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# 2013 Wild Blueberry Project Reports

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# 2013 Wild Blueberry Project Reports

## January 2014

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**FOOD SCIENCE AND NUTRITION**

**INVESTIGATORS**: Vivian Wu, Associate Professor of Food Safety and Microbiology, School of Food and Agriculture, University of Maine

**1. TITLE**: Development of effective intervention measures to maintain and improve food safety for wild blueberries.

**METHODS**: We have developed a comprehensive investigation in studying the effectiveness of various chemical sanitizers combined with low temperature frozen storage in inactivating foodborne pathogens on wild blueberries. Two strains of *Escherichia coli* O157:H7 (ATCC 35150 and ATCC 129000), *Salmonella* Typhimurium (ATCC 6962 and ATCC 14028) were used to inoculate the surface of blueberries by a dipping method. To prepare a cocktail mixture, two suspensions of each pathogen with equal populations were combined. Twenty five gram of blueberries without prior washing or decontamination were placed on sterile petri dish and inoculated with 2.5ml of bacterial cell suspension for each pathogen by the dipping method. The inoculated blueberries were placed on sterile glass rods and dried for 2h in a laminar flow hood. The initial concentration of *E. coli* O157:H7 and *S.* Typhimurium on blueberries was approximately around 7 log CFU/g after completion of inoculation. Fresh solutions of chemicals in distilled water were prepared the same day of each experiment. The treatments tested included: chlorine ($Cl_2$, 100, 150 and 200ppm), aqueous chlorine dioxide ($ClO_2$, 2.5, 5, 10 and 15ppm), lactic acid (1% and 2%), and peroxyacetic acid solution (1% and 2%). Control treatment was a water wash. Inoculated blueberries were spread on sterile wire screens using sterile forceps. Blueberry samples were sprayed with 250 ml of sterile distilled water (control) or different chemical solutions at different concentrations for different contact times ($ClO_2$, (2.5ppm,5ppm,10ppm and 15ppm for 10s, 1, 5 and 10min) $Cl_2$ (100ppm,150ppm and 200ppm for 10s, 1, 5 and 10min), lactic acid (2% and 1% for 5, 10 and 20min) and peroxyacetic acid (2% and 1% for 5, 10 and 20min) as shown in Figure 1. At the end of each treatment time, one set was kept in freezer at -15°C for 1 week and the other set was immediately proceeded for bacterial enumeration. For chlorine and chlorine dioxide treatments, visual quality testing and also these chemical residues left on these chemicals were also tested.

**RESULTS:** Freezing had significant impact on the reduction of *E. coli* O157:H7 and *S.* Typhimurium inoculated on blueberries. These chemical treatments, at any concentrations after freezing showed increased decontamination efficiency.

*Effect of aqueous chlorine dioxide treatment on pathogens:*

The effect of $ClO_2$ treatment combined with and without freezing on the reduction of *E. coli* O157:H7 and *S.* Typhimurium inoculated on blueberries are presented in Table 1. For *E. coli* O157:H7, $ClO_2$ at 10ppm concentration and 10 min treatment time caused the greatest reduction before freezing (1.87 log CFU/g) and after freezing (3.66 log CFU/g). However, the reduction of *E. coli* O157:H7 by 10 and 15ppm $ClO_2$ treatments were not significantly different. This means that 10 ppm treatment of $ClO_2$ has better application value in the food industry than that of 15 ppm for *E. coli* O157:H7. The overall *E. coli* O157:H7 decontamination at different $ClO_2$ concentrations at various time periods after freezing is shown in Figure 2. After freezing, with

the highest $ClO_2$ concentration (15ppm) and longest treatment (10min) there was an additional log reduction of 2.56 log CFU/g.

For *S.* Typhimurium, the highest $ClO_2$ concentration (15ppm) and the longest treatment (10min) had the greatest reduction before freezing (1.45 log CFU/g) and after freezing (4.93 log CFU/g) (Table 1). In our studies, aqueous $ClO_2$ was more effective in reducing *S.* Typhimurium than in reducing numbers of *E. coli* O157:H7 before and also after freezing. The overall *S.* Typhimurium decontamination at different $ClO_2$ concentrations at various time periods after freezing is shown in Figure 3.

*Effect of chlorine treatment on pathogens:*

The effect of $Cl_2$ treatment combined with and without freezing on the reduction of *E. coli* O157:H7 and *S.* Typhimurium inoculated on blueberries are presented in Table 2. For *E. coli* O157:H7, $Cl_2$ at 150ppm concentration and 10 min treatment time caused the greatest reduction before freezing (0.90 log CFU/g) and after freezing, the highest concentration (200ppm) and 1min treatment time showed greatest reduction (3.25 log CFU/g). Ten ppm treatment of $ClO_2$ has better reduction of *E. coli* O157:H7 than $Cl_2$. The Overall *E. coli* O157:H7 decontamination at different $Cl_2$ concentrations at various time periods after freezing is shown in Figure 4. After treatment combined with freezing, with the highest $Cl_2$ concentration (200ppm) and 1min treatment time there was an additional 0.82 log CFU/g reduction.

For *S.* Typhimurium, the highest $Cl_2$ concentration (200ppm) and longest treatment (10min) had the greatest reduction before freezing (1.22 log CFU/g) and after freezing (5.42 log CFU/g). $Cl_2$ was more effective in reducing *S.* Typhimurium than in reducing the numbers of *E. coli* O157:H7 before and also after freezing. This shows that $Cl_2$ treatment combined with freezing was slightly more effective in eliminating *S.* Typhimurium, with around 5 log CFU/g reductions, than $ClO_2$ combined with freezing which has 4.93 log CFU/g reductions. The overall *S.* Typhimurium decontamination at different $Cl_2$ concentrations at various time periods after freezing is shown in Figure 5.

Blueberries did not show any severe loss of visual quality after any of these chemical treatments and also there was no residue of these chemicals left on these blueberries.

*Effect of lactic acid treatment on pathogens:*

The effect of lactic acid treatment combined with and without freezing on the reduction of *E. coli* O157:H7 and *S.* Typhimurium inoculated on blueberries are presented in Table 3. For *E. coli* O157:H7, lactic acid at the highest concentration (2%) and 20 min treatment time caused the greatest reduction before freezing (1.98 log CFU/g) and also after freezing (4.41 log CFU/g). The overall *E. coli* O157:H7 decontamination at different lactic acid concentrations at various time periods after freezing is shown in Figure 6. After treatment combined with freezing, the highest lactic acid concentration (2%) and the longest contact time (20min) had additional 2.55log CFU/g reductions.

For *S.* Typhimurium, the highest lactic acid concentration (2%) and the longest treatment (20min) had the greatest reduction before freezing (2.24 log CFU/g) and also after freezing (4.73 log CFU/g). The overall *S.* Typhimurium decontamination at different lactic acid concentrations at various time periods after freezing is shown in Figure 7. Studies should be conducted to determine the visual quality of blueberries after treatment with lactic acid.

*Effect of peroxyacetic acid (PAA) treatment on pathogens:*

The effect of PAA treatment combined with and without freezing on the reduction of *E. coli* O157:H7 and *S.* Typhimurium inoculated on blueberries is presented in Table 4. PAA at both concentrations (2% & 1%) and at all treatment times caused a complete reduction after freezing with detection limit <1 log CFU/g for both pathogens (*E. coli* O157:H7 and *S.* Typhimurium*)* with original inoculation at approximately 7.0 log CFU/g. Before freezing, the maximum reduction achieved was around 2.72 log CFU/g with *E. coli* O157:H7 and 3.51 log CFU/g with *S.* Typhimurium. The overall *E. coli* O157:H7 decontamination at different PAA concentrations at various time periods after freezing is shown in Figure 8. Freezing alone contributed to additional 5.3log CFU/g reductions for *E. coli* O157:H7 and 4.2 log CFU/g for *S.* Typhimurium with both concentrations (1% and 2%).

The overall *S.* Typhimurium decontamination at different PAA concentrations at various time periods after freezing is shown in Figure 9. Studies should be conducted to determine the visual quality of blueberries after treatment with PAA and also to evaluate the residues of this chemical on these blueberries.

**DISCUSSION:** A significant reduction in pathogens can be achieved when any chemical treatment combined with freezing so, from this study we can say that the quality and safety of wild blueberries can be well maintained with efficient bacterial reductions when these chemical treatments are combined with freezing.

Aqueous chlorine dioxide treatment, even at low-concentrations and short exposure times, was more efficient in decreasing *E. coli* O157:H7 populations from blueberries than chlorine which has to be used at high concentrations like 200ppm. But, with *S.* Typhimurium, though $Cl_2$ treatment combined with freezing at highest concentration (200ppm) was slightly more effective in decreasing *Salmonella* counts from blueberries (with around 5 log CFU/g reduction) than $ClO_2$ combined with freezing (with around 4.93 log CFU/g reduction), there was no significant difference. Hence, for both the bacteria, chlorine dioxide at very low concentrations than chlorine when combined with freezing is effective in eliminating these pathogens from blueberries.

A complete bacterial reduction (with detection limit <1 log CFU/g) can be obtained on blueberries when peroxyacetic acid (15 or 2%) treatment is combined with freezing. However, further studies have to be conducted to look for visual quality and residues of this chemical on blueberries. Organic acids like lactic acid and peroxyacetic acid showed greater decontamination (though at longer time periods) in populations of *E. coli* O157:H7 and *S.* Typhimurium compared to other two chemicals ($Cl_2$ and $ClO_2$).

**FUTURE RESEARCH**: Further studies have to be conducted to look for the visual quality and residues of chemicals (lactic acid and peroxyacetic acid) on blueberries. We plan to extend the study to look at the effectiveness of all these chemical sanitizers [chlorine (100, 150 and 200ppm), aqueous chlorine dioxide (2.5, 5, 10 and 15ppm), lactic acid (1% and 2%), and peroxyacetic acid solution (1% and 2%)] on the reduction of inoculated *L. monocytogenes* on blueberries.

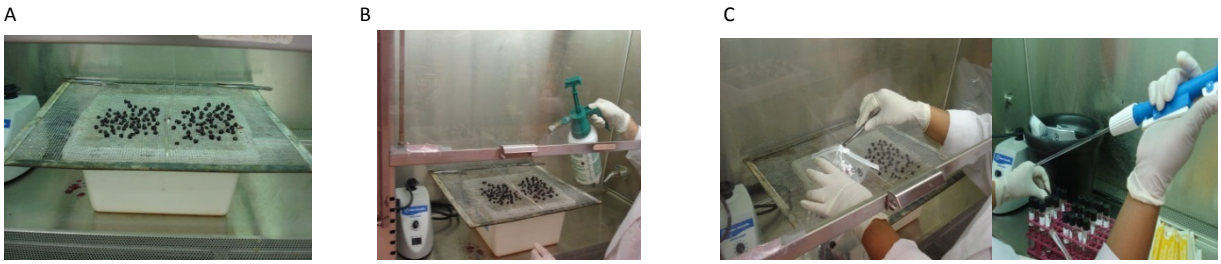



**Figure 1**: Blueberries chemical treatment. (A) Blueberries spread on sterile wire screens, (B) Blueberries sprayed with chemicals using home and garden sprayers modified with whirljet nozzle and left for various contact times, and (C) Blueberries were picked into sterile stomacher bag and after each treatment one set of each contact time was stored at -15°C for 1 week and the other set was subjected for serial dilutions and bacterial enumeration immediately.

**Table 1:** Reduction of *E. coli* O157:H7 and *S.* Typhimurium after treatment with aqueous chlorine dioxide at different concentrations and various contact times, before and after freezing at -15 $^{o}$ C/ 1week.

| *Pathogen* | *Treatment time $ClO_2$* | *Reduction (log CFU/g) before freezing* | | | | *Overall Reduction (log CFU/g) after freezing* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2.5ppm | 5ppm | 10ppm | 15ppm | 2.5ppm | 5ppm | 10ppm | 15ppm |
| *E. coli* O157:H7 | 10s | 0.42 | 0.53 | 0.95 | 1.14 | 1.67 | 2.61 | 2.57 | 2.37 |
| | 1min | 0.86 | 0.74 | 1.11 | 1.24 | 2.78 | 2.77 | 2.94 | 2.52 |
| | 5min | 0.61 | 0.83 | 1.25 | 1.34 | 1.81 | 3.24 | 3.08 | 3.10 |
| | 10min | 1.02 | 0.92 | 1.87 | 1.67 | 1.88 | 3.14 | 3.66 | 3.49 |
| *S.* Typhimurium | 10s | 0.79 | 0.86 | 0.99 | 1.04 | 3.94 | 3.98 | 3.98 | 4.14 |
| | 1min | 0.86 | 0.98 | 1.08 | 1.07 | 4.12 | 4.13 | 4.16 | 4.49 |
| | 5min | 0.95 | 1.05 | 1.22 | 1.30 | 4..28 | 4.32 | 4.44 | 4.70 |
| | 10min | 1.02 | 1.25 | 1.32 | 1.45 | 4.53 | 4.52 | 4.64 | 4.93 |

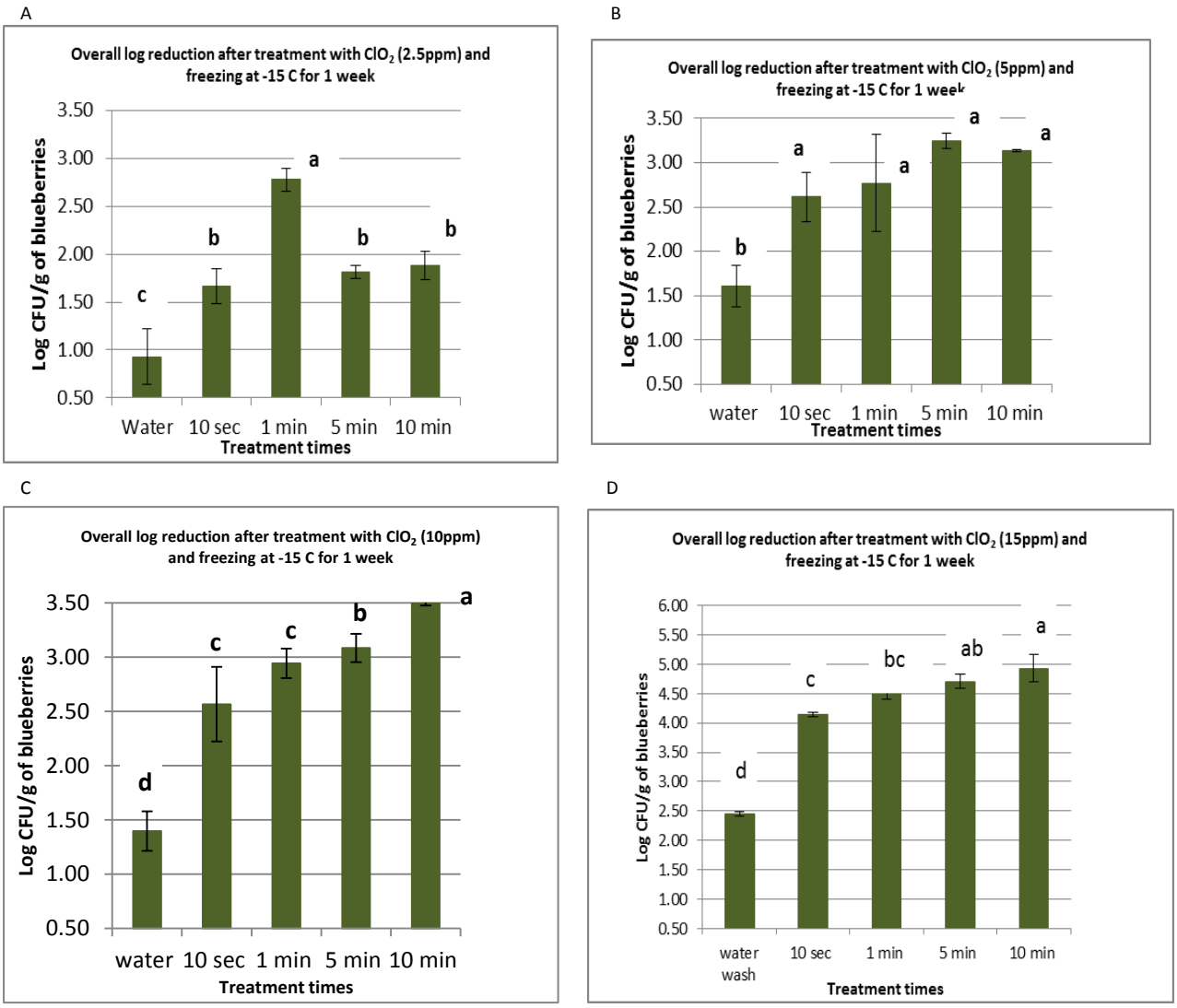



**Figure 2**: Log reduction of *E. coli* O157:H7 on blueberries at different contact times using a combination of aqueous chlorine dioxide followed by freezing at -15°C for 1 week. (A) 2.5ppm, freezing contributed to an average 1.04log CFU/g of this overall reduction and (B) 5ppm, freezing contributed to an average 1.7 6 log CFU/g of this overall reduction (c) 10ppm, freezing contributed to an average 2.30 log CFU/g of this overall reduction, and (D) 15ppm, freezing contributed to an average 2.30 log CFU/g of this overall reduction .
Concentrations of $ClO_2$ at each measurement time labeled with different letters are significantly different ($P<0.05$)

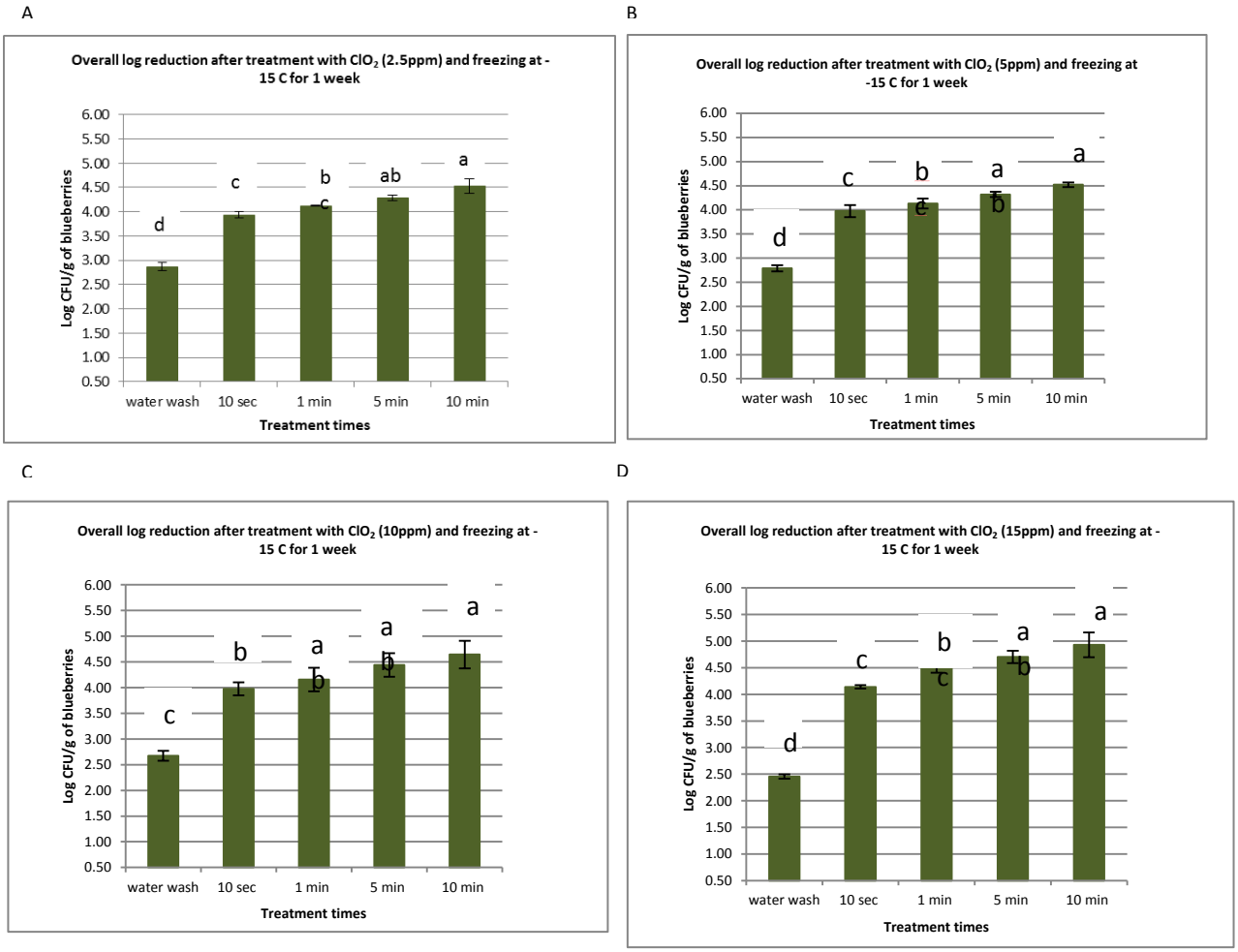



**Figure 3**: Log reduction of *S.* Typhimurium on blueberries at different contact times using a combination of aqueous chlorine dioxide followed by freezing at -15°C for 1 week. (A) 2.5ppm, freezing contributed to an average 1.6 log CFU/g of this overall reduction and (B) 5ppm, freezing contributed to an average 1.7 1 log CFU/g of this overall reduction (c) 10ppm, freezing contributed to an average 1.77 log CFU/g of this overall reduction and (D) 15ppm, freezing contributed to an average 2.84log CFU/g of this overall reduction .

**Table 2:** Reduction of *E. coli* O157:H7 and *S.* Typhimurium after treatment with chlorine at different concentration and various contact times, before and after freezing at -15 $^{o}$ C/ 1week

| Pathogen | Treatment time $Cl_2$ | Reduction (log CFU/g) before freezing | | | Overall Reduction (log CFU/g) after freezing | | |
|---|---|---|---|---|---|---|---|
| | | 100 ppm | 150ppm | 200ppm | 100 ppm | 150ppm | 200ppm |
| *E. coli* O157:H7 | 10s | 0.40 | 0.43 | 0.57 | 2.00 | 2.36 | 2.62 |
| | 1min | 0.45 | 0.49 | 0.70 | 2.14 | 2.46 | 3.25 |
| | 5min | 0.61 | 0.62 | 0.75 | 2.23 | 2.57 | 2.84 |
| | 10min | 0.79 | 0.90 | 0.89 | 2.40 | 2.63 | 2.96 |
| *S.* Typhimurium | 10s | 0.46 | 0.72 | 0.77 | 3.87 | 4.36 | 4.35 |
| | 1min | 0.52 | 0.82 | 0.83 | 3.99 | 4.55 | 4.59 |
| | 5min | 0.58 | 1.03 | 0.97 | 4.24 | 4.82 | 4.76 |
| | 10min | 0.70 | 1.13 | 1.22 | 4.36 | 5.10 | 5.42 |

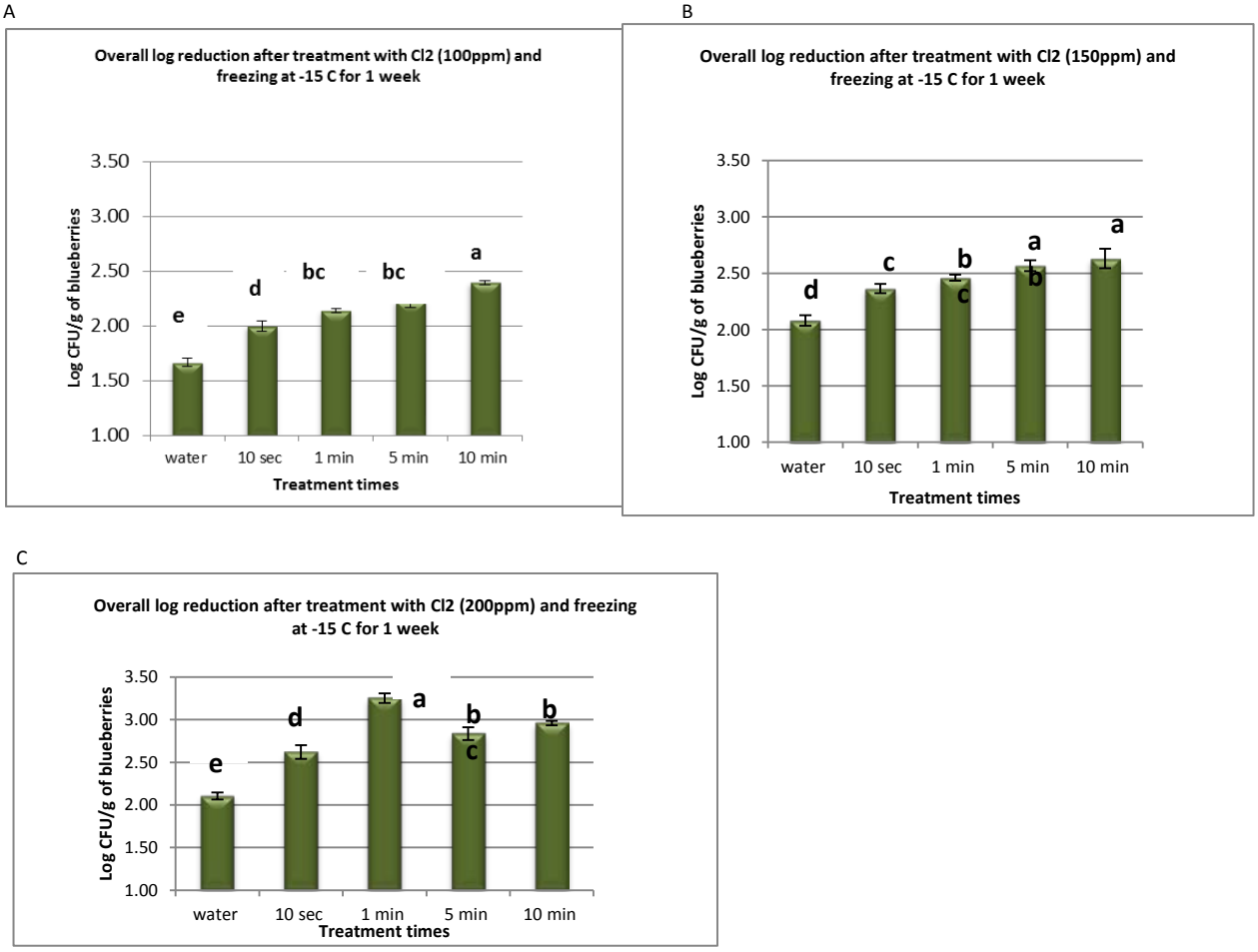



**Figure 4**: Log reduction of *E. coli* O157:H7 on blueberries at different contact times using a combination of chlorine followed by freezing at -15°C for 1 week: (A) 100ppm, freezing contributed to an average 0.60 log CFU/g of this overall reduction (B) 150ppm, freezing contributed to an average 0.56 log CFU/g of this overall reduction, and (C) 200ppm, freezing contributed to an average 0.53 log CFU/g of this overall reduction.

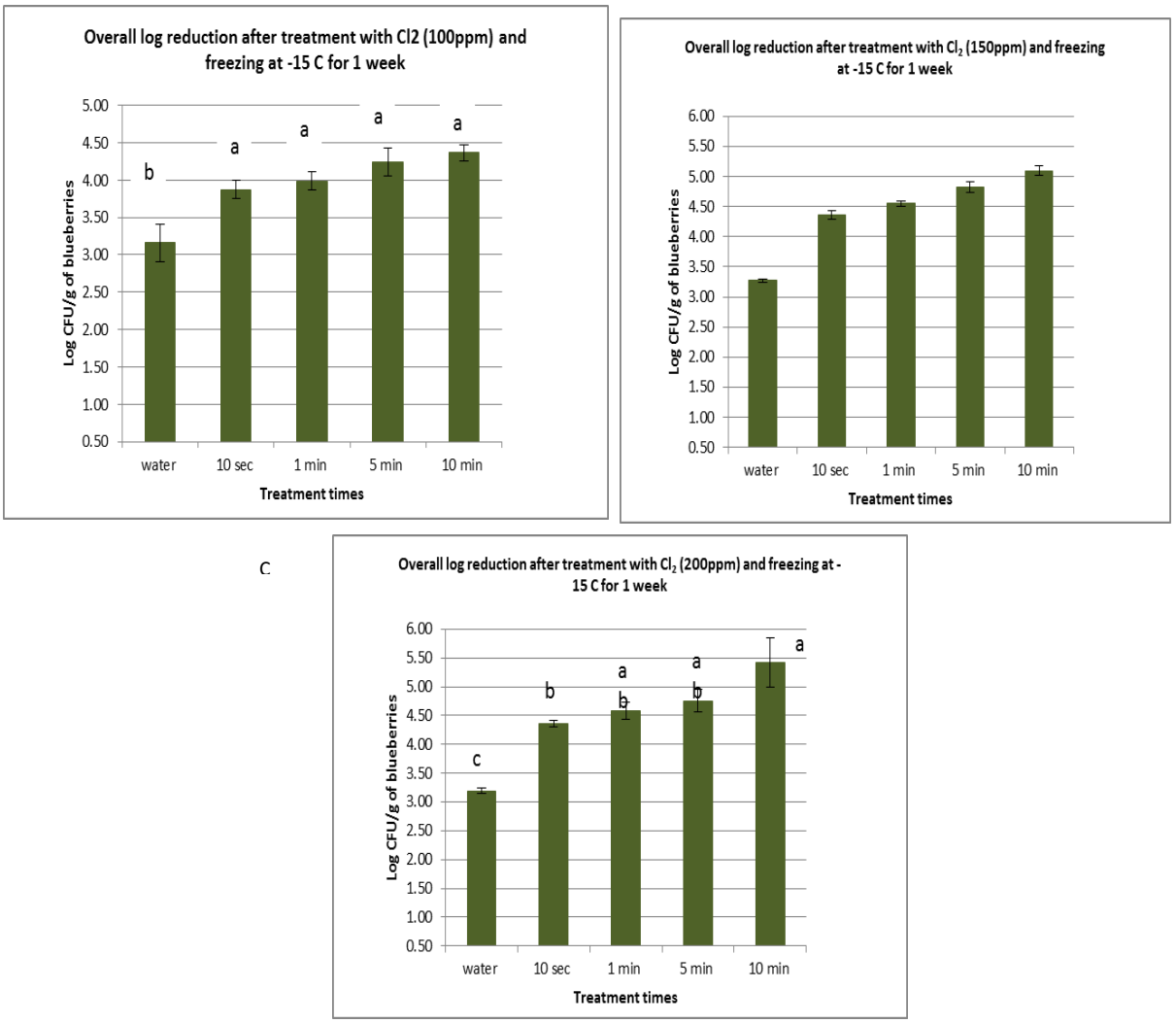



**Figure 5**: Log reduction of *S.* Typhimurium on blueberries at different contact times using a combination of chlorine followed by freezing at -15°C for 1 week: (A) 100ppm, freezing contributed to an average 1.37 log CFU/g of this overall reduction (B) 150ppm, freezing contributed to an average 2.05 log CFU/g of this overall reduction, and (C) 200ppm, freezing contributed to an average 2.46 log CFU/g of this overall reduction.

**Table 3:** Reduction of *E. coli* O157:H7 and *S.* Typhimurium after treatment with lactic acid at two different concentrations and various contact times, before and after freezing at -15 $^{o}$ C/ 1week.

| Pathogen | Treatment time Lactic acid | Reduction (log CFU/g) before freezing | | Overall Reduction (log CFU/g) after freezing | |
|---|---|---|---|---|---|
| | | 1% | 2% | 1% | 2% |
| *E. coli* O157:H7 | 5min | 1.17 | 1.44 | 3.48 | 3.66 |
| | 10min | 1.28 | 1.67 | 3.77 | 3.94 |
| | 20min | 1.67 | 1.98 | 4.19 | 4.41 |
| *S.* Typhimurium | 5min | 1.77 | 1.80 | 4.07 | 4.18 |
| | 10min | 1.92 | 2.01 | 4.32 | 4.47 |
| | 20min | 2.04 | 2.24 | 4.57 | 4.73 |

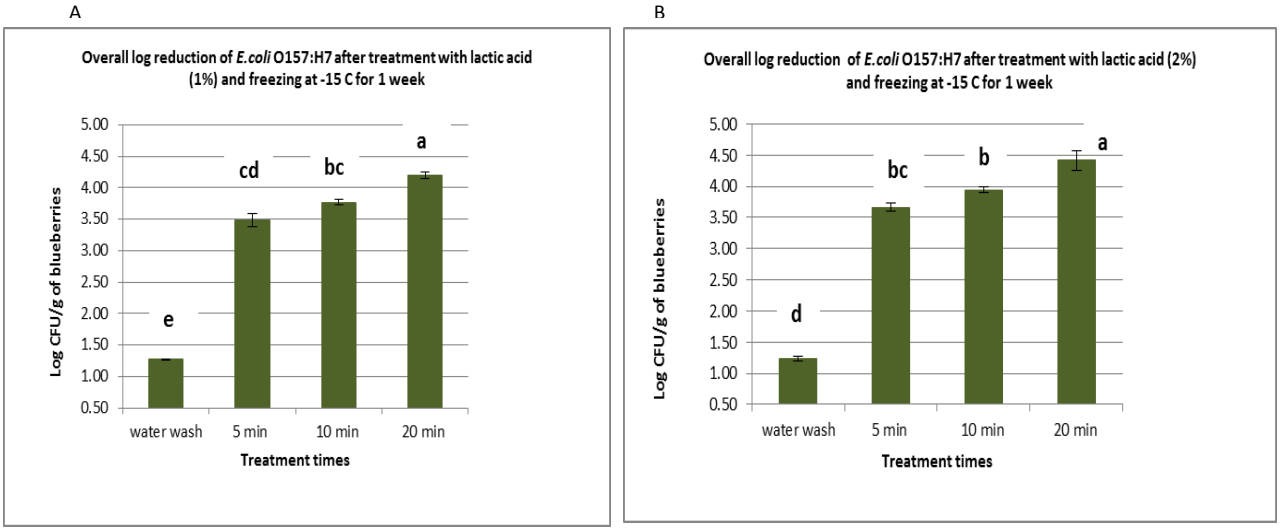
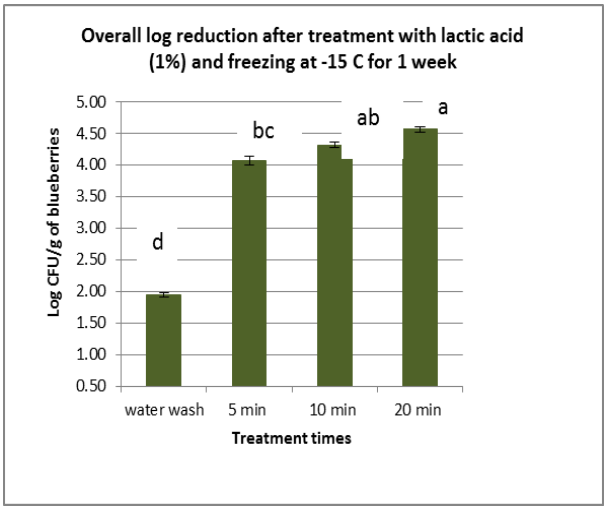



**Figure 6**: Log reduction of *E. coli* O157:H7 on blueberries at different contact times using a combination of lactic acid followed by freezing at -15°C for 1 week. (A)1% lactic acid, freezing contributed to an average 2.29 log CFU/g of this overall reduction and (B) 2% lactic acid, freezing contributed to an average 2.55 log CFU/g of this overall reduction.

A

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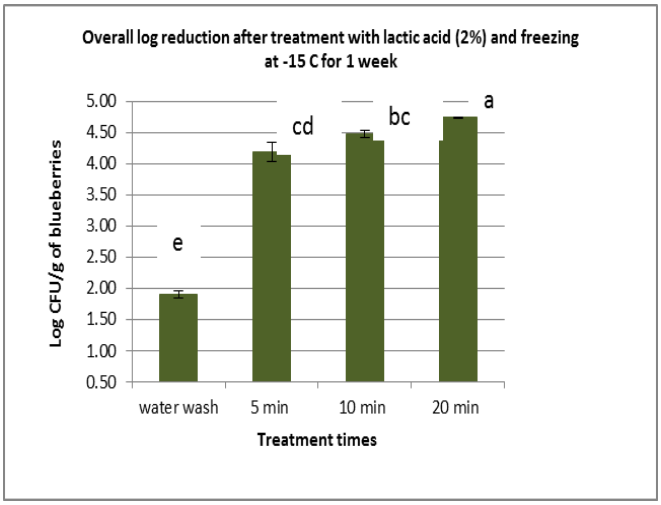



**Figure 7**: Log reduction of *Salmonella* on blueberries at different contact times using a combination of lactic acid followed by freezing at -15°C for 1 week. (A)1% lactic acid, freezing contributed to an average 1.61log CFU/g of this overall reduction (B) 2% lactic acid, freezing contributed to an average1.75 log CFU/g of this overall reduction.

**Table 4:** Reduction of *E. coli* O157:H7 and *S.* Typhimurium after treatment with peroxyacetic acid at two different concentrations and various contact times, before and after freezing at -15 $^{o}$ C/ 1week.

| Pathogen | Treatment time peroxyacetic acid | Reduction (log CFU/g) before freezing | | Overall Reduction (log CFU/g) after freezing | |
|---|---|---|---|---|---|
| | | 1% | 2% | 1% | 2% |
| *E. coli* O157:H7 | 5min | 2.15 | 2.25 | 7.25* | 7.28* |
| | 10min | 2.30 | 2.44 | 7.25* | 7.28* |
| | 20min | 2.58 | 2.72 | 7.25* | 7.28* |
| *S.* Typhimurium | 5min | 2.92 | 3.06 | 7.15* | 7.16* |
| | 10min | 3.01 | 3.29 | 7.15* | 7.16* |
| | 20min | 3.22 | 3.51 | 7.15* | 7.16* |

**Note**: * indicates complete reduction of pathogen from original inoculation (around 7.1-7.3 log CFU/g) with detection limit <1 log CFU/g.

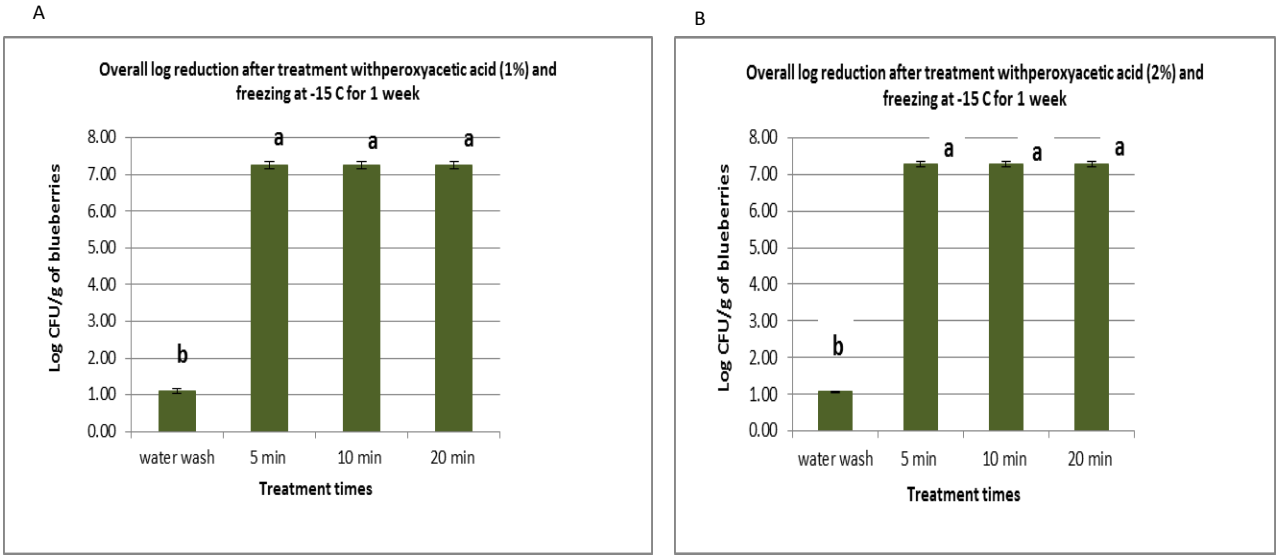



**Figure 8**: Log reduction of *E. coli* O157:H7 on blueberries at different contact times using a combination of peroxyacetic acid followed by freezing at -15°C for 1 week. (A) 1% PAA, freezing contributed to an average 5.38 log CFU/g of this overall reduction and (B) 2% PAA, freezing contributed to an average 5.36 log CFU/g of this overall reduction.

**Note**: After freezing, both the concentrations (1% and 2%) complete reduction from original inoculation (with original inoculum around 7.0log CFU/g) and detection limit <1 log CFU/g.

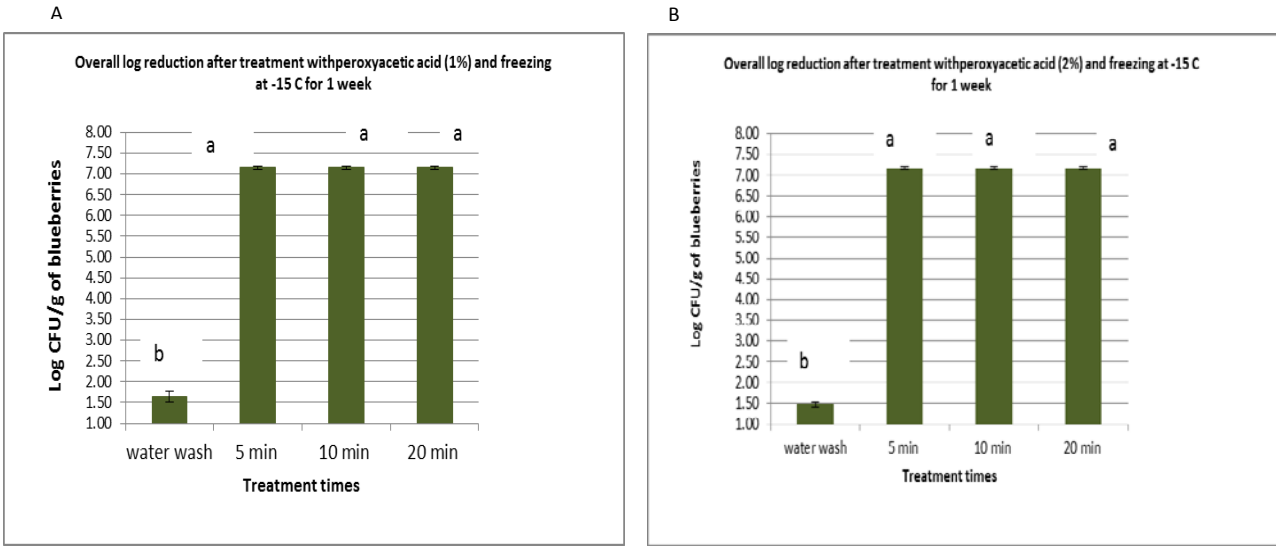



**Figure 9**: Log reduction of *Salmonella* on blueberries at different contact times using a combination of peroxyacetic acid followed by freezing at -15°C for 1 week. (A) 1% PAA, freezing contributed to an average 4.24 log CFU/g of this overall reduction and (B) 2% PAA, freezing contributed to an average 4.28 log CFU/g of this overall reduction. Note: After freezing, both the concentrations (1% and 2%) complete reduction from original inoculation (with original inoculum around 7 log CFU/g) and detection limit <1 log CFU/g.

**FOOD SCIENCE AND NUTRITION**

**INVESTIGATOR**: Dorothy J. Klimis-Zacas, Professor of Clinical Nutrition

**2. TITLE**: Do wild blueberries alleviate risk factors related to the Metabolic Syndrome?

**OBJECTIVES**: To investigate the ability of wild blueberries (WB) to alleviate pathologies associated with the Metabolic Syndrome (MetS) in the Obese Zucker Rat, an appropriate animal model for the human MetS. In particular, the effects of WB will be investigated on:
The goal of this project is to investigate the ability of a wild blueberry-enriched diet to improve parameters related to the pathogenesis of the metabolic syndrome in the obese Zucker rat.

In particular, the objectives of this project are to determine if consumption of wild blueberry will:
1) improve endothelial function, and specifically the functional arterial properties of the aortic vessel in response to phenylephrine induced vasoconstriction and acetylcholine induced vasodilation; and
2) influence the gene expression of inducible nitric oxide synthase (iNOS), prostacyclin $I_2$ ($PGI_2$) and cyclooxygenase-2 ($COX_2$) as related to endothelial function

**METHODS AND RESULTS**: 20 Obese Zucker rats and 20 Zucker lean littermate controls were randomly placed either on a control diet (AIN 93) or on an 8% wild blueberry diet (AIN 93 with 8% of freeze-dried wild blueberry powder). The rats were 8 weeks old at the beginning of the experiment, and remained on the diets for a total of 8 weeks before being sacrificed. At the end of the experimental period, serum was collected and stored at -80°C for subsequent analyses. Liver and adipose tissues were excised, snap-frozen in liquid nitrogen and stored at -80°C until further analysis. The thoracic aortae were harvested and immediately used for the functional arterial properties evaluation.

a) Functional arterial properties: arterial vasodilation
   An acetylcholine dose-response curve was generated using four aortic rings from each animal to evaluate vasodilation. Rings were precontracted with one maximal phenylephrine dose ($10^{-6}$ M) for 10 minutes, until the contraction curve reached a plateau. The aortic rings were subsequently exposed to six cumulative acetylcholine doses (from $10^{-8}$ to $3x10^{-6}$ M), and allowed to reach maximum vasorelaxation force for 6 minutes after each dose. The relaxant effect to each dose of acetylcholine was expressed as a percentage vasorelaxation of the maximum phenylephrine-induced precontraction force. The effective concentration of agonist at which 50% vasorelaxation is obtained (EC50) was determined for each ring, as well as vessel sensitivity to acetylcholine (pD2, -log10 $EC_{50}$).
   Obese Zucker rats exhibited a reduced vasoconstrictor response to Phe and an exaggerated vasorelaxant response to Ach. The WB diet partially restored Phe-induced constrictor responses and attenuated Ach-induced relaxant responses in OZR

b) Plasma Nitric Oxide (NO)
   Nitric Oxide metabolites in plasma were measured using the Nitric Oxide Metabolite

Detection Kit (Cayman), a nitrate/nitrite colorimetric assay, following the instructions provided by the manufacturer, with modifications.
NO levels in OZR were on average higher compared to LZR, independent of diet. WB diet resulted in increased NO in LZR, and decreased NO in OZR (22.1±1.1 μmol/L, WB vs 25.6±1.4 μmol/L, C, $p \leq 0.05$) with the WB diet.

c) Prostacyclin I2 (PGI2) in the aorta
Aorta was incubated in a 2 mL Radnoti tissue bath containing PSS at 37 °C and aerated with 95% $O_2$ / 5% $CO_2$. Tissue was allowed to equilibrate for 20 min before adding phenylephrine ($10^{-6}$ M for 10 min) followed by acetylcholine ($10^{-5}$ M for 10 min). The effluent was collected and PGI2 levels in the aortic effluent were determined using the enzyme immunoassay 6-keto-PGF1α EIA Kit (Cayman), following the instructions provided by the manufacturer, with modifications. 6-keto-PGF1α is a metabolite of non-enzymatic hydrolysis of PGI2.
PGI2 levels were higher in OZR compared to LZR, and significantly increased (766.5±92.2 pg/mg aorta in the WB vs 571.7±37.8 pg/g aorta in the C group, $p \leq 0.05$) following WB consumption in the OZR.

d) Thromboxane A2 (TXA2) in the aorta
TXA2 levels in the aortic effluent were determined using the enzyme immunoassay Thromboxane B2 EIA Kit (Cayman), following the instructions provided by the manufacturer, with modifications. TXB2 is a metabolite of non-enzymatic hydrolysis of TXA2.
TXA2 levels in the aortic effluent were found to be similar across both groups, and not affected by WB consumption.

Downregulation of iNOS and COX-2 expression in the OZR aorta was observed in the WB diet group.

**SIGNIFICANCE**: In conclusion, 8 weeks wild blueberry consumption altered and normalized the biomechanical properties of the obese Zucker rat aorta by partially restoring the impaired Phe-induced constrictor responses, and attenuating the exaggerated response to Ach-induced vasorelaxation. Additionally, our results suggest that both the COX2 and the NOS pathways contribute for the observed responses. This may have beneficial implications on the Metabolic Syndrome (MetS), a major public health problem in the U.S., characterized by vascular dysfunction along with central obesity, dyslipidemia, insulin resistance, glucose intolerance, hypertension, and a prothrombotic and a proinflammatory state, resulting in increased risk of Cardiovascular Disease and Diabetes Mellitus.
Results from this work can be of benefit not only to the scientific community but also the Food Industry and especially the Wild Blueberry Association of North America. Wild blueberries may not only be promoted as a food to prevent MetS, but they may also be included and strongly recommended for patients who already suffer from MetS. These patients may be able to see improvement on a diet rich in blueberries without suffering from the harmful side effects and financial burden of traditional pharmacotherapies. This research may positively influence the health of our population as well as further aid economically the wild blueberry producers in Maine.

**FOOD SCIENCE AND NUTRITION**

**INVESTIGATOR:** Dorothy J. Klimis-Zacas, Professor of Clinical Nutrition

**3. TITLE:** Wild Blueberry consumption and exercise-induced Oxidative Stress: Inflammatory Response and DNA damage.

**GOALS AND OBJECTIVES:** Strenuous and unfamiliar exercise substantially increases levels of reactive oxygen species (ROS) resulting in significant oxidative stress and elevated inflammation with potential for systemic damage beyond the working muscle. Wild blueberries exhibit both antioxidant and anti-inflammatory properties. This study examined the effect of wild blueberries on the oxidative stress/inflammatory response to exercise in untrained individuals.

**METHODS**: Ten sedentary males (21 – 26 years of age) completed a single bout of treadmill exercise at 70% of their $VO_{2max}$ before and after consuming 300 g of wild blueberries daily, for eight weeks. Biomarkers for oxidative stress, inflammation and DNA damage; TNFα (tumor necrosis factor-alpha), Mn-SOD (manganese-superoxide dismutase), IL-6 (interleukin-6), and DNA damage were measured pre-exercise, and at 0 minutes, 30 minutes, one hour, three hours and six hours post-exercise, before and after wild blueberry consumption.

**RESULTS**: A significant increase in IL-6 was observed for time (at 30 minutes and one hour), both pre- and post- exercise, while no significant changes were detected following the intervention. A significant interaction between pre- and post-intervention over time was detected in plasma Mn-SOD concentration. Post hoc comparisons showed no significant differences between time points pre- and post-intervention, although plasma Mn-SOD concentration tended to decrease 30 minutes after exercise with wild blueberry consumption. No significant differences were seen in DNA damage or TNFα either with exercise or the intervention.

**CONCLUSIONS**: This study demonstrates that a single bout of exercise at 70% of $VO_{2max}$ is sufficient to cause a significant increase in inflammation in untrained individuals as evidenced by plasma levels of IL-6. The small decrease in Mn-SOD concentration after exercise post-intervention indicates a potential benefit of wild blueberries to increase antioxidant capacity and reduce oxidative stress.

## ENTOMOLOGY

**INVESTIGATORS:** F. A. Drummond, Professor of Insect Ecology/Entomology
J. A. Collins, Assistant Scientist of Insect Pest Management

**4. I. TITLE:** Control Tactics for Blueberry Pest Insects, 2013

### *Study 1. Field control of blueberry tip midge on wild blueberry (pruned year) with foliar application of insecticides*

**METHODS:** There were four replications of each treatment plus six non-treated checks. Each plot measured 7 x 20 ft. A foliar application of Assail 30SG (acetamiprid) and Imidan 70WP (phosmet) was applied on 17 Jun to a pruned-year field in Deblois, ME. Both materials were applied in 25 gallons of water-mixture per acre with a $CO_2$-propelled, 80-inch boom sprayer (76-inch swath) equipped with four, flat-spray, 8002VS TeeJet® nozzles operating at 35 psi and at a slow walking speed. Walking speed for each application was regulated using a metronome.

On 17 and 25 Jun, and 1 and 8 Jul, the number of blueberry stems with tip midge damage as evidenced by curled leaves was determined from each of three, $m^2$ samples per plot. No symptoms of phytotoxicity were observed in any plot.

**RESULTS:** Multiple and Univariate Analyses of Variance (ANOVA & MANOVA, CRD) were used to compare mean number of curls among the treatment plots. Means separation was by Least Square Means. Subplots were pooled within main plots. Data were transformed by the square root to stabilize variance prior to analysis. Assessment of treatments via ANOVA suggested no significant difference among the treatments on 17 Jun (Prespray) (Table 1). Assail and Imidan were both ineffective in suppressing tip midge as evidenced by leaf curls. Postspray populations in the treated plots were either higher (1 Jul) or not significantly different (25 Jun and 8 Jul) than the non-treated checks (Fig. 1). MANOVA also revealed no treatment differences ($F_{(2,11)} = 1.589$, $P = 0.247$) and no time X treatment interaction ($F_{(6,18)} = 1.283$, $P = 0.313$), but a significant time effect ($F_{(3,9)} = 31.134$, $P < 0.0001$). This suggests that there was a continual decline of tip midge curls through the beginning of July and then resurgence by 8 Jul independent of treatment.

**Table 1.** Field control of tip midge with insecticides, summary.

| | | Mean curls/$m^2$ (SE) | | | |
|---|---|---|---|---|---|
| | Amt. | Prespray | Postspray | | |
| Material | form./acre | 17 Jun | 25 Jun | 1 Jul | 8 Jul |
| Assail 30SG | 5.3 oz | 30.0 (2.2) a | 9.5 (1.5) a | 3.0 (1.5) a | 21.0 (7.2) a |
| Imidan 70WP | 21.3 oz | 33.0 (6.3) a | 14.0 (4.2) a | 10.8 (3.4) b | 19.8 (7.0) a |
| Non-treated check | - | 21.7 (4.8) a | 7.8 (0.9) a | 3.2 (0.5) a | 15.5 (3.3) a |
| *P* = | | 0.2286 | 0.2405 | 0.0504 | 0.7883 |

Means within columns followed by the same letter are not significantly different (LSD, $P \leq 0.05$). Data were transformed by sqrt prior to analysis.

**Fig. 1.** Mean number of curls/$m^2$; data from 2013 trial.

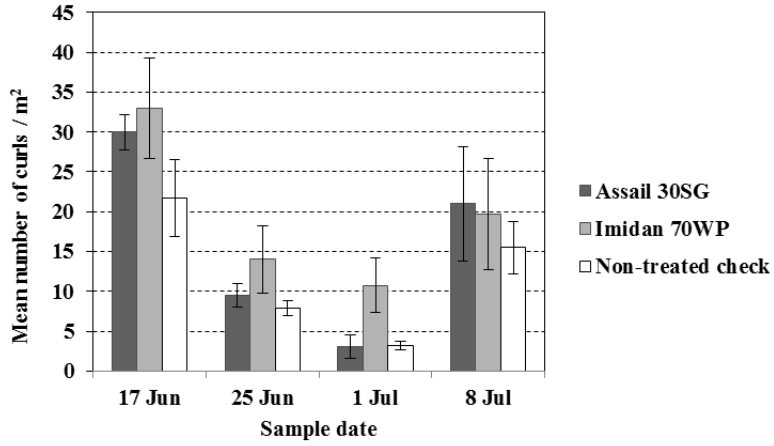
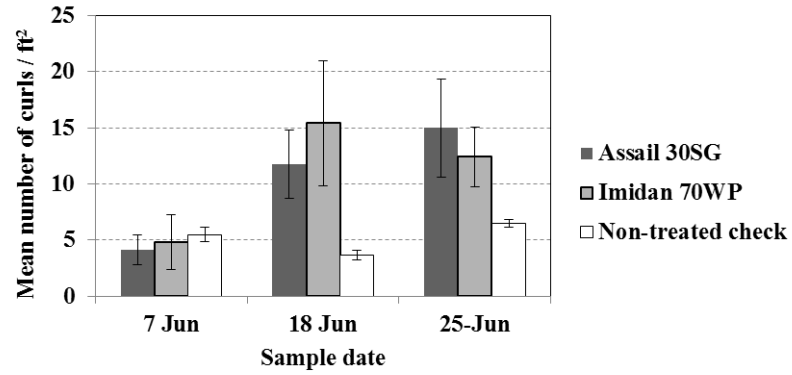



**Fig. 2.** Mean number of curls/$ft^2$; data from 2012 trial.




**CONCLUSIONS:** We have now completed two trials (2012 and 2013) with Imidan and Assail against blueberry tip midge. Similar results were observed in both years; populations of tip midge appeared to increase in plots treated with insecticides (Figs. 1 & 2). Although the reason for increasing populations in the treated plots is unclear, it is possible that the applications had a depressing effect on native predators of the tip midge, thus insecticide treatment might exacerbate a tip midge outbreak.

### *Study 2. Field control of spotted wing drosophila (SWD) on wild blueberry (crop-year) with insecticides*

**METHODS:** There were four replications per treatment. Each plot measured 14 x 60-ft. There were two applications (9 and 17 Sep). Each material was applied in 25 gallons of water-mixture per acre with a $CO_2$-propelled, 80-inch boom sprayer (76-inch swath) equipped with four, flat-spray 8002VS TeeJet® nozzles operating at 35 psi and at a slow walking speed. Speed was regulated using a metronome.

On the dates indicated in the table three fruit samples were taken from each plot. Each sample was approximately 2/3 cup or an average of 359 berries. The average number of berries was determined by counting the number of berries in each of four samples. Sample #2 (17 Sep) was collected prior to the second application. No evidence of phytotoxicity was observed in any plot.

**RESULTS:** Analyses of Variance (ANOVA, CRD) were used to compare mean number of SWD larvae in fruit samples among the treatments for each date. Subplots were pooled within main plots. Data were transformed by the square root to stabilize variance prior to analysis.

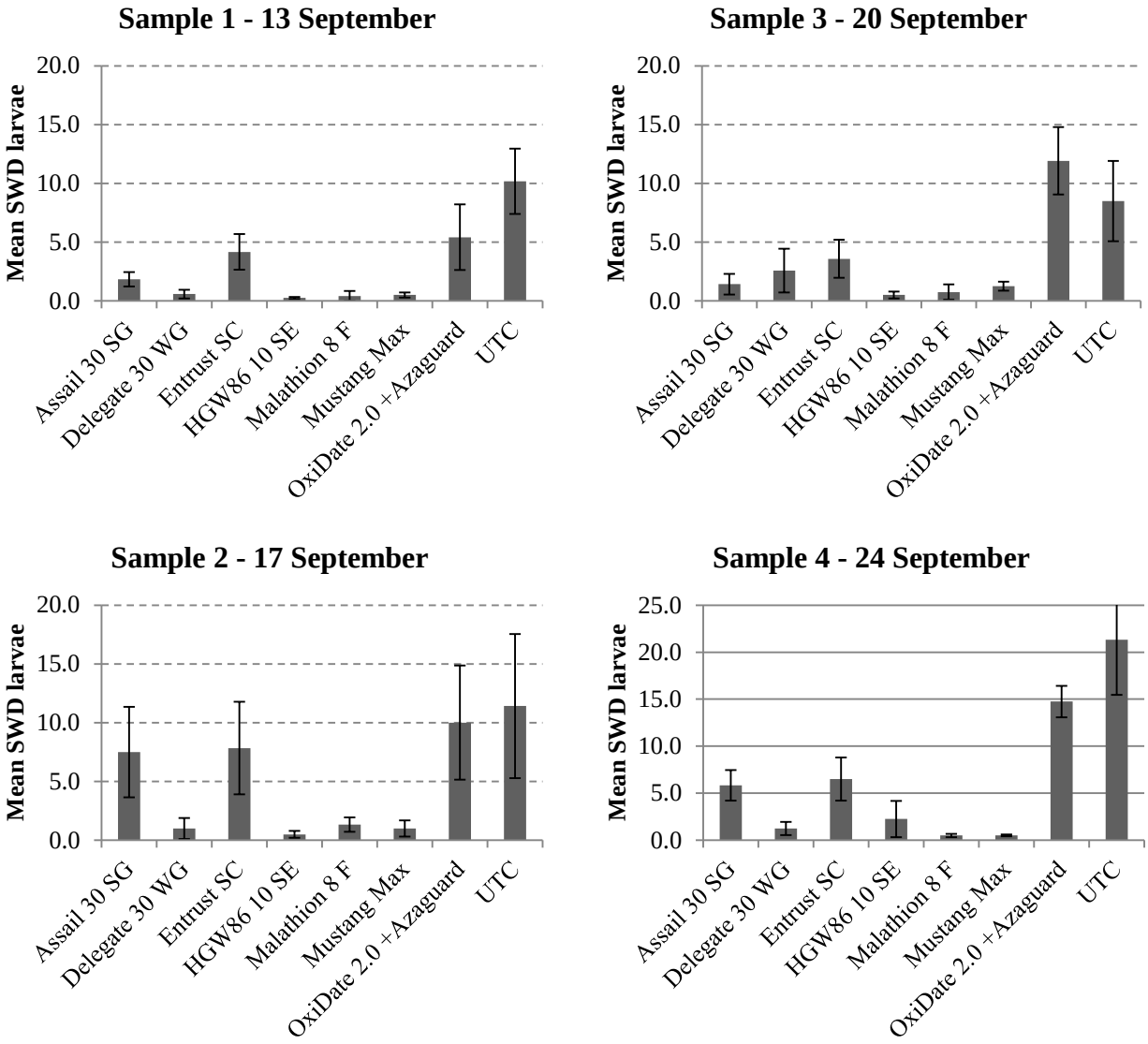
Two trials of Delegate, Malathion, Mustang Max and Cyazypyr all provided very good control of SWD and significantly reduced fruit infestation in comparison with the non-treated checks (Table 1 and Fig. 1). Assail and Entrust also showed some activity; although, the residues of these materials were much shorter. Assail and Entrust both significantly reduced fruit infestation 4 days after the first application on 9 Sep (Sample 1). However, by the second sample date (17 Sep), 8 days post, SWD infestation levels had increased in plots treated with Assail and Entrust. A similar result was observed after the second application on 17 Sep; Assail and Entrust were both effective 3 days later (Sample 3); however, populations appeared to again be on the increase by 24 Sep (7 days post). The combination of the fungicide OxiDate and AzaGuard did not provide consistent control in either trial. It had been theorized that these two materials together give a synergistic effect and enhance control.

**Table 1.** Field control of SWD with insecticides, summary.

| Material | Amt. form./ acre | SWD larvae/sample (SE) 13 Sep (4)* | 17 Sep (8) | 20 Sep (3) | 24 Sep (7) |
|---|---|---|---|---|---|
| Delegate 30WG | 6.0 oz | 0.6 (0.4) c | 1.0 (0.9) b | 2.6 (1.9) c | 1.2 (0.7) b |
| Assail 30SG | 5.3 oz | 1.8 (0.6) bc | 7.5 (3.8) ab | 1.4 (0.9) c | 5.8 (1.6) b |
| Entrust 2SC | 4.0 oz | 4.2 (1.5) bc | 7.8 (3.9) ab | 3.6 (1.6) bc | 6.5 (2.3) b |
| Mustang Max EC | 4.0 oz | 0.5 (0.2) c | 1.0 (0.7) b | 1.2 (0.4) c | 0.5 (0.1) b |
| Malathion 8F | 40.0 oz | 0.4 (0.4) c | 1.3 (0.6) b | 0.8 (0.6) c | 0.5 (0.2) b |
| Cyazypyr 10SE | 20.5 oz | 0.2 (0.1) c | 0.5 (0.3) b | 0.5 (0.3) c | 2.2 (1.9) b |
| + nonionic surfactant | .25% | | | | |
| OxiDate 2.0 + AzaGuard | 16 + 8 oz | 5.4 (2.8) b | 10.0 (4.9) a | 11.9 (2.9) a | 14.8 (1.7) a |
| Non-treated check | - | 10.2 (2.8) a | 11.4 (6.1) a | 8.5 (3.4) ab | 21.3 (5.9) a |
| % infestation range† | | 0.05 – 2.8% | 0.1 – 3.2% | 0.1 – 3.3% | 0.1 – 5.9% |
| *P* = | | 0.0001 | 0.0012 | 0.0003 | < 0.0001 |

Means within each column followed by the same letter(s) are not significantly different (LSD; $P \leq 0.05$).
* numbers in parentheses: days after application for trial 1 (application = 9 Sep) and trial 2 (application = 17 Sep).
† percent infestation based upon average of 359 berries per sample date per treatment.

**Fig. 1.** Density of SWD larvae, by sample date; note second application on 17 Sep was made after the sample was collected.




**CONCLUSIONS:** Insecticides that appear to offer 7-8 day protection are Mustang Max, Malathion 8F, cyazypyr (HGW86 10SE), and Delegate 30WG. Assail 30SG and Entrust SC provided good control for 3-4 days, but increased maggot infestation resulted by 7-8 days. The combination of the fungicide OxiDate 2.0 and Azaguard did not provide effective control during either trial. As fly pressure increased during the second trial, Oxidate and Azaguard resulted in maggot infestation not different from the non-treated checks, both at 3 days and 7 days after application.

***Study 3. <u>Laboratory control of spotted wing drosophila.</u>***

**METHODS:** In order to evaluate control of spotted wing drosophila (SWD) adults by insecticides in the laboratory, treated blueberry stems were collected from field plots established as part of a field control experiment (See Study 2) of this report. Seven different materials were evaluated in comparison with non-treated checks. Each material was applied on 9 Sep in 25 gallons of water-mixture per acre with a $CO_2$-propelled, 80-inch boom sprayer (76-inch swath) equipped with four, flat-spray 8002VS TeeJet® nozzles operating at 35 psi and at a slow walking speed. Speed was regulated using a metronome.

Fruit-bearing blueberry stems were cut from treated plots at three post-treatment timings (0 days, 3 days, and 7 days post application), brought into the laboratory, set in water in small glass beakers sealed with Parafilm®, and placed in small plastic containers. The 0 day collection was ca. 2 hours post application after the materials had dried on the foliage. On each collection day, 13-26 laboratory reared SWD adults (a mix of males and females) were added to the cages. After 24 hours the number of dead SWD adults was determined from each cage. This data was used to calculate percent mortality (Table 1).

**RESULTS:** It is interesting to note that the results of this laboratory trial appear somewhat in contrast to the results observed in the associated field control trial. In the field trial, Mustang Max EC, Malathion 8F, Cyazypyr 10SE, and Delegate 30WG all appeared to offer 7-8 day protection. In this laboratory trial, Delegate, Malathion, and Cyazypyr provided some control at 0 and 3 days post, but were less effective after 7 days. And, Mustang Max was ineffective. Results for Assail 30SG and Entrust 2SC were similar in both the field and laboratory; Assail and Entrust provided good control for 3-4 days, but increased infestation resulted by 7-8 days. The combination of the fungicide OxiDate 2.0 and AzaGuard did not provide effective control during either trial. However, the laboratory control study only evaluated direct toxicity of insecticide residues on leaves and fruit to the adult SWD, while the field study evaluated a combination of effects on both adult mortality and disruption of oviposition, and mortality in the egg stage.

**Table 1**. Summary, percent mortality of SWD adults.

| Material | Amt. form./ acre | % mortality after 24 hrs | | |
|---|---|---|---|---|
| | | 0 day | 3 day | 7 day |
| Delegate 30WG | 6.0 oz | 57.1 | 40.0 | 5.9 |
| Assail 30SG | 5.3 oz | 94.1 | 64.7 | 6.7 |
| Entrust 2SC | 4.0 oz | 100.0 | 94.4 | 0.0 |
| Mustang Max EC | 4.0 oz | 6.7 | 6.7 | 16.7 |
| Malathion 8F | 40.0 oz | 52.9 | 47.4 | 6.7 |
| Cyazypyr 10SE + nonionic surfactant | 20.5 oz .25% | 62.5 | 66.7 | 30.8 |
| OxiDate 2.0 + AzaGuard | 16 + 8 oz | 0.0 | 6.7 | 5.3 |
| Non-treated check | - | 0.0 | 6.3 | 50.0 |

**CONCLUSIONS:** This laboratory study shows that Delegate, Malathion, and Cyazypyr provide limited efficacy against adults; although, these compounds are effective in protecting fruit in the field (see Study 2 of this report). We also show that OxiDate 2.0 + AzaGuard have almost no activity against adults and so their ability to prevent larval infestation of the fruit is probably due to a repellent effect on the adult females or a physiological disruption to ovarian development, both of which have been reported in the literature for other insects. Entrust does have fairly long residual mortality effects on adults which was borne out in the field study showing this organically approved material to be quite effective in fruit protection.

### *Study 4. Field control of blueberry maggot fly on wild blueberry (crop year) with Sivanto® 200SL*

**METHODS:** Sivanto 200SL (14 oz/acre) was applied on 12 Jul (berries 15-20% ripening and turning blue) to four, 100 x 80 ft plots in a non-managed, fruit-bearing field in Township 19, ME. A CIMA® P55D Atomizer L.V. sprayer was used to apply the material in 20 gallons of water per acre. Pre- and postspray populations of BMF adults were monitored with baited, yellow Pherocon® AM traps. One trap was placed in each plot. Efficacy was further evaluated based on the number of BMF pupae collected from fruit samples.

On 30 Jul, we raked four quarts of berries from each treated plot. To collect BMF pupae, the berries from each plot were combined and distributed in a 1 to 2-inch deep layer in screened boxes suspended over ca. 2 inches of fine sand. Hardware cloth (1/4 in) was used as a screening material. In late-Oct, BMF pupae were separated from the sand. No symptoms of phytotoxicity were observed in any plot.

**RESULTS:** Analysis of Variance (ANOVA, RCB) was used to compare the change in mean number of adults captured between treated and non-treated check plots, seasonal density of BMF adults, and number of pupae per quart of fruit. Data were transformed by the square root prior to analysis to stabilize variance.

There was no significant difference in numbers of adults captured between the treatments prior to the application of Sivanto ($F_{(1,3)} = 3.99$, $P = 0.1397$ on 8 Jul and $F_{(1,3)} = 2.41$, $P = 0.2183$ on 12 Jul). Seasonal density of adults is in Figure 1 and Table 1. Although there was a trend towards more adults in the non-treated check, the difference was not significant ($F_{(1,3)} = 2.58$, $P = 0.2068$). Percent change between pre and postspray adult captures is shown in Figure 2. Figure 3 shows fruit infestation as measured by number of pupae collected per quart of fruit. Sivanto significantly reduced larval infestation in comparison with the non-treated check plots ($F_{(1,3)} = 73.25$, $P = 0.0034$).

**Table 1.** Field control of blueberry maggot fly with Sivanto insecticide, summary.

| Material | Amt. form./ acre | Adult seasonal . density (SE) | Pupae/qt (SE) |
|---|---|---|---|
| Sivanto 200SL<br>+ Dyne-amic nonionic surfactant | 14.0 oz<br>0.25% v/v | 2.6 (0.9) a | 1.0 (0.2) a |
| Non-treated check | | 5.4 (1.0) a | 8.2 (1.0) b |

Means within each column followed by the same letter are not significantly different (ANOVA, $P \leq 0.05$).

**Fig. 1.** Seasonal density of BMF adults.

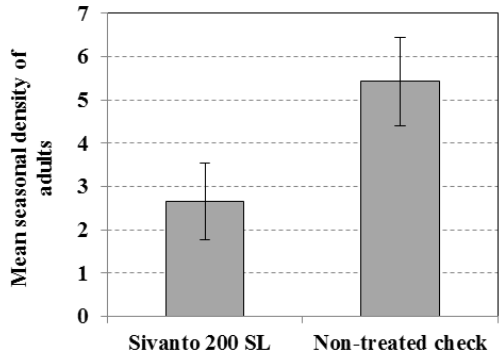



**Fig. 2.** Percent reduction in number of BMF adults following treatment.

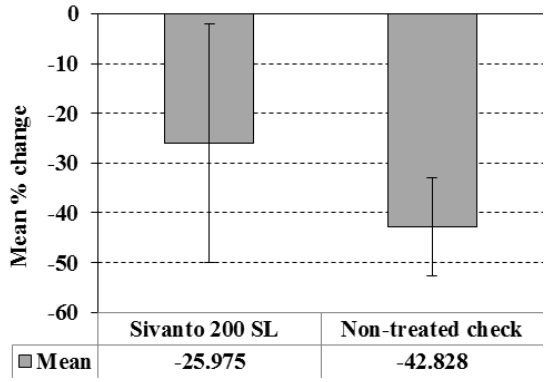
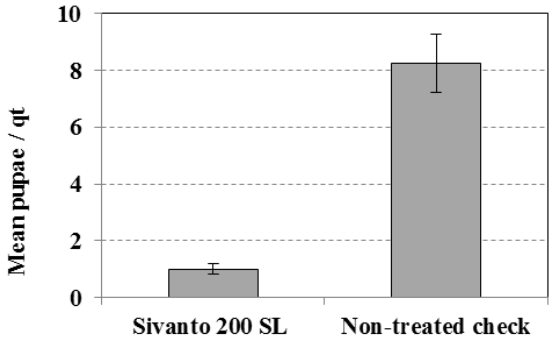


**Fig. 3.** Mean number of BMF pupae collected per quart of fruit.




**CONCLUSIONS:** We have now conducted four years of trials with Sivanto 200SL at the 14 oz rate. In three of the four years, this high rate of Sivanto did provide control of BMF. In our trial in 2012 the material was not effective. And, in 2012 there was significant phytotoxicity in the form of leaf drop and leaf spotting. No phytotoxicity was observed in any other trial including this 2013 trial. It should be noted however, that 2012 was the only trial where a boom sprayer was used for the application as well as the first time multiple applications were made to the same area. It was also the first time this material has been applied to commercial blueberry land. All other trials were conducted on non-managed land.

The material does appear to have promise as a new insecticide for control of the blueberry maggot fly. While this insecticide does not reduce adult populations it does protect the fruit from maggot infestation. Because Sivanto is promoted as "bee friendly" we will recommend this insecticide for blueberry maggot fly management when it becomes registered for wild blueberry. In addition, we will evaluate this insecticide for thrips, blueberry tip midge, and spotted wing drosophila management.

### *Study 5. Phytotoxicity of Cyazypyr™ on wild blueberry*

**METHODS:** There were three replications per treatment. Each plot measured 7 x 20 ft. Materials were applied on 15 May, 10 Jun, and 17 Jun. On each date, HGW86 10SE (cyazypyr) was applied in 25 gallons of water-mixture per acre with a $CO_2$-propelled, 80-inch boom sprayer (76-inch swath) equipped with four, flat-spray 8002VS TeeJet® nozzles operating at 35 psi and at a slow walking speed. Speed was regulated using a metronome. Application rates are in Table 1.

**Table 1.** Application rates.

| Material | Rate /acre |
|---|---|
| HGW86 10SE | 20.5 oz |
| HGW86 10SE | 41.0 oz |
| HGW86 10SE | 20.5 oz |
| + Damoil | 64.0 oz |
| HGW86 10SE | 41.0 oz |
| + Damoil | 64.0 oz |
| Damoil | 64.0 oz |

Phytotoxicity was evaluated by rating the percent of leaf drop and leaf spotting and blossom browning in each of three, sq ft quadrats per plot + three non-treated check plots. Rankings were 0-25% = 1, 25-50% = 2, 50-75% = 3, 75-100% = 4.

**RESULTS:** No evidence of phytotoxicity was noted following any of the three applications. Ratings were made on 27 May, 17 Jun, and 30 Jun.

