Research Proposal for the California Strawberry Commission (Fall 2008)

**Project Title:** Optimizing Anaerobic Soil Disinfestation for Non-Fumigated Strawberry Production in California

**Type of Proposal:** Continuing  
**Funding Amount for 2009:** $50,000  
**Year of Project:** 2nd year of 3 year project

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**Summary:**  
Considerable financial resources are being invested in the search for alternatives to methyl bromide, but these studies are evaluating primarily chemical fumigants. However, current re-registration and regulation processes may severely limit the sustainability of a fumigant-dependent production system. Soilborne disease management without use of chemical fumigants is one of the greatest challenges in strawberry production in California. Anaerobic soil disinfestation (ASD) was developed in the Netherlands and Japan as an ecological alternative to methyl bromide fumigation. ASD integrates principles from solarization and flooding for fields where these methods are unfeasible or ineffective. In Japan, hundreds of farmers use ASD to control soilborne pathogens (including *Verticillium dahliae*) and nematodes in strawberries and vegetables. The goal of this project is to optimize ASD for strawberry production in coastal California. In the first year, we conducted a series of incubation experiments and found: 1) regular plastic tarp for bed mulching worked well for creating anaerobic conditions and suppressing *Verticillium dahliae*, 2) ASD’s ability to suppress weed germination in coastal California may be limited and species dependent, 3) onion skin waste from Ventura and diluted ethanol have potential as carbon sources for ASD. Based on these results, we began on-farm experiments in Ventura, Moss Landing and Salinas to test ASD in varying soil types, carbon sources, and seasons. To further optimize ASD for California strawberries, we propose to continue our experiments for a second year. As the first year, the proposed funds from the Commission will enable us to repeat the study at a third field site in Salinas, in addition to the two locations funded through the USDA-methyl bromide alternative program.
Justification

Over the last four decades, California’s annual strawberry production system has relied upon preplant fumigation with methyl bromide (MeBr) (Martin and Bull, 2002; Wilhelm et al., 1961); however MeBr is a class 1 ozone depletter and is being phased out through the Montreal Protocol. Considerable resources are being invested to develop alternatives to MeBr by using other chemical fumigants, yet the most promising options, such as chloropicrin, also have potentially negative health effects and their continued use is uncertain since they are undergoing re-registration (US-FPA, 2006). This underscores the critical need for development of a wider range of alternative practices (Carpenter et al., 2001).

Soil disinfestation methods using anaerobic decomposition of organic matter were developed in the Netherlands (Blok et al., 2000; Goud et al., 2004) and Japan (Shinmura, 2000, 2004) as an ecological alternative to MeBr. Anaerobic soil disinfestation (ASD) integrates principles behind solarization and flooding to control nematodes and pathogens in situations where neither are effective or feasible. ASD works by creating anaerobic soil conditions by incorporating readily available carbon-sources into topsoil that is irrigated to saturation (not flooded) and subsequently covered with a plastic tarp impermeable to oxygen. The tarp is then left in place to enable anaerobic decomposers to respire using the added carbon and produce anaerobic by products that are toxic to pathogens, but that are degraded rapidly once the tarp is removed.

Results to date

The goal of the first year (2007-2008) was to test different management options to optimize the performance of an ASD option for strawberry systems in the central and south coasts of California. Prior to initiating field experiments, a series of pot experiments were conducted to examine the effect of ASD with varying plastic tarp, soil types, carbon sources, and temperature conditions on weed seed germination and survival of the soil-borne pathogen, Verticillium dahliae.

Effect of tarping material and soil type

A randomized block experiment with type of tarp (no tarp, green, white/black (both 1.25 mil standard polyethylene films), VIF (1.25 mil embossed black tarp), or pit tarp (8 mil black/white tarp)), and soil types (Watsonville sandy clay loam or Moss Landing sandy loam) as treatments, was performed with two replicates. Soil was mixed with 10 ton/ha equivalent of wheat bran and packed into a PVC pot (15 x 20 cm). Two nylon mesh packets containing 50 weed seeds of common California weeds, annual bluegrass (Poa annua; PA), or common purslane (Portulaca oleracea; PO), and another bag containing 50 grams of Verticillium dahliae naturally infested soil, were placed at 15 cm depth in each pot. Water was applied to saturate the soil and the excess allowed to drain through the bottom holes of the pots. After covering the soil surface with a plastic tarp, pots were placed in 25 deg C incubators for three weeks. Soil Eh1 and temperature at 15 cm depth in each pot were monitored continuously. After three weeks, buried weed seeds and Verticillium inocula were retrieved. Weed seed germination was visually assessed at 24 hours, three days, and seven days after retrieval and placement on wet filter paper at 25 °C. Retrieved Verticillium inocula were air dried for 4 weeks and assayed for active V. dahliae microsclerotia population on NP10 selective medium using the modified Anderson sampler (Koike et al., 1994).

