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2014 Wild Blueberry Project Reports

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2014 Wild Blueberry Project Reports

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TABLE OF CONTENTS

INPUT SYSTEMS STUDY – SCRI GRANT **PAGE**

FOOD SAFETY AND NUTRITION

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

OBJECTIVE: This study evaluated the effectiveness of two practical "processes" in combination (chemical sanitizers and freezing) in inactivating foodborne pathogens including *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* from the surface of wild blueberries. This is the final report of this study.

METHODS: This comprehensive investigation in inactivating foodborne pathogens on wild blueberries used two strains each of *E. coli* O157:H7 (ATCC 35150 and ATCC 129000), *S.* Typhimurium (ATCC 6962 and ATCC 14028) and *L. monocytogenes* (ATCC 19115 and ATCC 49554) to inoculate the surface of blueberries by a dipping method and then the effectiveness of various chemical sanitizers combined with low temperature frozen storage was studied. Initially, a cocktail mixture was prepared by mixing two suspensions of each pathogen with equal populations, and 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. The inoculated blueberries were placed on sterile glass rods and dried for 2h in a laminar flow hood. The initial level of inoculum on surface of inoculated blueberries was approximately 7log CFU/g for *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*. 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), and lactic acid (1% and 2%). The control treatments include distilled water wash and un-treated inoculated blueberries. 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 concentrations for 10s, 1, 5 and 10min treatment times), Cl_2 (100ppm, 150ppm and 200ppm concentrations for 10s, 1, 5 and 10min), and lactic acid (2% and 1% concentrations for 5, 10 and 20min) as shown in Figure 1. At the end of each treatment time, one set was kept in freezer at -12°C for 1 week and the other set was immediately processed for bacterial enumeration. For all these chemical treatments, visual quality testing was performed and also for chlorine and chlorine dioxide treatments, chemical residues left on these chemicals were tested.

RESULTS: The efficacy of all these sanitizers used in this study increased significantly ($p < 0.05$) in inactivating foodborne pathogens, when combined with freezing. Treatment with sterile deionized water did not significantly reduce the levels of the three pathogens ($p > 0.05$) as compared with all sanitizer treatments, and the reductions are only in the range of 1.0-2.5 log CFU/g when combined with freezing for all three pathogens.

Efficacy of chemical sanitizers in combination with freezing in reducing E. coli O157:H7:
The population reductions of *E. coli* O157:H7 inoculated on the surface of blueberries after treatment with different concentrations of Cl_2, ClO_2, and lactic acid and frozen storage at -12°C for 1 week is presented in Table 1. The highest overall reduction in *E. coli* O157:H7 (4.41 log CFU/g) was observed with lactic acid treatment (2% for 20min) in combination with freezing. ClO_2 in combination with freezing contributed to a maximum reduction of 3.66 log CFU/g while Cl_2 wash resulted in 3.25 log CFU/g of maximum reduction in combination with freezing. Freezing alone contributed to a log reduction in the range of 0.2-2.5 log CFU/g of the overall log reduction that was achieved with different chemicals treatments. Among different chlorine treatments, 200ppm concentration for 10 min in combination with freezing resulted in highest overall reduction (3.25 log CFU/g) of *E. coli* O157:H7. There is a significant difference ($p < 0.05$) in the reduction of *E. coli* O157:H7 populations from 2.5ppm ClO_2 concentration to 10 and15ppm concentrations. The combination of lactic acid treatment and freezing resulted in more than 4log CFU/g in *E. coli* O157:H7 counts with both 1% and 2% concentrations. Chlorine treatment was least effective in reducing *E. coli* O157:H7 levels from the surface of blueberries followed by ClO_2 and lactic acid.

Efficacy of chemical sanitizers in combination with freezing in reducing S. Typhimurium:
The population reductions of *S.* Typhimurium on blueberries after treatment with different concentrations of Cl_2, ClO_2, and lactic acid and frozen storage at -12°C for 1 week is presented in Table 2. These chemical treatments without combination of freezing showed only around 0.4-2.2 log CFU/g reduction in counts of *S.* Typhimurium while freezing acted as a second barrier and contributed to an additional log reduction ranging from 0.8-2.4 log CFU/g with Cl_2 wash, 1.0-2.8 log CU/g with ClO_2 and 1.1-1.7 log CFU/g with lactic acid. The highest overall log reduction of *S.* Typhimurium was observed with Cl_2 wash in combination with freezing (5.4 log CFU/g) and the lowest overall reduction (4.7 log CFU/g) was with lactic acid in combination with freezing. More than 5 log CFU/g reduction in *S.* Typhimurium was achieved with Cl_2 at 150 and 200ppm concentrations for 10min treatment time in combination with freezing. Treatment with 2.5 and 5ppm ClO_2 concentrations for 10min resulted in similar log reductions (4.5 log CFU/g) of *S.* Typhimurium. Reduction levels of *S.* Typhimurium did increase significantly between concentrations of 1% and 2% lactic acid. Chlorine wash was more effective in reducing *S.* Typhimurium from the surface of blueberries followed by chlorine dioxide and lactic acid.

Efficacy of chemical sanitizers in combination with freezing in reducing L. monocytogenes:
The population reductions of *L. monocytogenes* from the surface of blueberries after treatment with different concentrations of Cl_2, ClO_2, and lactic acid and frozen storage at -12°C for 1 week is presented in Table-3. Log reduction of *L. monocytogenes* was in the range of 0.2-3.1 log CFU/g when blueberries were treated with different chemical sanitizers alone without freezing, while after freezing combination, the reduction levels significantly increased ($p<0.05$) which were in the range of 2.2-6.9 log CFU/g. Treatment with Cl_2 (at 200ppm for 5 or 10min), ClO_2 (15ppm at 5 and 10min), and lactic acid (1% and 2% for 10 and 20min) in combination with freezing was able to ensure complete elimination of *L. monocytogenes* from the surface of blueberries (with detection limit <1 log CFU/g).

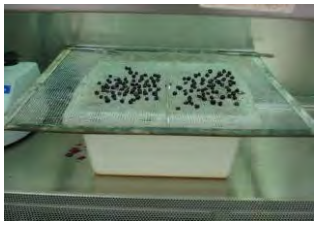
Effect of chemical sanitizers on visual quality of blueberries:
None of the chemical treatments showed any significant damage to visual quality of blueberries. All the appearance scores were above 8 (like very much) after chemical spray and with freezing combination. The blueberries treated with different concentrations of Cl_2 did not show any difference from untreated control. With ClO_2 treatments, though there was no significant difference between the exposed blueberries and control, concentrations higher than 2.5ppm showed slightly wrinkled and lighter skin colored blueberries compared to control. After lactic acid treatment (1% and 2% concentrations), a slight acidic odor was noticed on the treated blueberries at all contact times but, this odor was not noticed anymore after freezing treatment. The residues of Cl_2 and ClO_2 on treated blueberries were also analyzed and results showed that these chemical treatments did not leave any residues on blueberries.

CONCLUSIONS: According to this study, a significant reduction in pathogens can be achieved when chemical treatments tested combined with freezing, hence the quality and safety of wild blueberries can be well maintained with efficient bacterial reductions when these chemical treatments are combined with freezing. Though chlorine treatment and freezing together effectively reduced foodborne pathogens (with a minimum of 2.9 log CFU/g reduction) from the surface of blueberries, it has less affectivity compared to other two sanitizers and since chlorine can decompose rapidly in the presence of organic matter and it can produce harmful by-products, which is a limiting factor. ClO_2 being efficient in inactivating all the three foodborne pathogens compared to Cl_2, it can be a best alternative to Cl_2 for food processing industries. From these experimental results, lactic acid demonstrated good performance in substantially reducing all the three pathogenic microorganisms from the surface of blueberries. The efficacy of these chemical sanitizers on bacterial reduction with maintaining visual quality may be promissory to frozen food industries.

RECOMMENDATIONS: The bacterial reductions obtained in this study indicate that these interventions may be considered for frozen fruit industries such as blueberries, where the combination can be easily incorporated to possibly eliminate the pathogens effectively.

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.

A



B

C

Table 1: Reduction in *E. coli* O157:H7 from the surface of blueberries treated with different chemical sanitizers with or without freezing combination.

Chemical Treatment	Concentration	Reduction (log CFU/g) after chemical spray				Overall Reduction (log CFU/g) after chemical spray with freezing combination			
		Treatment times				Treatment times			
		10s	1min	5min	10min	10s	1min	5min	10min
Chlorine	100ppm	D 0.40b	C 0.45b	B 0.61b	A 0.79a	D 2.0c	BC 2.14c	BC 2.23c	A 2.4c
	150ppm	BC 0.43b	B 0.49b	AB 0.62b	A 0.90a	C 2.36b	BC 2.46b	AB 2.57b	A 2.63b
	200ppm	C 0.57a	B 0.70a	B 0.75a	A 0.89a	D 2.62a	A 3.25a	BC 2.84a	B 2.96a
	2.5ppm	C 0.42d	AB 0.86b	BC 0.61c	A 1.02b	B 1.67b	A 2.78a	B 1.81b	B 1.88c
Chlorine dioxide	5ppm	C 0.53c	B 0.74b	AB 0.83b	A 0.92b	A 2.61a	A 2.77a	A 3.24a	A 3.14b
	10ppm	C 0.95b	BC 1.11a	BC 1.25a	A 1.87a	C 2.57a	BC 2.94a	AB 3.08a	A 3.66a
	15ppm	B 1.14a	AB 1.24a	AB 1.34a	A 1.67a	C 2.37a	BC 2.52a	AB 3.10a	A 3.49a
		5min	10min	20min		5min	10min	20min	
Lactic acid	1%	B 1.17b	B 1.28b	A 1.67b		CD 3.48b	BC 3.77b	A 4.19b	
	2%	BC 1.44a	BC 1.67a	A 1.98a		BC 3.66a	B 3.94a	A 4.41a	

Note: Reductions (log CFU/g) were determined by subtracting the populations recovered from blueberries receiving chemical treatment from those of blueberries receiving no chemical treatment (control).
Overall reductions (log CFU/g) were calculated by subtracting the populations recovered from the blueberries after combination treatment from those of blueberry samples receiving no chemical treatment (control) before freezing.
Mean values in the same row with different capital letters (A through D) are significantly different among various treatment times of the same concentration of the chemical treatment.
Within same chemical treatment, mean values in the same column with different lowercase letter (a through c) are significantly different among various concentrations for each treatment time.

Table 2: Reduction in *S.* Typhimurium from the surface of blueberries treated with different chemical sanitizers with or without freezing combination.

Chemical Treatment	Concentration	Reduction (log CFU/g) after chemical spray				Overall Reduction (log CFU/g) after chemical spray with freezing combination			
		Treatment times				Treatment times			
		10s	1min	5min	10min	10s	1min	5min	10min
Chlorine	100ppm	AB 0.46b	AB 0.52b	AB 0.58b	A 0.70b	A 3.87b	A 3.99b	A 4.24b	A 4.36b
	150ppm	B 0.72a	AB 0.82a	AB 1.03a	A 1.13a	C 4.36a	BC 4.55a	AB 4.82a	A 5.10a
	200ppm	C 0.77a	BC 0.83a	B 0.97a	A 1.22a	B 4.35a	AB 4.59a	AB 4.76a	A 5.42a
	2.5ppm	B 0.79b	AB 0.86b	AB 0.95c	A 1.02b	C 3.94b	BC 4.12b	AB 4.28c	A 4.53c
Chlorine dioxide	5ppm	B 0.85b	AB 0.98ab	AB 1.05b	A 1.25ab	C 3.98c	BC 4.13c	AB 4.32b	A 4.52b
	10ppm	A 0.99a	A 1.08a	A 1.22ab	A 1.32ab	B 3.98b	AB 4.16b	AB 4.44b	A 4.64ab
	15ppm	B 1.04a	B 1.07a	AB 1.30c	A 1.45a	C 4.14a	BC 4.49a	AB 4.70a	A 4.93a
		5min	10min	20min		5min	10min	20min	
Lactic acid	1%	BC 1.77b	AB 1.92b	A 2.04b		BC 4.07b	AB 4.32b	A 4.57b	
	2%	C 1.80a	B 2.01a	A 2.24a		CD 4.18a	BC 4.47a	A 4.73a	

Note: Reductions (log CFU/g) were determined by subtracting the populations recovered from blueberries receiving chemical treatment from those of blueberries receiving no chemical treatment (control).
Overall reductions (log CFU/g) were calculated by subtracting the populations recovered from the blueberries after combination treatment from those of blueberry samples receiving no chemical treatment (control) before freezing.
Mean values in the same row with different capital letters (A through D) are significantly different among various treatment times of the same concentration of the chemical treatment.
Within same chemical treatment, mean values in the same column with different lowercase letter (a through c) are significantly different among various concentrations for each treatment time.

Table 3: Reduction in *L. monocytogenes* from the surface of blueberries treated with different chemical sanitizers with or without freezing combination.

Chemical Treatment	Concentration	Reduction (log CFU/g) after chemical spray				Overall Reduction (log CFU/g) after chemical spray with freezing combination			
		Treatment times				Treatment times			
		10s	1min	5min	10min	10s	1min	5min	10min
Chlorine	100ppm	D 0.27b	C 0.37b	B 0.61b	A 0.76b	A 2.20c	A 2.37c	A 2.54c	A 2.83c
	150ppm	C 0.25b	B 0.42b	A 0.65b	A 0.70b	A 2.47b	A 2.64b	A 2.49b	A 2.65b
	200ppm	CD 0.52a	BC 0.70a	AB 0.96a	A 1.28a	C 3.31a	BC 3.73a	*A 5.12a	*A 5.12a
	2.5ppm	C 1.23b	BC 1.28b	AB 1.34c	A 1.39b	D 2.5a	CD 2.63b	B 2.95b	A 3.38b
Chlorine dioxide	5ppm	D 1.16b	CD 1.27ab	BC 1.34bc	A 1.57ab	D 3.21ab	BC 3.39b	AB 3.57b	A 3.77b
	10ppm	B 1.34a	AB 1.4a	AB 1.51ab	A 1.72ab	C 3.33ab	BC 3.40b	BC 3.65b	A 4.07ab
	15ppm	D 1.31a	C 1.47a	B 1.72a	A 2.07a	C 3.75a	BC 3.96a	*A 6.13a	*A 6.13a
		5min	10min	20min		5min	10min	20min	
Lactic acid	1%	A 1.76a	A 1.82a	A 1.96b		B 4.27a	B 4.6b	*A 6.71b	
	2%	B 2.58a	B 2.75a	A 3.19a		B 5.06a	*A 6.96a	*A 6.96a	

Note: Reductions (log CFU/g) were determined by subtracting the populations recovered from blueberries receiving chemical treatment from those of blueberries receiving no chemical treatment (control).
Overall reductions (log CFU/g) were calculated by subtracting the populations recovered from the blueberries after combination treatment from those of blueberry samples receiving no chemical treatment (control) before freezing.
Mean values in the same row with different capital letters (A through D) are significantly different among various treatment times of the same concentration of the chemical treatment.
*Indicates complete elimination of *L. monocytogenes* with detection limit <1log CFU/g. The original population of *L. monocytogenes* on blueberries was in the range of 5.1 – 7.0 log CFU/g.
Within same chemical treatment, mean values in the same column with different lowercase letter (a through c) are significantly different among various concentrations for each treatment time.

FOOD SAFETY AND NUTRITION

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

2. TITLE: Role of wild blueberries on lipid metabolism and inflammation as related to obesity and the Metabolic Syndrome.

OBJECTIVES: Recently, the Metabolic Syndrome (**MetS**) is becoming a major public health problem in the United States and is expected to increase dramatically in the coming years in parallel with the rising obesity epidemic. This syndrome is characterized by central obesity, dyslipidemia, insulin resistance, glucose intolerance, hypertension, a pro-thrombotic and a pro-inflammatory state and thus an increased risk of Cardiovascular Disease and Diabetes Mellitus. Considering the magnitude of MetS as a major health problem in the US and as a precursor for Cardiovascular Disease and Diabetes Mellitus, dietary strategies to prevent or ameliorate the symptoms that cause and/or promote its development without the deleterious effects of pharmacotherapy is of highest priority and importance to public health.
Blueberries rich in antioxidants and anthocyanins have been reported to offer many health and disease-prevention benefits. **My past work on the role of wild blueberry-enriched diets on endothelial function and dysfunction, vasomotor function and metabolism as related to hypertension and Cardiovascular Disease has resulted in elucidating the beneficial role of**

wild blueberries in preventing and normalizing the above. Unfortunately little research – especially little *in vivo* research – on the role of dietary blueberries to reverse the symptoms of MetS exists. During 2013-2014 academic year the following objectives were addressed related to the ability of dietary blueberries to relieve the metabolic abnormalities associated with MetS *in vivo*.

Objectives: **Do wild blueberries normalize:**

1. **Lipid metabolism** and gene expression in the hepatic and adipose tissues, and
2. **Systemic and local inflammation** by measuring cytokine and adipokine concentrations and their gene expression in hepatic and adipose tissues in the obese Zucker rat, a model of the Metabolic Syndrome?

To answer the above objectives, genetic models of obesity and the metabolic syndrome (Obese Zucker rat (**OZR**) and its Lean littermate (**LZR**)) will be used to assess the role of wild blueberries in the above ***in vivo.***

METHODS:

Animals and diets:

Male OZR and their lean littermates, LZR (10-13 weeks old and 2 weeks old) were randomly assigned to two diet groups (n=12, for each group, two sets of experiments). The diets were as follows: a Control (**C**, AIN93M), a Wild Blueberry-enriched diet (**WB**) (8% w/w substituting for dextrose in the C diet). These levels are the equivalent of 2 cups of WB equivalent to human consumption respectively. The animals were fed the diets for eight weeks. Animals were weighed weekly and food intake was measured.

Blood and tissue collection:

At the end of the experimental period, animals were anesthetized with 95% CO2/5% O2 for two minutes. They were quickly exsanguinated by cardiac puncture and blood was collected for immediate plasma separation, collection and storage at -80°C until subsequent analysis. The liver, part of the visceral adipose tissue, a section of the aorta were excised, immediately snap-frozen in liquid nitrogen and stored at -80°C until further analysis. The liver and visceral adipose were placed in ice-cold lysis buffer (Roche Diagnostics), homogenized, sonicated for 10 seconds and centrifuged at 10,000 g for 5 minutes. The supernatant was collected and measurement of lipid and inflammatory markers and their gene expression thereof was conducted.

1. Lipid profiles and gene expression as related to lipid metabolism

Blood lipids (total serum cholesterol, LDL-C, HDL-C and triglycerides were measured with commercial kits) and expression of genes involved in lipid metabolism were also targeted. In particular, expression of fatty acid synthase (**FAS**), lipoprotein lipase (**LPL**), hormone sensitive lipase (**HSL**) and ATP-binding cassette transporter (**ABCA1**) were evaluated in both liver and the adipose tissues, and

2. Concentrations of pro-inflammatory markers and their gene expression in liver and the visceral adipose tissues

The following markers were determined by means of commercially available rat-specific enzyme-linked immunosorbent assay (**ELISA**) kits, following the instructions provided by the

manufacturers: TNF-α; Interleukin-6; Interleukin-.8; Adiponectin (R&D Systems); e-NOS; PAI-1; COX-2; and NFκB (MyBioSource).
Expression of pro-inflammatory markers in liver and adipose tissues: mRNA from liver and adipose tissue was isolated, retro-transcribed to cDNA and subjected to quantitative Real Time **PCR** (polymerase chain reaction) amplification using rat-specific primer sequences.

RESULTS:
1. Lipid profiles and gene expression as related to lipid metabolism
Plasma triglyceride and total cholesterol were significantly lower in OZR following WB (422·8 ± 47·1 and 228·7 ± 12·5 mg/dL respectively) compared to the C group (547·5 ± 31·5 and 263·1 ± 12·9 mg/dL, $P<0.05$), while there was no change in HDL cholesterol. No significant effects were observed for plasma lipids in LZR. Following the WB diet, expression of transcription factors PPARα and PPARγ in OZR was induced in AAT, while SREBP-1 expression was attenuated in liver and AAT. Fatty acid synthase expression significantly decreased in both tissues, and expression of ATP-binding cassette transporter 1 was induced in AAT following WB consumption.
Thus, WB consumption improves lipid profile and modulates the expression of key enzymes and transcription factors of lipid metabolism in severely dyslipidemic rats Zucker rats, models of the Metabolic Syndrome.

2. Concentrations of pro-inflammatory markers and their gene expression in liver and the visceral adipose tissues
In the OZR, WB consumption resulted in decreased plasma concentrations of pro-inflammatory markers i.e. tumor necrosis factor (**TNF**)-α (−25.6%), interleukin (IL)-6 (−14.9%) and C-reactive protein (**CRP**) (−13.1%) and increased the anti-inflammatory marker adiponectin concentration (+21.8%, Pb.05). Furthermore, expression of IL-6, TNF-α and nuclear factor (**NF**)-kB was down-regulated in both the liver (−65%, −59% and −25%, respectively) and the abdominal adipose tissue (−64%, −52% and −65%), while CRP expression was down-regulated only in the liver (−25%). In the abdominal adipose tissue, similar trends were also observed in LZR following WB treatment, with decreased liver expression of NF-kB, CRP, IL-6 and TNF-α (−24%, −16%, −21% and −50%) and increased adiponectin expression (+25%).
Thus, results of this study suggest that wild blueberry consumption exerts an overall anti-inflammatory effect in the OZR, a model of the metabolic syndrome.

SIGNIFICANCE: Obesity rates in the US have increased exponentially during the last 10 years. Inflammation is the consequence of increasing adipose mass with detrimental consequences on the blood vessels, liver and most organ systems that eventually leads to chronic diseases such as diabetes and cardiovascular disease. Thus, this area is of highest priority and extremely important for public health.
Results from last year's experiments document that wild blueberries significantly normalize blood lipids and their gene expression and reduce inflammation in a model of the Metabolic Syndrome. This work is of interest not only to the scientific community but also the Food Industry and especially the Wild Blueberry Association of North America. By studying the ways in which blueberries can mitigate MetS and its symptoms we will be able to manipulate the diet to enhance these effects. So, 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. 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 be able to positively influence public health as well as further aid economically the wild blueberry producers in Maine.

ENTOMOLOGY

INVESTIGATORS: F. A. Drummond, Professor of Insect Ecology/Entomology
J. A. Collins, Assistant Scientist of Insect Pest Management
E. Ballman, Research Associate in Invasive Species / Entomology

3. I. TITLE: Control Tactics for blueberry pest insects, 2014.

Study 1. Field control of blueberry tip midge on wild blueberry, 2012-2014

METHODS: Trials were completed in 2012, 2013, and 2014. For all three trials, 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 various sample dates as indicated in the tables, damage was assessed by counting the number of blueberry stems with and without tip midge damage as evidenced by curled leaves from each of three, ft^2 (2012) or m^2 (2013 and 2014) samples per treated plot. There were 4 replications of each material and 4 (2012 and 2014) or 6 (2013) non-treated checks. Plot size for all trials was 7 x 20-ft.
In 2012 (10 Jun) and 2013 (17 Jun) we made foliar applications Assail® 30SG (acetamiprid) and Imidan® 70WP (phosmet) in pruned-year fields after tip midge damage was evident (curled leaves on stems).
In 2014 Rimon® 0.83EC (novaluron), Success® 480SC (spinosad), and Entrust® SC (spinosad) were applied on 11 Jun to a pruned-year field. A second application of each material plus a first application of Assail 30SG (acetamiprid) and Mustang Max® (zeta-cypermethrin) was made on 19 Jun. Blueberry stems were scattered and < 1 inch tall on 11 Jun and 1.5 to 3 inches tall on 19 Jun. Larval infestation was assessed on 26 Jun, and 18 Jul. In addition, three yellow bowl traps filled with water and 1 drop of unscented dish soap were placed in the trial area to monitor for the presence of adult tip midge. The bowls were checked periodically and any adult midges were removed and preserved in 70% ethyl alcohol for future confirmation of ID.

RESULTS: Subplots were pooled within main plots. Data were transformed by the square root to stabilize variance prior to analysis.

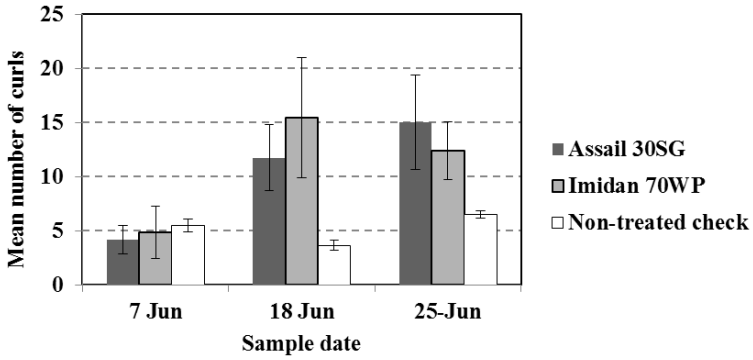
2012: Analysis of Variance (**ANOVA**, RCB) and LSD ($P \leq 0.05$) were used to compare mean number of curls among the treatment plots. Assail and Imidan were both ineffective in suppressing tip midge as evidenced by leaf curls (Table 1 and Fig. 1). Postspray populations in the treated plots were higher than the non-treated control.

Table 1. Field control of tip midge with insecticides, 2012 summary.

Material	Amount product/acre	Prespray 7 Jun	Mean curls/ft^2 Postspray 18 Jun	Postspray 25 Jun
Assail 30SG	5.3 oz	4.17 a	11.75 a	15.00 a
Imidan 70WP	21.3 oz	4.83 a	15.42 a	12.42 ab
Non-treated check	-	5.50 a	3.67 b	6.50 b
P =		0.5915	0.0345	0.1112

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

Fig. 1. Mean number of curls/ft^2 (lines are standard error of the mean). Data from 2012 trial.



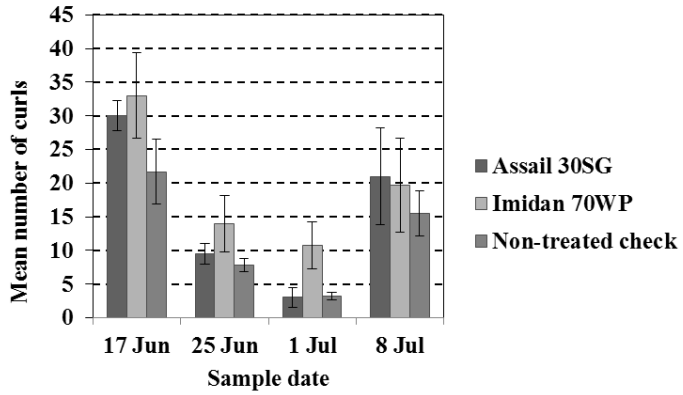
2013: A similar result was observed in 2013. Multiple and Univariate Analyses of Variance (ANOVA & **MANOVA**, CRD) and LSD ($P \leq 0.05$) were used to compare mean number of curls among the treatment plots. Assessment of treatments via ANOVA suggested no significant difference among the treatments on 17 Jun (Prespray). Assail and Imidan were both ineffective in suppressing tip midge as evidenced by leaf curls (Table 2 and Fig. 2). 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. MANOVA, also revealed no treatment differences ($F_{(2,11)} = 1.59$, $P = 0.247$) and no time x treatment interaction ($F_{(6,18)} = 1.283$, $P = 0.313$), but a significant time effect ($F_{(3,9)} = 31.13$, $P < 0.0001$). 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 2. Field control of tip midge with insecticides, 2013 summary.

Material	Amount product/acre	Prespray 17 Jun	Mean curls/m^2 Postspray 25 Jun	1 Jul	8 Jul
Assail 30SG	5.3 oz	30.0 a	9.5 a	3.0 a	21.0 a
Imidan 70WP	21.3 oz	33.0 a	14.0 a	10.8 b	19.8 a
Non-treated check	-	21.7 a	7.8 a	3.2 a	15.5 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. 2. Mean number of curls/m^2 (lines are standard error of the mean); data from 2013 trial.



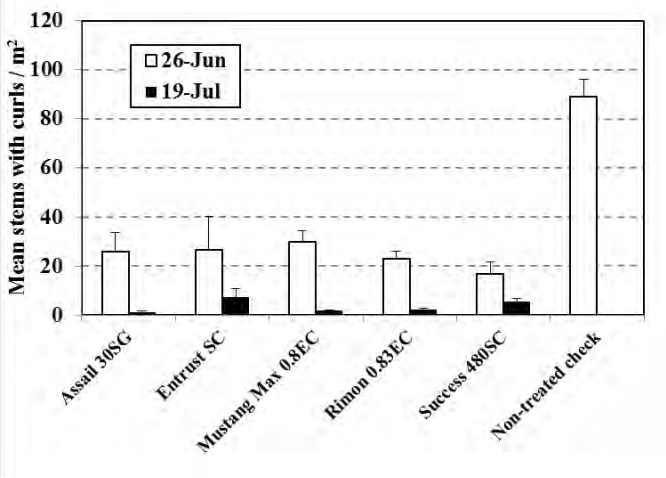
2014: Analysis of Variance (ANOVA, RCB) and LSD ($P \leq 0.05$) were used to compare mean number of curls among the treatment plots. In 2014 two applications of Rimon, Success, and Entrust or one application of Assail and Mustang Max were all initially effective in suppressing tip midge infestation. Significantly fewer damaged stems were found in the treated plots compared with the non-treated checks on the first sample date on 26 Jun ($F_{(5,15)}$, = 7.50, P = 0.0010)(Table1 and Fig. 1). Although the number of damaged stems in all the plots was much lower by the second sample date on 18 Jul, it does appear that tip midge populations in the treated plots had rebounded somewhat. Plots treated with Entrust, Success, Rimon, and Mustang Max all had significantly MORE damaged stems than the non-treated check plots (($F_{(5,15)}$, = 7.16, P = 0.0013) (Table 3 and Fig. 3).

Table 3. Field control of tip midge with insecticides, summary, 2014.

Material	Amount product/acre	Mean curls/m^2 26 Jun	18 Jul
Assail 30SG	5.3 oz	26.00 b	1.00 cd
Entrust SC	6.0 oz	26.42 b	7.50 a
Success 480SC	6.0 oz	16.92 b	5.50 ab
Rimon 0.83EC	12.0 oz	22.83 b	2.08 bc
Mustang Max 0.8EC	4.0 oz	29.84 b	1.75 c
Non-treated check	-	89.09 a	0.25 d

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

Fig. 3. Mean number of stems with curls/m^2 (lines are standard error of the mean), data from 2014.



CONCLUSIONS/RECOMMENDATIONS: Insecticide control has not provided adequate control of blueberry tip midge over the last three years. In fact in all three years, but especially in 2012 and 2014, the insecticide-treated plots ended up with more tip midge damage (stems with leaf curls or stem hooking) than the non-treated check plots. This has not occurred in any other insect pest spray trials in past years. We believe that blueberry tip midge might be under biological control that contains their damage a small amount, but when insecticides are applied and these agents are eliminated then the sprayed plots receive higher degrees of damage. In 2015 we intend to evaluate a range of spray timings as well as attempt to determine if a biological control agent exists.

Study 2. <u>Laboratory control of spotted wing drosophila</u>

METHODS: AzaGuard® (azadirachtin) and Assail® 30SG (acetamiprid) were evaluated in the laboratory to assess their potential to control spotted wing drosophila (**SWD**). The products were tested with and without added sugar at rates indicated in Table 1. Laboratory-reared SWD adults (6-10 per cage) were placed in plastic cages (9 x 4.38 x 4.13in) with 15-20 highbush blueberries treated with the insecticides. Prior to introduction of the SWD into the cages, the fruit was treated by mixing the various rates in 200ml water in a misting spray bottle set to the finest mist possible. Two applications (enough to wet the surface) were applied to the fruit that was spread out in a single layer in an open petri dish. There were three replications of each rate and three non-treated checks. SWD were introduced into the cages after the material had dried on the fruit (1-hr post application). The AzaGuard trial was repeated three times.
In a related trial Entrust® SC was applied to fruit in which SWD adults had been allowed to oviposit. For each treatment, 25 SWD (a mix of males and females) and 50 highbush blueberries were held in a plastic cage for 8 days. Water-soaked cotton balls were added as a source of moisture. After 8 days, the adults were removed and the berries were treated by spraying them, as outlined above, with either water (non-treated check) or Entrust SC (4 oz/acre). Ten berries were placed in each of 5 plastic drosophila tubes (28.5 x 95mm) per treatment. The tubes were observed daily the presence of emerging adult SWD. ANOVA (RCB) was used to assess the treatment effects in all of the lab trials, where each replication represented a statistical block. In the AzaGuard study each trial was treated as a block for the analysis (RCB). In all studies data were transformed by the square root prior to analysis. Mean separation was by Least Square Means.

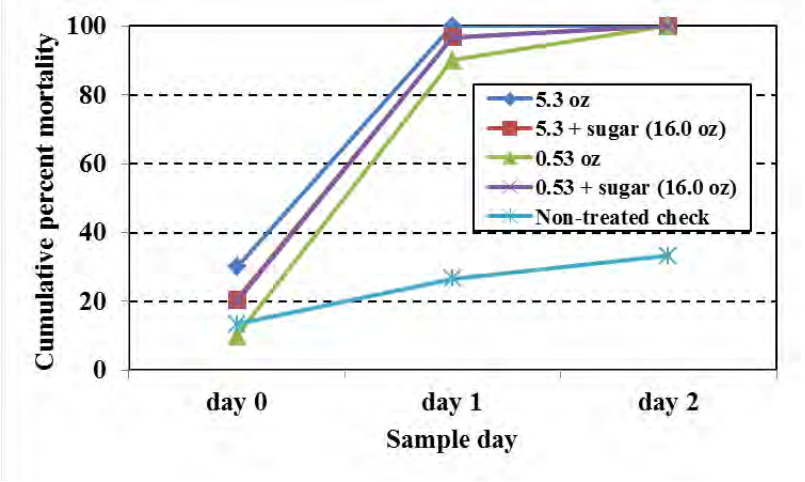
RESULTS: Entrust was effective in suppressing adult emergence in previously infested fruit. No SWD were observed in any of the tubes containing fruit that was treated with Entrust. Adults were seen in all the non-treated check tubes.
The results for the Assail trial are shown in Table 1 and Figure 1. Assail provided excellent control of SWD adults. After 4hrs, there was no significant difference between any of the treatments and the non-treated check ($F_{(2,4)} = 57.14$, $P = 0.723$). However, high mortality was observed in all the treatments after 24hrs (day 1) and; by day 2, 100% mortality was observed in all the treated cages compared with 33% mortality in the non-treated cages. It is interesting to note that 10% of the recommended field rate was as effective in this laboratory setting as the recommended field rate of 5.3 oz/acre. In 2013 field trials Assail 30SG at the 5.3 oz/acre rate provided good control for three to four days, but maggot infestation was seen after seven to eight days post treatment.

Table 1. Laboratory control of SWD with Assail, summary.

Treatment	Rate oz/acre	Cumulative % mortality day 0*	day 1	day 2
Assail 30SG	5.3	30.0 a	100.0 a	100.0 a
Assail 30SG + Sugar	5.3 + 16.0	20.6 a	96.7 a	100.0 a
Assail 30SG	0.53	10.0 a	90.0 a	100.0 a
Assail 30SG + Sugar	0.53 + 16.0	20.0 a	96.7 a	100.0 a
Untreated check	-	13.3 a	26.7 b	33.0 b

* Observations made 4 hours after adding SWD adults

Fig. 1. Percent mortality of SWD exposed to Assail over time.



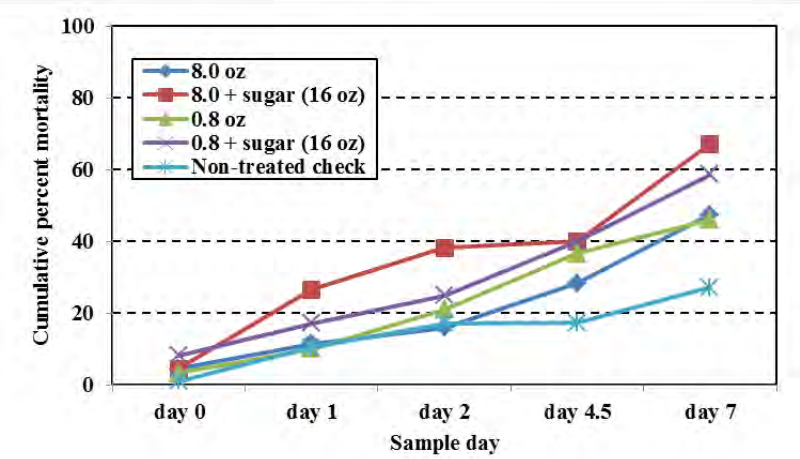
AzaGuard did provide control, but was less effective than Entrust or Assail. One interesting result is that better control was obtained using a lower rate of AzaGuard. By day 7, a 0.8 oz/acre rate of AzaGuard with and without sugar resulted in 58.6 and 67.2% mortality, respectively. And, mortality in these treatments was significantly higher than in the untreated checks. Mortality at the recommended field rate (8.0 oz/acre) with and without added sugar was 47.5 and 46.0%, respectively ($F_{(2,4)} = 2.97$, $P = 0.089$)(Table 2 and Fig. 2).

Table 2. Laboratory control of SWD with AzaGuard, summary.

Treatment	Rate oz/acre	Cumulative % mortality day 0*	day 1	day 2	day 4-5	day 7
AzaGuard	8.0	4.4	11.6	16.0	28.3	47.5 ab
AzaGuard + Sugar	8.0 + 16.0	3.3	10.0	21.1	36.7	46.0 ab
AzaGuard	0.8	4.4	26.5	38.1	40.0	67.2 a
AzaGuard + Sugar	0.8 + 16.0	8.3	17.2	24.9	40.0	58.6 a
Untreated check	-	1.1	10.5	17.1	17.4	27.1 b

* Observations made 4 hours after adding SWD adults

Fig. 2. Percent mortality of SWD exposed to AzaGuard over time.



CONCLUSIONS/RECOMMENDATIONS: Entrust will kill larvae inside the berries. This characteristic is probably found in other insecticides. This is a positive attribute because it means that when using action thresholds to time the first insecticide application against SWD, a very light initial infestation will not result in crop loss. Assail does appear to be a good option for SWD management. The addition of sugar does not seem to enhance the effectiveness of its action in killing adult SWD; although, mortality is slightly higher, numerically but not significantly, when sugar is added to the formulation. This is the case for both Assail and AzaGuard. Repellency of an insecticide can reduce the effectiveness of an insecticide, especially one that requires oral entry such as AzaGuard. In highly replicated laboratory trials in 2014 (3 trials each with 3 replicates) we found that repellency of AzaGuard was so high at the recommended application rate of 8.0 oz / acre that even reducing the rate by 10 times (0.8 oz / acre) resulted in the same amount of mortality to adult SWD. Now, it should be said that repellency is still a hypothesis since we did not directly measure it. It is also possible that AzaGuard is so highly biologically active that even a drop of exposure by 10X will result in a high mortality. This winter we intend to test the repellency hypothesis of AzaGuard.

Study 3. <u>Field control of spotted wing drosophila on wild blueberry (crop-year) with insecticides</u>

METHODS: There were four replications per treatment. Each plot measured 20 x 50-ft. There were two applications (4 and 19 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.

RESULTS: Logistic regression analyses were used to compare number of SWD larvae in fruit samples among the treatments for each date. Although infestation levels were very low in this trial some trends are apparent. With the possible exception of AzaGuard, all the materials appeared to have some suppressing effect on SWD fruit infestation (Table 1). Imidan and Mustang Max gave 7 and 15-day protection, while Success and Delegate gave between 3 and 6-7 day protection.

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

Material	Rate oz/acre	Prespray 3 Sep	SWD larvae/sample (odds ratio*) 7 Sep (3)**	10 Sep (6)	23 Sep (4)	26 Sep (7)	4 Oct (15)
Delegate 25WG	6.0 oz	0.25	0.00 (0.02)	0.25 (ns)	0.25 (0.06)	0.25 (0.04)	0.25 (ns)
Success 480SC	6.0 oz	0.08	0.00 (0.02)	0.08 (ns)	0.33 (0.02)	0.00 (<0.001)	0.25 (ns)
Mustang Max	4.0 oz	0.08	0.08 (ns)	0.25 (ns)	0.00 (<0.001)	0.17 (<0.001)	0.00 (0.05)
Imidan 70WP	21.3 oz	0.00	0.00 (0.02)	0.00 (<0.001)	0.17 (0.01)	0.17 (<0.001)	0.17 (0.08)
AzaGuard	8.0 oz	0.50	0.17 (ns)	0.67 (ns)	1.17 (ns)	1.17 (ns)	0.25 (ns)
Non-treated check	-	0.33	0.33 (NA)	0.67 (NA)	1.42 (NA)	1.33 (NA)	0.67 (NA)
P =		0.098	0.054	0.013	<0.0001	<0.0001	0.103

* odds ratio: likelihood that insecticide treatment has lower maggot infestation than check.
** numbers in parentheses: days after application for trial 1 (application = 4 Sep) and trial 2 (application = 19 Sep).

CONCLUSIONS/RECOMMENDATIONS: Two years of field trials (2013 and 2014) demonstrated that the insecticides recommended for control of SWD by the University of Maine Cooperative Extension provide excellent protection. Imidan and Mustang Max provided 7 to 15 day protection in 2014, while Delegate and Success provided only 3 to 6-7 day protection. AzaGuard provided 3-day control in 2013 under light SWD pressure, but did not provide control over a 3-day interval in 2014.

Study 4. <u>Determining the LC50 and LC90 of Beauveria bassiana for adult spotted wing drosophila. Report from Gabriel Alnajjar (Master's Candidate) and Dr. Frank Drummond</u>

OBJECTIVE: Spotted wing drosophila (**SWD**) is an exotic pest species in North America. Given its high affinity for targeting a variety of soft skinned, fleshy fruits, SWD management has become a necessity for mitigating yield losses of such crops. Entomopathogenic fungi are naturally occurring insect parasites that may be easily obtained for applications in large and small scale crop systems. These fungi are relatively harmless if ingested by humans and target a wide range of insect species. *Beauveria bassiana* strain GHA is one such fungus that has already been shown to induce mortality in SWD adults following mass exposure. Thus, the objective of this study was to determine the **LC50** (Lethal Concentration at which 50% of target die) and **LC90** (Lethal Concentration at which 90% of target die) of GHA for adult SWD.

METHODS: Twenty adult SWD were collected after a three-day eclosion (the act of emerging from the pupal case or hatching from the egg) period and used for each replicate of this assay. To accomplish this, thriving SWD cultures were vacated of all flies and juvenile stages were allowed to develop over three days. On the third day, flies were immobilized with CO_2 and transferred to culture tubes containing freshly prepared drosophila media. The assay was carried out the next day. Five *Beauveria bassiana* GHA suspensions were prepared with 10^5, 10^6, 10^7, 10^8 and 10^9 conidia (asexual spores)/mL + 0.01% tween surfactant. Fungal cultures used in this assay had 95% conidia viability and were stored at 4ºC. The control suspension had 0.01% tween only. A Burkard® automatized sprayer provided uniform application of a single suspension onto 0.22 um GV millipore filter paper. To ensure adequate hydration of conidia during the exposure period, each millipore filter paper was placed on top of a moistened piece of medium porosity filter paper prior to spraying. Each run on the Burkard automized sprayer also included a petri plate with agar to calculate spore density after deposition of the suspension onto a surface. All treatments were replicated five times.
Twenty flies were immobilized with CO_2 and released in each of the petri dishes containing sprayed millipore filter paper. These exposure chambers were then placed in plastic bags with dampened paper towels. The exposure period lasted for 24 hours and took place in dark growth chambers set at 25ºC ± 1ºC. Following 24 hours of exposure, flies were again CO_2 immobilized and placed into culture tubes they inhabited the previous day. Cultures were then placed back in the growth chamber with a 12 hour **L/D** (light/dark) cycle.

Data collection occurred for three days following initial exposure. Fly cadavers within the cultures were surface sterilized in zephiran chloride, rinsed in dH_2O, blotted dry on a kimwipe® and placed in well plates. Plastic bags containing well plates and a moistened paper towel were placed in the same growth chamber as the culture tubes. Sporulation on cadavers was monitored over a two week period. A logit analysis was conducted in JMP in order to calculate the LC50 and LC90 for GHA.

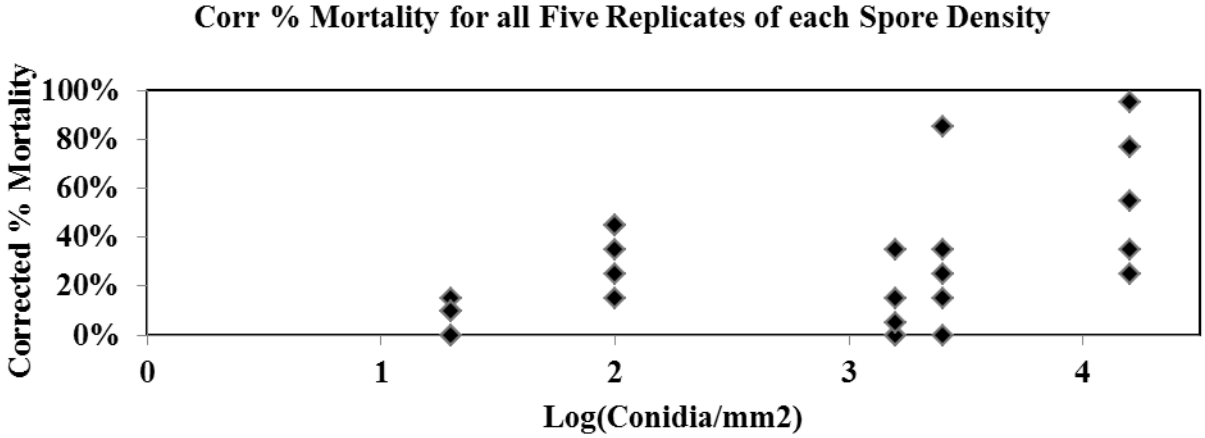
RESULTS: Table 1 shows the spore density to two significant figures, average corrected percent mortality on the third day for the five replicates, and standard deviation of corrected percent mortality for each experimental group. Figure 1 shows corrected percent mortality for each replicate after three days. Mortality of SWD adults was significantly different between replicates ($X^2_{(4)} = 23.04$, $P = 0.0001$). Furthermore, conidia concentration had a statistically

significant effect on fly mortality ($X^2_{(1)} = 78.69$, $P < 0.0001$). The LC50 and LC90 in this analysis were estimated to be $10^{4.1}$ and $10^{7.6}$ conidia/mL, respectively.

Table 1. Relationship between dose (concentration) and corrected percent mortality for SWD adults inoculated with *Beauveria bassiana*, GHA strain.

Conidia/mL 0.01% Tween	Spore density (Conidia/mm^2)	Total corrected % mortality	SD
10^5	22	7	7
10^6	98	33	13
10^7	1700	11	15
10^8	2400	32	32
10^9	17000	57	29

Fig. 1. Corrected percent mortality after three days of exposure. Calculated conidia densities on sprayed surfaces are log transformed on the x-axis. The corrected % mortality on day three is represented in this graph for each replicate, giving a total of five data points at each spore density.



CONCLUSIONS/RECOMMENDATIONS: Fungal entomopathogens may have a place in blueberry IPM against SWD. This study provides evidence of a dose / mortality response to the fungus *Beauveria bassiana* (GHA strain). To kill 90% of the flies should require a log dose of 7.6 conidia / ml, or 8 x $10^{11.6}$ conidia / acre. There are 1 x 10^{13} conidia / qt of formulated *B. bassiana* in Botanigard®. Field tests will be conducted during the summer of 2015.

ENTOMOLOGY

INVESTIGATORS: F. A. Drummond, Professor of Insect Ecology/Entomology
J. A. Collins, Assistant Scientist of Insect Pest Management
E. Ballman, Research Associate in Invasive Species / Entomology

4. II. TITLE: Pest biology and IPM, 2014.

Study 1. <u>Long-term trends in parasitism of the blueberry maggot fly</u>

METHODS: Diet cups containing blueberry maggot fly (**BMF**) pupae (66 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 collections of pupae from fruit and from trap captures of adult flies.

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. There was a sharp increase in parasitism of pupae collected in 2013 (23.0%). 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%. Parasitism rate increased sharply in 2013 to 23.0%. However, there does not appear to be a tight linkage between fly trap captures and the parasitism rates over time (Fig. 2). Although upon visual inspection of figure 2, one can see that whenever parasitism rates peak, usually a decline in fly number occur the year or two following. Modeling fly rate of increase as a function of parasite density suggests that a possibility *($F_{(1,14)}$ = 7.249, P = 0.018)* 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 2014 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. One can see that at as parasitoid numbers increase fly reproduction falls precipitously and that only a small window of very low parasitoid density allows positive blueberry fly increase in numbers from one year to the next; although, only 34.1% of the variation in fly increase is explained by parasitoid numbers. 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 blueberry maggot fly population ($F_{(1,14)}$ = 16.378, P = 0.001, r^2 = 0.539). 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.

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

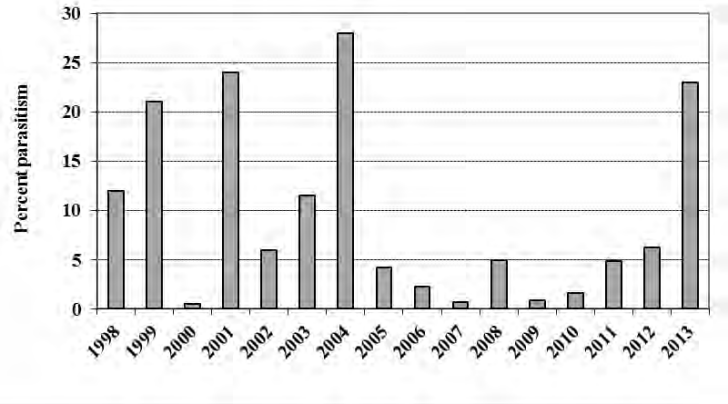


Fig. 2. Relationship between relative density of flies and % parasitism over time. Horizontal line depicts the average fly abundance over the period from 1998 – 2014.

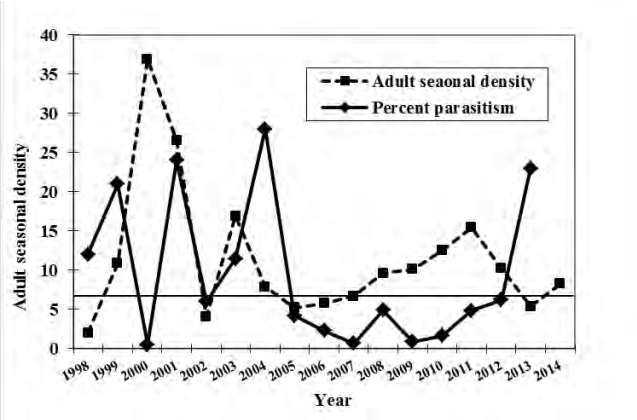


Fig. 3. Relationship between fly population increase and parasitoid density the previous year.

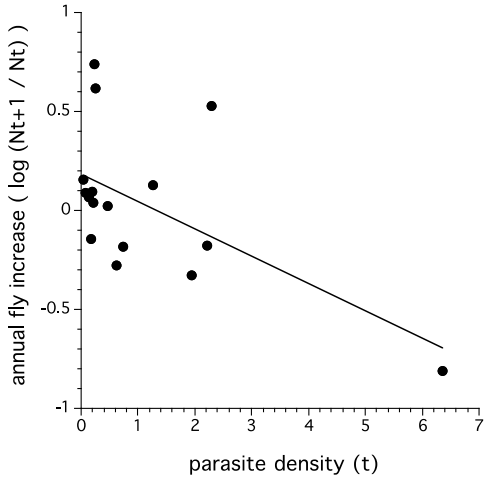
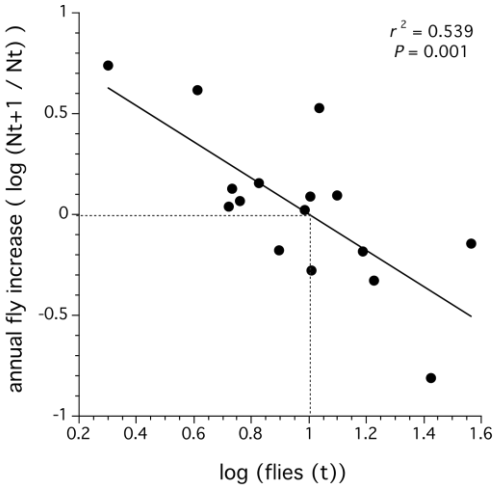


Fig. 4. Relationship between fly population increase and fly density the previous year. Dotted line demarks point of zero population increase.



CONCLUSIONS: This study is the only long-term monitoring of the blueberry maggot fly in North America. It shows that in 17 years there have been roughly 3 outbreaks of the blueberry maggot fly. Each outbreak takes 2-3 years to peak and then fly densities fall precipitously after the peak.

Study 2. <u>Attractiveness of two new synthetic lures to blueberry maggot fly</u>

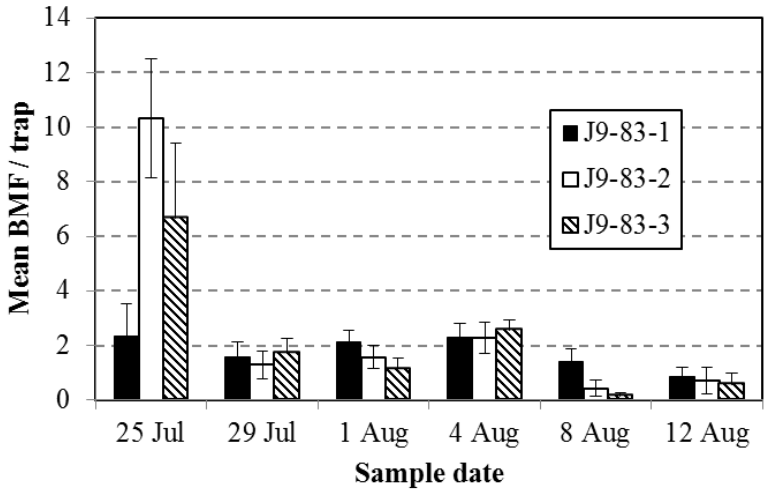
METHODS: The purpose of this trial was to field test two new lures for blueberry maggot fly; a blueberry synthetic blend and a white oak synthetic blend. The trial also included a check. There were seven replicates of each treatment labeled as: J9-83-1, J9-83-2, and J9-83-3 and set as a complete randomized 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 40ft 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 5 day intervals and any BMF were counted and removed. The experiment ran from 18 Jul until 12 Aug. Three replications were set on 18 Jul; four additional replications were set on 25 Jul.

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 ($F_{(2,6)} = 1.74$, $P = 0.2531$) or treatment x date interaction ($F_{(2,10)} = 0.1.73$, $P = 0.1203$). Data were transformed by the square root prior to analysis.

CONCLUSIONS: This is the second year of testing with these synthetic lures**.** Similar results were observed in the 2013 trial when 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. Lines are standard errors of the mean.



Study 3. <u>Long-range, within-field, movement of blueberry maggot fly in wild blueberry: A release/recapture study</u>

METHODS: This trial is the continuation of a study begun in 2013 to assess the long-range movement patterns of blueberry maggot fly (**BMF**). BMF were collected as pupae from infested blueberries in 2013. The wintering cups of pupae were separated into four equal groups. A small paint brush was used to layer orange, 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.
A line transect of 60 baited, yellow, Pherocon® AM traps was set along one edge of a pruned year blueberry field in Winterport, ME with 10ft between traps. Ammonium acetate superchargers were attached to every other trap to enhance attractiveness. On 21 Jul, the marked blueberry maggot flies were released at a point 200 feet (60m) across the pruned field from trap number 30 (the middle of the transect); ca. 700 flies were released. Traps were checked daily for 7 days and periodically for an additional 7 days; thereafter. All BMF were removed from the traps, brought back to the laboratory, and examined under UV light (Black-Ray® Longwave Ultraviolet Lamp) for the presence of fluorescent dye.

RESULTS: We recaptured a total of 33 marked flies (4.7%); no flies were recaptured after seven days from release; 23 of those were recaptured within the first four days after release (Table 1). The furthest distance that any marked fly traveled was 352.3ft on day 3. On day 1, two BMF traveled 320.2ft.
In 2013 we recaptured seven of 1000 marked flies (0.7%) that were released 328ft (100m) from a similar trap transect. Three flies were recaptured within two 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. The furthest any

BMF traveled was 406.4ft for a fly captured on day 7 after release. In 2013, we also released 1000 BMF at a point 1312ft (400m) from the transect. Only one fly (0.1%) was recaptured (on day 7 after release); that fly traveled 1380.7ft.

Table 1. Recapture rates and maximum distance traveled by marked flies.

Release distance	Trial year	Fly re-capture rate (%)	Furthest distance traveled (ft)
100ft (60m)	2014	4.7	352.3 (day 3)
328ft (100m)	2013	0.7	406.4 (day 7)
1312ft (400m)	2013	0.1	1380.7 (day 7)

CONCLUSIONS: A 1,000 foot dispersal in seven days suggests that flies in a split 20 acre field can easily make it from a previous cropped field into the far edge of a subsequent crop field the following year. This experiment will be repeated in 2015 by releasing a large number of flies and assessing their movement out to 5,000 ft.

Study 4. Survey of the weed, St. John's wort, and its natural enemies. 2013 - 2014

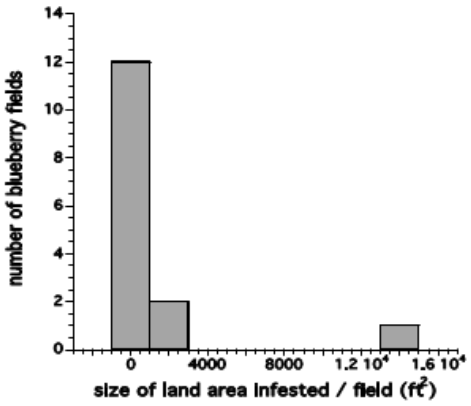
OBJECTIVE: This study was initiated in July 2013. The purpose is to assess the spread of the invading weed St. John's wort, *Hypericum perforatum* L. and the subsequent colonization of this weed's natural enemies. Both exotic natural enemies released by the USDA for biological control of this weed and endemic Maine herbivores or parasites were surveyed. In western North America three beetles *Chrysolina quadrigemina*, *Chrysolina hyperici*, and *Agrilus hyperici* have been introduced as biocontrol agents. In addition, a fungal parasite, *Fusarium oxysporum,* was also introduced to control this noxious weed.

METHODS: In August of 2013 and 2014, 15 fields were surveyed (5 in 2013, 10 in 2014) for St. John's wort. The fields were visually inspected for St. John's wort and patches of the weed were measured for their area. Field size, and surrounding landscape were assessed; and the latitude and longitude of fields were determined so that a geographic information system (**GIS**) analysis could be conducted at a later date. In one to five patches of St. John's wort in each field insect associates were collected and taken back to the laboratory. In addition, symptoms of foliar and stem pathogens were noted, as well as observations of dead or dying plants.

RESULTS: Sixty percent of the fields surveyed over the two years were infested with St. John's wort. Figure 1 shows the frequency distribution of land area infested. The smallest infestation was 75ft^2 and the largest was approximated at 13,000ft^2. The average infestation size over the two years was 1151ft^2. The size of these infestations will be affected by the weed management on each field. However, organic fields did not appear to have more St. John's wort

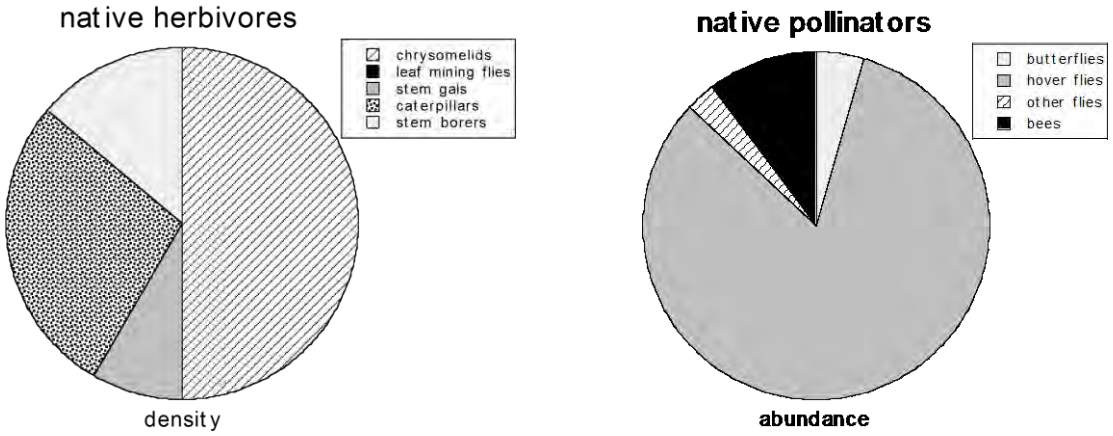
than conventional fields; although, the sample size at this point is too small to test this hypothesis.

Fig 1. Frequency distribution of St. John's wort densities in 15 blueberry fields.



Pathogens were few during the sample period. Only 2.3% leaf infections occurred with 0.7% stem lesions and no dead plants observed. Few insect herbivores were found associated with St. John's wort in Maine blueberry fields. One of the species of biological control agents that has been released in the western U.S., *Chrysolina quadrigemina* (0.53 / field) was found. Native insect herbivores were dominated by chrysomelid leaf beetles and caterpillars (Fig 2a). Native pollinators were dominated by hover flies followed by bees (Fig 2b).

Fig. 2. Densities of native insect herbivores (a) and relative abundance of native pollinators (b) on St. John's wort in blueberry fields.



CONCLUSIONS: This study is designed as a minimum labor study with the objective of monitoring an invasive pest weed as it colonizes Maine. A second objective is to see if biological control agents for this weed which were released in the western U.S. will follow St. John's wort into Maine and if natural herbivores will also help suppress its numbers. The flowers are known to be attractive to pollinators and so we will also monitor pollinator visitation.

Study 5. <u>Pest potential of a new invasive insect, winter moth in wild blueberry, 2013-2014. Report from Kaitlyn O'Donnell (Master's Candidate) and Dr. Eleanor Groden, School of Biology and Ecology</u>

OBJECTIVE: The winter moth, *Operophtera brumata*, is a European invasive caterpillar pest that was first discovered in Maine wild blueberry fields in 2012. Its current distribution in Maine is along the coast from York County to Mt. Desert Island. A two-year study initiated in 2013 in Harpswell, ME had two objectives. They were: 1. determine larval density on target host plants in the field throughout the feeding period, and 2. determine differential survival of caterpillars and pupae on target host plants in lab and field settings. This caterpillar pest species is an early spring defoliator and bud herbivore that has potential to become a significant pest. A very unusual aspect of its life cycle is that mating and egg laying takes place in the winter, November – January in Maine, with caterpillars hatching from eggs in April – May.

METHODS:

<u>Objective 1.</u>

Determine larval density on target host plants in the field throughout the feeding period. Six sites in Harpswell, ME were selected for study. In the spring, commencing with hatch, the number of larvae and buds were counted per 10cm of stem on seven host plants: white oak, apple, red maple, white birch, pin cherry, highbush blueberry and wild blueberry. The sampling was conducted May through June two times per week. Analysis of variance was used to determine density differences among the seven host plants in the field.

<u>Objective 2.</u>

Determine differential survival on target host plants in lab and field setting. This study was conducted by collecting caterpillars weekly from the seven host plants (three weeks in 2013 and four weeks in 2014). Ten caterpillars were put into petri dishes, five replicates per host plant. In 2013 the dishes were maintained in a growth chamber and in 2014 the dishes were maintained in an outdoor insectary. Leaf material and filter paper were replaced every 2-3 days and all dead caterpillars were removed and the numbers recorded. Survival to the pupa stage was also studied on the seven host plants. Caterpillars were hatched from eggs in the lab in 2013 and in the lab and field in 2014. The newly hatched caterpillars were set up on respective caged host plants in the field. Caterpillars were set up in cages on three dates in 2013 and two dates in 2014. The proportion of caterpillars pupating in the cages was recorded. Logistic regression was used to assess host plant effects on caterpillar survival and pupation.

RESULTS: While winter moth caterpillars feed on wild blueberry, based upon caterpillar densities, it was not found to be a good host plant for this invading insect (Fig. 1 and Fig. 2).

Fig. 1. Winter moth caterpillar densities (caterpillars observed per bud) on seven host plants in 2013. Bars with different letters are significantly different from one another ($P \leq 0.05$).

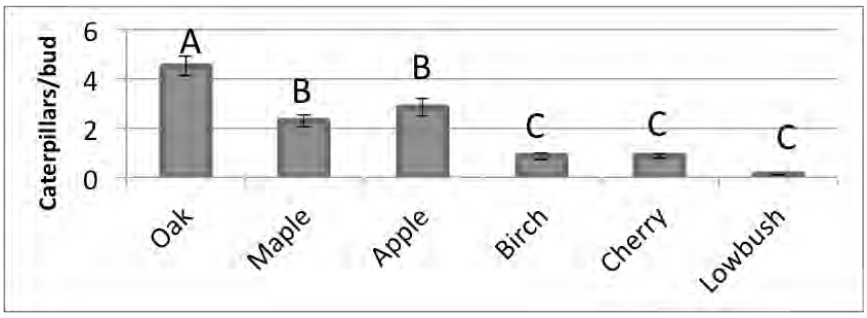
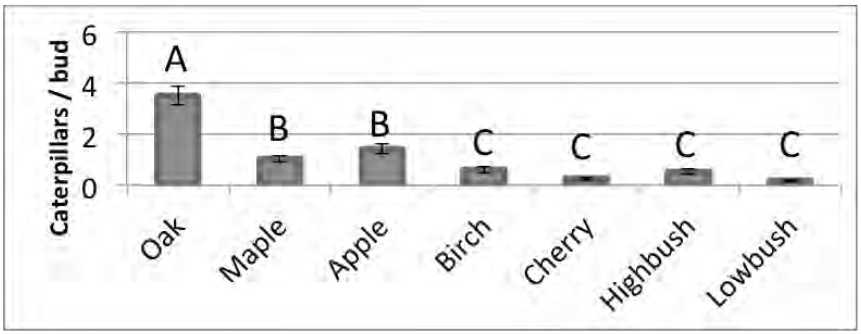


Fig. 2. Winter moth caterpillar densities (caterpillars observed per bud) on seven host plants in 2014. Bars with different letters are significantly different from one another ($P \leq 0.05$).



In both 2013 and 2014, survival of the caterpillars in petri dishes showed that white oak was a significantly better food than wild blueberry, about 43% higher survival. This relative survival among oak and wild blueberry reflects the densities observed in the field (Figs. 1 & 2).
A similar pattern emerged with pupation. The highest pupation rates were observed on white oak and the lowest on wild blueberry (Figs. 3 and 4).

Fig. 3. Winter moth caterpillar pupation rates observed on seven host plants in 2013. Bars with different letters are significantly different from one another ($P \leq 0.05$).

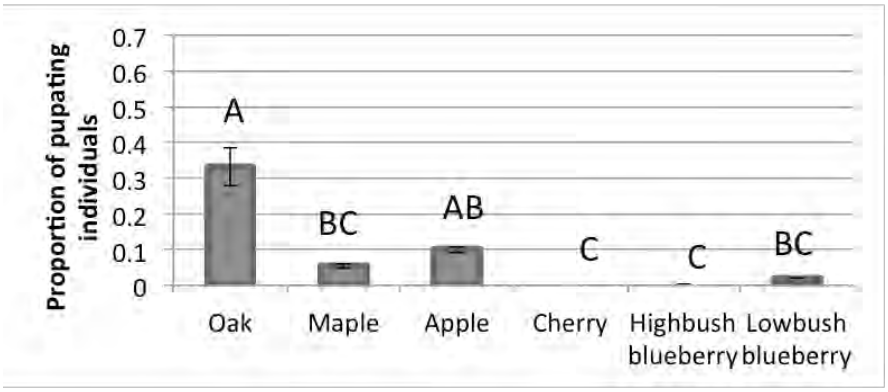
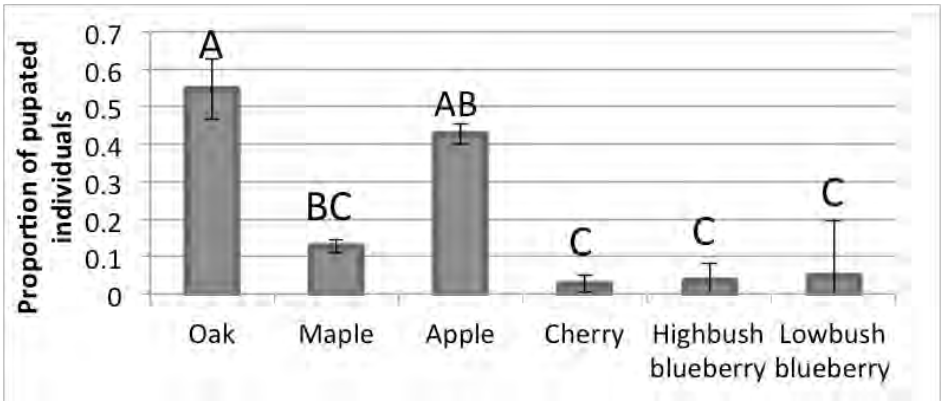


Fig. 4. Winter moth caterpillar pupation rates observed on seven host plants in 2014. Bars with different letters are significantly different from one another ($P \leq 0.05$).



CONCLUSIONS: In both years, white oak appears to be the predominant host plant. All three measures of host plant suitability: natural field densities, caterpillar survival rates, and pupation rates, showed a similar pattern of white oak being a superior host compared to wild blueberry. This has important implications for pest outbreaks and management.

RECOMMENDATIONS: We plan on continuing the study on long-term trends in parasitism of the blueberry maggot fly for another 3-5 years so that a sound basis of this pest's population dynamics can be acquired. The study of long-range movement of BMF is not directly applicable to management of the blueberry fly, but it does help explain what regulates its densities such as parasitoids, the frequency of outbreaks, and the relationship of threshold fly captures and the population growth of this very important pest. St. John's wort is not only a threat to the blueberry industry as a weed that competes for space, water, and nutrients; but it also is a threat to mammal herbivores. Ingestion by livestock or wildlife can cause photosensitization, central nervous system depression, spontaneous abortion, and can lead to death. Blueberry fields, while not supporting high densities of winter moth may be invaded by high populations that develop in Oak. Therefore, blueberry fields surrounded by oak forests should be surveyed for winter moth caterpillars in the spring. At this point there have been reports of winter moth found in wild blueberry fields, but not at high population densities causing defoliation.

ENTOMOLOGY

INVESTIGATORS: F. A. Drummond, Professor of Insect Ecology/Entomology
J. A. Collins, Assistant Scientist of Insect Pest Management
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5. III. TITLE: Biology of spotted wing drosophila, 2014.