**RECOMMENDATIONS:** The recommendations that have come out of our studies are as follows. First, blueberry tip midge control is not recommended with the standard insecticides that are used in wild blueberry control. One reason is that they have not been found to be effective. Another reason is that for two years it appears that insecticides might enhance tip midge infestation. The only reason that this would be the case is that these insecticides might be killing natural enemies that exert a level of control on this pest. This is only speculation at this point. In addition, a survey conducted in several blueberry fields over the past two years suggest that damage levels are extremely low, ranging from 0.004 – 0.15% infestation, and on average would not warrant control. Nevertheless, there are some growers that have moderate levels of tip midge and so in 2014 we will trial some other insecticides that have shown some promise for blueberry tip midge control.

The recommendations for SWD control are Delegate, Malathion, Mustang Max and Cyazypyr. All of these insecticides performed consistently well. Entrust gave moderate control, acceptable for organic growers.

## ENTOMOLOGY

**INVESTIGATORS:** F. A. Drummond, Professor of Insect Ecology/Entomology
J. A. Collins, Assistant Scientist of Insect Pest Management

**5. II. TITLE:** Pesticide Residues on Wild Blueberry, 2013

***Study 1. Residues of insecticides on wild blueberry***

**METHODS:** We evaluated residues of four insecticides on wild blueberry fruit. Each material was evaluated at five timings; 1, 3, 5, 10, and 15 days PHI. All materials were applied in 25 gallons of water-mixture per acre with a $CO_2$-propelled, 80-inch boom sprayer (76-inch swath) equipped with four, flat-spray 8002VS TeeJet® nozzles operating at 35 psi and at a slow walking speed. Plot size was 14 x 20 ft. Speed was regulated using a metronome. Treatments rates are in Table 1. The application for the 1, 3, and 5 day timings was 4 Aug; 10 and 15-day timings were applied on 25 Jul.

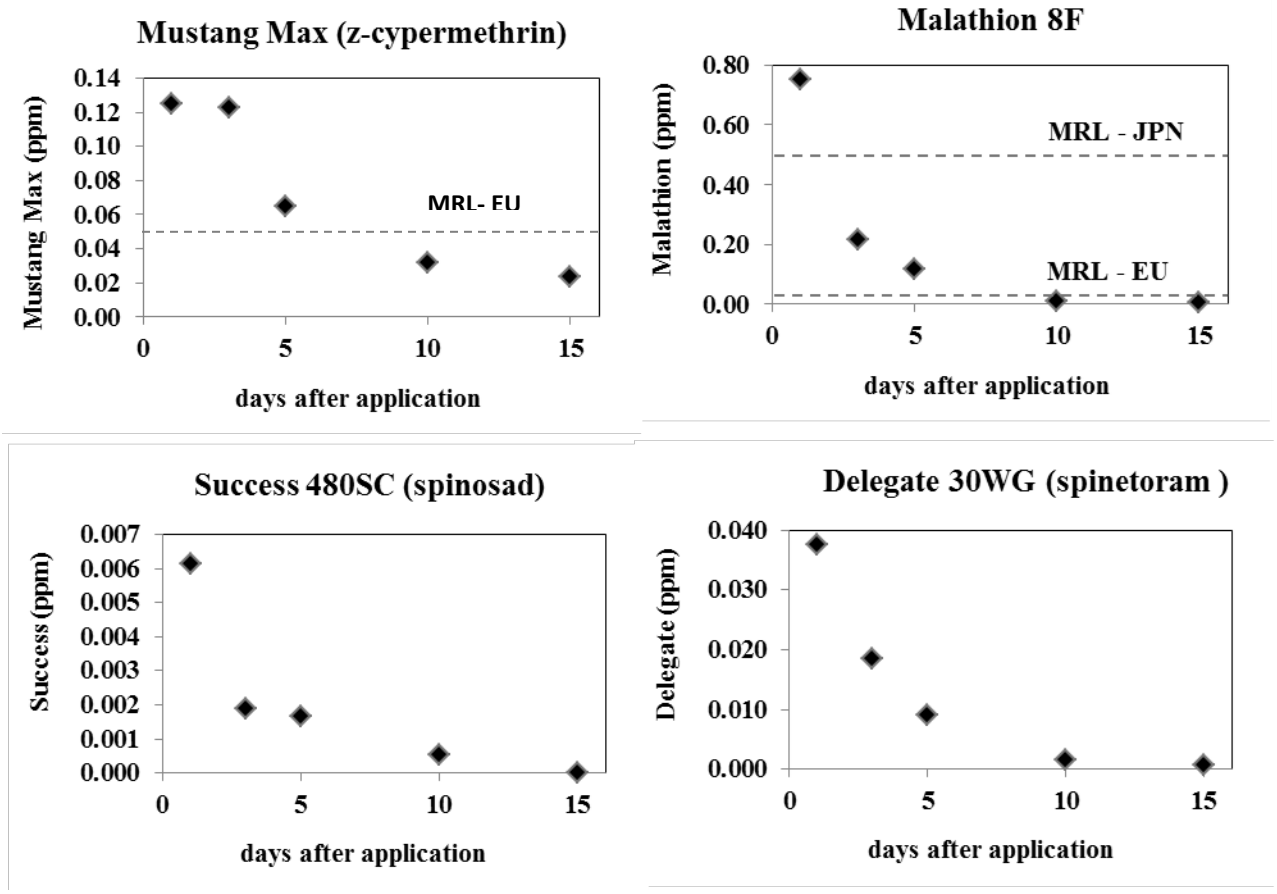
A clean commercial blueberry rake was used to harvest ca.1 lb of fruit for each treatment and timing. Each fruit sample was washed by agitating for 30 seconds in clean tap water, drained, placed in a zip-lock bag, placed in a cooler with blue ice, and delivered to Dr. Lawrence LeBlanc at the University of Maine for residue analysis.

**Table 1.** Summary of treatments and current MRLs (from USDA/FAS).

| Trt # | Treatment | Rate/acre | MRL (ppm) US | EU | JPN |
|---|---|---|---|---|---|
| 1 | Success 480SC | 6.0 oz | 0.25 | 0.4 | 0.3 |
| 2 | Mustang Max EC | 4.0 oz | 0.8 | 0.05 | 0.5 |
| 3 | Malathion 8F | 40.0 oz | 8.0 | 0.02 | 0.5 |
| 4 | Delegate 30WG | 6.0 oz | 0.25 | 0.2 | 0.5 |

**RESULTS:** Residues for the four materials are shown in Figure 1. Current MRLs (ppm) for the US, EU, and JPN are in Table 1. MRLs for Mustang Max range from 0.05 (EU) to 0.8 (US); Malathion from 0.02 (EU) to 8 (US); Delegate from 0.2 (EU) to 0.5 (JPN); and Success from 0.25 (US) to 0.4 (EU). Residues for Delegate and Success were below current MRLs for the duration of the study. Malathion residues were below the MRL for Japan (0.5) by day 3, but did not fall below the MRL for the EU (0.02) until day 10. Mustang Max MRLs were below current tolerances for the US and JPN at day 1 but did not fall below the MRL for the EU until day 10.

**Fig. 1.** Residues of insecticides; dashed lines are MRL.




**CONCLUSIONS:** Our results suggest that all insecticides are suitable for the U.S. and Japanese markets (Table 1 and Fig. 1). Malathion can be safely harvested by day 3 after application. The European Union market however, is more restrictive. Mustang Max would not be ready for harvest until after day 5 (-day 10) and Malathion would not be ready until after day 1 (-day 5). However, both Success and Delegate would be very safe to harvest 1 day after application. This trial should be replicated next year in order to provide the variation that naturally occurs in residues on fruit due to differences in weather.

### *Study 2. Residues of Duet® insecticide on wild blueberries*

**METHODS:** On 10 Aug 2013**,** Duet insecticide (prallethrin + phenothrin + piperonyl butoxide) (1.24 oz/acre diluted in mineral oil) was applied to a fruit-bearing wild blueberry field in Jonesboro, ME using a SOLO® 450 mist blower. On each sample date a clean commercial blueberry rake was used to collect three, 1 lb samples from the treated area. Samples were representative of the entire plot and were collected by harvesting in a swath diagonally across the plot. Fruit was air-winnowed into clean, plastic containers, placed in zip-lock bags, and frozen prior to chemical residue analysis by Dr. Lawrence LeBlanc, University of Maine, School of Marine Sciences.

**Table 1.** Sample collection.

| Sample ID | Date Collected | Days post application [1] |
|---|---|---|
| 1 | 10 Aug | 1 (12 hrs) |
| 2 | 11 Aug | 2 |
| 3 | 12 Aug | 3 |
| 4 | 14 Aug | 5 |

[1]Application on 10 Aug.

From each sample container (i.e., ziploc bag) 100 grams was weighed out. This 100 gram sample was homogenized completely, using a Brinkman polytron homogenizer/extractor (Brinkman instruments, Westbury, NY). From this homogenate 10 gram samples were weighed into extraction vessels. The QuEChERS method was employed for extraction and sample cleanup - which involves extraction with acetonitrile solvent, followed by:

1. elimination of water and isolation of the acetonitrile extract by addition of various salts (magnesium sulfate and sodium acetate) followed by centrifugation;
2. cleanup of extract by addition of solid phase extraction materials (PSA - primary secondary amine and graphitized carbon) followed by centrifugation; and
3. volume reduction of sample (to 500 uL for GC/MS analyses) by nitrogen evaporation

Instrumental analysis was performed using gas chromatography/mass spectrometry. In addition to traditional electron ionization (EI), a second analysis was done using chemical ionization - which uses methane gas to fragment the ions - this is a much less energetic method of ionizing the analyte molecules for identification and quantitation - and particularly suited to analysis of pyrethroid compounds - which tend to dissociate into small ion fragments under the more energetic EI conditions, which makes them less distinct from background noise. In chemical ionization - one gets the molecular ion which is unique to the chemical compound. In addition, monitoring for negative ions (called negative ion CI) is similar in sensitivity to an electron capture detector - which is particularly sensitive to chlorinated compounds. Therefore the quantitations made used negative ion CI, with positive ion CI and "normal" EI analyses run for confirmatory purposes.

Samples were quantified using an internal standard. For the Duet analysis deccachlorobiphenyl was used. A five-point standard curve was run prior to each analysis and showed decent linearity; $r^2$ values were generally 0.99.

**RESULTS/CONCLUSIONS:** Table 2 shows the residues of Duet (in ppm) on the fruit for 1, 2, 3, and 5 days after application. By the last day of the trial residues of prallethrin and phenothrin were not detectable. And, there was no evidence of residual piperonyl butoxide in any of the samples. Ions were present, but they were "in the grass" and so not detectable. Because the default MRL level for the European Union, Japan, and most other countries is 0.01 ppm, contamination of blueberries from a mosquito application with Duet would not pose a problem

with prallethrin even 1 day after application. However, phenothrin residues were still above a default MRL until 3 days post-application. Our recommendation would be that blueberry growers in areas that receive mosquito treatments of Duet would be wise to wait 3 days to harvest their crop after the insecticide application, especially if they are not sure if spray drift occurred onto their crop.

**Table 2.** Residues (ppm) of prallethrin and phenothrin post application.

| | Prallethrin | | Phenothrin | |
|---|---|---|---|---|
| Days* | Mean | Std. Dev. | Mean | Std. Dev. |
| 1 | 0.00116 | 0.00071 | 0.12181 | 0.01949 |
| 2 | 0.00001 | 0.00002 | 0.02727 | 0.02317 |
| 3 | 0.00004 | 0.00000 | 0.00909 | 0.00120 |
| 5 | ND | NA | ND | NA |

* Days after application

**RECOMMENDATIONS:** We now have several years of residue data for several insecticides. Year to year variation is high. Since Japanese and EU MRLs also vary, growers will need to be conservative in their post-harvest intervals. In general, malathion-treated crops can be harvested 3 days after application and Delegate and Success or Entrust-treated crops can be harvested 1-2 days after application. Mustang Max-treated crops appear to need at least 5 days post-application for harvest. We intend to put together a table of residues and MRLs so that growers and processors can be more informed regarding the 2014 harvest.

## ENTOMOLOGY

**INVESTIGATORS:** F. A. Drummond, Professor of Insect Ecology/Entomology
J. A. Collins, Assistant Scientist of Insect Pest Management

**6. III. TITLE:** Biology of Pest Insects and IPM, 2013.

***Study 1. Notes on parasitism of blueberry maggot fly***

**METHODS:** Diet cups containing blueberry maggot fly (BMF) pupae (72 cups of 50 pupae each) from various studies were maintained in the laboratory for a minimum of four weeks following the last observed emergence of BMF adults. Parasitic wasps were observed in the rearing cages. The wasps were collected and an estimate was made of percent parasitism. An estimate of relative size of blueberry maggot populations from year to year was obtained from both pupal collections from fruit and from trap captures of flies in control plots of annual insecticide trials.

**RESULTS:** This study is a continuation of our effort to assess the relationship between BMF population increase from year to year and parasitism. Figure 1 shows the time series of blueberry maggot percent parasitism from 1998 to 2013. Upon inspection of this graph it is apparent that percent parasitism fluctuates from year to year, ranging from a low of 0.5% to a high of 28.0%. However, there does not appear to be a tight linkage between fly trap captures and the parasitism rates over time (Fig. 2). Modeling fly rate of increase as a function of log parasite density suggested that a possibility ($F_{(1,13)}$ = 3.783, $P$ = 0.074) exists that a parasitic wasp (presumably *Opius sp.*) is important in regulating fly numbers and that steps should be taken to conserve its numbers. Also, based upon data collected from 1998 through 2013 and plotted in Figure 3 it appears that parasitism behaves as a density dependent factor that controls fly abundance from one year to the next. Figure 4 shows the relationship between the logarithm of fly abundance in year t versus the log rate of increase from year t to year t+1 (Log(Nt+1 /Nt)). The linear relationship suggests that a density dependent relationship exists between fly abundance and the next year's increase or decrease in the BMF population ($F_{(1,13)}$ = 15.527, $P$ = 0.002, $r^2$ = 0.544). In addition, inspection of figure 4 suggests that a seasonal fly abundance of 10 is the threshold for increase. Below a density of 10 the population will increase and above a seasonal density of 10 the population will decrease. What is particularly interesting about this threshold is that this is the threshold used for making decisions regarding insecticide control. Additional data was collected in 2013 to verify our hypotheses regarding the dynamics of BMF populations.

**Fig. 1.** Percent parasitism of blueberry maggot fly pupae.

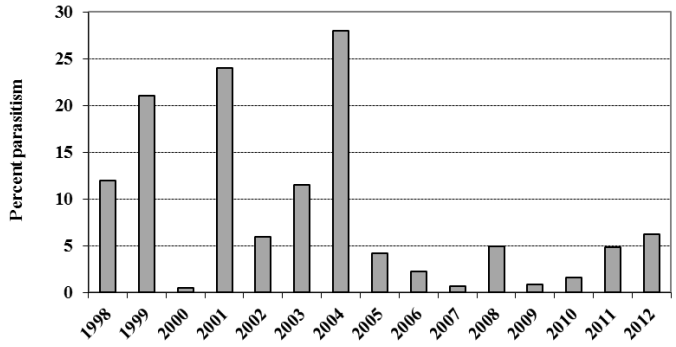



**Fig. 2.** Relationship between relative density of flies and % parasitism over time.

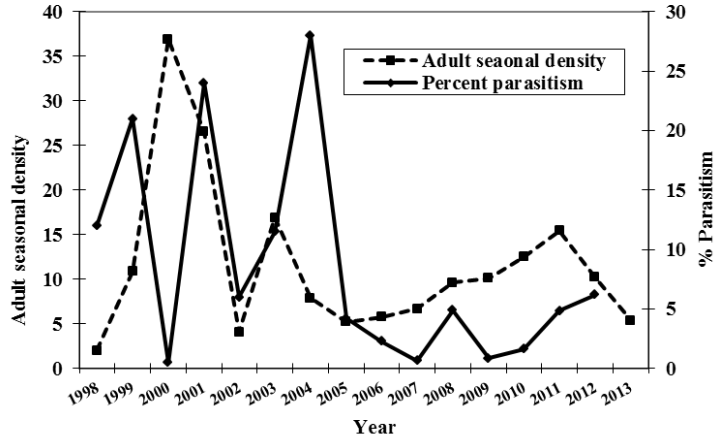


**Fig. 3.** Relationship between fly population increase and parasitoid density the previous year.

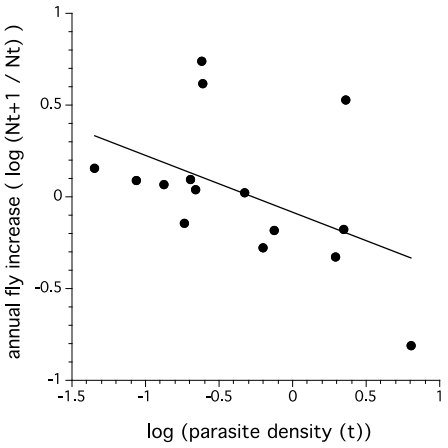
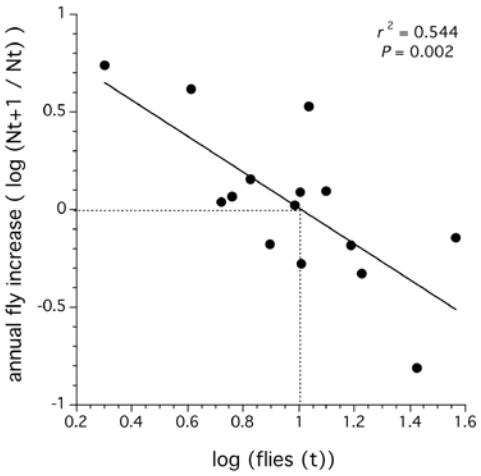



**Fig. 4.** Relationship between fly population increase and fly density the previous year. Dotted line demarks point of zero population increase.




**CONCLUSIONS:** At this point we have fairly strong evidence that BMF populations are under regulation and are being governed by factors that are influenced by fly population density. The factor most likely explaining this phenomenon is parasitism. A small parasitic wasp belonging to the family Braconidae, in the genus *Opius* is most likely the factor that regulates BMF populations. Those fields that do not have insecticides applied at a time that would impact the natural buildup of these parasites will tend to have fewer BMF problems over time.

### *Study 2. Long-range, within-field, movement of blueberry maggot fly in wild blueberry: A release/recapture study*

**METHODS:** The purpose of this trial was to continue a study of the long-range movement patterns of blueberry maggot fly (BMF). BMF were collected as pupae from infested blueberries in 2012. The wintering cups of pupae were separated into four equal groups. A small paint

brush was used to layer green, DayGlo® dye on top of the vermiculite in each of the cups. The cups were placed in cages and flies were allowed to emerge. Following emergence, the flies were fed honey and yeast for one week prior to release.

Marked BMF adults were released at two sites. At each site a line transect of 100 baited, yellow, Pherocon® AM traps was set along one edge of a pruned year blueberry field with 10 ft between traps. The pruned edge was abutted by a fruit-bearing field. Ammonium acetate superchargers were attached to every third trap to enhance attractiveness. On 24 Jun, the marked blueberry maggot flies were released at a point 100m (site 1) or 400m (site 2) across the pruned field from the trap transect; ca. 1000 flies were released at each site. Traps were checked daily for 8 days and periodically for an additional 7 days, thereafter. All BMF were removed from the traps, brought into the laboratory, and checked for dye.

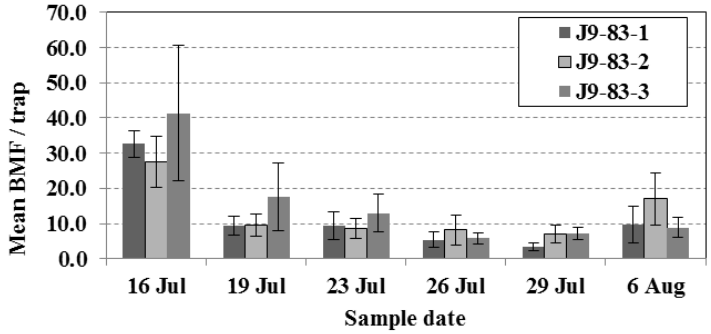
**RESULTS/CONCLUSIONS:** We recaptured seven marked flies on the 100m trap transect. Three flies were recaptured within 2 days (one on day 1 and two on day 2); four additional flies were recaptured 6 (n=3) or 7 (n=1) days after release. Only one fly was recaptured at the 400m site (on day 7 after release). These low recaptures are not unexpected. We have previously shown that BMF move 10m / day in a random direction and that movements of distances out to 100m are uncommon. Therefore, it appears that there is a 0.7% likelihood of flies moving 100m and only a 0.1% likelihood of flies moving 400 or more meters. These data suggest that colonization of new fields from previously infested fields will not occur at a significant rate if fields are more than 500m from each other. This field trial will be repeated in 2014.

### *Study 3. Attractiveness of two new synthetic lures to blueberry maggot fly*

**METHODS:** The purpose of this trial was to field test two new lures for blueberry maggot fly; a blueberry synthetic blend and a white oak synthetic blend. The trial also included a check. There were five replicates of each treatment labeled as: J9-83-1, J9-83-2, and J9-83-3 and set as a complete randomized block design. For each replicate (block), the traps were placed in a straight line transect along the edge of a fruit-bearing wild blueberry field and 40 ft apart. Each trap was baited with one of the three treatments. The chemical blends were formulated in centrifuge tubes with cotton balls and placed in plastic bags that were hung with clips from one corner of an unbaited, yellow, Pherocon® AM trap. The centrifuge tubes were left open. Traps were checked at 3 to 7 day intervals and any BMF were counted and removed. The experiment ran from 9 Jul until 6 Aug.

**RESULTS:** A Repeated-Measures ANOVA with "treatment" being the between subject factor, "block" the subject factor, and "date" the within-subject factor, was used to analyze the data. There was no significant treatment ($F_{(2,12)} = 0.12$, $P = 0.885$) or treatment x date interaction ($F_{(2,10)} = 0.70$, $P = 0.7249$). Since there was no evidence of a treatment effect, it can be concluded that none of the baits were attractive (different from the unbaited control).

**Fig. 1.** Bar graph showing mean BMF adults per treatment over each sample date.



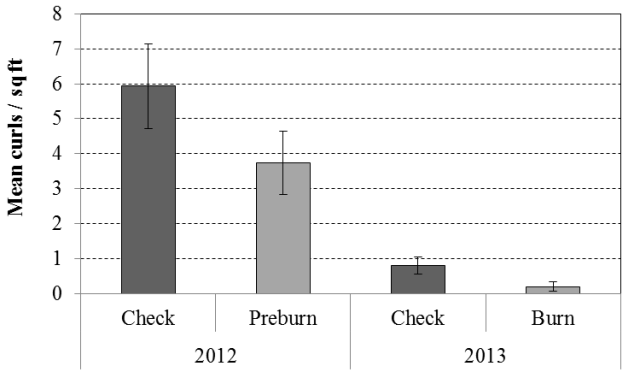

***Study 4. <u>Impact of a late spring burn on blueberry tip midge populations</u>***

**METHODS:** In order to determine the effectiveness of a late spring burn (after leaf curl formation) on blueberry tip midge populations a ca. 20 x 100 ft area was burned (oil burn) at Blueberry Hill Farm, Jonesboro, ME on 11 Jun 2012 (Fig. 1). Preburn populations of blueberry tip midge were estimated on 8 Jun 2012 by counting the number of blueberry stems with tip midge damage as evidenced by leaf curls in each of 15, sq ft quadrats. An additional 15 quadrats were evaluated in an adjacent unburned area. On 8 Jul 2013 we again estimated tip midge populations in these areas to see what, if any, impact the late burn had on subsequent populations. The number of blueberry stems with tip midge damage was counted in each of 10 sq ft quadrats per treatment.

**Fig. 1.** Experimental design.

**RESULTS:** Data were transformed by the square root prior to analysis. There was no significant difference in the number of stems with tip midge damage in the burned vs unburned check area (ANOVA, $F_{(1,46)} = 0.001$, $P = 0.9661$)(Fig. 2) BEFORE the area was burned. Populations of tip midge were much lower overall in 2013 than 2012 ($F_{(1,46)} = 44.230$, $P < 0.0001$). Although tip midge populations were low in 2013, there was a decrease in tip midge populations in the burned area ($F_{(1,46)} = 2.912$, $P = 0.0393$)(Fig. 2).

**Fig. 2.** Mean number of stems with tip-midge damage before (2012) and after (2013) burning.




**CONCLUSIONS:** Burning may be a non-insecticide option for tip midge suppression. However, the decline in the untreated plots from 2012 to 2013 was 86%, compared to a 95% decline in the burned plots over the same time period. Therefore, while this difference in declines was significant there was not a large decrease in tip midge due to burning over the check and so this tactic may not be economical unless a very high tip midge population exists.

### *Study 5. Impact of blueberry tip midge on flower-buds and subsequent flower development*

This trial concludes a three-year study to determine the impact tip midge has in Maine.

**METHODS:** On 7 Jun 2012 at Orland and 11 Jun 2012 at Jonesboro, we marked 100 stems per site, 50 with tip midge infestation (red flags) as evidenced by leaf curls and 50 without infestation (white flags). On 8 Oct 2012 at Orland and 15 Oct 2012 at Jonesboro we cut 25 stems from each treatment, brought them into the laboratory and counted the number of flower-bud clusters on each stem. Twenty-five marked stems of each treatment were left in the field at each site. On 27 May 2013 the stems were cut and brought into the laboratory to determine the number of flowers that developed from individual flower-bud clusters.

**RESULTS:** We have so far demonstrated that blueberry plant response in flower-bud production can be quite variable. In 2010-2011 trial we found NO difference in flower-bud clusters per stem due to blueberry tip midge (Fig. 1)($F_{(1,48)} = 0.01$, $P = 0.9054$); however, stems with blueberry tip midge infestation developed significantly fewer flowers then those without tip midge infestation (Fig. 2)($F_{(1,48)} = 17.46$, $P < 0.0001$)

As in the first trial, there was no significant difference in the number of flower-bud clusters ($F_{(1,48)} = 0.16$, $P = 0.6897$) in our 2011-2012 trial (Fig. 1). When individual flowers were counted in 2012, there appeared to be a trend towards more flowers on tip-midge damaged stems; however, the difference was not significant ($F_{(1,48)} = 2.83$, $P = 0.0967$)(Fig. 2). In both our trials begun in 2012 there was a significant difference in the number of flower-bud clusters (ANOVA, CRD; $F_{(1,48)} = 5.0$, $P = 0.03$, Jonesboro; $F_{(1,48)} = 4.22$, $P = 0.0454$, Orland) per stem between stems with and without tip midge damage (Fig. 1). Stems without damage had significantly more flower-bud clusters. And, stems with tip-midge damage developed fewer

flowers than undamaged stems; although, at our Jonesboro site the difference was not significant ($F_{(1,48)} = 2.73$, $P = 0.1050$, Jonesboro; $F_{(1,48)} = 6.18$, $P = 0.0164$, Orland)(Fig. 2).

The question that needs to be asked is…does blueberry tip midge result in potential crop loss when all of the four studies are combined. A MANOVA repeated measures was used to assess this question, the dependent variables being flower clusters / stem and also flower buds / stem. Two separate ANOVA analyses were also conducted to see if either bud clusters / stem or flower buds / stem were reduced by tip midge attack (sqrt transformed data). The MANOVA results and the ANOVA results suggest that blueberry tip midge did not reduce overall yield over the four trials ($F_{(1,3)} = 0.529$, $P = 0.297$; and $F_{(1,3)} = 1.365$, $P = 0.327$; $F_{(1,3)} = 1.476$, $P = 0.311$; for MANOVA and ANOVA for flower bud clusters / stem and flower buds / stem; respectively). Therefore, while individual years have shown an effect of tip midge on flower production, the overall response over the three years and four trials suggests that tip midge is not a consistently damaging pest. However, if the 2011-2012 year is left out of the analysis, then tip midge infestation does have an effect for the three of four trials ($F_{(1,3)} = 35.939$, $P = 0.014$; and $F_{(1,3)} = 4.696$, $P = 0.163$; $F_{(1,3)} = 20.914$, $P = 0.044$; for MANOVA and ANOVA for flower-bud clusters / stem and flower buds / stem respectively).

**Fig. 1.** Bar graph comparing mean number of flower-bud clusters between stems with and without tip-midge damage. Data collected from trials conducted in 2010-2011, 2011-2012, and 2012-2013.

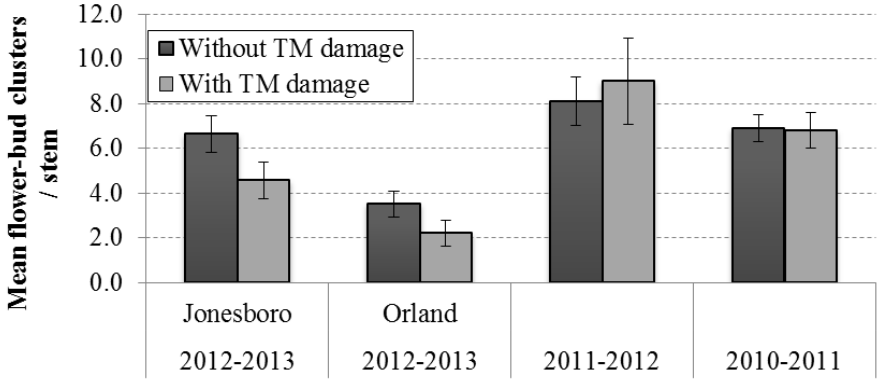
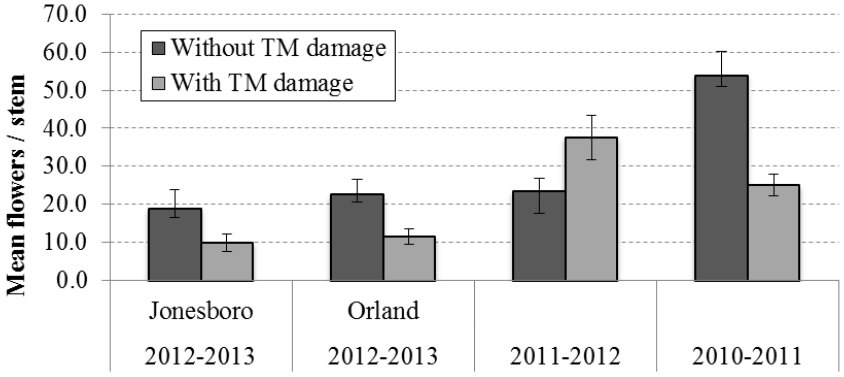



**Fig. 2.** Bar graph comparing mean number of individual flowers per stem between stems with and without tip-midge damage. Data collected from trials conducted in 2010-2011, 2011-2012, and 2012-2013.



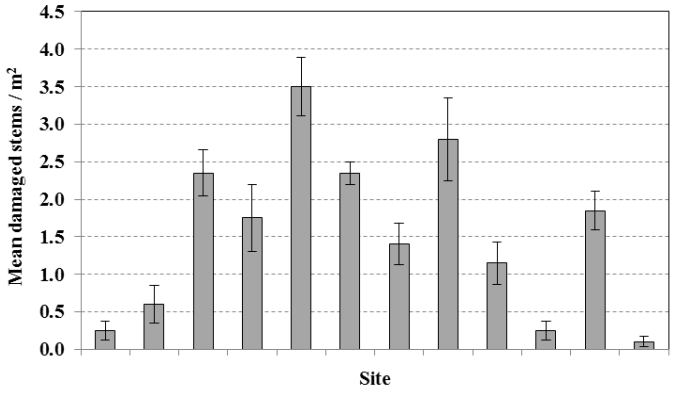
**CONCLUSIONS:** The results from the last three years of research suggest that tip midge can be damaging and that potential yield loss can be as high as 50%. However, because stem infestation rate is usually less than 5%, there is no evidence that control of blueberry tip midge is a justified expense. This may not be the case if tip midge infestation continues to increase as it appears to have done over the past decade. Just in case, we will continue to research the blueberry tip midge ecology, effects on plant yield loss, and management to be able to offer a contingency management plan if blueberry tip midge increases in severity.

***Study 6. <u>Survey of blueberry tip midge damage in wild blueberry fields</u>***

**METHODS:** Twelve pruned-year blueberry fields were sampled for tip midge in 2013. Damage was assessed by counting the number of blueberry stems with damage as evidenced by curled leaves in each of ten, $m^2$ subplots field.

**RESULTS/CONCLUSIONS:** Infestation levels were generally low; average numbers of infested stems in a field ranged from 0.1 to 3.5 damaged stems/$m^2$ (Fig. 1). A survey of stem density in 2013, based upon 16 fields suggests that stem density averages 1203.9 stems/$m^2$. At this density, % damage from tip midge resulting from the survey would range from 0.004 – 0.15%. This is well below any economic threshold that would require treatment.

**Fig. 1.** Bar graph showing mean number of stems per $m^2$ with tip midge damage in each of twelve fields.




**RECOMMENDATIONS:** The only recommendations that stem from the 2013 field season are as follows. Blueberry tip midge populations can be reduced by an early burn; although, more trials have to be conducted over the next few years to see if this is a consistent management tactic. At this point we recommend burning particularly heavily infested tip midge patches if they are found prior to June 15. Our survey suggests that blueberry tip midge outbreaks serious enough to warrant control are still uncommon. Therefore, we do not recommend control in most fields unless previous years appear to have resulted in significantly reduced flower production.

## ENTOMOLOGY

**INVESTIGATORS:** F. A. Drummond, Professor of Insect Ecology/Entomology
J. A. Collins, Assistant Scientist of Insect Pest Management

**7. IV. TITLE:** Biology of Blueberry, Beneficial Insects, and Blueberry Pollination.

***Study 1.*** ***Impact of floral bud thinning on whole-plant physiology and phenology as well as fruit chemistry and development.***
***Report from Alex Bajcz (Master's student) and Dr. Frank Drummond.***

This study assessed the potential for gains in plant health and fruit quality from a controlled thinning of floral buds prior to flowering in wild blueberry.

**METHODS:** During the first week of May, 2013, 15 blueberry clones were selected at Blueberry Hill Farm in Jonesboro, ME. In each clone, four 1/8$m^2$ plots were delineated (60 total plots), and, in each plot, all floral buds on all stems were counted. In two of these four plots (hereafter "experimental" or "X" plots), floral buds were thinned by approximately 70%, while in the other two plots (hereafter "control" or "C" plots), no floral buds were removed.

Whole stems were harvested from all 60 plots during three collection periods—during flowering (31 May), during fruit initiation (3 Jul), and during fruit ripening (30 Jul through 1 Aug). Ten stems were harvested from each plot during the first two periods and all remaining stems were harvested during the last period. Only stems with at least one intact reproductive structure were included. All harvested stems were separated into three portions: old stems, vegetative structures (leaves plus new stems), and reproductive structures (flowers or fruits). The three portions were then weighed separately. For the last two collections, the number of intact buds and fruits were also counted for each plot, and, for the third collection, ripe and unripe fruits were counted separately. Ripe fruits were analyzed for their anthocyanin pigment content using the AOAC pH differential method. Plot canopy development was measured at three time points (27 May, 13 Jun, and 2 Jul) using a light meter.

**RESULTS:** Results from this assessment come in two classes. For the first class, the null hypothesis is that control and experimental stems or plots will not differ significantly. This applies to all stem and vegetative measurements as well as some reproductive measurements. These results are presented first.

For the second class, the null is that measures taken from experimental stems or plots will be approximately 30% of those from control plots, reflecting the size of the initial treatment. For example, all other things being equal, experimental plants should produce only 30% as many fruits as control plants, on average, because approximately 70% of the floral structures of experimental plants have been removed. If this percentage is significantly higher than 30%, then treatment plants will have "exceeded expectations" relative to control plants. These results will be presented second.

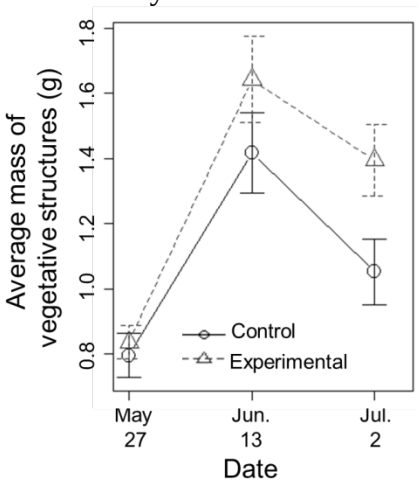
*Class 1*.

Blueberry canopies got significantly fuller over time as measured by both light transmittance through the canopy ($P < 0.0001$) and by Leaf Area Index (LAI; $P < 0.0001$).

While experimental plots had somewhat thicker canopies than control plots, this difference was not significant for either transmittance ($P = 0.32$) or LAI ($P = 0.14$).

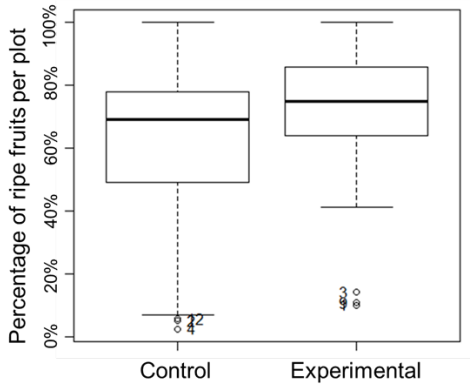
Average old stem weight per stem was not significantly related to date of harvest ($P = 0.44$) or treatment ($P = 0.37$). Vegetative weight per stem was significantly related to both date ($P = 0.008$) and treatment ($P = 0.0002$), with experimental stems having significantly more leaf mass than control stems, and this effect increasing over time (Fig. 1). Average fruits per bud were negatively related to date ($P < 0.0001$) but not affected by treatment ($P = 0.16$). Average fruit mass per bud was positively affected by both treatment ($P = 0.023$) and date ($P < 0.0001$), with experimental stems producing substantially more fruit mass per bud than control stems with this effect increasing over time. Average mass per fruit was positively affected by date ($P < 0.0001$) and marginally positively affected by treatment ($P = 0.06$). Fruit anthocyanin content was positively correlated with fruit mass ($P < 0.0001$) but not with treatment ($P = 0.92$). However, because experimental fruits were significantly larger than control fruits (next paragraph), experimental fruits had higher anthocyanin contents.

**Fig. 1**. Plot of means showing the change in average vegetative (leaves and new stems) mass over time between control and experimental plots of wild blueberry. Data from a 2013 study.




Differences were especially pronounced during the third (fruit ripening) collection period. The percentage of fruits that were ripe was significantly higher in experimental plots ($P = 0.01$) by approximately 8.3% (Fig. 2). The number of ripe fruits per bud was also substantially higher in experimental plots ($P = 0.0002$) by about a third of a fruit per bud during this period, and the average fruit was also substantially heavier for experimental plants ($P < 0.001$) by approximately 29 mg. The average fruit mass per bud during this period was also significantly higher ($P = 0.0001$) for experimental plants by approximately 124 mg.

**Fig. 2**. Boxplots comparing the percentage of fruits that were ripe at tissue harvest (30 Jul-1 Aug) between control and experimental plots of blueberry. Data from a 2013 study.




*Class 2*.

Table 1 summarizes the results of this class with respect to measurements taken from within a collection period. During the first collection period, experimental plants had flower weights per stem much higher than expected ($P$ = 0.001) based on control plant performance. During the second collection period, experimental plants significantly exceeded expectations in fruit weight per stem ($P$ < 0.0001), buds per stem ($P$ < 0.0001), and total fruits per stem ($P$ < 0.0001). During the third collection period, experimental plants significantly exceeded expectations in the number of buds per stem ($P$ < 0.0001), fruit weight per stem ($P$ < 0.0001), total fruits produced per stem ($P$ < 0.0001), and both unripe ($P$ = 0.03) and ripe ($P$ < 0.0001) fruits per stem.

**Table 1.** Fruit production outcomes during three tissue collection periods for experimental plots of wild blueberry relative to "expected" values (30% of control plot production). Plants in experimental plots had 70% of their initial floral buds removed prior to flowering. Data from a 2013 study.

| Collection | Measure | C | X | X % of C Expected | X % of C Observed | P value (Obs. – Exp.) |
|---|---|---|---|---|---|---|
| 1 | Flower mass/stem (mg) | 0.4 | 0.28 | 30% | 70% | **0.001** |
| 2 | Fruit mass/stem (mg) | 0.57 | 0.31 | 30% | 54.4% | **<0.0001** |
| 2 | Fruits/stem | 12.94 | 6.84 | 30% | 52.9% | **<0.0001** |
| 2 | Buds/stem | 3.89 | 1.98 | 30% | 51% | **<0.0001** |
| 3 | Fruit mass/stem (mg) | 1.32 | 0.93 | 30% | 70.5% | **<0.0001** |
| 3 | Unripe fruit/stem | 4.02 | 1.58 | 30% | 39.3% | **0.03** |
| 3 | Ripe fruit/stem | 4.14 | 2.87 | 30% | 69.3% | **<0.0001** |
| 3 | Total fruit/stem | 8.17 | 4.45 | 30% | 54.5% | **<0.0001** |
| 3 | Buds/stem | 2.77 | 1.45 | 30% | 52.3% | **<0.0001** |

**CONCLUSIONS:** This assessment strongly indicates that blueberry plants can "compensate" for the loss of some floral buds by investing more heavily into the floral buds that remain, as evidenced by much greater than expected fruit production and yield in experimental plots. Moreover, there appears to be substantial gains to plant health when initial floral bud number is reduced—experimental plots had significantly greater foliar mass than control plots.

Blueberry plants lose a significant portion of their initial reproductive structures (approx. 15-30%) before these structures become ripe fruits. These lost structures represent wasted plant (and grower) resources. This assessment suggests that gains in both fruit quality and quantity as well as in overall plant health may be obtained with a minor thinning (i.e. much less than the 70% thinning assessed here) of floral buds prior to bloom. If true, this may mean that fruit quality, yield, and ripening time could conceivably be *improved*, somewhat counter-intuitively, through a small *reduction* in the starting number of floral buds, as has been observed in other fruit crops, such as apple.

### *Study 2. Seasonal variation in cold hardiness of wild blueberry.*
### *Report from Lee Beers (Ph.D student) and Dr. Frank Drummond*

Wild blueberries (*Vaccinium angustifolium*) grown in Maine are genetically diverse with variation in color, yield, and response to the environment. The variation in cold hardiness among clones can be readily seen after a spring frost; some clones are damaged while others appear to be unharmed. The goal of this study was to identify the variation in cold hardiness during different growing seasons.

**METHODS:** Cold hardiness trials of closed flower buds were carried out in between October 2012 and March 2013. Stems containing healthy flower buds were collected from 10 wild blueberry clones at the Blueberry Hill Research Farm in Jonesboro, ME. Stems sections containing 8-10 total terminal flower buds were trimmed to ~5cm in length from each clone and placed in a damp paper towel. The damp paper towel was then sealed in a plastic bag. This was repeated 12 times. Bags containing terminal buds were placed in a programmable freezer at 4°C and acclimated for 3 hours. After the initial acclimation, the temperature in the freezer was dropped at a rate of 1°C/hour until the temperature reached -3°C to promote ice formation. Temperature was further decreased at a rate of 3°C/hour to target temperature of -21°C and then 5°C/hour to -40°C. Flower buds were removed at 4, -3, -6, -9, -12, -15, -18, -21, -25, -30, -35, and -40°C. Following exposure to freezing temperatures the terminal buds were incubated at 23°C for 48 hours. Terminal flower buds were dissected to observe damage to floral tissue. Damage was visually assessed based on percent necrosis (browning) of tissue compared to control stems (4°C). Any damage above 50% browning was considered to be significant damage and the flower was no longer viable.

Frost hardiness trials of open flower buds were conducted in April, May and June 2013. Generally, the methods for frost hardiness followed that of the cold hardiness trials with the exception of the exposure temperatures. Open terminal flower buds followed the same acclimation period followed by a 1°C/hour temperature decrease to 0°C followed by a 2°C/hour decrease to -16°C. Control stems were held at 23°C. Stems were removed at 0, -2, -4, -6, -8, -10, -12, -14, and -16°C and incubated at 23°C for 48 hours. Dissection methods were the same as the cold hardiness trial.

**RESULTS:** Significant variation ($P \leq 0.00001$) in floral damage was found among the ten clones tested at all dates. Generally, clones increased in hardiness until mid-winter and steadily exited the dormant state until a baseline cold hardiness of -6°C was achieved in late spring. Clones sampled in October had a wide range of hardiness values (Fig. 1A). The hardiest clone did not experience significant damage until -30°C while the least hardy clone had significant damage at -13°C. When clones were sampled again in December, there was a trend of increased hardiness among all clones (Fig. 1B). At this time point the most cold hardy clone survived -40°C (the lowest temperature tested) and the least hardy clone had damage at -18°C.

Another increase in cold hardiness was observed in February 2013 among the 10 clones sampled (Fig. 2A). Multiple clones had no significant damage to the flower buds at -40°C and others did not have significant damage until exposure to -35°C. When sampled in March 2013, the flower buds appeared to be de-hardening as there was a general decrease in cold hardiness observed (Fig. 2B). Again, multiple clones had no significant damage to flower buds at -40°C, but some clones had significant damage at -30°C. This was a decrease in hardiness of 5°C over 4 weeks.

**Fig. 1**. A) Cold hardiness of clones sampled October 26, 2012. All clones survived temperatures to -12°C before significant damage occurred at -13°C for the least hardy. The most cold hardy clone survived exposure to -30°C. B) A general increase in cold hardiness is seen in the clones sampled December 12, 2012. Significant damage does not occur until -18°C while the hardiest clone survives to -40°C.

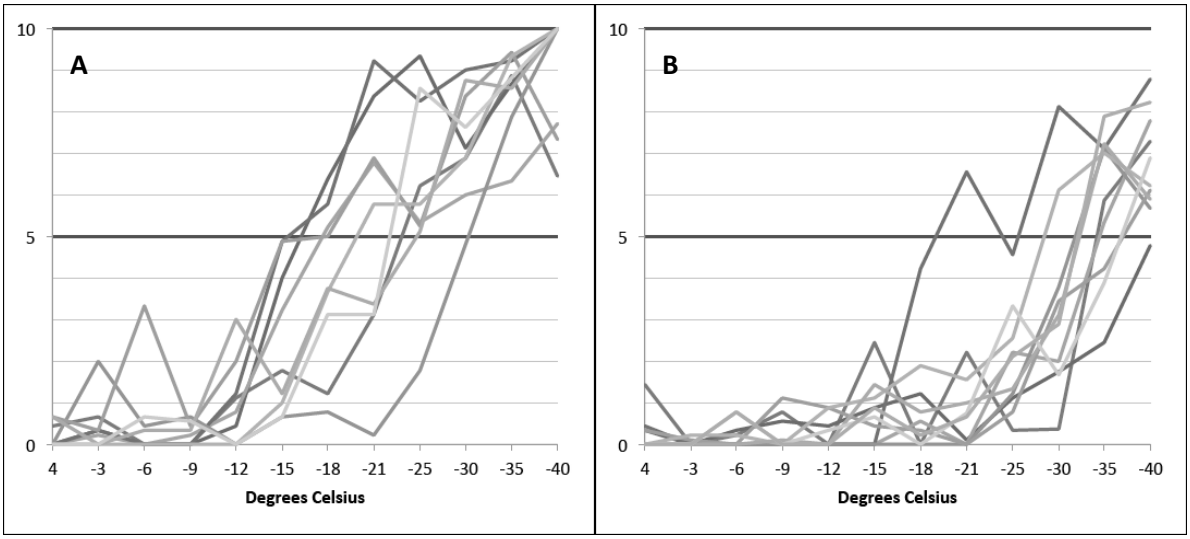
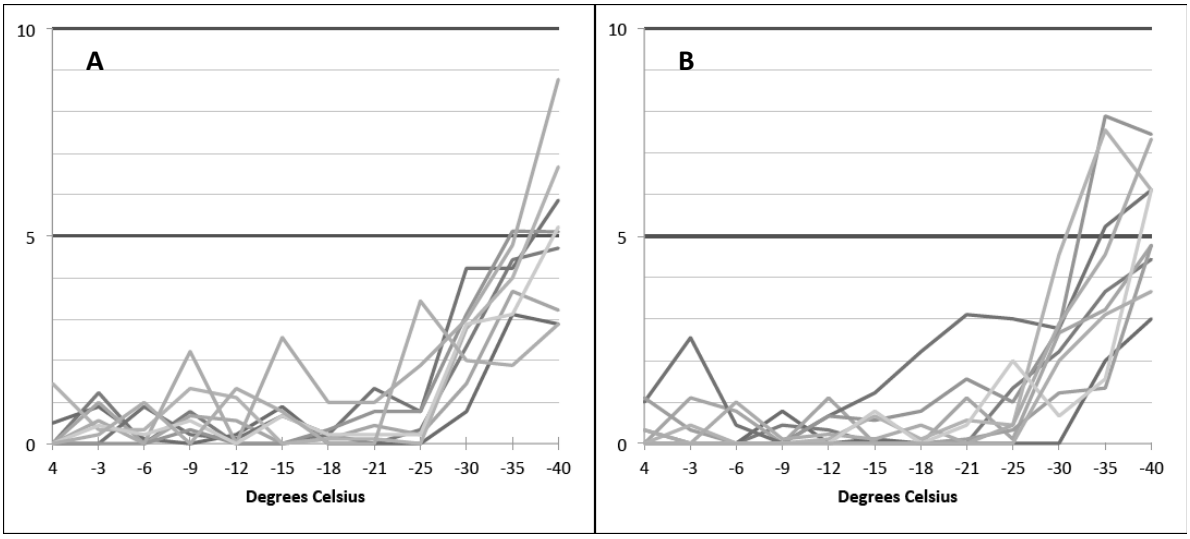
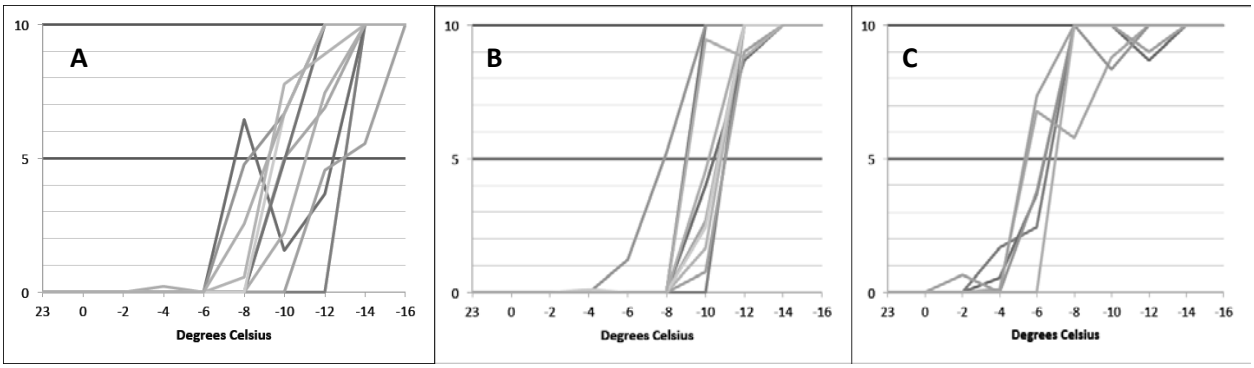


**Fig. 2**. **A)** Cold hardiness of clones sampled February 19, 2013. Cold hardiness increased relative to December 12. **B)** Some clones begin to deharden by March 15, 2013. Hardiness decreases by 5°C.




Dehardening occurred rapidly as flower buds developed and hardiness of open flower buds was lower compared to closed buds. Flower buds in tight cluster were sampled in April 2013 and were found to have hardiness between -6° and -12°C (Fig. 3A). Hardiness decreased again as flowers entered loose cluster (Fig. 3B) and finally at full anthesis (Fig. 3C). Flowers reached a stable hardiness between -4° and -6°C at full anthesis that continued into the immature fruit (data not shown).

**Fig. 3**. **A)** Frost hardiness of clones sampled April 19, 2013. Hardiness of tight cluster flower buds ranges between -7° and -12°C. **B)** Frost hardiness of loose cluster flowers sampled May 8, 2013 is decreased relative to April. **C)** Frost hardiness of flowers at full anthesis May 14, 2013.




**CONCLUSIONS:** Variation in cold hardiness was observed in 10 wild blueberry clones at Blueberry Hill Farm in Jonesboro, ME. Clones increase in cold hardiness throughout the fall, reaching maximum hardiness in mid-February before de-hardening in early spring. This suggests that unseasonably low temperatures can damage flower buds in the fall and spring.

Hardiness levels may vary from year to year due to different environmental conditions that influence the physiological changes necessary for cold hardiness. `Further evaluation in multiple years will be needed to provide a predictive model for potential frost damage to flower buds.

### *Study 3. Characterization of native pollinator habitat in electric transmission easements in Washington County, Maine*

***Report from Brianne Looze (Ph.D Student), Dr. Cyndy Loftin (USGS Coop Research Unit and Professor WLE), and Dr. Frank Drummond***

This pilot study is the basis for a dissertation on pollinator/landscape relationships around wild blueberry.

**METHODS:** Two studies were completed: a pollinator community survey and a bumblebee movement experiment. Pollinator community surveys were conducted three times: in July, August, and September 2013 at six sites along a large electric transmission easement in Washington County, ME. Three sites were near blueberry fields and three sites were isolated in forest to assess the effect of landscape cover on bee communities. Both active and passive survey methods were used: each survey consisted of one hour of live-netting bees and a 24-hour deployment of bowl traps. All bees were collected for identification to genus in the lab. The bumblebee movement experiment began on August 15, 2013. Three Koppert® bumblebee quads were placed at the interface of the power line and blueberry field. Six surveys of bee communities were conducted: one before placement of the quads and five after. Each survey consisted of one hour observing and tallying bees by type. Four, 25 m vegetation transects were also surveyed to account for available floral resources.

**RESULTS:** A t-test indicates there is a significant difference in Shannon diversity of live-netted bees between sites near blueberry fields (H=1.84) and sites isolated in forest (H=1.77) ($P$ = 0.01). The bumblebee movement experiment indicates that bumblebees do respond to forage present in an electric transmission easement. Bumblebees began to increase in number within the easement within one week of placement at the site. They continued to increase with increasing blooms, and then dropped off drastically as blooms decreased (Fig. 1). A linear regression shows that this relationship is statistically significant (Fig. 2).

**CONCLUSIONS:** Live-netting sampling is biased toward catching larger bees such as *Bombus sp*. and *Apis sp*., which made up much of the samples. Bowl trapping is biased toward catching smaller bees; identifying those samples will provide a more complete picture of pollinator communities in electric transmission easements. The bumblebee movement experiment demonstrates that temporal heterogeneity may play a strong role in the diversity and abundance of native pollinators within these easements. Bees responded strongly to an increase in forage; more sampling will determine if this is a universal trend. The pollinator community survey will be conducted five times at each site next summer, each month from May-Sep. Vegetation surveys will be incorporated to assess floral resources. We will establish a network of sites in the Midcoast region to compare effects of landscape complexity. The bumblebee movement experiment will be conducted in conjunction with the blueberry bloom to determine if mass flowering crops influence flight through a power line corridor.

**Fig. 1.** Time-series of numbers of bumblebees and blooms over the six-week sampling period of the bee movement experiment in Deblois, ME in 2013.

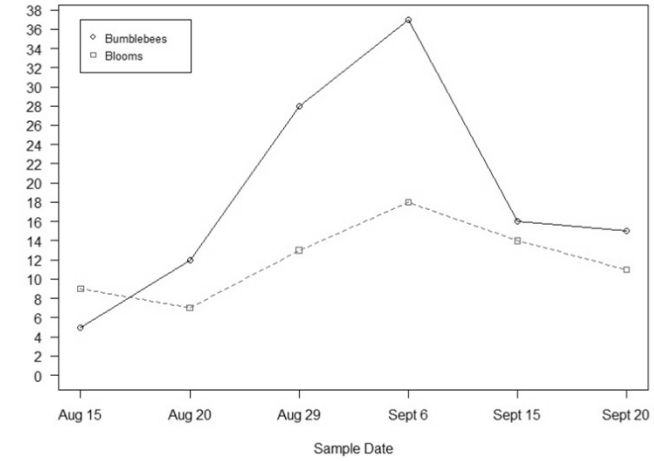
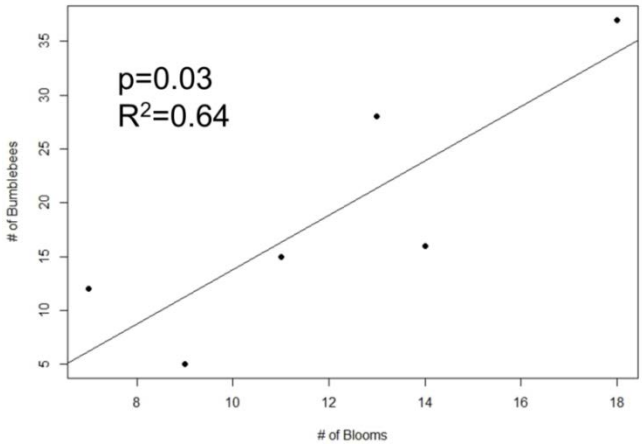



**Fig. 2.** Linear regression of number of bumblebees vs. number of blooms for the bee movement experiment in Deblois, ME, August-September, 2013.




***Study 4.*** ***<u>Pollinator Project Annual Report: Farm Economics, 2013</u>***
***<u>Report from Aaron Hoshide, Adjunct Assistant Professor / Faculty Associate</u>***
***<u>School of Economics, University of Maine</u>***

**INTRODUCTION:** As honey bee hive prices increase as U.S. supply of hives decline due to Colony Collapse Disorder (CCD), wild blueberry, cranberry, apple, and squash producers and researchers in the Northeast are evaluating alternative pollination options that rely on locally owned honey bee hives and renting bumblebees, as well as providing forage and habitat to enhance native bees. The current demand for honey bee hives by many specialty crop producers is not very responsive to escalating prices so adoption of these alternatives has not been widespread. However, a diverse group of Maine wild blueberry growers as well as Northeast cranberry, apple, and squash producers have been experimenting with one or more of these alternative pollination strategies. In the case of providing forage and habitat for local (managed

and wild native) bees this has been accomplished by 1) letting marginal areas in and around fields revert back to native perennial flowering plants or 2) planting wildflower and/or clover pasture mixes using either tilled or no-till methods. Recent historical production and value of Maine wild blueberries as well as the prevalence and economics of all these pollination alternatives are evaluated to determine which pollination strategy is more favorable under different production scenarios and scales.

**METHODS:** Historical data on rented hive imports to Maine (Personal correspondence with Dave Yarborough) as well as Maine wild blueberry production and value (USDA, NASS 1998-2012) were summarized in Excel. The use of pollination alternatives by Maine wild blueberry growers was based on a July 18, 2012 survey at the Blueberry Research Farm in Jonesboro, Maine, which involved blueberry growers predominantly from Downeast Maine (n=48). To increase the number of observations for wild blueberry growers from other areas in Maine, thirty-two additional growers were surveyed on-farm or on the phone between July 23, 2012 and July 1, 2013 to bring the total number of surveys to n=80. Survey data were entered into SPSS for statistical analysis. Economics of pollination alternatives such as owning honey bee hives and renting bumblebees were contrasted to renting honey bee hives in Excel. Budgets for bee pasture strips were created in Excel and were based on research plot data in Maine.

**RESULTS AND DISCUSSION:** According to the 2007 Census of Agriculture, Maine's total blueberry acreage involved in a two-year cycle was 44,462 acres. Of these total acres, 22,747 acres were harvested in 2007. After adjusting for inflation, the average real price of wild blueberries received by growers in Maine from 1998 to 2012 was $0.73 per pound with substantial price volatility fluctuating from $0.48 to $1.11 per pound over these fifteen years. Crop yield has averaged 3,305 pounds per acre over this time with an average annual crop value of $55,622,419 (Table 1). This total value of crop production would approximate the value of pollination if there was a complete crop loss due to a lack of pollination. While this is not likely due to many growers experiencing some level of background pollination (about 40% of fruit set) from native bees (Table 2), it does represent a maximum estimate of the value of pollination of wild blueberries in Maine.

A minimum estimate of the value of pollination would be the value of replacing hives lost to CCD at 2012 market prices. While many wild blueberry growers need to order rented honey bee hives six to twelve months in advance of the May pollination window in Maine, some growers base final payments for rented hives on third party inspections, so a "substitute" value of pollination based on current prices and use of rented honey bee hives is a valid estimate if hives are available. The average price of $104.20 per rented hive paid by surveyed wild blueberry growers in 2012 when multiplied by the estimated 75,000 hives used in Maine (Personal Correspondence with Dave Yarborough) results in a total value of rented honey bee hives of $7,814,668 or $344 per acre of harvested crop. Average rented honey bee hive stocking density for Maine equals 75,000 hives divided by 22,747 harvested acres which is about 3.3 hives per acre. This is less than the average hive stocking density (3.83) from surveyed growers (Table 1).

Rented honey bee hives are the predominant means of pollinating wild blueberries in Maine with 76% of surveyed growers renting hives. Owning honey bee hives (17.5%) and renting bumblebee quads (8.75%) are used by a much lower percentage of growers. Even strategies such as reducing hive use due to spillover pollination from neighboring growers (16.25%) as well as limiting floral competition of surrounding plants that flower at the same time

as wild blueberries (16.25%) were not as prevalent as renting honey bee hives. However, even though pollination is required by many growers to achieve commercially acceptable fruit set and yields, 23.75% (n=19) of the 80 surveyed growers rely exclusively on native bees with 36.25% that actively monitor their native bees in some way. Additionally, the fruit set attributed to native bee pollination estimated by participating producers ranged from 5% to 100% with an average of about 40% (Table 2). Native bees' contributions to crop pollination range from serving as a supplemental "insurance policy" to rented honey bees to being the only alternative used. A couple of surveyed growers have isolated fields that maintain favorable crop yields with no spillover pollination and reliance on just native bees. However, both cases have sizeable areas of bee forage surrounding fields so reliance solely on native bees is logistically possible though not probable for most producers in the industry.

Surveyed Maine wild blueberry growers also participated to varying degrees in five practices that enhanced native bees. The three most commonly used by surveyed growers were less time and resource intensive, namely leaving standing deadwood for wood-nesting native bees (65%), altering pesticide use in some way such as evening spraying to avoid bees (58.75%), and avoiding the mowing of wildflowers to insure adequate forage for native bees (52.5%). The other two practices involving actively constructing native bee nest sites (20%) and planting native bee pastures (13.75%) were less popular. Over three-quarters of surveyed growers were willing to invest in native bee enhancements assuming such pollination improvement strategies were responsible for ensuring 50% fruit set. Hypothetical farmer investment per acre ranged from \$0 to \$250 per acre with an average willingness to invest of \$31.73 per acre (Table 2).

The annual costs of pollination alternatives are summarized in Table 3. Assuming a honey bee hive rental price rounded down to \$100 per hive, pollination alternatives such as owning honey bee hives and renting bumblebee quads were generally less economically favorable alternatives. For example, the total costs per acre of owning four hives (\$536) were greater than renting four hives (\$400). The higher costs were dependent on replacing bees. Since rented honey bee hives are pollinating crops further to the south of Maine prior to the May pollination window for wild blueberries, they are considered to be stronger than local hives. Assuming two locally-owned hives equals one rented hive, the annual costs per acre of owning eight hives (\$1,072) is even greater than the cost of renting four hives (\$400) per acre. Renting bumblebee quads is three-quarters of the cost only if one quad (\$300) has the equivalent pollination potential of four rented honey bee hives (\$400). However, many growers surveyed did not believe bumblebees were that efficient feeling that one quad was more equivalent to one rented hive. If this is true then renting four bumblebee quads (\$1,200) is three times more expensive than renting four honey bee hives (\$400).

Annual total (variable plus fixed) costs are also compared for establishing native bee pastures. As shown in Table 3, tilling up areas to plant pollinator wildflower and/or clover pasture mixes that have a three to five year stand life costs three to six times more (\$600 to \$1,030 per acre) than letting marginal areas on the farm revert back to native perennial flowering plants (\$180 per acre). If areas are not mowed, costs are just the fixed cost of the land plus taxes and insurance estimated at about \$100 per acre (data not shown). If the land is already paid off, then the annual cost is just taxes and insurance at roughly \$50 per acre (data not shown). While it may be less costly for wild blueberry growers to rely on such low-management bee pastures, actively planted wildflower strips may provide better floral density and duration for local bees which may have a positive impact on fruit set and crop yield.

Native bee pasture establishment costs per acre can be more easily covered by profits from wild blueberries if the ratio of blueberries to tilled pasture strip is closer to 10:1 or 20:1 (Table 3). At a 20:1 ratio, the cost per acre covered by blueberries for tilled bee pasture strips ($32/acre) and no-till bee pasture strips ($40/acre) assuming a five year stand life are comparable to the average surveyed responses of willingness to pay (WTP) for native bee habitat enhancement ($31.73/acre). A wild blueberry to bee pasture ratio of 1:1 may be challenging due to the lack of availability of marginal land around fields as well as the annual costs for bee pastures ($640 to $1,030) covered by an acre of wild blueberries exceeding the crop's net profits. No-till bee pasture strips have been used by a couple of Massachusetts cranberry growers. Establishment of the no-till bee pasture involves four to six monthly applications of Roundup® (6.25% in solution) during the summer and fall, raking back dead perennial plant residue, followed by late-fall hydro-seeding of the flower seed mix. Subsequent years of the stand life for the no-till bee pasture involve a decreasing frequency of mowing to control weeds.

**Table 1.** 1998-2012 Maine Wild Blueberry Value of Pollination.

| | | -------- 2012 Rented Hives[a] -------- | | | ---------- 1998-2012 Crop Years[e] ---------- | | | |
|---|---|---|---|---|---|---|---|---|
| **Measure** | **Statistic** | **Amount** | **Cost ($/hive or $/acre)** | **Value ($)** | **Value ($ or $/acre)** | **Price ($/lb)[f]** | **Production (lb)** | **Harvested Acres[g]** |
| Total or | Average | 75,000[b] | $104.20 | $7,815,000[b] | $55,622,419 | $0.729 | 76,541,533 | 23,189 |
| Per Unit | Min | - | $75 | - | $30,034,602 | $0.476 | 46,000,000 | 22,747 |
| | Max | - | $125 | - | $88,715,078 | $1.110 | 110,990,000 | 24,943 |
| | Std. Dev. | - | $14.57 | - | $19,094,001 | $0.213 | 15,766,836 | 707 |
| Per Acre | Average | 3.83 | $398.58[c] | $344[d] | $2,408 | - | 3,305 | - |
| | Min | 0 | $286.90 | - | $1,306 | - | 2,009 | - |
| | Max | 8 | $478.16 | - | $3,900 | - | 4,630 | - |
| | Std. Dev. | 1.7 | - | - | $855 | - | 690 | - |

[a] From wild blueberry grower survey conducted from July 2012 to July 2013 throughout Maine's wild blueberry production regions. Hive stocking density and price was share weighted by Maine participating growers' wild blueberry acreage.

[b] Rented honey bee value estimated by multiplying 75,000 rented honey bee hives for 2013 (Personal correspondence with Dave Yarborough) by share-weighted average price ($104.20/hive) from 2012-2013 grower survey.

[c] Calculated by multiplying surveyed growers share-weighted honey bee hive stocking density and rented hive price so not the same as when estimated by dividing total rented hive value by crop acreage.

[d] Rented honey bee value per acre estimated by dividing rented hive value by 2007 harvested wild blueberry crop acreage from most recent Census of Agriculture (USDA, NASS 2007).

[e] From USDA, NASS (1998-2012) and Census of Agriculture (USDA, NASS 1997, 2002, 2007).

[f] Real crop prices are adjusted for inflation.

[g] Maine wild blueberry acreage not annually reported so annual acreage estimated by linear interpolation of harvested acreages of 25,429, 23,000, and 22,747 reported in 1997, 2002, and 2007 Census of Agriculture respectively. Census of Agriculture historically has only reported harvested acreage for Maine wild blueberry with the exception of the 2007 Census of Agriculture where total crop acreage for Maine was 44,462 acres which included both harvested (fruiting) and non-harvested (prune) land involved in what is predominantly a two-year cropping cycle.

**Table 2.** 2012-2013 Maine Wild Blueberry Grower Pollination Practices Surveys.

| | | | ME Wild Blueberry ---------- n=80 ---------- | | |
|---|---|---|---|---|---|
| **Pollinator** | **Pollination Option** | **Grower Pollination Practice or *Characteristic*** | **Grower Response[a]** | **Percent of Sample** | **Response Value** |
| Honey bees | Rented Hives | Stock Rented Honey bees | 61 | 76.25% | - |
| | Owned Hives | Just Own Hives | 5 | 6.25% | - |
| | | Own & Rent Hives | 5 | 6.25% | - |
| | | Own Hives & Use Quads | 3 | 3.75% | - |
| | | Own & Rent Hives & Use Quads | 1 | 1.25% | - |
| | Reduce Hives | Use Less Hives due to Spillover | 13 | 16.25% | - |
| | Enhance Hives | Limit Floral Competition | 13 | 16.25% | - |
| Bumblebees | Bumblebee Quads | Just Use Quads | 2 | 2.5% | - |
| | | Use Quads & Rent Hives | 5 | 6.25% | - |
| Native Bees | Native Bees | Just Use Native Bees | 19[b] | 23.75% | - |
| | | Monitors Native Bees | 29 | 36.25% | - |
| | | *Percent Fruit Set from Native Bees*[c] | *76* | *95%* | *39.9%* |
| | Enhance Native Bees | Leave Standing Dead Wood | 52 | 65% | - |
| | | Alter Pesticide Use | 47 | 58.75% | - |
| | | Avoid Mowing Wildflowers | 42 | 52.5% | - |
| | | Provide Nest Sites | 16 | 20% | - |
| | | Plant Bee Pasture | 11 | 13.75% | - |
| | | Invest Native Bee Forage/Habitat[d] | 62 | 77.5% | $31.73/acre |

[a] The 80 wild blueberry growers in Maine sampled was about 20% of the total population. Percent of total crop acreage not disclosed to maintain grower anonymity. Maine wild blueberry acres (44,462) from USDA, NASS (2007) Census of Agriculture includes both prune and fruit acres over a two-year cycle.
[b] Maine wild blueberry growers using just native bees were distributed between IPM (6), organic (6), traditional (5), and no spray (2), compared to the 80 growers surveyed that were IPM (48), traditional (14), organic (14), and no spray (4).
[c] Grower estimate of percent of fruit set just from native bees with range of 5% to 100% and standard deviation of 30.69% for Maine wild blueberry growers.
[d] Grower investment per acre in native bee forage and habitat assumed native bees responsible for 50% of fruit set. Grower investment ranged from $0 to $250 per acre for Maine wild blueberry growers.

**Table 3.** Economic summary of pollination options & insurance alternatives for wild blueberry.

| Pollination Management | Stand Life | Acres to One Acre of Wild Blueberries | Number per Acre | Unit Price ($/unit) | Cost per Acre of Berries |
|---|---|---|---|---|---|
| Rented Honey bee Hives | - | - | 2 | $100 | $200 |
| | - | - | 4 | $100 | $400 |
| Owned Honey bee Hives | - | - | 2 | N/A | $268 |
| | - | - | 4 | N/A | $536 |
| | - | - | 8 | N/A | $1,072 |
| Bumblebee Quads | - | - | 0.5 | $300 | $150 |
| | - | - | 1 | $300 | $300 |
| | - | - | 2 | $300 | $600 |
| | - | - | 4 | $300 | $1,200 |

| Pollinator Forage Management | Stand Life | Acres to One Acre of Wild Blueberries | Seed (lb) per Acre | Seed Price ($/lb) | Cost per Acre of Berries |
|---|---|---|---|---|---|
| Untilled Natural Wildflowers | Lifetime | 1 | 0 | $0 | $180 |
| Tilled Established Clover Mix | 5 | 1 | 54 | $7 | $640 |
| Tilled Established Wildflower | 5 | 1 | 12 | $40 | $700 |
| No-Till Established Wildflower | 5 | 1 | 12 | $40 | $800 |
| Tilled Established Clover Mix | 3 | 1 | 54 | $7 | $920 |
| Tilled Established Wildflower | 3 | 1 | 12 | $40 | $1,020 |
| No-Till Established Wildflower | 3 | 1 | 12 | $40 | $1,030 |
| Untilled Natural Wildflowers | Lifetime | 0.05 | 0 | $0 | $9 |
| Tilled Established Clover Mix | 5 | 0.05 | 54 | $7 | $32 |
| Tilled Established Wildflower | 5 | 0.05 | 12 | $40 | $35 |
| No-Till Established Wildflower | 5 | 0.05 | 12 | $40 | $40 |
| Tilled Established Clover Mix | 3 | 0.05 | 54 | $7 | $46 |
| Tilled Established Wildflower | 3 | 0.05 | 12 | $40 | $51 |
| No-Till Established Wildflower | 3 | 0.05 | 12 | $40 | $52 |

**FUTURE RESEARCH:** While pollination alternatives such as owning local honey bees or renting bumblebees may be less economically favorable than renting honey bee hives, this may not be the case if rented honey bee hive prices continue to increase. However, owning hives may involve steep learning curves as well as detract from management time devoted to adequately managing wild blueberries. Although bumblebees are more efficient pollinators compared to honey bees, wild blueberries have a lot of flowers making it difficult to sufficiently pollinate all blossoms without rented honey bees. However, bumblebee quads can seed bumblebee populations which could increase native pollinator populations which may stabilize fruit set and

yield. So even though owned honey bee hives and renting bumblebees may currently be more costly compared to renting honey bees, this may not be the case in the future.

While the costs of natural, un-planted pasture strips are lower than those established with either tilled or no-till methods, the relative efficacy of these three bee pasture systems in supporting native bees needs to be evaluated. Tilled and no-till pasture strips may provide a higher density of floral resources over a more extended season. If this is the case then the additional costs may be worth the positive impacts on native bee populations and subsequently improvements in blueberry fruit set and yields. However if the benefits of tilled and no-till pasture strips are marginally better or similar to natural strips, the higher costs associated with getting perennial crop producers to actively plant bee pastures may be prohibitive to adoption.

Wild blueberry growers and other perennial crop producers such as those in the Massachusetts cranberry industry may favor no-till establishment of native bee pastures. Unlike growers raising row crops (i.e. squash), wild blueberry farmers along with other perennial crop producers do not usually have the tillage and seeding equipment necessary for tilled pasture strips which may impede the adoption of this particular pollination enhancement strategy. However these growers typically have sprayers and Roundup® that could be used for no-till bee pasture establishment. Diversified wild blueberry farmers that also grow vegetables have tillage equipment so tilled establishment of bee pasture strips may be more suitable for these types of growers. For all growers, bee pasture strip establishment assumes land availability.

Larger farms with more acreage would have such land base needed for natural strips with presumably lower floral density, while smaller farms may need to use actively established strips to get enough forage resources for native pollinators. There is another more recent challenge to alternative pollination that has emerged. Unlike cranberries, wild blueberries are a soft-skinned small fruit that is susceptible to spotted wing drosophila (SWD), an invasive pest species that has become pervasively present in Maine and eastern Canada. Any type of insecticide used for pre-harvest control of SWD has negative impacts on bees (managed or native), which makes the pollination alternatives discussed more challenging to adopt. However, if growers can plant bee pastures in concentrated "refuge" areas as well as continue to time sprays when bees are not active, non-target impacts on local bees can be reduced. The wild blueberry industry has historically reduced pesticide applications to control blueberry maggot fly by timing sprays, using softer insecticides, and converting fields to a single cropping cycle (rather than a split field). Although SWD being a generalist pest rather than a specialist (maggot fly) limits the effectiveness of single cropping cycles, other integrated pest management strategies developed by growers, processors, and researchers can insure that pollination alternatives maintain their effectiveness while protecting wild blueberries from unacceptable losses from SWD.

