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2004 Wild Blueberry Project Progress Reports

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FOOD SCIENCE AND BIOSYSTEMS ENGINEERING

INVESTIGATORS: Alfred A. Bushway, Professor of Food Science
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1. TITLE: Determination of Pesticide Residue Levels in Freshly Harvested and Processed lowbush Blueberries.

OBJECTIVES:

- Determine pesticide levels in freshly harvested and processed lowbush blueberries.
- Develop new and more efficient analytical methods for pesticide analysis.
- Develop new analytical methods using GC/MSD instrumentation.
- Adjust analysis to target pesticides used more frequently by growers.

PROGRESS: Fifteen IQF blueberry samples were submitted by Maine growers for the analysis of residual pesticides. The only residues detected were the insecticide, phosmet and the fungicide, chlorthalonil. All pesticide residues were well below Federal Tolerance levels (Table 1).

Table 1.

2003 Blueberry Pesticide Results

	Phosmet ppm or ug/g	Posmet Oxon ppm or ug/g	Guthion ppm or ug/g	Chlorthalonil ppm or ug/g	Fenbuconazole ppm or ug/g	Hexazinone ppm or ug/g	Propiconizol ppm or ug/g	Captan ppm or ug/g	Terbacil ppm or ug/g
Detection limit	0.001	0.01	0.001	0.001	0.005	0.02	0.005	0.005	0.005
Sample									
Blueberry-1	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blueberry-2	**0.144**	ND	ND	ND	ND	ND	ND	ND	ND
Blueberry-3	**0.09**	ND	ND	ND	ND	ND	ND	ND	ND
Blueberry-4	**0.011**	ND	ND	ND	ND	ND	ND	ND	ND
Blueberry-5	**0.028**	ND	ND	ND	ND	ND	ND	ND	ND
Blueberry-6	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blueberry-7	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blueberry-8	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blueberry-9	ND	ND	ND	**0.09**	ND	ND	ND	ND	ND
Blueberry-10	ND	ND	ND	**0.025**	ND	ND	ND	ND	ND
Blueberry-11	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blueberry-12	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blueberry-13	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blueberry-14	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blueberry-15	ND	ND	ND	ND	ND	ND	ND	ND	ND

FOOD SCIENCE AND BIOSYSTEMS ENGINEERING

INVESTIGATORS: Alfred A. Bushway, Professor of Food Science
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Vivian Wu, Assistant Professor of Food Science
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2. TITLE: Effect of Wild Blueberry Products on Physical, Chemical, Microbiological and Sensory Quality of Soy-Based and Ground Beef Patties

METHODS: Ground beef patties were processed from 90% lean ground beef with varying concentration of blueberry concentrate (1.0, 2.5, 5.0 or 10.0%) and blueberry powder (1.0, 2.5, 5.0 or 10.0%) on a wt/wt basis. Untreated ground beef patties were prepared to serve as the negative control. Ground beef with and without wild blueberry products were formed into 25 g patties, which were than inoculates with 1 x 10^6 cells of a mixed culture of four strains of *E. coli* 0157:H7. Patties were stored at 3.5º C. Samples were analyzed for *E. coli* 0157:H7 one hour after processing and at 1, 3, 5,10 and 14 days of refrigerated storage. Samples were also analyzed for total aerobic plate counts. The experiment was replicated twice. A second series of experiments were performed with precooked ground beef patties (5 and 10% wild blueberry concentrate and 5% wild blueberry powder). Patties were broiled to an internal temperature of 75 C, cooled and than surface inoculated with 1 x 10^6 cells of a mixed culture of four strains of *E. coli* 0157:H7. Precooked patties were analyzed for *E. coli* 0157:H7 and total aerobic plate counts one hour after processing and at 1, 3, 5,10 and 14 days of refrigerated storage. The experiment was replicated twice.

Currently, experiments are in progress to examine the effect of 15% wild blueberry puree on the fat content, warmed-over flavor development in precooked beef patties formulated stored under refrigeration (4-5 C). Lipid oxidation is being evaluated by two chemical methods [Thiobarbaturic acid (TBA) reactive substances and hexanal production at 0, 3, 7, and 10 days of storage. A colorimetric method is being used for TBA analyses, and GC headspace analysis is being used for hexanal.

Research looking at the formulation of a soy- wild blueberry veggie burger is just starting.

RESULTS: Experiments completed at the end of last year demonstrated that incorporation of blueberry concentrate and powder into liquid systems (sterile water or Brain Heart Infusion Broth) containing *E. coli* 0157:H7 resulted in the decrease in the cell numbers when compared to the control (Figures 1-6). Experiments were performed at 3.5º C and 37 º C and each experiment was replicated. A portion of the reduction in the sterile water was probably related to the pH changes associated with the addition of the wild blueberry concentrate or powder to the water. Fifteen percent wild blueberry concentrate and 5, 10 and 15% blueberry powder significantly ($P \leq 0.05$) reduced the levels of º C *E. coli* 0157:H7 in raw ground beef patties stored at 3.5º C. (Figures 7 & 8). The effect of wild blueberry products on total aerobic plate counts was not as dramatic. Post processing inoculation of ground beef patties containing wild blueberry products demonstrated that heating patties the wild blueberry products to an internal temperature of 75º C

destroyed their antimicrobial activity (Data not shown). Changes in the phenolic acids and anthyocyanins when subjected to high temperatures resulted in this loss in activity.

RECOMMENDATIONS: The research on use of wild blueberry products to reduce and/or inhibit warmed-over flavor development in meat based food systems will be completed at the end of this year. In October, the USDA did serve ground beef patties with wild blueberry puree to students in a Texas elementary school. Their approach is to provide healthier choice for the school lunch program. Dr. Vivian Wu received a Maine Agriculture Center grant to examinee the effect of wild blueberry products in combination with other treatments in inhibiting the growth of several human pathogens in food systems. She will be requesting additional support from the Maine Wild Blueberry Commission to support her research.

Figure 1. The Mean *E. coli* O157:H7 (Log CFU/mL) in Sterile Distilled Water Treated with Blueberry Concentrate Incubated at 37ºC

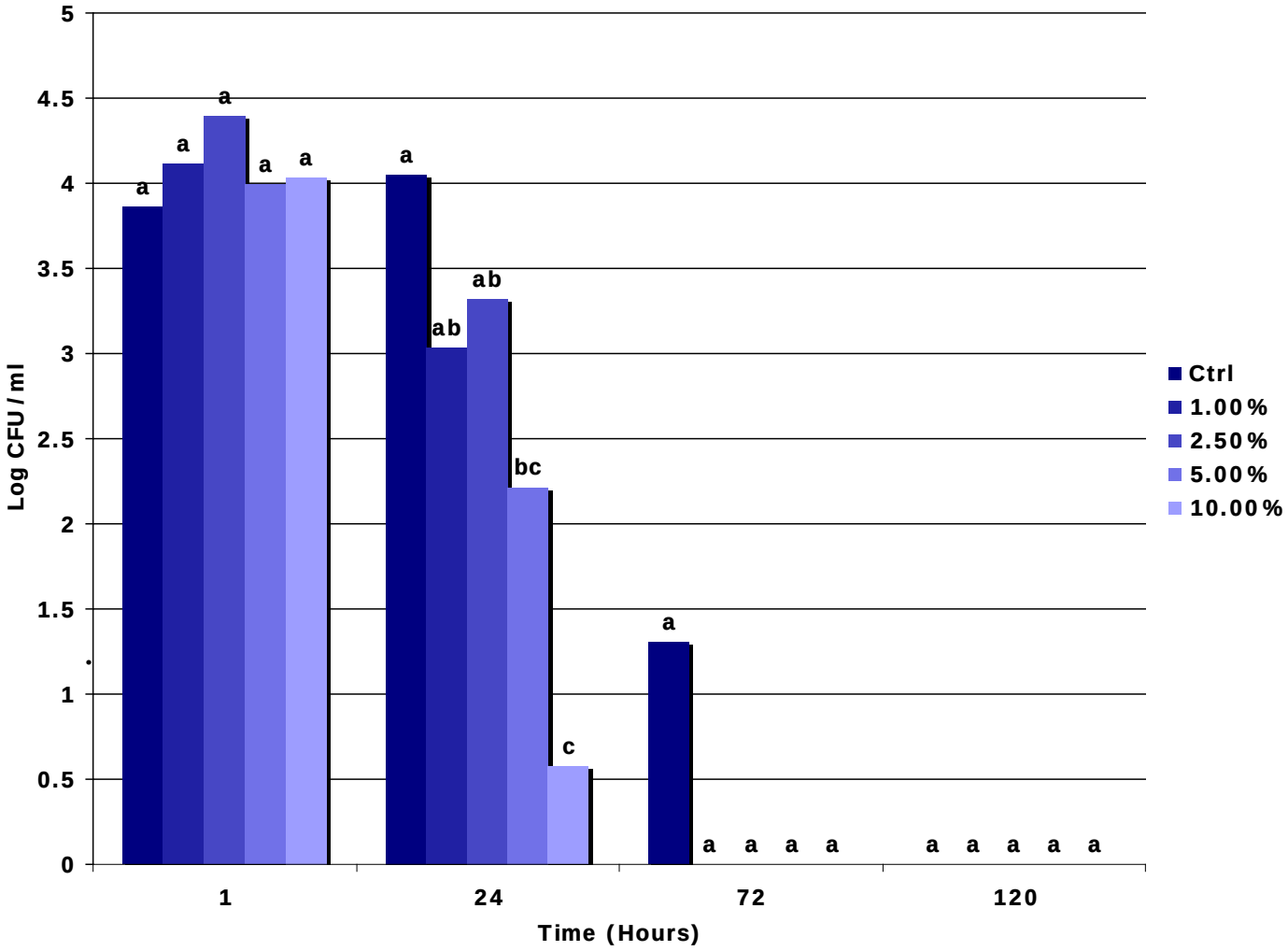


Figure 2. The Mean *E. coli* O157:H7 (Log CFU/mL) in Sterile Distilled Water Treated with Blueberry Powder Incubated at 37ºC

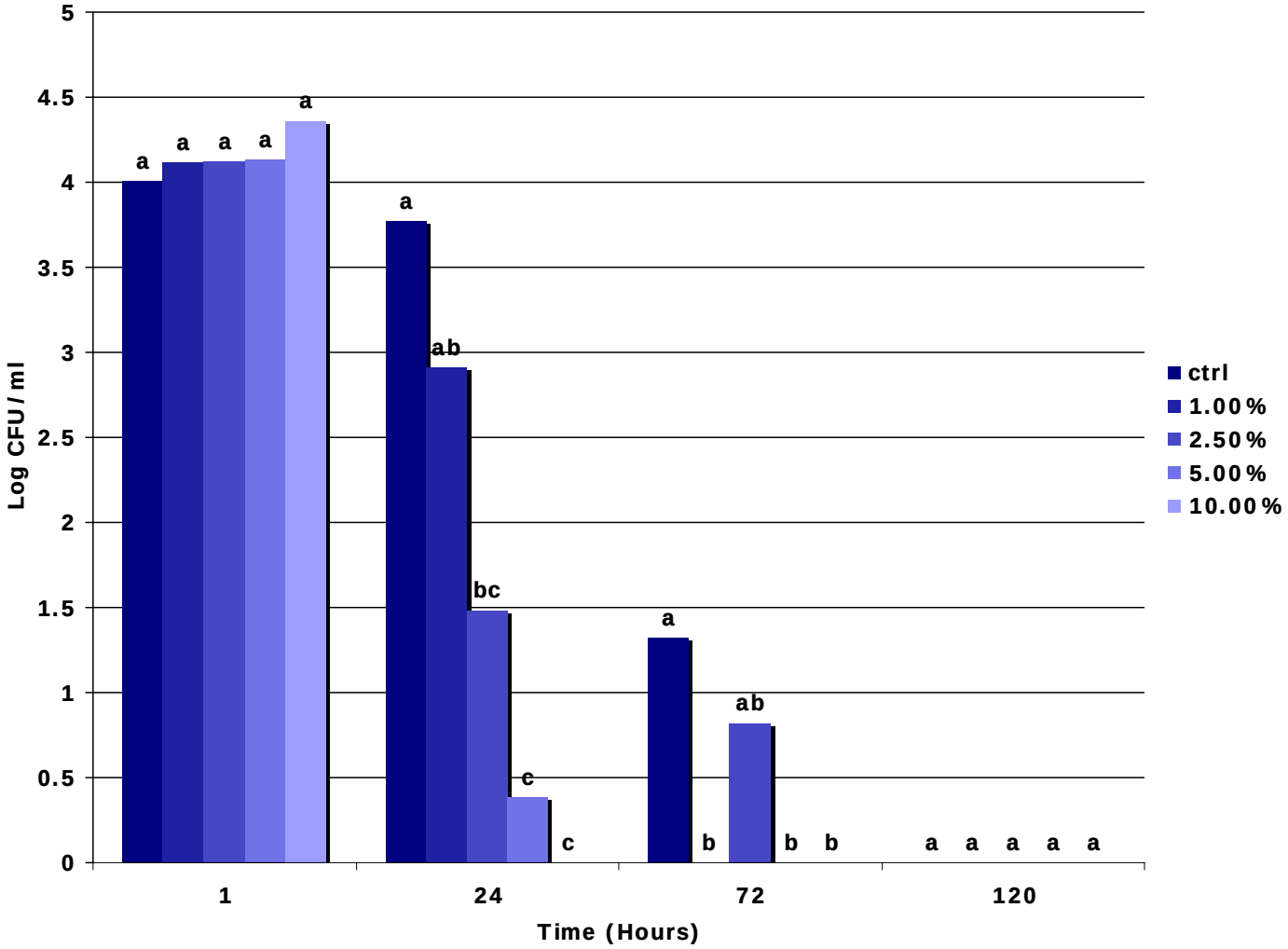


Figure 3. The Mean *E. coli* O157:H7 (Log CFU/mL) in BHI Broth Treated with Blueberry Concentrate Incubated at 37ºC

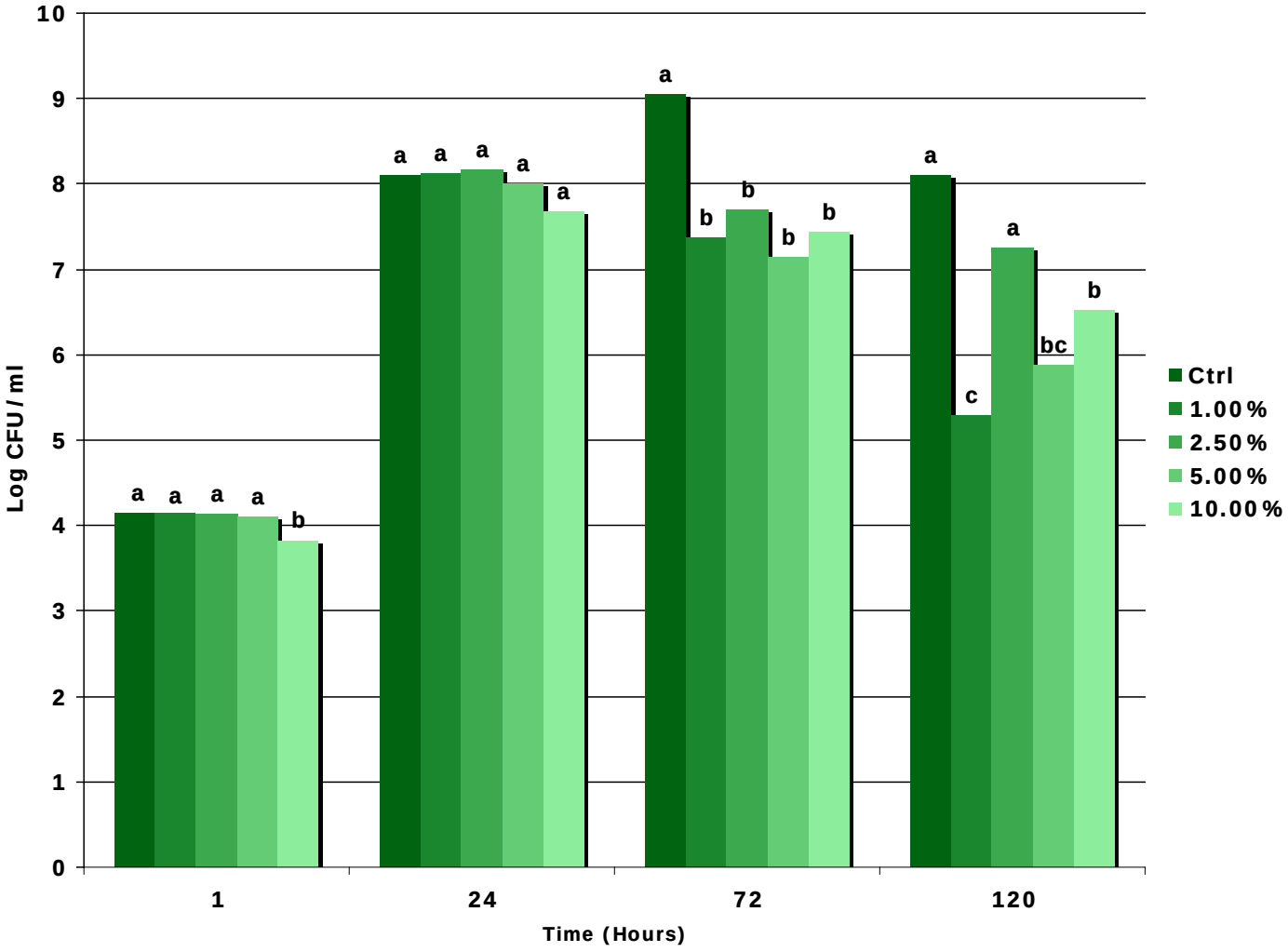


Figure 4. The Mean *E. coli* O157:H7 (Log CFU/mL) in BHI Broth Treated with Blueberry Powder Incubated at 37ºC

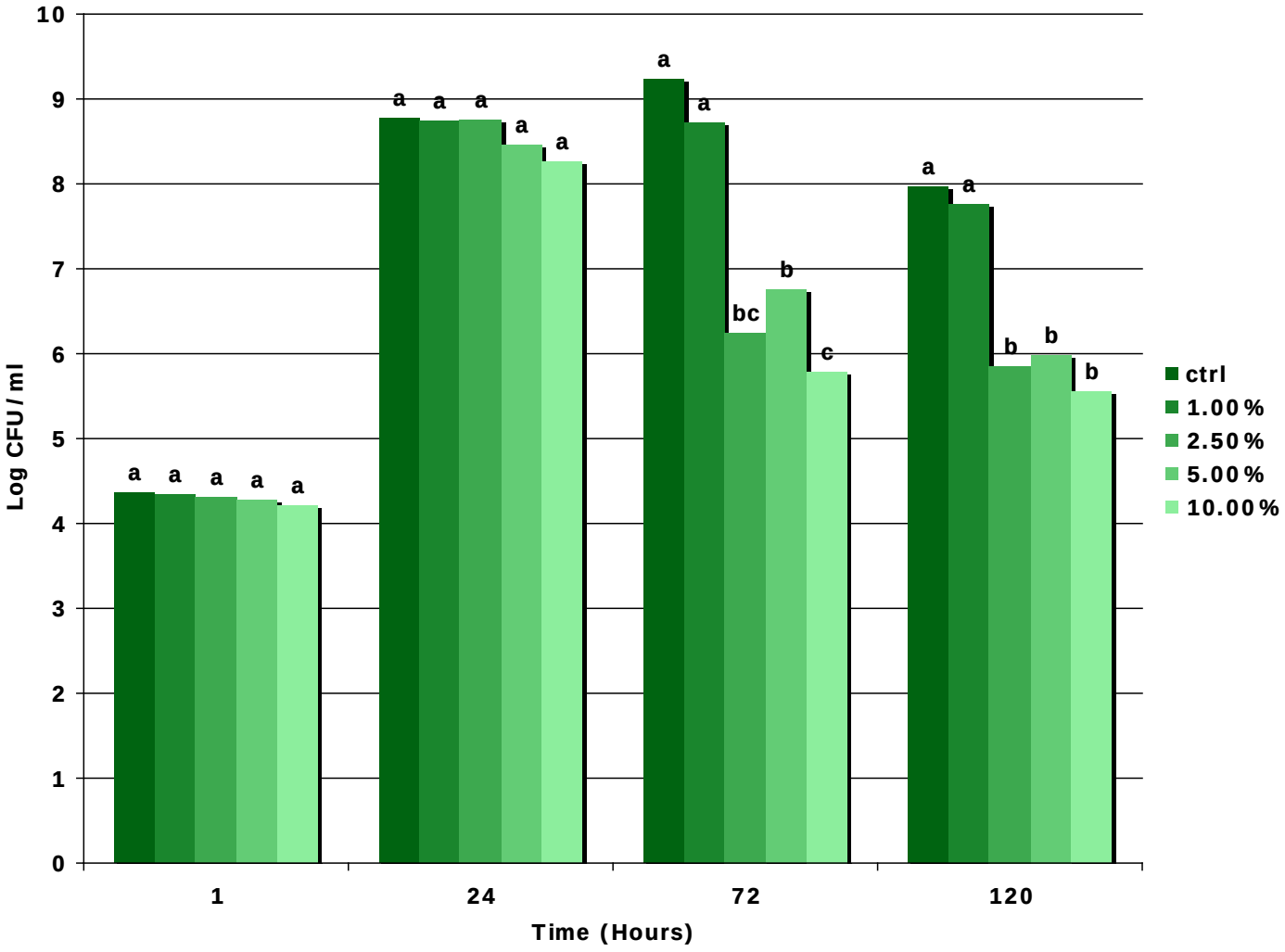


Figure 5. The Mean *E coli* 0157:H7 (Log CFU/ml) in BHI Broth Treated with Blueberry Concentrate Refrigerated at 3.5ºC

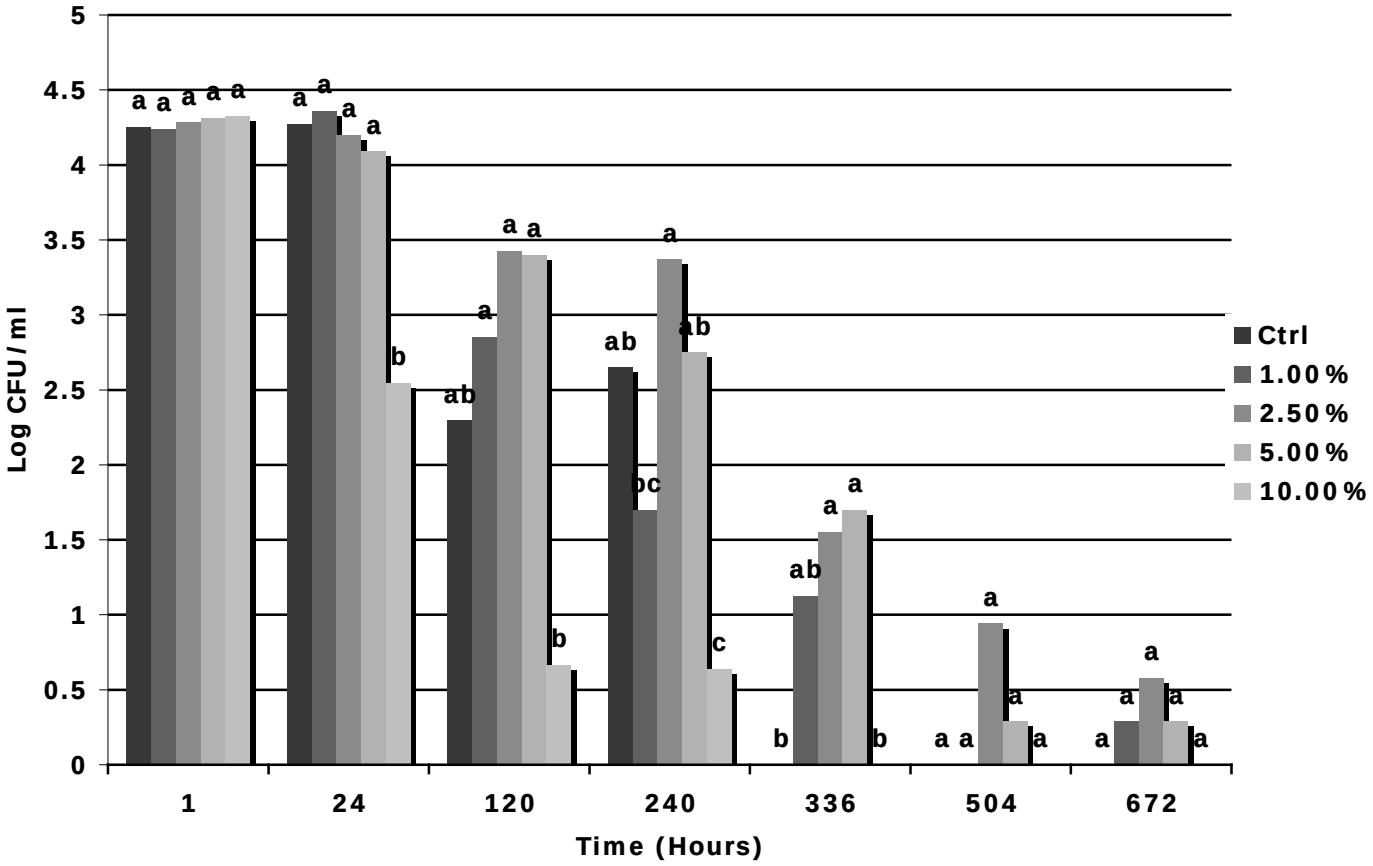


Figure 6. The Mean *E. coli* O157:H7 (Log CFU/mL) in BHI Broth Treated with Blueberry Powder Refrigerated at 3.5ºC

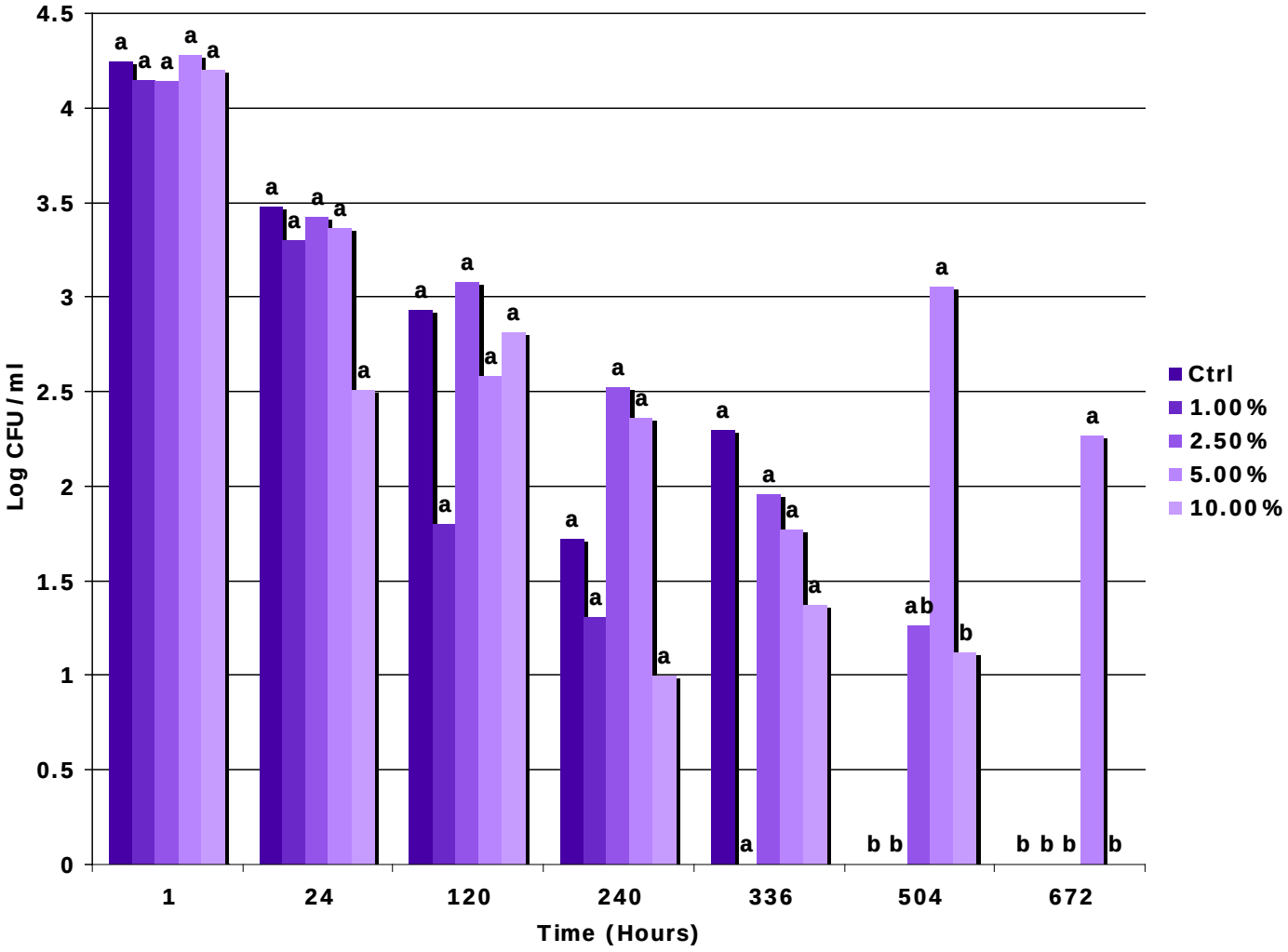


Figure 7. The Mean *E. coli* O157:H7 (Log CFU/g) in Raw Ground Beef Patties Treated with Blueberry Concentrate Refrigerated at 3.5ºC

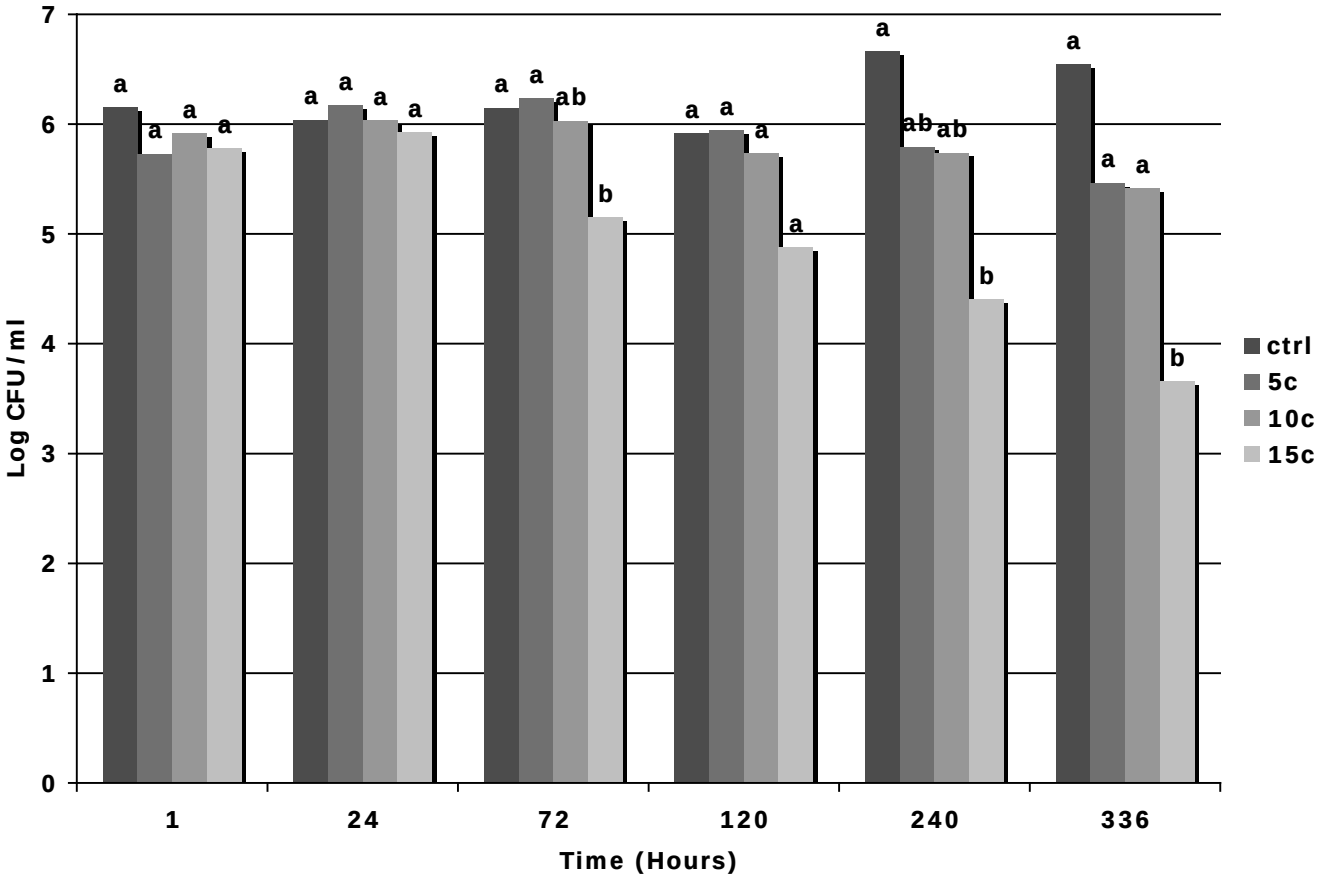
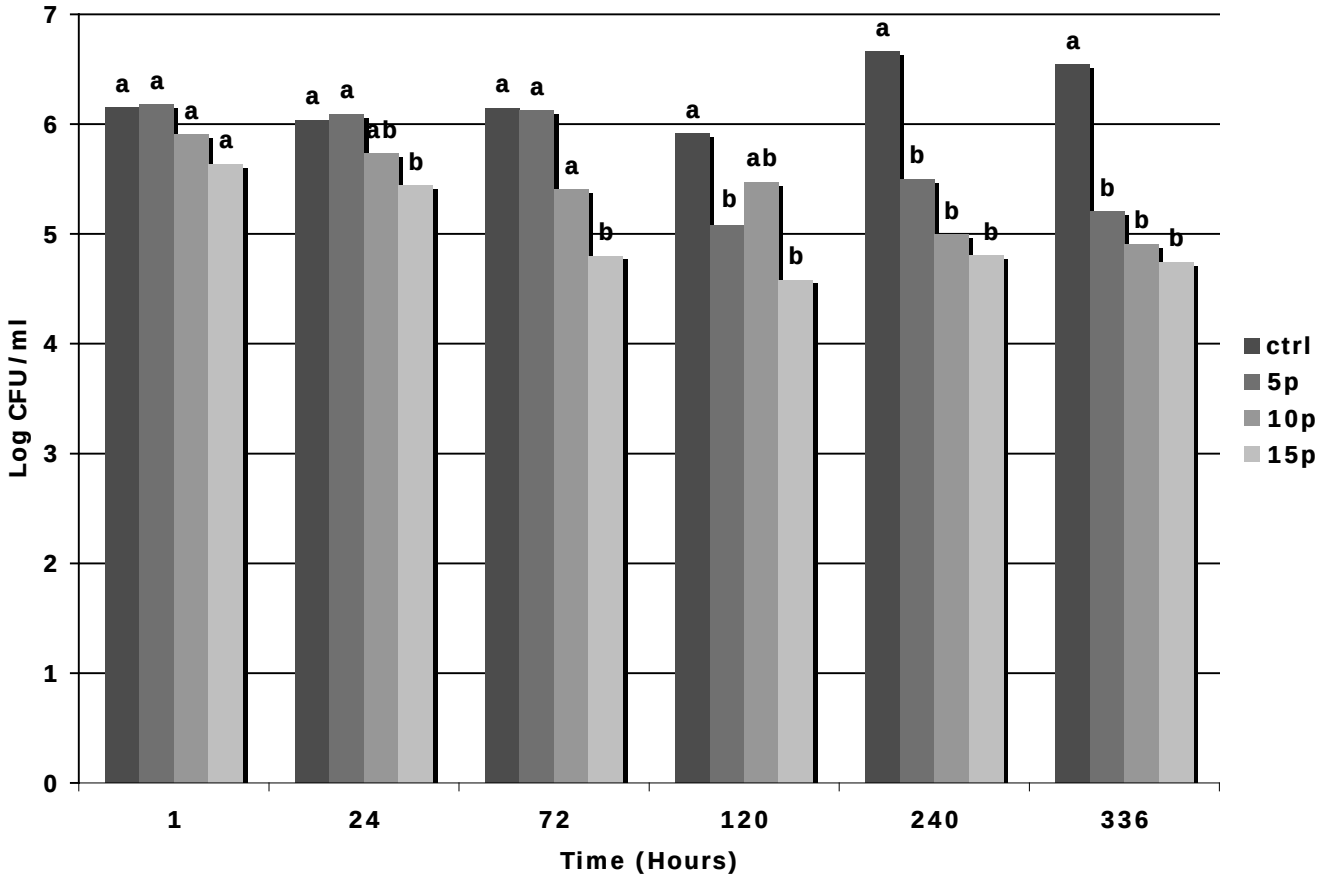


Figure 8. The Mean *E. coli* 0157:H7 (Log CFU/g) in Raw Ground Beef Patties Treated with Blueberry Powder Refrigerated at 3.5º C



FOOD SCIENCE AND BIOSYSTEMS ENGINEERING

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

METHODS: Plots were staked out on commercially productive blueberry land in Deblois, ME. Samples were collected and assayed immediately after initial treatment with Imidan WP (phosmet). Sampling and analysis continued every week for three weeks. Freshly harvested berries were transported to the University of Maine and subjected to sprays of pilot plant water, 100-ppm chlorine, 1.0 hydrogen peroxide, 1.0% hydrogen peroxide/UV, ozone/UV, 1.7-2.0 ppm ozone, 2.5-3.0 ppm ozone or ozone/1% hydrogen peroxide/UV before analysis for phosmet residues. Contact times were 60 and 120 sec. Hydrogen peroxide is classified by the U.S. Food and Drug Administration as Generally Recognized as Safe (GRAS) for certain specified food applications (21CFR184.1366). A recent action by the U.S. Environmental Protection Agency exempts use of $\leq$ 1% hydrogen peroxide applied to all post-harvest agricultural food commodities from the requirement of a tolerance (40CFR180.1197). Therefore, if a treatment containing 1% hydrogen peroxide proved to be efficacious in inactivating surface microorganisms and human pathogens on lowbush blueberries, post-harvest applications of hydrogen peroxide would be beneficial to the blueberry industry in improving product quality. Additionally, several studies have reported that applications of hydrogen peroxide and hydrogen peroxyacetic acid are capable of reducing certain pesticides and chemical residues in solution; therefore, if hydrogen peroxide treatments are capable of reducing residual phosmet, blueberry processors in Maine would further benefit from this combination approach to improving product quality. All samples used in this study were extracted by an internally validated laboratory protocol and were analyzed using a gas chromatograph equipped with an atomic emission detector (GC/AED). Samples of 50 g were taken initially and after each processing step. Microbiological analyses of total aerobes, yeast, coliforms and *E. coli* were conducted using FDA Standard Methods. Appropriate decimal serial dilutions were prepared and samples were plated in duplicate. Total aerobic plate counts were performed using Plate Count Agar. Yeast counts were conducted using Acidified Potato Dextrose Agar (FDA, Bacteriological Analytical Manual, 7th ed., 1992). Coliforms and *E coli* were determined by Most Probable Number (MPN).

All tests and treatments were performed in triplicate and plated in duplicate.

RESULTS: Significant advances have been made this year in verifying the efficacy of 1% hydrogen peroxide treatments applied post-harvest to improve the overall microbial quality of commercial blueberries. As the data indicates, hydrogen peroxide applications result in greater microbial reductions than the industry standard, chlorine, when allowed a 60 second contact time. Although the antimicrobial activity of chlorine increased when allowed a 120 second contact time, these results are not typical. Previous research indicates that a 1-log reduction is

the most that can be expected when chlorine is applied in concentrations $\leq$ 100ppm to lowbush blueberries for 60 or 120 seconds. All spray treatments applied this year were conducted using the spray/conveyor system. This equipment allowed researchers to apply treatment volumes in a manner similar to current industrial processes. The use of this equipment further validates the results of the study should the industry begin using hydrogen peroxide in post-harvest processing facilities. Incorporation of this technology should take place without extensive alterations to existing processing lines.

As governmental agencies such as the FDA Center for Food Safety and Applied Nutrition continue to mandate action plans to minimize food-borne illness, the validation of hydrogen peroxide's antimicrobial activity when applied to lowbush blueberries illustrates maximum progress towards this goal. In addition to the success of hydrogen peroxide spray treatments, photochemical treatments also showed promising results in this year's study. The addition of UV to chemical treatments, especially hydrogen peroxide, resulted in significant log reductions in total aerobic plate counts and yeast counts. Almost a 4-log reduction in bacterial populations was observed on blueberries treated with 1% hydrogen peroxide/UV for 60 seconds. When results were compared to those obtained over the previous 4 years, this treatment resulted in the single greatest reduction achieved through use of post-harvest treatments in concentrations allowed by the U.S. Environmental Protection Agency. In addition to chemical (1% hydrogen peroxide) and photochemical oxidation processes (1% hydrogen peroxide/UV), the antimicrobial effectiveness of UV alone also shows promising results for use in the fresh pack market. On individual samples treated with UV for 60 seconds, reductions in total aerobic counts were between 1 and 1.5 logs. Since log reductions were achieved without the addition of any liquid treatment to the samples, this treatment shows potential for use on fresh pack blueberries. Furthermore, it was observed that the bloom on all UV-treated berries remained intact throughout treatment and storage.

Preliminary experiments have shown the phosmet application has resulted in a reduction in the microbial population on the blueberries. These are very interesting results and are being further investigated with the expectations of submitting a USDA-NRI seed grant.

Although significantly effective treatments were previously discussed, numerous other treatments were also investigated, and results are reported in Table 1 and 2. Promising results were observed on blueberries treated with ozone and ozone/UV; however, modifications to the existing ozone system are needed before additional studies can be initiated to validate these results. These modifications are currently being made, and will be ready for next year's research project.

Table 1
Microbial Results of Treated Blueberries - Crop Year 2004

Reported as Log CFU/g[z] (Mean ± SD) and Log Reduction[y] Following Treatment Application

	APC	Yeast	Mold
Control – 60 Sec	**4.05 ± 0.15**	**4.53 ± 0.29**	**4.70 ± 0.22**
100ppm Cl_2	0.84	1.23	1.63
1% H_2O_2	1.27	1.54	1.19
1% H_2O_2/UV	3.70	1.62	0.40
O_3/UV (1.7-2.0ppm)	-0.56	1.23	0.23
Plant Water	0.48	0.20	0.20
O_3 (1.7-2.0ppm)	0.48	1.30	0.31
O_3 (2.5-3.0ppm)	1.40	0.15	0.03
O_3/1% H_2O_2/UV (60sec each)	1.79	0.73	0.20
Control – 120 Sec	**4.05 ± 0.15**	**4.53± 0.29**	**4.70 ± 0.22**
100ppm Cl_2	1.62	1.32	1.30
1% H_2O_2	1.25	no value post-treatment contamination	0.38
1% H_2O_2/UV	2.10	1.54	0.49
O_3/UV (1.7-2.0ppm)	0.77	1.25	0.31
Plant Water	0.89	0.58	0.33
O_3 (1.7-2.0ppm)	0.44	1.40	0.25
O_3 (2.5-3.0ppm)	1.49	0.14	0.41

[z] Treatments were performed in duplicate and plated in duplicate. All values obtained from analysis were converted to log CFU/g blueberries.

[y] Log reduction is the difference between microbial counts before and after treatment.

Table 2
Treatments Resulting in Significant Results in Bacterial Counts

Reported as Log CFU/g[z] (Mean ± SD) of Unwashed Control Samples and Log Reduction[y] in Total Aerobic Bacterial Populations Following Treatment Application

Treatment	**Contact Time 60 sec**	**Contact Time 120 sec**	**Contact Time 180 sec**	Contact Time 300 sec
Control	4.05 ± 0.15	4.05 ± 0.15	4.05 ± 0.15	**4.05 ± 0.15**
100ppm Cl_2	0.84	1.62		
1% H_2O_2	1.27	1.25		
1% H_2O_2/UV (60sec)	3.70	2.10		
UV	1.19		0.95	**0.78**
Plant Water	**0.48**	**0.89**		

[z] Treatments were performed in duplicate and plated in duplicate. All values obtained from analysis were converted to log CFU/g blueberries.

[y] Log reduction is the difference between microbial counts before and after treatment.

Color analysis using the Hunter LabScan Spectrocolorimeter, showed only small differences in L, a, or b-values among treatments.

Samples for residual pesticide analyses were extracted and extracts stored at –30 C. Analyses were completed last week and preliminary data is shown in Table 3. Based on this preliminary data the extraction procedure and analytical method need some further development.

Table 3
Phosmet Degradation Resulting from Post-harvest Treatment Applications
Unwashed Control - 22ppm phosmet

	60 Second Contact Time		120 Second Contact Time	
	Phosmet (ppm)	**Oxon (ppm)**	**Phosmet (ppm)**	Oxon (ppm)
100ppm Cl_2	1.22	0.31	1.40	**0.42**
1% H_2O_2	31.9	0.18	14.7	**0.19**
1% H_2O_2/ UV	59.3	0.25	24.1	**0.16**
Plant Water	16.3	0.07	15.3	**0.06**
	Phosmet (ppm)	**Oxon (ppm)**		
UV 60sec	32.3	0.12		
UV 180sec	30.3	0.13		
UV 300sec	**31.7**	**0.18**		

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

4. TITLE: Detection of Infested Blueberries using Near-Infrared Spectroscopy-Spectra Collection

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

METHODS:

1.Field and sample preparation

After fruit set, during July, 2004, Dr. Drummond identified areas where blueberry stems could be harvested for placement in fly cage systems for artificial laboratory infestation.

2. Artificial laboratory infestation and preparation

As laboratory-raised flies hatched they were released into insect cages in the biological engineering laboratory. Blueberry maggot adults were reared from pupae collected in 2003. As they emerged, adults were placed in ovipostion 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 allow access to cage and 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 fly population 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 (see Figure 1 for a flowchart description).

3. Near-infrared spectroscopy (NIRS) scanning and analysis

Once removed from cages (see section IV.2 above), usually once per week, the berries that were damaged during maturation were discarded. Usable blueberries assigned names according to their origin (e.g., "Jonesboro") and the batch number corresponding to the week in which berries were removed from the infestation cages. Each batch was separated in two to six subsets of 120 berries each and designated with a letter (A, B, C, D, E and F). These berries were then counted and recorded on data sheets. Each scannable berry was further processed as described here.

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 no longer than 4 days at laboratory refrigerator at ca. 4°C until they were scanned using one of the two NIRS systems. All berries in a single set were scanned on the same day and under the same conditions. Figure 2 gives a schematic of the basic overall berry scan procedure for both UMaine and USDA-ARS-Kansas State University (USDA-KSU) and set up differences are described below.

At UMaine, the berries were scanned with a UV/NIR system from Ocean Optics, Inc. (Dunedin, FL). A wide-spectrum (200 – 1200 nm) halogen light source was focused onto the individual berry at a distance from the culminating lens of approximately 25 mm. A culminating 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 550 and 1100 nm was graphed via the associated software program (OOIBase32, Ocean Optics, Inc.). Three replicate scans of each berry were collected using the reflectance chamber.

At USDA-KSU, two detectors (a silicon detector at 400-950 nm and an InGaAS detector for 950-1700 nm) were used. The USDA-KSU receiving fiber was at 360 (or 0) degrees (right beside) the excitation light and approximately 11 mm from the berry surface. All berries were scanned with stem and calyx end facing the light source. At the beginning of each scan set, two reference spectra (complete light and dark) were taken and saved for later validation. After completing the scanning, all berries were dissected to determine if a maggot was present. Individual berries were placed in an aluminum plate, dissected and examined under a light microscope (Olympus Model H011, Olympus, Inc., Japan) at 10X magnification and it was recorded on the datasheet whether a maggot was present. For preliminary data analysis of the scan information, the following protocol (see section 4 below) was used as suggested by Dowell (pers. comm., 2001).

Similar to 2003, in 2004 a series of experiments were carried out at USDA-KSU to investigate the effects of freezing on infestation prediction. Four sets of berries of 120 berries each were first scanned fresh. Then they were held in a freezer at -18°C overnight and scanned again the next day while still frozen.

4. Prediction model analysis

First, individual spectra were imported into the modeling tool (either GRAMS®, version 6.00, Themo Galactic, Salem, NH or MATLAB, version 5.3, MathWorks, Natick, MA) and standard spectral image files (proprietary SPC file type or MAT file) were created from the raw scan data files. Training (data) sets were built from the individual spectra in each set of 120 samples. The individual spectral files were examined for anomalies, potential outlier samples or particular wavelengths to study in further detail. This information was used when creating the calibration model. The observed anomalies in the raw spectra were compared later with outlier spectra identified by statistical tests on the residuals (error terms) from Partial Least Squares (PLS) models.

PLS analysis was carried out the data from 2004 using GRAMS software. PLS is a spectral decomposition technique that takes advantage of the correlation relationship between the spectral data and the constituent (infestation) information. This involves regression of the independent variations contained in the spectra against the constituent concentrations. All

independent variations are captured in separate factors called latent variables. Each factor 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 0 and 1 respectively (called constituent values). The threshold value was calculated as the arithmetic mean of the assigned arbitrary constituent values for each data set. Samples were considered infested if predicted constituent values were greater than the rejection threshold, and all others were considered non-infested.

Different types of preprocessing and data transformations were examined to determine the best approach for successful prediction by PLS. Preprocessing methods that were tested included mean centering, variance scaling, normalization and light scatter correction methods. Mean centering involves calculating the average spectrum of all the spectra in the training set and then subtracting the result from each spectrum. In addition, the mean concentration for each constituent is calculated and subtracted from the concentrations of each sample. Variance scaling is calculated by dividing the response at each spectral data point by the standard deviation of the responses of all training spectra at that point. The scatter correction methods tested were Multiplicative Scatter Correction (MSC) and Standard Normal Variate (SNV). MSC attempts to remove the effects of light scattering by linearizing each spectrum to the average spectrum while SNV does that by normalizing each spectrum by the standard deviation across the spectral range. First and second derivative were also used for preprocessing. First derivative is often used to correct for baseline shift and to separate overlapping peaks. These methods are often used in spectroscopic data analysis (Delwiche and Reeves, 2004; Walsh et al., 2004; Chen et al., 2002; Dardenne et al., 2000; Lammertyn et al., 2000) as they further enhance the PLS model calibration.

Data with replicate samples scanned at UMaine were transformed by averaging. Spectral data sets from the same batch scanned with the same instrument and settings were joined together after averaging across replicates. PLS was performed on these large joint data sets as well as on single data sets from the same batch and results were compared. Scatter correction and derivative were tested for all models.

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. It removes consecutively a sample or a group of samples from the training set and uses the remaining samples to predict the concentrations of the removed sample(s). Then standard error of cross validation (prediction), SECV, is calculated by comparing the predicted and actual constituent values for each sample. This is repeated until all samples have been left out and predicted at least once. A cumulative SECV value is returned as result indicating the success of prediction. The recommended number of PLS factors is based on the reduction in SECV. Another method for measuring the error of prediction is Prediction Residual Error Sum of Squares (PRESS) where the relationship between PRESS and SECV is PRESS = $SECV^2$*(Number of samples). Cross validation was performed for all models removing consecutively segments of 3 to 20 samples depending on the size of the data set. SECV values were used for identifying the number of factors giving the best data fit by the model as well as to compare results from applying preprocessing within the same data sets or between data sets with similar size.