Study 1. Comparison of adult abundance of spotted wing drosophila with larval fruit infestation

METHODS: To assess SWD adult infestation early in the season, on 20 or 21 May, three traps were set in each of 14 fruit-bearing blueberry fields. After one week the traps were removed and evaluated for the presence of spotted wing drosophila (**SWD**) adults. This was repeated in mid-June (traps set between 16 and 20 Jun). Beginning in early July, traps were placed in fields in Washington and Hancock Counties and monitored ca. weekly for the presence of SWD adults. With the exception of the Blueberry Hill Farm site, all sites had three traps; the Blueberry Hill Farm site had 7 traps. All traps were constructed from 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. Bait consisted of live yeast (1tbsp) + sugar (4tbsp) + 12oz water (makes enough for 4 traps). The traps were hung 1-2 feet above the top of the canopy using 36' plant stands. Throughout the study and on each sample date, traps set the previous week were collected and returned to the laboratory where male, female, and total abundance of SWD adults were determined and recorded. Using this data we calculated the mean SWD males per trap captured from each site for each date and the mean cumulative number of males over the collection period. The number of field sites being monitored ranged from a low of one site to a high of 14 sites on any given sample date.
To compare adult abundance with larval infestation, fruit samples were taken on various dates from mid-Jul until early Oct and processed using the Salt Extraction Method. Each sample consisted of 2/3 cup samples collected from the vicinity of the adult trap. There were ca. 359 berries per 2/3 cup sample. Using this data we calculated the mean number of maggots collected from each site on each sample date. This data was compared with the adult abundance data collected over the same time period.

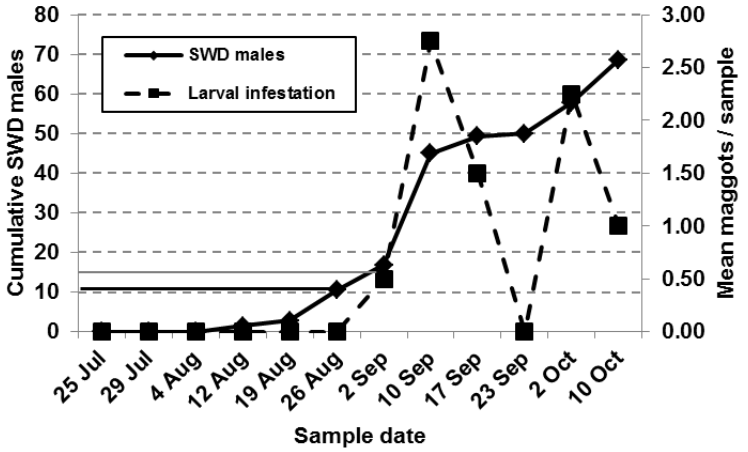
RESULTS: Ten of 14 fields that were sampled for male SWD ended up having infested fruit before they were harvested and so these were used in the following analysis. The normal distribution fit the frequency of male SWD captures at first fruit infestation. This theoretical model can be used to assess the cumulative trap capture when the average field will first detect infestation (16.4 cumulative male flies) and when 70% of fields will first detect infestation (6.1 cumulative male flies), or when 90% of the fields will first detect infestation (1 male fly per field). However, a MORE CONSERVATIVE and less risky approach is use the threshold of male trap captures the sample date PRIOR to any detection of SWD infestation of fruit. This threshold would be 10.0 cumulative flies per trap for the average field and 2.0 cumulative male SWD caught / trap to represent 70% of all fields.

Table 1. Summary, comparison of cumulative number of male SWD with date of first maggots in fruit samples, average = 15.75. Cumulative males prior to first infestation, average = 10.0.

Field ID	Sample date[1]	Cumulative SWD males with infestation	Sample date[2]	Cumulative SWD males prior to infestation
BBH Garden	2-Sep	16.8	10 Sep	10.5
BBH Sec 4	17-Sep	39.0	23 Sep	30.0
BBH Sec 8	2-Sep	19.5	10 Sep	9.5
Jonesboro	26-Aug	9.7	26 Aug	6.7
Cherryfield	26-Aug	14.7	26 Aug	8.7
Sedgwick	25-Aug	26.0	25 Aug	17.0
Otis	26-Aug	12.3	26 Aug	2.7
Orland	19-Aug	6.7	19 Aug	4.3
Stockton Springs	25-Aug	2.7	25 Aug	1.7
Penobscot	19-Aug	17.0	19 Aug	9.0

[1] Date of first maggots in fruit samples.
[2] Date prior to first infestation.

Fig. 1. An example of the relationship between sample date, cumulative male fly trap capture and SWD infestation of fruit (Blueberry Hill Farm, Jonesboro).



CONCLUSIONS/RECOMMENDATIONS: In 2013, 16 blueberry fields were monitored for SWD fly captures and fruit infestation. In 15 of 16 cases the fields were harvested prior to infestation of the fruit. The one field that SWD fruit infestation was detected had a cumulative male SWD trap capture of 20 flies just prior to fruit infestation. The results of the 2014 research showed that the 20.0 male SWD may be too high and that 10.0 male SWD is possibly a better estimate. We plan on repeating this study in 2015 to test this action threshold.

Study 2. <u>Attractiveness of two types of traps to spotted wing drosophila: A greenhouse release study</u>

OBJECTIVE: Two greenhouse release trials were conducted in order to assess the potential effectiveness of an easier to use sticky wing trap in comparison with the currently recommended red-cup trap.

METHODS: Two traps were tested 1) Red, plastic Solo® cup with ca. 4oz of standard yeast bait (1tbsp yeast + 4tbsp sugar + 12oz water), and 2) Red Sentry® wing trap with standard yeast bait (bait in diet cup with mesh lid and placed on sticky liner inside trap.
Laboratory-reared flies of various ages and sexes were dyed with Orange DayGlo® powdered fluorescent dye. Empty plastic drosophila tubes (28.5 X 95mm) were coated with a light layer of the dye. Excess dye was tamped out of the tubes. 100 flies from our lab-reared colony were added to each tube and then gently rolled in the dye. The dyed flies were then immediately transferred to tubes containing standard Drosophila media. The flies were allowed to groom for 24 hours before the release to remove any excess dye. This was repeated until there was a total of 500 orange-dyed SWD adults. In addition to the 500 dyed SWD, we also released 500 undyed adults.
Five traps of each type were placed in the greenhouse. Traps were spaced in an alternating pattern ca. 10ft apart in a grid: Rows 1 and 2 had four traps; row 3 had two traps.
To release, flies were tapped out into petri dishes placed at the four corners of the grid and one in the center of the greenhouse. Any flies that remained in the dishes after 10min were returned to the lab and counted. Unreleased fly totals were subtracted from the total number dyed to get a count of the number of flies released.
The traps were checked at 1-3 day intervals for 9 days. At each check the traps were brought back to the lab where the flies examined under UV light (Black-Ray® Longwave Ultraviolet Lamp) for the presence of fluorescent dye.

RESULTS: A total of 442 dyed and 468 undyed SWD adults were released. Recapture rates are shown in Table 1. A Two proportion Hypothesis Test using Fisher's Exact was used to evaluate the data. Red cups were the most effective in recapturing SWD adults; 86 (19.5%) dyed and 165 (35.3%) undyed SWD were recaptured in the cups compared with only 20 (4.5%) dyed and 24 (5.1%) undyed SWD recaptured in sticky wing traps. The difference was significant for both dyed ($P < 0.0001$) and undyed ($P < 0.0001$) flies for SWD captures between red cups and wing traps.
In addition, there was a difference in recapture rates of dyed compared with undyed flies. Significantly more dyed SWD were captured in Red cups compared to undyed SWD ($P < 0.0001$). There was no significant difference in captures of dyed compared to undyed SWD in the wing traps ($P = 0.7578$). When both trap types were combined, there was a significant difference in captures of dyed vs. undyed flies ($P < 0.0001$).
The majority of the recaptures occurred early in the trial. For red cup traps, 75.6% of the dyed and 70% of the undyed SWD that were recaptured were found on day 3 after the release (the first check date). For wing traps, those percentages were 65.0% and 58%, respectively.

Table 1. Recapture of dyed and undyed SWD on two trap types..

	Number recaptured			Percent Re-captured of SWD released		Percent of SWD re-captured that were dyed
	dye	no dye	Total	dye	no dye	
Red cups	86	165	251	19.5	35.3	34.3
Wing traps	20	24	44	4.5	5.1	45.4
Overall totals (all traps combined)	106	189	295	24.0	40.4	35.9

CONCLUSIONS/RECOMMENDATIONS: Earlier lab studies have shown that DayGlo dyes did not have any impact on mortality; however, their influence on fly behavior is generally unknown and warrants further investigation. This greenhouse study suggests that marked or dyed flies are recaptured in traps at a lesser rate than non-dyed flies. This is important as it suggests that our marker system for studying the movement of SWD in the field is not unbiased and that our recaptures may underestimate the travel distance of SWD released at the edge of blueberry fields. The results of this greenhouse study and a 2014 field study evaluating trap types do not suggest that a more easily used trap such as the Red Sentry® wing trap has potential in the field for monitoring SWD adults.

Study 3. Attractiveness of two types of traps and two baits to spotted wing drosophila: a field study

METHODS: There were five replications (blocks) of each of four combinations of baits and traps (Table 1). Traps within a block were placed a minimum of 30ft apart along the edge of a fruit-bearing blueberry field. Traps were hung from 3-ft tall plant stands. Each block was set in a different field. At each 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 2 and 4 are commercially available (Great Lakes IPM) and were either suspended from the lid of the red cup trap over the drowning solution (Trt #2) or attached to the edge of the wing trap (Trt #4)(Fig.1). Wing traps were red, plastic delta-style traps with replaceable sticky liners. The liners, yeast bait, and apple cider vinegar solution were replaced at each check date. Trece® lures were replaced after the second sample date.

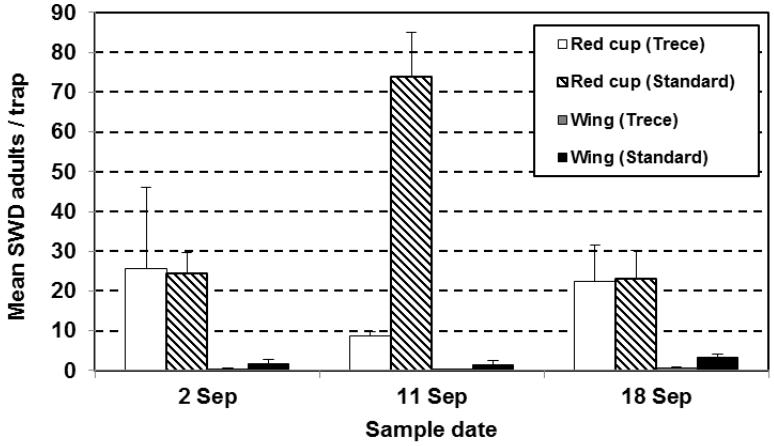
Table 1. Treatments.

Trt #	Trap description
1	Red, plastic Solo® cup with ca. 4oz of standard yeast bait (1tbsp yeast + 4tbsp sugar + 12oz water)
2	Red, plastic Solo® cup with Trece® bait and drowning solution (Apple cider vinegar)
3	Red Sentry® wing trap with standard yeast bait (bait in diet cup with mesh lid and placed on sticky liner inside trap
4	Red Sentry® wing trap with Trece® bait

Fig. 1. Red, plastic Sentry® trap with Trece® lures attached.

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. Data were transformed by the square prior to analysis. There was a significant difference in trap captures among the treatments. Both red cup treatments were significantly more effective in capturing SWD adults than either wing trap treatment ($F_{(3,16)} = 46.90$), $P = < 0.0001$). And, from inspection of the graph in figure 2, it can be seen that for two of the three sample dates, there was no significant difference between red cups baited with the standard yeast bait and cups baited with the Trece® lures hung over a drowning solution of apple cider vinegar. During sample date 2, the standard yeast bait caught significantly higher numbers of flies than the Trece® bait in the red cups ($F_{(3,12)} = 42.949$, $P < 0.0001$; mean separation by Student's t, $P \leq 0.05$). It should be noted that traps were baited with new yeast bait and apple cider vinegar solution at each check date. New Trece® lures were used on the first and third sample dates. It appears that the older Trece® lure used for the second sample date was not as attractive as new yeast bait solution.

Fig. 2. Bar graph showing mean SWD adults per treatment on each sample date. Lines are standard errors of the mean.



CONCLUSIONS/RECOMMENDATIONS: This bait trial was conducted to determine if a more efficient bait and trap combination could be found for monitoring SWD. From this trial, it appears that delta-style wing traps are not a viable alternative to the liquid-baited red cup traps currently recommended to growers. Captures of SWD adults in the wing-traps were very low. These traps also attracted a large number of non-target species which tended to obscure the SWD adults on the traps.

Study 4. Within-field movement of spotted wing drosophila

OBJECTIVE: Spotted wing drosophila (**SWD**) is of particular concern to Maine growers of wild lowbush blueberries as it readily infests this important commodity. During the summer, the flies are found virtually everywhere across the blueberry growing region including in the fields and along the wooded edges that border many blueberry fields. We sought to quantify how far and how quickly these flies are able to travel. We hope the results of this study can be used to understand the risk of SWD re-infesting a field after control materials are applied or for predicting when SWD may move into a certain area. Previous lab work studied the best dyes for marking SWD. We found that powdered fluorescent dyes are the easiest and least toxic to flies out of what we tested.

METHODS: Laboratory-reared flies of various ages and sexes were dyed with either Neon Red or Arc Yellow DayGlo® powdered fluorescent dye. Empty plastic drosophila tubes (28.5 X 95mm) were coated with a light layer of the dye. Excess dye was tamped out of the tubes. 100 flies from our lab-reared colony were added to each tube and then gently rolled in the dye. The dyed flies were then immediately transferred to tubes containing standard Drosophila media. The flies were allowed to groom for 24 hours before the release to remove any excess dye. This was repeated until there was a total of 1500 flies dyed red and 1500 dyed yellow.
This trial was replicated four times during the summer, three times in a pruned blueberry field in Winterport, ME and once in a fruit-bearing field in Jonesboro, ME. For all trials, flies were released along the edge of the blueberry field. Details of the release are outlined below. Flies

were tapped out into petri dishes set along the field edge and given 10 minutes to fly off. Any flies that remained in the dishes after this time period were returned to the lab and counted. Unreleased fly totals were subtracted from the total number dyed to get a count of the number of flies released.
For all trials, a recapture grid was arranged using plant stands approximately 2.5 feet tall. Red Solo® cups (16oz) containing 4oz standard yeast bait with holes punched around the rim and a red shade cover on top were set into the plant stands. The cups were replaced daily. Each day the cups were brought back to the lab where the flies were strained out of the liquid and examined under UV light (Black-Ray® Longwave Ultraviolet Lamp) for the presence of fluorescent dye. The cups were checked daily until no marked flies were captured.

Pruned field trials
Flies were released along a wooded edge during three different time periods (8 Jul, 7 Aug, or 1 Oct) and trials ran for five, seven, and nine days respectively.
Five transects were set in the field. Each transect began at the field edge and ran out into the field. Transects were 50ft apart from one another. Within each transect, the cups were placed 12.5, 25, 50, 100, 200, and 400 feet from the field edge/fly release point for a total of 30 cups. During the first release in the pruned field, we also released undyed flies. Wild SWD had not yet been observed in any of the fields we monitored so we were confident that any captured undyed SWD would be ones that we released. Subsequent releases utilized only dyed flies because wild SWD had begun showing up in traps across the state and there would be no way to distinguish wild from lab-reared SWD if they were not dyed.
In the first trial (8 Jul), undyed flies were released at each transect (0 ft), red flies were released at the second transect, and yellow flies at the fourth transect. For the other two trials (7 Aug and 1 Oct), red flies were released at the first transect and yellow flies were released at the fifth transect. The first trial released 1481 undyed, 235 red, and 178 yellow flies. The second trial released 1443 red and 1360 yellow, and the third trial released 182 red and 926 yellow flies.

Fruit-bearing field trial
The release trial in the fruit-bearing field began on 28 Aug and ran for 8 days. Because of space constrictions, there were only two transects. Transects were 50 feet apart and had the same cup spacing as described above with cups at 12.5, 25, 50, 100, 200, and 400 feet. Red flies were released at the first transect and yellow flies at the second transect.

RESULTS:
Trials in pruned fields
The first trial had an overall recapture rate of 5.76%. We recaptured a total of 109 flies (91 undyed, 11 red, and 7 yellow). We recaptured 6.14% of released undyed flies. The second trial had a recapture rate of 2.18%. We recaptured a total of 61 flies (26 red and 34 yellow). Recapture rate in the third trial was 4.87%. We recaptured a total of 54 flies (7 red and 47 yellow).
In the first trial, the furthest distance flies flew was 141.4 feet for red flies and 158.1 feet for yellow flies. The greatest distance traveled by the undyed flies was 100.0 feet. In the second trial, the greatest distance traveled by the red flies was 282.8 feet and the greatest distance traveled by the yellow flies was 400.0 feet. In the third trial, the greatest distance traveled by the red flies was 282.8 feet and the greatest distance traveled by the yellow flies was 400 feet.

Across all trials, the greatest distance traveled by day for a dyed fly was 206.2 feet on the first day, 250.0 feet on the second day, and 201.6 feet on the third day, and 400.0 feet by the fourth day (Table 1).

Trial in fruit-bearing field

There was an overall recapture rate of 0.24%; we recaptured only 5 marked flies (2 red and 3 yellow). The greatest distance recorded was 12.5 feet by the red flies and 70.7 feet for the yellow flies (Table 1).

Table 1. Recapture rates and maximum distance traveled by marked flies during each release and recapture period.

Trial	Overall dyed fly recapture rate (%)	Red fly recapture rate (%)	Yellow fly recapture rate (%)	Red fly furthest distance (ft)	Yellow fly furthest distance (ft)
Pruned #1	5.76	4.68	3.93	141.42	158.11
Pruned #2	2.18	1.80	2.50	282.84	400.00
Pruned #3	4.87	3.85	5.08	141.42	200.00
Fruit-bearing	0.24	0.20	0.29	12.50	70.71

CONCLUSIONS/RECOMMENDATIONS: We observed a low recapture rate across all prune field trials. However, this is not unusual for insect mark recapture field trials. Our previous blueberry maggot fly release and recapture studies generally average below 20% and sometimes less than 10%. The recapture rate in the fruit-bearing field was much lower compared to the pruned field. This is probably explained by ripe fruit competing with our baited cup traps, making the traps much less effective in attracting and recapturing flies. If low recapture was indeed a function of competition with ripe fruit, it highlights the relatively poor attractive nature of the red cup trap and bait.

Within 24 hours, a single fly can travel at least 200 feet in a pruned blueberry field. Within four days, a fly can travel at least 400 feet. We did not set up any baited cups past the 400 foot mark. It is possible flies traveled distances greater than 400 feet. This gives us some baseline physiological possibilities of the fly's flight capabilities. It would be interesting to see if this trend holds in a fruit-bearing field. It may be that with all of the available fruit, flies are less likely to continue traveling within the field for great distances. Unfortunately, with such low recapture rates, it is difficult to study this trend without releasing much greater numbers of flies. In order to reach the same recapture numbers as in our pruned field, we would have to release over 17 times the number of flies that were released in our pruned field (roughly 36,000 flies). However, even a total travel distance of 70ft and 56ft in a single day may preclude any effectiveness of a perimeter insecticide treatment tactic. Further research should continue to study how dye impacts recapture rates and flight distances. The only trial with dyed vs. undyed flies did not have a large difference in the recapture rates or distance traveled. Dye can interfere with normal behavior if the particles impact antennae sensing, wing movement, or act as an irritant. Earlier lab studies revealed that these DayGlo dyes did not have any impact on mortality, but further research is needed to determine if it can impact fly behavior. Overall,

DayGlo dyes seem to be a promising means to study adult SWD dispersal and movement within blueberry fields.

Study 5. Assessing the effectiveness of varying trap density on mitigating Spotted wing drosophila infestations in Maine lowbush blueberry
Report from Gabriel Alnajjar (Master's Candidate) and Dr. Frank Drummond

OBJECTIVE: Spotted wing drosophila (*Drosophila suzukii*) is an invasive species from Asia. Since spreading to the northern east coast, wild blueberries have become one of many fruit crops targeted by ovipositing females. Given the increasing efforts to reduce reliance on insecticides in pest management, whenever possible, it is important to explore alternative management strategies. One such strategy involves the use of attractive baits to trap out adult SWD in an attempt to lessen the severity of fruit infestations. This study was conducted in order to assess the effects of trapping out on SWD larva abundance in wild blueberry, and also to determine the effects of varying trap density on SWD infestations.

METHODS: On 27 Aug, twelve 30 x 30 ft study grids were set up in fruit-bearing fields in Jonesboro, ME. Three replicates for each treatment were assembled with trap densities of 3, 6 and 10 ft. spacing between traps. All the grids of each replicate were grouped together in the same crop section and positioned at a minimum of 30ft from one another. Each trap consisted of a red, 16 oz. Solo® cup positioned on a 2½ ft tall post. Experimental traps were filled with approximately 2 inches of liquid bait made of yeast, sugar and water with 1tbsp. yeast: 4tbsp. sugar: 12oz of water. Each trap also had a light exclusion lid in order to prevent excessive fermentation of the yeast bait. Control grids consisted of lids on red cups filled with water instead of bait, and control traps received 6ft of spacing. I n addition, the external surface of each experimental trap was sprayed with a mixture of 10 grams boric acid per liter 25% (*w/v*) sucrose/water solution. All traps were replaced weekly and sprayed, with three traps from each grid collected to serve as sub-samples. Sampled traps were taken back to the lab where male, female and total abundance of SWD adults was recorded.
Samples of blueberries were gathered weekly from random areas inside each study grid. Each blueberry sample was weighed, then crushed in a plastic bag and placed in a 10% saline solution. Crushed samples were allowed to sit in solution for roughly 30 minutes to induce disassociation of SWD larvae from the fruit pulp. SWD larvae were then filtered from the pulp and counted in a metal tray with water. Analysis of variance (RCB) was used to determine effects of mass trapping by the end of the season.

RESULTS: Table 1 summarizes the average number of adults and larvae counted in each of the grids in the field study. Adults were positively attracted by the sugar and yeast bait, with no flies captured in control traps. Figure 1 shows the abundance of larvae and adults found in baited and unbaited grids from each block. There was no statistically significant difference in larval abundance over time in blueberry samples collected from control and experimental grids with 6 ft of spacing ($F_{(1,19)} = 0.12$, $P = 0.973$). This trend was consistent between control and experimental groups within each replicate (Fig. 1). However, varying trap spacing of experimental grids did have a significant effect on the larval infestation in blueberry samples ($F_{(1,30)} = 15.00$, $P = 0.001$), but not on the number of flies captured in traps ($F_{(1,30)} = 0.74$, $P = 0.402$). Furthermore, the data suggest high variation in SWD abundance between replicates

(Fig. 2), reflecting the aggregated spatial distribution of SWD adults in fields. Field edges tend to have higher numbers of flies than field interiors.

Table 1. Average abundance of both larvae and adults in the study replicates for each of the grids. Letters in this table (C, H, M, and L) were assigned for the different treatments and represent the density of traps in the grid. C = Control grid with 6 feet of trap spacing. H = High density experimental grid with 3 feet of trap spacing. M = Medium density experimental grid with 6 feet of trap spacing. L = Low density experimental grid with 10 feet of trap spacing.

Statistical block	Treatment	Flies captured / trap	Larvae / cup of berries
1	C	30	0
2	C	0	0
3	C	5	0
1	H	20	28
2	H	3	4
3	H	29	33
1	M	22	43
2	M	3	2
3	M	2	7
1	L	3	11
2	L	1	6
3	L	1	23

Fig. 1. SWD adult and larval abundance in each of the three control grids (a) and experimental grids (b). N represents the average abundance of larvae and flies sampled throughout the field season.

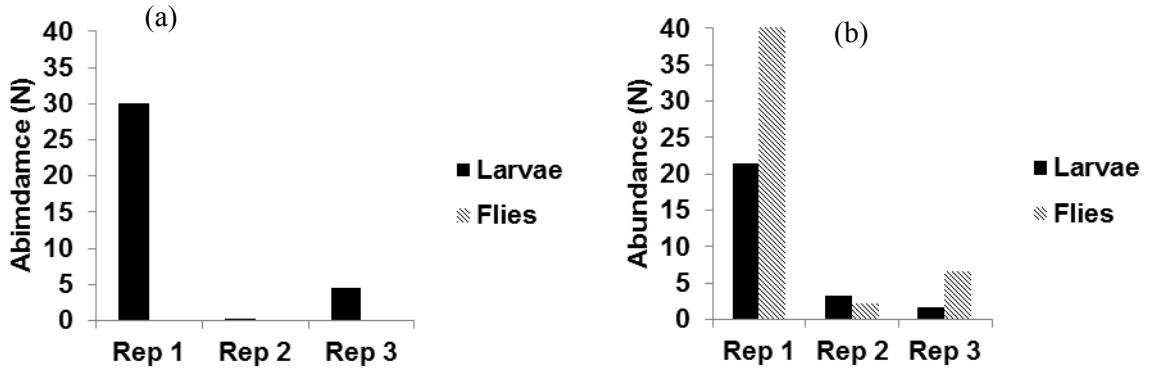
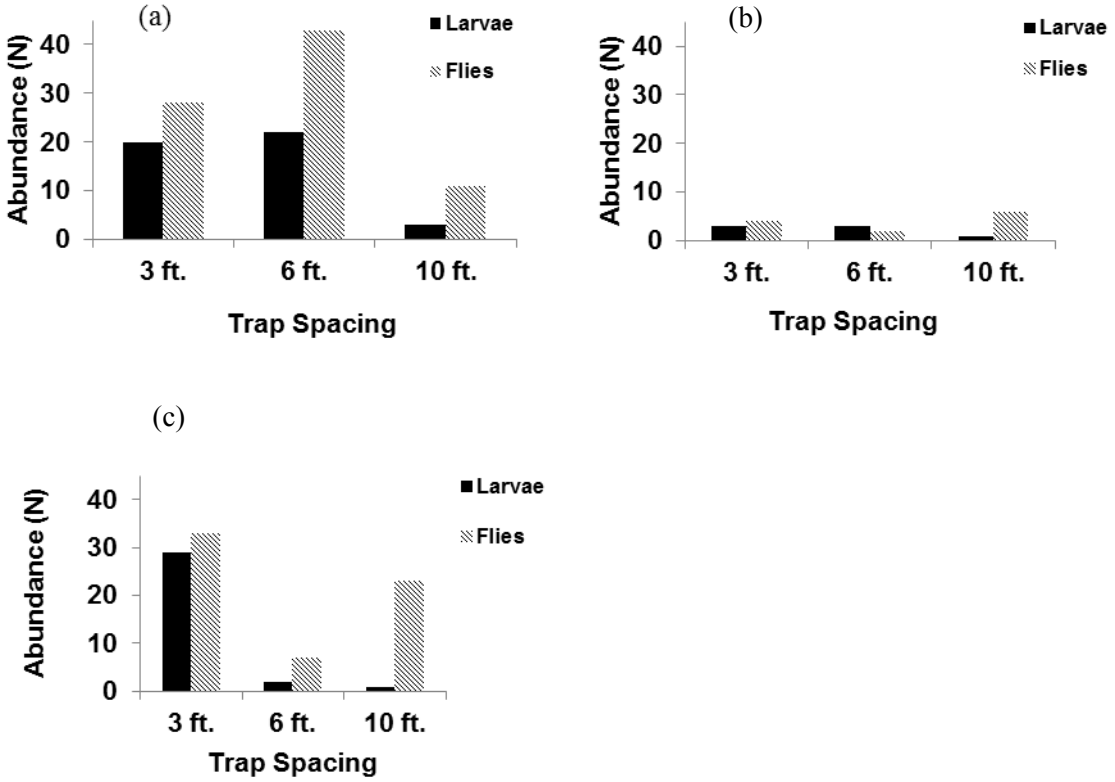


Fig. 2. Average SWD adult and larval abundance for experimental grids of replicate 1 (a), replicate 2 (b) and replicate 3 (c). N represents the average abundance of larvae and flies sampled throughout the field season. Grids positioned on the edge of the crop in replicate 1 include the high and medium density grids (3ft and 6ft of trap spacing), with high and low density grids positioned on the crop edge in replicate 2 (3ft and 10ft of trap spacing).



A mass trapping approach to managing SWD infestations does not appear to be an effective strategy for commercial blueberry growers. Baited field plots from each replicate of the study did not show any significant reduction of SWD larval infestation when compared with non-baited control plots (Fig. 1). This could be due to attracting high numbers of SWD adults to areas with baited traps. While some females will be captured before having a chance to oviposit, a significant number might not become captured before egg deposition. Thus, the traps are not removing enough reproductively active females to effectively reduce larval infestations. SWD overwinter as adults. In colder regions it is thought that they seek out shelter when temperatures drop to threshold levels that mark the onset of quiescence. This could help explain why grids positioned near forests and manmade structures generally experienced a higher number of adults captured and larger abundance of larvae in fruit samples. That is, simply because these areas of the crop are closest to SWD adults as they exit their state of dormancy. Perhaps over time, SWD abundance from grids positioned deep within the crop will reach a higher level of infestation, comparable to that of grids positioned on the periphery. As more fruits in the edge of the crop sections become infested, adult females will presumably be forced to travel farther into a crop to effectively search for healthy fruits for oviposition.

In follow up studies, it would be beneficial to minimize the variation between replicates and among grids within replicates (i.e., making sure that all grids in a replicate are positioned in areas with similar surroundings). Additional insights could also be obtained if SWD abundance in grids near potential overwintering areas becomes a variable of interest, compared with the abundance of SWD from grids surrounded only by crop vegetation. If adults are captured and prevented from traversing deeper into the crop, then the peripheral edge of a field might be a potential attraction buffer zone to restrict infestation. This may necessitate longer stretches of trapping grids, and while such a strategy will inevitably require some sacrifice of yield it could potentially work to prevent unmanaged infestations.

CONCLUSIONS/RECOMMENDATIONS: Mass trapping in wild blueberry may not be an effective tactic for managing SWD in wild blueberry until more attractive baits are found. However, our study conducted in 2013 and this study in 2014 suggests that mass trapping may have potential in the future. In 2013, a mass trapping study in Jonesboro resulted in reduced SWD larval infestation of fruit relative to a non-trapped control. The infestation was reduced by about 50%. However, we had only one replicate of a paired trapped and non-trapped study. In 2014, we designed a fully replicated experiment (3 replicates per treatment). While we did not show a reduction in SWD larval infestation between plots containing traps with water compared to plots with baited traps, we did find a decreasing larval infestation in plots as trap density increased. We found no such decrease in SWD adults as a function of trap density. Therefore, it appears that even though traps do not necessarily catch more flies per trap they do catch more flies per plot when trap number increase. Therefore, since more flies are being captured at a high density of traps, larval infestation was decreased in the plots with the higher number of traps.

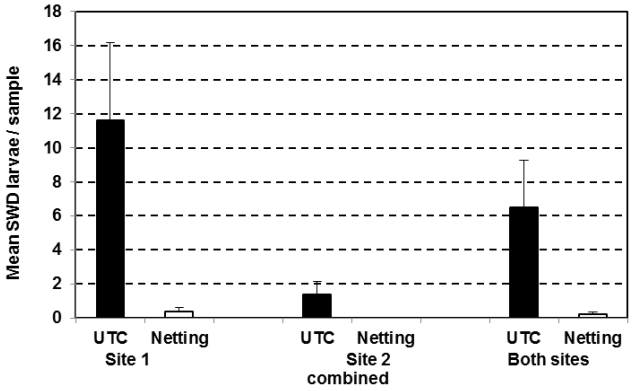
Study 6. Exclusion netting as an alternative method for control of spotted wing drosophila.

METHODS: Exclusion netting was evaluated to determine its effectiveness in preventing infestation of fruit by spotted wing drosophila (**SWD**). Anti-Insect Netting, (25 Mesh – 13' wide x 50' long) was placed in two fruit-bearing wild blueberry fields at Jonesboro, ME. One replication in time was set on 9 Jul and the second on 18 July. Fruit was just beginning to ripen. Red cups with standard yeast/bait solution were placed in each trial area to monitor for the presence of SWD adults. Fruit samples were taken periodically to determine the beginning of the fruit infestation period. On 17 Sep the exclusion netting was removed and within each of five blocks within each time replication, five fruit samples were taken from areas protected by the netting and paired areas not protected by any netting. Each sample was ca. 2/3 cup of ripe fruit. The fruit was processed to determine maggot infestation using the Salt Extraction Method

RESULTS/CONCLUSIONS: The statistical analysis relied upon a nested randomized block design (multiple blocks nested within each of two time replications). Data were transformed by the square root. In this study, exclusion netting appeared to be an effective means of suppressing fruit infestation in a limited area (Fig. 1). We found that there was a time replication effect ($F_{(1,9)} = 3.877$, $P = 0.081$), suggesting that the first time replication (site 1) had higher SWD infestation than the second time replication (site 2). We did not find any evidence for a block effect ($F_{(4,9)} = 0.713$, $P = 0.604$) or a treatment by block interaction ($F_{(4,9)} = 0.858$, $P = 0.524$). However, we did find a significant treatment effect ($F_{(1,9)} = 5.478$, $P = 0.044$). Significantly fewer maggots

were found in fruit samples collected from the treated (net covered) areas compared with the unprotected check areas (UTC). Where infestation was high (Site 1), < 1 maggot / sample was found; two of five samples had one maggot each. At lower infestation levels (Site 2), no maggots were found in any fruit sample from the protected area.

Fig. 1. Bar graph showing mean SWD larvae per treatment. Lines are standard errors of the mean.



Study 7. <u>Predation by ground beetles on spotted wing drosophila pupae, a laboratory study</u>

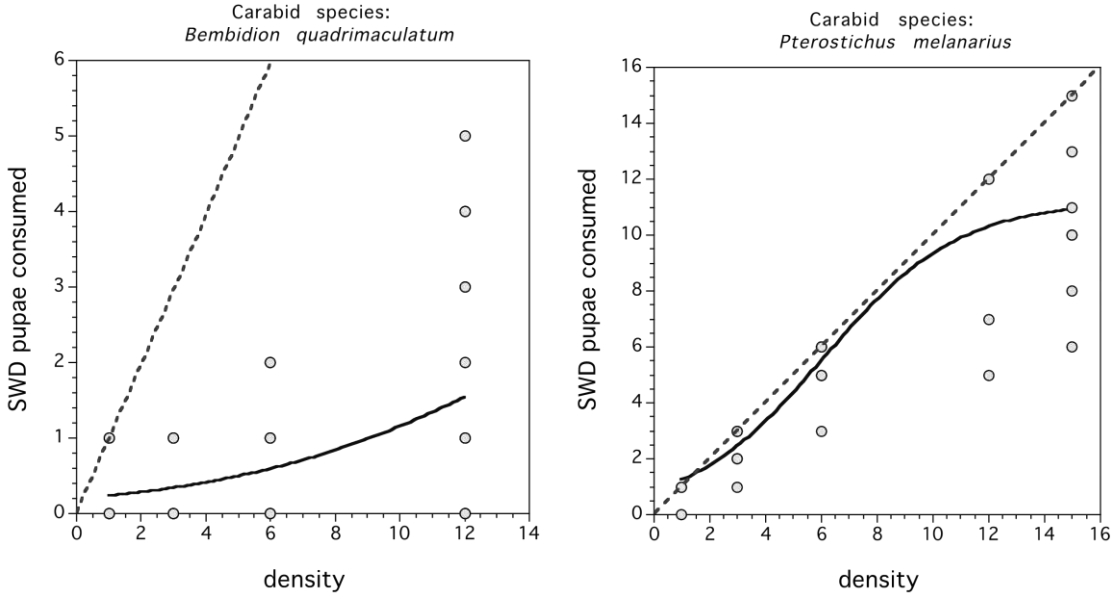
OBJECTIVE: The spotted wing drosophila (**SWD**) invaded Maine in October 2011. Little is known about its ecology in Maine. Predation and parasitism are key life history processes (or lack of) that often affect the population levels of invasive species. In 2014 we assessed predation by natural enemies in the field and in the laboratory. This report summarizes our laboratory studies on one of the most common predators in blueberry fields, ground beetles.

METHODS: Ground beetle adults from two genera (*Bembidion quadrimaculatum* and *Pterostichus melanarius*) were collected in pitfall traps and brought into the laboratory where they were fed one of 5 densities of SWD pupae (1, 3, 6, 12 or 15). The predation arena consisted of a petri dish with moistened filter paper and a small piece of wet sponge, SWD pupae, and one ground beetle adult. Ground beetles were stored in plastic containers with moist paper towels in the refrigerator prior to the study. SWD pupae were laboratory reared and taken from an established colony. Ground beetles were left in the petri dish for 24 hours then removed and the number of SWD pupae remaining in the petri dish was counted and recorded. The ground beetles were starved for 1-3 days and the trial was repeated with the same beetles. There were five replications of each SWD density. The experiment was replicated with each of the two genera

RESULTS: Both ground beetle species attacked and consumed spotted wing drosophila pupae. An analysis of variance provided evidence suggesting that the larger ground beetle, *Pterostichus melanarius,* consumes SWD at a higher rate (4.59 / day) than the smaller ground beetle species, *Bembidion quadrimacula* (0.75 / day)($F_{(1,134)} = 313.78$, $P < 0.0001$). The rate of consumption is a function of SWD pupal density ($F_{(1,134)} = 347.51$, $P < 0.0001$); the higher the density of SWD pupae in the arena, the higher the pupal consumption. But consumption rate as influenced by pupal density is also determined by species ($F_{(1,134)} = 271.96$, $P < 0.0001$). The rate of SWD pupal consumption was much higher for each increase in pupal density for the larger ground

beetle species (Fig. 1). The one-one line or ceiling on both figures suggests that only the large ground beetle species has a high discovery and subsequent consumption rate of the SWD pupa prey.

Fig. 1. The functional response of predation for two species of common ground beetles found in blueberry fields. Dashed line is the one-one predation line where predation occurs at the level of density in the arena.



CONCLUSIONS/RECOMMENDATIONS: Ground beetles appear to be ferocious predators of SWD. Whether the rate of predation observed in the laboratory is what occurs in the field is unknown. The pupae occur both in the fruit and in the duff layer of the soil. We did conduct a field predation study to determine what predation levels are in a more natural setting. At this point we recommend that growers attempt to minimize insecticide applications whenever possible so that ground beetle predators can be preserved in blueberry fields.

Study 8. Location of spotted wing drosophila pupation, a field study

OBJECTIVE: An experiment was designed to determine the location of pupating spotted wing drosophila (**SWD**). We wanted to determine where SWD pupate after the larvae live and feed inside blueberry fruit. We sought to determine if they pupate in the fruit or in the substrate.

METHODS: Wild lowbush blueberries were collected from Blueberry Hill Farm in Jonesboro and rinsed thoroughly before use. Between 16 and 21 berries were weighed, misted with water, and then added to a 9.3 X 2.6cm clear plastic drosophila culture tube. This was repeated 30 times; 15 female and 5 male SWD adults were added to each tube of blueberries. These flies remained in the tubes and were allowed to oviposit for seven days after which time flies were removed and discarded.
Pupation chambers were constructed from round 9 X 4cm plastic cups. A thin layer of sandy substrate was added to 30 pupation arenas. Ten of those arenas had an additional leafy duff layer

added on top of the substrate to imitate blueberry field conditions, and ten had a coarse mesh platform raised 1 inch above the sandy substrate to imitate fruit hanging from a plant (Fig. 1). The substrate was moistened with water. A single tube of SWD larvae infested fruit were set up in each of the 30 pupation chambers. For chambers without the mesh platform, the fruit was set directly on the substrate. The arenas were covered in a very fine mesh to prevent any SWD maggots from entering or exiting the arena. The arenas were set in a fruit bearing blueberry field in Jonesboro, ME on 10 Oct. Six days after placement in the field all arenas were misted with water and one half of the arenas were covered with a white, 9cm, painted petri dish to prevent any rain from collecting inside the dishes. Dishes were checked periodically for the presence of SWD pupae or adult flies, and once pupae were observed, the experiment was dismantled on 21 Oct to count the number of pupae and maggots in each arena.

Fig. 1. SWD ovipositing on blueberries and the three treatment arenas: sandy substrate, fruit elevated above sandy substrate, and sandy substrate covered in a leafy duff layer.

18 days after setting up the fruit in pupation chambers, the chambers were dismantled and the pupae in each location were counted. Fruit was examined externally and then dissected to look for pupae on the outside and inside of the fruit. The substrate was carefully examined to look for pupae on top of the substrate and then flooded with water to float the pupae within the substrate to the top of the water. The numbers of maggots in the fruit were also quantified. A one logistic regression was used to analyze the differences in the percentage of pupae in the substrate vs. fruit.

RESULTS: The arenas without rain covers filled at least halfway up with water after a heavy rain storm and so they were not included in the final results. This left us with a total of 5 arenas per treatment type. We located a total of 108 pupae across all three treatments. 105 were in the substrate, and three were inside of the fruit. No pupae were seen sitting on top of the substrate after a careful examination of the top layer of substrate. Pupae were only recovered in fruit in the arena with the duff layer; all SWD in the other two treatments pupated in the substrate (Table 1). There was no significant difference in the percentage of SWD that pupated in the substrate vs. fruit across the three treatments ($X^2 = 1.067$, df = 2, $P = 0.586$).

Table 1. Percentage of pupae located in fruit and the substrate.

Treatment	Total Pupae	Percent in fruit	Percent in substrate
Fruit on sand	62	0.0	100.0
Fruit suspended above sand	13	0.0	100.0
Fruit on duff and sand	32	9.4	90.6

Nine maggots were recovered from the arenas with fruit directly on the sandy substrate, one was recovered from the arenas with the fruit suspended above the substrate, and 21 maggots were recovered from the arenas with the duff layer.

Very few maggots pupated in the fruit compared to the substrate. These results are very different from a similar study that was conducted in the lab. In the lab study, the experiment was set up and run much the same way, the only differences being that the arenas were kept inside at room temperature under ambient light and we used commercial highbush blueberries. In that study, there was a mix of pupae in the substrate and fruit with more pupae in the substrate for the plain sand substrate and the duff layer substrate. In the lab experiment, the arena with the fruit elevated above the substrate had roughly equal numbers of pupae in the fruit and substrate.

In this field experiment, arenas with fruit suspended above the sandy substrate had the lowest numbers of pupae and maggots. This may have resulted from the berries drying out more when suspended compared to sitting on a damp substrate. If berries dried out, they might have not been able to support as many maggots compared to berries that did not dry out as quickly.

Although 105 pupae were recovered from the substrate, none of them were observed sitting on top of the substrate. It appears that SWD bury within the substrate. This may make it more difficult for predators and parasitoids to locate them.

One limitation of this experiment is that maggots are confined to a relatively small area and that may impact their natural pupation site selection behavior. This experiment also does not tell us how far maggots travel before pupating. Different species of flies, or even the same species raised under different conditions, can vary widely in the distance traveled to reach a pupation site. It would be beneficial to conduct additional experiments that measure how far SWD travel to reach a pupation site.

CONCLUSIONS/RECOMMENDATIONS: We plan on conducting predator trials in 2015 to see if SWD larvae burying into the substrate for pupation reduces predation by natural enemies such as ground beetles. Another hypothesis is that air temperature or maggot number relative to the size of infested fruits might affect the choice of pupation location. Laboratory studies designed to assess the plausibility of these hypotheses may be initiated during the winter of 2015. Another reason that this is important is that current research on biological control of SWD might be affected by where the larvae reside just prior to pupation.

Study 9. <u>Field predation of spotted wing drosophila pupae</u>

OBJECTIVE: Spotted wing drosophila (SWD) is a new pest in Maine, first detected in 2011. Until recently, this species had no described natural enemies in Maine (Study 7 of this report) and are capable of building up to very high numbers during the late summer months in Maine's wild blueberry fields. The eggs are laid inside fruit and the larvae spend a large part of their life also inside the fruit. These life stages are therefore fairly well protected from potential predators or natural enemies. The pupae are thought to pupate outside of the fruit, and present an easier target for predators. This experiment sought to quantify the potential number of pupae consumed by predators and to determine the types of predators that feed on them by utilizing different sized exclusion cages.

METHODS: Spotted wing drosophila pupae were collected from a lab colony. The pupae were examined under magnification to verify the presence of a live developing insect. Pupae were frozen for 24 hours to kill them so that they did not continue to develop and emerge during the duration of the experiment. Twenty freshly killed pupae were adhered to two, 7 cm pieces of double sided tape on top of a 9cm Petri dish.
The petri dishes were set out in the field under one of three treatments. One treatment comprised dishes that were surrounded by a very coarse weave wire cage (15 X 10cm). This cage was used to prevent larger predators, such as birds, access to the pupae. The second treatment had dishes that were uncaged and provided access to any potential predators. The control treatment consisted of dishes surrounded by the same size coarse cage, but the cage was covered in a very fine mesh to prevent all predators from gaining access to the pupae (Fig. 1). The controls were deployed in the field to determine if pupae were disappearing for reasons other than predators such as being dislodged from the tape from rain or weather events.

Fig. 1. Three treatments, from left: control with fine mesh surrounding the pupae, open dish not enclosed by any cage, and a coarse screen cage.

There were ten replicates for the caged and open treatments, and three replicates for the control for a total of 460 pupae per trial. This experiment had two trials, one at Blueberry Hill Farm in Jonesboro, Maine starting on 10 Sep and one at a commercial blueberry field in Sedgwick, ME on 22 Sep. The dishes were arranged in two transects within a fruit-bearing blueberry field with roughly 3 meters between transects. The dishes were arranged randomly within each transect and were 3 meters from each other. The dishes were checked every other day for missing pupae

starting two days after placing the dishes in the field and ending six days later for a total of three checks.
For the analysis, we compared predation rates from the open and coarse caged treatments. For the first trial in Jonesboro, data were transformed using Abbott's formula to subtract the experimental error from the treatment data (based on our control data). The transformed data were then analyzed using a repeated measures ANOVA. The data for the second trial in Sedgwick were non-normal and were analyzed using a logistic regression.
To sample arthropod predators present, we set up 12 pitfalls spaced 3 meters apart in a transect within the blueberry field. Pitfalls consisted of placing a 20 oz (6.35 X 11.43cm) deli cup so that the lip was at ground level and were covered with a 17 X 17cm tin rain cover propped up by nails. The deli cups were filled half way with propylene glycol to preserve any insects that fell into them. The pitfalls were left out for six days for both experiments.

RESULTS: In both experiments more pupae were missing in the open treatments, followed by the coarse caged treatments and very few pupae were missing from the control treatments (Table 1). In the first and second trial, there were never more than three pupae missing from any control dish. In the first trial, although there was a greater number of pupae missing from the open treatments compared to the coarse caged treatments this difference was not significant ($F_{(1, 14)}$ = 1.66, P = 0.22). In trial two, there was a significant difference of missing pupae between the two treatments (χ^2 =191.14, df=8, P > 0.0001).

Table 1. Percent of pupae missing across three dates for both trials.

Location	Treatment	Percent Missing Pupae Check 1	Percent Missing Pupae Check 2	Percent Missing Pupae Check 3	Corrected Final Predation rates*
Jonesboro	Caged	43.13	46.25	49.38	42.69a
Jonesboro	Open	51.88	56.25	68.75	64.62a
Jonesboro	Control	10.00	10.00	11.67	---
Sedgwick	Caged	18.13	28.75	43.75	---
Sedgwick	Open	73.13	88.13	95.63	---
Sedgwick	Control	0.00	0.00	5.00	---

*Data were corrected using Abbott's formula to remove experimental error from the treatments.

The most common predators/scavengers trapped in our pitfalls that may be capable of consuming SWD pupae were crickets, beetles, harvestmen, and ants (Table 2). Our pitfalls captured more predators at the field in Sedgewick compared to the field in Jonesboro.

Table 2. Total numbers of predators and scavengers collected in all pitfall traps by field site.

Predators/Scavengers	Sedgwick	Jonesboro
Crickets	235	41
Beetles	237	4
Harvestmen	93	2
Ants	31	23

By the end of both trials, an average of 1.67 pupae were missing from the control dishes indicating that some pupae were lost due to weather events such as rain or wind. This experimental error was subtracted from the treatment data using Abbott's formula to more accurately reflect the number of pupae missing from predation versus other random events in the first trial. More pupae disappeared from open treatments compared to coarse caged or control treatments. This indicates that larger animals, such as birds, may feed on the pupae in addition to predatory arthropods. By the end of trial one 10% of observed predation was attributed to vertebrates, and by the end of trial two 26% of the observed predation was attributed to vertebrates. A large number of pupae disappeared from the course caged treatments which indicate arthropods that are small enough to fit through the cage will also feed on the pupae. Examining the pitfalls revealed there are a large number of predatory insects found in blueberry fields. There was a much larger difference between treatments in the second trial. This may highlight the diverse and fluctuating populations of predators between field sites.
It is not well understood where SWD pupate. Lab studies (see Study 8 of this report) have shown that they often pupate outside of the fruit in the substrate. It is not known whether they pupate on top of the substrate or if they bury themselves first. This distinction can have large implications on predation rates. Our pupae were set out in the open on a petri dish. Predation rates will probably be much higher in an artificial situation like this where predators do not have to dig for them. In this field trial predators consumed large numbers of SWD pupae when they were presented with them, especially pupae that were simultaneously accessible to both arthropods and larger predators.

Fig. 2. Percent of pupae missing over time from the first trial in Jonesboro.

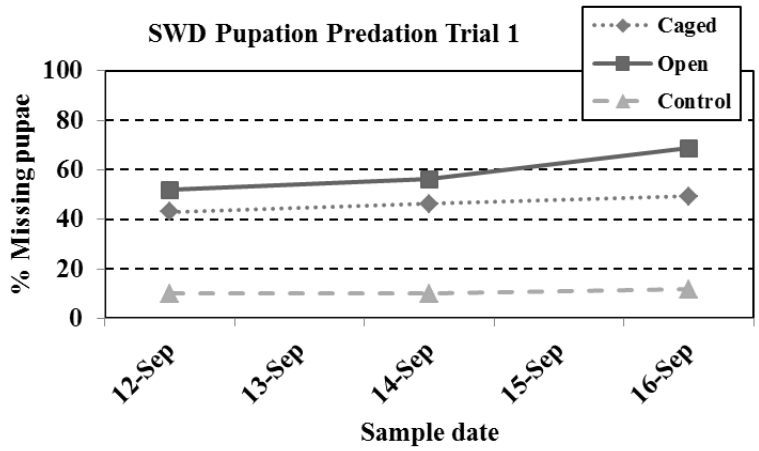
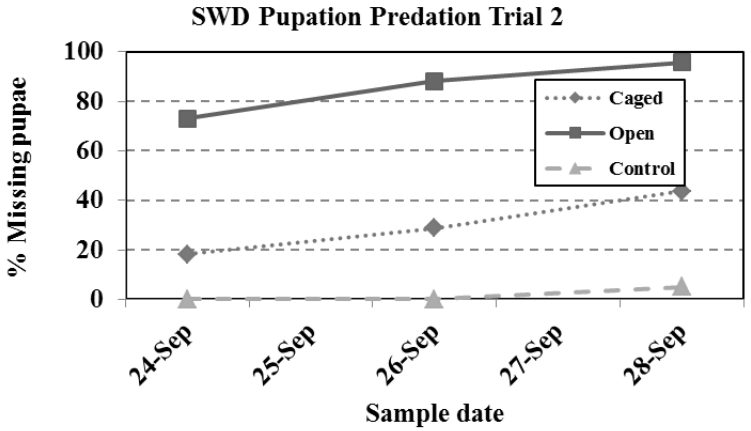


Fig. 3. Percent of pupae missing over time from the second trial in Sedgwick.



CONCLUSIONS/RECOMMENDATIONS: Predation appears to be heavy in blueberry fields. At the end of a four day experimental period predation in the open treatment was 70% in trial 1 (Fig. 2) and almost 100% in trial 2 (Fig. 3). This does not mean that SWD infestations are less likely to occur in blueberry fields, but that the rate of fruit infestation may not be as steep in future years as in the first two years of the SWD invasion. It might be very important to protect natural enemies so that SWD are not able to explode in the absence of predators.

Study 10. Survey of parasitoids of spotted wing drosophila

OBJECTIVE: Spotted Wing Drosophila (**SWD**) is an invasive pest in Maine with no known native, natural enemies. Surveys in other states have found parasitoids that were able to successfully attack and develop using SWD as their host. The SWD larval stage lives most of its life inside the fruit, but at least some pupae are located on the outside of the fruit or in the substrate, and thus may be more susceptible to parasitism. In 2013 we surveyed several wild blueberry fields in Maine to explore if parasitoids were attacking SWD. We found a few species of hymenopteran parasitoids in blueberries, but we were unable to confirm if they were attacking SWD. Therefore in 2014 we expanded the study to determine what the parasitoid species might be and the extent of their distribution.

METHODS: Parasitoid sampling began when SWD maggots appeared in blueberries at the field sites. We believed we were more likely to find parasitoids associated with the pupal stage, which is less protected than the larval stage. To guard against missing early pupae, sampling began when larvae were first detected in the fruit. This allowed us to sample continuously while pupae were present.
Sampling began in Hancock County on 20 Aug and in Washington County on 14 Sep. In Hancock County, five fields were sampled weekly until fruit was harvested. Two fields were sampled four times, two fields were sampled three times, and one field was sampled twice. One cup of fruit was collected from several randomly selected locations within each field. When present, fallen fruit and some of the surrounding duff layer were also collected. Wild fruits that are preferred hosts of SWD, such as raspberries and blackberries, found within or along the field

edge were also collected. In Washington County, the commercial fields were sampled weekly until harvested with two fields sampled twice, and one field sampled three times. The University-owned blueberry research field in Washington County left a portion of the site not harvested, allowing for continuous sampling of fruit for eight weeks. At the University research field, three cups of fruit were collected during each sample. The fruit samples were returned to the lab and held at room temperature to check for parasitoid emergence.
At two sites, we also set out cages that contained SWD infested fruit that contained both larvae and pupae. To infest the fruit, a mixture of bananas, strawberries, and wild blueberries were exposed to SWD in the lab for one week to allow the flies to oviposit. The fruit was then set in plastic Tupperware® (15 x 8 x 10cm) with coarse mesh on the sides to allow small insects access to the infested fruit. The fruit was then set out at two field sites: one in a non-harvested field in Hancock County, and the other at the University-owned blueberry field site in Washington County. Six fruit traps were placed at the Hancock County field site 1 Oct, and were left for two weeks. Six fruit traps were placed at the Washington County field site weekly from 10 Sep through 21 Oct. The fruit traps were randomly dispersed within a fruit-bearing field. Half of the traps were picked up after one week and the other half were picked up after two weeks. The fruit traps were returned to the lab, stored in cages at room temperature, and watched for parasitoid emergence.
All sampled fruit and fruit traps were held at room temperature inside plastic cages and lightly misted with water twice a week, to prevent desiccation. The samples were checked for parasitoid emergence for three and a half months before discarding.

RESULTS: We were unable to locate any parasitoids at any of the sampled fields in 2014. This may be because we do not have any parasitoids that are currently attacking SWD, or the populations of these parasitoids are highly fragmented and difficult to detect. Most farms harvested their fruit early, before SWD were present in high numbers, which limited the number of fields we were able to sample. A large number of SWD emerged from all fruit samples, which indicate that the fruit was infested when it was collected. Last summer, we found parasitic wasps in a blueberry fruit sample but were unable to confirm if they were attacking SWD. This wasp was not found this year, despite sampling from the same field it was located in last year. It is possible that much larger samples across more sites are needed to locate potential parasitoids.

CONCLUSIONS/RECOMMENDATIONS: Establishment of parasitoids could be extremely important and lead to a decline of the spotted wing drosophila through natural parasitism. We will continue to survey for naturally occurring parasitoids next year as well as attempt to obtain parasitoids from the USDA to release in Maine.

Study 11. Cold tolerance of spotted wing drosophila adults

OBJECTIVE: Little is known about the ability of spotted wing drosophila to overwinter in Maine. It has been suggested that cold winter temperatures exclude the possibility of winter survival in fields, and that yearly infestations are due to winter survival in warmer, man-made habitats or from seasonal dispersal and re-infestation. Although *Drosophila suzukii* prefers a moderate climate it can also survive in cold conditions. The flies are most active at 20°C (68°F).

Activity becomes reduced at temperatures above 30°C (86°F) or below freezing. However, *D. suzukii* is firmly established on the island of Hokkaido in Japan where winters average -4 to -12°C, suggesting the possibility of its establishment in cooler climates. Research from Oregon suggests that *D. suzukii* larvae, pupae, and adults have the potential to survive fluctuating overwintering conditions for periods up to 60 days. Adults are able to withstand longer periods of cold conditions than larvae or pupae.
This laboratory study attempts to provide a preliminary assessment of the potential for spotted wing drosophila to overwinter in and around blueberry fields in Maine.

METHODS: In order to obtain cold and non-cold tolerant adult SWD of known ages, SWD (a mix of males and females) were allowed to oviposit on instant drosophila media for 24 hrs at room temperature (ca. 20°C). The adults were then removed and the tubes were held at room temperature for 5 days. To obtain cold-tolerant adults, the tubes were placed in a growth chamber at 15°C. For non-cold tolerant adults, the tubes were held continuously at room temperature. All tubes were observed daily for adult emergence. Emerging adults were removed and placed in vials with new media. We tested 3, 5, and 7-day old adults. There were four replications of each age and treatment timing. For each replication, 9-11 SWD were placed in vials with new medium and help in a freezer at -16.5°C. The vials were left in the freezer for the treatment time specified (1/2, 2, 5, or 10hrs) then removed to room temperature (21.8°C). After two hours the number of live SWD in each vial was recorded. Using this information we calculated mean percent mortality for each age and "time in freezer" for cold tolerant and non-cold tolerant SWD adults.

RESULTS: ANOVA ($P \leq 0.05$) was used to determine the effect of treatment (cold vs non-cold tolerant), age, and time in the freezer. Examination of the data showed that there was a significant treatment*time interaction ($F_{(1,4)} = 5.91$, $P = 0.0003$)(Fig. 1). Mortality of cold tolerant adults was less than that of non-cold tolerant adults over time. And, there was also a significant age* time interaction ($F_{(1,8)} = 3.73$, $= P = 0.0008$)(Fig.2). The older flies (7-day old) died at a faster rate than the younger flies (3-day old), with the 5-day old being much more variable. No adults survived after 5hrs in the freezer regardless of treatment or age.

Fig. 1. Interaction between treatment (cold tolerant/non-cold tolerant) and time in the freezer.

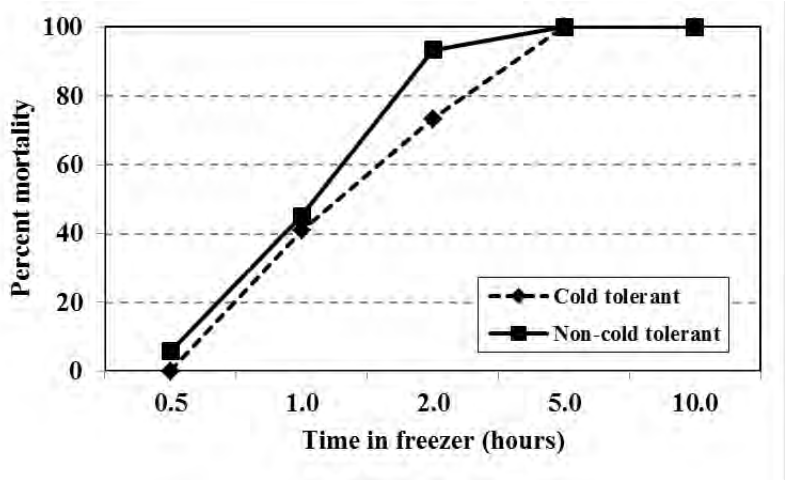
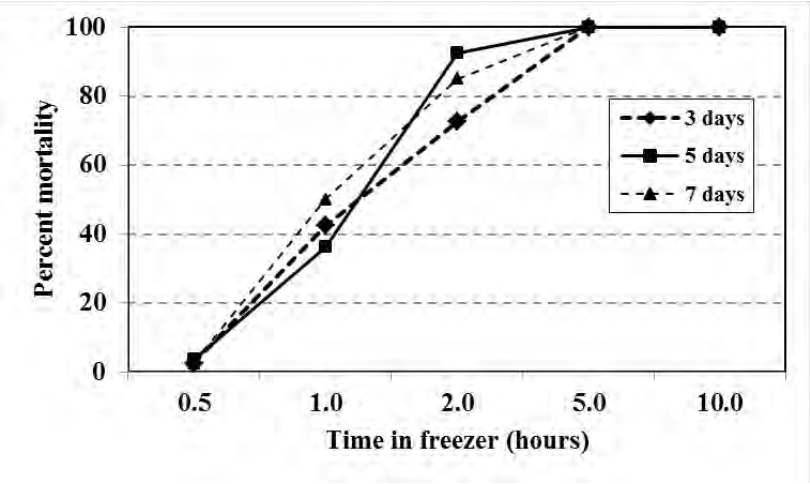


Fig. 2. Interaction between age of SWD and time in the freezer.



CONCLUSIONS: Spotted wing drosophila overwinter as adults. It is suspected that acclimation occurs during the fall prior to overwintering. Our data show that this is the case, as flies exposed to cool temperatures prior to freezing temperatures lived longer than non-cooled flies. No one knows how successfully SWD overwinter in Maine. It is thought that they have low hardiness and that almost all of the flies die over the winter. Because of this, fall production of billions of flies affects the number that will start the population growth the following summer.

ENTOMOLOGY

INVESTIGATORS: F. A. Drummond, Professor of Insect Ecology/Entomology
J. A. Collins, Assistant Scientist of Insect Pest Management
E. Ballman, Research Associate in Invasive Species / Entomology

6. IV. TITLE: Biology of blueberry, beneficial insects, and blueberry pollination.

Study 1. Pollination project

METHODS:

Blueberry flower-counts and subsequent fruit-set

In mid-May (peak bloom), six blueberry clones were selected within each of twelve fruit-bearing blueberry fields. Six of the fields were located in Hancock Co. and six in Waldo or Knox Co. 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, three marked stems from each clone were cut, placed in individual zip-loc bags, and brought into the laboratory where fruit-set was evaluated by counting the number of developing fruit on each stem**.** This was repeated in mid-July with the remaining three marked stems. Sample dates are given in Table 1.

Soil and foliage samples

Soil samples were collected from each site. Samples were sent to the Maine Soil Testing Service for Analysis. Soil pH was measured in distilled water. Organic matter was measured by loss on ignition (**LOI**) at 375°C. Nutrients were extracted in pH 4.8 ammonium acetate (modified Morgan extract). P was determined colorimetrically by Ion Analyzer. All other nutrients were measured by ICP-OES. Effective cation exchange capacity (**ECEC**) is calculated by summation of base cations plus readily exchangeable acidity. Unless otherwise specified, all nutrients are expressed as parts per million in the dry soil. The results of the soil analysis are shown in Table 2. Samples of the foliage were collected from each clone at peak bloom. Ten stems per clone were randomly collected from twelve clones, flowers were removed and the leaves were collected and dried for analysis. The results of the foliage analysis by field are in Table 3. Additional information collected on each site included yield, number of honeybee hives and pesticide inputs (insecticides, fungicides, herbicides). This information is in Table 4.

Table 1. Sample dates.

County	Site	Number of honeybee hives	Visual estimate of bee density Sample #1	Sample #2	Sample #3	Colored bowl traps Sample #1	Sample #2	Flower counts	Fruit set Early	Late
Hancock	1	0	21-May	29-May	9-Jun	30 May	11-Jun	30-May	27-Jun	23-Jul
	2	16	22-May	29-May	9-Jun	30 May	11-Jun	3-Jun	27-Jun	25-Jul
	3	0	22-May	29-May	9-Jun	30 May	11-Jun	3-Jun	27-Jun	25-Jul
	4	20	21-May	29-May	9-Jun	30 May	11-Jun	3-Jun	27-Jun	29-Jul
	5	1 [a]	21-May	29-May	9-Jun	30 May	7-Jun	2-Jun	27-Jun	25-Jul
	6	48	22-May	29-May	9-Jun	30 May	11-Jun	3-Jun	27-Jun	23-Jul
Knox/Waldo	7	0	21-May	30-May	6-Jun	30 May	7-Jun	27-May	24-Jun	21-Jul
	8	0	21-May	30-May	6-Jun	30 May	7-Jun	2-Jun	16-Jun [b]	21-Jul
	9	24 (4 BB quads)	21-May	30-May	6-Jun	30 May	7-Jun	2-Jun	24-Jun	[c]
	10	28	21-May	30-May	6-Jun	30 May	7-Jun	2-Jun	24-Jun	22-Jul
	11	35 BB quads	21-May	30-May	6-Jun	30 May	7-Jun	2-Jun	24-Jun	29-Jul
	12	30	21-May	30-May	6-Jun	30 May	7-Jun	27-May	24-Jun	21-Jul

a 1 top bar hive only

b Field covered with mesh netting by grower; many flags missing. Collected as many stems as could be found.

c Site harvested; no stems collected

Table 2. Soil sample analysis by field.

Field ID	soil pH	% LOI	ppm Ca	K	Mg	P	Al	B	Cu	Fe	Mn	Na	S	Zn	meq/ 100g ECEC
1	4.7	21.1	618	173	119	8.2	251	0.34	0.32	39	95	41	44	6.5	9.4
2	5.0	16.1	855	111	128	5.3	259	0.36	0.22	11	21	61	38	4.9	9.3
3	4.5	14.9	179	85	34	5.6	378	0.27	0.16	23	30	32	92	2.2	6.0
4	4.8	13.8	452	86	70	5.4	354	0.30	0.22	31	18	47	44	6.5	7.2
5	4.1	13.3	221	92	59	2.6	369	0.25	0.39	36	37	172	163	1.9	7.5
6	4.9	13.8	494	138	86	7.1	283	0.26	0.12	15	19	32	35	4.6	7.2
7	4.5	13.7	180	109	49	1.6	406	0.32	0.22	48	23	31	105	1.8	6.1
8	4.6	10.6	106	70	19	5.4	327	0.21	0.29	34	19	24	109	1.4	4.4
9	4.4	10.1	122	71	27	5.5	300	0.20	0.21	27	24	16	107	1.8	5.0
10	4.7	11.9	406	115	72	3.9	309	0.27	0.22	15	43	34	51	3.9	6.9
11	4.7	11.5	172	75	33	3.4	334	0.22	0.21	32	27	29	79	2.6	4.9
12	4.5	9.2	128	59	19	2.8	376	0.28	0.19	17	16	32	121	2.8	4.7
measured ->	6.0	4.5	1064	240	113	14.7	41	0.36	0.93	1.9	34	11	14	2.3	
OK range->	*5.9-6.1*	*4.3-5.1*	*1060-1160*	*225-255*	*112-125*	*14-17*	*41-52*	*0.2-0.4*	*0.8-1.0*	*1.8-2.2*	*28-34*	*9-13*	*12-14*	*2.3-2.7*	

Table 3. Foliar analysis by field.

Field ID	N %	Ca %	K %	Mg %	P %	Al ppm	B ppm	Cu ppm	Fe ppm	Mn ppm	Zn ppm
1	1.91	0.319	0.562	0.155	0.160	44.2	20.3	4.77	37.28	1310	15.4
2	1.94	0.278	0.598	0.141	0.210	54.1	19.1	5.42	44.78	965	21.7
3	1.83	0.325	0.541	0.120	0.191	49.1	18.1	6.02	45.78	1349	19.3
4	1.84	0.317	0.603	0.142	0.201	53.3	17.8	6.10	45.13	795	22.6
5	1.94	0.351	0.602	0.126	0.216	69.2	18.5	8.25	65.20	2115	25.0
6	1.91	0.315	0.602	0.143	0.186	81.8	20.8	5.59	75.48	1051	20.7
7	1.83	0.409	0.549	0.163	0.180	63.7	15.9	5.72	52.73	1381	18.1
8	1.92	0.335	0.578	0.138	0.197	87.7	16.8	6.74	95.86	1335	23.7
9	1.92	0.321	0.546	0.135	0.206	51.8	17.8	5.70	45.43	1446	20.3
10	1.91	0.336	0.602	0.139	0.196	48.1	17.6	6.01	49.23	1231	25.8
11	2.09	0.268	0.553	0.126	0.209	48.3	15.0	6.12	60.96	969	20.6
12	2.00	0.368	0.556	0.143	0.209	142.4	19.2	6.70	138.75	1961	24.0

Table 4. Additional site information; yield and pesticide inputs.

Site #	Yield (lbs/acre)	Fungicides	Herbicides	Insecticides
1	3100	no pesticides applied		
2	no information available at this time			
3	3000		Tilt (2X)	Malathion
4	2621			Imidan, Mustang Max
5	1721	no pesticides applied		
6	0 [a]			Imidan, Mustang Max
7	1000	no pesticides applied		
8	no information available at this time			
9	2840		Tilt, Bumper	Imidan
10	2170		Tilt (2X)	Imidan
11	3412		Tilt	Imidan
12	5487	Pristine	Tilt (2X)	Imidan (2X)

[a] Crop destroyed by hail

Bee abundance and pollination

Two different methods were utilized to study bee abundance: colored bowl traps and visual estimates. On sample dates indicated in the table blue, yellow, and white plastic cups were placed in each plot. Bees were sampled using colored bowl traps on two dates; peak bloom (29-30 May) and late bloom (7 or 11 Jun). For each sample date, there were three replications of each color in each field. 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 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) was counted in each of 15, m^2 quadrats per site. For each sample we counted the number of bees observed in 1 minute. This was repeated on each of three dates as indicated in Table 1. The data from all three dates was combined as "total bees/m^2/min" for the analysis.

RESULTS: As expected, percent fruit set measured in June (Fig. 1) was higher than percent fruit set measured in July (Fig. 1). The lower fruit set in July can be attributed to a number of factors including insect and disease damage and fruit drop. There was no significant difference ($F_{(1,10)} = 0.35$, $P = 0.5691$) in early fruit set (which is a measure of percent pollination) between sites located in Hancock Co. (64.4%) compared with the six sites located in the Knox/Waldo Co. area (67.4%)(Fig. 2).

Fig. 1. Percent fruit set for stems sampled in June or July.