### *Study 5. Abundance and diversity of native bees in relation to farm management*
### *Report from Dr. Sara Bushmann and Dr. Frank Drummond.*

The purpose of this project is to determine if farm management practices are related to the composition of wild, native bee communities found in and around blueberry fields and to determine if those bees contribute to crop yield. The study began in 2010 and ran for three consecutive years. This report provides results not previously published in the Wild Blueberry Reports.

**METHODS:** The research took place in 40 blueberry fields located in Hancock and Washington Counties. Management practices ranged from conventional with differing levels of inputs to organic. The fields differed with respect to the surrounding landscape and level of isolation from other agricultural fields. The fields were all less than 40 acres and so, this study represents "small" blueberry field dynamics of pollination. From the beginning of May until the end of June, wild native bees were collected from each field using both active and passive capture in soapy water traps. The active capture involved timed "bee hunts" within patches of flowers. The common name of the flower each bee was caught on was recorded. When enough pollen was present, the pollen was removed from the bee body and analyzed for the percent of blueberry content. The soapy water traps were 3.5 oz plastic cups painted either florescent yellow or blue or left an opaque white. The cup traps were not expected to catch bumblebees and honey bees in numbers that reflect their density in the field. Abundance of these two kinds of bees, therefore, was estimated through timed counting periods.

The following data were recorded for each field: 1) field size, 2) fruit set, 3) soil particle size (percent sand, silt, clay), 4) percent non-blueberry forage (flowers) in the fields during the months of May and June, 5) the abundance of honey bees, bumblebees, and other wild bees foraging during blueberry bloom, 6) the proportion of land surrounding the field within 500 meters (about 547 yards) of the field edge that is other blueberry growing land, 7) the proportion of land surrounding the field within 500 m of the field edge that is clear cut forest, 8) the proportion of land surrounding the field within 500 m of the field edge that is deciduous forest, and 9) the pesticide and pruning history of the previous crop cycle.

**RESULTS:** Neither field size, soil particle size, percent non-blueberry forage (flowering weeds in the field), pesticide (herbicides, insecticides, and fungicides) use, nor pruning method related to the abundance of bumblebees or other wild bees. The year of the study did relate to wild bee abundance per soapy water cup, wild bees per minute of hand catching, or bumblebees per minute of counting (Fig. 1). For all measures, the wild bee abundance was more similar in the first two years of the study than in the third. The abundance of bumblebees dropped precipitously in 2012. This was not unexpected since previously we have noted that wild bee populations fluctuate from year to year. This study only corroborates the fact that if one is depending "entirely" on native bees for pollination, large fluctuations in yield will result over time.

The abundance of bumblebees and all other wild bees was negatively related to the percent of blueberry land surrounding the study blueberry field. This means that fields in areas where large areas of blueberry are managed tend to have fewer bees than isolated fields. The abundance of wild bees was positively related to the proportion of clear-cut or deciduous forest surrounding the study blueberry field. This reflects the positive role that cleared or hardwood forests have on bee populations by providing them extra nectar and pollen outside of blueberry bloom.

The abundance of both honey bees and bumblebees determined the amount of fruit set and the abundance of honey bees and total wild bees also directly determined yield. The impact of bumblebees and total wild bees; however, was greater than the impact of honey bee abundance, on a per bee basis.

Five species of wild bees, all of the genus *Andrena*, carried enough pollen to determine the percent of blueberry pollen carried by the bee (Table 1). These five species were widespread and commonly caught in the fields. On average *A. carlini* represented about 12% of all bees

caught, *A. rufosignata* represented 4.25%, *A. vicina* represented 3.3%, *A. bradleyi* represented 3%, and *A. carolina* represented 2.3%.

**Fig. 1**. Three bee abundance measures by year for 40 fields; 2010-2012. Within each abundance measure, the years marked with a letter indicate values more similar to each other than the unmarked year. The measures from 2010 and 2011 are significantly different from 2012 for every measure. n = 40 fields (12 different fields each in 2010 and 2011, 16 fields in 2012).

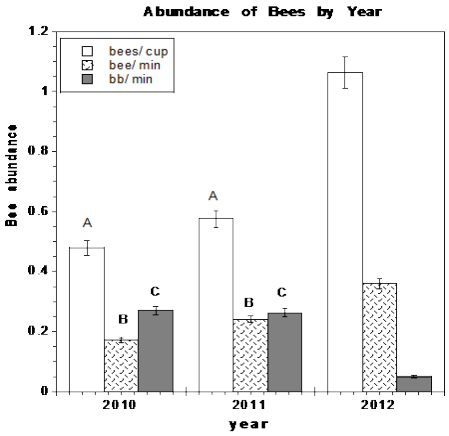



**Table 1.** The mean percent of ericaceous pollen carried by five species of *Andrena*. The same letter designates means that are not significantly different. All individuals (n = 101) caught while foraging on blueberry (*Vaccinium angustifolium*) flowers.

| Species | | Sample size | Mean % ericaceous pollen (standard deviation) |
|---|---|---|---|
| *A. carolina* | a | (n = 13) | 99.23 (1.96) |
| *A. bradleyi* | a | (n = 16) | 98.75 (1.98) |
| *A. rufosignata* | ab | (n = 13) | 89.03 (13.76) |
| *A. vicina* | bc | (n = 19) | 74.64 (33.11) |
| *A. carlini* | c | (n = 40) | 67.58 (38.07) |

ANOVA: $F_{(4,96)} = 5.541$, $P = 0.0005$

**CONCLUSIONS:** Farm management (only in terms of pesticide use and pruning method applied during the growing cycle) did not affect wild bee abundances in blueberry fields during the months of May and June. This study did not look at possible direct effects of summer applications of insecticides that are commonly applied for blueberry maggot fly or spotted wing drosophila control. Therefore, this study cannot provide information about if or how bee species that emerge in the summer months beyond blueberry bloom are affected by pesticide use.

Field characteristics such as soil particle size, field size and the percent of non-blueberry forage within the field did not relate to bee abundance. However, the proportion of the landscape

surrounding the blueberry field that was other blueberry land was negatively related to total wild bee abundance (all wild bees and bumblebees). The largest field for this study was about 40 acres. The results of this study support a prediction that increasing field size is associated with decreasing wild bee populations.

Given that wild bee abundance was positively related to the proportions of clear cut forest and deciduous forest surrounding the field, this study also suggests these landcover types provide habitat for wild bees. The landcover data supplied by Shannon Chapin of the University of Maine Wildlife Department is based on data from a 2004 landcover assessment, which is over 10 years prior to this study. Future forest research should monitor forest regrowth in order to track habitat generation for pollinators.

The pollen analysis suggests that bees of the genus *Andrena* have an affinity for blueberry flowers and may be significant pollinators of the crop. Several species are quite commonly found foraging on blueberry flowers. This study provides direct evidence of the importance of bumblebee abundance on fruit set and wild bee abundance on crop yield. This suggests that wild bee conservation is important for those growers relying primarily on wild bees for pollination.

### *Study 6. **The health of native bumblebees in blueberry fields in Downeast Maine and the effects of dietary imidacloprid on managed B. impatiens colonies. 2013***
### ***Report from Kalyn Bickerman (Ph.D. student) and Dr. Frank Drummond.***

**METHODS:**

*Native bumblebee health*

From 14 Jun 2013 to 2 Oct 2013, a total of 19 field sites were visited intermittently throughout the season in Downeast Maine. Two or three researchers spent 20 minutes at each site as a measure of sample effort and collected as many bumblebees that were not obvious queens as possible at each site of any species. Researchers split up at field sites to minimize the possibility that collected bumblebees were all from the same colony. Specimens were marked with the date, field site, and the common name of the flower on which they were collected (if known) then brought back to the lab and placed in a -20º C freezer to freeze-kill. Each bee was identified to the species level.

Specimens will be dissected to assess macroparasite presence or absence (conopid fly larvae) and their ages will be estimated using a four-point scale (0-3) based on wing wear and their intertegular spans will be measured as a proxy for individual size. Gut contents will be removed for examination under a phase contrast microscope and remaining body parts will stored in the -80ºC freezer. Five minutes will be spent on each slide of gut tissue to determine presence or absence of any pathogenic organism. Specimens were considered to be positive if two or more pathogenic spores were seen of *Nosema bombi*.

*The effect of dietary imidacloprid on the health and colony development of commercial B.impatiens*

40 small (~30 individuals per colony) colonies were ordered from Koppert Biological Systems (Romulus, MI) and delivered to Maine on 9 May 2013. The colonies were divided into six groups of six (with four extra) and each group was given a range of imidacloprid treatments added into their only food source. The doses ranged from the control (0 ppb) up to 125 ppb of added imidacloprid in the form of Admire Pro®. The bees were allowed to feed on the food for two weeks in the lab and colonies and their food bags were weighed daily to monitor growth and track food consumption. After this two-week period, each group was placed into one of six blueberry fields, around Waldo and Hancock Counties. These fields had management practices that ranged from small and organic (two fields), medium input (one field), and high input (three fields). Colonies were then weighed once a week and foraging workers were captured upon their return to their colonies in order to analyze the pollen they carried to ascertain floral resources. Counts of foraging workers entering and exiting each colony were also made in five minute intervals to estimate colony activity.

Colonies were collected on 10 July 2013 and frozen the same day. Final counts of workers, drones, and queens along with estimated of brood area were made in the following weeks. 50 individuals from each colony were chosen at random and the intertegular widths measured to estimate average worker size of each colony. Workers are currently being dissected in the same manner as the wild caught bees to look for conopid parasitism and *Nosema* infection. Immune analysis will also be performed to estimate immune strength of each colony, along with PCR identification of *Nosema* infection.

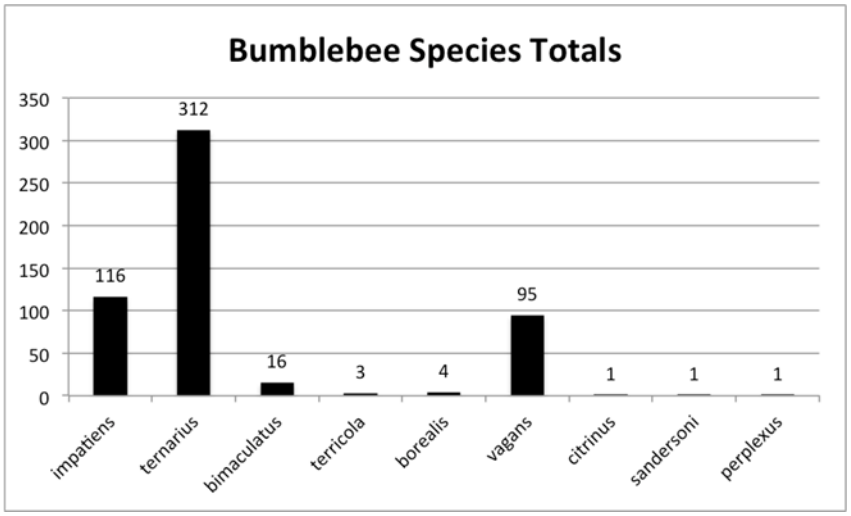
**RESULTS/CONCLUSIONS:**

*Native bumblebee health*

Statistical tests have not been performed at this stage but the breakdown of the species of the 549 individuals caught can be seen in figure 1. Of those caught, dissections have been performed on only 55 individuals and *Nosema* analysis on five of those. Thus far, there has been one positive visual identification of *Nosema bombi* infection in a *B. terricola* individual, five *B. ternarius* with conopids, and seven *B. vagans* with conopid parasitization. Of these, one *B. ternarius* was seen to be parasitized by two larvae and one *B. vagans* was also parasitized by a mermithid nematode along with the conopid larva.

The results of this year's dissections will be compared to those in 2012 as well as to following field seasons, which will allow us to look for year effects that may be weather-related. Possible plans for research include: comparing conventional fields to organic fields and using immune response as a measure of immune strength and health in different fields. This summer, as compared to last, had increased overall bumblebee abundance; these numbers represent the relative abundances of each species in the field sites and can be tracked in future years to observe changes. All specimens have been saved for immune response analysis, as well as for molecular identification of *Nosema bombi* using PCR.

**Fig. 1.** Breakdown of number of individuals of each species captured between 14 June and 2 October 2013.




*The effect of dietary imidacloprid on the health and colony development of commercial B.impatiens*

Statistical analyses have not been performed and dissections still have yet to be done, but there is preliminary data related to the average weight (Fig. 2), average colony size (Fig. 3) and average worker size (Fig. 4) of each colony based on imidacloprid dosage and date. The preliminary measurements demonstrate that there appears to be an effect of imidacloprid treatment on colony weight, colony size, and individual bee size by the end of the season. An analysis of least squares shows that there is no significant correlation between field site and colony size, but there is between treatment and colony size ($P = 0.0023$). This relationship was significant in both a linear ($P < 0.0001$) and curvilinear ($P < 0.0001$) treatment. However, there was no statistically significant correlation between field site placement or treatment group for the size of individuals in each colony. There did appear to be a slightly significant ($P = 0.0456$) linear, as well as curvilinear relationship ($P = 0.049$), between treatment group and individual size. Finally, there is a significant effect between colony weight at the end of the season and treatment group ($P < 0.0001$). This appears to be a linear ($P < 0.0001$) and curvilinear ($P < 0.0001$) relationship.

Future directions include: examining whether there is a field effect with these results; evaluating the possibility that treatment level affects susceptibility to parasites and pathogens through dissection and molecular identification of *N. bombi*; and analyzing the immune strength of individuals from each treatment to determine if their immunological systems are altered by imidacloprid exposure. This experiment will be continued next summer with the main difference being that the experiment will continue later into the season to ensure that the colonies reach their senescence points where queens and males are produced so that reproductive production may be evaluated in these treatments.

**Fig. 2.** Average colony weight of each dosage (ppb of imidacloprid) group through the season. There was a significant correlation between treatment group and colony weight at the end of the season ($P < 0.0001$).

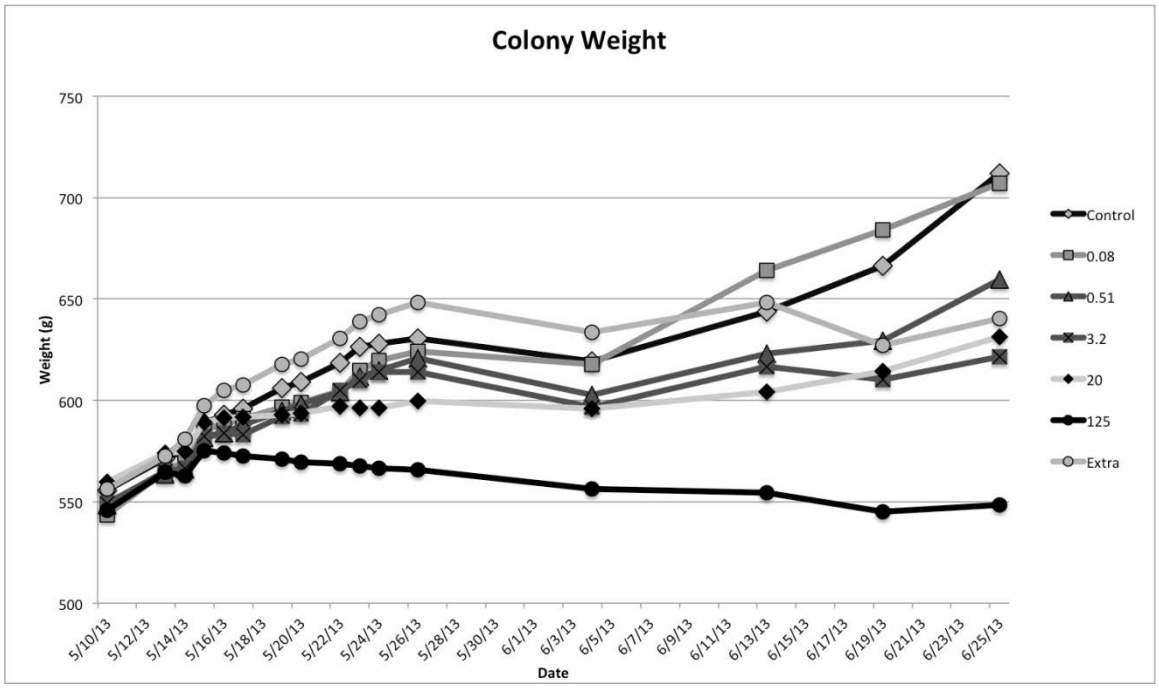



**Fig. 3.** Average colony size in number of workers of each dosage (ppb) group at the end of the season. Treatment group was the determining factor for final colony size ($P = 0.0023$).

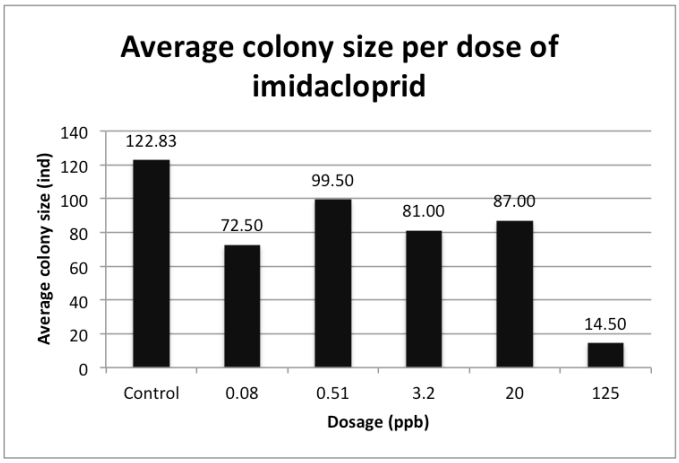
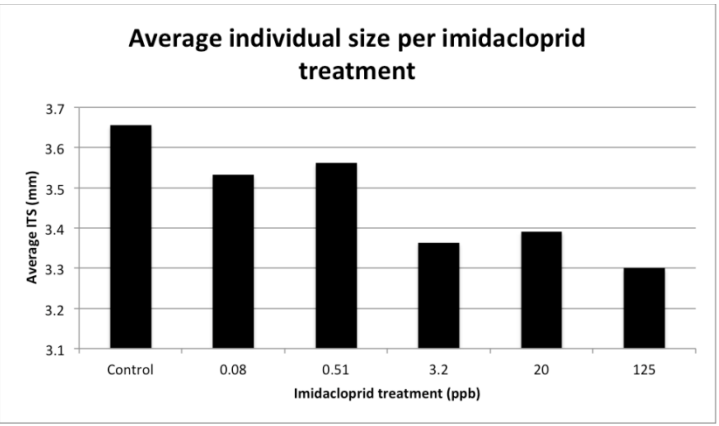


**Fig. 4.** The average intertegular space (mm) of individuals in each treatment group (not including the queens) at the end of the season. There was no significant correlation between field site or treatment group for individual size, although there was a significant linear ($P = 0.0456$) and curvilinear ($P = 0.049$) relationship.




***Study 7.*** ***<u>What do bees want? Testing bee preferences for flowers at four Maine sites in 2012-2013</u>***

***<u>Report from Dr. Alison Dibble (Research Scientist SBE), Dr. Frank Drummond, and Dr. Lois Berg Stack (Professor Univ. Maine Coop Extension),</u>***

Bees depend upon flowers for their food. To help expand pollinator security in wild blueberry agriculture, we are testing plants that could be used to support bees in pollinator plantings. Bee plants recommended from other parts of the U.S. may be suitable here, but more data are needed regarding bee plants for Maine farms. In the first two years of a five year study, we tested bee flower choice with the goal of improving plantings that attract native bees and honey bees, provide a succession of flowers through the entire growing season, and offer nectar and/or pollen to support pollinators when few plants are in flower around field edges.

**METHODS:** The design of the Bee Module experiment allows us to switch plant subjects in and out of four gardens each year. Two University of Maine-owned farms -- Rogers Farm in Old Town, Blueberry Hill Farm in Jonesboro -- and two privately owned blueberry farms in Blue Hill are the study sites, each either organic or with low inputs. We are testing more than 60 species (Table 1) of native and introduced wildflowers, shrubs, bedding plants, cover crops, and herbs, each within a 1 m sq patch separated by 1 m wide landscape fabric-covered walkways. Plantings are mostly replicated across all four gardens. Plants were selected from lists of recommended pollinator plantings, requests by growers, and our observations. Among bedding plants, we compare wild types to related fancy cultivars. We prioritize season-extender resources for pollinators, and de-emphasize plants that might become weedy, or that flower at the same time as wild blueberry. During good weather, observers count insects that land on flowers during three one-minute periods per plant, note their presence, but do not capture the bees. Bees are recognized as *Apis* (honey bee), *Bombus* (all bumblebees except orange-banded), *B. ternarius* (orange-banded bumblebee, Fig. 1a), Halictid (sweat bees), and Other Bee (all others).

**Table 1.** Plant subjects by common name, and number of minutes of bee observations per year over all sites. Total for 2012 is 2404 minutes and for 2013 is 1841.

| Common name | 2012 | 2013 |
|---|---|---|
| Alyssum Snow Flake | 93 | 0 |
| Bebb's willow | 0 | 9 |
| Blanketflower Sundance Bicolor | 0 | 74 |
| Blanketflower wild type | 0 | 71 |
| Blue cowslip | 0 | 11 |
| Borage-blue | 98 | 0 |
| Borage-white | 119 | 0 |
| Buckwheat | 96 | 0 |
| Butter-and-eggs | 0 | 65 |
| Butterfly milkweed | 45 | 63 |
| Calendula Dark Orange | 52 | 0 |
| Catmint | 101 | 62 |
| Coneflower | 15 | 73 |
| Coreopsis Roulette | 0 | 55 |
| Coreopsis wild type | 0 | 89 |
| Cosmos Double Click | 68 | 0 |
| Cosmos Mix | 37 | 0 |
| Cosmos Seashells | 86 | 0 |
| Cosmos Sensation | 31 | 0 |
| Crimson clover | 80 | 0 |
| Giant Hyssop | 111 | 0 |
| Hairy vetch | 32 | 0 |
| Japanese willow | 0 | 5 |
| Lavender | 18 | 0 |
| Lingonberry cultivars | 0 | 39 |
| Marigold Disco | 113 | 104 |
| Marigold Inca Yellow | 0 | 96 |
| Marigold wild type | 0 | 20 |
| Marigold Bonanza | 108 | 0 |
| Meadowsweet | 90 | 36 |
| Mealy sage-purple | 97 | 0 |
| Mealy sage-white | 103 | 0 |
| Mountain cranberry | 0 | 9 |
| Mustard-Ida | 88 | 15 |
| Mustard-Pacific | 124 | 15 |
| N. Bush honeysuckle | 16 | 51 |
| NEST2 nest habitat | 0 | 101 |
| Oregano Greek | 90 | 26 |
| Oregano Red | 0 | 36 |
| Pasture rose | 3 | 38 |
| Pink snapdragon | 0 | 69 |
| Platycodon blue | 0 | 114 |
| Platycodon white | 0 | 102 |
| Poppy Calif | 126 | 0 |
| Poppy corn red | 20 | 0 |
| Poppy Iceland | 65 | 67 |
| Poppy Iceland wild | 0 | 105 |
| Purple raspberry | 41 | 63 |
| Regent serviceberry | 0 | 3 |
| Snapdragon Fantasy Yellow | 0 | 67 |
| Summersweet Hummingbird | 45 | 21 |
| Sunflower Little Becka | 34 | 0 |
| Sunflower Teddy Bear | 27 | 0 |
| Sunflower wild type | 0 | 21 |
| Sunflower Zebulon | 25 | 18 |
| White wood aster | 106 | 15 |
| Winter Vetch | 1 | 0 |
| Yellow sweet clover | 0 | 13 |

***Data analyses***. More than 9300 observations of all insects for 2012-2013 are newly available for analysis. Sampling effort by plant subject and year is shown in Table 1. Some plants were much more intensively sampled than others because they have a long bloom season and flowered in both years, while others flowered quite early, only briefly and/or were included in only one of the years. Column graphs of average bees per minute (per plant subject, per farm, on a given day) reveal where bee activity was greatest.

**RESULTS:** We sampled insects on flowers for at least 2404 minutes in 2012 and 1841 minutes in 2013. We observed 3092 bees, of which *Bombus* was most abundant (Table 2), and many other kinds of insects (not reported here).

**Table 2.** Bees observed over two years in the Bee Module experiment at four sites. In addition, 18 sightings of the European wool carder bee were noted at two sites in 2012.

| Year | Total bees | *Apis* | *Bombus* | *B. ternarius* | Halictid | Other Bee |
|---|---|---|---|---|---|---|
| 2012 | 1681 | 499 | 513 | 147 | 396 | 126 |
| 2013 | 1411 | 245 | 339 | 182 | 321 | 328 |
| Totals | 3092 | 744 | 852 | 329 | 717 | 454 |

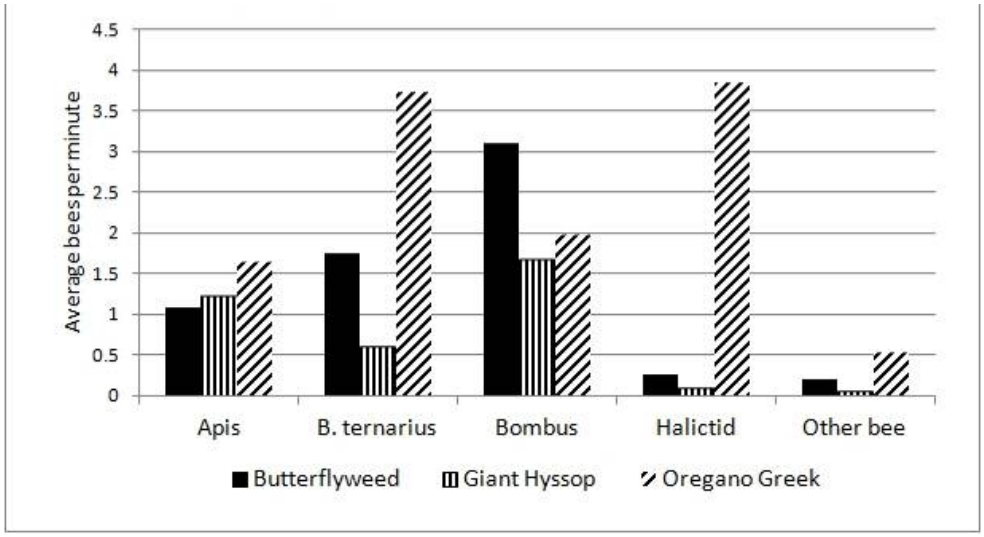
Bees were highly attracted to flowers of some plants, but not to those of others. Among our top bee plants were (1) Willow (*Salix bebbiana* at four farms and *Salix chaenomeloides* at one farm) which attracted miner bees or sand bees (*Andrena*, Fig. 1b), and bloomed early for only a few weeks when there was almost no other forage; (2) Greek oregano (*Origanum vulgare* ssp. *hirtum*) flowered both years over many weeks, with bees usually present on flowers during our observations (Fig. 2); (3) Butterfly milkweed (*Asclepias tuberosa*) attracted bumblebees at a high rate in 2013 (Fig. 2); (4) Summersweet (*Clethra alnifolia* var. 'Hummingbird') was much visited by honey bees and native bees, though inconsistently at four sites (Fig. 3). Bumblebees (*Bombus*) favored a suite of different plants than those visited by *B. ternarius*, with some overlaps.

**Fig. 1** (a) Orange-banded bumblebee (*Bombus ternarius*) on goldenrod, and (b) Miner bee (*Andrena*) on pussy willow in early April, both in Blue Hill, Maine.

**a**

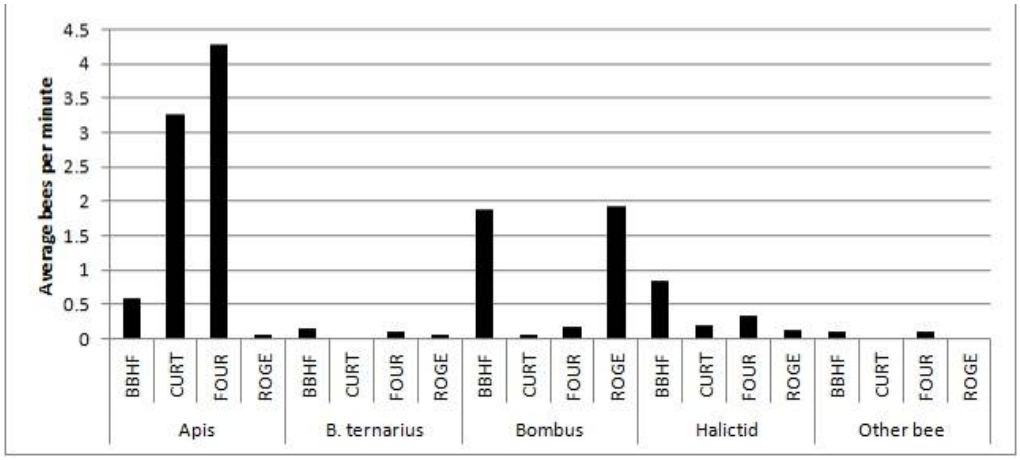
**b**

**Fig. 2.** Bar graph comparing bee activity by bee group on butterfly milkweed (2013), Greek oregano (2012-2013), and Giant hyssop (2012), over four sites.



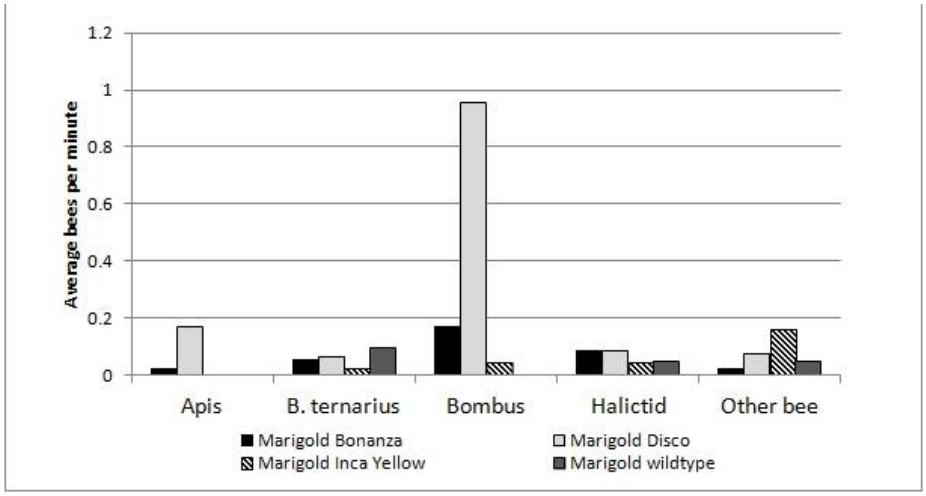

Bees were not consistent in their preferences across all farms. Summersweet is an example (Fig. 3). Other floral resources available at a given site could influence variability in bee activity on flowers within the Bee Module.

**Fig. 3**. Bar graph showing bees per minute on flowers of Summersweet cultivar Hummingbird at four farms (BBHF = Jonesboro, CURT and FOUR = Blue Hill, ROGE = Old Town.




To understand whether bees favor fancy cultivars over simpler related plants, we tested pairings of bedding plants. For example, among four marigold cultivars, the single yellow marigold Disco Yellow attracted more bees than petal-rich double cultivars (Fig. 4).

**Fig. 4.** Bee foraging rates on four marigold cultivars over four sites in 2012-2013. 'Disco Yellow' is single yellow, the others produce mostly double yellow flowers.




**CONCLUSIONS:** We found that bees came readily to flowers at all four sites in both years, perhaps due to the low- or no-insecticide environment at the farms. We identified bee forage plants that grew well in the Bee Module experiment, and must conduct further analyses to explain some of the patterns we found.

**FUTURE RESEARCH:** We do not know if bee populations will increase over time, given the additional food resources we offer. By 2015 we may be able to assess changes in bee abundance. We can address floral attributes that help explain why bees visit one plant over another regarding flower diameter, corolla tube length, flower color, detectable fragrance, plant height, and floral density. In 2014 and 2015 we expect to find more plant species that bees visit, that provide early season and late season forage for bees, and are easy to grow. We are testing an artificial ground nest environment as bee nest habitat feature. We will include these aspects in future reports.

**RECOMMENDATIONS:** At present there are no recommendations from these studies that will improve wild blueberry production practices. In the very near future, economic modeling of pollination will be undertaken. This will involve modeling both honey bee and native wild bee densities and the resulting expected fruit set and subsequent yields. This data has been collected since 1993 and includes 7 years of data from more than 96 blueberry fields. This will be a focus of 2014.

**ENTOMOLOGY**

**INVESTIGATORS:** F. A. Drummond, Professor of Insect Ecology/Entomology
J. A. Collins, Assistant Scientist of Insect Pest Management

**8. V. TITLE:** Biology of Spotted Wing Drosophila, 2013.

***Study 1.*** ***<u>Assessing the effectiveness of trapping-out on spotted wing drosophila</u>***
***<u>Report from Gabriel Alnajjar (Master's Candidate) and Dr. Frank Drummond</u>***

**ABSTRACT:** Spotted wing drosophila (*Drosophila suzukii*) is an invasive species from Asia that has become an agricultural pest in the United States. Since spreading to the northern east coast, wild blueberries have been a significant target of egg laying females. Given the increasing eagerness to adopt decreased reliance on insecticides in pest management, there is a desire to devise methods of capture before infestation with minimal impact on the environment. This study was conducted in order to provide observations on bait attraction of SWD and determine the effectiveness of the bait in deterring oviposition in wild blueberries.

**METHODS:** On 25 Jul, a 25 x 16 ft study area was set up using 12: red, 16 oz plastic cups as traps; traps were positioned on single poles in a 3 x 4 grid with approximately 5-8 feet between each trap. Bait for the traps consisted of a yeast and sugar mixture with ratios of 1 tbsp. yeast: 4 tbsp. sugar: 12 oz of water. Approximately 1½ inch of each cup was filled with the bait, and covered with a light-blocking lid. Collections occurred at 6 to 10 day intervals in which traps set from the previous week were numbered and collected. After collection, freshly baited traps were set in the grid. Gathered traps were taken back to the laboratory where male, female, and total abundance of SWD were determined and recorded. On the final collection date, 14 Sep, two samples of blueberries were gathered from random areas inside and outside the grid to determine female SWD oviposition activity in blueberries. Each blueberry sample was weighed first, then crushed in a plastic bag and placed in a salt water mixture to induce the exit of SWD larvae from the fruit. Samples were then filtered for SWD larvae and their abundance was determined and recorded.

Similar replicates of the trapping out experiment were set up at commercial blueberry farms in Jonesboro and Cherryfield, ME. However, fruit samples were not collected upon completion of these two replicates since farmers wanted to complete harvesting the fruits before any SWD larvae were found in the blueberries. Therefore, only one replicate of this study is presented.

**RESULTS:** SWD were found to be attracted to the traps throughout the study, with a higher number of individuals captured as the study progressed (Table 1). More captures were made in early – mid-September than in collections prior to that time span, with a high capture ratio of female to male SWD. Blueberry analyses showed that the collected blueberries had a weight difference of 0.8 grams, and that a higher number of SWD larvae were found in blueberries outside of the study grid; there was 1 SWD larva/7.9g blueberries inside the grid compared to 1 SWD larva/4.3g blueberries outside the grid. The results for blueberry weight and SWD larvae abundance are graphed in Figures 1 & 2, respectively.

**CONCLUSIONS:** SWD female capture in the bait was found to be higher than SWD male capture throughout the study. Given the egg laying behavior of females, this could be due to the use of yeast containing baits and therefore the occurrence of sugar fermentation, a known attractant for *Drosophila* species. Also notable is the fact that there were a larger proportion of individuals captured as the summer progressed, which can be seen in Table 1. This suggests that reproduction rates for this species increase late in the summer, from late August well into September. The study was concluded before SWD numbers began to decline. Therefore it cannot be determined when the species decreases in reproductive activity.

In figure 2, it can be seen that there were almost twice as many SWD larvae found in blueberries collected outside of the study grid in comparison to blueberries collected inside the grid. As seen in figure 1, differences in weight of blueberries collected from each area are negligible since there was only a 0.8 gram difference. Therefore, it can be concluded that the traps in this study were effective in decreasing SWD blueberry infestation.

**Table 1**. SWD abundance for males (M), females (F), and total (T).

| Trap# | 2-Aug M/F/T | 15-Aug M/F/T | 22-Aug M/F/T | 28-Aug M/F/T | 4-Sep M/F/T | 14-Sep M/F/T |
|---|---|---|---|---|---|---|
| 1 | 0/0/0 | 4/8/12 | 3/7/10 | 12/29/41 | 5/24/29 | 15/130/145 |
| 2 | 1/1/2 | 3/8/11 | 3/12/15 | 5/23/28 | 5/13/18 | 37/600/637 |
| 3 | 0/0/0 | 1/4/5 | 1/8/9 | 13/27/40 | 11/36/47 | 32/654/686 |
| 4 | 0/0/0 | 3/7/10 | 1/3/4 | 0/10/10 | 1/7/8 | 15/327/342 |
| 5 | 1/1/2 | 0/4/4 | 2/8/10 | 5/16/21 | 9/23/32 | 26/436/462 |
| 6 | 0/1/1 | 1/3/4 | 1/1/2 | 2/4/6 | 2/10/12 | 31/450/481 |
| 7 | 0/0/0 | 0/1/1 | 0/6/6 | 0/2/2 | 5/6/11 | 14/350/364 |
| 8 | 0/1/1 | 0/3/3 | 0/4/4 | 5/10/15 | 1/6/7 | 11/436/447 |
| 9 | 0/0/0 | 1/1/2 | 4/0/4 | 0/6/6 | 2/12/14 | 23/207/230 |
| 10 | 0/0/0 | 2/2/4 | 0/4/4 | 1/9/10 | 3/8/11 | 17/400/417 |
| 11 | 0/2/2 | 1/0/1 | 0/2/2 | 2/10/12 | 4/18/22 | 13/121/134 |
| 12 | 0/0/0 | 0/1/1 | 1/7/8 | 5/16/21 | 1/7/8 | 17/108/125 |

**Fig. 1.** Weight (grams) of blueberries collected from inside and outside of study grid.

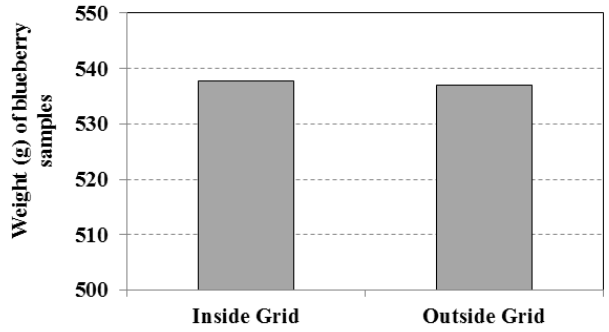



**Fig. 2.** Number of SWD larvae found infesting blueberries from samples collected on 14 Sep.

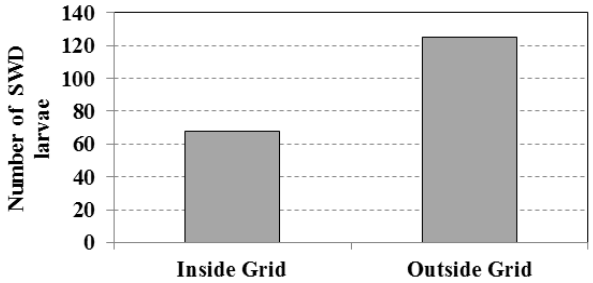
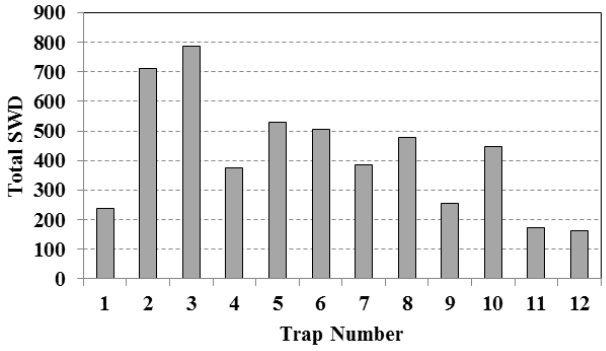



**Fig. 3.** Total number of SWD per trap captured through study.




***Study 2.*** ***<u>Exclusion netting as an alternative method for control of spotted wing drosophila</u>***

**METHODS:** An attempt was made to evaluate the effectiveness of netting as an exclusion method to prevent infestation of fruit by spotted wing drosophila. Anti-Insect Netting, (25 Mesh - 176" wide x 50’ long) was placed in a fruit-bearing wild blueberry field at Stockton Springs, ME. Trapping in the previous year had shown an adjacent field to be infested with spotted wing drosophila.

**RESULTS/CONCLUSIONS:** No results were obtained. The study area was harvested prior to the first appearance of SWD larvae in fruit samples. This study will be repeated in 2014 at Blueberry Hill Farm in Jonesboro, ME.

***Study 3. <u>Timing of oviposition by spotted wing drosophila</u>***

**METHODS:** In order to determine the preferred oviposition time of spotted wing drosophila (SWD), twenty laboratory-reared SWD adults (a mix of males and females) were placed in oviposition cages. Each cage consisted of an inverted, 8 x 9-inch, clear plastic hamster cage attached to a piece of plywood as a base. A Plexiglas® shield was attached to the plywood inside the cage to allow easier cleanup. A service hole, 6 inches in diameter, was cut in one end of the container and a cloth sleeve attached to allow access and to prevent flies from escaping. Each cage contained a diet cup with a cotton ball soaked with a solution of sugar and yeast (4 tbsp sugar / 1 tbsp yeast / 12 oz water) for moisture and nourishment.

The flies were allowed acclimate to the cages for one day and then stems with ripe blueberries were placed in the cages. The sugar/yeast solution was removed so as not to interfere with attraction of the SWD to the ripe fruit. The stems were in small glass beakers with water. The cages were placed outside in order to simulate natural conditions (lighting and temperature). The old stems were removed and new stems were placed in the cages at various times throughout the day (8 am-12 noon, 12 noon-4 pm, 4 pm-8pm and 8 pm-8 am). Old stems were held at room temperature (ca. 20$^{o}$C) in the laboratory for six days to allow development of any eggs. After six days, the fruit was removed from the stems, counted, and placed in petri dishes lined with filter paper. After an additional three days, the fruit was processed for SWD larvae using the Salt Extraction Method.

**RESULTS:** The mean number of SWD / berry was determined for each timing (4 replications [cages] per timing). Data were transformed by the square-root prior to analysis. There was a significant difference between the time periods. Most SWD oviposition activity occurred during the day; there was no significant difference among the 3 daylight periods (8 am-12 noon, 12 noon-4 pm and 4 pm-8 pm; however, all three were significantly higher than the evening (8 pm-8 am) period (ANOVA, RCB; $F_{(3,9)} = 9.57$, $P = 0.0037$)(Table 1 and Fig. 1). However, when a trend analysis was performed (single degree of freedom contrast) a continuing decreasing trend in egg laying is supported with the highest in the morning decreasing throughout the day and then being minimal throughout the evening ($F_{(1,8)} = 22.104$, $P = 0.0015$, Fig. 2).

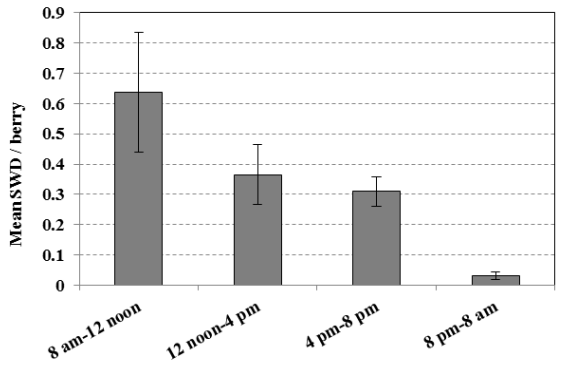
**CONCLUSIONS:** Based upon this experiment it appears that SWD is a diurnal creature, meaning that it is active during daylight hours with limited activity during the evening. However, during the daylight hours a trend exists of reduced oviposition (egg laying) throughout the morning and afternoon until reaching almost no activity in the evening. This has important implications. It suggests that the most likely way to maximize exposure of insecticides to SWD is to apply insecticides in the very early morning. This may not make that much of a difference to insecticides that have a long persistence of 5-7 days, but for those insecticides with a very short persistence (24 hrs or less), a morning vs an afternoon application might make a difference.

**Table 1.** Summary, oviposition timing of SWD.

| Timing | SWD/berry |
|---|---|
| 8 am-12 noon | 0.64 (0.20) a |
| 12 noon-4 pm | 0.36 (0.10) a |
| 4 pm- 8 pm | 0.31 (0.05) a |
| 8 pm-8 am | 0.03 (0 01) b |

Means followed by the same letter are not significantly different (LSD; $P \leq 0.05$).

**Fig. 1.** Bar graph showing mean number of SWD per berry from each of four oviposition timings.

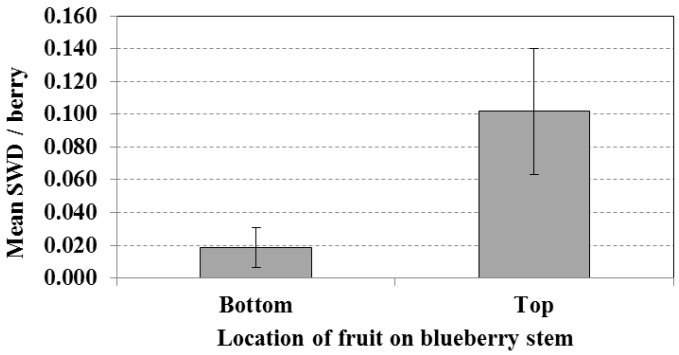



***Study 4. <u>Preferred oviposition site of spotted wing drosophila on blueberry stems</u>***

**METHODS:** On 12 Sep berries were collected from 20 stems at Blueberry Hill Farm. The fruit was in an area that adult monitoring and fruit sampling had shown to be heavily infested with SWD. For 10 stems, the fruit was taken from the unshaded top of each stem; for the 10 additional stems, fruit was collected from areas of the stems sheltered by foliage. The fruit from each stem was held in filter-paper lined petri dishes for one week and then counted and processed for SWD larvae.

**RESULTS/CONCLUSIONS:** Fruit collected from the top of 7 of 10 stems were found to be infested with SWD; infested fruit was found on the bottom of only 2 of 10 stems. The mean number of SWD per berry was 0.10 (top) and 0.02 (bottom). Despite the low number of larvae, there was a significant difference in the mean number of SWD per berry between fruit collected from the top and bottom of stems (ANOVA, $F_{(1,18)} = 5.89$, $P = 0.026$)(Fig. 1).

**Fig. 1.** Bar graph showing mean number of SWD per berry; fruit collected from top or bottom of blueberry stems (lines are standard error of the means).




It appears from these results, that fruit near the top of blueberry stems is more susceptible to damage by SWD than fruit located on the more protected and shaded lower portion of stems. Practical implications of the results of this study are that insecticide coverage may not have to be uniform. In fact, an application where only the tops of blueberry bushes receive a high dose of insecticide may perform very well in protecting the fruit. This suggests that low volume sprayers such as mist sprayers may be effective at managing SWD.

### *Study 5. Attractiveness of baits to spotted wing drosophila: a regional/national experiment*

**METHODS:** This trial was part of a regional/national spotted wing drosophila (SWD) trapping experiment designed to compare available SWD baits and/or lures using unified methodology over a range of crops, regions, seasons, and environmental conditions. Only the results for wild blueberry are presented in this report. There were six replications (blocks) of each of six baits or lures. Traps within a block were placed 30 ft apart with 100 ft between the blocks. Each trap consisted of a 32 fl. oz clear plastic cup hung ca. 2 ft above the crop canopy from a wooden post. Traps were placed along the edge of a fruit-bearing field in Stockton Springs, ME on 3 Jul and checked and baits changed weekly. At each weekly trap check, the position of the traps within a block was rotated to the adjacent position to reduce position effects. The Trece® lures included in treatments 5 and 6 are commercial lures in development in the US and were suspended from the lid over the drowning solutions, not placed in the solutions, and were changed bi-weekly. Treatment 3 (a trapping system developed in the Northeastern US) required the addition of a 4 oz specimen cup with mesh glued into the lid to exclude fly entry. Treatment 4 is a bait developed in Europe. In order to minimize potential differences in attraction due to head space, 150 ml of each liquid or drowning solution was used per trap. Data collected included – male and female SWD per trap and non-SWD drosophilids per trap.

**Table 1.** Composition of bait solutions.

1. Apple cider vinegar + unscented soap (4 ml/gal)
2. Yeast and sugar bait (2 tbsp yeast + 8 tbsp sugar + 24 fl oz water + 0.76 ml unscented soap)
3. Fermenting bait (69 g whole wheat flour + 100 ml water + 8 sugar + 4 ml apple cider vinegar + 1.3 g yeast); bait in specimen cup with mesh lid + drowning solution (apple cider vinegar + unscented soap)
4. DroskiDrink (450 ml Apple cider vinegar +150 ml red wine +12 g Muscovado sugar)
5. Trece® lure suspended over apple cider vinegar + unscented soap
6. Trece® lure suspended over drowning solution (600 ml water + 6 g Borax + 0.24 ml unscented soap)

**RESULTS:** A Repeated-Measures ANOVA with "treatment" being the between subject factor, "block" the subject factor, and "date" the within-subject factor, was used to analyze the data. Analysis of the SWD data included only the last four collection dates of the trial beginning on 7 Aug. The results are given in figures 1 (SWD) and 2 (non-SWD). For SWD trapping, a trt X date interaction was found to be significant ($F_{(15,51)} = 4.77$, $P < 0.0001$). Inspecting figure 1 it can be seen that the interaction involves apple cider vinegar being relatively attractive early (13 Aug), but less so 20 and 27 Aug. In addition, the yeast/sugar bait becomes more and more attractive compared to the other baits over time. The fermenting bait also increases in attractiveness to SWD, but not in a comparable way to the yeast/sugar bait.

**Fig. 1.** Bar graph showing mean SWD adults per treatment over each sample date.

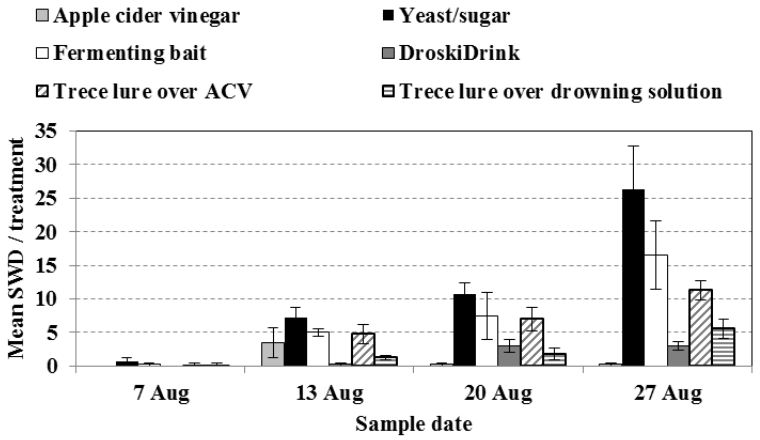
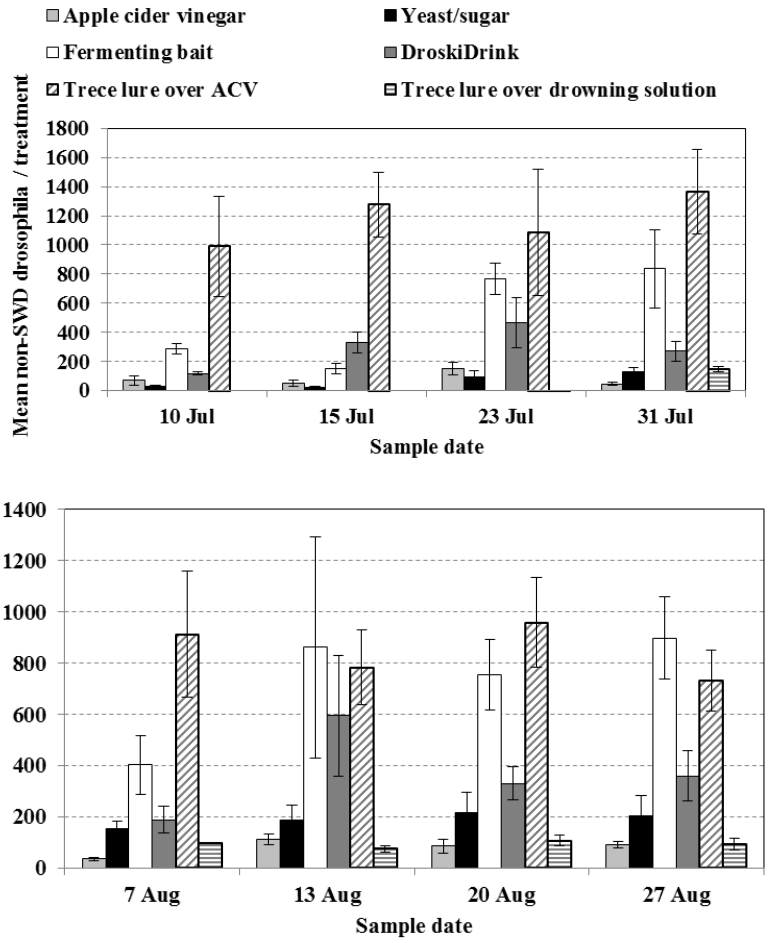


**Fig. 2.** Bar graph showing mean non-SWD adults per treatment over each sample date.




The non-SWD trap captures also demonstrated a trt X date interaction $F_{(35,112)} = 2.24$, $P = 0.0008$)(Fig. 2). This can be explained by the season long attractiveness by the Trece® lure in relation to the increasing attractiveness of the fermenting bait over the season. By far the best baits for non-SWD are the Trece® lure hung over apple cider vinegar and the fermenting bait.

**CONCLUSIONS:** This bait trial was conducted to determine if a more efficient bait could be found for monitoring SWD and at the same time to find a bait that reduced capture of non-SWD drosophila that could be confused with SWD. The bait that has already been adopted by our research lab, the yeast/sugar bait was confirmed as being the best available bait for SWD captures AND for reducing non-SWD. Our recommendation for 2014 is for growers to use the yeast/sugar bait in the red Solo® cup.

***Study 6. <u>Attractiveness of baits to spotted wing drosophila</u>***
***<u>Report from Gabriel Al-Najjar (Master's student) and Dr. Frank Drummond</u>***

Spotted wing drosophila (*Drosophila suzukii*) has become a significant pest of Maine wild blueberry. It is a native fly from Asia that lays its eggs in soft, fleshy fruits. This species was introduced to California in 2008 from where it has spread across the country and become a major concern for farmers of such crops. Due to its relatively recent development as an agricultural pest and the growing concern for environmental awareness, not much is known about the ecology of spotted wing drosophila (SWD) or potential methods of ecologically friendly control. This study was conducted to collect preliminary observations on SWD bait attraction and use these findings to determine the potential methods of capture that prevent fruit infestation and minimize ecologically invasive methods of control.

**METHODS:** This study was conducted on Blueberry Hill Farm, Jonesboro, ME. Traps consisted of red, plastic, 16-oz. cups numbered 1-36. There were three trials with 12 traps per trial; each trap had holes punched down three equally spaced lines until about two inches from the bottom of the cup. Holes were about a half centimeter in diameter. Half of the traps had a black ring painted around the rim; baits used were either water or an apple cider vinegar/ethanol solution, alone or in combination with a yeast solution to produce three different treatments with four traps per treatment. For each treatment, one black painted and one unpainted red cup was set with a yellow sticky card hung from the lid. The apple cider vinegar solution was a combination of 90% apple cider vinegar and 10% ethanol with a drop of unscented dish detergent. The yeast mixture was 355 ml of water, 16 ml of apple cider vinegar, 4 tbsp. of whole wheat flour, 4 tbsp. of white sugar, and 1 tbsp. of yeast. Traps containing the apple cider vinegar solution were filled with about an inch of the mixture. Traps containing the yeast mixture in combination with the apple cider vinegar solution had an additional, yeast-containing miniature cup within the larger red cup. These smaller cups were sealed with fine mesh tops in order to allow sufficient diffusion of the attractant while also preventing insects from crawling into the yeast mixture.

Beginning in mid-Aug the numbered traps were hung from a fence along the edge of a pruned-year field. Traps were placed in order of bait contents (i.e, all cups with water, all cups with vinegar solution only and all cups containing vinegar solution with yeast mixture). Individual cups were spaced out approximately 30 feet apart along the fence. SWD collections from cups were made at 4 to 10 day intervals for this 6 week experiment. Fresh baits were applied before resetting the cups on the fence in their original positions. All non-spotted wing arthropods were disregarded; SWD male, female and total abundances were determined and recorded. MANOVA was used to assess trap type, bait, and presence of sticky card and all of the interactions over time (weekly sampling). ANOVA was used to assess total trap capture over the season.

**RESULTS:** Throughout the experiment, there was a notable preference for traps containing both the yeast and vinegar solutions as opposed to the vinegar solution alone; SWD were not attracted to traps containing only water. The results of the six week collections can be observed in Table 1. For each week of collection, the recorded number of SWD males and females found in traps with the designated mixture were summed from all three trials. The

number of captured flies was almost always higher in the yeast containing cups, with exceptions in collections on 23 Aug and 4 Sep.

Multiple analysis of variance (MANOVA) provided evidence to suggest a bait effect ($F_{(2,22)} = 7.76$, $P = 0.003$), a time effect on SWD captures ($F_{(1.7,37.4)} = 6.154$, $P = 0.007$), and a bait X time effect ($F_{(3.4,37.4)} = 4.05$, $P = 0.019$). The interaction of bait X time suggests that as the season progressed SWD was more and more attracted to the most attractive bait, the combined ethanol, apple cider vinegar, and yeast. The analysis of variance (ANOVA) on total SWD throughout the entire season showed that bait was the only significant effect explaining trap captures ($F_{(2,28)} = 9.003$, $P = 0.001$). A Tukey's LS means HSD test provided evidence that the combined ethanol, apple cider vinegar, and yeast was significantly more attractive (43.2 SWD / trap) than water (0.02 SWD / trap) and the ethanol, apple cider vinegar (9.75 SWD / trap), which were not different from one another.

**Table 1.** SWD collection results. Males and females were counted separately. Abbreviations are as follows: VA = apple cider vinegar + ethanol solution (90%/10%); y = yeast mixture; R = red cup; Rb = red cup with black painted rim; s = yellow sticky card.

| | 16-Aug M/F/Total | 23-Aug M/F/Total | 31-Aug M/F/Total | 4-Sep M/F/Total | 14-Sep M/F/Total | 21-Sep M/F/Total |
|---|---|---|---|---|---|---|
| Bait: Water | | | | | | |
| R | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 |
| R+s | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 |
| Rb | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 |
| Rb+s | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 |
| Bait: VA | | | | | | |
| R | 0/3/3 | 0/2/2 | 4/8/12 | 12/22/34 | 9/11/20 | 55/44/99 |
| R+s | 3/3/6 | 3/1/4 | 3/5/8 | 10/26/36 | 23/44/67 | 35/42/77 |
| Rb | 2/5/7 | 1/1/2 | 1/6/7 | 14/18/32 | 6/29/35 | 50/65/115 |
| Rb+s | 0/0/0 | 1/3/4 | 0/1/1 | 12/15/27 | 10/25/35 | 34/35/69 |
| Bait:VA+y | | | | | | |
| R | 6/1/7 | 3/2/5 | 6/11/17 | 13/10/23 | 16/74/90 | 326/642/968 |
| R+s | 5/1/6 | 7/7/14 | 12/10/22 | 24/32/56 | 33/80/113 | 191/151/342 |
| Rb | 3/11/14 | 0/1/1 | 10/13/23 | 17/34/51 | 75/192/167 | 213/384/597 |
| Rb+s | 6/8/14 | 2/6/8 | 6/7/13 | 19/37/56 | 41/66/107 | 161/136/297 |

**CONCLUSIONS:** This study shows that there is a preference of SWD for yeast-containing baits over those that do not have any yeast. There is no clear preference of red cups or red and black striped cups, nor do the data suggest that the yellow sticky cards have an effect on fly capture early in the season. For future evaluations of bait preference, it could be advantageous

to deploy traps at different heights and to employ solid baits as well as liquid baits since several growers have complained about the use of liquid baits. The sticky card captures were consistent with timing of male SWD trap captures in the liquid bait portion of the combined traps. This suggests that sticky cards CAN BE USED to monitor FIRST trap capture of male SWD. This may offer growers an easier template for identification of SWD; although, sticky cards have their own problems for identification. The orientation of the fly on a sticky card is not always optimal for identification of females; males will be less of an issue regarding this constraint.

### *Study 7. Colonization of wild blueberry fields by spotted wing drosophila*

Two trials were completed in 2013 to assess movement patterns of spotted wing drosophila (SWD) in wild blueberry fields.

*Trial 1: Re-infestation of blueberry fields by spotted wing drosophila following application of insecticides.*

**METHODS:** Delegate 30WG (6 oz/acre) was applied on 15 Aug to a ca. 6 acre, fruit-bearing field at Blueberry Hill Farm, Jonesboro, ME. The material was applied with A CIMA® P55D Atomizer L.V. sprayer in 20 gallons of water per acre. Following the application SWD monitoring traps were placed 20 ft apart in a 200 x 200 ft grid. The grid was set so that one edge was along the field border and ca. 20 ft from the edge of the woods. There was a minimum of 200 ft from the trial area to the other three field boundaries. A dirt field road surrounded the area. Experimental design is shown in figure 1. Traps were constructed from Solo®, 16 fl. oz, red polystyrene cups with clear lids. Seven to 10, 3/16-inch holes were punched on the side of each container near the top, evenly spaced around the rim. Bait consisted of live yeast (1 tbsp) + sugar (4 tbsp) + 12 oz water (makes enough for 4 traps). The traps were hung 1-2 ft above the top of the canopy using 36" plant stands. Each trap was numbered, and the traps were checked daily for four days. Any SWD adults were counted and removed.

**RESULTS:** Daily trap captures are shown in figure 2. It can be seen that adult SWD moved into the field very quickly following the application of Delegate. The area treated with insecticide was approximately 6.0 acres. The trapped area was in the edge of the treated area of approximately 1 acre (200 x 200 ft). Therefore, if most trap captures resulted from flies moving into the treated area from outside the 6.0 acre field, then we demonstrate that colonization can occur at a very fast linear rate.

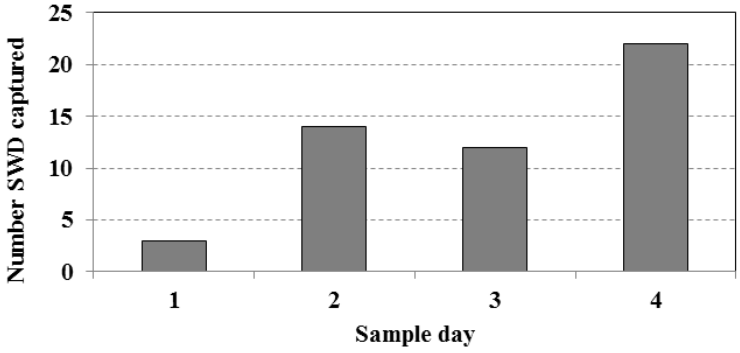
**Fig. 1.** Design of experiment showing location of traps within the field.

Woods

Dirt road surrounding field; ca 20 ft between woods edge and first row of traps; 20 ft between traps

| | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 20 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| 40 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 |
| 60 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 |
| 80 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 |
| 100 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 |
| 120 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 |
| 140 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 |
| 160 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 |
| 180 | 100 | 101 | 102 | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 |
| 200 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 | 121 |

**Fig. 2.** Summary of SWD captures; total SWD captured per day



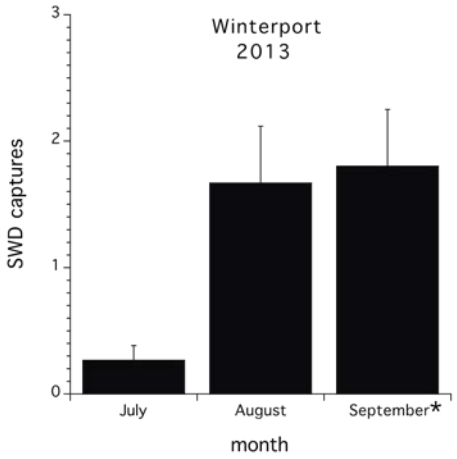

*Trial 2: <u>Movement of spotted wing drosophila into wild blueberry fields.</u>*

**METHODS:** Traps were placed in transects running into each of two fruit-bearing wild blueberry fields, one in Winterport and one in Jonesboro. There were three transects at each site. For each transect, one trap was placed at the field edge; additional traps were plated at 30, 50, 100, and 200 ft. Traps consisted of SOLO®, 16 fl. oz, red polystyrene cups with light-blocking lids. Seven to 10, 3/16-inch holes were punched on the side of each container near the top, evenly spaced around the rim. The traps were baited with Apple cider vinegar and hung 1-2 ft above the top of the blueberry canopy using 36" plant stands. Each trap was numbered, and the traps were checked daily periodically. Any SWD adults were counted and removed.

**RESULTS:** Multiple Analysis of Variance (MANOVA) provides evidence of a slow buildup of SWD in the transect/trap study in Winterport during the field season ($F_{(2,12)}$ = 5.072, $P$ = 0.025). Figure 3 depicts this buildup. However, there was no distance ($P$ = 0.859) or distance

x month interaction ($P$ = 0.542) suggesting that SWD does not diffuse into blueberry field interiors from the edge (see Fig. 1). In Jonesboro, the transect/trap study that was set up on 22 July only yielded 3 SWD captures by the time the study was dismantled due to harvest. Therefore, there was not sufficient data to determine if SWD colonizes fields from the edges.

**Fig. 3.** Seasonal captures of spotted wing drosophila (* denotes that captures for September are actually from September and the first two weeks of October).




**CONCLUSIONS:** The results of Trial 1 (Re-infestation following insecticide application) and Trial 2 (Transect Study) both suggest that perimeter field treatments of insecticide may not be an effective means of suppressing infestation of fruit by SWD. However, mark recapture studies need to be performed next year to verify the movement rates of adults in commercial blueberry fields.

***Study 8. <u>Collection of drosophila parasitoids. Report from Elissa Ballman</u>.***

**METHODS:**

*<u>Collected parasitoids from field efficacy trial</u>*

During a field efficacy trial at Blueberry Hill Farm, three, 2/3 cup samples of blueberries were collected from each of 32 plots. Fruit were collected on 20 Sep and placed into quart sized Ziploc® bags. The samples were held at room temperature for four days before inspection. During inspection for SWD larvae on 24 Sep, a single sample from plot "10" (plot treated with Delegate 30WG) contained parasitic wasps. The parasitoids were aspirated from the bag and placed into a vial with a 1:1 honey/water mixture on a cotton plug for 24 hours. Seven parasitoids were placed into a vial with an established SWD population, three parasitoids were placed in a vial that contained five SWD larvae of varying ages, and seven parasitoids were placed into a vial with 25 SWD pupae. Parasitoids were observed with the larvae and pupae for 20 min to try to confirm oviposition.

*<u>Fruit collection</u>*

One and a half gallons of blueberries were collected from Blueberry Hill Farm in Jonesboro, ME. Fruit was primarily wild blueberries; although, some highbush blueberries

were also collected. Fruit was collected from plants as well as fallen fruit from the ground. Fruit was brought back to the lab and placed into plastic dishes with a thin layer of sand on the bottom. These dishes were held in sleeve cages at room temperature and monitored daily for parasitoid emergence.

*Compost collection*

Fruit traps made from lidded plastic containers (16 x 11.5 x 7.5 cm) with coarse screening on the sides were filled with an assortment of fruit including bananas, melon, apple, and mango. Two fruit traps were set out by each compost pile in Orono, Argyle, Winterport, and Greenbush, ME on 27 Sep. At the same time and sites, two 50 ml centrifuge tubes containing Drosophila media were also set out by the compost piles. Both traps were left out until Drosophila pupae were observed, roughly three weeks. On 17 Oct, two 50 ml centrifuge tubes containing Drosophila colonies that had a mixture of larvae and pupae were set out by each compost pile in Argyle, Winterport, and Greenbush, ME. On this same date, four round plastic containers (9 x 4 cm) filled with sand and SWD larvae and pupae were set out in Greenbush. The colony tubes and larvae and pupae containers from Argyle and Greenbush were brought back one week later, and the colony tubes from Winterport were brought back two weeks later. All containers were placed into transparent sleeve cages and held at room temperature for one month and checked daily for parasitoid emergence.

**RESULTS:**

*Collected parasitoids from field efficacy trial*

The parasitoids were keyed to the family Pteromalidae using the Chalcidoidea key in Hymenoptera of the World: An Identification Guide to Families. No oviposition was noted during the 20 min observation period. Parasitoids lived for several days in the vials with larvae and pupae; whereas, the parasitoids placed into the SWD colony tube died within the first day due to the sticky walls of the vials. No parasitoids emerged from any of the SWD life stages.

*Fruit collection*

Many of the fruit samples had very high numbers of SWD while others had no fly emergence, which is probably indicative of the fragmented distribution of this fly in the field. No parasitoids were seen in any of the fruit samples.

*Compost collection*

Although a large number of various Drosophila species emerged from the fruit traps and colony tubes, no parasitoids were observed. The plain Drosophila media tubes proved much less attractive to ovipositing flies in the field compared to the fruit traps as very few larvae or pupae were seen in these tubes.

**CONCLUSIONS:** Fruit collections resulted in large numbers of SWD. In order to have better chances of locating associated parasitoids, fruit samples should be taken more often during the late summer. A weekly collection from multiple spots in the field would increase the chance of locating any parasitoids. The fruit traps were much more successful at recruiting Drosophila than the media tubes, but no parasitoids were found associated with these traps. Weekly trap

deployments would again increase the chance of capturing Drosophila parasitoids. Deploying traps earlier in the season may also increase the likelihood of catching parasitoids.

We do not have conclusive evidence to determine whether or not the parasitoids collected from fruit harvested from the field efficacy trial were attacking SWD in the field. They did not attack SWD in the lab, but this could be because we used artificial media instead of fruit which may interfere with their host finding abilities. It is also possible these wasps were utilizing a different host in the field. The wasps are being identified to species by Gary Gibson of the Canadian National Collection of Insects.

### *Study 9. Mark-recapture optimum dye for mark/recapture of spotted wing drosophila Report from Elissa Ballman.*

The goal of this project is to determine the best type of dye to mark spotted wing drosophila so that they can be released into the field, and recaptured. This information will tell us how and where they are moving in a field. To determine fly movement in a field, the dye must be highly visible, long lasting, and not cause significant mortality or alter behavior. In this study we are testing three colors of external dye, and three colors of internal dye for retention and mortality.

**METHODS:**

*External Dye Trial*

The dyes used to mark adult and pupae of spotted wing drosophila were Day-Glo® Orange, Blue, and Red. The walls of a 50 mL plastic tube were coated with a very thin layer of each dye type. Small amounts of sand were added to each color tube and gently shaken to remove excess dye clinging to the walls. A control was set up by coating the tube with undyed sand. Forty adult flies between one and six days old were added to each colored dye tube and left for two hours. After the two hours, six flies of each color were set up in tubes with standard drosophila media. This was replicated six times per treatment color. Every other day for eleven days the six flies from a replicate of each dye color were checked for mortality, dye retention, and the sex of the flies. The flies were examined under ultraviolet light for the presence of fluorescent dye.

The experiment was repeated with the same dye colors, only pupae instead of adults were exposed to the dye. A dye-sand mixture was created by mixing 40 parts sand to one part dye by weight; 1.5g of this sand was added to a single vial per dye color. 74 pupae were added to each dyed sand vial including a control that was only sand and no dye. All pupae were examined under a microscope to verify the presence of a developing fly. The cotton tube plug was dipped in water to increase humidity inside the tubes. The vials were checked daily for adult emergence and where adults were found, they were moved to a new 50 mL tube with standard drosophila media. The vials were checked daily for two weeks. Flies were checked every other day post emergence for dye retention and morality. Both living and dead flies were crushed on filter paper with a blunt probe dipped into acetone. The crushed flies were examined under ultraviolet light for the presence of fluorescent dye.

*Internal Dye Trial*

We attempted to dye flies internally by feeding the flies three different dyes: Sudan Black B, Rhodamine B, and Fluorescein. Each dye was fed at three concentrations of 2, 3, and 4 g/L. The dyes were dissolved in water (Sudan Black was dissolved in a small amount of oil first) and half an ounce of the dye mixture was added to the same amount of standard drosophila media in 50 mL tubes. A control was also set up with undyed standard drosophila media. 36 flies between one and six days old were added to each dye tube and left for 24 hours. After this time period, the flies from each dye tube were divided into six 50 mL tubes with standard drosophila media. A single tube from each dye concentration was checked every other day for dye retention, sex of the fly, and mortality. Dye retention was verified by crushing the flies on filter paper with a blunt probe dipped in acetone. The presence of Sudan Black was verified by inspecting the crushed fly under magnification with ambient light, while the other dyes were examined under ultraviolet light.

**RESULTS:** Results from this trial are still being analyzed.

**RECOMMENDATIONS:** Only two years have elapsed since the invasion of the spotted wing drosophila into Maine. The year 2012 resulted in an estimated 20% crop loss due to this pest. This pest was quite delayed in its phenology in 2013 and with coinciding insecticide applications and early harvests, most growers escaped any losses from SWD. At this point the recommendations are for growers to be vigilant and monitor for SWD adults in the summer. Upon the first capture of a male, we recommend a weekly (4-7 days depending upon the persistence of the insecticide) application of insecticides until harvest to protect the crop. Future research will focus on developing a more sustainable and economic IPM program for this pest's management.

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**DISEASE MANAGEMENT**

**INVESTIGATORS:** Seanna Annis, Assoc. Professor, School of Biology and Ecology
Caleb Slemmons, Blueberry Disease Research Assistant, School of Biology and Ecology

**9. TITLE:** Maine Wild Blueberry –mummy berry research and extension.

**OBJECTIVE:** Deploy and operate a fully operational disease forecasting system for mummy berry disease.

**METHODS:** In early April 2013, the 11 weather stations were deployed in blueberry growers' fields around Maine from West Rockport in Knox County to Meddybemps in northern Washington County (see Fig. 1). Stations consisted of Watchdog® data loggers and cellular telemetry allowing remote monitoring of air and soil temperature, soil moisture and leaf wetness at 15 minute intervals. In addition, relative humidity was monitored at the sites but these data were collected in monthly downloads. The station located at Blueberry Hill Research Farm was a Davis weather station that was configured with the same sensors but access was through a different website. In September and October 2013, we put out new mummy berry plots for the next season in some grower fields and retrieved the weather stations for winter

storage. We also sent out information to growers on how to put out their own mummy berry plots for next year.

**RESULTS:** In 2013, the weather stations performed without problems, except for one station where minor adjustments had to be made to get a consistent cellular signal. Data from the stations were used for the mummy berry forecast and weather data was collected throughout the season until mid September to mid October. These data are also being used to determine if bloom and Botrytis blight forecast models from Nova Scotia and insect emergence models for Spotted Wing Drosophila and Blueberry Maggot Fly were suitable for Maine weather conditions. Eight out of the 11 stations had mummy berry plots, but at two of those sites the mummy berries did not successfully germinate. We had numerous growers and members of the Blueberry Hill Research Farm who monitored mummy berry plots twice a week during the disease period. Throughout the disease risk season from mid-April to mid-May, we were able to provide multiple forecast reports on mummy berry disease, as well as, the occurrence of frost for most of the blueberry growing areas. In May and June, we were able to provide some information on Botrytis blight risk to the growers. The forecast reports were provided in messages sent out to an email list, posted on the Wild Blueberry extension blog (http://mainewildblueberries.blogspot.com/) and recorded as answering machine messages. In addition, weather station data was made available to growers in real time via a website linked from the Extension Wild Blueberry site. For next year, we are currently testing out and optimizing a remote camera that will allow more detailed observations of disease phenology at established plots.

