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2006 Lowbush Blueberry Project Reports

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2006 Wild Blueberry Project Reports

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2006 LOWBUSH BLUEBERRY PROJECT REPORTS

FOOD SCIENCE AND BIOSYSTEMS ENGINEERING

IRRIGATION

ENTOMOLOGY – INSECT PEST MANAGEMENT

DISEASE MANAGEMENT

PLANT NUTRITION

WEED MANAGEMENT AND FIELD COVER

EXTENSION

FOOD SCIENCE AND BIOSYSTEMS ENGINEERING

INVESTIGATORS: Kristi Crowe, Graduate Student & Postdoctoral Student
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Vivian Wu, Assistant Professor of Food Science
Brain Perkins, Research Laboratory Manager

TITLE: Evaluation of Emerging Disinfection Technologies for Wild Blueberry Processing.

METHODS: The effect of treatment of wild blueberries with chlorine or ozone on the microbial population (total aerobes, yeasts and molds) at time zero and following frozen storage was evaluated. Standard methods of analysis were used to enumerate total aerobes, yeasts and molds). As part of the research on the evaluation of emerging disinfection technologies for wild blueberry processing experiments were conducted to examine the influence of postharvest treatments on the color quality of lowbush blueberries. Color analyses were conducted on all treated blueberry samples following postharvest treatment application of chemical and photochemical oxidation processes. Color was evaluated using a Hunter LabScan XE Colorimeter (Hunter Associates Laboratory, Reston, VA) standardized against a white tile. *L, a,* and *b* values were measured for all samples from which hue angle ($\tan^{-1}$ b/a) was calculated to further assess color changes. The monomeric anthocyanin content was estimated using the pH differential method as described by Giusti and Wrolstad (2000). The anthocyanin absorbance of commercial blueberries was analyzed post-treatment by an Ocean Optics spectrophotometer (Ocean Optics Inc., Dunedin, FL) at 515 and 700 nm in pH 1.0 and 4.5 buffers. The absorbance was calculated based on cyanidin-3-glucoside (c3g) with a molar extinction coefficient of 26,900 according to the following formula: $A = [(A_{515} - A_{700})_{pH\ 1.0} - (A_{515} - A_{700})_{pH\ 4.5}]$. Results were expressed as mg c3g/g fresh weight.

RESULTS: Results of experiments examining the effect of chlorine and ozone on the total aerobic plate count, yeasts and molds on wild blueberries at harvest and following frozen storage are in progress. Data has been generated for wild blueberries at harvest and at one and two months of frozen storage (Figure 1). The response of fresh blueberries to postharvest oxidants for 60 sec is detailed in Table A1. Lengthening the exposure time of treatments did not significantly impact ($p<0.05$) color quality as evidenced by hue angle and the color parameters of *L, a,* and *b* (Table A2). Although all treated samples were not significantly different ($p<0.05$) than unwashed controls in terms of relative lightness or darkness, fluctuating *L* values reflect a change in color intensity in response to treatment. Chlorinated water sprays reduced pigment intensity resulting in a lighter pigment on blueberry skins whereas blueberries treated with ozonated water exhibited pigment darkening. Values of *L* resulting from chlorine and ozone treatments were significantly different ($p<0.05$) from each other with respect to relative lightness or darkness of blueberries. Except for chlorine and hydrogen peroxide/UV, all treatments contributed slightly to pigment darkening. According to the Judd-Hunter color solid, the color parameter depicted by the letter *a* reflects changes in the degree of redness exhibited by the sample with *–a* values indicating greenness and *+a* indicating redness. Statistical differences ($p<0.05$) in the degree of redness did not exist among all treated samples and the control; however, all treatments maintained the relative redness of blueberries as indicated by *+a* values except chlorine and chlorine/UV treatments which resulted in a shift in color pigments toward greenish-red. Comparison of *b* values for treated samples reflects a shift in the degree of blueness resulting from chlorinated water

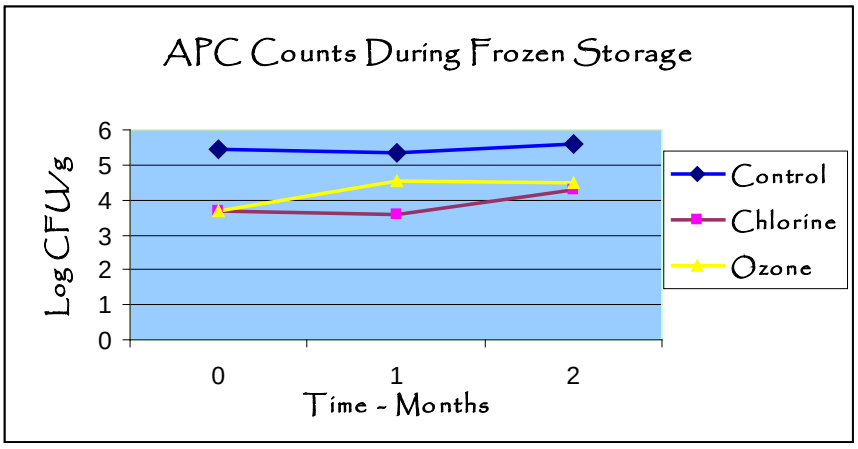
sprays. The shift was significantly different ($p<0.05$) than the calculated shift in blueness observed on blueberries exposed to other oxidants although not significantly different from the blueness exhibited by unwashed control berries. Results of hue angle calculations reveal shifts in hue ranging from 4.9° below to 6.0° above the mean hue angle of 82.90° for unwashed control samples. Nevertheless, calculations of hue angle from all treatments remained within the same color quadrant as the control. Blueberry hue was not significantly influenced ($p<0.05$) by treatment as evidenced by the relatively small degree of shift observed among treated samples. Overall, the impact of compounded oxidants was minimal and did not result in significant differences ($p<0.05$) in any of the parameters evaluated. The monomeric anthocyanin content of unwashed and processed lowbush blueberries ranged from 1.18 to 2.92 mg c3g/g with unwashed control berries containing 1.66 mg c3g/g (Table B1). Blueberry samples treated with a photochemical combination of chlorine/UV (2.92 mg of c3g/g) resulted in a significantly greater ($p<0.05$) anthocyanin content compared to unwashed controls; furthermore, anthocyanin oxidation was observed on samples treated with 1% hydrogen peroxide as evidenced by a reduction in anthocyanin concentration. Among all chemical and photochemical treatments evaluated, only blueberry samples treated with 1% hydrogen peroxide resulted in a decreased anthocyanin content compared to the control.

Typically, the reported anthocyanin content of blueberries, regardless of species variation, falls in the range of 1-2 mg/g fw (Ehlenfeldt and Prior, 2001; Moyer et al., 2002; Zheng and Wang, 2003). Unwashed control berries fell within this range (1.66 mg c3g/g) although variances in the concentration of anthocyanins were observed on all samples regardless of treatment. Among treatments, the photochemical combination of chlorine/UV resulted in a significantly elevated ($p<0.05$) anthocyanin concentration in excess of the anthocyanin content of control samples. Additionally, hydrogen peroxide induced oxidation of anthocyanins although the anthocyanin content of hydrogen peroxide-treated blueberries was not significantly different from the control. Catalyzed degradation of anthocyanins by decomposition of hydrogen peroxide has been reported by Sondheimer and Kertesz, 1952; Sapers & Simmons, 1998; De et al., 1999; Oskan, 2002). Except for hydrogen peroxide, the concentration of anthocyanins in all treated samples resulted in minimal increases in anthocyanin values compared to the control. These variances may be explained as treatment-induced effects on anthocyanin structure and stability as well as variations in the anthocyanin concentration of blueberry cultivars present in the sampling field.

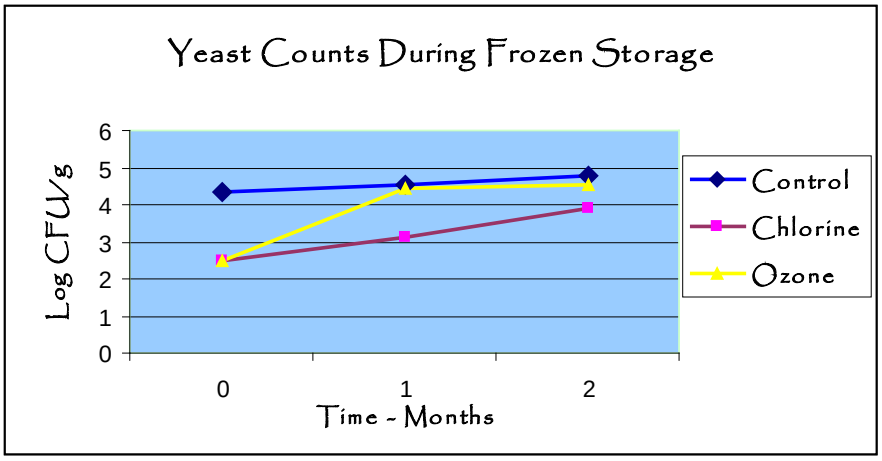
RECOMMENDATIONS: This completes the current research on new disinfection technologies for use on IQF lowbush blueberries. Based on our research ozone has been shown to reduce microbial load as well as pesticide levels on the fruit. Levels of 1 ppm ozone with a contact time of 60 sec were the optimum for reduce microbial load and pesticide residues. Future research should examine the use of gaseous ozone as a means of reducing total microbial load and pesticide residues on wild lowbush blueberries which will be marketed fresh.

Figure 1
Long Term Storage Evaluation – Microbial Analysis of Blueberries Treated with 100ppm Chlorine and 1ppm Ozone

a



b



c

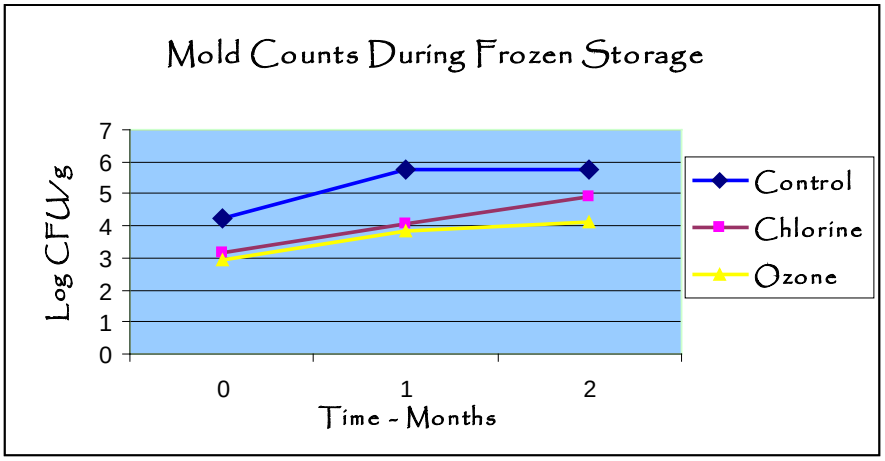


Table A1. Effect of Postharvest Treatments on Color Quality* of Lowbush Blueberries Following Treatment Exposure for 60 Sec

Treatment	*L*	*a*	*b*	*Hue Angle***
Unwashed Control	26.42[abc]	0.60[a]	-4.99[ab]	82.90[a]
100ppm Cl_2	31.15[a]	-0.71[a]	-6.96[b]	84.15[a]
1% H_2O_2	23.35[abc]	0.88[a]	-4.54[a]	78.75[a]
1ppm O_3	20.36[c]	0.95[a]	-4.45[a]	78.00[a]
UV	24.26 [abc]	0.46[a]	-5.07[ab]	84.80[a]
Plant Water	24.55[abc]	0.23[a]	-4.66[a]	86.95[a]
100ppm Cl_2/UV	22.96[abc]	-0.07[a]	-4.69[a]	88.90[a]
1% H_2O_2/UV	26.59[abc]	0.83[a]	-4.48[a]	79.05[a]
1ppm O_3/1% H_2O_2/UV	20.45[bc]	0.40[a]	-4.63[a]	84.95[a]

*L, a, and b parameters of the Judd-Hunter color system measuring degree of lightness or darkness, redness or greenness, and blueness or yellowness, respectively.

**Hue angle = $\tan^{-1}$ b/a

[a-c]Mean of three replicates per treatment. Means within columns not sharing similar superscripts are significantly different at $p < 0.05$ according to Tukey's HSD Multiple Comparison.

Table A2. Effect of Postharvest Treatments on Color Quality* of Lowbush Blueberries Following Treatment Exposure for 120 Sec

Treatment	*L*	*a*	*b*	*Hue Angle***
Unwashed Control	26.42[a]	0.60[a]	-4.99[a]	82.90[a]
100ppm Cl_2	25.07[a]	-0.38[a]	-4.48[a]	85.30[a]
1% H_2O_2	25.45[a]	0.17[a]	-5.11[a]	86.55[a]
1ppm O_3	21.95[a]	-0.25[a]	-5.02[a]	87.15[a]
UV	25.60 [a]	0.64[a]	-5.24[a]	82.85[a]
Plant Water	24.92[a]	0.15[a]	-5.11[a]	88.35[a]
100ppm Cl_2/UV	25.75[a]	0.37[a]	-5.19[a]	85.85[a]
1% H_2O_2/UV	26.72[a]	0.14[a]	-5.77[a]	85.10[a]
1ppm O_3/1% H_2O_2/UV	21.09[a]	0.16[a]	-4.54[a]	87.90[a]

*L, a, and b parameters of the Judd-Hunter color system measuring degree of lightness or darkness, redness or greenness, and blueness or yellowness, respectively.

**Hue angle = $\tan^{-1}$ b/a

[a-c]Mean of three replicates per treatment. Means within columns followed by different superscripts are significantly different at $p < 0.05$ by the Tukey's HSD Multiple Comparison.

Table B1. Effect of Chemical and Photochemical Oxidation Treatments on the Anthocyanin Content* of Commercial Lowbush Blueberries *(Vaccinium angustifolium)*

Treatment	*Anthocyanins (mg c3g/g) 60 sec Exposure*
Unwashed Control	1.66 ± 0.35^{ef}
100ppm Cl_2	2.32 ± 0.13^{abcde}
1% H_2O_2	1.18 ± 0.06^{f}
1ppm O_3	1.84 ± 0.05^{def}
UV	2.00 ± 0.43^{abcdef}
Plant Water	2.12 ± 0.23^{abcde}
100ppm Cl_2/UV	2.92 ± 0.12^{a}
1% H_2O_2/UV	1.87 ± 0.28^{cdef}
1ppm O_3/1% H_2O_2/UV	1.86 ± 0.28^{cdef}

*Anthocyanin content expressed as milligrams of equivalent cyaniding-3-glucoside per gram of fresh weight calculated from the mean of three replicates per sample; means within columns followed by different superscripts are significantly different at $p < 0.05$ by the Tukey's HSD Multiple Comparison test.

References

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I**NVESTIGATORS:** Alfred A. Bushway, Professor of Food Science
Rodney J. Bushway, Professor of Food Science
Brian Perkins, Research Laboratory Manager
Pam Small, Graduate Student

TITLE: Incorporation of wild blueberry puree into a soy-based burger and its effect on sensory and chemical properties of the broiled burgers.

METHODS: Two prototype soy- wild blueberry burgers have been prepared according to the formulations in Table 1. A control containing only soy was also prepared. Samples were broiled on an EmberGlo E24 electric charbrioler at 200 C to an internal temperature of 160 C. Sensory evaluation was performed the day following processing and after six months of frozen storage. The total phenolic acid content of the control and wild blueberry containing burgers was determined the day following processing and after six months of frozen storage. Mineral analyses have been completed on the three veggie burgers and results are currently being analyzed for statistically significant differences. Nutritionist 4 was used to determine the nutritional content of the control and the soy- wild blueberry burgers. An additional sensory evaluation will be performed at the Natural Living Center in Bangor, ME. The Natural Living Center caters to consumers who are vegans and purchase organic foods. In addition, experiments to determine if addition of wild blueberry puree will prevent the formation of heterocyclic aromatic amines (HA) during charbroiling are in progress. Solid phase extraction followed by high performance liquid chromatography will be used to isolate and identify heterocyclic aromatic amines (Toribio et al. 1999).

RESULTS: Results from the sensory evaluation indicated that soy burgers formulated with 10 or 15% wild blueberry puree were preferred by the panelists (Figures 1-4). Even after 6 months of frozen storage the soy-wild blueberry burgers were still preferred over the soy burgers. Research to determine if wild blueberry puree can inhibit (HA) formation is currently on going. The food matrices of the soy-based burger have required that the published method be modified. Research with HAs will also be preformed in ground beef burgers formulated with wild blueberry puree.

RECOMMENDATIONS: Research on development of a soy-wild blueberry burger has been completed. Two sensory evaluations have been completed (day following processing, after six months of frozen storage with a third to be performed at the Natural Living Center in Bangor, ME). Based on the sensory evaluations, the soy- wild blueberry burger containing 15% wild blueberry puree was rated as being significantly better than the soy burger without blueberry puree. There is an opportunity to expand into the vegetable burger market with a product containing wild blueberry puree. Such a product would take advantage of the potential health benefits from the isoflavones in the soy and the anthocyanins and phenolics in the wild blueberries.

TABLE 1
Soy-Wild Blueberry Burger Formulations

Ingredient	10% Puree	15% Puree
Texturized soy protein	125.7g	108.6g
Wild blueberry puree	34.1g	51.1g
Canola oil	89.1ml	89.1ml
Soy sauce	59.4ml	59.4ml
Chopped garlic	14.2g	14.2g
Dried onion	14.2g	14.2g
Sesame oil	3.7ml	3.7ml
Guar gum	7.1g	7.1g

Figure 1
Sensory Evaluation Day 1

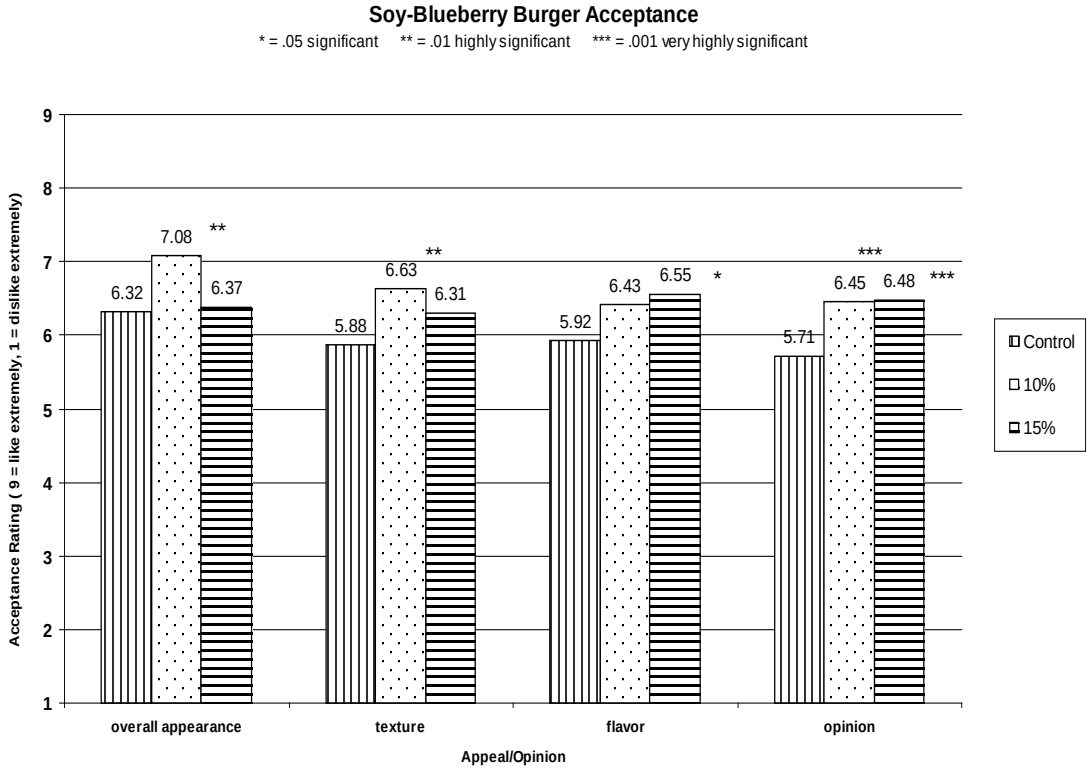


Figure 2
Sensory Evaluation Day 1 Preference Ranking
Soy-Blueberry Burgers

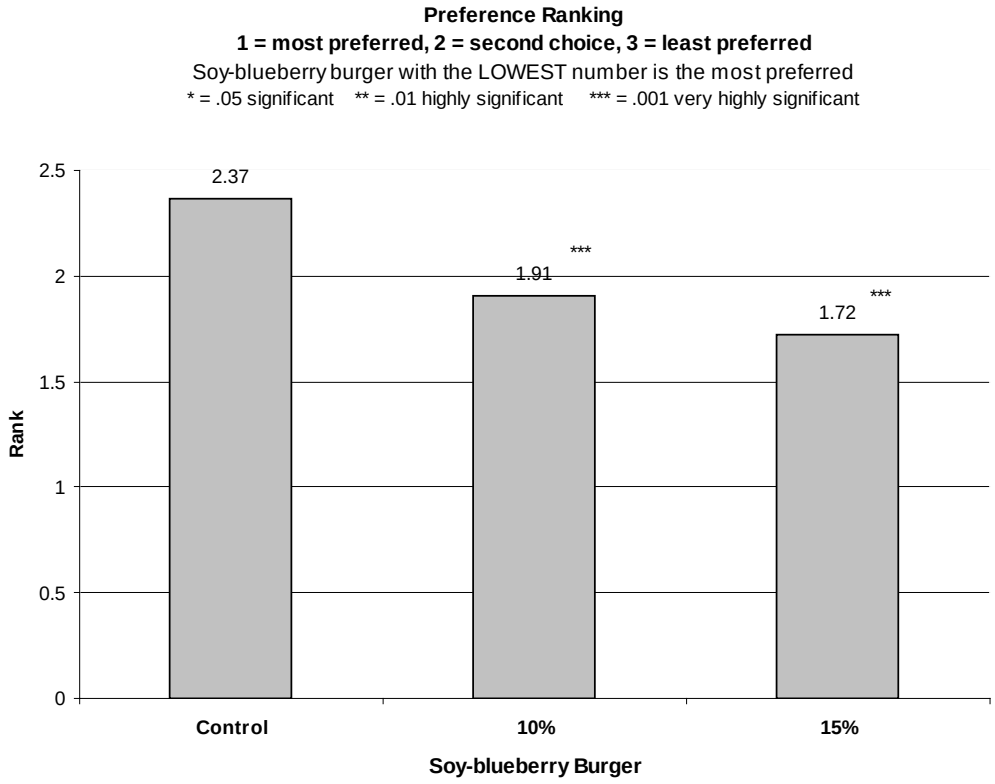


Figure 3
Sensory Evaluation Following Six Months Frozen Storage

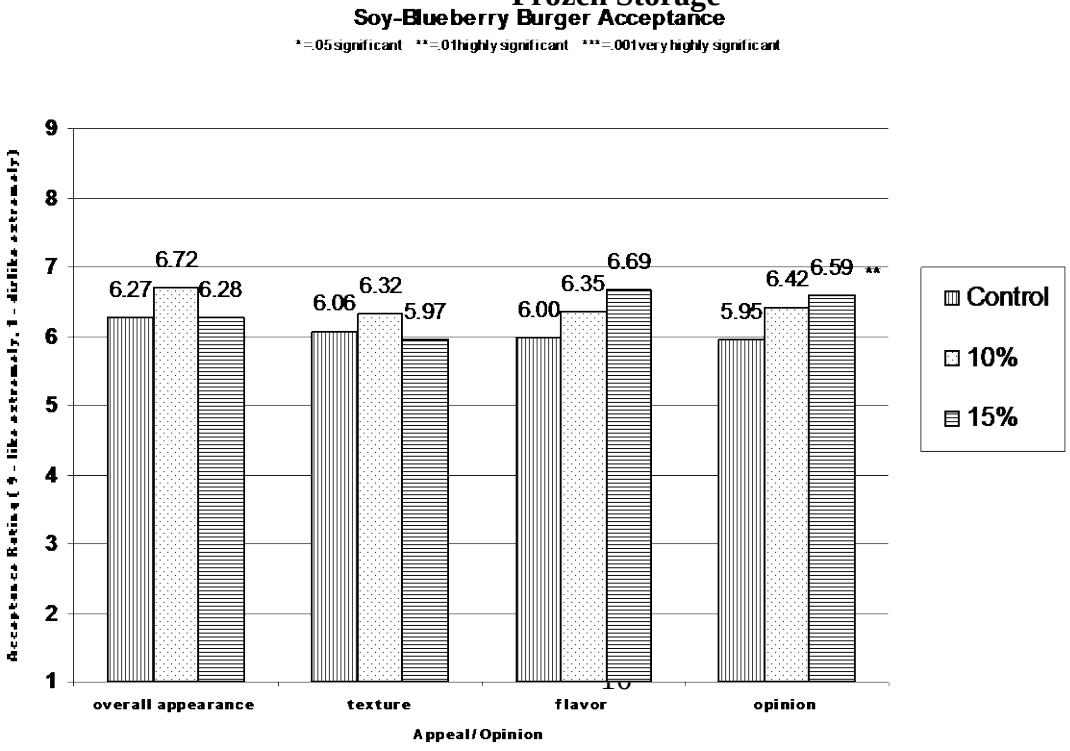
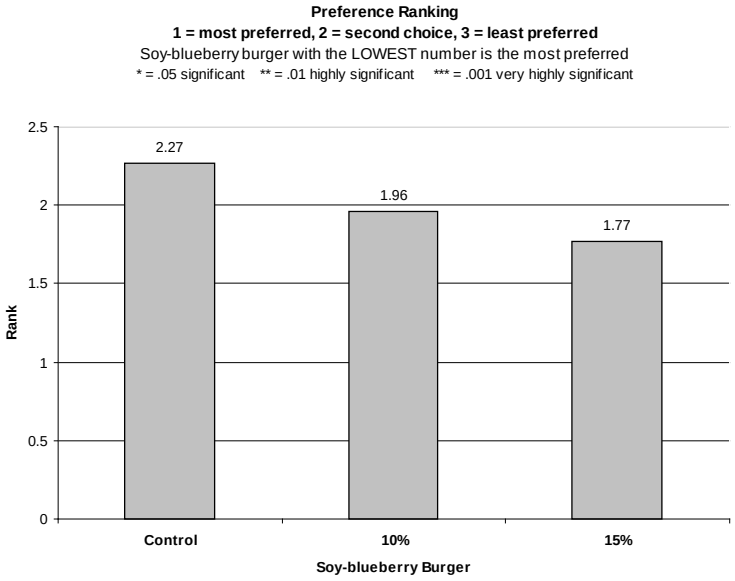


Figure 4
Sensory Evaluation Following Six Months Frozen Storage
Preference Ranking Soy-Blueberry Burgers



Nutrition Facts
Serving Size 4.00 ounce(s) (113g)

Amount Per Serving			
Calories 239	**Calories from Fat** 76		
			% Daily Value
Total Fat 8.4g			13 %
Saturated Fat 1.0g			5 %
Trans Fat 0.0g			
Cholesterol 0.0mg			0 %
Sodium 836.5mg			35 %
Total Carbohydrate 20.2g			7 %
Dietary Fiber 13.8g			55 %
Sugars 2.5g			
Protein 28.7g			
Vitamin A	0 %	Calcium	18 %
Vitamin C	11 %	Iron	29 %

Soy blueberry burger control

¼ lb burger = 4 oz = 113.4g

Ingredient	Amount	Unit
Textured soy protein	5.850	oz
Tap water	5.500	Fluid oz
Soy sauce	1.000	oz
Vegetable oil	1.000	oz
Chopped garlic	0.875	oz
Dehydrated minced onion	0.875	oz
Guar gum	0.563	oz
Sesame oil	0.156	oz

Nutrition Facts

Serving Size 4.00 ounce(s) (113g)

Amount Per Serving			
Calories 236	**Calories from Fat** 76		
			% Daily Value
Total Fat 8.5g			13 %
Saturated Fat 1.0g			5 %
Trans Fat 0.0g			
Cholesterol 0.0mg			0 %
Sodium 809.1mg			34 %
Total Carbohydrate 20.8g			7 %
Dietary Fiber 13.4g			54 %
Sugars 3.4g			
Protein 26.9g			
Vitamin A 1 %		Calcium	17 %
Vitamin C 12 %		Iron	27 %

Soy blueberry burger 10%
10% blueberry puree by weight
¼ lb burger = 4 oz = 113.4g

Ingredient	Amount	Unit
Textured soy protein	5.450	oz
Tap water	4.450	Fluid oz
Blueberry puree	1.5	oz
Soy sauce	1.000	oz
Vegetable oil	1.000	oz
Chopped garlic	0.875	oz
Dehydrated minced onion	0.875	oz
Guar gum	0.563	oz
Sesame oil	0.156	oz

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Cholesterol 0.0mg			0 %
Sodium 809.4mg			34 %
Total Carbohydrate 21.5g			7 %
Dietary Fiber 13.6g			54 %
Sugars 3.9g			
Protein 27.0g			
Vitamin A 1 %		Calcium	17 %
Vitamin C 12 %		Iron	27 %

Soy blueberry burger 15%
15% blueberry puree by weight
¼ lb burger = 4 oz = 113.4g

Ingredient	Amount	Unit
Textured soy protein	5.455	oz
Tap water	3.725	Fluid oz
Blueberry puree	2.250	oz
Soy sauce	1.000	oz
Vegetable oil	1.000	oz
Chopped garlic	0.875	oz
Dehydrated minced onion	0.875	oz
Guar gum	0.563	oz
Sesame oil	0.156	oz

Publications

- Crowe, K.M., **A.A. Bushway**, R.J. Bushway, and R.A. Hazen. Evaluation of chemical and photochemical oxidation methods for degradation of phosmet on lowbush blueberries (*Vaccinium angustifolium*) J. Agric. Food Chem. In press.

- Crowe. K.M., **A.A. Bushway**, R.J. Bushway, K. Davis-Dentici, and R.A. Hazen. A comparison of single oxidants versus advanced oxidation processes as chlorine-alternatives for wild blueberry processing (*Vaccinium angustifolium*). Int. J. Food Microbiol. In press.

- Crowe. K.M., **A.A. Bushway**, R.J. Bushway, and K. Davis-Dentici. Microbial degradation of Phosmet on blueberry fruit and in aqueous systems by indigenous bacterial flora on lowbush blueberries (*Vaccinium angustifolium*). Submitted J. Food Sci.

FOOD SCIENCE AND BIOSYSTEMS ENGINEERING

INVESTIGATOR: Dr. Darrell Donahue, Chemical and Biological Engineering-UMaine
COLLABORATORS: Dr. Frank Drummond and Judy Collins, Biological Sciences-UMaine
Dr. Floyd Dowell, USDA-ARS-Kansas State University

TITLE: Infestation Detection using NIRS

OBJECTIVE: Exploratory research examining Near-Infrared Spectroscopy (NIRS) as a method to detect maggot-infested blueberries in an IQF processing line.

METHODS:
1. Sample preparation. In 2006 we had an ample supply of laboratory-raised flies and were able to use fly chambers for artificial laboratory infestation of blueberries. We achieved infestation ratio of up to 39% per batch of berries compared to 4-10% natural infestation in 2005.
Laboratory-raised flies were kept into temperature and humidity controlled fly chambers in the University Research Farm laboratory in Jonesboro, Maine. Blueberry maggot adults were reared from pupae collected in 2005. As they emerged, adults were placed in oviposition cages in the laboratory. Each cage consisted of a rectangular acrylic container measuring 7”x13.5”x10” covered with a composite wood board measuring 12”x17”. A service hole ca. 6 inches in diameter was cut in the side of each container and plugged with a cotton cloth sleeve to prevent flies from escaping. Each cage also contained a Petri dish with 6-7 cotton balls soaked with water as a source of moisture. Excess water was wrung out of the cotton balls. To provide nourishment, feeding stations were made for each cage by cutting a large hole in the cover of a 100 x 10 mm Petri dish. Nylon screening was cemented over the hole. The underside of the screening was then smeared with honey. Fleischmann’s dry yeast was used as a source of protein.

The flies were allowed to mature for ca. 5-7 days at ca. 23-25^{O}C. Once sexual development of female flies was determined, blueberry stems with mature berries were placed in the cage. The stems were in small vials with water and stoppered with laboratory film. Stems were then removed on a weekly basis in order to collect eggs and larvae within the fruit. This task was performed to artificially inoculate the blueberries with maggots in a laboratory setting. The berries were left in the cages for approximately

one week. At that time the blueberries were removed and replaced with freshly harvested blueberry stems. This protocol was followed for four weeks or until the maggot flies expired. The blueberries were taken from the cages and placed in a cool laboratory (approximately 22 °C) for one week to allow for development of the maggot egg into the larval stage. These blueberries were observed every other day to assess deterioration. At the appropriate time the blueberries were prepared for near-infrared scanning as described below.

2. Near-infrared spectroscopy (NIRS) scanning and analysis. Samples were assigned names according to their origin (e.g., "Jonesboro") and the batch number corresponding to the week in which berries were picked. Each batch was separated in one to six subsets of 120 berries each and designated with a letter (A, B, C, D, E and F).

The first step of the NIRS process was sizing the individual berries. Employing a sizing template device the berries were sized, stem side up, by fitting it through the appropriate slot indicating berry diameter in mm. Berries that were under 6 mm were not used. Each berry was sized and placed in an individually labeled tray, which depicted the date, batch number, set letter and berry number. Once these steps were completed the berries were held at laboratory refrigerator at ca. 4°C until they were scanned using two NIRS systems. All berries in a single set were scanned on the same day and under the same conditions. Figure 1 gives a schematic of the basic overall berry scan setup for both NIR systems at UMaine.

During sizing, berries were graded by visual assessment using a scale of 1 to 5. Grading was based on ripeness and softness/deterioration where 1 was equal to lowest level and 5 to the highest level of these characteristics. Laboratory personnel were trained for visual assessment. These data were recorded in the data sheets and used as reference in the model building.

All sized berries were scanned at the Chemical and Biological Engineering laboratory at UMaine, with a prototype UV-NIR system from Ocean Optics, Inc. (Dunedin, FL) and a NIR system from Control Development, Inc. (South Bend, IN). In both cases a wide-spectrum (200 – 1700 nm) halogen light source was focused onto the individual berry at a distance from the culminating lens of approximately 25 mm and 70 mm for Ocean Optics NIRS (OO) and Control Development NIRS (CD) respectively. A collimating lens mounted at a 45 degree angle from light incidence allowed collection of light reflected from the berry; the reflected light was directed to an A/D converter via a fiber optic cable. After digital conversion, the sample data between 650 and 1100 nm (OO) or 900 – 1600 nm (CD) were graphed via the associated software program (OOIBase32, Ocean Optics, Inc. and Spec32, Control Development, Inc.). Replicate scans of each berry were collected and each set of berries was consecutively scanned on the two systems (see Figure 1 for a schematic).

After NIR scanning all berries were dissected under a light microscope to determine maggot presence. When found, maggots were classified based on their length and these data were included in data sheets for later modeling.

3. Spectral Subtractions

3.1. Subtractions of averaged spectra

Two sets of spectra from non-infested berries and two sets of spectra from infested berries were randomly selected and averaged resulting in two averaged spectra from non-infested and infested berries respectively. These averaged spectra were subtracted to get a difference.

3.2. Stem and calyx end spectra subtractions from the same berry

One stem spectrum and one calyx spectrum from the same berry were subtracted to determine if when these spectra are subtracted the result will be similar to a maggot-only spectrum (Figure 1). Subtractions are done to remove background signal and expose any underlying signal coming from the maggot.

4. Prediction model analysis

First, individual spectra were imported into the modeling tool (GRAMS®, version 6.00, Thermo Galactic, Salem, NH) and training data sets were built from each set of 120 samples. Before building calibration models, the individual spectra were examined for anomalies, potential outlier samples or particular wavelengths of interest. Observed anomalies in the raw spectra were compared later with outlier spectra identified by statistical tests on the residuals (error terms) from the Partial Least Squares (PLS) models.

In order to build balanced sample sets having approximately the same number of infested and non-infested samples, the spectra from all sets in a batch (picked and scanned during the same week) were combined together and equal number of infested and non-infested sample spectra was selected for building calibrations. PLS analyses were carried out on all spectra from previous years and on a number of data sets from 2006. PLS involves regression of the independent variations contained in the spectra against the measured reference data (infestation, size, water content, etc.). All independent variations are captured in separate factors which may represent different physical or chemical properties of the samples such as water or sugar content, color, size etc. The first factors isolated during PLS modeling usually represent the largest variation contribution in the spectral data.

For developing calibrations, non-infested and infested blueberries were arbitrary assigned a value of -1 and +1 respectively (called constituent values). The cut-off value was calculated as the arithmetic mean of the assigned arbitrary constituent values and in this case equal to zero (0). Samples were considered infested if predicted constituent values were greater than zero, and all others were considered non-infested.

Preprocessing methods that were used included mean centering, variance scaling, light scatter correction methods, and 1^{st} and 2^{nd} derivatives over 5 points. These methods are often used in spectroscopic data analysis as they further enhance the PLS model calibration (see Delwiche and Reeves, 2004; Walsh et al., 2004; Chen et al., 2002; Dardenne et al., 2000; Lammertyn et al., 2000).

Data with replicate scans were transformed by averaging across replications. Spectral data sets from the same batch scanned with the same instrument and settings were joined to yield combined data sets with large number of spectra. PLS was performed on these large combined data sets as well as on single data sets from the same batch and results were compared. Cross validation was used in the analysis to estimate the robustness of the models. This algorithm attempts to predict unknown samples by using the training data set itself. The reduction in the standard error of cross validation (prediction), SECV,

was used to determine the recommended number of PLS factors to include in the model. Spectral and concentration outliers were identified based on the residual plots after calculating the PLS models. Beta (calibration) coefficients from PLS were used to test for absorbance bands sensitive to differences between infested and non-infested berries.

4.1. Test of different cut-off values used in the PLS prediction models

The choice of a cut-off value has a direct impact on the percent of correctly predicted samples in the evaluation of model performance. This value is equal to the mean of the arbitrary values assigned for maggot presence or absence (-1 and +1) and in our case is set to 0. In order to test the effect on prediction ratios, cut-off values were varied from 0 to 0.8 in steps of 0.2 and prediction ratios for infested and non-infested berries were calculated at each step for several models. These models were compared to the original models with cut-off values of 0. Our goal was to achieve higher prediction ratio of infested berries while keeping the prediction ratio of non-infested berries at acceptable level.

5. Water content equilibration

In order to obtain equal moisture content in each berry, two sets of 120 berries were placed in a humidity chamber (AEWC, UMaine) for 24 h. The temperature was 25 °C and the relative humidity was maintained at 98 % during the first treatment to promote water uptake until all blueberries had approximately equal water content. The second treatment of the same berries was at 25 °C and 68% RH. All berries were scanned by NIR before and after each humidity chamber treatment and dissected after the last NIR scanning to determine infestation.

6. Freeze-drying

A set of 96 fresh berries were individually weighted and scanned by NIR. Then all berries were immersed in liquid nitrogen to initiate quick freezing. They were then freeze-dried under vacuum for 24 h. All berries were weighted again after drying was complete and moisture content was calculated for each berry. They were scanned again by NIR immediately after freeze drying. Spectra of fresh and freeze dried berries were compared for each berry and models were evaluated.

7. Firmness tests

Using rebound height evaluation, the firmness of two sets of 120 berries was measured after NIR scanning. Berries were dropped from approximately 40 cm and the rebound height was measured. Three replications were made for each berry. Then all berries were dissected to determine infestation. Firmness and infestation data were included into PLS models to test for correlations between these factors.

8. Soluble solids measurements

Total soluble solids (SS) were measured in each blueberry after NIR scanning using a light refractometer. Individual berries were homogenized after adding 1:1 (w/w) deionized water in microcentrifuge tubes. After thorough mixing, the tubes with the samples were centrifuged for 15 min at 10,000 rpm to remove any fruit debris and obtain clear solutions for refractometer measurements. The supernatant from each tube was used for three repeated SS measurements. The SS data were added to infestation data for PLS model evaluation.

9. Amount of reflected light test

The amount of reflected NIR light from a sample is directly proportional to the intensity of the NIR signal. By increasing the area on the surface of the blueberry from which reflected and diffuse light is collected, the intensity of the NIR signal is increased. Larger area of signal collection also improves the probability to detect a maggot in the blueberry. The amount of reflected light was varied by moving the detector fiber and collimating lens from our standard distance of 7 cm to a longer distance of 10 cm from the berry. This resulted in increasing the area from which signal is collected by approximately 25%. A caveat of this treatment is that by increasing the amount of incident light, the signal to noise ratio is decreased. A set of 96 berries were scanned with these two settings (closer and further distance) and two PLS models were calculated for comparison.

10. Total protein concentration

Proteins were extracted from infested blueberries (with the maggot removed), non-infested blueberries and from maggots. The extraction methods consisted of modified standard procedures for protein extraction, precipitation and purification. The method for protein extraction was based on Fils-Lycaon et al. (1996). General protein content was measured on the final purified extracts by Coomassie assay (Pierce Chemicals, Woburn, MA). To build a standard curve, a series of standard bovine serum albumin (BSA) dilutions were made ranging from 2.5 µg/ml to 2,500 µg/ml. Absorbance was measured at 595 nm fixed wavelength on UV/VIS spectrometer after adding the Coomassie dye to the standard solutions and samples. Protein concentrations of the samples were calculated from their absorbencies at 595 nm using the standard curve of protein concentration vs. absorbance.

11. Prediction of new samples with a saved model

After a calibration model was refined and validated it was saved and a number of spectra which have not been used for building this model were run through the model for infestation prediction. One of our calibration models was tested with 96 new spectra with 50% infested samples. Similar to calibration model evaluation, all predicted infestation values were compared to the actual values. The cut-off value was equal to zero and all predicted values less than 0 were considered non-infested and all greater than zero were considered infested.

RESULTS

1. Artificial laboratory infestation and preparation

The laboratory experiment to artificially inoculate berries with maggot larvae was successful this season. Average maggot infestation rate of samples sets during the 2006 season was 15 %, ranging from 0 to 37%. Lower percentages were due to increased mortality rate among mature laboratory raised flies towards the end of the season. However, for the purpose of developing prediction models data sets with high infestation ratios of above 30% were used and spectra were selected and combined resulting in data sets with approximately 50% infestation ratio.

2. NIRS: modeling and analysis

Based on analysis of preprocessing methods, mean centering and variance scaling were applied to all 2006 PLS models and multiplicative scatter correction, 1st and 2nd derivatives were tested. However, preprocessing had varied affects on different models and led to insignificant improvement of the prediction results of about 0-3%; our findings are similar to these of other researchers (Delwiche and Reeves, 2004).

3. Spectral subtractions

3.1. Subtractions of averaged spectra

By examining typical absorption spectra from both infested and non-infested samples, a region of interest was determined; 1350-1700 nm. These observations were confirmed by spectral subtractions of averaged non-infested and infested spectra (Figure 3). This is the wavelength band where proteins absorb, however, this is also the band of a major water peak. The difference spectrum from the two non-infested spectra shows random noise around zero indicating that non-infested spectra are very similar. However, there were differences seen after the subtraction of averaged spectra from infested and non-infested berries. Most pronounced these differences were for wavelengths between 1400 and 1700 nm. This band coincides with the position of the highest peak in sample spectra we have taken of larvae suggesting that by developing classification algorithms we should be able to classify blueberries according to infestation.

3.2. Stem and calyx end spectra subtractions from the same berry

After subtracting stem and calyx spectra from the same berry the resulting spectra for infested and non-infested berries were compared. There were no visual differences between these spectra and all subtracted spectra from infested and non-infested berries overlapped. These results could be due to large variations between scans for different orientation of the same berry. The calyx has very rough surface compared to the stem end, therefore simple subtraction might not achieve the goal of background signal removal to reveal possible underlying maggot signal. Another possible reason is that NIR is not detecting signal coming directly from the maggot but rather chemical and physical changes due to the maggot presence which is affecting the whole volume of the berry. Thus, subtraction spectra will display differences in berry orientation rather than detected chemical differences, which would be present in the spectra from any orientation of the berry.

4. Prediction model analysis

The preliminary results from 2006 PLS models confirm findings from previous years, i.e., models with small number of samples and low infestation ratio do not provide satisfactory infestation prediction. The higher percentage of infested berries in 2006 (37%) than in 2005 (0-10%) provided sufficient data for building and testing PLS prediction models. PLS prediction results in 2006 were generally similar to results from 2002 through 2005 (see Table 1). Total infestation prediction for the full models was between 74 and 88% with average prediction ratios equal to 80%.

In order to determine the effect of maggot size on prediction results as well as to find maggot size detection limit, sample spectra from berries and maggots with similar size were selected and models were computed similar to previous years. During dissections maggots were separated in three categories according to their length: large = 4 mm; medium = 3 mm and small < 2 mm. Models after maggot size separations showed differences in the prediction ratio of large and medium, 69-83% versus small maggot size 53-75% (see Table 1). Selecting large and medium size maggot samples generally yielded better prediction than small maggots and this prediction was similar to the prediction of the full model. Therefore, we can conclude that the detection limit for the NIR method is maggot length of 2 mm. This detection limit is similar to the visual detection limit of the standard USDA boil test.

4.1. Test of different cut-off values used in the PLS prediction models

The goal of this test was to increase the cut-off values in the analysis of the actual versus predicted infestation values in order to correctly predict higher percentage of infested samples. However, this analysis showed that there is a significant overlap of the predicted values of infested and non-infested samples. In order to obtain approximately 90% correct prediction of infested blueberries, the correct prediction of non-infested berries had to be decreased to an unacceptable level of 16-50%. Therefore, we concluded that a simple cut-off value adjustment does not have feasible application in improving model prediction.

5. Water content equilibration

Similar to the results in 2005, there were no visible differences in the raw spectra before and after each treatment. The standard deviations in the spectra from individual blueberries before and after humidity chamber treatment are presented in Figure 4. The standard deviations did not change significantly after 98% RH treatment when water is being absorbed by the berries. The standard deviations increased after 68% RH treatment when water is removed from the berries; normal natural water content is approximately 87% (Duke J.A., 2005). These results indicate that water is removed at different rates from each berry at 68% RH. PLS models showed slight improvement of 2-4% after treatment at 98% RH compared to no humidity treatment.

6. Freeze-drying

Spectra from blueberries before and after freeze drying had identical peaks and features. There was no improvement in model performance after including the water content data from this experiment in the PLS models. PLS models predicting the water content of each blueberry were tested. The PLS model predicted water content with $R^2 = 0.46$ (Table 2) which once again confirmed that water is a major source of noise in NIR, usually adversely affecting the NIR prediction.

7.Firmness

The results of PLS models predicting firmness are summarized in Table 2. Including firmness data in the PLS models did not provide an improvement of infestation prediction. The correlation values are relatively low, $R^2 = 0.61$.

8. Soluble solids

Including SS data in the PLS models did not provide an improvement of infestation prediction. The correlation values are relatively low; $R^2 = 0.60$ (Table 2).

9. Amount of reflected light test

PLS models for two different light detection areas on the surface of the berry were computed and compared. After increasing the area by 25%, the correct prediction improved by only 1.7% (from 75% to 76.7%). This can be explained by the fact that by moving the detector fiber further away from the berry expands the collection area but at the same time decreases the signal to noise ratio. Therefore, the detector fiber was kept in the default closer position (7 cm) for all other experiments.

10. Total protein concentration

Protein extracts from non-infested blueberries, infested blueberries and from maggots were prepared as described in the methods section. The total protein concentration in the protein extracts, measured by Coomassie protein assay, were found to be 2.1 µg/ml for the non-infested blueberry and 3.5 µg/ml for

the infested and 3.4 mg/ml for the maggot sample and are significantly different ($\alpha = 0.05$) The protein concentrations of the infested berry extracts were proven higher. However, these protein concentrations are lower than the 1 % theoretical detection limit for NIR spectroscopy. Therefore, the PLS models are most likely detecting variation due to other factors than only changes in the protein concentration in the blueberries. The next step in this research is identifying more compounds and their concentrations (other factors) by LC/MS which can potentially be sources for variation detected by our PLS prediction models.

11. Prediction of new samples with a saved model

After refining and validating the prediction models in 2006, one model was selected and tested with 96 spectra that have not been included in the calibration step. The model yielded approximately 83% correct prediction. This demonstrates that such prediction models are robust and capable of correctly predicting infestation in about 83% of the samples in processing environment. This success ratio is similar to the human operator rate of defect detection at lighted pick-over tables in the blueberry packing plants (Guyer, D, personal comm.., 2002).

CONCLUSIONS

Spectral subtractions of averaged spectra from infested and non-infested berries show differences in the protein absorption band; 1350- 1700 nm coinciding with a peak in a maggot only spectrum. However, the stem/calyx spectral subtractions from infested berries did not result in a maggot only spectrum indicating that the PLS prediction models are detecting variations due to other components in addition to the maggot presence.

The detection limit for our NIR and PLS method based on maggots size is approximately 2 mm maggot length, which is similar to the visual USDA boil test. PLS prediction models consistently provide approximately 80% correct prediction of infestation. Prediction of unknown samples yielded 83% correct prediction confirming that our models are robust. Including additional data in the models such as firmness, SS and water content did not lead to significant model improvement. This is evidence that water is a major source of noise in NIRS. Increase of the amount of reflected light led to a slight improvement of infestation prediction of about 2% due to trade-off between increased light intensity and signal to noise ratio decrease. Protein concentrations in infested berries are significantly higher than in non-infested berries indicating that infestation leads to increase in the concentration of protein and protein derivatives in the blueberries.

RECOMMENDATIONS

Our NIR combined with PLS method is a feasible technique for detection of fruit fly maggots in Maine wild blueberries. The validation and testing of the method showed that 83% of unknown samples can be predicted correctly. This ratio is similar to the ratio of defect detection by human operators at lighted pick-over tables in the blueberry packing plants.

The major advantage of the NIR method is that it is automated, non-invasive and non-destructive to sample. All these factors allow for its implementation on-line at a packing plant processing line. Further tests are necessary to determine method robustness in industrial conditions as well as model performance and transferability to an on-line detector. The high speed NIR detectors available today

make it possible to scan all berries on a processing line instead of just a sample which is advantageous to industry quality control.

A possible implementation of the NIR/PLS method will include a series of detectors and control points in sequential order that will be capable of detecting more infested berries. Assuming that the non-infested berries will be predicted correctly by each detector and the software model has a prediction ratio of 80%, and then each detector would be capable of correctly detecting an additional 16% of the berries misclassified by the preceding detector. Thus, theoretically, a series of detectors will be capable of detecting more than 90% of all infested berries.

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Table 1. Infestation prediction for PLS models based on maggot size from 2002 to 2006

Year	2002	2003	2004	2005	2006
Prediction for large maggots, %	**-**	**-**	**76**	**75**	**82**
Prediction for medium maggots, %	**-**	**-**	**75**	**69**	**83**
Prediction for small maggots, %	**-**	**-**	**53**	**75**	**71**
Overall correct prediction, %	**88**	**74**	**80**	**80**	**80**

Table 2. PLS prediction of rebound height (firmness), Brix (SS) and water content in fresh blueberries scanned on the Control Development spectrometer.

	Rebound height	Brix	Water content
Number of PLS factors	8	10	10
Total number of spectra	681	681	201
R^2	0.61	0.60	0.46

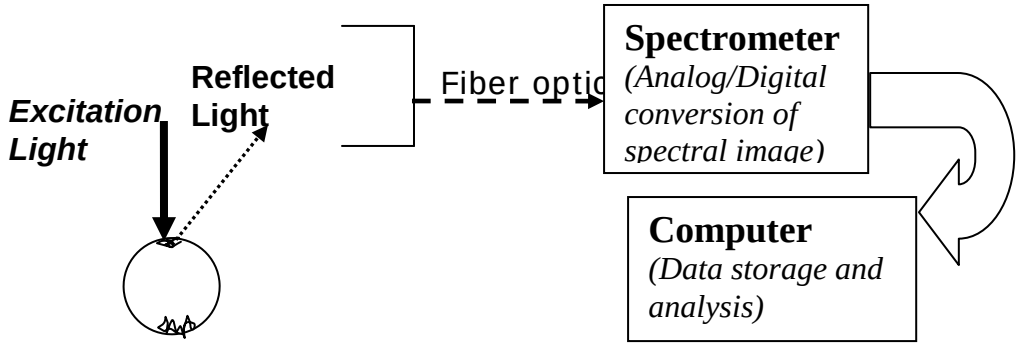

Figure 1. Flow schematic of equipment, light capture, spectrometer and computer. Reflected light will be at 45 degrees angle measured from the excitation light.

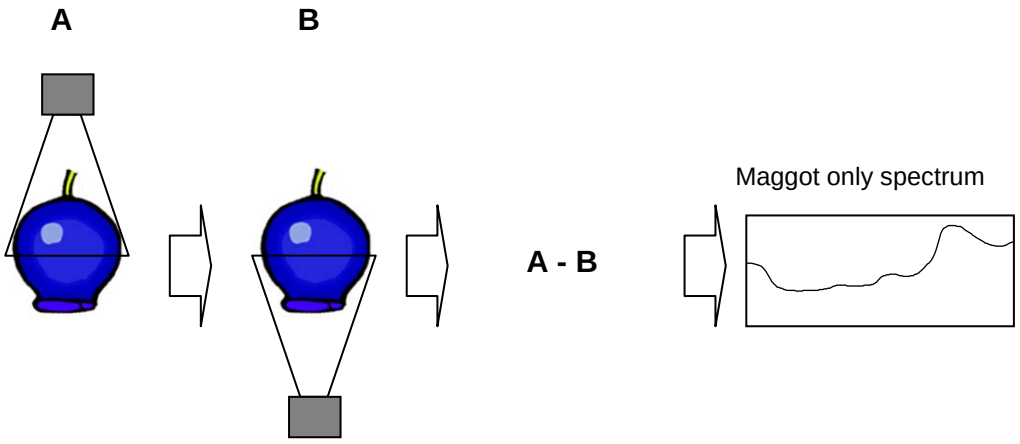

Figure 2. Subtractions of stem and calyx end spectra from the same blueberries. If a maggot is detected in one of the scans the difference spectrum will be similar to maggot only spectrum.

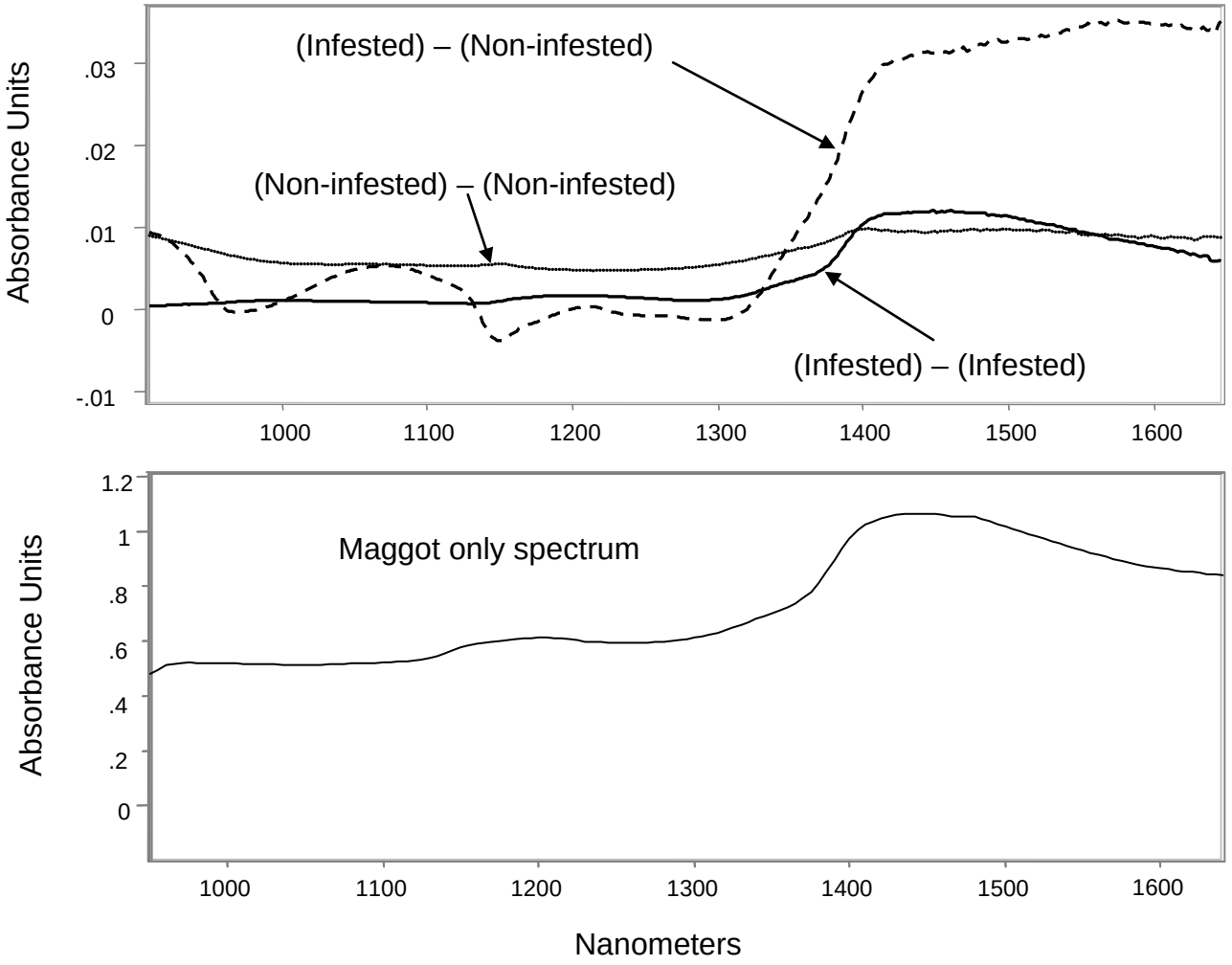

Figure 3. Subtractions of averaged NIR spectra from infested and non-infested blueberries and a maggot only spectrum (Control Development instrument).

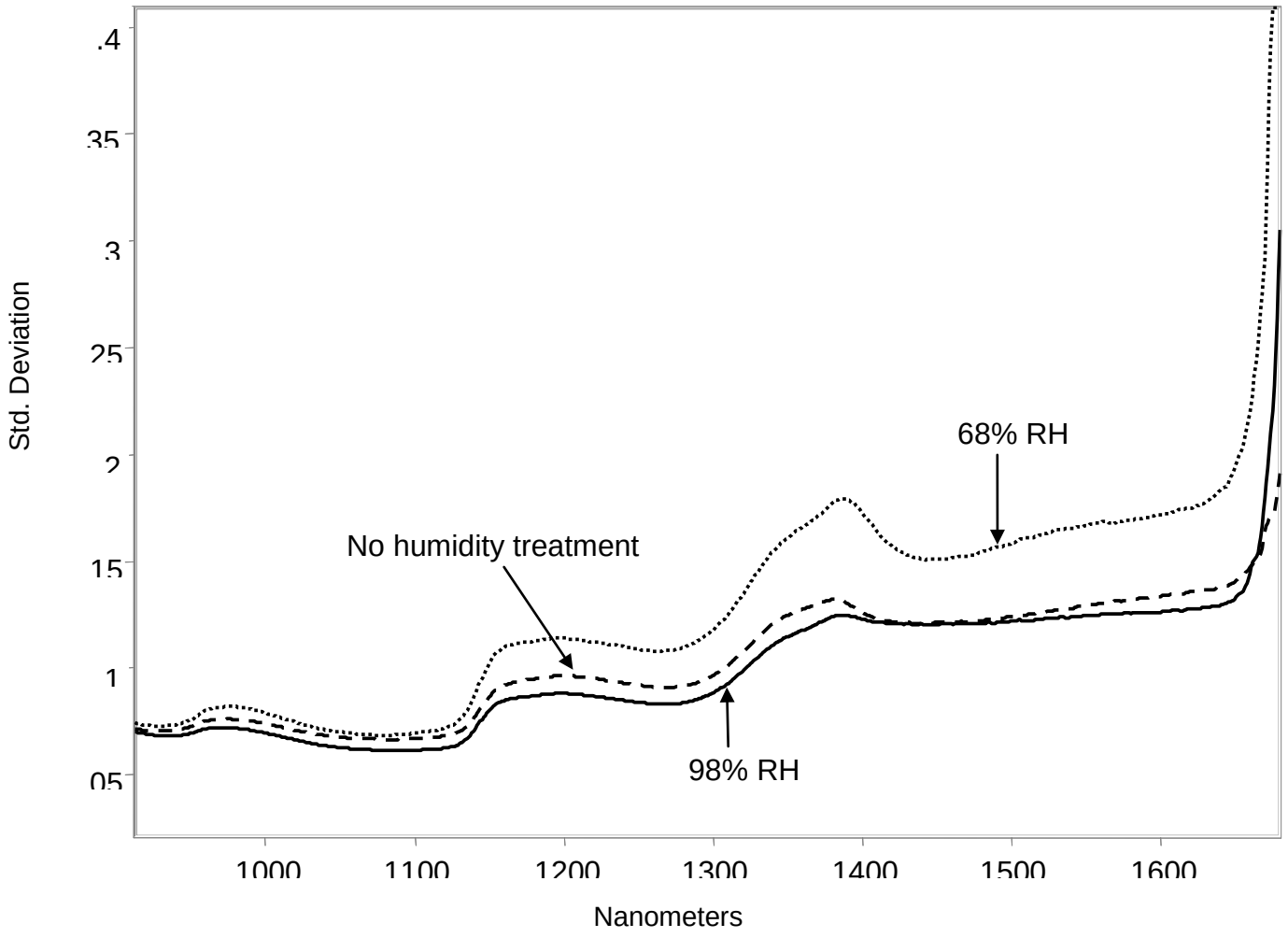

Figure 4. Standard deviations for all spectra before and after humidity chamber treatments at 68% and 98 % RH (Control Development instrument).