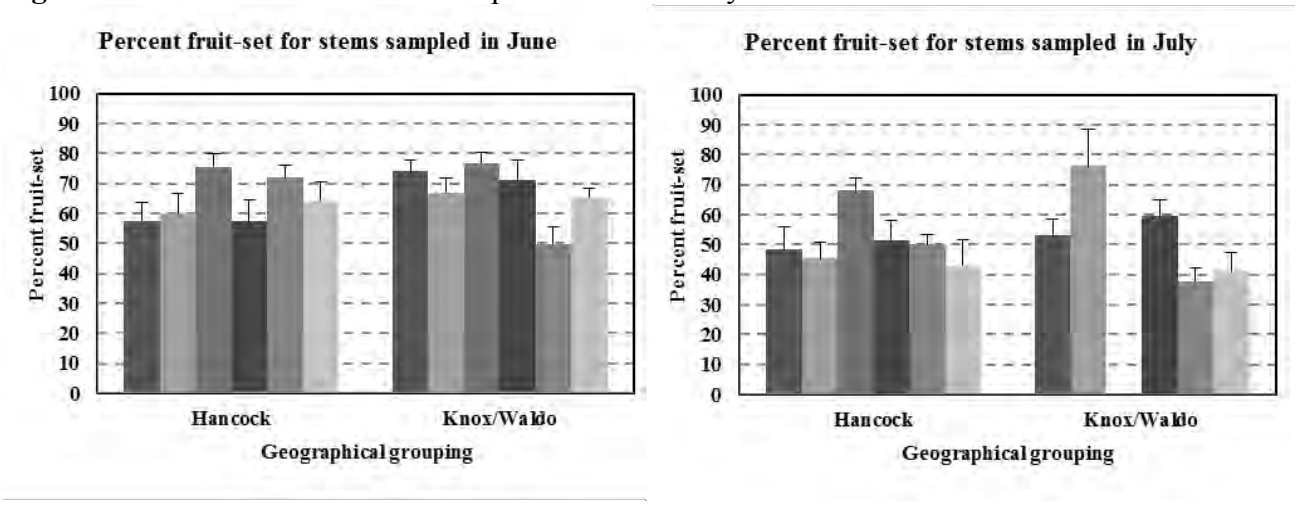


Fig. 2. Percent fruit set in fields sampled in Hancock Co. compared with fields sampled in Knox/Waldo Cos.

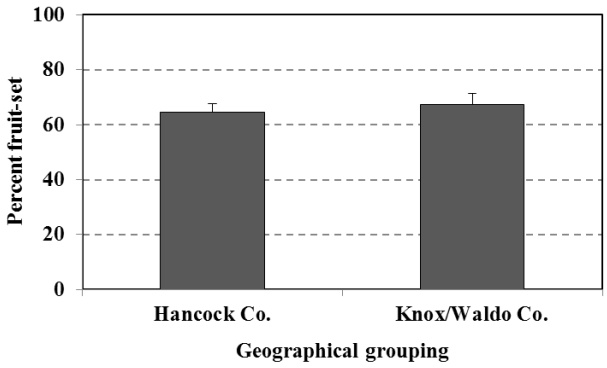
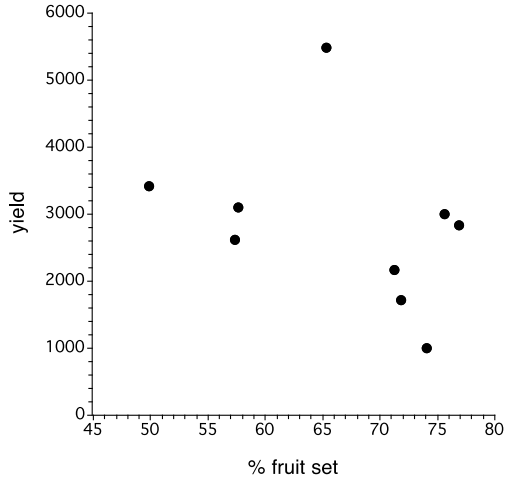


Figure 3 shows the relationship between yield and early fruit set as a measure of percent pollination. There is no significant relationship between fruit set and yield ($F_{(1,7)} = 1.031$, $P = 0.344$). This underscores the fact that much of the variation in yields in lowbush blueberries fields is NOT due to pollination. Diseases, weeds, insect pests, and management effects such as fertilizer all likely play important roles in determining yields. This phenomenon of not observing a relationship between fruit set and yield is not a frequent observation, but is a frequent occurrence.

Fig. 3. Relationship between yield and fruit-set (percent pollination).



Bee bowls of different colors were not equally attractive to the bee community. For honeybees, white bowls were the most attractive and yellow bowls were the least attractive trap ($F_{(1,2)}$ = 4.15, P = 0.02). All bowl colors were equally attractive to native bees ($F_{(1,2)}$ = 0.64, P = 0.531, Fig. 4). There were differences between the sample periods (peak vs. late bloom) for both honeybees and native bees. More honeybees were captured in bowl traps during the late bloom sample ($F_{(1,2)}$ = 8.99, P = 0.004), while native bees were more abundant in the peak bloom samples ($F_{(1,2)}$ = 7.54, P 0.008, Fig. 5).

Fig. 4. Honeybee and native bee abundance relative to bowl color.

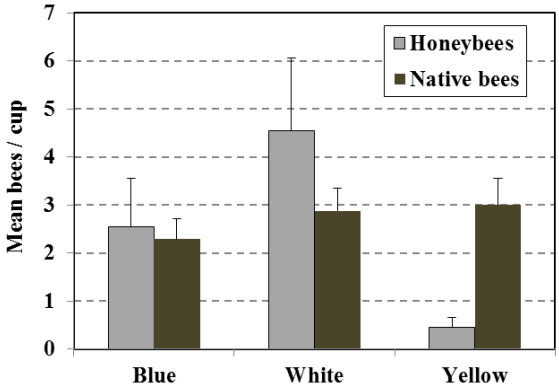
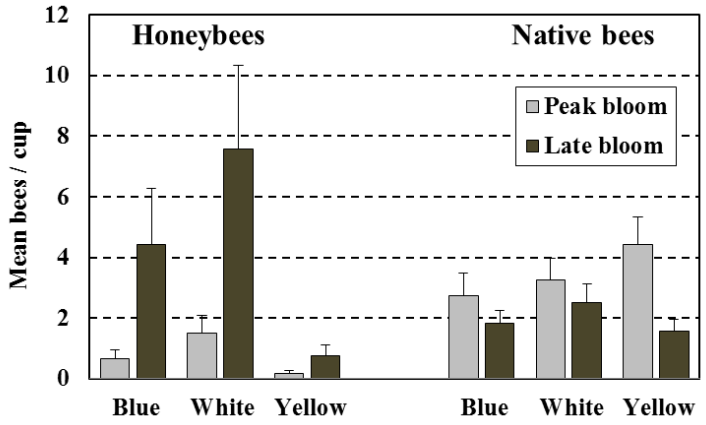


Fig. 5. Honeybee and native bee relative abundance for samples collected at peak and late bloom.



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 ($F_{(1,11)} = 0.04$, $P = 0.850$, Fig. 6). Honeybee numbers in fields did not appear to be related to the number of hives assigned to each field site (Fig. 7). This is a significant finding because it is often stated that honeybee presence can be detrimental to native bee abundance. This has not been the case historically in wild blueberry and as can be seen from Fig. 6 and Fig. 7, it was not the case in the 12 fields sampled in 2014.

Fig. 6. Relationship between numbers of honeybees and numbers of native bees captured in colored bowl traps.

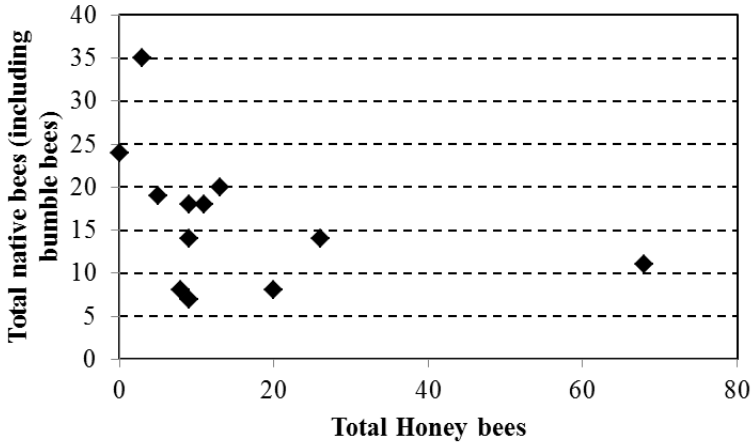
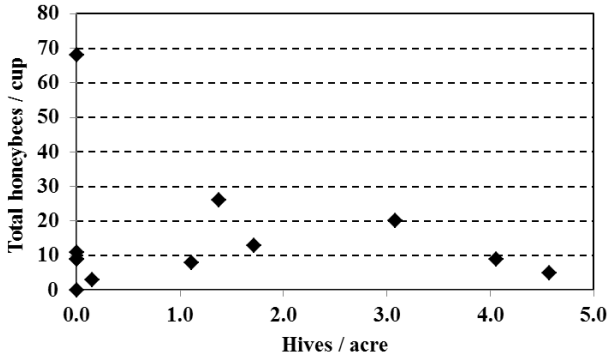
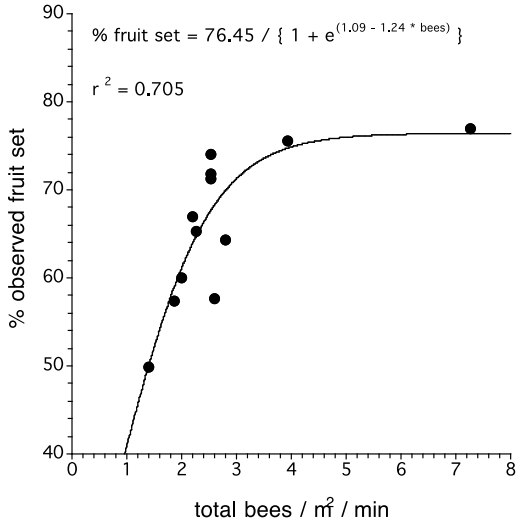


Fig. 7. Relationship between hive number at each field site and honeybee abundance from bowl trap collections. Data from both samples (peak and late) combined.



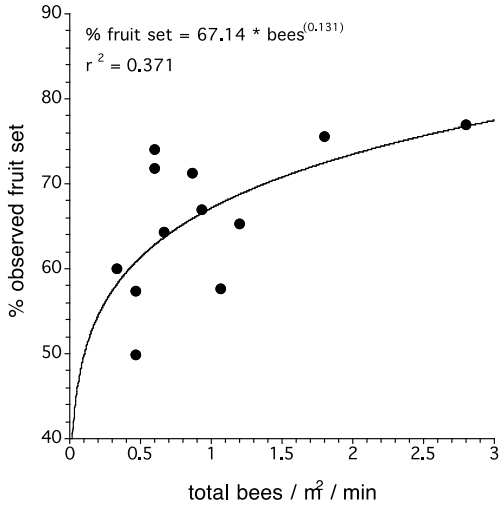
The relationship between fruit set and bee abundance (as measured by bee visitation of blueberry flowers in quadrats) was analyzed by analysis of variance. The linear relationship between bee density and fruit set was significant for early fruit set ($F_{(1,10)} = 4.766$, $P = 0.053$), but not for the late measured fruit set ($F_{(1,10)} = 1.054$, $P = 0.329$). The best predictor of early fruit set was the total number of bees foraging/m^2/min ($P = 0.019$) and the foliar Calcium content ($P = 0.014$). These two factors explained 67.6 % of the variation in fruit set among the 12 wild blueberry fields. Yield was best modeled by foliar iron (Fe) content ($P < 0.0001$), soil phosphorous content ($P < 0.001$), and foliar boron content ($P = 0.002$). As mentioned previously bee density at bloom did not predict yield in 2014. The model did explain 98% of the variation in yield among the 12 blueberry fields.
Both the seasonal density of bees (the sum of all the bees observed per m^2 per minute, (Fig. 8) throughout bloom and just at peak bloom (Fig. 9) was assessed as predictors of fruit set.

Fig. 8. Relationship between percent pollination (early fruit set) and the resulting bee densities throughout bloom in each field.



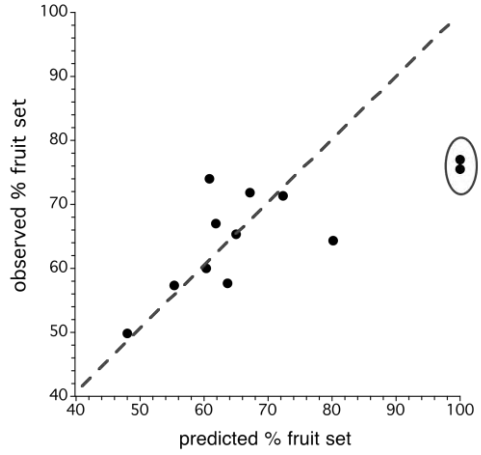
While both measures of bee density are significant ($P < 0.05$) predictors of fruit set, it can be seen that the measure of bee density throughout the season (Fig. 8) is a better predictor of fruit set than the single measure of bee density at peak bloom (Fig. 9), explaining 70.5% of the variance in fruit set for the seasonal measure vs 37.1% of the variance for bees at peak bloom.

Fig. 9. Relationship between percent pollination (early fruit set) and the resulting bee densities at peak bloom in each field.



The measured bee density can also be used to estimate fruit set using the model: % fruit set = 14.5 + 7.7 * honeybees / m^2 / min + 17.8 * native bees / m^2 / min. Figure 10 shows the relationship between the observed early fruit set and the predicted fruit set (based upon seasonal honeybee and native bee densities). Except for two fields that had the highest observed fruit set, the predictive mode shows excellent average prediction of fruit set (aligned along the 1:1 line of equivalency between observed and predicted fruit set).

Fig. 10. The relationship between observed and predicted fruit set, based upon observed bee density throughout the season. The dashed line is the 1:1 correspondence line and the circle around two fields depicts outliers that do not match well with the observed fruit set data.



CONCLUSIONS: The 2014 pollination study involving 12 wild blueberry fields showed that early fruit set was better predicted by bee density than late fruit set. Yield did not correlate well with fruit set. This demonstrates that post pollination factors such as disease or insect pests can reduce the projected yield from a high level of initial fruit set. Bee density did predict fruit set and along with phosphorous provided the best linear predictive model. A non-linear model of seasonal total bee density and early fruit set explained 70.5% of the variation in fruit set among fields.

Study 2. Influence of landscape type on bee diversity and abundance

OBJECTIVE: This study sought to quantify bee abundance and diversity across several habitat types commonly found in Maine. A spatially explicit landscape model, InVest®, was developed to predict bee diversity and abundance across real geographic landscapes. This model has been used with Maine bee data to predict bee diversity and abundance in blueberry growing regions of the state. The InVest model is currently being used in the development of a novel web-based tool for blueberry growers to visualize estimated bee abundance associated with varying land cover types surrounding their crop fields. However, the original implementation of the InVest model relied on rankings of landscapes within Maine based upon "Expert Opinion" (bee ecologists, botanists and natural history scientists). The study described here is the first year of an effort to determine if the "Expert Opinion" yields similar rankings to what might be natural rankings based upon native bee community data. Bee abundance and diversity will be assessed in eight landscape types (Table 1) over the next two years.

Table 1. Classification of landscapes.

Agriculture/field
Developed
Emergent wetlands/scrub
Wetlands/water
Commercial blueberry field
Coniferous forest
Deciduous/mixed forest edge
Deciduous/mixed forest interior

METHODS: Two different methods (bowl traps and live-netting) were utilized to study bee abundance over three sampling periods; spring (Jun), summer (Jul), and fall (Sep). Sample sites, landscape type, and sampling dates are in Table 2. Bees were sampled using colored bowl traps (blue, white, and yellow). For each sample date, there were ten replications of each color in each field for a total of 30 traps per field. The traps were arranged in groups of three bowls (one of each color) ca. 10m apart in a straight line transect. Traps were set at the top of the surrounding vegetation, either directly on the ground if vegetation was low, or when vegetation was taller, they were set on top of a urine cup, or attached to the top of a 3-ft plant stake. 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 24hrs and then traps from each site

were pooled and brought back to the laboratory where they were stored in 70% ethyl alcohol prior to pinning for future species identification.
Each hand collection consisted of 30 minutes of live-netting from flowering plants. Bees nearly caught, but escaped were recorded as honeybee, bumble bee or other native bee. Queen bumble bees were caught, identified to species and released (in spring only). For forest edge sites, collections were made within 10-15ft of either edge (into forest, or into open lawn). There was no hand collection at sites that did not have flowering plants.

Table 2. Location, landscape type, and sampling dates.

Site #	Location	Landscape type	Sample period	Collection date Hand	Bowl
1	Belmont	Blueberry Field	Summer	21-Jul	22-Jul
2	Lincolnville	Developed	Spring	11-Jun	11-Jun
	Lincolnville	Developed	Summer	21-Jul	22-Jul
	Lincolnville	Developed	Fall	8-Sep	9-Sep
3	Orono	Coniferous forest	Spring	7-Jun	7-Jun
	Orono	Coniferous forest	Summer	24-Jul	24-Jul
	Orono	Coniferous forest	Fall	24-Sep	25-Sep
4	Orono	Developed	Spring	7-Jun	7-Jun
	Orono	Developed	Summer	24-Jul	24-Jul
	Orono	Developed	Fall	24-Sep	25-Sep
5	Orono	Deciduous/mixed forest edge	Spring	7-Jun	7-Jun
	Orono	Deciduous/mixed forest edge	Summer	24-Jul	24-Jul
	Orono	Deciduous/mixed forest edge	Fall	24-Sep	25-Sep
6	Orono	Deciduous/mixed forest interior	Spring	7-Jun	7-Jun
	Orono	Deciduous/mixed forest interior	Summer	24-Jul	24-Jul
	Orono	Deciduous/mixes forest interior	Fall	24-Sep	25-Sep
7	Orono	Agriculture/field	Spring	7-Jun	7-Jun
	Orono	Agriculture/field	Summer	24-Jul	24-Jul
	Orono	Agriculture/field	Fall	24-Sep	25-Sep
8	Orono	Wetlands/water	Spring	7-Jun	7-Jun
	Orono	Wetlands/water	Summer	24-Jul	24-Jul
	Orono	Wetlands/water	Fall	24-Sep	25-Sep
9	Orono	Emergent wetlands/scrub	Spring	7-Jun	7-Jun

	Orono	Emergent wetlands/scrub	summer	21-Jul	22-Jul
	Orono	Emergent wetlands/scrub	Fall	24-Sep	25-Sep
10	Searsmont	Wetlands/water	Spring	11-Jun	11-Jun
	Searsmont	Wetlands/water	Summer	21-Jul	22-Jul
	Searsmont	Wetlands/water	Fall	8-Sep	9-Sep
11	Stock. Sprg.	Blueberry Field	Fall	24-Sep	25-Sep
	Stock. Sprg.	Blueberry Field	Summer	24-Jul	24-Jul
12	Stock. Sprg.	Deciduous/mixed forest edge	Spring	11-Jun	11-Jun
	Stock. Sprg.	Deciduous/mixed forest edge	Summer	21-Jul	22-Jul
	Stock. Sprg.	Deciduous/mixed forest edge	Fall	8-Sep	9-Sep
13	Stock. Sprg.	Deciduous/mixed forest interior	Spring	11-Jun	11-Jun
	Stock. Sprg.	Deciduous/mixed forest interior	Summer	21-Jul	22-Jul
	Stock. Sprg.	Deciduous/mixed forest interior	Fall	8-Sep	9-Sep
14	Warren	Blueberry Field	Summer	21-Jul	22-Jul
	Warren	Blueberry Field	Fall	8-Sep	9-Sep
15	Warren	Coniferous forest	Spring	11-Jun	11-Jun
	Warren	Coniferous forest	Summer	21-Jul	22-Jul
	Warren	Coniferous forest	Fall	8-Sep	9-Sep
16	Warren	Agriculture/field	Spring	11-Jun	11-Jun
	Warren	Agriculture/field	Summer	21-Jul	22-Jul
	Warren	Agriculture/field	Fall	8-Sep	9-Sep
17	Warren	Emergent wetlands/scrub	Spring	11-Jun	11-Jun
	Warren	Emergent wetlands/scrub	Summer	21-Jul	22-Jul
	Warren	Emergent wetlands/scrub	Fall	8-Sep	9-Sep

RESULTS: This is a preliminary report. Bee species are currently being identified by Dr. Sara Bushmann. For the purposes of this report, bees were identified and classified as honeybees and other bees (including bumble bees)**.** Data for average number of bees (honeybees, other bees, and all bees) from bowl trap collections and live netting was calculated for each landscape type and sample period (spring, summer, and fall). Data were further separated by general geographic region (mid-coast or central Maine). The results were given a ranking with 1 being the most bees collected and 8 the least. Table 1 is the ranking of bee abundance for all landscape types across both regions. Bowl trapping and live-netting data were combined to give one ranking of bee abundance. Over the entire season, blueberry fields, developed areas, and agricultural field edges generally had the most bees, while coniferous forests, wetlands/water, emergent wetland/scrub, and deciduous/mixed forest interiors had fewer total bees.

Table 1. Ranking of bee abundance for all sites and sample dates, combined. Number in parentheses is mean number of bees collected using bowl traps and live netting.

Landscape	Ranking (mean ± SE)
Blueberry Field	1 (1.20 ± 0.34)
Developed	2 (0.73 ± 0.22)
Agriculture/field	3 (0.47 ± 0.12)
Deciduous/mixed forest edge	4 (0.42 ± 0.06)
Emergent wetland/scrub	5 (0.31 ± 0.11)
Wetlands/water	6 (0.25 ± 0.15)
Deciduous/mixed forest interior	7 (0.19 ± 0.06)
Coniferous forest	8 (0.02 ± 0.01)

Table 2 is a comparison of the rankings between sites located in the mid-coast and central regions for all sampling periods, combined. Blueberry fields and developed areas had the most bees regardless of geographic grouping, while coniferous forest habitat was generally ranked lower in bee abundance. The differences between the geographic groupings can most likely be attributed to variation among the individual sites. Similar results were seen when the data were ranked within sampling periods (Table 3).

Table 2. Ranking of bee abundance for honeybees and other bees between mid-coast and central regions over the season. Number in parentheses is mean number of bees collected using bowl traps and live netting.

	Ranking (mean ± SE)	
Landscape	Mid-coast	Central [a]
Blueberry Field	1 (1.53 ± 0.60)	1 (0.87 ± 0.33)
Developed	2 (0.92 ± 0.44)	2 (0.54 ± 0.12)
Agriculture/field	3 (0.67 ± 0.15)	5 (0.27 ± 0.07)
Wetlands/water	4 (0.49 ± 0.24)	7 (0.01 ± 0.01)
Deciduous/mixed forest edge	5 (0.44 ± 0.07)	3 (0.39 ± 0.12)
Emergent wetland/scrub	6 (0.36 ± 0.21)	4 (0.27 ± 0.10)
Mixed Forest Interior	7 (0.12 ± 0.09)	4 (0.27 ± 0.07)
Coniferous forest	8 (0.02 ± 0.02)	6 (0.02 ± 0.01)

[a] Same ranking indicates a tie.

Table 3. Ranking of bee abundance for honeybees and other bees within each sample period. Number in parentheses is mean number of bees collected using bowl traps and live netting.

Landscape	Ranking (mean ± SE) Spring	Summer	Fall
Blueberry Field	na	1 (1.67 ± 0.47)	1 (0.73 ± 0.20)
Developed	2 (0.35 ± 0.05)	2 (1.27 ± 0.50)	2 (0.57 ± 0.13)
Agriculture/field	1 (0.37 ± 0.00)	5 (0.49 ± 0.36)	3 (0.55 ± 0.25)
Deciduous/mixed forest edge	1 (0.37 ± 0.20)	4 (0.50 ± 0.07)	4 (0.39 ± 0.05)
Wetlands/water	7 (0.02 ± 0.02)	6 (0.37 ± 0.37)	5 (0.37 ± 0.37)
Emergent wetland/scrub	4 (0.12 ± 0.02)	3 (0.62 ± 0.15)	6 (0.20 ± 0.00)
Deciduous/mixed forest interior	3 (0.22 ± 0.83)	7 (0.22 ± 0.15)	7 (0.15 ± 0.15)
Coniferous forest	5 (0.05 ± 0.17)	8 (0.00 ± 0.00)	8 (0.02 ± 0.02)

CONCLUSION: It is too early to evaluate the rankings that are based upon sampling the bee community in 2014. Additional sampling in 2015 will allow us to determine if the "Expert Opinion" of local scientists reflects the sampled data. A preliminary opinion of the author is that the data does coincide very well with the "Expert Opinion" rankings.

Study 3. Bee preferences for alternative forage resources
Report from Dr. Alison C. Dibble, Dr. Lois Berg Stack and Dr. Frank Drummond

OBJECTIVE: In the face of increasing costs for honeybee hive rental on the wild blueberry crop, native wild bees have been proposed to take on more of the pollinator services required by the crop. Growers have become increasingly interested in bee gardens (also called Pollinator Strips or Bee Forage Plots). Lists are available from which one can select plants to provide forage for bees but many of the plants have not yet been tested with repeated observations. This is a wildlife behavior study with the purpose of determining bee visitation rate upon flowers, the relative frequency of such visits, and the abundances of bees in various broad groups (e.g., honey bee, bumble bee, and others) upon the flowers.

METHODS: The 5-year experiment, started in 2012, involves four test gardens: two are at University of Maine farms (Jonesboro, Old Town), and two are at organic or low-input farms in Blue Hill. At each garden 36 one-meter square quadrats are planted each with a different herb, bedding plant, perennial, cover crop, or shrub, including native species and cultivars. Some of the plant subjects are observed every year while others are tested for only one season. To date, 70 plant species and cultivars have been included. Field data consist of a set of three, 1-min observations per plant subject in good weather when plants are in flower. Any insect that is on flowers or arrives within a timed 1-min observation period is counted and categorized to a coarse level (for bees: honeybees, orange-banded bumble bee, all other bumble bees, sweat bees, and other bees). Bee species will be documented in 2015 but insects were not captured in 2012-2014

to reduce possible impacts on the findings. The plants were measured, including aspects of floral display that might be perceived by bees such as total number of flowers, height above the ground, density of flowers in 3-dimensional space, flower diameter, and corolla tube depth. Since 2012, we have made 11449 one-minute observations. At least one bee was seen during the one-minute period in 45.2 percent of all observations, and 11563 total bees were seen across all three years. Forty percent of the total was bumble bees (not including orange-banded), 30% were honeybees, and 13% were the orange-banded bumble bee. Sweat bees accounted for 12%, and other bees (various, some are important blueberry pollinators) for 10%. Average bees per minute, within a single plant subject, ranged from 0 to 6, while the raw count of bees per minute ranged as high as 35 for butterfly milkweed. Visitation rate varied according to bee group (Fig. 1), with honeybee, bumble bee, and orange-banded bumble bee each favoring some plants more than others and no consistency across all bee groups or plant subjects.

The top bee plants include butterfly milkweed, Greek oregano, summersweet (*Clethra alnifolia*, we tested cultivar 'Hummingbird'), giant hyssop, borage (both white and blue), and bee's friend. Some easily grown cover crops such as buckwheat, some legumes (including yellow sweet clover and berseem clover), and bee's friend attracted bees. Visitation rate for short-tongue bees such as sweat bees was especially high on Greek oregano, blanket flower and tickseed. For honeybees, visitation rate was especially high on northern bush honeysuckle (Fig. 2), yet this native shrub is not much visited otherwise. Not shown: honeybee also favored giant hyssop, summersweet, and California poppy.

In a comparison of "plain" wild types versus some "fancy" cultivars with double flowers, or bright colors, or larger flowers, we found that when plant subjects are grouped with others in their wild type vs. cultivar broad categories, bumble bees tend to favor wild types, with some exceptions among the snapdragons (Fig. 2).

Fig. 1. Average bees per minute, comparison of honeybee (*Apis*), orange-banded bumble bee (*B. ternarius*), and all other bumble bees (*Bombus*) on plants that are wild at edges of blueberry fields or can be easily grown nearby.

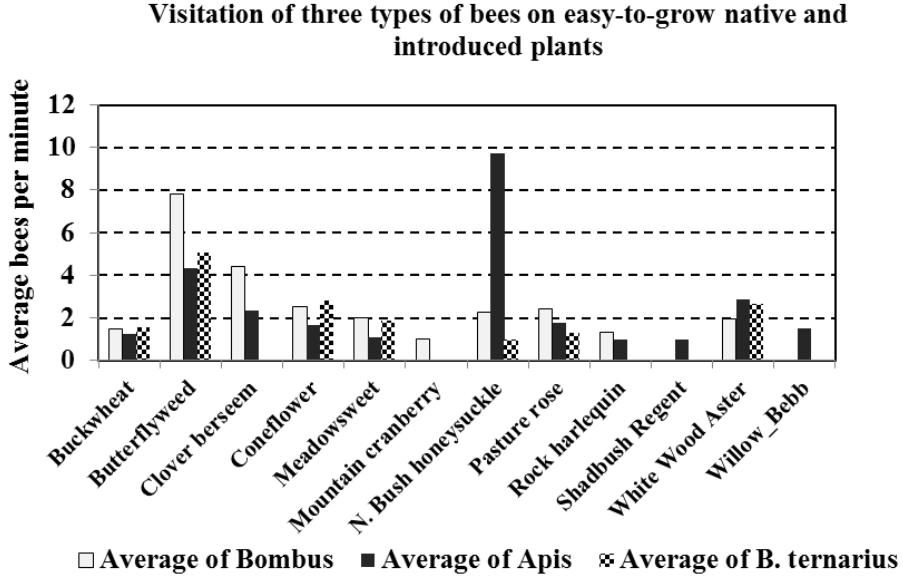
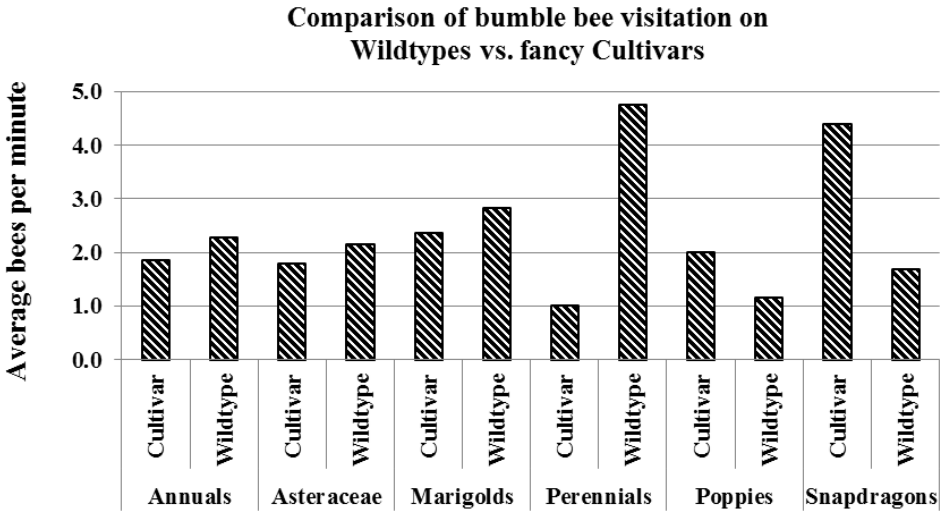


Fig. 2. Average bumble bees per minute on flowers of plants categorized as "Wildtype" or "Cultivar".



RECOMMENDATIONS: Early summaries of the 2012-2014 data indicate that if bee forage is increased at the farm, native bees are likely to find and then concentrate at the bee gardens, and honey bees will have support over a longer season. Growers interested in bee gardens will find that many easy-to-grow plants can be relied upon to improve pollinator habitat. Planting of pollinator gardens is a hedge against uncertainties of honeybee availability and will have best chance of success if the garden sites are away from spray drift or other pesticide exposure. Field edges, equipment storage areas, the headquarters area, and unproductive low ground might be considered for adding pollinator habitat. Many more details on plant selection and improving bee habitat will be available soon.

Study 4. Alternative bee forage in and around lowbush blueberry fields

OBJECTIVE: Wild blueberries are reliant on insects to pollinate the flowers in order to produce fruit. Native bees make up a major portion of blueberry pollinators, especially for many smaller farms that sometimes rely exclusively on native pollinators and do not use honeybees. We sought to quantify the types and amount of flowering plants that are available to pollinators during bloom and during early fruit set in blueberries.

METHODS: We sampled flowering plants along the edges and within twelve blueberry fields. Six fields were sampled in Hancock County and six fields were sampled in Knox/Waldo Counties. The Knox/Waldo County fields were sampled three times: once during early bloom, once during peak bloom and finally during the early fruit ripening stage. The fields in Hancock County were sampled twice, once during early bloom and once during early fruit ripening. To sample the field, we walked the perimeter of the fields and recorded the types of plants in flower. We also quantified the amount of alternative forage by estimating the total percentage of the landscape that was covered in flowering plants.

RESULTS: In Hancock County the early sample recorded seven types of flowering plants. Both the edges and interior of the fields had less than 1% of the landscape in bloom though the range varied from 0 to 5% in bloom (Table 1). The second sample recorded 21 different flowering plants along and within the blueberry fields. The average land cover in bloom was less than 1% for both within and along the field edges with a range of 0 to 10% in bloom. In Knox/Waldo Counties the first sample recorded five species of flowering plants, 14 species of plants in the second sample, and 17 species of plants in the third sample along and within the blueberry fields (Table 2). Each sample period had an average of less than 1% of the landscape in bloom for the edges and interior of the fields. Each sample date had a bloom range from 0 to 5% of the landscape in bloom.

Table 1. Hancock County flowering plants.

	Flowering Plants Sample 1	**Flowering Plants Sample 2**
Flowering Plants	Bluets, bunchberry, cherry, dandelion, forsythia, strawberry, violets	Bladder campion, bluets, buttercup, Canada toad flax, chickweed, cinquefoil, clover, cow vetch, daisy, dandelion, hawkweed, iris, lupine, maiden pink, daisy, pea, raspberry, roses, sheep sorrel, wild mustard, yarrow
Average % of Edge Landscape in Bloom	Less than 1% in bloom	Less than 1% in bloom
Average % of Field Interior Landscape in Bloom	Less than 1% in bloom	Less than 1% in bloom

Table 2. Knox/Waldo Counties flowering plants.

	Flowering Plants Sample 1	**Flowering Plants Sample 2**	**Flowering Plants Sample 3**
Flowering Plants	Allegheny serviceberry, bluet, dandelion, strawberry, violet	Bluet, bunchberry, Canadian mayflower, cherry, chickweed, dandelion, hawkweed, honeysuckle, lilac, onion, sheep laurel, strawberry, violet, wild pansy	Blackberry, bluet, bluntleaf sandwort, bunchberry, chickweed, cinquefoil, clover, daisy, dandelion, false baby's breath, grass-like starwort, dogwood, hawkweed, narrow leaf plantain, pea, sandwort, yarrow
Average % of Edge Landscape in Bloom	Less than 1% in bloom	Less than 1% in bloom	Less than 1% in bloom
Average % of Field Interior Landscape in Bloom	Less than 1% in bloom	Less than 1% in bloom	Less than 1% in bloom

Violets were the most common flowering plant species being found in all twelve of the fields we sampled followed by dandelions (10), bluets (9), bunchberry (8), clover (8), and cinquefoil (7). When plant species were assessed as a proportion of total bloom, violets were again the most prevalent followed by dandelion, clover, strawberry, bluet, and cherry (Table 3).

Table 3. Most prevalent plant species as a proportion of total bloom.

Species	Proportion of bloom
Violets	0.969
Dandelion	0.967
Clover	0.944
Strawberry	0.774
Bluets	0.757
Cherry	0.626

We also found that field edges had significantly more flowering plants then field interiors ($F_{(1,11)}$= 80.51, P = <0.0001)(Fig. 1). And, there was a significant positive correlation between species richness (number of flowering plant species) and diversity of flowering plants (Fig. 2).

Fig. 1. Comparison of species richness along field edges and within field interiors.

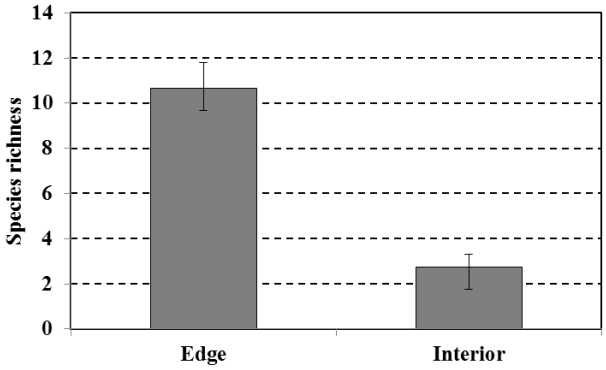
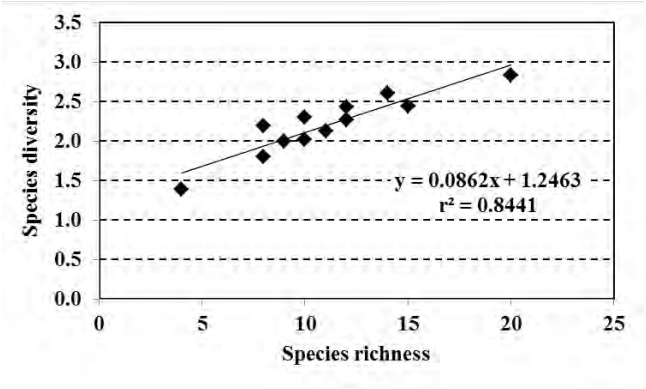
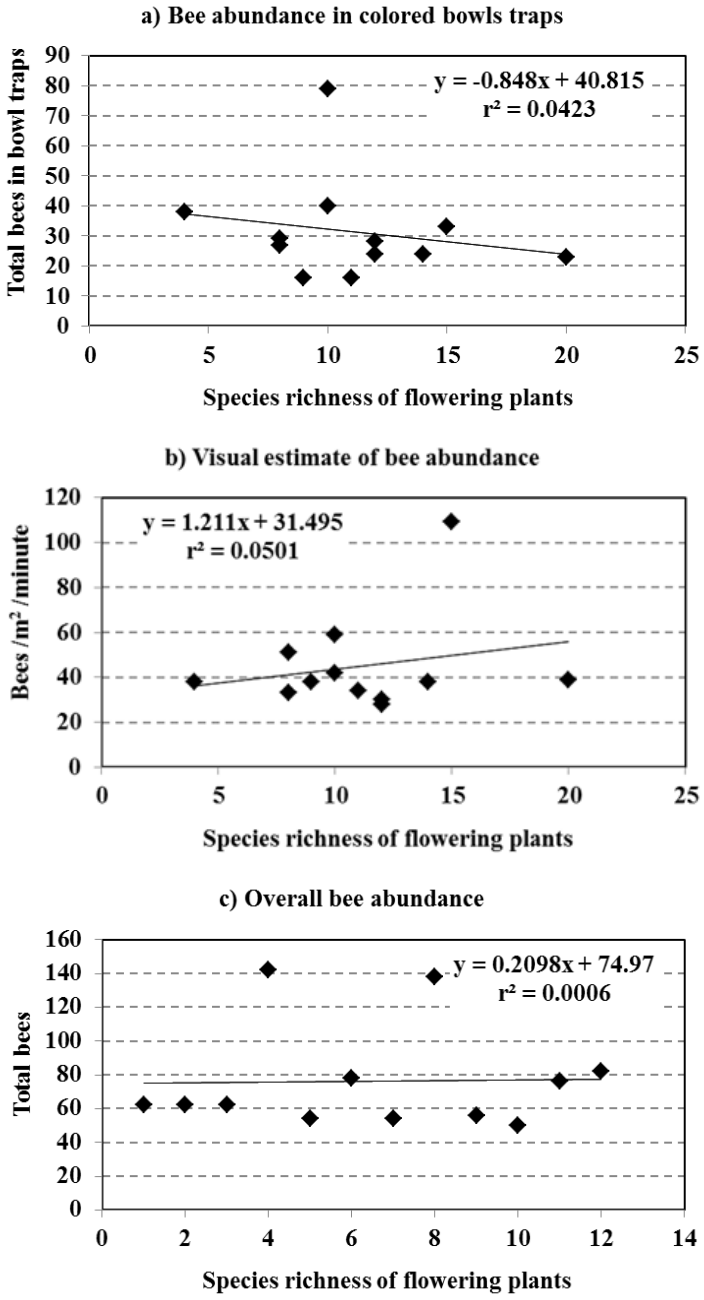


Fig. 2. Relationship between species richness and species diversity of flowering plants.



Finally, species richness was compared with bee abundance. Two different methods were utilized to study bee abundance, colored bowl traps and visual estimates. None of the relationships between floral richness or diversity and bee abundance was significant; although, the relationship between bees/m^2/min and species richness of flowering plants showed a positive trend ($P = 0.212$).

Fig. 4. Comparison of species richness of flowering plants and bee abundance as measure by a) colored bowl trap, b) visual observation, and c) both methods, combined.



CONCLUSIONS: Flowering plant diversity increased over the season with more types of flowering plants during fruit development than during blueberry bloom. This is a crucial period for bees because this is when blueberries stop flowering and they must seek out other flowering plants. Having additional flowering plants available to native bees after and even during blueberry bloom can help sustain a large, diverse native bee population. Even though there were a number of different flowering plants, they made up a small percentage of the total landscape. There were a few fields with flowering plants making up 10% of the landscape, but most field edges had considerably smaller percentages of flowering plants. The relationship between

within-field flowering plant diversity and bee abundance has been investigated previously in Maine blueberry fields (Bushmann 2013). She also did not find a strong relationship between bee abundance and flowering plant diversity or richness within a field, except for bumble bee queens, where she did find a positive significant relationship. She did find that habitat surrounding blueberry fields was a determinant of bee richness and abundance. All of these findings suggest that wild flowers in and at the edge of blueberry fields are not strong factors in affecting wild bee abundance in blueberry fields, but habitat outside blueberry fields is a determinant of abundant bee communities.

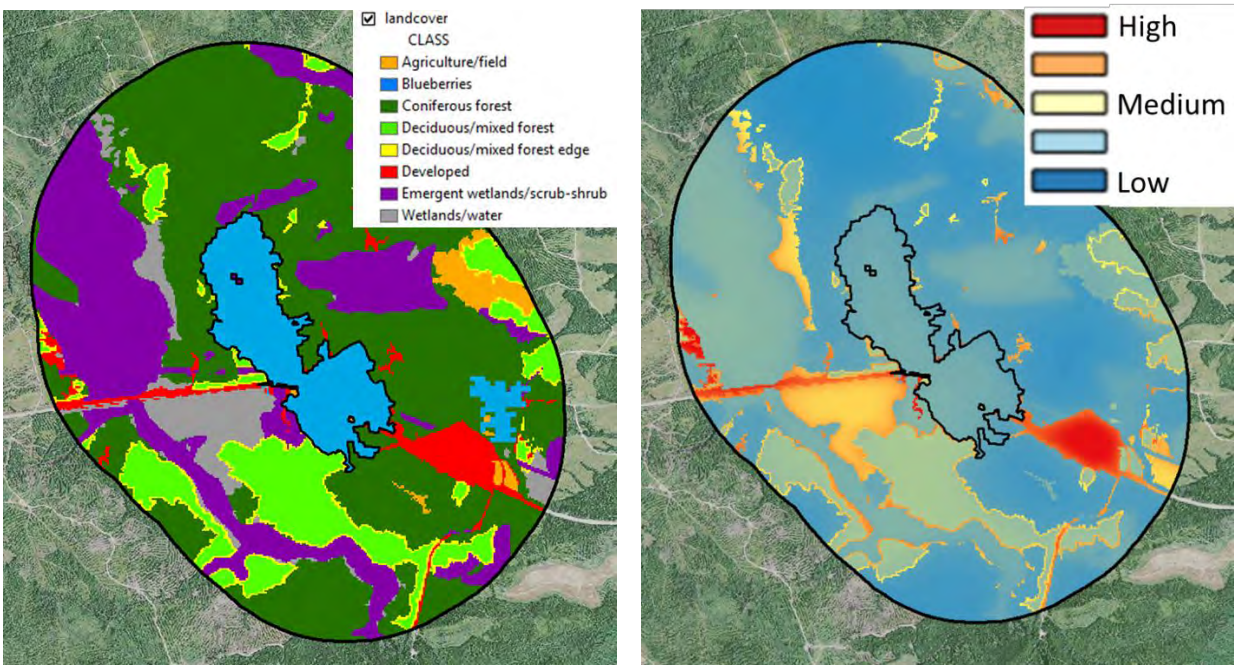
Study 5. Development of a web-based tool for grower assessment of native bee abundance in the wild blueberry production landscape
Report from Brianne Du Clos (Ph.D Student); Dr. Samuel Hanes, Department of Anthropology; Dr. Cyndy Loftin, USGS Coop Research Unit and Professor WLE; and Dr. Frank Drummond

OBJECTIVE: This study aims to share landscape-scale ecological model output with wild blueberry growers in a useful and meaningful way.

METHODS: Wild bees are an important source of pollination, and growers that contribute to wild bee conservation near their fields will benefit from increased crop pollination. We are developing a novel web-based tool for stakeholders to visualize estimated bee abundance associated with land cover in the landscape around focal crops. This tool aims to show growers where their conservation efforts are best focused at the landscape scale. Development of the web-based tool includes an iterative, participatory process that will incorporate grower feedback about the tool's design. We presented an early version of the web tool at the annual Wild Blueberry Commission Advisory Board meeting (November 2014), and anticipate three further opportunities for grower feedback in early 2015: in-depth one-on-one sessions with six key informant growers chosen for their knowledge of different growers groups, a hands-on workshop with growers identified based on their interest in bee conservation, and two workshops at Blueberry Field Schools.

RESULTS: An early version of the tool was presented at a meeting of the Wild Blueberry Commission Advisory Board in November 2014, where feedback was collected from wild blueberry growers and researchers. The tool will be accessible over the internet. Once in the tool, growers will locate their blueberry field using aerial photography and other visual navigation aids such as roads, rivers, and town boundaries. Growers will then display two circular buffers around the field representing the area from which small solitary bees or large bumblebees can reach the blueberry field in the surrounding landscape. Growers can display land cover—the types of land found around the blueberry field—and the bee abundance map within the foraging distance of large and small bees (Fig. 1). Coniferous land cover harbors bee communities with low abundance; areas around blueberry fields that are highly coniferous may benefit from efforts to enhance bee communities. Wetlands and deciduous/mixed forest edge harbor more abundant bee communities and may benefit future bee communities by being conserved. Further information on pollinator conservation practices will be linked to the web tool on the University of Maine Cooperative Extension wild blueberry web site.

Fig. 1. Landcover and bee abundance within the foraging distance of a large bumblebee around a blueberry field in the web tool.



CONCLUSIONS: We will continue to develop the web tool and seek feedback from wild blueberry growers throughout the tool development process. The final version of the tool will help growers visualize the contribution of the landscape surrounding their fields as wild bee habitat and inform their decisions about land management to enhance crop pollination as well as wild bee conservation.

Study 6. <u>Survey findings from two lowbush blueberry pollination workshops</u>
<u>Report from Kourtney K. Collum (Ph.D student) and Dr. Samuel Hanes, Department of Anthropology</u>

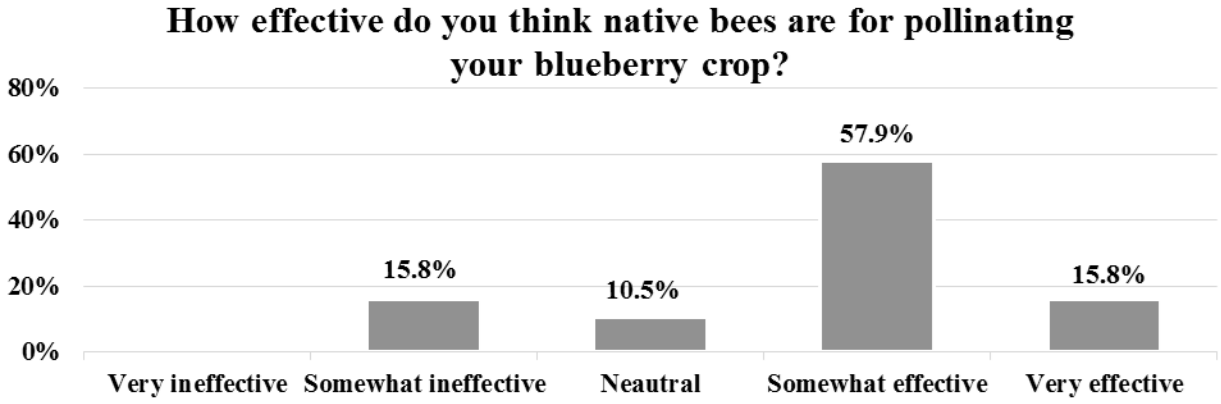
OBJECTIVE: In May and June of 2014, two free pollination workshops were offered for lowbush blueberry growers, one at Blueberry Hill Farm in Jonesboro and one at Seven Tree View Farm in Warren (funded by SARE grant #GNE13-055, Collum and Hanes co-PIs). The workshops provided information on bees' life histories, bee identification, and how to assess bee abundance and contribution to fruit set and yield. Dr. Frank Drummond conducted the workshops. Dr. Samuel Hanes and graduate student Kourtney Collum administered a survey to workshop participants to learn about their pollination practices and perceptions of native bees. Summarized below are key findings from the survey.

METHODS: Eight people attended the workshop at Blueberry Hill Farm and 18 people attended the workshop at Seven Tree View Farm. Participants included blueberry growers and representatives from agricultural agencies such as Cooperative Extension and the Natural Resources Conservation Service. In total, 19 growers completed a survey and one declined to

participate. Ninety-five percent of respondents (n=18) said they regularly attend Cooperative Extension meetings or workshops, and of those, 78% (n=14) said they attend three or more meetings or workshops per year. Given this high rate of participation at Cooperative Extension events, we classify workshop participants as potential early adopters of agricultural innovations. Thus, we assume that respondents are more likely than the average grower to use a diversity of pollination management practices and to adopt new pollination management practices in the future.

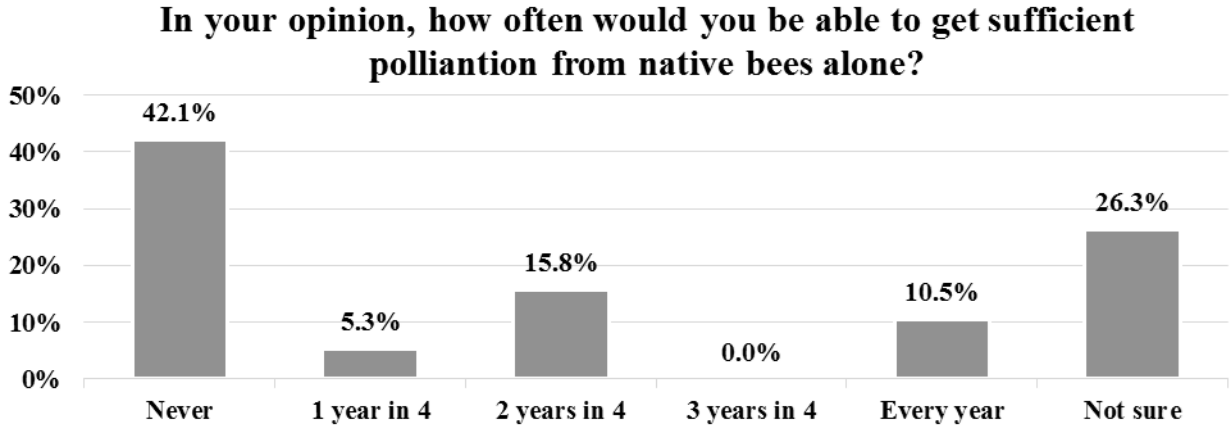
RESULTS: Respondents were asked how effective they think native bees are for pollinating their crop, on a five-point scale from *very ineffective* to *very effective* (Fig. 1). Overall, 74% of respondents said they think native bees are *somewhat effective* to *very effective*.

Fig. 1. Response to the question "How effective do you think native bees are for pollinating your crop?"



Despite positive perceptions of native bees' effectiveness, more than 40% (n=8) of respondents felt they would never be able to get sufficient pollination from native bees alone, and only 11% (n=2) felt they could get sufficient pollination from native bees alone every year (Fig. 2).

Fig. 2. Response to the question "In your opinion, how often would you be able to get sufficient pollination from native bees alone?"



The survey contained a list of pollination management practices—other than stocking commercial honeybees or bumble bees—and respondents were asked to indicate whether they regularly use each practice, whether they tried the practice in the past but discontinued it, or whether they never used the practice at all. They were also asked to indicate which practices they planned to use in 2014 (Fig. 3). The most commonly used practices were: altering pesticides to avoid harming pollinators (88.2%; n=15); avoiding mowing wildflowers to provide food for pollinators (41.2%; n=7); and leaving standing deadwood for pollinators (38.9%; n=7). More than half of respondents (53.3%; n=8) said that they planned to identify different kinds of native bees in their fields next season, and 40% (n=6) said they planned to estimate bees' contribution to fruit-set in their crops next year. These two management practices were the focus of the pollination workshops. Aside from these two practices, intention to use the pollination management practices listed on the survey was low among respondents (Fig. 3).

Fig. 3. Respondents' past, current, and planned use of nine pollination management practices.

Pollination Management Practice	Regularly Use	Tried & Discontinued	Never Used	Planned to use in 2015
Identify different kinds of native bees in my fields	38.9% (n=7)	5.6% (n=1)	55.6% (n=10)	53.3% (n=8)
Monitor the size of the native bee population in my fields in any way	5.6% (n=1)	11.1% (n=2)	83.3% (n=15)	26.7% (n=4)
Estimate bees' contribution to fruit-set in my crops	11.8% (n=2)	11.8% (n=2)	76.5% (n=13)	40% (n=6)
Use leaf cutting bee nest boxes or bumblebee nesting items	5.6% (n=1)	16.7% (n=3)	77.8% (n=14)	6.7% (n=1)
Avoid mowing wildflowers to provide food for pollinators	41.2% (n=7)	5.9% (n=1)	52.9% (n=9)	13.3% (n=2)
Plant wildflowers or bee meadows specifically for pollinators	11.8% (n=2)	0% (n=0)	88.2% (n=15)	33.3% (n=5)
Leave standing deadwood for pollinators	38.9% (n=7)	0% (n=0)	61.1% (n=11)	26.7% (n=4)
Alter pesticide application to avoid harming pollinators	88.2% (n=15)	0% (n=0)	11.8% (n=2)	N/A
Limit floral competition during bloom by cutting wildflowers or other blooming plants	17.6 (n=3)	0% (n=0)	82.4% (n=14)	20% (n=3)

Respondents were also asked to indicate how easy or difficult it would be to identify native bees, monitor the size of native bee populations, and estimate bees' contribution to fruit-set, on a scale from *very easy* to *very difficult* (Fig. 4). The high number of "*neutral*" and "*not sure*" responses suggests that some growers are uncertain about the time or skill required to implement these management practices. Furthermore, approximately 47% (n=9) of respondents indicated that estimating bees' contribution to fruit-set would be *difficult* or *very difficult*, and 42% (n=8) said the same of monitoring the size of the native bee population in their fields.

Fig. 4. Respondents' perceptions of the difficulty of identifying bees, monitoring bees, and estimating bees' contribution to fruit-set.

Pollination Management Practice	Very Easy	Easy	Neutral	Difficult	Very Difficult	Not Sure
Identifying different kinds of native bees in my field(s)	5.3% (n=1)	26.3% (n=5)	31.6% (n=6)	26.3% (n=5)	0% (n=0)	10.5% (n=2)
Monitoring the size of the native bee population in my field(s)	0% (n=0)	15.8% (n=3)	31.6% (n=6)	26.3% (n=5)	15.8% (n=3)	10.5% (n=2)
Estimating bees' contribution to fruit-set in my crop(s)	0% (n=0)	10.5% (n=2)	26.3% (n=5)	42.1% (n=8)	5.3% (n=1)	15.8% (n=3)

CONCLUSIONS/RECOMMENDATIONS: Stocking commercial bees during bloom remains the dominant pollination management strategy practiced by more than three quarters of Maine wild blueberry growers (Hanes et al. 2013, Rose et al. 2013). Findings from the workshop survey and previous research suggests that—beyond stocking commercial honeybees or bumble bees—few growers are implementing other pollination management practices, such as monitoring their bee populations or actively managing their fields to enhance wild bees. Respondents did express interest in some of the alternative practices, yet expressed uncertainty about the value of the practices and time and skill required to implement them. We conclude that further outreach and training is needed for growers who wish to implement additional practices to improve their decision-making about pollination management. Specifically, we recommend further training on: (1) monitoring native bee populations, and (2) estimating wild and commercial bees' contribution to fruit-set. We are in the process of writing a Cooperative Extension fact sheet on the workshop's contents that will include short profiles of blueberry and apple growers' who are successfully implementing alternative or diversified pollination strategies. The fact sheet will be available by spring of 2015.

Study 7. Adoption of innovation in response to crises in the wild blueberry industry, a historical study

Report from Kourtney K. Collum (Ph.D student) and Dr. Samuel Hanes, Department of Anthropology

OBJECTIVES: This study examines the history of the Maine blueberry industry's response to crisis. Crisis often drives change and innovation; the goal is to better understand how and why innovations spread in agriculture and in the wild blueberry industry in particular, so as to better understand the adoption of innovations in pollination strategies today. We do not assume there is a crisis in pollination, but rather that crisis can teach us much about the adoption of innovation in general.

METHODS: Historical resources were consulted to determine timing of and responses to crises in the wild blueberry industry. The most significant resource was the Maine Agricultural Experiment Station Bulletins. David Yarborough's anthology of wild blueberry history contained several important resources, including Clarence Day's history of the industry. Day was an Agricultural Editor for the University of Maine's College of Agriculture. David Smith's (a University of Maine historian) history of the University of Maine's Agricultural Experiment Station was an important resource on the founding of Blueberry Hill Farm.

RESULTS: Our search of historical records indicated two years we labeled as crises: 1923 and 1944. In 1923, there was a large blueberry maggot fly infestation. Other states threatened to use the U.S. Pure Food and Drug Act to ban Maine blueberries. Increasing competition made the crisis more acute. Although Maine still led the nation in blueberry production in 1923, significant cultivated blueberry production had begun in New Jersey and Michigan. Canners responded by building wire mesh tubes to wash and sort fruit, and by organizing for state inspections and labeling. Other products around the U.S. were already beginning inspection and labeling; these served as successful models for the wild blueberry industry. This strategy was effective and timely: in 1924 there was another large infestation and other states did not ban Maine blueberries (although a great deal of the crop was never marketed). The industry also responded by finding ways to reduce blueberry maggot flies in the field, including more complete burning, putting whole fields on the same cycle, and developing pesticides. These field-based practices spread more slowly but were effective as well.
In 1944 the crisis was an armyworm outbreak. Other factors also contributed to lower yields, which dropped from 17 million pounds in 1942 to 3.5 million pounds in 1944. Competition was again increasing during this time, making the crisis worse. The industry responded by asking the State Legislature to (1.) found the blueberry tax to support scientific research and to (2.) purchase an experiment station (Blueberry Hill Farm). Again, there were successful models of both taxes to support agriculture research and experiment stations. The State Legislature had purchased Highmoor Farm in 1907, with an emphasis on apple experiments, and Aroostook Farm in 1913 for potato and wheat growers in Northern Maine. The industry and Legislature's investment in research contributed to steadily increasing yields after the 1940s. Also, the industry founded a committee in 1944 to find a suitable location for the farm and to advise the Legislature on how to spend the tax to meet industry needs. This committee became the Wild Blueberry Commission of Maine. Industry trade organizations that work in concert with state and federal agencies were common at this time and so again the industry had ready models.

CONCLUSIONS/RECOMMENDATIONS: Crisis has driven major innovations in the wild blueberry industry and competition from other industries, particularly cultivated blueberry, has been important as well. The wild blueberry industry responded in 1923 and 1944 by adapting successful responses from other industries. Given this history, growers interested in innovations in pollination strategies may benefit from models of successful pollinators use and conservation in other industries and locations. Social science research is currently being carried out in Prince Edward Island, Canada looking at growers' pollination innovations in response to the province's ban on honeybee importations and these results will be communicated to Maine growers.

Study 8. <u>The health of native bumblebees in blueberry fields in Downeast Maine and the effects of dietary imidacloprid on managed B. impatiens colonies</u>
<u>Report from Kalyn Bickerman (Ph.D Student) and Dr. Frank Drummond</u>

METHODS:

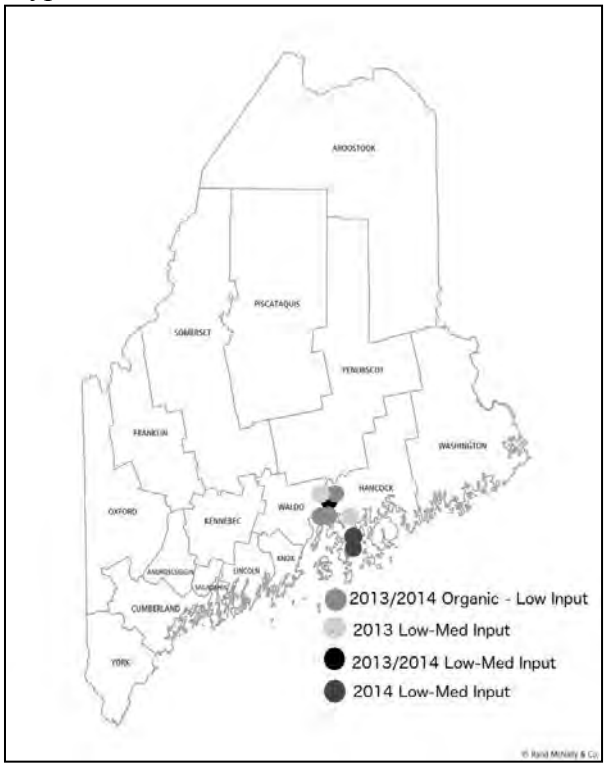
<u>Native bumblebee health</u>

From 14 Jul 2014 to 24 Sep 2014, a total of six field sites were visited intermittently throughout the season in the Mid-coast and Downeast regions of Maine (Fig. 1). Two to three researchers spent either an hour or collected 20 bumblebees at each site as a measure of sample effort. Obvious queens were not collected. 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 -20ºC freezer to freeze-kill. Each bee will be 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 will be considered to be positive if two or more pathogenic spores are seen of *Nosema bombi*.

Similar collections and dissections of native bumblebees have also been performed in 2012 and 2013.

Fig. 1. Locations of field sites of bumblebee collections and imidacloprid experiment in 2014, separated by management type. Several of these sites were also used in 2013 for collections.



The effect of dietary imidacloprid on the health and colony development of commercial Bombus impatiens

Twenty four small (~30 individuals per colony) colonies were ordered from Koppert Biological Systems (Romulus, MI) and delivered to Maine on 15 May 2014. The colonies were divided into six groups of four and each group was given a range of imidacloprid treatments as added into their only food source (a bag of Koppert "Bee Happy" food). The doses ranged from the control (0 ppb) up to 125ppb of added imidacloprid in the form of AdmirePro®. The bees were allowed to feed on the dosed food *ad libitum* 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, samples from each food bag and five individuals from each colony were collected and frozen at -20°C for chemical analysis to analyze actual dosage. Each group was placed into one of six blueberry fields around Waldo and Hancock Counties (Fig. 1), the same sites where wild bumblebee collections took place. These fields had management practices that ranged from small and organic-low input (three fields) to low-medium input (three fields). Colonies were placed > 10m apart to mitigate bees switching between colonies and were weighed once a week to monitor their growth.

Colonies were picked up from the fields on 29 and 30 July 2014 and placed into a 5.5°C cold room overnight. Final colony weights were taken and then colonies were moved to a -20°C freezer to freeze-kill. Final counts of workers, drones, and queens along with estimated of brood area were made in the following weeks. The intertegular widths of all individuals from each colony were measured to estimate average worker size of each colony. Workers will be 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.

A study of conopid fly parasitism of bumblebees

From 17 Jul to 10 Sep, approximately 20 wild *Bombus* were collected weekly from two blueberry fields, one in Waldo County with an organic-low input management type and the other in Hancock County with a low-medium input. Ten individuals of *Bombus ternarius* (the dominant species in nearly all blueberry fields in Downeast Maine) and 10 individuals of any other species were collected from each field. Bees were placed in 10 gallon glass terrariums (20" x 10" x 12") with 5cm of loose soil and fed a 1:2 sucrose solution *ad libitum*. Each enclosure contained bees from only one field and collection date.

The bees remained in the enclosures until death whereupon death site (above or below soil) and position were recorded. Bees were identified to species, measured (intertegular span, **ITS**), and the abdomen was dissected. If a conopid pupa was found, the length, width, and mass of the pupa were recorded and the pupa was placed into a urine cup of vermiculite. The presence of larvae was also recorded and the larvae were placed into vials containing 70% EtOH. Pupated conopids were kept at room temperature (≈ 21°C) until Nov 2014 then placed into a growth chamber with a photoperiod of 9**L**:15**D** (light/dark) and temperature of 12°C. Bees will then be moved to a 4°C refrigerator to "overwinter" the pupae until early summer of 2015 whereupon they will be kept at room temperature until adult emergence. Emerged individuals will be used for species identification.

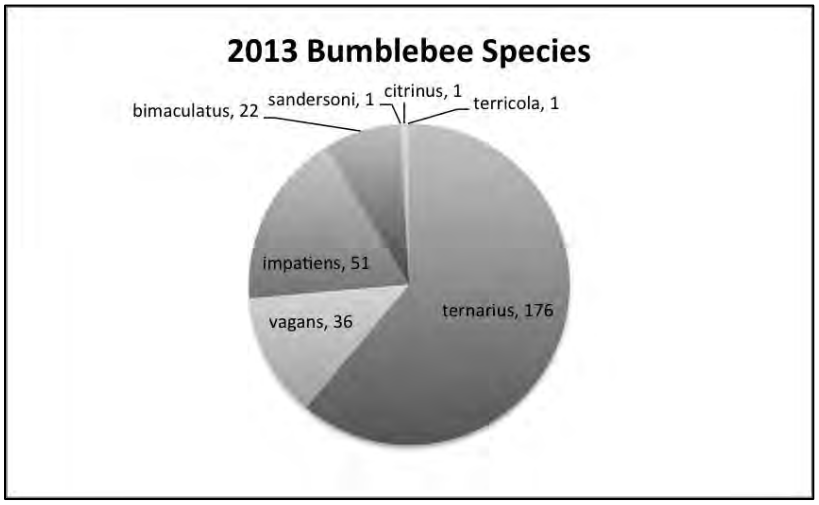
RESULTS:

Native bumblebee health

Although 2014 collection specimens have yet to be processed, dissections have been performed on 288 bumblebees collected from 19 field sites in 2013. Although these samples are continuing to be processed, over 60% of the bees collected and processed have been *B. ternarius* thus far (Fig. 2).

One hundred sixty five of these samples have been visually analyzed for the microsporidian pathogen *Nosema bombi* and two have been positive: one *B. vagans* and one *B. ternarius*, both collected during the month of July. Eight bees spanning collection dates through August and September have presented with conopid larvae in their abdomens. The results of 2013's and 2014's specimen dissections will be compared to those in 2012 to 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.

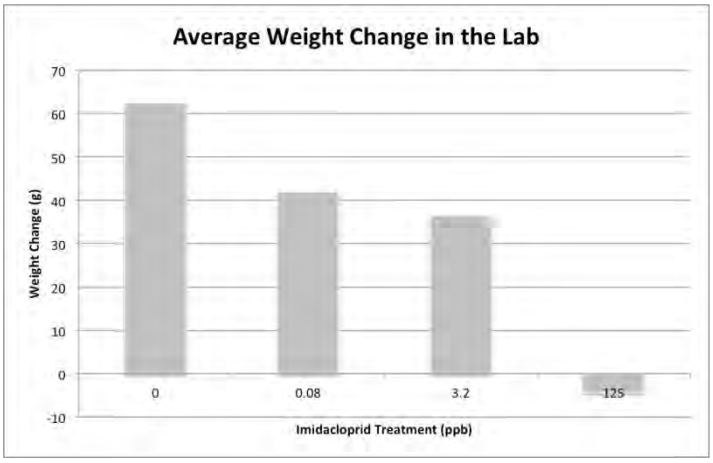
Fig. 2. Breakdown by species of 2013 specimens dissected to date.



The effect of dietary imidacloprid on the health and colony development of commercial Bombus impatiens

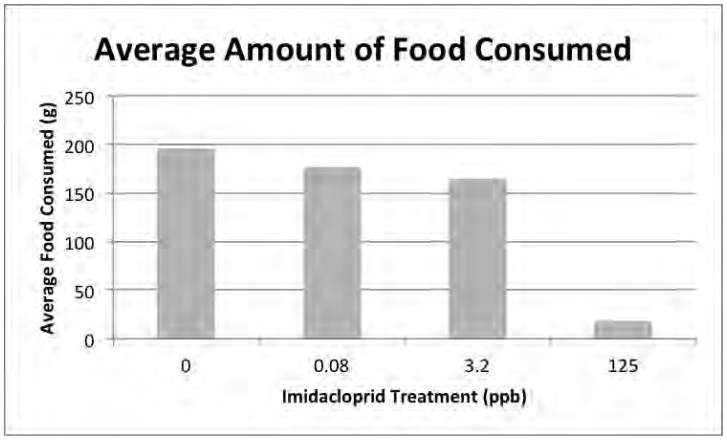
There was a significant effect of the imidacloprid treatment on the weight gain or loss of the colonies in the lab ($P < 0.00$) (Fig. 3). The higher the amount of imidacloprid each colony was given, the less weight the colony gained during the two weeks in the lab. All of the colonies given 125 ppb imidacloprid lost weight during that time.

Fig. 3. Average colony weight gain by dosage (ppb of imidacloprid) group through the season. There was a significant effect of treatment group on colony weight change in the lab during the dosing period ($P < 0.0001$).



However, the above figure only takes into account the amount of imidacloprid in the food and not the actual amount of imidacloprid the bees were consuming. During the dosing period, a feeding aversion was observed that resulted in the colonies with the higher given doses of imidacloprid consuming significantly less of the food ($P < 0.0001$) (Fig. 4).

Fig. 4. Average amount of food consumed by colonies during the dosing period. There was a significant effect of treatment (ppb) on the amount of food consumed by each colony ($P < 0.0001$).



Therefore, it was necessary to calculate an "index dose" of imidacloprid consumed per colony using the amount of food consumed and the dose to get a more accurate representation of the amount of imidacloprid consumed per colony (Fig. 5). A logarithmic curve best fits the data ($r^2 = 0.855$), indicating that the higher the imidacloprid concentration in the feed, the less food the bees would eat. From this calculation, we replaced the treatment (ppb) with this index dose for each individual colony. For example, using the calculated index dose in place of the given dose

for the weight difference of the colonies in the lab gives a significant effect of index dose on the weight difference at the end of the two-week dosing period (Fig. 6).

Fig. 5. Calculated "index dose" based on initial imidacloprid concentration in the food and food consumption over the two-week dosing period.

