The CERES Trust Organic Research Initiative Final Report

**Project Title: Development and delivery of a comprehensive orchard floor management tactics to promote natural pest management and enhanced soil quality.**

**Principal Investigator:** Dr. Matthew J. Grieshop, Associate Professor of Organic Pest Management, MI State University, Dept. of Entomology, 578 Wilson Road 205 CIPS, East Lansing, MI 48824, email: grieshop@msu.edu, Phone: 517-432-8034, Fax: (517) 353-5598

**Report Prepared By:** Jason M. Matlock, Project Doctoral Student and Matthew Grieshop

**Executive Summary:** Management of orchard floors presents unique opportunities for organic apple producers. The objective of our multiyear project was to develop and evaluate comprehensive floor management practices that optimize pest and nutrient management for organic apple producers. Initially our research focused on the utilization of mechanical implements to manipulate overwintering leaf cover –with the objective of minimizing apple scab and disturbing pest insect overwintering sites. We also evaluated whether buckwheat cover crops can modify orchard microclimatic conditions to help avoid frost damage. The biggest outcome from our studies so far is cultivation timed around key pest life cycle stages –especially late in the growing season– reduces early season pest damage. A second finding of this study is that early season physical manipulation of orchard floors is greatly complicated when faced with wet springs. Abnormally high precipitation events delayed our early season cultivation and physical floor material manipulation by nearly 2 months in 2014 and 1 month in 2015. Thus, in 2015 we evaluated how late season nitrogen applications in the form of amino acids affect orchard floor dynamics with a focus on the reduction of overwintering apple scab (the primary pest of apples in North Eastern production) and changes in the detritivore community utilizing metagenomic techniques. Results from this line of inquiry suggest that nitrogen applications may impact orchard decomposer invertebrates but not nearly as much as overall management practices. While just a first step in unraveling this complex system, the data we have generated in this area represents the most complete evaluation of the invertebrate detritivore community of apple orchards to date. We presented project results at 9 extension and scholarly events including a field day at AlMar Orchards. Data from this project is expected to result in 2-3 scholarly publications.

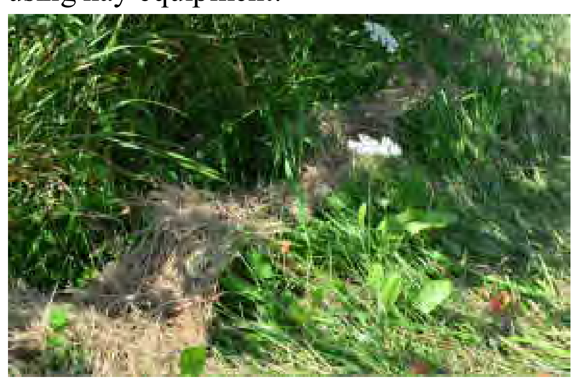
**Summary of Project Findings by Objectives:**

1) Determine soil and pest management and ecosystem service impacts of integrated floor management practices.

**Floor Litter Management Experiment:** Beginning in the Spring of 2014 We initiated a *Floor Litter Management Experiment* which involved moving ground litter and grass cuttings in and/or out of the drip line of the trees at specific times of the year utilizing a specialized hay rake called a hay bob. The goal of this study was to evaluate whether the addition and removal of leaves and other organic material (*e.g.* grass clippings) could limit the overwintering success of apple scab (*Venturia inaequalis*) and insect pests (Fig. 1). Five blocks were established in an organic apple orchard at Jim Koan's AlMar Orchards in Flushing, MI. Each block consisted of ten rows of high-density apple trees. The blocks were divided into four, ½-acre plots. Each plot was randomly assigned to one of four treatments as per a randomized complete block design. The four treatments were: Add: After each mowing, the organic residue from the drive row was pushed underneath the trees; Remove: At the end of the Fall and the beginning of the Spring, organic residue was pulled out from underneath the trees and moved into the drive row; Add-Remove: Combined the practices of both the Add and Remove treatments; Control: Used the Grower's standard practice of leaving organic residue from mowing in the drive row and leaf litter underneath the trees.

*Floor Litter Management Results:* Unfortunately, extremely wet field conditions in both 2014 and 2015 limited our ability to cultivate and haybob prior and during the primary scab infection period. Results from activities performed in 2014 indicated that late season floor management was unlikely to induce detectable changes in the rate of apple leaf decomposition, the status of apple scab or impacts on soil nutrients. Thus, we developed a new approach based on the recent availability of OMRI compliant, high nitrogen content, amino acid based fertilizers and a study site that would allow comparison between herbicide managed ground cover vesus cultivation managed ground cover.

**Figure 1.** Cut grass thrown under trees using hay equipment.



**Take Home Message:** *We saw no significant differences in any of the damage categories in either the early or late evaluation periods for spring 2015. This applies to both the frequency and severity of damage. This is likely because we were unable to apply cultivation or add/remove treatments in the spring of 2014 and 2015 due to unusually wet Spring conditions leading to impassable soils. Our collaborating grower, Jim Koan of AlMar orchards has indicated that based on these occurrences, early season physical floor management is not viable.*

**Orchard Floor Ecology Experiment:** Our revised floor litter management experiments evaluated the use of Fall nitrogen applications to enhance dormant season breakdown of leaf litter that harbors overwintering scab spores. In addition to measuring the breakdown of apple leaves, we also developed a Next Generation Sequencing approach towards to identification invertebrate detritivores.