Spectral and concentration outliers were identified based on the residual plots after calculating the PLS models. They were removed from the data sets and the models were

recalculated without the outlying samples. Concentration residuals, representing the prediction error for each sample, are the differences between the actual and predicted concentration values. Spectral residuals are the differences between each spectrum and the model-reconstructed spectrum, which is what the sample spectrum should look like, determined by the PLS model.

Beta (calibration) coefficients from PLS were used to test for absorbance bands sensitive to differences between infested and non-infested berries. For any given wavelength, the absolute value of the beta coefficient indicate how important that wavelength was for prediction, where a beta coefficient of 0 suggests no importance for prediction.

RESULTS/CONCLUSIONS:

1. Artificial laboratory infestation and preparation

The laboratory experiment to artificially inoculate berries with maggot larvae was successful this season. An approximate 20 % maggot infestation rate was achieved during the 2004 season, which was lower than previous years. This was due to increased mortality rate among mature laboratory raised flies later in the season. However, for the purpose of developing prediction models data sets with higher infestation ratio of 30% to 50% were used. In order to guarantee high maggot counts for use in evaluating the NIRS method of detection, these laboratory artificial infestation cage experiments must be continued to yield high portions of infested berries. Therefore, work by Drummond should continue in this area.

2. NIRS: data preprocessing, modeling and analysis

Data processing. By examining raw spectra, differences were found between stem and calyx scans. The regions where the two resulting spectra differed were 700-800 nm for the UMaine spectra and 1400 - 1600 for the USDA-KSU spectra. These differences are potentially interesting for identification of the position of the berry by NIRS.

When comparing raw spectra collected during 2002, 2003 and 2004 seasons no significant differences were found related to season. Spectra obtained by the same spectrometer showed the same peaks and features. There were some differences observed in USDA-KSU spectra from 2003 compared to the other two seasons. These differences were in the form of a baseline shift and increased noise level, which were primarily due to a longer distance from the sample to the detector. Therefore, shorter distance of 11 mm was used in 2004 scans, regardless of the need to discard a small number of larger size samples. The spectral data from these three seasons in some cases yielded prediction models with significantly different performance, which is discussed in the PLS modeling section below.

When comparing the effects of preprocessing techniques on the result of the PLS models we concluded that mean centering and variance scaling on average improved prediction with 1 – 2 %. The scatter correction methods, MSC and SNV, improved prediction levels 2 to 4 % with each method having different effects when modeling different training sets. On average, both of these techniques had the same contribution to prediction. Normalization did not improve prediction significantly. Derivatives led to improvement in the prediction ratio with 2 to 4 % and also to reduction in the number of factors. Therefore, mean centering and variance scaling were applied to all 2004 PLS models and scatter correction and derivatives were also tested. However, preprocessing rarely led to very significant improvement of the prediction results as has been suggested by other researchers (Delwiche and Reeves, 2004).

Spectral subtraction. By examining typical absorption spectra from both infested and non-infested samples, a region of interest was determined – 1350-1700 nm. This is the wavelength band where most protein compounds absorb (see Fig. 3). However, this is also the

band of a major water peak. These observations were confirmed by spectral subtractions of averaged non-infested and infested spectra (Fig. 4). Two different sets of non-infested spectra were randomly selected, averaged and subtracted from each other. A similar size set of infested spectra were selected and averaged and one of the non-infested averaged spectra was subtracted. The difference spectrum from the two non-infested spectra shows random noise around zero indicating that the non-infested spectra are very similar. However, there were differences seen between the two spectral subtractions from infested and non-infested berries. The most pronounced differences were for wavelengths between 1400 - 1700 nm, similar to the wavelengths reported by other researchers working with insect infestation (Perez-Mendoza et al., 2003). This band coincides with the position of the highest peak in sample spectra we have taken of a maggot (see Fig. 4) suggesting that with further analysis and developing classification algorithms we should be able to classify blueberries according to infestation level. In order to better understand these differences between spectra from infested and non-infested sample a chemical composition analysis has to be carried out.

Modeling and analysis. Initial comparisons between PLS models of berries scanned with stem and calyx end facing the detector showed that for the majority of the cases level of prediction was similar, but slightly higher for the stem sets in 2003 data. The reason for this is the higher degree of light scatter caused by the rough surface of the calyx. Therefore, current analysis efforts on the 2004 data are concentrated towards modeling stem sets. Previous results have shown that prediction of infestation is better for data sets with larger number of samples. Therefore, in our analysis efforts we used larger number of samples by combining several sets of 120 samples together.

PLS models, combined data sets. The results from PLS models based on data from UMaine and USDA-KSU are summarized in Table 1. The dataset scanned at UMaine is much larger than the set from USDA-KSU since three replicate scans were taken at each berry at UMaine. All data were mean centered and variance scaled and 1st derivative was tested in two models. The highest level of correct prediction (80.7 %) was achieved with the USDA-KSU combined dataset using 1st derivative. The increase due to this preprocessing was not significant since it improved prediction with only 0.6 % for the USDA-KSU model. The correct prediction for UMaine data was 75.7 % without using derivative and 72.4 % after taking 1st derivative. It is clear that, in the UMaine data taking a derivative leads to a 3.3 % reduction in the prediction ratio, which is not significant. These prediction levels are similar to the levels of 2002 models and the prediction of USDA-KSU models is significantly higher in 2004 than in 2003. These results suggest that deviations in the distance of the sample to the detector have a strong effect on model performance. This has to be taken into consideration when designing a prototype instrument and the optimal distance from the detector to the processing line has to be determined. Generally, the prediction levels for 2002 and 2004 data were similar at this stage of analysis, suggesting that season variations can be compensated for by the PLS models and ~80 % prediction ratio can be obtained consistently.

PLS models, water spectrum subtraction. Since the major absorbance peaks of water and of the pure maggot appear in the same wavelength range (Fig. 1 and Fig .2), removing the water signal from the data by subtraction could result in better prediction. Blueberries have high percent of water, which to great extent interferes with the NIR signal since water has high absorbance. The purpose of this analysis was to remove some of the water signal, which dominates the absorbance from the other components. PLS models from USDA-KSU data were computed after water spectrum subtraction and the results for one of them are presented in the

last column of Table 1. The prediction ratio for this model was 80.7 %, which is insignificant improvement compared to the 80.1 % of the basic model. These results indicate that the water content variation contribution in the spectra is accounted for by the PLS model.

PLS models, comparison between fresh and frozen. During this season the tests with frozen and blueberries were continued. The preliminary modeling results with frozen berries show a level of prediction of 68 to 82 % that is comparable and in many cases higher than the prediction levels with fresh berries. These data show that the difference in prediction level between frozen and fresh berries was between 2 and 8 %, which is similar to 2003 results. Therefore, infestation in frozen berries can be predicted as well and in many cases better than in fresh berries. This is important for the processing industry since blueberries are most often handled frozen.

PLS models, berry and maggot size separation. In order to determine the effect of maggot and berry 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. The results of nine models with different combinations of berry and maggot size are summarized in Fig. 5. Blueberries were separated in two size categories: 6-7 mm and 8-11 mm. During dissections maggots were separated in three categories according to their length: large ~ 4 mm; medium ~ 3 mm and small < 2 mm. Models after berry size separation did not show significant differences, which confirms that the PLS algorithm usually compensates for sample size by adding more factors. Maggot size separations, however, showed major differences in the prediction ratio of large and medium versus small maggot size. As seen in Fig. 5, selecting large maggot samples yielded ~80 % prediction, which was similar but not higher than the prediction of the full model. Similar prediction was obtained for medium maggots. Models with small maggot samples yielded prediction of ~55 % which means that the prediction model is failing. Therefore, we conclude that the detection limit for the NIR method is maggot length of approximately 2 mm. It has to be noted that this limit is similar to the detection limit of the standard USDA test, which is a visual test by the inspector (Donahue, unpublished data).

Additional studies involving chemical composition analysis are needed in order to better explain these findings. In order to determine which chemical compounds in the blueberry and in the maggot (or metabolite) are detected by NIR, chemical separation analysis such as LC-MS has to be carried out. Such study will help us correlate the absorption bands in NIR spectra to the chemical species in the samples, which is essential for model accuracy and reliability. This will help us answer the question whether NIR is detecting species due to maggot presence or species, which are correlated to some extent to infestation such as variation in water or carbohydrate content.

Overall results in 2004 indicate that the USDA-KSU spectrometer provides better results than the UMaine spectrometer due to longer wavelength range. Models on berry calyx and stem show similar classification results. Spectral subtraction show differences between infested and non-infested samples and define the region of interest; 1350-1700 nm. However, PLS models on selected wavelengths do not lead to improved classification. Detection limit based on maggot size is ~ 2 mm. Berries with maggots 2 mm and smaller are not classified as successfully. The analysis of the models from 2004 and previous years suggests that comparable level of prediction can be achieved regardless of field and season variation. In addition, the USDA-KSU work indicates that frozen berries can be modeled and their concentrations predicted as successfully as fresh ones, which is important for process-line applications.

RECOMMENDATIONS: Continue the study using NIRS during the 2005 field season. The laboratory inoculation/infestation method (lead by Dr. Drummond) of assuring a high percentage of maggot-infested berries will be used as a primary source of berries for these studies. Dr. Drummond will work to optimize the parameters associated with this portion of the study.

Dr. Donahue will continue to evaluate the NIRS systems in the VIS region (600-1100 nm) at UMaine and in the NIR (700 – 1700 nm region) through collaboration with USDA-KSU laboratories in Manhattan, Kansas (Dr. Floyd Dowell). Future work will include model design and validation in order to determine consistent classification ratio and reliability using data from all seasons. Chemical composition analysis study (LC-MS) will help to determine which compounds in the maggot (or metabolite) are detected by NIR spectroscopy, this work is essential for prediction model accuracy. Detection limit based on maggot size will be investigated further. Tests will be carried out of other known classification techniques (Neural Networks, Discriminant analysis) in attempt to improve infestation prediction ratio.

Dr. Donahue will also begin the initial phases of design of a prototype instrument for in-process-line separation in conjunction with Dr. Bruce Segee, UMaine electrical engineer, to compare with the current separation methods described herein. Funding for this study will be sought from the Maine Technology Institute and other external sources with the help of the Wild Blueberry Commission of Maine.

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Table 1. **PLS results of models on combined data sets with samples from Jonesboro, Maine, scanned at UMaine and USDA-KSU. Outliers were removed before the calculation of the model and all models were cross validated, mean centered and variance scaled. Models with 1st derivative pretreatments were tested on data sets from UMaine and USDA-KSU**

Instrument	**UMaine (triplicates)**	**UMaine (triplicates)**	**USDA-KSU**	**USDA-KSU**	**USDA-KSU Water spectrum subtraction**
Wavelength range, nm	650 – 1100	650 – 1100	550 – 1690	550 – 1690	550 – 1690
Preprocessing and number of PLS factors	None 11	1st derivative 8	None 8	1st derivative 6	1st derivative 6
Total number of spectra (non-infested, infested)	2114 (1269, 845)	2114 (1269, 845)	553 (398, 155)	553 (398, 155)	552 (399,155)
Total correct classification, %	75.7	72.4	80.1	80.7	80.7

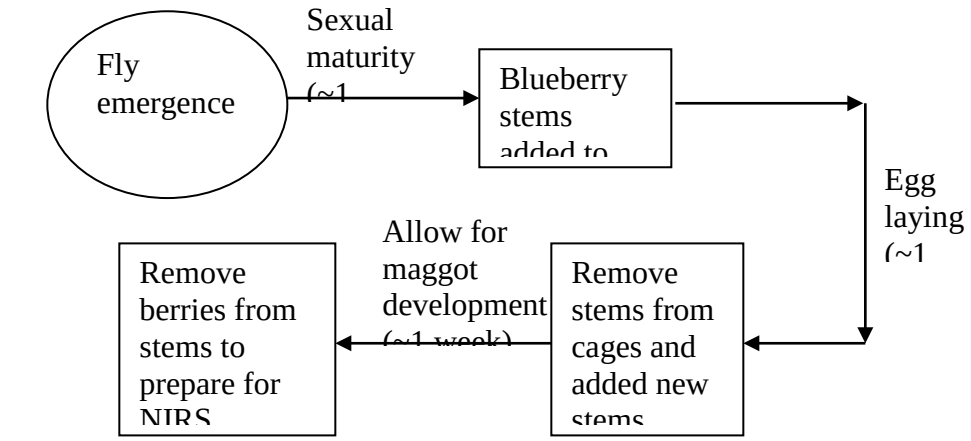

Figure 1. Schematic of the laboratory artificial infestation and preparation for NIRS scanning

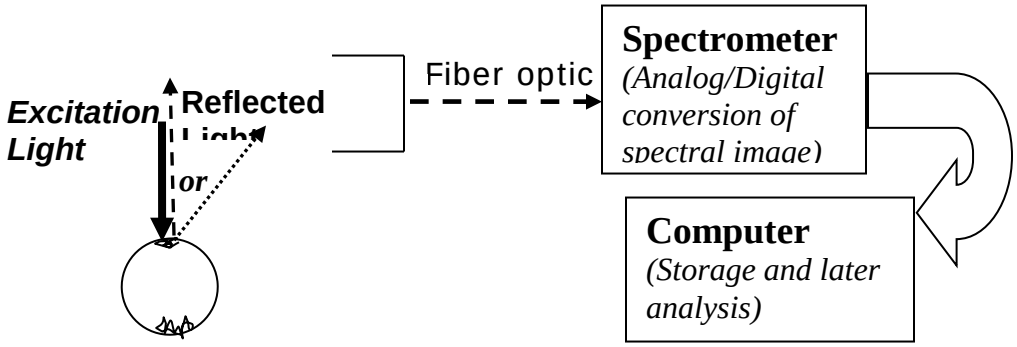

Figure 2. Flow schematic of equipment, light capture, spectrometer and computer. Reflected light will be at 45 or 360 degrees angle measured from the excitation light.

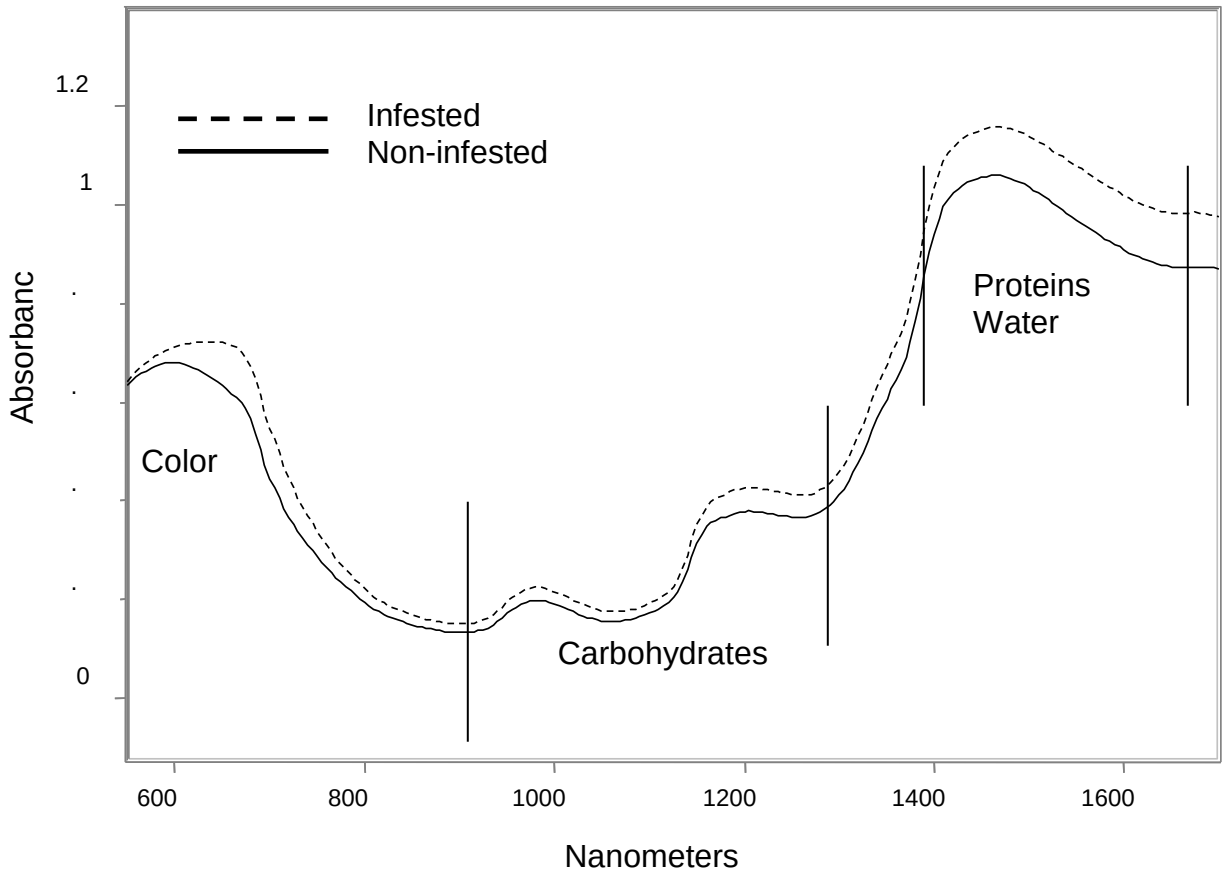

Figure 3. **Raw spectra scanned at USDA-KSU with wavelength regions of interest (color, < 750 nm; carbohydrates, ~900 - 1300 nm; and proteins and water 1350 – 1700 nm)**

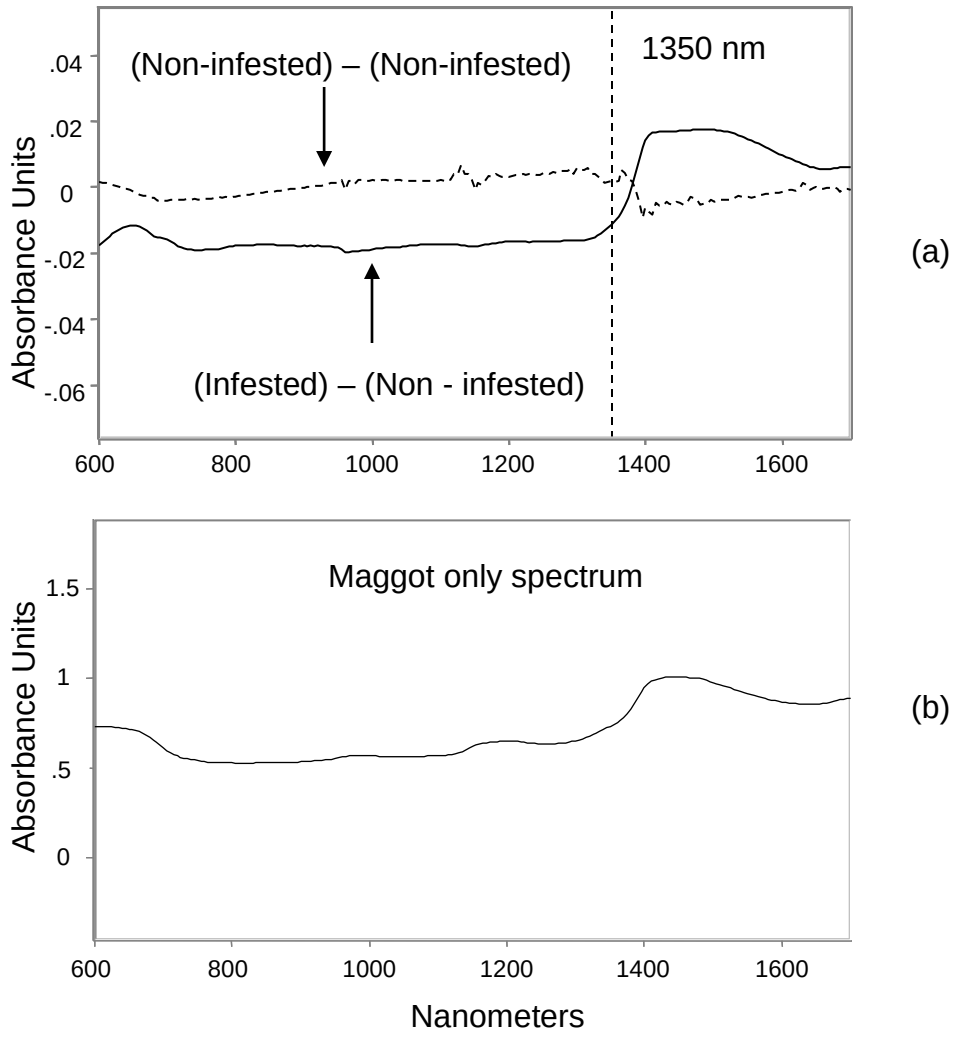

Figure 4. Subtractions of averaged spectra scanned at USDA-KSU (a) and a spectrum of a blueberry maggot (b)

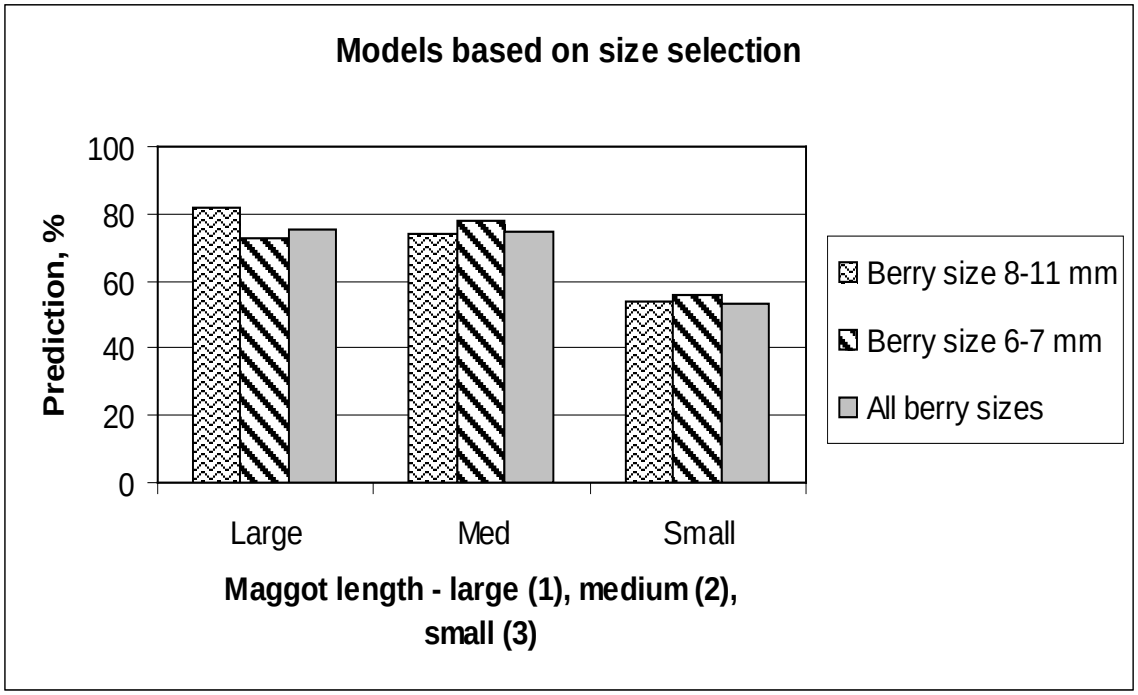

Figure 5. Model prediction after sample selection based on berry and maggot size (USDA-KSU data only)

FOOD SCIENCE AND BIOSYSTEMS ENGINEERING
INVESTIGATOR: Mary Ellen Camire, Dept. Of Food Science & Human Nutrition

5. TITLE: Health Claims for Wild Blueberries

OBJECTIVE: The purpose of this project was to review published literature and recommend strategies to the industry for successful health claim approval in the near future.

METHODOLOGY: Literature on wild blueberry health research was reviewed and summarized. Recommendations for future were developed.

RESULTS: Despite extensive publicity about the health benefits of wild blueberries, there is still insufficient scientific evidence to petition the U.S. Food and Drug Administration (FDA) for a qualified health claim for the fruit. Animal and *in vitro* studies may be submitted to FDA, but human research is essential. Health claims for foods no longer must have significant scientific agreement.

Claims with less supporting data can be used with specific warnings & qualifiers. FDA assigns letter grades of B-D for qualified claims. For example, a claim for soy-based phosphatidylserine was approved based on 15 intervention studies with humans. One form of the approved claim is: a consumption of phosphatidylserine may reduce the risk of cognitive dysfunction in the elderly. Very limited and preliminary scientific research suggests that phosphatidylserine may reduce the risk of cognitive dysfunction in the elderly. FDA concludes that there is little scientific evidence supporting this claim. This is not the type of claim that the wild blueberry industry would want to promote. While it is not clear how important health claims are to the general public, claims do seem to convey credibility.

Antioxidant Activity

While the publicity campaign for a number one antioxidant fruit has been fairly successful, it may be time to focus on other messages. There is no official antioxidant method approved by FDA or USDA for food labeling purposes. At this point in time foods may only make an antioxidant content claim based on vitamin C (ascorbic acid), vitamin, beta-carotene, or selenium content. Anthocyanins and other phenolic compounds are not recognized by FDA as dietary antioxidants because the National Academy of Sciences refused to consider them. FDA regards antioxidant activity as a nutrient content, not a health claim. There is no legal basis for labeling wild blueberries a re a good source of antioxidants. Development and use of the ORAC test for antioxidant activity by USDA does not make that method a gold standard for the food industry.

I strongly urge the industry to avoid challenging other commodities on the number one antioxidant claim. Bickering at professional meetings weakens the credibility of WBANA. Prior and co-workers published a paper in 2004 that updated previous work. Although lowbush blueberries had a high total ORAC per gram, the value was based on one sample only. Results based on a single sample are rarely considered valid. Efforts should be made to provide Dr. Prior with multiple samples for future projects. Since no comparative statistics were published, it is simply not good science to claim that one value is higher or lower than another in that paper. Claims that lowbush blueberries are the highest antioxidant food based on typical serving size are not defendable.

Aging

FDA does not regard aging as a disease, thus no health claim can be made for retarding or reversing aging. Aging does fall into the category of structure-function claims, however. If aging improvements could be related to a nutrient, then a structure-function claim could be made. Benefits do seem to be based on the anthocyanins and other phenolic compounds. Ironically, a dietary supplement made from blueberries could claim that it reverses or retards aging, but the berries themselves may not bear such a claim.

Brain Function

James Joseph and his colleagues have demonstrated a variety of benefits for animal models. To date, only one human study has been conducted and those findings are still being analyzed. That study, conducted at the University of Maine, used an organic wild blueberry extract supplement, not whole berries. Joseph's work has spanned many issues including Alzheimer's models, normal aging, and abnormal oxygen conditions. In some studies, lowbush blueberries did not perform as well as did cultivated berries, specifically Tif-blue berries. There is insufficient data at this time to pursue a health claim for blueberries' protection against Alzheimer's disease.

Cardiovascular Health

FDA has approved qualified health claims for reducing risks for coronary heart disease and vascular disease. In general, at least 5-6 human studies are needed to obtain a AB level of qualified claim. None of the published studies involving human subjects and blueberries have specifically addressed cardiovascular health. The majority have focused on changing antioxidant capacity in the serum. As previously mentioned, antioxidants are a gray area for claims. A recent review (Arts and Hollman, 2005) reviewed epidemiology studies and concluded that consumption of polyphenols has beneficial effects on cardiovascular diseases, presumably due to the antioxidant effects of the polyphenols. However, Madamanchi et al. (2005) stated that despite the growing evidence for a role of reactive oxygen species in the development of cardiovascular diseases, it does not appear that consumption of antioxidants can prevent death from those diseases.

Mazza et al. (2002) fed five men a freeze-dried blueberry powder along with a high-fat meal. Serum ORAC values increased significantly after the meal. Kay and Holub (2002) also found significant increases in serum ORAC values after eight middle aged men were fed a high fat meal with 100 grams of freeze-dried wild blueberry powder. In the only study published on the effects of cultivated blueberries on antioxidant status, consumption of commercial organic blueberry juice by nine women did not result in higher serum antioxidant levels, but cranberry juice did (Pedersen et al., 2000). None of these studies were long-term and all involved just a few people.

Other studies have examined the role of anthocyanins in controlling reactive nitrogen species, which are involved with the oxidation of LDL-cholesterol and atherosclerosis (Xu et al., 2004; Ichiyanagi et al., 2004). These *in vitro* studies support the animal research of Dorothy Klimis-Zacas, who fed rats freeze-dried wild blueberries and found improved blood vessel elasticity in the berry-fed rats (in press).

No evidence is yet available for reduction in other cardiovascular risks such as high serum LDL and inflammation levels. The study we are starting this year will examine the effects of consumption of one cup of frozen wild blueberries on serum cholesterol, high sensitivity C-

reactive protein, antioxidant activity and blood pressure in adults, compared to a control group following the American Heart Association's Therapeutic Lifestyle Change (TLC) diet. Approximately thirty people will be recruited for the study. Additional studies will be necessary to develop an adequate portfolio of studies to submit to FDA for a qualified health claim.

Other Health Issues

Anthocyanins may have a role in protecting liver function and preventing diabetes. These issues will be discussed in the full report.

RECOMMENDATIONS: The wild blueberry industry should focus research funds on human studies and support efforts of researchers to gain USDA and NIH funding for human studies.

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FOOD SCIENCE AND BIOSYSTEMS ENGINEERING
INVESTIGATOR: Dorothy J. Klimis-Zacas

6. TITLE: Wild blueberries and Arterial Functional Properties

OBJECTIVE: to study the role of wild blueberry consumption on the maximum force of relaxation (Fmax) of intact aortic rings when challenged with the endothelium-depended vasorelaxant, acetylcholine, and to measure the activity of the eNOS enzyme in aortic rings from Sprague-Dawley rats fed control and blueberry-enriched diets.

METHODS AND RESULTS: Weanling Sprague-Dawley rats were randomly fed two different diets (n=10 per group), a control diet (AIN '93) (C), and a blueberry diet (B) for thirteen weeks. 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 precontracted with 10^{-6} M Phe and relaxed with cumulative concentrations of acetycholine (10^{-9} to $3x10^{-6}$ M). The maximal force of relaxation was measured (Fmax) to determine the effect of blueberries on endothelium NO-mediated vasodilation of intact aortic rings. The activity of eNOS (endothelial nitric oxide synthase) in the aortic rings was determined by western blots in the aortas of rats fed control and blueberry-enriched diets. No significant differences in the maximal force of relaxation (0.997+0.087 vs. 0.957+0.086, p=0.445) were detected in the intact aortic rings between the C and B diets respectively. Thus there were no differences in percent relaxation between the control (99.722%) and the blueberry-enriched diets (95.745%) in response to acetylcholine, a vasodilator which requires the endothelium to employ its effect and its action is mediated through NO.
The eNOS activity assay was tested and validated this Fall. At this point we are repeating experiments and will be reporting results on eNOS next Summer (2005).

In other experiments, weanling male Sprague Dawley rats were randomly assigned to either a control (C) and blueberry (B) diet. After 13 weeks, aortic tissue glycosaminoglycans (GAGs) were isolated with papain digestion, alkaline borohydride treatment and anion-exchange chromatography. Cellulose acetate electrophoresis and treatment of the fractions with specific lyases showed the presence of three GAG populations, i.e. hyaluronan (HA), heparan sulfate (HS) and galactosaminoglycans (GalAGs). Disaccharide composition was determined by high-performance capillary electrophoresis (HPCE) following enzymatic degradation. A 13% increase of the amount of total GAGs in aortas of blueberries-fed rats was attributed to increased GalAGs. Capillary electrophoretic determination of the variously sulfated disaccharides showed an overall decrease of oversulfated disaccharides in both HS and GalAG populations in the aortas of the blueberry group. The undersulfation of the GAG chains underscores the importance of wild blueberries in protecting the endothelium from Low Density Lipoprotein (LDL) retention and may thus be atheroprotective when added to the diet.

CONCLUSION AND SIGNIFICANCE: Our studies in the past documented that wild blueberries affect the contractile machinery of the smooth muscle cell by decreasing arterial contractility in response to the stress hormone, epinephrine. From the present experiment 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 do not seem to have an effect at least when we compare animals fed normal diets to blueberry-enriched diets. The effect of blueberries on vasorelaxation may be able to be detected when animals that are under oxidative stress (Spontaneously Hypertensive or Manganese deficient) are placed on normal or blueberry enriched diets. Another possibility may be that wild blueberries operate through alternate pathways to affect arterial contractility and that new experiments should test compounds that inhibit different pathways in the endothelial and/or the smooth muscle cell layer of the artery. Thus, our pilot studies on endothelium-dependent relaxation need to be repeated and confirmed with additional experiments utilizing acetylcholine as an endothelium-dependent agonist but also with other endothelium-independent agonists such as nitroprusside, endothelin and KCL to pintpoint the mechanism of the previously observed effect of blueberries in preventing aortic vasoconstraction when challenged with L-Phe.

Our results on the effect of wild blueberries on aortic GAG structure demonstrate for the first time that a blueberry-rich diet induces structural changes in rat aortic tissue GAGs. These changes may affect many protein-protein interactions and could have major consequences for the biological function of GAG molecules within the vascular environment and consequently on the process of atherosclerosis.

RECOMMENDATIONS: Experiments will be repeated using the Spontaneously Hypertensive rat, a model for oxidative stress, and eNOS activity and expression will be studied in the above model.

Following are the publications that have resulted from the wild blueberry research:

Refereed Publications

Norton, C., Kalea, A.Z. and Klimis-Zacas, D. The effect of whole blueberries on arterial biomechanical properties in the Sprague Dawley rat. *Journal of Medicinal Food, 2004* (*In press-accepted Aug 2004-expected publication date March 2005*)

Kalea, A.Z. Lamari F., Theocharis A.D., Kordopatis P., Karamanos N.K., Klimis-Zacas, D. Dietary blueberries affect the composition and structure of rat aortic extracellular matrix, *Journal of Nutritional Biochemistry* (*under review*)

Published abstracts:

Kalea, A.Z., Norton, C., Harris, P.D. and Klimis-Zacas, D. Whole wild blueberries suppress $\alpha_1$adrenergic agonist induced contraction in rat aorta, *FASEB J.* 17(4): A334, 2003.

Norton, C., Kalea, A.Z., Harris, P.D. and Klimis-Zacas, D. Whole wild blueberries affect the vascular contractile machinery in the Sprague-Dawley rat, *FASEB J.* 16(4): A334, 2003.

Kalea, A.Z. Lamari F., Theocharis A.D., Karamanos N.K., Klimis-Zacas, D. Dietary blueberries affect the composition and structure of rat aortic extracellular matrix (*submitted for poster presentation in the FASEB-Experimental Biology 2005 Meeting, San Diego, April 2nd 2005*)

IRRIGATION

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

7. TITLE: Irrigation Water Use in Wild Blueberry Production

INTRODUCTION: 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 2002-2004 have substantially confirmed these beliefs. However, research is needed to make better recommendations on irrigation water applications. Research has progressed significantly since the inception of the project, and the 2004 dataset is the most complete of any thus far. Our preliminary analysis of 2004 data on water use generally confirms results presented in previous annual reports. In this report, we discuss progress on sites and methods, revisit results from 2002 and 2003 to provide background information, and then give a discussion of some unique features and initial analysis of the 2004 dataset.

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 three additional sites. In 2004, installations in both crop and prune years were used at Blueberry Hill and Deblois and an installation in crop only was used at Kelley Point. 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 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 is clearly 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, soil moisture tension sensors (heat dissipation sensors) have been installed just below the soil surface to measure an expected decrease in tension if water is being adsorbed from atmospheric vapor or drip from the plants.

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.

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

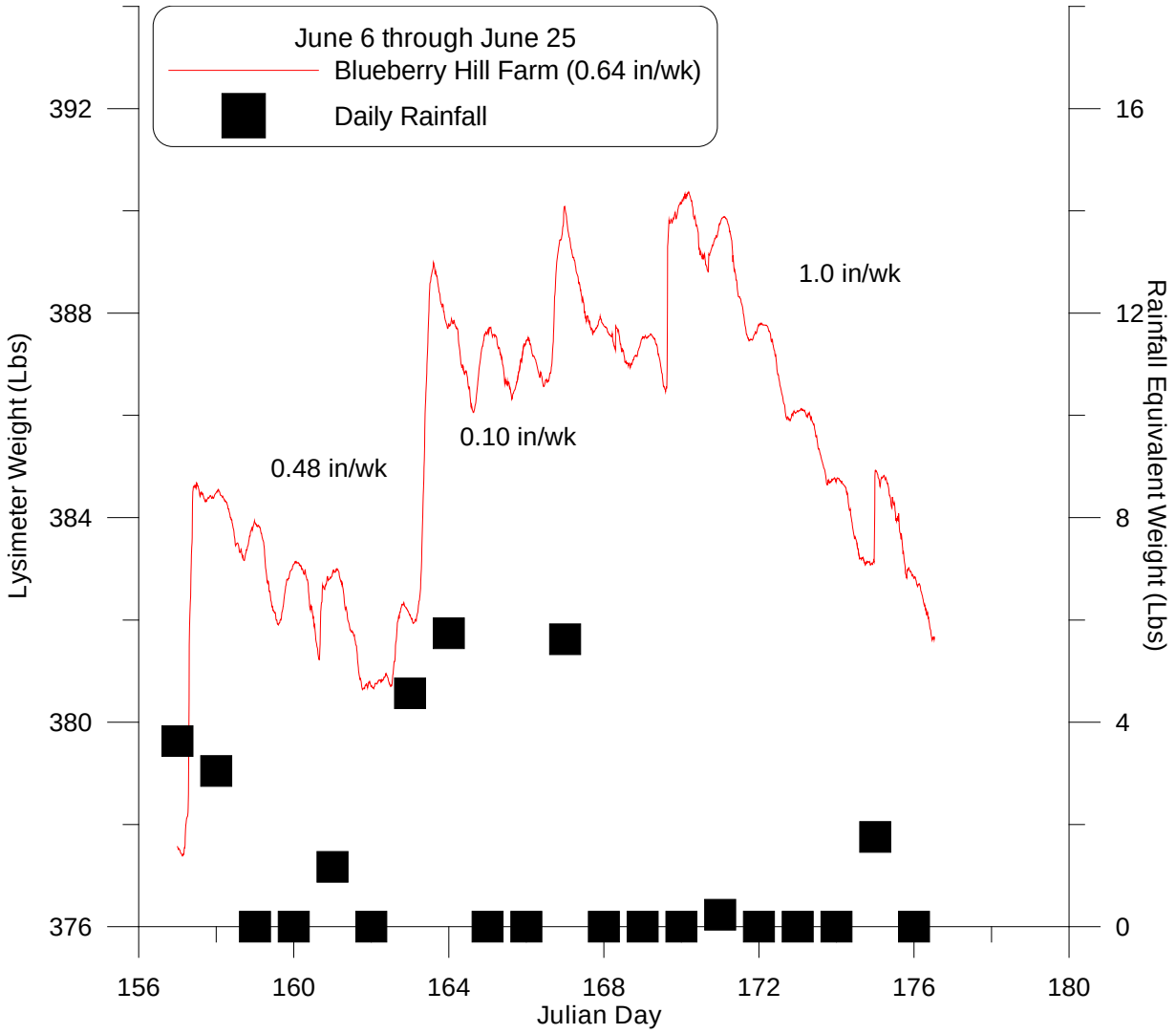


Figure 2. Rainfall equivalent weight and lysimeter weight versus the day of year (Julian Day) from June 6 through 25, 2002.

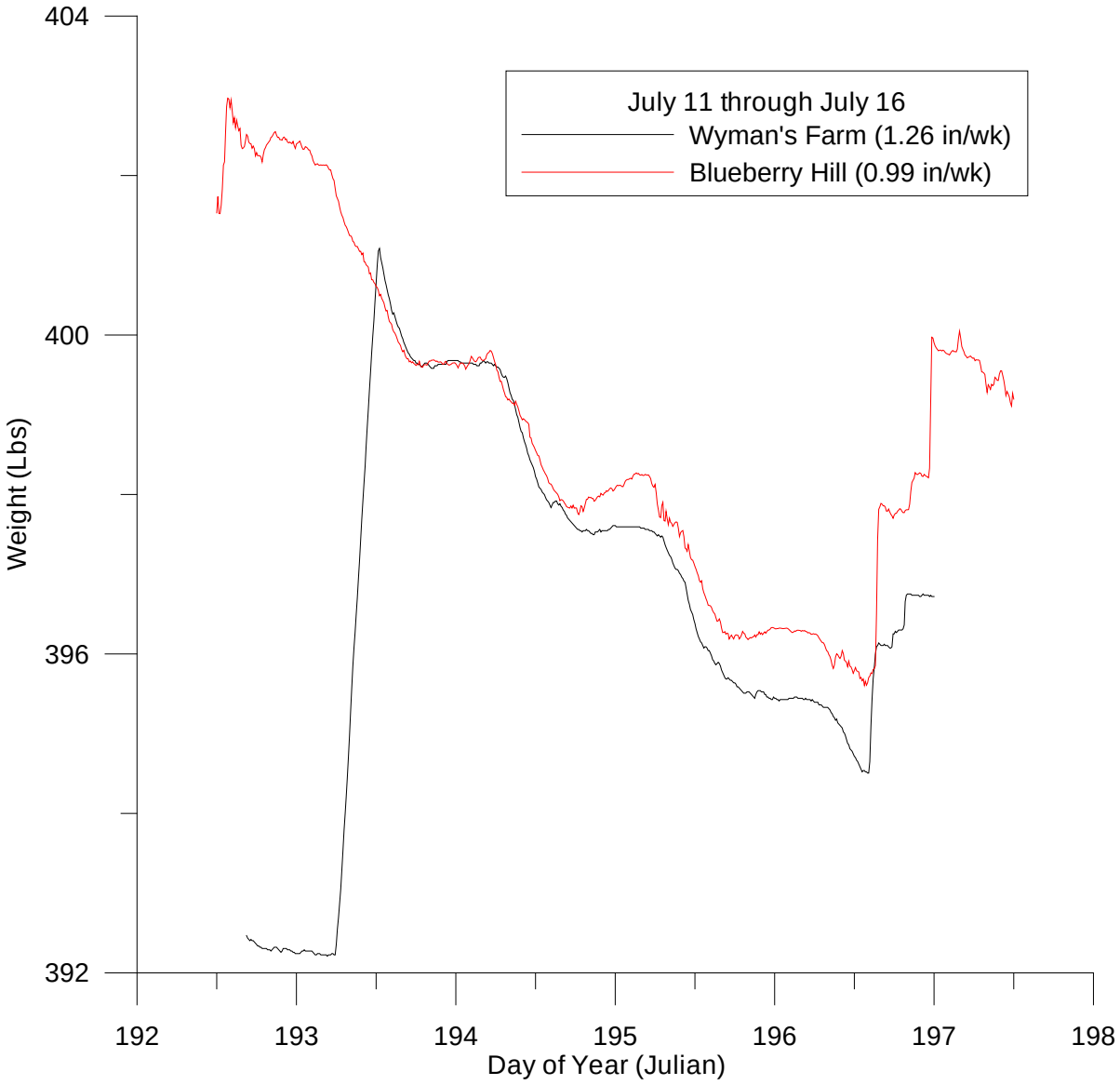


Figure 3. Lysimeter weight over time comparing Blueberry Hill and Wyman's Farm in 2002.

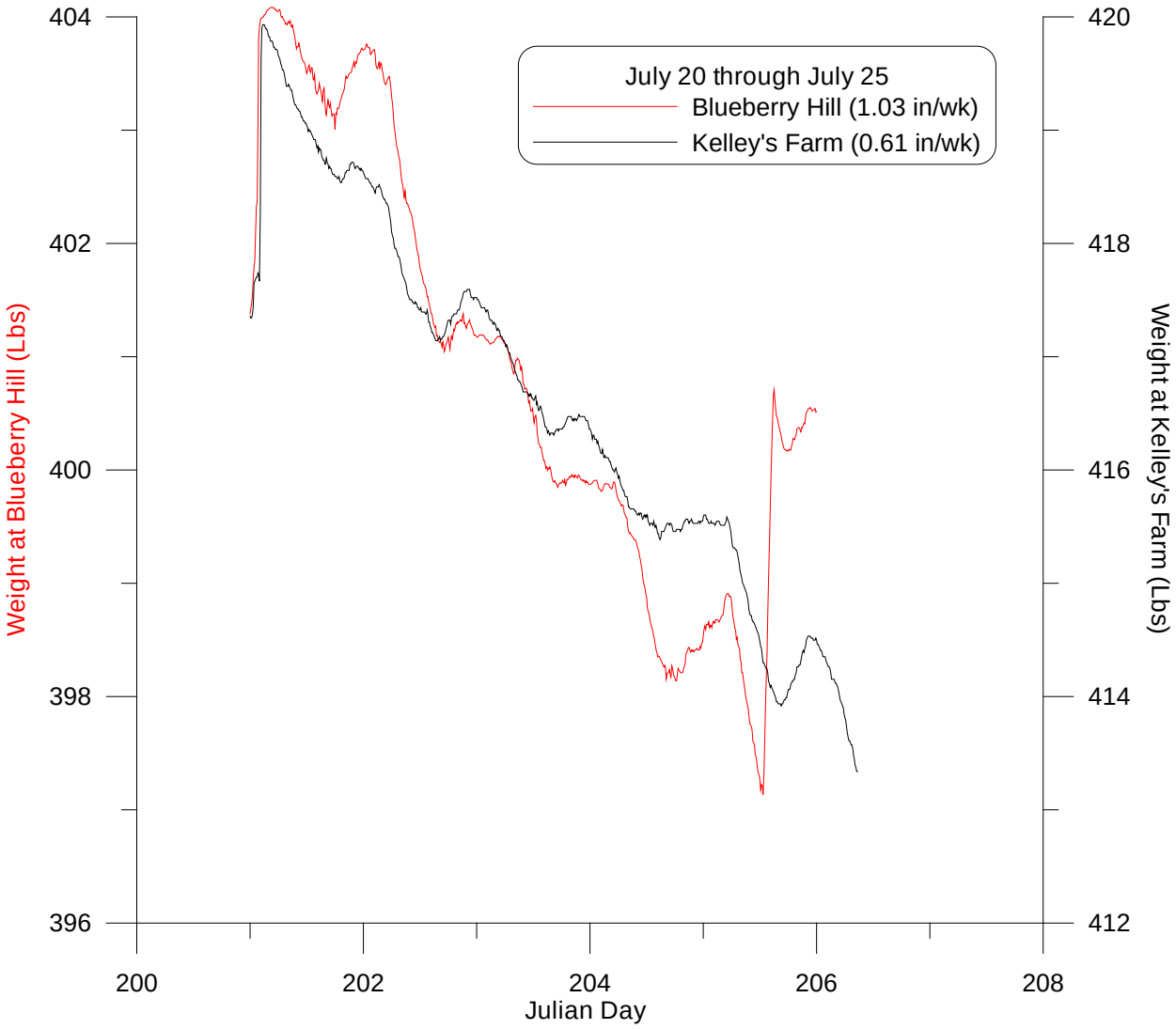


Figure 4. Lysimeter weight over time comparing Blueberry Hill and Kelley's Farm

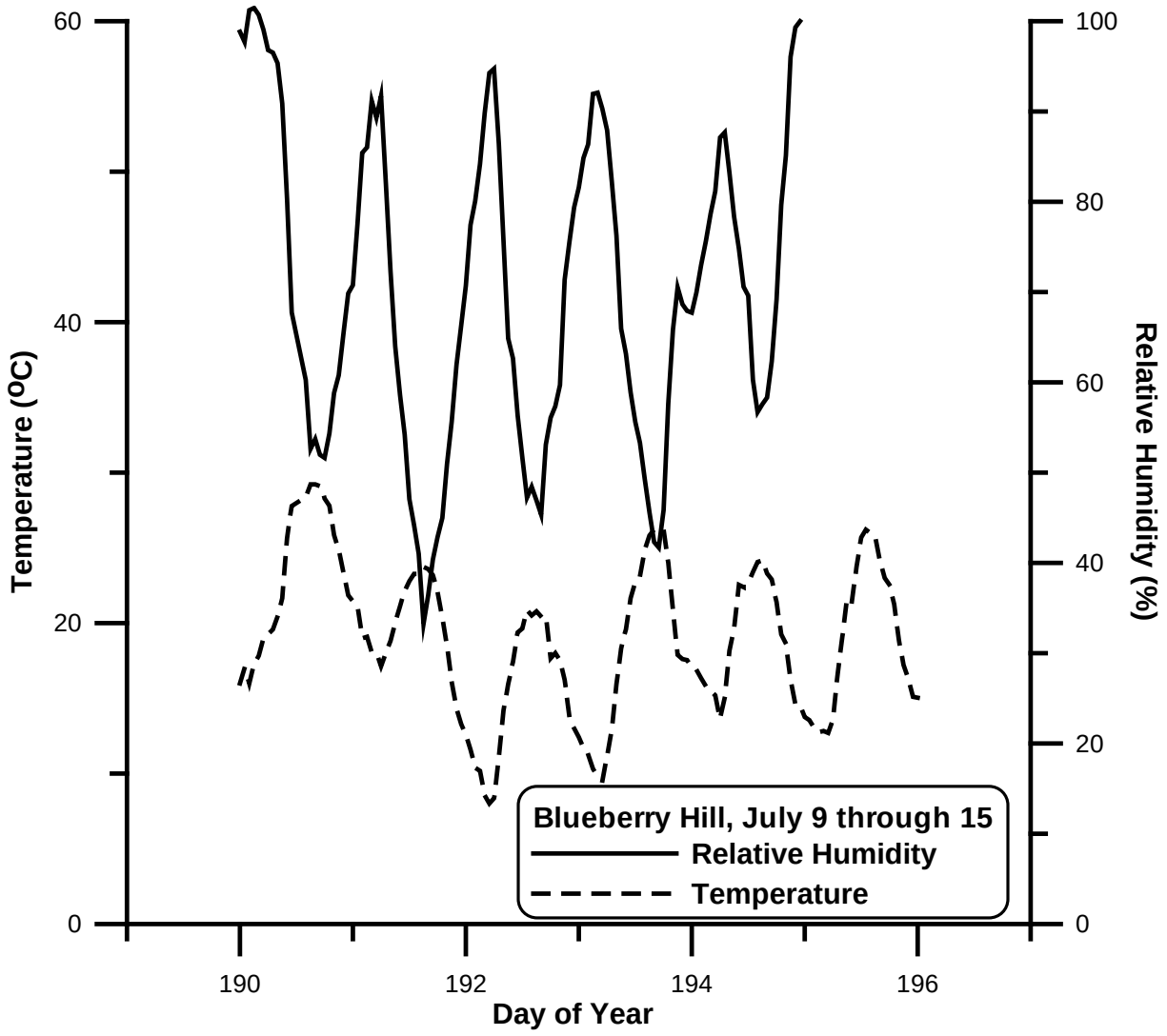


Figure 5. Daily temperature and relative humidity patterns at Blueberry Hill in Mid July, 2002.