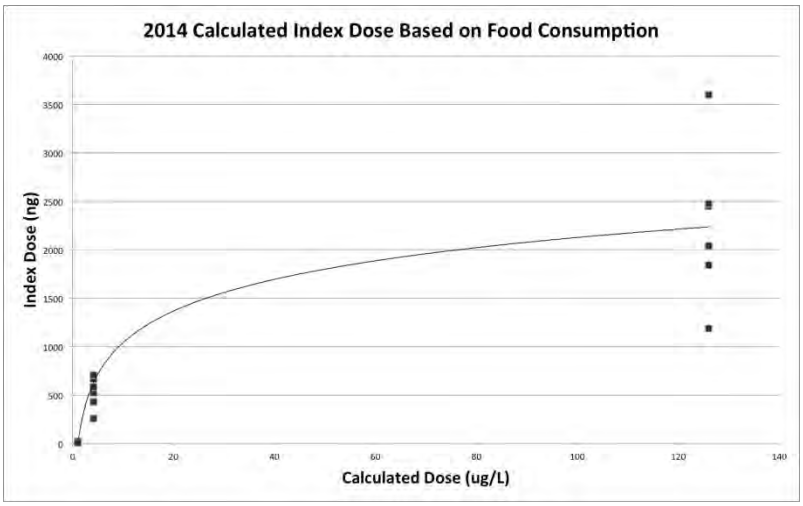
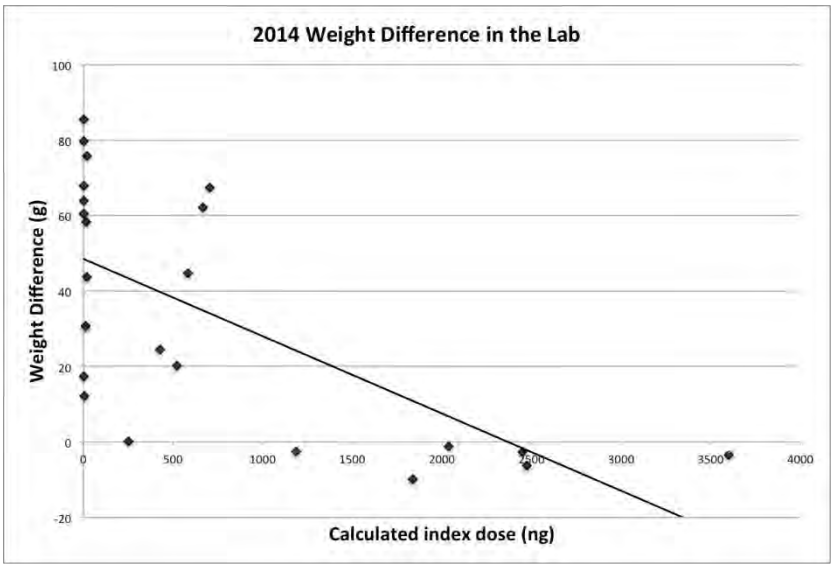


Fig. 6. The significant effect of index dose on the weight change of each colony in the lab ($P < 0.0004$).



Measurements of the ITS of workers of each colony taken at the end of the season showed no significant effect of either field management or index dose on final worker size (Fig. 7). Numbers of bees (drones and queens included) counted at the end of the season in each colony also display a downward linear trend with increasing index dose (Fig. 8), with a nearly significant effect of index dose on bee number ($P = 0.10$). Index dose had a marginally

significant effect ($P = 0.073$) on brood weight taken at the end of the season, but interestingly field management had a significant effect ($P = 0.036$) (Fig. 9) where the low-med input fields produced colonies with more brood mass than the org-low input fields.

Fig. 7. The effect of index dose on each colony on average worker size at the end of the season. Although there was no significant effect of index dose ($P = 0.64$) or field management ($P = 0.44$) on final worker size, a slight downward linear trend can be observed.

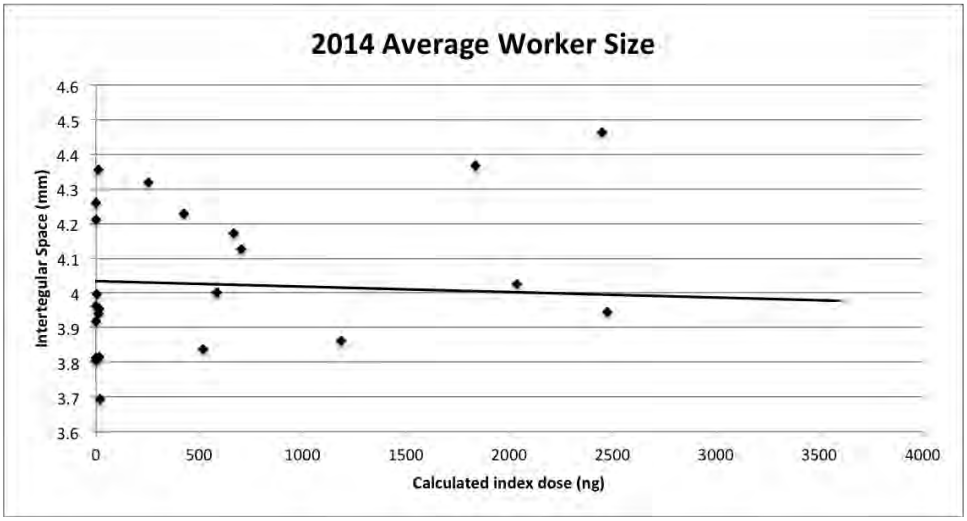


Fig. 8. The relationship between index dose and number of bees in each colony at the end of the season. There was no significant effect of management type on the number of bees ($P = 0.43$), but there was a near significant effect of index dose ($P = 0.10$).

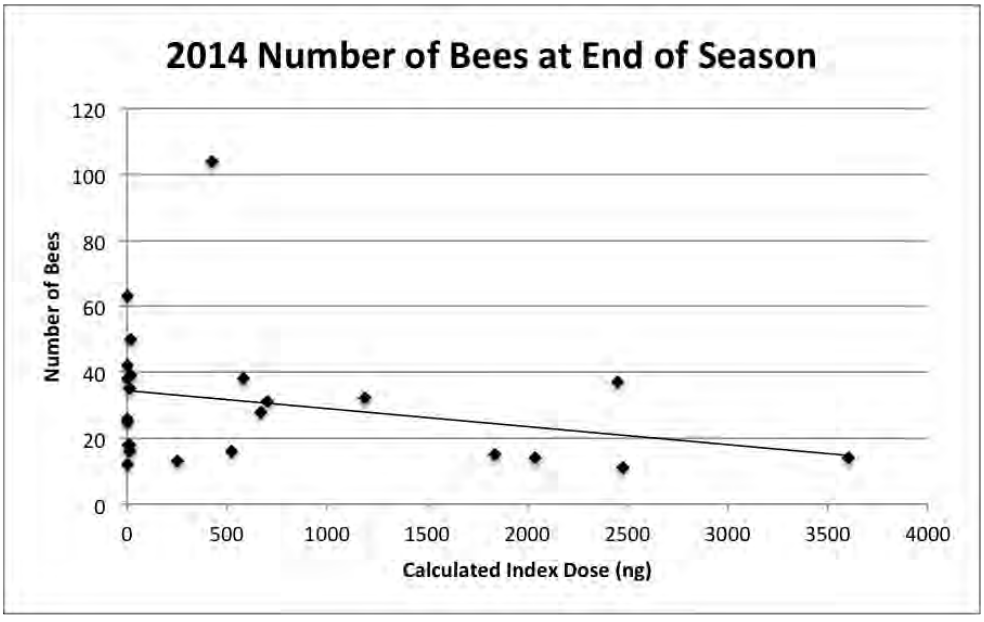
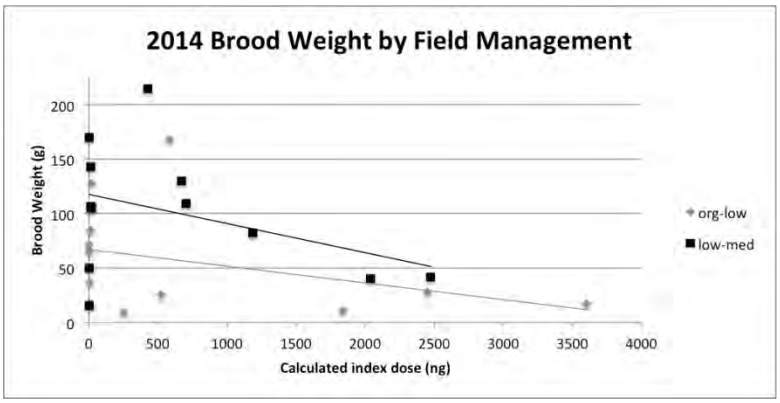


Fig. 9. The effect of index dose ($P = 0.073$) and field management type ($P = 0.036$) on brood mass at the end of the season.



A study of conopid fly parasitism of bumblebees

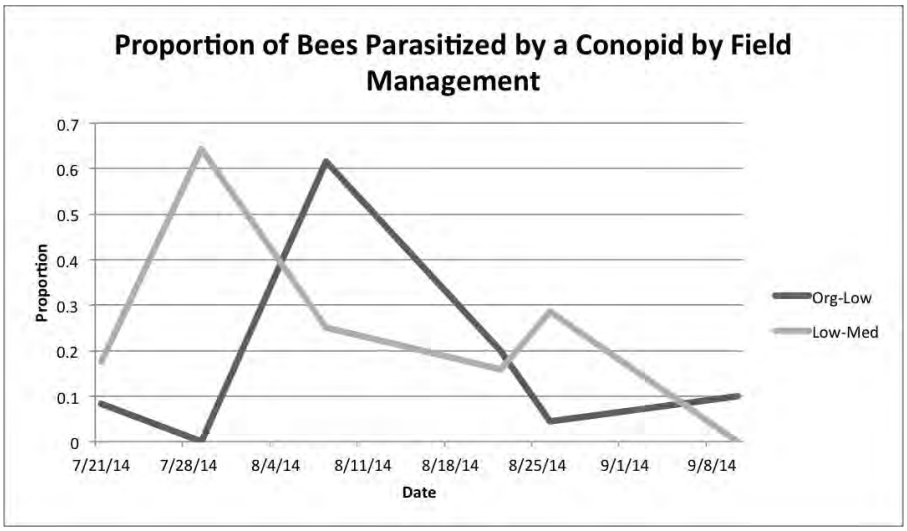
A total of 210 bees were dissected for conopid pupae in the summer and fall of 2014. Of these, 40 were observed to have conopid pupae (19%) and 4 (1.9%) were found to have unpupated larvae in their abdomens where the bee had died before pupation was able to occur (Table 1). Four of the conopid pupae were broken during dissection and preserved in 70% EtOH and the remaining 36 were kept for the overwintering protocol. Although approximately 20 bees were captured each week, not all bees were dissected due to not being able to locate them in the terrarium.

Table 1. Collection dates of bees and conopid prevalence.

FIELD MANAGEMENT	DATE	#BEES DISSECTED	#CONOPIDS	PREVALENCE
Org-Low	7/17/14	12	1	8.3%
Low-Med	7/21/14	18	3	16.7%
Org-Low	7/29/14	16	0	0%
Low-Med	7/29/14	14	9	64.3%
Org-Low	8/8/14	14	8	61.5%
Low-Med	8/8/14	28	7	25%
Org-Low	8/22/14	20	4	20%
Low-Med	8/22/14	19	3	15.8%
Org-Low	8/26/14	23	1	4.35%
Low-Med	8/26/14	21	6	28.6%
Org-Low	9/10/14	20	2	10%
Low-Med	9/10/14	5	0	0%

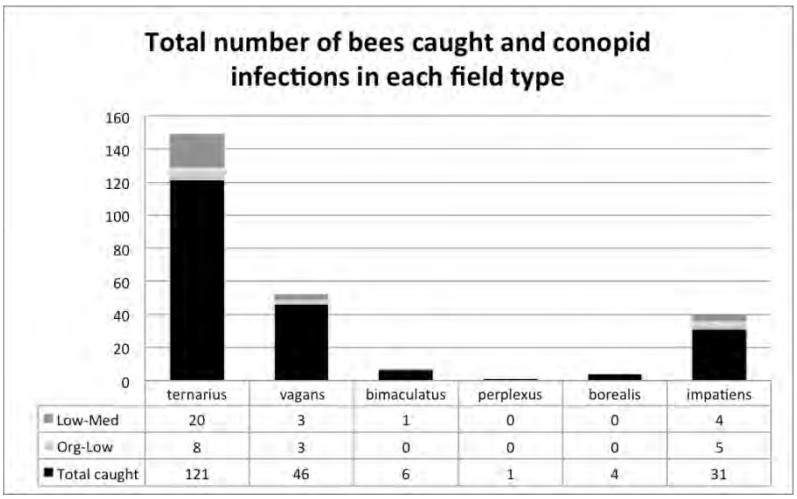
Both the org-low input field and the low-med appeared to have peaks in the proportion of parasitized bees from late Jul through mid-Aug, with rates tapering off after that point (Fig. 10). The peak for the org-low field seems to be a couple weeks after the peak for the low-med fields, which may be an indicator of conopid phenology differences by geographical location; although, with only two fields to compare this cannot be said definitively. The org-low field is located approximately 18.5km from the low-med field as the crow flies.

Fig. 10. The proportion of bees dissected that were parasitized by conopids by field management type and date. Peak for conopid activity appears to be in late July through mid-August.



In total, six different species were captured during the duration of the experiment (Fig. 11) with 105 bees dissected from the org-low field and 105 from the low-med field. Sixteen bees from the org-low field had a conopid larva or pupa (15.24%) and 28 bees from the low-med field (26.67%) presented with a conopid.

Fig. 11. The total numbers of each species caught and the numbers of each species presenting with a conopid pupa from each field type.



	ternarius	vagans	bimaculatus	perplexus	borealis	impatiens
Low-Med	20	3	1	0	0	4
Org-Low	8	3	0	0	0	5
Total caught	121	46	6	1	4	31

Death location recorded for each bee, above or below the soil, was found to be predominately above the soil. 16.67% of all bees were found to be buried below the soil, but when the bees are separated by conopid infection status, it appears that parasitized bees are over twice as likely to be found below the soil surface as non-parasitized bees (29.41% vs. 13.70%; Fig. 12, Table 2). This finding supports what previous studies have suggested: conopid parasites may be able to manipulate their bumble bee hosts into demonstrating a "burying" behavior, which could prove advantageous to the overwintering conopid pupa.

Fig. 12. Percentage of parasitized and non-parasitized bees that were found above or below the soil surface. Numbers above bars indicate number of individuals in each category.

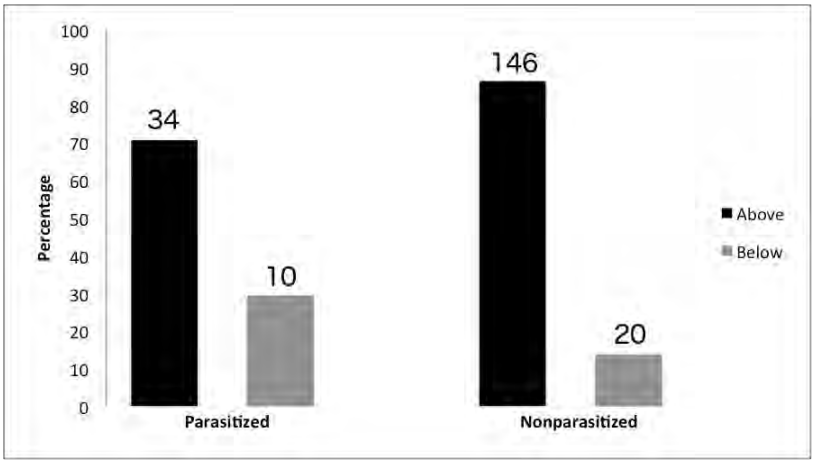


Table 2. The death locations of all bees, parasitized bees, and non-parasitized bees.

Location	All bees	Parasitized bees	Non-parasitized bees
Above soil	180	34	146
Below soil	30	10	20
Percent below soil	16.67%	29.41%	13.70%

CONCLUSIONS: Imidacloprid has been observed to affect bumble bees in our studies. Low levels are not obviously harmful, but high doses do impact bumble bees. Based upon these findings we recommend that growers minimize exposure of bumble bees to neonicotinoids, especially imidacloprid. Future directions in our research include evaluating the possibility that treatment level affects susceptibility to parasites and pathogens through dissection and analyzing the immune strength of individuals from each treatment to determine if their immunological systems are altered by imidacloprid exposure.
Naturally occurring pathogens and parasites also negatively affect native bees. Our research with the bumble bee shows that pathogens can be as, or more, devastating than exposure to pesticides. Future directions in our research include comparing conopid infection differences between species and between age (wing wear) and size (ITS) of bumblebees to investigate individual differences that may render an individual bee more susceptible to conopid fly attack.

Study 9. The status and health of migratory honeybee colonies brought to the Maine blueberry barrens in 2014

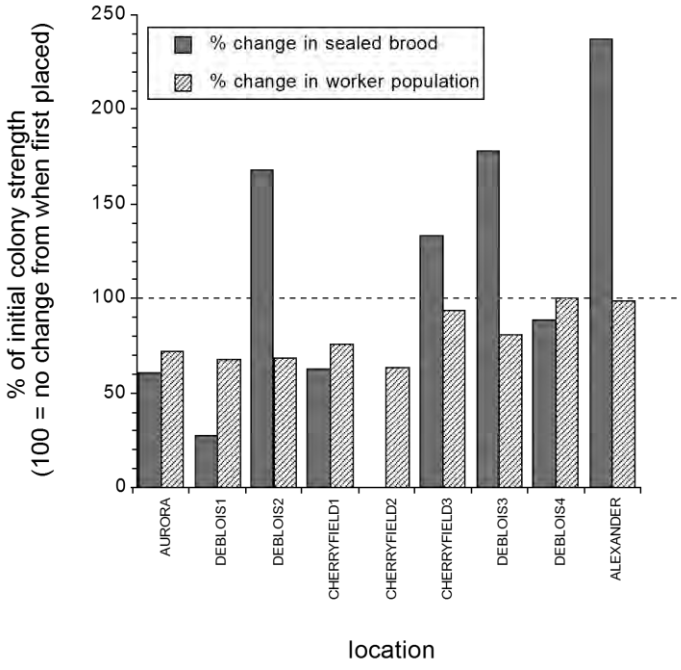
OBJECTIVE: Since 2006 honeybee colony health has been marginalized and colony losses are at an all-time high, averaging 30-40% per year. Causes for the decline in colony health and survival have been attributed to many factors such as pathogens, mite parasitism, exposure to pesticides, low genetic diversity, poor nutrition, and stress during movement. Honeybee colony health has not been documented in the Maine wild blueberry crop, which brings approximately 75,000 colonies to Maine during bloom between mid-May and mid-June. This study documents a 3-week study of colony health at nine locations in Downeast Maine during bloom in 2014.

METHODS: Between 18 and 22 May, 2014; nine groups or drops of hives were identified for a colony health survey. These groups or clusters of hives ranged from 60 to 200 hives at a single drop. The hive drops that were sampled were located in the towns of Aurora, Alexander, unorganized township T-22, Deblois, and Cherryfield, ME. Sampling started on 22 May during early bloom, just a few days after the colonies had been placed on the blueberry fields. At each drop, three random colonies were marked for sampling during the bloom period. An early bloom sampling of the colonies was conducted early in the morning before significant foraging occurred. I measured and recorded the number of hive bodies and supers and their respective sizes, presence and status of a laying queen (eggs and uncapped brood present), if re-queening was underway, if feeding syrup and/or pollen patties was being provided, population size of

workers and sealed brood (% of comb area covered with workers or sealed brood), and presence of any disease or mite parasite symptoms if observed. Wax comb samples were taken from the brood rearing area. At peak bloom (3 – 5 June) pollen traps were attached to each of the sampled colonies for two days. The pollen was collected and stored in the freezer at the University of Maine until it was sent out for pesticide analysis. The pollen was sent to Dr. Brian Eitzer at the Connecticut Agricultural Experiment Station, New Haven, CT. A second sampling was conducted 11-13 June just prior to the end of bloom. This late sampling included the measures mentioned for the early sampling, but in addition, wax comb was sampled from the brood area, 200 young nurse workers were collected for pesticide analysis from each hive and 200 older foragers were collected from each hive for pathogen detection and quantification of *Varroa* mite (mites / 100 workers) and tracheal mite infestation (dissection of 50 foragers) in the laboratory at the University of Maine. Pathogen detection (6 viruses: Deformed wing (**DWV**), Black queen cell (**BQCV**), Sacbrood (**SBV**), Israeli acute (**IAPV**), Kashmir (**KBV**), and Chronic (**CBV**); new trypanosome pathogen, and fungus: *Nosema ceranae*) was based upon the use of molecular markers (100 foragers from each hive) that were processed using quantitative polymerase chain reaction (**PCR**) by Dr. Jay Evans at the USDA Bee pathology Lab in Beltsville, MD. *This report is an interim report and does not include the pesticide analysis. As soon as the pesticide analysis is received, I will send out an updated report.*

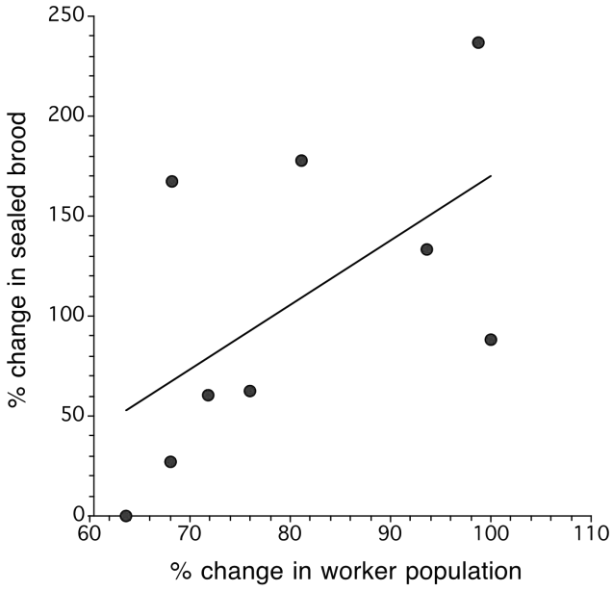
RESULTS: The colonies sampled in 2014 all represented migratory colonies with origins from outside the state of Maine. At the start of bloom, all of the hive drops, except one, provided the honeybee colonies with both syrup and pollen patties. Three of the hive drops had colonies that were not re-queened during bloom, while the other six drops re-queened at least some of the colonies within a group of colonies. Only 11% (3 out of 27) of the colonies sampled lacked brood, most had at least some brood, either uncapped or capped. The health of colonies was determined by assessing the percent change from the beginning of bloom until the end of bloom for capped brood and worker populations. Figure 1 depicts the rate of growth or decline of each of the nine hive drops. This figure represents no change throughout the bloom period at a rate of 100%. Hive locations less than 100% for either capped brood or workers experienced a decline in population; whereas, any hive locations greater than 100% experienced a population increase. When looking at sealed or capped brood, only four locations (averaged over the three colonies sampled at each location) experienced increased colony populations: Deblois-2, Deblois-3, Cherryfield-3, and Alexander. Deblois-4 experienced an essentially unchanged sealed brood population. The locations that had stable maintenance in worker bee populations were: Cherryfield-3, Deblois-3, Deblois-4, and Alexander.

Fig. 1. Colony health measures reflecting the rate of change in sealed brood and worker populations from the beginning of blueberry bloom until the end of bloom, 2014.



There is a significant correlation between worker population and sealed brood populations in 2014. Figure 2 shows this relationship. This relationship suggests that colony health is constituted by both strong sealed brood and strong worker populations. However, sealed brood population rate of change is much more variable than worker rate of change.

Fig. 2. The relationship between the rates of change of workers and sealed brood at the nine hive locations sampled in 2014.



The coefficient of variation is a measure of this variation standardized by the average of a measure. In 2014, the coefficient of variation for the worker populations was 17.5%, while for the sealed brood the coefficient of variation was 73.5%. This suggests that factors affecting the brood more than the workers might be operating during the period of bloom; although, it is well recognized that the development time of sealed brood is much shorter than the average longevity of workers. This discrepancy in the "residence" time of the two life stages results is a problem in trying to assess what life stage is suffering more during bloom when using sequential sampling as a methodology.

One hypothesis is that a decline in colony health in blueberry during bloom in 2014 could be due to pathogens and mite parasites. It would be expected that these agents would have been picked up by the colonies well before coming to Maine. Figure 3 shows the level of pathogens per colony, averaged to yield a location mean. The trypanosome, SBV, DWV, and *Nosema ceranae* made up the most prevalent of the viruses. Chronic virus, Israeli acute paralysis virus, and Kashmir Virus, were uncommon detections. Tracheal mites were detected at low levels across most of the locations. *Varroa* mite infestations demonstrated high variation among hive drops with some drops having no detectable *Varroa* mite and other drops having mite infestation levels above the treatment threshold of 8 mites / 100 honeybee workers. Figure 4 depicts the parasitic *Varroa* mite levels among the hive drops.

Fig. 3. Pathogen incidence for the nine sampled blueberry field locations in 2014.

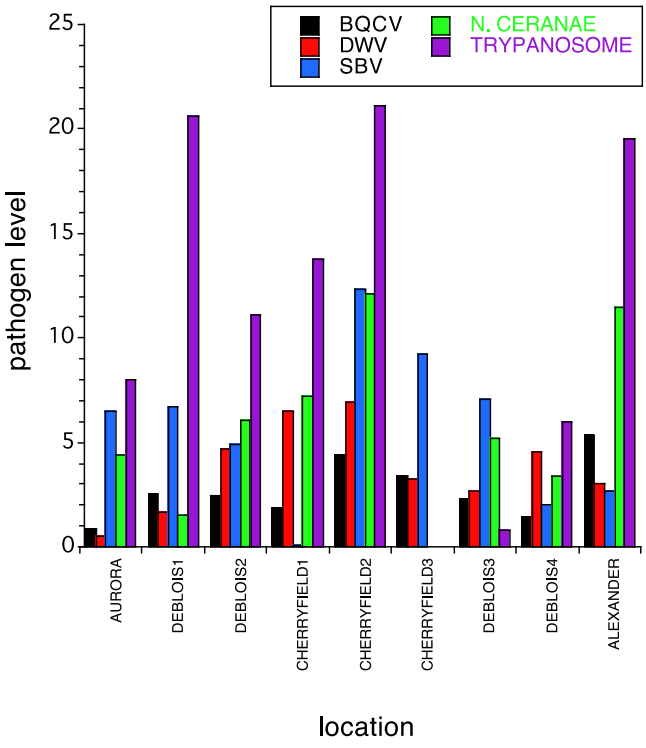
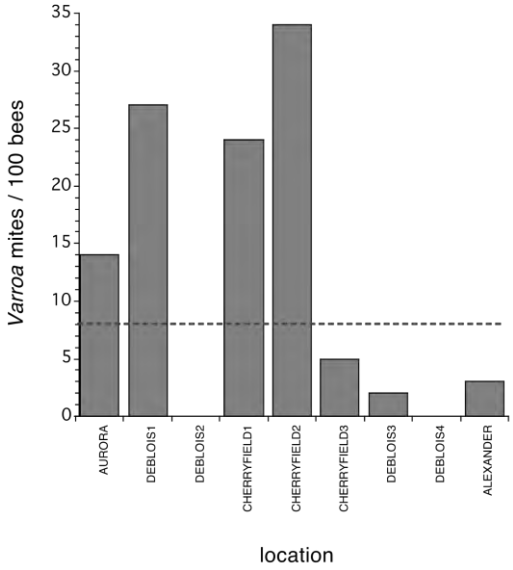
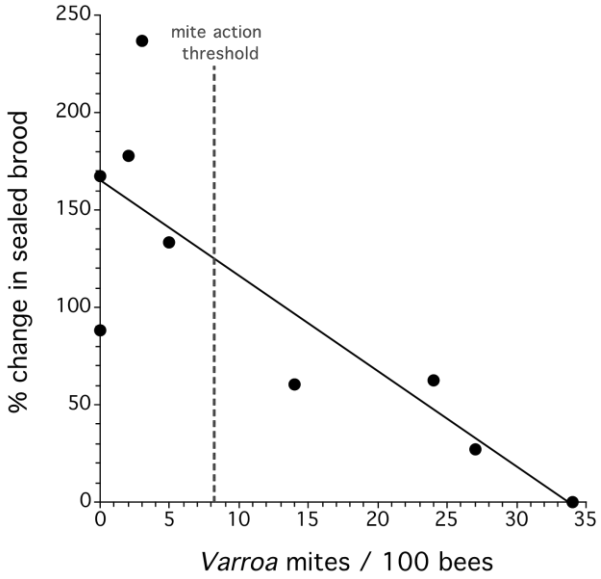


Fig. 4. *Varroa* (mites / 100 bees) mite infestations at nine sampled locations in 2014. Dashed line is action threshold for *Varroa* mite.



Stepwise linear regression was used to determine the relationship between all of the pathogens and the parasitic mites and colony health measures. Only *Varroa* mite was found to be a potential causative effect of the decline in health across the nine sites sampled. *Varroa* mite was negatively related to honeybee colony health ($P < 0.05$), explaining 68% and 44% of the variation in sealed brood rate of change and worker rate of change, respectively. It can be difficult to determine the causal agent when several factors might be correlated. Figure 5 depicts the negative relationship between *Varroa* mite and the rate of change in sealed brood. It is remarkable that with such a small sample size, the action threshold for *Varroa* mites appears to separate the colonies with a positive rate of change and those colonies that declined during bloom. The sampling shows moderate levels of mites in four hive drops, even though it appeared that all beekeepers were treating for *Varroa* mite. This is alarming and suggests that *Varroa* mite is still a major concern during the early spring pollination season.

Fig. 5. Relationship between *Varroa* mite sampling and sealed brood population percent rate of change during blueberry bloom in 2014.



CONCLUSIONS: This preliminary study into the health of honeybee colonies brought to Maine wild blueberry will have to be updated as soon as the pesticide analysis of honeybee foragers, pollen, and wax is complete. However, a preliminary conclusion is that 4 of the 9 sites sampled were characterized, on average, by colonies that declined in either sealed brood or worker populations over the three-week pollination period. Only 3 of the 27 colonies sampled lost their queens, despite re-queening efforts. Pathogen levels are closely related to *Varroa* mite infestations. A multivariate analysis of all the pathogens and the mite parasites only provided evidence for one causal factor of the honeybee colony decline. The factor was *Varroa* mite. This was surprising knowing that *Varroa* mite management is a primary focus of beekeepers; although, high levels of control of *Varroa* mite can be difficult to obtain, even with effective miticides. It has been shown that additional stressors such as pesticide exposure can affect colony susceptibility to pathogens and parasitic mites. An analysis of the pesticide exposure data may shed light on additional impacts once this data is received.

Study 10. Effectiveness of a joint pollination strategy, honeybees and bumble bees in the same blueberry field

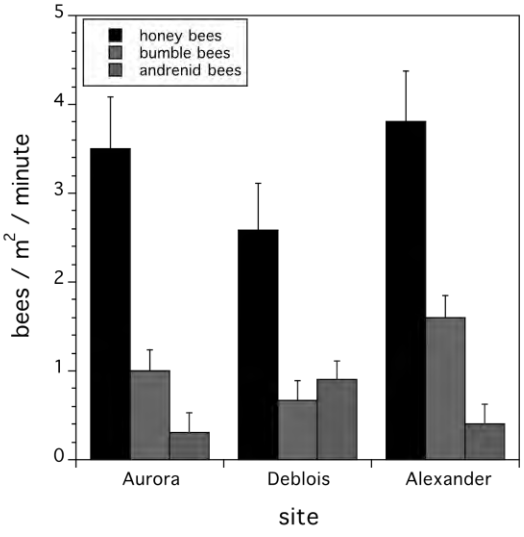
OBJECTIVE: Bumble bees have been used for pollination by wild blueberry growers since 1997. Many growers have used bumble bees either as the sole commercial bee in a field or in combination with honeybees. Studies by Stubbs and Drummond (2000, 2001) and Drummond (2012) have shown them to be effective, but at the same time colony strength of purchased bumble bee colonies has been observed to be highly variable from field to field and year to year. In addition, Drummond et al. (2001) caution about the aggressive nature (robbing of nectar and pollen) of honeybee colonies toward bumble bee colonies under certain conditions (low nectar flow). In addition, some growers have not had high levels of pollination from bumble bees. The

focus of this study was to ascertain if a mixed strategy of honeybees and bumble bees deployed in the same field can result in high levels of pollination and yield.

METHODS: Three wild blueberry fields were selected for study. These fields were located in Aurora, Deblois, and Alexander, Maine. In each field clusters or sets of bumble hives and honeybee hives were set out at opposite ends of each field. Transects were laid out between the bumble bee and honeybee sets. Spaced along the transects, 6-10 sampling stations were deployed. Each sampling station was comprised of a $1m^2$ quadrat where replicated 1 minute observations of bee visitation to blueberry bloom could be made. In addition, to the quadrat, 5 stems were marked for fruit set estimation. On each stem flowers were counted at the beginning of bloom and then set flowers or green fruit were counted two weeks after bloom had ended (early fruit set) and then five weeks after bloom (late fruit set). A measure of foraging activity was made 2-3 times during bloom. This consisted of counting the numbers of honeybees and bumble bees returning to their respective hives for a 1 minute period. A measure of foraging efficiency was also made 2-3 times during bloom. This measure consisted of counting the number of bumble bees and honeybees returning to their respective hives with and without pollen on their hind legs. A proportion of bees returning with blueberry pollen was then calculated from this data. During bee observations, measures of air temperature, relative humidity, and wind speed were recorded. Linear regression and analysis of variance were used to provide evidence for pollination performance.

RESULTS: There were significant differences in bee foraging (bees/ m^2/min) among the sites for bumble bees ($F_{(2,29)} = 4.085$, $P = 0.027$), but not for honeybees ($F_{(2,29)} = 1.343$, $P = 0.277$) or native Andrenid solitary bees ($F_{(2,29)} = 2.312$, $P = 0.117$). Figure 1 depicts the bee foraging densities during peak bloom.

Fig. 1. Average foraging abundance during peak bloom of honeybees, bumble bees, and Andrenid solitary bees at three blueberry fields in 2014.

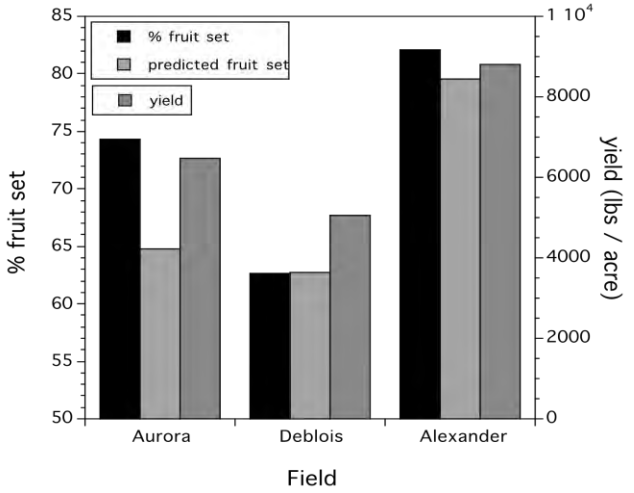


The lower bumble bee density in the Deblois field is correlated to the distance the bumble bee colonies were from the nearest honeybee colonies and the resulting aggression or attempted

robbing of bumble bee colonies by honeybee foragers. At the Deblois field bumble bee colonies were only a hundred feet or so from the honeybee colonies and robbing was much more intense at this site than the other two sites (3.1 honeybees per bumble bee quad entrance / min at Deblois vs 0.67 honeybees per bumble bee quad entrance / min at the other two sites). This supports my previous conjecture that mixing of honeybees with bumble bees works well, but only if the two sets of colonies are separated by a significant distance.

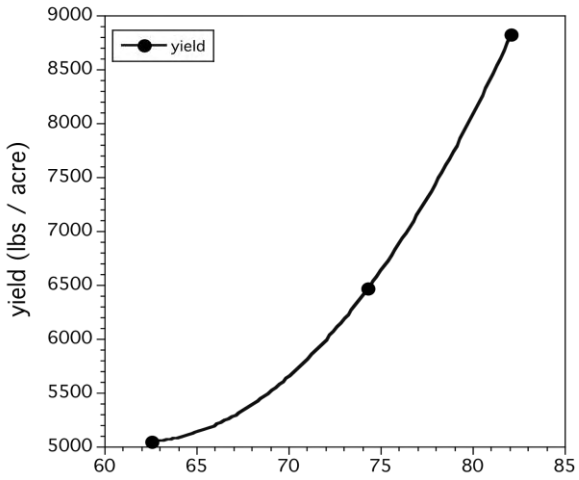
Fruit set differed among the three fields, a marginal difference for early fruit set, but a strong difference for late fruit set (Early fruit set: $F_{(2,22)} = 3.055$, $P = 0.068$; and Late fruit set: $F_{(2,22)} = 4.348$, $P = 0.026$) as shown in Figure 2**.** Predicted fruit set is similar to that observed.

Fig. 2. Observed and predicted % fruit set (predicted estimated from sampled bee foraging density at peak bloom) and yield for three field sites in 2014.



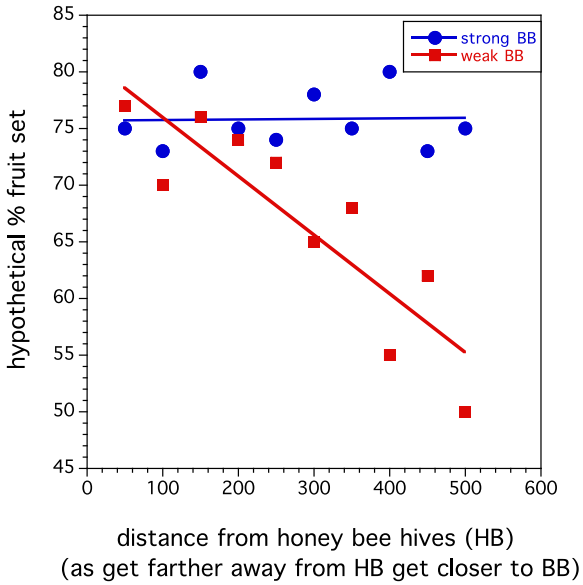
I predicted fruit set from the observed foraging bees sampled during peak bloom using the formula in Drummond (2002). The low fruit set and yield in the Deblois field is due to the low number of foraging bees compared to the other sites. However, some of the reason for low fruit set in Deblois could also be due to a frost that occurred during early bloom throughout the Downeast region, but differentially hit fields depending upon their elevation and air drainage. The observed and predicted fruit sets are very similar for two of the field sites, but are different for the Aurora site. This can only be explained in that I must have underestimated the bee foraging density. This of course can happen when sampling the field only two times during bloom. It was observed that % fruit set was highly related to yield. This is what one hopes for when investment in bees is high, although it does not always occur because of negative effects of weather and pests or the positive effects of fertilization and irrigation. Figure 3 shows the relationship between % fruit set and yield for the three sites.

Fig. 3. Relationship between observed fruit set and yield reported by farm manager.



The main objective for conducting this study was to determine if the mixed pollination strategy of combining honeybees and bumble bees was efficient. I recorded bee foraging both for honeybees and bumble bees and fruit set across the transect between the honeybee and bumble bee hives. My premise for designing the study in this way is that IF bumble bees were not as efficient in the stocking density that they were deployed at compared to honeybees one would see a decline in fruit set as one moved from the honeybee hive set to the bumble bee hive set and measured fruit set. Figure 4 shows this hypothetical outcome. In Figure 4 the blue line shows equally strong pollination from bumble bees and honeybees; whereas, the red line depicts what one might expect if bumble bees were not doing a good job of pollination compared to the honeybees.

Fig. 4. Hypothetical response where have equally strong or an unequal pollination in a field when honeybees are placed at one end of the field and bumble bees are placed at the other end of the field. Distance from honeybee hives represents a transect between the honeybee hives and the bumble bee hives.



The fruit set data collected along the transect in the three fields in 2014 showed a significant difference among fields as described earlier (Deblois has lower fruit set than Alexander), but the fruit set as one moved away from honeybee hives toward bumble bee hives did not differ significantly for either early or late fruit set (Early fruit set: $F_{(1,22)} = 0.848$, $P = 0.367$; late Fruit set: $F_{(1,22)} = 1.273$, $P = 0.271$). Figure 5 shows the data (distance was transformed to a rank distance since the distance between honeybee and bumble bee colonies differed for each field) for % early fruit set and transect distance from the honeybee hives. It can be seen that even though no statistically significant downward trend in the line was observed, there is a slight downward trajectory in fruit set for the largest field (Aurora). However, there was no statistical interaction between field site and the regression between fruit set and distance from the honeybee hives ($F_{(2,22)} = 0.154$, $P = 0.858$) which suggests that all three fields had the same pattern and there was not a weak pollination output from the bumble bee hives. This is supported by the expected fruit set predicted by our fruit set model based upon the observed foraging bees in the field. For instance at the Alexander field honeybee density was 3.8 bees / m^2 / min and bumble bees were 1.6 bees / m^2 / min. This resulted in the predicted fruit set by honeybees alone to be 36.9% and bumble bees to be 35.6 %.

Fig. 5. Observed fruit set across fields between honeybee and bumble bee hive sets. As the distance increases on the x-axis, the bumble bee hives get closer to the fruit set sampled and farther away from the honeybee hive set.

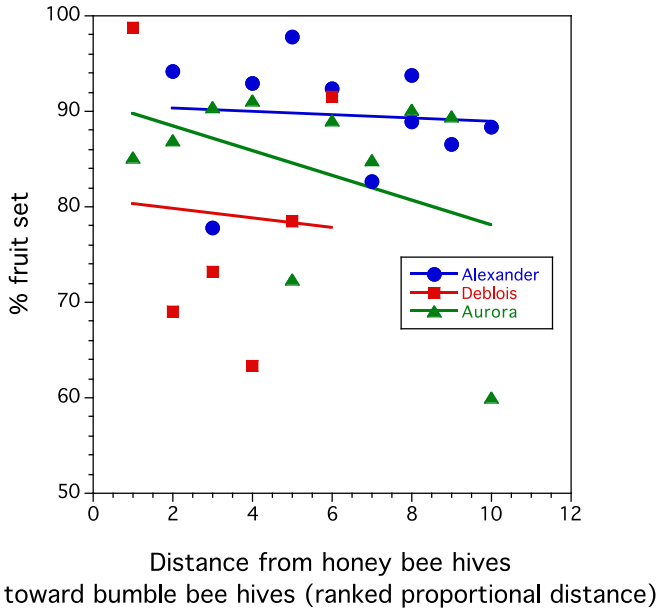
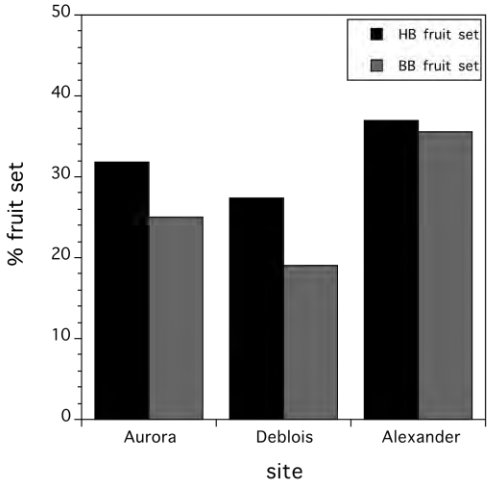


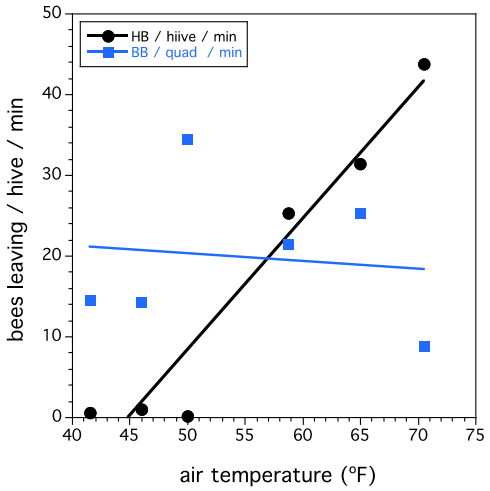
Figure 6 depicts the predicted fruit set for honeybees and bumble bees based upon the sampled bee foraging densities in each field (shown in Fig 1.).

Fig. 6. Predicted independent % fruit set due to honeybees and bumble bees in each field.



Weather can dramatically affect bee foraging. I measured the air temperature and recorded the number of bees leaving / minute from each of 5 honeybee hives and 5 bumble bee quads at each of the three sites on May 25 and then again on May 29. Figure 7 shows the reduced foraging of honey bees below 50ºF and then the increased foraging as temperature increased. The bumble bees did not show this increased foraging over the range of temperatures we observed. It is known that high temperatures will negatively affect bumble bees much more than honeybees (Stubbs et al. 2001).

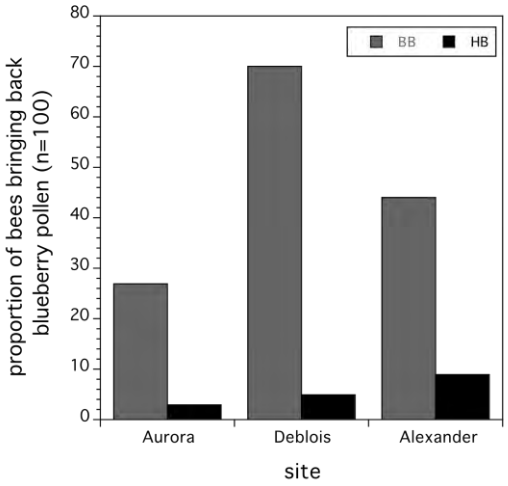
Fig. 7. Honeybees leaving the hive or bumble bees leaving the quad to forage on blueberry bloom on May 25 and May 29, 2014 (n= 5 for each bee species x temperature datum).



The last observation that I made in the field was on successful pollen foraging in blueberry. On May 29, a sunny warm day in which both honeybees and bumble bees were foraging, the proportion of bees for each species at each site (n=100 bees per site and per bee species) that brought back blueberry pollen to the hive or quad was recorded. A logistic regression supports the observation that bumble bees brought back a significantly higher proportion of blueberry pollen, on a per bee basis than honeybees ($X_{(1)}^2 = 150.491$, $P < 0.0001$). The model suggests that

bumble bees are 16.96 times more efficient than honeybees at extracting pollen from blueberry flowers. This higher efficiency of foraging bumble bees is not new and has been observed over several years in blueberry fields. However, the sheer massive abundance of honeybees offsets the efficiency on a per bee basis by numerically making up for any lack in efficiency. Figure 8 demonstrates the difference in pollen extracting efficiency of the two bee species. It can be seen that there is also differences between fields in the proportion of bumble bees that bring back blueberry pollen. This might be due to other flowering sources around fields that bees will visit during blueberry bloom.

Fig. 8. The proportion of honeybees and bumble bees that bring back blueberry pollen back to the hive or quad, May 29, 2014.



CONCLUSIONS: It was found that a mix of honeybees and bumble bees in 2014 provided good pollination and yields in 2014. This conclusion is only based upon 3 fields, and the pollination and yields were quite variable (although they ranged from a very good yield of over 5,000 lbs / acre to an extremely high yield of more than 8,000 lbs / acre). However, there was no evidence to suggest that bumble bees were performing poorly, resulting in an uneven systematic fruit set as sampling progressed adjacent to honeybee hive sets toward bumble bee quad sets. The cool days that were experienced during the beginning of bloom demonstrate that a mixed pollination strategy of bumble bees and honeybees can result in low level foraging by the bumble bees. Whether these results will be consistent across years is not known, but as long as honeybee hives are not placed in a close proximity to the bumble bee quads, the two species of bees should coexist and pollinate wild blueberry.

Study 11. Genetic diversity of Vaccinium angustifolium in managed and non-managed populations throughout its geographic range
Report from Lee Beers (Ph.D student) and Dr. Frank Drummond

OBJECTIVE: Previous studies on lowbush blueberry (*Vaccinium angustifolium*) have found high levels of genetic diversity among clones within a field and between populations. These studies were isolated to plants growing in areas of primary commercial blueberry harvesting,

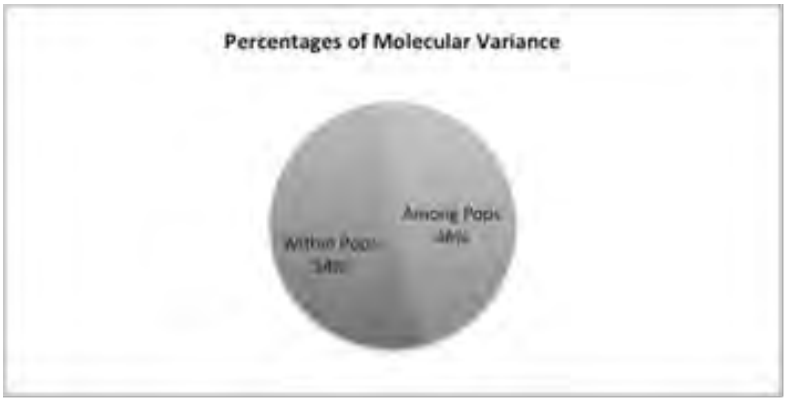
Maine and New Brunswick. The goal of this study is to assess the genetic diversity of lowbush blueberry in not only Maine, but throughout its natural growing range south to Virginia.

METHODS: Lowbush blueberry leaf samples were collected from 16-24 individual plants in several Maine locations representing managed and non-managed growth habitats (Sebago, Old Town, Jonesboro, Salem, Winterport, and Lubec). Leaf samples were also collected from several non-managed populations in VT, MA, NY, PA, MD, VA, and WV (Fig. 1). Sampled sites represent a range in cold hardiness zones from 4b (-31.7°C) to 7a (-15°C). Populations were separated by a minimum of 35km between the two closest populations and 1600km between the most distant. A total of 291 individual lowbush blueberry plants were sampled in this study.
Leaf material was used for DNA isolation using the Qiagen DNeasy Plant Mini Kit or a CTAB extraction protocol. Targeted regions of the genome were amplified using 24 expressed sequenced tagged polymerase chain reaction (**EST-PCR**) molecular markers that had been previously identified to produce repeatable polymorphic bands suitable for relationship analysis. DNA fragments were separated using gel capillary electrophoresis then viewed and sized with ProSize software. Size of the polymorphic bands was determined using a standard 100 base pair ladder reference and bands that fell within ±5% of the targeted size were included. Polymorphic bands were scored as dominant markers (homozygous); present or absent. Genetic distance, geographic genetic distance, AMOVA, spatial structure, and principal coordinate analyses were calculated using the Genalex 6.5 population genetics plugin for Microsoft Excel. Additional analyses (perMANOVA and MRBP) were calculated using the PC-ORD 6 software package.

Fig.5. The geographic distribution of *Vaccinium angustifolium* is shown in the gray shaded region. It extends from Quebec in the north to North Carolina and west to Minnesota. Populations sampled for this study are shown with black dots.

RESULTS: The 24 EST-PCR molecular markers yielded 202 polymorphic bands for each individual that could be used for analysis. The AMOVA for all sampled populations revealed higher variance within populations (54%) compared to among populations (46%) and overall found no significant similarities between populations ($P = 0.001$)(Fig. 2).

Fig. 2. Variance associated with and among populations with AMOVA.



Pairwise analysis between all populations also showed no significant similarity with the exception of the wild population of Lubec, ME and the managed population of Jonesboro, ME ($P = 0.141$). Our analysis also shows that lowbush blueberry populations have no spatial structure at the field level but it does appear when populations are separated by 10km to 25km (Fig. 3). This is consistent with previous findings that showed spatial structure appearing at 12km for Maine populations. Principal coordinate analysis (**PCoA**) of all populations show that the managed populations of Maine (plus the wild Lubec population) are more closely related than those of the non-managed populations even though the populations may be separated by a greater distance (Fig. 4). The remaining populations group together quite differently, the non-managed populations cluster together, the Mt. Elam, MA population does not cluster with any other populations and the remaining non-Maine populations cluster together.

Fig. 3. Spatial structure appears between 10km and 25km for lowbush blueberry populations.

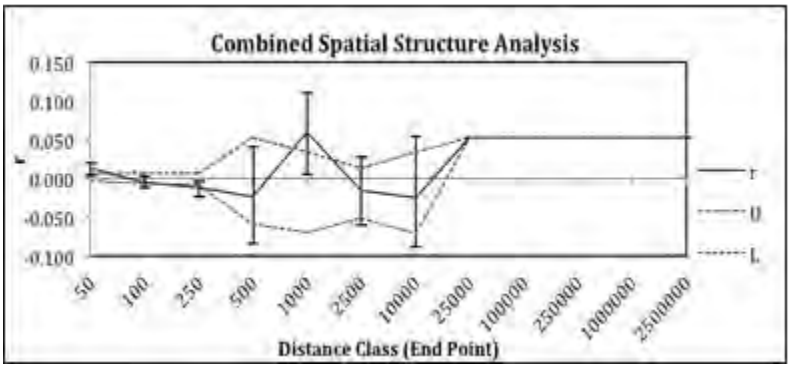
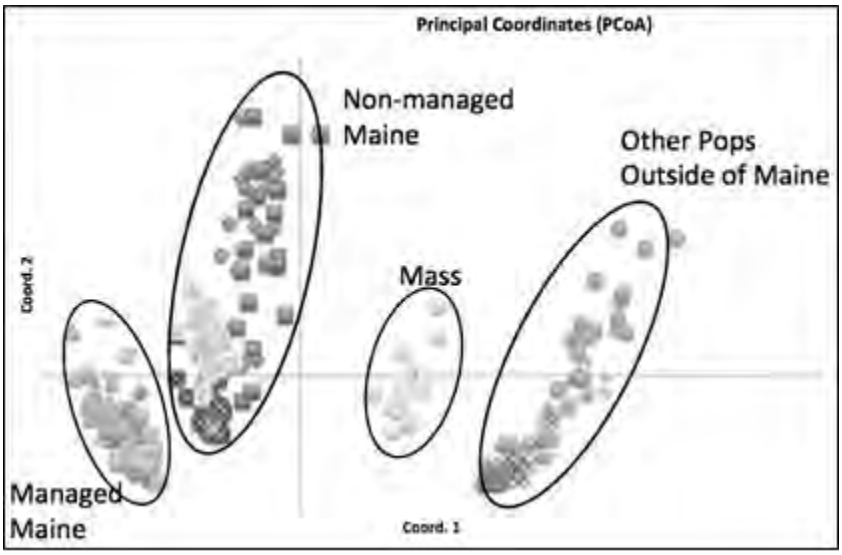
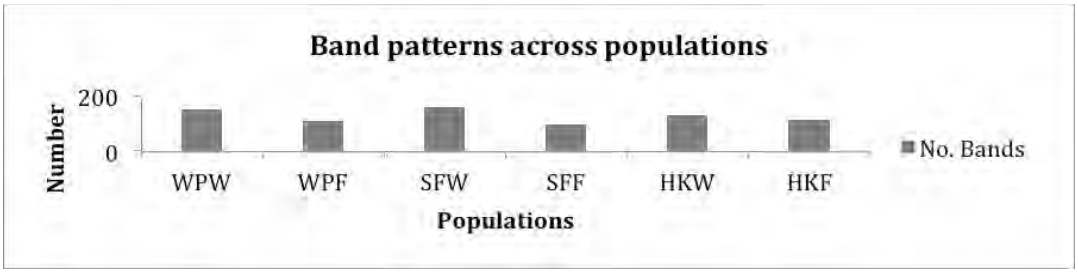


Fig. 6. PCoA for all sampled lowbush blueberry populations. Managed Maine populations cluster together while other populations cluster based on location.



Another goal of this study was to determine if management of lowbush blueberry for commercial harvest alters the genetic diversity of the plants. To do this we compared the genetic diversity of plants within a managed field to the genetic diversity of non-managed plants growing nearby that did not have a history of commercial harvesting. Non-managed populations were typically found in mature mixed forests around the edge of the managed field. Three paired managed/non-managed sites were used for analysis, Old Town, Winterport, and Jonesboro, ME. Initial multiple response blocked paired (**MRBP**) analysis found no significant difference ($P = 0.116$) between the managed populations and the neighboring non-managed plants. Further AMOVA and PERMANOVA tests showed significant differences between managed and non-managed populations ($P = 0.010$ and $P = 0.0002$, respectively) which contradicts the previous MRBP findings. Further analysis comparing managed and non-managed fields focused on the number of polymorphic bands found within each management type. There is a trend of fewer polymorphic bands in managed populations when compared to the non-managed populations but the trend is not significant (Fig. 5). However, if the Jonesboro populations (HKF) are removed from the analysis there is a significant difference in the number of polymorphic bands between managed and non-managed populations for the remaining samples.

Fig. 5. Number of polymorphic bands in managed and non-managed populations. Managed populations include WPE, SFF, and HKF. Non-managed populations include WPW, SFW, and HKW.



CONCLUSIONS: Results from this study have shown that the level of genetic diversity in lowbush blueberry is quite high. Significant differences in genetic diversity can be found between individuals and between most populations. Despite the significant differences between populations there does appear to be a higher level of relatedness for populations within Maine when compared to populations outside of the state. This, in part, could be due to the relatively high density of lowbush plants in Maine, which allows for outcrossing to numerous individuals. Populations with a lower density of plants across a landscape may have fewer chances for outcrossing and could become less related to other populations over time.
Management of lowbush blueberry for commercial harvesting does influence the genetic diversity of plants. Common practices utilized in the management of lowbush blueberry such as burning and the use of herbicides places selective pressure upon the population and plants that are not able to survive those events are removed. The remaining plants have fewer polymorphic bands and are significantly different from neighboring wild populations. Currently, we are adding another paired population to this study for future analysis.

Study12. Assessment of the mechanisms behind performance changes in Maine's lowbush blueberry induced by flower thinning

Report from Alex Bajcz (Ph. D. candidate) and Dr. Frank Drummond

OBJECTIVE: This study assesses if previously demonstrated changes in Maine wild blueberry performance brought about by a flower thinning treatment can be altered by three subsequent treatments, each reflecting a possible underlying mechanism for why flower thinning induces performance changes.

METHODS: During the first weeks of May, 2014, 22 lowbush blueberry clones were selected at Blueberry Hill Farm in Jonesboro, ME. In each clone, eleven 1/8m^2 plots were delineated (242 total plots), and, in all plots, all flower bud clusters on all stems were hand-counted. In seven of these eleven plots in every clone, approximately 70% of the clusters were removed from each stem via hand-pinching (hereafter "X" plots) while, in the other four plots (hereafter "C" plots), no clusters were removed. Because of inter- and intra-clonal differences in average numbers of flower bud clusters per stem, actual removal rates per plot varied between ~45% to ~70%. In four of the eleven plots in every clone (two X plots and two C plots), no further manipulations were performed; these plots served as internal controls. The remaining seven plots in every clone received one (and only one) additional manipulation: flower bud cluster thinning that was biased by position on the stem (two X plots), foliar fertilization (one C plot and one X plot), or leaf clipping (two X plots and one C plot). Each of these treatments was designed to interact with the effects of flower thinning by affecting thinned plots more or less than unthinned ones. Should such an interactive effect in the appropriate direction be found, this supports the hypothesis that the performance changes brought about by flower thinning may be explained by one or more proposed underlying biological mechanism(s).
Two X plots in every clone received their inflorescence bud thinning in a biased fashion; in one plot ("B" plots), top-most buds were preferentially removed while, in the other plot ("A" plots) bottom-most buds were preferentially removed. Two other plots in every clone, one X plot and one C plot, received foliar fertilization in the form of five doses of Coron® nitrogen fertilizer applied at a rate of 6 pounds of nitrogen per acre or, equivalently, ~1.2 mL Coron per dose per

plot. The Coron fertilizer was dispersed in filtered water, and each dose was applied approximately every 1.5 weeks starting on 23 Jun and ending on 31 Jul.
The remaining three plots in every clone, one C plot and two X plots, received leaf clipping between 24 and 26 Jun, approximately two weeks before the second collection period (see below). In one of the two X plots ("S" plots), all but the three top-most new vegetative branches were removed from each stem via hand-pinching. In the other two plots, all but the single top-most new vegetative branch was removed from each stem ("M" and "H" plots for the remaining X plot and the C plot, respectively). In every case, the removed vegetative mass was bagged and later weighed. Stems that were not reproductively active in these plots (i.e. they had no reproductive clusters) were clipped as normal, but their vegetative mass was not maintained.
Soil and tissue samples were collected from every clone for later environmental and genetic analyses. In addition, canopy development rate was monitored over the course of the growing season using a light meter. The rest of the data collected was derived from whole stems that were harvested from every plot at each of three collection points—peak bloom, the unripe fruit stage, and just prior to commercial fruit harvest. Five stems with active reproductive clusters were taken from each plot at the first two collection points (plots that had fewer than 20 anticipated reproductively active stems were skipped) and all remaining reproductively active stems were harvested at the last collection point. The number of remaining reproductive clusters was counted on each harvested stem, and then these stems were separated into three tissue types: 1) old stems, 2) new vegetative tissues (leaves plus new stems), and 3) reproductive tissues (flowers and/or fruits). These three tissue types were then bagged and weighed separately before being frozen for later analyses.
Several measures of plant performance will be compared across all plots; using mixed-effects regressions, we will identify treatments and/or treatment combinations that most strongly impact performance. The measures of plant performance surveyed will include:

1. Total vegetative, reproductive, and stem mass per plot, within and across collections.
2. Rates of reproductive failure at the stem, inflorescence, and individual flower/fruit levels.
3. Mass of reproductive structures per inflorescence.
4. Individual fruit size, shape, and mass.
5. Fruit ripening rate.
6. Total marketable fruit yield per unit area.
7. Leaf length and area, within and across collections.
8. Individual leaf and fruit wet and dry masses, as well as the wet:dry ratio thereof.
9. Canopy photosynthetic rate.
10. Canopy development rate as measured by leaf area index.
11. Ripe fruit titratable acidity and fruit pulp pH.
12. Fruit and leaf total soluble solids content.
13. Ripe fruit anthocyanin pigment content.
14. Leaf chlorophyll a and carotenoid content.
15. Seed set and number of mature seeds per ripe fruit.
16. Leaf tissue nutrient concentrations.

Dependent variables that are not dependent on the starting number of reproductive clusters will be treated as is in the regression models. However, for variables that are dependent on the starting number of clusters (i.e. measures of *absolute* reproductive output), values for X plots would be inherently "handicapped" relative to C plots by the extent of flower removal they have

received. In these cases, in order for X and C plots to be fairly compared on a relative reproductive output basis, values for C plots will be mathematically scaled down by the fraction by which the plot would have been flower-thinned, had it instead been an X plot. A significant difference between X and C plots in this case would reflect a difference between "real" versus "mathematical" flower removal.

The data listed above taken from within a collection period will be analyzed using linear mixed-effect models (**LMM**s) with individual plots as replicates and with a random intercept term for each clone to minimize the influence of pseudoreplication (R function *lmer*). For data listed above taken from multiple collection periods, LMMs with random intercepts for each clone, date, and plot will be employed. Every model will include the following fixed effects: 1) reproductive cluster removal (**X**) versus no removal (**C**); 2) biased cluster removal (**A** & **B** plots) versus haphazard removal (other X plots); 3) bottom cluster removal (**A** plots) versus top cluster removal (**B** plots); 4) fertilizer dosage (1.2 mL N per dose per plot vs. 0 for unfertilized plots); and 5) vegetative mass removed (in grams). Additionally, the models will include two two-way interaction terms: 6) cluster removal by fertilizer dosage (#1 x #4); and 7) cluster removal by leaf mass clipping (#1 x #5).

Because actual inflorescence removal rates (in the case of dependent variables that don't depend on starting reproductive cluster number) and actual plus mathematical removal rates (in the case of measures of absolute reproductive output) differed between plots, 8) the removal rate will be included in each model as a covariate to adjust for varying treatment strength. Lastly, because one observer's stem and bud counts tended to differ from all other observers, 9) a binary observer factor will be included in all models to account for this discrepancy.

Significance for factor #1 above will be interpreted as evidence of performance changes induced by flower thinning while significance (in the appropriate direction) for factors #2, #3, #6, and #7 will be interpreted as potential support for a mechanistic explanation for why these performance changes occurred. *P* values less than 0.05 and values between 0.05 and 0.1 will constitute significance and marginal significance, respectively. Model fit and validity will be accessed graphically (R function *mcp.fnc*) and quantitatively (R function *summary*). When appropriate, dependent variable transformations will be used to ensure models meet the requisite assumptions of homoscedasticity and normality.

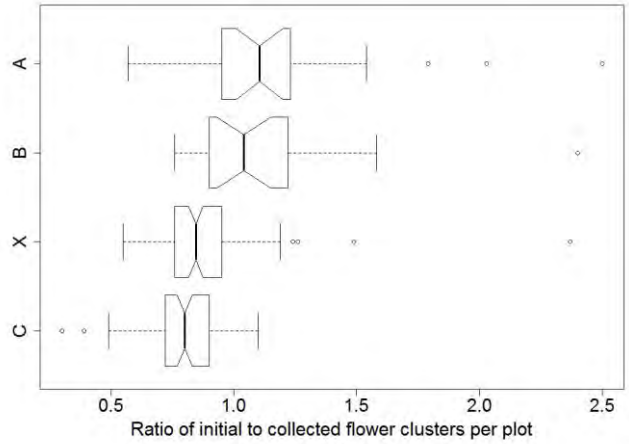
RESULTS: All the results presented below are from mixed-effects models with log-transformed dependent variables.

Flower thinning did not significantly correlate with the proportion of stems per plot that retained at least one active reproductive cluster until harvest ($P = 0.192$). However, plots that were flower thinned in a positionally biased manner showed significantly greater reproductive stem retention than those that were haphazardly thinned ($P = 0.006$; Fig. 1). Thinned plots did have a much greater proportion of total reproductive clusters that were retained until harvest than unthinned plots did ($P < 0.0001$). Moreover, bias-thinned plots showed greater reproductive cluster retention than haphazardly thinned plots did ($P = 0.006$). A significant thinning-by-fertilization interaction was observed for cluster retention; fertilized plots that were thinned had significantly higher retention than fertilized, unthinned plots ($P = 0.003$). However, this result runs counter to the expectations of the underlying proposed mechanism.

The rest of the results reported here are relative to starting reproductive cluster number. The average number of flowers per stem at peak bloom was significantly higher in thinned plots than in non-thinned plots ($P = 0.013$). However, none of the treatment-by-thinning interactions were

significant. At the unripe fruit stage, the average number of fruits per stem was also significantly higher in thinned plots than in non-thinned plots ($P < 0.0001$). A marginally significant thinning-by-leaf-clipping interaction was observed; the relationship between the amount of vegetative mass removed and the average number of fruits per stem was significantly more positive for non-thinned plots than for thinned plots ($P = 0.091$), which again runs counter to expectations.

Fig. 1. Proportion of reproductive stems per plot that retained at least one active reproductive cluster until harvest grouped by plot type. C plots were not flower-thinned; all other plot types were thinned in a consistent manner. X plots represent those thinned haphazardly, while A and B plots were those that were thinned from the bottom up and the top down, respectively. Notches represent 95% conference intervals



The average number of fruits (ripe and unripe) per stem at harvest time was significantly greater for thinned plots than for non-thinned plots ($P = 0.001$), and there was a marginally significant tendency for bottom-thinned (A) plots to outperform top-thinned (B) plots ($P = 0.088$). Thinning and fertilization significantly interacted, with thinned plots responding more positively to fertilization than non-thinned plots did ($P = 0.009$), a result which is opposite to expectations (Fig. 2). Thinning also significantly interacted with vegetative removal, with thinned plots performing better under comparable levels of clipping than non-thinned plots ($P = 0.039$), a result which supports the proposed underlying mechanism (Fig. 3).

Fig. 2. Average number of fruits (ripe and unripe) collected at harvest time grouped by fertilization and flower thinning treatment. C = not flower-thinned, X = flower-thinned. U = unfertilized, F = fertilized. Fertilized plots received five doses of 1.2 mL Coron foliar nitrogen fertilizer over the course of the season. Plots labeled X include values from A and B plots. Notches represent 95% conference intervals. Values for C plots have been adjusted to reflect a level of reproductive output relative to starting reproductive capacity to allow for a fairer comparison.

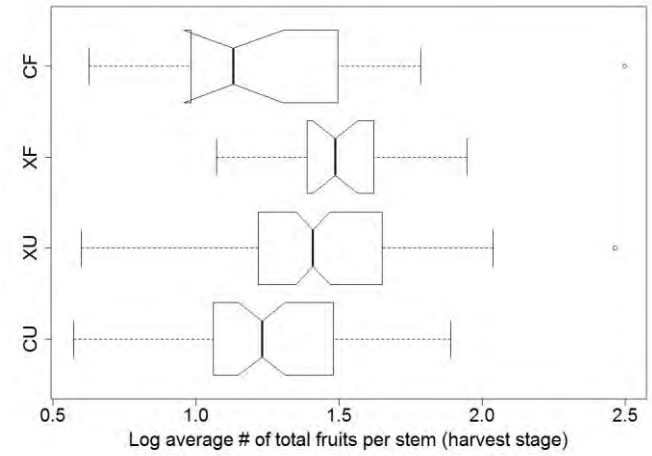
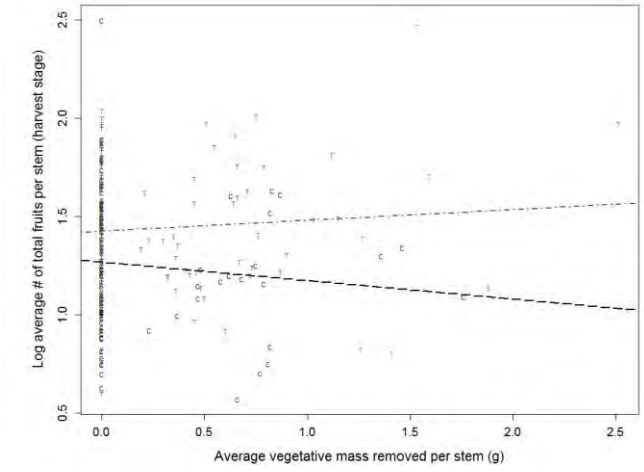


Fig. 3. Average number of fruits (ripe and unripe) per stem per plot at the harvest stage plotted against the amount of leaf and new stem tissue removed from the reproductively active stems in each plot (in grams). Point labels represent plot type; C plots were not flower-thinned, while T plots were all flower thinned in a consistent manner. Values labeled as T include data from X, A, and B plots. The top and bottom lines represent approximations of the best-fit lines for the T and C data, respectively. Y values for C plots have been adjusted to reflect a level of reproductive output relative to starting reproductive capacity to allow for a fairer comparison.



The average number of reproductive clusters per stem at bloom was increased by flower thinning ($P = 0.05$), but there were no notable treatment-by-thinning interactions. At the unripe fruit stage, the average cluster number was also increased by thinning ($P < 0.0001$), and there was a

marginal thinning-by-leaf-clipping interaction. Thinned plots had fewer reproductive clustered at comparable levels of clipping than non-thinned plots had ($P = 0.071$), which does not support the proposed underlying mechanism. At harvest, the average cluster number was increased by thinning ($P < 0.0001$), and this increase was higher in bottom-thinned (A) plots than in top-thinned (B) plots ($P = 0.049$; Fig. 4). There were also two further significant interactions. Thinned plots again responded more positively to fertilization than non-thinned plots did ($P = 0.002$), which is contrary to the expected result, while thinned plots endured clipped better than non-thinned plots did at comparable clipping levels ($P = 0.05$), which supports the proposed underlying mechanism.