FOOD SCIENCE AND BIOSYSTEMS ENGINEERING

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

TITLE: Mechanism of Action through which Wild Blueberries affect Arterial Functional Properties in Normotensive and Spontaneously Hypertensive Rats

OBJECTIVE: To study the role of wild blueberry consumption on *vasoconstriction* during exposure to agonists such as phenylephrine (an endothelium-dependent vasoconstrictor) and pintpoint the biochemical pathway by which wild blueberries may be acting after inhibition of the NO and COX pathways in young Sprague-Dawley and Spontaneously Hypertensive Rats (SHR).

METHODS AND RESULTS: Weanling male Sprague-Dawley (SD) and Spontaneously Hypertensive (SHR) (ten in each group) were placed on the following diets for 6 weeks.

1. Control diet and
2. Control diet and blueberries

Rat weights and food intakes were measured throughout the experiment and rats were fed the above diets for 6 weeks. Rats were anaesthetized, blood and arteries were removed and arterial rings prepared. Aortae were excised, rings were prepared, and were immersed in tissue baths containing physiological saline solution (PSS) at 37 C, aerated with 95% O2 and 5% CO2 (pH 7.4). Following equilibrium and preconditioning under 1.5gm preload, rings were pre-contracted with a maximal dose of the alpha-1 adrenergic agonist, L-Phenylephrine (L- Phe, $3x10^{-7}$). Dose-response curves were generated with cumulative concentrations of L-Phenylephrine (10^{-8} to $3x10^{-6}$ M), a vasoconstrictor which requires the endothelium to employ its effect and its action is mediated through NO. After washout, one ring was contracted with L-Phe in the presence of the NOS (nitric oxide synthase) inhibitor, L-NMMA, one with MFA, a COX pathway inhibitor, and one with both L-NMMA and MFA.

The maximal force of contraction was measured (Fmax) to determine the effect of blueberries on endothelium NO- and COX- mediated vasocontraction. The maximum vasocontraction of SD and SHR aortae to L-Phe as a percent of the initial precontraction before and after treatment with inhibitors was studied. Concentration-response curves were determined for L-Phe and after NOS inhibition by L-NMMA, as well as after COX pathway inhibition with Mefenamic acid (MFA). Additionally, the pD_2 value for each ring was calculated as the negative log of EC_{50}, a measure of vessel sensitivity to the the alpha-1-adrenergic receptor response. Thus the specific pathways that blueberries exert their action on the artery both in young normotensive (Sprague-Dawley) and in hypertensive animals (SHRs) were identified.

CONCLUSION AND SIGNIFICANCE: Our studies in the past documented that wild blueberries affect the contractile machinery of the smooth muscle cell in the normotensive animal by decreasing arterial contractility in response to the vasoconstrictor hormone, epinephrine. Additionaly, we determined that when acetylcholine (which needs the intact endothelium for its action and operates through increasing the release of NO) is used as the compound to affect vasorelaxation, wild blueberries seem to potentiate greater vasorelaxation in the aortas of the animals that are under oxidative stress, the hypertensive animals, as compared to hypertensive animals fed normal diets.

With the present experiment, we examined the effect of wild blueberries on endothelial vasoconstriction in the young hypertensive animal and documented that feeding blueberries for only six weeks to weanling normotensive animals, the force of contraction developed by the aorta when challenged with L-Phenylephrine, was decreased. This effect is due to a possible potentiation of NO release and/or preservation of NO bioavailability as previously observed. Additionally, we discovered that blueberries in the normotensive animal, act to potentiate the production of COX-derived vasodilators, which inhibit vasoconstriction. In the Spontaneously Hypertensive animal that was fed the blueberry-enriched diet for 6 weeks we did not see an effect of blueberries on basal NO release but we documented that blueberries function through the COX pathway to possibly inhibit the production of a COX-produced vasoconstrictor(s) and thus decrease vasoconstriction when the artery is challenged by L-Phe. Thus blueberries in the young hypertensive animal operate through alternate pathways to affect arterial vasomotor tone.

RECOMMENDATIONS:
Future experiments will address the effect of blueberries on older animals (20 week old) that have developed full-blown hypertension to determine whether blueberries may reverse blood pressure elevation. The involvement of blueberries on the COX pathway will be further verified by utilizing inhibitors targeting specific enzymes in the COX pathway and measuring the concentration and activity of prostanoids such as TXA_2 and PGH_2.

FOOD SCIENCE AND BIOSYSTEMS ENGINEERING

INVESTIGATORS: Vivian C. H. Wu, Ph.D., Assistant Professor, Dept. of Food Science & Human Nutrition
Alfred A. Bushway, Ph.D., Professor, Dept. of Food Science & Human Nutrition

TITLE: Practical Microbial Control Approach and Antimicrobial Properties Study for Wild Blueberries

OBJECTIVE:
1. To develop chlorine dioxide pouch method for sanitation and microbial control for Maine wild blueberries.
2. To study the functionality of Maine wild blueberries and expend international market of Maine wild blueberries.

METHODOLOGY:

Objective 1

Aqueous chlorine dioxide (ClO_2) was studied for its effectiveness in controlling foodborne pathogens such as *Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella* Typhimurium, *Staphylococcus aureus,* and *Yersinia enterocolitica* as well as natural flora on blueberries such as yeasts and molds. All five pathogens were spot- inoculated on the skin surface of blueberries. A sachet (2g size) containing all necessary chemicals for generation of ClO_2 was used to provide approximately 320ppm of ClO_2 in 7.6 liter of distilled water. The efficacy of different concentrations (1, 3, 5, 10, and 15ppm) of ClO_2 and various contact times (10 sec, 1, 5, 10, 20, 30 min, 1 h, and 2 h) were studied.

Objective 2

We added the berry concentrate mixture [half blueberry and half cranberry concentrate] 0, 2.5, 5, 7.5% in ground beef (90% lean), respectively. Ground beef were then cooked in electric skillets to internal temperature around 71^{o}C (160^{o}F). To prevent cross contamination, treatment and control (0 %) were cooked in different skillets. 1.27cm^2 will be served to panelists during the sensory evaluation. A 9-point hedonic test was performed. Fifty panelists evaluated the appearance, flavor, texture, and overall acceptability of burgers added with cranberry concentrate

RESULTS:

Objective 1

The results of this study have been accepted for publication in International Journal of Food Microbiology. The results are also indicated in Table 1 and Figure 1. Reductions of all pathogens inoculated on blueberries were achieved by the application of ClO_2 treatments. Aqueous ClO_2 was most effective in reducing *L. monocytogenes* (4.88 log CFU/g) as compared to the other pathogens. *P. aeruginosa* was reduced by 2.16 log CFU/g ($P < 0.05$) after 5min when treated with 15ppm of ClO_2. Relatively short treatment time (20 or 30 min) was more effective in reducing *S.* Typhimurium than longer treatment time (1 or 2 h) for most concentrations. The highest reduction (4.67 log CFU/g) in the population of *S. aureus* was achieved with a 15ppm treatment of ClO_2 for 30 min. When treated for 2 h with 5ppm ClO_2, the reduction of *Y. enterocolitica* (3.49 log CFU/g) was not significantly different than the reductions at 10 and 15ppm (3.70 and 3.54 log CFU/g, respectively). In general, longer treatment times did not significantly reduce pathogen counts ($P > 0.05$) as compared to shorter treatments. Fifteen ppm of ClO_2 reduced natural yeasts and molds by 2.82 log CFU/g after 1 h. Results indicate that aqueous ClO_2 shows promise as a sanitizer for reducing foodborne pathogens as well as yeasts and molds. In addition, concentrations of ClO_2 were shown to decrease over time when stored at room temperature. When exposed to blueberries, ClO_2 concentrations were further reduced, showing significant degradation ($P < 0.05$) and suggesting a need for further study of the effect of organic materials on aqueous ClO_2 residues after treatments.

Objective 2

Our sensory evaluation results indicated that consumers prefer combination of cranberry and blueberry concentrate rather than cranberry or blueberry concentrate alone, when compared to previous studies. Results showed that no differences were found among burgers with 0%, 2.5%, and 5% cranberry concentrate for flavor, texture, and overall. Burgers with 2.5% cranberry and blueberry concentrate had the highest score among other concentrations for appearance, flavor, texture, and overall acceptability.

CONCLUSIONS: Aqueous chlorine dioxide (ClO_2) can be used as a sanitizer for controlling foodborne pathogens as well as yeasts and molds on blueberries. We expect that brief treatment intervals and degradation of ClO_2 over time and by organic materials (blueberries) provide advantages to food processors who seek to preserve the appearance of their product and reduce ClO_2 residues while enhancing food safety with novel sanitization procedures. The synergistic effects of wild blueberries and cranberries have heath benefits, significant antimicrobial effects, and potential applications in food system. They have multiple functions and can be considered for food applications.

RECOMMENDATIONS: Further studies in comparison of chlorine and chlorine dioxide and the synergistic effects of both low concentrations on microbial decontamination should be conducted. Developing real-time identification method for microbial contaminations is also needed to prevent

foodborne outbreak such as *E. coli* O157:H7 linked to Maine wild blueberries.

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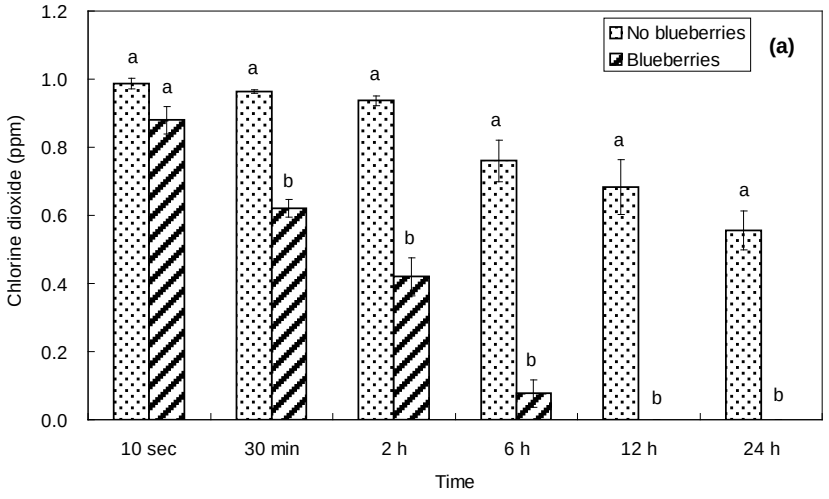
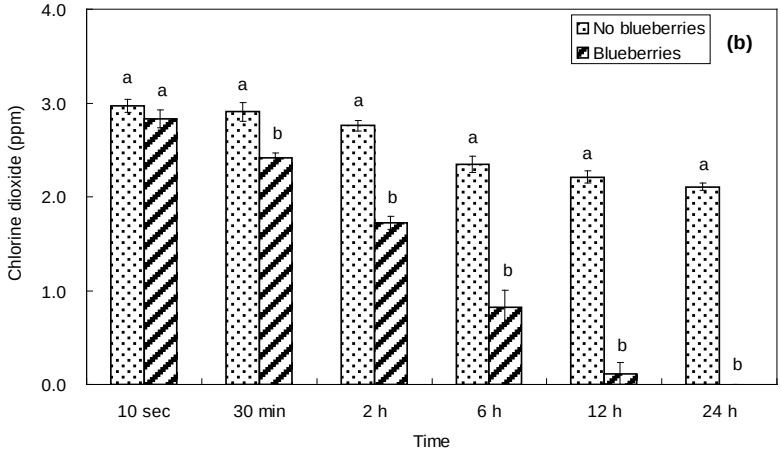
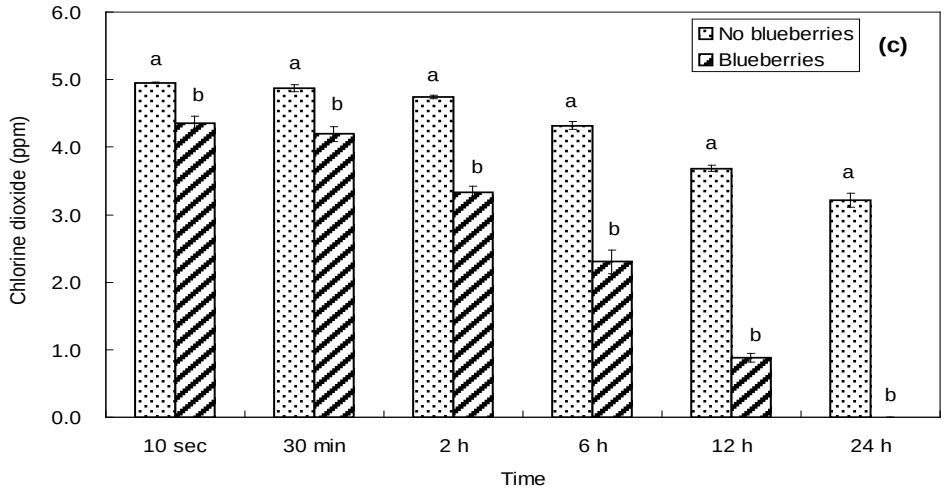
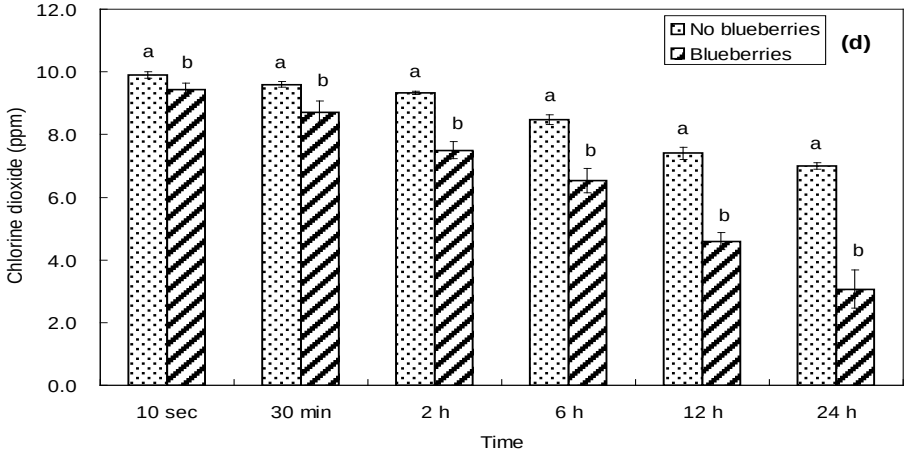
Fig. 1. Decrease of chlorine dioxide (ClO_2) concentrations over time with blueberries or without. (a) 1ppm, (b) 3ppm, (c) 5ppm, (d) 10ppm, and (e) 15ppm of ClO_2.
Concentrations of ClO_2 at each measurement time labeled with different letters are significantly different ($P < 0.05$) **(Kim and Wu, 2007)**.






(c)
No blueberries
Blueberries
Chlorine dioxide (ppm)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
a b a b a b a b a b a b
10 sec
30 min
2 h
6 h
12 h
24 h
Time


(d)
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Blueberries
Chlorine dioxide (ppm)
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10.0
8.0
6.0
4.0
2.0
0.0
a b a b a b a b a b a b
10 sec
30 min
2 h
6 h
12 h
24 h
Time

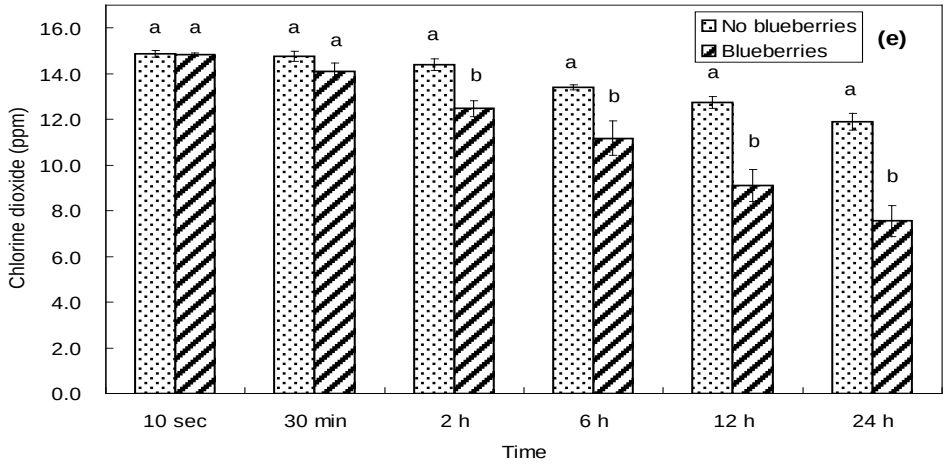

(e)
No blueberries
Blueberries
Chlorine dioxide (ppm)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
a a a a a b a b a b a b
10 sec
30 min
2 h
6 h
12 h
24 h
Time

Table 1. Reductions of *L. monocytogenes*, *P. aeruginosa*, *S.* Typhimurium, *S. aureus*, *Y. enterocolitica*, and yeasts and molds after treatment with aqueous chlorine dioxide (ClO_2) **(Kim and Wu, 2007).**

Pathogen	Treatment time	Reduction (log CFU/g)				
		1ppm[f]	3ppm	5ppm	10ppm	15ppm
Listeria monocytogenes	10 sec	A[g] 0.03 a[h]	C 0.04 a	D 0.08 a	D 0.04 a	F 0.07 a
	1 min	A 0.00 a	C 0.00 a	D 0.00 a	D 0.12 a	F 0.17 a
	5 min	A 0.07 c	C 0.05 c	D 0.31 c	C 0.87 b	E 1.39 a
	10 min	A 0.19 d	C 0.03 d	C 1.00 c	B 2.38 b	D 3.16 a
	20 min	A 0.17 c	C 0.58 c	B 1.61 b	B 2.66a	CD 3.46 a
	30 min	A 0.02 e	B 1.30 d	A 2.24 c	A 3.46 b	BC 3.95 a
	1 h	A 0.16 e	B 1.44 d	A 2.31 c	A 3.28 b	AB 4.25 a
	2 h	A 0.19 e	A 2.07 d	A 2.57 c	A 3.57 b	A 4.88 a
Pseudomonas aeruginosa	10 sec	A 0.02 a	B 0.06 a	C 0.11 a	D 0.24 a	D 0.15 a
	1 min	A 0.03 a	B 0.06 a	C 0.13 a	D 0.25 a	D 0.08 a
	5 min	A 0.05 c	B 0.18 c	BC 0.31 c	C 0.99 b	C 2.16 a
	10 min	A 0.24 b	B 0.12 b	ABC 1.50 a	B 2.20 a	C 2.36 a
	20 min	A 0.06 d	A 1.34 c	ABC 1.81 bc	A 2.96 ab	B 3.54 a
	30 min	A 0.17 d	A 1.31 c	AB 2.09 bc	A 2.98 ab	AB 3.85 a
	1 h	A 0.22 c	A 1.52 b	ABC 1.80 b	A 3.03 a	AB 3.81 a
	2 h	A 0.41 d	A 1.39 cd	A 2.36 bc	A 3.01 b	A 4.48 a
Salmonella Typhimurium	10 sec	B 0.00 a	AB 0.10 a	C 0.00 a	E 0.00 a	D 0.12 a
	1 min	B 0.00 a	B 0.00 a	C 0.00 a	E 0.00 a	D 0.23 a
	5 min	AB 0.24 b	AB 0.43 b	C 0.27 b	DE 0.60 b	CD 1.16 a
	10 min	AB 0.21 b	AB 0.66 ab	BC 0.57 ab	CD 1.55 ab	BC 2.03 a
	20 min	B 0.09 c	A 1.57 bc	A 1.93 ab	AB 2.86 ab	A 3.32 a
	30 min	AB 0.25 c	AB 1.52 bc	A 1.80 ab	A 3.21 a	AB 3.13 a
	1 h	AB 0.29 c	AB 1.41 bc	AB 1.47 bc	A 3.11 a	AB 2.43 ab
	2 h	A 0.42 c	AB 1.02 bc	AB 1.58 ab	BC 1.86 a	ABC 2.28 a

Pathogen	Treatment time	Reduction (log CFU/g)				
		1ppm[f]	3ppm	5ppm	10ppm	15ppm
Staphylococcus aureus	10 sec	B[g] 0.03 a[h]	C 0.10 a	C 0.27 a	D 0.19 a	D 0.21 a
	1 min	AB 0.15 c	C 0.18 c	C 0.50 bc	CD 1.01 a	D 0.98 ab
	5 min	AB 0.06 c	BC 0.39 bc	BC 1.06 ab	BC 1.85 a	CD 1.47 a
	10 min	B 0.01 b	AB 1.53 ab	AB 2.55 a	B 2.20 ab	BC 2.71 a
	20 min	B 0.02 d	AB 1.66 cd	AB 2.36 bc	A 3.46 ab	AB 4.24 a
	30 min	AB 0.09 d	A 1.92 c	AB 2.70 bc	A 3.92 ab	A 4.56 a
	1 h	A 0.26 d	A 1.73 c	A 3.07 b	A 3.82 ab	AB 4.37 a
	2 h	AB 0.19 d	A 2.06 c	A 3.11 b	A 3.87 ab	AB 4.33 a
Yersinia enterocolitica	10 sec	A 0.33 a	C 0.09 b	F 0.10 b	CD 0.40 a	CD 0.36 a
	1 min	A 0.12 a	C 0.11 a	F 0.06 a	D 0.15 a	D 0.18 a
	5 min	A 0.16 b	C 0.11 b	F 0.21 b	D 0.22 b	BC 0.86 a
	10 min	A 0.27 b	C 0.51 b	E 0.82 ab	C 0.86 ab	B 1.35 a
	20 min	A 0.17 c	C 0.64 bc	D 1.38 b	B 2.38 a	A 3.25 a
	30 min	A 0.19 c	C 0.62 c	C 1.97 b	A 3.17 a	A 3.33 a
	1 h	A 0.30 d	B 1.75 c	B 2.91 b	A 3.63 a	A 3.69 a
	2 h	A 0.12 c	A 2.88 b	A 3.49 ab	A 3.70 a	A 3.54 a
Yeasts and Molds	10 sec	A 0.26 a	A 0.27 a	C 0.20 a	D 0.41 a	D 0.57 a
	1 min	A 0.25 b	A 0.25 b	BC 0.67 b	D 0.54 b	C 1.82 a
	5 min	A 0.33 b	A 0.46 b	AB 0.85 b	CD 0.87 b	C 2.07 a
	10 min	A 0.26 d	A 0.52 cd	AB 0.85 bc	BCD 1.13 b	C 1.79 a
	20 min	A 0.16 c	A 0.57 c	AB 1.14 b	ABC 1.70 a	C 1.89 a
	30 min	A 0.34 b	A 0.43 b	AB 1.20 ab	ABC 1.92 a	BC 2.21 a
	1 h	A 0.40 d	A 0.52 cd	AB 1.17 c	AB 1.95 b	AB 2.82 a
	2 h	A 0.29 c	A 0.81 c	A 1.43 b	A 2.42 a	A 2.86 a

[f] Concentrations of ClO_2.

[g] Within the same microorganism, mean values in the same column with different capital letters (A through F) are significantly different among various treatment times for each ClO_2 concentration. [h] Within the same microorganism, mean values in the same row with different lowercase letters (a through e) are significantly different among various concentrations for each treatment time.

FOOD SCIENCE AND BIOSYSTEMS ENGINEERING

INVESTIGATOR: Mary Ellen Camire

TITLE: Wild Blueberry Consumption and Risks for Cardiovascular Disease

METHODOLOGY: Twenty-six adults with elevated low-density lipoprotein (LDL) cholesterol greater than 3.4 mmol/L (130 mg/dL) and less than 4.9 mmol/L (189 mg/dL) were recruited for this randomized control study. Weight, blood pressure, and fasting blood analyses were obtained at weeks 0, 4, and 8 of intervention. Blood analyses included cholesterol (total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein, and triglycerides), high sensitivity C - reactive protein, and total antioxidant status. Subjects were randomly assigned to a control or blueberry group; nine control subjects and 17 blueberry subjects completed the study Both groups were asked to follow the National Heart, Lung, and Blood Association's (NHLB) Therapeutic Lifestyle Changes (TLC) Diet personalized by subject gender, age, Body Mass Index, and activity level. The blueberry group was also asked to consume two half-cup servings (~64 grams each) per day of frozen blueberries provided by the Wild Blueberry Commission of North America. Three-day food records were collected at 4 and 8 weeks to estimate compliance.

RESULTS: No significant differences between groups were observed for serum total cholesterol, LDL cholesterol, total antioxidant status or blood pressure. Significant difference resulted for serum triglycerides, HDL cholesterol, and total cholesterol to HDL ratio. High-sensitivity C-reactive protein levels were inconsistent, suggesting that infection or other inflammatory factors confounded treatment effects. A few subjects lost weight during the study but most did not illustrate a weight change. Compliance with the TLC diet was poor and contributed to limited improvement in CVD markers.

CONCLUSIONS: Consumption of one cup of wild blueberries per day is insufficient to modulate risk factors for cardiovascular disease.

RECOMMENDATIONS: Efficacious doses of wild blueberries based on body mass should be investigated. Wild blueberries may play a role in weight reduction.

IRRIGATION

TITLE: Irrigation Water Use in Wild Blueberry Production

INVESTIGATORS: Gordon Starr, Soil Scientist/Hydrologist
David Yarborough, Professor of Horticulture

EXECUTIVE SUMMARY

Grower experience indicates that the wild blueberry crop requires somewhere near one inch per week of water and that fog and dew aid production by supplying water. Results obtained in the period of 2002-2006 have substantially confirmed these beliefs. However, research is needed to improve the amount and timing of irrigation water applications across the blueberry growing region of southeast Maine. Research has progressed significantly since the inception of the project in 2002, and the 2006 dataset is the most complete of any thus far. In this report, we discuss progress on sites and methods, revisit results from 2002 and 2004 to provide background information, and then give a discussion of some unique features of the 2005-2006 datasets.

BACKGROUND: Diminishing supplies and competing interests for water have resulted in severe irrigation water supply shortages for the Wild Blueberry Industry. A meeting was held in November, 2001 with industry representatives, growers, and researchers to determine water related research priorities of the Wild Blueberry Industry. A collaborative study was initiated in 2002 by the USDA-ARS and University of Maine with funding provided by the Wild Blueberry Commission and field support from collaborating farmers. This ongoing study has the following objective:

OBJECTIVE: Develop efficient water management practices for wild blueberry production.

IMPACT OF RESEARCH/BENEFIT TO INDUSTRY: Growers will have better information and techniques for making proper irrigation decisions. Water will be conserved and made more available for other users that compete with irrigators for this valuable and limited water resource.

SITES AND METHODS

Weighing lysimeters (Figure 1) and devices for measuring soil water tension, soil water content, and meteorological variables have been used since 2002 for studying crop water use at Blueberry Hill farm in Jonesboro, ME and these have been complemented by lysimeters four additional sites. Installations in both crop and prune years were used at Jonesboro and Deblois, ME and an installation in crop only was used in Jonesport, ME. These sites were chosen to give a range of climate to evaluate fog and temperature effects on water use of wild blueberries as they vary with distance from the Atlantic Coast for the dominant sandy soils of the blueberry growing region. An installation located in Addison, ME was completed in 2004 and another completed in 2005 in Northfiled, ME. Both the Addison and Northfield installations are on finer textured soil.

2002 RESULTS: Evapotranspiration was determined by measuring the change in lysimeter weight per day for a 24 hour period from midnight to midnight on days having no rain and expressing this as an equivalent depth of water per week. This is illustrated in Figure 2 which shows daily rainfall and average lysimeter weight versus time from June 6 through June 25,

2002 at the Blueberry Hill site. The ET for days 159 and 160 averaged 0.48 in/wk whereas days 164 and 165 averaged only 0.10 in/week. On all four of these days, strong increases in nighttime lysimeter weight were evident. By contrast, the nighttime rise in lysimeter weight was not as pronounced for days 172 through 174 and ET averaged 1.0 in/wk.

The nighttime increases in lysimeter weight were a persistent feature seen in the data, particularly at the two sites nearest the coast. Figure 3 compares Blueberry Hill and Wyman's farm from July 11 through July 16. For days 194 and 195, it is the nighttime rise in weight that appears to make the difference between the 0.99 in/wk recorded at Blueberry Hill and the 1.25 in/wk (3.2 cm/wk) recorded at Wyman's farm. The difference in ET between Blueberry Hill (1.0 in/wk) and Kelley Point (0.61 in/wk) could not be entirely explained by nighttime rises in lysimeter weight (Figure 4). The nighttime rises were evident at both sites yet Blueberry Hill still had much higher ET (Figure 4). The daytime temperature has a strong effect on ET and the Kelley Point site is persistently much cooler than either of the other sites located further inland.

The nighttime rise in weight appears to be a significant flux of water and should be studied further. Researchers in Europe saw similar effects in their weighing lysimeters containing bare soil near the Mediteranean coast and attributed them to influxes of cool, moist air from the sea. The water vapor from the air was thought to adsorb directly into the soil. Increases in relative humidity characteristically accompanied decreases in air temperature (Figure 4) at the Blueberry Hill site, so it is reasonable to suspect the same phenomena are at work. The lysimeters in this study contain lowbush blueberry plants that will frequently collect heavy dew as moist evening air condenses on leaves and stems. It is not clear how much of the water deposited on the lysimeters at night comes from dew and how much (if any) is directly adsorbed into the soil. In an attempt to resolve this question in the future, leaf wetness sensors are being installed to determine the presence of dew deposition.

Initial results for this study suggest that water was being supplied to the crop at night through direct condensation on the plants and adsorption into the soil. This effect was more prevalent at the sites near the coast. Several years of additional data are needed to quantify water use of the crop over time and throughout the two year cropping cycle. However, the initial results suggest that water demand of wild blueberries will be greater at inland locations where temperature is greater, humidity is less, and coastal fog is less prevalent.

2003 RESULTS: In 2003, the measured parameters included: vapor deposition (VD), vapor uptake (VU), evapotransporation (ET), rainfall (R), drainage (D), relative humidity (RH), solar radiation (SR), air temperature (T), visibility (V), wind speed (W), and volumetric soil water content (θ_v) at Blueberry Hill. Changes in weight averaged over the four lysimeters on an hourly basis were used to determine vapor transfers. The VD (hourly increase in weight) or VU (hourly decrease in weight) were calculated for only those hours when R = 0, D = 0, and irrigation = 0. Daily evapotranspiration was calculated using three different definitions: (1) daily change in weight (expressed as equivalent water depth) on days where R = 0, D = 0, and irrigation = 0; (2) depth equivalent daily change in weight minus daily R on days where D = 0 and irrigation = 0; (3) daily sum of VU minus sum of VD for all days.

It was a concern that only 74 of the total 115 days could be used with definition (1) and this might inject bias into the ET measurement. The ET was also calculated using definition (2) for 103 days and definition (3) for all 115 days. Using definitions (1), (2), and (3), ET averaged 0.31 cm, 0.27 cm, and 0.26 cm. Definition (1) gives a slightly higher average than definition (2) or (3), probably because by only using days with no rain, it represents a dry weather estimate for ET. Similary, by throwing out all hours with rainfall and irrigation,

definition (3) may understate true evapotranspiration because it does not accurately quantify the rapid evaporation period immediately following wetting events.
Initial data from a study of soil water uptake and deposition indicate that vapor deposition accounts for about 22% of the total water uptake and 28% of ET (calculated using definition 2) at the blueberry hill site. The supplemental irrigation to provide a constant weekly rate (1 inch/week) matched measured crop year water requirements through about day 235 after which ET fell rapidly and 1 inch/week would be excessive. Given the high rates of water deposition in the absence of rainfall it is important to have further studies of these phenomena as it may confound traditional irrigation scheduling. The VD may have a profound influence on ET, both over time and spatially at varying distances from the coast. Daily composite data indicated net deposition was greatest between 7:00 and 9:00 a.m. Vapor deposition was weakly correlated with changes in soil water storage suggesting that deposition may be directed into the soil and not merely in the form of dew deposition on plants. Day to day variation in water uptake (ET) rates was substantial and was clearly related to the maximum daily temperature and solar radiation.

2004 RESULTS: In 2004 we had relatively complete datasets on plant water uptake at the three coarse textured sites. Thus, our initial analysis of these data focuses on dry weather plant water uptake (ET as calculated using definition 1) comparing crop and prune year data at various distances from the coast. Water uptake depended fairly predictably on distance from the coast (Figure 6). The inland site (Deblois) had the highest uptake, followed by Blueberry Hill, and the lowest was the coastal Kelley Point site. Uptake for all sites and years fell off fairly rapidly after the beginning of August. The prune (solid lines) vs. crop (dashed lines) comparison showed that prune water uptake was lower than crop at both blueberry hill and Deblois. As the season progressed, the two phases of the growing cycle approached one another and by the latter part of August were nearly identical.

Based upon these data, it is suggested that the prune year water requirements were in the range of 0.8 to 0.9 inches per week over most of the growing season (higher at Deblois than Blueberry hill). However, water requirements were reduced after mid August and reached values as low as 0.5-0.7 inches per week by mid September. Crop year water requirements were considerably higher at Deblois than elsewhere from mid June through the latter part of July. There was a peak in crop year water uptake in late June at Deblois and Kelley point. The year 2004 is the first year that a full compliment of data is available for comparing water use at the various crop years and sites. Thus, future research is needed to confirm these results and establish long-term averages of crop water usage. Also, it should be cautioned that rainy days were not used in the 2004 calculations. As observed previously, rainy days, foggy days, and days with dew formation have generally lower water uptake and significant vapor deposition. Analysis is currently being conducted to establish corrections to water uptake curves to account for these phenomena.

2005-2006 RESULTS: The 2005 and 2006 crop years were the first in which data from a completed battery of research sites including sandy and finer textured soil are available. These data are currently being analyzed for crop water uptake rates and irrigation requirements. Two publications (one extension and one peer reviewed) are in preparation on this subject and these will be provided to the research committee. The analysis is being focused upon providing information on plant water demands that can be used in sizing and operating irrigation systems for maximum efficiency. In addition, the relationship between

sod depth and water holding capacity has been investigated using soil water mapping technology and our lysimeters that cover a range of soil types and sod depths. Testing of water redistribution through the sod and rhizome network has been done by studying redistribution along a drip-tube transect. Irrigation water application amounts, crop water stress (by the canopy temperature method), and soil water content have been measured across transects as the water redistributes following irrigation. Results from this study suggest that lateral redistribution through rhizomes will not greatly improve the water distribution of the irrigation system.

Both 2005 and 2006 were comparatively wet years. A general concern for the derivation of long term average water usage from the database (years 2004 through 2006 give complete complement of data on the sites) is that these include no drought years or even periods with very substantial dry weather. Thus, there is a need to continue monitoring to obtain representative long-term averages. Although it is difficult to set forth a minimum data set for deriving long term average crop water demands, five years of data should be obtained including a range of growing seasons from wet to dry.

Figure 1. A weighing lysimeter containing newly transplanted blueberry sod.

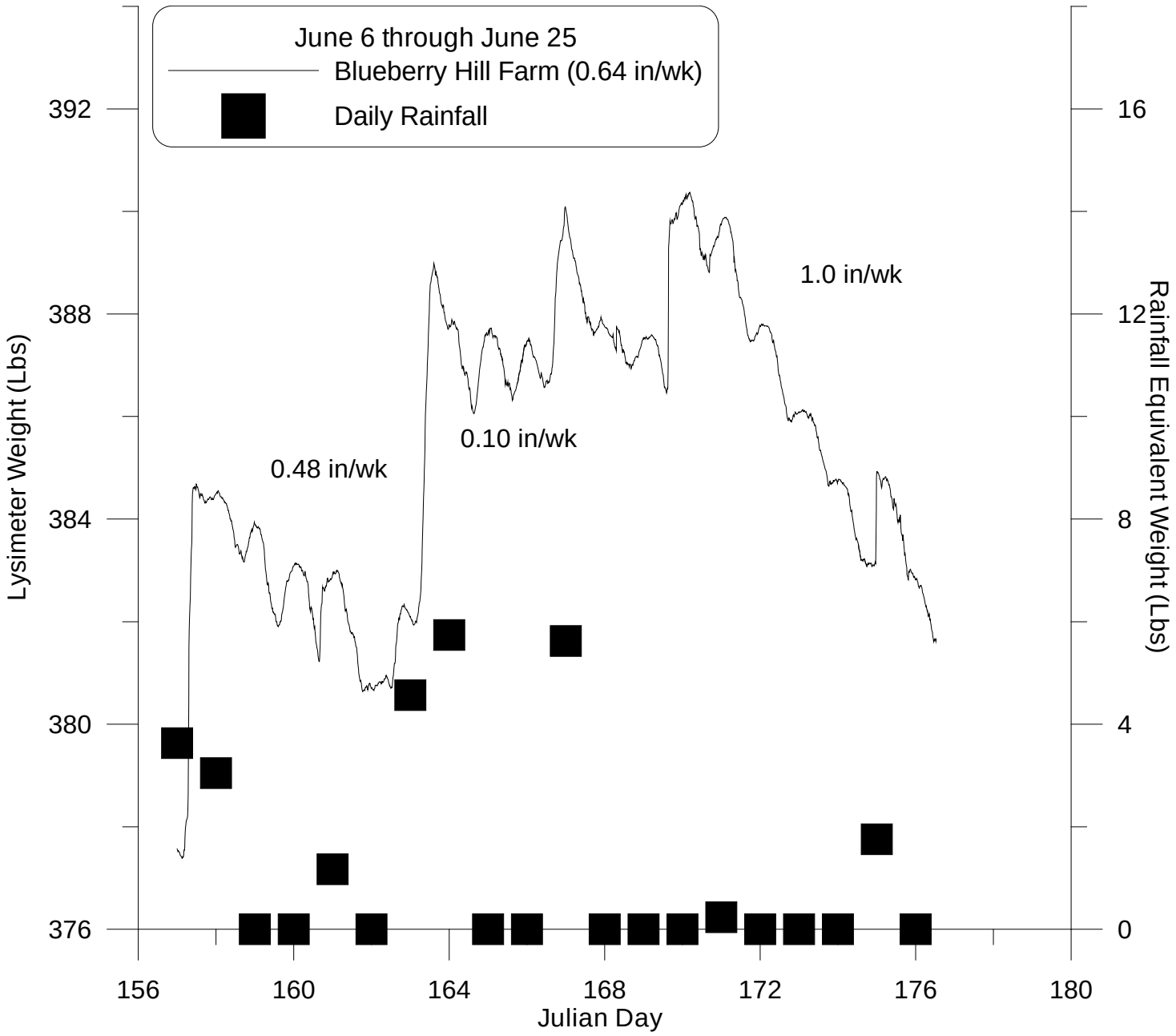


Figure 2. Rainfall equivalent weight and lysimeter weight versus the day of year (Julian Day) from June 6 through 25, 2002.

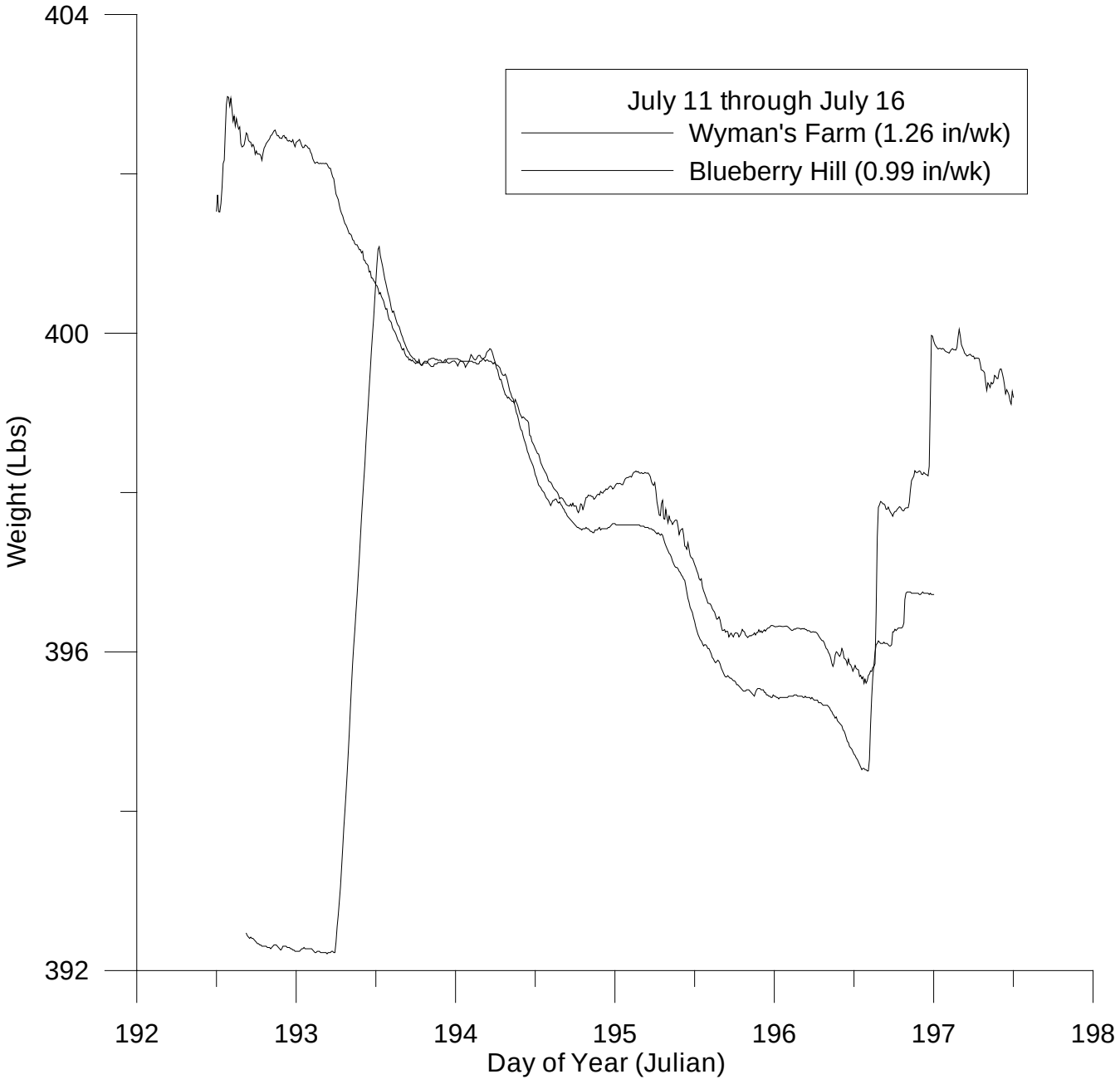


Figure 3. Lysimeter weight over time comparing Blueberry Hill and Wyman's Farm in 2002.

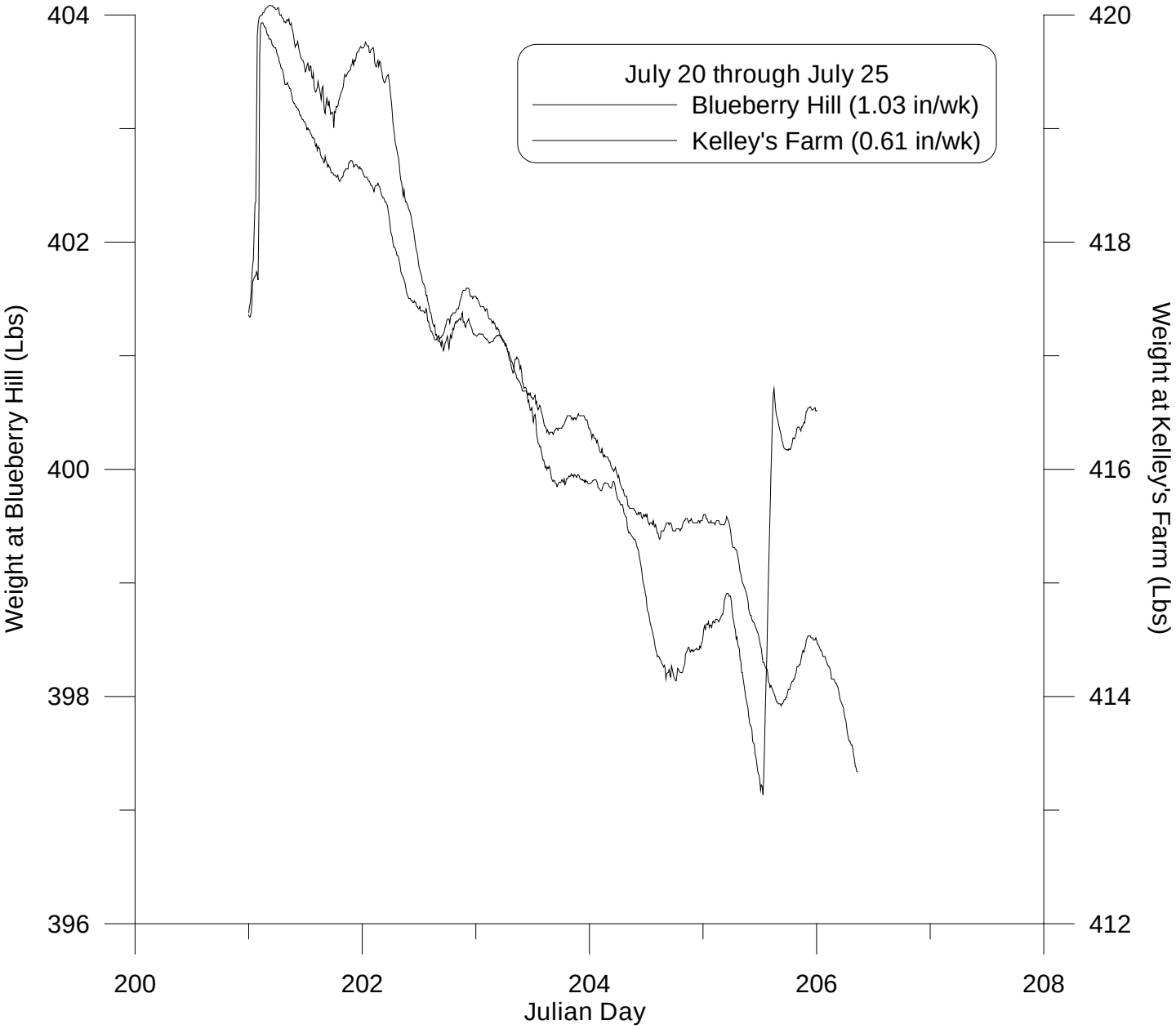


Figure 4. Lysimeter weight over time comparing Blueberry Hill and Kelley's Farm

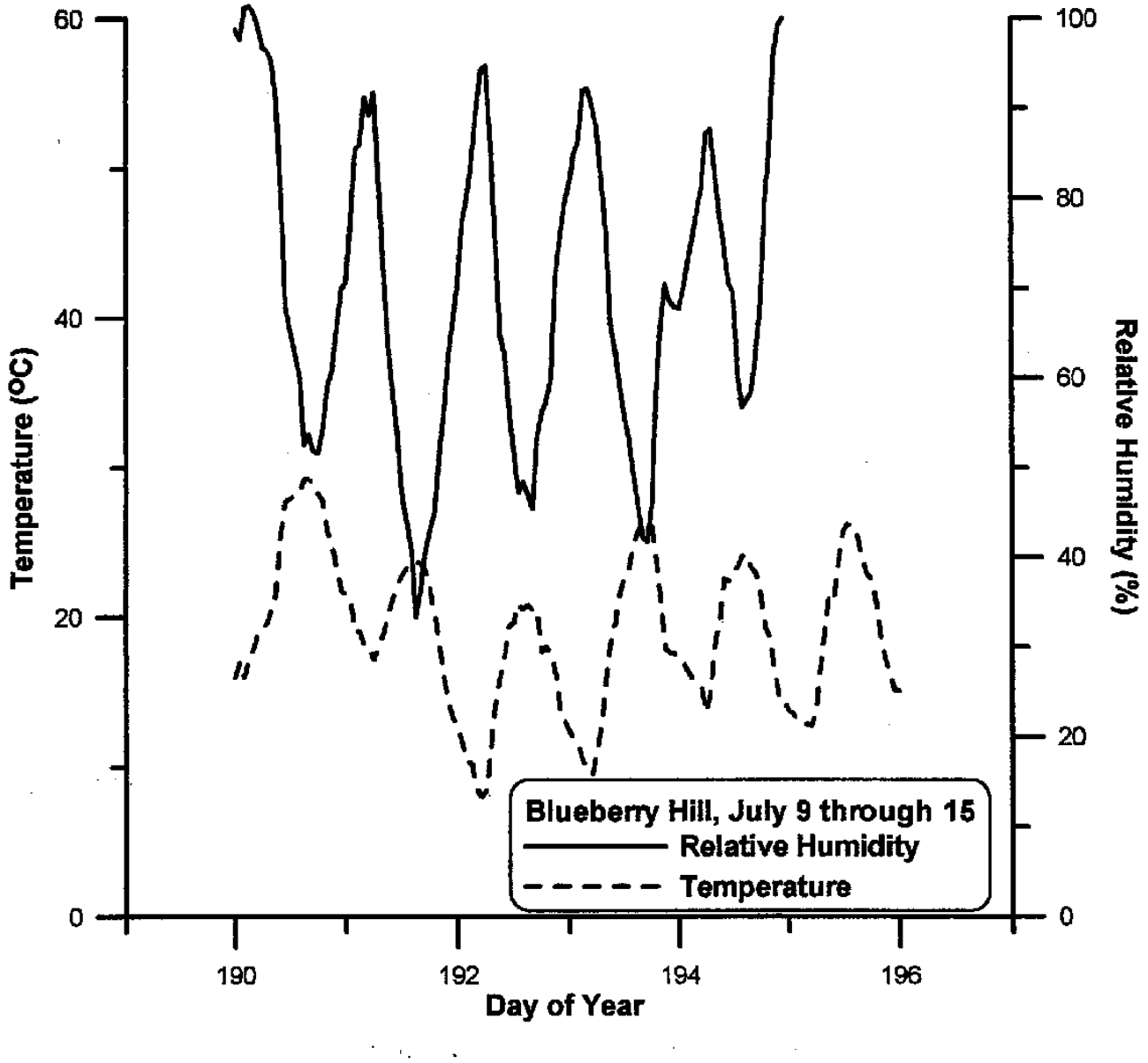


Figure 5. Daily temperature and relative humidity patterns at Blueberry Hill in Mid July, 2002.

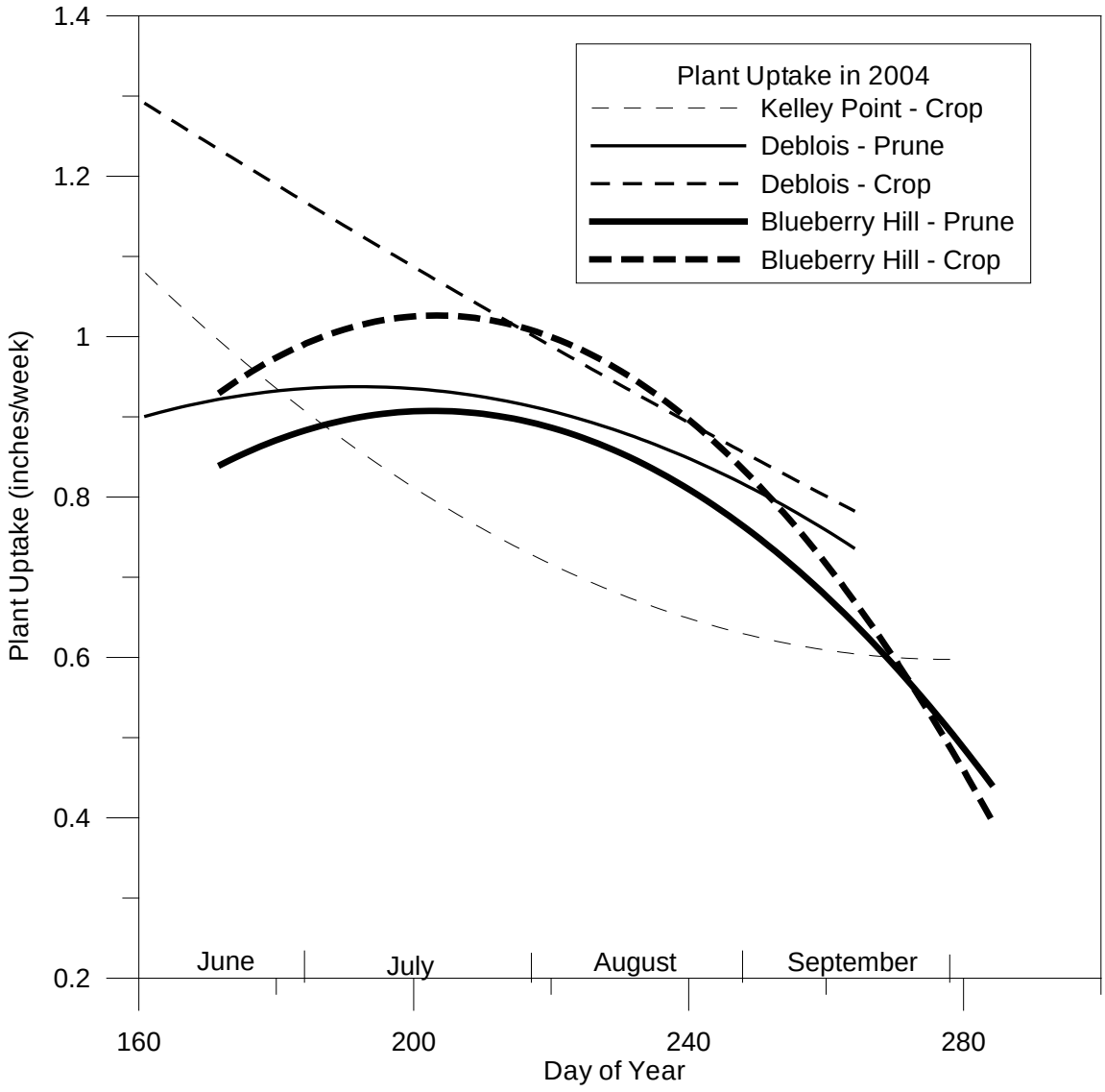


Figure 6. Plant water uptake curves for three sandy sites in prune and crop years.

ENTOMOLOGY

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

TITLE: Control Tactics for Blueberry Pest Insects, 2006

1. Laboratory screening of insecticides

METHODS: Each treatment 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. Walking speed was regulated using a metronome. The materials were allowed to dry on the foliage. Field-collected, late-instar blueberry flea beetle larvae or adults (FB) were placed in 3.5 inch diameter plastic cups with petri dish lids. One treated stem with foliage (collected from the field soon after application) was cut and placed in each cup. The cups were held at room temperature and assessed for mortality at one- or two-day intervals for five days. Untreated blueberry foliage was added in successive days during the experiment to each cup as needed.

RESULTS: Mean days to death of each treatment was compared to the untreated check within each trial (Table 1). Assail® 30 SG, Avaunt® 30 WG (both rates), Imidan® 70 WP, and SpinTor® 2 SC were all significantly different from the untreated check.

Figures 1, 2, and 3 are % survival of FB larvae or adults fed foliage treated with each material. In trial #1 against FB larvae, SpinTor and Assail both provided excellent control; and both rates of Avaunt also performed very well. Orbit® fungicide had no apparent insecticidal affects. In trials #2 and 3, Assail, Avaunt, Imidan and SpinTor also gave excellent control of FB adults.

Table 2 gives the rate/acre and the approximate cost/volume, unit cost, and cost/acre of the materials used in these trials. Imidan 70 WP is included as the material most commonly used in commercial production.

Table 1. Laboratory screening of insecticides, mean days to death.

Material	Rate (oz/acre)	Mean days to death *	*Prob Chi*^{2} $\leq$ 0.05 ***
Trial #1 – Blueberry flea beetle larvae			
Assail 30 SG	5.3 oz	1.0	< 0.0001
Avaunt 30 WG	4.0 oz	2.0	< 0.0001
Avaunt 30 WG	6.0 oz	4.0	0.0002
SpinTor 2 SC	6.0 oz	1.0	< 0.0001
Orbit fungicide	6.0 oz	**	0.5882
Untreated check	-	**	NA
Trial #2 – Blueberry flea beetle adults			
Assail 30 SG	5.3 oz	1.0	< 0.0001
Avaunt 30 WG	6.0 oz	1.0	< 0.0001
Imidan 70 WP	21.3 oz	1.0	< 0.0001
SpinTor 2 SC	6.0 oz	1.0	< 0.0001
Untreated check	-	**	NA
Trial #3 – Blueberry flea beetle adults			
Avaunt 30 WG	4.0 oz	1.0	< 0.0001
Avaunt 30 WG	6.0 oz	1.0	< 0.0001
SpinTor 2 SC	6.0 oz	1.0	< 0.0001
Untreated check	-	**	NA

* Biased estimate due to censored observations (live larvae) at end of study.
** Not possible to estimate because of > 50% survival.
*** Probability of treatment not different from untreated check.
NA Not applicable.

Table 2. 2006 costs ($) of insecticides (From Maine Potato Growers).

Material	Rate/acre	Cost /vol.	Unit cost	Cost/acre
Assail 30 SG	5.3 oz	15.26/oz	15.26/oz	80.88
Avaunt 30 WG	4.0 oz – 6 oz	5.70/oz	5.71/oz	22.84-34.26
SpinTor 2 SC	6.0 oz	595.36/gal	4.65/oz	27.90
Orbit fungicide	6.0 oz	103.00/qt	3.22/oz	19.32
Imidan 70 WP	1.3/lbs	9.20/lb	9.20/lb	11.96

Fig. 1. Percent survival of blueberry flea beetle larvae, trial #1.

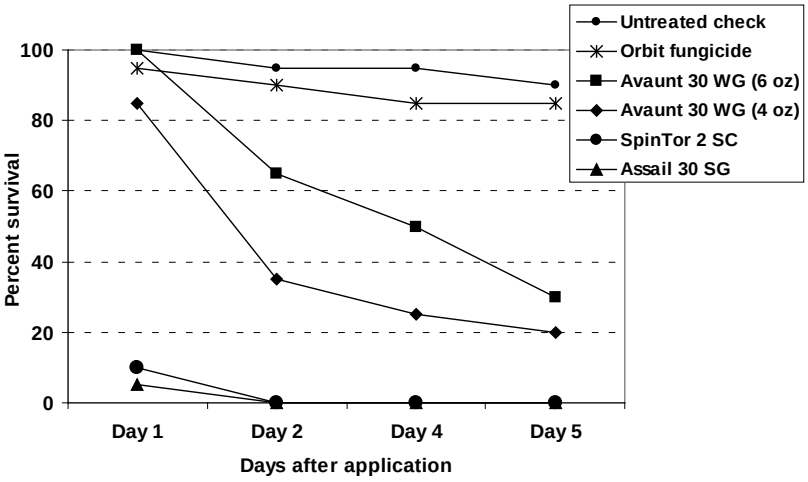


Fig. 2. Percent survival of blueberry flea beetle adults, trial #2.

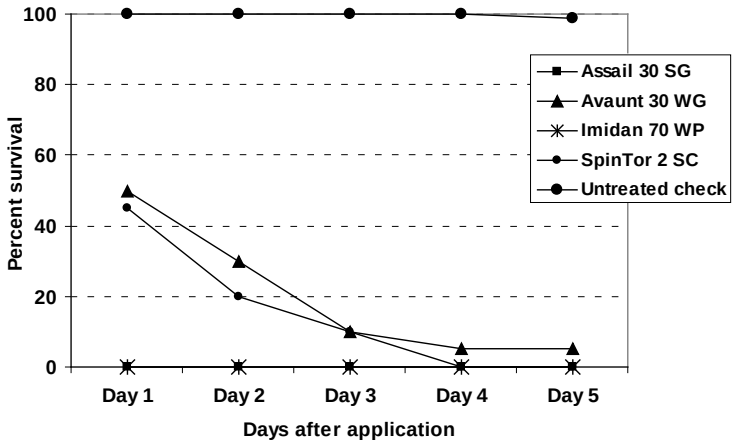
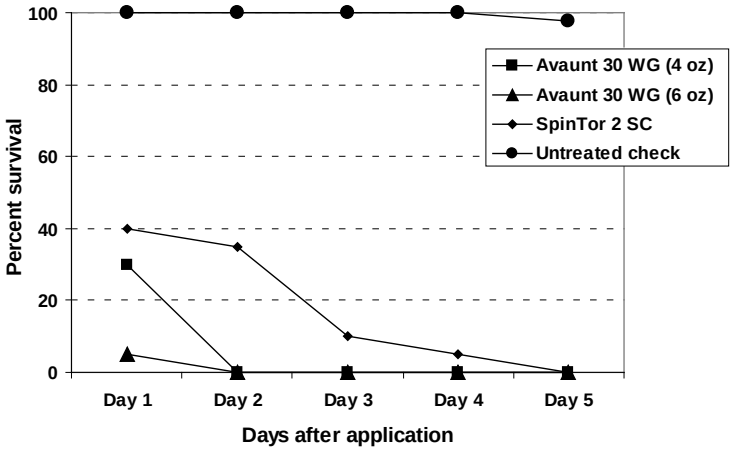


Fig. 3. Percent survival of blueberry flea beetle adults, trial #3.



CONCLUSIONS: All of the insecticides that were evaluated for control of flea beetles in the laboratory were effective relative to the check treatment of no insecticide. Assail and Avaunt took 1-2 days longer to reduce the populations of FB adults to negligible levels compared to SpinTor and Imidan. These later insecticides appear to reduce populations almost immediately after application of the insecticides to blueberry foliage. Future objectives will be focused upon evaluating reduced rates of some of these insecticides to establish if many of these reduced risk materials can be more economical relative to older materials such as Imidan, especially Assail (@ $80.88 / acre).

2. Field evaluation of insecticides for control of secondary pest insects

BLUEBERRY THRIPS

METHODS: Two trials were completed against blueberry thrips (BT). In trial #1, Admire Pro® and Provado Pro® were applied as sprays to the soil in a pruned field prior to stem emergence; Assail 30 SG were applied as a foliar spray timed to stem growth. Efficacy was evaluated according to the number of blueberry stems with and without thrips' damage as evidenced by curled leaves.

