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2012 Wild Blueberry Project Reports

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2012 Wild Blueberry Project Reports

January 2013



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FOOD SCIENCE AND NUTRITION

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

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

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

In particular, the objectives of this project were to determine if consumption of wild blueberry will:

1) improve endothelial function, and specifically the functional arterial properties of the aortic vessel in response to phenylephrine induced vasoconstriction and acetylcholine induced vasodilation;

2) influence the expression of genes related to inflammation and lipid metabolism in the adipose tissue and/or in the liver; and

3) the gene expression of inducible nitric oxide synthase (iNOS), prostacyclin I_2 (PGI₂) and cyclooxygenase-2 (COX₂)

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

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

Data are currently being analyzed.

b. Adipocyte size and number

Adipose tissue samples were collected and fixed for microscopy analysis. Adipose cells size and number were determined by count using light microscopy. **Data are currently being analyzed.**

c. Plasma Nitric Oxide (NO)

Nitric Oxide metabolites in plasma were measured using the Nitric Oxide Metabolite Detection Kit (Cayman), a nitrate/nitrite colorimetric assay, following the instructions provided by the manufacturer, with modifications.

NO levels in OZR were on average higher compared to LZR, independent of diet. WB diet resulted in increased NO in LZR, and decreased NO in OZR.

d. Prostacyclin I2 (PGI2) in the aorta

Aorta was incubated in a 2 mL Radnoti tissue bath containing PSS at 37 °C and aerated with 95% $O_2 / 5\%$ CO₂. Tissue was allowed to equilibrate for 20 min before adding phenylephrine (10⁻⁶ M for 10 min) followed by acetylcholine (10⁻⁵ M for 10 min). The effluent was collected and PGI2 levels in the aortic effluent were determined using the enzyme immunoassay 6-keto-PGF1 α EIA Kit (Cayman), following the instructions provided by the manufacturer, with modifications. 6-keto-PGF1 α is a metabolite of non-enzymatic hydrolysis of PGI2.

PGI2 levels were higher in OZR compared to LZR, and significantly increased following WB consumption in OZR.

e. Thromboxane A2 (TXA2) in the aorta

TXA2 levels in the aortic effluent were determined using the enzyme immunoassay Thromboxane B2 EIA Kit (Cayman), following the instructions provided by the manufacturer, with modifications. TXB2 is a metabolite of non-enzymatic hydrolysis of TXA2.

TXA2 were found to be similar across both groups, and not affected by WB consumption.

f. Gene expression as related to inflammation

Expression of CRP, IL-6, TNF-a, adiponectin and Nf-kB genes was evaluated in both liver and adipose tissue.

mRNA from liver and adipose tissues was isolated, retro-transcribed to cDNA and subjected to quantitative Real Time PCR amplification using rat-specific primer sequences for the CRP, IL-6 and TNF-a genes. mRNA from frozen fat fragments was isolated using the RNeasy Lipid Tissue Mini Kit (Quiagen, CA), while mRNA from liver was isolated using the High Pure RNA Isolation Kit (Roche). cDNA was synthesized from mRNA using oligo-dT and Superscript II Reverse Transcriptase (Life Technologies). cDNA was analyzed by RT-PCR on a qPCR System using SYBR Green Master Mix and rat-specific primer sequences targeting the CRP, IL-6 and TNF-a genes. Relative expression of these genes was determined by the $\partial \partial Ct$ method, relative to a housekeeping gene (beta-actin).

Expression of IL-6, TNF- α and Nf-kB was downregulated both in the liver and the abdominal adipose tissue, while CRP expression was downregulated only in the liver. 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 , and increased adiponectin expression.

- g. Gene expression as related to lipid metabolism
- Expression of genes involved in lipid metabolism was also targeted. In particular, expression of fatty acid synthase (FAS), lipoprotein lipase (LPL), and the ATP-binding cassette transporter 1 (ABCA1) was evaluated in both liver and the adipose tissue.
 Following WB, fatty acid synthase expression significantly decreased in both OZR and LZR, while lipoprotein lipase increased in LZR only. ATP-binding cassette transporter 1 expression was induced in both groups following WB treatment.

SIGNIFICANCE: Results of this study suggest that wild blueberry consumption exerts an overall anti-inflammatory effect and it appears to have a significant positive impact on the abnormal lipid profile in the OZR, a model of the metabolic syndrome. Additionally, expression of key enzymes involved in inflammation and lipid homeostasis appears to be favorably altered. This is the first study that has clearly documented the effects of wild blueberries on an animal model of the MetS and results have been incorporated into two manuscripts that have been accepted and will be published soon. These results are extremely important in light of the MetS being a major public health problem in the United States that is expected to increase dramatically in the coming years. Dietary strategies such as adding 1-2 cups of wild blueberries to your daily consumption will prevent or ameliorate the symptoms that cause and/or promote the development of MetS without the deleterious effects of pharmacotherapy. This is of highest priority and importance to public health and to our State's economy.

FOOD SCIENCE AND NUTRITION

- **INVESTIGATOR**: Vivian C. H. Wu, Ph.D., Associate Professor, Dept. of Food Science & Human Nutrition
- **2. TITLE**: Development of effective intervention measures to maintain and improve food safety for wild blueberries.

METHODS: A cocktail mixture of E. *coli* O157: H7 was made and the blueberries samples were inoculated with this cocktail by using the dipping method. After completion of dippinginoculation, an initial level of approximately 7 log CFU/g of inoculum was achieved on blueberries. Two hundred ppm chlorine (Cl₂) and 3ppm chlorine dioxide (ClO₂) solutions were freshly prepared for each trial. A set of 25g of inoculated blueberry samples were placed on a sterile wire screen and 250ml of sterile distilled water (control), 200ppm Cl₂ or 3pm ClO₂ was sprayed and left for various contact times (10 sec, 1 min, 5min, and 10 min). To check the efficiency of these chemicals combined with freezing, after each treatment one set of each contact time was stored at -15°C for 1 week and bacterial enumeration was done later after freezing. The other set was subjected for bacterial enumeration immediately. For bacterial enumeration all the samples were diluted using 25ml 0.1% peptone water and subjected to shaking for 5min at 200rpm. Then, a series of dilutions were prepared for plating. **RESULTS:** So far, besides preliminary studies, we successfully obtained two trials for 200ppm Cl₂ and for 3ppm ClO₂. All our trials showed that for both the chemicals (Cl₂ and ClO₂), treatments combined with freezing had best reduction in E. coli O157:H7. Figure 1 shows recovery of E. coli O157:H7 after treatment with trial-1 200ppm Cl₂ treatment alone without freezing. Maximum of around 0.8 log CFU/g reduction of E. coli O157:H7 was achieved after treatment but not combined with freezing (Figure 2). Recovery of E. coli O157:H7 after treatment combined with freezing was shown in Figure 3. After freezing at -15°C for 1week; there was an additional log reduction which was around 0.7 log CFU/g (Figure 4). The overall log reduction after freezing was around 3.2 Log CFU/g (Figure 5). Trial-2 of 200ppm Cl₂ treatment had similar results like trial-1. Ten minute contact time had the least recovery of E. coli O157:H7 (Figure 6). Similar like trial-1 there was a maximum reduction of around 0.8 Log CFU/g of E .coli O157:H7 after treatment but without freezing (Figure 7). Recovery of *E. coli* O157:H7 after treatment combined with freezing was shown in Figure 8. In trial-2 after combining the treatment with freezing there was an additional log reduction of around 0.9 Log CFU/g (Figure 9). The overall log reduction after freezing was around 3.3 Log CFU/g (Figure 10). For 3ppm ClO₂ treatment, in trial-1 though the least recovery of E. coli O157:H7 was achieved with 10 minute contact time there was no significant difference in recovery for any of the contact times (Figure 11). In this trial of $3ppm ClO_2$, around 0.9 log CFU/g reduction was achieved after treatment but not combined with freezing (Figure 12) but after freezing, there was an additional reduction with a maximum of around 0.9 log CFU/g (Figure 13). Figure 14 shows the recovery of E. coli O157:H7 after treatment combined with freezing. The overall log reduction achieved with 3ppm ClO₂ trial-1 after freezing was around 2 log CFU/g (Figure 15). For trial-2 of 3ppm ClO₂, the least recovery of E. coli O157:H7 was achieved with 1 minute contact time (Figure 16). In this trial, the treatment alone without freezing had a reduction of around $1.0 \log CFU/g$ (Figure 17) while the treatment combined with freezing had an additional reduction of around 2.0 log CFU/g (Figure 18). The recovery of E. coli O157:H7 after freezing was shown in Figure 19. The overall reduction achieved after treatment combined with freezing for trial-2 ClO₂ was around 2.9 Log CFU/g (Figure 20). In all our trials for Cl_2 and ClO_2 , there was no overall significant difference between the contact times in log reduction of E. coli O157:H7 for both with and without freezing treatment.

CONCLUSIONS: Overall any chemical treatment combined with freezing had increased the decontamination efficiency. With 200ppm Cl₂ treatment (the highest concentration) the maximum reduction of *E. coli* O157:H7 achieved was around 3.3 log CFU/g while with only 3ppm ClO₂ concentration, the best reduction of *E. coli* O157:H7 achieved was 2.9 log CFU/g. At this point of study, though, more concentrations of Cl₂ and ClO₂ have to be tested. A conclusion can be made that ClO₂ has better efficiency in killing *E. coli* O157:H7 from blueberries compared to Cl₂ because ClO₂ at the lowest concentration (3ppm) showed almost the same reduction as the Cl₂ which was at higher concentration (200ppm).

FUTURE RESEARCH: The efficiency of Cl_2 at concentrations of 100 and 150 ppm and ClO_2 at concentrations of 5, 10, and 15ppm will be studied in the future. Also efficiency of other chemicals like lactic acid and peroxyacetic acid at different concentrations (1% and 2%) will be studied.

Figure 1: Trial-1 recovery of *E.coli* O157:H7 after treating the blueberries with 200ppm Cl₂ alone without combining freezing are compared with inoculated blueberry control and distilled water washed blueberries.

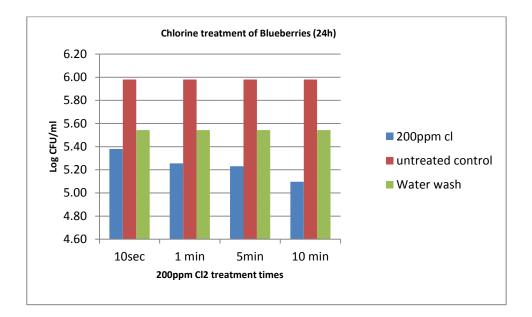


Figure 2: Trial-1 log reduction (CFU/g) of *E.coli*O157:H7 after treating the blueberries with 200ppm Cl_2 but, without freezing.

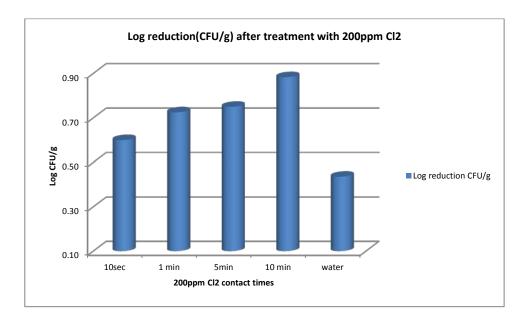


Figure 3: Trial-1 recovery of *E.coli* O157:H7 after treating the blueberries with 200ppm Cl_2 and freezing them at -15 ° C/ 1week are compared with inoculated frozen blueberry control and frozen distilled water washed blueberries.

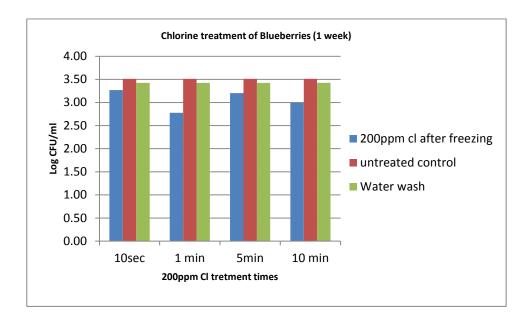


Figure 4: Trial-1 additional Log reduction (CFU/g) with 200ppm Cl_2 treated blueberries after freezing at -15 ° C/ 1 week.

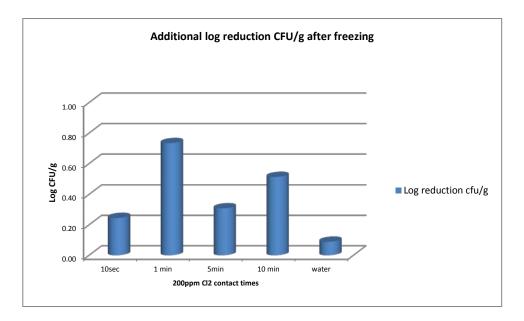


Figure 5: Trial-1 overall Log reduction (CFU/g) of *E. coli* O157:H7 after treating the blueberries with 200ppm Cl_2 and after freezing at -15 ° C/1 week.

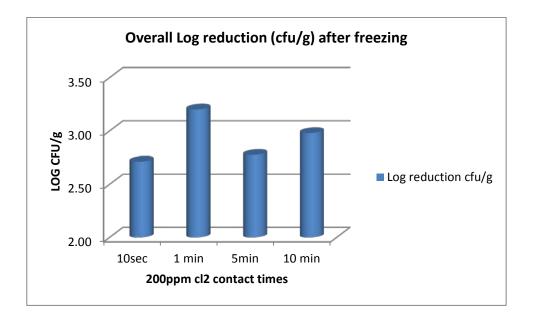


Figure 6: Trial-2 recovery of *E. coli* O157:H7 after treating the blueberries with 200ppm Cl_2 alone without combining with freezing are compared with inoculated blueberry control and distilled water washed blueberries.

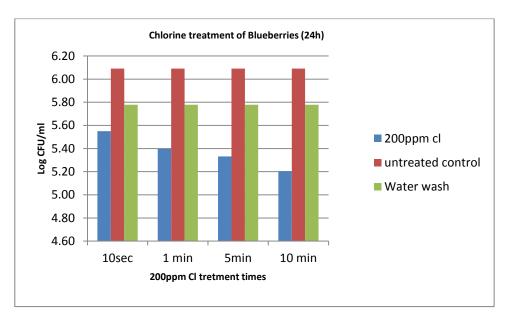


Figure 7: Trial-2 log reduction (CFU/g) of *E. coli* O157:H7 after treating the blueberries with 200ppm Cl_2 but, without freezing.

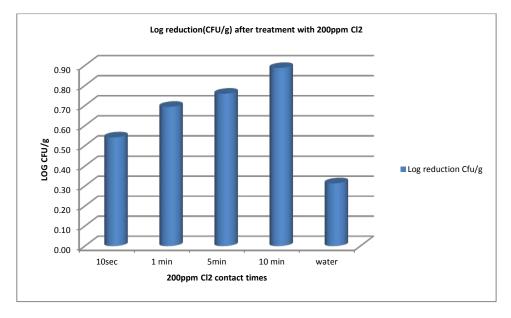


Figure 8: Trial-2 recovery of *E.coli*O157:H7 after treating the blueberries with 200ppm Cl_2 and freezing them at -15 ° C/ 1week are compared with inoculated frozen blueberry control and frozen distilled water washed blueberries.

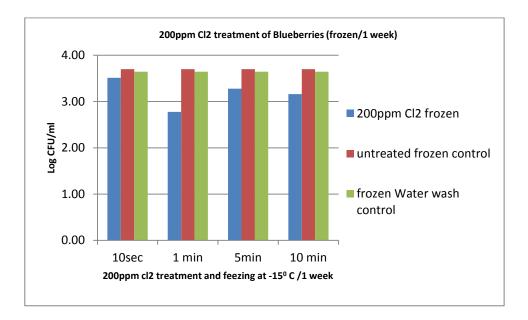


Figure 9: Trial-2 additional Log reduction (CFU/g) with 200ppm Cl_2 treated blueberries after freezing at -15 ° C/ 1 week.

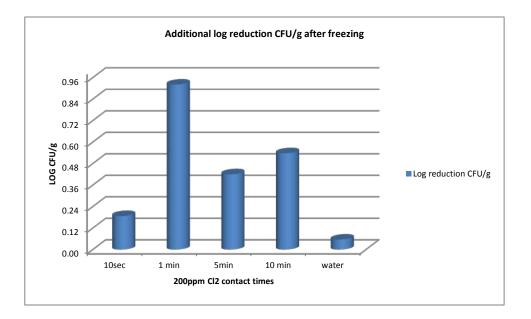


Figure 10: Trial-2 overall Log reduction (CFU/g) of *E. coli* O157:H7 after treating the blueberries with 200ppm Cl_2 and after freezing at -15 ° C/1 week.

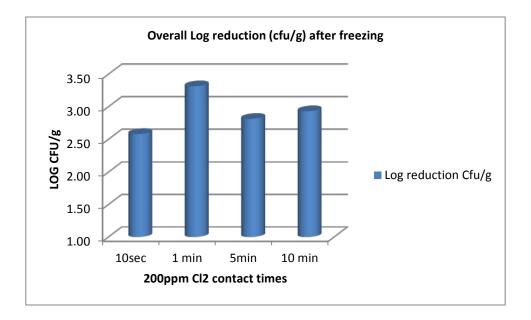


Figure 11: Trial-1 recovery of *E. coli* O157:H7 after treating the blueberries with 3ppm ClO₂ alone without combining with freezing are compared with inoculated blueberry control and distilled water washed blueberries.

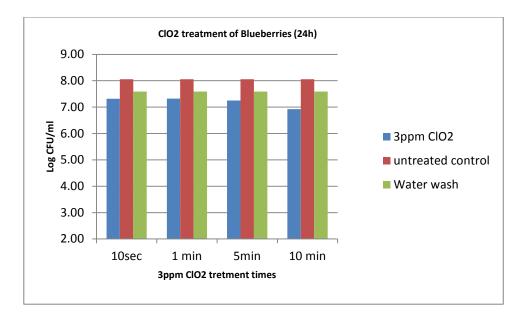


Figure 12: Trial-1 log reduction (CFU/g) of *E.coli* O157:H7 after treating the blueberries with 3ppm ClO_2 but, without freezing.

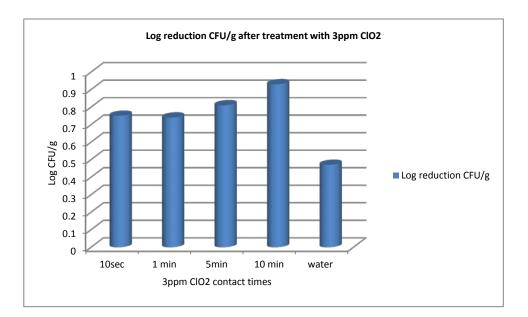


Figure 13: Trial-1 additional Log reduction (CFU/g) with 3ppm ClO₂ treated blueberries after freezing at -15 $^{\circ}$ C/ 1 week.

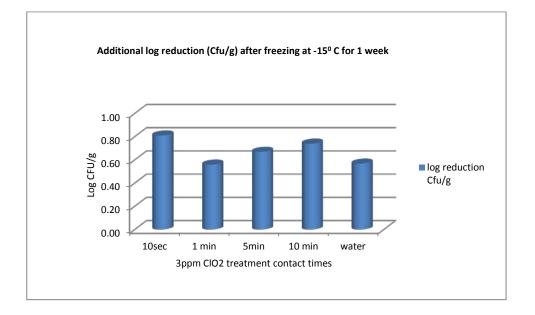


Figure 14: Trial-1 recovery of *E. coli* O157:H7 after treating the blueberries with 3ppm ClO_2 and freezing them at -15 ° C/ 1week are compared with inoculated frozen blueberry control and frozen distilled water washed blueberries.

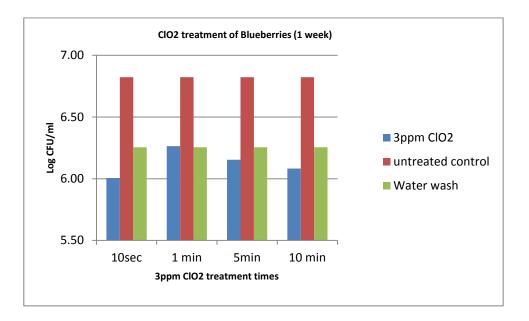


Figure 15: Trial-1 overall Log reduction (CFU/g) of *E. coli* O157:H7 after treating the blueberries with 3ppm ClO₂ and after freezing at -15 $^{\circ}$ C/1 week.

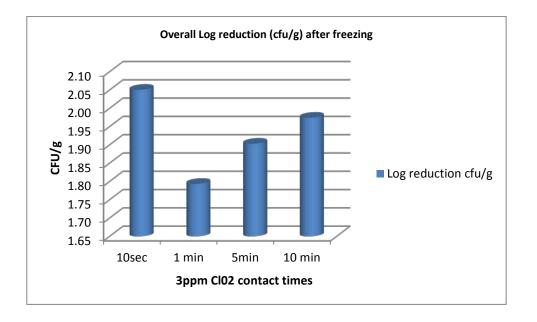


Figure 16: Trial-2 recovery of *E. coli* O157:H7 after treating the blueberries with 3ppm ClO_2 alone without combining with freezing are compared with inoculated blueberry control and distilled water washed blueberries.

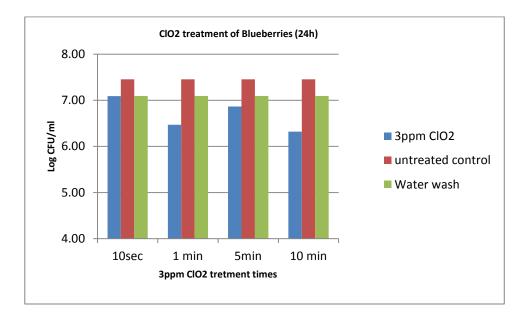


Figure 17: Trial-2 log reduction (CFU/g) of *E. coli* O157:H7 after treating the blueberries with 3ppm ClO_2 but, without freezing.

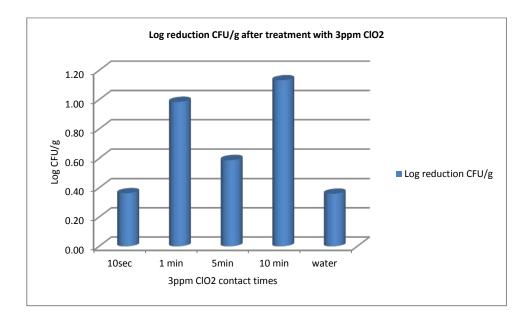


Figure 18: Trial-2 additional Log reduction (CFU/g) with 3ppm ClO₂ treated blueberries after freezing at -15 $^{\circ}$ C/ 1 week.

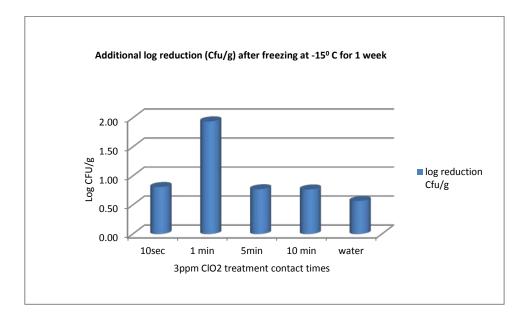


Figure 19: Trial-2 recovery of *E. coli* O157:H7 after treating the blueberries with 3ppm ClO_2 and freezing them at -15 ° C/ 1week are compared with inoculated frozen blueberry control and frozen distilled water washed blueberries.

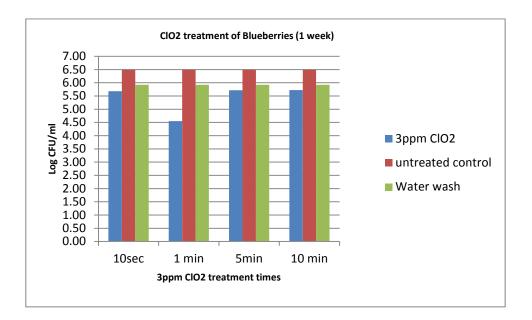
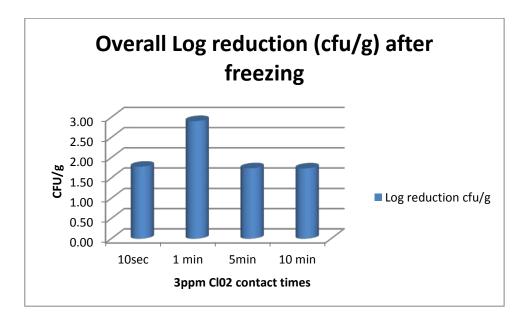


Figure 20: Trial-2 overall Log reduction (CFU/g) of *E.coli*O157:H7 after treating the blueberries with 3ppm ClO₂ and after freezing at -15 $^{\circ}$ C/1 week.



ENTOMOLOGY

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

3. TITLE: I. Control Tactics for Blueberry Pest Insects, 2012.

Study 1. <u>Field control of blueberry tip midge on wild blueberry (pruned year) with foliar</u> <u>application of insecticides</u>

METHODS: There were four replications of each treatment plus four non-treated checks. Each plot measured 7 x 20 ft. A foliar application of Assail 30 SG (imidacloprid) and Imidan 70 WP (phosmet) was applied on 10 Jun to a pruned-year field in Orland, ME. Both materials were applied in 25 gallons of water-mixture per acre with a CO_2 -propelled, 80-inch boom sprayer (76-inch swath) equipped with four, flat-spray, 8002VS TeeJet[®] nozzles operating at 35 psi and at a slow walking speed. Walking speed for each application was regulated using a metronome.

On 7, 18, and 25 Jun, the number of blueberry stems with and without tip midge damage as evidenced by curled leaves was determined from each of three, sq ft samples per plot.

RESULTS AND CONCLUSIONS: Analysis of Variance (ANOVA, RCB) and LSD ($P \le 0.05$) were used to compare mean number of curls among the treatment plots (Table 1). Subplots were pooled within main plots. Data were transformed by the square root to stabilize variance prior to analysis. Assail and Imidan were both ineffective in suppressing tip midge as evidenced by leaf curls (Figure 1). Post-spray populations in the treated plots were higher than the non-treated control. Although the reason for increasing populations in the treated plots is unclear, it is possible that the applications had a depressing effect on native predators of the tip midge, thus insecticide treatment might exacerbate a tip midge outbreak.

			Mean curls/ft ²	
	Amt.	Prespray	Posts	pray
Material	form./acre	7 Jun	18 Jun	25 Jun
Assail 30 SG	5.3 oz	4.17 a	11.75 a	15.00 a
Imidan 70 WP	21.3 oz	4.83 a	15.42 a	12.42 ab
Non-treated chec	-k	5.50 a	3.67 b	6.50 b

Table 1.	Field control	of tip mid	ge with insecticide	es, summary.
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Means within followed by the same letter(s) are not significantly different (LSD, $P \le 0.05$).

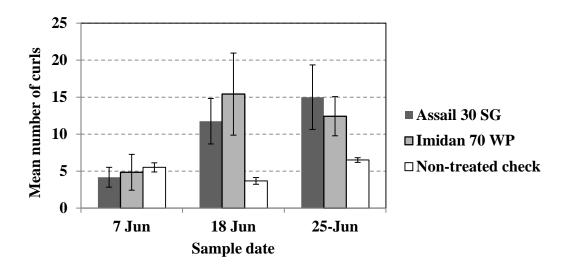


Fig. 1. Mean number of $curls/ft^2$ (lines are standard error of the mean).

Study 2. Field control of blueberry maggot fly on wild blueberry (crop year) with insecticides

METHODS: Each of two materials were applied on 9 Jul (berries 15-20% ripening and turning blue) to three, 100 x 80 ft plots in an unmanaged, fruit-bearing field in Township 19, ME. A CIMA[®] P55D Atomizer L.V. sprayer mounted on an Agco Allis[®] 6670 tractor was used to apply each material in 20 gallons of water per acre. The materials applied are listed in Table 1.

Pre- and post-spray populations of BMF adults were monitored with baited yellow Pherocon[®] AM traps. One trap was placed in each plot. Efficacy was further evaluated based on the number of BMF pupae collected from fruit samples.

On 23 Jul, we raked four quarts of berries from each treated plot. To collect BMF pupae, the berries from each plot were combined and distributed in a 1 to 2-inch deep layer in screened boxes suspended over ca. 2 inches of fine sand. Hardware cloth (1/4 in) was used as a screening material. In mid-Oct, BMF pupae were separated from the sand.

RESULTS: Analysis of Variance (RCB) and Tukey's HSD ($P \le 0.05$) were used to compare the change in mean number of adults captured between treated and non-treated check plots, seasonal density of BMF adults, and number of pupae per quart of fruit. Data were transformed by the square root prior to analysis.

Seasonal density of adults is in Figure 1 and Table 1. There was no significant difference between the treatments (P = 0.6275). Percent change between pre and post-spray adult captures is shown in Figure 2. Figure 3 shows fruit infestation as measured by number of pupae collected per quart of fruit. Assail and the 14.0 oz rate of Sivanto both significantly reduced infestation in comparison with the non-treated check plots (P = 0.0109).

Material	Amt. form./ acre	Adult seasonal . density	Pupae/qt
Assail 30 SG	5.3 oz	6.5 a	4.2 b
Sivanto 200 SL + Dyne-amic nonionic surfactant	10.5 oz 0.25% v/v	7.8 a	23.4 ab
Sivanto 200 SL + Dyne-amic nonionic surfactant	14.0 oz 0.25% v/v	6.8 a	5.0 b
Non-treated check		10.2 a	45.3 a

Table 1. Field control of blueberry maggot fly with insecticides, summary.

Means within each column followed by the same letter(s) are not significantly different (Tukey's HSD, $P \le 0.05$).

Fig. 1. Seasonal density of BMF adults.

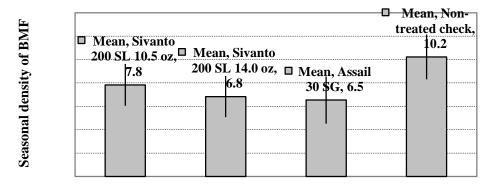


Fig. 2. Percent reduction in number of BMF adults following treatment.

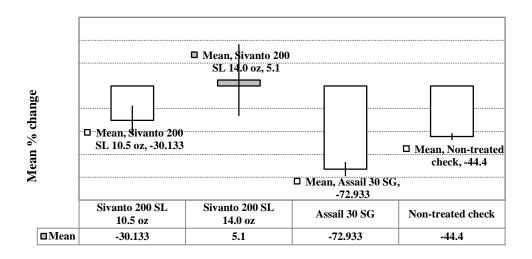
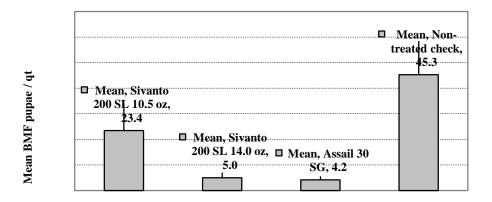


Fig. 3. Mean number of BMF pupae collected per quart.



CONCLUSIONS: This was the third trial in three years in which a high rate of Sivanto (formerly BYI-02960) 200 SL has proven effective in control of blueberry maggot fly. The performance of the high rate of Sivanto was not significantly different from the control observed with Assail. Both Assail and Sivanto did not reduced fly numbers, but had considerable effect on maggot infestation.

Study 3. Field control of blueberry maggot fly on wild blueberry with Sivanto 200 SL

METHODS: Sivanto 200 SL (14.0 oz/acre) was applied on 13 Jul (berries 20% ripening and turning blue) and 20 Jul (berries 85% ripening and turning blue) to a 60 x 200 ft area at Blueberry Hill Farm. A 20-ft boom sprayer equipped with 80015 TeeJet[®] nozzles was used to apply each material in 20 gallons of water per acre.

Pre- and post-spray populations of BMF adults were monitored with baited yellow Pherocon[®] AM traps. Two traps were placed in the treated area. Two additional traps were placed in non-treated areas. Efficacy was further evaluated based on the number of BMF pupae collected from fruit samples.

On 20 Jul and again on 3 Aug, we raked four quarts of berries from each of four subsites within the treated and non-treated check areas. To collect BMF pupae, the berries from each subsite were combined and distributed in a 1 to 2-inch deep layer in screened boxes suspended over ca. 2 inches of fine sand. Hardware cloth (1/4 in) was used as a screening material. In mid-Oct, BMF pupae were separated from the sand.

Phytotoxicity

Leaf drop and leaf spotting in the treated area was noted on 27 Jul. Phytotoxicity was evaluated by rating the percent of leaf drop and leaf spotting in each of 20 sq ft quadrats. Rankings were 0-25% = 1, 25-50% = 2, 50-75% = 3, 75-100% = 4. An additional 20 quadrats were sampled in the non-treated check area. Analysis of Variance (ANOVA) was used to compare damage (rating) between the treatments. Data were not transformed; analysis was performed on ranks. There was a significant difference in both leaf spotting (P = < 0.0001) and leaf drop (P = 0.0003). The area treated with Sivanto had significantly more damage.

RESULTS: Analysis of Variance was used to compare the seasonal density of BMF adults and number of pupae per quart of fruit. Data were transformed by the square root prior to analysis. There was a 26% increase in numbers of BMF adults captured on traps in the non-treated check area compared with the Sivanto-treated area which had a 35% increase in adult captures. However, there was no significant difference in the seasonal density of BMF adults between the two treatments (P = 0.3235) (Table 1). There was also no significant difference in the number of pupae per quart of fruit between the treatments for either sample date (Sample 1, 20 Jul, P = 0.5307; Sample 2, 3 Aug, P = 0.8685) (Fig. 1).

Material	Amt. form./ acre	Percent change	Adult seasonal density
Sivanto 200 SL + Dyne-amic nonionic surfactant	14.0 oz 0.25% v/v	- 34.9	2.9 a
Non-treated check	-	26.3	3.6 a

Table 1. Field control of blueberry maggot fly with insecticides, summary.

Means followed by the same letter are not significantly different (ANOVA).

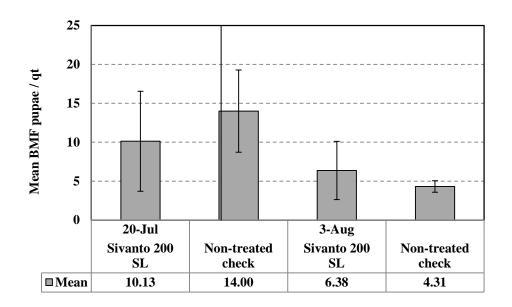


Fig. 1. Bar graph showing mean number of pupae per quart for each of two sample dates.

CONCLUSIONS: In previous trials, this high rate of Sivanto did appear to provide control of BMF. The material was not effective in this trial. However, infestation was generally low and so it was difficult to detect treatment effects due to the low power of the test.

This is the third year of testing for Sivanto 200 SL (previously identified as BYI-02960) but the first year we have seen evidence of phytotoxicity. Although fruit appeared to be unaffected, the lack of leaves could have a detrimental effect on berry quality due to loss of shading and moisture.

This was the first trial where a boom sprayer was used for the application as well as the first time multiple applications were made to the same area. It was also the first time this material has been applied to commercial blueberry land. All previous trials were conducted on unmanaged land.

RECOMMENDATIONS: There are currently no insecticides that have been shown to be effective against blueberry tip midge. We will continue to assess control strategy for this pest even though its populations are not widespread throughout the state; it does appear to cause damage during the prune year in some years.

Sivanto 200 SL at the 14 oz rate does appear to have promise as a new insecticide for control of the blueberry maggot fly. While this insecticide does not quickly reduce adult populations it does protect the fruit from maggot infestation. We will continue to investigate the consistency of this insecticide.

ENTOMOLOGY

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

4. TITLE: II. Development and implementation of a wild blueberry thrips IPM program, 2012.

OBJECTIVES: The objectives of this study were two-fold and build upon 23 years of research:

<u>Objective 1</u>: To develop an integrated pest management (IPM) program for thrips control in wild blueberry in Maine including:

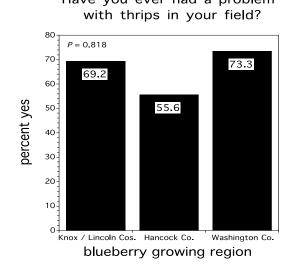
- a) the integration of monitoring and sampling methods to establish the extent of infestations and proper timing of control tactics;
- b) integration by growers of cultural and insecticide control tactics; and prior to the development of the integrated pest management plan, two large scale commercial field trials will determine the efficacy of novel control tactics for managing blueberry thrips that have been shown to be promising from multiple years of small scale experimental plot experiments.

Objective 2: Communicate the pest management strategy to wild blueberry growers in Maine, evaluate adoption of this new production practice among growers, and identify any impediments to adoption.

a) a survey was conducted to assess understanding of thrips biology and management by growers.

GROWER SURVEYS: Grower surveys were conducted in three locations in Maine (Waldoboro, Jonesboro, and Orland) on May 29, 30, and 31. In addition, surveys were conducted in Blue Hill at the annual Organic Blueberry Grower's Field Day on 12 Jun. The survey was given in paper form and growers were asked to fill out the survey/questionnaire prior to a discussion of thrips biology and management. The survey consisted of eight questions (see Appendix).

A total of 74 growers answered the survey/questionnaire (approximately 13% of the grower community). Figure 1 shows that thrips infestations have been experienced by most growers and that there appears to be no difference in grower perception that thrips are problematic for their production as a function of geographic region (P = 0.818). Most growers felt that thrips outbreaks were infrequent; although, almost 20% did not know how frequent thrips outbreaks were (Fig. 2). Only 15% perceived that they had frequent problems with thrips. However, if this survey is representative of the industry, then this could represent a fairly large acreage that is frequently under attack. When asked what damage that thrips cause (Fig. 3) and how one recognizes a thrips infestation (Fig. 4), most growers (78%) knew what characterizes thrips infestations, but only 16.2% understood the relationship between thrips are best controlled in the prune year of crop production (Fig. 5), and a slightly lower proportion (54%) knew that $\frac{1}{4}$ - $\frac{1}{2}$ inch shoot height is the phenological plant stage for best timing of insecticide applications (Fig. 6).



Have you ever had a problem

Fig. 1. Are thrips a problem?

Fig. 2. What is the frequency of the problem?

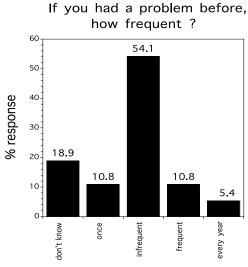
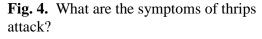
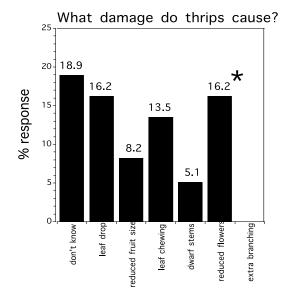
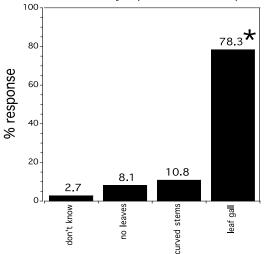


Fig. 3. What damage do thrips cause (* = correct)?





What are the symptoms of thrips attack



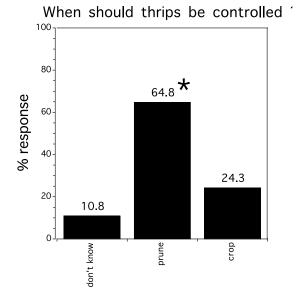


Fig. 7. What insecticides should be used?

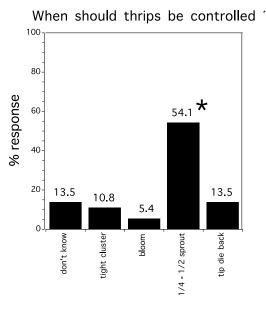
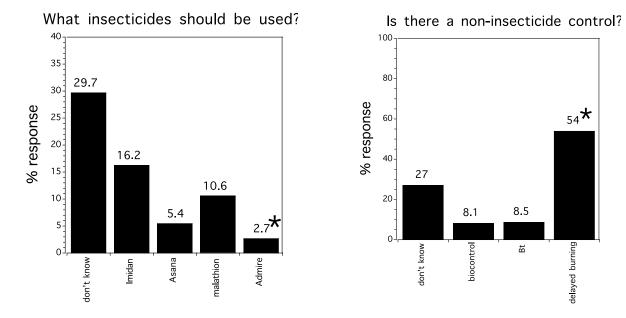


Fig. 8. Are there any non-insecticide controls?



Almost all of the growers were unfamiliar with a suitable insecticide for thrips control; only 2.7% of the growers correctly selected the recommended insecticide (Fig. 7). However, half the growers did know that delayed burning of an infested area is a viable option for management of this pest (Fig. 8). Conclusions from this survey are that a comprehensive fact sheet has to be written to provide a factual account of the thrips biology and management that can easily be accessed by growers, as there is currently a high degree of misunderstanding about thrips.

Fig. 5. In what crop cycle should thrips be controlled? Fig. 6. What plant stage is best for control?

CONTROL TRIALS: Controlled replicated efficacy studies were performed during the spring of 2012 on growers' fields in the Downeast region. Three experiments were conducted. The first experiment (referred to as "**pre-emergence**") was to confirm our findings from the past 5 years that the neonicotinoid insecticides, imidacloprid and acetamiprid, are effective insecticide tactics for applications when made prior to emergence of thrips from the soil AND before plant stems have emerged. The second experiment (referred to as "**emergence**") was focused on control prior to thrips emergence, but applications are made just as the shoots are emerging at a height of ¹/₄ to 1¹/₂ inch. The third experiment (referred to as "**post-emergence**") involved evaluating foliar sprays applied after thrips emergence AND after shoots and leaves are emerged. This tactic has been shown to be less optimal in the past than either pre-emergence window (shoot height ¹/₄ - 1¹/₂ inches) to still have some recourse for control. This third experiment represents the third replicate trial in the last three years.

Pre-Emergence

Insecticides were applied BOTH prior to thrips emergence AND blueberry shoot emergence. The experiment was conducted in Harrington, ME and the trial area was burned in the fall of 2011. A replicated RCB design was used with four blocks and three subplots within each block. There were three treatments, Admire Pro 4.6 F (7.0 oz/acre), Assail 30 SG (5.3 oz), and a non-treated control. Treatments were applied prior to thrips and blueberry shoot emergence on 2 May 2012. All materials and rates were applied in 25 gallons of water-mixture per acre with a CO₂-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. The number of blueberry stems with and without thrips damage as evidenced by curled leaves was determined from each of three, sq. ft samples per plot. Blueberry stems were 2 to 4 inches tall. No symptoms of phytotoxicity were observed in any plot. Analysis of covariance (ANCOVA) was performed with stem density / subplot as the covariate (P = 0.0005). The adjusted least-square means were assessed with Tukey's post-hoc means comparison test. There was a significant difference in the number of stems with damage as evidenced by curled stems among the treatments ($F_{(2,23)} = 6.348$, P =0.006), (Table 1 and Fig. 9). The pre-emergent application of Admire resulted in significantly less curls than was observed in Assail-treated plots or in the non-treated control plots.

<u>Emergence</u>

There were four blocks and in each block there were three replications of each treatment. Each plot measured 7 x 10 ft. The trial area was burned in the fall of 2011. Four rates of HGW86 10 SE (10.1, 13.5, 20.5, + 0.25% v/v Dyne-Amic non-ionic surfactant) and 13.5 oz - no surfactant) and Admire Pro 4.6 F (2.1 oz) were applied on 18 May when blueberry stems were 0.25 to 0.50 inches tall and again on 1 Jun when blueberry stems were 1 to 1.5 inches tall. HGW86 is a new insecticide that has much better bee safety than imidacloprid. All materials and rates were applied as described above. The number of blueberry stems with and without thrips damage as evidenced by curled leaves was determined from each of three, sq. ft samples per plot. Blueberry stems were 2 to 4 inches tall. No symptoms of phytotoxicity were observed in any plot. Analysis of covariance (ANCOVA, replicated RCBD) was used to compare percent infestation among the treatment plots. Data were transformed by the arcsine

of the square root of proportion curls to stabilize variance prior to analysis. Mean arcsine transformed % curls were adjusted by the significant covariate (stem density / subplot, P = 0.0013), but there was no significant difference in adjusted % leaf curls due to treatment ($F_{(5,47)} = 1.413$, P = 0.236). However, it appears that thrips pressure was very low in 2012 as only 13.7% of the stems had leaf curls in the non-treated check plots.

Material	Amt. form./acre	Avg. # stems/ ft ² (SE)	Avg. % stems with curls/ft ² (SE)
PRE-EMERGENCE APP	LICATION		
Admire Pro 4.6 F	7.0 oz	108.6 (12.3)	8.7 (2.3) b
Assail 30 SG	5.3 oz	112.7 (14.9)	15.6 (4.8) a
Non-treated check	-	99.5 (10.5)	13.7 (3.9) a
EMERGENCE APPLICA	TION		
Admire Pro 4.6 F	2.1 oz	104.7 (14.1)	14.5 (3.2) a
HGW86 10 SE	10.1 oz	127.3 (27.7)	16.1 (4.9) a
HGW86 10 SE	13.5 oz	128.5 (9.9)	12.9 (2.3) a
HGW86 10 SE (no surfact	ant) 13.5 oz	92.1 (15.9)	20.2 (2.5) a
HGW86 10 SE	20.5 oz	88.1 (9.6)	17.0 (1.2) a
Non-treated check	-	99.5 (10.5)	13.7 (3.9) a

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

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

reicent leuucile		ider of cur	is in com	parison w	iui uie no	n-nealeu (IIECK.
Percent reduction							
Perc	Admire Pre- emerg. Applic.	Admire Timing Applic.	Assail 30 SG	HGW86 10.1 oz	HGW86 13.5 oz	HGW86 - 13.5 oz no surf.	HGW86 20.5 oz
■% reduction	36.50	-5.50	-12.20	-14.90	5.80	-32.20	-19.40

Fig. 9. Percent reduction in number of curls in comparison with the non-treated check.

Post-emergence

Foliar applications of Admire Pro 4.6 F (2.1 oz) and Assail 30 SG (5.3 oz) were applied on 1 Jun to a pruned-year field in Harrington, ME after both thrips and blueberry shoots and foliage had emerged. Both materials were applied in 25 gallons of water-mixture per acre with a CO₂propelled, 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 1 (prespray), 8, 16, 20, and 27 Jun, 5 curls were collected from each plot, brought into the laboratory, and dissected to determine the number of thrips. Each plot measured 7 x 10 ft. There were four replications of each treatment. Analysis of Variance (ANOVA) and LSD ($P \le 0.05$) were used to compare infestation as measured by thrips per curl among the treatment plots. Admire Pro 4.6 F and Assail 30 SG both seemed to be effective in controlling thrips. For all dates combined, there was a significant difference in the number of thrips per curl among the treatments (P < 0.0001, Table 2). More thrips were found in the non-treated check curls then in curls treated with either Admire Pro or Assail. There was a dramatic increase in the number of thrips in the non-treated check curls on the final sample date (27 Jun), suggesting that both insecticide foliar treatments kept thrips levels quite low through the growing season.

	Amt.	Prespray_		Mean the Posts	-		
Material	form./acre	1 Jun	8 Jun	16 Jun	20 Jun	27 Jun	All dates
Admire Pro 4.6 F Assail 30 SG Non-treated check	5.3 oz	0.5 0.2 1.9	0.5 0.5 2.2	0.1 0.5 0.3	0.3 0.2 3.3	1.2 0.4 21.7	0.50a 0.35a 5.89b

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

LARGE-SCALE GROWER STUDIES: Two grower studies were conducted in 2012. The first was an evaluation of a post-emergence treatment of Assail. It is also referred to in the grant proposal as the commercial field demonstration. The second study involved 16 commercial blueberry fields with the intent of assessing blueberry production practices on thrips abundance.

Post-emergence grower treatment

In June of 2012, a blueberry grower in the Hancock County region noticed an extensive thrips infestation in a 10 acre field after the optimal shoot emergence timing for treatment. On 14 Jun the grower applied Assail 30 SG at 5.3 oz / acre to the entire field with a boom sprayer. The field was monitored for thrips using four 3" x 5" yellow sticky cards and by collecting 10 random thrips curls throughout the field. Sticky traps were collected and counted on 19, 24, and 30 Jun and 6 Jul. The curls were collected and brought back to the lab for dissection and microscopic examination on 15, 24, and 30 Jun. Figure 10 shows that the single application of

Assail might have delayed thrips increase, but it did not reduce thrips densities to acceptable levels.

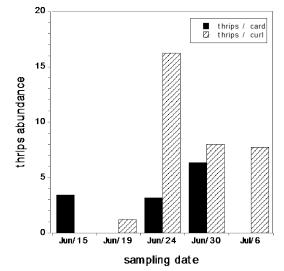


Fig. 10. Thrips abundance increase after a commercial Assail application.

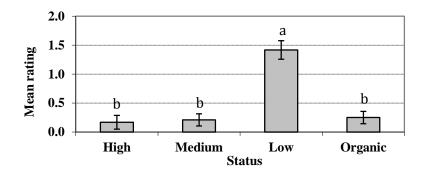
Production System Study

Sixteen blueberry fields during their prune cycle were sampled for thrips in 2012. Four production systems (Low, Medium, High input, and Organic) were represented by four fields each in three counties (Waldo, Hancock, and Washington). Between 18 and 22 Jun, thrips damage was measured along each of three 100 ft transects per field. 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 3.

Table 3. Rating system for thrips damage assessment.

