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Anaerobic soil disinfestation (ASD): a strategy for control of soil borne diseases in strawberry production

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Abstract

Anaerobic soil disinfestation (ASD), a biological alternative to soil fumigation, has been shown to control a range of soil-borne pathogens and nematodes in numerous crop production systems. Current work is focusing on how to optimize ASD (in terms of carbon source used, temperature and degree of anaerobiosis attained) to control specific sets of pathogens for California strawberries. A series of on-going field and greenhouse trials are testing the effect of soil temperature, carbon source (e.g., rice bran, grape pomace, molasses) and cumulative anaerobic conditions (Eh in mV h below 200 mV) on suppression of *Verticillium dahliae*, *Macrophomina phaseolina* and *Fusarium oxysporum*. Earlier work found that numbers of viable microsclerotia of *V. dahliae* in soil can be reduced with ASD using rice bran as a carbon source by 80 to 100% providing soil temperatures are above 18°C and a cumulative Eh of 50,000 mV h below 200 mV is achieved. Yields equivalent to those achieved with soil fumigants can be obtained, and use of ASD with rice bran (RB) eliminates the need for pre-plant fertilizer in strawberry systems. Shifts in soil microbial communities and associated production of organic acid and volatiles during the anaerobic phase may all be associated with disease suppression. In an organic field trial, we observed that ASD not only reduced *V. dahlia* severity in strawberry plants, but also resulted in lower numbers of microscleotia in the soil almost two years after treatment following an untreated lettuce crop. Temperature and Eh thresholds for other pathogens are being determined. When soil temperatures are high, ASD with RB as a carbon source shows promise for reducing *Fusarium* and *Macrophomina*, but did not provide complete control in field trials. Trials are being repeated with refinements to see if greater pathogen control can be achieved. In 2014-15, 400 ha of commercial strawberry and raspberries in California were planted using ASD, and in one field demonstration organic strawberry yields were doubled using ASD relative to grower standard practices.

Keywords: soilborne diseases, crop rotation, soil biology

INTRODUCTION

California organic strawberry producers face the challenge of controlling soil borne diseases, notably Verticillium wilt caused by *Verticillium dahlia*e, Fusarium wilt caused by *Fusarium oxysporum* (Koike et al., 2009) and charcoal rot caused by *Macrophomina phaseolina* (Koike, 2008). The main approach used to control these pathogens is crop rotation, however, many of the vegetable crops that can economically be rotated with strawberry are also hosts to one or more of the pathogens. *Brassica* species, notably broccoli have been found to suppress *V. dahliae* (Muramoto et al., 2014; Subbarao et al., 2007), but other key crops like lettuce are hosts. Furthermore, microsclerotia of *V. dahliae* can persist in the soil for many years. There is a clear need for additional disease control strategies and two that have shown promise are anaerobic soil disinfestation (Shennan et al., 2014) and use of mustard seed meals (Mazzola and Brown, 2010).

Anaerobic soil disinfestation (ASD) was developed in Japan (Momma, 2008) and The

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Netherlands (Blok et al., 2000) and has been shown to control soilborne pathogens and nematodes in strawberries. Previous studies conducted were aimed at optimizing ASD for California strawberry systems, and in conventional systems ASD was shown to be consistently effective at suppressing *Verticillium dahliae* in coastal California when 20 t ha^{-1} of rice bran (RB) was pre-plant incorporated and 75 to 100 mm of irrigation was applied in sandy-loam to clay-loam soils (Shennan et al., 2014). California berry growers have started to adopt ASD at a commercial scale with ASD acreage increasing from 2 ha to over 400 ha in the last four years (Farm Fuel Inc., pers. commun.), the majority in organic production.

Mustard seed meal (MSM) has been successfully used for controlling the replant disease complex of apples (Mazzola and Brown, 2010; Mazzola et al., 2015). It has also been evaluated in strawberry systems and showed neutral to positive effects on fruit production and disease suppression when used alone or in combination with other non-fumigant approaches such as ASD. Here we report on a recent rotation experiment testing ASD and MSM in a long term organic field site, and some large scale field demonstrations carried out in two different regions of California.