When assessed over all collection dates, the average number of flowers/fruits per stem was much higher in thinned versus non-thinned plots ($P < 0.0001$; Fig. 5), but there were no further substantial interactions. The average number of reproductive clusters per stem across all time points was greater in thinned plots than in non-thinned plots ($P < 0.0001$), and cluster number was marginally higher in bias-thinned plots than in haphazardly thinned plots ($P = 0.083$).

Fig. 4. Average number of reproductive clusters per stem per plot at the harvest stage grouped by plot type. C plots were not flower-thinned; all other plot types were thinned in a consistent manner. X plots represent those thinned haphazardly, while A and B plots were those that were thinned from the bottom up and the top down, respectively. Notches represent 95% conference intervals. Values for C plots have been adjusted to reflect a level of reproductive output relative to starting reproductive capacity to allow for a fairer comparison.

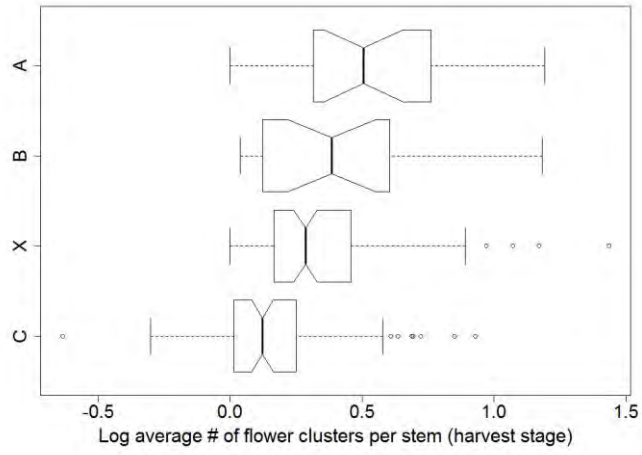
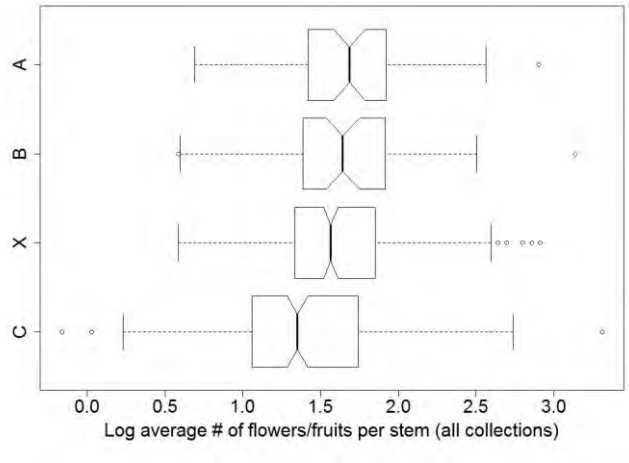


Fig. 5. Average flower/fruit number per stem per plot across all collections (peak bloom, unripe fruit stage, and harvest stage combined) grouped by plot type. C plots were not flower-thinned; all other plot types were thinned in a consistent manner. X plots represent those thinned haphazardly, while A and B plots were those that were thinned from the bottom up and the top down, respectively. Notches represent 95% conference intervals. Values for C plots have been adjusted to reflect a level of reproductive output relative to starting reproductive capacity to allow for a fairer comparison.



CONCLUSIONS: Currently, there are nine popular hypotheses in the plant biology literature for why, and under what circumstances, flower thinning should induce performance changes. Of these, three are thought to be the most comprehensive, i.e. able to explain both vegetative and reproductive changes. These are: 1) the (short-term) resource limitation hypothesis; 2) the spatio-temporal dominance hypothesis; and 3) the compound interest effect hypothesis. Each hypothesis basically suggests that flower thinning acts by "correcting" some underlying biological "problem" that limits performance to some extent.

The short-term resource limitation hypothesis contends that nutrient mobilization within plants is slow and that stunting and abortion of structures can occur even when the resource deficiencies responsible are transient and localized rather than systematic. By removing some proportion of structures, flower thinning may make these resource deficiencies less likely to occur for the remaining structures. Because nitrogen is the most crucial plant nutrient for growth and development, foliar fertilization with nitrogen should also reduce nitrogen deficiencies, mimicking the action of flower thinning and making it less "beneficial" to plant performance. This hypothesis is supported when fertilized plots, both thinned and non-thinned, perform better than their unfertilized counterparts but with a greater margin of improvement for non-thinned plots than for thinned ones.

The spatio-temporal dominance hypothesis argues that stunting and abortion of structures can occur when resources are distributed inefficiently based on a dominance hierarchy rather than based on need. These dominance hierarchies can be either spatial, temporal, or a mixture of the two. Spatial hierarchies arise when structures in certain spatial locations on the plant (e.g. those nearer to the stem or those insulated from frosts) have better access to resources and thus command a greater share of them largely for that reason. Temporal hierarchies arise when structures that happen to develop first or more quickly take a greater share of total resources simply because they have first access.

Flower thinning may reduce the level of antagonism between structures for resource access to a point at which even subordinate structures can get sufficient resource access. If dominance is related to location on the stem (top-most or bottom-most reproductive clusters are dominant), removal of mostly subordinate buds will reduce antagonism much less than removal of dominant buds would, in which case one direction of biased thinning would outperform the other.

Lastly, the compound interest effect hypothesis stipulates that all structures, vegetative and reproductive, initially develop each season from the same stored resource pool, and that these two types of structures compete for these resources in an antagonistic fashion. That is, if reproductive structures take more resources and develop more quickly, vegetative structures will take fewer resources and develop more slowly, and vice versa. However, eventually, reproductive structures will become dependent on vegetative ones for their resource supply as stored resources dwindle. As a result, rapid early reproductive development can stunt later reproductive maturation by impeding early vegetative development. Flower thinning can help prevent this by shifting early development back in favor of vegetative structures. If a large fraction of vegetative structures are removed around the time point when reproductive structures become dependent on them, this action should hurt both thinned and non-thinned plots, but it should hurt non-thinned plots to a significantly greater degree.

With the exception of the proportion of initial to recovered reproductive stems per plot, thinned plots outperformed non-thinned plots in all cases assessed so far *in terms of relative reproductive output*. In many cases, this performance difference was substantial—Figure 5 provides a good example.

Very little evidence for the short-term resource limitation hypothesis has been found so far in this study. In three cases (ratio of initial to recovered reproductive clusters per plot and average number of reproductive clusters and fruits per stem at harvest), a significant fertilization-by-thinning interaction was found, but, in each case, the result was contrary to expectations. In fact, while fertilization improved performance in thinned plots, it actually appeared to *decrease* performance in non-thinned plots (e.g. Fig. 2).

Some evidence has been found thus far for the existence of a spatial dominance hierarchy in lowbush blueberry. For both fruits and reproductive clusters per stem at harvest, plots with only top clusters remaining (A plots) outperformed plots with only bottom clusters remaining (B plots; Fig. 4). In both cases, biased thinning (A and B plots) did not outperform haphazard thinning (other X plots). This result indicates that bottom reproductive clusters may be dominant in blueberry because performance in top clusters increased more when total antagonism was reduced than performance in bottom clusters increased. However, alternative explanations for this result exist and need to be considered before conclusions are drawn. In three instances, support for the existence of a middle-cluster- and/or temporal-dominance hierarchy was found in the form of a significant performance increase in biasedly thinned plots over haphazardly thinned ones (ratio of initial to recovered reproductive stems and clusters and the average number of reproductive clusters per stem across all collections; Fig. 1). Past blueberry researchers have suggested that middle reproductive clusters may be dominant in blueberry; this would be the first experimental evidence we are aware of for the existence of such a hierarchy.

The support generated thus far by this study for the compound interest effect hypothesis, while mixed, is somewhat favorable. In two instances (the average number of reproductive clusters and fruits per stem at the unripe stage), marginally significant interactions in the opposite direction were observed, while in another two instances (the same two metrics at the harvest stage), significant interactions in the appropriate direction were seen. In the latter case, thinned

plots tolerated a comparable amount of leaf clipping to a much greater extent than non-thinned plots did, as the hypothesis predicts (Fig. 3). The former result might be explained by noting that the leaf clipping treatment did not occur until only two weeks prior to the second collection. As such, the unexpected and contrary results found at that time point may reflect the short window in which the plants had to respond to the treatment, which was likely highly stress-inducing. It may have taken X plots longer to cope with this second intense removal treatment than it took C plots, for whom this was the first such treatment. Moreover, the timing of the treatment perhaps also explains why no significant treatment-by-leaf-clipping interaction was observed when the flower/fruit and cluster data were assessed across collection periods but one was observed within the last collection period.

DISEASE MANAGEMENT

INVESTIGATOR: Seanna Annis, Associate Professor, Associate Extension Professor, School of Biology and Ecology

7. TITLE: Research and control of mummy berry disease.

OBJECTIVE: Improve control of mummy berry and *Botrytis* blight through research and the deployment and operation of a disease forecasting system using weather stations

METHODS: In April 2014, twelve weather stations were deployed in blueberry growers' fields around Maine from Dresden Mills, Knox County to Wesley, northern Washington County (Fig. 1). Ten locations also had mummy berry plots that growers monitored through April and into the beginning of May. 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. Data was available on the Maine mummy berry forecast website (http://www.grovision.com/AgriNET/ComServer/UofMaine/DashboardFrameset.htm). In addition, relative humidity was monitored at the sites but these data were collected hourly and at the end of the season. The station located at Blueberry Hill Research Farm was a Davis weather station that was configured with the same types of sensors but access was through a different website. We contracted with Skybit to get virtual data for 10 locations where we also had real weather stations.

Data from the stations were used for the mummy berry forecasts starting in April and extending through May, and then for *Botrytis* reports in May and June. Throughout the disease risk season from early April to June, forecast reports were provided on mummy berry disease, as well as reporting the occurrence of frost and *Botrytis* infection for most of the blueberry growing areas. The forecast reports were delivered in three ways: 1. in email messages sent out to an email list, 2. posted on the Wild Blueberry extension blog (http://umaine.edu/blueberries/blog/), and 3. recorded as answering machine messages.

In September and October 2014, we put out new mummy berry plots for the next season in some grower fields and retrieved the weather stations for winter storage.

In August 2013, we set up a field experiment to look at timing and variation of germination of pseudosclerotia (mummy berries) from three different fields. Soil from Blueberry Hill Research Farm was placed in a plot with removed grass sod, in a field plot at the University of Maine

Orono campus. In a randomized block design, we set up 30 pseudosclerotia of each field in a plot in each block with 4 blocks. In April 2014, we monitored the plots every other day for germination of the pseudosclerotia. Using pseudosclerotia that were placed outside in the plots and dug up in November 2014, we set up an incubator experiment to look at chilling hour requirements for pseudosclerotia germination.

RESULTS: In 2014, we had unexpected problems with the weather stations after putting them out. The software running the stations unexpectedly quit and was diagnosed eventually as needing to be upgraded and reprogrammed, probably due to new software implemented by the cellular network. This unfortunately happened in April and May in the mummy berry season, making it necessary to leave some field locations out of the forecast, move working weather stations around and to delay deploying some stations. All weather stations have now been upgraded and reprogrammed. We also had a few stations with inconsistent cellular signal. This problem was rectified at two locations by putting the antenna on a pole, but some sites may need to be moved next year to get consistent 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. The virtual and real weather data are being compared to see how well they correspond using the mummy berry and *Botrytis* blight forecast models from Nova Scotia. We will compare virtual and real data for another year in 2015.

Ten out of the 12 stations had mummy berry plots, but at one site we did not get germination and at another we were not able to get the weather station out during the mummy berry season. 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.

We had cooler conditions in April this year which delayed the start of mummy berry season until mid to late April (Table 1). The apothecia (cups) started to develop mid to late April, but most fields did not have susceptible plants until late April and early May. The season was about three weeks in most areas with the last possible infection periods near to bloom about May 22nd. It was difficult to determine when the apothecia were gone this year since by May 16th most sites appeared to only have dried up apothecia but then on May 17th and 19th growers found more mature cups in some plots. Most growers reported using at least two applications of fungicides to control disease this year. Control was good in most fields with less than 5% disease, but some fields with inadequate control had up to 34% of stems with disease.

The field experiment looking at pseudosclerotia germination had very few apothecia produced and with these low numbers we could not determine whether there were differences among pseudosclerotia from the original four locations. We are still examining the weather data to determine how it relates to apothecia production. We have also set up a repetition of this experiment in August of 2014.

We did find from setting up our incubator experiment that from 50 to 60% of pseudosclerotia were lost from August to November when placed in the soil. A graduate student will be following up on this observation with an experiment to determine what is happening to these pseudosclerotia. The incubator experiment revealed that more apothecia develop from pseudosclerotia with more accumulated chill hours. Low numbers of pseudosclerotia for this

experiment did not allow us to compare between field locations. This experiment is being repeated in the winter of 2014-2015.

RECOMMENDATIONS: We recommend continuing to monitor conditions for mummy berry infection with the weather stations at this time. 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 will also continue our study comparing virtual and real weather station data to see if the virtual data is suitable as a substitute in the future.

Figure 1. - Locations of mummy berry forecast stations and mummy berry plots for 2014.

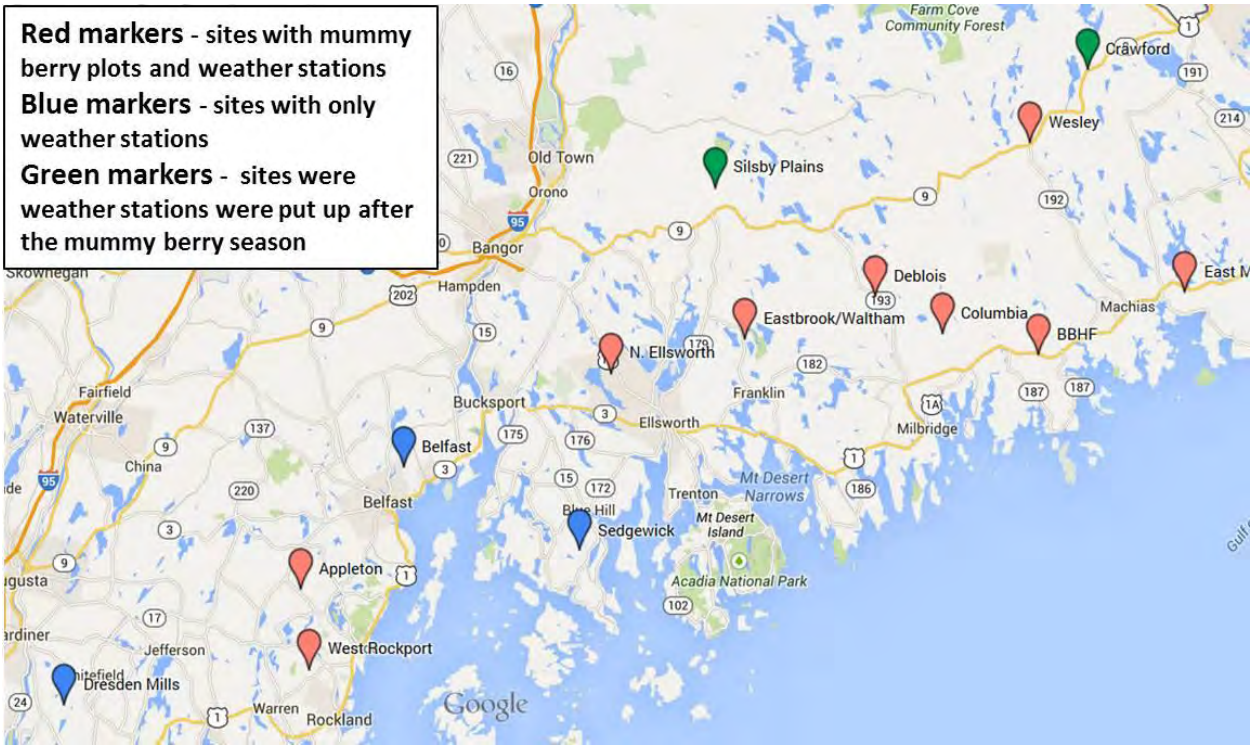


Figure 2. – Comparison between infection periods in 2013 (top) and 2014 (bottom) at the Deblois site. Air temperature and leaf wetness were used to determine infection periods (green bars) for *Monilinia vaccinii-corymbosi.* Blue bars represent when apothecia were present in the fields.

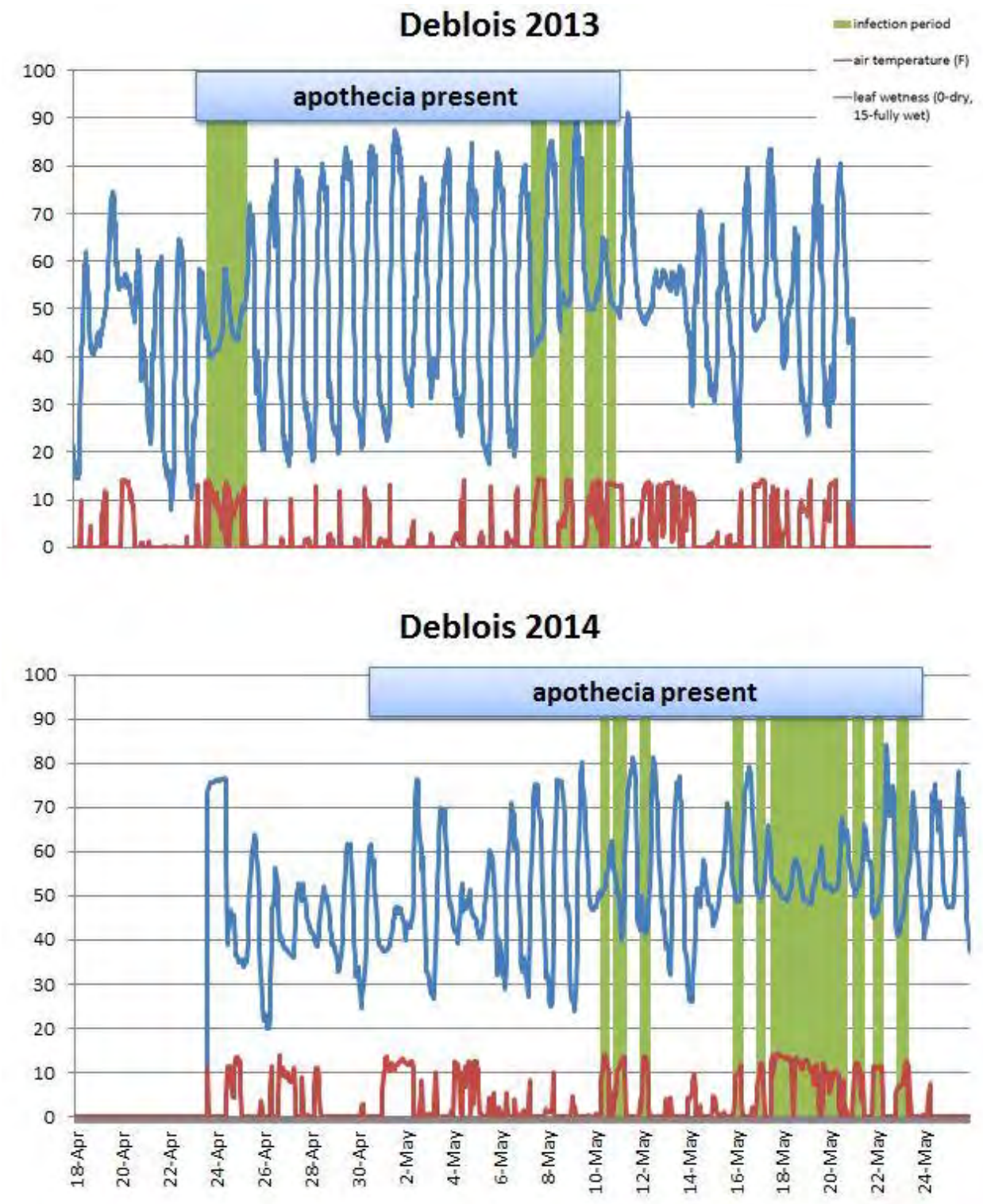


Table 1. - Estimated time of mummy berry cup production and infection periods for weather stations in 2014.

Weather station location	Start of Pinheads	Start of cups	End of Cups	Number of infection periods
Dresden Mills	N/A[1]	N/A	N/A	5
West Rockport	April 27	April 30	May 16	6
Appleton	April 27?	April 30	May 16	6
Belfast	N/A	N/A	N/A	7
Sedgewick	N/A	N/A	N/A	10
North Ellsworth	April 30	May 5	?	9
Eastbrook/Waltham	?	April 30	?	9
Deblois	April 28	April 30	May 22	9
Columbia/Cherryfield	May 1	N/A	May 22	7
Jonesboro	April 28	May 10	May 20	5
East Machias/Whiting	April 30	4-May	May 20	9
Wesley	N/A	2-May	May 20?	At least 7

[1] N/A = not available

DISEASE MANAGEMENT

INVESTIGATORS: Dr. Seanna Annis, Assoc. Professor, School of Biology and Ecology
Jennifer Cote, Asst. Scientist, School of Food and Agriculture

8. TITLE: Evaluation of fungicides for control of mummy berry on lowbush blueberry (2014).

OBJECTIVE: To evaluate control of the primary infection stage of mummy berry, causal agent *Monilinia vaccinii-corymbosi,* on lowbush blueberry (*Vaccinium angustifolium*).

MATERIALS AND 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 7 blocks per field. Fungicide applications were timed using the Mummy berry disease forecast[1] according to locally monitored conditions of fungal and plant development (Fig. 5 a and b), and weather conditions favoring disease development (Fig. 1). Fungicides were applied on May 12 in the Deblois and Township 19 fields. A second application planned for May 21 was not applied due to the resignation of the blueberry disease research assistant in early May, illness of the replacement applicator and backup person and inability to find a replacement person on short notice. 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 TeeJet tips and 50 mesh screens applied. Appropriate surfactants were added as recommended by the manufacturer (Table 1) and the control plots received no spray applications.
Disease assessments in both fields occurred on May 30 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 (Fig. 5d) was stretched along the transect and the stem closest to each marking was inspected for disease symptoms on flowers or leaves (Fig. 5c and d). 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 on August 12, 2014. Harvesting occurred in a 2 foot strip down each plot center with a mechanical harvester and fresh weight was measured.

[1]More information about the mummy berry forecast method can be found in UMaine Cooperative Extension Bulletin #217 (http://umaine.edu/blueberries/factsheets/disease) and the forecasts for last year are available at http://umaine.edu/blueberries/blog/.

RESULTS: This year was cooler at the start than 2013 so the apothecia were delayed compared to 2013 and the plants were also slower to develop. *Monilinia* apothecia appeared in Deblois and Township 19 fields around the end of April (Fig. 1, Fig. 5b), but the plant buds were not open enough to provide enough susceptible tissue for infection (Fig. 5a) at that time. The first infection period was the evening of May 10th, and due to scheduling difficulties from having to find a replacement person to apply fungicide applications, the fungicides were applied early on May 12th. A second application was planned on May 21st but this was not applied due to sudden, unexpected illness of the pesticide applicator and their back up person and lack of availability of other pesticide applicators.

Table 1. - Fungicides tested in 2014 for control of mummy berry.

Treatment (Trade Names)	Material	Manufacturer	FRAC group	EPA Registration Number	Registered on Blueberries
Protexio – Low 14.4 oz/a	fenpyrazamine	Valent USA	17	59639-179	No
Protexio – High 19.2 oz/a	fenpyrazamine	Valent USA	17	59639-179	No
1st spray Protexio – High (19.2 oz/a), 2nd spray **(not applied)** Quash 50% WDG	fenpyrazamine and metaconazole	Valent USA	17 and 3	59639-179 and 59639-147	No
Quash 50% WDG 2.5 oz/a	metaconazole	Valent USA	3	59639-147	Yes

Treatment (Trade Names)	Material	Manufacturer	FRAC group	EPA Registration Number	Registered on Blueberries
Proline 5.7 oz/a	prothioconazole	Bayer Crop Science	3	264-825	Yes
Proline 5.7 oz/a and Dyne-Amic (surfactant) 0.25% v/v	prothioconazole	Bayer Crop Science (Helena Chemical Company)	3	264-825 (5905-50071-AA)	Yes
Serenade Optimum 20 oz/a and Dyne-Amic (surfactant) 0.25% v/v	*Bacillus subtilis*, bacteria	Bayer Crop Science (Helena Chemical Company)	none	264-1160 (5905-50071-AA)	Yes
Positive Control – Tilt 6 oz/a	propiconazole	Syngenta	3	100-617	Yes

We had good control of mummy berry with Proline with no surfactant and Tilt in both fields. In the Township 19 field, which had higher levels of disease, we also had good control with Quash and the Proline with surfactant (Fig. 2a and b). The Protexio and Serenade materials did not decrease disease levels below the check in both fields. The 7 to 10 day period of protection after application would have extended from May 12th to May 19th (7 days) or May 22nd (10 days) and should have covered the bulk of the infection periods, from May 16th to May 23rd. None of the materials were applied before the first infection periods on May 10th and May 11th, and these early infection periods may have contributed to the disease levels found in these treatments, but do not explain the lack of difference between the check and these treatments unless infection did not occur in the later infection periods. In future trials, applications of these materials as protectants before infection periods may produce better results.

In the Township 19 field, the Quash and Proline treatments had significantly higher yield than the check plots (Fig. 3a), but no significant differences were found in the Deblois field (Fig. 3b) for yield.

There were no significant differences in the levels of frost among the treatments (Fig. 4), and no phytotoxicity effects were seen on the plants.

RECOMMENDATIONS: Proline and Quash will be recommended as a fungicide to control mummy berry disease on lowbush blueberries after 2 years of successful trials. It is recommended that Protexio and Serenade be tested again next year and applied before infection periods to determine their effectiveness to control mummy berry.

Figure 1. – Comparison between infection periods in 2013 (top) and 2014 (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.

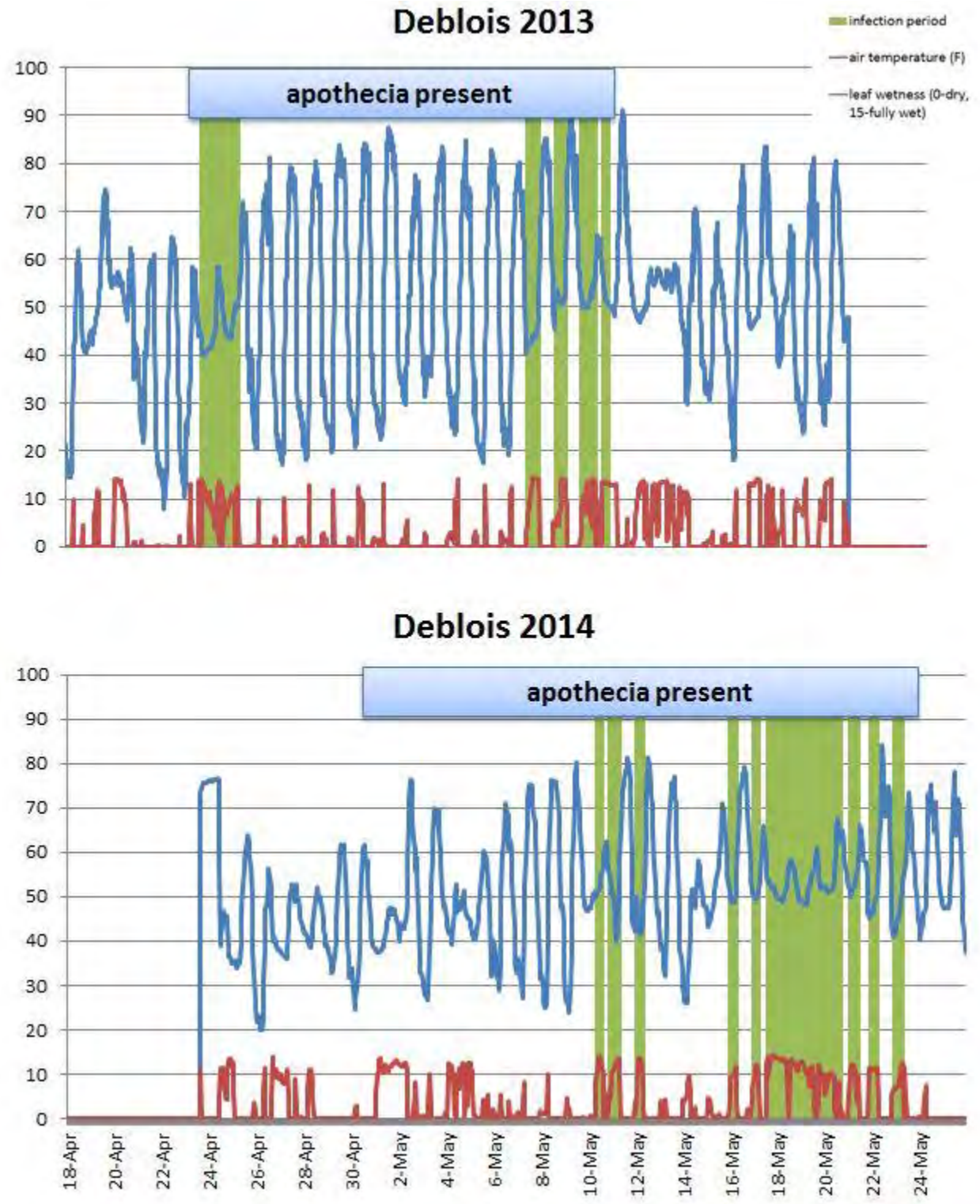


Figure 2. – Average percentage of stems with symptoms of mummy berry disease in fungicide trials at A) Township 19 and B) Deblois fields. Error bars represent standard error of the mean of 7 replicates. Bars with different letters were significantly different at $p<0.05$ within the fields.

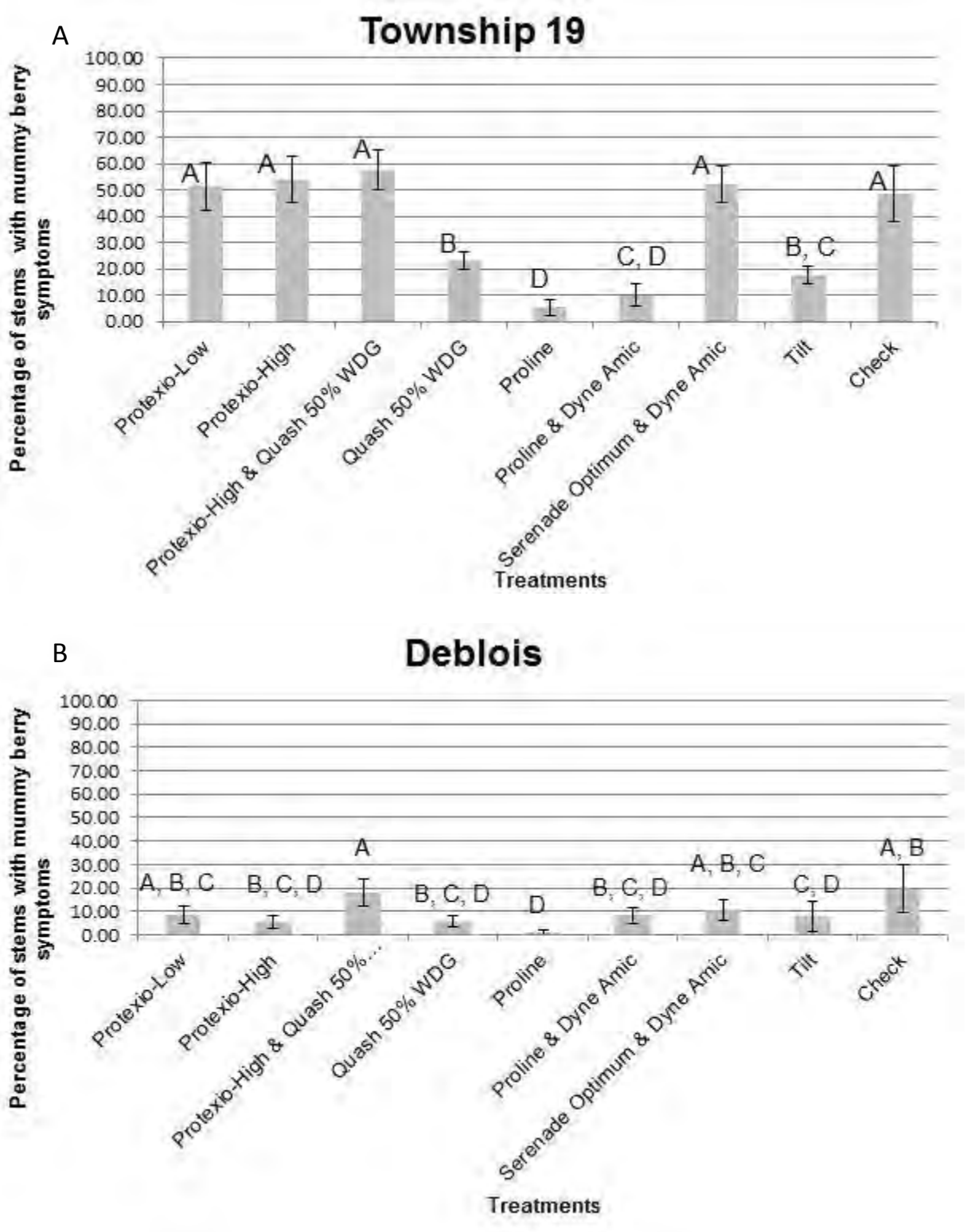
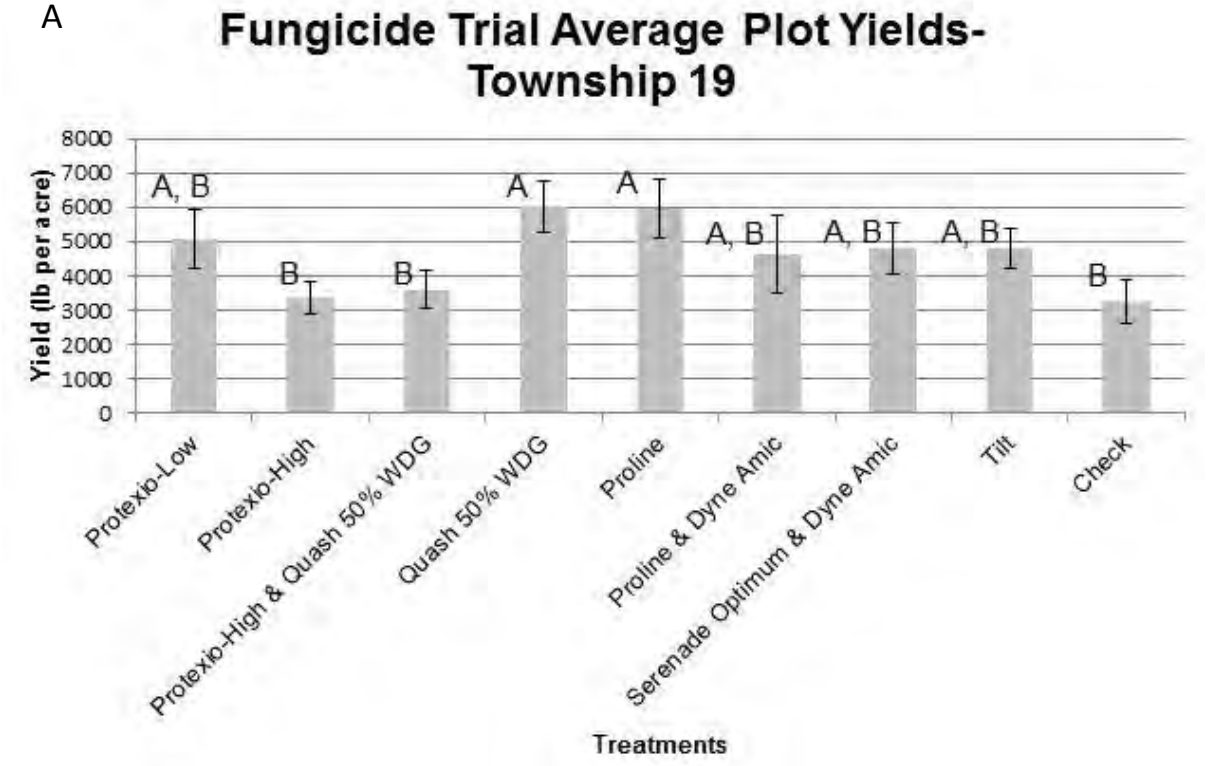


Figure 3. – Average blueberry yield in pounds per acre for treatments in fungicide trials at A) Township 19 and B) Deblois fields. Error bars represent standard error of the mean of 7 replicates. Bars with different letters were significantly different at $p<0.05$ within the Township 19 field. There were no significant differences among the treatments within the Deblois field.

A



B

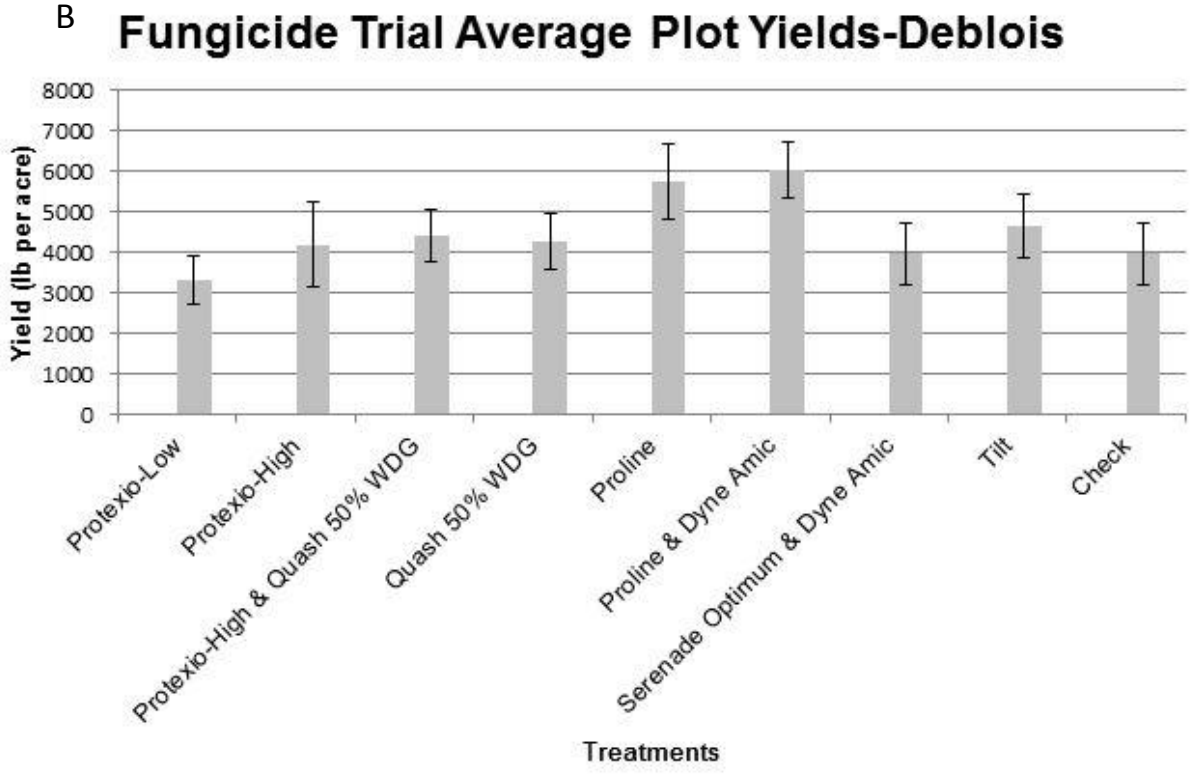


Figure 4. – Average percentage of stems with symptoms of frost damage in fungicide trials at Township 19 and Deblois fields. Error bars represent standard error of the mean of 7 replicates. There were no significant differences among the treatments within a field.

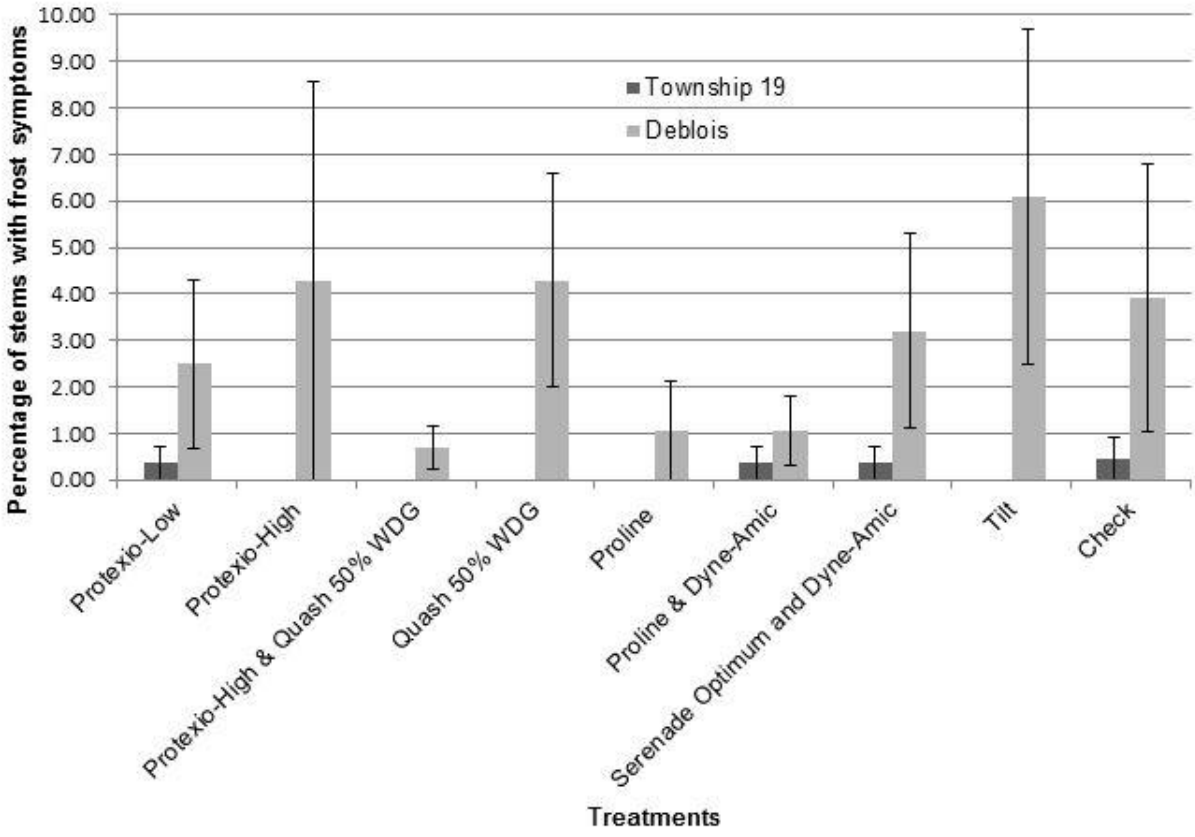
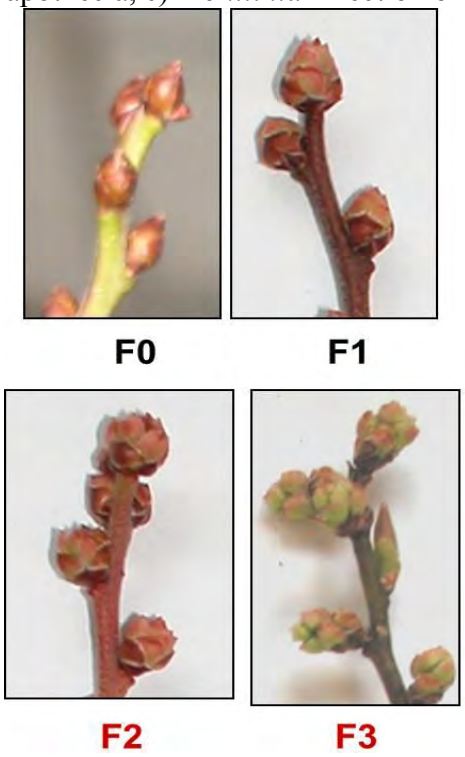


Figure 5. – Pictures showing a) flower bud development stages, b) development of *Monilinia* apothecia, c) *Monilinia* infection of leaves, d) assessment of *Monilinia* flower infection.



a. Flower bud development stages. F0 and F1 are not susceptible; F2 and all stages afterwards are susceptible.

b. *Monilinia* apothecia development from pinhead (i), to mature apothecia producing spores (iii) to old apothecia (iv).

c. *Monilinia* leaf infection, early stages

d. assessment of *Monilinia* infection (flowers)

WEED MANAGEMENT

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

9. TITLE: A 2014 preliminary trial for a Callisto-Matrix tank mix versus a traditional wild blueberry herbicide spray regimen.

OBJECTIVE: Matrix (rimsulfuron) is labeled for use in wild blueberry, but as an early postemergence application. Grower applications have gone on as a late application in combination with Callisto. We collected data from this application to evaluate phytotoxicity and effectiveness of this treatment to an adjacent preemergence application.

METHODS: In 2014, the field manager for Coastal Blueberries told us that he had sprayed a late post-emergence Callisto-Matrix tank mix on some of his fields, and that he had gotten the same or better weed control with this tank mix compared to Callisto and Arrow. We evaluated a Hart's Blueberries field in Union, ME which is adjacent to a Coastal field that had received the Callisto-Matrix tank mix, by assessing ten 1-m^2 plots in each field on 21 July 2014 to compare effects on wild blueberry cover and phytotoxicity, broadleaf weed cover, and grass cover. The Coastal field received a combination of Callisto at 3 oz/a plus Matrix 2 oz/a, Arrow 8 oz/a and a LI-700+Choice at 1 pt/100 gal on 17 June 2014. The Hart's field received Callisto 6 oz/a, Sinbar 2 lb/a and diuron 1.6 qt/a on 31 May 2014.
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). The two fields were compared using t-tests (α=0.05).

RESULTS: The Hart's field had almost continuous wild blueberry cover, and was significantly higher than the Coastal (Callisto/Matrix/Arrow) field which was more rocky (Figure 1, Photos 1-2). Phytotoxicity was significantly higher in the Coastal field, but at 4% minor chlorosis versus 0% it was acceptable. Broadleaf weed cover was extremely low (<2%) and both fields were comparable. There were no grasses assessed in either field, and so results are not graphed here.

Figure 1. Wild blueberry cover and phytotoxicity, and broadleaf weed cover in the Coastal field which received Callisto, Matrix, and Arrow versus the Hart's field which received Callisto, Sinbar and diuron (α=0.05; different letters denote significance).

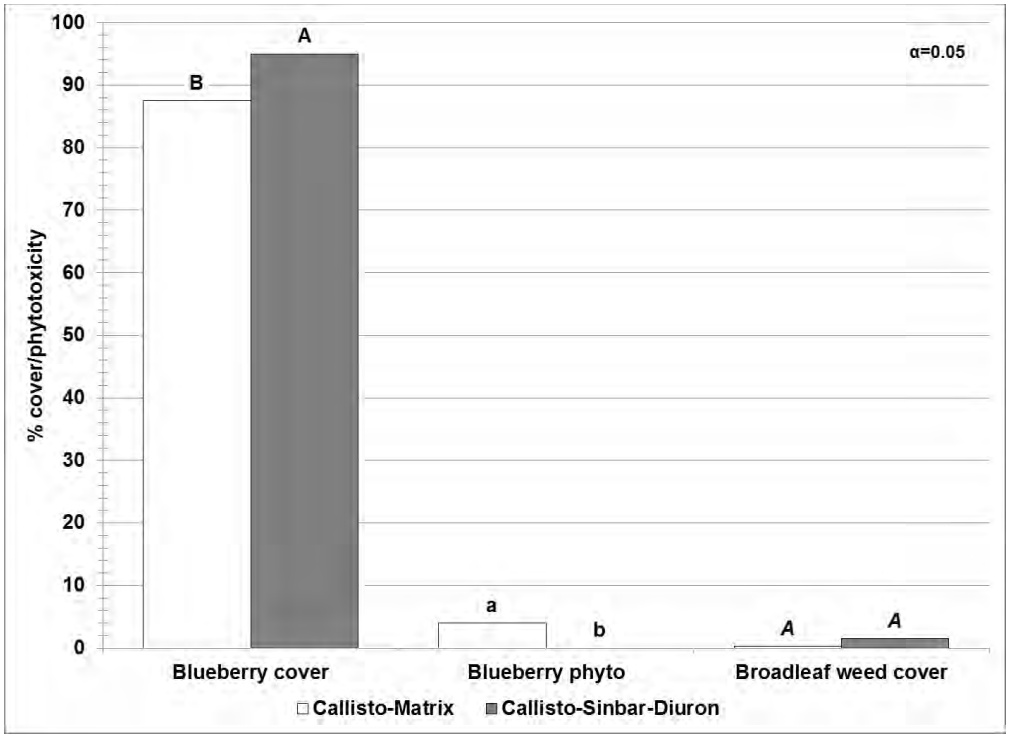


Photo 1. Example of a plot assessed in Hart's field where Callisto, Sinbar and diuron were sprayed in 2014.

Photo 2. Example of a plot assessed in Coastal's field where Callisto, Matrix and Arrow were sprayed in 2014; note that the field is rockier than Hart's field in Photo 1.

CONCLUSIONS: In these two fields, using a Callisto/Matrix/Arrow tank mix did not adversely affect wild blueberry cover or cause unacceptable injury, and it controlled weeds as well as the more "traditional" tank mix in Hart's field. Therefore, we could conclude that using a late postemergence Matrix in a tank mix with Callisto and Arrow could be an effective alternative to the more common groups of herbicides. Matrix is a group 2 herbicide; the only other group 2 herbicide currently used in wild blueberry is Express, which is specifically labeled to control bunchberry in the fall and is not widely used. Sinbar is group 5, the same group as Velpar, which uses are now resulting in resistant weed populations. Diuron is group 7 and is also widely used among growers.

RECOMMENDATIONS: Continue to evaluate a late postemergence application of a combination of Callisto/Matrix/Arrow but compare it within the same field to a check and preemergence application to access its relative effectiveness.

EXTENSION

INVESTIGATOR: David E. Yarborough, Extension Blueberry Specialist

10. TITLE: Wild Blueberry Extension Education Program in 2014.

OBJECTIVE: To provide educational programing to bring research based knowledge to wild blueberry growers in Maine. To collaborate with Canadian researchers and provide relevant information to Maine growers.

METHODS: 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/, through telephone and correspondence, and to 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 Research and Extension priorities, 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, Spring Grower meetings at three locations and a pesticide training session and license exam in Machias, field demonstration sessions conducted three times in three counties and the annual field day at Blueberry Hill Farm where we had a Wild Blueberry Association of North America Marketing Update from Ethos and ongoing research presentations. I also participated in a Barrens tour and educational sessions for a Korean delegation and Bloggers that Ethos had brought to Bar Harbor and participated at the 17th Wild Blueberry Health Summit in Bar Harbor.

Meetings attended:

68th Annual Meeting WSSA and Northeastern Weed Science Society Baltimore, MD February 6-9, 2014.

Blueberry Open House (Rutgers University NJ grower meeting), Hammonton, NJ, March 6, 2014.

North American Research and Extension Workers Conference, Atlantic City, NJ June 24-26, 2014.
17th Wild Blueberry Health Summit, Bar Harbor, ME September 17-19, 2014.
Wild Blueberry Association of North America and Wild Blueberry Research and Extension Workers Annual Meeting, Quebec City, Quebec, October 22-23, 2014.

Presentations:
Evaluation of pre-emergence herbicide combinations to prevent weed resistance in wild blueberry fields in Maine, the Northeastern Weed Science Society Annual Meeting, Philadelphia, PA, January 7-9, 2014.
Wild Blueberry Pest Management Update, Augusta Agricultural Trade Show, Augusta, ME January 9, 2014.
Preventing Weed Resistance in Wild Blueberry Fields and Weed Management, NRCS/Extension in-Service Training and Program Update, Bangor, ME January 16, 2014.
Systems Project Results for Weeds and Fertilizer, Wild Blueberry Spring Meetings, Waldoboro, Ellsworth, Machias, March 18,20,22, 2014.
Wild Blueberry Pesticide License Training, University of Maine, Machias, ME, March 22, 2014.
A systems approach to improving the sustainability of wild blueberry production - NIFA project 2009 – 2014. School of Food and Agriculture Seminar, Orono, ME April 14, 2014.
Maine's Wild Blueberry Industry, Gorham Elementary school 4th Grade Class, Gorham, ME May 9, 2014.
Wild Blueberry Research on Managing Herbicide Resistance, Wild Blueberry Summer Field Day, Jonesboro, ME July 16, 2014.
Maine's Wild Blueberry Industry, Eagle Hill Institute, Stuben, ME August 30, 2014
Maine's Wild Blueberry Industry, New England Guild of Book Workers, Cobscook Community Learning Center, Trescott, ME, September 13, 2014.
Wild Blueberries. Eastern States Expo, Springfield, MA September 26-28, 2014.
Maine's Wild Blueberry Industry. Go Away tours, Bar Harbor, ME, October 13, 2014.
A systems approach to improving the sustainability of wild blueberry Production. Wild Blueberry Association of North America and Wild Blueberry Research and Extension Workers Annual Meeting, Quebec City, Quebec October 22-23, 2014.

Publications:
Wild Blueberry Fact Sheets – 2014:
New:
Fact Sheet #194 Beneficial Insect Series 3: Dung Beetles

Revised:
Fact Sheet #209 (UMCE #2001) 2014 Insect Control Guide for Wild Blueberries
Fact Sheet #239 (UMCE #2025) 2014 Weed Control Guide for Wild Blueberries
Fact Sheet #219 (UMCE #2000) 2014 Disease Control Guide for Wild Blueberries
2014 Maine Wild Blueberry Pesticide Chart 1 of 3 Insecticides
2014 Maine Wild Blueberry Pesticide Chart 2 of 3 Fungicides
2014 Maine Wild Blueberry Pesticide Chart 3 of 3 Herbicides
A Pocket Guide to IPM Scouting in Wild Blueberries, 2nd Edition

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 117,153 page views in 2014 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 (mummy berry monitoring) posts at: http://mainewildblueberries.blogspot.com/

Other program activities:
I am the principle investigator for the NIFA *Sustainable Production of Wild Blueberries*, which provided 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 am the principle investigator on a Specialty Crop Block Grant (SCGB) *Integrated pest management to address weed control resistance in the Maine wild blueberry crop* (2013-2014) and co-investigator with Frank Drummond and Seanna Annis on a SCGB project: *Improving Integrated Pest Management Practices for the wild blueberry crop in Maine* (2014-2015).
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 serve as chair for all full professor promotions. I also serve on the graduate committee of: Alex Bajcz PhD student, Major advisor F. Drummond 2013 –present; Evan Amabile, Honors Thesis, Major advisor Seanna Annis 2014-2015, and supervised an intern from France, Martin Le Tors De Crecy. I serve on the faculty senate for NSFA, and Co-chair of the Service and Outreach Committee and have been 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.

RECOMMENDATIONS: Continue to support the Extension program to provide for the continuation of research based knowledge to be delivered to wild blueberry growers in Maine. Growers benefit in maintaining efficient production practices that allow them to be competitive with cultivated and Canadian production and the public will benefit from production practices that allow growers to produce wild blueberries at an affordable price and volume so that consumers will be able to afford to eat more healthy wild blueberries. The benefits of a healthier society are incalculable.

INPUT SYSTEMS STUDY – *SCRI GRANT OVERVIEW*

11. TITLE: Systems approach to improving the sustainability of wild blueberry production, Year Five of a six-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 four years of the study are presented in Report #19 of the 2010 Project Reports, Report #15 of the 2011 Project Reports, Report #13 of the 2012 Project Reports, and Report #12 of the 2013 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. The second crop cycle had issues with grower cooperation and grower data submission. Therefore, the principal investigators decided a third crop cycle was necessary, and the study was extended for an additional crop cycle of data collection with some growers replaced.

In the second 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.

In the third cycle, the original two Low fields from the first cycle were replaced by two fields managed by a grower in the Union area; the two fields from the second cycle were retained. The Medium system retained the two original sites of the first cycle, but the sites added in the second cycle were replaced by two fields managed by two growers in the Union area. Three Organic growers were replaced due to management and record-keeping issues, leaving one grower common to all three cycles. There were no High input growers in mid-Maine, so all High sites

remained the same as in the second cycle. The "typical" management input parameters for each system, as determined at the start of the project, are presented in Table 1.

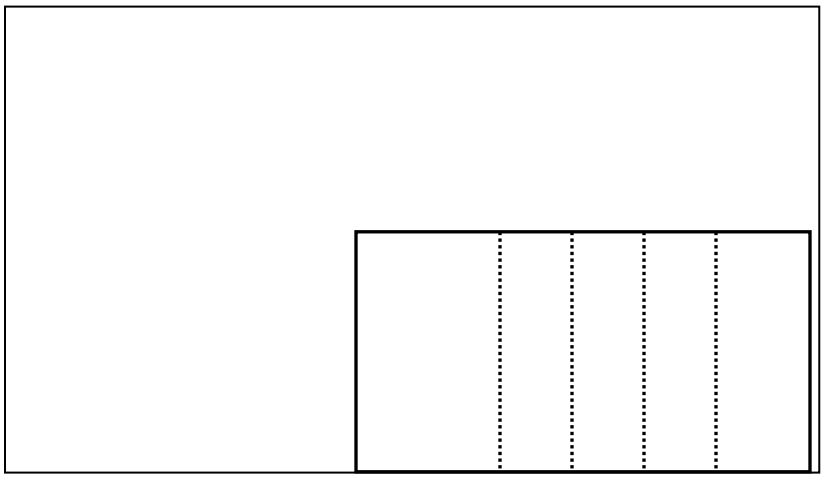
Table 1. Typical levels of inputs in four management systems for the production of wild blueberries.

	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. The sites in the second cycle were numbered 1-16; replacement sites were numbered from #17:
Organic: Fields 1, 17, 18, 19;
Low input: Fields 7, 8, 20, 21;
Medium input: Fields 9, 10, 22, 23;
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 grower inputs were made to each system in 2014 and are found in Table 2.

Sampling

Weed cover assessment along the transects was conducted in September only in 2014. In previous years two assessments were made, but in this prune year the early season evaluation was eliminated because the late season evaluation was a better indicator of weed pressure and effects on yield in the crop year. Leaf and soil sampling for overall nutrient analysis occurred at tip die-back (early July) across the entire block, and disease assessments and insect sampling were also conducted over the entire block. In the fall/winter of 2014, all growers were contacted for their prune year inputs and costs in order to build a preliminary partial budget spreadsheet for the two-year crop cycle.

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

Table 2. 2014 prune year inputs by input system.

Input	Site	Prune	pH	Fertility	Pest control	Disease control	Weed control	Irriga-tion
Organic	1	Mow	Sulfur 200 lb/a	None	None	None	Weed whacking	No
	17	Mow (burn every 3rd cycle, not 2014)	Spot spread over sedges/ grasses	None	Scouting	Scouting	Mulch bare spots Scything Hand weeding Brush saw perimeter	No
	18	Burn + Mow	None (2011)	None	None	None	Hand weeding Mulch with sawdust	No
	19	Mow	None	None	None	None	Hand weeding	No
Low	7	Burn + Mow	None	MAP 200 lb/a Black Label 1 gal/a	Assail 5.3 oz/a	Bravo 3 pt/a	Diuron 1.6 qt/a Sinbar 2 lb/a Velossa 3.2 pt/a w/Grounded Spot/wiper: Glystar 7.5 gal	No
	8	Mow	None	MAP 150 lb/a	None	None	Arrow 6 oz/a Parrot 1.5 qt/a Sinbar 1 lb/a Velossa 0.5 gal/a	No
	20	Burn	None	7-22-5 2 gal/a Black Label 1 gal/a 16-34-4 123 lb/a	None	Initiate 1 qt/a	Callisto 3 oz/a (2x) Diuron 0.4 gal/a Matrix 2 oz/a Velpar 0.5 gal/a w/Choice 4.8 oz/a w/LI-700 4.8 oz/a	No
	21	Burn	None	16-34-4 122 lb/a Black Label 1 gal/a	None	Bravo 1 qt/a	Arrow 7.5 oz/a Callisto 3 oz/a (2x) Diuron 0.4 gal/a Intensity 8 oz/a Velpar 0.5 gal/a w/Credit 1.7 oz/a w/Choice 1.5 oz/a spot: Arrow 1 oz + COC 4 oz in 2 gal water	No

Input	Site	Prune	pH	Fertility	Pest control	Disease control	Weed control	Irriga-tion
Medium	9	Mow	None	16.5-34.5-4.5-0.3B 289 lb/a	Scouting Imidan 1 lb/a	None	Callisto 5 oz/a Clethodim 8 oz/a Velossa 0.4 gal/a Spot: clethodim 100 oz	No
	10	Mow	None	16.5-34.5-4.5-0.3B 300 lb/a	Scouting Imidan 1 lb/a	Bravo Ultrex 2.4 lb/a	Clethodim 8 oz/a (2x) Velossa 0.4 gal/a	No
	22	Burn + Mow	None	16.5-34.5-4.5-0.3B 160 lb/a	Assail 5.25 oz/a Provado 8 oz/a	None	Bush-hog {Diuron 1.6 qt/a Velpar 0.5 gal/a}- not in plots Spot: Callisto+ Poast+ COC Wiper: glyphosate + Credit Extra	No
	23	Mow + Burn edges by wall	None	16-34-04-0.5B 200 lb/a	None	None	Callisto 6 oz/a Diuron 1.6 qt/a Sinbar 2 lb/a Velpar L 1 lb/a	No
High	13	Mow	None	AMS 16.5-34.5-4.5-0.3B 484 lb/a	Scouting	4.25 pt/a Bravo 3 oz/a Proline	Diuron 1 lb/a Sinbar 1 lb/a Velpar 1 lb/a	Yes
	14	Mow	None	AMS 16.5-34.5-4.5-0.3B 484 lb/a	Scouting	Bravo 4.25 pt/a Proline 3 oz/a	Diuron 1 lb/a Matrix 4 oz/a Velpar 1 lb/a Spot/ac: Callisto 3 oz+ LI-700 2 pt+ Request 2 pt	Yes
	15	Mow	None	AMS 16.5-34.5-4.5-0.3B 484 lb/a	Scouting	Bravo 4.25 pt/a Proline 3 oz/a	Diuron 1 lb/a Sinbar 1 lb/a Velpar 1 lb/a Spot/ac: Callisto 3 oz+ LI-700 2 pt+ Request 2 pt	Yes
	16	Mow	None	AMS 16.5-34.5-4.5-0.3B 484 lb/a	Scouting	Bravo 4.25 pt/a Proline 3 oz/a	Diuron 1 lb/a Sinbar 1 lb/a Velpar 1 lb/a Spot/ac: Callisto 3 oz+ LI-700 2 pt+ Request 2 pt	Yes

INPUT SYSTEMS STUDY

FOOD SAFETY & NUTRITION: Vivian Wu, Professor of Food Safety and Microbiology, School of Food and Agriculture
RESEARCH ASSOCIATES: Shravani Tadepalli, Special Project Assistant, School of Food and Agriculture

12. TITLE: Food safety- Prevalence study of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. on lowbush blueberries (*Vaccinium angustifolium*) (*Bacteriological analysis 2010-11 and 2012-13 crop cycles*).

OBJECTIVE: We conducted bacteriological analysis to evaluate the presence or absence of potential foodborne pathogens associated with two crop cycles using traditional culture methods and also alternative PCR screening methods on harvested lowbush blueberry samples.

METHODS: For 2010-2011 crop cycle, a total of 40 blueberry samples from four management systems (organic, low, medium and high) of 8 different farms were evaluated and for 2012-2013 crop cycle, a total of 32 samples from 16 locations of 9 different farms were evaluated. For the 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** (polymerase chain reaction) 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:

Crop cycle 2010-2011:

No *E. coli* O157:H7 and *L. monocytogenes* were isolated either through culture methods or PCR screening from any of the forty harvested blueberry samples. *Salmonella* spp. was isolated from nine out of forty blueberry samples through culture methods (Table 1) while through PCR screening, eleven samples out of forty blueberry samples were screened to be positive (Table 2). Overall there are five samples which were common positives for *Salmonella* spp. with both culture and PCR methods (Figure 1). Later, in order to identify the false positives from these culture and PCR positives, they all were subjected for serological confirmatory testing and we found out that culture methods showed one false positive with eight out of nine positives confirmed as *Salmonella* serological positive, and PCR method showed 5 false positives with only six out of eleven samples confirmed as *Salmonella* serological positive. Since traditional culture methods have fewer false positives compared to PCR method, we can conclude that eight samples which are culture positive and also serology positive as *Salmonella* positive (Table 3).

Crop cycle 2012-2013:

L. monocytogenes was not isolated either through culture methods or PCR screening from any of these 32 harvested blueberry samples. *Salmonella* spp. was isolated from 5 out of 32 blueberry samples through culture methods (Table 4), while through PCR screening, 6 samples out of 32 blueberry samples were screened positive (Table 5). Overall, there were three samples which were common positive for *Salmonella* spp. with both culture and PCR methods. Figure 2 shows the number of *Salmonella* positives obtained from each management system (out of four management systems-low, medium, high, and organic inputs). However, after serological confirmation for these PCR and culture positives, all 5 culture positives were confirmed as *Salmonella* serological positive while with PCR only 4 out of 6 are confirmed as *Salmonella* serological positive. Since the traditional culture method has no false positives we can conclude all the culture positives (5 samples) as *Salmonella* positive. 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 non-O157 *E. coli* positive. It was suspected as shiga toxin -producing *Escherichia coli* (**STEC**). Since STEC is a raising food safety concern these days, another PCR screening test was done to detect *stx*1 and *stx*2 virulent genes for STEC. It was found positive for these two virulent genes, indicating that it was presumptive shiga toxin -producing *E. coli* (STEC). Table 6 indicates the steps involved in non-O157 STEC detection for the positive blueberry sample.

CONCLUSIONS: Traditional culture methods have fewer false positives compared to PCR. Though PCR provides faster results, culture methods are more accurate in detecting *Salmonella* from blueberries. In crop cycle 2010-2011, contamination levels of *Salmonella* occurred mostly in the organic and medium input samples. In crop cycle 2012-2013, the high and medium input management systems were found to have positives for *Salmonella* spp. and organic input was positive for STEC. The differences of fertilizer application may be the reason for contamination levels of *Salmonella*. Though the incidence of *Salmonella* spp. in these blueberries was quite low (20% in crop cycle 2010-11 and 15.6% in crop cycle 2012-13), they still represented a risk to the consumer in regard to foodborne disease. Finding of non-O157 STEC (3.1% incidence) in these blueberries is important, taking into consideration that there is increased incidence of STEC outbreaks during the past decade.

RECOMMENDATIONS: Potential contamination of blueberries is possible and can be introduced through human management activity from pre-harvest production through post-harvest handling and processing. Since production practices such as crop inputs, soil fertility management, and water quality can influence the risk of contamination, care should be taken to prevent foodborne outbreaks.

Table 1: Blueberry samples positive for *Salmonella* spp. through traditional culture methods in 2010-2011 crop cycle.

SCRI ID	Culture or Enterotube test status for *Salmonella* spp.
Field#14, High, block#3	Culture positive
Field#9, Medium, block#1	Culture positive
Field#9, Medium, block#2	Culture positive
Field#10, Medium, block#5	Culture positive
Field#10, Medium, block#6	Culture positive
Field#10, Medium, block#7	Culture positive
Field#1, Organic, yes mulch, block#6	Culture positive
Field#1, Organic, yes mulch, block#5	Culture positive
Field#1, Organic, no mulch, block#3	Culture positive

Table 2: Blueberry samples positive for *Salmonella* spp. through PCR methods in 2010-2011 crop cycle.

SCRI ID	PCR status
Field#10, Medium, block#6	Salmonella PCR positive
Field#10, Medium, block#7	Salmonella PCR positive
Field#1, Organic, Yes mulch, block#5	Salmonella PCR positive
Field#1, Organic, Yes mulch, block#6	Salmonella PCR positive
Field#2, Organic, No mulch, block#3	Salmonella PCR positive
Field#1, Organic, Yes mulch, block#7	Salmonella PCR positive
Field#1, Organic, No mulch, block#7	Salmonella PCR positive
Field#1, Organic, yes mulch, block#8	Salmonella PCR positive
Field#2, Organic, yes mulch, block#1	Salmonella PCR positive
Field#2, Organic, No mulch, block#2	Salmonella PCR positive
Field#2, Organic, No mulch, block#4	Salmonella PCR positive

Figure 1: Number of blueberry samples that are *Salmonella* PCR and culture positives from different cropping systems in 2010-2011 crop cycle.

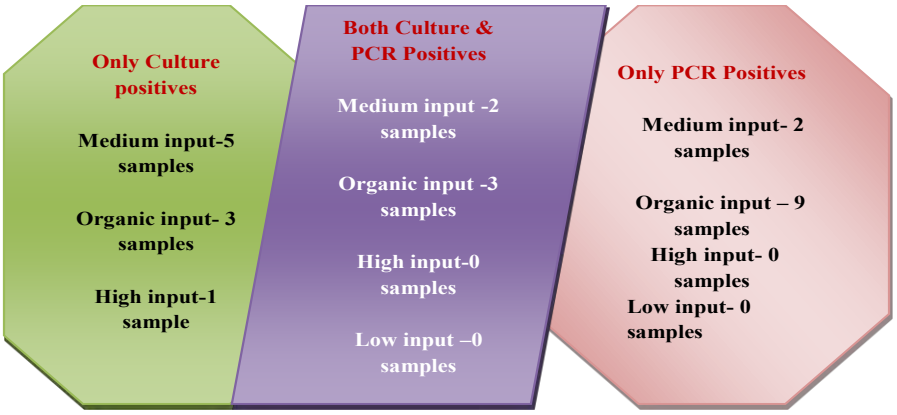


Figure 2: Number of *Salmonella* positives from different cropping systems in 2012-2013 crop cycle.

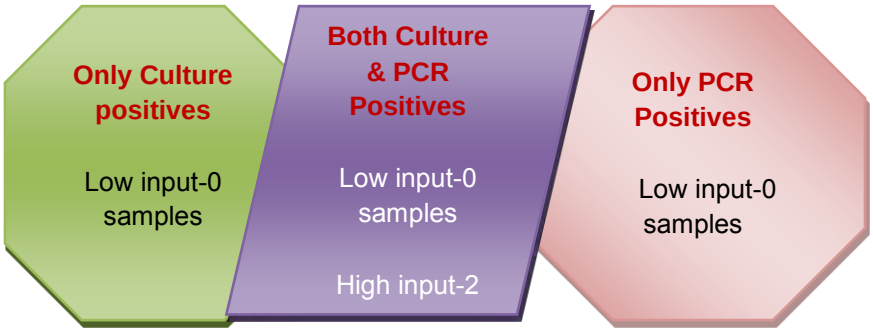


Table 3: List of blueberry samples confirmed to have *Salmonella* spp. in 2010-2011 crop cycle.