We had dry, warm conditions at the beginning of the production of pinheads in mid-April so there was delayed development of the apothecia (cups). The season was approximately three weeks in most areas. There were weather conditions to allow infection around April 24$^{th}$ to 25$^{th}$, but the majority of the plants did not have 30% bud opening and so were not susceptible. Most growers did not apply fungicide this early and had little disease except in very early developing clones. Most growers waited until the next set of infection periods around May 7$^{th}$ to apply fungicide and found one application provided protection through the rest of the mummy berry season. Many growers only applied fungicide once in 2013 for mummy berry control, and we typically found less than 5% of stems infected with Monilinia.

**RECOMMENDATIONS:** We recommend to continue to monitor conditions for mummy berry infection with the weather stations. Weather stations will be set up at 15 locations next year with mummy berry plots at as many sites as possible with growers willing to monitor them. We will continue with the disease forecast and will improve the website for the weather stations by including a current prediction of infection for each monitored field. We hope to increase participation of growers in the future and are actively looking for additional cooperators willing to monitor mummy berry plots. We hope to include data on bloom and Botrytis blight forecast models from Nova Scotia and insect emergence models for Spotted Wing Drosophila and Blueberry Maggot Fly next year.

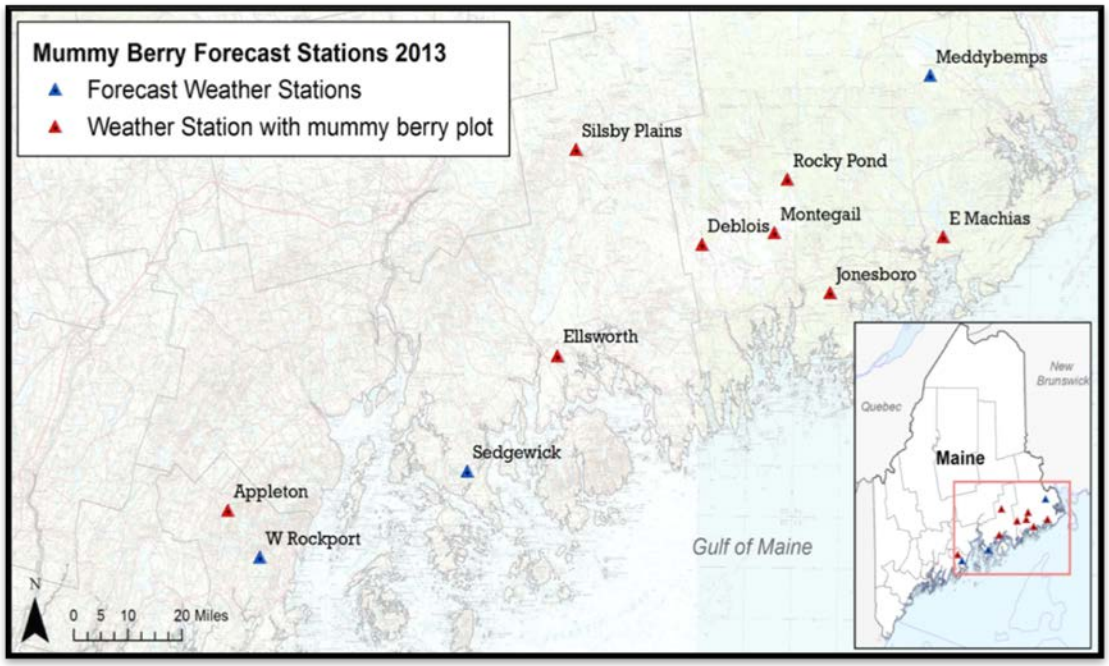


**Figure 1**. Locations of mummy berry forecast stations and mummy berry plots for 2013.

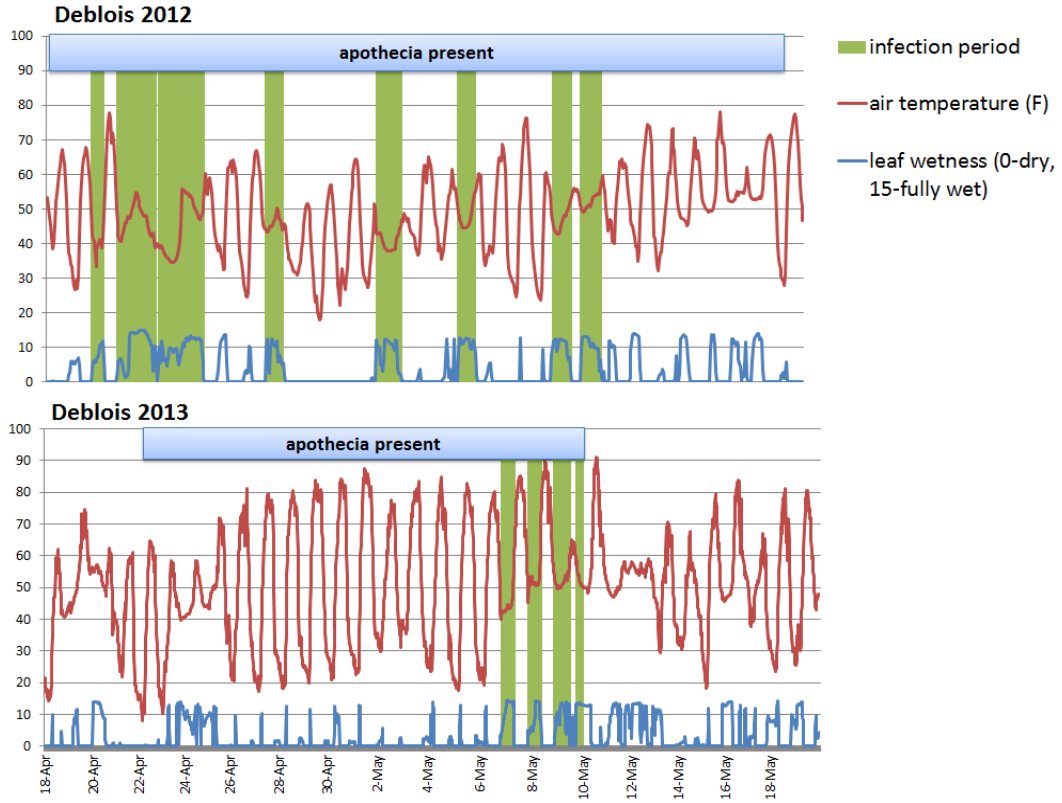


**Figure 2.** Comparison between infection periods in 2012 (top) and 2013 (bottom) at the Deblois site. Air temperature and leaf wetness were used to determine infection periods (green bars) for *Monilinia vaccinii-corymbosi.* Blue bars represent when apothecia were present in the fields.

**Table 1.** Estimated time of mummy berry cup production and infection periods for weather stations in 2013.

| Weather station location | Start of Pinheads | Start of cups | End of Cups | Number of infection periods |
|---|---|---|---|---|
| West Rockport | N/A[1] | N/A | N/A | 5 |
| Appleton | 18-Apr | 25-Apr | 9-May | 5 |
| Liberty | 17-Apr | 23-Apr | 7-May | N/A |
| Sedgewick | N/A | N/A | N/A | 7 |
| Ellsworth | 19-Apr | | 10-May | 6 |
| Silsby Plains | N/A | N/A | N/A | 7 |
| Deblois/Pineo | 22-Apr | | 16-May | 7 |
| Montegail | 19-Apr | 26-Apr | 16-May | 7 |
| Rocky Pond | 22-Apr | 26-Apr | 17-May | 8 |
| Jonesboro | 17-Apr | 23-Apr | 7-May | 6 |
| East Machias | 19-Apr | 25-Apr | 8-May | 5 |
| Meddybemps | N/A | N/A | N/A | 8 |

[1] N/A = not available

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## DISEASE MANAGEMENT

**INVESTIGATORS:** Dr. Seanna Annis and Caleb Slemmons, School of Biology and Ecology
Dr. David Yarborough and Jennifer L.D. Cote, School of Food and Agriculture

**10. TITLE:** Evaluation of fungicides for control of mummy berry on lowbush blueberry (2013).

**METHODS**: Complete randomized block experiments were established in two lowbush blueberry fields with histories of mummy berry disease. One field was near Deblois and the other in Township 19, Maine. Fungicides (Table 1) were randomly assigned to 6' x 30' plots with a 3' buffer lane between each plot and replicated in 8 blocks per field. Fungicide applications were timed using the Mummy Berry disease forecast (UMaine Cooperative Extension Bulletin #217 (http://umaine.edu/blueberries/factsheets/disease)) according to locally monitored conditions favoring disease development (Fig. 2 of Report #9). Fungicides were applied on May 7 in the Deblois field and on May 8 in the Township 19 field. Fungicides were applied at volumes equivalent to 20 gallons per acre at 35 psi with a $CO_2$ backpack sprayer equipped with a 4 nozzle boom, 8002VS T Jet tips and 50 mesh screens applied. Appropriate surfactants or adjuvants were added as recommended by the manufacturer and the control plots received no spray applications.

**Table 1.** Trial treatments for control of mummy berry disease.

| Trade Name(s) | Application Rate (per acre) | Component(s) | Company | Label |
|---|---|---|---|---|
| Fontelis (LEM17) + 0.25% v/v Silwet 77* | 16 ounces | penthiopyrad | DuPont | Blueberries |
| Fontelis (LEM17) + 0.25% v/v Silwet 77* | 24 ounces | penthiopyrad | DuPont | Blueberries |
| Proline 0.25% v/v Silwet 77* | 5.7 ounces | prothioconazole | Bayer Crop Science | For 2014 |
| Proline 0.25% v/v Silwet 77* | 5 ounces | prothioconazole | Bayer Crop Science | For 2014 |
| V10135 4SC | 16 ounces | fenpyrazamine | Valent Ag Products | Not Yet Labeled |
| Quash | 2.5 ounces | metconazole | Valent Ag Products | Supplemental Label For Blueberries |
| Positive Control - Tilt | 6 ounces | propiconazole | Syngenta | Blueberries |

*Surfactant

Disease assessments in both fields occurred on May 23 and consisted of presence/absence of the disease symptoms on 40 blueberry stems along a transect through the middle of each plot. A rope with evenly spaced markings was stretched along the transect and the stem closest to each marking was inspected for disease symptoms on flowers or leaves. In addition, the number of markings at bare places (missing data) and frost damaged stems was recorded. The percentage of infected stems was the number of counted infected stems divided by the total number of rated stems (40 minus the number of bare locations) for each plot. Phytotoxicity was also rated at the same time disease assessments were made.

Blueberries were harvested in a 2 foot strip down each plot center with a mechanical harvester and fresh weight was measured. Berries were harvested on August 1st for the Township 19 field and on August 6th for the Deblois field.

**RESULTS**: There was low disease pressure for mummy berry disease this year due to the dry warm conditions while the plant were susceptible and the *Monilinia* apothecia were present in the fields. While there was adequate inoculum in each field, weather conditions were favorable for fungal infection for only four infection periods at the end the period of time that apothecia of the fungus were present in the fields. Compared to 2012, we had lower levels of mummy berry disease throughout Maine this year due to the fewer infection periods.

In the Township 19 field, we did see a reduction in *Monilinia* infection in all of the fungicide treatments compared to the check (Fig 1). The fungicide treatments of the high rate of Fontelis and high rate of Proline provided significant reduced disease compared to the check at the Township 19 field. At the Deblois site, disease levels ranged from 0.9 to 5.6% infected stems, and we did not see an effect of the fungicide treatments on disease levels due to the low levels of disease (Fig. 1). Levels of disease recorded were very low with 2.8% at Deblois and 8.8% at Township 19 for the check plots.

Frost damage was found in both fields. The Township 19 field had up to 10% of stems affected by frost and the Deblois field had up to 5.6% (Fig. 2). There was no effect of fungicide

treatment on level of frost damage and no phytotoxicity was seen with any of the treatments. There was no significant effect of fungicide treatments or levels of frost on the yield from either field (Fig. 3). Yields ranged from approximately 7200 to 8600 lbs./acre at Township 19 field and approximately 4600 to 6100 lbs./acre at the Deblois field.

**RECOMMENDATIONS**: Fontelis will be recommended as a fungicide to control mummy berry disease on lowbush blueberries after 2 years of successful trials. It is recommended that Quash, Proline and fenpyrazamine be tested again next year for confirmation of effectiveness.

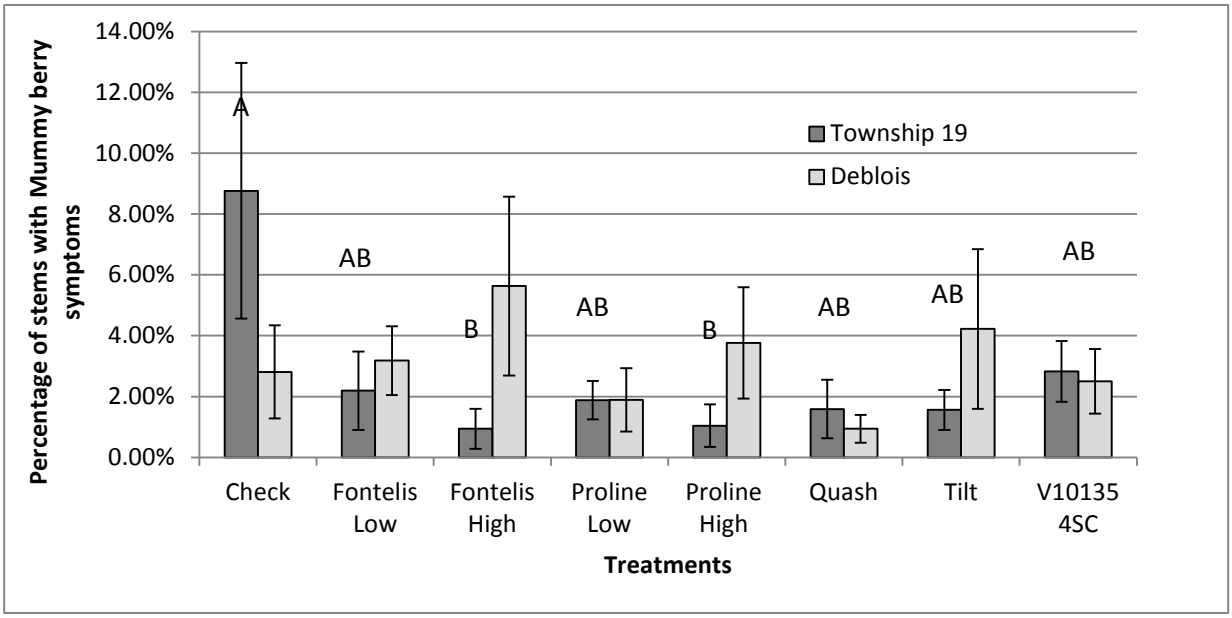



**Figure 1.** Average percentage of stems with symptoms of mummy berry disease in fungicide trials at Deblois and Township 19 fields. Error bars represent standard error of the mean of 8 replicates. Bars with different letters were significantly different at $p<0.05$ within the Township 19 field. There were no significant differences among treatments in the Deblois field.

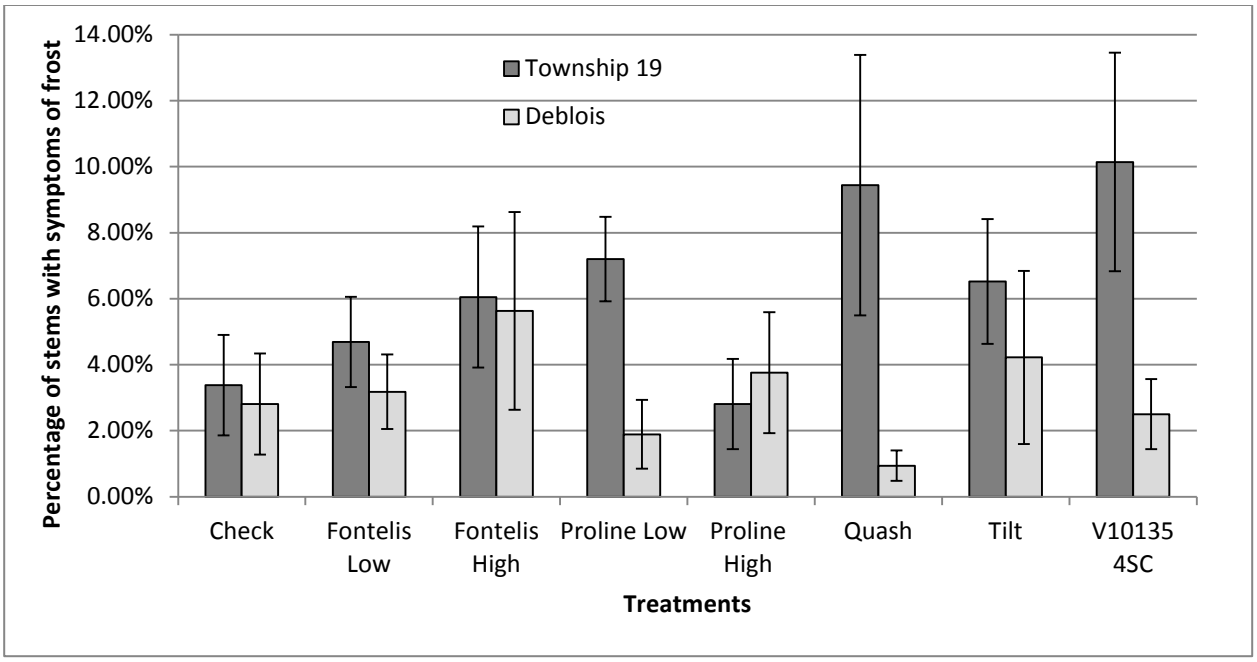



**Figure 2.** Average percentage of stems with symptoms of frost damage in fungicide trials at Deblois and Township 19 fields. Error bars represent standard error of the mean of 8 replicates. There was no significant difference among the treatments within a field.

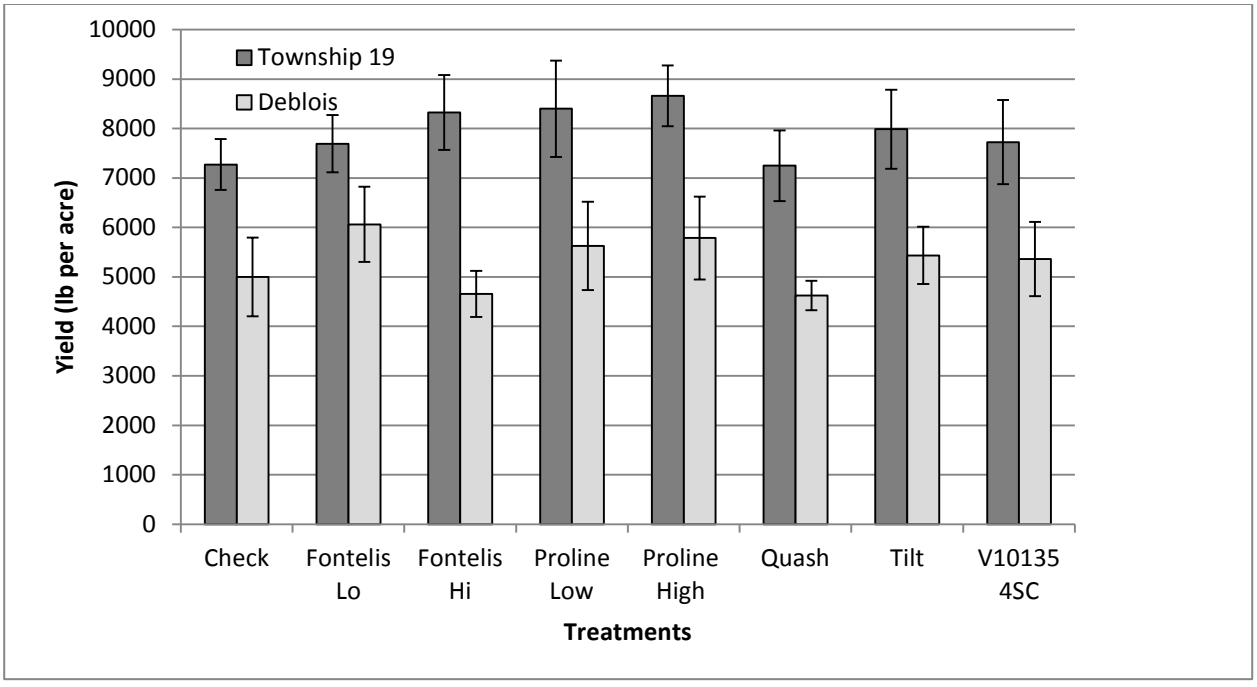



**Figure 3.** Average blueberry yield in pounds per acre for treatments in fungicide trials at Deblois and Township 19 fields. Error bars represent standard error of the mean of 8 replicates. There was no significant difference among the treatments within a field.

**EXTENSION**

**INVESTIGATOR:** David E. Yarborough, Extension Blueberry Specialist

**11. TITLE:** Wild Blueberry Extension Education Program in 2013.

**OBJECTIVES:** Conduct an educational program that will stress the use of best management practices in an integrated crop management program, which will improve the efficiency of culture and minimize the use of unnecessary pesticides and fertilizers. Conduct spring grower meetings and field days to introduce and reinforce the use of best management practices, integrated crop management and sound business management principles. Provide management information through the blueberry newsletters, fact sheets in the wild blueberry grower's guide both in print form and on the web at http://extension.umaine.edu/blueberries/, telephone and correspondence, and conduct field visits as appropriate. Cooperate with County Educators and provide support for blueberry initiatives requested by the County office. Cooperate with the Blueberry Research Advisory Committee, the Wild Blueberry Commission of Maine and the Wild Blueberry Association of North America on blueberry related matters. Cooperate with county (Soil and Water Conservation Districts), state (Department of Agriculture, Board of Pesticides Control) and federal agencies (USDA, IR-4) on blueberry related matters. Needs are determined from the Blueberry Advisory Committee long-range plan, Wild Blueberry Newsletter survey, and from individual client contacts. The advisory committee gave priority to grower outreach, IPM, pesticide recommendations for weeds, insects and diseases, food safety and groundwater. Needs identified by the survey include weed management, economics/ marketing, pest management, general information and fertilization. Needs identified by individual grower contact reinforce those previously identified, but also added the need for blueberry quality and groundwater concerns.

**RESULTS:**

*Educational Activities:*

This year the Blueberry Integrated Crop Management (ICM) program consisted of a presentation at the Agricultural Trade Show, a winter meeting at Blueberry Hill Farm, Spring Grower meetings at three locations, field demonstration sessions conducted three times in three counties and the annual field day at Blueberry Hill Farm where we had a guest speaker from Nova Scotia discuss red sorrel biology and control and I discussed weed resistance and new control options.

We continued our emphasis on our new pest, the Spotted Wing Drosophila (SWD). Growers were informed of how to identify this new pest, how to build new traps and their placement in the field and what were effective control measures at the Agricultural Trade Show in Augusta in January, at the Wild Blueberry Spring meetings in Waldoboro, Ellsworth and Machias in March, at ICM scouting sessions in Warren, Orland and Jonesboro in May and June and at the annual wild blueberry growers field day in July. New fact sheets on Identification, trapping and control measures were sent by list serve or mailed to growers and posted on the wild blueberry web site and the updated wild blueberry pesticide chart identified the most effective control measures for the SWD. 24C use labels were obtained for a higher rate of Malathion from the Maine Board of Pesticides Control to provide growers a more effective control measure for this pest.

I had Dr. Charles Forney, a postharvest physiologist for Agriculture and Agri-Food Canada from Kentville, NS a speaker come to Maine to give fresh pack growers advice on how to improve the quality of their pack. The session will took place at the Hancock County Extension Office in Ellsworth on Thursday March 21, 2013.

*Meetings attended:*

67th Annual Meeting Northeastern Weed Science Society Baltimore, MD February 4-7, 2013
Washington-Hancock County Farm Bureau Grower Meeting, Blueberry Hill Farm, Jonesboro, ME February 11, 2013
Journée Bleuet (Quebec Wild Blueberry Growers Annual Meeting), Dolbeau-Mistassini, Quebec, February 22, 2013
IR-4 National Education Conference in San Antonio, Texas, February 27-28, 3013
Blueberry Open House (Rutgers University NJ grower meeting), Hammonton, NJ March 14, 2013
Blueberry Information Day & PEI Wild Blueberry Growers' Association Annual General Meeting, Cornwall, PEI March 26, 2013
NB Blueberries Annual General Meeting, Moncton, NB April 6, 2013
2013 Berry Health Benefits Symposium, Charlotte, NC, June 18-20, 2013
16th Wild Blueberry Health Summit, Bar Harbor, ME August 14-16, 2013
IR4 Project Northeast Region Priority Setting Meeting, Albany, NY August 20, 2013
Wild Blueberry Association of North America and Wild Blueberry Research and Extension Workers Annual Meeting, Bangor, ME, October 24-25, 2013

*Presentations:*

Wild Blueberry Pest Management Update. Augusta Agricultural Trade Show, Augusta, ME January 10, 2013
Effect of timing and combinations of preemergence herbicides for weed control in wild blueberry fields. 67th Annual Meeting Northeastern Weed Science Society Baltimore, MD February 4-7, 2013
Pre- and Post-emergence Applications of Herbicides for Control of *Festuca filiformis* in Wild Blueberry (*Vaccinium angustifolium*) Fields. 67th Annual Meeting Northeastern Weed Science Society Baltimore, MD February 4-7, 2013
Preventing weed resistance in wild blueberry fields. Washington-Hancock County Farm Bureau Grower Meeting, Blueberry Hill Farm, Jonesboro, ME February 11, 2013
La production mondiale du bleuet and Comment ameliorer Jes rendements de votre bleuetiere at Journée Bleuet (Quebec Growers Annual Meeting), Dolbeau-Mistassini, Quebec, February 22, 2013
Wild Blueberry Production. MES 101 class. University of Maine, Orono, ME February 25, 2013
Wild Blueberry Production. PSE 110 class. University of Maine, Orono, ME March 18, 2013
Increasing Your Wild Blueberry Yields to 20,000 lb/a? Wild Blueberry Spring Grower Meetings in Waldoboro, Ellsworth and Machias, ME on March 19, 21, and 23, 2013
What Does It Take To Produce 20,000 lbs / Acre. Blueberry Information Day & PEI Wild Blueberry Growers' Association Annual General Meeting, Cornwall, PEI March 26, 2013
Producing 10,000 lbs/ac. NB Blueberries Annual General Meeting, Moncton, NB April 6, 2013
Girl Scout Jamboree. Freeport, ME May 18, 2013

Maine's Wild Blueberry Industry. Union Historical Society, Union, ME June 5, 2013
Maine's Wild Blueberry Industry. Lincoln House, Newcastle, ME July 1, 2013
Maine's Wild Blueberry Industry. Claremont Lecture Series, Southwest Harbor, ME July 11, 2013
Preventing Weed Resistance in Wild Blueberry Fields and Weed Management Research for 2013. Wild Blueberry Field Day, Jonesboro, ME July 17, 2013
Wild Blueberry Production and IPM. Wild Blueberry Legislative Tour, Jonesboro, ME August 22-23, 2013
Wild Blueberries. Eastern States Expo, Springfield, MA September 27-29, 2013
Maine's Wild Blueberry Industry. Go Away tours, Bar Harbor, ME, October 14, 2013
Maine's Wild Blueberry Industry. Central Maine Garden Club. Waterville, ME October 15, 2013
Wild Blueberry Production. BIO 342 class. University of Maine, Orono, ME November 5, 2013

*Publications:*

Rose, A., F. Drummond, D. Yarborough, and E. Asare. 2013. Maine Wild Blueberry Growers: A 2010 Economic and Sociological Analysis of a Traditional Downeast Crop in Transition, MAFES Miscellaneous Report 445, Orono, ME.

Yarborough, D.E. and J. D'Appollonio-Cote. 2013. Effect of timing and combinations of preemergence herbicides for weed control in wild blueberry fields. Proceedings of the Northeastern Weed Science Society 67:48.

Yarborough, D.E. and J. D'Appollonio-Cote . 2013. Pre- and Post-emergence Applications of Herbicides for Control of *Festuca filiformis* in Wild Blueberry (*Vaccinium angustifolium*) Fields. Proceedings of the Northeastern Weed Science Society 67:128.

Farooque A.A., Q.U. Zaman , D. Groulx, T. Nguyen-Quang , D. Yarborough, A.W. Schumann, Y. K. Chang, and T. J. Esau. 2013. Effect of Ground Speed and Header Revolutions on the Picking Efficiency of Wild Blueberry Harvester. 2013 ASABE Annual International Meeting, Kansas City, Missouri, July 21 – 24, 2013.

*Wild Blueberry Fact Sheets – 2013:*

*Revised:*

Fact Sheet #209 (UMCE #2001) 2013 Insect Control Guide for Wild Blueberries
Fact Sheet #239 (UMCE #2025) 2013 Weed Control Guide for Wild Blueberries
Fact Sheet #219 (UMCE #2000) 2013 Disease Control Guide for Wild Blueberries
Fact Sheet #202 (UMCE #2373) Blueberry Thrips
2013 Maine Wild Blueberry Pesticide Charts – 1. Insects, 2. Diseases, 3. Weeds

*New:*

Fact Sheet # 210-Spotted Wing Drosophila: Pest Biology and IPM Recommendations for Wild Blueberries
Fact Sheet # 196-Beneficial Insect Series 2: Carabidae (Ground Beetles) on Maine Farms.

*Wild Blueberry Website:*

The Wild Blueberry website found at http://www.wildblueberries.maine.edu continues to be updated and has been revised to comply with the University of Maine content management system. It received 118, 410 page views in 2013 and so is well used world-wide. The wild

blueberry blog is being used to update growers on current activities including insect (both pollinator and SWD), and disease (mummyberry monitoring) posts at: http://mainewildblueberries.blogspot.com/.

*Other program activities:*

I am the principle investigator for the NIFA *Sustainable Production of Wild Blueberries*, which provides funds for a five year (2009-2014) multidisciplinary systems approach project for wild blueberries. I am responsible for obtaining, compiling and producing the proposals and reports and providing summaries for the REEport on-line database.

I serve as the liaison for Maine in the IR-4, Minor Use Registration Program and convey project needs for all crops, as well as conduct projects. The objective of the program is to register least toxic alternative pesticides to replace materials that have been canceled so that our growers will be able to keep the minor crop production practices viable in Maine. I report on the wild blueberry crop to the New England Agricultural Statistics Service (NAAS) on a weekly basis during the wild blueberry-growing season. NAAS uses the information to provide updates on the web for the wild blueberry crop for all who are interested.

I serve on the peer review committee for the School of Food and Agriculture and the joint peer review committees of Renae Moran and Mark Hutton. I also serve on the graduate committee of: Sara Bushmann, Ph.D. student, Major advisor F. Drummond 2008 – 2011; Alex Bajcz Ph.D. student, Major advisor F. Drummond 2013 –present; Jennifer Denso, M.S. student, Major advisor M.E. Camire 2013- present. I serve on the faculty senate for NSFA, and Chair of the Service and Outreach Committee and a member of the Executive Committee from 2012 to 2015.

**CONCLUSIONS:** Growers are participating in IPM programs in the four primary wild blueberry growing counties: Washington, Hancock, Knox and Lincoln. The skills survey results indicate that growers are learning new skills and making positive changes in their management practices. A high percentage of participating growers indicated they had learned new skills and changed their practices in calibration, thereby reducing the rate of hexazinone used, being able to control blight, identifying and control weeds, being able to detect and control insects and the blueberry maggot fly, and using soil and leaf samples to determine fertilizer rates. Adoption of these management practices will enable growers to improve the efficiency of blueberry culture by reducing unnecessary pesticides and fertilizers. Developing alternative strategies for control of resistant weeds is necessary to prevent future losses in yield from weed competition. The introduction of the new pest, the spotted wing drosophila, will present additional challenges in monitoring, identification and control to prevent losses from this pest. These practices are essential to counter the perception of the anti-pesticide and the anti-aerial spray protests that have taken place and intensified in recent years.

The most recent survey conducted from the newsletter mailing list indicates that growers need the information provided by the meetings, fact sheets and newsletters. It also indicates that many growers are using integrated management techniques. Adoption of Best Management Practices will enable growers to improve the efficiency of blueberry culture by reducing unnecessary pesticides and fertilizers. More efficient management will result in greater returns and a stable, sustainable industry.

**INPUT SYSTEMS STUDY – *OVERVIEW***

**12. TITLE:** Systems approach to improving the sustainability of wild blueberry production, Year Four of a four-year study – experimental design.

**EXPERIMENTAL DESIGN***:* In spring of 2010, a four-year study of the effects of different blueberry cropping input systems on a. crop growth, yield, quality and food safety, b. pest levels/dynamics and level of risks to growers, c. soil health, and d. economic and ecological costs/benefits was initiated. Overviews of the first three years of the study are presented in Report #19 of the 2010 Project Reports, Report #15 of the 2011 Project Reports, and Report #13 of the 2012 Project Reports. In 2012, the study design was changed slightly for the second crop cycle of the project in order to give better representation of the ranges in variables examined in each management system, as well as greater statistical power. In this cycle, two one-acre blocks in four input systems (Organic and Low, Medium and High input conventional systems) were set up at four sites per management system for a total of eight blocks per system. We used the same two sites per system as in the first crop cycle but eliminated two blocks each; the two remaining blocks retained the original block designations. The other four blocks were set up two each on two additional sites, and growers were asked to perform their usual activities within these plots as part of the larger field landscape. The "typical" management input parameters for each system, as determined at the start of the project, are presented in Table 1.

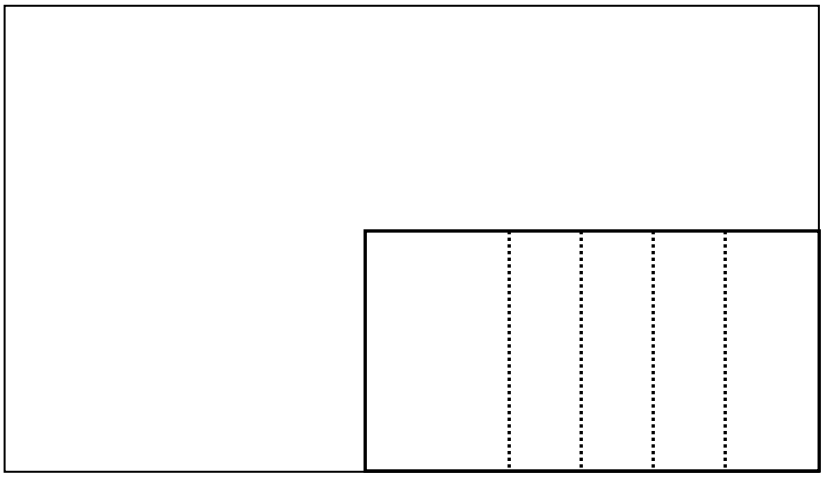
**Table 1.** Typical levels of inputs in four management systems for the production of wild blueberries.

| | **Management input systems** | | | |
|---|---|---|---|---|
| **Production Factors** | **Organic** | **Low Input** | **Medium Input** | **High Input** |
| **Pruning** | Burned | Burn | Mowed | Mowed |
| **Land leveling** | Not land leveled | Not land leveled | Land leveled | Land leveled |
| **pH management** | pH managed | No pH management | pH managed | pH managed |
| **Fertility** | No fertilizer | No fertilizer | Reduced Fertility (every other cycle) | Fertility optimal |
| **Pest, disease, and weed control** | Cutting woody weeds | Herbicide, blueberry maggot, mummyberry control with standard pesticides | Scouting, standard and reduced risk pesticides | Scouting, reduced risk pesticides |
| **Treatment of bare spots** | Mulch | No mulch | No mulch | Mulch |
| **Irrigation** | No irrigation | No irrigation | No irrigation | Irrigation |
| **Pollination** | Bees 2 hives/acre | No added bees | Bees 2 hives/acre | Bees 6 hives/acre |
| **Harvest method** | Hand raked | Hand raked | Mechanical Harvest | Mechanical Harvest |

Two one-acre blocks each were maintained on the following sixteen sites:
**Organic**: Fields 1-4.
**Low input**: Fields 5-8;
**Medium input**: Fields 9-12;
**High input:** Fields 13-16;

Each one-acre block contained a "sub-block" with four transects (Figure 1):

**Figure 1.** Example layout of an acre block, sub-block 15 x 30 m and 15 m transects.



**METHODS**: The following inputs were made to each system in 2013 and are found in Table 2. In-depth methods are located in each researcher's respective report(s).

*Sampling*
Sampling completed in 2012 is found in the Experimental Design report for 2012. In 2013, blueberry and weed cover assessment was conducted in early June and late July/early August in 1 $m^2$ plots along the 15 m transects. In mid-April and mid-May, stems were cut from each block for length, density, flowerbuds, flowers, fruit-set and bee abundance measures. Insect sweeps were conducted across the entire block in May, blueberry maggot fly sampling occurred over the 2013 growing season, and spotted wing drosophila (SWD) sampling occurred from July to September. Disease sampling took place along the transects and in 0.25 $m^2$ plots in May and September/October. Soil samples were taken from each block in 2013 to examine interactions between phosphorus and organic matter. Finally, in the fall of 2012 and 2013, all growers were contacted for their prune year and crop year inputs and costs in order to build a preliminary partial budget spreadsheet for the two-year crop cycle.

*Yield*
All sites were harvested between 30 July and 6 August 2013 in order to minimize potential effects on harvest from SWD. The blocks were harvested along the 15 m long transects, and went through the 1 $m^2$ weed cover plots. The Medium and High sites were harvested using two walk-behind harvesters with 2' wide heads, and the Low and Organic sites were harvested by hand using rakes of the same width as the harvesters. The berries were weighed on-site using

two analog tray scales, and were not winnowed prior to weighing. Yields were converted to lbs/a; yield by system was analyzed using a Tukey's test ($\alpha$=0.05), while yield by site was compared using the Standard Error of the Mean.
A composite subsample of berries (1 kg/block) for each site was winnowed and brought back to UMaine for taste, color, nutrient analysis and for pathogens by the Food Science Department. Results are presented in individual researchers' reports.

**RESULTS/CONCLUSIONS:**

*Sampling*

The results of each researcher's assessments are presented in their respective individual reports.

*Yield*

The yield results by system and site are presented in Figures 2-3. Some researchers have also presented yield results in their individual reports as they relate to the researchers' respective project aspects (weeds, insects, disease, etc.).
Overall, yields in the High and Medium systems were significantly higher than those in the Low and Organic systems, and the Low input system yield was also significantly higher than that of the Organic system (Figure 2). Several factors contributed to these differences. Irrigation was not a driving factor this year, since the summer was wet in general, and the region received sufficient rainfall during critical periods of berry development. However, pollination appeared to play a larger role in yield differences in 2013 due to many cool rainy days during pollination. Supplementing native bees with commercial hives allowed more flowers to be pollinated during the short stretches of good pollination weather. The wet spring also led to unusually high levels of Botrytis blight compared to most years; some growers who did not spray fungicides, such as the Organic growers, saw some flower loss. Organic grower #3 only harvested one acre of the five crop acres because the amount of berries on the remaining four acres was too low to justify the cost of harvesting. Organic grower #4 stopped managing their fields altogether this cycle, so the only management the site received for this crop cycle was the weed-whacking of the sub-blocks by UMaine personnel. Organic grower #1's fields were attacked by SWD and so lost several acres of berries to the pest. Low field #5 is a very rocky field with shallow soils on a steep slope; this site has very short plants and has had leaf drop every summer including 2013, due both to drought and disease (although fungicide was sprayed twice this May). These issues are reflected in the yields by site seen in Figure 3. Otherwise, site yields within each system were comparable to each other.
Trends of all aspects examined during this project (e.g. pest management, soil nutrition, pollination, etc.) need to be assessed in conjunction with each other, but the ultimate goal of managing blueberry fields is to maximize profit. The lack of a difference in yield between the Medium and High input systems suggests that there is a point of diminishing returns where adding more inputs to a system no longer results in significantly improved yield, and that this point lies somewhere in the range of inputs between these two systems.

**Figure 2.** 2013 wild blueberry yields by input system (α=0.05).

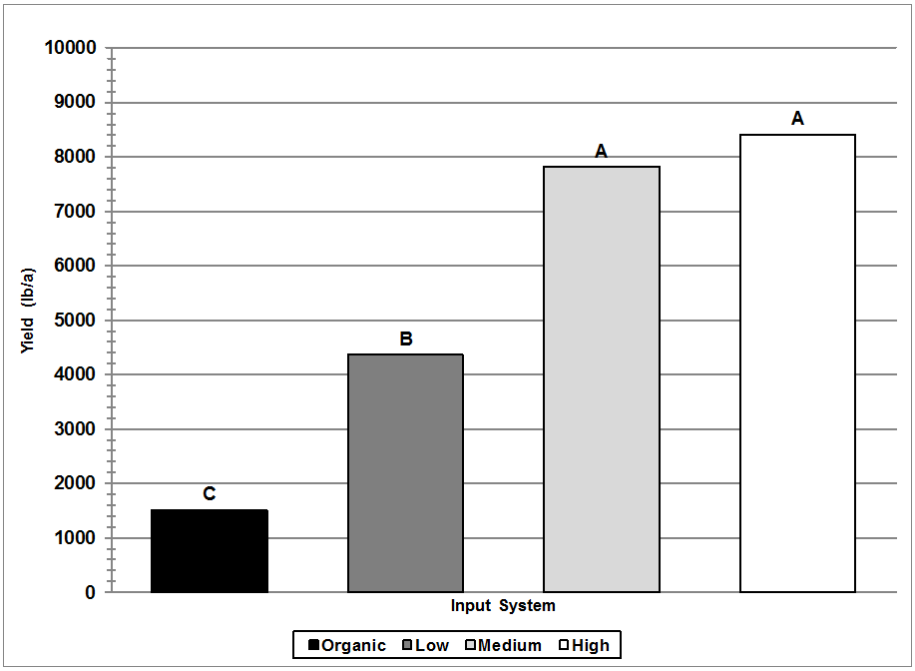



**Figure 3.** 2013 wild blueberry yields by site (Std. Error of Mean).

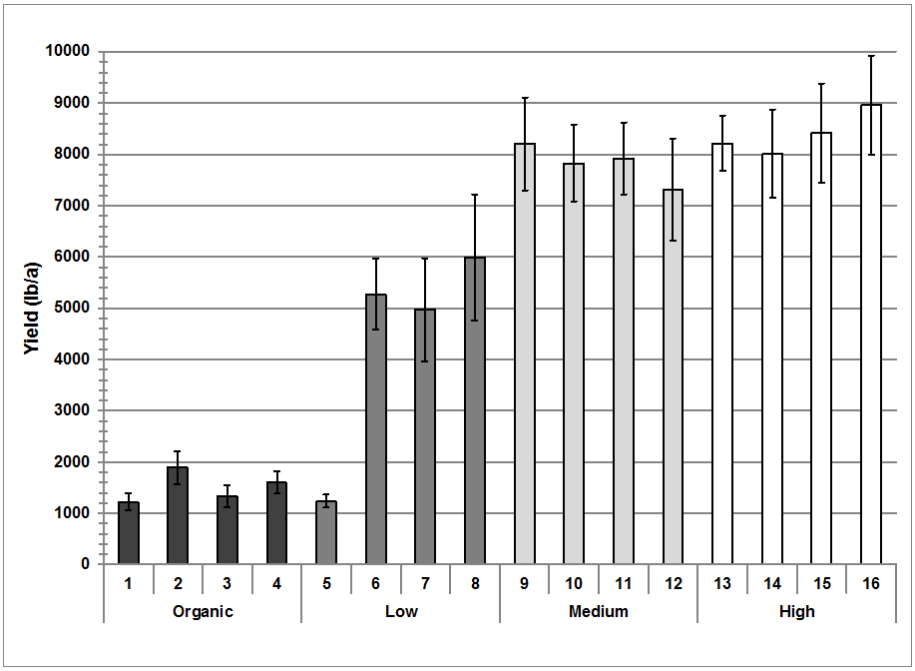


**Table 2.** 2013 crop year inputs by input system (NA = not applicable; N/A = not available).

| Input | Site | pH | Fertility | Pest control | Disease control | Weed control | Pollination |
|---|---|---|---|---|---|---|---|
| Organic | 1 | NA | NA | NA | NA | NA | 2 hives/11 a |
| | 2 | N/A | N/A | N/A | N/A | N/A | N/A |
| | 3 | NA | NA | NA | NA | NA | 5 hives/a |
| | 4 | NA | NA | NA | NA | NA | NA |
| Low | 5 | NA | 07-22-05 5 gal/a<br>Avail 0.015 gal/a<br>Black Label<br>1 or 2 gal/a (2x) | Imidan 1.31 lb/a, 1.28 lb/a, or 1.33 lb/a (3x)<br>Malathion 0.32 gal/a | Fitness 6 oz/a (2x)<br>Initiate 4 pt/a | Diuron 0.373 gal/a Velpar 0.75 gal/a | 2.2 hives/a |
| | 6 | NA | Black Label<br>1, 2, or 3 gal/a (3x) | Imidan 1.23 lb/a<br>Malathion 0.31 gal/a | Fitness 6 oz/a (2x)<br>Initiate 4 pt/a | Credit 32 oz/a<br>Diuron 1.5 qt/a<br>Sinbar 2 lb/a<br>Velpar 1 gal/a | 3 hives/a |
| | 7 | NA | NA | Imidan 1.3 lb/a<br>Malathion 8F 2.5 pt/a<br>Mustang Maxx 4 oz/a (2x) | Bravo 3 pt/a<br>Bumper 6 oz/a | NA | 2.4 hives/a |
| | 8 | NA | NA | NA | NA | NA | 1.7 hives/a |
| Medium | 9 | NA | DAP+ m.p. 100 lb/a | Scouting<br>Imidan 1 lb/a (2x) | Bravo Ultrex 2.4 lb/a<br>Tilt 6 oz/a (2x) | Poast 2.5 pt/a | 2 hives/a |
| | 10 | NA | DAP+ m.p. 100 lb/a | Scouting<br>Imidan 1 lb/a | Bravo Ultrex 2.4 lb/a<br>Tilt 6 oz/a (2x) | Poast 2.5 pt/a | 2 hives/a |
| | 11 | NA | DAP+ m.p. 100 lb/a | Scouting<br>Imidan 1 lb/a | Bravo Ultrex 2.4 lb/a<br>Tilt 6 oz/a (2x) | Poast 2.5 pt/a (2x) | 2 hives/a |
| | 12 | NA | DAP+ m.p. 100 lb/a | Scouting<br>Imidan 1 lb/a (2x) | Bravo Ultrex 2.4 lb/a<br>Tilt 6 oz/a (2x) | Poast 2.5 pt/a | 2 hives/a |
| High | 13 | NA | NA | Scouting<br>Assail 4.25 oz/a<br>Imidan 1.3 lb/a<br>Success 5 oz/a | Pristine 20 oz/a<br>Quilt Xcel 14 oz/a<br>Tilt 6 oz/a | NA | 4.7 hives/a |
| | 14 | NA | NA | Scouting<br>Assail 4.25 oz/a<br>Imidan 1.3 lb/a<br>Success 5 oz/a | Pristine 20 oz/a<br>Quilt Xcel 14 oz/a<br>Tilt 6 oz/a | NA | 7.6 hives/a |
| | 15 | NA | NA | Scouting<br>Assail 4.25 oz/a<br>Imidan 1.3 lb/a<br>Success 5 oz/a (2x) | Pristine 20 oz/a<br>Quilt Xcel 14 oz/a<br>Tilt 6 oz/a | Poast 1 qt/a | 6.5 hives/a |
| | 16 | NA | NA | Scouting<br>Assail 4.25 oz/a<br>Imidan 1.3 lb/a<br>Success 5 oz/a (4x) | Pristine 20 oz/a<br>Quilt Xcel 14 oz/a<br>Tilt 6 oz/a | NA | 6.8 hives/a |

**INPUT SYSTEMS STUDY**

**FOOD SCIENCE & NUTRITION**: Vivian Wu, Associate Professor of Food Safety and Microbiology, School of Food and Agriculture

**13. TITLE**: Food safety- Prevalence study of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. on lowbush blueberries (*Vaccinium angustifolium*).

**METHODS**: We conducted the bacteriological analysis for 2012-2013 crop cycle. A total of 32 harvested lowbush blueberry samples from four management systems (organic, low, medium and high) and from 16 locations of 9 different farms in Maine are evaluated for the presence or absence of potential foodborne pathogens using traditional culture methods and also alternative PCR screening methods. For culture methods, isolation and detection of three major foodborne pathogens, *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. were conducted based on the methods recommended by the U.S Food and Drug Administration with few modifications. Two blueberry subsamples of 25g each were aseptically weighed and subjecting to a sequence of steps including pre-enrichment, enrichment, selective-differential plating and biochemical characterization. *E. coli* O157:H7 and *Salmonella* culture positives were further screened using Enterotube test. Later all these positives were confirmed using serological testing. For PCR screening, DNA was extracted from the overnight enrichment broth and later screened for the target pathogen using specific primers ST-11 & ST-15 (amplified a 429 bp fragment of a cryptic 2.3kb chromosomal fragment of *Salmonella*) for *Salmonella* spp. and genes that target *eae*A gene in *E. coli* O157:H7, *prf*A gene for *L. monocytogenes.*

**RESULTS:** *L. monocytogenes* was not isolated either through culture methods or PCR screening from any of these 32 harvested blueberry samples from the 2012-13 crop cycle. *Salmonella* spp. was isolated from 5 out of 32 blueberry samples through culture methods (Table 1), while through PCR screening 6 samples out of 32 blueberry samples were screened to be positive (Table 2). Overall there are three samples which were common positives for *Salmonella* spp. with both culture and PCR methods. Figure 1 shows the number of *Salmonella* positives obtained from each management system (out of four management systems-low, medium, high, and organic inputs).

Though no *E. coli* O157:H7 serotype was isolated from any of these samples, in screening process, one out of these 32 samples found to be positive for non-O157 *Escherichia coli.* It was suspected as Shiga toxin -producing *Escherichia coli* (STEC). Since it is raising food safety concerns these days, another PCR screening test was done to detect *stx*1 and *stx*2 virulent genes. It was found to be positive for these two virulent genes, indicating that it is presumptive shiga toxin -producing *Escherichia coli* (STEC). Table 3 indicates the steps involved in non-O157 STEC detection for the positive blueberry sample.

**CONCLUSIONS**: Though the occurrence of *Salmonella* spp. in these blueberries was quite low, they still represent a risk to the consumer in regard to foodborne disease. Finding of non-O157 STEC in blueberry samples (organic input) is interesting, taking into consideration of the fact that there is increased incidence of STEC contamination over the past decade.

**RECOMMENDATIONS**: The use of either method in isolation would have resulted in the failure to detect *Salmonella* in some of positive samples. Therefore, it may be pertinent to use a combination of the PCR and culture methods in order to maximize the detection of *Salmonella*. Among four managements, only low input did not show any microbial contamination, referring potential contamination could be introduced through human management activity.

**Table 1.** Blueberry samples positive for *Salmonella* spp. through the traditional culture method.

| **SCRI ID** | **Culture status for *Salmonella* spp.** |
|---|---|
| Field#10, Medium, block#8 | Culture positive |
| Field# 9, Medium, block#3 | Culture positive |
| Field# 9, Medium, block#4***** | Culture positive |
| Field#15, High, block#1***** | Culture positive |
| Field#15, High, block#3***** | Culture positive |

* indicates positives with both culture and PCR methods

**Table 2.** Blueberry samples positive for *Salmonella* spp. through the PCR method.

| **SCRI ID** | **PCR status for *Salmonella* spp.** |
|---|---|
| Field#2, Organic, block#3 | positive |
| Field# 3Organic, block#1 | positive |
| Field# 9, Medium, block#4***** | positive |
| Field# 12, Medium, block#1 | positive |
| Field#15, High, block#1***** | positive |
| Field#15, High, block#3***** | positive |

* indicates positives with both culture and PCR methods

**Table 3.** Steps involved in interpretation of non-O157 *Escherichia coli* (STEC).

| **SCRI ID** | **Culture status** | **Serological confirmation status** | **PCR for eae A gene detection** | **Stx1 & Stx2 PCR status** | **Final interpretation** |
|---|---|---|---|---|---|
| **Field # 3, Organic, Block# 1** | Positive for *E. coli* O157:H7 | O157 negative and H Positive indicating that it might be non-O157 STEC | Positive for eae A gene | Positive for both *Stx*1 and *Stx*2 indicating it is shiga toxin producing *E. coli* | Non O157 shiga toxin - producing *Escherichia coli* (STEC) |

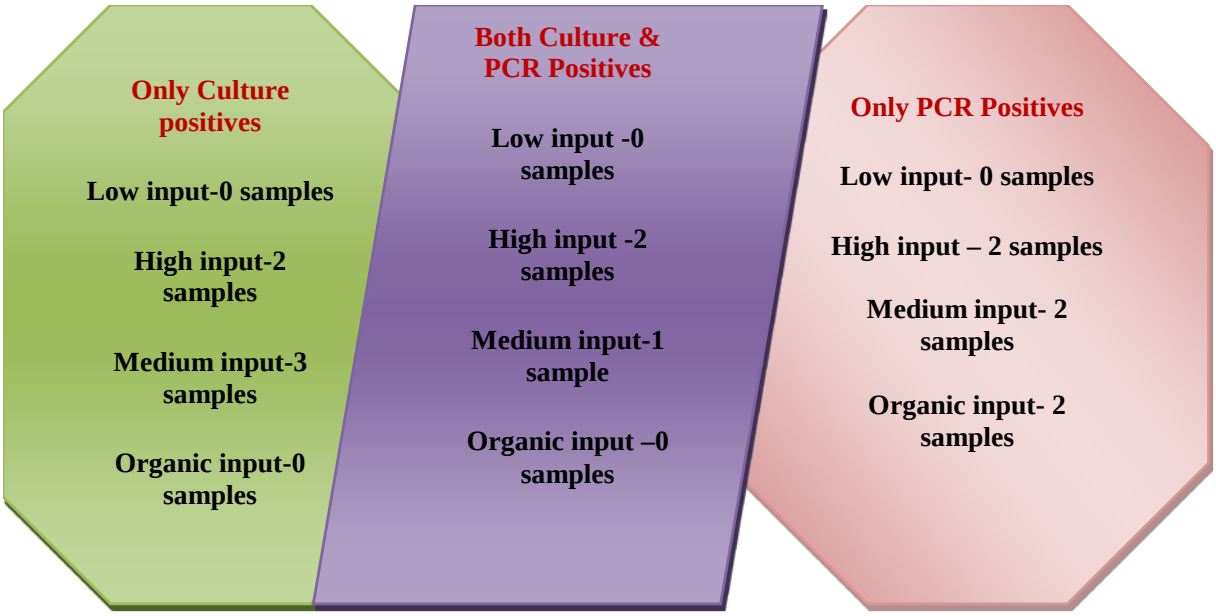



**Fig 1:** Number of *Salmonella* positives from different cropping systems.

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**INPUT SYSTEMS STUDY**

**FOOD SCIENCE & NUTRITION:** Mary E. Camire, Ph.D., Professor of Food Science and Human Nutrition
David E. Yarborough, Ph.D., Professor of Horticulture
Jennifer R. Chadbourne, RD, LD, Graduate Research Assistant
Michael Dougherty, Research Associate
Katherine Davis-Dentici, Research Assistant

**14. TITLE:** Agronomic Input Effects on Sensory Quality and Chemical Composition of Wild Maine Blueberries.

**METHODS**: Our objectives of this study were to determine the effects of four agronomic input levels (organic, low, medium and high) on the sensory and microbial quality and chemical composition of wild Maine blueberries. Blueberry samples were harvested from sixteen farms throughout Maine with four farms designated for each treatment group to capture the diverse nature of wild blueberries. Representative samples from each lot were winnowed and hand sorted to remove the imperfect fruit then stored at 4°C in preparation for analysis. Duplicates from each plot were prepared in triplicate to improve statistical power and significance of the results. Ten-gram samples were extracted with a 70% acetone extraction solvent acidified with 0.1% HCl. The blueberry extracts were then diluted and randomized for anthocyanin content, total phenolic content and antioxidant capacity analysis using pH differential, Folin-Ciocalteu, and ORAC assays. A sensory test was also completed to determine participant acceptability of the wild blueberries from each respective treatment group. Sensory evaluation was completed using a 9-

point hedonic scale with anchor terms ranging from "Dislike Extremely" to "Like Extremely." Approval from the University of Maine Institutional Review Board was obtained for testing with human subjects. Recruitment criteria excluded those who were under eighteen, allergic to blueberries, did not consume fresh blueberries at least twice a year, were smokers or had a cold or other health condition that affected their sense of taste. Fifty participants rated the 15 g blueberry samples for appearance, color, size, flavor, texture, and overall acceptability. The sample presentation order was randomized and balanced. Samples for mineral microbial, physical properties, and general chemistry were analyzed as previously reported by Professor Emeritus Alfred Bushway. ANOVA and Tukey's Honest Significant Difference (HSD) were used to identify significant differences between mean values based on $p \leq 0.05$. Pearson correlation statistics were also performed to identify inter-variable relationships.

**RESULTS**:

*Moisture*

The treatment averages for moisture content ranged from 86.4-87.3% (Table 1). The high input blueberries held the most moisture of the four treatment groups followed by the low input blueberries. Dry weight was calculated based on the mean percent moisture for each treatment group.

**Table 1**. Percent moisture of wild blueberries.

| Agronomic Input Level | Percent Moisture[a] |
|---|---|
| High | 87.3 ± 0.6 a |
| Medium | 86.5 ± 0.8 b |
| Low | 86.9 ± 1.1 ab |
| Organic | 86.4 ± 0.8 b |

[a] Means sharing a letter are not statistically significant from each other (Tukey's HSD, $p \leq 0.05$)

*Anthocyanin Content*

Anthocyanin content varied significantly between each treatment group. The average anthocyanin values between the four treatment groups ranged from 82.1 to 106.0 malvidin-3-glucoside equivalents mg/100g fresh weight (Table 2). Significantly higher levels of anthocyanins were extracted from low input blueberries compared to high, medium and organic input blueberries. The medium input blueberries produced the lowest levels of anthocyanins followed by the high input blueberries. Variations were also observed between the anthocyanin content of blueberries from different plots within each agronomic input level (Table 3). The medium input plots were the only treatment group to not statistically differ from one another. Anthocyanin content was positively correlated with the berry count per 50 g of fresh wild blueberries indicating that smaller fruit size was correlated with greater anthocyanin content.

**Table 2.** Anthocyanin content of wild blueberries.

| Agronomic Input Level | M3GE mg/100 g fw[a] | M3GE mg/100 g dry wt |
|---|---|---|
| High | 88.4 ± 10.1 c | 101.3 |
| Medium | 82.1 ± 10.1 d | 94.9 |
| Low | 106.0 ± 9.4 a | 122.0 |
| Organic | 100.0 ± 11.3 b | 115.7 |

[a] Malvidin-3-glucoside equivalents. Means sharing a letter are not statistically significant from each other (Tukey's HSD, $p \leq 0.05$)

*Total Phenolic Content*

Mean total phenolic content varied from 599.2 to 679.5 chlorogenic acid equivalents mg/100 g fresh weight. Total phenolic content was significantly higher in blueberries from the low and organic input management systems (Table 4). Several variations were observed between field plots within each agronomic input level, but the high input management system appeared to produce the least variability between plots (Table 5).

*Antioxidant Capacity*

Total antioxidant activity was significantly higher in the low and organic input systems compared to the medium input system (Table 6). Antioxidant capacity averages ranged from 5359.9 to 6196.0 μM trolox equivalents per 100g fresh weight between agronomic input levels. The high input system did not significantly differ from the other input systems. Antioxidant capacity was consistent between high input plots, and the greatest variation of antioxidant capacity between plots was observed in the organic input management systems (Table 7).

**Table 3.** Anthocyanin content of wild blueberries.

| Agronomic Input Level | Field Plot | M3GE mg/100 g fw[a] |
|---|---|---|
| High | H7, H8 | 96.9 ± 7.8 a |
| | H2, H4 | 80.8 ± 6.8 b |
| | H5, H6 | 94.0 ± 7.6 a |
| | H1, H3 | 81.9 ± 7.3 b |
| Medium | M7, M8 | 79.7 ± 6.3 a |
| | M3, M4 | 83.5 ± 9.0 a |
| | M1, M2 | 79.8 ± 6.0 a |
| | M5, M6 | 85.3 ± 15.9 a |
| Low | L5, L6 | 100.2 ± 5.6 b |
| | L7, L8 | 100.9 ± 9.1 b |
| | L1, L2 | 113.3 ± 11.0 a |
| | L3, L4 | 109.4 ± 2.6 a |
| Organic | O2, O3 | 100.7 ± 8.4 b |
| | O6, O7 | 92.8 ± 5.4 c |
| | O5, O8 | 91.7 ± 5.5 c |
| | O1, O4 | 114.9 ± 6.2 a |

[a]Means sharing a letter are not statistically significant from each other (Tukey's HSD, $p \leq 0.05$)

**Table 4.** Total phenolic content of wild blueberries.

| Agronomic Input Level | CAE mg/100 g fw[a] | CAE mg/100 g dry wt[b] |
|---|---|---|
| High | 612.0 ± 61.1 b | 701.0 |
| Medium | 599.2 ± 83.5 b | 692.7 |
| Low | 679.5 ± 64.6 a | 781.9 |
| Organic | 649.8 ± 61.7 a | 752.1 |

[a] Means sharing a letter are not statistically significant from each other (Tukey's HSD, $p \leq 0.05$)

**Table 5.** Phenolic content of wild blueberries.

| Agronomic Input Level | Field Plot | CAE mg/100 g fw[a] |
|---|---|---|
| High | H7, H8 | 670.6 ± 77.0 a |
| | H2, H4 | 584.9 ± 47.4 b |
| | H5, H6 | 621.4 ± 20.0 ab |
| | H1, H3 | 571.3 ± 30.2 b |
| | | |
| Medium | M7, M8 | 573.4 ± 35.1 bc |
| | M3, M4 | 610.3 ± 29.8 b |
| | M1, M2 | 532.3 ± 52.3 c |
| | M5, M6 | 680.9 ± 109.0 a |
| | | |
| Low | L5, L6 | 611.4 ± 23.5 c |
| | L7, L8 | 737.7 ± 36.6 a |
| | L1, L2 | 689.9 ± 68.2 ab |
| | L3, L4 | 678.9 ± 49.2 b |
| | | |
| Organic | O2, O3 | 660.4 ± 57.8 b |
| | O6, O7 | 624.8 ± 29.0 bc |
| | O5, O8 | 592.8 ± 30.0 c |
| | O1, O4 | 721.4 ± 35.5 a |

[a] Means sharing a letter are not statistically significant from each other (Tukey's HSD, $p \leq 0.05$)

**Table 6.** Antioxidant capacity of wild blueberries.

| Agronomic Input Level | µM TE/100g fresh wt[a] | µM TE/100g dry wt |
|---|---|---|
| High | 5796.8 ± 1133.6 ab | 6640.1 |
| Medium | 5359.9 ± 1204.5 b | 6196.4 |
| Low | 6196.0 ± 1173.2 a | 7130.0 |
| Organic | 6195.8 ± 1471.7 a | 7171.1 |

[a] Means sharing a letter are not statistically significant from each other (Tukey's HSD, $p \leq 0.05$)

**Table 7.** Antioxidant capacity of wild blueberries.

| Agronomic Input Level | Field Plot | µM TE/100g fresh wt[a] |
|---|---|---|
| High | H7, H8 | 6436.6 ± 1510.4 a |
| | H2, H4 | 5869.2 ± 518.9 a |
| | H5, H6 | 5615.6 ± 1032.6 a |
| | H1, H3 | 5265.8 ± 1038.5 a |
| | | |
| Medium | M7, M8 | 5006.6 ± 976.5 ab |
| | M3, M4 | 5418.5 ± 1512.6 ab |
| | M1, M2 | 4730.4 ± 804.0 b |
| | M5, M6 | 6284.0 ± 898.4 a |
| | | |
| Low | L5, L6 | 5653.6 ± 1128.9 b |
| | L7, L8 | 7040.2 ± 1114.7 a |
| | L1, L2 | 5995.6 ± 993.7 ab |
| | L3, L4 | 6094.5 ± 1106.0 ab |
| | | |
| Organic | O2, O3 | 7262.4 ± 1220.6 a |
| | O6, O7 | 5356.6 ± 1116.3 c |
| | O5, O8 | 5419.2 ± 963.7 bc |
| | O1, O4 | 6744.8 ± 1610.5 ab |

[a] Means sharing a letter are not statistically significant from each other (Tukey's HSD, $p \leq 0.05$)

*Consumer Acceptability*

Twenty-three males and 26 females participated in sensory evaluation; one participant did not specify gender. The majority of participants were 18 to 34 years old, however the ages included person aged 65 years or older. Over 30% of the participants reportedly consume wild blueberries at least once per week during the summer. Less than 15% of participants reported consumption of all blueberries once per week or more throughout the year for both fresh and frozen blueberries (Figure 1). Participants were also asked where they most often purchase fresh blueberries. The majority of participants reportedly purchase blueberries from the grocery store most often followed by pick-your-own farms. The remaining responses were divided between farmers markets, farm stands, and superstores such as Wal-Mart and Sam's Club (Figure 2). Figure 3 shows the importance of buying organic and local as reported by participants. Very few participants found buying organic or local produce to be "somewhat important" or "not important". The majority of participants rated both categories as "important".

**Figure 1.** Reported consumption of frozen and fresh blueberries throughout the year compared to wild blueberry consumption in summer.

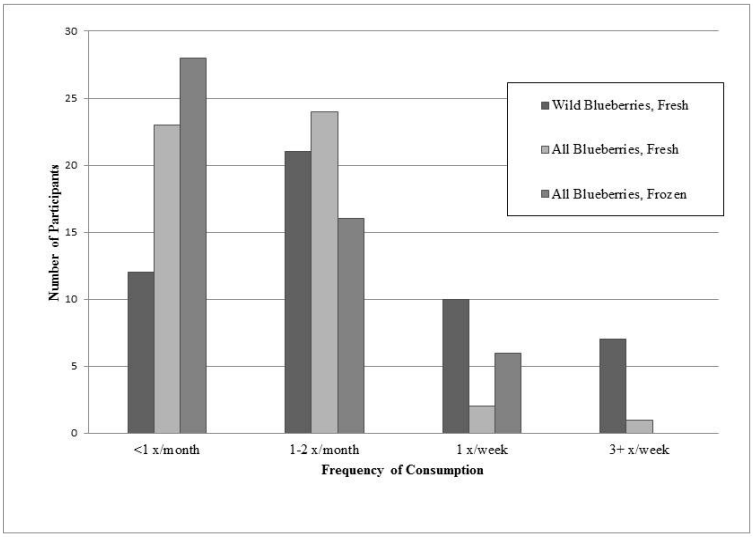



**Figure 2**. Participants' purchasing habits for fresh blueberries.

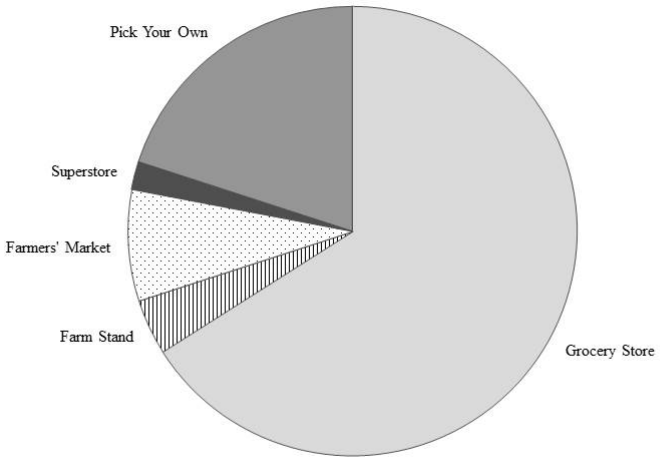


**Figure 3**. Reported importance of buying local and organic produce.

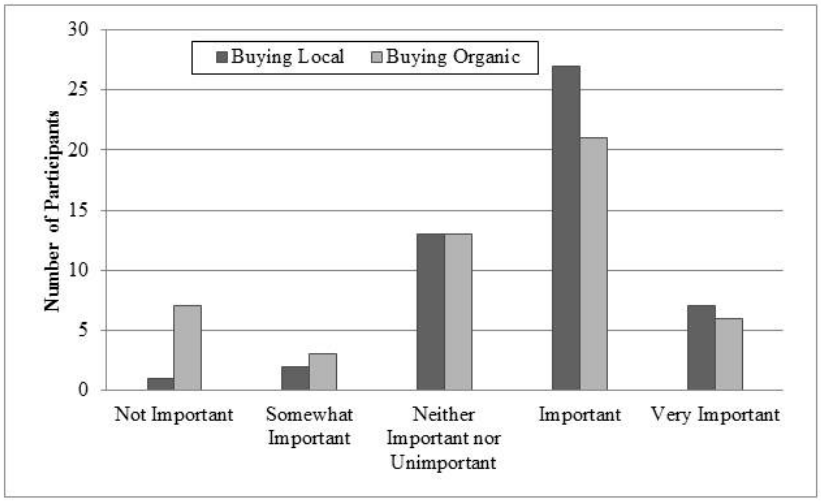



Table 8 shows significance among mean ratings for appearance, color, size, flavor, and texture. Overall liking of the organic, low input and medium input wild blueberry samples was significantly higher than overall liking of the high input samples (Table 9). Additionally, the liking scores received for size and flavor of the high input wild blueberries were significantly lower than the other three treatment groups. These results were also reflected in comments left by participants who described the high input blueberries as "flat", "watery", "bland" and "tasteless". As noted previously, the high input blueberries also had a larger moisture percentage, which participant appeared to notice. Participants rated the low and medium input wild blueberries significantly higher than the high input, but not the organic input, samples for appearance. There was no significant difference noted for color or texture between samples. All ratings for the organic, low and medium input berries were greater than 7 on the 9-point scale.

**Table 8**. Attribute ratings (n=50) of wild blueberries grown with different agronomic input levels based on a 9 point Hedonic Scale.

| Agronomic Input Level | Appearance[a] | Color[a] | Size[a] | Flavor[a] | Texture[a] |
|---|---|---|---|---|---|
| High | 6.74 ± 1.6 b | 7.28 ± 1.1 a | 6.10 ± 1.8 b | 6.14 ± 1.7 b | 6.64 ± 1.5 a |
| Medium | 7.44 ± 0.9 a | 7.54 ± 1.1 a | 7.44 ± 1.1 a | 7.22 ± 1.1 a | 7.30 ± 1.2 a |
| Low | 7.44 ± 0.8a | 7.42 ± 1.3 a | 7.10 ± 1.1 a | 7.24 ± 1.5 a | 7.34 ± 1.4 a |
| Organic | 7.18 ± 1.6 ab | 7.28 ± 1.2 a | 7.24 ± 1.0 a | 7.16 ± 1.4 a | 7.04 ± 1.4 a |

[a] Means sharing a letter are not statistically significant from each other (Tukey's HSD, $p \leq 0.05$).
1 = dislike extremely; 5 = neither like nor dislike; 9= like extremely.

**Table 9**. Participant overall liking ratings (n=50) of wild blueberries grown with different agronomic input levels based on a 9 point Hedonic Scale.

| Agronomic Input Level | Overall[a] |
|---|---|
| High | 6.66 ± 1.3 b |
| Medium | 7.36 ± 0.9 a |
| Low | 7.52 ± 1.1 a |
| Organic | 7.26 ± 1.2 a |

[a] Means sharing a letter are not statistically significant from each other (Tukey's HSD, $p \leq 0.05$)

Berries from the High input sites were expected to have the largest size, but the 2013 crop did not meet expectations. Medium input sites had the largest berries (lowest berry count) but differences among other treatments were not found (Table 10). The force in Newtons required to compress berries were fairly similar (Table 10). Low input berries required more force than did medium input berries. Organic and low input berries were lighter (Table 11), and medium and high input berries were more red. The b* values indicated that all berries were blue but high input berries were more blue than the others were. Chroma and hue angle values are derived from L*a*b* values.

**Table 10**. Berry count and texture.

| Agronomic Input Level | Berry Count/50 grams[a] | Texture (Newtons) |
|---|---|---|
| Organic | 145.8±24.1 a | 1.5±0.5 ab |
| Low | 137.1±30.5 a | 1.6±0.5 a |
| Medium | 109.4±8.4 b | 1.4±0.5 b |
| High | 132.4±8.9 a | 1.5±0.6 ab |

[a] Means within the same column with the same letter are not significantly different (Tukey's HSD, $p \leq 0.05$). N= 16 for count / 50g; N=200 for texture.

**Table 11**. Berry color.

| Agronomic Input Level | L*[a] | a* | b* | Chroma | Hue Angle° |
|---|---|---|---|---|---|
| Organic | 17.1±0.9 a | 0.2±0.2 b | -2.3±0.3 b | 3.9±0.5 a | 274.4±4.9 c |
| Low | 17.2±1.3 a | 0.1±0.1 c | -2.2±0.5 b | 3.6±0.8 b | 272.9±4.7 c |
| Medium | 16.6±1.3 b | 0.3±0.4 a | -2.2±0.4 b | 3.6±0.8 b | 278.8±10.4 b |
| High | 15.7±1.0 c | 0.3±0.2 a | -1.8±0.4 a | 3.2±0.6 c | 281.3±8.0 a |

[a] Means within the same column with the same letter are not significantly different (Tukey's HSD, $p \leq 0.05$, N= 144). L* = lightness (0 = black, 100= white); a* = red/green; b* = blue/yellow.

The mean pH of medium input berries was significantly higher than the values got organic and low input, but all berries were still in the high acid range. Brix, or soluble solids, readings were highest for organic and medium input berries. Titratable acidity was not different among treatments.

**Table 12.** Acidity and soluble solids.

| Agronomic Input Level | pH | °Brix | % TA[a] |
|---|---|---|---|
| Organic | 3.39±0.1 a | 10.4±0.6 ab | 0.37±0.08 |
| Low | 3.39±0.1 a | 9.9±1.0 bc | 0.33±0.05 |
| Medium | 3.31±0.1 b | 10.5±0.9 a | 0.33±0.08 |
| High | 3.36±0.1ab | 9.5±0.6 c | 0.36±0.04 |

[a] Means within the same column with the same letter are not significantly different (Tukey's HSD, $p \leq 0.05$). N= 24 for pH, % Brix and % Titratable Acidity.

General microbiological counts did not follow consistent trends. Standard deviations were quite high due to variation from site to site. Although mean aerobic plate counts for organic berries were statistically higher, one cannot conclude that the berries were at greater risk for spoilage or food safety problems since problematic species were not identified. Researchers working with other crops have not identified any difference in microbial loads due to agronomic practices. Any future work should re-evaluate sampling plans for microbial work to ensure that a representative sample is collected aseptically to prevent contamination from extraneous sources.

**Table 13.** Total microbial plate counts.

| Agronomic Input Level | Total Count (cfu/g) | | |
|---|---|---|---|
| | Aerobic Plate | Yeast | Mold |
| Organic | 82625.0 ± 88041.0 a | 22365.6 ± 33823.1 ab | 10146.9 ±13588.8 b |
| Low | 1698.4 ±1749.4 b | 14300.0 ± 9525.4 b | 13503.1 ± 13352.0 ab |
| Medium | 1478.3 ±1598.5 b | 11207.2 ±12540.4 b | 20905.9 ± 21960.7 a |
| High | 3197.5 ± 3322.7 b | 37106.3 ± 40547.1 a | 5003.1 ± 4690.4 b |

Means within the same column with the same letter are not significantly different (Tukey's HSD, $p \leq 0.05$) n=32

**CONCLUSIONS**: Agronomic inputs at low and organic levels produced the highest total phenolic content, anthocyanin content and antioxidant capacity. High input blueberries received significantly lower ratings for flavor, size and overall acceptability than did other treatments. Consumers rated the low and medium input blueberries higher than the high input samples for appearance. There were no differences in texture or color. Hedonic ratings were greater than 7 in all categories for the organic, low and medium input blueberries, indicating that berries were acceptable.