METHODS

Rotation trial

In June 2011, a 4 replicate randomized block split-plot experiment with crop rotation (broccoli-strawberry, cauliflower-strawberry, or fallow-strawberry) as the main plots and ASD, mustard seed meal (MSM) (*Brassica juncea*:*Sinapis alba* = 1:1 by weight), ASD+ MSM, and untreated control (UTC) as sub plots was established at the Center for Agroecology and Sustainable Food Systems (CASFS) organic farm on the University of California, Santa Cruz (UCSC) campus. After strawberries, a legume/cereal mix winter cover crop was planted and followed with summer lettuce in all treatment plots. For management details see Zavatta et al. (2014). Briefly broccoli 'Gypsy' and cauliflower 'Snow crown' were grown from June to September 2011 as main plots which were split prior to planting strawberries. For ASD plots, 20 t ha^{-1} of rice bran (RB) was applied to the bed surface and rototilled to 15 cm depth. For MSM plots 3.4 t ha^{-1} MSM was incorporated. For ASD+MSM plots, 16.9 t ha^{-1} of rice bran and 3.4 t ha^{-1} of MS were applied. ASD and ASD+MSM plots were drip irrigated to create and sustain anaerobic conditions for 3 weeks with a total of 108 mm of water applied. Strawberry plants 'Albion' were transplanted in November 2011, and fruit yield monitored from 20 plants bi-weekly from April to September 2012. A legume/cereal cover crop (45% bell beans (*Vicia faba*), 45% vetch (*Vicia sativa*), 10% rye (*Secale cereal*, 'AGS104') was planted in all plots in the fall 2012 and grown until the following spring at which time they were mowed and incorporated into the soil. Romaine lettuce 'Salvius' was grown in the summer 2013.

The numbers of viable *V. dahliae* microsclerotia in 0-15 cm of soil were estimated using a modified Anderson sampler and NP10 selective medium (Koike and Subbarao, 1994) before and after each crop, and pre- and post-ASD treatment. *V. dahliae* infection on strawberry plants was evaluated at the end of the growing season for four plants per plot. A split plot ANOVA was used for statistical analysis.

On-farm demonstrations

Two non-replicated large-scale ASD demonstration trials were conducted at a grower's farm in Watsonville, California during the 2012-2013 growing season. One trial was on conventionally farmed land and the other in a nearby organic field. Main treatments were ASD using rice bran (RB) at 20 t ha^{-1} or RB 10 t ha^{-1} + Molasses (Mol) 10 t ha^{-1}, and sub-treatments with or without pre-plant fertilizer. For the conventional field, a 673 kg ha^{-1} of slow release fertilizer (18-6-12) was added pre-plant, and for the organic site feather meal (12-0-0) at a rate of 1,122 kg ha^{-1}. For ASD, RB 20 or 10 t ha^{-1} was broadcast onto the assigned plots and rototilled to a depth of 15 cm. Beds were formed, drip tapes and plastic mulch applied, and the first irrigation began two days later. Mol was diluted with water at 1:2 to 1:5 in a water tank prior to application, then 6.5 t ha^{-1} was applied through the drip tapes. Seven days later, the remaining Mol (3.5 t ha^{-1}) was applied in the same manner. All plots were intermittently drip irrigated for three weeks from the first irrigation, with total irrigation amount of 60-75 mm. Soil Eh at 15 cm depth was monitored continuously using ORP (oxidation-reduction potential) sensors connected to a datalogger. Strawberries were harvested from 4 sections of each plot (20 plants section^{-1}) bi-weekly from March 19 to October 28, 2013.

A second strawberry demonstration trial was established in Oxnard, CA, in August 2014 with unreplicated 0.4 ha blocks of treatments of ASD RB 20 t ha^{-1}, ASD RB 15 t ha^{-1}, MSM 5.6 t ha^{-1} and grower standard practices. A 2.24 t ha^{-1} of pre-plant organic fertilizer (10-10-2.5) was applied only to the grower standard plot. The same treatments, except for the RB 15 t ha^{-1} plot, had been applied to the same plots for the previous strawberry crop which was followed by a wheat cover crop incorporated in early August 2014. Instead of RB 15 t ha^{-1}, the previous treatment had been ASD using RB 6.7 t ha^{-1} plus MSM 4.5 t ha^{-1} as C-sources. RB and MSM were both applied on August 25 by broadcaster then beds shaped, drip lines added and TIF (totally impermeable film) plastic tarp laid down. Approximately 200 mm of water was applied during ASD. Anaerobic conditions and soil temperatures were measured during ASD as above, and strawberries were harvested by the grower from January to May 2015. Plant mortality was measured by counting dead or nearly dead plants in each plot. Soil samples were taken from 0 to 6 inch depth in all plots post-treatment for microbial analysis by USDA-ARS WA using real-time quantitative PCR (RT-PCR) and terminal restriction fragment length polymorphism (T-RFLP) analysis.

