Final Report

Integrated Pesticide Reduction Strategies for Insect and Disease Management in Cole Crops

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Summary

Chemical fumigants and organophosphates for cole crop production in California are being targeted for reduced use due to stringent regulations and concerns regarding air and water quality. A team of researchers and growers examined and developed integrated pesticide reduction strategies in cole crop production using Brussels sprouts and broccoli in the central coast as model crops. We tested anaerobic soil disinfestations (ASD) and mustard seed meal (MSM) application to control plant parasitic nematodes (PPN), club root disease (CR), and cabbage maggot (CM) in Brussels sprouts. A series of IPM tools, including effective monitoring technologies, economic damage thresholds and alternative chemical strategies were also developed to help growers implement IPM for control of CM in broccoli production. Pest population reduction, damage severity, soil-plant N dynamics, and crop yields were monitored and net returns determined for strategies developed based on multi-year replicated field trials.

Although it provided good control of club root disease and cabbage maggots, ASD did not always provide comparable yield with fumigants and proved to have the lowest partial net returns among treatments tested due to the combination of higher cost and lower yields in some trials. Yields with grape pomace ASD could be limited by early season N availability due to immobilization, or inadequate field aeration. Further work is needed to optimize fertility management and field preparation with ASD in different soil types before the technique can be recommended. Nonetheless, ASD if optimized further, could be an effective way of controlling CR and a viable alternative to PCNB in highly infected fields.

MSM, on the other hand, appears to have a potential as an alternative to fumigant and PCNB though its effect on club root control was not as good as ASD and it had no effect on cabbage maggots. Further work with powdered MSM with and without using a roller merits further study. For club root disease control, maintaining soil pH to 7.2 or above should be integrated with any other approaches. Should club root disease pressure increase, sprouts growers may need to consider crop rotation with non-brassica crops as a part of IPM.

Future work is needed to find a more precise way to assess treatment effects on PPN populations, and a burial/retrieval method should be tested. Our work pertaining to IPM of CM in broccoli indicates that egg sampling via sand floating is the best means of monitoring for CM. We determined that 1 week old broccoli transplants with 6 CM eggs/plant or higher will show a significant decline in floret size. Additionally data indicates that in organic broccoli fields as CM pressure increases so does the rate of parasitism. A study of the management practices used in organic fields and their relationships to increased rates of parasitism could identify potential effective IPM strategies for the control of CM in broccoli production. Overall, the results from this study if combined with future research geared in the directions discussed, show that effective and viable biological alternatives to chemical fumigants in Cole crop production may be attainable in the future.

Introduction

For California's production of 90% of the nation's cole crops, growers depend on pesticides now known to produce unanticipated harm to public health and the environment, such as soil fumigants for control of plant parasitic nematodes (PPN), the organochlorine pentachloronitrobenzene (PCNB) for control of club root (CR), and the organophosphates diazinon and chlorpyrifos (CPF) for cabbage maggot (CM). Usage of registered soil fumigants, such as 1,3-dichloropropene and methyl isothiocyanate generators such as metam-sodium has increased rapidly, as has their generation of volatile organic compounds (VOC) that react with nitrogen-oxides to form ground-level ozone, a potential cause of

respiratory problems in humans (Lee et al. 2002). CDPR released new recommended permit conditions that will lead to greater restrictions on fumigant use on land adjacent to urban areas. PCNB is relatively persistent in soil and water as a pollutant, and has encountered serious problems due to impurities such as dioxin, formed during the manufacturing process, including a temporary stop order from the EPA for California in 2010 (USEPA, 2010). Chlorpyrifos, a common source of pesticide residue (Lozowicka et al. 2012), has been implicated in negative effects on humans, from cancer to semen quality to neurobehavioral development (e.g. Meeker et al. 2004, Lee et al. 2002, 2004, Ventura et al. 2012, USEPA 2000) and as a frequently detected and hazardous pollutants in surface water (a risk score of 100; Zhan and Zhang 2012). Further, CPF and diazinon, both used extensively in Brussels sprouts and broccoli are being specifically targeted for reduced use due to pollution of surface waters in the central coast region. New total maximum daily loads have been introduced for CPF and diazinon for the lower Salinas Valley which sets numerical targets for reduced levels in surface water by 2025. No IPM guidelines have been developed to reduce organophosphate use for controlling CM in this region, potentially resulting in higher applications than optimal.

Sixty eight percent of US Brussels sprouts harvested fields were located in the three California counties Monterey, Santa Cruz, and San Mateo in 2007 (USDA-NASS, 2009). Among a number of soilborne diseases in California cole crops, club root (*Plasmodiophora brassicae*) and those caused by PPN (primarily cyst nematodes, *Heterodera* spp., and root-knot nematodes *Meloidogyne* spp.) are of particular concern as they are highly pathogenic and can persist in host-free field soil for several years. There are no economically feasible treatments to eliminate these pathogens from infested fields. In heavily infested soil, the pathogens are capable of causing tremendous yield loss. When cyst nematodes attack seedlings, the entire Brussels sprouts planting may be ruined economically (USDA, 1999). In heavily *P. brassicae*-infested soil, the disease is capable of destroying a crop before it reaches market maturity. Therefore growers rely heavily on soil fumigants to control soilborne pathogens and PPN. In 2010, 88% (165,294 lbs/A) of the total pesticide input in CA Brussels sprouts production consisted of three soil fumigants (1,3D, metam sodium and metam potassium); in addition, 5750 lbs of PCNB were applied to mitigate CR in San Mateo County (CDPR, 2012). According to the growers collaborating in this project, PPN problems are wide spread across the Brussels sprouts production regions in San Mateo, Santa Cruz, and Monterey Counties, whereas to date CR has been limited to San Mateo County.

The California Central Coast is a major broccoli growing region: Monterey County alone produces nearly 43% of the state's crop (CDFA 2011). The cabbage root maggot (CM) (Diptera: Anthomyiidae: *Delia radicum* L.) is a specialist pest of cole crops, including broccoli, and is common in coastal California. Adult female flies lay well over 100 eggs at the base of cole crop plants (Biron et al. 2002), which provide emerging larvae with access to feeder roots and taproots. Their damage to the vascular tissues consequently interferes with proper plant development and reduces yield potential. Moderate temperatures along the coast allow CM to be active almost year-round.

Because California broccoli growers lack CM monitoring techniques and economic threshold guidelines (Natwick 2009), both critical components of integrated pest management, growers employ sub-optimal CPF applications either designed to provide prophylactic protection against CM establishment in the soil at transplant or for mobile CM adults later in the season. Both of these approaches lack precision and can promote CPF overuse. Consequently, cole crops are responsible for the majority of CPF use on the central coast: 82% of applied CPF AI in Monterey County in 2002 was directed towards cole crops; 56% of CPF use was in broccoli alone (Spurlock 2004). In turn, CPF surface water pollution has become

increasingly problematic, with a majority of water column samples exceeding CPF maximum allowances in the Lower Salinas River Watershed (CRWQCB 2011).

The project addresses two priority areas that are impacted by the current management of soilborne diseases and arthropod pests in California cole crop production; namely **to reduce the use of field agricultural fumigants**, and **organophosphate insecticides used on cole crops in the Central Coast**. Specifically we tested promising alternatives to soil fumigation in Brussels sprouts for control of PPN, CR and CM and developed IPM tools for growers to use in control of CM in broccoli. A number of field trials were established in growers' fields in San Mateo, Santa Cruz and Monterey Counties over three years. For Brussels sprout production we tested anaerobic soil disinfestation (ASD) and mustard seed meal (MSM) application for their ability to control soilborne diseases, PPN and CM, and compared them against fumigant and untreated controls in terms of yield and treatment costs. Carbon sources for ASD and mustard seed meal can provide significant amount of pre-plant N, impacting Brussels sprouts growth. Therefore, we also monitored inorganic N dynamics throughout the trials. In broccoli we compared, developed and implemented optimal methods of CM monitoring, actionable thresholds and CPF alternatives in Santa Cruz and Monterey Counties; all designed to improve the precision of CM management, thereby reducing both crop damage and CPF application rates. This integrated approach is designed to better target CM pressure in both time and space, and therefore should be very competitive when compared with either foliar CPF applications (the grower's standard) or untreated controls.

Brussels sprouts production in California is built around the use of soil fumigants to control soilborne disease and PPN, which has allowed growers to grow Brussels sprouts continuously on the same ground. Three promising alternative strategies are tested here and compared against fumigation. ASD has been shown to effectively control a range of soilborne pathogens and PPN in a wide variety of locations and production systems, including vegetable and strawberry production in Japan (Shinmura, 2000, 2004; Momma, 2008, Momma et al., 2010), the Netherlands (Blok et al, 2000, Goud et al, 2004, Messiha et al, 2007, Lamers et al, 2010), Florida (Butler et al., 2011, 2012) and California (Shennan et al., 2010, 2011, 2014). The ASD process creates anaerobic soil conditions by incorporating readily available carbon sources into topsoil that is irrigated to saturation and subsequently covered with a plastic tarp. Naturally occurring microbial anaerobic decomposers respire using the added C and produce various metabolites that have biocidal activity and thereby reduce the population of plant pathogens and PPN (Momma et al., 2006, Katase et al., 2009, Runia, et al., 2014). The metabolites are rapidly degraded when the soil is cultivated for planting allowing for short plant back times. In California trials with ASD have consistently achieved 80-100% reductions of *Verticillium dahliae* microsclerotia in soils and produced comparable yields of strawberries with those obtained in fumigated soil (Shennan et al., 2011, 2012). A Japanese study showed that ASD suppressed club root disease on broccoli (Yogo et al., 2012). There is no information currently assessing the ability of ASD to control CM.

Numerous studies have also demonstrated the suppressive impact of Brassica plant residues and seed meals on soilborne pathogens (incl. PPN) (Brown and Morra, 1997, Borek et al., 1997, Ploeg and Stapleton, 2001, Cohen et al., 2005, Valdes et al., 2012). Plants in the genus Brassica contain glucosinolates that in contact with the enzyme myrosinase hydrolyze to volatile isothiocyanates (ITCs), nitriles, and thiocyanates. The ITCs in particular have general biocidal properties; thus the method is named biofumigation (Angus et al, 1994, Kirkegaard and Sarwar, 1998). The microbial community is also changed by addition of the seed meal and has been shown to contribute to suppression of different pathogens and PPN that form the complex that causes apple replant disease (Mazzola and Manici, 2012, Mazzola and Brown, 2010). Because CM is a specialist pest of Brassica, ITCs could influence oviposition

and all treatments may disrupt natural enemies. Studies showed rye cover crop incorporation can suppress PPN in soil (Zasada et al.,2007, McBride et al., 2000, McSorley, 2011), and rotations with grain reduce the build-up of CM but interfere with biological control by rove beetles (Dosdall et al. 2012).

Our overall goal is to test different strategies for reducing use of soil fumigants (1-3D, metam sodium); fungicides (PCNB) and pesticides (CPF) in two important cole crops, Brussels sprouts and broccoli. Specifically, our objectives were to;

Objective 1. Optimize ASD, MSM and rye cover crop incorporation, alone or in combination, against CR, and PPN for CA Brussels sprouts production.

Objective 2. Test the efficacy of ASD in reducing CM damage in Brussels sprouts.

Objective 3. Develop transferable CM monitoring techniques that will be based on evaluating various trapping technologies and sampling strategies for broccoli

Objective 4. Develop economic threshold for CM based on optimized monitoring technique

Objective 5. Test the susceptibility of broccoli to CM damage at various growth stages

Objective 6. Test the efficacy of Chlorpyrifos granules in broccoli plug-transplants in reducing CM damage.

Over the course of this study Objective 1 was changed to the optimization of ASD and MSM application only and not of rye cover crop due to conflicts with growers' planting schedules and the accidental fumigation of parts of the field. Objective 6 was also changed to test the efficacy of Ecotec G, a promising organic insecticide/deterrent, in reducing CM damage in turnips coupled with a field trial funded by UCSC to test a mixed crop assemblage with lettuce and carrot on CM damage to turnip. Throughout the past three years we have taken many steps to achieve these objectives. Further explanation and details of the activities and findings of this study are provided below as well as suggestions for future work to develop effective biological alternatives to fumigants and a fungicide in Cole crop production.

Project Activities and Accomplishments

Objectives 1 and 2: Brussels Sprout

Trial design:

In order to optimize ASD and MSM application for the control of PPN and clubroot disease as well as test the efficacy of ASD to reduce CM survivorship in Brussels sprouts, we conducted a total of 13 replicated on-farm trials in Half Moon Bay, Santa Cruz and Watsonville, the major Brussels sprout production areas in coastal California, from the 2013 season (Year 1) through the 2015 season (Year 3). Although some of the project members have been working on optimizing ASD and MSM for California strawberries for many years (Shennan et al., 2007), this was our first efforts to test these non-fumigant alternatives for other crops. Brussels sprout production differs from strawberry production in many ways. In terms of optimizing ASD and MSM, the lack of use of plastic tarp and drip irrigation, and much lower fumigation costs ($450 - 800/ac for Brussels sprout vs. $1,500 (bed fumigation) - $3,800 (flat fumigation) for strawberries) in Brussels sprout production are the main challenges we had to deal with. This required us to work with the growers to adapt the management approaches from one year to the next, based on what was learned from the previous season's experiments.

Table 1 summarizes the series of field experiments undertaken, the treatments applied at each location and other conditions such as soil temperature, extent of anaerobic conditions achieved with ASD and length of ASD treatment. Basic trials in which ASD (carbon source 9t/ac), MSM (1t/ac, tarped),

fumigation, and untreated check were compared (SC1, Wat1 and HMB1 in year 1, SC2, SC3, Wat2, and HMB3 in year 2, and SC4 and Wat3 in year 3) and C-source rate/tarp trials where we examined effects of carbon source rates and types in ASD, rates of MSM, or use of tarp, roller or non-tarp in ASD and MSM treatments against fumigation and un-treated check (HMB2 in year 1, HMB4 in year 2, and HMB5 and HMB6 in year 3). All trials were designed as completely randomized block experiments with 4 replications. Each plot was 24' x 24' consisting of 8 beds. In all ASD plots, C-source was applied and incorporated to a depth of ~6" with a rototiller, water was sprinkler irrigated for 2 to 3 hours (2-3 ac-inches), and the plots were covered by clear VIF or TIF the next day. The tarp was kept for 9 to 21 days depending on growers planting schedule (Table 1) during which soil temperature and soil Eh, an indicator of level of anaerobisis, were monitored at 6" depth using automated data loggers. For MSM plots, MSM was broadcasted and incorporated to a depth of ~6" with a rototiller, water was sprinkler irrigated for 2 to 3 hours (2-3 ac-inches), and the plots were covered by clear VIF or TIF the next day.

In year 1, grape pomace was used in SC1 due to its lower costs (approximately $300/ton for rice bran vs. $210/ton for grape pomace during the project period), local availability (in CA, 200,000 tons/yr for rice bran vs. 400,000 tons/yr for grape pomace) and its higher C/N ratio (18 for rice bran vs. 23 for grape pomace) which reduces the potential for early season nitrogen leaching. Additionally at SC1, instead of tarping the entire area of the ASD plots (flat treatment), we tarped individual beds leaving the furrow area untarped (bed treatment). However, for the rest of trials in year 1 and in all trials in year 2, we used rice bran as the carbon source for ASD and flat treatment as opposed to bed treatment because we found these methods to be more effective at creating anaerobic conditions than grape pomace and bed tarping. At HMB4 we compared different rates of MSM with and without tarp and both rice bran and grape pomace for ASD with and without tarp (Table 1). Additionally SC2 and HMB3 trials were conducted in early May, as opposed to early/mid June to see if ASD can be done under early season planting conditions. In year 3, additional treatments of no tarp but rolled flat in ASD and MSM were examined in HMB5 and HMB6. We switched back to grape pomace for ASD due to promising initial results that showed greater control of club root with this carbon source and its lower costs. Also we used powdered MSM instead of pelleted MSM this year, since it is reported to release active ingredients (allyisothiocyanates, AITCs) faster than the pelleted form (Mazzola, unpublished).

Table 1. Summary of Brussels sprout field trials and ASD treatment conditions

Year	Field (Trial name: soil type)	Planting - Harvest date (Variety)	Treatments	ASD treatment conditions			
				Cumulative Eh mV hrs <200mV	Average soil temp °F z)	Min-Max soil temp °F z)	Begin-End date (days)
Y1 2013-2014	Santa Cruz **(SC1:** Elkhorn sandy loam	6/18-11/18 (Confident)	1. ASD 9t/ac-grape pomace tarped 2. MSM 1t/ac tarped 3. Untreated check 4. Fumigation-Telon II 8gal/ac	1: 4,958	75	65 - 92	6/1-6/13 (13 days)
	Watsonville **(Wat1:** Baywood loamy sand**)**	7/3-12/19 (Cobus)	1. ASD 9t/ac-rice bran tarped 2. MSM 1t/ac tarped 3. Untreated check 4. Fumigation-Telon II 16gal/ac	1: 10,976	84	70-101	6/12-6/21 (9 days)
	Half Moon Bay **(HMB1:** Farallone coarse sandy loam**)**	7/16-1/3 (Topline)	1. ASD 9t/ac-rice bran tarped 2. MSM 1t/ac tarped 3. Untreated check 4. Fumigation-Vapam 20gal/ac Blocker4F 5gal/ac	1: 71,679	81	65 - 98	6/15-7/5 (20 days)
	Half Moon	7/1-1/6	1. ASD 4.5 t/ac-rice bran tarped	1: 9,071	1: 83	1: 67 -106	6/15-6/26

	Bay: Rate/tarp trial **(HMB2:** Botella clay loam**)**	(Genius)	2. ASD 6 t/ac-rice bran tarped 3. ASD 9 t/ac-rice bran tarped 4. ASD 9 t/ac-rice bran non-tarped 5. MSM 0.5 t/ac tarped 6. MSM 0.75 t/ac tarped 7. MSM 1 t/ac tarped 8. MSM 1 t/ac non-tarped 9. Untreated check 10. Fumigation - Vapam 20gal/ac	2: 10,487 3: 6,342 4: 3,542	9: 66	9: 59 - 78	(11 days)
Y2 2014-2015	Santa Cruz: Early planting **(SC2:** Watsonville loam**)**	6/3-11/10 (Confident)	1. ASD 9t/ac-rice bran tarped 2. MSM 1t/ac tarped 3. Untreated check 4. Fumigation-Telon II 8gal/ac	1: 60,199	79	68 - 88	5/2-5/12 (10 days)
	Santa Cruz **(SC3:** Watsonville loam**)**	6/18-11/21 (Confident)	1. ASD 9t/ac-rice bran tarped 2. MSM 1t/ac tarped 3. Untreated check 4. Fumigation-Telon II 8gal/ac	1: 60,188	77	67 - 88	6/6-6/17 (11 days)
	Watsonville **(Wat2:** Elder sandy loam**)**	7/10-1/9 (Cobus)	1. ASD 9t/ac-rice bran tarped 2. MSM 1t/ac tarped 3. Untreated check 4. Fumigation-Telon II 16gal/ac	1: 17,095	82	69 - 93	6/19-7/7 (18 days)
	Half Moon Bay: Early planting **(HMB3:** Farallone coarse sandy loam**)**	5/29-11/3 (Confident)	1. ASD 9t/ac-rice bran tarped 2. MSM 1t/ac tarped 3. Untreated check 4. Fumigation-Kapam 15.5gal/ac Blocker4F 5gal/ac	1: 113,331	84	65 - 100	5/3-5/23 (20 days)
	Half Moon Bay: Rate/tarp/C-source trial **(HMB4:** Farallone coarse sandy loam**)**	7/15-1/14 (Topline)	1. ASD 9 t/ac-rice bran tarped 2. ASD 9 t/ac-rice bran non-tarped 3. ASD 9 t/ac-grape pomace tarped 4. ASD 9 t/ac-grape pomace non-tarped 5. Mustard seed meal 1 t/ac tarped 6. Mustard seed meal 1 t/ac non-tarped 7. Mustard seed meal 0.5 t/ac tarped 8. Untreated check (UTC) 9. Fumigation- Kpam 15.5gal/ac Blocker4F 5gal/ac	1: 164,568 2: 34,706 3: 22,513 4: 1,576	1: 82 8: 69	1: 67-98 8: 62-78	6/10-7/2 (21 days)
Y3 2015-2016	Santa Cruz **(SC4:** Watsonville loam**)**	6/18-12/7 (Confident)	1. ASD 9t/ac-grape pomace tarped 2. MSM 1t/ac tarped 3. Untreated check 4. Fumigation-Telon II 8gal/ac	1: 31,124	78	64 - 97	6/8-6/18 (10 days)
	Watsonville **(Wat3:** Elder sandy loam**)**	6/13-12/14 (Cobus)	1. ASD 9t/ac-grape pomace tarped 2. MSM 1t/ac tarped 3. Untreated check 4. Fumigation-Telon II 16gal/ac	1: 93,302	82	70 - 96	5/21-6/9 (19 days)
	Half Moon Bay: Tarp vs roll trial **(HMB5:** Denison loam**)**	7/7-12/15 (Genius)	1. ASD 9 t/ac-grape pomace tarped 2. ASD 9 t/ac-grape pomace rolled non-tarped 3. MSM 1 t/ac tarped 4. MSM 1 t/ac rolled non-tarped 5. Untreated check 6. Fumigation- Telon II 12gal/ac Blocker4F 5gal/ac	1: 11,952 2: 2,034	1: 77 2: 71	1: 64 – 91 2: 61 - 82	6/12-7/2 (20 days)
	Half Moon	6/29-11/19	1. ASD 9 t/ac-grape pomace tarped	1: 11,211	1: 81	1: 65 – 90	6/3-6/19

	Bay: Tarp vs roll trial **(HMB6:** Tunitas clay loam**)**	(Confident)	2. ASD 9 t/ac-grape pomace rolled non-tarped 3. MSM 1 t/ac tarped 4. MSM 1 t/ac rolled non-tarped 5. Untreated check 6. Fumigation- Vapam 20gal/ac Blocker4F 5gal/ac	2: 4,071	2: 69	2: 62 - 77	(16 days)
Reference: for the control of *Verticillium dahliae* in strawberries (Shennan et al., 2009)				>50,000	> 68 during the first week of ASD treatment		>21 days

[z)] At 6" depth.

ASD conditions:

Both anaerobisis and soil temperature above threshold levels are required to achieve effective *Verticillium dahliae* control by ASD (Shennan et al., 2014). However, thresholds appear to be pathogen specific and those for cyst nematode and club root disease are unknown. Cumulative Eh mV hours below 200 mV (Cum Eh), an indicator of intensity and duration of anaerobisis (the greater the number, the stronger the anaerobic condition), and soil temperature during ASD treatment at 6" depth were monitored at each trial. The average Cum Eh of ASD rice bran or grape pomace 9 tons/ac tarped plots across all 13 trials were 50,533 mV hrs (min. 4,958 mV hrs, max. 164,569 mV hrs). Only six out of 13 trials had the average Cum Eh above 50,000 mV hrs, the threshold for controlling *Verticillium dahliae* (Shennan et al.,2009). Overall strong coastal winds, typical in May to June in the region, made tarp maintenance extremely challenging. Tarps were occasionally blown off by the strong wind interrupting anaerobic condition. Also ASD treatment period averaged only 15 days (min. 9 days, max 21 days), which was shorter than a typical 21 day-ASD treatment for strawberries. Collaborating growers and researchers did their best to extend the treatment period, but pre-determined planting dates and other work schedule issues often hindered a longer treatment period. Rice bran-based ASD tended to create a stronger anaerobic condition than grape pomace-based ASD (see Cum Eh values of HMB4 trial in table 1) though the latter was more effective in reducing club root in HMB4 trial (see club root section). Without tarp, it was difficult to maintain a strong anaerobic condition (see HMB4 in table 1) even with soil compaction by a roller (see HMB5 and HMB6 in Table 1). Soil temperature at 6" depth during ASD averaged 80 °F (min. 75 °F – max. 84°F) across all trials including the ones initiated in early May. Use of clear tarp in all trials raised soil temperature 10 °F or more compared to non-tarped plots due to its solarization effect. Correlations between Cum Eh, soil temperature, and PPN and CR reduction are discussed later.