**RECOMMENDATIONS**: Wild Maine blueberries produced within low or organic input management systems can provide a desirable chemical composition and acceptable sensory quality. Comparison of the two crop years is needed to determine whether trends were consistent.

**INPUT SYSTEMS STUDY**

**ENTOMOLOGY:** F. A. Drummond, Professor of Insect Ecology/Entomology
J. A. Collins, Assistant Scientist of Insect Pest Management

**15. TITLE:** Systems approach to improving the sustainability of wild blueberry production, Year four of a four-year study – Reports from Frank Drummond

*Blueberry stem measurements*

**METHODS:** In mid-April, all the stems from each of ten, 15.2 x 15.2 cm (6 x 6 in) quadrats per site (5 per block) were cut at ground level, brought into the laboratory, and counted to determine stem density, stem length, and branching. Ten stems were also randomly selected from each sample to determine the number of flower-bud clusters and flowers per stem.

Analysis of Variance (CRD) and LS Means Differences ($P \leq 0.05$) were used to compare stem density, stem length, flower-bud clusters per stem, and branching among the treatments. Subplots were pooled within main plots. Data were transformed by the square root to stabilize variance prior to analysis.

**RESULTS:** Stem density did not vary significantly among the production system treatments ($F_{(3,12)} = 1.26$, $P = 0.332$)(Figs. 1 & 2). However, there were significant differences among production system treatments in the other stem measurements. Significantly more flower-bud clusters were found on stems from the high and medium input sites compared with the low and organic sites ($F_{(3,12)} = 25.35$, $P = < 0.0001$)(Figs. 3 & 4). And, high input sites were significantly greater than medium input sites. There was also a significant difference in stem length (Figs. 5 & 6). Stems from high and medium input sites were taller than those from low or organic input sites ($F_{(3,12)} = 4.89$, $P = 0.019$). There was no significant difference in branching ($F_{(3,12)} = 2.20$, $P = 0.141$)(Figs. 7 & 8).

**Fig. 1**. Bar graph showing mean stem density, by site, for each production system. Lines are standard error of the mean.

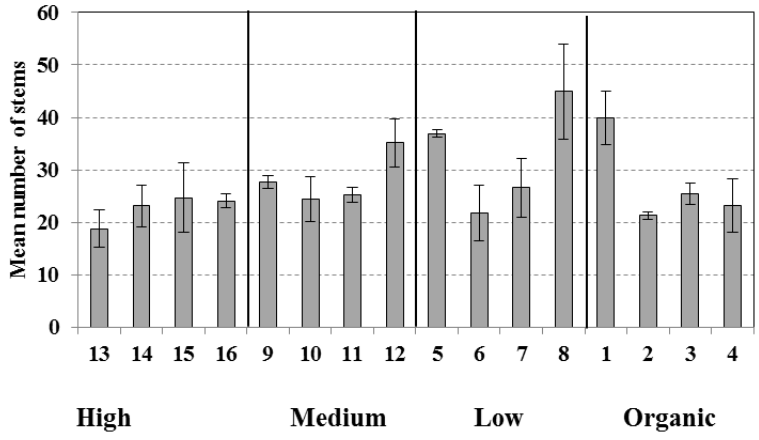


**Fig. 2.** Bar graph showing mean stem density, by production system. Lines are standard error of the mean. No significant differences among the treatments.

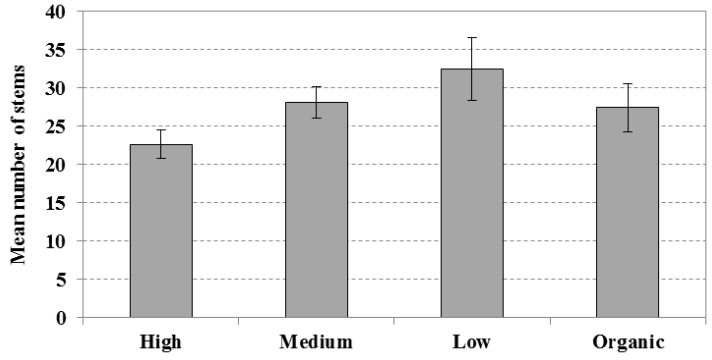



**Fig. 3**. Bar graph showing mean number of flower-bud clusters per stem, by site, for each production system. Lines are standard error of the mean.

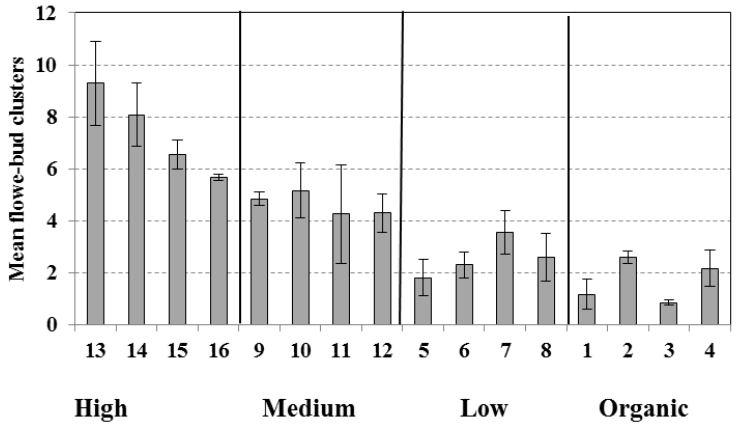



**Fig. 4.** Bar graph showing mean number of flower-bud clusters per stem, by production system. Lines are standard error of the mean.

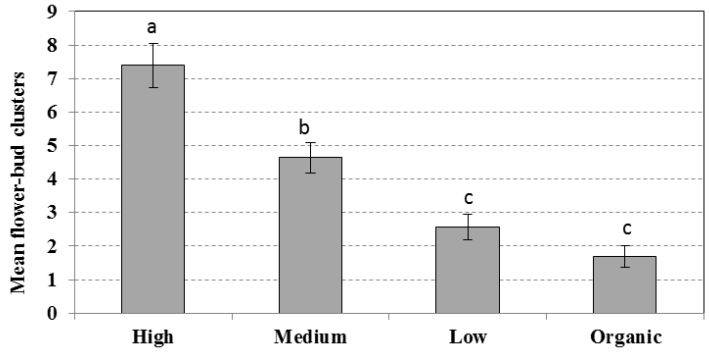


**Fig. 5**. Bar graph showing mean stem length (cm), by site, for each production system. Lines are standard error of the mean.

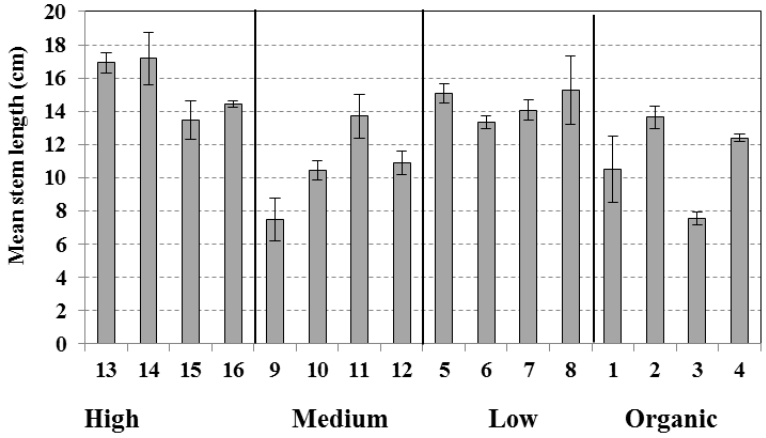



**Fig. 6.** Bar graph showing mean stem length (cm), by production system. Lines are standard error of the mean.

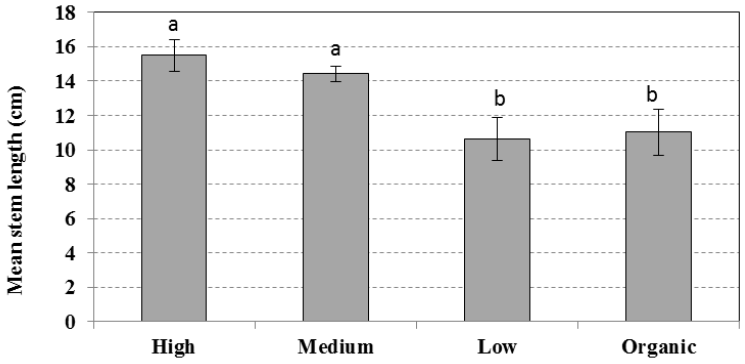



**Fig. 7**. Bar graph showing number of branches per stem, by site, for each production system. Lines are standard error of the mean.

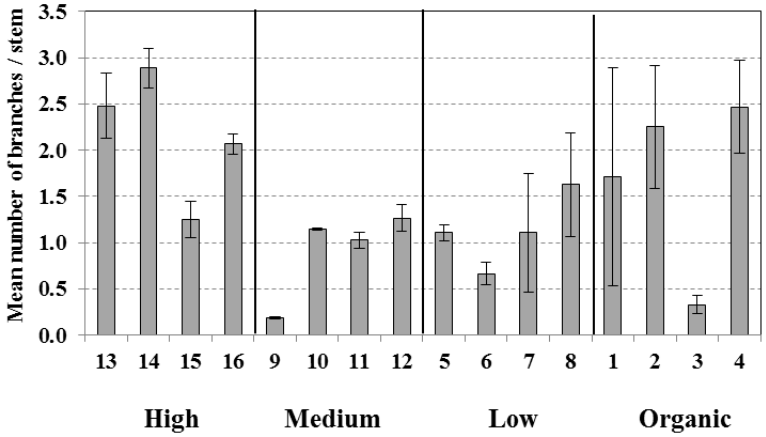
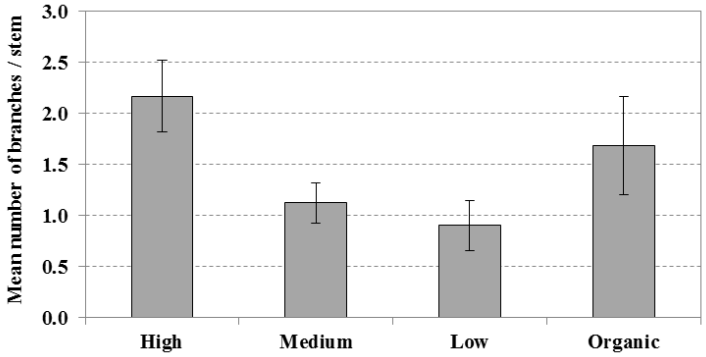


**Fig. 8.** Bar graph showing mean number of branches per stem, by production system. Lines are standard error of the mean. No significant differences among the treatments.




*Blueberry flower-counts and subsequent fruit-set; bee abundance and pollination*

**METHODS:** In mid-May (peak bloom), six blueberry clones were selected within each plot. For each clone, we counted the number of flowers on each of six stems. The stems were marked with numbered metal plant tags. We also recorded stocking density of honeybees for each site. In late June the stems were cut, placed in individual zip-lock bags, and brought into the laboratory where fruit-set was evaluated by counting the number of developing fruit on each stem**.**

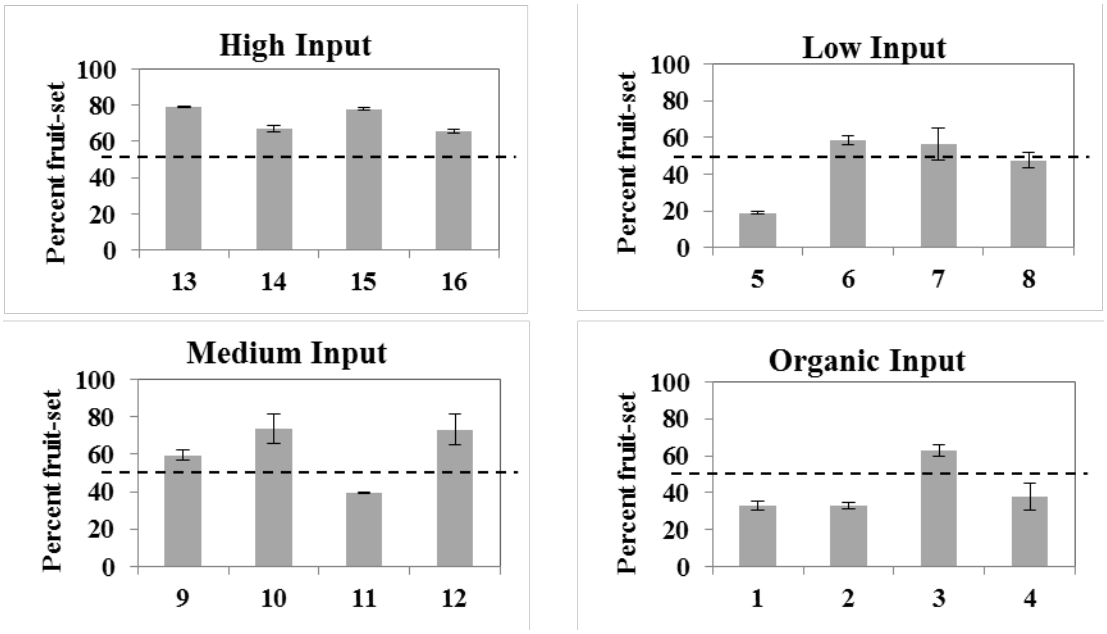
Two different methods were utilized to study bee abundance, colored bowl traps and visual estimates. On three sample dates (17 May, 30 May, and 5 Jun) blue, yellow, and white plastic cups were placed in each plot. There were three replications of each color per plot (total 9 traps per block, 18 per site). Cups were placed such that the top of the cup was even with the top of the blueberry canopy. Each cup was filled ¾ full with water. A drop of unscented dish-washing detergent was added to the water to break the surface tension. Traps were left in the field for 24 hrs. At collection, traps from each block at each site of the same color were pooled and brought back to the laboratory where they were placed in urine cups with 70% ethyl alcohol for sorting and identification. To visually estimate bee abundance, the number of bees (honeybees, bumble bees, and other native bees were counted in each of 16, $m^2$ quadrats per site. For each sample we counted the number of bees observed in 1 minute.

**RESULTS:** There was a significant difference in fruit-set ($F_{(3,12)} = 3.82$, $P = 0.0393$) among the production systems. Figure 1 shows fruit-set for each site within a production system. The results suggest that the organic and low input systems had lower fruit-set than the high input system. In addition, it can be seen that only the high and medium input sites had fruit-set levels higher than expected from background native pollinators alone.

Figure 2 shows the relationship between yield (lbs/acre) and percent fruit-set. A strong relationship exists between fruit-set and yield ($F_{(1,14)} = 15.586$, $P = 0.002$). More than 50% of the variation in yield is explained by fruit-set ($r^2 = 0.526$). However, a central point is that almost 50% of the variation in yield is NOT due to pollination, but most likely disease, weeds, insect pests, and management effects such as fertilizer. It is apparent from figure 3 that yield followed a similar pattern in response to production system as did fruit-set. The high and

medium input systems had significantly higher yields than the organic and low input systems ($F_{(3,12)}$ = 33.52, $P$ = <0.0001).

**Fig. 1.** Percent fruit-set measured in 2013, dashed line is expected background pollination.




When production system and fruit set are used to model yield, 90% of the variation in yield is accounted for ($r^2$ = 0.905). Thus, an additional 35% of the variation in yield is accounted for by adding the type of production system used to produce the yield. Future analyses will attempt to tease out specific factors that might be accounted for by production system, but also others that might be independent of production system such as soil type, etc. It is interesting that including flower-bud clusters/stem* stems/area, as a measure of potential yield and multiplying this by fruit-set did not increase the explained variation in yield compared to just using fruit-set. This suggests that other factors such as disease may be playing a more important role in final yield.

**Fig. 2**. Relationship between yield and fruit-set (percent pollination).

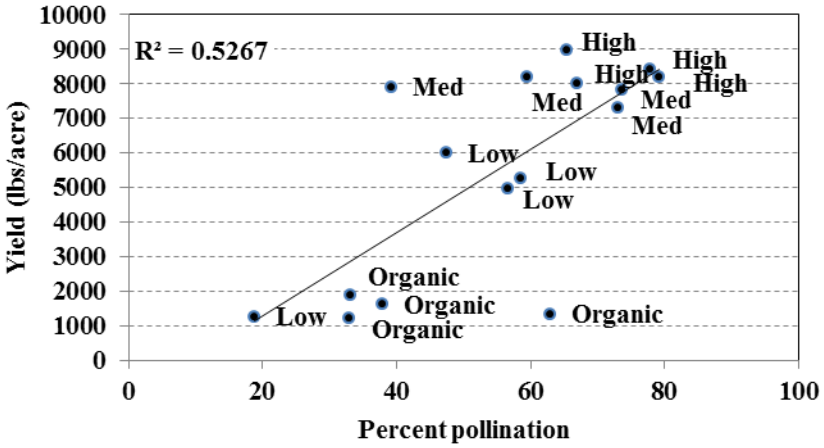
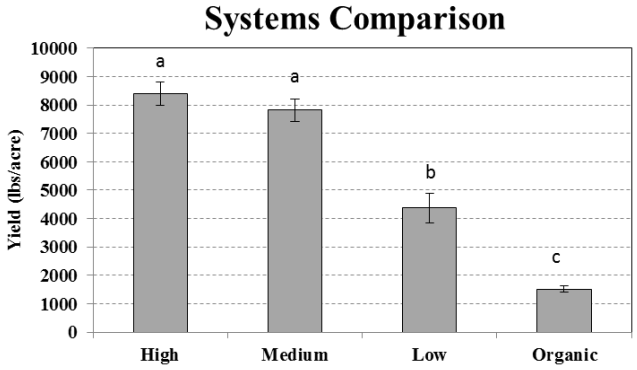


**Fig. 3.** Relationship between yield and production system.




The bee community appears to be sampled at a higher resolution using the bowl traps than when making visual counts in a square meter quadrat for a time period of one minute. This conjecture is arrived at due to the lack of native bees in some fields when visual counts were used to quantify bee communities compared to the bowl trapping. Bee bowls of different colors were not equally attractive to the bee community. For both honeybees and native bees, white bowls were the most attractive and yellow bowls were the least attractive trap ($F_{(2,28)} = 31.92$, $P < 0.05$; $F_{(2,28)} = 31.92$, $P = 0.061$; respectively for honeybees and native bees). However, the quadrat counts provide information that growers can utilize to estimate fruit-set and yield. When looking at the bee bowl trap data we found no evidence to support that importation of honeybees are detrimental to native bee abundance. First, honeybee capture in bowls was independent of native bee capture ($F_{(1,13)} = 0.537$, $P = 0.477$). Honeybee abundance was affected by production system ($F_{(3,11)} = 52.667$, $P < 0.0001$); whereas, native bees were not ($F_{(3,11)} = 1.962$, $P = 0.178$) as shown in figure 4. Honeybee numbers in fields were related to the number of hives assigned to each field ($F_{(1,12)} = 12.703$, $P = 0.004$)(Fig. 5).

**Fig. 4.** Honeybee and native bee relative abundance across the four production systems.

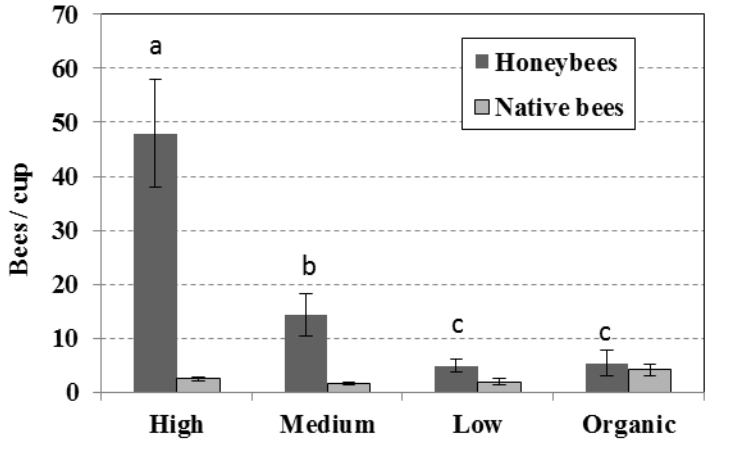
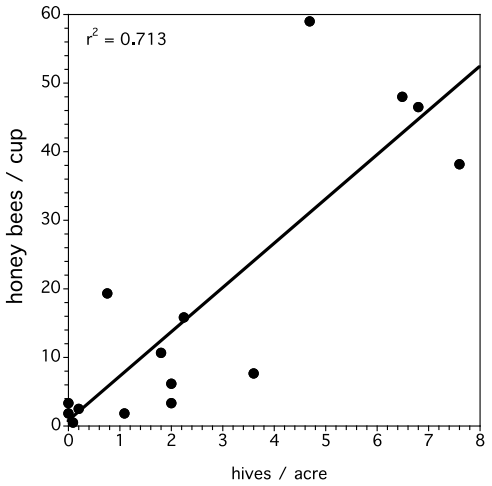
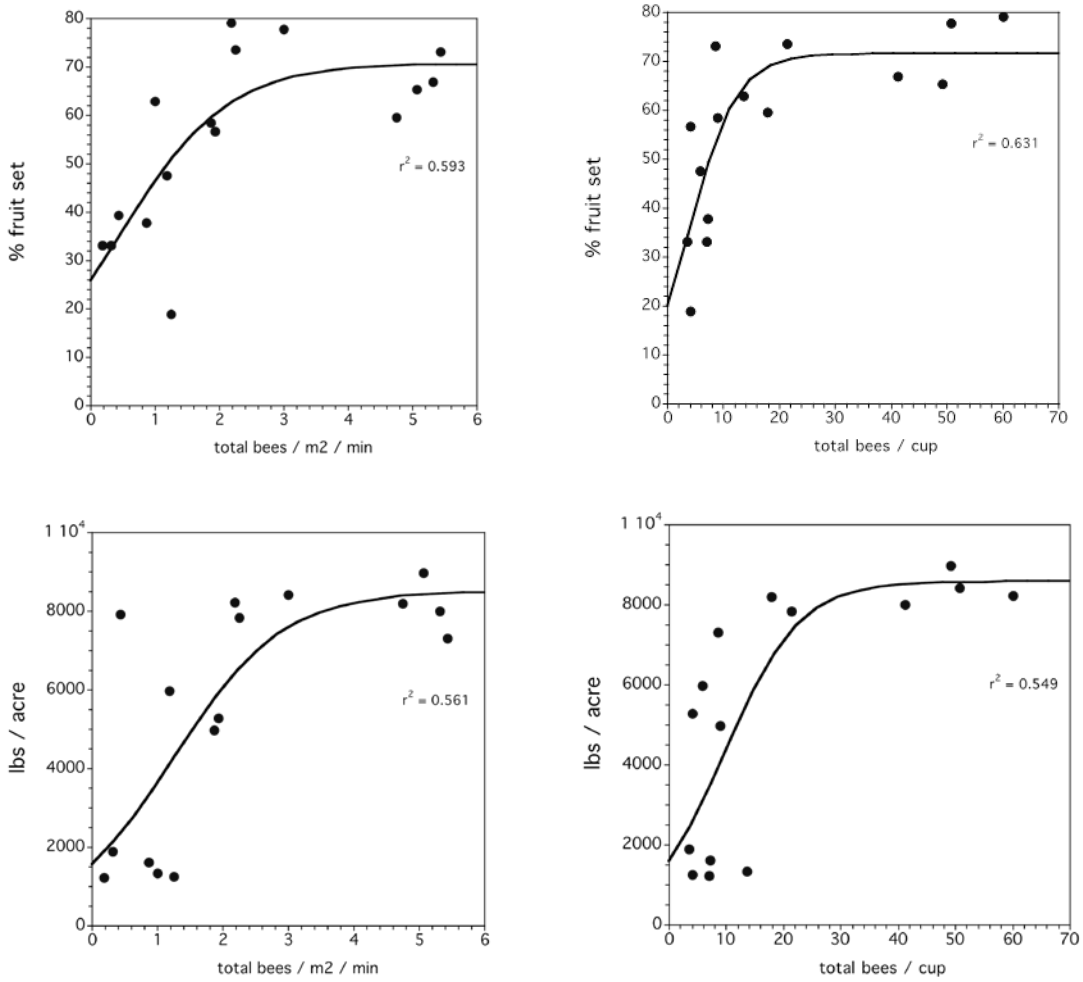


**Fig. 5.** Relationship between hive number at each field site and honeybee abundance in field.




Both bee abundance as measured with cups and bee abundance measured by visual assessment for one minute in $m^2$ quadrats resulted in significant relationships with fruit-set (Fig. 6). Using either measure of bee density results in an asymptotic relationship between fruit-set and yield. This is what would be expected when maximum yield is obtained under field conditions.

**Fig. 6.** Relationship between fruit-set and yield and the resulting bee densities in each field. Graphs on the left use visual counts of bees /$m^2$/min, while graphs on the right use bees captured/bowl in 24 hrs.




Initial stem density did not vary by production system. However, flower-bud clusters/stem and stem length were a function of production system; medium and high input had significantly greater flower-bud clusters/stem and greater stem length than the other two production systems. Pollination level, as measured by fruit-set, was seen in 2013 to be a function of production system and bee abundance or density in fields. A little more than 50% of the variation in yield was accounted for by fruit-set; however, incorporating the number of flower-bud clusters per field did not improve the prediction of yield from fruit-set. Taking into account the differing numbers of flower buds per unit area did not improve the prediction of yield from fruit-set. Modeling yield using both fruit-set and production system yielded a highly predictive model that production system variation, such as differences in disease, insect pests, weeds and fertility; may account for the differences in yield. Bee density did explain a significant amount of the variation in both fruit-set and yield. This is important as 2013 will be an additional year that can be used to model the economics of bee density, hive stocking rates, and resulting yield.

*Abundance of natural enemies and pest insects; sweep-net survey*

**METHODS:** Because of unusually wet and cold temperatures, only seven of the 16 sites were sampled (three organic and four low input). Samples were taken on 10 or 16 May. Five sets of ten sweeps each were taken from each block (two blocks/site) with a 12-inch diameter sweep net. Samples were distributed through the block with one sample being taken from each quadrant and one from the middle area. The number of insects and spiders of each species was counted and then returned to the same plot. Sweep-net data was analyzed using Analysis of Variance ($P \leq 0.05$). Subplots were pooled within main plots.

**RESULTS:** Ants and spiders were the most abundant natural enemies (Fig. 1). There was no significant difference in the number of ants or spiders between the low input and organic production system treatments ($F_{(1,5)} = 1.46$, $P = 0.2810$, ants; $F_{(1,5)} = 0.24$, $P = 0.6469$, spiders). Pest abundance was very low, with no pest exceeding threshold numbers during the season. The most abundant pest insects found in sweep-net samples were blueberry spanworm larvae, strawberry rootworm adults, and grasshoppers (Fig. 2). The differences were not significant ($F_{(1,5)} = 0.89$, 0.49, and 0.51; $P = 0.3885$, 0.5136, and 0.5084; respectively). Small numbers of tarnished plant bugs, leaf beetles, and cutworms were also found in the samples.

**Fig. 1.** Relative abundance of natural enemies in sweep-net samples.

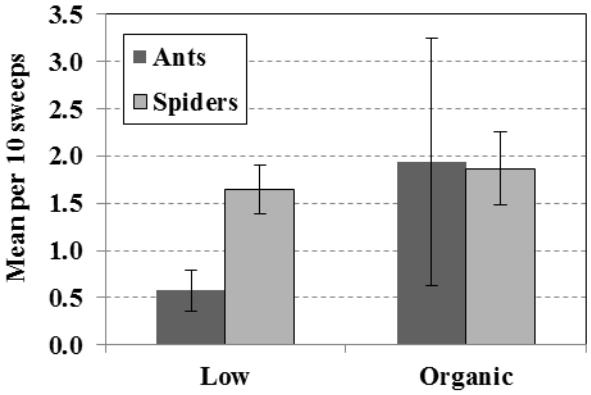
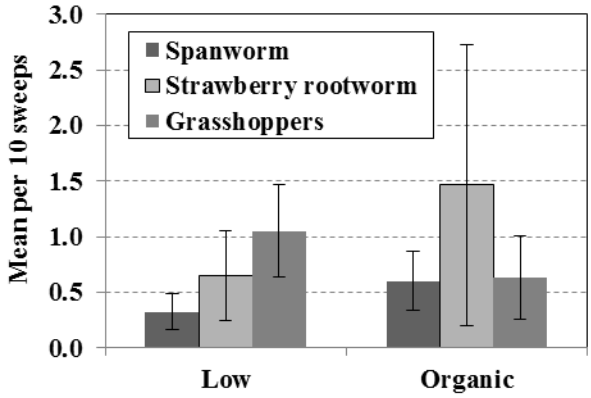



**Fig. 2.** Relative abundance of pest insects in sweep-net samples.



*Blueberry maggot fly monitoring*

**METHODS:** To monitor blueberry maggot fly (BMF), one baited yellow Pherocon® AM trap was placed in each block. Traps were checked at 3 to 7 day intervals. Any captured BMF were counted and removed from the traps. To measure fruit infestation, we raked four quarts of berries from each block. To collect BMF pupae, the berries were combined and distributed in a 1 to 2-inch deep layer in screened boxes suspended over ca. 2 inches of fine sand. Hardware cloth (1/4 in) was used as a screening material. In late-October, BMF pupae were separated from the sand. Analysis of Variance (CRD) and LS Means Differences ($P \leq 0.05$) were used to compare seasonal density of adults and the number of pupae per quart of fruit among the treatments.

**RESULTS:** There was a significant difference in the seasonal density of adults (integration of abundance over the season) with organic sites being the highest ($F_{(3,12)} = 5.10$, $P = 0.0166$)(Fig. 1). This also held true for fruit infestation. Organically-managed sites had significantly more pupae per quart of fruit ($F_{(3,12)} = 5.69$, $P = 0.0116$)(Fig. 2).

**Fig. 1**. Bar graph showing seasonal density of adults, by production system. Lines are standard error of the mean.

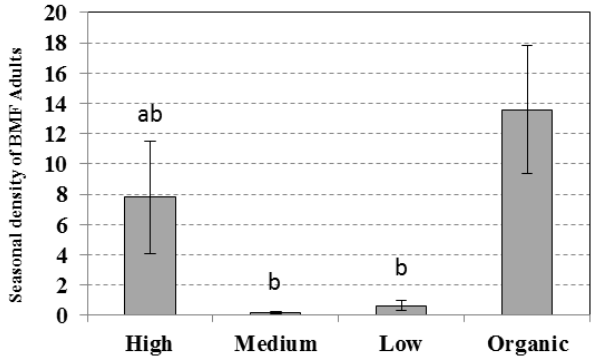
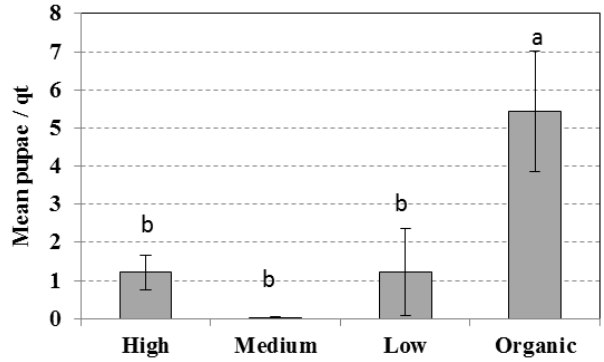



**Fig. 2**. Bar graph showing mean BMF pupae, by production system. Lines are standard error of the mean.



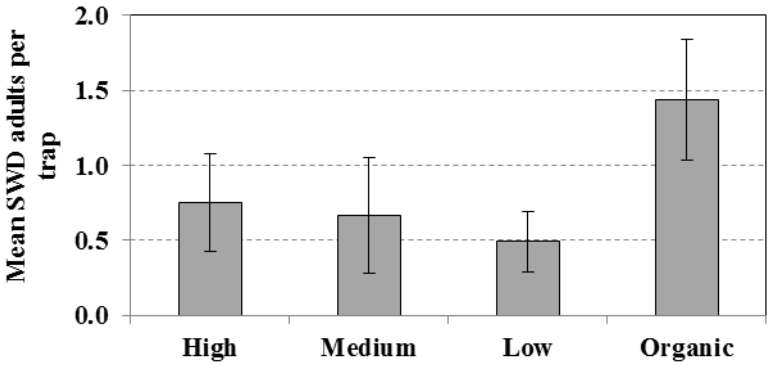

*Influence of production system on spotted wing drosophila abundance*

**METHODS:** Beginning on 3 or 5 Jul and continuing through Aug, one trap was set in each block per field (2 traps at each site) and monitored weekly for the presence of spotted wing drosophila (SWD) adults. Two additional trap sites were set on 5 Jul at Blueberry Hill Farm.

Traps were constructed from Solo®, 16 fl. oz, red polystyrene cups with clear lids. Seven to 10, 3/16-inch holes were punched on the side of each container near the top, evenly spaced around the rim. Bait consisted of live yeast (1 tbsp) + sugar (4 tbsp) + 12 oz water (makes enough for 4 traps). The traps were hung 1-2 ft above the top of the canopy using 36' plant stands. On each sample date, traps set from the previous week were collected and returned to the laboratory where male, female, and total abundance of SWD were determined and recorded. Using this data we calculated the mean SWD per trap captured from each site between 9 or 10 Jul and 13 Aug (n= 10 or 12 sample dates).

**RESULTS:** Figure 1 shows the effect of production system on adult SWD captures. Although there appears to be a trend towards more SWD in organically-managed fields, the difference was not significant (ANOVA, CRD, $P = 0.2651$). This does not take into account the effect of any insecticide applications. Information on applications is still being collected.

**Fig. 1.** Bar graph showing SWD adult abundance from 9 or 10 Jul until 13 Aug. Lines are standard error of the mean.



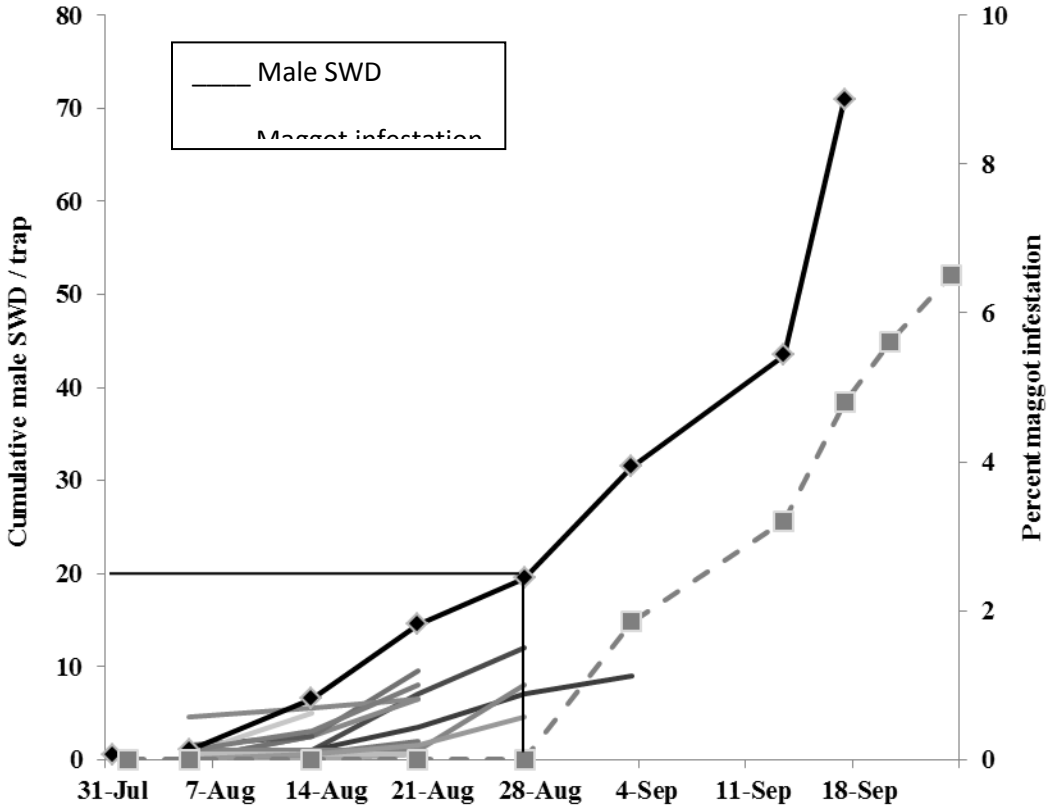

*Comparison of adult abundance of spotted wing drosophila with larval fruit infestation*

**METHODS:** To compare adult abundance with fruit infestation, samples were taken on various dates from late Jul until early Sep and processed for larval infestation using the Salt Extraction Method. Each sample consisted of 2 or 3: 2/3 cup fruit taken from each block per site as well as from an organic field in Cherryfield, and from Blueberry Hill Farm. There were ca. 359 berries per 2/3 cup sample.

**RESULTS:** A comparison of SWD male abundance and percent fruit infestation is shown in figure 1. It appears from these data that one can wait until 20 cumulative male SWD captures/trap before getting any larval infestation. This level was only reached at one of 18 sites. In 2012, we found no significant difference in SWD populations among production systems. In 2013, the pattern appears to be the same; although, some of the higher populations were from organic fields. This may be due to the intensive insecticide control program that was implemented in many of the conventional blueberry farms.

The data assessing action thresholds are certainly preliminary, but in 2013, it appears that trap captures of up to 20 cumulative male SWD can be tolerated before larval infestation begins to be detected. In the next several years we will continue to refine the action threshold. However, it certainly appears that a threshold of a single fly is on the conservative side.

**Fig. 1.** Comparison of SWD male abundance and percent fruit infestation (dashed line is maggot infestation and solid lines reflect SWD adult captures).




**CONCLUSIONS:** At this point we plan an integrated analysis of the data. Plans for such an analysis will be conducted in the near future. The approach will be to assess the interactions between plant growth, soil fertility, disease, weeds, and insect pests.

**INPUT SYSTEMS STUDY**

**DISEASE:** Seanna Annis, Assoc. Professor, School of Biology and Ecology
Edward Bernard, Blueberry Disease Research Assistant
Caleb Slemmons, Blueberry Disease Research Assistant
Tamara Levitsky, Research Assistant

**16. TITLE:** Systems approach to improving the sustainability of wild blueberry production, Year 4 of a four-year study, disease management results.

**METHODS:**

*Disease Sampling*

Diseases were rated by block twice during the season, once in May from 5/21 to 5/30 and a second time in the fall from 9/17 to 10/2. In May, mummy berry (*Monilinia*) was rated by 2 surveyors within each site and in each block. Mummy berry was rated on 30 random stems along four transects of 45 ft within each block. The number of frost damaged stems was also counted. The percentages of stems with mummy berry disease and frost damage were calculated. In the fall, leaf spot and stem diseases were rated within each block in the following manner: 5 sampling plots of $0.25m^2$ were rated by at least 2 surveyors visually estimating percentages of blueberry coverage, blueberry leaf loss, blueberry stems with Phomopsis, and blueberry leaf area with the following leaf spot diseases: Septoria leaf spot, powdery mildew, red leaf, leaf rust and false Valdensinia. Fall disease ratings were averaged across the surveyors by sampling plot and then across all 5 sampling plots within a block before analysis.

Data were analyzed at the management input level for differences in blueberry cover, disease coverage and leaf loss in SAS (Statistical Analysis Software - SAS Cary, NC) using mixed model procedures (PROC GLIMMIX). Blueberry cover, powdery mildew, and leaf rust were logit ($\log(x/(1-x))$ transformed, Septoria leaf spot and Phomopsis disease measures were arcsine square root transformed, and blueberry leaf loss data were normal and therefore did not require transformation. Least Square means were used to determine specific differences among system types ($\alpha = 0.05$). Data were analyzed at the block and field level with untransformed data for correlations amongst different measures of disease, blueberry cover and leaf loss using Spearman's rank correlation in SAS (PROC CORR). Untransformed data is shown in all graphs.

**RESULTS AND DISCUSSION:** Mummy berry disease was found in all of the fields, with the exception of one field under the organic management system (O3). The majority of the fields had low levels of mummy berry disease which ranged from 0 to 32 % of stems affected among the fields, although most fields fell within the 1 to 12% range (data not shown). The organic and medium input management systems demonstrated significantly lower percentages of stems affected by mummy berry than both the low and high input systems (Fig. 1).

Blueberry cover was significantly lower in the organic input system when compared to all other management systems (Fig. 2). However, leaf loss was also significantly lower in the organic management system than all other systems (Fig. 3). The highest levels of leaf loss were found in the high input system. False Valdensinia was not detected in any fields in 2013, however this may be due to the extensive leaf loss observed. For all leaf spot diseases, the percentage of plots affected by a particular disease was not significantly different between input management types

in 2013 (Figs. 4A, 5A, 6A, 7A). However, there were significant differences in the percent of leaf area coverage by these diseases by management system.

Red leaf disease levels were quite low in 2013, ranging from no red leaf detected (0%) to 1.5% and red leaf was only detected in 5 of the 16 fields in 2013 (data not shown). However, leaves affected by red leaf usually fall off in September or October, making red leaf disease assessments later than ideal. Mid-July would have been the ideal time to rate for red leaf disease, but this was pre-harvest and it was not possible to access the fields at this time. Despite the organic input system fields demonstrating significantly higher levels of red leaf than the high input system fields (Fig. 4B), the difference between the organic input system and the high input system is likely due to the fact that three of the five fields where red leaf was detected were under the organic input system (O2, O3, and O4), which demonstrated lower levels of leaf loss and probably allowed the disease to be detected more readily.

Powdery mildew was detected in all but one field in 2013 (O4). Though a large percentage of plots had powdery mildew (Fig. 5A), there was a low overall percentage of leaf area affected by this disease. The percentage of leaf coverage by powdery mildew (Fig. 5B), like the percentage of plots affected by powdery mildew, was not significantly different among the input systems.

Septoria leaf spot, like powdery mildew, were more prevalent in 2013 than the other measured diseases. Septoria was detected at a level of at least 60% in all fields in 2013 (data not shown). Input system also appeared to have a significant effect on Septoria leaf spot, with Septoria leaf coverage lower in the high input system than any other system (Fig 6B). Septoria leaf spot was highest in both the organic and low input systems, which typically do not spray fungicides for this disease.

Leaf rust was detected in all fields in 2013, but with a much wider range (10%-100%) of plots affected than what was found for Septoria. Percent coverage of leaf rust was significantly lower in both the organic and medium input systems than either the high or low input systems (Fig. 7B).

Phomopsis stem disease was found in all medium input fields in 2013, three out of the four low and high input fields, but only one field under the organic management input (O1) had Phomopsis present. This is reflected in the percent coverage by Phomopsis, which was significantly different among all of the management input systems with organic system fields have the lowest levels (Fig. 8). The percentage of stems with Phomopsis was not related to average stem densities or stem lengths as measured in the fields in April (data not shown; stem density and length data measured by F. Drummond's lab).

There were no significant correlations among mummy berry disease with yield, blueberry cover, and leaf loss which were all measured later in the season in 2013. Preliminary analyses of leaf spot diseases have suggested that there may be a correlation between decreased Septoria leaf spot levels and increased yield (Fig. 9), but decreased Septoria leaf spot levels also correlated with increased numbers of flower buds and longer stem lengths. There may not be a direct effect of Septoria leaf spot on yield and may rather be an effect of plant health on leaf spot levels. Further analysis will try to determine the effects of management practices on disease levels.

**CONCLUSIONS AND RECOMMENDATIONS:** Management inputs can affect the level of leaf and stem diseases present during the crop year. For instance, percentage of stems with mummy berry disease was lower in the organic and medium input systems when contrasted with the low and high systems. Leaf rust also followed this trend, while effects on other diseases were a bit more complicated. Whether or not these effects may translate into measurable

increases in yield is still under investigation. Furthermore, the levels of stem and leaf diseases in the prune year must be taken into account when considering effects of management practices on increasing yield and reducing disease. Once these relationships are better understood, recommendations will be made accordingly.

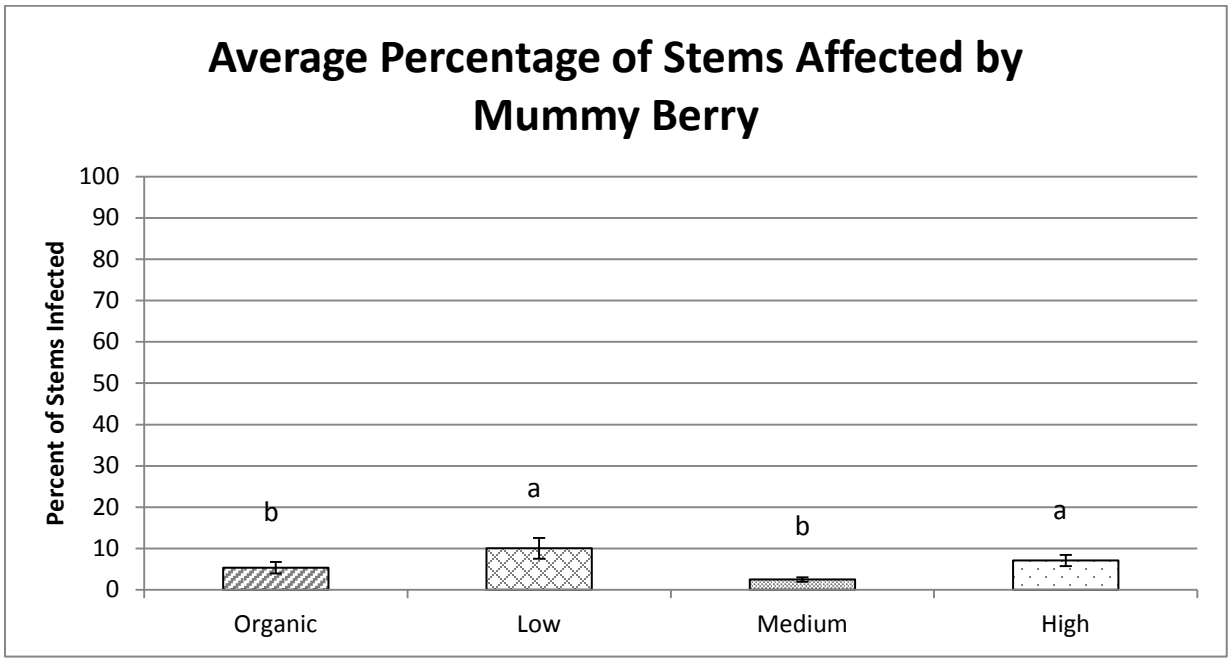



**Fig. 1 –** Average percentage of stems affected with mummy berry disease by management input type for 2013. Error bars indicate standard error of the mean. Bars with different letters indicate statistically significant differences at $\alpha = 0.05$.

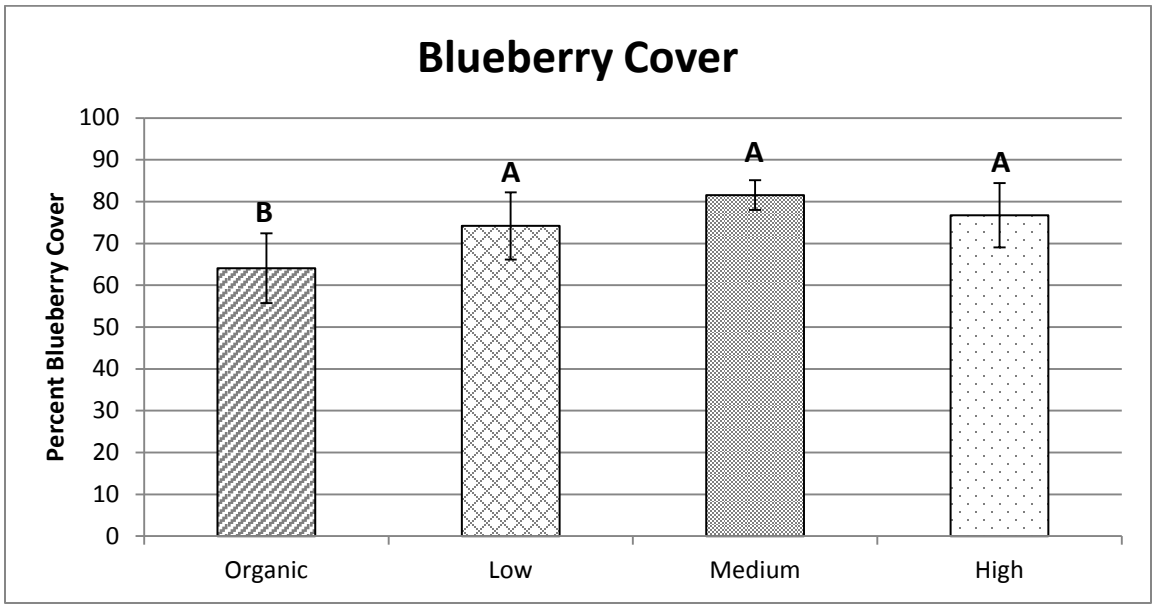



**Fig. 2 -** Average percent of blueberry cover by management input types for 2013. Error bars indicate standard error of the mean. Bars with different letters indicate statistically significant differences at $\alpha = 0.05$.

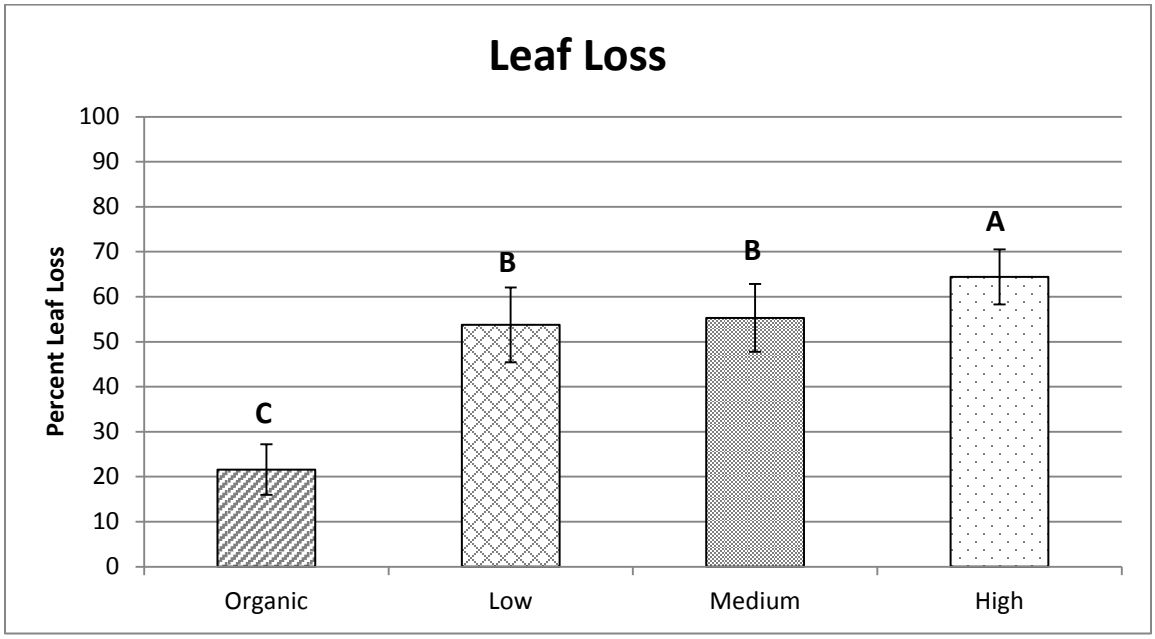



**Fig. 3 –** Average percent leaf loss by management input types for 2013. Error bars indicate standard error of the mean. Bars with different letters indicate statistically significant differences at $\alpha = 0.05$.

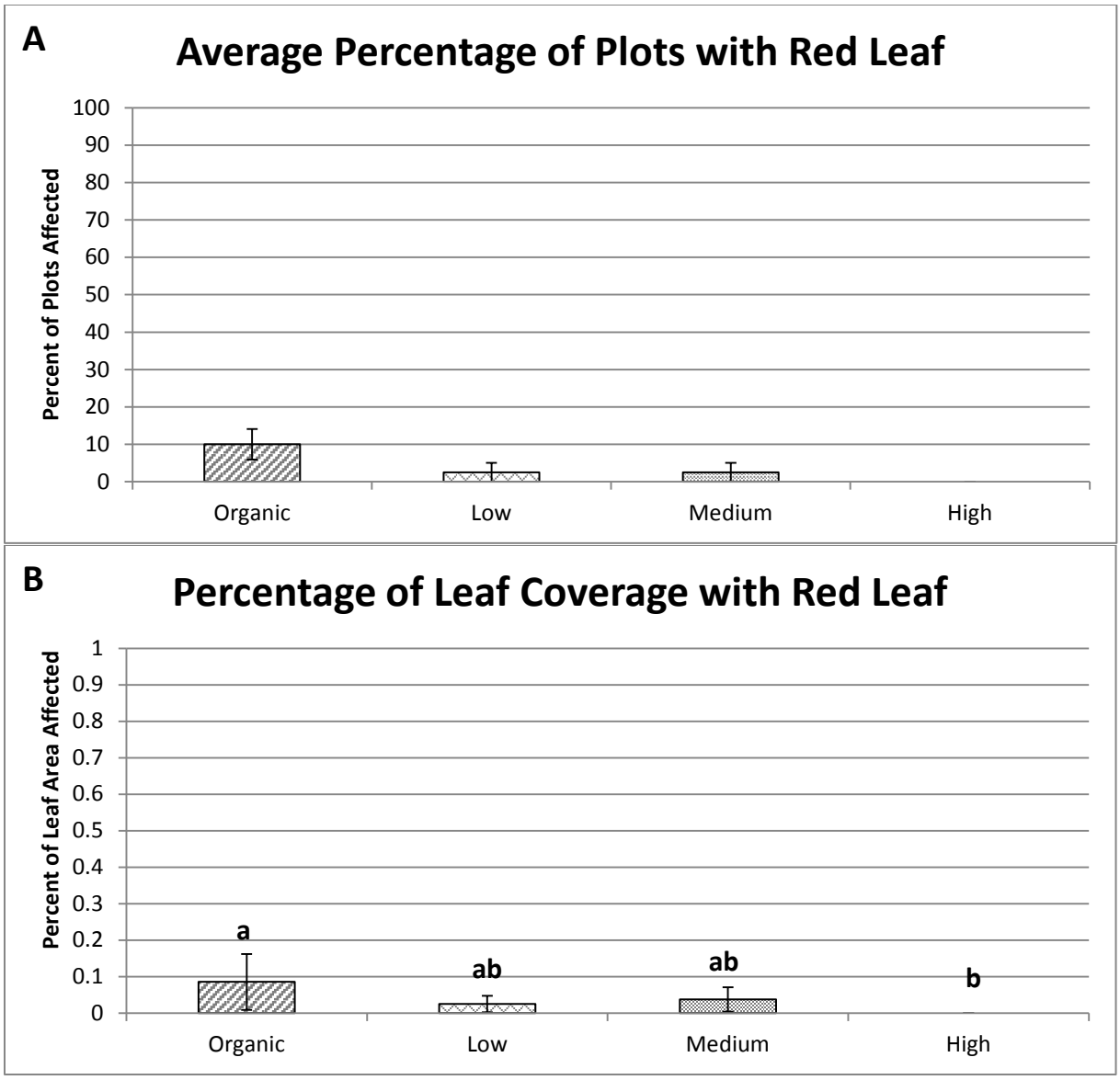



**Fig. 4 –** Average percent of plots affected by red leaf (A) and average percent of leaf coverage with red leaf disease (B) by management input types for 2013. Error bars indicate standard error of the mean. There were no significant differences in the percent of plots affected by red leaf by management type. Bars with different letters within graph indicate statistically significant differences at $\alpha = 0.05$. Note the change in scale from (A) to (B).

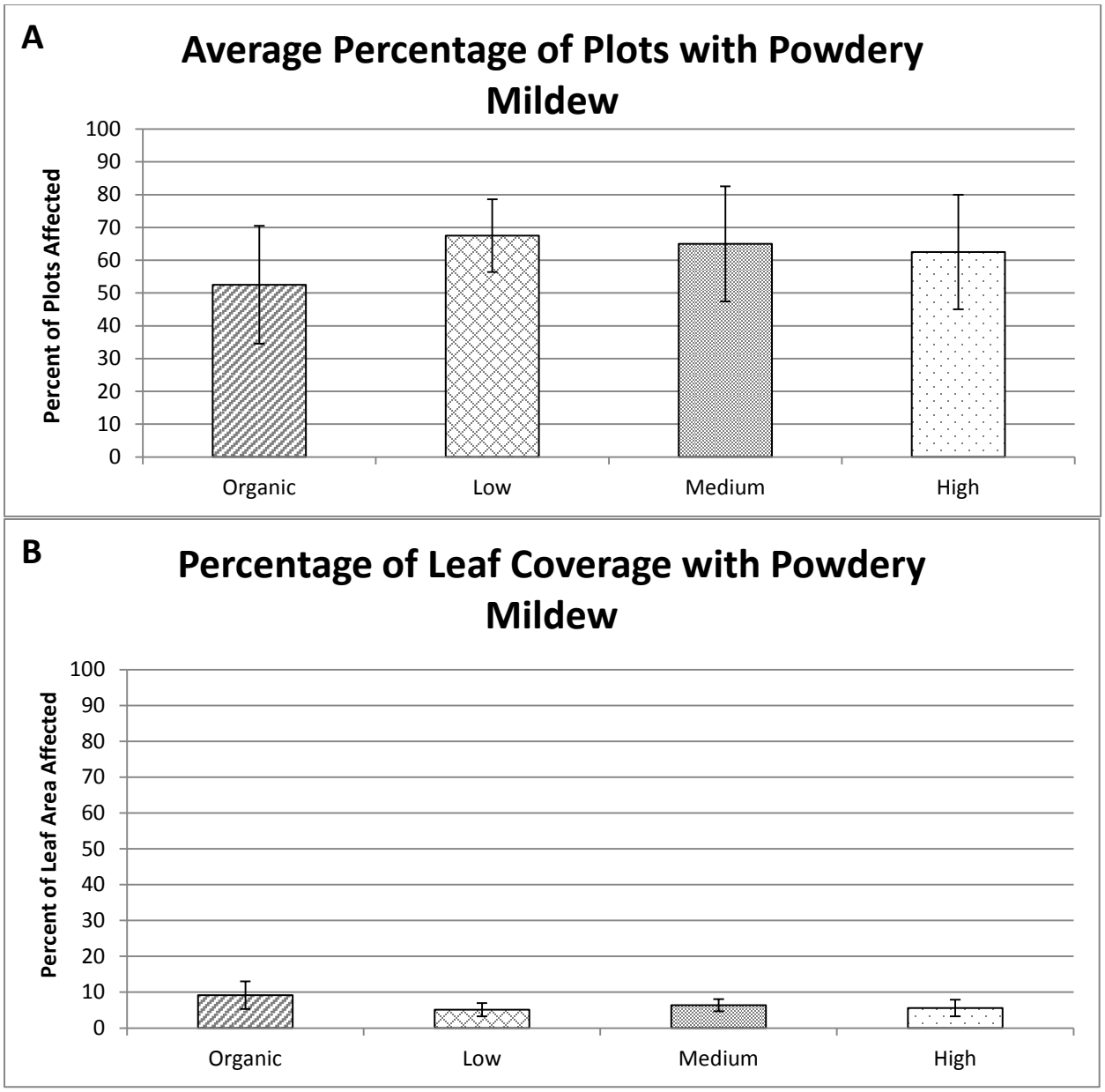



**Fig. 5 –** Average percent of plots affected by powdery mildew (A) and average percent of leaf coverage with powdery mildew disease (B) by management input types for 2013. Error bars indicate standard error of the mean. There were no significant differences in percent of plots affected by powdery mildew or in the percentage of powdery mildew leaf coverage between management inputs.

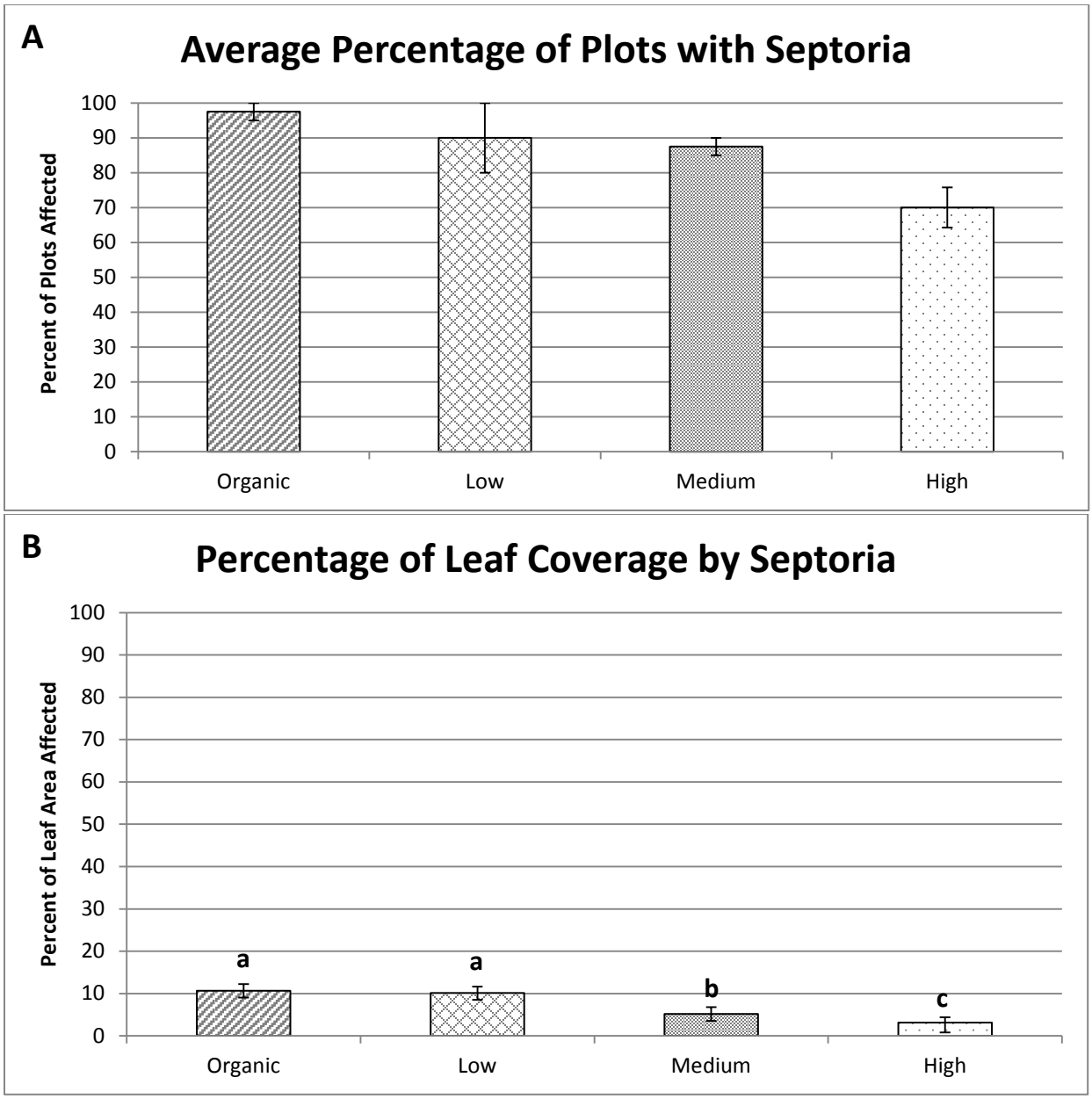



**Fig. 6 -** Average percent of plots affected by Septoria (A) and average percent of leaf coverage with Septoria disease (B) by management input types for 2013. Error bars indicate standard error of the mean. There were no significant differences in percent of plots affected by Septoria. Bars with different letters indicate statistically significant differences at $\alpha = 0.05$.

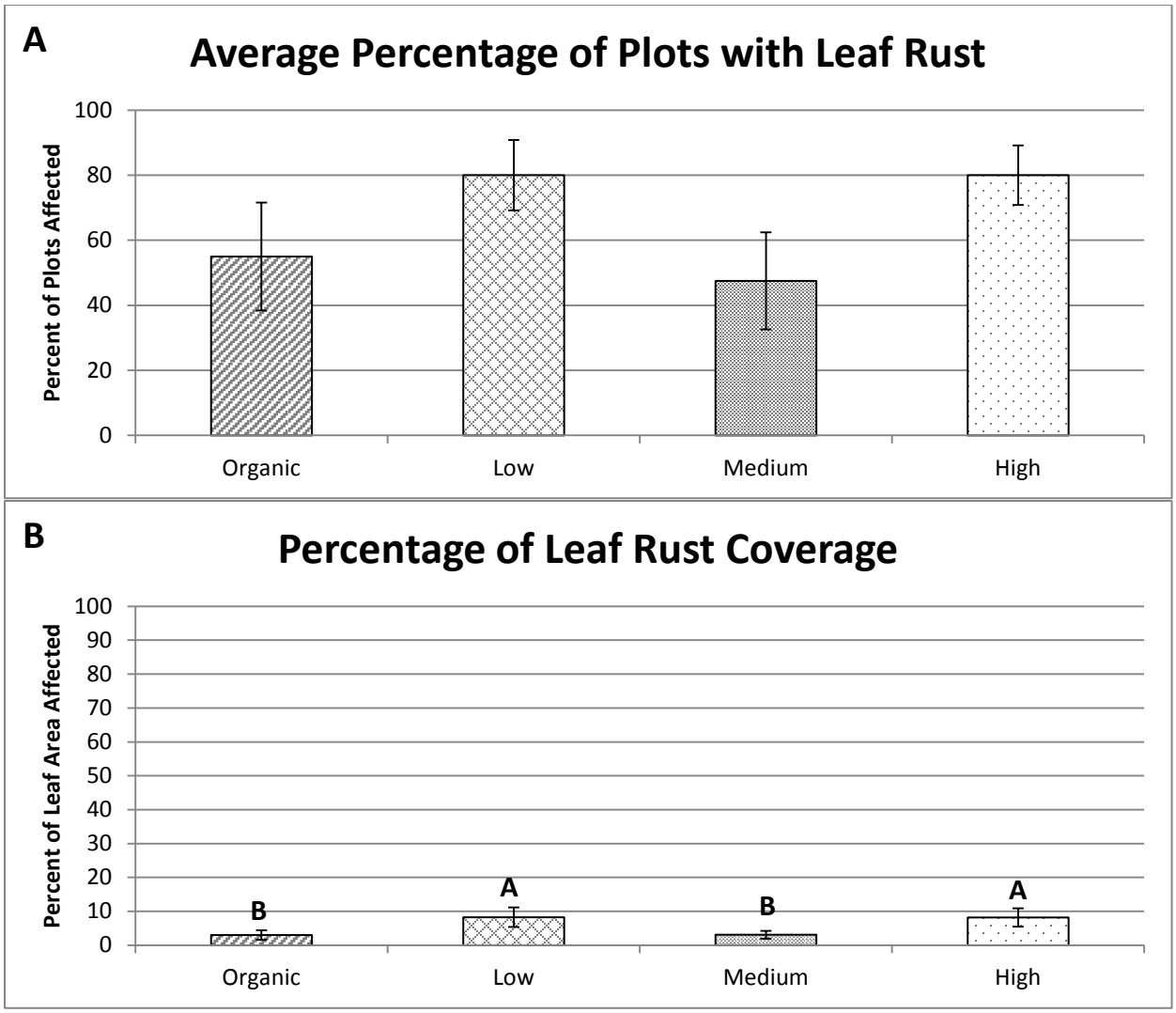



**Fig. 7 -** Average percent of plots affected by leaf rust (A) and average percent of leaf coverage of leaf rust disease (B) by management input types for 2013. Error bars indicate standard error of the mean. There were no significant differences in percent of plots affected by leaf rust. Bars with different letters indicate statistically significant differences at $\alpha = 0.05$.

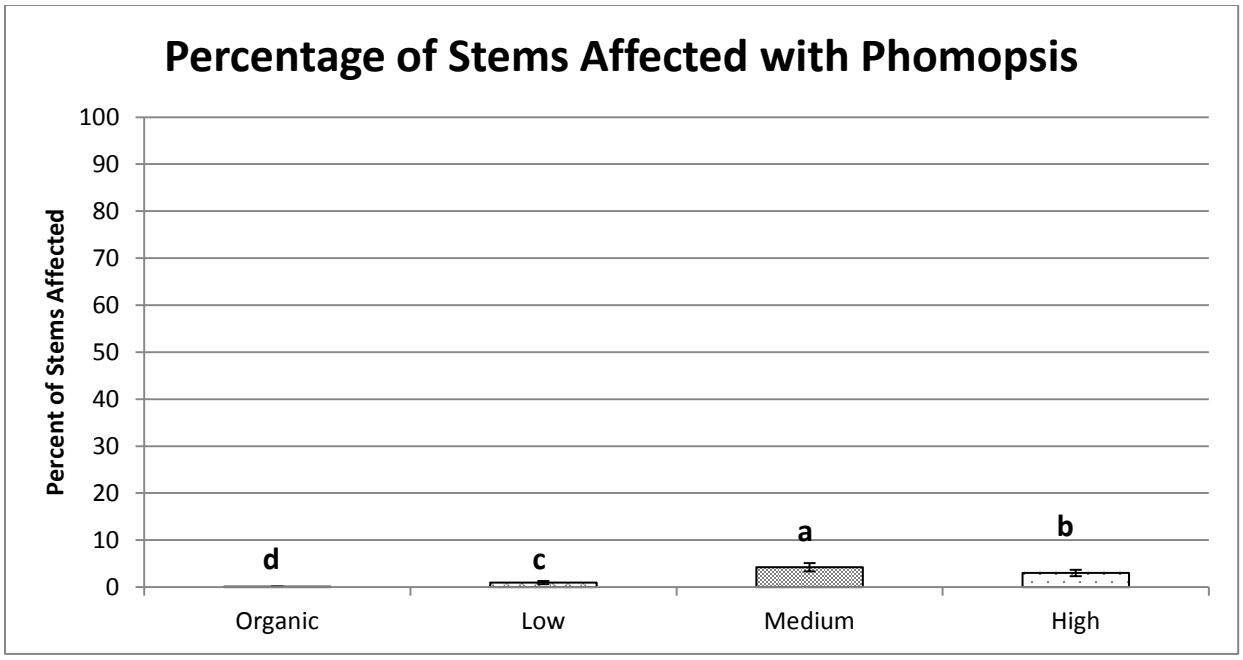



**Fig. 8 -** Average percent of stems affected by Phomopsis disease by management input types for 2013. Error bars indicate standard error of the mean. Bars with different letters indicate statistically significant differences at $\alpha = 0.05$.

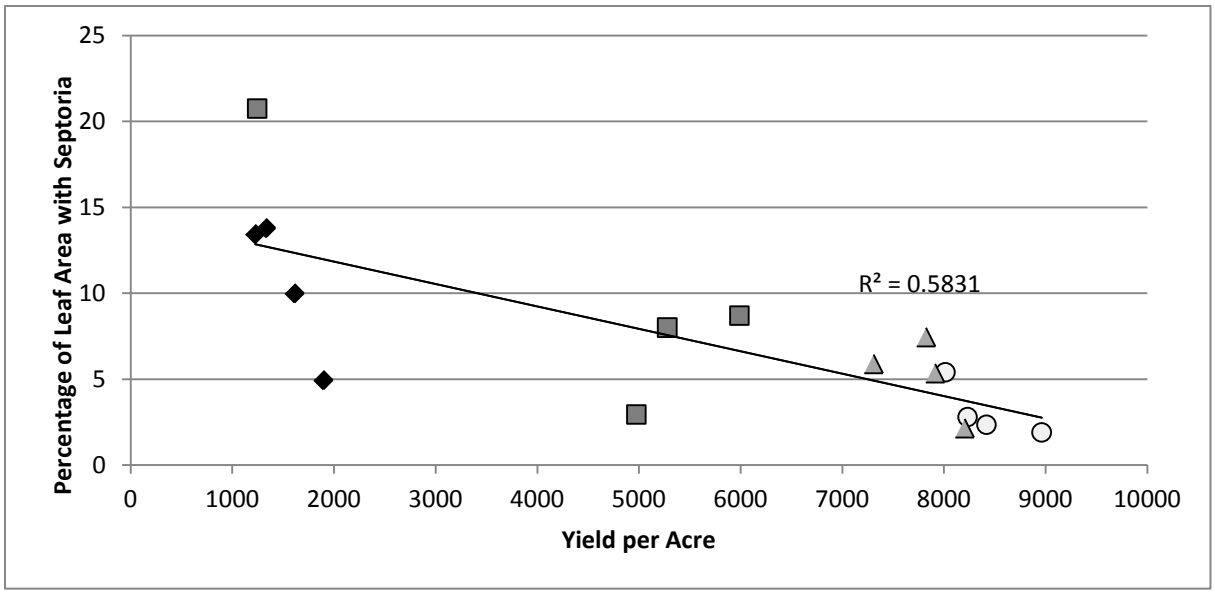



**Fig. 9 –** Average percentage of leaf area affected with Septoria versus yield by management input system. The solid line represents a best fit linear regression line for all management inputs ($R^2 = 0.5831$). Diamonds represent organic input systems, squares represent low input systems, triangles represent medium input systems and circles represent high input systems.

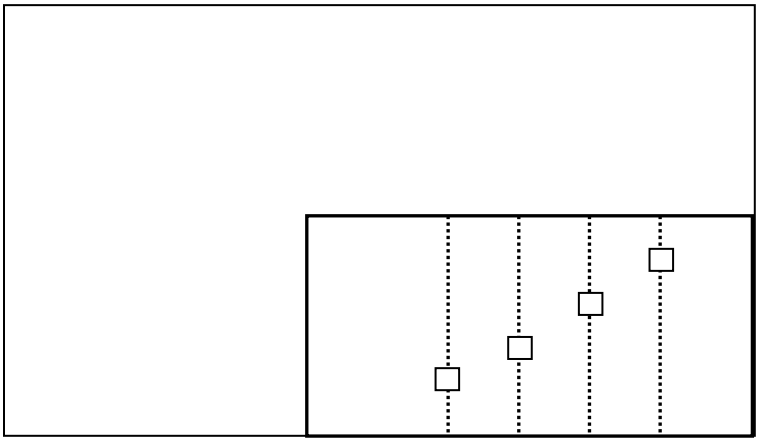
**INPUT SYSTEMS STUDY**

**WEED MANAGEMENT:** David E. Yarborough, Professor of Horticulture
Jennifer L.D. Cote, Assistant Scientist

**17. TITLE:** Systems approach to improving the sustainability of wild blueberry production, Year Four of a four-year study, weed management results.

**METHODS**: The second crop cycle study design and 2013 crop year inputs are listed in Table 2 of the overall Experimental Design (Report #12). In 2012 16 trial sites were set up containing two 1 acre blocks each with 15 x 30 m sub-blocks; along the 30 m baseline (the outer long edge of the block) of each sub-block, four transects were located 5 m apart in order to set up 1 $m^2$ sample plots to assess weed cover. One 1 $m^2$ sample plot was staked on each transect 3 m apart so that the sample plots ranged diagonally across the subplot (Figure 1). In this cycle, the Organic system sample plots were not paired with mulched plots as was done in the previous cycle.