SCRI ID	Culture status	Final interpretation
Field#9, Medium, block#1	Culture positive	Confirmed *salmonella*
Field#9, Medium, block#2	Culture positive	Confirmed *salmonella*
Field#10, Medium, block#5	Culture positive	Confirmed *salmonella*
Field#10, Medium, block#6	Culture positive	Confirmed *salmonella*
Field#10, Medium, block#7	Culture positive	Confirmed *salmonella*
Field#1, Organic, yes mulch, block#6	Culture positive	Confirmed *salmonella*
Field#1, Organic, yes mulch, block#5	Culture positive	Confirmed *salmonella*
Field#1, Organic, no mulch, block#3	Culture positive	Confirmed *salmonella*

Table 4: Blueberry samples positive for *Salmonella* spp. through the traditional culture method in 2012-2013 crop cycle.

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

Table 5: Blueberry samples positive for *Salmonella* spp. through the PCR method in 2012-2013 crop cycle.

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

Table 6: 2012-13 crop cycle- List of samples showing STEC positives.

SCRI ID	Culture status	Serological confirmation status	PCR for eae A gene detection	Stx1 & Stx2 PCR status	Final interpretation
Filed # 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)

INPUT SYSTEMS STUDY

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

13. TITLE: Systems approach to improving the sustainability of wild blueberry production, Year 5 – Reports from Frank Drummond.

METHODS:

Blueberry stem measurements

In April 2015, all the stems from each of ten, 15.2 x 15.2 cm (6 x 6 in) quadrats per field will be cut at ground level, brought into the laboratory, and counted to determine stem density, stem length, and branching. Ten stems will also be 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 ($P \leq 0.05$) will be used to compare stem density, stem length, flower-bud clusters per stem, and branching among the treatments. Subplots will be pooled within main plots. Data will transformed by the square root to stabilize variance prior to analysis.

Abundance of natural enemies and pest insects

Sweep-net survey

Samples were taken between 17 and 23 Jun. Ten sets of ten sweeps each were taken from each field (two, 1 acre blocks/field) with a 12-inch diameter sweep net. Samples were distributed through the blocks 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. Data were analyzed using Analysis of Variance (CRD)($P \leq 0.05$). Mean separation was by Least Square Means. Subplots were pooled within main plots. Data were transformed by the square root prior to analysis.

Thrips Survey

Between 17 and 23 Jun, thrips damage was measured along each of six 100 ft transects per field (3 per block). Damage was rated by assessing the amount of damage as evidenced by curled leaves. Damage was rated on a scale of 1 to 5 as outlined in Table 1, below.

Table 1. Rating system for thrips damage assessment.

0	no damage
1	a few curls widely scattered
2	Curls along < ½ of the transect, but no patches
3	Scattered curls along > ½ the transect
4	1-2 patches ≥ 2 ft^2 + scattered curls
5	3 or more large patches + scattered curls

Tip Midge Survey

Tip midge damage was assessed between 17 and 23 Jun by counting the number of blueberry stems with damage as evidenced by curled leaves in each of ten, m^2 subplots per field (5 per

block). Data were analyzed using Analysis of Variance (CRD)($P \leq 0.05$). Mean separation was by Least Square Means. Subplots were pooled within main plots. Data were transformed by the square root prior to analysis.

RESULTS:

Sweep-net survey

Ants and spiders were the most abundant natural enemies (Fig. 1). There was a significant difference in the number of ants and spiders between the production system treatments ($F_{(3,28)}$ = 5.00, P = 0.007, ants; $F_{(3,28)}$ = 4.34, P = 0.012, spiders). Significantly more ants were found in the organic compared to low, medium, and high-input systems. And, the organic system had more spiders than the high or low systems; more spiders were also found in the medium-input compared with the low-input system.

Pest abundance was very low, with no pest exceeding threshold numbers during the season. The most abundant pest insect found in sweep-net samples was grasshoppers (Fig. 2). Although an ANOVA indicated no significant difference, LSD mean separation did indicate that significantly (at the $\alpha = 0.1$ level) more grasshoppers were present in the high compared with the low-input system. ($F_{(3,28)}$ = 2.19, P = 0.112). Small numbers of blueberry spanworm larvae, strawberry rootworm adults, blueberry leaf beetles, tarnished plant bugs, and unidentified caterpillars were also found in the samples.

Fig. 1. Relative abundance of natural enemies in sweep-net samples (bars are means and error line segments represent standard errors of the mean).

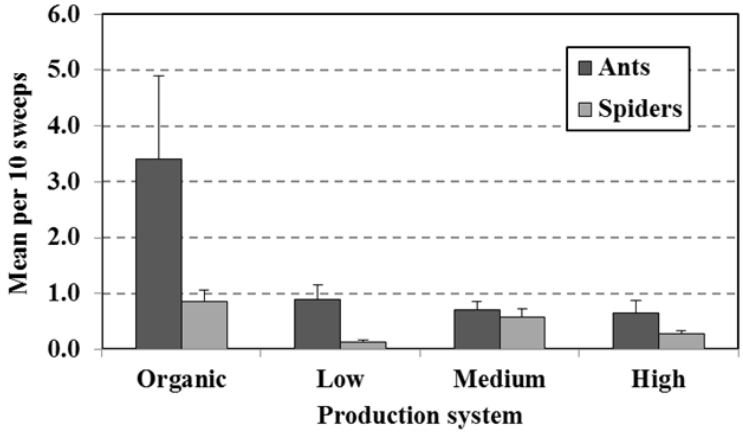
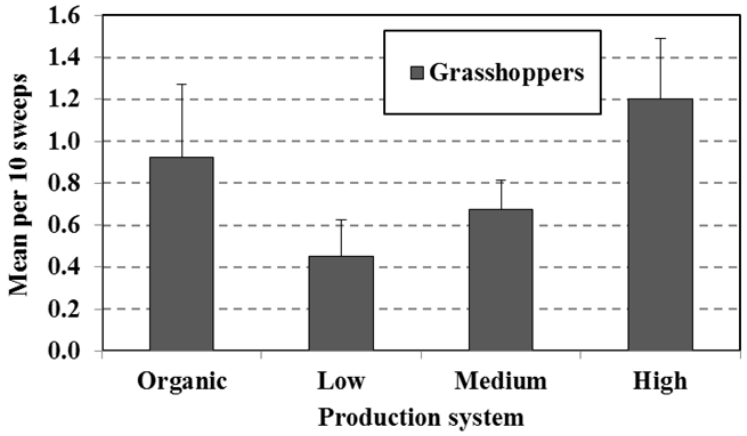


Fig. 2. Relative abundance of pest insects in sweep-net samples (bars are means and error line segments represent standard errors of the mean).



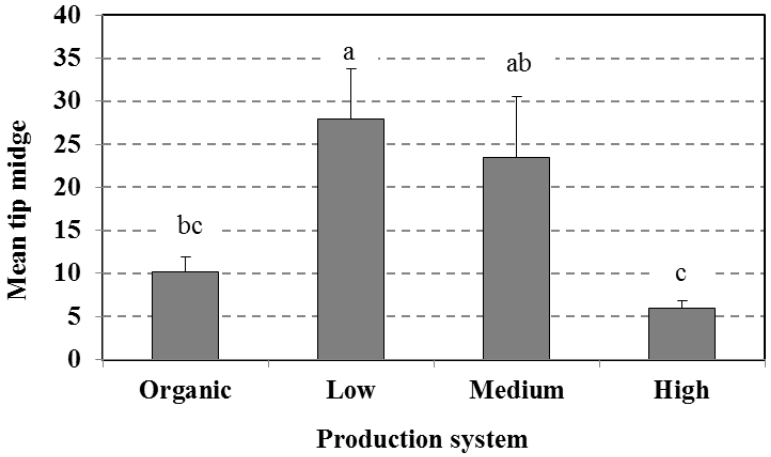
Thrips Survey

Thrips populations were very low in 2014. Only one small patch with a few widely scattered stems with curls (rated 1) was found among all the samples of 16 fields. The one infestation was in an organically-managed field.

Tip Midge Survey

There was a significant difference in damage by tip midge as evidenced by stem with curls. Significantly more tip midge were found in low and medium production sites compared with high production sites ($F_{(3,28)} = 6.13$, $P = 0.002$) (Fig. 3).

Fig. 3. Abundance of tip midge, mean per production system (bars are means and error line segments represent standard errors of the mean, different letters represent significantly different means).



CONCLUSIONS: The 2014 year of the systems study represents the 5th year of a six-year study. In previous years we also found that natural enemies of insect pests of blueberry, mostly spiders and ants, are more abundant in organic crop systems than low, medium, or high input conventional production systems. In accordance with these findings on natural enemies we also have observed no greater abundance of blueberry insect pests in organic production systems compared to conventional systems. While grasshopper densities appeared high in organic fields in 2014, the only differences seen were between low and high input systems. Tip midge has been a phenomenon in the recent past two decades that continues to perplex us. Insecticide control has been somewhat ineffective of this pest and in addition, it is suspected that insecticide applications may intensify its densities. In 2014 we found that densities were significantly greater in low and medium input systems compared to high input and organic systems. A similar pattern of this infestation was also observed in 2010 and 2012. After year six of the systems project it is hoped that a statistical analysis can provide greater insight into some of these findings. At this point we feel that evidence suggests from these field studies that natural enemies provide a high degree of pest insect regulation in wild blueberry fields.

INPUT SYSTEMS STUDY

DISEASE MANAGEMENT: Seanna Annis, Assoc. Professor, School of Biology and Ecology
Rachael Martin, Research Asst., School of Biology and Ecology
Tamara Levitsky, Research Asst., School of Biology & Ecology

14. TITLE: Systems approach to improving the sustainability of wild blueberry production, 2014, Year 5 of a six-year study, disease management results.

OBJECTIVE: In 2014 we continued to examine differences in leaf loss and disease levels between field management types (organic, low, medium and high input).

METHODS: Diseases were rated by block in each field between September 17 and 29, 2014. Within each block, 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, and leaf rust. Any red leaf and false Valdensinia disease were also noted. 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 blueberry cover, disease coverage and leaf loss, and the number of plots with each disease in **SAS** (Statistical Analysis Software - SAS Cary, NC) using mixed model procedures (PROC GLIMMIX). Percentage data was arcsin transformed prior to analysis. Least Square means were used to determine specific differences among system types ($\alpha = 0.05$). Data were analyzed at the 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: Blueberry cover in September was significantly higher in the medium and high input systems than in the organic and low input systems (Fig. 1) as seen in previous years with a partially different set of fields. There was a significant interaction of management type and field. Fields of different management types were not always similar to other fields of the same management type. The blueberry cover in Field 19, an organically managed system, and Field 8, a low input system, were not significantly different from high or medium input fields and were significantly different from the other low and organic fields.
There were significantly higher levels of leaf loss in the medium and organic input systems in the prune year compared to the low and high input systems (Fig 2). The range of leaf loss amongst fields within each system type was variable and overall leaf loss ranged from (0 to 73%) with field 9 in the medium input system having significantly higher levels of leaf loss than all other fields.
For all leaf spot diseases, the percentage of plots with a specific disease was not significantly different between the management systems (Fig. 3A, 4A, and 5A). There was also no significantly difference in the number of plots with symptoms of *Phomopsis* stem blight (Fig. 6A). There were significant differences amongst the management systems in the percentage of leaf area with different leaf spot diseases and the levels of *Phomopsis* leaf blight found. Powdery mildew symptoms were found in every field but symptoms did not affect a large percent of the leaf area and ranged from 1.5 to 17.5%. The levels of powdery mildew were

significantly lower in the high input system compared to the other systems (Fig 3B.) The levels of Septoria leaf spot symptoms were also very low with fields ranging from 0 to 7% of leaf area. The low and organic input systems had significantly higher levels of leaf area with Septoria symptoms than the medium and high input systems (Fig. 4B).
Rust levels were significantly lower in the high input systems than in the other systems (Fig. 5B). Rust affected from 0 to 34% of the leaf area in the various fields. There was a significant interaction of management type and field.
Phomopsis levels were significantly higher in high input systems than in medium, low or organic systems (Fig. 6) which is different than in previous years where the high and medium input fields had higher levels of *Phomopsis* than the low and organic input fields. This difference in may be due to the switching of fields since two medium input fields were switched with ones outside Washington county. In 2014, *Phomopsis* affected stems were found in ten of the 16 fields. The unaffected fields were organic or low input and one of the medium input fields examined. The levels of *Phomopsis* may be affected by the geographic location more than the levels of field inputs.
Preliminary analyses of leaf spot diseases found there was a significant correlation between increased levels of rust and increased levels of leaf loss (Fig. 7) as was found with fields in the last prune year (2012) examined in the **SCRI** (Specialty Crop Research Initiative) study. Rust levels may be a factor in leaf loss but is not the only one, since some fields with high levels of rust had moderate levels of leaf loss and some fields with moderate levels of leaf loss had lower levels of rust. Multiple factors, including diseases, soil, nutrition and water, are probably affecting leaf loss. Further analysis will try to determine the effects of management practices on disease levels.

CONCLUSIONS/RECOMMENDATIONS: Management inputs can affect the level of leaf and stem diseases present during the prune year and may affect crop production in the following year. Since early leaf loss can affect production of flower buds, control of leaf loss is a priority for growers. Further analysis of the levels of stem and leaf diseases in the prune year must be taken into account along with the levels of disease in the crop year when considering effects of management practices on yield.

Figure 1. - Average percent of blueberry cover by management input types for September 2014. Error bars indicate standard error of the mean. Bars with different letters indicate statistically significant differences at α =0.05.

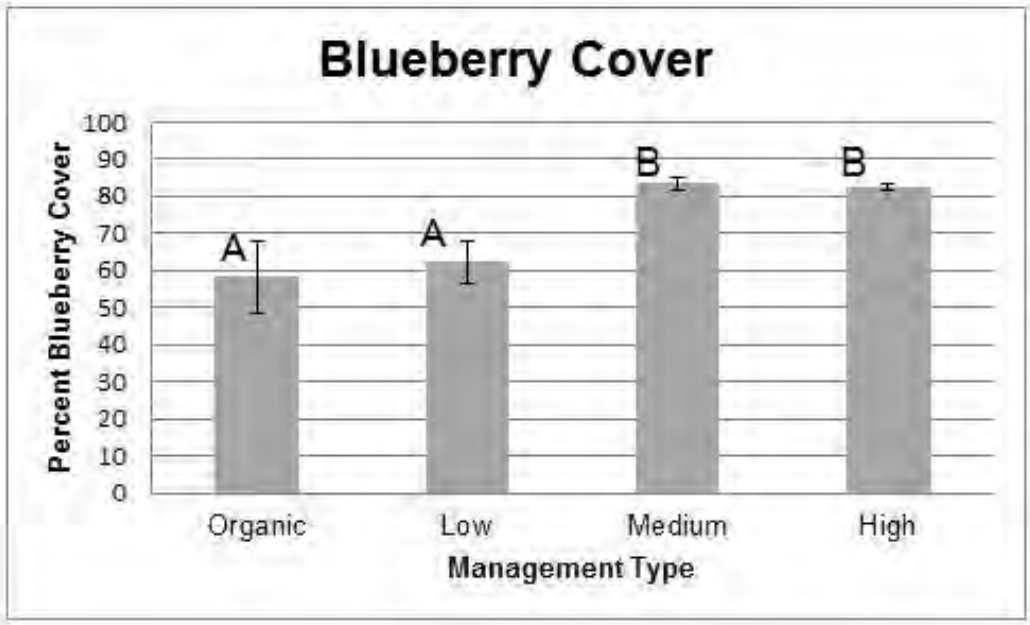


Figure 2. – Average percent leaf loss by management input types for September 2014. Error bars indicate standard error of the mean. Bars with different letters indicate statistically significant differences at α =0.05.

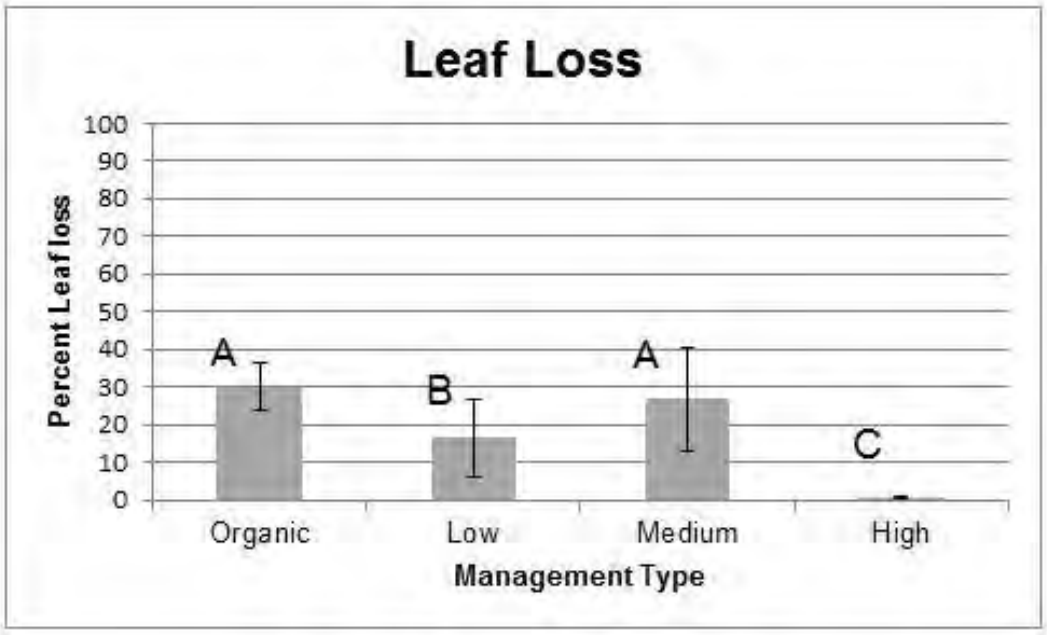
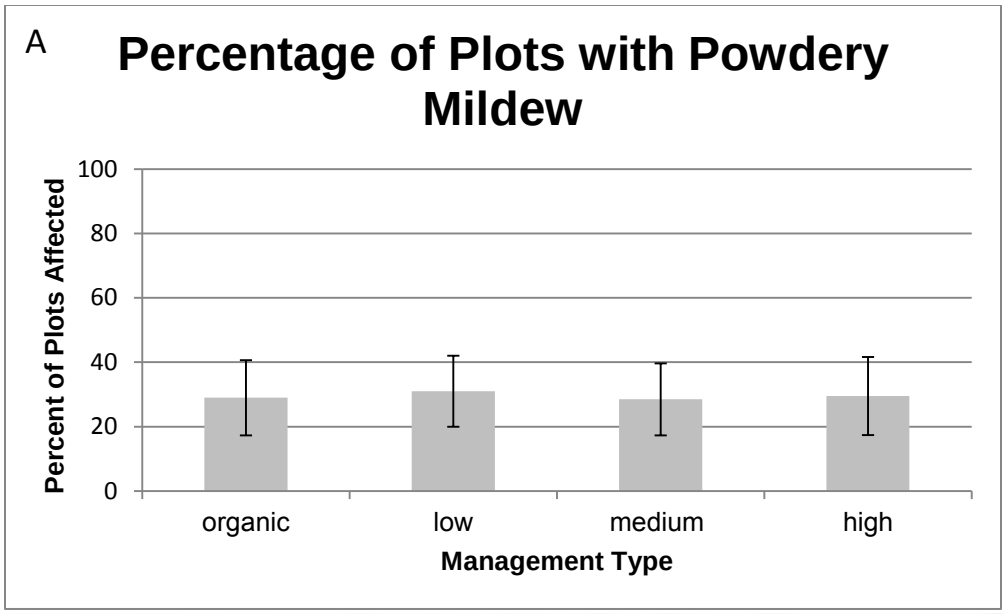


Figure 3. -Average percent of plots with Powdery Mildew (A) and average percent of leaf area affected by Powdery Mildew (B) by management input types for September 2014. Error bars indicate standard error of the mean. Bars with different letters indicate statistically significant differences at $\alpha = 0.05$.



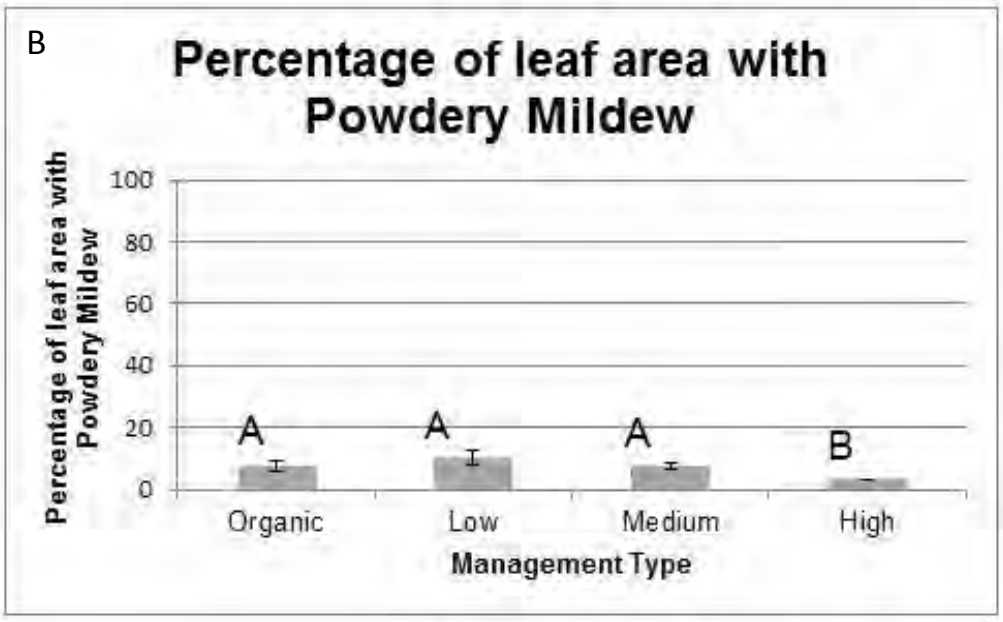
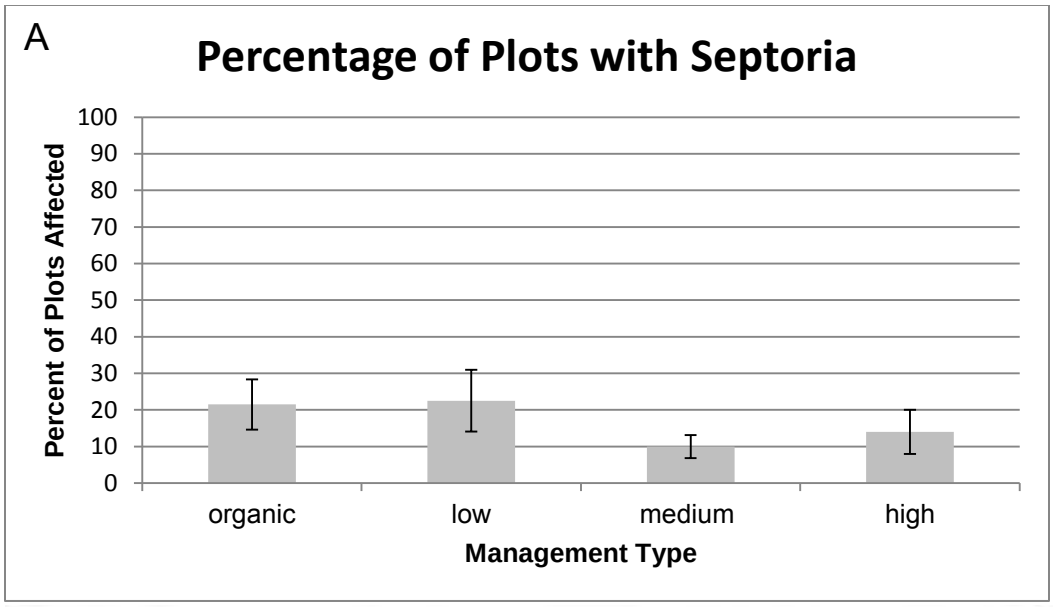


Figure 4. -Average percent of plots with Septoria (A) and average percent of leaf area affected by Septoria (B) by management input types for September 2014. Error bars indicate standard error of the mean. Bars with different letters indicate statistically significant differences at α=0.05.



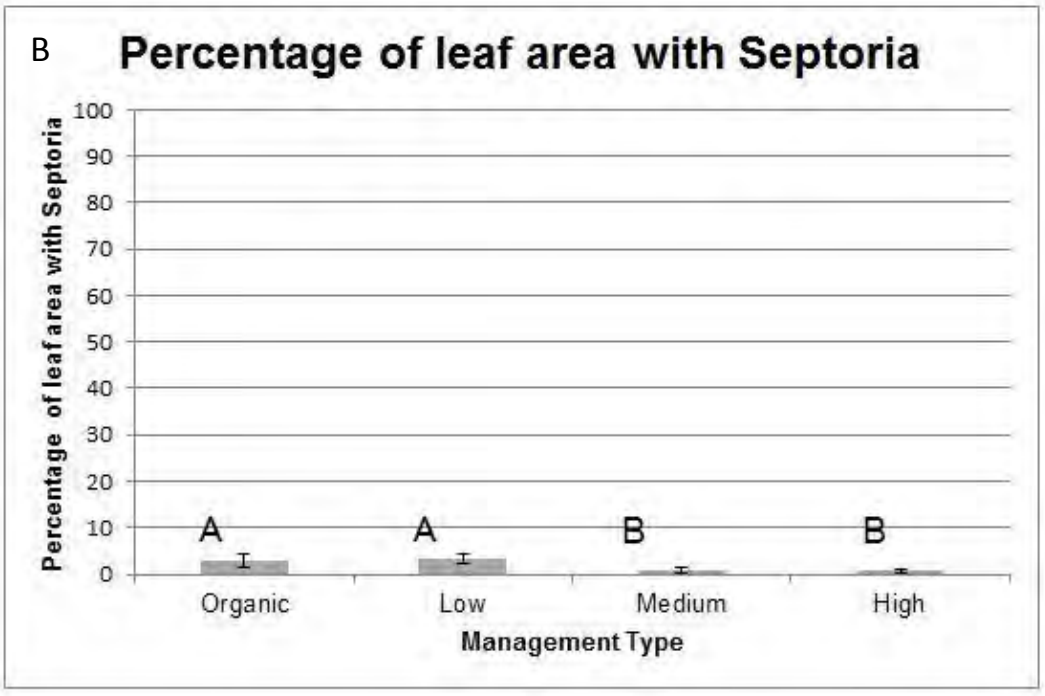
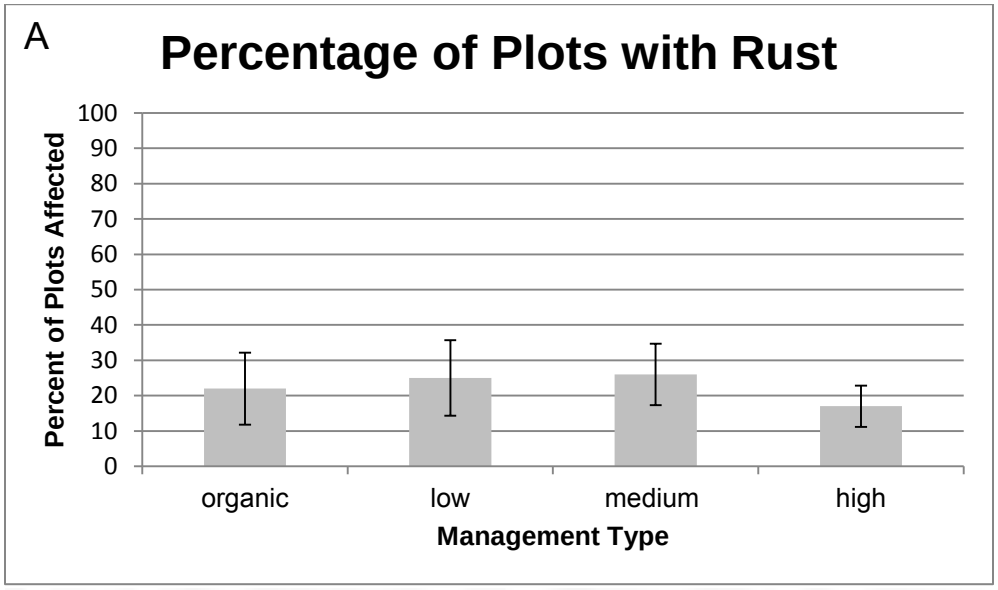


Figure 5. – Average percent of plots with leaf rust (A) and average percent of leaf area affected by rust (B) by management input types for September 2014. Error bars indicate standard error of the mean. Bars with different letters indicate statistically significant differences at α =0.05.



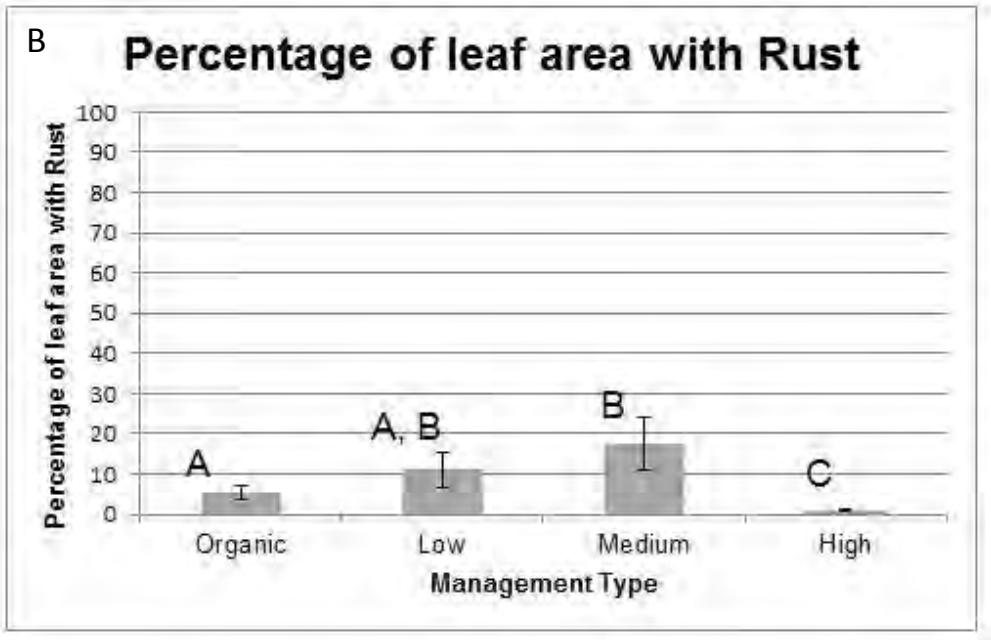
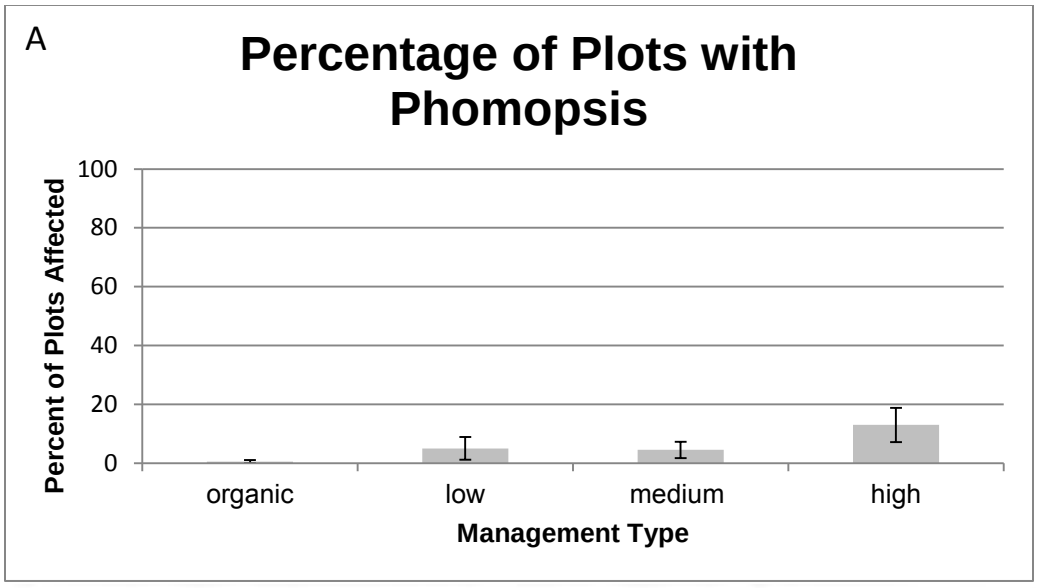


Figure 6. - Average percent of plots with *Phomopsis* (A) and average percent of leaf area affected by *Phomopsis* (B) by management input types for September 2014. Error bars indicate standard error of the mean. Bars with different letters indicate statistically significant differences at $\alpha = 0.05$.



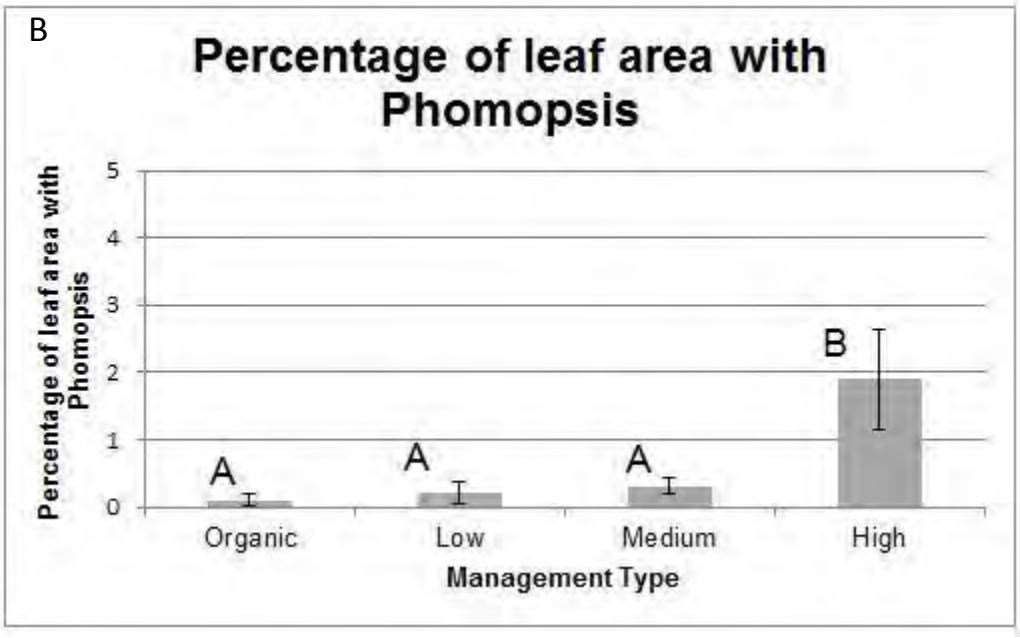
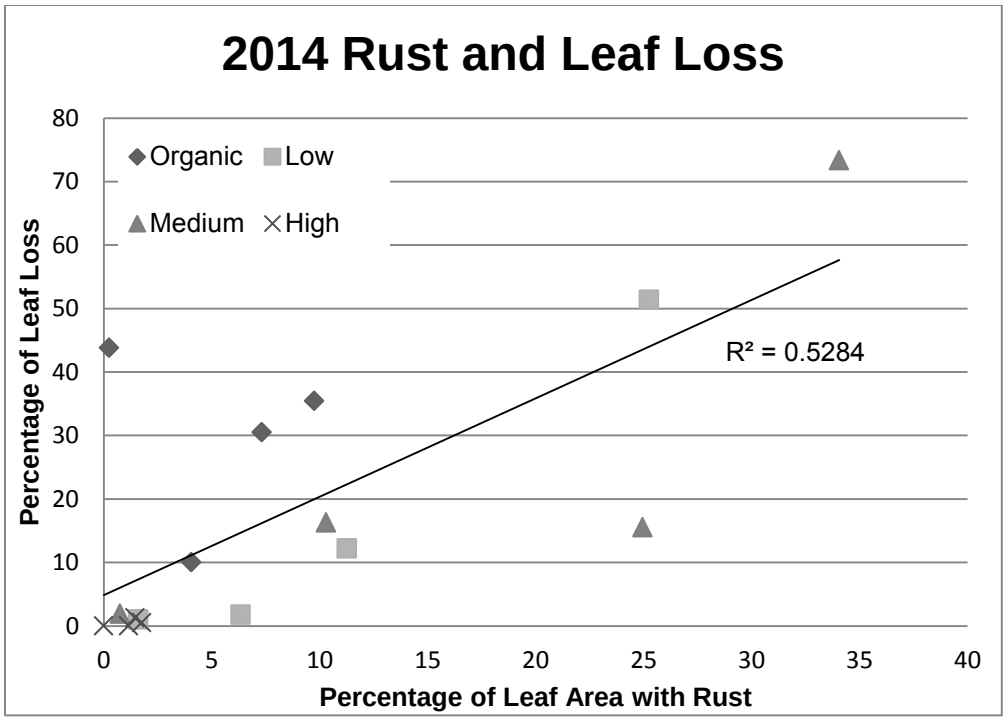


Figure 7. - Average percentage of leaf area with rust plotted with the average leaf loss per field. The solid line represents a best fit linear regression line for all management inputs ($R^2 = 0.5284$). Diamonds represent organic input systems, squares represent low input systems, triangles represent medium input systems and Xs represent high input systems.



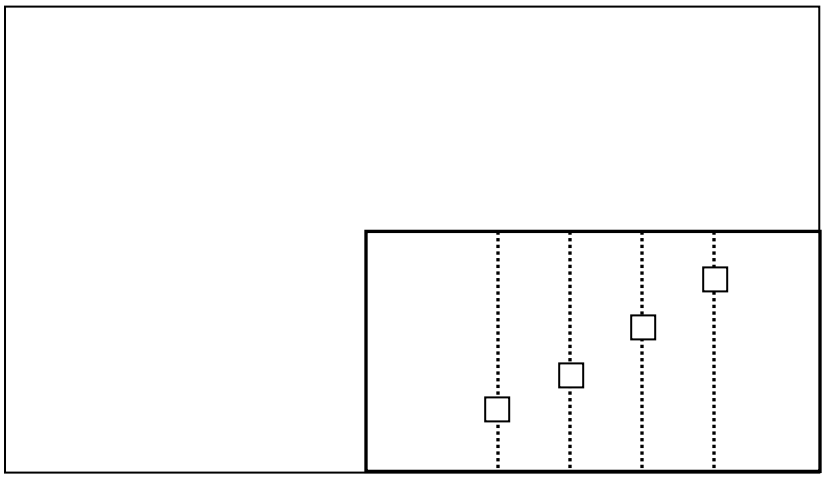
INPUT SYSTEMS STUDY

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

15. TITLE: Systems approach to improving the sustainability of wild blueberry production, Year Five of a six year study, weed management results.

METHODS: The third crop cycle study design and 2014 prune year inputs are listed in Table 2 of the overall Experimental Design (Report #11). In 2014 16 trial sites were once again 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, seven sites were replaced due to either a lack of grower cooperation, or in order to encompass a wider geographical range.

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



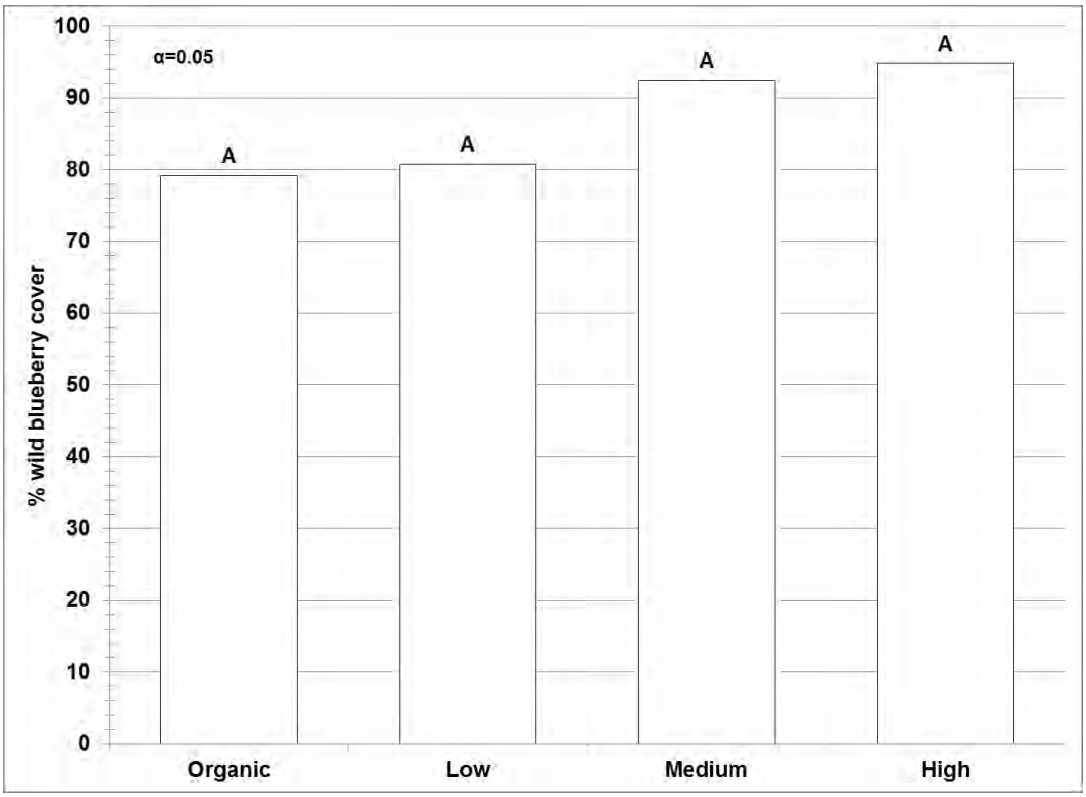
Blueberry cover, woody weed cover, broadleaf weed cover and grass cover were assessed in all 1 m^2 sample plots on 4-9 September 2014. In the two previous cycles, two weed evaluations were conducted in the prune year; we eliminated the early season evaluation because the late season evaluation was a better indicator of weed pressure and effects on yield in the crop year. Cover was assessed using the Daubenmire Cover Class scale, which were converted to percent cover; 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 (α=0.05). Overall blueberry cover and weed cover comparisons were made among all four input systems.

RESULTS:

Blueberry and weed cover

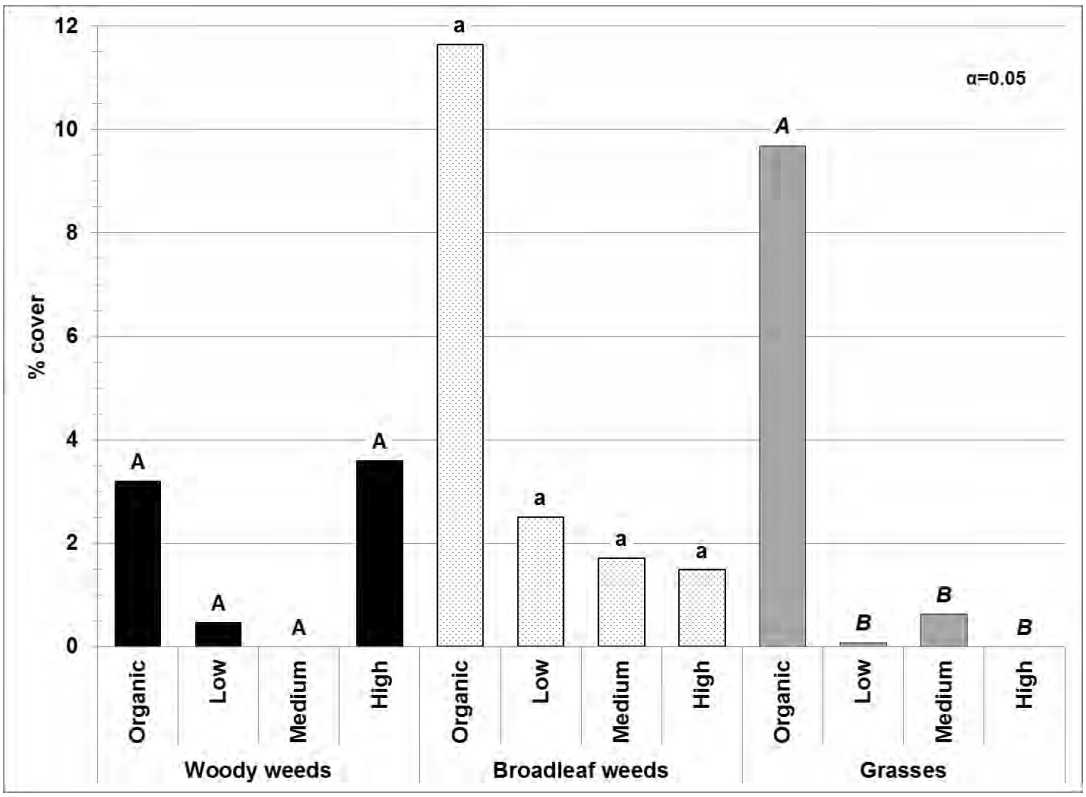
There were no significant differences in wild blueberry cover among the four systems (Figure 2). In 2014 blueberry cover followed a slightly different trend compared to 2011-13. In previous years, by August the Medium system had the highest cover, followed by the High system. This year, the High system had the highest cover. The difference between this year and previous years is that in previous crop cycles, all Medium sites were managed by one company and were located Downeast; in this cycle, they were managed by three companies and ranged from the Union area to Downeast.

Figure 2. Wild blueberry cover by input management system in prune year 2014 (Tukey's HSD, different letters denote significance at α=0.05).



Woody weed cover was very low overall (<4 %), and there were no significant differences among systems (Figure 3). Broadleaf weed cover followed the same trend as in previous cycle; although there were no significant differences, the Organic system had the highest broadleaf weed cover, while the conventional management systems were around 4 % cover and under (Figure 3). Finally, grass cover also followed the trend seen in the previous cycle, where the Organic system had significantly more grasses than the conventional management systems (Figure 3).

Figure 3. Woody weed, broadleaf weed and grass cover among input systems in prune year 2014 (Tukey's HSD, different letters denote significance at α=0.05).



CONCLUSIONS: In this crop cycle, wild blueberry cover and weed cover followed the same basic trends as the previous two cycles. Wild blueberry cover was comparable among systems, and overall weed cover was highest in the Organic system compared to the conventional management systems (Photos 1A-4), with the exception that woody weed cover in the High system was equal to the Organic system this year when it was lower than the Organic system last cycle. There was less grass cover in the Organic system this prune year compared to the last crop cycle (Photo 1A-B); the difference is likely due to the fact that in the last crop cycle one organic grower did not manage his field at all over either year, while a second grower did minimal weed control in the prune year. None of the organic growers conducted weed control in the 2013 crop year. In addition, and we were unable to gather data on crop input costs from two growers. Therefore, three organic growers were replaced this cycle with growers who managed their fields more intensively in the prune year and/or had better recordkeeping.
The Low system continued to have the most variability in wild blueberry cover among sites (Photo 2A-B), but because of the replacement of two sites with two new sites which had good blueberry cover, overall cover in the Low system improved from last cycle.

Photo 1. (A) Example of blueberry and weed cover in one of the new organic plots in September, showing better grass control compared to **(B)** a plot from an organic grower in crop year 2013.

Photo 2. Example of wild blueberry and weed cover in the Low system in September, showing variability in cover among sites: **(A)** high cover at new site, **(B)** patchy cover at old site.

Photo 3. Example of wild blueberry and weed cover in the Medium system in September.

Photo 4. Example of wild blueberry and weed cover in the High input system in September.

RECOMMENDATIONS: None at this time. This study has been extended a third cycle, to allow for additional data collection and for more accurate analysis of system differences and the costs and returns. After this crop cycle phase is completed in 2015, the results will be compared to the previous two cycles.

INPUT SYSTEMS STUDY

PLANT NUTRITION: David E. Yarborough, Professor of Horticulture
Jennifer L.D. Cote, Assistant Scientist

16. TITLE: Systems approach to improving the sustainability of wild blueberry production, Year Five of a six year study, plant productivity.

METHODS: See report no. 11 for an overview of the Input Systems Study. In the third crop cycle of the study, each input management system was represented by four sites per system with two one-acre blocks per site. In this cycle, seven sites were replaced due to either lack of grower cooperation or in order to spread sites out over a larger geographical area (sites 17-23). In early July 2014, soil and leaf samples were collected from each block. In each block two transects were set diagonally from corner to corner, and twenty soil cores were taken at regular intervals along each transect for a total of forty cores. The soil cores were collected using a standard soil sample tube removing a 0.8 inch diameter core to a depth of 3 inches. All forty cores were mixed in a bucket and a composite sample was removed for analysis. The samples were analyzed for soil pH (water), organic matter **(OM)** (%), and nutrients (parts per million or **ppm**). OM was measure by loss on ignition (**LOI**) at 375°C. Nutrients were extracted in pH 4.8 ammonium acetate (Modified Morgan method) and measured by plasma emission. Leaf tissue samples were collected along the same transects at the same intervals; at each sampling point, two stems at tip dieback were cut below the lowest leaf for a total of 80 stems. Leaf samples were prepared according to the methods of Kalra and Maynard (1991), analyzed for leaf nutrients (% or ppm) and compared to the standards and minimum/maximum ranges set forth in Wild Blueberry Fact Sheet No. 223 (Yarborough and Smagula 2013). Both soil and leaf samples were submitted to the University of Maine Soil and Plant Tissue Testing Laboratory for analysis. Soil pH and OM, and soil and leaf nutrients, were analyzed both across management systems and across sites using Tukey's HSD test (α=0.05).

RESULTS:

Soil characteristics

Soil pH was not significantly different among management systems in this cycle (Table 1). When sites were compared individually, there were no significant differences within systems with the exception of the Organic system, and the High sites tended to have lower pH overall (Table 2). Overall % OM did not differ among systems or sites, but when all sites were compared, the Medium system tended to have lower levels of OM and the Organic system contained both the highest and lowest values for OM (Table 2).

Because the soil nutrients don't necessarily reflect leaf or fruit nutrient levels, in-depth discussions of the results in Tables 1 and 2 are not presented here, but there were a few trends worth mentioning. The Organic system tended to have the most extremes (aka highest and

lowest values) in both system and site soil nutrient levels. It is unclear why the Organic system had such high aluminum (**Al**) levels, but the high sulfur (**S**) may have been because many of the organic growers apply S to their fields to reduce pH and control weeds. The Low system had the highest values of all systems for eight out of fourteen parameters. One Low site, L20, also had the highest level of all sites for calcium (**Ca**; over 3x other sites), potassium (**K**), magnesium (**Mg**) and sodium (**Na**); this site is a rocky burned sloping site and is a new site for this cycle. The High system was particularly high in boron (**B**) due to the application of micro-nutrient packs, but was lowest overall in Al, copper (**Cu**), manganese (**Mn**), Na, and S – the latter three of which also contained the lowest site values.

Table 1. Soil characteristics of the input management systems compared across systems in 2014. The highest value of each parameter is in **bold**; the lowest value of each is in *italics*.

Management system	pH (water)	Organic Matter (%)	Ca (ppm)	K (ppm)	Mg (ppm)	P (ppm)	Al (ppm)	B (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Na (ppm)	S (ppm)	Zn (ppm)
Organic	4.24a	12.5a	*162a*	*66.8a*	*31.5a*	*13.7b*	**346a**	*0.18b*	**0.26a**	39.3a	36.2a	13.5a	**119.1a**	*1.93a*
Low	**4.25a**	**13.9a**	**297a**	74.0a	**56.3a**	20.5a	276ab	0.23b	0.20ab	**40.1a**	**39.6a**	**16.5a**	91.6ab	**3.16a**
Medium	4.19a	*12.0a*	185a	70.0a	44.4a	**20.7a**	254bc	0.21b	0.14bc	*24.3a*	27.9ab	13.9a	84.8ab	2.56a
High	*4.06a*	13.5a	223a	**75.0a**	49.6a	16.8ab	*185c*	**0.34a**	*0.09c*	28.1a	*8.9b*	*11.8a*	*47.4b*	2.65a

Means across management systems with the same letter are not significantly different. Mean separation by Tukey's HSD test, α=0.05

Table 2. Soil characteristics of the input management systems compared across sites in 2014. The highest value of each parameter is in **bold**; the lowest value of each is in *italics*.

Management system	Site	pH (water)	Organic Matter (%)	Ca (ppm)	K (ppm)	Mg (ppm)	P (ppm)	Al (ppm)	B (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Na (ppm)	S (ppm)	Zn (ppm)
Organic	1	4.05cd	13.1a	114.0b	77.5a	27.5bc	14.8bc	**376a**	0.26bcde	0.26abc	**67.5a**	31.0bcd	13.0ab	151.0ab	1.65de
	17	4.10bcd	**17.2a**	196.0b	73.5a	35.5bc	*12.5c*	338a	0.19bcde	0.14bcd	40.0ab	17.2cd	17.5ab	101.0bcde	1.85de
	18	**4.65a**	10.5a	239.5b	59.0a	40.0bc	13.1bc	313ab	*0.12e*	0.24abcd	22.0b	24.0bcd	13.5ab	51.0de	*1.30e*
	19	4.15bcd	*9.3a*	*96.5b*	57.0a	*23.0c*	14.4bc	360a	0.16cde	**0.40a**	27.5ab	**72.5a**	10.1b	**173.5a**	2.90bcde
Low	7	4.15bcd	11.5a	97.0b	*52.5a*	23.5c	13.2bc	342a	0.18bcde	0.16cde	42.0ab	15cd	13.0ab	107.0bcd	1.40e
	8	4.25bcd	13.5a	220.5b	82.5a	50.5bc	**27.9a**	272abcd	0.18bcde	0.22bcd	26.0ab	52.5abc	10.6b	84.5cde	**4.75a**
	20	4.45ab	17.1a	**726.0a**	**103.5a**	**123.0a**	20.9abc	188de	0.26bcde	0.15bcd	38.0ab	63.0ab	**29.5a**	51.0de	4.55ab
	21	4.15bcd	13.4a	142.5b	57.5a	28.0bc	20.1abc	301abc	0.32abc	0.28ab	54.5ab	28.0bcd	13.0ab	124.0abc	1.95cde
Medium	9	4.10bcd	12.4a	232.5b	59.5a	52.5bc	20.5abc	*158e*	0.13de	0.08d	*18.5b*	12.0cd	15.0ab	42.5e	2.95bcde
	10	4.05cd	12.5a	210.0b	68.5a	52.5bc	20.1abc	197cde	0.26bcde	*0.07d*	23.0b	10.5d	14.0ab	57.0de	3.20abcd
	22	4.40abc	11.7a	193.5b	71.0a	48.0bc	20.6abc	322a	0.18bcde	0.19bcd	30.5ab	46.0abcd	12.0b	80.0cde	2.40cde
	23	4.20bcd	11.5a	103.0b	81.0a	24.5c	21.7ab	340a	0.26bcde	0.21bcd	25.0ab	43.0abcd	14.5ab	159.5ab	1.70de
High	13	4.15bcd	11.6a	176.5b	62.0a	31.0bc	14.5bc	209bcde	0.31abcd	0.09cd	26.0ab	6.4d	13.5ab	*42.0e*	2.30cde
	14	4.00d	15.6a	223.0b	84.0a	51.5bc	18.5bc	169.5de	**0.45a**	0.08d	24.5ab	*5.9d*	*9.8b*	50.5de	2.60cde
	15	*3.95d*	15.2a	288.0b	91.0a	74.5b	19.7abc	168.0de	0.36ab	0.11bcd	30.5ab	8.2d	14.0ab	48.0de	3.65abc
	16	4.15bcd	11.5a	206.0b	63.0a	41.5bc	14.6bc	193.5de	0.27abcde	0.11bcd	31.5ab	14.5cd	10.1b	49.0de	2.05cde

Each site contains 2 blocks. Means across management systems with the same letter are not significantly different. Mean separation by Tukey's HSD test, α=0.05.

Plant leaf nutrient concentrations

Leaf nitrogen (% **N**) was significantly higher in the Medium system compared to the High system, and all three conventional management systems were significantly higher than the Organic system, which was also the only system deficient (i.e. below the "optimum"/standard) in % N (Figure 1). When % leaf phosphorus (**P**) was examined, the Low and Medium systems exceeded the maximum recommendation of 0.143 %, the High system was at the standard of 0.136 %, and the Organic system was deficient but not below the minimum recommendation of 0.111 % (Figure 2). The significant differences in leaf P among systems mirror the differences in leaf N. All systems were above the 0.44 % standard for leaf K; the Low and Medium systems were significantly higher than the Organic and High systems (Figure 3).

Figure 1. Leaf nitrogen (%) compared across input management systems (different letters denote significance at α=0.05).

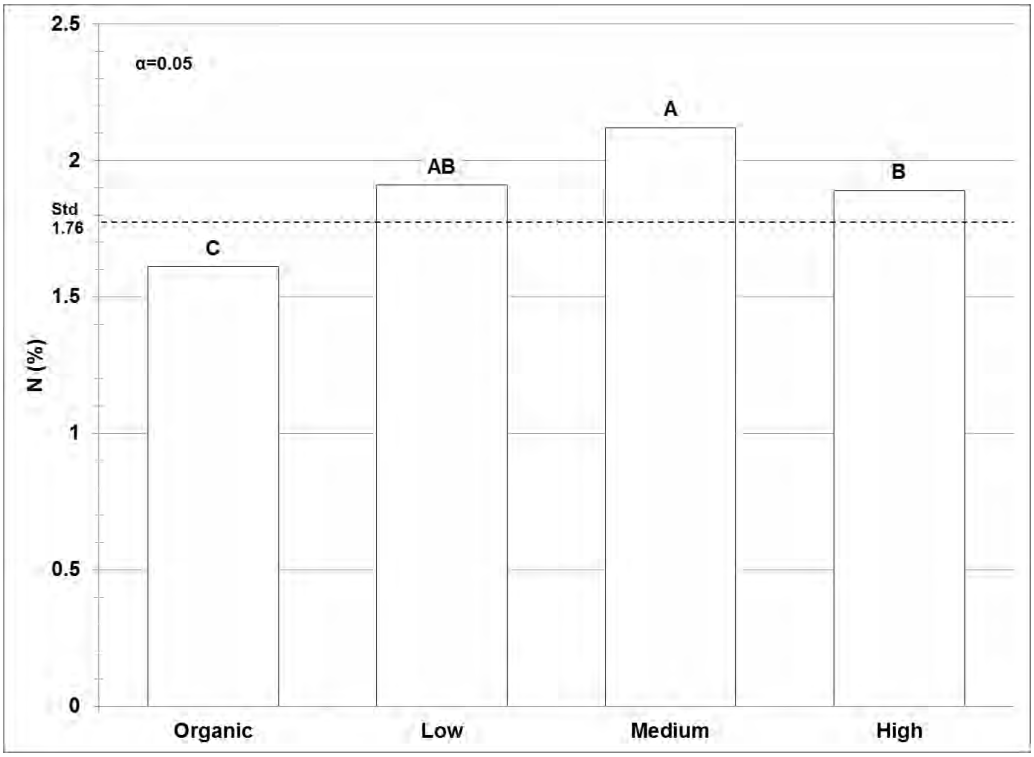


Figure 2. Leaf phosphorus (%) compared across input management systems (different letters denote significance at α=0.05).

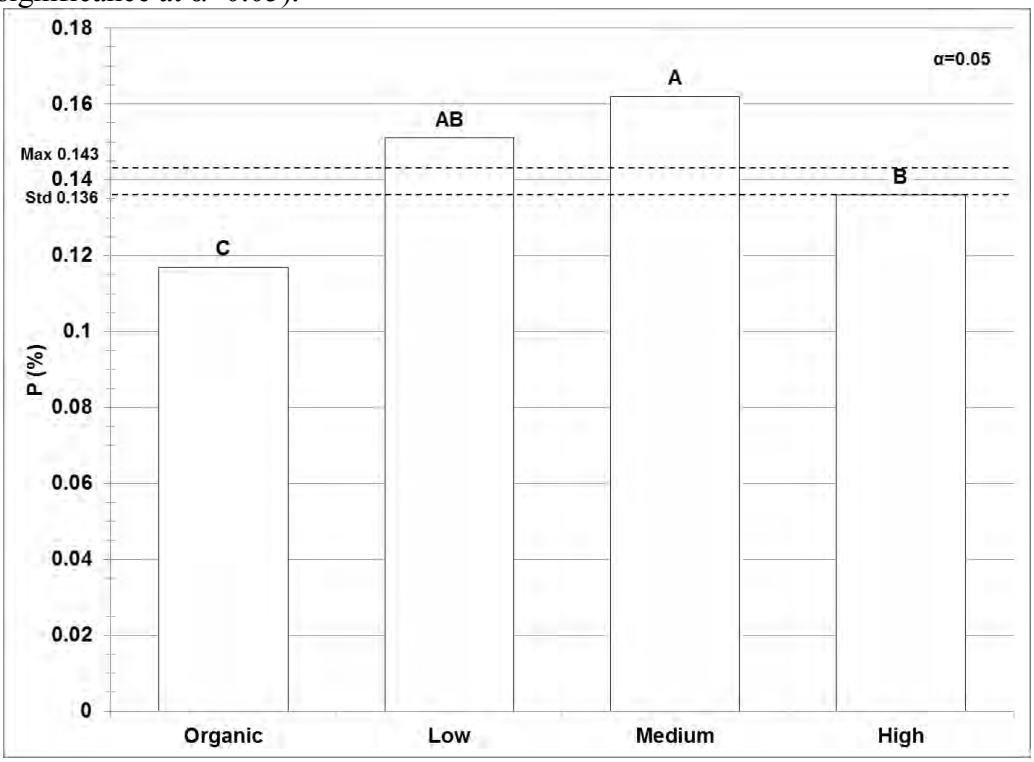
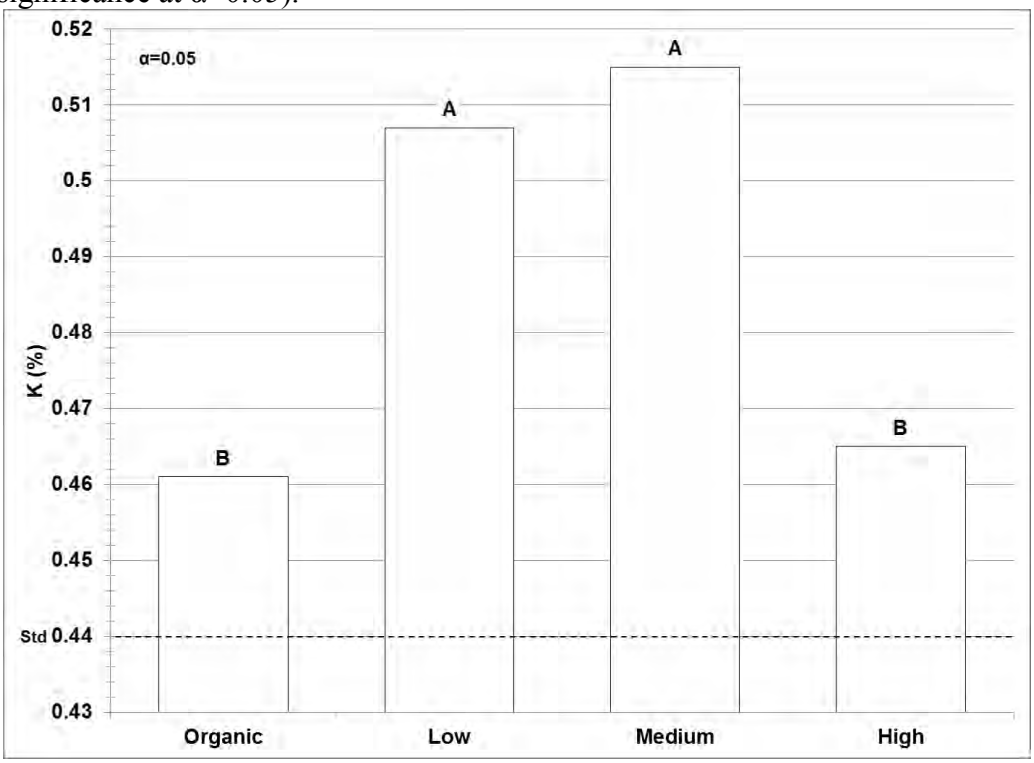


Figure 3. Leaf potassium (%) compared across input management systems (different letters denote significance at α=0.05).



By contrast, only the Organic system was above the 0.38 % standard for leaf Ca and it was significantly higher than the conventional management systems (Figure 4). Of the conventional systems, only the Low system was above the minimum recommendation of 0.31 % Ca. It should be noted that the unusually high Ca seen in site L20 was not reflected in the leaf Ca (Table 3). All systems were deficient in % leaf Mg, and were below the minimum recommendation of 0.16 % (Figure 5). Although the systems were not significantly different, the Organic system was slightly less deficient. Leaf Al levels were quite low; only the Organic system exceeded the minimum recommendation of 98 ppm (Figure 6), and this appears due to the high Al at site O19 (Table 3). However, there were no significant differences among systems. An analysis of leaf B revealed that the Organic system was the only system within the standard range; the three conventional management systems were well above the maximum recommendation of 44 ppm (Figure 7). The Medium and High systems were significantly higher than the Organic system, while the Low system was not significantly different from any other system. Leaf Cu was above the standard in all systems and the High system was significantly lower compared to the other systems (Figure 8). The min-max range of leaf Fe is quite narrow (34-37 ppm). All systems greatly exceeded the maximum recommended level, but were not significantly different from each other (Figure 9). By contrast, leaf Mn has a very large min-max range (710-2637 ppm). The Organic, Low and Medium systems exceeded the standard of 963 ppm; the Organic and Low systems were significantly higher than the High system which was deficient below the minimum recommendation (Figure 10). Finally, leaf zinc (**Zn**) exceeded the maximum recommended level of 15 ppm in all systems, with the High system being significantly higher than the other systems (Figure 11).

Figure 4. Leaf calcium (%) compared across input management systems (different letters denote significance at α=0.05).

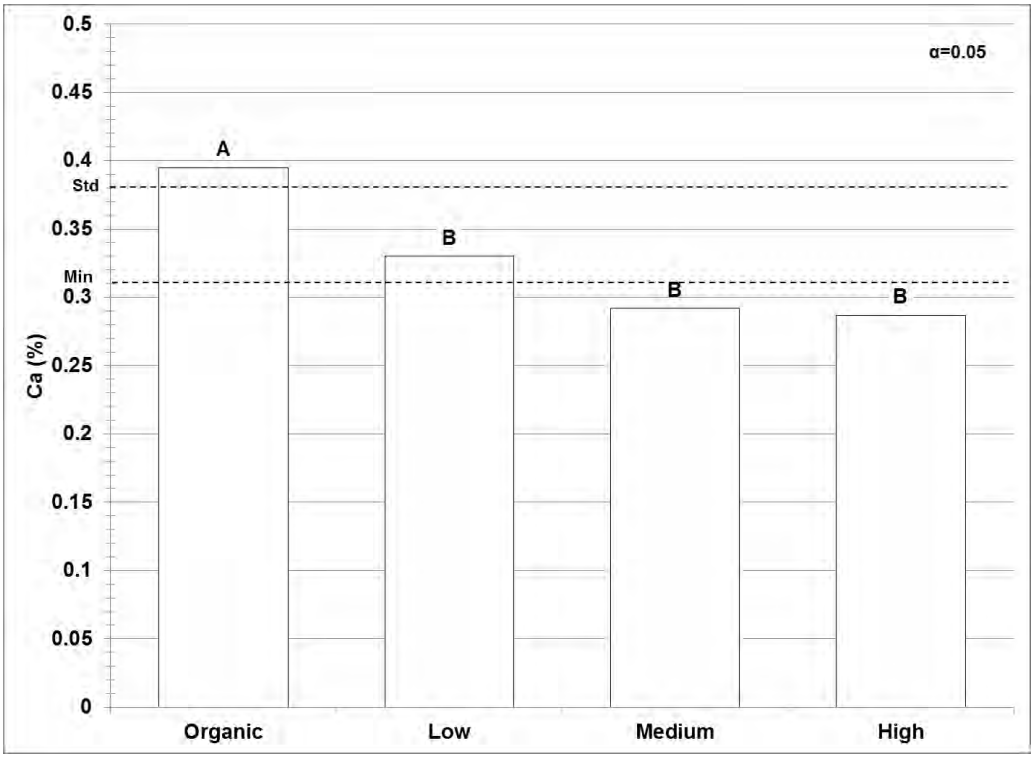


Table 3. Leaf characteristics of the input management systems compared across sites in 2014. The highest value of each parameter is in **bold**; the lowest value of each is in *italics*.

Management system	Site	N (%)	Ca (%)	K (%)	Mg (%)	P (%)	Al (ppm)	B (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Organic	1	1.57ef	0.41ab	0.46a	0.13abcde	0.113ef	94a	32ef	4.5abc	36a	1965ab	15ef
	17	*1.52f*	0.39abc	0.44a	0.15abc	0.111f	*58a*	37ef	4.4abc	*31a*	1003cde	*13f*
	18	1.52f	0.36abcd	0.44a	**0.16a**	*0.110f*	83a	*27f*	4.7abc	35a	689de	15ef
	19	1.83cdef	**0.42a**	0.51a	0.13abcde	0.135bcdef	**158a**	38ef	**5.4a**	**152a**	**2760a**	20cd
Low	7	1.73def	0.39ab	0.51a	0.12cde	0.138bcdef	100a	42def	5.2ab	43a	1765bc	20cd
	8	1.79cdef	0.38abc	0.51a	0.14abcd	0.137bcdef	90a	57cdef	4.3abc	42a	1260bcd	17cdef
	20	1.90bcde	0.25f	0.48a	0.14abcde	0.163abc	100a	53def	4.9abc	67a	533de	21c
	21	2.21ab	0.29cdef	0.53a	0.12cde	0.165ab	96a	122ab	4.9abc	54a	1610bc	19cde
Medium	9	20.9abc	0.26ef	0.52a	0.14abcde	0.164abc	*58a*	44def	4.8abc	69a	497de	16def
	10	2.00abcd	0.28def	0.51a	0.13abcde	0.160abc	65a	98abc	3.9abc	42a	687de	19cde
	22	2.07abc	0.32bcdef	0.51a	0.16ab	0.150abcd	92a	69cdef	5.1ab	56a	1060cde	16def
	23	**2.32a**	0.31bcdef	**0.53a**	*0.11e*	**0.175a**	86a	**140a**	4.8abc	35a	2015ab	17cdef
High	13	1.99abcd	0.28def	0.46a	0.13bcde	0.139bcdef	70a	82bcd	5.0abc	36a	372e	27b
	14	1.91bcd	0.35abcde	0.49a	0.15abc	0.143bcde	65a	122ab	*3.5c*	38a	*312e*	**35a**
	15	1.87cde	0.26def	0.47a	0.12cde	0.134cdef	108a	75cde	3.7bc	78a	350e	16def
	16	1.81cdef	*0.25f*	*0.44a*	0.12de	0.128def	104a	55cdef	4.0abc	74a	585de	28b

Each site contains 2 blocks. Means across management systems with the same letter are not significantly different. Mean separation by Tukey's HSD test, α=0.05.