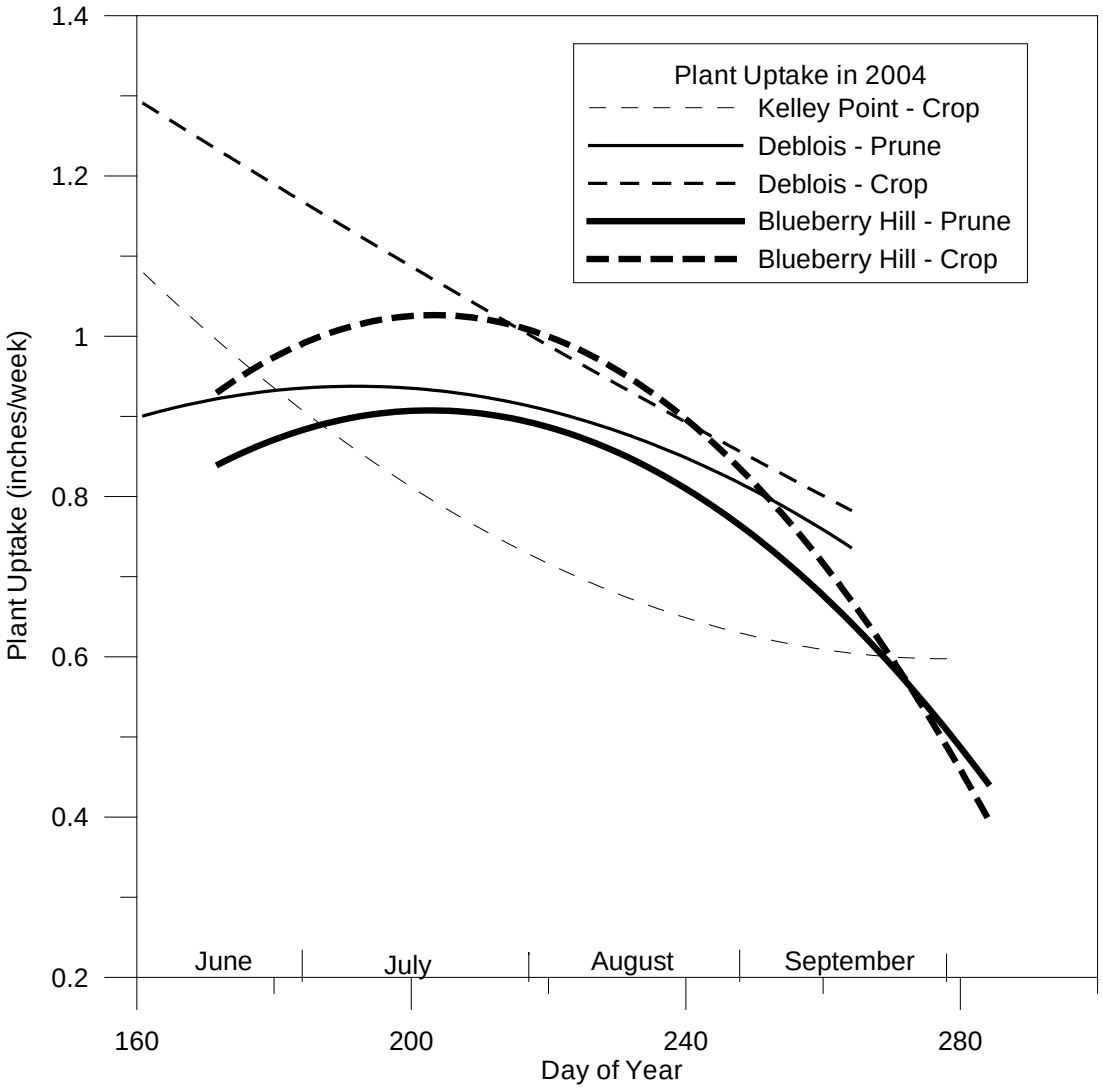


Figure 6. Plant water uptake curves for three sandy sites in prune and crop years.

ENTOMOLOGY

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

8. TITLE: Insect Control Tactics for Blueberry Pest Insects & Program Base

1. Laboratory screening of insecticides.

METHODS: The efficacy of two rates of Fermented Salmon, All-purpose Liquid Organic Fertilizer® was evaluated against blueberry flea beetle larvae (BF). In a second trial, red-striped fireworm larvae (RSFW) were allowed to feed on treated blueberry foliage. In both trials, insects were provided with insecticide-treated stems that were cut and brought into the laboratory.

RESULTS/CONCLUSIONS: Neither a 4 nor 8 oz rate of fermented salmon product was effective in controlling FB larvae (Table 1). In the RSFW trial, application of Intrepid 2 F® resulted in 98% mortality of RSFW larvae within 10 days of the application (Fig. 1). Although mean time to death with Mycotrol ES® was significantly different from the control, it resulted in only 19% mortality by the end of the trial; 14.3% mortality was obtained with Aza-Direct®.

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

METHODS: Trials were completed against blueberry spanworm (SW), blueberry flea beetle (FB) strawberry rootworm (SRW), red-striped fireworm (RSFW) and blueberry thrips (BT). The tests against blueberry spanworm and blueberry flea beetle were applied as foliar sprays to fruit-bearing fields. Pre- and postspray sweep-net samples were used to estimate control. In the FB trials, *Beauveria bassiana* mortality was determined by holding field-collected cohorts in the laboratory and observing them for sporulation.

The field used for the RSFW trial was pruned by mowing in the spring of 2004 and was heavily infested with RSFW as evidenced by webbed-together leaves on blueberry stems. Following the application, we collected 30 infested stems (as evidenced by webbed-together leaves) from each plot, brought them into the laboratory, and examined them for the presence or absence of live or dead larvae. *B. bassiana* mortality was determined by holding field-collected RSFW in the laboratory and observing them for sporulation. Several weeks later, we also counted all stems with webbed-together leaves in five, sq ft frames per plot.

In the BT trial, Admire® was applied as a spray to the soil in a pruned field prior to stem emergence; GF-968 was 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 the SRW trial, application was made in a pruned blueberry field with areas heavily defoliated by feeding. Efficacy was based on blueberry stem recovery as indicated by of stem height and % cover.

RESULTS/CONCLUSIONS: Imidan® (both formulations), GWN-1975 (both rates), GF-968 (all three rates), and SpinTor® all provided excellent seasonal control of SW larvae. The 9.6 oz rate of Novaluron® gave some control, while the 38 oz rate was not effective (Fig. 2).

Imidan 2.5 EC®, GWN-1975 2.5 EC, and Entrust 80 WP® also gave excellent control of FB larvae, and when data were adjusted to account for mortality due to *B. bassiana*, Mycotrol ES® also gave very good control (Fig. 3). In a second trial against BF larvae, a half rate of Mycotrol® + a half rate of Entrust® was not significantly different from the untreated check on day 7 of the trial. However, over the entire season, Mycotrol® + Entrust® did significantly reduce FB larval populations in comparison with the untreated control (Table 2). Three rates of GF-968 also performed well.

For the SRW trial, there was no significant difference in stem height or % cover in plots treated with SpinTor®, Imidan®, or Admire® (Table 3).

The pre-emergence application of Admire® resulted in a 64% reduction in the average % stems with thrips curls in each sq ft sample (Table 4). A reduction of 38% was observed following a similar application in 2000 and 26% in 2003. A 30% reduction was observed with GF-968. There was no significant difference in the average number of stems per sample.

Mycotrol® and Intrepid® appeared to be the most effective treatments against RSFW larvae. Survival was lowest in the Intrepid-treated plots compared to the untreated control plots (Table 5). It is also interesting to note that there was a higher percentage of empty curls in plots treated with Mycotrol® or Intrepid®.

3. Control of blueberry maggot with ground application of insecticides.

METHODS: We conducted three trials against blueberry maggot (BMF). All materials were applied in 6 gallons of water-mixture per acre using a SOLO® 450 mistblower. 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.

RESULTS/CONCLUSIONS: In Trial #1, only Imidan 2.5 EC® and Provado 1.6 F® significantly reduced the seasonal density of BMFadults in comparison with the untreated controls (Table 6). These results must be interpreted with caution, since a possible "overfly with Imidan 70 WP® may have occurred on 26 July. A commercial aerial application was made to the area; however, the proximity of our test plots to the tree-lined perimeter may have protected them from the application. There was a subsequent reduction in larval infestation as measured by number of pupae collected from berry samples in the Imidan-and Provado-treated blocks. There was also a reduction in number of pupae collected from plots treated with Aza-Direct®. Although fly numbers were not reduced, this material may inhibit egg-laying activity.

Two applications of Entrust 80 WP® had no significant effect on the seasonal density of BMF adults in comparison with the untreated check blocks in Trial #2. GWN-1975 2.5 EC was only slightly nonsignficant. Both materials were ineffective in reducing the number of pupae collected from the berries. There was no significant reduction in the seasonal density of BMF adults following 2 applications of Novaluron 10 EC®. There was also no reduction in infestation.

4. Control of blueberry maggot with ground application of 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.

RESULTS/CONCLUSIONS: 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 controls (seasonal density = 4.4 ± 1.2 vs. 7.8 ± 1.8, respectively. GF-120 NF is a short-residual material and may require frequent applications to maintain control. This is well demonstrated in Fig. 4. Following each application, there is a drop in average BMF captures followed by a recovery in fly numbers until the next application. There was also a significant reduction in fruit infestation. On average, only 5.3 ± 2.2 pupae were found in treated berries compared to 21.0 ± 7.0 in the untreated checks.

5. <u>Effect of formulation on decay rate of phosmet applied to lowbush blueberry in the crop year.</u>

METHODS: Imidan 2.5 EC® and Imidan 70 WP® were each applied as foliar sprays with a CO2-propelled boom sprayer at the precise rate of 1 lb ai per acre At 1, 7 and 14-days after the applications, 1 quart of berries was raked from each plot. The fruit was frozen and sent to the University of Maine Food Science Chemistry laboratory for analysis of phosmet and the phosmet oxygen analog residues.

RESULTS/CONCLUSIONS: There was a significant difference in persistence of phosmet between the two formulations. The application of Imidan 2.5 EC® resulted in significantly more phosmet residues (Fig. 5). Only one day after application, there was about three times more phosmet on the fruit from the 2.5 EC formulation than from the 70 WP formulation. However, by day 7 phosmet residues from both applications were similar.

Phosmet oxygen analog residues were also significantly higher for Imidan 2.5 EC® (Fig. 6). There were also significant Time*Treatment interactions for phosmet residues and phosmet oxygen analog residues. This indicates that phosmet degradation over time was not equal for the two formulations. Oxygen analog residues for Imidan 2.5 EC® were high on day 1 (5.1 ug/g) and day 7 (4.1 ug/g), than decreased by day 14 (0.3 ug/g); Imidan 70 WP® oxygen analog residues were low throughout the trial (day 1 = 0.4 ug/g, day 7 = 0.4 ug/g, day 14 = 0.8 ug/g).

RECOMMENDATIONS: Since there was strong evidence to support our conclusion that salmon fertilizer does not have insecticidal activity against blueberry insect pests, despite the manufacturer's claim to the contrary, we will not recommend this fertilizer be used as a means of controlling lowbush blueberry pests.

Our field trial and laboratory investigations with the red-striped fireworm this past year did suggest that the reduced-risk insecticide, Intrepid®, does offer promise for management of this pest. We plan on repeating evaluations of Intrepid® over the next two years to determine whether this insecticide has a place in lowbush blueberry IPM. For the organic grower, Mycotrol ES®, does offer some, but limited control of the red-striped fireworm. Aza-Direct® is another possible management tool for red-striped fireworm for the organic grower, but more evaluation of this insecticide needs to be conducted since this past summer was the first time that we investigated its use.

Our field studies did suggest that the EC formulation of phosmet (Imidan®) had a slightly higher persistence on the berries than the WP formulation for one day, but this difference was not seen at 7 days post-application. Insecticide evaluation studies showed no difference in performance between EC and WP formulations of Imidan®, so because of this we do not feel that there is any need to recommend one formulation over another, they both give excellent long-season control of blueberry spanworm, blueberry flea beetle, and blueberry maggot.

The reduced-risk insecticide, SpinTor®, continues to provide excellent control of the blueberry spanworm and will be recommended for control of this insect pest. Trials for flea beetle control suggest that SpinTor®, Entrust®, Imidan®, and Mycotrol® all perform very well. All of these insecticides are registered and currently recommended for use. We did have very good success with a new reduced-risk insecticide, GWN-1975. We plan to continue field trials with this material in the future. Blueberry thrips remain a problem for control. We did have better results this year with Admire®, a 64% reduction in leaf curls. This may be one of our better materials in the long-term, since Diazinon® will not be available in the future.

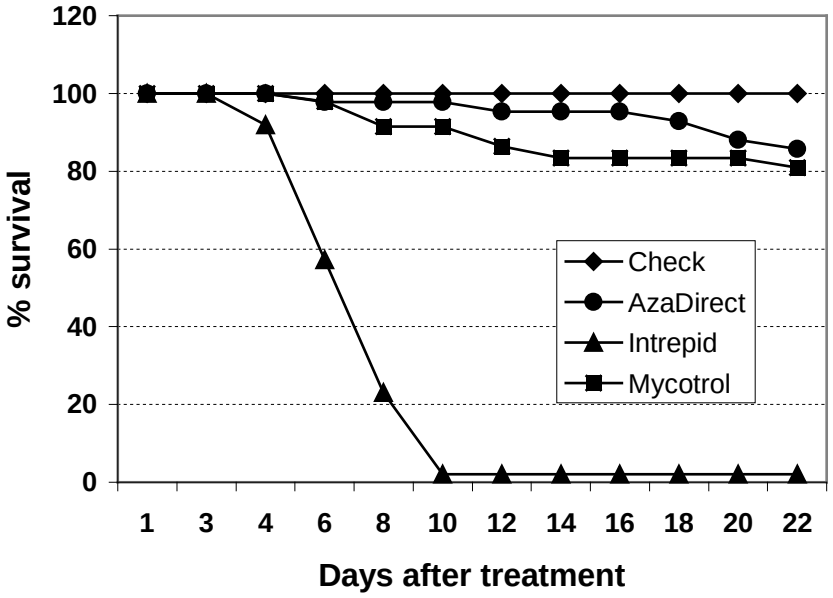
New blueberry maggot fly insecticides show promise, while the standard, Imidan®, continues to provide excellent season long control. There is promise for low toxicity insecticides for blueberry maggot control. Aza-Direct® did not reduce the number of flies, but did significantly reduce the level of infested berries. GF-120 NF®, spinosyn in a sugar bait, applied along the perimeter of fields was very effective at reducing fly numbers and infestation of berries. Both of these materials will be evaluated again next year to determine if they can provide consistent control.

1. <u>Laboratory screening of insecticides.</u>

Table 1. Laboratory screening of Fermented Salmon, All-purpose Liquid Organic Fertilizer against blueberry flea beetle.

Material	Rate (oz/acre)	Number dead (% Mortality) Days after application 1	3	5	8	12	14
Fermented salmon	4 oz	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.0)	1 (8.0)	1 (8.0)
Fermented salmon	8 oz	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.0)	1 (8.0)	1 (8.0)
No insecticide	-	0 (3.4)	1 (3.6)	1 (3.6)	1 (3.6)	1 (3.6)	2 (7.1)

Fig. 1. Laboratory screening of insecticides against red-striped fireworm.



<u>Field evaluation of insecticides for control of secondary pest insects.</u>

Fig. 2. Field Control of Blueberry Spanworm Larvae with Insecticides.

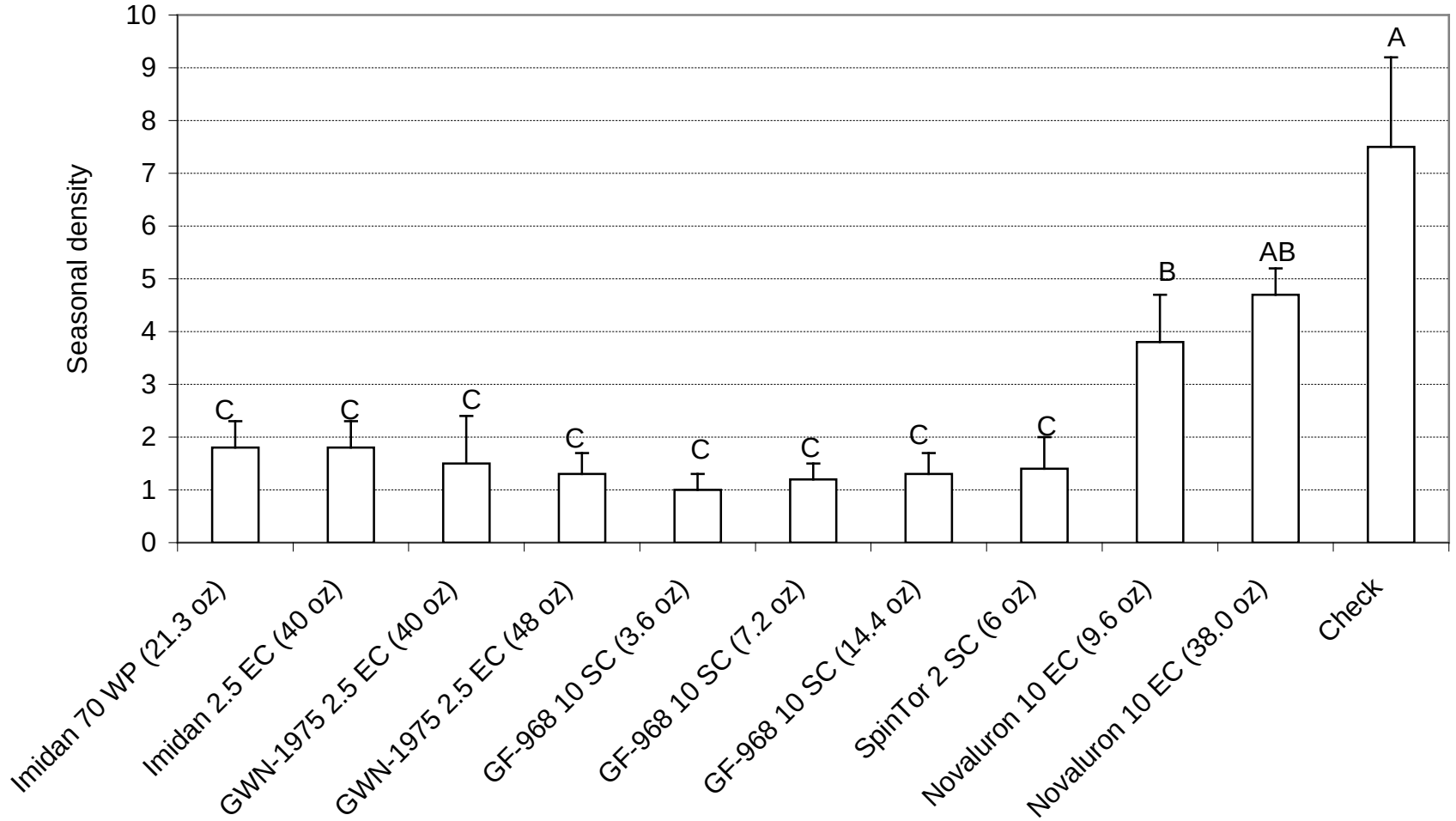
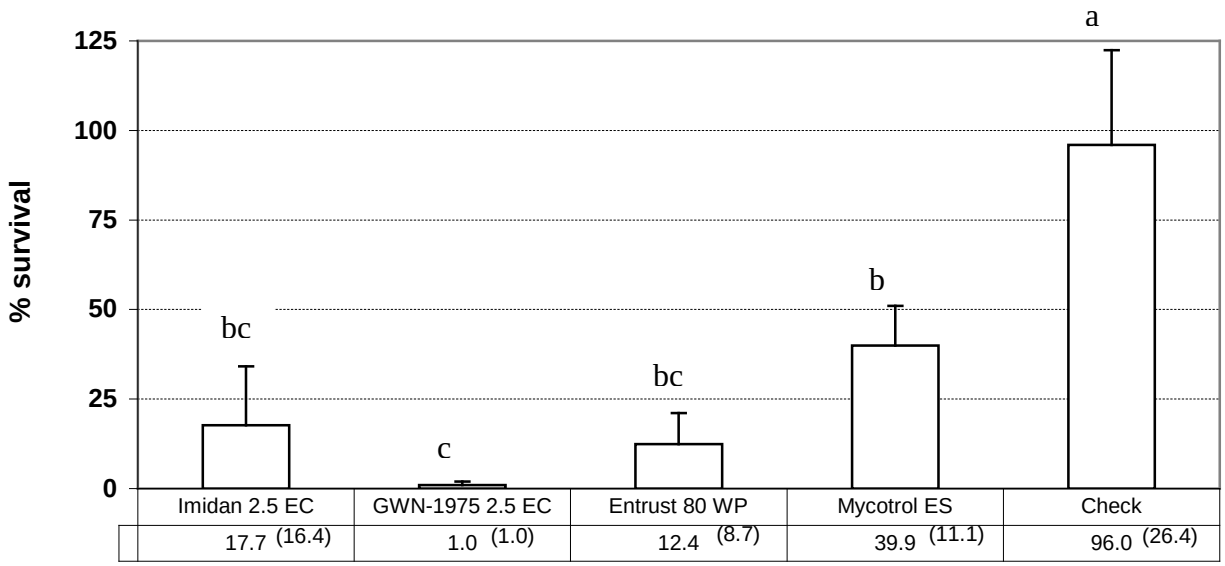


Fig. 3. Control of Flea Beetle Larvae with Insecticides, % survival (Standard error) 15 days after application.



Means followed by the same letter(s) are not significantly different ($P < 0.05$, Tukey's).

Table 2. Field control of blueberry flea beetle larvae with insecticides.

	Amt.	Larvae/10sweeps				
	Form./	Prespray	Postspray			Seasonal
Material	acre	3 June	8 June	10 Jun	16 Jun	density
GF-968 10 SC	2.6 oz	32.5 a	2.0	1.0	0.3	6.3 b
GF-968 10 SC	7.2 oz	32.0 a	0.3	0.3	0.0	5.6 b
GF-968 10 SC	14.4 oz	32.5 a	0.3	0.5	0.0	5.6 b
Mycotrol ES	16.0 oz	30.8 a	8.3	8.8	0.0	10.1 b
+ Entrust 80 WP	1.0 oz					
No insecticide	-	35.8 a	26.8	19.0	1.3	19.2 a

Seasonal densities are trapezoidal integrals of densities over the season divided by the number of day's duration of the experiment. Means followed by the same letter(s) are not significantly different ($P < 0/05$, SNK)

Table 3. Field control of strawberry rootworm with insecticides.

Material	Amt. form./acre	Avg. stem height (in.) 14 Jul	Avg. stem height (in.) 12 Aug	% cover 14 Jul	% cover 12 Aug
SpinTor 2 SC	6.0 oz	4.0 a	5.0 a	38.3 a	93.3 a
Imidan 70 WP	21.3 oz	4.3 a	6.0 a	50.0 a	85.0 a
Admire 2 F	4.3 oz	4.3 a	5.3 a	40.0 a	85.0 a
No insecticide	-	4.7 a	6.3 a	46.7 a	83.3 a

Means among the treatments on each sample date followed by the same letter are not significantly different ($P < 0.05$, SNK).

Table 4. Field control of thrips with insecticides.

Material (SE)	Amt. form./acre	Avg. # stems/ ft^2 (SE)	Avg. % stems with curls/ft^2
Admire 2 F (pre-emergence)	16.0 oz	148.3 (30.0) a	3.8 (0.01) b
GF-968 10 SC (21.26 g ai/A)	7.2 oz	94.9 (2.1) a	7.4 (2.9) a
No insecticide	-	154.0 (22.5) a	11.5 (0.7) a

Means followed by the same letter are not significantly different ($P < 0.10$, SNK).

Table 5. Field control of red-striped fireworm with insecticides.

Material	Amt. form./ acre	Avg. # stems (SE) with [1] webbed-together leaves/ft^2 25 Aug	10 Sep	% survival all stems	% empty [2] curls	% survival [3] adjusted for empty curls
Intrepid 2 F	16 oz	3.5 (0.3)	3.1 (0.4)	19.2 b	65.8 a	6.6 c
Mycotrol ES	32 oz	3.9 (1.2)	2.4 (1.0)	54.2 a	45.8 b	29.4 b
Aza-Direct	32 oz	3.5 (0.5)	3.5 (0.7)	61.7 a	37.5 c	38.7 a
No insecticide	-	3.5 (0.5)	4.7 (1.3)	63.3 a	36.7 c	40.8 a

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

[1] 25 Aug = Prespray, 10 Sep = Postspray.

[2] Examined 30 stems per treatment for presence or absence of larvae.

[3] % survival of red-striped fireworm larvae was adjusted to account for empty leaf curls.

[4] % survival of red-striped fireworm larvae was adjusted to account for empty leaf curls and for *Beauveria bassiana* mortality.

3. Control of blueberry maggot with ground application of insecticides.

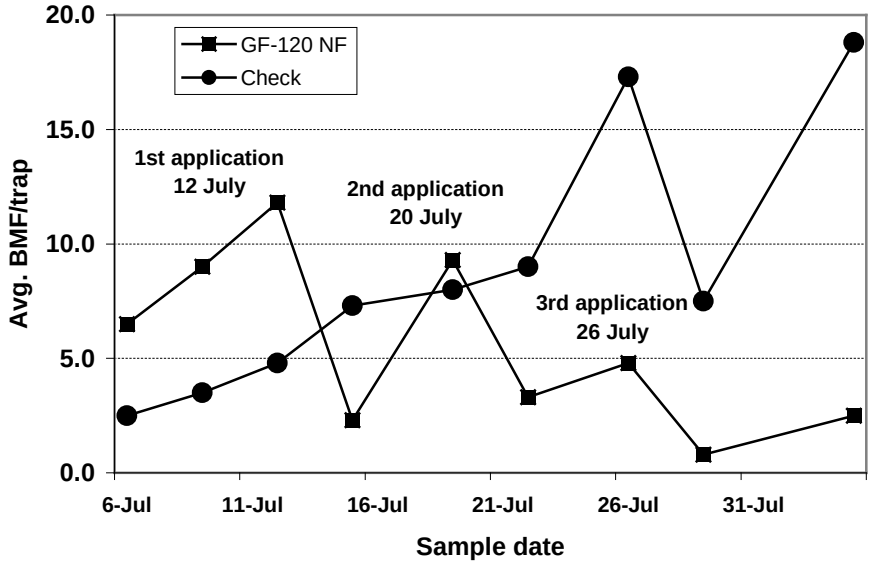
Table 6. Field control of blueberry maggot with insecticides.

	Amt. form./acre	Avg. pupae/5 qts	Adults/trap seasonal density
Trial #1			
Pyganic 1.4 EC	32 oz	14.0 (5.5) d	16.4 (0.4) ab
Aza-Direct	64 oz	1.3 (0.9) cd	26.6 (4.5) a
Provado 1.6 F	8 oz	0.3 (0.3) b	2.6 (0.9) c
Imidan 2.5 EC	40 oz	0.3 (0.3) b	2.5 (0.3) c
Untreated check	-	10.3 (7.8) cd	7.6 (2.0) b
Trial #2			
GWN 1975 2.5 EC	40 oz	15.0 (7.4) a	5.4 (1.2) a
Entrust 80 WP	2 oz	32.7 (7.1) a	7.9 (2.2) a
Untreated check	-	43.0 (12.3) a	8.5 (0.8) a
Trial #3			
Novaluron 10 EC	19 oz	9.0 (6.9) a	11.8 (3.2) a
Untreated check	-	21.0 (7.0) a	7.8 (1.7) a

Seasonal densities are trapezoidal integrals of densities over the season divided by the number of days duration of the experiment.

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

Fig. 4. Average BMF captures, by date.



5. Effect of formulation on decay rate of phosmet applied to lowbush blueberry in the crop year.

Fig. 5. Phosmet Residue (ug/g)

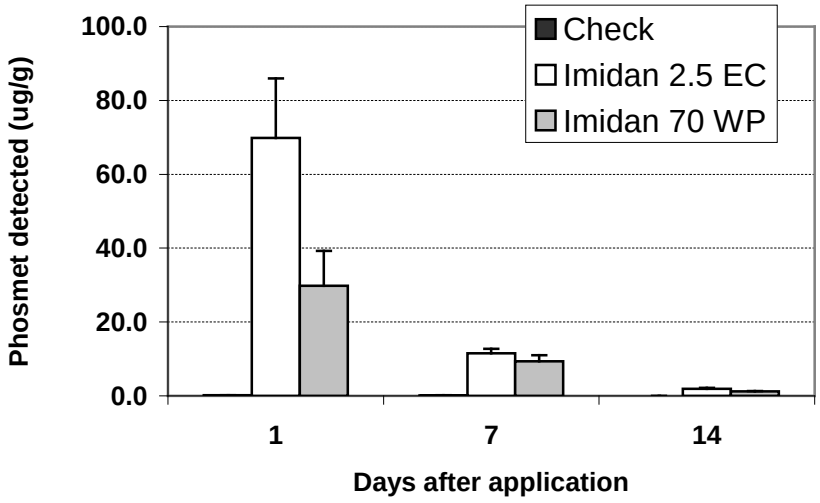
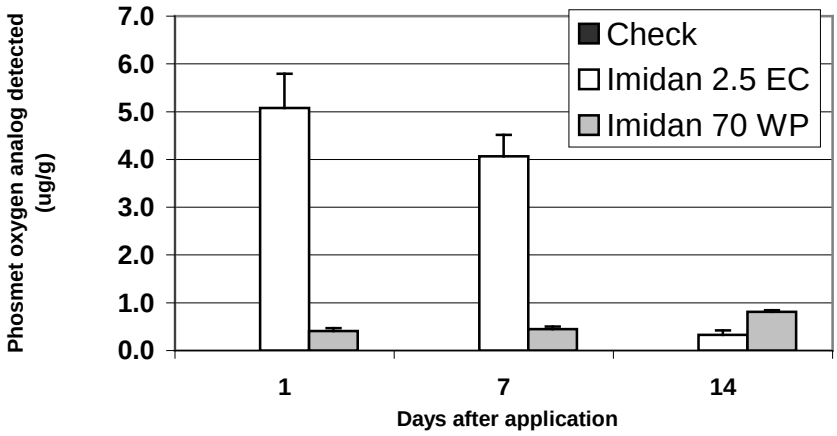


Fig. 6. Phosmet Oxygen Analog Residue (ug/g)



ENTOMOLOGY

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

9. TITLE: Integrated Pest Management (IPM) Strategies

BLUEBERRY SPANWORM

1. <u>Evaluation of feeding damage by blueberry spanworm larvae in the pruned year.</u>

METHODS: In May 2004, five replications (blocks) were established in a pruned field at Blueberry Hill Farm. The field was burned in the fall of 2003. Four, 4-ft^2 plots were set in each block and one of four different densities of blueberry spanworm larvae was placed in each plot (0, 25, 50, or 100 larvae). Two blocks were set on 14 May using 1st to 4th instar larvae collected from an infested field. Two additional blocks were set on 17 May with 2nd to 5th instar larvae, and one block was set on 19 May with 2nd to 5th instar larvae. Each plot was covered with a mesh cage and sealed with sand around the bottom to prevent movement of the larvae out of the plots.

On 9 June, the mesh cages were removed and the number of blueberry stems growing within each 4-ft^2 plot was counted and compared with the initial larval density.

In the spring 2005, 50 stems within each plot will be cut and brought into the laboratory. The number of flower buds/stem will be recorded at each density. Analysis of Variance and Regression analyses will be conducted comparing average flower buds/stem with initial larval density.

RESULTS/CONCLUSIONS: Fewer stems were found in plots with low initial spanworm larval densities; however, the difference was not significant (ANOVA, P = 0.4569). Figure 1 shows the relationship between average number of stems and initial spanworm larval density. There was no significant quadratic (P = 0.3253) or linear trend (P = 0.1030).

2. <u>Evaluation of flower-bud development subsequent to feeding damage by blueberry spanworm in the pruned year.</u>

METHODS: In May 2003, six replications (blocks) were established in a pruned field at Blueberry Hill Farm. The field was flail-mowed in the fall of 2002. Five, 4-ft^2 plots were set in each block and one of five different densities of blueberry spanworm larvae was placed in each plot (0, 10, 20, 40, or 60 larvae). Three blocks were set on 8 May using 1st and 2nd instar larvae collected from an infested field. Three additional blocks were set on 28 May with 2nd to 4th instar larvae. Each plot was covered with a mesh cage and sealed with sand around the bottom to prevent movement of the larvae out of the plots.

On 12 June, the mesh cages were removed and the percentage of blueberry cover within each 4 ft^2 plot was estimated, converted to % defoliation, and compared with the initial larval density.

On 20 April 2004, 50 stems within each plot were cut and brought into the laboratory. The number of flower buds/stem was recorded at each spanworm density. An Analysis of Variance (ANOVA) was conducted comparing average flower buds/stem with initial larval density.

RESULTS/CONCLUSIONS: An Analysis of Variance of the data in 2003 revealed no significant difference between the blocks (ANOVA, P = 0.0795) and no difference between blocks set in early compared to late May 2003 (ANOVA, P = 0.1570). There was a significant difference in % cover (ANOVA, P = 0.0106) and consequently in % defoliation among densities (ANOVA, P = 0.0013). Defoliation increased with increasing spanworm densities. Table 1 summarizes the % blueberry leaf cover and the resulting % defoliation at each initial spanworm larval density. The analysis of subsequent flower-bud production in 2004 indicated that the plants recovered from the defoliation. There was no significant difference in flower-bud production among the treatments (ANOVA, P = 0.9533)(Table 1).

3. <u>Effect of fertilizer application on flower-bud development in late-emerging blueberry stems subsequent to blueberry spanworm infestation.</u>

METHODS: The "upper field" of Blueberry Hill Farm was flail-mowed in the fall of 2002. Portions of the field were heavily infested with blueberry spanworm larvae in the spring of 2003. The subsequent feeding damage resulted in late emergence of the blueberry stems.

On 25 July, DAP (10-10-10) fertilizer was applied by hand to 2-m^2 plots located in the defoliated area. Following the applications, each plot was irrigated with a hand-held hose. Two rates were applied, 122 lbs and 244 lbs per acre. Four replications were set in each of three areas of the field for a total of 12 replications of each rate plus 12 untreated checks. On 22 April, 50 stems within each 2-m^2 plot were cut and brought into the laboratory. The number of flower buds/stem was recorded at each fertilizer rate. An Analysis of Variance was conducted comparing average flower buds/stem with fertilizer rate.

RESULTS/CONCLUSIONS: The Analysis of Variance showed no significant difference in the number of flower buds developing on untreated plots and plots receiving 122 or 244 lbs of DAP fertilizer (ANOVA, $P = 0.6253$)(Table 2). However, an extreme cold spell during the first two weeks of January 2004 resulted in stem death and flower bud necrosis. Because of this, our results must be interpreted with caution.

4. <u>Effect of late-emergence of blueberry stems on flower-bud development subsequent to feeding by blueberry spanworm larvae.</u>

METHODS: The "upper field" of Blueberry Hill Farm was flail-mowed in the fall of 2002. Portions of the field were heavily infested with blueberry spanworm larvae in the spring of 2003. The subsequent feeding damage resulted in late emergence of the blueberry stems.

The defoliated area was observed at ca. weekly intervals beginning on 4 June 2003. On each of 5 observation dates (4, 13, 23 June and 7, 9 July), an estimate was made of blueberry plant development. Two to five, m^2 plots were established in areas judged to have reached 100% canopy coverage since the date of the previous observation. In the spring of 2004, 50 stems within each m^2 plot were cut, brought into the laboratory, and a count was made of the number of flower buds/stem. An Analysis of Variance was conducted comparing average flower buds/stem with the date of 100% cover.

RESULTS/CONCLUSIONS: Two of the seven blocks were found to have very high numbers of flower buds across all densities. It is possible that these 2 blocks were set in blueberry clones that were not typical of the site. Therefore, 2 separate analyses were performed either including or excluding these blocks. The results of both analyses are presented in Table 3. The Analysis of Variance showed no significant difference in flower-bud production among any of the sample dates when the high-count blocks were excluded (ANOVA, $P = 0.2535$). When the high-count blocks were included, the difference was only slightly nonsignificant (ANOVA, $P = 0.0987$).

Despite the relative lateness of 100% cover (9 July), due to feeding by blueberry spanworm larvae, the late-emerging blueberry stems apparently still had sufficient time to recover from the defoliation and produced a similar number of flower-buds as earlier emerging stems.

BLUEBERRY FLEA BEETLE

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

METHODS: On 20 May 2004, three replications (blocks) were established in a pruned field at Blueberry Hill Farm. The field was burned in the fall of 2003. Three, 4-ft^2 plots were set in each block and one of four different densities of mid-instar blueberry flea beetle larvae was placed in each plot (0, 25, 50, or 100 larvae). Each plot was covered with a mesh cage and sealed with sand around the bottom to prevent movement of the larvae out of the plots.

On 14 June, the mesh cages were removed and the number of blueberry stems growing within each 4-ft^2 plot was counted and compared with the initial larval density.

In the spring 2005, 50 stems within each plot will be cut and brought into the laboratory. The number of flower buds/stem will be recorded at each density. Analysis of Variance and Regression analyses will be conducted comparing average flower buds/stem with initial larval density.

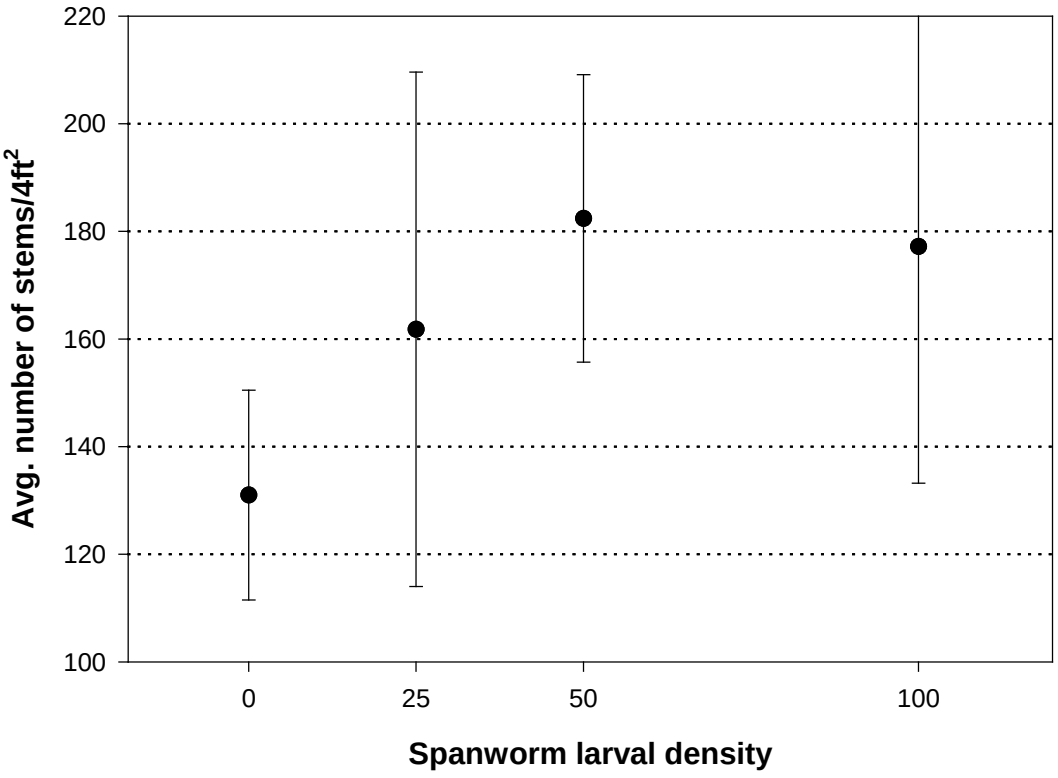
RESULTS/CONCLUSIONS: There was no significant difference in the number of stems among the densities (ANOVA, $P = 0.6683$). Figure 2 shows the relationship between average number of stems and initial flea beetle larval density. There was no significant quadratic ($P = 0.6282$) or linear trend ($P = 0.5758$). The data were transformed by $\log_{10}(X + 0.1)$ prior to analysis.

RECOMMENDATIONS: Our focus for the past several years has been on the impact of blueberry defoliating insect pests such as blueberry spanworm and blueberry flea beetle on the yield of the lowbush blueberry plant. In 2004, we found that total defoliation of prune cycle blueberry plants did not reduce potential yield (blueberry flower buds) in the following year. Four different experiments suggest that blueberries can tolerate complete defoliation so long as it is not too late in the season. In addition, we saw no benefit to adding fertilizer to defoliated areas for the purpose of stimulating plant growth and increasing flower bud development. Plants also appear to be quite tolerant to defoliation by blueberry flea beetle. Densities up to 25 larvae per square foot did not significantly reduce yield. Therefore, we will focus on setting more liberal thresholds for control of these pests in prune cycle fields.

BLUEBERRY SPANWORM

1. <u>Evaluation of feeding damage by blueberry spanworm larvae in the pruned year.</u>

Fig. 1. Relationship between initial spanworm larval density and number of blueberry stems.



2. Evaluation of flower-bud development subsequent to feeding damage by blueberry spanworm in the pruned year.

Table 1. Percent of blueberry leaf cover and defoliation as a result of spanworm larval feeding, and subsequent flower-bud development.

Initial spanworm larval density	% cover [1]	% defoliation [2]	Avg. flower buds/ Stem [3]
0	20.0 (6.2)	0.0	8.24 (5.26) a
10	13.3 (7.6)	37.5	7.79 (4.75) a
20	10.3 (7.0)	65.0	8.40 (5.42) a
40	8.7 (6.7)	67.1	8.14 (4.90) a
60	8.3 (5.6)	66.7	7.41 (4.16) a

[1] Mean % cover ± standard error, 12 June 2003.

[2] % defoliation = (% cover at 0 density - % cover at selected density) / % cover at 0 density)) * 100, 12 June 2003.

[3] Avg. flower buds ± standard error, 20 April 2004. Means followed by the same letter are not significantly different ($P < 0.05$, SNK).

3. Effect of fertilizer application on flower-bud development in late-emerging blueberry stems subsequent to blueberry spanworm infestation.

Table 2. Effect of different rates of DAP fertilizer on flower-bud production.

Rate lbs DAP/acre	Mean flower buds/stem (SE)
122 lbs	1.43 (0.12) a
244 lbs	1.45 (0.13) a
0 lbs	1.69 (0.14) a

Means followed by the same letter are not significantly different ($P < 0.05$, SNK).

4. <u>Effect of late-emergence of blueberry stems on flower-bud development subsequent to feeding by blueberry spanworm larvae.</u>

Table 3. Average number of flower buds/stem vs. date of 100% cover in late emerging blueberry stems.

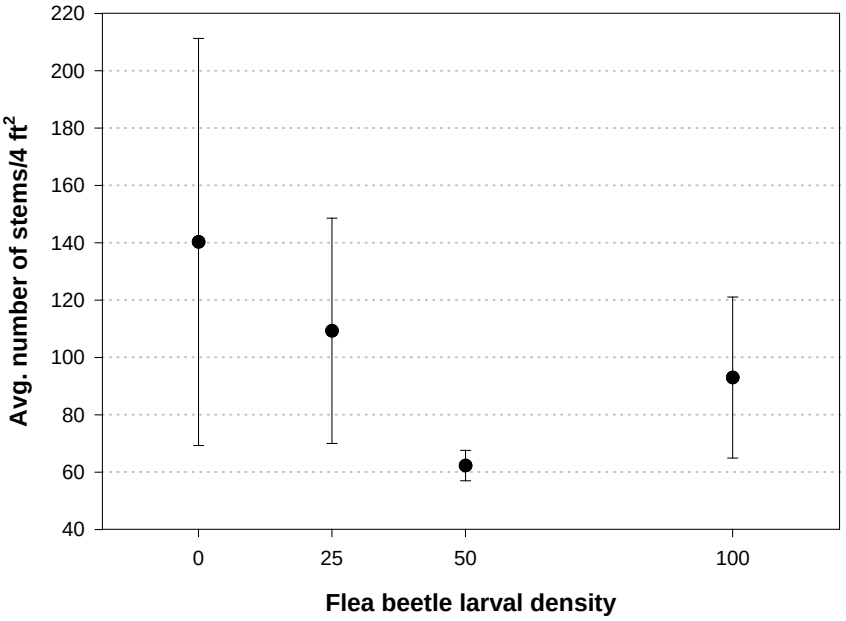
Date100% cover Reached	Mean flower buds/stem (SE)
4 June	1.46 (0.57)
13 June	2.22 (0.95)
23 June	3.80 (0.58)
2 July	2.98 (0.58)
9 July	1.90 (0.38)
Control [1]	8.24 (5.26)
Control [2]	2.97 (0.66)

[1] Includes 2 blocks with high flower-bud counts.
[2] Excludes 2 blocks with high flower-bud counts.

BLUEBERRY FLEA BEETLE

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

Fig. 2. Relationship between initial flea beetle larval density and number of blueberry stems.



ENTOMOLOGY

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

10. TITLE: Biology and Ecology of Blueberry Insect Pests

BLUEBERRY SPANWORM

1. Growth and development of blueberry spanworm in the laboratory.

METHODS: Blueberry spanworm eggs were collected in the summer of 2003. In late April, the eggs were removed from the refrigerator and reared at one of four different temperatures (15, 20, 21 or 25 ^{o}C). Emerging larvae were placed in individual plastic diet cups with fresh blueberry buds and foliage. Larval instar as determined by head capsule width was recorded at 1 to 3 day intervals for each larva. Larvae were also collected from plates wintered in the field at Blueberry Hill Farm. Weight and sex were recorded for insects reaching the pupal and adult stages, respectively.

RESULTS/CONCLUSIONS: The average number of days required for each immature life stage to complete its development at each temperature is shown in Table 1. Pupal weight is in Table 2, and % survival between life stages is in Table 3. These data will be the basis of a computer simulation model used to investigate the optimal timing for insecticide controls. The data collected in 2004 shows considerable difference from data collected in 1999 and 2002 suggesting that there may be other factors besides temperature, such as viral load or clone phenotype, which might influence development.

2. Development of blueberry spanworm on different blueberry phenological plant stages.

METHODS: Blueberry spanworm larvae were collected from an infested field site, brought into the laboratory and allowed to feed on blueberry flower buds in various stages of development (swollen bud, tight cluster, loose cluster, or bloom). The larvae were reared individually in diet cups maintained at ca. 20^{o}C and provided with new plant material of the appropriate stage as needed. Survival of larvae feeding on each stage was assessed at regular intervals.
Weight and sex were recorded for insects reaching the pupal and adult stages, respectively.

RESULTS/CONCLUSIONS: When 1st and 2nd instar blueberry spanworm larvae were fed blueberry flower buds in the swollen bud stage, only 9 of 26 larvae or 35% survived to the pupal stage. In contrast, 27 of 30 (90%) and 21 of 28 (75%) of 1st and 2nd instar larvae survived to the pupal stage when reared on flower buds in the tight cluster and loose cluster stages, respectively. Only 7 of 28 or 25% of 3rd and 4th instar larvae survived when fed flower buds in the bloom stage (Fig. 1). The Product-Limit Survival

(Kaplan-Meier) Method and Wilcoxon Mean Separation (*Prob > x^2 > 0.05)* were used to test for differences in survival among larvae reared on flower buds at the swollen bud, tight cluster or loose cluster stages. Bloom data was not included in the analysis; older instar SW larvae were used for that portion of the trial. Survival was significantly better on both loose cluster (x^2 = 0.0005) and tight cluster (x^2 = < 0.0001) compared to swollen bud. There was no significant difference between tight and loose cluster (x^2 = 0.1702).

Analysis of Variance and Tukey's Mean Separation showed no differences in the weights of male and female pupae (ANOVA, *P* = 0.1101). Female pupae weighed, on average, 0.0475 grams; males weighed 0.0441 grams. Across the treatments, there was a significant difference in the weight of pupae developing from larvae fed flower buds of different stages. Pupae from loose cluster (0.04622 grams) and tight cluster (0.0470 grams) were significantly heavier then those developing on swollen buds (0.0358 grams)(ANOVA, *P* = 0.0067).

3. <u>Development of blueberry spanworm in the field.</u>

METHODS: Blueberry spanworm eggs were collected in the summer of 2003. Eggs were wintered in petri dishes placed outside in the field at Blueberry Hill Farm. A shallow hole was excavated and the dishes were placed in the hole in a single layer to that the top cover of each dish was at ground level. The dishes were then covered with a 1-2 inch layer of bark mulch. On 7 April, 21 dishes of eggs were removed from the field and held in the refrigerator. Batches of dishes were returned to the field (7 on 10 May, 9 on 24 May and 5 on 2 June and monitored at 1-3 day intervals for egg hatch. The remaining 41 dishes were left in the field and also monitored at 1-3 day intervals for larval hatch. A HOBO® temperature monitor was placed with each batch of eggs.

RESULTS/CONCLUSIONS: Predictions lagged behind the observed 50% egg hatch for all four trials. Trials 1, 3, and 4 resulted in predictions that were fairly close to the observed (3-5 days)(Table 4). The second trial resulted in a very large discrepancy that cannot be explained. The soil temperature used for predicting egg hatch was monitored at ca 5 cm beneath the soil surface. It is expected that most wintering eggs will be located between 0 and 2 cm soil depth. These soil depths would most likely be more variable than the deeper soil temperatures and on average much warmer, closer to the air temperatures. Unfortunately due to the malfunctioning of the Blueberry Hill farm temperature probe, the theft of our soil temperature probe and the missing data from the irrigation project weather database, we could not test the predictive egg hatch model with temperatures that the eggs would have experienced. However, our predictions do indicate that the degree model is worth trying to validate next spring.

BLUEBERRY MAGGOT FLY

1. Wild blueberry maggot fly emergence in fruit bearing, wooded, and pruned habitats.

METHODS: On 29 June, emergence cages were placed in, and adjacent to, three commercially managed wild blueberry fields in Washington Co. Fifteen cages were set at each site. Five cages were set along the field edge in a fruit-bearing area of the field. Five cages were set along the field edge in a nearby pruned section. The remaining five cages were placed in an adjacent wooded area with unmanaged blueberries in the under story. A Pherocon® AM trap was placed with each set of 5 emergence cages to monitor for the presence of blueberry maggot fly (BMF). The emergence cages and AM traps were checked periodically from 5 July to 3 August and any BMF were counted and removed. All AM traps were replaced on 19 and 27 July.

RESULTS/CONCLUSIONS: BMF were found in emergence cages at only one site in 2004. No flies were captured from cages set in fruit-bearing habitats. In the four years that this study has been replicated, only 3 BMF have been captured from an emergence cage set in a fruit-bearing area (3 from one cage on 15 July 2002). For all four years combined, captures from woods and prune traps were 25.3% and 70.7%, respectively (Table 5 and Fig. 2). Analysis of Variance revealed significant differences among the treatments (ANOVA, $P = 0.0295$). Significantly more BMF were collected from emergence cages set in pruned areas than in fruit-bearing areas (Table 5).

Although more BMF were captured on yellow sticky traps set in fruit-bearing fields, there was no significant difference in seasonal density among the treatments for all years, combined (ANOVA, $P = 0.7937$)(Table 5 and Fig. 3). This demonstrates that emerging flies often move rapidly through the landscape searching for mating and oviposition sites.