0	no damage
1	a few curls widely scattered
2	Curls along $< \frac{1}{2}$ of the transect, but no patches
3	Scattered curls along $> \frac{1}{2}$ the transect
4	1-2 patches $\geq 2 \text{ ft}^2 + \text{scattered curls}$
5	3 or more large patches + scattered curls

Analysis of variance (ANOVA, CRD) was used to compare thrips abundance (rating) among the treatments (High, Medium, Low, or Organic). Data were not transformed, analysis was performed on ranks. In general thrips abundance was low; all transects sampled had ratings of 3 or less. There was a significant difference in mean density rating of thrips due to production system ($F_{(3,12)} = 23.579$, P < 0.0001). Significantly more thrips were found on low input farms (Fig. 11). This is the second year that similar results have been observed. In 2010, Low input fields also had significantly higher thrips abundance than Organic and Medium and High input fields. The reason for this striking result is not clear, but clearly needs further investigation. Fig. 11. Abundance of thrips, mean rating.



OTHER CONTRIBUTIONS: The project objectives and goals for 2012 were accomplished. An evaluation of the delayed burning cultural control was not performed since this tactic was evaluated a decade ago and has been widely adopted by several growers in Maine. Dr. David Yarborough (Co-PI) on this project, however, did present the findings of seven years of our data on delayed pruning and its effects on yield at the International *Vaccinium* Meeting in the Netherlands, June 2012. A manuscript is currently in press on this topic in the Small Fruits Journal. Another objective that was not listed in the proposed research was confirmation of the thrips complex in wild blueberry. Since the 1950s it has been conjectured that thrips species that attack wild blueberry are comprised of three cryptic species. Until recently identification of these species has been tenuous at best. Molecular markers have facilitated identification. Thrips were collected from several fields in Maine in 2012 and sent for identification to the USDA Systematics Laboratory. It is anticipated that species identifications will be sent to the University of Maine by the end of 2012.

CONCLUSIONS AND RECOMMENDATIONS: Research on the biology and management of blueberry thrips has been conducted by the PI since 1990. Since 2000, several new insecticides have been registered for use in wild blueberry that made insecticide tactics for control of thrips more feasible. Research into the biology and management of thrips is now at a point where a definitive integrated pest management program can be constructed and discussed with growers. The following points are the center of a pest management program for thrips.

- 1. Most thrips infestations are aggregated in a spatial pattern.
- 2. Sampling is critical to early detection.
- 3. Delayed pruning is effective for thrips control, BUT 10-25% loss.
- 4. A pre-emergent control tactic works, but requires a soil drench approach, so recommended as a spot treatment, over 8 trials since 2000:
 - i. 6 of 8 suggest imidacloprid is effective,
 - ii. 3 of 3 suggest acetamiprid is effective
- 5. An emergence control tactic is the optimal insecticide tactic and has the greatest consistency. Since 2000: 10 of 12 trials have been effective (imidacloprid and acetamiprid).

- 6. Sampling thrips with 3" x 5" sticky cards has been found to be a very effective means of ensuring that thrips emergence is synchronized with shoot and leaf emergence.
- Post-emergence control has not been consistent (looked at since 2006, although in 2011 & 2012 acetamiprid was effective), tend to get resurgence, BUT will be recommended for growers that find large infestations late.
- 8. Biocontrol has been tested, but results observed were not promising.
- 9. A fact sheet for thrips has been written, edited, and submitted for publication for grower use. A copy of the factsheet is included below:



Wild Blueberry Fact Sheet

Insects - 202 - Blueberry Thrips

Fact Sheet No. 202, UMaine Extension No. 2373

Description

Thrips are very small (1/8 to 1/4-inch long) and difficult to see. Uncurling the rolled up leaves of actively infested plants will reveal small, slender, lemon-yellow thrips (Photo 1). This problem is more readily identified by the presence of very tightly rolled-together leaves and twisted stems on blueberry plants beginning early in the season (late May or early June) (Photo 2). Also, infested leaves often turn bright red and are quite conspicuous (Photo 3). Another pest, the blueberry tip midge (Dasineura oxycoccana (Johnson)), also causes rolled leaf galls. However, the leaf galls caused by the blueberry tip midge are loosely curled and more "football" shaped compared to the tighter and more slender "cigar" shaped galls caused by thrips. In addition, the galls caused by the blueberry tip midge are only at the terminal end of the stem and are usually green and dimpled; whereas, the galls caused by the blueberry thrips can envelope the entire stem and are smooth and are often red. Two species of thrips, Frankliniella vaccinii Morgan and Catinathrips kainos O'Neill, attack wild blueberries. There are slight differences in the phenology or seasonality of their life cycle, but in general their biology is the same and the damage that they cause is identical.

Life Cycle

Blueberry thrips winter in the soil as adults. They overwinter in soil depths deep enough so that burning does not appear to affect their survival. Thrips emerge and feed on tender new plant material in late May to early June. Eggs are laid in developing leaf veins, and in the spring young, immature insects can be found within the curled leaves (curling results as a response to the damaged veins from egg laying). During the growing season, thrips develop through two larval stages that actively feed and then metamorphose into a non-feeding prepupal stage and a subsequent non-feeding pupal stage. New adults are found within the leaf galls in late July or early August. Typically in late summer, mature adult females (and some males), leave the plant, and move back into the soil.

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Damage and Economic Importance

Thrips damaged plants can be found in both vegetative and fruit-bearing fields. However, it is harder to detect thrips infestations in fruit-bearing fields since the foliage can obscure the leaf galls. Greater densities of galls are found in vegetative fields possibly because more young and tender foliage is available for attack during the time when adult female thrips emerge from the soil. Economically important damage occurs in vegetative fields. Leaves infested with feeding thrips curl around the stem starting at the stem tip and then, as successive leaves emerge and are attacked, leaves curl around the stem from the top to the base of the stem. The infested leaves remain tightly curled around the stem of the plant throughout the growing season and shield the developing flower-bud tissue from the light so that flower buds do not develop. Heavily infested stems will have no flowers the following spring. Damage is usually confined to small, isolated, heavily infested patches ranging from 100 square feet to one half acre or more in size. However, it is also common to have individual thrips-injured plants scattered throughout a field.

Pest Management Strategies

The blueberry thrips can be controlled with insecticides but also with cultural methods based upon pruning by burning infested patches. Timing of application is very important for effective management of this pest. Research over the past 20 years has identified three strategies for blueberry thrips control that have been shown to be frequently effective; although, it should be noted that this insect pest can be difficult to manage because it spends much of its time protected from both natural predators and insecticides inside the leaf curls.

I. CULTURAL CONTROL

Delayed Pruning. In most years female thrips emerge from the soil from late May to early June and continue to emerge for the next month; although, the majority of the population has emerged by mid-June. The concept behind managing these pests by pruning is to allow as great a

proportion of the population to emerge and become established within the leaf curls as possible and then kill them with a deadly burn before they can produce offspring. The best strategy would be to wait until mid-July in the prune year when every last thrips has emerged and colonized blueberry leaves and then to kill them. The area to be burned should encompass the area that has abundant leaf curls plus a10 yard buffer area with few curls that will also be burned as a safety margin. This strategy poses two problems. The first is that as one waits the emerging blueberry stems become abundant in lush green leaves. It can be difficult and/or extremely time consuming to burn fully foliated stems. The second problem associated with this strategy is that the LONGER one waits for more of the thrips population to emerge from the protected environment of the soil, the SHORTER the remaining length of the growing season, resulting in fewer flower buds formed for the following year. We have conducted research on this issue for more than five years and have established dates that can help growers make the decision of when to burn. An inspection of the table below shows that a reasonable pruning window is between June 15 and June 30. In most infestations while a delayed burn prune might result in as much as 50% flower bud loss, if the infested area only makes up 5% of the field, then total field flower bud loss would only be 2-3 % total loss, but still minimize the chances of worse infestations in following years.

Delayed Pruning Date	Average flower bud loss (%)	Range in percent bud loss (%)*
May 25	16.7	0 - 33
June 1	20.7	5 - 35
June 10	27.6	12 - 42
June 15	32.3	17 - 47
June 20	37.8	23 - 53
June 25	43.7	31 - 59
July 1	51.3	39 - 63
July 10	62.7	52 - 73
July 20	74.1	66 - 82

Table 1. Spring and summer pruning dates and the average yield loss expected at each date.

* this range represents what is expected in 70% of the fields that would experience this loss over all types of spring and summer weather and pest conditions experienced in Downeast Maine.

II. INSECTICIDE CONTROL

There are three tactics for controlling thrips with insecticides. They are described below. Specific insecticide recommendations and rates can be found in the current University of Maine Cooperative Extension Wild Blueberry Factsheet 209.

 <u>Pre-emergence</u>. The neonicotinoid insecticides provide a systemic mechanism for protecting the blueberry plant as it is growing and during the window of attack. A soil application or drench has been tested and been found to be effective. The drench tactic should ONLY be used in the pruned year and because of the amount of water needed it is not practical for a whole-field application, but only as a spot-treatment. In order to use a pre-emergence tactic it is critical that in the previous year, when the crop is being harvested, the perimeter of the infested patch is marked with stakes. In the spring after pruning an application of a recommended neonicotinoid insecticide (see Factsheet 209) can be applied to the soil in a high volume of water (refer to the product label). The closer the application date is to sprout emergence, the better. A soil application should NOT be made when the soil is still frozen in the early spring, but it should be made just prior to the estimated sprout emergence time so that the roots are likely to take up the insecticide. In addition, the site should be level so that runoff of the application is minimized. Our studies have shown that when the neonicotinoids: imidacloprid or acetamiprid, are applied in the spring of the prune year there will be no contamination of pollen or nectar the following year during bloom. Only one application is recommended. It is a good practice to monitor the planting after application through sprout emergence to determine if the systemic activity of the insecticide was enough to provide control. If control is poor, then a delayed prune tactic or one of the other insecticide tactics described below can be considered.

2. <u>Emergence</u>. Thrips emergence and sprout emergence of a pruned crop often coincide. Many years of study has shown that in most years timing of an insecticide when the sprouts are just starting to emerge provides optimal control. Two applications of an insecticide (see Factsheet 209), the first when shoots are ¼ to ½ inches in length and then again at 3/4-1 inches, is the optimal timings. In some years, the thrips emergence can be a bit delayed compared to sprout emergence. This can be more critical if a non-systemic (one that does not move into the inside of the plant and distribute itself) insecticide is used.

In order to improve the timing of thrips and sprout emergence we suggest that you monitor for thrips emergence by placing two or three, 3 x 5 inch YELLOW sticky cards on stakes just above the soil surface in the infested area. Checking the sticky cards twice a week will allow you to determine when thrips emergence begins. You will need a good hand lens (15-20X) to see the thrips on the cards. When thrips begin emerging AND sprouts are emerging then begin the two application tactic. If thrips begin to emerge BEFORE sprout emergence, WAIT. Only start the thrips control tactic of two consecutive applications when both thrips and sprouts are emerging. This tactic is the most consistent and reliable of the insecticide control tactics that we have evaluated.

3. <u>Post-emergence</u>. Thrips may emerge and cause substantial leaf curling before being noticed. This can happen in late June or early July. If this does happen, not all is lost. An application of a neonicotinoid insecticide (see Factsheet 209) can be targeted at the leaf curls. Because of the locally systemic activity of these insecticides to move into the leaf tissue, called translaminar activity, mortality of thrips can still result. Such a tactic will only arrest the thrips buildup and not prevent flower bud losses that might have already occurred. However, it may reduce the number of thrips that are produced for overwintering and that will contribute to a buildup of the population over time. Our experience is that one application does lower thrips populations, but in some years the population can recover by the end of the summer. Therefore, we recommend one application if the thrips infestation is small, but two applications separated by a 2 week interval if the infestation is large. This tactic can be used in either the prune or the crop cycle. Of the four tactics outlined in this factsheet, the post-emergence tactic is the least efficacious and least reliable. It should ONLY be considered only as a "fallback" emergency option.

III. BIOLOGICAL CONTROL

Inundative release. Biological control has been tested previously with commercially available parasites (2001, 2006). The entomopathogenic fungus, *Beauveria bassiana*; and the entomopathogenic nematode, *Heterorhabditis marelatus*, have been evaluated in wild blueberry in Maine. However, the results have not been promising. Therefore, biological control is not currently recommended. Continued research into this tactic will be evaluated if new options appear. Biological and cultural control strategies are usually the best tactics for minimizing harmful control tactics to pollinators, other beneficial organisms and non-target wildlife.

For additional information on monitoring and control, refer to Wild Blueberry Fact Sheet 204 and 209, which may be found at <u>www.wildblueberries.maine.edu</u> or contact the lowbush blueberry specialist, University of Maine Cooperative Extension, 1-800-897-0757 (toll-free in Maine) or 207-581-2923.

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Prepared by Francis A. Drummond, Professor of Entomology and Judith A. Collins, Assistant Scientist, in cooperation with David Yarborough, Extension Blueberry Specialist, The University of Maine, Orono, ME 04469. Revised December 2012.

Appendix 2012 Thrips survey

1.	Have you ever	had a problem	with thr	ips?	NO		YES	
2.	If you have ha ONCE	d a problem wi INFREQUEN	-		•		nly been ST EVERY YEA	R
3.	What damage	do thrips cause	?					
LEAF	DROP	REDUCED FI	RUIT SĽ	ZE LEAF	F CHEW	ING		
DWAI	RF STEMS	REDUCED FI	LOWER	BUDS	EXTI	RA BRA	NCHING	
4.	How do you re	ecognize a thrip	os proble	m?				
NO LE	EAVES	CURVED STI	EMS	LEAF	GALL	(CURL)		
5.	What crop cyc	le should thrips	s be cont	rolled?	CROP]	PRUNE	
6.	When should t	hrips be contro	olled?					
TIGH	Γ CLUSTER	BLOOM	1⁄2" SPR	OUT	TIP DI	E BACK		
	What insectici		-					
IMIDA	AN	ASANA	1	ASSAIL	ADMI	RE		
	Is there a non-	insecticide con	trol for tl BT	-	AYED B	URNINO	3	

ENTOMOLOGY

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

5. TITLE: III. IPM

Study 1. <u>Effect of late pruning on lowbush blueberry development and flower-bud</u> production

METHODS: On 17 Jun 2011, a flail-mower mounted behind an ATV was used to mow three replicated, 2-m² blocks. The area was fruit-bearing at the time of pruning. On 24 Oct, 100 stems within each block were cut, brought into the laboratory and evaluated for flower-bud production by counting the number of flower-bud clusters per stem. On 2 Nov an additional 100 stems were cut from the non-treated area immediately adjacent to each flail-mowed block (non-treated checks). Between 18 and 24 May 2012, 25 stems were cut, brought into the laboratory, and the total number of individual flowers per stem was determined for each block and from the non-treated area immediately adjacent to each block. Stem density was

determined on 18 May 2012 by counting the number of stems within each of three, sq. ft quadrats per block. A randomized complete block (RCB) analysis of variance (ANOVA) was used to analyze the non-transformed count data.

RESULTS AND CONCLUSIONS: In the fall of 2011 there was no significant difference in the number of flower-bud clusters between the treatments (ANOVA, P = 0.5847) (Fig. 1). However, blocks mowed in mid-Jun appeared to have fewer and shorter stems then the nontreated check blocks. This was confirmed in 2012 (Fig. 2). Late-pruned blocks had significantly fewer stems (ANOVA, P = 0.0139) than non-treated check blocks. The 2012 count of individual flowers indicated a highly significant difference; late pruned blocks had significantly fewer individual flowers per stem (ANOVA, P = 0.0014) (Fig. 3). The number of flowers per stem shown in Figure 3 is considerably less than an estimated reduction of flowers / stem of 40% from a predictive model recently published by Drummond and Yarborough (2013) based upon calendar day of pruning (see Fig. 3, solid line). However, the number of degreedays after pruning is also very important and it was very cold and wet after the date that the plots were pruned. Using a model also developed by Drummond and Yarborough (2013) it can be seen that our observed reduction in flower buds / stem (at ca. 80%) is very close to the predicted reduction in flower buds / stem from the degree-day model (at 72%) published by Drummond and Yarborough (2013) (see Fig. 3, dashed line; and Fig. 4, predictive model).

Fig. 1. Comparison of mean number of flower-bud clusters between the treatments (Fall 2011) (lines on each bar represent standard errors of the mean).

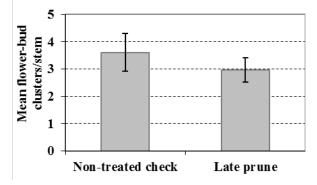


Fig. 2. Comparison of stem density between the treatments (May 2012).

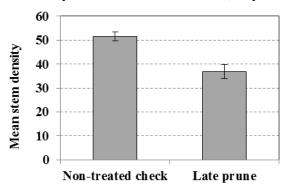


Fig. 3. Mean number of individual flowers per stem between the treatments May 2012 (solid line is estimate of flower reduction from a model using day of prune as a predictor and dashed line is estimate of flower reduction from a model using degree days since prune date as a predictor (see figure 4, Drummond and Yarborough (2013)).

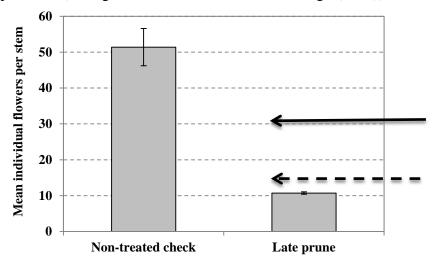
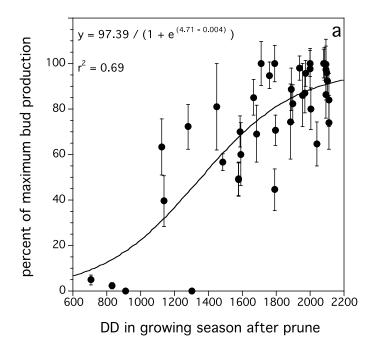


Fig. 4. Predictive model for estimating loss of flower buds per stem based upon accumulated degree days during the growing season after pruning in the vegetative year (Drummond and Yarborough (2013).



Literature Cited

Drummond, F.A. and D.E. Yarborough. 2013. Growing Season Effects on Wild Blueberry (*Vaccinium angustifolium*) in Maine and Implications for Management. Acta Hort. *In press.*

Study 2. Impact of blueberry tip midge on flower-buds and subsequent flower development

Blueberry tip midge is becoming a significant problem in New Brunswick, Canada. Population densities and infestation are increasing along with resulting crop loss. This is the third year of a study to determine what impact tip midge may be having in Maine.

METHODS: On 12 Jul 2011, colored flags were used to mark 50 vegetative lowbush blueberry stems with tip midge infestation (green flags) and 50 stems without tip midge infestation (white flags) as evidenced by the presence or absence of leaf curls. On 2 Nov 2011 we collected 10 stems from each treatment, brought them into the laboratory and counted the number of flower-bud clusters and branches per stem. A non-branched stem was counted as having one branch. Forty marked stems of each treatment were left in the field. On 15 May 2012 the stems were cut and brought into the laboratory to determine the number of flowers that developed from individual flower-bud clusters.

RESULTS: Data were transformed by square root prior to analysis. The 2011 count of flower-bud clusters showed there was no significant difference in the number of flower-bud clusters (Completely Randomized Design, ANOVA. P = 0.6897) or branches (P = 0.3796) per stem between the treatments (Fig. 1) (stems that had been infested by blueberry tip midge in the prune cycle compared to non-infested stems of the same clone). However, we did note that four of the 10 stems damaged by tip midge had deformed terminal ends ("crooks"). There was no significant difference in the number of flower-bud clusters on stems with "crooks" compared with those without such damage (P = 0.2237). When individual flowers were counted in 2012, there did appear to be a trend towards more flowers on the tip midge-damaged stems; however, the difference was not significant (P = 0.0967) (Fig. 2). This is not an unexpected result as sometimes insect damage can stimulate greater growth and partitioning of resources to reproductive structures. However, we have so far demonstrated that blueberry plant response in flower-bud production can be quite variable. In the spring of 2009-2010 we found NO difference in flower-buds per stem due to blueberry tip midge and yet in the 2010-2011 trial flower-bud clusters on stems with blueberry tip midge infestation developed significantly fewer flowers then those without tip midge infestation (P < 0.0001) (Fig. 2).

Fig. 1. Bar graph comparing mean number of flower-bud clusters and mean branches per stem between stems with and without tip-midge damage (Nov 2011).

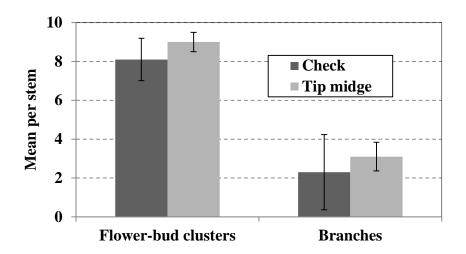
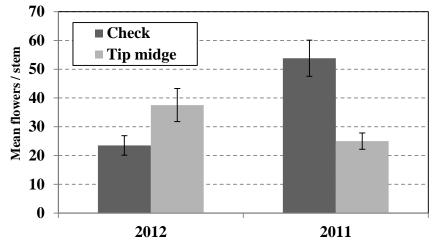


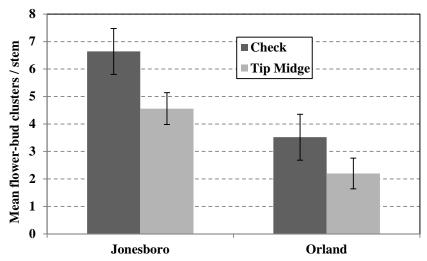
Fig. 2. Bar graph comparing mean number of individual flowers per stem between stems with and without tip-midge damage. Samples collected from trials conducted in 2011 and 2012.



An effort will be made to verify our results. This trial will be repeated in 2012-13. On 7 Jun at Orland and 11 Jun at Jonesboro, we marked an additional 100 stems per site, 50 with tip midge infestation (red flags) as evidenced by leaf curls and 50 without infestation (white flags). On 8 Oct at Orland and 15 Oct at Jonesboro we cut 25 stems from each treatment, brought them into the laboratory and counted the number of flower-bud clusters and branches per stem. A non-branched stem was counted as having one branch. Twenty-five marked stems of each treatment were left in the field at each site.

In both our trials begun in 2012 there was a significant difference in the number of flower-bud clusters (P = 0.03, Jonesboro; P = 0.0454, Orland) per stem between stems with and without tip midge damage (Fig. 3). In trials in both 2010 and 2011, we saw no significant difference in the number of flower-bud clusters. Three and four of the 25 stems damaged by tip midge had deformed terminal ends at Orland and Jonesboro, respectively.

Fig. 3. Bar graph comparing mean number of flower-bud clusters between stems with and without tip-midge damage at Jonesboro and Orland, ME (Oct. 2012).



Study 3. <u>Control of blueberry tip midge populations with late spring burn</u>

METHODS: In order to determine the effectiveness of a late spring burn (after leaf curl formation) on blueberry tip midge populations a ca. 20 x 100 ft area was burned (oil burn) at Blueberry Hill Farm on 11 Jun. Pre-burn populations of blueberry tip midge were estimated on 8 Jun by counting the number of blueberry stems with tip midge damage as evidenced by leaf curls in each of 15, sq ft quadrats. An additional 15 quadrats were evaluated in an adjacent non-burned area.

RESULTS AND CONCLUSIONS: There was no significant difference in the number of stem with tip midge damage in the burned vs. non-burned check area (P = 0.1551) (Fig. 1) BEFORE the area was burned; data were transformed by square root prior to analysis. In the late spring of 2013 we will again estimate tip midge populations in these areas to see what, if any, impact the late burn had on tip midge populations.

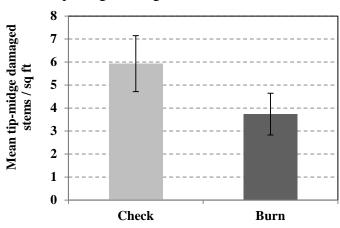


Fig. 1. Pre-burn estimate of tip midge damage.

RECOMMENDATIONS: Delayed pruning is an effective control tactic for managing both blueberry thrips and blueberry tip midge outbreaks. However, it must be realized that costs can be great if the prune is conducted in the very late spring. June 1 is the recommended last date for using this tactic. As we demonstrate in this study a delay of pruning as late as June 17 (mid-June) can result in drastic loss of flower buds the subsequent year. However, even this level of flower bud loss may be tolerable if the thrips or tip midge infestation area is small relative to the size of the non-infested section of the field. The models for predicting flower loss vs. both date of pruning and degree-days accumulated during the prune year, post-prune date, will be published in a new blueberry thrips management wild blueberry fact sheet planned for 2013.

At this point it is not possible to conclude that blueberry tip midge is a consistent pest of concern. We plan on conducting several more years of research in order to assess the risk that growers face when this pest builds up to noticeable levels.

ENTOMOLOGY

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

6. TITLE: IV. Biology of blueberry and pest insects, 2012.

Study 1. Notes on parasitism of blueberry maggot fly

In 2010 a modeling approach was taken to assess the relationship between BMF population increase from year to year and parasitism.

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

RESULTS AND DISCUSSION: Figure 1 shows the time series of blueberry maggot percent parasitism from 1998 to 2011. Upon inspection of this graph it is apparent that fly numbers fluctuate from year to year, ranging from a low of 0.5% to a high of 28.0%. However, there does not appear to be a tight linkage between fly trap captures and the parasitism rates over time (Fig. 2). Modeling fly rate of increase as a function of log parasite density suggested that a possibility ($F_{(1,11)} = 2.970$, P = 0.112) 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 2012 and plotted in Figure 3 it appeared that parasitism behaves as a density dependent factor that controls fly abundance from one year to the next. Figure 4 shows the relationship between the logarithm of fly abundance in year t versus the log rate of increase from year t to year t+1 (Log(Nt+1/Nt)). The linear relationship suggested that a density dependent relationship exists between fly abundance and the next year's increase or decrease in the blueberry maggot fly population ($F_{(1,11)} = 13.453$, P = 0.004, $r^2 = 0.57$). 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. Additional data were collected in 2012 to verify our hypothesis.

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

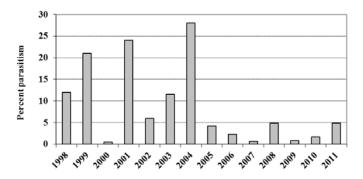


Fig. 2. Relationship between relative density of flies and % parasitism over time.

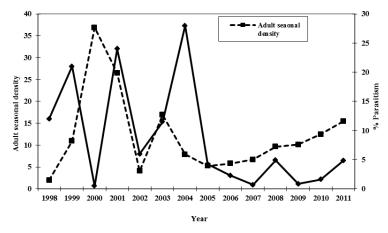


Fig. 3. Relationship between fly population increase and parasitoid density the previous year.

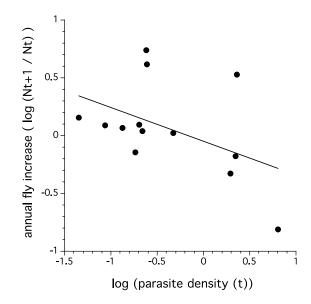
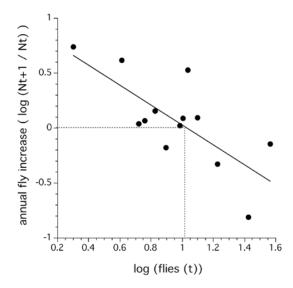


Fig. 4. Relationship between fly population increase and fly density the previous year. Dotted line demarks point of zero population increase.



Study 2. Impact of spotted-wing drosophila on lowbush blueberry

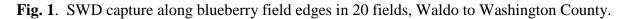
The objective of these four studies was to elucidate the biology of spotted-wing drosophila (SWD) in lowbush blueberry.

METHODS: Traps were constructed from Solo[®], 18 fl. oz, red polystyrene cups with clear lids. Seven to 10, 3/16-inch holes were punched on the side of each container near the top, evenly spaced around the rim. We left a section of the rim diameter without a hole to allow pouring out of used bait solution. The type of bait varied according to the objective of the trial. A yellow sticky card was hung inside each trap. After a week in the field, all traps were examined in the laboratory under magnification to determine the number of male and female SWD on the yellow sticky card and in the bait solution.

1. <u>Seasonal occurrence of SWD in lowbush blueberry fields</u>

Twenty commercial blueberry fields were sampled with several traps both in the field interior and along the field perimeter in the shrub and tree line. On average 4 traps were deployed in each field (although some fields had 5 traps), two in the field interior within the lowbush blueberry plant canopy (traps placed upon the ground) and two traps placed at a ca. 4 ft height hung from trees or shrubs along the field edge. Traps were initially deployed in 9 fields in Waldo and western Hancock County on 8 Jun. On 30 Jun traps were deployed in 11 more fields in eastern Hancock and Washington County. Traps were replaced weekly and the collected traps were taken back to the laboratory for SWD assessment. SWD were sexed and counted on both the sticky cards and in the fluid. Graphs were made of the trap captures throughout the season for the 9 fields in Waldo and western Hancock County and the 11 fields in eastern Hancock and Washington County. In addition, the trap captures were grouped as to their location, field interior and field edge. The field interior traps in the eastern Hancock County and Washington County were pulled from the field immediately prior to harvest and before SWD adults were caught in any traps. Therefore, the field interior traps really represented traps along the field edge, but positioned on the ground.

RESULTS AND CONCLUSIONS: Flies were not captured until 27 Jul in Waldo and western Hancock Counties and 9 Aug in eastern Hancock County and Washington Counties (Fig. 1). In both regions SWD was trapped 7-10 days earlier in traps along the field edge than in the field interior. A steep rise in trap captures occurred within a week after first trap capture in both regions and continued to increase geometrically throughout the late summer and fall. Figure 2 shows that significantly more SWD were captured in traps placed along the field edge (P = 0.0001), and that traps deployed on the ground independent of whether the traps were located in the field or along the field edge caught very few SWD.



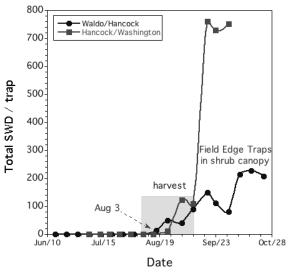
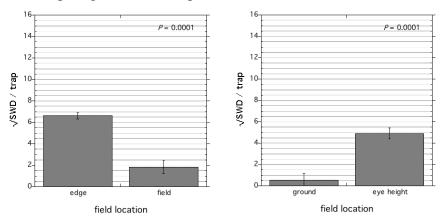


Fig. 2. Abundance of SWD in field edges compared to traps placed within the field, and impact of trap height on SWD captures.



2. Comparison of numbers of SWD in fruit-bearing and pruned fields

Ten isolated lowbush blueberry fields were selected for this study, 5 fruit-bearing and 5 pruned. At each commercial field, three traps were hung ca. 4 ft high and 30 ft apart along the edge of the blueberry field. Traps were constructed as described above. Apple cider vinegar (2-3 oz/trap) was used as bait. Traps were deployed on 11 Aug and collected on 18 Aug; during this

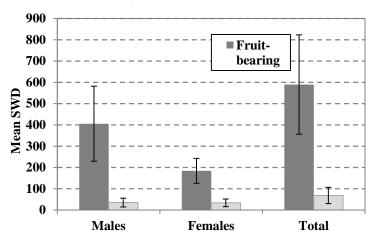
time SWD had been observed in most fruit-bearing fields that we had previously sampled in Hancock and Washington Counties.

RESULTS AND CONCLUSIONS: Analysis of Variance (RCB) was used to compare the number of SWD captured in fruit-bearing vs. pruned fields. Data were transformed by square root prior to analysis. Significantly more SWD were captured in fruit-bearing fields. This was true for males (P = 0.0444), females (P = 0.0466), and total SWD (P = 0.0431) (Table 1 and Fig. 3).

		Mean SWD per trap)	
Treatment	Males	Females	Total	
Fruit-bearing	405.47	184.33	589.81	
Pruned	35.07	33.60	68.73	

Table 1. Number of SWD captured in fruit-bearing and pruned lowbush blueberry fields.

Fig. 3. Number of SWD captured in fruit-bearing vs pruned lowbush blueberry fields (lines are standard errors of the mean).



The results of the prune vs. crop study suggest that overwhelming more flies occur in fruitbearing fields. Although, a fair number of both male and more importantly, female flies, were caught in prune or non-fruit bearing fields. This finding is very important because it suggests that the population finds wild fruits and/or flowers to reproduce on and thus maintain its population even when a crop is not present.

3. <u>Attractiveness of three baits to SWD</u>

Two trials were completed to determine the effectiveness of three baits in attracting SWD. The baits are described in Table 2. For each trial, four traps per treatment were hung ca. 4 ft high and 30 ft apart along the edge of the field. Two trials of this experiment were conducted. Traps were deployed for one week (Trial #1 - 15-22 Aug, Trial #2 - 11-17 Sep).

Table 2. Composition of bait solutions.

- 1. Live yeast (1 Tbsp) + sugar (4 Tbsp) + 12 oz water; makes enough for 4 traps
- 2. Apple Cider Vinegar (commercially available, 3 oz per trap)
- 3. Cowles SWD bait 57% white grape juice from concentrate, 37% apple cider vinegar, 6% 95% ethyl alcohol

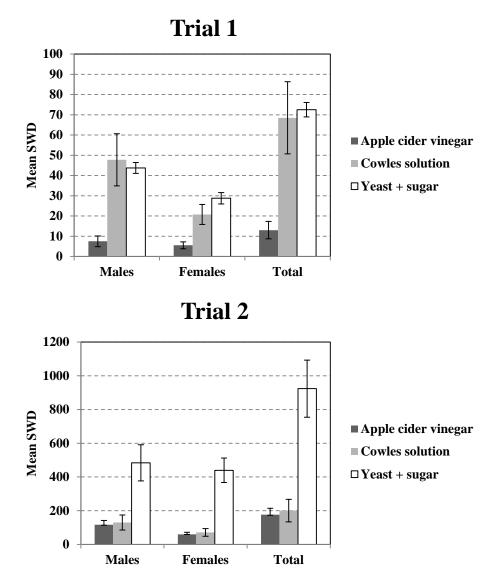
RESULTS: Analysis of Variance (RCB) was used to compare the number of SWD captured in traps with different baits. Data were transformed by sq rt prior to analysis. For trial #1, traps baited with Cowles SWD bait or Yeast + sugar captured significantly more SWD than traps baited with Apple cider vinegar. This was true for males (P = 0.0107), females (P = 0.0123) and total SWD (P = 0.0112) (Table 3 and Fig. 4). In trial #2, significantly more SWD were captured in traps baited with Yeast + sugar than either Cowles SWD bait or Apple cider vinegar. Again, this was true for males (P = 0.0002), females (P = 0.0001) and total SWD (P =0.0003). A three-way Analysis of Variance (RCB) was conducted in order to test if the treatment effects had different relative attractiveness from trial 1 to trial 2. When both trial and bait treatment were considered we found that for males there was a significant trial x treatment interaction described by the apple cider vinegar being the worst attractant bait in trial 1; the yeast + sugar bait and the Cowles bait were equivalent. But in trial 2, apple cider vinegar and the Cowles bait were the least attractive, both being outperformed by the yeast + sugar bait (P =0.007). The females and total trap capture reflected the same pattern as observed with the male trap captures (P = 0.0001, P = 0.0008; females and total flies, respectively).

	Mean SWD per trap					
Treatment	Males	Females	Total			
<u>Trial 1</u>						
Yeast + sugar	43.75 a	28.75 a	72.50 a			
Apple Cider Vinegar	7.50 b	5.50 b	13.00 b			
Cowles SWD Bait	47.75 a	20.75 a	68.50 a			
Trial 2						
Live Yeast + sugar	484.00 b	439.75 b	923.75 b			
Apple Cider Vinegar	116.50 a	60.25 a	176.75 a			
Cowles SWD Bait	130.00 a	71.00 a	201.00 a			

Table 3. Comparison of number of SWD captured in traps with different baits.

Means within each trial and column followed by the same letter are not significantly different (Tukey's HSD ($P \le 0.05$).

Fig. 4. Average number of SWD captured in traps with different baits (bars are standard errors of the mean).



CONCLUSIONS: The results of our study suggest that baits CHANGE in their attractiveness throughout the season. However, the most critical time for detection of SWD is in the early season just prior to harvest. Based upon our experiment we suggest that either the yeast + sugar or the Cowles bait be used. Growers that have a long harvest season might be better off using the yeast + sugar bait as this bait never lost its attractiveness throughout the trapping period.

4. SWD in response to blueberry production management

The 20 fields monitored for SWD in Waldo, Hancock, and Washington counties were categorized as to their management, standard or organic. Three fields were organically managed and 17 fields were standard or conventional management. Starting on 15 Aug, when SWD populations were found throughout all regions, and continuing until 8 Oct an unbalanced

non-replicated two-way analysis of variance was conducted (CR design) to estimate the effects of sample date and blueberry management system (standard vs. organic) on the total SWD captured in traps suspended in shrub and tree canopies along the edge of each field (logarithm transformed).

RESULTS: We found that there was a date effect (P < 0.0001), suggesting that populations continued to build in all fields between 15 Aug and 8 Oct (see Fig. 2). There was NO management effect (P = 0.713) (Fig. 5); however, mean total SWD captured (raw, non-transformed data) in standard fields over the sampling period was 149.1 flies / trap (SD = 20.6) and 155.4 flies / trap (SD = 45.1) in organic fields.

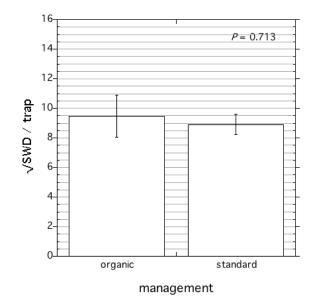


Fig. 5. Abundance of SWD in response to management practice.

CONCLUSIONS: At this early stage of our investigations on SWD in wild blueberry we have few recommendations, except that standard and conventional growers are likely to have similar risk in fruit damage. The standard fields sampled were not treated with insecticides for SWD prior to harvest and so our results are not a reflection of any failure in control tactics.

Study 3. <u>The genetic diversity of Vaccinium angustifolium clones in Maine</u> <u>Report from Lee Beers (Ph.D student) and Dr. Frank Drummond</u>

METHODS: Populations of lowbush blueberry (*Vaccinium angustifolium*) were sampled in a north south transect spanning 1,360 km (845 miles)(Lubec, ME to New Castle, VA) that included non-managed (wild) and managed populations. Genetic diversity among and within the sampled populations was assessed using expressed sequence tag polymerase chain reaction (EST-PCR) molecular markers. A total of 202 bands were produced from 23 primer pairs. Primers were selected that have previously been shown to produce polymorphic bands as well as primers designed from stress and metabolism related genes.

The effect of management for crop production on the genetic diversity of lowbush blueberry was evaluated in three paired populations in Maine. Each population contained both non-

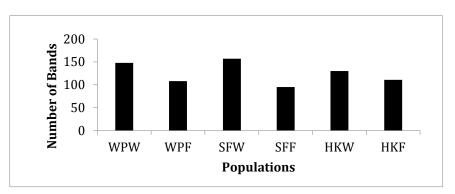
managed and managed plants growing in close proximity (< 500 m, 1640 ft). Comparisons between the managed and non-managed growing conditions with multi-blocked pairwise permutations (MRBP) showed there were no significant differences between the growing habitats (P = 0.1155). Further analysis with non-parametric ANOVA and analysis of molecular variance (AMOVA) found that there are highly significant differences between the managed and non-managed plants (P = 0.0002 and P = 0.001, respectively) (Table 1). Although the results are contradictory, the highly significant results of the perMANOVA and AMOVA, and weak significance of the MRBP analysis, suggest that there is a difference between managed and non-managed plants.

Table 1. (Left) perMANOVA table for managed and non-managed plants. There is a significant difference between growth, location of paired populations, and the interaction. (Right) AMOVA pairwise comparison between managed and non managed plants in a paired population. Significant differences are seen between the management practices.

Source	d.f.	SS	MS	F	р*	Pop1	Pop2	PhiPT	P(rand >= data)
Location	2	2.4406	1.2203	7.2873	0.000200	WPW	WPF	0.288	0.001
Growth	1	4.8538	4.8538	28.985	0.000200	SFW	SFF	0.257	0.001
Interac.	2	2.1084	1.0542	6.2953	0.000200	HKW	HKF	0.150	0.001
Residual	114	19.090	0.16746						
Total	119	28.493							

Frequency of bands present in managed and non-managed plants were counted. Although not statistically significant, a trend of decreased genetic diversity can be seen in the managed populations relative to the non-managed neighbors. There is approximately a 25% decrease in genetic diversity when lowbush blueberries are managed for fruit production (Fig. 1). The non-managed populations also had a higher number of bands associated with stress related genes than the managed plants, but the numbers of bands were similar for both growing habitats.

Fig. 1. Number of bands from neighboring plants that were managed for fruit production (WPF, SFF, HKF) or not managed (WPW, SFW, HKW).



Expanding the geographic range, comparisons were made between non-managed populations sampled from Maine to Virginia. Pairwise comparisons of non-managed populations in AMOVA found significant differences (P = 0.001) between all populations. Greater percentage of variance was found within populations (57%) compared to among populations (43%). Managed populations in Maine were analyzed separately and found similar variance patterns

within (70%) and among (30%) the populations. Managed populations were found to be significantly different in pairwise comparisons (P = 0.001) (Fig. 2).

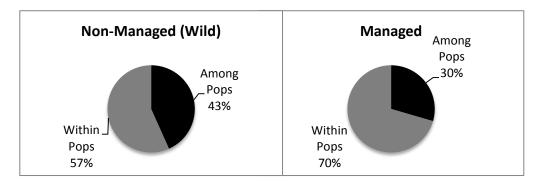
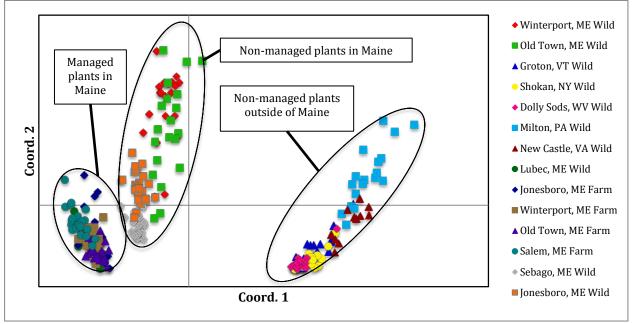


Fig. 1. Percentage of molecular variance for non-managed and managed plants.

Principle coordinate analysis (PCA) separated the sample populations into three groups (Fig. 3). Managed populations of lowbush blueberry cluster together despite a maximum distance of 214 km (133 miles) between populations. Also clustering with the managed plants is the non-managed population in Lubec, ME. The remaining non-managed populations separated into two clusters, one representing the Maine populations and the second representing all non-managed populations sampled outside of Maine.

Fig. 2. Principle coordinate analysis of all sampled populations including managed (farm) and non-managed (wild) growing habitats.



RECOMMENDATIONS: The parasitoid wasp of the blueberry maggot fly may have a key role in regulating pest populations and outbreaks. However, since 2005 parasitoid populations have been very low and blueberry maggot fly numbers have been steadily increasing. Conservation of this parasitoid can be enhanced by avoiding sprays one month after the initial emergence of the blueberry fly or after the date of August 1.

Spotted wing drosophila (SWD) is a new pest in Maine and we have few control recommendations. The insecticides that are effective against the blueberry maggot fly also appear to be effective against SWD, except for the neonicotinoids: acetamiprid and imidacloprid. We have found that the SWD infest fields starting in mid-August and increase geometrically from that time. Crop and prune fields have SWD populations suggesting that next year's crop fields are likely to be attacked. The best bait for monitoring appears to be a sugar syrup and yeast solution. Future studies will focus on insecticide efficacy, mass trapping as a means of control, and trap catch as a means of timing insecticide applications.

ENTOMOLOGY

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

7. TITLE: V. Biology of beneficial insects and blueberry pollination, 2012.

Study 1. <u>The economics of bee pollination and the yield of blueberries.</u> <u>Report from Eric Asari (Master's Candidate) and Dr. Frank Drummond.</u>

Blueberry yield is influenced by a number of factors. The primary factors are pollination; fertilization; weed control; irrigation; insect and pest management; pH management; pruning; and weather, including precipitation, solar radiation, wind speed and temperature. Bee pollination is perhaps the most important, since without pollination yields would be negligible. This study's objective is to estimate a lowbush blueberry yield model to quantify the effects of the factors affecting the yield of blueberry farms in Maine. Also, a fruit set model will be estimated to explain the factors affecting fruit set (a proxy for bee pollination). Again, the last phase of the study will simulate and quantify the effect of the state of bees (specifically, honeybee collapse, native bee availability and the price of honeybees) in Maine on the benefit-cost position of a blueberry farm in Maine.

METHODS: The study uses an unbalanced panel data (1993, 1997, 1998 and 2011) of blueberry farms in Maine. The number of farms in 1993 was 34, 18 in 1997, 16 in 1998 and 12 in 2011. The number of farms also differed in terms of whether it was a low input, medium input, high input or organic farm. In 1993, there were 24 low input farms, 8 medium input farms, 2 high input farms and 0 organic farms. In 1997, there were 6 low input farms, 6 medium input farms, 4 high input farms and 2 organic farms. There were 5 low input farms, 6 medium input farms, 4 high input farms and 1 organic farm in 1998. For 2011, there were 3 low input farms, 3 medium input farms, 3 high input farms and 3 organic farms. A definition table of the predictors (Table 1) is given below. A log-linear model is used to estimate the fruit set model, using an ordinary least square regression approach. Also, a log-log model is used to estimate the yield model, using an ordinary least square regression method.

RESULTS AND CONCLUSIONS: The results of the models show that the density of native bees have a positive and statistical significant (P = 0.01) effect on fruit set. The relationship between fruit set and native bees is shown in Figure 1. The model explained about 46.5 percent

variation in fruit set. A low input farm (P = 0.02), medium input farm (P < 0.001) and high input farm (P < 0.001) have significant and positive effects on fruit set compared to an organic farm. The density of honeybees also has a positive, but not significant (P = 0.18) relationship on fruit set at the 10 percent level. The relationship between fruit set and the density of honeybees is shown in Figure 2. However, the non-significant effect of honeybees is due to the inclusion of production intensity (low, medium, and high input) in the model. In fact, the production intensity is a reflection of the stocking density of honeybees deployed on the farm. If farm production intensity is not included in the model, then honeybee density in the field, as well as native bee density, is highly significant (P < 0.001) in explaining fruit set.

The blueberry yield model showed that, fruit set has a positive and significant effect (P < 0.001) on the yield of blueberries. Also, low input farms, medium input farms and high input farms all had positive and significant effects (P < 0.001) on fruit set compared to an organic farm. The model explained about 61 percent of the variation in yield.

The density of native bees and fruit set (a proxy for pollination) are very important for blueberry fruit set and yield respectively. Efforts are being made to improve on the result of the models. For instance, the inclusion of number of pollination days as an explanatory variable in the fruit set model is expected to improve the performance (fit) of the model. Also, including a winter kill variable in the yield model might improve the fit of the model. After estimating reasonable fruit set and yield models, the second phase of this study will be devoted to establishing the impact of pollination on blueberry yields through a simulation analysis using excel and @Risk. The goal is to develop a template, with which the blueberry farmer can use to know beforehand the expected impact of his/her use of honeybees and native bees on the net farm profit level, given the other variables. This will give the farmer a sense of the risk associated with using honeybees or native bees.

Variable	Definition	Unit	Formula/Comment
Fruit Set	The percentage of	Percentage	(Total number of blueberry
	flowers that turn		fruits)/Total number of flowers) * 100
	into blueberry		
	fruits		
Yield	Blueberry yield	Kg/Ha	Harvested yield of blueberries/farm
			size(hectares)
Honeybee	Honeybee density	-	One time count of honeybees during
			peak bloom
Native Bee	Native bee density	-	One time count of native bees during
			peak bloom
Hives/Ha	Number of hives	Hives/Ha	Number of hives employed divided by
	per hectare		farm size (hectares)
Square Root	Square root	-	Square root of honeybee
Honeybee	transformation of		
	honeybees		
Square Root	Square root	-	Square root of native bee
Honeybee	transformation of		
	native bees		

Table 1. Variable definitions.

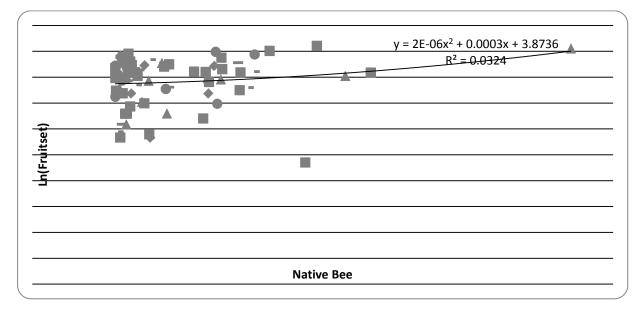
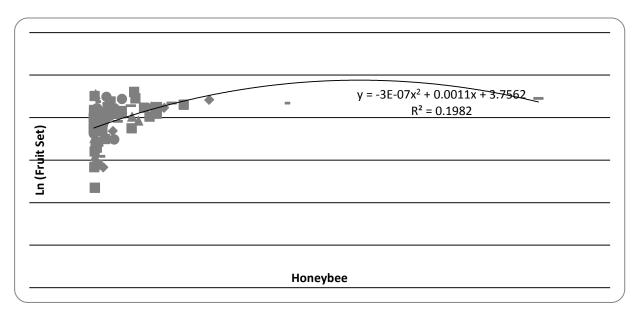


Fig. 1. Relationship between Ln (fruit set) and native bee.

Fig. 2. Relationship between Ln(fruit set) and honeybee.



Study 2. <u>Understanding the contributions to variation in seed number, size, and viability,</u> from individual clones, stems, stem positions, and fruits in lowbush blueberry. Report from Alex Bajcz (Ph.D student) and Dr. Frank Drummond.

