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2016 Wild Blueberry Project Reports

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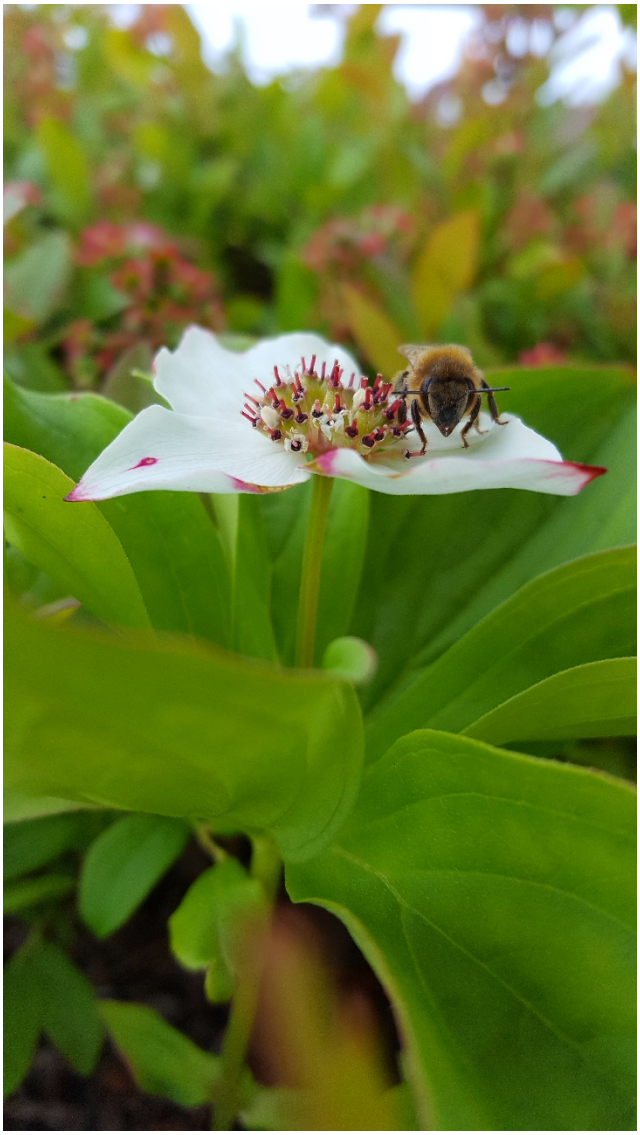

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FOOD SCIENCE AND NUTRITION

INVESTIGATOR: Vivian Wu, Ph.D. Adjunct Professor of Food Safety and Microbiology, University of Maine
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1. TITLE: Increasing the food safety margin of wild blueberries through improved intervention measures.

OBJECTIVES:
1. To evaluate the effectiveness of different chemical sanitizers using short contact times in combination with freezing on microbial reduction; and
2. To develop an effective double spraying system for microbial reduction by combining two chemical sanitizers along with freezing.

METHODS: A bacterial cocktail with two strains each of *S.* Typhimurium (ATCC 6962 and ATCC 14028) and *L. monocytogenes* (ATCC 19115 and ATCC 49554) was used to inoculate the surface of blueberries. The initial level of inoculum on surface of inoculated blueberries was approximately 7 log CFU/g. Fresh solutions of chemicals in distilled water were prepared the same day of each experiment. The treatments tested included: chlorine (Cl_2, 200ppm), aqueous chlorine dioxide (ClO_2, 15ppm), and lactic acid (2%) for objective-1 and combination chemical sanitizers treatments including chlorine (100ppm) and lactic acid (2%), aqueous chlorine dioxide (10ppm) and lactic acid (2%), chlorine (200ppm) and aqueous chlorine dioxide (15ppm), chlorine (100ppm) and aqueous ozone (5ppm), aqueous chlorine dioxide (10ppm) and aqueous ozone (5ppm), and lactic acid (2%) and aqueous ozone (5ppm) for objective-2 double spray system. The control treatments include distilled water wash and un-treated inoculated blueberries. To imitate industrial setup similar to those used in blueberry processing, a portable conveyer belt with a fixed overhead double spray was designed and used for the treatments. Inoculated blueberry samples were spread on the conveyer belt and 150ml sterile distilled water (control) or different individual chemical solutions or combination sanitizers for double spray were sprayed from a designated height while the berries were rotated and moved on the conveyer belt. The treated blueberries were left on the conveyer belt for different contact times (10sec, 1min and 3min). To evaluate the efficiency of these chemicals combined with frozen storage, after each treatment time, one set of blueberries was stored at -17°C for 1 week. Bacterial enumeration was conducted before and after freezing.

RESULTS:

Objective-1: Evaluating the effectiveness of different chemical sanitizers using short contact times in combination with freezing on microbial reduction.

Efficacy of chemical sanitizers in reducing L. monocytogenes in combination with freezing at -17°C for 1 week:

Cl_2 wash at 200ppm with 3 min contact time when combined with freezing showed > 5 log reduction of *L. monocytogenes*. Though there is increase in log reduction with increase in contact time, the 1min contact time did not shown any significant difference ($p > 0.05$) from 10 sec treatment time. Treatment with aqueous chlorine dioxide (ClO_2) for 3 min resulted in 2.7 log

reduction before freezing and when combined with freezing the log reduction significantly increased to 5.5log CFU/g. Exposure time of 1 min with ClO_2 in combination with freezing showed > 5.0 log CFU/g reduction indicating shorter exposure times of ClO_2 are equally effective. With lactic acid treatment in combination with freezing, at 3min contact time, > 6.0 log CFU/g reduction was noted. Log reduction > 4.5 log CFU/g was achieved with lactic acid treatment in combination with freezing at shorter contact times indicating lactic acid is very effective even for shorter exposure times.

Efficacy of chemical sanitizers in reducing S. Typhimurium in combination with freezing at -17°C for 1 week:

Treatment with Cl_2 wash (200ppm with 3min contact time) in combination with freezing resulted in 5.3 log CFU/g reduction of *S.* Typhimurium. Contact times of 10 sec and 1 min resulted in only 3.7 and 4 log CFU/g reduction, respectively, indicating there is no significant difference ($p > 0.05$) between them. Treatment with aqueous chlorine dioxide (ClO_2-15ppm) for 3 min when combined with freezing showed a log reduction of 4.8 log CFU/g. Contact time of 1 min with ClO_2 in combination with freezing showed 4.6 log CFU/g reduction and 10 sec showed only 4.4 log CFU/g reduction. Lactic acid treatment in combination with freezing, at 3min contact time resulted in 4.5 log CFU/g reduction. Though there is no significant difference in log reductions of 10 sec and 1 min after freezing, they resulted in < 4.0 log CFU/g reduction.

Objective- 2: Developing an effective double spraying system for microbial reduction by combining two chemical sanitizers along with freezing.

Efficacy of different combination double spray treatments in reducing L. monocytogenes with freezing:

The double spray treatments including chlorine and lactic acid, chlorine and chlorine dioxide, and chlorine dioxide and lactic acid resulted in complete elimination of *L. monocytogenes* (with detection limit < 1 log CFU/g) with 3 min contact time from the surface of blueberries. Double spraying of ozone with chlorine, chlorine dioxide, and lactic acid with freezing combination showed 5.3, 5.7, and 5.6 log CFU/g reduction of *L. monocytogenes,* respectively. Also, though double spray of chlorine (200ppm) and chlorine dioxide (15ppm) are used at slightly higher concentrations than rest of chemical combinations, it showed significantly higher reduction ($p < 0.05$) than ozone combinations and significantly equal reductions ($p = 0.05$) as of lactic acid combinations.

Efficacy of different combination double spray treatments in reducing S. Typhimurium with freezing:

The synergistic effect of chlorine when coupled for double spray treatments with lactic acid, and chlorine dioxide, and in combination with freezing reduced the populations of *S.* Typhimurium to undetectable levels with both 1 min and 3 min contact times. With chlorine dioxide and lactic acid double spray treatment in combination with freezing , the reduction of *S.* Typhimurium though did not show complete eliminations like *L. monocytogenes,* it still resulted in >5.0 log CFU/g reductions including short exposure times like 10 sec contact time and showed 6.0 log CFU/g reduction with 3 min time. Ozone and lactic acid double spray with freezing treatment resulted in 5.8 log CFU/g and 5.23 log CFU/g reduction of *S.* Typhimurium with 3 min and 1 min contact times, respectively, whereas ozone in synergistic effect with

chlorine and chlorine dioxide showed 4.9 log CFU/g and 5.3 log CFU/g, respectively, with 3 min contact times.

CONCLUSIONS: This study showed an effective sanitization protocol which uses low-dosages of multiple sanitizer "hurdles" together with the standard industry use of individual quick freezing process without adverse effect on quality of produce. The efficacy of these sanitizers is increased significantly in inactivating foodborne pathogens, when combined with freezing. The food safety margin of blueberries can be increased by incorporating this sanitization strategy into the existing processing protocols.

RECOMMENDATIONS: Our approach, which imitates industrial conditions, is worth to be considered by the Maine wild blueberry industry, where these conditions can be easily incorporated to possibly eliminate pathogens more effectively.

ENTOMOLOGY

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

2. I. TITLE: Control tactics for blueberry pest insects, 2016.

Study 1. Field control of blueberry tip midge on wild blueberry (pruned-year) with insecticides.

METHODS: Materials were applied in 25 gallons of water-mixture per acre with a CO_2-propelled, 80-inch boom sprayer (76-inch swath) equipped with four, flat-spray, 8002VS TeeJet® nozzles operating at 35 psi and at a slow walking speed. Walking speed was regulated using a metronome. There were four replications of each treatment, and plot size was 7 x 20-ft. Assail® 30SG (acetamiprid), Mustang Maxx® (zeta-cypermethrin), and Success® 2SC (spinosad) were applied on 26 May to a pruned-year field in Jonesboro, ME. Blueberry stems were scattered and < 1 inch tall. On 12, 19, and 26 June, the number of blueberry stems with tip midge damage as evidenced by curled leaves was determined from each of three, m^2 samples per plot.

RESULTS AND CONCLUSIONS: Analysis of Variance was used to compare mean number of curls among the treatment plots. Means separation was by Least Square Means. Subplots were pooled within main plots. Tip midge populations in all plots (including the untreated check) were extremely low in 2016. There were very few infested stems in the study area and no significant differences among the treatments on any sample date (Table 1).

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

Material	Amt. form./acre	Mean curls/m^2 12-Jun	19-Jun	26-Jun
Assail 30SG	5.3 oz	0.165	0.248	0.250
Mustang Maxx	4.0 oz	0.250	0.165	0.165
Success 2SC	6.0 oz	0.165	0.083	0.168
Untreated check	-	0.250	0.083	0.250
P =		0.8668	0.5896	0.9458

Study 2. <u>Field control of blueberry spanworm larvae on wild blueberry</u>.

METHODS: Two rates of Belt® 400SC - flubendiamide (1.5 and 2.4 oz/acre) and one rate of Assail® 30SG – acetamiprid (5.3 oz/acre) were applied on 20 May. Blueberry plants were at ca 20% bloom and spanworm larvae were mid-instars (3rd-5th). There were four replications per treatment. Each plot measured 20 x 20-ft with a minimum 5-ft untreated buffer zone around each plot. Each material was applied in 25 gallons of water-mixture per acre with a CO_2-propelled, 80-inch boom sprayer (76-inch swath) equipped with four, flat-spray 8002VS TeeJet® nozzles operating at 35 psi and at a slow walking speed. Speed was regulated using a metronome. On the dates indicated in the table, 10 sweeps with a standard 12-inch diameter sweep net were taken systematically through the center area of each plot avoiding plot boundaries. After larvae were counted, they were distributed back into the same plot.

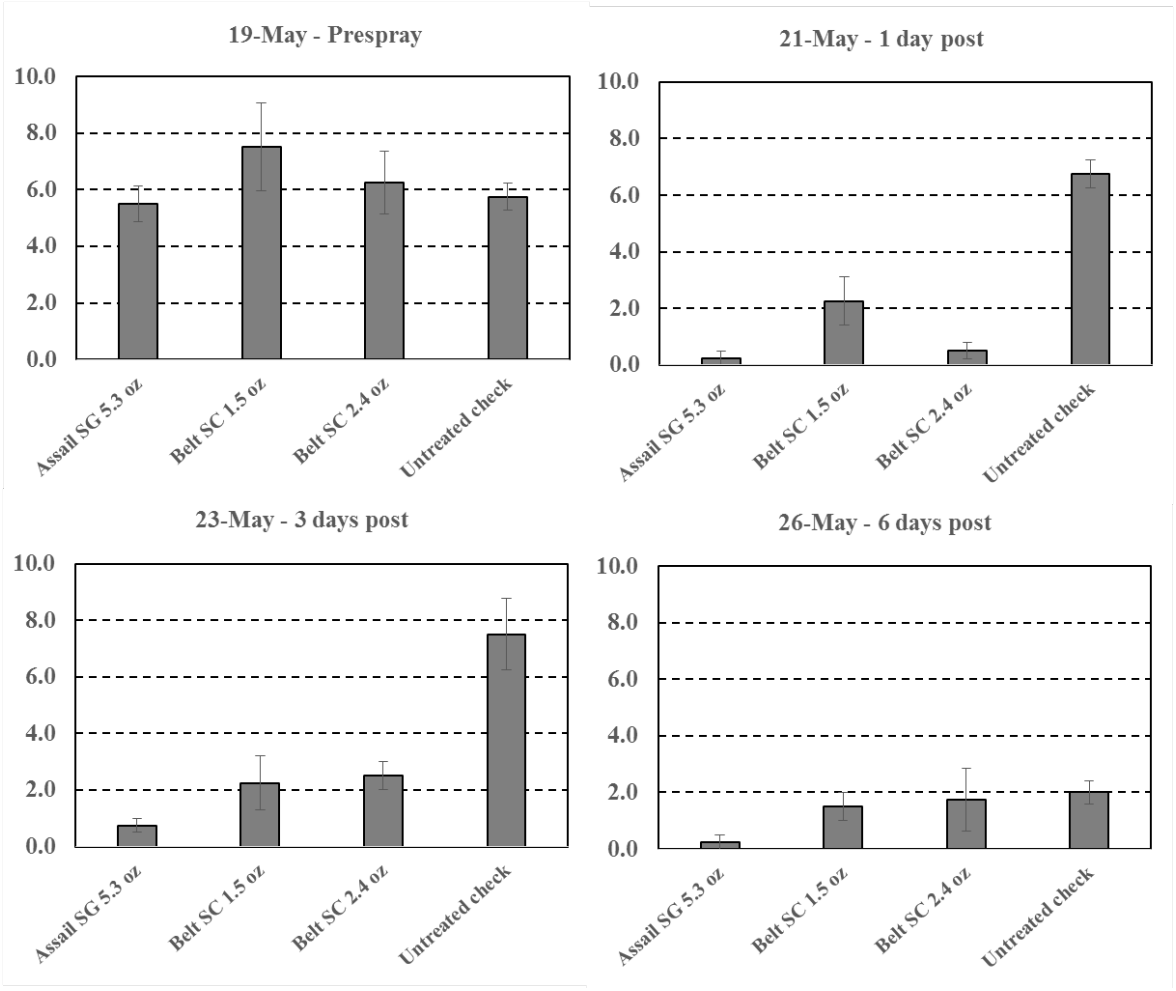
RESULTS: Analysis of Variance and Tukey's post-hoc test ($P \leq 0.05$) were used to compare numbers of spanworm larvae captured in sweep-net samples among the treatments for each date. Data were transformed by the square root to stabilize variance prior to analysis. Pre-spray populations were not significantly different among the treatments ($P = 0.382$). All materials initially provided excellent control of blueberry spanworm larvae. After one day (21 May), numbers of blueberry spanworm larvae in plots treated with Assail 30SG and both rates of Belt 400SC were significantly lower than the untreated check ($P = 0.002$). This trend continued through day three post-spray (23 May). However, by day five (25 May), Belt (both rates) was not significantly different than the untreated check; but Assail was significantly different than the untreated check even at day six (26 May).

Table 1. Field control of blueberry spanworm larvae with insecticides, summary.

Material	Amt. form./ acre	Prespray 19 May	Larvae/10 sweeps Post spray 21 May	23 May	25 May	26 May
Assail 30SG	5.3 oz	5.5 a	0.3 c	0.8 c	0.8 b	0.3 b
Belt 400SC	1.5 oz	7.5 a	2.3 b	2.3 bc	3.8 ab	1.5 ab
Belt 400SC	2.4 oz	6.3 a	0.5 bc	2.5 b	3.3 ab	1.8 ab
Untreated check	-	5.8 a	6.8 a	7.5 a	7.5 a	2.0 a

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

Fig. 1. Density of blueberry spanworm larvae, by sample date.



CONCLUSIONS: The new insecticide, Belt, was effective against blueberry spanworm larvae resulting in significantly lower larval densities than the untreated check for three days. However, Assail was superior to Belt and maintained significantly lower larval densities than the untreated check through six days after application.

RECOMMENDATIONS: Since it was not possible to draw any conclusions from our tip midge trial, we will attempt to repeat this trial in 2017. In addition, we will monitor infestation by collecting curls and examining for immatures at weekly intervals.

It should be noted that the U.S. Environmental Protection Agency decided in August to pull the conditional registration for Belt 400SC insecticide. The EPA has ruled that farmers can use up their existing stockpiles of flubendiamide. Therefore, we cannot recommend the use of this product. Flubendiamide is used on a variety of crops including alfalfa, almonds, vegetables, cotton and walnuts.

ENTOMOLOGY

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

3. II. TITLE: Pest biology and IPM, 2016.

Study 1. Long-range, within-field, movement of blueberry maggot fly in wild blueberry: A release/recapture study.

OBJECTIVES: This trial is the continuation of a study begun in 2013 to assess the long-range movement patterns of blueberry maggot fly (BMF). The central question being…how far does an isolated field need to be from a larger blueberry production area with multiple fields to enjoy the benefit of reduced blueberry maggot fly pressure?

METHODS: BMF were collected as pupae from infested blueberries in 2015. The wintering cups of pupae were separated into six equal groups and placed in cages where flies were allowed to emerge. Following emergence, the flies were fed honey and yeast for one week prior to release.

Three line transects of 20 baited, yellow, Pherocon® AM traps was set in a pruned year blueberry field adjacent to a fruit-bearing field. Transects were set parallel with traps set at 330ft (100m), 820ft (250m), and 1640ft (500m) from a release point. Within each transect, traps were spaced 10ft apart. Ammonium acetate superchargers were attached to every other trap to enhance attractiveness. Approximately 1500 blueberry maggot fly adults were released on 16 June. Traps were checked daily for 10 days. We calculated the distance each captured fly travelled per day from the release point.

RESULTS: In 2016 we recaptured a total of 19 flies (1.3%) (Table 1) over all three transects. The first fly was recaptured 335.4ft from the release point on day 2 after release; subsequently,

flies were captured beginning on day 4 at 821.5ft and day 5 at 1641.1ft. Over all three transects, the furthest distance travelled by any individual fly was 328.22ft/day (range 56.59 to 328.22ft) (Table 1 and Fig. 2).

In 2015, we recaptured a total of seven marked flies (3.5%); no flies were recaptured after 10 days from release; the first fly was recaptured four days after release. The mean distance traveled per day by the seven recaptured flies was 288.6ft. The furthest distance travelled by any individual fly was 453.35ft/day (Table 1 and Fig. 2).

In a similar trial in 2014 we recaptured a total of 33 marked flies (4.7%) that were released at a point 200ft (61m) from the trap transect; no flies were recaptured after seven days from release; 23 were recaptured within the first four days after release. The furthest distance that any marked fly traveled was 336ft on day 1. On day 1, two BMF traveled 320.2ft. Mean travel distance per day was 113.3ft (Table 1 and Fig. 2).

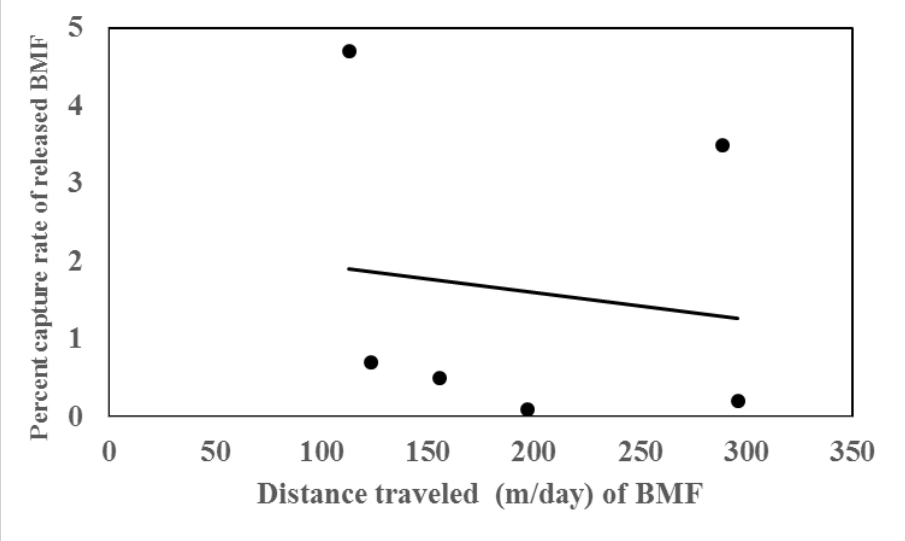
In 2013 we recaptured seven of 1000 marked flies (0.7%) that were released 328ft (100m) from a similar trap transect. Three flies were recaptured within two days (one on day 1 and two on day 2); four additional flies were recaptured six (n=3) or seven (n=1) days after release. The mean distance traveled per day by the seven recaptured flies was 123.8ft. The furthest any BMF raveled was 328ft for a fly captured on day 1 after release. In 2013, we also released 1000 BMF at a point 1312ft (400m) from the transect. Only one fly (0.1%) was recaptured (on day 7 after release); that fly traveled 197.2ft/day (Table 1 and Fig. 2).

Table 1. Recapture rates and average distance traveled by marked flies per day.

Release distance	Trial year	Fly recapture rate (%)	Mean distance per day (n)
330ft (100m)	2016	0.6	83.2ft (25.3m) (9)
820ft (250m)	2016	0.5	155.8ft (47.5m) (7)
1640ft (500m)	2016	0.2	296.0ft (90.5m) (3)
1800ft (549m)	2015	3.5	288.6ft (88.0m) (7)
200ft (61m)	2014	4.7	113.3ft (34.5m) (33)
328ft (100m)	2013	0.7	123.8ft (37.7m) (7)
1312ft (400m)	2013	0.1	197.2ft (60.1m) (1)

n = number of flies captured.

Fig. 2. Distance (meters) of daily travel of blueberry maggot fly (BMF) adults over pruned field landscapes.



CONCLUSIONS: Despite the low recovery in captures, we conclude that there is no relationship between capture rate out to 400m and likelihood of trap capture. This suggests that BMF can easily travel well over 400m to neighboring fields.

Study 2. <u>Long-term trends (19 years) in parasitism of the blueberry maggot fly</u>.

METHODS: Diet cups containing blueberry maggot fly (BMF) pupae (90 cups of 50 pupae each) were maintained in the laboratory for a minimum of four weeks following the last observed emergence of BMF adults. The pupae were collected from infested fruit. Parasitic wasps were observed in the rearing cages. The wasps were collected and an estimate was made of percent parasitism. An estimate of relative size of BMF populations from year to year was obtained from both collections of pupae from fruit and from trap captures of adult flies and added to our database of the relationship between BMF population increase from year to year and parasitism (2016 data).

RESULTS: Figure 1 shows the time series of BMF percent parasitism from 1998 to 2015 (2015 parasitism is estimated one year later in 2016). Parasitism rates continued to drop (6.01%) following the sharp increase in parasitism of pupae collected in 2013 (23.0%) (Fig. 1). However, there does not appear to be a tight linkage between fly trap captures and the parasitism rates over time (Fig. 2). Although, upon visual inspection of figure 2, one can see that whenever parasitism rates peak, usually a decline in fly number occur the year or two following. Modeling fly rate of increase annual fly population growth as a function of parasite density suggests that a possibility exists that a parasitic wasp (presumably *Opius* sp.) is important in regulating fly numbers and that steps should be taken to conserve its numbers ($F_{(1,15)} = 0.743$, $P = 0.0145$). Also, based upon data collected from 1998 through 2016 and plotted in figure 3 it appears that parasitism behaves as a density dependent factor that controls fly abundance from one year to the next. One can see that as parasitoid numbers increase fly reproduction falls precipitously and that only a

small window of very low parasitoid density allows positive BMF increase in numbers from one year to the next; although, only 33.7 % of the variation in fly increase is explained by parasitoid numbers. Figure 4 shows the relationship between the logarithm of fly abundance in year t versus the log rate of increase from year t to year t+1 (Log(Nt+1 /Nt)). The linear relationship suggests that a density dependent relationship exists between fly abundance and the next year's increase or decrease in the BMF population ($F_{(1,15)} = 1.193$, $P = 0.0008$). In addition, inspection of figure 4 suggests that a seasonal fly abundance of 10 is an appropriate threshold for increase since it is the phase shift point of subsequent year increase or decrease in the population. Below a density of 10 the population will increase and above a seasonal density of 10 the population will decrease. What is particularly interesting about this threshold is that this is the threshold that we suggest be used for making decisions regarding insecticide control of BMF.

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

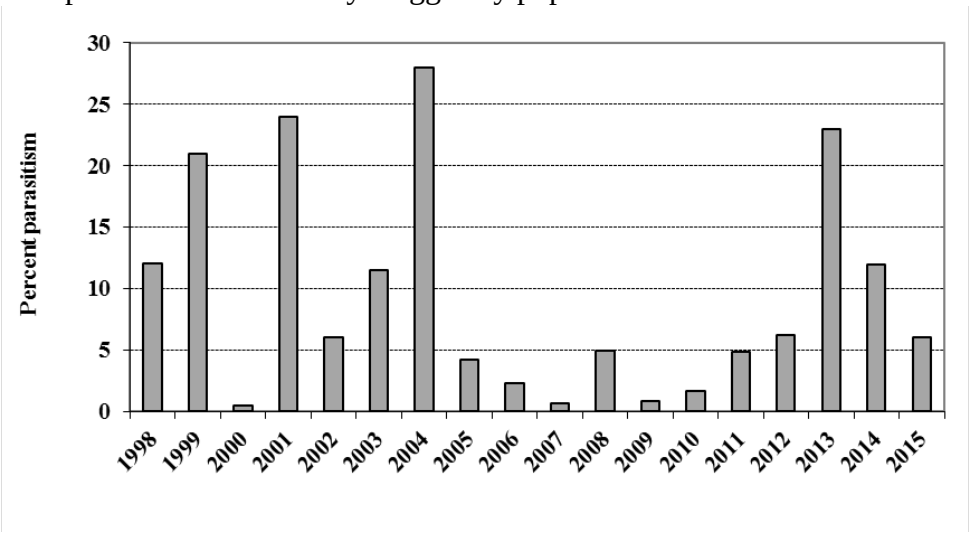


Fig. 2. Relationship between relative density of flies and % parasitism over time. Horizontal line depicts the average fly abundance over the period from 1998 – 2016.

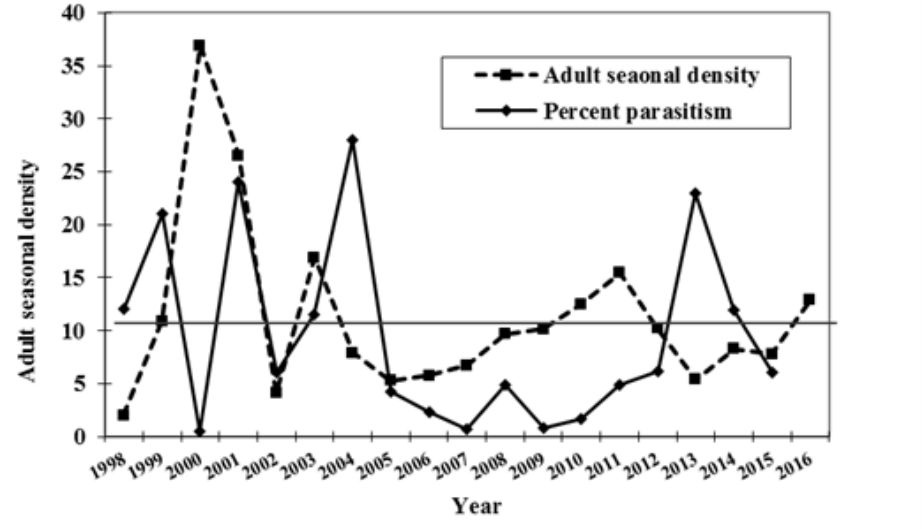


Fig. 3. Relationship between fly population increase and parasitoid density the previous year (1998-2016). Square symbol is 2016 data.

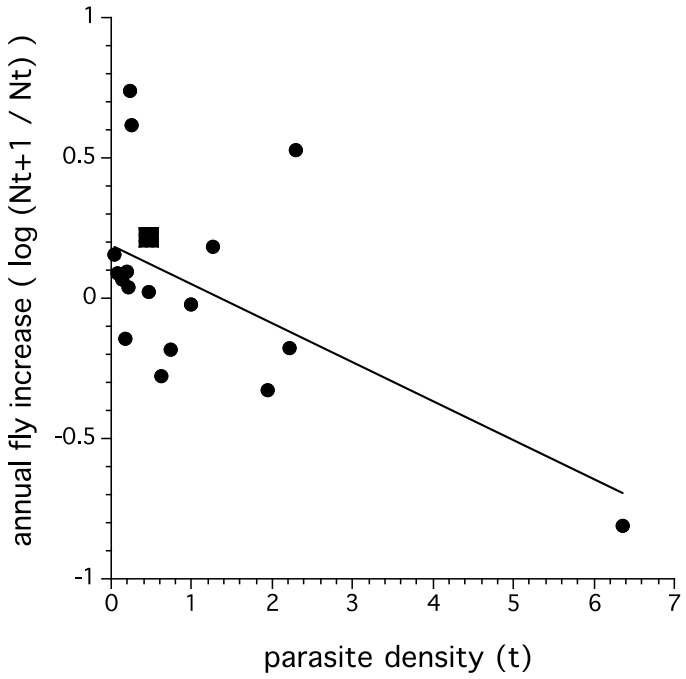


Fig. 4. Relationship between fly population reproduction and fly density the previous year. Dotted line demarks point of zero population increase (1998-2016). Square symbol is 2016 data.

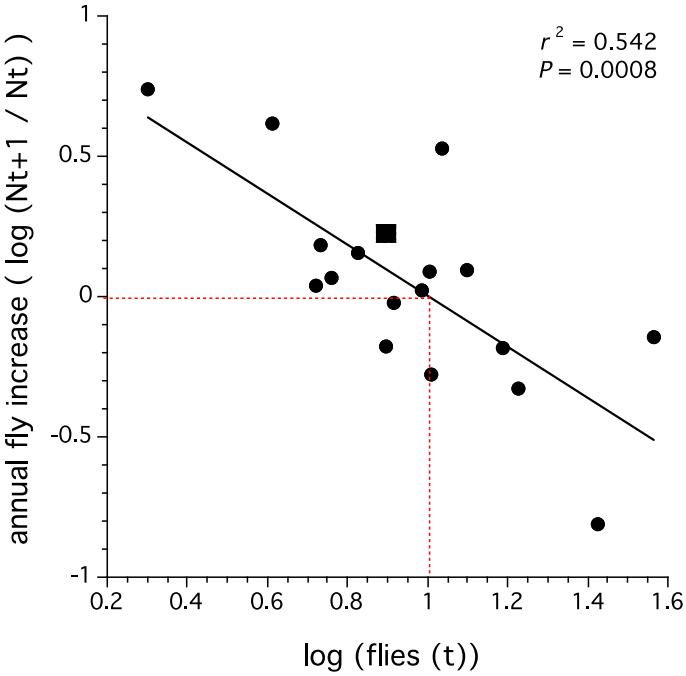
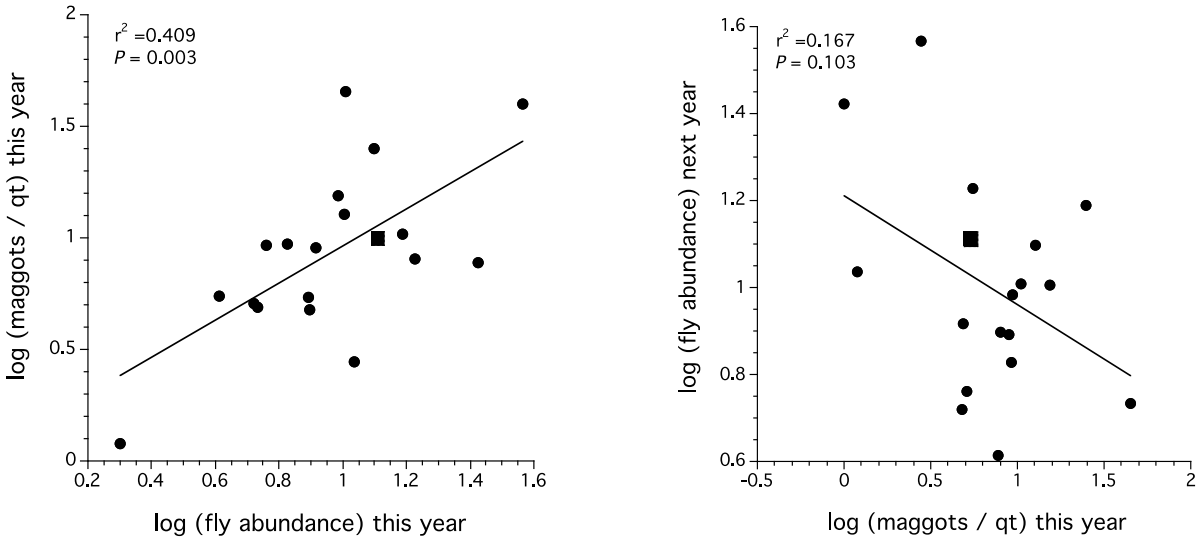


Fig. 5. Relationship between this year's flies and this year's maggots in fruit (left), and the relationship between this year's maggots in fruit and next year's fly population (right) (1998-2016). Square symbol is 2016 data point.



Two other relationships have emerged from the long-term BMF data. First the data documents that while an increase in the number of flies result in an increase in maggot infestation of fruit (Fig. 5), there is a noisy relationship suggesting that other factors affect infestation levels of fruit. The most obvious factor is insecticide application. The other relationship shows that the current year's infestation of fruit is related to the subsequent year fly abundance. However, this relationship is an inverse relationship suggesting that parasites, predators, and fly dispersal from out of the field that they originated in to new fields are density dependent processes and as maggot infestation increases the following year's flies are reduced. However, this relationship is also very noisy and barely significant at the 10% level ($P = 0.103$).

CONCLUSIONS: This study is the only long-term monitoring of the blueberry maggot fly in North America. It shows that in 19 years there have been roughly three explosive outbreaks of the blueberry maggot fly in Downeast Maine. Each outbreak has 2-3 years to peak and then fly densities fall precipitously after the peak.

Study 3. ***Influence of fertility and disease management practices on sap-feeding insects, premature flowering, stem characteristics, leaf spot, leaf retention, and foliar nutrients in wild blueberry.***

METHODS: In 2015, five replicated plots (20 x 20-ft) were set in a field that was in the prune cycle. The fungicides applied were Pristine® (18.5 oz/acre), Pristine (18.5 oz/acre) + DAP fertilizer (200 lbs/acre), and Bravo® (56 oz/acre). All fungicides and DAP were applied on 23 June 2015 at recommended rates. Materials were applied in 25 gallons of water-mixture per acre with a CO2-propelled, 80-inch boom sprayer (76-inch swath) equipped with four, flat-spray, 8002VS TeeJet® nozzles operating at 35 psi and at a slow walking speed. Walking speed was

regulated using a metronome. DAP was applied using a shaker can to spread the material evenly over the plot. An untreated control (UTC) was also included in the experiment. In the spring of 2016 (27 June), a second application of Pristine and Bravo were made to the same plots; the Pristine + DAP application was not repeated. To evaluate potential yields, we counted the number of buds and flowers on each of 10 randomly collected stems per plot. Stems were collected on 23 May 2016. We also determined actual yields. On 8 August 2016, yields were determined by raking a diagonal swath across each plot and weighing the harvested fruit. Sweep sampling for sap-feeding insects was conducted on 5, 15, and 23 May, and 12 and 27 June 2016. The trial above was repeated in 2016. The same materials were applied on 23 June 2016. For the trial initiated in 2016, on 8 August and 5 October 2016, twenty stems were randomly selected from each plot and rated for percent leaf spot fungi. Leaf retention was also evaluated on 5 October and 5 December 2016; the number of leaves retained and fallen was determined for each of 20 randomly selected stems. Sweep sampling for sap-feeding insects was conducted on 23 June, 11 and 25 July, and 1 and 9 August 2016. Ten sweeps with a standard 12-inch diameter sweep net were taken systematically through the center area of each plot avoiding plot boundaries. Tip midge infestation was assessed on 25 July 2016. Premature flowering in the prune crop was assessed on 5 October 2016. Plots were rated as either with or without stems with flowers present. Stems were also collected on 5 October 2016 to assess stem density, length, branching, and number of flower bud clusters. All stems within two, sq. ft. quadrats per plot were cut and brought into the laboratory. To evaluate foliar nutrients, 10 stems were randomly collected from each plot on 5 July 2016; leaves were collected and dried for analysis.

RESULTS: This was the second year of a three-year study (two replicated trials) designed to evaluate potential effects of summer applications of fungicide. Our analysis of flower-bud clusters in 2015 suggested that Bravo might increase potential yield, but that Pristine will only increase potential yield when nitrogen fertilizer is applied. This did not appear to be the case when plots were re-sampled in 2016. Despite the significant difference in numbers of flower-bud clusters/stem noted in the fall of 2015 ($F_{(3,12)} = 4.69$, $P = 0.022$)(Fig. 1), there was no significant difference in the subsequent crop year in number of flowers/bud ($F_{(3,12)} = 1.10$, $P = 0.3868$) or in yields among the treatments ($F_{(3,12)} = 1.86$, $P = 0.1909$)(Fig. 2). In 2016, there was no significant differences number of flower-bud clusters/stem ($F_{(3,12)} = 0.1.86$, $P = 0.1864$).

Fig. 1. Mean flower-bud clusters/stem. Lines are standard error of the mean. Letters which are different denote significant differences ($P < 0.05$). Data from 2015 trial.

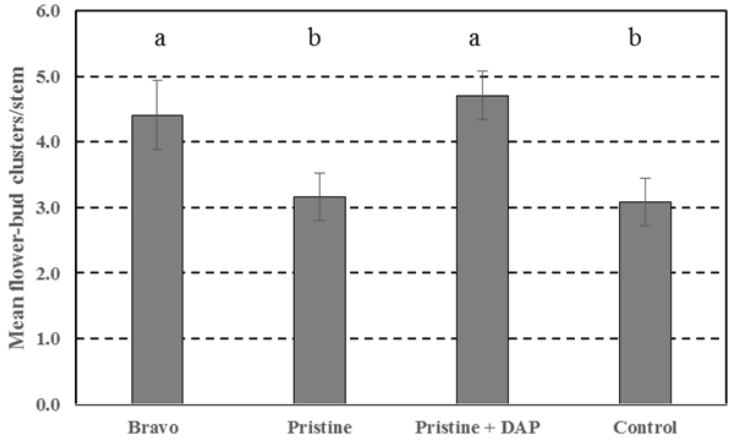
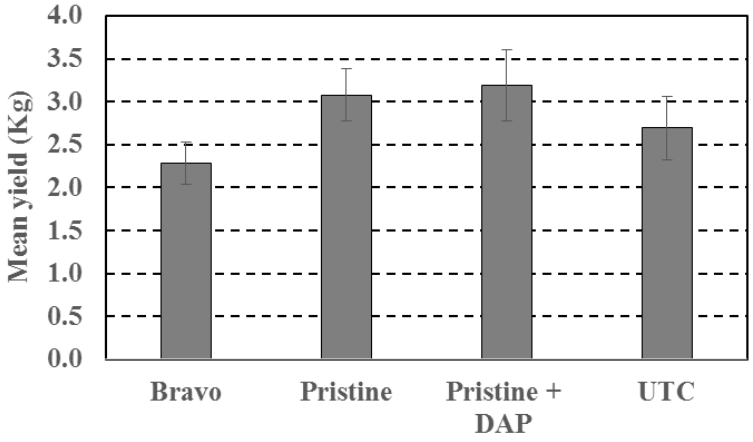


Fig. 2. Mean yield (kg). Lines are standard error of the mean. Data from 2015 trial.



We have also evaluated various plant measures. Analysis of Variance (ANOVA) and LSD ($P \leq 0.05$) were used to compare leaf retention, incidence of leaf spot, foliar nutrients, and stem measures including length, branching, and stem density. Subplots were pooled within main plots. In 2016, there were no significant differences in stem density ($F_{(3,12)} = 0.32$, $P = 0.8083$), or branching ($F_{(3,12)} = 1.86$, $P = 0.1896$). The only difference was in stem length ($F_{(3,12)} = 14.33$, $P = 0.0003$). Plants treated with Pristine + DAP had significantly longer stems than those treated with Bravo or the untreated controls. Plants treated with Pristine only also had longer stems than those in the untreated control plots (Fig. 3). Similarly to the 2015 trial, we observed no fungicide effects on premature flowering.

There were some other differences between the two replicated trials. In 2015, the plots treated with Bravo had significantly more leaves in October than those treated with Pristine + DAP ($F_{(3,12)} = 7.049$, $P = 0.006$)(Fig. 4). This was not the case in 2016 when we observed no significant differences in leaf retention among the treatments ($F_{(3,12)} = 1.11$, $P = 0.3838$)(Fig. 5).

Fig. 3. Mean stem length (cm). Lines are standard error of the mean. Letters which are different denote significant differences ($P < 0.05$). Data from 2016 trial.

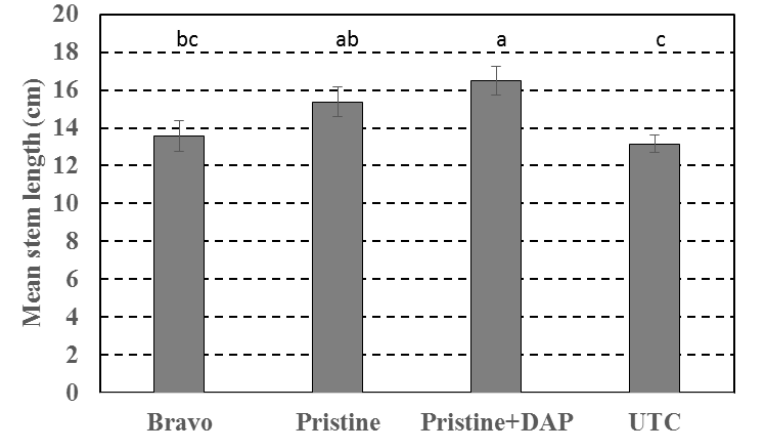


Fig. 4. Mean percent leaf retention. Lines are standard error of the mean. Data from 2015 trial.

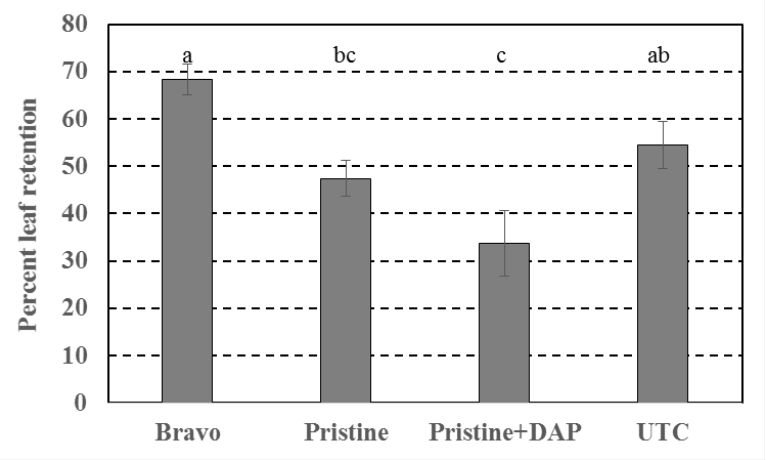
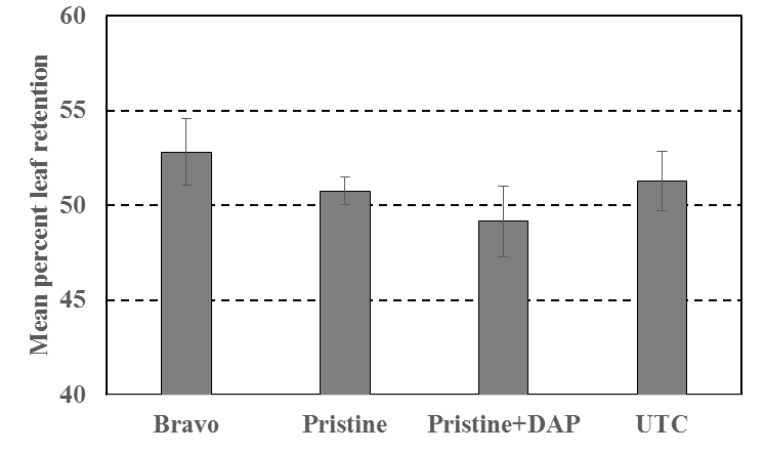


Fig. 5. Mean percent leaf retention. Lines are standard error of the mean. Data from 2016 trial.



As far as fungal induced leaf spot, in 2015 we did observe a treatment effect ($F_{(3,12)}$ = 3.32, P = 0.0569). Figure 6 shows that Pristine + DAP fertilizer resulted in more leaf spot than either Bravo or Pristine without fertilizer. A similar result was observed in 2016 when Pristine + DAP-treated plots had more leaf spot than Bravo-treated plots ($F_{(3,12)}$ = 2.90, P = 0.0787)(Fig. 7).

Fig. 6. The effects on the fungicide treatments applied in 2015 on fungal leaf spot incidence. Letters which are different denote significant differences (P < 0.05). Data from 2015 trial.

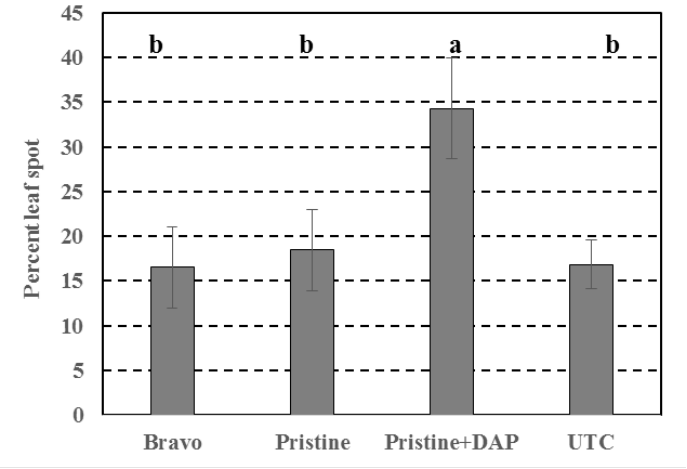
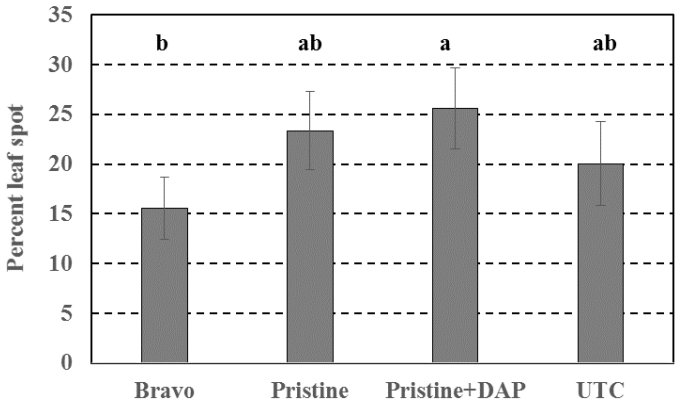


Fig. 7. The effects on the fungicide treatments applied in 2016 on fungal leaf spot incidence. Letters which are different denote significant differences (P < 0.05). Data from 2016 trial.



Species in five groups of sap feeding insects were collected in sweep samples in 2016. The most abundant group was leafhoppers (five species). We also collected tarnished plant bugs, aphids, lygaeids, and weevils in sweep samples. There were significant differences among the treatments. Plots treated with Pristine + DAP had significantly more leaf hoppers ($F_{(3,12)}$ = 6.84, P = 0.0061)(Fig. 8) and tarnished plant bug ($F_{(3,12)}$ = 4.70, P = 0.0216)(Fig. 9) than Pristine alone or Bravo. Aphids were most abundant in plots treated with Bravo ($F_{(3,12)}$ = 4.67, P = 0.022); while lygaeids were most abundant in plots treated with Pristine ($F_{(3,12)}$ = 1.91, P = 0.1820) (Fig. 9). There was no significant difference in the number of weevils.

Fig. 8. Effect of fungicide treatment on leafhopper abundance. Letters which are different denote significant differences ($P < 0.05$). Data from 2016 trial.

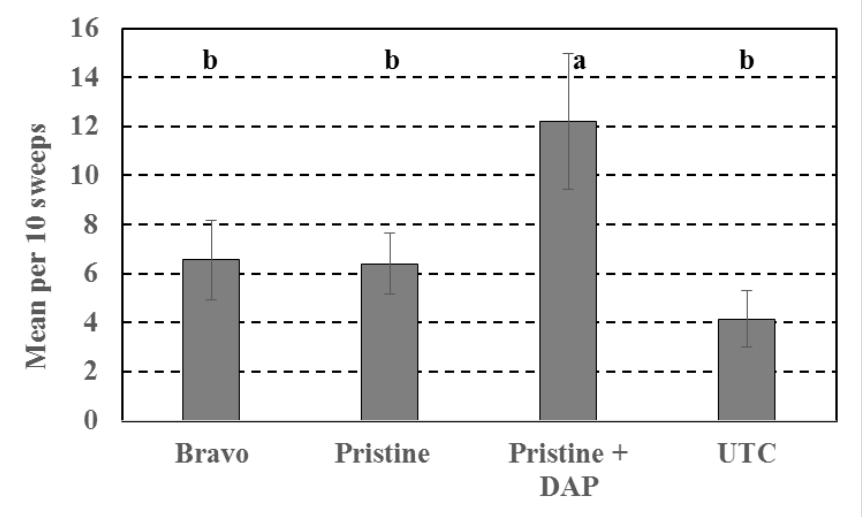
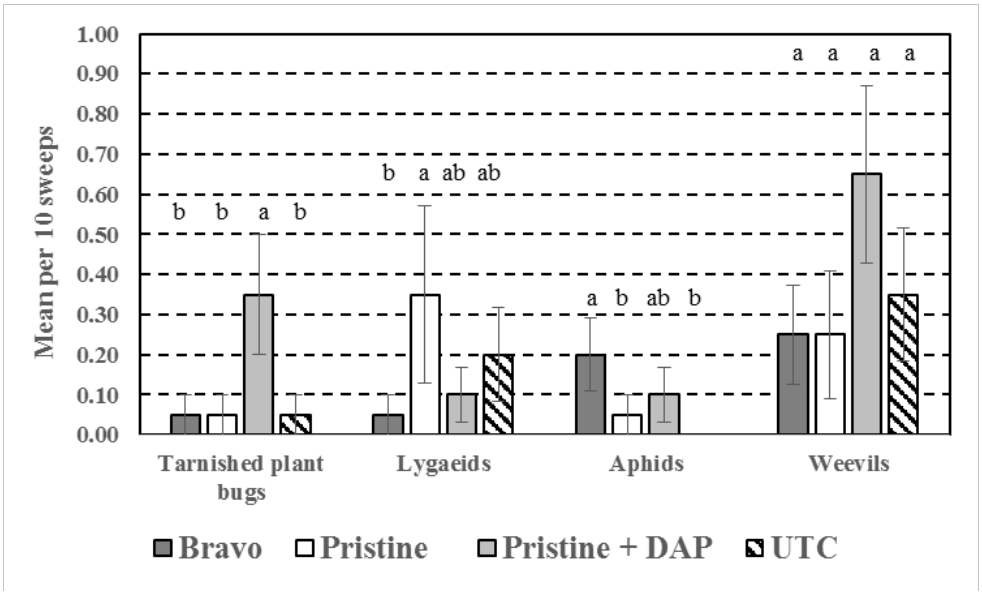
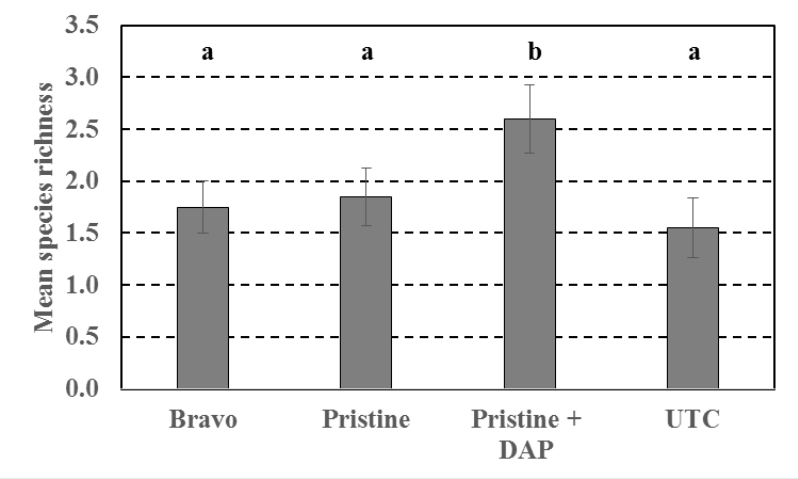


Fig. 9. Effect of fungicide treatment on abundance of tarnished plant bug, aphids, lygaeids, and weevils. Letters which are different denote significant differences ($P < 0.05$). Data from 2016 trial.



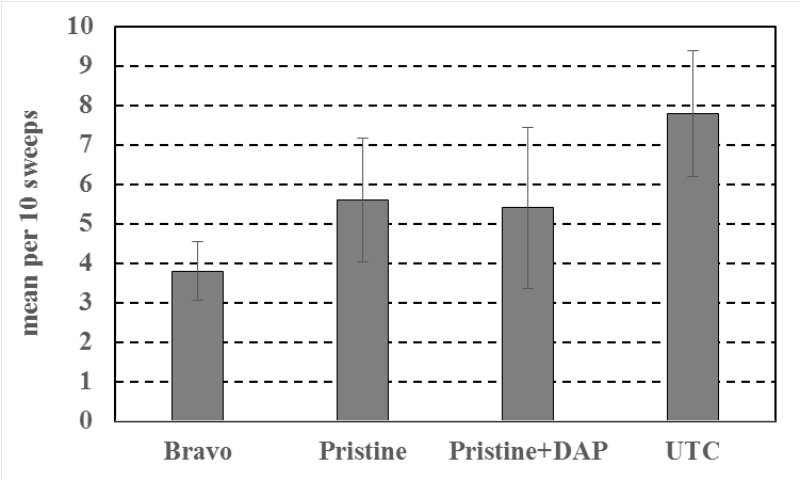
In regards to sap-feeding insect species richness, plots treated with Pristine + DAP had a significantly greater number of species captured than Bravo, Pristine alone, or the untreated control plots ($F_{(3,12)} = 5.16$, $P = 0.0161$)(Fig. 10).

Fig. 10. Effect of fungicide treatment on the number of sap-feeding insect species captured (richness). Letters which are different denote significant differences ($P < 0.05$). Data from 2016 trial.



These results are in contrast to our 2015 results; we did not observe any effects of the fungicide treatments ($F_{(3,12)} = 2.294$, $P = 0.129$)(Fig. 11). It is interesting, that although not significant, there were more of the four species of sap-feeding insects in the non-treated control plots than the fungicide treated plots in 2015.

Fig. 11. Effect of fungicide treatment on abundance of sap-feeding insects. Lines are standard error of the mean. Data from 2015 trial.



The results of the analysis of foliar nutrients are in Table 1 and are somewhat different than what we observed in 2015. In 2016 there were no significant differences noted in levels of nitrogen, calcium, potassium, or phosphorus. There were significant differences in levels of aluminum ($F_{(3,12)} = 5.82$, $P = 0.0108$) and iron ($F_{(3,12)} = 10.35$, $P = 0.0012$). Plots treated with Pristine alone had significantly more foliar aluminum and iron than Bravo or Pristine + DAP-treated plots and mean separation showed more magnesium ($F_{(3,12)} = 2.85$, $P = 0.082$) in plots treated with Pristine compared with those treated with Pristine + DAP.

In 2015 there were significant differences in levels of nitrogen, calcium, potassium, phosphorus, and aluminum among the treatments. Pristine + DAP treatments had significantly more nitrogen ($F_{(3,12)} = 8.44$, $P = 0.0028$) and phosphorus ($F_{(3,12)} = 8.08$, $P = 0.0033$) than the other treatments. There was also a significant difference in levels of potassium in Bravo-treated plots compared with plots treated with Pristine ($F_{(3,12)} = 4.719$, $P = 0.0304$), and mean separation indicated more aluminum in Bravo-treated plots compared with plots treated with Pristine + DAP ($F_{(3,12)} = 2.00$, $P = 0.1675$) and higher levels of calcium in the Pristine + DAP treatment than Pristine alone ($F_{(3,12)} = 2.67$, $P = 0.0951$)(Table 1).

Correlation analysis was conducted to determine if associations exist between leaf nutrients and pests. As far as inter-nutrient associations go we found a significant ($P < 0.05$) negative correlation between boron and magnesium and phosphorus (r = -0.485, -0.629; respectively. Positive associations were observed between boron and aluminum, iron, and manganese (r = +0.456, +0.521, +0.556; respectively). A negative association was observed between manganese and phosphorus (r = -0.477), but a positive association between manganese and aluminum (r = +0.494). A positive association was observed between zinc and copper (r = +0.631).

A positive association was observed between leaf spot intensity and boron (r = +0.628) and a negative association between leaf spot intensity and calcium (r = -0.572). A negative relationship was observed between manganese and weevil incidence (r = -0.448), and between tarnished plant bug and magnesium (r = -0.468). There was also a negative association between aphids and lygaeids (r = -0.511), but a positive association between leafhoppers and tarnished plant bugs (r = +0.540).

CONCLUSIONS: Pristine and DAP in both prune years appears to enhance leaf spot disease; although, we cannot determine at this point if yield is also depressed by the combination of Pristine and DAP until the end of 2017. We found a significant and diverse community of sap-feeding bugs colonizing blueberry. Fungicide applications affected sap-feeding bug incidence in 2016, but not in 2015. In 2015, the Pristine and DAP treatment again enhanced pests, this time sap-feeding bugs.

Table 1. Foliage analysis.

Treatment	(%) N	(%) Ca	(%) K	(%) Mg	(%) P	(ppm) Al	(ppm) B	(ppm) Cu	(ppm) Fe	(ppm) Mn	(ppm) Zn
2015											
Pristine	1.54b	0.40b	0.46b	0.20a	0.12b	80.46ab	25.40a	4.04a	36.70a	917.40a	12.60a
Pristine + DAP	1.87a	0.49a	0.49ab	0.19a	0.16a	61.42b	23.54a	3.72a	40.84a	1040.20a	14.02a
Bravo	1.62b	0.43ab	0.52a	0.18a	0.13b	91.52a	25.72a	4.30a	44.24a	1197.20a	15.10a
UTC	1.59b	0.46ab	0.48b	0.21a	0.12b	75.82ab	25.26a	4.18a	38.12a	947.60a	14.56a
2016											
Bravo	1.73a	0.36a	0.49a	0.17ab	0.13a	75.76b	20.76a	5.42a	29.16b	369.00a	12.76a
Pristine	1.83a	0.36a	0.51a	0.18a	0.14a	93.28a	22.24a	6.29a	35.26a	380.80a	17.86a
Pristine + DAP	1.82a	0.33a	0.50a	0.16a	0.14a	73.62b	20.68a	16.16a	30.86b	361.80a	16.12a
UTC	1.82a	0.36a	0.50a	0.16a	0.14a	70.72b	19.86a	5.60a	31.20b	296.00a	13.68a

Study 4. <u>Biology of blackheaded fireworm in Maine wild blueberry</u>.

OBJECTIVES: Blackheaded fireworm (*Rhopobota naevana*) is a serious pest of cranberry production. In recent years, Maine blueberry growers have become concerned that this pest may also be a problem in wild blueberry production. In cranberry, the larvae feed on both foliage and fruit and, if left uncontrolled, can significantly reduce yield. In cranberry, there are two separate generations of larvae (maybe even three) each season, and the 2nd generation is the more damaging one since their numbers are normally much greater and the plants are developing their fruit at that time.

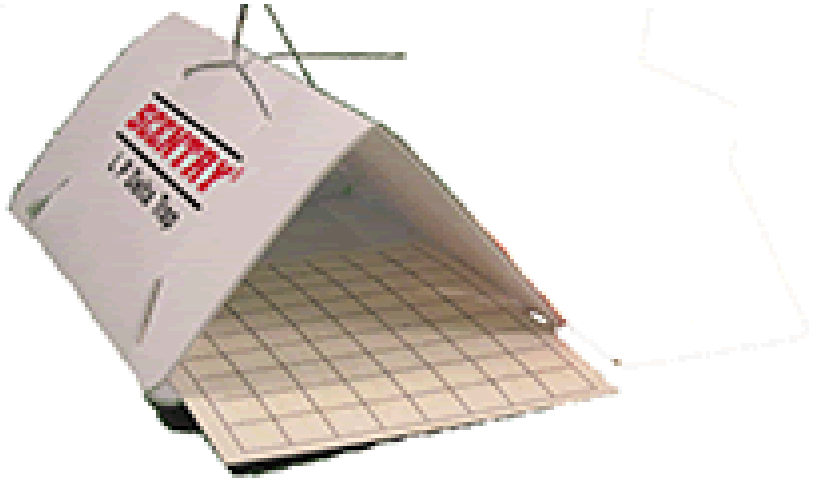
In cranberry, larvae are active from mid- to late-May until mid- to late-June, and again starting generally in mid- to late-July when a 2nd generation begins. The damaging stage of the blackheaded fireworm is the larval stage. Larvae are green-yellow or pale yellow, and their heads are black and shiny (Fig. 1). When mature (2 to 3 weeks after hatching), they measure roughly 1/3-inch in length. Adults (Fig. 2) are very small, only about 1/4-inch in length, dark in color, with alternating light and dark gray-brown bands on their forewings. Very little is known about the biology of this pest in wild blueberry. The purpose was to begin to fill in the gaps in our understanding particularly concerning the number of generations.

Fig. 1. Blackheaded fireworm larva on cranberry.

Fig. 2. Blackheaded fireworm adult.

METHODS: Beginning on 16 June, Sentry® traps baited with a pheromone (Fig. 3) were distributed in six fields in Washington Co. which grower reports had indicated had been infested with larvae in early spring. There was one trap per field. Traps were checked weekly for seven weeks for adults.

Fig. 3. Sentry® trap baited with pheromone for blackheaded fireworm.



RESULTS AND CONCLUSIONS: Over the seven week sampling period, only one possible adult blackheaded fireworm was seen in a pheromone trap. The specimen was embedded in Tanglefoot® making it difficult to make a confirmed species ID. However, during our trapping, it did appear that only one larval generation occurred, with a summer flight of moths occurring in June.