**Figure 1.** Example layout of a block, sub-block, transects and weed sample plots (not to scale).



Blueberry cover, woody weed cover, broadleaf weed cover and grass cover were assessed in all 1 $m^2$ sample plots on 4-5 June 2013 and late July/early August (24 July-5 August; two Organic sites could not be evaluated until day of harvest). Covers were assessed using the Daubenmire Cover Class scale, which were converted to percent; weed species were also identified. The data were analyzed using the Nested General Linear Model (SAS 9.4) and Tukey's HSD tests for significant differences ($\alpha$=0.05). Overall blueberry cover and weed cover comparisons were made among all four input systems.

All sites were harvested between July 30 to August 5 to compare yields among input systems. Detailed methods and general comparisons are included in the overall Experimental Design Report. Weed cover as a determining factor for yield is presented two different ways in the Results section. Woody, broadleaf and grass weed covers were combined for an overall % weed cover (if the sum was >100, then the value was 100%). First, a contrast comparison was performed on the weed cover (x) vs. yield (y) for each input system, to determine what type of relationship, if any, existed between weeds and yield (e.g. linear, quadratic, cubic, etc.). Weed cover was also arcsine transformed to normalize the data for analysis, and compared to yield to

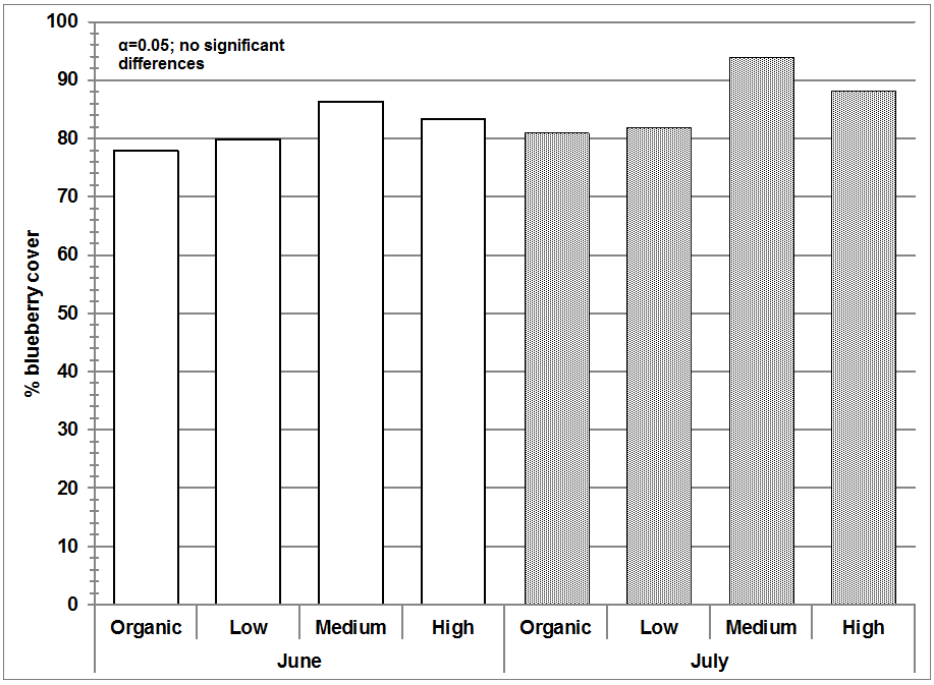
determine how much variation in yield was due to input system and how much was due to weed cover. For the purposes of this report, the arcsine-transformed weed data are graphed as percent cover.

**RESULTS**:

*Blueberry and weed cover*

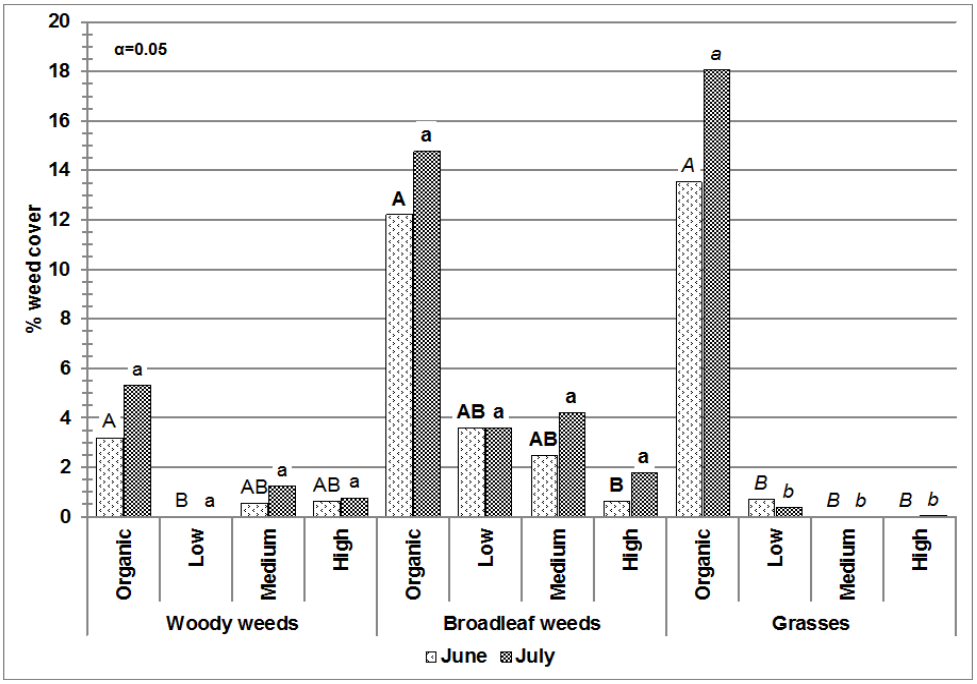
There were no significant differences in blueberry cover among the four systems at either evaluation (Figure 2). However, in 2013 blueberry cover followed a similar trend as in 2011 and 2012; by the second evaluation, the Medium input system had the highest blueberry cover, followed by the High input system.

**Figure 2**. Wild blueberry cover by input system in crop year 2013 (Tukey's HSD, α=0.05).




Woody weed cover was low overall; cover in the Organic system was significantly higher than in the Low system in June, while the Medium and High systems were not different from any other system (Figure 3). By July, there were no significant differences among systems. Broadleaf weed cover remained about the same level as 2012 overall. Percent cover was highest in the Organic system at both evaluations, but was only significantly higher than the High system in June. The three conventional systems were not different from each other. Grasses were also highest in the Organic system at both evaluations, and at both evaluations the Organic system was significantly higher than the conventional systems which were not different from each other. Grass cover in crop year 2013 was roughly the same as in prune year 2012.

**Figure 3**. Woody weed, broadleaf weed and grass cover among input systems in crop year 2013 (Tukey's HSD, different letters denote significance at α=0.05).




*Yield vs. weeds*

When weed cover by input system was compared to yield to find out what type of relationship weeds and yield had (e.g. linear, quadratic, cubic, etc.), there were no significant relationships (Figure 4). However, when input system and weed cover were both used as independent predictor variables in a regression to predict what was driving variations in yield, there was a negative relationship between weed cover (arcsine transformed but presented as % cover) and yield (Figure 5). Input system was significant at $\alpha<0.0001$, while weed cover was not significant at $\alpha=0.10$. Input system accounted for 56 % ($R^2=0.555$) of the variation in yield, while weed cover accounted for 18 % ($R^2=0.184$).

**Figure 4**. The relationship between weed cover by input system and yield (α=0.05; no significant relationships).

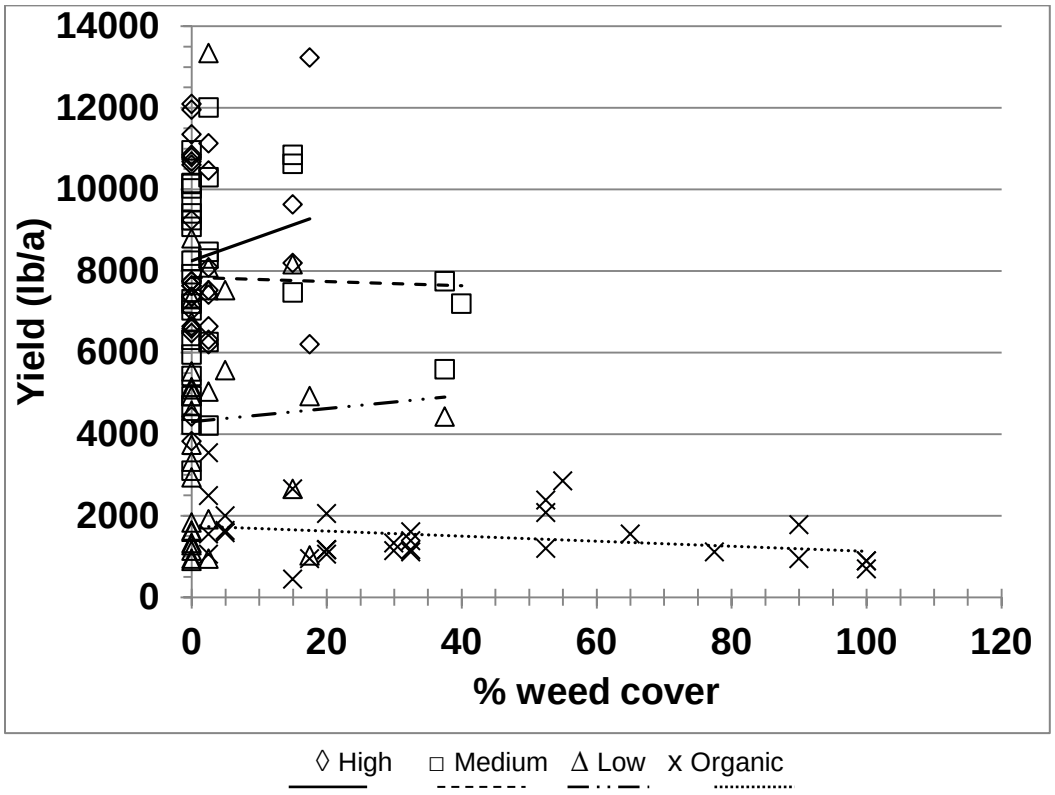
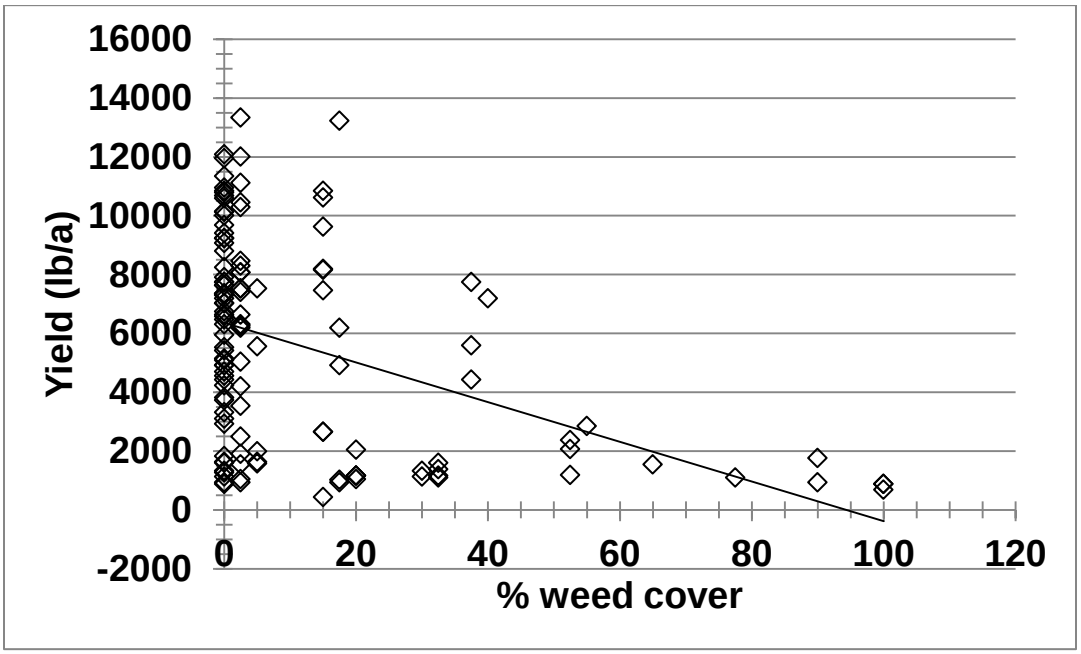



**Figure 5.** Weed cover and input system as predictors of variation in yield (overall $R^2$=0.747; Input system $R^2$=0.555; Weed cover $R^2$=0.184).



**CONCLUSIONS**:

*Blueberry and weed cover*

As in 2011 and 2012, higher inputs resulted in more blueberry cover later in the growing season, but the differences were minimal and non-significant. The Organic input system had the highest levels of weeds overall, just as it did in 2011 and 2012, again most likely due to limited or no control measures being implemented (Photo 1). One Organic grower did not manage their fields whatsoever over this cropping cycle, while others did not manage their fields in the crop year beyond providing bee hives. The Low input growers did not manage their fields as intensively as the Medium and High growers, but their fields had comparable blueberry and weed cover in general. However, while the reduced level of management was not readily apparent regarding mean percent blueberry cover, it resulted in significantly reduced yields compared to the Medium and High systems (see Photos 2-3, Yield section and Experimental Design Report).

**Photo 1.** Example of blueberry and weed cover in the Organic input system at the end of July.

**Photo 2.** Example of blueberry and weed cover in the Medium input system at the end of July.

**Photo 3.** Example of blueberry and weed cover in the High input system at the end of July.

*Yield vs. weeds*

The Low input system had the greatest variation among plots and fields. One field looked like a Medium or High field, with continuous blueberry cover and very few weeds. Two fields had good blueberry cover where there was blueberry, but both fields had extensive bare areas (Photo 4). In addition, these two fields contained several clones with lots of vegetative growth but few berries. The fourth Low field (Field 5) was sloped and very rocky with shallow soils; the blueberry plants were quite short, and the field had extensive leaf drop from Botrytis and/or lack of water due to the site conditions (see Photo 5). This site variation, especially the low yield from Field #5, pulled the mean yield for the Low input system down, as can be seen in the system and site yield graphs in the Experimental Design Report.

The results of the weeds versus yield analyses, both with and without input system as a predictor variable, suggest that the variation in yield among systems is mainly a response to input system, not weed cover. At this point, it is unclear what factors of each input system are significantly driving the differences in yield, but we will have a better understanding once the data from all years and all aspects (e.g. insects, disease, pollination, etc.) are examined together.

**Photo 4.** Example of blueberry and weed cover in the Low input system at the end of July.

**Photo 5**. Low field #5 showing extensive leaf drop and loss of flowers/berries.

**RECOMMENDATIONS**: None at this time. The four years of data will be combined and discussed for weed, insect, disease, fertility, etc. input effects on yield and profitability. This project may be extended for an additional cycle. After this data collection phase is complete, the results will be compared to the previous two cycles to confirm the results.

**INPUT SYSTEMS STUDY**

**SOIL HEALTH & CHEMISTRY:** Tsutomu Ohno, School of Food and Agriculture

**18. TITLE:** Phosphorus and Organic Matter Interactions on Short-Range Ordered Minerals in Acidic Barren Soils.

**ABSTRACT:** The effects of organic and conventional blueberry production on the chemical speciation of soil phosphorus were investigated with $^{31}$P-NMR and on soil organic matter (OM) composition with ultrahigh resolution mass spectrometry. Regression analysis indicated that water-extractable P was preferentially adsorbed to short-range ordered (SRO) Al minerals while OM was preferentially adsorbed to SRO-Fe minerals. The $^{31}$P-NMR results show that organic management soils had lower inorganic to organic ratios (0.9) as compared to the three conventional management soils (4.6). Principal components analysis shows that orthophosphate and the carboxylic-rich alicyclic molecules (CRAM)/lignin components of OM were associated with SRO-Al minerals and the aromatic OM components and monoester-P species were associated with the SRO-Fe minerals. It is speculated that soil amendments with OM containing greater CRAM/lignin content would be more effective in increasing soil P bioavailability which may reduce the quantity of P fertilizer used.

**INTRODUCTION:** The concentration of orthophosphate-P in soil solution, the dominant factor controlling the bioavailability of soil P[1,2], is typically determined in acid soils by adsorption/desorption reactions of P onto short-range ordered (SRO) Fe and Al minerals. These SRO minerals with their preponderance of surface hydroxyl groups have high affinity sorption sites for P and organic matter (OM)[3,4]. In acid soils without significant exchangeable hydrolyzed aluminum, P may be adsorbed by a ligand exchange reaction involving the exchange of a phosphate group for a surface hydroxyl group[5]. Although soil P bioavailability is likely to be affected by P-OM interactions, studies linking the two soil constituents with spectroscopic techniques which can provide chemical speciation information have been limited. Using $^{13}$C- and $^{31}$P-NMR methods to determine soil C and P speciation interrelationships, P mineralization was directly coupled to litter decomposition in a mixed-conifer forest soil[6] and P sequestration was linked to soil carbon humification in wetland soils[7]. Molecular-scale characterization of OM can also be obtained with electrospray Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) with its capability of resolving up to dozens of compounds per nominal mass and is ideal for molecular-level examination of soil OM[8-10].

The acidic and sandy glacial outwash barren soils in the northeastern United States and eastern Canada often support the growth of the woody perennial lowbush blueberry (*Vaccinium angustifolium*). Lowbush blueberry production is unique because it is not grower-planted, but is actively managed where there is sufficient native plant density to produce economically viable yields. The soils typically have well-developed organic layers, and are uncultivated which gives these acidic soils more forest soil characteristics than those of agricultural soils[11]. Managed production usually involves N and P fertilization to obtain higher fruit yields[12]. However, studies have shown that P fertilization may not increase yields suggesting that the P requirement can be met by native soil P[11,13]. The lack of a positive yield response to added P suggests that reduced or elimination of P fertilization would lessen the environmental risk of adverse P runoff impacts on surface waters in the catchments where lowbush blueberries are being commercially

managed. Competition between P and OM for sorption sites on SRO mineral surfaces could be important in determining P bioavailability.

Here we study the relationships between soil P and C speciation obtained using $^{31}$P-NMR and FT-ICR-MS of 12 soils from a blueberry systems study which included organic management and low-, medium- and high-input conventional management. A previous study reported that the management treatments did not affect total soil C and P content, or the oxalate- and modified Morgan-extractable soil P contents[14]. We postulate that $^{31}$P-NMR and FT-ICR-MS analysis, in combination with principal component analysis (PCA)[15], can provide a molecular-level details of the interactions of soil P and OM with SRO surfaces present in these acidic soils. This molecular-level understanding of soil P and OM relationships may offer insights on how to manage blueberry production for economically viable yields, while maintaining the environmental sustainability of this unique agro-ecosystem.

**METHODS:**

*Field Sites and Sample Collection*

An agro-ecosystems study was initiated using growers' fields to investigate the effects of blueberry production systems on soil quality. Details about the management practices can be found elsewhere[14]. Briefly, the four management treatments included an organic (O-1, O-2, O-3) system and low- (L-1, L-2, L-3), medium- (M-1, M-2, M-3), and high- (H-1, H-2, H-3) input conventional systems. All field sites had a minimum of 10 years of the prescribed management system. The organic and low-input fields received no fertilizer inputs. The medium-input fields received 31 kg ha$^{-1}$ N and 57 kg ha$^{-1}$ P fertilizer inputs, whereas the high-input fields received 80-90 kg ha$^{-1}$ N and 74-83 kg ha$^{-1}$ P of inputs. Organic and low-input sites were on non-leveled fields and used burning for the biennial pruning of the plants. The medium- and high-input fields were leveled allowing for the use of flail mowing for pruning. Soil to 10 cm depth were sampled from each site and sieved fresh through a 4-mm sieve, and air-dried at room temperature.

*Soil Characterization*

Water extractable P (WEP) was determined by extracting 1 g of soil with 10 mL of deionized water for 30 min, centrifuging at 900 X *g*, and filtered through 0.4 µm filter. Inductively coupled plasma-atomic emission spectrometry (ICP-AES) was used to determine P concentration of the extract. SRO content was determined using 0.2 M, pH 3 ammonium oxalate which selectively removes amorphous forms of Fe and Al[16.] Total C was determined using a LECO CN-2000 analyzer and total P was determined using HCl and $HNO_3$ with microwave heating[14].

*$^{31}$P NMR Spectroscopy*

Five g of soil was extracted with 100 mL of 0.25 M NaOH + 0.05 M EDTA for 16 h at room temperature[17]. The extracts were centrifuged for 30 min at 900 X *g* and filtered through Whatman 42 filter paper. A small aliquot was taken for P analysis by ICP-AES and the remaining solution was frozen and freeze-dried. For the NMR spectra acquisition, 100 mg of the sample was dissolved in 0.6 mL of 1 M NaOH in 10% $D_2O$. Solution $^{31}$P NMR spectra were collected on an Alpha 600 FT NMR spectrophotometer (JEOL, Tokyo, Japan) with a 5-mm probe. Spectra were recorded at 242.85 MHz employing a pulse width of 10.00 µsec (90º), an acquisition time of 0.4522 sec, and a pulse delay time of 2.0000 sec, with broadband proton decoupling at 30ºC. Each spectrum was scanned 30,000 times and a broadening factor of 5.00 Hz was employed in the Fourier transform procedure. Chemical shifts (ppm) were determined with

respect to 85% $H_3PO_4$ solution (0 ppm). The $^{31}P$ NMR spectra were divided into four phosphorous compositional classes[18]: orthophosphate (typically observed at around 6.0 ppm), pyrophosphate (-4.1 ppm), DNA phosphate (-0.4 ppm), and phosphate monoesters (5.6 to 3.4 ppm) as a mixture of signals with extreme broadening). The total signal intensity and the fraction contributed by each of the four classes of P compounds were calculated by integration of the spectral signals using ACD/NMR Processor (ACD Labs, Toronto, Canada). The integrated NMR signals were designated into chemical classes manually.

*ESI-FT-ICR-MS Analysis*

A 16-hour hot-water (80º C) pre-extraction was used to remove the water-extractable organic matter pool prior to the 4-hour 0.125 M sodium pyrophosphate extraction to obtain the adsorbed, stable pool of OM. The extracts were centrifuged and vacuum filtered through 0.4 µm polycarbonate filters. The dissolved organic carbon (DOC) concentration was determined using a Shimadzu 5000 analyzer (Shimadzu Scientific, Braintree, MA). The extracts were de-salted using Agilent PPL solid phase extraction cartridges[19]. The samples were diluted with LC-MS grade methanol to give a final sample composition of 50:50 (v/v) methanol:water. In order to increase the ionization efficiency, ammonium hydroxide was added immediately prior to ESI to bring the pH up to 8. Samples were introduced by a syringe pump providing an infusion rate of 120 µL hr-1 and analyzed in negative ion mode with electrospray voltages optimized for each sample. Ions (in the range of 200-2000 m/z) were accumulated in a hexapole for 1.0 sec before being transferred to the ICR cell. Exactly 300 transients, collected with a 4 MWord time domain, were added, giving about a 30 min total run time. The summed free induction decay signal was zero-filled once and Sine-Bell apodized prior to fast Fourier transformation and magnitude calculation using the Bruker Daltonics Data Analysis software. Prior to data analysis, all samples were externally calibrated with a polyethylene glycol standard and internally calibrated with naturally present fatty acids within the sample. Only m/z values with a signal to noise above 5 were used in the molecular formula calculation. The assigned formula list was passed through a MATLAB script to constrain the formulas to chemically feasible organic matter molecules using the following criteria: O/C < 1.2, H/C < 2.25, H/C > 0.3, N/C < 0.5, S/C < 0.2, P/C < 0.1, (S+P)/C < 0.2, and double bond equivalents (DBE) ≥ 0 and must be a whole number[20]. The script also parsed the assigned peaks into the appropriate van Krevelen space which consisted of four discrete regions[10]: aliphatic, DBE< 0.3 and H/C > 1.5; carboxylic-rich alicyclic molecules (CRAM), DBE/C 0.30-0.68, DBE/H 0.20-0.95; and DBE/O 0.77-1.75; aromatic, AI > 0.5; and condensed aromatic, AI > 0.67, where AI = aromaticity index = (1+C-O-S-0.5H)/(C-O-S-N-P). Further post-processing details can be found elsewhere[21].

**RESULTS AND DISCUSSION:**

*P-OM-SRO Interactions*

Selected soil chemical properties of the 12 soils are shown in Table 1. These acidic soils contained high amounts of SRO-Al (2.29 to 8.06 mol $kg^{-1}$ soil) and SRO-Fe (1.66 to 4.53 mol $kg^{-1}$ soil) and their important role in OM and P soil reactions are shown in Fig. 1. WEP concentration decreased significantly ($p = 0.05$) with increasing SRO-Al content, but was not related ($p = 0.18$) to SRO-Fe content suggesting that WEP is preferentially adsorbing to SRO-Al minerals (Fig. 1a,c). This supports earlier chemical speciation findings that these 12 soils were in equilibrium with gibbsite and undersaturated with respect to crystalline and amorphous forms of varisite[14]. A study with grassland soils also reported stronger correlation of P extractions with

SRO-Al than with SRO-Fe[22]. In contrast to WEP, the PyEOM fraction was not significantly ($p = 0.30$) related to SRO-Al, but was significantly ($p = 0.015$) related to SRO-Fe minerals (Fig. 1b,d). These results suggest that there may be different affinities of the P and OM for the Al and Fe SRO minerals present in the soils.

*$^{31}$P-NMR Speciation*

The average NaOH-EDTA P extraction efficiency was 79% (Table 1 ) which was in the range (66%-79%) reported for agricultural soils[23,24]. Representative $^{31}$P-NMR spectra from each of the four management treatments are shown in Fig. 2. The $^{31}$P-NMR analysis indicated the presence of orthophosphate, orthophosphate monoesters, DNA-P, and pyrophosphate species in the 12 soils. Across all soils, the inorganic orthophosphate and organic monoester forms were the two dominant P forms observed with the DNA-P and pyrophosphate- P form being minor components (Fig. 3a). For the organic management soils, the orthophosphate form of P averaged 48% of the total extracted P (range 31-61%) and the organic monoester form averaged 49% (range 37-61%). The three conventional management soils were dominated by orthophosphate-P with an average of 77% (range 64-91%) with monoester-P comprising an average of 21% (range 8-35%). The orthophosphate-P content was significantly ($r = 0.75$, $p = 0.05$) correlated with total soil P for the medium- and high-input conventional management subset of soils which received P fertilization indicating that orthophosphate-P content is controlled by the P fertilizer amendment. Phosphate monoesters are likely to be derived from plant and microbial residues[25] and DNA-P and pyrophosphate-P have been attributed to microbial origins[26]. The average inorganic P:organic P ratio was 0.9 for the organic system soils as compared to 4.6 for the conventional input system soils. The greater relative organic P content of the organic system soils suggests they may have higher capacity to mineralization of orthophosphate from organic P forms through microbial processes, while the conventional input systems are likely to be more dependent on added fertilizer P input to supply crop needs. The organic managed soils have an average of 246 mg $kg^{-1}$ monoester-P (total P times %monoester-P) as compared to 181 mg $kg^{-1}$ monoester-P for the conventionally managed soils which corresponds to 35% more P in the organic pool for potential P mineralization.

*ESI-FT-ICR-MS Characterization*

The van Krevelen diagrams of the four representative samples are shown in Fig. 4. Visually the medium input and high-input soil diagrams show more formula assignments in the aliphatic ($H/C > 1.5$) than those for the organic and low-input soils. This may be due to the greater blueberry plant densities in the medium- and high-intensity fields resulting in higher levels of root exudate inputs to these soils. Root exudates components include carbohydrates, amino acids, organic acids, and fatty acids which would all reside in the aliphatic van Krevelen diagram space[27]. These compounds are readily available to microbial consumption, but their presence in the pyrophosphate-extractable pool indicates that some fraction of these compounds and/or the aliphatic byproducts of microbial processing are stabilized by adsorption processes. The distributions of the van Krevelen classifications for all soils are shown in Fig. 3b. CRAM/lignin was the dominant class comprising of 49-66% of the assigned formulas. Aromatic and condensed aromatic classes jointly accounted for 18-36% of the formulas, with the remaining aliphatic class consisting of 9-21% of formulas. The dominance of the CRAM/lignin for this data set likely reflects the high quantities of SRO minerals present in these soils.

*Principal Components Analysis*

Multivariate statistical methods, such as PCA, are ideal for identifying patterns and visually highlighting relationships in higher dimensional datasets[15]. PCA was conducted on the P and OM species distribution data (Fig. 2a,b) and the SRO data (Table 1) and the resulting loadings plot is shown in Fig. 5. The first two principal components accounted for 77% of the variance in the data. The first component (PC 1 axis) can be interpreted as separating the SRO-Al and SRO-Fe minerals while the second component (PC 2 axis) separates the OM classes based on their aliphatic/aromatic nature. Orthophosphate-P loading maps very closely with SRO-Al loading supporting the results shown in Fig. 1 where WEP is significantly related to SRO-Al content. Monoester-P and SRO-Fe loadings were closely mapped indicating that the monoester-P form likely has preferential affinity for SRO-Fe minerals. The DNA-P and pyrophosphate-P were not related to SRO-Al or SRO-Fe minerals in this study. The PCA diagram also shows that CRAM/lignin compounds are associated with SRO-Al minerals while the aromatic and condensed aromatic compounds are associated with SRO-Fe minerals.

Our findings profoundly illustrate how molecular-scale studies can enhance our understanding of the strong interactions that P and OM have with ubiquitous SRO minerals present in soils. First, the data presented in this study suggests that it is the CRAM/lignin component of OM that competes with orthophosphates for adsorption. Second, orthophosphate and CRAM/lignin molecules preferentially for adsorb to SRO-Al minerals. This may help explain the chemical basis for the differential effectiveness of OM from varying sources[28]. Thus, we speculate that one effective management strategy for increasing the bioavailability of native soil P would be to utilize organic amendments that have water-soluble OM with a high percentage of CRAM/lignin components which could effectively compete with orthophosphate for sorption sites resulting in higher orthophosphate concentrations in soil solution.

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**Table 1. Selected Soil Chemical Properties of the Soils[1].**

| soil | total soil C | PyEOM | total soil P | NMR extract | WEP | SRO Al | SRO Fe |
|---|---|---|---|---|---|---|---|
| | g $kg^{-1}$ | g $kg^{-1}$ | mg $kg^{-1}$ | %recovery | mol $kg^{-1}$ | mol $kg^{-1}$ | mg $kg^{-1}$ |
| O-1 | 29.4 | 8.71 | 490 | 96 | 5.63 | 2.62 | 0.98 |
| O-2 | 73.1 | 11.07 | 602 | 78 | 3.49 | 3.80 | 2.22 |
| O-3 | 86.5 | 8.63 | 451 | 67 | 2.29 | 4.53 | 3.82 |
| L-1 | 50.3 | 8.66 | 749 | 74 | 3.43 | 3.46 | 1.71 |
| L-2 | 67.0 | 10.03 | 1363 | 75 | 4.87 | 3.79 | 2.32 |
| L-3 | 49.6 | 9.07 | 643 | 103 | 8.06 | 4.40 | 1.18 |
| M-1 | 49.4 | 7.48 | 1257 | 68 | 6.03 | 2.52 | 3.10 |
| M-2 | 49.3 | 7.58 | 1198 | 87 | 6.06 | 2.73 | 2.57 |
| M-3 | 15.1 | 4.43 | 554 | 59 | 7.24 | 2.03 | 0.52 |
| H-1 | 32.9 | 7.88 | 631 | 83 | 7.75 | 2.77 | 0.68 |
| H-2 | 26.0 | 4.99 | 505 | 97 | 4.08 | 1.66 | 0.66 |
| H-3 | 32.8 | 6.42 | 858 | 55 | 6.96 | 3.88 | 0.63 |
| | | | | | | | |

[1] Total soil C, SRO-Al and SRO-Fe data from reference (14).

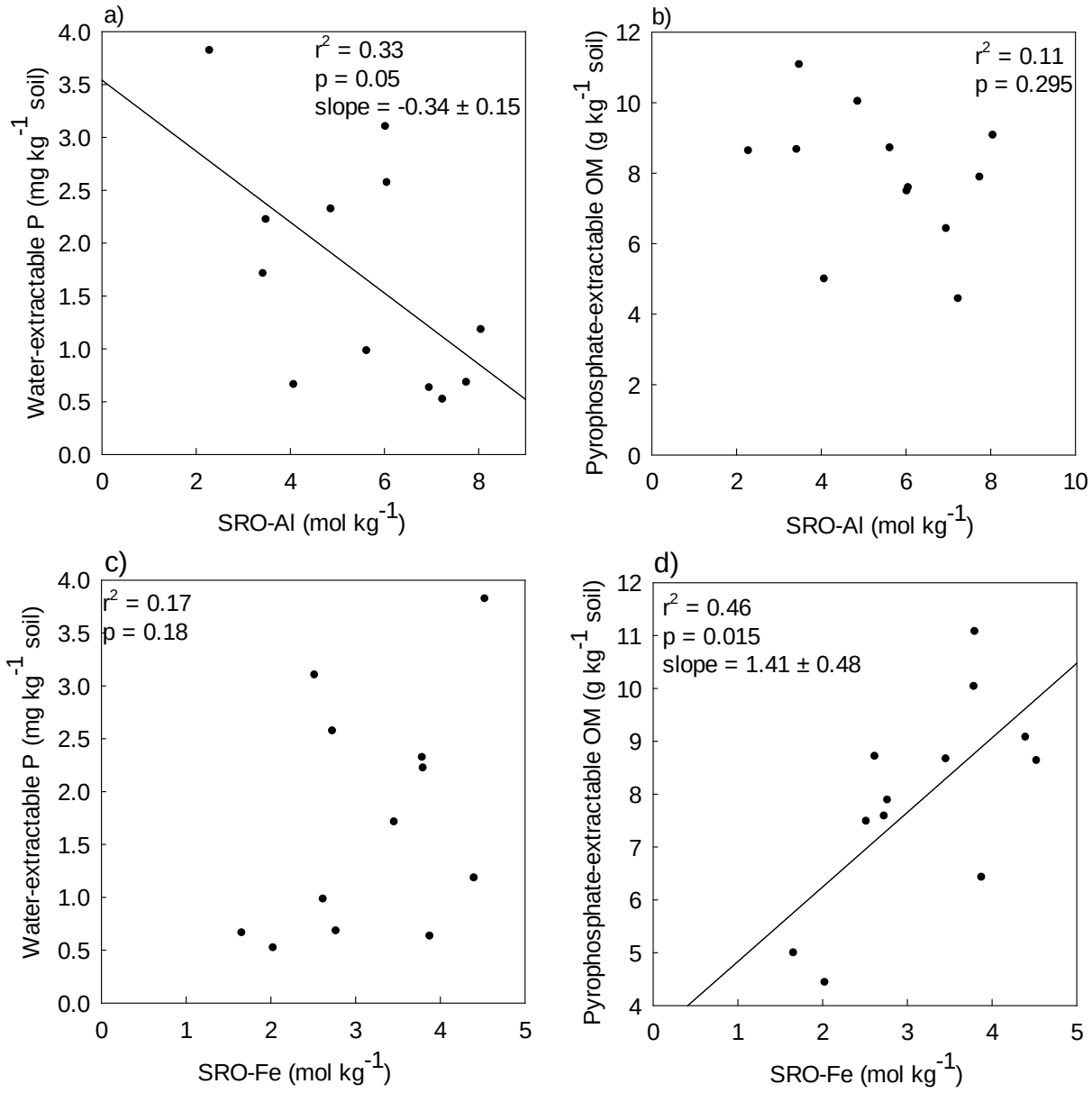


**Fig. 1.** Water-extractable phosphorus and pyrophosphate-extractable organic matter as a function of short-ranged order aluminum and iron content.

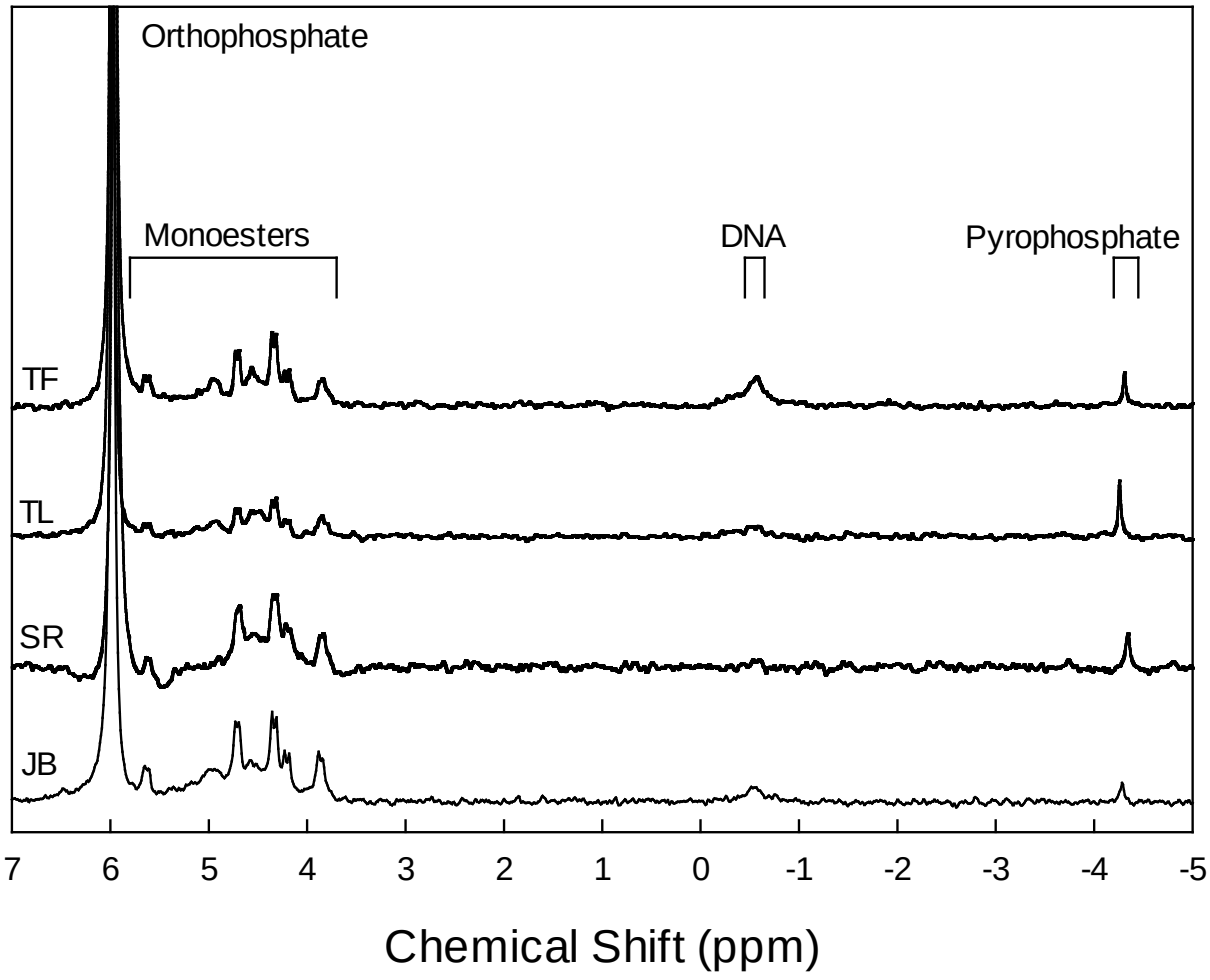


**Fig. 2.** Solution $^{31}$P NMR spectra of NaOH-EDTA extracts of JB, SR, Tl, and TF soils. The peak regions of the orthophosphate-P, monoester-P, DNA-P, and pyrophosphate-P species are shown.

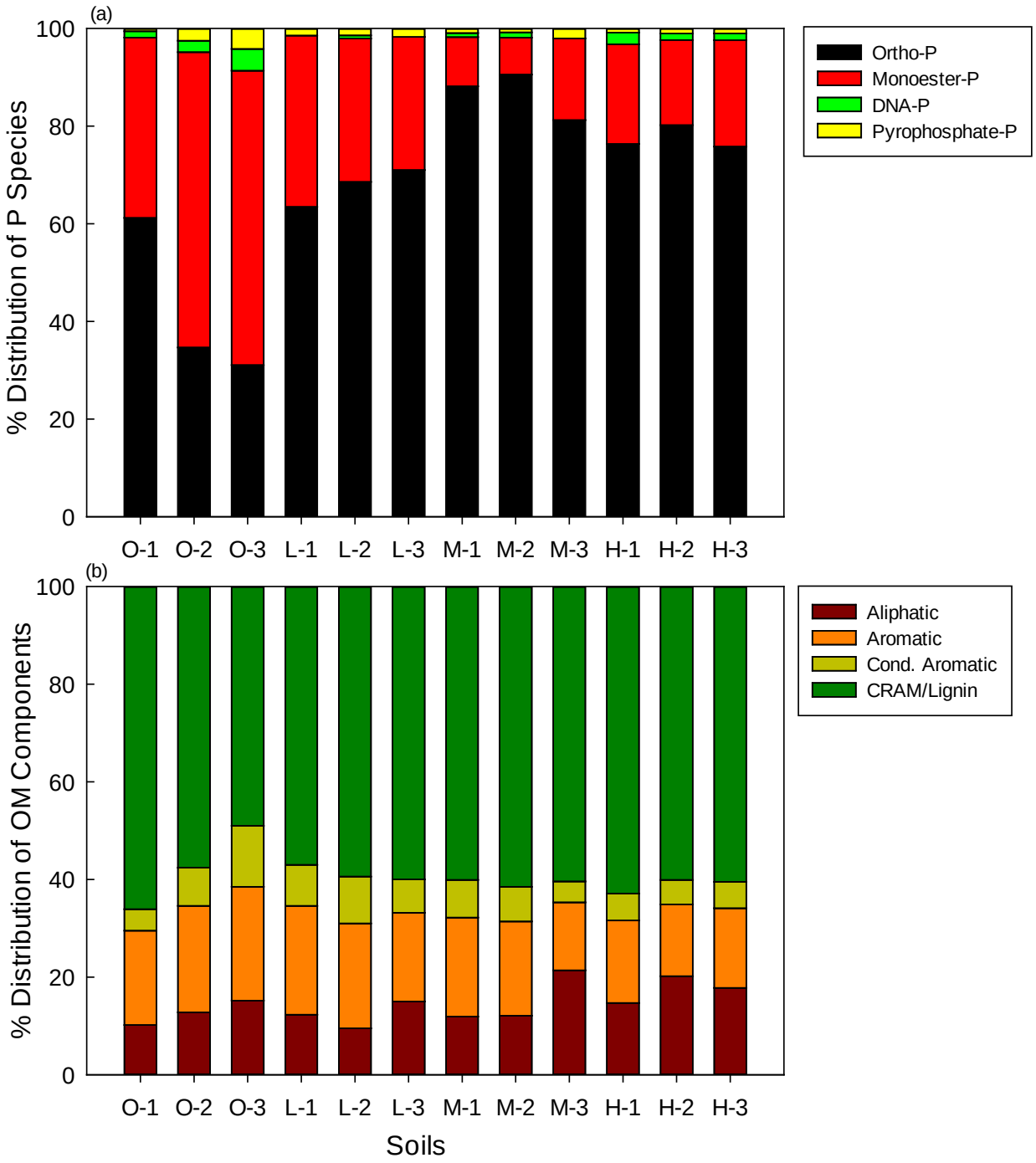


**Fig. 3.** Distribution of phosphorus species and organic matter components in the 12 soils investigated.

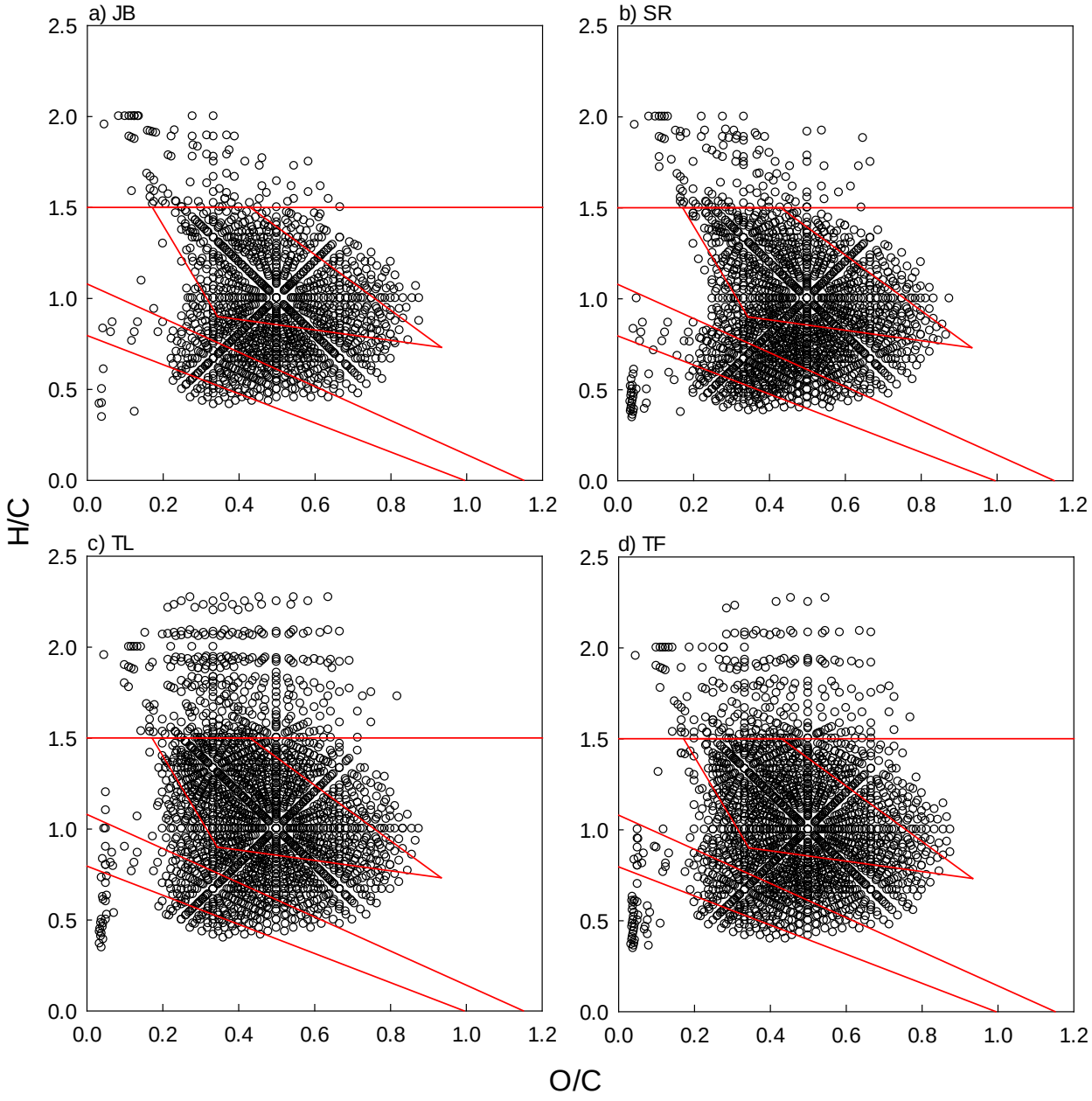


**Fig. 4.** van Krevelen diagrams for all assigned formulas for pyrophosphate-extractable organic matter of the JB, SR, TL, and TF soils. The region above the H/C = 1.5 is classified as aliphatic, the polygon is classified as CRAM/lignin, the area between the two diagonal lines are classified as aromatic, and the region below the bottom diagonal line is classified as condensed aromatic.

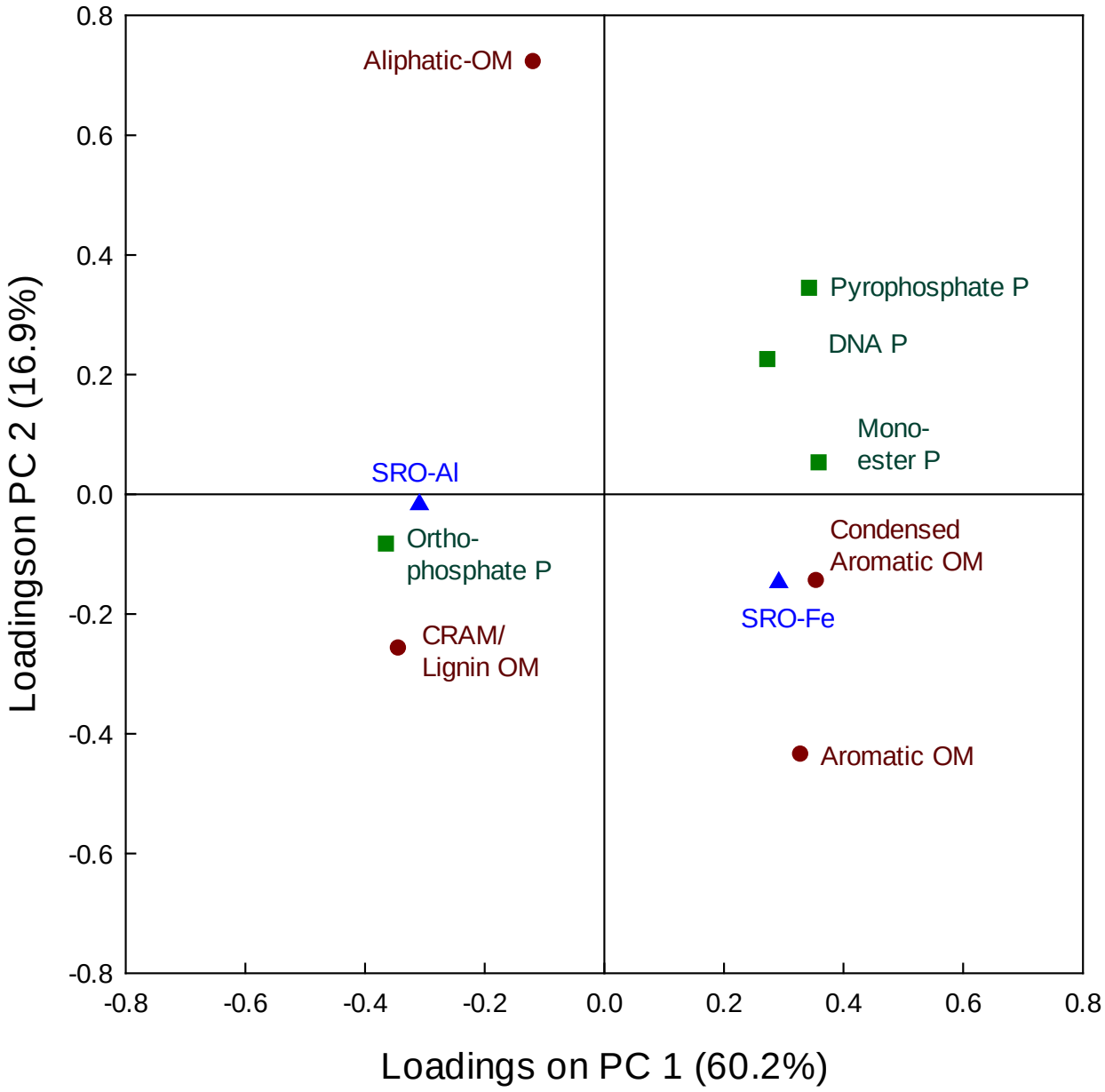


**Fig. 5.** Principal component analysis of phosphorus species and organic matter components of the 12 soils investigated.

**INPUT SYSTEMS STUDY**

**ECONOMICS:** David E. Yarborough, Professor of Horticulture
Jennifer L.D. Cote, Assistant Scientist

**19. TITLE:** Systems approach to improving the sustainability of wild blueberry production, preliminary economic comparison for 2012-13.

**METHODS:** Project layout is described in the overall Experimental Design Report. The number of acres, wild blueberry yields and cost of harvest, and all variable input costs were obtained from the cooperators for each input level and location for the 2012-2013 crop cycle. The Organic 4 grower stopped managing their fields after the trial site was set up; therefore, we were unable to procure input costs and the site is omitted from the budgets. Although we contacted Organic grower 2 several times, we were unable to get a response for 2013 inputs and harvest costs. Their 2012 input costs were minimal. The 1-acre blocks were accidentally pruned by a fire that swept through the fields, which also destroyed two of the four permanent on-site hives. The blocks were weeded by browsing goats or by family members at no cost; there were no other inputs in 2012. Therefore, this site is also omitted from the budgets, but will be added later if the grower responds. Field prices per pound were obtained from all growers except the High input sites, which were estimated at $0.73. For the purposes of this report, the figures for capital and fixed costs were also estimated. All of these figures were put into a partial budget Excel spreadsheet that is available to wild blueberry growers on the www.wildblueberries.com website to determine the cost and returns per acre and per pound.

**RESULTS:** The preliminary per acre costs for the two organic fresh pack sites we have data for were $965.37 and $1835.73. Organic grower #1 received an average of $4.60/lb for the berries but lost several acres to SWD. Organic grower #3 received $5.20/lb but only harvested one acre of his five crop acres because yield was so low on the remaining acreage that it wasn't cost effective to harvest them. Only one Low input site (#8) returned a profit to the company, even though that site had fewer inputs than the other three sites. Harvest costs for the Low sites were comparable to Organic sites in this cycle and yields were somewhat higher, but the greater input costs and lower berry prices contributed to negative returns. The Medium input sites had a range of returns from $2600.75 to $2917.01/a, while the High input sites had a range of returns from $1145.02 to $2719.23/a. However, per acre input costs for the Medium sites were a little over half that of the High sites, and the average per acre return was greater at $2,600.75 to $2,917.01. The differences in per acre costs were largely due to greater High system input costs for pollination, pest monitoring, insect and disease control, and irrigation.

**CONCLUSIONS/DISCUSSION:** As was mentioned in the Experimental Design report regarding inputs versus yield, there also appears to be a point of diminishing returns regarding per acre input costs versus per acre returns to the company. The difference between the organic and conventional systems was not as great as would be expected, but this is due to the fact that the organic growers can make much more profit on a pound of fresh pack berries compared to the conventional growers' processed berry price. The large difference in returns between the Low and Medium/High input systems shows that to a point, increasing inputs does result in increasing returns to the company. However, the lack of overall improvement in profit in the

High input system compared to the Medium input system suggests that although the High growers' increased inputs did result in slightly increased yield; the increase in yield (and therefore profit) was negated by the increased input costs.

**RECOMMENDATIONS**: No recommendations at this time. The data will be analyzed by the project Economist, and final interactive budget spreadsheets will be generated for growers to use.

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **Organic 1** | | |
| | | |
| Number of Acres (Crop) | 11.00 | |
| Yield (Lbs./Acre) | 574.91 | |
| Price/Lb. ($) | 4.60 | |
| REVENUE/ACRE ($) | 2,644.59 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 0.00 | 0.00 |
| Mowing | 34.20 | 0.06 |
| Average Pruning | 34.20 | 0.06 |
| Weed Control | 627.75 | 1.09 |
| Fertilization | 0.00 | 0.00 |
| Pollination | 2.73 | 0.00 |
| Pest Monitoring | 0.00 | 0.00 |
| Insect Control | 0.00 | 0.00 |
| Blight Control | 0.00 | 0.00 |
| Irrigation | 0.00 | 0.00 |
| Sulfur (pH) | 0.00 | 0.00 |
| Harvest: | | |
| Raking | 0.00 | 0.00 |
| Mechanical | 247.21 | 0.43 |
| Average Harvest | 247.21 | 0.43 |
| Packing and Marketing | 6.57 | 0.80 |
| Interest on Capital | 42.60 | 0.07 |
| Blueberry Tax | 4.31 | 0.0075 |
| TOT. VARIABLE COSTS | 965.37 | 1.68 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 1,058.30 | 1.84 |
| | | |
| RETURNS ABOVE COSTS SHOWN | 1,586.28 | 2.76 |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **17,449.13** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **Organic 3** | | |
| | | |
| Number of Acres (Crop) | 1.00 | |
| Yield (Lbs./Acre) | 750.00 | |
| Price/Lb. ($) | 5.20 | |
| REVENUE/ACRE ($) | 3900.00 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 245.00 | 0.33 |
| Mowing | 0.00 | 0.00 |
| Average Pruning | 245.00 | 0.33 |
| Weed Control | 60.00 | 0.08 |
| Fertilization | 132.50 | 0.18 |
| Pollination | 550.00 | 0.73 |
| Pest Monitoring | 0.00 | 0.00 |
| Insect Control | 0.00 | 0.00 |
| Blight Control | 0.00 | 0.00 |
| Irrigation | 0.00 | 0.00 |
| Sulfur (pH) | 375.00 | 0.50 |
| Harvest: | | |
| Raking | 340.00 | 0.45 |
| Mechanical | 0.00 | 0.00 |
| Average Harvest | 340.00 | 0.45 |
| Packing and Marketing | 85.00 | 0.11 |
| Interest on Capital | 42.60 | 0.06 |
| Blueberry Tax | 5.63 | 0.0075 |
| TOT. VARIABLE COSTS | 1,835.73 | 2.45 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 1928.66 | 2.57 |
| | | |
| RETURNS ABOVE COSTS SHOWN | 1971.35 | 2.63 |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **1971.35** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **Low Input 5** | | |
| | | |
| Number of Acres (Crop) | 29.00 | |
| Yield (Lbs./Acre) | 388.45 | |
| Price/Lb. ($) | 0.65 | |
| REVENUE/ACRE ($) | 252.49 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 564.43 | 1.45 |
| Mowing | 0.00 | 0.00 |
| Average Pruning | 564.43 | 1.45 |
| Weed Control | 118.66 | 0.31 |
| Fertilization | 33.97 | 0.09 |
| Pollination | 206.68 | 0.53 |
| Pest Monitoring | 0.00 | 0.00 |
| Insect Control | 30.08 | 0.08 |
| Blight Control | 14.53 | 0.04 |
| Irrigation | 0.00 | 0.00 |
| Sulfur (pH) | 0.00 | 0.00 |
| Harvest: | | |
| Raking | 75.75 | 0.20 |
| Mechanical | 0.00 | 0.00 |
| Average Harvest | 75.75 | 0.20 |
| Packing and Marketing | 0.00 | 0.00 |
| Interest on Capital | 42.60 | 0.11 |
| Blueberry Tax | 5.83 | 0.0150 |
| TOT. VARIABLE COSTS | 1,092.53 | 2.81 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 1,185.46 | 3.05 |
| | | |
| RETURNS ABOVE COSTS SHOWN | (932.96) | (2.40) |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **(27,055.96)** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **Low Input 6** | | |
| | | |
| Number of Acres (Crop) | 20.00 | |
| Yield (Lbs./Acre) | 1,927.60 | |
| Price/Lb. ($) | 0.65 | |
| REVENUE/ACRE ($) | 1,252.94 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 476.97 | 0.25 |
| Mowing | 0.00 | 0.00 |
| Average Pruning | 476.97 | 0.25 |
| Weed Control | 228.78 | 0.12 |
| Fertilization | 38.79 | 0.02 |
| Pollination | 281.46 | 0.15 |
| Pest Monitoring | 0.00 | 0.00 |
| Insect Control | 35.71 | 0.02 |
| Blight Control | 14.88 | 0.01 |
| Irrigation | 0.00 | 0.00 |
| Sulfur (pH) | 0.00 | 0.00 |
| Harvest: | | |
| Raking | 375.14 | 0.19 |
| Mechanical | 0.00 | 0.00 |
| Average Harvest | 375.14 | 0.19 |
| Packing and Marketing | 0.00 | 0.00 |
| Interest on Capital | 42.60 | 0.02 |
| Blueberry Tax | 28.91 | 0.0150 |
| TOT. VARIABLE COSTS | 1,523.24 | 0.79 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 1,616.17 | 0.84 |
| | | |
| RETURNS ABOVE COSTS SHOWN | (363.23) | (0.19) |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **(7,264.68)** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **Low Input 7** | | |
| | | |
| Number of Acres (Crop) | 30.00 | |
| Yield (Lbs./Acre) | 1,971.05 | |
| Price/Lb. ($) | 0.65 | |
| REVENUE/ACRE ($) | 1,281.18 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 476.58 | 0.24 |
| Mowing | 0.00 | 0.00 |
| Average Pruning | 476.58 | 0.24 |
| Weed Control | 151.89 | 0.08 |
| Fertilization | 165.21 | 0.08 |
| Pollination | 221.76 | 0.11 |
| Pest Monitoring | 0.00 | 0.00 |
| Insect Control | 100.37 | 0.05 |
| Blight Control | 61.33 | 0.03 |
| Irrigation | 0.00 | 0.00 |
| Sulfur (pH) | 101.33 | 0.05 |
| Harvest: | | |
| Raking | 512.47 | 0.26 |
| Mechanical | 0.00 | 0.00 |
| Average Harvest | 512.47 | 0.26 |
| Packing and Marketing | 0.00 | 0.00 |
| Interest on Capital | 42.60 | 0.02 |
| Blueberry Tax | 29.57 | 0.0150 |
| TOT. VARIABLE COSTS | 1,863.11 | 0.95 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 1,956.04 | 0.99 |
| | | |
| RETURNS ABOVE COSTS SHOWN | (674.86) | (0.34) |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **(20,245.66)** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **Low Input 8** | | |
| | | |
| Number of Acres (Crop) | 7.00 | |
| Yield (Lbs./Acre) | 2,403.00 | |
| Price/Lb. ($) | 0.70 | |
| REVENUE/ACRE ($) | 1,682.10 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 140.71 | 0.06 |
| Mowing | 24.29 | 0.01 |
| Average Pruning | 165.00 | 0.07 |
| Weed Control | 152.00 | 0.06 |
| Fertilization | 50.00 | 0.02 |
| Pollination | 140.57 | 0.06 |
| Pest Monitoring | 0.00 | 0.00 |
| Insect Control | 0.00 | 0.00 |
| Blight Control | 0.00 | 0.00 |
| Irrigation | 0.00 | 0.00 |
| Sulfur (pH) | 0.00 | 0.00 |
| Harvest: | | |
| Raking | 0.00 | 0.00 |
| Mechanical | 336.42 | 0.14 |
| Average Harvest | 336.42 | 0.14 |
| Packing and Marketing | 0.00 | 0.00 |
| Interest on Capital | 42.60 | 0.02 |
| Blueberry Tax | 36.05 | 0.0150 |
| TOT. VARIABLE COSTS | 922.64 | 0.38 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 1,015.57 | 0.42 |
| | | |
| RETURNS ABOVE COSTS SHOWN | 666.54 | 0.28 |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **4,665.75** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **Medium Input 9** | | |
| | | |
| Number of Acres (Crop) | 44.00 | |
| Yield (Lbs./Acre) | 5,955.00 | |
| Price/Lb. ($) | 0.73 | |
| REVENUE/ACRE ($) | 4,347.15 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 0.00 | 0.00 |
| Mowing | 90.75 | 0.02 |
| Average Pruning | 90.75 | 0.02 |
| Weed Control | 143.81 | 0.02 |
| Fertilization | 147.15 | 0.02 |
| Pollination | 200.00 | 0.03 |
| Pest Monitoring | 13.45 | 0.00 |
| Insect Control | 52.78 | 0.01 |
| Blight Control | 99.46 | 0.02 |
| Irrigation | 0.00 | 0.00 |
| Sulfur (pH) | 0.00 | 0.00 |
| Harvest: | | |
| Raking | 0.00 | 0.00 |
| Mechanical | 774.15 | 0.13 |
| Average Harvest | 774.15 | 0.13 |
| Packing and Marketing | 0.00 | 0.00 |
| Interest on Capital | 42.60 | 0.01 |
| Blueberry Tax | 89.33 | 0.0150 |
| TOT. VARIABLE COSTS | 1,653.48 | 0.28 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 1,746.41 | 0.29 |
| | | |
| RETURNS ABOVE COSTS SHOWN | 2,600.75 | 0.44 |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **114,432.78** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **Medium Input 10** | | |
| | | |
| Number of Acres (Crop) | 77.00 | |
| Yield (Lbs./Acre) | 6,389.00 | |
| Price/Lb. ($) | 0.73 | |
| REVENUE/ACRE ($) | 4,663.97 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 0.00 | 0.00 |
| Mowing | 90.75 | 0.01 |
| Average Pruning | 90.75 | 0.01 |
| Weed Control | 105.92 | 0.02 |
| Fertilization | 147.15 | 0.02 |
| Pollination | 200.00 | 0.03 |
| Pest Monitoring | 15.36 | 0.00 |
| Insect Control | 26.39 | 0.00 |
| Blight Control | 99.46 | 0.02 |
| Irrigation | 0.00 | 0.00 |
| Sulfur (pH) | 0.00 | 0.00 |
| Harvest: | | |
| Raking | 0.00 | 0.00 |
| Mechanical | 830.57 | 0.13 |
| Average Harvest | 830.57 | 0.13 |
| Packing and Marketing | 0.00 | 0.00 |
| Interest on Capital | 42.60 | 0.01 |
| Blueberry Tax | 95.84 | 0.0150 |
| TOT. VARIABLE COSTS | 1,654.04 | 0.26 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 1,746.97 | 0.27 |
| | | |
| RETURNS ABOVE COSTS SHOWN | 2,917.01 | 0.46 |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **224,609.39** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **Medium Input 11** | | |
| | | |
| Number of Acres (Crop) | 36.00 | |
| Yield (Lbs./Acre) | 5,918.00 | |
| Price/Lb. ($) | 0.73 | |
| REVENUE/ACRE ($) | 4,320.14 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 0.00 | 0.00 |
| Mowing | 90.75 | 0.02 |
| Average Pruning | 90.75 | 0.02 |
| Weed Control | 143.54 | 0.02 |
| Fertilization | 147.15 | 0.02 |
| Pollination | 200.00 | 0.03 |
| Pest Monitoring | 13.83 | 0.00 |
| Insect Control | 26.39 | 0.00 |
| Blight Control | 99.46 | 0.02 |
| Irrigation | 0.00 | 0.00 |
| Sulfur (pH) | 0.00 | 0.00 |
| Harvest: | | |
| Raking | 0.00 | 0.00 |
| Mechanical | 769.34 | 0.13 |
| Average Harvest | 769.34 | 0.13 |
| Packing and Marketing | 0.00 | 0.00 |
| Interest on Capital | 42.60 | 0.01 |
| Blueberry Tax | 88.77 | 0.0150 |
| TOT. VARIABLE COSTS | 1,621.83 | 0.27 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 1,714.76 | 0.29 |
| | | |
| RETURNS ABOVE COSTS SHOWN | 2,605.38 | 0.44 |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **93,793.68** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **Medium Input 12** | | |
| | | |
| Number of Acres (Crop) | 146.00 | |
| Yield (Lbs./Acre) | 5,917.00 | |
| Price/Lb. ($) | 0.73 | |
| REVENUE/ACRE ($) | 4,319.41 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 0.00 | 0.00 |
| Mowing | 90.75 | 0.02 |
| Average Pruning | 90.75 | 0.02 |
| Weed Control | 105.92 | 0.02 |
| Fertilization | 147.15 | 0.02 |
| Pollination | 200.00 | 0.03 |
| Pest Monitoring | 14.24 | 0.00 |
| Insect Control | 52.78 | 0.01 |
| Blight Control | 99.46 | 0.02 |
| Irrigation | 0.00 | 0.00 |
| Sulfur (pH) | 0.00 | 0.00 |
| Harvest: | | |
| Raking | 0.00 | 0.00 |
| Mechanical | 769.21 | 0.13 |
| Average Harvest | 769.21 | 0.13 |
| Packing and Marketing | 0.00 | 0.00 |
| Interest on Capital | 42.60 | 0.01 |
| Blueberry Tax | 88.76 | 0.0150 |
| TOT. VARIABLE COSTS | 1,610.87 | 0.27 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 1,703.80 | 0.29 |
| | | |
| RETURNS ABOVE COSTS SHOWN | 2,615.62 | 0.44 |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **381,879.79** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **High Input 13** | | |
| | | |
| Number of Acres (Crop) | 112.54 | |
| Yield (Lbs./Acre) | 6,123.00 | |
| Price/Lb. ($) | 0.73 | |
| REVENUE/ACRE ($) | 4,469.79 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 0.00 | 0.00 |
| Mowing | 71.00 | 0.01 |
| Average Pruning | 71.00 | 0.01 |
| Weed Control | 160.59 | 0.03 |
| Fertilization | 202.23 | 0.03 |
| Pollination | 517.00 | 0.08 |
| Pest Monitoring | 84.00 | 0.01 |
| Insect Control | 169.53 | 0.03 |
| Blight Control | 236.93 | 0.04 |
| Irrigation | 60.00 | 0.01 |
| Sulfur (pH) | 0.00 | 0.00 |
| Harvest: | | |
| Raking | 0.00 | 0.00 |
| Mechanical | 649.04 | 0.11 |
| Average Harvest | 649.04 | 0.11 |
| Packing and Marketing | 0.00 | 0.00 |
| Interest on Capital | 42.60 | 0.01 |
| Blueberry Tax | 91.85 | 0.0150 |
| TOT. VARIABLE COSTS | 2,284.77 | 0.37 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 2,377.70 | 0.39 |
| | | |
| RETURNS ABOVE COSTS SHOWN | 2,092.10 | 0.34 |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **235,444.37** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **High Input 14** | | |
| | | |
| Number of Acres (Crop) | 63.29 | |
| Yield (Lbs./Acre) | 6,946.00 | |
| Price/Lb. ($) | 0.73 | |
| REVENUE/ACRE ($) | 5,070.58 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 0.00 | 0.00 |
| Mowing | 71.00 | 0.01 |
| Average Pruning | 71.00 | 0.01 |
| Weed Control | 160.59 | 0.02 |
| Fertilization | 189.19 | 0.03 |
| Pollination | 836.00 | 0.12 |
| Pest Monitoring | 84.00 | 0.01 |
| Insect Control | 169.53 | 0.02 |
| Blight Control | 236.93 | 0.03 |
| Irrigation | 60.00 | 0.01 |
| Sulfur (pH) | 0.00 | 0.00 |
| Harvest: | | |
| Raking | 0.00 | 0.00 |
| Mechanical | 736.28 | 0.11 |
| Average Harvest | 736.28 | 0.11 |
| Packing and Marketing | 0.00 | 0.00 |
| Interest on Capital | 42.60 | 0.01 |
| Blueberry Tax | 104.19 | 0.0150 |
| TOT. VARIABLE COSTS | 2,690.31 | 0.39 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 2,783.24 | 0.40 |
| | | |
| RETURNS ABOVE COSTS SHOWN | 2,287.34 | 0.33 |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **144,765.75** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **High Input 15** | | |
| | | |
| Number of Acres (Crop) | 82.11 | |
| Yield (Lbs./Acre) | 7,681.00 | |
| Price/Lb. ($) | 0.73 | |
| REVENUE/ACRE ($) | 5,607.13 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 0.00 | 0.00 |
| Mowing | 71.00 | 0.01 |
| Average Pruning | 71.00 | 0.01 |
| Weed Control | 221.59 | 0.03 |
| Fertilization | 202.23 | 0.03 |
| Pollination | 715.00 | 0.09 |
| Pest Monitoring | 84.00 | 0.01 |
| Insect Control | 232.22 | 0.03 |
| Blight Control | 236.93 | 0.03 |
| Irrigation | 60.00 | 0.01 |
| Sulfur (pH) | 0.00 | 0.00 |
| Harvest: | | |
| Raking | 0.00 | 0.00 |
| Mechanical | 814.19 | 0.11 |
| Average Harvest | 814.19 | 0.11 |
| Packing and Marketing | 0.00 | 0.00 |
| Interest on Capital | 42.60 | 0.01 |
| Blueberry Tax | 115.22 | 0.0150 |
| TOT. VARIABLE COSTS | 2,794.98 | 0.36 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 2,887.91 | 0.38 |
| | | |
| RETURNS ABOVE COSTS SHOWN | 2,719.23 | 0.35 |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **223,275.56** | |

| **Wild Maine Blueberry Budget** | | |
|---|---|---|
| **High Input 16** | | |
| | | |
| Number of Acres (Crop) | 132.19 | |
| Yield (Lbs./Acre) | 5,256.00 | |
| Price/Lb. ($) | 0.73 | |
| REVENUE/ACRE ($) | 3,836.88 | |
| | | |
| VARIABLE COSTS | **($/Acre)** | **($/Pound)** |
| | | |
| Pruning: | | |
| Burning | 0.00 | 0.00 |
| Mowing | 71.00 | 0.01 |
| Average Pruning | 71.00 | 0.01 |
| Weed Control | 160.59 | 0.03 |
| Fertilization | 202.23 | 0.04 |
| Pollination | 748.00 | 0.14 |
| Pest Monitoring | 84.00 | 0.02 |
| Insect Control | 357.60 | 0.07 |
| Blight Control | 236.93 | 0.05 |
| Irrigation | 60.00 | 0.01 |
| Sulfur (pH) | 0.00 | 0.00 |
| Harvest: | | |
| Raking | 0.00 | 0.00 |
| Mechanical | 557.14 | 0.11 |
| Average Harvest | 557.14 | 0.11 |
| Packing and Marketing | 0.00 | 0.00 |
| Interest on Capital | 42.60 | 0.01 |
| Blueberry Tax | 78.84 | 0.0150 |
| TOT. VARIABLE COSTS | 2,598.93 | 0.49 |
| | | |
| FIXED COSTS: | | |
| Machinery & Equipment | 60.10 | 0.01 |
| Taxes | 32.83 | 0.01 |
| | | |
| TOTAL FIXED COSTS | 92.93 | 0.02 |
| | | |
| TOTAL COSTS | 2,691.86 | 0.51 |
| | | |
| RETURNS ABOVE COSTS SHOWN | 1,145.02 | 0.22 |
| | | |
| AVERAGE TOTAL ANNUAL RETURN TO MANAGEMENT | | |
| ($/FARM) | **151,360.19** | |

**INPUT SYSTEMS STUDY – *ANCILLARY STUDY***

**DISEASE:** Seanna Annis, Assoc. Professor, School of Biology and Ecology
Erika Lyon, MS graduate student, School of Biology and Ecology

**20. TITLE:** Ancillary projects in disease research.

**OBJECTIVES:**
- Determine possible sources and methods of spread of Valdensinia; and
- Determine causal agent of root rot disease of lowbush blueberries.

**METHODS:** Newly reported fields with Valdensinia leaf spot in 2013 were visited in July to August survey for disease. If the field showed Valdensinia leaf spot symptoms, 5 to 7 stems in diseased areas about 10 ft apart were collected and placed in individual plastic bags. Isolates were obtained by surface sterilizing ten infected leaves per sample and plating them out on half-strength oatmeal medium amended with antibiotics. Plated leaves were incubated at 17°C under 12 hr light to induce spore formation by Valdensinia. Valdensinia spores were transferred to new plates and put into storage for genetic analysis.
Isolates of the organisms isolated from roots showing symptoms of root rot were grown on plates and induced to produce spores. Potted seedlings were inoculated by soaking the soil of the potted plants in a spore solution for 24 hrs. The plants were then grown in the lab and watered when needed except for two drought periods applied at approximately one and two months after inoculation. The plant roots will be collected and plated out to re-isolate the original organisms.

**RESULTS:** We identified five new fields with Valdensinia leaf spot. Three of the fields were in Waldo county, and one each in Washington and Hancock counties. The areas ranged from a single area approximately 10 ft by 10ft to a couple of acres and were in one crop field and four prune fields. Leaves were collected from different infection sites in the field and plated out to collect isolates. Fifty-seven new isolates were collected for a total of 156 isolates from Maine fields. A collaboration with Dr. Jim Polaschock identified microsatellite DNA markers for DNA fingerprinting the *Valdensinia* isolates. Erika Lyon, a MS graduate student, has extracted DNA from most of the isolates and is testing microsatellite primers for DNA fingerprinting of the isolates. No suitable fields were discovered early enough in the year to set up fungicide trials for Valdensinia leaf spot or burn trials.
We have performed one experiment testing organisms isolated from diseased roots for their ability to cause disease. We used 5 year old potted plants and found plants inoculated with one isolate had smaller root masses than the controls or the other isolate. We plated out the diseased roots but found numerous fungi and had difficulty re-isolating the organisms. We set up a second experiment on younger potted plants and will be isolating the roots in early January.