Plant parasitic nematode (PPN):

PPN samples were taken pre- and immediately post-treatment at each trial. Twenty sub-samples at 0' – 12" depth moist soil were randomly collected from each plot to form a composite sample per plot. To reduce spatial sampling errors, we introduced a grid sampling method in year 2. In theory, this method allows us to take pre- and post-treatment soil samples at virtually the same location reducing sampling errors caused by the special variability of PPN population. We used this method for all PPN samplings thereafter. The soils were shipped to the Nematology lab at UC Riverside where cysts of both sugar beet cyst nematode (*Heterodera schachtii*) and cabbage cyst nematode (*H. cruciferae*) were extracted from 500 g air-dried soil using a Fenwick can (Fenwick, 1940), and they were further separated from soil debris using a glycerine-ethanol mixture. Cysts were then collected and counted under a dissecting microscope at 40 x magnification. Five cysts were handpicked at random, squashed individually, and

number of viable eggs/larvae estimated. Data were expressed as PPN eggs and larvae number per 100 ml of dry soil. To evaluate treatment effect, ANCOVA analysis was conducted for Log (x+2) transformed post-treatment PPN data using similarly transformed pre-treatment PPN data as a covariate. Table 2 shows a summary of PPN analysis including results of ANCOVA. Results of ANOVA of pre- and post-treatment samples were also included as references.

Overall, PPN data were highly variable. Pre-treatment PPN data revealed that low (0/100 ml dry soil) to extremely high (23,958/100 ml dry soil) PPN populations existed across all trial sites (Table 2. Average 3,619/100 ml dry soil). Westerdahl et al (1998) suggested Brussels sprouts' damage threshold by sugarbeet cyst nematode being 2,000/100 ml dry soil. According to this threshold, assuming cabbage cyst nematodes can cause similar damage with sugar beet cyst nematodes on Brussels sprout, 8 sites (SC1, SC2, SC4, Wat1, Wat2, Wat3, HMB5 and HMB6) out of 13 sites had a damaging level of average PPN population at pre-treatment. Although the average was numerically lower than pre-treatment PPN population, post-treatment PPN populations were also highly variable; average: 1,950/100 ml dry soil, min.: 0 and max.: 13,951. Unfortunately, no consistent treatment effect was observed for post-treatment PPN populations even in the fumigation treatment (Table 2). Across all 13 trials, ANCOVA of post-treatment PPN was significant only in 2 trials; SC3 and HMB4. In SC3, MSM-tarped treatment had significantly lower PPN than UTC ($P<0.05$. Fumigation plots were not included in the analysis since pre-treatment samples were not collected at fumigated plots due to the schedule conflict). In HMB4, UTC had lower PPN than ASD-rice bran-tarped plots and fumigated plots ($P<0.10$). No treatment effects were found even when data were combined in each year, each location and across all three years from all locations (Table 2), and there was no correlation between post PPN populations and Brussels sprout relative total yields ($P<0.61$).

Soil PPN assessment is notorious for being highly variable due to sampling errors, patchy spatial distribution of nematodes, and the fact that cysts themselves can remain in soil for a long time even in absence of host plants and/or fumigation. We introduced the grid method in year 2 but it did not reduce variability of PPN data to the extent that we could observe consistent treatment effects. Increasing the number of sub-samples could reduce the variability further but it may be labor-prohibitive. More studies on PPN sampling methods are needed. At the same time, future studies may want to use a burial/retrieval approach using PPN inocula of known population size in order to evaluate treatment effects. Also long-term (multiple year) monitoring may be necessary to detect any treatment effects since PPN numbers may change over time as a result of treatment effects on nematode multiplication (i.e. Cysts that are killed because of some treatments will still be present in soil and counted, but no J2 will hatch, and therefore longer term nematode build-up would be reduced.

For ASD plots, Cum Eh and PPN ratio (log-transformed post-treatment PPN/ log-transformed pre-treatment PPN) were not correlated when data from all trials and years are combined (Figure 1A). However, when data from all Watsonville trials are used, it showed a clear trend indicating a threshold of around 50,000 mV hours (Figure 1B. $P=0.0003$***). Future studies using pot and field experiments should be conducted to confirm this trend.

Table 2. Summary of soil PPN population pre- and post-treatments in all trials

Year	Trial	Treatment	Cyst nematode egg/larvae population (#/100ml dry soil)	
			Pre-treatment	Post-treatment
Yr 1	SC1	UTC	3995	5888
		ASD-GP-tarped	5451	3724

		MSM1-tarped	1960	3403
		Fum	7919	4804
		ANOVA (P)	*0.072*	*0.82*
		ANCOVA (P)	-	*0.87*
	Wat1	UTC	4625	4888
		ASD-RB-tarped	14113	7383
		MSM1-tarped	9291	4182
		Fum	15548	2579
		ANOVA (P)	*0.39*	*0.37*
		ANCOVA (P)	-	*0.12*
	HMB1	UTC	774	714
		ASD-RB-tarped	679	585
		MSM1-tarped	894	205
		Fum	1723	628
		ANOVA (P)	*0.96*	*0.17*
		ANCOVA (P)	-	*0.14*
Yr 2	SC2	UTC	6031	2377
		ASD-RB-tarped	4061	3217
		MSM1-tarped	5346	4350
		Fum	2270	731
		ANOVA (P)	*0.28*	*0.07*†
		ANCOVA (P)	-	*0.10*
	SC3	UTC	2104	2397 A*
		ASD-RB-tarped	1540	1214 AB
		MSM1-tarped	1752	599 B
		Fum	no sample	(2219)
		ANOVA (P)	*0.72*	*0.25*
		ANCOVA (P)	-	*0.01**
	Wat2	UTC	1982	1599
		ASD-RB-tarped	4852	2522
		MSM1-tarped	2691	463
		Fum	3160	1100
		ANOVA (P)	*0.28*	*0.099*†
		ANCOVA (P)	-	*0.14*
	HMB3	UTC	2450	929
		ASD-RB-tarped	1483	2041
		MSM1-tarped	1040	1813
		Fum	1340	1249
		ANOVA (P)	*0.80*	*0.78*
		ANCOVA (P)	-	*0.51*
	HMB4	UTC	1614 A†	786 B†
		ASD-RB-tarped	2637 A	2350 A
		MSM1-tarped	507 B	1706 AB
		Fum	2373 A	2252 A
		ANOVA (P)	*0.003***	*0.063*†
		ANCOVA (P)	-	*0.093*†
Yr 3	SC4	UTC	2755	468
		ASD-GP-tarped	1881	762
		MSM1-tarped	2569	1541
		Fum	3010	446
		ANOVA (P)	*0.93*	*0.24*
		ANCOVA (P)	-	*0.25*
	Wat3	UTC	989 B*	708
		ASD-GP-tarped	4319 AB	269
		MSM1-tarped	3501 AB	1624
		Fum	6307 A	1750

		ANOVA (P)	*0.039**	*0.25*
		ANCOVA (P)	-	*0.54*
	HMB5	UTC	5410	1387
		ASD-GP-tarped	1266	1283
		ASD-GP-rolled	1044	1098
		MSM1-tarped	2491	596
		MSM1-rolled	1526	818
		Fum	2062	161
		ANOVA (P)	*0.28*	*0.44*
		ANCOVA (P)	-	*0.61*
	HMB6	UTC	4464 A*	1873
		ASD-GP-tarped	4751 A	4713
		ASD-GP-rolled	7446 A	1345
		MSM1-tarped	7194 A	1034
		MSM1-rolled	4325 A	3630
		Fum	1056 B	990
		ANOVA (P)	*0.0017***	*0.23*
		ANCOVA (P)	-	*0.48*
All sites Year 1 (n=3)		UTC	3131	3830
		ASD-GP/RB-tarped	6748	3897
		MSM1-tarped	4049	2597
		Fum	8397	2670
		ANOVA (P)	*0.24*	*0.35*
		ANCOVA (P)	-	*0.22*
All sites Year 2 (n=4)		UTC	2836	1618
		ASD-RB-tarped	2914	2269
		MSM1-tarped	2267	1786
		Fum	2286	1510
		ANOVA (P)	*0.31*	*0.72*
		ANCOVA (P)	-	*0.97*
All sites Year 3 (n=4)		UTC	3405	1109
		ASD-GP-tarped	3054	1757
		MSM1-tarped	3939	1199
		Fum	3109	837
		ANOVA (P)	*0.57*	*0.98*
		ANCOVA (P)	-	*0.92*
SC all years (n=3)		UTC	4260	2911
		ASD-GP/RB-tarped	3797	2568
		MSM1-tarped	3292	3098
		Fum	4400	1994
		ANOVA (P)	*0.99*	*0.38*
		ANCOVA (P)	-	*0.22*
HMB all years (n=5)		UTC	2943	1138
		ASD-RB/GP-tarped	2163	2194
		MSM1-tarped	2425	1071
		Fum	1711	1056
		ANOVA (P)	*0.51*	*0.44*
		ANCOVA (P)	-	*0.55*
Wat all years (n=3)		UTC	2532	2399
		ASD-RB/GP-tarped	7761	3392
		MSM1-tarped	5161	2090
		Fum	8338	1810
		ANOVA (P)	*0.12*	*0.76*
		ANCOVA (P)	-	*0.93*
All trials, all years (n=11)		UTC	3190	1965
		ASD-RB/GP-tarped	4136	2623

	MSM1-tarped	3408	1901
	Fum	4252	1517
	ANOVA (P)	*0.68*	*0.76*
	ANCOVA (P)	-	*0.89*

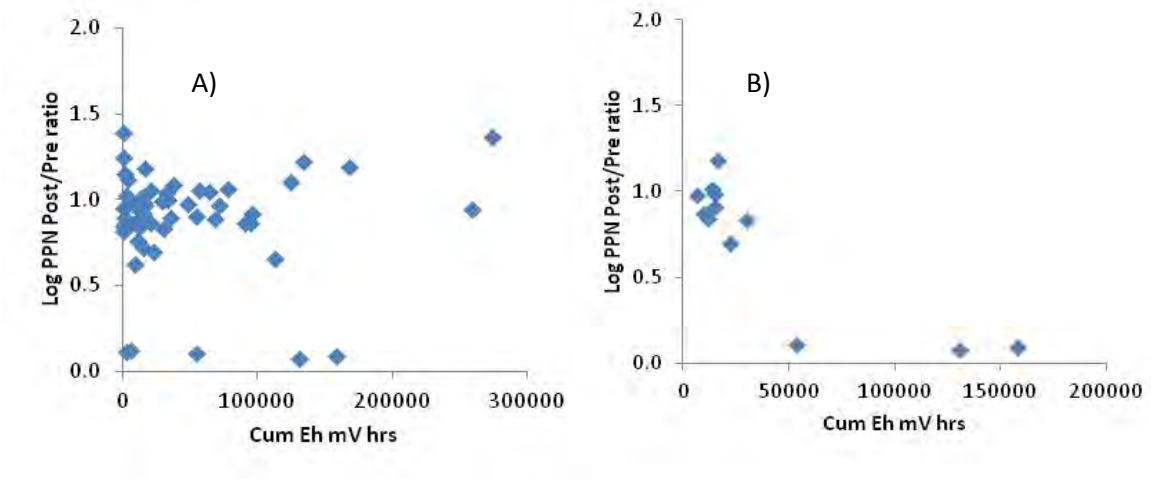


Figure 1. Correlations between cumulative Eh (mV hours) below 200 mV and plant parasitic nematode (PPN) ratio (Log (2+post-plant PPN)/Log (2+pre-plant PPN)) in all ASD plots across all trials (A) and ASD plots in 3 Watsonville trials only (B).

Club root disease:

Club root disease levels were visually evaluated at harvest for both tap roots using 1 (healthy) to 4 (worst) scale and feeder roots using 1 (healthy) to 5 (worst) scale of 20 plants per plot. Among 13 trials, club root disease was found at 5 trials; HMB, HMB3, HMB4 and HMB6, where PCNB was applied to the fumigation plots, and SC4 where there was no history of the disease and PCNB was not applied. Results of the disease scores for tap roots and feeder roots, together with ANOVA results and P values for correlations between club root damage scores and total Brussels sprout yields are summarized in Table 3.

Significant difference in treatments in club root scores were found in 3 out of 5 trials with the disease. HMB1 site showed that ASD rice bran 9 t/ac with tarp and MSM 1ton/ac with tarp treatments were as effective a control for club root disease on tap roots as PCNB-added fumigation treatment compared to UTC ($P<0.05$). In HMB6, although no difference was found in tap root disease scores, ASD with grape pomace 9 tons/ac with tarp treatment had the lowest feeder root club root score and the fumigated treatment was the worst ($P<0.10$). In SC4, ASD using grape pomace 9 tons/ac with tarp treatment had the best tap root score and was significantly better than UTC ($P<0.10$). Overall, ASD using grape pomace or rice bran at 9 tons/acre with tarp appears to be the best alternative to PCNB treatment among treatments tested in terms of club root disease suppression. However, comparison of MSM 1 t/ac, ASD grape pomace 9 t/ac tarped or compacted with a roller merits further investigation. Club root disease generally affected Brussels sprout yield negatively in all but one trial; In HMB3 tap root club root scores had a slightly positive correlation with total yield for unknown reason. When all club root scores are combined, tap root scores showed much stronger negative correlation with the relative total yield (Figure 2A) compared to feeder roots (Figure 2B). This may be because tap root infection occurs earlier than

feeder root infection, and that in general levels of feeder root infection were low. For ASD plots, no significant correlation was found between Cum Eh and club root scores of either tap roots or feeder roots (data not shown).

Table 3. Summary of club root damage on Brussels sprout roots (club root infested trials only).

Trial	Treatment	Club root damage	
		Tap roots (1: healthy – 4: worst)	Feeder roots (1: healthy – 5: worst)
HMB1	UTC	2.4 A*	1.5
	ASD-RB-tarped	1.1 B	1.1
	MSM-tarped	1.1 B	1.2
	Fumigant+PCNB	1.0 B	1.7
	ANOVA (P)	*0.014**	*0.16*
	R^2 (P) vs.Total yield	*0.006***	*0.20*
HMB3	UTC	1.9	2.0
	ASD-RB-tarped	2.7	1.7
	MSM-tarped	2.5	2.1
	Fumigant+PCNB	2.9	2.3
	ANOVA (P)	*0.27*	*0.18*
	R^2 (P) vs.Total yield	*0.07†*	*0.39*
HMB4	UTC	1.5	1.2
	ASD-RB-tarped	1.3	1.2
	ASD-RB-nontarped	1.3	1.2
	ASD-GP-tarped	1.1	1.1
	ASD-GP-nontarped	1.5	1.1
	MSM 0.5-tarped	1.2	1.1
	MSM1-tarped	1.1	1.1
	MSM1-nontarped	1.5	1.2
	Fumigant+PCNB	1.0	1.0
	ANOVA (P)	*0.56*	*0.30*
	R^2 (P) vs.Total yield	*0.003***	*0.048**
HMB6	UTC	2.6	1.7 AB†
	ASD-GP-tarped	1.9	1.3 B
	ASD-GP-rolled*	2.6	1.6 AB
	MSM-tarped	1.9	1.5 AB
	MSM-rolled*	2.3	1.6 AB
	Fumigant+PCNB	2.4	2.1 A
	ANOVA (P)	*0.78*	*0.13*
	R^2 (P) vs.Total yield	*0.0013***	*0.19*
SC4	UTC	1.9 A†	1.3
	ASD-GP-tarped	1.1 B	1.0
	MSM-tarped	1.3 AB	1.1
	Fumigant	1.4 AB	1.1
	ANOVA (P)	*0.085†*	*0.16*
	R^2 (P) vs.Total yield	*0.087†*	*0.25*
All trials	**UTC**	**2.1**	**1.5 AB***
	ASD-tarped	**1.6**	**1.2 B**
	MSM-tarped	**1.6**	**1.4 AB**
	Fum	**1.7**	**1.6 A**
	ANOVA (P)	***0.25***	***0.03****
	R^2 (P) vs.Total yield	***4.4E-06*****	***0.0017*****

Means with the same letters do not have significant difference at *P*=0.05 (*) or 0.10 (†) according to Tukey's HSD test.

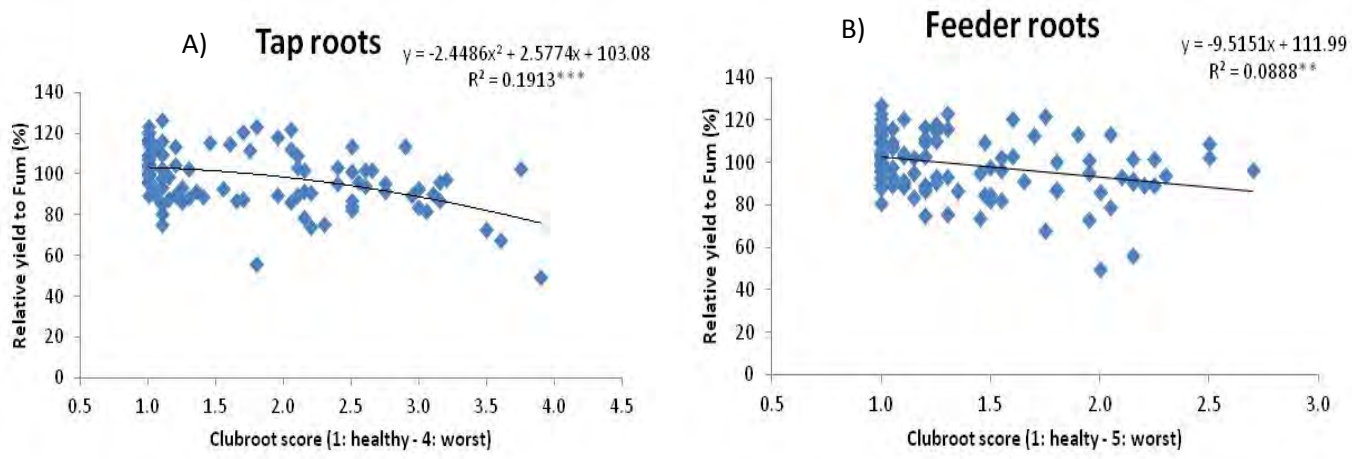


Figure 2. Correlations between club root scores and relative total yield of Brussels sprout. A: tap root club scores, B: feeder root club root scores. Data from 5 trials with club root disease were used. Total yield of each plot of each trial was standardized by dividing with the average yield of fumigation treatment of each trial and expressed as a relative yield to fumigation plot (%).

Cabbage maggot (CM):

Root Damage Scores. In Y1 to test the efficacy of ASD to reduce damage to Brussels sprouts caused by CM we assessed the roots for CM tunneling at harvest and assigned scores of 1: none - 3: severe for 5-10 plants per plot. We did not find any statistically significant differences in damage score among treatments (Figure 3). Furthermore we did not see any correlation between damage score and yield. One possible reason for this is that by late season it is likely the ASD treatment effect has worn off and any late season tunneling, although presenting as a high damage score, most likely had minimal impact on the growth of the plant. Thus a high damage score does not necessarily correlate to lower yields. Due to the lack of correlation and extremely difficult nature of cleaning and scoring hundreds of roots this measurement is not a good indicator of CM damage and we decided to forego CM root damage score at harvest for Y2 and Y3.

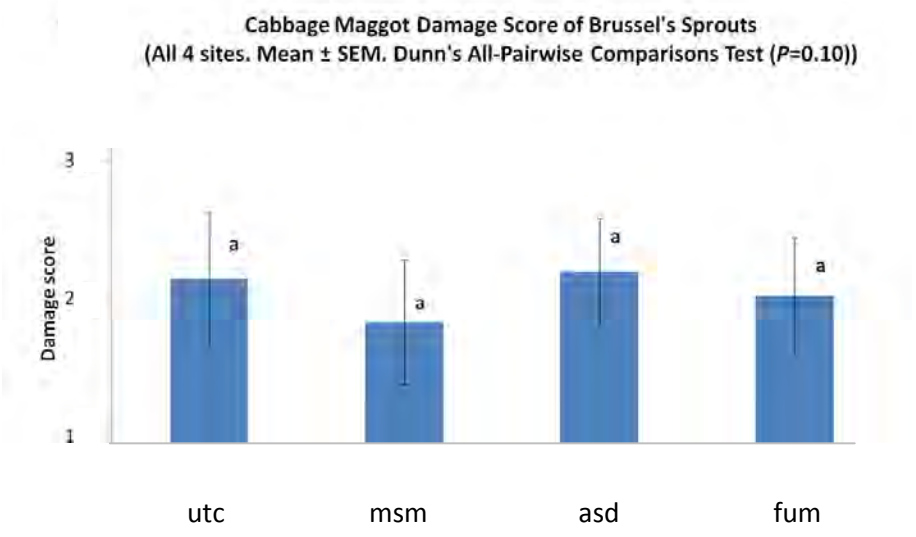


Figure 3. Y1 CM damage score of all 4 sites combined.

Deterrence of Egg Laying. Another way we tested the ability of ASD and MSM application to reduce CM damage in Brussel sprouts was by doing egg counts post treatment to see if there was deterrence in cabbage root fly egg laying caused by treatment. To do this we placed sand traps at the base of the plants, left them for a week, then retrieved them and counted the number of eggs laid. This sampling technique was developed as part of Objective 3 and will discussed in more detail later. In Y1 we only saw a treatment effect at one site, HMB1. Our findings show deterrence in egg laying for up to two weeks post ASD treatment. In Y2 when looking at all 5 sites combined we again see the same egg laying deterrence in ASD treated plots (Figure 4). In Y3 however, both separately and when looking at all 4 sites combined we did not see the same trend as Y1 and Y2. Unlike HMB1 and trials in year 2, grape pomace, instead of rice bran, was used as the carbon source for ASD in year 3. Thus, type of carbon source may have effect on deterrence of egg laying by ASD.

Pupae Burial Another way we tested the efficacy of ASD to reduce CM damage in Brussel sprouts was by burying CM pupae pre-treatment and retrieving/rearing them to emergence post treatment. In Y1 we had a difficult time successfully rearing CM to emergence post treatment. We mostly lost pupae to desiccation and hadn't accounted for large amounts of parasitism in the field collected pupae. Going into Y2 we fine-tuned our rearing technique to include daily maintenance of pupae and maintained a colony of CM so as to not have to worry about parasitism. Maintaining a colony had its own set of difficulties, however, and after several colony crashes and barely having enough CM pupae for the experiments we decided to test housefly (HF) pupae in conjunction with CM pupae so as to use HF as surrogates for CM. HF pupae can easily be purchased from an insectary at reasonable prices making it an ideal surrogate if they proved to behave similarly to CM. To test the comparability of HF to CM we conducted an in lab potted experiment where we buried both HF pupae and CM pupae in soil in ASD treated pots and untreated control pots at a low temperature of 64 degrees Fahrenheit and a high temperature of 75 degrees Fahrenheit. Data from this study showed good indication that HF performs comparably to CM, and also that ASD treatment reduces pupae emergence relative to the untreated control (Figure 5).