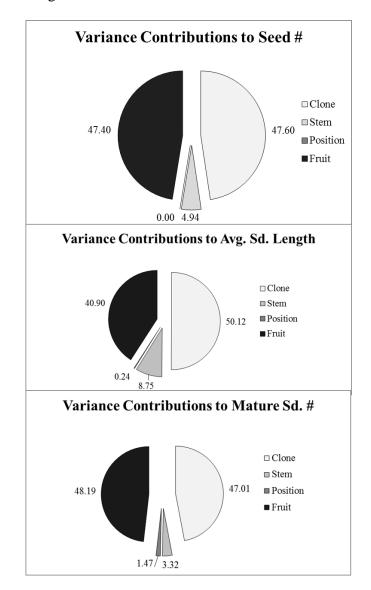
METHODS: During summer 2012, several different lowbush blueberry clones from within an organic blueberry farm were chosen *ad hoc* for study. From each of these clones, 10-18 berries were picked from three different haphazardly-selected stems in each clone and two different positions on each of those stems (top and bottom), for a total of six fruit samples per clone. These fruit samples were sorted into cups and frozen until the fall. In fall 2012, all six fruit

samples from three of these clones, as well as one sample each from three other clones, were randomly-chosen for further analysis. Each fruit from these samples was skinned, measured for equatorial diameter (in millimeters), and macerated. Then, the number and length of all seeds in each fruit were recorded using a Dinolite[®] camera microscope and software. Seeds were sorted considered mature and likely viable if they were >1mm in diameter, in accordance with previously-published research. A hierarchical linear mixed-effects regression and variance components analysis were performed on the total seed number, the total number of likely viable seeds, and on average seed length using clone, stem, and fruit as hierarchical random factors and stem position and fruit size as fixed factors.

RESULTS: When included as a fixed factor, stem position was never found to have a significant linear relationship with any of the three seed measurements studied here (P > 0.05). As a result, it was converted to a random factor nested in between stem and fruit in the hierarchical structure. Fruit size had a significant positive linear relationship with total seed number (B = 2.40, P < 0.001), the total number of class 3 mature seeds (B = 5.07, P < 0.001), and average seed length (0.05, P < 0.001). However, these linear trends are only noticeable after random variation due to differences between clones, stems, stem positions, and fruits has been accounted for; models between fruit size and these seed measurements show very poor fit otherwise ($\mathbb{R}^2 < 0.05$). Variance components analysis revealed that much of the variation in all three seed measurements assessed here are due to clone-by-clone and fruit-by-fruit differences, with <10% of the variation in these seed measurements explained by stem-within-clone differences or position-on-stem differences (Figs. 1A-C).

DISCUSSION/CONCLUSIONS: We looked at seeds per fruit as a measure of blueberry fitness because this study was initiated to look at the evolutionary forces that shape the reproductive biology of lowbush blueberry. However, fruit size is positively related to these three seed characteristics of individual fruits, and so the study also has direct relevance to blueberry production. Our preliminary results suggest that while fruits from any random position on a stem or from any random stem within a clone are likely to be comparable to fruits from any other stem position or stem in that same clone, different individual fruits, as well as fruits from different clones, are likely to be very different in terms of seed characteristics. This work suggests that experimenters interested in explaining variation in individual blueberry fruit seed characteristics can pull fruits from any stem position from any stem within a clone without ballooning their data's variance. However, many fruits per clone, as well as many clones, will need to be included in such a study to get enough data to see through seed-measurement variation at these levels.

Figs. 1A-C. Most of the variation in seed number per fruit, average seed length per fruit, and number of mature (class 3) seeds per fruit comes from differences in individual fruits and in individual clones. Fruit seed characteristics vary much less between stems in the same clone and almost not at all between locations on the same stem as a percentage of total variation.



Study 3. <u>The health of bumblebees in blueberry fields in Downeast Maine.</u> Report from Kalyn Bickerman (Ph.D student) and Dr. Frank Drummond.

METHODS: From 26 Jul and 2 Oct 2012, 8-11 field sites were visited approximately once a week in Downeast Maine. The goal was to collect a total of five bumble bees at each field of any species or caste, with around 200 bumble bees caught in total over the entire summer. 20-30 minutes were spent at each field as a measure of sample effort and different portions of fields were visited in order to limit the possibility that specimens were from the same colony. Specimens were place in a -20°C freezer to freeze-kill and store until dissection.

For dissection, bees were identified to species (in most cases without ambiguity), aged on a four-point scale (0-3) based on wing wear and the thorax was measured with digital calipers for bee size. The abdomen was opened on the ventral side to inspect the interior for possible presence of macro-parasites, particularly dipteran larvae. If a parasite was found, it was preserved in 95% ethanol. Gut contents were removed for examination under a phase contrast microscope and other body parts were stored in the -80°C freezer. Five minutes was spent on each slide of gut tissue to determine presence or absence of any pathogenic organism. Specimens were considered to be positive if two or more organisms were seen (*Nosema bombi* or *Crithidia bombi*). Infection intensity was measured using a Neubauer Hemocytometer. Any gut tissue that showed presence of disease was preserved in 25% glycerol then placed in the -80°C freezer. 130 samples have been processed in this manner thus far.

RESULTS AND CONCLUSIONS: Although statistical tests have not been performed at this stage, the breakdown of species caught and their infection can be seen in Table 1.

Species	Total	Dipteran parasitoid	N. bombi	C. bombi	Mermithid nematode
B. ternarius (70%)	91	25.6% (23)	5.5% (5)	2.2% (2)	
<i>B. vagans</i> (2.3%)	19	26.3% (5)			5.3% (1)
B. bimaculatus (6.9%)	9	22.2% (2)	22.2% (2)		
B. terricola (3.8%)	5	20% (1)	60% (3)		
B. perplexus (2.3%)	3	33.3% (1)	33.3% (1)		
B. impatiens (14.6%)	3				
Total prevalence	130	24.6% (32)	10% (13)	1.5% (2)	0.7% (1)

Table 1. Parasite and pathogens found in specimens from Summer 2012, along with their total and relative prevalence by species.

Although specimens from this field season are still being processed, it would seem that the presence of a Dipteran parasitoid is equally like among all species, hovering around 25%. However, for *N. bombi*, which only has a total prevalence of 10% in this sampling, it seems more likely that some species (*B. terricola*) are more likely to be infected. This warrants more consideration in the coming field seasons, especially in light of *B. terricola's* recent observed population declines.

Possible plans for research include: comparing conventional fields to organic fields; using immune response as a measure of immune strength and health in different fields; and pesticide trials in relation to immune response. In a summer of decreased overall bumble bee abundance, these numbers represent the relative abundances of each species in the field sites and can be tracked in future years to observe changes. Specimens have been saved for immune response analysis.

Study 4. <u>Abundance and diversity of native bees in relation to farm management.</u> <u>Report from Sara Bushmann (Ph.D student) and Dr. Frank Drummond.</u>

The purpose of this project is to determine if farm management practices are related to the composition of wild, native bee communities found in and around blueberry fields. The study began in 2010 and is expected to run for three consecutive years. This report summarizes the third year of the study.

METHODS: Sixteen blueberry fields located in Hancock and Washington County were identified for the third and final year of this study. These fields have never been previously used in this study. Management practices ranged from conventional with differing levels of inputs to organic. The fields differed with respect to the surrounding landscape and level of isolation from other agricultural fields. From the beginning of May until the end of June, wild bees were collected from each field using both active capture and passive capture in soapy water traps. The active capture involved timed "bee hunts" within patches of flowers. The common name of the flower each bee was caught on was recorded. The traps were 3.5 oz plastic cups painted either florescent yellow or blue or left an opaque white. The cup traps are not expected to catch bumble bees and honeybees in numbers that reflect their density in the field. Density of these two kinds of bees, therefore, was estimated through timed counting periods.

The alternative flowering vegetation was recorded by species or genus throughout the blueberry flowering season to one week beyond bloom. The data collected will provide a measure of the abundance of non-blueberry floral vegetation for each field. A measure of fruit set was obtained for nine clones in each field. Soil tests to determine percent composition of sand, silt, and clay have been completed. These tests will potentially give an indication of suitability of the soil as a nesting substrate for soil nesting bees. Finally, all bees were washed, dried, and pinned and most were given a tentative identification. In November of 2012 all bees caught were taken to Sam Droege of the USGS for confirmation of identification.

RESULTS: Two thousand one hundred seventy-one (2,171) bees were either collected by hand or trapped from the 16 blueberry fields throughout May and June, 2012. Most of these bees are identified to species. A few are identified to only genus since the species designation proved impossible to determine. Fewer than 100 individuals of the genera *Nomada* and *Sphecodes* still need final identification. Bees from these two genera are not included in the following discussion.

In 2012, 81 species representing 15 genera were identified. Including the 2010 bees (n=761) and those from 2011 (n=1550), the total number of bee species caught in blueberry fields totals 108. When the two parasitic genera are included this number will increase.

Not all species are equally represented by the 2012 total catch. Six species account for 54.73% of the bees caught (Table 1). The traps caught more bees (64.0%) than were hand caught. And the blue painted cups caught more bees (41.0% of the cup catch) than either the yellow or white cups (28.0% and 31.0% respectively). The cup traps, however, are a passive form of bee capture. They are placed in a field for 24-48 hours and are able to catch many (10- 40 maximum) bees during that period. Any bee that is foraging in or around the field might be attracted to the cup and get trapped in the soapy water. These bees may or may not visit blueberry flowers.

A more rigorous indicator of which species contribute to pollination of blueberry flowers would be the identification of those caught actively foraging on blueberry flowers. We caught 401 bees foraging on blueberry flowers. Sixty-three percent of these bees are represented by five species (Table 2), and one of these species, *Andrena carlini*, was caught in all 16 fields. Further analysis concerning the importance of wild bee pollinators will focus the distribution and abundance of these species. Preliminary analysis reveals a non-statistically significant, yet positive relationship between *Andrena* relative abundance and the percent mature berries found in the fields. The average percent of mature berries in 2012 is 43.64%, yet ranges from 23-71 percent.

The *Andrena* also appear to prefer soils with the silt-loam classification, which includes those fields with less than 40% sand particles in the soil sample (Fig. 1). On average, the 16 study fields measured as 58.4% sand, 33.5% silt, and 8.1% clay particles.

Yet to be analyzed is the alternative floral resources found in the field. Final analysis will consider the multiple field characteristics (e.g. size, chemical management, yield, soil types, etc) in relation to the wild bee abundance and diversity.

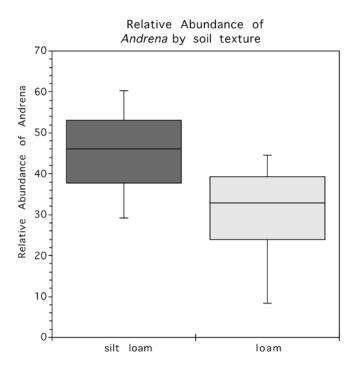
Table 1.	Six most abundant bee species caught by both hand collection and trapping in 16
	blueberry fields in Maine, May-June 2012.

Species	Relative Abundance	No. of fields with species
Andrena carlini	17.20%	16
Augochlorella aurata	15.68%	16
Lasioglossum cressonii	8.77%	16
Lasioglossum leucocomum	5.05%	14
Lasioglossum albipenne	4.26%	13
Andrena vicina	3.77%	14

Table 2.	Five most common	bee species	caught while	foraging of	on blueberry flowers.

Species	Relative Abundance
Andrena carlini	34%
Andrena vicina	13%
Colletes inaequalis	10%
Andrena rufosignata	9%
Andrena carolina	7%

Fig. 1: Relative abundance of all *Andrena* species in each field in relation to soil particle size. Silt-loam classification (n= 3 fields) has less than 40% sand in the sample. Loam classification, n= 13. Boxes represent 50% of the variation; lines indicate upper and lower measures.



Study 5. <u>Assessing the effect of landscape pattern and arrangement on native bee</u> <u>abundance in Maine's lowbush blueberry fields.</u> <u>Report from Shannon Chapin (Master's Candidate), Dr. Cyndy Loftin (USGS</u> <u>Coop Research Unit and Professor WLE), and D</u>r. Frank Drummond.

It is estimated that seventy-five percent of the world's crops rely at least partly on successful pollination by animals (Klein et al. 2007). Bees are considered the most important insect pollinator. Wild lowbush blueberries, a leading industry in Maine, are one such crop that requires insect pollination. Maine is the world's largest producer of wild blueberries and the country's second largest importer of non-native honeybees for pollination, with more than 50,000 hives deployed yearly (USDA 1999). The decline of honeybee populations has increased the cost of hive rentals (Pennsylvania State University Extension 2011). Focus has now turned to partially relying on and improving populations of native pollinators. Native pollinators are a freely available ecosystem service that have coevolved with wild blueberries and are adapted to forage in lower light levels and cooler temperatures common where blueberries grow.

There are over 270 native bee species, in six families, in Maine. More than 40 bee species have been documented foraging in lowbush blueberries in Maine; though, it is estimated that there are more associated species, as over 60 species have been recognized on blueberries in Nova Scotia (Drummond and Stubbs 2003). While the six bee families present in Maine exhibit various life history traits, all require at a minimum, two key components to survive: suitable nesting habitat and floral resources for forage.

Recent studies have shown that there is a link between the proportion of natural habitat surrounding a crop field and pollination by bees, as natural habitat can be synonymous with the two key components mentioned above. The definition of natural habitat varies by geographic location and simply includes environments that offer shelter, nesting grounds and food resources. Natural habitat, as it relates to bees, fits the description of different land cover types in Maine, including (but not limited to) deciduous forest, deciduous edge, scrub/shrub and herbaceous wetlands, and old fields and grasslands.

This relationship has been incorporated into a crop pollination model, InVEST, as part of the Natural Capital Project (Lonsdorf et al. 2011). The aim of InVEST is to remotely map the relative abundance of pollinators across a landscape. The model derives per-cell pollinator abundance, based on nesting resources within the cell and floral resources surrounding the cell within the confines of the modeled bee's foraging range, with a single spatial landcover dataset and user estimated parameters as model inputs. InVEST is a generic model that can be adapted to model any crop; however, it requires validation.

It has been predicted that not only landscape composition, but also the pattern and arrangement of the surrounding landscape affect the abundance of pollinators. Studies of these effects have not yet been published. I will investigate relationships of landscape composition, pattern, and analysis scale with the InVEST model combined with neutral landscape models (NLMs). NLMs often are considered the "null hypothesis" in the field of landscape ecology. Ecological processes in real landscapes can be compared against a randomly created NLM to reveal the effects of landscape pattern on process.

OBJECTIVES: There are three main objectives of my research:

- 1. Investigate the feasibility of using the InVEST model to understand relationships between landscape pattern and abundance of Maine's blueberry pollinators. Specifically, does the InVEST pollination model provide a good fit for predicting native bee abundance in Maine's blueberry fields?
- 2. Conduct a sensitivity analysis of the InVest parameters to identify relationships between user-provided parameters (e.g. foraging distance, forage availability, nesting habitat availability) and validity of the InVEST model.
- 3. Assess landscape configuration (composition, arrangement, scale) effects on pollinator services with NLMs to reveal relationships between landscape pattern and pollination services.

METHODS: Model parameters will be derived with a combination of expert opinion and available literature. Bee foraging distances will be based off of the predictive regression equation provided by Greenleaf et al. (2007) using measurements obtained from locally caught specimens. GIS data will include SPOT imagery, Maine Landcover Dataset 2004, USDA Croplands Dataset 2010, and ancillary data (National Wetlands Inventory, Maine Department of Transportation e911 roads and railroads, and heads up digitizing of powerline right-of-ways).

I will run at least two simplified models initially, and increase model complexity in subsequent model runs. I will conduct a model sensitivity analysis on the parameters in the simplified model. InVEST model output will be compared to field-collected bee abundance data from 2010, 2011, and 2012. Regression models will be developed to relate the field-collected data to the InVEST model output.

NLMs will be created with the program QRULE (Gardner 1999). Output NLMs will be used as the input landscape layer for the InVEST pollination model. I will compare results of the pollination model output on both the real landscape and the NLM produced landscapes. Specifically, I will compare the relationship between the dependent pollinator abundance values and independent landscape type specific metrics. Landscape metrics, including the number of patches of a particular land cover, average patch size and a measurement of patch compactness will be analyzed with a combination of FRAGSTATS v4 (McGarigal et al. 2012) and QRULE.

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Study 6.Bee preferences for alternate forage resources regarding lowbush blueberry in
Maine.
Report from Dr. Alison Dibble (Resarch Scientist SBE), Dr. Frank Drummond,
Dr. Lois Berg Stack (Professor Univ. Maine Coop Extension), and Eric Venturini
(Master's Candidate).

This report covers the first year of a 5-year study by Drs. A. C. Dibble, F. A. Drummond, L. Berg Stack, and Mr. E. Venturini, as part of the Pollination Security project funded by the USDA and the University of Maine. The plight of the honeybee due to Colony Collapse Disorder has led to concern for native bees, which pollinate economically important crops like wild blueberry in Maine, and fruits and vegetables throughout New England. Farmers and gardeners want to plant bee gardens, but recommendations for what to plant include species that have not been used much in Maine. We look at how blueberry growers in Maine can maximize a bee garden by including plants that do not (much) overlap the crop in flowering period, with the intent that over time the native bee populations will expand. We also tested some fancy annual bedding plants that are of interest to the greenhouse growers industry. We call the experiment the Bee Module because plant material can be replaced, switched in and out, and tested in small patches.

METHODS: We included 29 different plants in four replicated gardens by counting bee visits on flowers. Our focus is plants and insects, not garden design, so the gardens might seem oddly arranged upon first sight. In spring 2012 we established gardens at these sites: Blueberry Hill Farm in Jonesboro, ME, Rogers Farm in Old Town, ME, and two privately-owned blueberry farms in Blue Hill, ME, one certified organic and one low-spray. The plant species were selected based on published recommendations, our informal observations around Maine, ease of cultivation, and potential long bloom time. Each plant was afforded 1 m sq in area (though some sprawled well beyond this). We visited weekly or more often, during good weather, as plants came into flower. Per visit, we observed insects on flowers in 1 min. periods, with three periods per plant species (Fig. 1). Deadheading and weeding were necessary to keep the plants in flower longer.

Insects were categorized into groups of bees (e.g., honeybee, native bees such as bumblebee, miner bee, sweat bee (Fig. 2), leaf-cutting bee, and so forth), or into wasp, fly, butterfly, moth, etc. A few insects were recorded to species if their identification on the wing is straightforward, such as the honeybee and orange-banded bumblebee. Layout of the garden differed by site but all the same plants were present at each of the four sites.

Fig. 1. Dr. Alison Dibble checks on sweet alyssum at the field site in Jonesboro, Maine.



Fig. 2. A small native bee collects nectar at white-flowered borage in October 2012. This plant was the top bee-attracting annual species across all four gardens.



RESULTS: Borage, especially white borage (Fig. 2, bar graph in Fig. 3) was our top bee plant for visits of all bee species.

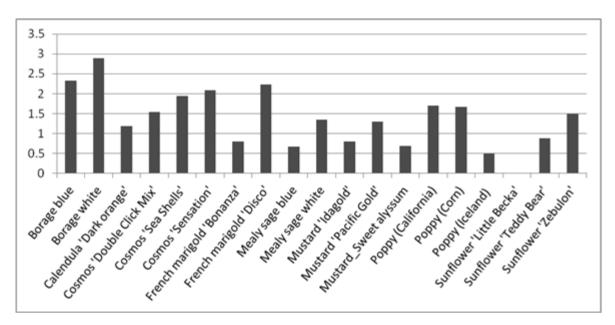
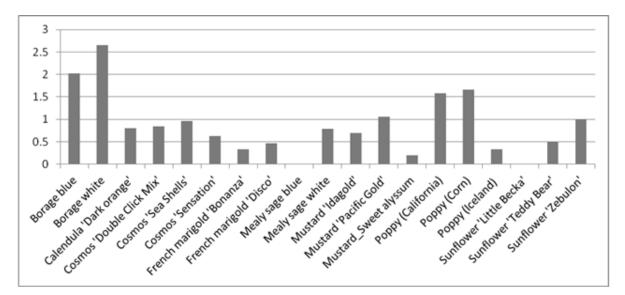


Fig. 3. Average number of bees per minute on flowers across four sites.

Fig. 4. Average number of honeybees per minute on flowers in the Bee Module experiment, across four sites.



Honeybees were kept on farms where the Bee Module experiment was set up, and are expected to benefit from any bee garden a grower might create. Honeybees preferred white borage flowers above all other plant species we tested (Fig. 4). Our categorization into groups of bees might mask some species- or genus-specific trends, as we found a different result for the orange-banded bumblebee (Fig. 6) than for all bumble bees (Fig. 5).

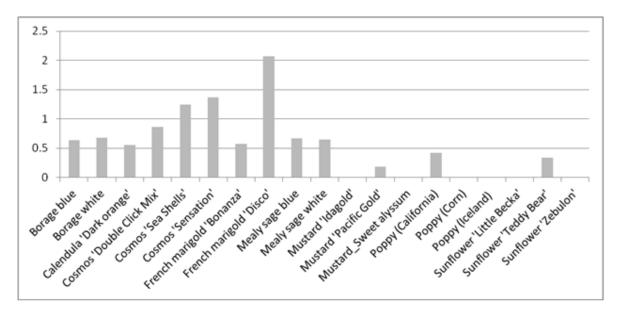
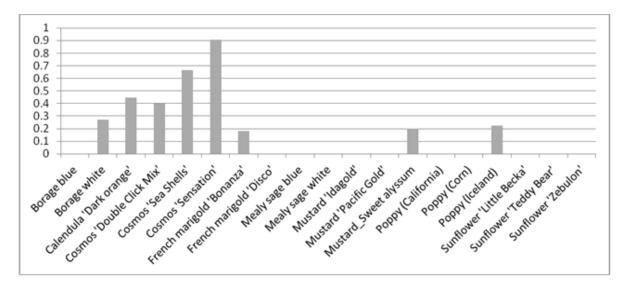


Fig. 5. Average number of bumblebees per minute (includes orange-banded bumblebee)

Fig. 6. Average number of orange-banded bumblebees on flowers per minute.



Some bees are difficult to recognize on the wing because they move quickly, or sense the presence of the observer and move off before we can get a photo or confirm our identification to group. Might these bees then be under-represented in the data? They could be included as "unknown bee". An example of this, is the leaf-cutting bee, see Fig. 7.

Fig. 7. A small native leaf-cutting bee (*Osmia* species) visits Calendula 'Dark Orange' in late summer.



DISCUSSION/CONCLUSIONS: These preliminary results may help growers and home gardeners choose plants for the bee garden. In a nutshell, a variety of plantings is likely to assure greater diversity of native bees, so try to find plants for early (before blueberry comes into flower), mid-season, and later parts of the season.

White-flowered borage was the top bee plant among all the annual flowers we tested. Single-flowered 'Sensation' cosmos attracted more bees than double-flowered 'Double Click'. Tubular-flowered 'Sea Shells' attracted bees well, too. Bumble bees preferred single French marigold 'Disco' compared to the double-flowered 'Bonanza'. Honeybees favored poppies especially, while bumble bees favored several other species, with some overlap. Orange-banded bumble bee (one of Maine's most common bumbles) differed from other bumble bees in some of its floral preferences.

Some annuals that attract us (like double French marigolds) might not have much appeal to bees (which prefer single marigolds). Native bees vary from one place to another. Our results are most applicable to the Northeast. Bee plant lists from other parts of the country are not always applicable. We'll have more data in the coming years, as we test some plants another season, and add other plants to the study.

Acknowledgments

We thank the USDA, New England Floriculture, Inc., and New England Grows for their support. Thanks to our Maine farmer-partners, University of Maine Blueberry Hill Farm staff, and our associated faculty, graduate students and undergraduate field assistants who helped install the bee plantings, collected insect observation data, and helped get this 5-year project off to a great start. All photos taken Dr. A. C. Dibble.

Study 7. <u>Honeybee exposure to the fungicide propiconazole in lowbush blueberry, year II.</u> <u>Report from Dr. Frank Drummond.</u>

Honeybees are the mainstay of lowbush blueberry pollination in Maine. Since 2006 honeybees have been under much stress, and severe colony losses have been experienced by beekeepers throughout the U.S. and Europe. While much of this decline has been attributed to diseases, there is still widespread belief that exposure of honeybees to sub-lethal concentrations of pesticides are also an important contribution to colony losses. This study was designed to assess the effects of honeybee exposure to typical applications of propiconazole (Orbit) on lowbush blueberry just prior to bloom. This report is a replication of a similar field trial conducted during the 2011 field season.

METHODS: Seventeen honeybee colonies were selected from colonies that were started from packages in mid-April 2011. The bees and queens were obtained from a package producer in Georgia. Nine colonies were randomly designated to an untreated check treatment and eight colonies were designated to a fungicide exposure treatment. All colonies were kept at the University of Maine in Orono until just before blueberry bloom. The colonies were inspected on 6 May and colony strength of workers and capped brood was recorded. Throughout the spring prior to moving them to blueberry bloom they were fed sugar syrup as needed. No medications or pesticides were placed in the hives for bee parasite or pathogen control. On 11 and 12 May hives were moved in groups to two blueberry fields in early bloom. The untreated check group was moved to an organic field in Stockton Springs and the fungicide-exposed group was moved to a blueberry field in Deblois that had propiconazole applied the previous day. Two hundred flowers from each field were harvested on the day that the hives were moved to the fields and frozen at -20°C; at the end of the summer the flowers were shipped on dry-ice to the analytical laboratory at the Connecticut Agricultural Experiment Station for determination of the residue levels on the flowers. Hives were moved back to Orono after bloom (10 Jun) and monitored throughout the rest of the summer. In the fall all surviving hives were wrapped and fed for overwintering. Hives were monitored for colony strength and bee samples were collected from each hive for disease and mite density assessment on 25-26 May, 13-14 Jun, 27-31 Jul, and 29 Aug – 7 Sept. In addition, queen egg-laying was assessed on 21 Jun by confining 5 randomly selected queens in a queen excluding cage for 2 days within each of five hives at each field location. Also, on 1 Jul full frames of brood were taken from 5 random hives from each experimental group and stored in mesh cages in an environmental chamber at 30°C. Emergence rate of the workers (brood survival) and longevity of the newly emerged workers was assessed by setting up 3-4 replicates of 15 workers from each emerged frame in a wire cage with access to sugar syrup. Daily assessment of worker longevity was recorded. The last measure to determine potential physiological effects of propiconazole is dissection of the newly emerged workers (8 per hive) and measurement of the size of the hypopharyngeal gland. This gland is an important endocrine gland in the honey bee. The dissections are planned for Jan 2013. Analysis of variance and non-parametric life-table analysis was used to analyze the data.

RESULTS: During the summer 0% (n = 17) of the hives were lost. Colony strength measured as both worker population and brood population were not affected by fungicide exposure over the course of the entire spring and summer ($F_{(1,15)} = 0.140$, P = 0.713 and $F_{(1,15)} = 0.129$, P =

0.185; workers and brood respectively). However, there was a significant change in the difference between the two treatments over time ($F_{(4,12)} = 4.787$, P = 0.015 and ($F_{(4,12)} = 7.170$, P = 0.003; workers and brood respectively). Figure 1 shows that colonies that were not exposed to propiconazole built up worker populations more quickly during bloom than the colonies exposed to propiconazole, but declined in worker populations after bloom. Figure 2 shows the same pattern as in the worker populations (Fig. 1), but it is even more extreme with the brood populations. Queen supercedure rates were not affected by exposure to fungicide ($\chi^2 = 0.008$, P = 0.929).

Queen egg-laying was significantly affected by exposure to propiconazole. However, we saw an unexpected result. Queens exposed to fungicides actually had a higher rate of oviposition than those not exposed ($F_{(1,8)} = 5.373$, P = 0.049, non-fungicide = 1109 eggs / 48 hrs and fungicide = 1765 eggs / 48 hrs). It is hard to explain these results; although, sub-lethal exposures to toxins can sometimes stimulate an over-compensation in physiological and/or behavioral responses. We also looked at the maladapted behavior of laying multiple eggs in a cell. We found no difference in multiple egg laying ($F_{(1,8)} = 0.460$, P = 0.348). In addition, we measured worker deposition of nectar in cells in the brood area. We found no evidence to support a significant difference in nectar deposition ($\chi^2 = 0.00004$, P = 0.995).

Larvae reared during blueberry bloom did not experience differential survival due to fungicide exposure ($F_{(1,10)} = 2.818$, P = 0.124). However, there was a trend towards greater survival in larvae that were not exposed to fungicides. Mean survival for brood reared in the non-sprayed field was 48.8% while those reared on the fungicide-exposure field had a survival rate of 71.1%. The longevity of young worker bees that were reared as larvae when the hives were on the blueberry fields and then emerged in the laboratory did appear affected by fungicide exposure (Mantel test: $\chi^2 = 41.840$, P = 0.0001). Figure 3 shows the longevity of the bees in the two groups. The median (50%) longevity is different (fungicide = 3 days, non-fungicide = 7 days). It can be observed in Fig. 3 that the bees that foraged on the non-sprayed field lived several days longer than the bees exposed to fungicide. On average a bee that foraged on the fungicide sprayed field had a relative risk of dying at a rate 1.91 times that of a bee that had foraged on the non-sprayed field.

Other data that we collected have not been analyzed yet: fungicide residues on blueberry flowers, stored nectar, pollen; and disease levels in worker bees. The results of these data are being analyzed at the Connecticut Agricultural Experiment Station (chemical residues) and the USDA/ARS Beltsville Honey Bee Research Laboratory.

We did measure *Varroa* mite infestation in June and September and we found no significant differences in parasitic mite levels due to fungicide exposure (MANOVA: $F_{(1,10)} = 0.142$, P = 0.645). Figure 4 shows the mite densities for the two sample dates.

Fig. 1. Worker honeybee strength per colony (measured as the cumulative percent comb area occupied by workers and then converted to numbers of workers per hive) for the two treatment groups. Shaded rectangle represents period of time when bees were moved to their respective blueberry foraging fields.

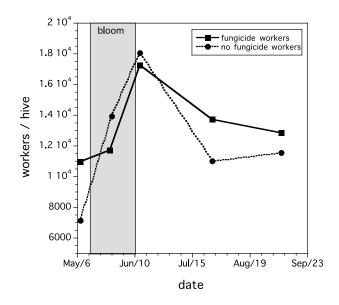


Fig. 2. Honeybee brood strength per colony (measured as the cumulative percent comb area occupied by capped brood and then converted to numbers of capped brood per hive) for the two treatment groups. Shaded rectangle represents period of time when bees were moved to their respective blueberry foraging fields.

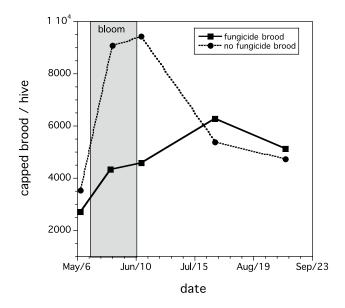


Fig 3. Longevity of newly emerged worker bees held in the laboratory.

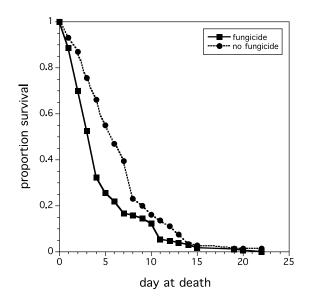
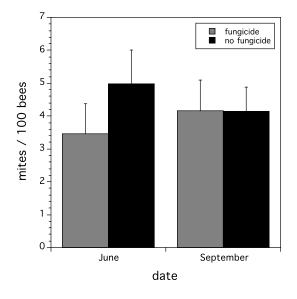


Fig. 4. Varroa mite infestation (per 100 bees) sampled in June and September.



CONCLUSIONS: Our second year of research shows that the honeybee health effects of the commonly used fungicide propiconazole are not entirely consistent between years. Although, we can conclude that negative effects are suggestive. We found that overall exposure of honeybee foragers to residues on flowers does not reduce colony strength of worker or capped brood populations. Queen laying and capped brood survival also does not appear to be affected by exposure to sub-lethal doses of this fungicide. We did find evidence in both years to suggest that workers reared as larvae during bloom result in young nurse bees whose longevity is reduced. Other data for 2012 has not been collected yet and so it is not possible to make firm conclusions until all data are analyzed.

Study 8. <u>Assessing pollination strategies and perceptions of native pollinators.</u> <u>Report from Dr. Sam Hanes (Research Asst. Professor Dept. Anthropology).</u>

METHODS: This study has employed two major methods so far: a grower survey and indepth interviews. The survey was conducted 18 Jul 2012 at the Wild Blueberry Field Day in Jonesboro, ME. The survey gathered information on growers' pollination practices and their perceptions of native pollinators. The main aim was to see whether perceptions of the effectiveness of native pollinators presented an obstacle to their greater utilization. Secondary aims included getting baseline information on pollination practices and seeing whether grower characteristics correlated with practices and perceptions. Survey data was entered into SPSS and analyzed using analysis of variance (ANOVA) and Chi-Square.

Interviews began in September. The main goals of the interviews are to 1) assess growers' pollination strategies and reasons for them, 2) explore growers' interactions with native pollinators, and 3) learn growers' concerns about pollination. Interviews are semi-structured; there are pre-set questions on a checklist the interviewer always covers but interviewees are encouraged to direct the conversation as much as possible. They usually last an hour. Digital recordings are made during each interview and these will be transcribed and coded in the database software NVivo. In coding, the researchers attach descriptive codes to portions of interviews to aid in analysis and retrieval. Sixteen interviews with 19 growers have been completed. Interviews will continue throughout the winter.

In addition, an intercept survey of home gardeners was conducted at the Common Ground Fair on 22 Sep 2012. The goal was to assess home gardeners' interactions with pollinators. Data has been entered in SPSS and will be analyzed over winter break.

RESULTS AND CONCLUSIONS:

Pollination strategies

75% of respondents rent or own bees, and 66% use rented or owned bees on all of their property. All respondents who manage over 25 acres rent or own bees, whereas growers with fewer than 5 acres are significantly less likely to rent or own bees (P = 0.001). 47% of those who rent bees reported stocking 1-2.5 hives per acre, and 23% reported stocking over 2.5 hives per acre. The average among growers who rent bees was 2.28 hives per acre. Stocking density was correlated with increased yield (P = 0.008), and the number of extension meetings the grower attended (P = 0.036).

Perception of native pollinators' effectiveness

Growers who rent bees feel they get between 5-80% of their fruit set from native pollinators. The median is 26% from native pollinators. When asked how much their fruit set would decline if they rented no bees at all, growers who currently rent bees said it would decline between 0-75%, with the median being 49%. This latter number indicates that growers who rent bees see native pollinators as a significant fallback mechanism. Growers were also generally uncertain about native pollinators' contribution to fruit set, only 23% of respondents said they were *certain* or *very certain* about native pollinators' contribution to fruit set; although, growers who classified themselves as IPM or "traditional" were more certain (P = 0.034), as were growers who attended more extension meetings (P = 0.047).

Perception of native pollinators' habitat around fields

Growers were asked to check boxes indicating the type of landscape and vegetation within a half mile of their fields, which is roughly the maximum distance a large native bee can travel for food. They were then asked to rate the quality of their overall native bee habitat. No growers felt they had *very poor* habitat, and only 6.1% saw their habitat as *poor* (Fig. 1).

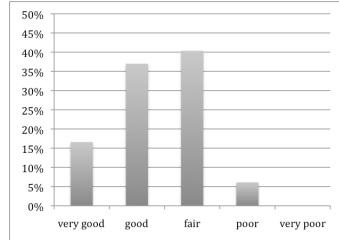


Fig. 1. Responses to "How would you rate the quality of habitat for native pollinators within a half mile of your fields?"

Use of native pollinator habitat enhancements

89% of growers reported utilizing at least one of the native pollinator enhancements listed on the survey. The most commonly used enhancements were, alternating pesticide use (73.1% of respondents), leaving deadwood standing (68.3%), and avoiding mowing wildflowers (59.7%). Organic and "no spray" growers tended to use more of these enhancements (P = 0.011).

Identification and monitoring

Growers felt it would be easier to identify native pollinators than it would be to monitor native pollinator populations, but in neither case did a majority see these as difficult (Figs. 2 and 3). Growers who stated that they already do some identification of native pollinators were significantly more likely to respond that it would be *somewhat easy* or *very easy* to identify native pollinators (P = 0.001), so the perceived ease of this task may increase with experience.

Concerns

Growers were asked to list or describe their main concerns about pollination. The most common responses were weather (29% of respondents), bee health (24%), bee quality (24%), fruit set or yield (22%), and cost (16%).

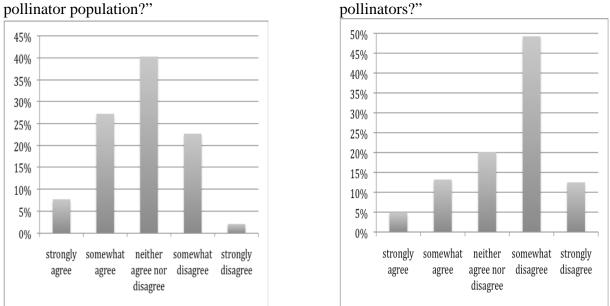


Fig. 3. Responses to "Do you feel it would

be difficult to *identify* your native

Fig. 2. Response to "Do you feel it would be easy to *monitor the size* of your native pollinator population?"

CONCLUSIONS: These results indicate that perceptions of effectiveness are unlikely to be an obstacle to greater use of native pollinators. This does not imply native pollinators are seen as a viable replacement, and "greater use" may simply mean employing habitat enhancement strategies such as bee nesting boxes. The same is true for perceptions on habitat, monitoring, and identification. Current widespread use of habitat enhancement strategies corroborates these findings.

Study 9. <u>Pollinator Project Annual Report: Farm Economics. 2012.</u> <u>Report from Dr. Aaron Hoshide (Adjunct Assistant Professor / Faculty Associate School of Economics)</u>

As honeybee hive prices increase as U.S. supply of hives decline due to Colony Collapse Disorder (CCD), specialty crop producers and researchers in the Northeast are evaluating alternative pollination options that rely on native bees. In wild blueberries, cranberries, apples, and squash these options require providing forage and habitat for native bees. The current demand of honeybee hives by Maine blueberry producers is not very responsive to escalating prices so adoption of alternative pollinators such as managed bumble bees and creating environments more supportive of native bees has not been widely adopted. However, some Maine blueberry growers have been experimenting with providing forage and habitat for native bees by either 1) letting marginal areas revert back to native perennial flowering plants or 2) tilling up areas to plant pollinator wildflower and/or clover pasture mixes. The efficacy and economics of both strategies are being evaluated to determine which native bee forage enhancement strategy is more favorable under different production scenarios and scales.

METHODS: A pollinator practices, demand, and valuation survey was designed, pre-tested, and implemented by Pollinator Project team members at The University of Maine and The University of Massachusetts. Pre-testing with blueberry growers was conducted in the spring of 2012. The survey was administered to blueberry growers predominantly from Downeast Maine (n=48) and Canada (n=10) on 18 Jul at the Blueberry Research Farm in Jonesboro, Maine. To increase the number of observations for U.S. wild blueberry growers from other areas in Maine, twenty additional growers were surveyed on-farm or on the phone between 23 Jul and 3 Aug to bring the total number of U.S. surveys to n=68. Although the number of observations was lower than a previous mail survey, farm size and type (traditional, no spray, organic) were similar. Survey data were entered into SPSS for statistical analysis. То determine Maine blueberry growers' responsiveness to the rising price of honeybee hives, a honeybee hive demand elasticity analysis was conducted (n=40) for surveyed growers currently in the market for hives. Growers' hypothetical consumption of hives was surveyed for a range of price level scenarios (\$40, \$120, \$200, \$280). Budgets for pollinator pasture strips were created in Excel based on research plot data.

RESULTS AND DISCUSSION: The demand elasticity for honeybee hives was found to be inelastic with an estimated elasticity coefficient of 0.0107. This confirms that honeybee hives are a necessity to many blueberry growers in Maine for commercial production due to relatively few substitutes (i.e. bumble bee quads) and constituting a manageable proportion of both costs and farm income, at least for now. Demand elasticity is defined as the responsiveness of changes in quantity demanded to changes in price. Elasticity results suggest that Maine blueberry growers would only cut hives 1.07% even if hive prices increased 100%. Due to this extremely inelastic demand, the total revenue maximizing price for hives that bee keepers should theoretically charge was found to be higher than the highest price level surveyed (\$280/hive). Like gasoline, honeybee hives have the potential for price gouging. This may be mitigated with credible shifts to substitutes if hive prices get too high.

Key considerations for shifting to such substitutes for managed honeybee hives are both effectiveness and cost (opportunity and direct). While bumble bee quads were used by 26% of surveyed producers, only 10% relied exclusively on quads for managed pollination. Bumble bees have been shown to be very efficient pollinators. For example, one bumble bee quad (800-1000 foraging pollinators) has comparable pollination efficiency to four honeybee hives (200,000-240,000 bees of which 48,000-60,000 are foragers). This is based on current University of Maine Cooperative Extension recommended stocking densities. In our survey, the average price paid for bumble bee quads (\$252) is about two-thirds the average price paid for four honeybee hives (\$96 x 4 = \$384). While bumble bees are efficient pollinators and are relatively cheaper to stock for pollination, many Maine blueberry growers are not used to quads or have had bad experiences with bumble bees. This has prevented growers from directly substituting into quads. However, this may change as honeybee hives become increasingly expensive due to more frequent splitting and starting of new hives due to CCD.

For Maine wild blueberry growers, bumble bees are a direct substitute for honeybees. However, growers not renting bees consider native pollinators a direct substitute for hives while for growers renting bees, natives are viewed at best as a favorable "insurance policy." About 35% of growers in our survey rely exclusively on native bees and do not rent honeybee hives or bumble bee quads. For organic growers, this is a higher percentage (73%) where 8 of the 11 organic growers surveyed rely exclusively on native bees for pollination. Growers (n=24) that

rely on native pollinators to completely substitute for rented hives were willing to pay \$55 an acre on habitat and forage modifications that would ensure native pollinators would adequately pollinate 100% of their crop. They also thought that native bees contributed to 61% of fruit set. Growers who rent bees (n=44) were willing to pay only \$39 an acre for native pollinator enhancement and felt native bees only contributed to 29% of fruit set. Growers that rent bees felt that their fruit set would decline by 37% if they did not rent hives/quads. This background native pollination would not be productively acceptable and would be viewed as insurance (37% percent fruit set reduction while bad is better than no fruit set).

An essential consideration when it comes to native bee habitat and forage enhancement by growers is not only the direct cost of such enhancements but their opportunity cost as measured in management time. In other words, many growers spend endless hours on crop management and harvest and have limited time to devote to such native bee enhancements. For example, 63.3% of surveyed growers enhance native bee habitat by leaving standing deadwood (relatively low opportunity cost for "passive" management) compared to only 19.1% who hang leaf-cutting bee nest boxes or provide bumble bee nests (relatively higher opportunity cost for more "active" management). It is similarly not surprising that a higher percentage of surveyed producers "passively" avoid mowing wildflowers (54.4%) compared to those that "actively" plant wildflower bee meadows (17.6%). Planting bee meadows requires more management time in addition to land availability and row crop equipment, seed, and fuel.

In addition to lower opportunity costs, there are also lower direct costs of letting marginal areas on the farm revert back to native perennial flowering plants compared to tilling up areas to plant pollinator wildflower and/or clover pasture mixes. The annual cost of letting sub-prime production areas on the farm revert back to native wildflowers with annual mowing is \$184 per acre of pollinator forage. If areas are not mowed, costs are just the fixed cost of the land plus taxes and insurance estimated at \$98 per acre. If the land is already paid off, then the annual cost is just taxes and insurance at \$48 per acre (Table 1). These annual costs would be higher if land has to be cleared to create areas for pollinator forage as a cooperating producer had to recently do. Several Maine blueberry producers surveyed have historically left areas to revert back to early succession of perennial plants and wildflowers. If the blueberry cash crop has to cover the cost of native pollinator enhancement, such a lower cost and more "passive" management approach is about one-third to one-sixth the cost of bee pasture strips that are more "actively" tilled and seeded (Table 2).

Although there is currently Federal financial support for farmers installing native bee pasture strips, it is practical to assume that this subsidization will not continue indefinitely. There are also limitations to the extent that Maine blueberry growers will be able to cover the costs of native pollinator enhancements by cutting managed bee hives. This is especially true for traditional growers that do not rely exclusively on native bees and that are more input intensive seeking to maximize yields by using hives, controlling pH, and using chemical herbicides, fertilizers, and pesticides. While some traditional blueberry growers in areas of more diversified habitat account for higher native bee populations by cutting hive density to two to three hives per acre, others in areas of less diversified habitat follow or exceed the UMaine Extension recommendation of four hives per acre. Due to such limitations, many growers have to rely on the blueberry cash crop to cover the cost of pollinator pastures and habitat improvements. A higher ratio of blueberry acreage to pasture strip lowers the per acre cost of these pasture strips that has to be covered by the blueberry cash crop.

	Untilled		- Tilled Pasture St	rip Cost (\$/a	acre)
Annual	Pasture Strip		Life $= 5$ years	± ,	ife $= 3$ years
Production Costs	Cost (\$/acre)	Clover	Wildflower	Clover	Wildflower
Variable Costs					
Lime	\$0	\$135	\$135	\$225	\$225
Seed	\$0	\$28	\$87	\$47	\$145
Labor	\$35	\$237	\$246	\$380	\$395
Fuel & Lube	\$4	\$40	\$40	\$57	\$57
Maintenance	\$25	\$27	\$27	\$27	\$27
Equip. Rental	\$0	\$15	\$15	\$24	\$24
Total Variable Costs	\$64	\$482	\$550	\$760	\$87 <i>3</i>
Fixed Costs					
Depreciation					
Structures	\$4	\$4	\$4	\$4	\$4
Equipment	\$2	\$37	\$37	\$37	\$37
Tractor/ATV	\$16	\$16	\$16	\$16	\$16
Land	\$50	\$50	\$50	\$50	\$50
Taxes & Insurance	\$48	\$48	\$48	\$48	\$48
Total Fixed Costs	\$120	\$155	\$155	\$155	\$155
TOTAL COSTS	\$184	\$637	\$705	\$915	\$1,028

Table 1. Annual production costs for native bee pasture strips for 3-year and 5-year stand life.^a

^a Production cost line items rounded to sum correctly.

Table 2. Annual cost per acre of native bee pasture strips covered by wild blueberry cash crop for 3-year and 5-year stand life assuming different ratio of blueberries to pasture strip.^a

Ratio of Blueberry	Untilled	Tilled Pasture Strip Cost (\$/acre)			
(BB) Acres to	Pasture Strip	Stand Life $= 5$ yrs.		Stand Life = 5 yrs. Stand Life =	
Pasture Strip Acres	Cost (\$/acre)	Clover	Wildflower	Clover	Wildflower
BB 1: Pasture Strip 1	\$184	\$637	\$705	\$915	\$1,028
BB 5: Pasture Strip 1	\$37	\$127	\$141	\$183	\$206
BB 10: Pasture Strip 1	\$18	\$64	\$70	\$92	\$103
BB 20: Pasture Strip 1	\$9	\$32	\$35	\$46	\$51

^a Production costs per acre rounded.

Tilled pasture strips being trialed at University of Maine research farms and at two cooperating farms involve tilling up areas to seed down perennial mixes of clover or wildflowers. It is assumed that the stand life will range from three to five years before the pasture strip has to be re-established. The total cost of establishing these strips is higher for a lower stand life since most of the variable costs of establishment such as lime, seed, fuel and lube, and equipment rental are annualized over a smaller number of years (Table 1). If one acre of tilled pasture strip is required for each acre of blueberry, the costs are prohibitive since the costs per acre that have to be covered by blueberries (\$637 to \$1,028) exceed or come close to exceeding the net

profits per acre for blueberry enterprise budgets provided by the industry assuming yields of 3,000 lb and 6,000 lb per acre. Native bee pasture establishment costs per acre are more manageable if the ratio of blueberries to tilled pasture strip is closer to 10:1 or 20:1 (Table 2). At this 20:1 ratio, the cost per acre covered by blueberries (\$32 to \$51/acre) for tilled pasture strip matches the surveyed responses of willingness to pay (WTP) for native bee habitat enhancement (\$39 to \$55/acre). For untilled passively-managed strips, the ratio of blueberries to strip can be as low as 4:1 to match such grower WTP.

FUTURE RESEARCH: While the direct and opportunity costs of untilled pasture strips are lower than tilled, the relative efficacy of supporting native bees needs to be evaluated. Tilled pasture strips may provide a higher density of floral resources over a more extended season. If this is the case then the additional costs may be worth the positive impacts on native bee populations and subsequently improvements in blueberry fruit set and yields. However if the benefits of tilled pasture strips are marginally better or similar to untilled strips, the higher costs and time associated with getting perennial crop producers to adopt semi-annual cropping may be prohibitive to adoption. Additionally, the optimal ratio of blueberries to pasture strip needs to be determined for blueberry production areas in Maine that have more and less diversified native bee habitat and forage. Growers would then be able to balance the effectiveness of using enough pasture strips with their cost as covered by the blueberry crop enterprise. Current economic analyses assume a capital bundle for a small to medium size farm with one acre of pasture strip. Future analyses will evaluate managing pasture strips at more efficient scale, as well as the added costs of land clearing for growers with limited open areas for these strips. Similar farm economics need to be conducted for different whole-farm combinations of pasture strips and blueberries for traditional, low-input, and organic farms at different sizes (small, medium, large) in addition to cranberries, apples, and squash in the Northeastern U.S.