**CONCLUSIONS:** Valdensinia leaf spot is still spreading among lowbush blueberry fields. Wet weather conditions around bloom provide an early start for this disease. Extra care must be taken to wash equipment and remove all leaves before moving equipment among fields. \

**RECOMMENDATIONS:** The recommendations for dealing with Valdensinia include: avoid walking or travelling through areas infected with Valdensinia, perform a hard burn of

Valdensinia-infected areas to destroy all of the leaf litter as soon as possible, and continue monitoring infected areas in years following attempted eradication of disease. Clean shoes and field equipment of leaves before moving between fields. If possible, field experiments will be set up in 2014 to test fungicides for their effectiveness for controlling Valdensinia leaf spot and to test methods and timing of burning for eradicating the disease.

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**INPUT SYSTEMS STUDY – *ANCILLARY STUDY***

**WEED MANAGEMENT:** David E. Yarborough, Professor of Horticulture
Jennifer L. D. Cote, Assistant Scientist

**21. TITLE:** Systems approach to improving the sustainability of wild blueberry production – Ancillary land-leveling study, Year Three of a four-year study.

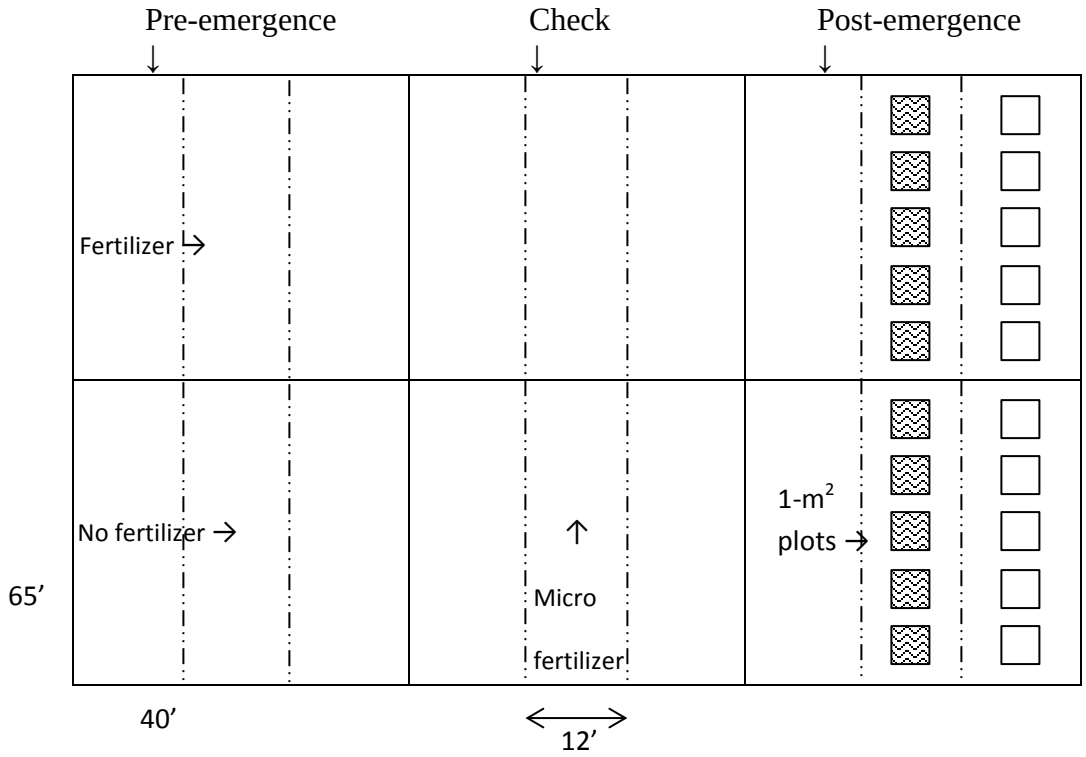
**METHODS**: In 2011 we established a study to determine the optimal combination of fertilizer, mulch and herbicides (weed control) that would allow land leveled wild blueberry stands to reestablish in the shortest time frame. By the end of the 2012 growing season it was evident that the piece of land used for the trial was new land recovered from forest and had too much weed pressure to be controlled with our treatments. We had wanted to assess the effects of the treatments on an established field which was de-rocked and leveled in the first year. The Barren Pond Lot used in 2011-12 had reverted from forest and was cut and leveled just prior to our study. The wild blueberry plants were not established enough to provide the data we needed. Therefore, in 2013 an established field that had been de-rocked and slightly leveled just prior to research plot set-up was used. The new site was located on the west side of Rt. 1 in Jonesboro, ME. A Randomized Complete Block Design using split/nested treatments with three 40' x 130' blocks was established in early spring 2013. The previous cycle's data showed that mulch did not significantly aid re-establishment; therefore, that treatment was omitted this cycle, and was replaced with a nested micro-fertilizer treatment.

Each block received one of three herbicide treatments: check; pre-emergence Velpar L 1 lb/a + Sinbar WDG 2 lb/a + Direx 4L 2 lb/a tank mix; or a post-emergence Callisto 3 oz/a + Select 6 oz/a + COC 1% v/v tank mix applied twice. The pre-emergence treatment was applied on 15 May by the Blueberry Hill Farm (BHF) crew using a tractor mounted boom sprayer. The post-emergence treatments were applied on 10 June and 25 June, also by the BHF.

The 130' block length was split at right angles into two 65' sections, and the section further from the access road had DAP+0.5% B fertilizer applied at 200 lb/a by the BFF with a fertilizer spreader on 15 May. Within each block was nested a 12'x130' strip which had two micro-fertilizers applied at different timings. The first micro-fertilizer, Bio-Forge was applied at 1 pt/a on 21 May. The second, X-tra Power, was applied to the same strips at 4 pt/a on 10 June. The result was six 40'x65' blocks and a total of 12 herbicide/fertilizer/micro-fertilizer combinations. Within each 40'x65' block we set up ten 1-$m^2$ plots – five within the micro-fertilizer strip and five outside of it (Figure 1). The plots were assessed for blueberry cover and phytotoxicity, broadleaf weed cover, and grass cover on 9 July and 7 August. Covers were determined by using a Daubenmire Cover Class scale, which were converted to percent for analysis. Blueberry phytotoxicity was evaluated on a scale of 0-10, which was converted to percent injury (0=none and 10=100% injury/dead). Only main effects were examined using t-tests ($\alpha$=0.05) with

Bonferroni adjustment for herbicide main effects (α=0.0167). There was extremely low (≤ 4 %) to no blueberry phytotoxicity at either evaluation, so the results are not presented here.

**Figure 1**. Site layout (not to scale).




**RESULTS**:

*Herbicides*

Pre-emergence herbicide application resulted in the highest blueberry cover, both in July and August (Figure 2). In July, the check was significantly lower than pre-emergence, and post-emergence was significantly lower than both. By August, the check and pre-emergence were no longer different. There were no significant differences among treatments for broadleaf weed cover at either evaluation. Grasses were initially significantly higher than the two herbicide treatments in the check, but by August all treatments were comparable and grass cover was quite low (<10 %).

*DAP Fertilizer*

Blueberry cover for the check versus DAP 200 lb/a was comparable at both evaluations (Figure 3). Broadleaf weed cover was also comparable at both evaluations, and there was not a large increase in cover between the two evaluation dates. Grasses in the check were actually significantly higher in July, but by August grass cover in the check had dropped so that the two treatments were comparable; grass cover in the DAP treatment remained constant over time.

**Figure 2.** Main effects of herbicide application on wild blueberry, broadleaf weed and grass cover (α=0.0167).

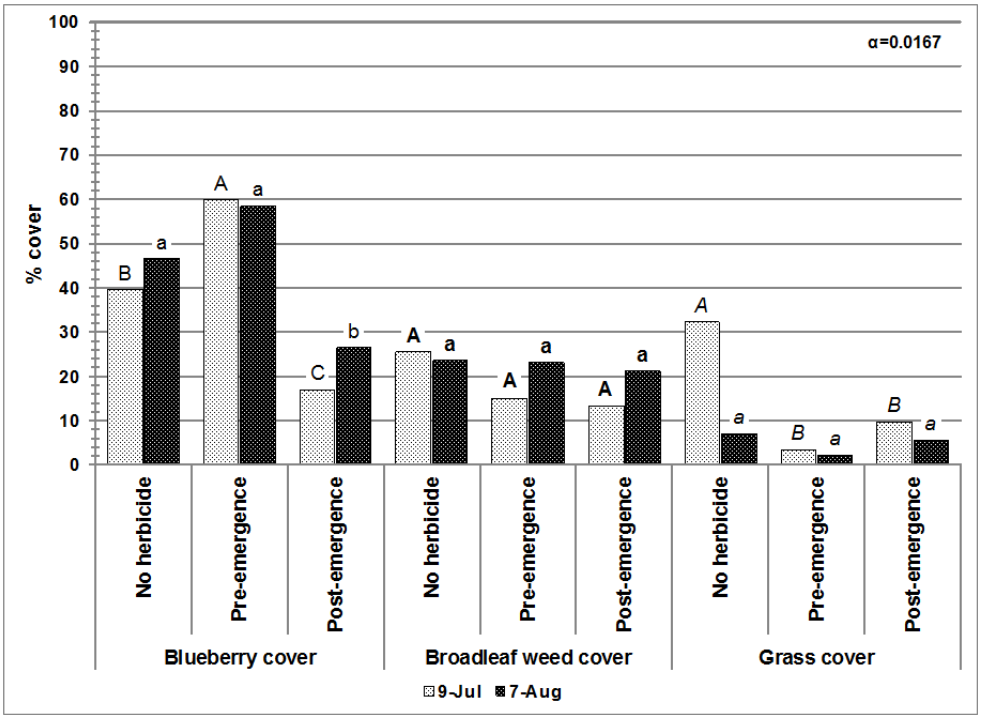
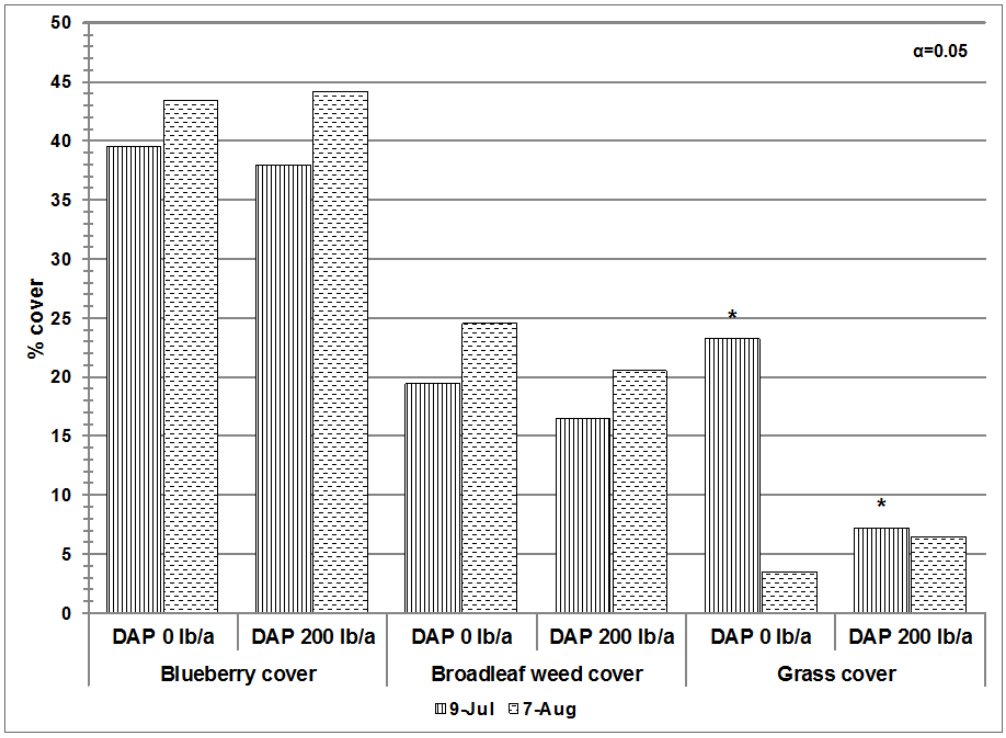


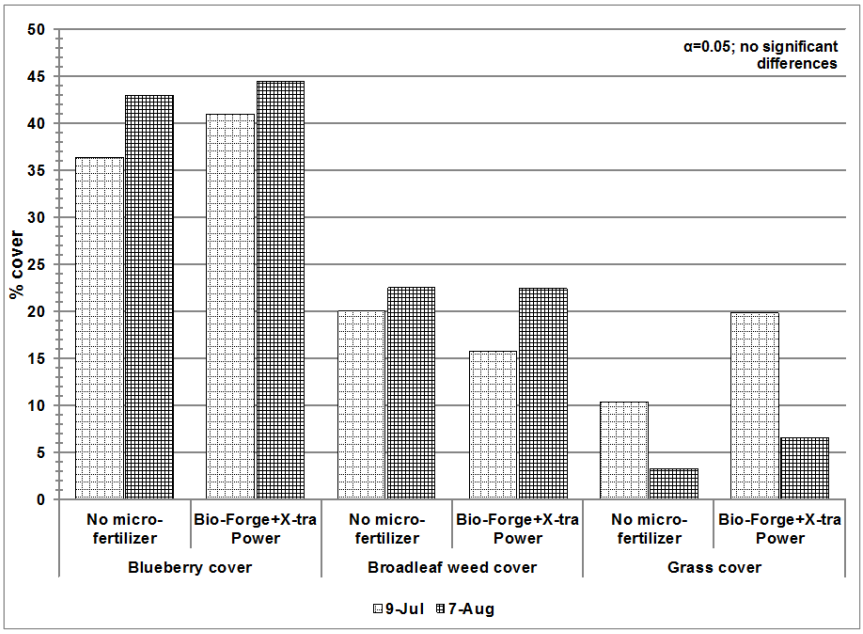

**Figure 3.** Main effects of DAP fertilizer application on wild blueberry, broadleaf weed and grass cover (α=0.05).



*Micro-fertilizer*

There were no significant differences between the check and the micro-fertilizer treatments at either evaluation for blueberry cover or weed cover (Figure 4). In fact, while blueberry cover and broadleaf weed cover increased slightly over time, grass cover in both treatments actually decreased over time.

**Figure 4.** Main effects of micro-fertilizer application on wild blueberry, broadleaf weed and grass cover (α=0.05).




**CONCLUSIONS**: The unusually low blueberry cover in the post-emergence herbicide treatment block compared to the check and pre-emergence blocks appears to be largely due to placement of the trial site. The latter two treatment blocks were located on relatively level ground, while the post-emergence herbicide block was located on the foot of a slope. We believe that the rock removal/leveling activity in this block cut deeper into the soil because of the slope, which resulted in fewer intact rhizomes left to fill back in. Large bare patches were noted in this block over the growing season, and they were not filling in with blueberry at the same rate as in the other two blocks (Photos 1-4).

There was also a lack of greater weed cover in the post-emergence herbicide block due to weeds taking advantage of the bare spots; we believe this may be due to weed root systems and seeds also being removed during the leveling process. The weed species at the site were largely typical of Downeast wild blueberry fields, but there were a few atypical species such as corn spurry (*Spergula arvensis*) and what appeared to be escaped domesticated common oat (*Avena sativa*). Weed cover in general was sparser at the site than expected, and there was also an unexpected lack of response to herbicide application or fertilizers. This may be because the trial was set up almost immediately after rock removal/leveling, thereby removing some of the existing weed seedbank and perennial weeds, so there could be more of a carry-over effect next year once the seedbank begins to be replenished.

**Photo 1.** Bare spots and slope in the post-emergence herbicide treatment block.

**Photo 2.** Example of plot in the post-emergence herbicide treatment block, showing areas of bare ground with dead wild blueberry rhizomes.

**Photo 3.** The pre-emergence herbicide treatment block, showing filling in by wild blueberry.

**Photo 4.** Example of plot in the pre-emergence herbicide treatment block, showing filling in of bare ground with wild blueberry rhizomes.

**RECOMMENDATIONS:** No recommendations at this time. The trial will continue through 2014 and will be evaluated for blueberry and weed covers as well as yield.

**INPUT SYSTEMS STUDY – *ANCILLARY STUDY***

**WEED MANAGEMENT**: David E. Yarborough, Professor of Horticulture
Jennifer L.D. Cote, Assistant Scientist

**22. TITLE**: Pre-emergent combinations of herbicides for weed control in wild blueberry fields – 2013 results from the 2012 trial.

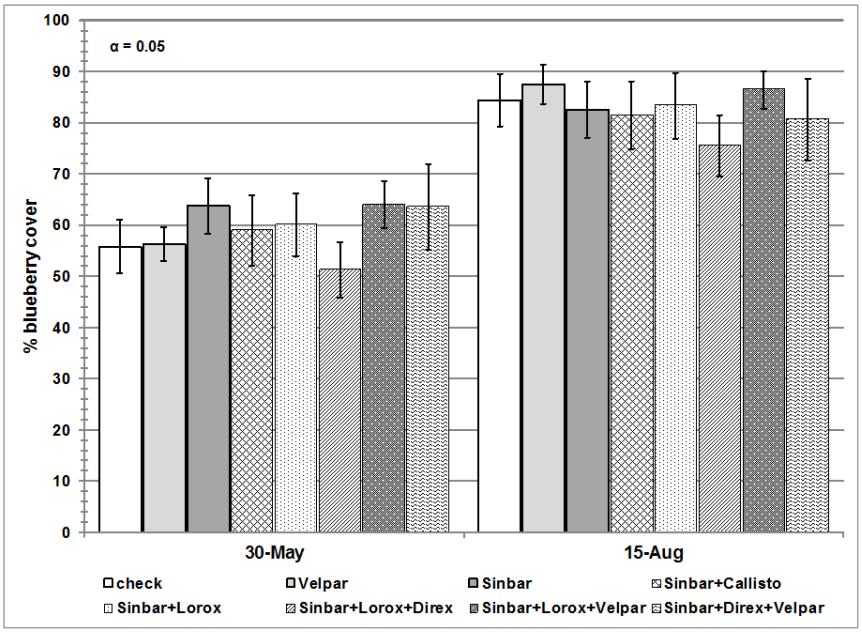
**METHODS**: In 2013 we continued a trial conducted in 2012 to continue the research begun in 2011 to assess the effects of pre-emergence application of herbicides on herbaceous broadleaf weed and grass cover, wild blueberry cover, and injury to blueberry as compared to a check and the industry standard Velpar. Six replications of blocks containing 6'x 40' plots of eight treatments were located in a non-crop field at Blueberry Hill Farm in Jonesboro, ME. Pre-emergence treatments were applied on 7 May 2012 at the following rates:

1. Velpar L 2 lb/a (hexazinone)
2. Sinbar WDG 2 lb/a (terbacil)
3. Sinbar WDG 2 lb/a + Callisto 6 oz/a (mesotrione)
4. Sinbar WDG 2 lb/a + Lorox DF 2 lb/a (linuron)
5. Sinbar WDG 2 lb/a + Lorox DF 2 lb/a + Direx 4L 2 lb/a (diuron)
6. Sinbar WDG 2 lb/a + Lorox DF 2 lb/a + Velpar L 1 lb/a
7. Sinbar WDG 2 lb/a + Direx 4L 2 lb/a + Velpar L 1 lb/a
8. Untreated check.

Plots were assessed for blueberry cover/phytotoxicity and weed cover three times in 2012 by sampling two $1m^2$ quadrats per plot. In 2013, the plots were assessed again for blueberry and weed cover on 30 May and 15 August. Covers were evaluated using a Daubenmire Cover Class scale, which were converted to percent. Blueberry phytotoxicity was evaluated on a scale of 0-10, which was converted to percent injury (0=none and 10=100% injury/dead). The 2013 data were compared using the Standard Error ($\alpha$=0.05) and each treatment was compared individually to the check and to Velpar. The plots were machine harvested on 21 August 2013 and yields were converted to lbs/acre for analysis via Duncan's Multiple Range Test ($\alpha$=0.05).

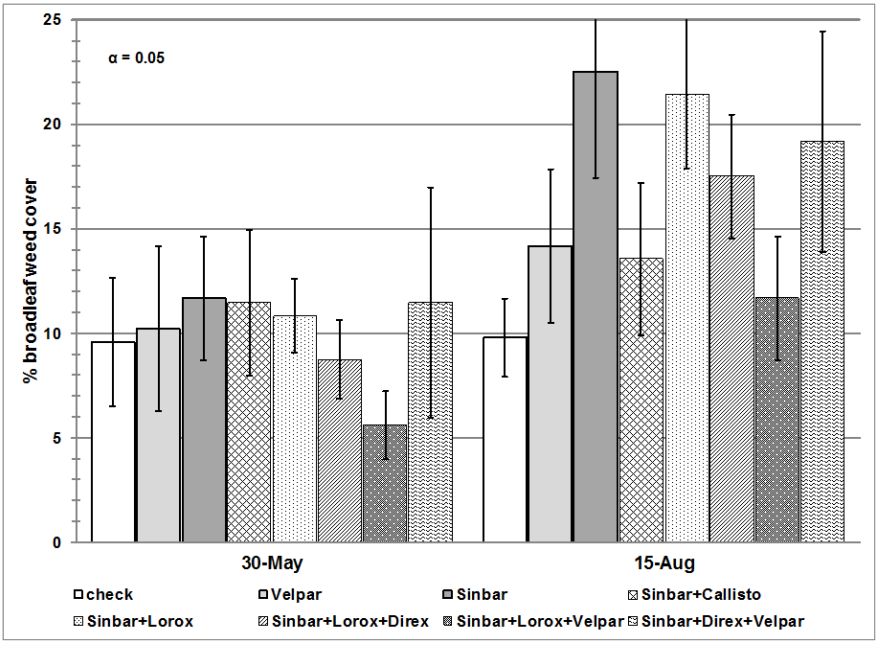
**RESULTS**: In both May and August, although the Sinbar+Lorox+Direx treatment had the lowest blueberry cover (Figure 1) this was not significantly lower than the check or Velpar, and by August the blueberry cover in the Velpar treatment had become higher. There were no other differences between the Sinbar treatments and the check or Velpar. However, at both evaluations, Sinbar+Lorox+Velpar had the highest blueberry cover of the Sinbar treatments.

**Figure 1**. Wild blueberry cover in 2013 following 2012 pre-emergence Sinbar combination treatments.



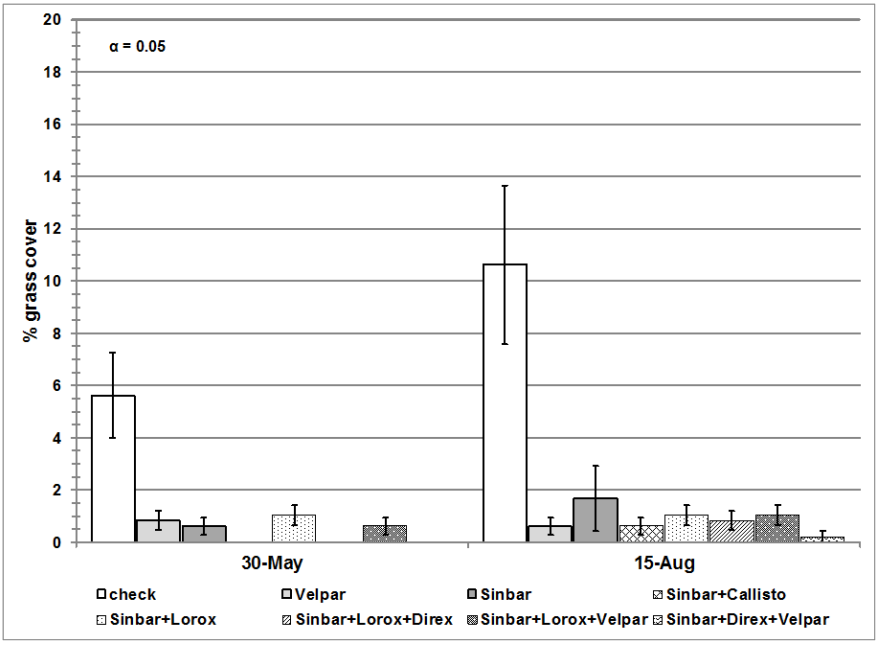

In May, no Sinbar treatment was significantly different from the check or Velpar for broadleaf cover, but only the Sinbar+Lorox+Direx and Sinbar+Lorox+Velpar treatments had less broadleaf weed cover than the check or Velpar (Figure 2). In August, broadleaf weed cover in the check remained constant but all other treatments it increased. Sinbar, Sinbar+Lorox, Sinbar+Lorox+Direx, and Sinbar+Direx+Velpar were all significantly higher compared to the check but no Sinbar treatment combination was different from Velpar.

**Figure 2**. Broadleaf weed cover in 2013 following 2012 pre-emergence Sinbar combination treatments.



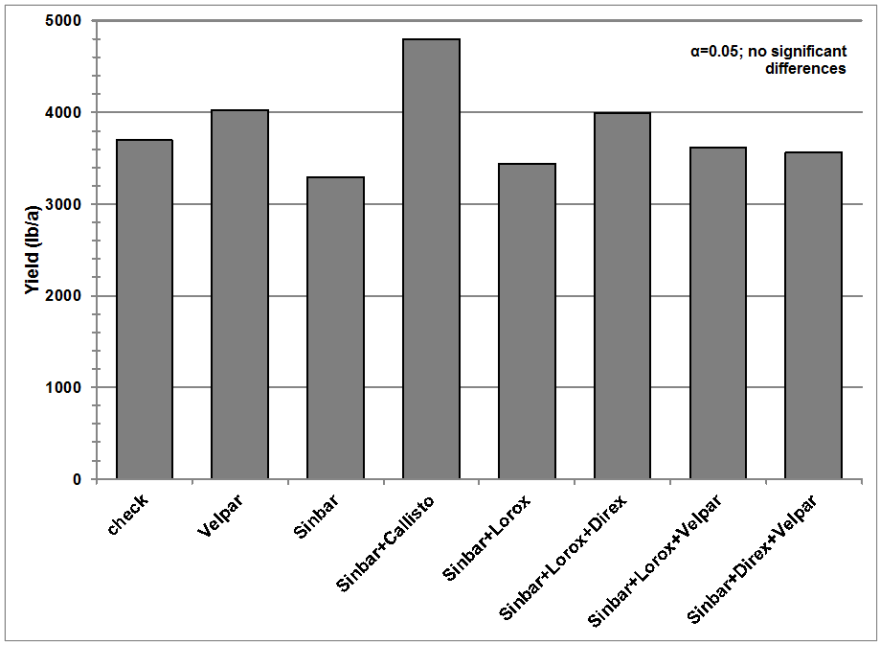
Grass cover was low overall; in May, all treatments were below 6% grass cover (Figure 3). Sinbar+Callisto, Sinbar+Lorox+Direx, and Sinbar+Direx+Velpar had no grass, while all other Sinbar treatments were comparable to Velpar and lower than the check. By August, grass cover had doubled in the check but remained very low (<2%) in all other treatments. All Sinbar treatments were significantly lower than the check and comparable to Velpar.

**Figure 3**. Grass cover in 2013 following 2012 pre-emergence Sinbar combination treatments.




There were no significant differences for yields among treatments (Figure 4). Sinbar+Callisto had the highest yield at almost 5,000 lb/a, while Sinbar alone resulted in the lowest yield at 3,300 lb/a. The remaining treatments ranged from approximately 3,450 to 4,000 lb/a.

**Figure 4**. 2013 wild blueberry yield (lb/a) following 2012 pre-emergence Sinbar combination treatments.




**CONCLUSIONS**: No prune year Sinbar combination resulted in reduction of blueberry cover in the crop year, and overall cover was slightly higher in the crop year compared to the prune year (average 80 percentile versus 70 percentile, respectively). Yields were not reduced by any Sinbar combination but were increased with the addition of Callisto. This may be due to the fact that Callisto controlled broadleaf weeds better long-term than many other Sinbar combinations, and was comparable to Velpar over time in both years. The effectiveness of the Sinbar treatments in controlling grasses released broadleaf weeds in several treatments. Lorox did not appear to control broadleaf weeds unless combined with Sinbar and Velpar, and Direx also did not provide broadleaf weed control in any combination. This supports the 2012 results, in which Lorox and Direx showed the same trends. It should be noted that in both years, Sinbar+Lorox+Velpar had the lowest broadleaf weed cover of the Sinbar combinations at all evaluations. The results of this trial indicate that blueberry growers may want to rethink the use of Trimix (Sinbar+Direx+Velpar) combination if Lorox is registered for use on wild blueberry. The application of Lorox in combination with Sinbar and Velpar consistently provided equivalent control of grasses and better control of broadleaf weeds with no appreciable phytotoxicity, and comparable yields.

**RECOMMENDATIONS**: Recommend that Lorox be registered for use on wild blueberry in the prune year, and specify that it should be used in combination with Velpar *and* Sinbar, since use with Sinbar alone or with Direx was not nearly as effective in weed control over time.

**INPUT SYSTEMS STUDY – *ANCILLARY STUDY***

**WEED MANAGEMENT:** David E. Yarborough, Professor of Horticulture
Jennifer L.D. Cote, Assistant Scientist

**23. TITLE:** Evaluation of herbicides for 2012 prune year control of fineleaf sheep fescue in wild blueberries – 2013 crop year results.

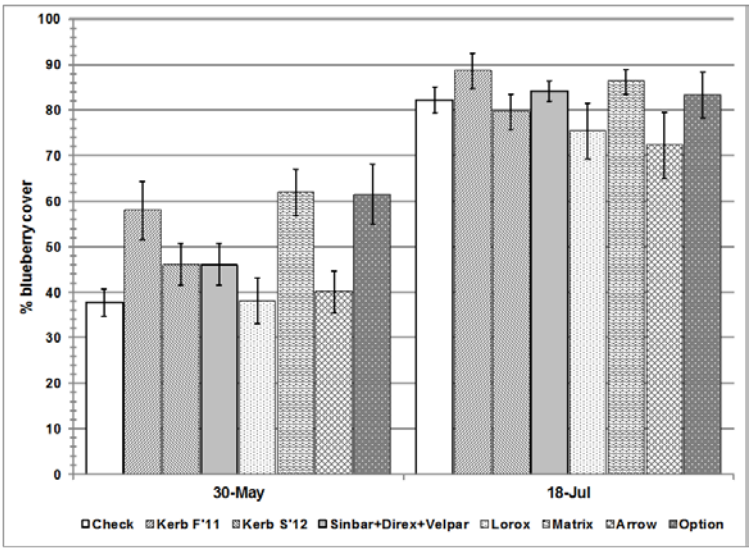
**METHODS**: The experimental site was established in 2011 on a commercial blueberry field on Mason's Bay Road in Jonesport that had an extensive cover of fineleaf sheep fescue (*Festuca filiformis*) which was not controlled by either pre-emergent applications of Velpar or post-emergent applications of Poast. Plots were 6 x 40 feet in a randomized complete block design with six replications. Kerb 50W at 2 lb/a was applied post-pruning on 10 November 2011 when the soil temperature was below 50°F, and it rained the same day. Pre-emergence treatments applied on 20 April 2012 were:
- untreated check
- Kerb 50W 2 lb/a (fall and spring) (pronamide)
- Sinbar WDG 2 lb/a (terbacil) + Direx 4L 2 lb/a (diuron) + Velpar L 1 lb/a (hexazinone) = "Trimix"
- Matrix SG 4 oz/a (rimsulfuron)
- Lorox DF 2 lb/a (linuron)

Post-emergence treatments applied twice on 24 May and 8 June 2012 were:
- Arrow 8 oz/a (clethodim) + NIS 0.25% v/v
- Option 1.5 oz/a (foramsulfuron) + MSO 1.5 pt/a + AMS 1 qt/100 gal

Blueberry phytotoxicity, blueberry cover, fescue grass cover, other grass cover and broadleaf weed cover were assessed three times in 2012. In 2013, blueberry cover and weed cover were assessed on 30 May and 18 July using the Daubenmire Cover Class scale, which were converted to percent; weed species were also identified. The 2013 data were compared using Standard Errors ($\alpha$=0.05).

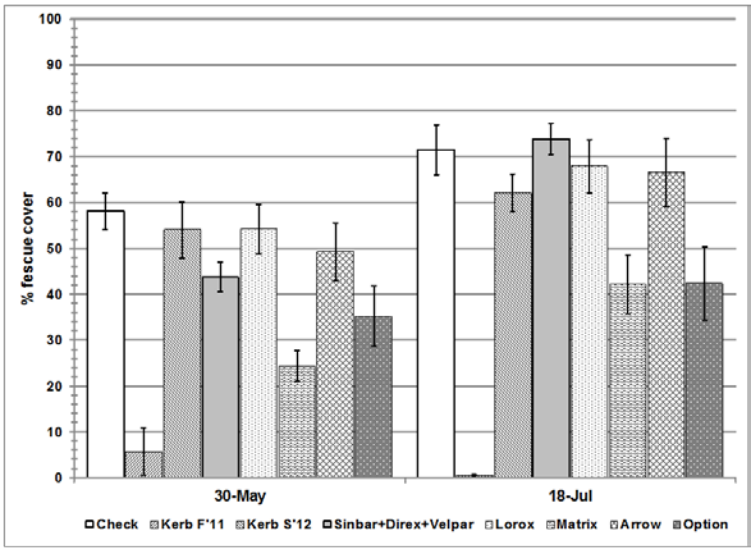
**RESULTS**: In May, the Lorox and Arrow treatments had blueberry cover comparable to the check; the rest of the treatments were significantly higher (Figure 1). By July, blueberry cover in all treatments had increased, but Lorox and Arrow still had the lowest percent cover. Cover in the Fall Kerb treatment was significantly higher than in the Spring Kerb, Lorox, and Arrow treatments; cover in the Matrix and Trimix treatments were also significantly higher than Lorox or Arrow. This is consistent with the 2012 cover assessments, in which Arrow and Lorox consistently had lower blueberry cover.

**Figure 1**. 2013 wild blueberry cover following pre- and post-emergence herbicide applications in 2011-12.




Fall Kerb treatment was by far the most effective in controlling fineleaf sheep fescue over time (Figure 2, Photo 1). Fescue cover in the Fall Kerb treatment was significantly lower than all other treatments at both evaluations; by July, cover was <1%. No other treatment came close except for Matrix at the May evaluation, and the effect did not last over the growing season. However, Matrix and Option did continue to significantly suppress fineleaf sheep fescue compared to the check and the remaining treatments. Spring application of Kerb was not effective in controlling fescue (Photo 2). In 2012, Fall Kerb was by far the most effective in controlling fescue, and Matrix was also quite effective. Fescue control by Option was essential equally effective in 2012 compared to 2013; however, control in 2012 was less effective compared to Matrix while it was comparable to Matrix in 2013.

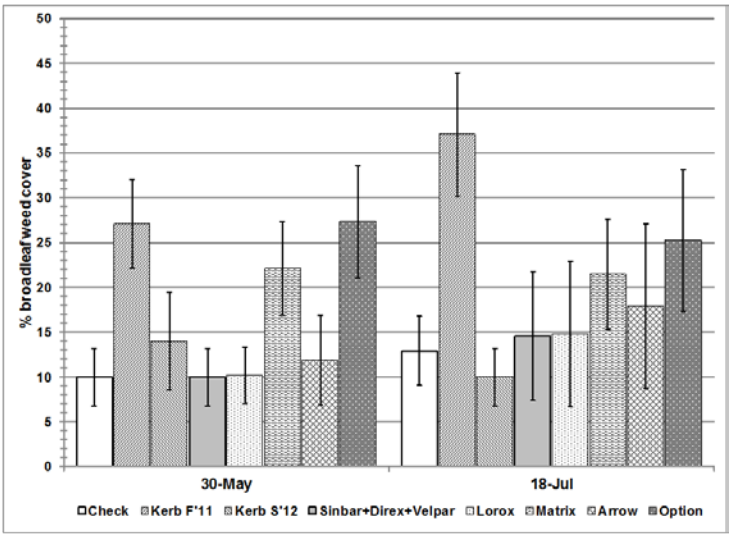
**Figure 2**. 2013 fineleaf sheep fescue cover following pre- and post-emergence herbicide applications in 2011-12.



**Photos 1-2**. Fineleaf sheep fescue control in the crop year; Fall Kerb (L) and Spring Kerb (R).

By contrast, broadleaf weed cover was significantly higher in the Fall Kerb treatment compared to the Spring treatment at both evaluations; it was also higher than all other treatments except for Matrix and Option in May, and all except Option in July (Figure 3). By July, all treatments except Fall Kerb were comparable, with Spring Kerb being most effective on broadleaf weeds. This contrasts with the 2012 trends, in which the Kerb treatments were comparable to all other treatments, Option resulted in the least control, and Trimix resulted in the most control. In some treatments, although fineleaf sheep fescue was controlled, other broadleaf weeds were released. For example, the Matrix controlled fescue fairly well, but it released broadleaf weeds such as violets (Photos 3-4).

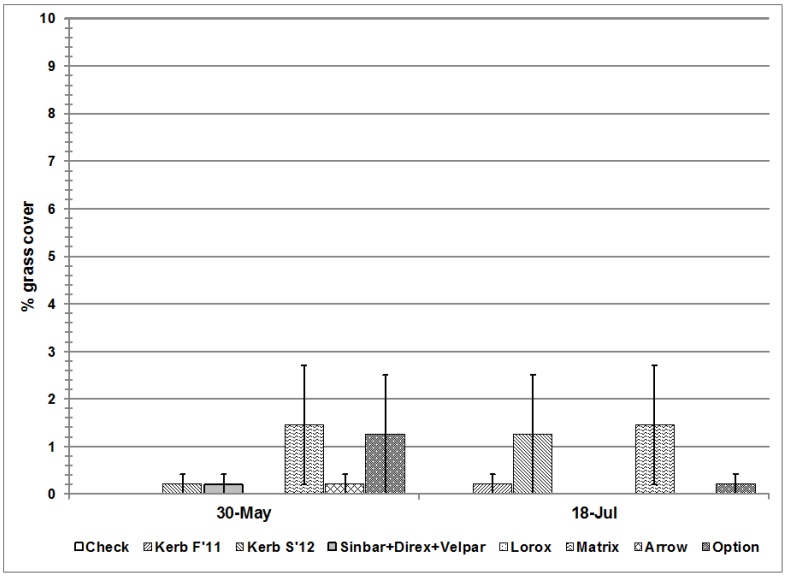
**Figure 3**. 2013 broadleaf weed cover following pre- and post-emergence herbicide applications in 2011-12.



**Photos 3-4.** The Matrix treatment controlled fineleaf sheep fescue, but released other weeds such as violets.

Percent cover of other grasses was very low overall (<2%); the check and Lorox treatments had no other grasses at either evaluation (Figure 4). There were no significant differences among the treatments containing other grasses. Other grass cover was also very low in 2012 and there were no significant differences.

**Figure 4**. 2013 grass cover following pre- and post-emergence herbicide applications in 2011-12.



**CONCLUSIONS**: Fall application of Kerb was extremely effective in controlling fineleaf sheep fescue, but it also released broadleaf weeds more so than any other treatment in this trial. If a grower were to apply Kerb in the fall, they would need to follow it up with a spring application of broadleaf weed herbicide. By contrast, spring application of Kerb was quite effective in controlling broadleaf weeds, but was ineffective on fescue. Because this product is expensive and requires specific environmental conditions to be effective, e.g. low soil temperature and rainfall to incorporate it into the soil soon after application, it is better used as a tool to control resistant fescue. The other two treatments effective on fineleaf sheep fescue, Matrix and Option, exhibited the same release of broadleaf weeds as Spring Kerb. Use of either of these products would also require an additional broadleaf weed herbicide application and could be done in a tank mix with Matrix. Option may not be the best option because it had to be applied twice for effective control of the fescue. In other trials, Matrix at 4 oz/a (the rate used in this trial) was not significantly better at controlling weeds compared to the 2 oz/a rate but it is not known if control of resistant fescue would be as effective at the lower rate.

**RECOMMENDATIONS:** The most cost effective measure is to tank mix Velpar and Matrix to prevent resistant populations of this grass from developing.

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**INPUT SYSTEMS STUDY – *ANCILLARY STUDY***

**WEED MANAGEMENT**: David E. Yarborough, Professor of Horticulture
Jennifer L.D. Cote, Assistant Scientist

**24. TITLE**: 2012 pre-emergence application timing and rate of Alion and Sandea in combination with Velpar or Sinbar – 2013 yields.

**METHODS**: In the spring of 2012, a trial was initiated to continue the assessment of two herbicides under consideration for registration for use on wild blueberry. In 2011 and 2012, indaziflam (Alion) and halosulfuron (Sandea) were tested for weed control and potential injury to wild blueberry. Phytotoxicity to blueberry was observed in 2011 and was associated with late timings of applications; therefore, in 2012 the effects of rate and application and timing for Alion and Sandea alone and in combination with hexazinone (Velpar) or terbacil (Sinbar) were assessed.

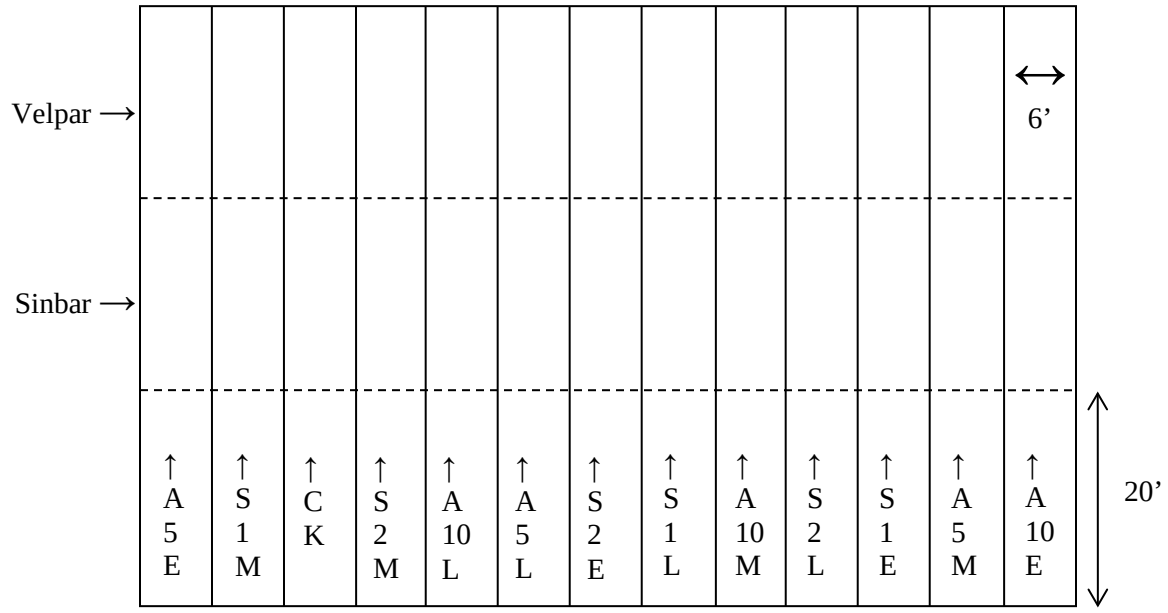
The trial was established on a non-crop field at Blueberry Hill Farm in Jonesboro, ME. The trial consisted of a Randomized Complete Block split plot design with six replications. The Alion and Sandea treatments were applied to 6'x 60' plots, with the timing as follows: the "early" treatments were applied on 18 April, "mid" treatments on 2 May, and "late" treatments on 24 May at the cusp of emergence (budbreak). This resulted in thirteen main treatments:

1. check
2. Alion 5 oz/a early
3. Alion 5 oz/a mid
4. Alion 5 oz/a late
5. Alion 10 oz/a early
6. Alion 10 oz/a mid
7. Alion 10 oz/a late

8. Sandea 1 oz/a early
9. Sandea 1 oz/a mid
10. Sandea 1 oz/a late
11. Sandea 2 oz/a early
12. Sandea 2 oz/a mid
13. Sandea 2 oz/a late

The 6'x 60' plots were split into three 20' lengths with Sinbar at 2 lb/a or Velpar at 1 lb/a applied at the Early timing at right angles to the main treatments. This resulted in 39 total treatments, as seen in Figure 1.

**Figure 1.** Example block layout (A=Alion, S=Sandea, E=early, M=mid, L=late; number indicates rate in oz/a).




When the results of wild blueberry cover/phytotoxicity and weed cover assessments were examined in 2012, it was found that Sandea 2 oz/a resulted in unacceptable phytotoxicity regardless of timing. Sandea 1 oz/a also resulted in unacceptable phytotoxicity when applied at the late timing. Late application timing of Alion also resulted in unacceptable injury to blueberry regardless of rate, but there were no issues at the early or mid-timing. Blueberry cover and weed control at the early and mid-timings were comparable for Alion 5 oz/a vs. Alion 10 oz/a (see the 2012 Wild Blueberry Commission report for detailed discussion of 2012 results).

On 14 August 2013, blueberries were harvested from all 39 treatments (the 6' x 20' plots) and yields (in oz/plot converted to lb/a) were compared using Fisher's protected LSD test ($\alpha$=0.05) to account for unequal treatment cell size. Alion and Sandea were analyzed separately, and the two rates of each herbicide were also analyzed separately. Prior to final analysis, at each application timing (early, mid, or late) the herbicide alone was compared to the herbicide+Sinbar and herbicide+Velpar (n=6 each). If there were no significant differences, the data were pooled into early, mid, or late (n=18 each). The data for both rates of Alion at all timings were able to be pooled, so that the early, mid and late timings were compared to the check, Sinbar alone and

Velpar alone. The same was true for Sandea 1 oz/a; however, the data for the early timing of Sandea 2 oz/a could not be pooled. Therefore, those data were kept separate for final analysis.

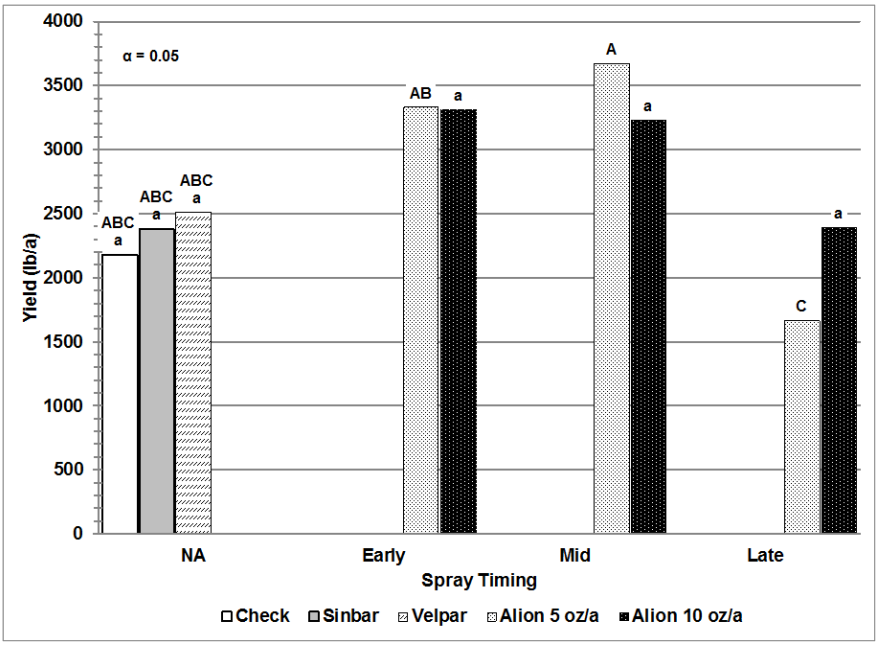
**RESULTS/DISCUSSION**:

*Alion*

The early, mid and late timings for Alion 5 oz/a were compared to each other and the check, Sinbar alone and Velpar alone. The mid timing resulted in the highest yield, while the early timing resulted in the second highest yield; both were significantly greater than the late timing, but no timing was significantly different from the check, Sinbar or Velpar (Figure 2).

The early, mid and late timings for Alion 10 oz/a were also compared to each other and the check, Sinbar alone and Velpar alone. Yields for the early and mid-timing were almost identical to the 5 oz/a rate, but the late timing had a higher yield than that of the 5 oz/a rate (Figure 2). None of the 10 oz/a timing yields were significantly different from each other or the check, Velpar or Sinbar.

**Figure 2.** Effects of Alion rate & spray timing in 2012 prune year on 2013 yield (α=0.05).



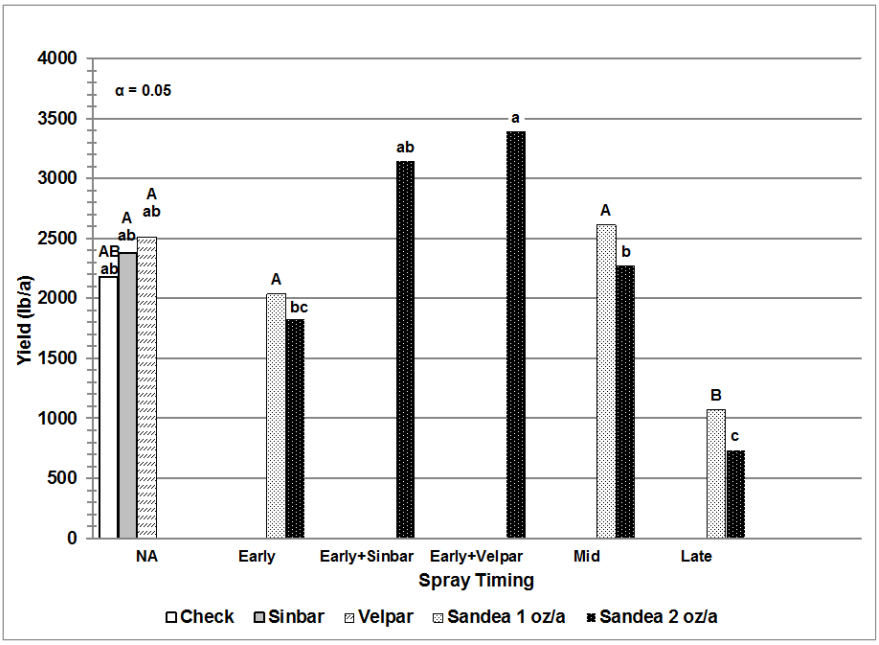

*Sandea*

The early, mid and late timings for Sandea 1 oz/a were compared to each other and the check, Sinbar alone and Velpar alone. The mid-timing for Sandea alone resulted in the highest yield (Figure 3). Yield for all treatments except for the check were significantly higher than the late Sandea timing, but were not significantly different from each other. The yield in the late timing treatment was severely reduced compared to all other treatments.

As previously mentioned, the data from the early timing of Sandea 2 oz/a could not be pooled; therefore, those data were kept separate and were compared to the check, Velpar alone and Sinbar alone, as were the pooled data for the mid and late timings. The early+Velpar treatment resulted in the highest yield, followed closely by early+Sinbar (Figure 3). The early+Velpar

treatment yield was significantly greater than the early, mid and late timings, while the early+Sinbar treatment was only significantly greater than the late timing treatment. As with the lower rate of Sandea, the late timing treatment for Sandea 2 oz/a was greatly reduced compared to all other treatments.

**Figure 3.** Effects of Sandea rate & spray timing in 2012 prune year on 2013 yield (α=0.05).




**CONCLUSIONS/RECOMMENDATIONS**:

*Alion*

The prune year blueberry and weed cover data for this trial suggested that one rate was not consistently better than the other in regards to broadleaf weed or grass control, and that although phytotoxicity was not an issue at the early and mid-timing, there was unacceptable injury to blueberry at the late timing for both rates. The yield data appears to bear this out, as yield for the late timing was less than half that of the early or mid-timings. Furthermore, the 10 oz/a rate yields were roughly equal to the 5 oz/a yields at the early and mid-timings. Therefore, the 5 oz/a rate is sufficient for controlling broadleaf weeds and grasses without reducing yield of wild blueberry. Alion controls broadleaf weeds and grasses in the prune year better in a tank mix with Velpar or Sinbar, so if this product is registered for use on wild blueberry, we will recommend that it be used as a tank mix and applied no later than early May.

*Sandea*

In the prune year, Sandea exhibited unacceptable injury to wild blueberry at the 2 oz/a rate, and at the 1 oz/a late timing. However, it did provide good grass control in combination with Sinbar. When used with Velpar, there was initial broadleaf weed control but it did not last long-term. This year's yields for the 2 oz/a rate, however, do not appear to be affected by the early or mid-timings, as they are comparable to the check, Velpar and Sinbar. The yields for the 1 oz/a early and mid-timings are also comparable to the check, Velpar and Sinbar. Yields for the late timing

of both rates were greatly reduced due to prune year phytotoxicity. When the results of both years' data are examined together, we ultimately do not recommend applying Sandea at 2 oz/a because of unacceptable injury to blueberry in the prune year coupled with the lack of long-term weed control. We believe that Sandea at 1 oz/a, in combination with Sinbar, can be an effective tool for grass control, but even when used with Velpar it did not provide any significant broadleaf weed reduction. Sandea must be applied no later than the beginning of May (or earlier in a warm spring) in order to avoid unacceptable injury to wild blueberry. If this product is registered, we would recommend it as a tank mix to improve grass control when applied as an early pre-emergence treatment.

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**INPUT SYSTEMS STUDY – *ANCILLARY STUDY***

**WEED MANAGEMENT:** David E. Yarborough, Professor of Horticulture
Jennifer L.D. Cote, Assistant Scientist

**25. TITLE:** Pre-emergence Sinbar combinations for weed control in a non-crop wild blueberry field, 2013-2014.

**METHODS**: A trial was initiated in 2013 to continue the work of previous years in looking for new combinations of pre-emergence herbicides with Sinbar, in order to control a variety of weeds while maintaining resistance management. We also continued to assess whether the 4 oz/a rate of Matrix was more effective in controlling weeds compared to the labeled 2 oz/a rate. The trial was set up as a Randomized Complete Split Block Design with six replications on non-crop wild blueberry in the upper field at Blueberry Hill Farm in Jonesboro, ME. Each plot was 6' x 60'; Sinbar combinations were applied along the length of the plots, the plots were divided into three 20' sections, and two rates of Velpar were applied at right angles. The treatments were applied on 21 May 2013 (at the cusp of emergence due to rain and wind issues) as follows for a total of 21 treatments (Figure 1):

*Main*

1. untreated check
2. Sinbar WDG 1 lb/a (Sinbar-1)
3. Sinbar 2 lb/a (Sinbar-2)
4. Sinbar 1 lb/a + Direx 4L 1 lb/a (Sinbar-1+Direx-1)
5. Sinbar 2 lb/a + Direx 2 lb/a (Sinbar-2+Direx-2)
6. Sinbar 1 lb/a + Matrix 2 oz/a (Sinbar-1+Matrix-2)
7. Sinbar 1 lb/a + Matrix 4 oz/a (Sinbar-1+Matrix-4)

*Split*

8. Velpar L 1 lb/a (Velpar-1)
9. Velpar L 2 lb/a (Velpar-2)

**Figure 1**. Example layout of a block (not to scale).

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Check → | | | | | | | |
| Velpar 2 lb/a | | | | | | | |
| Velpar 1 lb/a | Sinbar 1 lb+ Direx 1 lb | Sinbar 2 lb | Sinbar 1 lb+ Matrix 4 oz | Sinbar 2 lb+ Direx 2 lb | Check | Sinbar 1 lb+ Matrix 2 oz | Sinbar 1 lb |

Wild blueberry cover and phytotoxicity, broadleaf weed cover and grass cover were assessed on 19 June and 28 August. Cover data were gathered using the Daubenmire Cover Class system converted to percent; phytotoxicity data were gathered using a scale of 0-10 (0=no damage, 10=100% damaged/dead) converted to percent. The data were analyzed using a non-parametric one-way exact median test ($\alpha$=0.05). All treatments were compared individually to the check. All treatments containing Sinbar 1 lb/a were compared individually to Sinbar 1 lb/a alone; treatments containing Sinbar 2 lb/a were compared individually to Sinbar 2 lb/a alone; treatments containing Velpar 1 lb/a were compared individually to Velpar 1 lb/a alone; and treatments containing Velpar 2 lb/a were compared individually to Velpar 2 lb/a alone.

**RESULTS:**

*Wild blueberry cover and phytotoxicity*

When compared to the check, the only significant difference at the June evaluation was that Sinbar-1 + Matrix-2 was significantly lower than the check (Figure 2). However, all treatments were comparable at the August evaluation (Figure 3). There were also no significant differences at either evaluation when the Sinbar 1 lb/a combinations were compared to Sinbar 1 lb/a alone (Figures 2-3), or when the Sinbar 2 lb/a combinations were compared to Sinbar 2 lb/a alone (Figures 2-3). However, there was a slight trend in that the Sinbar + Matrix 4 oz/a treatments resulted in slightly higher blueberry cover than Sinbar + Matrix 2 oz/a treatments, regardless of evaluation date and presence or rate of Velpar (Figures 2-3). When treatments containing Direx were examined, no such trend was observed. Out of the Velpar comparisons, only the Sinbar-1 + Direx-1 + Velpar-1 treatment resulted in higher blueberry cover than Velpar 1 lb/a alone, in August (Figure 3). Phytotoxicity in June was 6 % or lower, and there was no phytotoxicity in August, so these data are not presented here.

**Figure 2.** Wild blueberry cover at the June evaluation for all treatments compared to the check and to Sinbar 1 lb/a, Sinbar 2 lb/a, Velpar 1 lb/a or Velpar 2 lb/a (α=0.05).

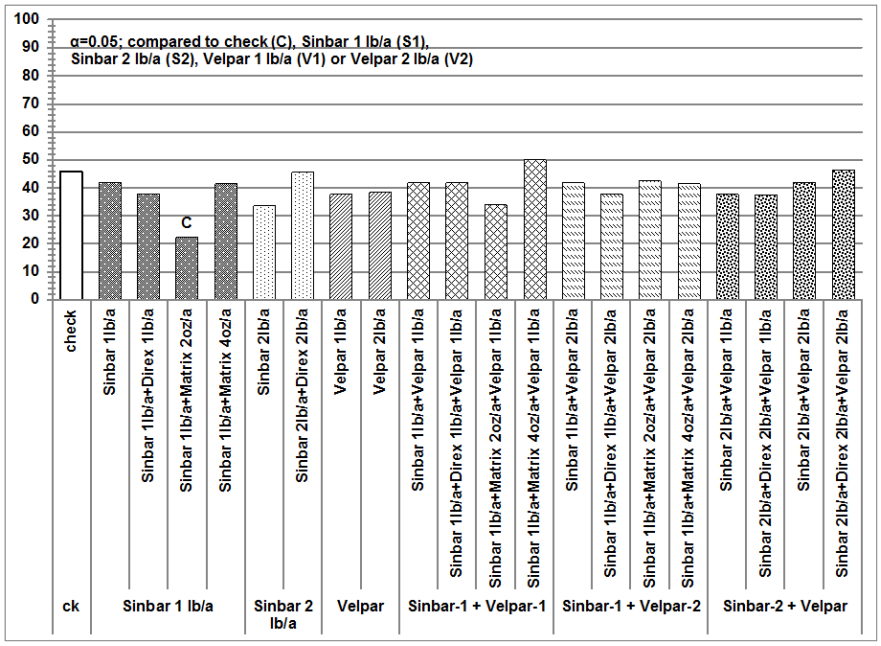
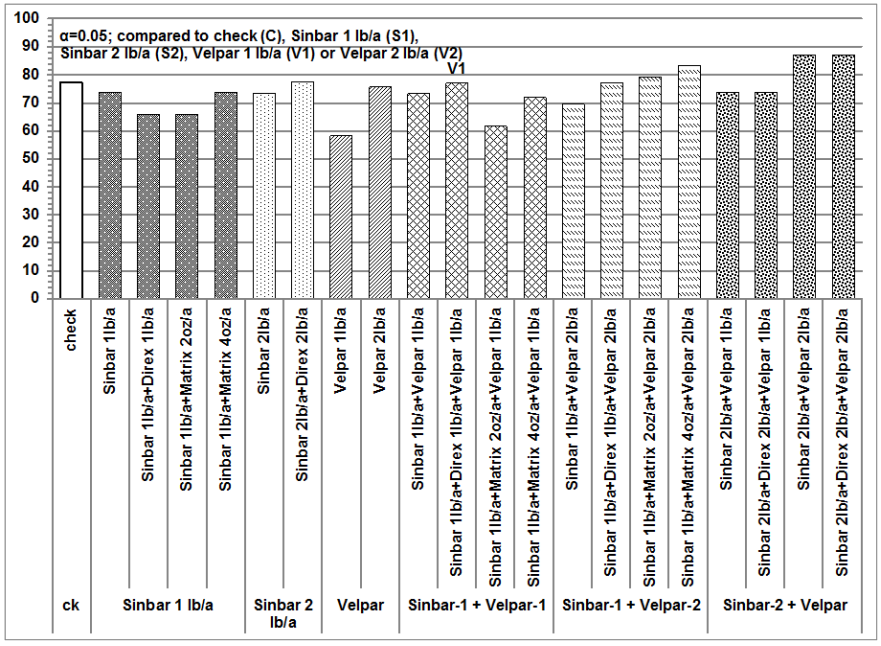



**Figure 3.** Wild blueberry cover at the August evaluation for all treatments compared to the check and to Sinbar 1 lb/a, Sinbar 2 lb/a, Velpar 1 lb/a or Velpar 2 lb/a (α=0.05).



*Broadleaf weed cover*

When compared to the check, no treatment was significantly different in June (Figure 4). In August, Sinbar-1 + Velpar-1 and Sinbar-1 + Velpar-2 had significantly lower broadleaf weed cover than the check, while Sinbar-1 + Matrix-4 + Velpar-1 had significantly higher cover (Figure 5). When compared to Sinbar 1 lb/a, Sinbar-1 + Matrix-2 + Velpar-2 initially had lower broadleaf weed cover but by August there was no longer a significant difference (Figures 4-5). In August, Sinbar-1 + Direx-1 and Sinbar-1 + Matrix-4 + Velpar-1 had broadleaf weed cover 3x that of Sinbar 1 lb/a (Figure 5). Broadleaf weed cover in the Sinbar 2 lb/a combinations had no significant differences compared to Sinbar 2 lb/a at either evaluation. There were also no significant differences at either evaluation when treatments containing Velpar 1 lb/a were compared to Velpar 1 lb/a alone, while in August Sinbar-2 + Velpar-2 had significantly higher cover than Velpar 2 lb/a alone (Figure 5).

**Figure 4.** Broadleaf weed cover at the June evaluation for all treatments compared to the check and to Sinbar 1 lb/a, Sinbar 2 lb/a, Velpar 1 lb/a or Velpar 2 lb/a (α=0.05).

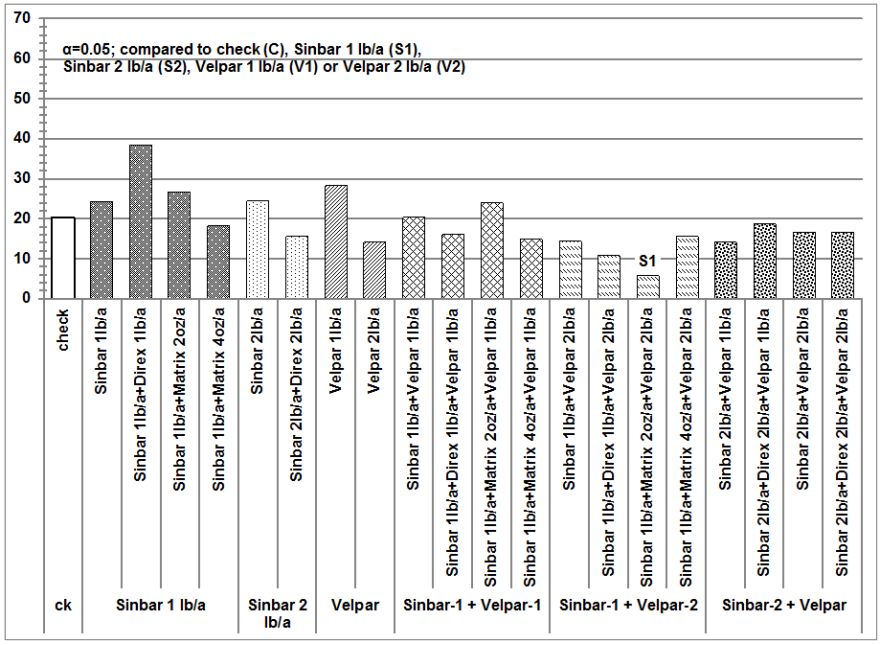
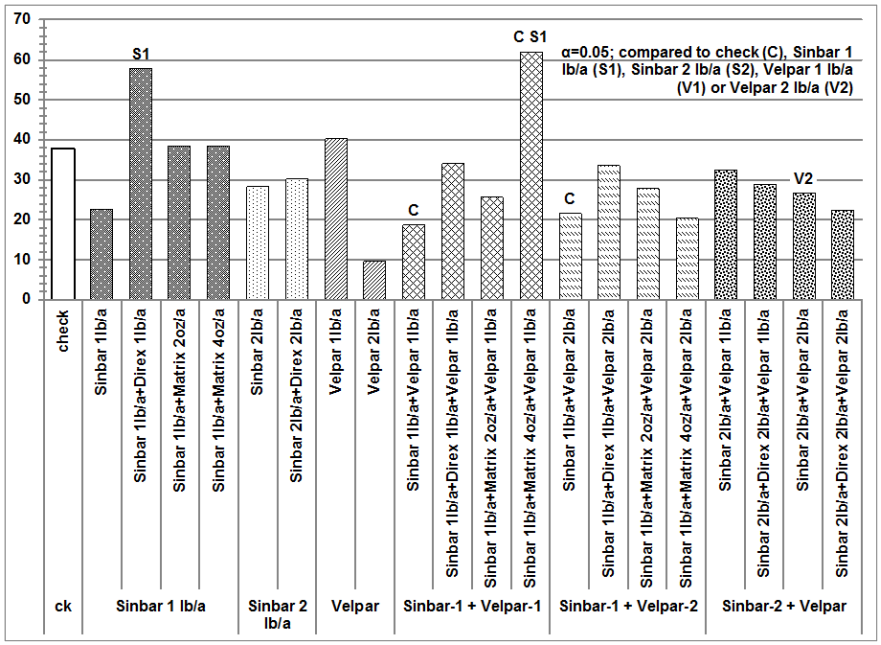


**Figure 5.** Broadleaf weed cover at the August evaluation for all treatments compared to the check and to Sinbar 1 lb/a, Sinbar 2 lb/a, Velpar 1 lb/a or Velpar 2 lb/a (α=0.05).




*Grass cover*

In June, all Sinbar combination treatments except Sinbar-1 and Sinbar-1 + Velpar-1 had significantly less grass cover than the check (Figure 6). However, none were significantly different from Sinbar 1 lb/a or 2 lb/a alone. In August, Sinbar-2 + Velpar-2 grass cover had increased so that it was no longer different from the check, as well as Sinbar-1 + Velpar-2, Sinbar-1 + Direx-1 + Velpar-1, Sinbar-1 + Direx-1 + Velpar-2, and Sinbar-1 + Matrix-2 + Velpar-2 (Figure 7). No Sinbar 2 lb/a combination was different from Sinbar 2 lb/a alone in August, but Sinbar-2, Sinbar-1 + Direx-1 and Sinbar-1 + Matrix-4 + Velpar-2 had less grass cover compared to Sinbar 1 lb/a alone (Figure 7).

When the Velpar 1 lb/a treatments were compared to Velpar 1 lb/a alone, in June only Velpar-2 and Sinbar-1 + Velpar-1 were not significantly lower (Figure 6). By August, only Sinbar-1 + Matrix-2 + Velpar-1 remained significantly lower (Figure 7). When the Velpar 2 lb/a combinations were compared in June, both Direx treatments and Matrix treatments had significantly less grass, but by August only Sinbar-2 + Direx-2 + Velpar-2 and Sinbar-1 + Matrix-4 + Velpar-2 remained significant (Figures 6-7).

**Figure 6.** Grass cover at the June evaluation for all treatments compared to the check and to Sinbar 1 lb/a, Sinbar 2 lb/a, Velpar 1 lb/a or Velpar 2 lb/a (α=0.05).

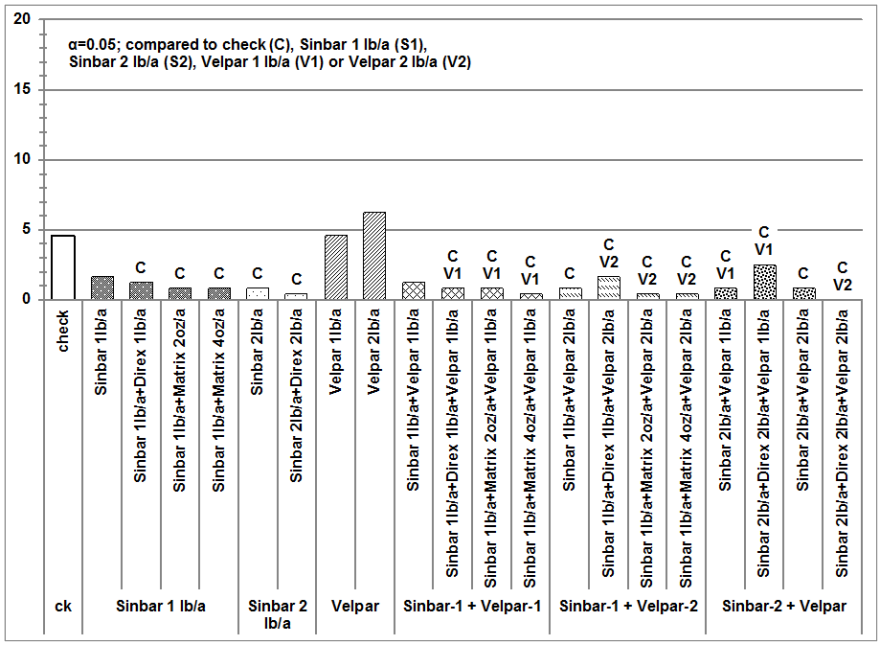
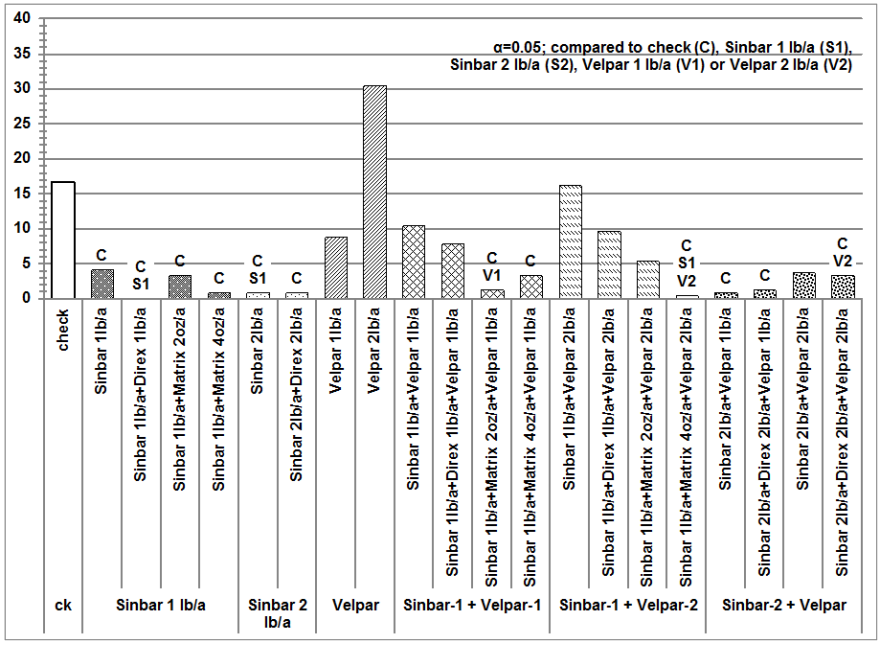



**Figure 7.** Grass cover at the August evaluation for all treatments compared to the check and to Sinbar 1 lb/a, Sinbar 2 lb/a, Velpar 1 lb/a or Velpar 2 lb/a (α=0.05).



**CONCLUSIONS**: It is difficult to draw definitive conclusions from this trial for two main reasons. First, the inherent variability in weed populations, and the use of non-parametric statistical analysis due to the non-normal distribution of weed data, resulted in large Standard Errors for some treatments. This led to some treatments not being significantly different despite having greater differences than some treatments which were significantly different. Second, there were inconsistent responses to a certain herbicide or rate of herbicide.

For example, when Velpar was added to Sinbar-1 + Matrix-2 (Figures 4-5), broadleaf weed control was improved. However, the higher rate of Matrix did not improve broadleaf weed control (Photos 1-2), and when Velpar 1 lb/a was added control was greatly reduced but when 2 lb/a was added it was slightly improved. We have demonstrated in other trials the lack of improved control in using 4 oz versus 2 oz of Matrix, but Velpar is mainly a broadleaf weed herbicide so adding it should not have released broadleaf weeds (it was also not due to suppressing grasses because grass cover was similar to the other aforementioned treatments). We also do not see the same relationship when looking at Sinbar-1 + Velpar-1 and Sinbar-1 + Velpar-2 without Matrix. In general, by late in the growing season varying the rate of Sinbar, Velpar, Direx and/or Matrix still resulted in similar levels of broadleaf weed cover, with two exceptions. Sinbar + Direx was not effective whatsoever on broadleaf weeds without the addition of Velpar; and not only did increasing the rate of Matrix fail to significantly improve broadleaf weed control, but the high rate combined with the low rates of Velpar and Sinbar actually increased weed cover.

The same inconsistencies can be seen in Figures 6 and 7 for grass cover. When Velpar 1 lb/a was added to Sinbar-1 + Matrix-2, grass control was improved, but control declined when Velpar 2 lb/a was added. Conversely, when Velpar 1 lb/a was added to Sinbar-1 + Matrix-4, grass control was reduced but was improved by adding Velpar 2 lb/a. In general, Sinbar + Direx controlled grasses well over time compared to the check or Velpar, but did not significantly improve control over Sinbar alone; increasing the rate of Direx did not result in significant improvement of grass control either. Similarly, Sinbar + Matrix also controlled grasses well compared to the check, but was inconsistent compared to Velpar. Only Sinbar with the high rate of Matrix combined with the high rate of Velpar significantly improved grass control over Sinbar alone.

That being said, some general conclusions can be made. None of the treatments negatively affected wild blueberry cover long-term. Direx did not appear effective in combination with Sinbar 1 lb/a in controlling broadleaf weeds (Photos 1-2); control was slightly improved when used with Sinbar 2 lb/a but no combination was significantly better than Sinbar or Velpar alone. Sinbar 1 lb/a or Sinbar + Velpar was not as effective on grasses as when Matrix or Direx was added, but the high rate of Sinbar controlled grasses as well as the tank mixes.