2. Diurnal movement of blueberry maggot fly.

METHODS: The purpose of this trial was to study the daily movement patterns of blueberry maggot fly (BMF) in order to determine time of day of greatest fly activity and where along blueberry field borders BMF are most likely to travel. Three fields were selected, two in the Blue Hill area and one at Blueberry Hill Farm in Jonesboro. Ten sets of four baited, yellow, Pherocon® AM traps were placed along one border in each field (nine sets of four traps in Jonesboro). For each set, one trap was placed 3 m outside the field edge, at the field border but elevated about 2 m, 3 m inside the field edge, and 10 m inside the field edge. The sets of traps were separated by 20 m. Except for the slightly elevated trap located at the field's border, the traps were placed on metal stakes and elevated just above the blueberry foliage.

Trials were run for three, 5-day periods (weeks of 12 and 27 July, and 9 August. During each trial, traps were checked four times a day, 4:00 am, 9:00 am, 3:00 pm, and 8:00 pm). Any captured flies were collected, soaked for 24 hrs in kerosene to remove any sticky residue, and stored in 70% ethyl alcohol (ETOH) prior to inspection in the

laboratory to determine gender and oviposition status. Traps were not changed during each 5-day trial.

RESULTS/CONCLUSIONS: Analysis of the data is ongoing. The initial analysis shows that when all three sample weeks are combined, significantly more BMF were observed on the traps at 3 and 8 pm. Very few flies were captured between 8 pm and 4 am. The results were similar at all three sites (Table 6)(Fig. 4). These results may have ramifications in regards to timing of control applications; especially for applications of short-residual materials. Late morning and afternoon appear to be when most flies are active and foraging in blueberry fields.

Further analysis will reveal the sex of the fly in relation to time caught and the numbers of flies caught at each trap placement, as well as any differences among the three sample weeks. Future studies of this kind will be used to determine the size of the trap's attraction zone and the movement of young, sexually immature flies.

BEE FORAGING ACTIVITY

1. <u>Bee foraging patterns during bloom.</u>

METHODS: Bee foraging patterns were observed and recorded on 9 and 10 June at three sites (Blueberry Hill Farm (BBH), T-18 and T-19). Individual bees were followed and the number of flowers visited per stem, the distance between stems visited, and the angle flown from one stem to the next stem were all recorded. The primary bee species sampled was the honeybee, *Apis mellifera*. These data will be used to construct a database from which a bee foraging computer simulation model can be assembled.

RESULTS/CONCLUSIONS: Because of the small number of bees at two of the sites (4 at T-18 and 5 at T-19; 18 honeybees were followed at BBH), data from the three sites was pooled together. Figure 5 shows the frequency distribution of number of stems visited. Honeybees visited an average of 5.9 ± 1.6-stems/foraging bout. In a similar study in 2003, honeybees foraging at Blueberry Hill visited 7.8 ± 1.7 stems/bout while those at Clary Hill in Union visited 18.4 ± 3.4 stems/bout.

Figure 6 is the frequency distribution of the number of flowers visited/stem. The average was 1.6 ± 0.4 flowers/stem. In 2003, 2.0 ± 0.1 flowers/stem were visited at Blueberry Hill than, 1.4 ± 0.1 at Clary Hill and 1.6 ± 0.1 at a site in Columbia.

Overall, honeybees traveled an average of 6.2 ± 2.4 inches between stems (Fig. 7). Finally, the angle of departure from one stem to the next stem in a single foraging trip that could be followed in the field was determined (Fig. 8). Honeybees seemed most likely to travel North or East.

ROLE OF PREDATORS IN REGULATING ABUNDANCE OF BLUEBERRY PEST INSECTS

METHODS: Four organic blueberry fields were used to sample ground-dwelling

arthropods using pitfall traps. In each of the four fields, 6 pitfall traps were placed in the ground; 3 at the field's edge, adjacent to the woods, and 3 in the middle of the field. Once per month (June, July, and August), the traps were left out for one week to capture ground-dwelling arthropods. .

In the same four organic fields, blueberry spanworm (SW) eggs and blueberry maggot fly (BMF) pupae were set out to see if they would be eaten under varying conditions. BMF pupae were set out for one week, concurrent with the second round of pitfall trapping. Ten pupae were placed next to each of the 6 traps, either out in the open, under a wire cage (mesh diameter about 5 mm), or 1-2 mm under the duff. After one week, the remaining pupae were collected and counted. Pitfall traps were also removed at that time so we were able to assess what potential predators were most abundant in the immediate area. BMF pupae were also set out in 6 additional pruned fields, 3 that were managed under biofriendly reduced-risk (RR) conditions (not organic) and 3 that were managed in the conventional manner (typically organophosphate insecticides) termed grower standard (GS). Data for the BMF pupal predation study was analyzed using nested Analysis of Variance (ANOVA). The data were transformed (sqrt (X + 0.05)) prior to analysis.

Blueberry spanworm (SW) eggs were placed out in the same four organic blueberry fields concurrent with the third round of pitfall trapping. Due to a limited number of eggs, only 20 eggs, laid on cheesecloth by female SW, were placed in each field. Ten of the eggs were left out in the open and 10 were placed under a wire cage (mesh diameter about 2 mm). The eggs were laid next to 2 of the 3 edge pitfall traps, removed after one week and counted. As with the BMF pupal predation study, SW eggs were also placed in 3 RR and 3 GS fields during the same period. The data were analyzed using Pearson X^2

RESULTS/CONCLUSIONS: Most of the SW eggs were missing from all fields one week after they were placed. When comparing fields that had some eggs remaining and those that had none remaining, there were more organic fields than RR or GS fields that had all eggs predated (x^2 = 0.0177). In fact, all of the 60 SW eggs set out in 3 organic fields were gone one week after they were deployed (Fig. 9).

There was no difference between RR and GS fields or the location of the eggs (uncovered or under a cage) when comparing treatments with eggs remaining vs. no eggs remaining (x^2= 0.903 and 0.249, respectively)(Fig. 10). Although, RR fields tended to have more eggs remaining than (GS) fields both in uncovered and caged locations.

In all fields, ants were the most common natural enemy predator captured in pitfall traps set next to the eggs during the third round of trapping, with spiders being the next most common predator. Carabid and staphylinid beetles and phalangids were less common (Fig. 11).

There were no significant differences in the number of BMF pupae that were missing or damaged in any organic, RR, or GS field after one week (P = 0.564). There were significantly more pupae remaining under the duff than were remaining in the cages or out in the open (P = 0.012). In general, there were very few BMF pupae missing or

damaged when they were placed under the duff, and none of the pupae were missing from the duff treatment in organic and RR fields (Fig. 12).

Regarding BMF pupae placed in RR and GS fields, there was no significant difference in the number of pupae remaining after one week due to crop cycle (pruned or crop) or treatment (RR or GS)($P = 0.949$ and $P = 0.118$, respectively). There were however, significantly more BMF pupae remaining under cages in RR fields than there were under cages in GS fields ($P = 0.0409$). Numbers of pupae remaining under cages in RR fields were similar to numbers remaining under the duff and uncovered in both RR and GS fields (Fig. 13).

In all the fields, the most common natural enemies during the second round of trapping were ants and spiders. There were a few carabids and phalangids, but they were less common. Staphylinid beetles were rare. In general, there were similar numbers of natural enemies in all the fields, with the exception of ants that were more abundant in organic fields than in GS fields (Fig. 14).

RECOMMENDATIONS: Our biology studies in 2004 suggest several avenues for continued research. We will continue to refine our estimates of blueberry spanworm egg and larval development as affected by temperature and host plant quality. A validation of the temperature dependent predictive model will be attempted again in 2005 with better coverage of soil depths. Our results in 2004 suggest that our model has considerable potential for estimating egg hatch in the field based upon soil temperature. A refined estimate of larval development will allow later development of a computer simulation model of blueberry spanworm populations. It is hoped that this model can be used to explore the subtleties of spray timing in the spring.

Other studies that will be continued are the daytime foraging behavior of blueberry maggot flies and the characterization of between-flower and between-stem foraging behavior of honeybees and native bees. Quantification of these behaviors will allow us to develop greater insight into the timing of non-persistent insecticides for the control of blueberry maggot flies and the efficacy of pollination by different species of bee. The exploration of natural enemies in the blueberry system will be continued so that we can develop guidelines for assessing the amount of natural control that growers may have in their fields in the absence of insecticides. However, in order to get to this point, it is important to first understand which species are the dominant predators in Maine blueberry fields.

BLUEBERRY SPANWORM

1. Growth and development of blueberry spanworm in the laboratory.

Table 1. Development of blueberry spanworm at 15, 20, 21, or 25ºC.

Insect growth stage	Days development at each temperature (SE) 15	20	21	25
Egg	10.7 (1.49)	5.6 (1.24)	3.8 (0.59)	3.0 (0.00)
1st instar	9.2 (0.71)	5.4 (0.54)	5.2 (1.73)	5.0 (0.80)
2nd instar	6.6 (1.19)	3.4 (0.49)	4.3 (0.71)	3.3 (0.63)
3rd instar	8.6 (1.99)	3.5 (0.70)	3.8 (0.79)	3.8 (0.97)
4th instar	7.6 (2.47)	3.0 (0.60)	4.7 (1.39)	5.5 (0.40)
5th instar	13.2 (0.97)	5.3 (0.88)	7.0 (1.30)	6.0 (0.80)
Pupa	22.5 (1.09)	12.3 (0.35)	10.7 (0.59)	9.0 (0.80)

Table 2. Weight of male and female blueberry spanworm pupae reared at one of four temperatures.

Temperature (ºC)	Weight in grams (SE) Males	Females	Both
15	0.0365 (0.0028)	0.0320 (0.0016)	0.0343 (0.0024)
20	0.0434 (0.0030)	0.0492 (0.0039)	0.0463 (0.0038)
21	0.0440 (0.0020)	0.0503 (0.0068)	0.0476 (0.0053)
25	0.0500 (0.0000)	-	0.0500 (0.0000)

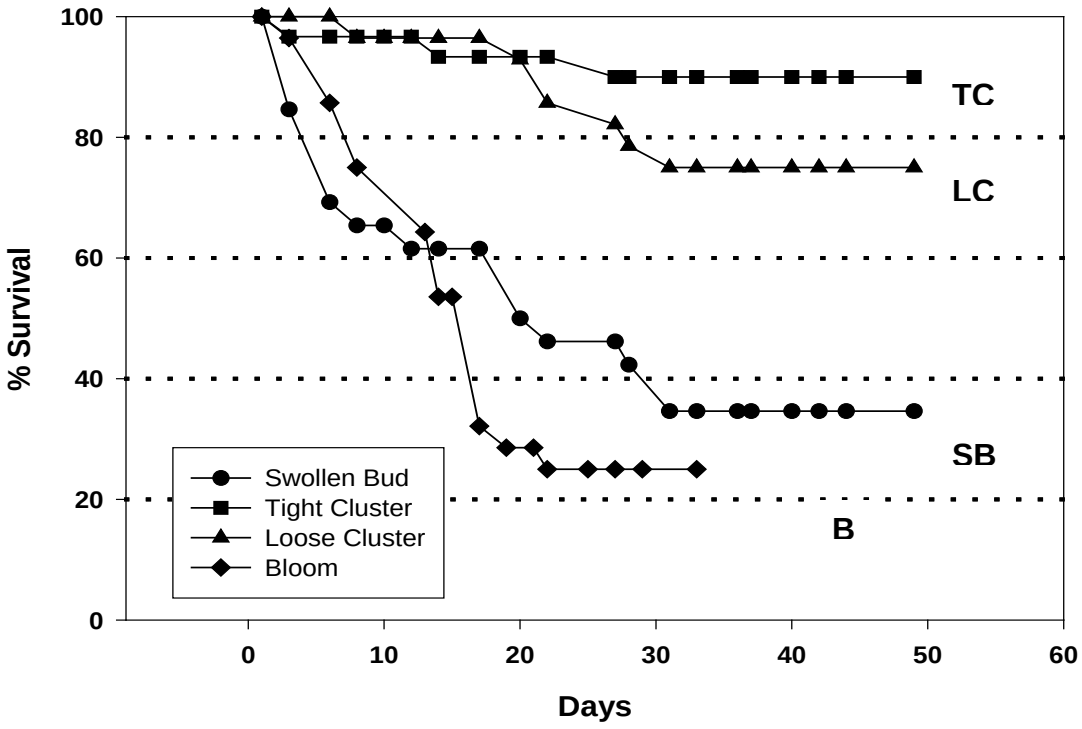
Table 3. Percent survival at each life stage of blueberry spanworm reared at one of four different temperatures.

Insect growth stage	% survival at each temperature (#) [1] 15	20	21	25
1st instar	47.4 (18)	92.6 (25)	100.0 (10)	100.0 (7)
2nd instar	50.0 (9)	100.0 (25)	100.0 (10)	57.1 (4)
3rd instar	77.8 (7)	96.6 (25)	90.0 (9)	50.0 (2)
4th instar	57.1 (4)	100.0 (24)	77.8 (7)	100.0 (2)
5th instar	100.0 (4)	100.0 (24)	100.0 (7)	100.0 (2)
Pupa	100.0 (4)	100.0 (24)	100.0 (7)	50.0 (1)

[1] # = number of individuals completing life stage.

2. Development of blueberry spanworm on different blueberry phenological plant stages.

Fig. 1. Percent Survival of Blueberry Spanworm Larvae Reared on Different Blueberry Flower-Bud Stages.



3. Development of blueberry spanworm in the field.

Table 4. Egg hatch predictive model.

Trial	# eggs deployed	Observed	Predicted*	Error
1	381	May 10	May 15	+ 5 days
2	84	May 13	June 3	+ 21 days
3	103	June 6	June 10	+ 4 days
4	50	June 8	June 11	+ 3 days

* According to accumulated degree-day model with a threshold temperature of 13ºC and an accumulated number of degree-days required for 50% hatch at 36 DD.

BLUEBERRY MAGGOT FLY

1. Wild blueberry maggot fly emergence in fruit bearing, wooded, and pruned habitats.

Table 5. Blueberry maggot fly emergence in fruit bearing, pruned and wooded habitats from 2001-2004.

Treatment	Seasonal Density (SE)	Emergence Trap Captures (SE)
2001		
Fruit-Bearing	5.9 (1.2)	0.0
Pruned	8.1 (3.4)	0.7
Wooded	13.9 (6.1)	1.3
2002		
Fruit-Bearing	28.0 (14.0)	0.2
Pruned	7.3 (4.0)	0.3
Wooded	18.2 (0.9)	0.0
2003		
Fruit-Bearing	0.4 (0.2)	0.0
Pruned	4.1 (1.4)	0.1
Wooded	5.2 (3.1)	1.3
2004		
Fruit-Bearing	38.8 (4.1)	0.0
Pruned	3.0 (0.5)	1.3
Wooded	6.1 (2.4)	0.1
All years, combined		
Fruit-Bearing	18.3 (9.1) a	0.05 (0.05) b
Pruned	5.6 (1.2) a	0.91 (0.27) a
Wooded	10.8 (3.1) a	0.36 (0.32) a

Seasonal densities are trapezoidal integrals of densities over the season divided by the number of day's duration of the experiment. Means followed by the same letter(s) are not significantly different ($P < 0.05$, SNK).

Fig. 2. Emergence Trap Captures of Blueberry Maggot Fly.

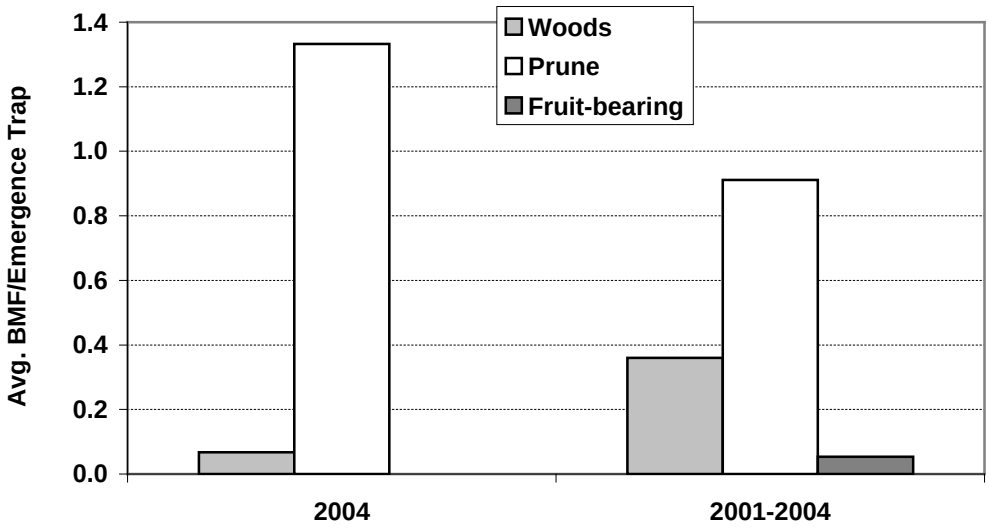
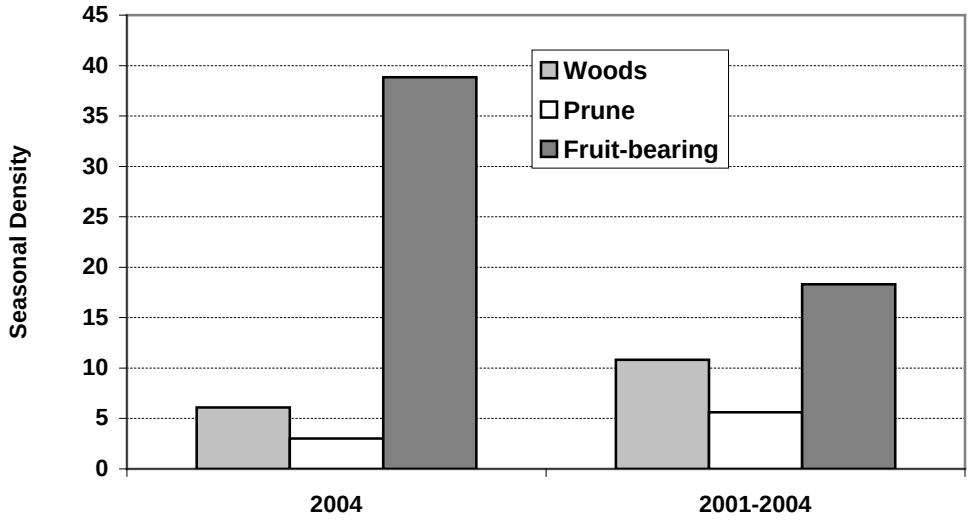


Fig. 3. Seasonal Density of Blueberry Maggot Fly.



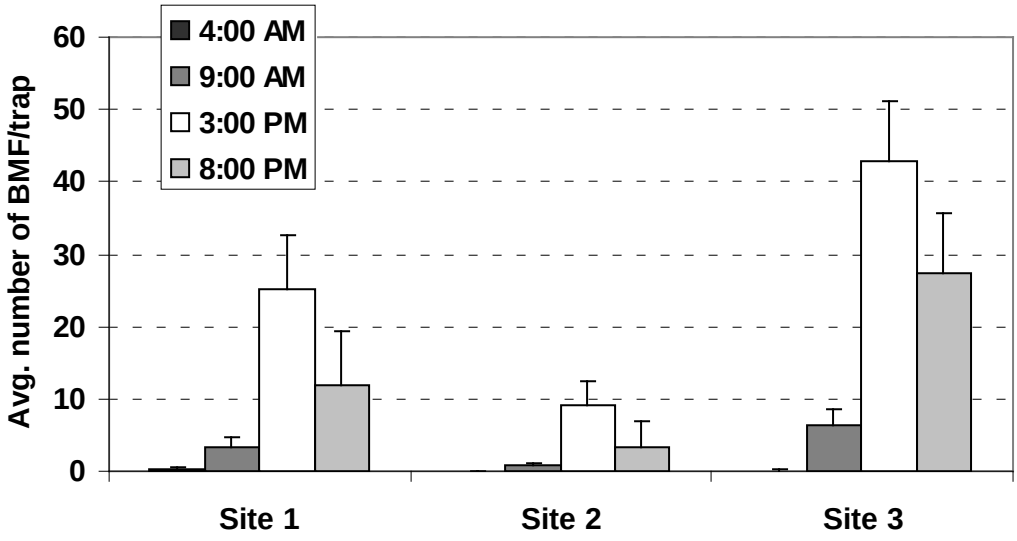
2. Diurnal movement of blueberry maggot fly

Table 6. Diurnal movement of BMF, average trap captures at each observation.

Observation time	Avg. BMF/time
4 am	0.13 (0.09) -
9 am	3.50 (1.26) b
3 pm	25.73 (6.40) a
8 pm	14.29 (4.02) a

Means followed by the same letter are not significantly different ($P < 0.05$, LS means. Data for 4 am were not used in the analysis because of the large number of zeros in the dataset.

Fig. 4. Capture of Blueberry Maggot Fly, by Time, at Each Field Site.



BEE FORAGING ACTIVITY

1. Bee foraging patterns during bloom.

Fig. 5. Number of Stems Visited During Foraging Trips.

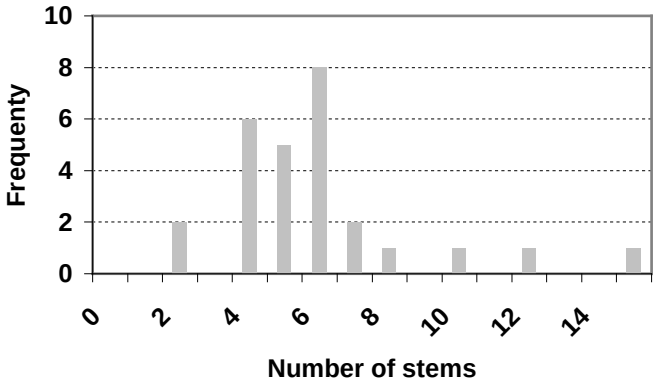


Fig. 6. Number of Flowers Visited/Stem.

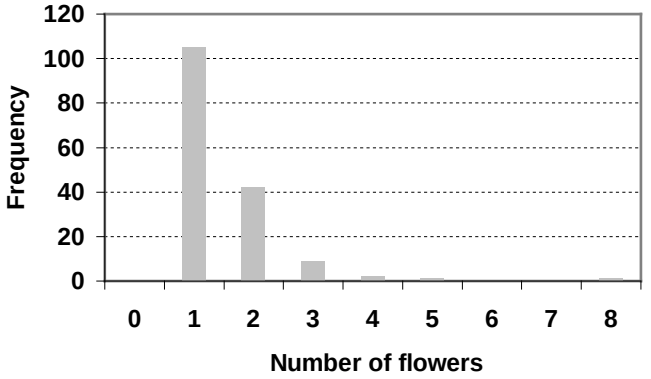


Fig. 7. Distance Traveled Between Stems (inches).

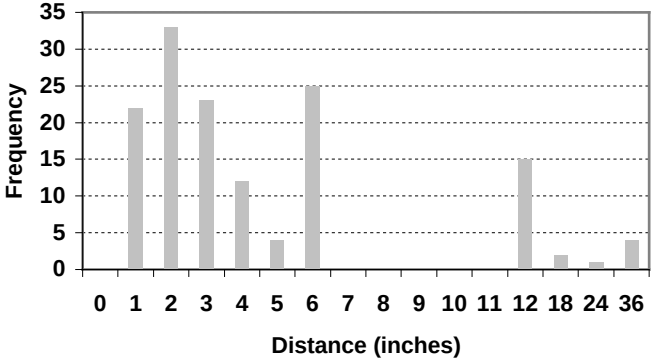


Fig. 8. Angle of Flight From One Stem to the Next.

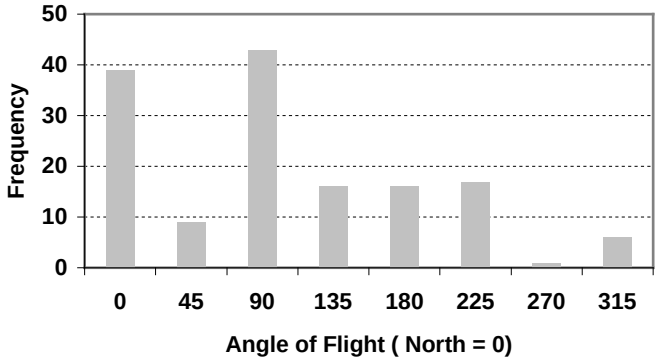


Fig. 9. Mean (± SE) number of SW eggs remaining in each treatment (cage or out in the open) in organic, RR, and GS fields after one week.

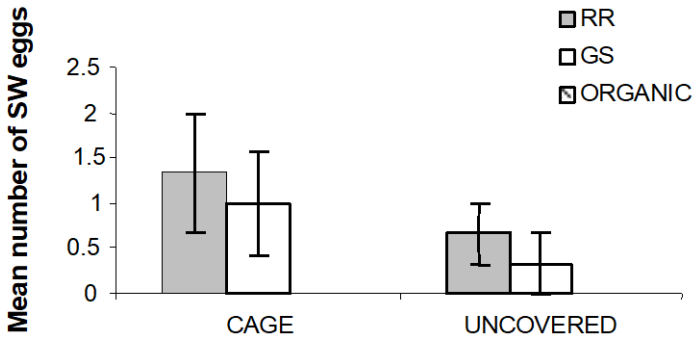


Fig. 10. Mean (± SE) number of SW eggs remaining in RR, GS, crop, and pruned fields after one week.

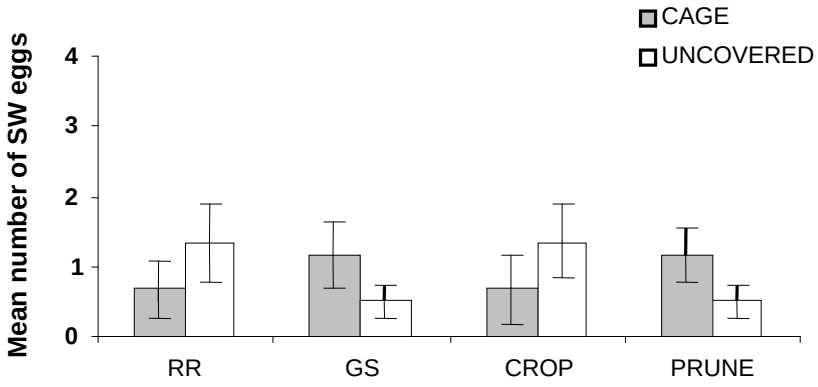


Fig. 11. Mean number of each natural enemy captured in pitfall traps placed in organic, RR, and GS fields during the third round of trapping.

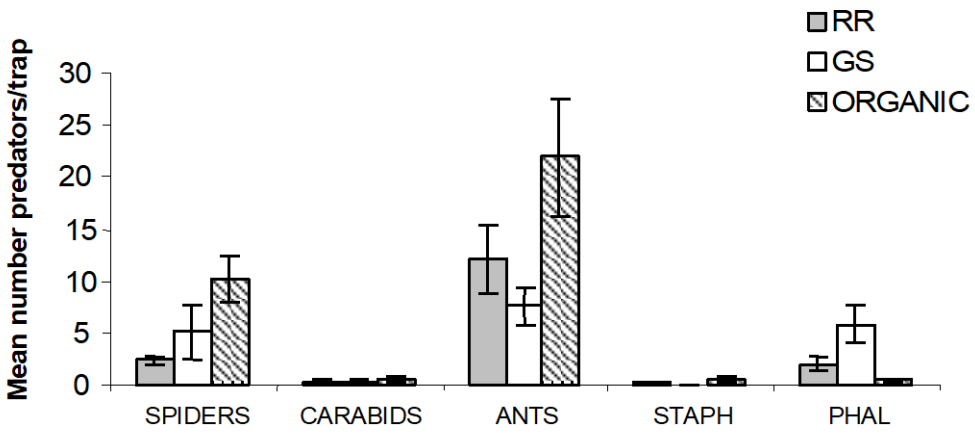


Fig. 12. Mean (± SE) number of BMF pupae remaining in each treatment (cage, out in the open, or under the duff) after one week.

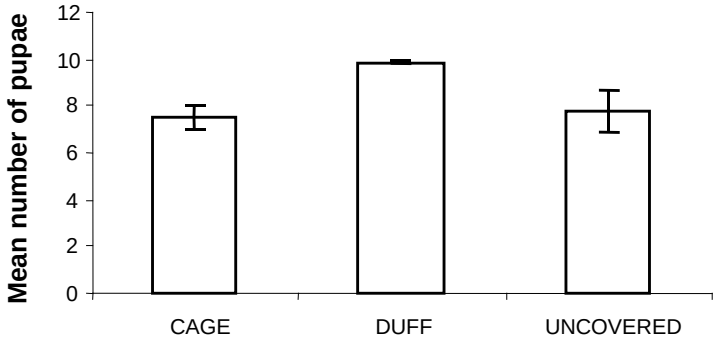


Fig. 13. Mean (± SE) number of BMF pupae remaining under cages, in the duff or out in the open (uncovered) in RR and GS fields after one week.

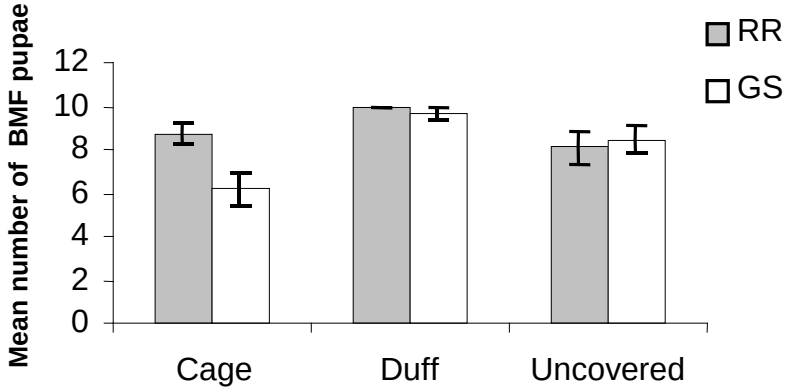
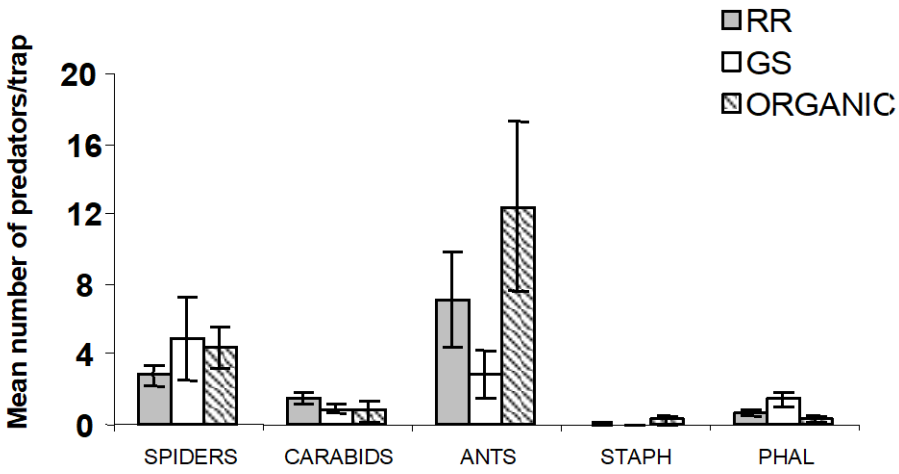


Fig. 14. Mean number of each natural enemy captured in pitfall traps placed in organic, RR, and GS fields during the second round of trapping.



DISEASE MANAGEMENT

INVESTIGATORS: S.L. Annis, Biological Sciences
C.S. Stubbs, Biological Sciences
COOPERATOR: D. Yarborough, Blueberry Extension Specialist
K.F. Lough, Research Assistant

11. TITLE: Stem Blight/Dieback and Leaf Spot Diseases in Wild Blueberry Fields

METHODS:

1) The effect of the onset of disease in stems during in the prune year on the yield in the subsequent crop year.

In August 2003, 20 0.25m^2 plots were established in six fields, which had been pruned for 2003. All diseased stems (including dead stems) within each plot were tagged in August 2003. Samples of leaf and stem litter were collected and plated on water agar to determine if crop residue could be a source of stem pathogens. The plots were examined and any newly diseased or dead stems since the previous observation period were tagged in November 2003, late April 2004 (prior to bloom), June 2004 and late July 2004. In late July 2004, tagged stems and all newly diseased or dead stems were collected, and plots were assessed for leaf spot. Leaf spot incidence was assessed by estimates of the % of stems with leaf spot and ranked on a scale of 0 to 4 (0 indicated no leaves had spots-4 indicated all the leaves had spots). Winterkill of stem tips was assessed in April 2004. To assess the effect of stem disease on yield, stem density was measured by counting all of the stems in each 0.25 m^2 plot in all fields in late April – early May 2004. The incidence of dead stems and winterkill was the proportion of dead stems or winterkilled stem tips, respectively, in each plot. From each field, stem samples for each plot were sorted by location of the disease on the stem and the disease symptoms were recorded. Stems were surface sterilized in 10% bleach and plated on water agar. Fungi isolated from the stems are being identified to genus.

2) The aggressiveness of different strains of disease-causing fungi to lowbush blueberry and the susceptibility of different blueberry clones.

Fungi of genera commonly found on diseased stems and leaves were isolated from diseased stem and leaf tissue plated out in the winter of 2004. Multiple isolates of each genus were selected and maintained. Fungal cultures were maintained on malt-yeast extract or V8 media at 20 C. Spore suspensions were made with one plate culture for each isolate and with sterile water with 2% Tween-20.

Individual wild blueberry plants grown in 6-inch pots were placed into cold storage (40C) for vernalization for 3 months. After removal from cold storage in early June 2003, the plants were placed outdoors. Plants were randomly selected to get different inoculation treatments: spores of *Pestalotia* or *Sphaeropsis*, and a water control. Two tagged stems on each plant were wounded with a sterile razor producing a wound of approximately 1.5 mm x 2—6 mm. Plants were wounded and inoculated on August 9, 2004. Each wound was treated with 20 µl of 1-to 5 x10^5 spores/ml or with 20 µl of water as a control. After inoculation, plants were gently misted with water and covered in plastic bags for 18 hours.

Observations of inoculations to note changes in lesion color, length and width were begun on July 11, 2004, 2 days after inoculation and made every 2-4 days until the stems were collected on September 12, 2004. Stems were photographed and then plated on water agar.

3) Assess the effectiveness of Bravo and other fungicides for decreasing the incidence of leaf spot (with D. Yarborough).

This was a replicated block design with 8 blocks per treatment conducted at two field sites. Treatments were Bravo at a rate of 4 pts/acre, Abound at a rate of 15.5 oz/acre and Cabrio at 16 oz/acre applied with a CO_2 backpack sprayer at 20 gpa with 80002VS Tjet nozzles to 12' x 50' plots. Each treatment was applied on June 20 2003 and July 1 2003 when the plants were in their prune year. Leaf spot incidence was assessed on October 3 2003 by estimates of the % of stems with leaf spot and also ranked on a scale of 0 to 4 (0 indicated no leaves had spots-4 indicated all the leaves had spots). Leaf spot was again assessed on Aug. 16, 2004 in two 6" x 36" sampling areas per plot using the methods described previously. Each sampling area was raked and berry weights per sampling area determined.

RESULTS:

1) The effect of the onset of disease in stems during the prune year on the yield in the subsequent crop year.

Dead stems were the most prevalent disease symptom in all 6 fields over the entire sampling period (Fig. 1). The second most prevalent was tip death (Fig. 1). The incidence of symptoms at the middle and base of the stems was much lower. The incidence of dead stems varied by sampling date among fields with no consistent trend among all fields (Fig.2). Two out of four fields (3 and 32) had high mortality of stems from late Dec. 2003 to the sampling in April 2004. The proportion of stems lost to disease ranged from 1.3% to 2.9 % for different fields (Fig.3). Winterkill of stem tips was particularly severe in four out of six fields with greater than 10% of stems affected (Fig. 4). In some fields it was noted that there was less winterkill in stems near windbreaks compared to stems in the middle of the fields (approximately 40 m from a windbreak).

The incidence of leaf spot was significantly different among fields, as measured by leaf spot rank (Fig. 5) and by the percentage of stems with leaf spots (Fig. 6) ($p < 0.0001$, Kruskal-Wallis). The two measures of leaf spot, rank and percentage of stems with leaf spots, were consistent within a field.

All fungi isolated from stem and leaf litter were saprophytic, with *Aureobasidium* being the most common genus isolated.

2) Aggressiveness of different strains of disease-causing fungi to wild blueberry and susceptibility of different blueberry clones.

Lesion appearance, width and length did not differ among treatments, including the check. *Sphaeropsis* and *Pestoltia* did not grow from inoculated stems when they were placed on nutrient medium. The lack of disease symptoms and inability to isolate the

inoculated fungus indicates that our inoculation technique needs refinement. Another set of plants will be inoculated with suspected strains of pathogenic fungi using a modified inoculation technique in the winter 2005 in the greenhouse.

3) The effectiveness of Bravo and other fungicides for decreasing the incidence of leaf spot (with D. Yarborough).

The plots in the two fields used in this study had similar levels of leaf spot in the control plots in 2003. Bravo was the only fungicide that significantly decreased the average percentage of stems with leaf spot compared to the control plots at both field sites in 2003 (Fig. 7 - 10). In 2004, there were no significant differences among treatments for the control of leaf spot in either field (Figs. 7- 10). The Township 19 field site had an average of 21 to 24% weed cover in the plots which was much higher than that found at the Deblois site. The weed cover, particularly tall weeds, may have affected the spray coverage of the fungicides, the microclimate of the plants and the horizontal spread of fungi causing leaf spot. There was more leaf spot in 2004 than 2003 in some treatments but there was no significant difference between treatments in 2004 (Figs.7-10). There was no significant effect on yield among the treatments (Figs. 11-12) Berry weight per berry also was not significantly different between treatments and ranged from 0.34 - 0.42 grams.

CONCLUSIONS: The most abundant disease symptom on stems from prune year to crop year was death of the entire stem, with from 1 to 3% of stems dying. In two out of 6 fields, the highest number of stems died over the winter, which may have been due to environmental factors, disease or interactions between both factors. The extensive winterkill in some blueberry clones indicated that many plants were severely stressed over winter, but this does not account for all of the dead stems. The cause of stem death in the prune year and after overwintering needs to be further examined. The studies on aggressiveness of fungal genera need to be continued with improved inoculation techniques. There was no effect of control of leaf spot in the prune year on the levels of leaf spot or yield found in the following crop year. A wet cool spring did contribute to increased levels of mummy berry blight found in the barrens and may have affected the yield of all field trials.

RECOMMENDATIONS: Continue to evaluate fungal and environmental causes for stem death during the prune year. Control of leaf spot by fungicide applications in the prune year is not recommended at this time.

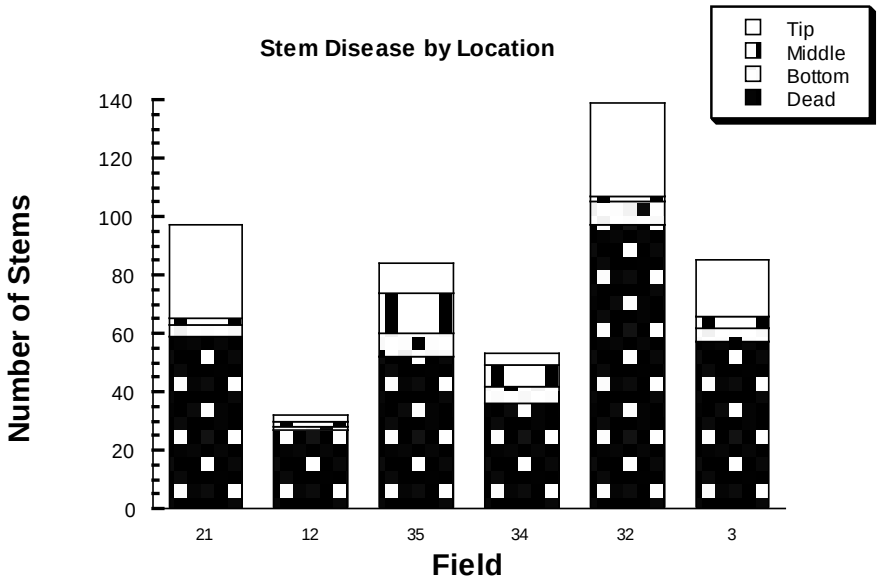


Figure 1. Location of disease symptoms on all stems collected in 6 lowbush blueberry fields

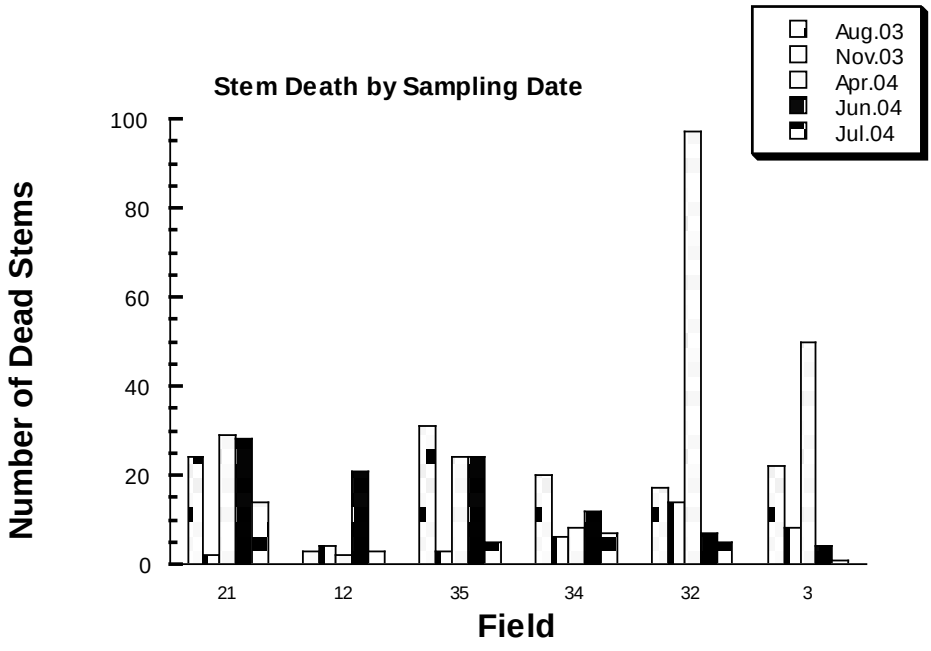


Figure 2. Number of stems that have died since the previous evaluation recorded at each evaluation time.

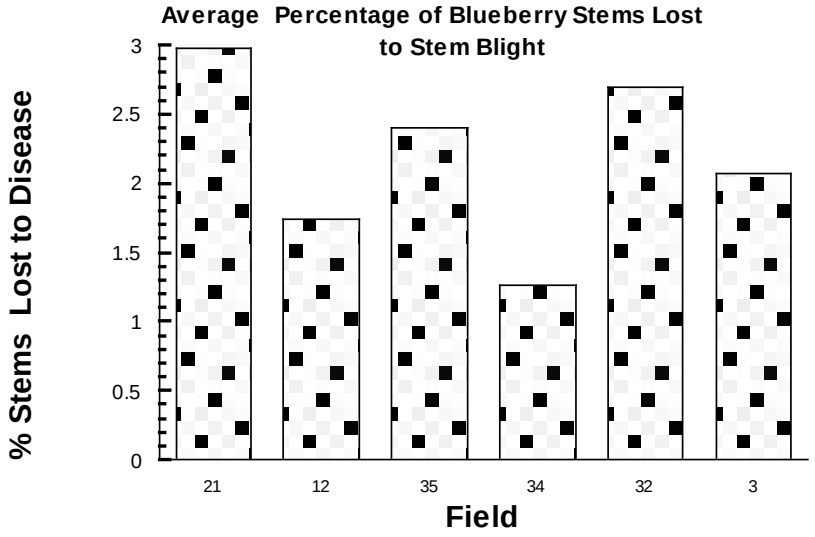


Figure 3. The average percentage of blueberry stems lost to stem blight within a 0.25m^2 plot for 6 wild blueberry fields.

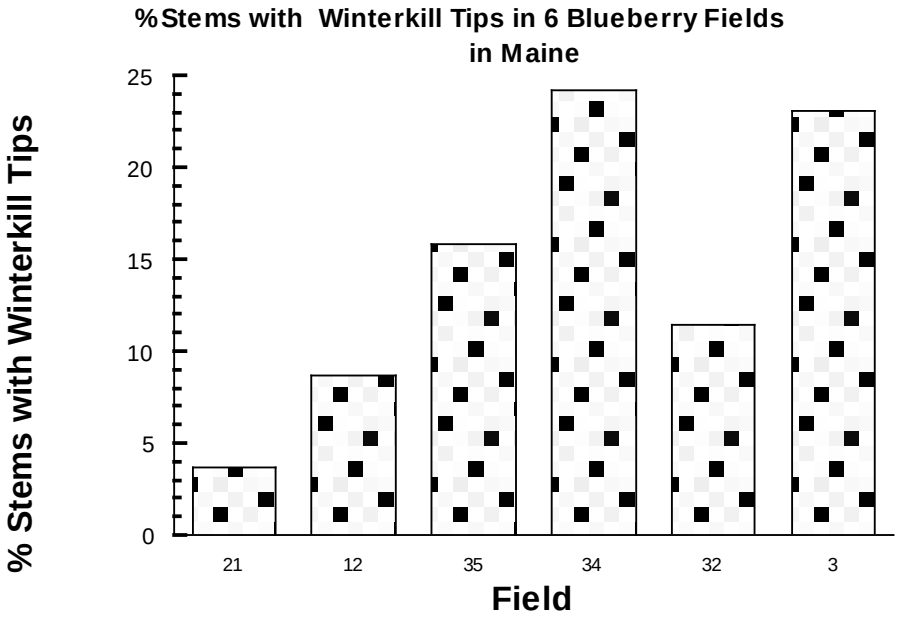


Figure 4. The average percentage of blueberry stems affected by winterkill within a 0.25m^2 plot for 6 wild blueberry fields.

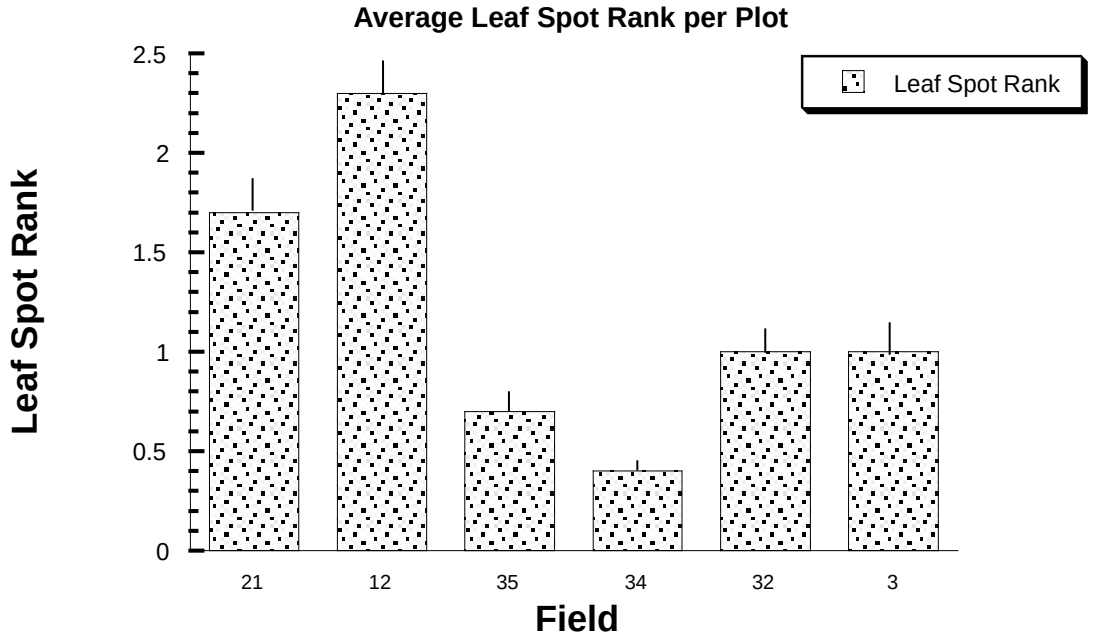


Figure 5. The average ranking for leaf spot for 6 wild blueberry fields. Leaf spot rank was 0 for no spots and 4 for severe spotting. Bars represent the standard error of the means.

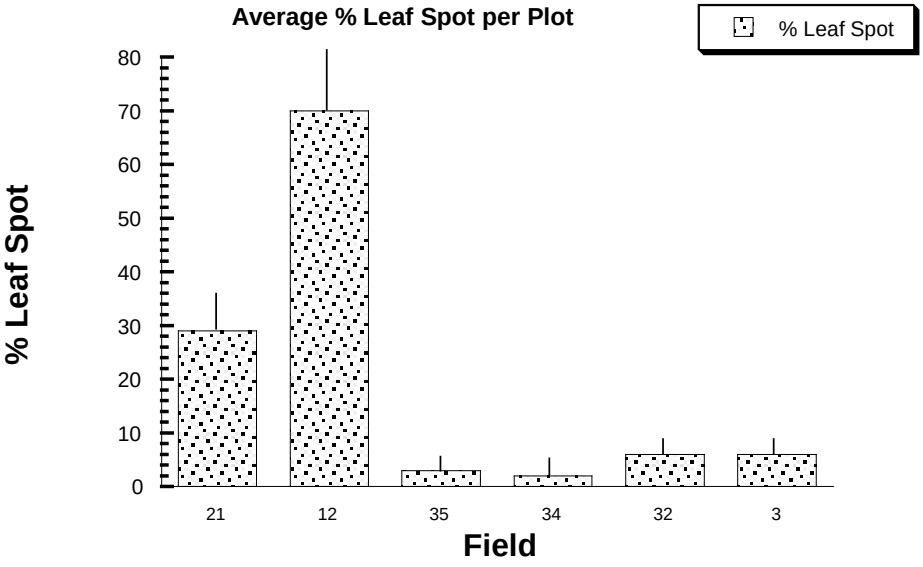


Figure 6 . The average percentage of stems with leaf spot in a 0.25m^2 plot for 6 wild blueberry fields. Bars represent the standard error of the means.

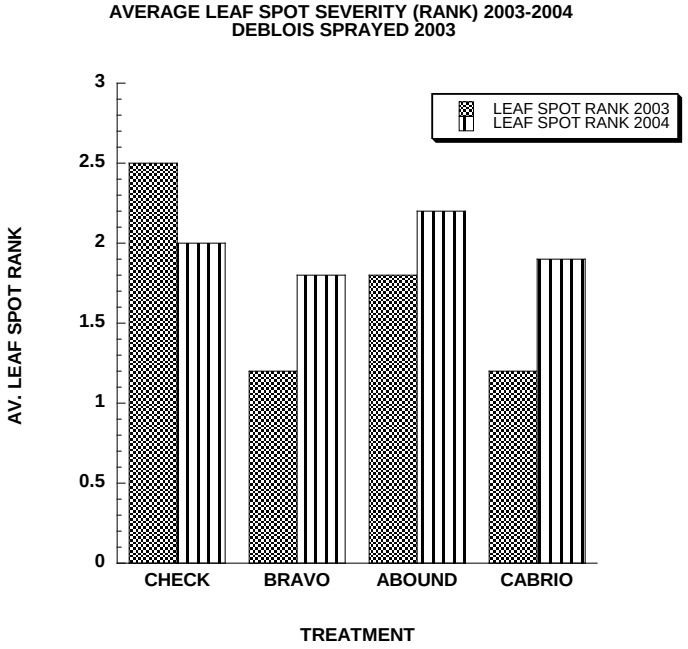


Figure 7. Average ranking of leaf spot severity evaluated in 2003 and 2004 for Deblois field sprayed in with fungicides in 2003.

