provided a high level of suppression of *Verticillium* sp. which resulted in increased strawberry yields (Shennan *et al.* 2009).

Cover crops have been used as the C-source in ASD trials in the Netherlands (Blok *et al.* 2000), Florida (Butler *et al.* 2012b, 2014), and Tennessee (McCarty *et al.* 2014). These crops include 4-week-old broccoli (Blok *et al.* 2000), cowpea (Butler *et al.* 2012b), sunn hemp (Butler *et al.* 2012b), pearl millet (Butler *et al.* 2012b), sorghum-sudangrass (Butler *et al.* 2012b), mustard and arugula (McCarty *et al.* 2014), fresh grass clippings (van Overbeek *et al.* 2014), ryegrass (Goud *et al.* 2004; Korthals *et al.* 2014), and apple orchard grass residues (*Dactylis glomerata* L., Hewavitharana *et al.* 2014). Application rates vary based on the seeding rates for each crop. Even though similar anaerobic levels (i.e., similar cumulative Eh levels; Butler *et al.* 2012b) were achieved using these cover crops, C-source composition appeared to affect overall ASD efficacy, as pearl millet was less effective than molasses at suppressing *F. oxysporum*

and *M. inocognita* (Butler *et al.* 2012b). VOCs produced by decomposing cruciferous tissues have been attributed to suppression of *V. dahliae* and Verticillium wilt (Subbarao *et al.* 1999). However, Blok *et al.* (2000) reported that both broccoli and ryegrass applied at similar fresh weights suppressed *F. oxysporum* f. sp. *asparagi*, *R. solani*, and *V. dahliae* populations to equivalent levels. McCarty *et al.* (2014) also reported similar suppression of *R. solani* with ASD treatments using mustard/arugula or cereal rye.

5. Liquid C-source amendments

In ASD trials, molasses has been applied through both irrigation lines (Muramoto *et al.* 2014) and as a dilute spray on the soil surface (Butler *et al.* 2012b). In these trials, molasses was applied at the start of ASD treatment and either sprayed onto the soil surface (8.2 t ha^{-1}) and covered with a plastic tarp for 3 weeks (Butler *et al.* 2014), or pumped through irrigation lines (10–20 t ha^{-1}) once or twice after the soil was covered (Muramoto *et al.* 2014). ASD using molasses significantly reduced populations of *F. oxysporum*, root knot nematodes, and galling in eggplant (Butler *et al.* 2012a).

While anaerobic conditions were maintained throughout the 3-week ASD treatment in Florida trials using molasses as the sole C-source (Butler *et al.* 2012a, 2014), the molasses-generated anaerobicity was only sustained for 2–3 days in California strawberry trials (Muramoto *et al.* 2014). In preliminary ASD trials in the California Central Valley (Browne, personal communication), molasses applied through the irrigation lines only slightly enhanced anaerobic conditions, measured at a depth of 30 cm, for less than 12 hours in plots which had rice bran previously incorporated. These differences may be attributed to the different application methods, as application through irrigation lines may not provide an even distribution of the C-source compared to spraying (Muramoto *et al.* 2014). In addition, soil type between these studies varied, with the Florida (Butler *et al.* 2012a) and California Central Valley (Browne, personal communication) studies conducted in sandy soils while the California strawberry trials were performed in coastal soils with a higher clay component (Shennan *et al.* 2010). However, as both the Florida and California Central Valley studies were conducted in sandy soils, it seems unlikely that soil type alone contributes to the differences in molasses effectiveness. Differences in the metabolic capability of the indigenous microbial communities at these different sites also may play a role in the ability of the community to generate sustained anaerobic conditions during ASD. Additional research examining the interaction between soil type, C-source, molasses type, and incorporation method is needed.