In trial #2, Admire Pro and Provado Pro were both applied as foliar sprays late in the season after monitoring indicated the presence of thrips as evidenced by leaf curls and yellow monitoring traps. Efficacy in this trial was evaluated by counting the number of thrips found in curls.

RESULTS: The pre-emergence application of Admire Pro resulted in a 75% reduction in the average percent stems with thrips curls. An 87% reduction was obtained with Provado Pro (Table 1). The results were significant ($P = 0.0008$) and are similar to those obtained in 2005 when a pre-emergence application of Admire 2 F (2.0 lbs ai/gallon) resulted in a 68% reduction in the average percent stems with thrips curls. There were also significantly fewer percent stems with thrips curls in the plots treated with two applications of Assail 30 SG (3.5%) than in the untreated check plots (25.4%). This is an 86% reduction; a 57% reduction was obtained with one application of Assail in 2005.
Foliar applications of Admire Pro and Provado Pro also appeared to have some affect. Curls in plots treated with both Admire Pro and Provado Pro had significantly fewer thrips on the second post treatment sample date ($P = 0.0218$) (Table 2).

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

Material (SE)	Amt. form./acre	Avg. # stems/ ft^2 (SE)	Avg. % stems with curls/ft^2
Admire Pro (pre-emergence)	7.0 oz	70.6 (10.8) a	6.3 (3.1) b
Assail 30 SG	5.3 oz	79.2 (12.0) a	3.5 (2.0) b
Provado Pro (pre-emergence)	16.0 oz	83.7 (8.9) a	3.4 (1.3) b
No insecticide	-	86.0 (2.0) a	25.4 (11.0) a

Means within each column followed by the same letter are not significantly different. Data for average number of stems per ft^2 was separated with SNK, $P \leq 0.05$. Data for average percent stems with curls was transformed by arcsine prior to analysis and separated with LS Means Differences Tukey's HSD, $P \leq 0.05$.

Table 2. Field control of thrips with foliar application of insecticides, summary.

		Avg. thrips/curl (SE)		
	Amt.	Prespray	Postspray	
Material	form./acre	19 Jun	26 Jun	2 Jul
Admire Pro	7.0 oz	1.18 (0.28) a	0.85 (0.40) a	1.08 (0.71) a
Provado Pro	16.0 oz	2.62 (1.73) a	1.10 (0.11) a	0.85 (0.45) a
No insecticide	-	1.00 (0.38) a	1.30 (0.41) a	7.05 (3.48) b

Means within each column followed by the same letter are not significantly different (LS Means Differences Tukey's HSD, $P \leq 0.05$).
Data for thrips per curl was transformed by $\log_{10} (X + 0.1)$.

RED-STRIPED FIREWORM

METHODS: Each material was applied in 25 gallons of water-mixture per acre with a CO_2-propelled, 80-inch boom sprayer (76-inch swath) equipped with four, flat-spray 8002VS TeeJet nozzles operating at 35 psi and at a slow walking speed. Speed was regulated using a metronome.

On dates indicated in the table, we collected 10 infested stems (as evidenced by webbed-together leaves) from each plot and examined them for the presence or absence of larvae. On 23 and 30 August (1 and 7 days post-treatment, respectively), any larvae found in the stems collected from the plot treated with Mycotrol® ES were brought to the laboratory, placed in individual plastic cups, and assessed for *Beauveria bassiana* mortality by observing them for sporulation.

RESULTS: There was some indication that Entrust® 80 WP had an effect on RSFW ($P = 0.09$) (Table 3). There was a 50% reduction in the number of stems found to contain larvae after one week (30 Aug); 33% of stems treated with Entrust were empty after 2 weeks (5 Sep). There was also a date effect on the number of stems with missing larvae ($P = 0.01$) and this effect was different for the Entrust treatment (number of missing larvae increasing over time) compared to what was observed in the Mycotrol and check treatments (number of missing larvae being constant over the experimental duration) ($P = 0.02$), i.e. the treatment by date interaction effect. None of the larvae brought into the laboratory and observed for sporulation had died after 2 weeks. And, no evidence of dead, infected larvae was found in any leaf curl.

Table 4 gives the rate/acre and the approximate cost/volume, unit cost, and cost/acre of the materials used in this trial. The cultural treatment of burning is included since it is the treatment currently recommended for control of this pest insect.

Table 3. Field control of red-striped fireworm with insecticides, summary.

Material	Amt. form/ acre	% empty curls (% reduction) Prespray 22 Aug	Postspray 23 Aug	30 Aug	5 Sep	Avg.
Entrust 80 WP	2 oz	40 (50)	30 (0)	100 (50)	90 (33)	65 a
Mycotrol ES	32 oz	30 (0)	60 (17)	40 (0)	50 (0)	45 b
No insecticide	-	20 (0)	50 (0)	50 (0)	60 (0)	45 b

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

Table 4. 2006 costs ($) of insecticides (From Maine Potato Growers).

Material	Rate/acre	Cost/vol.	Unit cost	Cost/acre
Entrust 80 WP	2.0 oz	350/lb	21.88	43.75
Mycotrol ES	32.0 oz	**		
Burning *				200.00

* Personal communication Dave Yarborough, UM Blueberry Extension Specialist.
** Rights to this product have recently been sold to a new manufacturer; no up-to-date pricing information was available.

GRASSHOPPERS

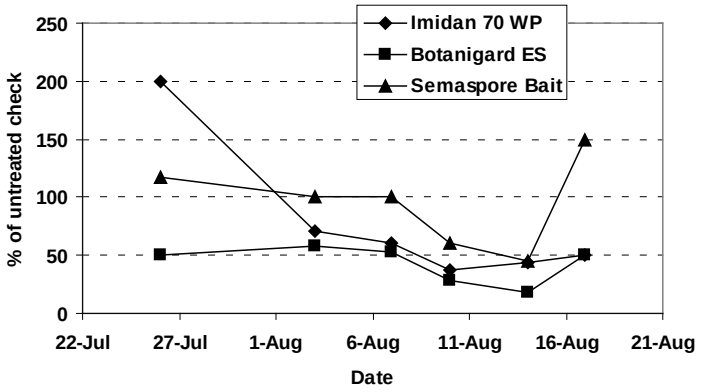
METHODS: Imidan® 70 WP and Botanigard® ES were applied in 20 gallons of water per acre using a CIMA® P55D Atomizer L.V. sprayer mounted on an Agco Allis® 6670 tractor. Speed, psi, and nozzle orientation were adjusted to provide coverage to a 50-ft swath. Semaspore® Bait was applied using a granular spreader calibrated to deliver 1 lb/acre. On the dates indicated in the figure, 7 sets of 10 sweeps each were taken systematically through the center area of each plot with a standard 12-inch diameter sweep net. Grasshoppers (nymphs and adults) were counted and then distributed back into the same plot. On 3 and 7 August (3 and 7 days post-treatment, respectively), an additional 10 grasshoppers were collected from the areas treated with Botanigard ES or Semaspore Bait and from an untreated check area, brought to the laboratory for observation to assess mortality due to infection.

RESULTS: Post spray counts of grasshoppers were generally low in all plots. However, some trends are apparent. Both Imidan 70 WP and Botanigard ES significantly reduced the seasonal density of grasshoppers in comparison with adjacent untreated check plots (P = 0.0008). Semaspore Bait was less effective.

Figure 1 shows populations in the treated areas as a percentage of populations in the untreated check areas for each treatment on each sample date. July 27th represents the pre-

treatment counts. After this date one can see that the application of Imidan 70 WP resulted in a significant drop in the populations of grasshoppers relative to the untreated check (ca. 4X) and populations stayed down for the duration of the experiment. A similar phenomenon occurred with the Botanigard ES application, except that the decline in the grasshopper population occurred 7-10 days later (as predicted from previous laboratory studies). The degree of population decline was about 50% which is consistent with the laboratory estimates of mortality due to *B. bassiana* infection (60% and 40%, respectively for the two collection dates). The application of Semaspore bait also resulted in a 50% population drop. But populations in both the Botanigard-treated plot and the Semaspore-treated plot appeared to rebound toward the end of the experiment.

Fig. 1. Grasshopper populations in treated areas as a percentage of the untreated check.



CONCLUSIONS: All three materials tested against blueberry thrips show potential and warrant further testing in 2007. Admire Pro is a new formulation of imidacloprid containing 4.6 lbs ai/gallon; while Provado Pro has 1.6 lbs ai/gallon.

As an organic management strategy, Entrust did appear to offer some control of RSFW compared to the untreated check or Mycotrol. However, the level of control as a result of an application of Entrust would not appear to justify the expense of the insecticide material cost used in an application.

The application of Imidan 70 WP resulted in the best control (ca. 75% population reduction) of grasshopper populations. The mean densities of grasshoppers in the treated and check plots were not significantly different before the application of Imidan, but they were different (less in the Imidan plots) after application. An application of Botanigard ES also resulted in a significant drop in the grasshopper population relative to the untreated check; however, the population did rebound at the end of the experiment suggesting that a second application might be needed if this organic tactic is to be considered for grasshopper management. Semaspore Bait showed a similar trend (a 50% reduction), but the overall population differences were not significantly different between the check and the treated plot after application.

3. <u>Control of blueberry maggot with ground application of insecticides.</u>

METHODS: Three trials were conducted in fruit-bearing lowbush blueberry fields. Plot size, number of replications, materials, rates, and application dates are given in Table 1.

Populations of blueberry maggot flies (BMF) within each plot were monitored with baited, yellow Pherocon® AM traps before and after treatment applications. The materials were applied in 5.4 gallons of water-mixture per acre using a SOLO® 450 mist blower. Swath width was 40 ft. Efficacy was further evaluated based on the number of BMF pupae collected from berry samples.

Table 1. Experiment design of BMF trials.

Trial #	Plot size (ft)	Reps	Treatment/formulation	Rate-Amt. product/ acre	Application date
1	40 x 100	4	Provado Pro 1.6 F	4.0 oz	6, 14, and 18 Jul
			Provado Pro 1.6 F	6.0 oz	6, 14, and 18 Jul
			Untreated check	-	
2	40 x 100	4	Provado 1.6 F	6.0 oz	12 and 17 Jul
			Calypso® 480 SC	3.0 oz	12 and 17 Jul
			Aza-Direct®	32.0 oz	12 and 17 Jul
			Untreated check	-	
3	40 x 150	4	Assail 30 SG	5.3 oz	6 Jul
			Avaunt 30 WG	6.0 oz	6 Jul

RESULTS: The seasonal density of BMF adults captured on Pherocon AM traps between treated and untreated check plots was compared for all trials. In trial #1, there was no significant difference among the treatments ($P = 0.3109$) (Table 2). And, significantly more pupae were found in the untreated check plots than in plots treated with Provado Pro at the 6 oz rate ($P = 0.0455$).

The effectiveness of Provado 1.6 F, Calypso 480 SC and Aza-Direct were evaluated in trial #2. There was no significant difference in the seasonal density of BMF adults among the treatments ($P = 0.3679$). Only Provado significantly reduced the number of pupae found in berry samples compared to the untreated check at $P = 0.076$.

The effectiveness of Assail 30 SG and Avaunt 30 WG were evaluated in trial #3. Between 17 and 20 July, a large section of the field encompassing ca. 75% of the treatment area for this trial was either sprayed directly or contaminated by drift from a commercial application of Imidan 70 WP. Data collected after the commercial application of Imidan was excluded from the analysis. Although there was no significant difference in seasonal density of adults among the treatments ($P = 0.741$), both Assail and Avaunt did reduce adult populations immediately after the application. Between 5 July (prespray) and 10 July (1st post-spray sample date) there was an average reduction of 69.2 and 60.2% in the number of adults captured on Pherocon AM traps in the Assail and Avaunt-treated plots, respectively compared to a 7.5% increase in populations in the untreated check. However, there was no significant effect on these levels of reduction ($P = 0.1337$). Populations had rebounded by 17 July (Fig. 1). The need for an additional application is also reflected in the high level of pupal infestation in both treatments ($P = 0.9763$) (Table 2).

Table 3 gives the rate/acre and the approximate cost/volume, unit cost, and cost/acre of the materials used in this trial. Imidan 70 WP is also included as the control material most commonly used by growers in commercial production.

Table 2.

Treatment/ formulation	Rate Amt. product/acre	Avg. pupae/qt	Adults/trap seasonal density [a]
Trial #1			
Provado Pro 1.6 F	4.0 oz	1.2 ab	4.20 a
Provado Pro 1.6 F	6.0 oz	1.0 b	4.33 a
Untreated check	-	9.8 a	5.87 a
Trial #2			
Aza-Direct	32.0 oz	5.4 a	10.30 a
Calypso 480 SC	3.0 oz	2.2 ab	7.85 a
Provado 1.6 F	6.0 oz	1.0 b	4.96 a
Untreated check	-	4.3 a	8.46 a
Trial #3			
Assail 30 SG	5.3 oz	5.2 a	6.9 a
Avaunt 30 WG	6.0 oz	4.6 a	9.0 a
Untreated check	-	5.0 a	7.2 a

Seasonal densities of adults are trapezoidal integrals of densities over the season divided by the number of day's duration of the experiment.
Means within each column and trial followed by the same letter(s) are not significantly different. Mean separation of seasonal density was by LS Means Differences Tukey's HSD, $P \leq 0.05$. Comparison of mean pupae/qt was by Friedman's Two-way Nonparametric ANOVA, $P \leq 0.05$, trial #1, LSD (T), $P \leq 0.05$, trial #2, and LS Means Differences Tukey's HSD, $P \leq 0.05$, trial #3.

Fig. 1. Captures of BMF adults by sample date, trial #3.

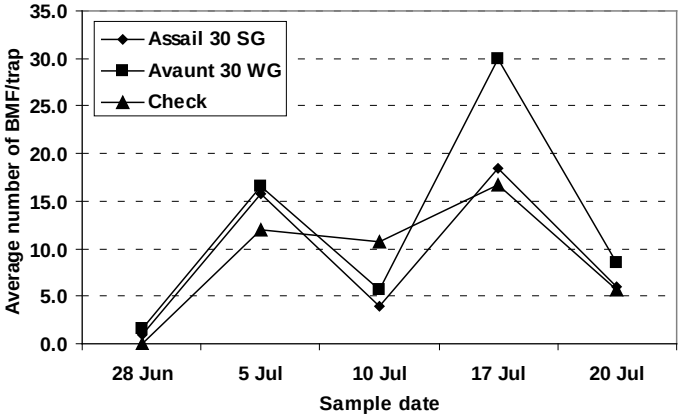


Table 3. 2006 costs ($) of insecticides (From Maine Potato Growers)

Material	Rate/acre	Cost/vol.	Unit cost	Cost/acre
Assail 30 SG	5.3 oz	15.26/oz	15.26/oz	80.88
Avaunt 30 WG	6.0 oz	5.70/oz	5.70/oz	34.20
Aza-Direct	32.0 oz	175.00/gal	1.37/oz	43.75
Calypso 480 SC	3.0 oz	827.00/gal	6.46/oz	19.38
Provado 1.6 F	6.0 oz	450.00/gal	3.52/oz	21.12
Imidan 70 WP	1.3 lb	9.20/lb	9.20/lb	11.96

CONCLUSIONS: This is the third year of trials with Provado 1.6 F (imidacloprid). It has proven effective in all three years. However, inconsistent results have been obtained using a low (4 oz) rate. In 2005, one of four blocks treated with the 4 oz rate had a high rate of infestation (7 pupae/qt). A similar result was seen this year; the rate of infestation was high (3.2 pupae/qt) in one of four blocks. The range in the remaining blocks was 0 to1.2 pupae/qt.

Provado provided the best control of blueberry maggot; although, as Table 3 demonstrates, it is expected to cost almost double the amount per application as the current standard insecticide, Imidan. Aza-Direct exhibited no evidence of control despite more promising results in previous years with the same active ingredient, azadirachtin.

The accidental contamination by Imidan 70 WP impacted the outcome of the trial with Assail and Avaunt since it resulted in a sharp decrease in the number of BMF captures on yellow sticky traps (20 July) and precluded a second application of either material.

4. Control of blueberry maggot with GF-120® NF Fruit Fly Bait

METHODS: An ATV-mounted sprayer was used to apply 2:20-ft perimeter swaths of GF-120 NF Fruit Fly Bait at a rate of 1:5 v/v with water. Pre- and postspray populations of BMF adults were monitored with baited yellow Pherocon AM traps. Efficacy was further evaluated based on the number of BMF pupae collected from berry samples. There were

three treatments plus an untreated check with four replications per treatment set in a complete block design as outlined below.

1. Weekly whole-plot applications of GF-120 NF Fruit Fly Bait applied in 5:20-ft wide swaths.
2. One, mid-season, whole-plot application
3. Weekly applications applied in alternating, 20-ft, treated and untreated strips.
4. Untreated check.

RESULTS: The effectiveness of applying GF-120 NF with different timings and methods was evaluated. There was no significant difference in the seasonal density of BMF adults captured on Pherocon AM traps between treated and untreated check plots ($P = 0.6305$) (Table 1). Although the number of flies captured was generally low, there were some trends apparent. GF-120 is a short-residual material and may require frequent applications to maintain control. This is well demonstrated in Fig. 1. Following each application, there was a drop in average BMF captures followed by a recovery in fly numbers until the next application.

One check plot had only a small number of pupae (0.20/qt)) in comparison with the other three check plots (4.8, 12.0, and 22.4 pupae/qt). This is unusual since under high BMF colonization, fruit infestation is expected. When this low infestation check sample was included in the analysis, there was no significant difference among the treatments ($P = 0.2366$). When this sample was excluded from the analysis, there was a significant difference among the treatments ($P = 0.012$) (Table 1 and Fig. 2).

Table 1. Field control of blueberry maggot with GF-120 NF Fruit Fly Bait, summary.

	Avg. pupae/qt	Avg. pupae/qt	Adults/trap seasonal density (SE)
1. Weekly whole-plot	1.65 a	1.65 b	1.53 (0.60) a
2. Whole-plot 1X	0.93 a	0.93 b	1.15 (0.51) a
3. Weekly alternating strip	0.80 a	0.80 b	1.21 (0.53) a
4. Untreated Check	9.85 a *	13.07 a **	1.51 (0.57) a

* Data for number of pupae/qt (all replications included in analysis)

** Data for number of pupae/qt (check replication #3 excluded from analysis).

Seasonal densities of adults are trapezoidal integrals of densities over the season divided by the number of day's duration of the experiment.

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

Fig. 1. Capture of blueberry maggot fly adults on each sample date.

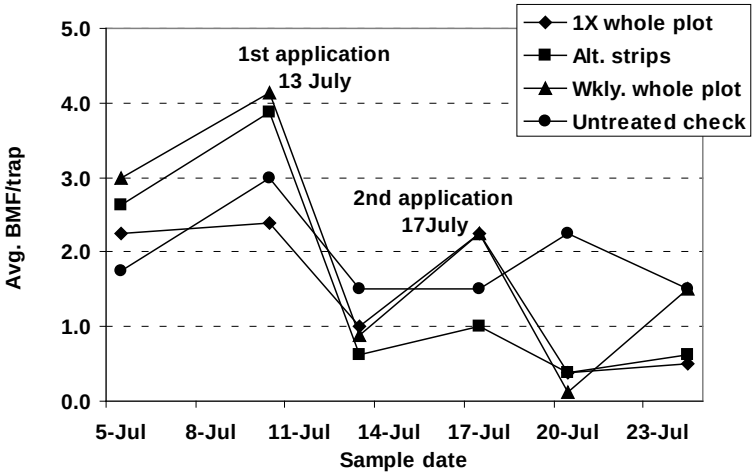
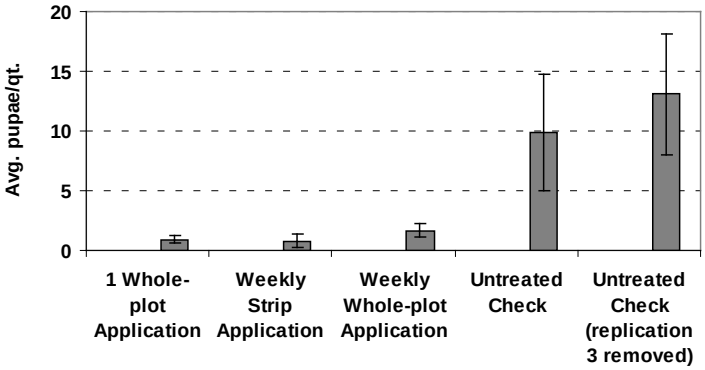


Fig. 2. Fruit infestation by blueberry maggot fly pupae.



CONCLUSIONS: In 2003, one, late-season application of GF-120 Fruit Fly Bait resulted in a significant reduction in the number of BMF captured on AM traps from the treated compared to the check areas ($P = 0.0007$). No infestation data was collected in 2003. In the 2004 trial, three applications of GF-120 NF Fruit Fly Bait resulted in a significant reduction in the seasonal density of BMF adults in comparison with the untreated checks (seasonal density = 4.4 vs. 7.8 BMF/trap, respectively) ($P = 0.0049$). There was also a significant reduction ($P = 0.0262$) in fruit infestation. On average, only 1.1 pupae/qt were found in treated berries compared to 4.2/qt in the untreated checks. Despite the promising results obtained in 2003 and 2004, GF-120 NF Fruit Fly Bait was ineffective in 2005.

In our 2006 trial application of GF-120 resulted in significantly fewer infested fruit (when the anomalous low infested check plot was eliminated from analysis) in treated plots compared to the untreated check plots. There was no difference in whole plot treatments compared to strip application treatments.

RECOMMENDATIONS: There are several recommendations that have come out of the 2006 insecticide efficacy trials. Most of these recommendations are also based upon several

additional prior years' trials. The first recommendation is that some of the new less toxic insecticides do not appear to be as efficacious for flea beetle control as the currently recommended insecticides such as Imidan and SpinTor or Entrust. Materials such as Assail and Avaunt kill flea beetle adults well, but are less effective on larvae. Their activity is deceiving since it takes significantly longer to kill than SpinTor or Imidan. In general, these two materials will not be recommended for flea beetle control unless we find that lower rates are also effective in future trials.

The new thrips insecticides that we tested all looked very good: Admire, Assail, and Provado. Another year of testing these compounds should yield a recommendation for their use if results are as good as those we obtained in 2006. Red-striped fireworm still remains a problem for insecticide control, especially for organic growers. Our trials suggest that there are no good alternatives and that burning is the only alternative for heavily infested fields at this point.

Grasshoppers can easily be controlled by many of our standard insecticides. There are few organic options; however, 2006 showed that Mycotrol is a good control measure and yields similar results to Imidan.

Blueberry maggot research is still focused on possible long-term replacements for Imidan and options for organic production. After several years of testing we have found Provado 1.6 F is a very good material for maggot control. It is effective for control at the 4 oz rate and currently we are pursuing a section 24(c) registration for its use in Maine. Naturalyte, GF-120, has been tested for several years as an option for organic growers. It is currently registered for BMF and we are going to recommend its use with the proviso that it does quite often result in very good control, but that it is not always consistent. We will continue to assess its effectiveness, especially as an evening application, a time where degradation of the insecticide by sunlight is at a minimum and foraging movement of blueberry maggot flies are at a maximum.

INVESTIGATORS: F. A. Drummond, Professor of Insect Ecology/Entomology
J. A. Collins, Assistant Scientist of Insect Pest Management
S. Hoffmann, Research Associate of Biological Sciences

TITLE: IPM strategies, 2006

1.<u>Evaluation of feeding damage by blueberry flea beetle adults in the pruned year</u>

METHODS: On 29 June, three replications (blocks) were established in a pruned-year blueberry field at Blueberry Hill Farm. The field was burned in the fall of 2005. Five, 4-ft^2 plots were set in each block and one of five different densities of field-collected, blueberry flea beetle (FB) adults was placed in each plot (0, 25, 50, 75, or 100 adults). Each plot was covered with a mesh cage (2.0 x 2.0 x 1.5 ft) and sealed with sand around the bottom to prevent movement of the adults out of the plots.

On 2 July and again on 25 July, 10 stems were observed from each cage. The stems were selected randomly and each stem was evaluated as either with or without feeding damage as evidenced by chewed foliage. On 22 November, 50 stems within each plot were cut and

brought into the laboratory. The number of flower buds/stem was recorded at each flea beetle density. Analysis of Variance (ANOVA) and LS Means Differences Tukey's HSD ($P \leq 0.05$) were used to compare initial FB adult density with percent of stems with feeding damage and subsequent flower-bud production.

RESULTS: There was a significant difference in the percentage of stems with feeding damage as evidenced by chewing among the densities on both sample dates (12 July, $P = 0.0001$; 25 July, $P = 0.0003$) (Table 1 and Fig. 1). There was also a significant linear ($P < 0.0001$) and quadratic ($P < 0.0001$) trend on both dates. The quadratic regression for 25 July is in Fig. 2. As FB density increased, there was an increase in observed damage.

Table 1 and Fig. 3 compare FB adult density with the average number of flower buds/stem. There was a significant difference ($P = 0.0017$). And, there was a significant linear ($P = 0.0006$) and quadratic ($P = 0.0003$) trend.

Table 1. Comparison of percent stems with feeding damage and subsequent flower-bud production to FB adult density.

Initial flea beetle adult density	Percent stems with feeding damage (SE) 12 July	25 July	Flower-bud production
0	20.0 (5.8) a	33.3 (12.0) a	3.05 (0.20) a
25	66.7 (8.8) b	66.7 (8.8) ab	2.72 (0.17) a
50	66.7 (6.7) b	93.3 (6.7) b	1.21 (0.11) b
75	93.3 (6.7) b	100.0 (0.0) b	1.16 (0.10) b
100	96.7 (3.3) b	100.0 (0.0) b	1.15 (0.11) b

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

Fig. 1. Relationship between FB adult density and percent stems with feeding damage on each of two sample dates.

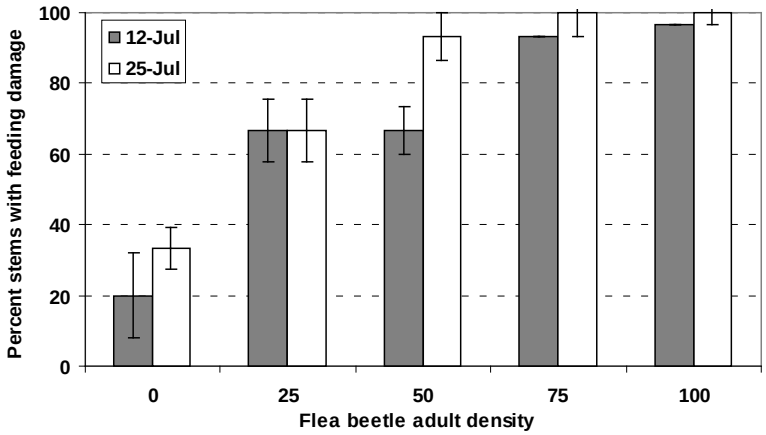


Fig. 2. Graph of the quadratic regression for 25 July comparing initial adult FB density with percent stems with feeding damage.

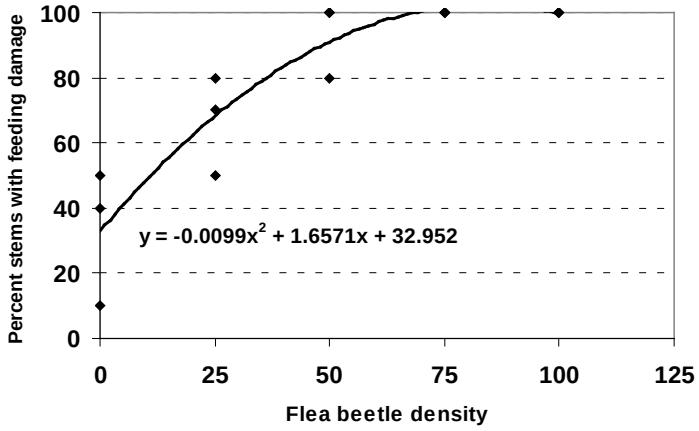
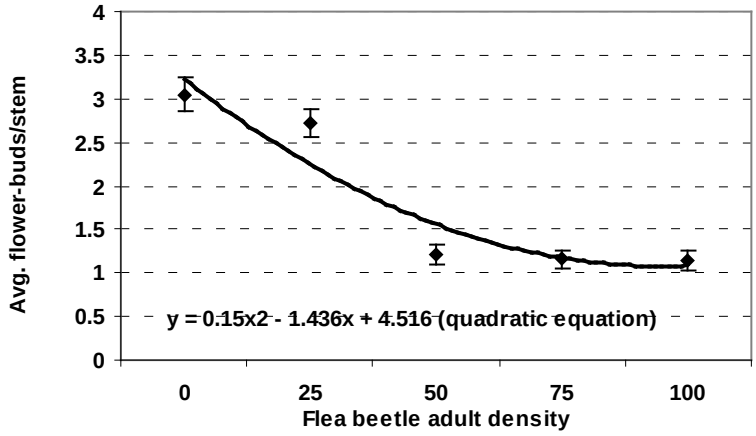


Fig. 3. Relationship between FB adult density and average flower buds/stem.



CONCLUSIONS: As is the case with flea beetle larvae, increases in adult density also result in increased foliar feeding and damage. However, this is expected and we have shown similar relationships in previous years. What was most striking is that there was also a subsequent decrease in flower-bud production, especially at higher levels of adult infestation. In fact, it would be expected that adult defoliation might be more severe than larval defoliation due to the later timing of defoliation by adults resulting in less time in the growing season for the plant to recover.

2. Attractiveness of Sentry Bee-Scent® to pollinators

METHODS: Two rates of Bee Scent® (Sentry Biologicals, Inc.)(2 and 4 qts/acre) were applied at 8 am on 25 May and again on 30 May to a crop-year blueberry field. There were four replications per treatment + four untreated check plots set in a block design. All treatments in a block were set within the same blueberry clone. Each plot measured 20 x 20-ft. Weather conditions were clear and dry on both application/sample dates. Blueberry plants were at full bloom.

Following each application, counts were made of the number of bee pollinators (honey bees, bumble bees and solitary bees) observed over a 15-second period in each of three, m^2 sub-samples per plot. Counts were made at various times on the day of application (25 and 30 May). On 7 August, yield within each plot was determined by harvesting and weighing all berries within two, m^2 quadrats per plot.

RESULTS: Very few bees were observed at any sample time on either date (< 1 per 15 sec on any date at any time) (Table 1). Bee type was pooled on each date and analyzed using Analysis of Variance (Complete Block Design, $P \leq 0.05$). Data for bees observed/15 sec were transformed by $\log_{10}(X + 0.1)$ prior to analysis. Neither the recommended rate of 2 qts/acre nor a double rate of 4 qts/acre demonstrated any apparent attractiveness to bees in this trial. And, there was no significant difference in yield among the treatments (ANOVA, $P = 0.8747$).

Table 1. Mean number of bees observed by sample time for both sample dates, and average yield.

	Avg. number of bees observed/15 sec			
	Observation time			
Rate/acre	9 am	12:50 pm (25 May) 11:15 am (30 May)	2 pm	Yield (lbs)
25 May				
4 qts	0.25 a	0.00 a	-	-
6 qts	0.17 a	0.08 a	-	-
Untreated	0.08 a	0.08 a	-	-
30 May				
4 qts	0.58 a	0.42 a	0.08 a	2.095 a
6 qts	0.17 a	0.25 a	0.75 b	2.097 a
Untreated	0.33 a	0.25 a	0.42 ab	2.106 a

Means within each column and date followed by the same letter(s) are not significantly different (LS Means Differences Tukey's HSD, $P \leq 0.05$).
Data for number of bees was transformed by $\log_{10}(X + 0.1)$.

CONCLUSIONS: Bee-Scent® attractant is a pheromone-based liquid formulation containing attractants that are designed to direct honey bees to treated blossoms for improved crop pollination. We were not able to demonstrate any increased bee activity in plots that were treated with Bee-Scent. The weather was suboptimal for honey bee foraging on both of the application dates. Average temperature was 54.5°F and 60.1°F on 25 and 30 May, respectively. This may be responsible for the low levels of bee foraging observed on the dates of the experiment.

3. Comparison of captures of blueberry maggot fly with Pest Barrier® Sticky Glue and Tangle foot®

METHODS: On 19 July, unbaited Pherocon® AM traps coated with either Pest Barrier® Sticky Glue or Tanglefoot® were placed in a crop-year lowbush blueberry field which prior monitoring had shown to be heavily infested with blueberry maggot fly (BMF). There were eight replications of each treatment. The traps were placed alternating 10 ft apart along the perimeter of the field and 10-20 ft in from the edge. On each of three dates, we counted and removed any adult flies.

RESULTS: Analysis of variance ($P \leq 0.05$) was used to compare the number of adults captured between the treatments. Tanglefoot consistently captured more adults, and the difference was significant on two of the three sample dates. There was no interaction between treatment and sample date ($P = 0.3639$). When all three dates were combined the result was that Tanglefoot was a superior adhesive for holding flies on traps ($P = 0.024$).

Table 1. Summary.

	Avg. number of adult BMF captured		
Treatment	20 Jul	24 Jul	27 Jul
Pest Barrier	2.6 (0.46) a	5.8 (0.77) a	6.8 (0.80) a
Tanglefoot	5.1 (1.22) b	8.6 (1.65) a	12.8 (2.25) b
	$P = 0.021$	$P = 0.144$	$P = 0.017$

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

CONCLUSIONS: If Pest Barrier sticky glue is to be used as an adhesive, the grower might want to half the thresholds since total trap captures represent roughly half (57%) of the captures resulting from the use of Tanglefoot.

4. Use of Pherocon® AM traps to prevent immigration of blueberry maggot flies into crop-year blueberry fields

METHODS: Baited, yellow, Pherocon® AM traps were evaluated for their effectiveness as a barrier to immigration of blueberry maggot fly (BMF) into lowbush blueberry fields. Trial sites were established in three, crop-year blueberry fields (blocks) in Washington Co. Plot size in each field was ca. 100 x 100 ft. The traps were hung from metal poles, 6-10 inches above the blueberry canopy and 5-ft apart in a square pattern with at least one side of the square along a field edge close to a wooded area from which BMF were most likely to colonize. An adjacent area of each field was left unprotected as an untreated check.

In each treated area, six traps were placed within the perimeter-trap barrier. Three traps were placed inside the perimeter closest to the woods (edge) and three traps were placed across the middle (middle) of each plot. Six additional traps were placed in a similar pattern in the adjacent non-treated check area. The traps were checked twice per week from 29 June to 24 July. All BMF were counted

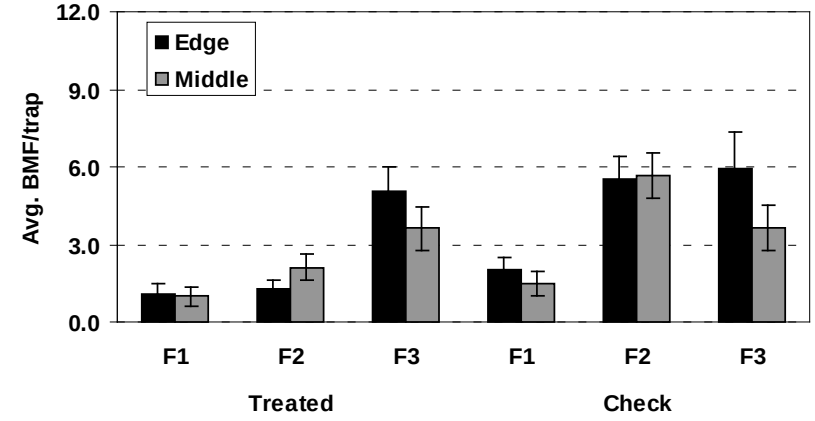
and removed from the traps. All the traps were replaced on 13 July. The data was analyzed using a split-plot design pooled over time and sub-sample within plots. Effectiveness was further evaluated by counting the number of BMF pupae found in berry samples.

RESULTS: There was no significant difference between fly captures in treated vs. untreated check plots ($P = 0.395$). There was also no significant difference in the number of flies captured between the edge and middle of plots ($P = 0.413$). A given field had anywhere from 1 to 6 BMF/trap on a sample date with Field #3 having the most overall flies (Fig. 1a). At all locations, flies were most abundant in early-mid July. In check plots, there was another smaller peak in abundance in mid-late July (Fig. 1b).

Overall, there were more pupae found at the edge of treated and check plots than in the middle (an average of 8.6 vs. 5.3 pupae per quart of berries, respectively). However in treated plots, there were slightly more pupae collected in the middle (8.3/qt) than at the edge (6.4/qt); whereas, there were more pupae collected at the edge of check plots (10.0/qt) than in the middle (2.2/qt) (Fig. 2). There were also slightly more pupae in treated plots (7.4/qt) than in check plots (6.1/qt). There were varying numbers of pupae found in the three fields, ranging from 1 to 19.3/qt.

Fig. 1. (a) Average number of BMF, by field, captured on Pherocon AM traps placed at the edge and middle of fields and (b) average number of BMF, by date, for Pherocon AM traps placed at the edge and middle of fields.

A



B

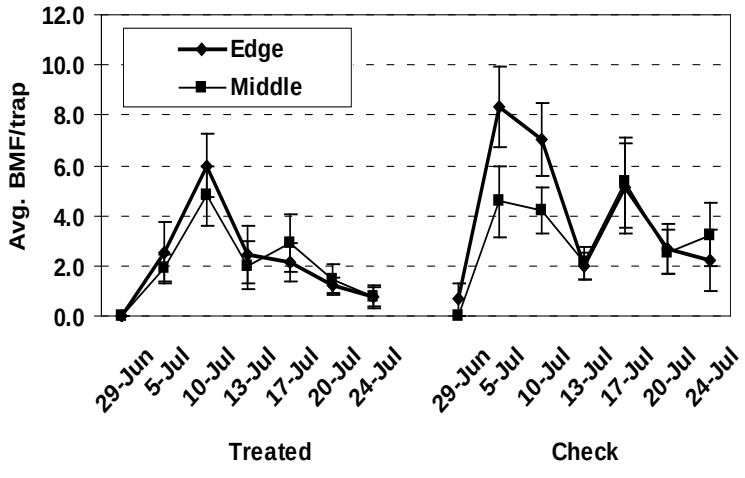
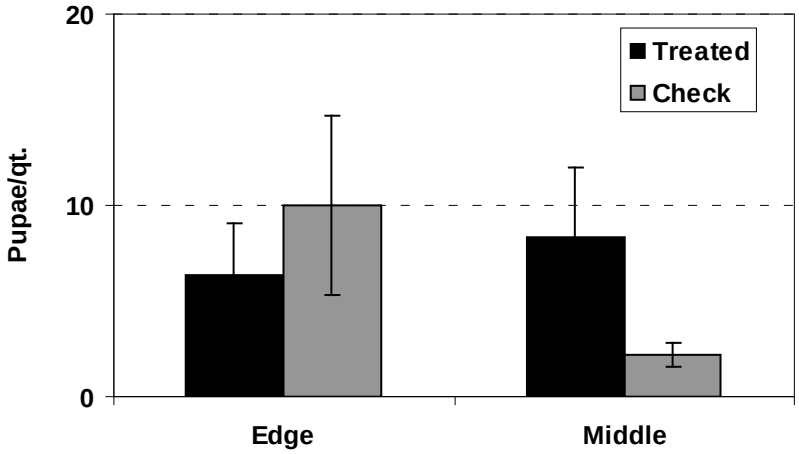


Fig. 2. Average number of BMF pupae/qt of blueberries at the edge and in the middle of treated and check plots for all fields.



CONCLUSIONS: Pherocon AM traps are meant to provide an estimate of the population abundance of BMF immigrating into fields from the surrounding forest and other blueberry fields. However, we tested the hypothesis that by deploying numerous traps in a field it would be possible to reduce the number of BMF and the resulting maggot infestation in a field. Given that there were more pupae found in treated plots than check plots, and that within treated plots there were more pupae found in the middle than around the edge, we can conclude that the traps were ineffective at keeping blueberry maggot flies from penetrating into the blueberry fields and laying their eggs in the fruit. Therefore, while Pherocon AM traps are indicators of BMF population density and damage potential, they are not useful as a control tool, even at trap intervals of one trap every 5 ft.

5. <u>Effect of date of pruning on flower-bud production in lowbush blueberry</u>

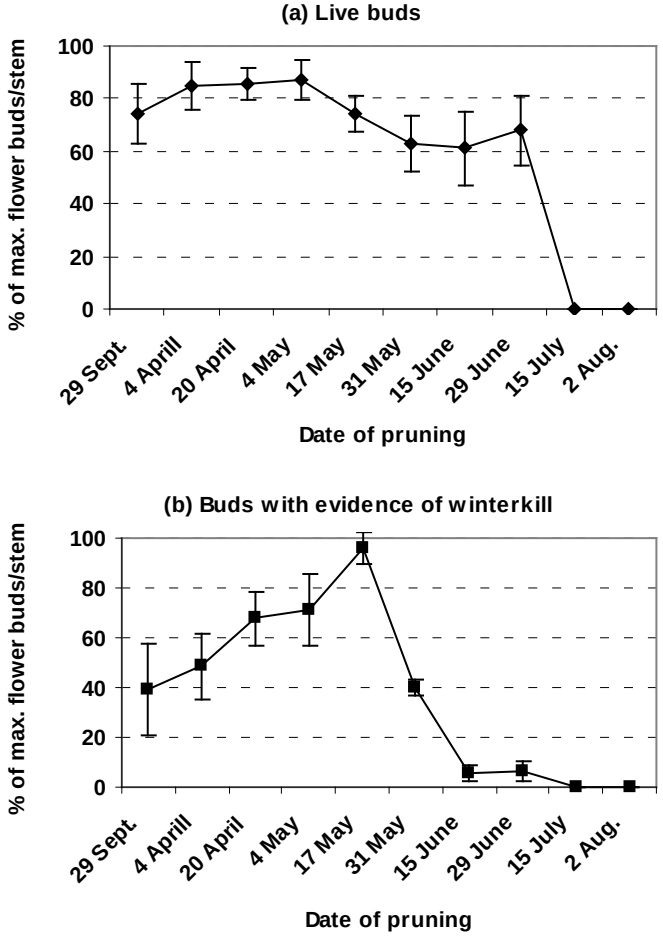
METHODS: 2004-2005. In the fall of 2004, seven lowbush blueberry clones (blocks) were selected and set with markers in a crop-year blueberry field. The minimum size of each block was 20 x 20 ft (ca. 6 x 6 m). On 29 September 2004, a flail-mower mounted on an ATV was used to mow a minimum 2-m^2 plot within each block. In the spring and summer of 2005, nine additional plots were mowed within each block. Treatment dates were: 5 and 20 April; 4, 17, and 31 May; 15 and 29 June; 15 July; and 2 August 2005. In May 2006, 50 stems within each plot were cut and brought into the laboratory. The number of live flower buds/stem and the number of buds showing evidence of winter damage was recorded for each pruning date. Flower buds with green or swollen tissue were categorized as "live buds". Any dead or shriveled flower buds were categorized as "winter-killed".

The trial was repeated in 2005-2006. Pruning dates were 5 October 2005; 4 and 21 April; 5, 7, and 30 May; 16 and 28 June; 14 July; and 1 August 2006. On 17 October, 50 stems within each plot were cut, brought into the laboratory and evaluated for flower-bud production by counting the number of flower buds per stem. The same blocks and plots will be maintained until 2007-08 when the trial will be repeated to study the effect of late pruning of the same plants in multiple cycles. The trial will be repeated a third time in 2006-2007. The first

pruning date was 17 October 2006. Nine additional plots will be mowed within each block in the spring and summer of 2007.

RESULTS: 2004-2005. Figure 1 shows the percent of maximum flower-buds per stem for a) live buds, b) buds with evidence of winter-kill, and c) total flower buds (live + winter-killed) on each pruning date and for all 7 blocks, combined. It should be noted that stems cut from plots pruned prior to 31 May were generally taller with more total buds and a greater number of buds with winter-kill. Stems cut from plots pruned on 31 May, and 15 or 29 June, appeared somewhat shorter with both fewer total buds and less winter-kill. Plots pruned in July and August had only short, scattered stems with few if any buds. The percent of maximum flower-buds per stem for the trial year 2005-2006 is in figure 2.

Fig. 1. Percent of maximum flower buds per stem, 2004-2005.



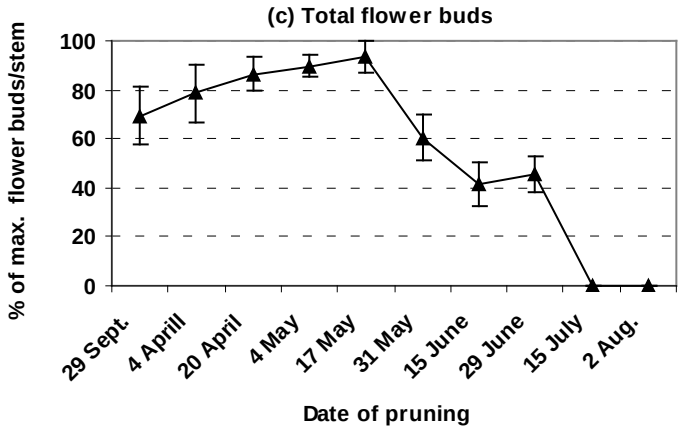
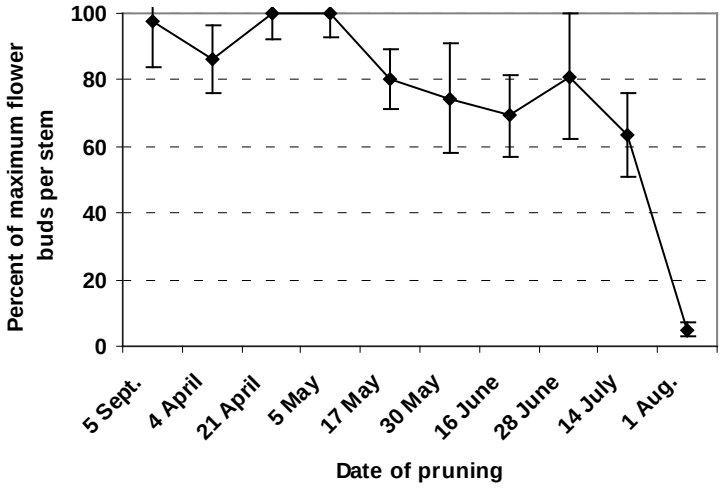


Fig. 2. Percent of maximum flower buds per stem, 2005-2006.



CONCLUSIONS: Data collected in both the 2004-2005 pruning season and the 2005-2006 pruning season suggests that little loss in potential yield occurs if pruning occurs prior to early to mid-May (ca. 10% loss in flower buds). Pruning that occurs between mid-may and mid-June results in losses that range from 20-40%. Pruning after the end of June in 2005 and after mid-July in 2006 resulted in almost total crop loss as measured by potential flower buds formation. This data can also be used to predict crop loss due to insect defoliation during the prune year. Therefore, we might expect that defoliation that occurs before mid-May will have little effect. However, defoliation occurring during the month of June is expected to result in crop loss between 20-40%. Late defoliation occurring in July will have the expected result of 100% crop loss for the subsequent year.

6. Monitoring populations of thrips in wild blueberry (pruned year) fields

METHODS:

Yellow sticky cards

On 8 May, three yellow sticky cards were placed in a pruned blueberry field that had been infested with thrips in 2005. Each card measured 3 x 5 inches and was hung just above the ground from a wooden lathe. No blueberry plants had emerged at the time the cards were distributed in the field. All the cards were replaced at weekly intervals from 12 May to 7 August. The number of thrips on each card was counted using a dissecting microscope.

Examination of leaf curls

At weekly intervals beginning on 25 May when curls were first observed, 10 leaf curls were collected the same field and brought into the laboratory. The curls were examined and the number of thrips per curl was recorded.

To monitor winter soil temperatures, a HOBO® Tidbit temperature logger was buried 1-inch deep in the field on 8 November 2005.

RESULTS: Peak captures of blueberry thrips on yellow sticky cards were recorded in mid- and late June. A second peak occurred in late July. The highest number of thrips in curls was in early to mid-July (Fig. 1). In three of the four years this study has been repeated, there has been a delay between the first appearance of thrips on yellow sticky cards and first appearance of damage as evidenced by leaf curls (Fig. 2). In 1999 there was a three week delay. No delay was observed in 2000. There was a one week delay in 2005 and again in 2006.

Fig. 1. Comparison of numbers of thrips captured on yellow sticky cards and thrips found in leaf curls.

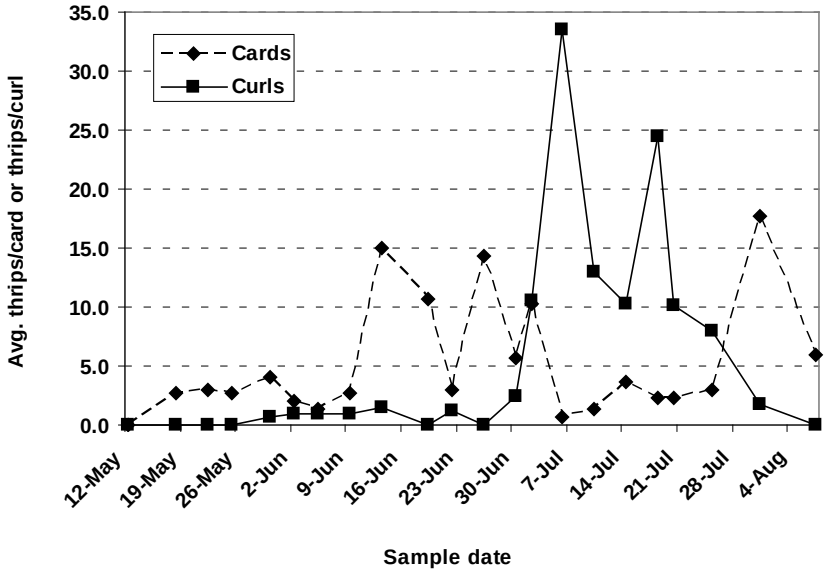
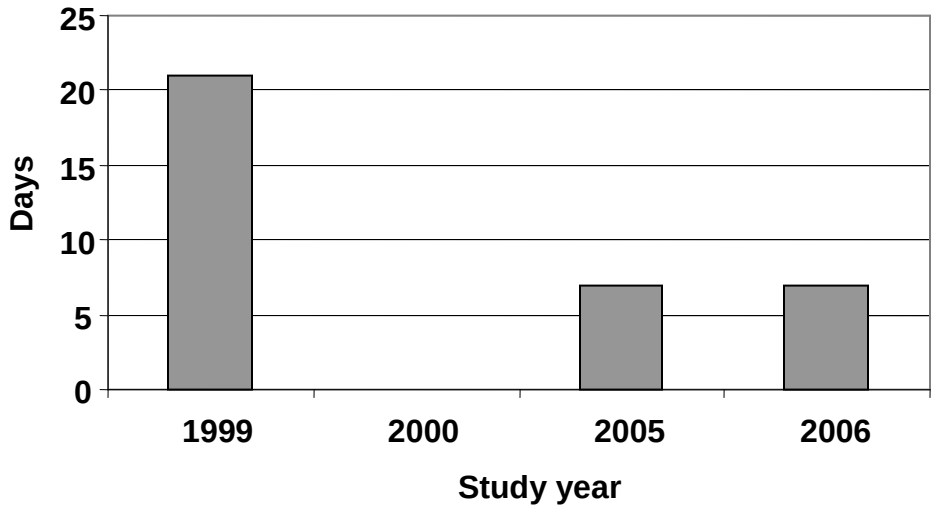


Fig. 2. Number of days between first appearance of thrips on yellow sticky cards and first appearance of damage as evidenced by leaf curls in the field.



CONCLUSIONS: These results suggest that yellow sticky cards are an effective early warning monitoring technique for blueberry thrips. If deployed early, sticky cards will give growers at least a one-week warning and provide time for the application of the first insecticide. We also formulated a preliminary model for predicting thrips emergence from the soil temperature data collected in 2006. We estimate that 94 degree days (base 50ºF) accumulated from April 1 is necessary for the beginning of thrips emergence. This model needs to be evaluated over the next two-three years to determine whether it is accurate.

RECOMMENDATIONS: The studies conducted in 2006 under the IPM program provide information to support several recommendations. The first one is that yellow sticky cards are very effective for monitoring blueberry thrips emergence. Our data over the past four years show that they generally allow the detection of thrips 1-3 weeks prior to the onset of leaf curling (in 3 of 4 years). This early warning provides ample time for a well timed insecticide application.

The second recommendation is that perimeter deployment of yellow sticky traps for control of blueberry maggot fly is not effective. This study confirms the results of a study conducted 20 yrs ago by Dr. Dutch Forsythe that baited AMF Pherocon® traps are not effective control tools. They are however, very effective population monitoring tools allowing growers to make decisions on population levels that may warrant insecticide applications. Some organic growers may want to use these traps with a natural adhesive such as Pest Barrier® Sticky Glue. Our recommendation for the use of yellow sticky traps that use Pest Barrier® Sticky Glue instead of the standard Tanglefoot® adhesive is that thresholds of BMF should be decreased by half since they appear to be only half as effective at capturing and holding flies upon the trap.

Our data on defoliation of blueberry by flea beetle adults suggest that even moderate densities can result in serious reduction in the following year's flower bud number. This crop loss is due to the mid-summer emergence of adults resulting in little growing season after defoliation for the recovery of the blueberry plant. Therefore, our recommendation is

that fields which produce large numbers of flea beetle adults, due to a lack of control of larvae, should be treated with insecticides immediately after adult emergence to minimize the impact of feeding.

The pruning study is in its second year. It is too early to make recommendations regarding the deleterious impact of late pruning. Two more years should provide a data set that will enhance our understanding of blueberry plant growth and development. It appears at this point we can suggest that little loss in potential yield occurs if pruning occurs prior to early to mid-May (ca. 10% loss in flower buds). Pruning that occurs between mid-May and mid-June results in losses that range from 20-40%. Pruning after the end of June in 2005 and after mid-July in 2006 resulted in almost total crop loss as measured by potential flower buds formed the following year.

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TITLE: Biology and Ecology of Blueberry Insects, 2006

1. Vertical distribution of blueberry maggot flies within different tree species

METHODS: On 22 June, baited yellow Pherocon® AM sticky traps were hung from twelve trees adjacent to a fruit-bearing wild blueberry field in Washington Co. There were four replications of each of three types of trees (birch, pine, and poplar). In each tree, the traps were hung 5, 10, 15, and 20 ft above the ground on a rope attached to a pulley to allow easier monitoring. Another trap was hung 6-10 inches above the ground (0 ft) from a separate pole. On 12 July, an additional trap was hung, 5-ft high, within the canopy of each tree; or if the sample tree had no canopy at the 5-ft height, the trap was hung from a nearby tree of the same species which had an appropriate canopy. All traps were checked periodically beginning on 29 June and continuing until 10 August. Captured blueberry maggot flies (BMF) were collected and will be examined this winter to determine gender and oviposition status. All the traps were changed on 12 July and 3 August.

RESULTS: Analysis of Variance (ANOVA) and LS Means Differences Tukey's (HSD), $P \leq 0.05$ were used to evaluate the effect of tree species, height, and canopy on captures of BMF over the season. Data for BMF captures were transformed by $\log_{10}(X + 0.1)$ prior to analysis.

Significantly more BMF were captured on traps hung in birch trees then from pine or popular ($P = 0.0003$) (Fig. 1); there was no significant difference observed between pine and popular. For all three species combined, there was also a significant difference among the heights ($P = 0.011$) (Fig. 2 and Table 1); however, there was no significant interaction between species and height ($P = 0.642$).

The presence or absence of a canopy also had a significant effect on captures; more BMF were captured on traps placed within the canopy at a height of 5 ft then those placed in areas with no canopy at the same height ($P = 0.037$) (Fig. 3). There was no significant interaction between tree species and the presence or absence of a canopy ($P = 0.201$).

Table 1. Comparison of trap height.

Height (ft)	Avg. BMF/trap
0	4.61 (0.73) a
5	2.41 (0.35) ab
10	1.39 (0.23) b
15	0.59 (0.12) b
20	0.80 (0.17) b

Means followed by the same letter (s) are not significantly different (LS Means Differences Tukey's HSD, $P \leq 0.05$).
Data for BMF captures were transformed by $\log_{10}(X + 0.1)$ prior to analysis.

Fig. 1. Effect of tree species on BMF captures, by species for all dates combined.

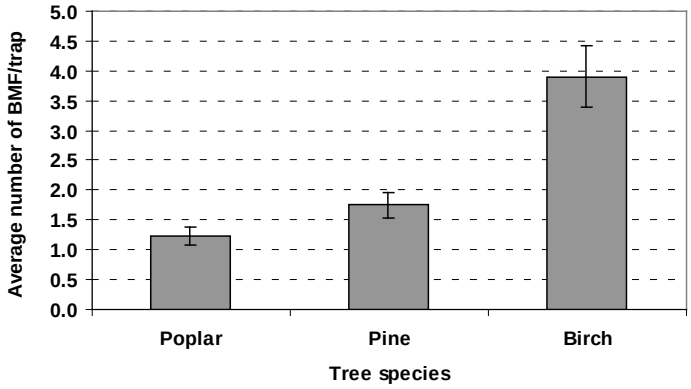


Fig. 2. Effect of trap height on BMF captures, by species for all dates combined.

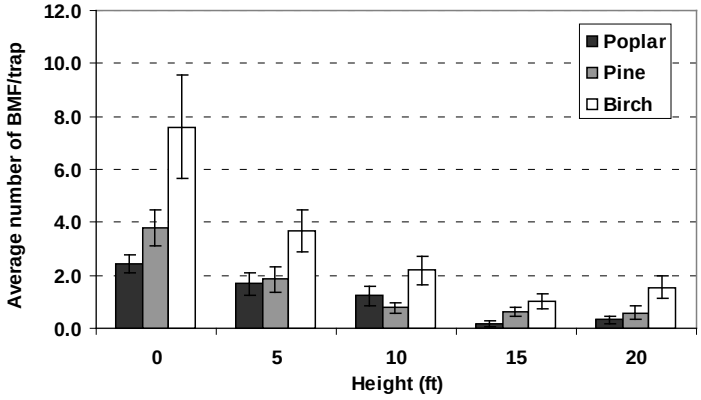
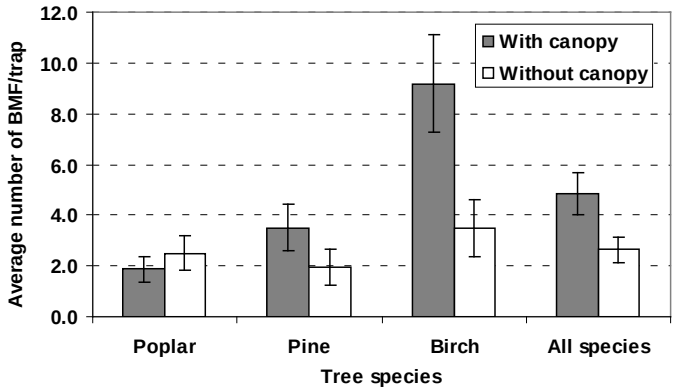


Fig. 3. Effect of canopy on BMF captures, by species for all dates combined. Traps placed at 5 ft.



CONCLUSIONS: This study was conducted to begin to assess the tree colonizing behavior of BMF. We believe that this behavior might be especially important in fields that are targeted for perimeter insecticide treatments since flies dispersing into blueberry fields from the tops of tall trees may clear the perimeter treated edges. Our research has found that BMF do make choices in colonizing trees. Birch trees tend to be selected in greater frequency than poplar or pine trees. In addition, trees with a canopy are colonized by flies more than trees lacking a canopy. However, in 2006 we found that fly abundance dropped off considerably with increasing height in the tree. This implies that less than 10% of the flies may actually reach heights that would be significant in allowing them to disperse across the field perimeter. These results are quite different from previous years' results. Research in 2007 will be focused on factors that might result in tree species choice in BMF colonization and the relationship between height that flies are released in trees and their success in avoiding capture in field perimeters.

2. Notes on parasitism of blueberry maggot fly

METHODS: Cups containing blueberry maggot fly (BMF) pupae were maintained in a growth chamber at 20^{o}C and 60-70% relative humidity for four weeks following the last observed emergence of BMF adults. Parasitic wasps (presumably *Opius sp.*) were observed in the rearing cages. The wasps were collected and will be pinned for future identification. An estimate was made of percent parasitism in each cage

RESULTS/CONCLUSIONS: Parasitism of pupae collected in 2005 was fairly low (Table 1). The data compares to previous parasitism rates of 6% in 2002, 12% in 2003, and 28% in 2004. This data will be incorporated into our long-term parasitism dataset for the blueberry maggot fly. We hope to be able to build a model for predicting levels of parasitism and possibly use these predictions to predict low and high density years of blueberry maggot fly.

Table 1. Percent parasitism of blueberry maggot fly in 2006.

Cage #	# pupae	# wasps	% parasitism
1	1150	33	2.9
2	1150	67	5.8
3	1050	42	4.0

3. Release/recapture of blueberry maggot flies

METHODS: In 2005 and again in 2006, adult blueberry maggot flies (BMF) were marked and released to evaluate fly movement into fields. Marked BMF were collected as pupae from infested blueberries in 2004 and 2005. BMF were marked on the dorsal side of the thorax with a dot of Tester's® brand model paint and put into cages that were hung at a height of either 5 ft or 20 ft from a tree located ca. 10 ft from the edge of fruit-bearing blueberry fields. Flies were released at two sites in each year. Each cage had 100 marked flies (200/site), corresponding to 400 total marked flies. One day after the flies were released, the containers were checked for fly mortality.