Research beginning in the early 1960s and continuing into the current decade has shown that late season applications of nitrogen to apple orchards can reduce the incidence of spring apple scab spores by up to 95%. Urea has been the nitrogen source typically used to achieve this reduction in spore production and the suspected mechanism is an accelerated breakdown of the overwintering leaves that harbor overwintering apple scab. The advantage of a nutrient based (versus a physical management based) reduction of leaves is that orchard floor passability is typically very good in the Fall whereas our experiences have shown that this can be a serious problem in the Spring. Unfortunately, the NOP compliant nitrogen sources available until recently have not had sufficient nitrogen content to affect reductions in spring inoculum. Furthermore, the actual mechanisms behind scab reductions including the identity of key players in the detritivore food web are unknown. The field experiment that we initiated in Fall 2015 addressed both of these objectives.

Experimental plots were established at The Country Mill Orchard in Charlotte, MI. The orchard was under both USDA Certified Organic and Conventional management. We established experimental blocks in Red Delicious and Jonogold plantings, both of which are susceptible to apple scab infection. Experimental units were 8 trees long by 4 rows wide, approximately one-tenth of an acre. One block was established per management style for each cultivar. We included apples under conventional management not to compare organic to conventional practices but because we felt that this was the best way to elucidate the mechanisms at play in this system. This rational can be summarized by two points: 1) floor management is vastly different between organic and conventional apples with organic orchard floors typically having a much more diverse plant community (conventional orchard floors are typically barren beneath the trees); 2) we felt that it was imperative that we include a Urea control as this has been the standard in previous literature but could not do this on certified organic apples. Treatments in Conventional Management are Urea, Amino Acid, and Control. Treatments in Organic Management are Amino Acid and Control. The Urea treatment is a single application of a 5% urea solution. The Amino Acid treatment is a single application of a 5% solution of Solu-Nitrogen (Compostwerks LLC, Mt.Kisco NY). Solu-Nitrogen is an OMRI-listed blend of soluble, free amino acids derived from soy protein and has been available for less than two years. The Control treatment is a single application of water. All treatments were applied on the same day, November 11th, at a rate of 300 gallons per acre. The application date corresponded was within a few days of the first hard fall frost and represented 75% average leaf fall across the orchard. Applications were made to leaf litter on the ground, with the exception of areas in which trees had achieved <50% leaf fall. In these areas, applications were split evenly between the canopy and the litter. This experiment involved two sampling regimes: tracking changes in the invertebrate community structure of the soil-litter interface and tracking the progress of physical leaf degradation.

*Invertebrate Community Methods :*Samples for invertebrate community structure were taken by placing a 1 sq ft quadrat in the center of an experimental unit, vacuuming the area to capture any fast moving organisms, capturing any ground cover and remaining litter, and removing the top 1-cm of soil. Four subsamples were taken per experimental unit. The material was then processed in the lab using Berlese funnels to extract soil invertebrates. The first round of samples was taken 3 days after application of treatments on 11/28/2015, with subsequent samples taken on 12/18/2015, 2/24/2016, and 4/06/2016. The samples were then frozen at -80 C prior to DNA extraction, amplification, and identification using Next Generation Sequencing. Invertebrate samples were hand-sorted to obtain clean specimens and DNA was extracted using a CTAB-PVP DNA extraction technique w/bead homogenization. DNA was amplified using universal invertebrate CO1 PCR primers and submitted for MiSeq sequencing. Sequences were run through a custom bioinformatics pipeline to process sequencing data. Data were then GenBank and BOLD genomic databases. Once invertebrates were identified we used Non-Metric Dimensional Scaling (NMDS) to visualize our data and evaluate the similarity/dissimilarity of our communities.

*Leaf Decay Methods:* Leaf Samples consisted of leaves collected from the center of each experimental unit periodically throughout the experiment and quantifying the degree of degradation by measuring *Specific Leaf Area*. Specific leaf area is defined as the dry mass of a leaf divided by its surface area. Collected leaves are rinsed free of debris over a sieve, flattened in a leaf press for 24 hours, and then laid out on a sheet of white paper (Fig. 2). The leaves and paper are photographed. The area of the paper is known, which allows the surface area of the leaves to be determined using the pixel count of the photo:

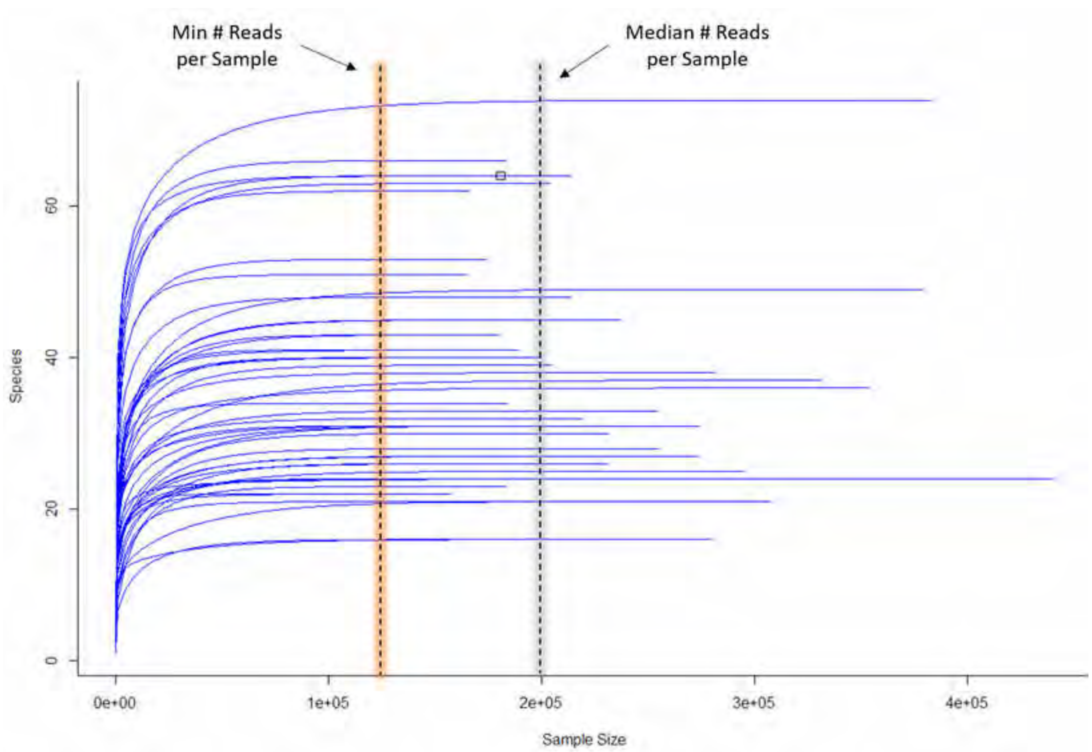
Leaf Surface Area =[ (Total Pixels-White Pixels)/Total Pixels] x Surface Area of Paper