A strong to moderate anaerobic condition (Eh -200 to 100 mV) was developed within one week in all treatments except for the no tarp treatment. Cumulative Eh values below 200 mV during the entire incubation period were similar among all tarp types. Further, active V. dahliae microsclerotia population in inocula retrieved from standard tarp plots were significantly lower.

1 Eh, also known as redox potential, is an indicator of anaerobiosis. The lower the Eh, the stronger the anaerobic condition.
than one from none tarp plot, and numerically lower than VIF and pit tarp plots (Fig. 1). This indicates that standard plastic film for strawberry mulch may be sufficient for ASD.

![Graph showing microsclerotia number per gram of soil for different tarp conditions](image)

**Figure 1 Verticillium dahliae** population in retrieved inocula after three weeks of ASD treatment with varying plastic tarp types. See text for detailed information on each tarp. Values are back-transformed means ± SEMs. No significant difference was observed between factors with the same letter by Tukey's HSD test at the $P=0.05$.

Germination rates of retrieved seeds were also similar across varying tarp types; 32 to 36% for PA (~40% reduction from no tarp treatment) and 62 to 69% for PO (23% increase from no tarp treatment), as opposed to 57% for PA and 53% for PO in no tarp treatment. This suggests that the potential of ASD in controlling common weeds in California strawberry fields may be limited and species dependent, but further repetitions of the experiment are needed to draw more robust conclusions. No significant effects of soil type on both active *V. dahliae* microsclerotia population and weed germination were detected.

Other incubation experiments suggested that onion skin waste from onion processing company in Ventura and diluted ethanol (Uematsu et al., 2007) were also promising as carbon-sources for ASD (data not shown).

**On-farm trials** Three field trials with randomized block designs (4 replicates) were initiated in summer/fall 2008. The first trial was in the Hansen Trust farm (clay loam soil) in Santa Paula, Ventura County on 6/19/08. Treatments were rice bran (14 t/ha), 1% ethanol (2.3 acre-ins), onion skin waste (28 t/ha dry matter) and a control (no added carbon). Soon after carbon-source incorporation and bed listing, all plots were covered with a standard plastic film and drip-irrigated with 2.3 ac-ins. The second trial began on 8/16/08 at a sandy loam field in Moss landing, Monterey County. Treatments were rice bran (10 t/ha and 20 t/ha), 1% ethanol (2.1 acre-ins), and control (no carbon source or irrigation for soil saturation). 2.1 (ethanol) to 3.2 (rice bran 20 tons per hectare plot) acre-inches of water were drip-irrigated after bed listing and mulch application. The third trial began on 10/13/08 in a sandy loam field in Salinas, Monterey County, with rice bran (10 t/ha) and a control (no added carbon or irrigation) as treatments. As of 10/26/08 a total of 7.3 ac-ins of water has been added via intermittent drip-irrigation. Irrigation amounts were used to saturate the bed soil, but not collapse the beds. Two to 4 bags of *V. dahliae* inocula (all sites) and *Phytophthora cactorum* inocula (Ventura site only) were buried in each plot at 15 cm depth and retrieved when tarpers were removed after 3 weeks (results pending). Soil Eh, temperature, and water content or water potential was continuously monitored at 15 cm depth.

Over the three weeks soil temperatures were highest in Ventura (min. 22 to max. 37 deg C), followed by Moss Landing (16 to 29 deg C) and Salinas (14 to 26 deg C). Soil water increased during irrigation and subsequently decreased to field capacity.
Figure 2. Changes in soil Eh at 15cm depth during three weeks of ASD treatment in a: Ventura, b: Moss Landing, and c: Salinas sites. Average of four replicates is shown for each treatment in each site. In Salinas, one of the replicates in rice bran plot is also shown.

In the Ventura trial, onion waste and rice bran treatments created strong anaerobic conditions, whereas ethanol only produced weak anaerobic conditions little different from the control (Fig 2a). At the Moss Landing site, rice bran at both 10 and 20 t/ha plots kept relatively strong anaerobic conditions for 7 days, whereas ethanol only achieved weak anaerobic conditions for a shorter time (Fig 2b). In Salinas, only weak anaerobic conditions were achieved with rice bran, probably because of the rapid drainage observed across most of the field and cooler soil temperatures. However, one of the replicates had more tractor traffic and here strong anaerobic condition occurred, suggesting moderate soil compaction may improve ASD in this site (Fig 2c).