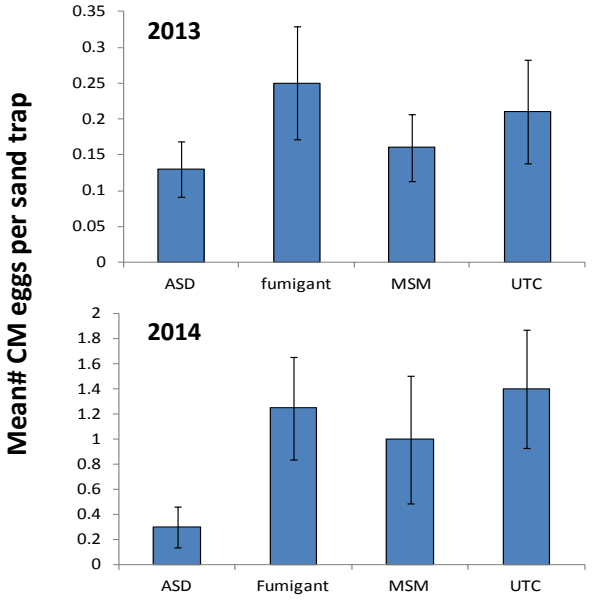


Figure 4. Top-Y1 mean # of cabbage maggot eggs per sand trap at HMB1. Bottom-Y2 mean # of cabbage maggot eggs per sand trap at all 5 sites combined.

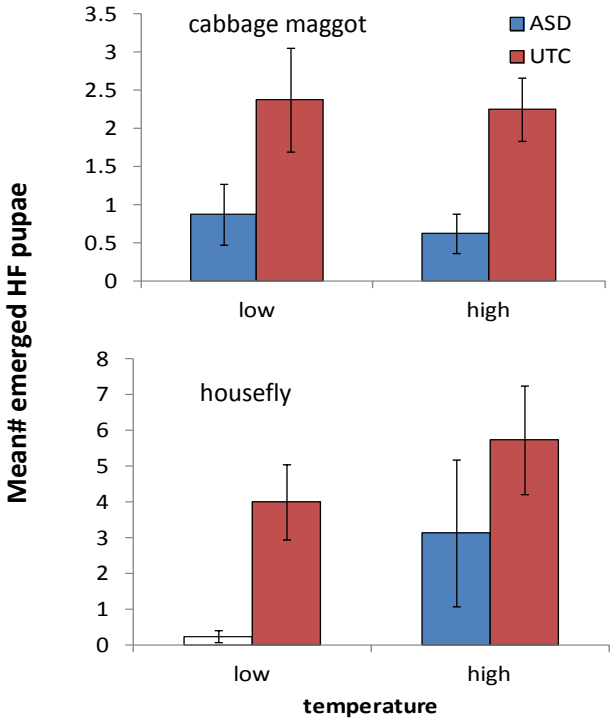


Figure 5. Mean # of emerged flies (top-cabbage maggot, bottom-housefly) from incubated pot trial at 2 temperatures.

In Y2 we did not have enough CM pupae to place in the field trials due to an ill-timed colony crash. Given the promising results from our pot study we decided to go forward with using HF pupae as surrogates for CM. Results from Y2 again showed reduced emergence of ASD treated pupae (Figure 6).

In Y3 our colony was productive enough for us to put CM pupae in the field trials but we decided to continue to use HF to further test their comparability. Results from Y3 data again indicated reduced emergence of ASD treated pupae relative to untreated control pupae and comparable responses between HF and CM pupae. Overall with the 3 years combined data our findings indicate that ASD effectively reduces emergence of CM adults to 20%, whereas the untreated control showed an emergence rate of 50% (Figure 7). Additionally our findings indicate that HF behave comparably to CM in regards to ASD trials and can therefore be used as a surrogate. Future work aimed at testing the efficacy of ASD to control for CM in Brussels sprouts should look at ASD in combination with trap crops to see what can be done to mitigate against the re-entry of CM into a field that has had ASD applied to it.

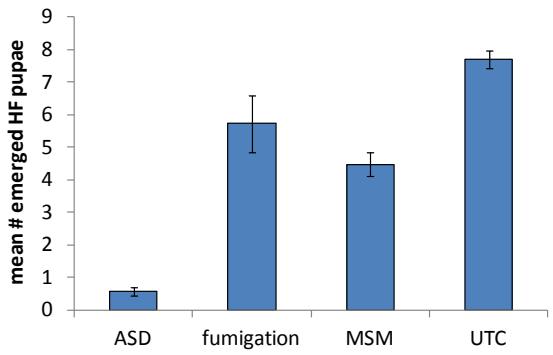


Figure 6. Mean # of emerged houseflies from all Y2 fields.

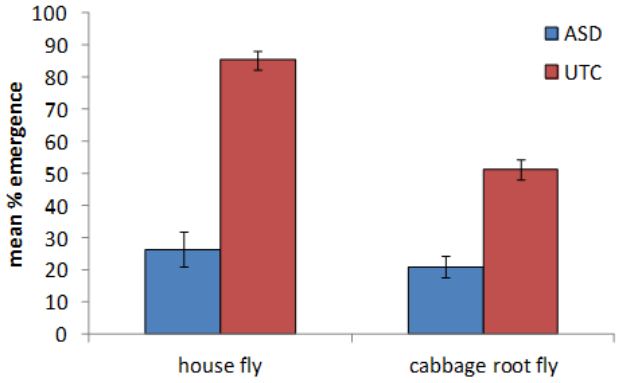


Figure 7. Three years of combined data for mean % CM and HF fly emergence in ASD treated and untreated controls

Soil inorganic N:

To examine the effect of ASD and MSM on N dynamics in Brussels sprout production, soil inorganic N contents in top soil (0”-6” depth) were monitored as nitrate (NO_3-N) and ammonium (NH_4-N) pre- and post-treatments, and then monthly during the cropping season in most trials. Ten to twenty sub samples were collected from each plot to form a composite sample and ammonium and nitrate were extracted using 2M KCl solution and determined by FIA methods (Lachat instruments, 1993, 1993).

Generally, effects of ASD and MSM on soil N dynamics appeared for a short period of time after treatment application (Figure 8 A to V). For rice bran-based ASD, the effect was an increase of ammonium (10 to 55 mg/kg) immediately post-treatment (Wat1 (Figure 8D), HMB1 (Figure 8F), SC2 (Figure 8H), SC3 (Figure 8J), Wat2 (Figure 8L), and HMB4 (Figure 8P)). In some cases, a decrease of nitrate also occurred (HMB1 (Figure 8E), SC2 (Figure 8G), Wat2 (Figure 8K), and HMB4 (Figure 8O)). This trend usually disappeared within a month. This result is similar to the trend found in flat ASD treated strawberry systems where, compared to bed ASD treatment, much less effect on soil N dynamics was observed (Muramoto et al., 2015).

Grape pomace-based ASD reduced nitrate levels (SC1 (Figure 8A), SC4 (Figure 8Q), Wat3 (Figure 8S) and HMB5 (Figure 8U)) and only minimally increased of ammonium (<10 mg/kg) (SC1 (Figure 8B), SC4 (Figure 8R), Wat3 (Figure 8T) and HMB5 (Figure 8V)). Such difference in the effect of carbon sources on N dynamics has been observed in ASD in strawberry systems (Zavatta et al., 2014) and is attributed to the difference in C/N ratio of the carbon sources (18 for rice bran vs. 23 for grape pomace). N immobilization by grape pomace lasted for another month after ASD treatment in SC4 (Figure 8Q), Wat3 (Figure 8S) and HMB5 (Figure 8U), which might have contributed to the lower yield found in this treatment in some trials. Notably, both rolled and tarped ASD grape pomace treatments showed N immobilization in HMB5 (Figure 8U) though yield was significantly lower in tarped ASD grape pomace plots than rolled. There might be some other factors that negatively affect sprout growth in ASD (see yield section for further discussion).

Effects of MSM on soil N dynamics were to increase nitrate (SC1 (Figure 8A), SC2 (Figure 8G), Wat 3 (Figure 8S), and HMB5 (Figure 8U)) and moderately increase ammonium (Wat1 (Figure 8D), HMB1 (Figure 8F), SC3 (Figure 8J), Wat2 (Figure 8L), and SC4 (Figure 8R)) immediately post-treatment. The effect did not show up for the rest of the growth period in most trials except high nitrate in MSM plots at HMB5 lasted for 2 more months (Figure 8U)). Soil nitrate dynamics in SC2 (Figure 8G) were rather unique. This heavy soil took about a month to dry out after ASD and other treatments, which delayed transplanting, slowed down the nitrification process, and resulted in a lower overall sprout yield (see table 5).

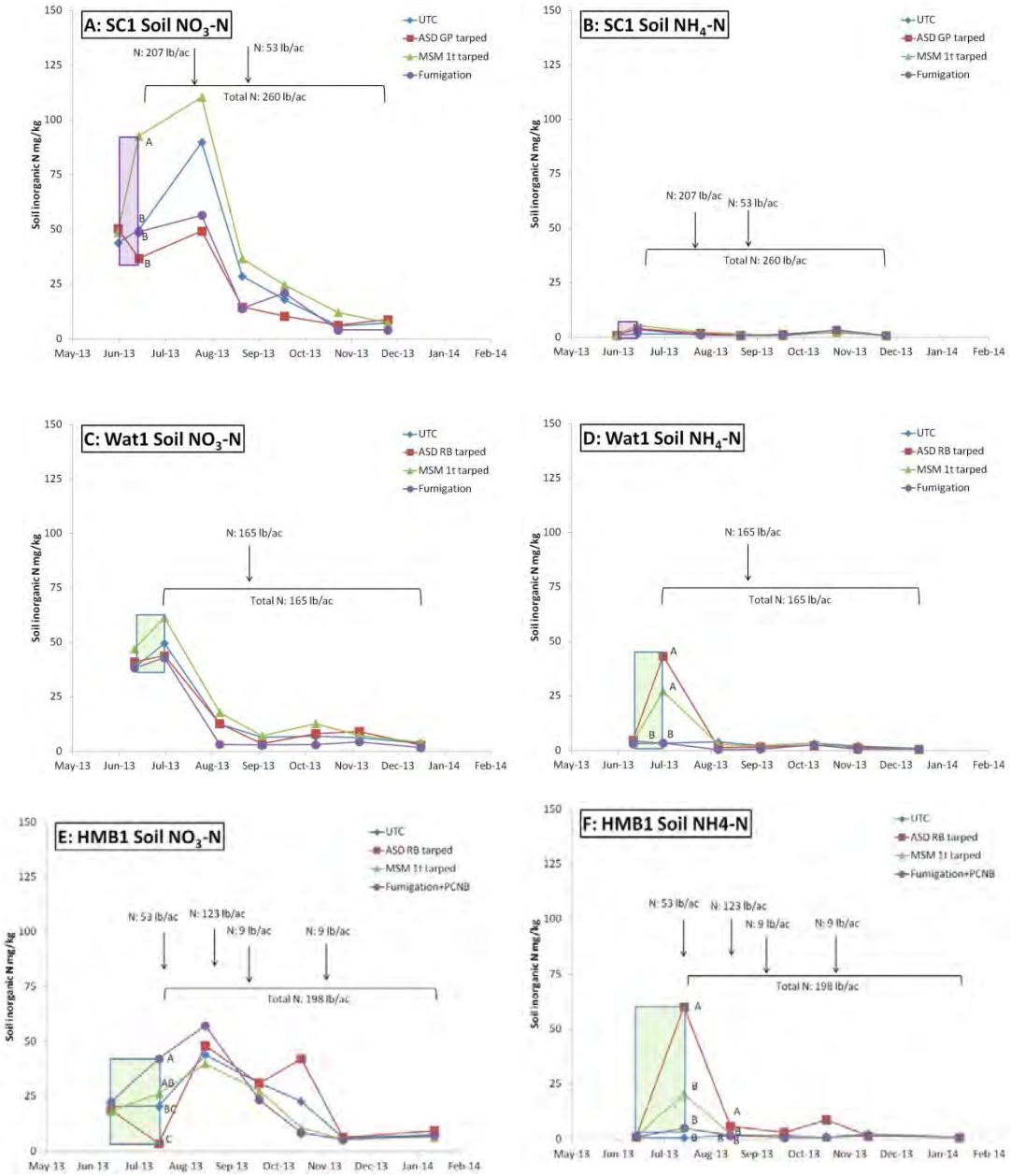
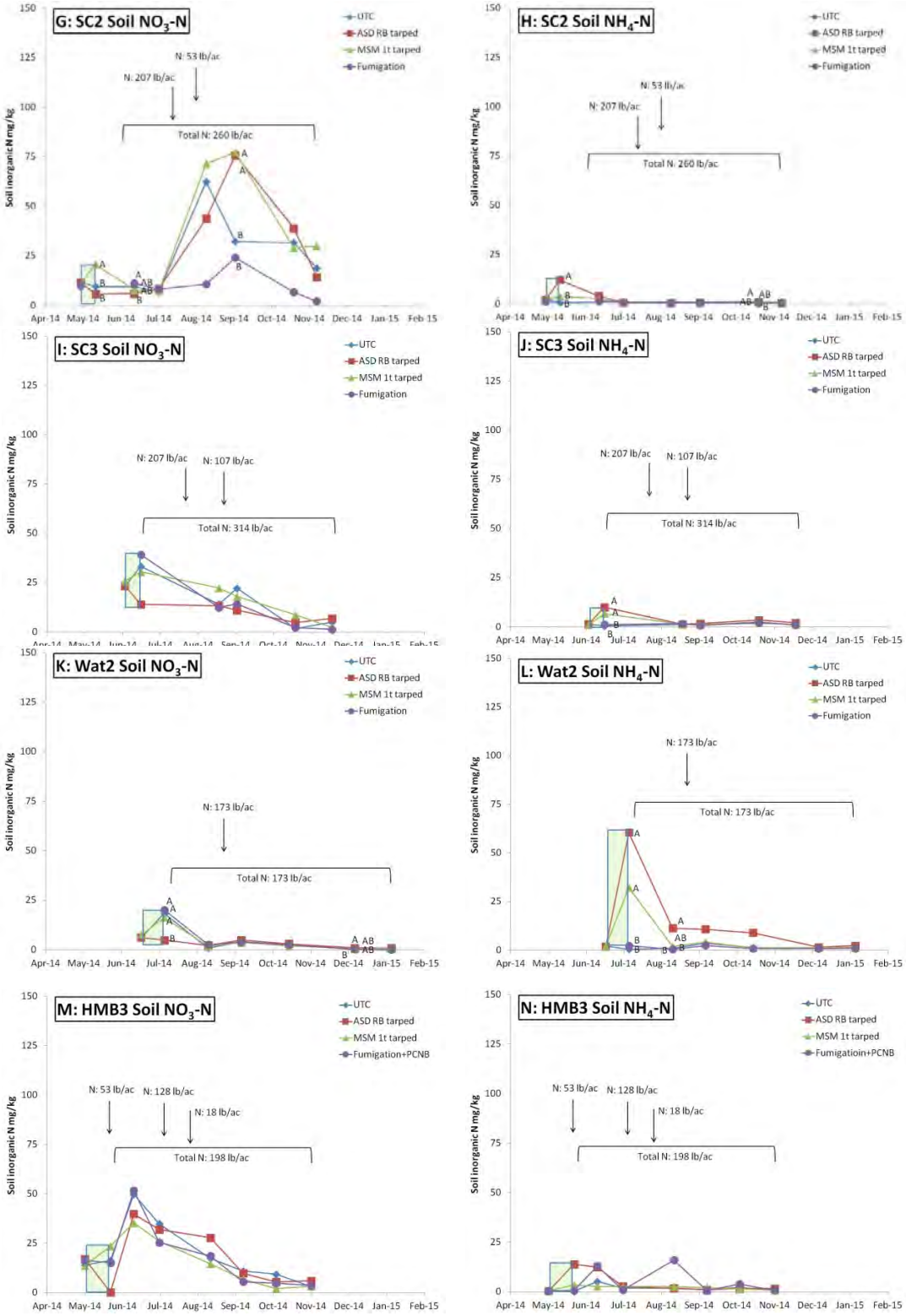


Figure 8A-F. Soil inorganic N dynamics at 0”-6” depth in Brussels sprout field trials in year 1. Bracket indicates growth period of Brussels sprouts. Each arrow shows time that indicated rate of N was applied. Rectangle indicates tarped period for ASD and MSM treatments. Green and purple rectangle shows rice bran-based ASD and grape pomace-based ASD, respectively. Means with the same letters on the same date are not significantly different according to Tukey’s HSD test at P=0.05. ASD: anaerobic soil disinfestation. MSM: mustard seed meal. RB: rice bran 9t/ac rate. GP: grape pomace. Tarped: covered with clear TIF.


G: SC2 Soil NO_3-N
H: SC2 Soil NH_4-N
I: SC3 Soil NO_3-N
J: SC3 Soil NH_4-N
K: Wat2 Soil NO_3-N
L: Wat2 Soil NH_4-N
M: HMB3 Soil NO_3-N
N: HMB3 Soil NH_4-N
UTC
ASD RB tarped
MSM 1t tarped
Fumigation
Fumigation+PCNB
Fumigatioin+PCNB
N: 53 lb/ac
N: 207 lb/ac
N: 107 lb/ac
N: 173 lb/ac
N: 128 lb/ac
N: 18 lb/ac
Total N: 260 lb/ac
Total N: 314 lb/ac
Total N: 173 lb/ac
Total N: 198 lb/ac
Soil inorganic N mg/kg
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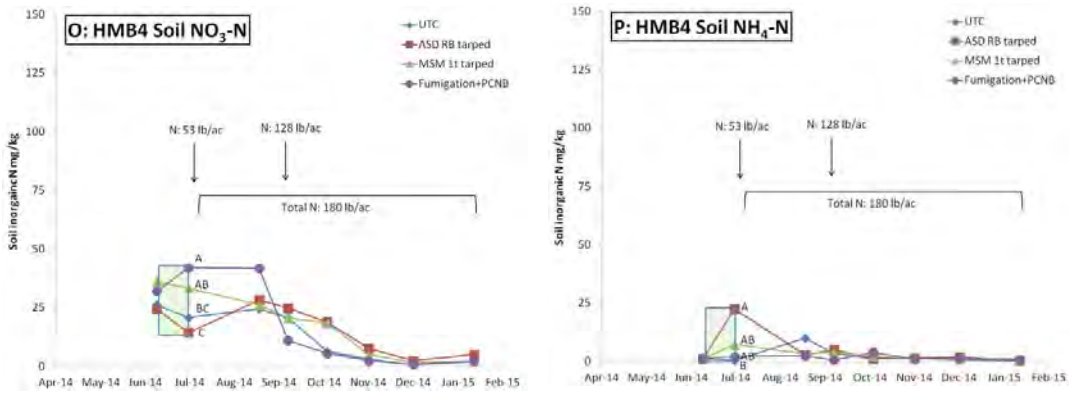

Figure 8G-P. Soil inorganic N dynamics at 0”-6” depth in year 2. See legends for Figure 8A-F.

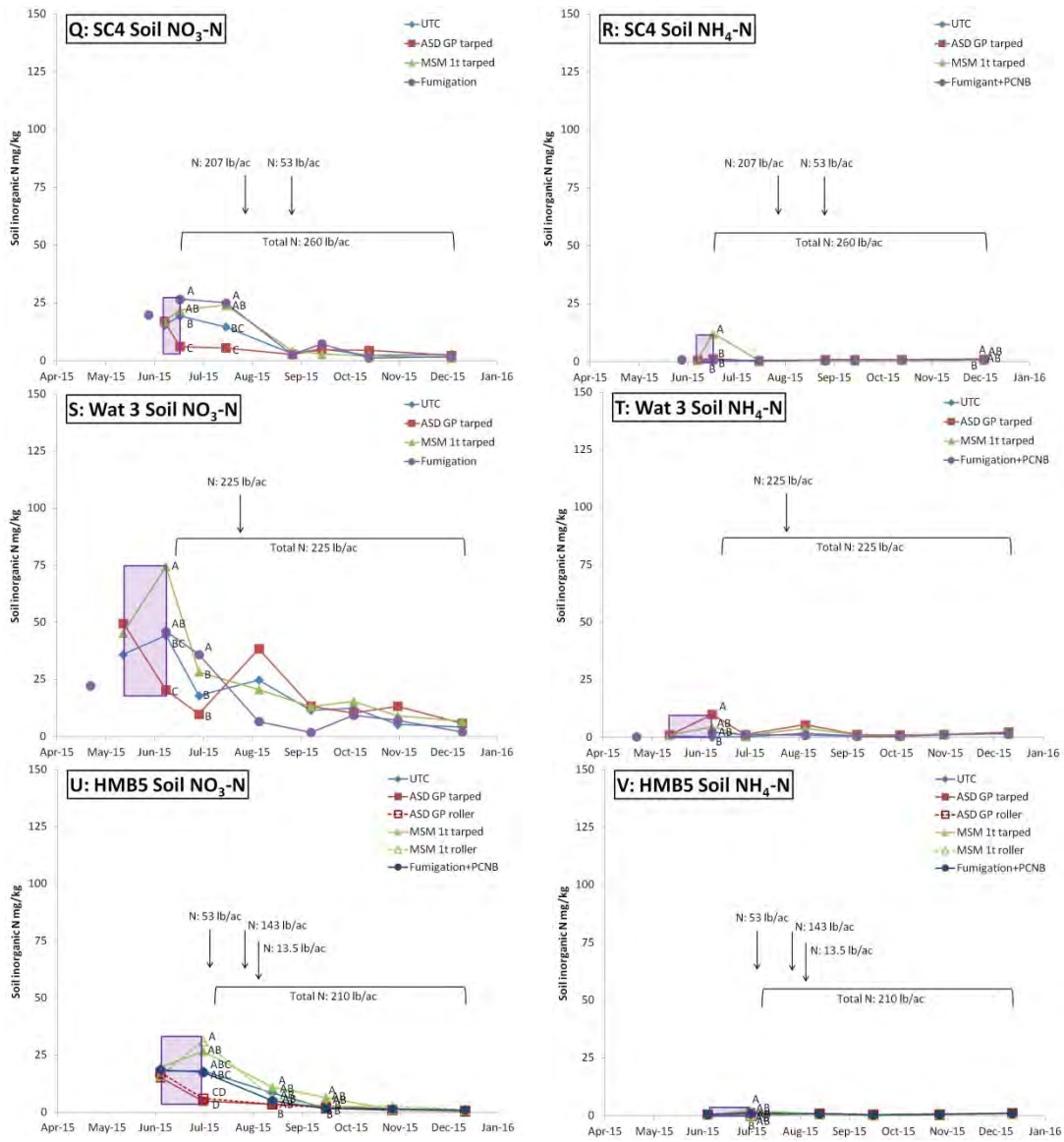

Figure 8Q-V. Soil inorganic N dynamics at 0”-6” depth in year 3. See legends for Figure 8A-F.

Soil pH:

Maintaining soil pH equal or above 7.2 is recommended to control club root disease in brassica crops (Heinrich and Stone, 2013). Thus sprout growers in this region lime their fields every spring. ASD can lower soil pH due to acids produced in the process (Momma et al, 2006; Muramoto et al., 2016). Thus effect of ASD on soil pH in these trials was examined. We took 0”- 6” depth soil samples as described previously at harvest at ASD, MSM, fumigation and UTC plots in selected trials (SC1, SC2, SC3, SC4, Wat2, Wat3, HMB4, HMB5, and HMB6) and measured pH (1:1) (USDA, 1998). In average, ASD treatment reduced soil pH by 0.1 from fumigated control (Table 4).