RESULTS AND DISCUSSION

Rotation study

There was no significant effect of previous crop on the growth and yield response of strawberries irrespective of the disease management strategy used (see Zavatta et al., 2014, for details). However, when averaged across all previous crop treatments (fallow, broccoli and cauliflower) yields were highest in the ASD treatment with RB+MSM as the carbon source and ASD with RB only (Figure 1A). This was likely due to a combination of enhanced fertility in the early season and disease suppression in the later season. Indeed disease severity was significantly reduced with the ASD treatments (Zavatta et al., 2014). What is particularly interesting, however, is that the numbers of *V. dahliae* microsclerotia present in the soil were still lower in the ASD treatments almost 2 years later (Figure 1B). This is despite a cover crop being grown and incorporated and a lettuce crop produced in the interim, and suggests a long term suppression of disease by the ASD treatment. This agrees with observations by Goud et al. (2004) that ASD provided long term suppression of *V. dahliae*, but in their case the soil was undisturbed during the intervening 3 years. As discussed below, microbial community changes associated with ASD have been found to persist for many months and may be responsible for the longer term suppression.

Watsonville demonstration

Excellent anaerobic conditions were created in this field demonstration (with unreplicated large blocks in each treatment), in both the organic and conventional fields (Figure 2) and were associated with excellent strawberry yields (Figure 3). Previous work found that around 50,000 mV h below 200 mV was necessary for good control of *V. dahlia* (Shennan et al., 2009) and this threshold was greatly exceeded in both fields. In the conventional fields yields were equivalent to the adjacent fumigated areas, and in both cases there was little or no additional yield benefit from adding pre-plant fertilizer. Rice bran (2% N) and MSM (6% N) both contain large amounts of nutrients which can substitute for pre-plant fertilizer, however, current work is focusing on either reduced rates of application or alternative carbon sources to avoid potential excess losses of nutrients into the environment.

Treatments connected by a line are not significantly different at $p<0.05$ (Tukey's). Main plot treatments are previous crop: fallow, broccoli or cauliflower; and sub plots: ASD=ASD 20 t ha^{-1} rice bran, ASD+MC=ASD 16.9 t ha^{-1} rice bran + 3.4 t ha^{-1} mustard seed meal, MC=3.4 t ha^{-1} mustard seed meal, UTC = untreated control.

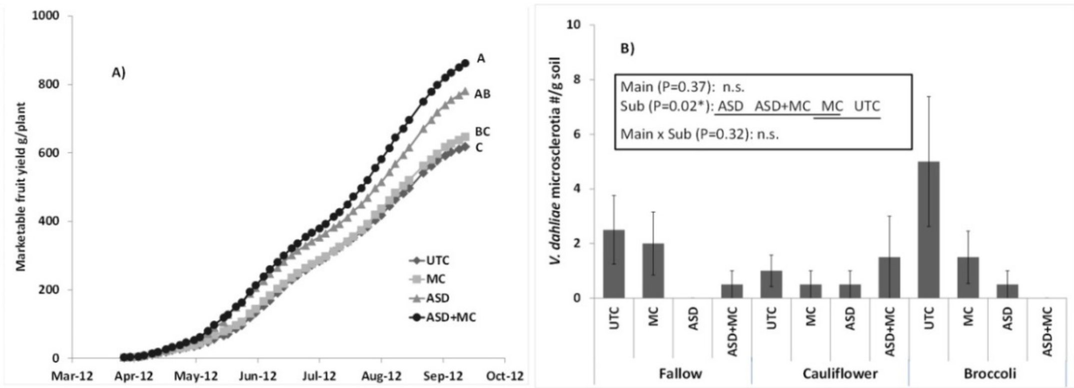

Figure 1. A) Cumulative strawberry marketable yields from UCSC rotation study 2012 (treatments with the same letter are not significantly different), and B) number (#) of viable *V. dahlia* microsclerotia in the top 15 cm of soil depth following lettuce harvest in 2013.