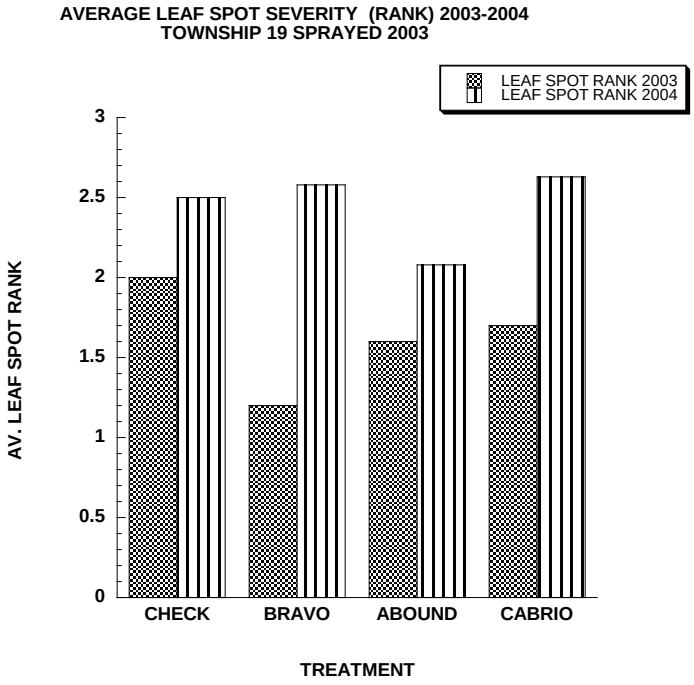


Figure 8. Average ranking of leaf spot severity evaluated in 2003 and 2004 for T19 field sprayed in with fungicides in 2003.

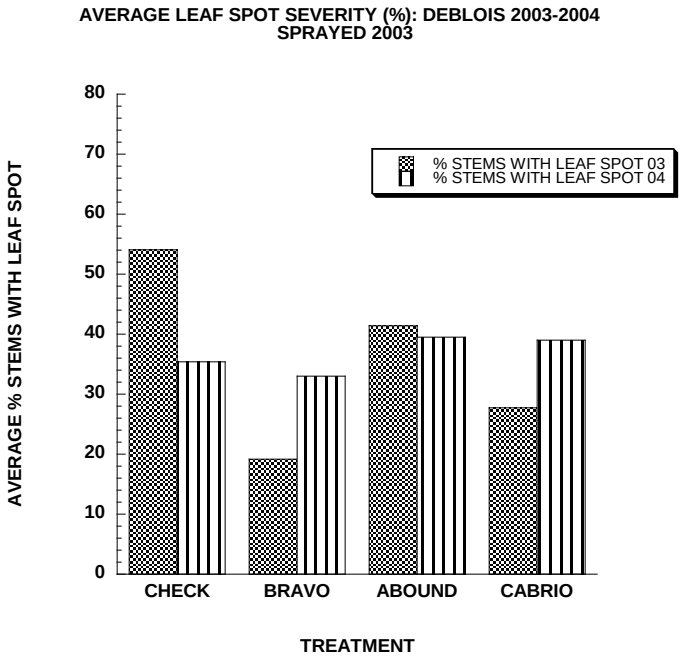


Figure 9. Average estimate of the percentage of stems with leaf spot evaluated in 2003 and 2004 for Deblois field sprayed in with fungicides in 2003.

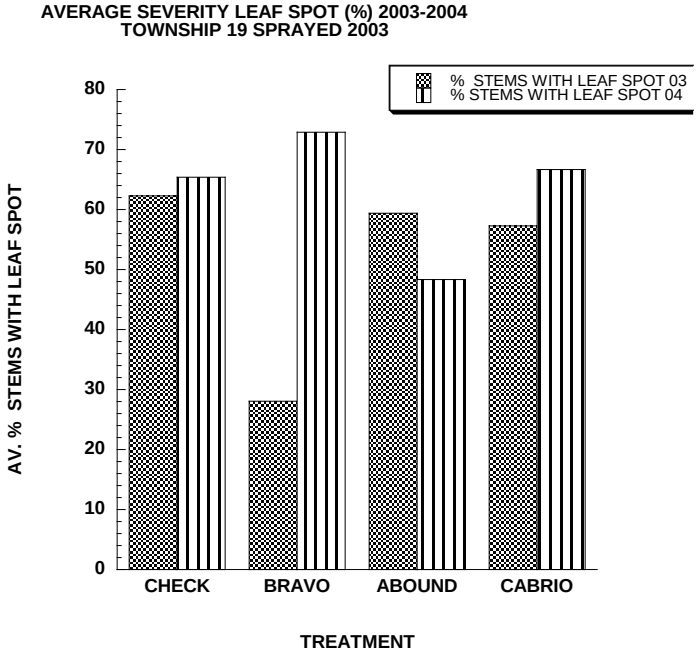


Figure 10. Average estimate of the percentage of stems with leaf spot evaluated in 2003 and 2004 for T19 field sprayed in with fungicides in 2003.

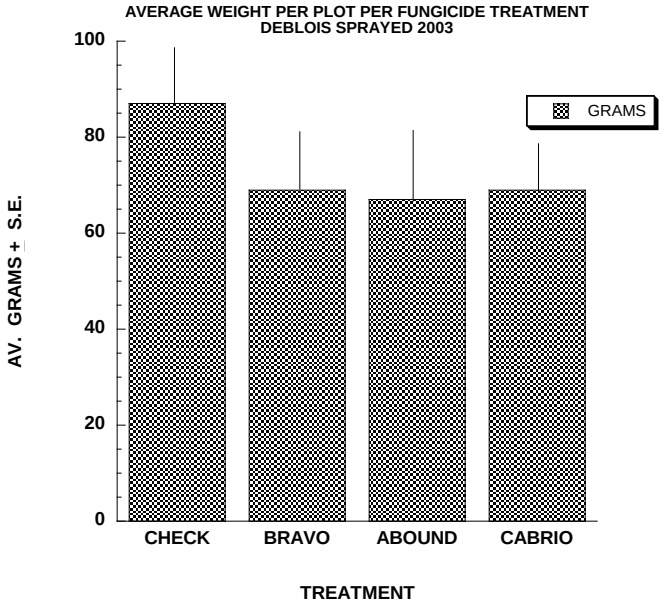


Figure 11. Average weight of blueberries raked within a sample area in 2004 for Deblois field sprayed in with fungicide treatments in 2003.

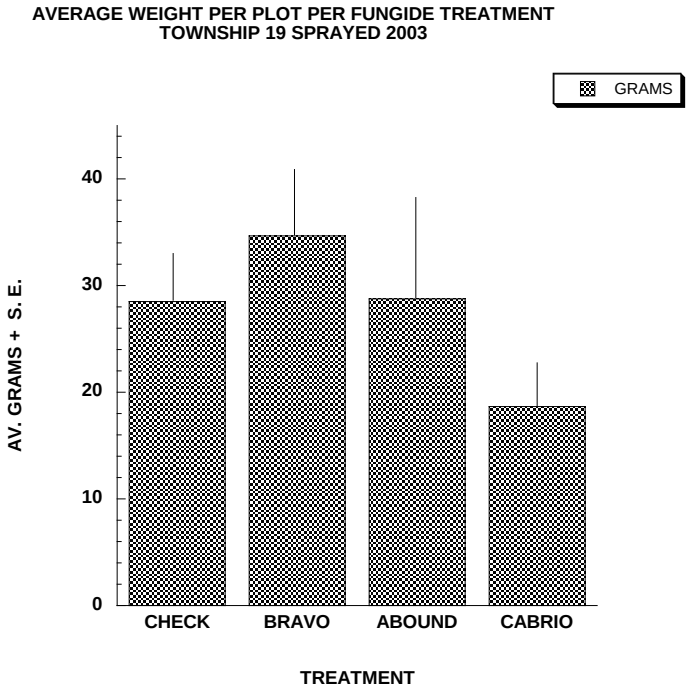


Figure 12 Average weight of blueberries raked within a sample area in 2004 for T19 field sprayed in with fungicide treatments in 2003.

DISEASE MANAGEMENT

INVESTIGATORS: D. E. Yarborough, Professor of Horticulture
S. L. Annis, Biological Sciences
C. S. Stubbs, Biological Sciences
K. F. Lough, Research Assistant

12. TITLE: Evaluation of fungicide control of mummy berry blight in wild blueberries: a) ground application and b) aerial application

METHODOLOGY: a) A randomized complete block design study was conducted at two sites, T19 and Deblois**.** Study plots of 6' x 30', replicated 4 times at each site, were treated with fungicides. Treatments included an untreated control, Elevate 1.5 lb/a, Indar 2 oz/a, Orbit 6 oz/a, Switch 9 oz/a, Pristine 18.5 oz/a, V116 at 6.1 oz/a, and Omega at 0.33 lb/a and 0.65 lb/a. The fungicides were applied on 12 May, 19 May and 27 May, and 3 June 2004. Plots were evaluated for *Monolinia* blight on 10 and 11 June 2004. The incidence of disease was the average percentage of stems with mummy berry blight in 4 sample areas of 6" x 18" for each plot. In early August, leaf spot disease was estimated for severity and incidence in two 6" x 36" sampling areas per plot. Leaf spot incidence was assessed on October 3 2004 by estimates of the % of stems with leaf spot and also ranked on a scale of 0 to 4 (0 indicated no leaves had spots-4 indicated all the leaves had spots). Each sampling area was also raked and berry weights recorded.

b) Aerial applications of fungicides were applied to a site in Deblois. Treatments included an untreated control, Indar 2 oz/a, and Pristine at 18.5 oz/a. Treatments were applied on 12 May and 27 May 2004. *Monilinia* was evaluated on 11 June 2004 and a harvest sample was taken on 4 August 2004. The incidence of disease was measured as the average percentage of stems infected with mummy berry for 10 sample areas of 6" x 18" within each plot. Blueberry yield was estimated from the average weight of berries raked from 10 sample areas of $1m^2$ for each treatment..

RESULTS: a) Results were variable between the two field sites. On the T19 field (Figure 1), plots treated with Indar, Elevate, Switch and the higher rate of Omega had similar or slightly higher incidences of disease than the untreated control and V116 treatment had significantly lower incidence of disease than Indar and Elevate but not the control. On the Deblois plots (Figure 1), Orbit and Indar treatments had significantly lower incidences of disease than both rates of Omega and Pristine, which also had higher, but not significant, incidences of disease than the untreated control. Treatments with V116, Elevate, and Switch also had lower, but not significant incidences of disease than the check. The fungicide treatments also had no significant effect on the average percentage of stems with leaf spot or the leaf spot severity compared to the control (Table 1). Yield per plot (weight of berries from raking a 6" by 36" sample) was not significant among treatments for either field (Table 1). The Deblois field had a higher average yield than the T19 field. The weight per berry ranged from 0.27 to 0.42 in T19 and from 0.32

to 0.38 in Deblois fields, indicating that the differences in yield between the fields is due to the number of berries rather than the size of the berries.

b) Plants treated with Indar had a significantly lower incidence of disease than the untreated control (Figure 2). Treatment with Pristine also had a lower incidence of disease than the control, but was not significantly different from the control or Indar. There was no difference in the blueberry yields among the three treatments (Figure 3).

CONCLUSIONS: The wet spring and winterkill on blueberry plants contributed to the variability among results at the two experiment sites. Orbit and V116 exhibited the best potential to decrease *Monilinia* infections on blueberry plants. Pristine also showed some suppression but was less effective than Indar on the aerial test plots.

RECOMMENDATIONS: Continue to evaluate fungicides for mummy berry control, including organic materials such as Serenade and SulfurX.

Figure 1. Effect of Fungicide Application on Monolinia Infection Rate

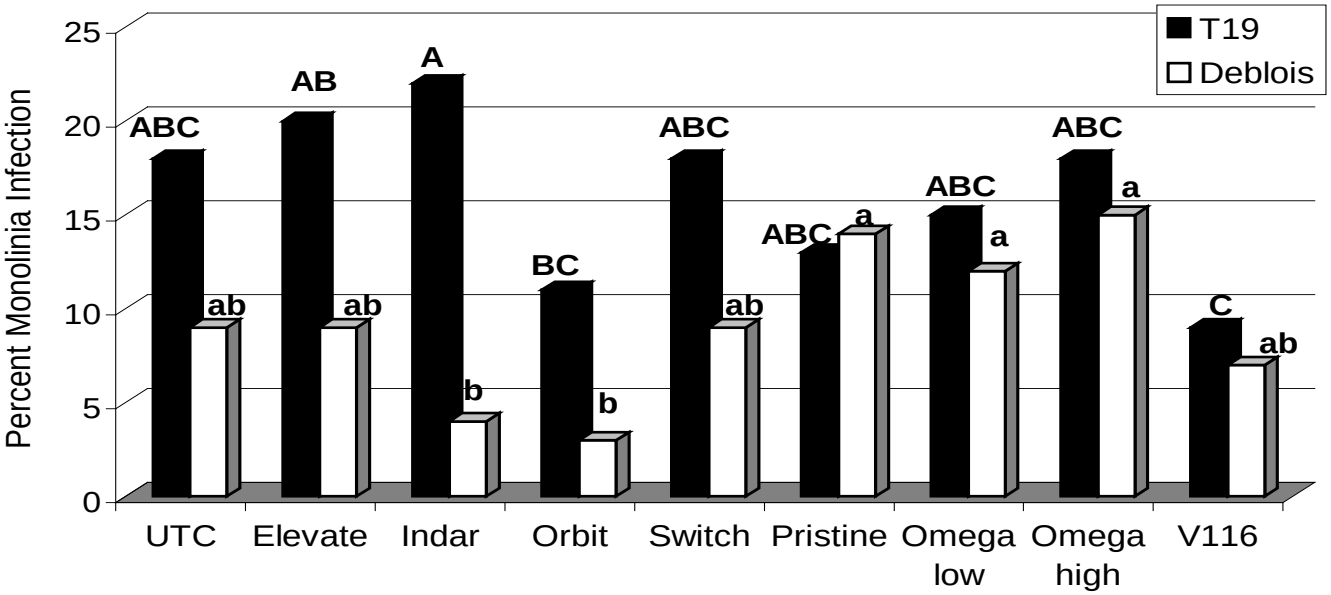


Table 1. Leaf spot in plots treated with fungicides for mummy berry control.

Field	Treatment	Average Leaf Spot Rank	Average % stems with leaf spot
T19	Control	2 (0.3)	63.1 (13.7)
	Elevate 1.5 lb/a	2.6 (0.3)	69.4 (9.5)
	Indar 2 oz/a	1.6 (0.4)	46.9 (12.0)
	Orbit 6 oz/a	1.9 (0.3)	66.9 (9.3)
	Switch 9 oz/a	2.1 (0.3)	58.8 (11.0)
	Pristine 18.5 oz/a	2 (0.4)	46.3 (10.1)
	V116 at 6.1 oz/a	2.5 (0.5)	50.9 (13.2)
	Omega at 0.33 lb/a	2.4 (0.4)	63.1 (13.3)
	Omega 0.65 lb/a.	2.4 (0.4)	75.6 (6.0)
Deblois	Control	2.4 (0.3)	74.3 (9.4)
	Elevate 1.5 lb/a	3 (0.4)	77.5 (7.8)
	Indar 2 oz/a	2.1 (0.3)	55.6 (10.6)
	Orbit 6 oz/a	1.7 (0.3)	46.9 (10.3)
	Switch 9 oz/a	2.3 (0.3)	67.5 (9.1)
	Pristine 18.5 oz/a	2 (0.3)	55 (11.6)
	V116 at 6.1 oz/a	2.1 (0.2)	58.8 (7.7)
	Omega at 0.33 lb/a	1.9 (0.4)	45 (13.1)
	Omega 0.65 lb/a.	2 (0.4)	53.1 (10.4)

Figure 2. Monolinia Infection after Aerial Application of Fungicides

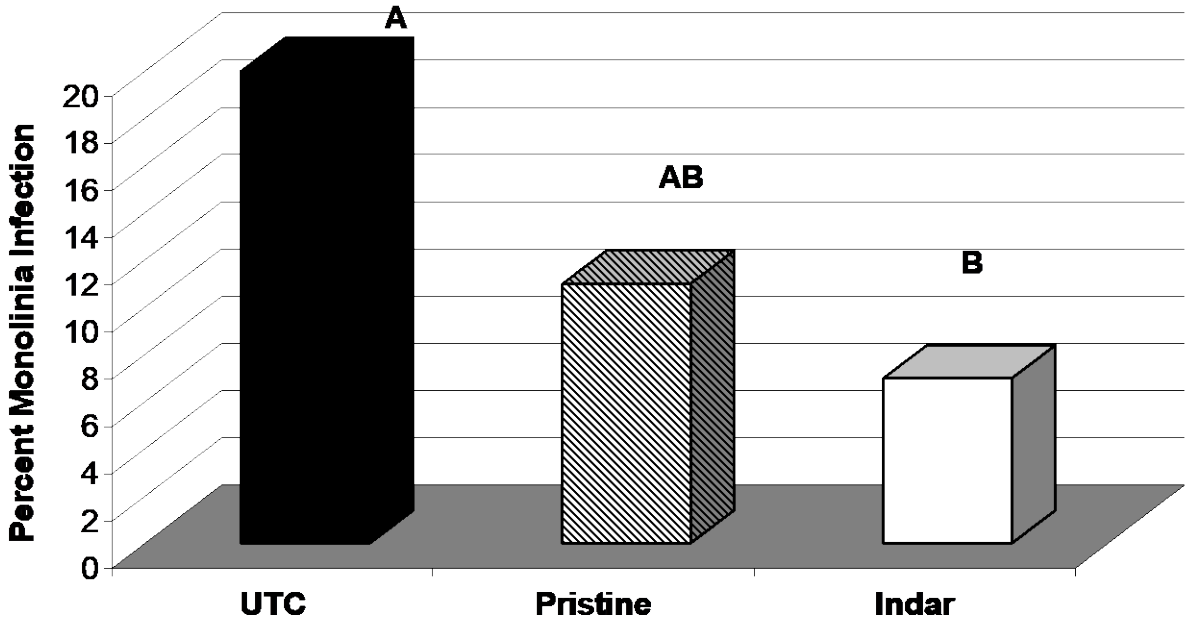
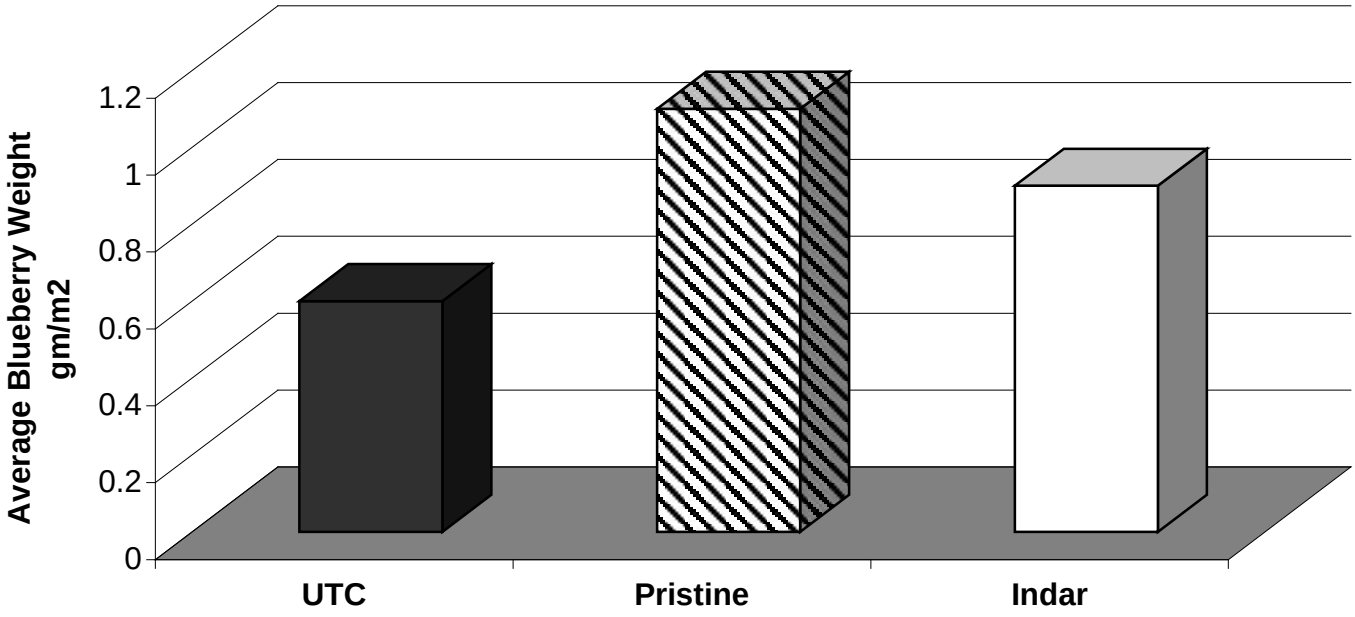


Figure 3. Blueberry Yield after Aerial Fungicide Application



PLANT NUTRITION AND FERTILITY

INVESTIGATORS: John M. Smagula, Professor of Horticulture
Ilse W. Fastook, Scientific Technician

13. TITLE: Effect of Foliar Copper Application on Growth and Yield of Wild Blueberries

OBJECTIVE: Determine the effect of raising foliar copper (Cu) concentrations on growth and yield of wild blueberries.

Brief Justification:

The standard set for Cu by Trevett in 1972 is 7 ppm. Many fields have leaf Cu concentrations below 7 ppm, so raising the leaf Cu concentration to above the standard will test the accuracy of the standard and provide growers with information about methods to raise leaf Cu concentrations. Since Cu is a component of many enzymes and is one of the electron carriers in photosynthesis, we anticipate an increase in growth and flower bud formation with the prune year application of Cu. Fruit development and yield may be enhanced by the prune year application of Cu. A 2000 study using 0.5 lb Cu Chelate/acre had no effect on leaf Cu concentration. A different product will be tried with concentrations up to 2 lb Cu/acre.

METHODS: A commercial wild blueberry field with leaf Cu concentrations below 7 ppm was selected for this study. Cu Keylate (Stoller Enterprises, Inc.) containing 5% Cu was applied as a foliar spray in a volume of 67 gals/acre. Ammonium sulfate at 2.8 lbs/acre was added to the solution to enhance uptake of the Cu chelate. Since several growers are using a product called Micromate calcium fortified mix (Stoller Enterprises, Inc.) to supply secondary and micronutrients along with N and P through diammonium phosphate (DAP), we decided to include this as an additional treatment at the rate they were using. Micromate is a homogeneous granule containing calcium (10%), magnesium(5%), sulfur (1%), boron (1%), iron (2%), manganese(1.5%), zinc (3%) and Cu (0.3%). Treatment plots measuring 6 ft x 50 ft received the following foliar treatments and Micromate on June14, 2001:

1. Control
2. Cu Keylate at 0.5 lb Cu/acre
3. Cu Keylate at 1.0 lb Cu/acre
4. Cu Keylate at 1.5 lb Cu/acre
5. Cu Keylate at 2.0 lb Cu/acre
6. Micromate at 0.04 lb Cu/acre

These treatments were randomly assigned to treatment plots in a randomized complete block design with 7 blocks. Soil samples and composite leaf tissue samples were taken July 13, 2001 from each treatment plot. Stem samples from 4 randomly placed, 1/4 ft^2 quadrats were collected November 6, 2001 for measurement of stem length and flower bud formation. Yield was determined August 9, 2002.

Because 2001 leaf samples indicated that N and P were deficient and could have masked the effect of corrected Cu deficiency, the plots were maintained through another cropping cycle and treatments were reapplied with or without DAP to correct N and P deficiencies. The blocks were split, creating two 25 ft x 6 ft plots. One half of each block received 400 lbs diammonium phosphate (DAP) per acre on May 19, 2003 to correct the N and P deficiency and the same Cu Keylate rates as in 2001 were applied on June 17, 2003. Composite leaf tissue samples were taken July 22, 2003. Soil samples were taken July 29, 2003. Stem samples were taken on November 17 and 18, 2003 for growth and potential yield measurements. Yield was taken on August 9, 2004.

RESULTS: Leaf N concentrations were below the standard (1.6%) and were not affected by any treatment (Fig. 1). Leaf P concentrations were also below the standard (0.125%) (Fig. 2) and was unaffected by treatments. Leaf Cu concentrations increased linearly with increasing Cu rate but Micromate had no effect on leaf Cu concentration, compared to the control (Fig. 3). The level of leaf Cu concentration in the controls indicated a deficiency. The lowest rate of Cu Keylate® (0.5 lb Cu/acre) raised the leaf Cu concentration to above the 7 ppm standard.
The soil analysis indicated that the pH averaged 4.4 across all plots and the organic matter content (loss on ignition) averaged 9.9 %. Soil Cu concentration was not affected by any treatment (Fig. 4).
Stem density, average stem length (Fig. 5), and number of branches (Fig.6) were not influenced by Cu treatments. Branch length was not meaningfully affected by the Cu treatments (Fig.6). Flower buds per stem (Fig. 7), flower bud density (flower buds per unit area) (Fig.8), and berry yield (Fig. 9) were not influenced by any treatment.

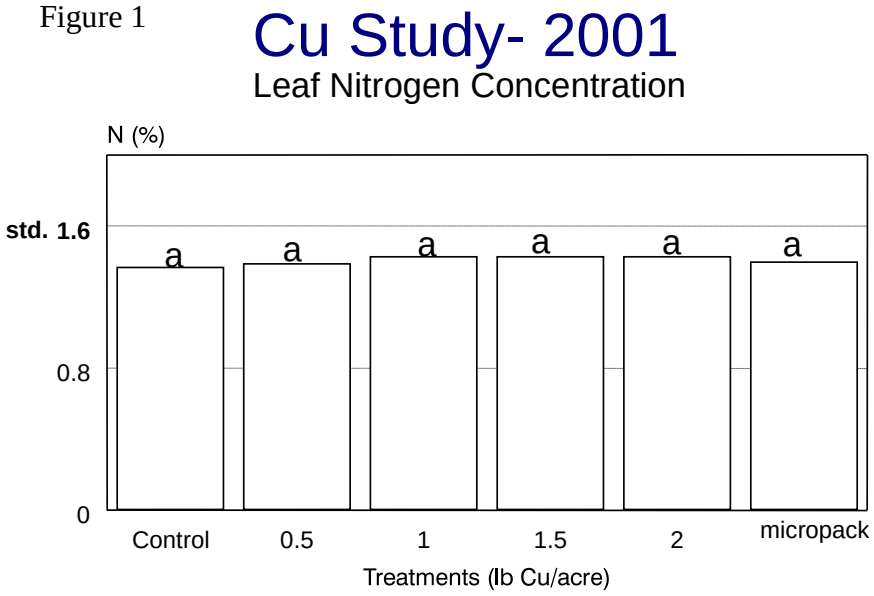
Leaf samples taken in 2001 indicated that N and P were deficient and could have masked the effect of corrected Cu deficiency. The Cu treatments in 2001 were very effective in raising leaf Cu concentration but stem characteristics, including flower bud formation and yield, were not affected. In 2003, the Cu treatments were reapplied in a split block design with the Cu treatment as the main plots and the DAP as the split plots. DAP increased leaf N (Fig.10) and leaf P (Fig. 11) concentrations. The Cu treatments in 2003 did not raise leaf Cu concentrations to those levels observed in 2001 (Fig. 12). The effect of DAP partially contributed to the lower leaf Cu concentrations (Fig. 13); perhaps, by stimulating more growth and larger leaves, causing a dilution effect. Over all, plots treated with DAP had significantly lower leaf Cu concentrations, compared to those that received no DAP (Fig. 14). A similar dilution effect of DAP on leaf concentrations was observed for iron and boron. Treatments of 1 and 1.5 lbs Cu/acre resulted in a slight increase in soil Cu concentrations (Fig. 15). DAP increased the soil P concentrations (Fig. 16). Stem density and stem length (Fig. 17) were not meaningfully influenced by the Cu treatments. Stem branching and branch length (Fig. 18) were not affected by Cu treatments. Average number of flower buds per stem or per unit area were not affected by Cu treatments (Figs. 19 and 20). **DAP did not affect the following characteristics of unbranched stems: density, length, and number of flower buds. DAP did, however, increase the density, length, number of branches, flower buds per stem and flower**

bud density of branched stems (Figs 21-23). There was no effect of copper treatments on yield (Fig.24). Although DAP increased flower bud density the average yield for plots with or without DAP were about the same, 6,727 and 6,035 lbs/acre, respectively.

CONCLUSIONS: Cu Keylate was effective in raising leaf Cu levels to a sufficiency level in 2001. The deficiency of N and P, however, may have compromised the test of the Cu standard in 2001 and 2002. Micromate provided inadequate amounts of Cu to raise leaf Cu concentrations above the levels found in the controls. In 2003, leaf N and P concentrations were raised by DAP at 400 lb/acre, but leaf Cu concentrations were lower than in 2001 even though the same rates were applied. DAP application reduced the levels of leaf Cu in the plots receiving the foliar Cu applications and in the control plots. Cu treatments elevated leaf Cu concentrations in 2003 but did not have an affect growth or yield.

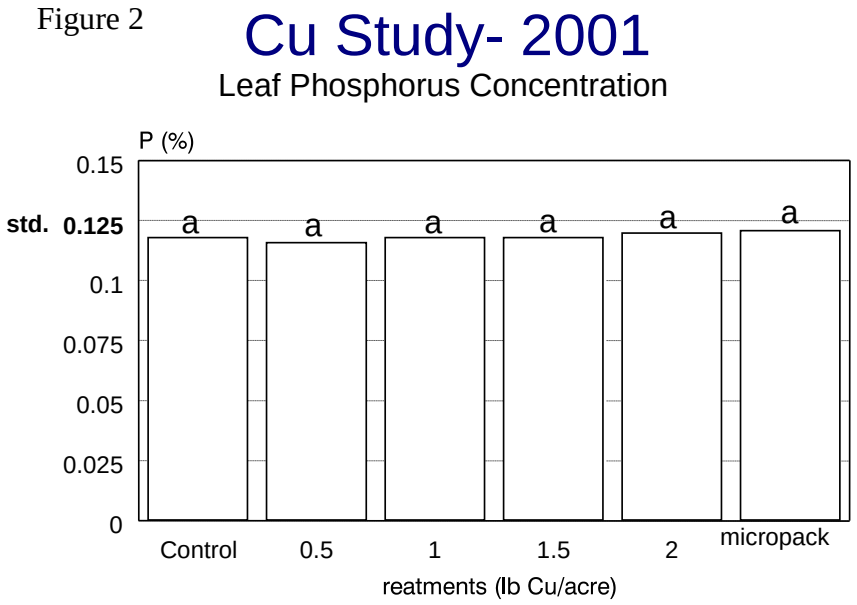
RECOMMENDATIONS: No recommendations for Cu fertilization can be made to growers at this time.

Figure 1



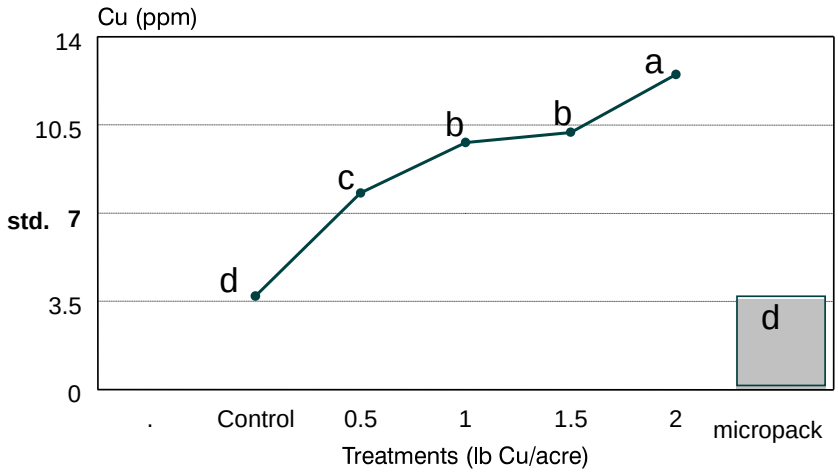
Mean separation by Duncan's Multiple range test, 5% level.

Figure 2



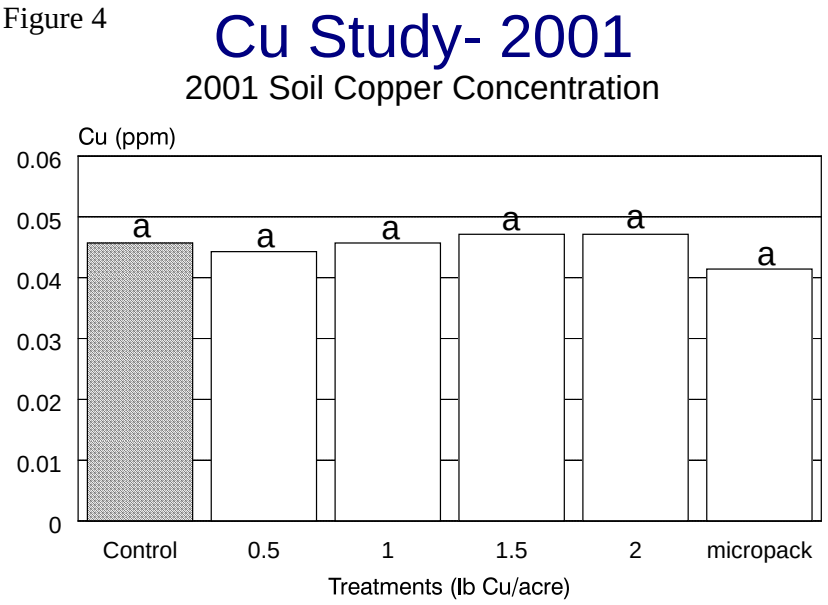
Mean separation by Duncan's Multiple range test, 5% level.

Figure 3



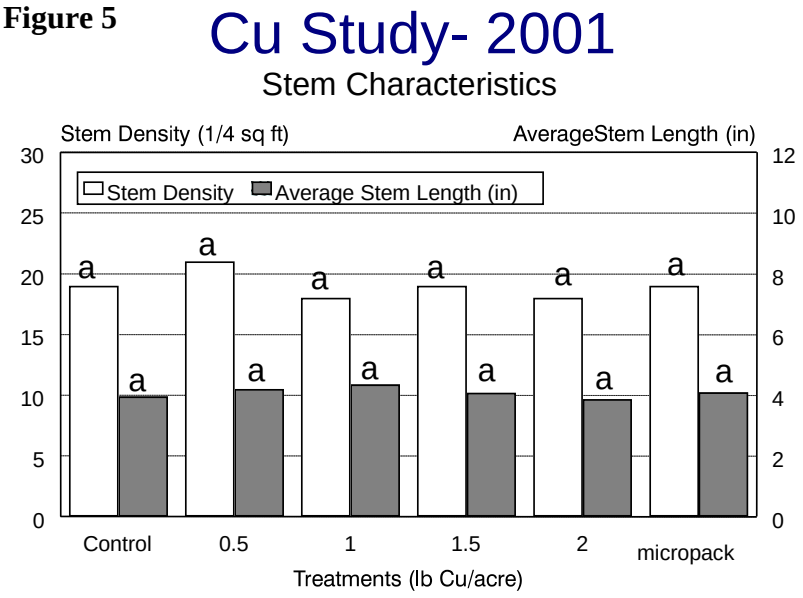
Mean separation by Duncan's Multiple range test, 0.01% level. Significant linear increase in leaf Cu concentration with increasing foliar Cu rate, 0.01% level.

Figure 4



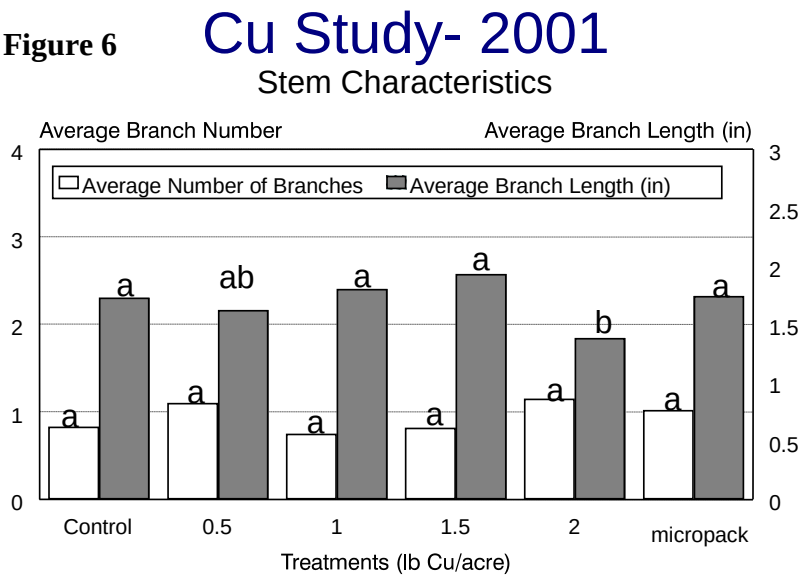
Mean separation by Duncan's Multiple range test, 5% level.

Figure 5



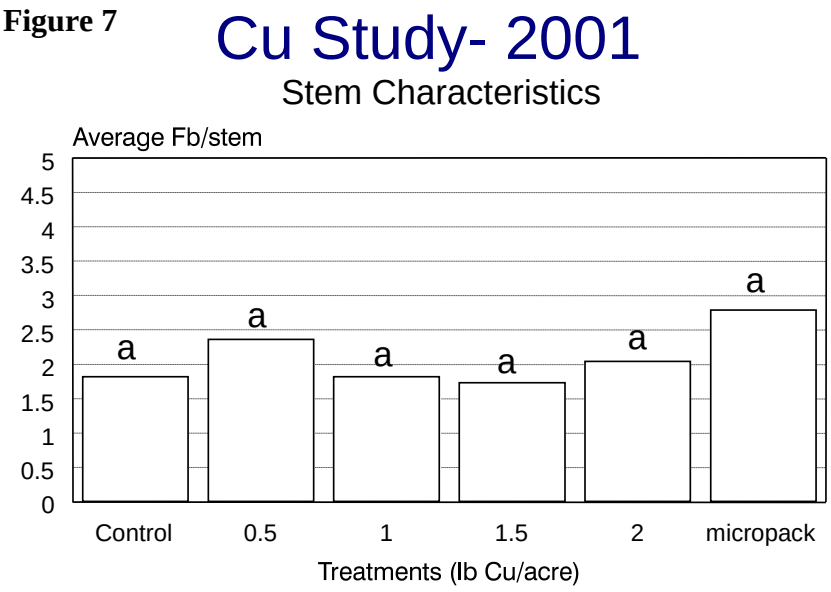
Mean separation by Duncan's Multiple range test, 5% level.

Figure 6



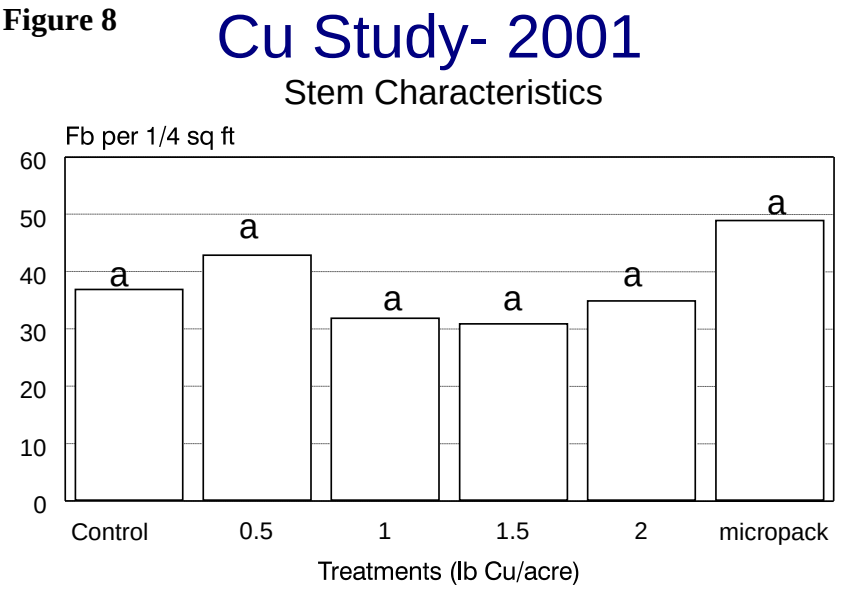
Mean separation by Duncan's Multiple range test, branch number 5% level, average branch length, 0.01% level.

Figure 7



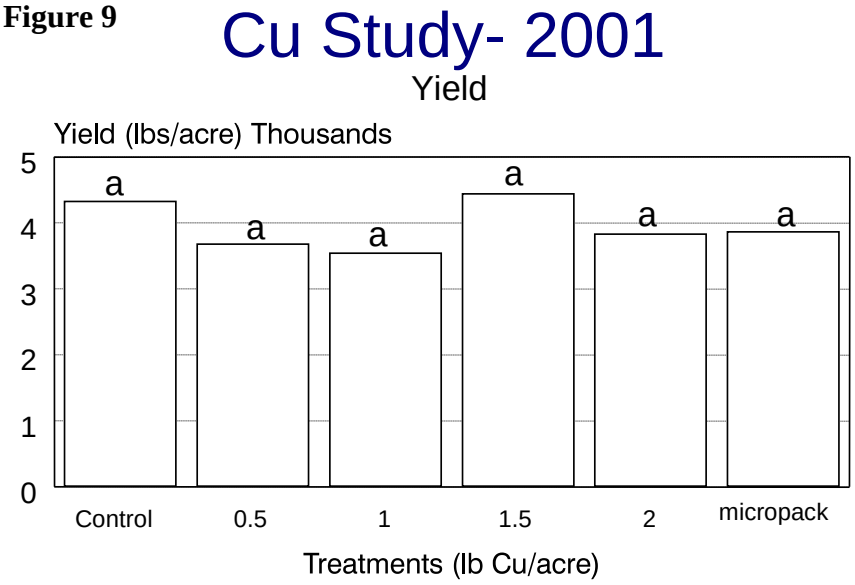
Mean separation by Duncan's Multiple range test, 5% level.

Figure 8



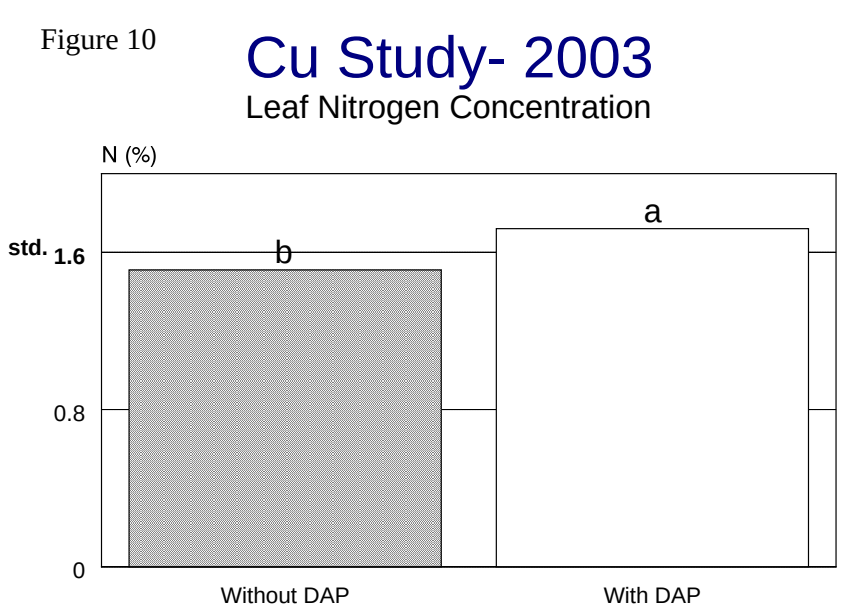
Mean separation by Duncan's Multiple range test, 5% level.

Figure 9



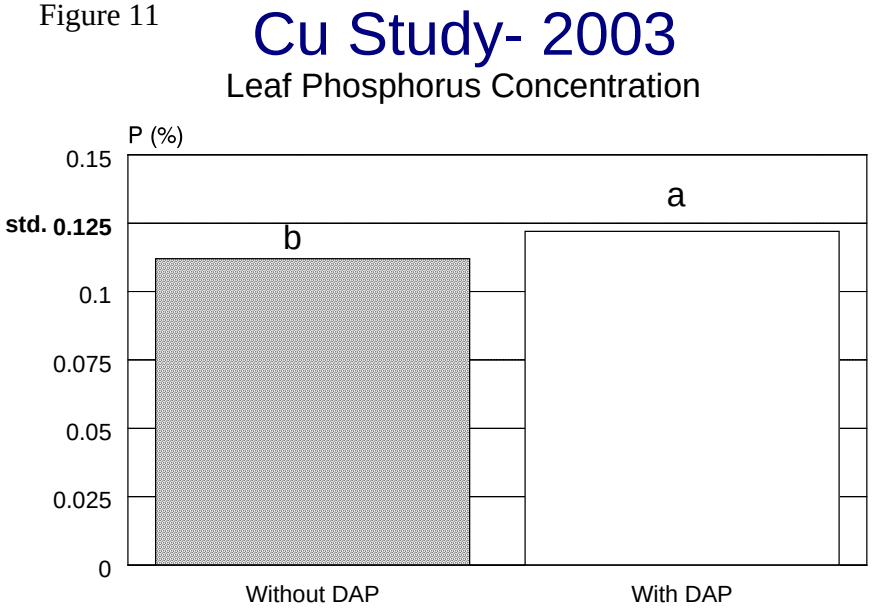
Mean separation by Duncan's Multiple range test, 5% level.

Figure 10



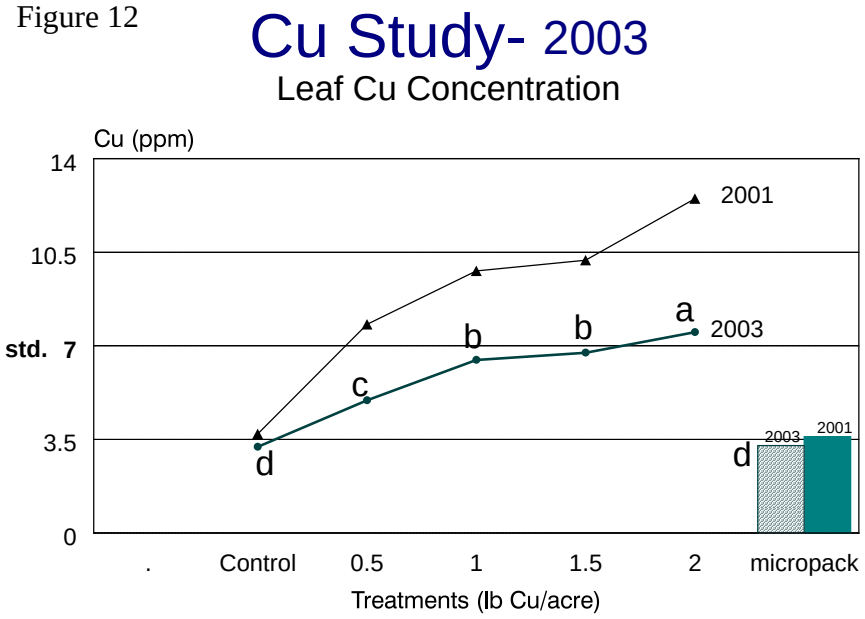
Mean separation by Duncan's Multiple range test, 1% level.

Figure 11



Mean separation by Duncan's Multiple range test, 1% level.

Figure 12



Mean separation by Duncan's Multiple range test, 0.1% level. .