Study 10. <u>Dung Beetles' (Coleoptera: Scarabaeidae) potential to suppress pathogenic E. coli</u> 0157:H7 in the agricultural arena: a laboratory study. <u>Report from Matthew Jones (Master's Candidate) and Dr. Frank Drummond.</u>

METHODS: During the summer of 2012, the ecological role of a generalist dung beetle species, *Onthophagus hecate*, was explored as a potential natural biological control agent of feces-borne pathogens, and alternatively as a pathogen vector between feces and food. Understanding the ecological role of dung beetles in agriculture has strong implications for any system in which food crops are at risk of wildlife fecal contaminations. We conducted a laboratory study to elucidate dung beetle feeding ecology as it relates to suppression and/or transmission of *E. coli* 0157:H7 from white tailed deer feces to pre-harvest lowbush blueberry fruit. Dung beetles were fed white tailed deer scat, intentionally inoculated with *E. coli* 0157:H7, within a microcosm including fruiting lowbush blueberry plants. In addition, independent control treatments including non-inoculated scat as well as inoculated scat sans beetles allowed for the comparison of all variables with one another. Beetles were allowed 10 days to feed on/bury scat, after which the harvestable fruit, soil, and feces were tested for the pathogen of concern.

RESULTS AND DISCUSSION: Data indicate that beetles buried the same percentage of feces whether or not it was inoculated with the pathogen ($F_{(1,6)} < 0.0001$; P = 0.999; Fig. 1). Beetles were found to vector NO amount of the pathogen to the fruit. Lastly, dung beetles

lowered the amount of pathogenic *E. coli* persisting in the soil (Fig. 2). Data suggest that the generalist dung beetle species, *Onthophagus hecate*, when present in agroecosystems, has the potential to suppress *E. coli* 0157:H7.

Fig. 1. ANOVA comparing the % feces removal based on whether or not feces were inoculated with pathogenic *E. coli* 0157: H7, or not. NSS ($F_{(1,6)} = 0.00$; P = 0.99).

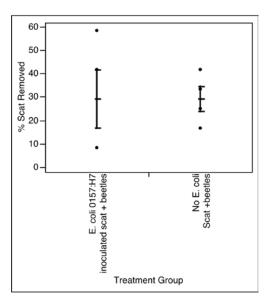
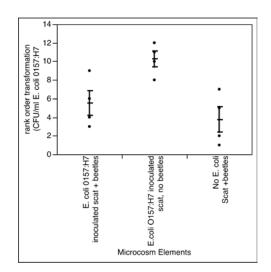


Fig. 2. ANOVA comparing bacteria (CFU/ml, values rank transformed) from soil samples subjected to different treatment combinations of beetles, and deer feces. Soil from microcosms including dung beetles had significantly fewer colonies of *E. coli* 0157:H7 than soil from microcosms without beetles ($F_{(2,9)} = 7.76$; P = 0.05). Comparison of *E. coli* inoculated scat + beetles and No *E. coli* scat + beetles was NSS ($F_{(2,9)} = 7.76$; P = 0.58). Soil from *E. coli* inoculated scat and NO beetles had significantly greater CFU, than the No *E. coli* + beetles treatment ($F_{(2,9)} = 7.75$; P = 0.01).



CONCLUSIONS: Preliminarily, dung beetles are important to be aware of. In the future, growers may consider conservation management practiced to heighten dung scavenging activity in their fields, as they seem to be offering a very beneficial ecosystem service. More research, specifically field research, must be done to confirm these preliminary data. As food safety regulations become tighter, knowing whether or not specific farms foster an abundance of dung beetles may have implications on how confident a grower (or processor) could be in relying on their ecosystem services.

Study 11. <u>Risk analysis of wildlife scat within the Maine lowbush blueberry agroecosystem;</u> <u>is Escherichia coli 0157:H7 a food safety concern here?</u> <u>Report from Matthew Jones (Master's Candidate) and Dr. Frank Drummond</u>

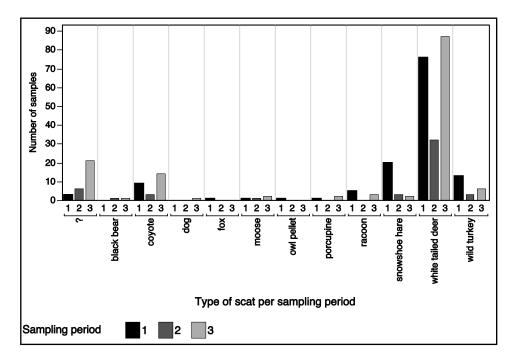
Wild and domestic animal feces are a threat to agricultural production of safe food. In the recent decade several incidence have occurred in the U.S. that have resulted in contamination of the U.S. food supply of the pathogen, *Escherichia coli* 0157:H7. This pathogen is commonly found with animal feces and can be transmitted to crops by physical association. We initiated a study to determine how common this threat might be in wild blueberry.

METHODS: Twelve fields were sampled 3 times for wildlife feces in 2012 (March/April, June, and September). Field edges, randomly assigned "W transects" within fields (Wheater, Bell, & Cook, 2011), and any obvious wildlife paths leading into adjacent forests (approx. 50 meters in length) were observed in search of wildlife fecal material. All scats found, both old and fresh, were collected and stored using sterile methods. Detailed, scaled photos of each scat were taken prior to collection to aid in scat identification.

In the lab, scat samples were identified using photos and field guides. Each sample was then weighed. Currently, microbial analysis is in progress in order to determine which, if any, samples are contaminated with *E. coli* 0157:H7.

RESULTS AND DISCUSSION: In total, 318 wildlife feces samples were collected. During the spring, summer, and fall 130, 49, and 139 samples were found, respectively. Sixty-one percent of the samples (n=195) were identified as white tailed deer feces. Figure 1 shows a full break down of the samples, including origin and sample period during which they were collected. Thirty samples remained unidentifiable. Samples could only be collected within the blueberry field itself during the spring collection period (period 1), as plants leaf out making it impossible to observe the ground beneath the canopy. No data is yet available regarding microbial or biochemical analyses.

Fig. 1. Preliminary results regarding wildlife feces samples collected seasonally during; (1=spring, 2=summer, 3=fall)



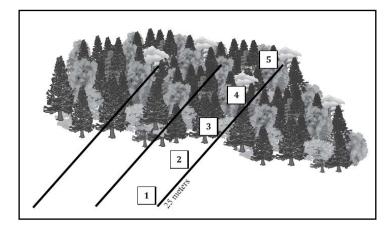
<u>Literature Cited</u>

Wheater, P., J. Bell, and P. Cook. 2011. *Practical Field Ecology: a Project Guide*. West Sussex: Wiley-Blackwell.

Study 12. <u>The effects of forest edge habitat and field management on select insect-mediated</u> <u>ecosystem services within the Maine lowbush blueberry agroecosystem.</u> <u>Report from Matthew S. Jones (Master's Candidate) and Dr. Frank Drummond.</u>

METHODS: Predators and scavengers can play a vital role in regulating pest insect and weed populations and are especially important to organic agricultural production. Additionally, they have the ability to degrade vertebrate feces at high rates. From a food safety standpoint, it is also becoming increasingly important to examine the role of coprophagous (feces eating) insects in the agricultural arena. The objectives of this study were to quantify how different pest management strategies and surrounding forest composition interact to drive insect predator and scavenger mediated ecosystem services in the Maine lowbush blueberry ecosystem. Sentinel substrates (insect pupae, weed seeds, and deer scat) were used to explore resource removal in 12 fields and surrounding forest habitat. Figure 1 shows the field site set-up. In addition to the removal rate study, pitfall trapping and time-lapse, macro-video footage were used to better understand the composition of the arthropod community responsible for providing these services.

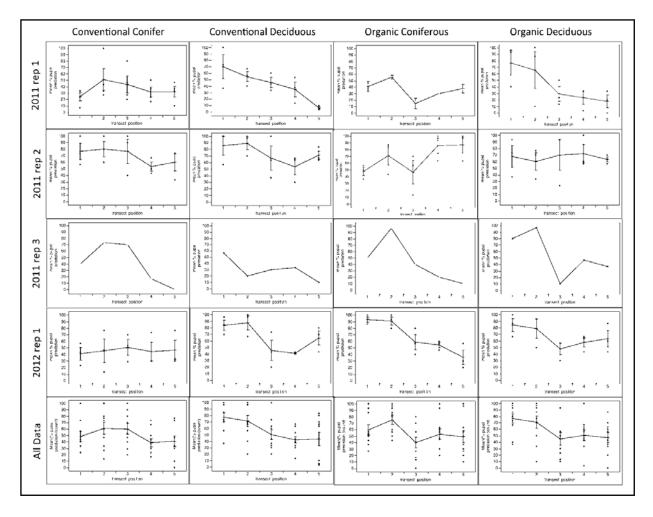
Fig. 1. Field site with 3, 100 m transects spanning blueberry field/forest interface. Each contains 5 bait stations, spaced 25 m apart.



RESULTS AND DISCUSSION: A split-split plot ANOVA model was used to understand the predation and scavenging data. Experiment-wide, within exclosures, 47.8% of insect pupae were removed while outside exclosures 55.0% were removed. This indicates that while vertebrates are providing some ecosystem services, invertebrates are the main drivers. A significant "transect position" effect ($F_{(4,76)} = 16.36$, P < 0.001) is driven by the distinct pattern seen in insect predation where, consistently, there are higher predation values within fields than at the field/forest interface and within forests. Independent of treatment combination and cage treatment, removal values for each transect position are as follows: position 1(field center) = 62.2%, position 2 = 67.4%, position 3 = 45.4%, position 4 = 41.1%, position 5 (within forest) = 41.0%.

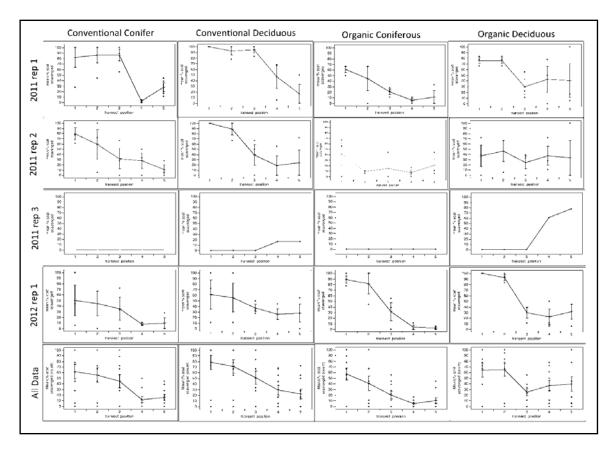
Throughout both years of the study, predation of insects across transects, in all treatment combinations is being driven by arthropods; however, periodically, vertebrates are contributing significantly to predation services. The most pronounced trend seen through the insect predation study was the location in the field. Predation was nearly always significantly higher in the two field locations than at the forest edge or within the forest. This is directly contradictory to the findings of other current literature regarding edge-effects on insect recolonization. In general it is understood that insect diversity and abundance is highest, and insect mediated ecosystem services are most prominent around field perimeters. Additionally, field treatment combinations were shown to interact significantly with transect position in multiple repetitions indicating that the composition of the surrounding forests AND the way a field is managed can work synergistically to determine where, relative the field's edge, the most insect predation can be expected to occur. Refer to Figure 2 for presentation of entire study's scavenging trends. Experiment-wide, there were low levels of seed predation with no obvious trends to expound on.

Fig. 2. Relative predation of insect pupae (*Musca domestica*) relative to management type/forest edge treatment combinations and field location (1=field interior, 5=forest interior). Data displayed are from outside exclosures indicating total predation.



When our model was run on the scavenging data from 2011 and 2012, the was a significant transect position effect ($F_{(4,76)} = 20.83$, P = 0.00) which can be explained by the severe difference in scavenging values occurring within the field as opposed to on the field edge or within adjacent forests. Removal rates, experiment-wide, independent of treatment combination and cage variables are as follows: position 1 = 61.6%, position 2 = 57.8%, position 3 = 33.4%, position 4 = 18.5%, position 5 = 21.2%. There was also a treatment combination effect ($F_{(3,19)} = 3.553$, P = 0.034) which is driven by higher amounts of scavenging in fields surrounded by deciduous forest edges (45.6 % removal) than in fields with coniferous edges (31.3 % removal), independent of "management type," field location, and a "cage" treatment. Refer to Figure 3 for presentation of entire study's scavenging trends.

Fig. 3. Relative scavenging of white-tailed deer (*Odocoileus virginianus*) scat relative to management type/forest edge treatment combinations and field location (1=field interior, 5=forest interior). Data displayed are from outside exclosures indicating total scavenging.



Pitfall trapping and motion sensing video footage (Fig. 4) indicated ants, crickets, and ground beetles to be the main predators of insect pupae (Fig. 5) while dung beetles predominantly fed on wildlife feces.

Fig. 4. Modified motion sensing/time lapse camera and pictures taken (dung beetle burying/moving deer scat circled in white)



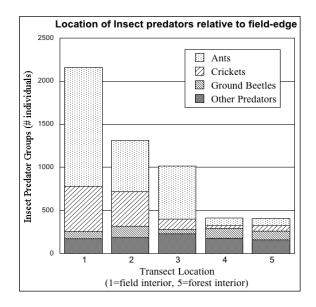


Fig. 5. Insect predator group composition along field/forest interface transects.

CONCLUSIONS: Blueberry fields are highly active environments for insects independent of whether a field is organic or conventional in management. We did find very low seed predation compared to predation of insect pupae. A major seed predator in agricultural systems, the ground beetle, *Harpalus rufipes*, can be found in fairly high abundance in blueberry fields, but their impact appears to be minimal. Insect predators which lower densities of blueberry insect pests and dung beetles which lower the incidence of scat in blueberry fields appear to be influenced by the composition of the forest edge. These two beneficial insect communities are also in higher abundance in the interior of the blueberry field than in the forest habitat surrounding blueberry fields.

Study 13. Landscape ecology of Maine blueberry pollinators.

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

A large body of work connecting pollinators to the landscape exists, but there are gaps in knowledge that could be filled. The findings have been summarized in a concept map that shows just how interconnected this line of research is (Fig. 1). The basic dissertation concept aims to examine pollinator/landscape interactions for most, perhaps all, of the focal crops in this study, with focus on the effects of landscape pattern. We would specifically like to address any effects of linear features (roadsides, power lines) on native pollinators in Maine.

Working dissertation concept: Effects of landscape pattern (emphasizing shape and arrangement) on native pollinators

Ideas for further exploration:

1) Translate pollinator community and forage plant field-based sample data into landscape models.

- Assess relationship of shape of forage planting areas (e.g., linear vs. circular) and pollinator abundance for various crops
- Assess relationship of plant species composition and arrangement/shape of forage planting areas on pollinator abundance (expanding on mixes used in current field trials) for various crops
- Examine shape and composition of forage plantings and crops in MA and CT: examine landscapes along a gradient of crop isolation and patterns (e.g., patch size, shape, composition arrangements; context)

2) Compare context and landscape structure of different crop systems and locations (ME, CT, MA, and NY).

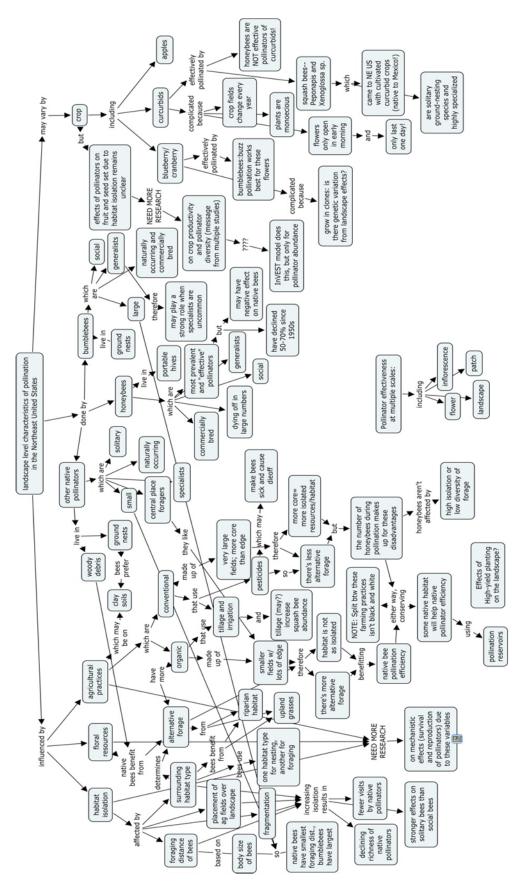
- Describe and compare landscape structure and composition surrounding blueberries in ME, cranberries in MA, squash in CT; apples in NY
 - If NY has spatial info, could translate it to ME's apple orchards for comparison
- Apply InVEST model to cranberries, squash/pumpkins, and apples in Maine and these crops and blueberries in CT, NY, MA to assess relationship of landscape structure and composition to pollinator abundance ; narrow this approach based on pollinator abundance data from each crop and state; target particular landscape arrangement/composition/pattern in each state/crop

3) Value of linear habitat elements (power lines, roadsides, and right of ways) for Maine's native pollinators.

- May provide connectivity and alternative forage between patches of a specific crop
- Assess shape of patches and connectors—may be a network of linear features between scattered patches
- May need to do field work to assess bee communities in specially selected power line or roadside connectors
- Combine field assessment with InVest model manipulation and neutral landscape models to examine effects of linear elements on pollinator abundance (complements item #1 above)

4) Landscape effects on diversity of pollinator communities.

- Pollinator diversity decreases as you move north
- There may be more short-distance foraging pollinators in northern landscapes
- Patchiness may be beneficial
- These factors may be part of assessment of landscapes examined in item #2 above





Study 14. <u>Growing wildflowers for native bee habitat: Effects of mowing, a nurse crop, and</u> <u>a fescue on successful wildflower establishment adjacent to lowbush blueberry.</u> <u>Report from Eric Venturini (Master's Candidate) and Dr. Frank Drummond.</u>

METHODS: Study plots are adjacent to a conventionally managed lowbush blueberry field (Vaccinium angustifolium) at Blueberry Hill Farm, the University of Maine's research farm in Jonesboro, ME. Blueberry Hill Farm grows several acres of lowbush blueberries for research purposes and commercial sale. To achieve a pH of 6.0 from a starting pH of 4.7, on 18 May, 2012 we applied lime to our plots at a rate of 7000 lbs/acre using a Gandy[®] T36 drop spreader and tilled the soil to a depth of about 3 inches using a 6 foot wide Cutter[®] 2 tiller. Two full passes with the tiller resulted in a reasonably smooth surface. After waiting approximately 2 weeks to allow weed seed germination we tilled again on 31 May to a depth of 3 inches. On 8 Jul we raked the plots smooth and broadcast seeded using an Earthway[®] hand crank seeder. Seeds were bulked with vermiculite and well mixed before and during broadcasting to ensure an even distribution of seeds. Immediately after all seeds were broadcasted the entire plot was rolled with a 5 ft wide pull-behind 2,600 lb compacter. The entire study site was irrigated so that in total, each plot received 1 inch of rain per week. If rain fell equal to or in excess of 1 inch per week in a given week, no additional irrigation was applied. Each block (4 total) contains 5 treatments with an additional split-plot variable. Treatment plots are 4 x 8 m. For a list of treatments see Table 1. For a list of seed, and seeding rates see Table 2.

On 13 - 14 Aug we counted stems and flowers of all seedlings in 4 randomly selected 30 cm² quadrats per sub-plot for a total of 40 sub-plots sampled. We considered only the central square meter of each sub-plot when randomly placing the 30 cm² quadrats in order to minimize any edge effects. The same 30 cm² area was never counted twice. Specimens of each herbaceous plant recorded were collected and identified to the level possible. Many of the perennials were still seedlings and so were impossible to identify to species, instead we identified them to the genus or family level. For analysis, grasses and sedges were grouped as graminoids. After the sampling, treatments 2 and 4 were mowed in all blocks.

RESULTS: Overall establishment looked very good. Very few flowers were counted during the August sampling but by early September the annuals *C. tinctoria*, *H. annuus*, and *G. pulchella* were in full bloom. Preliminary analysis of the 2012 season results indicate no significant differences between treatments (Fig. 1). Counts of weed, graminoid, and wildflower density were all very similar between plots. We were unable to accurately identify sheep fescue during the sampling. This may be due to low germination rates or because the seedlings were too young to identify.

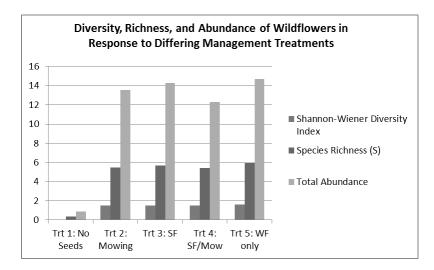
If differences exist between treatments, 2013 survey data will have a greater chance of detecting them. The effects of mowing and the diversity of the perennials will be more evident with time. Weed pressure at the site was low and mowing was not needed until August. Therefore, no mowing was done in 2012 until after the first survey was completed. Possible problems with treatments include poor fescue establishment and a low oat seeding rate. Oat germination rates also differed widely between sub-plots.

Treatment 1	Control, No wildflower seed
Treatment 2	Mowed in August 2012 to a height of 12 inches
Treatment 3	Over-seeded with sheep fescue as a weed suppressant
Treatment 4	Mowed (same as Trt. 2) x sheep fescue (same as Trt 3)
Treatment 5	No treatment, only sown with wildflower seeds
Split-plot treatment	Over-seeded with nurse crop of oats, 10 lbs/acre

Table 1. Establishment study treatments.

		No. live seeds/ sq	No. live seeds per
Common Name	Species	foot	acre
Annuals			
Plains Coreopsis	Coreopsis tinctoria	9.55	416,000
Indian Blanket	Gaillardia pulchella	7.66	333,600
Sunflower	Helianthus annuus	2.75	120,000
	Totals	19.96	869,600
Perennials			
Lavender Hyssop	Agastache foeniculum	5.29	230,400
Lance-Lvd. Coreoopsis	Coreopsis lanceolata	5.79	252,000
Canada Tick Trefoil	Desmodium canadense	1.82	79,200
Purple Coneflower	Echinacea purpurea	4.69	204,400
Common Boneset	Eupatorium perfoliatum	3.53	153,600
Bergamot	Monarda fistulosa	4.01	174,720
New-England Aster	Symphyotrichum novae-angliae	4.52	196,800
	Totals	29.64	1,291,120
Nurse Crop and Weed Suppression	I	lbs/acre	
Sheep Fescue	Festuca ovina	10.00	
Oats	Avena sativa	10.89	

Fig. 1. Shannon-Wiener diversity index, species richness, and total abundance of sown wildflowers in five treatments with different management strategies.



Study 15. <u>Enhancing native pollinators in Maine's lowbush blueberry fields.</u> <u>Report from Eric Venturini (Master's Candidate) and Dr. Frank Drummond</u>

METHODS:

<u>Experimental Design</u>

Pollinator plantings were established in the summer of 2012 at four sites in Maine to assess any influence on native pollination services over time. Three of four sites are commercial blueberry operations with several acres of harvestable blueberries each year. Roger's farm (ROGE), a University of Maine research farm is mostly wheat, corn and mixed vegetables. Treatment sites are located in Old Town, Jonesboro, and Blue Hill. We paired each of the three commercial blueberry treatment sites with a nearby control field. ROGE has no control field. Control fields were selected on the basis of proximity to treatment sites, similarity of management, and willingness of the grower to participate. At all sites except ROGE, we sampled fruit set by flower and berry counts, yield by stratified sampling, and visitation to blueberry blossoms by 1-minute observations of 15, 1 meter quadrats per field. At all sites we measured bee diversity by fatally sampling and in two sampling periods per field also collected 15 bumble bees carrying pollen. The bumble bees (*Bombus spp.*) were collected to assess percent composition of pollen loads. The numbers of honeybee and/or *Bombus* quads stocked in fields was also noted.

At each of the four treatment sites we maintained plots with three different treatments: natural regeneration, clovers, and wildflowers. For species composition and seeding rates of wildflower and clover treatments see Table 1. Both the clover and the wildflower mixes were planted with a nurse crop of oats, *Avena sativa*. The natural regeneration treatment was prepared with lime and tilling but no seeds were sown. Each treatment measures 3.33×50 m. At all sites but one, total plot area is 500 m^2 .

Establishing Plantings

Between 18 May and 1 Jun 2012, we prepared seed beds for plantings at all treatment sites. Soil tests run at the University of Maine's soil laboratory revealed pH levels at commercial blueberry sites between pH 4.5 and 5.4. Lime was applied at all sites between 18 May and 1 Jun 2012 at the recommended rates using a Gandy[®] T36 drop spreader to achieve a pH of 6.0. On the same date at each site we tilled plots to a depth less than or equal to 3 inches using a 6 foot wide Cutter[®] 2 tiller. Plots at ROGE had a pH of 6.1 and required no lime. To "stale seed bed" the plots, we returned to each site between 31 May and 20 Jun 2012, about 2 weeks after the initial tilling. After the second tilling, we raked the beds smooth, and broadcasted seeds using an Earthway[®] hand crank seeder. We bulked clover and wildflowers and a 14:1 ratio for clovers. Oats were seeded by hand without a bulking agent. On the same day that seeds were broadcast, we compacted each site with a weighted roller. Final planting dates ranged from 8 to 20 Jun 2012.

During dry periods plots were occasionally watered using a hose with one exception. Plots at BBHF were drier than the other sites so we set up an irrigation system from late Jul to the end of Aug. BBHF was irrigated at a rate of about 1 inch per week. Plots at all sites were cut with a weed-whacker when weeds were significantly higher than seedlings. The frequency of weed whacking differed between sites due to differing types and amounts of weed pressure.

Alternative Forage

From May through early Sep 2012 at all treatment sites, trained observers measured the abundance and native bee visitation to alternative forage. Two observers visited each field weekly to bi-weekly and independently walked in and around the field for 30 minutes recording estimated patch sizes of all plants in flower. These 30 minutes were spent at a brisk walk with the intent of recording as many of the blooming plants in the field as possible. We selected the top three plants per site in terms of in-field subjective estimates of the relative floral abundance, density, and bloom period. Three, 1-minute observations of pollinator visitation per top three flowering plants were conducted in 1 m quadrats intentionally placed in the densest flower patch. Within each quadrat, we recorded pollinator visitation/minute, floral density by nearest neighbor, bloom diameter, corolla tube length, and minimum and maximum flower heights. Bee observations were only made when the following conditions were met: temperatures greater than 10 C°, wind speed less than 4.5 m/s, no rain, and time between the hours of 0930 and 1600 hours.

RESULTS:

Pollinator Plantings

Overall, wildflower establishment was very successful. By August of 2012, many of the eight perennial plants seeded were identifiable in the understory of the three flowering annuals, *Coreopsis tinctoria, Gaillardia pulchella,* and *Helianthus annuus*. At two out of four treatment sites the bloom of these three annuals was prolific with plants often up to 1 meter in height. First year blooming annuals at the other two sites were scattered, probably due to the combination of weediness and increased frequency of mowing required for controlling weeds at these two sites. We suspect that in the summer of 2013 many if not all of the perennials will start to flower at all four sites.

Any effects that the pollinator plantings may have on native bee communities and pollination services will only be evident over time. Plantings were seeded in June and will require several generations of native bees using the new floral resources before possible increases in bee abundance and/or diversity can be measured.

Alternative Forage

From May to Aug, 2012 we made a total of 389, one minute observations of 67 alternative forage plant species. During these observations we recorded 817 insect visits including 273 solitary bees, 181 honeybees, and 90 bumble bees. The 67 flowering plants were grouped into 43 categories. Plants are grouped by species or genus, or where this resolution was not possible, to family.

For increased power, bees were grouped into *Apis mellifera*, solitary bees, and *Bombus* spp. Within sites functional bee groups exhibited different foraging patterns although some overlap is present. Plant groups that ranked in the top three for visitation rates across multiple bee groups were *Rumex acetosella*, *Rubus* spp., *Prunus* spp., *Solidago* spp., *Taraxacum offinalis*, *Galium* spp., *Trifolium repens*, and *Cirsium* spp. Twelve other plant groups ranked in the top three for only one functional bee group.

To assess bees' true preferences for flowers while accounting for differing amounts of each flower group in the landscape, we calculated a visitation/floral abundance index of all native bees. Both native bee visitation and floral abundance scores were indexed by dividing visitation rates (or abundance scores) per flower by the highest visitation rate (or abundance

score) recorded for all flowers in a given site (Fig. 2). We then divided the resulting indices (visitation index/abundance index) to get a value that provides an indication of true preference. If a plant scores > 1 its visitation rate is proportionally higher than its abundance, suggesting a relatively strong preference. Inversely, a score < 1 indicates no preference. *Spiraea alba, Hypericum* spp., *Galium* spp., and *Anthemis cotula* scored highest but all of these scored highly only at one site even though all but *Anthemis cotula* were present at every site.

We wanted to explore the relationships between blueberry yield, native bee visitation on alternative forage, and plant richness. Three out of four study sites are blueberry fields; of those, one rents commercial honeybees. We eliminated this field from this comparison because comparing fields with and without honeybees would cloud any relationship between yield, wild bees, and plant richness. Although not significant and based only on two sites, the three factors may be related. Yields increased as both native bee visitation to alternative forage and plant richness increased (Fig. 1).

		No. live	No. live
		seeds/ sq	seeds per
Common Name	Species	foot	acre
Annuals			
Plains Coreopsis	Coreopsis tinctoria	9.55	416,000
Indian Blanket	Gaillardia pulchella	7.66	333,600
Sunflower	Helianthus annuus	2.75	120,000
	Totals	19.96	869,600
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Canada Tick Trefoil	Desmodium canadense	1.82	79,200
Purple Coneflower	Echinacea purpurea	4.69	204,400
Common Boneset	Eupatorium perfoliatum	3.53	153,600
Bergamot	Monarda fistulosa	4.01	174,720
New England Aster	Symphyotrichum novae-		
New-England Aster	angliae	4.52	196,800
	Totals	29.64	1,291,120
Nurse Crop		lbs/acre	
Oats	Avena sativa	10.89	
Clovers		lbs/acre	
Crimson Clover	Trifolium incarnatum	7.00	
Medium Red Clover	Trifolium pratense	5.00	
Sweet White Clover	Melilotus officinalis	6.00	

 Table 1.
 Species planted and seeding rates. Wildflower seeding rates courtesy of Applewood Seed Company.

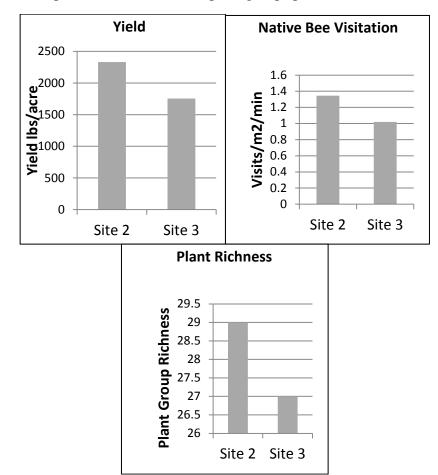
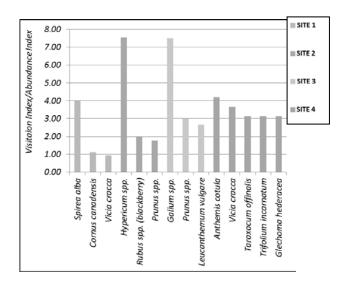


Fig. 1. Blueberry yield as measured by nine, 1 m² samples per site, average visitation by native bees over all flowers per site, and richness of plant groups per site.

Fig. 2. Top three preferred plants by preference index. Visitation/abundance index for native bee preferences. High scores (>1) reflect less abundant flowers and high visitation rates. A measure of true preference.



RECOMMENDATIONS: The results of our beneficial insect and pollinator studies are for the most part preliminary and still underway. This is especially true of the research focused on native pollinators and pollination. A recommendation from our research on honeybee exposure to the fungicide propiconazole is that whenever possible minimizing this exposure is paramount. While the effects of propiconazole have not been shown to have strong negative impacts on colony strength, we have collected evidence that there are negative impacts on larval and adult worker survival. Mummyberry is a serious and devastating disease, but pollination is also a very important part of blueberry production. Therefore, extreme caution should be used in applying fungicides for management of this disease. Our recommendation is that flowers should not be sprayed when the danger of infection does not exist.

Beneficial insects that predate insect pests, weed seeds, and decompose feces of wildlife tend to be abundant in blueberry fields and field edges relative to the surrounding forest habitat. Forest habitats can be a source of recolonization of these beneficial insects, but adoption of insect pest management strategies that reduce unnecessary insecticide applications or promote perimeter applications or spot treatments are recommended. While many of these beneficial insects do not in themselves control pest insects and weeds, they do contribute to reducing their numbers and resulting crop loss in fields. Therefore, whenever these free ecosystem services can be taken advantage of or encouraged, a more sustainable production system should result.

ENTOMOLOGY

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

8. TITLE: VI. Pesticide residues on lowbush blueberry, 2012.

Study 1. <u>Residues of malathion on lowbush blueberry</u>

METHODS: Residues of malathion were determined on lowbush blueberry fruit collected from small research plots and from a full-field commercial application. The purpose of the study was to evaluate the utility of using malathion as a control tactic for the spotted-wing drosophila just prior to harvest.

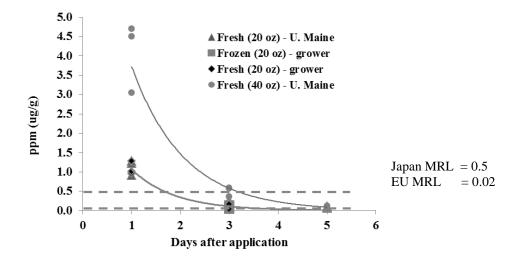
On 13 Aug, Malathion 5 E was applied in 25 gallons of water-mixture per acre with a CO_2 -propelled, 80-inch boom sprayer (76-inch swath) equipped with four, flat-spray 8002VS TeeJet[®] nozzles operating at 35 psi and at a slow walking speed. Speed was regulated using a metronome.

A clean commercial blueberry rake was used to harvest ca.1 lb of fruit for each of four timings (0, 1, 3, and 5 days after the application) and two rates (20 and 40 oz/acre). Two non-treated check samples were also collected. Each fruit sample was washed by agitating for 30 seconds in clean tap water, drained, placed in a zip-lock bag, placed in a cooler with blue ice, and delivered to Dr. L. Brian Perkins at the University of Maine for residue analysis.

A grower in Washington Co. also collected samples for residue analysis following the application of malathion (20 oz/acre) to a commercial blueberry field. Residues were evaluated on both fresh and frozen fruit run through a processing line. Fresh samples were collected 1 and 3 days after the application. Frozen samples were collected 3 days after the application.

RESULTS AND CONCLUSIONS: The results of the two trials are shown in Figure 1. The first thing that is apparent is that the University of Maine small plot results are in-line with the commercial field results and that there is little difference between fresh and frozen fruit residues. The estimated regression lines are based upon an exponential model of insecticide decay in the environment, an accepted model for such estimates. The conclusions from this graph suggest that 3 but better yet 4 days are needed for residues to fall below both the EU and Japanese MRLs at the 20 oz / acre rate of application. The 40 oz/acre rate requires 5 days to be below both the EU and Japanese MRLs; however, MRL levels are constantly changing.

Fig. 1. Malathion (ppm) from residue analysis of small-plot and full-field application.



Study 2. <u>Residues of Belay (clothianidin) insecticide on lowbush blueberries</u>

METHODS: On 14 Jul 2011, Belay insecticide (clothianidin) (4 oz/acre) was applied to a fruit-bearing lowbush blueberry field in 20 gallons of water per acre using a CIMA[®] P55D Atomizer L. V. sprayer mounted on an Agco Allis[®] 6670 tractor. On each sample date a clean, commercial blueberry rake was used to collect two, 1-2 lb samples from the treated area; only one sample was collected on 14 Jul (Table 1). For the 14 Jul sample the material was allowed to dry on the plants prior to collection. Samples were representative of the entire plot and were collected by harvesting in a swath diagonally across the plot. Fruit was air-winnowed into clean, plastic containers, placed in zip-lock bags, and frozen prior to shipment for chemical residue analysis.

Sample ID	Date Collected	Days post application ¹
1	14 Jul	0
2A and 2B	21 Jul	7
3A and 3B	28 Jul	14
4A and 4B	4 Aug	21
5A and 5B	11 Aug	28
6A and 6B	18 Aug	35
UTC (control)	14 Jul	NA
UTC	21 Jul	NA
UTC	18 Aug	NA

 Table 1.
 Sample collection.

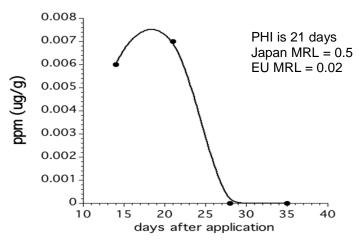
1 Application on 14 Jul.

RESULTS: Table 2 and Figure 1 show the residues of clothianidin on the fruit for 14, 21, 28, and 35 days after application. Residues were below the US (0.01 ppm) and EU (0.02 ppm) MRLs from day 14 through day 35 after application.

Table 2.	Residues	of clothiani	idin post a	pplication.
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	Sample #1	Sample #2	Average
14 DALA* 21 DALA 28 DALA	0.011 ppm 0.006 ppm < 0.005	< 0.005 ppm 0.007 ppm < 0.005	0.006 ppm 0.007 ppm 0
35 DALA Limit of dete * Days after	< 0.005 ction = 0.005 ppm application	< 0.005	0

Fig. 1.	Clothianidin	(ppm) from	residue	analysis.



RECOMMENDATIONS: The label PHI for Belay (clothianidin) is 21 days; however, such a long PHI interval for wild blueberries precludes this insecticide's use close to harvest despite the low residues. Belay might have a niche for use in thrips or tip midge control during the crop year. In 2011 significant honeybee kills resulted from clothianidin used in corn at time of planting. This material is highly toxic to honeybees if the exposure is significant. Unless it can be applied in the crop year in the spring with minimal risk of exposure, we will not test it against spring defoliating pests and focus more on leaf curl pests such as thrips and tip midge.

DISEASE

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

9. TITLE: Maine wild blueberry –mummy berry research and extension.

OBJECTIVE: Implement a fully operational mummy berry disease forecasting system (MBFS).

METHODS: We accomplished our target of setting up eleven weather stations for mummy berry forecasting for the spring of 2012. In late March to early April, the weather stations were deployed in blueberry growers' fields around Maine from Appleton in Knox County to Meddybemps in northern Washington County (Fig. 1). New stations consisted of Watchdog® data loggers and cellular telemetry allowing remote monitoring of air and soil temperature, soil moisture and leaf wetness at 15 minute intervals. In addition, relative humidity was monitored at the sites but this data was collected in monthly downloads. In August and September 2012, we put out new mummy berry plots for the next season and retrieved the weather stations for winter storage. We also sent out information to growers on how to put out their own mummy berry plots for next year.

RESULTS: Due to the delay in obtaining funds, there was no time to test the weather stations before deploying them. We had to troubleshoot how to set up the weather stations and deal with solar heating effects on the air temperature probes and long periods of cloudy weather on solar charging of the batteries. We had one station which had to have a reconfigured cellular antenna to get more consistent signal. The weather stations generally performed well even with these problems and provided timely and accurate data to use for the forecasts.

Six of the stations had mummy berry plots and three of the locations had plots where mummies did not successfully germinate. We had seven 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 early April to mid-May, we were able to provide 20 forecast reports on mummy berry disease, as well as, the occurrence of frost for most of the blueberry growing areas. The forecast reports were provided in messages sent out to an email list, posted on the Wild Blueberry extension blog (http://mainewildblueberries.blogspot.com/) and recorded as answering machine messages. In addition, weather station data was made available to growers in real time via a website linked from the Extension Wild Blueberry site.

We are currently testing out and optimizing a remote camera that will allow more detailed observations of disease phenology at established plots.

Mummy berry cups (apothecia) started to emerge in Appleton, the earliest field monitored, on April 10th, and cups continued to emerge with the latest field monitored, Wesley/Meddybemps, starting on April 20th (Table 1). The mummy berries continued to produce cups in each field for approximately a month, which is longer than in recent years. This was a long and early season compared to recent years as seen in data for cup emergence from Deblois fields (Fig. 2). Five to six infection periods occurred in the Waldo County and from 10 to 12 infection periods in Hancock and Washington counties this year putting the plants under heavy disease pressure.

RECOMMENDATIONS: We recommend continuing monitoring conditions for mummy berry infection with the weather stations. Weather stations will be set up at 11 locations next year with viable mummy berry plots and growers willing to monitor them. We will continue with the disease forecast and will improve the website for the weather stations by including a current prediction of infection for each monitored field. We hope to increase participation of growers in the future and are actively looking for additional cooperators willing to monitor mummy berry plots.

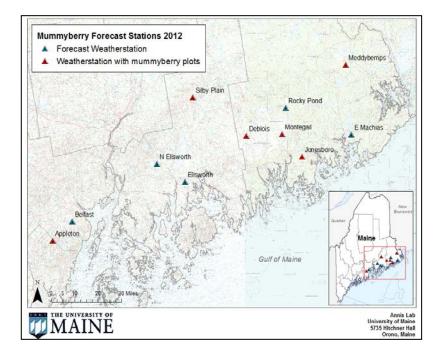


Fig. 1. Locations of mummy berry forecast stations and mummy berry plots for 2012.

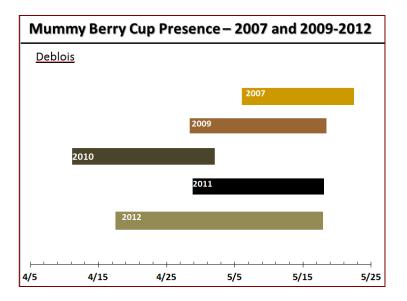


Fig. 2. Mummy berry cup presence monitored at Deblois plots: 2007 and 2009-2012.

Table 1 . Estimated time of mummy berry cup production and infection periods for weather	
stations in 2012.	

Weather Station Location	Estimated Date of Cups start	Estimated Date of Cups End	Number of infection periods
Appleton, Waldo	4/10	5/6	5
Belfast, Waldo	4/10	5/6	6
N. Ellsworth, Hancock	4/16	5/18	17
Ellsworth*, Hancock	4/16	5/18	13
Silby Plain, Hancock	4/18	5/18	12
Deblois*#, Washington	4/18	5/18	11?
Jonesboro, Washington	4/18	5/18	8
Montegail*, Washington	4/18	5/18	12
Rocky Pond, Washington	4/19	5/18	11
E. Machias, Washington	4/19	5/18	8
Meddybemps*, Washington	4/20	5/18	11

*Deblois, Ellsworth, Meddybemps, Montegail-some 15 min. interval data are missing and infection periods are estimated from hourly data

Deblois station was down for May 10th through May 13th

WEED MANAGEMENT

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

10. TITLE: Efficacy of Apogee growth regulator for stimulating rhizome growth into bare spots in wild blueberry fields.

METHODS: A study was initiated in 2011 to examine the efficacy of Apogee (prohexadione) to increase the spread of wild blueberry clones into bare spots. Apogee is a growth regulator used in apple production to reduce vegetative growth temporarily (2-5 weeks). In wild blueberry the potential benefit would be to reduce vegetative growth of shoots, which would allow the plants to devote more resources to rhizome growth, thereby filling in bare spots more quickly.

A site with several bare spots was chosen on Wyman's Airport 4 lot in Deblois, ME. Forty 1m² plots were set up in the bare spots, with at least one margin along the border of a clone. Plots were set up in pairs; one plot of each pair received 3" of mulch, while the other was left bare (Photos 1-2). Each pair was associated with one clone, but if the clone was large, multiple pairs were situated along its edges. Ten plot pairs were randomly chosen and on 6 June 2011 a 6' swath spanning from the clones' borders toward the interiors was sprayed with Apogee 18 oz/a at approximately 2 mph (2.8 ft/sec) using a boom sprayer. Application began approximately 6' before the first plot in a pair encountered on the clone edge and ended about 6' beyond the second plot in the pair.

Blueberry cover was assessed on 16 September 2011 and again on 31 July 2012. Differences were analyzed using t-tests (α =0.05) to compare changes in blueberry cover from 2011 to 2012 in a plot pair, differences between spread in sprayed and unsprayed clones at each evaluation, and differences in mulched versus unmulched plots at each evaluation. The interaction between mulching and Apogee at each evaluation was analyzed using Tukey's HSD test (α =0.05).

RESULTS: There were no significant differences in spread of blueberry plants into the uncovered area on the clone edge in sprayed versus unsprayed clones when compared to each other in either 2011 and 2012 or between years (Figure 1). There were also no significant differences between mulched and unmulched plots to each other or between years (Figure 2). When the interaction between mulching and Apogee was examined, there were also no significant differences in 2011 or 2012 (Figure 3).

CONCLUSIONS: Apogee did not appear effective in stimulating rhizome spread into bare areas. The addition of mulch alone or with the Apogee application did not significantly improve rhizome spread either. Mulching also does not appear to significantly stimulate rhizome spread into bare areas within one year, although the benefits over time have been demonstrated in other studies.

RECOMMENDATIONS: This treatment was not effective in stimulating spread of wild blueberry clones into bare spots, so no more research trials are planned.

Figure 1. Clones sprayed with Apogee versus unsprayed clones – differences in percent blueberry cover in 2011 and 2012 for plots placed along clone edges.

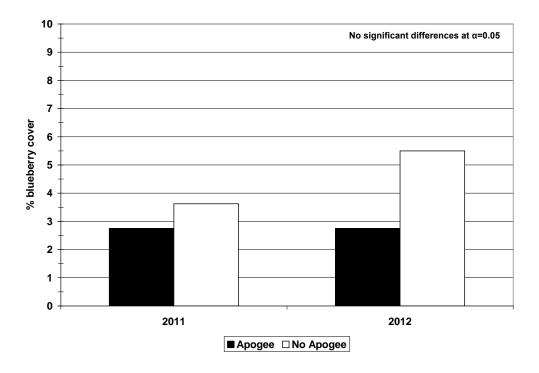


Figure 2. Mulched plots versus unmulched plots – differences in percent blueberry cover in 2011 and 2012 for plots placed along clone edges.

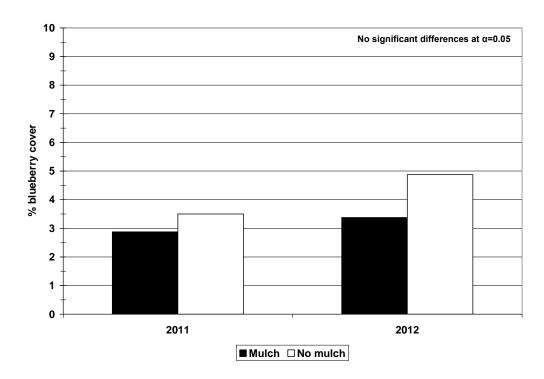


Figure 3. Interaction between Apogee application and mulching on percent blueberry cover in 2011 and 2012 for plots placed along clone edges.

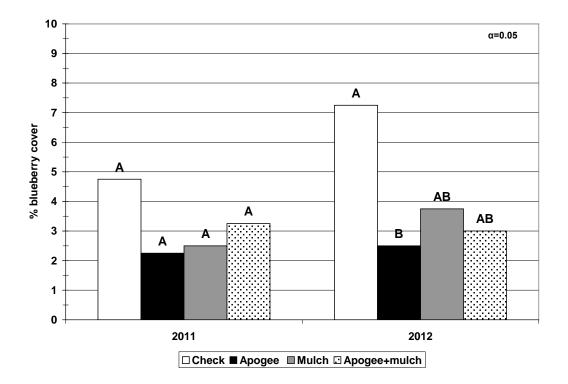


Photo 1. Example of an unmulched plot in 2012.



Photo 2. Example of a mulched plot in 2012.



WEED MANAGEMENT

INVESTIGATOR: David E. Yarborough, Professor of Horticulture Jennifer L. D'Appollonio-Cote, Assistant Scientist

11. TITLE: Velpar by Matrix pre and post-emergence applications - demonstration plots.

OBJECTIVES: Matrix was labeled for use on wild blueberries in 2012 but there was little information on what weeds were controlled and how it would perform in combination with existing herbicides, so three demonstration plots were established to evaluate the effect of Matrix with or without Velpar on wild blueberry cover, phytotoxicity and on broadleaf and grass weed cover.

METHODS: Experimental plots were established in Orland and Warren in commercial fields and at BBHF MAFES in Jonesboro, ME. The plot size was 12 by 12 feet as a Split-Split-Block design with the three sites serving as replications. On 26 April or 2 May (Jonesboro), Velpar was applied preemergence at 0, 1 and 2 lb/a and at right angles Matrix was applied preemergence at 0, 2 and 4 oz/a to give a total of nine Velpar by Matrix combinations. Matrix was applied post-emergence on May 30 or 31 (Jonesboro) at 4 oz/a to the fields that was treated with Velpar or Velpar-Diuron-Callisto (Jonesboro) to give a total of 10 treatments. Plots were evaluated on June 27-28 and August 21 or 29 (Jonesboro) for blueberry cover, phytotoxicity, grass and broadleaf weed control using a Daubenmire cover scale and converting the values to percent. Data were analyzed by Duncan's NMR test (α =0.05). **RESULTS**: Wild blueberry cover was higher on the herbicide treated plots and varied from 60 to 88% but was not significantly different for the herbicide applications (Figure 1). The Matrix at 4 oz/a had considerably more injury than the untreated or Velpar treated plots and delayed the growth and stunted the blueberries, especially with the post-emergence treatment, in the early season (Figure 2, Photo 1). There was more grass cover and blueberry phytotoxicity on the Jonesboro site (Figure 3). Broadleaf cover was reduced most by the 4 oz/a Matrix treatments with and without Velpar (Figure 4, Photos 2-4). Grass cover was reduced with the 4 oz/a of Matrix and was lower with 2 oz/a Matrix and 1 lb/a of Velpar than it was with 2 lb/a of Velpar, and none of the treatments controlled the woody perennials (Figure 5, Photo 5).