**Photo 1**. Weed cover in the Sinbar 1 lb/a + Matrix 2 oz/a treatment, August.

**Photo 2**. Weed cover in the Sinbar 1 lb/a + Matrix 4 oz/a treatment, August.

**Photo 3**. Broadleaf weed cover in the Sinbar 1 lb/a treatment, August.

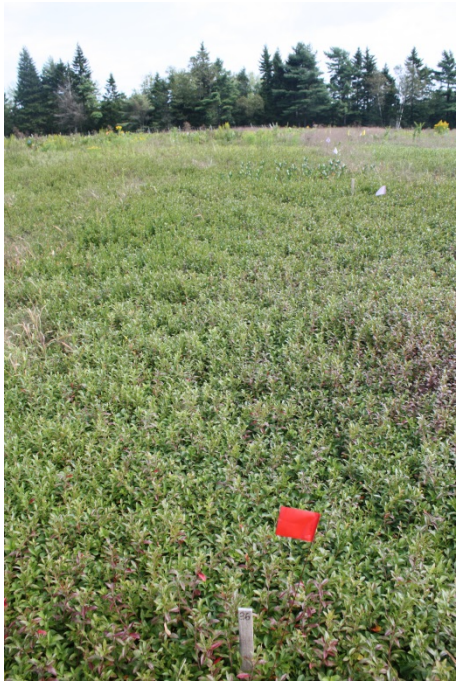


**Photo 4**. Broadleaf weed cover in the Sinbar 1 lb/a + Direx 1 lb/a treatment, August.

**RECOMMENDATIONS**: Velpar alone especially with the higher rate increased grass cover, so the addition of Sinbar or Matrix is needed in combination to provide control. Matrix at the higher rate when combined with both Sinbar and Velpar at the higher rate did provide better broadleaf and grass control. Sinbar alone does not control broadleaf weeds so the addition of Velpar, Direx or Matrix is needed provide the additional control. Some weeds such as ferns are not controlled by any of the treatments and would require spot treatment of Asulox.

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**INPUT SYSTEMS STUDY – *ANCILLARY STUDY***

**WEED MANAGEMENT:** David E. Yarborough, Professor of Horticulture
Jennifer L.D. Cote, Assistant Scientist

**26. TITLE**: Evaluation of three pre-emergence herbicides alone and in combination with Velpar or Sinbar for effects on wild blueberry productivity and weed control.

**OBJECTIVE**: In 2013 we continued to assess three new herbicides for efficacy in weed control as well as effects on blueberry growth and yield. The first, Matrix (rimsulfuron), was labeled for use on wild blueberry in 2012, while Alion (indaziflam) and Sandea (halosulfuron methyl) are not currently registered.

**METHODS**: In spring 2013, a trial was set up at nine sites across the blueberry growing regions of Maine (Mid-coast to Downeast), representing a range of soils, weeds, grower management techniques and climate conditions: Appleton, Hope, Union, Ellsworth, Orland, T-19, Jonesboro, Northfield and Wesley. At each site, three 18'x72' plots were set up and sprayed pre-emergence with Velpar 1 lb/a, Sinbar 2 lb/a, or nothing (check). The plots were split at right angles by four 18'x54' plots which were sprayed pre-emergence with Alion 5 oz/a, Matrix 2 oz/a, Sandea 1 oz/a, or nothing (check). In addition, the Sandea plot and split check plot were extended an additional 54' (final size 18'x108' each) to compare the grower's weed management spray regimen combined with Sandea ("grower Sandea") and without ("grower check") to herbicides used in the trial. The resulting treatments are as follows (18'x18' except for grower check and grower Sandea which are 18'x54'): Check, Velpar, Sinbar, Alion, Velpar+Alion, Sinbar+Alion, Matrix, Velpar+Matrix, Sinbar+Matrix, Sandea, Velpar+Sandea, Sinbar+Sandea, grower Sandea, grower check (Figure 1). The sites were set up and sprayed between 15 April and 1 May 2013. All sites were evaluated for wild blueberry cover and phytotoxicity; broadleaf weed cover and grass cover twice, on 13-20 June and on 14-28 August 2013. Cover was assessed using a Daubenmire cover scale converted to percent, and phytotoxicity was assessed using a scale of 0-10 (0=no damage, 10=dead) converted to percent. Data were analyzed using a non-parametric one-way exact median test ($\alpha$=0.05) to compare each herbicide of interest to the check, as well as the herbicide combinations to Velpar or Sinbar alone. Soil samples were taken to characterize site differences in pH, OM and soil texture (Table 1). A list of herbicides, fertilizer and/or sulfur applications by the growers (e.g. for grower check, grower Sandea) is presented in Table 2.

**Figure 1**. Example of plot layout (not to scale).

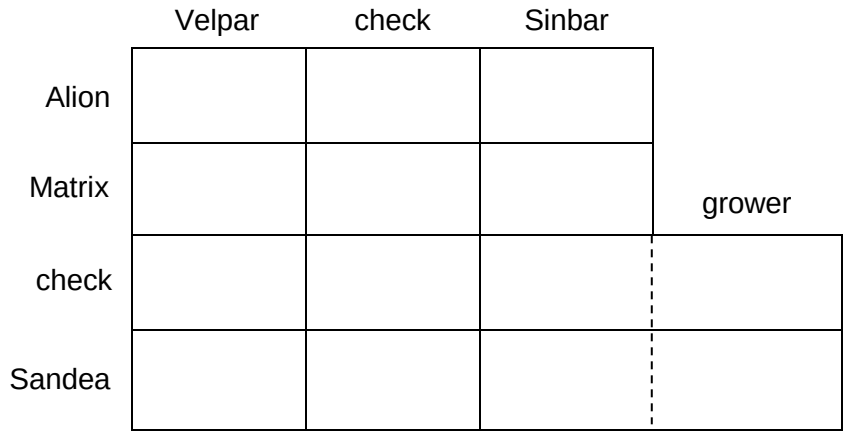



**Table 1**. Soil characteristics on the sites used in the trial.

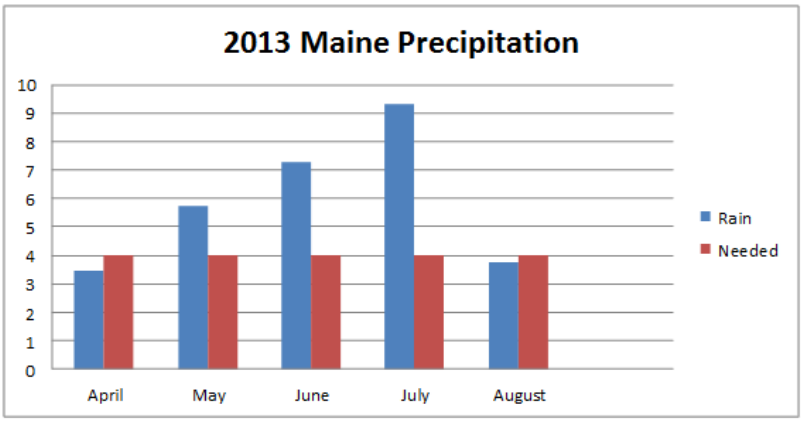
| **County:** | **Knox-Lincoln** | | | **Hancock** | | **Washington** | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Town:** | **Appleton Ridge** | **Hope** | **Waldoboro** | **Ellsworth** | **Orland** | **Jonesboro** | **North-field** | **T-19** | **Wesley** |
| **pH** | 3.6 | 4.1 | 4.2 | 4.2 | 4.6 | 4.3 | 4.5 | 3.9 | 4.5 |
| **% OM** | 16.8 | 8.4 | 7.1 | 6.8 | 12.4 | 9.8 | 11.7 | 14.2 | 14.2 |
| **% sand** | 41 | 42 | 60 | 71 | 48 | 69 | 63 | 58 | 53 |
| **% silt** | 49 | 45 | 36 | 13 | 40 | 20 | 26 | 38 | 33 |
| **% clay** | 10 | 13 | 4 | 16 | 12 | 11 | 11 | 4 | 14 |
| **texture** | loam | loam | sandy loam | sandy loam | loam | sandy loam | sandy loam | sandy loam | sandy loam |

A supplemental demonstration block was set up on a burned field at Blueberry Hill Farm in Jonesboro, and was sprayed with Alion 5 oz/a, Matrix 2 oz/a and Sandea 1 oz/a on 19 November 2012. Velpar 1 lb/a and Sinbar 2 lb/a were sprayed at right angles on 15 May 2013, so that the resulting treatment combinations were the same as above (excluding the grower check and grower Sandea treatments). Statistical analyses were not performed on these data, but wild blueberry cover/phytotoxicity, broadleaf weed cover and grass cover data taken on 20 June and 7 August 2013 were examined using the Standard Errors for comparison.

**Table 2**. Grower herbicide and/or fertility site applications in 2013.

| Site | Date | Product | Rate |
|---|---|---|---|
| **Appleton Ridge** | 5/3 | Velossa<br>Diuron<br>Callisto<br>Grounded<br>Black Label | 0.5 gal/a<br>0.4 gal/a<br>3 oz/a<br>1 pt/a<br>1 gal/a |
| | 6/1 | Sinbar | 3 lbs/14 a spot |
| | 6/20 | Credit w/COC | 32 oz/14 a spot |
| | 6/20 | Callisto<br>Poast<br>LI700<br>Black Label | 3 oz/a<br>1 pt/a<br>4.8 oz/a<br>1 gal/a |
| **Hope** | 5/10 | Velpar<br>Diuron | 0.5 gal/a<br>1.6 qt/a |
| | 6/6 | Poast<br>Callisto | 2 pt/a<br>3 oz/a |
| | 6/14 | Fertilizer 16.6-34.5-4.5 + 0.3 B | 170 lb/a |
| | 7/18 | Sulfur | 730 lb/a |
| **Waldoboro** | 5/8 | Velpar L<br>Diuron<br>Callisto | 6 pt/a<br>2 lb/a<br>6 oz/a |
| | 6/2 | MAP | 200 lb/a |
| **Ellsworth** | 4/29 | Velossa<br>Diuron<br>Sinbar<br>Grounded | 0.4 gal/a<br>0.4 gal/a<br>2 lb/a<br>0.17 gal/a |
| | 6/18 | Black Label | 1 gal/a |
| | 7/29 | TigerSul sulfur<br>DAP | 147 lb/a<br>100 lb/a |
| | 9/16 | Arrow<br>Boost | 8 oz/a<br>6.4 gal/a |
| | 9/26 | Glystar | 5 gal spot |
| **Orland** | 5/22 | MAP | 150 lb/a |
| **Jonesboro** | None | | |
| **Northfield** | Pre-emergence | Velossa<br>Callisto | 1.5 lb/a<br>6 oz/a |
| | Post-emergence | Arrow | 8 oz/a |
| **T-19** | 5/31 | Velpar L<br>Sinbar<br>Diuron 4L | 6 pt/a<br>1.5 lb/a<br>1.5 qt/a |
| | 7/3 | AmSul fertilizer | 424 lb/a |
| **Wesley** | 5/4 | Velossa | 4.8 pt/a |
| | 5/13-14 | DAP+Velpar | 200 lb/a |
| | 6/4-5 | Arrow<br>Callisto | 8 oz/a<br>6 oz/a |
| | 8/13 | Arrow<br>Callisto | 8 oz/a<br>6 oz/a |

**Figure 2.** 2013 needed versus actual precipitation (inches) in Maine.




**RESULTS**: Precipitation for the summer of 2013 from Blueberry Hill Farm Experiment Station was more than adequate and resulted in good blueberry plant growth and weed pressure but weeds varied considerably from site to site. Site differences showed a range in pH from 3.6 to 4.5 and OM from 7.1 to 16.8 %; texture varied from a loam to sandy loam. When Alion, Matrix, Sandea, grower Sandea and the grower check were compared to the check, there were no significant differences in wild blueberry cover in June or August (Figure 3). In June, the grower check had significantly higher phytotoxicity compared to the trial check; this was observed mainly as chlorosis with scattered necrosis (Figure 3). Both the Sandea and grower Sandea treatments also had significantly higher phytotoxicity (stunting and delayed growth) compared to the check in June (Photos 1-2). However, in August there was no appreciable injury to blueberry still evident (for any treatment or combination - data not shown). There were also no significant differences in broadleaf weed or grass covers for the aforementioned treatments compared to the check in June or August (Figures 4-5).

**Figure 3**. Wild blueberry cover and phytotoxicity for Alion, Matrix, Sandea, and the growers' spray regimen (alone and with Sandea) vs. no treatment (α=0.05).

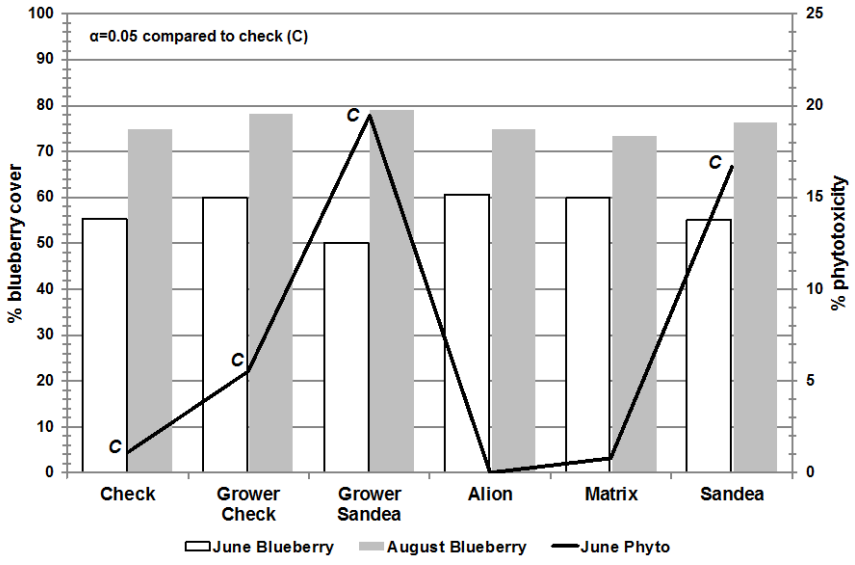


**Figure 4.** Broadleaf weed cover for Alion, Matrix, Sandea, and the growers' spray regimen (alone and with Sandea) vs. no treatment (α=0.05).

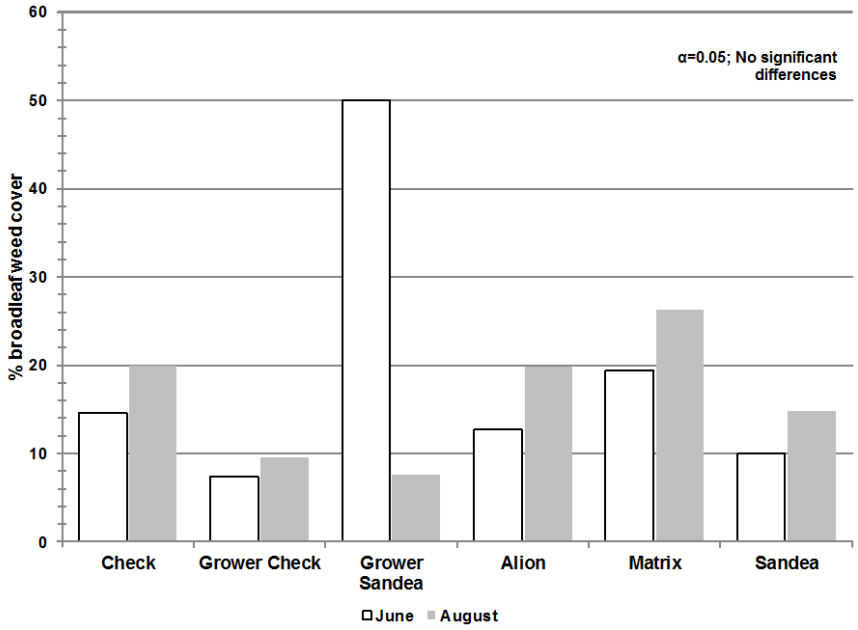



**Figure 5**. Grass cover for Alion, Matrix, Sandea, and the growers' spray regimen (alone and with Sandea) vs. no treatment (α=0.05).

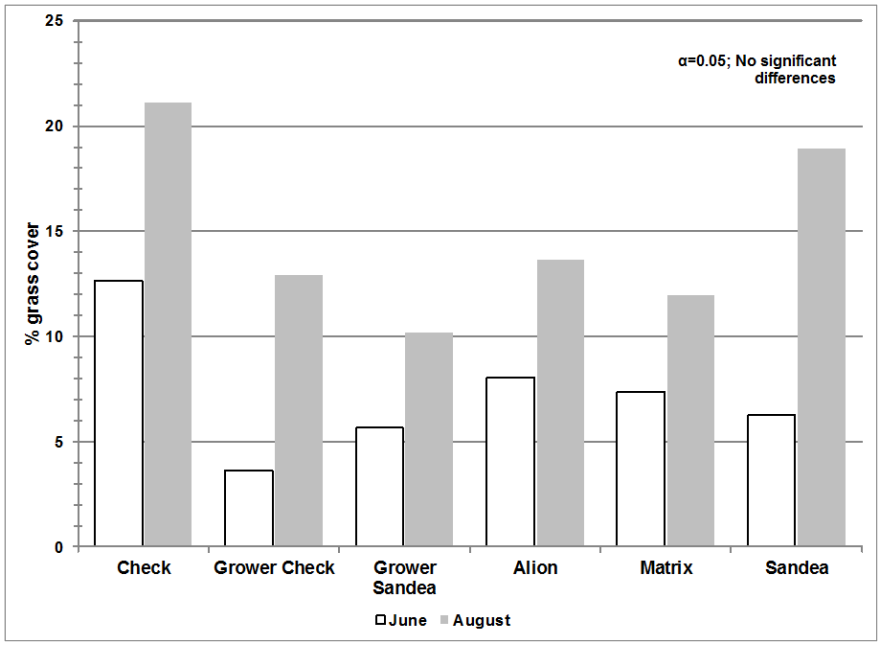


**Photo 1.** Phytotoxicity in June in the Sandea treatment, Orland.

**Photo 2**. The untreated check in June, Orland (Sandea phyto to rear left).

Alion, Matrix and Sandea combined with Velpar were compared to the check and Velpar alone. In June, Sandea+Velpar had significantly less blueberry cover compared to Velpar, but no treatment was different from the check (Figure 6). In August there were no significant differences in blueberry cover. In June, the Sandea+Velpar treatment also had significantly more phytotoxicity (stunting and delayed growth) compared to the check or Velpar alone (Figure 6), but the plants had recovered by August. Broadleaf weed cover for the Velpar combinations was not significantly different from the check or Velpar in June or August (Figure 7). In June, grass cover in the Sandea+Velpar treatment was significantly lower compared to Velpar alone, but by August all Velpar combinations were comparable (Figure 8).

**Figure 6**. Wild blueberry cover and phytotoxicity for Alion, Matrix, and Sandea combined with Velpar vs. Velpar alone or no treatment (α=0.05).

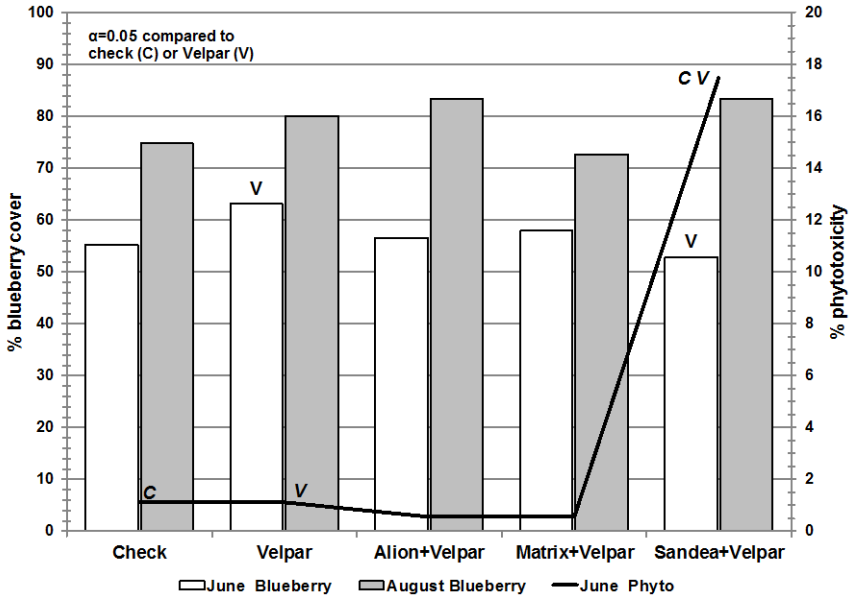



**Figure 7**. Broadleaf weed cover for Alion, Matrix, and Sandea combined with Velpar vs. Velpar alone or no treatment (α=0.05).

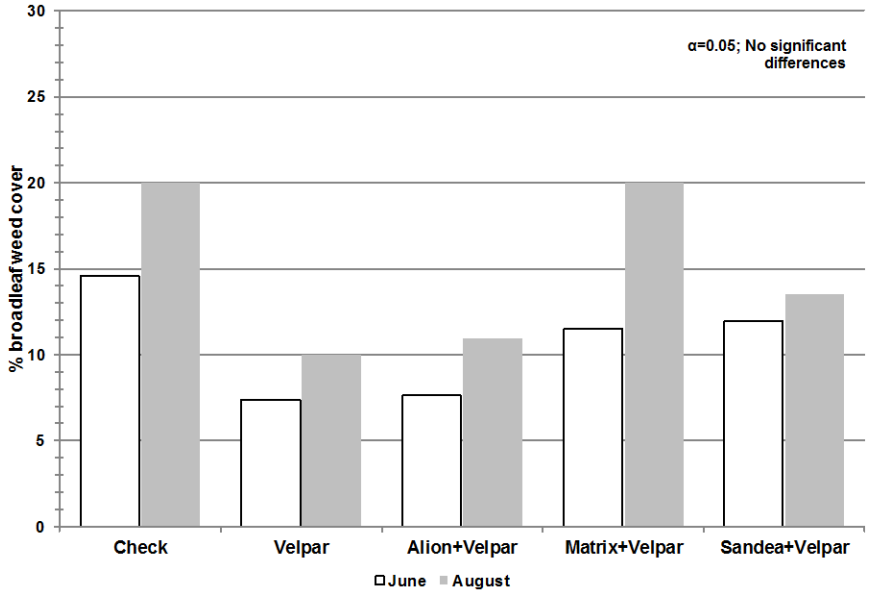
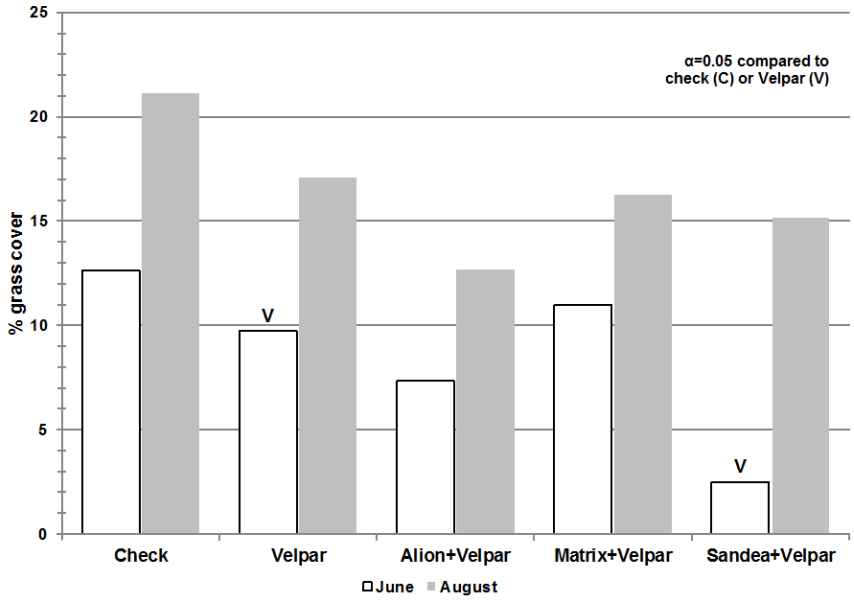


**Figure 8**. Grass cover for Alion, Matrix, and Sandea combined with Velpar vs. Velpar alone or no treatment (α=0.05).




Alion, Matrix and Sandea combined with Sinbar were compared to the check and Sinbar alone. In June, Sandea+Sinbar had about three-fold less blueberry cover compared to both the check and Sinbar alone, but again, by August there were no significant differences for any treatment (Figure 9). As with the other Sandea treatments, Sandea+Sinbar showed significant phytotoxicity as stunting and delay in growth compared to the check and Sinbar in June, but by August there was no appreciable phytotoxicity in any treatment. The Sinbar combinations were not significantly different from the check or Sinbar at either evaluation regarding broadleaf weed cover (Figure 10). However, in June, both the Alion+Sinbar and Matrix+Sinbar treatments significantly reduced grasses compared to the check (but not Sinbar alone) (Figure 11). In August, Matrix+Sinbar continued to significantly reduce grasses compared to the check (Photos 3-4), but not Sinbar.

**Figure 9**. Wild blueberry cover and phytotoxicity for Alion, Matrix, and Sandea combined with Sinbar vs. Sinbar alone or no treatment (α=0.05).

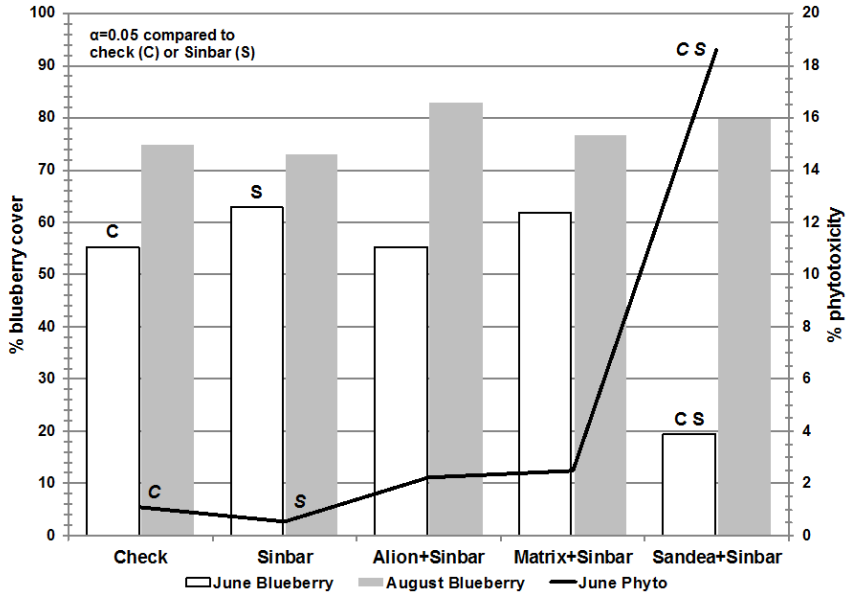



**Figure 10**. Broadleaf weed cover for Alion, Matrix, and Sandea combined with Sinbar vs. Sinbar alone or no treatment (α=0.05).

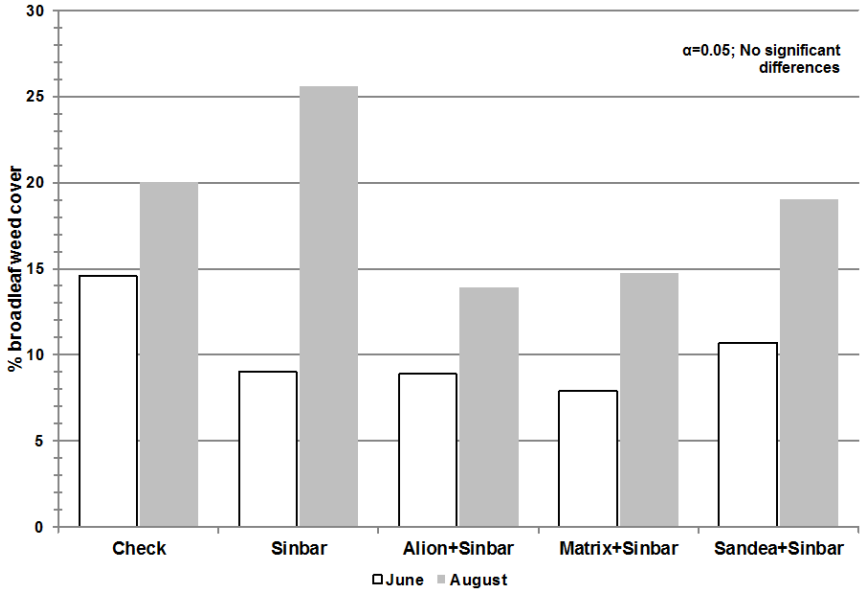


**Figure 11**. Grass cover for Alion, Matrix, and Sandea combined with Sinbar vs. Sinbar alone or no treatment (α=0.05).

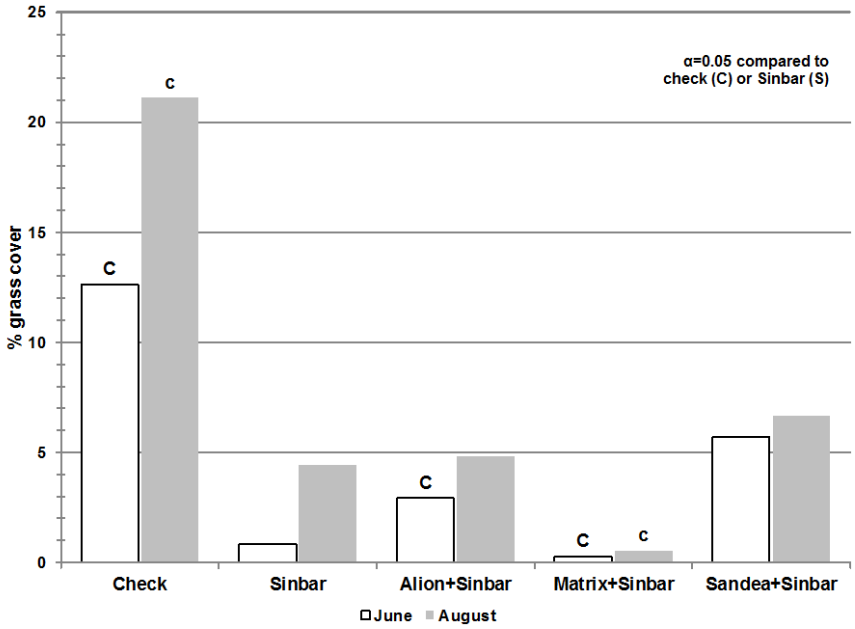



**Photo 3.** Matrix + Sinbar plot in August, Hope.

**Photo 4.** The untreated check in August, Hope.

When the data from the fall application demonstration plots were examined, blueberry cover for Sandea alone and combination treatments were lowest of all treatments in June and August (Figure 12-14). Sandea+Velpar was also the only treatment to exhibit 10% phytotoxicity in June, (Figure 14); there was no phytotoxicity in August (data not shown). Of the herbicides alone, Matrix had the best long-term broadleaf weed control (Figure 15), but Alion showed the best long-term broadleaf control when combined with Sinbar (Figure 16), but was greater than the check as Sinbar released the broadleaf weeds. Alion and Sandea showed the best control when combined with Velpar (Figure 17). Of the herbicides alone, Alion exhibited the best long-term grass control (Figure 18). All Sinbar combinations had comparable grass control (Figure 19), while the three Velpar combinations exhibited better grass control than Velpar alone (Figure 20).

**Figure 12**. Wild blueberry cover and phytotoxicity for fall application of Alion, Matrix and Sandea vs. no treatment (± Std. Error).

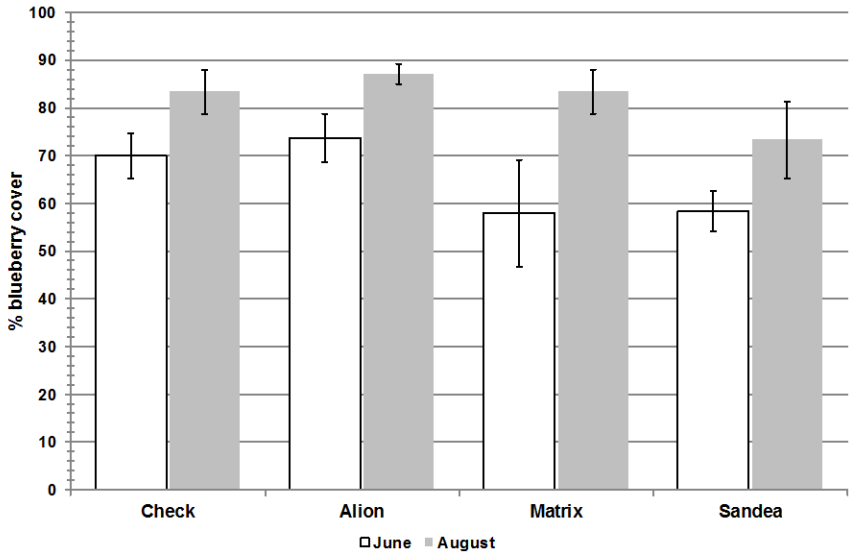


**Figure 13**. Wild blueberry cover and phytotoxicity for fall application of Alion, Matrix and Sandea combined with Sinbar vs. Sinbar alone and no treatment (± Std. Error).

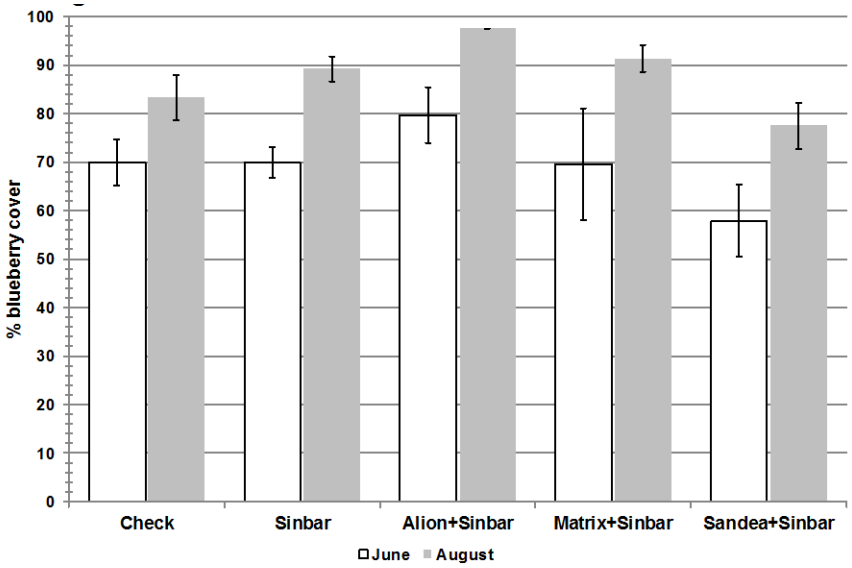



**Figure 14**. Wild blueberry cover and phytotoxicity for fall application of Alion, Matrix and Sandea combined with Velpar vs. Velpar alone and no treatment (± Std. Error).

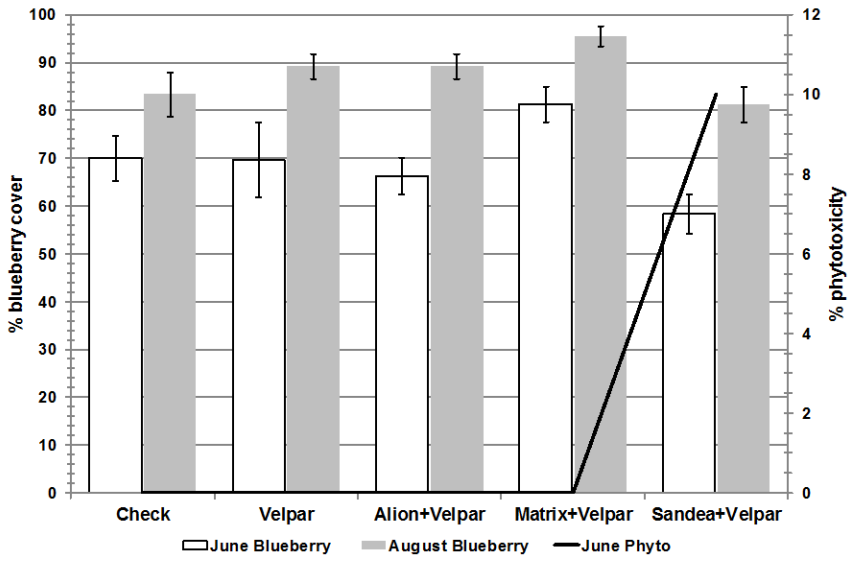


**Figure 15**. Broadleaf weed cover for fall application of Alion, Matrix and Sandea vs. no treatment (± Std. Error).

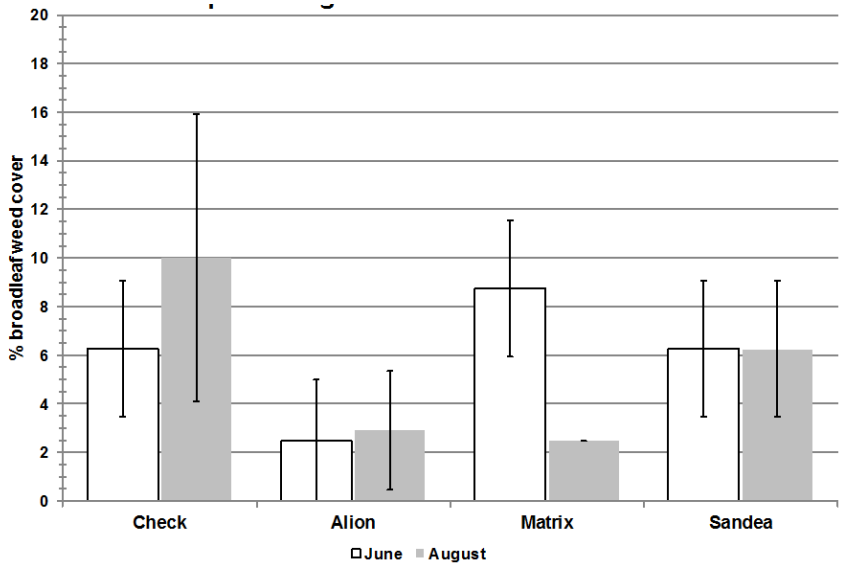



**Figure 16**. Broadleaf weed cover for fall application of Alion, Matrix and Sandea combined with Sinbar vs. Sinbar alone and no treatment (± Std. Error).

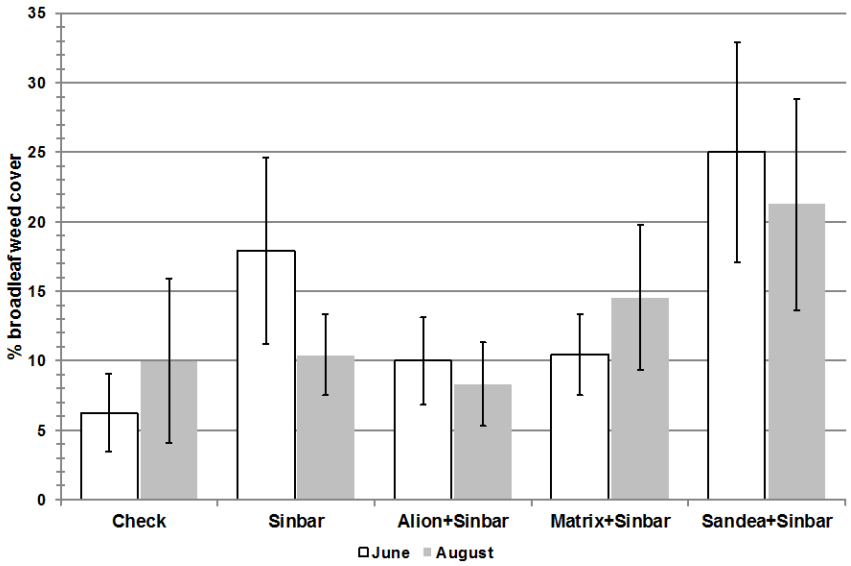


**Figure 17**. Broadleaf weed cover for fall application of Alion, Matrix and Sandea combined with Velpar vs. Velpar alone and no treatment (± Std. Error).

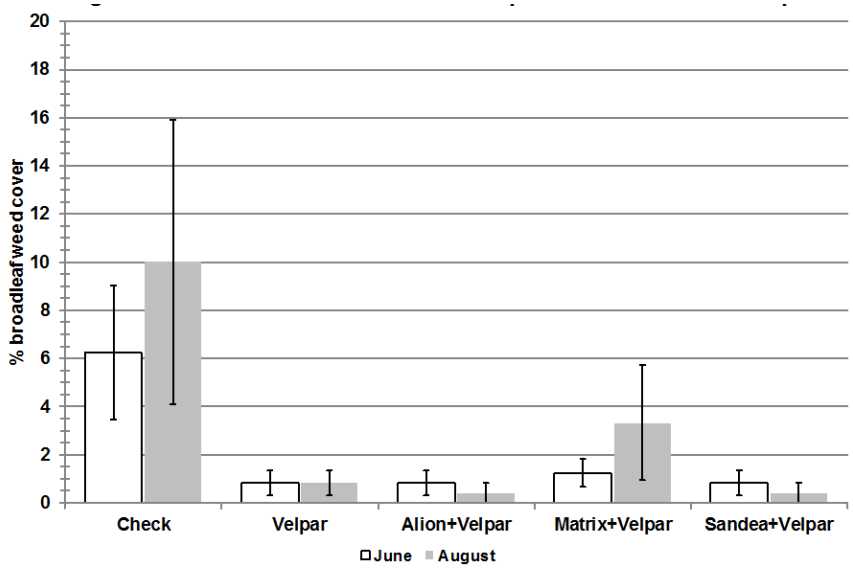



**Figure 18**. Grass cover for fall application of Alion, Matrix and Sandea vs. no treatment (± Std. Error).

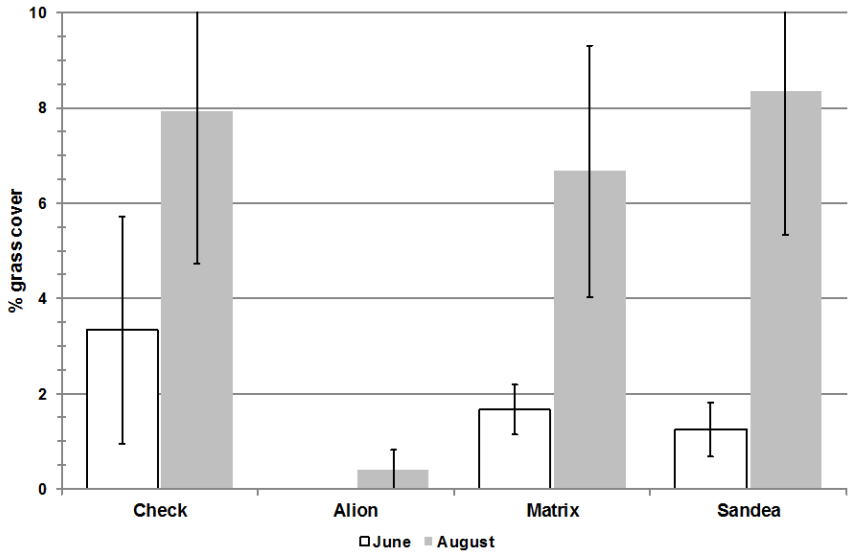


**Figure 19**. Grass cover for fall application of Alion, Matrix and Sandea combined with Sinbar vs. Sinbar alone and no treatment (± Std. Error).

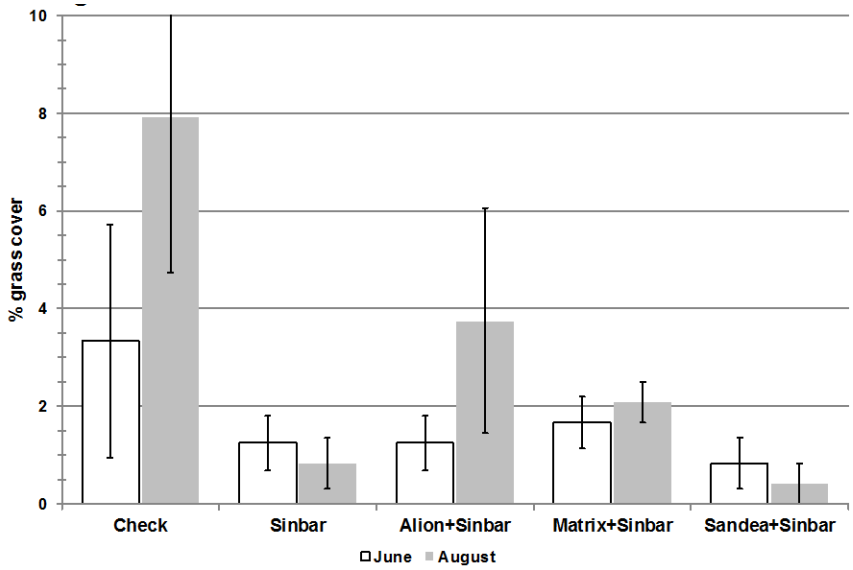
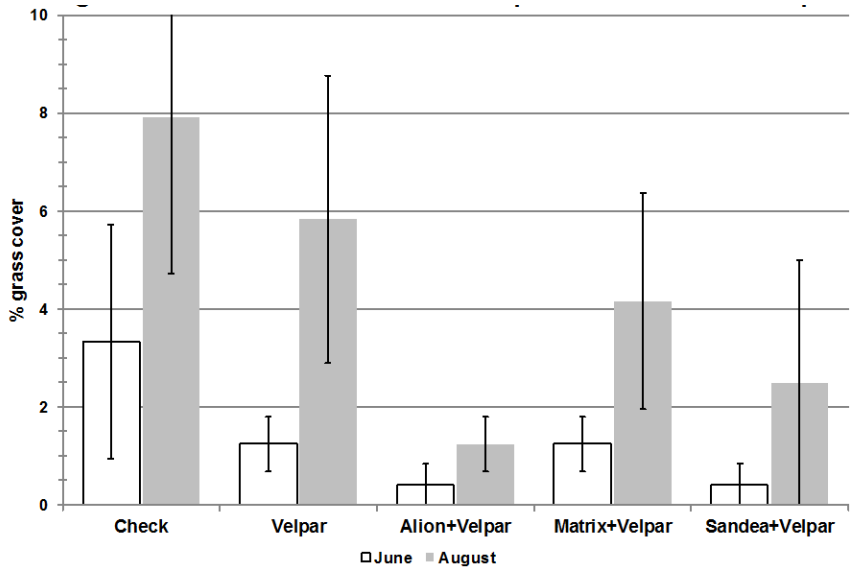



**Figure 20**. Grass cover for fall application of Alion, Matrix and Sandea combined with Velpar vs. Velpar alone and no treatment (± Std. Error).




**CONCLUSIONS**: Alion and Matrix, whether alone or in combination with Velpar or Sinbar, did not reduce wild blueberry cover or result in unacceptable phytotoxicity. Sandea alone or in combination did show an initial stunting and delay in growth, but the plants recovered over the growing season. This is borne out by the initial reduction but subsequent increase in blueberry cover for the Sandea combinations. Although the results were not significant, the growers' spray regimen alone, Sandea alone, or the combination had an effect in suppressing broadleaf weeds over the long-term, as did Velpar, Alion+Velpar, Sandea+Velpar, Alion+Sinbar and

Matrix+Sinbar. Grasses were initially controlled by Sandea+Velpar, the growers' regimen (not significant), Sinbar, Alion+Sinbar and Matrix+Sinbar. In addition, although in August only Matrix+Sinbar continued to control grasses, all of the Sinbar combinations also continued to maintain grass cover about 5% or below.
Applying Alion, Matrix and Sinbar in the fall appeared to slightly improve long-term blueberry cover overall, as well as reduce the early Sandea phytotoxicity. Broadleaf weed and grass pressure was low overall at this site, showed reduction in weed cover in the treatments compared to the check. Fall application appeared to improve broadleaf weed control in general compared to spring application when combined with spring Velpar. Grass control was improved by fall application of Alion with spring Velpar, and to a lesser extent Sandea+Velpar and Sandea+Sinbar grass control improved as well when compared to Sandea applied in spring.

**RECOMMENDATIONS**: This trial will be continued through 2014 to assess effects on wild blueberry yield. In November 2013 nine more plots were set out on sites in the same areas as the spring 2013 sites. Since the Matrix, Alion and Sandea do not leach readily and the Velpar and Sinbar do, the first three herbicides were applied in the late fall and the latter two will be applied in the late spring of 2014. This should maximize the effectiveness of the applications.

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**INPUT SYSTEMS STUDY – *ANCILLARY STUDY***

**WEED MANAGEMENT:** David E. Yarborough, Professor of Horticulture
Jennifer L.D. Cote, Assistant Scientist

**27. TITLE:** Post-harvest control of red sorrel in a non-crop blueberry field, 2012-2014.

**METHODS**: In the fall of 2012, we initiated a trial to determine whether red sorrel control in wild blueberry fields could be achieved by treating the weed after post-harvest pruning. The trial was set up on Wyman's Station Road Lot in Centerville, ME. The plots were set out in a Completely Randomized Design with ten 1-$m^2$ replications per treatment, which were as follows:

1. Untreated check
2. Hand-held backpack oil burner
3. Roundup 2% v/v
4. Reglone 2 pt/a + NIS 0.25% v/v

The oil burner plots were burned on 16 November 2012, and the herbicides were applied on 19 November 2012 using a backpack boom sprayer with a single nozzle. Because we wanted to assess whether the above treatments would control red sorrel when combined with a grower's regular spray regimen, we asked Wyman's to spray their herbicide treatments on the plots as well over the 2013 growing season. Their treatments were as follows: 5/7/13 – Velossa 0.4 gal/a; and 6/14/13 – Arrow 2EC 8 oz/a + Callisto 6 oz/a + COC 12 oz/a.
The plots were evaluated for wild blueberry cover and phytotoxicity, broadleaf weed cover, grass cover, and red sorrel cover on 1 July and 9 September 2013. Cover data were determined by using the Daubenmire Cover Class system converted to percent; phytotoxicity data were

gathered using a scale of 0-10 (0=no damage, 10=100% damaged/dead) converted to percent. The data were analyzed using t-tests with a Bonferroni adjustment (α=0.0125).

**RESULTS:** There were no significant differences among treatments for any cover or phytotoxicity. There was no grass in the plots in July, and only three plots contained grass (<5 % cover) in September so the results are not presented here. It should be noted that the lack of differences was not due to the stringent alpha level, since there would have been no differences at the 5 % significance level either.

Wild blueberry cover was low in all treatments because the field had many bare spots, and the red sorrel was colonizing the bare spots first and then moving in under the blueberry canopy. In July, all treatments ranged within 10 % cover values of each other, but by September blueberry cover was reduced slightly in the Reglone treatment (Figure 1). In July, minor phytotoxicity was observed as chlorosis, but the effect was field-wide and appeared to be due to the June Callisto application (Figure 2, Photo 1); there was no phytotoxicity by September.

**Figure 1.** 2013 wild blueberry cover following fall 2012 treatments for red sorrel control.

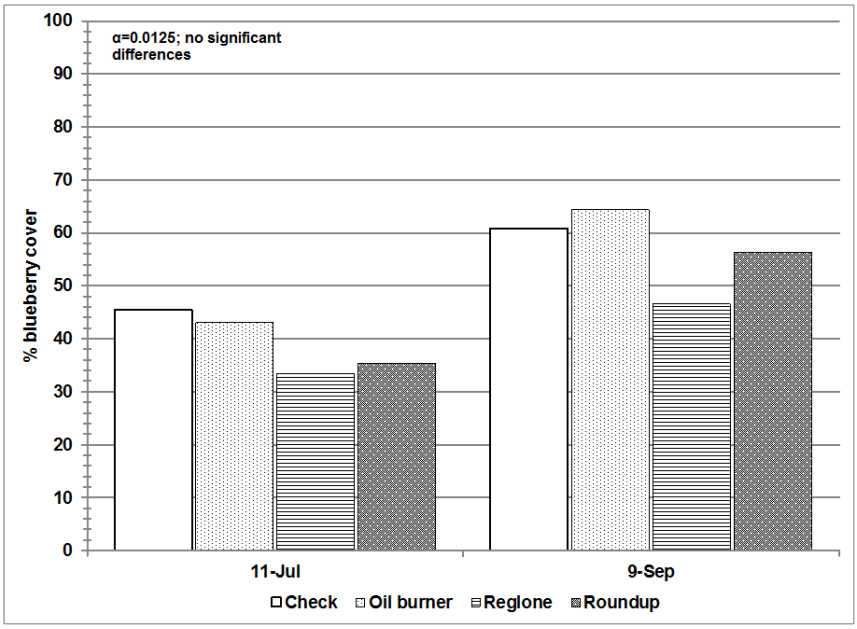
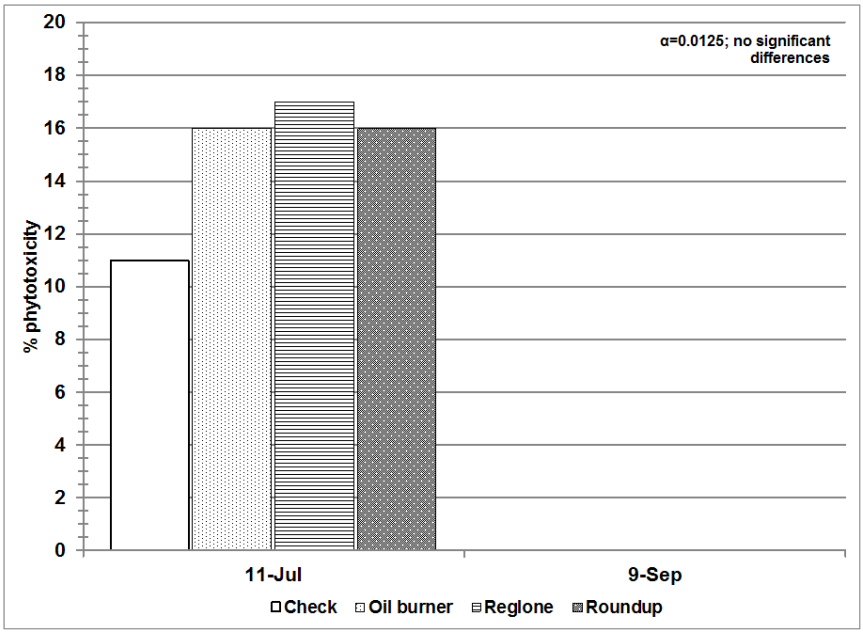


**Figure 2.** 2013 wild blueberry phytotoxicity following fall 2012 treatments for red sorrel control.




There were no significant differences in red sorrel cover at either evaluation, but it was still clear that the Roundup treatment was most effective on red sorrel (Figure 3, Photo 1). By September, red sorrel cover in the Roundup treatment was half that of the other treatments (Photos 2-4). Both the Reglone and burner treatments did not provide effective control on red sorrel long-term.

**Figure 3.** 2013 red sorrel cover following fall 2012 treatments for red sorrel control.

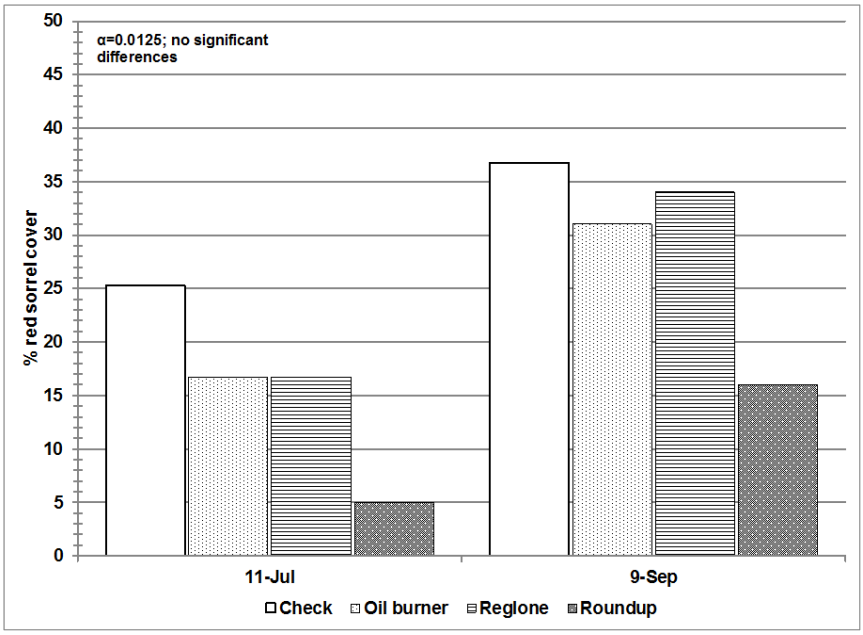


**Photo 1.** Wild blueberry and red sorrel cover in the Roundup treatment.

**Photo 2.** Wild blueberry and red sorrel cover in the check (red border surrounds blueberry).

**Photo 3.** Wild blueberry and red sorrel cover in the oil burner treatment (red border surrounds blueberry).

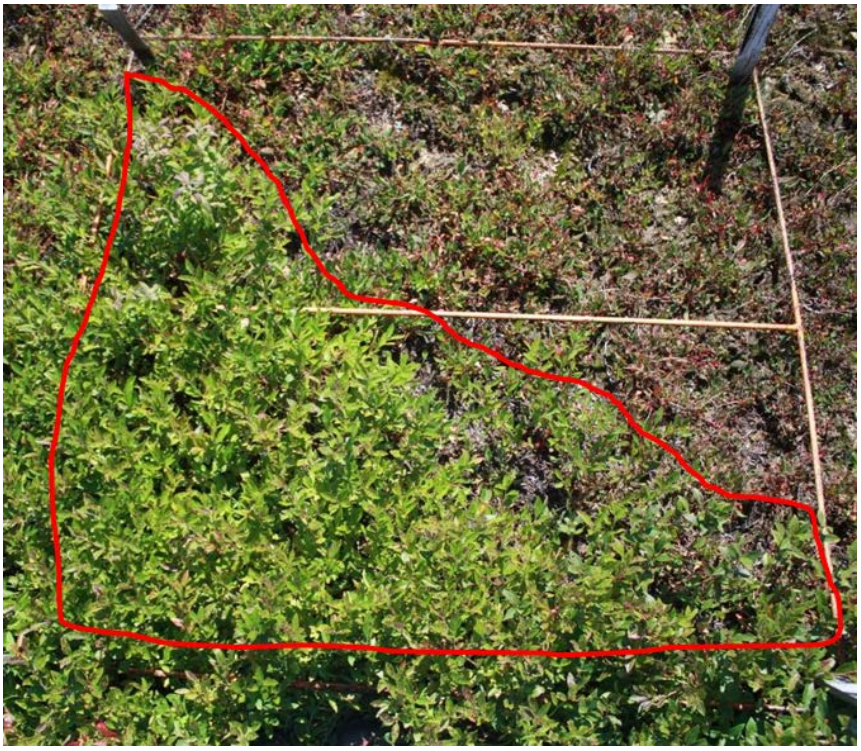
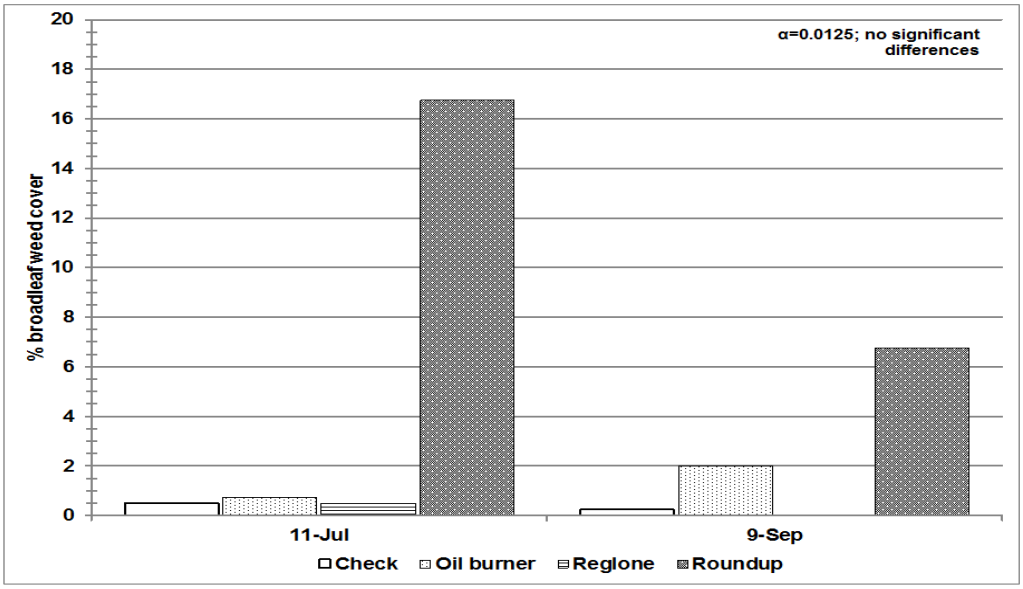


**Photo 4.** Wild blueberry and red sorrel cover in the Reglone treatment (red border surrounds blueberry).

Although Roundup controlled red sorrel it also released other broadleaf weeds, namely blue toadflax (*Nuttallanthus canadensis*) and spreading dogbane (*Apocynum androsaemifolium*), which are also problem weeds in wild blueberry fields and neither were controlled by the grower's herbicide applications. Whereas broadleaf weed cover in the other treatments remained at 2 % cover or less, broadleaf weed cover in the Roundup treatment was almost 20 % in July and over 6 % in September, even after blue toadflax and dogbane had senesced (Figure 4).

**Figure 4.** 2013 broadleaf weed cover following fall 2012 treatments for red sorrel control.




**CONCLUSIONS:** The only treatment in this trial to show promise in controlling red sorrel when applied in the fall was Roundup. Roundup was not a complete success because it did not eradicate red sorrel, and it also released other problem weeds. Reglone, a desiccant, did not provide long-term control, and burning as a cultural weed management tool was also not effective.

**RECOMMENDATIONS:** This trial will continue through August 2014, at which time the plots will be harvested and yields compared. Based upon the results of this trial, an additional fall red sorrel control trial was initiated in late October 2013. Roundup was sprayed in the fall and glufosinate, a burn-down product which has shown promise in Canada, will be applied in the spring and the grower will apply their regular herbicide applications over the 2014 growing season.

**INPUT SYSTEMS STUDY – *ANCILLARY STUDY***

**SOIL HEALTH & CHEMISTRY:** Ellen Mallory, Assistant Professor of Sustainable Agriculture
Katie McPhee and Hannah Griffin, Research Associates

**28. TITLE**: Compost and mulch effects on soil health and nutrient dynamics in wild blueberry.

**INTRODUCTION:** The potential for poor soil health to limit wild blueberry production has long been recognized. Adding stabilized organic matter in the form of seafood-waste compost could enhance the soil organic mat while providing a slow-release source of nutrients. Compost nutrient concentrations are low relative to fertilizers but they match the low nutrient requirements of wild blueberries. Currently, most organic producers in Maine rely on expensive bagged organic fertilizers that comprise 20-50% of production expenses. Bulk compost, available locally, may be a cheaper source of nutrients. A study was initiated in 2010 on a commercial wild blueberry farm in Township32, Maine, USA to evaluate seafood-waste compost for its impacts on soil quality, soil fertility, and crop yield. These plots will be monitored over four years. A second set of plots were established in 2012 to repeat the study.

This report covers the yield results at both sites from 2013. Comprehensive soil and plant tissue results for the entire four years of the trial are forthcoming. For complete results from 2010-2012, please see our 2012 report which was based on the following manuscript that was accepted but still awaiting publication: Mallory, E.B. and J.M. Smagula. In press. Effects of seafood-waste compost and mulch on soil health and soil nutrient dynamics in wild blueberry (*Vaccinium angustifolium* Ait). Acta Horticulturae.

**METHODS**:

*Experimental design*

The study site was a commercial, low-input wild blueberry field in Township32, Maine, USA on an Adams loamy sand soil. The experiment was repeated twice on two adjacent half-acre areas of the field. The first set of plots was established in 2010 and the second set was established in 2012, both in the spring during non-crop years. The field owners applied a selective herbicide as usual but no fertilizer. A randomized complete block design was used with a split plot arrangement of treatments. Mulch (with and without) was the main plot factor and soil amendment (five levels) was the split-plot factor. The soil amendment treatments were: compost, bagged organic fertilizer (Pro-Holly 4-6-4, North Country Organics®, Bradford, VT), synthetic fertilizer (diammonium phosphate, DAP, 18-46-0, Northeast Agricultural Sales, Detroit, ME) at a rate of 1x (222 lbs acre$^{-1}$) or 2x (444 lbs acre$^{-1}$), and a control treatment. All treatments were replicated six times. Plots were 6’ x 30’ in size. A 5’ alley was established between plots to prevent cross contamination by soil amendments (Figure 1) and a 5’ or 10’ alley separated blocks.

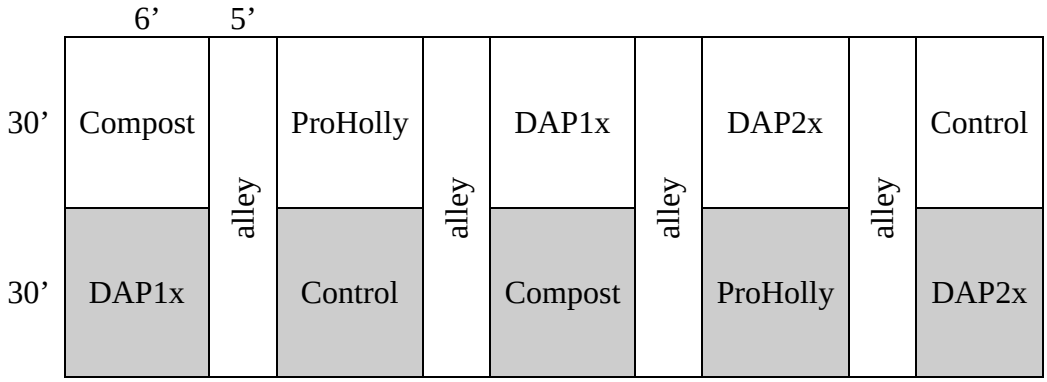



**Figure 1.** Example of the treatment layout in one block at Sunkhaze Blueberry Farm, 2010. Shaded area represents the mulched plots.

In 2010, seafood-waste compost (Sunrise Seafood Compost, Addison, ME) was applied at a rate of 4.3 t acre$^{-1}$ (fresh weight), which supplied approximately 38 lb acre$^{-1}$ of plant available nitrogen (N), assuming 10% of the compost total N was available during the first year after application (Gale et al., 2006; Laos et al., 2000). The seafood-waste compost also supplied 245 lb acre $^{-1}$ of phosphorus (P), 22 lb acre $^{-1}$ of potassium (K), and 10,598 lb acre $^{-1}$ of calcium (Ca). Application rates for Pro-Holly (0.8 t acre$^{-1}$) and DAP1x (222 lb acre $^{-1}$) were calculated to match the estimated available N supplied by the seafood-waste compost and assure sufficient P. First-year availability from the Pro-Holly fertilizer was assumed to be 57% of total N (100% of inorganic N and 30% of organic N, Gale et al., 2006). Pro-Holly also supplied 49 lb acre $^{-1}$ of P, 64 lb acre $^{-1}$ of K, and 135 lb acre$^{-1}$ of Ca. DAP1x supplied 45 lb acre$^{-1}$ of P. The DAP2x treatment was included as a nutrient response check. Fertility treatments were applied on 10 May 2010. Softwood bark mulch was applied the next day to create approximately 5cm of cover.

In 2012, the DAP1x and Pro-Holly treatments received additional applications of their respective fertilizers at the same rates as in 2010. The compost and DAP2x treatments received no further applications to investigate the possibility of maintaining adequate crop production with less frequent applications.

This process was repeated starting in 2012 at a second site (Site 2) to establish a second set of plots. In this case, beef manure compost (Coast of Maine Organic Products, Portland, ME) was used to avoid high calcium levels typical of seafood-waste compost.

*Soil and plant tissue sampling*

Leaf and soil samples were collected on 21 July 2010 and 24 July 2012 after the terminal growing point died. Thirty stems exhibiting tip dieback were cut below the lowest growing leaf, dried (60 C) and ground (Wiley Mill, 20 mesh). In each plot, soil cores (19 mm dia.) were collected to a 10 cm depth at six random locations, bulked, mixed, and sieved to 2 mm. The samples were air-dried and analyzed by the Maine Agricultural and Forest Experiment Station Analytical Laboratory. Tissue samples were analyzed for mineral concentrations by dry-ashing and inductively coupled plasma (ICP) analysis, and soil samples were analyzed for pH, organic matter by loss on ignition, P by ICP, and other nutrients and cation exchange capacity by Modified Morgan extraction (McIntosh, 1969).

*Stem measurements*

After leaf drop (17-19 November 2010 and 6 November 2012), blueberry stems from two 15cm x 15cm quadrats per plot were clipped at the stem etiolation point. Samples were stored at room temperature until stem number, branch number, and flower buds per stem were counted. In mid-May 2011 and 2013, 10-15 stems per plot with 3-9 flower buds were identified, marked with colored wire, and number of buds per stem was recorded. Flower numbers per stem were counted in early June and fruits per stem were counted in early August in 2011 and 2013.

*Fruit set*

Fruit yield fresh weight was determined by harvesting the center 0.61 x 8.5 m strip of each plot using a mechanical harvester and cleaner, and weighing (17 August 2011 and 7 August 2013). A 550-g subsample was collected, separated into edible and inedible (unripe, squashed, and diseased) fractions, and weighed. Berry weight (per 300 berries) was determined. Nutrients were analyzed for a 2-g subsample of pureed edible berries by the Maine Agricultural and Forest Experiment Station Analytical Laboratory by dry-ashing and ICP.

*Data analysis*

Data were analyzed using mixed model analysis of variance following verification of normality and equality of variance assumptions (JMP, Version 9.0, SAS Institute Inc., Cary, NC, 2010). Treatment effects were separated by Tukey's Highly Significant Differences test at a 5% significance level.

**RESULTS:** A summary of the results for soil organic matter and chemistry, leaf tissue nutrient concentrations, and blueberry growth measures over the two sites and four years are forthcoming. Results for these measures for the first two years of this trial can be found in our 2012 report.

*Blueberry yield*

At Site 1, all soil amendment treatments performed equally in terms of the edible fruit yield of the first crop after amendment application and all exceeded the control treatment by 70% on average (Table 1). For the second crop at this site, all of the amendment treatments except DAP2x produced equivalent yields that were again 70% higher than the control treatment. The DAP1x and Pro-Holly treatments received a second application of fertilizer in 2012 before the second crop year, whereas the compost and DAP2x treatments received only the initial application made in 2010. Compost is known to release nutrients gradually over many years. In this trial it appears to have provided nutrients during the second crop cycle after application. In contrast, any excess nutrients after the first crop cycle of the high DAP rate treatment appear to have been lost. At the second site, only the DAP2X treatment produced significantly higher yields than the control. The mulch treatment had no effect on fruit yields at site 1 and a weak effect at site 2.

**CONCLUSIONS:** At Site 1, seafood-waste compost applied every other crop cycle was equally effective as a fertility source for wild blueberries as Pro-Holly and DAP applied at typical rates every crop cycle. The costs of the soil amendment treatments, based on prices that growers would have paid at the time of the study, are as follows: seafood-waste compost, \$2000 $ac^{-1}$; Pro-Holly \$680 $ac^{-1}$; DAP1x \$82 $ac^{-1}$; DAP2x, \$164 $ac^{-1}$; control, \$0. Compost is much more

expensive than these other fertility sources but its effects appear to endure over time. Applying compost every other crop cycle at slightly lower rates would be cost-effective compared with the bagged fertility sources available to organic farmers in Maine.

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**Table 1.** Edible fruit yield as affected by soil amendment and mulch treatments after. At Site 1 yields were measured for two subsequent crop years.

| | Trial Site 1 | | Trial Site 2 |
|---|---|---|---|
| | First crop | Second crop | First crop |
| Treatments | 2011 | 2013 | 2013 |
| | ---------- lb acre$^{-1}$ ---------- | | |
| Soil amendment[1] | | | |
| Compost | 2960 a | 2325 ab | 2955 b |
| Pro-Holly | 2791 a | 2425 ab | 2658 b |
| DAP1x | 2650 a | 2706 a | 2602 b |
| DAP2x | 2600 a | 1706 bc | 3972 a |
| Control | 1610 b | 1460 c | 2232 b |
| Mulch | | | |
| Mulch | 2620 | 1951 | 3092 |
| No Mulch | 2431 | 2297 | 2676 |
| | ---------- ANOVA Results ---------- | | |
| Amendment (A) | *** | *** | *** |
| Mulch (M) | NS | NS | † |
| A x M | NS | NS | NS |

[1]Site 1 received seafood-waste compost, which consisted of salmon, sea urchin, sea cucumber, mussel culls, and lobster bodies mixed with sawdust. Site 2 received beef manure compost. Compost was applied only in 2010. Likewise the DAP2x treatment received one application in 2010. In contrast, the DAP1x and Pro-Holly treatments received fertilizer in 2010 and 2012.

NS, not significant ($p>0.05$).

†, *, **, ***, significant at the 0.10, 0.05, 0.01, 0.001 levels of probability, respectively.

**INPUT SYSTEMS STUDY – *ANCILLARY STUDY***

**PLANT NUTRITION:** Marianne Sarrantonio, Associate Professor of Sustainable Agriculture

**29. TITLE:** Evaluation of conventional and organic fertilizers on blueberry growth and yield.

**OBJECTIVE:** To sample and measure plant and soil parameters to determine the fate of applied nutrients in different formulas; to determine whether there is a relationship between nutrient type and rate applied and blueberry plant development and yield.

**METHODS:** Field trials were initiated at 2 sites in Maine in early May, 2013 to look at nutrient management in wild blueberries using conventional fertilizers. The objective of these studies for this first year was to determine whether N added as DAP (diammonium phosphate) at the rate of 80 lb N/ac would affect stem growth, leaf number, leaf tissue nutrient concentration or bud formation in the prune year of the 2-yr blueberry growth cycle. The 2 sites where identical treatments were established were the University of Maine Blueberry Hill Experimental Farm in Jonesboro, ME (5/7/13) and the Wyman's farm in Deblois, ME (5/8/13). A third trial was established at the property of Aram Calhoun and Malcom Hunter in Amherst, ME on June 6, 2103 to test the effects of organic fertilizers on wild blueberry growth. At each site, three plots measuring 6.5 ft x 3.25 ft (1m x 2m) were established on each of 8 clones, which served as replications. One plot on each clone received 80 lb/ac of N as DAP, and another plot received 80 lb/ac of N as DAP (40 lb) + Pro-Holly (40 lb). Pro-Holly is a fertilizer formulated for acid-loving plants and includes a range of other nutrients in addition to N and P, such as potassium, calcium, magnesium and several micronutrients including zinc and copper. The third plot on each clone received no fertilizer and was used as the control treatment. On June 28 (Blueberry Hill) and July 3 (Wymans) stem density was measured in 50 $in^2$ quadrats in each plot (2 quads/plot). Stem samples were collected (20/plot), and 8 soil cores were taken in each plot to a depth of 6". Samples were cooled and taken to the University of Maine for measurement and nutrient analyses. This sampling was repeated in late July at Blueberry Hill and Wymans, as well as at the organic trial in Amherst on July 31. A final sampling for the season was done in mid-October at all three sites to sample plant stems to assess bud formation and to take soil samples for end of season nutrient analyses.

**RESULTS:** There were no significant differences in plant growth measurements between any of the fertilizer treatments at any of the sites. Fertilizer treatments did promote stem growth as compared to the no-fertilizer control. Stem length averaged 20% higher in fertilized plots than non-fertilized plots at all three sites. There were also 35% more buds/stem on fertilized plants than unfertilized control plants (7.3 buds/stem in fertilized plots vs 5.5 in the control plots) . There were also significant differences between the three sites. Blueberries growing at Wymans had the thickest organic pad (1.2" ave), the tallest stems and the most flower buds/stem of the three sites. Blueberry soil at the Amherst site had a very shallow organic pad (0.5"). Stems were were short (ave. 7.5"vs. 17.2" at the other sites) and had the fewest buds/stem of the three sites.

**CONCLUSIONS/RECOMMENDATIONS:** This was the first year of this study and it was prune year, so there are no conclusions or recommendations from this work yet.