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

METHODS: On 15 July 2015 at Jonesboro, we marked 100 stems per site, 50 with tip midge (TM) infestation (pink flags) as evidenced by leaf curls and 50 without infestation (yellow flags). On 5 October 2015 we cut 25 stems from each treatment, brought them into the laboratory and counted the number of flower-bud clusters on each stem. Twenty-five marked stems of each treatment were left in the field at each site. On 20 May 2016 the remaining stems from our 2015-2016 trial were cut and brought into the laboratory to determine the number of flowers that developed from individual flower-bud clusters. The trial will be repeated in 2016-2017. Stems were selected and marked on 1 August 2016. Stems were cut for analysis of flower-bud clusters on 5 October 2016. The remaining stems will be evaluated for development of individual flowers in the spring of 2017.

Another focus of our investigations has been the development of economic thresholds. Based upon the amount of crop loss from TM infestations estimated over four previous years (2010-2011 and 2015-2016). An estimate of the percent of stems infested relative to the cost of crop loss and the cost of insecticide control was determined to assess economic thresholds. These relationships were for this 2016 report, but will be updated when the flower number per stem are estimated in the spring of 2017.

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

There was no significant difference in the number of flower-bud clusters ($F_{(1,48)} = 0.16$, $P = 0.6897$) in our 2011-2012 trial (Fig. 1). When individual flowers were counted in 2012, there appeared to be a trend towards more flowers on tip-midge damaged stems; however, the difference was not significant ($F_{(1,48)} = 2.83$, $P = 0.0967$)(Fig. 2). In both our trials begun in 2012 there was a significant difference in the number of flower-bud clusters (ANOVA, CRD; $F_{(1,48)} = 5.0$, $P = 0.03$, Jonesboro; $F_{(1,48)} = 4.22$, $P = 0.0454$, Orland) per stem between stems with and without tip midge damage (Fig. 1). Stems without damage had significantly more flower-bud clusters. And, stems with tip-midge damage developed fewer flowers than undamaged stems; although, at our Jonesboro site the difference was not significant ($F_{(1,48)} = 2.73$, $P = 0.1050$, Jonesboro; $F_{(1,48)} = 6.18$, $P = 0.0164$, Orland)(Fig. 2).

The count of flower-bud clusters in our 2015-2016 trial showed there was a significant difference in the number of flower-bud clusters (ANOVA, CRD; $F_{(1,48)} = 5.56$, $P = 0.0225$). Stems without damage had significantly more flower-bud clusters than non-infested stems of the same clone. And, stems with blueberry tip midge infestation developed significantly fewer flowers then those without tip midge infestation (ANOVA, CRD; $F_{(1,48)} = 13.53$, $P = 0.0006$)(Fig. 2).

The initial evaluation from the current 2016-2017 trial again showed no significant difference in the number of flower-bud clusters (ANOVA, CRD; $F_{(1,48)} = 0.43$, $P = 0.5172$)(Fig. 1) between infested compared to non-infested stems.

Fig. 1. Bar graph comparing mean number of flower-bud clusters between stems with and without tip-midge damage. Data collected from trials conducted in 2010-2011, 2011-2012, 2012-2013, 2015-2016, and 2016-2017.

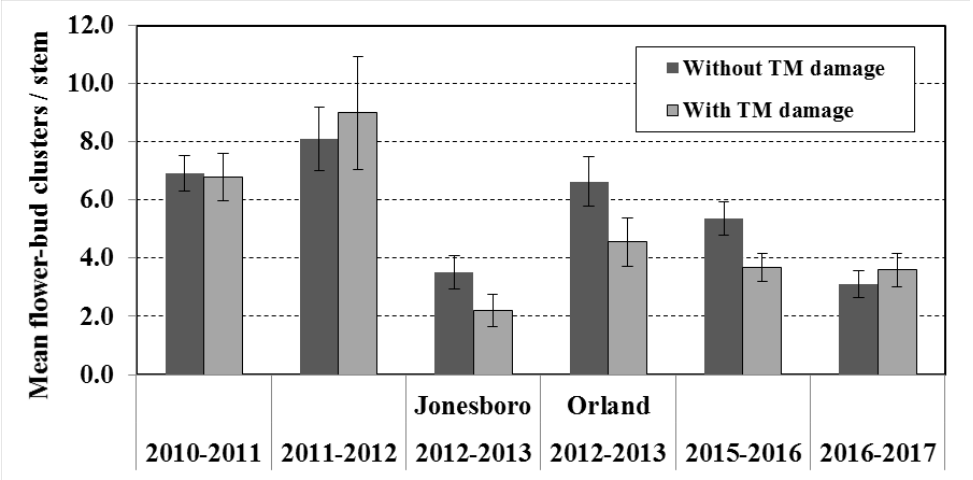
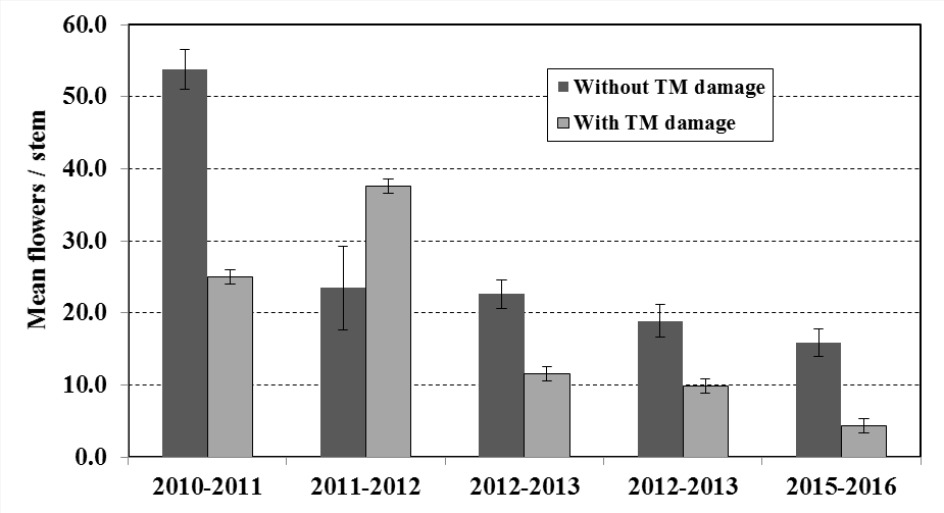
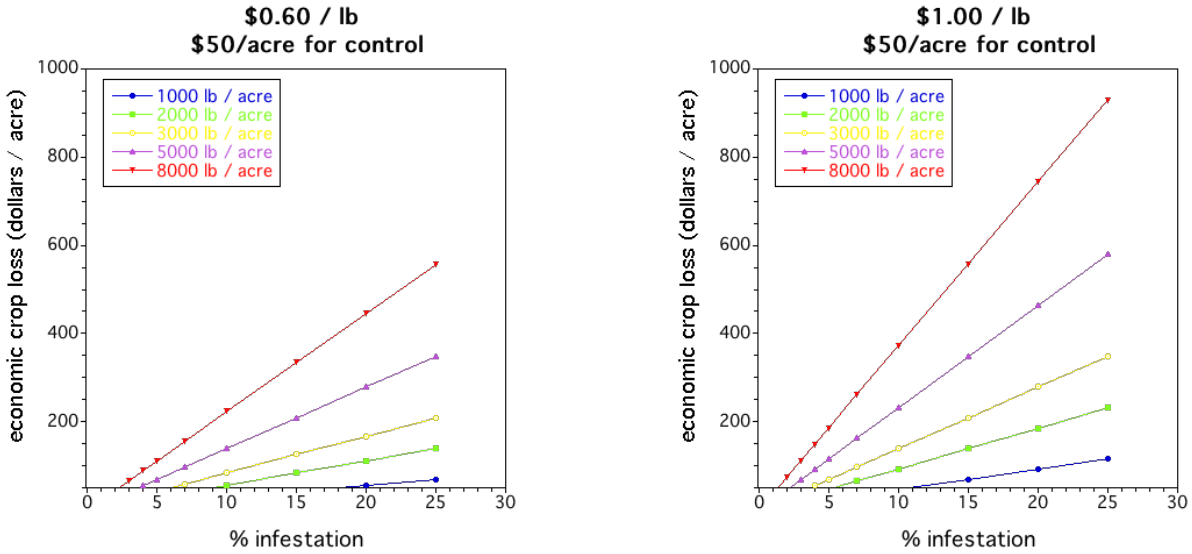


Fig. 2. Bar graph comparing mean number of individual flowers per stem between stems with and without tip-midge damage. Data collected from trials conducted in 2010-2011, 2011-2012, 2012-2013, and 2015-2016.



The results of our economic threshold analysis are shown in figure 3. The graphs show economic thresholds if, on average, crop loss due to infested stems is 44.3% (2010-2011 and 2015-2016). It can be seen that for the average level of production currently in Maine (3,000 lbs/acre) the economic injury level is about 7-10% infestation (the level where the cost of control equals the cost of crop loss). This analysis will be finalized when the flower number per stem crop loss is estimated (in spring of 2017) from the 2016 field season.

Fig. 3. Economic threshold calculations for control costs of $50.00/acre and two prices for the crop ($0.60/lb and $1.00/lb) under varying levels of production.



Study 6. <u>Insect survey on the weed, St. John's wort, in wild blueberry fields.</u>

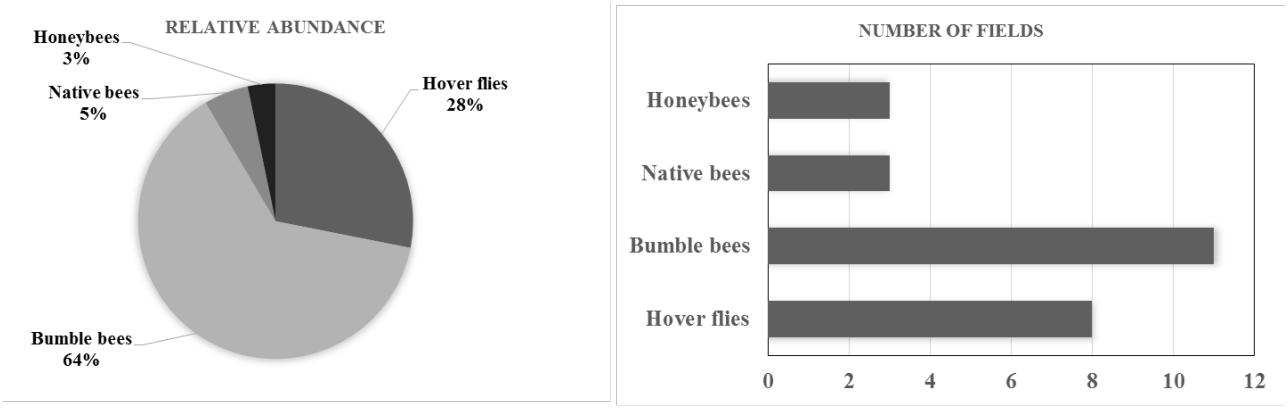
OBJECTIVES: This was the 4th year of a study initiated in July 2013. The purpose is to assess the spread of the invading weed St. John's wort (**SJW**), *Hypericum perforatum* L. and the subsequent colonization of this weed's natural enemies.

METHODS: We examined 15 fruit-bearing wild blueberry fields for the presence of flowering SJW. Our survey fields were located in the Mid-coast (6) and Downeast (9) blueberry growing regions. Mid-coast sites were sampled on 7 July; Downeast sites were sampled on 25 July. At each site, we recorded the number and type of pollinators (honeybees, bumble bees, other native bees, and hover flies) present on each of three to five plants by recording visitation on each plant in one minute of observation. We also collected three plants from each field by quickly and carefully bagging the stalks which were then frozen for future evaluation of predators and herbivores. At each field, we characterized the percentage of the field with SJW and rated abundance as scattered or clumped. Field size, and the latitude and longitude of fields were determined so that a geographic information system (GIS) analysis can be conducted at a later date.

RESULTS: Of the 15 crop fields we examined, 11 (73.3%) contained SJW. In fields with flowering SJW, SJW was well under 5% of the total vegetation in all fields examined and plants were scattered in the field or along field edges. Mean patch size was 3.2 ft^2. There was a linear positive relationship between % cover of St. John's wort and the average patch size (r = +0.614, P = 0.015). This means that higher infestations of this weed are characterized by larger patch sizes.

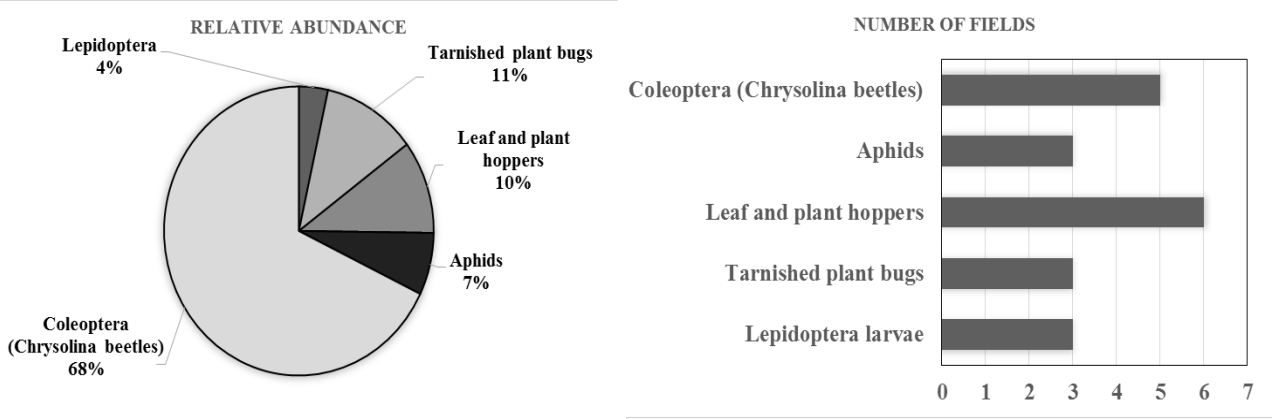
One minute observations for pollinators and other beneficial insects were completed for 45 plants. The most common native pollinators were hover flies and bees (primarily bumble bees, but also other native bees, and honeybees) (Fig. 1). Hover flies were seen on 22 of 45 plants (43 specimens from 8 fields). A total of 97 bumble bees were noted on 21 of 45 plants; bumble bees were seen in all 11 fields.

Fig. 1. Relative abundance pollinators on St. John's wort in blueberry fields (native bees refer to native bees other than bumble bees).



To assess herbivores and predators, thirty-three plants were collected from 11 fields. The most commonly associated insects were those in the order Coleoptera with 82 specimens found on 13 plant stalks (Fig. 2). It is interesting to note that 78 of them were beetles in the genus *Chrysolina*. This genus contains biocontrol agents that have been specifically released to control SJW. One species, *Chrysolina quadrigemina,* has been released in the western US as a biological control agent. They were seen on plants in five of 11 fields, all in the mid-coast region. Of the 78 specimens, 63 came from one field. Native insect herbivores were dominated by Hemiptera and included aphids (four in three fields), plant hoppers and leaf hoppers (12 in six fields), and tarnished plant bugs (13 in three fields) (Fig. 2).

Fig. 2. Relative abundance and number of sampled fields with herbivores.



Spiders were the most commonly collected predators with a total of 19 spiders collected from 11 stalks. We also collected a small number of ants (5).

CONCLUSIONS: Both exotic natural enemies released by the USDA for biological control of this weed, and endemic Maine herbivores or parasites, were surveyed. St. John's wort is not only a threat to the blueberry industry as a weed that competes for space, water, and nutrients; but it also is a threat to mammal herbivores. Ingestion by livestock or wildlife can cause photosensitization, central nervous system depression, spontaneous abortion, and can lead to death. On average, St. John's wort is in very low densities in blueberry fields, either due to intensive herbicide programs or because of herbivory by introduced and natural enemies.

RECOMMENDATIONS: The study of long-term trends in parasitism of BMF is not directly applicable to management of the blueberry fly, but it does help explain what regulates its densities such as parasitoids, the frequency of outbreaks, and the relationship of threshold fly captures and the population growth of this very important pest. We plan on continuing this study for ONE MORE year so that a sound basis of this pest's population dynamics can be acquired after 20 years of study.

We have conducted this study of long-range movement BMF for four years. Next year, 2017, will be the last year of this study. We hope to assess movement out to distances of one to two miles.

Our data suggest that it is the fertilizer that is causing the disease and sap-feeding bug enhancement and not the fungicide applications. Next field season will allow us to finish this study and make firm conclusions about the effects of foliar applied fungicides on leaf spot and

sap-feeding insect pest incidence. Nutrients that might cause these pest enhancements are a lack of calcium and an excess in boron for the case of leaf spot disease; and a lack of manganese and magnesium for enhancement of weevils and tarnished plant bug.

We will continue to study the biology of blackheaded fireworm in 2017. We will attempt to distribute our pheromone traps in more fields in order to pick up the adults in the traps.

Our studies of blueberry tip midge have demonstrated that blueberry plant response in flower-bud production can be quite variable often with no significant difference in flower-bud production on infested compared to non-infested stems. In three of the six studies (five years, six locations) in which we have conducted this trial, there was a significant reduction in the numbers of flower-bud clusters produced on infested stems. However, when flower number per stem was assessed in five studies (four years, five locations), four of five studies showed a significant potential crop loss in terms of reduced numbers of flowers per stem. We also found that for an average level of production currently in Maine (3,000 lbs/acre) the economic injury level is about 7-10% infestation (the level where the cost of control equals the cost of crop loss). This is currently a much greater an infestation level than is generally found in Maine when considering tip midge infestation over an entire field.

We have refined our search for a good bee colony level detection unit. Research in 2017 will be to develop a portable unit that farmers and beekeepers can use. We will design an activity monitor based on the 10.5 GHz radar. This will involve measuring signal strength with a simple rectifier circuit recording data on a simple data logger. There will be no need for signal processing (computers). We estimate the cost of the prototype will be less than $50.

We have no recommendations for biocontrol of St. John's wort since it appears to be naturally taking place. However, since the weed is not in high infestation rates it should also be recognized that this weed does provide a food resource for bees, especially bumble bees after bloom.

ENTOMOLOGY

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4. III. TITLE: Biology of spotted wing drosophila, 2016.

Study 1. ***Optimal sample size for determining fruit infestation by spotted wing drosophila.***

OBJECTIVES: Current sampling of fruit for the detection of **SWD** (spotted wing drosophila) larvae has been based upon taking a representative sample of fruit, usually three cups in the field. The fruit samples are then processed for the presence of larvae using the saltwater crush test. A study was conducted in 2016 to determine what the optimal number of 1-cup samples might be to accurately assess a field for infestation.

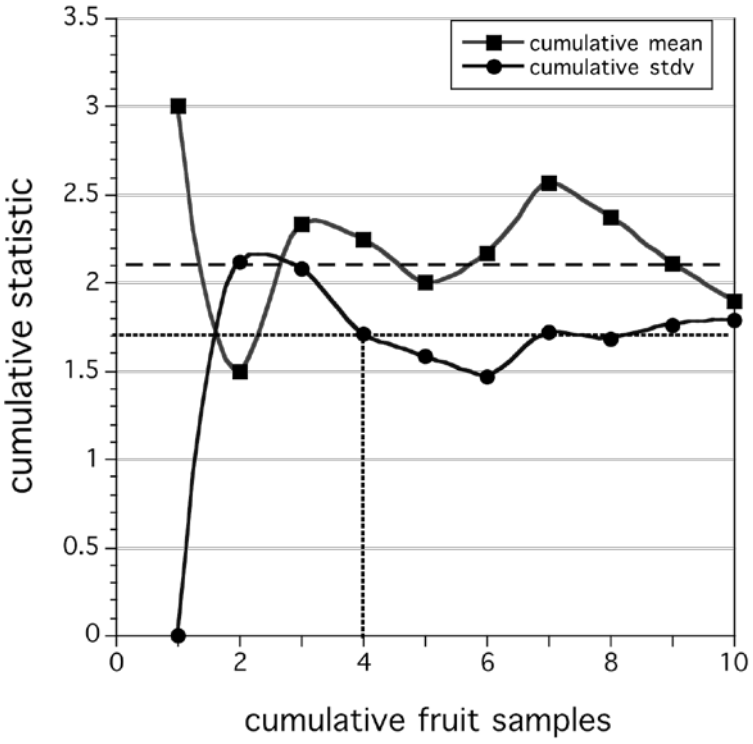
METHODS: Ten sequential 1-cup samples of fruit were sampled in a wild blueberry field during a period when SWD larval infestation in the fruit had been detected. Each sample was

then assessed for the number of larvae using the saltwater crush test delineated in the Maine wild blueberry factsheet # 210-Spotted Wing Drosophila: Pest Biology and IPM Recommendations for Wild Blueberries (https://extension.umaine.edu/blueberries/factsheets/insects/210-spotted-wing-drosophila/). The cumulative mean and standard deviation was then plotted against sequential number of samples taken. This plot was then visually inspected to assess an optimal sample size suggested by a stabilization of the standard deviation.

RESULTS: Figure 1 shows the results of the sequential sampling effort conducted in 2016. The plot suggests that the standard deviation stabilizes with a minimum of four 1-cup samples of fruit. This optimal sample size will vary according to the level of larval infestation in the field.

CONCLUSIONS: The pattern usually seen is that the lower the infestation rate, the more fruit samples are needed to estimate the larval infestation with a fixed level of precision. However, for our research sampling a sample size of four 1-cup samples will be used for 2017. Also, in 2017, we plan to sample several fields throughout the season in order to complete an optimal sample size criterion.

Fig. 1. Cumulative mean and standard deviation from sequential sampling of infested fruit.



Study 2. <u>Survey of parasitoids of spotted wing drosophila</u>.

OBJECTIVES: We have found that predators are very active in wild blueberry fields, consuming up to 100% of the SWD pupae on the soil in a field (see Study 3 of this report). However, we have not found parasitoids of SWD naturally occurring in wild blueberry fields. In

2016 we continued to search of parasitoids that might attack SWD and parasitize the immature stages.

METHODS: We set out dishes filled with SWD-infested fruit in three fields across Maine including Blueberry Hill Farm in Jonesboro, a commercial field in Waldoboro, and an organic field in Stockton Springs. The infested fruit dishes consisted of a 7 x 3-cm cup filled with sand and lowbush blueberries. The cups were placed into a sleeve cage with adult SWD where they were allowed to oviposit for one week before the cups were placed in the field. Every two weeks, four fruit cups were set out at each location and were left in place for two weeks. The cups were set along wooded field edges. Three of the cups were set on the ground and the fourth was elevated 2.5 feet above the ground using our SWD trap stands. All of the cups were inside of Tupperware® containers with coarse mesh screening on the sides and a red shade cloth covering the lid to prevent overheating. After removing them from the field, the cups were placed inside of Tupperware® containers with small pin holes for air circulation and placed inside a tote with damp paper towels. The first infested fruit traps were set outside on 1 August in Stockton Springs, 2 August in Jonesboro, and 4 August in Waldoboro. Jonesboro had traps until 12 October, Stockton Springs until 12 September, and Waldoboro until 17 August. A total of 56 infested fruit cups were deployed during the course of this study; 24 at Jonesboro, 32 at Stockton Springs, and 8 at Waldoboro. All infested fruit cups were kept in the lab for at least two months after being brought back from the field.

RESULTS: Unlike 2014 and 2015, we had parasitoids emerge from several of our traps. A total of four traps from Waldoboro and Stockton Springs had parasitic Hymenoptera. No parasitoids emerged from any traps in Jonesboro. The parasitoids from Stockton Springs were caught in traps that were picked up on 29 August and 13 September and parasitoids from Waldoboro were from traps picked up on 17 and 29 August.

CONCLUSIONS: We will send off our samples of parasitoids for species identification so that we might learn more about their biology. We still do not know if these parasitoids were a result of attacking SWD or native drosophila that were using wild blueberries as a host. If we continue to catch these parasitoids in future years we may be able to determine if SWD is the host.

Study 3. Field predation of spotted wing drosophila pupae.

OBJECTIVES: This is the third and final year of a study that has been quantifying the natural predation of SWD pupae in wild blueberry fields.

METHODS: Spotted wing drosophila pupae were collected from our laboratory colony and examined under a microscope to verify the presence of living pupae. We froze and killed all living pupae for 24 hours prior to using them in our experiments. The freshly killed pupae were attached to two, 7cm strips of double-sided tape on top of a 9cm Petri dish. Each strip of tape had 10 pupae on it for a total of 20 pupae per plate.

In the field, the plates of pupae were set up in transects 3m apart from other plates and each transect was set 3m apart from the other transects. The plates were set up under one of three different treatments with two different controls (Fig. 1). The first treatment was a plate

covered in a duff layer under a coarse diameter wire cage to (1 x 1-cm openings) (cage treatment). The second treatment was also covered in a duff layer but without a cage (open duff treatment). The third was a dish set out in the open without a duff layer or cage which allowed access from all predators (open treatment). Our first control was a plate set inside a coarse cage covered with fine mesh to prevent access from any predators (mesh control). This last treatment acted like a control to verify that wind and rain were not dislodging the pupae. The second control was an open plate design but the pupae were glued to a piece of string with Elmer's® glue (string control). This allowed us to see if the pupae were actually being eaten or just moved off the plate. The open, cage, and open duff treatments were replicated ten times per field, and the controls were replicated three times per field each. A total of 720 pupae were inspected, set up, and monitored for predation at each field. The plates were picked up after two days and all remaining pupae were counted.

In addition to the plates with SWD pupae, we also set up 12 pitfalls traps to examine potential predators. The pitfall traps were arranged in a line transect with 3m between traps. The holes were dug using a post-hole digger and then a 20oz (6.35 x 11.43-cm) deli cup was set into the hole so that the lip was at ground level. The pitfalls were filled half way with soapy water and covered with a 17 x 17-cm tin rain cover propped up by nails. The soapy water was changed after two days and then picked up after five days.

This trial was replicated at two field sites. One site was located in a commercial organic blueberry field in Stockton Springs. The trial was set up on 17 August in a pruned field separated from a fruiting field by a rock wall. The second trial was set up on 24 August at the University of Maine's Blueberry Hill Farm in Jonesboro in a fruiting field.

RESULTS: As in previous years, pupae predation rates were high in both fields (Table 1, Table 2). In Jonesboro, predation was much higher in the open plates compared to the duff open and caged treatments. In this site, 100% of the pupae were eaten in the open treatments but only 33% of pupae were missing from the open duff plates, and only 11.5% of pupae were missing from the caged treatment. In Stockton Springs, the treatments had higher predation rates. 100% of pupae were missing from the open plates, 77% from the open duff, and 58% missing from the caged plates. All pupae were eaten from the string controls. Small pieces of the pupal cases were attached to the string which indicates the pupae were eaten and not just moved off the plate. No pupae were missing from the mesh controls at either site. However, the mesh came unglued for two of our controls in Stockton Springs and predators were seen inside the mesh; therefore, those were excluded from the results and only the intact control was used in the analysis.

The most common predators observed in our pitfall traps were ants, crickets, beetles, and harvestmen (Table 3). Figure 1 shows that these predator groups vary in relative abundance by year and location. Crickets were captured and used in laboratory trials to assess the daily SWD consumption rate by these potential predators.

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

Location	Treatment	Percent missing pupae
Jonesboro	Caged	11.5
Jonesboro	Open	100
Jonesboro	Open Duff	33
Jonesboro	Mesh Control	0
Jonesboro	String Control	100
Stockton Springs	Caged	58
Stockton Springs	Open	100
Stockton Springs	Open Duff	77
Stockton Springs	Mesh Control	0
Stockton Springs	String Control	100

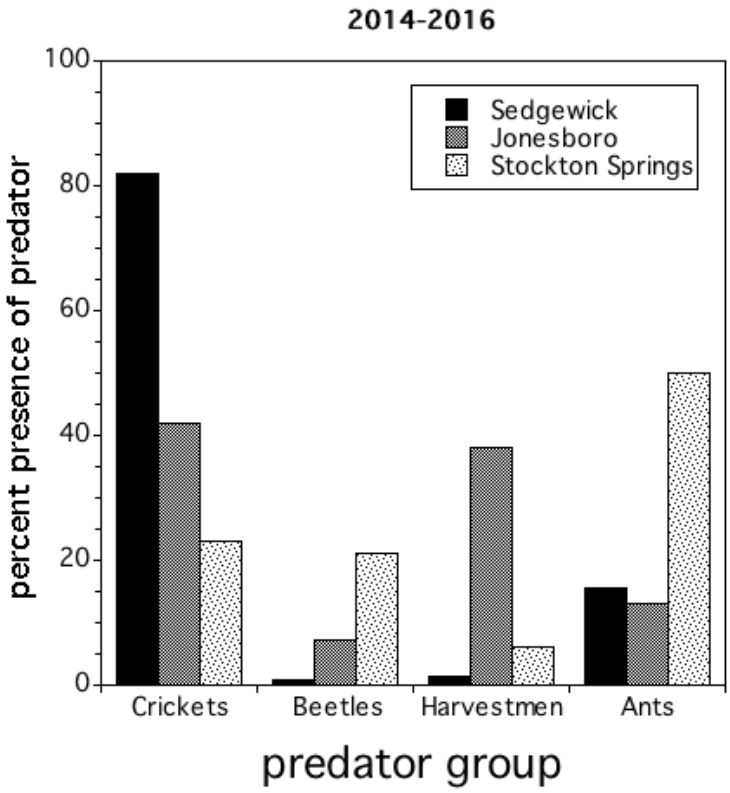
Table 2. SWD pupal predation in open environments (2014-2016).

Site	Year	Percent predation estimate
Jonesboro	2014	64.6
Sedgewick	2014	95.6
Jonesboro	2015	94.0
Stockton Springs	2015	100.0
Jonesboro	2016	100.0
Stockton Springs	2016	100.0

Table 3. Total numbers of predators and scavengers collected in all pitfall traps by field.

Predators/Scavengers	Stockton Springs	Jonesboro
Crickets	35	28
Beetles	2	17
Harvestmen	0	41
Ants	660	40

Fig. 1. Relative abundance of predator groups over the course of the three years of this study (2014-2016).



CONCLUSIONS: Natural predation does appear to be very high in wild blueberry fields. This predation is not enough to protect the crop from attack by SWD, but it probably does dampen population increases over time.

Study 4. <u>Predation on spotted wing drosophila pupae by crickets – a laboratory experiment</u>.

OBJECTIVES: Spotted wing drosophila (SWD) is a relatively new pest to the state of Maine. Since its introduction into the state, it has become a major pest of lowbush blueberries. Little is known about what predators may be utilizing them in Maine. Previous field studies we conducted have shown that large amounts of SWD pupae can be consumed in a short period of time. One of our previous lab studies documented beetles in the family Carabidae consuming up to 4.59 pupae per day. Crickets are a common scavenger in blueberry fields and so we tested if they were capable of consuming SWD pupae.

METHODS: Spotted wing drosophila pupae were collected from our lab colony and examined under a microscope to verify the presence of living pupae. We froze and killed all living pupae for 24 hours prior to using them in our experiments. Crickets were collected from a garden in Greenbush, ME 48 hours prior to the start of the experiment. They were held at room temperature in 1,100 ml deli cups with damp paper towels until the experiment began.

We ran three different trials in the lab. In the first trial, a single cricket was given 1, 3, 6, or 36 pupae. In the second trial, crickets were given 20, 40, 60 or 80 pupae. In the final trial crickets were given 60, 80, 100 or 150 pupae. Each pupal density was replicated five times per trial. Pupae were attached to 7cm strips of double-sided tape on top of a piece of filter paper. One piece of filter paper with pupae attached was place inside a Tupperware® container (10 x 6.5-cm) along with a 2cm square of damp sponge. A single cricket was added to each experimental arena. After 24 hours, the crickets were removed from the experimental arenas and all remaining pupae were counted. Pupae that were chewed, but not entirely consumed were counted as having been predated since they were no longer viable.

RESULTS: Crickets ate a large number of pupae in each of the trials (Table 1, Fig. 1). When given 60 pupae or less, crickets were capable of eating or destroying nearly every pupa. In one replicate, a single cricket was able to consume 91 pupae within 24 hours. We determined that crickets display a Type I functional response (Fig. 2). This suggests that crickets, despite their large consumption rate of SWD pupae, will probably not be a predator that regulates the population density over a long time horizon. This is not surprising, considering the omnivorous nature of crickets, characterized by prey switching. However, the consumption rate of crickets is much higher than two species of ground beetles that we tested (Fig. 3). Figure 3 shows consumption rates of SWD pupae over a range of SWD pupal densities by two species of ground beetles (these laboratory studies conducted in 2014). Another predator group that may provide a large degree of population regulation are ants. These predators are common and diverse in wild blueberry fields.

Table 1. Percent of pupae missing across all trials.

Number of pupae given	Trial	Percent missing pupae
1	1	100
3	1	100
6	1	100
36	1	98.89
20	2	100
40	2	100
60	2	97
80	2	92
60	3	91.67
80	3	73.44
100	3	61.2
120	3	45.20

Fig. 1. Pupae consumption by crickets across all trials.

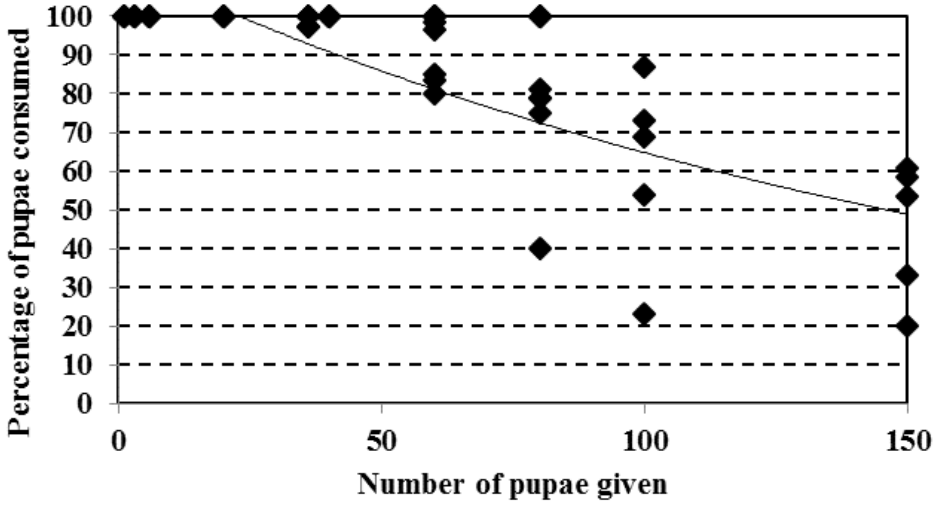


Fig. 2. Type I functional response of crickets to SWD prey.

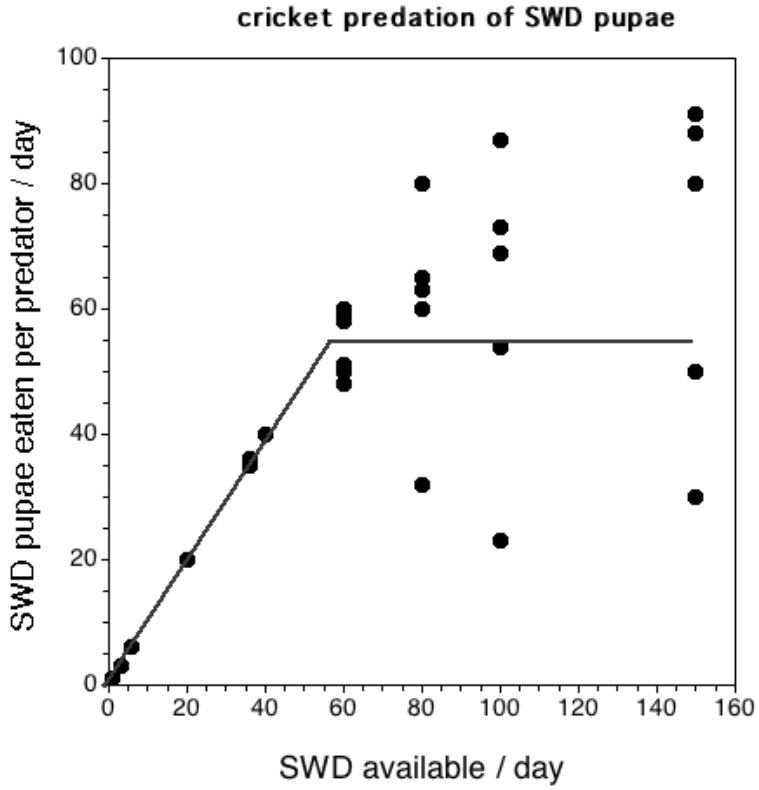
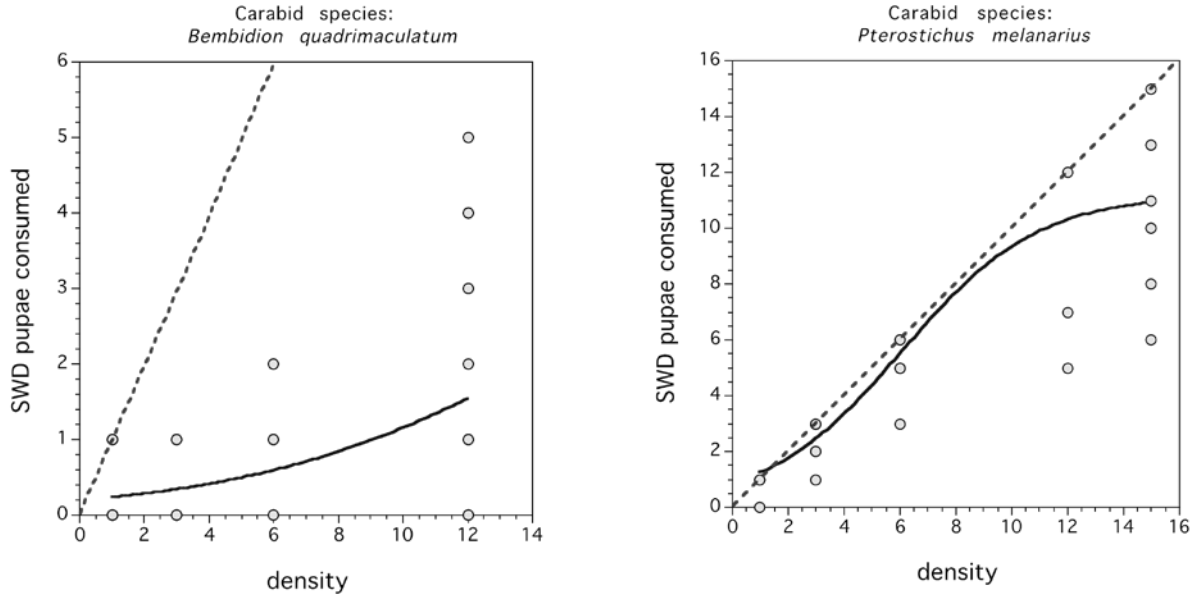


Fig. 3. Functional responses of two ground beetle species common in Maine wild blueberry fields. Data from 2014.

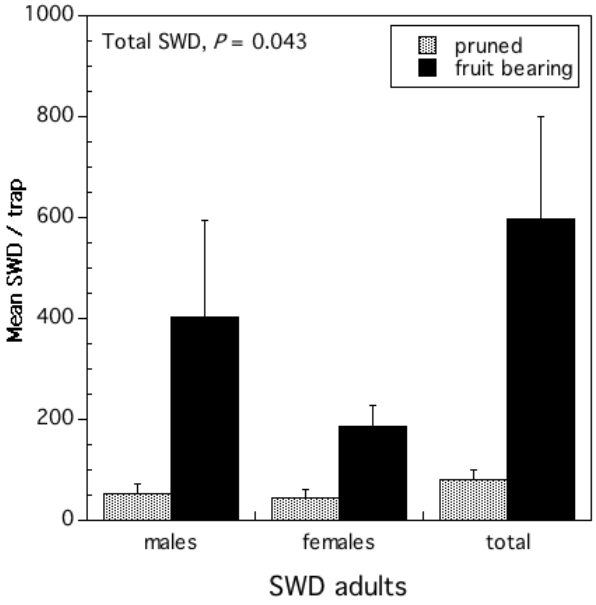


CONCLUSIONS: Many naturally occurring predators of SWD pupae characterize the wild blueberry agro-ecosystem. We suspect that the diversity of predators is very high and that this complex of species or community varies from field to field and even within a field, from year to year (see Study 3 of this report).

Study 5. <u>Infestation of wild fruit by spotted wing drosophila</u>.

OBJECTIVES: Spotted wing drosophila (SWD) is in invasive insect that attacks wild blueberries in Maine. In 2012, we found that SWD were common in prune fields, although not as abundant as in crop fields (Fig. 1). So, a question that arose is, how can SWD proliferate in prune fields. Use of alternative wild fruits was our first hypothesis. SWD has a very large host range which has helped make it a widespread and successful invader. Surveys from other states in the country have found dozens of different cultivated and wild hosts. Known wild hosts include blackberry, cherry, elderberry, and honeysuckle to name a few. We attempted to survey alternative wild SWD fruit hosts in Maine by focusing on wild fruit found adjacent to wild blueberry fields in 2015 and 2016.

Fig. 1. Abundance of spotted wing drosophila flies in prune and crop fields in 2012.



METHODS: In 2015 we collected non-blueberry wild fruit during a one-month period along 15 different blueberry fields. We tried to collect fruit at an additional 16th field, but no wild fruit could be found in the vicinity. We began sampling on 4 August 2015 which coincided with the beginning of our first SWD fly captures in blueberry fields. We collected wild fruit in each field until that field was harvested or until wild fruit could not be located. Our final wild fruit collection was 1 September 2015.

In 2016, we collected wild fruit during a six week period along nine different blueberry field edges. SWD generally first arrive in Maine in late July through August so we began sampling on 21 July. Our final wild fruit collection was 14 September.

In both years, after a wild fruit was collected, it was placed into a one ounce Solo® cup with a lid. It was labeled with a unique identifier and placed into a tote filled with dampened paper towels to provide humidity. The fruit was checked every other week for SWD development and emergence. In addition to collecting wild fruit from each of these sites, in 2015 we also deployed four SWD adult traps and took four ½ cup blueberry samples from each field weekly to check for SWD maggots in blueberries.

RESULTS: We collected a total of 1,817 fruits in 2015 and 2,246 fruits in 2016. Of those, 100 individual fruits had confirmed SWD infestation in 2015. This suggests an overall infestation rate of 5.50%. In 2016, we had 125 fruits infested yielding an overall infestation rate of 5.57%. In 2015 we found the following fruit bordering lowbush blueberry fields: blackberry (*Rubus fruticosus*), bunchberry (*Cornus canadensis*), mountain holly (*Ilex mucronata*), cherry (*Prunus* sp.), chokeberry (*Aronia melanocarpa*), dewberry (*Rubus flagellaris*), honeysuckle (*Lonicera* sp.), Juneberry (*Amelanchier canadensis*), raspberry (*Rubus* sp.), rose hips (*Rosa* sp.), and wild raisin (*Viburnum cassinoides*) (Fig. 2). In 2016, we collected all of those fruit except Juneberry and mountain holly and in addition, also collected a *Cornus* species, baneberry (*Actaea* sp.), bittersweet nightshade (*Solanum dulcamara*), Canada-mayflower (*Maianthemum canadense*), Virginia creeper (*Parthenocissus quinquefolia*), and winterberry (*Ilex verticillata*). No SWD

infestations were found in baneberry, chokeberry, Juneberry, rose hips, Canada-mayflower, Virginia creeper, winterberry, or wild raisin in either year. Infestation in cherry was only documented in 2015. In 2015 raspberry had the highest overall SWD infestation rate with nearly 11% of raspberries collected infested and blackberry followed with just over 9% infestation (Table 1). In 2016, blackberry and honeysuckle had the highest SWD infestation rate with 24.3% of blackberries collected infested and 22.34% of honeysuckle infested. The first recorded incidence of SWD infestation in wild fruit started on 11 August in 2015 and 4 August in 2016. SWD infestation increased during the course of this study (Fig. 3 & 4). We recalculated infestation rates by fruit type starting with the first date of SWD infestation and included all proceeding dates. These late infestation rates were much higher, with roughly 30% of raspberries and bunchberries infested in 2015 and with 30.43% of blackberries infested and 53.28% of invasive honeysuckle infested in 2016. The first adult SWD were captured in these fields starting on 21 July and 27 July for our southern and Downeast fields respectively in 2015. In 2016, we found our first adults on 26 July and 20 July for our southern and Downeast fields, respectively. We found the first SWD maggot infested blueberry samples on 1 September in 2015 and 17 August in 2016.

Table 1. *Infestation rates of wild fruit.*

Fruit	**Infestation rate 2015**	**Infestation rate 2016**	**Combined infestation rate (%)**	**# sites where fruit collected**
Baneberry	n/a	0	0	1
Bittersweet nightshade	n/a	1.25	1.25	1
Blackberry	9.05	24.40	17.16	7
Bunchberry	5.05	3.25	4.06	12
Mountain holly	4.40	n/a	4.40	1
Cherry	5.74	0	3.06	12
Chokeberry	0	0	0	11
Cornus sp.	n/a	4.38	4.38	2
Dewberry	6.52	3.54	4.40	5
Honeysuckle	1.89	22.34	15.11	6
Juneberry	0	n/a	0	3
Canada-mayflower	n/a	0	0	1
Raspberry	10.95	0.76	6.93	8
Rose hips	0	0	0	3
Virginia creeper	n/a	0	0	1
Wild raisin	0	0	0	4
Winterberry	n/a	0	0	2

Fig. 2. Distribution of wild fruit.

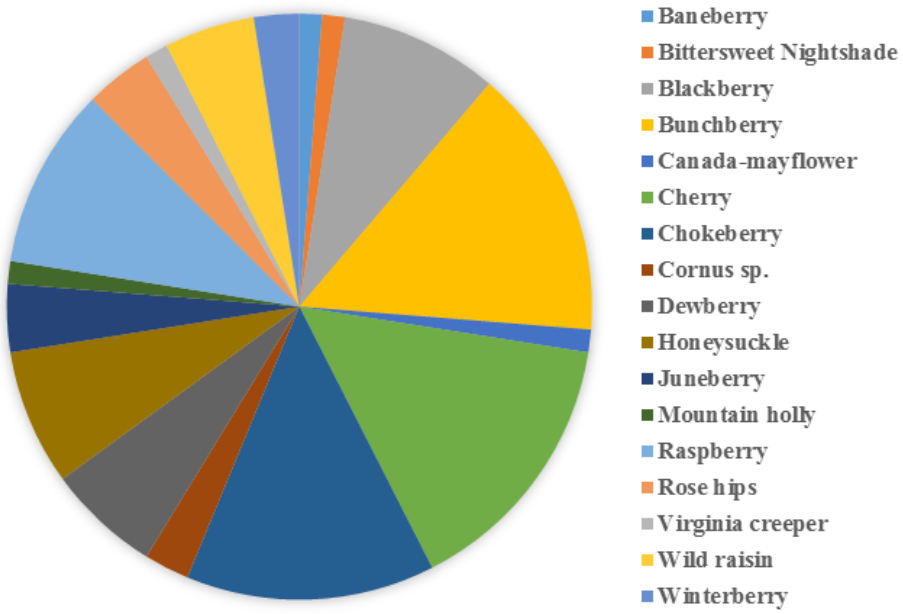


Fig. 3. Spotted wing drosophila infestation in wild fruit increased as the sampling period progressed in 2015. Dashed arrow is date when SWD infestation of blueberry started to increase rapidly.

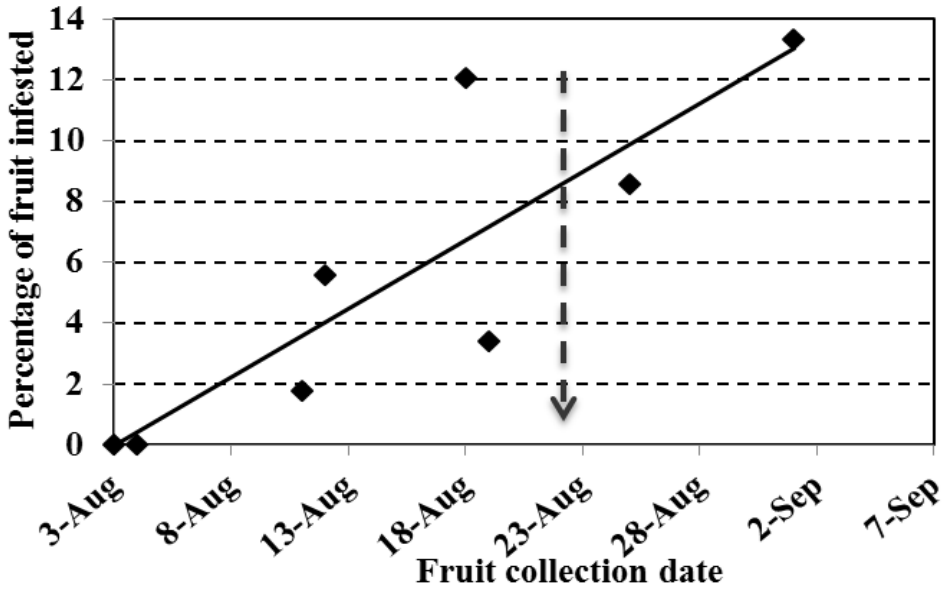
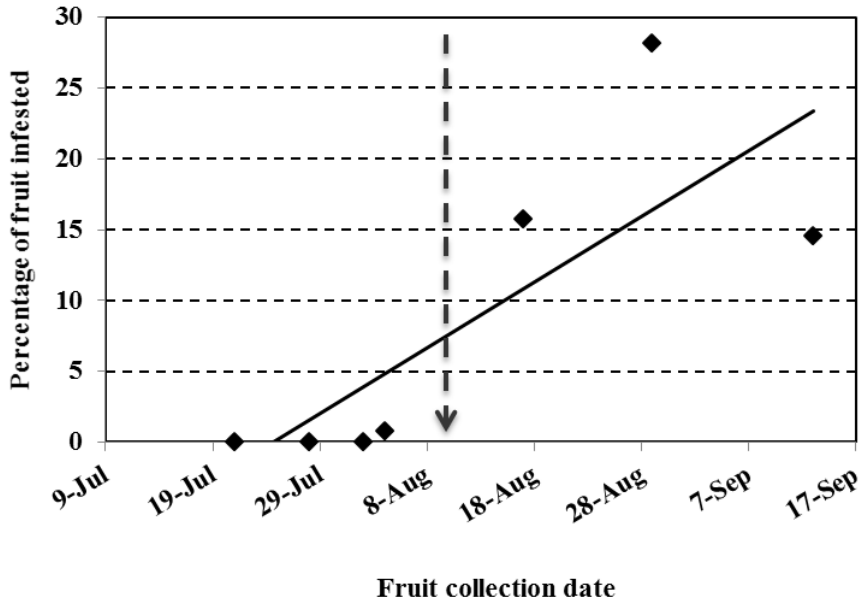


Fig. 4. Spotted wing drosophila infestation in wild fruit increased as the sampling period progressed 2016. Dashed arrow is date when SWD infestation of blueberry started to increase rapidly.



CONCLUSIONS: Blueberry field edges are commonly wooded edges that contain a variety of alternative fruit host plants for SWD. These field edges provide not only egg laying sites, but also shade and moisture that SWD need. In addition, these field edges may act as sources of infestation that can allow SWD to move into blueberry fields in higher numbers. Infestation rates were high in several species, especially raspberries, blackberries, honeysuckle and surprisingly, bunchberries. Raspberries and blackberries are known popular hosts, but bunchberries were a previously unrecorded host. This is of particular interest because bunchberry is commonly distributed in blueberry fields. However, SWD did not use bunchberry as a host until roughly the same time as blueberries, so although it is a common plant, it may not help SWD build up in high numbers prior to blueberry infestation. Infestation in wild fruit began several weeks before infestation in blueberries, despite blueberries being ripe and vulnerable to SWD attack. In 2015 the earlier infestation of wild fruits compared to blueberry was more pronounced than in 2016 Overall, however, this phenological difference in attack suggests that SWD prefer these wild fruit earlier in the season. This preference may help to increase SWD densities before they move into blueberries.

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

METHODS: Exclusion netting was evaluated for a third year to determine its effectiveness in preventing infestation of fruit by spotted wing drosophila (SWD). Agribon® + AG-19 Row Cover (commonly referred to as "Reemay") was placed in two areas of a fruit-bearing wild blueberry field at Jonesboro, ME on 14 July (Fig. 1).

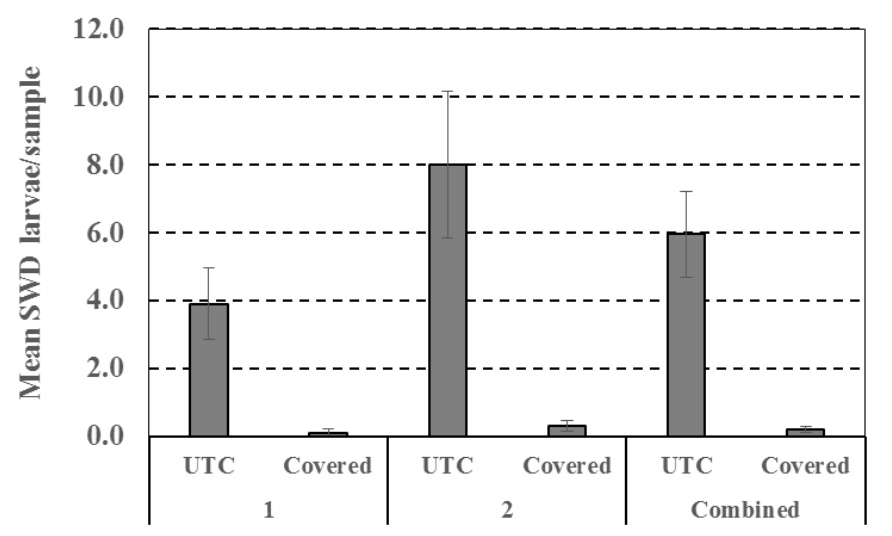
Fig. 1. Exclusion netting covering wild lowbush blueberry.

There were two replications each 50' wide x 150' long. Blueberries were <5% ripening and turning blue at the time of deployment. Two, red cup traps with standard yeast/bait solution were placed in each trial area to monitor for the presence of SWD adults. Fruit samples (two, 1 cup samples from each replication) were taken periodically to determine the beginning of the fruit infestation period. Between 29 and 31 August the exclusion netting was removed and ten fruit samples were taken from areas protected by each block of netting. Five samples were taken near the edge of the netting and five along the midline. Ten additional samples were collected from areas not protected by any netting. Each sample was ca. 1 cup of ripe fruit. The fruit was processed to determine maggot infestation using the procedure outlined in the factsheet published by Drummond and Yarborough (http://umaine.edu/blueberries/factsheets/insects/210-spotted-wing-drosophila/).

RESULTS: First maggots were observed in fruit from samples taken outside the treated (net-covered) area on 22 August and the results are discussed in Study 7 of this report comparing adult population density with larval infestation.

Analysis of variance was used to evaluate the effectiveness of the netting in reducing larval infestation of fruit. In this study, exclusion netting was an effective means of suppressing fruit infestation in limited areas. We did not find any evidence for a block effect ($F_{(1,1)} = 3.18$, $P = 0.0831$) or any treatment by block interaction ($F_{(1,1)} = 2.61$, $P = 0.1147$). However, we did find a significant treatment effect ($F_{(1,1)} = 22.72$, $P < 0.0001$)(Fig. 2). There was also no evidence of an edge effect; i.e. more larvae found in fruit close to but under the edge of the netting ($F_{(1,18)} = 1.20$, $P = 0.2878$)

Fig. 2. Bar graph showing mean SWD larvae per treatment. Lines are standard errors of the mean. 2016 data.



These results support the results of our 2014 and 2015 trials where significantly fewer maggots were found in fruit samples collected from the treated (net covered) areas compared with the unprotected check areas (UTC) in both 2014 (nested randomized block (multiple blocks nested within each of two time replications))($F_{(1,9)} = 5.478$, $P = 0.044$) and 2015 (logistic regression, $\chi^2 = 15.851$, $P < 0.0001$)(Figs 3 & 4).

Fig. 3. Bar graph showing mean SWD larvae per treatment. Lines are standard errors of the mean. 2014 data.

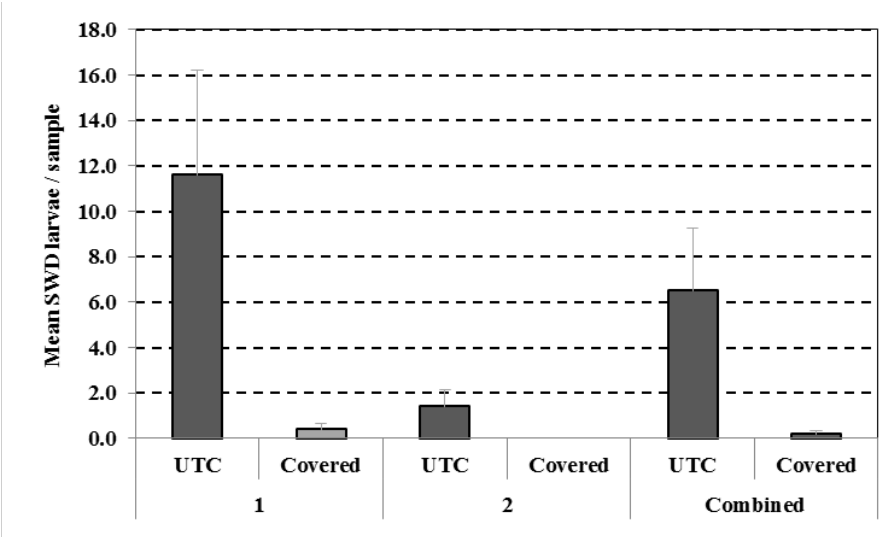
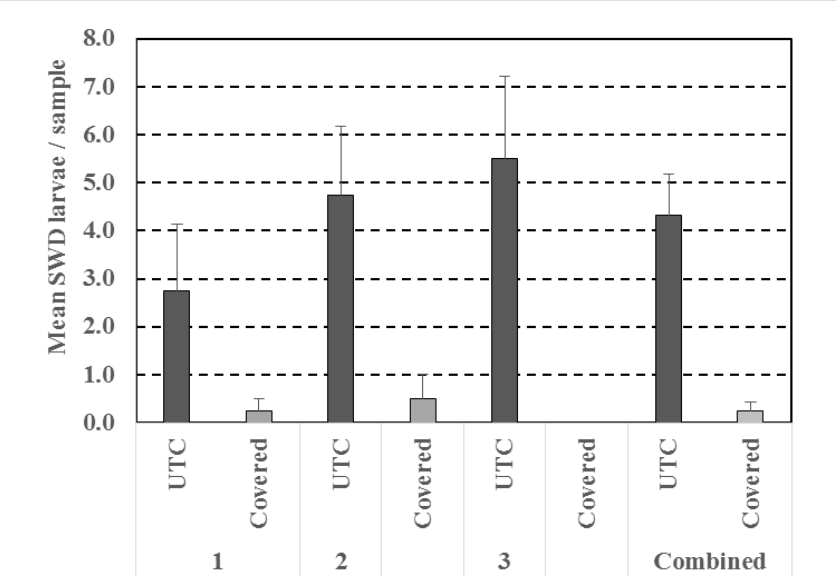


Fig. 4. Bar graph showing mean SWD larvae per treatment. Lines are standard errors of the mean. 2015 data.



CONCLUSIONS: This was the third year in which we evaluated exclusion netting as a possible alternative control method against SWD, but the first year evaluating the Agribon row cover (Reemay). In both 2014 and 2015 we used 25 mesh Anti-Insect Netting. Although the Reemay proved highly effective, it was difficult to deploy as the cloth fabric was easily torn. In addition, fruit appeared to have been knocked off stems possibly due to excessive movement of the fabric in high winds, and some fruit was discolored; although, there was no evidence of disease (Fig. 5). Sand bags were the most economical and effective means of anchoring the fabric netting; however, the result was dead areas under the sandbags (Fig. 6). Metal or wire stakes were not suitable for this purpose as the wind would easily tear the fabric away from the stakes. The initial estimated lifespan of six years is also unrealistic for wild blueberry; growers will be fortunate to get two years of service. The fabric we used was very brittle by the end of the trial period.

Fig. 5. Photograph showing discolored fruit under netting.

Fig. 6. Dead areas where sandbags were placed.

Study 7. <u>Early harvest and action thresholds for spotted wing drosophila</u>.

OBJECTIVES: Immediately after the first year of invasion by the spotted wing drosophila many processors initiated a tactic of "early harvest". The concept behind this tactic is to harvest as many fields as possible prior to buildup of SWD adults and the initiation of fruit infestation. In 2012 we started collecting data on the relationship between male SWD fly capture in traps and the infestation levels of fruit throughout the growing season until harvest. In 2015 we estimated a set of action thresholds based upon average cumulative capture of male flies. These action thresholds ranged from a conservative action threshold of 1.0 cumulative males per trap to a less conservative action threshold of 3.0 males per trap, to a liberal action threshold of 10.0 male flies per trap. These action thresholds are linked to the likelihood of experiencing SWD fruit infestation the following week. In 2016 we evaluated these thresholds on 14 farms and we also evaluated the cost (in terms of unripe fruit) to the "early harvest" tactic.

METHODS: Prior to the 2016 summer several wild blueberry growers were contacted and asked if they wanted to be part of this study. Cooperating growers allowed us to trap adult SWD and sample fruit for larval infestation on a weekly basis. The fields were maintained by the growers using typical wild blueberry production practices.

Beginning in mid-July before any SWD had been captured and continuing until fields were harvested, traps were placed in 14 wild blueberry fields in Downeast and mid-coast, Maine. Traps were monitored at 5 to 7 day intervals for the presence of SWD adults. Three traps were placed at each site. All traps were constructed from Solo®, 16 fl. oz, red polystyrene cups with light-blocking lids. Seven to 10, 3/16-inch holes were punched on the side of each container near the top, evenly spaced around the rim. Bait consisted of live yeast (1 tbsp) + sugar (4 tbsp) + 12 oz water (makes enough for four traps). The traps were hung 1-2 ft. above the top of the canopy using 36' plant stands. Throughout the study and on each sample date, traps set the previous week were collected and returned to the laboratory where male, female, and total abundance of SWD adults were determined and recorded. Using this data we calculated the

mean SWD males per trap captured from each site for each date and the mean cumulative number of males over the collection period.

To compare adult abundance with larval infestation, fruit samples were taken from the 14 wild blueberry fields on various dates from mid-July until field was harvested and processed using the procedure described in the factsheet published by Drummond and Yarborough (http://umaine.edu/blueberries/factsheets/insects/210-spotted-wing-drosophila/).

Each sample consisted of 1-cup samples collected from the vicinity of each adult trap (three traps per field). We also calculated the percentage of unripe fruit in the last sample collected prior to harvest. Using this data we calculated the mean number of maggots collected from each site on each sample date. This data was compared with the adult abundance data collected over the same time period.

Utilizing data from the 14 sites, we also attempted to determine the effectiveness of four different grower IPM strategies; 1) early harvest before SWD are caught, 2) harvest as soon as possible after 1st male fly is caught in field trap, 3) wait until an average of 3 males per trap is captured then harvest, and 4) wait until an average of 9 males per trap is captured then harvest. The number of fields that had detectable fruit infestations the week following the detection of a range of male SWD trap captures are shown in figure 1. From this data a probability of infestation in a field based upon the previous week's male SWD cumulative trap capture can be derived (Table 1).

Fig. 1. The number of blueberry fields that have infestation the week following detection of SWD males.