Specific leaf area was then be determined by oven drying the leaves from each page and dividing the calculated area by the dry weight. This metric allows us to detect changes in leaf composition even when leaves are highly degraded and piecemeal. We had 8 sampling dates corresponding to Days 0, 10,20,40,60,100,120, and 140 after treatment. This sampling regime covered the time from application all the way to the start of primary scab infection in the Spring of 2016.

**Figure 2.** Litter Decomposition Photos. Note: (LEFT) Original photo of flattened, degraded leaves; (RIGHT) Transformed photograph read for pixel counting

*Invertebrate Community Results:* The first step in post-processing analysis of sequence data is ensuring that sequencing depth sufficient to ensure detection of the organisms within each sample was employed. Rarefaction is a bootstrapping method that evaluates how many organisms become detectable at different levels of sequencing. The goal is for a rarefaction curve to flatten out before it reaches the experimental sampling level. Our rarefaction analysis indicates that we achieved sufficient sequencing depth. Figure 3 shows that we achieved

**Figure 3.** Rarefaction Curves Showing Successful Detection of Invertebrates in Samples with Sequences with less than 5 reads removed. Horizontal curve between dotted lines indicates optimal sampling.



acceptable sequence depth when sequences occuring less than 5 times were removed from our data set. This is well within the heuristic value of 10 copies per sequence used by most metagenomic studies.

Interpretation of ecological data is complicated by having many responses (multivariate) that change values with time and treatments. Identifying important trends can be challenging if numerical methods are used to explore the data. It is helpful to visualize data first, and then validate visual trends numerically. We used Non-Metric Dimensional Scaling (NMDS) to visualize our data. NMDS takes multivariate, multifactor data and plots it such that the relative distance between points represents the cumulative difference resulting from the combined experimental factors. Points that cluster together are related, and clusters that are separate from each other are different. The axes have no units. Overlaying circles that correspond to the space of each treatment allows us to discern associative relationships between our experimental factors and the data clusters.

Our data suggest that the detritivore communities of organic and conventionally managed orchards have distinctly different compositions throughout the overwintering period (Fig. 4). We also observed a shift in community structure resulting from the addition of urea, which may indicate that a subset of the conventional community responds to that addition. The lack of consistent differences between nitrogen treated and untreated samples suggests that potential shifts in these communities due to urea applications are mediated by weather conditions or some other extrinsic factor. Investigation not the diversity of species within the 20 arthropod families within organic and conventional plots revealed some striking differences (Fig. 5). Organic plots had a higher diversity of Carabidae (ground beetles) Entomobryidae (springtails), Julidae (millipedes) and Cicadellidae (true bugs). Conventional had higher diversity of Pyralidae(moths), Tomoceridae (springtails), Formicidae (ants), Sciritidae (March Beetles) and Cecidomyiidae (midges).

The results collected in this study represent one of the largest studies to date using metagenomic approaches to identifying invertebrate detritivore communities. The next steps will be to identify key indicator taxa associated with these changes, and to associate known ecological function with those key taxa in order to understand how these changes are impacting ecosystem services. These activities are being carried out in ongoing leveraged projects funded by the Michigan Apple Committee including a study using molecular methods to detect which invertebrate taxa consume apple scab.

**Figure 4**. Exploration of Sequence Data using NMDS Ordination where each red dot is a single record of an individual invertebrate family or genus. O_C indicates organic management, C_C indicates conventional management without urea and C_U indicates conventional management with urea. Overlaying circles indicate 95% confidence intervals of association among red dots. Note that the circle marked O_C is always seperated from the ovals marked C_C or C_U. In contrast C_C and C_U circles overlap, especially on 12/18/2015 and 02/24/2016.