Ethanol is commonly used as a C-source for ASD in Japan, where it is diluted and applied through irrigation lines at a rate of 100 L m^{-2} at a 1–2% concentration (Momma *et al.* 2013). ASD utilizing ethanol in both open fields and plastic houses suppressed *M. incognita*, *F. oxysporum*, and *R. solanacearum* (Momma *et al.* 2013). In pot experiments, ASD using ethanol suppressed *P. penetrans*, *P. ultimum*, *F. oxysporum*, and *R. solani* AG-5 (Hewavitharana *et al.* 2014). Due to the potential changes in soil nutrient concentrations through the addition of most other ASD C-sources, ethanol's lack of nitrogen (N) and ease of application makes it an appealing C-source for use in ASD (Momma *et al.* 2010).

6. Combinations of dry and liquid amendments

A key concern after ASD treatment is the effect on soil nutrient availability prior to planting. In ASD treatments using molasses, soil NH_4-N and NO_3-N was similar to untreated or chemically fumigated soils (Butler *et al.* 2014). Despite no significant difference in available soil inorganic N between no-treatment controls and ASD-treated soils, bell peppers planted in the ASD-treated soils exhibited leaf N deficiency (Butler *et al.* 2014). Using composted poultry litter (Butler *et al.* 2012b, 2014) and molasses together in the ASD treatment significantly increased soil inorganic N concentrations. However by mid-season with bell peppers and eggplants, there was no difference in soil inorganic N (Butler *et al.* 2014). Applying a combination of a dry and liquid C-source, such as rice bran and molasses, may provide both a lower N level and decreased cost for ASD treatment. However, compared to no-treatment controls, *F. oxysporum* populations increased in California ASD trials where molasses and rice bran were combined and used as the C-source (Shennan *et al.* 2014).

Compost addition post-ASD treatment could also assist in pathogen suppression, as compost-only amendments have suppressed pathogens such as *Pythium* spp. (Darby *et al.* 2006), *A. tumefaciens* (Strauss *et al.* 2015), and *V. dahliae* (Termorshuizen *et al.* 2006), and compost combined with solarization increased pathogen suppression (Gamliel *et al.* 2000). However, Butler *et al.* (2012a, 2014) found that root galling by *Meloidogyne* spp. was similar in ASD molasses treatments regardless of the addition of compost, and improvements in eggplant yields in compost amended plots were likely due to improved soil nutrient conditions and increased water holding capacity of the sandy soil. Additional work is needed to clearly define the efficacy of introducing organic amendments to ASD treated soil to enhance suppression of microbial pathogens.

7. Plastic tarps

The use of clear (Butler *et al.* 2012a; Strauss *et al*. unpublished) or opaque (black: Blok *et al.* 2000; or green: Shennan *et al.* 2010; McCarty *et al.* 2014) plastic tarps for ASD depends on both the climate and crop. In the Netherlands, black plastic was used to ensure high soil temperatures (Blok *et al.* 2000). Opaque plastic has been the standard for raised strawberry beds especially in organic systems to control weeds, and in some locations, there was no difference in marketable yield in ASD trials conducted using either opaque or clear plastic (Shennan *et al*. 2010). However, clear plastic facilitates solarization which elevates soil temperatures up to 10°C greater than under opaque plastic (Shennan *et al.* 2010). For example, *V*. *dahliae* populations were significantly lower under clear plastic compared to opaque plastic at one strawberry test site (Shennan *et al.* 2010). Covering clear plastic with opaque plastic at the end of ASD under clear plastic, as performed in Florida trials (Butler *et al.* 2012b, 2014), facilitated greater soil temperatures, which enhanced disease and weed suppression under clear plastic. The cooler soil temperatures under opaque plastic are needed for crop production when a grower will be planting through the plastic tarp in warm regions such as Florida.