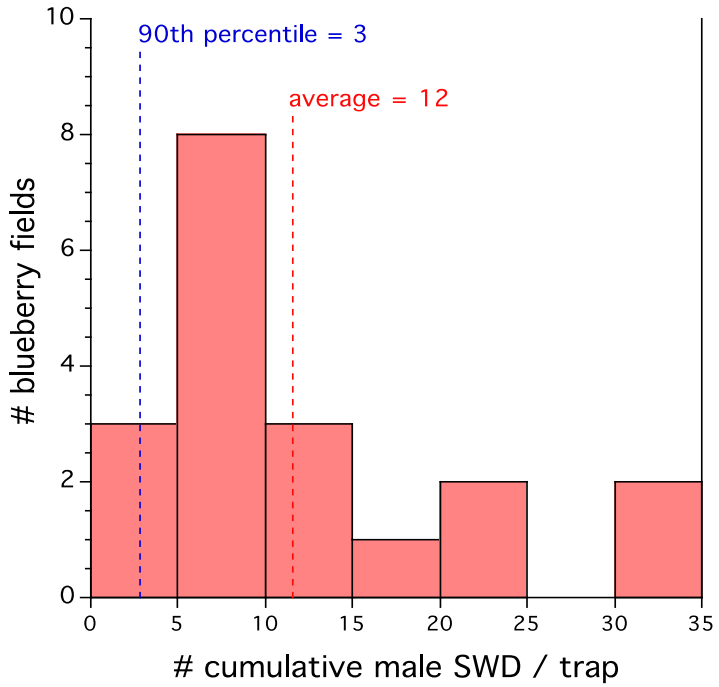


Table 1. Action thresholds for spotted wing drosophila in Maine wild blueberry (based upon field data collected 2012-2015).

Cumulative male SWD captured / trap (based upon 3 traps / field)	Probability of infestation the week following cumulative male SWD trap capture
0	0.1%
1	0.3%
3	9.7%
9	25.0%
12	50.0%

RESULTS: Our 2016 sampling of 14 blueberry fields and sampling conducted by Dr. David Handley in both the Mid-coast and Western regions of Maine allowed us to observe the population increase in 2016 and compare it to the previous year. Overall population patterns over the season in 2016 differed from 2015 (Figs. 2 and 3). The growth rate in the Downeast wild blueberry region was slower and did not reach the high abundance in 2016 as was observed in 2015. In the south, mid-coast and western regions, the rate of population increase, indicated by trap capture was higher in 2015 compared to 2016, but trap captures in 2016 appeared to vary more from week to week and location to location than we had seen in the previous year. This allowed growers in some locations to delay insecticide sprays and spray less frequently in 2016 than in previous years. However, populations did reach damaging levels early enough to pose a significant threat to late ripening blueberries and fall-bearing raspberries (Fig. 3).

The capture of male SWD does not necessarily mean that infestation of fruit will immediately follow. This is shown in Table 1, but also of the total of 14 wild blueberry sites sampled in 2016, nine were harvested prior to fruit infestation. Nine of the 11 sites included in the "Grower IPM" study were harvested prior to fruit infestation. Fifteen of 17 sites studied in 2015 were harvested prior to fruit infestation. Four of 14 fields that were sampled for male SWD in 2014 ended up having no infested fruit before they were harvested. In 2013, 16 blueberry fields were monitored for SWD fly captures and fruit infestation. The majority (21 fields) were characterized by very low male trap captures ($\leq$ 4 SWD males/trap). However, 7 fields had more than 15 male SWD and still did not have infested fruit. Figure 4 shows the distribution of SWD males in those fields that did **not** have infested fruit prior to harvest in 2014-2016.

Fig. 2. Adult SWD captures for 2015 in wild blueberry fields in Downeast, Maine and highbush blueberry, wild blueberry, and raspberry in Southern, Mid-coast, and Western Maine.

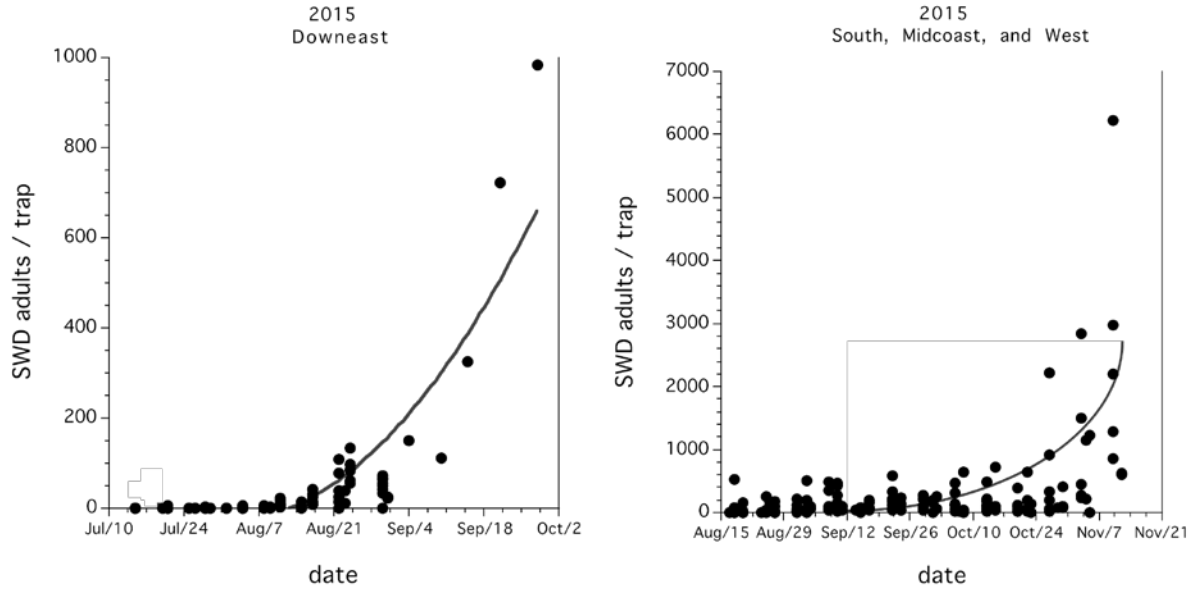


Fig. 3. Adult SWD captures for 2016 in wild blueberry fields in Downeast, and highbush blueberry, wild blueberry, and raspberry fields in Southern, Mid-coast, and Western Maine.

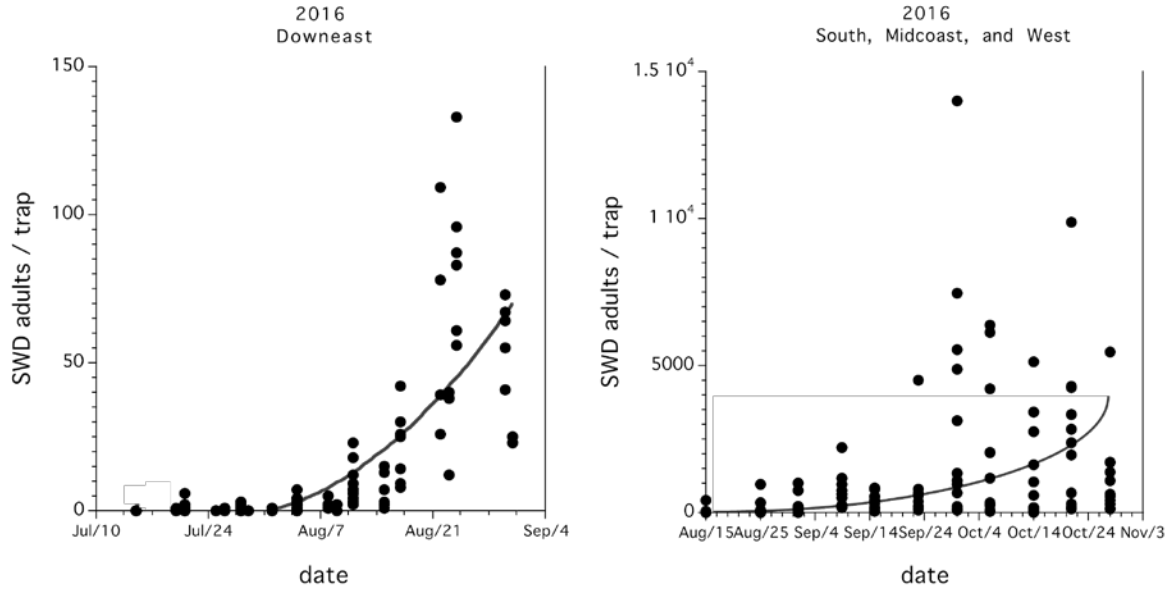
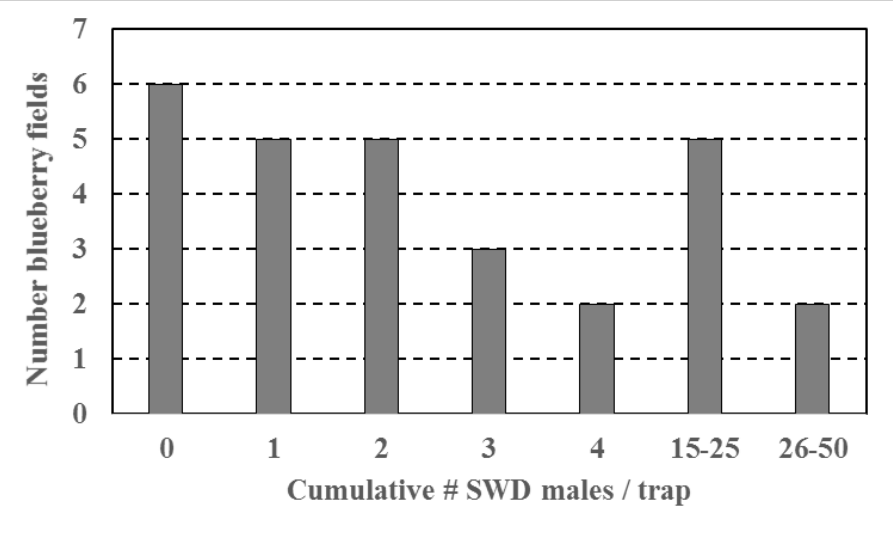
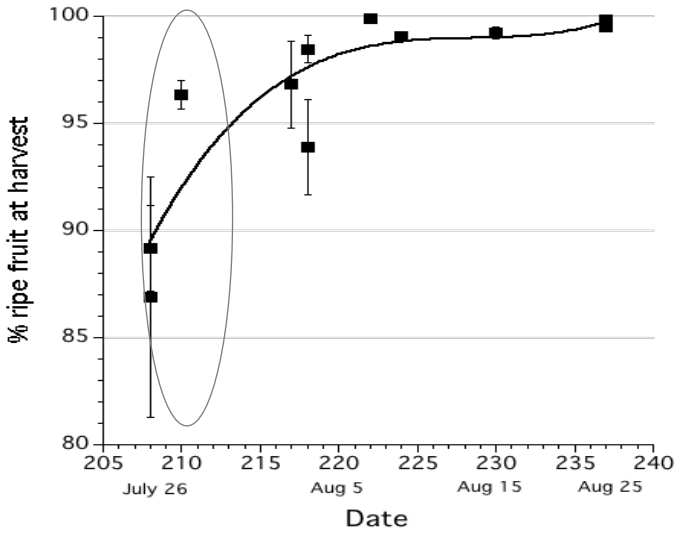


Fig. 4. The frequency distribution of fields with various levels of male SWD/trap not infested by the time of harvest in 2014, 2015 and 2016.



In 2016 the three "early harvest" fields were harvested successfully prior to any SWD infestation of fruit. However, there is a cost involved in this tactic. The benefit, of course, is the avoidance of infested fruit and minimization of insecticide residues on the harvested fruit. The cost is a higher percentage of the fruit is not ripe and thus of no monetary value. This in some ways is equivalent to a crop loss. Figure 5 demonstrates this dynamic in the fields sampled in 2016. The earlier a field is harvested, the more likely it is that green fruit becomes a significant cost. It can be seen that in 2016 that the earliest harvested fields incurred between 10 and 15% reduction in yield due to green fruit. Later harvested fields are associated with a higher percentage of ripe fruit, but also a potentially higher risk of SWD infestation.

Fig. 5. Percentage of ripe fruit; 2016 grower IPM study. Ellipse identifies the early harvest fields.



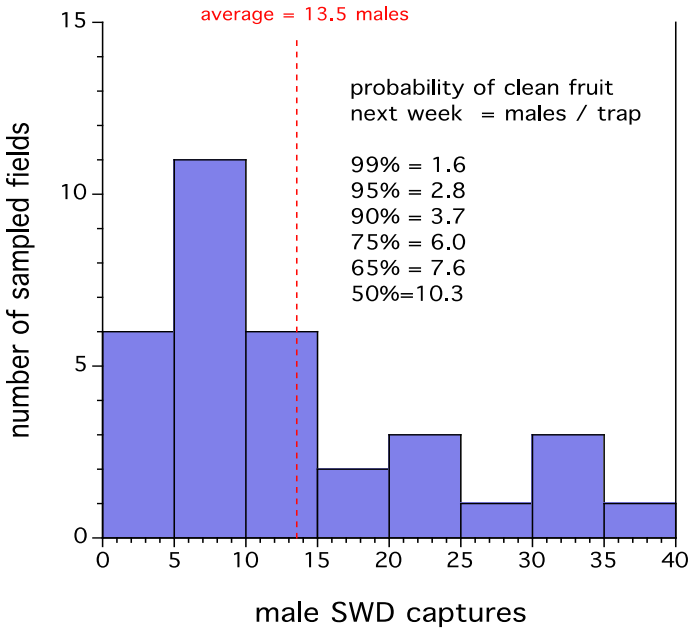
In 2016 the action threshold results were fairly successful (Table 2). The fields that used low levels of male SWD action thresholds escaped without any infestation the following week that they were harvested. Only the fields that accumulated the higher level of male SWD, 9 accumulated males, experienced infestation in the week following this action threshold; and only 40% or two of the five fields had infestations.

Table 2. Results of 2016 action threshold study (percentages were estimated as if every larva detected came from a separate fruit, a liberal or upper bound estimate).

Action threshold	SWD infestation the following week / field
0 male SWD (Early harvest fields, n=3 fields)	0, 0, 0%
1 male SWD (n=3 fields)	0, 0, 0%
3 male SWD (n=3 fields)	0, 0, 0%
9 male SWD (n=5 fields)	0, 0, 0, 0.3, 0.5%

Incorporating the 14 fields sampled in 2016, the new frequency distribution of fields that are associated with a given cumulative capture and the following week being infested is shown in figure 6. A theoretical log-normal distribution allowed estimates of the likelihood or probability of the field having infested fruit the following week and from this estimate we calculated the likelihood of "clean non-infested fields the following week. Therefore, with this NEW five-year data set we can see that an average capture of **1 male SWD** (actually 1.6 males) results in a **99% chance of no infestation** the following week. An average of **3 male SWD** (actually 2.8) results in a **95% chance of no infestation** the following week. An average of **6 male SWD** results in a **75% chance of no infestation** the following week. The addition of the new data has lowered the likelihood of infestation for the action thresholds of 1 and 3 cumulative male SWD, but increased the likelihood for a higher action threshold.

Fig. 6. Data from 2012-2016 that show the frequency (# fields) of cumulative SWD male captures in fields that the following week had infested fruit.



CONCLUSIONS: In conclusion, we have been developing a database of male fly captures and the associated likelihood of infestation by SWD the following week from given capture rates. These data have allowed us to estimate likely thresholds for varying levels of accepted risk. Our field trial in 2016 suggests that action thresholds will work and that the more data we have, the more certain we will be of the risk level for specific thresholds.

Study 8. Spotted wing drosophila winter survival.

OBJECTIVES: The severity of SWD in wild blueberry most likely depends upon what proportion of the population in the fall is able to survive the winter in Maine. While scientists are almost certain that SWD flies can overwinter successfully in southern New England and Michigan, it is not known whether they can overwinter in a more northern climate such as Maine. We are testing the overwintering survival capability of spotted wing drosophila as part of a multi-state project. This is an-ongoing project that will extend into March 2017.

METHODS:

Creating winter morphs

Adult SWD acquired from our lab colony were moved to new SWD tubes (25 x 95mm) with commercial media (Carolina formula 4-24® Instant Drosophila Medium). Approximately 50 flies were allowed to oviposit for 24 hours under summer conditions (25°C, 16:8 L:D) in each tube. After 24 hours, adult flies were removed from tubes and tubes containing eggs were moved to a cooler chamber (15°C, 12:12 L:D). Vials were inspected every 48 hours to look for adult emergence. As adults emerged from the cool chamber tubes, the adults were removed to fresh tubes of media and held at 10°C, under a 12:12 L:D cycle until their field release. Flies were held at 10°C a minimum of one week prior to their release.

Field set up

A field site was set up along a wooded edge at the University owned Roger's Farm in Old Town, ME. Twenty-four holes were dug using a fence post digger. The soil plugs were placed into 32oz deli cups leaving 1-2cm at the top open. The empty space at the top of the deli container was filled with organic matter from the site. A piece of apple was set on top of the organic matter and 50 chilled males and females (total of 100 flies) were added to the organic matter and covered in 2-3cm of leaves. A double layer of 1mm mesh was secured over the top of the deli cup and held in place with rubber bands. The deli cup with flies was then placed into the hole so that the lip of the deli cup was level with the ground. The mesh was tucked into the ground around the cup and then was covered with surrounding leaf litter.

Every two weeks, a set of four pots were dug up, transported back to the lab and held in mesh cages at room temperature. The apple piece was discarded, the mesh was removed, and all dead flies were counted in the cage after a two week holding period. The first set of pots were brought back the same day the experiment was launched. This experiment will last for 10 weeks beginning on 15 November 2016.

Temperature at the field site was recorded using Hobo® data loggers. A set of loggers were suspended from a nearby tree branch to measure air temperature, and a set of loggers were placed in-between the soil and leaf litter in the pots to measure the temperature SWD flies were

exposed to. Data loggers recorded temperatures every hour for as long as the pots remained in the field.

Monitoring wild SWD in the field

Two types of traps were deployed along the field edge to monitor for wild SWD adults. Adults were live trapped during warm days above 10°C by using a red Solo® cup elevated three feet above ground and baited with 60% merlot wine and 40% apple cider vinegar mixture. The bait was in a one ounce portion cup covered with 1mm mesh netting and placed inside the red solo cup. The live traps were checked the following morning and all living flies were aspirated and frozen at -80°C. Live flies were collected throughout the duration of the experiment and sent to Dalila Rendon at Oregon State University to analyze nutritional content of flies.

Flies were also trapped during the experiment using a Sentry® commercial baited trap. Two traps were deployed along the field edge at the start of the experiment and were checked every two weeks for SWD. The traps had a drowning solution that was comprised of 90g salt, 250 ml water and one drop of unscented dish soap. The flies were strained out of solution and preserved in ethanol before shipping to Dr. Greg Loeb at the Cornell University for morphological measurements. The drowning solution was changed every two weeks and the commercial baits were replaced every six weeks.

RESULTS: Results are forthcoming in 2017 after the project is completed.

Study 9. Novel lure testing for spotted wing drosophila.

OBJECTIVES: Spotted wing drosophila (SWD) is an invasive insect in Maine that lays its eggs in lowbush blueberries and other soft fruits and is a major concern for blueberry growers. In Maine, SWD first appears during the late summer in late July or early August. SWD damage can be greatly reduced when using economic thresholds based on field trap catches to time field treatments and harvesting. Traditionally, growers and scientists use a homemade yeast-sugar bait to capture SWD in monitoring traps. Previous studies have shown that this bait only captures a small percentage of SWD in fields, and some find it awkward to use. Novel lures were developed by Dr. Cesar Rodriguez-Sonoma at Rutgers University and tested across multiple states including Maine, Michigan, New Jersey, New York, North Carolina, and Oregon. This study sought to determine which of these new synthetically produced lures are most effective in capturing SWD and other drosophila. An effective trap should catch high numbers of SWD while limiting bycatch such as other drosophila which make it harder for growers to see and identify SWD. We hope the results of this study can be used to determine which lures capture the most SWD while limiting bycatch.

METHODS: The trial was conducted at the University of Maine's Wild Blueberry Farm in Jonesboro, Maine and at an organic commercial blueberry farm in Stockton Springs, Maine. Five different lures were used in this study in addition to a water control (Table 1). Two replicates of each trap type were deployed at each location for a total of 28 traps. Traps were a commercial yellow jacket trap (Victor Model # M362; 1,100 capacity). Traps with a lure had the lure snapped into the inside of the trap lid. Lures were changed in the traps every two weeks and the drowning solution was collected and changed on a weekly basis. Insects were drained out of the drowning solution and stored in 70% ETOH (ethyl alcohol) until they could be sorted

and counted at the end of the season. Traps were deployed in Stockton Springs on 1 August and were deployed in Jonesboro on 2 August. The traps in Jonesboro were left out for seven weeks with the final trap collected on 19 September. The traps in Stockton Springs were left out for six weeks with the final trap collected on 13 September.

For each trap catch, the number of male and female SWD and all non-SWD drosophila were counted. Many of the fermentation and yeast sugar traps collected large numbers of flies during the latter half of the trial and so subsampling was used on traps that had too many flies to reasonably count. The insects were drained out of the ethanol and rinsed through a coarse sieve. The smaller insects that flowed through the sieve were collected and strained. These strained insects contained drosophila and other small insects. These sieved samples were then weighed. Three subsamples around 1.5g were removed from the sieved trap catch and also weighed. These subsamples were counted and used to calculate how many male, females, and non-SWD drosophila were captured.

Table 1. Trap lure treatments.

Treatment	Drowning solution per trap
Fermentation lure	456 ml water, 8 ml acetic acid, 36 ml ethanol, 1 drop of natural liquid dish soap
Leaf lure	500 ml water, 1 drop of natural liquid dish soap
Fruit lure	500 ml water,1 ml acetic acid, 1 drop of natural liquid dish soap
Yeast Lure	500 ml water, 1 drop of natural liquid dish soap
Yeast Sugar	5.07 g of dry active yeast, 25.35 g of sugar, 450 ml of water, 1 drop of natural liquid dish soap
Water Control	500 ml water, 1 drop of natural liquid dish soap

RESULTS: The control, leaf lure, and yeast lure caught no or very few SWD and non-SWD drosophila. The fermentation lure, fruit lure, and yeast sugar bait all caught substantial numbers of SWD and other drosophila (Figs. 1 & 2). On average across all sites and dates, the fermentation lure and yeast sugar bait both caught substantial numbers of SWD, but the fermentation lure caught nearly six times the number of non-SWD drosophila. The fruit lure caught substantially fewer SWD and drosophila compared to the fermentation lure and yeast sugar bait, but caught roughly equal number of SWD and non-SWD drosophila (Table 2). As expected, the number of SWD caught in traps increased during the season (Figs. 3 & 4).

Table 2. Average trap catches across all dates and sites, letters designate statistical differences.

Treatment	Male SWD	Female SWD	Total SWD	Non-SWD drosophila
Control	0.04	0	0.02 d	0.12 e
Fermentation Lure	329.77	259.82	364.90 a	986.23 a
Fruit Lure	34.43	31.07	34.25 c	61.08 c
Leaf Lure	0.036	0.62	0.35 d	0.81 d d
Yeast Lure	0	0	0 d	0.23 e
Yeast Sugar Bait	191.44	342.47	282.52 b	165.77 b

Fig. 1. The average number of male and female SWD and non-SWD drosophila caught over the season in Stockton Springs.

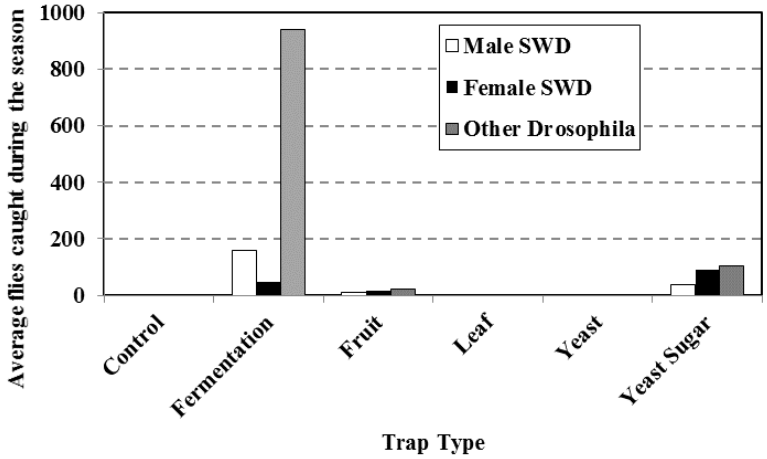


Fig. 2. The average number of male and female SWD and non-SWD drosophila caught over the season in Jonesboro.

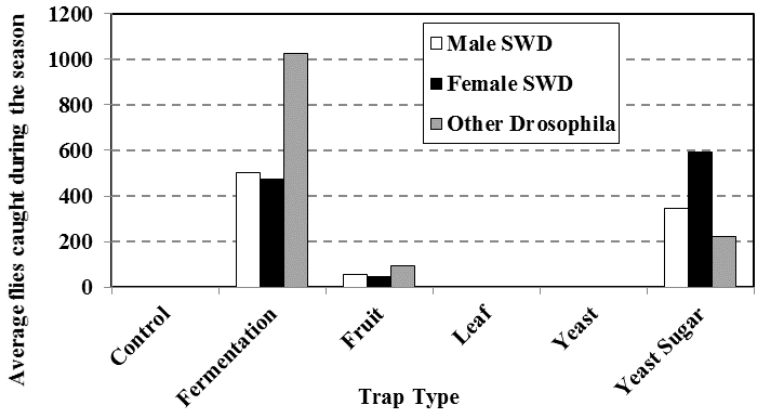


Fig. 3. The average number of SWD (male and female combined) caught over the season in Jonesboro.

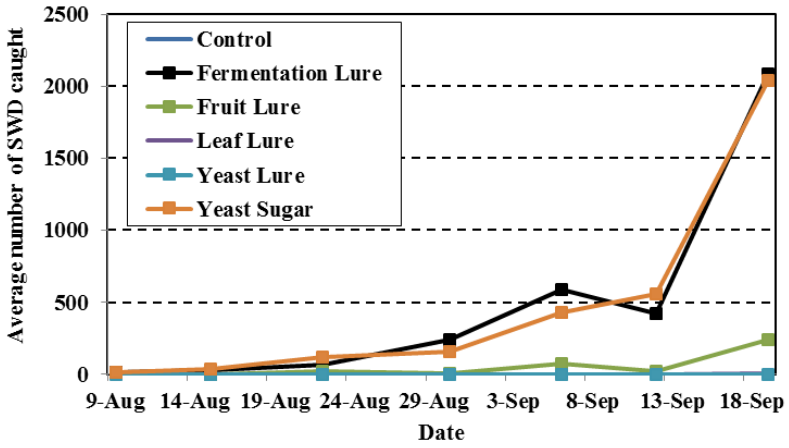
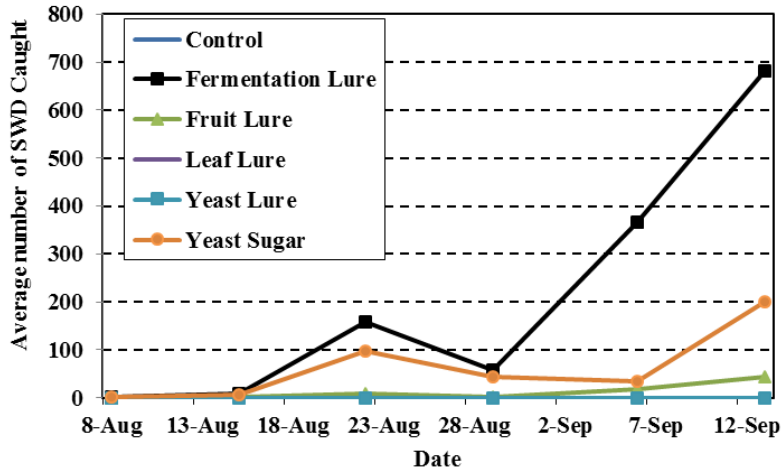


Fig. 4. The average number of SWD (male and female combined) caught over the season in Stockton Springs.



CONCLUSIONS: Of the five lures and baits tested, three show promise for trapping SWD. The leaf and yeast lures caught very few SWD and do not appear to be promising choices. The fermentation lure is very effective at trapping SWD, but unfortunately it also traps a very large number of non-SWD drosophila. Having additional fly bycatch in the traps makes it harder to see and identify SWD. If these lures are to be used by growers to monitor their fields, the fermentation lures may not be the best choice. The fruit lure did not catch the large amount of non-SWD drosophila like the fermentation lure, but it was also less effective at catching SWD. The standard yeast sugar bait that we have used and recommended to growers in Maine appears to still be the most effective bait for catching large numbers of SWD while minimizing non-SWD bycatch.

Study 10. Genetics of spotted wing drosophila.

OBJECTIVES: The spotted wing drosophila was detected in Maine wild blueberry in October 2011, and the first crop loss due to this new invasive pest was experienced during the summer of 2012. However, we are not sure how homogeneous the population is in Maine and whether it might have originated from a single introduction south or west of Maine or if the population presently in Maine is the result of several distinct introductions. A genetic analysis of samples of flies collected throughout the wild blueberry growing regions may help answer this question.

METHODS: Spotted wing drosophila were collected from infested wild fruit and sent off for molecular analysis. This project is in collaboration with Joanna Chiu at the University of California-Davis and whose purpose is to study dispersal of flies between geographic sites. Wild fruit was collected along field edges to determine when, what species, and how much wild fruit is infested with SWD (see Study 5 of this report). Wild fruit was held and checked for SWD emergence in the lab. Sixteen live female SWD flies were collected from the fruit, placed into 70% ETOH, and held at 4^{o}C. Flies included in this study were collected from two commercial

blueberry fields in Union, ME. Flies were collected from blackberry, honeysuckle, and dogwood.

RESULTS: Results are forthcoming in 2017 after molecular analyses have been conducted in California to determine relatedness of flies within Maine, between different blueberry growing regions; and between different states.

Study 11. Within-field movement of spotted wing drosophila.

OBJECTIVES: Spotted wing drosophila (SWD) is an invasive insect in Maine that lays its eggs in lowbush blueberries and is a major concern for blueberry growers. This fly is ubiquitous across all blueberry growing regions of Maine. Flies are usually first detected along wooded edges which are thought to be places of refuge. In Maine, SWD first appears during the late summer in late July or early August and are present throughout the fall, at least until frost. Previous studies determined that flies can travel up to 400m within a few days but those studies were conducted without using dye so flies were released early in the season before wild SWD appeared. This year we conducted our study while wild SWD were present so a powdered dye was used to differentiate between wild and released flies. This study was replicated several times during peak infestation periods in Maine. We hope the results of this study can be used to further understand the movement of SWD during peak infestation periods.

METHODS: Flies were dyed by using a powdered fluorescent Day-Glo® orange, or pink powdered fluorescent dye. One hundred flies (50 male, 50 female) were anesthetized and held in 540 ml deli cups on ice while the dye was administered. A thin layer of dye was applied by spraying a puff of dye from a bulb duster (Punchau® Pest Control Bulb Duster). A moist cotton ball and pea-sized amount of commercial Instant Drosophila Medium formula 4-24 (Carolina Biological Supply Co., Burlington, N.C.) was added to each deli container. The dyed flies were held overnight to allow them to groom excess dye off their bodies.

The trial was conducted in a pruned field at the University of Maine's Blueberry Hill Farm in Jonesboro, ME. The recapture grid was comprised of two, 100m length transects (Labeled A & B in Table 2) that crossed in the middle of the field. Flies were released at the middle intersection of the two transects. Recapture cups were spaced at 2.5, 5, 10, 30, and 50m from the release point for a total of 20 recapture points. At each recapture point, red Solo® cups were filled with 4oz standard SWD bait (4:1:24 sugar: yeast: water), set on a plant stand 2.5 feet tall. The cups had holes punched around the rim to give flies an access point and had a red shade foam glued to the lid to prevent overheating inside the cup.

This trial was replicated three times during the field season on 26 June, 19 August, and 7 September. Roughly 2,000 flies were released for each date (Table 1). Containers holding the flies were opened and flies were given 20 minutes to fly out. Containers were closed after this 20 minute period and all remaining flies were counted and recorded as non-responsive which determined our number of released flies.

The recapture cups were changed every other day for approximately one week. The bait was drained through a mesh sieve and all captured flies were examined under UV light (Black-Ray® Longwave Ultraviolet Lamp) under magnification to detect dye.

RESULTS: The first trial had an overall recapture rate of 3.62%, the second trial 2.91%, and the third trial had a recapture rate of 2.44% (Table 1). In all three trials, at least one fly had flown the furthest distance from the release point (50m) within two days of its release (Table 2).

Table 1. Summary of the three 2016 release trials.

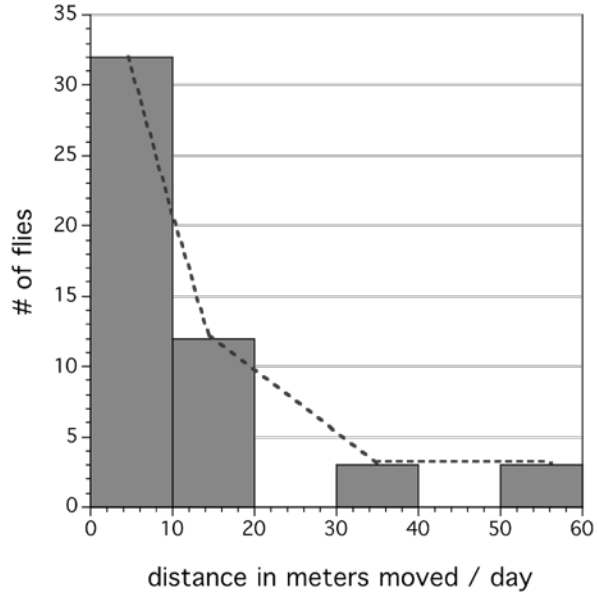
Release date	Dye color	Number of flies released	Number of flies recaptured	Recapture rate
26 June	Pink	2458	89	3.62%
19 August	Pink	1891	55	2.91%
7 September	Orange	1846	45	2.44%

Table 2. The number of combined SWD marked flies recaptured at each sample point over all three trials in 2016.

Cup number (distance from release point)	Transect A	Transect B
1 (50m)	0	6
2 (30m)	1	7
3 (10m)	8	6
4 (5m)	10	13
5 (2.5m)	5	22
6 (2.5m)	19	8
7 (5m)	13	1
8 (10m)	10	8
9 (30m)	7	3
10 (50m)	39	7

The distances flown were adjusted by dividing the distance by the number of days between release and capture. This adjustment provides a rough estimate of the distance SWD flies moved per day. Figure 1 shows these distances plotted against the number of flies that were recorded flying those distances. It can be seen by inspection of figure 1 that most SWD only move approximately 10m or less per day. This distance of daily dispersal matches a perimeter spray tactic; however, it appears that some flies may move up to 40-60m. This would make a perimeter treatment less effective. We need more data to assess what proportion of an SWD population at the field edge would move farther than a perimeter treatment of 30m or so.

Fig. 1. Probability (frequency) of distance (m) moved per day by SWD adults.



CONCLUSIONS: We have only begun to assess fly movement and it will take several years to determine how far a SWD population can move and how this knowledge might be used to develop sound management tactics.

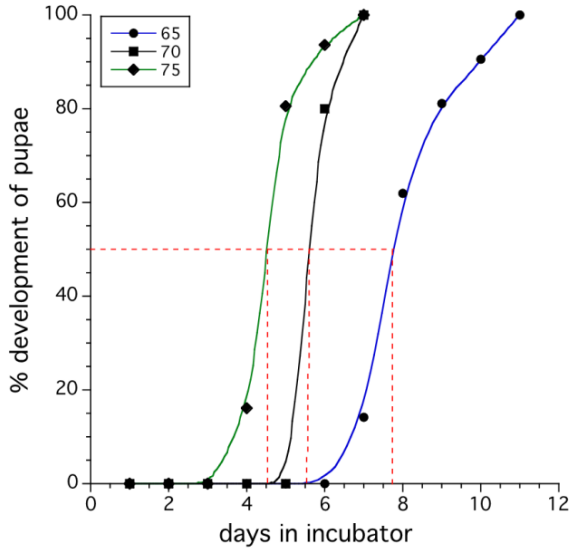
Study 12. <u>Development of spotted wing drosophila pupae at different temperatures</u>.

OBJECTIVES: This study is part of on-going trials designed to study environmental factors influencing the biology of spotted wing drosophila. In this particular study the focus was on determining the length of time under different temperature regimes that SWD pupae are available for attack by natural enemies.

METHODS: All adults and pupae were removed from established spotted wing drosophila rearing vials. The vials were held at room temperature (ca. 70^{o}F) and checked daily for new pupae. Any pupae were removed and placed in new media-filled vials. These vials with pupae were then held in a growth chamber at one of three temperatures (65, 70, or 75^{o}F). A minimum of 20 pupae was placed at each temperature. All vials were checked daily for adult emergence; any adults were counted and removed from the vials.

RESULTS AND CONCLUSIONS: Development was generally faster at higher temperatures. Pupae held at 65^{o}F took 7-11 days (days to 50% = 7.75) to develop to adults. At 70^{o}F development took between 6 and 7 days (days to 50% = 5.5); while development at 75^{o}F took 4 to 7 days (50% = 5.5, Fig. 1).

Fig. 1. Percent of pupae developing at three different temperatures. Dashed line indicates time needed for 50% of pupae to emerge as adults.



RECOMMENDATIONS: Based upon the 5-year database, we are now planning on using 1, 3, and 6 cumulative male SWD for 2017 action thresholds. Caution is still advised for following these thresholds because the last two years of study have been particularly dry and not optimal for SWD population growth. At least two more years are warranted for testing and refining SWD action thresholds.

Although we tested a variety of baits and lures, the standard yeast sugar bait that we have used and recommended to growers in Maine appears to still be the most effective bait for catching large numbers of SWD while minimizing non-SWD bycatch. In 2017, multiple infested fields will be sampled so that an optimal sample size can be determined over a range of larval infestation rates.

Exclusion netting was evaluated for a third year to determine its effectiveness in preventing infestation of fruit by SWD. Although a more economical alternative (based on initial cost) than the Anti-insect netting previously tested, the negatives may outweigh the positives for Reemay. The initial monetary investment of Anti-insect netting is more expensive, but will also last much longer (at least 3 years).

Our recommendation is that blueberry fields with abundant non-blueberry wild fruit shrubs and trees along field edges might be considered for an alternative fruit host removal effort. We have no evidence that this will reduce SWD infestation or the timing of infestation in wild blueberry fields, but perhaps this is a grower on-farm research project that could be initiated and evaluated.

This is the third and final year of a study that has been quantifying the natural predation of SWD pupae in wild blueberry fields. Both predators and parasitoids may end up being important natural enemies that dampen the explosive potential of this invasive pest. We recommend that growers use thresholds for management of SWD not only to be much more efficient and effective with insecticide applications, but also to preserve these important natural enemies. A priority for future research should be in tactics for minimizing pesticide exposure to these natural enemies.

ENTOMOLOGY

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5. IV. TITLE: Biology of blueberry, bees, and blueberry pollination.

Study 1. Predicting the bloom period in Maine wild blueberry.

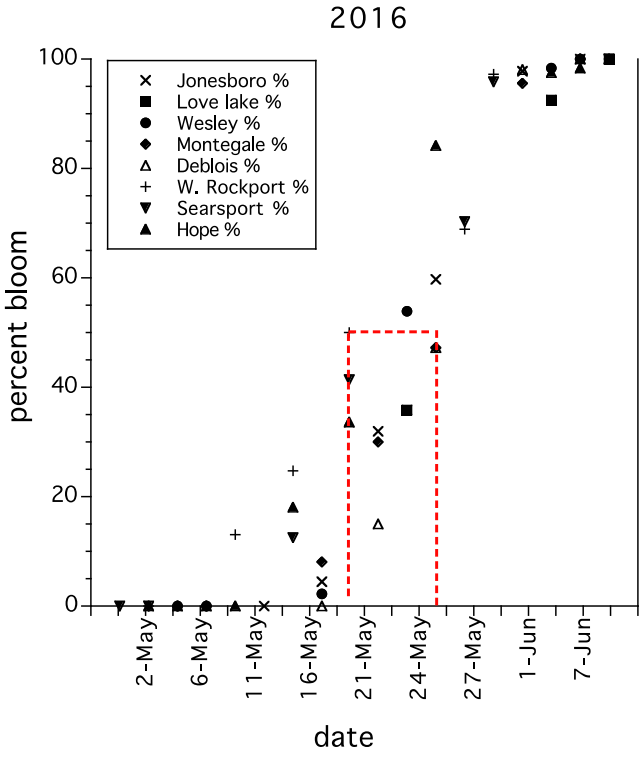
OBJECTIVES: We initiated an extensive data collection process in 2015 to develop a more appropriate model for Maine under conditions where plants do not begin to develop until at least March. Validation of this model was conducted in 2016 by sampling a second set of wild blueberry fields to estimate the progression of bloom.

METHODS: In 2015 we visited 18 wild blueberry fields in the mid-coast (11 fields) and Downeast (7 fields) growing regions of Maine. At each visit we collected 10 stems and counted the number of open and closed flower buds and calculated the proportion of flowers in the field in bloom. To develop a degree-day model for Maine we collected daily maximum and minimum air temperatures from local weather stations in the vicinity of each field. Using this data the number of degree-days was calculated for threshold base temperatures from 32-50ºF (every 2 degrees) and for 55 and 60ºF using the formula: degree-days = (average daily air temperature – threshold base temperature), where average temperature is: [(maximum air temperature + minimum air temperature) / 2]. Using this information we determined the precision of each degree-day (**DD**) model base for estimating three endpoints: initial bloom (1%), mid-bloom (50%), and full bloom (99%). The measure of precision used was the standard error to mean DD ratio. Using this approach we developed a preliminary degree-day model with a base of 45ºF where prediction of 1% bloom was 147 DD, 50% bloom was 228 DD, and 99% bloom was 404 DD.

In 2016 we visited eight wild blueberry fields and repeated the calculations outlined above. We first used the degree-day model developed in 2015 to predict the periods of 1, 50, and 99% bloom for the 2016 sampled fields. Then we reassessed other base temperatures and determined which base temperature minimized the error in prediction for BOTH the 2015 and 2016 datasets.

RESULTS AND CONCLUSIONS: Figure 1 shows the bloom progression in the eight fields that we sampled in 2016 as a function of date. It can be seen that there is a large spread of time at approximately 10% bloom (10 days), about a week at 50% bloom, and about 12 days at > 95% bloom. This is not surprising given the geographic range represented by the sampled blueberry fields.

Fig. 1. Progression of observed bloom (2016 data). Dashed lines show the variation in dates for 50% bloom.



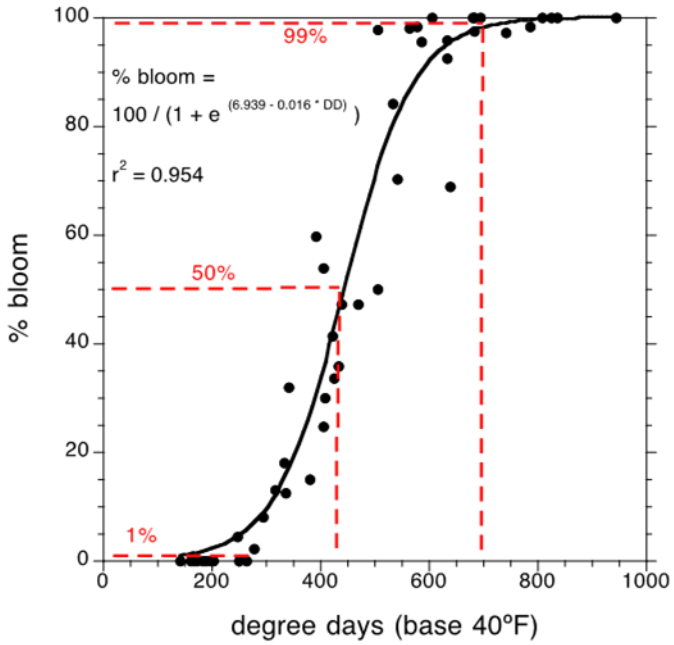
When we applied the 2015 degree-day model (base 45ºF) to the 2016 data, the model performed well for estimating the 50% and 99% levels of bloom (no significant difference in means for each year, $P < 0.05$); the estimations for 1% bloom were significantly different ($P < 0.05$). The average degree-days for 1% bloom in 2015 was 147.43 $\pm$ 6.13 (se), while the average for 1% bloom in 2016 was 94.97 $\pm$ 4.99 (se). In order to search for a more optimal model we assessed the difference in degree-day accumulations for base temperatures ranging from 32º - 60ºF. We found that a base of 40ºF minimized the error for all three bloom prediction levels (1, 50, and 99%). Table 1 shows the results of the optimal 40ºF base degree-day model. The precision is extremely high given that this model is predicting bloom over two years and two different growing regions.

Table 1. Degree-day model developed for both 2015 and 2016 (n=24 fields).

% bloom	Mean DD	Standard error (se)	% Precision (se/mean)	Lower 95% confidence interval	Upper 95% confidence interval
1	284.542	12.188	3.876	260.167	308.918
50	434.297	18.779	4.344	396.738	471.857
99	694.854	27.345	3.951	640.164	749.544

Figure 2 depicts the observed progression of bloom over degree-days and the predicted average cumulative degree-days for bloom in the 2016 sampled fields (indicated by dashed lines).

Fig. 2. The relationship between cumulative degree-days (base 40ºF) and the progression of bloom for 2016. The dashed lines show the estimated model predictions.



Study 2. ***<u>Computer simulation model of wild blueberry pollination.</u>***
<u>Report from Hongchun Qu (Chongqing University of Posts and Telecommunications) and F. Drummond</u>

OBJECTIVES: Wild blueberry (*Vaccinium angustifolium* Aiton), a unique crop species native to eastern North America, is an economically important fruit crop. Yield depends heavily on cross-pollination (allogamy) that requires insects, primarily bees. However, wild blueberry yield is not always a simple linear relationship to bee density; it may also be subject to variation of weather and spatial factors. Temperature and rainfall can change blueberry bloom initiation and duration as well as bee foraging activity, which makes interactions between the two organisms too complicated to be predictable in a straight-forward manner. Increasing yield, fruit quality, and economic stability in wild blueberry production requires better understanding of the fundamental ecological processes of cross-pollination and how bee species abundance, plant clone (genet) spatial pattern, and weather conditions interact with each other. Although some mechanisms that drive these ecological processes have been studied, we still lack a holistic understanding of how these dynamic interactions affect pollination efficiency under the influence of changing weather conditions, particularly in a complex context of varying spatial, temporal, taxonomic, and genetic scales. Modeling wild blueberry pollination allows us to decipher hidden relationships between organisms to better understand the ecological processes of the wild

blueberry cropping system under climate change and, to develop improved management strategies for achieving optimal trade-offs between investment and yield.

METHODS: We modeled wild blueberry pollination in five stages: 1) identified entities and processes guided by domain experts; 2) represented entities as agents in a spatially-explicit environment; 3) scheduled agents in appropriate temporal scales; 4) parameterized agents with empirical data; and 5) built the graphic user interface.

Entities and processes were defined by identification of relevant organisms and decomposing their interactions into key ecological processes. The model consists of two types of entities: 1) regular entities, e.g., blueberry fields, blueberry clones, stems and flowers, and bee pollinators that are visual objects in real world; and 2) virtual entities, marked as gray rectangles, such as the environment, system scheduling, weather, and phenology that provide spatial and temporal reference that coordinates interactions among regular entities (Fig. 1).

Wild blueberry clones and bees are the two primary organisms whose key ecological processes are represented. The clone processes modeled are vegetative growth (including clone rhizome horizontal spatial expansion) and reproductive growth and phenology (including physiological timing of bloom, production of pollen within flowers on a per stem basis, acquisition of pollen on floral stigmas, compatible pollen recognition, pollen tube fertilization of ovules, and seed production). The bee processes modeled are floral search behavior, flower visitation (including pollen extraction and pollen deposition on stigmas), and return to and occupation of nest site (Fig. 2). The interactions between these ecological processes may be spatial, temporal, taxonomic, and genetic or weather relevant, e.g., a flower might accept pollen grains depending on its age (T) and pollen genotype (G); a bee will leave its nest to search for flowers according to the bee species-specific foraging activity (X) subject to weather conditions (W) when the bloom signal (T) has been received. The two organisms are connected via the temporal signaling for the beginning of bloom season, spatial sensing during flower searching, and pollen exchange via bee-flower contact.

Fig. 1. Entities and their interactions identified for wild blueberry pollination.

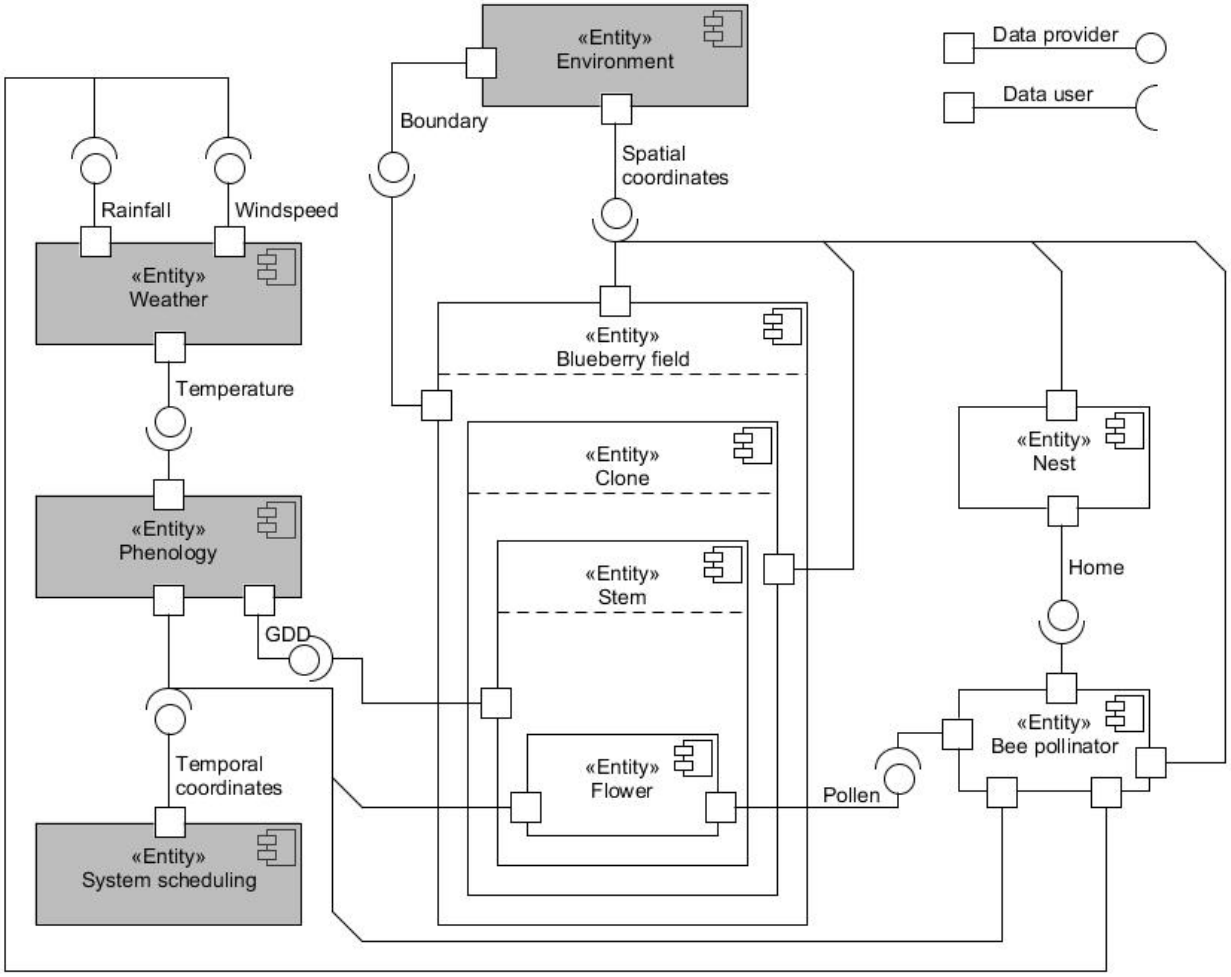


Fig. 2. Conceptual model of wild blueberry cross pollination composed of key ecological processes (ellipses) and their interactions (dashed arrows) that are considered to be spatial (S), temporal (T), taxonomic (X, bee taxa), plant genetic (G), or weather (W) relevant.

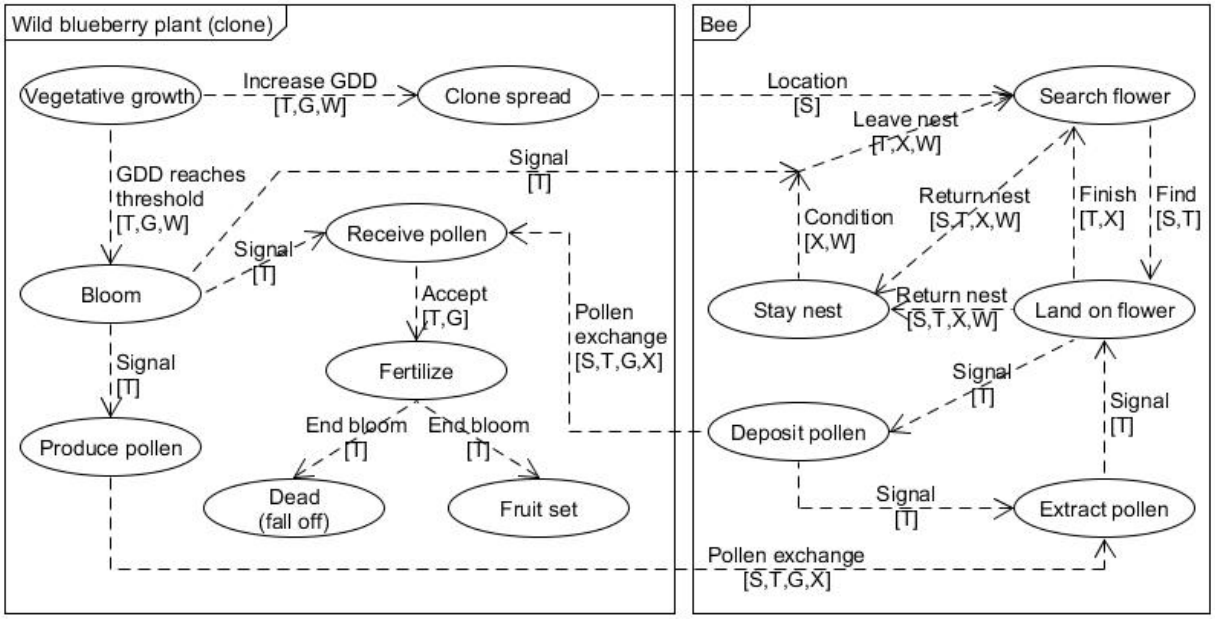
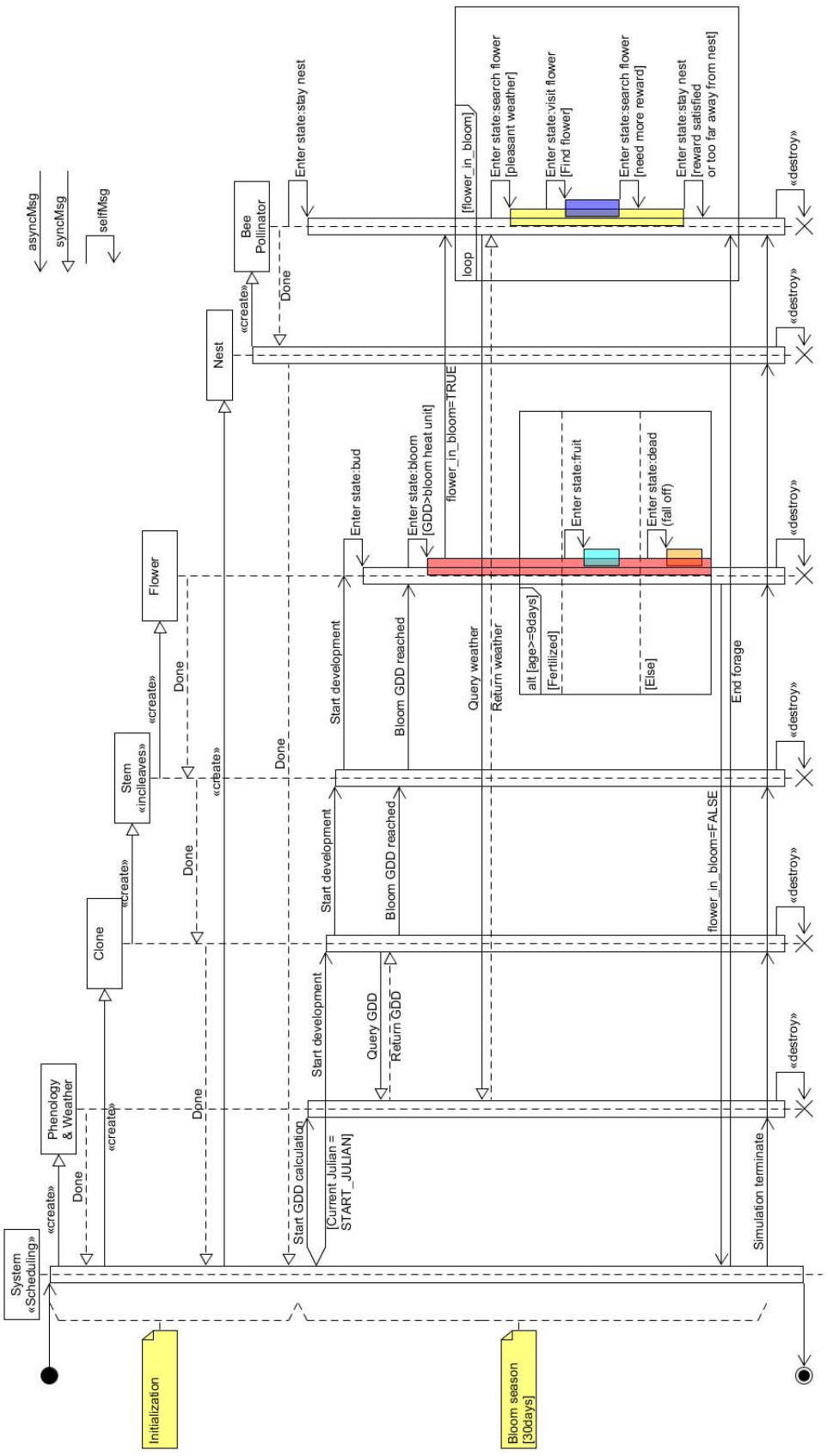


Fig. 3. Ecological processes scheduling.

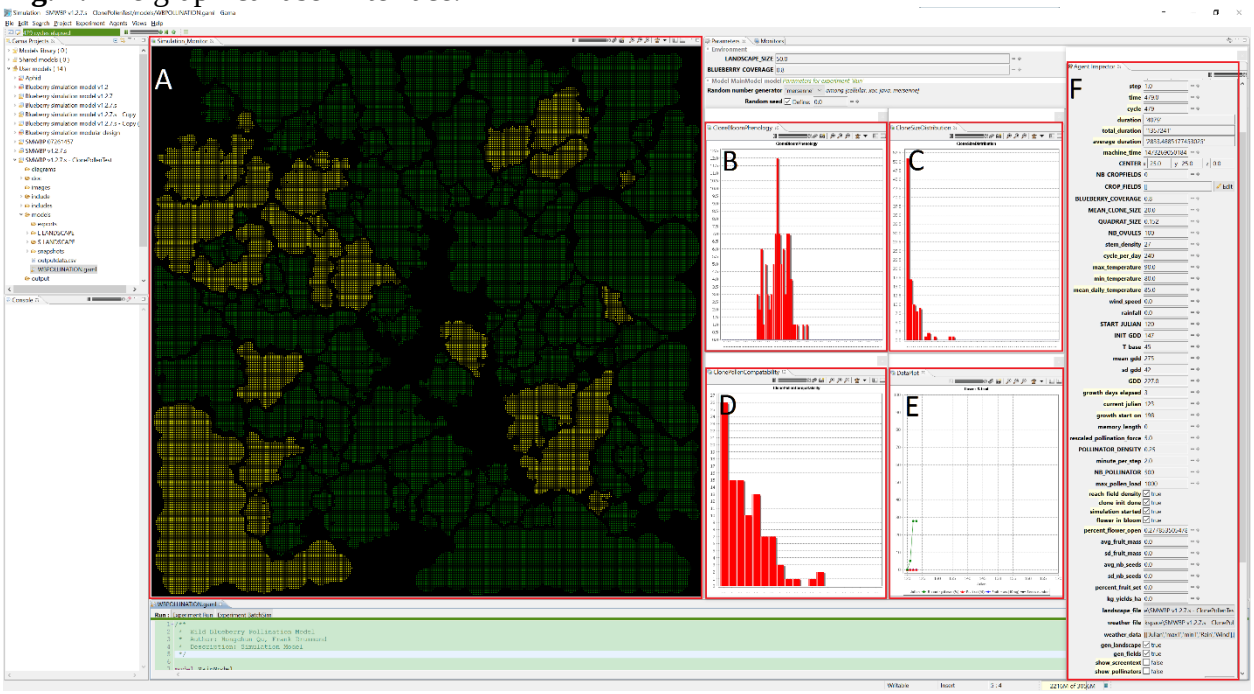


The entities are modeled as agents interacting with each other in a virtual environment. The environment is represented as continuous space and is referenced with a Cartesian coordinate system. A blueberry field is composed of a number of spatially scattered clones. Stems with leaves and flowers attached are randomly distributed within a clone. Bees are spatially initialized by nest site that might be arranged in specific locations in and around a field dependent upon the bee taxa. Bee foraging is modeled as searching and flower visiting from one flower to another that is determined by species-specific flight speed and heading. Pollination, the interaction between plant and bee, is defined as pollen exchange at one flower that is located on a specific stem in a specific clone.

The simulation of bloom season covers approximately 30~45 Julian days (a function of growing degree days (GDD)) after the end of the model initialization stage in which all entities are created and arranged. Then all agents are scheduled according to wild blueberry phenology on daily basis for blueberry development, or on minute basis for bee foraging (Fig. 3).

To incorporate the stochastic nature of organisms and their interactions with their environment, ecological processes of the model are linked to statistical models parameterized with empirical data. First, by using GLM regression (with data appropriate error distributions and linkage functions) and estimation of probability density functions from collected data, parameters and their sampling variance are determined to provide stochastic representation of mechanisms for ecological processes. Then the probability density functions for these processes are assigned to agents to formulate their behaviors by means of Monte-Carlo techniques.

Fig. 4. The graphical user interface.



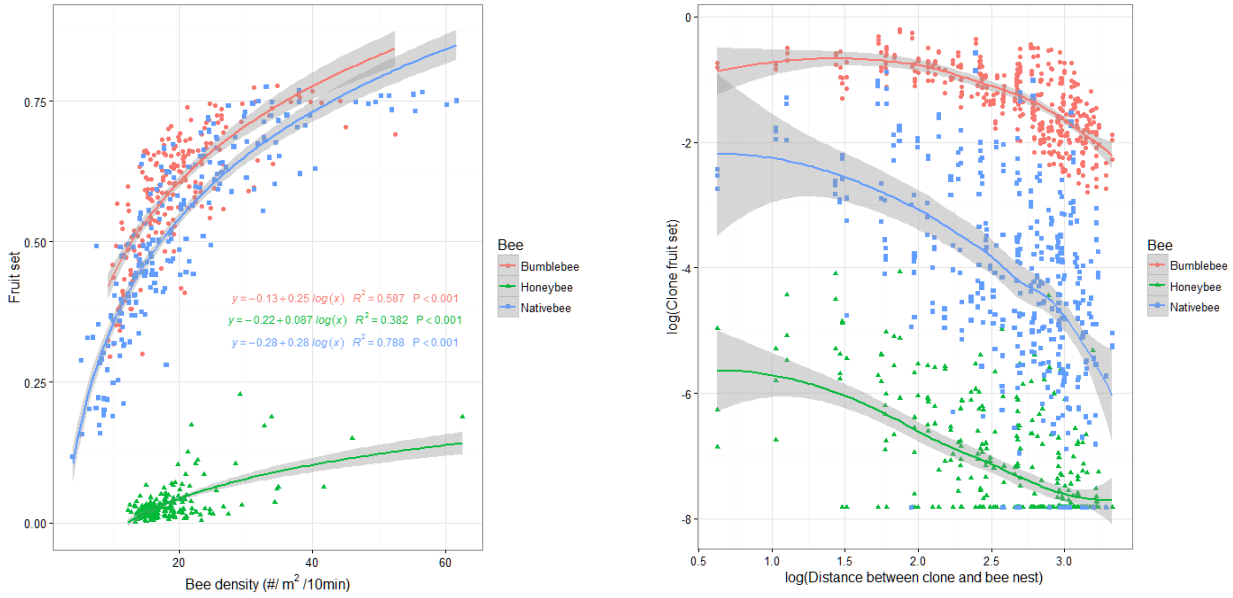
The simulation model has been implemented in GAML language using the open source GAMA modeling platform. Figure 4 shows the screen shot of the graphical user interface in which wild blueberry landscape (A), distribution of clone phenology (B), size (C), and pollen compatibility (D), time series data plot of fruit set, fertile seeds, and berry mass (E) as well as parameters and simulation inspection (F) are organized in the GAMA modeling environment.