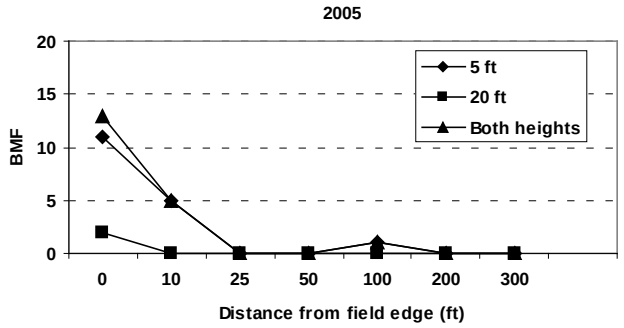
To recapture the marked BMF, three sets of seven baited, yellow, Pherocon® AM traps were placed in three transects running into the blueberry field adjacent to the release site. For each transect, one trap was placed at the field edge; additional traps were placed at 10, 25, 50, 100, 200, and 300 ft. Traps were checked periodically and any marked flies were counted and removed.

RESULTS: In 2005, 5.5% of released flies were recaptured (19/343). Of the 19 flies recaptured, 17 were released from the 5 ft height and 2 from the 20-ft height. Most flies were found on traps within 10 ft of the woods edge (18 of 19); 13 of the 18 were on traps at the woods edge, while one fly was found on a trap 100 ft from the edge of the woods. Both of the flies captured from the 20-ft release height were found on traps at the woods edge (0 ft from the woods) (Fig. 1A).

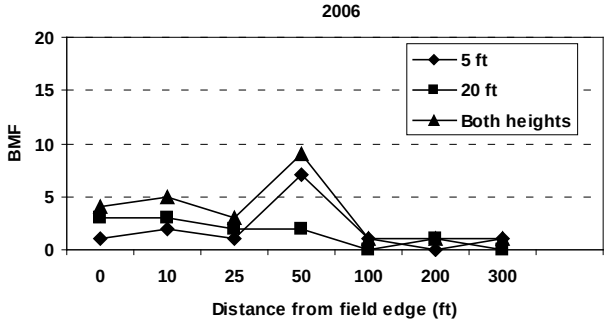
A similar number of BMF were recaptured in 2006 (6.0%, 24/400) (Fig. 1B); thirteen were released from 5-ft high and 11 from 20 ft. There was some indication that marked flies migrated further into the field in 2006. Twenty-one of the 24 (87.5%) BMF recaptured were found on traps as far out as 50 ft from the woods edge; 11 were released from a height of 5 ft, and 10 from the 20-ft height. Seven BMF released from a height of 5 ft were collected on traps set 50 ft into the field while single BMF released from 5-ft high was recaptured on traps set 100 and 300 ft into the field. One BMF released at 20-ft was found on a trap set 200 ft into the field. This would seem to indicate that height of release is not a significant factor in BMF immigration. For both years combined, the majority (90.1%) of recaptured BMF were found on traps within 50 ft of the field edge (Fig. 1C).

Fig. 1. Number of marked blueberry maggot flies recaptured at each distance. Flies released from heights of 5 or 20 ft in (A) 2005, (B) 2006, or (C) total for both years, combined.

A



B



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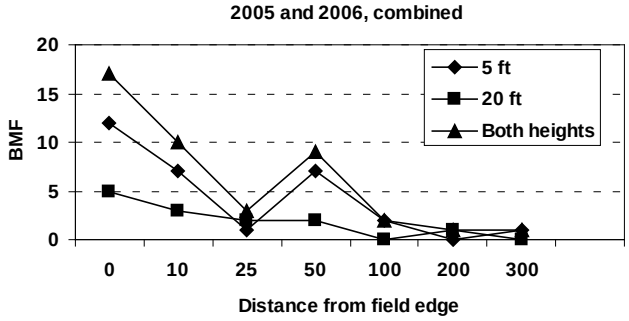


CONCLUSIONS: The two years of data do not support the initial hypothesis that blueberry maggot flies colonizing fields from high in the tree canopy would tend to land beyond a 75-100 ft perimeter, insecticide-treated zone at the edge of the field. In fact the opposite appears to be the case. This could be due to the flies dropping down from trees to the soil surface and then immigrating into the blueberry field.

4. Toxicity of insecticides to natural enemies

METHODS: All the materials were applied in 25 gallons of water-mixture per acre with a CO^2-propelled, 80-inch boom sprayed (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. The materials were allowed to dry on the foliage. Field-collected adult *Calosoma calidum* or *Harpalus rufipes* beetles were placed individually in 3.5-inch diameter plastic cups with petri dish lids. One treated stem with foliage (collected from the field soon after application) was cut and placed in each cup. The cups were held at room temperature (~22°C) and assessed for mortality at daily intervals. Untreated blueberry foliage was added in successive days during the experiment to each cup as needed. Results were analyzed using logistic regression models.

RESULTS: In Study 1, Provado® 1.6 F was significantly more toxic to *C. calidum* adults than either Assail® 30 SG or the untreated check ($P = 0.0007$). Only 39% of beetles exposed to foliage treated with Provado survived after eight days; 95% of both the Assail and untreated check beetles survived (Fig. 1).
Imidan® 70 WP was significantly more toxic to *C. calidum* than the untreated check ($P = 0.0368$) in Study 2. A total of 54% of beetles survived in the Imidan treatment; 91% survived in the untreated check (Fig. 2).

There was no significant difference in the percent survival of *H. rufipes* adults among the three treatments in Study 3. After eight days most *H. rufipes* were still alive; 93%, 80% and 83% for Provado, Assail, and the untreated check, respectively (Fig. 3). There was no need to run an analysis for Study 4 as 100% of *H. rufipes* exposed to foliage treated with Imidan 70 WP died within two days (88% died after one day) and 100% of *H. rufipes* exposed to SpinTor 2 SC, or untreated, were alive after eight days (Fig. 4).

Fig. 1. Percent survival of *C. calidum* adults exposed to foliage treated with Provado 1.6 F or Assail 30 SG.

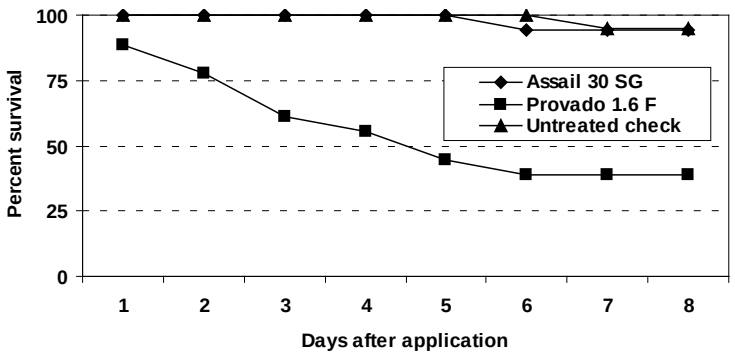


Fig. 2. Percent survival of *C. calidum* adults exposed to foliage treated with Imidan 70 WP.

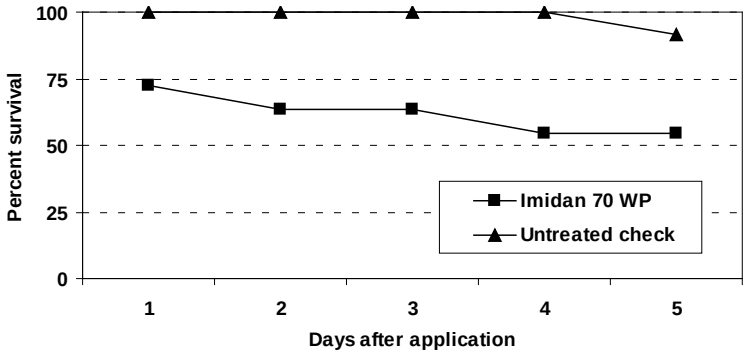


Fig. 3. Percent survival of *H. rufipes* adults exposed to foliage treated with Provado 1.6 F or Assail 30 SG.

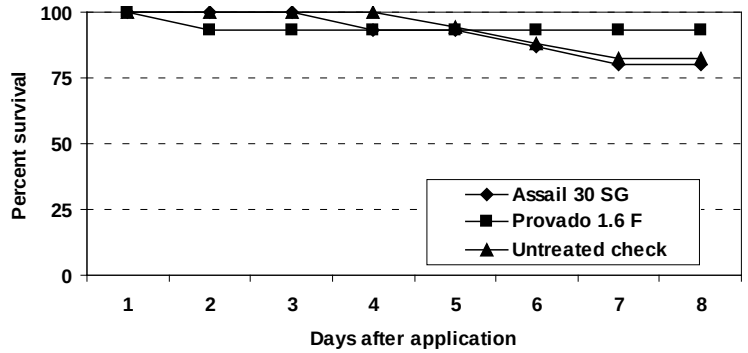
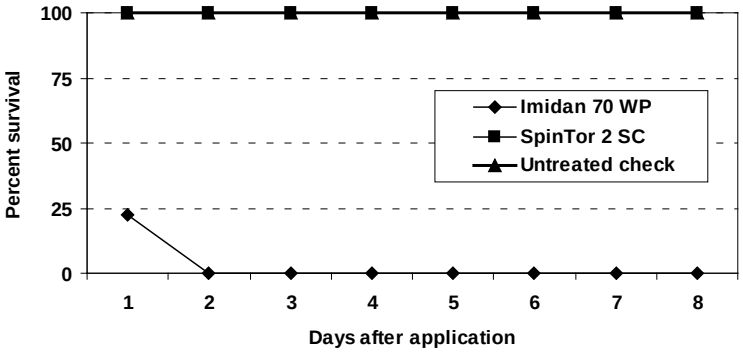


Fig. 4. Percent survival of *H. rufipes* adults exposed to foliage treated with Imidan 70 WP or SpinTor 2 SC.



CONCLUSIONS: *Calosoma calidum* is a large ground beetle predator. Mortality due to insecticide exposure tends to be a dose/mass relationship and so one might expect that *C. calidum* would be less susceptible to insecticides used in blueberry pest management than many of the smaller blueberry insect pests that it feeds upon. This is what we found except

that Imidan, a conventional organophosphate insecticide was less toxic than the reduced risk insecticide, Provado. The reduced risk insecticide, Assail, appeared to have no toxicity to *C. calidum.*

Imidan is highly toxic to *H. rufipes* beetles unlike what we found with *C. calidum*; whereas, the reduced risk insecticides, SpinTor 2 SC, Assail 30 SG, and Provado 1.6 F did not appear to result in any acute toxicity relative to the control.

5. Feeding trials with *Harpalus rufipes*

METHODS:
Choice feeding trial (2005)

The purpose of this study was to quantify preference for different life stages in predation of red-striped fireworm (RSFW) by *H. rufipes* beetles in a laboratory setting. In two laboratory trials, larvae, eggs, and seeds were set up in 14 cm diameter (4 cm depth) cups along with a moist cotton wick. Each cup contained one red-striped fireworm larvae (RSFW), 10 blueberry spanworm eggs (SW), five blueberry flea beetle eggs (FB) (eight in study 2), and 10 weed seeds. Each group of food items was placed in a small rubber circle at equidistant points from one another. One *H. rufipes* beetle adult per cup was placed between and equidistant from the food items. A different beetle was used for each replication. In trial #1, the cups were checked after 1 and 2 days and in trial #2 they were checked after 1, 2, and 3 days to see how many of each food item were consumed.

Feeding on exposed red-striped fireworm larvae

The purpose of this trial was to quantify predation of red-striped fireworm (RSFW) by *H. rufipes* beetles in a laboratory setting. According to several years of pitfall trap captures, *H. rufipes* is the most abundant ground beetle predator present during the larval life stage of RSFW.

RSFW larvae were set up in 14 cm diameter (4 cm depth) cups along with a moist cotton wick. Each cup contained 1, 3, 5, or 7 larvae, and an *H. rufipes* adult. There were five replications of each treatment. The cups were checked daily for seven days, and the results were analyzed using a linear regression model.

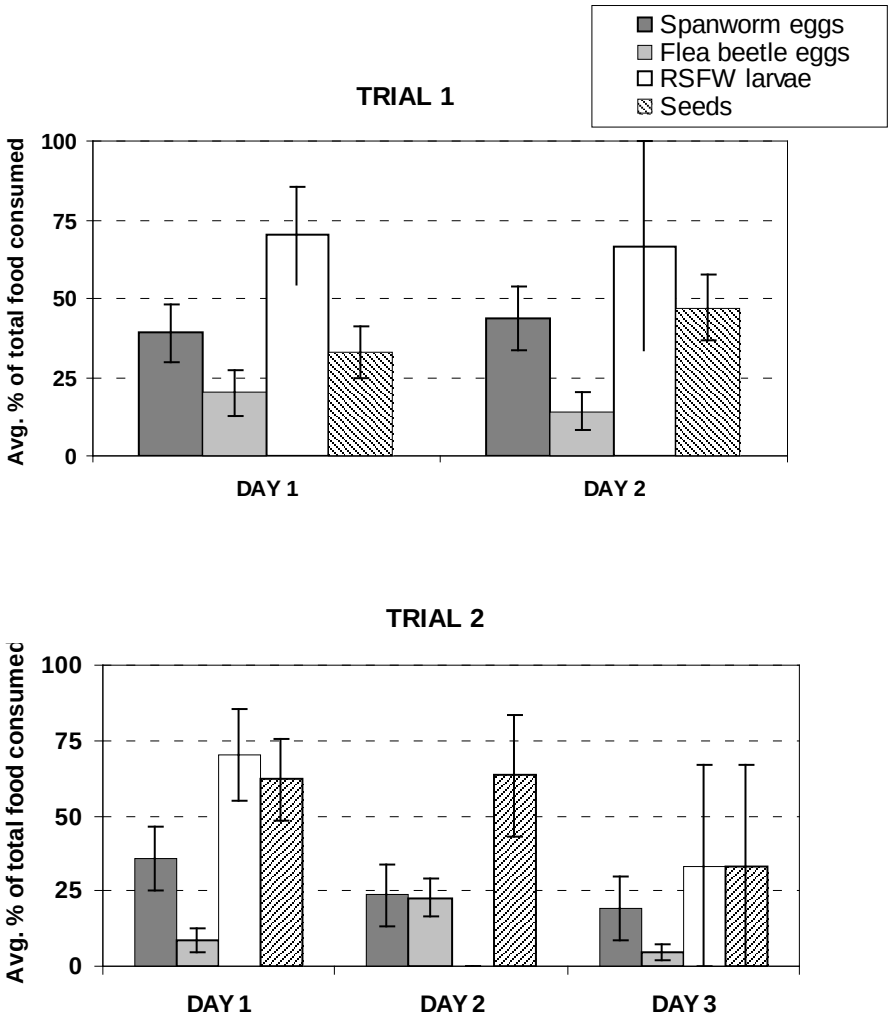
Feeding on red-striped fireworm larvae hidden within webbed blueberry leaves or exposed

This experiment was designed to determine if, in a laboratory setting, *H. rufipes* beetles can detect and prey upon RSFW larvae inside webbed leaves. Larvae that were to be presented to the beetles inside webbed blueberry leaves were placed in a 14 cm diameter cup with a fresh blueberry stem three days in advance of the study to provide them time to web leaves together. Once the RSFW had formed a webbed home, one *H. rufipes* beetle adult was placed in each cup along with a moist cotton wick. In addition, 12 *H. rufipes* beetles were individually placed in cups with one exposed RSFW larva and a moist cotton wick. The cups were checked after five days to determine whether the larva had been eaten. Results were analyzed using a logistic regression model.

RESULTS:
Choice feeding trial (2005)

Red-striped fireworm larvae were preferred over other prey items in both trials (Fig. 1). Larvae were consumed at the highest percentage (as a proportion of the total number of each prey item that was given) on days 1 and 2; 70% of the total given were consumed on day 1 and 67% of the total remaining were consumed on day 2 of trial #1. In trial 2, consumption of RSFW larvae was still high on days 1 (70%) and 3 (33%), but none were consumed on day 2. Seeds and SW eggs were generally equal in preference in trial #1. Both were less preferable than RSFW larvae, but still preferred over FB eggs. Between 33-47% of the total mass of SW eggs and seeds were consumed per day; similar percentages of food items were consumed on day 1 and day 2. Percentage of seed consumption was higher in trial #2 (62%, 63%, and 33% for days 1, 2, and 3, respectively). Percentage of SW egg consumption was slightly lower in trial #2 (36%, 24%, and 19% for days 1, 2, and 3, respectively. Flea beetle eggs had the lowest consumption rate in both trials; 20% on day 1 and 14% on day 2 of trial #1. In trial #2; 8, 23, and 5% were consumed on days 1, 2, and 3, respectively.

Fig. 1. Percent of total food item consumed per day for trial 1 and trial 2.



Feeding on exposed red-striped fireworm larvae

All but five RSFW larvae were eaten by *H. rufipes* adults within the first three days. After seven days, three larvae remained uneaten (Fig. 2). On day 1, there was a significant positive correlation between the amount of larvae given and amount eaten ($P < 0.0001$), i.e. the number of larvae consumed increased with the number of larvae provided. There was a similar trend on day 2, but the correlation was not significant ($P = 0.113$) (Fig. 3).

Fig. 2. RSFW larvae consumed per day by *H. rufipes* adults given 1, 3, 5, or 7 larvae.

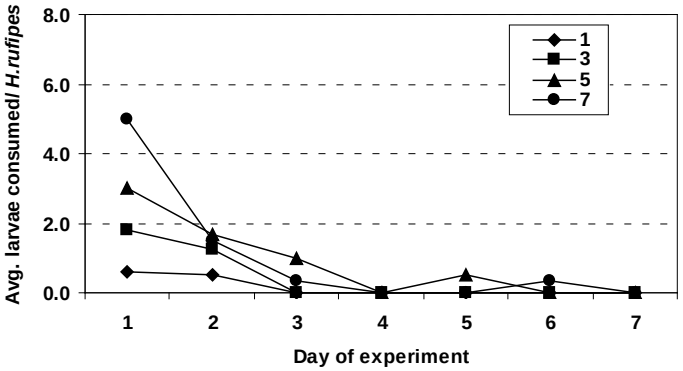
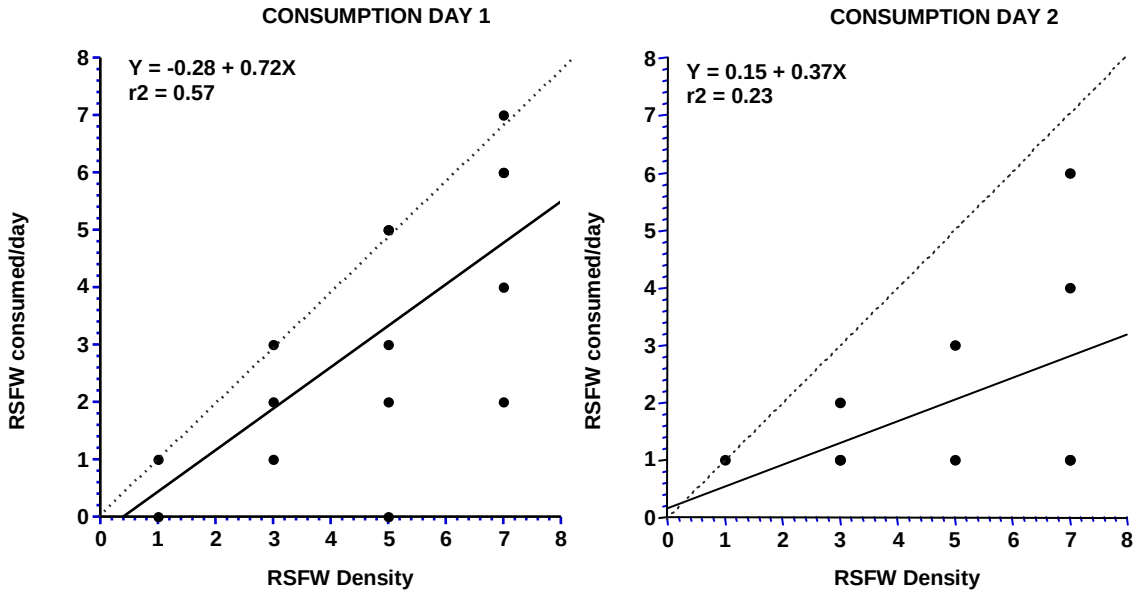


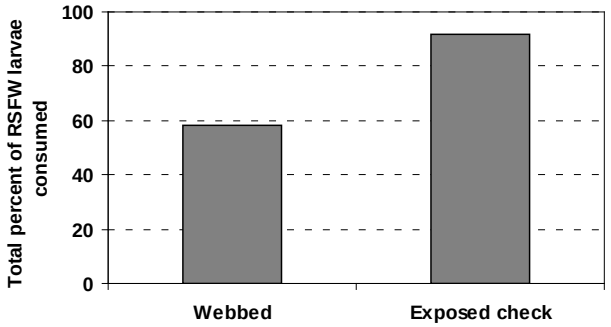
Fig. 3. Consumption of RSFW larvae by *H. rufipes* adults as a function of RSFW density. Dashed line is a slope of 1 and solid line is fitted linear regression; day 1 (n = 20), day 2 (n = 12).



Feeding on red-striped fireworm larvae hidden within webbed blueberry leaves or exposed

In general, *H. rufipes* adults ate more exposed RSFW larvae (92%) than those hidden between webbed leaves (58%); however, the difference was not significant ($P = 0.085$) (Fig. 4), indicating that *H. rufipes* is capable of finding a prey item that is hidden from view, at least in a laboratory setting.

Fig. 4. Percent of RSFW larvae consumed by *H. rufipes* when hidden between webbed blueberry leaves or exposed.



CONCLUSIONS: *Harpalus rufipes* is a commonly occurring carabid beetle spp. in lowbush blueberry. *H. rufipes* is a known omnivore, feeding on both weed seeds and arthropod prey. Based on pitfall trap captures, *H. rufipes* is most abundant in lowbush blueberry fields during the months of August and September, which coincides with the larval life stage of red-striped fireworm, the egg life stage of blueberry spanworm and blueberry flea beetle, and many species of weed seeds. *H. rufipes* is a potential natural enemy of these blueberry pests.

6. Feeding trials with *Calosoma calidum*

METHODS:

***Calosoma calidum* feeding on blueberry spanworm larvae**

In 2006 laboratory trials, blueberry spanworm larvae were set up in a 14 cm diameter (4 cm depth) cup with a blueberry stem. This is similar to how we hypothesize *C. caladium* would encounter the larvae in blueberry fields. Each cup contained 1, 3, 5, or 7 spanworm (SW) larvae, and one *C. calidum* beetle. The cups were checked after 1, 4, and 7 days to see how many SW larvae were consumed. The results were analyzed using a weighted linear regression model.

***Calosoma calidum* feeding on dead and live blueberry flea beetle larvae**

hile carabids are known predators, they are also usually omnivorous to one extent or another. The purpose of this study was to evaluate whether *C. calidum* beetles fed preferentially on live or dead prey items. Individual *C. calidum* beetles were placed in a 14 cm diameter cup (4 cm depth) with five live and five dead blueberry flea beetle (FB) larvae and a moist cotton

wick. The cups were checked for the number of whole live and dead larvae after 1, 2, 3, and 6 days.

RESULTS:

<u>*Calosoma calidum* feeding on blueberry spanworm larvae</u>

All of the SW larvae that were eaten were consumed one day after they were introduced to a *C. calidum* beetle (Fig. 1). And, there was a significant correlation between the number of larvae given and the number of larvae eaten on day one ($P = 0.0218$) (Fig. 2). There were only two replicates so further study should be done. One of the *C. calidum* beetles with seven larvae in its cup ate one larva on the first day. The other six larvae were dead from day 2 on, and were never consumed.

Fig. 1. Mean number of blueberry spanworm larvae consumed by *C. calidum* beetles after 1, 4, and 7 days.

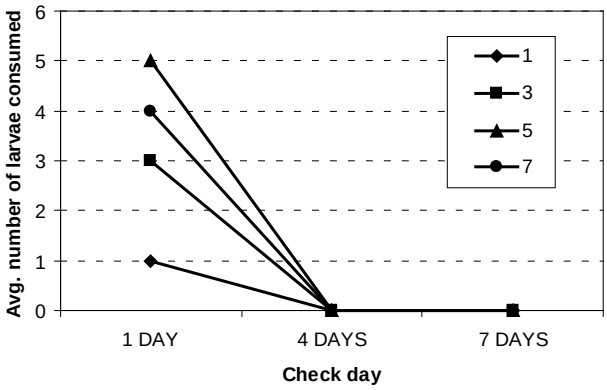
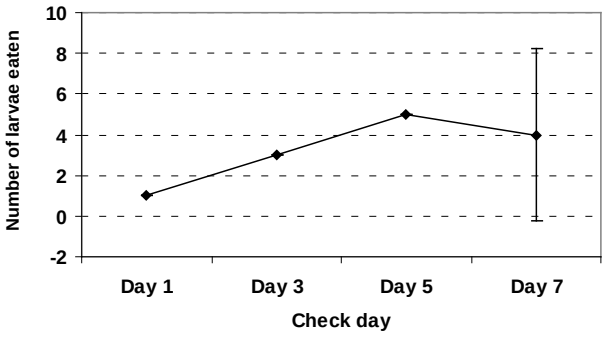


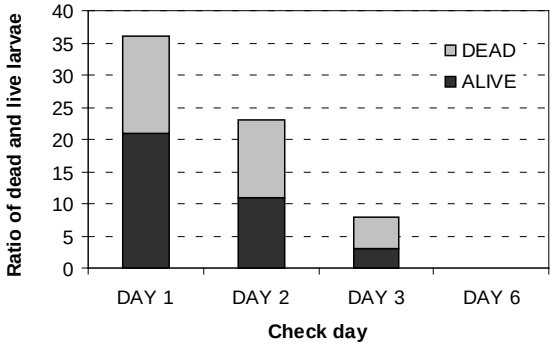
Fig. 2. Correlation between number of larvae given and number of larvae consumed on day one.



<u>*Calosoma calidum* feeding on dead and live blueberry flea beetle larvae</u>

On day 1, there were more live FB larvae remaining than dead ones (21 and 15, respectively). On days 2 and 3, there were similar numbers of live and dead prey remaining (11/12 and 3/5; live/dead respectively). There were no whole larvae, live or dead, on day 6 (Fig. 3). One thing to consider is that there is no way of telling if the whole, dead larvae were not once live and had somehow died, which may have artificially increased the number of dead larvae remaining.

Fig. 3. Number of whole live and dead FB larvae remaining after 1, 2, 3, and 6 days.



CONCLUSIONS: *Calosoma calidum* is another commonly occurring carabid species in lowbush blueberry. Based on pitfall trap captures, *C. calidum* is most abundant in lowbush blueberry fields during the months of June and July, which coincides with the larval life stage of blueberry spanworm and the blueberry flea beetle which means that *C. calidum* could potentially be a natural enemy of this pest and should be conserved whenever possible by selecting insecticides in the spring that are least toxic to this predator such as *Bt*, SpinTor®, or Entrust®.

7. Growth and development of blueberry flea beetle immatures in the laboratory

METHODS: Blueberry flea beetle eggs were collected in the summer of 2005 and wintered in the field. In late April or early May, the eggs were removed from the field and reared at one of three different temperatures (15, 20, or 25°C). Emerging larvae were placed in individual plastic diet cups with fresh blueberry buds and foliage and reared at 25°C. Larvae were also field-collected in early to mid-May as 1st instar larvae and reared at 15 or 25°C. Larval instar as determined by head capsule width was recorded at 1 to 3 day intervals for each larva.

RESULTS: The average number of days required for each immature life stage to complete its development is in Table 1. No larvae emerged from eggs incubated at 15°C, and no pupae survived to the adult stage. These data will be the basis of a computer simulation model used to investigate the optimal timing for insecticide controls.

Table 1. Rate of development of blueberry flea beetle immatures.

Insect growth stage	Days development (SE)	% survival (#)
15°C		
Egg	NA	
1st instar *	6.75 (0.43)	100.0 (20)
2nd instar	9.07 (0.48)	75.0 (15)
3rd instar	20.67 (1.45)	20.0 (3)
Pupa		0.0 (0)
20°C		
Egg	10.00 (0.38)	(55)
25°C		
Egg	6.96 (0.36)	100.0 (24)
1st instar *	3.88 (0.87)	70.8 (17)
2nd instar	4.29 (0.40)	70.8 (17)
3rd instar	9.18 (0.51)	70.8 (17)
Pupa	8.38 (0.20)	94.1 (16)

* Some Individuals were field collected as 1st instar larvae; therefore, data for "days development" as 1st instar larvae are incomplete and are an underestimate of development.

\# Number of individual completing life stage.

CONCLUSIONS: At this point few conclusions can be drawn from this study. We have conducted temperature dependent-development studies for blueberry maggot fly, blueberry spanworm, and we are initiating studies on blueberry flea beetle, and blueberry thrips. Once we have completed the study with the blueberry flea beetle we will have the basis for a computer simulation model that will allow us to build a tutorial simulator which growers and researchers can use to evaluate control strategies.

8. Evaluation of the Alleghany mound ant, *Formica exsectoides*, prey preference and preliminary developments in portable ant colonies

(Progress report by Beth Choate

Prey Preferences

METHODS: A total of eight active *F. exsectoides* mounds were identified within four lowbush blueberry fields. Two field sites were monitored during a single eight hour period by pairing sites within close proximity. Sites were located within Orland, Penobscot, Cherryfield, and Beddington, ME. In each field two mounds were monitored 1 d per week during 4-30 min. periods throughout the day. Monitoring was conducted by two researchers that were assigned a single mound within each field to ensure that monitoring was consistent throughout the experiment. A field was visited by the two researchers and mounds monitored for 30 min. after which they moved to the paired site. Movement between sites continued until each site was visited a total of four times throughout the day. Fields were visited twice in the AM and twice in the PM to determine if time of day impacted prey

preference by the ants and total number of prey collected.

Monitoring of mounds consisted of observing each ant forager entering the mound on previously identified active foraging trails. Researchers used soft forceps to pick up foragers as they entered the mound. The mandibles were then observed using a hand lens and if prey was present, it was removed from the mandibles and place in a vial of 75% ethanol. Samples were then brought back to the laboratory for identification.

In addition to monitoring mounds, *F. exsectoides* workers kept within the lab were fed known lowbush blueberry pests. These pests included red-striped fireworm larvae (within and removed from leaves) and all stages of blueberry flea beetle.

RESULTS/CONCLUSIONS: Identification of collected prey indicates *F. exsectoides* are generalist predators feeding on a variety of arthropod species. Table 1 displays the total number of prey brought to mounds. All prey were identified to order except members of the phylum Annelida (segmented round worms). Specimens listed as unknown were too damaged to identify. The majority of prey belonged to the orders Hymenoptera (ants, bees, and wasps) and Lepidoptera (caterpillars of the moths and butterflies), which accounted for 22% and 16% of the total specimens collected, respectively. When life-stage of specimens was evaluated 98% of Hymenoptera collected were adults; whereas, the majority of Lepidoptera collected (84%) were larvae. Seventy-three percent of hymenopterans were various species of Formicidae (ants); however, no *F. exsectoides* were identified as prey. Figure 1 displays the average number of each insect order collected during a single 8 hour period of monitoring two sites.

Figure 2 displays the number of prey brought to the nest during each monitoring period at all four sites. There is a distinct curve to this data, with a peak in foraging activity toward the end of July. Monitoring of mounds began in mid-June when foraging activity was first observed and continued into September until no workers were observed bringing prey to the nest. Knowledge of peak foraging periods is essential in developing methodology for the use of *F. exsectoides* colonies in controlling pest populations. Foraging was initiated in blueberry fields when flea beetle larvae and pupae are present in the field, peaked during the onset of blueberry maggot fly pupation and red-striped fireworm egg hatch.

Within the lab, workers were observed attacking and feeding on red striped fireworm when removed from blueberry foliage, as well as the egg, larval, pupal and adult stages of blueberry flea beetle.

Table 1. Total number of prey of each order brought to the nest by *F. exsectoides* workers during monitoring periods from June to September 2006.

Classification	Total	Percent
Hymenoptera	152	21.560
Lepidoptera	112	15.887
Unknown	111	15.745
Diptera	81	11.489
Homoptera	62	8.794
Coleoptera	50	7.092
Orthoptera	38	5.390
Annelida	30	4.255
Araneae	28	3.972
Hemiptera	24	3.404
Psocoptera	8	1.135
Collembola	4	0.567
Opiliones	3	0.426
Blattodea	2	0.284

Fig. 1. Avg. number of prey brought to the nest by *F. exsectoides* workers during each monitoring period.

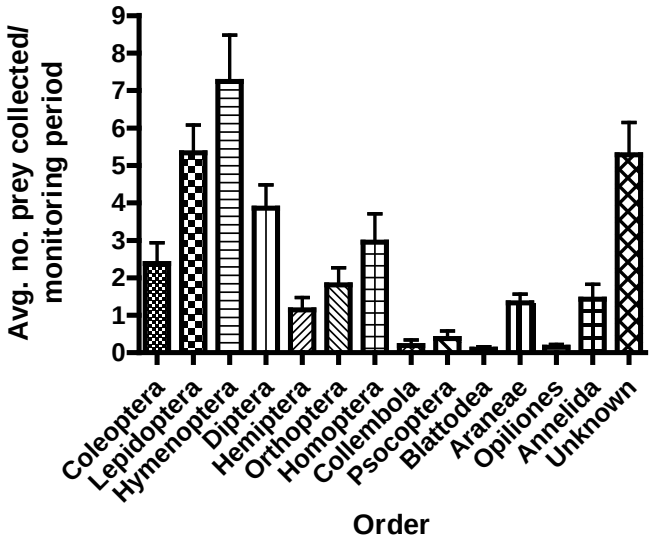
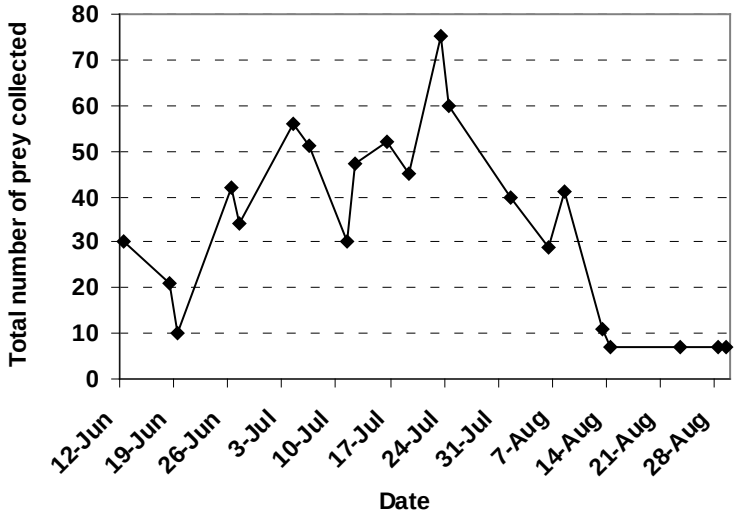


Fig. 2. Number of prey collected by *F. exsectoides* during each sampling period throughout the summer at four lowbush blueberry field sites in Downeast Maine.



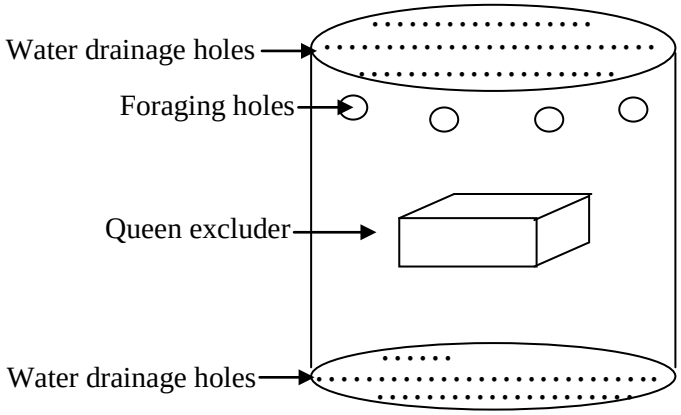
Portable Ant Colonies

METHODS: The basic design of the portable ant colony was taken from Campbell 1990; however, this publication did not indicate details of design, thus initial laboratory experiments were conducted to determine the sizes of holes for foraging, water drainage and the queen excluder. Initial prototypes placed foraging holes at the base of the container; however, it was observed that this was unnatural for the workers to exit from the bottom of the mound. Foraging holes 5/16 inch in size were placed at the top of the bucket. The plastic surface of the bucket was too slick for the workers to crawl down; thus tape was placed below the foraging holes to provide traction. Drainage holes (5/64 inch) were placed in the lid and bottom of the bucket so that the necessary moisture would be provided, yet the bucket would not flood (Fig. 3). A queen excluder was developed in the laboratory. The queen was placed in a 4.5 x 3.5 x 2 inch plastic container with 7/64 inch holes drilled into the top, sides and bottom. This hole size was small enough to keep the queen in and allow the workers to move in and out freely. This prevents the queen from leaving the mound, an event which would ultimately lead to the movement or death of the nest.

Two materials, rectangular plastic trash cans and 5-gallon buckets, were tested for use as the portable nest container. Nests containing 1500 workers and 200 brood were constructed from both materials and placed outside on 23 August 2006. Queens were not included due to the inability to locate them within nests this late in the season.

RESULTS/CONCLUSIONS: Mounds were monitored daily until mid-October. Workers were observed leaving both nests; however, only those within the bucket returned consistently. Studies with portable ant colonies will continue in the summer of 2007 using 5-gallon buckets as the nest material with an effort to collect queens early in the season and place them within queen excluders.

Fig. 3. Diagram of portable ant colony design.



9. Bee foraging patterns during bloom

METHODS: Bee foraging patterns were observed and recorded on 25 and 30 May, and 1 and 2 June at Blueberry Hill Farm. The purpose of this study was to evaluate the efficacy of different bee species in pollinating lowbush blueberry. 2006 represents the fourth year of this study. The analysis of this question involves first collecting data that describes the bee foraging behavior in the field and then involves simulating this behavior on the computer. Individual bees were followed and the number of stems, number of flowers visited per stem, the distance between stems visited, and the angle flown from one stem to the next were all recorded. We also recorded the average number of blossoms per stem within each blueberry clone visited by individual bees. The total number of open blossoms was recorded for each of three randomly selected stems per clone. The bee species sampled were honeybee, *Apis mellifera* and bumble bee, *Bombus impatiens*.

RESULTS: Observations were made for 113 honeybees and 36 bumble bees. Figure 1 shows the frequency distribution of the number of stems visited per foraging bout by each species. Honeybees visited an average of 6.6 stems per foraging bout while bumble bees visited 13.2 stems/bout. Honeybees visited an average of 7.8, 5.9, and 8.1 stems/bout in 2003, 2004, and 2005, respectively (Fig. 2).

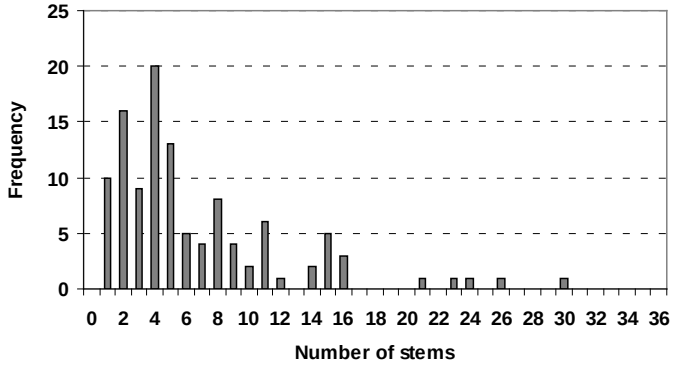
Figure 3 is the frequency distribution of the number of flowers visited/stem. The average for honeybees was 1.8 flowers/stem; honeybees visited 2.0, 1.6, and 1.9 flowers/stem in 2003, 2004, and 2005, respectively. Bumble bees visited 3.9 flowers/stem in 2006 (Fig. 4).

Distance traveled between stems is in figure 5. Bumble bees traveled 8.2 inches between stems. Honeybees traveled an average of 6.8 inches between stems. In 2005, bees traveled an average of 5.6 inches (Fig. 6). A similar result was obtained in 2004 when honeybees traveled an average of 6.2 inches between stems. Finally, the angle of departure from one stem to the next in a single foraging trip that could be followed in the field was determined (Fig. 7).

A preliminary computer simulation model has been built that simulates foraging of a honeybee colony within a field with five blueberry clones (Fig. 8). This model will be modified and used to explore the dynamics of pollination by different bee species

Fig. 1. Blueberry stems visited during foraging bouts, 2006.

Honeybees (n = 113)



Bumble bees (n = 36)

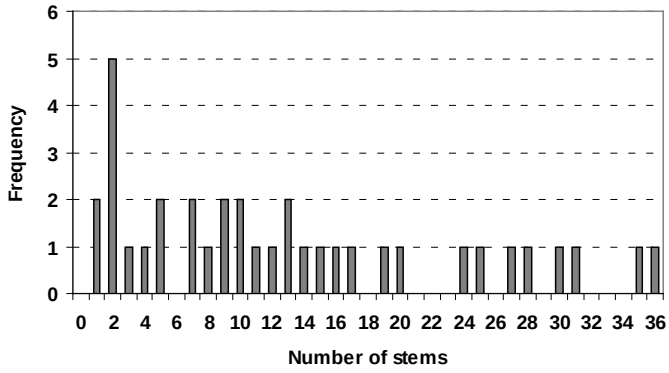


Fig. 2. Mean number of stems visited per foraging bout for study years 2003-2006.

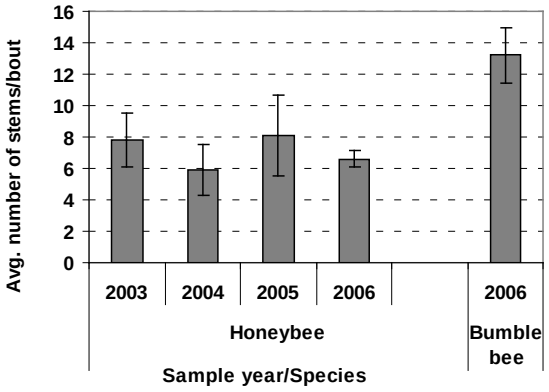
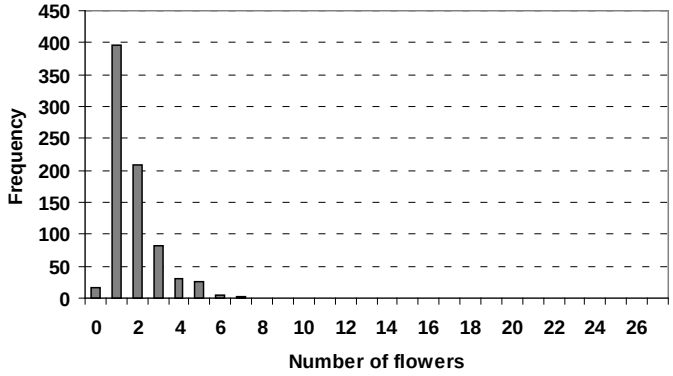


Fig. 3. Number of flowers visited per stem during foraging bouts, 2006.

Honeybees (n = 113)



Bumble bees (n = 36)

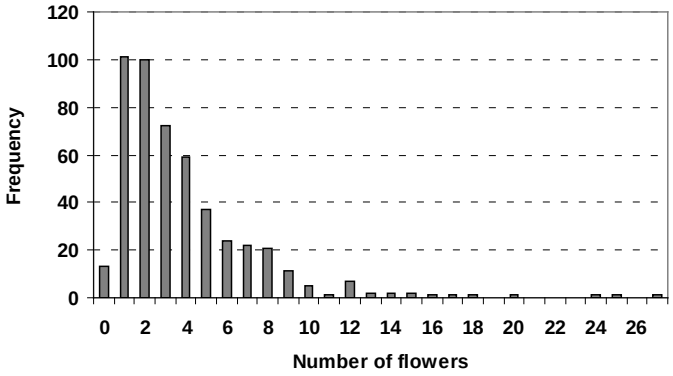


Fig 4. Mean number of flowers visited per stem during foraging trips for study years 2003-2006.

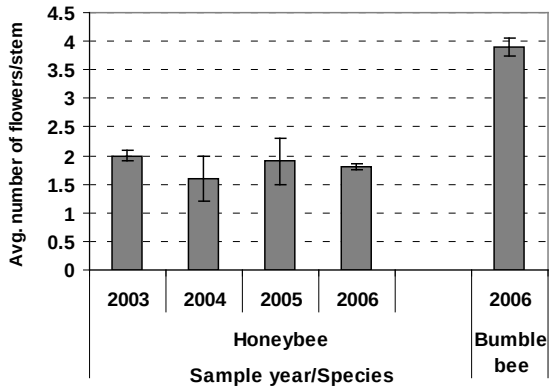
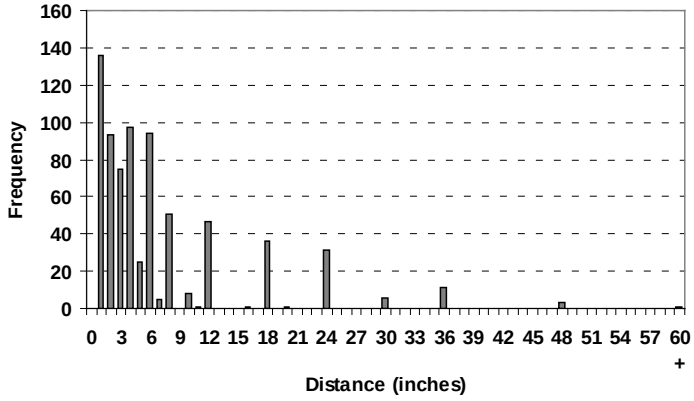


Fig. 5. Distance traveled between stems during foraging bouts, 2006.

Honeybees (n = 113)



Bumble bees (n = 36)

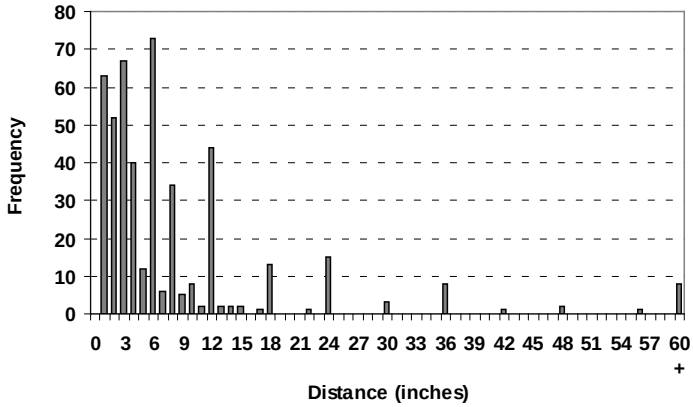


Fig. 6. Mean distance traveled between stems during foraging bouts for study years 2004-2006.

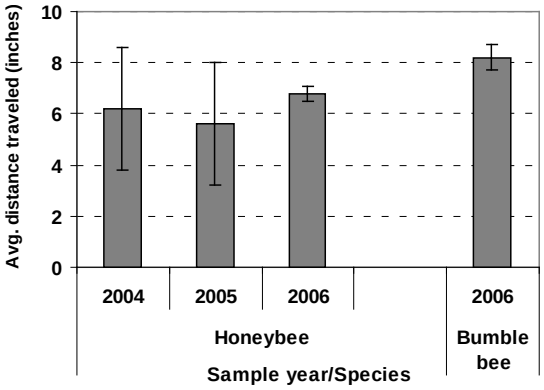
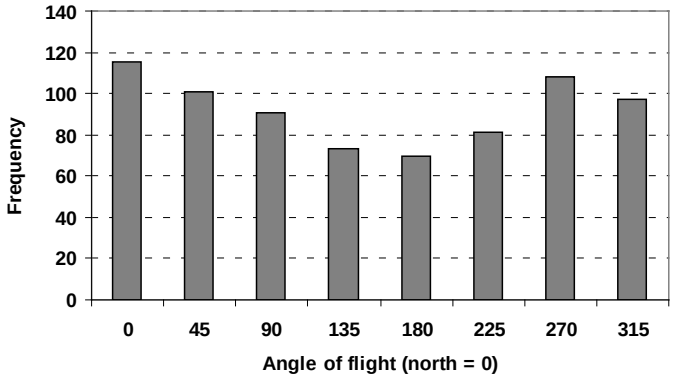


Fig. 7. Angle of flight from one stem to the next, 2006.

Honeybees (n = 113)



Bumble bees (n = 36)

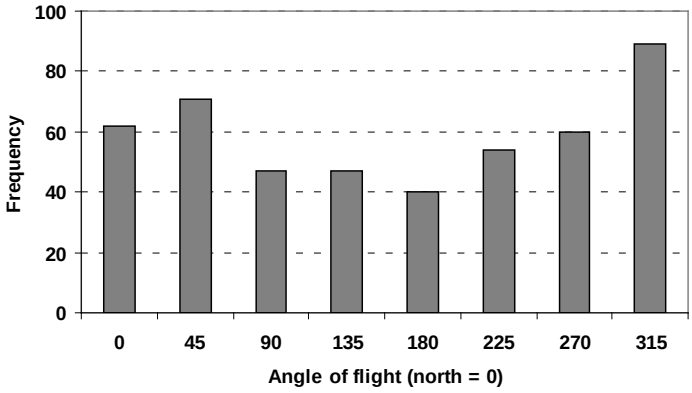
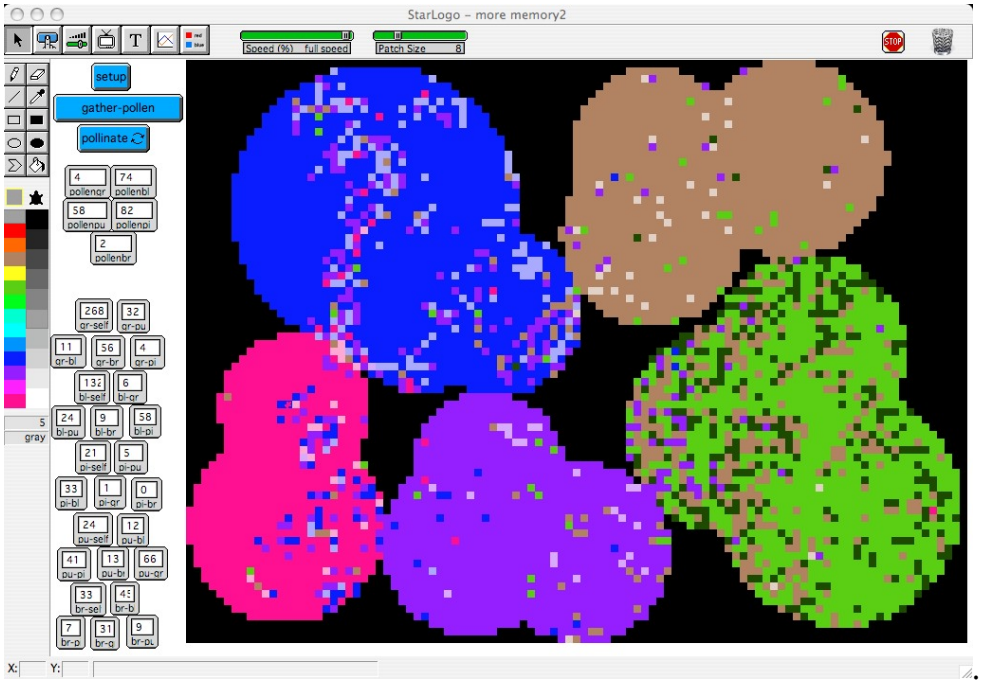


Fig. 8. The window of the preliminary pollination model that has been constructed to assess pollination efficacy of various bee species. The dots within each clone are flowers that have been pollinated, the color representing the source the clone from which the pollen came from.



CONCLUSIONS: This is the fourth year of a research project with the goal of constructing a computer simulation model of bee foraging on lowbush blueberry so that the amount of out-crossing can be estimated in a lowbush blueberry field. We have initiated a modeling project to simulate bee movement and pollination within lowbush blueberry fields. Much of our work on this project will be confined to the modeling component.

10. <u>Bumble bee pollination</u>

METHODS: A study was initiated in the early spring of 2006 in the Coastal Blueberry Region in Maine. Eight isolated lowbush blueberry fields were chosen as study sites. Three fields had honeybees deployed on them at a stocking density of 4 hives per acre and five fields had commercial bumble bee hives deployed in them roughly 3/4 quad / acre (GPS ground truthing of the fields remains to be conducted to calculate accurate stocking densities). Fifteen clones were selected in each field for study. The spatial layout of the clones was such that five clones along three linear transects were chosen at 10, 20, 30, 50, and 100 m from the hive clusters. In each clone five randomly chosen stems were marked with string collars and the number of flowers were counted before bloom and recorded. During bloom bee visitation (honeybees, bumble bees, and other pollinators) was assessed per minute in 1 m^2 plots adjacent to the selected clones. Two weeks after bloom an initial estimate of fruit set was made by counting all set fruit minus non-viable "pin heads" (primordial fruits with non-swollen calyx). There was considerable disease in most of the fields and so an analysis of fruit set was conducted with all of the marked stems and with only those stems that showed no visible symptoms of disease (corrected for disease). A harvest sample of fruit was also taken to assess the standing crop, berry size, berry weight,

the number and proportion of viable seeds/berry, and total yield/stem. In addition, bee foraging activity was recorded in the field and at hive entrances.

RESULTS: The 2006 bloom period was extremely rainy. In some areas of Maine, May 2006 was one of the wettest months on record, similar to the spring of 2005. This created ideal conditions for disease and less than ideal conditions for pollination. The data collected during this study (Table 1) suggests that the bumble bee fields had significantly less proportion fruit set compared to honeybee fields when disease was not taken into account (0.583 vs 0.672, $P = 0.035$ (nested ANOVA)…a 13.2% difference in fruit set overall). When disease was taken into account, bumble bee fields still had lower proportion fruit set but at a more marginal probability level (0.617 vs 0.705, $P = 0.060$ (nested ANOVA)…a 12.4% difference in fruit set overall). Therefore, either analysis suggests a small, but potentially important difference in fruit set, with the higher fruit set observed in honeybee fields. Figure 1 illustrates the individual initial field fruit set estimates (NOT corrected for disease). One can see that not all of the honeybee fields had greater proportion fruit set than the bumble bee fields. Figure 2 shows the proportion of fruit set just prior to harvest. This represents fruit set after fruit drop has occurred. There was no significant difference ($P = 0.746$) in fruit set between honeybee fields and bumble bee fields. There were also no differences observed in the proportion of ripe fruit (Fig. 3) nor berry weight (Fig. 4) between honeybee fields and bumble bee fields

There was no indication that fruit set or berry weight varied in relation to the distance of the blueberry plants from either honeybee hives or bumble bee quads. This relationship has been observed for honey bee pollinated fields previously. However, this is usually only observed in fields that have a suboptimal honeybee stocking density, thus suggesting that the fields in this study that received honeybees did so at an adequate stocking density.

Bee activity was assessed in a variety of ways during bloom. Bee visitation was measured directly by counting the number of honeybees, *Bombus impatiens* bumble bees, and native bees other than *B. impatiens* visiting a square meter of blueberry bloom. Eight-teen square meter plots (6 plots along three transects) were observed in each field for 15 seconds at 10, 20, 30, 50, 75, and 100 m from the honeybee or bumble bee colonies. These observations were conducted twice during bloom. In addition, the number of honeybees and bumble bees returning to a hive (honey bee hive or bumble bee colony) during a one minute period was observed for 3-6 hives twice during bloom in each field. Three to five sets of 10 honeybees and bumble bees were also collected with a sweep-net upon returning to the hive. Bees without pollen stores on the hind legs were released immediately. Bees possessing pollen were released after a sample of the pollen was taken for identification in the laboratory. Blueberry pollen can not be easily distinguished from other Ericaceous pollen. However, no Ericaceous plants, other than lowbush blueberry, were observed in bloom during our collections, thus we have assumed that any Ericaceous pollen was lowbush blueberry pollen. The pollen sampling was conducted during two times. A percentage of the bees returning to the hive with blueberry pollen were calculated.

Within a blueberry field there was no trend in the number of honeybees or bumble bees visiting blueberry bloom as a function of the distance the blueberry plants were away from the hives. This is in concordance with the similar relationship observed for fruit set. Overall differences in abundance of all bee pollinators averaged over the two observation dates can

be seen in figure 5. The data suggests that as overall pollinator abundance increases so did the fruit set in the fields. In addition, it can be seen that fields with lower abundances of bees (all species of bees) had lower levels of fruit set. Figure 6 shows the relationship between just the honeybee abundance (averaged over the two observation dates) and average fruit set per field. As similar pattern described for all bees is also characterized by the honeybee data, suggesting that fields with higher levels of honeybees had higher levels of fruit set. Unfortunately, some of the bumble bee fields also were observed to have moderate levels of honey bee visitation, presumably from nearby blueberry fields. This fact makes conclusions about the efficacy of commercial bumble bees as pollinators more difficult. However, figure 7 shows that fruit set in the bumble bee fields does increase with increased bumble bee abundance suggesting that the bumble bees were having a positive role in fruit set. This is not observed in the honeybee fields. Figure 8 shows the return rate to the hive for one minute periods. In general, honeybees were 20 times more abundant in returning to the hive than bumble bees. However, these data should only be compared between hives of the same bee species because the return rate will greatly be affected by the amount of time that an individual bee spends foraging in the blueberry field before the return. A bee that stays longer in the field will produce a lower return rate, all other factors being equal. The important aspect of the data in figure 8 is that the difference in honeybee activity between the three honeybee fields was 20%, but the difference between bumble bee hives was 400% (4x). Thus there appears to be a large difference in bumble bee foraging from the hives in different fields. The cause of this variability is not known, but it is disconcerting. The percent of bees returning to the hive with blueberry pollen reflects the efficiency of the two species of bee. Only about 3% of the honeybees returning to the hives possessed noticeable amounts of blueberry pollen while about 29% of the bumble bees observed during the same time of day were observed to bring back blueberry pollen.

Further analysis on the number of seeds/berry will shed light on the pollination efficacy characterizing each of these fields. Data collection is currently taking place, but it unfortunately involves the tedious task of dissecting a large number of fruit.

Table 1. Estimates of fruit set from fields that were stocked with honeybees and those that were stocked with bumble bees. The table summarizes estimates of proportion fruit-set without taking into account diseased stems, the proportion of stems with any disease symptoms, and the corrected fruit set calculated by only including stems with no visible symptoms of disease.

FIELD	TREATMENT	FRUIT SET	DISEASE	CORRECTED
Brown	Bumble bee	0.526	0.413	0.579
Hires	Bumble bee	0.535	0.533	0.600
Merrill 1	Bumble bee	0.547	0.213	0.567
Merrill 2	Bumble bee	0.634	0.20	0.660
Erskine	Bumble bee	0.672	0.227	0.680
Roche	Honeybee	0.611	0.253	0.683
Ford	Honeybee	0.622	0.267	0.649
Rte 3	Honeybee	0.783	0.013	0.783

Fig. 1. Initial proportion fruit set for 8 fields (3 with honeybees and 5 with bumble bees). The mean fruit set in honeybee fields was significantly higher in than in bumble bee fields (P = 0.035).

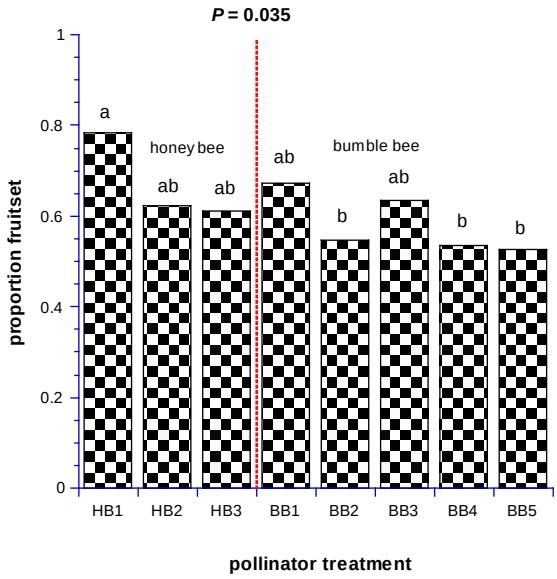


Fig. 2. Proportion fruit set prior to harvest for 8 fields (3 with honeybees and 5 with bumble bees). The mean fruit set in was NOT significantly higher in honeybee fields compared to bumble bee fields (P = 0.764).

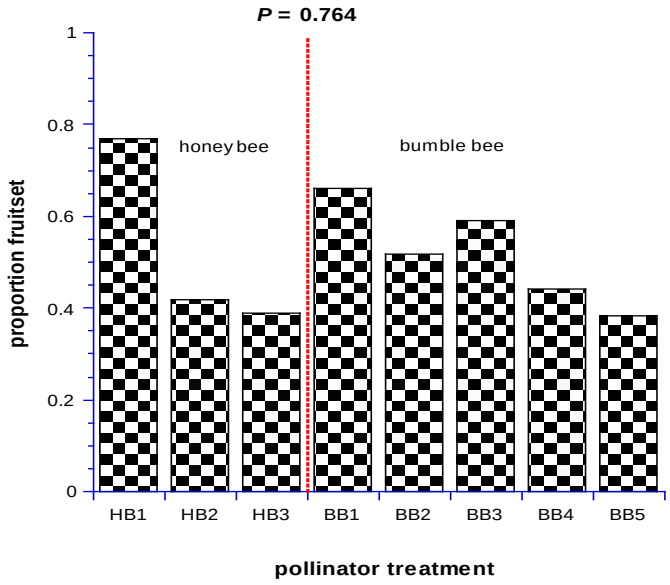


Fig. 3. Proportion ripe fruit prior to harvest for 8 fields (3 with honeybees and 5 with bumble bees). The mean proportion of ripe fruitwas NOT significantly higher in honeybee fields compared to bumble bee fields (P = 0.973).