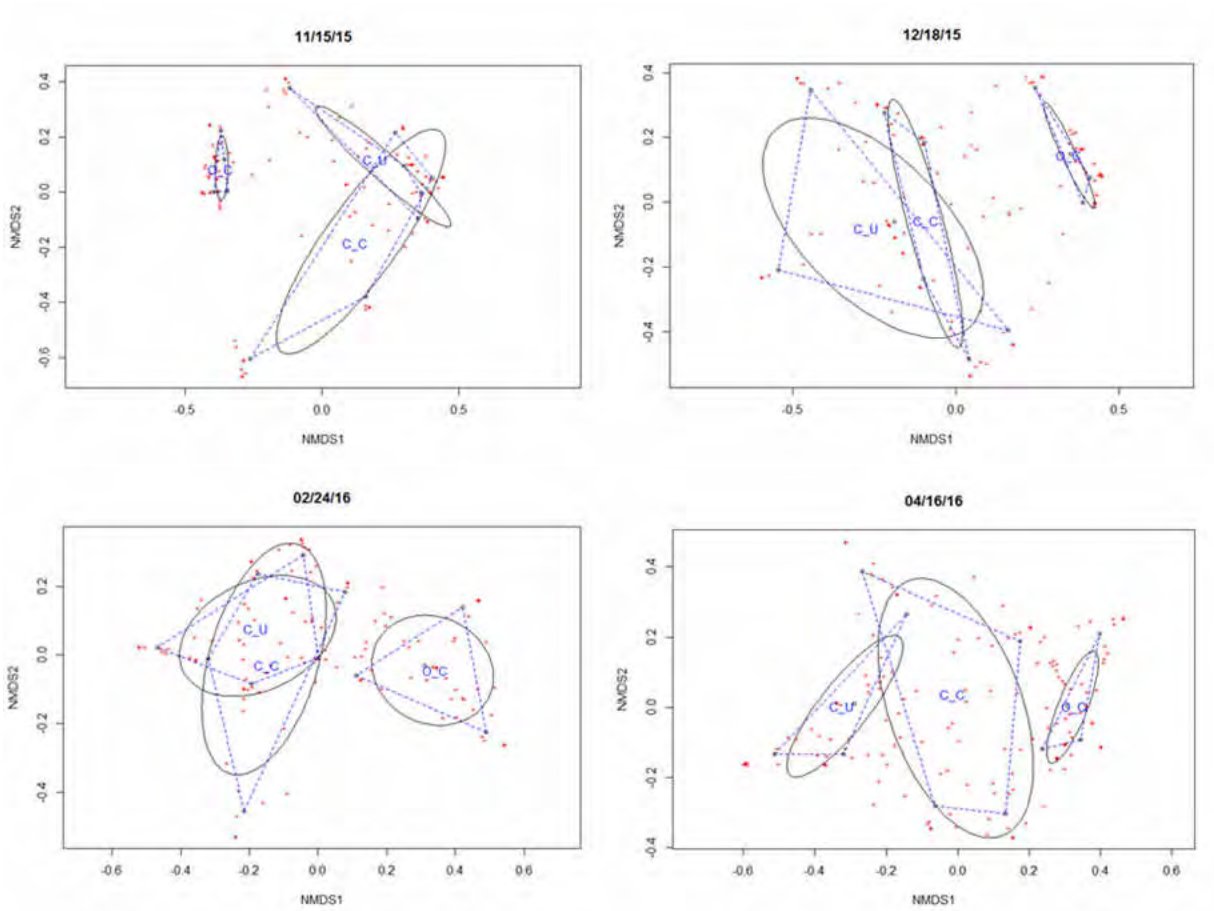
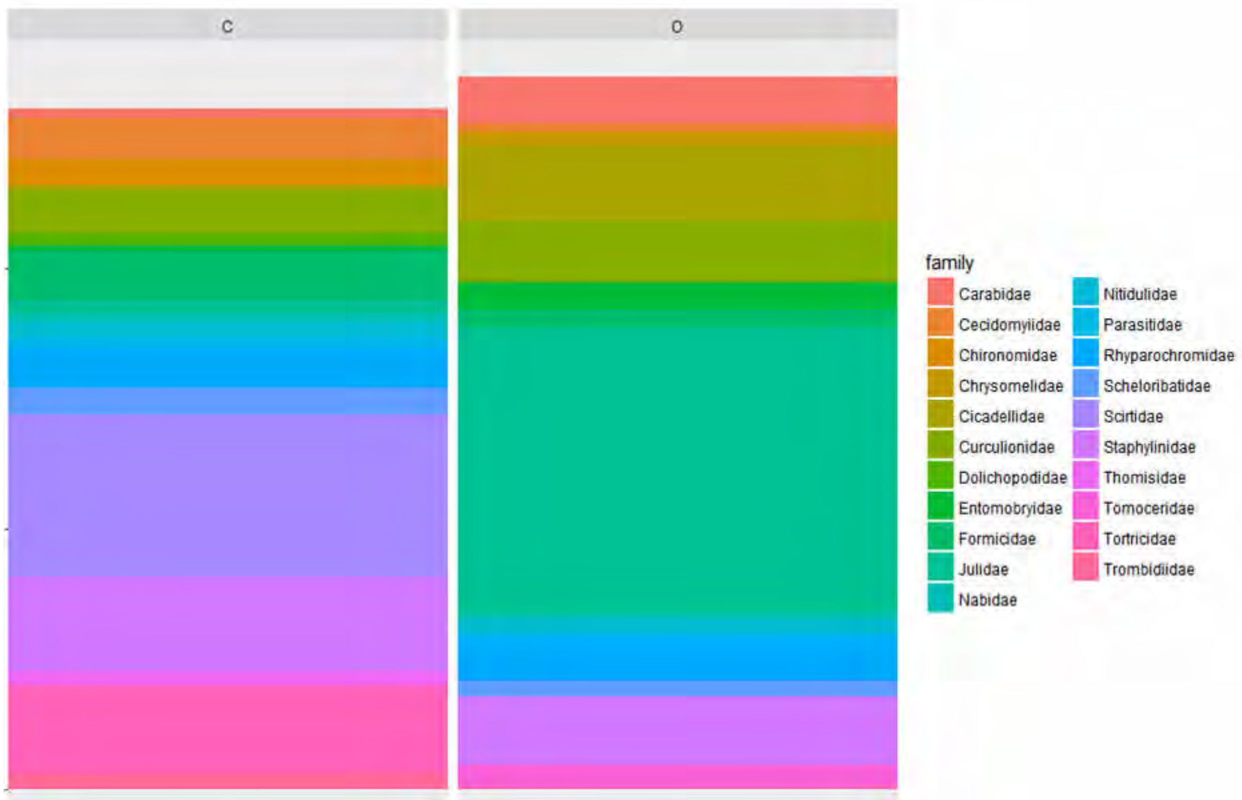
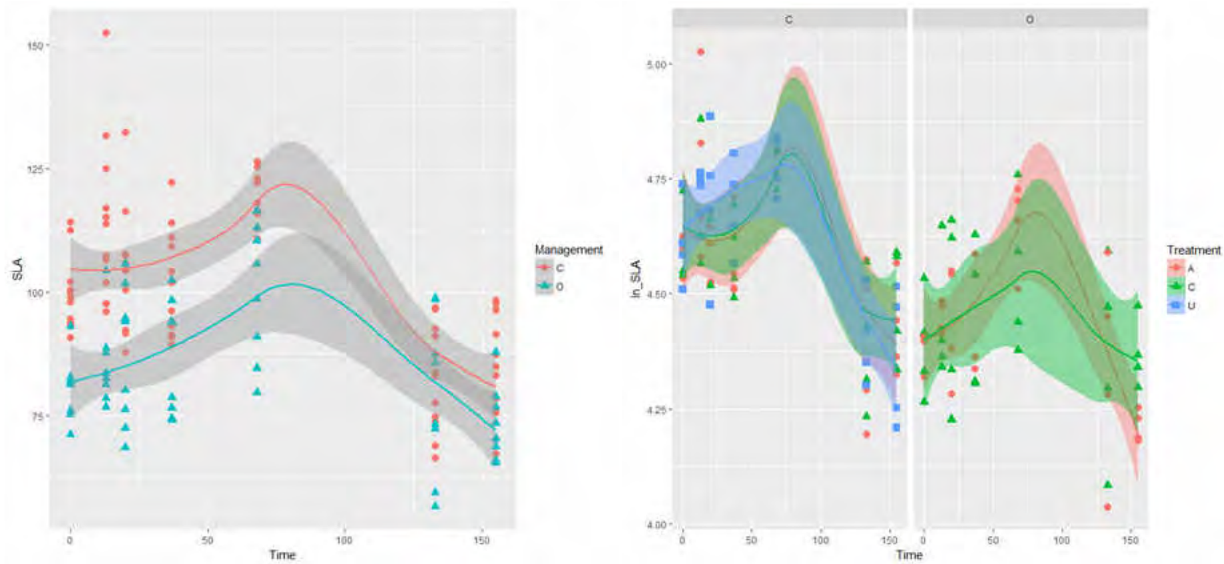