8. Contribution of solarization to ASD

In general, ASD is effective when temperatures under the tarp reach 25–30°C for at least 10 days (Katase *et al.* 2009). The disease suppression effects of solarization are well documented (Katan 1981; Gamliel *et al.* 2000), but solarization with an added C-source (i.e., ASD) resulted in significantly lower rates of root galling by *M*. *incognita* than solarization alone (Butler *et al.* 2014). However, a solarization component is not required for ASD-mediated pathogen suppression, as *R*. *solanacearum* and *G*. *pallida* were suppressed in ASD treatments that only reached a maximum of 16.4°C under the tarp (Blok *et al.* 2000; van Overbeek *et al.* 2014). In Japan, ASD is often performed in plastic houses where soil temperatures only reach 25–30°C (Momma *et al.* 2006, 2013), yet *M*. *incognita* and *R*. *solanacearum* populations are significantly reduced. In open field trials in Japan, ASD was only required for 14 days to suppress *F*. *oxysporum* when temperatures were greater than 30°C, but required greater time with lower temperatures (Yonemoto *et al.* 2006). The addition of a solarization component to ASD is generally dictated by environment and timing. ASD is limited to the summer months in the Netherlands, where soil temperatures only typically reach 17–20°C, while in California and Florida, soil temperatures are at least 17°C for 10 months of the year.

There has not been a comprehensive examination of the minimum ASD treatment time necessary for pathogen suppression, though this is likely to be pathogen and site dependent (Table 1). In our preliminary field trials, we found that populations of *A*. *tumefaciens* were reduced below detection limits within one week of the start of ASD treatment (Strauss *et al.* unpublished). However, ASD treatments tend to last 3–6 weeks (Table 1).

9. Future research directions

Now that ASD mediated suppression has been demonstrated for a variety of soilborne plant pathogens and weeds on a variety of hosts, cost and accessibility of required ASD components must be addressed. Trials have attempted to use local, readily available resources for C-sources, such as molasses, a readily available by-product of sugar production (Butler *et al.* 2012a). However, some resources that were initially readily available are now seeing increased demand that most likely will increase cost. Other agricultural by-products, such as almond hulls or grape pomace (Zavatta *et al.* 2014), may be effective, economical, and readily available alternative C-sources for ASD applications. We are currently testing a wide variety of these potential C-sources for use in ASD.

The depth of ASD treatment penetration also must be examined. Dry amendments are generally roto-tilled into the soil to a depth of approximately 15–20 cm. While this depth is sufficient to suppress many bacterial and fungal pathogens (Agrios 2005), it may be insufficient to suppress plant parasitic nematode populations below commercially viable levels. ASD suppressed root-knot nematodes in raised vegetable beds (Butler *et al.* 2012a), but were only assessed at a depth of 15 cm. This depth of phytoparasitic nematode control will not be sufficient for Central California tree-crop nurseries where it has been shown nematodes can migrate into the crop root zone from depths greater than 1.5 m (McKenry 2002). In our preliminary ASD field trials, we have found consistent pathogen suppression down to 46 cm and more limited suppression down to 76 cm (Strauss *et al.* unpublished). Additional measures such as deeper incorporation of dry C-sources and combining dry C-sources with delivery of soluble C-sources through buried irrigation lines may allow for deeper penetration of labile C, and generation of anaerobicity at greater depths.

ASD is clearly showing promise in a variety of agricultural systems around the world. However, for ASD to be widely adopted in the United States it is important that application costs per treated area be equal to, or less than, the costs for traditional chemical fumigation. To accomplish this goal, more work is needed to clearly define the mechanisms of ASD that will facilitate development of the most cost effective

and commercially viable approach.

Acknowledgements

This work was supported by the California Department of Food and Agriculture Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board, USA.

References

Agrios G N. 2005. *Plant Pathology*. Elsevier Academic Press, San Diego, CA.

Blok W J, Lamers J G, Termorshuizen A J, Bollen G J. 2000. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. *Phytopathology*, **90**, 253–259.

Browne G T, Lampinen B D, Holtz B A, Doll D A, Upadhyaya S K, Schmidt L S, Bhat R G, Udompetaikul V, Coates R W, Hanson B D, Klonsky K M, Gao S, Wang D, Gillis M, Gerik J S, Johnson R S. 2013. Managing the almond and stone fruit replant disease complex with less soil fumigant. *California Agriculture*, **67**, 128–138.