Table 4. Effect of ASD and MSM on soil pH at harvest in Brussels sprout field trials (9 trials from 3 years).

Year	Treatment	pH (1:1) at harvest
All 3 years (n=9)	UTC	7.36 AB
	ASD	7.28 B
	MSM	7.34 AB
	FUM	7.39 A
	ANOVA (*P*)	*0.03**

Means with the same letters do not have significant differences at *P*=0.05 according to Tukey’s HSD test.

Further, soil pH at harvest had a negative correlation with club root score for both tap roots (Figure 9A) and feeder roots (Figure 9B). Thus it is important to maintain pH above 7.2 by liming though the threshold appears to work well for feeder roots but less so for tap roots (Figure 9A and 9B). Reduction of pathogen population in soil by additional means may be necessary to completely control the disease.

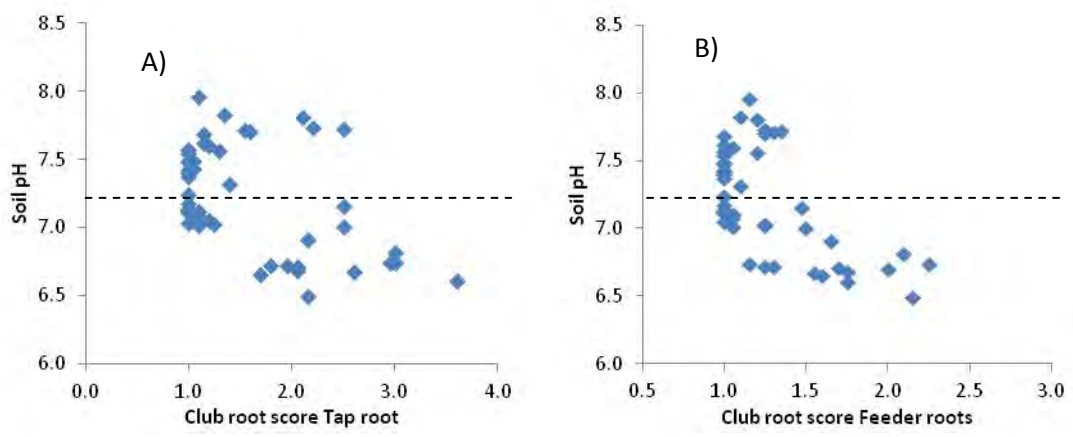

Figure 9. Relationships between soil pH and club rot scores. A: tap roots. B: Feeder roots. A dotted line in each figure indicates the pH threshold of 7.2 above which club root disease can be controlled (Heinrich and Stone, 2013).

Yield:

We evaluated Brussels sprout yield by manually harvesting sprouts from 5 plants per row x 4 middle rows in each plot (20 plants/plot) and weighing small (<1 3/8" diameter), standard (1 3/8" to 1 ½" diameter), and large (>1 ½" diameter) sprouts separately. Yield of each size and total yield were calculated and expressed as lb per plant. Non-marketable sprouts were excluded from yield count. Table 5 shows the results of ANOVA for small, standard, large and total sprout yield of 13 trials we conducted in 3 seasons.

Among these 13 trials, 10 trials showed significant treatment effects, but there was no consistent pattern across trials (Table 6). Nonetheless, two important patterns can be seen. First, ASD's ranks among the 4 treatments in each year tend to go down over time. In particular, the low yields with ASD in two trials in year 3 (Wat3 and HMB5) raises a concern. In the 3rd year we used grape pomace as a carbon source for all ASD treatment based on the lowest club root score on tap roots in HMB4 (see Table 3) and its lower cost. Part of the reason for the lower yield may be the lack of soil inorganic N during the early growth stage due to N immobilization by grape pomace (See Figure 8S-8T for Wat3, Figure 8U-8V for HMB5). If this is the case then supplemental pre-plant N application could improve yields. However, in HMB5, although both rolled ASD plots and tarped ASD plots showed similarly low soil inorganic N levels, rolled ASD plots had significantly higher yield than tarped ASD plots (Table 5) suggesting other yield limiting factors must have existed in tarped ASD plots. We observed stunted plants in these plots at Wat3 and HMB5 from their early growth stage. Brassica crops are known to favor well-aerated soil (Wolfe et al., 1995), so if ASD plots remained partially anaerobic at planting and beyond, this could reduce crop growth as it did in SC2. Therefore, when using ASD for Brussels' sprout and other brassica crops it may be important to ensure soil is sufficiently dry and re-cultivate the field before planting these crops. Contrary to this idea, however, there was no difference between rolled and tarped ASD plots in HMB6, despite it being a heavier soil. Furthermore, grape pomace ASD tarped also performed well in SC1, as did ASD with RB in a number of trials, **Clearly, more work comparing fumigants with rolled and tarped grape pomace ASD, with supplemental pre-plant fertilizer is needed before ASD can be recommended.**

On the other hand, MSM's rank has improved greatly during the course of trials especially in year 3; it had top yield among 4 treatments in 2 trials (HMB5 and HMB6). As mentioned earlier, for the 3rd year, we introduced powdered MSM instead of pelleted MSM. Powdered MSM is known to release active ingredients such as AITCs much faster compared to the pelleted form (Mazzola, unpublished). Further, rolled MSM had the highest total yield in HMB5 and the 2nd after tarped MSM in HMB6, which appears to be promising. **Future trials should compare the effect of rolled and un-rolled powdered MSM on Brussels sprout growth and club root contro**l.

Table 5. Summary of Brussels sprout yield in 13 trials conducted in 3 seasons.

Year	Trial	Treatment	Brussels sprout yield (lb/plant)			
			Small (< 1 3/8")	Standard (1 3/8" – 1 ½")	Large (> 1 ½")	Total
Yr 1	SC1	UTC	1.04	0.64 B*	0.73	2.42
		ASD-GP-tarped	0.91	0.96 A	0.76	2.63
		MSM1-tarped	1.08	0.78 AB	0.77	2.63
		Fum	1.05	0.92 A	0.83	2.80
		ANOVA (P)	*0.30*	*0.0098***	*0.97*	*0.31*
	Wat1	UTC	1.43	0.58 AB†	0.39	2.53
		ASD-RB-tarped	1.48	0.64 AB	0.41	2.60

		MSM1-tarped	1.50	0.45 B	0.34	2.45
		Fum	1.40	0.70 A	0.47	2.69
		ANOVA (P)	*0.81*	*0.10†*	*0.81*	*0.16*
	HMB1	UTC	1.83	0.47 B†	0.22	1.97
		ASD-RB-tarped	1.64	0.75 A	0.39	2.38
		MSM1-tarped	1.60	0.72 AB	0.40	2.35
		Fum	1.68	0.66 AB	0.29	2.40
		ANOVA (P)	*0.88*	*0.077†*	*0.25*	*0.12*
	HMB2	UTC	1.16 AB†	0.57	0.30	2.05
		ASD-RB4.5-tarped	1.26 AB	0.52	0.33	2.03
		ASD-RB6-tarped	1.32 AB	0.66	0.42	2.45
		ASD-RB9-tarped	1.55 A	0.71	0.20	2.51
		ASD-RB9-nontarped	1.32 AB	0.82	0.19	2.23
		MSM0.5-tarped	1.32 AB	0.99	0.49	2.26
		MSM0.75-tarped	1.34 AB	0.68	0.45	2.44
		MSM1-tarped	1.16 B	0.87	0.27	2.16
		MSM1-nontarped	1.40 AB	0.68	0.24	2.30
		Fum	1.40 AB	0.51	0.07	2.05
		ANOVA (P)	*0.19*	*0.36*	*0.18*	*0.13*
Yr 2	SC2	UTC	1.25	0.11	0.16	1.51
		ASD-RB-tarped	1.43	0.16	0.13	1.72
		MSM1-tarped	1.36	0.12	0.13	1.60
		Fum	1.47	0.13	0.11	1.70
		ANOVA (P)	*0.36*	*0.24*	*0.61*	*0.63*
	SC3	UTC	1.36 A*	0.34	0.59	2.29
		ASD-RB-tarped	1.21 B	0.46	0.76	2.43
		MSM1-tarped	1.22 B	0.43	0.72	2.37
		Fum	1.46 A	0.34	0.54	2.35
		ANOVA (P)	*0.0001****	*0.16*	*0.096†*	*0.70*
	Wat2	UTC	1.59 B*	0.18	0.18	1.95 B*
		ASD-RB-tarped	1.63 B	0.25	0.24	2.11 AB
		MSM1-tarped	1.63 B	0.22	0.20	2.05 AB
		Fum	2.03 A	0.22	0.21	2.45 A
		ANOVA (P)	*0.0033***	*0.28*	*0.63*	*0.0069***
	HMB3	UTC	1.14 B†	0.17	0.25	1.56 B†
		ASD-RB-tarped	1.30 AB	0.21	0.27	1.79 AB
		MSM1-tarped	1.52 A	0.20	0.34	2.06 AB
		Fum	1.52 A	0.31	0.40	2.24 A
		ANOVA (P)	*0.059†*	*0.20*	*0.39*	*0.078†*
	HMB4	UTC	1.62	0.03	0.00	1.65
		ASD-RB-tarped	1.72	0.06	0.00	1.78
		ASD-RB-nontarped	1.72	0.06	0.002	1.78
		ASD-GP-tarped	1.64	0.04	0.00	1.68
		ASD-GP-nontarped	1.66	0.05	0.00	1.70
		MSM1-tarped	1.61	0.05	0.00	1.66
		MSM1-nontarped	1.68	0.06	0.00	1.74
		MSM0.5-tarped	1.67	0.07	0.00	1.74
		Fum	1.62	0.02	0.00	1.64
		ANOVA (P)	*0.91*	*0.59*	*0.46*	*0.87*
Yr 3	SC4	UTC	1.62	0.38	0.18	2.29
		ASD-GP-tarped	1.63	0.47	0.17	2.37
		MSM1-tarped	1.57	0.35	0.22	2.23
		Fum	1.75	0.34	0.23	2.38
		ANOVA (P)	*0.60*	*0.28*	*0.58*	*0.62*
	Wat3	UTC	1.64 A*	0.19 A*	0.68	2.54 A*
		ASD-GP-tarped	1.22 B	0.13 B	0.53	1.92 B

		MSM1-tarped	1.55 A	0.16 AB	0.59	2.36 AB
		Fum	1.69 A	0.18 AB	0.62	2.52 A
		ANOVA (P)	*0.0006****	*0.052†*	*0.45*	*0.016**
	HMB5	UTC	1.51 AB*	0.024	0.0015	1.54 AB*
		ASD-GP-tarped	1.23 B	0.0008	0	1.24 B
		ASD-GP-rolled	1.61 A	0.024	0.00075	1.65 A
		MSM1-tarped	1.58 A	0.043	0	1.63 A
		MSM1-rolled	1.77 A	0.047	0.0028	1.85 A
		Fum	1.63 A	0.18	0	1.68 A
		ANOVA (P)	*0.0028***	*0.53*	*0.33*	*0.0035***
	HMB6	UTC	1.34 B†	0.12	0.15	1.72 B†
		ASD-GP-tarped	1.44 AB	0.08	0.13	1.80 AB
		ASD-GP-rolled	1.47 AB	0.13	0.16	1.86 AB
		MSM1-tarped	1.76 A	0.16	0.19	2.29 A
		MSM1-rolled	1.59 AB	0.13	0.16	2.03 AB
		Fum	1.59 AB	0.14	0.0	1.93 AB
		ANOVA (P)	*0.088†*	*0.23*	*0.17*	*0.079†*
Yr 1 (n=88)			1.37 B*	0.69 A*	0.41 A*	2.38 A*
Yr 2 (n=100)			1.52 A	0.17 B	0.21 B	1.90 B
Yr 3 (n=80)			1.56 A	0.16 B	0.21 B	1.99 B
ANOVA (P)			0.0001***	0.0001***	0.0001***	0.0001***
Yr 1 (n=4)		UTC	1.42	0.56	0.41	2.24 B†
		ASD-RB/GP-tarped	1.40	0.77	0.44	2.53 A
		MSM1-tarped	1.34	0.71	0.45	2.40 AB
		Fum	1.38	0.70	0.42	2.49 AB
		ANOVA (P)	*0.77*	*0.19*	*0.91*	*0.075†*
Yr 2 (n=5)		UTC	1.39 B*	0.16 B†	0.24	1.79 B*
		ASD-RB/GP-tarped	1.46 AB	0.23 A	0.28	1.97 AB
		MSM1-tarped	1.47 AB	0.20 AB	0.28	1.95 AB
		Fum	1.62 A	0.20 AB	0.25	2.08 A
		ANOVA (P)	*0.059†*	*0.061†*	*0.61*	*0.065†*
Yr 3 (n=4)		UTC	1.53 AB†	0.18	0.25	2.02
		ASD-GP-tarped	1.38 B	0.17	0.21	1.83
		MSM1-tarped	1.62 AB	0.18	0.25	2.13
		Fum	1.66 A	0.21	0.24	2.13
		ANOVA (P)	*0.064†*	*0.79*	*0.40*	*0.40*
Santa Cruz (n=4)		UTC	1.32	0.37 B†	0.42	2.13 B*
		ASD-RB/GP-tarped	1.30	0.51 A	0.46	2.29 A
		MSM1-tarped	1.31	0.42 AB	0.46	2.21 AB
		Fum	1.43	0.43 AB	0.43	2.31 A
		ANOVA (P)	*0.13*	*0.09†*	*0.71*	*0.02**
Watsonville (n=3)		UTC	1.55	0.31	0.42	2.34
		ASD-RB/GP-tarped	1.44	0.34	0.40	2.21
		MSM1-tarped	1.56	0.28	0.38	2.29
		Fum	1.70	0.37	0.43	2.55
		ANOVA (P)	*0.37*	*0.44*	*0.58*	*0.26*
Half Moon Bay (n=6)		UTC	1.47	0.23	0.15	1.75
		ASD-RB/GP-tarped	1.48	0.30	0.17	1.92
		MSM1-tarped	1.54	0.34	0.20	2.03
		Fum	1.57	0.30	0.14	1.99
		ANOVA (P)	*0.64*	*0.23*	*0.46*	*0.12*
All trials (n=13)		UTC	1.44 AB*	0.29 B†	0.29	2.00 B*
		ASD-RB/GP-tarped	1.41 B	0.38 A	0.31	2.10 AB
		MSM1-tarped	1.47 AB	0.35 AB	0.32	2.14 AB
		Fum	1.56 A	0.36 AB	0.30	2.22 A
		ANOVA (P)	*0.046**	*0.061†*	*0.68*	*0.026**

Table 6. Summary of ten trials with significant treatment effects (*P*<0.1). For mean separation, Ag: ASD with grape pomace, Ar: ASD with rice bran, F: fumigation, Mt: pelleted mustard seed meal, Mp: powdered mustard seed meal, U: un-treated check. Treatments are arranged in order of highest yield to lowest yield. Treatments on the same line do not have significant difference according to Tukey's HSD test at P indicated. †, *, ** and *** means significant at *P*<10%, 5%, 1% and 0.1%, respectively.

Year	Site (sprout size)	Mean separation[z]	*P*	Club root
Yr 1	SC1 (standard)	Ag F Mt U	**	no
	Wat1 (standard)	F Ar U Mt	†	no
	HMB1 (standard)	Ar Mt F U	†	yes
	HMB2 (small)[y]	Ar F U Mt	†	no
Yr 2	SC3 (small)	F U Mt Ar	***	no
	Wat2 (total)	F Ar Mt U	**	no
	HMB3 (total)	F Mt Ar U	†	yes
Yr 3	Wat3 (total)	U F Mp Ag	*	no
	HMB5 (total)[x]	Mp F U Ag	**	no
	HMB6 (total)[x]	Mp F Ag U	†	yes

[z] When significant treatment effect was found in certain size category yield and total yield, the result of total yield is shown in this table.
[y] ASD with rice bran tarped plots and MSM 1t tarped plots were selected here.
[x] ASD with grape pomace tarped plots and MSM 1t tarped plots were selected for this table.

Economic analysis:

To evaluate economic feasibility of alternatives tested, partial net return of each treatment was calculated. A partial net return is a balance between gross sales and input costs for each treatment assuming the rest of the production costs are the same across different treatments. To calculate gross sales, actual total yield data from the trials and the average price of Brussels sprout from the Packer website http://www.thepacker.com/vegetables/brussels-sprouts/commodity-pricing (F.o.b.s. Central district, CA. Medium size 25 lbs carton) at closest date of the harvest date was used. For the 3 year-average analysis, the average price during the actual harvest period of the trials were used. Cost of fumigation was based on the cost paid by the growers for the 2015 season ($500 for fumigation. $300 for PCNB). Rice bran and grape pomace costs were based on the price from Farm Fuel Inc ($300/ton for rice bran, $210/ton for grape pomace). Cost of tarping was based on the cost of tarp, glue, application and recovery in broadcast fumigation in strawberries in the 2015 season ($1,500/ac). Water

cost was based on the actual cost in 2015 for Pajaro Valley Water Management Association ($191/ac-ft) plus pump fee ($60/ac-ft). Cost of roller was not included in the analysis for HMB5 and HMB6.

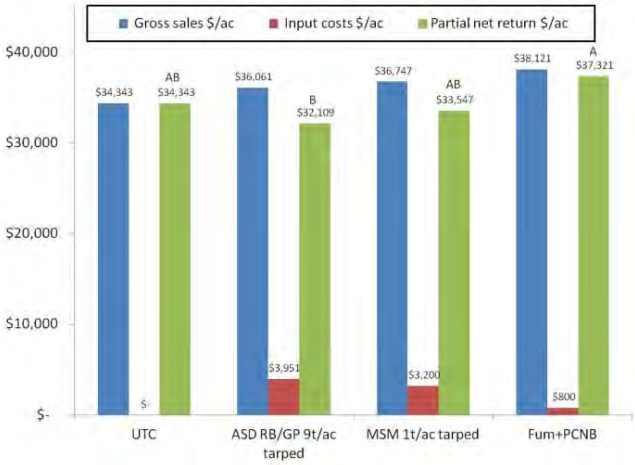

Figure 10. Average gross sales, input costs, and partial net return of 4 treatments for 13 trials conducted over 3 years. Partial net returns with the same letters are not significantly different at P<0.001 according to Tukey's HSD test.

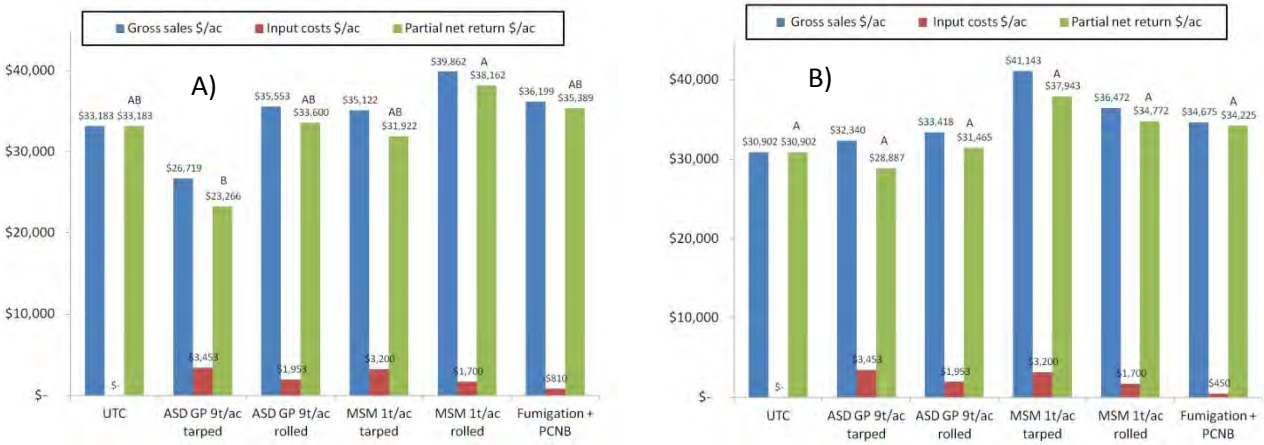

Figure 11. Gross sales, input costs, and partial net return of different treatments in HMB5 (A) and HMB6 (B). Partial net returns with the same letter are not significantly different at P<0.001 and P<0.10 for A) and B), respectively, according to Tukey's HSD test.

For the 13 trial average, partial returns were highest in the Fumigation treatment followed by UTC (-8% compared to Fumigation), MSM (-10%) and ASD was the lowest (-14%) though a significant difference was found only between Fumigation and ASD (*P*=0.001***) (Figure 10). To evaluate the effect of rolled powdered MSM treatment, partial net return of HMB5 and HMB6 were calculated. Figure 11A shows that in HMB5 MSM rolled treatment had 8% higher partial net return compared to fumigation+PCNB treatment though the difference was not statistically significant. Likewise, in HMB6, rolled MSM had comparable partial net return with fumigation + PCNB (Figure 11B).Use of powdered MSM as an alternative to fumigant and PCNB appears to warrant further studies. Also grape pomace – ASD could potentially be an option for fields with CR, providing supplemental pre-plant N is added to improve yields, as observed for strawberry production (Zavatta, et al., 2014).

Objectives 3-6: Broccoli

Objective 3

Trial Design. In Y1 in order to develop transferable monitoring techniques for trapping and sampling CM in broccoli production, we tried 3 methods of sampling; sand/soil floating, sticky traps and water traps. Sticky traps were placed at various locations around a broccoli field in Watsonville, CA. They were collected and replaced every week for up to 7 weeks. Water traps, which were gallon sized yellow buckets filled almost to the brim with water, were placed in the same locations as the sticky traps. Insects were collected from water traps and placed into a vial with alcohol every week for up to 7 weeks. To sand/soil float we placed sand at the base of 30 broccoli plants in a field in Watsonville, CA. The sand was placed to cover a 1.5-2" radius around the plant and was no more than 1cm in depth. Every week for up to 7 weeks we collected the sand from all 30 plants and floated it in water to count the eggs that float to the surface. We also collected soil from the base of 30 plants, at a similar radius and depth as the sand, in order to see if there was an egg laying preference between sand and soil.

Results. Of all three methods of monitoring CM we found sand traps to be the best measure of CM presence in a field. Both the sticky traps and water traps were problematic because it was very difficult to distinguish between the CM adult fly and other similar looking flies. We were advised from several experts that this would be an extremely difficult task. However we found identifying CM eggs to be very simple, and can even be done with a hand lens fairly quickly. From floating the sand and soil traps weekly the data indicates that CM flies prefer to lay their eggs in sand than in soil (figure 22). If a grower wanted to know whether or not they have CM in their field, placing sand at the base of plants and floating it a week later for eggs would be a good indication. Future work in developing monitoring techniques of CM could be aimed at easier methods to identify the adult flies. Although our best method of sampling removed the eggs from the plants such that we were not able to correlate egg abundance with an effect on yield or damage for this trial, this relationship is further explored in Objective 4 and 5.