Model assessment includes verification, validation and sensitivity analyses. First, all sub-models and their interactions were tested in terms of unit function examination to confirm that they were implemented as intended. Second, pattern oriented validation methods were used to confirm that the model predictions are visually or statistically consistent with pattern observed from field observations or lab experiments, which were different than the data sets employed to parameterize the model. Specifically, domain experts were invited to use their intuition and knowledge to examine model output. Then statistical "goodness-of-fit" tests were conducted to compare simulations with field observations.

In addition, to clarify how model parameters were chosen, their plausible ranges, as well as, how their uncertainty affect model output, the Morris' one-at-a-time method was employed for local sensitivity analysis. Parameters that cannot be determined from empirical data or literatures were chosen. Each parameter value was changed to values of 50% and 150% of the original quantified value of the parameter while holding all others constant. By running simulations, parameter variations were transmitted to model outputs in terms of causing fluctuation. Finally, sensitivities of model output were quantified and explained by perturbations in parameters.

RESULTS: We give an example to show how the simulation model can be used to 1) predict fruit set with specific bee densities for different bee taxa and 2) assess effects of clone distance to bee nest on expected fruit set. Three simulations were conducted in which three bee species: the honeybee (*Apis mellifera*), bumble bee queens (*Bombus* spp.) and solitary native bees (*Andrena* spp.) were tested under the same conditions. In each simulation, we used the same 2500m^2 field with 60% blueberry coverage (40% bare ground or weeds) and 201 clones in the field were generated with a mean clone size of 10.0 m^2. Pollination was performed by 2000 bees (0.8 bees/m^2) under optimal weather conditions. Other parameters were set as defaults. During bloom season, bee visits to each clone was recorded. Simulated fruit set (Fig.5A, honeybee) shows a good agreement with field observations (Fig. 5B). Bee density, bee taxon and their interactions are shown to significantly affecting fruit set (ANOVA, Density: $F = 1384.63$, $P < 0.001$; Bee taxon: $F = 3298.09$, $P < 0.001$; Density*Bee taxon: $F = 31.27$, $P < 0.001$). Clone fruit set is negatively correlated with distance to bee nests, but the correlation varies with bee taxon (ANOVA, Distance: $F = 419.5$, $P < 0.001$; Bee: $F = 1748.2$, $P < 0.001$; Distance*Bee taxon: $F = 201.5$, $P < 0.001$), which has also been observed from wild blueberry fields in Maine. Overall, results shows that bumble bee queens are the most effective pollen vector amongst the three bee species; native solitary bees of the genus *Andrena* show less efficiency than bumble bee queens, but is still much higher in efficiency than non-native honeybees, which are similar to empirical study findings.

Fig. 5. Effects of bee density (A) and distance between clone and bee nest (B) on clone fruit set for different bee taxa, simulated by the model.



(A) (B)

CONCLUSION: We modeled the spatial and genetic structure of wild blueberry plants and bee foraging behaviors for different species at the individual bee, flower, stem, and clone level. The interactions between individuals are scheduled by blueberry phenology and are linked to environmental conditions. We are able to simulate pollination efficiency varying factor combinations both within a field and at the field level.

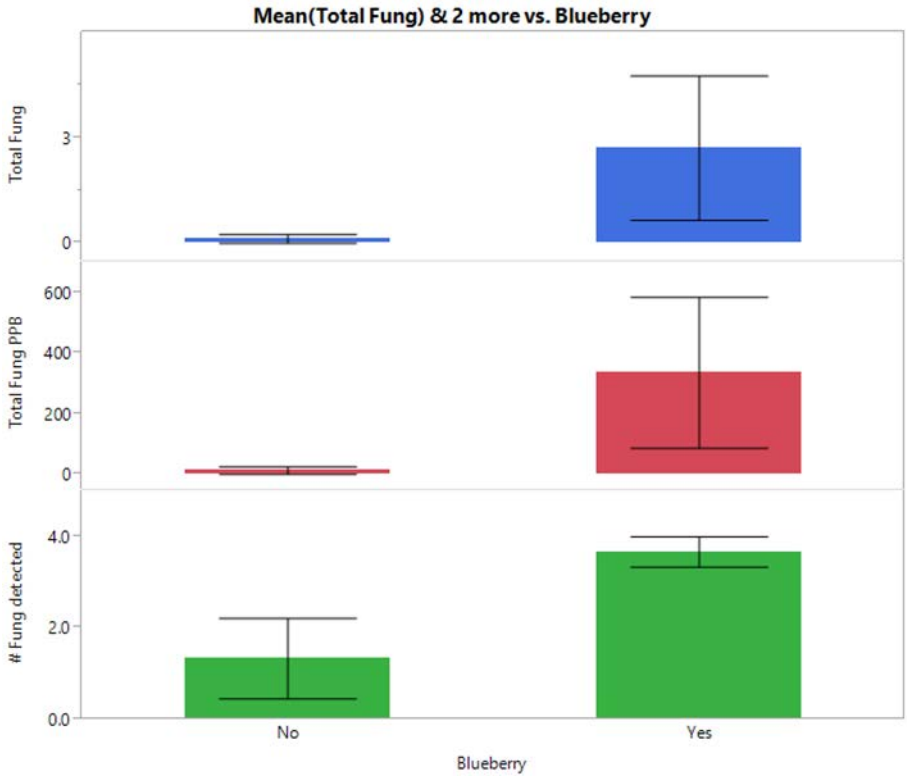
Study 3. ***<u>Honeybee colony health on the wild blueberry barrens during bloom.</u>***
<u>Report from D. vanEngelsdorp and A. Garavito (U. Maryland), and F. Drummond</u>

OBJECTIVES: Wild blueberry requires pollination to produce high, uniform yield, and so a viable and healthy supply of bees is essential. The Bee Informed Partnership has received numerous reports of commercial beekeeper colony losses during and shortly following blueberry pollination, which may be driving up pollination prices as beekeepers build compensation into their rates against potential pesticide losses. Emergency Response Kits evaluated on behalf of commercial beekeepers demonstrate elevated fungicide exposure during blueberry pollination (Fig 1). Currently we only have three samples per group, so although a clear trend is visible, the sample numbers are too small to provide statistical power for testing. While fungicides are currently deemed safe for honeybees, there is some evidence that suggests these fungicides could be detrimental to honeybee larvae and colony health. We thus proposed to follow colonies from three commercial operations before, during, and after blueberry pollination to evaluate the impacts of blueberry pollination on risk factors associated with poor colony health.

The objectives in this study, conducted in 2015, were to determine the risk wild blueberry pollination has on colony health by measuring impacts (if any) of blueberry pollination on

colony size and other colony health metrics or risk factors and by measuring and comparing pesticide residues in beebread before, during, and after blueberry pollination.

Fig. 1. Fungicide levels from Emergency Response Kit pollen pesticide analysis in colonies pollinating blueberries (right) compared to colonies not in blueberries (left). Blue = Hazard Quotient risk from all fungicide residues detected, red = fungicide residues in ppb, green = # of fungicide residues detected.



METHODS: We conducted an observational study of three commercial beekeeping operations that pollinate wild blueberries, evaluating colony strength and collecting samples before, during and after blueberry pollination. Samples included live bee samples to measure *Varroa* mite, *Nosema*, and viral disease loads, and 3-gram samples of beebread for pesticide residue analysis. Seventy-two colonies were selected (24 from each operation) and evaluated for colony strength using standard assessment methods. 36 colonies, 12 per operation were then randomly assigned to the wild blueberry exposure group (those moving into blueberry fields) while the remainders were moved to an organic blueberry field to act as our control. Two weeks after placement in blueberry fields, colony health was once again assessed and beebread samples were also taken. Finally, approximately two months later, and about six weeks after removal from the blueberry fields, colonies were tracked and resampled. Regrettably, one operation could not locate the experimental or control colonies and so only two operations could be sampled.

RESULTS: No colonies were lost over the course of the experiment. Wild blueberry exposure colonies were of equal sizes at the start of the study, and brood patterns did not differ. No differences in colony size and brood quality measures were detected when colonies were

sampled in wild blueberries (Figs. 2-4). However, colonies in control groups were significantly smaller (as measured by frames of bees (Fig. 2) and brood (Fig. 3)) and had poor brood patterns (Fig. 4) when sampled in July as compared to wild blueberry exposure groups. This result suggests that wild blueberry production in 2015 did not have any adverse effects on honeybee colony growth.

While *Varroa* mite levels, as measured by mites per 100 bees, did not differ between the wild blueberry exposure group before moving into blueberry fields, control groups had higher mite levels than the wild blueberry exposure hives (Fig. 5); this difference remained evident six weeks later. No measure of disease (*Nosema* loads, parasitic mite syndrome, DWV, Chalk brood, European foulbrood, queen condition) differed between control and wild blueberry exposure colonies. While beebread samples were collected from every sampled colony, to reduce expenses, beebread samples from colonies on the same pallet were combined before analysis. Of the 174 products and/or their metabolites tested, seven were found in at least one sample taken over the course of the study. These products included 2,4 Dimethylaniline and DMPF, both breakdown products of the varroacide Amitraz® found in some samples taken before, during, and post blueberry. Similarly the varroacide fluvalinate was found in samples taken at each sampling period. The insecticide phosmet (Imidan®) was found in thee samples collected from colonies placed in conventional blueberry fields (wild blueberry exposure group). The herbicide sethoxydim was found in two samples taken from colonies in conventional farms during blueberry pollination. Finally the varroacide Thymol® was found in all samples taken from one operator at all collection time points. When grouped by functional group, the number of insecticides found in samples taken while colonies were placed in blueberry fields to pollinate crops was higher in wild blueberry exposure hives (1±0.25) compared to control hives (0±0.0) (P = 0.027). The total Hazard Quotient, a simplified measure of a colony's pesticide exposure, was also higher in wild blueberry exposure colonies compared to control colonies during pollination (0.92±4.73 vs 13.6±4.73; P = 0.044). However, the HQ value in the most contaminated sample had an HQ of 16.0, which is less than 0.2% of the HQ score thought to possibly cause harm to colonies (HQ=1,000 represents 10% of a bees LD_{50} if she eats the pollen for 10 days).

Fig. 2. Worker bee populations in the wild blueberry group (red) and control group (blue).

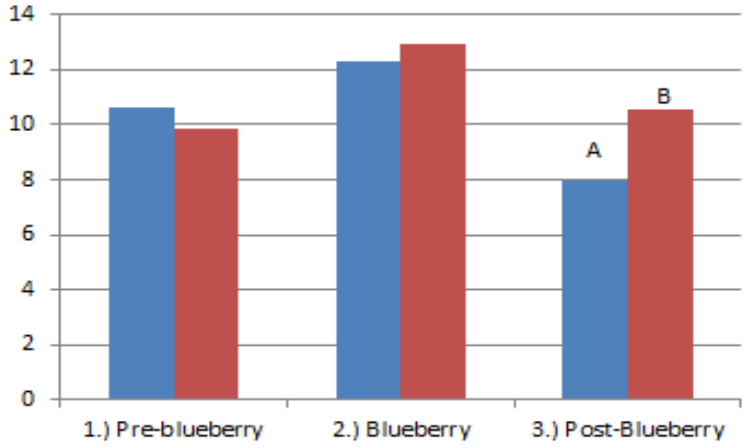


Fig. 3. Brood (larvae) populations in the wild blueberry group (red) and control group (blue).

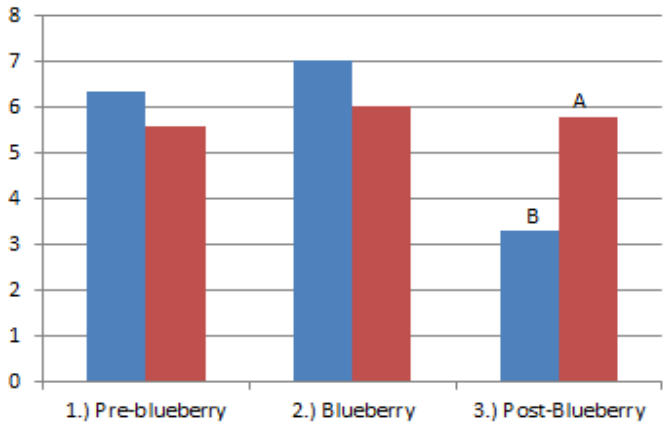


Fig. 4. Brood pattern in the wild blueberry group (red) and control group (blue).

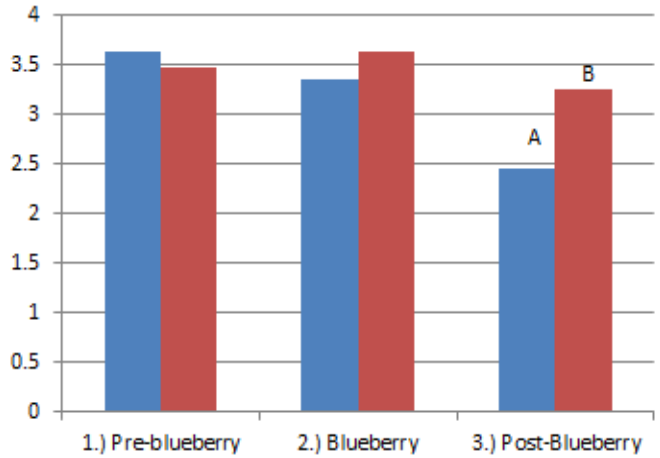


Fig. 5. *Varroa* mite infestation in the wild blueberry group (red) and control group (blue).

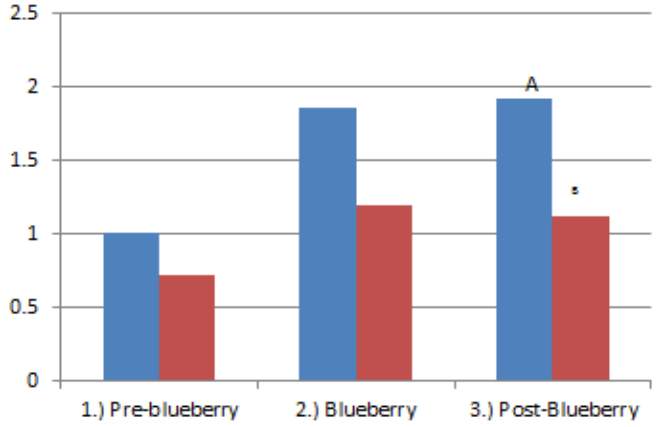
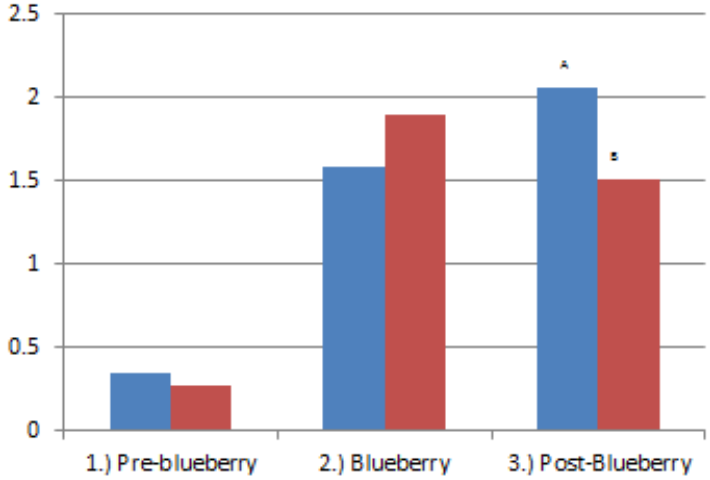


Fig. 6. *Nosema spp.* infection in the wild blueberry group (red) and control group (blue).



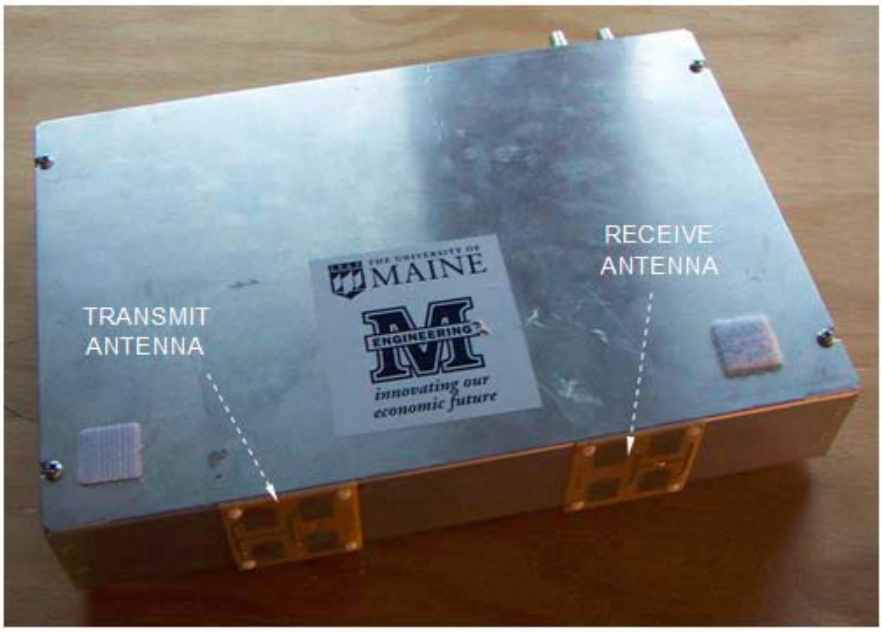
CONCLUSIONS: The lack of pesticide detections in samples taken during and after pollinating blueberry fields was a pleasant surprise. We contacted blueberry producers to see if there was a change in pesticide practice and we learned that the year our experiment was conducted, growers did not need to spray as many fungicides since mummy berry threats were low. While frustrating, this does allow us to tease apart some other issues as well as give us some insight on what is really happening in the field. One concern we have is that, when we separate out the pollen loads of bees by type, we often find pesticide loads in non-crop pollen and little in the crop pollen. This has led us to be concerned that some weedy species in the area of the crop are reservoirs for pesticide contamination. The current results don't support this theory, which is good to know and also encouraging as it suggests, for blueberries at least, reduced spray regimens have immediate effect in terms of the number of products found in colonies.

Study 4. ***Development of a Doppler radar microphone for honeybee colony health assessment.***
Report from Dr. N. Emanetoglu, Dr. H. Aumann, J. Lund, and F. Drummond

OBJECTIVES: Honeybee health monitoring requires intensive and sometimes invasive hive inspections. The hive inspections are very labor intensive and preclude assessment of a large number of hives in a small amount of time. In 2016, we conducted a second year of investigating RADAR techniques to monitor honeybee hives, with the aim to test different versions of the transmitter and receiver.

METHODS: Portable Doppler radar microphones were built and tested in the field. The modulation of the radar signal due to the mechanical bee vibrations emitted by bees living in the hive was measured and correlated with honeybee activity. The versions that were built were: 1) a 2.4 GHz Doppler radar, 2) a 5.8 GHz Doppler radar microphone (transmission), 3) a 5.8 GHz Doppler radar microphone (reflection), 4) a 5.8 GHz Doppler radar microphone (IQ), and 5) a 10.5 GHz Doppler radar microphone.

Fig. 1. Doppler radar microphone developed at the University of Maine for honeybee research.



Three units (2.4 GHz, 5.8 GHz (IQ), 10.5 GHz) were used near an observation hive at the Aumann Farm site. They were used to take measurements of the observation hive daily, from June through August, multiple times a day. The output was recorded using a laptop, in the WAV file format. Simultaneous recordings were also made using acoustic microphone, and these recordings were also saved in WAV file format. Three units; 5.8 GHz (reflection), 5.8 GHz (transmission), 10.5 GHz) were also used in experiments at the University of Maine Apiary at Roger's Farm, Stillwater, ME in June, July, and August. The transmit and receive antennas were placed on opposite sides of the hive. The 5.8 GHz radar used an aluminum U-frame to mount antennas & wooden legs (Fig. 2). The transmit and receive antennas were placed together, facing the same direction on the side of hive, facing the hive entrance, or facing away from the hive entrance. The reflected signal was modulated by bee motion: Bee flight →Doppler effect, Stationary vibrations →radar microphone effect. For the 5.8 GHz single channel radar, the protocol was as follows. Audio data was collected for 1 minute every 15 minutes, at 8 kS/s. Expected bee vibrations & Doppler shifts were measured between 10 Hz and 500 Hz, i.e. audio frequencies. RF signal attenuation level and temperature inside the data collection system enclosure was recorded as well. Figure 3 shows the single channel 5.8 GHz radar.

Fig. 2. Mounted frame for stabilization of the 5.8 GHz Doppler radar unit.

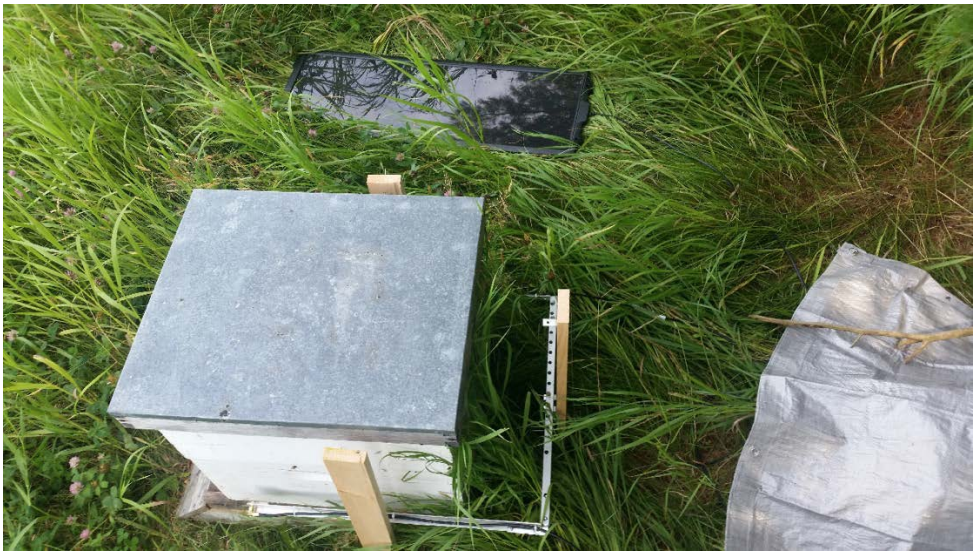
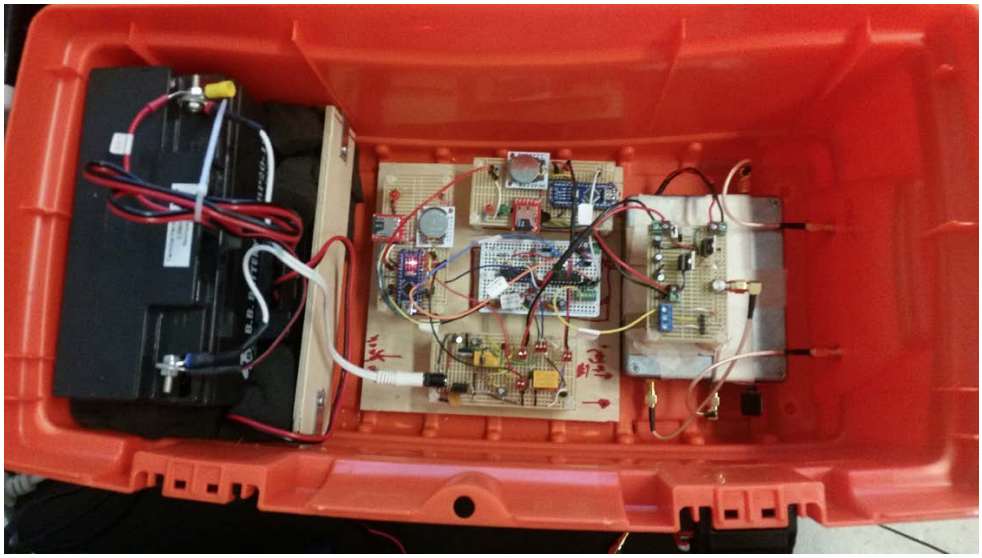


Fig. 3. University of Maine 5.8 GHz Doppler radar system.

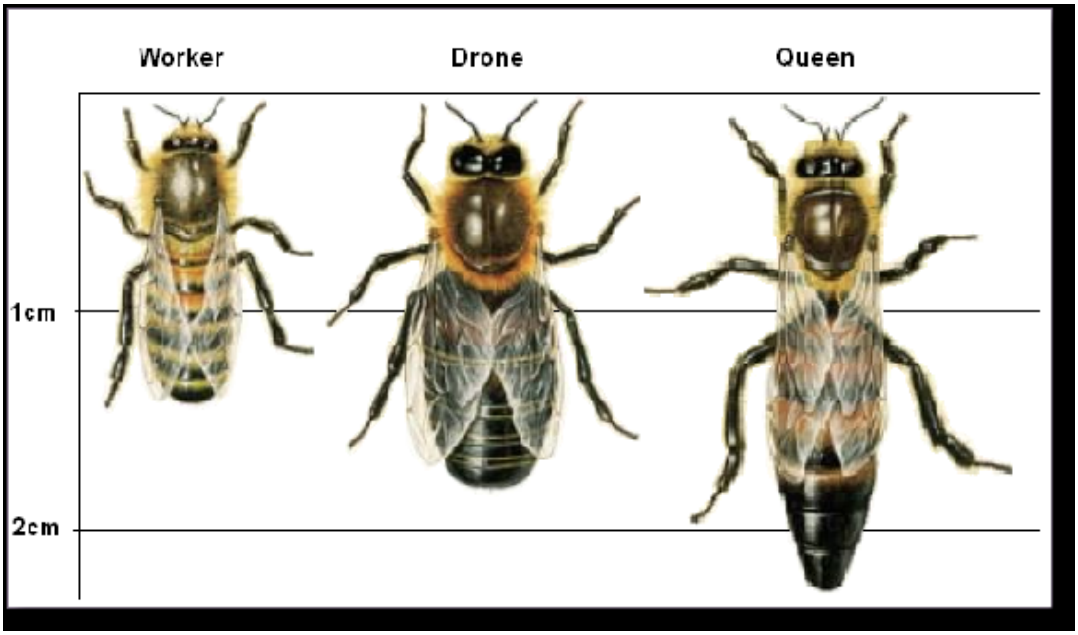


The radar frequency and wavelength determines if single bees can be detected by the radar. Table 1 and Figure 4 show that the 5.8 and 10.5 GHz radars can detect individual bees while the 2.4 GHz cannot. This is because wavelength (λ) is inversely proportional to frequency and resolution of the smallest object that can be resolved by the radar is ~ quarter wavelength (Rayleigh limit for optical resolution is λ/2).

Table 2. Frequency, wavelength, and resolution of the three radar units.

Frequency (GHz)	Wavelength (cm)	Resolution (cm)
2.4	12.5	3.2
5.8	5.2	1.3
10.5	2.9	0.71

Fig. 4. Honey bee worker and queen size (cm).



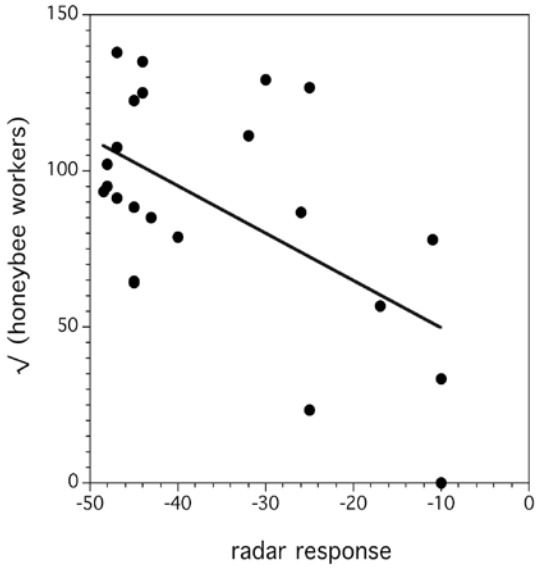
The hypotheses tested in 2016 were:

Transmission Mode Experiments
Hypothesis 1: The RF signal attenuation will be proportional to the 'fullness' of the hive and so can be used as a metric to determine amount of bees and/or honey.
Hypothesis 2: The "radar microphone" effect should also be detected in transmission mode and so can be used as a tool to separate bees from honey, and possibly quantify bee activity levels or colony size.

Reflection Mode Experiments
Hypothesis 1: Bee activity levels can be determined from Doppler data. Count number of flying bees or total signal strength
Hypothesis 2: The "radar microphone" effect can be used to listen to bees inside the hive. Bee vibration / noise should be related to colony strength and health. Bee activity such as fanning to circulate air could be detected.

RESULTS AND CONCLUSIONS: In 2015, we found that the Doppler microphone was able to predict worker number in each box fairly well (explaining 32% of the variance in worker bee numbers, $P = 0.005$, Fig. 5). Including the amount of honey in the hive, it was found that the Doppler microphone can predict hive strength much better when measured as BOTH worker bee numbers and the amount of honey. The overall percent variation in predicting colony strength increases to 51% of the variation explained ($P = 0.0003$). This is because both honey and honeybee workers are comprised of high levels of water and water is a good absorber of radio waves.

Fig. 5. Relationship between Doppler microphone readings and worker population per hive box.



In 2016 we found the following:
1) 2.4 GHz Transmission Mode Results. Approximately three months of data was collected. Antennas were positioned around the lower hive body. Large diurnal variation was detected in signal attenuation. This was larger at nighttime, indicating bee presence. Average attenuation increased approximately linearly in the long term, which might be an indicator of honey & brood. The acoustic signature due to bee activity was not detected because the electromagnetic wavelength was too large to resolve bees effectively (see Table 2 and Fig. 4).

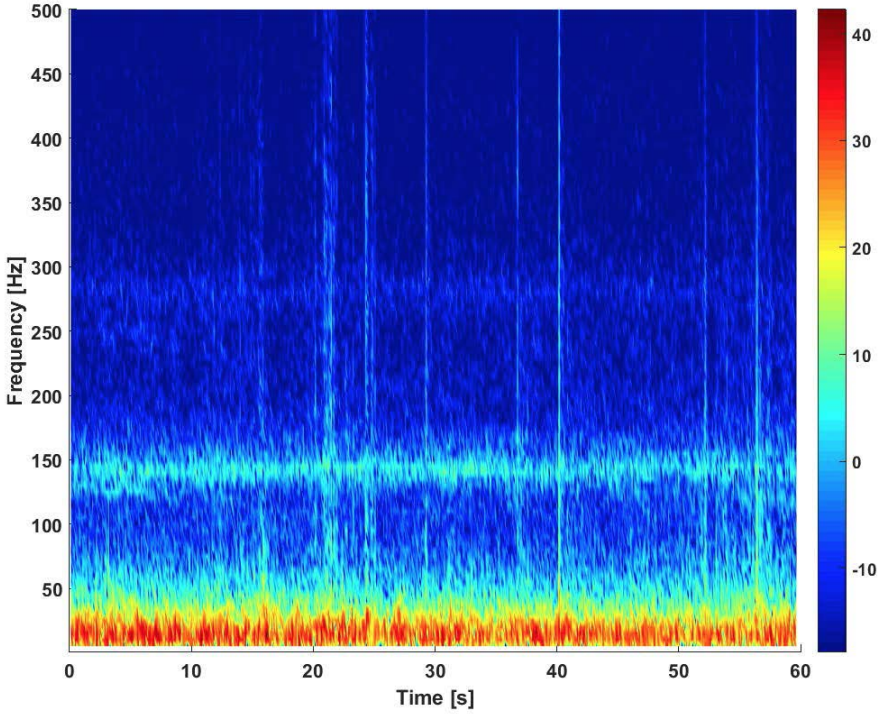
2) 5.8 GHz Transmission Mode Results. Approximately one week of data was collected. Antennas were positioned around the lower hive body. The signal was attenuated down to the detection limit from the beginning. The detection limit set by the noise floor, but this could be potentially improved by a factor 10 by redesigning the RF system. Over 400 audio files were collected. The acoustic signature due to bee activity was detected in five files. The RF attenuation was too high, so audio signals were below the noise floor in most cases. The acoustic signature that was detected was between 100-150 Hz. This is the suspected wing beat frequency of worker bees.

3) Reflection mode experiments: Acoustic signatures observed in most data files. The frequency range was 100 –150 Hz (Fig. 6). The signal strength could not be reliably related to time of day

or activity level.

4) Reflection mode at the hive entrance. The acoustic signatures were observed in almost all data files. When bees were flying: Doppler tracks < 100 Hz, audio of wings beating between 150 to 250 Hz. When bees were not flying the audio signature was in the 100 –150 Hz range (Fig. 7). Signal strength reliably related to time of day & activity level (Fig. 8).

Fig. 6. Reflection mode frequency (note the light blue frequency range of 100-150 Hz).



The 10.5 GHz reflection radar results turned out to be most sensitive to single bees due to the fact that the wavelength is smallest and highly directional. The Doppler shift was clearer. This was expected due to higher frequency. Again the localized 100-150 Hz vibration was detected by individual bee wing beat frequencies.

Fig. 7. Frequency reflection for bees flying (top) and bees at home in the hive (bottom).

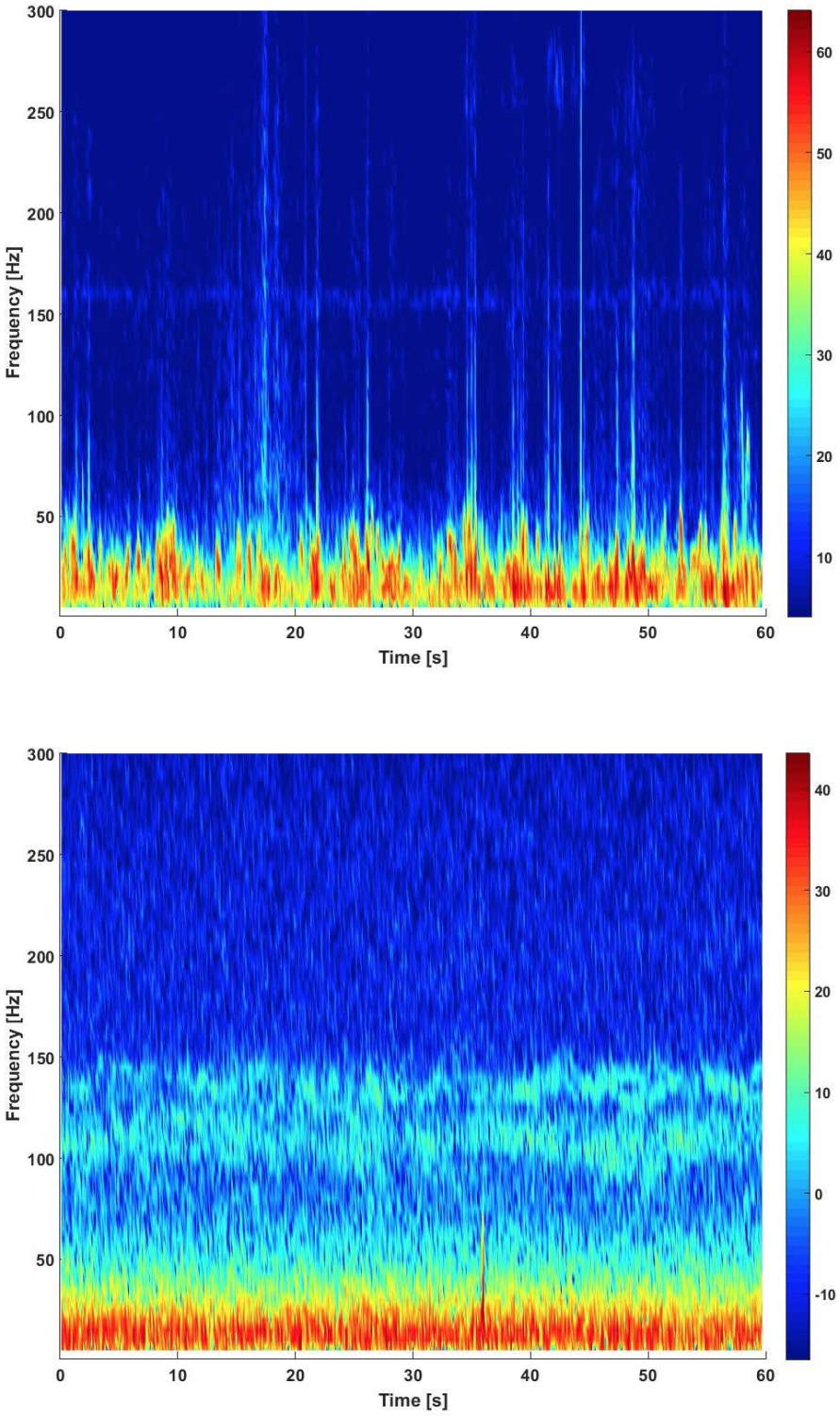
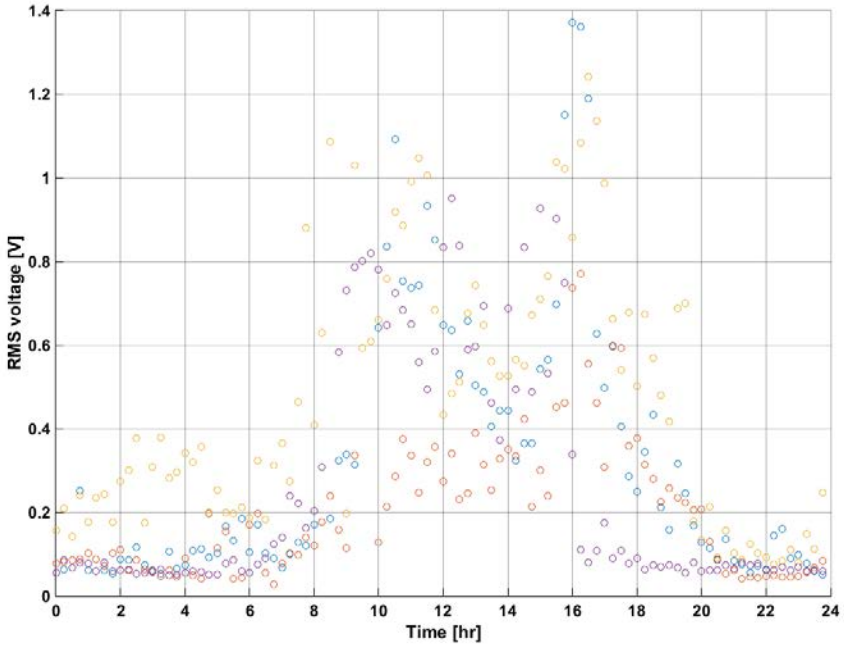


Fig. 8. Time of day and bee activity indicated by reflectance frequency change.



Study 5. ***<u>Potential lethal synergism of two commonly used pesticides: effects on honeybees and bumble bees</u>.***

OBJECTIVES: Acetamiprid (formulated as Assail®) is thought to be a "bee safe" insecticide. It is very effective against blueberry flea beetle, blueberry spanworm, and moderately effective against blueberry maggot fly and the spotted wing drosophila. However, over a decade ago, a study conducted in Japan in the laboratory showed that the simultaneous exposure of honeybees to acetamiprid and the fungicide propiconazole (commonly used by blueberry growers as Orbit® or Tilt® for control of mummy berry) was shown to synergize the toxicity of acetamiprid making it no longer "bee safe". We have been studying exposure to both bumble bees and honeybees to simultaneous exposure to both of these pesticides to determine if this form of synergism is observed under field conditions.

METHODS: Several experiments were conducted in the field in 2016. The first experiment was to determine if recommended application field rates (measured as the contact LD_{50}) for the pesticides propiconazole (Orbit®) and acetamiprid (Assail®) could be used for the Eastern bumble bee, *Bombus impatiens*. We conducted a cage study in the laboratory in a controlled temperature growth chamber maintained at 20ºC and 80-90% relative humidity. The cages were wire hardware cloth cylinders 12cm in diameter and 25cm high. Each cage had a plastic petri dish top as a top lid with a hole drilled to fit a syrup feeder. Bumble bee workers (8/cage) were taken randomly from two Koppert® commercial bumble bee colonies and topically dosed with either propiconazole, acetamiprid, both of the pesticides, or distilled water (control). The rates were 0.1x, 0.5x, and 1x (the honeybee contact LD_{50}). Survival was monitored for eight days.

A second experiment involved the use of 6 x 2 x 2-m field cages erected over wild blueberry in bloom at the University of Maine Blueberry Hill Experimental farm, Jonesboro, ME. The crop inside the cages were sprayed at 25% bloom with either water (control), acetamiprid (formulated as Assail®), or a combination of acetamiprid and propiconazole (formulated as Tilt®). The rates used were estimated to provide 10% mortality based upon the honeybee contact LD_{50} level. Immediately after pesticide application either a honeybee colony (started that spring from a package) or a commercial bumble bee colony (*B. impatiens* obtained from Koppert® Quads) were placed separately in each of three replicated cages for each treatment. Colonies were kept in the cages until the end of bloom and then transported to the University of Maine Witter Farm (Orono, ME) and monitored monthly for the rest of the spring, summer and fall.

The third experiment involved spring feeding bumble bee colonies (sugar syrup) and honeybee colonies (pollen patties) the following treatments: 1) no pesticide (control), acetamiprid (formulated as Assail®, concentration to result in 10% mortality), and acetamiprid and propiconazol (formulated as Tilt®, concentration for acetamiprid and propiconazol to result in 10% mortality). Three bumble bee colonies were fed each treatment for seven days and 12 honeybee colonies were fed each treatment in a formulated pollen patty (300gms) placed on the top bars of each colony. Both honeybee and bumble bee colonies were monitored monthly throughout the spring, summer, and fall. Appropriate statistical analysis was used to assess individual bee survival (experiment 1) or colony health (experiments 2 and 3).

RESULTS: The results of the first experiment can be seen in figure 1. None of the treatments resulted in mortality of worker bumble bees that were significantly different than mortality observed in the control. This suggests that Assail (acetamiprid) and the mixture of Assail and Orbit (propiconazole) do not result in any higher rate of mortality in the cages than what occurred in the control treatment. However, while not significantly different than the control, the full rate of Orbit did result in significantly higher mortality than the 0.5x rate of Orbit, suggesting that this fungicide can be toxic to bumble bee workers.

The results of the second experiment with bumble bees suggests no detectable differences between treatments in the abundance of total individuals (larvae and adults) ($F_{(15,19)} = 2.01$, $P = 0.14$); although, the P value suggests that caution should be exercised with this conclusion. Nor did the addition of pesticides to syrup result in reduced syrup consumption ($F_{(15,19)} = 0.32$, $P = 0.86$) or hive weight ($F_{(15,19)} = 1.47$, $P = 0.26$) as shown in Table 1. However, there was a difference observed in the number of gynes (potential queens for following year)($P = 0.049$) between hives fed syrup containing 0.1x Tilt and 1.0x Assail, but not between the control and Assail at 1x concentration (Fig. 2). Table 1 shows all of the bumble bee measures relative to the treatments.

Fig. 1. Mortality of Eastern bumble bee workers (*B. impatiens*) held in cages in the laboratory and exposed to field application rates of Assail® (acetamiprid), Orbit® (propiconazole), and a combination of both pesticides.

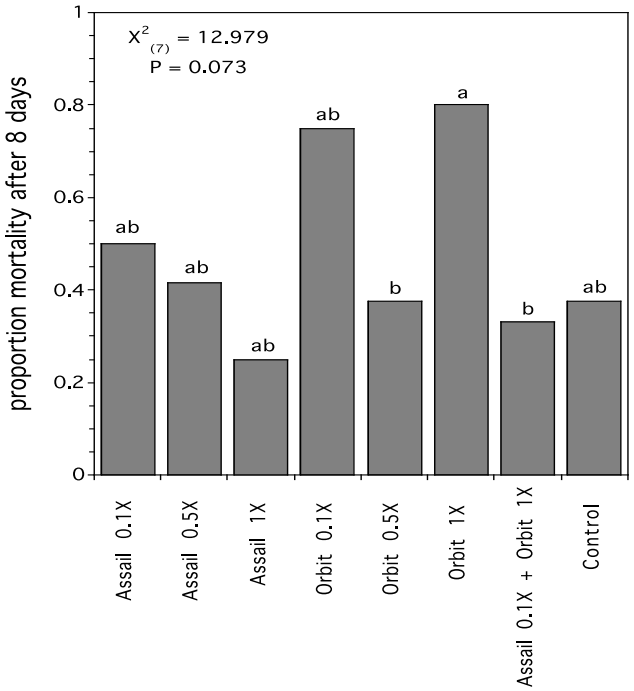


Fig. 2. Queen (gynes) production by end of experiment (error bars are se), treatments sharing the same letters are not significantly different from one another (Tukey post-hoc test, $P \leq 0.05$).

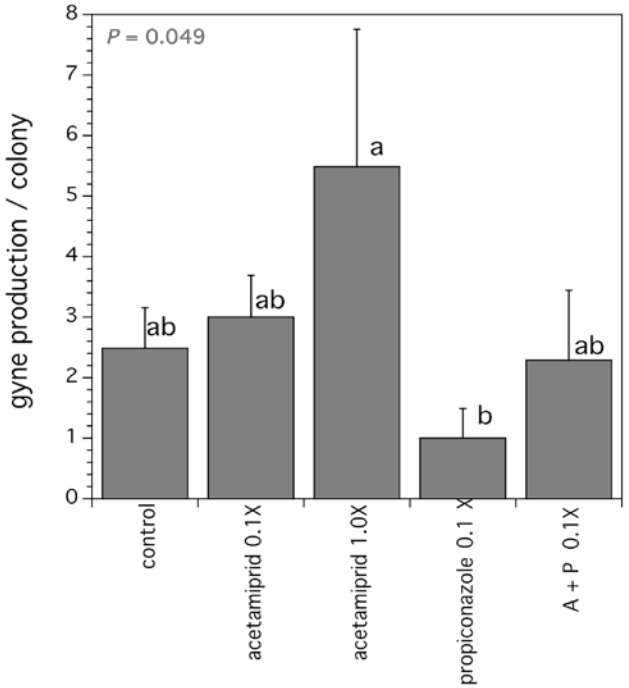
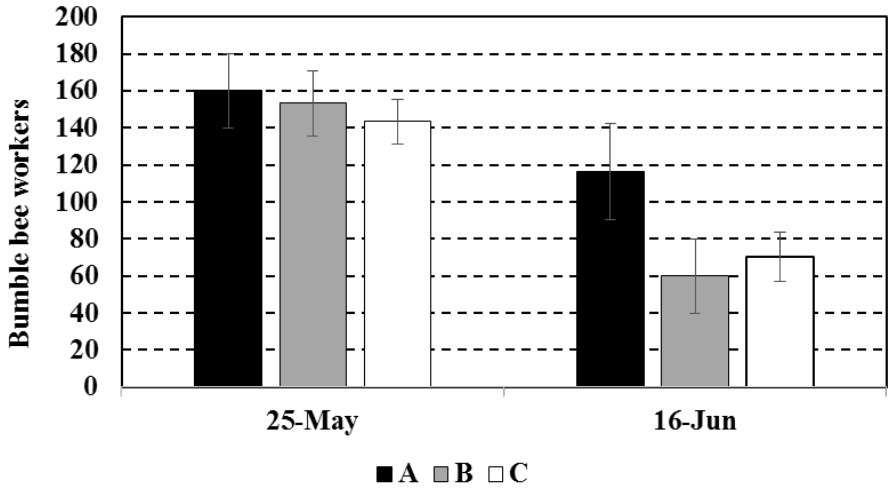


Table 1. Mean ± standard deviation for each dependent variable represented by treatment (A: acetamiprid, P: propiconazole). Only one statistically significant difference was detected in the abundance of gynes between hives fed syrup containing 1.0x Assail and hives a fed syrup mixture containing 0.1x Assail with 0.1x Tilt.

TMT	Total bees	Adults	*Gynes	Med Adults	Sm Adults	Pupae	Larvae	Honey Pots	% Δ Weight	% Δ Syrup
Control	180 ± 26	616 ± 103	2.5 ± 1.3	115 ± 25	62 ± 44	211 ± 101	225 ± 95	62 ± 19	52% ± 24%	67% ± 15%
0.1x A	157 ± 53	693 ± 234	3 ± 1.4	110 ± 42	44 ± 14	290 ± 118	246 ± 112	68 ± 20	42% ± 18%	69% ± 17%
1.0x A	172 ± 51	638 ± 97	*5.5 ± 4.5	117 ± 47	49 ± 10	225 ± 34	241 ± 88	72 ± 41	46% ± 11%	58% ± 16%
0.1x P	248 ± 45	1021 ± 260	*1 ± 0.8	173 ± 55	74 ± 11	369 ± 108	405 ± 112	79 ± 36	70% ± 27%	58% ± 37%
0.1x A + P	230 ± 92	938 ± 480	2.3 ± 0.5	174 ± 64	54 ± 29	420 ± 262	288 ± 149	49 ± 9	72% ± 30%	72% ± 22%

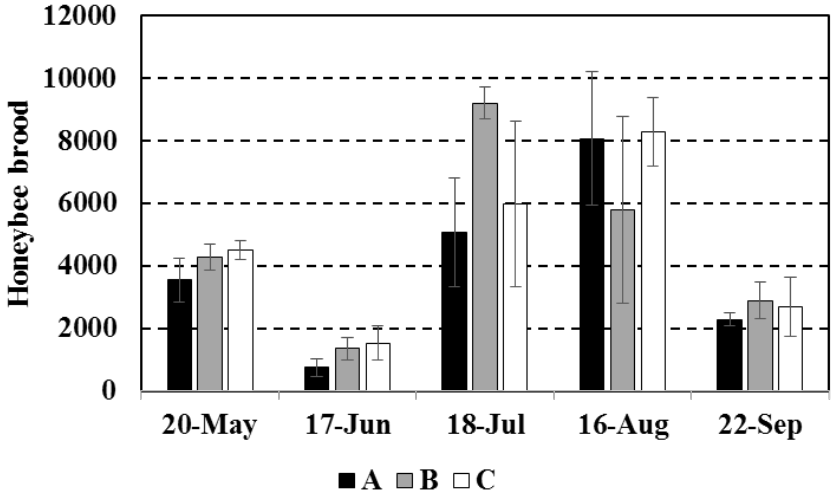
A second trial of the bumble bee cage study was compromised due to an early collapse of the bumble bees due to disease. However, results through to July (the time when colonies collapsed) suggested that there were no differences due to treatment for worker bumble bees ($F_{(2,6)} = 1.159$, $P = 0.375$) or brood (larvae) ($F_{(2,6)} = 1.207$, $P = 0.363$). Although a trend toward lower populations of adults and brood appears to exist with Assail and the Assail + Tilt mixture and the control, but not between Assail and Assail + Tilt mixture which suggests that Assail may reduce bumble bee populations in colonies, but there does not seem to be evidence that synergy between Assail and Tilt exists. Figure 3 shows this dynamic in worker bumble bees.

Fig. 3. Bumble bee workers in colonies exposed to the field cage study treatments A = control, B = Assail, and C = mixture of Assail and Tilt.



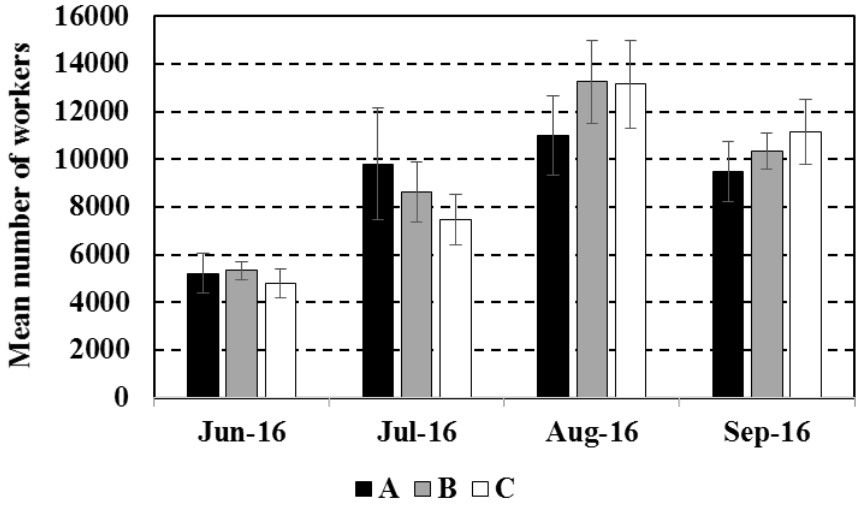
The results of the second experiment with honeybees is not complete yet since overwintering success will not be assessed until the spring of 2017. At this point we have not found treatment effects on worker bees ($F_{(2,6)} = 0.437$, $P = 0.665$); honeybee brood ($F_{(2,6)} = 0.384$, $P = 0.697$); queen egg laying (eggs/day)($F_{(2,6)} = 0.237$, $P = 0.865$); or *Varroa* mite parasitism ($F_{(2,5)} = 1.701$, $P = 0.273$). An example of the treatment response in brood production in the honeybee colonies is shown in figure 4.

Fig. 4. Honeybee brood (larvae) in colonies exposed to the field cage study treatments A = control, B = Assail, and C = mixture of Assail and Tilt.



The third experiment was only completed for the honeybee trial because disease in the bumble bees resulted in the entire experiment being compromised. For honeybees we observed no effects due to treatment for workers ($F_{(2,15)} = 0.0643$, $P = 0.938$); brood ($F_{(2,15)} = 0.006$, $P = 0.953$); egg laying by queens ($F_{(2,13)} = 0.145$, $P = 0.866$); the percent of non-emerged brood ($F_{(2,13)} = 0.549$, $P = 0.589$), or levels of the parasitic *Varroa* mite ($F_{(2,14)} = 0.031$, $P = 0.809$). Figure 5 illustrates the treatment effects on worker populations in the treated and non-treated colonies.

Fig. 5. Honeybee workers in colonies exposed to the feeding treatments A = control, B = Assail, and C = mixture of Assail and Tilt.



CONCLUSIONS: While our experiments suggest that field rate concentrations of Assail and a mixture of Assail and Tilt can result in a few detrimental effects to bumble bee and honeybee colonies, synergy characterized by high levels of mortality (800 times that observed with Assail alone in the laboratory) were not observed.

Study 6. Field exposure of honeybee colonies to pesticides in Maine.

OBJECTIVES: There is no baseline data for pesticide exposure to honeybees in Maine. We have conducted several studies over the past decade that have characterized exposure to honeybees through pollen, nectar, wax, and foragers; but all of these studies have been conducted in the wild blueberry agro-ecosystem. Therefore, little is known how these exposure rates compare to other agricultural or non-agricultural landscapes. In 2015, we were funded by the state of Maine and the USDA to initiate such a baseline for honeybee exposure in the state. We are reporting upon this study now because the pesticide chemistry analyses were not completed until the summer of 2016.

METHODS: During the winter of 2015, beekeepers throughout Maine were solicited to enlist in this project and volunteer their time to assist in trapping pollen throughout the state. Initially the selection of beekeepers was based upon geographic distribution, a beekeeper having more than two colonies, and beekeepers were selected who would represent a diversity of landscapes within which their apiaries were imbedded. However, poor overwintering success in many apiaries across the state necessitated finding additional volunteers just prior to the spring. In all, 26 volunteers/sites were involved in this project. In addition, six wild blueberry sites were sampled by Dr. Drummond and his lab for a total of 32 sites. Figure 1 depicts the geographic distribution of these sites and the breakdown between agricultural and non-agricultural sites.

Each volunteer was provided with a front entrance pollen trap (Fig. 2), instructions for use, and collecting cups. Instructions were to trap/collect pollen from a single colony for a week in the spring, summer, and fall. Pollen from the three collection periods was sent to the University of Maine, Orono where they were aggregated over sample date. The 32 samples were then sent to the Connecticut Agricultural Experiment Station where Dr. Brian Eitzer ran a screen for 192 different pesticides and metabolites using HPLC and mass spectrometry. After the results were sent to Dr. Drummond's lab, summary of the pesticide exposure by site was conducted.

Fig. 1. Honeybee colony apiary sites in 2015. Red dots are agricultural sites and green dots are non-agricultural sites.

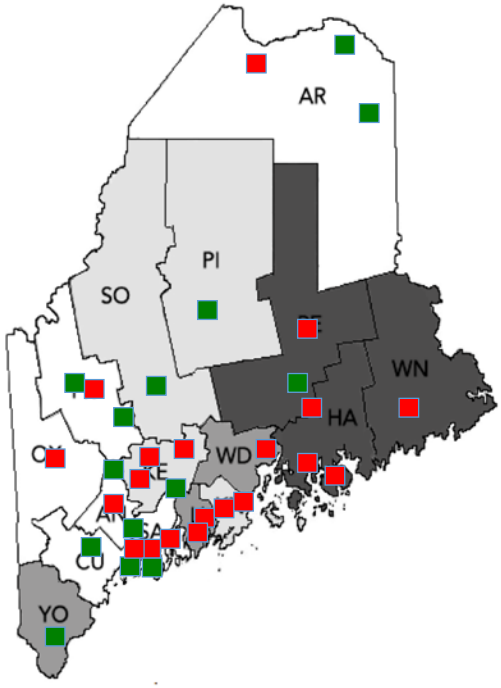
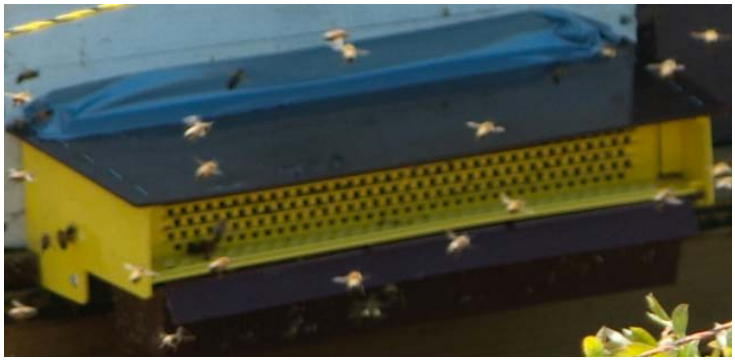
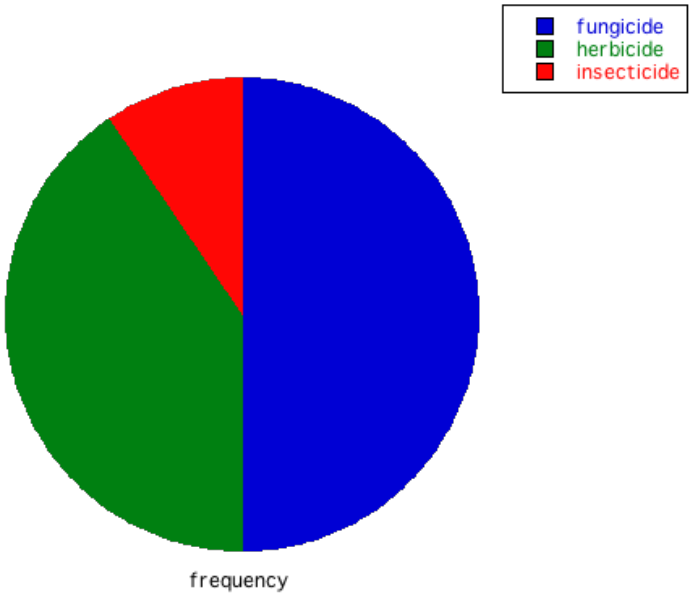


Fig. 2. Front entrance pollen trap that was distributed to volunteers.



RESULTS: In 2015, 25 pesticides were detected in pollen at the 32 sites. There were 94 total detections or 2.9 detections per site. The total concentration of pesticide detected in pollen samples aggregated over the entire spring – fall season per site was 864 ppb (part per billion). Detections by pesticide class are shown in figure 3. It can be seen that fungicides and herbicides constitute the majority of detections, while insecticides only make up about 11% of all detections. The top five pesticides detected (in terms of frequency of detections) are shown in figure 4. The fungicide, carbendazim, was the most commonly detected pesticide; followed by the herbicide, atrazine; the fungicide, propiconazole; the fungicide, pyraclostrobin; and the herbicide, pendimethalin. Of these, propiconazole is a common fungicide used in wild blueberry production, formulated as Orbit® and Tilt®.

Fig. 3. Frequency of detections of insecticides, fungicides, and herbicides.



When exposure was assessed in terms of concentration (ppb) and not detections, a slightly different conclusion emerges. Fungicides make up the majority of exposure with herbicides almost being imperceptible and insecticides still about 11% (Fig. 5).

Fig. 4. Most commonly detected pesticides in 2015 collected pollen throughout Maine.

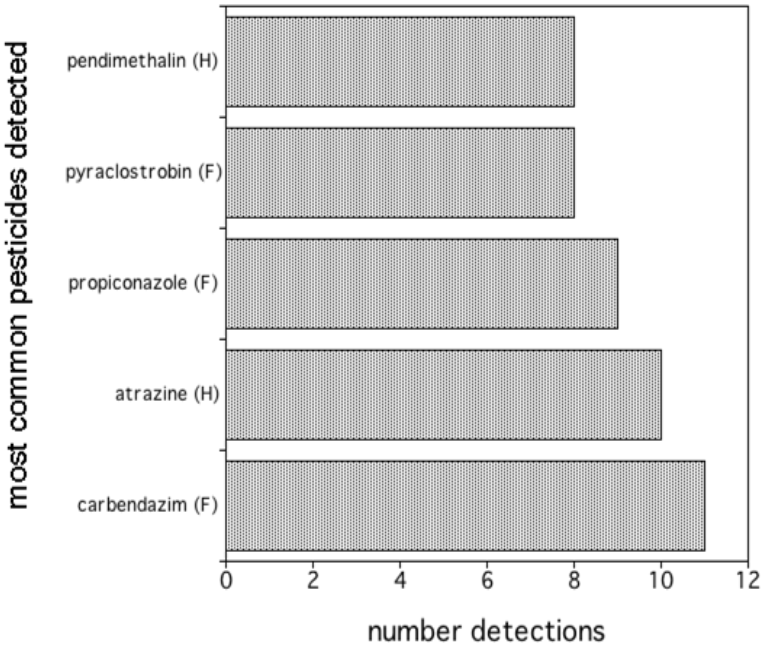
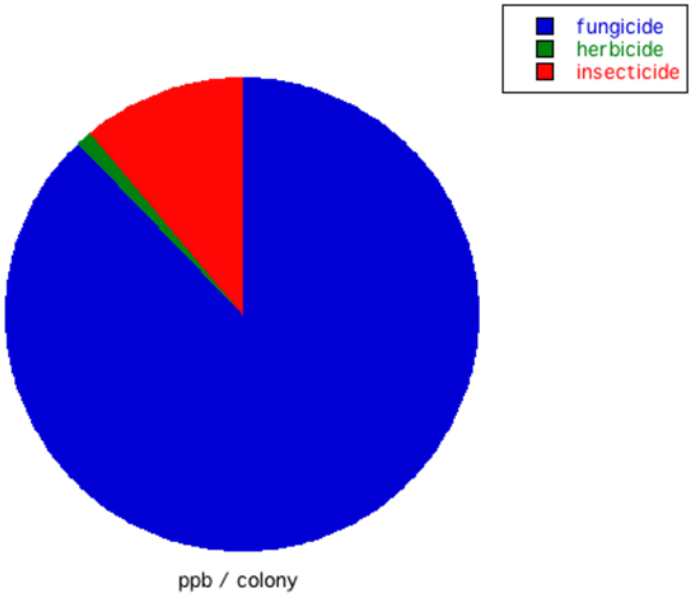


Fig. 5. Concentration (ppb) of pesticide contaminants in pollen.