Figure 5. Leaf magnesium (%) compared across input management systems (different letters denote significance at α=0.05).

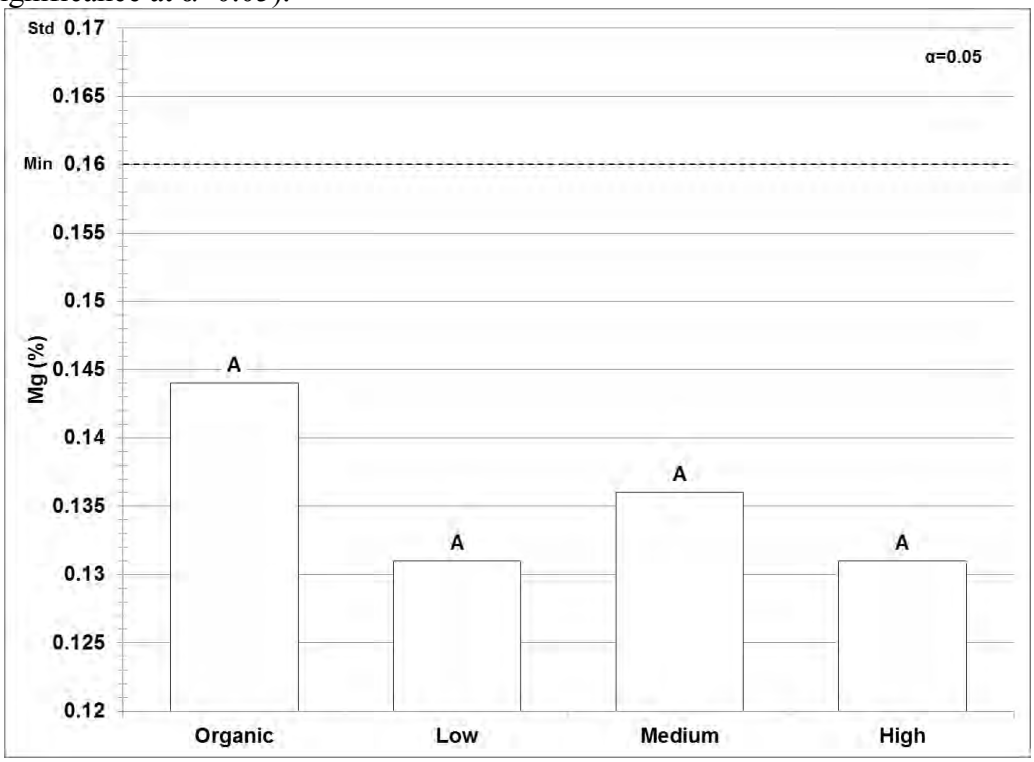


Figure 6. Leaf aluminum (ppm) compared across input management systems (different letters denote significance at α=0.05).

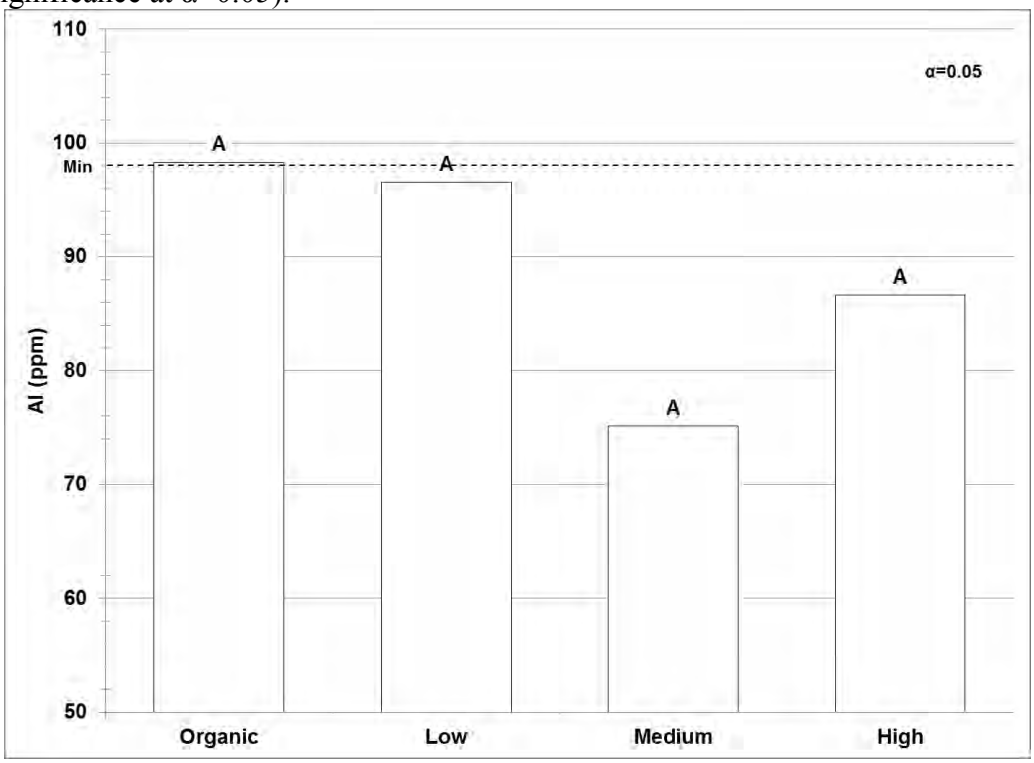


Figure 7. Leaf boron (ppm) compared across input management systems (different letters denote significance at α=0.05).

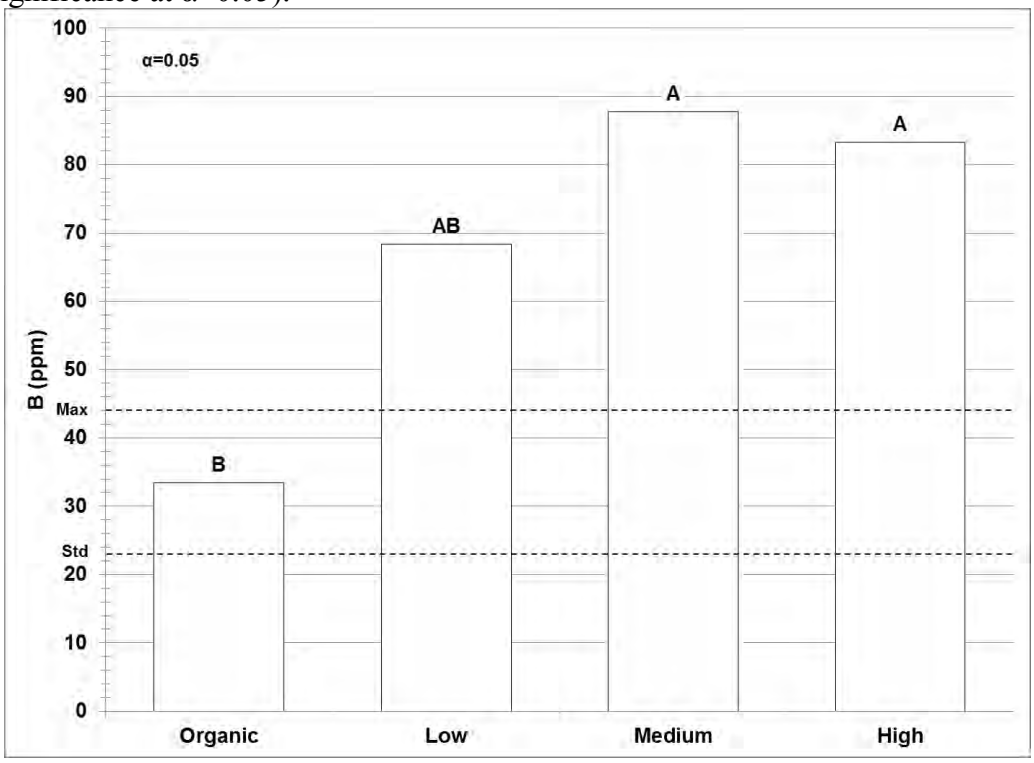


Figure 8. Leaf copper (ppm) compared across input management systems (different letters denote significance at α=0.05).

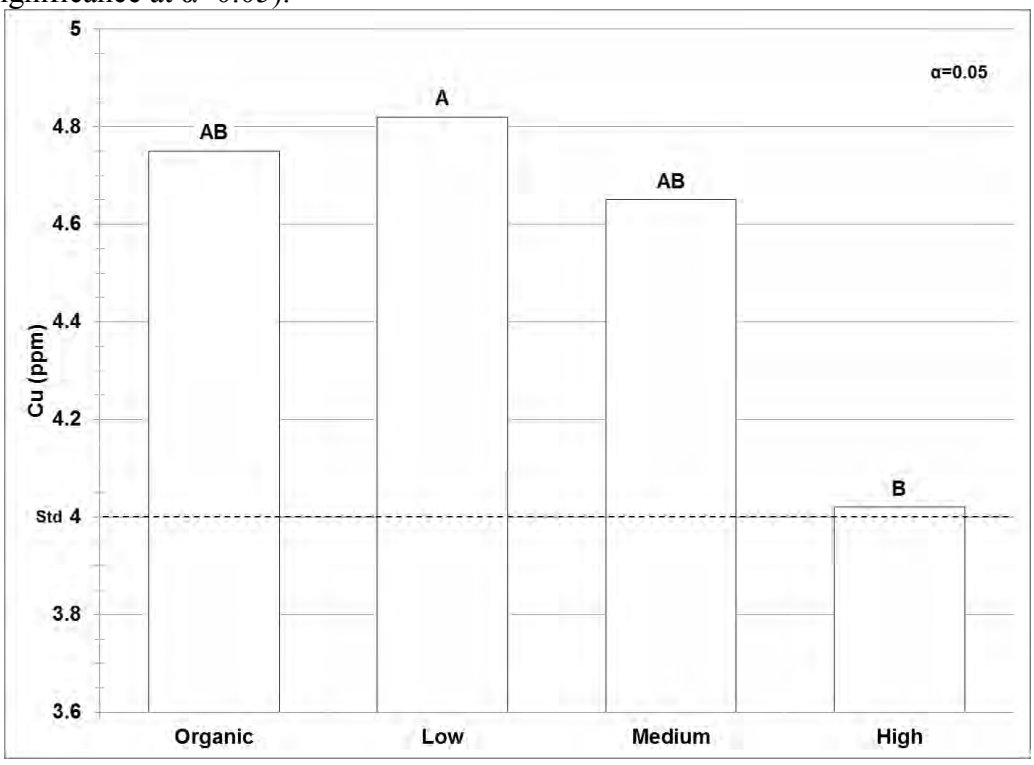


Figure 9. Leaf iron (ppm) compared across input management systems (different letters denote significance at α=0.05).

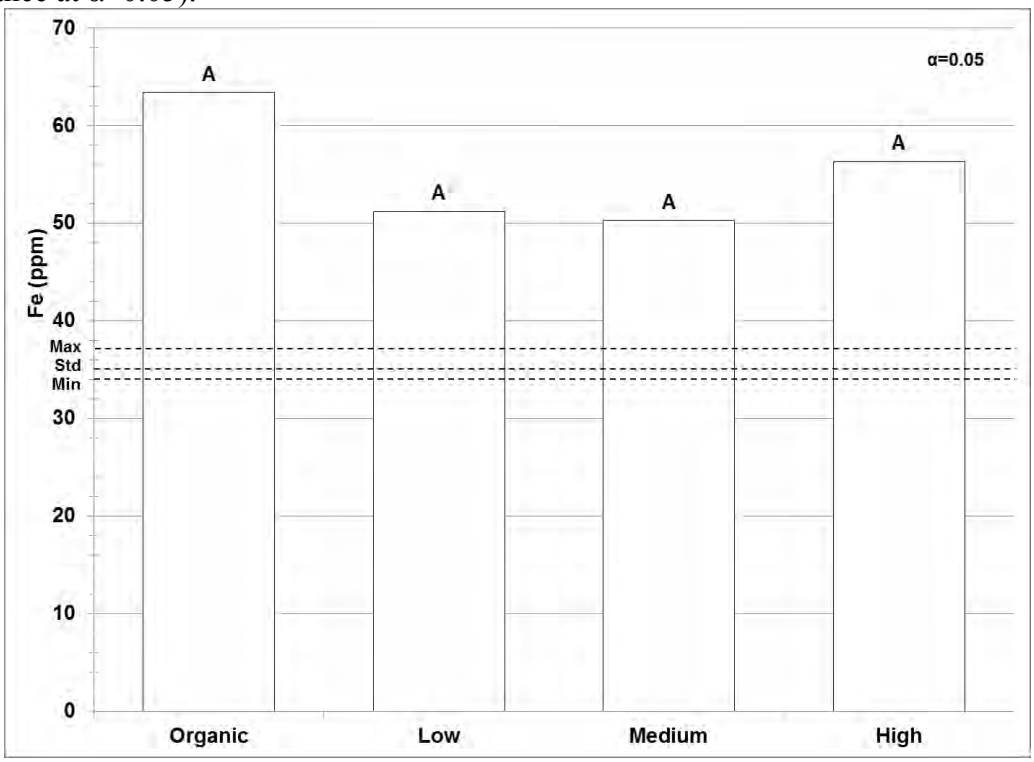


Figure 10. Leaf manganese (ppm) compared across input management systems (different letters denote significance at α=0.05).

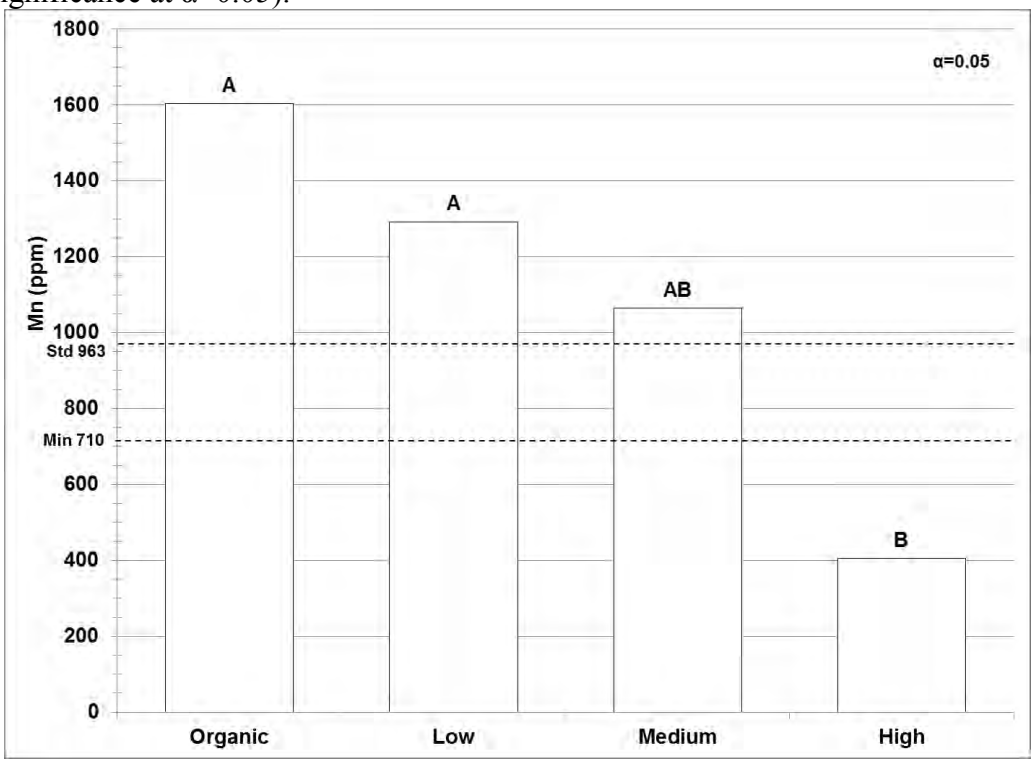
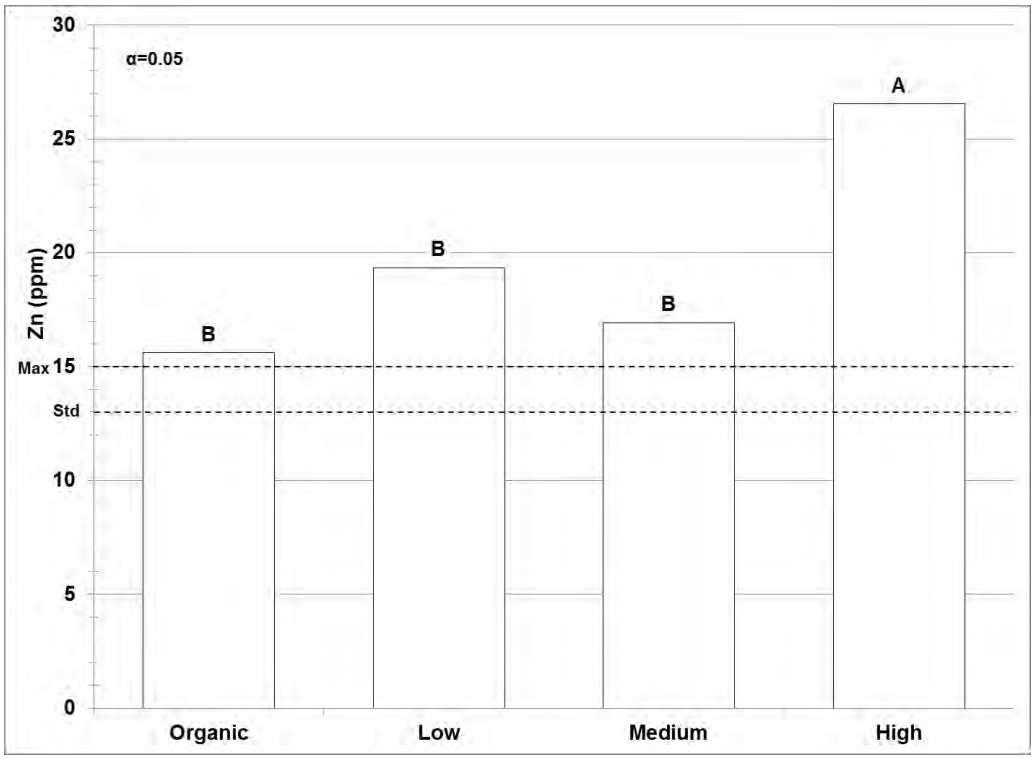


Figure 11. Leaf zinc (ppm) compared across input management systems (different letters denote significance at α=0.05).



CONCLUSIONS: Two of the Organic sites were above the 1.55 % minimum leaf N recommendation (one was also above the standard), and the plants in these fields appeared healthy. The other two sites were below the minimum leaf N recommendation, and this was reflected in shorter plants and sparser wild blueberry cover, which can be symptoms of N deficiency (Yarborough and Smagula 2013). Increasing leaf N should not only increase the vigor of the plants in these deficient organic fields, but will increase yield as well.
We know that lowering the soil pH makes Al more available for plant uptake. The Organic and Low systems had almost identical soil pH, and this is reflected in the very similar leaf Al levels. The High system had the lowest pH overall, but had over 10 ppm more Al than the Medium system; the reason behind this is not clear. The leaf B results are of concern because Yarborough and Smagula (2013) states that if applied beyond the level necessary for plant growth, boron can extensively damage blueberries. Table 3 shows that in the conventional management systems, all sites except M9 exceeded the maximum, in some cases by a factor of 2-3x. Copper is another micronutrient that can damage blueberries if too much is applied, but in this crop cycle all systems were below the maximum recommended level of 6 ppm. Yarborough and Smagula (2013) indicates that manganese deficiency can result in interveinal chlorosis. This was not observed in the High sites, so while the Mn levels should probably be increased (see Table 3), at this point the lack of Mn is not causing visible injury.

RECOMMENDATIONS: There are no soil nutrient recommendations for wild blueberry, and previous research has shown that soil nutrient levels do not necessarily reflect leaf or fruit nutrient levels, so there are no conclusions or recommendations regarding soil parameters

beyond pH and OM. In this case, soil pH and OM are within acceptable ranges for all sites (see Table 2), so there are no recommendations at this time.
The excessively high amounts of leaf B at the conventional management sites could lead to wild blueberry injury over time. We recommend that the Low, Medium and High system management companies in this trial reexamine their micro nutrient fertilizer application regimes and consider eliminating it if boron is in the micro-pack .
In the last crop cycle, we stated that we will research available organic fertilizers to give these growers tools to improve management (e.g. increase leaf N), and that the information would be used to in conjunction with the yield and economics data to provide growers with the options to decide which management system best fits their needs for sustainable production. This continues to be our recommendation in 2014.

Literature Cited

Kalra, Y.P. and D.G. Maynard. 1991. Methods manual for forest soil and plant analysis, Northwest Region. Information Report NOR-X319, Canadian Forest Service, Edmonton, Alberta.

Yarborough, D.E. and J.M. Smagula. 2013. Interpreting your leaf analysis results. UMaine Fact Sheet No. 223, UMaine Extension No. 2093. University of Maine, Orono, ME. 3 pp.

INPUT SYSTEM STUDY

ECONOMICS: George Criner, Professor School of Economics
David Yarborough, Professor of Horticulture/Ext. Blueberry Specialist

17. TITLE: 2014 economic analysis of Maine blueberry production systems including an introductory risk analysis.

OBJECTIVE: The objective is to create a set of budgets for each blueberry production system (organic, low input, medium input and high input). Each budget will contain estimates of variable and fixed costs. Fixed costs will include both annual fixed, such as insurance, and also longer term capital costs.
An extension will include an exploration of risks associated with blueberry production. There are many risks in farming, and the initial focus with this project will be yield risk, blueberry price risk, and selected input cost risk. Historic variation of yield, input prices and blueberry prices will be used to create sample distribution for risk simulations.
A goal is to create budgets which separate out utilization rates and prices. For example with field burning, rather than have a cost per acre, we will attempt to arrive at a fuel use per acre, which can be coupled with fuel prices to arrive at a field burning cost.
A final feature will be the development of budgets which are user friendly, so that producers can compare their costs with the based scenario estimated budget values.

METHODS: Standard budgeting on spreadsheets, and risk simulation techniques are used.

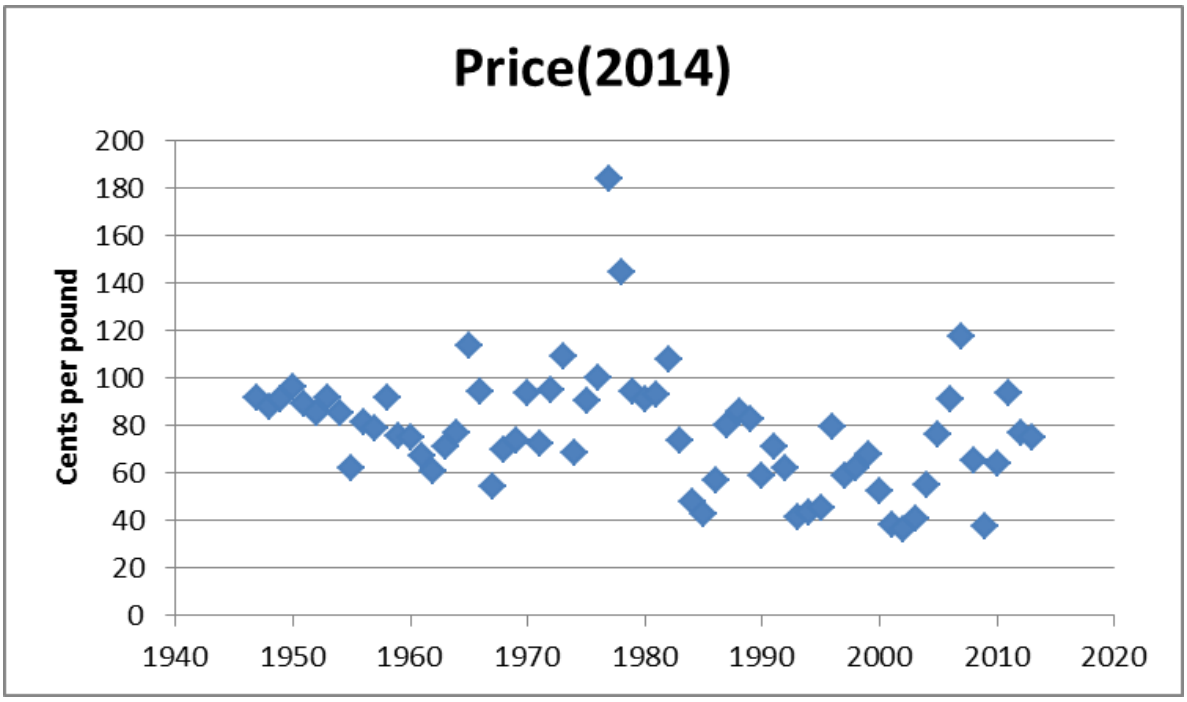
RESULTS: Initial budgets have been prepared and analyzed. These budgets show, given the yields in 2011 and 2013, that the medium input producers received higher net costs than the high

input producers. Analysis shows that for these two harvest years (2011 and 2013), yields were favorable for medium producers, who had yields similar to the high input producers. Our hypothesis is that the extra pollination and irrigation services, which the high input producers employ but the medium producers do not, were not needed given weather and pollination conditions. Thus, the extra cost for having the extra pollination and irrigation services resulted in lower net returns for the high input producers. Some of the findings are listed below. Note that the irrigation costs are estimates based on partial information, and future research will attempt to improve this cost estimate.

1. Pollination costs are three to four times higher for the high input producers;
    a) 3 to 4 cents/lb for medium input farms
    b) 11 to 14 cents/lb for high input farms
2. Irrigation in the model increased costs by $0.01/lb;
3. There was not much difference in Medium and High yields (2011 and 2013).

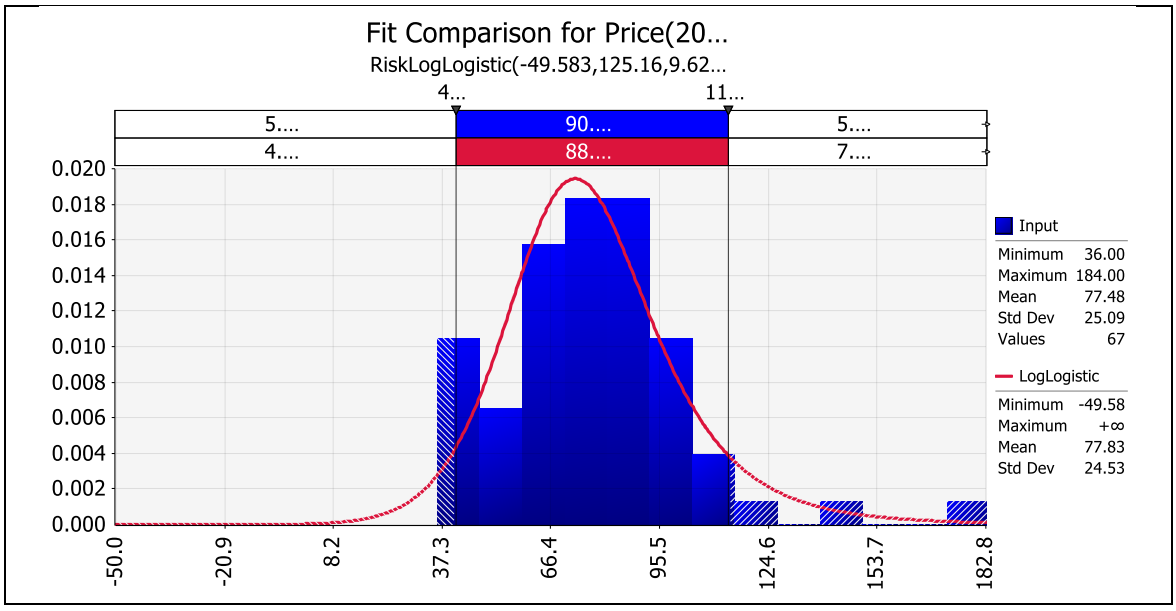
While the services of extra pollination and irrigation did not seem to be needed for the 2011 and 2013 crops, we know that in some years extra pollination and irrigation are needed as a result of weather conditions. This year to year yield variation, which to a large extent is driven by variation in weather, reflects the basic risk and uncertainty which blueberry producers face. In addition to weather variability, there is variability in costs and blueberry prices. For example, Figure 1 below show the variation in Maine blueberry prices in inflation adjusted prices (prices are in 2014 dollar values). Note that there are a few years where prices are notably higher, but the lower edge of prices, which appears to be trending downward, has fewer individual observations which stand out.

Figure 1. Real Maine blueberry prices.



Current research will attempt to model the variation in prices and other factors. Figure 2 below shows an example of modeling the variation in prices. The actual distribution of Maine blueberry prices are shown in the blue columns, and a hypothetical estimated price distribution is shown in the red line. Both of these distribution show that there is a 90% chance that the real Maine blueberry price in any year will be between 41 and 114 cents per pound.

Figure 2. Risk simulation.



CONCLUSIONS: Cost and other economic information can be important for producers in making informed decisions. This research, while still ongoing, shows that there are important and significant variation in prices and yields. Future research will include improvements with the budgets, and further development of the risk modeling.

RECOMMENDATIONS: Researchers and the industry should continue to analyze costs and other economic factors important to producers. Economic data on input usage and costs, as well as accurate yield data, are crucial for accurate economic analysis. We are hopeful that the industry will continue to help collect and share this information.

INPUT SYSTEMS STUDY – *ANCILLARY STUDY*

FOOD SAFETY & NUTRITION: Vivian Wu, Professor of Food Safety and Microbiology, School of Food and Agriculture

18. TITLE: Biosensor development for food safety.

OBJECTIVE: We developed a piezoelectic immunosensor for specific capture and enrichment of viable pathogens by quartz crystal microbalance sensor, followed by detection with antibody-functionalized gold nanoparticles.

METHODS: A sensitive real-time bacteria enrichment and detection system for viable *Escherichia coli* O157:H7 was developed using a piezoelectric biosensor-quartz crystal microbalance (**QCM**) with antibody-functionalized gold nanoparticles (**AuNPs**) used as detection verifiers and amplifiers. In the circulating-flow QCM system, capture antibodies for *E. coli* O157:H7 were first immobilized onto the QCM chip. The sample containing *E. coli* O157:H7 was circulated through the system in the presence of 10 ml of brain heart infusion (**BHI**) broth for 18 h. The cells of *E. coli* O157:H7 specifically captured and enriched on the chip surface of the QCM were identified by QCM frequency changes. *Listeria monocytogenes* and *Salmonella* Typhimurium were used as negative controls. After bacterial enrichment, detection antibody-functionalized AuNPs were added to enhance the changes in detection signal.

RESULTS:

Simultaneous enrichment and detection of viable E. coli O157:H7

To grow bacteria on the QCM chip surface and also in the circulated bacteria enrichment medium used in the circulating-flow QCM system, BHI broth was introduced into the QCM system in order to enrich the target bacterium and establish simultaneous enrichment and detection. The BHI broth and low concentration (0-1 log CFU/ml) of viable *E. coli* O157:H7 were circulated in the QCM system for 18 h. The bacteria were enriched from 0-1 log (1.5×10^0 - 2.6×10^1) to 8 log CFU/ml after 18 h circulation in the QCM system, hence more bacteria were captured on the chip surface. The dAb-functionalized AuNPs were then introduced into the QCM system, resulting in significant frequency decreases. The signal pattern from the detection of 1 log CFU/ml *E. coli* O157:H7 using the simultaneous enrichment and detection nanoparticle-functionalized piezoelectric biosensor-QCM system is shown in Fig. 1A and the pattern of the blank control is shown in Fig. 1B. The frequency changes after the addition of dAb-functionalized AuNPs in the developed QCM system are shown in Fig. 1C. The results show that after the addition of the dAb-functionalized AuNPs, frequency decreases of 125 ± 13 Hz and 110 ± 35 Hz were observed from initial concentrations of *E. coli* O157:H7 at 1 and 0 log CFU/ml respectively, while the frequency decrease observed from the non-bacteria sample was only 18 ± 10 Hz.

The specificity of the QCM system's simultaneous enrichment and detection was evaluated using mixtures of different bacterial strains (1 log CFU/ml). The results show that a frequency decrease of 125 ± 13 Hz was observed from the mixture of *E. coli* O157:H7 (including ATCC 35150, 12900, and 700594) after the addition of the dAb-functionalized AuNPs, while frequency decreases of 25 ± 13, 27 ± 12, 14 ± 8 and 18 ± 10 Hz were observed from the mixture of *L. monocytogenes* (including ATCC 49594 and 19115), the mixture of *S.* Typhimurium (including

ATCC 6962 and 072209), and the non-bacteria samples respectively. The frequency change caused by applying the mixture of *E. coli* O157:H7 into the QCM system was significant different ($p < 0.01$) from the frequency changes caused by the other bacteria (Fig. 1D).

Detection of E. coli O157:H7 in food samples

Wild blueberry samples were inoculated with viable *E. coli* O157:H7 at a final concentration of 1 log CFU/g. The results of signal patterns indicated that in the presence of viable *E. coli* O157:H7, frequency changes were observed during the BHI enrichment, whereas no frequency changes were observed when the viable cells were absent (data not shown). After the addition of dAb-functionalized AuNPs, significant frequency change ($p < 0.01$) was observed using the blueberry samples containing initial concentration of 1 log CFU/g mixture of *E. coli* O157:H7 when compared with the frequency changes from the blueberry samples containing initial concentrations of 1 log CFU/g mixture of *L. monocytogenes*, *S.* Typhimurium and non-bacteria (Fig. 2). A frequency change of 93 ± 24 Hz was observed from the blueberry samples containing 1 log CFU/g mixture of *E. coli* O157:H7, while the frequency decreases of 8 ± 4, 15 ± 14, and 7 ± 3 Hz were observed from the blueberry samples consisting of the mixtures of *L. monocytogenes* and *S.* Typhimurium, and non-bacteria. The results indicate that the QCM system developed in the present study is practical for the simultaneous enrichment and detection of viable *E. coli* O157:H7 in real food samples.

CONCLUSIONS: In this study, a simultaneous enrichment and detection nanoparticle-functionalized piezoelectric biosensor-QCM system was developed. The sensor utilized BHI broth to enrich the target samples in the system, hence confirming the viability. AuNPs serving as further detectors and signal amplifiers were used to improve the sensitivity of the sensor, thereby improving the detection limit of the QCM system. The detection limit was improved from 4 log to 0-1 log CFU/ml when the detection antibodies for *E. coli* O157:H7 conjugated-AuNPs were applied after the BHI enrichment. Moreover, the whole detection can be completed in one day without additional enrichment outside of the system. This study reports, for the first time, the enrichment and detection of viable bacterial cells in a nanoparticle-functionalized piezoelectric biosensor-QCM system, provides a more sensitive and specific immunosensor than those reported previously. By strategically combining culture methods, immunology, nanotechnology, and QCM sensing technology, QCM biosensors can be made more sensitive and specific than those previously reported, providing for future practical application in food safety inspections. The new trend of high-throughput multiple pathogen detection may also be archived by further studies with multiple antibody coating using QCM array.

RECOMMENDATIONS: The simultaneous enrichment and detection for *E. coli* O157:H7 established in the study could be used to detect viable bacteria at low concentration in food samples. The combination of both procedures in a nanoparticle-functionalized piezoelectric biosensor system indicates potential for the application of biosensors in real fields.

Figure 1. Detection of viable *E. coli* O157:H7 in the simultaneous bacterial capture and enrichment QCM system, followed by detection with dAb-functionalized AuNPs. (A) Frequency change was observed from a low concentration (1 log CFU/ml) of viable *E. coli* O157:H7 enriched in the QCM system in the presence of 10 ml BHI broth. (B) Little frequency shift was observed from non-bacteria. (C) After the BHI enrichment, the dAb-functionalized AuNPs were added as a signal amplifier and detection verifier. Relative frequency decreases were observed, where * indicates $p < 0.01$, *vs.* non-bacteria. (D) Detection specificity of the QCM system/dAb-functionalized AuNPs system, where mixtures of *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium at 1 log CFU/ml were used. * indicates $p < 0.01$ *vs.* mixtures of *L. monocytogenes* and *S.* Typhimurium, and non-bacteria.

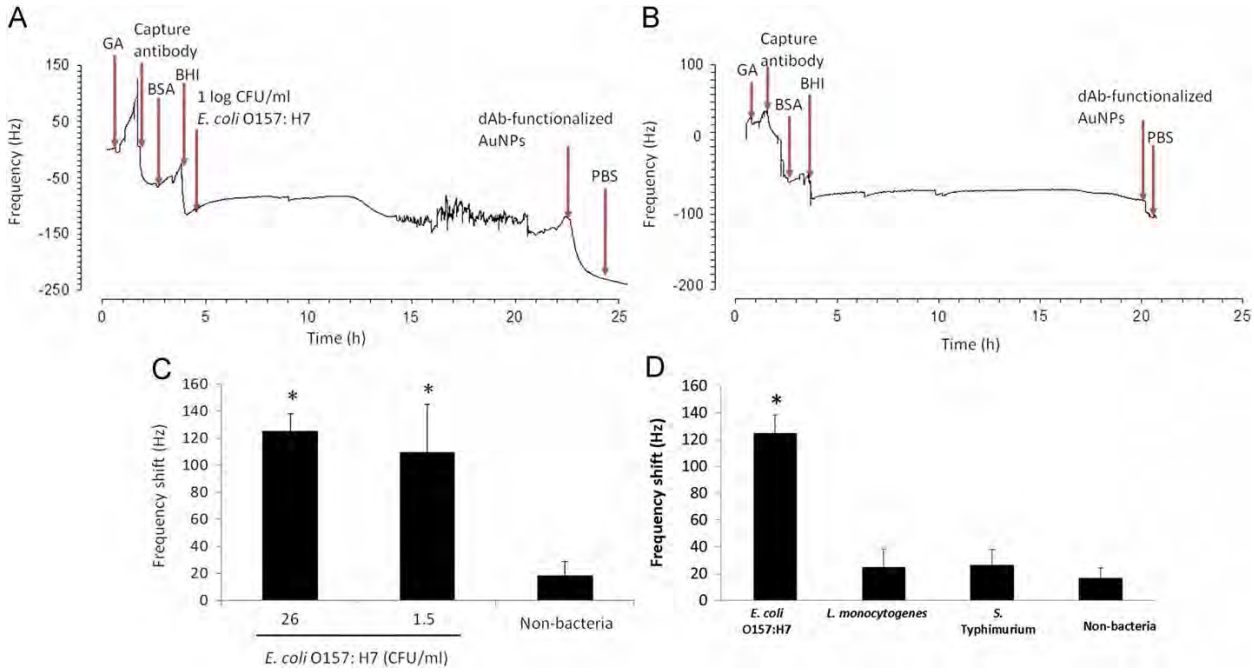
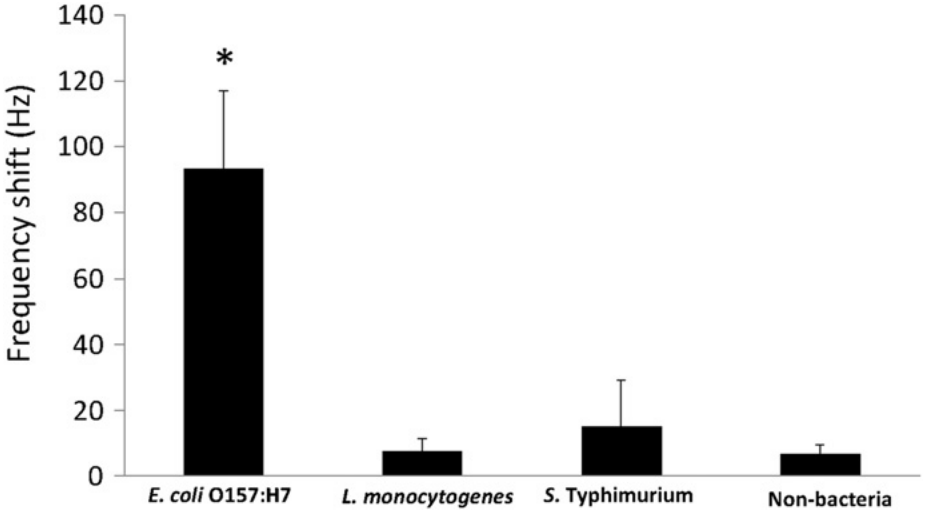


Figure 2. Specific detection of *E. coli* O157:H7 inoculated on blueberry samples by the QCM/dAb-functionalized AuNPs system. Mixtures of *E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium at 1 log CFU/g on blueberries were introduced into the QCM/dAb-functionalized AuNPs system. The blueberry samples with viable bacteria were enriched in the QCM system in the presence of 10 ml BHI broth for 18 h. * indicates $p < 0.01$, *vs.* blueberries with *L. monocytogenes*, blueberries with *S.* Typhimurium, and blueberries without bacteria inoculated.



INPUT SYSTEMS STUDY – *ANCILLARY REPORT*

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

19. TITLE: Ancillary projects in disease research.

OBJECTIVES:
- Determine possible sources and methods of spread of Valdensinia leaf spot;
- Determine causal agent of root rot disease of lowbush blueberries; and
- Determine the timing of spore release of leaf spot causing fungi using spore traps.

METHODS: Fields suspected to have Valdensinia leaf spot in 2014 were visited in July to August to 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 hours of light to induce spore formation by *Valdensinia*. *Valdensinia* spores were transferred to new plates and put into storage for genetic analysis. Fields previously known to have *Valdensinia* were visited in May to look for sexual structures on diseased leaves and later in the season to determine how well control methods were working.

New fields with symptoms of root rot were visited and sections of the possibly infected roots were surface sterilized and plated on nutrient medium with antibiotics. Previously collected isolates were grown and their DNA extracted and amplified for specific sections of DNA to identify them. The DNA was sequenced at the UMaine DNA sequencing facility.
Spore traps were placed in the prune and crop fields at Blueberry Hill Research Farm on Aug. 5th. We collected spore tapes containing the trapped airborne spores every week until Oct. 14th when leaf rust spores were difficult to find on the tapes. Plant samples with signs of powdery mildew and leaf rust were collected in August and September. Fungal structures for powdery mildews or rusts were scraped off of leaves and their DNA was extracted. Specific sections of this DNA will be sequenced and used to design primers specific for these fungal organisms.

RESULTS: This was not a very wet year during bloom when *Valdensinia* spores are usually first released. There was only one new field found with Valdensinia leaf spot in Washington County. Leaves were collected from different infection sites in the field and plated out to collect isolates. We now have a collection of 171 isolates collected from 21 fields in Maine.
Collaboration with Dr. Jim Polashock identified numerous potential microsatellite DNA markers for DNA fingerprinting the *Valdensinia* isolates. Erika Lyon, an MS graduate student, has screened over 30 sets of primers and has identified 8 microsatellite primer sets that will work for DNA fingerprinting of the isolates. We did not find any sexual structures in the plant debris examined this year. We will be repeating the survey in the spring of 2015. Fields that had been hard burned had lower levels of Valdensinia leaf spot. Two fields that had Valdensinia leaf spot in 2014 in the prune year were sprayed with Pristine early in the crop year by growers who obtained good control of the disease.
Using molecular techniques we have identified the organism associated with blueberry root rot as *Phytophthora cactorum*. We have isolated this organism from the roots from two new fields.
The tracking of spore release project is in its preliminary stages. Honors students have extracted DNA from samples of powdery mildew and leaf rust spores. In 2015, they will be amplifying specific DNA fragments and looking for unique sequences to design primers to identify these fungi.

CONCLUSIONS AND RECOMMENDATIONS: 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. Applications of Pristine can suppress the spread of the disease. *Phytophthora* appears to be attacking areas where the soil is saturated in the spring. Applications of materials to control this disease will be attempted in the spring.

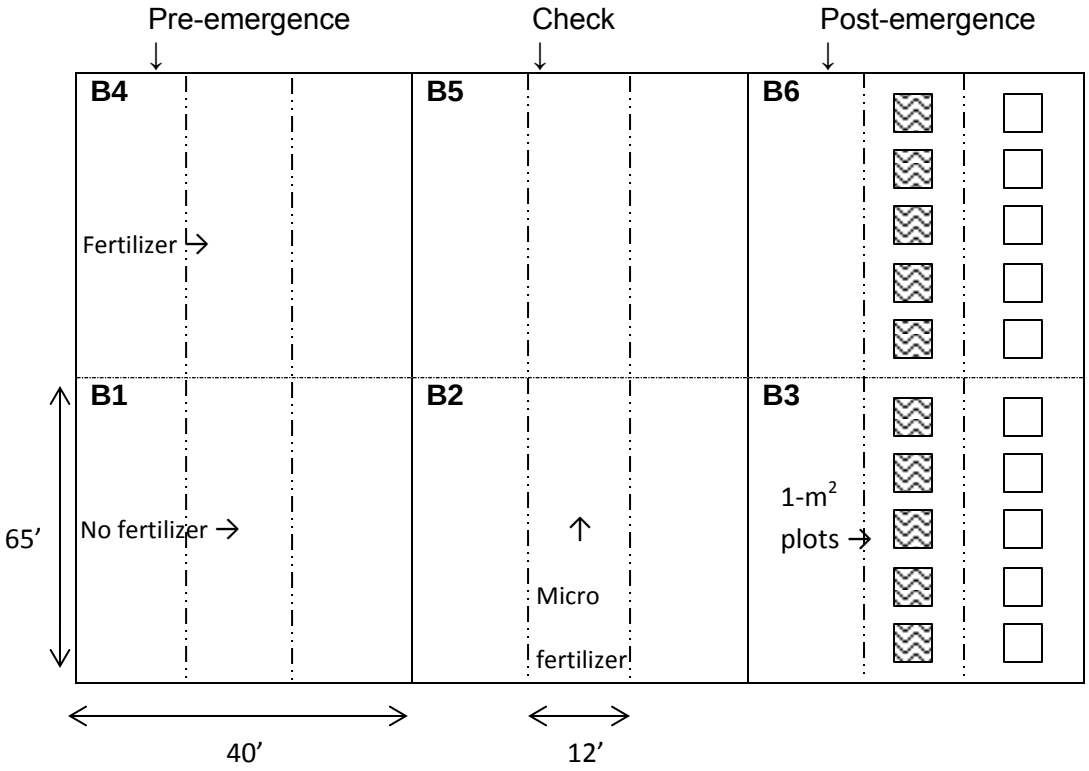
INPUT SYSTEMS STUDY – *ANCILLARY STUDY*

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

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

METHODS: In 2013 an established field in Jonesboro, ME that had been de-rocked and leveled just prior to research plot set-up was selected for the second cycle of the land-leveling study. 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, the mulch treatment was omitted this cycle, and was replaced with a nested micro-fertilizer treatment.
In 2013, 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 + crop oil concentrate (**COC**) 1% v/v tank mix applied twice. The pre-emergence treatment was applied on 15 May 2013 with a tractor mounted boom sprayer, and the post-emergence treatments were applied on 10 June and 25 June 2013. 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** (diammonium phosphate with boron) fertilizer applied at 200 lb/a on 15 May 2013. Within each block was nested a 12'x130' strip which had two micro-fertilizers applied at different timings: Bio-Forge was applied at 1 pt/a on 21 May 2013 and Xtra Power was applied to the same strips at 4 pt/a on 10 June 2013. The result was six 40'x65' blocks and a total of 12 herbicide/fertilizer/micro-fertilizer combinations. Each 40'x65' block contained 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, broadleaf weed cover, and grass cover on 31 July. Cover was determined by using a Daubenmire Cover Class scale, which was converted to percent for analysis. Wild blueberry yield was assessed on 7 August by machine harvesting 2-ft wide strips the length of each 65' block using a walk-behind harvester – one strip inside and one strip outside the nested micro-fertilizer strip in each block, through the 1-m^2 plots. Only main effects were examined for both cover and yield, using t-tests (α=0.05) with Bonferroni adjustment for herbicide main effects (α=0.0167).

Figure 1. Site layout (not to scale; B = Block).



RESULTS:

Herbicides

As in 2013, the pre-emergence herbicide application had the highest blueberry cover in 2014; however, in 2014 the differences were not statistically significant (Figure 2). As in 2013, there were no significant differences in broadleaf weed cover among treatments. Grass cover was comparable in the check and post-emergence herbicide treatments, but was significantly lower in the pre-emergence herbicide treatment.

DAP Fertilizer

Blueberry cover for the check versus DAP 200 lb/a was comparable in 2014 (Figure 3), but increased to an average of about 65% cover as opposed to 45% cover in 2013. Broadleaf weed cover increased overall from 2013, but the section with DAP had significantly fewer broadleaf weeds compared to the section without DAP. It is unclear as to why the addition of fertilizer would result in less broadleaf weed cover; it was not due to a corresponding increase in wild blueberry cover, but there was a non-significant increase in grasses in the DAP treatment which may have replaced some of the broadleaf weeds.

Figure 2. Main effects of prune year herbicide application on wild blueberry, broadleaf weed and grass cover in the crop year (α=0.0167, different letters denote significance).

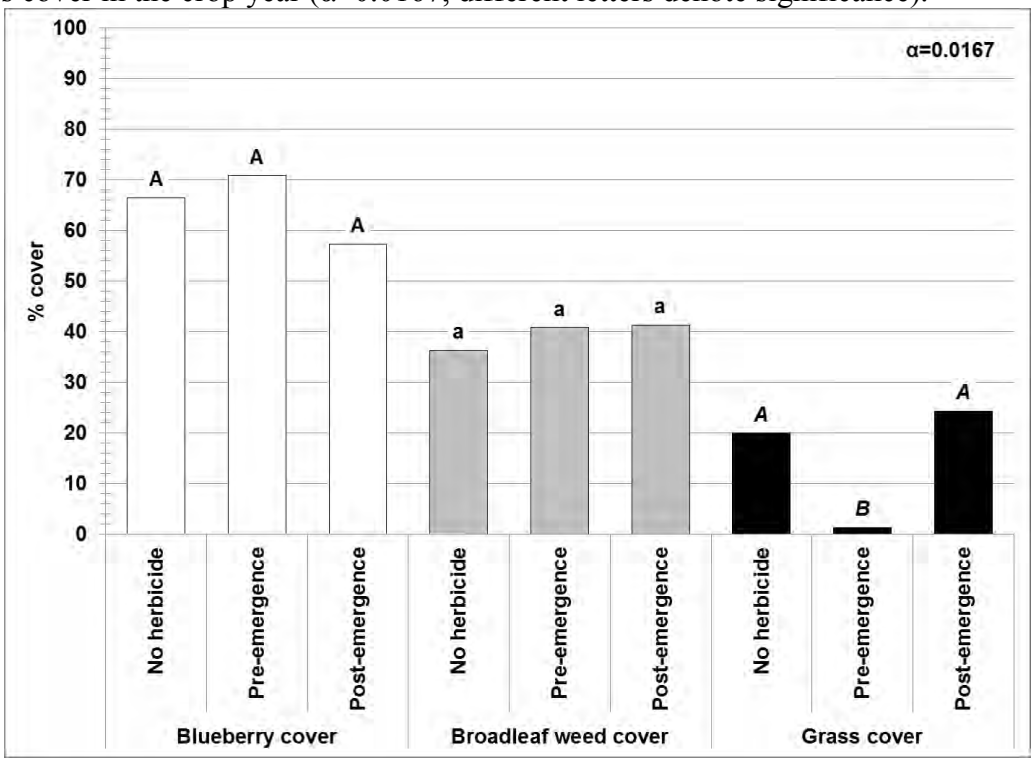
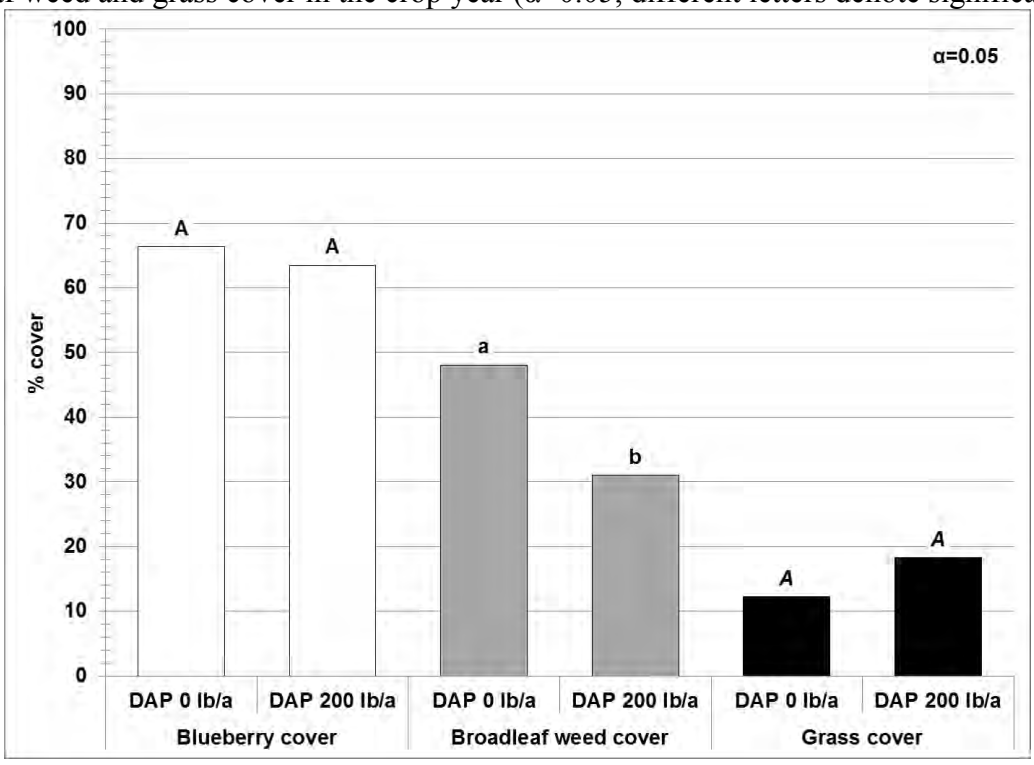


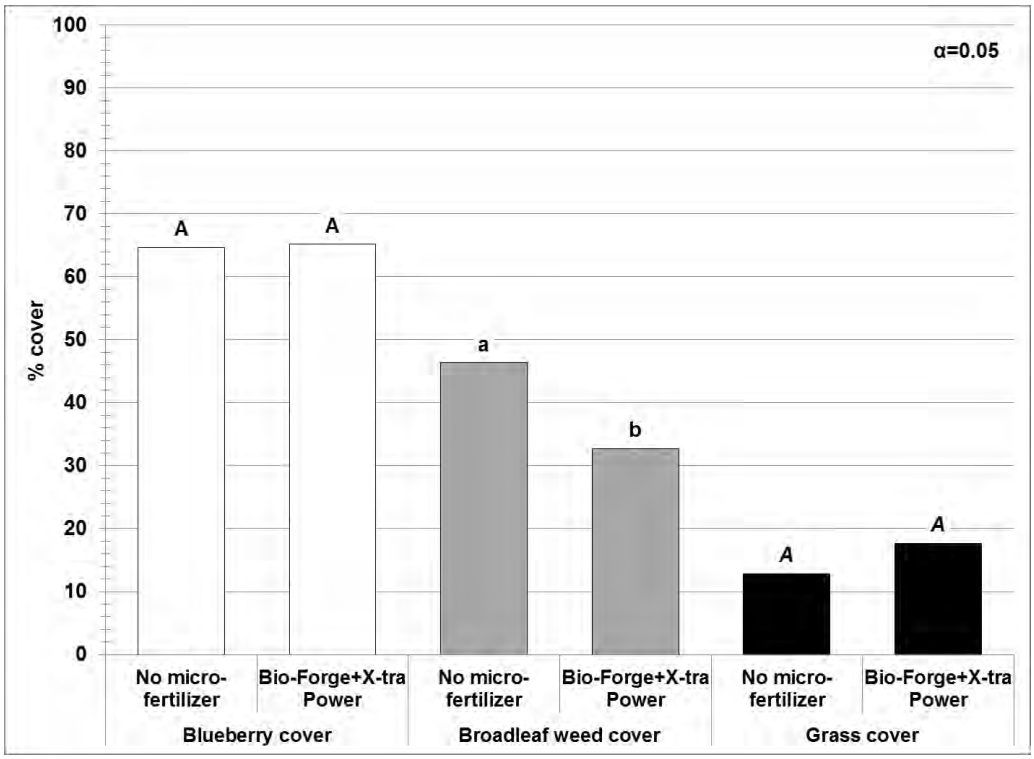
Figure 3. Main effects of DAP fertilizer application in the prune year on wild blueberry, broadleaf weed and grass cover in the crop year (α=0.05, different letters denote significance).



Micro-fertilizer

Wild blueberry cover was within one percent of each other for the micro-fertilizer treatment compared to no micro-fertilizer (Figure 4). Grass cover in the two treatments remained comparable as in 2013, but cover in both treatments roughly doubled between last August and this August. Broadleaf weed cover also increased from last year to this year, but there was significantly less cover in the micro-fertilizer treatment this year. As with the DAP treatment, it is unclear as to why the micro-fertilizer treatment would have fewer broadleaf weeds than the treatment without it. As with DAP, the difference does not appear due to a change in wild blueberry cover, but could be a result of increased grass cover suppressing the broadleaf weeds.

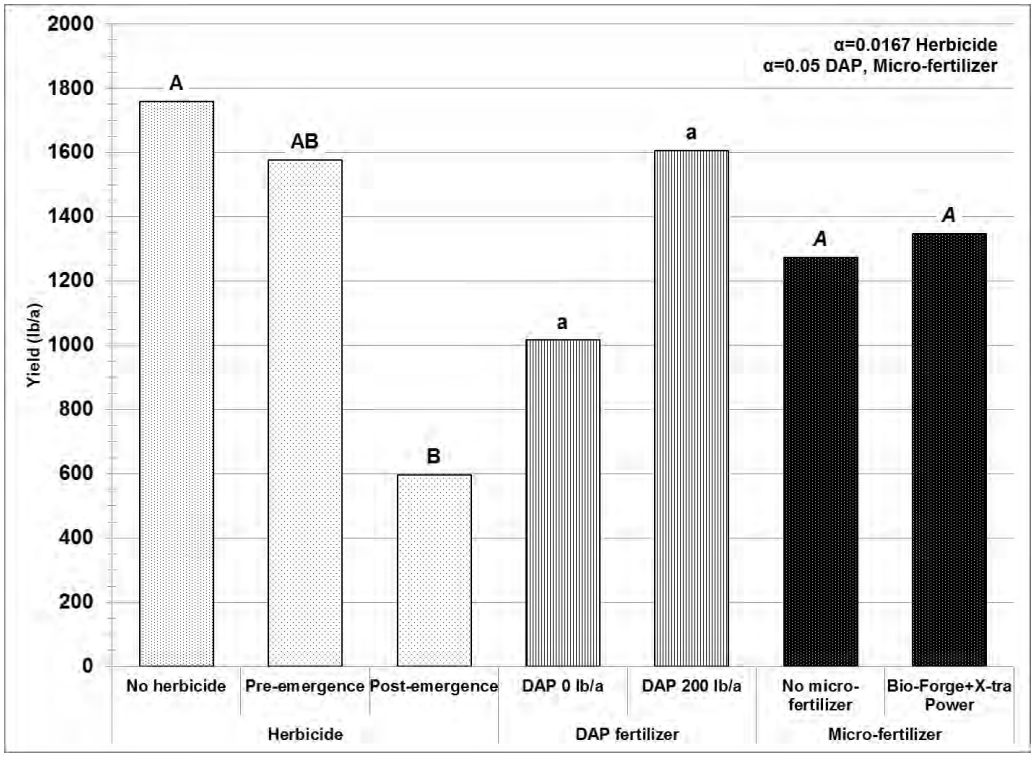
Figure 4. Main effects of micro-fertilizer application in the prune year on wild blueberry, broadleaf weed and grass cover in the crop year (α=0.05, different letters denote significance).



Yield

When yield among the herbicide treatments was compared, the pre-emergence treatment yield was not significantly different from either of the other treatments, and was within about 200 lb/a of the no-herbicide treatment. The no-herbicide treatment had significantly higher yield than the post-emergence herbicide treatment, which was 2-3x less than the other treatments (Figure 5). There was no difference in yield between DAP and no DAP, but the DAP treatment did result in a higher yield. Finally, there was also no difference in yield between micro-fertilizer and no micro-fertilizer, and yields were within 100 lb/a of each other. Overall yields were very low because of poor cover and perhaps the effect of the land leveling setting back the plants. This is observed on other sites that have been land leveled and it usually takes several production cycles to recover.

Figure 5. Main effects of herbicide, DAP fertilizer, and micro-fertilizer treatments in the prune year on wild blueberry yield (α=0.05 DAP and micro-fertilizer, α=0.0167 herbicide; different letters denote significance).



CONCLUSIONS: In 2013 we indicated we found unusually low blueberry cover in the post-emergence herbicide treatment block compared to the check and pre-emergence blocks which appeared 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 believed 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 the post-emergence herbicide treatment over the 2013 growing season, and they were not filling in with blueberry at the same rate as in the other two treatments. This lack of prune year cover seems to be the primary reason why yield in the post-emergence herbicide treatment was so low. Although Figure 2 shows that wild blueberry plants filled in the treatment somewhat in 2014, the new stems did not bear fruit (see Photos 1-2).

In 2013 there was also a lack of the expected greater weed cover in the post-emergence herbicide block due to weeds taking advantage of the bare spots; we believed this was likely due to weed root systems and seeds also being removed during the leveling process. The reduced roots and seedbank probably resulted in fewer perennial weeds, and the increase in grasses was largely due to the annual grass witchgrass (*Panicum capillare*), whose seeds could have come from the disturbed soil or blown in from outside the trial area.

As we had observed in 2013, this year there remained an unexpected lack of response to herbicide application or fertilizers (Photo 3). Last year we stated that we expected there would be more weeds this year as the seedbank was replenished after leveling. We did see an increase in weeds overall, but did not observe the flush of weeds we would expect from the addition of

macro- or micro-nutrients. The DAP and micro-nutrient treatments having fewer broadleaf weeds but more grasses than the treatments without them could also be from witchgrass and other grasses responding more quickly to nutrient additions and therefore competing more effectively with broadleaf weeds.

Photo 1. A plot treated with micro-fertilizer and post-emergence herbicide, showing filling in of bare spots with grasses, broadleaf weeds and new blueberry stems without fruit in 2014.

Photo 2. A plot treated with micro-fertilizer and pre-emergence herbicide, showing bunchberry but no other weeds, and almost continuous blueberry cover with berries in 2014.

Photo 3. The trial area, standing at block 1 (no DAP) looking toward block 4 (with DAP) in the background. The area to the left is outside the trial area.

RECOMMENDATIONS: This study illustrates the profound effects of land leveling on blueberry plants and indicates that additional herbicides or fertilizers will not overcome these effects in the first production cycle. It is recommended that weed control, either pre- or post-emergence applications, be made to prevent weed establishment and competition to allow the blueberry plants to recover and reestablish themselves in the field.

INPUT SYSTEMS STUDY – *ANCILLARY STUDY*

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

21. TITLE: 2013-14 evaluation of three pre-emergence herbicides alone and in combination with Velpar or Sinbar for effects on wild blueberry productivity and weed control – 2014 crop year results.

OBJECTIVE: In 2014 we assessed weed and yield effects of three new herbicides in the crop year. 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'x 72' 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'x 108' 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 (Table 1). The resulting treatments are as follows (18'x 18' except for grower check and grower Sandea which are 18'x 54'): 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. In 2014, sites were evaluated for wild blueberry cover, broadleaf weed cover and grass cover on 21-23 July. Cover was assessed using a Daubenmire cover scale converted to percent. Yield was assessed by hand-raking two 1-m^2 plots in each treatment and weighing the berries, on 30 July to 4 August 2014. Cover data were analyzed using a non-parametric one-way exact median test (α=0.05) to compare each herbicide of interest to the check, as well as the herbicide combinations to Velpar or Sinbar alone. Yields were converted to lb/a and all treatments were compared using Duncan's Multiple Range Test (α=0.05).

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

Table 1. Grower herbicide and/or fertility site applications in 2013.

Site	Date	Product	Rate
Appleton Ridge	5/3	Velossa Diuron Callisto Grounded Black Label	0.5 gal/a 0.4 gal/a 3 oz/a 1 pt/a 1 gal/a
	6/1	Sinbar	3 lbs/14 a spot
	6/20	Credit w/COC	32 oz/14 a spot
	6/20	Callisto Poast LI700 Black Label	3 oz/a 1 pt/a 4.8 oz/a 1 gal/a
Hope	5/10	Velpar Diuron	0.5 gal/a 1.6 qt/a
	6/6	Poast Callisto	2 pt/a 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 Diuron Callisto	6 pt/a 2 lb/a 6 oz/a
	6/2	MAP	200 lb/a
Ellsworth	4/29	Velossa Diuron Sinbar Grounded	0.4 gal/a 0.4 gal/a 2 lb/a 0.17 gal/a
	6/18	Black Label	1 gal/a
	7/29	TigerSul sulfur DAP	147 lb/a 100 lb/a
	9/16	Arrow Boost	8 oz/a 6.4 gal/a
	9/26	Glystar	5 gal spot
Orland	5/22	MAP	150 lb/a
Jonesboro	None		
Northfield	Pre-emergence	Velossa Callisto	1.5 lb/a 6 oz/a
	Post-emergence	Arrow	8 oz/a
T-19	5/31	Velpar L Sinbar Diuron 4L	6 pt/a 1.5 lb/a 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 Callisto	8 oz/a 6 oz/a
	8/13	Arrow Callisto	8 oz/a 6 oz/a

RESULTS: There were no significant differences between treatments for wild blueberry cover in 2014 (Figure 2). Wild blueberry cover in all treatments was within 10% of each other, with the exception of the untreated check, which was lower than all other treatments. The only significant differences in broadleaf weed cover were between the check and the two grower treatments, the grower treatments having significantly lower broadleaf weed cover (Figure 3). Sandea + Sinbar and Velpar alone resulted in approximately the same level of broadleaf weed cover as the grower treatments, but the differences were not significant. The Sandea + Sinbar treatment was slightly higher (14.2%) than the grower treatment (13.2%), but the Velpar treatment also had 13.2% cover, so a lack of significant difference may be due to greater variation (error) in this treatment. There were no significant differences in grass cover between treatments, with the exception that Matrix had significantly less grass cover compared to the check (Figure 4). The test herbicides alone resulted in the same or more grass cover than the untreated check, and Sinbar performed better alone than in combination with Alion or Sandea. However, the addition of Sandea to the grower treatment resulted in a reduction of grasses.

Figure 2. Wild blueberry cover in the crop year. All treatments are compared to the check; Sinbar combinations are compared to Sinbar alone, and Velpar combinations are compared to Velpar alone (α=0.05).

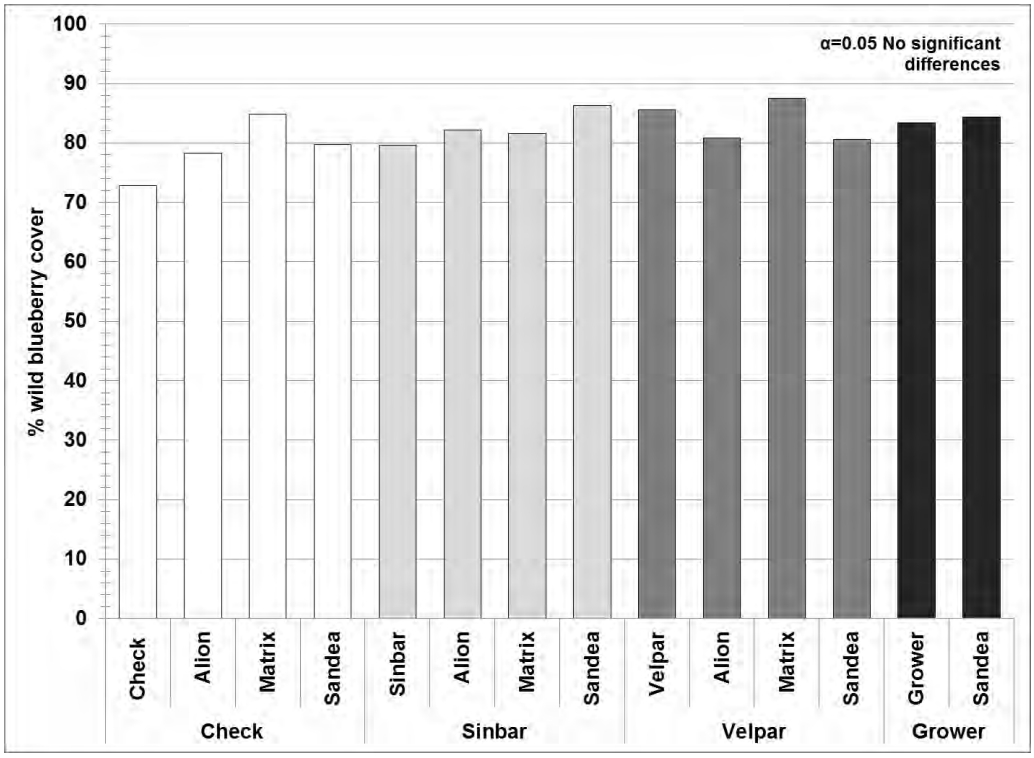


Figure 3. Broadleaf weed cover in the crop year. All treatments are compared to the check; Sinbar combinations are compared to Sinbar alone, and Velpar combinations are compared to Velpar alone (α=0.05).