CONCLUSIONS: When a single herbicide is used, for decades, such as the herbicide Velpar, resistance and weed shifts will occur. There is a need to use new herbicides with different modes of action to alternate or to use in combination with herbicides now used to prevent resistant weeds from developing. Matrix will provide a different mode of action than Velpar and its combination with Velpar will prevent resistant grasses from increasing in wild blueberry fields.

RECOMMENDATIONS: Continue to put out demonstration plots to illustrate the use of Matrix to encourage grower acceptance and use.

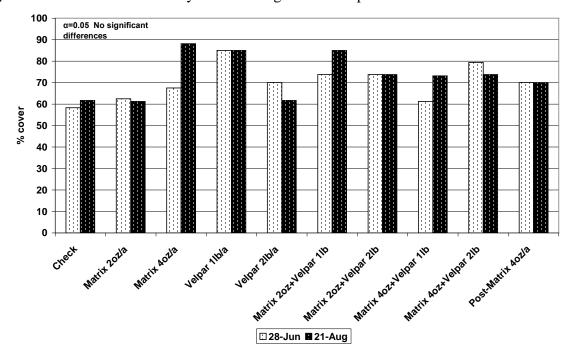


Figure 1. Percent wild blueberry cover among Matrix/Velpar combinations in 2012.

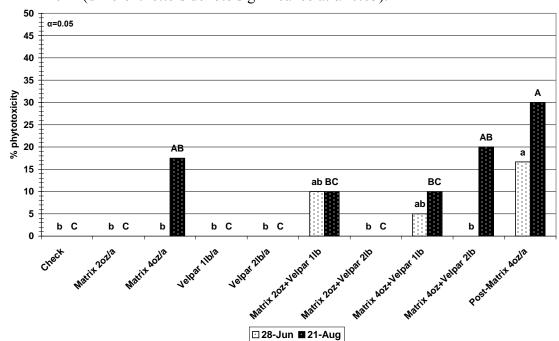


Figure 2. Differences in wild blueberry phytotoxicity among Matrix/Velpar combinations in 2012 (different letters denote significance at α =0.05).

Figure 3. Site differences in grass cover and wild blueberry phytotoxicity for Matrix/Velpar combinations in 2012 (different letters denote significance at α =0.05).

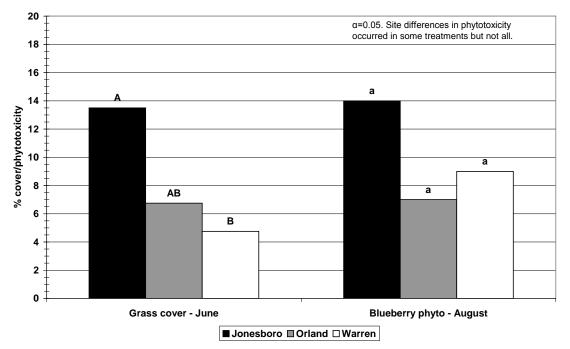


Figure 4. Broadleaf weed cover among Matrix/Velpar combinations in 2012 (different letters denote significance at α =0.05).

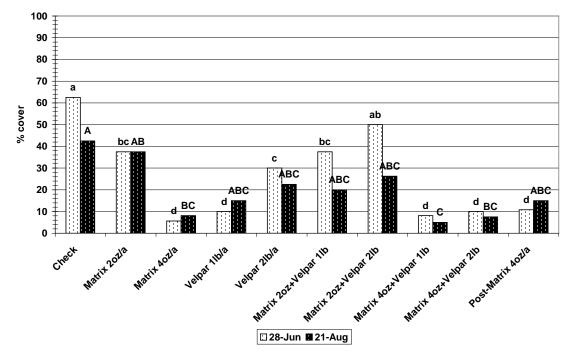


Figure 5. Grass cover among Matrix/Velpar combinations in 2012 (different letters denote significance at α =0.05).

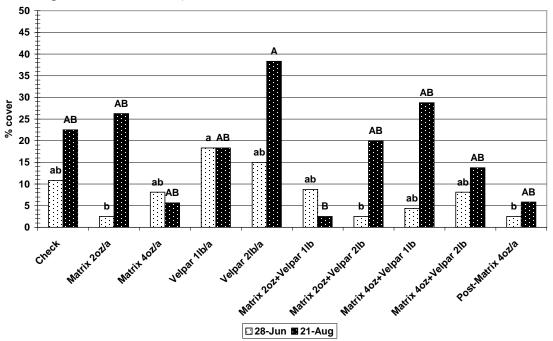


Photo 1. Matrix applied post emergence at 4 oz/a caused stunting and delayed emergence of blueberries.



Photo 2. Weedy Check plot.



Photo 3. Matrix 4 oz/a preemergence application.



Photo 4. Matrix applied at 4 oz/a post-emergence vs. untreated on right.



Photo 5. Matrix at 2 oz/a plus 1 lb/a Velpar preemergence gave better control than Velpar at 2 lb/a.



EXTENSION

INVESTIGATOR: David E. Yarborough, Extension Blueberry Specialist

12. TITLE: Wild blueberry Extension Education Program in 2012.

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

individual grower contact reinforce those previously identified, but also added the need for blueberry quality and groundwater concerns.

RESULTS:

Educational Activities:

This year the Blueberry Integrated Crop Management (ICM) program consisted of a presentation at the Agricultural Trade Show, Spring Grower meetings at three locations, field demonstration sessions conducted three times in three counties and the annual field day at Blueberry Hill Farm.

I also did a series of articles in the Wild Blueberry Newsletter on improving yield from March through September, see <u>http://umaine.edu/blueberries/newsletters/</u>.

This year an emphasis was made on the new pest, the Spotted Wing Drosophila. Growers were informed of how to identify this new pest, how to build new traps and their placement in the field, and what were effective control measures at the Agricultural Trade Show in Augusta in January; at the Wild Blueberry Spring meetings in Waldoboro, Ellsworth and Machias in March; at ICM scouting sessions in Warren, Orland and Jonesboro in May and June; and at the annual wild blueberry growers Field Day in July. New fact sheets on identification, trapping and control measures were sent by list serve or mailed to growers and posted on the wild blueberry web site. A section 18 emergency use label was obtained from the Maine Board of Pesticides Control to provide growers a more effective control measure for this pest.

Meetings attended:

66thAnnual Meeting Northeastern Weed Science Society Philadelphia, PA, January 3-6, 2012. International Workshop on Precision Agriculture Truro, NS, Canada, February 23-24, 2012.

10th International Symposium on *Vaccinium* and other Superfruits. Maastricht, Netherlands, June 18-22, 2012

15th Wild Blueberry Health Summit, Bar Harbor, ME, August 15-17, 2012

- Wild Blueberry Association of North America and Wild Blueberry Research and Extension Workers Annual Meeting, Fredericton, NB, Canada, October 24-26, 2012
- Wild Blueberry Producers Association of Nova Scotia Annual Meeting. Truro, NS, Canada, November 16-17, 2012

Presentations:

- Preemergent Combinations of Herbicides for Weed Control in Wild Blueberry Fields. 66th Annual Meeting of the Northeastern Weed Science Society, Philadelphia, PA, January 3-6, 2012
- Wild Blueberry Weed and Disease Management. Augusta Agricultural Trade Show, Augusta, ME, January 12, 2012

Wild Blueberry Production. PSE 110. University of Maine, Orono, ME, February 13, 2012

Benefits of Precision Agriculture Technologies for Wild Blueberries in Maine and Canada. International Workshop on Precision Agriculture. Truro, NS Canada, February 23-24, 2012

Integrated Crop Management Field Training Sessions, in Warren, Orland and Jonesboro, ME, April 24, 25, 26, May 29, 30, 31, June 26, 27, 28, 2012

- Growing Season Effects on Wild Blueberry (*Vaccinium angustifolium*) in Maine and Implications for Management. 10th International Symposium on *Vaccinium* and other Super fruits, Maastricht, Netherlands, June 18-22, 2012
- Improving Northern Bilberry (*Vaccinium uliginosum*) Production. 10th International Symposium on *Vaccinium* and other Super fruits, Maastricht, Netherlands, June 18-22, 2012
- Organic Weed Management, Blue Hill Berry Company. Penobscot, ME, June 12, 2012
- Weed Management Research for 2012. Wild Blueberry Field Day, Jonesboro, ME, July 18, 2012
- Improving Chinese Wild Blueberry (*Vaccinium uliginosum*) Production, 5th Annual meeting of the Small Fruit Branch of the Chinese Society of Horticultural Science. Yichun, China, August 2-4, 2012.
- Wild Blueberry Production and IPM. Wild Blueberry Legislative Tour, Jonesboro, ME, August 23-24, 2012
- Wild Blueberries. Eastern States Expo, Springfield, MA, September 27-29, 2012
- Wild Blueberry Production. Go Away tours, Bar Harbor, ME, October 15, 2012
- Growing Season Effects on Wild Blueberry (*Vaccinium angustifolium*) in Maine and Implications for Management. INT500 Seminar. Orono, ME, November 7, 2012.
- World and Maine Production 2012. Wild Blueberry Producers Association of Nova Scotia Annual Meeting. Truro, NS, Canada, November 16-17, 2012.
- You can get there from here or what does it take to get 20,000 pounds on your blueberry field. Wild Blueberry Producers Association of Nova Scotia Annual Meeting. Truro, NS, Canada, November 16-17, 2012

Publications:

- Drummond, F. and D.E. Yarborough. 2012. Growing Season Effects on Wild Blueberry (*Vaccinium angustifolium*) in Maine and Implications for Management. Acta Hort. In press.
- Drummond, F., S. Annis, J.M. Smagula and D.E. Yarborough. 2012. Organic Lowbush Blueberry Research and Extension in Maine. International Journal of Fruit Science 12(1-3): 216-231.
- McGovern, K., S. Annis, and D.E. Yarborough. 2012. Efficacy of organically acceptable materials for control of mummy berry disease on wild blueberries in Maine. International Journal of Fruit Science 12(1-3):188-204.
- Yarborough, D.E. 2013. Improving Northern Bilberry (*Vaccinium uliginosum*) Production. Acta Hort. In press.
- Yarborough, D.E. 2013. Establishment and Management of the Cultivated Lowbush Blueberry. International Journal of Fruit Science 12(1-3):14-22.
- Yarborough, D.E. and J.L. D'Appollonio. 2012. Preemergent Combinations of Herbicides for Weed Control in Wild Blueberry Fields. Proceedings of the Northeastern Weed Science Society. 66:77.

Wild Blueberry Fact Sheets – 2012:

Revised:

Fact Sheet #209 (UMCE #2001) 2012 Insect Control Guide for Wild Blueberries Fact Sheet #239 (UMCE #2025) 2012 Weed Control Guide for Wild Blueberries Fact Sheet #219 (UMCE #2000) 2012 Disease Control Guide for Wild Blueberries 2012 Maine Wild Blueberry Pesticide Chart *New:* Targeting the Prune Year Field for Blueberry Maggot Management Spotted Wing Drosophila Traps Spotted Wing Drosophila Update

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 80,618 page views in 2012 and so is well used world-wide. The wild blueberry blog is being used to update growers on current activities including insect (both pollinator and SWD), and disease (mummyberry monitoring) posts and the flamer demonstration this fall at BBHF: http://mainewildblueberries.blogspot.com/

Other program activities:

I am the principle investigator for the NIFA *Sustainable Production of Wild Blueberries*, which provides funds for a four year (2009-2013) multidisciplinary systems approach project for wild blueberries. I am responsible for obtaining, compiling and producing the proposals and reports both on paper and providing summaries for the Current Research Information System database on-line for both projects.

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 Policy Advisory Committee for Cooperative Extension and on the peer review committee for the Department of Plant, Soil and Environmental Sciences and the joint peer review committees of Renae Moran and Mark Hutton. I also serve on the graduate committees of: Sara Bushmann, PhD student, Major advisor F. Drummond 2008 – present; Jen Dezeno MS student Major advisor M.E. Camire 2012 – present.

CONCLUSIONS: Growers are participating in IPM programs in the four primary wild blueberry growing counties: Washington, Hancock, Knox and Lincoln. The skills survey results indicate that growers are learning new skills and making positive changes in their management practices. A high percentage of participating growers indicated they had learned new skills and changed their practices in calibration, thereby reducing the rate of hexazinone used, being able to control blight, identifying and control weeds, being able to detect and control insects and the blueberry maggot fly, and using soil and leaf samples to determine fertilizer rates. Adoption of these management practices will enable growers to improve the efficiency of blueberry culture

by reducing unnecessary pesticides and fertilizers. Developing alternative strategies for control of resistant weeds is necessary to prevent future losses in yield from weed competition. The introduction of the new pest, the spotted wing drosophila, will present additional challenges in monitoring, identification and control to prevent losses from this pest. These practices are essential to counter the perception of the anti-pesticide and the anti-aerial spray protests that have taken place and intensified in recent years.

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

INPUT SYSTEMS STUDY – SCRI GRANT

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

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

		Management	input systems		
Production	Organic	Low Input	Medium Input	<u>High Input</u>	
Factors					
Pruning	Burned	Burn	Mowed	Mowed	
Land leveling	Not land leveled	Not land leveled	Land leveled	Land leveled	
pН	pH managed	No pH	pH managed	pH managed	
management		management			
Fertility	No fertilizer	No fertilizer	Reduced	Fertility optimal	
			Fertility (every		
			other cycle)		
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	Hand raked	Hand raked	Mechanical	Mechanical	
method			Harvest	Harvest	

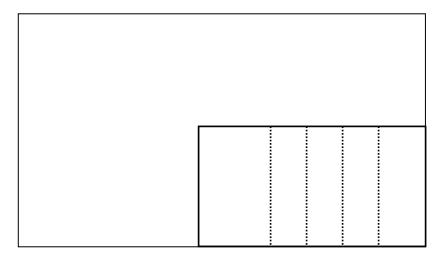
Table 1. Levels of inputs in four management systems for the production of wild blueberries.

Two one-acre blocks each were maintained on the following sixteen sites (*update 2015*: field names were replaced with numbers, so may be out of order in tables in this section): **High input:** Fields 13-16;

Medium input: Fields 9-12; Low input: Fields 5-8; Organic: Fields 1-4.

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

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



METHODS: The following inputs were made to each system in 2012 and are found in Table 2. <u>Sampling</u>

Weed cover assessment was conducted in late May and late July along the transects, and soil sampling for analysis of the organic pad was conducted along the transects as well. 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. The Organic sites' 15 x 30 m sub-blocks were weed-whacked by University personnel above the height of the blueberries in late June, July and August.

RESULTS/RECOMMENDATIONS/CONCLUSIONS:

<u>Sampling</u>

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

Input	Site	рН	Fertility	Pest control	Disease control	Weed control	Pruning
	Field 1	NA	NA	NA	NA	Woody weed cutting	Mowed (2 nd yr crop to be burned S'13)
Organic	Field 2	NA	NA	NA	NA	Hand weeding Goat browsing	Burned
	Field 4	NA	NA	NA	NA	Hand weeding	Mowed
	Field 3	Tiger Organic 750 lb/a	Bone char 100 lb/a Azomite 125 lb/a	NA	NA	Scythed bracken fern	Burned
	Field 5	NA	07-22-05 5 gal/a Xtra power 1.5 pt/a Interlock 6 oz/a Black Label 1 gal/a	NA	Initiate 4 pt/a	Velpar 0.75 gal/a Diuron 1.5 qt/a Grounded 1 pt/a	Burned
Low	Field 6	NA	Black Label 4 gal/a	NA	Initiate 4 pt/a	Velpar 1 gal/a Sinbar 2 lb/a Diuron 1.5 qt/a Credit Extra + Terramark + COC spot spray	Mowed + Burned
	Field 7	Tigersul sulfur 267 lb/a	DAP 300 lb/a Black Label 2 gal/a	NA	Initiate 3 qt/a	Sinbar 2 lb/a Callisto 6 oz/a Credit Extra 1 qt/a	Burned
	Field 8	NA	DAP 136 lb/a	NA	NA	Velossa 0.7 gal/a Sinbar 1 lb/a Direx 0.4 gal/a	Burned
Medium	Field 9	NA	MAP+ micronutrients 250 lb/a	Scouting	Bravo Ultrex 3.6 lb/a	Velossa 0.4 gal/a Callisto 6 oz/a Clethodim 8 oz/a	Mowed

Table 2. 2012 prune year inputs by input system (NA=Not Applicable).

Input	Site	pH	Fertility	Pest control	Disease control	Weed control	Pruning
	Field 10	NA	MAP+micronu trients 250 lb/a	Scouting	Bravo Ultrex 3.6 lb/a	Velossa 0.4 gal/a Clethodim 8 oz/a	Mowed
	Field 11	NA	MAP+micronu trients 250 lb/a	Scouting	Bravo Ultrex 3.6 lb/a	Velossa 0.4 gal/a Clethodim 8 oz/a	Mowed
	Field 12	NA	MAP+micronu trients 250 lb/a	Scouting	Bravo Ultrex 3.6 lb/a	Velossa 0.4 gal/a Clethodim 8 oz/a	Mowed
	Field 14	NA	N 16-34-4 70 lb N/a	Scouting	Bravo ZN 4.25 pt/a Tilt 3 oz/a	Velpar 1 lb/a Sinbar 1 lb/a Diuron 1 lb/a Asulox spot spray	Mowed
· · · · ·	Field 13	NA	N 16-34-4 80 lb N/a	Scouting	Bravo ZN 4.25 pt/a Tilt 3 oz/a	Velpar 1 lb/a Sinbar 1 lb/a Diuron 1 lb/a Asulox spot spray	Mowed
High	Field 15	NA	N 16-34-4 80 lb N/a	Scouting	Bravo ZN 4.25 pt/a Tilt 3 oz/a	Velpar 1 lb/a Sinbar 1 lb/a Diuron 1 lb/a Asulox spot spray	Mowed
	Field 16	NA	N 16-34-4 80 lb N/a	Scouting	Bravo ZN 4.25 pt/a Tilt 3 oz/a	Velpar 1 lb/a Sinbar 1 lb/a Diuron 1 lb/a Asulox spot spray	Mowed

INPUT SYSTEMS STUDY

FOOD SCIENCE & NUTRITION: Vivian C. H. Wu, Ph.D., Associate Professor, Dept. of Food Science & Human Nutrition

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

METHODS: The microbial quality of harvested blueberries from four management systems were evaluated for the presence or absence of potential foodborne pathogens using conventional culture methods and also alternative PCR screening method. For culture methods, isolation and detection of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. involved weighing two subsamples of 25g each and subjecting them to a sequence of pre-enrichment, enrichment, selective-differential plating and biochemical characterization. For PCR screening, DNA extraction was done from all the samples after overnight enrichment and later tested for the three target pathogens using specific primers. After traditional culture and PCR methods, all the positive samples were confirmed using serological confirmation tests. Note that the samples were taken in 2011 from the sites used in the first cycle of this study; therefore there are eight sites, and the organic sites contain mulched and unmulched plots.

RESULTS: From our study, no *E. coli* O157:H7 or *L. monocytogenes* was 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 are subjected for serological confirmatory testing and we found out that culture methods showed one false positive with eight out of nine positives with only six out of eleven samples were confirmed to have *Salmonella* spp. by serological confirmation (Table 4). Since traditional culture methods have less false positives compared to PCR method we can conclude that eight samples which are culture positive and also serology positive are confirmed to have *Salmonella* spp. (Table 5).

CONCLUSIONS: The differences of fertilizer application may be the reason for contamination levels of *Salmonella* in the organic and medium input samples. 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.

RECOMMENDATIONS: The use of either method in isolation would have resulted in the failure to detect *Salmonella* in some of positive samples. Therefore, it may be pertinent to use a combination of the PCR and culture methods in order to maximize the detection of *Salmonella*.

Table 1. Blueberry samples positive for Salmonella spp. through traditional culture methods

SCRI ID	Culture or Enterotube test status for Salmonella spp.
High input, Field 14 - 3	Culture positive
Medium input, Field 9 - 1	Culture positive
Medium input, Field 9 - 2 Medium input, Field 10 - 5	Culture positive Culture positive
Medium input, Field 10 - 6	Culture positive
Medium input, Field 10 - 7	Culture positive
Organic input, Field 1 Yes mulch-6	Culture positive
Organic input, Field 1 Yes mulch-5	Culture positive
Organic input, Field 2 No mulch-3	Culture positive

 Table 2. Blueberry samples positive for Salmonella spp. through PCR methods.

SCRI ID	PCR status for Salmonella spp.
Medium input, Field 10 - 6	Salmonella PCR positive
Medium input, Field 10 - 7	Salmonella PCR positive
Organic input, Field 1, Yes mulch-5	Salmonella PCR positive
Organic input, Field 1, Yes mulch-6	Salmonella PCR positive
Organic input, Field 2, No mulch-3	Salmonella PCR positive
Organic input, Field 1, Yes mulch-7	Salmonella PCR positive
Organic input, Field 1, No mulch-7	Salmonella PCR positive
Organic input, Field 1, Yes mulch-8	Salmonella PCR positive
Organic input, Field 2, Yes mulch-1	Salmonella PCR positive
Organic input, Field 2, No mulch-2	Salmonella PCR positive
Organic input, Field 2, No mulch-4	Salmonella PCR positive

Figure 1. Number of blueberry samples that are *Salmonella* PCR and culture positives from different cropping systems.

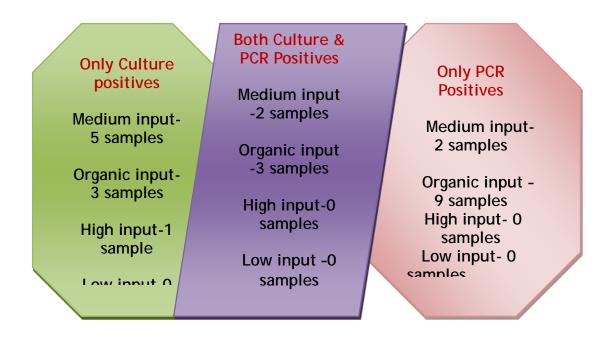


Table 3. Serological confirmatory status of blueberry samples positive for *Salmonella* spp. through culture methods with \bigstar indicating culture false positives.

SCRI ID	Culture status	Serological status	Final interpretation
High input, Field 14 - 3	Culture positive	O, Vi and H Antigen negative	Presumptive <i>salmonella</i> . ★ Culture false positive
Medium input, Field 9 - 1	Culture positive	O, Vi positive and H Antigen negative	Confirmed salmonella
Medium input, Field 9 - 2	Culture positive	O, Vi and H Antigen positive	Confirmed salmonella
Medium input, Field 10-5	Culture positive	O, Vi and H Antigen positive	Confirmed salmonella
Medium input, Field 10-6	Culture positive	O, Vi positive and H Antigen negative	Confirmed salmonella
Medium input, Field 10-7	Culture positive	O, Vi positive and H Antigen has slight agglutination- positive	Confirmed salmonella
Organic input, Field 1 yes mulch-6	Culture positive	O, Vi positive and H Antigen has slight agglutination- considered positive	Confirmed salmonella
Organic input, Field 1 yes mulch-5	Culture positive	O, Vi positive and H Antigen negative	Confirmed salmonella
Organic input, Field 2 no mulch-3	Culture positive	O, Vi positive and H Antigen positive	Confirmed salmonella

SCRI ID	PCR and culture status	Serological status	Final interpretation
Medium input, Field 10 - 6	Salmonella PCR positive and Culture positive	O, Vi positive and H Antigen negative	Presumptive <i>salmonella</i> . Only serology negative, but PCR and culture positive \bigstar
Medium input, Field 10 - 7	Salmonella PCR positive and Culture positive	O, Vi positive and H Antigen has slight agglutination- positive	Confirmed salmonella
Organic input, Field 1, Yes mulch-5	Salmonella PCR positive and Culture positive	O, Vi positive and H Antigen negative	Confirmed salmonella
Organic input, Field 1, Yes mulch-6	Salmonella PCR positive and Culture positive	O, Vi positive and H Antigen has slight agglutination- positive	Confirmed salmonella
Organic input, Field 2, No mulch-3	Salmonella PCR positive and Culture positive	Very light agglutination for O, Vi and H Antigen	Confirmed salmonella
Organic input, Field 1, Yes mulch-7	Salmonella PCR positive and Culture Negative	O, Vi and H Antigen negative	Presumptive <i>salmonella</i> . ★ PCR false positive
Organic input, Field 1, No mulch-7	<i>Salmonella</i> PCR positive and Culture Negative	O, Vi and H Antigen negative	Presumptive <i>salmonella</i> . * PCR false positive
Organic input, Field 1, Yes mulch-8	Salmonella PCR positive and Culture Negative	O, Vi and H Antigen negative	Presumptive <i>salmonella</i> . PCR false positive
Organic input, Field 2, Yes mulch-1	Salmonella PCR positive and Culture Negative	O, Vi positive and H Antigen slight agglutination – considered H positive	Confirmed salmonella
Organic input, Field 2, No mulch-2	<i>Salmonella</i> PCR positive and Culture Negative	O, Vi and H Antigen negative	Presumptive <i>salmonella</i> . PCR false positive
Organic input, Field 2, No mulch-4	Salmonella PCR positive and Culture Negative	O, Vi and H Antigen negative	Presumptive <i>salmonella</i> . * PCR false positive

Table 4. Serological confirmatory status of blueberry samples positive for *Salmonella* spp. through PCR methods with \bigstar indicating PCR false positives.

Table 5. List of blueberry samples confirmed to have Salmonella spp.

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

INPUT SYSTEMS STUDY

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

15. TITLE: Abundance of insect pest species and natural enemies in lowbush blueberry fields maintained under different management practices.

METHODS:

<u>Plot design</u>

Four cropping systems (Organic and Low, Medium and High levels of conventional inputs) indicative of the variation in management methods used by growers were established as oneacre plots. There were two, 1 acre blocks per site. All sites were maintained by the grower using IPM practices appropriate to the level of management. Ground-dwelling arthropods were evaluated using pitfall traps. The abundance of blueberry thrips and tip midge was also assessed.

Thrips Survey

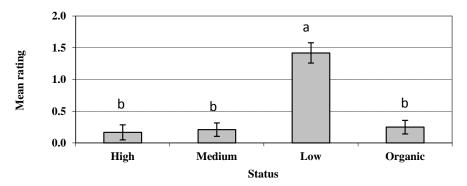
Sixteen blueberry fields during their prune cycle were sampled for thrips in 2012. Four production systems (Low, Medium, High input, and Organic) were represented by four fields each in three counties (Waldo, Hancock, and Washington). Between 18 and 22 Jun, thrips damage was measured along each of three 100 ft transects per field. 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.

Table 1. Rating system for thrips damage assessment.

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

Analysis of Variance (ANOVA, CRD) was used to compare thrips abundance (rating) among the treatments (High, Medium, Low, or Organic). Data were not transformed, analysis was performed on ranks. In general thrips abundance was low; all transects sampled had ratings of 3 or less. There was a significant difference in mean density rating of thrips due to production system (P < 0.0001). Significantly more thrips were found on Low input farms (Fig. 1). This is the second year that similar results have been observed. In 2010, Low input fields also had significantly higher thrips abundance than Organic and Medium and High input fields. The reason for this striking result is not clear, but clearly needs further investigation.

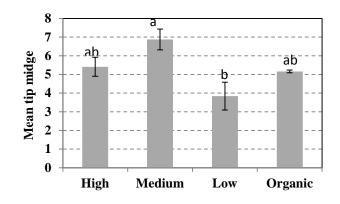
Fig. 1. Abundance of thrips, mean rating.



Tip Midge Survey

Tip midge damage was assessed between 11 and 18 June by counting the number of blueberry stems with damage as evidenced by curled leaves in each of ten, m^2 subplots per block. Analysis of Variance (CRD) was used to compare infestation among the treatments. Data were transformed by square root prior to analysis. No significant difference was observed between High, Low, and Organic sites; however, Medium sites had significantly more tip midge than Low input sites (P = 0.0137) (Fig. 2).

Fig. 2. Abundance of tip midge, mean per production system.



Pitfall trap survey

Pitfall traps were set out in each plot on two sample dates (Jun and Aug). The traps consisted of plastic cups 7.0 cm in depth with a top diameter of 10.5 cm filled 1/3-1/2 full with propylene glycol. A rain shield with four, 16 d nails as supports was placed over each trap and remained in place until traps were serviced. Five pitfall traps were placed in each plot. For each of the two collection periods the traps were left out for 7-8 days; than the contents of each trap were brought back to the laboratory where they were placed in urine cups with 70% ethyl alcohol for future sorting and identification. The abundance of ground-dwelling arthropods associated with lowbush blueberry fields under each of the four management systems was evaluated for both pest and beneficial species. Prior to analysis, abundance was converted to specimens / day. All data were transformed by the square root prior to analysis to stabilize variance. Analysis of Variance (RCBD) and LS Means Differences ($P \le 0.05$) were used to compare the abundance of natural enemies among the treatments.

The results of the first sample, collected in June, are in Figure 3. Ants, spiders, ground beetles (both adults and larvae), and harvestmen were the most abundant natural enemies captured in pitfall traps. Other natural enemies including tiger beetles, rove beetles, and centipedes were collected in small numbers and pooled together as "others" for the purpose of analysis.

For sample 1, there was no significant difference in the number of spiders among the treatments (P = 0.3763). Ants were most abundance in Organic fields (P = 0.0201) and ground beetles (carabids) were most common in High input fields (P = 0.0231). There was also a trend towards crickets being more abundant in Organic fields; however, the difference was not significant (P = 0.6033). Low and High input fields had the highest captures of harvestmen (P < 0.0001); captures of "other" natural enemies were highest in Organic and High inputs fields (P = 0.0198).

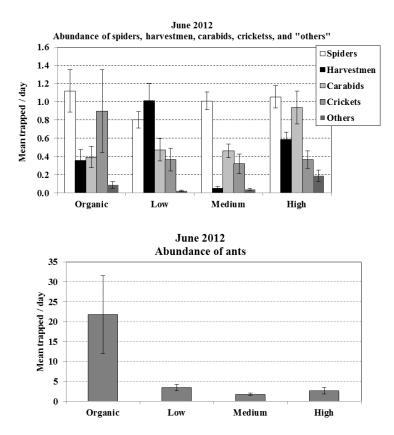


Fig. 3. Relative abundance of natural enemies in pitfall trap samples, Jun 2012.

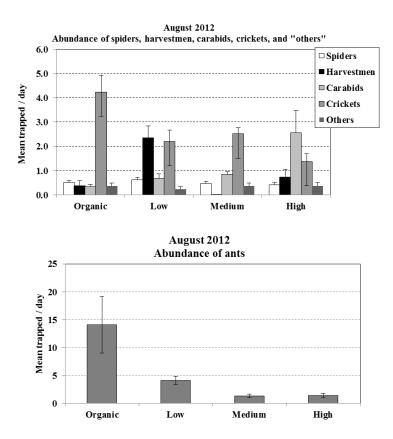


Fig. 4. Relative abundance of natural enemies in pitfall trap samples, Aug 2012.

CONCLUSIONS: The fourth year and final year of this study will be conducted in 2013. As one can see from the pest and natural enemy data presented, there are production system practices that significantly affect the populations of pest and beneficial insects. The fourth year of the study will allow us to determine if these effects result in subsequent effects on yield and if they are consistent over the two cropping cycles that were studied.

INPUT SYSTEMS STUDY

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

16. TITLE: Input Systems Study: Systems approach to improving the sustainability of wild blueberry production, Year 3 of a four-year study, disease management results.

METHODS: Diseases were rated by block twice during the season, the first time during the period from 7/11 to 7/20 (July) and again from 8/30 to 9/18 (September). Within each block, 10 sampling plots of $0.25m^2$ were rated by at least 2 surveyors visually estimating percentages of blueberry coverage, blueberry stems with Phomopsis, witch's broom or red leaf diseases, and blueberry leaf area with the following leaf spot diseases: Septoria leaf spot, powdery

mildew, leaf rust, and false Valdensinia leaf spot. Blueberry leaf loss was rated in both the July and September assessments. Ratings were averaged across all surveyors by plot before analysis.

Data were analyzed at the management input level for blueberry cover, disease coverage and leaf loss in SAS (Statistical Analysis Software - SAS Cary, NC) using mixed model procedures (PROC GLIMMIX). For July, blueberry cover and Septoria leaf spot were arcsine square-root transformed, and powdery mildew and Phomopsis disease measures were logit (log(x/(1-x))) transformed. For September data, blueberry cover was arcsine square-root transformed and leaf loss, powdery mildew, Phomopsis, rust and Septoria disease measures were logit transformed. Least Square means were used to determine specific differences among system types ($\alpha = 0.05$).

Data were analyzed at the block and field level with untransformed data for correlations amongst different measures of disease, blueberry cover and leaf loss using Spearman's rank correlation in SAS (PROC CORR).

RESULTS/DISCUSSION: Blueberry cover was similar in both July and September with the highest levels of cover in the Medium and High input systems and the lowest levels in the Organic and Low input systems (Fig. 1). In July, the only disease present in all fields was Septoria leaf spot; by the September evaluation, Septoria leaf spot, powdery mildew and rust disease were found in all fields. There were no significant correlations between the levels of diseases and blueberry cover.

Powdery mildew levels increased from July to September by being present in 11- 22% of plots in July to 61- 82% of plots in September (Fig. 2). There were no significant differences in the percentage of plots that had powdery mildew among the input systems. Even though there was a high percentage of plots with powdery mildew in September, there were typically low levels of leaf area affected by this disease (Fig. 3). There were significantly lower powdery mildew levels in the Low input systems than in the other management systems in the September rating.

Over 70% of plots in all management types had Septoria leaf spot in both July and September (Fig. 4). There was little difference among the management types in the average percentage of plots or the average percentage of leaf area with Septoria in either July or September (Fig. 5).

In July, leaf rust was found in one or two plots within two Medium input fields, (Fields 10 and 12) and one Organic field (Field 2) (data not shown). Leaf rust was detected in all fields in August and affected a significantly higher percentage of leaf area in the Organic fields than the other input types (Fig. 6).

In the July assessment, there was minimal leaf loss with only 3 plots in all the fields showing 5 to 10% leaf loss. In September, all of the fields had leaf loss and the Organic (11 to 19.8%) and Low (3.5 to 30%) input fields had significantly higher levels of leaf loss than the Medium (0.8 to 5.3%) and High (0.6 to 4.2%) input fields (Fig. 7). One Low input field (Field 8) had unusually high levels of leaf loss at 30.3% and also had comparatively high levels of blueberry cover at 86% when the other Low input fields ranged from 50 to 61% in their blueberry cover.

When we looked at the effect of the different leaf diseases on leaf loss, we found a significant effect of leaf rust and Septoria. In fields with higher levels of leaf rust and Septoria leaf spot, there was a significant correlation to higher levels of leaf loss (Figs. 8 and 9). The correlation between leaf loss and leaf rust is more robust than that between Septoria leaf spot and leaf loss. When Field 8 (with 30.3% leaf loss) is removed from the analysis using leaf rust, the correlation is still strong, but when this is done with Septoria leaf spot the resulting correlation

is much weaker and no longer significant. Other factors, such as water and heat stress, may also affect leaf loss and the level of leaf loss due to disease.

The leaf disease, False Valdensinia leaf spot, was only found in a few plots in two Organic fields (Fields 1 and 3) in July and in only five fields (one Organic, Field 2; one Medium, Field 11; and three High input fields, Fields 13, 15, 16) in September.

Stem diseases were not as prevalent as leaf spot diseases. Phomopsis stem disease was found in all fields in July except a Low input field (Field 5), and in all fields except Fields 5 and 2 (Organic field) in September. In both assessments, the level of Phomopsis disease was significantly higher in the Medium and High input fields than in the Organic and Low input fields (Fig. 10). Witch's broom was only found in one to two plots in one Low input field (Field 8) and two Organic fields (Fields 1 and 3). Red leaf disease was found in one to three plots in very low levels in at least one field of each management type and in fewer plots in August due the difficulty of detecting the disease from the drying up and drop off of affected leaves. The levels of red leaf among the input types ranged from 0.03 to 1.1% of stems affected with no significant differences among the management types (data not shown).

Analysis of the effects of individual management practices on disease levels is continuing.

CONCLUSIONS/RECOMMENDATIONS: Management inputs can affect the level of leaf diseases and stem diseases present during the prune year. Which management practices have the greatest effects on disease levels is being investigated. Once this analysis is completed, recommendations will be made.

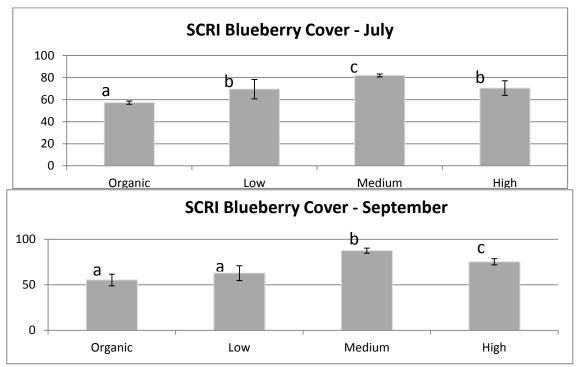


Fig. 1. Average percent of blueberry cover by management input types L to R Organic, Low, Medium, High for July (top) and September (bottom). Error bars indicate standard error of the mean. Bars with different letters within graph indicate statistically significant differences at α =0.05.

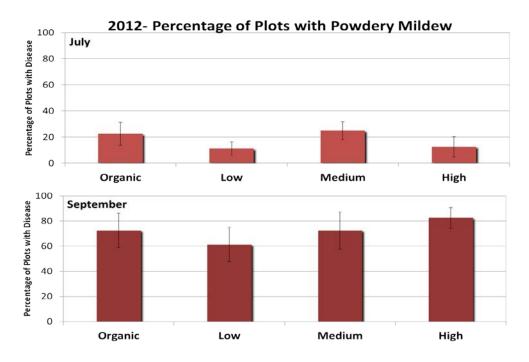


Fig. 2. Average percent of plots with powdery mildew by management input types for July (top) and September (bottom). Error bars indicate standard error of the mean. No significant differences were found among the input systems.

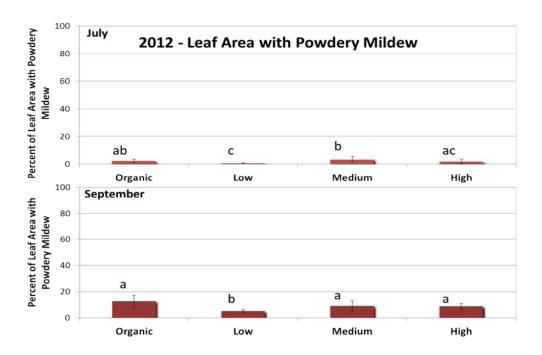


Fig 3. Average percent leaf area coverage of powdery mildew (\pm standard error) by management input types for July (top) and September (lower). Bars with different letters indicate statistically significant ($\alpha = 0.05$) differences.

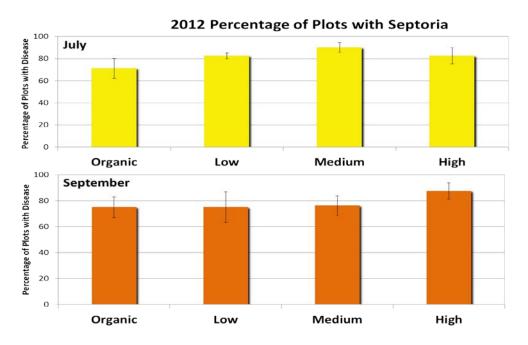


Fig. 4. Average percent of plots with Septoria by management input types for July (top) and September (bottom). Error bars indicate standard error of the mean. No significant differences were found among the input systems.

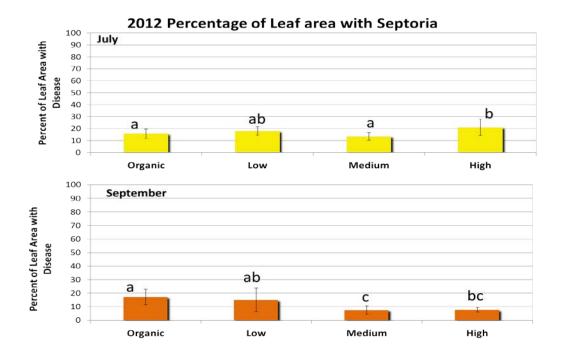


Fig 5. Average percent leaf area coverage of Septoria (\pm standard error) by management input types for July (top) and September (lower). Bars with different letters indicate statistically significant (α =0.05) differences.

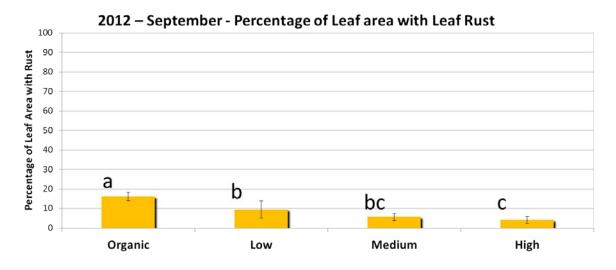
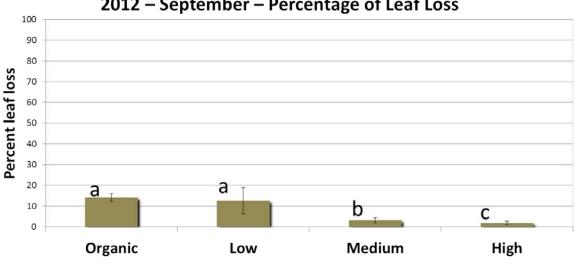


Fig 6. Average percent leaf area coverage of rust (\pm standard error) by management input types for September. Bars with different letters indicate statistically significant ($\alpha = 0.05$) differences.



2012 – September – Percentage of Leaf Loss

Fig 7. Average percent leaf loss (+ standard error) by management input types for September. Bars with different letters indicate statistically significant ($\alpha = 0.05$) differences.

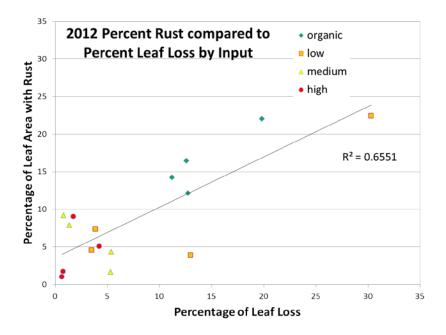


Fig. 8. Average percentage leaf area coverage of rust compared to average percent leaf loss for September by management input. The solid line represents a best fit linear regression line for all management inputs (R^2 =0.6551).

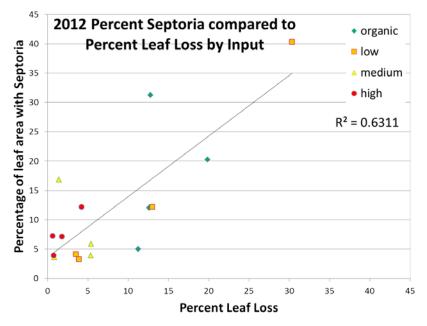


Fig. 9. Average percentage leaf area coverage with Septoria compared to average percent leaf loss for September by management input. The solid line represents a best fit linear regression line for all management inputs ($R^2=0.6311$).

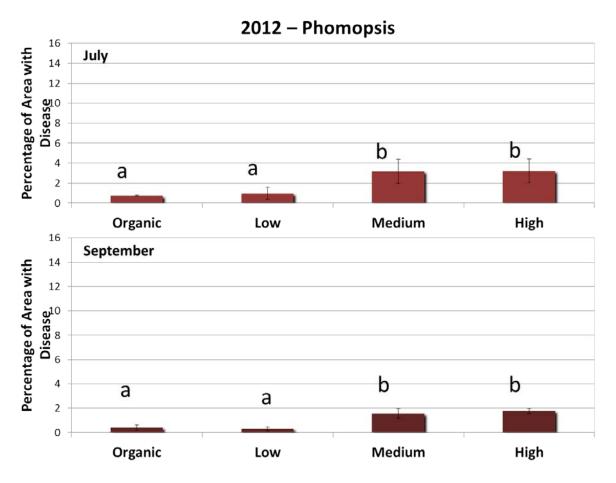


Fig. 10. Average percentage of stems with Phomoposis (+ standard error) for July and September assessments by management input. Bars with different letters indicate statistically significant ($\alpha = 0.05$) differences.

PLANT NUTRITION: David E. Yarborough, Professor of Horticulture Jennifer D'Appollonio-Cote, Assistant Scientist Caleb Slemmons, Research Assistant

17. TITLE: Plant productivity, Year Three of a four-year study.

METHODS: See report no. 13 for an overview of the Input Systems Study. In the second crop cycle of the study, two additional sites were selected to represent each input management system for a total of four sites per system. At each site there were two one-acre blocks. In early July, 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 pint composite sample was removed for analysis. The samples were analyzed for soil pH (water), organic matter (%), and nutrients (ppm). Organic matter 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 set forth by Trevett et al. (1968). 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

Overall soil pH's in the Organic and Low input management systems were significantly higher than in the Medium input system (Table 1). This follows the trend seen in the previous cycle, except that the High input system also had significantly lower pH than the Organic and Low systems (Table 1A). When all sites were compared individually, two of the Organic sites had the highest pH while two of the Medium sites had the lowest pH; the Low input management system was the only system where all pH values were above pH 4 (Table 2). Overall organic matter (OM) did not differ among systems, but when all sites were compared, the Medium management system tended to have higher levels of OM, while the Low and Organic systems tended to have lower levels and the Downeast Organic site (Field 2) had the lowest OM of all. This differs from last cycle, in which OM was significantly higher in the Organic and Low input management systems (Table 1A).

Soil calcium (Ca) was highest in the Medium input system, and was significantly higher than the Low input system (Table 1). Soil magnesium (Mg) and phosphorus (P) were also significantly higher in the Medium input system compared to the other systems. Soil aluminum (Al) was highest in the Organic input system; Al in the Low input system was significantly lower than in the Organic input system but significantly higher than in the Medium and High input systems. Soil copper (Cu), iron (Fe) and sulfur (S) were also highest in the Organic input system and significantly higher than the Medium or High input systems, but not significantly different from the Low input system. The Organic and Low input systems had almost identical levels of soil manganese (Mn), which were four times as great as in the High input system. Soil sodium (Na) in the Low input system was significantly higher than in all other systems. There were no differences in soil potassium (K) or zinc (Zn).

Soil Ca and K were particularly high in one of the Organic sites and one of the Medium input sites (Table 2). It is interesting to note that the same Organic site had the highest soil K, Cu and Mn, second highest Mg and third highest Al; this site was the most southerly of all sites. Another Organic site, the most easterly of all sites, had the lowest OM, soil Ca, K, Mg, P and Zn and second lowest Mn. One Organic site and one Low input site had exceptionally high soil Mn at 54 and 58 ppm respectively, while one High input site had exceptionally low Mn at 7 ppm. One Low input site had S applied to it in May 2012, and is reflected in the high soil S level comparable to the highest soil S level of all sites at one Organic site, which had S applied to it in 2010.

Plant nutrient concentrations

Leaf nitrogen (N) (%) was greatest in the High and Medium input management systems, and was significantly greater than in the Organic input system, which was also the only input

system deficient in N according to Trevett's standard (Figure 1). By contrast, leaf Ca (%) was greatest in the Organic system and was significantly greater than the Medium and High input systems (Figure 2). All input systems exceeded the 0.27 % standard for Ca, but the Organic system also exceeded the maximum recommended nutrient level of 0.52 %. Leaf P (%) was highest in the Medium input system, and was significantly higher than in the Organic or High input systems (Figure 3). The Organic system was also deficient in leaf P.

Aluminum (ppm) was highest in the Low input system, and was significantly more than in the Medium and High input systems, while the Organic system was significantly higher than in the Medium input system (Figure 4). However, not only were concentrations in all systems greater than the standard 50 ppm, they also exceeded the maximum level of 75 ppm specified by Trevett in 1968. Leaf boron (B) (ppm) was sufficient in all input systems, and in the High input system was significantly higher than in all other systems; the Medium input system also had a significantly higher B concentration compared to the Organic system (Figure 5). Conversely, leaf Cu concentrations were deficient in all input systems (Figure 6), and only the Organic system had significantly higher leaf Cu compared to the High input system, which had a concentration little more than half that of the standard minimum recommended level of 7 ppm. Leaf Fe concentration (ppm) in the Low input system was significantly greater than in all other systems, and was the only system to exceed the standard (Figure 7). Leaf Mn varied widely among systems, from a concentration deficient according to the standard minimum 750 ppm in the High input system, to a concentration exceeding the maximum standard level of 1490 ppm in the Organic system; the Low input system also exceeded the maximum (Figure 8). Leaf Mn concentrations in the Organic system were significantly higher than in the Medium and High input systems, while the Low input system was also significantly higher than the High input system. Finally, although there were no significant differences, leaf Zn was greater in the Low and High input systems and exceeded the standard, while leaf Zn concentrations in the Organic and Medium systems were deficient (Figure 9). There were no significant differences among systems for leaf Mg or K, and all concentrations were within the respective standards' ranges.

		Organic											
Management	pН	Matter		K	Mg	Р	Al	Cu	Fe	Mn	Na		
system	(water)	(%)	Ca (ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	S (ppm)	Zn (ppm)
Organic	4.13 a	13.69 a	281.5 ab	93.25 a	52.25 b	21.1 b	330.13 a	0.25 a	45.88 a	43.38 a	13.23 b	158.25 a	2.34 a
Low	4.09 a	13.69 a	205.63 b	75.13 a	39.25 b	26.73 b	267.13 b	0.17 ab	41.75 ab	46.63 a	21.25 a	111.38 ab	3.38 a
Medium	3.83 b	16.76 a	416.38 a	86.5 a	97.38 a	40.95 a	143.38 c	0.13 b	22.63 b	28.25 ab	15.1 b	59.5 b	4.24 a
High	3.93 ab	14.66 a	311.13 ab	101.0 a	70.25 ab	24.44 b	153.38 c	0.10 b	24.88 b	10.84 b	14.5 b	57.38 b	3.21 a

Table 1. Soil characteristics of the input management systems compared across systems.