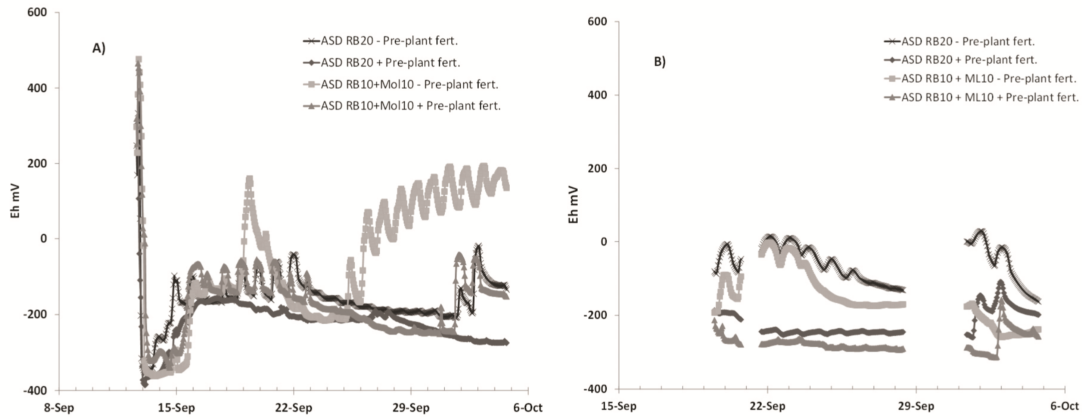

Figure 2. Oxidation-reduction potential (Eh) at 15 cm depth during ASD at the Watsonville demonstration site A) conventional field and B) organic field.

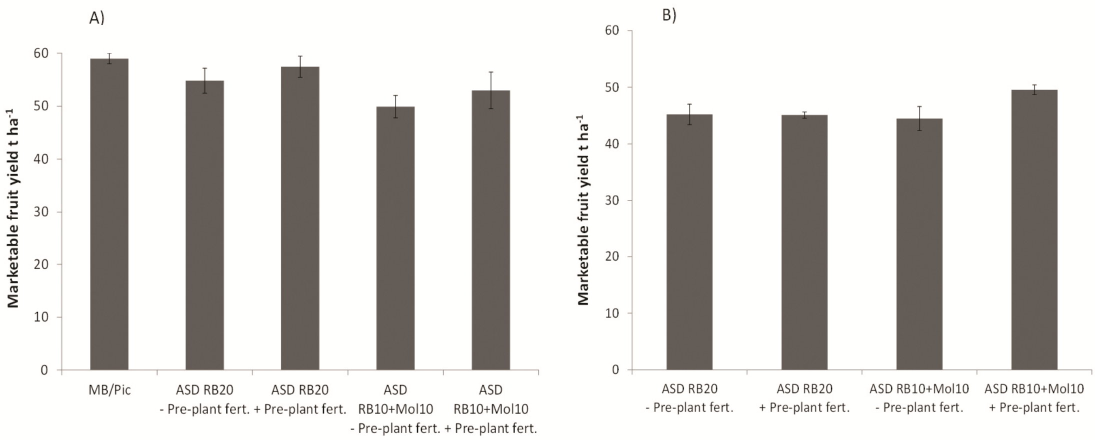

Figure 3. Strawberry marketable yields at the Watsonville demonstration site 2013, A) conventional field and B) organic field. Error bars represent SEM from sub sampled areas within each treatment block (treatments unreplicated).

Oxnard demonstration

Macrophomina phaseolina is present at this location and is a serious concern for strawberry production. In both years of the demonstration ASD greatly improved crops yields relative to the grower's standard practice (Figure 4A) and reduced disease severity as measured by crop mortality (Figure 4B). The second year trial again demonstrated that no pre-plant fertilizer was necessary at ASD RB 20 t ha^{-1} plot. There was a significant shift in fungal community composition in soils post ASD, relative to the MSM and the grower standard (Figure 5A). However, prior to treatment the following year and after an intervening wheat cover crop, the ASD RB9 (20 t ha^{-1}) treatment still clustered together and were distinct from the other plots (Figure 5B) again indicating longer term changes in the soil following ASD. Following the second year with ASD treatment soil fungal communities again showed very distinct clustering based on treatment (Figure 5C). This pattern has been observed in other field trials and we are in the process of identifying which species become more prevalent following ASD and if this is related to type of carbon source. Initial screening of NextGen sequencing data suggest that enhanced strawberry yields in response to ASD conducted using rice bran was associated with elevated detection of sequences representing various genera within the Flavobacteria known to have anti-fungal properties.