**Figure 5.** Relative Abundance of Top 20 Detected Families by Management. C = Conventional and O = Organic. Organic plots had a higher diversity of Carabidae (ground beetles) Entomobryidae (springtails), Julidae (Millipedes) and Cicadellidae(true bugs). Conventional had higher diversity of Pyralidae(moths), Tomoceridae (springtails), Formicidae (ants), Sciritidae (March Beetles) and Cecidomyiidae (midges).




*Leaf Decay Results:* The effect of floor management and nitrogen application on leaf decomposition rate was evaluated using a time series measurement of leaf specific area (Fig. 6). We saw that specific leaf area was greater in the conventionally managed plots than the organically managed plots during the late fall. Specific leaf area values converged to equivalency by the start of the following spring. This data suggests that 1) conventionally managed leaves had more solid content than organically managed leaves, though the reason for that remains unexplained; 2) the rate of leaf decomposition in conventionally managed plots is slightly faster than in organically managed plots. A possible explanation for the rate difference is that leaves are the only carbon source in the herbicide-managed floor, whereas organic orchards have a considerable carbon load from other herbaceous ground cover that competes with the leaves for decomposers. We did not see an appreciable effect of nitrogen application on leaf decomposition in either management system.

**Figure 6.** Changes in Leaf Specific Area Over Time. Shaded areas represent 95% confidence intervals (LEFT) Visualization of the impact of general floor management strategy. "O" is Organic-Certified management; "C" is conventional management (RIGHT) Visualization of the impact of fall nitrogen application. "A" is amino, "U" is urea, "C" is a water control