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

Table 1A. Soil characteristics of the input management systems compared across sites in 2010. The highest value of each parameterand those significantly different from it are in **bold**; the lowest value of each and those significantly different from it are in*italics*.

Management system	Site	pH (water)	Organic Matter (%)	Ca (ppm)	K (ppm)	Mg (ppm)	P (ppm)	Al (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Na (ppm)	S (ppm)	Zn (ppm)*
Organic	2	4.4 ab	10 d	172 d	54 d	25 de	3 e	314 b	0.10 bc	24 bcd	19 b	16 ab	134 b	
	1	4.2 bc	11 bc ⁺	189 b	96 a	27 cde	3 e	360 a	0.16 a	35 b	38 a	15 ab	207 a	
Low	6	4.4 a	11 cd	115 с	73 bc	22 e	6 d	315 b	0.09 bc	27 bc	17 b	17 a	59 c	
	5	4.2 bc	11 bcd	130 с	65 cd	32 cd	8 c	237 с	0.13 ab	57 a	19 b	13 bc	50 c	
Medium	9	4.0 de	11 bcd	245 a	66 cd	49 a	11 ab	138 e	0.07 c	15 d	10 с	14 ab	30 c	
weddulli	10	4.1 cd	13 ab	198 b	60 cd	39 b	12 a	175 de	0.07 c	21 cd	11 c	14 ab	31 с	
High	14	3.9 e	14 a	182 b	85 ab	32 cd	11 b	167 de	0.09 bc	18 cd	3 d	15 ab	50 c	
Ingli	13	4.1 cd	11 cd	176 b	69 cd	28 cde	9 c ⁺	190 d	0.06 c	23 cd	5 cd	11 с	57 c	

Site 1 contains blocks 1-4 and site 2 contains blocks 5-8. Means across management systems with the same letter are not significantly different. Mean separation by Tukey's HSD test, α =0.05. Bold indicates significantly different from highest value; italic indicates significantly different from lowest value (*missing variable; bold+italic = significantly different from both).

Table 2. Soil characteristics of the input management systems compared across sites in 2012. The highest value of each parameter and those significantly different from it are in **bold**; the lowest value of each and those significantly different from it are in *italics*.

			Organic											
Management		pН	Matter	Ca						Fe		Na		
system	Site	(water)	(%)	(ppm)	K (ppm)	Mg (ppm)	P (ppm)	Al (ppm)	Cu (ppm)	(ppm)	Mn (ppm)	(ppm)	S (ppm)	Zn (ppm)
	2	3.95 abc	9.70 c	90 b	46 b	19 e	17 b	319 а	0.12 c	44.5 ab	10.5 de	9.4 a	132.5 bcd	1.1 d
Organia	1	3.8 bc	14.10 abc	124 b	80 b	29 cde	23 ab	336 a	0.19 bc	69 a	27 cde	13 a	233 a	1.85 cd
Organic	4	4.35 ab	13.40 abc	292 ab	94 ab	48 abcde	20 ab	342 a	0.33 a	34.5 ab	54 abcd	14.5 a	92.5 cde	2.8 bcd
	3	4.4 a	17.55 abc	621 a	153 a	114 а	25 ab	324 а	0.37 a	35.5 ab	82 a	16 a	175 abc	3.6 abcd
	5	4.0 abc	11.60 bc	141 b	49 b	35 bcde	26 ab	233ab	0.17 bc	64.5 ab	22 cde	18.5 a	79.5 de	1.55 cd
Low	8	4.1 abc	16.95 abc	315 ab	111 ab	62 abcde	38 ab	222 ab	0.27 ab	27 ab	76 ab	16.5 a	84 de	6.95 a
LOW	7	4.05 abc	13.50 abc	220 ab	73 b	34 bcde	19 ab	309 a	0.12 c	43.5 ab	57.5 abc	24.5 a	204 ab	2.6 bcd
	6	4.2 abc	12.70 bc	148 b	68 b	27 de	24 ab	306 a	0.14 c	32 ab	31 bcde	25.5 a	78 de	2.4 bcd
	9	3.75 с	17.05 abc	414 ab	79 b	98 abcd	40 ab	107 bc	0.12 c	15.5 b	24 cde	14 a	59 de	3.8 abcd
Medium	11	3.8 bc	20.30 a	507 ab	103 ab	117 a	40 ab	94 c	0.15 c	15.5 b	23 cde	15.5 a	58.5 de	4.2 abcd
Wiedfulli	12	3.85 abc	14.75 abc	314 ab	66 b	76 abcde	30 ab	154 bc	0.10 c	29 ab	37.5 abcde	11.4 a	43 e	3.1 bcd
	10	3.9 abc	14.95 abc	431 ab	100 ab	100 ab	53 a	219 abc	0.16 bc	30.5 ab	28.5 cde	19.5 a	77.5 de	5.85 ab
	15	3.85 abc	18.05 ab	375 ab	119 ab	99 abc	28 ab	140 bc	0.14 c	25 ab	9.55 de	16 a	56 de	4.75 abc
High	14	3.85 abc	13.75 abc	261 ab	94 ab	62 abcde	23 ab	148 bc	0.10 c	19.5 b	6.75 e	13 a	52.5 de	2.5 bcd
High	16	4.1 abc	12.15 bc	312 ab	92 ab	64 abcde	20 ab	169 bc	0.10 c	33 ab	14.5 cde	15 a	59.5 de	2.8 bcd
	13	3.9 abc	14.70 abc	297 ab	101 ab	57 abcde	26 ab	157 bc	0.09 c	22 ab	12.55 cde	14 a	61.5 de	2.8 bcd

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. Bold indicates significantly different from highest value; italic indicates significantly different from both).

Management							Al		Cu	Fe		Zn
system	Site	N (%)	Ca (%)	K (%)	Mg (%)	P (%)	(ppm)	B (ppm)	(ppm)	(ppm)	Mn (ppm)	(ppm)
Organic	2	1.53 bc	0.47 a	0.42 a	0.13 c	0.11 a	100 ab	28 c	5.07 a	38 b	2270 ab	16 bc
	1	1.63 bc	0.52 a	0.50 a	0.15 abc	0.12 a	128 ab	25 c	5.66 a	40 b	3530 a	17 bc
	4	1.62 bc	0.56 a	0.45 a	0.16 abc	0.11 a	103 ab	26 c	4.61 a	38 b	2035 bcd	15 bc
	3	1.42 c	0.59 a	0.56 a	0.22 a	0.12 a	127 ab	27 с	5.19 a	39 b	1550 bcdef	16 bc
Low	5	1.58 bc	0.51 a	0.48 a	0.19 abc	0.13 a	117 ab	32 c	4.84 a	56 b	1450 bcdef	17 bc
	8	1.60 bc	0.50 a	0.51 a	0.16 abc	0.13 a	124 ab	32 c	4.42 a	56 b	1885 bcde	18 bc
	7	1.77 ab	0.44 a	0.52 a	0.14 bc	0.18 a	138 a	28 c	4.05 a	100 a	2080 bc	64 a
	6	1.80 ab	0.44 a	0.51 a	0.16 abc	0.14 a	123 ab	37 bc	5.40 a	58 b	1700 bcdef	22 abc
Medium	9	1.80 ab	0.44 a	0.51 a	0.19 abc	0.16 a	77 b	32 c	4.42 a	32 b	920 cdef	14 c
	11	1.76 ab	0.47 a	0.44 a	0.21 ab	0.16 a	93 ab	38 bc	4.30 a	44 b	945 bcdef	14 c
	12	1.75 ab	0.45 a	0.45 a	0.18 abc	0.16 a	90 ab	32 c	3.97 a	42 b	1430 bcdef	14 c
	10	1.75 abc	0.45 a	0.52 a	0.18 abc	0.18 a	82 b	38 bc	5.13 a	34 b	1105 bcdef	16 bc
High	15	1.72 abc	0.47 a	0.49 a	0.17 abc	0.14 a	111 ab	46 abc	4.71 a	56 b	575 ef	58 ab
	14	1.71 abc	0.40 a	0.50 a	0.16 abc	0.13 a	95 ab	55 ab	3.82 a	47 b	482 f	27 abc
	16	1.96 a	0.47 a	0.50 a	0.18 abc	0.14 a	87 ab	62 a	4.33 a	39 b	824 cdef	19 bc
	13	1.72 abc	0.50 a	0.47 a	0.16 abc	0.12 a	95 ab	46 abc	3.96 a	37 b	709 def	17 bc

Table 3. Leaf characteristics of the input management systems compared across sites. The highest value of each parameter and those significantly different from it are in **bold**; the lowest value of each and those significantly different from it are in *italics*.

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. Bold indicates significantly different from highest value; italic indicates significantly different from both).

Figure 1. Leaf nitrogen concentrations in the input management systems compared across systems (α =0.05).

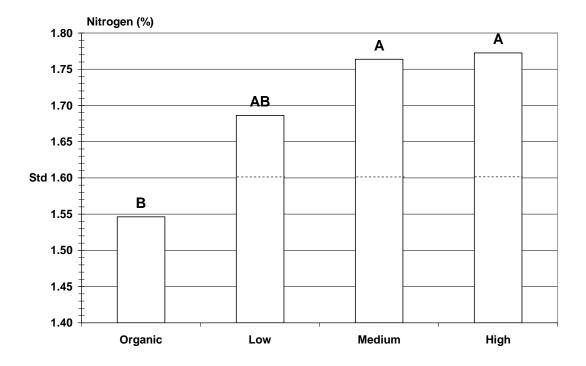


Figure 2. Leaf calcium concentrations in the input management systems compared across systems (α =0.05).

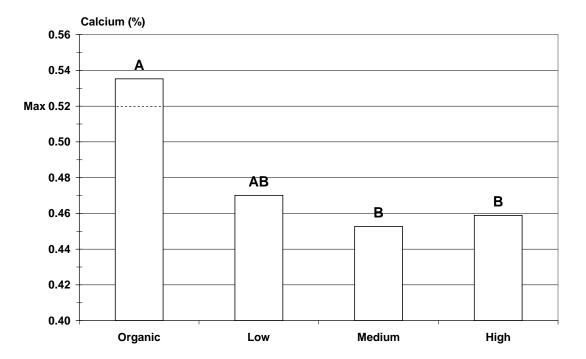


Figure 3. Leaf phosphorus concentrations in the input management systems compared across systems (α =0.05).

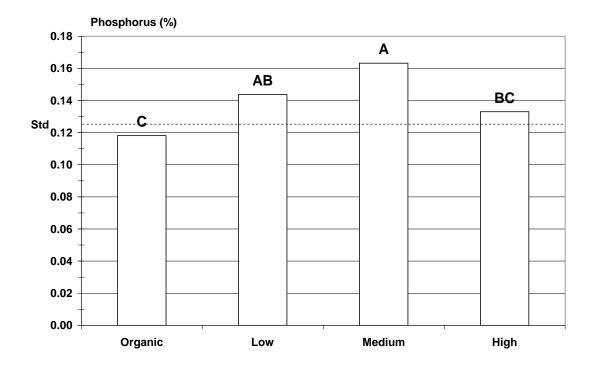


Figure 4. Leaf aluminum concentrations in the input management systems compared across systems (α =0.05).

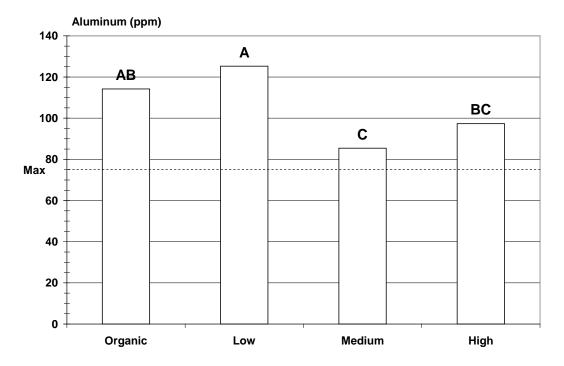


Figure 5. Leaf boron concentrations in the input management systems compared across systems (α =0.05).

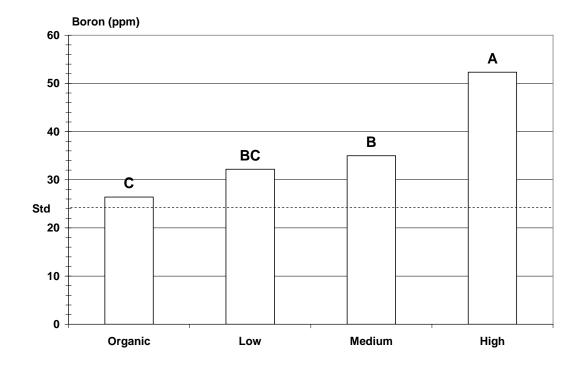


Figure 6. Leaf copper concentrations in the input management systems compared across systems (α =0.05).

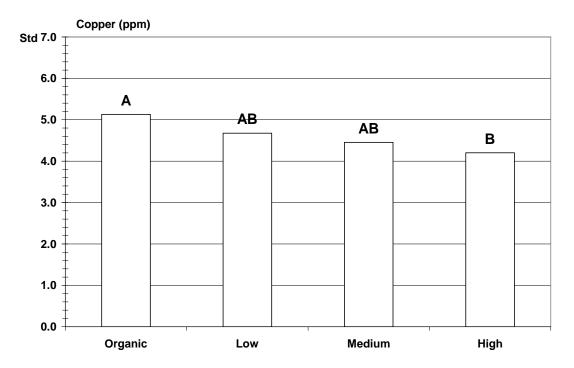
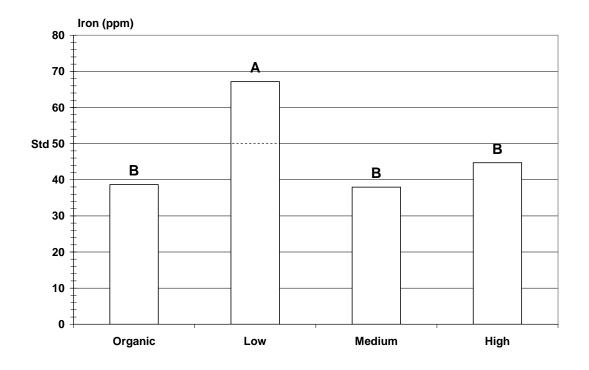
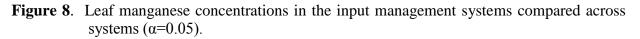


Figure 7. Leaf iron concentrations in the input management systems compared across systems $(\alpha=0.05)$.





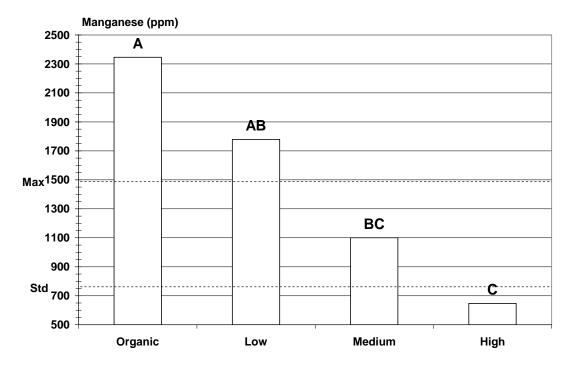
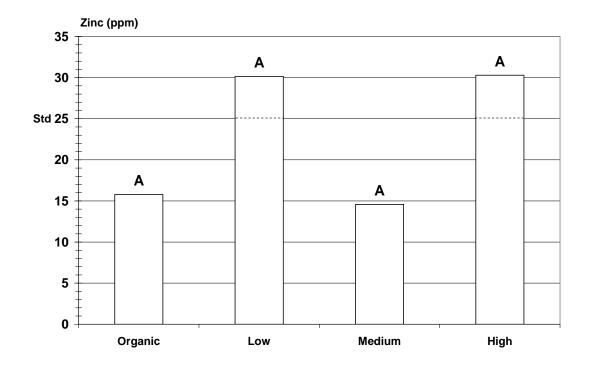


Figure 9. Leaf zinc concentrations in the input management systems compared across systems $(\alpha=0.05)$.



Leaf N was lowest overall at the Organic sites, with two of the three deficient fields in the Organic system (Table 3). The third deficient site was in the Low input system and was a rocky site with shallow soils. Leaf Ca varied with the management system but not among sites (Figure 2, Table 3), although the Organic sites had the highest Ca levels and contributed to the system-level differences. Conversely, Mg did not vary by system but did vary by site (Table 3), with both the highest and lowest values at the Organic sites. When P was compared among sites, all four Organic sites and one High input site were deficient. All sites had Al levels above Trevett's maximum recommended level. The Organic sites had the lowest levels of B, while the High input sites had the highest levels. All sites were deficient in Cu; although there were no differences at the site level, higher average values at the Organic sites had Mn levels above the recommended maximum, while all High input sites were deficient; three Low input sites were also above the maximum. All sites were deficient in Zn except for one Low and one High input site, which were over the maximum, and one High input site which was within the recommended range.

CONCLUSIONS: There are no soil recommendations so these are not used in management, but it is noted that the Low and Organic sites had higher levels of Mn in the fruit from previous organic studies by Yarborough et al. (2009) which is reflected in the higher soil levels. The Organic sites need to improve their leaf N levels to improve their low yields

RECOMMENDATIONS: The high Al leaf levels are an indication of the lower soil pH values in the field and do not appear to affect the yields, so perhaps this value needs to be

increased when the standards are revised. The low Cu and Zn levels also did not affect production and should also be reviewed when the leaf standards are revised. We will research available organic fertilizers to give these growers tools to improve management. This information will 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.

Literature Cited

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Trevett, M.F., P.N. Carpenter, and R.E. Durgin. 1968. A discussion of the effects of mineral nutrient interactions of foliar diagnosis in lowbush blueberries. Maine Agr. Expt. Sta. Bul.665.

Yarborough, D.E., J.M. Smagula, F.A. Drummond, and S. Annis. 2009. Organic Production of Wild Blueberries III. Fruit Quality. Acta Hort. 810:847-852.

INPUT SYSTEMS STUDY

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

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

METHODS: The second crop cycle study design and 2012 prune year inputs are listed in Table 2 of the overall Experimental Design (Report no. 13). As was set up in 2010, 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 m² sample plots to assess weed cover. One 1 m² sample plot was staked on each transect 3 m apart so that the sample plots ranged diagonally across the subplot (Figure 1). In this cycle, the Organic system sample plots were not paired with mulched plots as was done in the previous cycle.

Blueberry cover, woody weed cover, broadleaf weed cover and grass cover were assessed in all 1 m² sample plots in late May/early June (30 May-1 June) and late July/early August (26 July-2 August). Covers were assessed using the Daubenmire Cover Class scale, which were converted to percent; weed species were also identified. The data were analyzed using the Nested General Linear Model (SAS 9.3) 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.

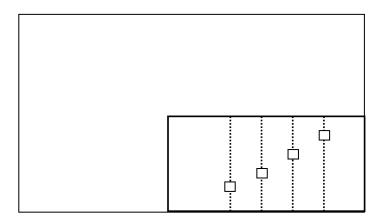
RESULTS: There were no significant differences in blueberry cover among the four systems at either evaluation (Figure 2). Regardless, this year blueberry cover followed the same trend as in 2011; by the second evaluation, the Medium input system had the highest blueberry cover, followed by the High input system. Woody weed cover also followed the same trend as 2011 with the same lack of significant differences among systems in June (Figure 3). In July 2011, the Organic system had significantly higher woody weed cover than the Medium input system,

and the Low, Medium and High input systems were not significantly different. In July 2012, the Organic system had significantly more woody weeds than the High and Low input systems, but the three conventional systems were still not significantly different. Broadleaf weed cover in June was not significantly different among systems, and followed the same pattern of cover as in 2011 (Figure 3, see also 2011 Report #21 Fig. 3). In July, broadleaf weed cover in the Organic system was significantly higher than in the High input system; otherwise, there were no other significant differences. In both June and July, grass cover in the Organic system was significantly higher compared to the three conventional systems, which followed the same pattern of percent cover as in 2011 as well (Figure 3, see also 2011 Report #21 Fig. 3).

CONCLUSIONS: As in 2011, higher inputs resulted in more blueberry cover later in the growing season. The Organic input system had the highest levels of weeds overall, just as it did in 2011, again most likely due to limited or no control measures being implemented. The fact that these trends were consistent with the last cycle despite the addition of two sites per system gives us greater confidence that certain types of management will influence blueberry and weed cover in predictable ways.

RECOMMENDATIONS: None at this time.

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



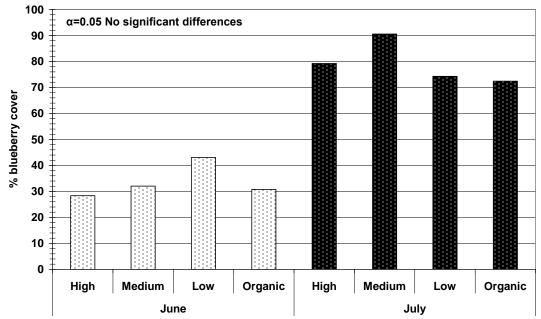


Figure 2. Wild blueberry cover by input system in prune year 2012 (Tukey's HSD, α =0.05).

Figure 3. Woody weed, broadleaf weed and grass cover among input systems in prune year 2012 (Tukey's HSD, different letters denote significance at α =0.05).

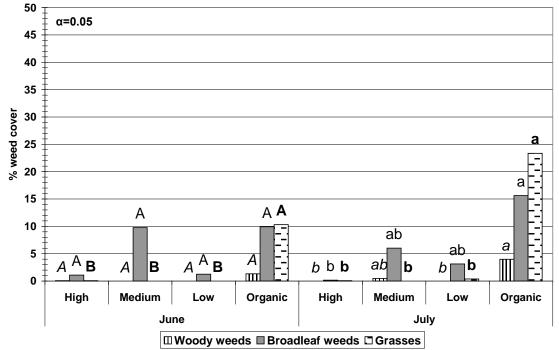


Photo 1. Example of blueberry and weed cover in the High input system at the end of July.



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



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



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



INPUT SYSTEMS STUDY

SOIL HEALTH & CHEMISTRY: Tsutomu Ohno, Professor of Soil Chemistry

19. TITLE: Effects of organic and conventional management systems on the phosphorus solubility of lowbush blueberry barren soils.

INTRODUCTION: Lowbush blueberry (*Vaccinium angustifolium*) is a native perennial woody species typically found on acidic, sandy glacial barren soils in the northeastern United States and eastern Canada. Unlike most agricultural and horticultural crops, lowbush blueberry is not grower-planted, but rather actively managed with fertilizer inputs and weed control in locations where there is sufficient plant density to produce economically viable yields. Almost all of the lowbush blueberry production in the United States takes place on 25,000 ha in the Maine barrens and producing in 2011 a 38 million kg crop with an economic value of \$71 million (USDA, 2012). There has been a recent expansion of lowbush blueberry production outside the traditional United States and Canadian regions and now includes China (Li & Hong, 2009), Japan (Tamada, 2009), and Europe (Abolins *et al.*, 2009).

The lowbush blueberry barrens are typically wide-open, flat to hummocky expanses of glacial outwash deposit terrain located in pristine environments. The soils are acidic and nutrient poor requiring diammonium phosphate fertilizer to increase the levels of available N and P to the blueberry crop for higher yields (Yarborough & Smagula, 1993). Lindsay (1979) has shown that pH 6 to 6.5 is the transition zone from the acid region where aluminum phosphate minerals are likely to control the solubility of soil P and the near-neutral to alkaline zone where calcium phosphate minerals control P solubility. The optimum soil pH for blueberry production is 4.0 (Yarborough, 2009) suggesting that soil Al chemical reactions will be important in controlling the fate of P fertilizer inputs. It has been shown that in acidic soil environments that P reactions with Al on the cation exchange sites of minerals or organic matter control P solubility (Traina *et al.*, 1986).

Organic matter (OM) is a complex, heterogeneous mixture of geo- and bio-molecules involved in important ecosystem processes such as metal solubility and plant nutrient bioavailability (Rember & Trefry, 2004; Ganor et al., 2009). Soil amendments rich in organic matter have been shown to increase soil P solubility (Iyamuremye & Dick, 1996; Easterwood & Sartain, 1990). Soil organic matter may increase P bioavailability through three mechanisms: 1) competing with P for adsorption sites; 2) complexation with surface-bound aluminum or iron to form soluble organic-metal compounds and releasing previously adsorbed P; or 3) adsorbing to soil particles at non-specific sorption sites which increases the surface negative charge of the particle which reduces electrostatic attraction of P to the soil surface (Traina *et al.*, 1986, Guppy *et al.*, 2005).

It is reasonable to hypothesize that the ability of organic matter to form stable complexes with metals such as Al will be involved in the control of P solubility in the acidic blueberry barren soils. Knowledge of the soil chemical reactions of added P fertilizer could significantly assist in the sound environmental management of the blueberry barren soils which are often located in pristine environments close to surface water which could be adversely affected by excessive P fertilization. The goal of this study is to evaluate the effects of an organic and three conventional blueberry production systems on the soil chemical properties related to P solubility. This was done to 1) determine the soil component controlling P solubility in these

soils and 2) to investigate the influence of OM on determining soil P solubility and adsorption processes.

METHODS:

Field sites and soil sampling

A study was initiated in Maine on grower sites to investigate the effects of different blueberry management systems on crop yield and soil quality. The four production management treatments included an organic system and three conventional production systems of low, medium, and high inputs. Organic and Low input sites were on non-leveled land and used burning for pruning the blueberry plants. The Medium and High input sites were on leveled land which allowed the use of flail mowing for pruning. There were no fertilizer inputs for the Organic and Low input treatments, while the High input sites received an optimal level of diammonium phosphate input, and the Medium input treatment received half of the optimal quantity of fertilizer application by limiting the addition to every other harvesting cycle. The growers performed their standard activities on these research plots as part of their management of the surrounding field in production.

The soils were collected from the 12 sites in 2011, the first crop year of the study. The management treatments and the sites were: Organic, Field 1 (F1), Field 2 (F2), and Sedgwick (SW); Low input, Field 5 (F5), Field 6 (F6), and Harrington (HT); Medium input, Field 9 (F9), Field 10 (F10), and Town Line (TL); and High input, Field 13 (F13), Field 14 (F14), and Montegail (MG). Soils were sampled from the surface-10 cm depth at three random locations from each site using a hand shovel. The soils were sieved through a 4-mm screen, and air-dried at room temperature.

Soil characterization

Soil pH was measured using distilled-deionized water (DI-H₂O) at a ratio of 5 g soil:10 mL DI-H₂O. The soils were extracted with the modified Morgan extraction (ammonium acetate, pH 4.8) to determine standard soil test nutrient contents (Wolf & Beegle, 1995). Ammonium oxalate (0.2 M, pH 3) was used to determine the amorphous Al and Fe oxide content of the soils (McKeague & Day, 1966). Sequential P extraction with DI-H₂O; pH 8.5, 0.5 M NaHCO₃; 0.1 M NaOH; and 1 M HCl was conducted to characterize the soil P pool (Tiessen & Moir, 2008). Elemental analysis was done by inductively coupled plasma-atomic emission spectrometry (ICP-AES) on all extraction solutions. The hygrometer method was used to determine soil texture (Gee & Bauder, 1979). Total C and N of the soils were determined using a LECO CN-2000 analyzer.

Water-soluble Ca, Mg, K, Al, Fe, P and dissolved organic carbon (DOC) was extracted by adding 25.0 mL of DI-H₂O to 2.5 g of soil in a 50-mL centrifuge tube. The suspensions were shaken on an orbital shaker for 30 min at room temperature (22 ± 1 °C), centrifuged at 2800 X g for 30 min, and filtered through 0.45 µm Acrodisk syringe filters. The extraction period was selected to minimize microbial alteration during extraction (Zhou & Wong, 2000). Elemental concentrations in the soil:water extracts were determined ICP-AES, except for P which was determined using a flow-through colorimetric analyzer. The DOC concentrations of the extracts were determined using a Shimadzu 5000 analyzer. The equilibrium chemical speciation of its thermodynamic database (J. P. Gustafsson, KTH Royal Institute of Technology, Sweden).

Phosphorus sorption in the presence of DOC was examined using a one-point P sorption index (Hunt *et al.*, 2007). The sorption of P (0.5 mg P g⁻¹ soil) was determined in the absence or presence of DOC (0, 64, or 128 mg DOC l⁻¹). The DOC was extracted from the organic pad present in the study sites to ensure that the DOC used would be representative of the dissolved organic matter found in the soils. Sorbed P was calculated as the initial P solution concentration minus the final P solution concentration.

RESULTS AND DISCUSSION:

Plant foliar nutrient content and fruit yield

Unlike most agronomic crops, fertilization recommendations for lowbush blueberry production are based on foliar analysis and not on soil testing (Yarborough & Smagula, 1993; Yarborough, 2009). The average nutrient foliar concentrations for the plants in the different management treatments are shown in Table 1, as well as the optimum foliar ranges as defined in Maine by Trevett (1972). The foliar nitrogen levels are below the optimum levels for all management treatments. The organic and high input foliar samples were below the concentration recommended for P content. The plants were in or above the range recommended for foliar Ca content for all management treatments. The plants were above the recommended Al levels for all four treatments indicating that plants may be under Al stress. The fertilization guidelines currently recommend 448 kg ha⁻¹ of diammonium phosphate if N level is below 1.6% and P level is below 0.13% (Yarborough & Smagula, 1993). These guidelines indicate that N and P fertilization with diammonium phosphate of the organic, low, and high management treatments may increase blueberry yields above current levels.

The average blueberry yields for the four management systems are shown in Fig. 1. There was a significant effect (p = 0.025) of management system with the high-input system yield being significantly greater than the organic and low- input yields. The % coefficient of variation for the organic (59%) and low-input systems (78%) were much greater than for the medium-input (18%) and high-input (34%) systems. This is likely to be due to the latter two systems being on land-leveled fields allowing for more consistency and uniformity in plant density and field operations in these fields.

Soil test characterizations

The general physical and chemical properties of the 12 soils are shown in Table 2. As typical for soils of the blueberry barrens, the soils were classified as sandy loam or loam with the average sand content in the 56% - 64% range across the management treatment sites. There were no statistically significant (p = 0.05) effects of management treatments on any of the soil chemical test parameters indicating that even the most intensive level of blueberry production practices are not likely to affect soil chemical properties. The soils were acidic with an average pH of 4.54 (Table 2). The exchangeable cation suite of the soils was dominated with Al, which accounted for 75% of the exchangeable cations, followed by Ca with 11% of the exchangeable cations. The low pH and dominance of Al on the exchange complex is likely to make Al the key cation involved in the interaction of the soil with added P fertilizer.

There was a general trend for exchangeable Ca, K, and Mg to be the highest in the organic input management soils and be the lowest in the high conventional input soils. The low levels of the base cations for the high input treatment are likely to be in part due to the greater crop removal as a result from the greater fruit yield for this management treatment (Fig. 1). The organic management treatment soils had the highest level of total C and declined as

management input levels increased (Table 1). This trend is likely to be due to past management histories involving the biennial pruning of the plants by burning. Pruning enhances the development of new stems from the rhizomes which increases yield. Although the medium and high input treatments now use flail mowing for pruning, burning was used in the past. Burning has been shown to lead to the loss of organic matter in soils (González-Pérez *et al.*, 2004).

Sequential soil extraction has frequently been used to fractionate soil P into empirical chemical classification pools (Chang & Jackson, 1957; Hedley et al., 1982). This approach has the potential to provide more information about the chemical nature of soil P than the traditional soil testing approach which is based on a single extraction. The averaged sequential waterextractable; pH 8.5, 0.5 M NaHCO₃-extractable; 0.1 M NaOH-extractable; and 1 M HClextractable P contents for the four management treatment classes are shown in Fig. 2. There was no significant effect of input treatment class on any of the four P fractions determined (p > 0.16) expressed as on either a content (mmol kg⁻¹) or % distribution basis. The smallest pool was the water-extractable fraction which averaged 0.8% of the total P extracted by the four extractants. The small size of this pool is likely to be due the low solubility of soil P compounds (Lindsay, 1979). The bicarbonate-extractable pool averaged 21% and this represents readily available soil P which is adsorbed to soil surfaces. The largest soil P pool was the NaOH-extractable fraction which averaged 67.2% and is a measure of the soil P associated with Fe- and Al-minerals (Tiessen & Moir, 2008). The HCl-extractable fraction is representative of Ca-bound soil P and averaged 11.0%.

The results from the standard soil testing procedures and the sequential P characterization show that current management practices, both organic and conventional, are not creating excessive levels of soil phosphorus. This suggests that there is approximate balance of the P addition (in the mid- and high-input conventional systems) and the amount removed by harvest. The currently recommend 448 kg ha⁻¹ of diammonium phosphate used in Maine may be a reference point to use in other worldwide regions where lowbush blueberry production is starting where the soils are acidic and low in native levels of N and P.

Principal components analysis

Principal component analysis was used to discern multivariate relationships among the 12 soil using soil chemical data shown in Table 1. The first and second principal components explained 44.9% and 24.7% of the variance, respectively (Fig. 3). The conventional management soils clustered in a narrow band and are shown along with its 95% confidence The scores for the three organically managed soils were located away from the band. confidence band of the conventionally managed soils indicating that they were differentiated from conventionally managed soils. This is likely to be due to the influence of the fertilization and other management actions which bring the soil chemical properties to reflect more of the inputs, rather than the native soil chemical properties. Furthermore, there was less similarity between the three organically managed soils than found for the conventionally managed soils as indicated by the non-clustering of the data points representing the organic soils. It is interesting to note that although none of the management treatments have significant effects on the chemical parameters individually, a multivariate data analysis reveals that the organically managed soils are significantly different from the conventionally managed soils.

Soil:water extract characterization

Unlike the modified-Morgan soil test data (Table 2), there were statistically significant effects of management treatment on the elemental concentrations present in the soil:water extracts (Table 3). This difference is likely to be due to the soil:water extracts approximating the nutrients present in soil solution while the soil test modified-Morgan extract is determining the nutrients present on the soil exchange complex in addition to that present in the soil solution. The soil:water extract concentration of nutrients is dynamic as it is determined by the balance between the plant nutrient uptake process which removes nutrients from the soil solution and mass-action process which replenishes the soil solution with nutrients from the soil exchange sites as nutrients are taken up by plants.

The base cations Mg^{2+} and Ca^{2+} were at higher concentration in the organic management treatment than in the three conventional input treatments. In addition, there was a higher level of water-extractable Al concentrations in the organic management soils which is likely a result of two of the three organic management sites having substantially lower pH values (3.96 and 4.11) as compared to the other ten fields. There is a trend in the soil:water extract DOC concentration being the highest in the organic management soils and lowest in the high input treatment soils (Table 3). The concentration of DOC in the 1:10 (m:v) soil:water extracts follows the trend of total soil C data (Table 2). Although the water-extractable soil C constituted an average of only 0.5% of the total soil C in these soils, it is the most reactive fraction of soil organic matter involved in important soil processes such as complexation of metals in soil solution (Zsolnay, 2003).

Equilibrium chemical speciation analysis

Gibbsite is the only $Al(OH)_3$ polymorph typically found in soils. Assuming that gibbsite controls the solubility of Al in soils, the dissolution reaction to express the relationship between pH and Al concentration is:

$$Al(OH)_3 + 3H^+ = Al^{3+} + 3H_2O.$$

This relationship is commonly expressed in logarithmic form as:

$$\log Al^{3+} = \log K_{SO} - 3pH.$$

The log K_{SO} (solubility product) values for crystalline gibbsite varies between studies and have been reported as 8.11 (May *et al.*, 1979), 7.74 (Palmer & Wesolowski, 1992), and 7.97 (Kittrick, 1966). Gibbsite in acid soil environments was reported to have a log K_{SO} of 8.77 (Sposito, 1989). Equilibrium chemical speciation of the data shown in Table 4 was used to calculate the activity of the uncomplexed Al^{3+} species as a function of soil pH for the 12 soils investigated with the stability lines for K_{SO} values 7.74, 8.11, and 8.77 forming a solubility range for crystalline and soil gibbsite (Fig. 4). Eight of the 12 soils are within the equilibrium window for gibbsite indicating that that these soils can be appropriately modeled with the assumption of gibbsite as the Al (oxy)hydroxide mineral where surface chemical reactions take place. One Organic treatment soils was undersaturated crystalline gibbsite. One High and two Medium input soils were slightly below the log K_{SO} 7.74 line indicating possible undersaturation with respect to gibbsite (Fig. 4).

Phosphate solubility in acid soils is typically controlled by sparingly soluble aluminum phosphate compounds (Lindsay, 1979; Pierzynski *et al.*, 1990). The stability diagrams for crystalline and amorphous of variscite (AlPO₄) as reported by Hetrick & Schwab (1992) is shown in Fig. 5. The calculated equilibrium activity of the $H_2PO_4^-$ species in the soil:water extracts from all 12 soils were undersaturated with respect to both crystalline and amorphorus

variscite. This suggests that adsorption reactions with the gibbsite surface were controlling $H_2PO_4^-$ activity, rather than formation of the sparingly soluble solid phase aluminum phosphate compounds through precipitation reactions.

Phosphorus-organic matter interactions

Organic matter can interact with soils to increase the solubility of P in soils (Guppy *et al.*, 2005). The regression fit (denoted by the solid line) of water-soluble P as a function of both DOC and water-soluble Al in this set of 12 soils are both statistically significant (p > 0.05) which suggests that soil organic matter and Al are important factors in determining the soil P solubility (Figs. 6a-b). However, the SS soil data point with the highest water-soluble P level at 16.5 µg L⁻¹ (denoted by the arrow in the figures) is a statistically significant outlier (p > 0.05, Grubbs' outlier test). Both regression equations become non-significant (denoted by the dashed line) with the SS soil data point removed (Figs. 6a-b) which suggests that organic matter does not affect the P solubility in these blueberry barren soils.

The role of DOC on P soil chemistry was further investigated by using a one-point P sorption study (Hunt *et al.*, 2007). There was no significant P sorption inhibition effect of the DOC at both concentration levels for the any of the four management level treatment soils (Fig. 7). This finding supports that the interpretation of Fig. 6a-b with the SS soil data removed as an outlier indicating that organic matter does not affect the water-soluble P concentration in these blueberry barren soils.

The lack of an organic matter effect on P sorption in this study may be due to the high noncrystalline Al (oxy)hydroxide mineral content of the soils as estimated from the oxalateextractable Al content of the soils (Table 2). There was no significant effect of management treatment on the degree of P saturation (DPS) calculated as oxalate-extractable [P/(Al+Fe)] * 100 averaged a relatively low 5.3% indicating there was a large excess of potential sites for P sorption in the soils (Fig. 8). Modeling of soil P adsorption using the non-ideal competitive adsorption model (Abou Nohra et al., 2007) and the Langmuir model (Jiao et al., 2007) have shown that soils have a high binding strength site with low binding capacity, and a low binding strength site with high capacity. The high binding strength site averaged 36% of the total binding sites in these two studies which suggests that with DPS < 9.8% in these blueberry soils, the P found in these soils are bound at the high binding strength sites. The lack of an organic matter effect on P solubility and sorption is likely to be due to the inability of the organic matter ligands to compete with the phosphate anion for the high energy binding sites of the gibbsite. These results suggest that amendment with carbon-rich materials such as compost is not likely to increase the P use efficiency of the fertilizer additions or lead to the increase the soil P bioavailability in the acidic blueberry barrens soil.

CONCLUSIONS: The results from this study show that lowbush blueberry management treatments do not significantly affect the soil chemical characteristics as measured by standard soil tests and sequential P extraction protocol. Equilibrium chemical speciation calculations showed that gibbsite was likely to be controlling the solubility of Al in these soils. Phosphorus was undersaturated with respect to amorphous $Al(OH)_2PO_4$ suggesting that adsorption reactions are controlling the orthophosphate solubility in these soils. The results of these equilibrium calculations suggest that adsorption reaction of phosphate, as well as other chemical amendments used in blueberry production should be focused on gibbsite as the reactive surface. Dissolved organic matter isolated from the organic pad of these blueberry

barren soils did not inhibit the sorption of P in a laboratory study which was likely due to P in these soils being held to the soil through high-energy bonds. Further studies with these soils are needed to gain insights into how management treatments may be used to increase the bioavailability of soil P in a sound manner to preserve the pristine environments frequently surrounding areas of commercial lowbush blueberry production.

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Table 1. Management system effects on the lowbush blueberry foliar concentration of N, P, Ca, and Al and their recommended optimum levels.^a

Element	Organic Input	Low Input	Medium Input	High Input	Recommendation Trevett (1972)
N, %	1.38	1.32	1.51	1.35	1.6-2.0
P, %	0.112	0.127	0.148	0.107	0.125-0.222
Ca, %	0.564	0.585	0.431	0.507	0.27-0.52
Al, ppm	111	114	77	82	50-75

^a Data from Drummond, F.A., and Collins, J., 2012. Abundance of insect pest species and natural enemies in lowbush blueberry fields maintained under different management practices. 2011 Wild Blueberry Project Reports, University of Maine. p. 106-115.

Table 2. Standard soil chemical and physical properties of the lowbush blueberry systems study soils.															
	Input					Total	Total	Soil	Soil	Soil	Soil	Soil	Soil	Oxa	Oxa
Soil	class	pН	Sand	Silt	Clay	С	Ν	Test ^a	Test	Test	Test	Test	Test	late	late
		-						Ca	Κ	Mg	Al	Fe	Р	Al ^b	Fe
					%		•		•		ol kg ⁻¹	•			
F2	Organic	4.11	72	15	13	2.94	0.152	1.23	0.89	0.26	16.2	0.27	0.27	313	70
F1	Organic	3.96	43	40	17	7.31	0.401	1.32	2.99	0.62	14.4	1.23	0.32	194	102
SW	Organic	4.68	56	31	13	8.65	0.332	9.27	1.82	2.52	10.0	1.48	0.24	127	122
F5	Low	4.65	60	23	17	5.03	0.264	1.29	1.66	0.54	13.2	0.36	0.42	191	93
F6	Low	4.65	50	33	17	6.70	0.362	1.09	2.01	0.52	13.2	0.34	0.64	271	102
HT	Low	4.86	61	23	16	4.96	0.240	1.32	1.35	0.48	17.1	0.32	0.39	448	118
F10	Medium	4.53	66	19	15	4.94	0.235	2.25	1.47	1.15	9.5	0.39	0.49	335	68
F9	Medium	4.53	64	22	14	4.93	0.250	2.82	1.17	0.97	9.2	0.29	0.42	337	73
TL	Medium	4.60	61	24	15	1.51	0.096	0.51	0.64	0.11	15.1	0.24	0.12	403	54
F13	High	4.74	62	23	15	3.29	0.172	0.81	1.00	0.23	17.1	0.53	0.29	431	74
F14	High	4.65	61	24	15	2.60	0.116	0.62	0.83	0.13	10.4	0.24	0.21	227	45
MG	High	4.55	46	41	12	3.28	0.166	0.59	0.93	0.22	16.8	0.59	0.39	387	104
	ANOVA ^c	0.09	0.74	0.71	0.21	0.20	0.18	0.41	0.22	0.42	0.55	0.11	0.27	0.32	0.15
1	ANOVA	0.09	0.74	0.71	0.21	0.20	0.18	0.41	0.22	0.42	0.55	0.11	0.27	0.32	0.15
	Average Organic	4.25	57	29	14	6.30	0.295	3.94	1.90	1.13	13.5	0.99	0.28	211	98
Ave	erage Low	4.72	57	27	17	5.56	0.289	1.23	1.67	0.51	14.5	0.34	0.48	303	104
	Average Medium	4.55	64	22	15	3.79	0.194	1.86	1.09	0.74	11.3	0.31	0.34	358	65
Ave	erage High	4.65	56	29	14	3.06	0.151	0.67	0.92	0.19	14.8	0.45	0.30	348	74

^a Soil test Ca, K, Mg, Al, Fe, and P were obtained by the modified (ammonium acetate, pH 4.8) ^b Oxalate-extractable Al, Fe, and P were obtained with 0.2 M ammonium oxalate, pH 3.
 ^c p value of one-way analysis of variance with management class as the factor.

Soil	Input Class	DOC	Ca ²⁺	Mg ²⁺	\mathbf{K}^+	Na ⁺	Al ³⁺	$\mathrm{NH_4}^+$	NO ₃ ⁻	PO ₄ ³⁻
			•	•	mg	g/L	•	•	•	μg/L
F2	Organic	14.5	6.14	1.23	7.48	1.01	2.95	0.444	0.305	7.48
F1	Organic	45.0	3.76	2.23	16.45	2.64	2.94	0.541	0.032	16.5
SW	Organic	42.4	2.24	0.79	6.53	4.03	1.26	0.202	0.026	6.53
F5	Low	20.5	0.73	0.57	6.85	1.47	0.77	0.493	0.204	6.85
F6	Low	24.7	0.39	0.43	7.82	1.80	0.63	0.294	0.090	7.83
HT	Low	21.1	0.43	0.37	6.14	1.65	0.54	0.541	0.215	6.14
F10	Medium	23.8	0.42	0.37	6.71	1.42	0.72	0.056	0.022	6.71
F9	Medium	24.6	0.42	0.30	5.63	1.02	0.63	0.220	0.058	5.63
TL	Medium	7.6	0.81	0.50	5.52	1.69	0.27	0.113	1.020	5.52
F13	High	12.7	0.61	0.43	6.03	1.44	0.37	0.107	0.121	6.03
F14	High	9.9	0.50	0.35	5.84	1.27	0.36	0.135	0.073	5.84
MG	High	11.9	0.31	0.34	6.24	2.39	0.35	0.118	0.080	6.24
1	ANOVA ^a	0.113	0.01	0.03	0.29	0.39	0.01	0.02	0.68	0.31
	Average Organic	34.0	4.05	1.42	10.2	2.56	2.83	0.396	0.121	10.2
Av	erage Low	22.1	0.52	0.46	6.94	1.64	0.65	0.443	0.170	6.94
	Average Medium	18.7	0.55	0.39	5.95	1.38	0.54	0.130	0.367	5.95
Ave	erage High	11.5	0.47	0.37	6.04	1.70	0.36	0.120	0.091	6.04

Table 3. Concentration of selected elements and dissolved organic carbon in 1:10soil:water (m:v) extracts of the lowbush blueberry systems study soils.

a p value of one-way analysis of variance with management class as the factor.

Figure Legends

- Effect organic and low-, medium-, and high-input conventional management system on the lowbush blueberry fruit yield. Figure modified from Drummond, F.A., and Collins, J., 2012. Abundance of insect pest species and natural enemies in lowbush blueberry fields maintained under different management practices. 2011 Wild Blueberry Project Reports, University of Maine. p. 106-115.
- 2. Effect of management treatment on the P pools as determined by sequential P extraction of the lowbush blueberry system study soils.
- **3.** Principle component analysis of soil chemical parameters of the lowbush blueberry system study soils.
- 4. Aluminum activity as a function of pH of the lowbush blueberry system study soils.
- 5. Aluminum phosphate solubilities of the lowbush blueberry system study soils.
- 6. Effect of dissolved organic carbon and Al on water-soluble P concentration in 1:10 soil:water extracts of the lowbush blueberry systems study soils. Dashed regression removes the SS outlier soil denoted with the arrow.
- 7. Percent sorption 0.5 mg P g^{-1} soil to the organic, low, medium, and high input conventional management soils in the presence of 0, 64, and 128 ppm dissolved organic carbon. The error bars indicate the standard deviation of the mean of the three soils in each management category.
- 8. Degree of phosphorus saturation of the organic, low, medium, and high input conventional management treatments.

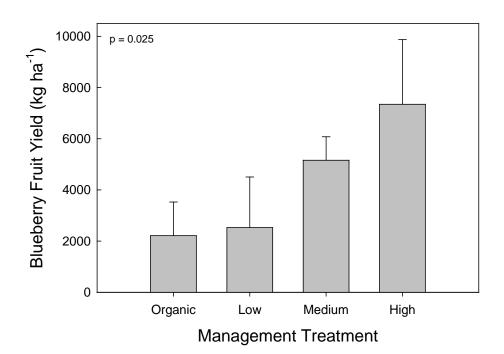


Fig. 1.

Fig. 2.

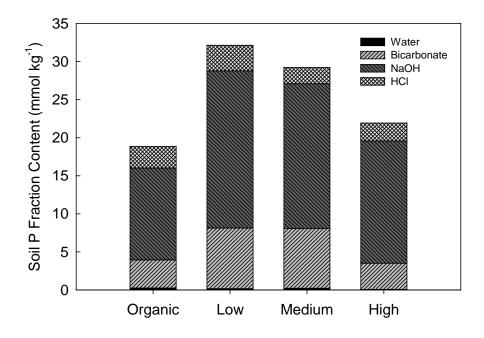


Fig. 3.

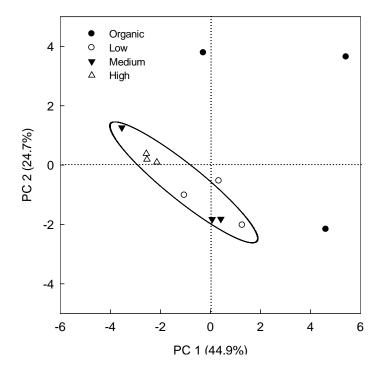


Fig. 4.