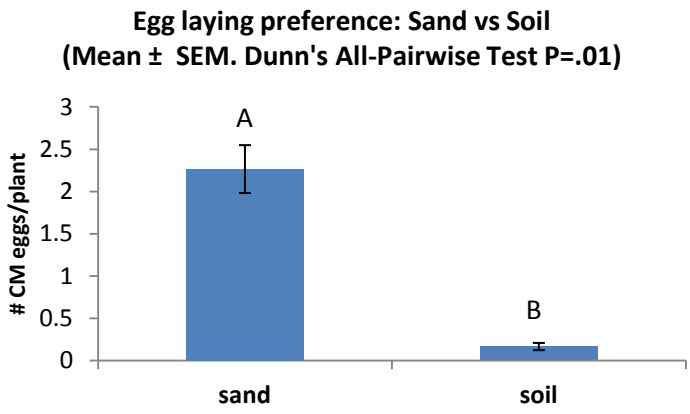

Figure 12. Comparison of # of CM eggs found in sand traps versus in soil.

Objectives 4 and 5

Trial Design. In order to develop an economic damage threshold that utilizes this egg counting monitoring technique as well as to test the susceptibility of broccoli to CM damage at various growth stages, we conducted an in lab potted experiment. We wanted to determine at what egg density and

growth stage of a plant might create damage that results in an economic loss. We looked at egg densities of 0,3,6,12 and 18 eggs per plant replicated 6 times each and at 1 week old, 3 week old and 5 week old plants (post transplant) for a total of 3 rounds of this experiment. Additionally to look to at the susceptibility of broccoli to CM damage at various growth stages we place sentinel pupae in the field early, mid and late season and collected and reared them to see levels of parasitism and predation. To compliment this data we also did destructive samples at the same locations and in early, mid and late season to see naturally occurring rates of parasitism and predation at these growth stages.

Results. For the 1 week old round of the potted experiment we saw at egg densities of 6 or higher there is a decrease in floret size of 40%-50% relative to the florets of plants with no eggs applied (figure 23). For the three and five week old plants there were no statistically significant treatment effects relating the number of eggs present and floret size. This indicates that plants are only vulnerable to a yield loss at very early growth stages and at rates of 6 eggs per plant or more. The next stage of this experiment would be to take it to the field level to see if these results are replicable outside of the lab. Additionally other egg densities could be tested.

For the work in parasitism and predation at various growth stages of the plant we found that in organic broccoli fields there are relatively fewer CM pupae present in early season than in mid and late season. However when pupae abundance picks up in mid and late season we also saw an increase in rove beetles and % parasitism of CM pupae by rove beetles (figure 14). This indicates that biological control in organic fields is occurring and future work in understanding the susceptibility of broccoli to CM damage would look at management practices of organic fields (e.g. tillage practices, trap crops, etc.) to see how they relate to effective control of the pests.

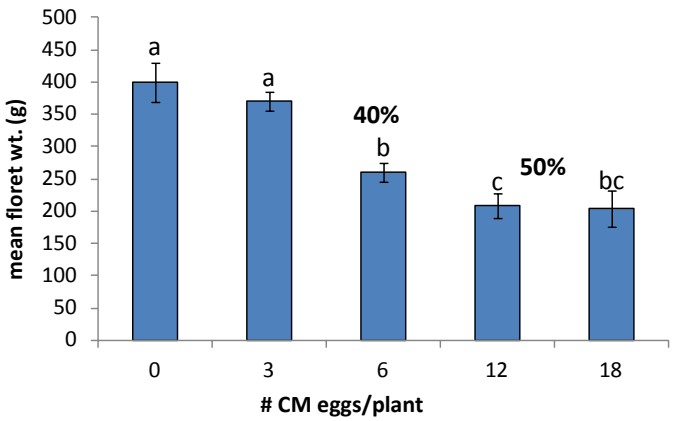

Figure 13. One week old economic damage threshold experiment at egg densities of 0, 3, 6, 12 and 18 eggs per plant.

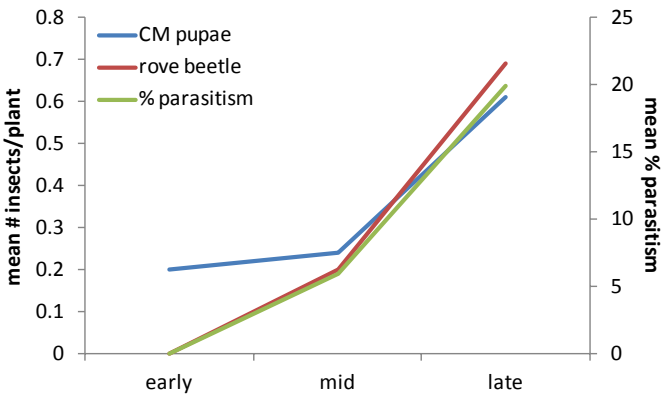

Figure 14. Year 2 CM presence and parasitism in organic broccoli fields at early, mid and late season.

Objective 6

Trial Design. Objective 6 originally was intended to test the efficacy of Chlorpyrifos granules in broccoli plug-transplants in reducing CM damage. Over the course of this study however objective 6 was changed to test the efficacy of Ecotec G, a promising organic insecticide/deterrent advertised as useful for CM control, in reducing CM damage in turnips. This volatile oil-based, botanical product seemed to work on turnips at the UCSC organic farm, to reduce damage in very susceptible varieties. We coupled the product in a split plot design with 3 replicates to test it in monoculture turnip and polyculture turnip intercropped with carrot along the center row after establishing baby lettuce on both sides of the bed. The pre-plant application of Ecotec G was applied to half of each plot. We conducted biweekly destructive samplings to assess damage caused by CM and measured ground predator activity and diversity in pitfall traps placed in each sub-treatment.

Results. Analysis of data showed no CM damage in any of the sub-treatments or controls after two weeks and no effect of Ecotec G or interplanting on CM damage or ground predator activity. Yield advantages in the intercropping were due to the inclusion of lettuce, not because of higher turnip yields. The validity of these results are in question because of the following problems and constraints on the experiments: 1) there were no CRF eggs or damage detected until the turnips were relatively large, which means that the Ecotec G may have already been dissolved or ineffective by the time its effects were testable, 2) the plot size was small compared to other such experiments in the literature so natural enemies and pests may have acted as though the whole field was intercropped, and 3) effects of the intercropping habitat were probably subdued, at least in the first two ground predator samples, because of ground squirrel herbivory. Future work in the efficacy of Ecotec G to control for CM in broccoli should use larger plot sizes and an additionally application of Ecotec G when CM presence is detected.

Conclusions

Table 7. Summary of alternatives evaluation for Brussels sprouts in Objectives 1 and 2

Treatment	Cyst nematode control	Club root control	Cabbage maggot control	Effect on yield	Partial net return	Over all potential
ASD rice bran 9t/ac, tarped	?	good	excellent	somewhat	negative	not recommended due to cost
ASD grape pomace 9t/ac, tarped	?	good	not tested	negative – due to early N deficiency?	negative	not recommended without further work
Powdered MSM, 1t/ac, tarped	?	somewhat	no	good	good	good
Powdered MSM, 1t/ac, rolled	?	somewhat	no	good	better	good
Fumigation with PCNB	?	somewhat	no	good	good	future availability uncertain
Un-treated	no	no	no	no	-	-

Given the widespread environmental and human health concerns associated with the use of chemical fumigants and lack of viable alternatives for the control of major pests in Cole crop production in central coastal California it was the goal of this study to increase knowledge and understanding of biological alternatives to fumigants, such as ASD, MSM application and IPM strategies.

Outcomes from studies for Objectives 1 and 2 are summarized in table 7. Although it showed efficacy in controlling club root disease and cabbage maggots, ASD did not always provide comparable yield with fumigant and proved to have the lowest partial net returns among treatments tested due to the combination of cost and lower yields in some trials. As discussed earlier, yields with grape pomace ASD could be limited by early season N availability due to immobilization, or inadequate field aeration. Further work is needed to optimize fertility management and field preparation with ASD in different soil types before the technique can be recommended. Nonetheless, ASD if optimized further, could be an effective way of controlling CR and a viable alternative to PCNB in highly infected fields.

MSM, on the other hand, appears to have a potential as an alternative to fumigant and PCNB though its effect on club root control was not as good as ASD and it had no effect on cabbage maggots. Further work with powdered MSM with and without using a roller merits further study. For club root disease control, maintaining soil pH to 7.2 or above should be integrated with any other approaches. Should club root disease pressure increase, sprouts growers may need to consider crop rotation with non-brassica crops as a part of IPM.

Future work is needed to find a more precise way to assess treatment effects on PPN populations, and a burial/retrieval method should be tested. Our work on Objectives 3-5 pertaining to IPM of CM in broccoli indicates that egg sampling via sand floating is the best means of monitoring for CM. Given this method of sampling we have determined that 1 week old broccoli transplants with 6 eggs/plant or higher will see a statistically significant decline in floret size. Additionally data indicates that in organic broccoli fields as CM pressure increases so does the rate of parasitism. Future work should look for a simpler means to monitor adult CM flight activity than keying out fly species from sticky traps or water traps. Also, an economic damage threshold experiment taken to the field level would be a great step forward and compliment the in lab pot trials. Lastly a study which looks into the management practices used in organic fields and their relationships to increased rates of parasitism would be essential for determining effective IPM strategies for the control of CM in broccoli production. Overall, with the work we have achieved in this study combined with future research geared in the directions discussed, effective, and viable biological alternatives to chemical fumigants in Cole crop production may be attainable in the future.

Acknowledgements

We thank our collaborative growers, Steve Bontadelli of Bontadelli, Inc., John Giusti of Giusti Farms, David Lea of Cabrillo Farms, and Danny Rodoni of Sunset Farms for their excellent cooperation for this project. Margherita Zavatta, Graeme Baird, and many student workers, interns and volunteers contributed to the field work and lab work for this project. Fred Crowder (San Mateo County Agricultural Commissioner/Sealer), Eric Lauritzen (Monterey County Agricultural Commissioner), Mary Lou Nicoletti (Santa Cruz County Agricultural Commissioner) and Jeena Andrews (CCA/PCA, Crop Production Services, Watsonville) helped construct the project. Finally, we thank funding from the California Department of Pesticide Regulation for this project.

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Appendix

The power point presentation for the final project meeting held at UCSC, April 20. 2016.

Integrated Pesticide Reduction Strategies for Insect and Disease Management in Cole Crops

~Final Project Meeting~
UC Santa Cruz, 4/20/2016

Carol Shennan, Deborah Letourneau, Joji Muramoto, Diego Nieto, Janet Bryer, Catlin Slay, and Lucinda Toyama, UC Santa Cruz

Steven Koike, UC Cooperative Extension, Salinas

Antoon Ploeg, UC Riverside

Funded by California Department of Pesticide Regulation

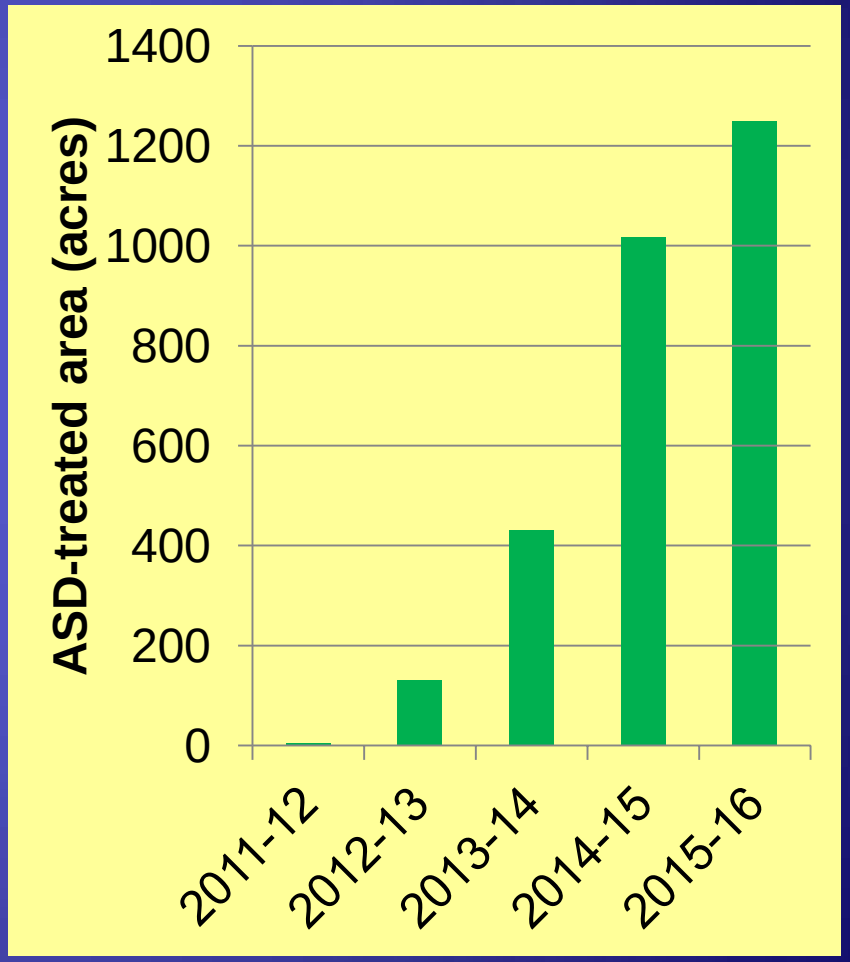
Grant Agreement No. 12-PML-R004

Background

- Plant parasitic nematodes (PPN. cyst nematodes, *Heterodera* spp., and root-knot nematodes *Meloidogyne* spp.) and club root (CR. *Plasmodiophora brassicae*) are serious soilborne pests and disease for Brussels sprouts
- Soil fumigants and PCNB have been used to control soilborne pathogens and PPN but future availability of these materials is uncertain due to increasingly stringent regulations
- The cabbage root maggot (CM) (*Diptera: Anthomyiidae*: *Delia radicum* L.) is a specialist pest of cole crops including broccoli
- No CM monitoring techniques and economic threshold guidelines have been established for broccoli causing over use of chlorpyrifos

Anaerobic Soil Disinfestation (ASD)

- Developed as alternative to Methyl bromide fumigation in the Netherlands (Blok et al., 2000) and Japan (Shinmura, 2000)
- Can control a range of soilborne pathogens and PPN by creating anaerobic condition via acid fermentation
- Commercially adopted by berry growers in CA



Mustard Seed Meal (MSM)

- Material left after extracting oil from mustard seeds
- N fertilizer (~6% N)
- Releases “isothiocyanates” (ITCs) that can suppress weeds and diseases
- ITCs affect associated soil microbial communities that can control diseases and weeds
- Can control weeds and root knot nematodes

Project objectives

1. Test ASD and MSM against club root, and plant parasitic nematodes for CA Brussels sprouts production
2. Test the efficacy of ASD in reducing CM damage in Brussels sprouts
3. Develop transferable CM monitoring techniques that will be based on evaluating various trapping technologies and sampling strategies for broccoli
4. Develop economic threshold for CM based on optimized monitoring technique
5. Test the susceptibility of broccoli to CM damage at various growth stages
6. Test the efficacy of Ecotec G in reducing CM damage in turnips

Project objectives

1. Test ASD and MSM against club root, and plant parasitic nematodes for CA Brussels sprouts production
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5. Test the susceptibility of broccoli to CM damage at various growth stages
6. Test the efficacy of Ecotec G in reducing CM damage in turnips

Objectives 1 and 2

- Completely randomized block designed on-farm trials with 4 replicates in Half Moon Bay, Santa Cruz, and Watsonville across 3 seasons (13 trials)
- Basic trials (9 sites): ASD (carbon source 9t/ac, tarped), MSM (1t/ac, tarped), fumigation, and untreated check
- C-source rate/tarp trials (4 sites): effects of carbon source rates and types in ASD, rates of MSM, or use of tarp, roller or non-tarp in ASD and MSM treatments against fumigation and un-treated check
- Fumigant used: Telon II 8-16 gal/ac, Vapam 20 gal/ac or Kpam 15.5 gal/ac
- Blocker F (PCNB): 5 gal/ac in HMB trials

Santa Cruz 1 (SC1)

Watsonville 1 (Wat1)

Half Moon Bay1 (HMB1)

Half Moon Bay 2 (HMB2)

Half Moon Bay 6 (HMB6)

Plant Parasitic Nematodes (PPN)

Trial	Treatment	Cyst nematode egg/larvae population (#/100ml dry soil)	
		Pre-treatment	Post-treatment
All trials, all years (n=11)	UTC	3190	1965
	ASD-RB/GP-tarped	4136	2623
	MSM1-tarped	3408	1901
	Fum	4252	1517
	ANCOVA (P)	-	*0.89*

Plant Parasitic Nematodes (PPN)

Trial	Treatment	Cyst nematode egg/larvae population (#/100ml dry soil)	
		Pre-treatment	Post-treatment
SC all years (n=3)	UTC	4260	2911
	ASD-GP/RB-tarped	3797	2568
	MSM1-tarped	3292	3098
	Fum	4400	1994
	ANCOVA (P)	-	*0.22*
HMB all years (n=5)	UTC	2943	1138
	ASD-RB/GP-tarped	2163	2194
	MSM1-tarped	2425	1071
	Fum	1711	1056
	ANCOVA (P)	-	*0.55*
Wat all years (n=3)	UTC	2532	2399
	ASD-RB/GP-tarped	7761	3392
	MSM1-tarped	5161	2090
	Fum	8338	1810
	ANCOVA (P)	-	*0.93*

Clubroot at HMB1

Photos by Steven T. Koike

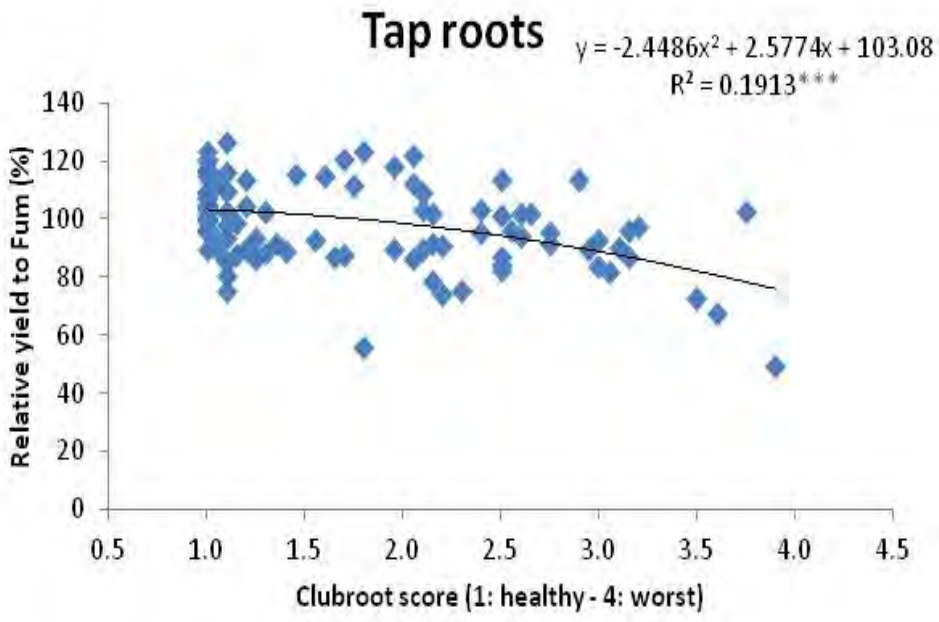
Club root disease

Trial	Treatment	Club root damage	
		Tap roots (1: healthy – 4: worst)	Feeder roots (1: healthy – 5: worst)
HMB1	UTC	2.4 A*	1.5
	ASD-RB-tarped	1.1 B	1.1
	MSM-tarped	1.1 B	1.2
	Fumigant+PCNB	1.0 B	1.7
	ANOVA (P)	*0.014**	*0.16*
HMB6	UTC	2.6	1.7 AB†
	ASD-GP-tarped	1.9	1.3 B
	ASD-GP-rolled*	2.6	1.6 AB
	MSM-tarped	1.9	1.5 AB
	MSM-rolled*	2.3	1.6 AB
	Fumigant+PCNB	2.4	2.1 A
	ANOVA (P)	*0.78*	*0.13*

Club root disease

Trial	Treatment	Club root damage	
		Tap roots (1: healthy – 4: worst)	Feeder roots (1: healthy – 5: worst)
All trials (n=5)	UTC	2.1	1.5 AB*
	ASD-tarped	1.6	1.2 B
	MSM-tarped	1.6	1.4 AB
	Fum+PCNB	1.7	1.6 A
	ANOVA (P)	*0.25*	*0.03**

Club root vs. Relative yield



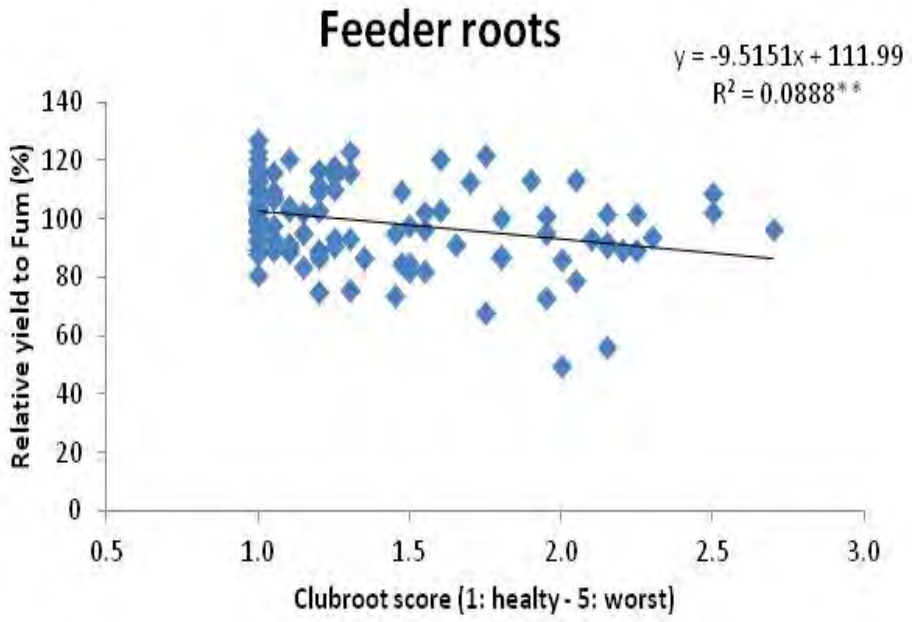
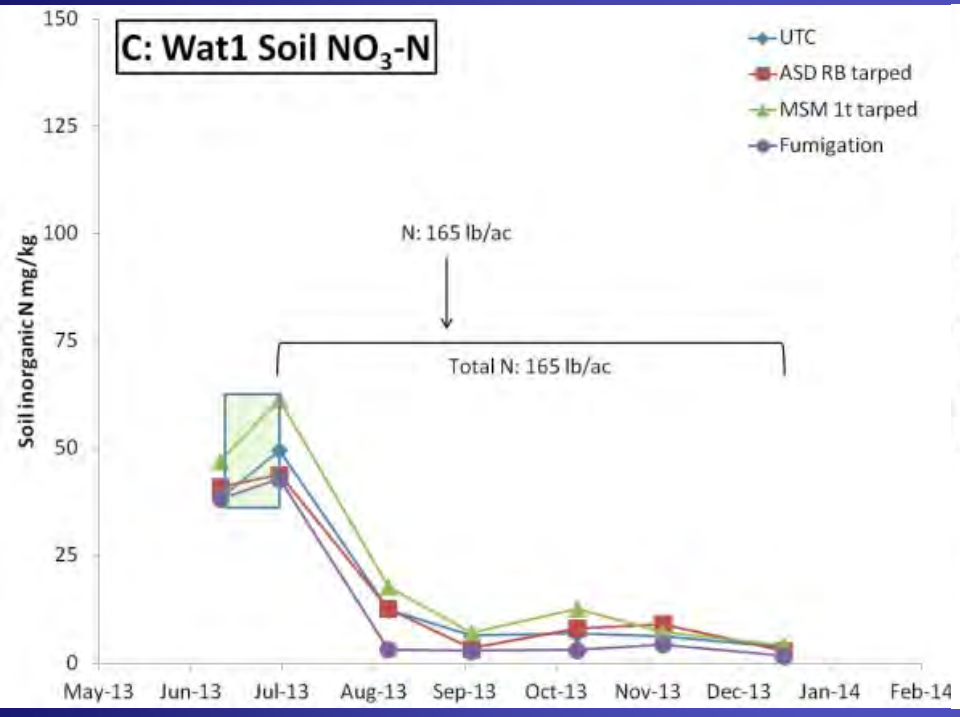


Figure . Correlations between club root scores and relative total yield of Brussels sprout. A: tap root club scores, B: feeder root club root scores. Data from 5 trials with club root disease were used. Total yield of each plot of each trial was standardized by dividing with the average yield of fumigation treatment of each trial and expressed as a relative yield to fumigation plot (%).