Butler D M, Kokalis-Burelle N, Albano J P, McCollum T G, Muramoto J, Shennan C, Rosskopf E N. 2014. Anaerobic soil disinfestation (ASD) combined with soil solarization as a methyl bromide alternative: Vegetable crop performance and soil nutrient dynamics. *Plant and Soil*, **378**, 365–381.

Butler D M, Kokalis-Burelle N, Muramoto J, Shennan C, McCollum T G, Rosskopf E N. 2012a. Impact of anaerobic soil disinfestation combined with soil solarization on plant-parasitic nematodes and introduced inoculum of soilborne plant pathogens in raised-bed vegetable production. *Crop Protection*, **39**, 33–40.

Butler D M, Rosskopf E N, Kokalis-Burelle N, Albano J P, Muramoto J, Shennan C. 2012b. Exploring warm-season cover crops as carbon sources for anaerobic soil disinfestation (ASD). *Plant and Soil*, **355**, 149–165.

Carpenter J, Lynch L, Trout T. 2001. Township limits on 1,3-D will impact adjustment to methyl bromide phase-out. *California Agriculture*, **55**, 12–18.

Darby H M, Stone A G, Dick R P. 2006. Compost and manure mediated impacts on soilborne pathogens and soil quality. *Soil Science Society of America Journal*, **70**, 347–358.

Ebihara Y, Uematsu S. 2014. Survival of strawberry-pathogenic fungi *Fusarium oxysporum* f. sp. *fragariae*, *Phytophthora cactorum* and *Verticillium dahliae* under anaerobic conditions. *Journal of General Plant Pathology*, **80**, 50–58.

Ebihara Y, Uematsu S, Nomiya S. 2010. Control of *Verticillium dahliae* at a strawberry nursery by paddy-upland rotation. *Journal of General Plant Pathology*, **76**, 7–20.

Fennimore S A, Serohijos R, Samtani J B, Ajwa H A, Subbarao K V, Martin F N, Daugovish O, Legard D, Browne G T, Muramoto J, Shennan C, Klonsky K. 2013. TIF film, substrates and nonfumigant soil disinfestation maintain fruit yields. *California Agriculture*, **67**, 139–146.

Gamliel A, Austerweil M, Kritzman G. 2000. Non-chemical approach to soilborne pest management — organic amendments. *Crop Protection*, **19**, 847–853.

Gao S, Hanson B D, Wang D, Browne G T, Qin R J, Ajwa H, Yates S R. 2011. Methods evaluated to minimize emissions from preplant soil fumigation. *California Agriculture*, **65**, 41–46.

Goud J K C, Termorshuizen A J, Blok W J, van Bruggen A H C. 2004. Long-term effect of biological soil disinfestation on Verticillium wilt. *Plant Disease*, **88**, 688–694.

Hanson B D, Gao S, Gerik J, Qin R, Cabrera J A, Jhala A J, Abit M J M, Cox D, Correiar B, Wang D, Browne G T. 2013. Preplant 1,3-D treatments test well for perennial crop nurseries, but challenges remain. *California Agriculture*, **67**, 181–189.

Hewavitharana S S, Ruddell D, Mazzola M. 2014. Carbon source-dependent antifungal and nematicidal volatiles derived during anaerobic soil disinfestation. *European Journal of Plant Pathology*, **140**, 39–52.

Katan J. 1981. Solar heating (solarization) of soil for control of soilborne pests. *Annual Review of Phytopathology*, **19**, 211–236.

Katase M, Kubo C, Ushio S, Ootsuka E, Takeuchi T, Mizukubo T. 2009. Nematicidal activity of volatile fatty acids generated from wheat bran in reductive soil disinfestation. *Japanese Journal of Nematology*, **39**, 53–62.