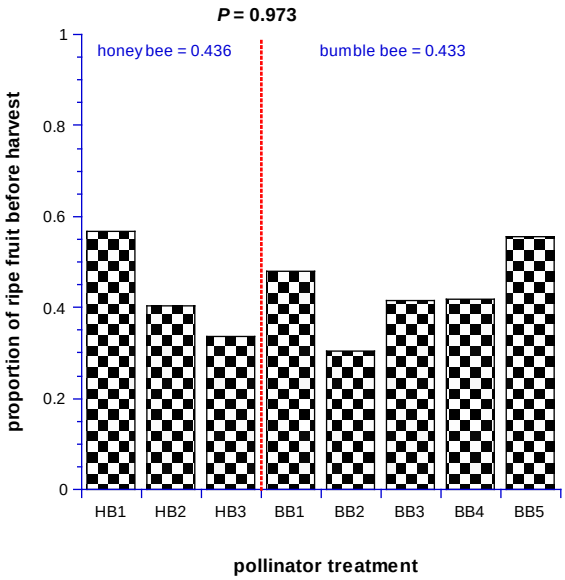


Fig. 4. Berry weight (gms/berry) at harvest for 8 fields (3 with honeybees and 5 with bumble bees). The mean berry weight was NOT significantly higher in honeybee fields compared to bumble bee fields (P = 0.238).

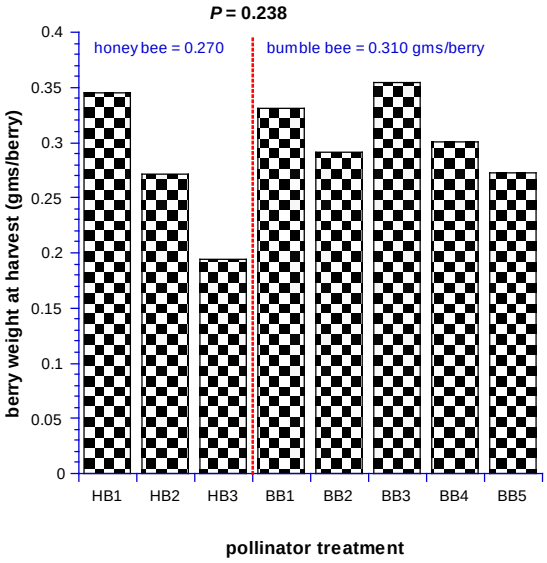


Fig. 5. The relationship between bumble bees (pollinators not including wasps, flies, etc.) and the proportion fruit set, B = bumble bee field, H = honeybee field.

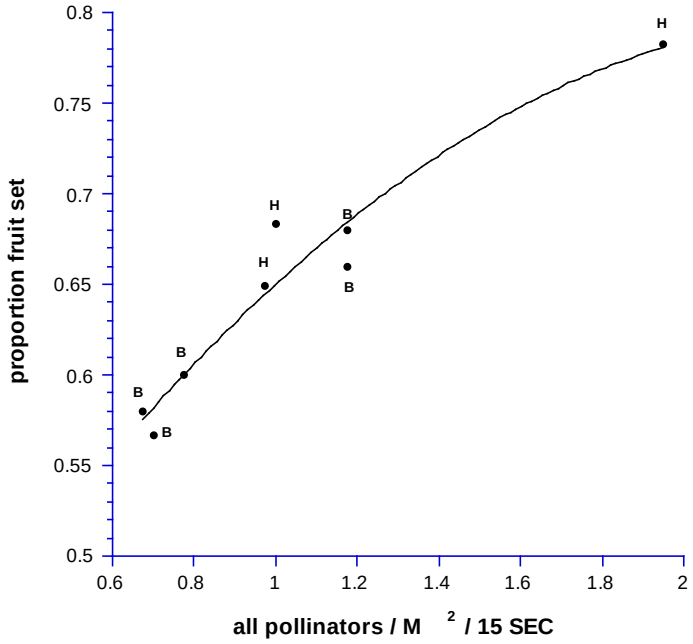


Fig. 6. The relationship between honeybee visitation and the proportion fruit set for each field, B = bumble bee field, H = honeybee field.

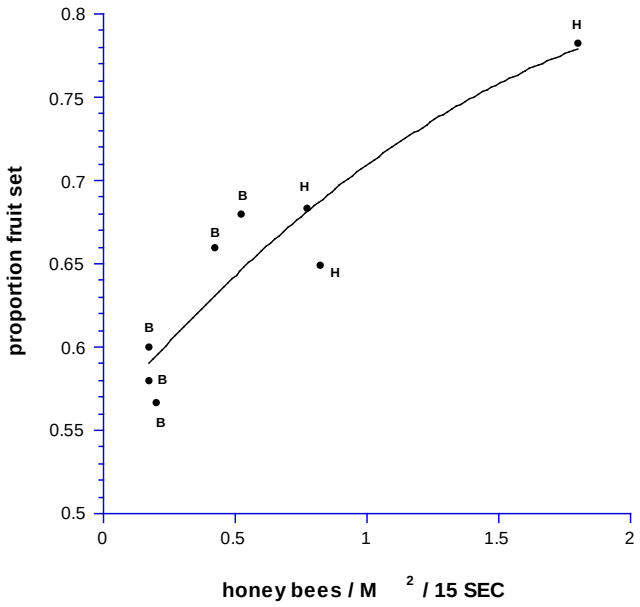


Fig. 7. The relationship between bumble bees of the species *B. impatiens* and the proportion fruit set for each field, B = bumble bee field, H = honeybee field.

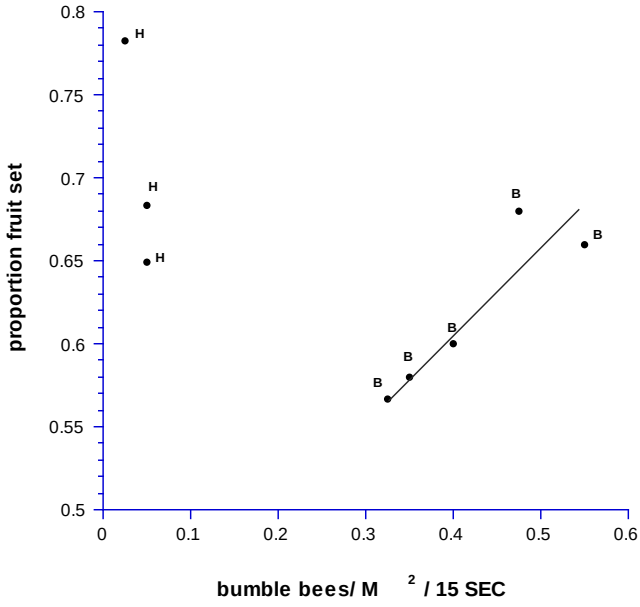


Fig. 8. The number of returning honeybees or bumble bees to a hive (for bumble bees this is one colony NOT a quad of four colonies).

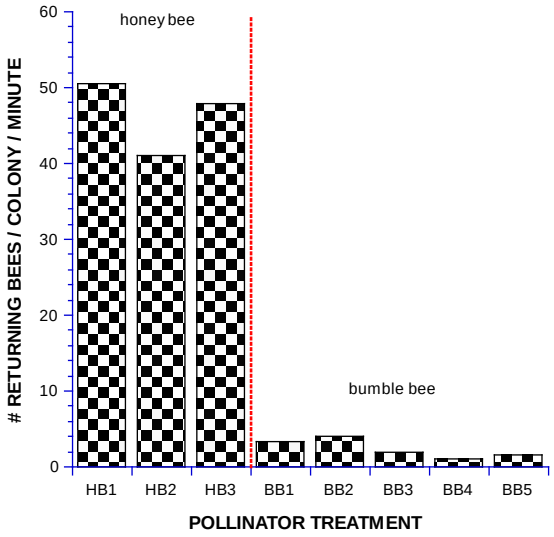
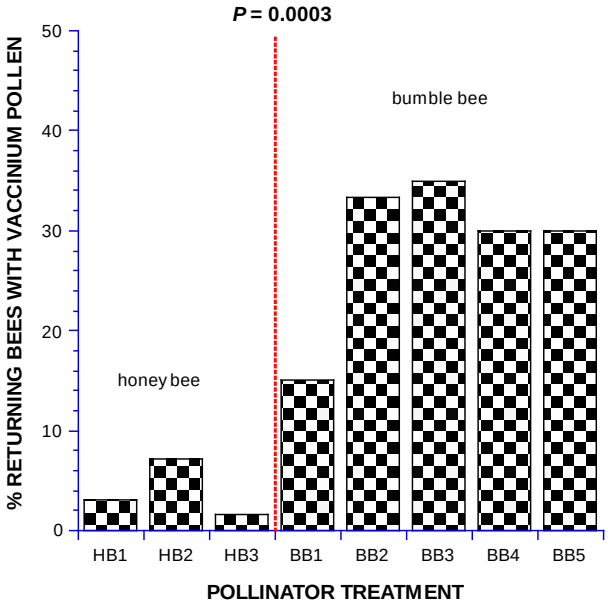


Fig. 9. The percent of bees returning to the hive (n = 10 for each set of collections) possessing what we assumed to be blueberry pollen.



CONCLUSIONS: In conclusion, the data collected in 2006 suggests that current recommended stocking rates for bumble bees will result in pollination rates (fruit set and berry weight) that is not significantly different from those observed with honeybees at the stocking density of 4 hives/acre. However, it is worth stating that the year 2006 was not representative of good pollination weather and our results could be interpreted as confounded since we did pick up some honeybees foraging in the "isolated" bumble bee fields.

11. Genotyping lowbush blueberry (*V. angustifolium*) using EST-PCR markers

Progress report by: Daniel J. Bell

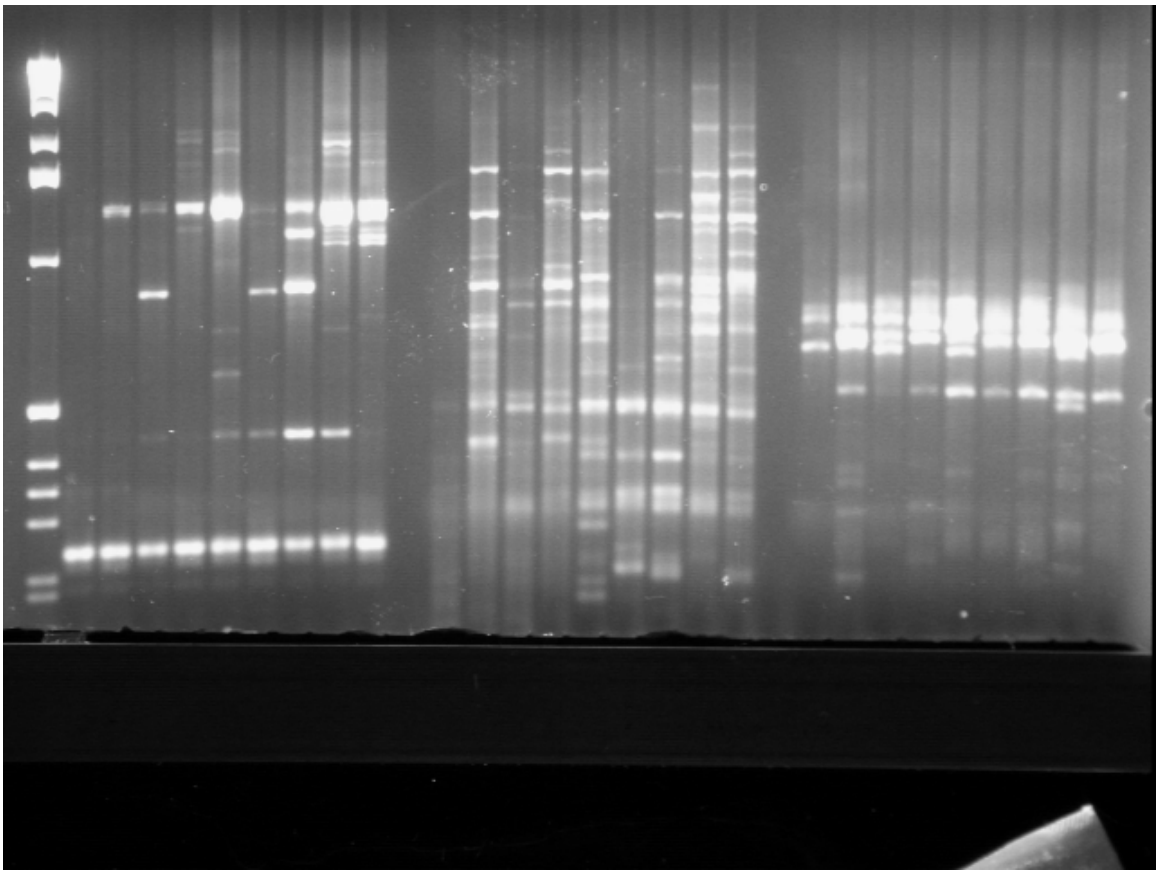
METHODS: In an effort to genotype genetic individuals in two populations plus a group of five clones used in hand pollination crosses in the summer of 2006, fresh leaf material was collected from 1 x 20=40, plus 5 individuals in the field at the Blueberry Hill Farm, Jonesboro, ME. A second population of 20 proximally growing clones was collected in Columbia, ME. Materials were sent down fresh on ice, as well as another shipment of frozen -80C on dry ice to the Fruit Labs of the U.S.D.A., Beltsville, MD. The principal investigator of that lab, Dr. Jeannine Rowland, is the designer of the EST markers used later in the PCR amplification process and is helping as collaborator in this ongoing effort.

During August, 2006 Mr. Bell traveled to the U.S.D.A. labs to work on DNA extractions and PCR amplifications using primers designed in Dr. Rowland's lab. The DNA extraction protocol is basically a modification of the Doyle and Doyle procedure which had previously been published. Dr. Rowland's modifications of this protocol were published some years back in a 'BioTechniques' article. The exact reference citation is being obtained.

RESULTS: During the first visit of one month duration 25 total extractions were attempted. All 25 were successful on first try as evidenced by a significant amount of high molecular weight DNA evidenced on agarose gels using ethidium bromide. Of these, 9 extractions worked flawlessly and repeatedly in subsequent PCR reactions using primers designed in Rowland's lab. Other extractions did not amplify in direct PCR reactions and needed to be adjusted by dilution techniques which were successful. As time was short in only having one month, 9 of the 25 extractions were used in generating polymorphic bands using EST primers. Thus, an attempt was made to completely screen to a suitable level for the stated purposes, 9 of the DNA extractions or clones.

Over the course of the last three weeks of stay, these 9 DNA samples were used in screening 24 total EST-PCR primers. PCR conditions used are those documented and published in other articles by Dr. Rowland. The identifications of these primers can be supplied and are basically just coded entries from Dr. Rowland's EST library of cDNA libraries generated from cold acclimated highbush blueberries. Of all 24 primers used, 21 showed repeatable (n=2) polymorphic bands across the 9 screened DNA samples. Polymorphic bands generated ranged from 1-6. Gels were photographed under U/V light and printed to photographic paper and scored across the samples. This scoring was done with the human eye. Mr. Bell did the first scoring and later coo borated my scoring with the chief support scientist under Dr. Rowland, Elizabeth Ogden. She agreed and coo borated my scoring with some exceptions as in adding bands I did not score. Thus, the band scoring was conservative in nature. This process basically results in marking bands that are polymorphic across the samples (thereby useful in discriminating relatedness among the clones). A band is tagged as being from a

given primer, and subscripted to show its number in the bands scored for that primer. Thus, a table was generated in which polymorphic bands are scored as either present or absent from a DNA sample. This presence/absence information when tallied across 79 different bands generates a matrix which can be input into a software program that generates trees of relatedness and relationship coefficients. The included picture is from a 9 x 3 primer set of reactions. Repeated runs were run on another gel and scored with these. If the reactions were not duplicated, they were not scored.

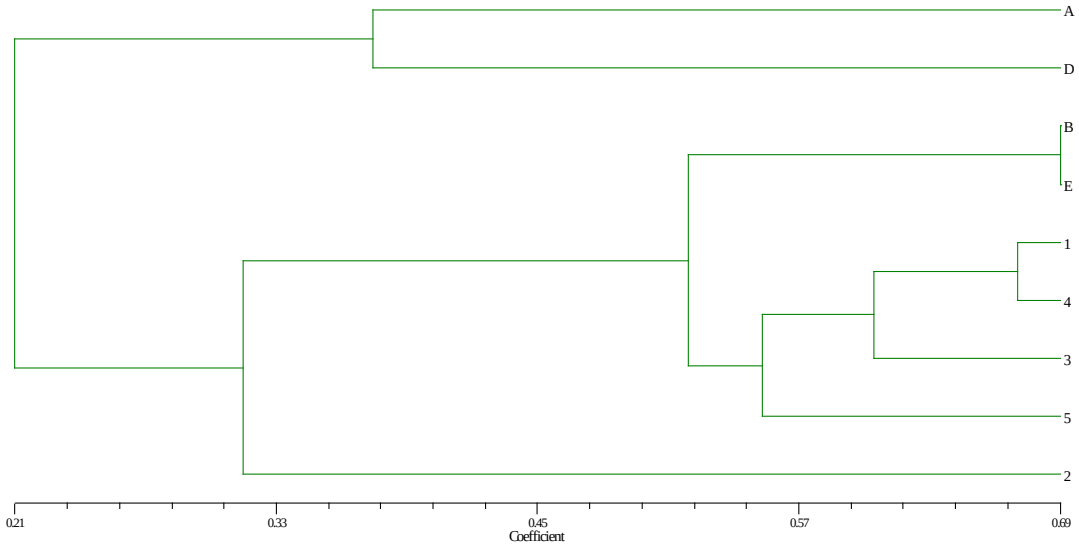


A gel run showing 3 primers screened against the 9 DNA samples. Lanes are: Low molecular weight ladder, and A,B,D,E,1,2,3,4,5 for each of the three primers used.

The software used was NTSYS v. 2.2 (Sokal). This software is used by the U.S.D.A. as well as by Professor Christopher Campbell of the University of Maine, Orono. The raw data of presence/absence is processed through a series of programs designed to output a tree of relatedness or dendrogram. This tree basically gives coefficients of similarity or by one step further in mathematical analysis and genetic distance measure between any two clones (or among the entire group), in this case nine.

The following tree chart or dendrogram is the direct output from NTSYS in the TREE module. This output was generated from 9 DNA extractions designated A, B, D, E, 1, 2, 3, 4, 5. What was provided as input into NTSYS was 9 extractions and a total of 79 polymorphic bands, either present or absent. Specifically, the SIMQUAL module was used, followed by the SAHN module using the DICE parameter (a frequently used method of calculating the matrix) followed by the TREE module which generates this actual tree.

The results so far, with 9 clones show thusly.



It can be seen that there are two groups relating overall at a similarity of approximately 21% (x-axis). From there I see two upper groups consisting of A and D, and E, B and 1,4,3,5, and 2 clustering. The highest order or similarity was between clones B and E at 69% similarity. GIS information on the exact location of the clones and their physical distance are also known but not shown. Basically, at this point, the lettered clones are from the upper fields at the Blueberry Hill farm with the numbered clones from the lower. It is too early to make definitive conclusions about the biology or evolutionary process that may be occurring but it is clear from this summer's advance that the EST primers are robust and polymorphic and do result in strong, repeatable data that can discern genetic relationships in this system. To date, this has not been possible in the lowbush blueberry.

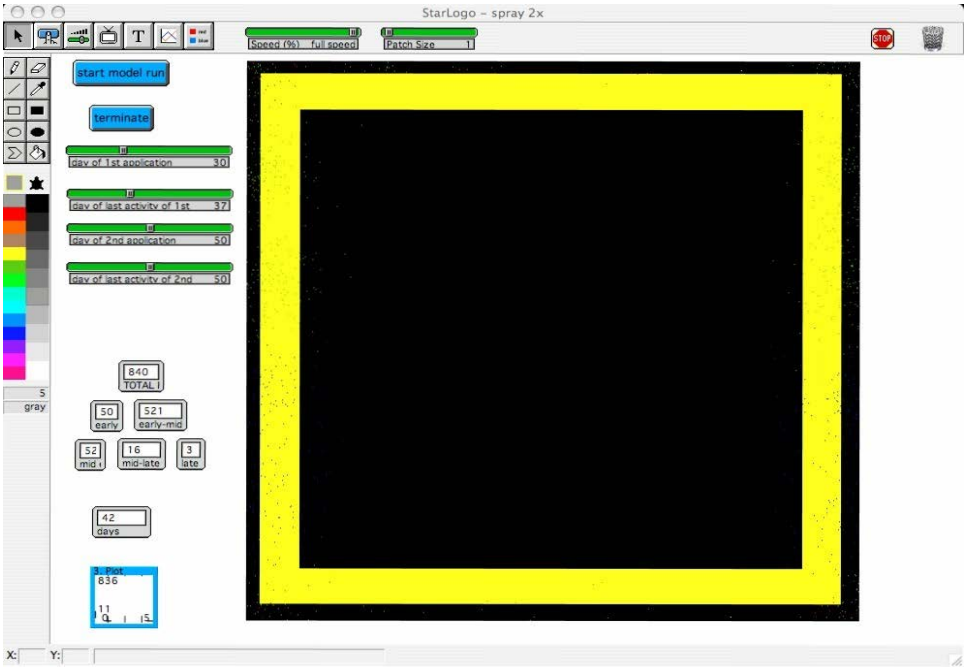
It is planned to continue extractions from the remaining population and perhaps add more as they related to compatibility studies that are ongoing. It may be that relatedness factors into compatibilities among clones and thus would be an important next step in managing this wild system in Maine.

12. A model of blueberry maggot fly colonization and strategies for insecticidal control

METHODS: I have been studying blueberry maggot fly movement in blueberry fields for several years. Since 1998 I have been marking flies with fluorescent powders and releasing them in blueberry fields. Recapture of these marked released flies has allowed the estimation of daily movement distances and directions. In addition I have deployed BMF traps along linear transects into blueberry fields from within the field forest edge. From these data I have developed a model for blueberry maggot fly colonization. This model is best described as a two-dimensional random walk model with a mean step of 10 m / day (distributed as a Poisson distribution). During the summer of 2006 I incorporated this model into a spatially explicit computer simulation model using the software platform Starlogo®. The BMF adults emerge from the soil over a period of a month and enter the blueberry field to mate and lay eggs in the fruit. I also incorporated an insecticide submodel that applies insecticide as a 33

m wide perimeter swath. The model can be set up to make two applications on any day during the growing season. In addition, the environmental half-life of the insecticide can be parameterized. The model window is illustrated in figure 1 below.

Fig. 1. User-friendly interface of the BMF colonization model. The black square is the simulated blueberry field and the yellow perimeter is the area of the perimeter treatment. The sliders (green bars) on the left are controllers for controlling the insecticide applications (timing and frequency).



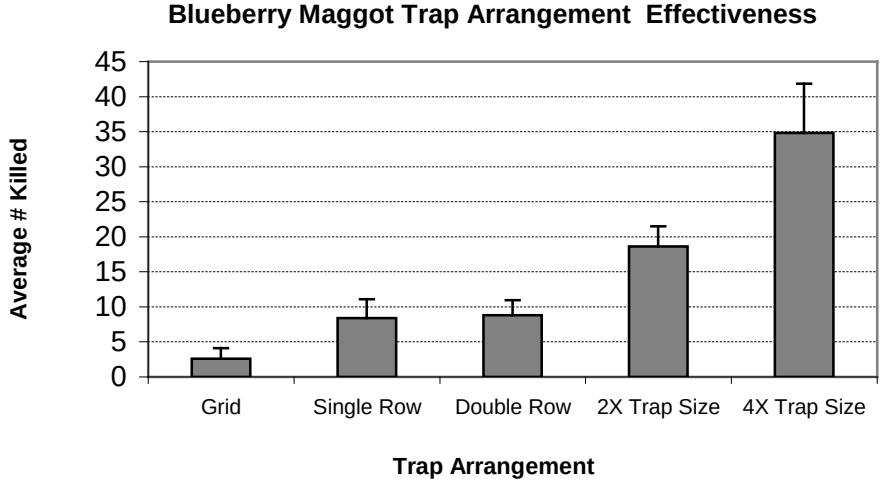
RESULTS: The results that I will present in this report are quite preliminary since I have only developed the initial prototype model. A more detailed model development and analysis will follow in the future.

Initial simulation investigations were as follows:1) potential for trapping out BMF as they colonize a field and 2) efficacy of a "Reduced Risk" insecticide used as a perimeter treatment compared to a conventional standard insecticide such as Imidan®.

The first study consisted of deployment of BMF yellow sticky traps in a blueberry field. A constant number of traps were used in each treatment condition. The treatments were: 1) around the perimeter of a blueberry field in a single row, 2) around the perimeter of a field in a double row, 3) a grid pattern of deployment throughout the entire field, 4) around the perimeter of the field in a single row but with double the trap attractiveness, and 5) around the perimeter of the field in a single row but with quadruple the trap attractiveness. In all simulation runs the field was a 10 acre field and the traps numbered 100. The number of flies colonizing a field were 100 females in total. The simulation was run for 30 days. The results are shown in Fig. 2. In general, it can be seen that trapping out flies is probably not going to be a viable strategy. Deployment of 100 traps in a grid throughout the field results in only 2.6 $\pm$ 1.5 (mean $\pm$ SD) % mortality. A single and double row perimeter deployment

of traps increases trap capture or mortality but only up to levels of 8-9%. Large increases in mortality or trap capture are only realized when the attractiveness of traps are doubled or quadrupled…in this case doubling and quadrupling the mortality. This suggests that chemical attractants would be a useful research direction to enhance the use of traps as a control tactic. However, it also suggests that current traps at low densities are only useful as monitoring tools for estimating the abundance of flies, but not for control.

Fig. 2. The results of five simulation runs for each BMF trap deployment treatment.



The second study involved comparing Imidan® perimeter sprays with SpinTor® perimeter sprays. Imidan was modeled with a 7 day residual activity and SpinTor was modeled with a 2 day residual activity. The first series or simulation runs represents a single application of either insecticide in a perimeter swath at various times in the season where day 1 is the first day of colonization (note the population of flies was 1000 and the emergence period was 30 days or 1 month). Fig. 3 shows the results of the simulation runs with Imidan. It can be seen that a single spray yield maximum kill half way through the emergence period (day 15). However, the best control is about 60%. This seemingly low level of control is because with a long period of emergence many flies are not exposed to the 7 day residual. Fig. 4 shows the results of a single SpinTor application. SpinTor is much less effective, resulting in only 38% maximum control and the best timing is delayed to day 19 which would be toward the end of July in a blueberry field in Maine. A scenario such as this would inevitably result in considerable maggot infestation (I have not modeled maggot infestation at this point, only adult survival and movement).

Fig. 3. The number of flies killed (out of 1000) with Imidan for the days 7-14, 11 - 18, 15 – 22, 19 – 26, and 30-37 (days that insecticide activity was sufficient to kill flies).

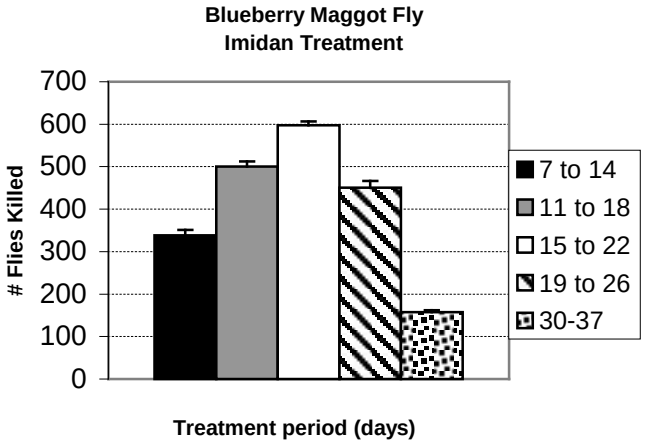
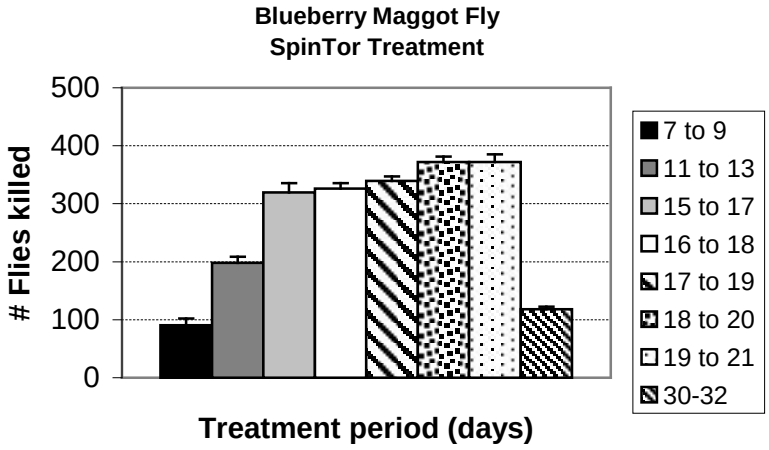


Fig. 4. The number of flies killed (out of 1000) with SpinTor for the days 7-9, 11 – 13, 15 – 17, 18 - 20, and 19 – 21, and 30 - 32 (days that insecticide activity was sufficient to kill flies).



When two perimeter applications were made with Imidan control was highest when at least one of the applications occurred during peak emergence and colonization into the field (Fig. 5). The highest mortality, 75%, occurred when applications were made at 12 and 24 days after the onset of emergence or colonization. Two perimeter applications of SpinTor did not result in mortality greater than 50% and the best control occurred with applications at 12 and 20 days, 16 and 20 days, 12 and 24 days, and 16 and 24 days after emergence (Fig. 6).

Fig. 5. The number of flies killed (out of 1000) with Imidan after two applications, colored bars represent first day of application and x-axis is the second day of application

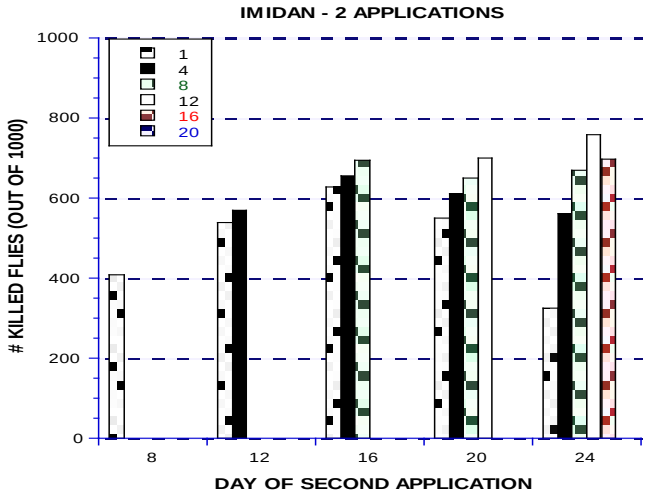
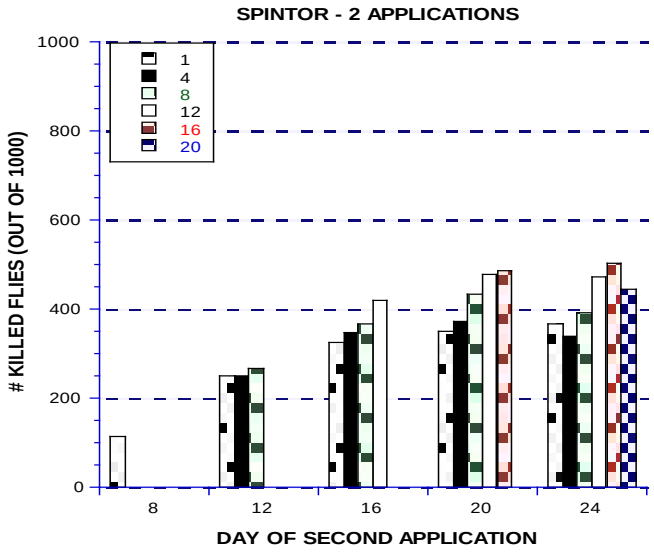


Fig. 6. The number of flies killed (out of 1000) with SpinTor after two applications, colored bars represent first day of application and x-axis is the second day of application



CONCLUSIONS: The development of a blueberry maggot fly movement model is not meant to offer predictions as to when or where BMF outbreaks will occur or provide tailored control strategies for specific blueberry fields. The intent of the model is to simulate the ecology of the BMF and thus provide a tool for evaluation of various novel control tactics that can be explored in the laboratory before taking them to the field. I believe that this simulation tool will become more useful as new insecticides or control tactics are developed for replacing our standard control tactics such as whole field Imidan applications.

RECOMMENDATIONS: The purpose of the basic biology studies is to increase our understanding of the behavior and ecology of pest and beneficial insects in blueberry fields. The ultimate goal is that an increased understanding will result in better pest management and pollination strategies. At this point we are still in the early phase of many of our studies and so there are few recommendations that have resulted.

The recommendation that has come out of the basic biology studies is a verification of the bumble bee stocking density. In the mid 1990s Dr. Constance Stubbs and I conducted research suggesting that a stocking density of 3/4 - 1 quad (4 colonies) / acre was sufficient for adequate pollination of lowbush blueberry. Since that time there has been some inconsistencies that have arisen with bumble bee pollination and so in 2005 and 2006 I conducted large scale field trials to compare honeybees stocked at the rate of 4 colonies / acre with bumble bees at the standard stocking rate. We found in both years that the bumble bee stocked fields had pollination levels that were not different from honeybee stocked fields. However, neither of these years was characterized by “good” pollination weather. Therefore, I recommend no change in the bumble bee stocking rates unless growers report dissatisfaction with bumble bee pollination in the future. In 2006, it was observed that there was a large difference in bumble bee foraging activity and pollination between the bumble bee fields. This might suggest that bumble bee colony quality could be an issue; although, I have not directly assessed this. Bumble bee colony quality could be an issue to investigate in the future.

INVESTIGATOR: S.L. Annis, Biological Sciences
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TITLE: Research on Wild Blueberry Diseases for 2006-2007

METHODS:

1) Developing a forecasting method for application of fungicides for control of mummy berry.

In the fall of 2005, mummies were collected from 2005 crop fields and from fields that would bear a crop in 2006. "Mummy grids" were constructed using "egg-crate" lighting panels with ½ inch squares which were open on both sides. Wire window screen was glued to the bottom of the grids. In December of 2005, in four different blueberry fields, 30-50 mummies were placed in each grid, and the grid was laid on ground that had been cleared of stems, and to which a ½ -1" layer of leaf litter was added, to allow more moisture to contact the mummies through the screen bottom. Another piece of screen was placed on top of the grid, and the whole grid was covered with more leaf litter to preserve moisture levels. In April of 2006, the upper leaf litter mulch was removed and the mummies were monitored for germination. The grids were removed from the fields in June. Data from nearby weather stations was to be used to correlate amount of chilling the mummies received with the timing of their germination.

2) The identification of fungi causing a leaf and stem blight in wild blueberry.

Eight fungi of known or suspected pathogenicity, which had been isolated from diseased stems and leaves in 2005, were cultured on malt yeast agar medium to produce spores. Pathogenicity of the fungi was tested on 15 blueberry stems per fungus (3 stems each from 5 different clones). Crop year stems were collected on March 7, 2006 at Blueberry Hill Farm in Jonesboro, ME. The stems were stuck into blocks of florist foam, fed with solutions of 1/16 Hoaglands solution + 10% Sprite® soda, and kept in a greenhouse until leaves sprouted and were large enough to be inoculated (2-5mm). Other experiments used prune-year stems sprouted in foil-wrapped test tubes in a 25°C incubator with a 9 hour photo-period to mimic spring conditions.

Inoculations were performed by spraying stems and leaves with spore solutions of specific concentrations and covering inoculated stems with plastic tents for 3 days to maintain high humidity. Disease symptoms and incidence were recorded for all treatments and compared to controls. Five to six weeks after inoculation, half the stems were placed in humidity chambers to produce fruiting structures and half were cultured on a potato dextrose agar medium. The resulting fruiting structures were identified and compared to the original inoculated fungi.

3) Possible organic controls of *Monilinia* blight in lowbush blueberries.

Two fields near Jonesboro, ME, were used to test the efficacy of organic treatments and cultural methods for mummy berry blight control. Seven treatments and a control were replicated in four blocks in each field. Treatment plots were 2m x 20m. Treatments included *Bacillus subtilis* (Serenade, AgraQuest), *Bacillus pumilis* (Sonata, AgraQuest), aerated compost tea, a garlic and yucca adjuvant (Biolink, Westbridge), neem oil (Trilogy, CertisUSA), water, and a peat moss mulch. The compost tea was produced using 272 g Coast of Maine Lobster Compost in 7570 ml distilled water and incubated with aeration for 2-4 days and then applied undiluted at a rate of 10.7 gal/acre (100 L/ha). The *Bacillus subtilis*, *Bacillus pumilis* and Neem oil were diluted to 4.29% in water and applied at a rate of 1 gal/acre. In addition, the bacterial and compost sprays were mixed with 2.15% garlic adjuvant (at a rate of 0.5 gal/acre). The garlic adjuvant was applied alone at the same rate as a control (2.15% at 0.5 gal/acre). The mulched plots were spread with 3 cm peat moss on April 24-25. Treatments were applied every 3 to 4 days. All treatments were sprayed with a CO_2 backpack sprayer at 20gpa with 80002VS Tjet nozzles during the period of leaf development (mid April to mid May) when blueberry is susceptible to infection by *Monilinia vaccinii-corymbosi.*

On June 2, each plot was assessed for mummy berry blight by determining the average proportion of infected stems in four random 16"x18" subsections per plot. In August, blueberries were harvested by hand-raking a 45 cm strip down the center of each plot and weighing total harvested berries. Equipment and personnel effects were minimized by having one person rake all treatments in a block.

4) Evaluation of new fungicides for control of mummy berry and effect on yield.

In the spring of 2006, 48-6'x20' plots were established in two crop-year fields. Six replications of 8 treatments were applied in a replicated block design: 1) control; 2) Orbit (6 oz/acre); 3) Orbit (6 oz/acre in 10 gal water); 4) Enable (6 oz/acre); 5) Enable (6 oz/acre with 1% crop oil concentrate (COC) as a surfactant); 6) Pristine (18.5 oz/acre); 7) Serenade (3lbs/acre); 8) Serenade (3 lbs/acre with surfactant). These were applied with a CO_2 backpack sprayer at a dilution rate of 20 gallons/acre (except treatment #3), with 80002VS Tjet nozzles on May 5 and May 15. Five replicates, instead of 6, of treatment 2 (Orbit, 20 gal) and treatment 5 (Enable + 1%COC) were sampled due to a labeling error. The proportions of stems with *Monilinia* blight and *Botrytis* infection were assessed in 4 random 6"x18" subsections of each plot on May 24. On July 24-25, yield was estimated by harvesting a 45cm rake-width down the center of each plot and weighing the berries.

RESULTS:

1) Developing a forecasting method for application of fungicides to small fields for control of mummy berry.

None of the mummies used in this experiment germinated, and most were found to be quite dry when checked in April, despite the attempt at maintaining ground contact and covering with leaf litter mulch. In addition, the weather station data that was accessible for determining chill hours proved inadequate since only one temperature reading was taken

every 24 hours. This experiment will be repeated with modifications to ensure direct ground contact by the mummies and taking hourly temperature readings.

2) The impact of leaf and stem blight on wild blueberry fields.

The level of disease incidence and success at re-isolating inoculated fungi are varied (Table 1). *Gloeosporium* and *Pestalotia* caused more necrotic tissue to leaves than the control treatments and were reisolated from after inoculations. *Phomopsis* was only used in one set of inoculations late in the year, but was isolated as a latent infection from many crop-year stems. These three fungi have been chosen for further study to identify them to species and determine control measures. The other fungi, *Acremonium, Cladosporium, Gliocladium* and *Bactrodesmium* did not consistently produce lesions on leaves or stems of inoculated blueberry plants, and are unlikely to be pathogens of blueberry.

3) Possible organic controls of *Monilinia* blight in lowbush blueberries.

The two fields used for this experiment showed remarkably different timing in leaf bud development. Field 1 was past the susceptible stage by April 30. There was significantly less incidence of mummy berry blight (90% confidence level) for the mulch treatment compared to the water treatment at Field 2 (Figure 1). Field 1 was probably mulched too late to show disease suppression, nevertheless the plants had an improved appearance relative to the control. For both fields, there was significantly less incidence of disease in plots treated with *Bacillus subtilis* (70% confidence level) compared to the water and no-spray controls. There was significantly less disease incidence for the compost tea treatment (75% confidence level) relative to the no-spray control at Field 1 only. The *Bacillus pumilus* treatment in Field 1 was the only treatment to have significantly higher yield than the control plants in each field (Figure 2).

4) Evaluation of new fungicides for control of mummy berry and effect on yield.

Monilinia blight incidence was significantly lower than the control only for the Enable and Enable + 1%COC treatments in the Township 19 field (Figure 3). There was no significant difference in *Monilinia* blight incidence between the control and any of the treatments in the Deblois field (Figure 4). There were extremely low levels of *Botrytis* blight in both fields so no differences were found among any of the treatments (Figure 5).

There was no significant difference in yield between the treatments and the control in the Township 19 field (Figure 6). Yield was significantly higher for both Orbit treatments and the Pristine treatment compared to the control in the Deblois field (Figure 6).

CONCLUSIONS: Mummy berry disease is a significant problem to the blueberry industry and evaluating new fungicides to control this disease must remain a priority. Some conventional and organic fungicide treatments should be re-evaluated next year to determine their effectiveness to control mummy berry disease since two years of data with different weather conditions will confirm efficacy or not of the treatments. The development and implementation of a forecasting system in Maine to determine the risk of infection by mummy berry blight is a priority for improving control of this fungus. Three of the fungi isolated from diseased leaves and stems in prune fields in 2005 are being further evaluated to

determine their identifications and which fungicides will be the best to try field trials for control.

RECOMMENDATIONS: Re-evaluate fungicides and organic methods for control of mummy berry disease. No organic method for control of mummy berry blight can be recommended at this time. Continue developing a forecasting system for risk of mummy berry blight. Determine the causal agent of stem blights observed in the prune year.

Table 1. Inoculations of blueberry stems with potential pathogenic fungi

Inoculated fungus	**Type of stem**	**Leaf symptoms (different from control)**	**Inoculated fungus re-isolated?**
Acremonium	Crop	Y	N
"	Crop	Y	N
"	Prune	--	N
"	Prune	N after 6 days	N
Gloeosporium	Crop	Y	**Y**
"	Crop	Y	**Y**
"	Crop	Y	N
"	Prune	--	N
"	Prune	N after 6 days	**Y**
Pestalotia	Crop	Y	**Y**
"	Crop	N	N
"	Prune	--	**Y**
Cladosporium	Crop	N	N
"	Crop	Y	N
Gliocladium	Crop	Y	N
"	Crop	Y	N
"	Prune	N after 6 days	Y
Bactrodesmium	Crop	N	N
Botrytis	Crop	N	N
Phomopsis	Prune	Y after 6 days	N

--some prune-year stems dried before leaf symptoms appeared

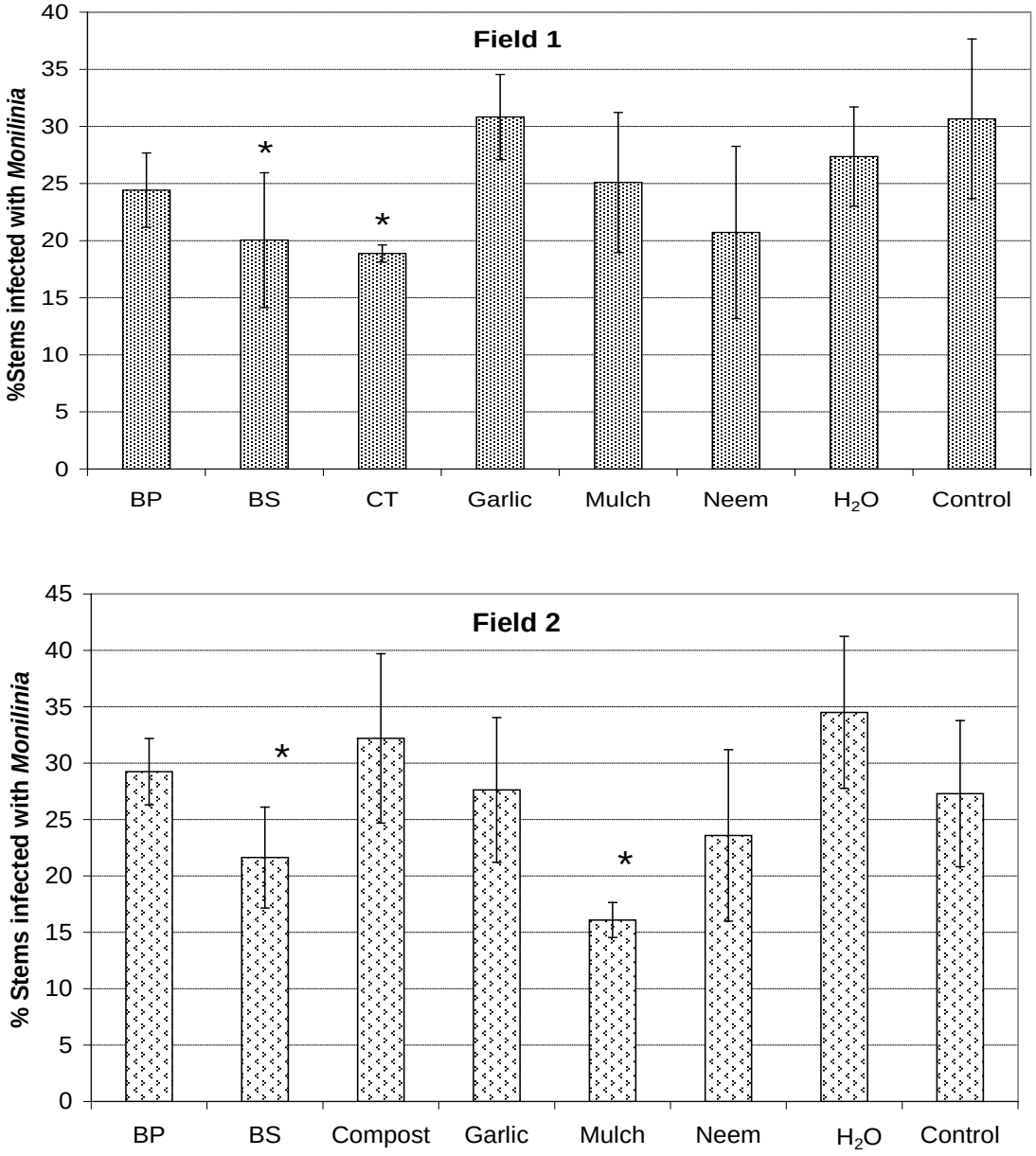
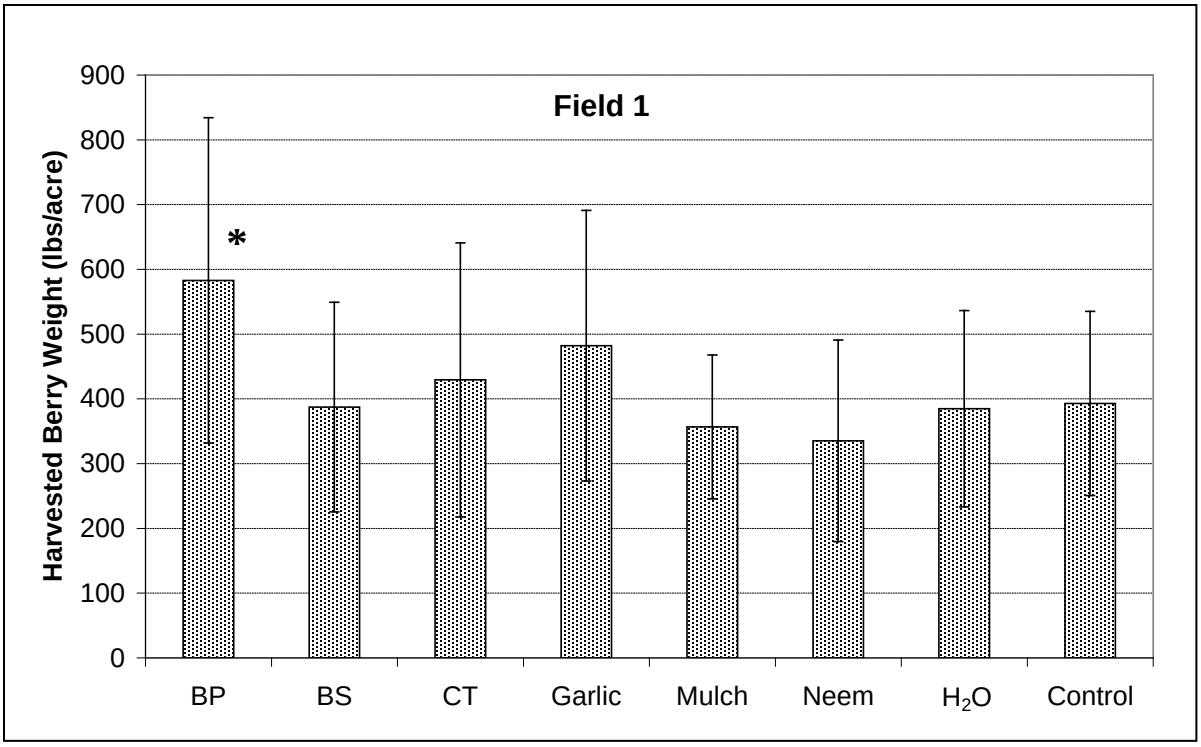


Fig 1. Effect of organic controls on mummy berry blight in two fields in 2006.
BP=*Bacillus pumilus*, BS =*Bacillus subtilis*, Compost =compost tea, Garlic=garlic adjuvant, Mulch=3 cm peat moss, Neem=Neem Oil, H_2O=water, Control=no treatment.
* Asterisk indicates significant difference from control at a >70% confidence level. Bars indicate standard error from the mean.



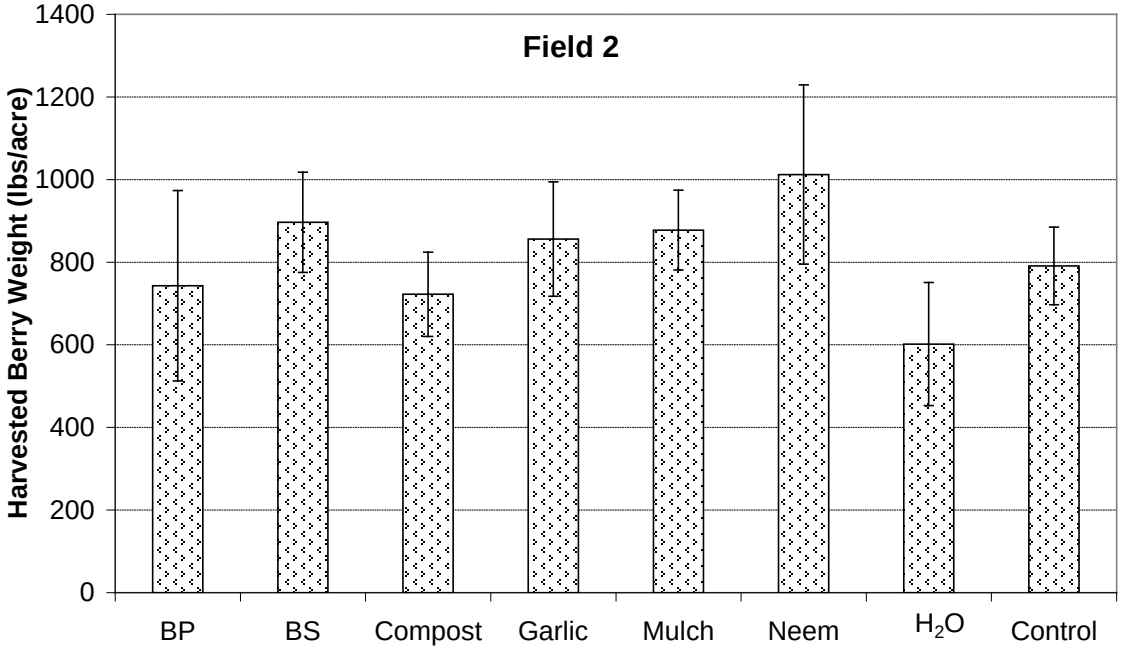


Fig. 2. Effect of organic fungal controls on berry yield in two fields in 2006. BP=*Bacillus pumilus*, BS =*Bacillus subtilis*, Compost =compost tea, Garlic=garlic adjuvant, Mulch=3 cm peat moss, Neem=Neem Oil, H_2O=water, Control=no treatment. * Asterisk indicates significant difference from control at a >70% confidence level. Bars indicate standard error from the mean.

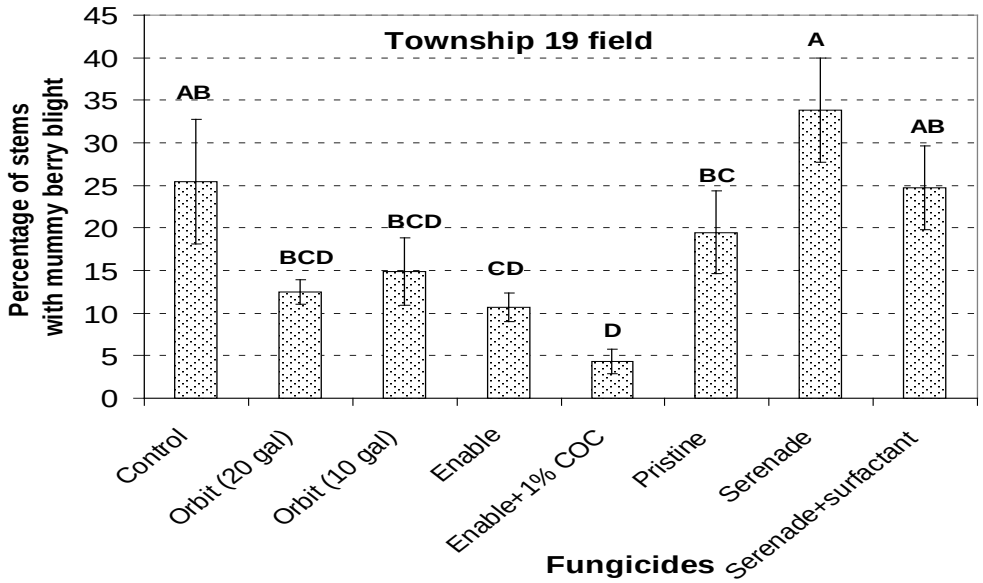


Fig 3. Control of mummy berry blight by fungicides in Township 19 field in 2006. Bars represent standard error of the mean. Treatments that are significantly different at p=0.05 are labeled with different letters.

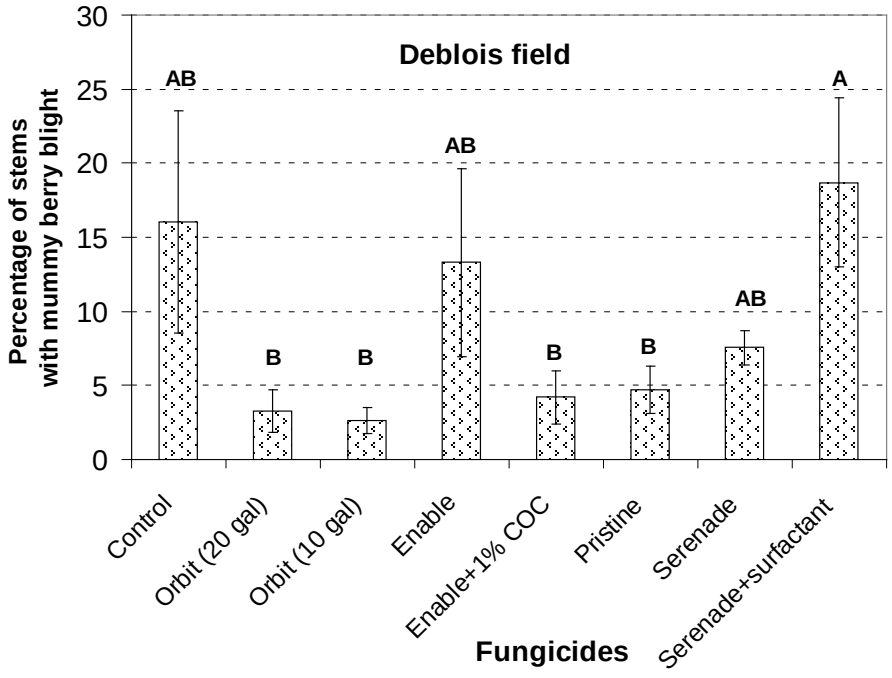


Fig 4. Control of mummy berry blight by fungicides in Deblois field in 2006. Bars represent standard error of the mean. Treatments that are significantly different at p=0.05 are labeled with different letters.

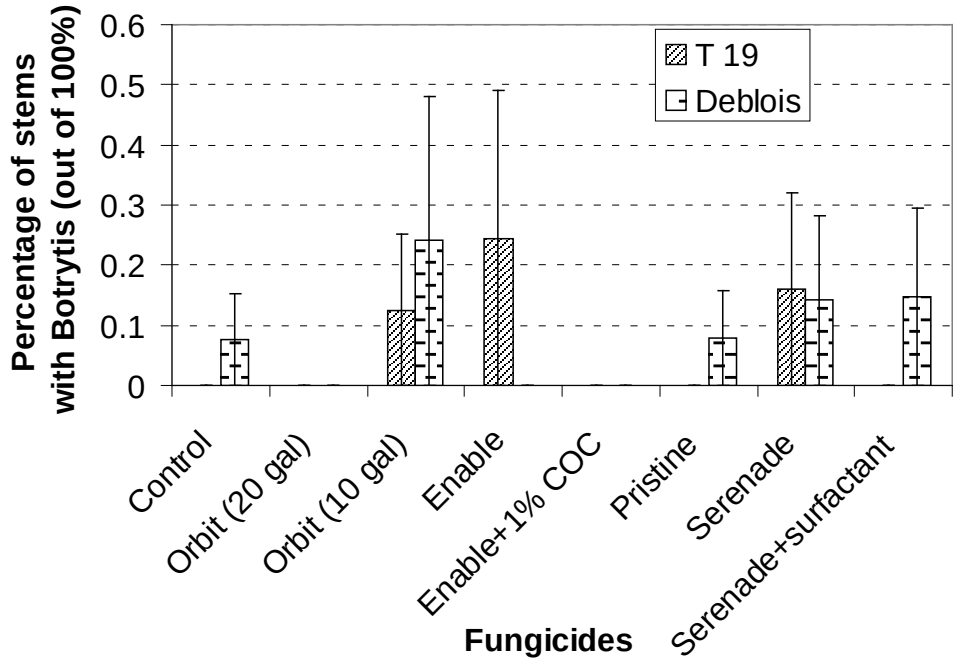
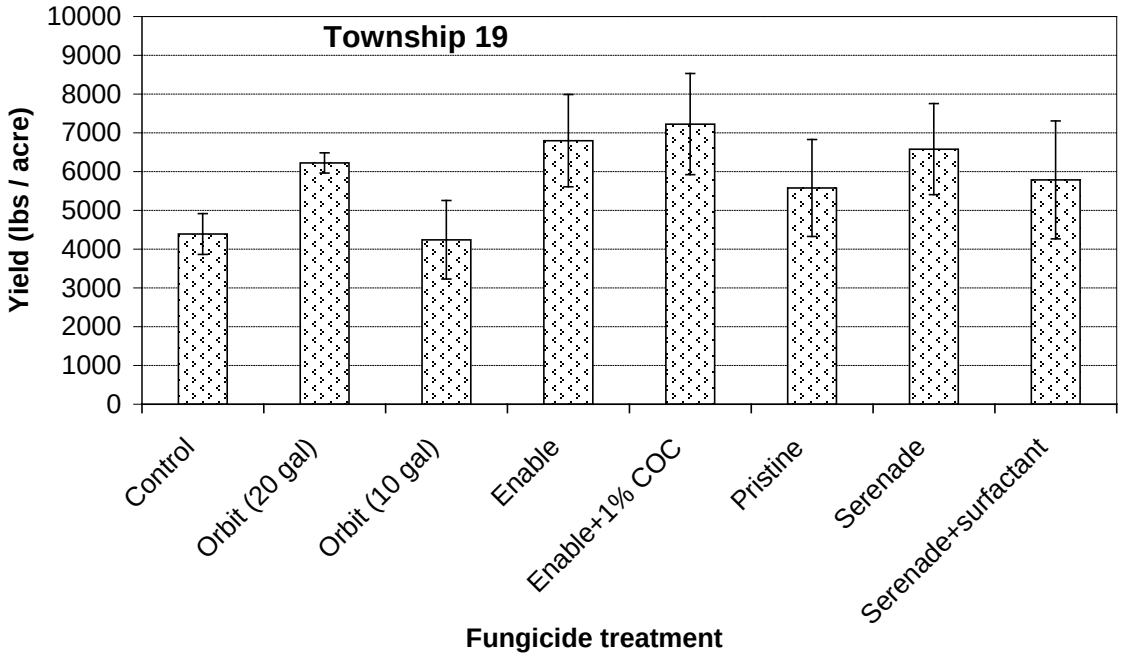


Fig 5. *Botrytis* blight in two fields, Township 19 and Deblois, 2006. Fungicide treatments were applied for control of mummy berry blight. Bars indicate standard error of the mean.