Soil Inorganic N



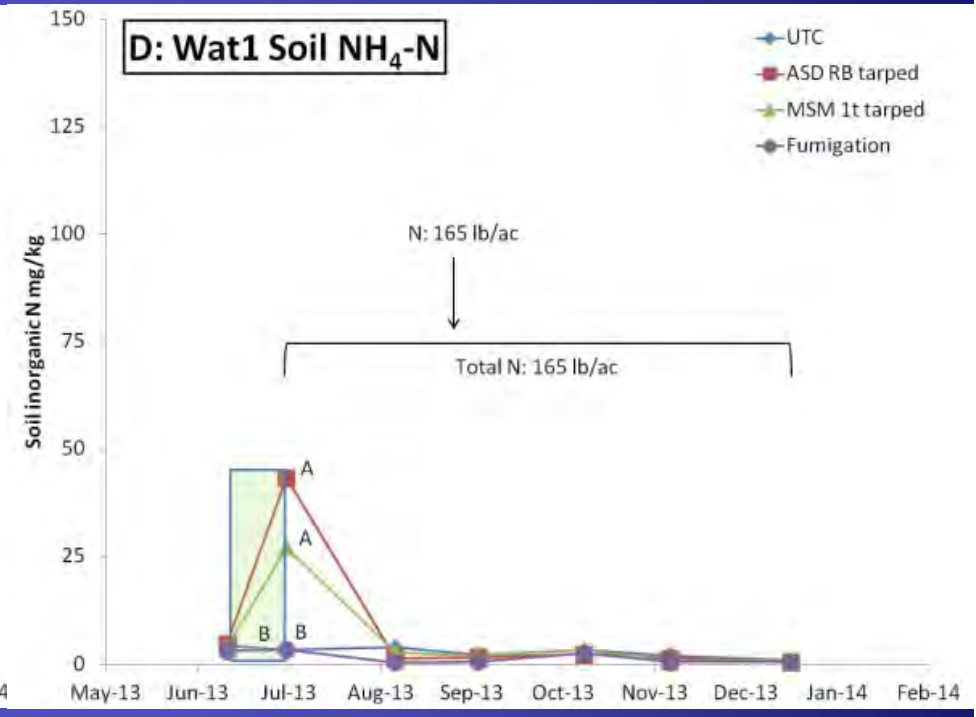
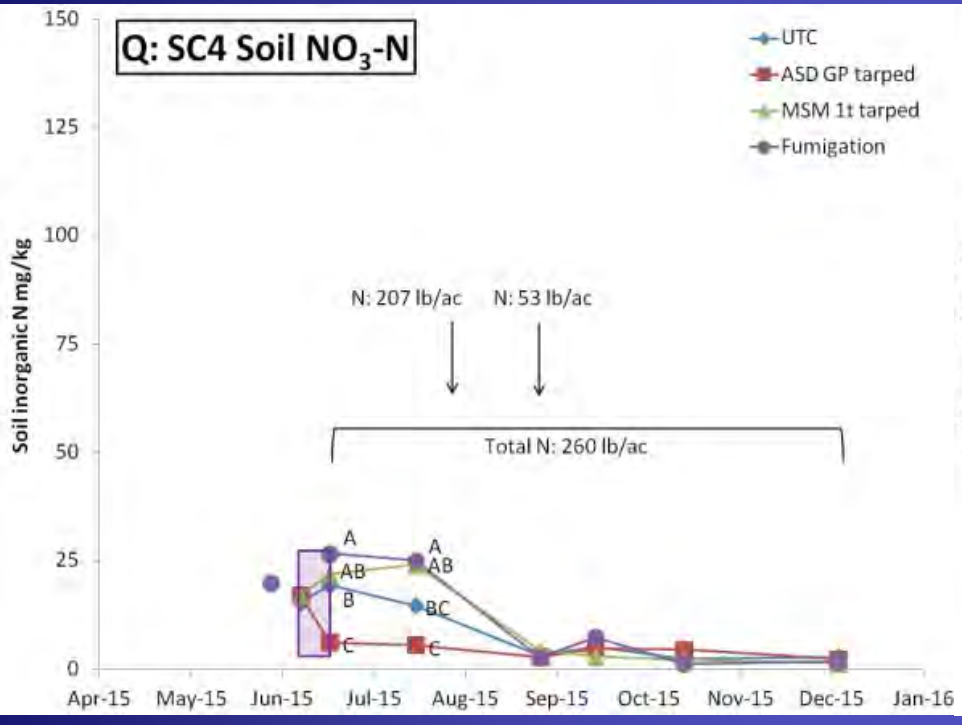


Figure . Soil inorganic N dynamics at 0”-6” depth at Wat1

Soil Inorganic N



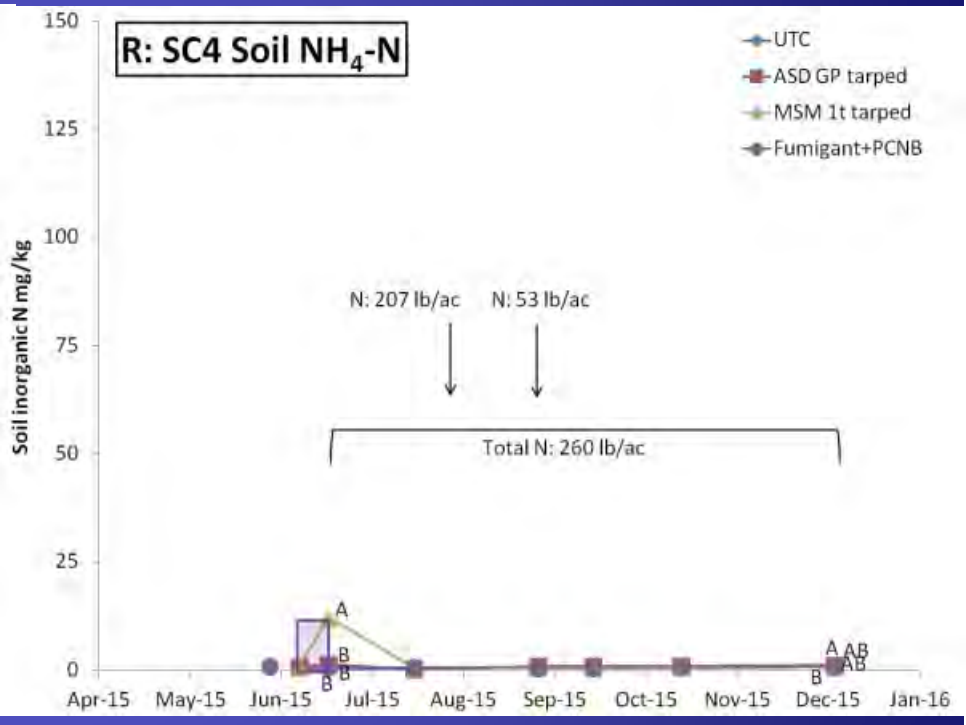


Figure . Soil inorganic N dynamics at 0”-6” depth at SC4

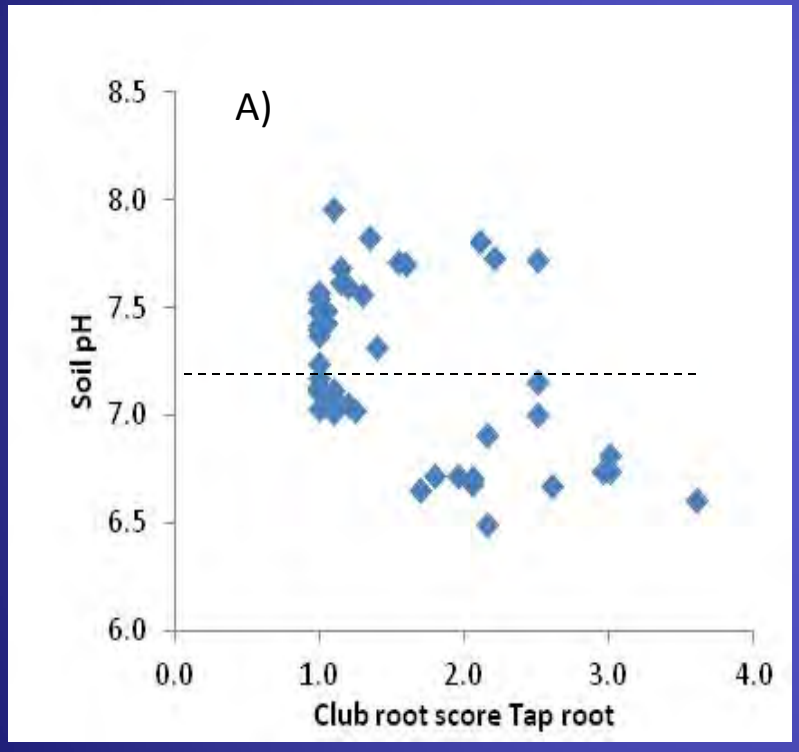
Soil pH

Effect of ASD and MSM on soil pH at harvest in Brussels sprout field trials (9 trials from 3 years).

Year	Treatment	pH (1:1) at harvest
All 3 years (n=9)	UTC	7.36 AB
	ASD	7.28 B
	MSM	7.34 AB
	FUM	7.39 A
	ANOVA (*P*)	*0.03**

Means with the same letters do not have significant differences at *P*=0.05 according to Tukey's HSD test.

Club root and soil pH



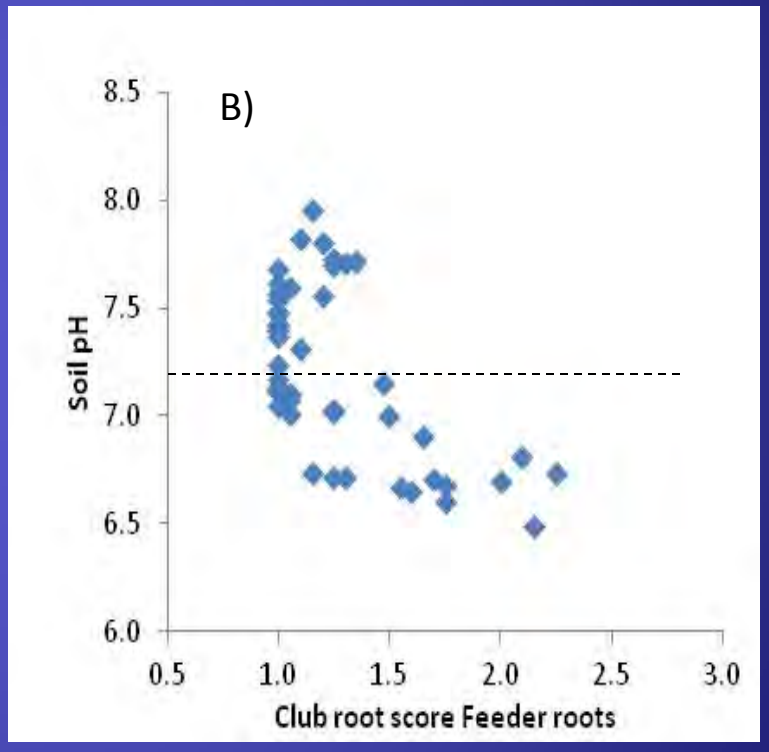


Figure . Relationships between soil pH and club rot scores. A: tap roots. B: Feeder roots. A dotted line in each figure indicates the pH threshold of 7.2 above which club root disease can be controlled (Heinrich and Stone, 2013).

Mean separation of sprout yield

Year	Site (sprout size)	Mean separation[z]	*P*	Club root
Yr 1	SC1 (standard)	Ag F Mt U	**	no
	Wat1 (standard)	F Ar U Mt	†	no
	HMB1 (standard)	Ar Mt F U	†	yes
	HMB2 (small)[y]	Ar F U Mt	†	no
Yr 2	SC3 (small)	F U Mt Ar	***	no
	Wat2 (total)	F Ar Mt U	**	no
	HMB3 (total)	F Mt Ar U	†	yes
Yr 3	Wat3 (total)	U F Mp Ag	*	no
	HMB5 (total)[x]	Mp F U Ag	**	no
	HMB6 (total)[x]	Mp F Ag U	†	yes

Mean separation of sprout yield

Year	Site (sprout size)	Mean separation[z]	*P*	Club root
Yr 1	SC1 (standard)	Ag F Mt U	**	no
	Wat1 (standard)	F Ar U Mt	†	no
	HMB1 (standard)	Ar Mt F U	†	yes
	HMB2 (small)[y]	Ar F U Mt	†	no
Yr 2	SC3 (small)	F U Mt Ar	***	no
	Wat2 (total)	F Ar Mt U	**	no
	HMB3 (total)	F Mt Ar U	†	yes
Yr 3	Wat3 (total)	U F Mp Ag	*	no
	HMB5 (total)[x]	Mp F U Ag	**	no
	HMB6 (total)[x]	Mp F Ag U	†	yes

Mean separation of sprout yield

Year	Site (sprout size)	Mean separation[z]	*P*	Club root
Yr 1	SC1 (standard)	Ag F Mt U	**	no
	Wat1 (standard)	F Ar U Mt	†	no
	HMB1 (standard)	Ar Mt F U	†	yes
	HMB2 (small)[y]	Ar F U Mt	†	no
Yr 2	SC3 (small)	F U Mt Ar	***	no
	Wat2 (total)	F Ar Mt U	**	no
	HMB3 (total)	F Mt Ar U	†	yes
Yr 3	Wat3 (total)	U F Mp Ag	*	no
	HMB5 (total)[x]	Mp F U Ag	**	no
	HMB6 (total)[x]	Mp F Ag U	†	yes

Economic Analysis

Partial Net Return

= Gross sales – Treatment costs

- Assuming the rest of production costs are the same across treatments
- Gross sales: price from the Packer and actual yield of each treatment
- Treatment costs: costs of materials, water, application based on interviews from local growers and suppliers

Price of Brussels Sprout (F.o.b.s.Central Coast, CA)

Period	Ave. Price $/25 lbs mid size carton*
Nov. 2013 - Jan. 2014	$28.74
Nov. 2014 - Jan. 2015	$24.98
Nov. – Dec. 2015	$40.62
Ave.	***$31.45***

* According to the Packer website.

Gross sales (e.g. Ave. of 13 trials)

Treatment	Total yield lb/ac	Gross sales $/ac
UTC	27,300	$34,343
ASD RB/GP 9t/ac, tarped	28,665	$36,061
MSM 1t/ac, tarped	29,211	$36,747
Fumigant + PCNB	30,303	$38,121

- Ave. plant density: 13,650 /acre

Treatment costs

Treatment	Material $/acre	Tarping $/acre	Water $/acre	Total $/ac
UTC	-	-	-	$0
ASD RB 9t/ac, tarped	$2,700	$1,500	$63	$4,263
ASD GP 9t/ac, tarped	$1,890	$1,500	$63	$3,453
MSM 1t/ac, tarped	$1,700	$1,500	$0	$3,200
MSM 1t/ac, rolled*	$1,700	$0	$0	$1,700
Fumigant + PCNB	$800	$0	$0	$800

* Does not include cost of rolling

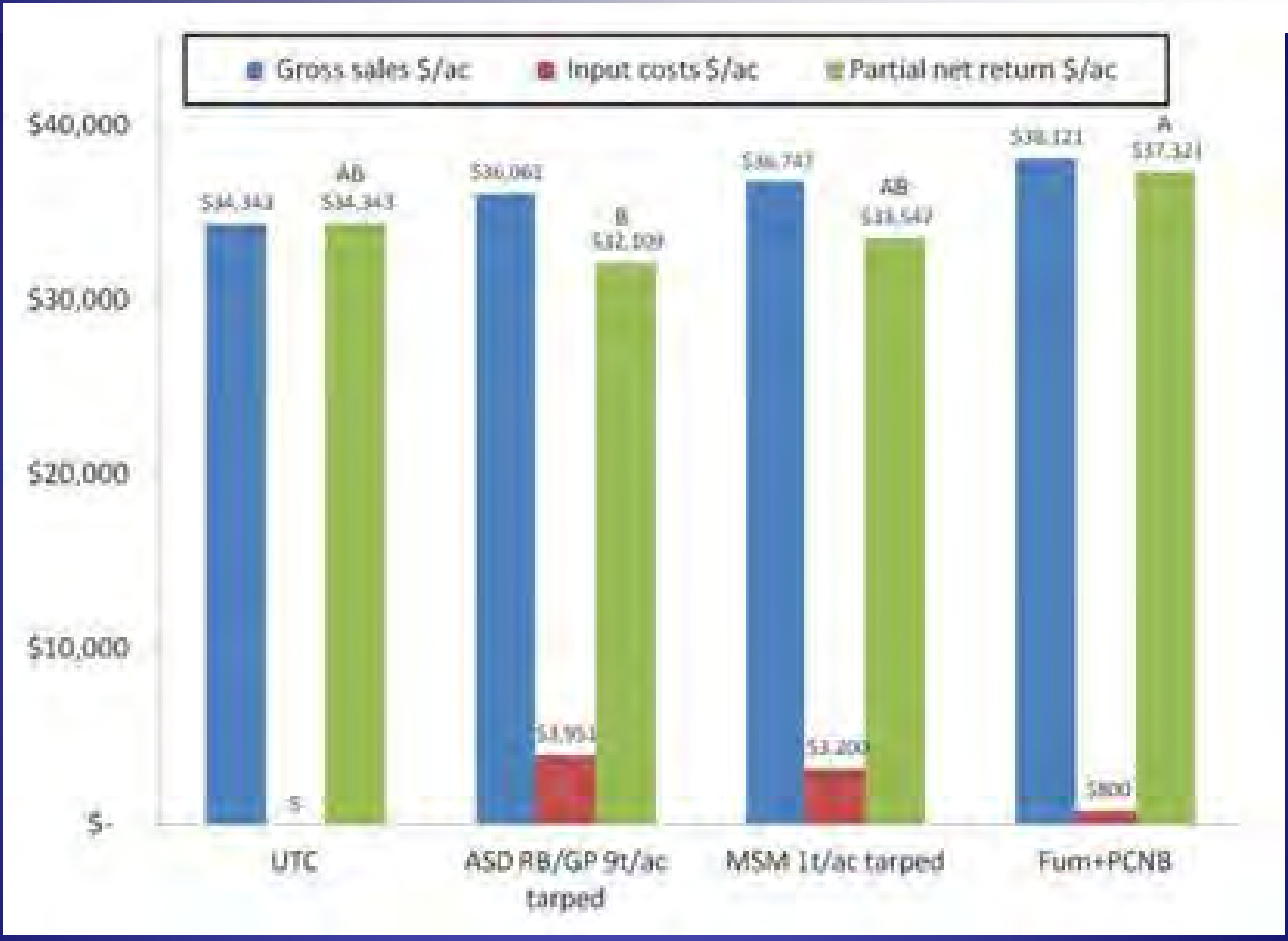


Fig. Average gross sales, input costs, and partial net return of 4 treatments for 13 trials conducted over 3 years. Partial net returns with the same letters are not significantly different at *P*<0.001 according to Tukey's HSD test.

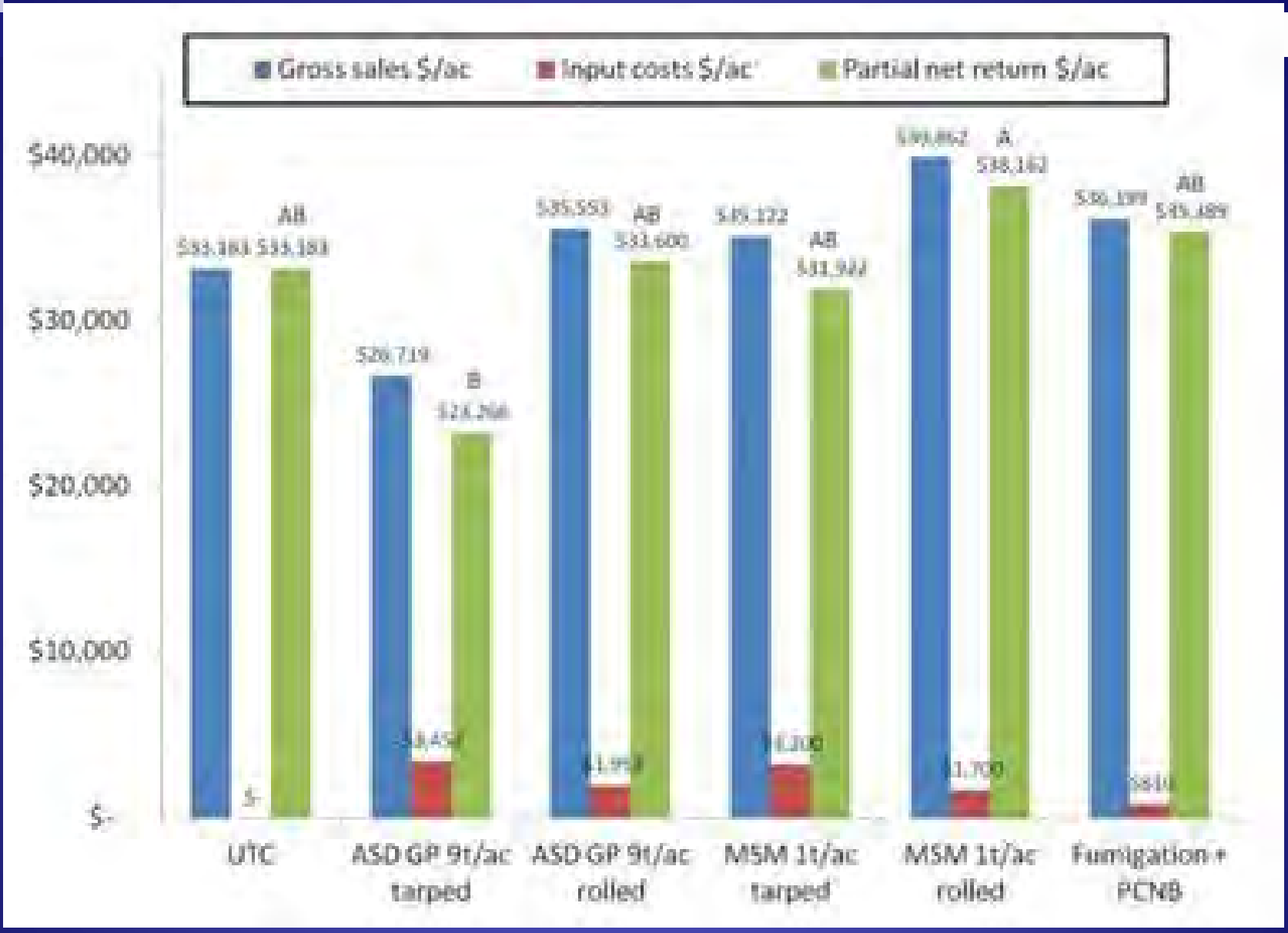


Fig. Gross sales, input costs, and partial net return of different treatments in HMB5. Partial net returns with the same letter are not significantly different at P<0.001 and P<0.10 for A) and B), respectively, according to Tukey's HSD test.