Figure 13

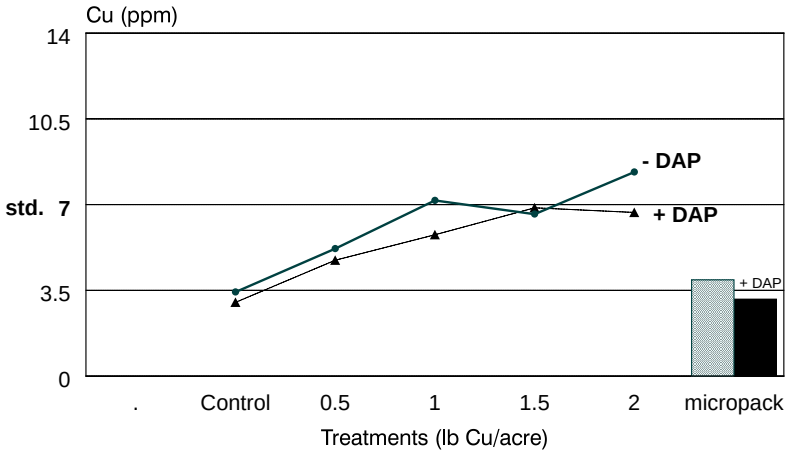
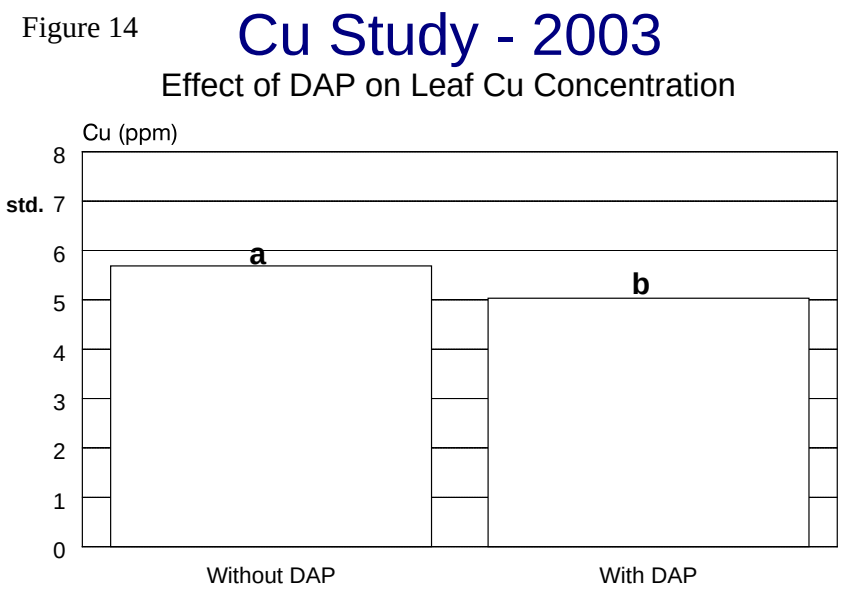
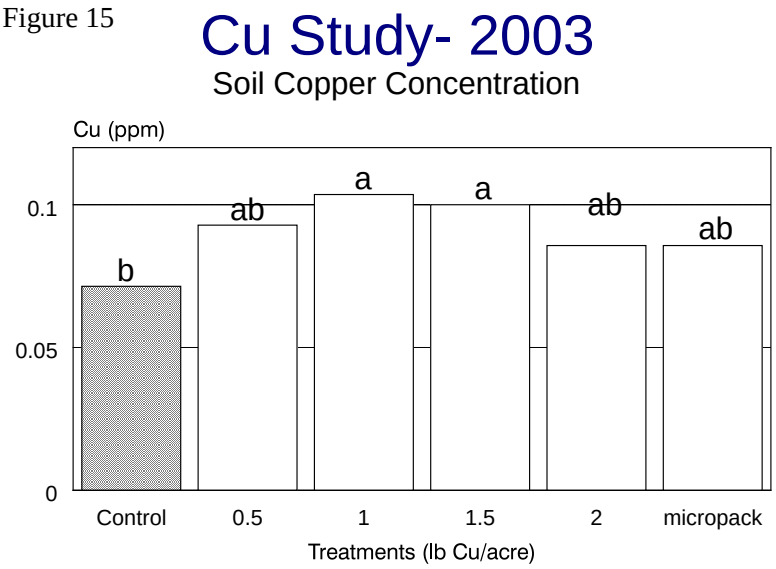


Figure 14



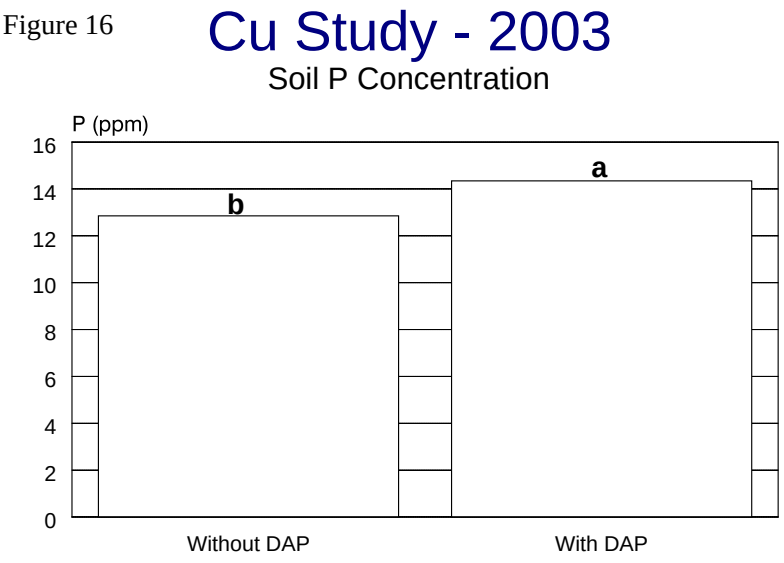
Mean Separation by Duncan's Multiple range test, 1% level

Figure 15



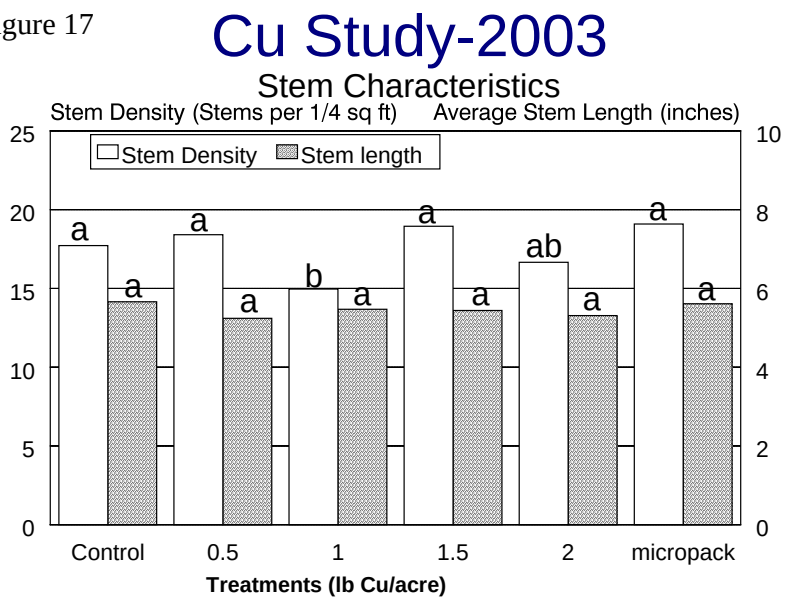
Mean separation by Duncan's Multiple range test, 5% level.

Figure 16



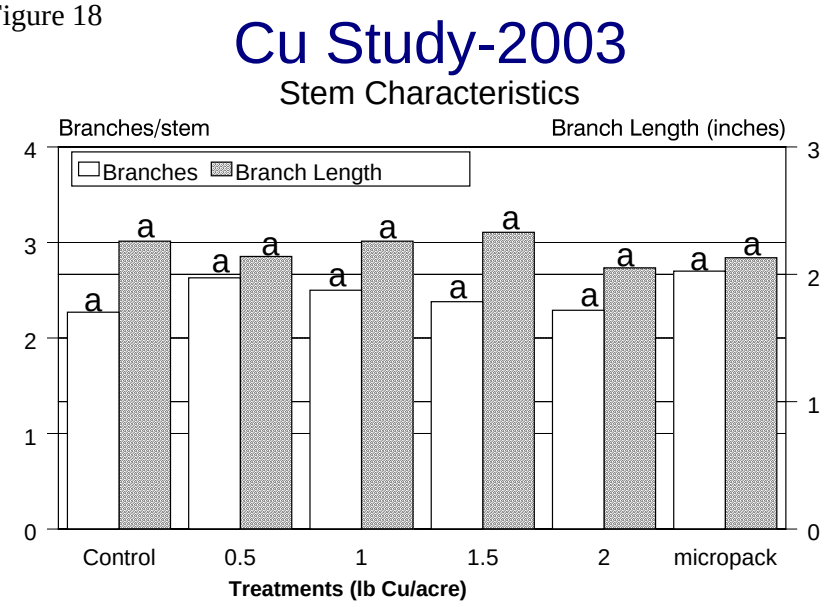
Mean Separation by Duncan's Multiple range test, 5 % level.

Figure 17



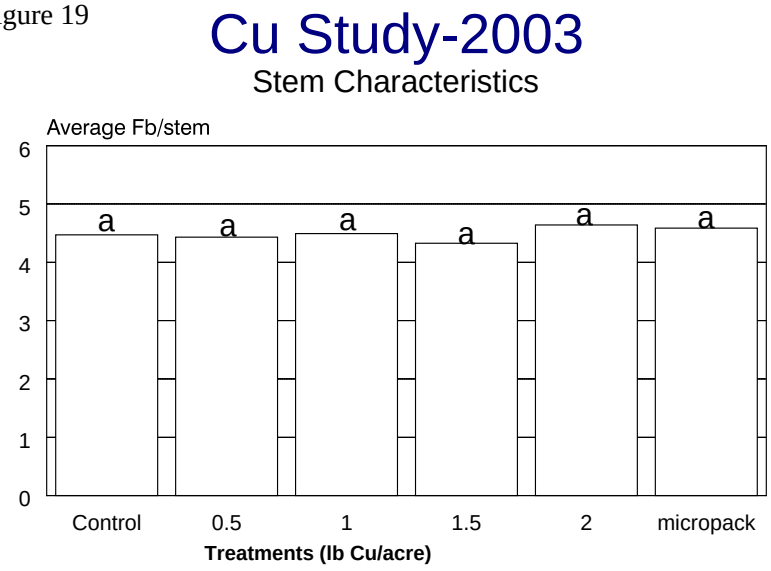
Mean separation by Duncan's Multiple range test, level 5%

Figure 18



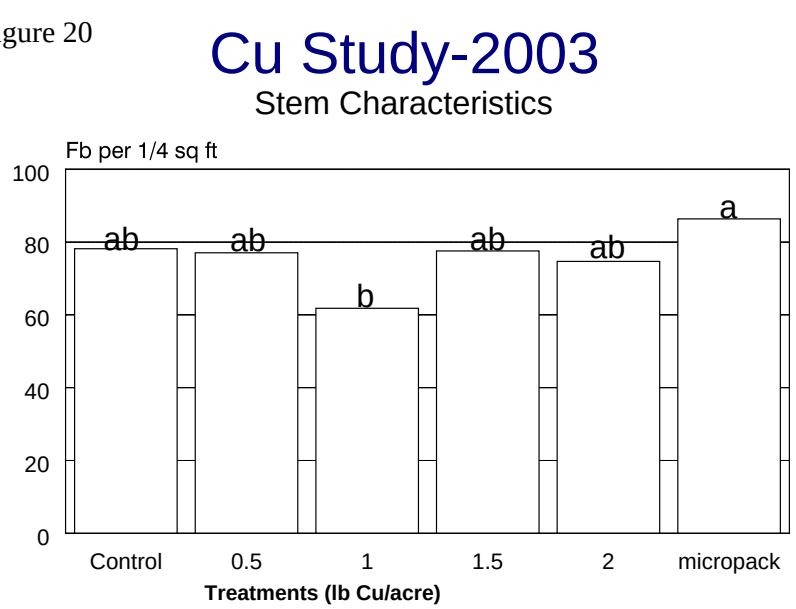
Mean separation by Duncan's Multiple range test, level 5%

Figure 19



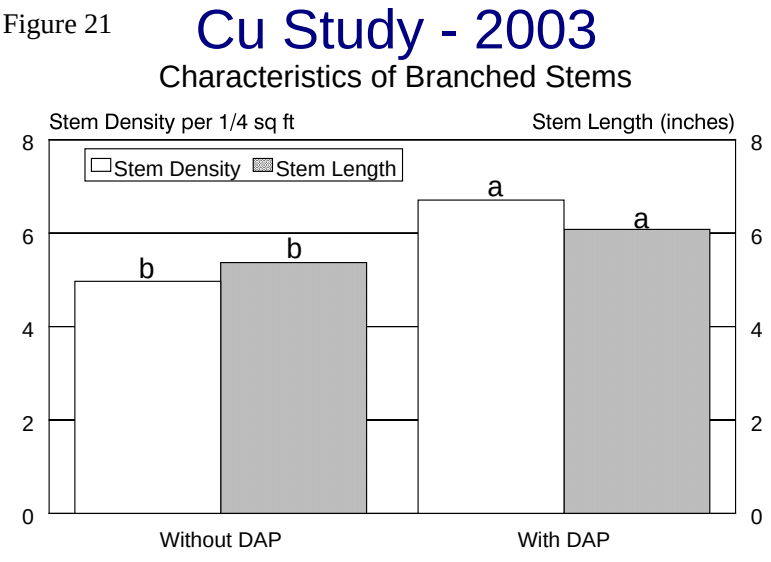
Mean separation by Duncan's Multiple range test, level 5%

Figure 20



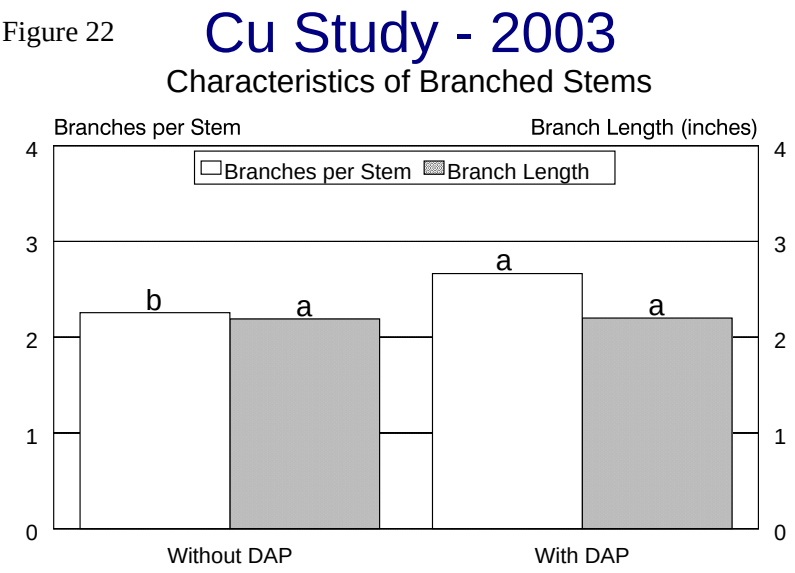
Mean separation by Duncan's Multiple range test, level 5%

Figure 21



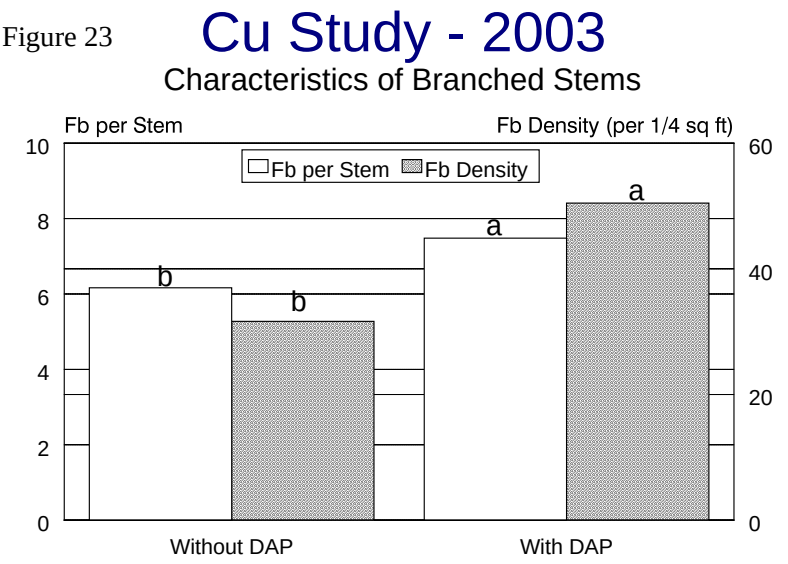
Means Separation by Duncan's Multiple range test, 0.01 % level.

Figure 22



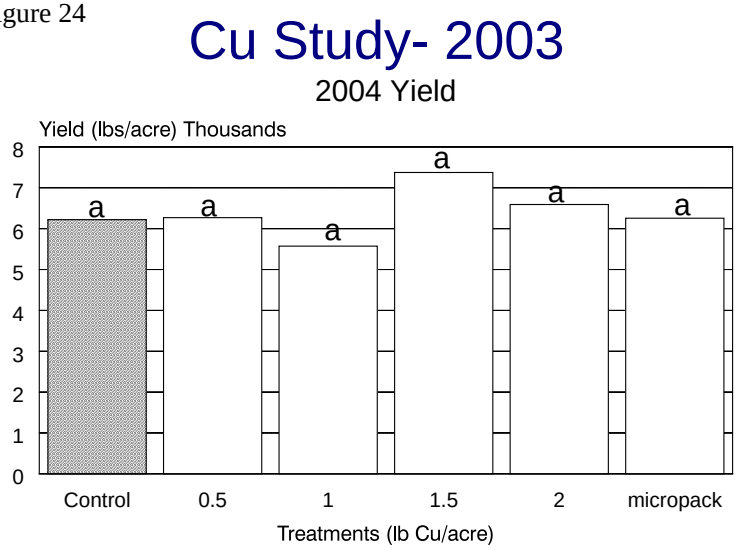
Means Separation by Duncan's Multiple range test, 1 % level.

Figure 23



Means Separation by Duncan's Multiple range test, 5 % level.

Figure 24



Mean separation by Duncan's Multiple range test, 5% level.

PLANT NUTRITION AND FERTILITY

INVESTIGATORS: John M. Smagula, Professor of Horticulture
Ilse W. Fastook, Scientific Technician

14. 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
2001 leaf nutrient concentrations

Treatment	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

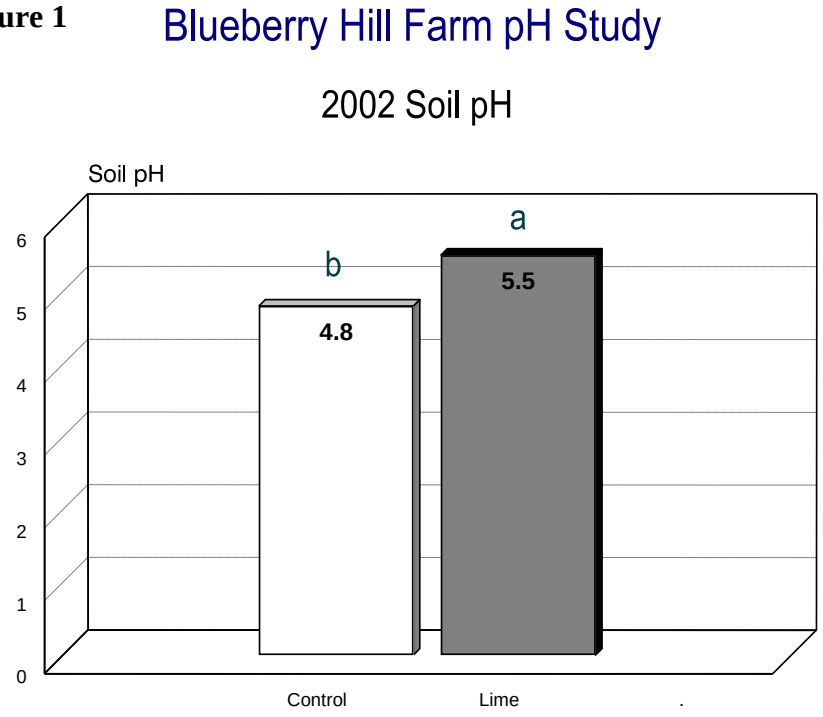
Crop year leaf samples (Table 3) showed different concentrations but similar trends to that found in 2001 prune year leaf samples. Leaf N, P, Zn, and Ca concentrations were not different between the control and limestone-treated plots, but leaf concentration of Mg increased and leaf K, B, Cu, Mn, Al,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

Table3
2002 leaf nutrient concentrations

Treatment	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
2002 soil nutrient concentrations

Treatment	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

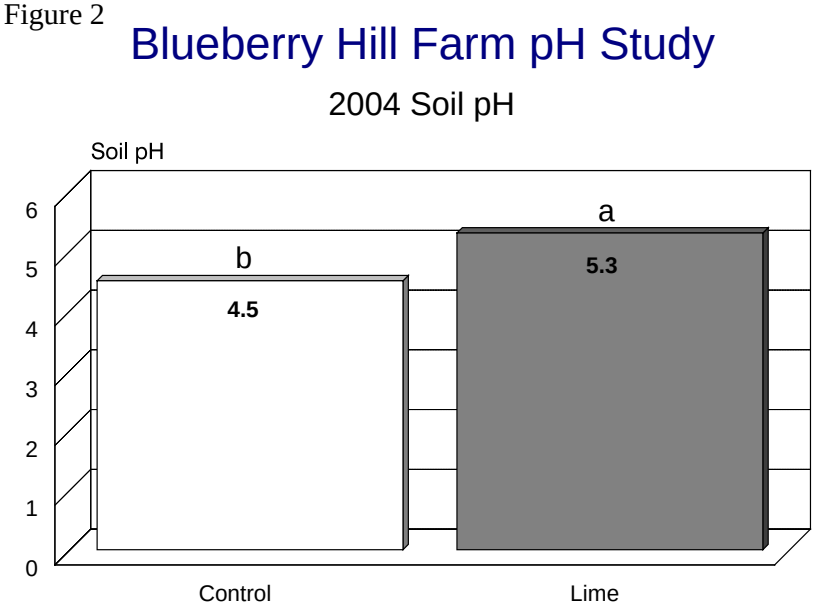
Prune year leaf samples (Table 5) showed different concentrations but trends did not reflect the data from 2002 (Table 3). Leaf N, Cu, Fe, Zn, concentrations were not different between control and limestone-treated plots, but leaf concentrations of Mg increased and leaf Ca, K, P, Mn, Al, and B concentrations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

Table 5
2004 leaf nutrient concentrations

Treatment	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.

Treatment	Table 6 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**

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 7). Yield was collected 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 7)**.** 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 1/9 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 be 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 7 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 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 8.

Table 8
2001 leaf nutrient concentrations

Treatment	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 9**.**

Table 9
2001 soil nutrient concentrations

Treatment	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, stem length, and flower buds per stem were not affected by treatments (Table 10).

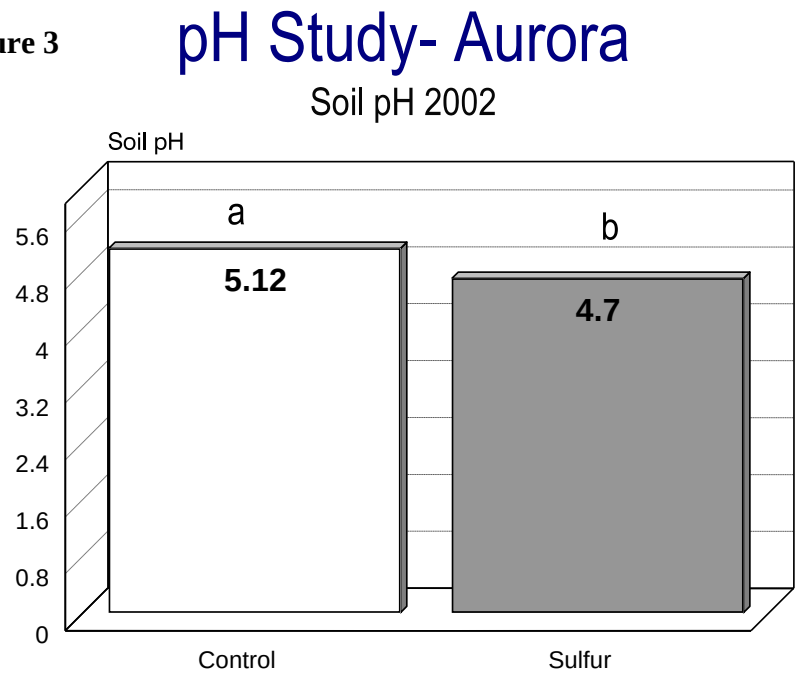
Table 10
2001 Stem Characteristics

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.3) 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 3

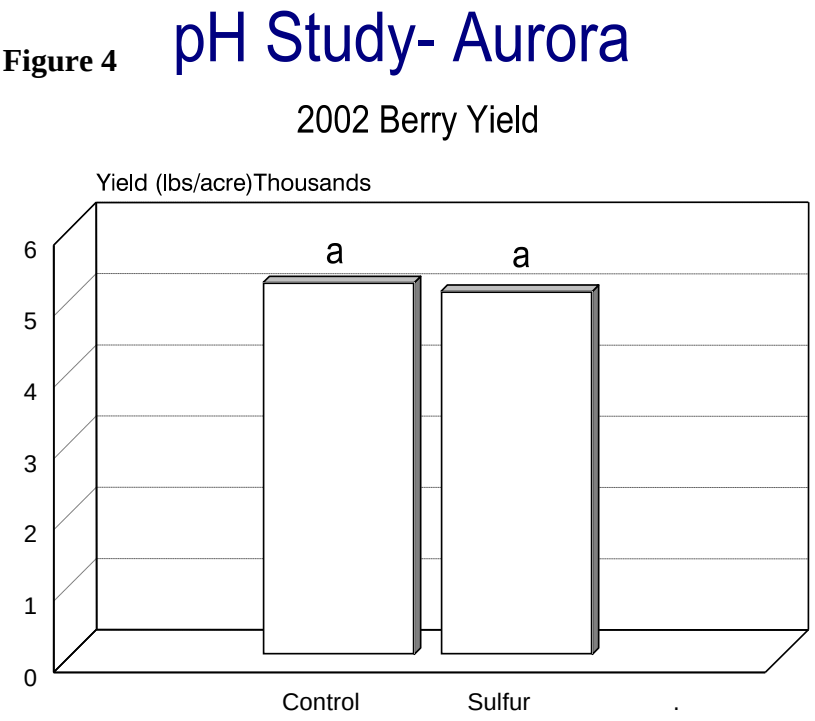


Treatment	Table 11 2002 soil nutrient concentrations							
	Ca (ppm)	K (ppm)	Mg (ppm)	P (ppm)	B (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
Control	**302a**	**83a**	**34a**	**6.4a**	**.06a**	**.17a**	**1.8a**	**5.8b**
Sulfur (S)	**331a**	**86a**	**37a**	**7.1a**	**.06a**	**.21a**	**2.2a**	**12.8a**

2002 Yield

Blueberry yield collected in August 7, 2002 was not affected by sulfur treatment (Fig. 4).

Figure 4



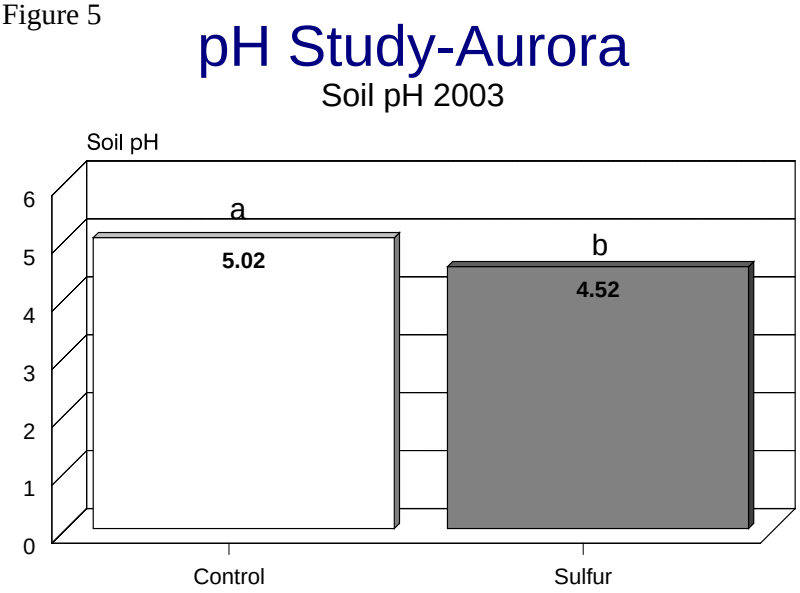
2003 Soil and Leaf Tissue Analysis

Prune-year leaf tissue levels were similar for control and sulfur-treated plots, except for leaf Mn concentrations (Table 12). Soil Mn concentrations were also higher in treatment plots receiving sulfur (Table 13). Soil pH values for treatment plots in 2003 (Fig. 5) were similar to those in 2002.

Treatment	Table 12 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

Treatment	Table 13 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 5

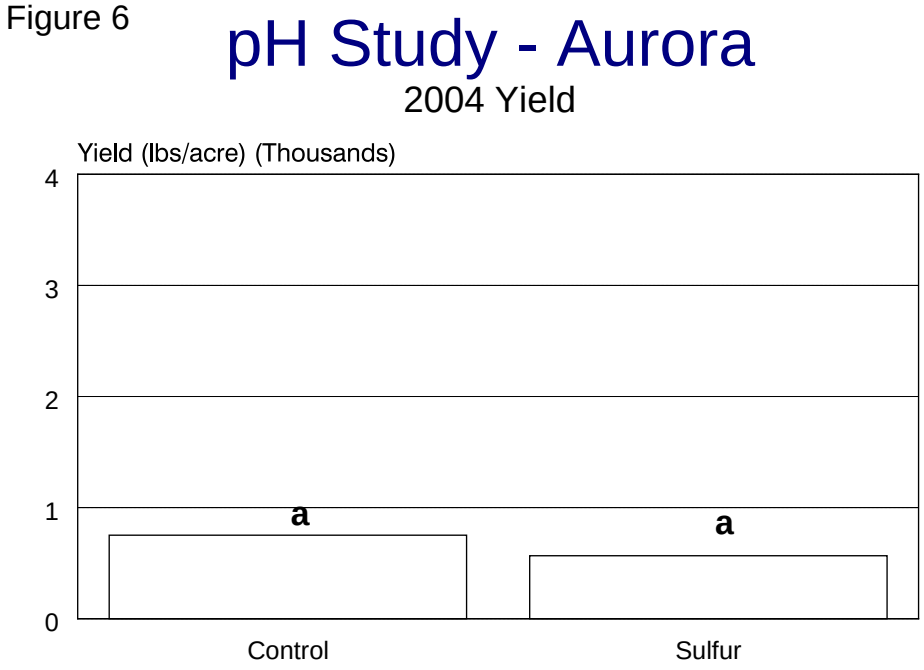


2003 Stem Characteristics

Stem density, length, branching and flower bud formation were not affected by soil pH (Table 14). 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. 6).

Treatment	Table 14 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 6



CONCLUSIONS: No conclusions can be made at this time.

RECOMMENDATIONS: No recommendations can be made at this time.

PLANT NUTRITION AND FERTILITY

INVESTIGATORS: John M. Smagula, Professor of Horticulture
Ilse W. Fastook, Scientific Technician

15. TITLE: Effect of Fertilizer Timing (prune year vs. crop year) on Wild Blueberry Growth and Productivity.

OBJECTIVES: To determine the effect of time of fertilizer application on nutrient uptake, soil nutrient availability, plant growth, and yield.

METHODOLOGY: A commercial wild blueberry field that had a history of low leaf nitrogen (N) and phosphorus (P) concentrations was used in this study. Diammonium phosphate (DAP) at 400lbs/acre (72 lbs N and 80 lbs P/acre) was applied to 6 ft x 50 ft treatment plots in the spring (pre-emergent) of the prune year (2003), or will be applied in the spring of the crop year (2004). A foliar application of N (CoRoN™) was also tested to determine if it will hasten the entry of N into the plant during the spring or crop year, compared to the soil applications in those cycles. CoRoN ™ (28% N) is a combination of polymethylene urea coupled with fast-release, low-biuret urea, designed to act as a slow-release foliar fertilizer. CoRoN ™ was applied June 13 of the prune year and June 26, about two weeks later. CoRoN™ will also be applied twice in the Crop year beginning when there is adequate foliage to absorb the spray, sometime in May. Some plots will receive DAP in the spring and crop year and some will receive just foliar sprays in the spring and crop year. A control plot received no fertilization. These 8 treatments (Table 1) were replicated 8 times in a randomized complete block design.

Table 1
Treatment Summary

Treatment 1	**Control**
Treatment 2	**DAP (400 lb /acre), spring of prune year**
Treatment 3	**DAP (400 lb /acre), spring of crop year**
Treatment 4	**DAP (400 lb /acre), spring of prune year +DAP (400 lb /acre), spring of crop year**
Treatment 5	**DAP (400 lb /acre), spring of prune year + two*foliar application of N in spring of crop year**
Treatment 6	**Two foliar application of N in spring of prune year**
Treatment 7	**Two *Foliar application of N in spring of crop year**
Treatment 8	**Foliar application of N in spring of prune year + two*Foliar application of N in spring of crop year**

*Due to severe winter injury, the crop-year foliar N treatments were not applied.

Composite leaf tissue samples were taken July 18, 2003 from each treatment plot. Soil samples were collected July 28, 2003. Stem samples from 4 randomly placed, 1/4 ft^2 quadrats were collected November 6, 2003 for measurement of stem density, stem length, stem branching and flower bud formation.

RESULTS:

Leaf Nutrient Concentrations

Leaf N concentrations were below the standard (1.6%) in control plots and were raised to sufficiency levels by DAP, but not by CoRoN ™ treatments (Fig. 1). Leaf P concentrations were also at less than sufficient levels in control plots and were raised only by DAP (Fig. 2). Leaf K levels were not meaningfully affected by treatments (Fig. 3). Often we see a lowering of some leaf nutrient concentrations in response to DAP through a dilution effect caused by increased growth and larger leaves stimulated by the N in the DAP. We apparently see this effect with leaf Ca and Mn concentrations (Figs. 4 and 5).

Soil Nutrient Concentrations

Soil P concentrations were raised by prune-year application of DAP, compared to the controls (Fig 6.). In low pH blueberry soils, some of the P from DAP is tied up as insoluble aluminum phosphate. In figure 7 we see this phenomenon, as soil Al is lowered by DAP.

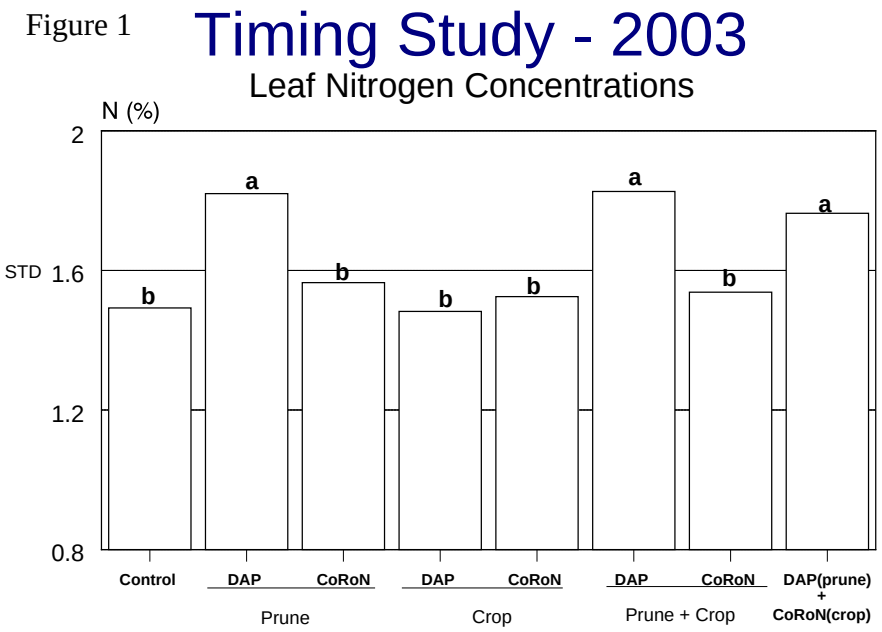
Stem Characteristics

Overall stem density was not affected by treatments of DAP or CoRoN ™ (Fig.8) but DAP resulted in more stem branching (Figs 9 and 10). This is also evident in figure 14, where the number of branches per stem is presented. The average stem length (height) of all stems indicates some growth response to DAP (Fig 11), but this is clearly a response of branched stems (Fig 12), rather than unbranched stems (Fig. 13). The DAP treatments stimulated more branching (Fig. 14), but had little effect on average branch length (Fig. 15). The potential yield is represented by the number of flower buds per stem. Averaged across branched and branched stems there is no effect of DAP or CoRoN ™ on flower bud formation. However, the average number of flower buds produced by branched stem was more than double than that on unbranched stems (Figs. 17 and 18). The flower bud density also suggests that DAP in increasing potential yield by its affect on branching. While there was no significant effect on total flower buds per unit area (Fig. 19), there is an indication that flower bud density of branched stems was increased compared to the controls (Figs 20 and 21). A positive correlation between stem length and flower bud formation has been reported. Perhaps the N contributed by DAP has become limiting at the time branches are developing, resulting in short branches.

Yield

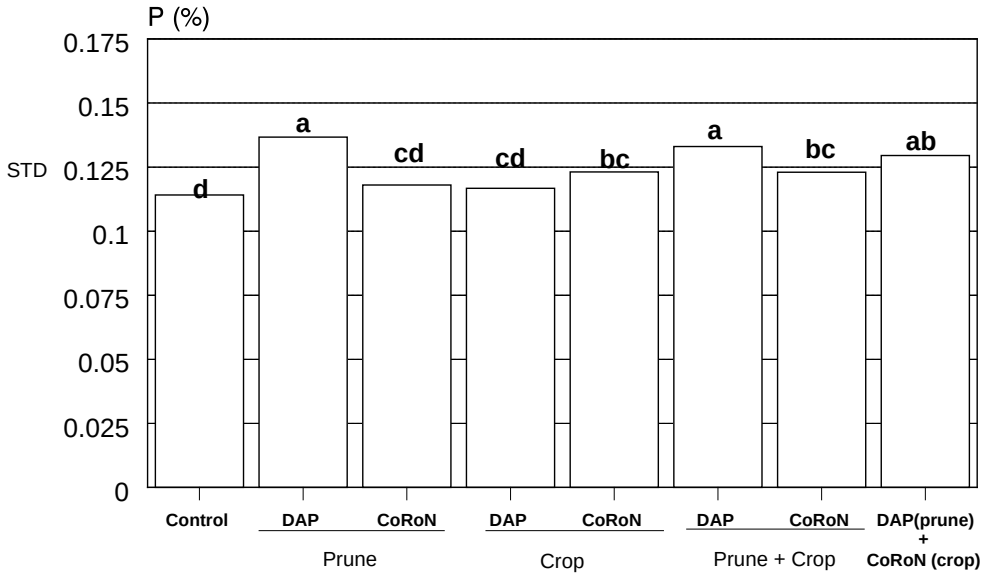
Due to winter injury in 2003/2004, yield data was compromised and is not shown.

Figure 1



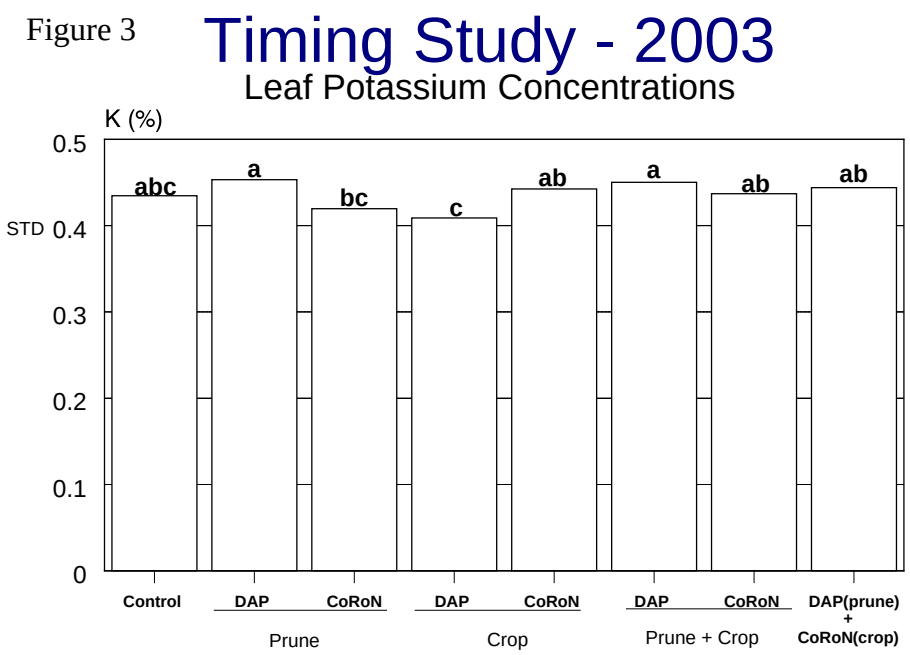
Soil-applied DAP 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 0.01% level.

Figure 2



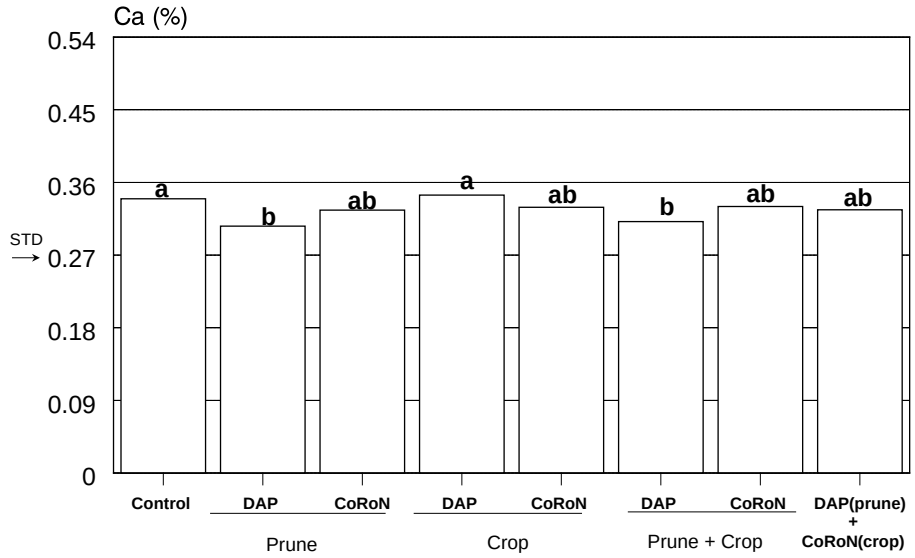
Soil-applied DAP 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 0.01% level.

Figure 3



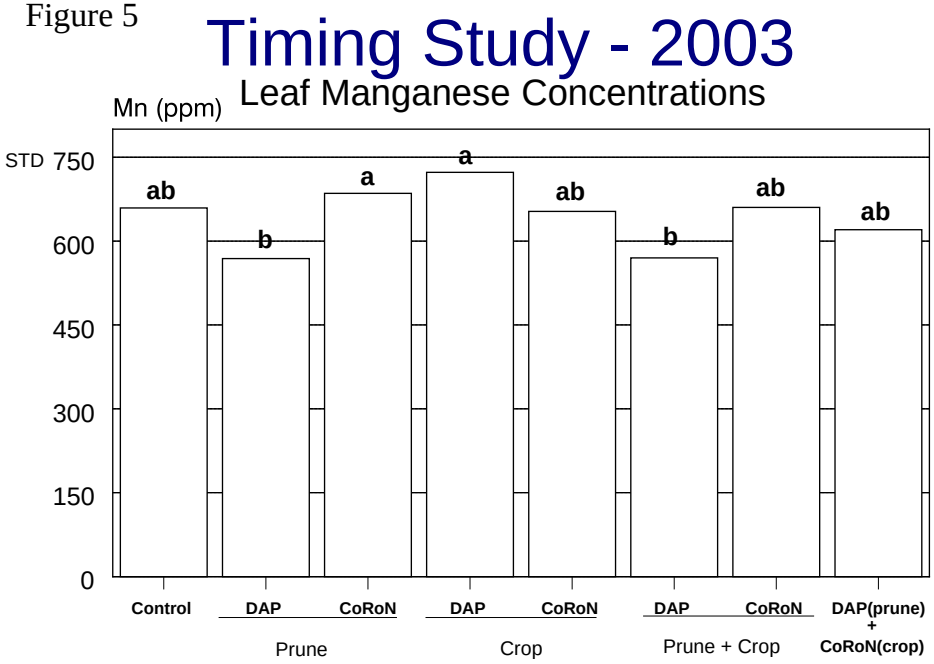
Soil-applied DAP 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 5% level.

Figure 4



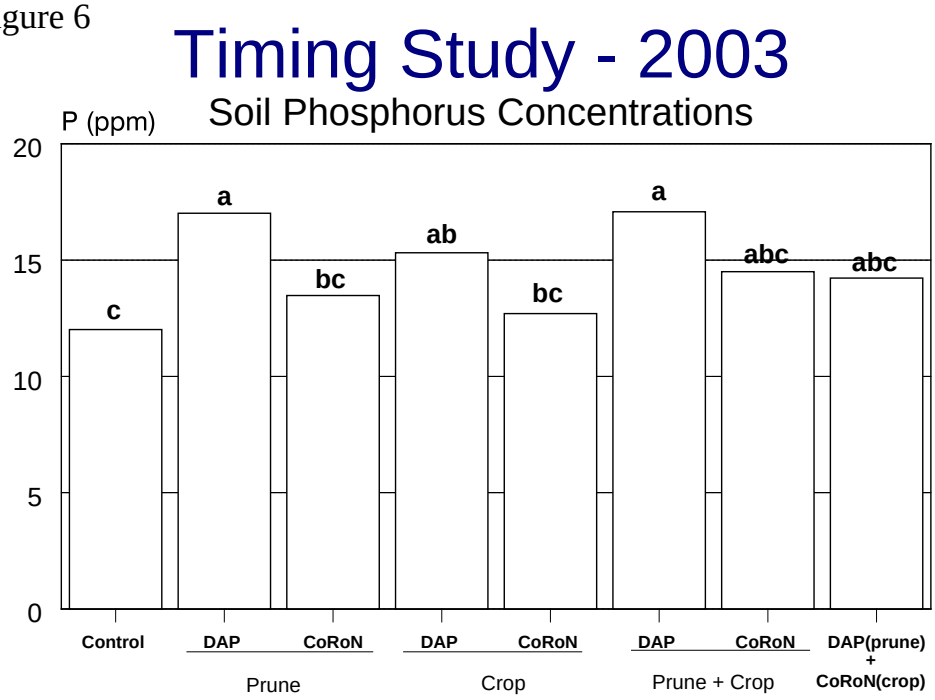
Soil-applied DAP 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Means Separation by Duncan's Multiple range test, 5% level.

Figure 5



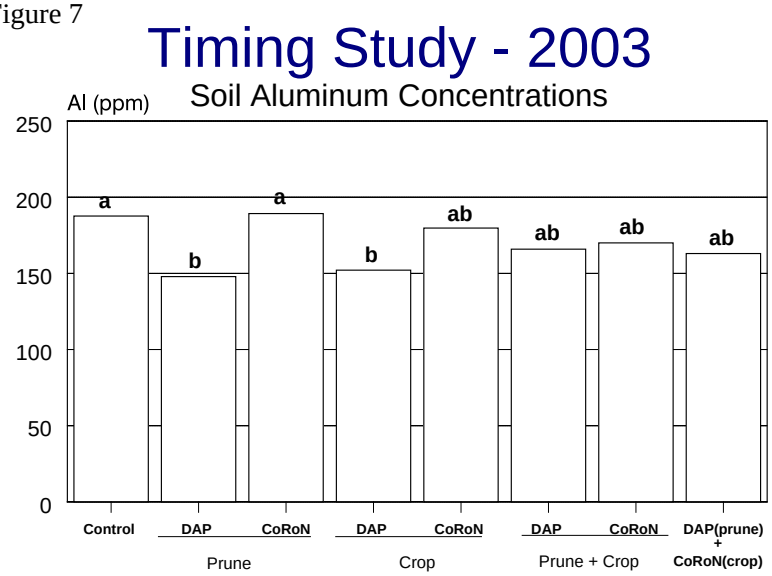
Soil-applied DAP 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 5% level.

Figure 6



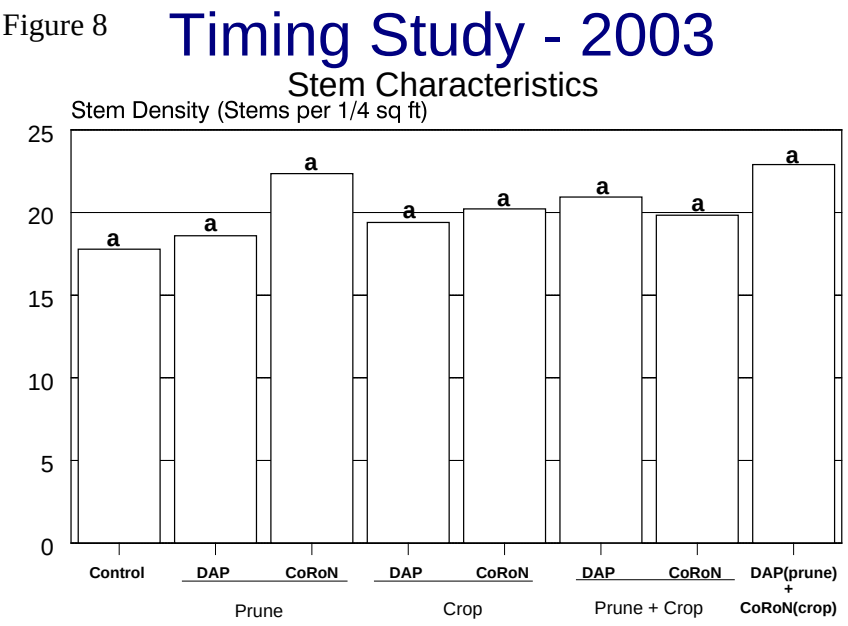
Soil-applied DAP 400 lb/acre. Foliar applied CoRoN at 2.97 lb N/acre in spring. Means Separation by Duncan's Multiple range test, 0.1 % level.

Figure 7



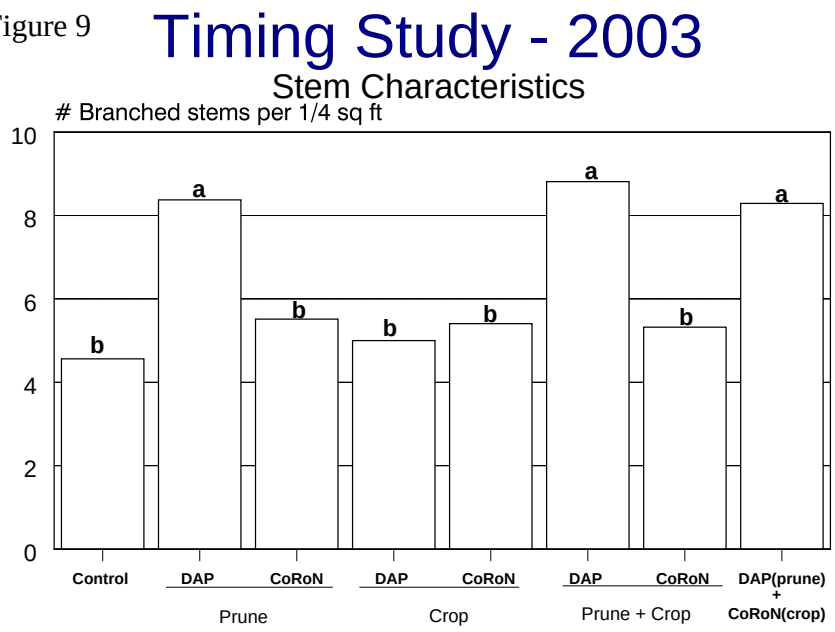
Soil-applied DAP at 400 lb/acre. Foliar applied CoRoN at 2.97 lb N/acre in spring. Means Separation by Duncan's Multiple range test, 5 % level.

Figure 8



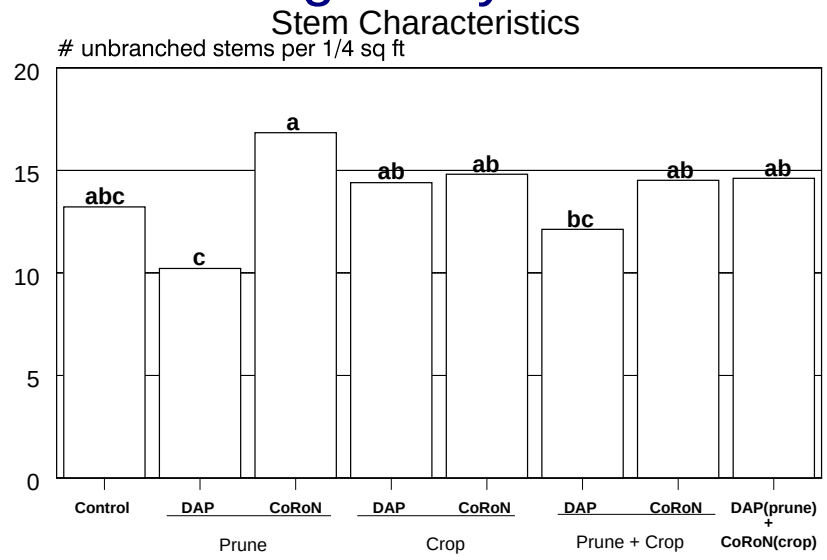
Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 5% level.