**Objectives:**

The overall project objectives are to:

1. Develop optimized ASD methods for suppressing *Verticillium dahliae* and selected weeds in strawberry fields in the central and the south coast of California; using a combination of greenhouse/incubator studies and on-farm trials where treatments and management are developed in collaboration with the growers.

2. Disseminate information about optimized ASD methods to strawberry growers in the central and the south coast of California through a combination of workshops, field trips and written materials.
These objectives match the first priority area of the current RFP: strawberry production without the use of fumigants.

For the second year specifically, we will focus on further optimization of the ASD methodology by a) continuing to test different C-sources alone and in combination, and b) improve field scale management techniques for different locations and soil conditions. The second-year of funding from the commission will allow us to continue working at an additional field site in the central coast to further optimize ASD across a range of soil types. Trials at the other Central Coast and Ventura field sites will again be funded through the grant from the USDA Alternatives to MeBr Program.

Methods:
This second year we will focus on testing different C-sources, and timing and lengths of tarping periods as key variables for ASD use (Table 1). We will continue a series of incubation experiment using similar methods to those used in the first year to identify the best efficacy, ease of use and cost/benefit of different carbon sources for ASD. See Table 2 for comparative costs of materials being studied. We are particularly interested in the potential for using waste products from other agricultural operations. For example, based on a preliminary survey of cost and availability, we anticipate that rice bran from northern California rice mills would be a good option as an organic material (see Table 2 for estimate costs of organic materials for ASD). On the other hand, although it costs more, molasses can be easily applied through drip tapes and can control pests in deeper soil layers (Shimamura 2004). Four acre-inches of 1% ethanol (Uematsu et al., 2007) was tested in the first year trials but it is cost prohibitive (~$8,000/acre) unless we could find a source of crude ethanol that is less expensive. We will also examine onion skins (Ventura) and grape pomace as carbon sources for ASD.

Table 1. Potential selection of ASD variables to be tested in this proposal*

<table>
<thead>
<tr>
<th>Target pest(s)</th>
<th>Pathogen: Verticillium dahliae and others**</th>
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<tr>
<td></td>
<td>Weeds: Stellarium media, Poa annua, Capsella bursa pastoris, Portulaca oleracea, Cyperus esculentus, and Malva parviflora</td>
</tr>
<tr>
<td>Carbon source to be tested</td>
<td>Rice bran, molasses, grape pomace, and onion waste (Ventura), with variable rates and combinations</td>
</tr>
<tr>
<td>Tarping period</td>
<td>3-6 weeks in summer to fall</td>
</tr>
</tbody>
</table>

* Potential applications of ASD are not limited to pests and a crop listed in this table.
** Diseases caused by other pathogens will also be monitored (see below).

Table 2. Cost of organic materials for anaerobic soil disinfection

<table>
<thead>
<tr>
<th>Organic material</th>
<th>Local price $/unit</th>
<th>Amount lbs/acre</th>
<th>Cost $/acre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice bran</td>
<td>$200/ton</td>
<td>8914*</td>
<td>809</td>
</tr>
<tr>
<td>Onion skin waste (Ventura)</td>
<td>Free</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grape pomace</td>
<td>Free</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molasses</td>
<td>$150/55 gallon</td>
<td>8023**</td>
<td>2188</td>
</tr>
<tr>
<td>MeBr fumigation</td>
<td>~2500</td>
<td></td>
<td></td>
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</table>

* Equivalent to 10 tons/ha (Shimamura 2004), ** Equivalent to 9 tons/ha (Shimamura 2004).

Based on results of on-going field trials, we plan to run some location specific short trials to test for the ability of different C inputs and management techniques (e.g. moderate compaction for rapid draining soils). Using this information and discussions with participating growers, we will design the next set of field experiments in Moss Landing, Salinas and Ventura areas by June 2009. Randomized complete block or split plot randomized block design with 4 replications will
be used and data analyzed using ANOVA or split-plot ANOVA methods as previously. Field methods will be used as described for year 1, namely, carbon sources such as rice bran will be broadcast and incorporated in the fields, according to the experimental design. Beds will be listed and buried drip tapes will be set. Plastic tarps of specific types (to be determined) will be applied on beds. Then between 2 to 4” of water will be irrigated by buried drip tapes depending on the soil type (with less water required for a clay soil than for a sandy soil). The total amount of irrigation water will be determined site-by-site basis by monitoring changes in soil water contents to saturate the bed soil. If decided, molasses of specific amounts will be applied with irrigation water through buried drip tapes. Plastic tarps will be kept in place as plastic mulch during the whole production period. After specific periods in a range of three to six weeks of ASD treatment, holes will be cut on tarped beds in which strawberry plants will be planted in October to November 2009.