Cabbage Maggot

Brussels sprouts

-test the efficacy of ASD to control for CM

Broccoli

-develop monitoring technique for CM

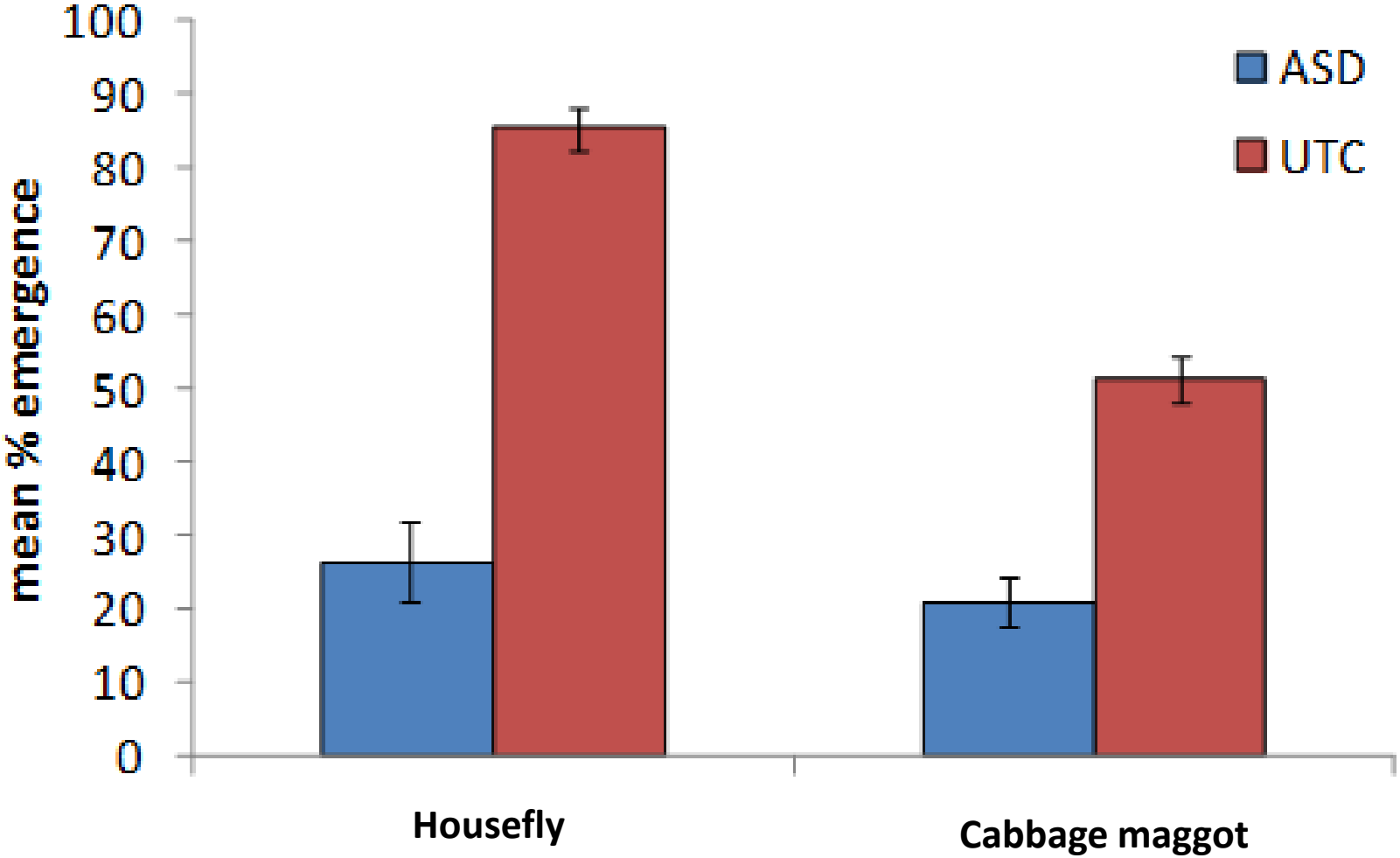
-develop an economic damage threshold based off of the monitoring technique

Brussels sprouts: ASD to control for CM

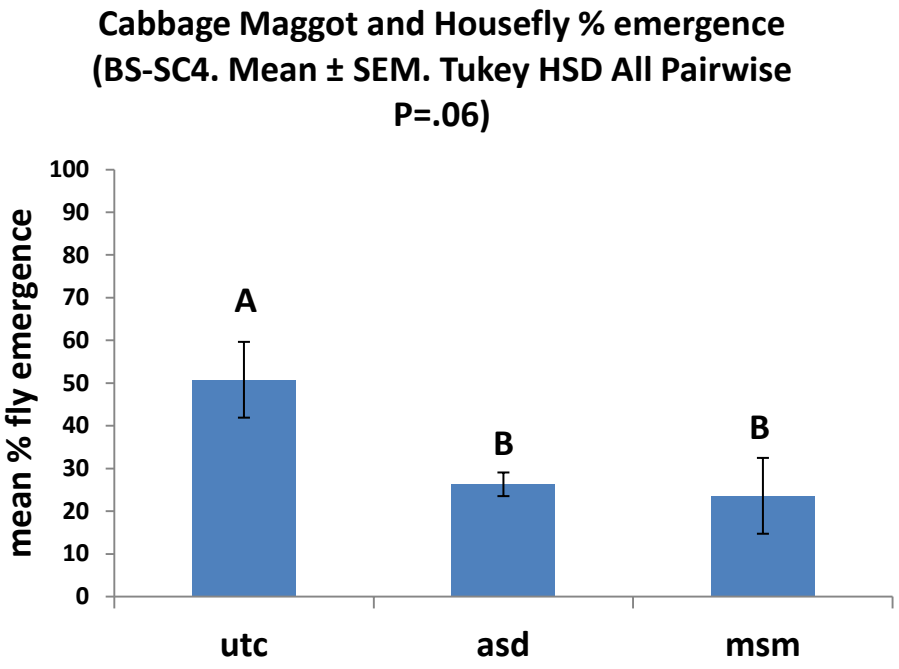
3 years of ASD field trials

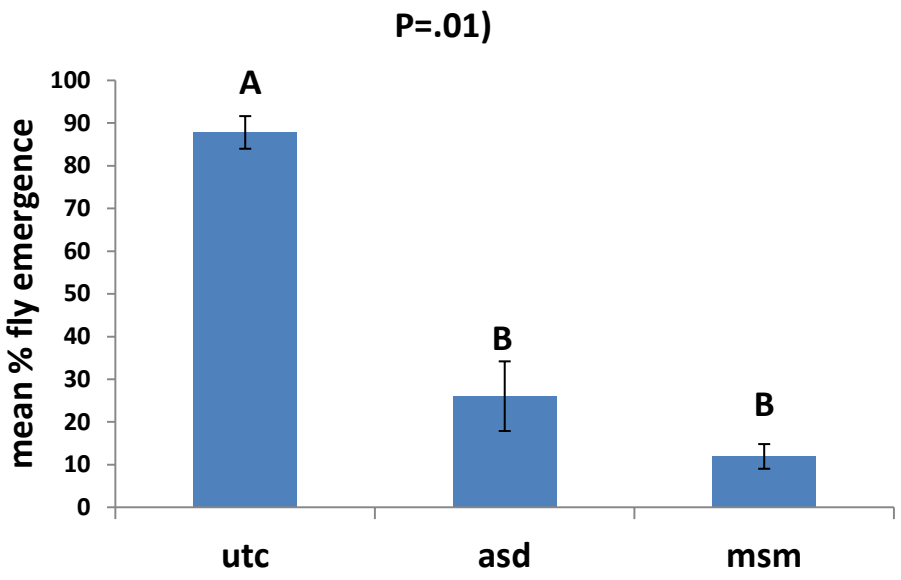
- CM and/or HF (housefly) pupal emergence post soil treatment
- deterrence of egg laying post soil treatment

Brussels sprouts: CM/HF pupal emergence



All Brussels sprouts ASD trials 2013-2016


Cabbage Maggot and Housefly % emergence
(BS-SC4. Mean ± SEM. Tukey HSD All Pairwise
P=.06)
mean % fly emergence
100
90
80
70
60
50
40
30
20
10
0
A
B
B
utc
asd
msm


Housefly % emergence
(BS-Wat3. Mean ± SEM. Tukey HSD All Pairwise
P=.01)
mean % fly emergence
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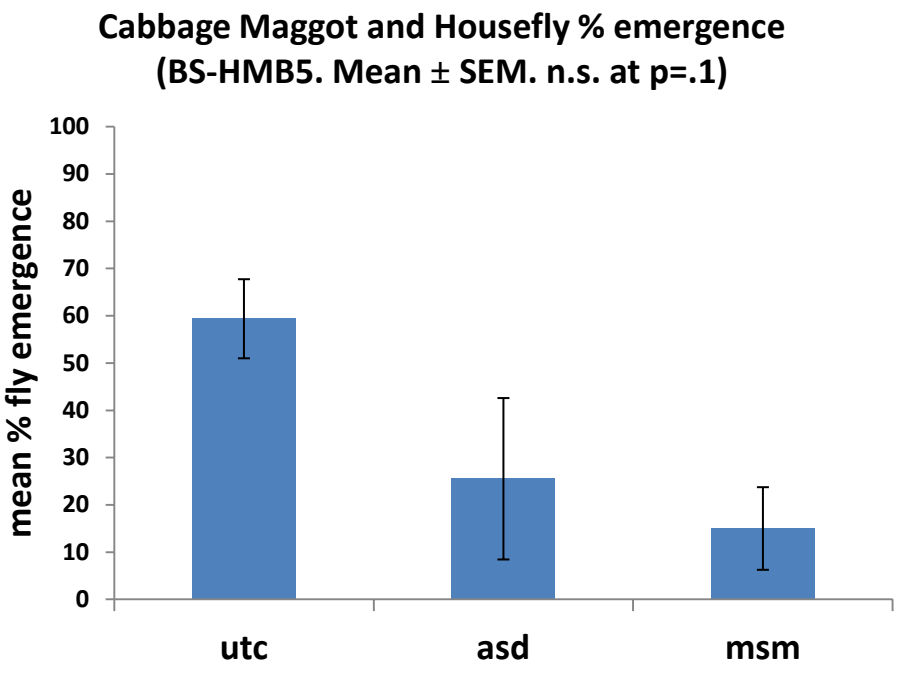
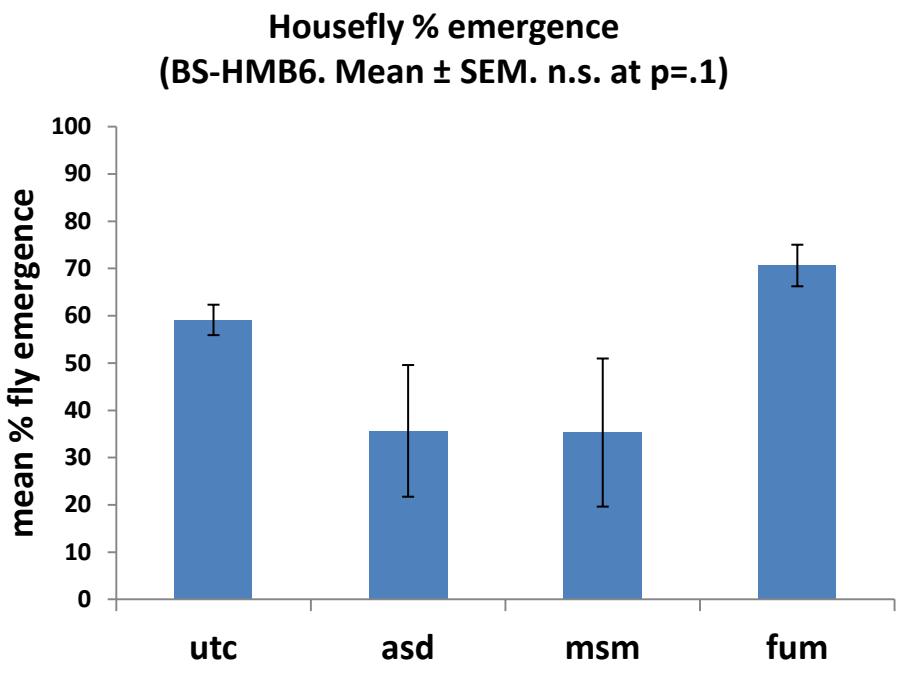
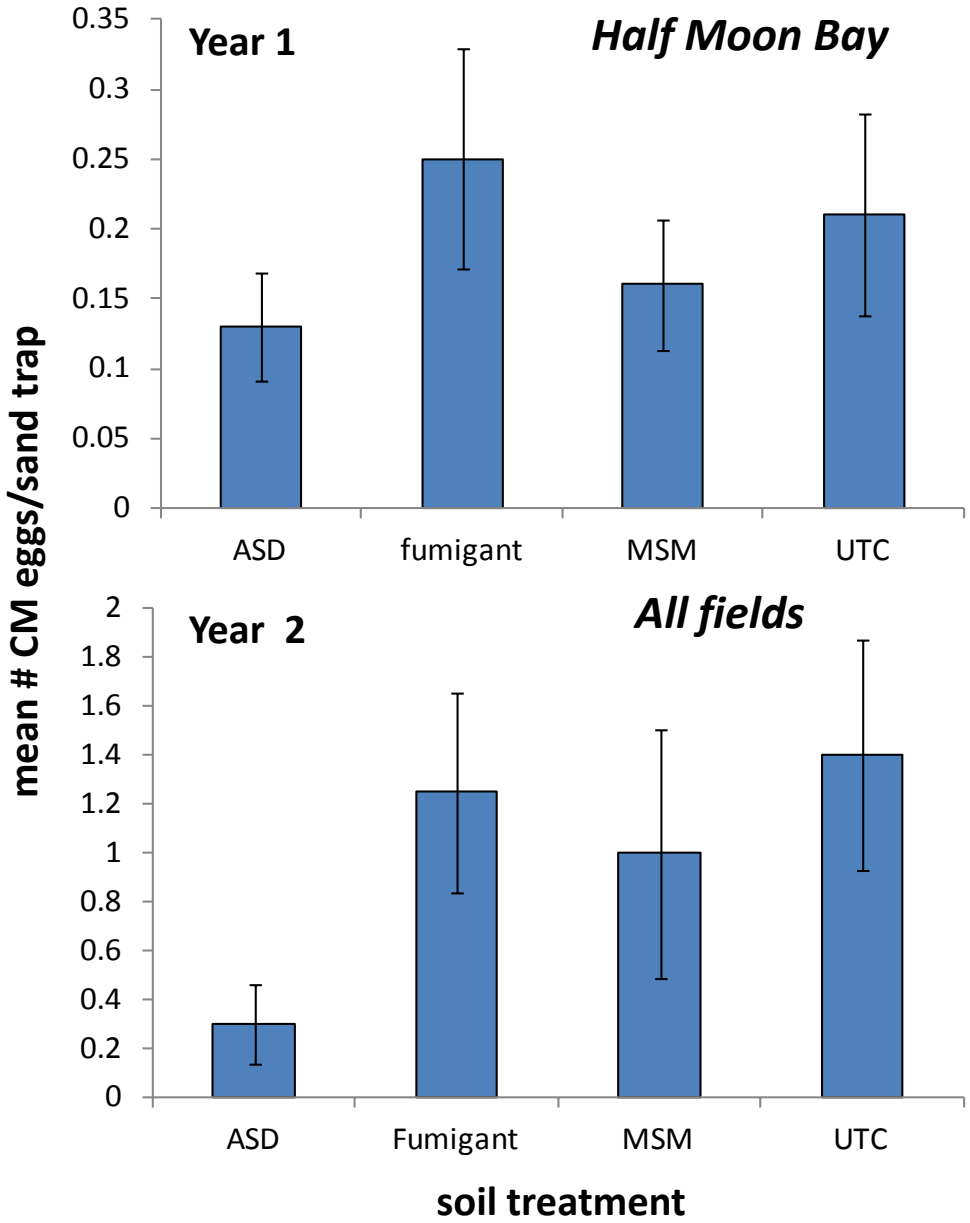
90
80
70
60
50
40
30
20
10
0
A
B
B
utc
asd
msm

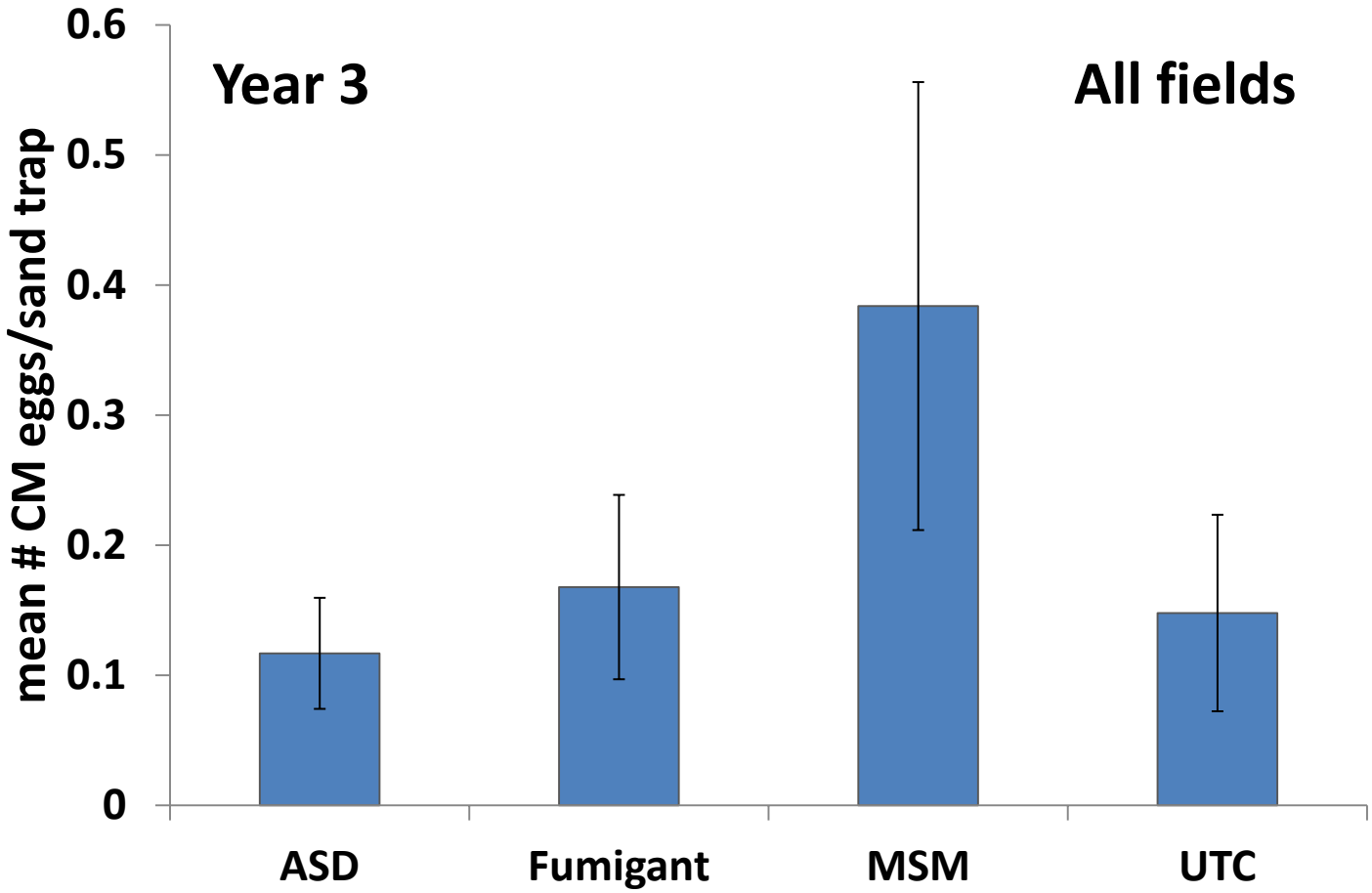

Cabbage Maggot and Housefly % emergence
(BS-HMB5. Mean ± SEM. n.s. at p=.1)
mean % fly emergence
100
90
80
70
60
50
40
30
20
10
0
utc
asd
msm


Housefly % emergence
(BS-HMB6. Mean ± SEM. n.s. at p=.1)
mean % fly emergence
100
90
80
70
60
50
40
30
20
10
0
utc
asd
msm
fum

Effects of ASD on CM egg-laying



CM Egg Density (1-2 weeks post-transplant)



CM Egg Density (1-2 weeks post-transplant)

Cabbage Maggot in Broccoli

-develop monitoring technique for CM

-develop an economic damage threshold based off of the monitoring technique

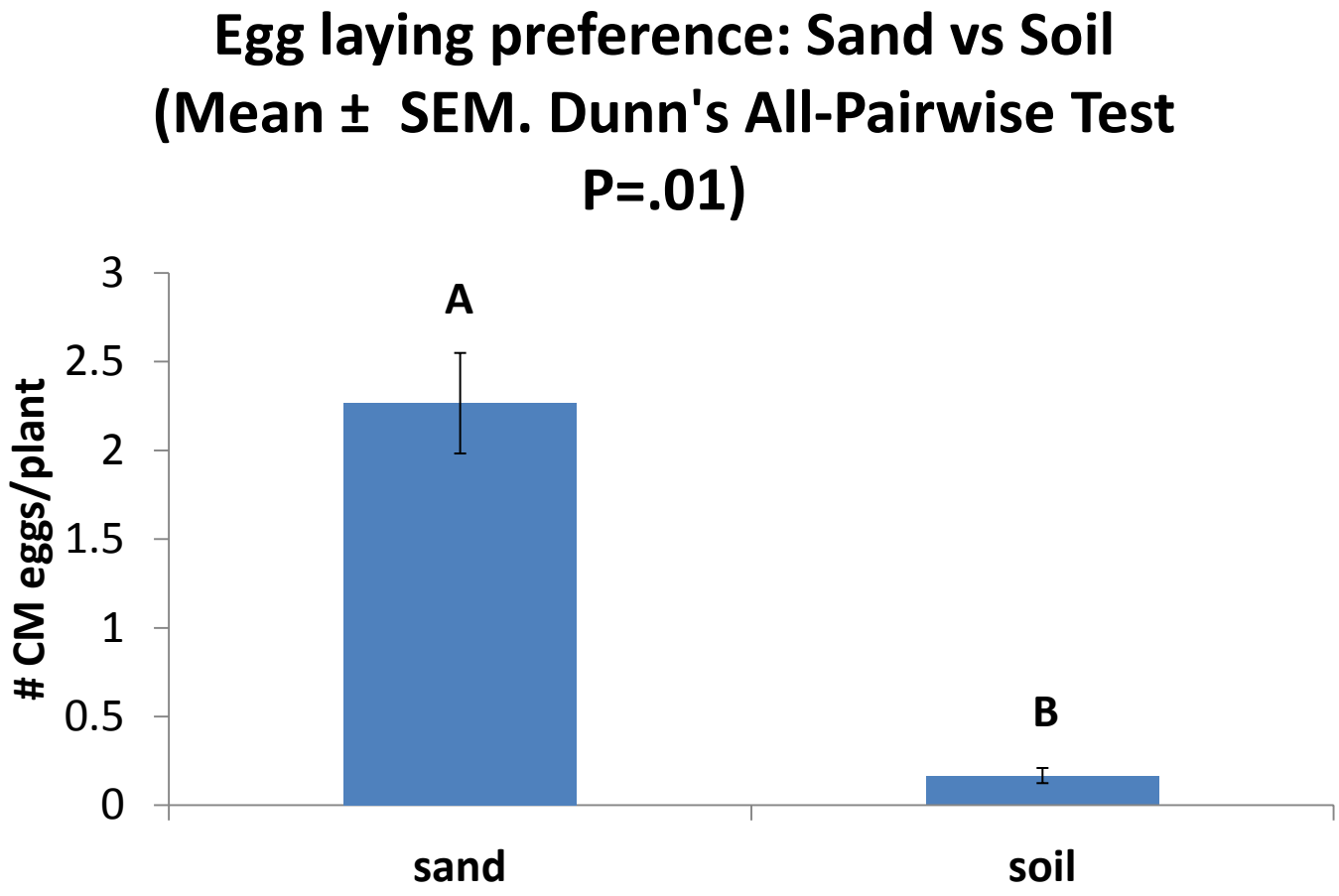
Broccoli:
Monitoring for CM

-sticky traps (monitors for CM adults)