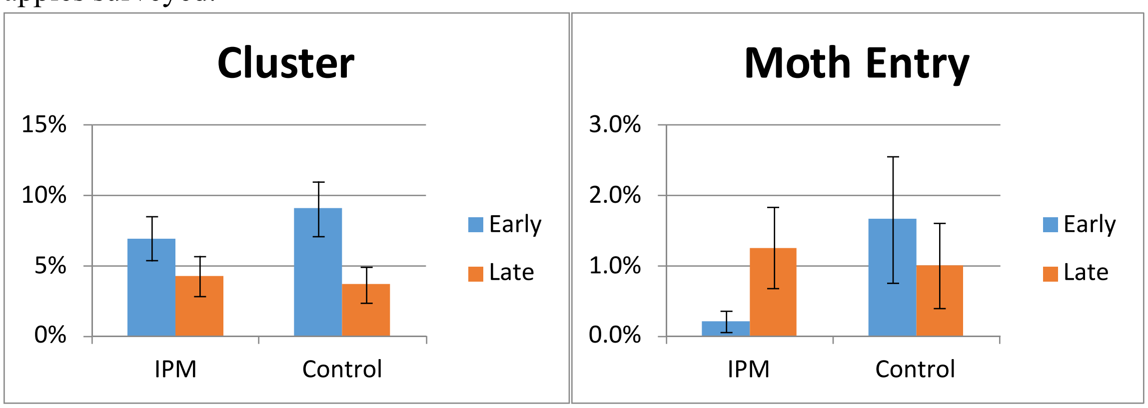

| |
|---|
| **Take Home Message:** *Management style but not nitrogen applications had a significant impact on leaf breakdown; surprisingly leaf decay was slower in organic managed orchards compared with conventional. Nitrogen applications did not significantly affect leaf breakdown in either system. Invertebrate detritivore communities showed significant differences between conventional and organically managed apples. Nitrogen appeared to affect these communities in the warmer sampling periods (fall and spring) but not in the winter. Based on these results we do not feel that organic nitrogen sources are adequate for scab management.* |

2) Determine impacts of optimized timings of cultivation and litter management on key orchard pests

**Targeted IPM Experiment:** This experiment was conducted to evaluate whether light cultivation and flail mowing of orchard floors could affect key apple pests including codling moth, Oriental fruit moth, plum curculio, stink bugs and apple scab. Five blocks were established in an organic apple orchard at Jim Koan's AlMar Orchards in Flushing, MI. Each block consists of ten rows of high-density apple trees. The blocks are divided into two, 1 acre plots. Each plot was randomly assigned to either the control or experimental treatment as per a randomized complete block design. The two treatments were: Targeted IPM: Fallen apples and litter are collected and moved into the drive row to be crushed with a flail mower in order to physically damage developing larvae. The events were timed around "June Drop" and the late larval/pupal stages of the codling moth using phonological models calibrated against insect traps and weekly fruit scouting and Control: The grower's standard management practices. The experiment was initiated in 2014 and damage data collected in early July and just prior to harvest in late August of 2015 and 2016.

*Targeted IPM Results:* In 2015 we observed few significant differences in any of our measured categories in terms of frequency or severity of damage. The two responses that varied however were early season apple scab lesions and fruit moth entries, both of significant concern to Michigan apple growers (Fig. 7). On average, apple scab lesions covered 2% less surface area per leaf cluster in the Targeted IPM treatment during the early evaluation period. The most significant finding in this study was a reduction in the number of fruit with codling or oriental fruit moth entries, from 1.5% of fruit observed to 0.5%.

**Figure 7.** Significant Findings from 2015 Damage Evaluations (Left) Severity of apple scab Infections on leaf clusters. Percentages represent the surface area infected by apple scab relative to the whole cluster. (Right) Frequency of apples discovered with signs of moth entry. Percentage represent the number of fruit infested with moth larvae relative to the total number of apples surveyed.




In 2016 significant differences in the presence of pest damage on fruit were detected for moth stings and stinkbugs during the Early July sampling period (Tables 1-2). All other observed differences were not statistically significant. The consistent reduction of early season fruit moth damage is encouraging. Codling moth and Oriental fruit moth are capable of traveling miles in a single night and second generation populations are typically higher than first generation populations. Thus, the fact that this effect consistently disappeared during the later season may be due to female moths immigrating into the treated acreage during the second generation. The fact that plum curculio damage was never reduced suggests that cultivation is not a viable management tool for this pest.

**Table 1.** Apple Fruit Pest Damage in Early July 2017 (400 fruit per orchard sampled)

| | Plum Curculio | Moth Sting | Moth Entry | Leaf Roller | StinkBug |
|---|---|---|---|---|---|
| **IPM** | 11.8 | ***4.6*** | 1.4 | 0.3 | ***2.4*** |
| **Control** | 12.8 | ***7.6*** | 1.3 | 0.2 | ***4.7*** |
| **P-value** | .4950 | ***.0055*** | .8463 | .6561 | ***.0061*** |

**Table 2.** Apple Fruit Pest Damage in Late August 2017 (400 fruit per orchard sampled)

| | Plum Curculio | Moth Sting | Moth Entry | Leaf Roller | StinkBug |
|---|---|---|---|---|---|
| **IPM** | 15.2 | 14.4 | 8.0 | 0.1 | 10.9 |
| **Control** | 16.8 | 15.0 | 7.6 | 0.5 | 9.1 |
| **P-value** | .3278 | .7041 | .7383 | .1405 | .1760 |

**Note:** Values are reported as percent of surveyed fruit displaying damage symptoms

**Take Home Message:** *These data are encouraging because they suggest that late season cultivation can have a detectable impact on early season insect and fungal pest damage. While it is unlikely that this impact will provide economic control of apple scab or fruit moths, incorporation of this tactic should contribute to resistance management and leverage both mating disruption as well as the limited pesticide options available to organic apple producers. It is important to note that these pest management services are in addition to the primary service of orchard floor weed management –thus they represent an added benefit of a standing orchard practice.*

3) Determine the thermal and biological effects of clean cultivation or buckwheat understory crop on orchard soil and canopy ecosystems.