During ASD treatment, soil Eh, temperature, and moisture at 15 cm depth will be continuously monitored in each treatment with sensors connected to an automatic data logging system (Campbell CR-1000 with AM 16/32 multiplexer). To examine the effects of ASD on soil fertility, soil inorganic nitrogen content pre- and post- ASD will be measured by 2M KCl extractions and the auto analyzer method (Lachat Instruments, 1993). Potential phytotoxicity of ASD to strawberry plants (note: this has not been observed in our preliminary studies) will be evaluated by comparing plant diameter 2 to 3 times during the growth period. Fruit yield of strawberries will be measured once/twice a week during the harvest season (April to September in the Central coast; January to June in the Ventura) from 20 marked plants in each plot. Experienced harvesters will weigh cull fruit and marketable fruit separately and count number of fruit for fruit size evaluation.

To assess soilborne disease suppression, we will focus on *V. dahliae* as a representative lethal pathogen on strawberries in the coastal California (Martin, 2003) at all sites, and *Phytophthora cactorum* in Ventura. The same buried inoculum retrieval method will be used as previously (see Justification for details). Buried inocula will be retrieved after ASD treatment and assayed for active *V. dahliae* microsclerotia population on NP10 selective medium using the modified Anderson sampler (Koike et al., 1994). Strawberries will be regularly examined and evaluated for the development of any soilborne disease. Disease severity and incidence will be noted. Affected plants will be collected and tested in the UCCE Diagnostic Lab in Salinas for the presence of soilborne pathogens. Foliar disease development will also be monitored in the event that treatments might influence the susceptibility of plants to foliar pathogens.

To assess weed suppression the same methodology will be used as before, but more weeds will be tested. namely: common chickweed, annual bluegrass, shepherd’s-purse, common purslane, little mallow and yellow nutsedge. Bags of seeds will be buried 2 inches deep in the soil before ASD and retrieved after tarp removal. Germination tests will be conducted as before and in addition non-sterminated seed will be stored at −20°C until tested for viability with tetrazolium salts. Weed biomass and weeding time will be compared after ASD against non-ASD controls 2 to 3 times as needed during the season.

**Proposed schedule of accomplishments:**
Year 2 (Feb. 2009 - Jan. 2010)*
May – June 2009: Determine experimental design, and preparations for the second round of field experiments.
June 2009 – September 2010: Strawberry field experiment in Central and South Coast comparing optimized ASD with methyl bromide control.
* Schedule for Year 3 will be determined based on results of Year 2.
Literature review:

Two methods, biological soil disinfestation (BSD) for open fields in the Netherlands (Blok et al., 2000; Coud et al., 2004; Messiha et al., 2007) and soil reduction sterilization (SRS) for greenhouses in Japan (Shinmura, 2000; Shinmura, 2004; Momma, 2008) were developed independently based on the same principles. SRS uses more water (4 to 6”) and organic matter input (wheat or rice bran 10 tons per ha) than BSD (2” of water and use freshly mowed plant residues 35-55 FW tons/ha) but SRS requires much shorter tarping period (3 weeks) than of BSD (10-15 weeks). The required tarping period also depends upon soil temperature. Both methods were effective in suppressing soilborne pathogens such as Fusarium oxysporum, Fusarium redolens, Verticillium dahliae, and Ralstonia solanacearum as well as plant-pathogenic nematodes such as Meloidogyne incognita and Pratylenchus fallax. Goud et al. (2004) observed that BSD reduced soil inoculum levels of Verticillium dahliae by 85% relative to the control and it did not increase for 4 years. Since its development, SRS has been rapidly gaining popularity among greenhouse owners throughout Japan as a method for disease and/or nematode control on tomatoes, welsh onion, spinach, eggplants and strawberries in different soil types including andisols (volcanic ash soil), sandy, loamy-clay, and heavy clay soils as an alternative to methyl bromide (Chiba Prefectural Agriculture Research Center, 2002; Shinmura, 2000; Tochigi Prefectural Agriculture Research Center, 2002). Potential disadvantages of this method may include N losses via denitrification during the anaerobic treatment, odor from anaerobic decomposition of organic matter, and the opportunity costs lost during tarping. Further, the system may be less effective in very well-drained soil (e.g. deep sandy soil) and cooler regions/seasons. Nevertheless, in Chiba, a leading vegetable production prefecture in Japan, more than 200 farmers practiced SRS in 2002 and 2003 (Takeuchi, 2003; Takeuchi, 2004).

Literature cited