-water traps (monitors for CM adults)

-sand and soil traps (monitors CM egg laying)

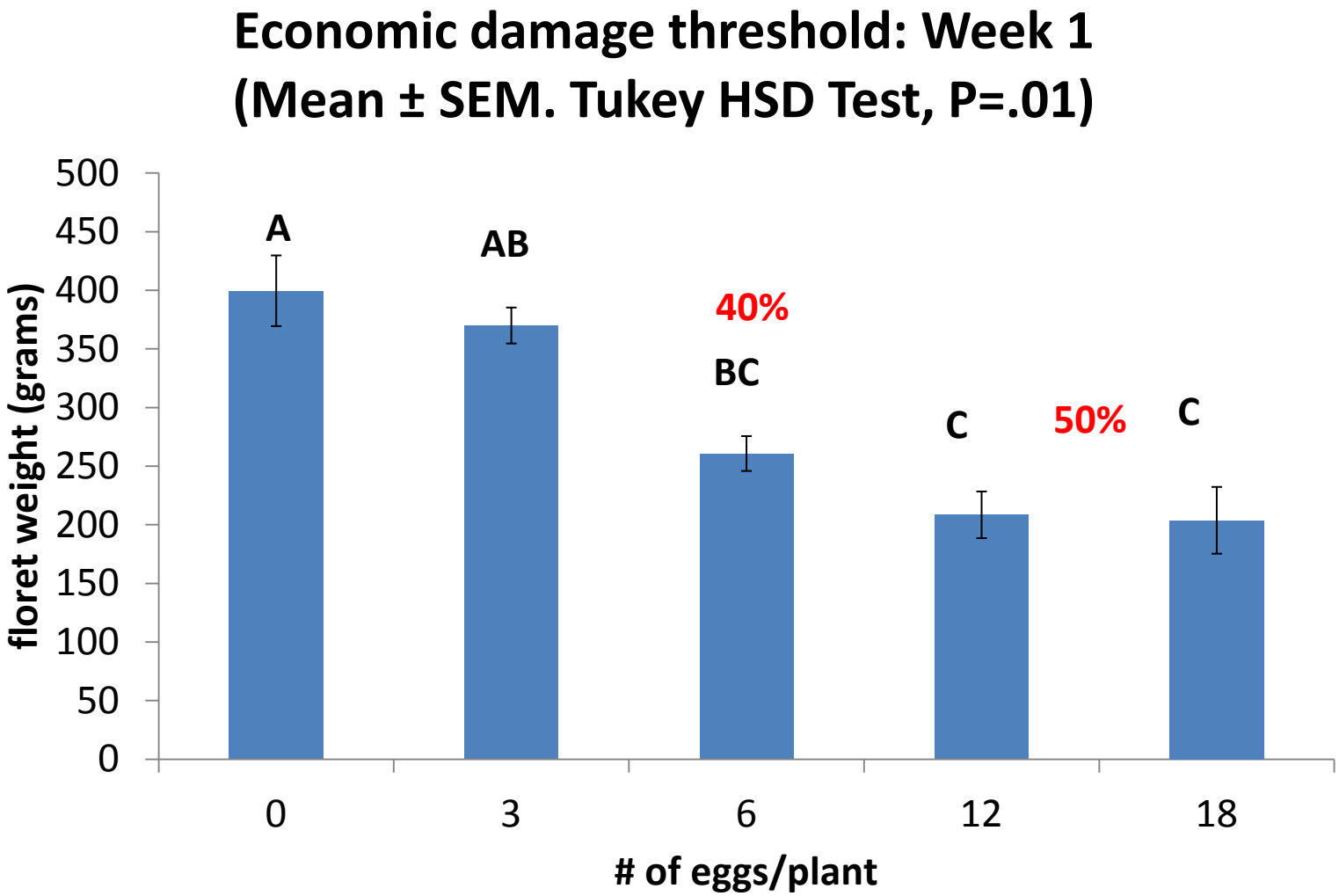
Broccoli: Monitoring for CM



Broccoli: Economic Threshold

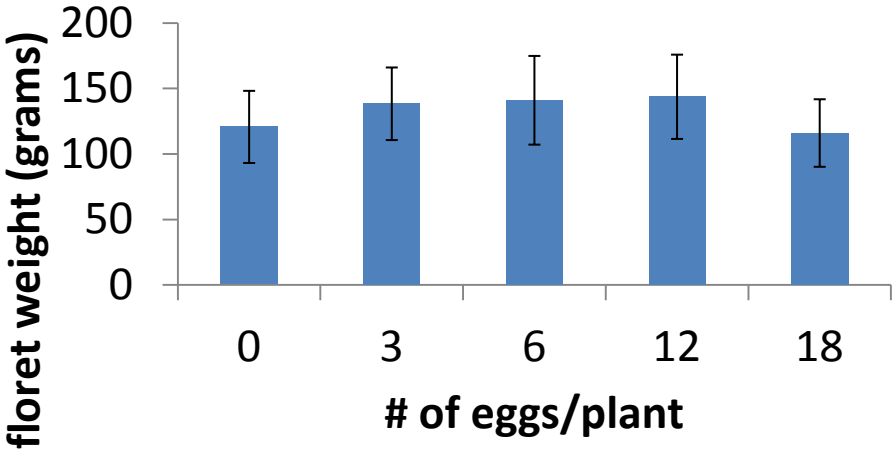
- Question: At what stage in plant growth, and at what level of egg density do you see an adverse effect on yield, or economic loss?
  - Plant growth stages: broccoli plants were treated with varying egg densities at 1, 3 and 5 weeks after transplant
  - Egg densities: 0, 3, 6, 12, and 18 eggs replicated 6 times each were placed on broccoli plants

Broccoli Economic Threshold: 1 week after transplant

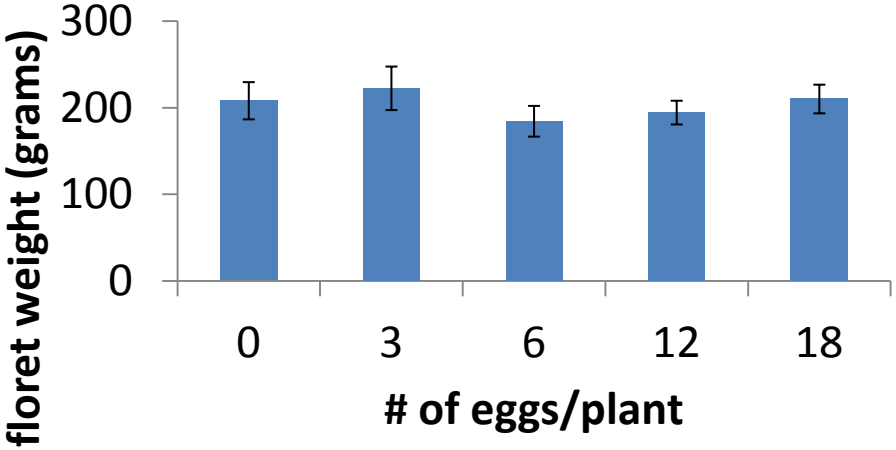


Broccoli Economic Threshold:

Economic damage threshold: Week 3 (Mean ± SEM. n.s at P=.1)



Economic damage threshold: Week 5 (Mean ± SEM. n.s at P=.1)



Cabbage Maggot Summary

Brussels sprouts

-ASD controls for CM pupal emergence, but does not deter egg laying post treatment

-an effective IPM approach would use ASD in combination with other tactics for the control of CM in Brussels sprouts

Broccoli

-most transferable technique for monitoring CM is sand traps

-our economic damage threshold study shows that at 1 week after transplant if there are 6 or more eggs on a single plant you could expect a decrease in floret size of ~40%

Summary of Brussels Sprout Trials

Treatment	Cyst nematode control	Club root control	Cabbage maggot control	Effect on yield	Partial net return	Over all potential
ASD rice bran 9t/ac, tarped	?	good	excellent	somewhat	negative	not recommended due to cost
ASD grape pomace 9t/ac, tarped	?	good	not tested	negative – due to early N deficiency?	negative	not recommended without further work
Powdered MSM, 1t/ac, tarped	?	somewhat	no	good	good	good
Powdered MSM, 1t/ac, rolled	?	somewhat	no	good	better	good
Fumigation with PCNB	?	somewhat	no	good	good	future availability uncertain
Un-treated	no	no	no	no	-	-

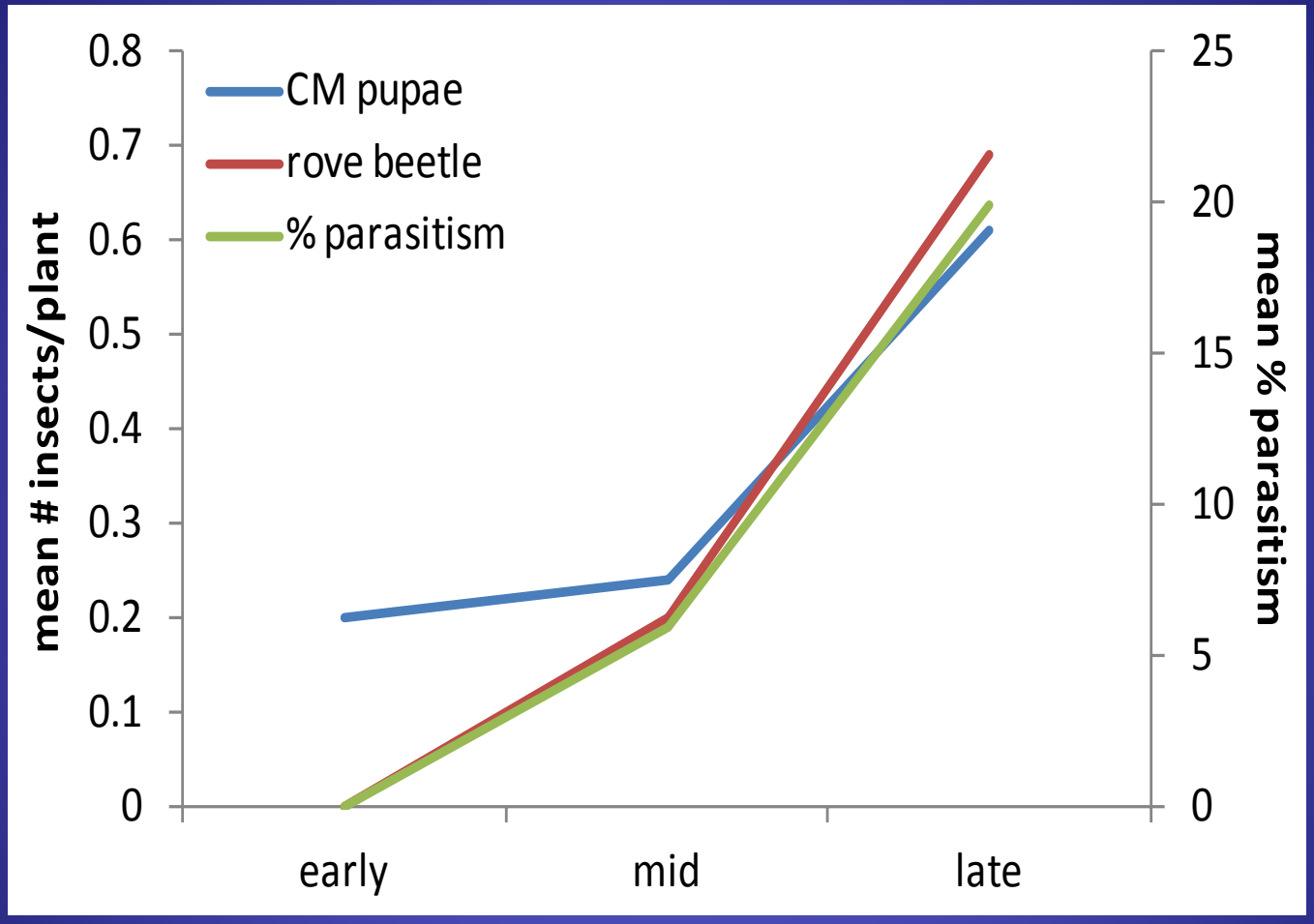


Figure Year 2 CM presence and parasitism in organic broccoli fields at early, mid and late season.

Objective 6

- Chlorpyrifos trial replacement with Ecotec-G
  - EPA proposed revocation of all tolerances
  - Observational data at UCSC farm
  - Trials in Salinas by Dr. Joseph for chlorpyrifos
- Ecotec-G active ingredients 2014, 15
  - Volatile oils / Neurotransmitter disruptors
  - Clove, Cinnamon and Thyme oils
  - Recommended for cabbage maggot, symphylans…
- Mono- versus polyculture trial 2015
  - Turnips (susceptible to CM)
  - Confusion with carrot and lettuces as non-hosts
  - Early season habitat for predaceous beetles, spiders

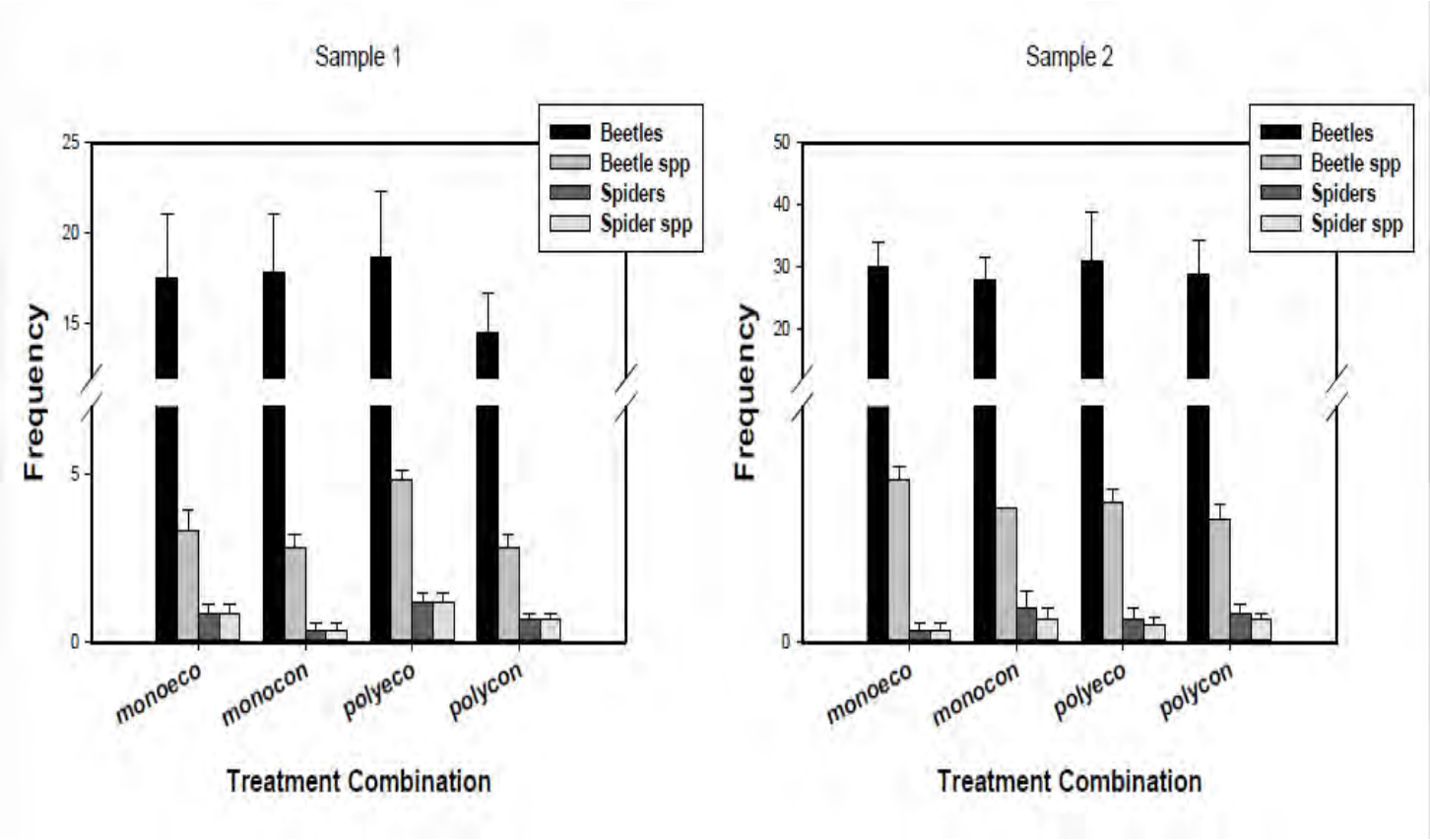
Ecotec-G
OMRI Listed
Ready-to-use Granular Broad Spectrum Insecticide
KEEP OUT OF REACH OF CHILDREN
CAUTION - PRECAUCIÓN
Active Ingredients
Clove Oil 4.00%
Cinnamon Oil 1.00%
Thyme Oil 0.66%
Other Ingredients* 94.34%
Total 100.00%
FIRST AID
PERSONAL PROTECTIVE EQUIPMENT (PPE)
USER SAFETY RECOMMENDATIONS
PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS
CAUTION
STORAGE AND DISPOSAL
BRANDT
Net Contents:
20 lbs
Packaged 2 x 20 lbs
Net Weight: 40 lbs

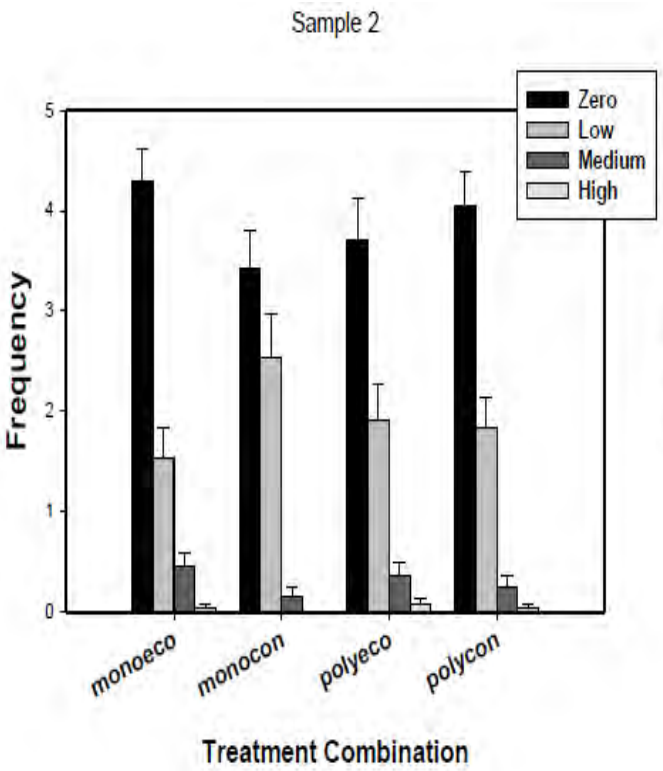
main treatment randomly assigned to 12 plots of 20' by 8 beds

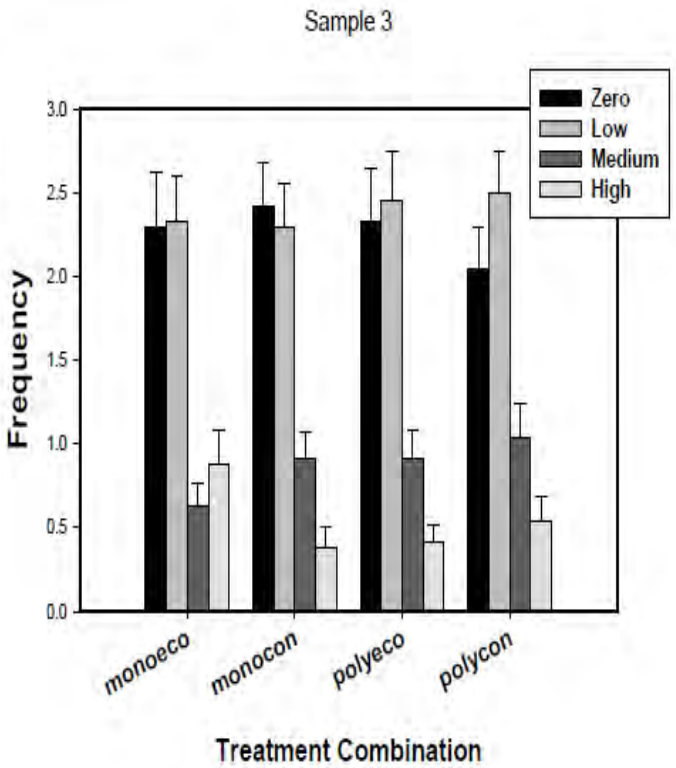
monoculture turnip

lettuce, carrot, turnip polyculture

split plots show Eco-tec G application




Sample 2
Zero
Low
Medium
High
Frequency
0
1
2
3
4
5
monoeco
monocon
polyeco
polycon
Treatment Combination


Sample 3
Zero
Low
Medium
High
Frequency
0.0
0.5
1.0
1.5
2.0
2.5
3.0
monoeco
monocon
polyeco
polycon
Treatment Combination

Market Yield Outcomes

Turnip	NS (Main and Sub)
Lettuce	NS (Sub)

TotYield Kruskall-Wallis X^2=5.1, P=0.0243 (Main)

Ecotec-G		Ecotec-G
10.3 Kg/plot		16.1 Kg/plot
Vs.		Vs.
8.6 Kg/plot		15.0 Kg/plot
$42.9 /plot	Vs.	$101.5/plot

Conclusions/Future work

- PPN….sampling method, burial/retrieval approach
- Club root….ASD, MSM, pH 7.2 or above, rotation
- ASD….further optimizing N management and soil prep
- Powdered MSM….roller vs. non-roller
- CM monitoring….egg sampling via sand floating
- CM threshold…. Above at 1 week old broccoli transplants with 6 eggs/plant -> decline floret size
- Simpler method to monitor adult CM flight activity needed
- Studying relationships between organic management practices and parasitism rates for IPM strategies for the CM control in broccoli production

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- Eric Lauritzen, Monterey County Agricultural Commissioner
- Mary Lou Nicoletti, Santa Cruz County Agricultural Commissioner
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Questions?