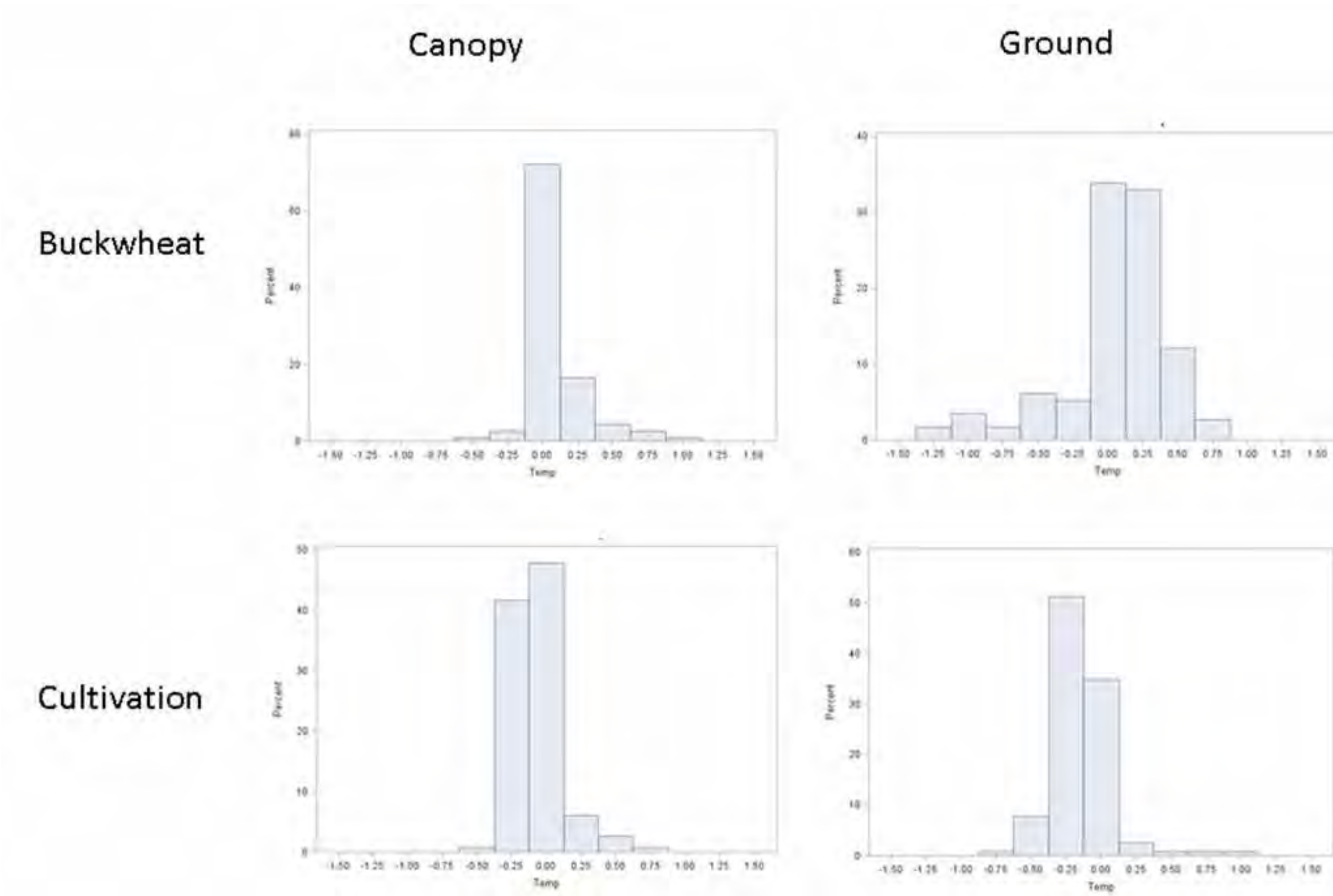
**Ground-Cover Thermal Regulation Experiment:** Five blocks were established in an organic apple orchard at Jim Koan's AlMar Orchards in Flushing, MI. Each block consists of three rows of high density, trellised apple trees. The blocks are divided into three, 100m long plots. Each plot was randomly assigned to one of three treatments as per a randomized complete block design. The three treatments were: Buckwheat where buckwheat seed is planted towards the latter part of growing season in the drip line of the tree row; Cultivation: Multiple cultivation passes are made after harvest through the late Fall in order to keep the drip line bare and Control: grower standard practices.

Buckwheat was planted on the 25th of August 2014 and 26th of July 2015 using a custom-built seed spreader (Fig. 8). Untreated buckwheat seed was purchased from the Mason Grain Elevator (Mason, MI). HOBO Pendant weatherproof temperature loggers were purchased from Onset Computer Corporation (Bourne, MA) and placed on tree trunks at two heights near ground level in early March of 2015 and 2016 to capture potential effects of floor management on ground level temperatures during critical frost events occurring as trees emerge from winter dormancy. We monitored 4 trees in each experimental unit. The trees were located in the center row of the experimental unit and evenly distributed across their lengths. Each tree had two data loggers placed 3" and 5' from the ground, respectively.