Risk can be measured as the concentration of a specific pesticide that is expected to kill 50% of the worker bees in a colony divided by the exposure concentration (ppb). Risk indices greater than 0.2 are of concern. A Risk Index of 0.2 suggests that that level of exposure to that particular pesticide is expected to kill 10% of the colony work force and a Risk Index of 1.0

suggests that exposure at this level is expected to kill 50% of the worker bees in a colony. We quantified Risk by exposure through contact with the outside body of the insect and also through feeding on contaminated pollen (oral). Figure 6 shows that both contact and oral risk is due to exposure, almost entirely to insecticides. This is important to realize, considering that figures 3 and 5 showed that detections and concentrations of pesticide residues in pollen were primarily driven by herbicides and fungicides.

Fig. 6. Contact and Oral Risk Index throughout the state by pesticide type.

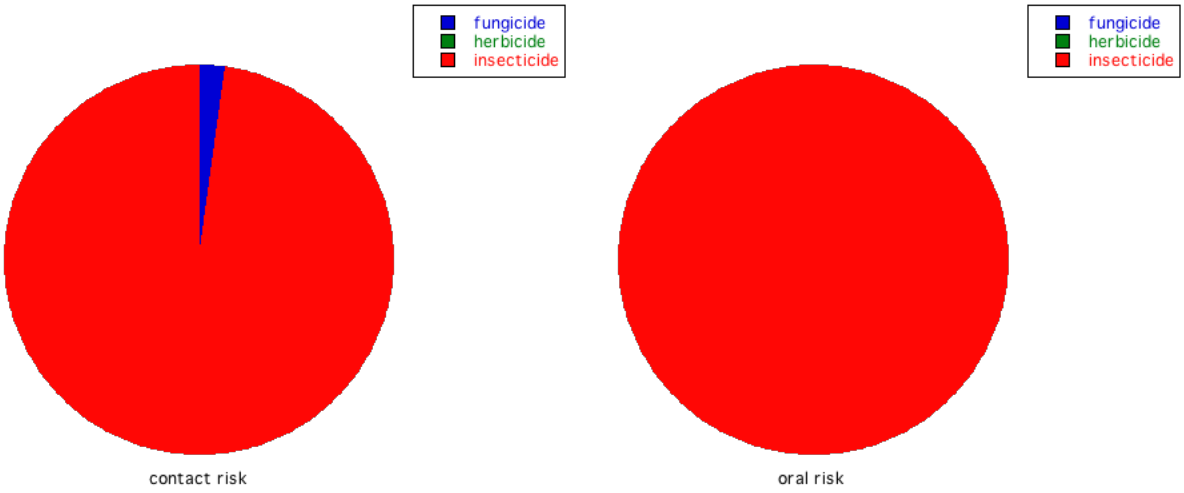
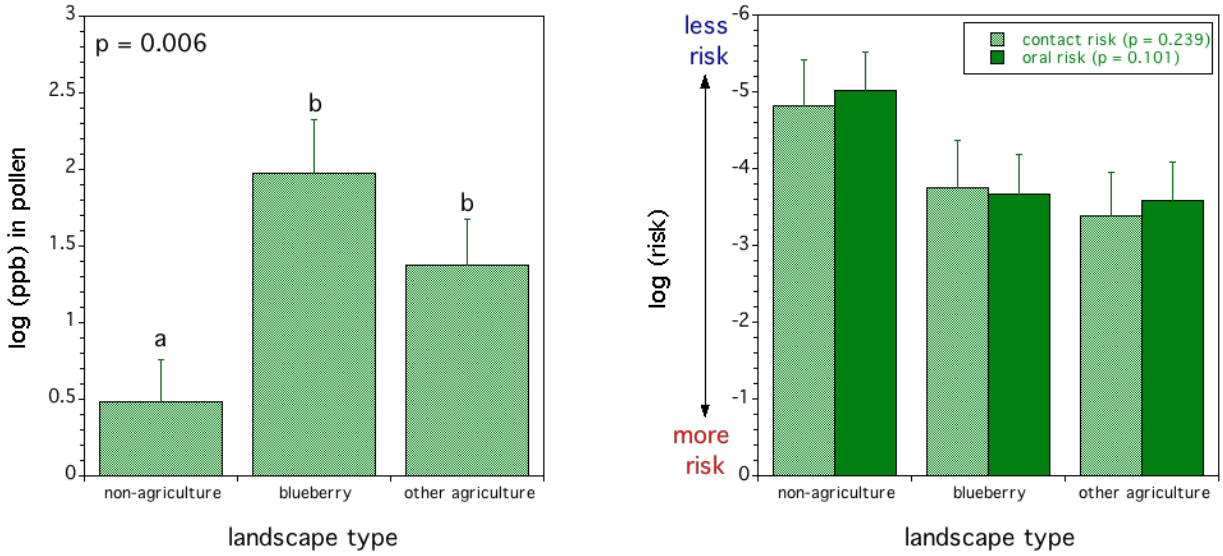


Table 1 shows that, on average, detections and concentrations were very low, resulting in a very low Risk, despite insecticides making up the majority of risk in the 2015 pollen samples. When concentration and Risk Index were summarized by landscape type (wild blueberry, other agriculture, and non-agriculture (Fig. 7), it can be seen that the concentration of pesticide residues in pollen was significantly greater in wild blueberry and other agricultural areas by an order of 1.5 magnitude difference ($P < 0.06$). Risk, both contact and oral were not significantly different between landscape types ($P > 0.10$); although, a trend in increasing Risk in agricultural landscapes compared to non-agricultural landscapes can be seen.

Table 1. Insecticide concentration and LD_{50} (oral) per site.

Insecticide	Detection frequency	Concentration (ppb)	LD_{50} (oral) ppb
Phosmet (Imidan®)	4	88.3	3,700
Carbaryl (Sevin®)	3	1.84	2,310
Indoxacarb (Steward®)	1	0.12	1,825
Acetamiprid (Assail®)	1	0.84	145,300
Imidacloprid (Mavrik®)	ND	ND	37

Fig. 7. Concentration (log ppb) (left) and Risk (log) (right) for the three landscape types (wild blueberry, other agriculture, and non-agricultural).



CONCLUSIONS: This study is one of the first in the U.S. that provides a baseline exposure to honeybees statewide. In general, the diversity of pesticides detected in pollen was 25, fairly high. However, detection rate and concentrations were low and were dominated by the less toxic fungicides and herbicides. Insecticides do constitute the highest risk of all three pesticide classes, but the individual site Risk of exposure was very low. There was only one site of 32 sites that resulted in Risk Indices that were of concern (0.22 contact, and 0.64 oral). This site was close to an apple orchard and exposure was relatively high to phosmet (Imidan®).

Study 7. ***The movement of a neonicotinoid pesticide, imidacloprid, through commercial Bombus impatiens colonies after foraging on treated lowbush blueberry.***

Report from K. Bickerman (Ph.D. candidate) and F. Drummond

OBJECTIVES: Currently, researchers know very little about the status of populations of native, wild bumble bees in the United States because there is no comparable baseline data such that exist in Europe. However, in the past decade evidence has surfaced of range reductions and species declines around North America. Proposed reasons behind these declines include habitat fragmentation, loss of forage resources, and the introduction of novel pathogens from commercial bumble bee colonies and migratory honeybee hives. Another possible contributing factor to bumble bee declines is the increased use of broad-spectrum insecticides on crops and in managed areas, with a particular focus on the neonicotinoids.

One neonicotinoid in particular, imidacloprid, is the largest selling pesticide in the world and has been the focus of many pollinator studies due to its widespread use in the United States. Conventional lowbush blueberry growers in Maine currently use imidacloprid in their fields in order to combat blueberry maggot and thrips. Although research has shown little direct effect on bee survival at the level of the colony, recent literature has suggested that imidacloprid, which is a neurotoxin, may affect colony development and reproductive output of bumble bee colonies. Recent research has demonstrated that dosed colonies have less success in producing

reproductives (particularly queens) at the end of the season, which can have detrimental impacts on populations in the following year, even if the pesticide does not directly kill the bees.

In this study, we sought to investigate the movement of imidacloprid over time through commercial bumble bee colonies from workers foraging on treated lowbush blueberry all the way into several colony components: wax/comb, nectar, stored pollen, larvae, and pupae. The results of this study will give us a more complete picture of what constitutes "field-realistic" exposure concentrations and possible implications to colony development.

METHODS: Four flight cages, each approximately 6 meters long, were set up in a lowbush blueberry field (*Vaccinium angustifolium*) in the beginning stages of bloom on 18 May 2015 at the University of Maine's Wild Blueberry Research Facility in Jonesboro, ME. On 27 May 2015 the blueberry clones in three of the flight cages were sprayed with Admire® Pro Systemic Protectant (42.8 wt% imidacloprid) at the rate of 2.8 oz/acre, the recommended spray rate for treatment of blueberry maggot fly and thrips, and allowed to dry. One flight cage was kept untreated as the control (Fig. 1).

Fig. 1. The field set up of the flight cages and spray regimen.

That same day, 12 colonies of commercial *Bombus impatiens* (Koppert® Biological Systems, Romulus, MI) were split into four groups of three and placed inside the flight cages. The boxes were opened and bees were allowed to forage on the blueberry flowers. One week post treatment (5 June 2015), one colony and flower samples were collected from each flight cage and immediately frozen. Three weeks post treatment (18 June 2015), one colony was again collected from each flight cage and frozen. Due to blueberry bloom ending, the remaining four colonies (one from each cage) were transported back to the Rogers Farm Forage and Crop

Research Facility in Stillwater, ME and allowed the forage there. These colonies were collected and frozen six weeks post spray (7 July 2015).

Additionally, on 4 June 2015, we removed the bags containing the Bee Happy® food from four extra colonies (DOSE1, DOSE2, DOSE3, DOSE4)and used the Admire® Pro to create a concentration of 20 ppb imidacloprid in each food bag. We weighed and replaced the food bags and kept the colonies outside on the University of Maine campus but closed, so the only option for the bees would be to consume the sugar syrup as their energy source. At the end of a two-week dosing period (18 June 2015) the dosed food bags were removed and weighed from the colonies and the bees were taken to Rogers Farm to forage until being frozen six weeks post-dosing (14 July 2015).

All colonies were removed from the freezer, photographed from above, and all parts (adults, pupae, larvae, pollen, nectar, and wax) separated into Falcon tubes for chemical analysis at the Connecticut Agricultural Experiment Station to ascertain concentrations of imidacloprid and common metabolites (imidacloprid-urea, imidacloprid-olefin, and OH-imidacloprid) throughout the colony components through time.

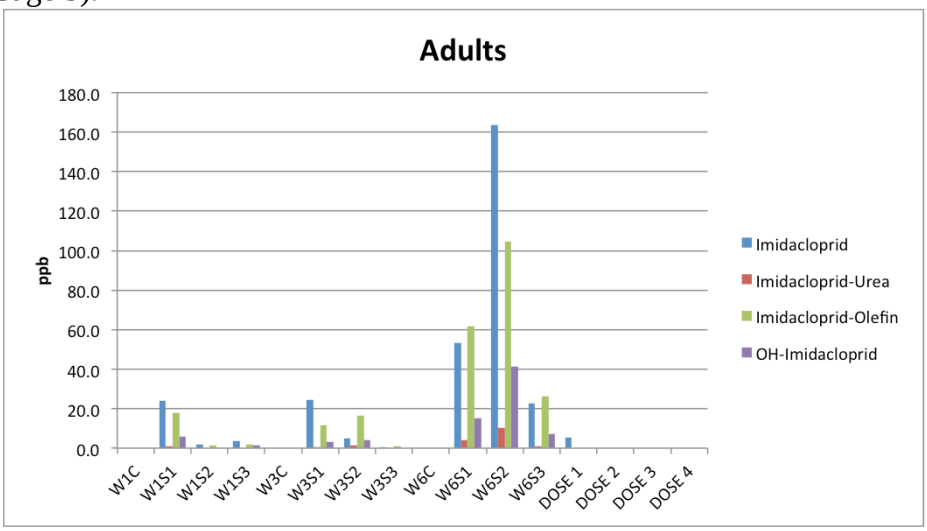
RESULTS: Analysis of the control and treated blueberry flowers showed high concentrations of imidacloprid and its metabolites in treated cages one week post treatment (Table 1) and no detectable (n.d.) imidacloprid in the control cage.

Table 1. Concentrations of imidacloprid in its metabolites in the leaves and flowers of lowbush blueberry one week post spray in the control and treated cages.

Leaves and Flowers	Imidacloprid	Imidacloprid-Urea	Imidacloprid-Olefin	OH-Imidacloprid
Inside Control Cage	n.d.	n.d.	n.d.	n.d.
Inside Treated Cage	1000 ppb	14 ppb	14 ppb	25 ppb

Concentrations of imidacloprid and its metabolites in adults varied between the replicated treatment cages and over time (Fig. 2). For the colonies fed the 20 ppb imidacloprid syrup, only the first replicate (DOSE1) showed a very small (5.2 ppb) concentration of imidacloprid in the adults after the six weeks. The control colonies had no detectable amount of imidacloprid. Concentrations of imidacloprid and metabolites appear to build up in the adults through time, with the colonies collected in the sixth week after spray having the highest amounts by far than previous weeks. Only the adults had measurable metabolites.

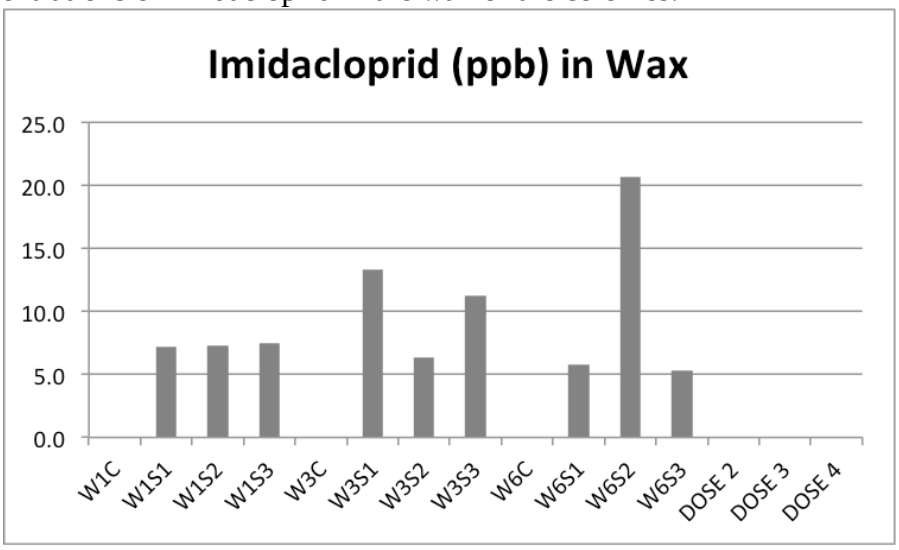
Fig. 2. Concentrations of imidacloprid and metabolites (ppb) in adult bumble bees. Colonies are separated by week (W1 = one week post spray, W3 = three weeks post spray, and W6 = six weeks post spray) and by treatment cage (Control = C, S1 = Spray Cage 1, S2 = Spray Cage 2, S3 = Spray Cage 3).



Pupae were not present in several of the colonies (W3C = three weeks post spray control; DOSE 1, DOSE 3, and DOSE 4 = colonies fed a concentration of 20 ppb imidacloprid) and therefore could not be tested. For the remaining colonies, only W3S1 had a measureable imidacloprid concentration (8.8 ppb); although, there is uncertainty with this measurement due to small sample size. No metabolites were detected in any colonies.

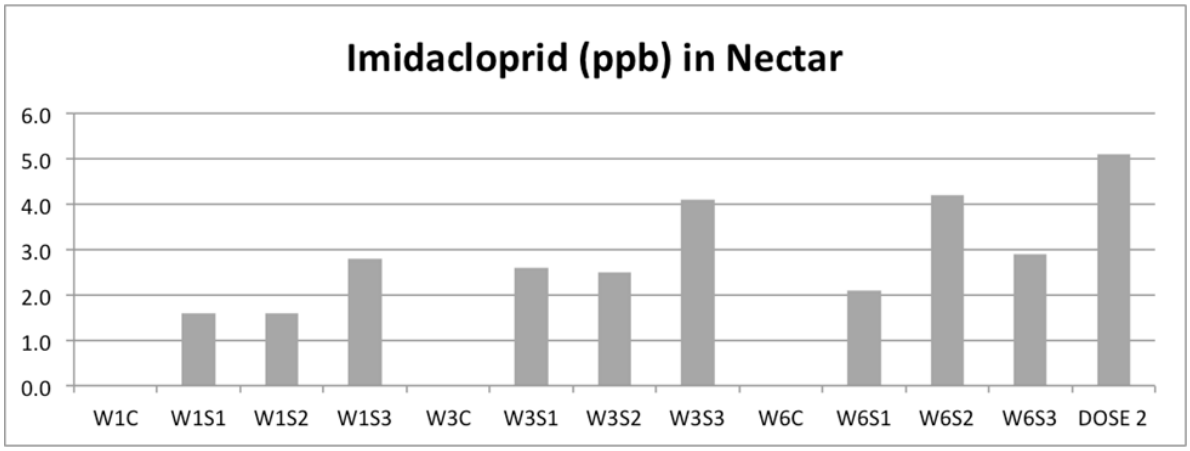
There were no measureable metabolites in the wax in the colonies, but there was imidacloprid except in the controls and the dosed food colonies (Fig. 3). No wax was present in DOSE 1. Imidacloprid concentrations were consistent across all three replicate cages in the first week after spraying and SC1 and SC3 had similar patterns of a slight increase three weeks post spray then a decrease in the sixth week. SC2 diverged from this pattern and the highest concentrations (although still only at approximately 20 ppb) were found six weeks post spray.

Fig. 3. Concentrations of imidacloprid in the wax of the colonies.



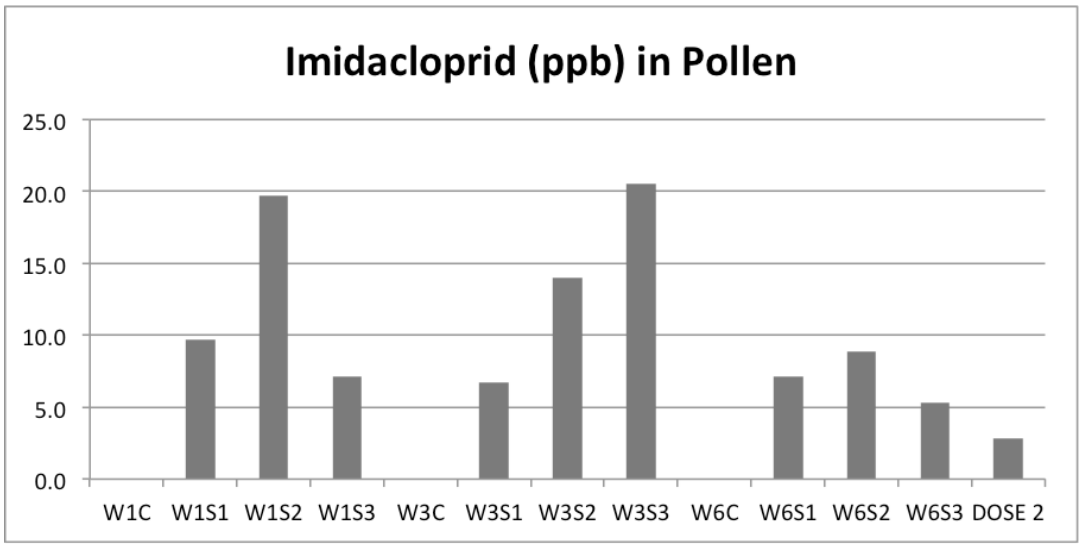
No measureable imidacloprid metabolite concentrations were found in the nectar and imidacloprid concentrations remained low (< 5 ppb) in the flight cage colonies and imidacloprid was only present in DOSE 2 for the dosed colonies at 5.1 ppb (Fig. 4).

Fig. 4. Imidacloprid concentrations in the nectar.



There were no measurable metabolites in any of the pollen from the colonies, and nothing was measureable from DOSE 1, DOSE 3, or DOSE 4. Imidacloprid concentration in the pollen was between ~5 ppb and 20 ppb one week and three weeks post spray in the treated flight cages, but declined to < 10 ppb in all colonies by the sixth week (Fig. 5).

Fig. 5. Concentrations of imidacloprid in the pollen.



In the larvae, measureable imidacloprid was only found in W1S1, W1S2, W3S1, and W6S2 (Table 2). No metabolites were found and no larval samples were available from W3C, W3S2, or DOSE 1. The sample of larvae from W3S1 was an extremely small sample size and therefore there could be uncertainty in the imidacloprid measurement.

Table 2. Imidacloprid concentrations in the larvae of the colonies.

Treatment	Imidacloprid concentration (ppb)
W1S1	14.8
W1S2	2.2
W3S1	33.4
W6S2	3.5

CONCLUSIONS: Concentrations of imidacloprid and its metabolites appeared to build up over time in the adults. Besides the adults, no measurable imidacloprid metabolites were found in any other colony component we measured. A similar study seeking to track the movement and degradation of imidacloprid within honeybee colonies was conducted where researchers fed colonies either dosed sucrose syrup (20 ppb imidacloprid) to simulate contaminated nectar or dosed diet patties (100 ppb) to simulate pollen, or both at once. Samples were then collected at 2, 4, and 6 weeks during exposure and then six weeks after exposure of adults, beebread, honey, and larvae. In this study, higher imidacloprid residues were found in colonies fed the 100 ppb diet patties as compared to the 20 ppb sucrose syrup with the highest levels being in the honey (average 6.5 – 7.2 ppb) with much lower levels in the adults (average 0.3 – 0.7 ppb) and beebread (average 0.9 – 1.0 ppb) and no metabolite residues were detected.

The authors concluded that the residue concentration was much lower in adults and in larvae due to the rapid metabolism of imidacloprid, which was not reflected in our study with the highest levels being found in bumble bee adults six weeks post spray. This may indicate that the metabolism of imidacloprid is very different between *Apis mellifera* and *Bombus impatiens* with the latter being less effective at detoxifying imidacloprid. This conclusion is supported by a 2012 paper that found that honeybees showed no response to dietary imidacloprid in any tested variable (locomotion, feeding, longevity); but bumble bees demonstrated a decline in feeding rate when fed 10 ppb imidacloprid in sucrose syrup. The authors speculated that honeybees may be pre-adapted to feed on nectars with synthetic alkaloids due to their tropical provenance as compared to temperate bumble bees.

Study 8. The Maine Bumble Bee Project.

Report from F. Drummond, E. M. Venturini, R. Butler (UM Farmington), J. Staples (USM), S. Dobrin (UM Presque Isle), and C. Lage (UM Augusta)

OBJECTIVES: Maine is home to 17 different species of bumble bees. These large-bodied social bees are the most efficient pollinators of wild blueberry. They are capable of buzz pollination, releasing large quantities of pollen from deep within the poricidal anthers of the blueberry flower. Once they leave the flower, much of the pollen remains and is now much more accessible to other flower visitors. In subsequent visits, honeybees and other pollinators pick up this pollen and transport it to other flowers. The fact is, honeybees and wild bees become more efficient, effective pollinators when bumble bees are abundant in blueberry fields.

Little is known about the status of bumble bees in our state. What we do know is often rather alarming. One species, the rusty-patched bumble bee, used to be one of the most common

species in the state. This year, it was proposed by the USF&WS to be listed as an endangered species. Another once common species, the yellow banded bumble bee, disappears relatively early in the season each year. Some surveys suggest that it is in great decline; however, in other years its numbers seem to rebound dramatically. We do not yet understand what drives these extreme population cycles. Other species, for example the orange-belted bumble bee, used to be rare, but is now very abundant throughout the state.

This study looks at bumble bee species in Maine with a broad brush. We seek to understand some of the major factors influencing these shifting bumble bee communities in our state and inform future research objectives. By understanding the factors affecting bumble bees, we may be able to offer recommendations to growers in terms of easy to employ strategies to help boost their numbers around crop fields.

Our objectives were to (1) assess the relative abundance of the bumble bee assemblage across Maine; (2) determine their plant foraging preferences; (3) measure their pathogen susceptibility, as determined by prevalence and diversity of pathogens; (4) assess their genetic diversity, as a measure of population health; and (5) measure their exposure and susceptibility to pesticides.

METHODS:

Objective 1 hypothesis: The relative abundance of bumble bees is biophysical zone and landscape dependent, with the occurrence of spatially rare and vulnerable (to extirpation) species being determined by geographic locale.

We collected bumble bees from five regions across Maine. Each region, comprised of multiple MEGIS defined Biophysical Regions, was represented by seven or more sites. Sites were visited monthly. Site visits were initiated on a site by site basis once sufficient numbers of bumble bees had emerged in the spring. Some sites were visited twice, others as many as five times from June to October. All sampling rounds were completed within the first two weeks of each month. All sampling was conducted on clear days between 8 am and 7 pm, with temperatures 16 - 27°C and wind speed <11 miles/hour. We used Kestrel® Weather Meters to record weather data at each site visit. During each site visit, we spent one person hour collecting bumble bees with a sweep net. During the first 30 minutes, we collected as many bumble bees as possible, prioritizing numbers of bees over rarity of bee species. In the second 30 minutes, we targeted rarer species, morphs, and males at the expense of numbers of specimens. Bees were typically collected on flowers (host plants). All host plants were either identified to species on-site, or later with the aid of detailed photographs.

Objective 2 hypothesis: Bumble bees utilize non-crop floral resources that require specifically adapted morphologies and behaviors that allow a competitive advantage in acquiring nectar and pollen.

At each site (40 total) used for specimen collections (Objective 1 above), we also sampled floral resources during each site visit in order to measure bumble bees' plant forage preferences. During each visit, workers walked representative transects through the site for a total of 15 minutes. While walking, we recorded all blooming flower species within view, and recorded each flower species' abundances according to both Level I and Level II rankings (Table 1). Each plant species recorded was identified to species. When field identification of plants was impossible, we took photographs for later identification.

Table 1. Flower species' abundances.

Level I Ranking:

Rank	Qualification
1	Single plant
2	Single cluster
3	2-20 plants
4	2-5 clusters
5	> 20 individual plants scattered through landscape
6	> 5 clusters scattered through landscape.

Level II Ranking:

Rank	Qualification
1	Flowering plant comprising trace (<1) % of land cover (trace or barely detectable)
2	1-5% of land area (low abundance)
3	6-10% (moderate abundance)
4	11-25% (common)
5	26-50% (high)
6	> 51% of land area (extremely high abundance)

Objective 3 Hypothesis: Pathogen prevalence and diversity will be greater in spatially rare bumble bee species compared to abundant species.

Bombus ternarius, an extremely abundant bumble bee across Maine, was selected as our representative 'abundant' species. The rare and possibly declining *Bombus terricola* represented a spatially 'rare' bumble bee species. This collection was a subset of bees captured for Objective 1. From each site where both the abundant and rare species were found, we collected up to five individuals of each species. At some sites where the rare species was not found, we collected the 10 or more abundant bees at each visit to contrast pathogen loads over time.

Specimens were kept cool and alive in the field so that pathogens did not degrade. All specimens were collected in sterile centrifuge tubes and stored at temperatures between -70 and -80^{o}C. Specimens are currently being analyzed genetically for pathogen prevalence and diversity.

Objective 4 hypothesis: Genetic diversity will be greater in bumble bee species that have been abundant historically compared to species that are currently expanding their range north into Maine and species that appear to be recovering from decline.

This collection was comprised of a subset of the bees caught for Objective 1. From each site where both *Bombus ternarius* (abundant) and *Bombus terricola* (rare) were found, we collected up to five individuals of each species. We identified bees as either *B. terricola* or *B. ternarius* in the field. Each collection team collected an additional 20 specimens of each of these two species. These voucher specimens will be identified by a bee taxonomist to vouch for our ability to identify specimens in the field. Specimens were captured in vials, and moved to vials of 95% ETOH later on the day of capture. Vials were stored in ETOH at -20^{o}C.

Objective 5 hypothesis: Pesticide exposure is relatively low in bumble bees relative to honey bees in Maine, but susceptibility to common pesticides in the environment is high.

To compare the prevalence of pesticides in bumble bees between abundant (*B. ternarius*) and rare (*B. terricola*) species, we analyzed both their pollen loads, and the bees themselves. We collected bees for this objective in a single, separate, sampling effort not linked to the sites mentioned in previous objectives. Bees were collected into centrifuge tubes; no sweep nets were used to minimize cross contamination. Specimens were only handled after thoroughly washing hands, and never after application of bug spray. All collectors abstained from using bug spray throughout the season to avoid contaminating the specimens.

Pollen loads from abundant and rare bumble bee species were pooled separately across the state to achieve a total of at least 1 gram of pollen from each of the two species. Pollen samples were sent to a pesticide residue lab for analysis.

RESULTS: In total, we collected 2,489 bumble bees across 40 sites and 5 sub-sites during our initial 30 minute collections at each site. These bees will be used to contrast bee relative abundance by region across the state. We captured an additional 698 bees during our second 30 minute collection at each site. These bees will help to inform our estimate of species richness at each site. 522 abundant (*B. ternarius*) and rare (*B. terricola*) bumble bees are currently being analyzed for pathogen prevalence and diversity. 274 abundant and rare bumble bees were analyzed for their genetic diversity. 158 pollen loads of *B. ternarius* and 70 pollen loads of *B. terricola* and 176 whole bumble bees are being analyzed for pesticide residue.

CONCLUSIONS: The data from this study are still being analyzed.

Study 9. Documenting the phenology of Maine wild bees.
Report from E. M. Venturini, S. Bushmann, K. Bickerman, B. DuClos, and F. Drummond

OBJECTIVES: A growing body of research is providing convincing evidence that populations of wild bees can be successfully managed for enhanced crop pollination. Fruit set in lowbush blueberry fields is increased when growers install pollination reservoirs, and these plantings are heavily used by bumble bees; on average provisioning 37% of bumble bee collected pollen. Another recent study highlights a new tool, designed to aid growers in predicting wild bee pollination in blueberry fields. This tool relies upon GIS map data and ranks habitat according to quality. Missing from this tool is phenology data that would make some habitats more or less useful, in accordance with the phenology matching between the habitat's flowering phenology and the phenology of key crop pollinators. To most successfully apply these tools and management strategies, we need to better understand the life-cycles of key crop pollinators in Maine. The primary objective of this project is to develop a robust catalogue of bee species active flight periods. We will also contrast bee phenology over time, which may signify bee species' adapting to a changing climate.

METHODS: This project compiles all available records of bee specimens in Maine. We scoured the Maine State Museum Archives, recording the capture date and species identification for every specimen with complete data. All co-authors, and some others, have contributed their

own robust data sets that contain bee species identifications and capture dates. We will compile these records by year and species, to develop a range of capture dates for each. These ranges represent the active flight periods of each species.

RESULTS: To date, we have compiled records of over 20,000 specimens, representing 252 different species. This is 91% of the total recognized number of bee species in Maine, and contains every species of bee known to be associated with the Maine wild blueberry agroecosystem. This study is not completed. Analysis is underway.

Study 10. Replanting a pollination reservoir at Blueberry Hill Farm.

OBJECTIVES: Our recent research findings suggest that pollination reservoirs, or set-aside areas of on-farm pollen and nectar rich flowering plants, can support wild bees. Pollination reservoirs in Maine's lowbush blueberry agroecosystem increased fruit, and were heavily used by bumble bees. On average, bumble bees foraging in fields with pollinator plantings, captured after blueberry bloom, were carrying 37% of pollination reservoir pollen. Pollination reservoirs were established at Blueberry Hill Farm, Jonesboro, ME as part of this project in 2012. Based upon our research findings, we redesigned the flower mixture and replanted a much larger area. In the lower field, which is harvested in odd years, we planted 0.29 acres to pollination reservoir. In the upper field, harvested in even years, we planted 0.58 acres to pollination reservoir. Our objectives are twofold: (1) To create full-scale pollination reservoirs as a demonstration to growers who are considering implementing this strategy; and (2) to create pollination reservoirs suitable for future long-term research on their effectiveness as a partial replacement for honeybee colony rental.

Study 11. Influence of landscape pattern and land cover type on wild bee abundance and diversity.

Report from Brianne Du Clos (Ph.D. candidate), C. Loftin, and F. Drummond

OBJECTIVES: Little is known about wild bee communities outside of wild blueberry fields in Maine. We are quantifying wild bee community richness and abundance in eight land cover types throughout Maine's wild blueberry growing region (Table 1). The growing region is split into two areas that differ in landscape pattern. The Downeast region is dominated by more coniferous forest and contains very large wild blueberry fields. The Mid-coast region is deciduous forest dominant with much smaller blueberry fields and more urban and other agricultural land cover. Research in other crop systems indicates that complex landscape pattern promotes bee abundance and diversity; therefore, we expect to see differences in bee communities between these two areas. The purpose of this project is to understand independent and interactive effects of landscape pattern and land cover type on wild bee communities in a heterogeneous landscape.

Table 1. Land cover types surveyed for wild bees.

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

METHODS: We established two blocks of 16 survey sites throughout Maine's wild blueberry production landscape: one in the Mid-coast growing region and one in the Downeast growing region. Each block consisted of two sites of each land cover type, for a total of 32 survey sites. We assessed nesting resources at each site by looking for standing dead wood, fallen dead wood, woody shrubs with hollow twigs, and open, sandy soils. We surveyed bee communities at each site in early, mid-, and late summer 2015. Each survey consisted of 30 minutes of live-netting bees and a 24-hour deployment of bowl traps. We also recorded blooming flowers and collected samples of flowers for identification. There was no live-netting of bees at sites that did not have blooming flowers. All bees were sent out to taxonomic experts for identification to species.

RESULTS AND CONCLUSIONS: We collected 1370 individual bees in 119 species and 23 genera. Two of the species we found were new state records: *Andrena personata*, a miner bee, and *Lasioglossum platyparium*, a small metallic sweat bee. Both of these species are common in the Mid-Atlantic, but this is the furthest northeast they have been recorded.

We found no significant difference in wild bee communities between the Downeast and Mid-coast areas (Fig. 1). However, bee communities did differ by cover type. Agriculture, blueberry, and the two types of wetland have similar bee communities, but urban areas and forest cover have distinct bee communities (Fig. 2). Further examination showed that the differences in bee communities by land cover type varied by growing region. Bees are more abundant in urban areas and more diverse in deciduous forest in the Mid-coast growing region (Fig. 3).

Fig. 1. Wild bee communities by wild blueberry growing region in Maine.

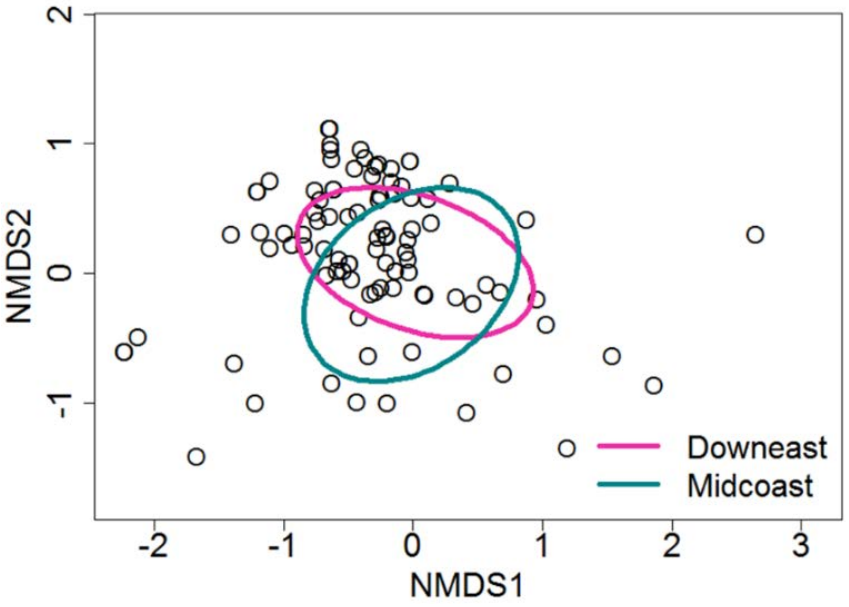


Fig. 2. Wild bee communities by land cover type in Maine's wild blueberry growing regions.

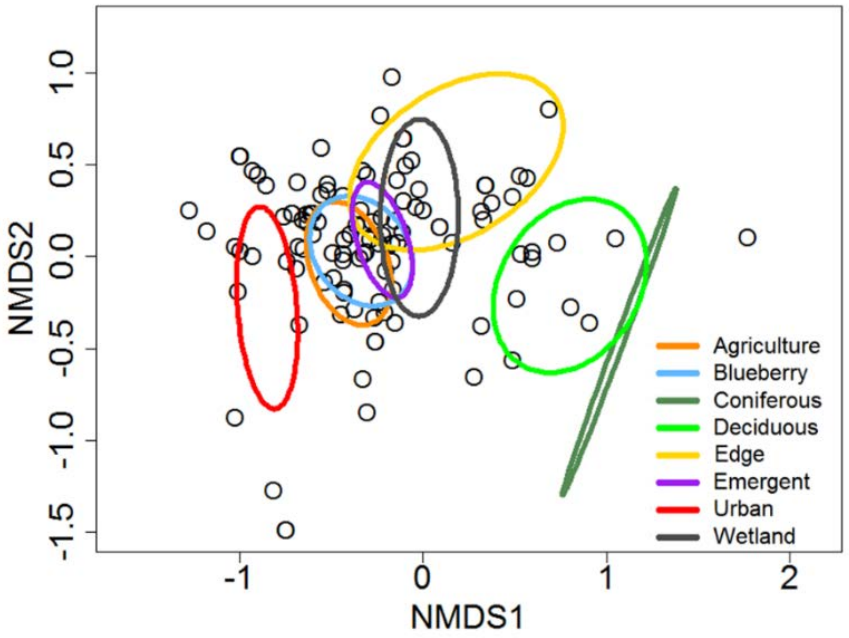
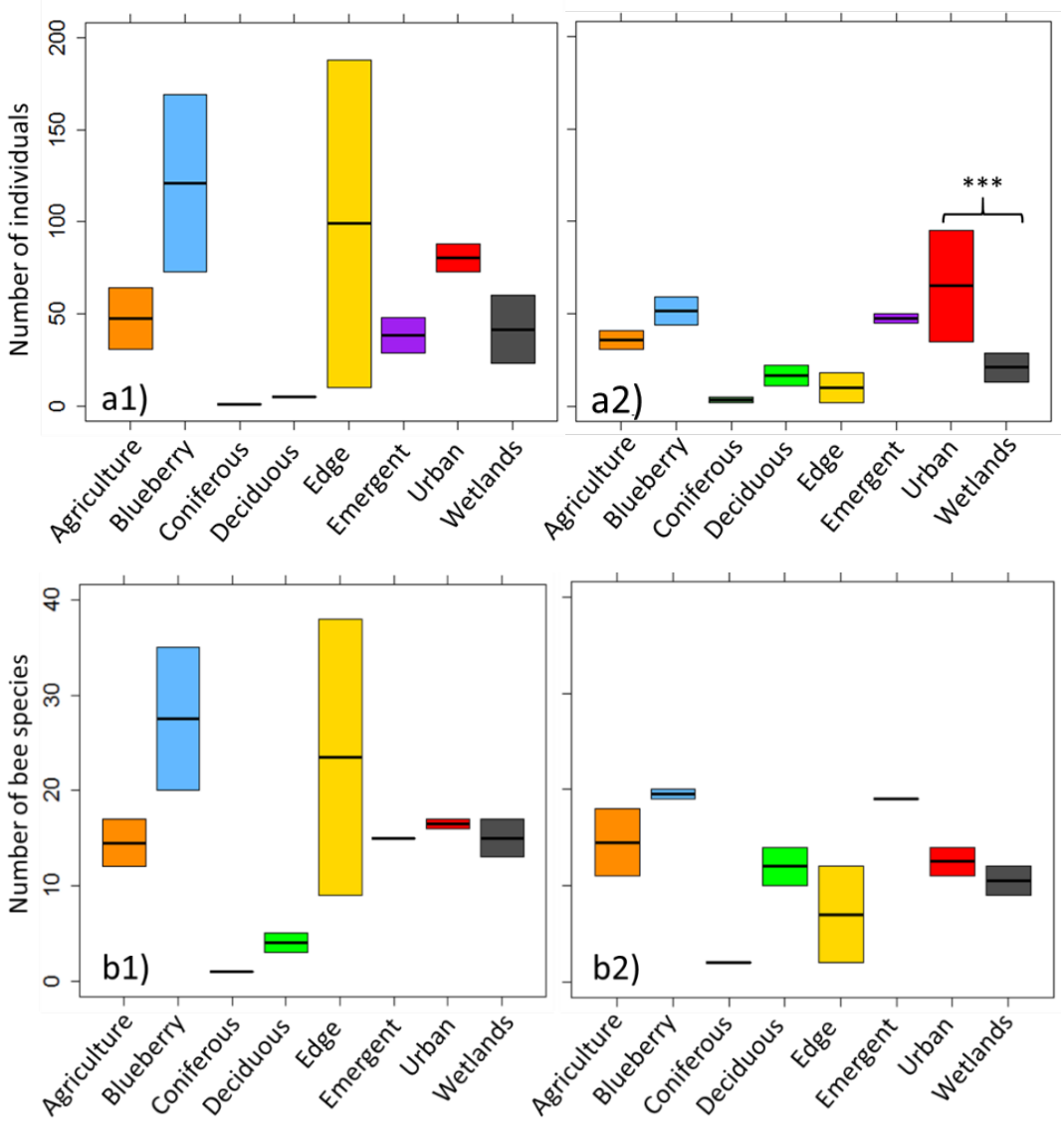


Fig. 3. Wild bee a) abundance and b) richness by cover type in the 1) Downeast and 2) Mid-coast wild blueberry growing regions of Maine.



Study 12. ***<u>Cyst-like bodies found in Mane honeybees.</u>***
<u>Report from Rebecca Rivernider, R. Simmons, and F. Drummond</u>

OBJECTIVES: In many fruit and nut crops throughout the world, honeybees are a primary source of pollination. Without honeybees many fruits and nuts would not be available to consumers. Honeybees are at risk due to colony collapse disorder (**CCD**) which currently results in 30-40% colony losses each year. It is believed that multiple causes are responsible, but parasites and pathogens are believed to a factor. Parasites such as *Apocephalus borealis*, a phorid fly infecting honeybees and bumble bees, are increasing in occurrence. Once a fly larva hatches, the larva travels through the honeybee consuming fatty tissue, eventually reaching and eating the brain. The honeybee then exhibits a strange behavior and flies far from the hive to

lights at night and eventually dies. Then the phorid larva emerges from the dead bee, metamorphoses into a pupa and then to an adult fly. The fly then searches for a new honeybee host on which to lay its eggs.

Phorid flies in honeybees have not been observed in Maine to this date (they have been documented in bumble bees). The pathogen, *Nosema* spp., has been a common occurrence in Maine honeybees. Nosema has been known to cause cysts and can also cause a shorter life span, less superseding queen cells, and queens that produce unviable eggs and larvae, contributing to CCD.

METHODS: We collected groups of 10 honeybees into 70% ethanol from several different hives on the blueberry barrens. Bee were then frozen at ca. -20^{o}C prior to analysis. When we were ready to dissect the honeybees we took them out of the freezer and used pinning needles to examine each bee for the presence of round solid white cyst-like bodies. Sometimes these could be seen through the bee without cutting it open. We put five bees that had the cyst-like bodies into a tube with a tissue-macerating metal ball and ground up the bees. We also took five bees without the cyst-like bodies into a separate tube and ground them with the same methods.

Once the bees were ground into a liquid state, we used the "power soil experienced user protocol" and kit to extract the DNA. Then we made sure the DNA was present in a large enough quantity to be amplified using a nano-sampler. We used *Nosema* primers on one sample and COI region phorid fly primers on the other and assessed the amplification of primers through electrophoresis. Finally, we cut the DNA from the agarose gel and sent it to the University of Maine Sequencing Facility to be sequenced for verification of our results.

RESULTS: After reviewing the PCR results, both experimental and control samples gave the same results. The CO1 primers were only successful in amplifying bee DNA, with 100% match to *Apis mellifera*. The *Nosema* primers successfully amplified *Nosema ceranae*, 100% matches were found as well.

CONCLUSION: In both control and experimental groups of honeybees we found negative results for infection by the phorid fly. We did obtain positive results for *Nosema* primers, specifically those indicating presence of the pathogen *Nosema ceranae*. The microsporidian pathogen *Nosema ceranae* is becoming more of a problem every year. It is a pathogen that was introduced from Asia in the early 1990s. This pathogen has been spreading throughout North America since its introduction. The cyst-like bodies we observed visually in honeybees are most likely *Nosema ceranae* and appear to be causing blockages in the gut tissue.

Study 13. Bee nutrition, does it determine bee visitation?

Report from M. Leach, B. Perkins, L. Berg-Stack, A. Dibble, and F. Drummond

OBJECTIVES: Little is known about plant selection by bees. Is it just flower display or the number of flowers on a plant that determine which plants bees visit; is it just random; or it affected by nutritional quality? This project seeks to answer this question experimentally.

METHODS:

Bee visitation

Four plant species were observed for bee visitation, these include *Gaillardia aristata* (blanketflower; Family: Asteraceae), *Helianthus annuus* 'Zebulon' (Sunflower 'Zebulon'; Family Asteraceae), *Borago officinalis* (Borage; Family Boraginaceae), and *Phacelia tanacetifolia* (Bee's friend; Family Boraginaceae). Four sites were planted with the selected plant species. These included Rogers Farm in Stillwater Maine, Curtis farms in Blue Hill Maine, Four Fields farm in Blue Hill Maine, and Blueberry Hill Farm Research Station in Jonesboro Maine. Plants were sowed in m^2 plots in previously established pollinator research plantings. These plantings have 36, m^2 plots, which are planted with pollinator plants or established as pollinator habitat. Landscape plastic covers the entire plot between plantings. *G. aristata* plants were started in the winter of 2015 and then transplanted in the first two weeks of June at the four field sites. Twelve *G. aristata* plants were transplanted at each site. *H. annuus* 'Zebulon' and *B. officinalis* seeds were evenly spaced in 12 spaces, and *P. tanacetifolia* seeds were broadcast at each of the four sites.

G. aristata, B. officinalis, and *P. tanacetifolia* began flowering at the end of July and observations began 29 July. *H. annuus* 'Zebulon' began flowering in August and observations began on 13 August. Bee visitation was observed for three, one minute intervals at each site once a week. In addition to visitation, floral characteristics and the number of open flowers were recorded. Four flowers were measured for corolla depth, flower diameter, and disc corolla depth (*G. aristata, H. annuus* 'Zebulon). Bee groups recorded during the one minute observations included *Apis mellifera*, *Bombus ternarius, Bombus* species (excluding *B. ternarius*), and other bees. Other bees included solitary and unidentifiable species.

Chemical Analysis

To collect pollen and nectar, large strips of each plant were planted at two of the observation sites, Blueberry Hill Farm in Jonesboro and Rogers Farm in Stillwater. Soil from each of the plots was tested and the appropriate amendments were made to produce enough flowers for pollen and nectar analysis. The entire plot measured 12 x 5.5-m and each strip measured 12 x 1.5-m. *G. aristata* plants were started in the winter of 2015 and transplanted the second week of June; plants were planted at 0.25m spacing in two rows. *H. annuus* 'Zebulon' seeds were planted at 0.4m spacing and 1.5cm depth in two rows, *B. officinalis* seeds were planted at 0.4m spacing and 0.75cm depth in two rows, and *P. tanacetifolia* seeds were broadcast throughout the strip. Plots were covered with straw after planting to reduce weed pressure and watered regularly. The plot in Jonesboro failed to produce enough flowers for pollen and nectar collection.

To collect pollen and nectar, flowers were covered with exclusion cages or bagged to prevent insect visitation and pollen/nectar contamination. Only unopened flowers were bagged and old flowers were removed under exclusion cages to prevent old pollen or nectar collection.

Pollen from *G. aristata* and *H. annuus* 'Zebulon' flowers were brushed with a clean paintbrush into glass vials. *B. officinalis* flowers were removed and placed over the opening of a glass vial then vibrated with a tuning fork to vibrate pollen out of the anthers. *P. tanacetifolia* anthers were removed from the plant and pollen removed from anthers after freeze drying by shaking glass vials so pollen would stick to the vial. All pollen and the anthers from *P. tanacetifolia* was stored in a -80^{o}C freezer until chemical analysis. Nectar was collected by removing flowers and centrifuging nectar into a 50 or 2.5mL centrifuge tube. Individual flowers from inflorescences of *B. officinalis*, *P. tanacetifolia*, and *H. annuus* 'Zebulon' were collected and placed in 2.5mL centrifuge vials upside down with glass wool to prevent pollen contamination. The vials were centrifuged at 1500 rpm for 30 seconds. *G. aristata* inflorescences were collected and placed upside down in 50mL centrifuge tubes with glass wool. The tubes were centrifuged at 2500 rpm for 30 seconds. Nectar was stored at -80^{o}C until sugar analysis could be completed. Amino acid analysis and sugar analysis was conducted under the guidance of Dr. Brian Perkins in the School of Food and Agriculture.

Statistical Analysis

All statistical analysis was completed in RStudio (RStudio Team 2015). Visitation observations were analyzed with negative binomial linear regression and ANOVA with Tukey post hoc tests. Separate analyses were conducted for each of the bee species/groups (Other, *A. mellifera*, *B. ternarius*, and *Bombus* spp.). Because *H. annuus* 'Zebulon' flowered later than the other three plant species, two separate analyses were conducted for the dates that *H. annuus* 'Zebulon' was not flowering (23 July to 28 August), and when it was (13 August to 16 August). There is overlap in the flowering and non-flowering dates because of observations made at four sites. The different flowering periods were also tested to determine any significant differences in bee visitation during the two separate flowering periods. Spearman correlation analyses were used to determine which flowering characteristics recorded were influencing bee visitation to flowers. Non-metric Multidimensional Scaling (**NMDS**) ordination was used to determine if specific amino acids are associated with each of the plant species pollen and correlation analyses were subsequently used to determine which amino acids were correlated with visitation for each bee group/species. Another correlation analysis was used to determine which amino acids are correlated with bee visitation for all bee groups/species. Only one site produced enough flowers for amino acid analysis, therefore no statistical analyses to determine significance were run on the data.

RESULTS: There was no significant difference in bumble bee (*B. ternarius*, *Bombus* spp.) or honeybee (*A. mellifera*) visitation between the two flowering periods. Other bees had significantly different visitation in the two flowering periods ($X^2_{(1)} = 7.395$, $P = 0.007$). The number of open flowers was not significantly different for both flowering periods. For the first flowering period, plant species was a strong predictor of *B. ternarius* ($X^2_{(2)} = 119.019$, $P < 0.0001$) and other bees ($X^2_{(2)} = 11.657$, $P = 0.003$, Fig. 1). Bumble bee, *B. ternarius*, had significantly higher visitation for *P. tanacetifolia* ($P = < 0.001$). Tukey post-hoc tests showed no significant different in visitation among the plant species.

The second observation/flowering period spanned from 13 August to 2 September. Plant species was a strong predictor of visitation for *Bombus* spp. ($X^2_{(3)} = 13.570$, $P = 0.004$), *B. ternarius* ($X^2_{(3)} = 100.894$, $P < 0.0001$), and *A. mellifera* ($X^2_{(3)} = 9.184$, $P = 0.03$, Fig. 2). The Tukey post-hoc test revealed no significant difference in *Bombus* spp. and *A. mellifera* visitation

among the four plant species. Bumble bee, *B. ternarius*, visitation was significantly higher for *P. tanacetifolia* than *G. aristata*, *H. annuus* 'Zebulon', and *B. officinalis* ($P < 0.001$, $P = 0.02$, and $P < 0.001$; respectively). Spearman correlation analyses showed a correlation only for *B. ternarius* with the number of open flowers ($P < 0.001$, r = 0.532), and the flower diameter ($P < 0.001$, r = -0.539, Table 1).

Non-metric multidimensional scaling ordination showed clear separation of amino acid composition for the plant species. Honeybees had the greatest number of amino acids correlations with visitation and was positively correlated with the amino acid, Asparagine ($P = 0.05$, r = 0.946), and negatively correlated with the amino acid, Glycine ($P = 0.03$, r = -0.966). Bumble bee, *B. ternarius*, visitation was positively correlated with Serine ($P = 0.02$, r = 0.978). For all other bumble bee species combined, *Bombus* spp., visitation was negatively correlated with Phenylalanine ($P = 0.05$, r = -0.949). Other bees had a positive correlation with total amino acids and percent of essential amino acids ($P = 0.001$, r = 1.00), and essential amino acids ($P = 0.0001$, r = 1.00). Amino acids correlated with visitation for all bees included Threonine ($P = 0.04$, R = 0.523), Tyrosine ($P = 0.04$, r = 0.523), and Leucine ($P = 0.04$, r = 0.523).

Fig. 1. Percent visitation for *A. mellifera*, *B. ternarius*, *Bombus* spp. and other bees on three flowering plant species, *P. tanacetifolia*, *B. officinalis*, and *G. aristata*. Observations began on 23 July and ended on 28 August.

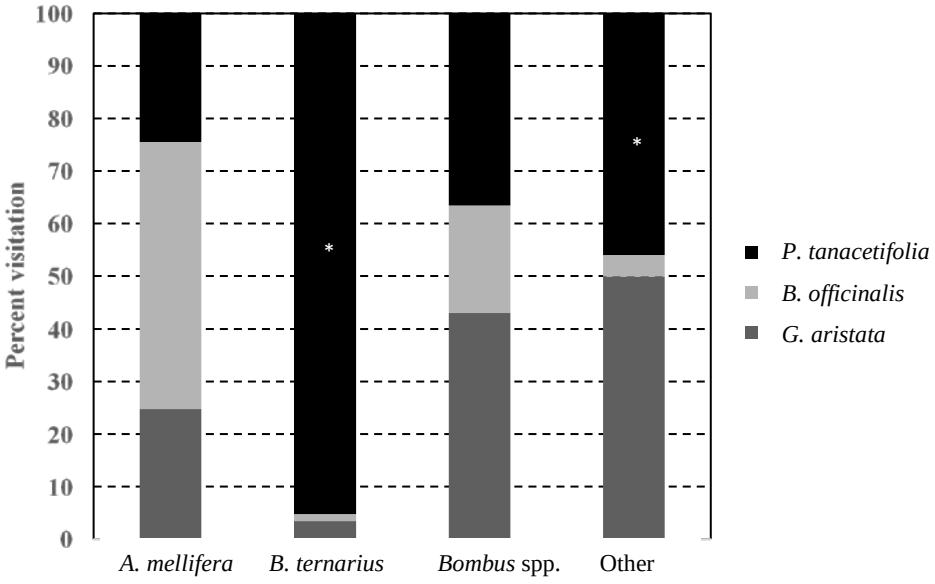


Fig. 2. Percent visitation for *A. mellifera*, *B. ternarius*, *Bombus* spp. and other bees on four flowering plant species *P. tanacetifolia, B. officinalis, H. annuus* 'Zebulon', and *G. aristata*. Observation dates span from 13 August to 2 September.

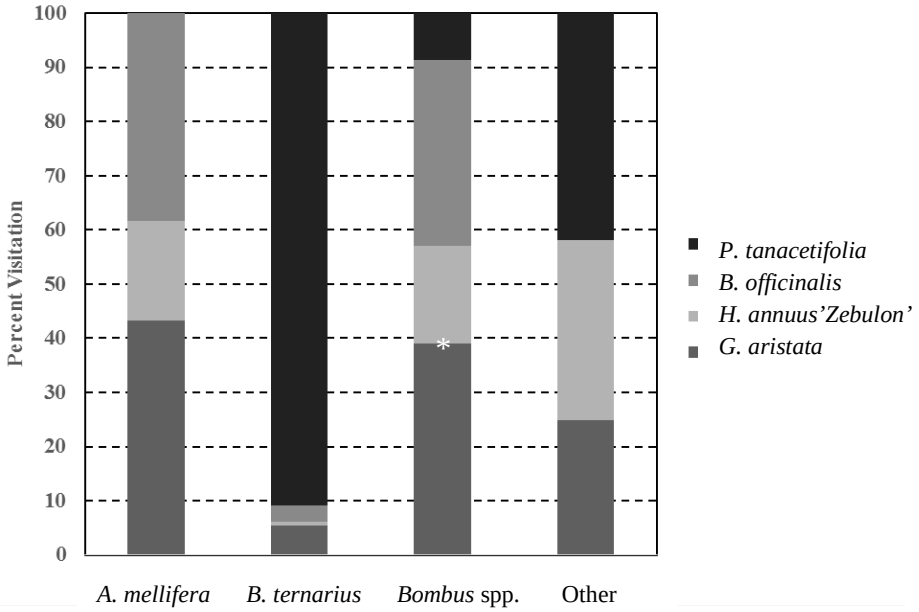


Table 1. Results of Spearman correlation analyses for floral characteristics and number of open flowers in observation plots.

	Open flowers		Flower diameter		Corolla depth		Disc diameter	
Species	**P value**	**r**	**P value**	**r**	**P value**	**r**	**P value**	**r**
Apis mellifera	0.947	-0.00901	0.3157	0.135	0.641	0.076	0.849	-0.0258
Bombus spp.	0.204	0.171	0.434	0.106	0.6486	-0.0617	0.821	0.0369
Bombus ternarius	**2.06E-05**	**0.532**	**1.54E-05**	**-0.539**	0.880	-0.0247	0.420	0.109
Other	0.8336	0.0285	0.38	0.118	0.994	0.00114	0.398	-0.114

CONCLUSIONS: We have only begun our analysis of floral pollen and nectar nutrition and bee visitation. We conducted another experiment that we conducted where nitrogen application was used to change the nutritional quality of pollen and nectar. However, we have not analyzed the data for this experiment yet. Our first experiment does suggest that nutritional content of pollen, in terms of amino acid concentration, may affect the visitation of honeybees and bumble bees, but that the number of open flowers and flower diameter also influences the visitation of the orange banded bumble bee, *B. ternarius*, to plants in bloom.

Study 14. <u>The effects of pruning method on mining bee tumuli.</u>

OBJECTIVES: In 2015 wild blueberry fields were sampled for soil temperature, mining bee nest density, and abundance. Noting that burned fields typically contained higher densities of mining bee nests (tumuli), we collected additional data in 2016 to better understand these effects.

Andrenids (also known as mining bees, sand bees, or spring bees) are possibly the most important group of wild blueberry pollinators. In fact, the four most abundant wild pollinators of Maine's wild blueberry are mining bees. Most mining bees are spring fliers whose active flight period overlaps with wild blueberry bloom in late May and early June. Like many insects, the timing of their emergence is likely variable and dependent upon weather conditions, and microclimatic gradients. Due their importance as a pollinators in blueberry, predicting whether or not mining bee emergence will be synchronous with the crop is useful. If pruning practice significantly influences mining bee abundance, it may be possible to make informed management decisions that influence mining bee emergence timing, or their abundance during crop bloom. Degree day accumulation can be used by growers to predict the emergence time of many pest insects. The emergence of some bee species also follows degree day accumulation, and mining bees may also time emergence with degree day accumulation. Degree days, or the number of days above a certain temperature threshold, vary with site conditions. For example, bare soil will heat up faster in the spring than soil with a thick layer of mulch. Dark, or charred soils, like those found in burned blueberry fields, may also influence soil temperature differently than mowed fields.

The objective of this study is to understand the role of prune method on soil temperature and mining bee tumuli densities. We hypothesize (H_1) that burned fields will have higher average soil temperatures than flail mowed fields; and (H_2) that mining bee tumuli will occur at greater densities in burned fields than in mowed fields.

METHODS: In 2015 we measured the soil surface temperature of five points in each field using an infra-red temperature gun. Temperature was measured in 10 flail mowed and 11 burned fields. In 16 fields (8 mowed and 8 burned), we counted all visible mining bee tumuli in a 1 x 100-m swath of each field. In no case were tumuli counts made after a heavy rain, as rain erodes the tumuli, making detection difficult.

In 2016, we placed Hobo® data loggers in three sets of paired flail mowed and burned fields, two data loggers per field. The temperature sensors were set 1/2" deep into the soil, and logged a temperature reading every hour from mid-April of 2016 to the termination of wild blueberry crop bloom (mid-June). In these same fields, we recorded soil surface temperatures with an infra-red temperature gun, once before bloom, and once after bloom. Temperature readings were made at 40 points at least five meters apart in each field. Twenty of these were in areas of low blueberry stem density, 20 were in areas of high stem density. We segregated our experimental design in this way to account for the possible confounding effect of stem density on soil temperature readings. Points designated as low stem density were bare or thinly populated by lowbush blueberry stems. Points designated as high stem density were readings taken from the middle of blueberry clones with high stem densities.

In 2016 we used the temperature gun at eight paired (burn vs. mow) and two unpaired fields for a total of 18 fields, 8 burned and 10 mowed; six of these fields contained the data loggers mentioned above. We collected soil samples from each field, and sent these away for laboratory analysis of soil composition and pH level. All sets of paired fields shared a border.

At each field we took 40 temperature gun readings in accordance with the methods outlined above. In these same fields, we replicated our 2015 mining bee tumuli counts. In an additional 16 fields, we counted mining bee tumuli but took no temperature readings. In no case were any measurements made in the same field in both years.

In a subset of the 2016 temperature gun fields we located a nearby (<50 m) blueberry field in early bloom (n=5). In these five fields we observed bee visitors to wild blueberry flowers, recording bees as mining bees, bumble bees, or honeybees. Observations were made in 1m quadrats for exactly one minute. In each field, we performed 20 replicated observations of bee visitation. A visit was counted when a mining bee touched a part of a flower that was within the quadrat. Individual bees were not counted more than once, even if they exited and returned to the quadrat. Observations were made between 9 am and 3 pm, with temperatures between 17-27°C, no precipitation, and a visible sun.

RESULTS: Our 2015 results are thoroughly documented in last year's blueberry reports. Here we will provide only a summary. Burned fields were slightly warmer than mowed fields, were more likely to contain nesting aggregations of mining bees, and contained a greater density of mining bee tumuli.

In 2016 we counted a total of 433 mining bee tumuli in 18 wild blueberry fields. In burned fields there were an average of 30.2 tumuli per 100 m^2 (SD = 32.17). In mowed fields tumuli density averaged 13.1 tumuli per 100 m^2 (SD = 17.18). We analyzed both years (2015 and 2016) together using Analysis of Variance (ANOVA) with model effects 'year,' 'prune method,' and 'year x prune method.' The model was significant ($F_{(3, 49)}$= 8.24, P = 0.0002). We used multiple student's t-test to compare means. Although tumuli densities were greater in burned fields in both years, this difference was only significant in 2015 (Fig. 1).