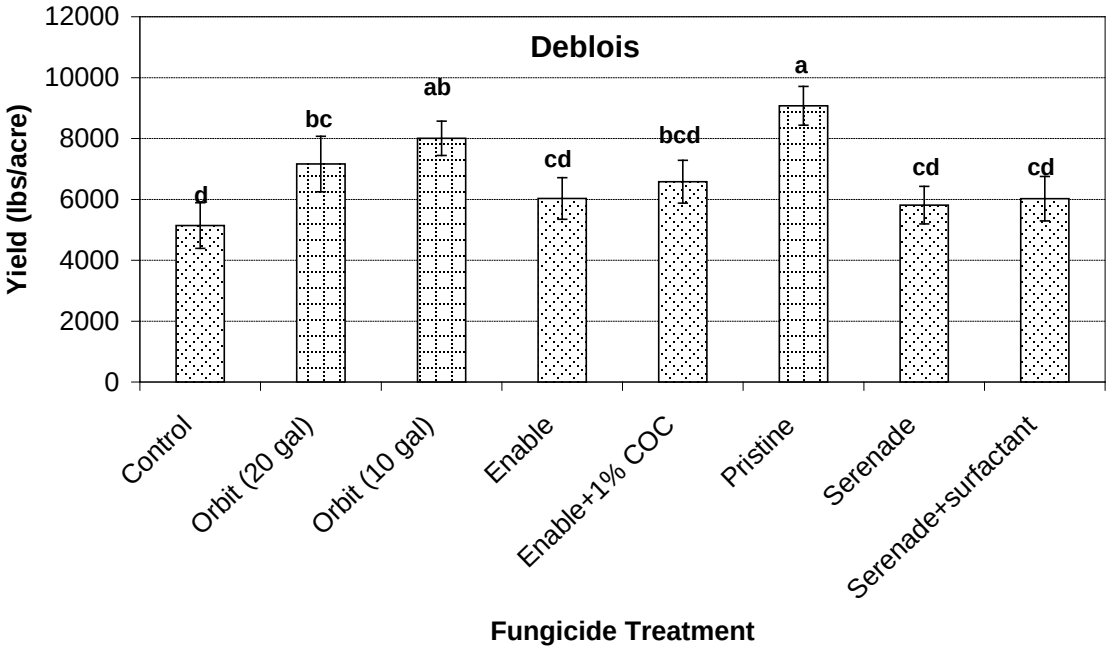


Fig 6. Effect of fungicide application on yield in Township 19 and Deblois fields in 2006. Bars indicate standard error of the mean. No significant difference among treatments at p=0.05.

PLANT NUTRITION

INVESTIGATORS: John M. Smagula, Professor of Horticulture
Loretta Kreider, Scientific Technician

TITLE: Effect of Soil pH on Nutrient Uptake.

OBJECTIVES: To determine the effect of soil pH adjustment on nutrient uptake, available soil nutrients, plant growth and yield.

Brief Justification: Many growers have soil pH values at the high end of the recommended pH range for growing wild blueberries yet they are recording high yields. They are reluctant to adjust their soil pH for fear of reducing yields. Yet, soil pH also has an effect on weed growth and lowering soil pH is recommended as a means of reducing weed pressure. These studies will provide data to support current recommendations for lowering soil pH to 4.6 or result in a reevaluation of these soil test recommendations.

pH Study - Blueberry Hill Farm

METHODS: Four clones were selected at Blueberry Hill Experiment Station Farm in Jonesboro. In each clone, eight 4 ft x 4 ft sections (plots) were identified for establishing four replications of two treatments. The perimeter of each plot was cut down to 6 inches to sever the rhizomes and isolate each plot. In August 1999, the plots were hand raked and the berry weight was not significantly different among potential treatment plots within each clone. Soil samples taken November 1999 from each clone indicated two had a pH of 4.5, one had 4.7 and one had a pH of 4.9. Since one ton of ground limestone will raise pH about 0.2 , treatment plots received an appropriate amount of limestone in May 2000 to adjust the soil pH to about 5.3 (Table 1). Control plots received gypsum ($CaSO_4$) to provide Ca in the amount that the limestone contributed**.**

Table 1
Treatment Summary

Clone	Treatment Number	Starting pH	Limestone $CaCO_3$ (lb/acre)	Gypsum $CaSO_4$ (lb/acre)
1	**1**	**4.7**	**0**	**6,693**
1	**2**	**4.7**	**7,000**	**0**
2	**1**	**4.9**	**0**	**4,784**
2	**2**	**4.9**	**5,000**	**0**
3	**1**	**4.5**	**0**	**8,608**
3	**2**	**4.5**	**9,000**	**0**
4	**1**	**4.5**	**0**	**8,608**
4	**2**	**4.5**	**9,000**	**0**

In this way, paired plots with the same plant material will have substantially different soil

pH. Plant and soil nutrients will be monitored by leaf tissue and soil analysis. Soil pH and leaf nutrient concentrations will be related to yield during the crop year. Within each treatment plots stems within randomly placed 1/6 ft^2 quadrats will be cut for stem density (stems/ft^2) and stem length, branching, and flower bud formation measurements.

RESULTS:

2001 Leaf Tissue Analysis

Treatment with limestone had an effect on a number of nutrient elements in leaf tissue samples taken July 2001 (Table 2**).** The leaf tissue concentrations of Ca, K, B, Cu , Zn and Mn were all lower in the plots receiving limestone ($CaCO_3$) compared to the control. Leaf Mg concentrations were raised by raising the soil pH. Control plot leaf Ca concentration was probably higher due to the greater solubility of $CaSO_4$ than $CaCO_3$.

Table2

Treatment	2001 leaf nutrient concentrations						
	Ca (%)	**K (%)**	**Mg (%)**	**B (ppm)**	**Cu (ppm)**	**Zn (ppm)**	**Mn (ppm)**
Control ($CaSO_4$)	**.721a**	**.481a**	**.208b**	**33a**	**4.2a**	**11.6a**	**1135a**
Limestone ($CaCO_3$)	**.676b**	**.451b**	**.256a**	**25b**	**4.0b**	**10.9b**	**629b**

2002 Leaf Tissue and Soil Analysis

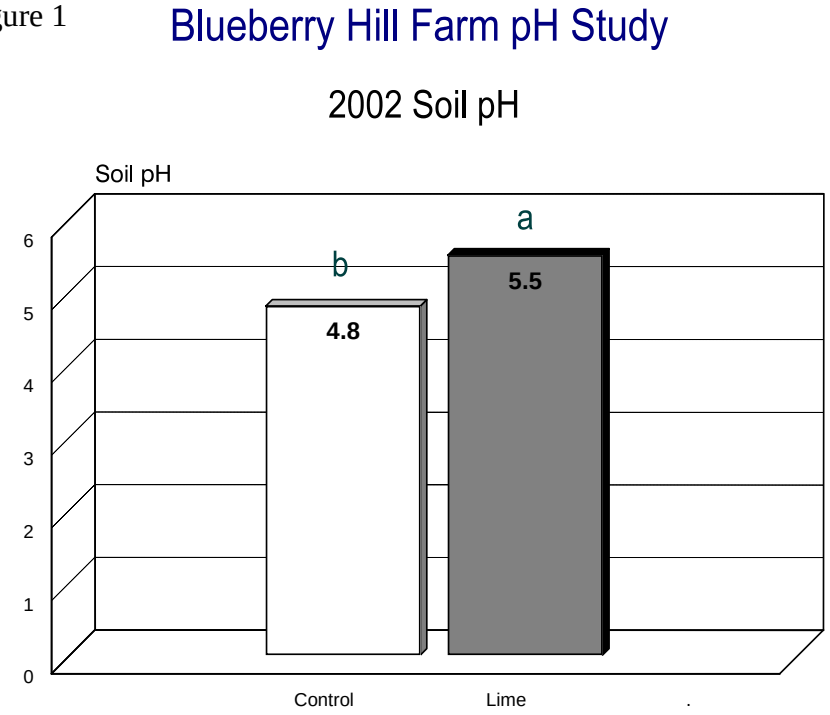
Crop year leaf samples (Table 3) showed different concentrations but similar trends to that found in 2001 prune year leaf samples. Leaf N, P, Zn, and Ca concentrations were not different between the control and limestone-treated plots, but leaf concentration of Mg increased and leaf K, B, Cu, Mn, Al, and Fe concentrations decreased in response to limestone application.

Table3

Treatment	2002 leaf nutrient concentrations						
	K (%)	**Mg (%)**	**B (ppm)**	**Cu (ppm)**	**Mn (ppm)**	**Al (ppm)**	**Fe (ppm)**
Control ($CaSO_4$)	**.398a**	**.150b**	**24a**	**4.42a**	**621a**	**80a**	**40a**
Limestone ($CaCO_3$)	**.380b**	**.168a**	**18b**	**4.19b**	**286b**	**71b**	**35b**

2002 Soil samples showed limestone treated plots had a higher pH than controls (Fig 1.).

Figure 1



Soil concentrations of Ca, Mg, B, Zn, and Mn were higher in the limestone-treated plots that had a higher pH compared to the control (Table 4). Liming resulted in a lower S soil concentration. Soil P, K, Cu, Fe, and Al were unaffected by the change in pH brought about by liming. Yield was not obtained in 2003 due to blossom damage and crop failure when a herbicide for grass control was applied to the field using the wrong oil adjuvant.

Table 4

Treatment	2002 soil nutrient concentrations							
	Ca (ppm)	**K (ppm)**	**Mg (ppm)**	**P (ppm)**	**B (ppm)**	**Cu (ppm)**	**Zn (ppm)**	**Mn (ppm)**
Control ($CaSO_4$)	**535b**	**51a**	**16b**	**7.2a**	**0.06b**	**0.13a**	**1.8b**	**4.68b**
Limestone ($CaCO_3$)	**1709a**	**54a**	**79a**	**6.9a**	**0.08a**	**0.10a**	**3.1a**	**6.83a**

2004 Leaf Tissue and Soil Analysis

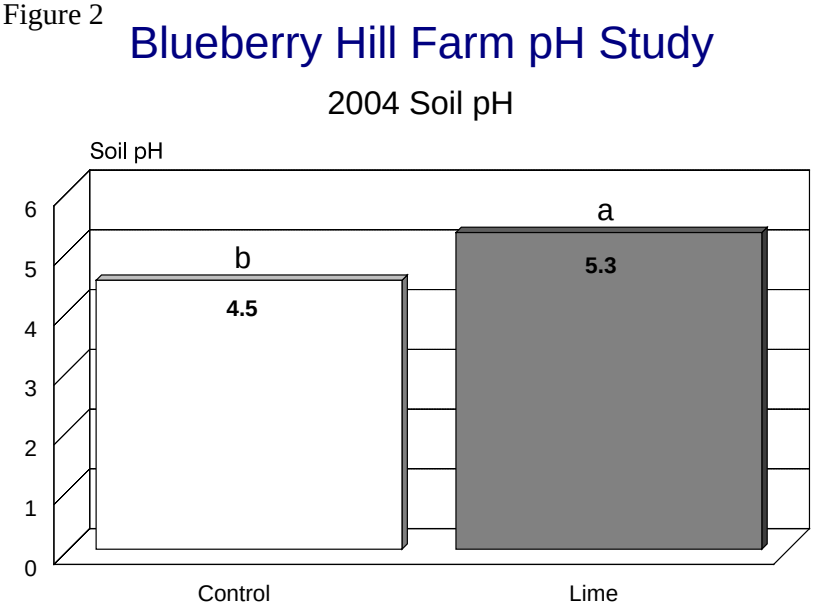
Prune year leaf samples (Table 5) showed different concentrations but trends did not reflect the data from 2002 (Table 3). Leaf N, Cu, Fe, Zn, concentrations were not different between control and limestone-treated plots, but leaf concentrations of Mg increased and leaf Ca, K, P, Mn, Al, and B concentrations decreased in response to limestone application.

Table 5

Treatment	2004 leaf nutrient concentrations						
	K (%)	Mg (%)	Ca (%)	P (%)	Mn (ppm)	Al (ppm)	B (ppm)
Control ($CaSO_4$)	.513a	.142b	.416a	.164a	561a	54a	23a
Limestone ($CaCO_3$)	.490b	.155a	.383b	.141b	210b	48b	17b

Soil samples taken in 2004 confirmed that limestone treated plots had a higher pH than controls. (Fig. 2)

Figure 2



Soil concentrations of Ca, Mg, B, Zn, and Mn were higher in the limestone-treated plots that had a higher pH compared to the control (Table 6). Soil K, P, Cu, Fe, and Al were unaffected by the change in pH brought about by liming.

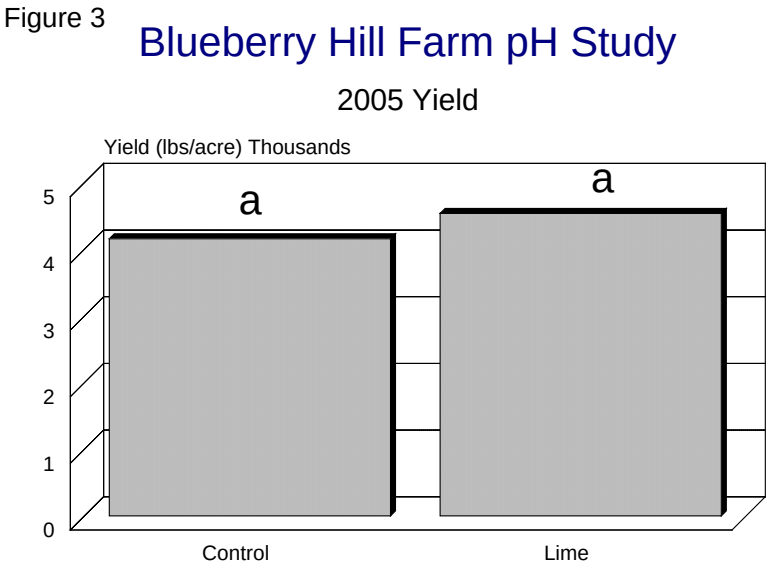
Table 6

Treatment	2004 soil nutrient concentrations							
	Ca (ppm)	K (ppm)	Mg (ppm)	P (ppm)	B (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
Control ($CaSO_4$)	511b	49a	30b	11.9a	0.15b	0.043a	1.6b	4.53b
Limestone ($CaCO_3$)	1578a	51a	86a	11.6a	0.22a	0.048a	2.3a	7.67a

2005 Blueberry Yield

The yield of fruit within the 4 ft x 4 ft plots was not significantly different between the control and the limestone treatment (Fig. 3).

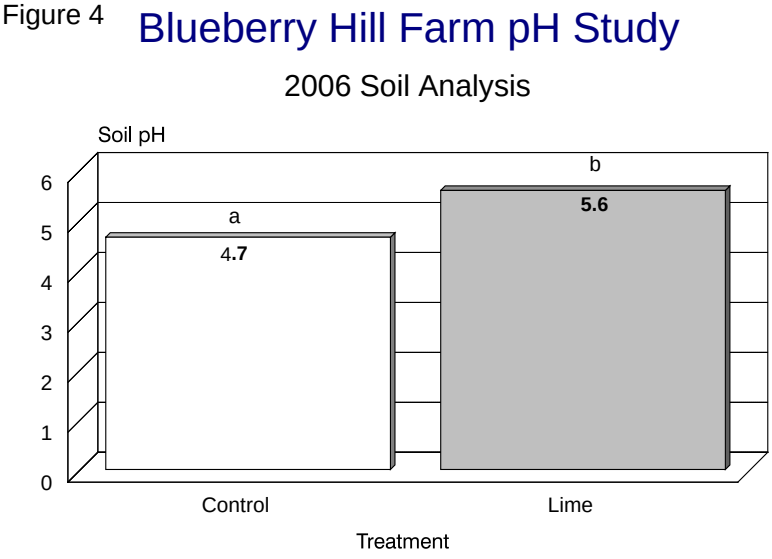
Figure 3



2006 Soil analysis

Soil pH was significantly higher in 2006 for treatment plots receiving lime (Fig. 4). Soil B analysis was not available in 2006. Soil concentrations of Ca and Mg were higher in the limestone-treated plots that had a higher pH compared to the control (Table 7). Soil Fe concentrations decreased by 10 ppm with liming, as did S which was 11.2 ppm in limed plots, compared to 18.6 ppm in control plots. Soil K, P, Zn, and Mn were unaffected by the change in pH brought about by liming.

Figure 4



Means with same letter are not significantly different at the 5% level.

Table 7
2004 soil nutrient concentrations

Treatment	Ca (ppm)	Fe (ppm)	K (ppm)	Mg (ppm)	P (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
Control ($CaSO_4$)	**380b**	**23a**	**41a**	**27b**	**6.4a**	**0.111a**	**1.6a**	**3.7a**
Limestone ($CaCO_3$)	**1301a**	**13b**	**42a**	**74a**	**7.6a**	**0.077b**	**1.3a**	**3.9a**

2006 Leaf Tissue analysis

Leaf Mg concentrations were increased by liming but leaf N, P, K, Ca, Cu Fe and Zn were unchanged (Table 8). Liming resulted in decreases of leaf Mn, B, and Al concentrations, presumably by increasing soil pH.

Table 8
2006 leaf nutrient concentrations

Treatment	K (%)	Mg (%)	Ca (%)	P (%)	Mn (ppm)	Al (ppm)	B (ppm)
Control ($CaSO_4$)	**.481a**	**.163b**	**.504a**	**.115a**	**498a**	**101a**	**23a**
Limestone ($CaCO_3$)	**.476a**	**.174a**	**.484a**	**.115a**	**154b**	**87b**	**19b**

pH Study - Aurora

METHODS: Five discrete clones were selected in a commercial blueberry field in Aurora. Two 4 ft x 4 ft treatment plots were established in each clone and the perimeter of each was cut with a spade to isolate each plot. Soil samples indicated that the soil pH under these clones ranged from 5.1 to 5.5 (Table 5). Yield was collected prior to sulfur treatment in August 2000 from each treatment plot within each clone and no difference was found between those randomly assigned treatment 1 (9,303 lbs/acre) or those assigned treatment 2 (9, 375 lbs/acre). Sulfur (S) was applied in June 2001 to plots assigned treatment 2 to adjust the soil pH down toward pH 4.6. This required from 550 to 990 lb S/acre, depending upon the pH under the specific clone (Table 5)**.** Soil and leaf samples were collected in July 2001 to establish base line data to compare changes as the soil pH changes. Stem samples were taken from each plot in October 2001 from a randomly placed 1/6 ft^2 quadrat for stem density, stem length and branching and flower bud formation measurements. Soil samples were taken July 22, 2002 to determine the effect on soil pH. Yield was collected August 7, 2002. The nutrient concentrations in leaf and soil samples collected each prune year will document changes during the extent of the experiment. Measurements made on stem samples collected in the fall of each prune year will indicate changes in growth and development. Yield will be collected each crop year.

Table 5 Treatment Summary			
Clone	**Treatment Number**	**Starting pH**	**Sulfur lb/acre**
1	**1**	**5.3**	**0**
1	**2**	**5.3**	**770**
2	**1**	**5.2**	**0**
2	**2**	**5.2**	**660**
3	**1**	**5.5**	**0**
3	**2**	**5.5**	**990**
4	**1**	**5.4**	**0**
4	**2**	**5.4**	**880**
5	**1**	**5.1**	**0**
5	**2**	**5.1**	**550**

RESULTS:

2001 Prune Year Leaf Tissue and Soil Analysis

Soil samples taken in July 2001 indicated that control and sulfur-treated plots had similar soil pH values of 5.18 and 5.16, respectively. Leaf nutrient concentrations were not significantly different between control and sulfur-treated treatment plots for all nutrients, except manganese (Mn). Leaf nutrients that might be expected to change with soil pH are given in Table 6.

Table 6

Treatment	2001 leaf nutrient concentrations						
	Ca (%)	**K (%)**	**Mg (%)**	**B (ppm)**	**Cu (ppm)**	**Zn (ppm)**	**Mn (ppm)**
Control	**.400a**	**.493a**	**.176a**	**28a**	**5.0a**	**15.0a**	**450b**
Sulfur (S)	**.412a**	**.471a**	**.174a**	**26a**	**5.2a**	**15.1a**	**580a**

Soil nutrient concentrations for control and sulfur-treated plots were not different for Ca, K, Mg, P, Al, B, Cu, Fe, Zn or Mn. The concentrations of most elements are presented in Table 7**.**

Table 7

Treatment	2001 soil nutrient concentrations							
	Ca (ppm)	**K (ppm)**	**Mg (ppm)**	**P (ppm)**	**B (ppm)**	**Cu (ppm)**	**Zn (ppm)**	**Mn (ppm)**
Control	**437a**	**96a**	**62a**	**9.4a**	**.17a**	**.11a**	**1.8a**	**12.4a**
Sulfur (S)	**524a**	**106a**	**77a**	**9.4a**	**.17a**	**.13a**	**2.1a**	**16.6a**

2001 Stem Characteristics

Stem density was higher in sulfur-treated plots, but stem length, and flower buds per stem were not affected by treatments (Table 8).

Table 8

2001 Stem Characteristics

Treatment	Density (Stems/ft^2)	Stem Length (in)	Branches (No)	Branch Length (in)	Flower buds/stem
Control	**34.8b**	**3.22a**	**1.76a**	**1.67a**	**1.42a**
Sulfur (S)	**53.8a**	**3.39a**	**0.77b**	**2.1a**	**1.42a**

2002 Crop-Year Soil Analysis

Soil pH was significantly lower in sulfur-treated plots one year after treatment (Fig.2) but only soil Zn, Mn, and S concentrations were higher in sulfur-treated plots (Table 9). S concentration was 190 ppm in sulfur-treated plots compared to 52 ppm for the controls.

Figure 2

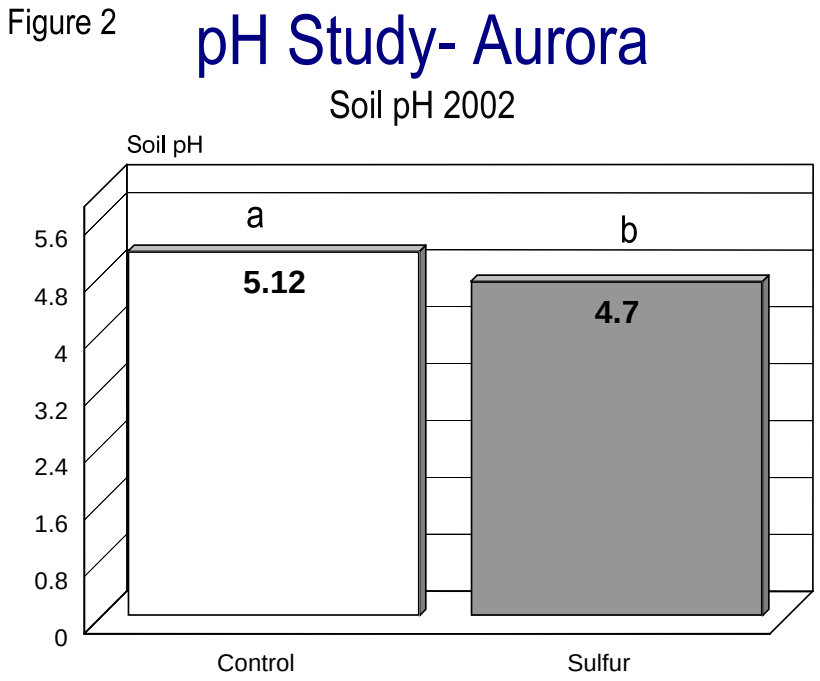


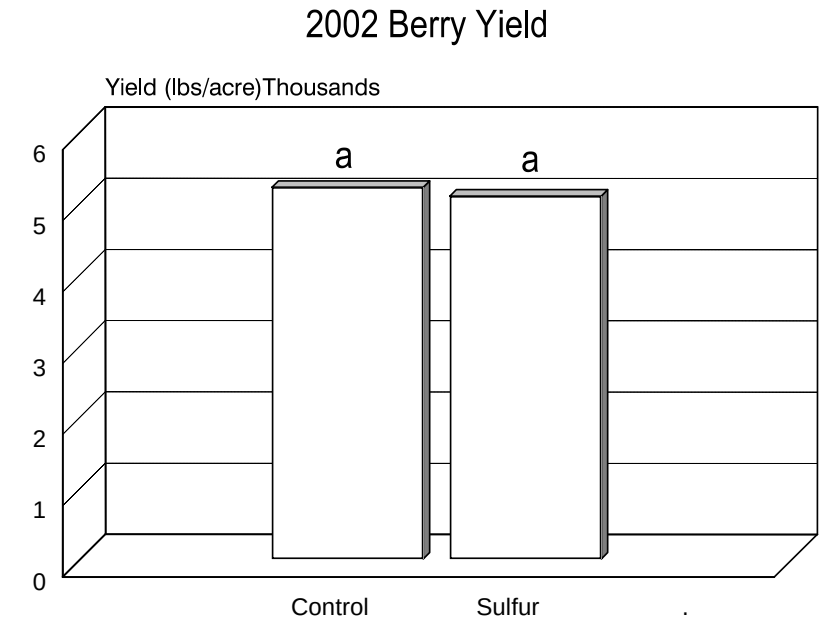
Table 9

Treatment	2002 soil nutrient concentrations							
	Ca (ppm)	K (ppm)	Mg (ppm)	P (ppm)	B (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
Control	**302a**	**83a**	**34a**	**6.4a**	**.06a**	**.17a**	**1.8a**	**5.8b**
Sulfur (S)	**331a**	**86a**	**37a**	**7.1a**	**.06a**	**.21a**	**2.2a**	**12.8a**

2002 Yield

Blueberry yield collected in August 7, 2002 was not affected by sulfur treatment (Fig. 3).

Figure 3 pH Study- Aurora



Prune-year leaf tissue levels were similar for control and sulfur-treated plots, except for leaf Mn concentrations (Table 10). Soil Mn concentrations were also higher in treatment plots receiving sulfur (Table 11). Soil pH values for treatment plots in 2003 (Fig. 4) were similar to those in 2002.

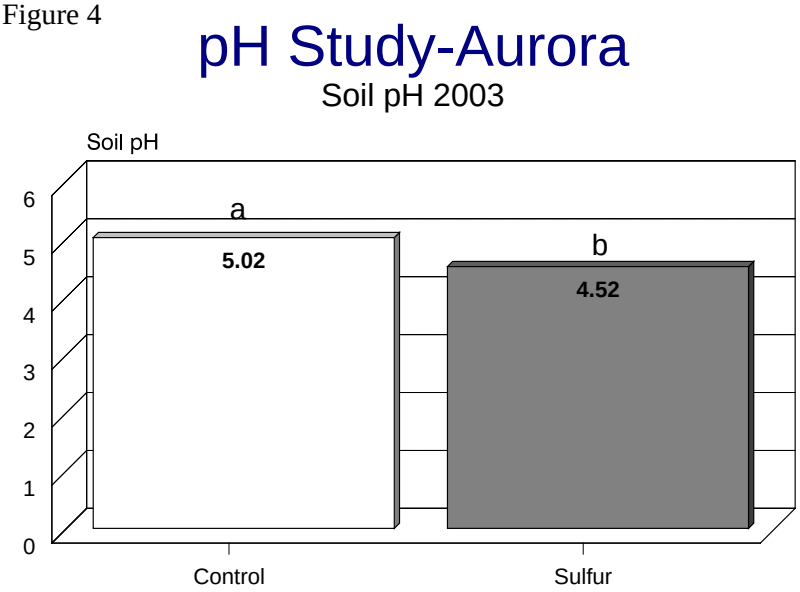
Table 10

Treatment	2003 leaf nutrient concentrations						
	Ca (%)	K (%)	Mg (%)	B (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
Control	**.503a**	**.447a**	**.179a**	**28a**	**4.2a**	**28.2a**	**632b**
Sulfur (S)	**.504a**	**.501a**	**.171a**	**27a**	**4.0a**	**31.8a**	**1098a**

Table 11

Treatment	2003 soil nutrient concentrations							
	Ca (ppm)	K (ppm)	Mg (ppm)	P (ppm)	B (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
Control	**452a**	**88a**	**53a**	**10.4b**	**.08a**	**.14a**	**2.1a**	**14.6b**
Sulfur (S)	**390a**	**83a**	**41a**	**12.1a**	**.07a**	**.16a**	**2.5a**	**21.2a**

Figure 4



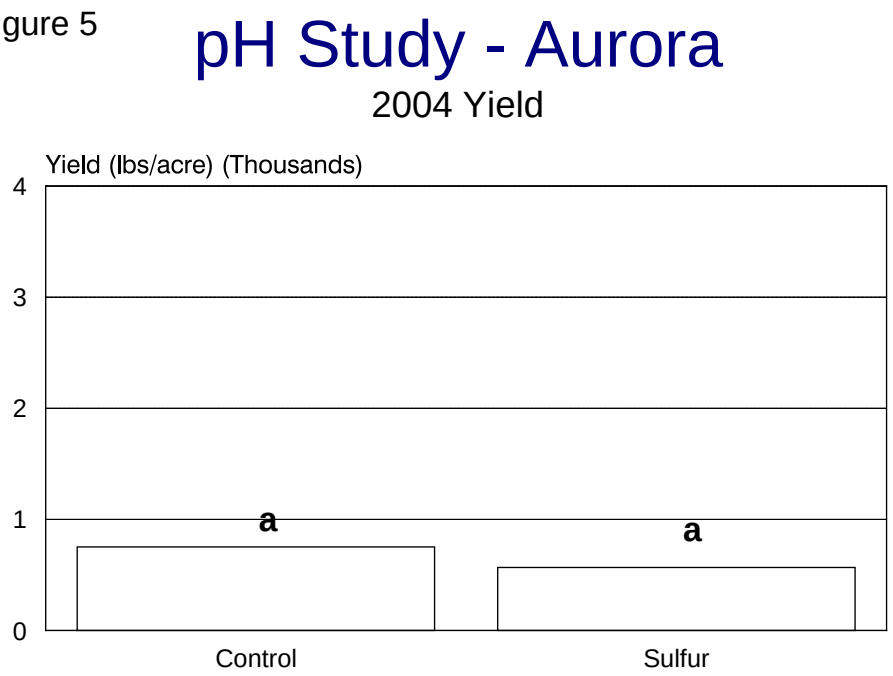
2003 Stem Characteristics and Yield

Stem density, length, branching and flower bud formation were not affected by soil pH (Table 12). Berry yield was extremely low due to severe winter injury across the state. There was no difference between the sulfur treatments and the controls (Fig. 5).

Table 12

Treatment	2003 Stem Characteristics				
	Density (Stems/ft^2)	Stem Length (in)	Branches (No)	Branch Length (in)	Flower buds/stem
Control	**40.27a**	**3.84a**	**0.54a**	**1.51a**	**0.85a**
Sulfur (S)	**38.38a**	**3.81a**	**0.57a**	**1.60a**	**0.85a**

Figure 5



2005 Soil and Leaf Tissue Analysis

Soil pH continues to show a difference between control and sulfur-treated plots (Fig. 6). Analyses of leaf tissue samples show Mn as the only nutrient that is different between the sulfur and control plots (Table 13). Nitrogen and P concentration, 1.36% and 0.096%, respectively, were below the satisfactory range of 1.6% (N) and 0.125% (P).

Figure 6

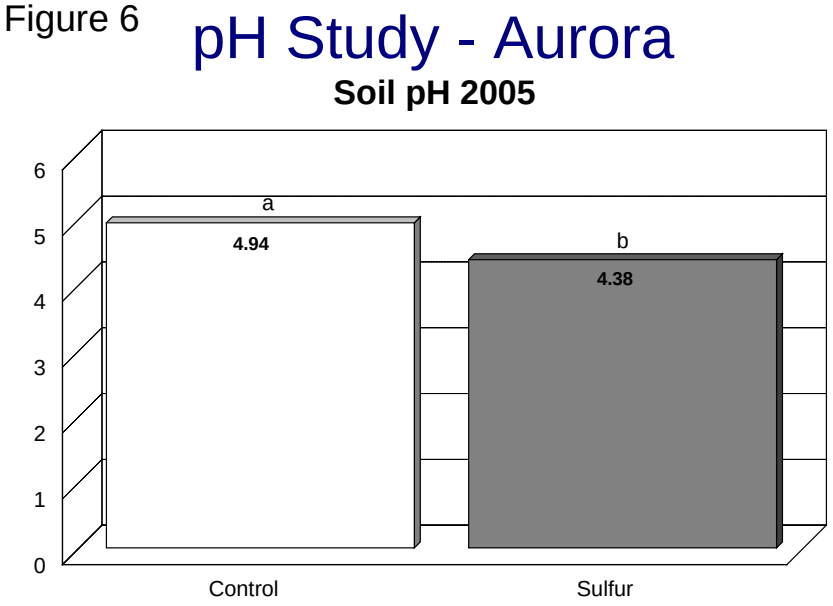


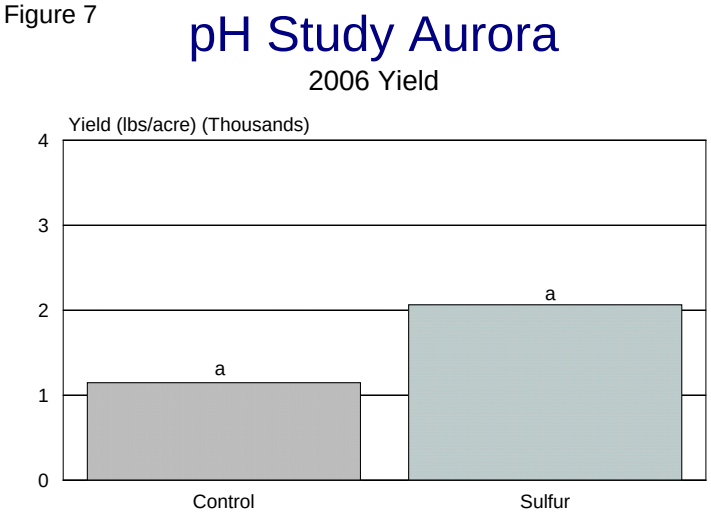
Table 13

Treatment	2005 leaf nutrient concentrations						
	Ca (%)	K (%)	Mg (%)	B (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
Control	**.489a**	**.466a**	**.183a**	**23a**	**4.9a**	**16.1a**	**560b**
Sulfur (S)	**.498a**	**.475a**	**.170a**	**27a**	**5.2a**	**15.9a**	**1220a**

2006 Yield

No significant difference was found between the control and sulfur-treated plots in 2006.

Figure 7



CONCLUSIONS: No conclusions can be made at this time.

RECOMMENDATIONS: No recommendations can be made at this time.

INVESTIGATORS: John M. Smagula, Professor of Horticulture
Loretta Kreider, Scientific Technician
Qian Wang, Graduate Student

TITLE: Effect of Manganese on Growth and Yield of Wild Blueberry

OBJECTIVES: To determine the effect of raising leaf manganese (Mn) concentration on growth and yield of wild blueberry

Brief Justification: The lowbush blueberry exhibits a tendency to be a manganese accumulator, with leaf Mn concentrations occurring above 1000 ppm with no apparent adverse affects. The leaf Mn concentrations reported in highbush blueberry leaves are usually under 300 ppm, and it has been suggested that concentrations above 500 ppm are associated with toxicity symptoms. This study will determine if raising low leaf Mn concentrations (<750 ppm) in a commercial lowbush blueberry field will result in more growth and yield and higher fruit Mn concentrations, compared to untreated controls.

METHODOLOGY: A commercial wild blueberry field that had a history of average leaf tissue Mn concentrations of < 750 ppm was used for this study. Eight discrete clones were selected in 2004 but one was eliminated from the study when it was found to be a mixture of two clones. Four treatments were replicated four times within each clone. The sixteen treatment plots (2 ft x 2 ft) were isolated from the rest of the clone by cutting through the blueberry sod to a depth of about six inches. Plots received a preemergent soil drench (750 ml/plot) containing 0, 1, 2, or 3 lbs Mn/acre from Citraplex (20% Mn) (Nortrace Co.). Composite leaf tissue samples were taken on July 6, 2004 from 15 stems in each treatment plot to determine leaf nutrient concentrations. Soil samples were also taken on July 6, 2004 to determine pH, organic matter content and nutrient concentrations. Stems were sampled November 26 and 27, 2004. All stems were cut at ground level in three randomly placed 1/9 ft^2 quadrats per plot to determine shoot number, length, and branching and the number of flower buds produced per stem. Berry yield was determined in August 2005 by hand raking each plot. Samples of berries were also be analyzed for Mn concentrations.

RESULTS: Changes in soil and leaf tissue Mn concentrations in response to Mn soil treatments showed a similar pattern (Fig. 1). There was a large variability even within clones and therefore changes in soil and leaf tissue Mn were not significant at the 5% level. At the 10% level, leaf Mn concentrations increased at the highest rate compared to the lowest rate. There were major differences in the leaf Mn concentration among clones (Fig 2), ranging from 588 to 1258 ppm.

Variation in leaf nutrient concentrations were found among the seven clones (Table 1). An interesting trend is observed when Mn concentrations are compared to other nutrients such as N (Fig. 3), P (Fig. 4), and K (Fig. 5); the clones having the lowest Mn concentrations had the highest N, P, and K concentrations. Although there was no significant difference among clones for soil Mn concentration, leaf Mn concentrations varied among the 7 clones (Fig. 5b and Table 1). This means that some clones were able to absorb and transport to their leaves more Mn from the soil.

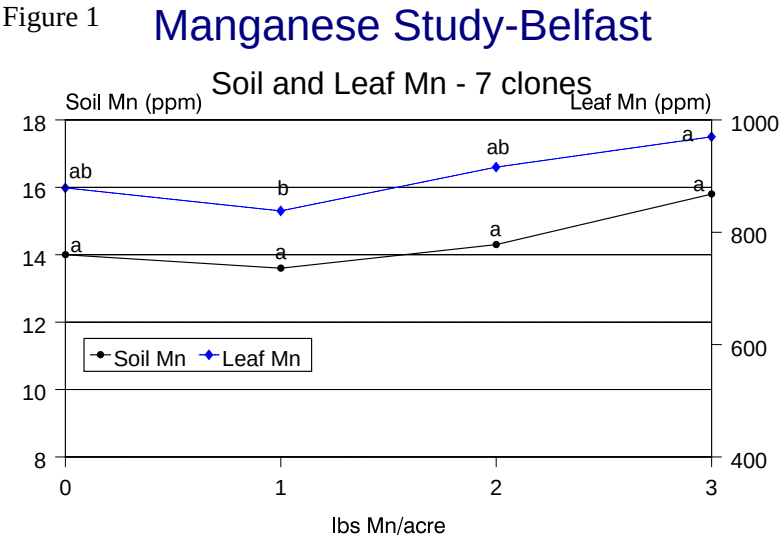
We measured the growth characteristics (Table 2) and the potential yield characteristics (Table 2b) of the seven clones and correlated them with leaf nutrient concentrations (Table 3). Positive correlations were found between leaf Mn concentration and flower bud density and flower buds per stem.

Berries were harvested from each plot, weighed for yield determination and the weight converted to the equivalent in lbs/acre. There was no significant difference among treatments (Fig. 6). The yield was low and differed dramatically among clones (Fig. 7). The leaf Mn concentrations are superimposed on the yield in figure 8 to show that there is a trend for clones with high leaf Mn to have a higher yield. Fruit Mn concentrations were significantly different among clones and there was a positive correlation between leaf Mn and fruit Mn concentrations among the clones (Fig. 9).

CONCLUSIONS: While we have not been very successful in raising leaf Mn concentrations through foliar or soil applications of Mn, there are indications that clones with higher leaf Mn concentrations have the potential for higher yields. Fruit Mn concentrations vary among clones and there is a positive correlation between leaf and fruit Mn concentrations.

RECOMMENDATIONS: No recommendations can be made for fertilization with Mn fertilizer.

Figure 1



Citraplex (20%Mn) applied to soil pre-emergent. Mean Separation By Duncan's Multiple Range test, 10% level.

Figure 2

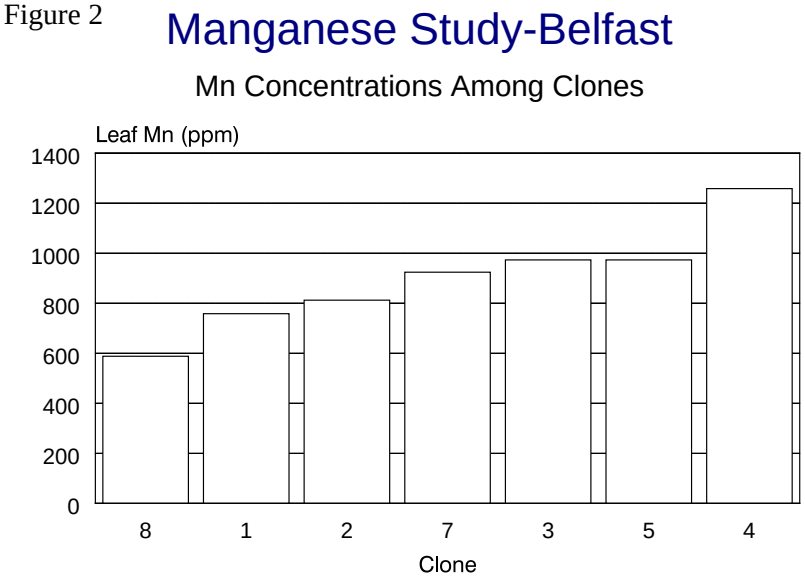


Figure 3

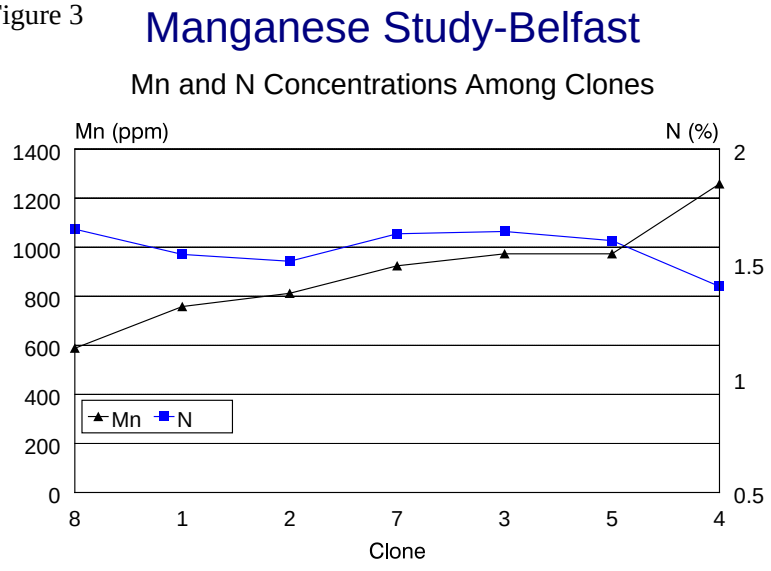


Figure 4

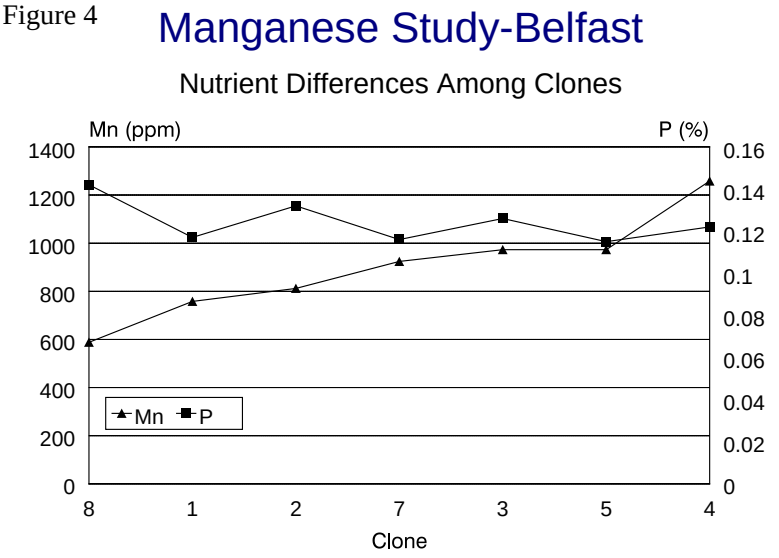


Figure 5

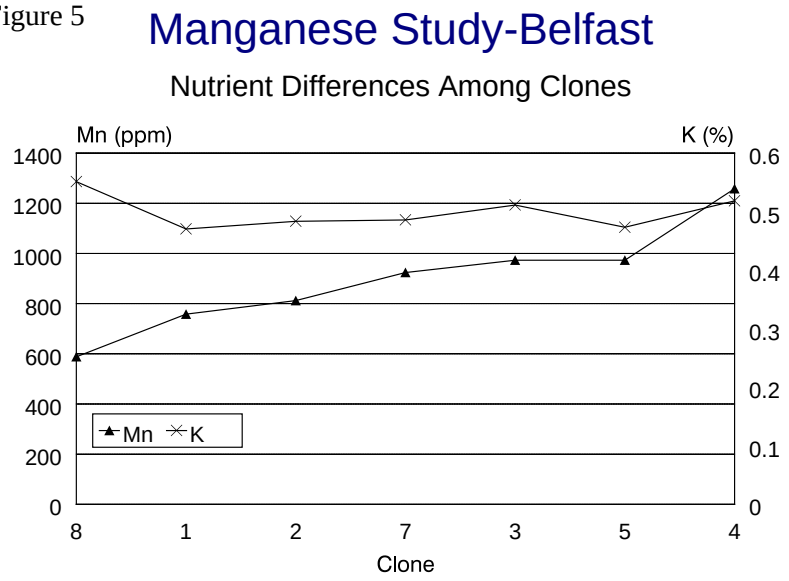
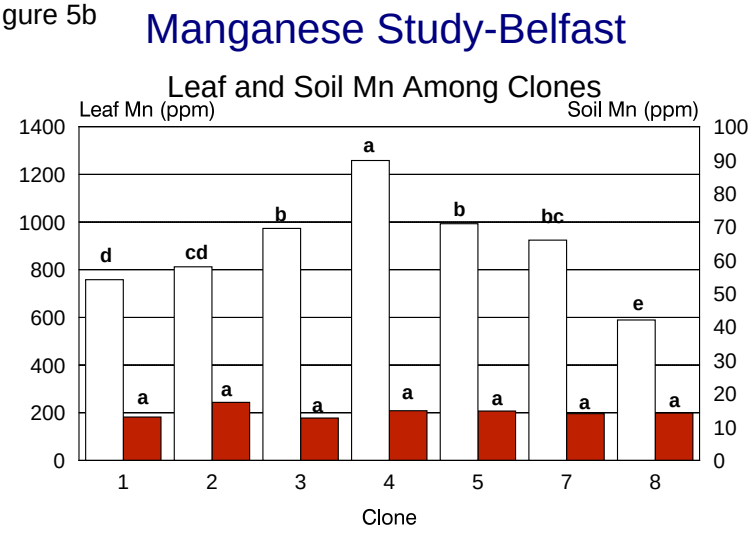


Figure 5b



Citraplex (20%Mn) applied to soil pre-emergent. Mean Separation By Duncan's Multiple Range test, 5 % level.

Table 1 Clonal differences in leaf nutrient concentrations averaged across all treatments

Clone	N	Ca	K	Mg	P	Al	B	Cu	Fe	Mn	Zn
	($g \cdot kg^{-1}$)					($mg \cdot kg^{-1}$)					
1	15.4 b	3.9 b	4.7 c	1.9 a	1.2 de	78 c	15.9 d	3.9 a	32.3 bc	758 c	13.9 c
2	15.1 b	3.8 b	4.8 c	1.6 e	1.3 b	95 a	22.7 b	3.7 ab	29.1 c	812 c	15.7 b
3	16.4 a	3.7 bc	5.1 b	1.8 b	1.3 c	96 a	18.9 c	3.4 abc	38.0 b	973 b	14.8 bc
4	14.0 c	3.6 c	5.2 b	1.5 e	1.2 cd	83 bc	23.4 b	3.2 bc	28.6 c	1258 a	14.7 bc
5	16.0 a	3.2 d	4.7 c	1.6 d	1.2 e	84 bc	15.7 d	3.3 bc	36.5 bc	993 b	17.9 a
6	16.3 a	3.8 b	4.9 c	1.6 de	1.2 e	80 c	23.8 b	3.0 c	38.3 b	924 b	17.5 a
7	16.5 a	4.1 a	5.5 a	1.7 c	1.4 a	91 ab	29.6 a	3.4 abc	54.3 a	559 d	17.3 a

Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

Table 2. Growth characteristics of clones in prune year.

Clone	Stem or branch density (No./16 in^2)				Stem or branch length (in)				Branching (branches/stem)
	Stm[z]	US[y]	BS[x]	Brc[w]	Stm[z]	US[y]	BS[x]	Brc[w]	BS[x]
1	13 b	13 ab	1 e	1 e	2.5 d	2.5 cd	1.3 d	0.4 d	0.6 c
2	17 a	15 a	2 d	2 de	2.2 e	2.1 e	1.9 c	0.67 c	1.0 b
3	16 a	14 a	2 cd	3 cd	2.4 d	2.4 cd	1.7 cd	0.67 c	1.0 b
4	15 ab	14 a	1 e	1 e	3.4 a	3.4 a	1.8 cd	0.70 c	0.7 bc
5	15 ab	10 c	5 a	8 a	2.8 c	2.6 c	3.3 a	1.18 ab	1.6 a
6	17 a	14 a	3 b	4 b	2.3 de	2.3 de	2.6 b	1.30 a	1.4 a
7	13 b	10 bc	3 bc	4 bc	3.0 b	2.9 b	3.1 ab	0.94 bc	1.4 a

[z]All the stems
[y]Unbranched stems
[x]Branched stems
[w]Branches
Means followed by different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$.

Table 2b. Potential yield characteristics of clones in prune year.

Clone	Flower bud density (FB/103.2 cm^2)					Flower bud per stem/branch					Flower bud ratio (FB/cm)		
	Stm[z]	US[y]	BS[x]	BSM[w]	Brc[v]	Stm[z]	US[y]	BS[x]	BSM[w]	Brc[v]	US[y]	BSM[w]	Brc[v]
1	10 c	9 c	1 c	0 c	1 c	1 cd	0.8 bc	0.6 bc	0.3 c	0.3 c	0.1 cd	0.0 cd	0.1 b
2	10 c	8 c	1 c	1 c	0 c	1 d	0.6 c	0.6 bc	0.4 bc	0.2 c	0.1 d	0.1 bc	0.0 b
3	15 b	13 b	2 c	1 c	1 c	1 bc	0.9 b	0.8 bc	0.4 bc	0.3 c	0.1 ab	0.1 b	0.1 b
4	19 a	17 a	2 c	1 c	1 c	1.3 a	1.2 a	1.1 b	0.6 b	0.3 bc	0.1 bc	0.0 bc	0.1 b
5	18 ab	9 c	9 a	5 a	4 a	1.2 ab	0.9 b	1.9 a	1.0 a	0.5 ab	0.1 cd	0.1 a	0.2 a
6	19 a	14 ab	6 b	3 b	3 b	1.2 ab	1.0 b	2.0 a	1.1 a	0.6 a	0.2 a	0.1 a	0.2 a
7	5 d	3 d	1 c	1 c	1 c	0.4 e	0.3 d	0.4 c	0.2 c	0.1 c	0.0 e	0.0 d	0.0 b

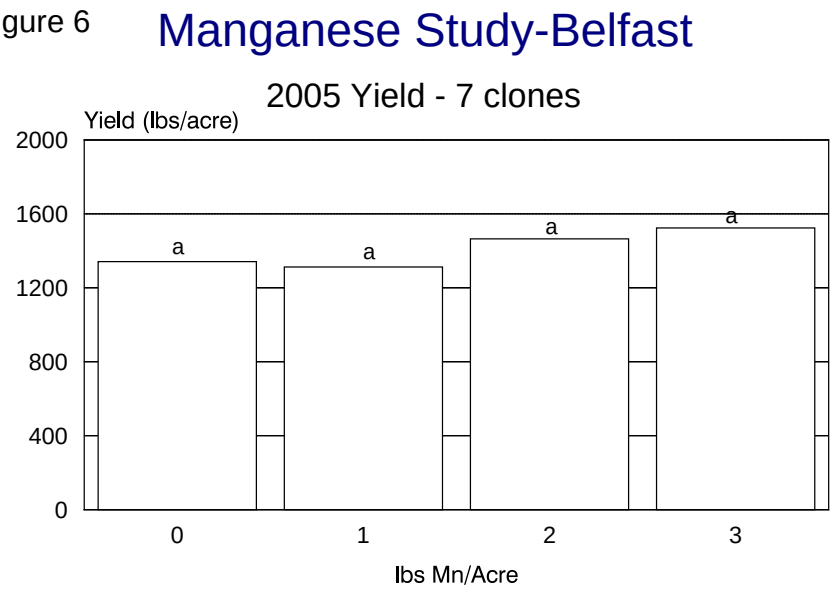
[z]All the stems
[y]Unbranched stem
[x]Branched stem
[w]Major stem of branched stem
[v]Branch
Means followed by different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$.

Table 3. Significant correlation coefficients between leaf nutrient concentrations and growth characteristics of the seven clones.

	Stem or branch density (No./103.2 cm^2)				Stem or branch length (cm)				Branching	Flower bud (FB) density (No./103.2 cm^2)					FB per stem or branch					FB ratio (FB/cm)		
	Stm[z]	Ubs[y]	Bs[x]	Brc[w]	Stm[z]	Ubs[y]	Bs[x]	Brc[w]	Bs[x]	Stm[z]	Ubs[y]	B[v]	Bs[x]	Brc[w]	Stm[z]	Ubs[y]	B[v]	Bs[x]	Brc[w]	Ubs[y]	Bs[v]	Brc[w]
N		-0.22	0.3	0.38	-0.2	-0.3	0.28	0.38	0.32		-0.26	0.24	0.2	0.26		-0.21						
Ca			-0.4	-0.38						-0.37		-0.48	-0.5	-0.37	-0.42	-0.28	-0.3	-0.32		-0.29		
K	-0.25				0.37	0.34				-0.25		-0.25	-0.24	-0.22	-0.21		-0.23		-0.26	-0.32		-0.27
Mg			-0.2	-0.23				-0.24		-0.28		-0.31	-0.35	-0.22	-0.29	-0.21	-0.25	-0.34				
P										-0.44	-0.32	-0.34	-0.32	-0.31	-0.42	-0.39	-0.24	-0.19	-0.25	-0.46		-0.25
Al										-0.3	-0.24	-0.19		-0.21	-0.3	-0.26	-0.22		-0.27	-0.22		-0.22
B			-0.2		0.27	0.26	0.19			-0.29		-0.32	-0.31	-0.27	-0.31	-0.24	-0.2		-0.25	-0.34		-0.25
Cu										-0.2					-0.19			-0.2				
Fe	-0.27	-0.34								-0.32	-0.37				-0.26	-0.32				-0.3		
Mn					0.36	0.37				0.46	0.49				0.47	0.52		0.23		0.37		
Zn			0.35	0.42			0.27	0.23	0.2			0.32	0.28	0.31								
Mn:Fe	0.25			-0.19	0.33	0.39	-0.21			0.46	0.57				0.42	0.51		0.19		0.34		

[a]All the stems
[y]Unbranched stems
[x]Branched stems
[w]Branches
[v]Major stem of branched stems
All correlations are significant at $P \leq 0.05$.

Figure 6



Citraplex (20%Mn) applied to soil pre-emergent. Mean Separation By Duncan's Multiple Range test, 5% level.

Figure 7

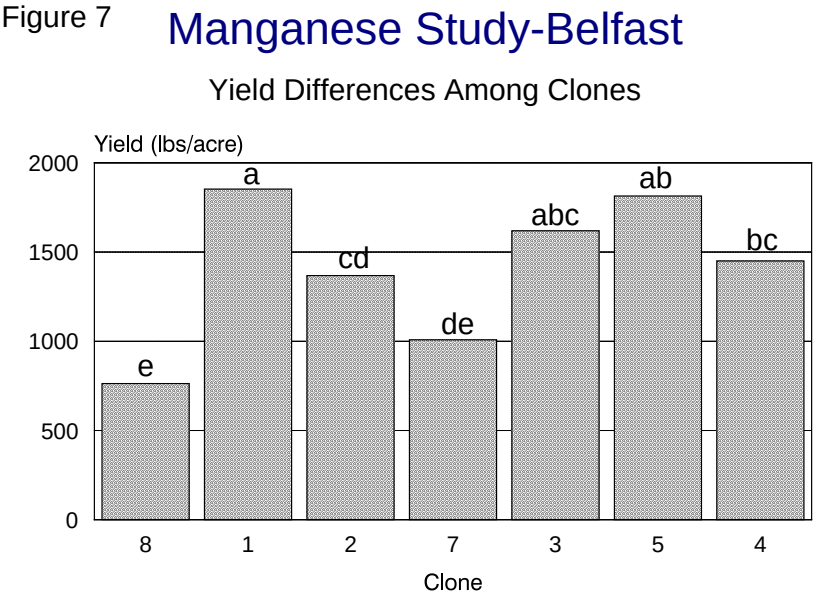


Figure 8

Manganese Study-Belfast

Leaf Mn and Yield Among Clones

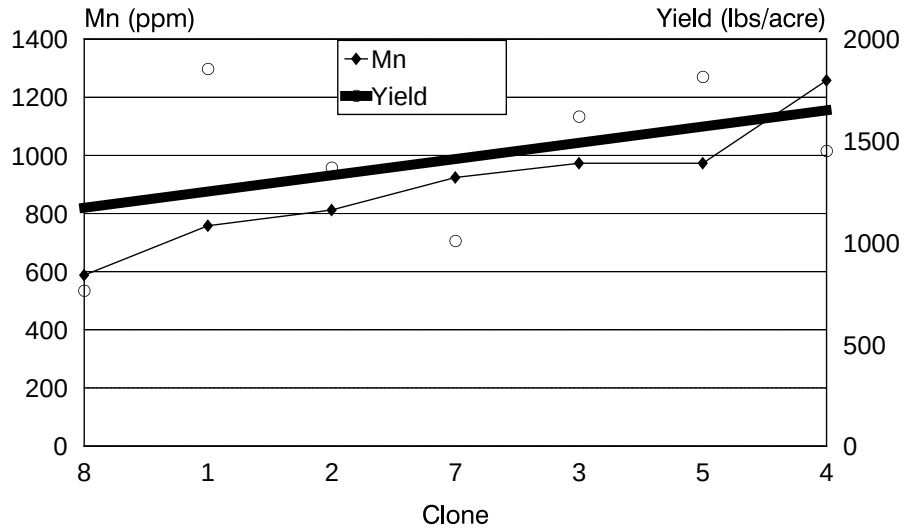
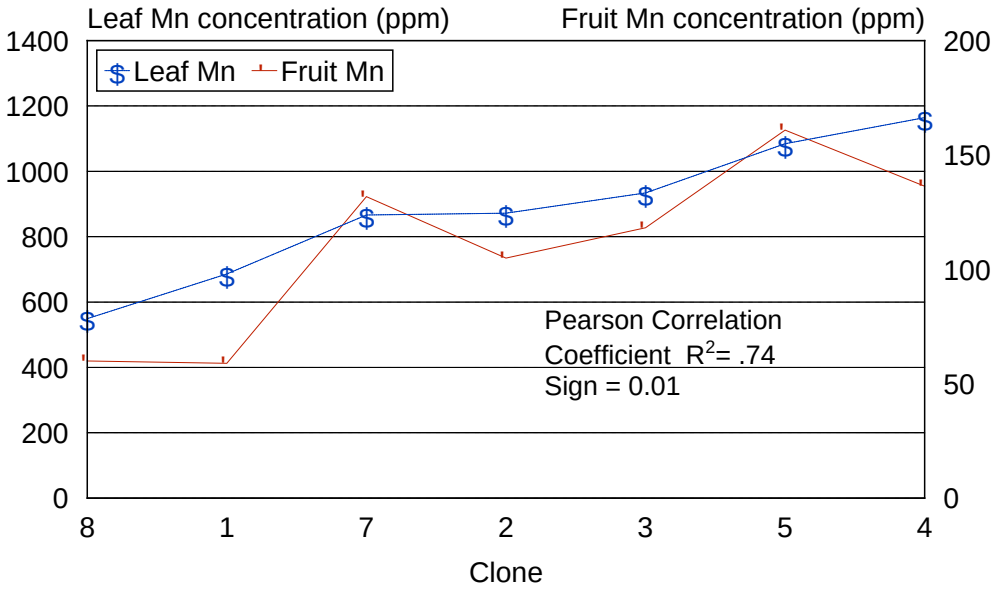


Figure 9

Manganese Study - Belfast

Positive Correlation of Clonal Leaf Mn and Fruit Mn



Values are averages of 4 replications of control plots in 7 clones

INVESTIGATORS: John M. Smagula, Professor of Horticulture
Loretta Kreider, Scientific Technician

TITLE: Effects of Summer Foliar Fertilization to Increase Branch Length and Flower Bud Formation in the Prune Year.

OBJECTIVES: Determine the effect of raising foliar nitrogen (N) and phosphorus (P) after initial tip dieback on growth and yield of wild blueberries.

IMPACT OF RESEARCH: At the time of tip dieback in early July, the lateral buds on emerging shoots can either develop into a flower bud or remain a vegetative bud. The vegetative buds can break and elongate to form lateral branches. We have found in recent studies that preemergent application of DAP in the prune year increases the number of these branches. These branches are usually short and produce only one or two flower buds. If they could be encouraged to grow longer, would more flower buds form? A positive correlation between stem length and number of flower buds of unbranched stems has been found in a number of studies. Nitrogen and phosphorus may be limiting factors affecting the length of lateral branches and the production of flower buds. Would application of fertilizer through foliar sprays, overcome nitrogen and phosphorus deficiency and increase the length of these branches and in turn result in more flower buds? Is N the only nutrient that is needed? What would be the optimum time for this foliar application? This study will answer these questions.