Korthals G W, Thoden T C, van den Berg W, Visser J H M. 2014. Long-term effects of eight soil health treatments to control plant-parasitic nematodes and *Verticillium dahliae* in agro-ecosystems. *Applied Soil Ecology*, **76**, 112–123.

Locascio S J, Gilreath J P, Dickson D W, Kucharek T A, Jones J P, Noling J W. 1997. Fumigant alternatives to methyl bromide for polyethylene-mulched tomato. *Hortscience*, **32**, 1208–1211.

Martin F N, Loper J E. 1999. Soilborne plant diseases caused by *Pythium* spp: Ecology, epidemiology, and prospects for biological control. *Critical Reviews in Plant Sciences*, **18**, 111–181.

Mazzola M. 2007. Manipulation of rhizosphere bacterial communities to induce suppressive soils. *Journal of Nematology*, **39**, 213–220.

McCarty D G, Inwood S E E, Ownley B H, Sams C E, Wszelaki A L, Butler D M. 2014. Field evaluation of carbon sources for anaerobic soil disinfestation in tomato and bell pepper production in tennessee. *Hortscience*, **49**, 272–280.

McKenry M. 2002. *Field Evaluations/Input for Grower Replant Settings and New Lines of Nematode Resistance. Walnut Research Reports*. pp. 509–516.

Menzies J D. 1963. Survival of microbial plant pathogens in soil. *Botanical Review*, **29**, 79–122.

Messiha N A S, van Diepeningen A D, Wenneker M, van Beuningen A R, Janse J D, Coenen T G C, Termorshuizen A J, van Bruggen A H C, Blok W J. 2007. Biological Soil Disinfestation (BSD), a new control method for potato brown rot, caused by *Ralstonia solanacearum* race 3 biovar 2. *European Journal of Plant Pathology*, **117**, 403–415.

Momma N. 2008. Biological soil disinfestation (BSD) of soilborne pathogens and its possible mechanisms. *Japan Agricultural Research Quarterly*, **42**, 7–12.

Momma N, Kobara Y, Momma M. 2011. Fe^{2+} and Mn^{2+}, potential agents to induce suppression of *Fusarium oxysporum* for biological soil disinfestation. *Journal of General Plant Pathology*, **77**, 331–335.

Momma N, Kobara Y, Uematsu S, Kita N, Shinmura A. 2013. Development of biological soil disinfestations in Japan. *Applied Microbiology and Biotechnology*, **97**, 3801–3809.

Momma N, Momma M, Kobara Y. 2010. Biological soil disinfestation using ethanol: Effect on *Fusarium oxysporum* f. sp. *lycopersici* and soil microorganisms. *Journal of General Plant Pathology*, **76**, 336–344.

Momma N, Yamamoto K, Simandi P, Shishido M. 2006. Role of organic acids in the mechanisms of biological soil disinfestation (BSD). *Journal of General Plant Pathology*, **72**, 247–252.

Mowlick S, Inoue T, Takehara T, Tonouchi A, Kaku N, Ueki K, Ueki A. 2014. Usefulness of Japanese-radish residue in biological soil disinfestation to suppress spinach wilt disease accompanying with proliferation of soil bacteria in the *Firmicutes*. *Crop Protection*, **61**, 64–73.

Mowlick S, Yasukawa H, Inoue T, Takehara T, Kaku N, Ueki K, Ueki A. 2013. Suppression of spinach wilt disease by biological soil disinfestation incorporated with *Brassica juncea* plants in association with changes in soil bacterial communities. *Crop Protection*, **54**, 185–193.

Muramoto J, Shennan C, Baird G, Zavatta M, Koike S T, Bolda M P, Daugovish O, Dara S K, Klonsky K, Mazzola M, Pugliese M. 2014. Optimizing anaerobic soil disinfestation for california strawberries. *Acta Horticulturae*, **1044**, 215-220.

van Overbeek L, Runia W, Kastelein P, Molendijk L. 2014. Anaerobic disinfestation of tare soils contaminated with *Ralstonia solanacearum* biovar 2 and *Globodera pallida*. *European Journal of Plant Pathology*, **138**, 323–330.