Considering the 2016 data independently, we accounted for differences in soil texture and pH by including data from our soil tests in the model. We tested the effects of these factors with ANOVA. Our main model effects were prune type, % silt, and pH level. According to a multivariate analysis of soil composition factors (% sand, silt, and clay, and pH), % silt and pH co-varied the least, and were therefore used in our model. Our whole model analysis was significant ($F_{(3, 18)}$ = 3.577, P = 0.0415). When accounting for the effects of soil composition and pH, burned fields had significantly greater tumuli densities than mowed fields (P = 0.0329). As the proportion of silt increased (from a low of 40% to a high of 65%) tumuli counts declined; although, this effect was only marginally significant (P = 0.0628). Soil pH had no effect.

To test the effects of pruning method and blueberry stem density on infra-red temperature gun soil surface temperatures, we ran a nested ANOVA, nesting stem density within prune method ($F_{(3, 38)}$ = 2.0111, P = 0.1309). Unlike our findings from 2015, pruning method had no influence on soil surface temperature (Fig. 2). Within fields, areas with higher wild blueberry stem densities had slightly lower soil surface temperatures, but this was only significant at the P = 0.10 level.

Data loggers gave us a much higher resolution look at treatment effects on temperature. We pooled data by week, contrasting treatment effects across nine weeks. Due to a low sample size (six fields) we used multivariate analysis of variance (MANOVA) to test temperature differences. Nesting stem density within prune type, our repeated measures MANOVA was not significant ($F_{(3, 8)}$ = 2.037, P = 0.1873). We then tested each week independently, running separate nested ANOVAs for each of the nine weeks. Pruning method did not influence soil

temperature (Fig. 3), but in weeks 3, 4, 5 and 8, field areas with low stem density experienced significantly lower daily low temperatures (Fig. 4).

Fig. 1. Effect of prune method on mining bee tumuli density in both years (49 fields).

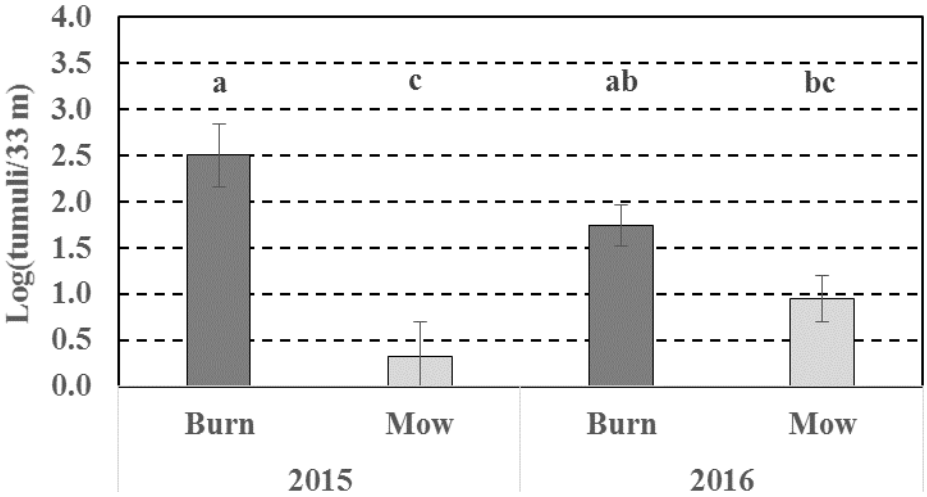


Fig. 2. Effect of pruning method and stem density (HD = high density, LD = low density) on soil temperature (n=16).

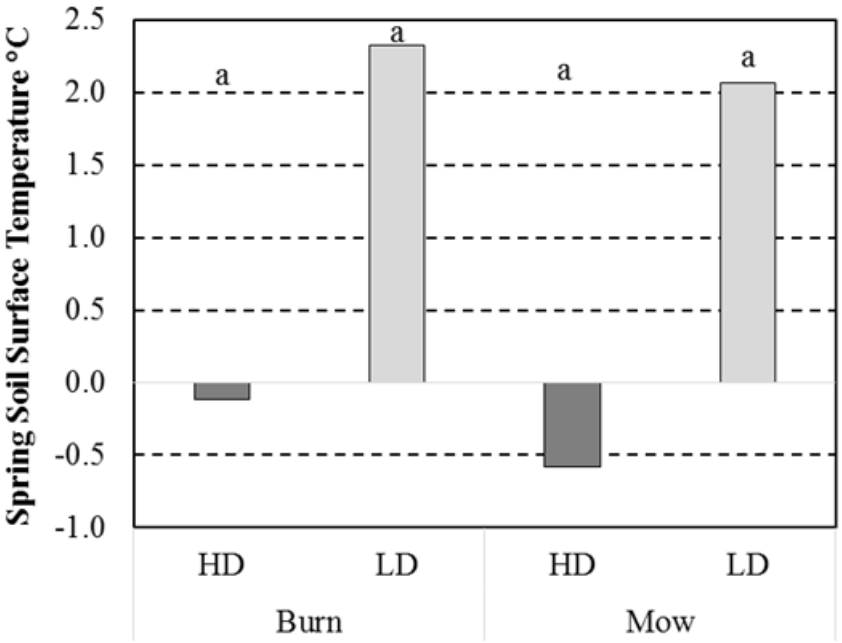


Fig. 3. Effect of pruning method on daily low soil temperatures as measured by data logger (n = 6). Pruning method had no significant ($P > 0.05$) effect on soil temperature.

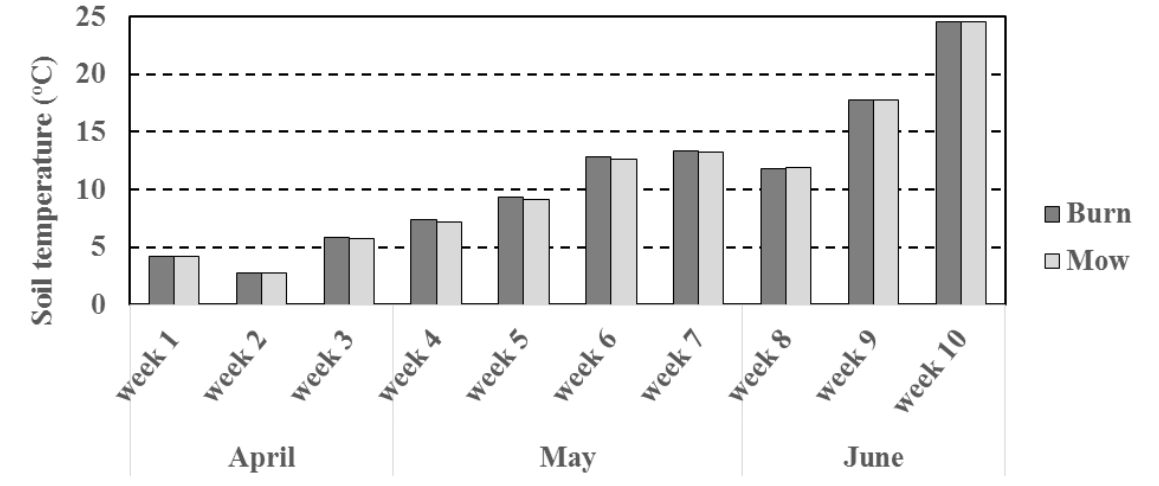
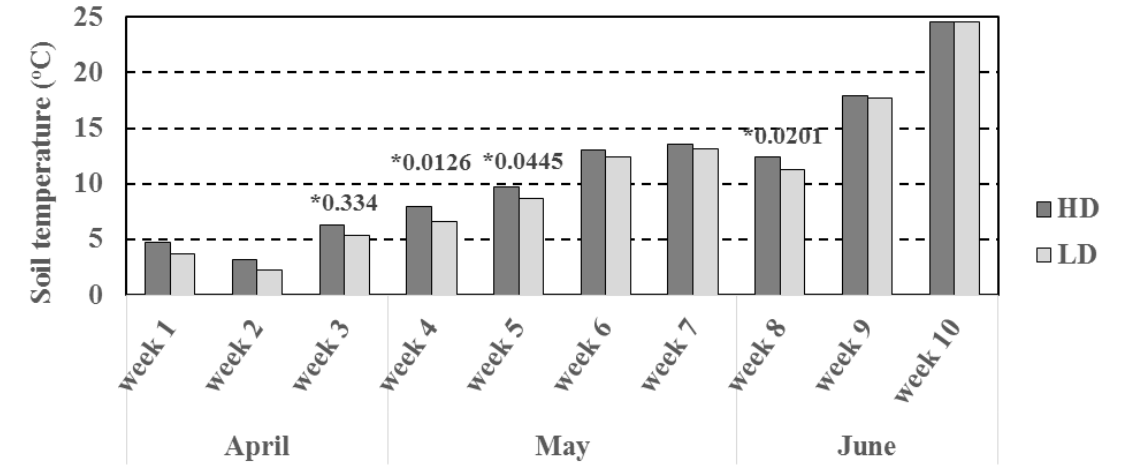


Fig. 4. Effect of wild blueberry stem density (HD = high density, LD = low density) on daily low soil temperatures as measured by data logger (n = 6). In weeks 3, 4, 5 and 8, field areas with low stem density experienced significantly lower daily low temperatures.



CONCLUSIONS: We hypothesized that soil surface temperatures in burned wild blueberry fields would be warmer than in mowed fields. Although our data provide evidence for this in 2015, we found no evidence to support this idea 2016, despite an 8-fold increase of in-field replication of temperature gun measurements from 2015 to 2016. Our temperature gun reads temperatures on the surface of the soil. Our data loggers, placed in six fields in 2016, were buried ½ inch below the soil surface and provided us with a virtually continuous reading of soil temperature throughout the spring. Despite this high level of resolution, we detected no differences in temperature between burned and mowed fields. Therefore, we can conclude that soil temperature is not the mechanistic explanation for the differences that we observed in mining bee tumuli densities.

Blueberry stem density on the other hand, does seem to play a role in soil temperatures. We provide some evidence that high stem density areas moderate temperature extremes. Studies

in other systems mirror these findings. Unfortunately, there are many unknowns. We do not know how deep into the soil this moderating effect penetrates, nor what effect if any it has on mining bees. Perhaps most likely, mining bees select bare areas of soil because it is *not* buffered from temperature extremes, and so heats up faster on cold spring mornings—enabling the bees to start foraging earlier. However, this temperature moderation effect could also mitigate the effects of earlier springs on phenology mismatches between bees and the crop. Considering the projected rise in extreme weather events, this may merit further research. This effect could be beneficial for overwintering bees, especially in years where extreme cold hits prior to adequate snow cover.

Study 15. Economics of blueberry pollination.

Report from E. Asare, A. Hoshide, G. Criner, X. Chen, and F. Drummond

OBJECTIVES: Wild blueberry production is dependent upon insect pollination and bees are the dominant insect pollinators. In the past 30 years we have identified more than 125 species of bees that are associated with wild blueberry pollination in Maine. Both native wild bees and commercially managed bees, honeybees and bumble bees, are common pollinators in Maine fields. Since 1993, bee populations have been sampled along with fruit set and yield. In total 162 wild blueberry fields were sampled. This database was used to investigate the economics of wild blueberry pollination.

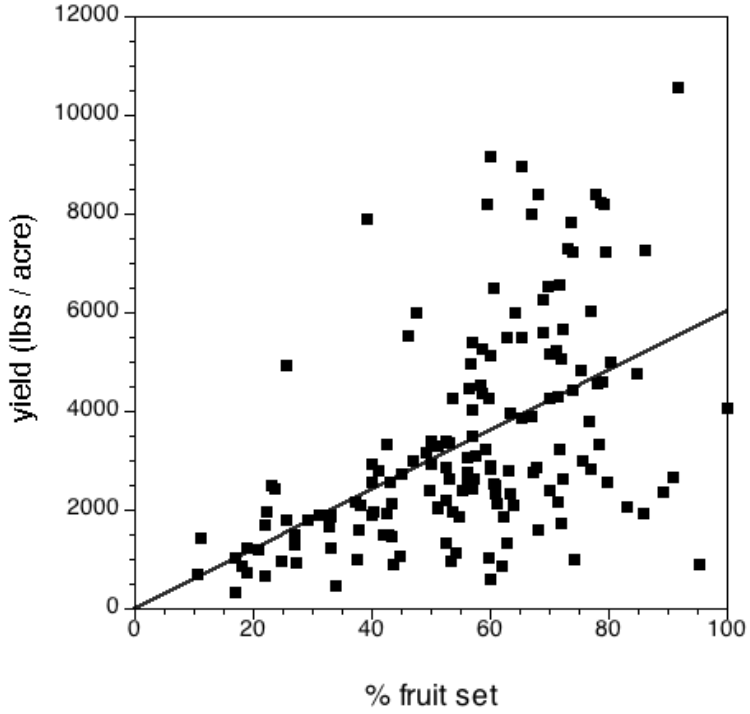
METHODS: This study uses cross-sectional data sampled from wild blueberry farms in Maine. The data collections were made in 11 years: 1993, 1997 to 1998, 2005 to 2007, and from 2011 to 2015. Wild blueberry production is grouped into organic, low-, medium-, and high-input farming systems. In these commercial blueberry fields, sampling was done for bee population abundances and density. An index of bee abundance was estimated for each field two to three times during bloom. At each visit from 1993-2015, 10 to 20, m^2 quadrats of blooming crop were arbitrarily selected, and for one minute the number of honeybees and wild native bees foraging on the bloom were counted and recorded. In 1993, for the first sampling period, five to seven, 100 x 1-m belt transects were used to estimate honeybee and native bee abundance. Bees were counted and the time to survey the transect length was recorded. The abundance of bees from these transects were converted to bees/m^2/min, so that they could be averaged with square quadrat counts.

Wild native bees were not identified to species or family, but in Maine wild blueberry, native bees comprise almost 100 species (ranging from 5 to 42 species per field) representing the families *Apidae*, *Andrenidae*, *Halictidae*, *Colletidae*, and *Megachilidae*. These quadrat counts were averaged for each visit and over all the visits for each field. The sampling of bee abundance was only conducted between 9am and 3pm and when the weather was at least partly sunny and warmer than 10ºC with average wind speed less than 30 km/hr. Yields were obtained for each field from wild blueberry producers and converted to kg harvested per ha.

RESULTS AND CONCLUSIONS: There is a positive relationship between fruit set and yield (Fig. 1). This is expected because fruit set is a determinant of the potential yield of wild blueberries. Also, there are positive relationships between fruit set and bees/m^2/min for honeybees, native bees, and total bees. The effect of the square root of native bee density on

fruit set is about 4.4% greater than that of the square root of honeybee density, suggesting that native bees on average are more efficient pollinators than honeybees on a per bee basis. This finding has been confirmed and quantified independently for several species of native bees and honeybees in flight cage and greenhouse studies.

Fig. 1. Relationship between percent fruit set and yield (1993-2015).



An analysis of variance (square root transformed honeybee densities ($F_{(3,158)} = 49.841$, $P < 0.0001$) shows that the high-input system had the highest mean count (14.68 bees/m^2/min) of honeybees, followed by the medium-input (3.5 bees/m^2/min). Low-input (1.1 bees/m^2/min), and organic at 0.33 bees/m^2/min were not significantly different from each other, but lower than the medium-input system. This is expected since theoretically high-input farms rely mostly on honeybees compared to the other crop systems. Organic at the other extreme does not typically use rented honeybees.

An analysis of variance conducted to determine the cropping system effect on native bee densities suggested that there was no significant effect ($P = 0.406$). Native bee counts for organic were 0.47/m^2/min, high-input were 0.52/m^2/min, medium 0.61/m^2/min, and low were 0.62/m^2/min. These observations are not consistent with theory for fruit production across the United States. It is generally expected that organic farms will have the highest count of native bee density per m^2, followed by low-, medium- and high-input systems mainly due to pesticide exposure and access to alternative pollen and nectar forage plants. However, this observed pattern of no effect of cropping system on native bee community density has been corroborated previously in wild blueberry. An exception to this is that large areas of contiguous wild blueberry fields, independent of the cropping system, tend to have significantly lower densities and richness of native bees.

Given that fruit set is an indicator of pollination service, it is not surprising that the high-input system has the highest mean fruit set (66.5%). An analysis of variance ($F_{(3,158)} = 5.374$, $P = 0.002$) showed that medium-input was not significantly different from the high-input (58.3%) and not significantly different from low-input (51.2%) and organic at 49.1% which were both different from high-input. Wild blueberry mean yield is highest ($F_{(3,154)} = 26.261$, $P < 0.0001$) for the high-input (6,167 kg/ha), followed by medium-input (4,610 kg/ha), and then by low-input (2,675 kg/ha) and organic (2,092 kg/ha) production systems which were not significantly different from one another.

Bees are related to percent fruit set (Fig. 2), but more specifically; year, cropping system, and native bee and honeybee density are significant predictors of fruit set ($F_{(16,145)} = 7.209$, $P < 0.0001$). The fruit set model indicates native bee and honeybee densities (square root transformed) both have positive and significant (at 1% confidence) effects on the fruit set of wild blueberries (Table 1).

Fig. 2. Relationship between total bee foraging force and fruit set.

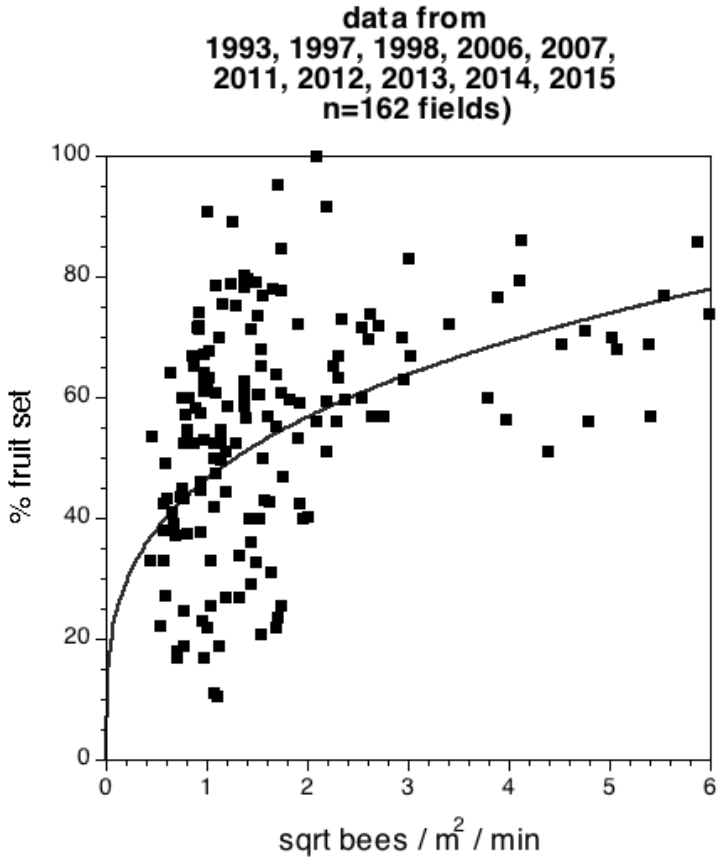


Table 1. Wild blueberry fruit set regression model.

Variable, Dep. Variable: Fruit Set (%)	Coefficient	Standard error
Intercept	37.0861***	4.189
Dummy_1993	1.2936	2.929
Dummy_1997	-14.1111***	4.597
Dummy_1998	-11.9996***	3.778
Dummy_2005	-15.0067**	5.979
Dummy_2006	17.5508***	5.268
Dummy_2007	1.4891	5.618
Dummy_2011	-2.051	4.214
Dummy_2012	-12.8323***	3.711
Dummy_2013	3.19	3.700
Dummy_2014	17.5065***	4.183
High Input System Dummy	4.1931	3.902
Low Input System Dummy	-4.5188	2.471
Medium Input System Dummy	4.6038**	2.052
Square Root Honey Bees (Count/m^2)	6.1217***	1.554
Square Root Native Bees (Count/m^2)	14.8804***	5.142
Interaction of (Native Bee)0.5 and (Honey Bees)0.5	-5.4018	3.147

Number of Observations = 162, Adjusted R-Square = 0.39

***, **,* denote significance at 1%, 5% and 10%, respectively

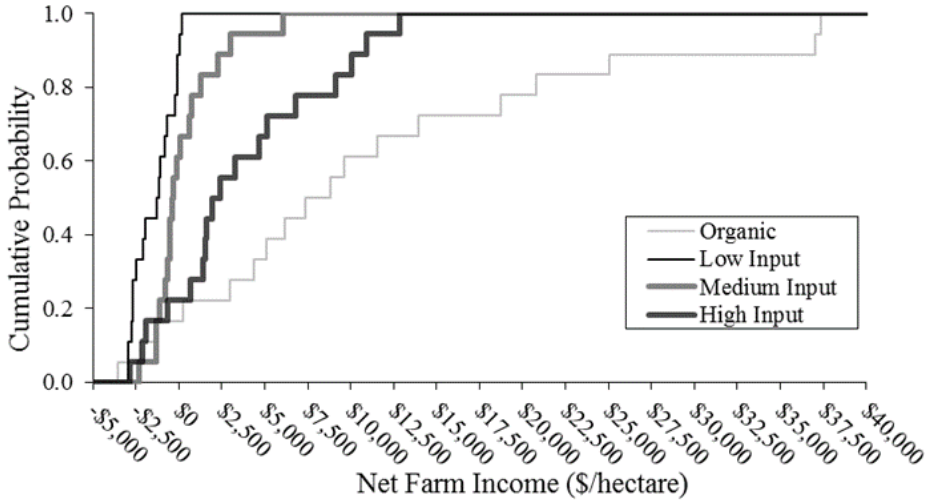
The impact of an individual native bee on fruit set is about 1.6 times greater than that of a honeybee. The model explains 39% of the variation in percent fruit set. The interaction term between native bees and honeybees is negative although not significant. It has been observed tin several studies that honeybees can suppress foraging of certain native bees such as bumble bees. Year is also significant (relative to the base year of 2015). One would expect year to have a large effect of fruit set since weather affects frost incidence, the number of suitable foraging days during bloom, nectar secretion, and length of stigma viability. In addition, because different fields were surveyed each year, the genetic structure of plants varies from field to field and this greatly affects fruit set. Cropping system also significantly affects fruit set. This is not surprising as stem density and flower density/stem varies by cropping system and the management of insect pests that attack flowers, mummy berry disease, and weeds that might compete for pollinators all vary by cropping system. Across all of the cropping systems the average portioned variance explained in fruit set (partial r^2) for year was 75.4%; for honeybees it was 16.4%, and for native bees it was 8.2%. Therefore, year can be seen to drive the variance in fruit set, and of course, bee foraging density is not totally independent of year as the model would suggest.

Bee foraging densities are not a good predictor of yield ($P > 0.05$). This is most likely due to the numerous other factors that affect yield. However, fruit set is a good predictor of yield as it is a requisite of yield. The best predictive model for yield included fruit set, year, and cropping system ($F_{(14,143)} = 20.691$, $P < 0.0001$). The yield model indicates fruit set has a

significant (1% level) positive effect on wild blueberry yield. Also, yields in high-input and medium-input farming systems are higher compared to organic. These yield differences are significant at the 1% confidence level. The model explains 63.7% of the variation (adjusted r^2) in wild blueberry yield (kg/ha). The partitioned variance explained in yield (partial r^2) was as follows: year, 39.3%; cropping system, 48.9%; and fruit set, 11.8%.

Comparison of agricultural production and profit risk for organic, low-input, medium-input, and high-input wild blueberry systems using yield estimates from our fruit set and yield models based on observed fruit set data (n=162) only show clear first-order stochastic dominance and thus economic preference of high-input and medium-input systems over low-input (Fig. 3) since cumulative distribution functions (**CDF**) for high- and medium-input are both to the right of that for low-input. Organic has higher average net farm income (**NFI** = $11,222/ha) than the other systems (-$1,434 to $3,610/ha) but a wider variance of profitability outcomes that suggest it may be riskier than any of the conventional systems. Likewise the high-input may be more risky than medium-input since the area under the high-input CDF is greater than the area under the medium-input CDF.

Fig. 3. Stochastic dominance comparisons of wild blueberry system models estimated solely on observed fruit set field data.



While 2012 wild blueberry prices received for organic fresh pack ($8.58/kg) are 434% higher than the typical conventional frozen price ($1.61/kg) for low-, medium- and high-input models based on our 2012-13 producer surveys; estimated yields for organic, based just on fruit set measured in the field, were not much lower (2,345 kg/ha) compared to the range of simulated conventional yields (2,998-6,912 kg/ha) for low-, medium- and high-input. Only one cooperating organic producer, of 12 producers interviewed, had a yield greater than this estimated yield for organic. This organic grower's high yield was due to meticulous hand control of weeds. The 10-yr average wild blueberry yield from surveyed organic producers was only 724 kg/ha. The 10-yr average yield from surveyed conventional producers was also lower than the yields that we measured in the field. These differences between measured in-field yields and grower survey estimated yields could be due to different efficiencies in harvesting fields, grower estimates based upon harvested berries winnowed in the field, or yields reported to growers by processors after sorting and culling fruit not meeting U.S. no. 1 grade.

Comparing stochastic simulations (1,000 iterations) of all four wild blueberry systems demonstrates clear first-order stochastic dominance of profitability distributions for conventional systems with increasing input use when comparing NFI per hectare (Figs. 4 & 5). The CDF for high-input is to the right of the CDF for medium-input which is to the right of the CDF for low-input which indicates conventional wild blueberry systems are more economically preferable with increasing levels of input (including rented honeybee hives) intensity. This was consistent for both simulations adjusted to represent mean crop yields (Figs. 4 & 5). Depending on the crop yield assumptions used, low-input systems have negative NFI across much if not all of their cumulative distributions, while medium- and high-input maintains positive NFI.

Organic wild blueberry, due to higher prices received per kg for fresh pack, is first-order stochastically dominant and thus economically preferable to all conventional systems assuming average yields based on field experimental data (2,345 kg/ha) as shown in figure 4. Organic has 90% of its simulated NFI between $12,383-$15,668/ha ($3.45-$3.72/dry liter) and only 5% of NFI values below $12,383/ha ($3.45/dry liter) as indicated by the light gray bars above the graph; high-input has100% of NFI values below $12,383/ha ($3.45/dry liter) as indicated by the black bars above the graph, while this is true for only 5% of organic NFI values.

Fig. 4. Stochastic dominance comparisons of wild blueberry system profitability ($/ha) simulated to represent mean crop yields estimated from field study data.

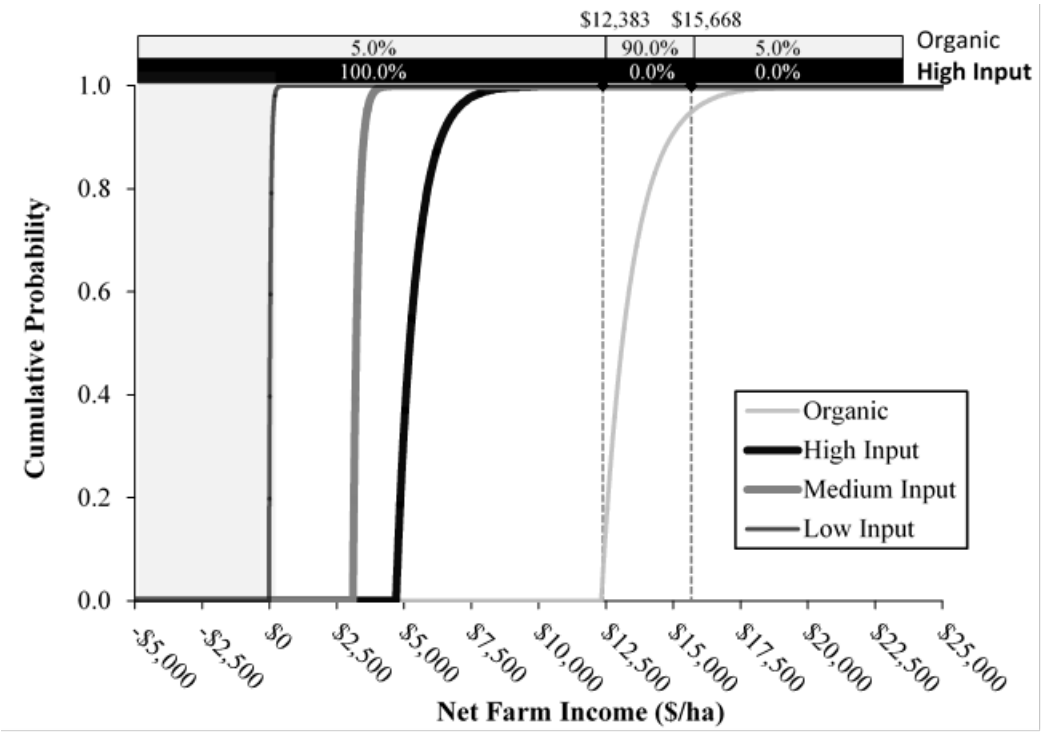
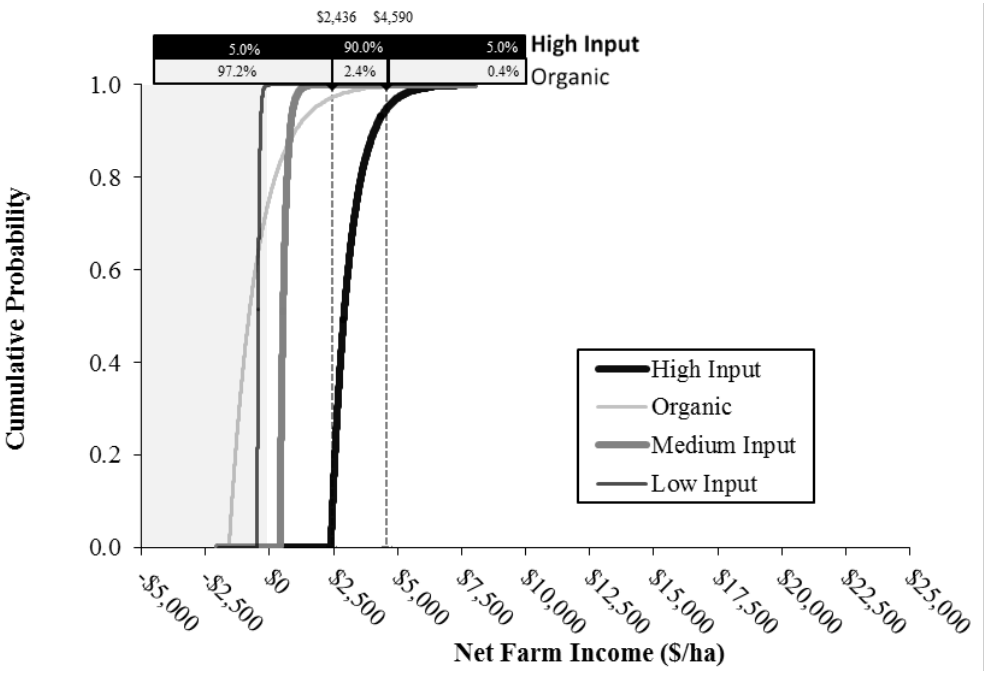


Fig. 5. Stochastic dominance comparisons of wild blueberry system profitability ($/ha) simulated to represent mean crop yields surveyed from producers.



RECOMMENDATIONS: A degree-day model for wild blueberry bloom in Maine is suggested based upon field sampling across the two major growing areas over two years. This model is different than a previous model developed in Nova Scotia since air temperatures are not accumulated until March. In addition, a 40ºF base is used instead of the 50ºF base used for Nova Scotia. The precision for two years and two regions is higher than the typical 10% precision often seen. We plan to sample a small number of fields in 2017 to validate this final model. The use of this model will mainly be for simulation modeling in order to assess mummy berry dynamics and climate change effects on pollination.

The simulation model of wild blueberry pollination takes advantage of field data accumulated over 30 years of wild blueberry pollination research in Maine and the Canadian Maritimes and provides a simulation tool that allows users to explore how various factors, including inter-clone compatibility, stem density within clones, flower density within clones, clone size frequency distribution, changes in weather conditions, bee species composition, bee density, and their foraging behavior as well as landscape spatial arrangement, in isolation and combination with other fields and landscapes, affect the pollination efficiency and yield; particularly in the case where logistical, spatial, and temporal limitations are encountered in large-scale experimentation. The graphical user interface lowers barriers to using simulation for researchers or interested growers with little or no experience in modeling, and enables them to test hypothesis, develop theories, and assess field management strategies for wild blueberry pollination. Further, the open-source feature allows further development for other blueberry related theoretical studies, e.g., mummy berry disease transmission vectored by bee pollinators. In addition, the general approach that we used has potential for translation to other crop pollination systems.

The big take home message from the study of honeybee colony health on the wild blueberry barrens during bloom is the transmission of *Varroa* in loading yards – as indicated by the spike of mites in the control apiaries. This suggests that perhaps beekeepers who use loading yards should leave several colonies in the holding yards deliberately until the end of colony movement. These last colonies should be treated after the others are moved out as they seem likely to act as a sink or repository for drifting *Varroa* and treating those colonies may prevent a *Varroa* explosion from being initiated in otherwise healthy colonies at a later date.

We have refined our search for a good bee colony level detection unit. Research in 2017 will be to develop a portable unit that farmers and beekeepers can use. We will design an activity monitor based on the 10.5 GHz radar. This will involve measuring signal strength with a simple rectifier circuit recording data on a simple data logger. There will be no need for signal processing (computers). We estimate the cost of the prototype will be less than $50.

Since application of Assail and Tilt are not recommended during bloom, we suspect that significant detrimental effects to colonies of honeybees and bumble bees will not result when these pesticides are used correctly. However, to be more confident with bumble bee exposure we need to conduct these trials again with healthy bumble bee colonies.

Our study of field exposure of honeybees to pesticides showed that non-agricultural sites did have significantly lower exposure concentrations of pesticide residues in pollen than agricultural landscapes; but Risk did not differ significantly due to the variability between sites. Neonicotinoids were not a threat to honeybees in 2015 and are probably not a threat most years in most parts of the state. Our recommendation for the wild blueberry industry is to reduce exposure whenever possible using good pest management practices.

Our wild bee surveys have created a robust data set of wild bees outside of wild blueberry fields in Maine. Wild bee communities do not differ between the wild blueberry growing regions, but they do differ by land cover type. However, the relationship between bee community and land cover type differs by growing region, suggesting that landscape pattern—the combination of cover type and shape—may have a role in determining the wild bee communities found in these areas. We need to better understand how landscape pattern affects the wild bee communities in the Downeast and Mid-coast growing regions, and we are incorporating measures of landscape pattern into our statistical models.

Colony collapse disorder (CCD) is becoming a problem for agricultural production all over the United States. Obtaining information on what the cyst-like bodies we observed in honeybees are might be a small step in alleviating losses from CCD. In October, a beekeeper sent Dr. Drummond pupae of what appear to be the phorid fly, *Apocephalus borealis*. These pupae were recovered from his honeybee hive. We are currently incubating them. If adult flies emerge then we can identify them. If these pupae are *Apocephalus borealis*, then this will be the first phorid fly confirmed parasitism of honeybees in Maine.

Findings from our study of mining bees support our hypothesis that the density of mining bee tumuli within wild blueberry fields is higher in burned fields; although, this effect was more pronounced in 2015. We offered one mechanistic explanation for these findings, a possible temperature difference between burned and mowed fields, but failed to find support for this mechanism in our data. It seems quite possible that the differences we did find in temperature in 2015 were more related to the underlying difference between most burned and mowed fields. Burning is a more expensive practice, so growers tend to use this pruning method only when necessary. Fields situated on slopes and strewn with boulders are very difficult, if not impossible to flail mow. It is these fields that are typically burned instead. Burned fields are generally

patchier where blueberry clones are interspersed with patches of bare soil and rocks. One explanation for our findings is that mining bees prefer to nest in burned fields, not because they are burned, but because they offer a more heterogeneous mixture of blueberry plants, bare soil, rocks, and weeds. These fields may serve as source populations of mining bees into surrounding homogenous fields. Considering the current practice of removing rocks and leveling fields to create a homogenous field more accessible to machinery, the idea of land-levelling and rock removal to increase mechanical harvest and mowing efficiency alone may not be the best decision for smaller undercapitalized growers who need to enhance their wild bee populations.

Our model of the economics of blueberry pollination can help wild blueberry producers know the risk associated with relying on native bees and honeybees on their crop's profitability. This can provide them with insight into managing these risks with appropriate pollination insurance measures such as alternative commercial pollinators: bumble bees, alfalfa leaf cutting bees, native and exotic *Osmia* spp. leafcutting bees; and actively installing pollinator plantings to enhance native pollinator populations. Such risk management can ensure reasonable certainties in their net profits or losses for future planning purposes. It could also assist policy makers and implementers of pollination security practices on farms to propose the appropriate policies and strategies to conserve bees and to sustain the wild blueberry industry in Maine.

DISEASE MANAGEMENT

INVESTIGATORS: Seanna Annis, Associate Professor and Associate Extension Professor
Rachael Martin, Research Assistant, School of Biology and Ecology
Tyler Case, MS Graduate Student, School of Biology and Ecology

6. TITLE: Research and control of leaf spot diseases.

OBJECTIVE: Improve control of various leaf spots, Septoria leaf spot (*Septoria* sp.), powdery mildew *(Erysiphe vaccinii*), and leaf rust (*Thekopsora vaccinii*) using field and lab research.

METHODS:

Survey of weather and levels of disease in wild blueberry fields

The fifteen fields with weather stations were rated for leaf spot diseases (powdery mildew, Septoria, and leaf rust), between September 20 and 30, 2016. Five sampling plots of $0.25m^2$ were rated by two surveyors visually estimating percentages of blueberry coverage, blueberry leaf loss, blueberry stems with Phomopsis, and blueberry leaf area with the following leaf spot diseases: Septoria leaf spot, powdery mildew, and leaf rust. Any red leaf and false Valdensia disease were also noted. Fall disease ratings were averaged across the surveyors by sampling plot and then across all five sampling plots within a field.

Spore dispersal measured by Burkard spore traps

In June 2016, spore traps were placed in two prune fields near Deblois and in a crop field at Blueberry Hill Research Farm (BBHF) in Jonesboro, ME. We collected spore trap tapes containing the trapped airborne spores every week until October 6, 2016. Spore trap tapes were cut in half and half was frozen for future DNA work and the other half was mounted on glass slides. Tapes were examined for rust spores at hourly intervals and the number of rust spores was

recorded. One of the spore traps (in a prune field near Deblois) was found to not be working properly and was brought in for repairs and then placed out in the prune field at BBHF and then subsequently returned to the lab when it was found to still not be working.
Disease assessments occurred weekly in the spore trap fields when the spore trap tapes were collected. Five sampling plots of $0.25m^2$ were rated by visually estimating percentages of blueberry coverage, blueberry leaf loss, blueberry stems with Phomopsis, and blueberry leaf area with the following leaf spot diseases: Septoria leaf spot, powdery mildew, and leaf rust.

Fungicide trial

A Complete Randomized Split-plot Design experiment was established in a lowbush blueberry field near Deblois, Maine. Fungicides (Table 1) were randomly assigned to 6' x 30' plots with a 3' buffer lane between each plot and replicated in 6 blocks. Plots were divided in half and treatments were randomly assigned to one of two application timings (June 16 or June 30, 2016). Fungicides were applied at volumes equivalent to 20 gallons per acre at 35 psi with a CO_2 backpack sprayer equipped with a 4 nozzle boom, 8002VS T Jet tips and 50 mesh screens applied. Control plots received no spray applications.

A rope with 20 evenly spaced markings was stretched along a transect through each plot and the stem closest to each marking was cut and bagged. The next day, stems were inspected for disease symptoms on the leaves. The total number of leaves, nodes lacking leaves (leaves fallen) and the estimated percent coverage of each disease was noted per stem. Phytotoxicity was also rated at the same time disease assessments were made. The first stems were collected July 21 and rated July 22 and 23. Stems were again collected on August 16 and rated August 16-18. A third collection occurred on September 12, and were rated on September 12-14. After leaf fall, stems were collected November 15th and were rated on November 15-17 for stem length, and number of leaf and flower buds. Data were analyzed by plot averages in SAS (Statistical Analysis Software - SAS Cary, NC) using split plot design in mixed model procedures (PROC GLIMMIX). Least Square means were used to determine specific differences among treatments ($\alpha = 0.05$).

RESULTS:

Weather station fields

Leaf spot symptoms were rated in September to early October, after harvest in crop fields where weather stations were located. There were low levels of Septoria (average 1%) seen at this time (Fig. 1), but this is probably too late to see peak Septoria levels which are more likely in July and August (Fig. 2 and 3). Higher levels of powdery mildew (average 11%) and rust (average 10%) than Septoria were observed (Fig.1). Humid weather with lack of rainfall may help in the spread of powdery mildew spores. The dry weather may also have affected the disease pressure for rust, since the average rust levels were 15% in 2015 which had wetter weather in August than this year.

Spore trap project

Of the three Burkard spore traps we placed in different fields to measure the presence of rust spores, it was found that one spore trap was not working all season. Attempts to fix this machine during the season were unsuccessful. We replaced a motor, but will need other parts. This machine is manufactured in Britain and communication with the company has been slow.

In the other two fields, ratings of Septoria symptoms, powdery mildew and rust were made each week starting June 23rd in the Spring Pond field and July 21st at BBHF. In Spring Pond (Fig. 2), low levels of Septoria symptoms were found starting June 30th and levels stayed moderate until early September. September ratings of leaf spots were difficult to determine if the symptoms were Septoria or early leaf rust since both types of spots are sunken black spots and are difficult to distinguish. With the rapid increase in rust symptoms (leaf spots clearly producing rust urediospores) starting Sept. 22, it is likely the leaf spot symptoms seen earlier in September are likely developing rust lesions. In BBHF (Fig. 3), the first rating on July 21st found low levels of Septoria symptoms that continued through to middle of September. The levels of Septoria were very high in July 28th and August 18th and lower on days before and after which may be due to variation among the random clones that were rated or possibly dropping of diseased leaves. In the future, leaf loss will also be rated.

Powdery mildew symptoms were low in both fields and did not show an overall increase in symptoms in either field. Leaf rust symptoms were clearly detected for the first time, by the production of urediospores in the lesions, on Sept. 22 in both Spring Pond and BBHF (Fig. 2 and 3, respectively). As mentioned earlier, leaf rust lesions may have started earlier in September but are difficult to distinguish from Septoria lesions at the early stage of leaf rust lesions. In 2015, clear leaf rust symptoms were observed a month earlier on Aug. 22nd in BBHF and another field near Wesley, ME. The later production of visible rust symptoms in 2016 may be due to the drier weather and a slower build-up of rust inoculum.

Spore trap tapes are still being examined. From preliminary data, rust spore levels are much lower than they were in 2015 at BBHF.

Fungicide trial

Five different materials were tested at two different application times for their efficacy in controlling leaf spots. None of the treatments caused visible phytotoxicity to the plants during any of the ratings. Leaf loss increased on average from 11 to 18 to 33% over the three rating times (Fig. 4, 5, and 6). The levels of Septoria leaf spot were low in all three ratings and ranged from 0.5 to 5.9% (Fig. 7, 8, and 9). Powdery mildew affected less than 2% of leaf area in the July and August ratings (Fig. 10 and 11) and increased in the September rating to 1.5 to 6.5% (Fig. 12). There was very little leaf rust this year. No rust was found in the July and August ratings. Some leaf rust was seen in the September ratings (Fig. 13).

July ratings: There were no significant effect of the treatments or timing of fungicide applications in the first rating on leaf loss or disease levels (leaf loss, Septoria and powdery mildew, respectively in Fig. 4, 7, and 10). August ratings: There were significant differences among the treatments in the level of Septoria leaf spot (Fig. 8) and leaf loss (Fig. 5). There were no significant effects of treatment or timing on powdery mildew levels (Fig. 11). There was no significant effect of timing of fungicide application and no significant interaction with treatments. Pristine and Proline treatments had significantly lower leaf loss than the negative control (Fig. 5). Daconil Ultrex and Luna Tranquility had lower levels of leaf loss than the control but along with the Double Nickel treatments, were not significantly different from the controls. When the treatments were compared within each timing, the significant differences among treatments was only seen with the early application timing (Fig. 8). Pristine was the only treatment with significantly less Septoria than the untreated control. September ratings: There was no significant effect of timing or interaction between timing and treatment for leaf loss in the September rating (Fig. 6). Daconil Ultrex, Pristine and Proline had significantly lower levels of

leaf loss than the untreated control. There was no significant effect of the timing or treatments on the level of Septoria, powdery mildew or leaf rust in September (Fig. 9, 12, 13). There were no differences in levels of flower buds among treatments (data not shown). November ratings: The number of leaf and flower buds and stem lengths measured in November were consistent across treatments and there was no significant effect of the treatments (data not shown).

RECOMMENDATIONS: We did not see a clear effect of timing of fungicide application on levels of leaf loss or disease levels. It was very dry year throughout the growing season, which likely would depress levels of disease due to lack of moisture for infection but also put the plants under drought stress which may increase leaf loss. Daconil Ultrex (chlorothalonil) is the most commonly used material to control leaf spots and was effective in decreasing leaf loss in September and Septoria levels in August, but did not decrease leaf loss in August or July below the non-treated control. Pristine and Proline treatments decreased leaf loss in August and September and Septoria levels in August. Luna Tranquility and Double Nickel (a biological control) did not decrease leaf spots and leaf loss compared to the untreated controls. These materials may need alternate timings or multiple applications to be effective. We will repeat this experiment next year and continue to look at timings of fungicide applications.

Table 1. Fungicides tested in 2016 for their efficacy to control leafspots.

Treat-ment (Trade Names)	Material	Application Rate (per acre)	Manufact-urer	FRAC group	EPA Reg. Number	Registered on Blueberries
Daconil (Bravo) Ultrex	Chlorothalonil (tetrachloroiso-phthalonitrile)	3.6 lbs/a	Syngenta	M5	50534-201-100	yes
Double Nickel	*Bacillus amyloliquefaciens* strain D747	2.1 qt/acre	Certis	Biologi-cal	70051-107	Biocontrol / Not applicable
Double Nickel	*Bacillus amyloliquefaciens* strain D747	1.06 qt/acre	Certis	Biologi-cal	70051-107	Biocontrol/ Not applicable
Luna Tranqui lity	fluopyram and pyrimethanil	16 fl oz/a	Bayer	7 9	264-1085	no
Pristine	pyraclostrobin and boscalid	18.5 oz/a	BASF Corp.	117	7969-199	yes
Proline	Prothioconazole	5.7 fl oz/a	Bayer Crop Science	3	264-825	yes

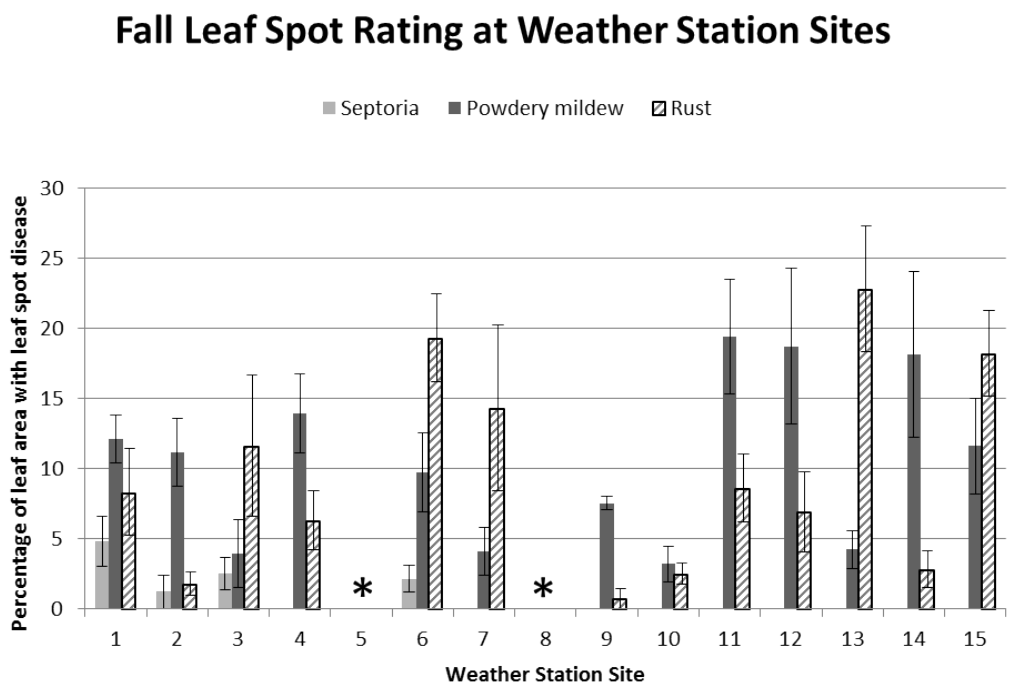


Figure 1. Percentage of leaf area with Septoria (light gray bars), powdery mildew (dark grey bars) and rust (striped bars) at each of the weather station fields. Error bars indicate standard error of the mean. *Two fields were not rated, one due to early pruning and the other due to early weather station removal.

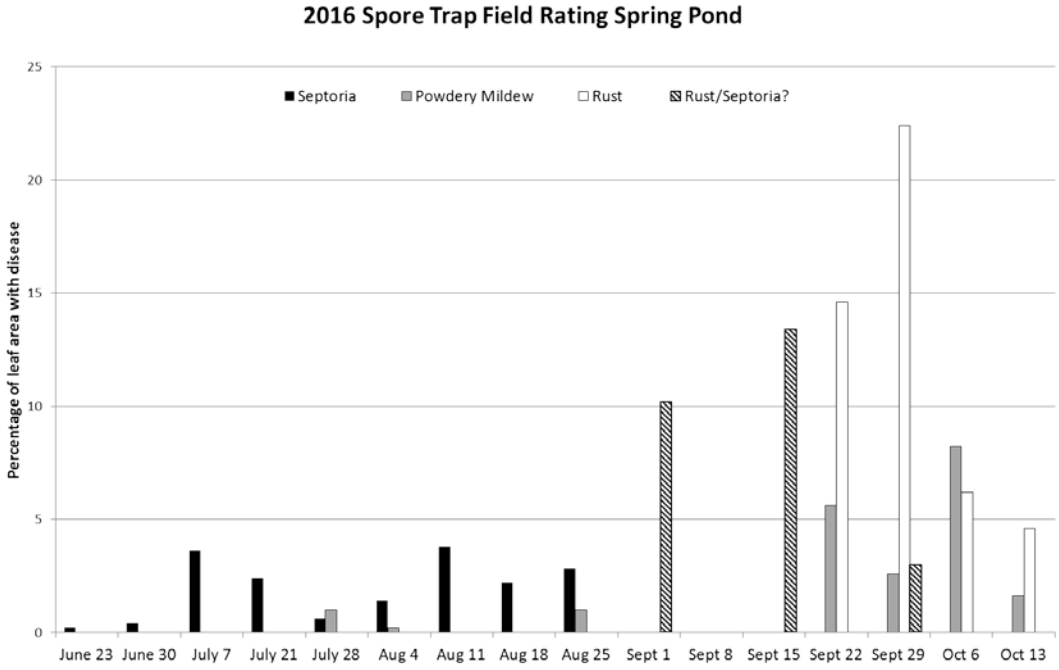


Figure 2. Symptoms of leaf spots, powdery mildew, Septoria and rust, rated each week in Spring Pond field near Deblois, ME where a spore trap was placed.

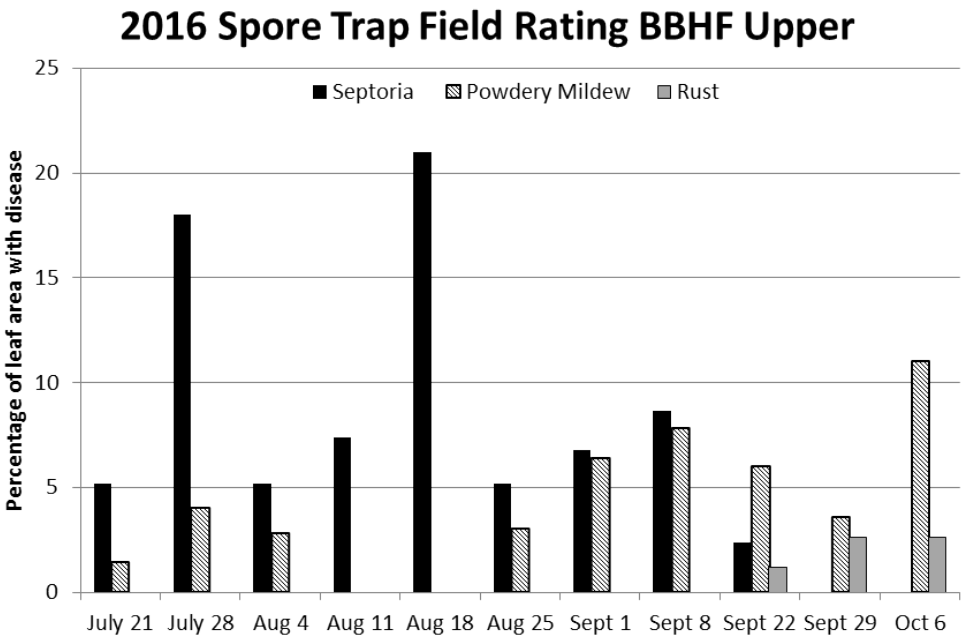


Figure 3. Symptoms of leaf spots, powdery mildew, Septoria and rust, rated each week in Blueberry Hill Farm upper field near Jonesboro, ME where a spore trap was placed.

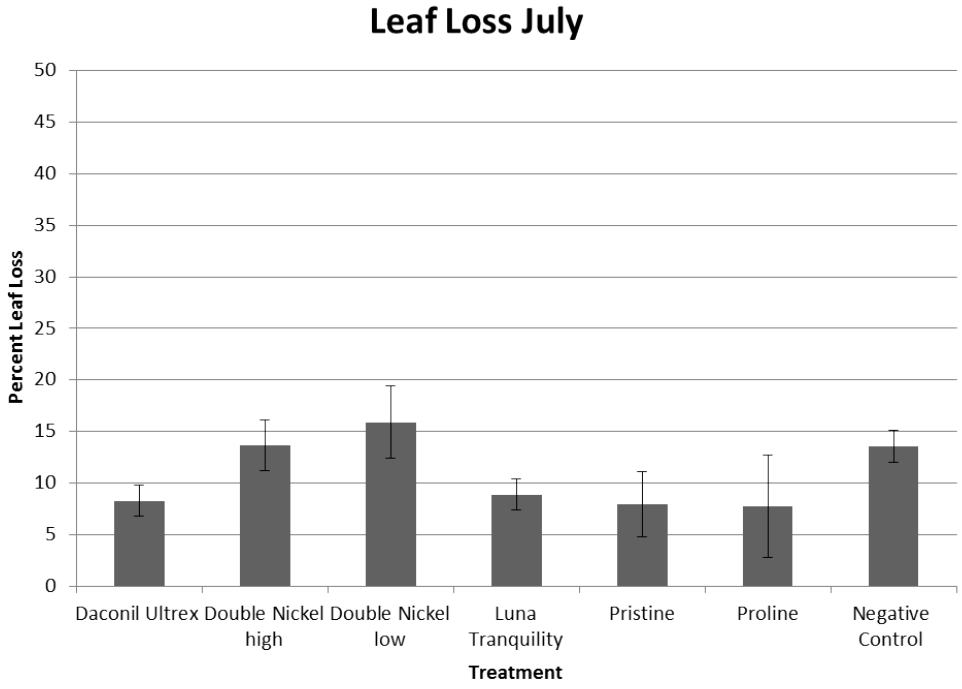


Figure 4. Percentage of leaf loss in July. There were no significant differences in the timing of treatment applications so the combined data is shown. There were no significant differences among treatments and no significant interactions.

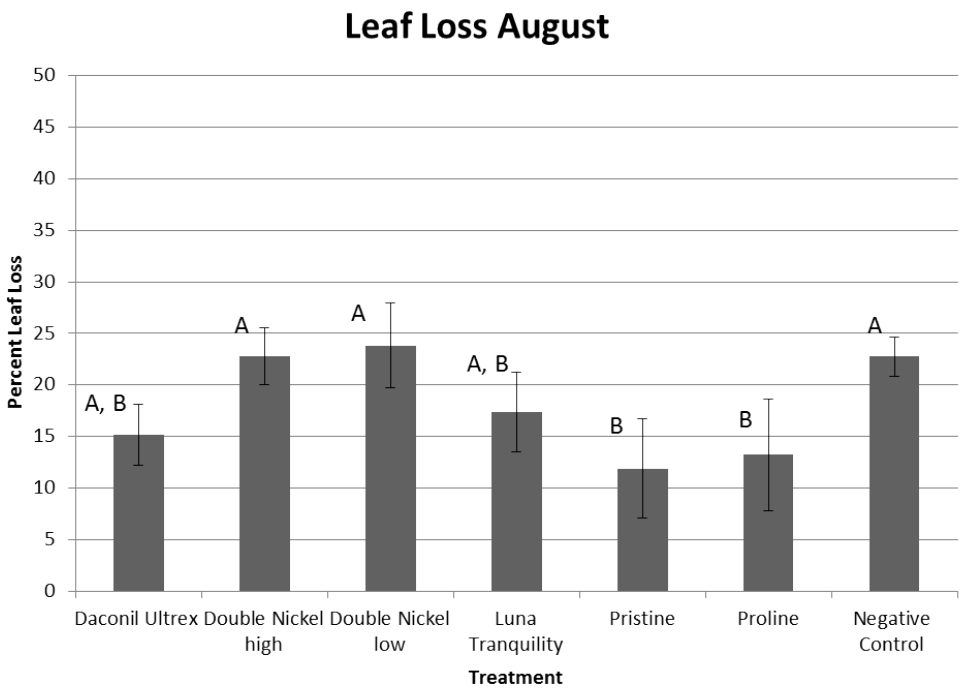


Figure 5. Percentage of leaf loss in August. There were no significant differences in the timing of treatments so the combined data is shown. There were no significant interactions. Bars with different letters indicate statistically significant differences at $\alpha = 0.05$.

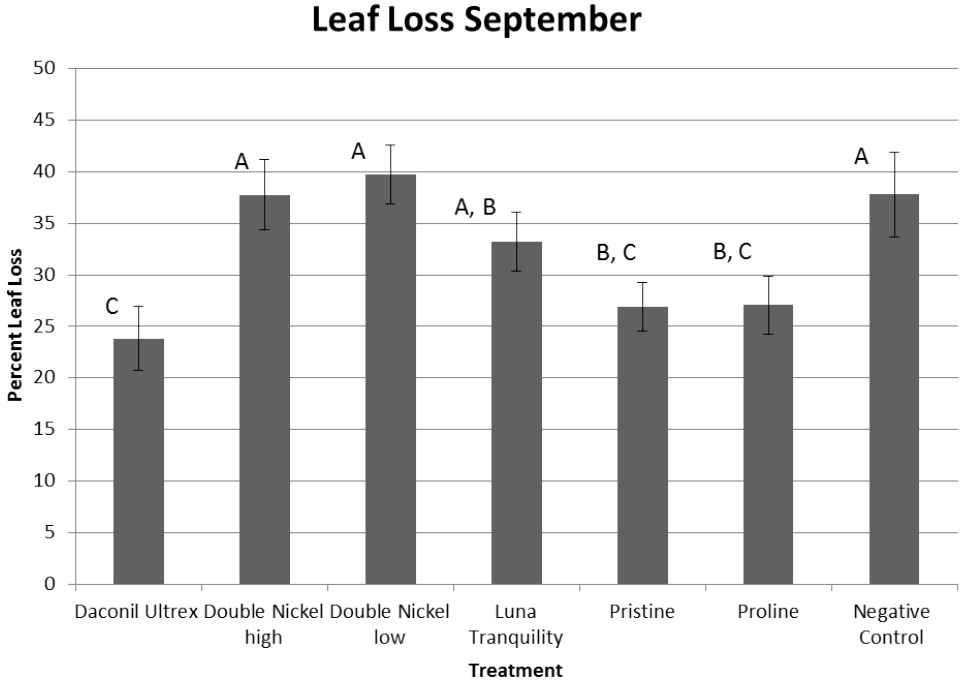


Figure 6. Percentage of leaf loss in September. There were no significant differences in the timing of treatments so the combined data is shown. There were no significant interactions. Bars with different letters indicate statistically significant differences at $\alpha = 0.05$.

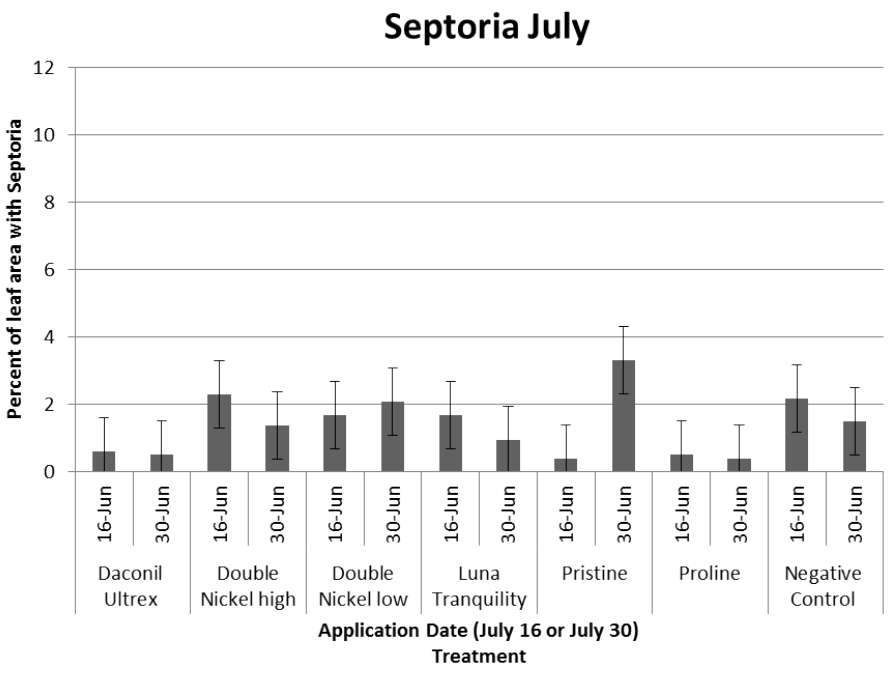


Figure 7. Percentage of leaf area with Septoria by treatment and application timing in July. There were no significant differences in treatments or treatment timing.

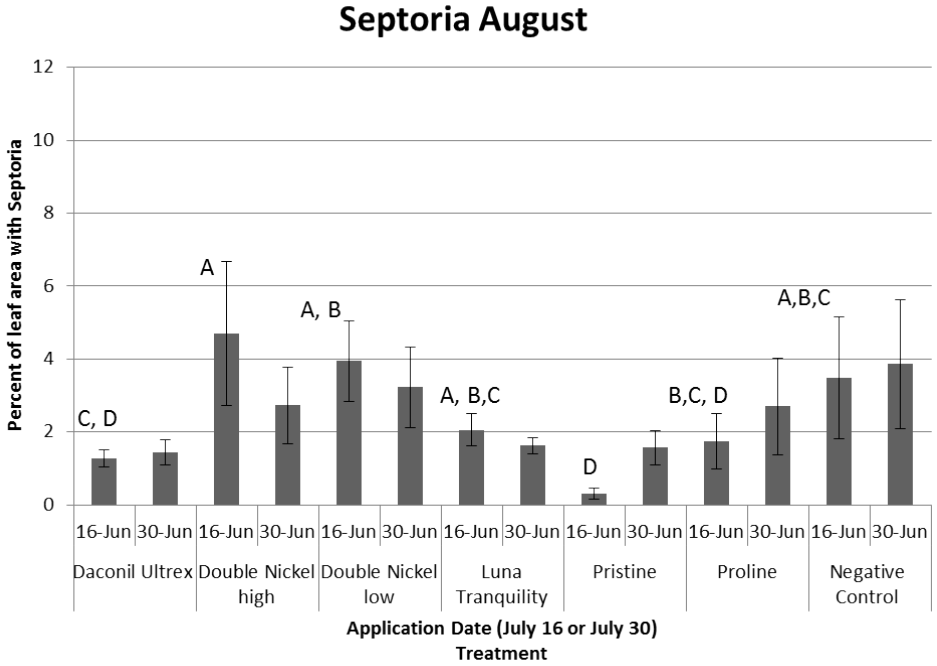


Figure 8. Percentage of leaf area with Septoria by treatment and application timing in August. There were no significant differences in the timing of treatments and no significant interactions. There were significant differences in treatments for the June 16 application. Bars with different letters indicate statistically significant differences at α=0.05. There were no significant differences in treatments for the June 30 treatments.