METHODOLOGY: A commercial blueberry field from which previous analysis of leaf samples indicated nitrogen and phosphorus deficiency was used to determine the most effective time to apply CoRoN™ in order to influence the branch length and flower bud formation. CoRoN™, containing 28% N, was used in a foliar spray volume of 67gal/acre. The highest rate of CoRoN™ without leaf burning (12 lbs N/acre) was determined in a greenhouse study using blueberry sods of two clones. We also studied a foliar fertilizer called "TKO" Phosphite (0-29-26), which contains mono- and di-potassium salts of phosphorous acid, and determined the highest safe rate was 12 lbs P_20_5/acre. In the field study, an application of foliar N following a soil application of DAP was evaluated to determine the affect on growth and development of branches and flower buds. A foliar application "TKO" Phosphite (0-29-26), which contains P and K, was also tested at 12 lbs P_20_5/acre, with or without the application of CoRoN™ at 12 lbs N/acre. The most beneficial time for the application of N, PK, or N + PK foliar sprays was studied by applying these treatments to plots at three-week intervals, beginning about four weeks before tip dieback, on June 9, 2005. Plots measuring 6 ft x 50 ft received the following prune-year treatments:

1. Control (no treatment)
2. DAP
3. DAP + CoRoN™ June 9
4. DAP + CoRoN™ + PK June 9
5. DAP + PK June 9
6. DAP + CoRoN™ June 28
7. DAP + CoRoN™ + PK June 28
8. DAP + PK June 28

9. DAP + CoRoN™ July 19
10. DAP + CoRoN™ + PK July 19
11. DAP + PK July 19
12. DAP + CoRoN™ August 8
13. DAP + CoRoN™ + PK August 8
14. DAP + PK August 8
15. DAP + CoRoN™ September 2
16. DAP + CoRoN™ + PK September 2
17. DAP + PK September 2
18. DAP + CoRoN™ September 22
19. DAP + CoRoN™ + PK September 22
20. DAP + PK September 22

Treatments were randomly assigned to plots in a randomized complete block design with 10 blocks. Sixteen stems in each treatment plot in the first 7 blocks were tagged to evaluate the effect of the early foliar sprays (prior to normal tip dieback) on time of branching. Two weeks after each treatment spray, stems were sampled from those plots as well as the controls to determine leaf nutrient concentrations. Leaf samples were ground and submitted to the Maine Analytical Lab for analysis of nutrients. Branching was evaluated weekly for 5 weeks, between 7/21 and 8/20 on the tagged stems in plots receiving foliar sprays before 8/12. In the spring 2006, the tagged stems were evaluated to determine the effect of treatments on flower development and fruit set, the percentage of blossoms on a stem that develop into fruit. To determine the effect of treatments on stem density, stem length, branching and branch length, stem samples from 4 randomly placed 1/4 ft^2 quadrats were collected in November 2005. The number of flower buds on each stem was also measured. Yield was determined in August 2006.

RESULTS:

Stem Branching

Branching on the 16 tagged stems was increased by DAP and DAP plus foliar sprays of N and NPK compared to the controls (Fig.1). DAP plus PK foliar spray had less of an effect than foliar treatments that included N. The August 20 measurement (Fig. 2) suggests that DAP increased branching by 11%, from 3.5% in control plots to 14.5% increase. Foliar nutrient sprays increased branching from 3-10 % higher than just the DAP alone.

Leaf nutrient concentrations

Leaf N was highest in leaf tissue for all treatments applied June 9 and sampled on June 23 (Fig. 3). Leaf N declined during the growing season (sample dates June 23 to October 5) for the control and the fertilizer treatments (Fig. 4). This decline has been reported and is the reason for taking leaf tissue samples at 90-100% tip dieback in early July; the change is minimal during this period and the standards are based on leaf tissue concentrations at this time. Leaves sampled on July12 (June 28 spray date) would correspond to the normal sample time, at about 90-100% tip dieback, and can be compared to the leaf standards established by Trevett. On July 12, leaves from control plots, DAP alone plots and those that received DAP plus foliar treatments of CoRoN (N) and CoRoN + PK phosphite and PK phosphite alone were sampled and their nutrient concentrations determined. N was below the sufficiency level of 1.6 % in leaves taken from the control plots (average of 10 plots) (Fig.5). DAP increased the leaf N concentrations to above the standard concentration. CoRoN did not

significantly increase the leaf N concentration compared to the DAP alone. The PK phosphite was not effective alone but in combination with CoRoN, leaf N concentrations were higher than the DAP. The trend for leaf P concentrations for all the treatments during the course of the summer was somewhat different; leaf P concentrations in all treatment plots declined until September, when they begin to increase (Fig. 6). Leaves in treatment plots receiving the PK foliar treatments had the highest P concentrations throughout the growing season. Leaves sampled on July 12 indicated that phosphorus was raised by DAP to above the standard of 0.125% (Fig. 7). The PK foliar treatments, however, raised the levels even higher, to about 0.168%. CoRoN had no effect on leaf P concentration, compared to the DAP alone. While potassium (K) was not deficient (below 0.400%) in the control plots, the foliar PK sprays significantly raised the leaf K concentrations (Fig.8).

Stem Characteristics

Stems were cut to ground level in four ¼ square foot quadrats per treatment plot. The number of stems per quadrat (stem density), as measured in the fall of 2005, suggests that DAP followed by foliar sprays may have increased stem density to a small degree (Fig. 9). The average percentage of branched stems in four ¼ sq ft quadrats per plot was not significantly higher in DAP treated plots compared to the controls (Fig. 10). Foliar treatments along with the DAP did result in more branched stems. The height of unbranched stems was not improved by foliar treatments compared to DAP alone (Fig 11). Hatched bars are significantly taller than the controls. DAP, however, clearly increased the height of branched stems, compared to the controls (Fig. 12). Foliar fertilizer treatments did not increase stem height compared to DAP alone. Average branch length on branched stems was increased by DAP, compared to the controls; but foliar fertilizer treatments did not enhance this branch length as anticipated (Fig. 13). Flower bud formation on unbranched stems was not increased by DAP alone (Fig. 14). Hatched bars indicate those treatments that were significantly different than the control or DAP alone. Flower buds on branched stems were higher than on unbranched stems, even for the controls, which averaged about 2.5 on unbranched and 4.3 on branched stems (Fig. 14 and Fig. 15). Flower bud formation more than doubled for stems that were branched, from less than 3 to almost 8 flower buds per stem for treatment plots receiving DAP alone or DAP plus foliar N, NPK or PK. Flower bud density, number per ¼ sq ft, also was increased by foliar fertilizer treatments, compared to the DAP alone (Fig. 16). Hatched bars indicate foliar fertilizer treatments that had significantly higher flower bud density than DAP alone. Berry yield was increased from about 5,400 lbs/acre in control plots to about 10,500 in the plots receiving DAP (Fig. 17). While there appeared to be an increase in potential yield based on flower bud counts, treatment plots receiving DAP plus foliar N, NPK, or PK, did not yield higher than those receiving DAP alone. Perhaps the yield potential of the clones in this field had been reached.

CONCLUSIONS: DAP at 400 lbs/acre corrected the N and P deficiency that was present in control plots, resulting in a doubling of yield from 5,000 to 10,000 lbs/acre. The yield increase was accounted for by increased branching and not an increase in flower buds on unbranched stems. There were more branches on stems tagged at tip dieback. The branched stems put on more growth and were taller in November when stems were cut in ¼ sq ft quadrats for measurements. The yield of plots was exceptionally high and even though there appeared to be an increase in potential yield (increased flower buds) due to foliar fertilization with TKO- Phosphite this potential was not realized as higher berry yield. Perhaps the plants could not maintain the extra fruit and they were aborted. Perhaps TKO – Phosphite would

have been shown more benefit in a field with lower yield or in a year when conditions were not as favorable.

Figure 1 Summer Foliar Fertilization Study

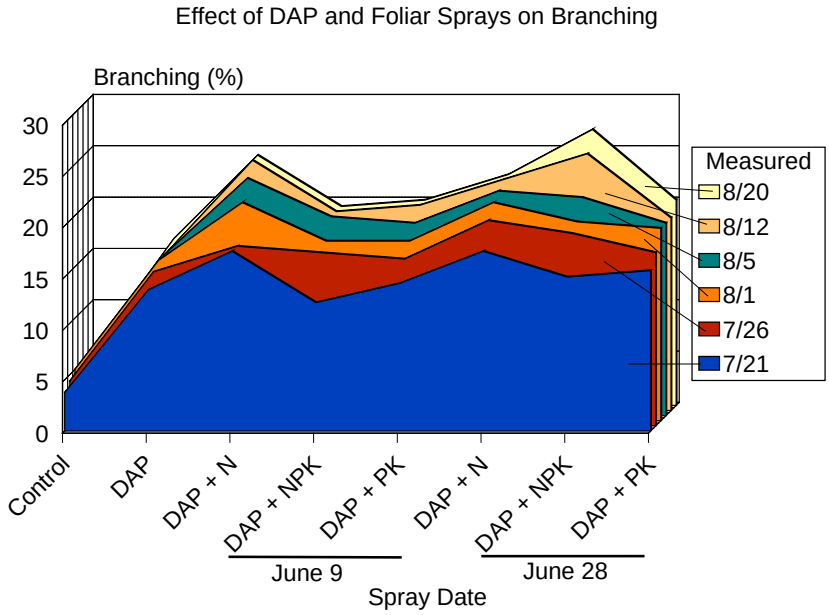
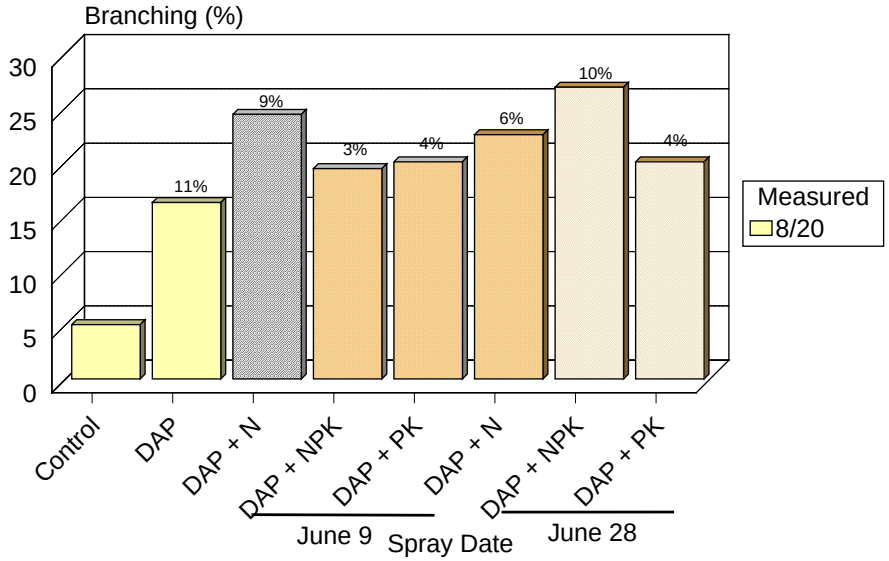


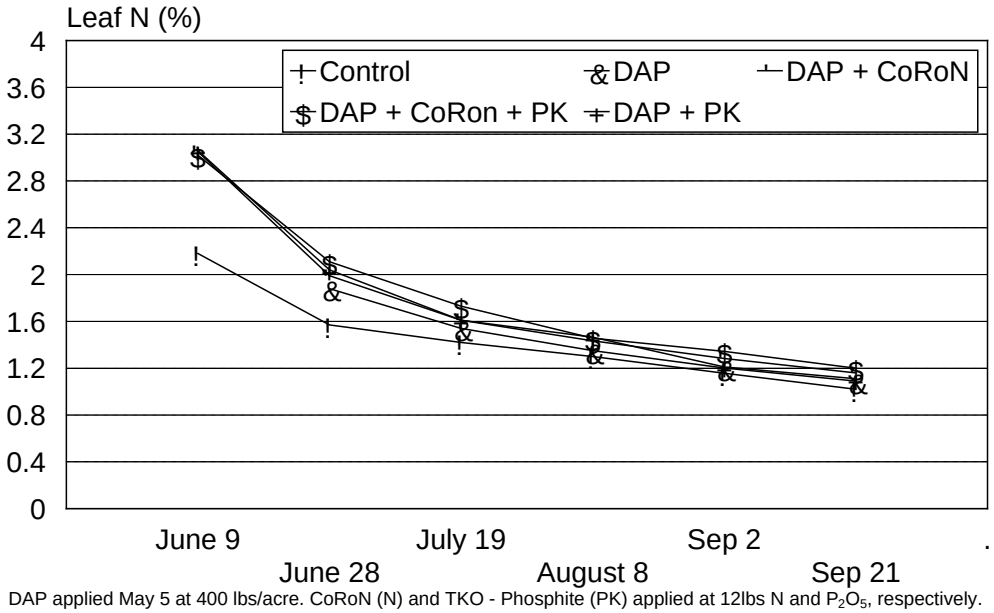
Figure 2 **Effect of DAP and Foliar Sprays on Branching of 16 tagged stems/plot**



Percentage above DAP is compared to control, above others is compared to DAP.

Figure 3

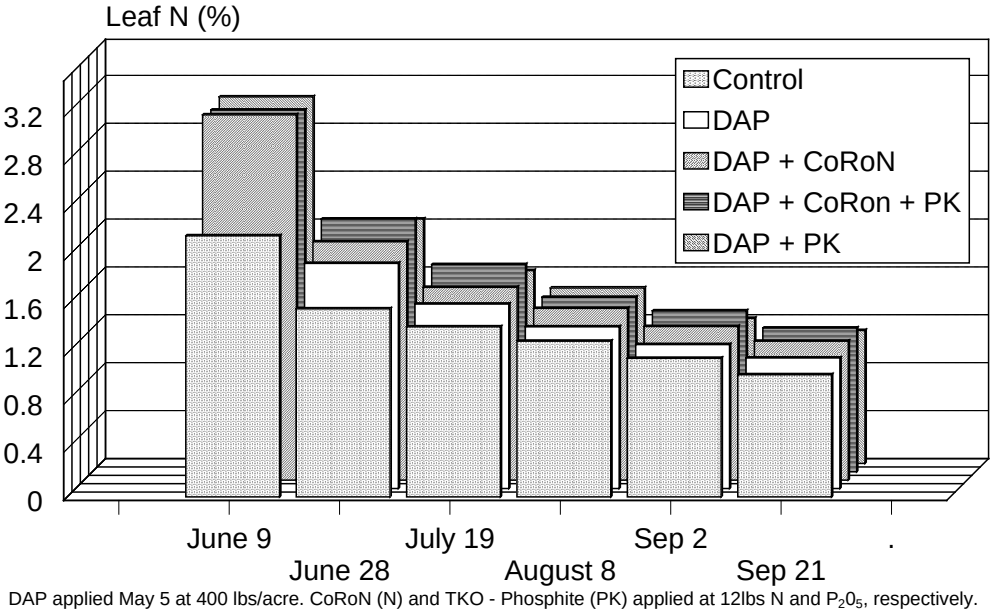
Leaf Nitrogen



DAP applied May 5 at 400 lbs/acre. CoRoN (N) and TKO - Phosphite (PK) applied at 12lbs N and P_2O_5, respectively.
Leaves sampled June 23, July 12, August 1, August 22, September 19, and October 5. Significant at 0.01% level.

Figure 4

Leaf Nitrogen

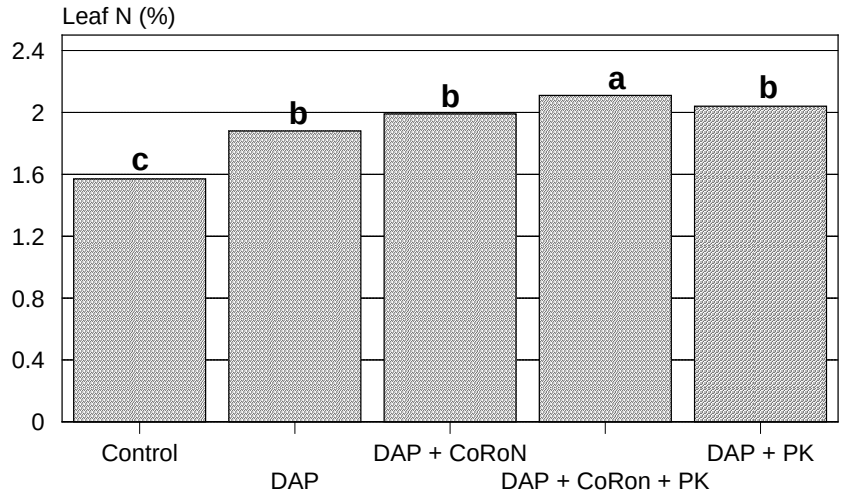


DAP applied May 5 at 400 lbs/acre. CoRoN (N) and TKO - Phosphite (PK) applied at 12lbs N and P_20_5, respectively.
Leaves sampled June 23, July 12, August 1, August 22, September 19, and October 5. Significant at 0.01% level.

Figure 5

Leaf Nitrogen

June 28 Spray- sampled July 12

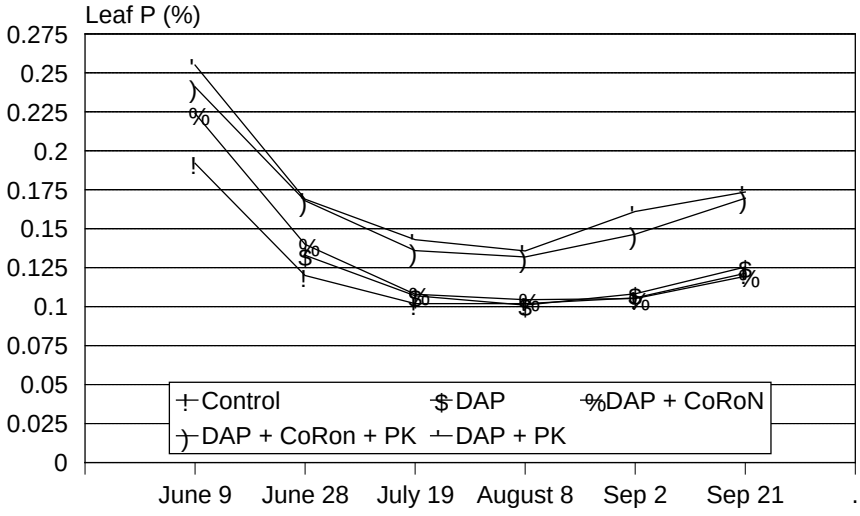


DAP applied May 5 at 400 lbs/acre. CoRoN (N) and TKO - Phosphite (PK) applied at 12lbs/a N and P_2O_5, respectively. Significant at 0.01% level.

Figure 6

Leaf Phosphorus

All Treatments

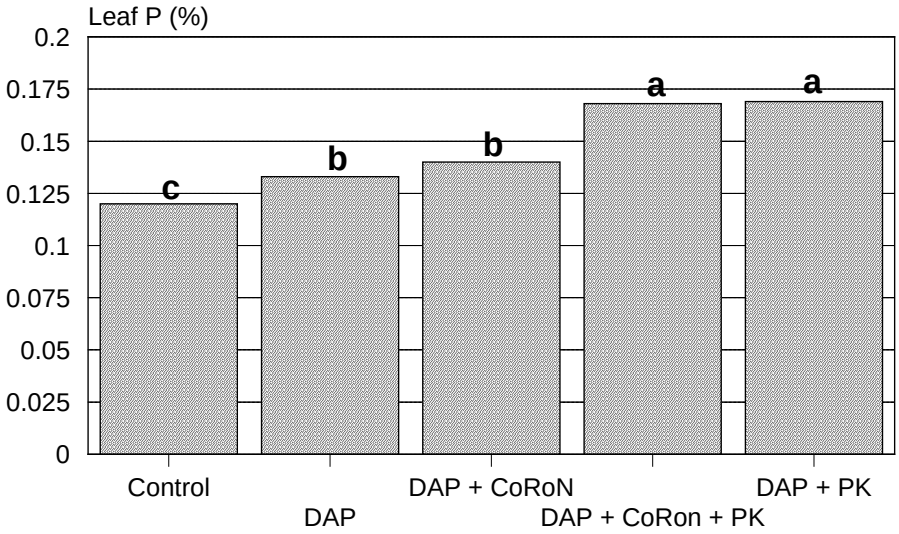


DAP applied May 5 at 400 lbs/acre. CoRoN (N) and TKO - Phosphite (PK) applied at 12lbs N and P2O5, respectively. Leaves sampled June 23, July 12, August 1, and August 22. Significant at 0.01% level.

Figure 7

Leaf Phoshorus

June 28 Spray - sampled July 12

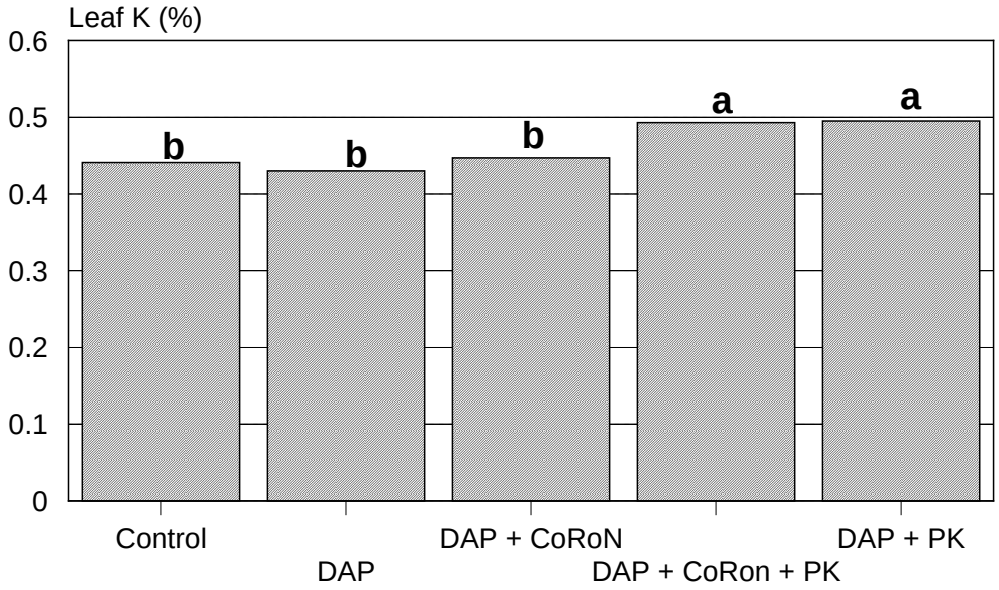


DAP applied May 5 at 400 lbs/acre. CoRoN (N) and TKO - Phosphite (PK) applied at 12lbs/a N and P2O5, Respectively. Leaves sampled July 12. Significant at 0.01% level.

Figure 8

Leaf Potassium

June 28 Spray - sampled July 12

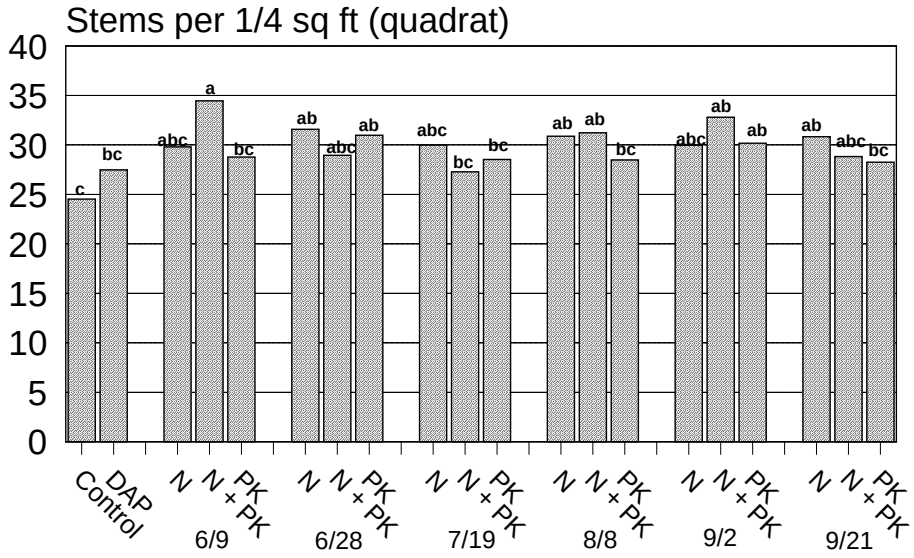


DAP applied May 5 at 400 lbs/acre. CoRoN (N) and TKO - Phosphite (PK) applied at 12lbs N and P2O5, respectively. Significant at 0.01% level.

Figure 9

Summer Foliar Study

Stem Density

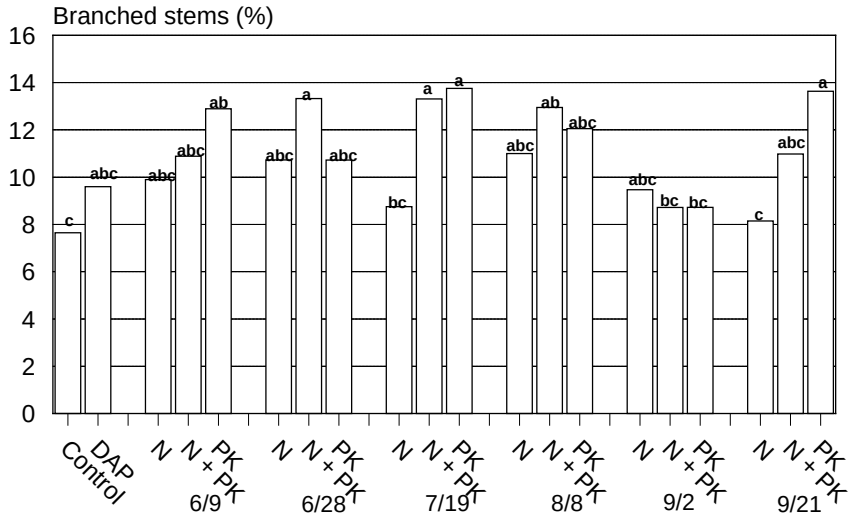


Values represent an average four quadrats/treatment plot for10 blocks and are significant at the 5% level. N, N+PK, and PK treatment plots also received preemergent DAP application.

Figure 10

Summer Foliar Study

Percent of stems branched

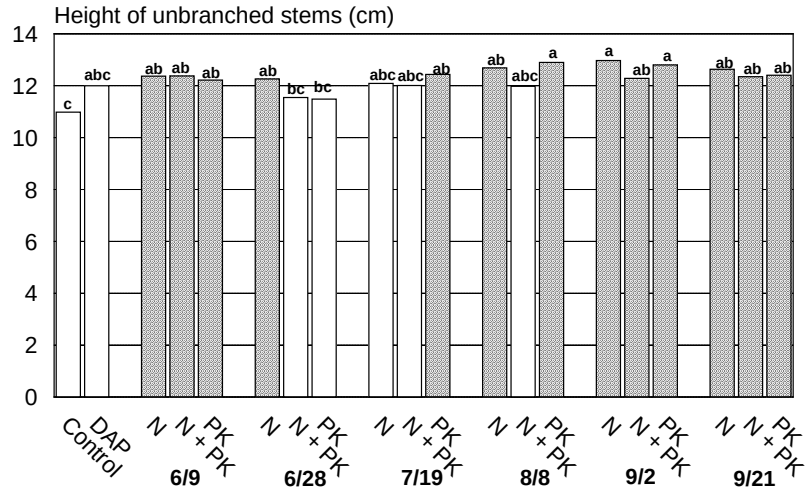


Branching of stems collected in November in four ¼ sq ft quadrats/ treatment plot. Values represent an average of 10 blocks and are significant at the 5% level. N, N+PK, and PK treatment plots also received preemergent DAP application.

Figure 11

Summer Foliar Study

Unbranched Stems

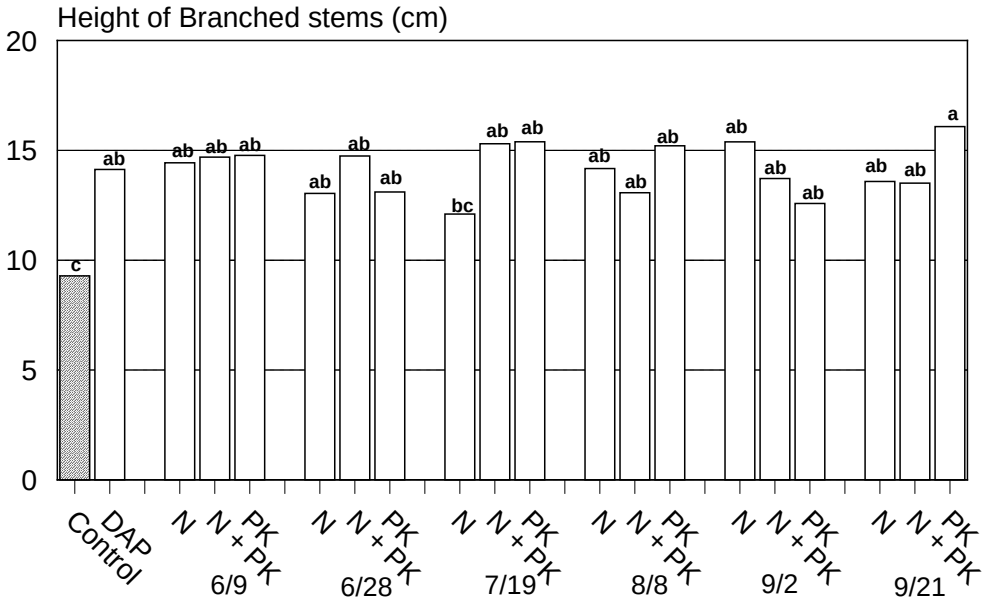


Values represent an average of 10 blocks and are significant at the 5% level. N, N+PK, and PK treatment plots also received preemergent DAP application.

Figure 12

Summer Foliar Study

Branched stems

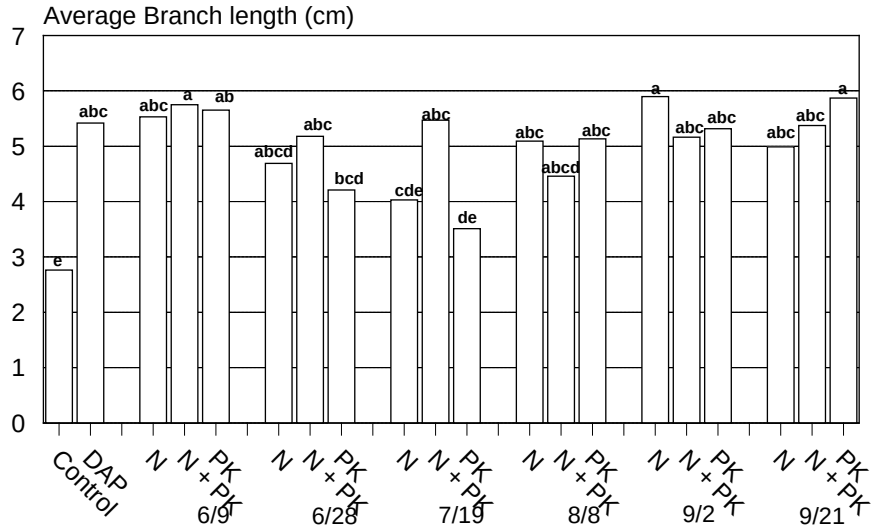


Values represent an average of 10 blocks and are significant at the 5% level. N, N+PK, and PK treatment plots also received preemergent DAP application.

Figure 13

Summer Foliar Study

Length of branches

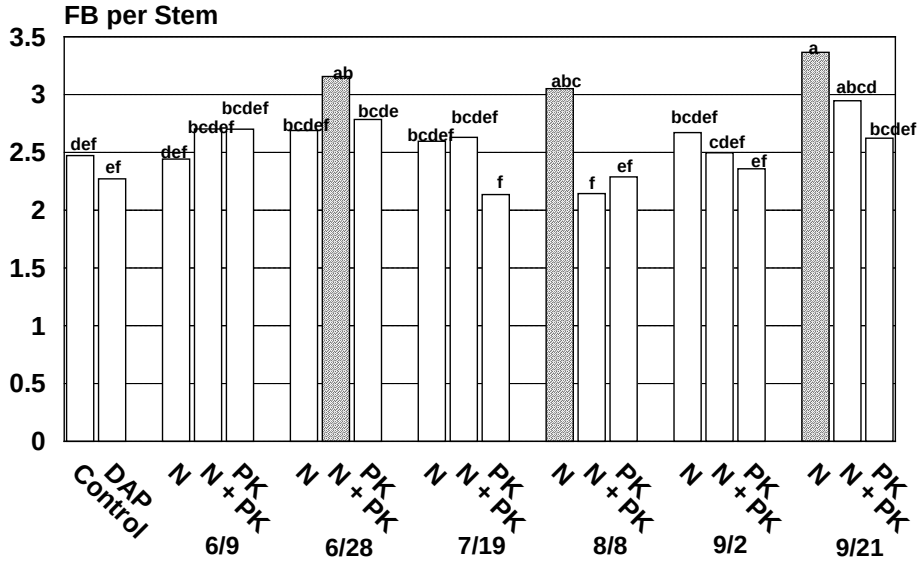


Values represent an average of 10 blocks and are significant at the 5% level. N, N+PK, and PK treatment plots also received preemergent DAP application.

Figure 14

Summer Foliar Study

Flowerbuds on unbranched stems

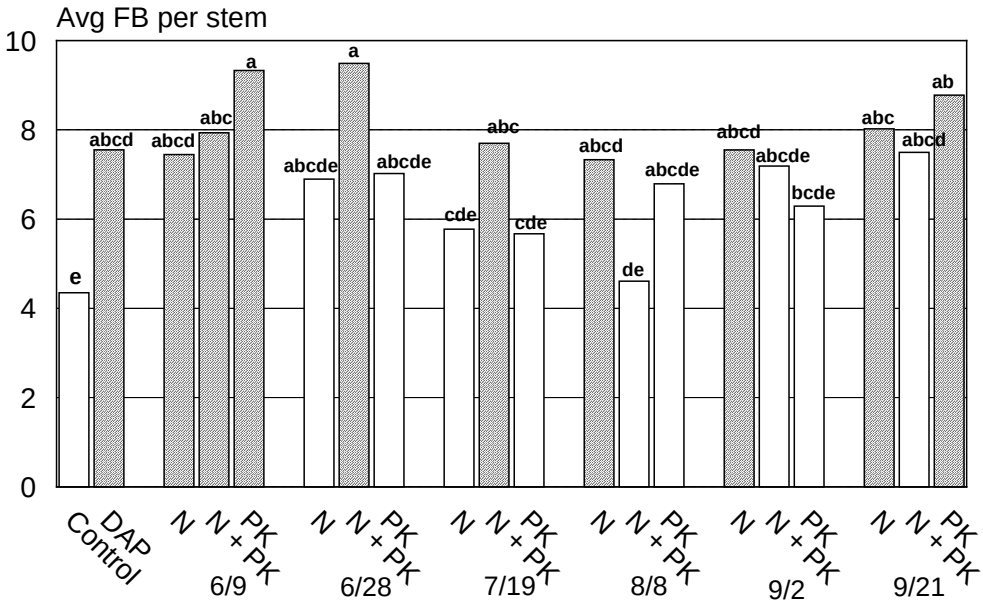


Values represent an average of 10 blocks and are significant at the 5% level. N, N+PK, and PK treatment plots also received preemergent DAP application.

Figure 15

Summer Foliar Study

Flower buds on Branched Stems

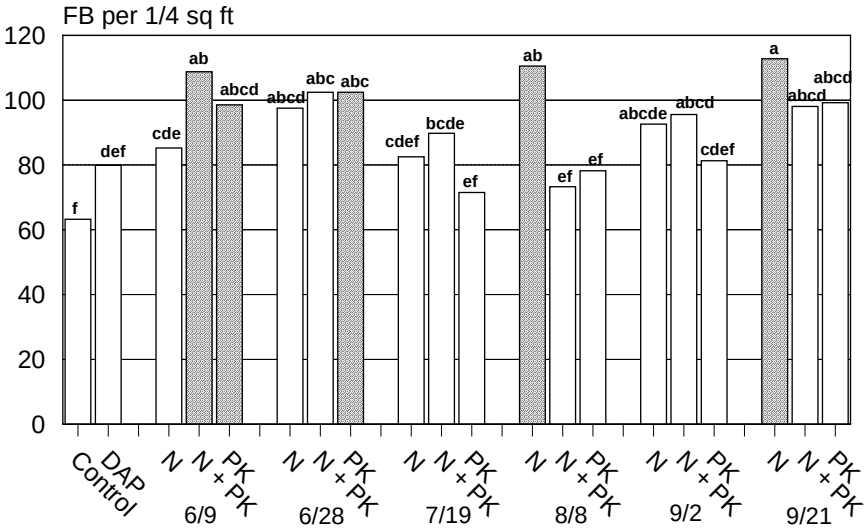


Values represent an average of 10 blocks and are significant at the 5% level. N, N+PK, and PK treatment plots also received preemergent DAP application.

Figure 16

Summer Foliar Study

Flower bud Density

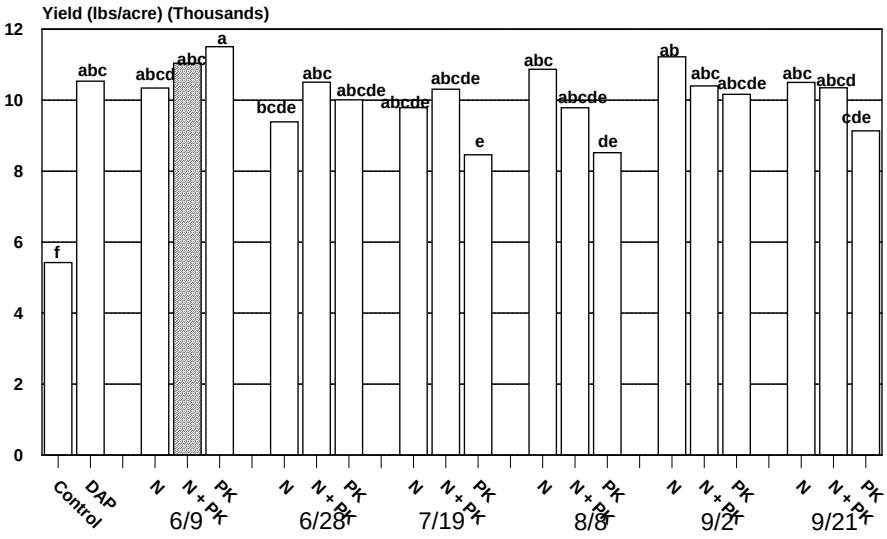


Values represent an average of 10 blocks and are significant at the 5% level. N, N+PK, and PK treatment plots also received preemergent DAP application.

Figure 17

Summer Foliar Study

Berry Yield



Values represent an average of 10 blocks and are significant at the 5% level. N, N+PK, and PK treatment plots also received preemergent DAP application.

INVESTIGATORS: John M. Smagula, Professor of Horticulture
Loretta Kreider, Scientific Technician

TITLE: Effects of Phosphite Foliar Fertilizers on disease control and fruit set of wild blueberry.

OBJECTIVES: Compare two commercial phosphite fertilizers on the growth, disease incidence (leaf spotting), fruit set and yield of wild lowbush blueberry.

IMPACT OF RESEARCH: There is evidence that foliar phosphite fertilizer application has an effect of enhancing leaf N concentrations. One explanation for this is that improved root growth enables more uptake of available N from the soil. The manufacturer of a commercial product that utilizes Ca and Cu salts instead of a K salt to deliver the phosphite suggests that improved fungal disease resistance results from its use. Plants are known to produce compounds called “phyto-alexins” to defend against stress or pathogens. Vigor-Cal-Phos™ (VCP™) is a nutrient fertilizer comprised of phosphorous salts of calcium and copper that has stimulated induced resistance and therefore improved fruit yield in a number of crops. Specifically for blueberry (presumably, highbush), they suggest that regular use of VCP™ prior to bud differentiation can “stimulate root development, phosphorus uptake and plant health during the critical bud differentiation time period”.

METHODOLOGY: A commercial lowbush blueberry field that has a history of low leaf N and P concentrations was used in this study. Eight 6 ft x 50 ft treatment plots with 5 ft alleys

between plots constituted one block. The experiment was replicated 6 times, using 6 blocks in a Randomized Complete Block design. The following treatments were randomly assigned to a treatment plot in each block:

1. Control (no fertilizer)
2. DAP (diammonium phosphate) (400 lbs/acre)
3. DAP + TKO – Phosphite (4 lbs P_2O_5/acre)
4. DAP + TKO – Phosphite (8 lbs P_2O_5/acre)
5. DAP + TKO – Phosphite (12 lbs P_2O_5/acre)
6. DAP + VCP - Phosphite (4 lbs P_2O_5/acre)
7. DAP + VCP - Phosphite (8 lbs P_2O_5/acre)
8. DAP + VCP - Phosphite (12 lbs P_2O_5/acre)

DAP was applied preemergent. The phosphite fertilizers were applied on June 23, 2006 in a volume of 67gals/acre. Due to rain shortly after the application of VCP – Phosphite, these plots were split into two 25 ft plots and the treatments were reapplied to one of the two plots on June 26, 2006. Leaf tissue samples were taken at tip dieback (July 10, 2006) and analyzed for leaf nutrient concentrations. Soil samples were taken after leaves were sampled to measure soil pH of control plots. Disease in each plot was assessed in August using a scale of 1-5, where 0 was no leaf spotting and 5 was severe leaf spotting with significant leaf drop. Stems were sampled from four ¼ square-foot quadrats randomly placed in each treatment plots in November, 2006 for determination of stem density, stem length, branching, branch length, and flower bud formation. Yield will be determined in August 2007.

RESULTS: Soil samples indicated that the average soil pH for plots receiving DAP were slightly lower than controls; pH 5.00 and 4.88 for control and DAP plots, respectively. Soil P concentrations were raised from 6.6 mg/kg in control plots to 10 mg/kg in DAP plots. Leaf samples taken from the site in 2004 had leaf N concentrations below the 1.6% standard. Leaf samples taken from control plots in 2006, however, had leaf N concentrations above the 1.6% level (Fig. 1). This can be explained by the fact that the site was treated with Velpar in 2006 which released weed pressure on the available soil N. Preemergent DAP application raised leaf N concentrations, but DAP plus TKO – Phosphite or VCP Phosphite did not raise leaf N concentrations above that of DAP alone. A second application of VCP - Phosphite, three days later, also did not enhance N uptake above that of DAP alone. Leaf P concentrations in control plots were below the standard (0.125%) and were raised by DAP alone (Fig 2). DAP plus the double application of VCP – Phosphite at the highest rate resulted in the highest leaf P concentrations. Leaf Cu concentrations (Fig. 3) suggest that the first VCP-Phosphite application was absorbed and not totally washed off since there was elevated Cu in the treatment plots receiving a single application at the highest rate. The second application of VCP-Phosphite shows a dramatic linear increase in leaf Cu concentration with increasing VCP-Phosphite concentrations.

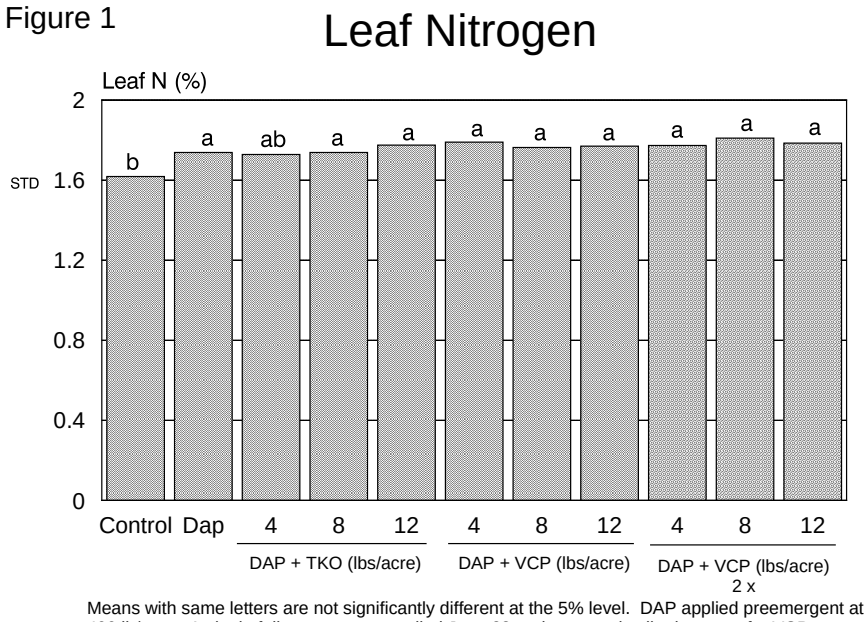
A leaf spot rating scale was established to determine the effect to treatments on condition of the blueberry foliage (Fig. 4). On this scale 1= little spotting and 5 = severe spotting with extensive leaf drop. Treatment plots were divided into approximately 4 sections and this area was evaluated and rated as to leaf spotting and leaf drop. Figure 5 shows the effect of all treatments on leaf spot rating. DAP alone resulted in lower leaf spot ratings compared to the control, but the ratings for plots receiving DAP plus TKO- Phosphite were even better

(lower) (Figs. 5 and 6). VCP-Phosphite was sprayed twice but the first spray alone was effective in reducing leaf spot (Figs. 5, 7, and 8).
Stems have been collected and are being measured for stem length, branching and flower bud formation.

CONCLUSIONS: Correcting P deficiency and raising leaf N concentrations with DAP resulted in healthier plants with less leaf spotting. DAP plus TKO – Phosphite or VCP – Phosphite resulted in a reduction of leaf spotting beyond that of DAP alone.

RECOMMENDATIONS: Rates and multiple applications of these products need to be further studied before a recommendation can be made.

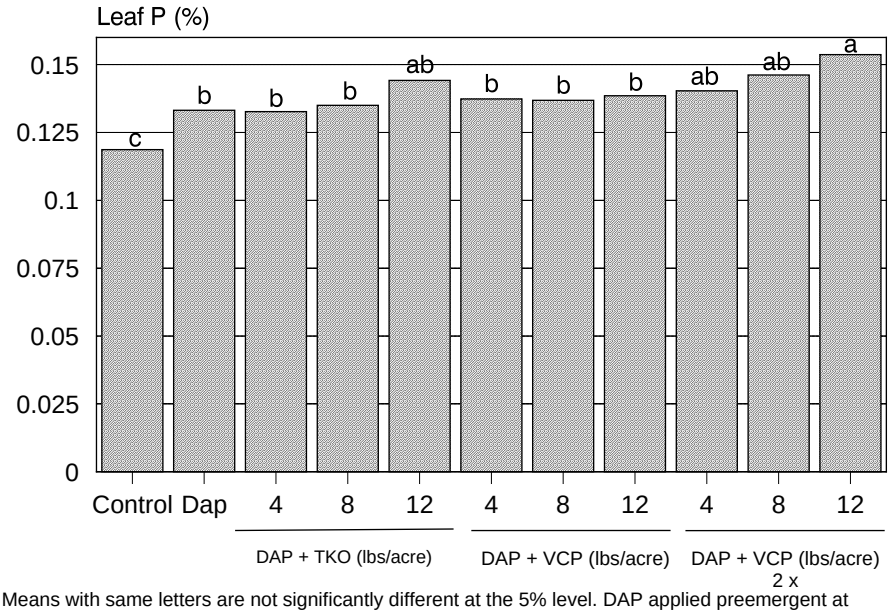
Figure 1



Means with same letters are not significantly different at the 5% level. DAP applied preemergent at 400 lb/acre. A single foliar spray was applied June 23 and a second split-plot spray for VCP - Phosphite on June 26.

Figure 2

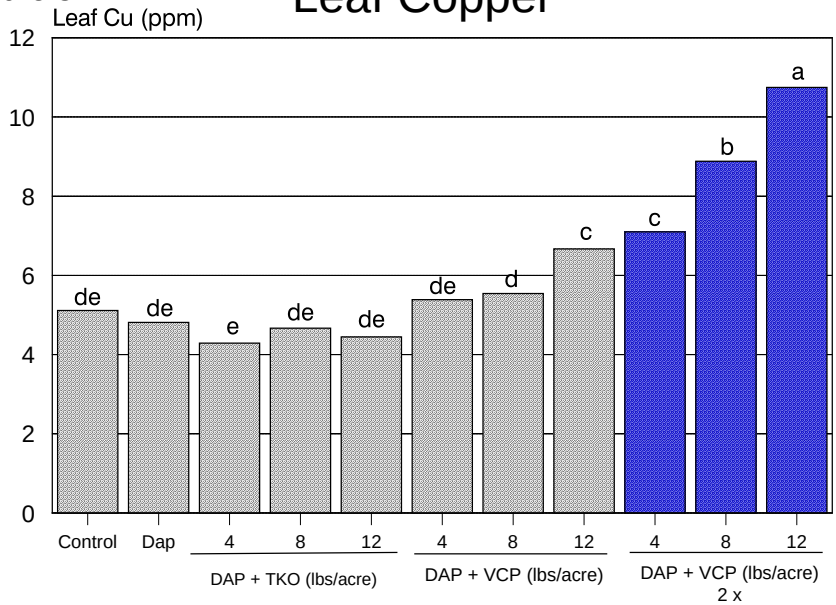
Leaf Phosphorus



Means with same letters are not significantly different at the 5% level. DAP applied preemergent at 400 lbs/acre. A single foliar spray was applied June 23 and a second split-plot spray for VCP - Phosphite on June 26.

Figure 3

Leaf Copper



Means with same letters are not significantly different at the 5% level. DAP applied preemergent at 400 lbs/acre. A single foliar spray was applied June 23 and a second split-plot spray of VCP-Phosphite was applied June 26.

Figure 4. Leaf spot rating scale.

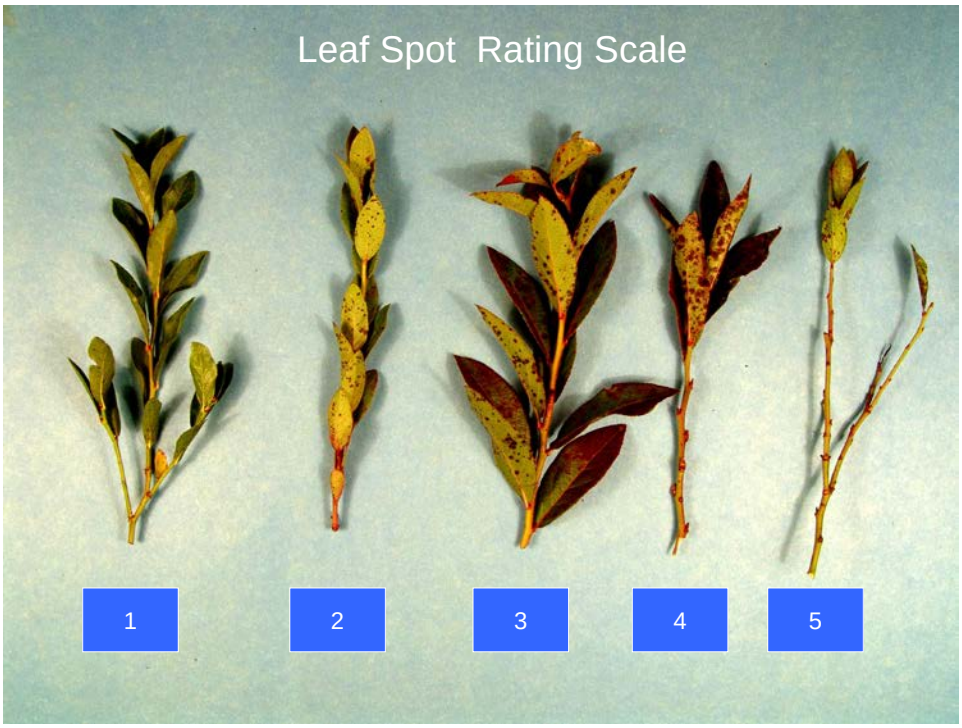
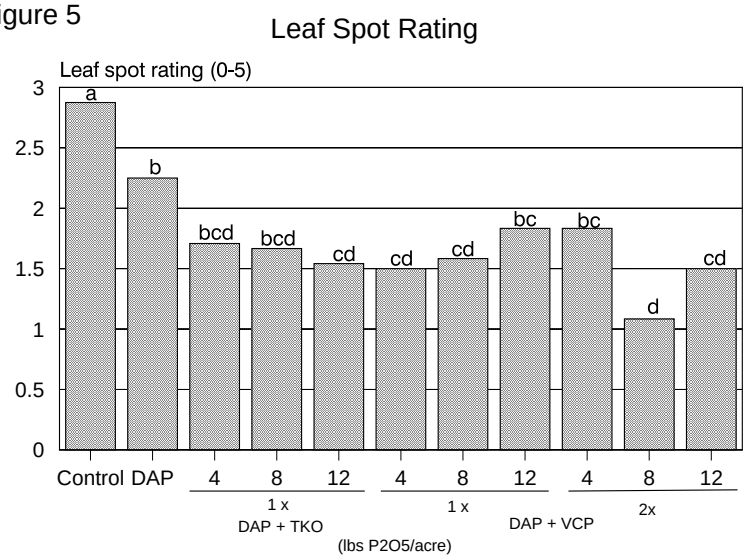


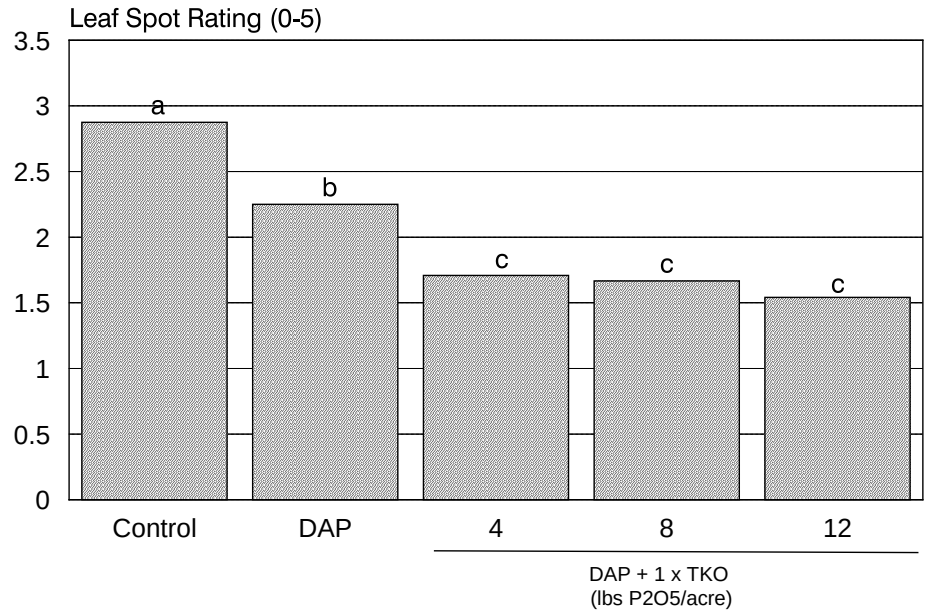
Figure 5



Means with same letter are not significantly different at the 5% level. DAP applied preemergent at 400 lbs/acre. TKO-Phosphite and VCP-Phosphite applied once (1x) on June 23 or twice (2x) on June 23 and June 26 at rates indicated.

Figure 6

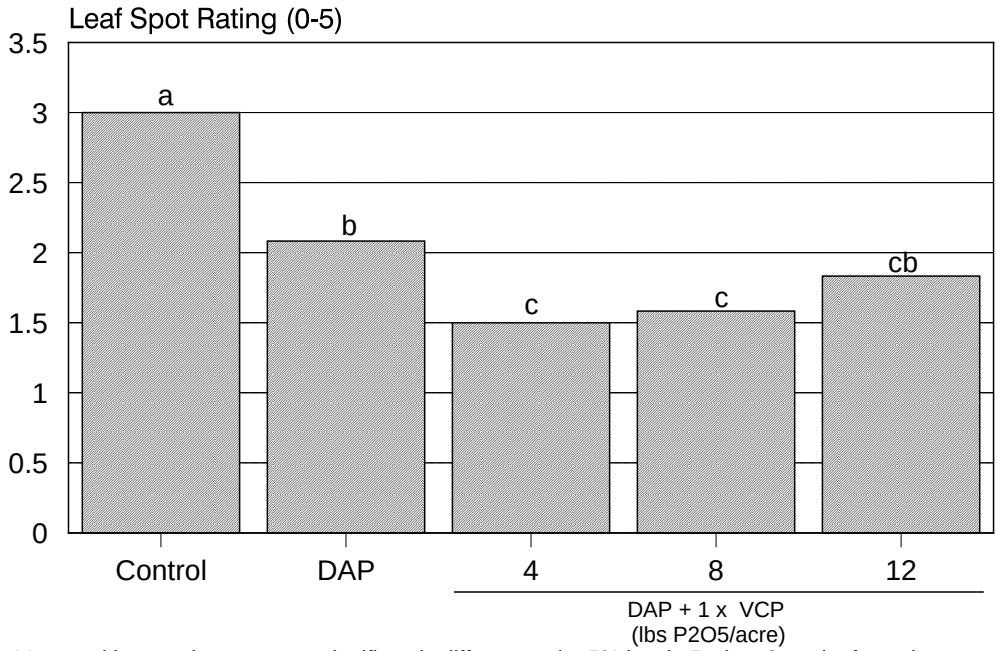
Leaf Spot Rating



Means with same letter are not significantly different at the 5% level. Rating: 0=no leaf spotting, 5=severe leaf spotting w/ leaf drop. Control, DAP, and TKO plots were not split plot in this analysis.

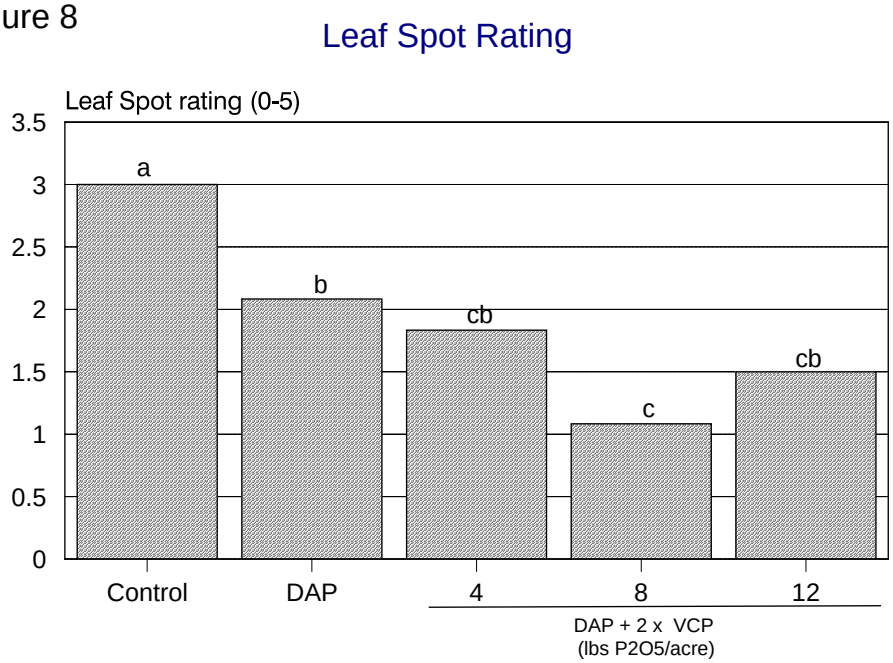
Figure 7

Leaf Spot Rating - VCP



Means with same letter are not significantly different at the 5% level. Rating: 0=no leaf spotting, 5=severe leaf spotting w/ leaf drop. Control, DAP, and 1 x VCP plots were not split plot in this analysis.

Figure 8



Means with same letter are not significantly different at the 5% level. Rating: 0=no leaf spotting, 5=severe leaf spotting w/ leaf drop. Control and VCP-Phosphite plots were analyzed as split plots in this anlaysis .

WEED MANAGEMENT

INVESTIGATOR: David E. Yarborough, Professor of Horticulture
Kerry F. L. Guiseppe, Research Assistant

TITLE: Assessment of Hexazinone Alternatives for Weed Control in Wild Blueberries

METHODOLOGY: a) A split block design was established on six wild blueberry fields throughout the state to obtain a diversity of soil types and weed species. A block was established in the Maine towns of Union, Belfast, Penobscot, Orland, Township 19 and at the Blueberry Hill Experimental Farm in Jonesboro. A 64' x 48' block was comprised of 12' X 48' treatment plots including an untreated control, mesotrione 6 oz/a (444 ml/ha) pre-emergence, 3 oz/a (222 ml/ha) pre-emergence and 3 oz/a (222 ml/ha) post-emergence (same plot), and 3 oz/a (222 ml/ha) post-emergence. At right angles a 24' X 64' plot of either untreated control or a hexazinone treatment at 64 oz/a (1 kg/ha) was applied to give a total of eight combinations. Pre-emergence treatments were sprayed on 8 May (Jonesboro), 9 May (Union and Belfast), 10 May (Penobscot and Orland), and 11 May 2006 (Township 19). Post-emergence treatments were sprayed on 6 June (Columbia Falls and Jonesboro), 7 June (Orland and Penobscot) and 9 June 2006 (Union and Belfast). Treatment effects were assessed for broadleaf, fern and grass weed cover and wild blueberry phytotoxicity from four 1m square subplots within each treatment. The first weed cover evaluation was on June 19 and 23, while the second occurred on August 14 and 23, 2006. A weed list of the species not controlled was recorded for each site. Species that were found for each treatment are listed in Table 1.

b) A carryover weed control assessment and yield samples were taken from the six sites treated in 2005. Weed assessment occurred on June 19 and 23. Plots were harvested using four 1m square subplots within each treatment and weights are in pounds per plot. Blocks were harvested on 26 July (Union and Northport), 1 August (Jonesboro), and 3 August (Lamoine and Township 19).

RESULTS: a) Blueberry cover was significantly affected by treatment type (Figure 1). Overall blueberry cover was rated lower in August than June. This is likely due to the emergence of thick weed patches in several of the treatments, which reduced the observed blueberry cover. Blueberry cover was the lowest in untreated controls for both the June and August evaluations. This is most likely due to the high weed cover in the untreated controls. The mesotrione 3 oz/a post-emergence treatment also had lower blueberry cover than the other treatments. A small amount of phytotoxicity (less than 1% cover) in the form of burned looking leaves was noted for this treatment in the June evaluation, but the weed cover was also high in this treatment which is more likely the cause for low blueberry cover. Grass cover (Figure 2) was highest in the control, post-emergence 3oz/a and pre-emergence 6 oz/a treatments for both evaluations. Hexazinone combined with the 3 oz/a post-emergence or 3 oz/a pre and 3 oz/a post-emergence mesotrione had the best control of grass cover in both evaluations. The 3 oz/a pre and 3 oz/a post-emergence mesotrione treatment without hexazinone on the second evaluation date was statistically the same as with hexazinone. Broadleaf weed cover (Figure 3) was highest in the untreated control and the 3 oz/a pre-emergence treatment. The combinations of hexazinone with mesotrione resulted in the lowest broadleaf cover ratings as did the 3 oz/a pre and 3 oz/a post-emergence treatment.