**Figure 8.** Custom built seed spreader in orchard row

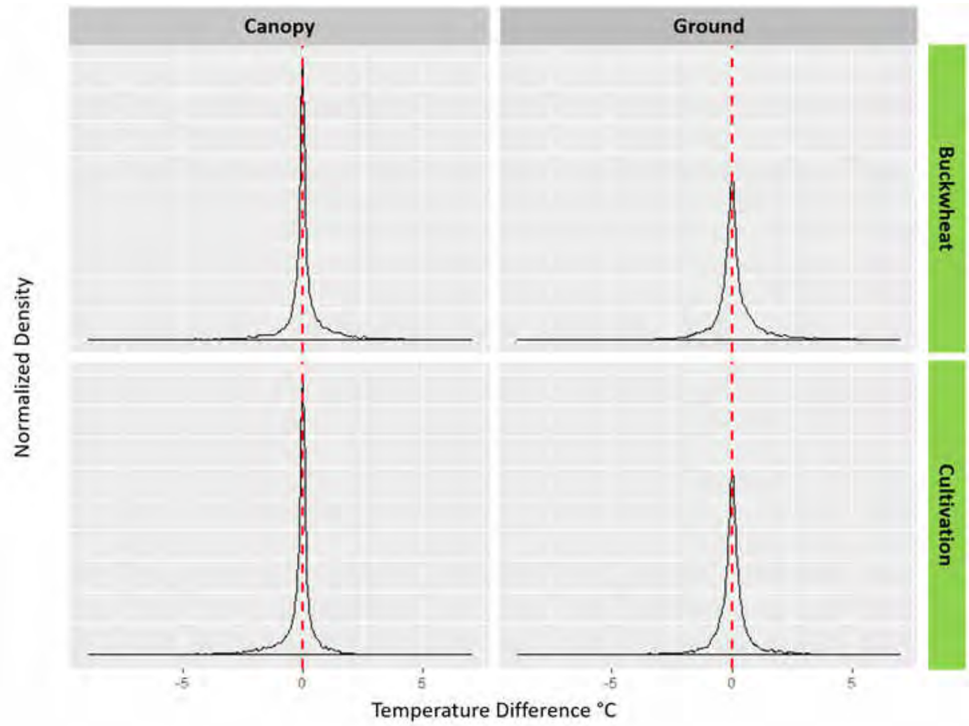
*Results for Ground-Cover Thermal Regulation Experiment:* In 2015 thermal data was restricted between April 15 and May 20$^{th}$ –the time when apple trees are most susceptible to frost damage. We then further refined the data set by using only time points at which the average temperature of at least one of the three treatments was at or below freezing. We then calculated the differences between the two experimental treatments (Buckwheat Cover Crop and Cultivation) and the control treatment (Grower's Standard Practices). For the majority of time points, temperature differences relative to the control fell between -0.25 and 0.25 degrees C for both experimental treatments. This was true of canopy and ground temperatures. Statistical analysis showed that the observed differences were non-significant (Fig. 9).

**Figure 9.** Temperature Differences of Experimental Treatments During Critical Frost Periods Relative to Temperatures observed in Areas under Standard Practices . Patterns indicate minimal differences due to floor canopy manipulation.




In 2016, the difference in temperature between each experimental treatment and the grower standard was calculated separately for Canopy and Ground values for each time point within each block. Data was trimmed to a date range from April 1st to May 20th to capture potential differences arising during the frost-risk period for this growing region. The distributions of temperature differences all centered sharply around zero, indicating no appreciable change resulting from treatment applications (Fig. 10).

**Figure 10.** Change in Ground and Canopy Temperatures Resulting from Application of Experimental Treatments. Dotted line denotes the mean temperature difference observed for the duration of the study.




| |
|---|
| **Take Home Message:** *Neither cultivation or buckwheat cover crops had an appreciable impact on canopy temperatures during the frost risk period. In addition, it was very difficult to establish a solid buckwheat canopy under the apple trees.* |

4) Deliver findings to the North Central region organic orchardist community

**Extension/Outreach:** We presented data/outcomes from our study at nine extension meetings including an overview of the project that was presented at the 2016 Pennsylvania Sustainable Agriculture Society (PASA) annual meeting and at a field day held at AlMar orchards on June 25, 2017. In addition we will be presenting a talk at the upcoming Entomological Society of America National Meeting in Denver, CO in early November 2017.

1. Grieshop, M.J. 2014. University of Wisconsin Department of Entomology Seminar Series, Madison WI, "Exploring floor management to enhance perennial crop IPM programs." (February 14, 2014).1.
2. Schmidt, J. (Presenter & Author), Grieshop, M.J. (Author Only). 2014, November. Effects of orchard floor management on disease and biocontrol. Poster session presented at Entomological Society of America Annual Meeting, Portland, Oregon. (November 18,

2014).
3. Matlock, J., Grieshop, M. J. (December 6, 2016) *Overwintering Apple Scab Inoculum and Invertebrate Detritivores: Progress and Challenges in Using Molecular Ecology to Explore Orchards*. Presented poster at Great Lakes Fruit and Vegetable Expo, Michigan State Horticultural Society, Grand Rapids, MI.
4. Grieshop, M.J., Baughman B. and Matlock, J. (January 12, 2016) *Alternative Floor Management in Orchards and Vineyards.* Presented paper at Northwest Orchard and Vineyard Show, MSUE. Acme MI.
5. Grieshop, M. J. 2015, June. Organic pest management in tree fruit. Student Organic Farm, East Lansing, MI. (June 10, 2015)
6. Matlock, J., Grieshop, M. J. Unraveling Decay Cycles in Orchard Soils. Abstract presented at Great Lakes Fruit and Vegetable Expo, Grand Rapids, MI. (December 10, 2015).
7. Grieshop, M. J., Koan, J. (February 5, 2016) *An Overview of Organic Apple Production... plus hogs!* Presented paper at PASA Annual Meeting, Pennsylvania Association of Sustainable Agriculture (PASA), State College, PA.
8. Grieshop, M. J., Koan, J. (February 5, 2016) *An Overview of Organic Apple Production... plus hogs!* Presented paper at PASA Annual Meeting, Pennsylvania Association of Sustainable Agriculture (PASA), State College, PA.
9. Grieshop, M. J., Koan, J. (June 24, 2017) 2017 AlMar orchards field day: *An Overview of Organic Apple Production* AlMar Orchards, Flushing, MI.