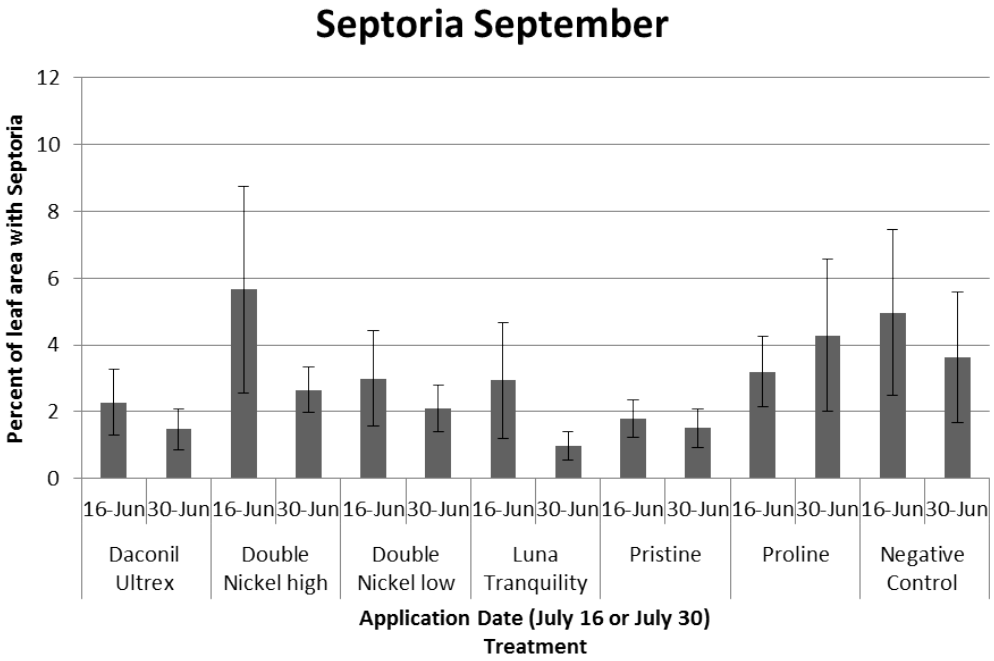


Figure 9. Percentage of leaf area with Septoria by treatment and application timing in September. There were no significant differences in treatments or treatment timing, and no significant interactions.

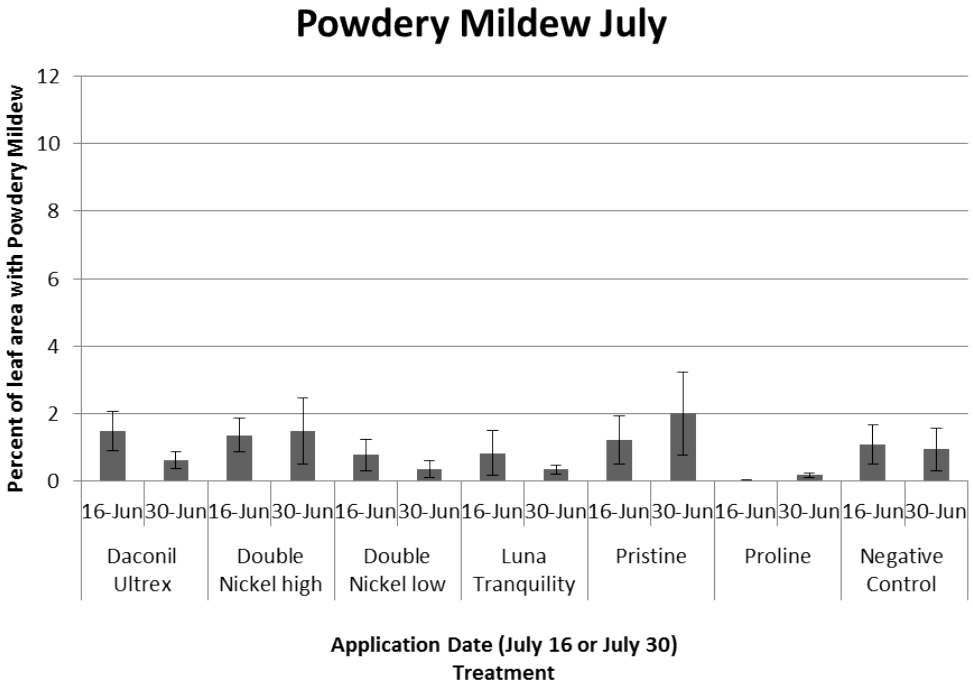


Figure 10. Percentage of leaf area with powdery mildew by treatment and application timing in July. There were no significant differences in treatments or treatment timing, and no significant interactions.

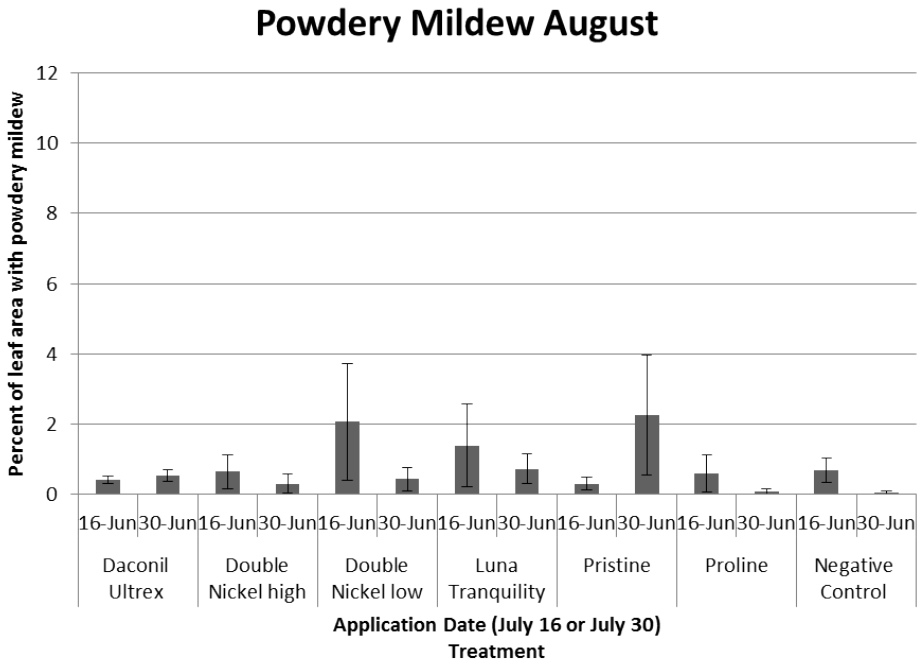


Figure 11. Percentage of leaf area with powdery mildew by treatment and application timing in August. There were no significant differences in treatments or treatment timing, and no significant interactions.

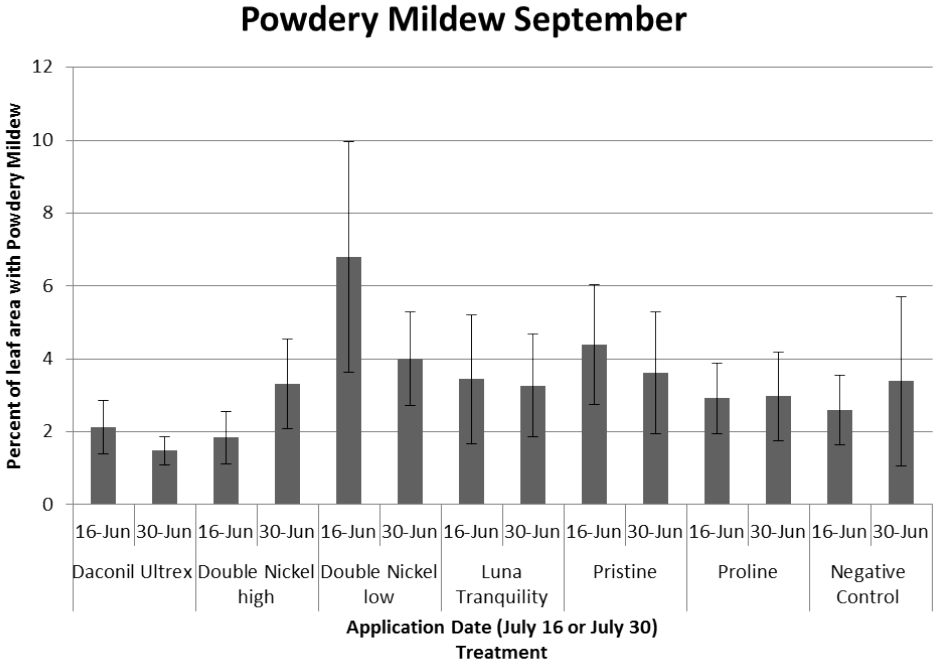


Figure 12. Percentage of leaf area with powdery mildew by treatment and application timing in September. There were no significant differences in treatments or treatment timing, and no significant interactions.

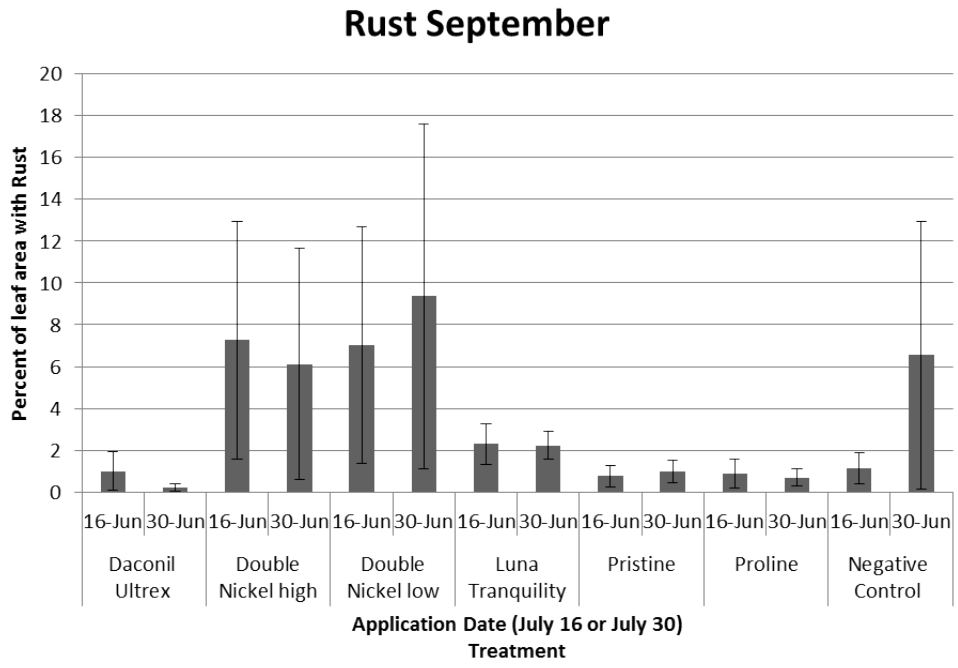


Figure 13. Percentage of leaf area with rust by treatment and application timing in September. There were no significant differences in treatments or treatment timing, and no significant interactions.

DISEASE MANAGEMENT

INVESTIGATORS: Seanna Annis, Associate Professor and Associate Extension Professor
Rachael Martin, Research Assistant, School of Biology and Ecology
Tyler Case, MS Graduate Student, School of Biology and Ecology

7. TITLE: Research and control of mummy berry and Botrytis blossom blight.

OBJECTIVE: Improve control of mummy berry, caused by *Monilinia vaccinii-corymbosi* (MVC) and Botrytis blight, caused by *Botrytis cinerea*, through research and the deployment and operation of a disease forecasting system using weather stations.

METHODS:

Weather stations and disease forecasting

From April 8 to April 25, 2016, fifteen weather stations connected to the internet via cellular modems were deployed in blueberry growers' fields around Maine from Waldoboro, Lincoln County to Crawford, northern Washington County (Fig. 1). We added improved heat shields to all of the weather stations, which should improve the accuracy of the air temperature data. Twelve locations also had MVC mummy berry (pseudosclerotia) plots that growers monitored through May. We contracted with Skybit to get virtual data for 10 locations where we also had real weather stations.

The fifteen fields with weather stations were rated for mummy berry between May 23 and June 2, 2016. Four 30m transects with 30 evenly spaced marks were randomly placed in the field around the weather station. The stem closest to each mark was inspected for mummy berry and Botrytis symptoms and the presence or absence of each disease was noted. Stems with top kill, frost, tip midge and red leaf were also recorded.

Fungicide efficacy trials

Field trials were set up in two lowbush blueberry fields with histories of mummy berry disease. One field was near Deblois and the other in Township 19, Maine. The plots were set up in a complete randomized block design of 8 blocks per field. Fungicides (Table 1) were randomly assigned to 6' x 30' plots with a 3' buffer lane between each plot and replicated in each of the 8 blocks per field. Fungicide applications were timed using the Mummy Berry disease forecast, which uses locally monitored fungal and plant development and weather, to identify conditions favoring infection by MVC (Fig. 2). Fungicides were applied on April 28, May 6, and May 13 in the Deblois and Township 19 fields as protectant applications before infection periods were expected to occur. Fungicides were applied at volumes equivalent to 20 gallons per acre at 35 psi with a CO_2 backpack sprayer equipped with a 4 nozzle boom, 8002VS T Jet tips and 50 mesh screens applied. Appropriate surfactants were added as recommended by the manufacturer (Table 1) and the negative control (check) plots received no spray applications. Weather stations are located within 3 miles of the test fields and measured air temperature and leaf wetness at approximately 4" off the ground, soil temperature at 1" below the surface and soil moisture at 1" to 5" below the surface where most of the blueberry roots are located.

Disease assessments in both fields occurred on May 31, 2016. In each field, ratings consisted of presence/absence of mummy berry symptoms on 40 blueberry stems in each plot. A rope with evenly spaced markings was stretched along a transect through the middle of the plot and the stem closest to each marking was inspected for disease symptoms on flowers or leaves. In addition, the number of markings at bare places (missing data) and frost damaged stems was recorded. The percentage of infected stems was the number of counted infected stems divided by the total number of rated stems (40 minus the number of bare locations) for each plot. Phytotoxicity was also rated at the same time disease assessments were made.

Blueberries were harvested on August 8, 2016. Harvesting occurred in a 2 foot strip down each plot center with a mechanical harvester and fresh weight was measured. In Township 19, 2 ft of one end of some plots were harvested accidentally when making a walkway. Yield data for those plots was for 28 ft and not 30 ft. Yields from the other plots were multiplied by 0.933 (percent that 28 ft is of 30 ft) to produce a comparable yield to mistakenly harvested plots.

Percent data was converted to proportion and then an arcsin transformation of the square root performed to attempt to normalize the data. Only the yield data had a normal distribution. Treatments were compared with the non-parametric Wilcoxon rank sums and a Kruskal-Wallis test using Chi-sq analysis in procNpar1way in SAS 9.4 (SAS institute Inc.).

Timing of ascospore release

Two spore traps were placed in a field in Deblois from May 6 to May 19, 2016. The spore traps were placed in a road running between a prune and crop field. One spore trap faced into the prune field, the other into the crop field. The number of *Monilinia* ascospores and conidia were counted hourly for each day the spore traps were in the field.

Effects of field edges on mummy berry incidence

Eleven fields adjacent to prune fields and which had mummy berry symptoms were rated for edge effects on disease incidence. Four transects were placed perpendicular to the field edge 1 m, 12 m, 24 m and 36 m from the edge. The transects were 30 m with 30 evenly spaced markings. The stem closest to each mark was inspected for mummy berry and the presence or absence of disease was recorded. The edge of the fields adjacent to a prune field and a forest edge (within approximately 20 ft) were rated. Percent data was converted to proportion and arcsin transformation of the square root performed to normalize the data. Mixed models using distance and least mean square comparison were used to compare locations in SAS 9.4 (SAS institute Inc.)

Effects of insects on pseudosclerotia in the field

Cage experiments were set up at Blueberry Hill Research Farm in Jonesboro from September 1-October 13, 2016 to investigate the potential interaction between ground dwelling invertebrates and overwintering pseudosclerotia from *Monilinia vaccinii-corymbosi*. A randomized block design was used with two replicates per treatment type, with two different treatment types (four blocks in total). The first treatment type was 1 m into the field edge, immediately adjacent to the surrounding forest. The second treatment type was located 50 m from the edge and roughly in the middle of the field. Each of the four blocks had eight treatments including four treatments with 30 pseudosclerotia in each of a full 1 mm mesh cage, 4 mm mesh cage, 14 mm mesh cage and a negative control with no cage. Four additional treatments were added to the design to see if there were any responses to increasing densities of pseudosclerotia. The four treatments used contained 3, 9, 27 and 81 pseudosclerotia with no cage. Cages were checked each week for number and condition of pseudosclerotia. Insects were captured in pitfall traps to inventory the existing population of invertebrates in the field that may be interacting with overwintering pseudosclerotia. Insects collected from pitfall traps were sorted and identified to family.

Pseudosclerotia germination in the lab

In November 2016, we set up an incubator experiment to look at chilling hour requirements for the germination of pseudosclerotia from five different fields (Hope, Spring Pond, Montegail, Junior Grant and Love Lake). This experiment is being repeated for a third year with slight modifications from last year. Three different soil moisture levels were used in 2015, approximately 50, 60 and 75% moisture. This year, all of the treatments were set up in saturated soil while accumulating varying amounts of chill-hours. Upon removing the treatments from their 4°C chill-hour accumulation, the soil was allowed to dry slowly by leaving the plastic bags open until the desired moisture level has been reached (50, 60 or 75% moisture). All moisture adjustments are based on weight, and incubation medium characteristics. In 2015, the treatments ranged from 800-2400 chill-hours; this year we increased the range from 1200-3000 chill-hours. Field chill-hour data was collected from five sites (two to three button loggers per site) during the winter of 2015/2016. The five fields used to collect these data are listed as follows with the average number of chill-hours from each field from 10/1/2015 thru 5/1/2016 in parenthesis: Little Oak (3238 hours), Montegail (2286 hours), Appleton (3121 hours), Crawford (3318 hours) and Wesley (2678 hours). All of the fields in this study have had three winter button loggers in them (from 10/2016-5/2017) to estimate field chill-hour conditions and to use that information to better reflect natural conditions in our controlled experiments.

RESULTS AND RECOMMENDATIONS:

Disease forecasting

We surveyed blueberry growers from February to March, 2016 in an on-line or mail-in paper survey on their use of the weather stations and their use of fungicides for control of diseases. We had 57 surveys returned and found 89% used the Mummy Berry Forecast reports and 75% used the Botrytis reports. 81% of the growers used fungicides to control mummy berry, and 72% used the forecast to help them time their applications. The most used fungicide for mummy berry control was propiconazole (78%), and more growers were rotating their fungicides than in the past (28%). We also found all weathers stations were used, and use varied from 4 to 18 growers for each location. There was also a desire for more stations in locations where we currently do not have stations. The survey information was used to plan where to put stations in 2016. In April 2016, fourteen cellular connected weather stations were deployed around blueberry growing regions and a wi-fi connected station at Blueberry Hill Research Farm, Jonesboro, ME was deployed (Fig.1)

We provided mummy berry reports every two to three days from mid-April to mid-May 2016, and Botrytis reports in May. Disease reports (15 reports) were provided via a blog on the Cooperative Extension blueberry website (https://extension.umaine.edu/blueberries/blog/), via email list, and as recorded phone messages. It was an unusually dry year with uncertainty about mummy berry apothecia production which involved more field visits and calls to growers than normal. Dry weather in early spring produced *Monilinia* infection conditions we have not seen in the last few years (Figure 2 compares 2015 to 2016). We had a very dry spring in 2016 with cool temperatures lasting into May. We also did not have a lot of snow pack and so had dry conditions leading into the field season (Fig. 2D). We had frequent rainfalls, but they only amounted to 0.1 to 0.25 inch of rain at a time with scattered showers (Fig. 2C). This lack of rain produced dry conditions where most of the time the soil appeared drier than in previous years and particularly the top layer of soil where apothecia are produced. This produced an interesting situation for the *Monilinia* fungus which produced apothecia starting around April 20th but all of those apothecia and immature cups dried up before we got our first infection period. The rain we got on May 2nd to May 4th would normally have caused infection periods for the fungus, but there were very few apothecia present and so few spores. The rain did produce another round of production of apothecia and there were some infections after May 5th. Most apothecia were drying up again by mid-May. In contrast, in 2015 (Fig. 2), we also had little rainfall, but had significant snow pack to provide soil moisture. The soil moisture in 2015 was relatively consistent and we had production of *Monilinia* apothecia throughout the mummy berry infection period.

At each weather station, symptoms of mummy berry, Botrytis (late May to early June), and leaf spots (September) were rated, using an appropriate method for each disease, in the fields around the weather stations. We measured low levels of mummy berry with an average of 10% of stems infected and varying amounts from none to 54% reported in fields (Fig. 3). Data on mummy berry trends were presented at the July Field day at the Wild Blueberry Hill Research Farm in Jonesboro, ME.

We ran into difficulty comparing the real and virtual data when we found the virtual data was estimated at 6 ft off the ground and the real weather stations were taking measurements approximately 4 inches off of the ground. This produced a large discrepancy in infection periods between the two types of data. We were able to get a correction for air temperature in the

summer of 2016 but not for leaf wetness. The Skybit company in now working on a correction factor for leaf wetness and we hope to compare the virtual and real weather station data from 2014, 2015 and 2016 seasons in early in 2017 so we can determine the effectiveness of using virtual data.

Fungicide trial

We tested two products in different FRAC groups from propiconazole and applied the materials as protectants before infection periods were expected. Due to the unusual weather and dry soil conditions, we made three applications of the products. We saw no phytotoxicity with any of the treatments. We had low levels of mummy berry disease this year in both of our trial fields (Fig. 4). We did not see any significant differences in disease levels with the treatments compared to the check. We did see higher levels of disease in the Torino 9 oz treatment in the Deblois field, but this was not seen in the Township 19 field. We did not see any significant effect of the treatments on yield (Fig. 5). We recommend retesting the Torino and Luna Tranquility fungicides next year. With more typical spring conditions, we get 25% to 50% of stems with *Monilinia* infection. This season was unusual with the very dry conditions producing little inoculum and the resulting low levels of disease.

Ascospore release

We set out Burkard spore traps to collect ascospores of *M. vaccinii-corymbosi* in two adjacent fields, one prune and one crop in early May 2016. We discovered one of the spore traps was not working properly and so we only have data from one field. We found a peak of spores was released on May 11th which was approximately 3 days after a heavy rainfall (Fig. 6). The peak in spore production probably also coincided with a new flush of apothecia reported by growers after the heavy rainfalls from May 3rd to May 8th. We also found most spores were released overnight, from midnight to 6am, when averaged over the 14 days we had the spore trap working (Fig. 7). We will repeat this experiment in 2017 with more working spore traps over a longer time period during ascospore discharge.

Mummy berry edge ratings

Stems 1 m into the crop field from the prune field border had significantly higher incidence of infection than stems located 12 m and further into the field (Fig. 8A). This suggests inoculum may be blowing into the crop field from the prune field, but that there is inoculum being produced within the crop field interior. There was no significant difference in the incidence of disease at among any of the distances from the forest edge (Fig. 8B). This experiment will be repeated next year with more transects covering a finer gradient of distances from the field edge.

Effects of insects on pseudosclerotia in the field

The proportion of pseudosclerotia (mummies) removed from the cages did not differ between cages set up at the edge of the field or in the interior (data not shown). There was also no difference in the proportion of pseudosclerotia lost when the number of pseudosclerotia varied from 3 to 81 (data not shown). The cage with the largest mesh, 14 mm, and the no cage treatments lost a similar proportion of pseudosclerotia over time and more pseudosclerotia than the full cages or 4mm cages (Fig. 9). A small portion of pseudosclerotia were lost in the full cage, suggesting that some pseudosclerotia breakdown from weather or microbial decay. Larger

insects, >4 mm, are probably responsible for most of the loss of mummies but some mummies may be attacked in place. Any pseudosclerotia that was no longer a full sphere was considered damaged (Fig. 10). This "damage" may occur during the maturation process as uninfected areas of the dried fruit decay, since even in full cages up to 20% of the mummies were damaged after 42 days. The most pseudosclerotia were damaged in the treatment without a cage (Fig. 10). The 14mm and 4mm cages had similar numbers of damaged mummies and more than the full cage, suggesting insects may be attacking the mummies.

The major groups of insects in the pit fall traps have been identified, with minor families still in the lab to be identified at a later date. In alphabetical order, the families captured over four weeks (total number of individuals in parentheses) were Carabidae (5), Gryllidae (38), Formicidae (48) and Scarabidae (1).

Pseudosclerotia germination in the lab

A set of lab experiments were set up to compare the germination of pseudosclerotia by varying the number of accumulated chill hours (from 800 to 2400 hr at 4˚C), soil moisture and temperatures during post-chill growth (50, 55 and 61˚F; 10, 13 and 16˚C) using pseudosclerotia from 4 fields. We had low levels of germination and were not able to compare among chill hours for some fields or among fields. We did observe a significant effect of soil moisture and post-chill growing temperature on germination when looking at the field with the highest levels of germination (Fig. 11). Germination was significantly greater at 16°C compared to 10°C and 13°C ($p < 0.05$) and significantly more pseudosclerotia germinated at 60% soil saturation than treatments at 50% and 75% saturation ($p < 0.05$) under all temperature conditions. There were no significant differences in germination across the chill-hours tested (1150, 1500 and 1850 chill-hours) in this experiment ($p > 0.05$). These experiments (Fig. 11) demonstrate the importance of soil moisture and air temperature in pseudosclerotia germination. This experiment will be repeated in the winter of 2016 to 2017.

Table 1. Fungicides tested in 2016 for control of mummy berry.

Treatment (Trade Names)	Application Rate (oz per acre)	Material	Manufacturer	FRAC group	EPA Reg. Number	Registered on Blueberries for mummy berry
Torino with Induce (surfactant)	6	cyflufenamid	Gowan	U6	8033-103-10163	No
Torino with Induce (surfactant)	9	cyflufenamid	Gowan	U6	8033-103-10163	No
Torino with Induce (surfactant)	12	cyflufenamid	Gowan	U6	8033-103-10163	No
Luna Tranquility	16	fluopyram and pyrimethanil	Bayer	7 / 9	264-1085	Yes
Positive Control - Bumper	6	propiconazole	Adama	3	66222-42	Yes

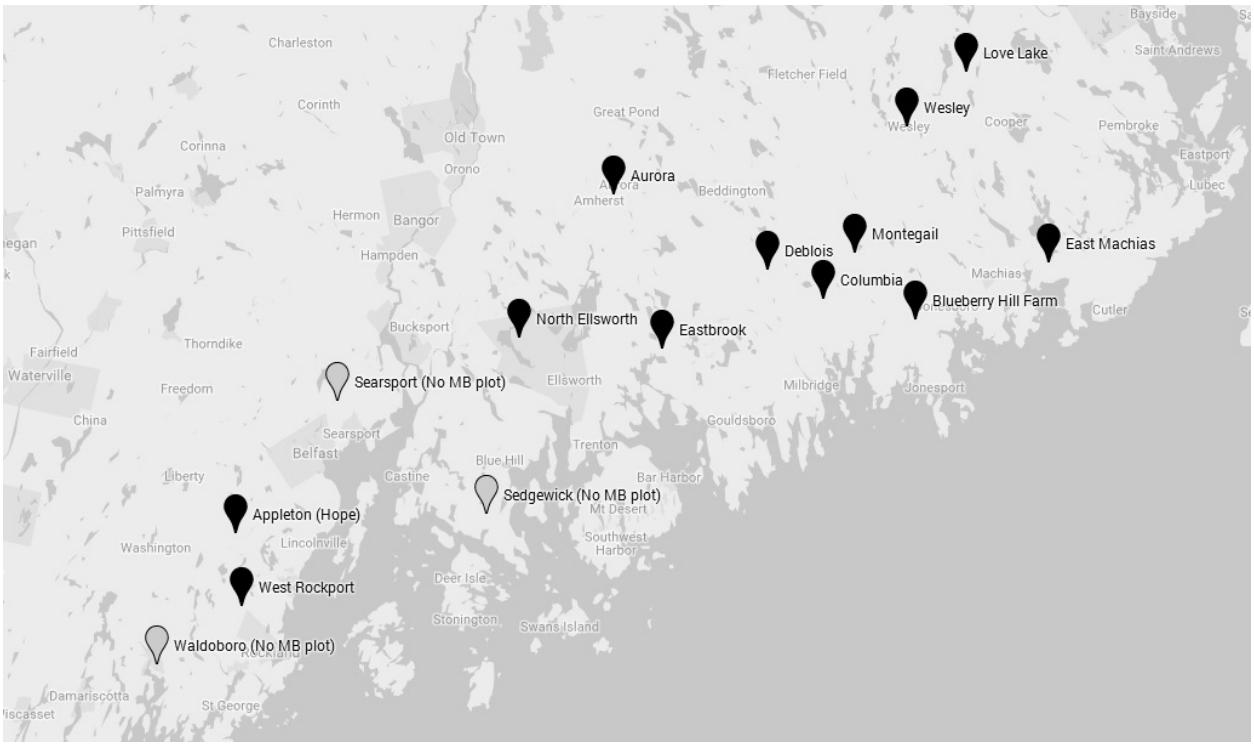


Figure 1. Locations of mummy berry forecast stations and mummy berry plots for 2016. Sites with black markers had a mummy berry plot; sites with gray markers did not.

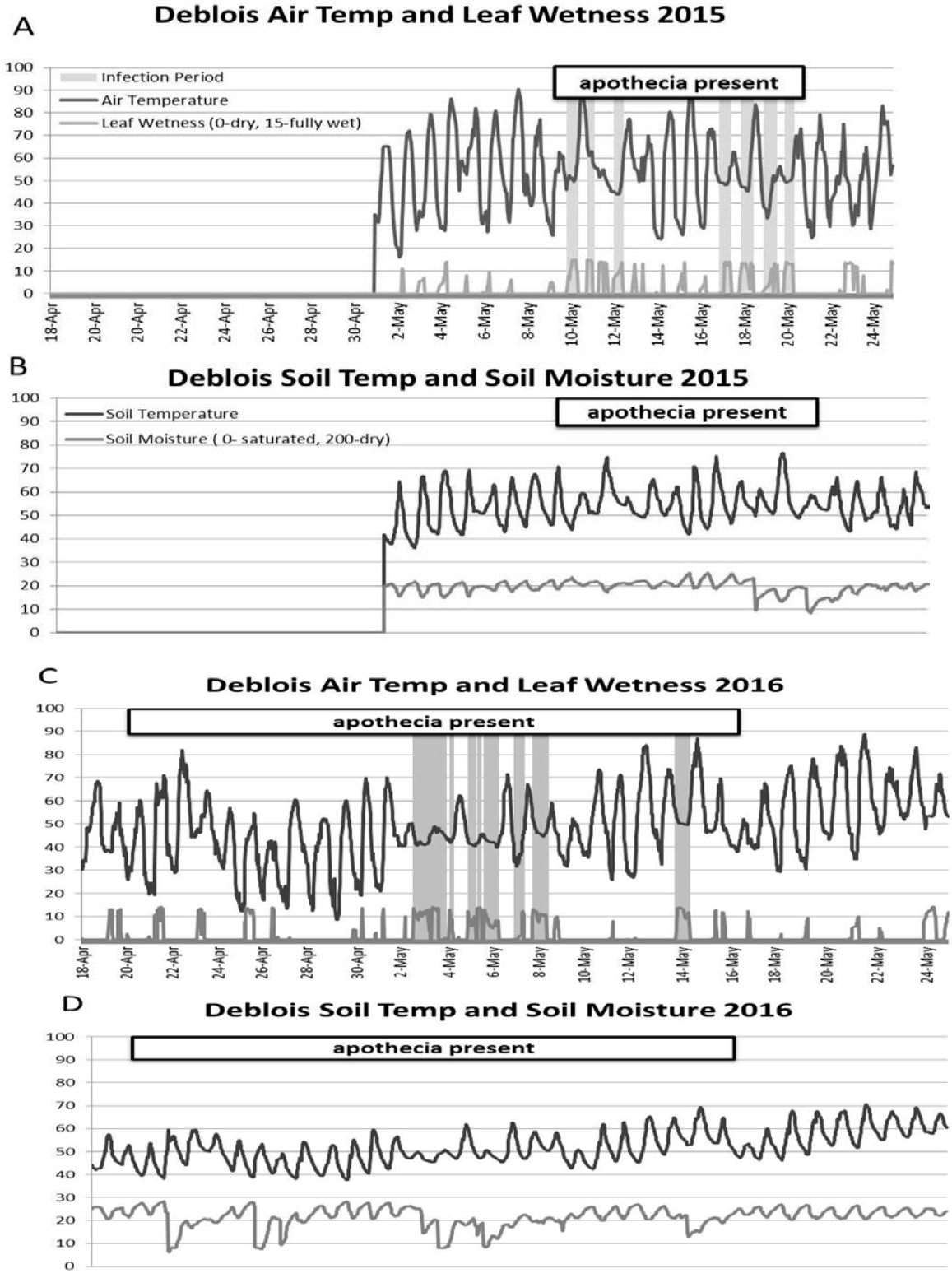


Figure 2. Comparison of infection periods from 2015 and 2016 at the Deblois site. Air temperature and leaf wetness (A and C) were used to determine infection periods (gray bars) for *Monilinia vaccinii corymbosi.* White bars indicate when apothecia were present in fields. Soil moisture and temperature for the same location is presented in B and D.

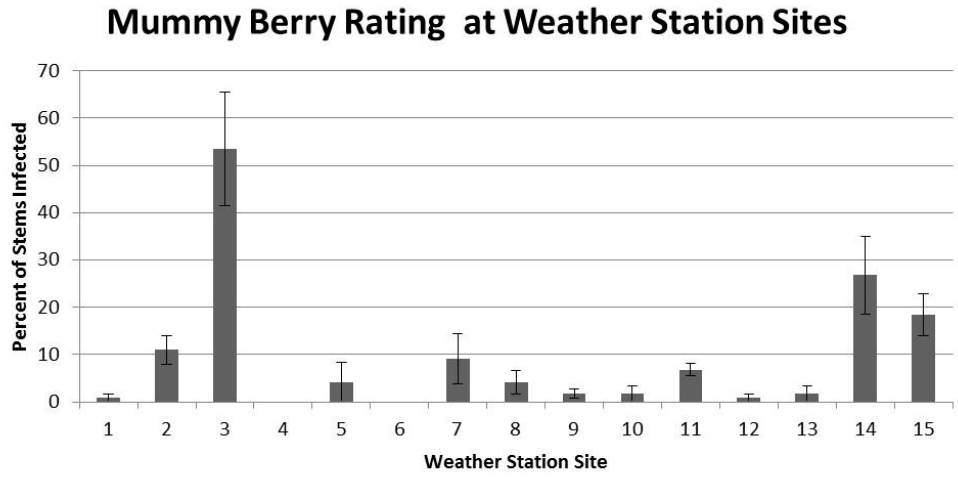


Figure 3. Percentage of stems infected with mummy berry at each of the fifteen weather station sites. Error bars indicate standard error of the mean.

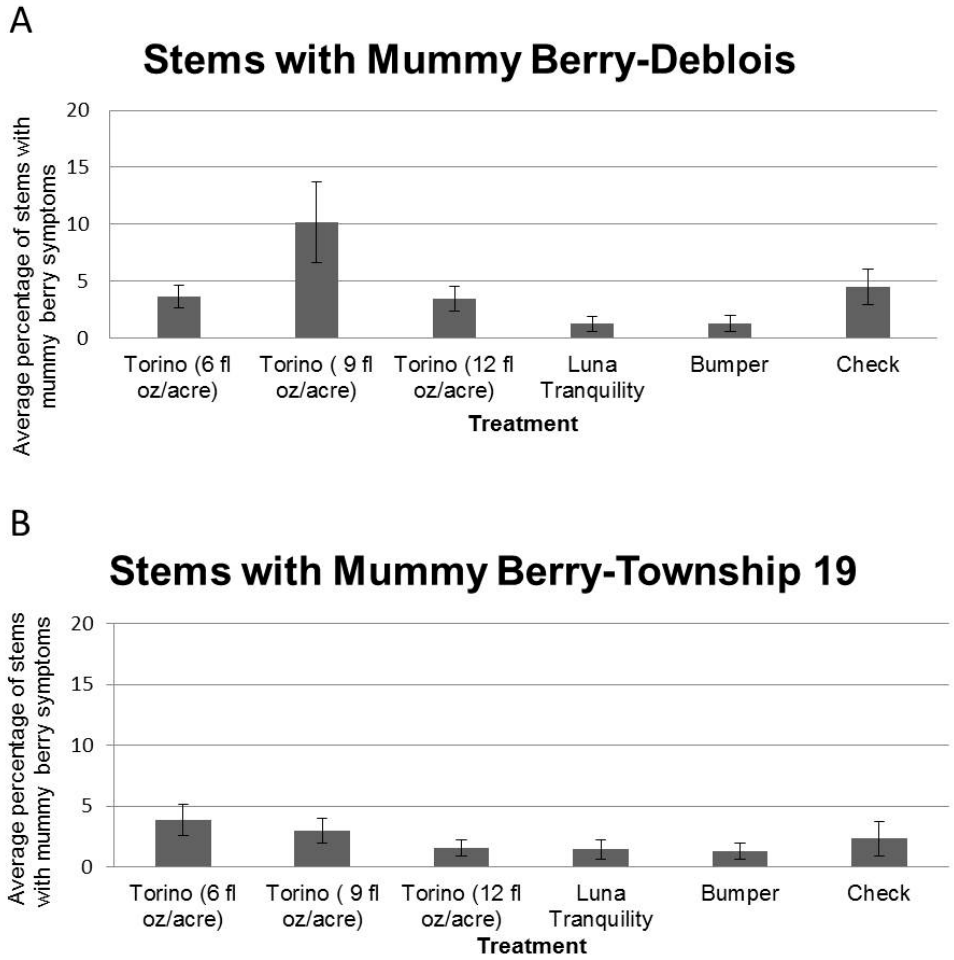


Figure 4. Average percentage of stems with symptoms of mummy berry disease in fungicide trials at A) Deblois and B) Township 19 fields. Error bars represent standard error of the mean of 8 replicates. There were no significant differences among the treatments within the Deblois and Township 19 fields.

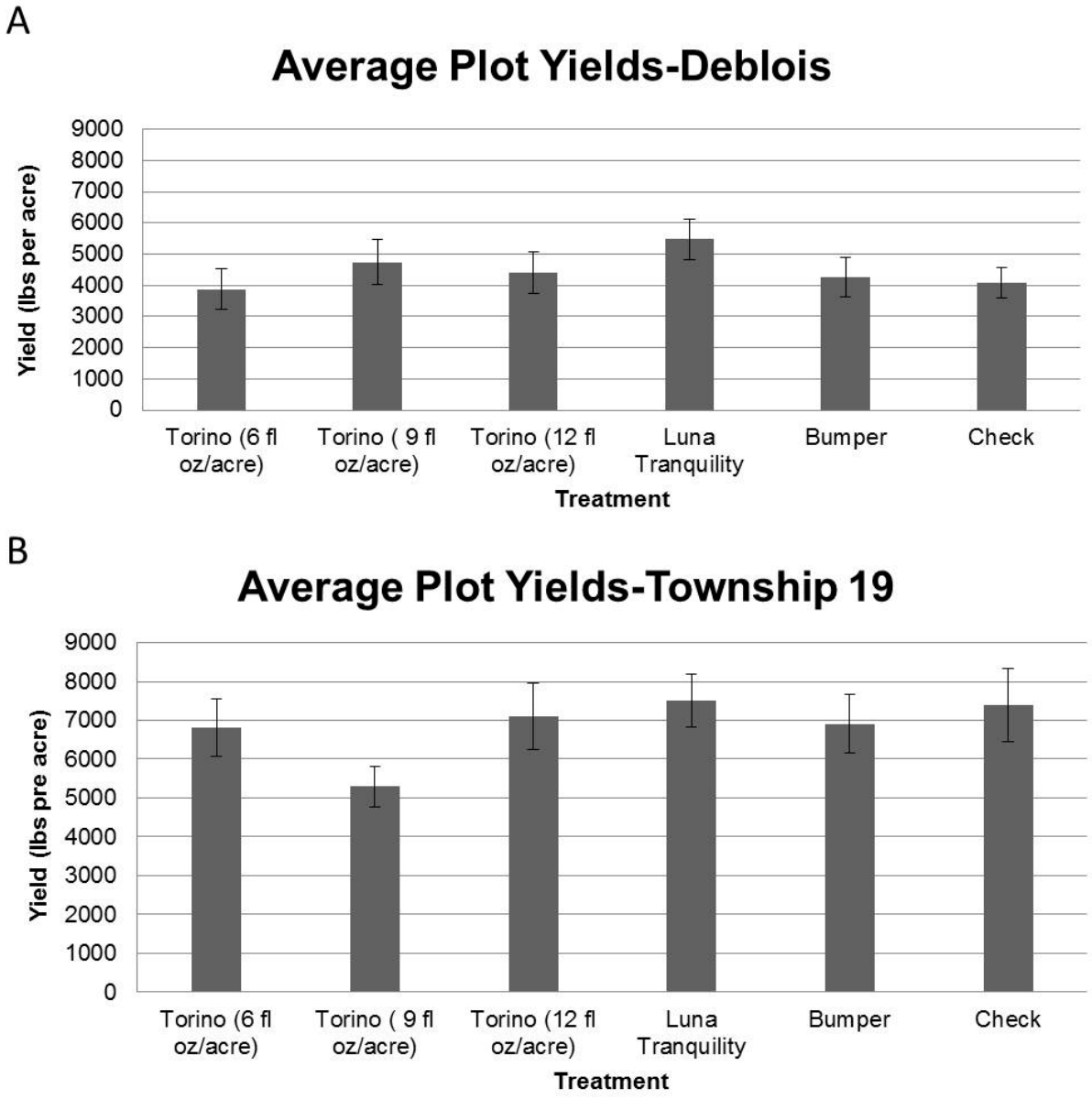


Figure 5. Average blueberry yield in pounds per acre for treatments in fungicide trials at A) Deblois and B) Township 19. Error bars represent standard error of the mean of 8 replicates. There were no significant differences among the treatments within the Deblois and Township 19 fields.

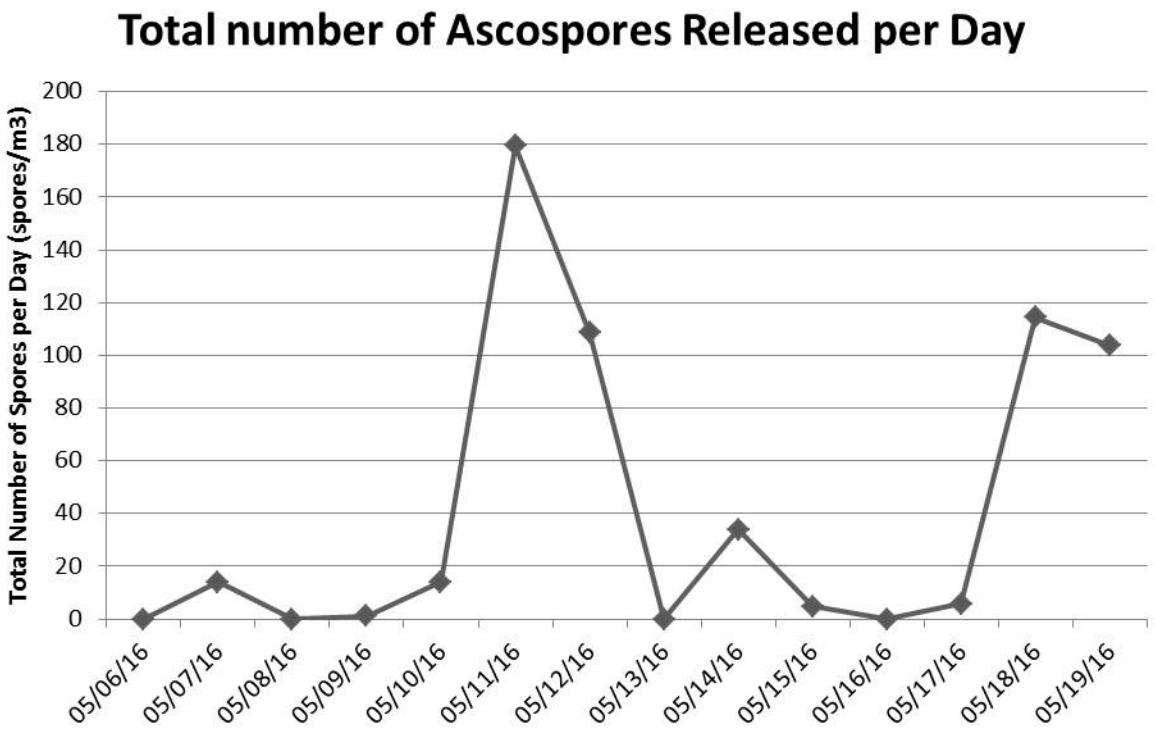


Figure 6. Total number of ascospores released per day from May 6 to May 19, 2016 in a prune field.

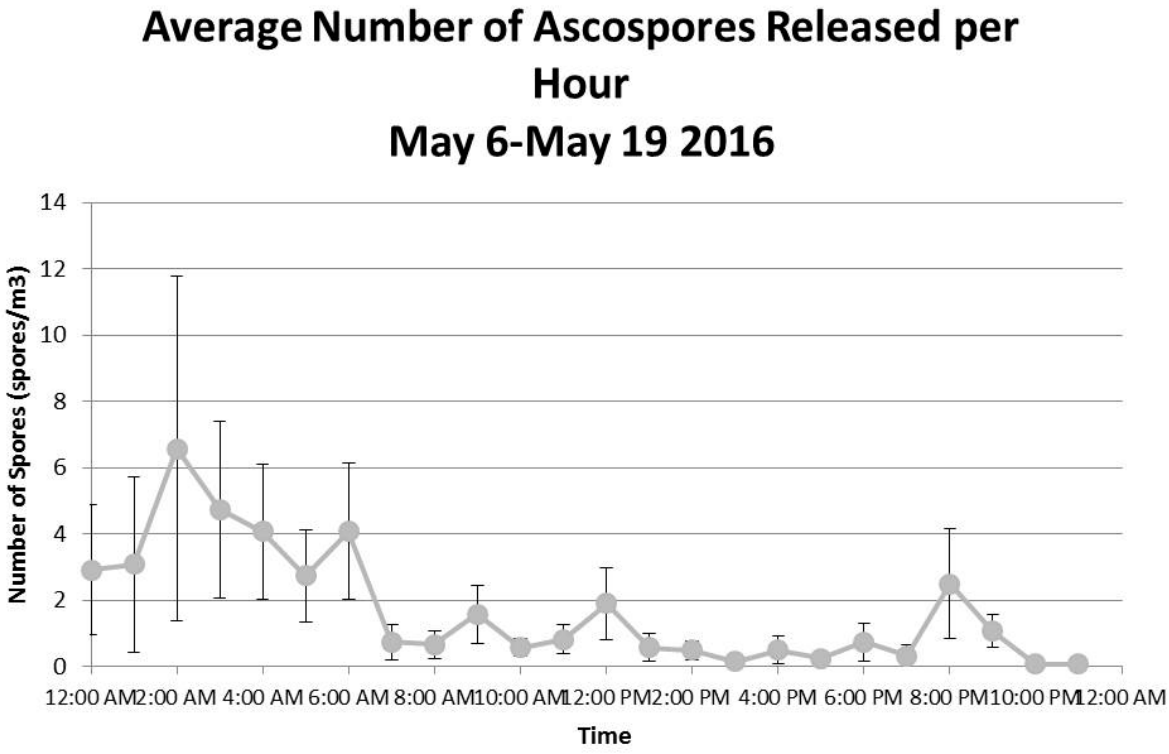


Figure 7. Average number of ascospores released per hour from May 6 to May 19, 2016 in a prune field. Error bars indicate standard error of the mean.

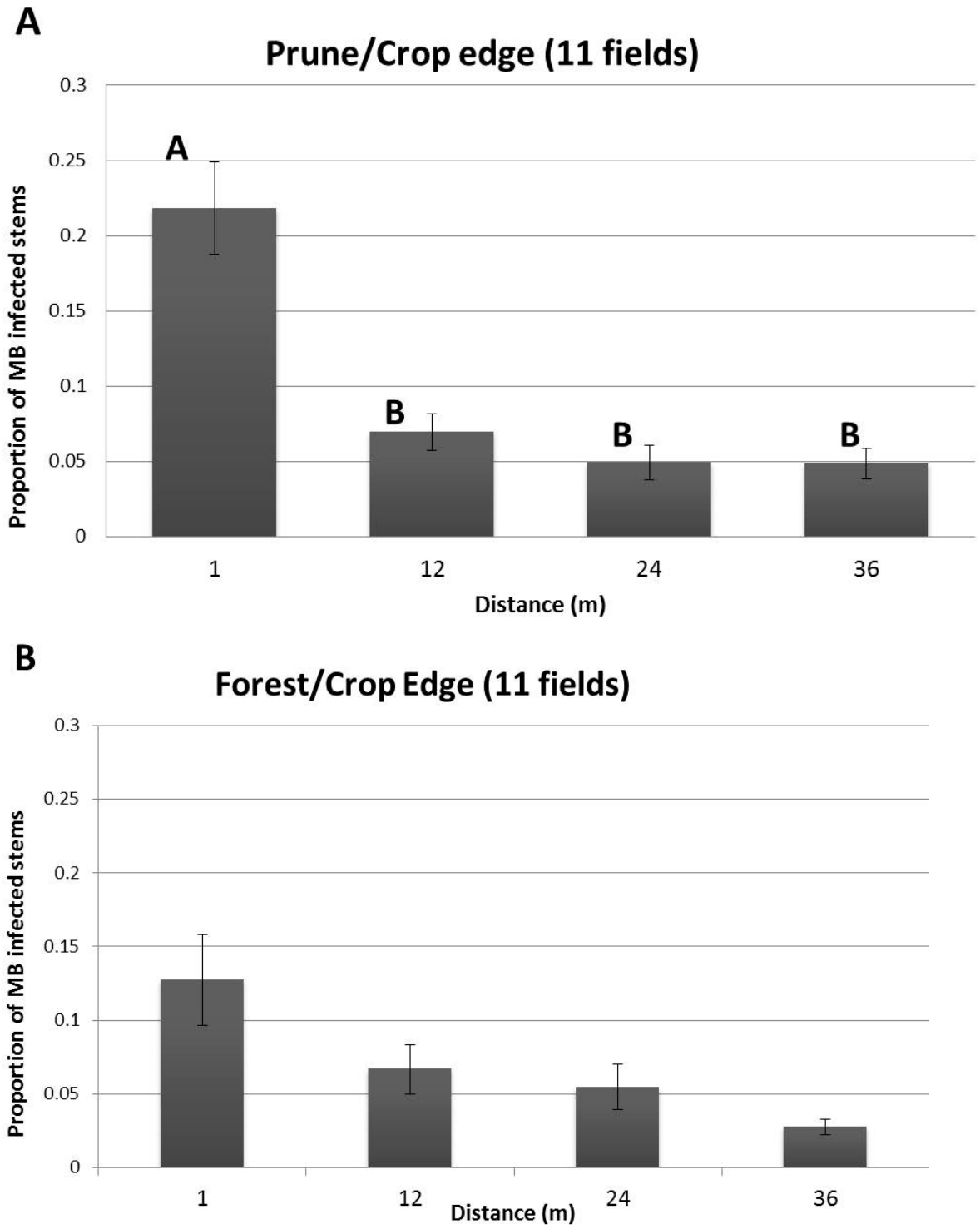


Figure 8. Proportion of stems infected with mummy berry along transects placed at 1, 12, 24, and 36m from the crop field edge in A) fields bordering prune fields and B) fields bordering forest edge. There was a significant difference between treatments in the crop/prune edge (A), bars with different letters were significantly different at $\alpha < 0.05$. There was no significant difference among locations between the crop and forest edge (B).

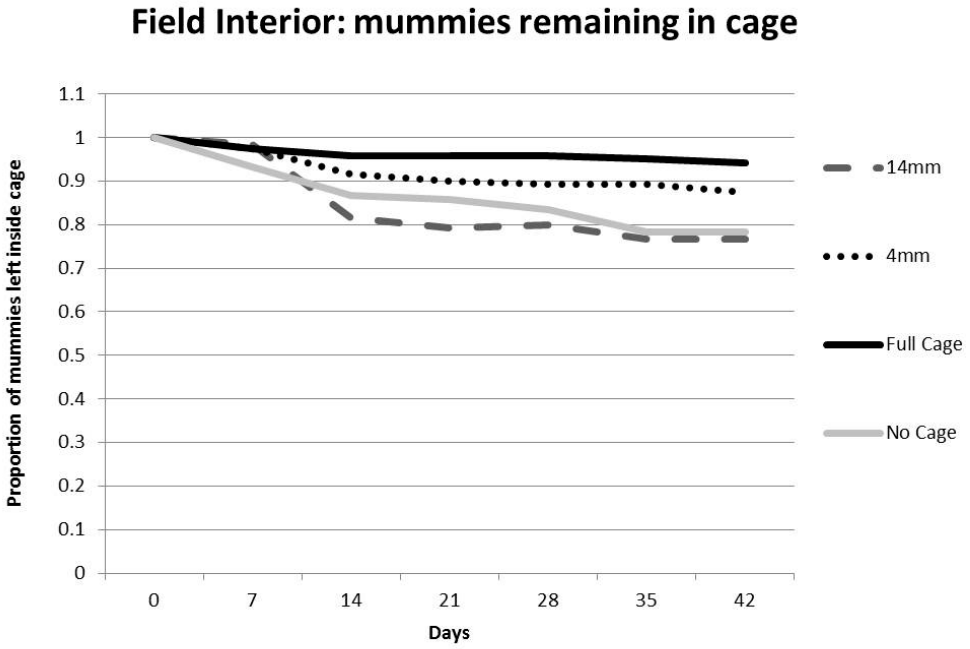


Figure 9. Proportion of pseudosclerotia remaining over time. 30 pseudosclerotia were placed in one of four cage treatments: 14mm mesh cage, 4mm mesh cage, full (1mm) cage, or no cage and counted every 7 days.

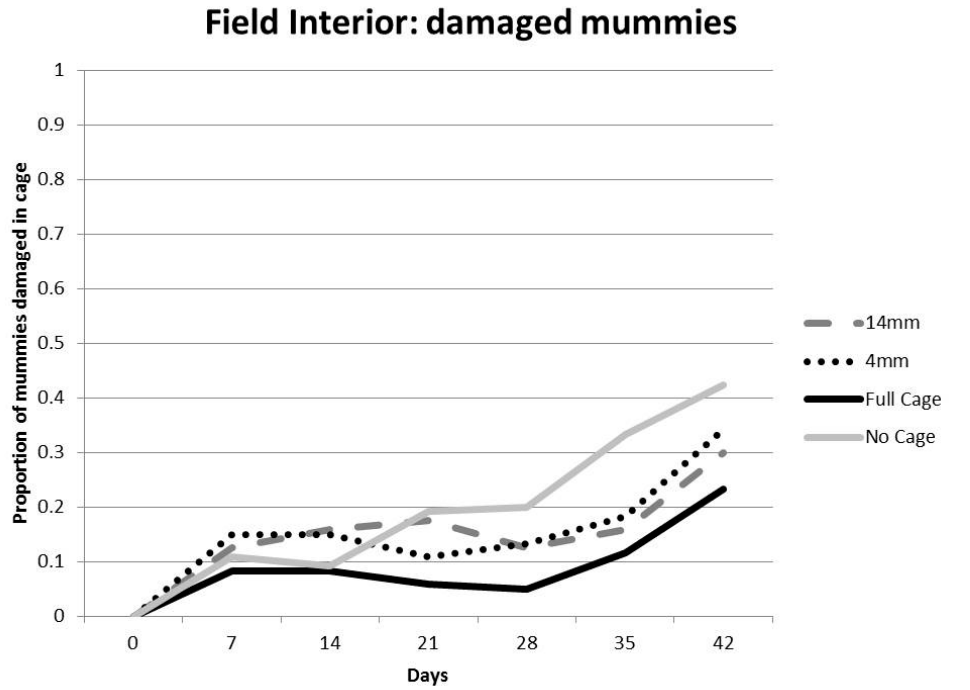


Figure 10. Proportion of mummies (pseudosclerotia) damaged over time. 30 pseudosclerotia were placed in one of four cage treatments: 14mm mesh cage, 4mm mesh cage, full (1mm mesh) cage, or no cage and assessed for damage every 7 days.

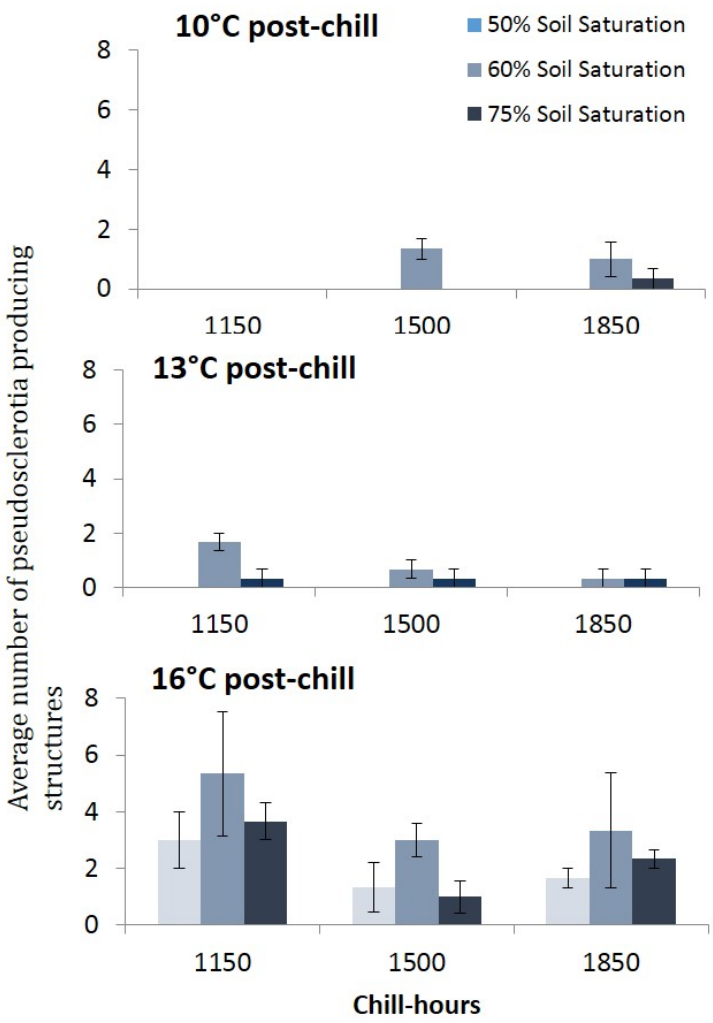


Figure 11. Pseudosclerotia germination under three soil moistures, three chill hours, and three post-chill temperatures in laboratory experiments (2015/2016).

DISEASE MANAGEMENT

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8. TITLE: Lab and field studies of mummy berry and interactions between the fungus and bees.

INTRODUCTION: Wild blueberries are an important economic crop in Maine. Mummy berry (*Monilinia vaccinii-corymbosi* Reade) is a blueberry fungal disease that can cause flower and leaf defoliation. Heavy mummy berry infection can result in up to 80% defoliation which greatly reduces fruit yield. Wind disperses the first ascospores that cause leaf and flower infection and death of the tissue. The second type of spores, conidia, are carried primarily by insects, including pollinators, and perhaps by wind and rain to healthy flowers. The infected flowers go through normal development except the fungus consumes the developing fruit and replaces it with an overwintering structure (pseudosclerotia), commonly called a mummy berry. Dead leaf and flower tissue produce compounds that entice bees to visit the necrotic plant tissue by mimicking the UV signature of a flower and possibly the sugar compounds of nectar. There are many questions on the details of this fungal life cycle and its interactions with bees. We set up a series of experiments and field measurements to fill in some of these gaps.

METHODS:

Mummy berry infection and conidia production

In a field near Hope, ME, 26 clones with mummy berry were selected and rated for mummy berry symptoms approximately twice a week from May 17th to June 6th. Twenty stems, randomly chosen along a transect, were rated for any mummy berry symptoms on each rating date. The percentage of stems with symptoms was noted and whether any conidial sporulation was occurring on the dead tissue. Six stems were randomly chosen from each transect and taken back to the lab, and the total number of flowers and leaves and infected plant parts were counted.

Clones were revisited on July 18 and July 26 to estimate the number of fruit and pseudosclerotia (mummy berries). Twenty random stems along a transect were chosen and on each stem the number of blue fruit, green fruit and mummy berries was counted.

Number of conidia required to infect a flower

Sods of wild blueberry plants were dug up from the University of Maine's wild blueberry farm in Jonesboro, Maine. The plants were held outdoor and mesh cages were erected around them to prevent visits from insects. Forty eight hours prior to the start of the experiment, the sods of blueberries were moved into the lab and all open flowers were cut from the plants. On the day of the experiment, flowers were trimmed until there was only a single newly-opened flower on each stem. These stems with single flowers were cut and placed into tubes of water.

Infected plant material was gathered from a commercial blueberry field in Hope, Maine. Blueberry plant stems with sporulating lesions were cut and the cut ends were wrapped in a damp paper towel and transported back to the lab. The cut stems were placed into beakers of water and the tops of the stems were covered in a large plastic bag to conserve humidity and they were stored in an incubator on a 12/12 hr light and dark cycle at 16˚C and used within 2 to 3 days.

Different sized insect pins (00, 1 and 3) were used to try to transfer different numbers of conidia to flowers. To determine the number of conidia picked up by the pins, each size of pin was used to touch sporulating infected tissue, and then count the number of spores on the pin. There were low numbers of spores so three individual pins of each size were used to touch infected tissue and then the tips were placed in 10 µl of 0.05% Tween 20 solution in a 1.5ml microfuge tube. The 10 µl of spore solution was then counted on a hemocytometer and the estimated average number of spores on each pin size was calculated.

With each size of pin, a pin tip was touched to sporulating tissue and then touched to the end of a stigma of a healthy flower. Stems were chosen with newly opened flowers. All unopened or previously opened flowers were removed the night before. The stem was placed in a small test tube with water and kept in the lab at room temperature. Ten flowers were inoculated with each insect pin size in two experiments. In the first experiment started on May 24, 2016, the flowers were approximately 4 days old when inoculated and were kept in the lab for 4 days before the style was collected and fixed in 0.5ml of 1M KOH in a 1.5ml microfuge tube. In the second experiment, the flowers were one day old and the inoculated flowers were fixed three days after inoculation. Styles were boiled in KOH by autoclaving for 15 min and then stored at room temperature until they could be examined. Styles were carefully removed from tubes, placed on a slide and then stained with aniline blue (0.05% aniline blue in 0.067M K_2HPO_4, pH 9). The styles were examined at 200x under fluorescence using an Olympus BH-2 microscope with a 360nm excitation filter, a 420nm transmission barrier and a UVA filter of 360nm to 380nm. The number of spores observed, germinated, and the number of spores with germ tubes growing down the style were counted.