There was zero fern cover in the June evaluation. In the August evaluation, 6 oz/a pre-emergence treatment had less than 1 % fern cover so the results are not presented.

b) For the six blocks treated in 2005, blueberry cover was significantly lower in untreated control and the post-emergence flumioxazin applications (Figure 4). Grass cover was the highest in the post-emergence flumioxazin treatment and lowest in the pre-emergence flumioxazin plus hexazinone treatment. Broadleaf cover was significantly higher in the untreated control (Figure 5). There were no significant differences in fern cover among the treatments. Blueberry harvest weight was significantly less in pre and post-emergence applications of flumioxazin, but was highest in post-emergence flumioxazin or mesotrione plus hexazinone (Figure 6).

CONCLUSIONS: The mesotrione applications at the higher rate preemergence or at the low rate per and post emergence gave equivalent control to the hexazinone application. When these applications were combined with hexazinone additional suppression of both grasses and broadleaf weeds was obtained. These treatments, except for the post emergence flumioxazin resulted in higher yields than the control and equivalent yields to the hexazinone standard treatment. Mesotrione may be used alone or in combination to provide weed suppression and increase in yields in lowbush blueberries.

RECOMMNEDATIONS: Continue to evaluate the successful treatments on more sites to obtain additional data over more sites and environmental conditions and submit data for IR-4 registration trials.

Table 1. Plant species list for pre and post-emergence mesotrione trial

First Evaluation- June
Untreated control - sheep sorrel, bunchberry, goldenrod, indian tobacco, fireweed, wild lettuce, goldenrod, common rush, wild oat grass, ragweed
Without hexazinone
3 oz/a pre and 3 oz/a post-emergence - bunchberry, goldenrod, dogbane, wild oat grass, sheep sorrel
6 oz/a pre-emergence - sheep sorrel, bunchberry, yellow cinquefoil, fireweed, birch, ragweed, indian tobacco, wild blue lettuce
3 oz/a post-emergence - wild oat grass, bunchberry, indian tobacco, sheep sorrel, birch, wild lettuce, ragweed, common rush, goldenrod
With Hexazinone
1 lb/a hexazinone - sheep sorrel, bunchberry, indian tobacco, fireweed, goldenrod, wild lettuce, ragweed
3 oz/a pre and 3 oz/a post-emergence - bunchberry, honeysuckle, dogbane, bunchberry, quack grass, ragweed, rose
6 oz/a pre-emergence - dogbane, bunchberry, fireweed, quack grass, ragweed
3 oz/a post-emergence - fireweed, ragweed, goldenrod, wild lettuce, wild oat grass, bunchberry
Second Evaluation – August
Untreated control - goldenrod, purple vetch, wild oat grass, bunchberry, sheep sorrel, birch, wild lettuce, black eyed susan, buttercup
3 oz/a pre and 3 oz/a post-emergence - goldenrod, wild oat grass, birch, wild lettuce, St. johnswort, quack grass, meadow sweet
6 oz/a pre-emergence - ragweed, goldenrod, dogbane, birch, quack grass, meadow sweet,
3 oz post-emergence - birch, wild lettuce, goldenrod, quack grass, wild oat grass, black eyed susan, meadow sweet, purple vetch
With Hexazinone
Hexazinone 1 lb/a - goldenrod, rose, wild oat grass
3 oz/a pre and 3 oz/a post-emergence - goldenrod, birch, wild lettuce, ragweed
6 oz/a pre-emergence - ragweed, dogbane, bunchberry, goldenrod, fireweed, St. johnswort
3 oz/a post-emergence - goldenrod, St. Johnswort, joe pie weed,

Figure 1. Blueberry cover following herbicide treatment, 2006

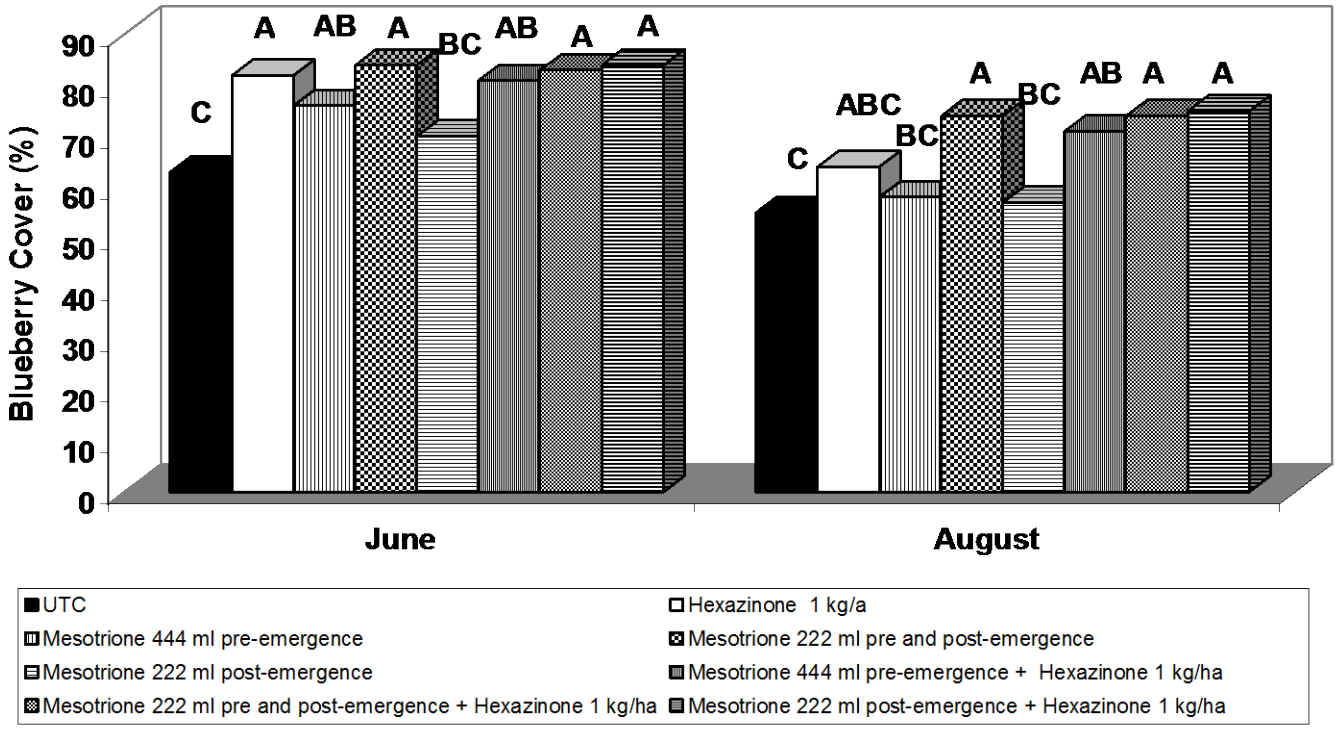


Figure 2. Grass cover following herbicide treatment, 2006

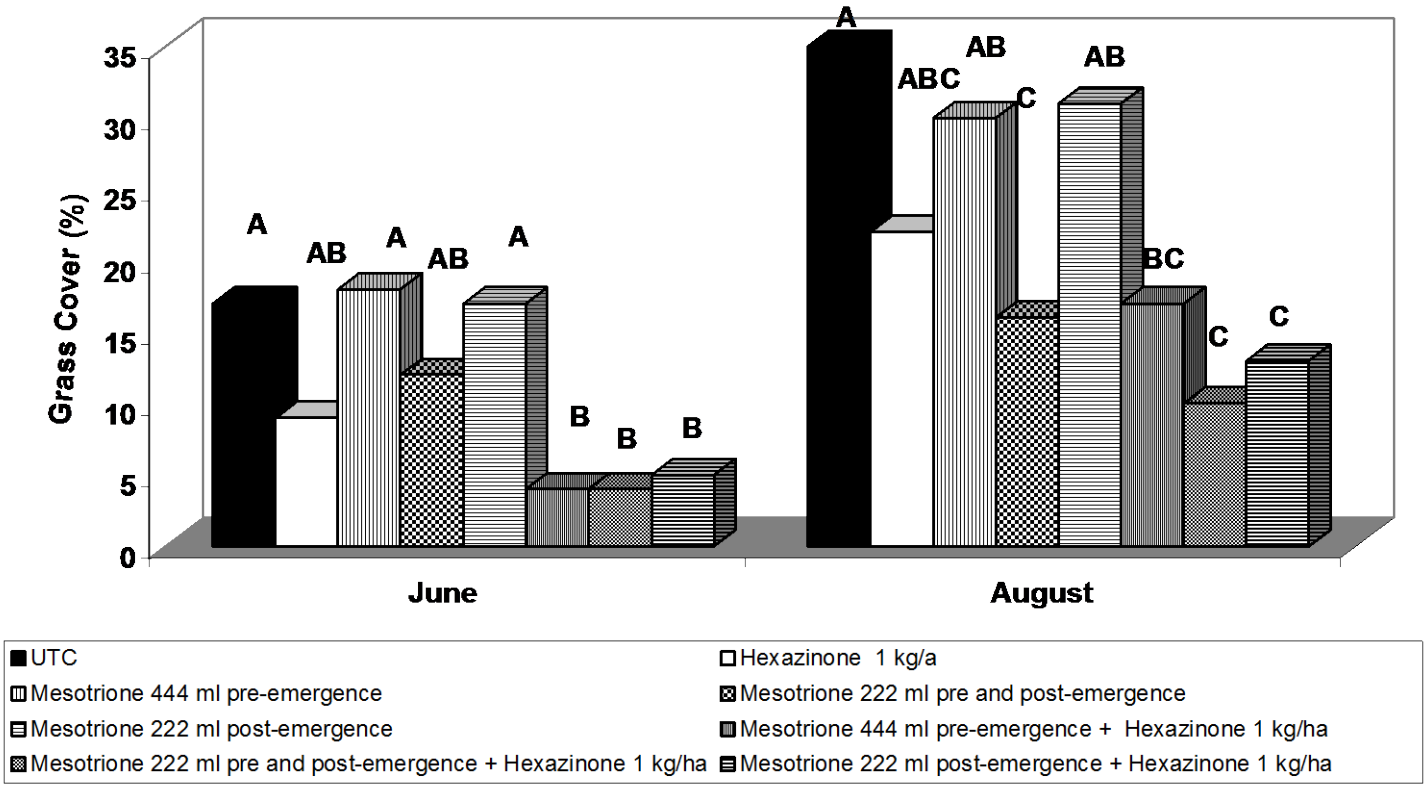


Figure 3. Broadleaf weed cover following herbicide treatment, 2006

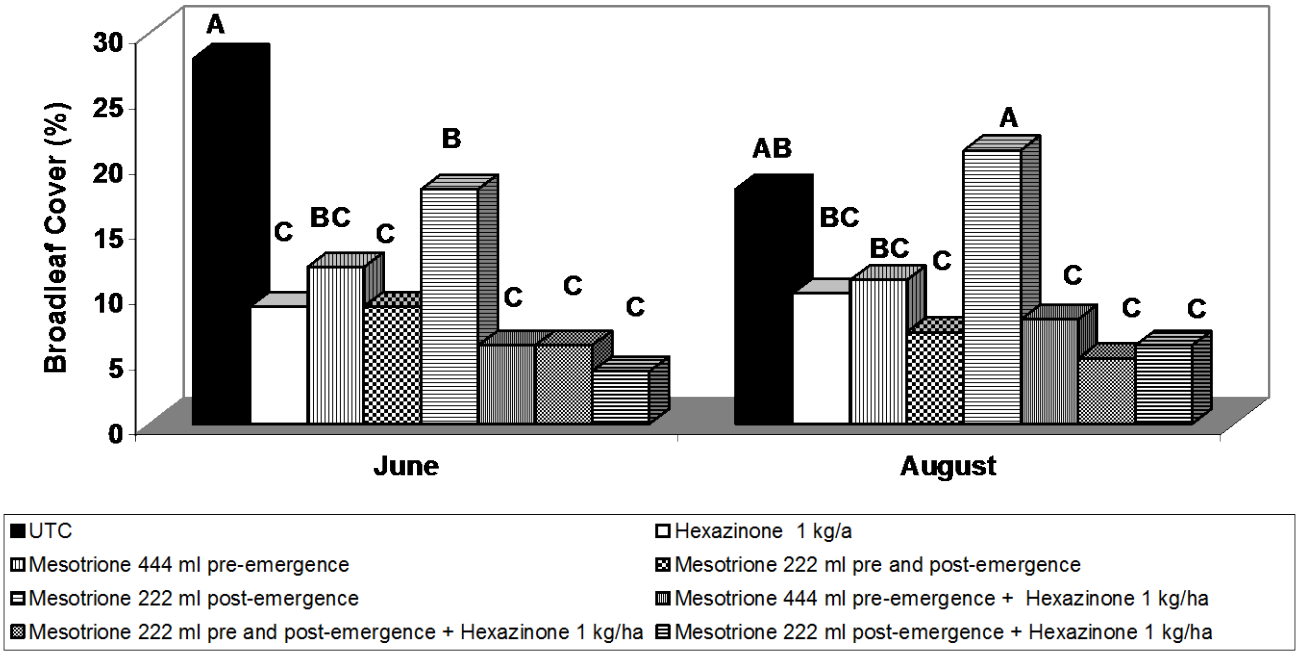


Figure 4. Blueberry and grass cover following 2005 herbicide treatment

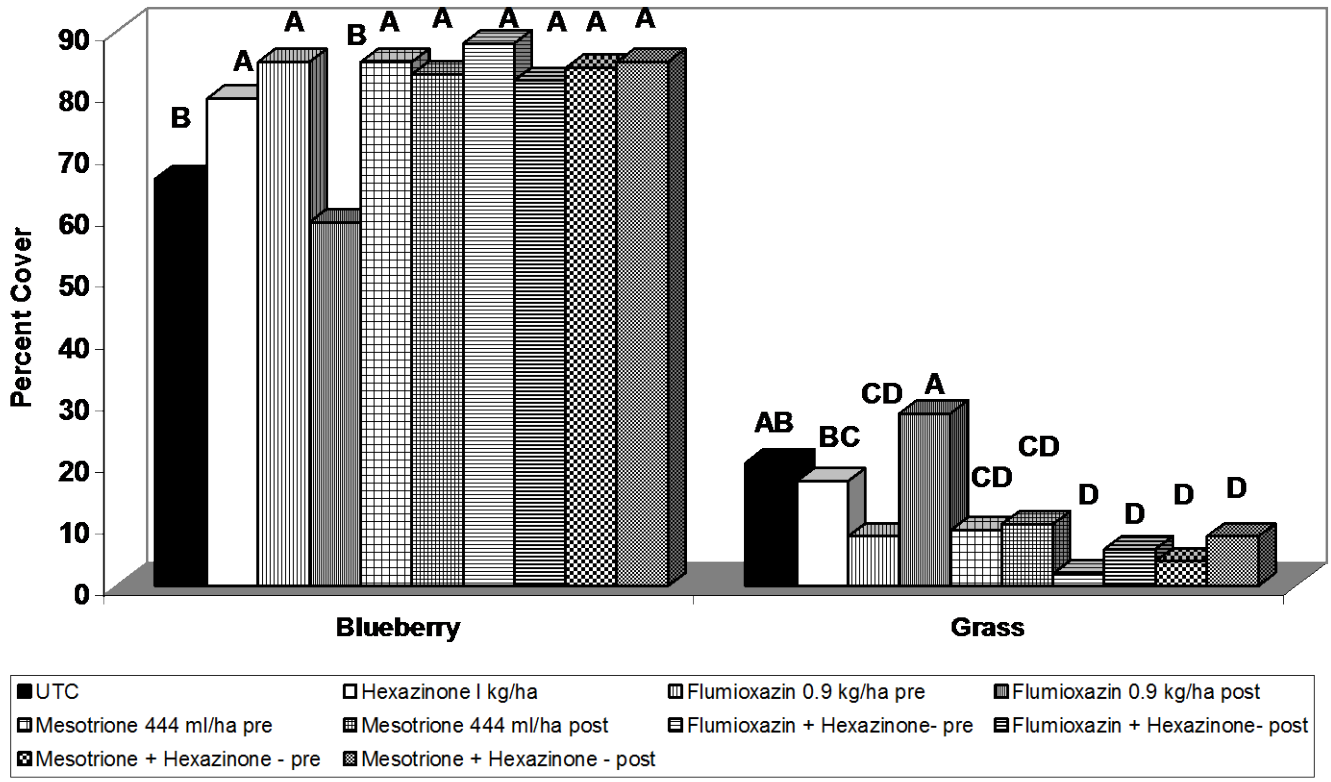


Figure 5. Broadleaf and fern cover following 2005 herbicide treatment

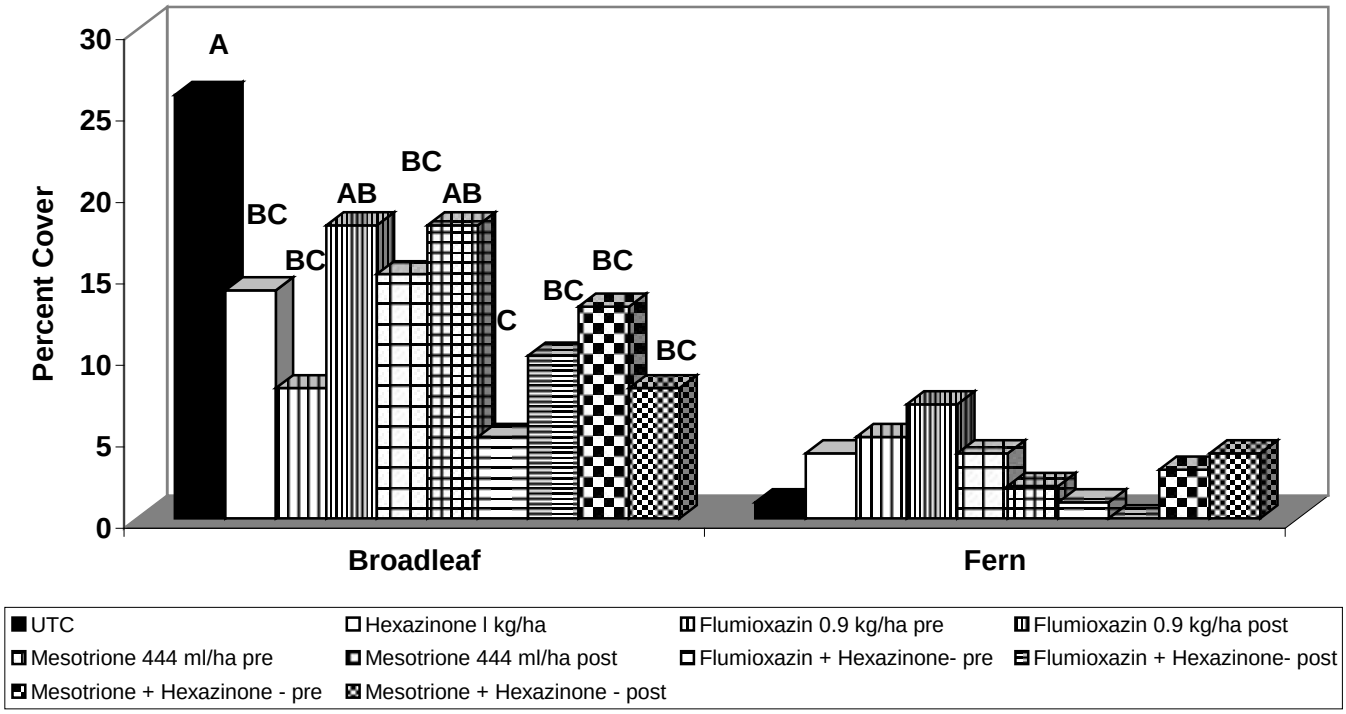
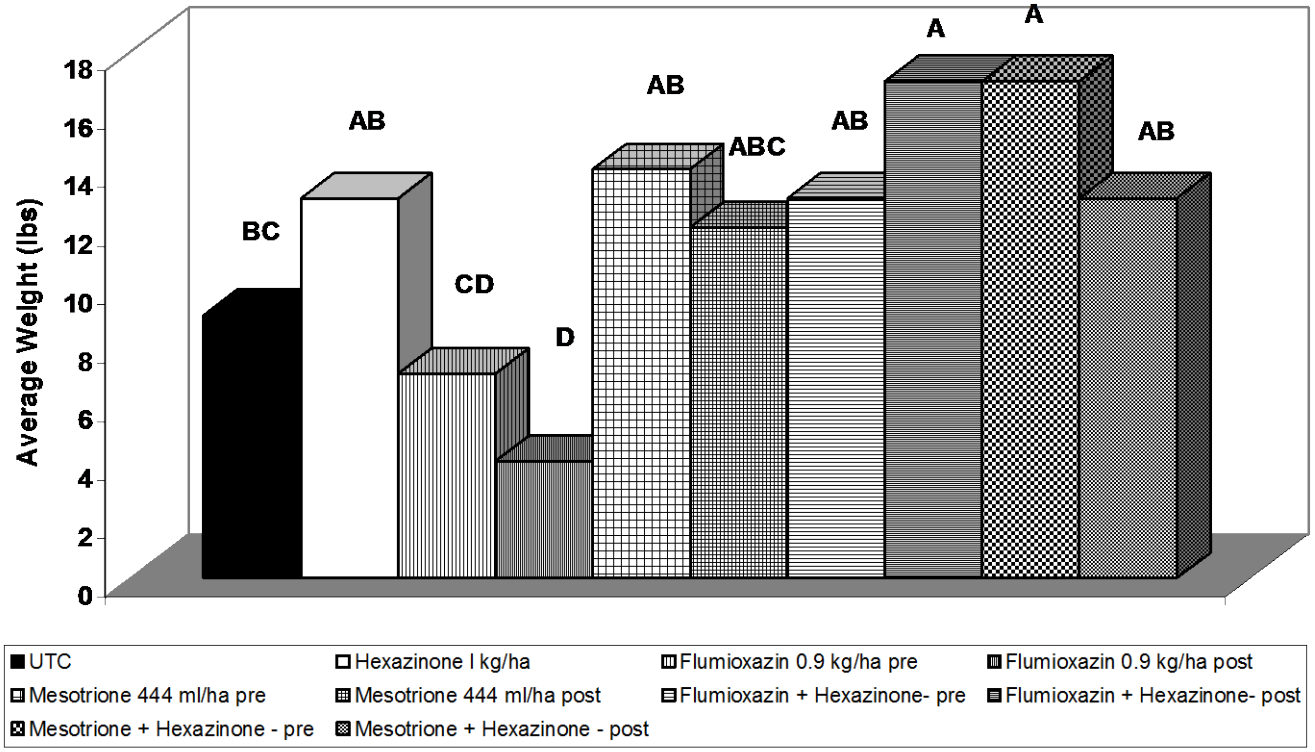


Figure 6. Blueberry harvest weight for 2005 herbicide treated sites.



INVESTIGATOR: David E. Yarborough, Professor of Horticulture
Kerry F. L. Guiseppe, Research Assistant

TITLE: Evaluation of Fall Applications of Tribenuron Methyl for Bunchberry Control in Wild Blueberries.

METHODOLOGY: Tribenuron methyl was applied with a surfactant on non-cropping fields at Blueberry Hill Experimental Farm in Jonesboro in the fall of 2006 to evaluate its effectiveness in controlling bunchberry. Treatments included an untreated control and tribenuron methyl treated on 29 August, 26 September, and 17 October 2006. Each treatment was replicated 10 times. Bunchberry and wild blueberry cover were rated before treatment on 29 August 2006 and will be rated in the summer of 2007.

RESULTS: Results from 2004 treatments presented in 2005 showed good suppression of bunchberry with minimal injury to blueberry. Results concerning the effectiveness of the treatments will be reported following evaluation of bunchberry and blueberry cover from this experiment in 2007 and results from Canadian studies.

CONCLUSIONS: Conclusions on the best timing and rate will be based on evaluations from 2005 and data obtained during the summer of 2007.

RECOMMENDATIONS: Continue to evaluate treatment to provide data to DuPont for registration of tribenuron methyl fall treatment.

INVESTIGATOR: David E. Yarborough, Professor of Horticulture
Kerry F. L. Guiseppe, Research Assistant

TITLE: Evaluation of spot treatments of Tribenuron Methyl, Ultim and Roundup for weed control in Wild Blueberries

METHODOLOGY: Tribenuron Methyl at 1 oz/gal with a surfactant was used to spot treat 10, 1-meter square plots with bracken fern, yellow loosestrife and purple vetch. Treatment occurred on 24 July 2006. An equal number of untreated plots were used as a control. On 15 June 2006. Ultim was applied at the rate registered in Canada, 4.2 g/100L water with a 0.25% surfactant and roundup at 2% v/v solution to spot treat 10 bulrush clumps with an equal number of untreated plots as a control. Efficacy of control and phytotoxicity to wild blueberries was rated on 17 August 2006.

RESULTS: The tribenuron methyl treatment significantly reduced bracken fern, yellow loosestrife and purple vetch cover (Figs 1-3). There were no significant reductions in blueberry cover. No blueberry phytotoxicity was observed. Bulrush was significantly lower in the treated plots (Figure 4) but the reduction in bulrush cover was not below 75%. No blueberry phytotoxicity was observed, though there was no blueberry cover on the untreated/treated bulrush plots.

CONCLUSIONS: Tribenuron methyl is a promising for spot treatment of brackenfern, loostrife and vetch. Ultim did not provide sufficient control of bullrush.

RECOMMENDATIONS: Continue to obtain more data to support registration of tribenuron methyl.

Figure 1. Bracken fern cover following July tribenuron methyl treatment

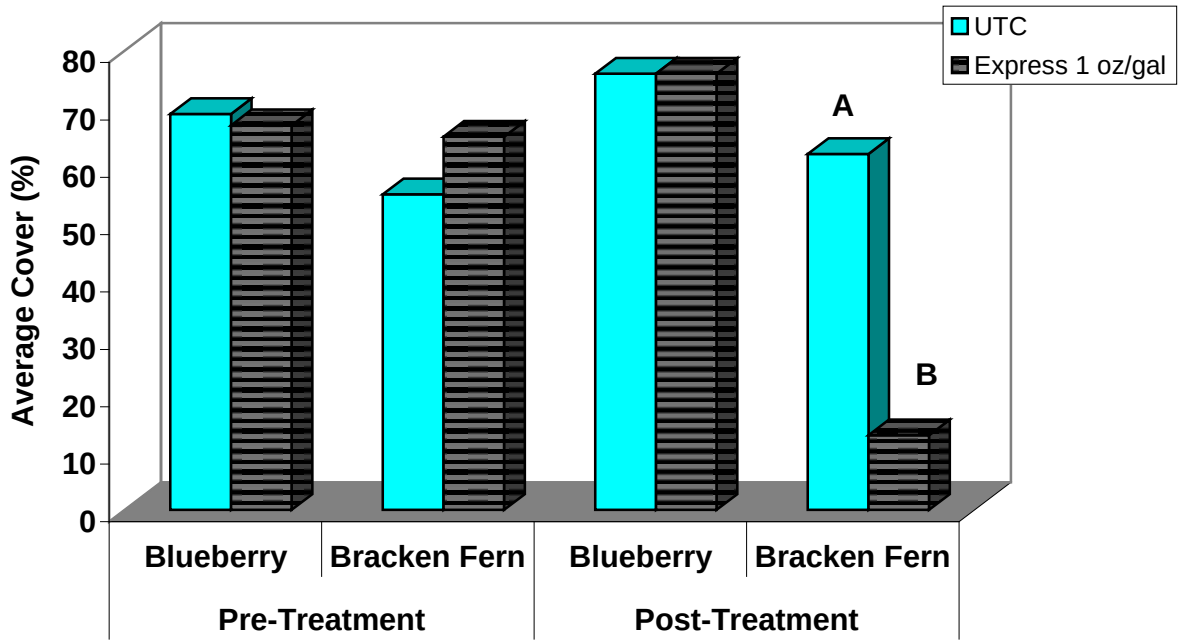


Figure 2. Yellow Loosestrife cover following July tribenuron methyl treatment

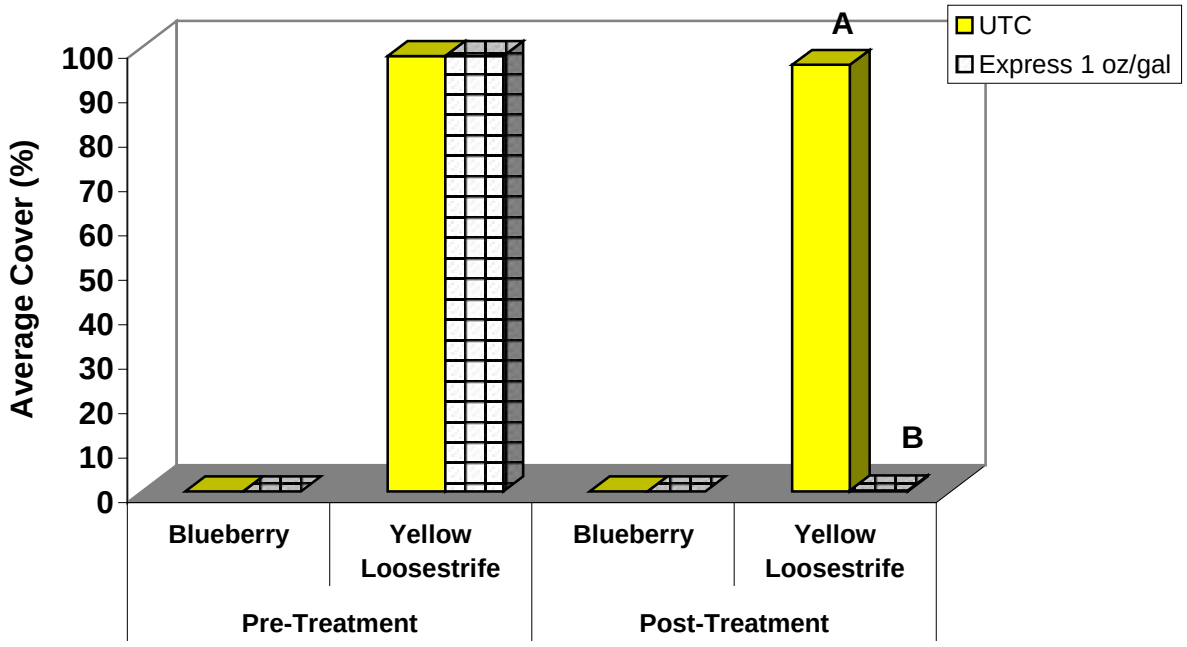


Figure 3. Purple Vetch cover following July tribenuron methyl treatment

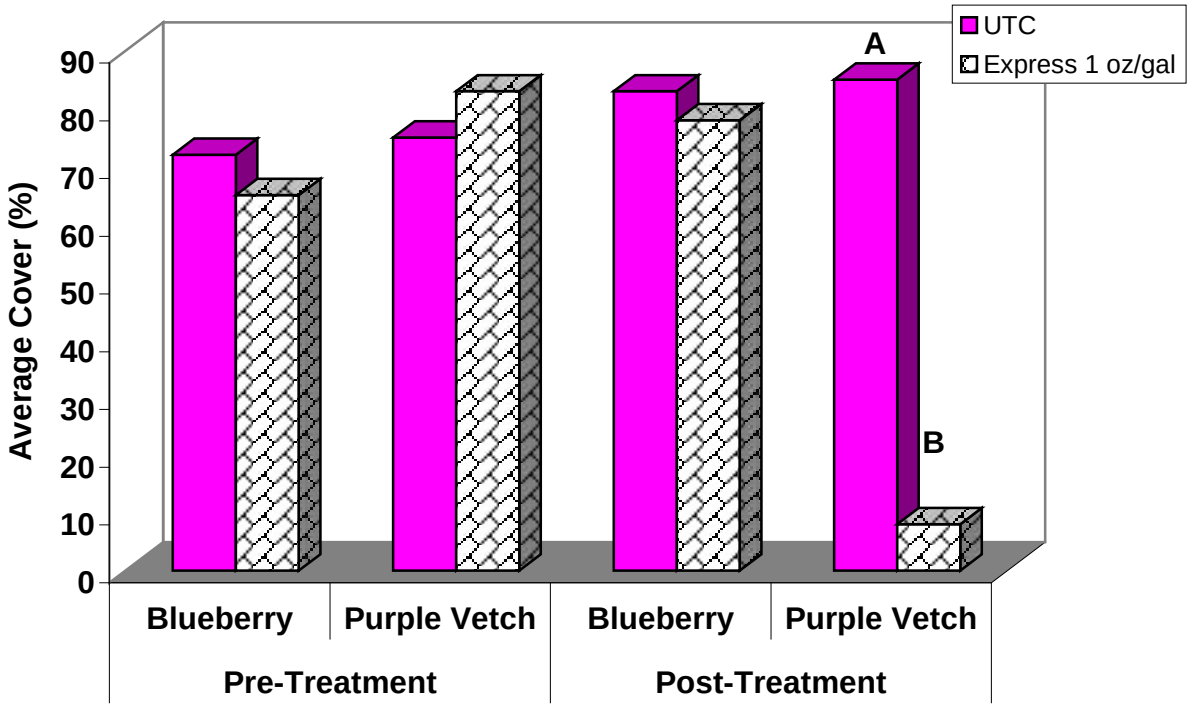
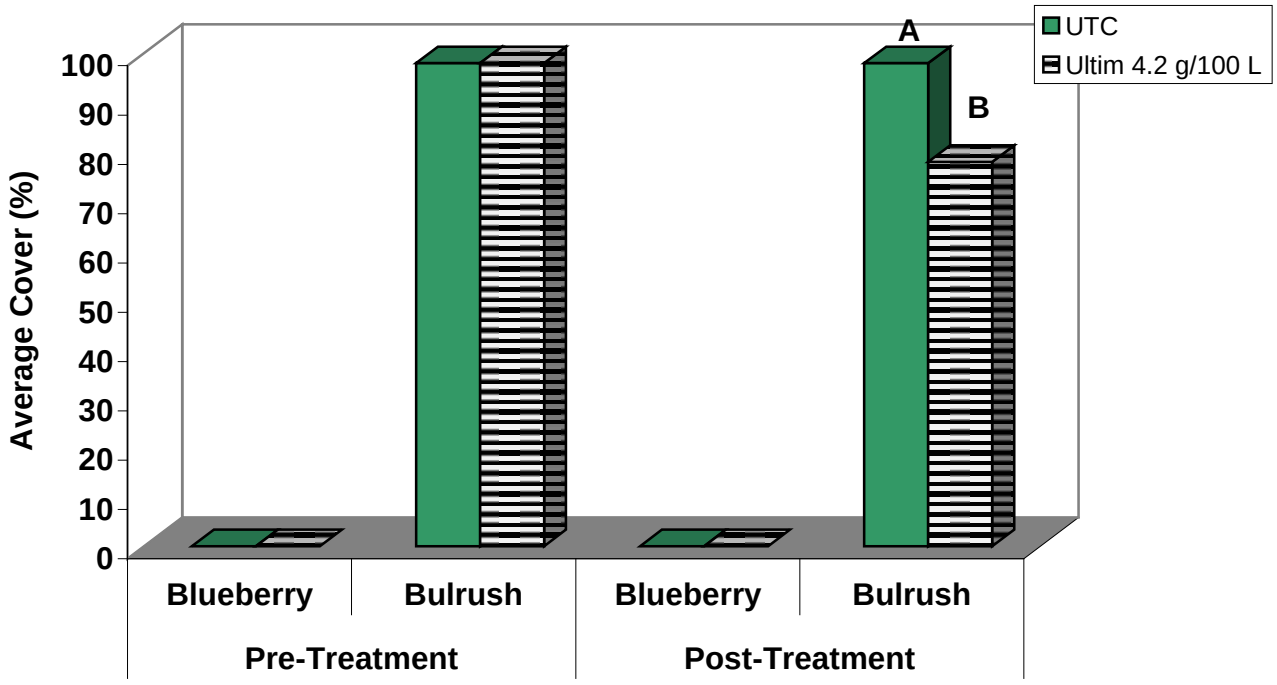


Figure 4. Bulrush cover following June Ultim treatment



EXTENSION

INVESTIGATOR: David E. Yarborough, Extension Blueberry Specialist

TITLE: Wild Blueberry Extension Education Program in 2006

METHODS: Conduct an educational program that will stress the use of best management practices in an integrated crop management program, which will improve the efficiency of culture and minimize the use of unnecessary pesticides and fertilizers. Conduct spring grower meetings and field days to introduce and reinforce the use of best management practices, integrated crop management and sound business management principles. Provide management information through the blueberry newsletters, fact sheets in the wild blueberry grower's guide both in print form and on the web at www.wildblueberries.maine.edu , 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 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 program consisted of field demonstration sessions conducted three times in three counties. Program requirements have been better defined over the past years, new fact sheets have been developed and better examples have been provided, such as weed mapping and explanation of decision making for blight control and perimeter spraying of insecticides for blueberry maggot fly control.

Note: On sabbatical leave September 2005- March 2006.

Meetings Attended:
IR-4 Regional Meeting, Geneva, NY, October 2-3, 2005.
National Berry Crop Initiative, Geneva, NY, October 25-26, 2005.
WBANA Canada, Saint Felicien, QU, October 28-29, 2005.
Northeastern Weed Science Society 60th Annual Meeting, Providence, RI. January 3-6, 2006.
IR-4 National Education Conference, Phoenix, AZ, February 28-March 2, 2005.
Syngenta Crop Protection Meeting, Dundee, NY, March 16-17, 2006.
10th North American Blueberry Research and Extension Workers Conference. Tifton, GA, June 4-8, 2006.

WBPANS field day, Debert, NS, July 22, 2006;
New Brunswick field day, Val-Doucet, NB, July 29, 2006.
Wild Blueberry Research and Extension Workers and WBANA Meeting, Moncton, NB, October 26-27, 2006.

Professional Improvement Activities:
Delivered the following talks at Professional Meetings:

Yarborough, D. and Guiseppe, K. 2006. Reducing soil pH to control weeds in wild blueberries. Proceedings of the 10th North American Blueberry Research and Extension Workers Conference. Tifton, GA. June 4-8, 2006 and Annual Meeting of the Research and Extension Workers and Wild Blueberry Association of North America. Moncton, NB, October 26-27, 2006.

Yarborough, D. and Guiseppe, K. 2006. Evaluating new pre and post-emergence herbicides for weed control wild blueberries. Proceedings of the 10th North American Blueberry Research and Extension Workers Conference. Tifton, GA. June 4-8, 2006.

Yarborough, D. and Guiseppe, K. 2006. An Assessment of mesotrione and hexazinone on weeds in wild Maine blueberries. Annual Meeting of the Research and Extension Workers and Wild Blueberry Association of North America. Moncton, NB, October 26-27, 2006.

Yarborough, D. and Guiseppe, K. 2006. An assessment of pre and post-emergence herbicide application on weeds in Maine wild blueberries. Northeastern Weed Science Society 60th Annual Meeting, Providence, RI. January 3-6, 2006. 60:21-23.

Grower meetings:

Wild Blueberry Spring Grower Meetings: Waldoboro, March 21; Ellsworth, March 22; Machias, March 25, 2006 (Frank Drummond coordinated).

Blueberry Hill Farm Annual Field Day on July 19, 2005.

ICM sessions:

ICM field training sessions: *Knox/Lincoln Counties:* May 2, May 30 and June 27; *Washington County:* May 3, May 31 and June 28; *Hancock County:* May 4, June 1 and June 29, 2006.

Teaching:

Taught PSE203 Weed Identification, 3 credits, Fall 2006 for Eric Galandt who is on sabbatical leave.

Extension Presentations:

Taming the Wild Blueberry and Upland Cranberry Production for LCH110 Horticultural Science class at Orono, ME April 7, 2006.

Wild Blueberry Production, Bar Harbor Health Summit Tour, Bar Harbor, ME August 9, 2006.

Explained Maine wild blueberry production to hundreds of attendants of the Big E Agricultural Fair in Springfield, MA on September 22-24, 2006.

Wild blueberry production and Wild Blueberry IPM, Maine legislative tour, Machias, ME August 17-18, 2006.

Wild Blueberry production, North American Strawberry Growers Association 9th Annual Summer tour, Grey, ME August 12, 2006.

Wild blueberry production and Wild blueberry IPM, North American Blueberry Council Fall Tour, October 4-5, 2006.

Taming the wild blueberry, Go away tour, Bar Harbor, October, 17, 2006.

Publications:

Perkins, B. L., D. Yarborough, K. Guthrie, and R. Bushway. 2006. Detection of Hexazinone in Maine's Groundwater- A Nine Year Study. Acta Horticulturae 715:329-335, MAFES 2708.

Starr, G. C. and D. E. Yarborough. 2006. Influence of Vapor Deposition on Wild Blueberry Water Requirements in a Humid Coastal Climate. Acta Horticulturae 715:323-328, MAFES 2706.

Yarborough, D. E. 2006. Innovations in Weed Management in Wild Blueberry Fields in Maine. Acta Horticulturae 715:197-202, MAFES 2701.

Yarborough, D. and Guiseppe, K. 2006. Reducing soil pH to control weeds in wild blueberries. Proceedings of the 10th North American Blueberry Research and Extension Workers Conference. Tifton, GA. June 4-8, 2006. pg 73-81. and Annual Meeting of the Research and Extension Workers and Wild Blueberry Association of North America. Moncton, NB, October 26-27, 2006. pg 5.

Yarborough, D. and Guiseppe, K. 2006. Evaluating new pre and post-emergence herbicides for weed control wild blueberries. Proceedings of the 10th North American Blueberry Research and Extension Workers Conference. Tifton, GA. June 4-8, 2006. pg 73-81.

Yarborough, D. and Guiseppe, K. 2006. An Assessment of mesotrione and hexazinone on weeds in wild Maine blueberries. Annual Meeting of the Research and Extension Workers and Wild Blueberry Association of North America. Moncton, NB, October 26-27, 2006. pg 7.

Television/radio/newspaper Interviews 2006:
Note: On sabbatical leave September 2005- March 2006.

The number of sources and multiple contacts are to illustrate that I am regarded as a reliable source in the media and that this interaction gives exposure and credibility to the University of Maine as a good, unbiased source of information.

Boston Globe: June 13
Chanel 2 Bangor: June 26
Ellsworth American: June 19, July 21, August 14, 22 October 25 (Editorial)
Fruit Growers News (MI): September 14
Maine Public Radio: June 13
Portland Press Herald: September 11
Seattle Times: June 19

Public testimony
Public testimony Maine Board of Pesticides Control, Augusta, ME: May 12, 2006.

Other program activities:
I am the principle investigator for USDA/CSREES *Lowbush Blueberry Research*, which provides funds for all aspects of wild blueberry production. 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.

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.

Since 1997, I have petitioned the Board of Pesticides Control each year to request a Section 18 for the use of the fungicide Orbit for the control of mummy berry disease in wild blueberry fields in Maine. I developed the original petition and continue to update it each year but 2006 is expected to be the last year for this label.

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 that are interested.

I serve on the peer review committee for the Department of Plant Soil and Environmental Sciences and the joint peer review committees of Renae Moran & Mark Hutton. These review activicties take one week a year.

I serve on the graduate committees of:
Theresa Thornton MS Student Major advisor L. Osher 2004 -August 2005
Kirsten McGovern MS student Major advisor S. Annis 2005 - present
Beth Ann Choate PhD student, Major advisor F. Drummond 2006 - present
Jesse Swift MS Student, Major advisor D. Yarborough 2006 - present

Wild Blueberry Fact Sheets – 2006

Revised
Fact Sheet #209 (UMCE #2001) 2006 Insect Control Guide for Wild Blueberries
Fact Sheet #239 (UMCE #2025) 2006 Weed Control Guide for Wild Blueberries
Fact Sheet #219 (UMCE #2000) 2006 Disease Control Guide for Wild Blueberries
Added on web site
Crop Statistics - Wild Blueberry Acres by Counties

CONCLUSION: Growers are participating in IPM programs in the four primary 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, reducing the rate of hexazinone used, being able to control blight, identifying and controlling weeds, being able to detect and control insects and the blueberry maggot fly and that they used 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. 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 hexazinone groundwater survey I have conducted from 1992 through 2004 provides information on the movement of this herbicide into the groundwater that is used at ICM meetings. This information has been used by the Department of Agriculture in both developing and in updating Best Management Practices and by the Board of Pesticides control in deciding to continue use of hexazinone in Maine. The most recent survey conducted from the newsletter mailing list indicates that grower's 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. 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.

RECOMMENDATIONS: Continue to support Extension educational program.

EXTENSION

INVESTIGATOR: David E. Yarborough, Extension Blueberry Specialist
Kerry F. L. Guiseppe, Research Assistant

TITLE: Cultural Weed Management Using pH.

OBJECTIVES: To evaluate the effect of lowering pH on weed populations in wild blueberry fields.

IMPACT OF RESEARCH/BENEFIT TO INDUSTRY: If weed pressure is reduced then fewer herbicide inputs will be needed. This will provide a cost effective means to reduce reliance on herbicide applications.

METHODOLOGY: Six sites were established in 2000 in Appleton, W. Rockport, Machiasport, Whiting and Wesley (2), four sites were established in 2001 in Union, Jonesboro and Wesley (2) and originally treated with either 0, 0.5, 1 or 2 lb ai/a Velpar (except for Sinbar on two sites) and with sulfur at 0, 500 or 1,000 lbs/a. Three more sites were established in 2003 at Eastbrook, Franklin and Blue Hill and were originally half treated with 0, 0.5, 1 or 2 lb ai/a Velpar and half treated with 0, 0.5, 1, or 2 lb ai/a Sinbar. The Whiting site was discontinued in 2002. Sites established in 2000 were retreated pre-emergence with either Sinbar or Velpar at 0, 0.5, 1, or 2 lb/acre depending on the original treatment. The Machiasport and Guptil sites were treated on 3 May 2006 and the Rockport and Appleton sites were treated on 9 May 2006. Soil samples were taken in each sulfur plot to determine the extent of pH change and weed cover was assessed on 15 August and 23 August 2006. Four herbicide plots by 3 sulfur plots provide 12-combination treatments/site. Stakes at the Appleton, West Rockport and Eastport sites were removed by someone during the season. Therefore, these three sites could not be sampled for soil pH nor could Appleton be assessed for weed cover.

RESULTS: The sulfur treatment significantly affected both grass and broadleaf weed cover (Figure 1). Grass cover was highest in the 500 lb/a treatment and lowest in the 1000 lb/a treatment. Neither Velpar nor Sinbar significantly reduced grass or broadleaf weed cover (Figure 2 and Figure 3). Blueberry cover was not affected by herbicide application. For plots treated in 2000 (Figure 4), ph levels ranged from 4.8-5.2 for 0 lbs/acre, 4.7-4.9 for 500 lbs/acre, and 4.6-4.8 for 1000 lbs/acre. For plots treated in 2001 (Figure 5), ph levels ranged from 4.8-5.2 for 0 lbs/acre, 4.5-4.8 for 500 lbs/acre, and 4.5-4.9 for 1000 lbs/acre. For plots treated in 2003 (Figure 6), ph levels were 5.1 for 0 lbs/acre, and ranged from 4.9-5 for 500 lbs/acre and 4.3-4.7 for 1000 lbs/acre.

RECOMMENDATIONS: This project is concluded. Results should be presented to growers and a fact sheet developed for the wild blueberry guide.

CONCLUSIONS: Sulfur applications reduce weed pressure and are a good cultural management tool to suppress weeds. Fields need to be soil sampled periodically and the pH reduced with sulfur to suppress weeds.

Figure 1. Evaluation of blueberry, grass, and broadleaf cover after sulfur application

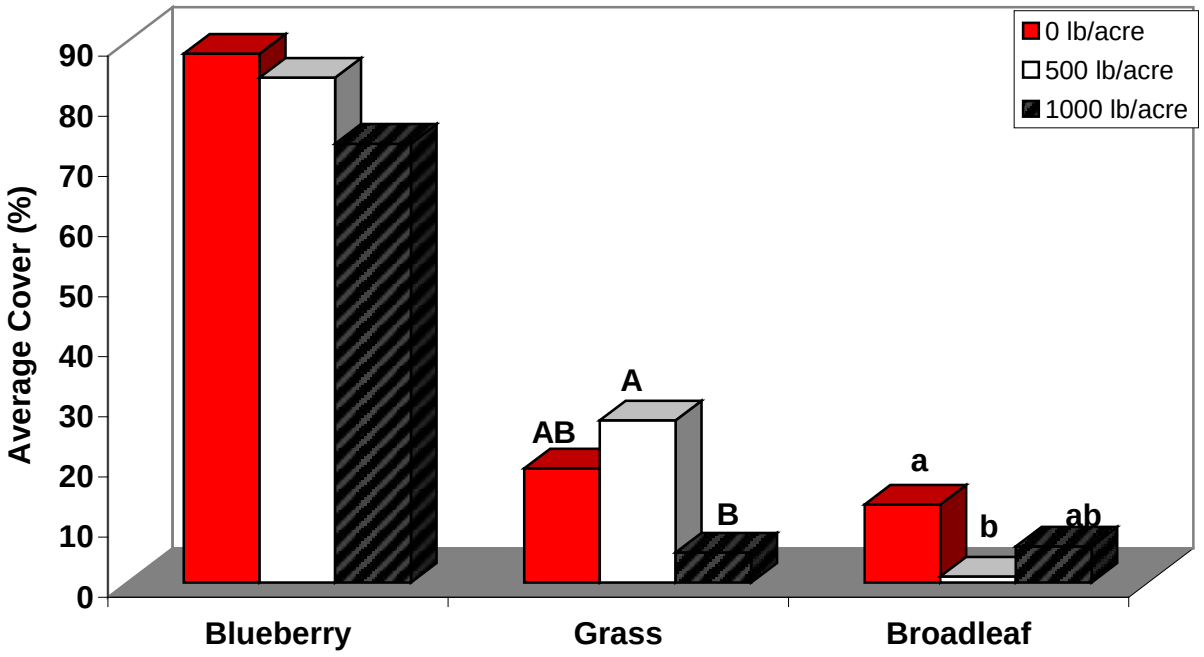


Figure 2. Evaluation of blueberry, grass, and broadleaf cover after Sinbar application

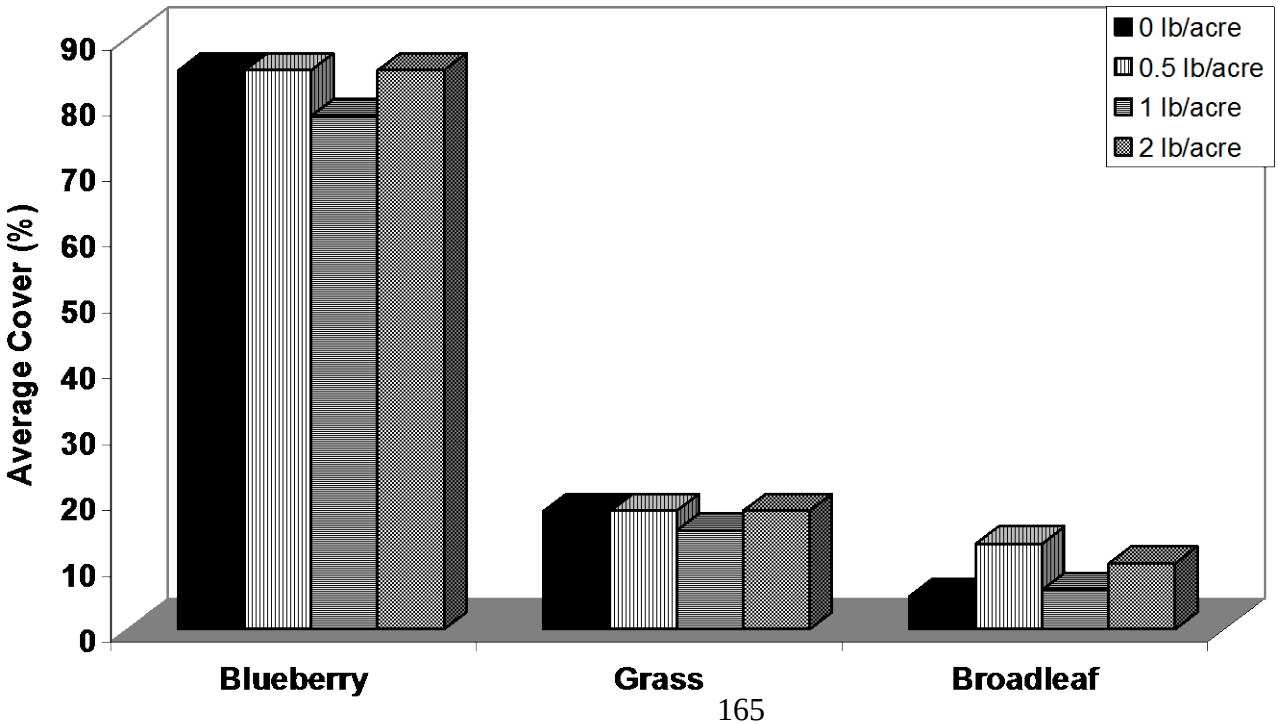


Figure 3. Evaluation of blueberry, grass, and broadleaf cover after Velpar application

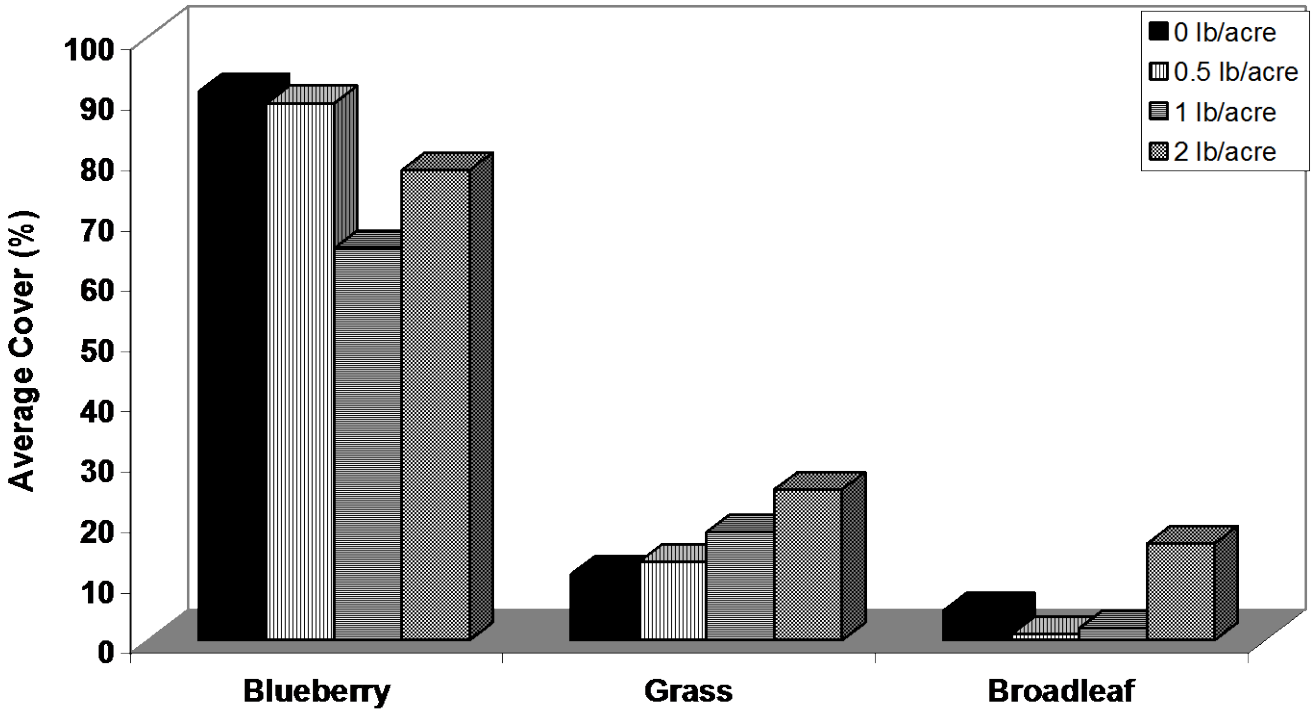


Figure 4. pH levels of sites treated with sulfur in 2000

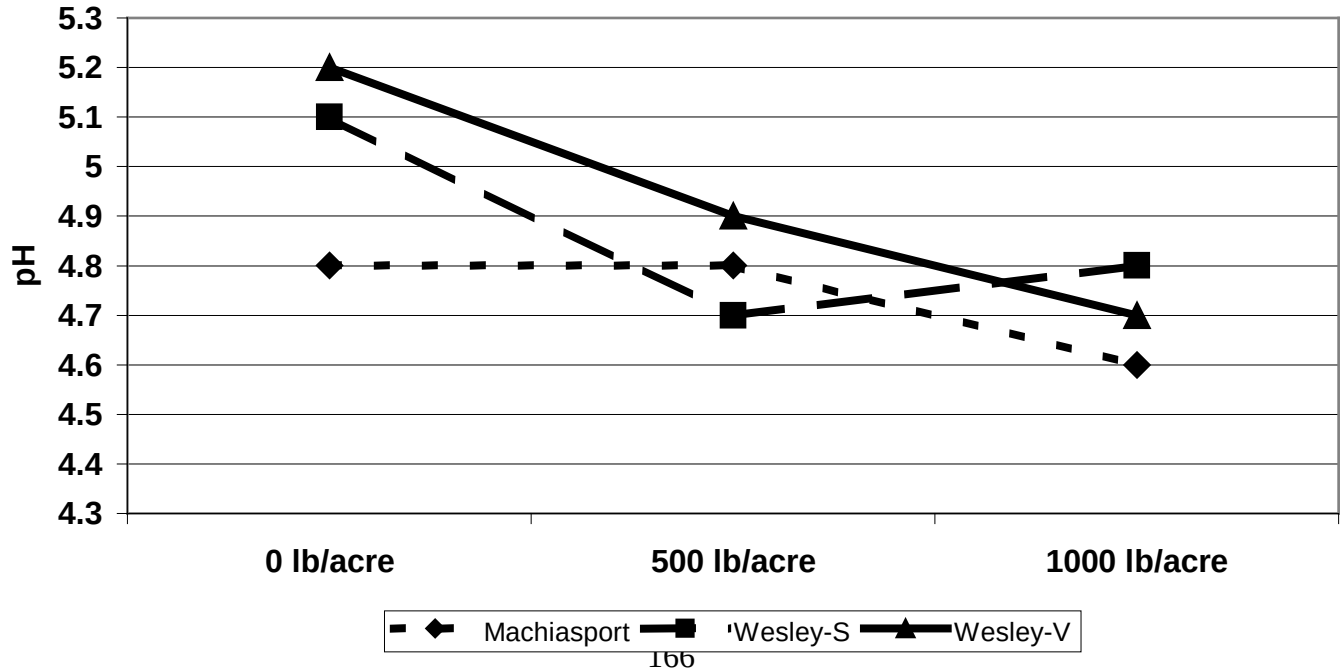


Figure 5. pH levels of sites treated with sulfur in 2001

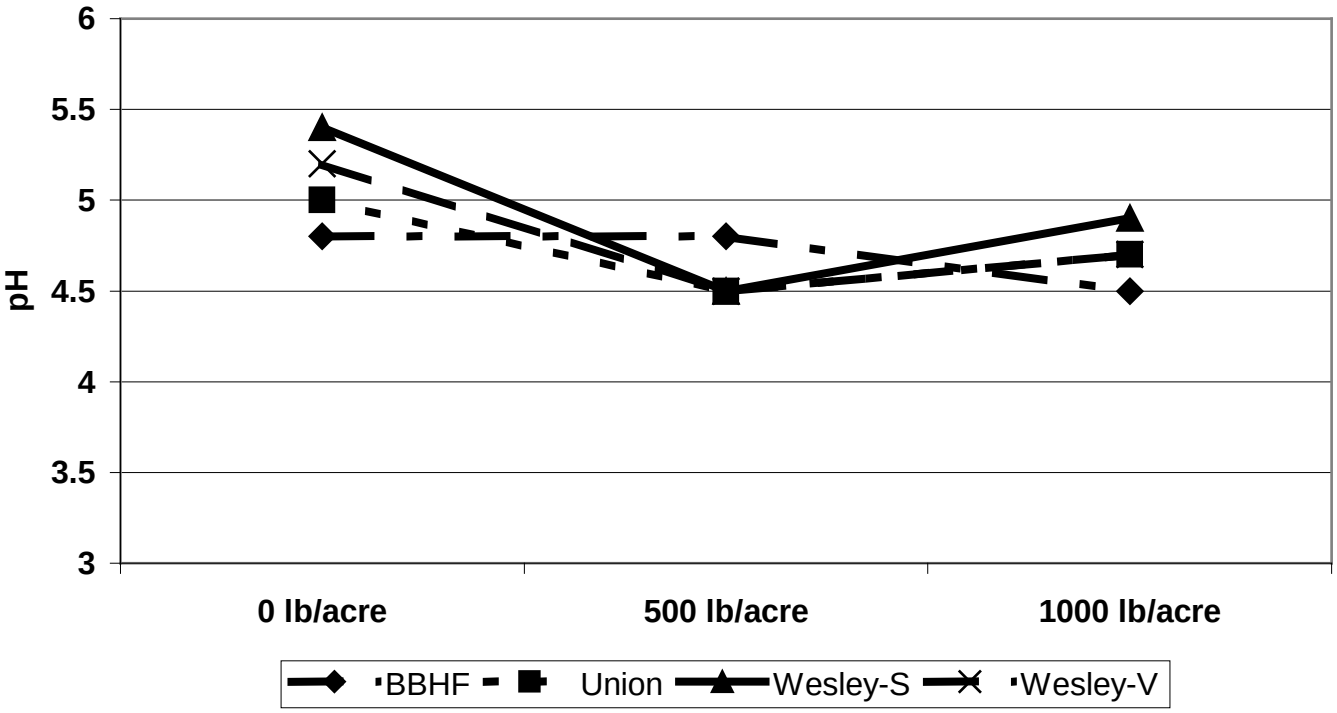


Figure 6. pH levels of sites treated with sulfur in 2003