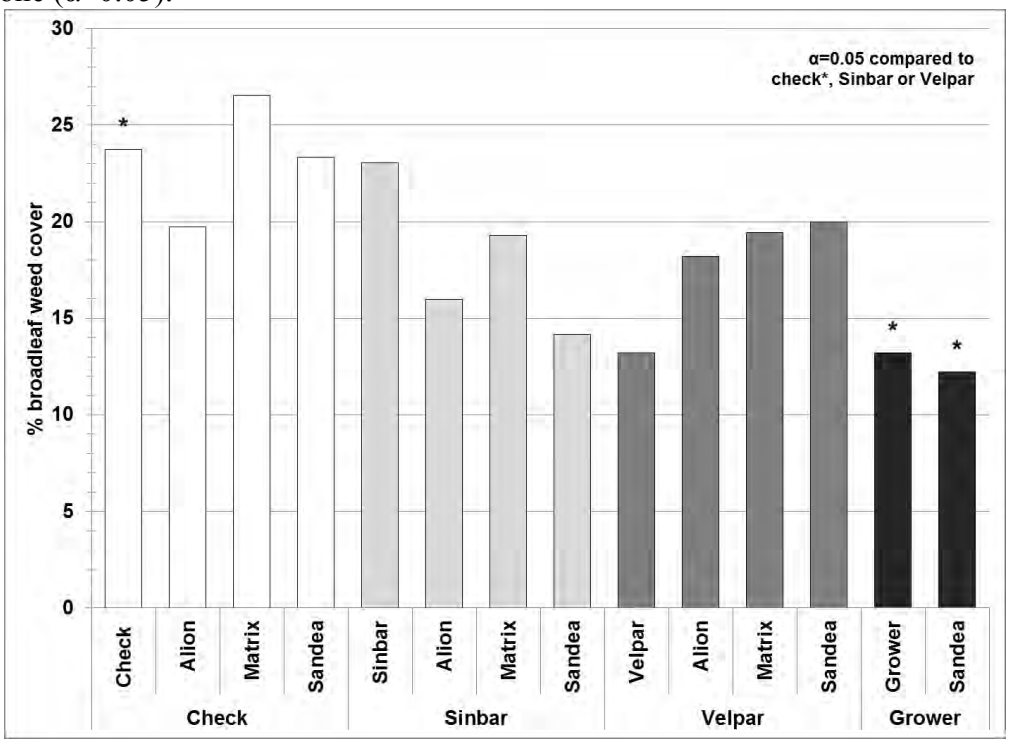
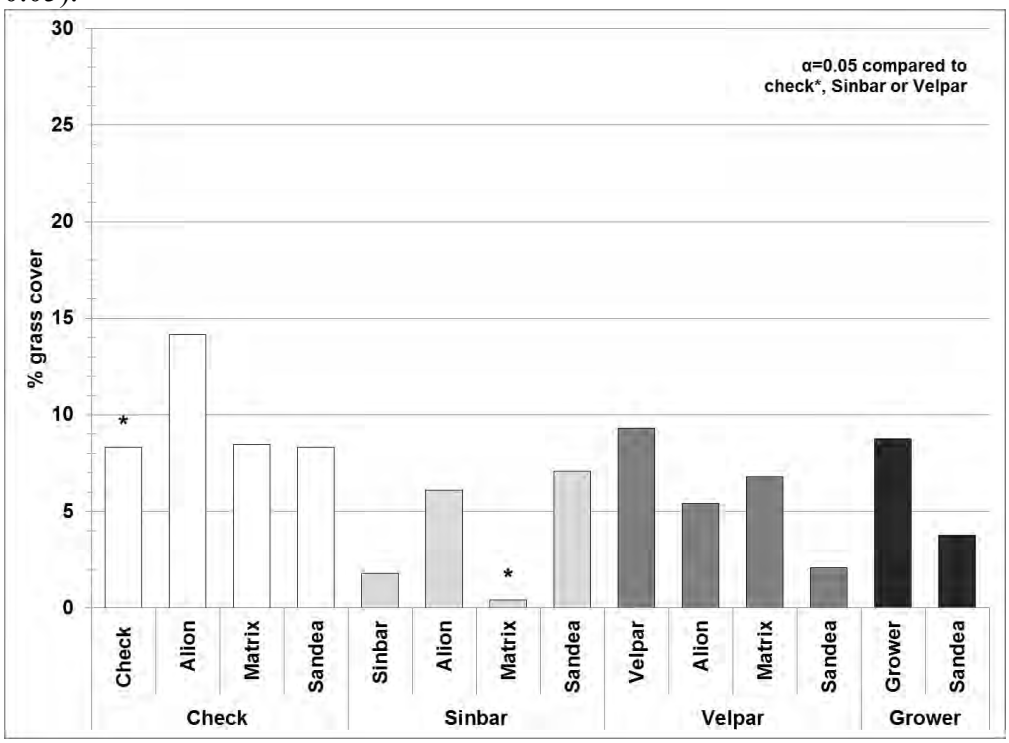
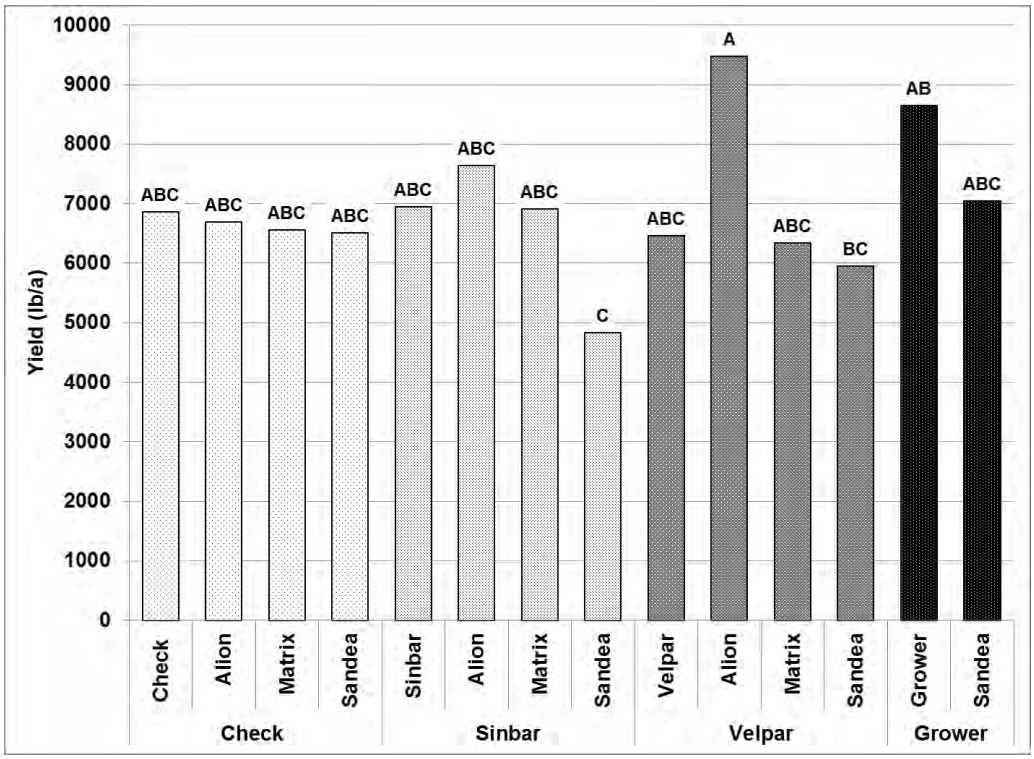


Figure 4. Grass cover in the crop year. All treatments are compared to the check; Sinbar combinations are compared to Sinbar alone, and Velpar combinations are compared to Velpar alone (α=0.05).



Although Alion+Velpar did not result in the best weed control over time, it did result in the highest yield of any treatment (Figure 5). Conversely, although Sandea + Sinbar was average in prune year weed control and among the best in crop year broadleaf weed control, it resulted in the lowest yield of all treatments. This appears due to phytotoxicity observed in the prune year. In June 2013, Alion + Velpar had <1% phytotoxicity; by contrast, Sandea + Sinbar had the most phytotoxicity of any treatment at 18.6%. In fact, all Sandea treatments showed 15-20% injury in 2013, and three Sandea treatments (all except Grower Sandea) resulted in the three lowest yields.

Figure 5. Wild blueberry yield (lb/a) for all treatments. Different letters denote significance (α=0.05).



CONCLUSIONS: There appeared to be a carryover effect of the herbicide regimens the growers used in the 2013 prune year. Table 1 shows that all but two growers used Velpar in 2013, and five out of nine sites had diuron and/or Callisto applied as well. This carryover effect on broadleaf weeds was slightly improved by the addition of Sandea; although not significantly so; the only two test herbicide treatments to show the same carryover effect were Velpar alone and Sandea + Sinbar. Therefore, adding Sandea to a grower's regular spray regimen may improve broadleaf weed control carryover into the crop year, as well as providing an alternative mode of action to manage resistance and suppress certain hard to control weeds.
In regard to grass control, the results shown in Figure 4 illustrate that these test herbicides alone are not sufficient to control grasses. They should be used in combination with Sinbar or Matrix, or controlled with postemergence applications of Poast, or with the use of sulfur and/or other products for grass control. This is illustrated by the combination of Sandea with the grower treatment, which had a reduction in grass cover beyond the grower treatment alone, albeit not

significant compared to the check. Table 1 shows that almost all growers used one or more of the aforementioned grass control agents.
In 2013, we stated that 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. 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. When weed control over both years is taken into account, it becomes clear that these test herbicides are a good addition to the grower "toolkit" to be used in combination with other weed control products, but are not sufficient to control weeds long-term by themselves.
Finally, the use of Alion and Matrix, whether alone or in combination, did not appear to negatively affect yield compared to the check or grower treatment. However, even when applied in fall which reduced injury to wild blueberry, Sandea phytotoxicity was still high enough to reduce yields. Sandea may be better recommended for areas with problem weeds, where the loss of yield due to weed pressure would be greater than the loss due to wild blueberry injury.

RECOMMENDATIONS: The 2013-15 follow-up trial to this trial is slated to be harvested in 2015 and assessed for effects on yield. In addition, a trial to assess the effects of Alion, Matrix, Sandea, Chateau and Trellis on horseweed (*Conyza canadensis*) was initiated in November 2014. Another trial looking at the effects of these herbicides on red sorrel (*Rumex acetosella*) will be initiated early next spring.

INPUT SYSTEMS STUDY – *ANCILLARY STUDY*

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

22. TITLE: Evaluation of fall and spring combinations of preemergence herbicides to prevent weed resistance in wild blueberry fields, 2013-15.

METHODS: In the fall of 2013, a trial was initiated to test the efficacy of several unregistered and/or untested herbicides on wild blueberries in Maine, in conjunction with the industry standards Velpar and Sinbar. Nine sites across the wild blueberry growing region of Maine were sprayed in November 2013 and/or preemergence in May 2014: Union, Appleton, Hope, Ellsworth, Penobscot, Wesley, Northfield, Jonesboro, and T19. The main 18'x54' treatments were as follows: Alion (5 oz/a fall and both in fall+spring; 6.5 oz/a spring), Sandea (1 oz/a fall), Matrix (2 oz/a fall), Trellis (1.33 lb/a spring) and an untreated check. In spring 2014 on 8'x72' strips of Sinbar (2 lb/a), Velpar (1 lb/a) or an untreated check were applied at right angles to the test herbicides for a total of 21 treatments (Figure 1). Wild blueberry cover and phytotoxicity, broadleaf weed cover, and grass cover were assessed by sampling two 1 m^2 quadrats per treatment in June and August 2014 for all treatments as well as the growers' spray regimes outside the trial areas. 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. Soil samples were also collected at each site and analyzed for percent organic matter, soil pH and soil texture (Table 1). The Wesley site was dropped after the first assessment because it was over-sprayed by the grower, and was not soil sampled.

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

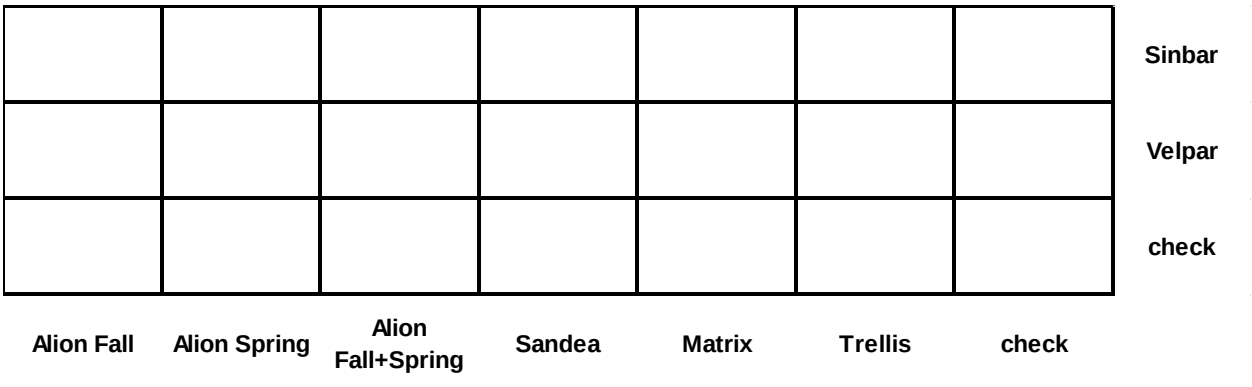


Table 1. Soil characteristics of the eight sites used in the initial data analysis.

County:	Knox-Lincoln			Hancock		Washington		
Town:	**Union**	**Hope**	**Appleton**	**Penobscot**	**Ellsworth**	**Northfield**	**Jonesboro**	**T19**
pH	4.1	4.1	4.4	4.3	4.4	5.0	4.3	4.3
% OM	12.1	10.6	11.0	12.3	8.1	11.5	8.9	9.5
% sand	52	55	42	43	62	59	73	57
% silt	37	36	49	47	29	30	21	32
% clay	11	9	9	10	9	11	6	11
texture	Sandy loam	Sandy loam	Silt loam	Loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam

The main effects of the test herbicides alone, with Velpar, or with Sinbar were compared to the untreated check or to the samples from the growers' fields using t-tests for pairwise comparisons (α=0.05). The test herbicide treatments were also individually compared to the check and the combinations to the check and the respective industry standard (e.g. Velpar combinations to the check and to Velpar) using a non-parametric one-way exact test (α=0.05). Six additional sites were excluded after data analysis indicated a lack of weeds across all treatments, leaving only the Northfield and Jonesboro sites for analysis because they were the only two sites which had at least 10% weed cover in at least half of the treatments.

RESULTS: The summer of 2014 was relatively cool and was not overly dry, so we would have expected more of both broadleaf weeds and grasses than was observed in the trial areas. It was noted that both the untreated check and the untreated trial area buffers at the dropped sites were almost weed-free. This indicates that there was likely a carryover effect from the consistent herbicide applications by the growers (Photo 1). Table 2 lists the herbicides and fertilizers that each grower used on the remainder of the field in which the trial area was located. Note that the Northfield and Jonesboro sites received the least herbicide and fertilizer of all sites. These two sites were also third and first, respectively, in terms of % sand, and Northfield also had the highest soil pH (Table 1). The Northfield site had been leveled just prior to the initiation of this trial, and mulch was put down in bare areas in fall 2013 and spring 2014. The leveling stimulated weed growth, but the mulch suppressed weeds so that site weed pressure was less than expected.

Table 2. Grower herbicide and fertilizer applications in 2014.

Site	Product	Rate	Date
Union	Sinbar	2 lb/a	4/30
	Diuron	1.6 qt/a	
	Callisto	6 oz/a	
	fert 16.5-34.5-4.5 w/0.3 B	200 lb/a	6/21
Hope	Velpar	2 qt/a	5/9
	Diuron	1.6 qt/a	
	Callisto	3 oz/a	
	fert L 7-22-5	1 gal/a	
	Initiate	8 oz/a	6/17
	Callisto	3 oz/a	
	Matrix w/choice, LI-700	2 oz/a	
	foliar fert 7-22-5	1 gal/a	
	DAP + Avail	120 lb/a	
Appleton	Velpar	2 qt/a	5/14-6/4
	Diuron	1.58 qt/a	
	16.5-34.5-4.5 w/0.3 B	150 lb/a	6/21,23
	Callisto	3 oz/a	7/1,3,6
	Poast	2 pt/a	
	w/COC	2 pt/a	
	w/Request pH adjuster	1 pt/a	
Penobscot	DAP	111 lb/a	5/23
	Sinbar	.918 lb/a	6/4
	Velossa	2 qt/a	
	Parrot	1.8 lb/a	
	Request	2.9 oz/a spot spray	7/12
	Callisto	1.68 oz/a spot spray	
	Arrow	3.7 oz/a	7/21
Ellsworth	Velossa	4.8 pt/a	5/8
	Callisto	6 oz/a	
	MAP	200 lb/a	6/2
	Black Label	1 gal/a	6/25
	Arrow 2EC	8 oz/a	7/29
	w/Boost	1 qt/100 gal	
	glyphosate	weed wiper	Aug/Sept
Northfield	Velpar	1 lb/a	6/1
	Callisto	6 oz/a	
	Matrix	2 oz/a	
	fert 7-21-4	5 lb/a	7/12
	w/Cu+Zn micropak	0.5 lb/a	
Jonesboro	none		
T19	Velpar	1 lb/a	5/24
	Callisto	3 oz/a	
	Callisto	3 oz/a spot spray	6/2
	Callisto	3 oz/a	6/7
	Poast	1 qt/a	

Photo 1. The untreated check at the dropped Penobscot site, showing a lack of weeds.

There were no significant differences for main effects of the test herbicides ("others") with or without the industry standards, when compared to the untreated check or grower spray regimens (Figure 2-3). For eight pairwise comparisons, if the variances had been equal there would have been a significant difference (as denoted by the symbols in parentheses), but the unequal variances and lack of replications negated the difference. There are some trends worth mentioning, however. June broadleaf weed cover was lower in the test herbicides alone plots and plots with Velpar. By August broadleaf weed cover had increased in the plots with test herbicides alone, with Sinbar and the grower treatments but not in the check or plots with Velpar; however, the grower treatments still exhibited a potentially significant reduction compared to the check. In June, grass cover in the plots with Sinbar was lower compared to the check or grower treatments, and remained so at the August evaluation; the grower treatments resulted in the same amount of grasses as the untreated check.

Figure 2. Main effects of the test herbicides alone, with Velpar or with Sinbar, for wild blueberry cover and phytotoxicity compared to the check or grower herbicide regimens (α=0.05).

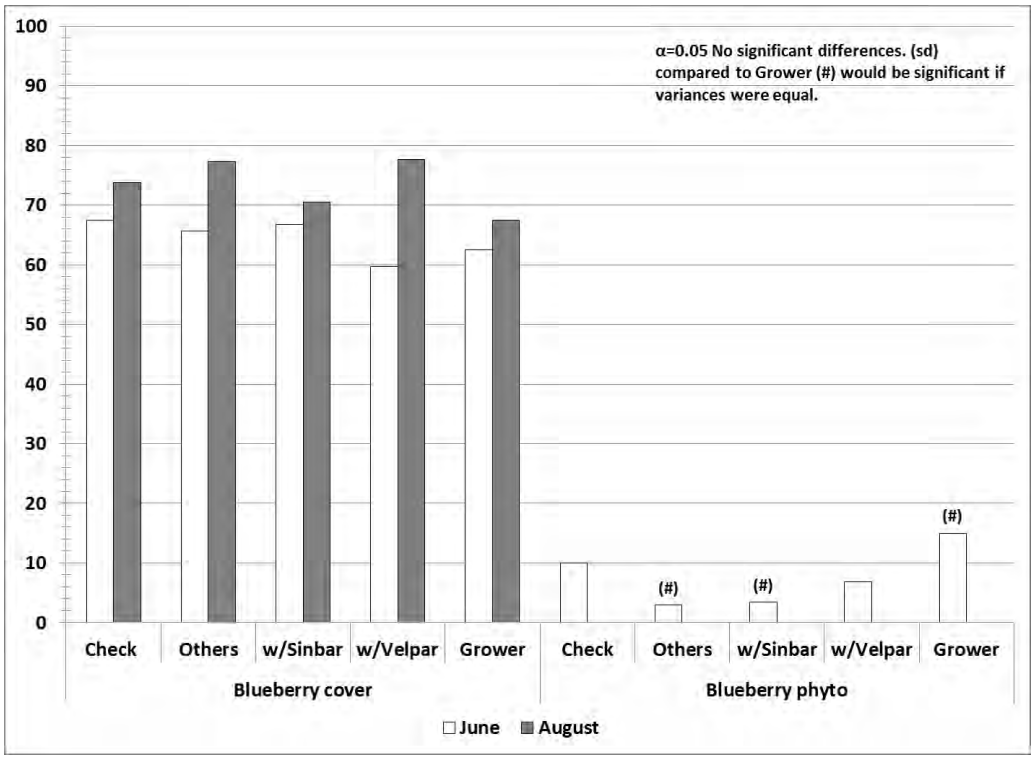
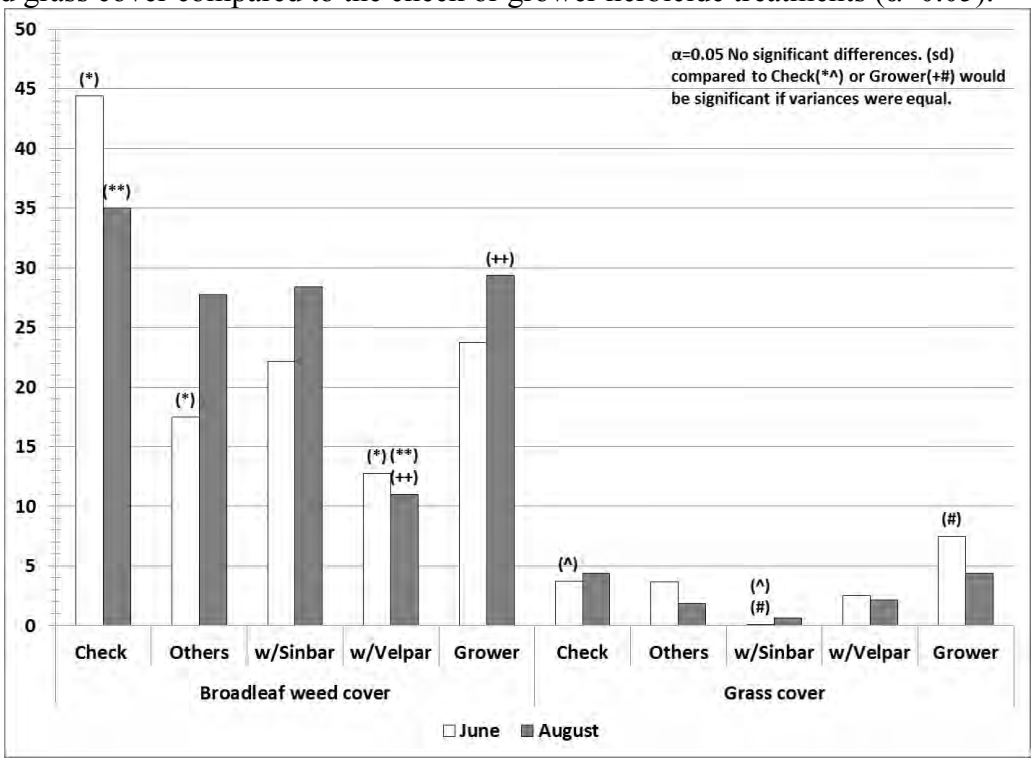


Figure 3. Main effects of the test herbicides alone, with Velpar or with Sinbar, for broadleaf weed and grass cover compared to the check or grower herbicide treatments (α=0.05).



There were no significant differences in blueberry cover among Alion treatments alone, with Sinbar or with Velpar at either assessment (Figure 4). Sandea and Sandea+Sinbar blueberry cover was significantly greater in August compared to the check or Sinbar alone, respectively; otherwise, there were no other significant differences in blueberry cover (Figure 5). Phytotoxicity was minimal in general, with the highest phytotoxicity seen in the grower treatments, and none was noted at the August assessment (Figures 6-7). It should be noted that blueberry in the check showed minor chlorosis in some clones. Because this could not be separated from chlorosis due to herbicide injury, it was recorded as a baseline to compare to other treatments.

Figure 4. Wild blueberry cover in the Alion treatments. All treatments are compared to the check; Sinbar combinations are compared to Sinbar alone, and Velpar combinations are compared to Velpar alone (α=0.05).

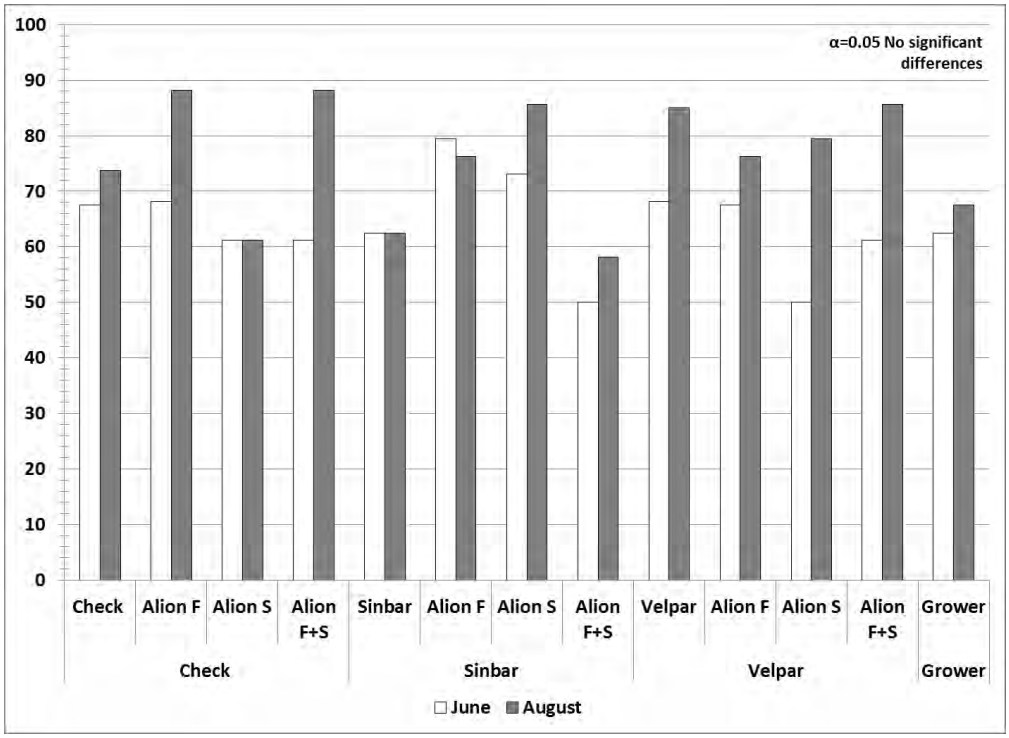


Figure 5. Wild blueberry cover in the Sandea, Matrix and Trellis treatments. All treatments are compared to the check*; Sinbar combinations are compared to Sinbar^ alone, and Velpar combinations are compared to Velpar alone (α=0.05).

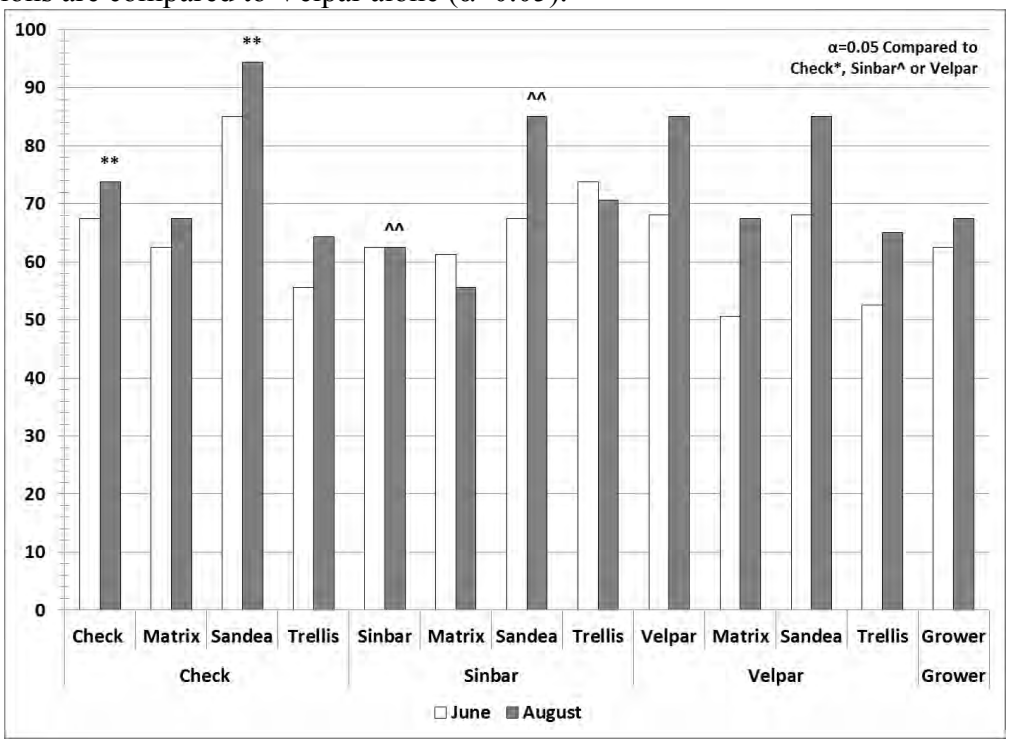


Figure 6. Wild blueberry phytotoxicity in the Alion treatments. All treatments are compared to the check; Sinbar combinations are compared to Sinbar alone, and Velpar combinations are compared to Velpar alone (α=0.05).

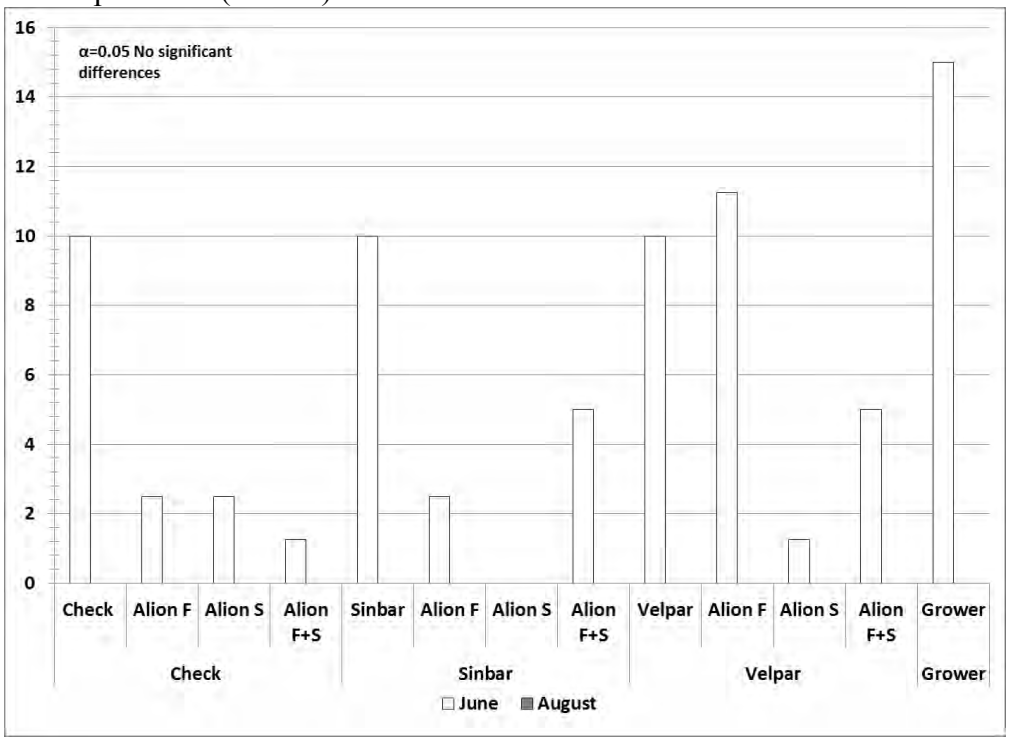
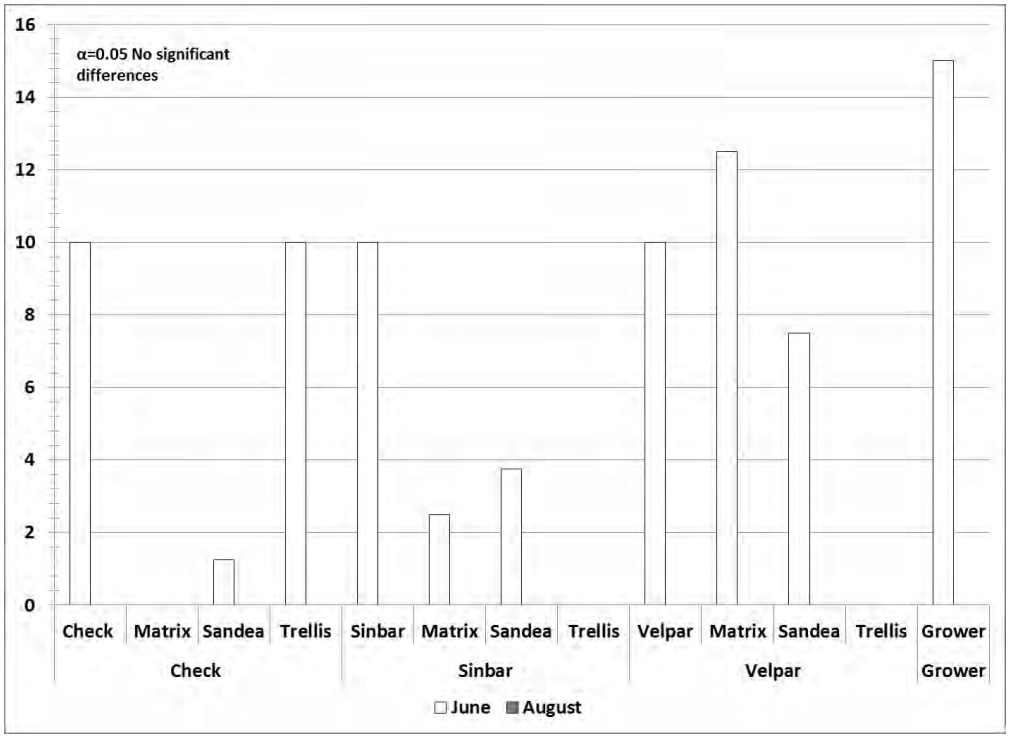


Figure 7. Wild blueberry phytotoxicity in the Sandea, Matrix and Trellis treatments. All treatments are compared to the check; Sinbar combinations are compared to Sinbar alone, and Velpar combinations are compared to Velpar alone (α=0.05).



As observed in previous trials, the use of Sinbar alone increased broadleaf weed cover compared to the untreated check. Conversely, the Alion, Sandea and Trellis treatments combined with Sinbar suppressed broadleaf weeds significantly more than Sinbar alone (Figures 8-9). However, in the Sandea+Sinbar treatment the significant suppression effect did not last long-term (Photo 2). Matrix, alone or in combination, did not control broadleaf weeds better than the industry standards alone (Photo 3). Alion+Sinbar, regardless of spray timing or frequency, also significantly suppressed broadleaf weeds compared to the check in June, but not in August. The spring application of Alion in combination with Velpar significantly reduced broadleaf weeds compared to the check in June, but by August the difference was no longer significant (Photo 4).

Figure 8. Broadleaf weed cover in the Alion treatments. All treatments are compared to the check*; Sinbar combinations are compared to Sinbar^ alone, and Velpar combinations are compared to Velpar alone (α=0.05).

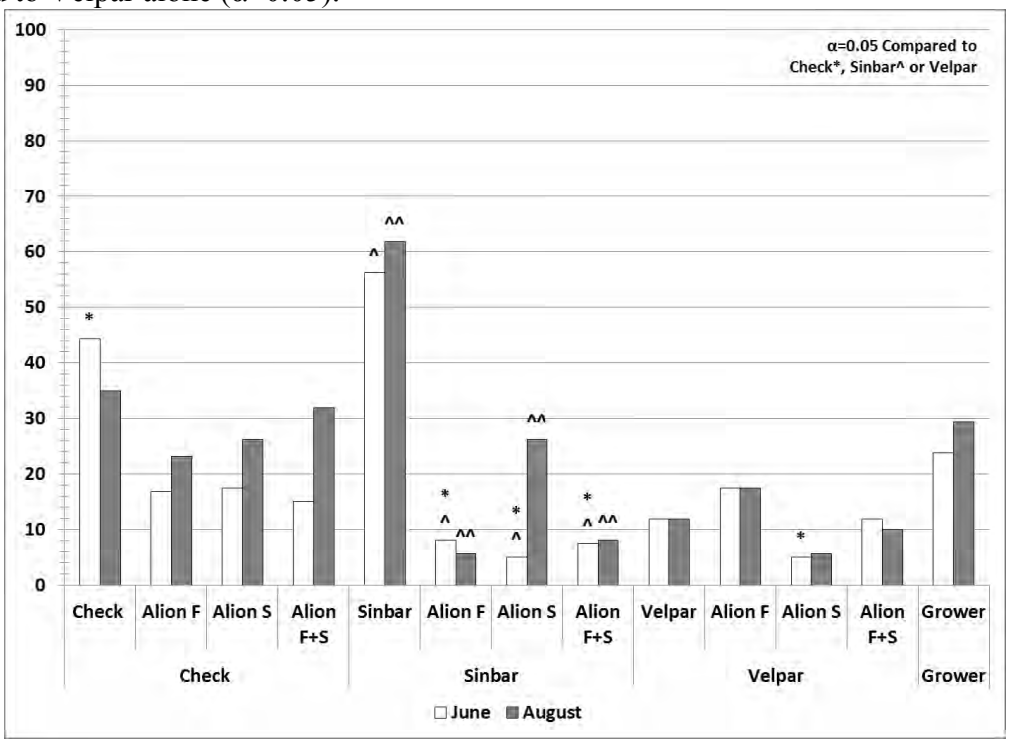


Figure 9. Broadleaf weed cover in the Sandea, Matrix and Trellis treatments. All treatments are compared to the check*; Sinbar combinations are compared to Sinbar^ alone, and Velpar combinations are compared to Velpar alone (α=0.05).

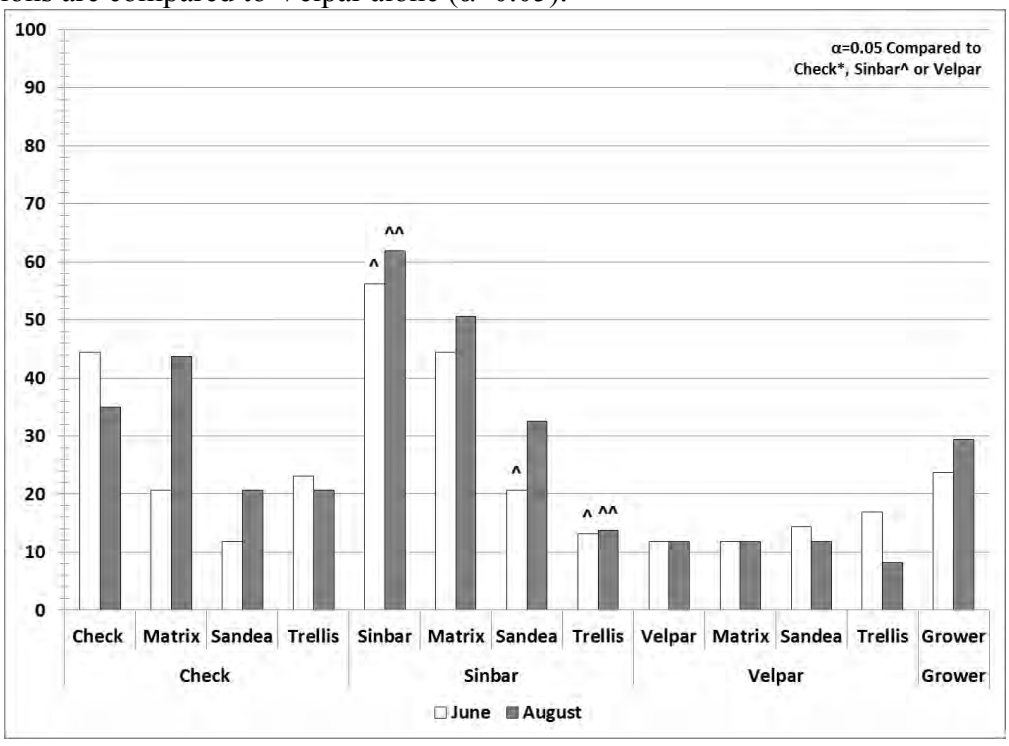


Photo 2. Sandea + Sinbar treatment in August, showing a lack of long-term broadleaf weed control.

Photo 3. Matrix + Sinbar in August. Broadleaf weeds were not well controlled; note the bunchberry present among the blueberry stems.

Photo 4. Spring Alion + Velpar in June, showing reduced weed pressure as compared to the check.

Grasses were essentially eliminated by Sinbar and the Sinbar combinations; otherwise, although grass pressure was very low there were no differences among the check and the herbicides alone, or Velpar and its combinations (Figures 10-11). It should be noted that although grass cover was very low and the differences were not significant, Trellis alone or with Sinbar, and Sandea with Velpar did see an increase in grasses from June to August. Grass cover in the grower treatment plots was twice as high compared to the untreated check in June, but by August grass cover was comparable.

Figure 10. Grass cover in the Alion treatments. All treatments are compared to the check; Sinbar combinations are compared to Sinbar alone, and Velpar combinations are compared to Velpar alone (α=0.05).

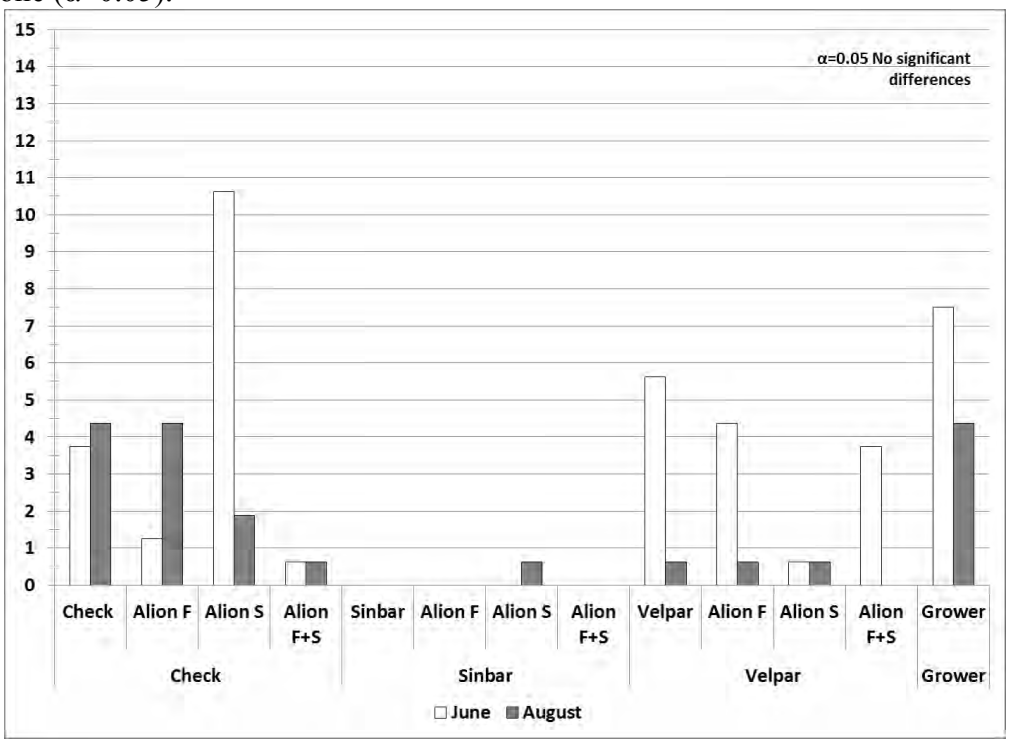
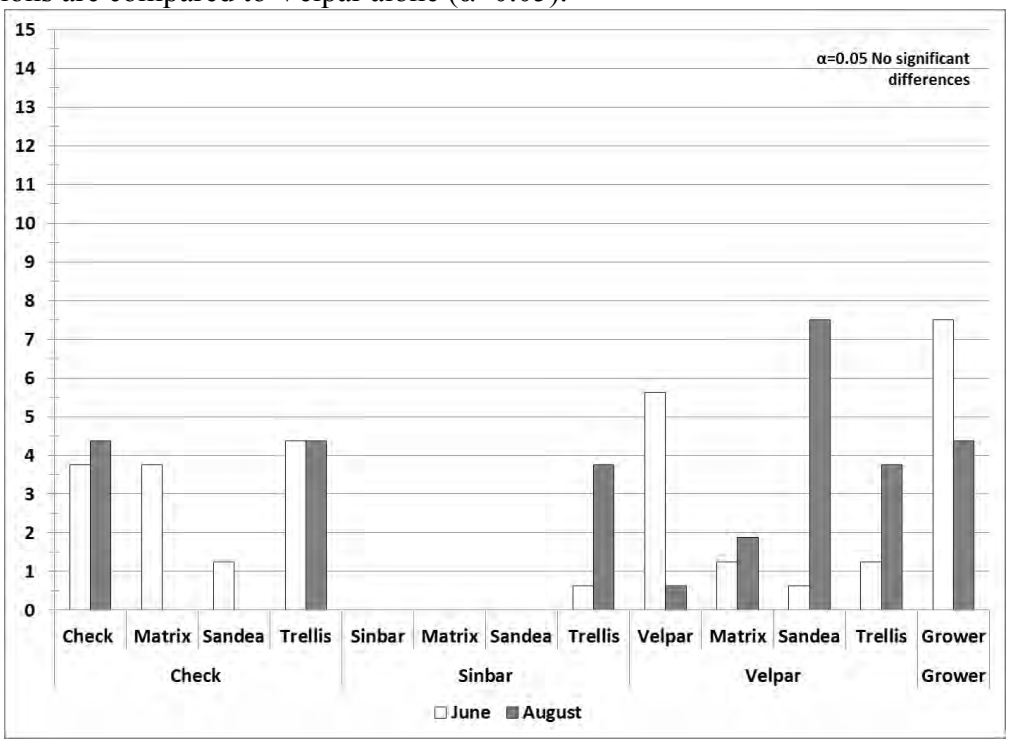


Figure 11. Grass cover in the Sandea, Matrix and Trellis treatments. All treatments are compared to the check*; Sinbar combinations are compared to Sinbar^ alone, and Velpar combinations are compared to Velpar alone (α=0.05).



CONCLUSIONS: Alion, in combination with Sinbar, is effective in controlling both broadleaf weeds and grasses whether applied in the fall or spring. Trellis also appears effective on both broadleaf weeds and grasses in combination with Sinbar. Even though grasses in the Trellis+Sinbar treatment increased from <1% in June to almost 4% in August, the level of grass cover remained so low that we considered this as being effective. Sandea alone or with Sinbar was effective on grasses, but in this trial did not offer long-term suppression of broadleaf weeds. Matrix exhibited fair control of weeds, but was not significantly more effective than the standards alone. The combinations of fall and spring treatments provided for improved weed control versus what growers are currently using.

The earlier spring spray timing and fall spray timing of Alion resolved the phytotoxicity issue observed in some previous trials. The fall application of Sandea also resolved phytotoxicity issues seen in previous trials even at early pre-emergence spray timing (Photo 5). The lack of long-term broadleaf weed control when applied with Sinbar also follows the trend seen in the 2013 trial. Finally, the lack of improved weed control by Matrix, compared to Velpar or Sinbar alone, also follow trends seen in previous trials.

Although not statistically analyzed here, a few other trends emerged that are consistent with previous trials. We saw that using Sinbar alone actually led to an increase in broadleaf weeds, indicating that growers need to use other products in conjunction with Sinbar if they want to control broadleaf weeds. When the grower treatments are examined, we see that growers' current practices often do not lead to large reductions in weed pressure compared to not treating at all (see check vs grower in Figures 8-11). Therefore, the use of the products tested here may be paramount to enabling growers to get ahead of certain hard to control and/or potentially resistant weeds.

Photo 5. Minimal phytotoxicity as stunting/delayed emergence in the Sandea treatment (left side of photo; untreated buffer to right) at the June evaluation.

RECOMMENDATIONS: This trial will be continued through 2015 to assess the effects on wild blueberry yield. Trials for the herbicides tested and with the addition of Chateau will also be established this fall and/or early next spring to target specific problem weeds such as red sorrel (*Rumex acetosella*), St. Johnswort (*Hypericum perforatum*) and horseweed (*Conyza canadensis*) in order to determine effectiveness of these new chemistries. A series of postemergence treatments in combination with Callisto will also be conducted in the late spring and early summer.

INPUT SYSTEMS STUDY – *ANCILLARY STUDY*

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

23. 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. 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 in the crop year for wild blueberry cover, broadleaf weed cover, grass cover, and red sorrel cover on 31 July 2014. Cover data were determined by using the Daubenmire Cover Class system converted to percent, and the data were analyzed using t-tests with a Bonferroni adjustment (α=0.0125). The plots were harvested on 7 August 2014 by hand raking and were weighed on-site in total ounces per plot. Because blueberry cover was uneven and there were extensive areas without plants blueberry yield was adjusted to 100% cover. The harvest weights were converted to lb/a and were compared using Duncan's Multiple Range Test (α=0.05).

RESULTS: There were no significant differences among treatments for wild blueberry cover or any weed cover (Figure 1). In 2013 there was no grass in the plots in July, and only three plots contained grass in September; in July 2014, only one plot had grass (<5 % cover). As in 2013, the lack of differences was not due to the significance level being 1.25 % instead of 5 %; even if the significance level had been 5 %, the only difference would have been in broadleaf weed cover between the check and Roundup. Wild blueberry cover remained somewhat low in all treatments in 2014 due to bare spots which were colonized by red sorrel. In September 2013, we

noted that blueberry cover in the Reglone treatment was slightly reduced compared to other treatments; this trend held true in 2014. Furthermore, in 2014 the Roundup treatment still had the least red sorrel of all treatments, as was observed in 2013 (in 2013, roughly 15% red sorrel compared to 31-38% for other treatments).

There were no significant differences in wild blueberry yield among treatments, and yield in the check, burning and Reglone treatments all were within 500 lbs of each other (yields were high because of the adjustment to 100% cover) (Figure 2). However, despite the fact that the Roundup treatment had approximately the same blueberry cover as the check and burning treatment, its yield was about 3000 lb/a less than the other treatments. This appears to be because although the yields were adjusted to reflect 100% cover due to the great variation in wild blueberry cover, the Roundup treatment also had two plots in which the blueberry plants had almost no berries. Photos 1-3 show an average check plot versus low-yielding plots in the Roundup treatment.

Figure 1. 2014 wild blueberry and weed cover following fall 2012 treatments for red sorrel control.

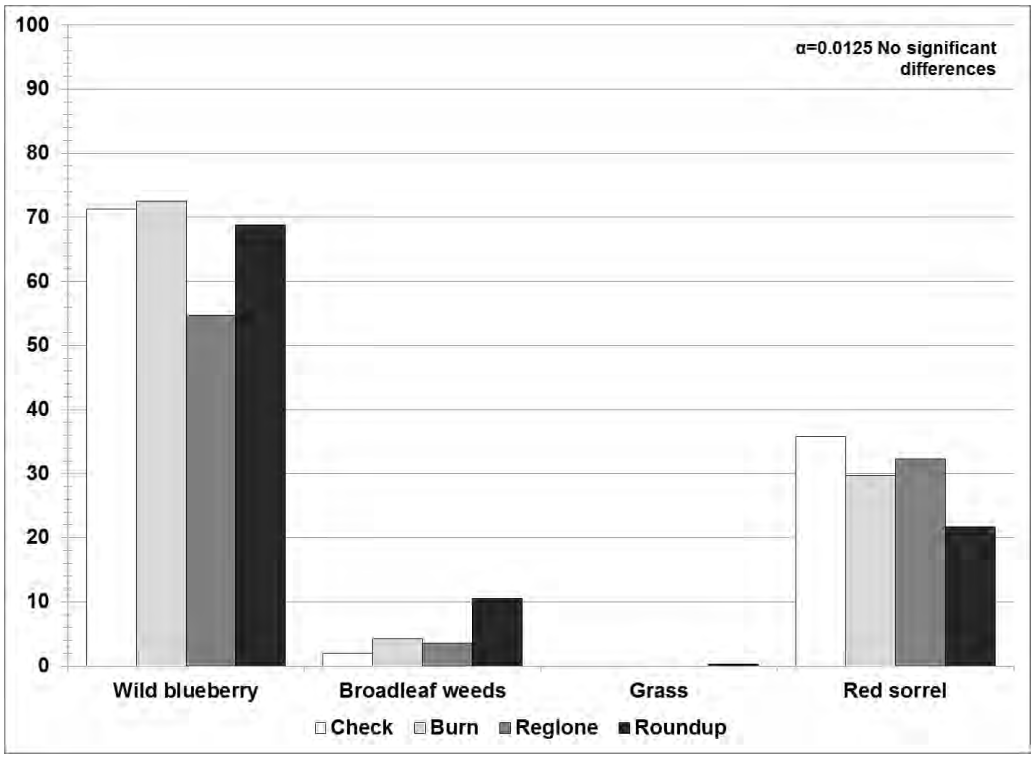


Figure 2. 2014 wild blueberry yield for all treatments, following fall 2012 treatments for red sorrel control (different letters denote significance at α=0.05).

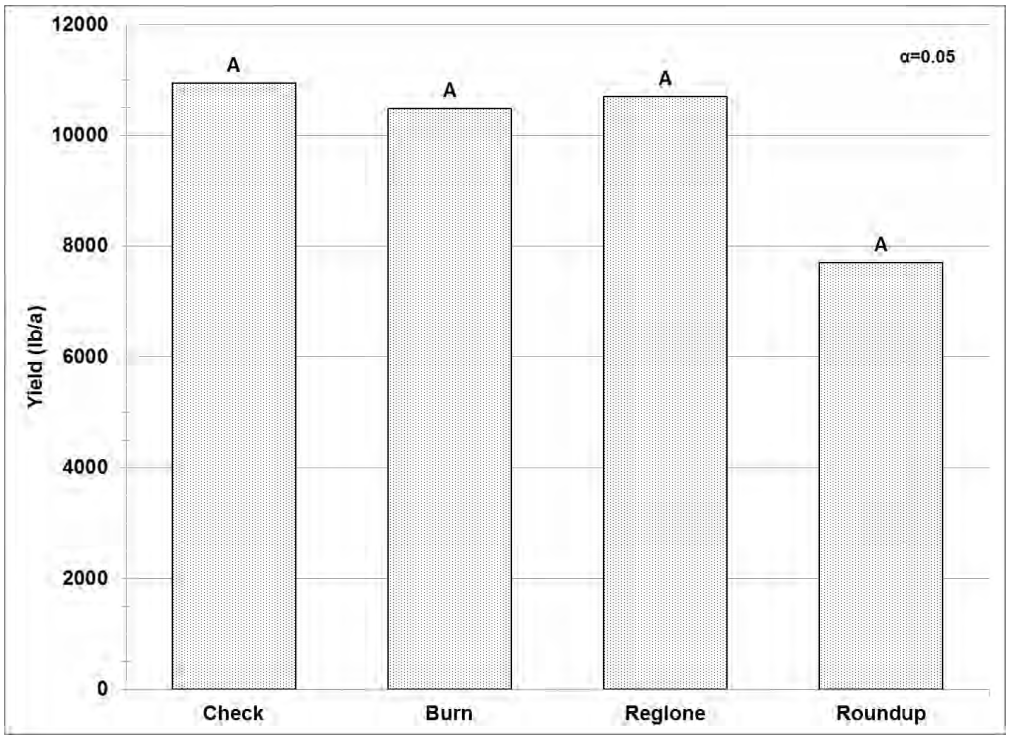


Photo 1. An average plot in the check treatment.

Photo 2. A plot in the Roundup treatment, containing <25% wild blueberry cover.

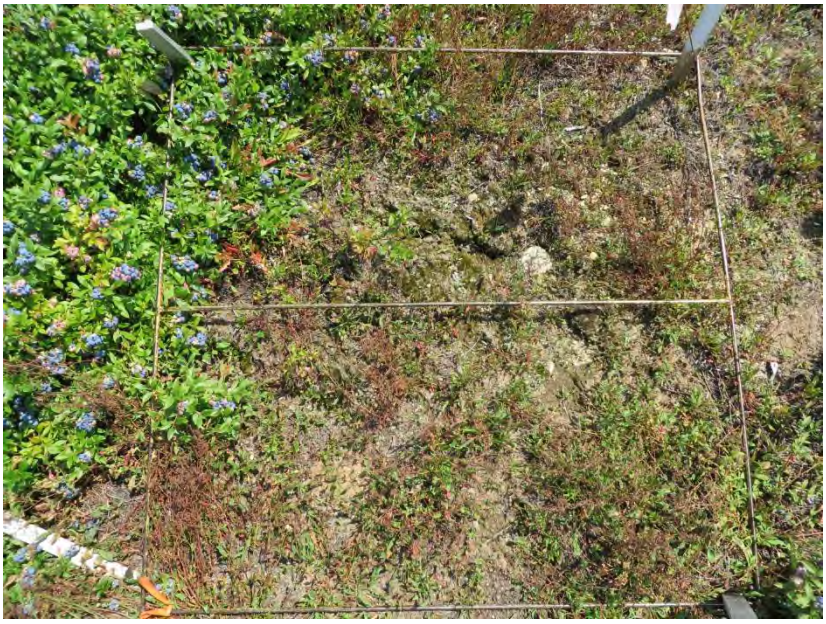


Photo 3. A plot in the Roundup treatment, containing wild blueberry plants with almost no berries.

In 2013, although Roundup controlled red sorrel it also released other broadleaf weeds, namely blue toadflax (*Nuttallanthus canadensis*) and spreading dogbane (*Apocynum androsaemifolium*). In 2014, the main weed present overall was blue toadflax. There was an area with additional weeds such as spreading dogbane and Canada St. Johnswort (*Hypericum canadense*), but this appeared due more to topography (a runoff gully down a slope) rather than treatment. The patch of weeds did not correspond with a lack of yield in the other treatment plots located along the gully; dogbane can shade blueberry to the point that fruit production is reduced, but we did not

see the same lack of berries in other treatment plots with dogbane in that area. In addition, there were a few plots in the other treatments which contained <25-50% wild blueberry cover, but plants in those plots had more berries and did not significantly drive down overall treatment yield. The low yield in the two Roundup plots may also have been due to being sited in a low-producing clone, or residual phytotoxicity from grower treatments upgradient. All of these theories are conjecture, as we could not determine a conclusive reason for the lower yield in the Roundup treatment.

CONCLUSIONS: In 2013, we stated that the only treatment in this trial to show promise in controlling red sorrel when applied in the fall was Roundup, but that it was not a complete success because it did not eradicate red sorrel, and it also released other problem weeds. In 2014, although Roundup continued to control red sorrel better than the other treatments, yield was reduced, although the reason for this is unclear. Therefore, we cannot make conclusive recommendations regarding Roundup at this time.

RECOMMENDATIONS: A follow-up red sorrel control trial was initiated in fall 2013 and will continue through 2015 for crop year cover and yield. We will make our final recommendations at that time. A third trial will be initiated in early spring 2015; this trial will examine the effects of Alion, Matrix, Sandea, Trellis and Chateau on red sorrel.

INPUT SYSTEMS STUDY – *ANCILLARY STUDY*

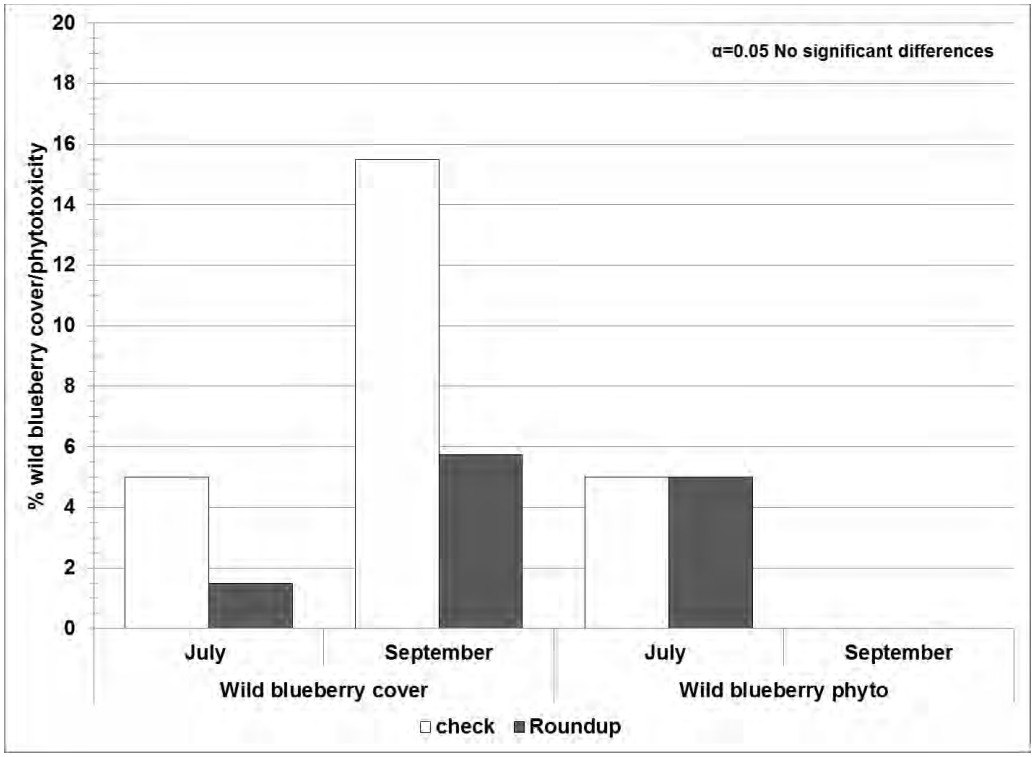
WEED MANAGEMENT: David E. Yarborough, Professor of Horticulture
Jennifer L.D. Cote, Assistant Scientist

24. TITLE: Post-harvest control of red sorrel in a non-crop blueberry field, 2013-2015.

METHODS: In the fall of 2013, we initiated a follow-up trial to the 2012-14 post-harvest red sorrel control 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 Camp Meadow Hill Lot off the Baseline Road in Deblois, ME. The plots were set out in a Completely Randomized Design with ten 3-m^2 replications per treatment, which were as follows: 1. Untreated check; and 2. Roundup 2% v/v. Only the Roundup treatment was carried over from the previous trial as it was the only one to exhibit any long-term control of red sorrel. The Roundup was applied on 31 October 3013 using a backpack boom sprayer with 20 GPA TeeJet nozzles. As in the previous trial, because we wanted to assess whether the Roundup treatment would improve control of 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 2014 growing season. Accordingly, they applied Sandea 1 oz/a and Velossa 1.5 lb/a in late April/early May. The plots were evaluated for wild blueberry cover and phytotoxicity, broadleaf weed cover, grass cover, and red sorrel cover on 2 July and 8 September 2014. 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 (α=0.05).

RESULTS: As in the previous trial, there were no significant differences between the treatments for any cover or wild blueberry phytotoxicity. Wild blueberry cover was very low (<16% overall), and phytotoxicity was 5% for both treatments in July and 0% in September (Figure 1). This was because we set the plots out after pruning in areas that we believed encompassed both wild blueberry and red sorrel (Photo 1); in this field, when the plants emerged in 2014 we saw that the red sorrel mostly occurred in bare spots and therefore did not contain many blueberry plants. The phytotoxicity in the treatments was due to the grower's Sandea application. In past trials assessing Sandea, phytotoxicity was consistently noted as also stunting and delaying wild blueberry growth, which may have also contributed to the low blueberry cover in the plots. However, this is unlikely considering the blueberry plants outside the trial area did not appear stunted or delayed in July or September.

Figure 1. 2014 wild blueberry cover and phytotoxicity following fall 2013 treatment for red sorrel control (α=0.05).

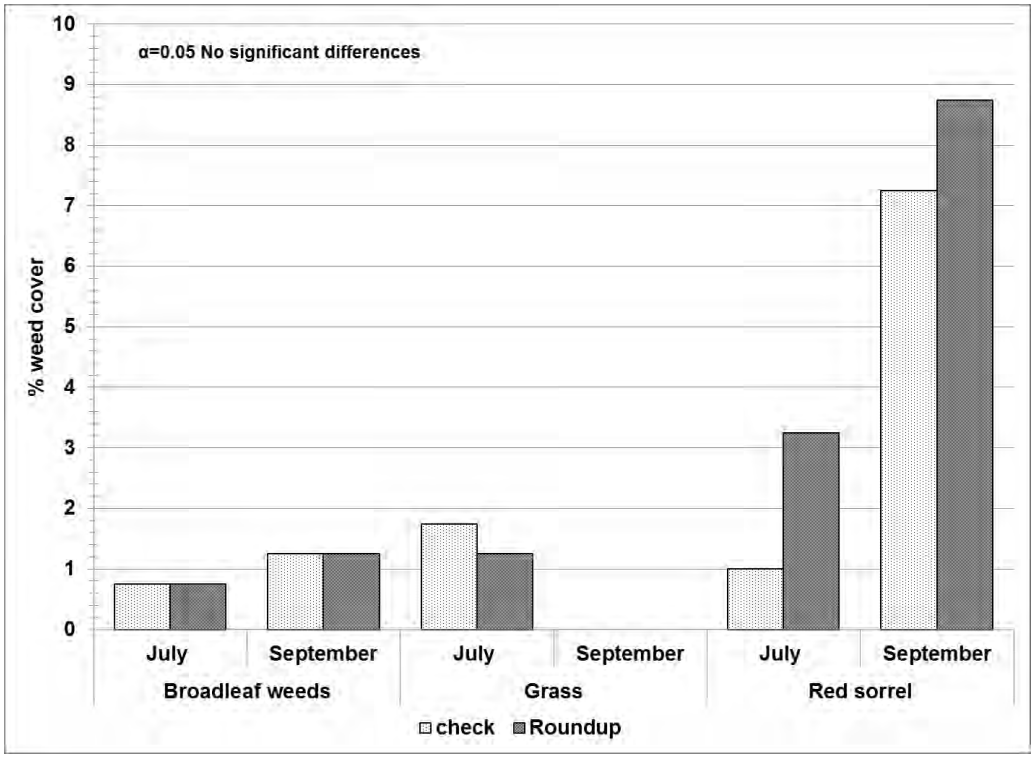


Broadleaf weeds and grasses were below 2% cover at both evaluations, and there were no significant differences between treatments (Figure 2). There was also no difference in red sorrel cover between the two treatments at either evaluation, and red sorrel cover remained below 10% at the September evaluation (Figure 2). We believe the low weed cover is also due to the grower's Velossa and Sandea treatments, because the check, plot buffers and the rest of the field outside the trial area was almost free of weeds (Photo 2).

Photo 1. Trial area at trial initiation on 31 October 2013.

Photo 2. Trial area with Roundup plot (orange flag) in the foreground. Note the lack of wild blueberry, few live weeds and many dead weeds in the trial area, as well as the lack of weeds among the blueberry plants in the background outside the trial area.

Figure 2. 2014 weed cover following fall 2013 treatments for red sorrel control (α=0.05).



CONCLUSIONS: The results of the Roundup treatment in this trial contrast with the 2012-14 trial, in which we noted that Roundup released other broadleaf weeds such as blue toadflax (*Nuttallanthus canadensis*) and spreading dogbane (*Apocynum androsaemifolium*). The difference between the previous trial and this trial is that in the previous trial, the grower applied Velossa, Arrow and Callisto. Therefore, the improved red sorrel and other weed control appears due to the addition of Sandea to the weed management regimen. In light of the combined trial results, we conclude that Roundup does not result in significantly more effective red sorrel control when used in conjunction with other herbicides.

RECOMMENDATIONS: This trial is slated to continue through 2015, at which time we normally would assess cover and compare yields. However, the low wild blueberry cover in 2014 may preclude being able to collect sufficient berries from the plots in order to accurately compare yields. We will assess wild blueberry and weed cover in summer 2015, and will make a determination at that time as to whether the plots will be harvested.
A third red sorrel control trial will be initiated in early spring 2015. This trial will examine the effects of fall applications of Alion, Matrix, Sandea, Trellis and Chateau on wild blueberry, red sorrel and other weeds both with and without the grower's regular spray regimen.

INPUT SYSTEMS STUDY – *ANCILLARY STUDY*

PLANT NUTRITION: Marianne Sarrantonio, Associate Professor of Soil Science

25. TITLE: Effect of soil nutrient amendments on growth and yield of wild blueberries in Maine.

OBJECTIVE: In 2014 my research focused on measuring the yields of wild blueberries in plots established in 2013 (prune year) under three different fertility regimes at three different sites in Maine to determine whether any of the treatments increased berry yields when compared to the unfertilized control plots, and to correlate the depth of the organic pad to berry yield. New plots with similar treatments were established in summer 2014 for berry harvest in late summer 2015.

METHODS: In late July, 2014 blueberries were harvested from sites in Jonesboro, ME (UMaine Blueberry Hill Experimental Farm), Deblois, ME, (Wyman's) and Amherst, ME (Calhoun/Hunter farm) from plots established in 2013. At Blueberry Hill there were 8 replications of each of three treatments in plots that measured 2 m^2 each. Treatments in 2013 included: DAP at 80 lb/ac N (trt 1), DAP + 40 lb/ac N + ProHolly at 40 lb/ac N (trt 2), and a control treatment with no additional amendments. At Wyman's, berries from 6 replications of the same 3 treatments established at Blueberry Hill in 2013 were harvested. At Amherst, 3 replications were harvested; each replication contained 3 treatments: fishmeal applied at 80 lb/ac N, blood meal applied at 80 lb/ac N and a control with no amendment. The Amherst site originally had 6 replications, but parts of the fields had been damaged by a hired manager who did not see the stakes indicating a research site.
Research plots were established at 1 new organic site in 2014, the Dickson Farm in Frankfort, ME, and a second set of plots were established at Blueberry Hill, and Amherst, replicating the 2013 experiments. These sites will be harvested in summer 2015, representing a total of 6 trials lasting 2 years each (Wyman's will not be utilized in 2015, due to logistical problems). Blueberries were harvested from a 1 m^2 area in the middle of each of the 2 m^2 plots at the three research sites established in 2013, using hand held blueberry rakes. Harvested berries were placed into 2 gal. plastic bins and brought back to the University of Maine and weighed within 6 hours of harvest, then frozen for nutritional analyses of the berries (sample preparations currently in progress).

RESULTS: Blueberry yield averages were highest from the Wyman's research plots, averaging the equivalent of 11,894 lb/ac (10,620 kg/ha) fresh wt. vs. roughly 6048 lb/ac (5400 kg/ha) at Blueberry Hill. Yields from plots at Amherst were very low and highly variable both between replications and within treatments. Yields were highest in the control treatment (no nutrients added) at all three sites, but the differences between treatments were not statistically significant at $p=0.05$. There was a significant relationship between the depth of the organic pad at each site and the overall berry yields. Plots with organic pad depth of over 1.5" (3.75 cm) averaged significantly higher yields than other plots with the same treatment. In an informal blind taste test of the blueberries harvested from each site in 2014, eight of the ten tasters thought the organically-grown blueberries were the sweetest and most flavorful, but most admitted that they would probably choose the somewhat larger berries grown on the conventional farms if they were purchasing wild blueberries at the supermarket, due to the additional cost of organic berries.

CONCLUSIONS: Neither inorganic (MAP, DAP) nor organic fertilizers (bloodmeal, fishmeal, Pro-Holly) led to any significant wild blueberry yield increases in any of the trials harvested in 2014. There was a noticeable correlation between the average depth of the organic pad at each of the research sites and the yields obtained from those sites (Table 1), but the correlation was not significant at p=0.05 within research sites. From this study, it appears that the depth of the organic pad may be an indicator of the yield potential in wild blueberry production, but more research is needed to verify whether the correlation between the depth of the organic pad and berry yield is consistent and significant.

RECOMMENDATIONS: Further study of the relationship between the depth of the organic pad and yields should include the nutrient holding capacity of the pad as a means of regulating cationic nutrients to the blueberry plants as well as examine more closely the role of rhizomes in storing plant nutrients.

Table 1. Average depth of organic pad and 2014 yields from 3 sites.

SITE/OP depth/yield	**Blueberry Hill**	**Wyman's (Deblois)**	**Amherst**
Ave. Depth Org. Pad (cm)	2.96 cm	5.40 cm	2.37 cm
Yield (kg ha^{-1})	7660 kg ha^{-1}	10,620 kg ha^{-1}	5260 kg ha^{-1}