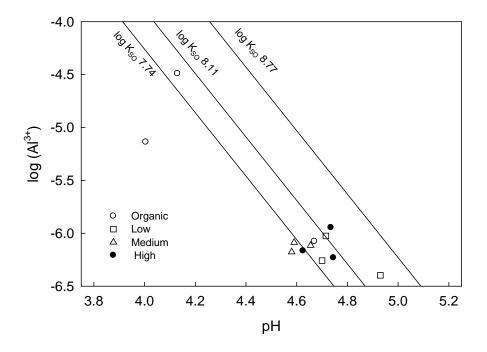


Fig. 5.

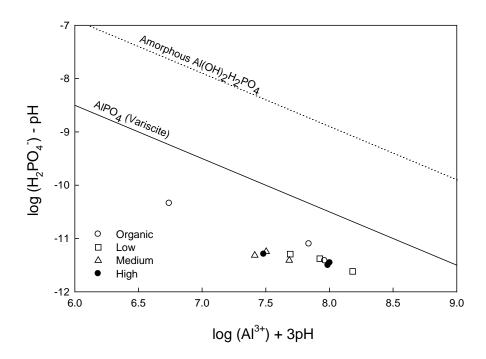
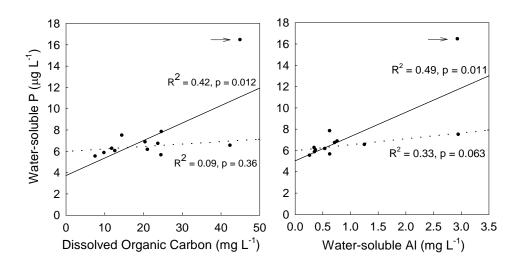
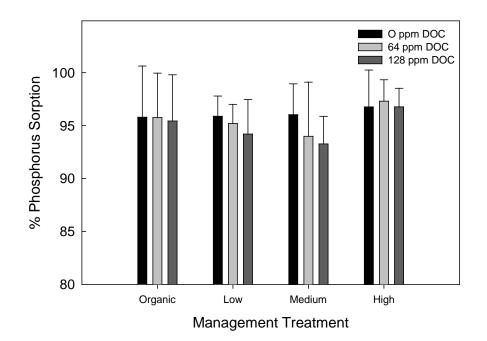


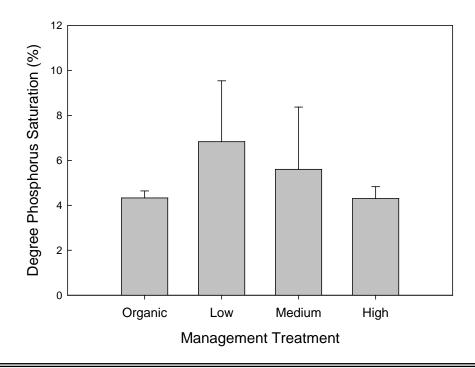
Fig. 6.











INPUT SYSTEMS STUDY

SOIL HEALTH & CHEMISTRY: Ellen Mallory, Assistant Professor of Sustainable Agriculture Hannah Griffin, Research Associate

20. TITLE: Systems approach to improving sustainability of wild blueberry production – soil health and chemistry measures.

METHODS: In spring of 2010, a four-year study was initiated on the effects of different wild blueberry input management systems on: crop growth, yield, quality and food safety; pest levels/dynamics and level of risks to growers; soil health; and economic and ecological costs/benefits. Four management input systems were evaluated, as shown in Table 1 of the overall Experimental Design for the 2012-13 cycle (see report no. 13).

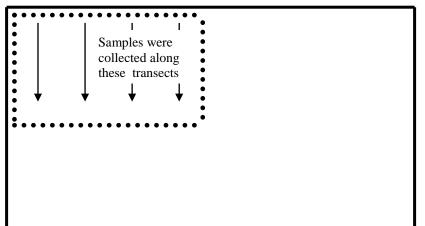
This report covers the soil health assessments that were conducted in 2010 and 2012 for these sites. In 2010, two fields were chosen for each of the four input systems. An additional set of fields was added for soil health characterization and insect monitoring. In the first pair of fields, four one-acre plots were located and flagged in areas with typical blueberry growing conditions. In the second set of fields, one one-acre plot was used per field. In 2012, an additional 2 fields per input level were added for monitoring using two one-acre sampling areas per site (Table 1).

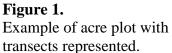
Year of				
Sampling	Organic	Low Input	Medium Input	High Input
2010	Field 1	Field 5	Field 9	Field 13
	Field 2	Field 6	Field 10	Field 14
	Amherst	Harrington	Townline	Montegail
2012	Field 1	Field 5	Field 9	Field 13
	Field 2	Field 6	Field 10	Field 14
	Field 3	Field 7	Field 11	Field 15
	Field 4	Field 8	Field 12	Field 16

Table 1. Sites sampled in 2010 and 2012 for each of the four management systems.

Within each one-acre plot, a subplot was staked out as follows: 30×30 m in the High and Medium input systems, and 15×30 m in the Low and Organic input systems (for the 2010-2011 cycle); in the 2012-2013 cycle, all subplots were 15×30 m. The fields were pruned in the 2010 and 2012 non-crop years, and any pH reduction was applied as necessary as sulfur at a rate of 1000 lb/a per pH unit to reduce soil pH to below pH 4 within two years.

Along the 30 m baseline (the outer long edge of the plot in the High and Medium input systems in 2010) of each subplot, four transects were located 5 m apart in order to set up $1-m^2$ sample plots to assess weed cover and soil health (Figure 1). One $1-m^2$ sample plot was staked on each transect in such a manner that the sample plots ranged diagonally across the subplot. For example, in a 15 x 30 m subplot, the transects were located at 5, 10, 15 and 20 m along the 30 m baseline; sample plot A was located at 3 m on the first transect, B at 6 m on the second, C at 9 m on the third and D at 12 m on the fourth. In 2010, the Organic system subplots also had a mulch treatment, consisting of four transects in each subplot containing mulch strips approximately 2 m wide, 15 m long and four inches deep. Each mulch transect was paired with a non-mulch transect 2 m away, for a total of four mulched $1-m^2$ sample plots and four unmulched $1-m^2$ sample plots per subplot.





Six soils cores were taken at random along each of the transects to a 10-cm depth using a standard soil coring device. A total of 24 soil cores were taken per plot. For each core, the organic soil layer was removed from the coring devise and placed in a plastic bag. The 24 organic samples for each plot were bulked in one bag. The depth of the remaining mineral layer was measured and recorded, and the mineral soil was placed in a separate plastic bag. Again, the 24 mineral samples for each plot were bulked. Organic and mineral samples were sieved through 2 mm screens, dried overnight at 60°C, and then weighed. Samples were submitted to the University of Maine Soil Analytical Laboratory for soil pH, organic matter, phosphorus, calcium, potassium, magnesium, aluminum, iron, sodium, sulfur, zinc, copper, manganese, total carbon and total nitrogen.

RESULTS: The organic pad is the upper-most layer of soil, which contains the most available nutrients and water holding capacity for plant growth and maintenance. Management system more often impacts the organic pad rather than the lower mineral layer. Organic pad depth results are presented with 2010 and 2012 results combined. A two-year site average was used for those sites that were sampled in both years. Average depth of the organic pad ranged from 1.6 cm to 2.2 cm over the four input management systems (Figure 2) but was extremely variable within input management systems (for example, the range of organic pad depths for individual soil cores in the Low system plots was 0.0 to 4.6 cm). Among the input management systems, the Medium system was significantly greater than the others. This system uses mowing to prune as compared with the Low and Organic systems, which use burning. However, the High input system also uses mowing but the organic pad depth was equal to that of the Organic and Low input system field and less than that for the Medium system fields.

Chemical analysis of the 2012 samples are not yet complete, so the results presented here are for the 2010 samples and are the same as those reported last year. Chemical content of the organic pad soil did differ among treatments (Table 2 and 3). Soil pH was highest in the Low input fields and lowest in the Medium and Organic fields. High and Medium input fields had greater CEC than Low and Organic. Organic fields had the highest aluminum and sulfur levels, as well as the lowest phosphorus levels. There were no significant differences among input systems for organic matter, nitrogen, potassium, calcium, copper, or magnesium.

Chemical content of the mineral layer soil also differed among input systems for some measures (Table 4 and 5). The High input fields had the lowest pH, while the Organic fields had the highest. There was also more phosphorus in the High and Medium input fields than in the Low and Organic input fields. Manganese was highest in the Low and Organic fields and lowest in the High and Medium treatments. As in the organic pad, aluminum and sulfur levels were high in the organic soils. As organic growers often treat their fields with sulfur to manage pH levels and weeds, this is not a surprisingly finding. There were no significant system treatment differences for organic matter, CEC, total nitrogen, potassium, calcium, copper, iron, magnesium, sodium, or zinc.

CONCLUSIONS: The thickness and amount by weight of the organic pad was unaffected by the pruning method, contrary to expectations, although the Medium input system did have a deeper organic pad than the other systems. Medium and Organic input systems had the lowest soil pH in the organic layer but High input fields had the lowest in the mineral layer. High and

Medium input systems had greater CEC in the organic layer. Aluminum and sulfur levels were highest in both soil layers in the organic fields.

The soil health and chemistry results should be combined with the results from the other monitoring of these sites (e.g. weed and pest pressure, crop yield, fruit quality) to give a broader comparison among the input management systems.

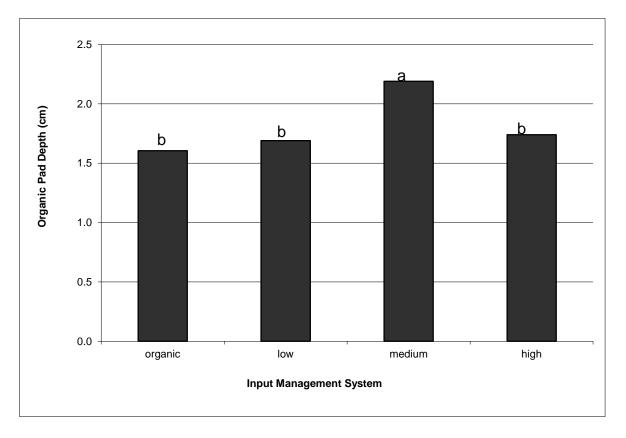


Figure 2. Averaged depth of the organic pad as affected by input management system. Results are from sites sampled in 2010 and 2012. For sites sampled in both years a site average was used. Treatments with no letters in common are significantly different at the 90% confidence level.

Input system	Organic Matter† %	Soil pH	CEC meq/100g	Total N	Phosphorus†† %	
High	30.6	3.94 ab	10.0 ab	0.82	35.5 ab	163
Medium	38.5	3.82 bc	10.9 a	0.96	49.9 a	154
Low	28.7	4.28 a	7.1 c	0.84	30.8 ab	191
Organic	21.0	3.45 c	8.6 bc	0.60	19.6 b	151
ANOVA Results						
Treatment	nsd	**	**	nsd	*	nsd
Site	**	**	**	***	***	***
<u>C.V. (%)</u>	34.4	7.7	18.6	27.7	45.8	36.0

Table 2. Organic layer soil characteristics and macronutrient concentrations as affected by input management system, for sites sampled in 2010.

[†], Organic matter determined by loss on ignition.

††, Phosphorus determined by ICP.

nsd, no significant difference.

*, **, ***, significant at the 0.05, 0.01, 0.001 levels of probability, respectively.

Table 3. Organic layer soil secondary and micronutrient concentrations as affected by input management system for sites sampled in 2010.

Treatments	Aluminum	Calcium	Copper	Magnesium	Sodium	Sulfur
			n	ng/kg		
High	80.1 b	568	0.239	96.8	20.5 a	55.2 b
Medium	75.6 b	584	0.348	147.4	20.5 a	51.4 b
Low	170.3 b	820	0.264	162.1	15.5 ab	50.8 b
Organic	326.6 a	500	0.209	71.7	11.3 b	764 a
ANOVA Results	5					
Treatment	**	nsd	nsd	nsd	*	***
Site	***	***	*	***	*	***
<u>C.V. (%)</u>	66.6	145.4	35.8	61.0	42.5	154.6

nsd, no significant difference.

*, **, ***, significant at the 0.05, 0.01, 0.001 levels of probability, respectively.

Input system	Organic Matter† %	Soil pH	CEC meq/100g	Total N	Phosphorus†† %	Potassium
High	9.48	4.27 b	5.64	0.252	8.23 a	42.8
Medium	7.70	4.53 ab	4.91	0.210	7.79 a	32.9
Low	9.48	4.48 ab	5.63	0.298	5.62 ab	46.1
Organic	8.62	4.67 a	4.30	0.278	2.27 b	52.2
ANOVA Results Treatment Site	nsd *	* nsd	nsd ***	nsd *	** **	nsd ***
C.V. (%)	16.1	5.00	21.7	20.5	46.7	34.7

Table 4. Mineral layer soil characteristics and macronutrient concentrations as affected by system treatment, sampled summer 2010.

[†], Organic matter determined by loss on ignition. Soil treatment averages from no mulch plots. ††, Phosphorus determined by Color.

nsd, no significant difference.

*, **, ***, significant at the 0.05, 0.01, 0.001 levels of probability, respectively.

Input system	Aluminum	Calcium	Copper	Iron	Magnesium	Manganese
			mg	/kg		
High	267 b	114	0.159	32.2	19.3	1.3 b
Medium	226 b	139	0.217	19.6	11.5	4.1 b
Low	257 b	130	0.192	28.3	13.0	11.9 a
<u>Organic</u>	449 a	145	0.175	20.6	8.3	16.8 a
ANOVA Results						
Treatment	**	nsd	nsd	nsd	nsd	***
Site	***	***	nsd	nsd	***	*
<u>C.V. (%)</u>	33.8	57.4	36.1	35.0	59.5	96.5

Table 5. Mineral layer soil secondary and micronutrient concentrations as affected by input management system, sampled summer 2010.

Input system	Sodium	Sulfur	Zinc	
		mg/kg		
High	9.1	41.9 b	1.87	
Medium	10.2	37.9 b	2.02	
Low	6.9	52.0 b	1.78	
Organic	8.6	193.3 a	1.59	
ANOVA Results				
Treatment	nsd	*	nsd	
Site	nsd	***	***	
<u>C.V. (%)</u>	22.4	98.4	37.1	

nsd, no significant difference.

*, **, ***, significant at the 0.05, 0.01, 0.001 levels of probability, respectively.

INPUT SYSTEMS STUDY – ANCILLARY STUDY

DISEASE: Seanna Annis, School of Biology and Ecology

David Yarborough, Plant Soil and Environmental Sciences Caleb Slemmons, Blueberry Disease Research Assistant Jennifer D'Appollonio-Cote, Blueberry Weed Assistant Scientist

21. TITLE: Evaluation of fungicides for control of mummy berry disease.

METHODS: Field trials were set up in complete randomized block designs in two lowbush blueberry fields, Deblois and Columbia Falls, with histories of mummy berry disease. Fungicides (Table 1) were each randomly assigned to a 6' x 30' plot within 8 blocks in total.

Three foot buffer rows were set up between each pair of treatment plots. Fungicide applications were timed using the mummy berry disease forecast¹ method using locally monitored weather to determine conditions favoring disease development (Fig. 1). Fungicides were applied on April 19, May 3 and May 13 via a CO_2 backpack sprayer equipped with a 4 nozzle boom, 8002VS T Jet tips and 50 mesh screens applied at 20 gpa at 35 psi (Fig. 6a). Appropriate surfactants or adjuvants were added as recommended by the manufacturer (Table 1) and the control plots received no applications.

Disease assessment occurred on May 23 and consisted of determining the presence or absence of mummy berry disease symptoms on 40 blueberry stems along a transect through the middle of each plot. A rope with evenly spaced markings was stretched along the transect and the stem closest to each marking was inspected for disease symptoms of the flowers or leaves (Fig. 6b and c). In addition, the numbers of markings that occurred at bare places (missing data) and frost damaged stems were recorded. The percentage of infected stems was the number of infected stems divided by the total number of stems rated for each plot.

Blueberries were harvested in a 2 foot strip down each plot center with a mechanical harvester (Fig. 6d) on August 9 and weighed. For each plot, a subsample of berries was collected and the number of marketable berries in 50 g was counted.

Data were analyzed using PROC GLIMMIX in SAS (Statistical Analysis Software - SAS Cary, NC). The percentage of infected stems data were arcsine-square root transformed and were compared, along with harvest data, using least square means to determine significant differences among treatments ($\alpha = 0.05$).

¹More information about the mummy berry forecast method can be found in UMaine Cooperative Extension Bulletin #217 (http://umaine.edu/blueberries/factsheets/disease)

Trade Name(s)	Application Rate	Active Ingredient(s)	Company
Fontelis (LEM17) + 0.25% v/v Silwet 77*	24 ounces per acre	penthiopyrad	DuPont
Indar $2F + 1\%$	6 ounces per acre	fenbuconazole	Dow
**COC			Agroscience
Propimax	6 ounces per acre	propiconazole	Dow
			Agroscience
Quilt Xcel	14 ounces per acre	azoxystrobin and propiconazole	Syngenta
Quilt Xcel	17.5 ounces per	azoxystrobin and	Syngenta
	acre	propiconazole	
Quash	2.5 ounces per	metaconazole	Valent Ag
	acre		Products
Positive Control -	6 ounces per acre	propiconazole	Syngenta
Tilt			

Table 1. Fungicide treatments tested for their control of mummy berry disease.

*Surfactant **Crop oil concentrate

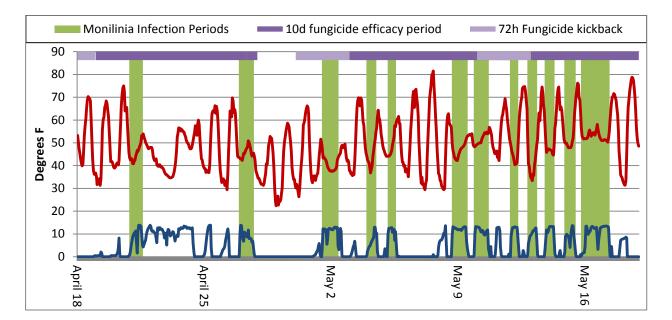


Figure 1. Columbia Falls air temperature (red line) and leaf wetness (blue line) with resulting *Monilinia* infection periods (green bars). Fungicide applications were on 4/19, 5/3 and 5/13 (start of dark purple bars which show a 10 d fungicide efficacy period) and light purple represents a 72 h kickback for certain fungicides.

RESULTS: Measured levels of disease were relatively low with 22.5% at Deblois and 15.9% at Columbia Falls for check plots and ranging from 0.4 to 4.9% for treated plots (Fig 2). This was unexpected since there were 11 to 12 possible infection periods this year (Fig. 1) which is higher than many years. Weather conditions of heavy rain and winds after the last fungicide application may have caused diseased leaves to fall off resulting in the lower detection of disease. Many of the symptoms found during rating were of leaves just starting to show infection (Fig. 6b). Future trials will also include an additional disease assessment approximately half way through the mummy berry infection season.

At both sites, all fungicides provided a significant reduction in *Monilinia* infection compared to the untreated check (Fig 2). All fungicides performed as well as Tilt which is our standard fungicide for comparison. There was slightly less disease in the Tilt and Indar treated plots at Deblois than in the Columbia Falls field. This was probably related to variation in distribution of inoculum and infection periods (at least 11 in Deblois, and 12 in Columbia Falls) within the two fields. A substantial amount of frost damage, up to 28% of stems, was evident in the plots in both fields (Fig. 3), but there was no effect of fungicide treatment on the level of frost damage.

Yields ranged from approximately 6,500 to 11,800 lbs/acre at Columbia Falls and 7,400 to 10,000 lb/acre at Deblois. Plots treated with Tilt, Indar, Propimax and Quash had, on average, significantly higher yield compared to the check at the Columbia Falls site but not at Deblois (Fig. 4). The yield was higher, but not significantly different, in the Quilt (both levels) and Fontelis treated plots at Columbia Falls. There was no significant difference in average berry weight between all of the fungicide treatments and the check (Fig. 5). Presumably, there was not severe enough disease pressure to see any effects upon berry weight.

CONCLUSIONS/RECOMMENDATIONS: Propimax, Quilt Xcel and Fontelis will be mentioned as possible fungicide controls for mummy berry disease in extension information in the future since they have two years of successful control of mummy berry disease. The fungicide Fontelis has a different mode of action than the currently used azole fungicides and so will be a good fungicide to include in rotations with propiconazole to prevent fungicide resistance. Next year, we will be examining fungicides with new chemistries and lower risk materials for rotation with current fungicides.

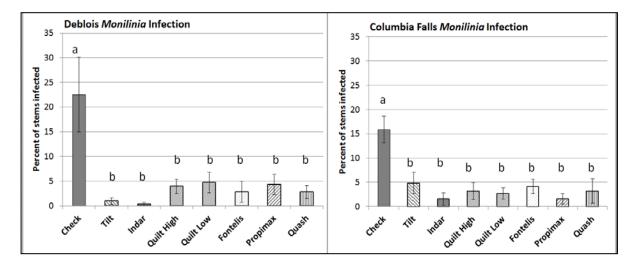


Figure 2. Disease assessment of mummy berry disease in fungicide trials at Deblois (left) and Columbia Falls (right) displaying the average percentage of *Monilinia* infected stems. Error bars represent standard error of the mean of 8 replicates. Bars with different letters were significantly different at p < 0.05.

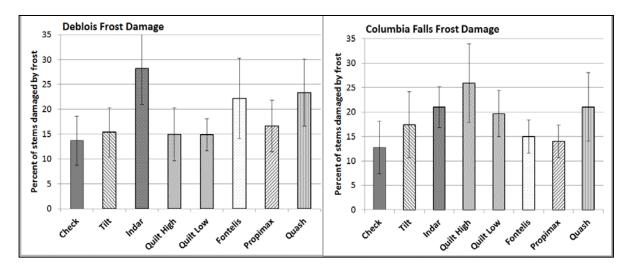


Figure 3. Average percent of stems with frost damage in fungicide trials at Deblois (left) and Columbia Falls (right). Error bars represent standard error of the mean of 8 replicates.

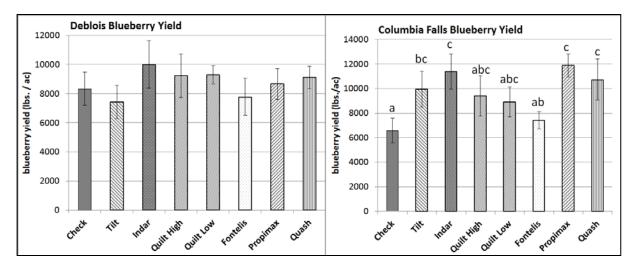


Figure 4. Blueberry harvest of fungicide treatments in fields in Deblois (left) and Columbia Falls (right). Harvest is shown in average yield (lbs/acre). Error bars represent standard error of the mean of 8 replicates. Bars with different letters were significantly different at p<0.05.

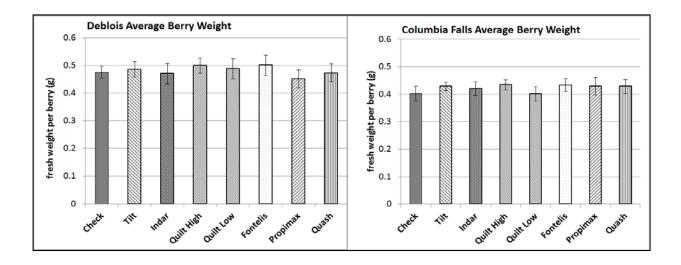


Figure 5. Average berry weight (grams) harvested from fungicide treatments in fields in Deblois (left) and Columbia Falls (right). Error bars represent standard error of the mean of 8 replicates. There were no significant differences among the treatments.



a. Fungicide application with CO₂ sprayer



b. Monilinia leaf infection



c. Assessment of *Monilinia* leaf and flower infection



d. Mechanical harvest, 2' swath through plot center

Figure 6. Pictures showing fungicide spray application, disease assessment and mechanical harvest.

INPUT SYSTEMS STUDY – ANCILLARY STUDY

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

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

METHODS: In order to determine the optimal combination of fertilizer, mulch and herbicides (weed control) that will allow land leveled wild blueberry stands to reestablish in the shortest time frame, an ancillary study was initiated in 2011. A field that had recently been leveled was located on Cherryfield Foods' Barren Pond Lot (BP_3B_7.6) in Township 24. A Randomized Complete Block Design using split/split/nested treatments with eight 60' x 60' blocks was established on 17 May 2011. The blocks were staked in a single line following an access road; on the first split 30' wide fertilizer application was applied in the half of each block further from the road. DAP+boron at a rate of 315 lb/a was applied over two dates, 27 May and 6 June 2011, by Blueberry Hill Farm using a spreader calibrated to a 30' width.

The second split was herbicide application at right angles to the fertilizer application. Each block was split into three 20' x 60' lanes from the road to the back edge of the block. One of three herbicide treatments – none, pre-emergence (1 time) or post-emergence (2 times) herbicide – was randomly assigned to each of the three lanes. The pre-emergence treatment of Velpar 2 lb/a + Sinbar WDG 2 lb/a + Direx 4L 2 lb/a was applied on 27 May 2011 by Blueberry Hill Farm using a tractor mounted boom sprayer. The post-emergence treatment of Callisto 6 oz/a + Arrow 6 oz/a + nonionic surfactant 0.25% v/v was applied in the same manner on 16 and 30 June 2011. This resulted in six 20' x 30' fertilizer/herbicide combinations per block. Within each combination were nested two 1-m² plots; one plot which received 2.5" of mulch on 10 June 2011. Therefore, there were a total of 12 treatments with eight replications each (Figure 1).

This is the first crop year for this project; the trial will continue for another full cycle. Wild blueberry cover, broadleaf weed cover and grass cover were evaluated on 7 June and 7 August 2012. Covers were determined by using a Daubenmire Cover Class scale, which were converted to percent for analysis. Blueberry phytotoxicity was evaluated on a scale of 0-10, which was converted to percent injury (0=none and 10=100% injury/dead). Only main effects were examined using t-tests (α =0.05) with Bonferroni adjustment for herbicide main effects (α =0.0167). The 1-m² plots were scheduled to be harvested in August 2012, but were not because a. weeds were so thick in some areas that twelve 1-m² plots could not be located, and b. nineteen plots were located in clones that had no fruit or so little fruit set that there would have been no measurable harvest. Therefore, yield was not assessed in 2012, but will be in 2014.

RESULTS: There were no significant differences in blueberry or weed cover between the fertilized vs. unfertilized plots in June or August (Figure 2). There were also no significant differences in broadleaf weed or grass cover between mulched plots and unmulched plots at either evaluation, but blueberry cover in the unmulched plots was significantly higher than the mulched plots at both evaluations (Figure 3). There were no significant differences in blueberry cover between the check and either herbicide treatment at either evaluation date

(Figure 4). At the June evaluation, broadleaf weed cover in the check was significantly higher than in either the pre-emergence or post-emergence herbicide treatments, but by August weed cover in the latter two treatments increased so that there were no longer significant differences (Figure 4, Photos 1-3). Grass cover was significantly lower in the post-emergence herbicide treatment, compared to the check, in June but by August there were no longer any significant differences among the treatments vs. the check (Figure 4).

CONCLUSIONS: In 2012 (the 2nd year after leveling), fertilizer no longer had an effect on blueberry or weed covers. The use of mulch continued to reduce blueberry cover, but had no effect on weed suppression. In other trials, we have seen that mulch no longer affected blueberry cover after the first year, but those trials were conducted in established fields which had not been recently leveled. The previous year's herbicide application did not influence blueberry cover in the crop year. As in 2011, the pre-emergence herbicide treatment appeared most effective in suppressing broadleaf weeds over time. The post-emergence treatment followed the same trend as in 2011; that the broadleaf weeds were reduced early in the growing season, but increased later in the season. Finally, in 2012 as in 2011, grass pressure was very low and although the post-emergence herbicide treatment appeared to suppress grasses in June, cover was too low to make any conclusions.

RECOMMENDATIONS: The trial will be continued for an additional cycle. The herbicide and fertilizer treatments will be repeated in spring 2013, blueberry and weed covers will be assessed in 2013 and 2014, and yield will be assessed in 2014. Due to the presence of hard-to-control weeds such as blackberry, sweet fern and spreading dogbane, a wiper treatment may be added in 2013.

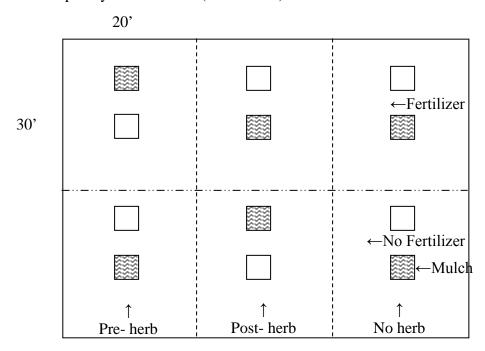


Figure 1. Example layout of a block (not to scale).

Figure 2. Wild blueberry and weed covers for fertilizer main effects – fertilizer versus no fertilizer, in Year 2 of a land-leveling study, 2012 (α =0.05).

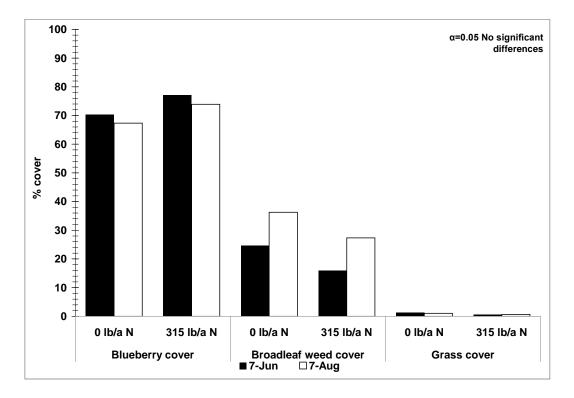


Figure 3. The main effects of mulch on wild blueberry, broadleaf weed and grass covers – mulch versus no mulch, Year 2 of a land-leveling study, 2012 (α =0.05).

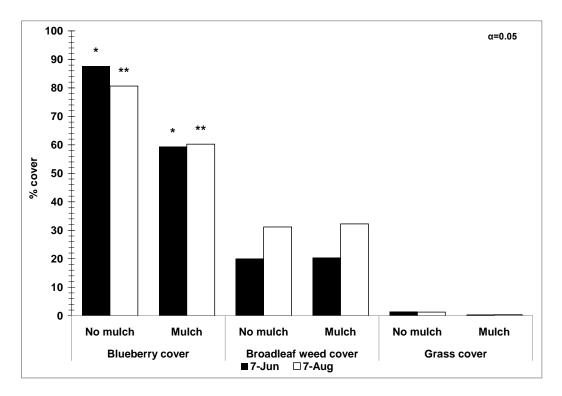


Figure 4. The main effects of herbicide on wild blueberry, broadleaf weed and grass covers – Year 2 of a land-leveling study, 2012 (Different letters denote significance at α =0.0167).

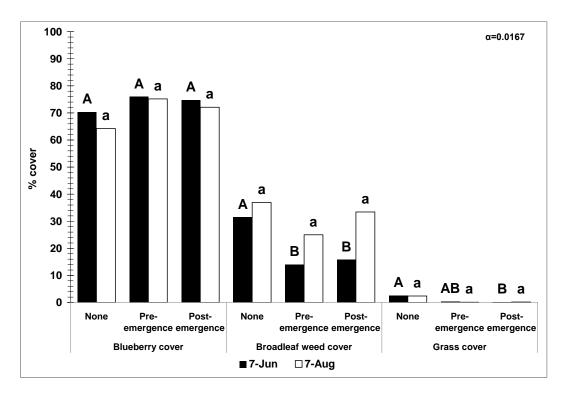


Photo 1. Weed cover in August in a plot with no mulch, fertilizer or herbicide (<5% blueberry cover under weeds).



Photo 2. Blueberry and weed covers in August in a plot with no mulch or fertilizer, and preemergence herbicide applied in 2011.



Photo 3. Blueberry and weed covers in August in a plot with no mulch or fertilizer, and postemergence herbicide applied twice in 2011.



INPUT SYSTEMS STUDY – ANCILLARY STUDY

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

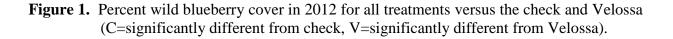
23. TITLE: Pre-emergent combinations of herbicides for weed control in wild blueberry fields – 2012 results from the 2011 trial.

METHODS: In 2011, A Randomized Complete Block Design study was conducted to assess the effects of pre-emergence application of herbicides on herbaceous broadleaf weed and grass cover, wild blueberry cover, and injury to blueberry as compared to a check and the industry standard hexazinone (as Velossa). Six replications of blocks containing 6'x 40' plots of six treatments were located in a non-crop field at Blueberry Hill Farm in Jonesboro, ME. Preemergence treatments were applied on 18 May 2011 at the following rates: Velossa 1 lb/a + Grounded surfactant 1 qt/a, Sinbar WDG 2 lb/a, Sinbar WDG 2 lb/a + Callisto 6 oz/a, Sinbar WDG 2 lb/a + Matrix 4 oz/a, Sinbar WDG 2 lb/a + Lorox DF 2 lb/a, Sinbar WDG 2 lb/a + Lorox DF 2 lb/a + Direx 4L 2 lb/a, Sinbar WDG 2 lb/a + Velpar L 1 lb/a + Direx 4L 2 lb/a, Sandea 1 oz/a, Matrix 4 oz/a, and Alion at 5 and 10 oz/a. On 10 April 2012 half of each Alion plot was retreated with the same rate of Alion as the original treatment. All plots were reassessed for percent covers by sampling two 1m² quadrats per plot on 20 August 2012, and the plots were harvested the next day. Cover was evaluated using a Daubenmire Cover Class scale, which were converted to percent. Blueberry phytotoxicity was evaluated on a scale of 0-10, which was converted to percent injury (0=none and 10=100% injury/dead). Yield was weighed in ounces and then converted to lb/a. Because the cover data failed the assumptions of the General Linear Model, the data were analyzed with a non-parametric one-way median exact test (α =0.05) and each treatment was compared individually to the check and to the Velossa. Percent cover in the retreated vs. not retreated Alion plots were compared using the t-test procedure (α =0.05). Yield was analyzed using the Least Significant Difference test (α =0.05) for differences among all treatments.

RESULTS/DISCUSSION: There were no significant differences in blueberry cover in 2012, except for the Alion 5 oz/a treatment, which was significantly lower compared to the check (Figure 1). Broadleaf weed cover was not significantly different from Velossa for any treatment; however, the Sinbar and Matrix treatments had significantly more broadleaf weeds compared to the check (Figure 2). Grass cover in all Sinbar treatments was significantly lower than the check, and was substantially lower than in the Matrix, Sandea or Alion treatments (Figure 3, Photo 1A-B). No treatment had significantly different grass cover compared to Velossa. When blueberry, broadleaf weed and grass covers were compared between the halves of the Alion plots retreated in 2012 and those that were not, there were no significant differences (Figure 4).

The Sinbar + Lorox treatment had the highest blueberry yield, but was not significantly different from any other treatment except for being significantly higher than the Sandea and the Alion 10 oz/a treatment (Figure 5). Site effects may have played a factor; Blocks 1 and 4 were less weedy overall, and yields were higher in Blocks 1-3 regardless of treatment. However, the results for Sandea and Alion indicated that a late application of either herbicide will stunt blueberry growth which has resulted in a reduction in yield.

CONCLUSIONS: We had re-treated the Alion plots in 2012 to determine if an additional preemergence application in the crop year would reduce weeds. A crop year application of Alion did not suppress the weeds present and so does not offer any significant benefit. The yield results for Sandea and Alion 10 oz/a show the carry over effect of the significant phytotoxicity seen in these two treatments in 2011. The plots were treated just before emergence in May, and the effect of the late pre-emergence application resulted in significantly reduced yields. We have a trial in progress for 2012-2013 looking at the effects of application date and rate of these two herbicides on percent cover (2012+2013) and yield (2013). The results of this trial confirm that late application of either herbicide will reduce blueberry cover. Once yield on the 2012 trial is analyzed in 2013, we will be able to make more definitive recommendations regarding the proper timing of these two herbicide products.



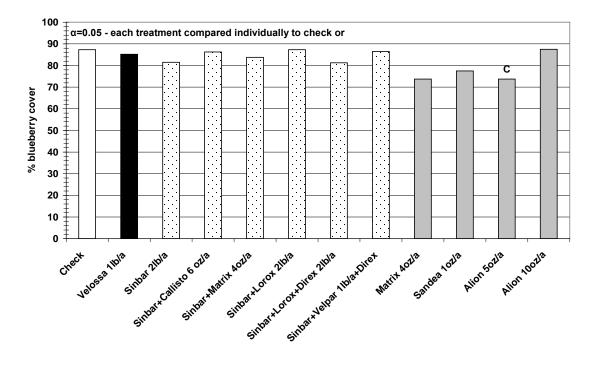


Figure 2. Percent broadleaf weed cover in 2012 for all treatments versus the check and Velossa (C=significantly different from check, V=significantly different from Velossa).

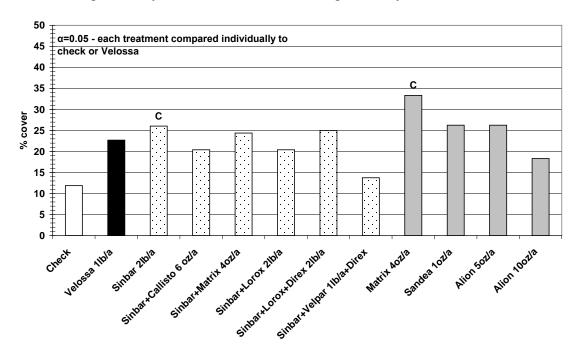


Figure 3. Percent grass cover in 2012 for all treatments versus the check and Velossa (C=significantly different from check, V=significantly different from Velossa).

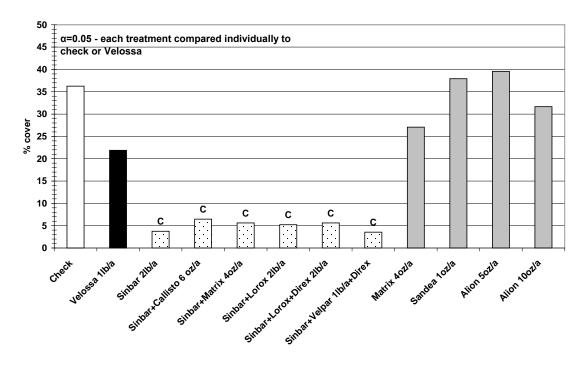


Figure 4. Percent wild blueberry, broadleaf weed, and grass cover in the Alion plots retreated in 2012 versus those that were not retreated.

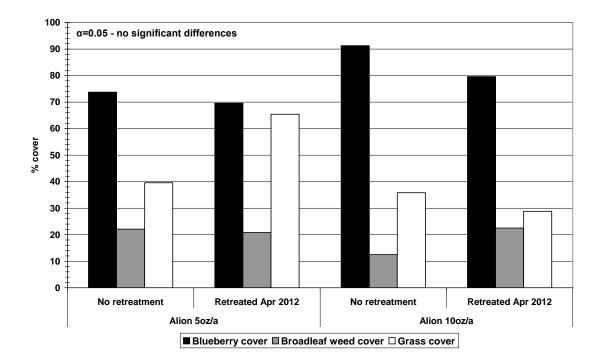


Figure 5. Blueberry yield (lb/a) in 2012 (different letters denote significance at α =0.05).

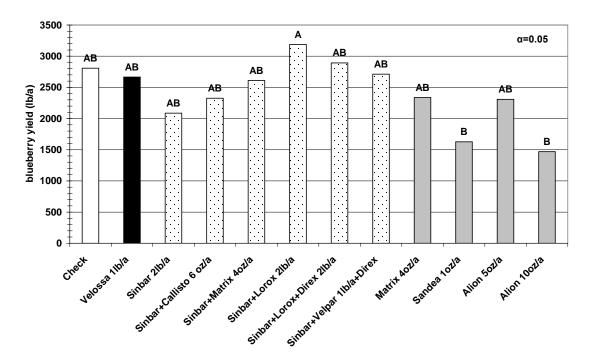


Photo 1A-B. Sinbar only treatment (A. Block 4) in early July 2012 from 2011 application; Sinbar + Matrix treatment (B. Block 1) in mid August 2012.



INPUT SYSTEMS STUDY – ANCILLARY STUDY

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

24. TITLE: Pre-emergent combinations of herbicides for weed control in wild blueberry fields – 2012 trial.

METHODS: A Randomized Complete Block Design study was conducted in 2012 to continue the research begun in 2011 - namely, to assess the effects of pre-emergence application of herbicides on herbaceous broadleaf weed and grass cover, wild blueberry cover, and injury to blueberry as compared to a check and the industry standard hexazinone (as Velpar). Six replications of blocks containing 6'x 40' plots of eight treatments were located in a non-crop field at Blueberry Hill Farm in Jonesboro, ME. Pre-emergence treatments were applied on 7 May 2012 at the following rates:

- 1. Velpar L 2 lb/a (hexazinone)
- 2. Sinbar WDG 2 lb/a (terbacil)
- 3. Sinbar WDG 2 lb/a + Callisto 6 oz/a (mesotrione)
- 4. Sinbar WDG 2 lb/a + Lorox DF 2 lb/a (linuron)

5. Sinbar WDG 2 lb/a + Lorox DF 2 lb/a + Direx 4L 2 lb/a (diuron)
6. Sinbar WDG 2 lb/a + Lorox DF 2 lb/a + Velpar L 1 lb/a
7. Sinbar WDG 2 lb/a + Direx 4L 2 lb/a + Velpar L 1 lb/a
9. Untrasted sheets

8. Untreated check.

Plots were assessed on 6 June, 9 July and 6 August 2012 by sampling two $1m^2$ quadrats per plot. Covers were evaluated using a Daubenmire Cover Class scale, which were converted to percents. Blueberry phytotoxicity was evaluated on a scale of 0-10, which was converted to percent injury (0=none and 10=100% injury/dead). The data were analyzed with a non-parametric one-way median exact test (α =0.05) and each treatment was compared individually to the check and to Velpar.

RESULTS/DISCUSSION: The Sinbar + Callisto treatment had significantly higher blueberry cover than the Velpar treatment in June, but in July and August there were no significant differences for any treatment (Figure 1). Phytotoxicity was very low in all treatments and even the Sinbar + Direx + Velpar treatment, which was significantly higher than the check, was less than 5% so there was no unacceptable injury (Figure 2).

Although Lorox is registered for control of selected broadleaf weeds, it did not appear effective in this trial, as the broadleaf weed cover in the Sinbar + Lorox treatment was significantly higher than the Velpar treatment in June, and both Velpar and the check in July (Figure 3, Photos 1-2). Reduction in weeds from July to August was most likely due to senescence of dominant weeds such as *Rumex acetosella* (red sorrel) and *Apocynum androsaemifolium* (spreading dogbane). Sinbar alone and Sinbar + Lorox + Direx also resulted in significantly higher broadleaf weed cover compared to Velpar. The addition of Velpar to Lorox and Sinbar resulted in the best broadleaf weed control of the Sinbar combinations (Photo 3). Sinbar + Callisto initially controlled broadleaf weeds almost as well as Velpar, but the effect was not long-term enough to control late season weeds. Suppression of grasses by the combinations can release broadleaf weeds, resulting in less control than a broadleaf weed herbicide alone.

Grass pressure in this trial was low. Initially, conditions were perfect for grass growth during the spring. However, although we had sufficient rain, summer temperatures were consistently too hot for optimal grass growth. Regardless, all Sinbar combinations except for Sinbar + Lorox significantly suppressed grasses compared to the check or Velpar (Figure 4). Germination of late season grasses such as *Panicum capillare* (witchgrass) resulted in an increase in grasses in the Sinbar + Lorox and Sinbar + Direx + Velpar treatments in August.

CONCLUSIONS/RECOMMENDATIONS: Sinbar + Lorox did not effectively control broadleaf weeds, and was the only Sinbar combination that did not consistently significantly reduce grasses compared to the check or Velpar. Therefore, Sinbar + Lorox was not effective as a combined application to control broadleaf weeds and/or grasses. However, the addition of Velpar to Sinbar + Lorox was the only combination to have broadleaf weed control comparable to Velpar alone, so Sinbar + Lorox + Velpar was effective for combined application to control both broadleaf weeds and grasses. Sinbar + Callisto was also effective for control of broadleaf weeds and grasses, but post-emergence application of a broadleaf weed herbicide may be necessary to control late season species. Sinbar + Direx + Velpar was also effective but Direx was not as effective in controlling broadleaf weeds as Lorox in combination with Sinbar and Velpar, and did not control late-germinating grasses as well.

Figure 1. Percent lowbush blueberry cover for all treatments versus the check and Velpar (C=significantly different from check, V=significantly different from Velpar).

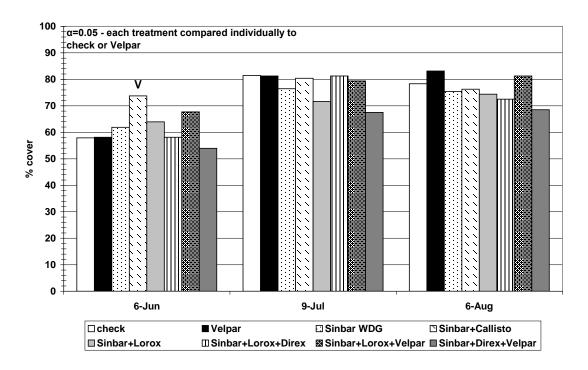


Figure 2. Phytotoxicity for all treatments versus the check and Velpar (C=significantly different from check, V=significantly different from Velpar).

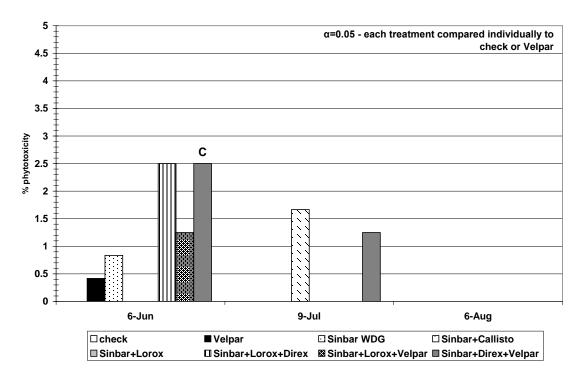


Figure 3. Percent broadleaf weed cover for all treatments versus the check and Velpar (C=significantly different from check, V=significantly different from Velpar).

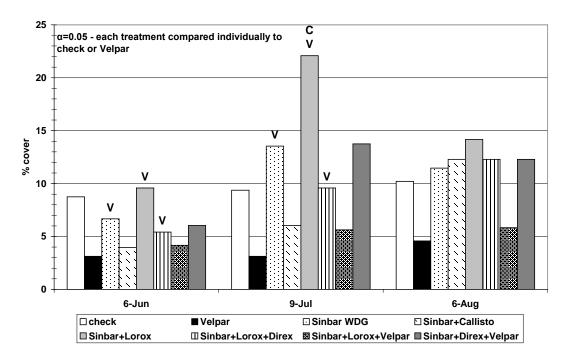


Figure 4. Percent grass cover for all treatments versus the check and Velpar (C=significantly different from check, V=significantly different from Velpar).

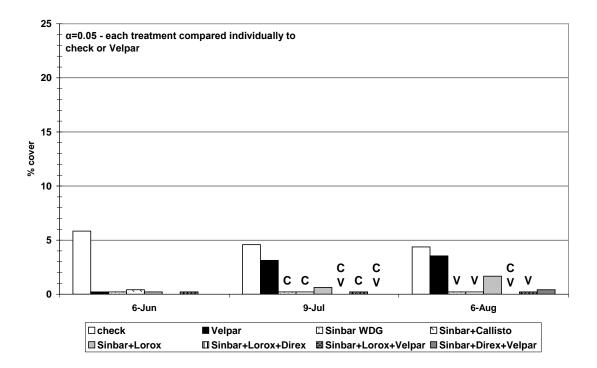


Photo 1. Broadleaf weeds in the check at the July evaluation.



Photo 2. Broadleaf weeds in the Sinbar + Lorox treatment at the July evaluation – more weedy than the check.



Photo 3. Broadleaf weeds in the Sinbar + Lorox + Velpar treatment at the July evaluation – less weedy than the check.



INPUT SYSTEMS STUDY – ANCILLARY STUDY

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

25. TITLE: Evaluation of herbicides for control of fineleaf sheep fescue for grass control in wild blueberries.

METHODS: The experimental site was established on a commercial blueberry field on Masons Bay Road in Jonesport that had an extensive cover of fineleaf sheep fescue (*Festuca filiformis*) which was not controlled by either pre-emergent applications of Velpar or post-emergent applications of Poast (Photo 1). Plots were 6 x 40 feet in a randomized complete block design with six replications. Kerb 50W at 2 lb/a was applied on 10 November 2011 when the soil temperature was below 50°F and rain was anticipated within the next day. Pre-emergence treatments applied on 20 April 2012 were: Kerb 50W at 2lb/a, Sinbar WDG 2 lb/a +Direx 4L 2 lb/a +Velpar L 1 lb/a = "Trimix", Matrix SG 4 oz/a, and Lorox DF 2 lb/a. Post-emergence treatments applied twice on 24 May and 8 June 2012 were: Arrow 8 oz/a + NIS 0.25% v/v, and Option 1.5 oz/a + MSO 1.5 pt/a + AMS 1 qt/100 gal.

Blueberry phytotoxicity (%), blueberry cover, fescue grass cover, other grass/sedge cover and broadleaf weed cover were assessed in all sample plots after the last post-emergence application on 28 June, and then on 12 July and 14 August. Blueberry and weed cover were assessed using the Daubenmire Cover Class scale, which were converted to percent; and weed

species were also identified. Data were analyzed using Duncan's Multiple Range Test with α =0.05.