Pullman G S, Devay J E, 1981. Effect of soil flooding and paddy rice culture on the survival of *Verticillium dahliae* and incidence of Verticillium wilt in cotton. *Phytopathology*, **71**, 1285–1289.

Ramos D E. 1998. *Walnut Production Mannual Publication 3373*. University of California Division of Agriculture and Natural Resources, Oakland, CA.

Runia W T, Thoden T C, Molendijk L P G, van den Berg W, Termorshuizen A J, Streminska M A, van der Wurff A W G, Feil H, Meints H, Pugliese M. 2014. Unravelling the mechanism of pathogen inactivation during anaerobic soil disinfestation. *Acta Horticulturae*, **1044**, 177–193.

Shennan C, Muramoto J, Lamers J, Mazzola M, Rosskopf E N, Kokalis-Burelle N, Momma N, Butler D M, Kobara Y, Pugliese M. 2014. Anaerobic soil disinfestation for soil borne disease control in strawberry and vegetable systems: Current knowledge and future directions. *Acta Horticulturae*, **1044**, 165–175.

Shennan C, Muramoto J, Koike S T, Daugovish O. 2009. Optimizing anaerobic soil disinfestation for non-fumigated strawberry prodution in California. In: *Annual International Research Conference on Methyl Bormide Alternatives and Emissions Reductions*. Methyl Bromide Alternatives Outreach, San Diego, CA.

Shennan C, Muramoto J, Koike S T, Daugovish O. 2010. *Optimizing Anaerobic Soil Disinfestation for Non-Fumigated Strawberry Production in California. California Strawberr Commission Annual Production Research Report 2010–2011*. pp. 113–121.

Stover R H. 1955. Flood-fallowing for eradication of *Fusarium oxysporum* F. *cubense*: III. Effect of oxygen on fungus survival. *Soil Science*, **80**, 397–412.

Strauss S L, Stover J K, Kluepfel D A. 2015. Impact of biological amendments on *Agrobacterium tumefaciens* survival in soil. *Applied Soil Ecology*, **87**, 39–48.

Subbarao K V, Hubbard J C, Koike S T, 1999. Evaluation of broccoli residue incorporation into field soil for Verticillium wilt control in cauliflower. *Plant Disease*, **83**, 124–129.

Termorshuizen A J, van Rijn E, van der Gaag D J, Alabouvette C, Chen Y, Lagerlof J, Malandrakis A A, Paplomatas E J, Ramert B, Ryckeboer J, Steinberg C, Zmora-Nahum S. 2006. Suppressiveness of 18 composts against 7 pathosystems: Variability in pathogen response. *Soil Biology & Biochemistry*, **38**, 2461–2477.

Wang Q, Garrity G M, Tiedje J M, Cole J R. 2007. Native bayesian classifer for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, **73**, 5261–5267.

Yakabe L E, Parker S R, Kluepfel D A. 2014. Incidence of *Agrobacterium tumefaciens* biovar 1 in and on 'Paradox' (*Juglans hindsii*×*Juglans regia*) walnut seeds collected from commercial nurseries. *Plant Disease*, **98**, 766–770.

Yonemoto K, Hirota K, Mizuguchi S, Sakaguchi K. 2006. Utilization of the sterilization by soil reduction in an open air field and its efficacy against Fusarium wilt of strawberry. In: *Shikoku Plant Defense*, Shikoku, **41**, 15–24. (in Japanese)

Zavatta M, Shennan C, Muramoto J, Mazzola M. 2014. Evaluating C-sources for anaerobic soil disinfestation. In: *Annual International Conference on Methyl Bromide Alternatives and Emissions Reductions*. Methyl Bromide Alternatives Outreach, Orlando, CA, USA.

(Managing editor WENG Ling-yun)