Figure 9



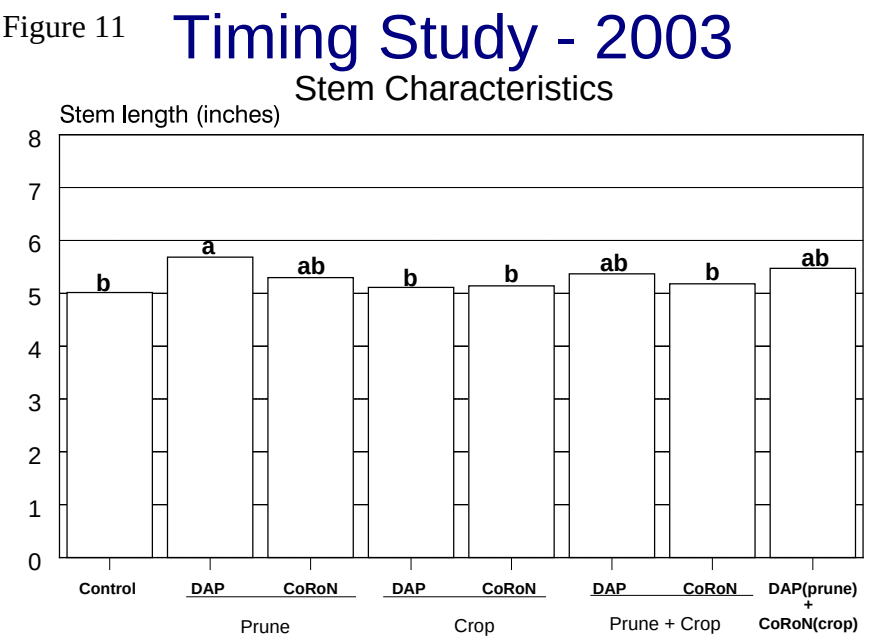
Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 0.01% level.

Figure 10



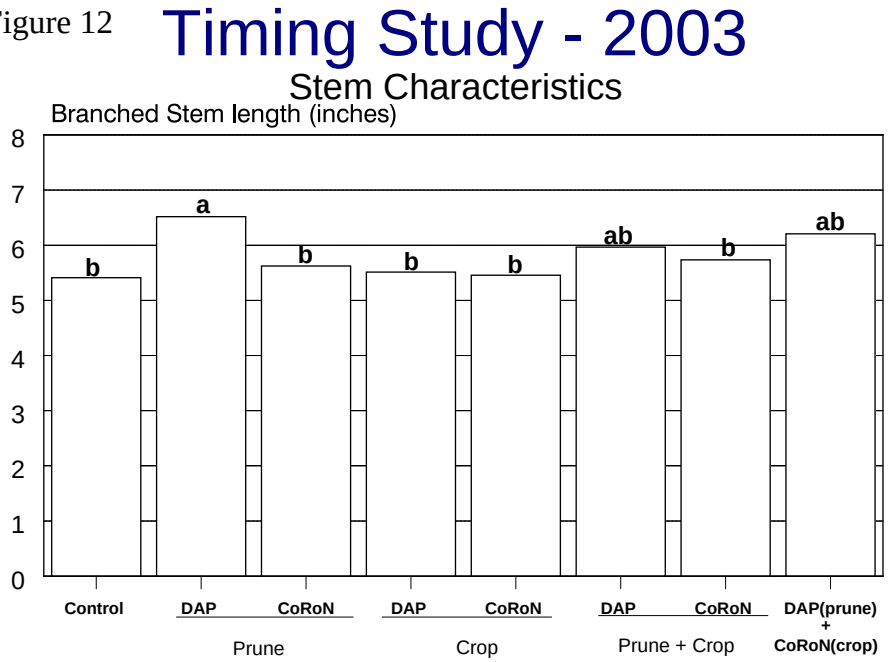
Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 0.1% level.

Figure 11



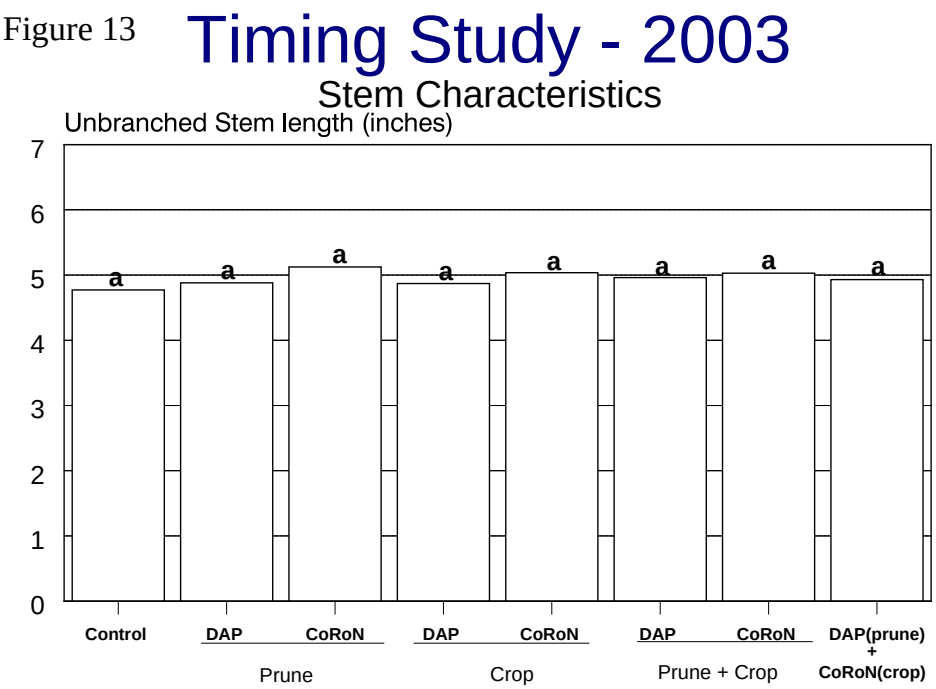
Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 5% level.

Figure 12



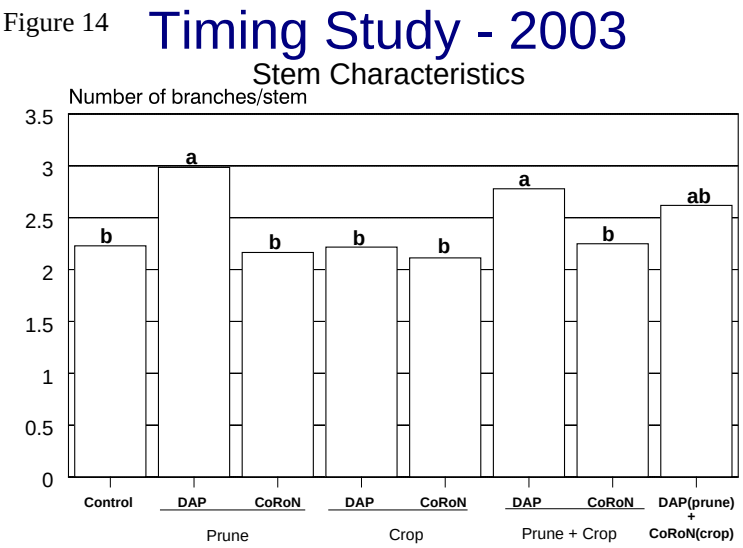
Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 0.1% level.

Figure 13



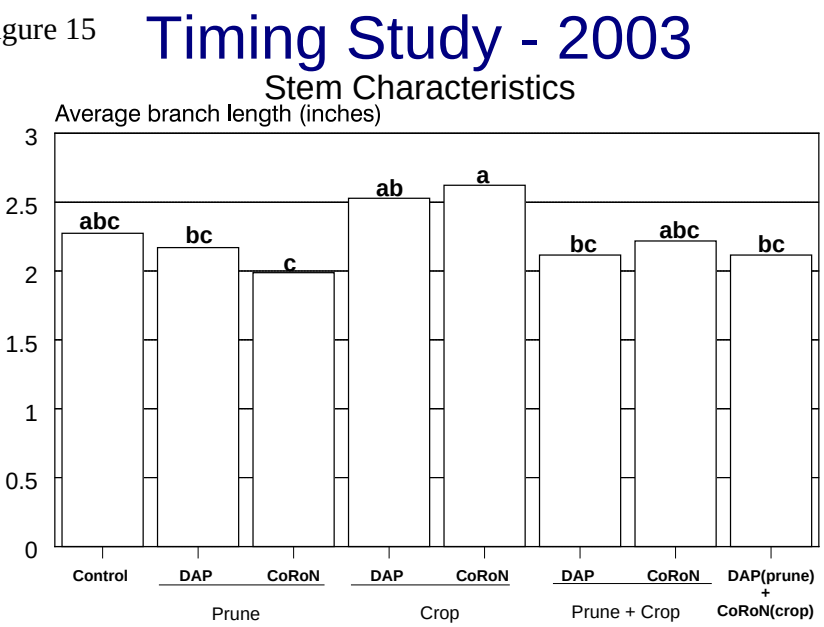
Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 5% level.

Figure 14



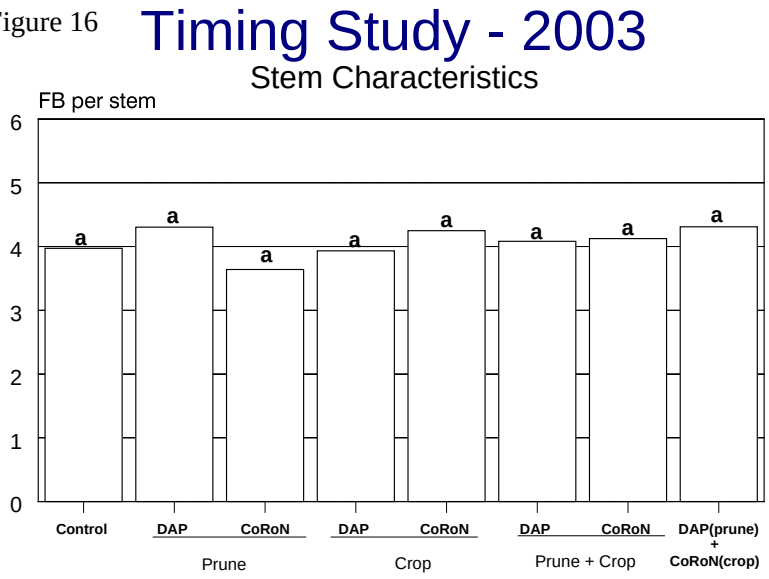
Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 0.1% level.

Figure 15



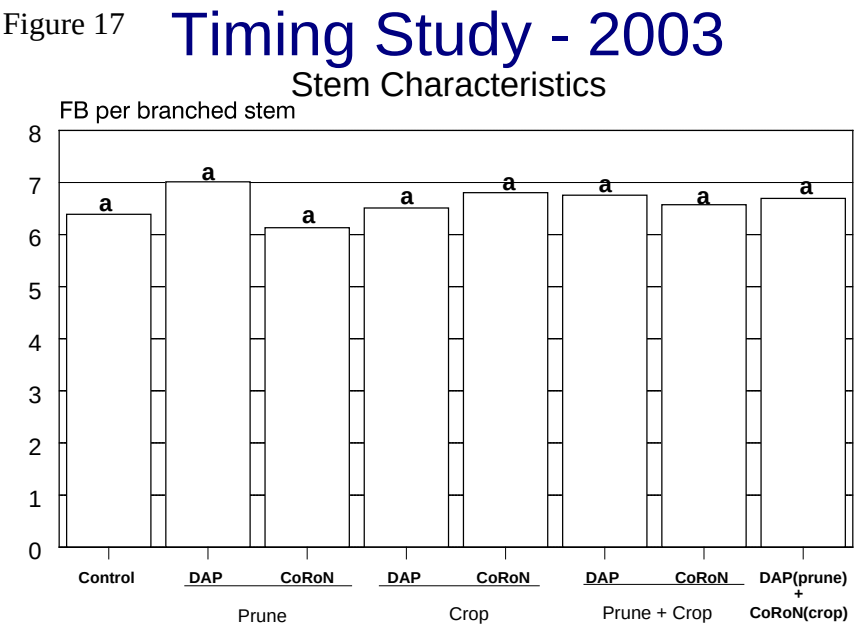
Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 5% level.

Figure 16



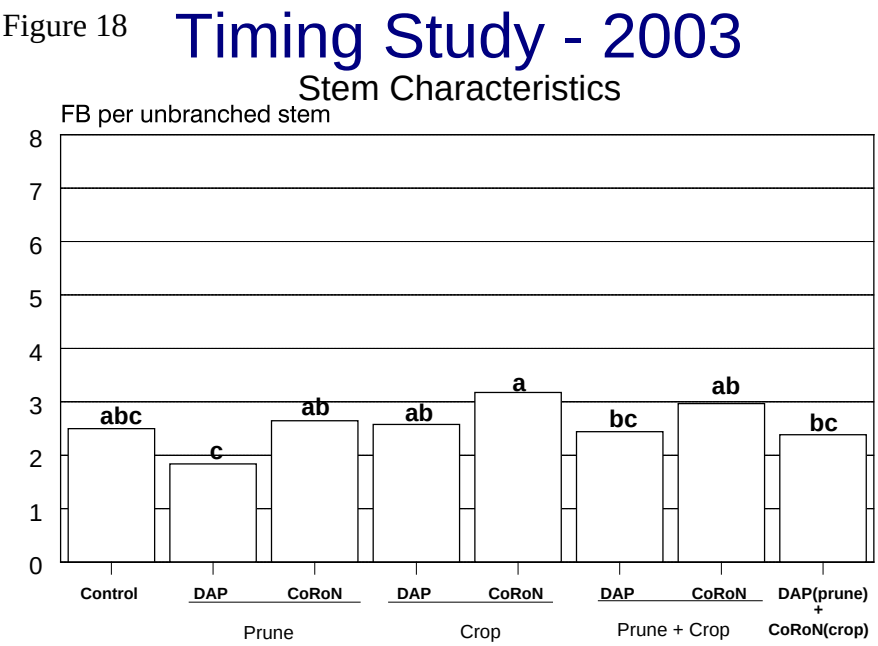
Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 5% level.

Figure 17



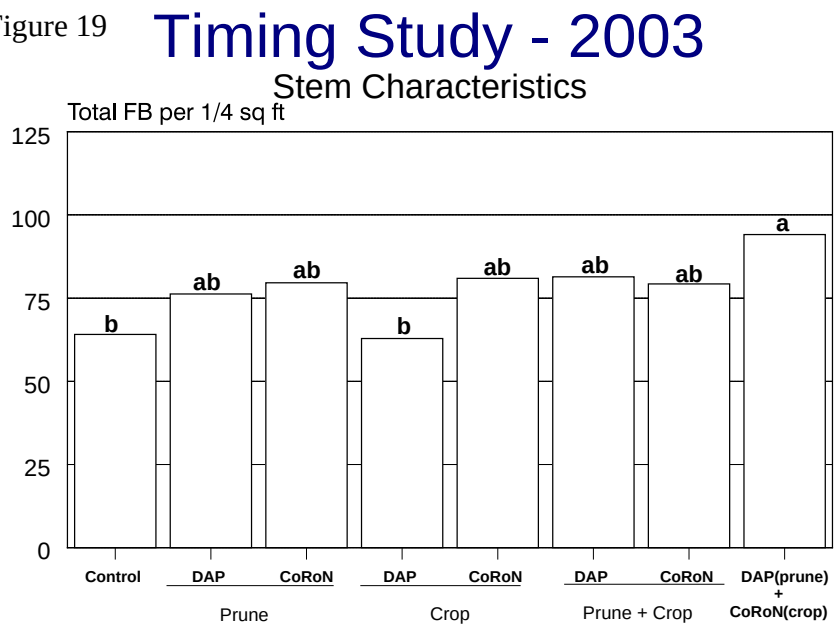
Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 5% level.

Figure 18



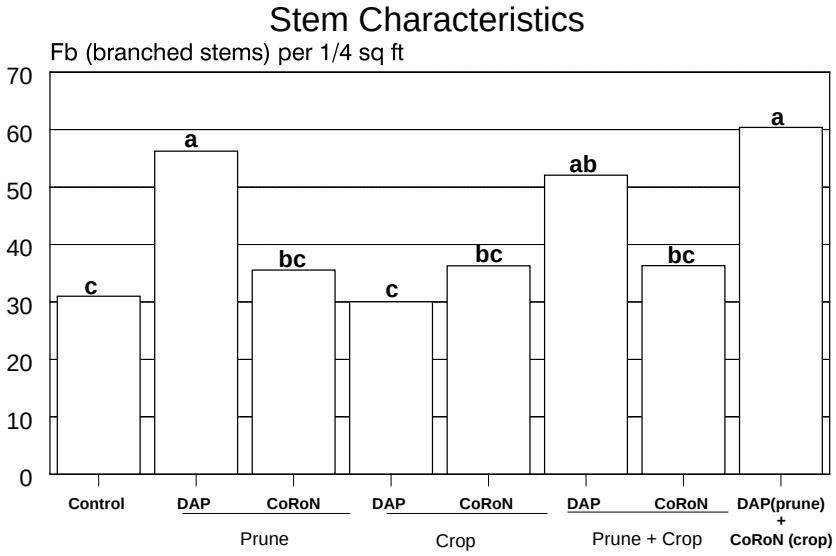
Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 1% level.

Figure 19



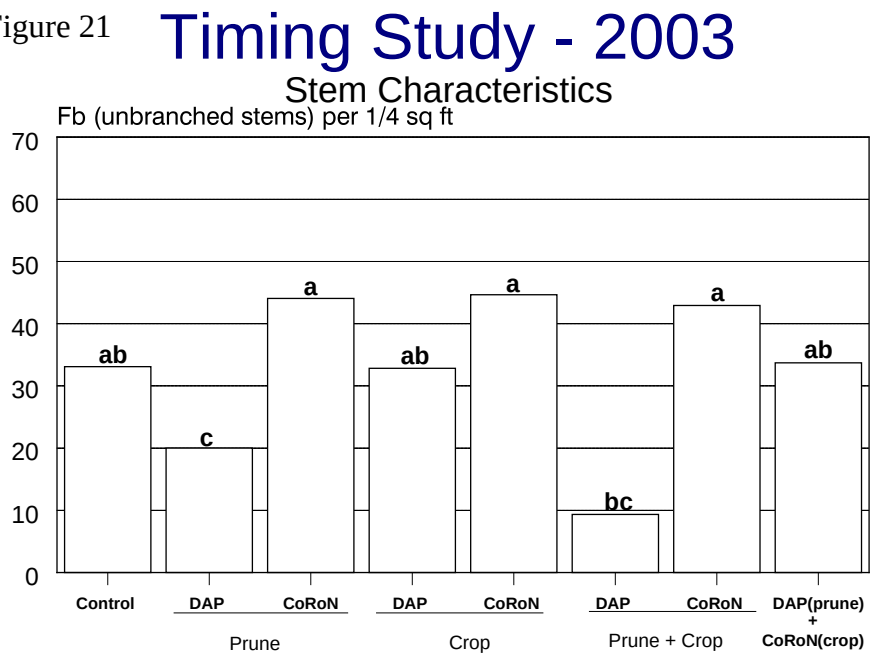
Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 5% level.

Figure 20



Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 0.1% level.

Figure 21



Soil-applied DAP at 400 lb/acre. CoRoN foliar applied twice at 2.97 lb N/acre. Mean Separation by Duncan's Multiple range test, 0.01% level.

CONCLUSIONS: Foliar application of N was not effective in raising leaf N concentrations at the rate and timing of applications. This method of applying N to raise leaf N concentrations needs further investigation in a separate study. This experiment should be repeated with more replication.

No conclusions can be made at this time regarding timing of fertilization.

RECOMMENDATIONS: No recommendations can be made at this time.

PLANT NUTRITION AND FERTILITY

INVESTIGATORS: John M. Smagula, Professor of Horticulture
Ilse W. Fastook, Scientific Technician

16. TTLE: Raising Foliar Nitrogen by Application of CoRoN™

OBJECTIVES: To determine an effective method of raising leaf Nitrogen concentrations that may increase growth and yield of wild blueberries.

METHODS: A commercial wild blueberry field that had a history of low leaf nitrogen (N) concentration was used in this study. CoRoN ™ (28% N) is a combination of polymethylene urea coupled with fast-release, low-biuret urea, designed to act as a slow-release foliar fertilizer. A Citrate-Phosphate buffer was used in treatments 2, 3, and 4 to control the pH during application. CoRoN ™ at 6lbsN/acre was applied to the entire 3 ft x 50 ft plots on June 12, 2004 of the prune year and to half the 3 ft x 50 ft plot on June 24, about two weeks later. Both Cu and Fe Keylate (Stoller Enterprises, Inc.) were added to CoRoN ™ treatments at 0.5 lbs/acre to see if they enhance N uptake. Ammonium Sulfate was added to the Cu/Fe solution at 0.7%, as suggested by the manufacturer. CoRoN ™ plus Cu and Fe without the Ammonium Sulfate was also tested. A control plot received no fertilization. These 9 treatments (Table 1) were replicated 6 times in a randomized complete block design.

Table 1 **Treatment Summary**	
Treatment 1	**Control**
Treatment 2	**CoRoN pH 5 using buffer**
Treatment 3	**CoRoN pH 6 using buffer**
Treatment 4	**CoRoN pH 7 using buffer**
Treatment 5	**CoRoN pH 8 using distilled H2O**
Treatment 6	**CoRoN pH 8, Cu, Fe, and Ammonium Sulfate (.7%)**
Treatment 7	**CoRoN pH 8 and Ammonium Sulfate (.7%)**
Treatment 8	**CoRoN pH 8, Cu, And Fe**
Treatment 9	**Ammonium Sulfate at 3lbs N/acre**

Composite leaf tissue samples were taken July 13, 2004. Soil samples were taken from control plots July 13, 2004. Stem samples were taken October 20 & 21, 2004 for growth and potential yield measurements. Yield will be taken in August 2005.

RESULTS:

2004 Leaf Tissue Concentrations

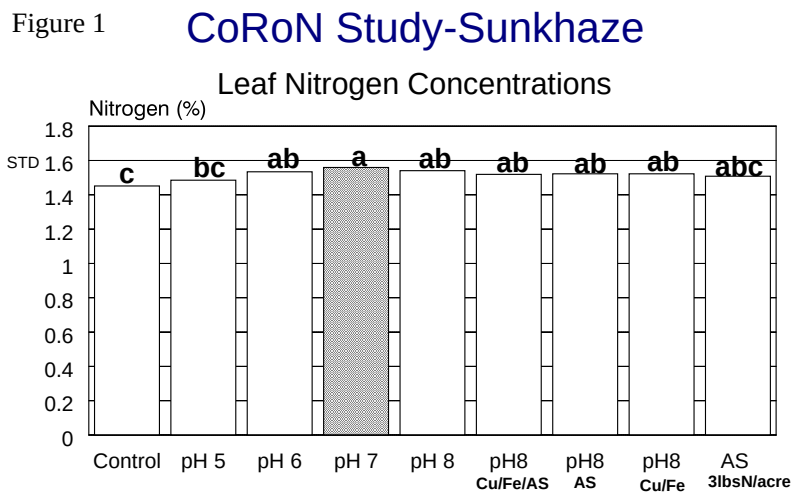
Treatments had no meaningful effect on P, K, Ca, Mg, Al, B, Mn, or Zn. Leaf N concentrations were raised by all CoRoN ™ treatments compared to the control, except that which was buffered at pH 5 (Fig. 1). Adding Cu/Fe, Cu/Fe plus ammonium sulfate, or ammonium sulfate did not improve the effectiveness of CoRoN ™ in raising leaf N concentrations. The leaf N concentrations in plots receiving ammonium sulfate at 3 lbs N/acre was not different from the controls. When the control and the four buffered sources were analyzed alone, there was a significant linear trend of increasing leaf N concentration with increasing pH (Fig. 2). The CoRoN ™ solution buffered at pH 7 appeared to be the best for raising leaf N concentration. Two applications of CoRoN ™ are slightly better than a single early application (Fig. 3). While adding Cu/Fe with or without ammonium sulfate did not enhance N penetration, the leaf Cu concentrations were raised to above the 7 ppm standard concentration (Fig. 4). Leaf Cu concentrations were also raised higher with two, compared to one application (Fig. 5). Leaf Fe concentrations were also raised by the CoRoN ™ and Cu/Fe solutions with or without the ammonium sulfate (Fig. 6). Two sprays were more effective than one (Fig. 7). The addition of ammonium sulfate to these solutions increased both the leaf Cu and leaf Fe concentrations.

2004 Soil Data- Soil pH of samples taken from controls on July 13, 2004 ranged from 4.3 to 4.5.

CONCLUSIONS: CoRoN ™ was effective in raising leaf N concentrations compared to the control; but the concentration was not raised to above the sufficiency level. Adding Cu/Fe with or without ammonium sulfate did not improve the penetration of N from CoRoN ™. Lowering the CoRoN ™ solution pH did not improve efficacy. Two applications appear to be better than a single early application.

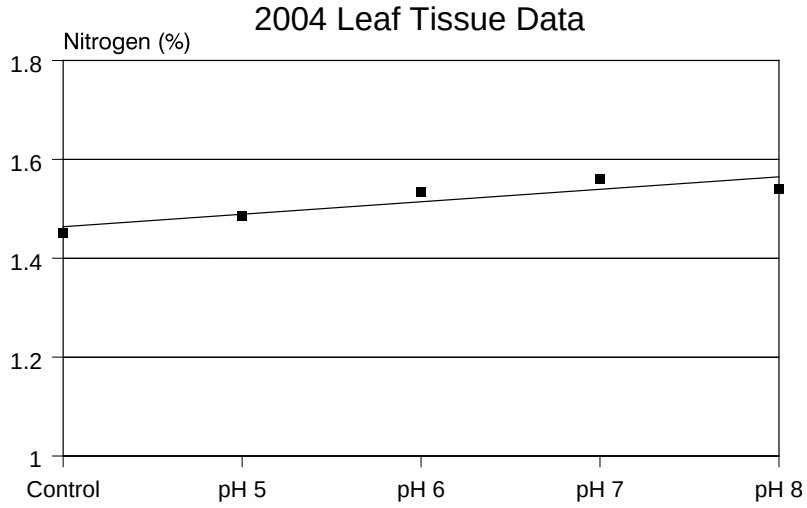
RECOMMENDATIONS: More work needs to be done on the most efficient rate of CoRoN ™ and the best time to apply CoRoN ™. Multiple applications should be further explored.

Figure 1



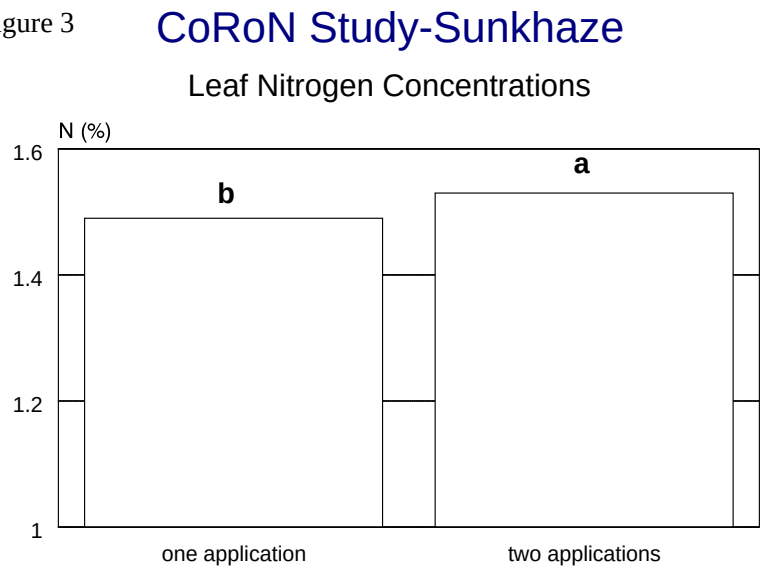
CoRoN applied at a rate of 6lbsN/acre,Copper and Iron each applied at 0.5 lbs/acre, Ammonium sulfate with Cu/Fe at 0.7% and alone at 3lbs N/acre. Mean Separation by Duncan's Multiple range test, 5% level.

Figure 2



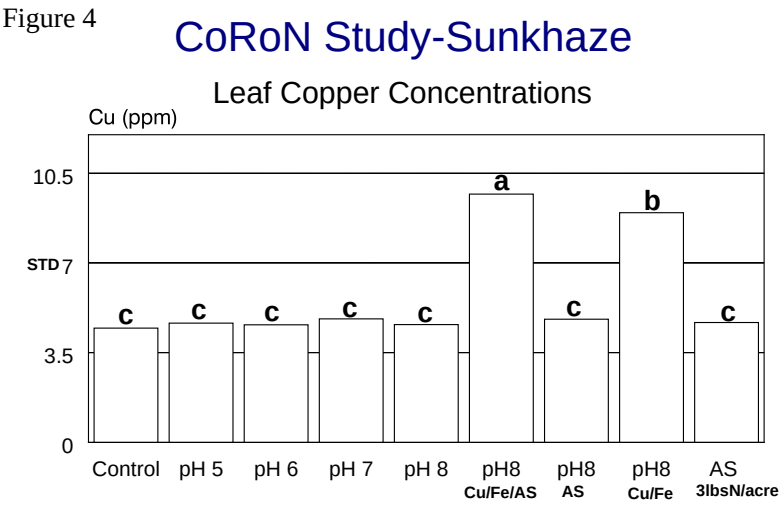
CoRoN applied at a rate of 6lbsN/acre.

Figure 3



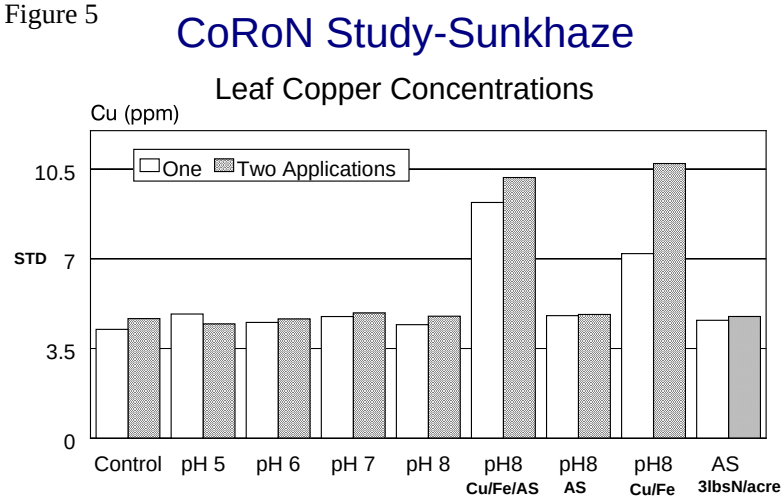
Means are average N concentrations across all treatments. Mean Separation by Duncan's Multiple range test, 7% level.

Figure 4



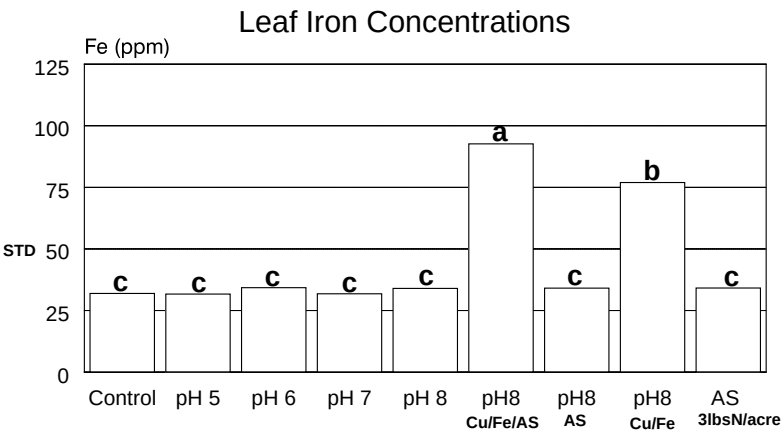
CoRoN applied at a rate of 6lbsN/acre,Copper and Iron each applied at 0.5 lbs/acre, Ammonium sulfatewith Cu/Fe at 0.7% and alone at 3lbs N/acre. Mean Separation by Duncan's Multiple range test, 0.01% level.

Figure 5



CoRoN applied at a rate of 6lbsN/acre,Copper and Iron each applied at 0.5 lbs/acre, Ammonium sulfate with Cu/Fe at 0.7% and alone at 3lbs N/acre. Mean Separation by Duncan's Multiple range test, 0.01% level.

Figure 6



CoRoN applied at a rate of 6lbsN/acre,Copper and Iron each applied at 0.5 lbs/acre, Ammonium sulfate with Cu/Fe at 0.7% and alone at 3lbs N/acre. Mean Separation by Duncan's Multiple range test, 0.01% level.

PLANT NUTRITION AND FERTILITYI
NVESTIGATORS: John M. Smagula, Professor of Horticulture
Ilse W. Fastook, Scientific Technician
Qian Wang, Graduate Student

17. 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.

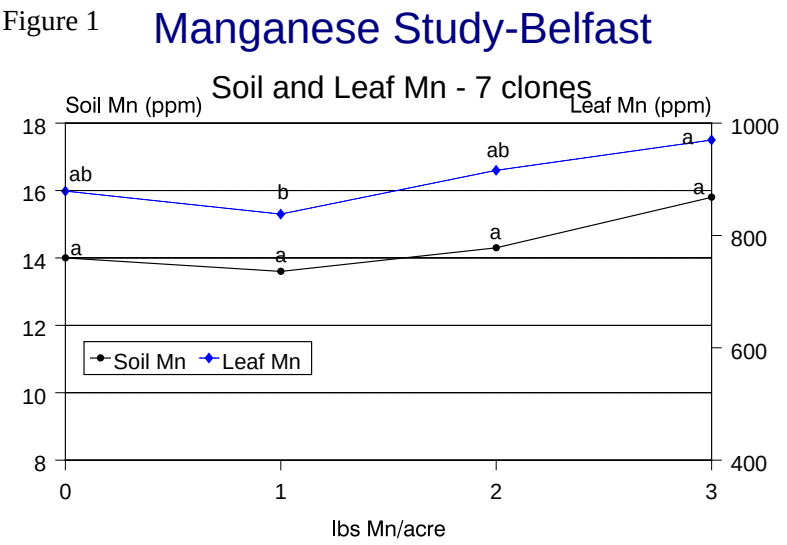
METHODOLOGY: A commercial wild blueberry field that had an average leaf tissue Mn concentration of < 750 ppm in a 2001 field sample was used for this study. Eight discrete clones were selected but one was eliminated from the study when it was found to be a mixture of two clones. Four treatments were 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 pre-emergent 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 will be determined in August 2005 by hand raking each plot. Samples of berries will 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 was major difference in the leaf Mn concentration among clones (Fig 2), ranging from 588 to 1258 ppm. 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 that had the lowest Mn concentrations had the highest N, P, and K concentrations.

CONCLUSIONS: No conclusions can be made at this time.

RECOMMENDATIONS: No recommendations can be made at this time.

Figure 1



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

Figure 2

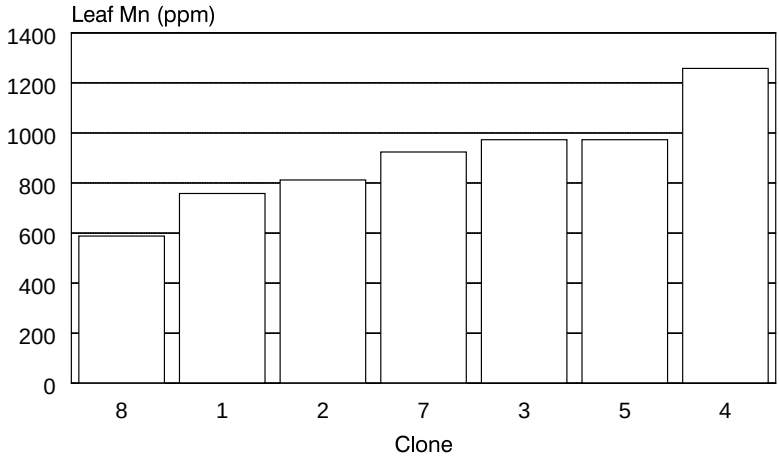


Figure 3

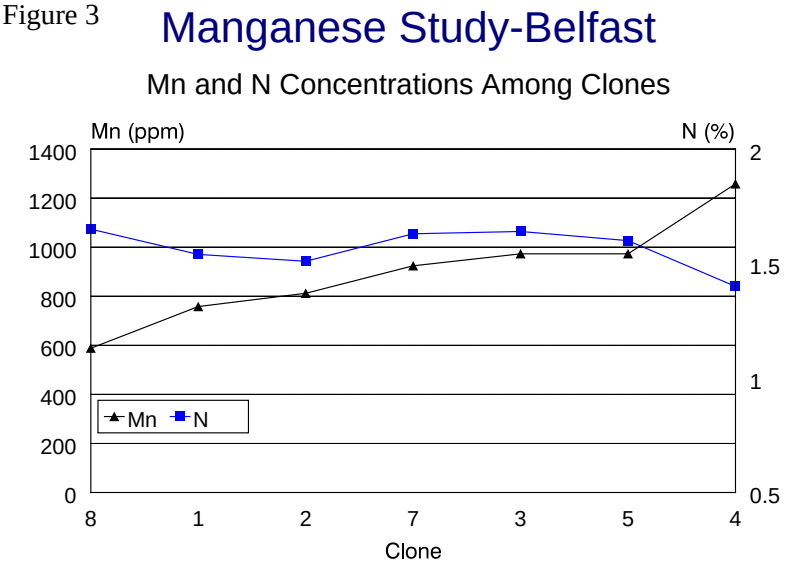


Figure 4

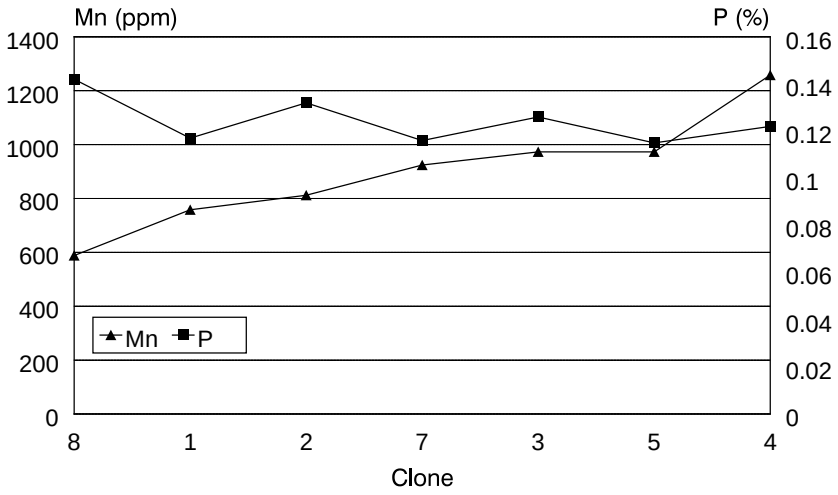
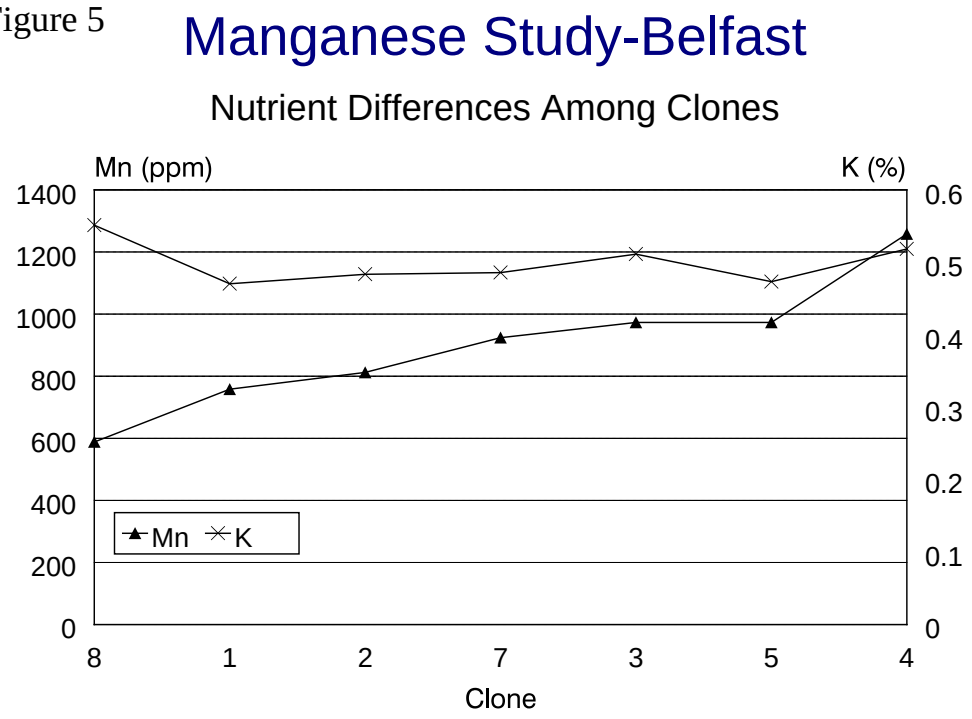


Figure 5


Manganese Study-Belfast
Nutrient Differences Among Clones
Mn (ppm)
K (%)
1400
1200
1000
800
600
400
200
0
0.6
0.5
0.4
0.3
0.2
0.1
0
Mn
K
8
1
2
7
3
5
4
Clone

WEED MANAGEMENT AND FIELD COVER

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

18. TITLE: Assessment of Hexazinone Alternatives for Weed Control in Wild Blueberries and Field Cover Program Base.

METHODOLOGY: The trial was conducted at Blueberry Hill Farm Research Station in Jonesboro, Maine. The experimental design was a randomized, complete block design with six replicates. Treatments consisted of an untreated check and herbicides including hexazinone as the standard at 1 lb/a, flumioxazin at 12 oz/a, and mesotrione at 2, 3 and 6 oz/a. Both the flumioxazin and mesotrione rates were applied pre and post-emergence. Treatments were applied using a hand held, CO_2 propelled boom sprayer. Hexazinone and flumioxazin were applied pre-emergence on 10 May; mesotrione was applied pre-emergence on 13 May 2004. A post-emergence application of flumioxazin and mesotrione was applied on 9 June 2004. Evaluation of blueberry cover, herbaceous weeds, grasses and ferns were made using a 1-6 Daubenmire cover class scale on 23 June and 18 August 2004. Data were transformed to percent cover and analyzed by the General Linear Model of SAS with significant means separated by a Duncan's multiple range test.

RESULTS: No significant reductions in cover or phytotoxicity of wild blueberries were noted for any of the treatments (Figure 1). Broadleaf cover averaged less than 20% (Figure 2) and some treatments were less than the untreated or hexazinone standard, several were greater, but none were statistically significant. Grass cover (Figure 3) appeared to be released, with both the post flumioxazin and mesotrione treatments showing an increase in cover. Fern cover also increased with the highest rate of both flumioxazin and mesotrione treatments but the effect was not significant (Figure 4). In all cases the hexazinone standard and check plot were not significantly different.

CONCLUSIONS: Broadleaf cover appeared to be controlled more at the highest pre-emergence mesotrione rate and for all mesotrione post-emergence timing. Whereas the grass cover was highest at the lowest rate of mesotrione in pre-emergence and the two highest rates of mesotrione in post-emergence application. For broadleaf, grass, and ferns, pre-emergence application of Flumioxazin resulted in lower weed cover than the post-emergence application, though not significantly. The variability in outcomes may be result of several factors. The test site was selected after the ground had been bi-annually burned and weed cover was unknown. After emergence, it became clear, from evaluating block differences and control plots, that weed cover was not consistent over the entire research plot. Further evidence of the unusual test site cover was indicated through inconsistencies in the effectiveness of hexazinone, which has proven to be an effective herbicide. This was most likely because of the random spread of seeds unintentionally mixed in with the straw fuel used to burn the plots, and the unusually cold and cloudy weather during the spring.

RECOMMENDATIONS: To determine if either flumioxazin or mesotrione can successfully be used as alternatives to hexazinone, further research is planned for the summer of 2005 on six different field sites with different soil types and weed populations located on wild blueberry fields in Maine.

Figure 1. Blueberry Cover after Pre and Post-Emergence Herbicide Application

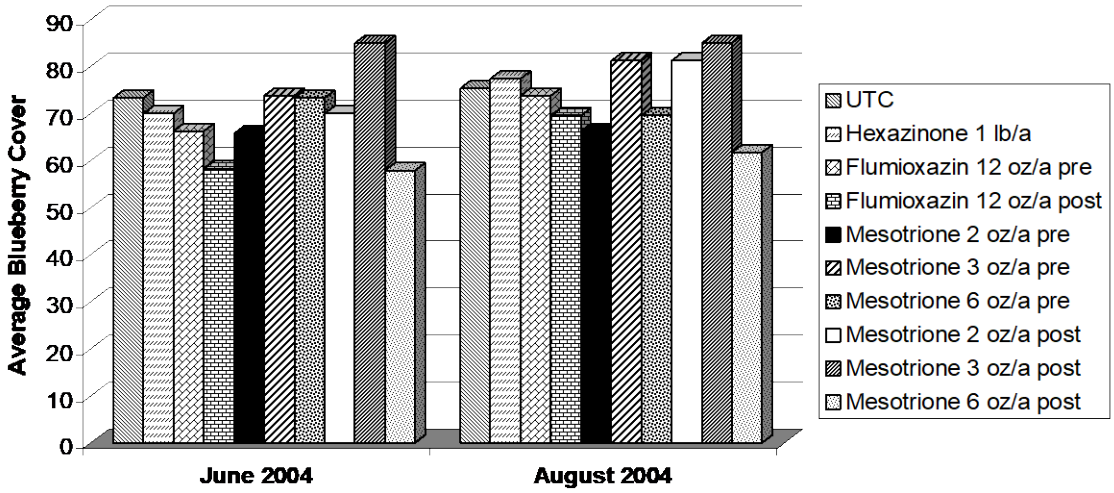


Figure 2. Broadleaf Weed Cover after Pre and Post-Emergence Herbicide Application

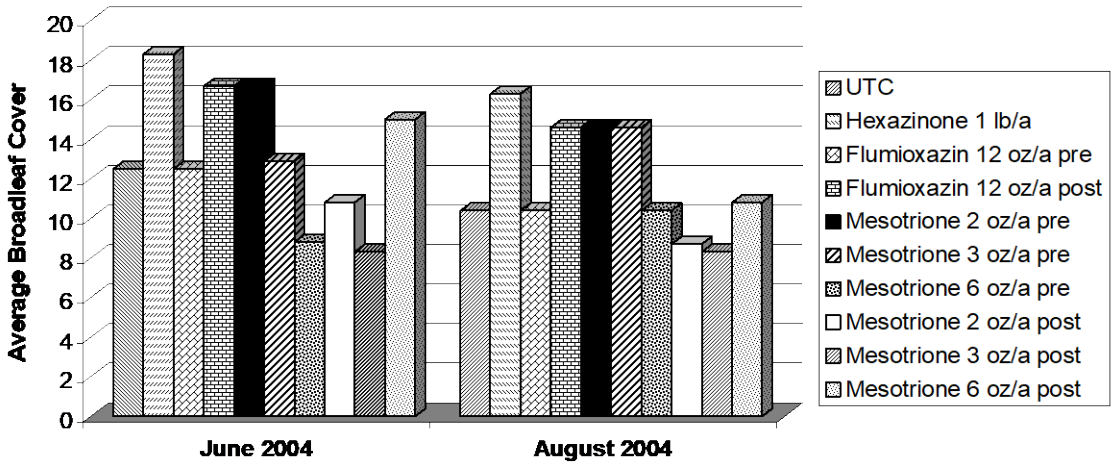


Figure 3. Grass Cover after Pre and Post-Emergence Herbicide Application

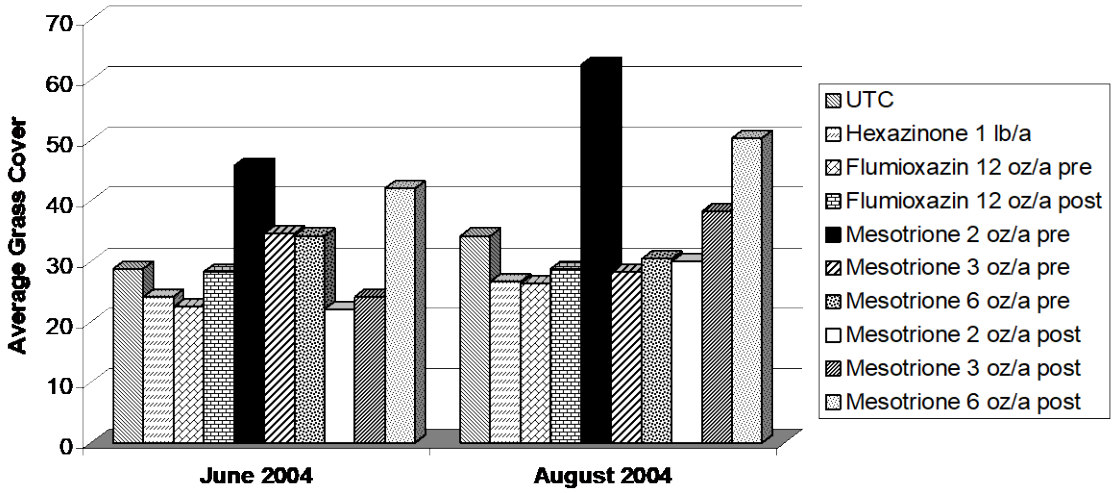
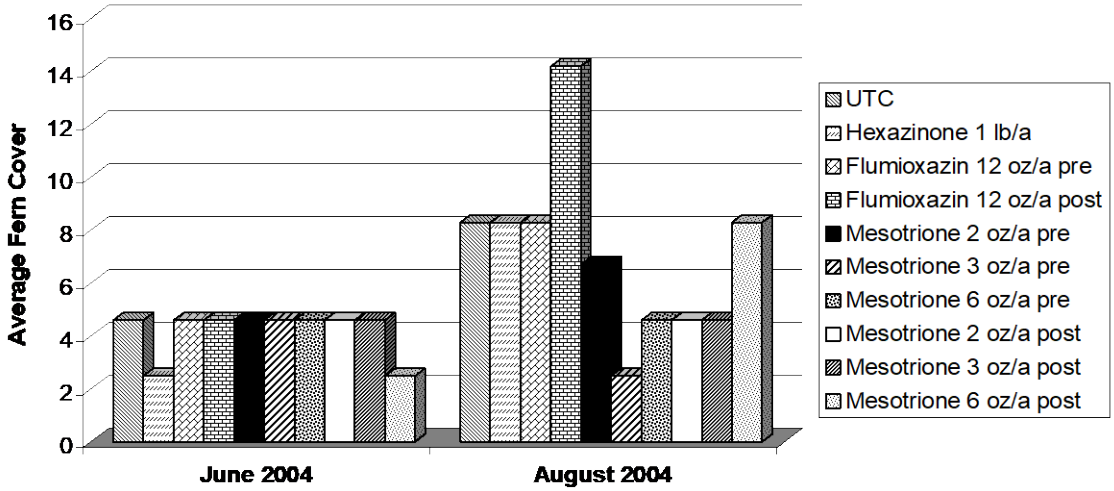


Figure 4. Fern Cover after Pre and Post-Emergence Herbicide Application



WEED MANAGEMENT AND FIELD COVER

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

19. TITLE: Evaluation of Fall Applications of Sulfonylurea Herbicides for Bunchberry Control in Wild Blueberries.

METHODOLOGY: The effectiveness of five herbicides applied at Blueberry Hill Experiment Farm in the 2003 trial was evaluated for effectiveness. Plots were 1 meter squared and each treatment was replicated 8 times. Treatments included an untreated control, tribenuron methyl at 1oz/a, and 2 oz/a, prosulfuron at 1 oz/a and 2 oz/a, rinsulfuron at 2 oz/a and 4 oz/a, triasulfuron at 1 oz/a and 2 oz/a, and halosulfuron at 1 oz/a and 2 oz/a. Treatments were applied on a cropping field 29 September 2003 and were burned in late October 2003. Plots were evaluated for blueberry and bunchberry cover on17 August 2004.