RESULTS: Initially, the Matrix treatment had the highest blueberry cover and was significantly higher than all other treatments except for the Fall Kerb treatment (Figure 1). The Fall Kerb treatment had increased blueberry cover and was the highest in July and August, but was not significantly higher than any treatment except for Lorox, and Arrow in July only. Blueberry phytotoxicity was not unacceptably high in most treatments, but the Lorox and Option treatments did have significantly more phytotoxicity than the others in June (Figure 2). Both treatments had significantly higher phytotoxicity in July, and Lorox continued to have phytotoxicity into August but was only at 5%, an acceptable level.

When broadleaf weed cover was assessed, the Kerb treatments were consistently lower than the check but were not significant (Figure 3). Option was the least effective on broadleaf weeds, but there were no significant differences found until the August assessment when Option became significantly higher than the Trimix treatment. Trimix resulted in the least broadleaf weed cover overall.

Fall Kerb and Matrix gave the best fineleaf sheep fescue control (Figure 4, Photo 2-3). Spring Kerb reduced fineleaf sheep fescue cover but did not have long-term control (Photo 4B), while Arrow (Photo 4A) and Lorox were not effective. Option and Trimix gave limited control; they were significantly lower than the check but significantly less effective than Fall Kerb or Matrix. In July and August, it was noted that although the Lorox and Option treatments did not eliminate fineleaf sheep fescue, the inflorescences were stunted (Photo 5A-B). Pressure from other grasses was very low (<10%) and there were no significant differences in control (Figure 5).

CONCLUSIONS/RECOMMENDATIONS: Kerb fall treatment was most effective in controlling fineleaf sheep fescue. Spring treatment had mediocre control of fescue and did not last long-term. Both treatments were fair in controlling broadleaf weeds. Arrow did not control broadleaf weeds or fescue. Lorox had good control of broadleaf weeds, and reduced the height of fescue inflorescences but did not control it. Matrix showed minimal control of broadleaf weeds but had excellent control of fescue, while Option had higher levels of broadleaf weeds than the check, but gave good control of fescue. Trimix was the most effective in controlling both broadleaf weeds while also exhibiting good control of fescue.

A fall Kerb application or pre-emergence Matrix application are the best materials to control herbicide-resistant fineleaf sheep fescue. Arrow, spring Kerb and Lorox are not effective on fields with resistant fescue populations. Option should not be used if there are also broadleaf weeds, but if tank mixed with an effective broadleaf herbicide such as Velpar, could be a possible solution. Finally, Trimix treatment could be sufficient on fields with low resistant fescue grass pressure.



Photo 1. Resistant fineleaf sheep fescue in a commercial blueberry field in Jonesport, ME.

Figure 1. Percent wild blueberry cover following application of pre- and post-emergence herbicides for the control of fineleaf sheep fescue.

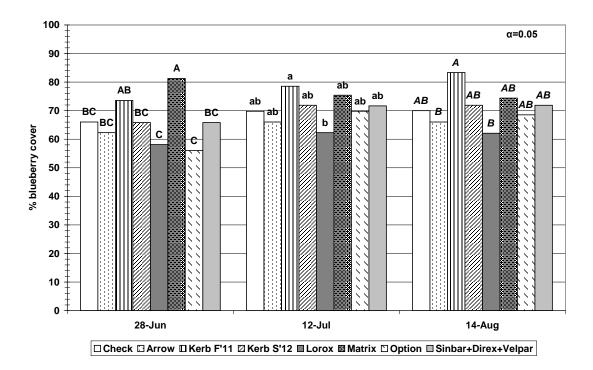


Figure 2. Wild blueberry phytotoxicity following application of pre- and post-emergence herbicides for the control of fineleaf sheep fescue.

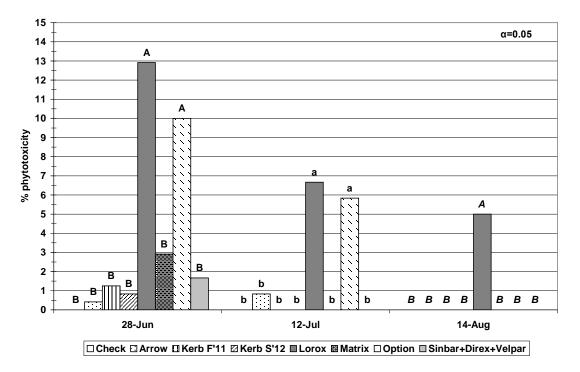


Figure 3. Percent broadleaf weed cover following application of pre- and post-emergence herbicides for the control of fineleaf sheep fescue.

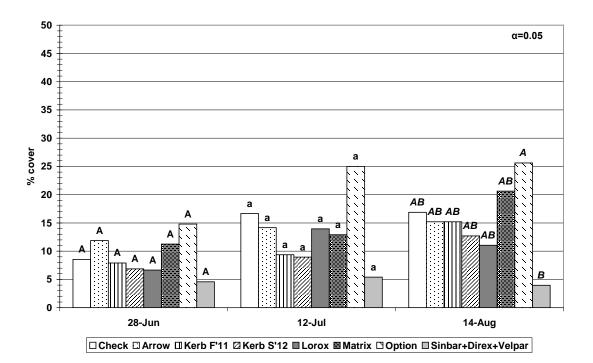


Figure 4. Percent fineleaf sheep fescue cover following application of pre- and postemergence herbicides for the control of fineleaf sheep fescue.

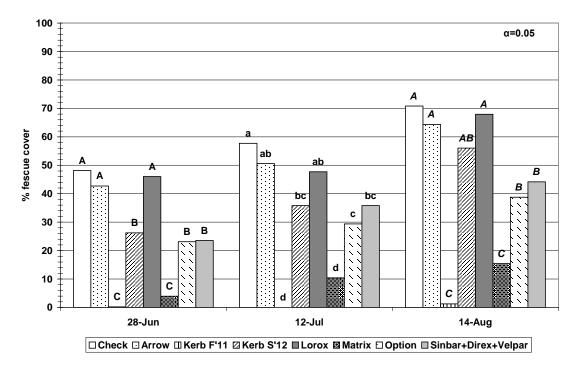


Figure 5. Percent cover of other grasses following application of pre- and post-emergence herbicides for the control of fineleaf sheep fescue.

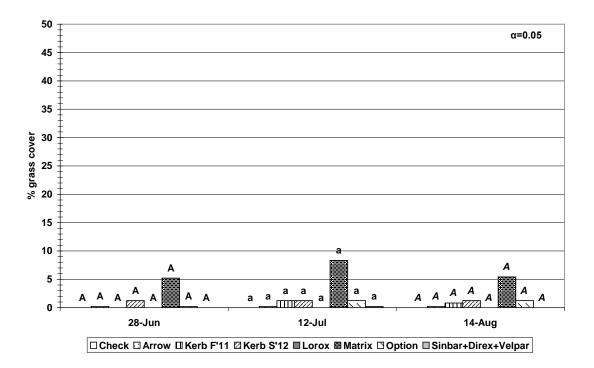


Photo 2. The Matrix treatment did not effectively control broadleaf weeds in this trial, but did control fineleaf sheep fescue.



Photo 3. Fall Kerb application resulted in the best fineleaf sheep fescue control.



Photo 4A-B. Arrow (A) did not control fineleaf sheep fescue, while spring Kerb (B) gave mediocre control.



Photo 5A-B. Lorox and Option did not control fineleaf sheep fescue but did reduce the height of inflorescences (Lorox treatment pictured here).



INPUT SYSTEMS STUDY – ANCILLARY STUDY

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

26. TITLE: Pre-emergence application timing and rate of Alion and Sandea in combination with Velpar or Sinbar on weed control and injury to wild blueberry.

METHODS: In the spring of 2012, we initiated a trial to continue the assessment of two herbicides under consideration for registration for use on wild blueberry. Last year, indaziflam (Alion) and halosulfuron (Sandea) were tested for weed control and potential injury to wild blueberry. Phytotoxicity to blueberry was observed and was thought to be a function of the timing of application; therefore, in this trial we evaluated the effects of applications at three timings and two rates for Alion and Sandea alone and in combination with hexazinone (Velpar) or terbacil (Sinbar).

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

- 1. check
- 2. Alion 5 oz/a early
- 3. Alion 5 oz/a mid
- 4. Alion 5 oz/a late
- 5. Alion 10 oz/a early
- 6. Alion 10 oz/a mid
- 7. Alion 10 oz/a late
- 8. Sandea 1 oz/a early
- 9. Sandea 1 oz/a mid
- 10. Sandea 1 oz/a late
- 11. Sandea 2 oz/a early
- 12. Sandea 2 oz/a mid
- 13. Sandea 2 oz/a late

The 6'x 60' plots were split into three 20' lengths with Sinbar at 2 lb/a or Velpar at 1 lb/a applied at right angles to the main treatments on 18 April. This resulted in 39 total treatments, as seen in Figure 1. There were 3' alleys between the plots to allow visual comparison of stems from the same clone inside vs. outside the plots.

Velpar →													↔ 6'	
Sinbar \rightarrow														
	↑ A 5 E	↑ S 1 M	↑ C K	↑ S 2 M	↑ A 10 L	↑ A 5 L	↑ S 2 E	↑ S 1 L	↑ A 10 M	↑ S 2 L	↑ S 1 E	↑ A 5 M	↑ A 10 E	20'

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

Plots were assessed on 19 June, 23-24 July and 22+24 August by sampling one 1-m^2 quadrat per 20' section. Covers were evaluated using a Daubenmire Cover Class scale, which were converted to percent. Blueberry phytotoxicity was evaluated on a scale of 0-10, which was converted to percent injury (0=none and 10=100% injury/dead). The data were analyzed with a non-parametric one-way median exact test (α =0.05) and each treatment was compared individually to the check.

RESULTS/DISCUSSION:

Alion combinations

Blueberry cover in the Alion combination treatments was comparable to the check, with two exceptions. At the June evaluation, the Late Alion 10 oz/a treatment had significantly less blueberry cover than the check (Figure 2). Conversely, at the July evaluation, the Early Alion 5 oz/a + Velpar treatment had significantly more blueberry cover compared to the check.

Phytotoxicity in the Alion combinations was observed mainly as stunting and delayed emergence of the blueberry plants compared to the same clones' height and percent cover in the adjacent alleys. Some chlorosis was also observed, but it was not the main component of phytotoxicity. Phytotoxicity to blueberry at the June evaluation was significantly higher than the check in all of the Late Alion treatments regardless of the rate or combination with Velpar or Sinbar (Figure 3). However, the 10 oz/a rate resulted in over twice the severity of phytotoxicity compared to the 5 oz/a rate regardless of combination with Velpar or Sinbar (Photo 1A-B). There was also residual stunting observed in the Late Alion 5 oz/a + Velpar treatment at the August evaluation, but at less than 5% it is not cause for concern.

Broadleaf weed cover in the Alion-only treatments was initially not significantly different from the check, with the exception of the Late 10 oz/a treatment, which was significantly lower (Figure 4). There were no significant differences at the July evaluation; by August, broadleaf

weed cover was higher in all treatments compared to the check, but only the Late 5 oz/a treatment was significantly so. When Alion was applied in conjunction with Sinbar, broadleaf weed cover was initially the same or lower than the check at the June evaluation, but only the Mid 5 oz/a and Late 10 oz/a treatments were significant (Figure 5). Broadleaf weed cover in the Alion + Sinbar treatments remained lower than the check in July, but by August all were higher than the check except for the Mid 10 oz/a treatment; but there were no significant differences. When Alion was applied in conjunction with Velpar, broadleaf weed cover in all treatment combinations were significantly lower than the check at the June evaluation, but in July and August there were no significant differences (Figure 6).

Grass cover in the Alion-only treatments was lower than the check at all three evaluations (Figure 7). In June, all treatments except the Mid 5 oz/a and 10 oz/a treatments had significantly lower grass cover compared to the check, while in July all did. In August, only the Late 5 oz/a and 10 oz/a treatments were significantly lower than the check. When Alion was applied in combination with Sinbar, grass cover was significantly lower compared to the check for all rates and application timings except for the Late 5 oz/a treatment in June and August (Figure 8). When Velpar was applied with Alion, a similar trend emerged. Grass cover was lower in all treatments compared to the check at all evaluations, and was significant except for the Late 5 oz/a treatment in July and August (Figure 9).

Sandea combinations

In June, percent blueberry cover was lower in all Sandea treatment combinations compared to the check, Sinbar, or Velpar (Figure 10). It was significantly lower than the check in all Late treatments, Early Sandea + Velpar at both 1 oz/a, 2 oz/a, and Mid Sandea 2 oz/a with Velpar and with Sinbar. Otherwise, there were no significant differences among treatments.

As in the Alion combinations, phytotoxicity in the Sandea combinations was observed mainly as stunting and delayed emergence of the blueberry plants compared to the same clones' heights/percent cover in the adjacent alleys, with some chlorosis also observed but not the main component of phytotoxicity. The Early and Mid Sandea 1 oz/a treatment combinations had very low levels of injury to wild blueberry (Figure 11). However, all three Late 1 oz/a combinations had significantly higher levels of phytotoxicity compared to the check, and reduced cover by about 40 % in June (Photo 2). The Early Sandea 2 oz/a + Velpar treatment also had significant phytotoxicity in June, but the plants had recovered by July. The three Mid 2 oz/a treatments had significant phytotoxicity ranging from 20-35 % in June, but also had recovered by July. In the Late 2 oz/a combinations, phytotoxicity in June was upwards of 50-60 % with significant reduction in plant cover (Photo 3A). Even as late as August, the phytotoxicity remained significantly higher than the check and the plants were still visibly shorter than those in the alleys (Photo 3B).

In general, there were no significant differences in broadleaf weed cover among the Sandeaonly treatments in June or July (Figure 12). The Early 2 oz/a treatment had the highest cover overall, which was significantly higher than the check in August. When Sinbar was applied with Sandea, again there were no significant differences except for the Late Sandea 2 oz/a + Sinbar treatment, which initially had significantly lower broadleaf weed cover but increased to have significantly higher cover by August (Figure 13). This is most likely a function of the higher rate initially controlling more emerged weeds at the late timing, but then the surviving/later-germinating weeds had less competition from the shorter, sparser blueberry plants and were able to increase. When used in conjunction with Velpar, the Mid and Late 1 oz/a as well as the Late 2 oz/a treatments initially resulted in significantly lower broadleaf weed cover (Figure 14). The Late 1 oz/a treatment was significantly lower in July, but by August there were no longer any significant differences. An interesting phenomenon was noted here – although broadleaf weed cover in the check decreased from July to August, perhaps from the hot and dry conditions, it increased in all Sandea combinations regardless of the rate or timing. It may be due to the weeds having less competition from the reduced blueberry cover early in the season, but the trend held true even in the Early treatments which had little to no phytotoxicity.

Grass cover in all Sandea-only treatments was lower than the check at all evaluations, with the Early and Late 2 oz/a treatments significant and were most effective in controlling grasses (Figure 15). Although the Early 1 oz/a and Mid 2 oz/a treatments were significantly lower at the July evaluation, the differences disappeared by August because of grasses declining in the check from the hot dry weather. The addition of Sinbar greatly improved grass control, bringing grass cover in all combinations below 5 % at all evaluations (Figure 16). All combinations were significantly lower than the check except for the Mid 2 oz/a + Sinbar treatment in June. By contrast, the addition of Velpar appeared to release grasses, most likely because both Sandea and Velpar reduced broadleaf weeds at least in June and July, thereby reducing competition for the grasses (Figure 17). In June, all combinations except Early and Late 2 oz/a + Velpar significantly reduced grasses compared to the check, but by July grass cover had doubled or tripled in most combinations, and only the Mid 1 oz/a treatment remained significantly lower than the check. Grass cover in that treatment increased in August with the rainfall so that it was no longer significantly different from the check, but cover in the Early 1 oz/a treatment decreased so that it became significantly different. In general, by August grass cover in the Sandea + Velpar combinations was two to three times greater than in the Sandea + Sinbar combinations, but had mixed results compared to Sandea alone.

CONCLUSIONS/RECOMMENDATIONS: All conclusions and recommendations presented here are preliminary and subject to change. The trial will continue through 2013, during which time blueberry and weed cover will be assessed again, and the plots will be harvested to evaluate effects on yield.

<u>Alion</u>

The indaziflam label indicates it will control annual grasses and some broadleaf weeds. According to these trial results, Alion did have good grass control, especially in combination with Sinbar (see Figure 8). However, it did not suppress broadleaf weeds well. Although Alion + Velpar resulted in lower broadleaf weed cover in this trial compared to Velpar alone, and the combination initially significantly reduced broadleaf weeds, weed control was not significant later in the year (see Figure 6). Late pre-emergence application should not be made because of unacceptable injury to the blueberry plants. At the Early and Mid timings (mid April to early May), blueberry cover was comparable and phytotoxicity was not an issue at either the 5 oz/a or 10 oz/a rate. One rate was not consistently better than the other in regards to broadleaf weed or grass control; therefore, the 5 oz/a rate could be used.

<u>Sandea</u>

In combination with Sinbar, Sandea also provided good grass control. (see Figure 16). However, if Sandea is used in combination with Velpar on fields with heavy grass pressure, it

will require the addition of a grass-specific herbicide. In this trial, Sandea alone or with Velpar generally did not control broadleaf weeds significantly better than the check. In combination with Velpar, Sandea did have some control broadleaf weeds initially (see Figure 14), but a second application would be necessary for hard-to-control and/or late-germinating weeds. Regarding timing of application, Sandea should be applied no later than the beginning of May (or even earlier during a warm spring) in order to avoid unacceptable injury to blueberry and reduction in cover, and the 2 oz/a rate should not be used as it will result in significant phytotoxicity even at very early application timing.

Figure 2. Percent wild blueberry cover for pre-emergence Alion rates and spray timing alone and in combination with Sinbar or Velpar.

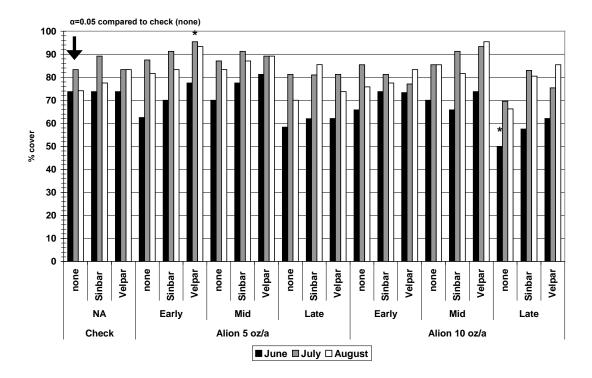


Figure 3. Percent wild blueberry phytotoxicity for pre-emergence Alion rates and spray timing alone and in combination with Sinbar or Velpar.

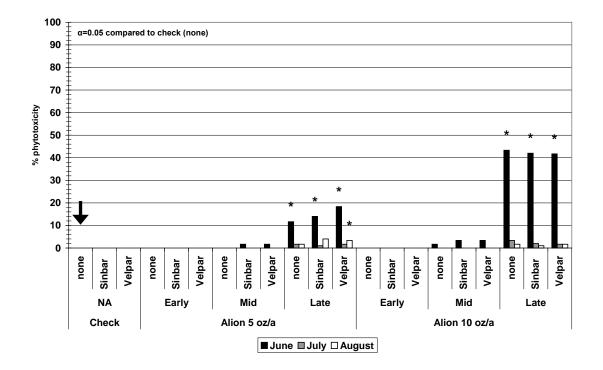


Figure 4. Effects of Alion rates and spray timing on percent broadleaf weed cover.

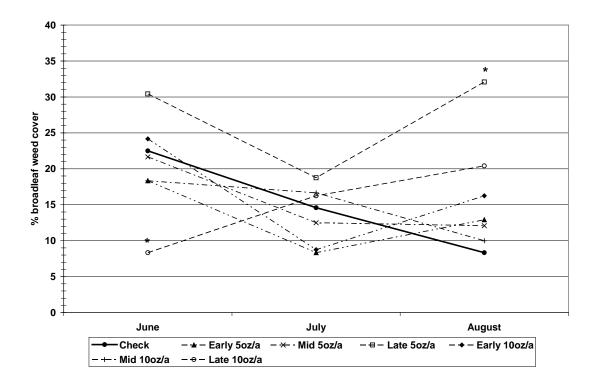


Figure 5. Effects of Alion rates and spray timing in combination with Sinbar on percent broadleaf weed cover.

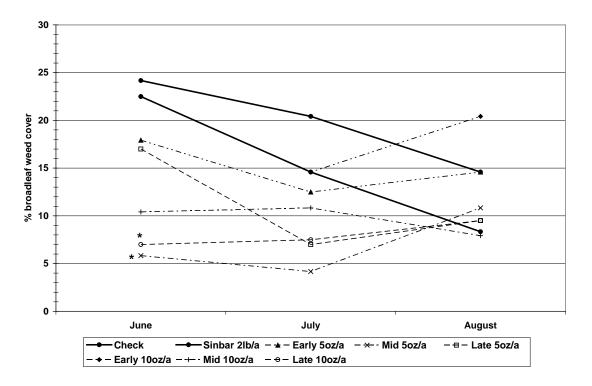
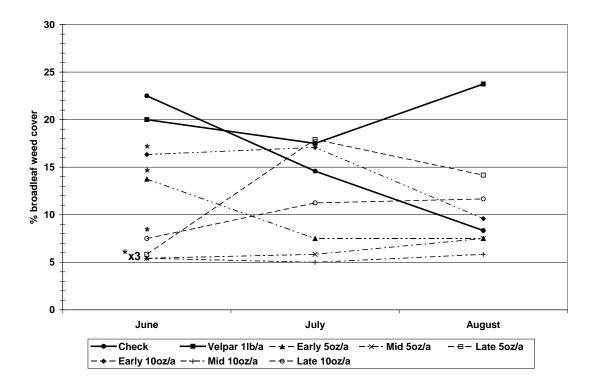


Figure 6. Effects of Alion rates and spray timing in combination with Velpar on percent broadleaf weed cover.



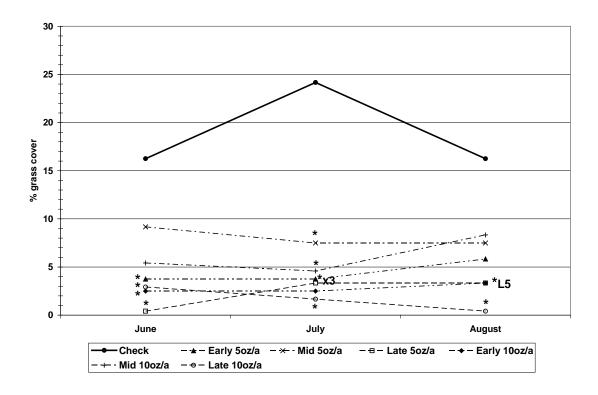
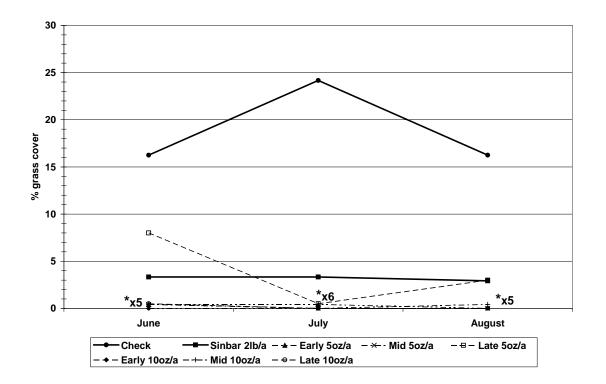


Figure 7. Effects of Alion rates and spray timing on percent grass cover.

Figure 8. Effects of Alion rates and spray timing in combination with Sinbar on percent grass cover.



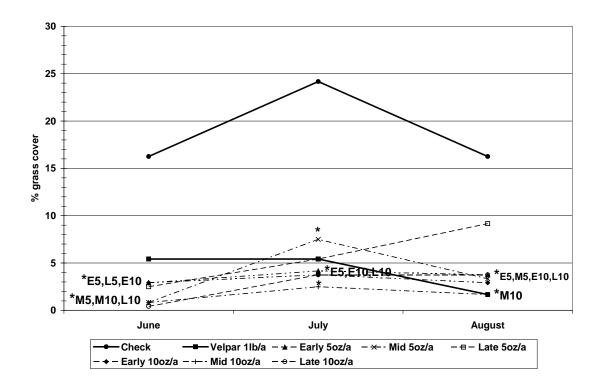


Figure 9. Effects of Alion rates and spray timing in combination with Velpar on percent grass cover.

Figure 10. Percent wild blueberry cover for pre-emergence Sandea rates and spray timing alone and in combination with Sinbar or Velpar.

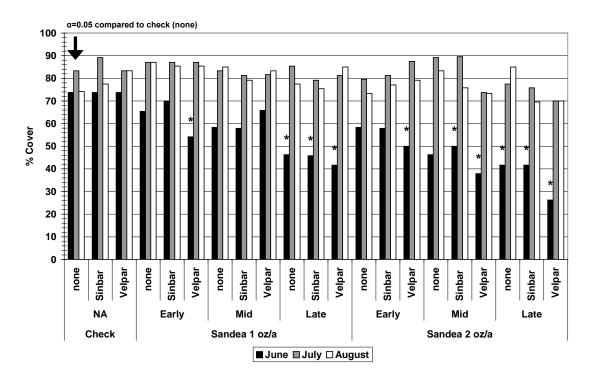


Figure 11. Percent wild blueberry phytotoxicity for pre-emergence Sandea rates and spray timing alone and in combination with Sinbar or Velpar.

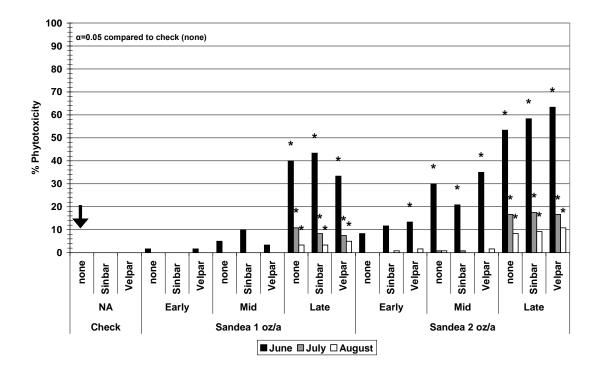


Figure 12. Effects of Sandea rates and spray timing on percent broadleaf weed cover.

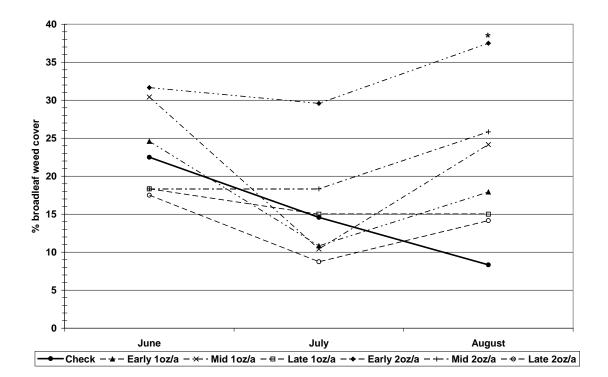


Figure 13. Effects of Sandea rates and spray timing in combination with Sinbar on percent broadleaf weed cover.

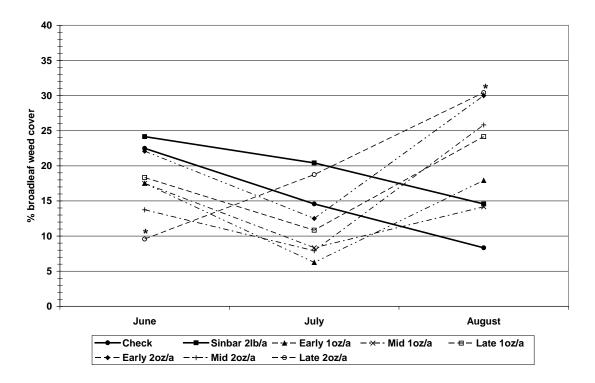
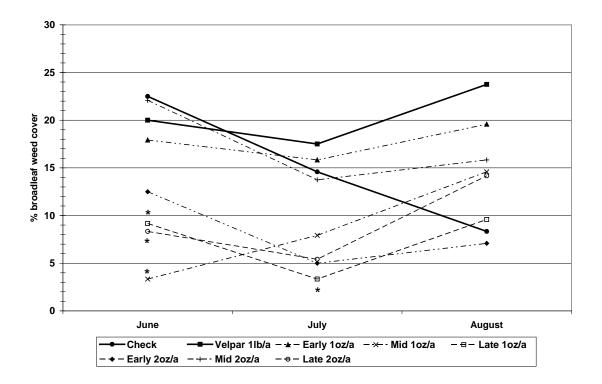


Figure 14. Effects of Sandea rates and spray timing in combination with Velpar on percent broadleaf weed cover.



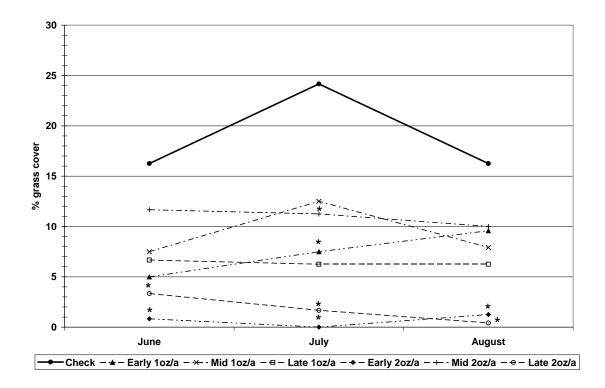


Figure 15. Effects of Sandea rates and spray timing on percent grass cover.

Figure 16. Effects of Sandea rates and spray timing in combination with Sinbar on percent grass cover.

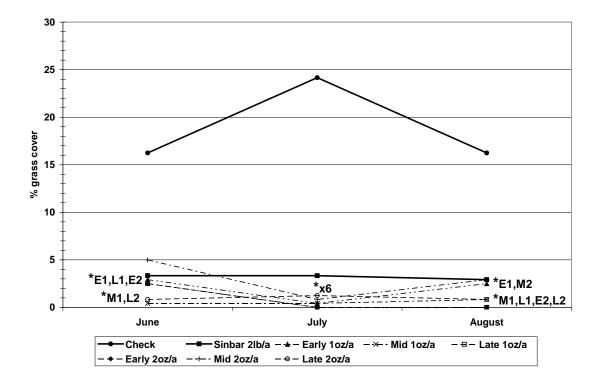


Figure 17. Effects of Sandea rates and spray timing in combination with Velpar on percent grass cover.

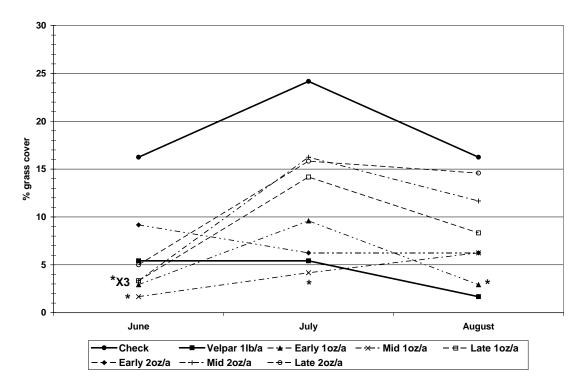


Photo 1A-B. Phytotoxicity at the June evaluation in the Late Alion 10 oz/a treatment (A) vs. the Late Alion 5 oz/a treatment.

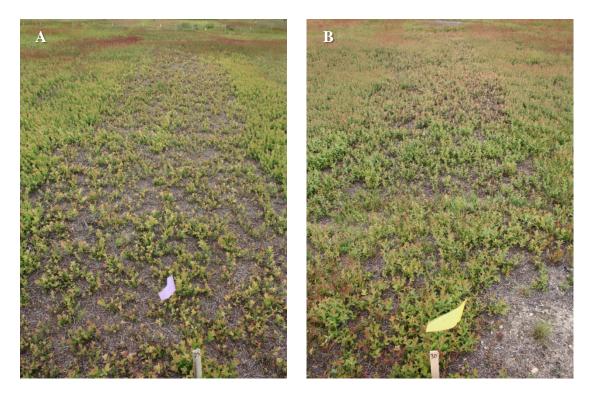


Photo 2. Phytotoxicity at the June evaluation in the Late Sandea 1 oz/a treatment.



Photo 3A-B. Phytotoxicity in the Late Sandea 2 oz/a treatment at the June evaluation (A) and the August evaluation (B).



INPUT SYSTEMS STUDY – ANCILLARY STUDY

SOIL HEALTH & CHEMISTRY: Ellen Mallory, Assistant Professor of Sustainable Agriculture Katie McPhee, Research Associate Hannah Griffin, Research Associate

27. TITLE: Compost and mulch effects on soil health and nutrient dynamics in wild blueberry.

INTRODUCTION: The potential for poor soil health to limit wild blueberry production has long been recognized. Adding stabilized organic matter in the form of seafood-waste compost could enhance the soil organic mat while providing a slow-release source of nutrients. Compost nutrient concentrations are low relative to fertilizers but they match the low nutrient requirements of wild blueberries. Currently, most organic producers in Maine rely on expensive bagged organic fertilizers that comprise 20-50% of production expenses. Bulk compost, available locally, may be a cheaper source of nutrients. A study was initiated in 2010 on a commercial wild blueberry farm in Township32, Maine, USA to evaluate seafood-waste compost for its impacts on soil quality, soil fertility, and crop yield. These plots will be monitored over four years. A second set of plots were established in 2012 to repeat the study. This report covers the first two years of the trial as results are pending for the 2012 field season. The report is based on the following manuscript that has been accepted for publication:

Mallory, E.B. and J.M. Smagula. *In press*. Effects of seafood-waste compost and mulch on soil health and soil nutrient dynamics in wild blueberry (*Vaccinium angustifolium* Ait). Acta Horticulturae.

METHODS:

Experimental design

The study site was a commercial, low-input wild blueberry field in Township32, Maine, USA on an Adams loamy sand soil. The experiment was repeated twice on two adjacent half-acre areas of the field. The first set of plots was established in 2010 and the second set was established in 2012, both in the spring during non-crop years. The field owners applied a selective herbicide as usual but no fertilizer. A randomized complete block design was used with a split plot arrangement of treatments. Mulch (with and without) was the main plot factor and soil amendment (five levels) was the split-plot factor. The soil amendment treatments were: compost, bagged organic fertilizer (Pro-Holly 4-6-4, North Country Organics[®], Bradford, VT), synthetic fertilizer (diammonium phosphate, DAP, 18-46-0, Northeast Agricultural Sales, Detroit, ME) at a rate of 1x (222 lb acre⁻¹) or 2x (444 lb acre⁻¹), and a control treatment. In 2010, seafood-waste compost (Sunrise Seafood Compost, Addison, ME) was used but in 2012 beef manure compost (Coast of Maine Organic Products, Portland, ME) was used to avoid the high calcium content typical of seafood-waste composts. All treatments were replicated six times. Plots were 6' x 30' in size. A 5' alley was established between plots to prevent cross contamination by soil amendments (Figure 1) and a 5' or 10' alley separated blocks.

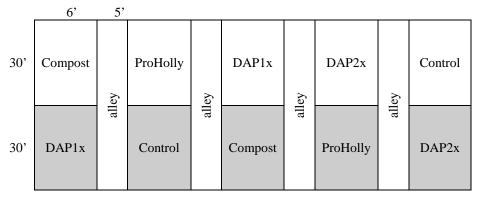


Figure 1. Example of the treatment layout in one block at a commercial wild blueberry farm, 2010. Shaded area represents the mulched plots.

In 2010, seafood-waste compost was applied at a rate of 4.3 t acre⁻¹ (fresh weight), which supplied approximately 38 lb acre⁻¹ of plant available nitrogen (N), assuming 10% of the compost total N was available during the first year after application (Gale et al., 2006). The seafood-waste compost also supplied 245 lb acre⁻¹ of phosphorus (P), 22 lb acre⁻¹ of potassium (K), and 10,598 lb acre⁻¹ of calcium (Ca). Application rates for Pro-Holly (0.8 t acre⁻¹) and DAP1x (222 lb acre⁻¹) were calculated to match the estimated available N supplied by the seafood-waste compost and assure sufficient P. First-year availability from the Pro-Holly fertilizer was assumed to be 57% of total N (100% of inorganic N and 30% of organic N, Gale et al., 2006). Pro-Holly also supplied 49 lb acre⁻¹ of P, 64 lb acre⁻¹ of K, and 135 lb acre⁻¹ of Ca. DAP1x supplied 45 lb acre⁻¹ of P. The DAP2x treatment was included as a nutrient response check. Fertility treatments were applied on 10 May 2010. Softwood bark mulch was applied the next day to create approximately 5cm of cover.

This process was repeated in 2012 to establish a second set of plots. In this case, beef manure compost was obtained from Coast of Maine Organic Products (Portland, ME) to avoid high calcium levels typical of seafood-waste compost.

Soil and plant tissue sampling

Leaf and soil samples were collected on 21 July 2010 after the terminal growing point died. Thirty stems exhibiting tip dieback were cut below the lowest growing leaf, dried (60° C) and ground (Wiley Mill, 20 mesh). In each plot, soil cores (19 mm dia.) were collected to a 10 cm depth at six random locations, bulked, mixed, and sieved to 2 mm. The samples were air-dried and analyzed by the Maine Agricultural and Forest Experiment Station Analytical Laboratory. Tissue samples were analyzed for mineral concentrations by dry-ashing and inductively coupled plasma (ICP) analysis, and soil samples were analyzed for pH, organic matter by loss on ignition, P by ICP, and other nutrients and cation exchange capacity by Modified Morgan extraction (McIntosh, 1969).

Stem measurements

After leaf drop (17-19 November 2010), blueberry stems from two 15cm x 15cm quadrats per plot were clipped at the stem etiolation point. Samples were stored at room temperature until stem number, branch number, and flower buds per stem were counted. In mid-May 2011, 10-

15 stems per plot with 3-9 flower buds were identified, marked with colored wire, and number of buds per stem was recorded. Flower numbers per stem were counted in early June 2011 and fruits per stem were counted in early August 2011.

<u>Fruit set</u>

Fruit yield fresh weight was determined by harvesting the center 0.61 x 8.5 m strip of each plot using a mechanical harvester and cleaner, and weighing (17 August 2011). A 550-g subsample was collected, separated into edible and nonedible (unripe, squashed, and diseased) fractions, and weighed. Berry weight (per 300 berries) was determined. Nutrients were analyzed for a 2-g subsample of pureed edible berries by the Maine Agricultural and Forest Experiment Station Analytical Laboratory by dry-ashing and ICP.

<u>Data analysis</u>

Data were analyzed using mixed model analysis of variance following verification of normality and equality of variance assumptions (JMP, Version 9.0, SAS Institute Inc., Cary, NC, 2010). Treatment effects were separated by Tukey's Honestly Significant Differences test at a 5% significance level. Soil was sampled in the new plots on 11 May 2012 before treatment initiation for baseline soil health and chemistry, including depth of organic pad layer, soil carbon parameters and nutrients. Using a ³/₄-inch core, 12 cores per main plot were taken to a depth of 10 cm. The depth of the organic pad was measured for each core. The soil from the 12 cores was bulked per main plot in two fractions, the organic pad fraction and the mineral soil fraction, and sieved through a 2mm screen. These were submitted separately to the University of Maine Analytical Laboratory for carbon content and nutrient analysis.

RESULTS:

Soil organic matter and chemistry

A one-year application of seafood-waste compost did not produce an increase in soil organic matter levels that was statistically significant in this trial (Table 1). The difference in organic matter concentrations between the seafood-waste compost and control treatments translates into approximately 5400 kg ha⁻¹ of soil carbon which is less than what was applied with the compost (7100 kg ha⁻¹), indicating that mineralization of compost carbon occurred before sampling.

Seafood-waste compost raised soil pH by almost 0.4 units compared with the control treatment. Warman et al. (2009) also observed soil pH increases of a similar magnitude associate with compost, but after 4 years of repeated application. The seafood-waste compost used in the present study contained mussel shells and thus had an unusually high Ca level and pH (7.2). Shells had not been observed in previous batches of seafood-waste compost from this supplier, and should be avoided for use in blueberries for their alkalinizing effect. The significant soil amendment x mulch interaction for soil Ca was due to a different magnitude of response to the compost in the mulch (788 ppm) vs. no mulch (1596 ppm) treatments. The increase in soil pH was accompanied by an increase in cation-exchange capacity and a reduction in soil aluminum concentration compared with the control. Again the significant soil amendment x mulch plots (7.47 vs. $11.45 \text{ meq } 100g^{-1}$, respectively).

Soil P was highest in the seafood-waste compost and DAP2x treatments due to having the highest application rates. Seafood-waste compost also increased soil test magnesium (Mg)

levels compared with all the other treatments, presumably through application in the compost, which added 194 kg Mg ha⁻¹, and possibly by increasing soil pH. The Pro-Holly treatment added the most K resulting in higher soil K levels compared with the other treatments. The mulch treatment had no effect on soil nutrients. The reduction in cation exchange capacity (CEC) with mulch is likely due to a sampling error whereby some of the organic pad was brushed away with the mulch before sampling.

Leaf tissue nutrient concentrations

Neither seafood-waste compost nor Pro-Holly affected leaf tissue nutrient concentrations compared with the control treatment despite changes in soil nutrient levels (Table 2). Warman et al. (2009) observed increases in N and K associated with municipal solid waste compost but after four years of repeated application. DAP2x raised leaf tissue N and both DAP treatments raised leaf tissue P compared with the control, consistent with prior studies with synthetic fertilizer (Smagula and Dunham, 1995; Smagula et al., 2004; Percival and Sanderson, 2004; Smagula, 2006; Smagula and Fastook, 2009). The DAP2x treatment also saw a reduction in leaf tissue Mg (and Ca although not significantly), which in other experiments has been associated with increased growth and dilution of those nutrients (Smagula, 2006). Mulch also reduced leaf tissue Mg levels, while increasing N and K.

Blueberry growth and yield

Soil amendment treatment did not affect stem density but did affect branches per stem (Table 3). Greater stem branching is typically associated with greater numbers of flower buds per stem, which in turn can lead to a higher fruit yield (Smagula and Dunham, 1995). While seafood-waste compost and DAP2x both increased branch density compared with the control, only seafood-waste compost increased the number of flower buds per stem and fruits per bud. Seafood-waste compost was the only treatment that increased the number of flower buds per stem over the control. In contrast, Pro-Holly showed a reduction in fruits per bud and fruit set compared with the control. However, all soil amendment treatments performed equally in terms of total and edible yield and exceeded the control treatment by 70% for both yield measures.

The mulch treatment significantly reduced stem density and increased buds per stem, but had no effect on fruit yields.

CONCLUSIONS: Seafood-waste compost was equally effective as Pro-Holly and DAP as a fertility source for wild blueberries. Seafood-waste compost affected soil nutrient concentrations compared with the control, but these changes were not reflected in differences in leaf tissue nutrient concentrations. Fruit yields were equal among all of the soil amendment treatments and 70% higher than the control treatment. One application of seafood-waste compost did not have a significant effect on soil organic matter, although a measurable increase would be expected with repeated applications.

The soil amendment treatment costs were estimated based on prices that growers would have paid at the time of the study as follows (in U.S.\$): seafood-waste compost, \$4940 ha⁻¹; Pro-Holly \$1680 ha⁻¹; DAP1x \$203 ha⁻¹; DAP2x, \$406 ha⁻¹; control, \$0. In this study, compost was applied at a high rate to affect quick changes in nutrient status. Similar results with lower rates may be possible and should be investigated. As well, compost may not need to be applied every crop cycle because nutrient release from compost occurs gradually over many years. In

this study, the effects from the single 2010 application of seafood-waste compost and DAP2x on crop performance will be followed through the 2014 crop cycle and compared to biannual applications of Pro-Holly and DAP1x. Reducing the compost application rate to 45 t ha⁻¹ and applying every other crop cycle would reduce the cost of using compost to \$1235 ha⁻¹, which, while still much more expensive than synthetic fertilizer, would be cost-effective compared with the bagged fertility sources available to organic farmers in Maine.

Acknowledgements

The authors gratefully acknowledge the help of Shannon and Steve Lion as grower cooperators and Katherine McPhee and Tom Molloy for technical assistance. The work was funded in part by the Hatch Act and by U.S.D.A. National Institute of Food and Agriculture, Specialty Crop Research Initiative Grant Award 2009-02548,"Systems approach to improving the sustainability of wild blueberry production." Maine Agricultural and Forest Experiment Station Publication Number 3279."

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<u>Tables</u>

	Organic		CEC	Total N	Р	К	Ca	Mg	Al	Zn
Treatments	matter (%)) pH	$(meq \ 100g^{-1})$	(%)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
Soil amendment		-								
Compost ¹	7.40^{2}	4.99 a	9.46	0.184	13.0 a	38.1 b	1190	62.7 a	255 b	2.08 a
Pro-Holly	7.20	4.53 bc	5.72	0.182	8.1 bc	49.4 a	209	30.1 b	285 ab	2.09 a
DAP1x	6.58	4.51 c	4.90	0.177	8.8 bc	34.4 b	168	27.0 b	266 ab	1.52 b
DAP2x	7.20	4.55 bc	5.38	0.174	10.1 ab	35.1 b	185	30.0 b	260 b	1.80 ab
Control	6.50	4.61 b	4.90	0.179	7.1 c	34.8 b	177	27.5 b	294 a	1.80 ab
Mulch										
Mulch	7.32	4.65	5.83	0.179	8.9	41.4	310	33.6	274	1.84
No mulch	6.91	4.58	6.32	0.179	9.9	35.3	462	37.3	270	1.88
					ANOV	A Results				
Amendment (A)	NS	***	***	NS	***	***	NS	***	**	*
Mulch (M)	NS	NS	**	NS	NS	NS	***	NS	NS	NS
A x M	NS	NS	***	*	NS	NS	**	NS	NS	NS

Table 1. Soil characteristics, macronutrient, and select micronutrient concentrations as affected by soil amendment and mulch treatments, sampled to a 10 cm depth at tip dieback, 21 July 2010.

¹Seafood-waste compost, which consisted of salmon, sea urchin, sea cucumber, mussel culls, and lobster bodies mixed with sawdust. ²Fertility treatment averages for organic matter are from no mulch plots only.

NS, not significant (p>0.05).

*, **, ***, significant at the 0.05, 0.01, 0.001 levels of probability, respectively.

Treatments	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Al (ppm)	Zn (ppm)
Soil amendment	• •				<u>.</u>		
Compost ¹	1.69 b	0.120 bc	0.444	0.395	0.165 a	72.1	14.7
Pro-Holly	1.73 b	0.123 bc	0.449	0.381	0.159 ab	68.2	14.0
DAP1x	1.67 b	0.132 ab	0.426	0.383	0.158 ab	71.1	13.5
DAP2x	1.83 a	0.140 a	0.433	0.365	0.150 b	65.9	13.4
Control	1.66 b	0.120 c	0.428	0.398	0.164 a	71.1	14.3
Mulch							
Mulch	1.79 a	0.131	0.446 a	0.367	0.152 b	70.4	14.0
No Mulch	1.64 b	0.125	0.426 b	0.399	0.166 a	69.0	14.0
			Al	NOVA Resul	ts		
Amendment (A)	***	***	NS	NS	*	NS	NS
Mulch (M)	*	NS	*	NS	*	NS	NS
A x M	NS	NS	NS	NS	NS	NS	NS

Table 2. Blueberry leaf tissue macronutrient and select micronutrient concentrations as affected by soil amendment and mulch treatments, sampled at tip dieback, 21 July 2010.

¹Seafood-waste compost, which consisted of salmon, sea urchin, sea cucumber, mussel culls, and lobster bodies mixed with sawdust. *, **, ***, significant at the 0.05, 0.01, 0.001 levels of probability, respectively.

NS, not significant (p>0.05).

	Stem density	Branch density	Flower buds per stem	Blossoms per bud	Fruits per bud	Fruit set	Total fruit yield	Edible fruit yield
Treatments	$(no. m^{-2})$	$(no. m^{-2})$	(no.)	(no.)	(no.)	(%)	(lb acre ⁻¹)	$(lb acre^{-1})$
Soil amendment								
Compost ¹	798	1365 a	5.7 a	3.9	2.5 a	63 a	3260 a	2960 a
Pro-Holly	737	1173 ab	3.0 ab	3.5	1.9 c	53 b	3100 a	2791 a
DAP1x	831	874 ab	4.6 ab	3.8	2.4 ab	64 a	2970 a	2650 a
DAP2x	863	1245 a	2.9 ab	3.7	2.3 ab	62 a	2870 a	2600 a
Control	933	605 b	2.5 b	3.4	2.0 bc	60 ab	1781 b	1610 b
Mulch								
Mulch	615 b	938	4.9 a	3.9	2.2	58	2920	2620
No Mulch	1050 a	1167	2.5 b	3.4	2.2	63	2670	2431
Amendment (A)	NS	**	*	NS	***	**	***	***
Mulch (M)	*	NS	*	NS	NS	NS	NS	NS
A x M	NS	NS	NS	NS	NS	NS	NS	NS

Table 3. Stem density, fruit yield components, and yield as affected by soil amendment and mulch treatments, sampled in fall 2010 for stem density, branch density and flower buds per stem, and in summer 2011 for all other measures.

¹Seafood-waste compost, which consisted of salmon, sea urchin, sea cucumber, mussel culls, and lobster bodies mixed with sawdust. NS, not significant (p>0.05).

*, **, ***, significant at the 0.05, 0.01, 0.001 levels of probability, respectively.