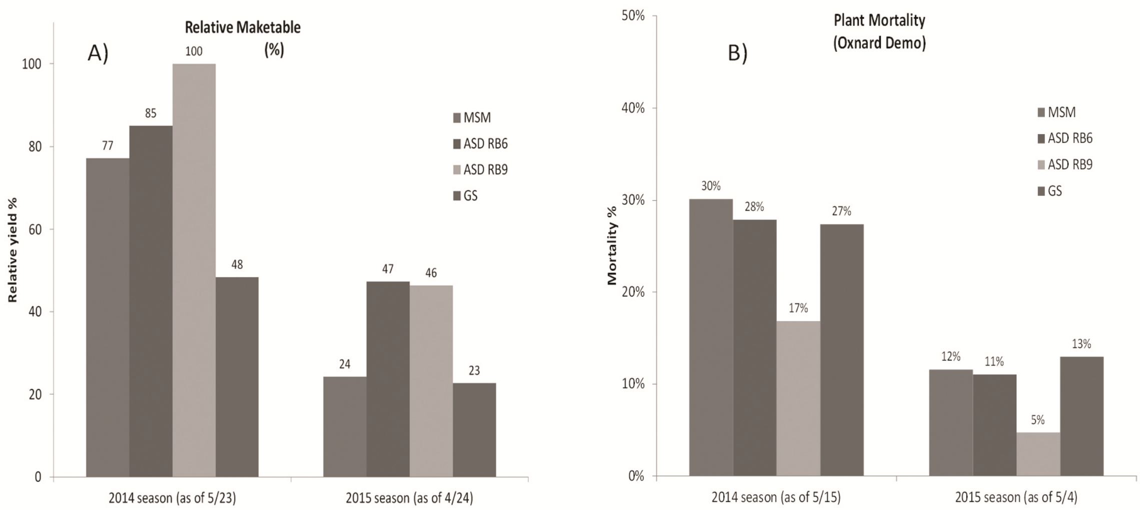

Figure 4. A) Relative strawberry yields and B) Plant mortality in the Oxnard demonstration trial. GS = grower standard, MSM = mustard seed meal 5.6 t ha^{-1}, ASD RB9 = ASD rice bran 20 t ha^{-1}, and ASD RB6 = rice bran 15 t ha^{-1}. (Note: in 2014 season second bar in each graph was ASD MSM = rice bran 6.7 t ha^{-1}+ mustard seed meal 5.4 t ha^{-1} not ASD RB6).

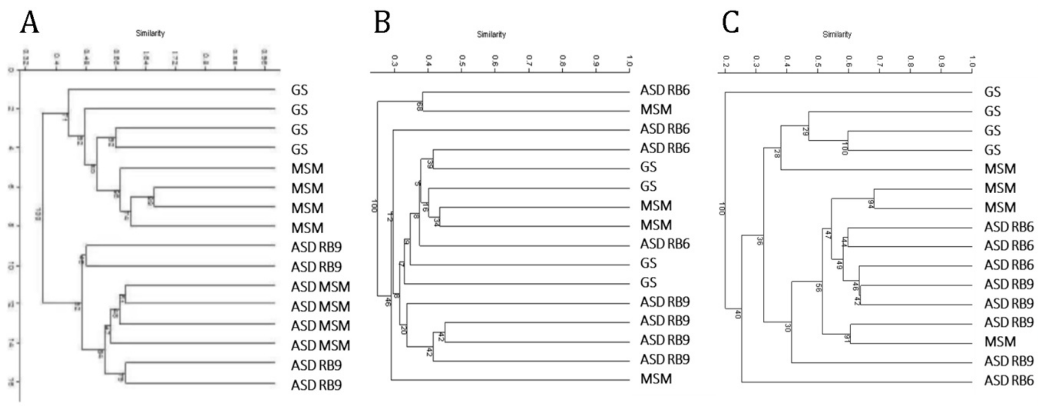

Figure 5. Fungal community similarity based on ITS T-RFLP analysis A) post-treatment October 2013, B) pre-treatment August 2014 and C) post-treatment September 2014 from the Oxnard demonstration trial. GS = grower standard, MSM = mustard seed meal 5.6 t ha^{-1}, ASD RB9 = ASD rice bran 20 t ha^{-1}, ASD MSM = rice bran 6.7 t ha^{-1} + mustard seed meal 5.4 t ha^{-1}, and ASD RB5 = rice bran 15 t ha^{-1}.

CONCLUSION

ASD reduced *V. dahliae* in strawberries and suppressed microsclerotia increase during growth of a host lettuce crop more than 18 months after the treatment. This long-term suppression may be due to soil microbial shifts observed in ASD treated soils when RB is used as a carbon source. ASD with 20 t ha^{-1} of RB provided excellent yields in two demonstration trials in California strawberries; one with low disease pressure in Watsonville and the other with high pressure from *M. phaseolina* in Oxnard. No pre-plant fertilizer with RB or RB+MSM ASD was necessary in both trials. Future studies should examine effects of different carbon sources for ASD on disease suppression and changes in fungal and bacterial communities in the soil to better understand the mechanisms of action of ASD in terms of immediate disease control and longer term suppression.

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