In 2004, tribenuron methyl was applied to plots on a non-cropping field at Blueberry Hill Experiment Farm to further assess its effectiveness and evaluate different timings. Plots were 1 meter squared and each treatment was replicated 10 times. Treatments included an untreated control, and two rates of tribenuron methyl at 16.2 g/a and 32.4 g/a applied at three different dates, 30, August 2004, 13 September 2004 and 6 October 2004.

RESULTS: There were no significant differences among the 2003 treatments for blueberry cover (Figure 1). Though it does appear that almost all of the treatments resulted in lower bunchberry levels, there were no significant differences among the treatments (Figure 2). It appears that tribenuron methyl at 1oz/a had the lowest bunchberry cover while rinsulfuron at 4 oz/a had the highest level.

CONCLUSIONS: Except for tribenuron methyl, there appeared to be differences in bunchberry cover depending on rate, but not significantly. Tribenuron methyl appears to have the highest potential for controlling bunchberries, as has also been shown in Canada. Evaluation and analysis of treatments applied this fall will further our understanding of the success of tribenuron methyl.

RECOMMENDATIONS: Dependent on evaluation of 2004 application of tribenuron methyl, if it looks promising we will continue to evaluate with different fall timings of application.

Figure 1. Blueberry Cover after Fall Application of Sulfonyl Urea Herbicides

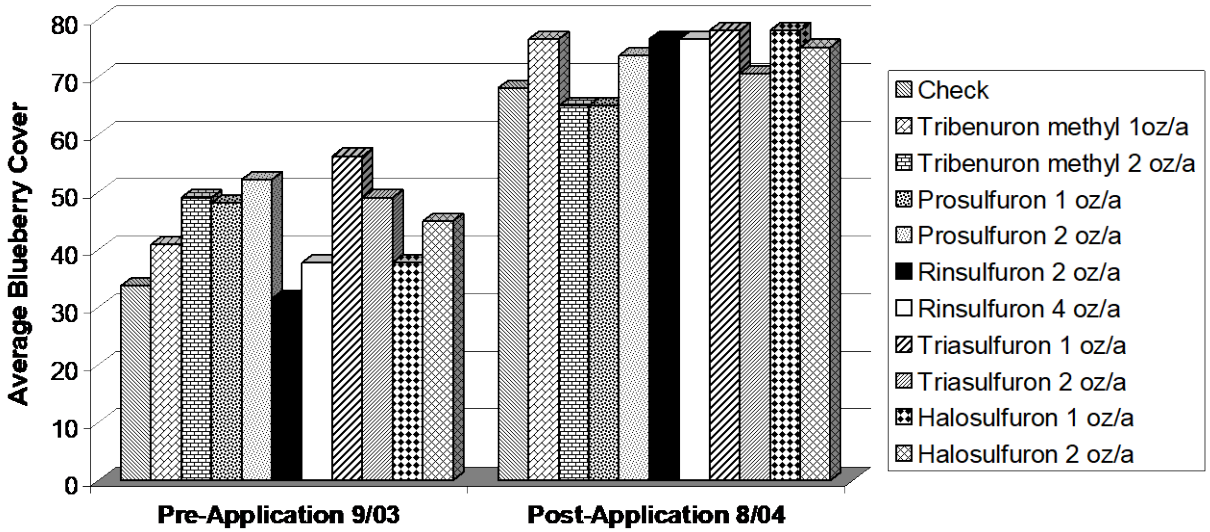
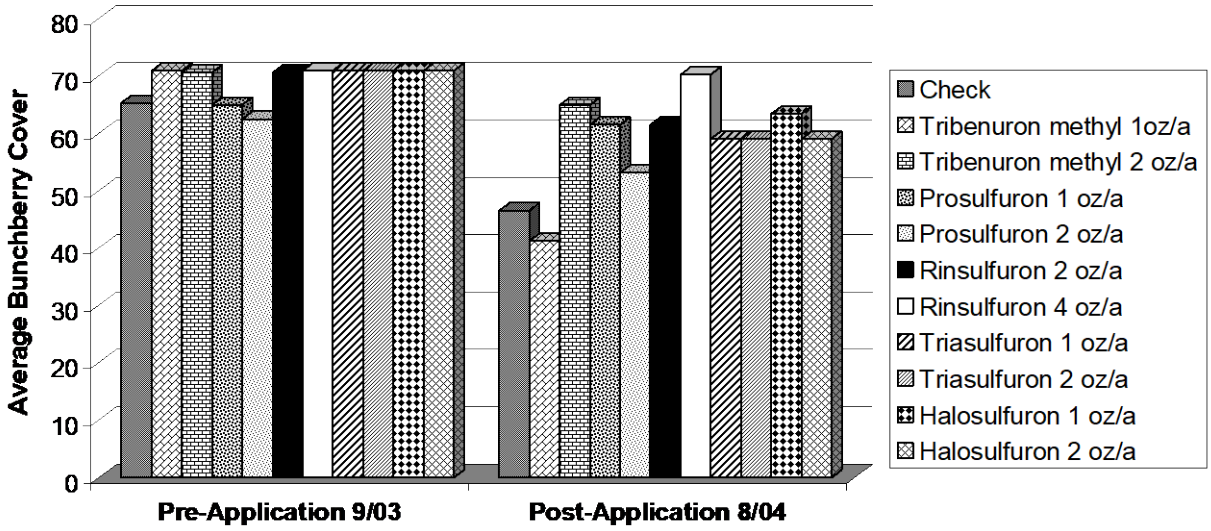


Figure 2. Bunchberry Cover after Fall Application of Sulfonyl Urea Herbicides



WEED MANAGEMENT AND FIELD COVER
INVESTIGATOR: David E. Yarborough, Professor of Horticulture

20. TITLE: Evaluation and Demonstration of Techniques for Filling in Bare Spots in Wild Blueberry Fields.

METHODS: Tissue culture wild blueberry plants were planted at a 1 foot spacing and mulched with three inches of bark. In 2000, in Aroostook County, one 40' x 40' plot was planted in an old potato field in Caribou and 2 lb/a Velpar and 1000 lb/a sulfur was added because the pH was 5.5. Another Aroostook site was established in Hamlin, in a field owned by Rene LeVasseur that had wild blueberry plants coming in naturally and so provided a good demonstration site. Soil analysis of the Hamlin site showed a pH of 4.7 and a sandy loam texture, both of which are suitable for blueberry growth. A 40' x 120' area in the field was mowed, Velpar applied at 2 lb/a and bark mulch spread at a depth of 3" in an 80' x 40' area. Blueberry plants were put in at 1' spacing over a 40' x 40' area. This site will serve as a demonstration on the feasibility of growing blueberry plants in Aroostook. For comparison purposes, plants were inter-planted in bare spots among the established clones at Blueberry Hill Farm, and at Guptill Farm by their wild blueberry freezer building in Wesley. In Wesley a 30'x30' plot with plants at a 1'x1' spacing was established by the freezer. In 2002 the Hamlin and Jonesboro locations were treated with 1 lb/a Velpar and the Wesley location received 10 lb/a Pronone. In 2003 the Hamlin location was treated with 1 lb/a Velpar and the Wesley location was weeded by hand in the 1'x1' area of the blueberry plant.

Wild blueberry plant survival and spread from 10, 1-foot square subplots in each area were measured using cover scale ratings taken in the summer of 2001, 2002, 2003 and 2004. The rating represents the mean cover plants spread in a one-foot square plot.

RESULTS:

Jonesboro was the only site that increased in cover in 2004 (Figure 1). The Pronone application from 2002 continued to kill blueberries in Wesley, the wet season increased weeds in Hamlin and Wesley but greatly but greatly increased blueberry spread in Jonesboro. Mortality in Wesley was 50%, but was only 10% in Hamlin and 20% in Jonesboro (Figures 2 & 3). Jonesboro and Hamlin had good growth on plants but there was less spread in Hamlin due to excessive weed pressure (Figure 4).

CONCLUSION: Effective weed control at Hamlin and Wesley are needed to continue the increases in blueberry cover. Although the Jonesboro site had a slow start it has the least mortality and best spread in year four.

RECOMMENDATIONS: Continue with the project for one more year, maintaining weed control for next year, and continue the evaluation of cover. I use these sites to demonstrate feasibility of inter-planting tissue culture wild blueberry plants.

Figure 1. Spread of plants planted in 2000 at three sites.

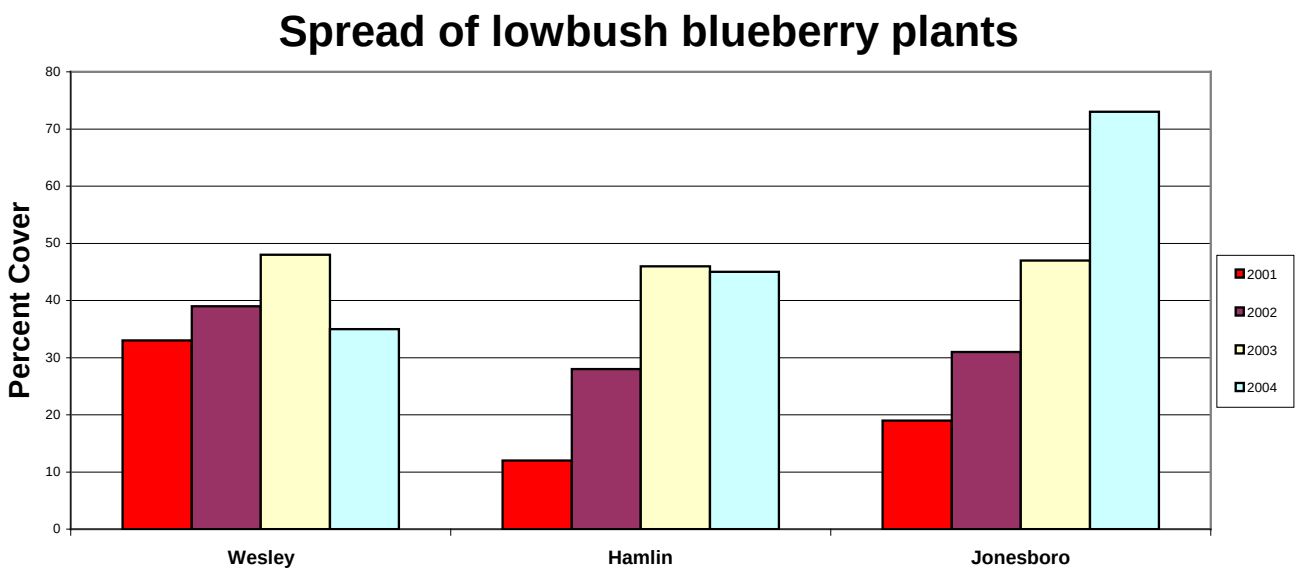


Figure 2. Rainfall produced excessive weed growth on heavier soils in Wesley and in Hamlin.

Figure 3. Wesley had good growth on surviving plants but high mortality at 50% from application of Pronone herbicide.

Figure 4. Jonesboro and Hamlin plant spread.

WEED MANAGEMENT AND FIELD COVER
INVESTIGATOR: David E. Yarborough, Professor of Horticulture
Kerry F. Lough, Research Assistant

21. TITLE: Assessment of Evitol for Sedge Control in Wild Blueberries

METHODOLOGY: A completely randomized block design was used to assess the effectiveness of Evitol and Kerb in grass and sedge control. Treatments included an untreated control, Evitol 80lb/a, and Kerb 4 lb/a. Plot sized varied between Evitol and Kerb treatments because a Gandy spreader was used to apply Evitol in plots 3.5' x 30' and Kerb was applied using a CO_2 propelled boom sprayer on 6' x 30' plots. The Evitol was applied on 1 November 2004 and the Kerb treatment was applied 4 November 2004. Treatments were not applied in the Spring 2004 as originally intended because of concerns of blueberry damage.

RESULTS: Treatment plots will be evaluated Spring 2005

CONCLUSIONS: None yet.

RECOMMENDATIONS: Continue to evaluate treatments in spring of 2005.

EXTENSION
INVESTIGATOR: David E. Yarborough, Extension Blueberry Specialist
Kerry F. Lough, Research Assistant

22. TITLE: Cultural Weed Management Using pH.

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 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 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®.

Sites established in 2000 were treated with 0, 0.5, 1 or 2 lbs Velpar® or Sinbar®. These sites were evaluated in August for weed cover density. Soil samples were taken in each sulfur plot to determine the extent of pH change. The Whiting site was discontinued in 2002.

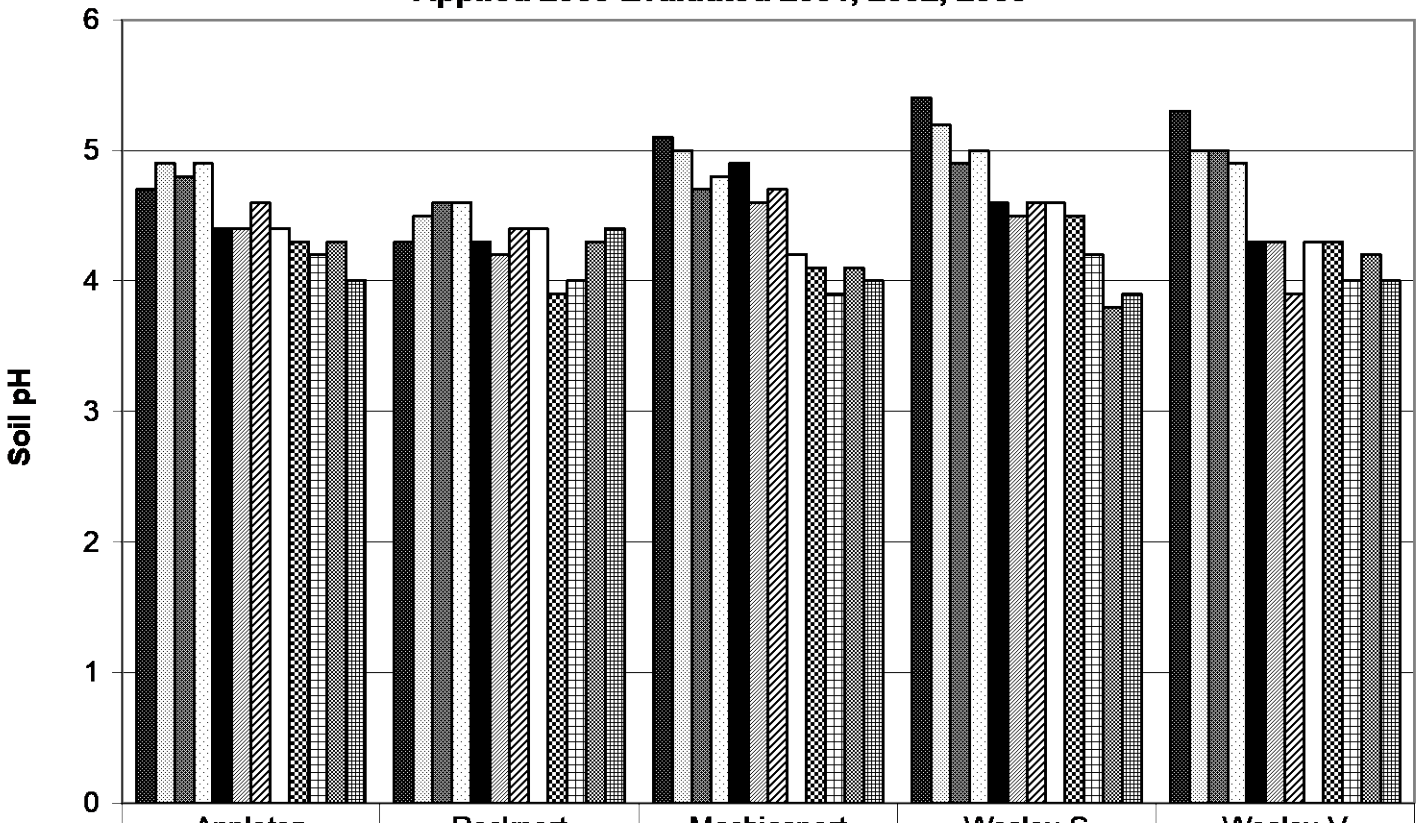
RESULTS: Soil pH reduction varied by site and year treated with some showing more or less than the 0.5 pH reduction with 500 or 1000lb/a sulfur application. There were small increases in pH at several sites (no more than 0.4) in 2004. For plots treated in 2000 (Figure 1), pH levels ranged from 4.6 – 5.0 for 0 lb/a, 4.2 – 4.6 for 500 lb/a and 3.9 – 4.4 for 1000 lb/a. For plots treated in 2001 (Figure 2), pH levels ranged from 4.6 – 5.0

for 0 lb/a, 4.0 – 4.5 for 500 lb/a, and 3.8 – 4.0 for 1000 lb/a. For plots treated in 2003 (Figure 3), pH levels ranged from 4.6 – 4.9 for 0 lb/a, 4.2 – 4.8 for 500 lb/a and 4.0 – 4.3 for 1000 lb/a. Neither sulfur nor herbicide application significantly reduced woody or herbaceous weed cover, though a decrease in grass was seen on plots treated with 500 and 1000 lb/a sulfur (Figures 4 – 6). Grass cover was significantly higher on untreated sites, established in 2000, with both sulfur application and herbicide application (Figures 4- 6).

CONCLUSIONS: As expected, the pH reduction among sites varied because of variations in factors such as soil CEC differences. There is a clear pattern of lower pH with sulfur application and a significant corresponding reduction in grass cover. Though not significant it also appears there is similar reduction of herbaceous and woody weed cover.

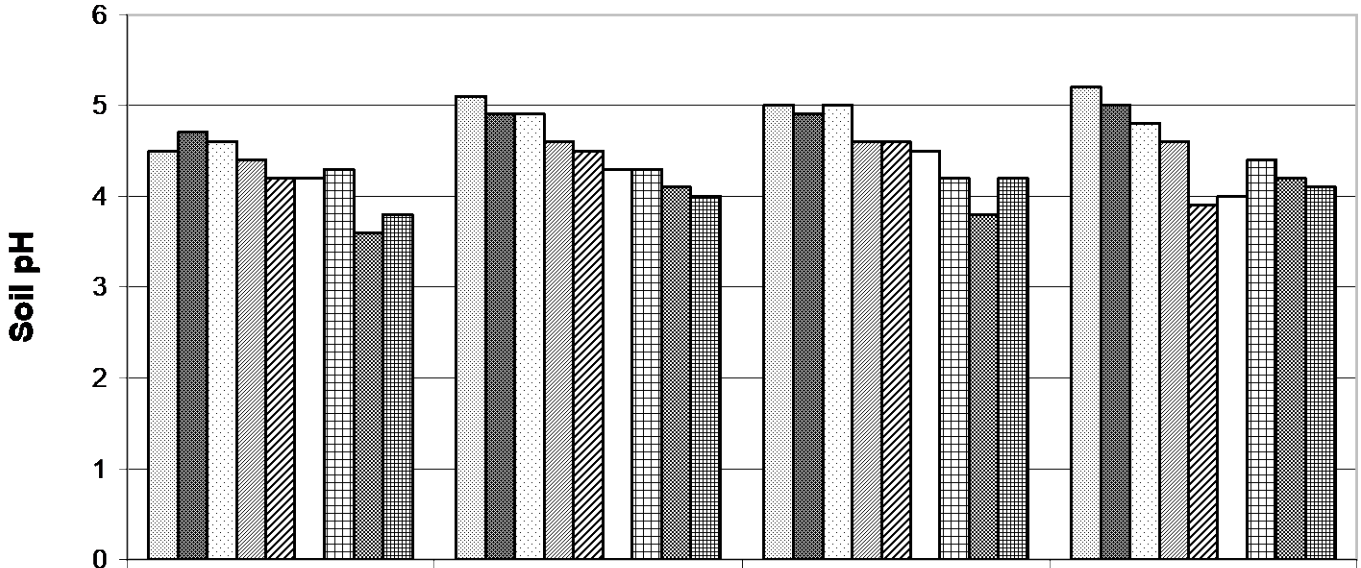
RECOMMENDATIONS: This project should be continued over at least three production cycles in order to document changes in weed composition associated with the decrease in pH.

Figure 1. Effect of Sulfur on Reducing Soil pH
Applied 2000 Evaluated 2001, 2002, 2003



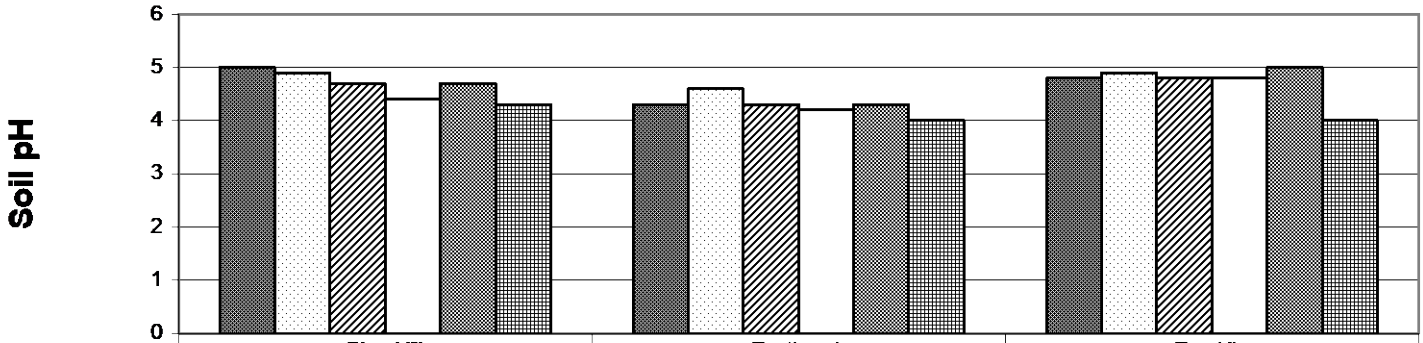
	Appleton	Rockport	Machiasport	Wesley-S	Wesley-V
0S-01	4.7	4.3	5.1	5.4	5.3
0S-02	4.9	4.5	5	5.2	5
0S-03	4.8	4.6	4.7	4.9	5
0S-04	4.9	4.6	4.8	5	4.9
500S-01	4.4	4.3	4.9	4.6	4.3
500S-02	4.4	4.2	4.6	4.5	4.3
500S-03	4.6	4.4	4.7	4.6	3.9
500S-04	4.4	4.4	4.2	4.6	4.3
1000S-01	4.3	3.9	4.1	4.5	4.3
1000S-02	4.2	4	3.9	4.2	4
1000S-03	4.3	4.3	4.1	3.8	4.2
1000S-04	4	4.4	4	3.9	4

Figure 2. Effect of Sulfur on Reducing Soil pH
Applied 2001 Evaluated 2002, 2003, 2004



	BBHF	Union	Wesley-S	Wesley-V
0S-02	4.5	5.1	5	5.2
0S-03	4.7	4.9	4.9	5
0S-04	4.6	4.9	5	4.8
500S-02	4.4	4.6	4.6	4.6
500S-03	4.2	4.5	4.6	3.9
500S-04	4.2	4.3	4.5	4
1000S-02	4.3	4.3	4.2	4.4
1000S-03	3.6	4.1	3.8	4.2
1000S-04	3.8	4	4.2	4.1

Figure 3. Effect of Sulfur on Reducing Soil pH
Applied 2003, Evaluated 2003, 2004



	Blue Hill	Eastbrook	Franklin
0S-03	5	4.3	4.8
0S-04	4.9	4.6	4.9
500S-03	4.7	4.3	4.8
500S-04	4.4	4.2	4.8
1000S-03	4.7	4.3	5
1000S-04	4.3	4	4

Figure 4. 2004 Weed Cover Evaluation for Five Sulfur Treated Plots

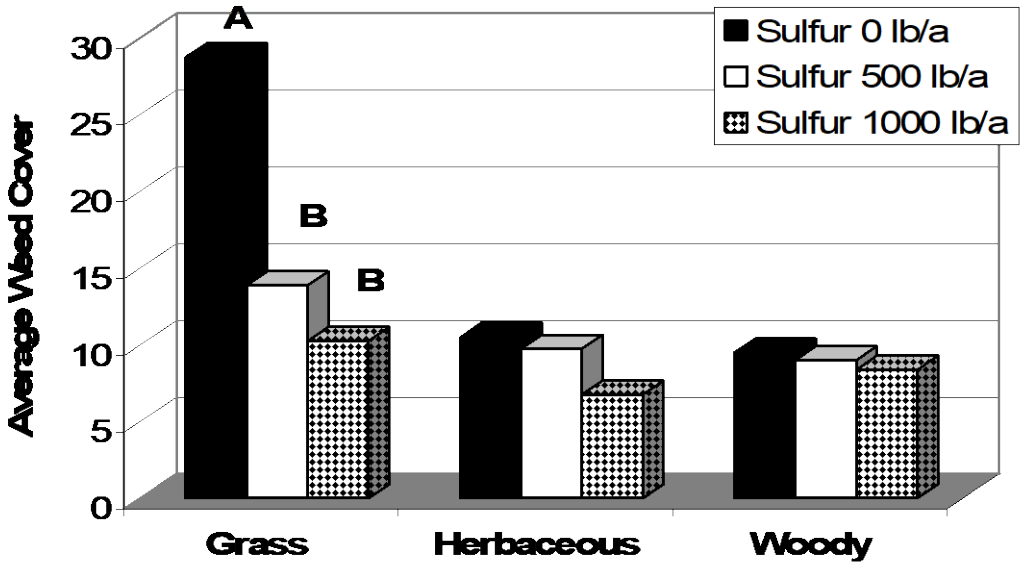


Figure 5. 2004 Weed Cover Evaluation of Four Sulfur and Velpar Treated Plots

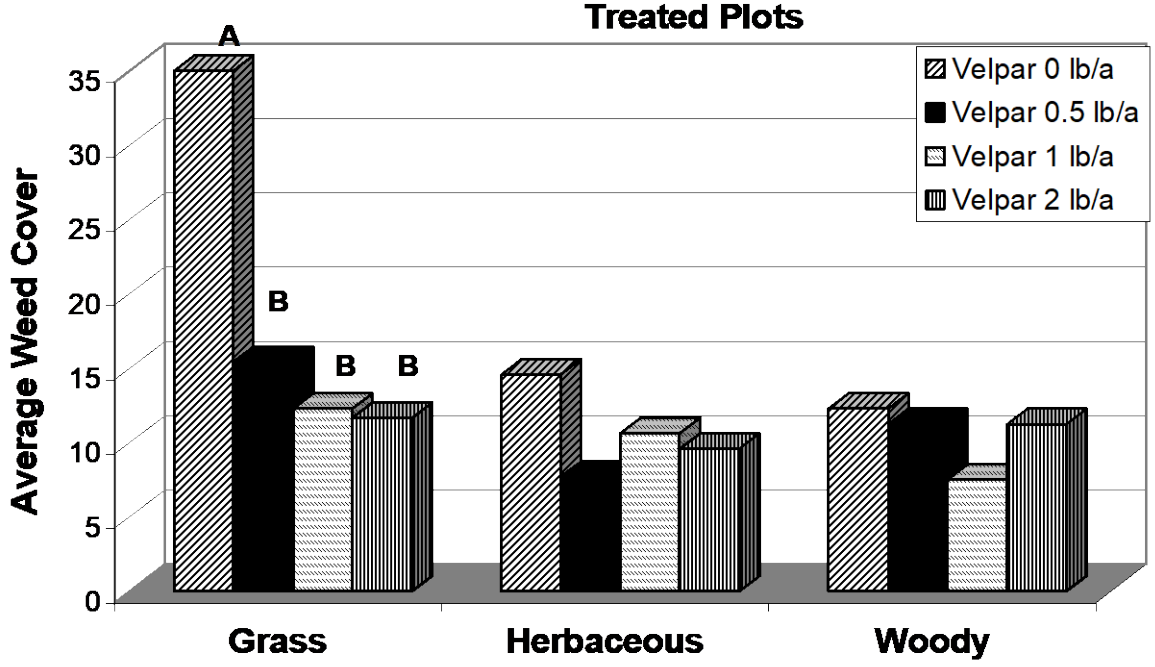
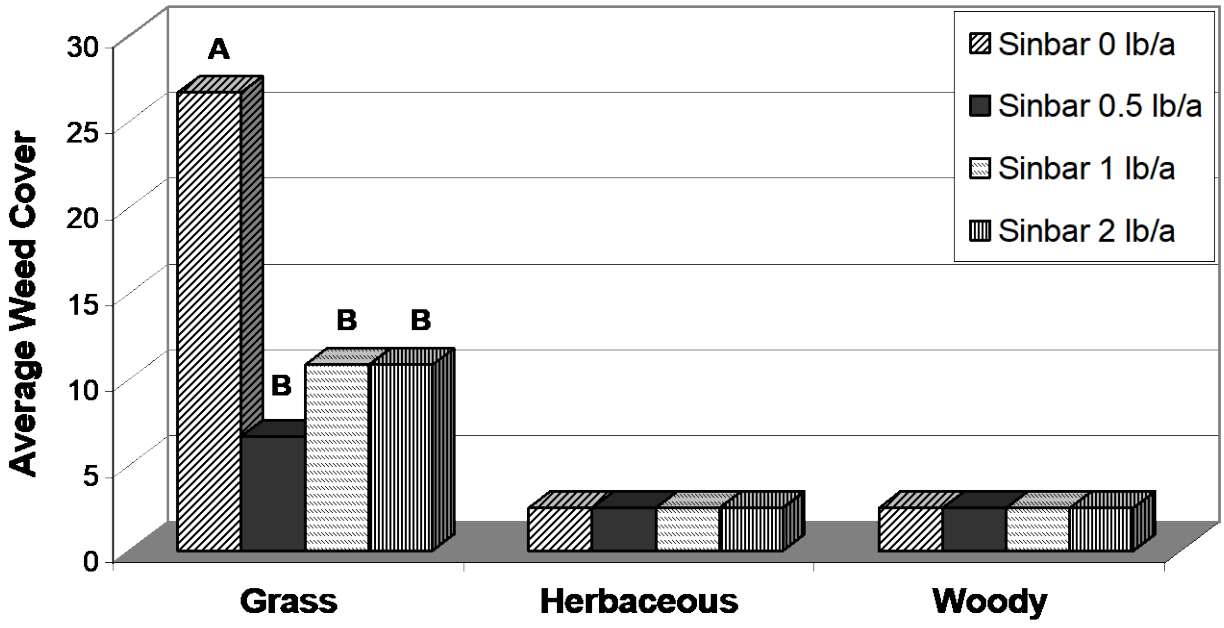


Figure 6. 2004 Evaluation of a Sulfur and Sinbar Treated Plot



EXTENSION
INVESTIGATOR: David E. Yarborough, Cooperative Extension blueberry specialist

23. TITLE: 2004 Pesticide Groundwater Survey

METHODS: Surveyed seven drilled wells, two test wells, and seven adjacent surface water samples taken in May, June, July, August and September to test if herbicides and a fungicide were present. The three test wells were put in by the Maine Department of Conservation in 1986 and the others were drilled. One new site with three samples was added in 2003. These samples include the Machias town water, a well adjacent to the town well, and a stream draining towards that well. Well sites were chosen on the basis of a high probability of finding hexazinone. Residue analysis of the water was performed at the University of Maine Food Science & Human Nutrition Department with a high pressure liquid chromatography which has a detection limit of 0.05 parts per billion (ppb). Tests serve to monitor effectiveness of *Hexazinone Best Management Practices* and to determine if the herbicides hexazinone, terbacil and the fungicides propiconazole and febunconazole are present in ground or surface water.

RESULTS: Hexazinone levels in water varied over the season (Figure 1 and 2) and were lower than those found last year. Hexazinone levels ranged from non-detect (ND) to 9.5 ppb (Table 1). The site with highest hexazinone level at 9.5 ppb was the well that had previous level of over 100 ppb from a point source spill. The town water supply (42T)

was steady at just over 1 ppb and the well adjacent to it (42) was also at or below 1 ppb, which, was much lower than last year. The management practices on the fields adjacent to the Machias town well indicate low rates and granular applications of hexazinone were made in compliance with best management practices. On the sites with test wells treated with terbacil, (9 & 11) levels below1 ppb were detected in the wells and adjacent surface waters. No fungicides were detected in either surface or groundwater sites. The hexazinone groundwater varied a few parts per billion but the trend line was very flat (Figure 3). The trend for the surface hexazinone data is a decrease in the levels in the spring, followed by a slight increase and a leveling off after applications were made (Figure 4).

CONCLUSION: These data further substantiate that the current use patterns are not resulting in any increase in hexazinone or terbacil levels in the groundwater. When alternative herbicides are used, some detections can be expected on sites with sandy soils and shallow water tables. No fungicide was detected in 2004. All detected levels were well below established EPA health advisory limits (HAL).

RECOMMENDATIONS: Sample data from 12 years have shown that the use of best management practices do not result in pesticide detection's above the HAL. Since these long-term data have shown consistent reduction in detections, it is not necessary to

continue to sample on a yearly basis. Sampling may be continued in the future if it is considered necessary. I will continue the educational program to emphasize best management practices to growers to increase awareness of the solubility of hexazinone and potential for well water contamination.

Figure 1. Hexazinone present in Ground Wells 2004

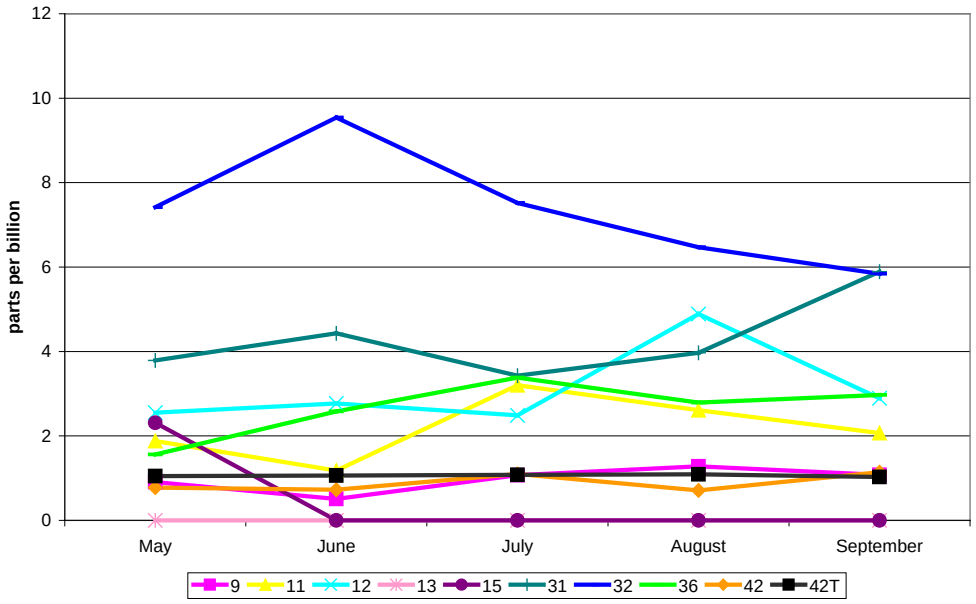


Figure 2. Hexazinone in Surface Samples 2004

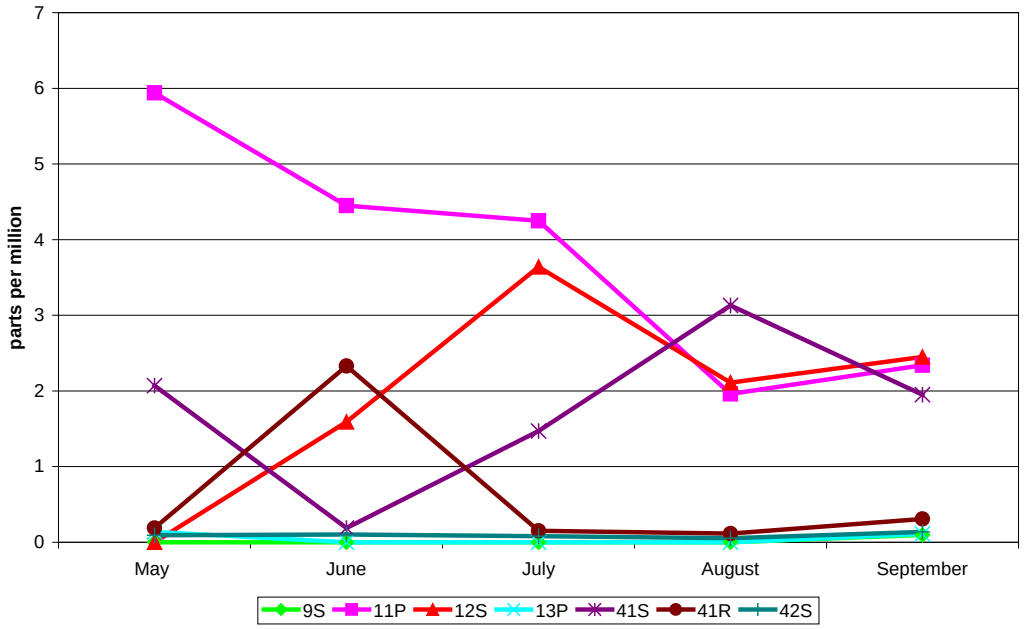
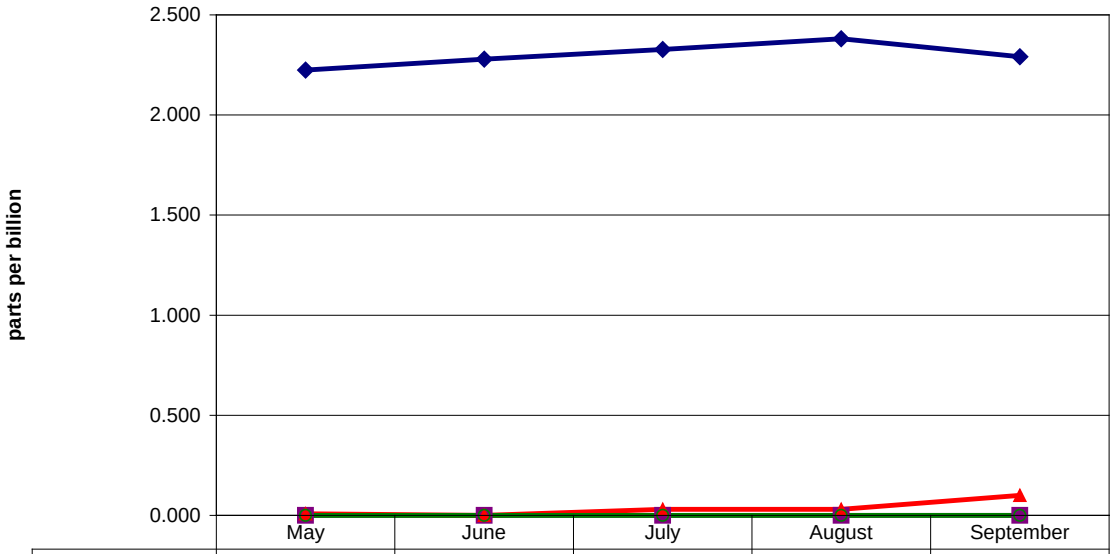
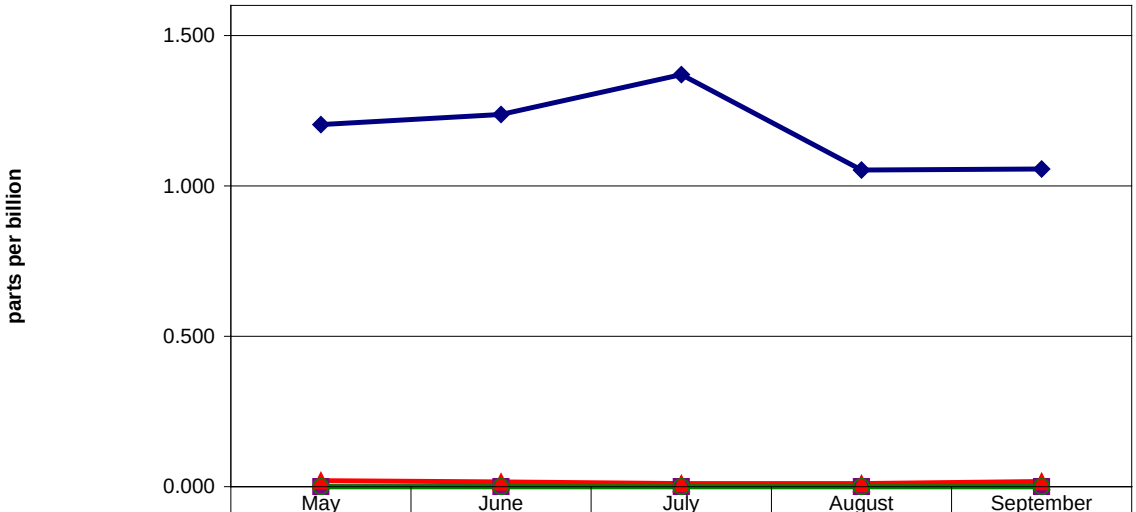


Figure 3. Average Ground Water Samples 2004



	May	June	July	August	September
Hexazinone	2.224	2.279	2.327	2.381	2.291
Febuncoazole	0	0	0	0	0
Terbacil	0.0076	0	0.03	0.03	0.1
Propiconazole	0	0	0	0	0

Figure 4. Average for Surface Samples 2004



	May	June	July	August	September
Hexazinone	1.204	1.238	1.370	1.052	1.056
Febunconazole	0	0	0	0	0
Terbacil	0.02	0.016	0.01	0.01	0.017
Propiconazole	0	0	0	0	0

Table 1. 2004 Groundwater Test Result Summary
University of Maine Well Water Survey
Hexazinone/Febunconazole/Terbacil/Propiconazole in parts per billion

Site well/ Hexazinone (H) /Diuron (D)/ Terbacil (T)/ Propicoanzole (P)	**May**				**June**				**July**				**August**				**September**			
Wells	H	F	T	P	H	F	T	P	H	F	T	P	H	F	T	P	H	F	T	P
9 test	0.907	ND	ND	ND	0.506	ND	ND	ND	1.07	ND	0.171	ND	1.28	ND	0.201	ND	1.08	ND	0.744	ND
11 test	1.88	ND	0.076	ND	1.18	ND	ND	ND	3.2	ND	0.152	ND	2.61	ND	0.137	ND	2.07	ND	0.308	ND
12 test	2.55	ND	ND	ND	2.77	ND	ND	ND	2.49	ND	ND	ND	4.89	ND	ND	ND	2.89	ND	ND	ND
13 drill	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
31 drill	3.79	ND	ND	ND	4.43	ND	ND	ND	3.43	ND	ND	ND	3.97	ND	ND	ND	5.89	ND	ND	ND
32 drill	7.42	ND	ND	ND	9.54	ND	ND	ND	7.52	ND	ND	ND	6.47	ND	ND	ND	5.84	ND	ND	ND
36 drill	1.56	ND	ND	ND	2.58	ND	ND	ND	3.38	ND	ND	ND	2.79	ND	ND	ND	2.97	ND	ND	ND
15 drill	2.31	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
42 drill	0.776	ND	ND	ND	0.727	ND	ND	ND	1.1	ND	ND	ND	0.707	ND	ND	ND	1.14	ND	ND	ND
42T drill	1.05	ND	ND	ND	1.06	ND	ND	ND	1.08	ND	ND	ND	1.09	ND	ND	ND	1.03	ND	ND	ND
Surface																				
9 stream	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.099	ND	0.064	ND
11 pond	5.94	ND	0.144	ND	4.45	ND	0.113	ND	4.28	ND	0.082	ND	1.96	ND	0.1	ND	2.34	ND	0.059	ND
12 stream	ND	ND	ND	ND	1.59	ND	ND	ND	3.64	ND	ND	ND	2.11	ND	ND	ND	2.45	ND	ND	ND
13 pond	0.137	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.11	ND	ND	ND
41 spring	2.07	ND	ND	ND	0.189	ND	ND	ND	1.47	ND	ND	ND	3.13	ND	ND	ND	1.95	ND	ND	ND
41 river	0.189	ND	ND	ND	2.33	ND	ND	ND	0.151	ND	ND	ND	0.114	ND	ND	ND	0.309	ND	ND	ND
42 stream	0.092	ND	ND	ND	0.104	ND	ND	ND	0.08	ND	ND	ND	0.053	ND	ND	ND	0.136	ND	ND	ND

ND=no detect to 0.05 PPB

EXTENSION
INVESTIGATOR: David E. Yarborough, Extension blueberry Specialist
24. TITLE: Wild Blueberry Extension Education Program in 2004

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.

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

Yarborough D.E. and K. Lough. 2004. Comparison of 'clean-cut‘ vs. Wiping and Cutting for Weed Control in Wild Blueberries. Northeastern Weed Science Society 58th Annual Meeting, Cambridge, MA. January 5-7, 2004

Yarborough D.E. and K. Lough. 2004. Comparison of 'clean-cut‘ vs. Wiping and Cutting for Weed Control in Wild Blueberries. Annual Meeting of the Research and Extension Workers and Wild Blueberry Association of North America. Bangor, ME. March 30, 2004.
Yarborough, D.E. 2004. Wild Blueberry Extension Update 2004. Annual Meeting of the Research and Extension Workers and Wild Blueberry Association of North America. Bangor, ME. March 30, 2004.

Yarborough, David E. Innovations in Weed Management in Wild Blueberry Fields in Maine. 8th International Symposium on Vaccinium Culture. Oeiras Portugal and Seville Spain, May 3-8, 2004.

Perkins, Brian, L., Yarborough, David E, Guthrie, Kelly and Bushway, Rod. “Detection of Hexazinone in Maine’s Groundwater- A Nine Year Study. 8th International Symposium on Vaccinium Culture. Oeiras Portugal and Seville Spain, May 3-8, 2004.

Starr, Gordon C and Yarborough, David E. Influence of Vapor Deposition on wild Blueberry Water Requirements in a Humid Coastal Climate. 8th International Symposium on Vaccinium Culture. Oeiras Portugal and Seville Spain, May 3-8, 2004.

Grower meetings:

'World Trade Situation and Outlook', 'Enterprise Budgets', 'Production Efficiencies', 'Improving Quality', and Marketing Opportunities' Presented for Trade Adjustment Act Program On February 11, 2004 in Ellsworth, ME, February 12, 2004 in Waldoboro, ME and February 14, 2004 in Machias, Maine .

Wild Blueberry Spring Grower Meetings: South Paris, March 15; Waldoboro, March 17;

Ellsworth, March 18; Machias, March 20, 2004.

Blueberry Hill Farm Annual Field Day on July 21, 2004.

Lowbush Blueberry Production Costs and Returns. Great Lakes EXPO – Mapping Your Route to the Future, Grand Rapids Michigan, December 9, 2004.

Lowbush Blueberry Production Trends. Great Lakes EXPO – Mapping Your Route to the Future, Grand Rapids Michigan, December 9, 2004.

ICM sessions:

Best Management Practices for Wild Blueberries in Maine. Augusta Trade Show, Augusta, ME. January 15, 2004.

ICM field training sessions: *Knox/Lincoln Counties:* April 27, June 1 and June 29;

Washington County: April 2*8*, June 2 and June 30; *Hancock County:* April 29, June 3 and July 1, 2004.

Extension Presentations:

Taming the Wild Blueberry and Upland Cranberry Production for LCH110 Horticultural Science class at UMaine, April 2, 2004.

Growing Wild Blueberries in the Home Garden, Spring Garden Celebration, Searsport High School, Searsport, ME, March 27, 2004.

Explained Maine wild blueberry production to hundreds of attendants of the Big E Agricultural Fair in Springfield, MA on September 20-21, 2003.

Publications:

Dalton T. J and D. Yarborough. 2004. The economics of supplemental irrigation on wild blueberries: A stochastic cost assessment. Small Fruits Review 3(1/2):73-86, MAFES 2571.

Jensen K.I.N. and D. E. Yarborough. 2004. An overview of weed management in the wild lowbush blueberry - past and present. Small Fruits Review 3(3/4):229-255.

Seymour, R. M., G. Starr and D. Yarborough. 2004. Yield and quality differences of lowbush blueberry (Vaccinium angustifolium) in irrigated and rain-fed conditions. Small Fruits Review 3(1/2):45-56.

Starr, G., R.M. Seymour, F. Olday, F and D. Yarborough. 2004. Determination of evapotranspiration and drainage in lowbush blueberries (Vaccinium angustifolium) using weighing lysimeters. Small Fruits Review 3(3/4):273-283.

Yarborough, D.E. 2004. Factors Contributing to the Increase in Productivity in the Wild Blueberry Industry. Small Fruits Review 3(1/2):33-43, MAFES 2569.

Television/radio/newspaper Interviews 2004:

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.

Bangor Daily: January 26, June 15, July 13
Christian Science Monitor: August 14
Ellsworth American: January 14, 22, March 22, July 12
Fortune Small Business Magazine: March 5
Lexus: June 10
Maine Public Radio: July 9, 22
New York Times: January 13
Portland Press Herald: August 25
Republican Journal: July 29
Sun Times: July 7, August 4
TV13: August 17
Successful Farming Magazine: May 13, 24
Village Soup: November 20

Public testimony
Public testimony Maine Board of Pesticides Control, Augusta, ME: January 23, December 17, 2004.

Other program activities:

I am the principle investigator for USDA/CSREES *Wild Blueberry Production and Processing Technologies*, 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.

Presented educational program for Trade Adjustment Act, federal program to pay growers to compensate for increased imports and decline in field price of wild blueberries. In conjunction with the University of Minnesota, I developed the *Wild Blueberry Technical Assistance Curriculum,* a 126 page resource guide and five Power Point presentations on *World Trade Situation and Outlook, Enterprise Budgets, Production Efficiencies, Improving Quality, and Marketing Opportunities.*

These have been produced as a web based course and may be found at http://www.agrisk.umn.edu/taa/Commodities/WildBlueberriesMaine/.

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.

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 am a service provider for the Farms for the Future Program, worked with The Farm in Rockport and Highland Blueberry Farm in Stockton Springs to improve and diversify their wild blueberry operations. I have worked the Coastal Land Trust in Camden to develop a management plan to improve production on the Beach Hill reserve.

I serve on the peer review committee for Cooperative Extension, the Department of Plant soil and Environmental Sciences and the joint peer review committees of Renae Moran & Mark Hutton. These review activicties take four weeks a year.

Wild Blueberry Fact Sheets - 2004

Revised
Fact Sheet #224 (UMCE # 2040) Commercial Pollinators 2004
Fact Sheet #209 (UMCE #2001) 2004 Insect Control Guide for Wild Blueberries
Fact Sheet #239 (UMCE #2025) 2004 Weed Control Guide for Wild Blueberries
Fact Sheet #219 (UMCE #2000) 2004 Disease Control Guide for Wild Blueberries
Fact Sheet #227 (UMCE #2253) Sources of Lowbush Blueberry Plants
Fact Sheet #237 (UMCE#2176) Glyphosate for Weed Control in Wild Blueberries
Fact Sheet #253 Cultural Management for Insects and Diseases in Wild Blueberries
Fact Sheet # 251 Best Management Practices for Wild Blueberry Production in Maine

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.

The hexazinone groundwater survey I have conducted from 1992 through 2004 continues to provide information on the movement of this herbicide into the groundwater. I have sampled test and drilled wells and surface water in blueberry fields over eleven years. 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 survey 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.

RECOMMENDATIONS: Continue to support Extension educational program.