Number of conidia bees pick up and efficiency of transmission to flowers

Bumble bees used in this study were commercially sourced *Bombus impatiens* (Koppert Biological Systems). The bumble bees were allowed to forage outdoors until 24 hours prior to the start of the experiment when their hive was closed. During the experiment, a single bee was placed into a 50ml centrifuge tube and a single stem of infected plant material was placed into the tube. The end of the tube was plugged with a piece of foam to prevent the bee from escaping. After the bee visited the infected sporulating tissue, the bee was moved to a clean tube with a new foam plug. The exposed bee was given a single stem that contained just a single unexposed flower that opened within the past 48 hours. After the bee visited the flower, the stem was placed into a small tube of water and held at room temperature. The bee was given up to 15 single stems of flowers, or as many as it would visit within an hour. We had 11 bees that visited all 15 flowers, and four bees that visited 1-3 flowers each.

After three days, the flower styles were collected and placed into microfuge tubes containing 1M KOH as describe above. The tissue was fixed, stained and examined as described above.

Number of conidia on bees collected in the field

We collected three different types of bees in a commercial wild blueberry field in Hope, Maine that was heavily infested with mummy berry. We focused on three common bee types found in blueberry fields during bloom: honeybees, bumble bees, and Andrenid bees. This experiment was conducted during May 2016 and had two different collection periods. During the first collection period, we collected 20 each of honeybees, bumble bees, and andrenids. During the second collection period, we collected 20 honeybees, 11 bumble bees, and 21 andrenids. We collected bees immediately after they landed on infected mummy berry plant tissue into 50ml centrifuge vials and put them in a cooler with ice. After bees were collected, they were held in a refrigerator overnight and then processed for conidia the following day.

We attempted to remove all conidia from the bees body by dunking the bees into solution and vortexing them. We added 4 ml of tween to the tubes of refrigerated bees. The bee was vortexed for 30 sec and then 1.4 ml of the vortexed solution was added to a 1.5ml centrifuge tube. The solution was centrifuged for 5 min at 13,000 rpm. 1,260 µl of the supernatant was discarded and 60 µl of 100% glycerol was added to the solution. This final solution of glycerol and pelleted spores was vortexed for 30 sec and then placed into a -80°C freezer.

After all bees and plants were processed, put into solution and frozen, we counted the number of conidia from each bee sample. The samples were defrosted, vortexed for 30 sec, and then two 10 µl drops were placed on a hemocytometer slide. The spores in each sample were subsampled by counting the spores in the each of the four corners plus the middle square of the grid and estimating the total number of spores per sample. If a sample had fewer than 10 spores in the five squares, spores in all squares were counted and the total number of spores was estimated. Each sample was counted twice and the number of conidia was averaged across the two counts.

Bee behavior with infected tissue in the field

We recorded the behavior of honeybees, bumble bees and andrenids during two sampling periods during blueberry bloom. On 18 May, we recorded the behavior of 20 honeybees, 35 andrenids and 20 bumble bees. On 27 May, we recorded the behavior of an additional 14 bumble bees. For each behavioral observation, a single bee was followed around the field until it flew out of our line of sight. During this period, we used a digital recorder to document the number of times it visited healthy flower and leaf clusters, and the number of times it visited infected flower and leaf clusters.

Bee taxa density assessment in the field

On 22 May, we estimated bee taxa density by counting and recording the number of honeybees, bumble bees, andrenids, *Osmia* leafcutting bees, and other native bees observed in m^2 sample quadrats for one minute. Bloom was approximately peak during this sample and the weather conditions were sunny, light wind and 25ºC air temperature. A total of twenty quadrats were sampled throughout the Hope, Maine study site.

RESULTS:

Timing of necrotic tissue and sporulation of conidia

The percentage of stems with infected tissue increased from 23% on May 17th to 67% on May 23rd and then stayed at a similar level between 60 and 70% until the last rating on June 7th (Figure 1). The percentage of sporulating tissue increased from approximately 13% on May 17th to 61% on May 23rd and then started to decrease by May 31st. The presence of conidia appears to be during a set period of time and follows a pattern of increasing to maximum level and then decreasing with time. The dead tissue increases and then remains on the plant until it is excised later and so the incidence of dead tissue does not correspond to the level of sporulation at the end of the sporulation period.

Initially there were more infected leaves (approximately 15 to 25% per stem) than flowers from May 18th to May 26th, but the level of leaf infection did not increase after May 26th (Figure 2). On May 31st and subsequent dates, the percentage of dead flowers was higher than that of leaves. This difference in infection incidence may be due to differences in the susceptibility of the two tissue types during infection periods. Within the field, there was a lot of variation amongst the clones on the level of incidence with clones ranging from 30 to 100% of sampled stems being infected (Figure 3). The level of secondary infection (mummy berries) ranges from 0 to 20% and had no correlation to the level of primary infection.

Number of conidia required to infect a flower

The number of conidia picked up by the smallest insect pin size was on average 104 conidia, and on pin size 1 and 3 were 330 and 437, respectively. In the first experiment, very few conidia were transferred to the flower styles, conidia only germinated and penetrated the style with transfers of spores with the smallest pin, 00, and only 2 out of 10 flowers were infected by germinating spores (Table 1). This may have been due to the older age of the flowers. In experiment 2, flowers were only 24 hours old and more conidia were successfully transferred in all of the treatments. There were over twice as many spores transferred, germinated and approximately twice as many infected flowers with the largest pin size compared to the smallest and medium sized pins (Table 1). As few as one spore observed on the stigma was enough to infect the stigma tip. We did not measure how long the fungal grew down the style or whether they were successful in infecting the ovaries in these experiments.

In experiments of transmission of conidia by bumble bees in the lab, it was difficult to get the bees to visit the infected tissue and then visit healthy flowers. We had 11 bees that visited all 15 flowers, and four bees that visited 1-3 flowers each. The majority of the bees did not transfer conidia to the first flower they visited (Table 2). The first flower had the highest number of transfers at four out of 15 attempts (Table 2). Which of the subsequent flowers was contaminated with conidia appeared to be random (Table 2). Transfer of conidia appeared to be rare and one or more spores could be transferred at a time. Only a few flowers had germinated spores. Two spores germinated in the first flowers, one spore in the second, and one in the tenth (Table 3). This, again, suggests the transfer and infection of flowers by bumble bees is a random event, transfer is unlikely after 13 flowers, and infection is rare.

Number of conidia on bees collected in the field

We found mummy berry conidia on all three bee taxa, but honeybees had significantly more spores (logarithm transformed) on their bodies compared to bumble bees and Andrenid bees appeared to be somewhat intermediate in conidia density ($F_{(2,109)} = 6.409$, $P = 0.002$, Fig.

4). The difference in spores is may be a result of bee behavior as bumble bees spent significantly less time on infected leaf clusters compared to honeybees and andrenids.

Bee behavior in the field

The frequency of visitation to sporulating infected and non-infected leaves and flowers varies between bee taxa (logistic regression analysis, $P = 0.031$). Figure 5 shows that bumble bees visit healthy (non-infected flowers) at a greater rate than honeybees or andrenids, while honeybees and andrenids visit and rest on non-infected leaves at a higher rate than bumble bees. All bee taxa visit sporulating infected flowers at the same frequency, but honeybees and andrenids visit sporulating infected leaves at a significantly higher rate than bumble bees. This increased frequency of visitation to sporulating infected leaves may also be a reason why honeybees have a higher abundance of spores on their bodies than bumble bees.

Bee taxa density in the field

Figure 6 shows the bee taxa specific densities that occurred in the Hope blueberry field in 2016 where the mummy berry and bee interaction studies were conducted. It can be seen that the majority of foraging bees in the field were honeybees. Thus, the potential for transmission was high only if the number of conidia per bee is directly related to floral transmission and infection, and this is not yet known.

CONCLUSIONS: Timing of bloom and production of conidia by the *Monilinia* fungus on infected tissue need to coincide to increase the chance of infection. As found in prior studies, the level of primary infection is not similar to that of secondary infection. As found in previous studies, flowers within one day of opening are more likely to be infected by conidia, but transfer of conidia is difficult even with high numbers of conidia present. Transfer of conidia by bumble bees is difficult and it appears to be a random event in which the flower is contaminated subsequent to the bee becoming contaminated with conidia. Previous studies have documented bees visiting sporulating plant tissue, but our study has shown that different bee taxa visit infected plant parts at different rates, especially leaves. Bumble bees spent less time on the infected plants and visited them less frequently compared to honeybees and andrenids. This behavioral difference might explain why bumble bees carry fewer conidia compared to honeybees (almost two orders of magnitude fewer spores). Although we did not record how long each bee spent on infected plants, visual observations suggest bumble bees spend very little time on these infected plants compared to honeybees. Honeybees were often seen resting, grooming and probing infected plant tissue. Further studies are needed determine if this dynamic among bee taxa in relation to acquiring mummy berry conidia is consistent between years and to better understand and predict the role bees serve in mummy berry disease epidemiology.

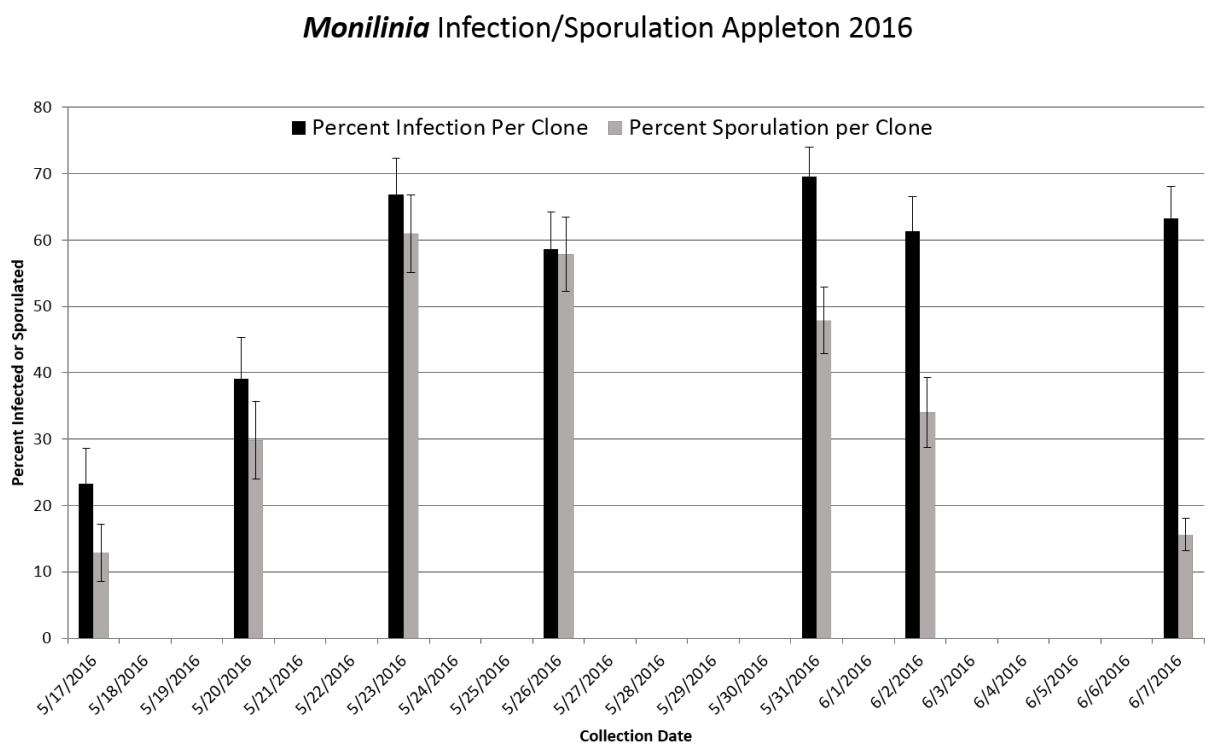


Figure 1. Percentage of infected and sporulating stems over time. Infection and sporulation data are averages of individual ratings of 26 wild blueberry clones.

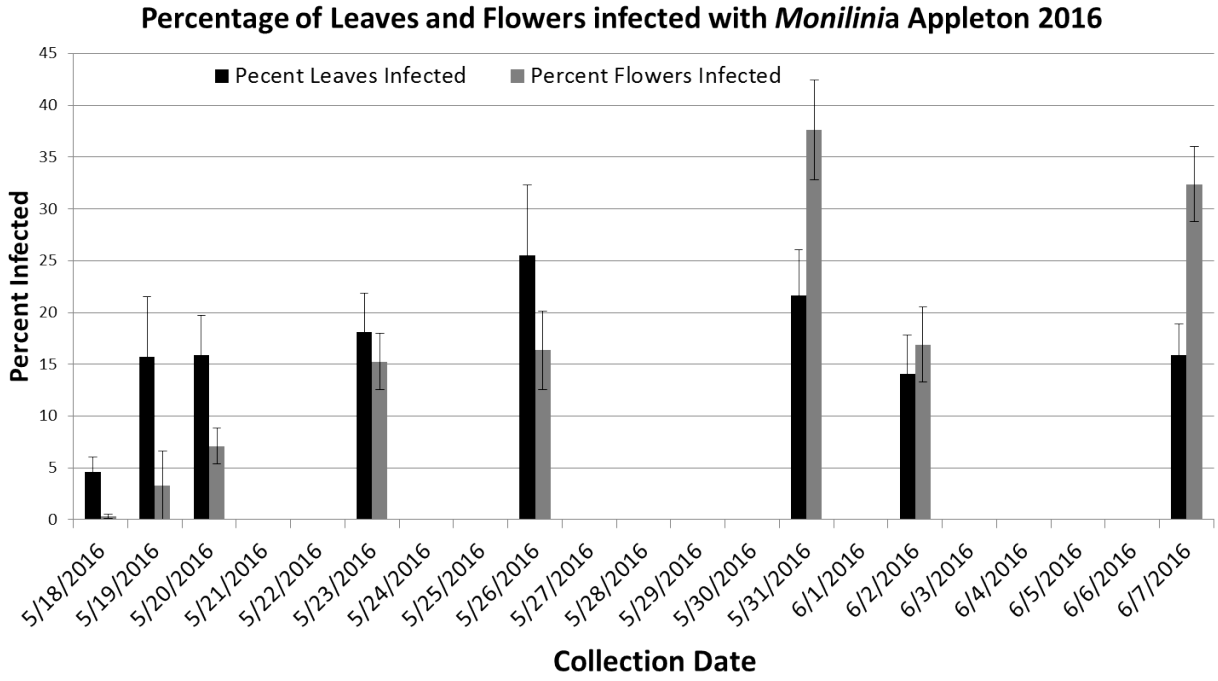


Figure 2. Percentage of infected leaves and flowers per stem over time from averages across 26 wild blueberry clones.

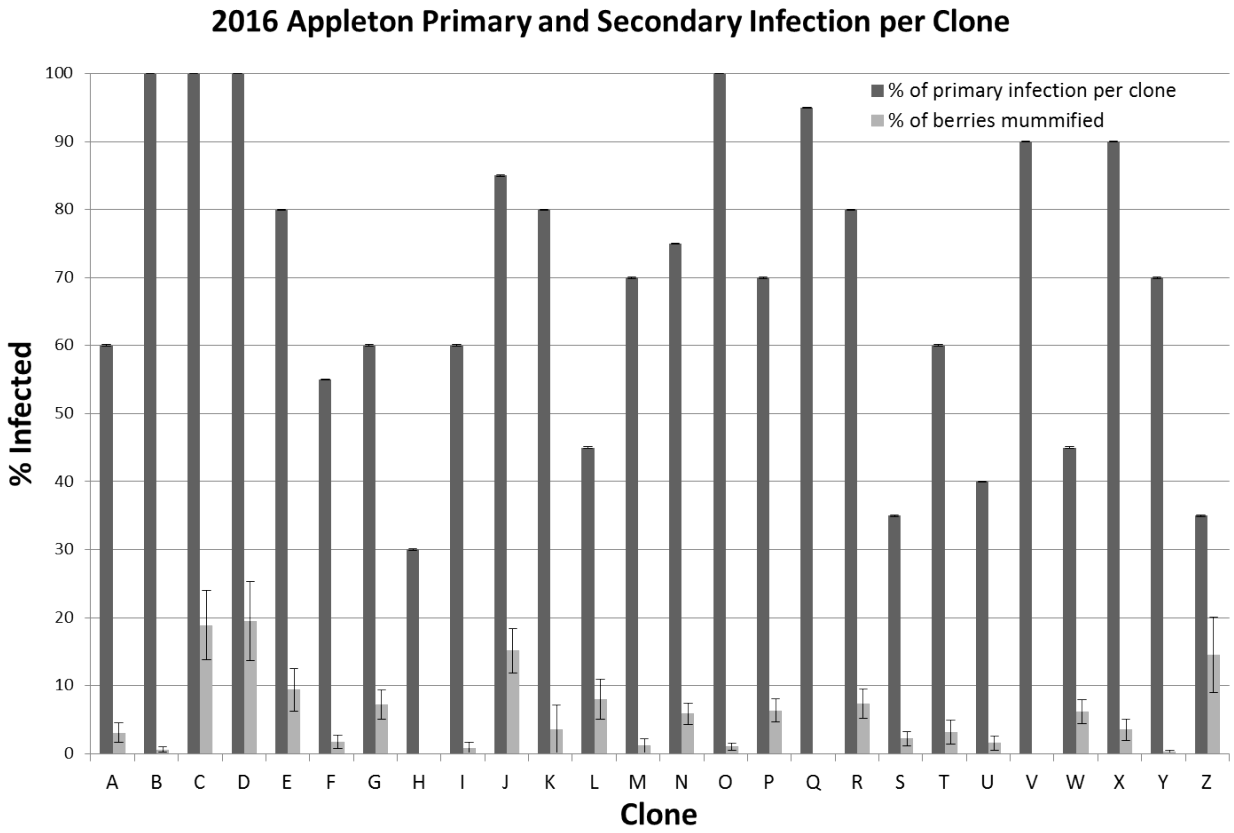


Figure 3. Percentage of primary infection (May 31, 2016) measured as incidence of disease and secondary infection (July 28, 2016) measured as percentage of mummy berries of total berries per clone.

Table 1. Results of inoculation of flowers with *Monilinia* conidia using different sized insect pins.

Date Flowers Infected	Date Style Fixed	Needle Size	Average Number of Spores Observed	Average Number of Spores Germinated	Proportion of Flowers Infected
Experiment 1 5/24/2016	5/28/2016	00	0.22	0.22	0.22
		1	0	0	0
		3	0.80	0	0
Experiment 2 6/1/2016	6/3/2016	00	0.80	0.20	0.10
		1	1	0.40	0.20
		3	2.7	1.1	0.40

Table 2. The number of times *Monilinia* conidia were transferred to flower stigmas after being visited by a bumble bee that first visited *Monilinia* infected tissue. Flower number are in the subsequent order of visits by the bumble bee.

	Flowers visited in subsequent order by a contaminated bumble bee														
Number of Spores on Flower Stigma	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
0	12	14	14	12	11	11	10	11	10	11	9	9	9	11	10
1	**1**	**1**	**1**	**1**	**1**	0	0	**1**	**1**	0	**1**	**2**	0	0	0
2	**1**	0	0	0	0	**1**	0	0	0	**1**	0	0	0	0	0
3	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0
>3	**2**	0	0	0	**1**	**1**	0	0	**1**	0	0	0	**1**	0	0
Total number of flowers contaminated	**4**	**1**	**1**	**1**	**2**	**2**	0	**2**	**2**	**1**	**1**	**2**	**1**	0	0

Table 3. The number of times *Monilinia* conidia germinated on flower stigmas on flowers visited subsequent to the bumble bee first visiting *Monilinia* infected tissue. Flower number are in the subsequent order of visits by the bumble bee.

	Flowers visited in subsequent order by a contaminated bumble bee														
Number of Spores Germinated on Flower Stigmas	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
0	14	14	15	13	13	13	10	13	12	11	10	11	10	11	10
1	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0
2	**1**	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
>3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total number of flowers with germinated spores	**2**	**1**	0	0	0	0	0	0	0	**1**	0	0	0	0	0

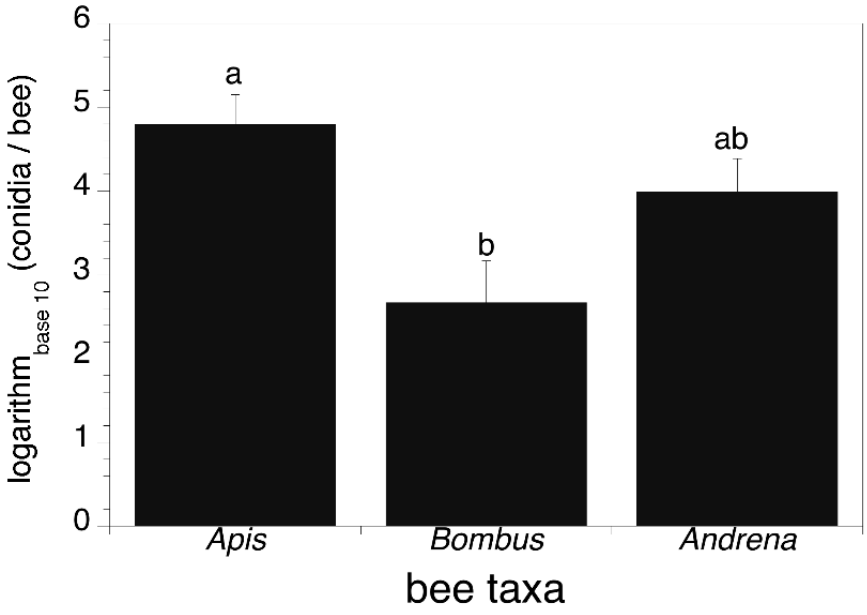


Figure 4. Number of mummy berry conidia found on the bodies of field collected bees represented by honeybees, bumble bees and Andrenid bees. Similar letters shared among bee taxa suggest no significant difference ($P > 0.05$).

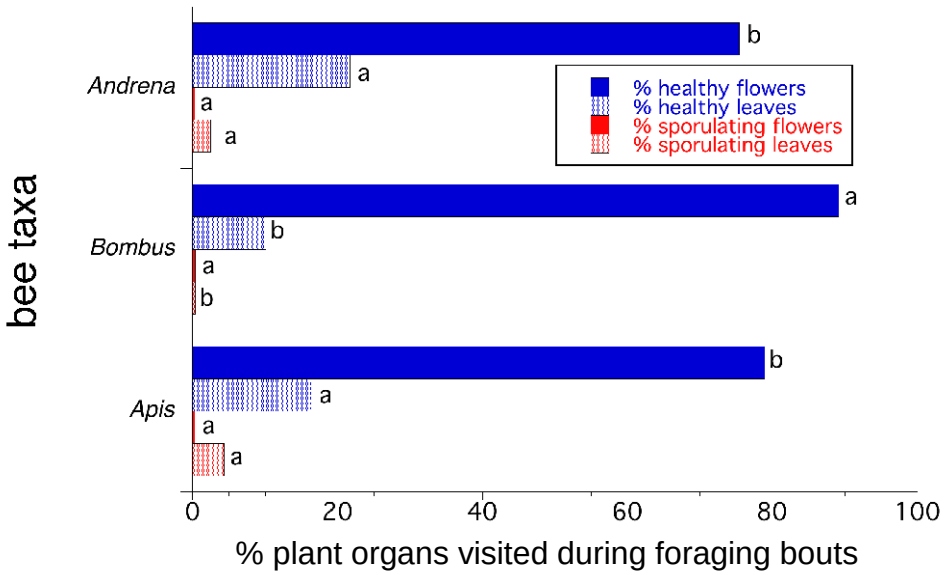


Figure 5. Frequency of visitation to healthy flowers, healthy leaves (bees rest and groom on leaves), sporulating flowers, and sporulating leaves during bee foraging bouts for honeybees, bumble bees and Andrenid bees.

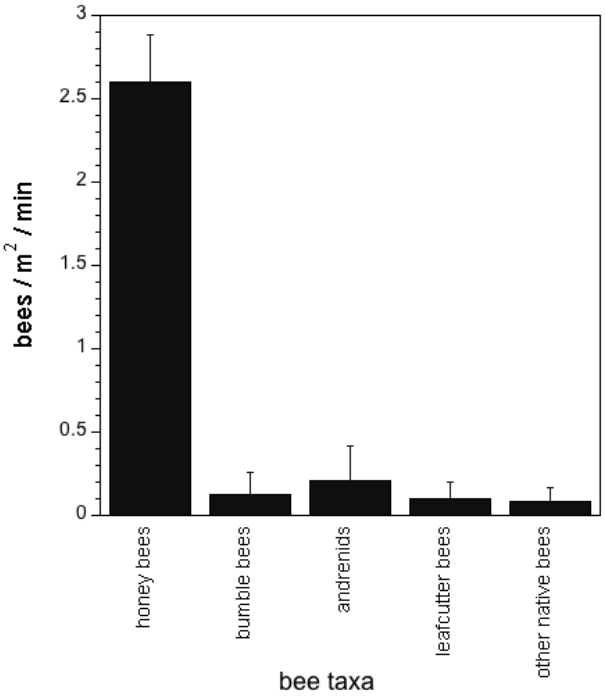


Figure 6. Average bee taxa densities on May 22, 2016 in the Hope, Maine study field.

WEED MANAGEMENT

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

9. TITLE: Pre-emergence and post-emergence applications of Zeus Prime XC for weed control in wild blueberry fields, 2016.

METHODS: Zeus Prime XC is a product which has a general blueberry label in the U.S., but has not been tested in lowbush blueberry. It is a Group 14 herbicide with carfentrazone and sulfentrazone as the active ingredients; we currently do not have any Group 14 products registered for lowbush blueberry, so Zeus has the potential to be a good fit for a resistance management program. In spring 2016 we set up a trial in two prune fields - at Blueberry Hill Farm in Jonesboro and at Wymans' No-Name Lot in Wesley - to test the effects of Zeus Prime XC at different rates and timings on blueberry and weeds, and to compare it to tank mixes with Solida (rimsulfuron, Group 2) as well as Solida plus Aim (carfentrazone alone).
A Randomized Complete Split Block Design was replicated four times on each site with 6'x40' plots split in half, with Velpar 1 lb/a applied randomly to one half of each block on 12 May 2016. The main treatments were as follows:

1. untreated check;
2. Zeus Prime XC 7.7 oz/a with COC 1% v/v **pre-emergence** *(Zeus Low)*;
3. Zeus Prime XC 12.5 oz/a with COC 1% v/v **pre-emergence** *(Zeus Mid)*;
4. Zeus Prime XC 15.2 oz/a with COC 1% v/v **pre-emergence** *(Zeus High)*;
5. Zeus Prime XC 7.7 oz/a with COC 1% v/v **pre-emergence** plus Zeus 7.5 oz/a with COC 1% v/v in **fall 2016 after leafdrop** *(Zeus pre+fall)*;
6. Zeus Prime XC 7.7 oz/a with COC 1% v/v **pre-emergence** plus Solida 4 oz/a **pre-emergence** *(Zeus+Solida pre)*;
7. Zeus Prime XC 7.7 oz/a with COC 1% v/v **pre-emergence** plus Solida 4 oz/a **post-emergence** *(Zeus pre+Solida post)*; and
8. Aim 2 oz/a **pre-emergence** plus Solida 4 oz/a **post-emergence** *(Aim+Solida)*.

The pre-emergence treatments were applied on 12 May 2016, the post-emergence treatments were applied on 10 June 2016, and the fall treatment was applied on 1 November 2016. It should be noted that this trial was conducted at the request of the FMC Corporation Agricultural Products Group and we applied the herbicides they sent, of which the Zeus Prime XC was packaged under a different name (Spartan Charge); however, the % active ingredients and EPA registration numbers were identical and so for the purposes of reporting we are using the tradename Zeus Prime XC. It should also be noted that Blueberry Hill Farm applied DAP fertilizer at 150 lb/a on 20 May 2016 in Jonesboro only, and the Wesley site did not receive fertilizer.

The plots were evaluated for wild blueberry cover and phytotoxicity, broadleaf weed cover and grass cover on 21 June and 1-2 August. Cover data were determined by using the Daubenmire Cover Class system converted to percent; phytotoxicity data were gathered using a scale of 0-10 (0=no damage, 10=100% damaged/dead) converted to percent. Upon preliminary analysis, we found a significant difference in overall broadleaf weed cover and grass cover

between the two sites due to site differences (see Table 1 for soil conditions); therefore, the sites were analyzed individually. The treatments were compared to each other with Velpar treatments and no-Velpar treatments analyzed separately using Tukey's tests (α=0.05). T-tests were also performed for significant differences between no-Velpar and Velpar for each main treatment (α=0.05). Finally, Zeus alone at the four rates (0 oz/a, 7.7 oz/a, 12.5 oz/a, 15.2 oz/a) and Zeus plus Velpar (with "0" being Velpar alone) were analyzed for the effect of rate on either broadleaf weed cover or grass cover and the nature of the relationship (linear, quadratic, etc.). Significant relationships were then analyzed using either a linear or polynomial regression (α=0.05).

Table 1. Differences in soil conditions between the two trial areas Jonesboro and Wesley.

	pH	**% OM**	**% sand**	**% silt**	**% clay**	**Soil texture**
Jonesboro	4.8	11.6	68	26	6	sandy loam
Wesley	5.0	15.3	48	42	10	loam

RESULTS:

All-treatment comparisons

When Tukey's tests were performed upon the data, it became apparent that some treatment differences were not captured due to variability in the data; this was because the sites had to be analyzed separately and four Degrees of Freedom were lost. Also, the Zeus Prime XC pre-emergence+fall treatment only reflects the results from the pre-emergence application, as the fall application was applied after leaf drop and so effects from the spring-fall combination will not be seen or discussed until next year. Nevertheless, some trends emerged.

In Jonesboro, there were no significant differences among treatments without Velpar at either evaluation on wild blueberry cover (Figure 1). With Velpar, in June the check and Zeus pre+fall had significantly higher cover compared to Zeus pre+Solida post but by August, these treatments were no longer different. In Wesley, there were also no significant differences in wild blueberry cover among treatments without Velpar at either evaluation (Figure 2). With Velpar, in June Velpar alone and Zeus pre+fall had higher blueberry cover than Zeus+Solida pre but by August the treatments were no longer different.

Phytotoxicity overall at both sites was low, with no treatment resulting in unacceptable injury to wild blueberry. In Jonesboro, phytotoxicity was only observed in June; without Velpar, the most phytotoxicity was observed in the Aim+Solida treatment and was significantly higher than all other treatments except Zeus pre+Solida post (Figure 3, Photos 1 and 3A). Zeus pre+Solida post and Zeus+Solida pre also had significantly more phytotoxicity than the remainder of the treatments, which had no phytotoxicity. With Velpar, Aim+Solida and Zeus pre+Solida post also had the most phytotoxicity in June and were significantly higher than all other treatments except Zeus+Solida pre and Zeus High. Again, no phytotoxicity was observed in August. Phytotoxicity in June in Wesley followed a similar trend, with Aim+Solida having the most phytotoxicity and being significantly higher than all other treatments except Zeus pre+Solida post (Figure 4). However, with Velpar the Aim+Solida treatment was only significantly higher than the check or Velpar alone. In August no-Velpar treatments, phytotoxicity was observed as minor chlorosis in the Zeus Low treatment, as drift from Wyman's herbicide spray in the Zeus+Solida pre treatment, and stunting in the Aim+Solida treatment (Photo 3B), but there were no differences. With Velpar, Aim+Solida also had stunting but there were no significant differences (Photo 3B).

Figure 1. Wild blueberry cover in Jonesboro following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida (only significant differences at α=0.05 denoted by different letters).

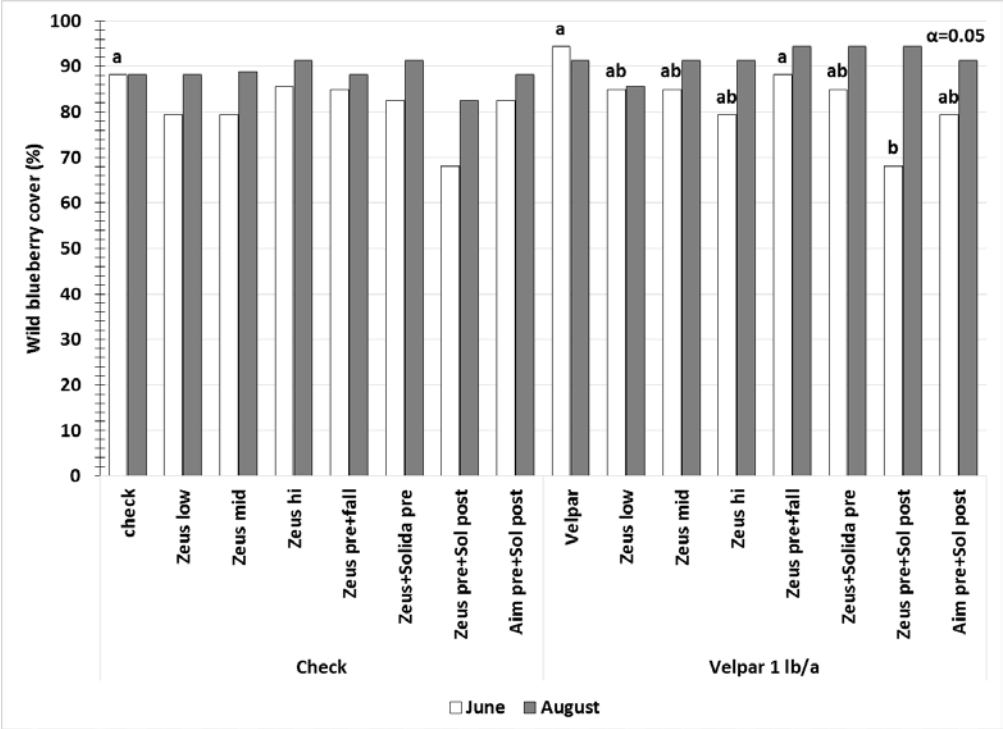


Figure 2. Wild blueberry cover in Wesley following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida (only significant differences at α=0.05 denoted by different letters).

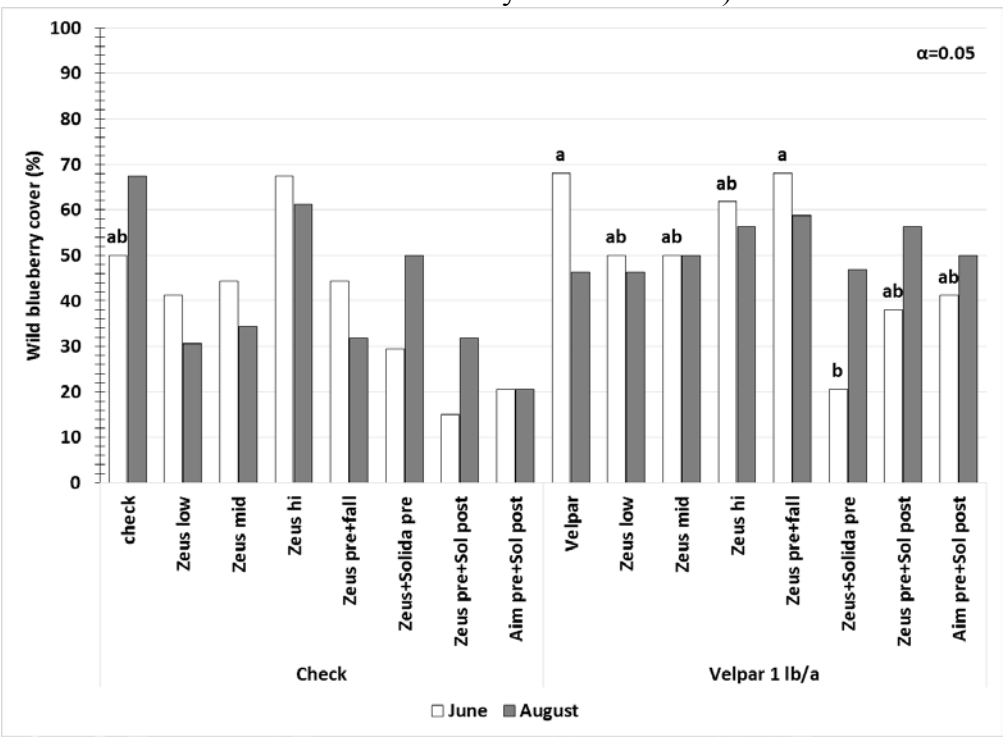


Figure 3. Wild blueberry phytotoxicity in Jonesboro following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida (only significant differences at α=0.05 denoted by different letters).

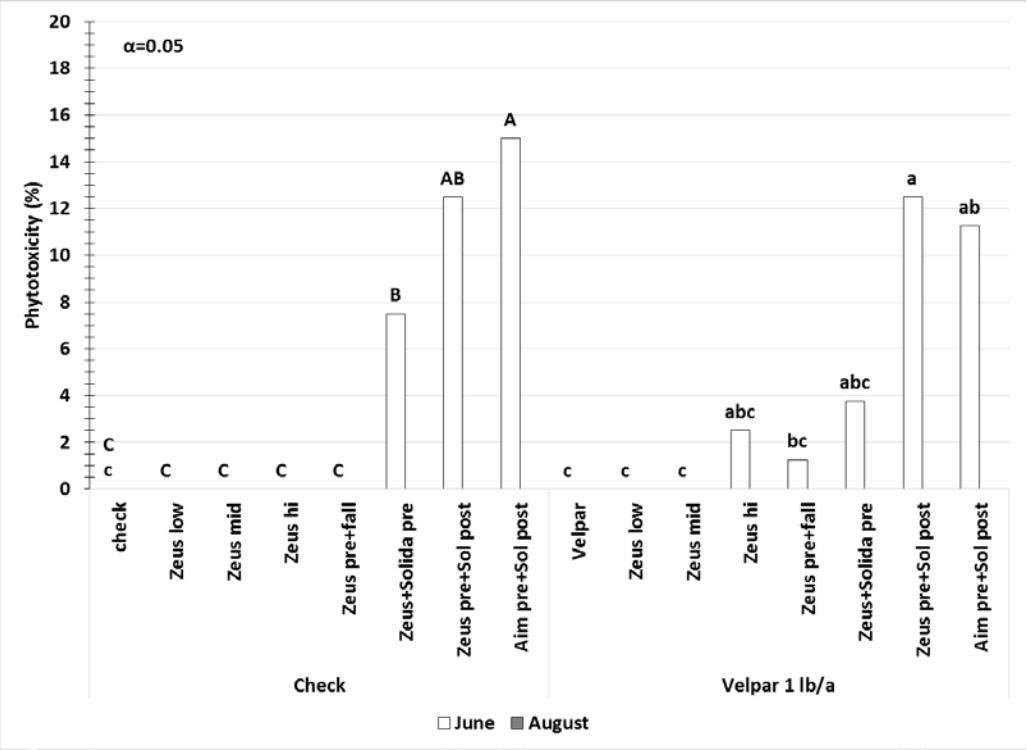


Figure 4. Wild blueberry phytotoxicity in Wesley following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida (only significant differences at α=0.05 denoted by different letters).

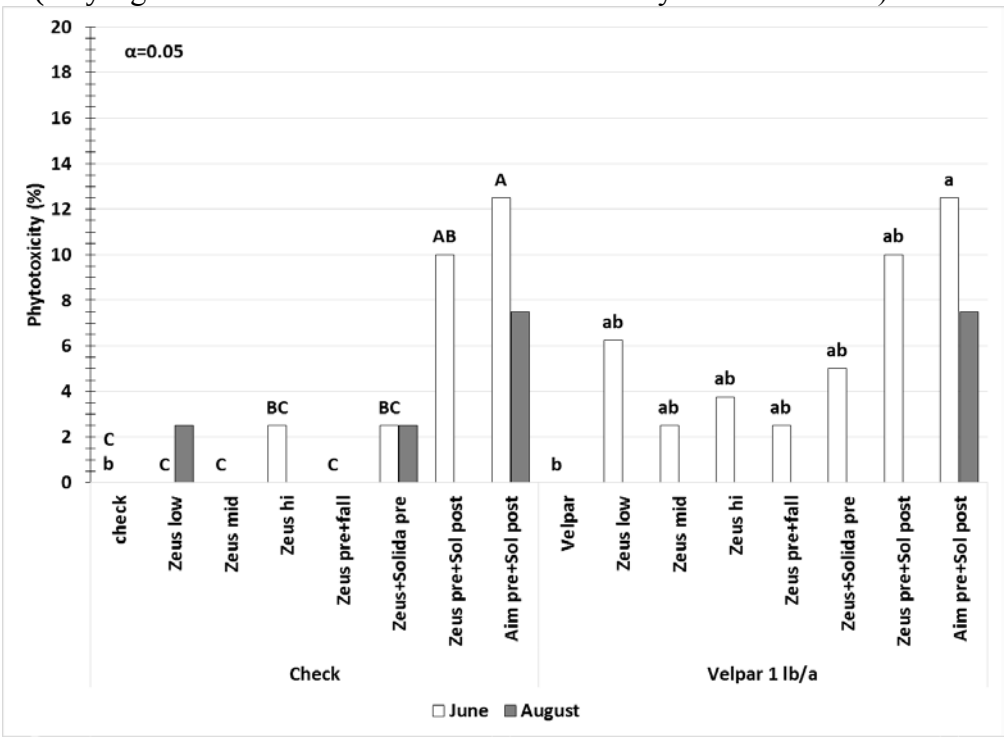


Photo 1. June phytotoxicity as stunting in the Zeus pre+Solida post treatment in Jonesboro (with Velpar, foreground).

The Wesley site had many more broadleaf weeds than Jonesboro. Dominant species included red sorrel (*Rumex acetosella*), bladder campion (*Silene vulgaris*), ragweed (*Ambrosia artemisiifolia*), lesser stitchwort (*Stellaria graminea*) and goldenrods (*Solidago* spp.), as opposed to Jonesboro which had species such as wild lettuce (*Lactuca canadensis*), tall blue lettuce (*Lactuca biennis*), violets (*Viola* spp.), bunchberry (*Cornus canadensis*) and goldenrods. In June in Jonesboro, the check was significantly higher compared to all other treatments, which were not different from each other, with or without Velpar (Figure 5). In August there were no differences among treatments without Velpar, but with Velpar the check remained higher than all other treatments except Velpar alone. In Wesley in June, there were no differences among treatments without Velpar, even though the Zeus pre+fall treatment had over 60% broadleaf weeds while Zeus High and Zeus+Solida pre had 23% (Figure 6). With Velpar, the check had significantly more broadleaf weed cover compared to Zeus High and the combination treatments, but the treatments were not different from each other or Velpar alone. In August, there were no differences among treatments with or without Velpar.

In Wesley, on-site visual observation showed Zeus pre+Solida post and Aim+Solida initially controlled red sorrel and bladder campion, but Zeus alone did not control bladder campion. By August, all treatments contained bladder campion, whether with or without Velpar. In August, it was also visually noted that Zeus High and Zeus+Solida controlled cow vetch (*Vicia cracca*), while Aim+Solida controlled ragweed and to some extent, goldenrods.

In Jonesboro, it was visually observed in June that control of tall/wild lettuce and fineleaf sheep fescue (*Festuca filiformis*) was driven by Velpar.

Figure 5. Broadleaf weed cover in Jonesboro following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida (only significant differences at α=0.05 denoted by different letters).

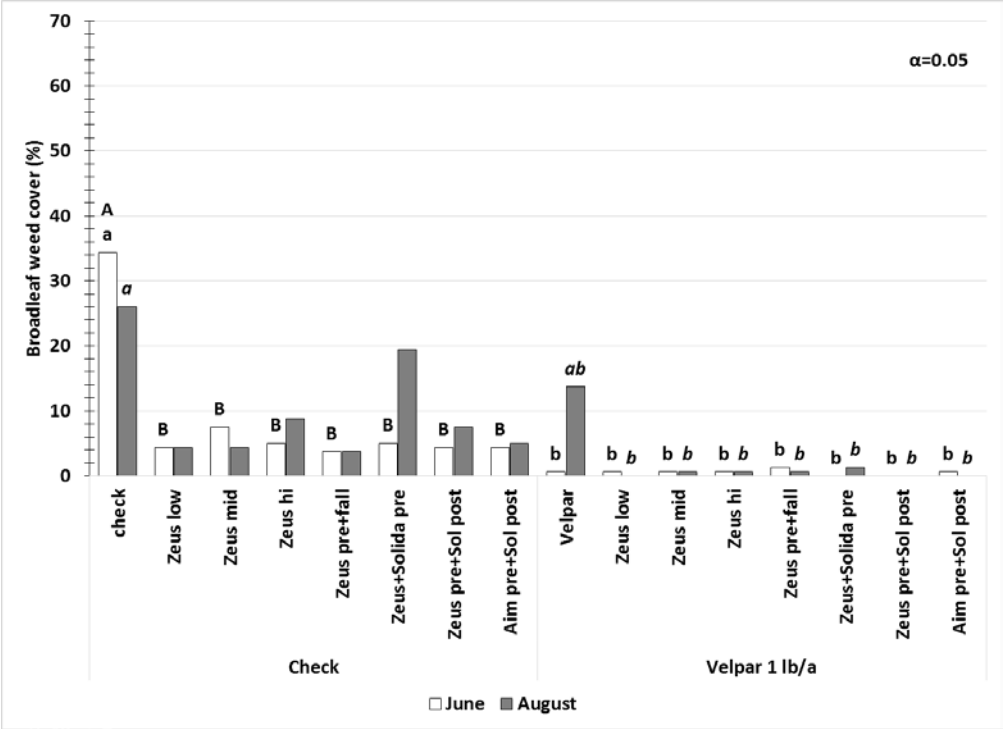
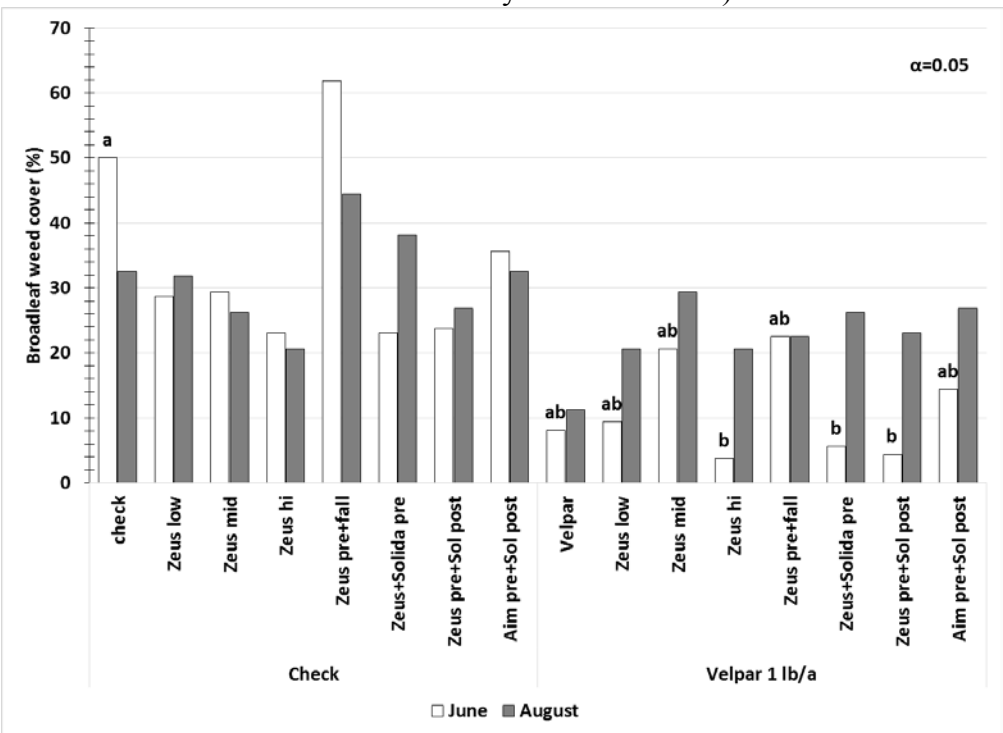


Figure 6. Broadleaf weed cover in Wesley following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida (only significant differences at α=0.05 denoted by different letters).



The Jonesboro site had many more grasses than Wesley. Dominant species included wild oatgrass (*Danthonia spicata*), Kentucky bluegrass (*Poa pratensis*), Canada bluegrass (*Poa compressa*), fineleaf sheep fescue and colonial bentgrass (*Agrostis capillaris*), compared to Wesley which had mostly witchgrass (*Panicum capillare*) with some yellow foxtail (*Setaria pumila*). In Jonesboro, there were no differences among treatments without Velpar at either evaluation; again, even though Zeus pre+fall was 50-60% cover compared to the three other treatments which were at 5-9% (Figure 7). Adding Velpar reduced grasses, but there were still no differences at either evaluation (Photo 2). In June there were little to no grasses in Wesley (Figure 8), most likely because witchgrass and yellow foxtail emerge later in the summer than several of the grass species at Jonesboro. The grass cover observed in August was primarily due to witchgrass. There were no significant differences among treatments with or without Velpar at either evaluation.

Photo 2. Treatment block at the Jonesboro site, looking across main treatments. No Velpar is to left, with Velpar is to right; note reduction in grasses.

Figure 7. Grass cover in Jonesboro following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida (α=0.05, no significant differences).

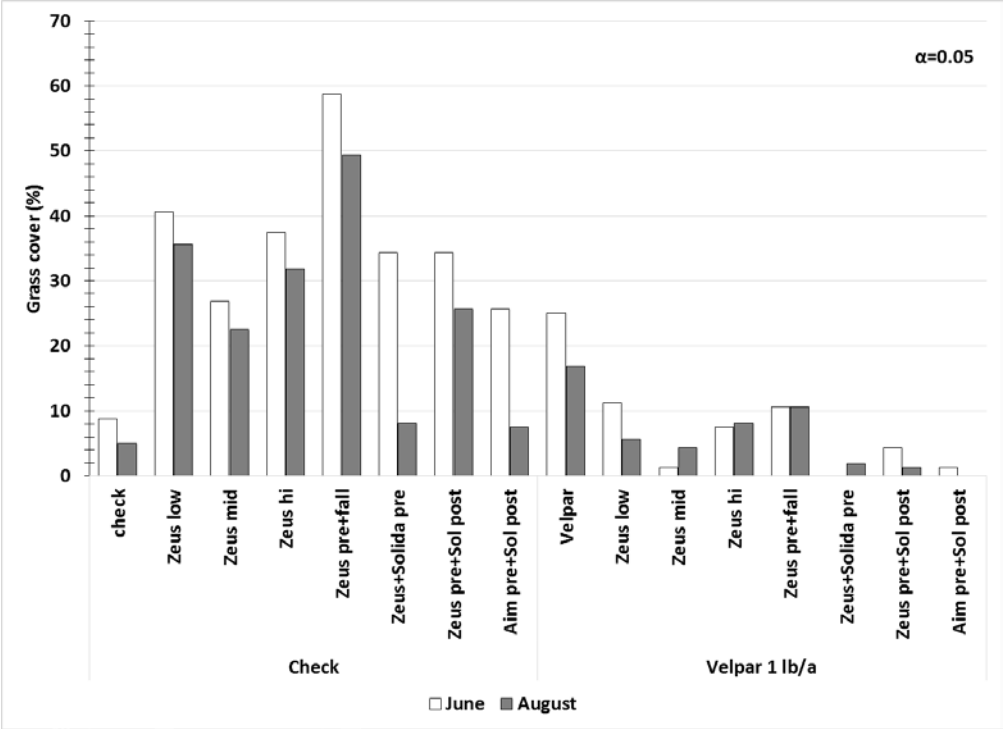
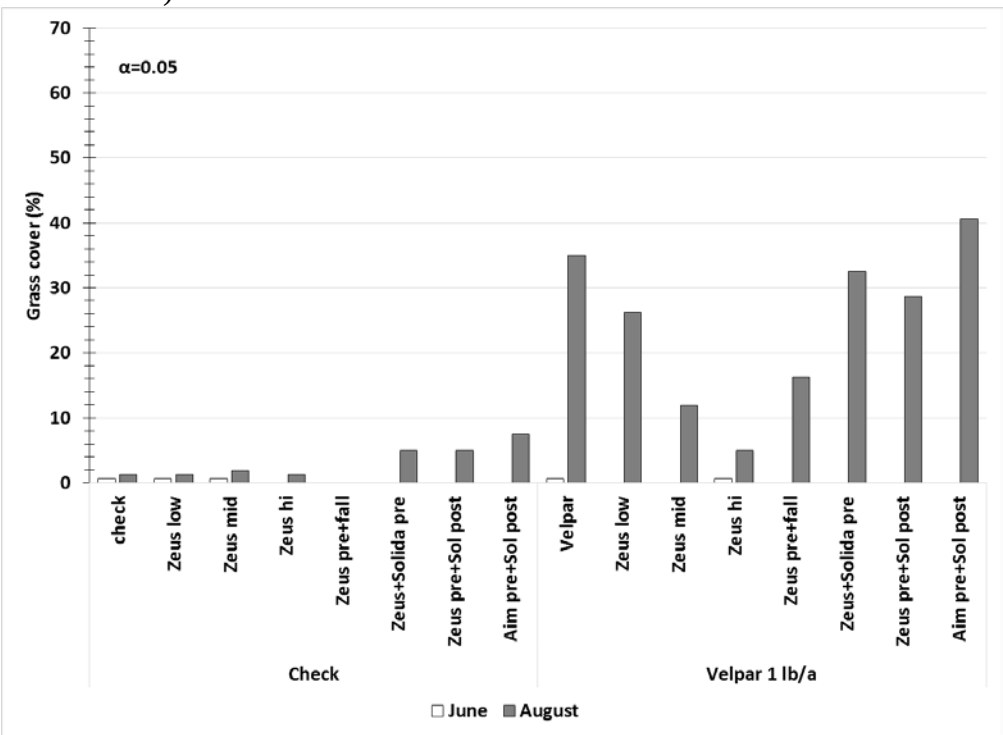


Figure 8. Grass cover in Wesley following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida (α=0.05, no significant differences).



T-tests

T-tests were performed for no Velpar versus Velpar within each main treatment at both sites; only the significant results are presented here. The lack of significant differences in some comparisons which had more data spread than the significant results is due to variability in the data coupled with the loss of four Degrees of Freedom from analyzing the sites separately.

In June in Wesley, there was a significant reduction in broadleaf weeds in the Velpar only treatment compared to the check (Figure 9); this was expected, as Velpar is primarily a product for broadleaf weed control. In August in Wesley, there was significantly higher wild blueberry cover in the Zeus pre+Solida post treatment with Velpar compared to no-Velpar (Figure 10). Overall grass cover in Wesley in August was higher in the Velpar plots for all treatments, indicating it released the grasses, and there were significantly more grasses in the Zeus Mid and Zeus pre+Solida post treatments (Figure 11).

Figure 9. Broadleaf weed cover in Wesley in June; a comparison of no Velpar versus Velpar within each main treatment (α=0.05, shaded "check" bar is Velpar only).

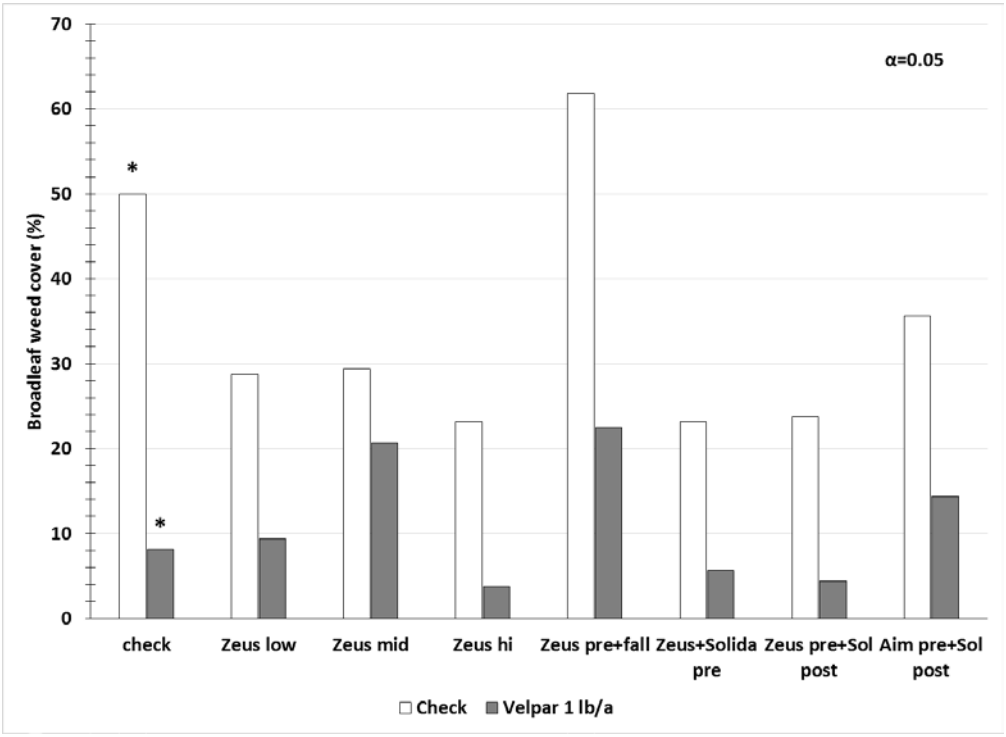


Figure 10. Wild blueberry cover in Wesley in August; a comparison of no Velpar versus Velpar within each main treatment (α=0.05; shaded "check" bar is Velpar only).

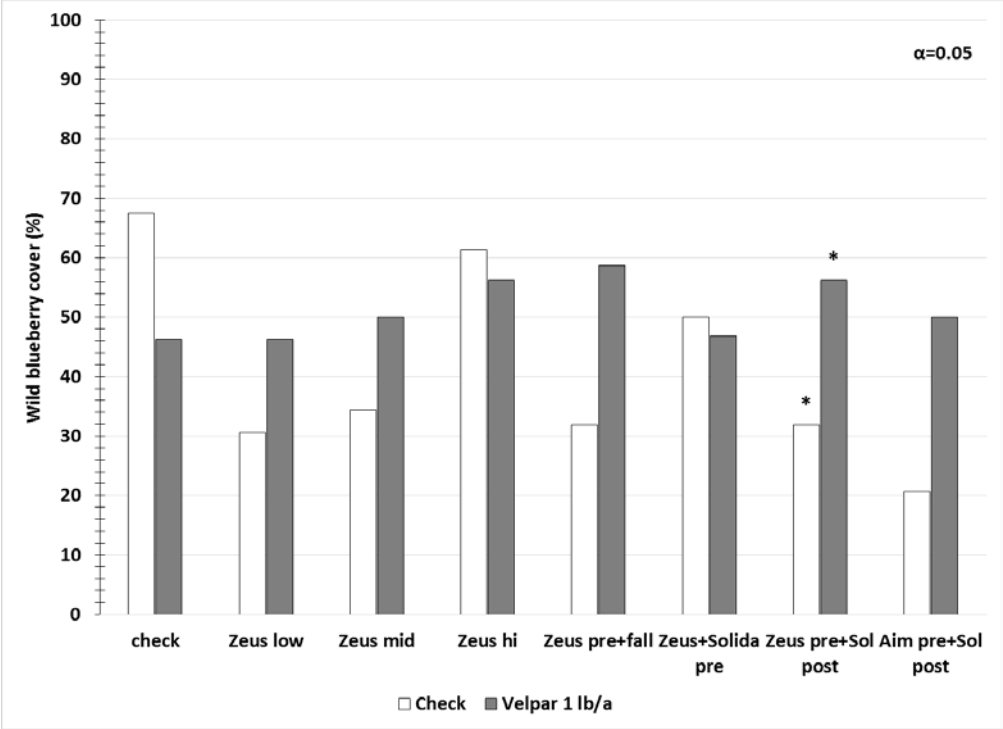
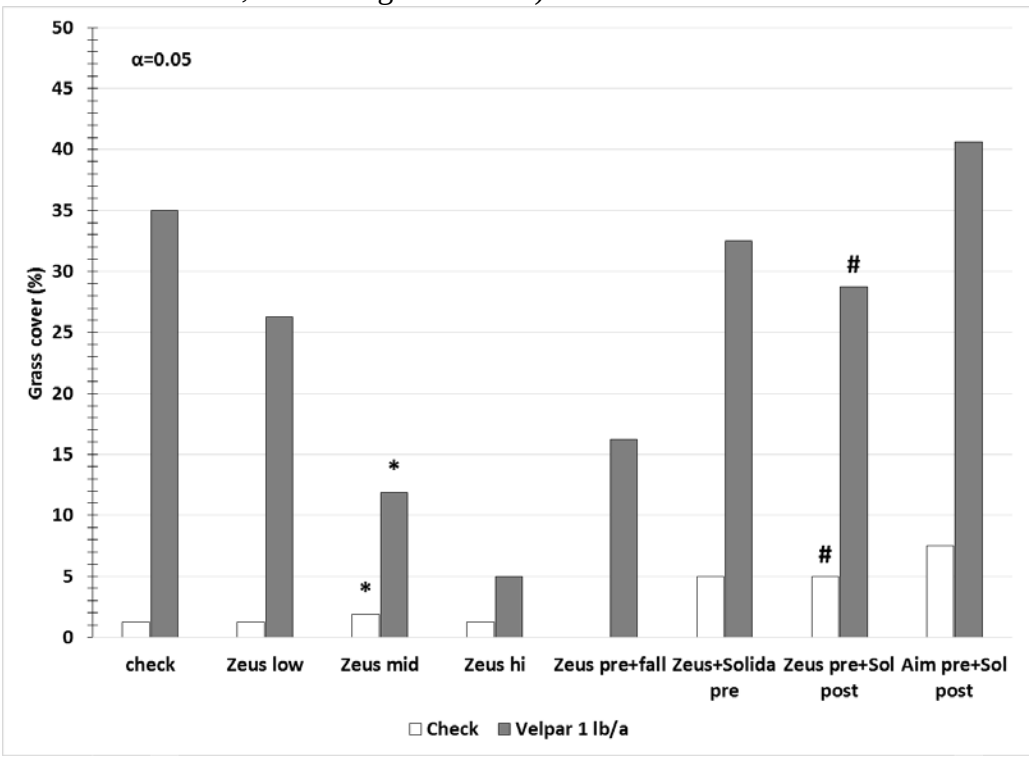


Figure 11. Grass cover in Wesley in August; a comparison of no Velpar versus Velpar within each main treatment (α=0.05; shaded "check" bar is Velpar only. Symbols represent significance within each main treatment, not among treatments).



Regression analyses

In Jonesboro, there was a quadratic trend of decreasing broadleaf weeds with an increase in the rate of Zeus alone in June and a linear trend of decreasing broadleaf weeds with an increase in Zeus alone in August (Figure 12). However, Zeus rate only explained 19.59% and 18.82% of the variation in broadleaf weed cover, respectively. There were no significant relationships between Zeus rate and broadleaf weed cover with or without Velpar in Wesley, and trends were both positive and negative (Figure 13). There were also no relationships between Zeus rate and grass cover with or without Velpar in Jonesboro (Figure 14) or Wesley (Figure 15). In Jonesboro, trends were both positive and negative; and in Wesley, there was a negative trend with Velpar in August, but no trend in June or without Velpar in August.

Figure 12. Regression analysis of broadleaf weed cover in Jonesboro for increasing rates of Zeus Prime XC with or without Velpar (0 oz/a, 7.7 oz/a, 12.5 oz/a and 15.2 oz/a; 0 oz/a with Velpar is Velpar alone). June and August with no Velpar were significant.

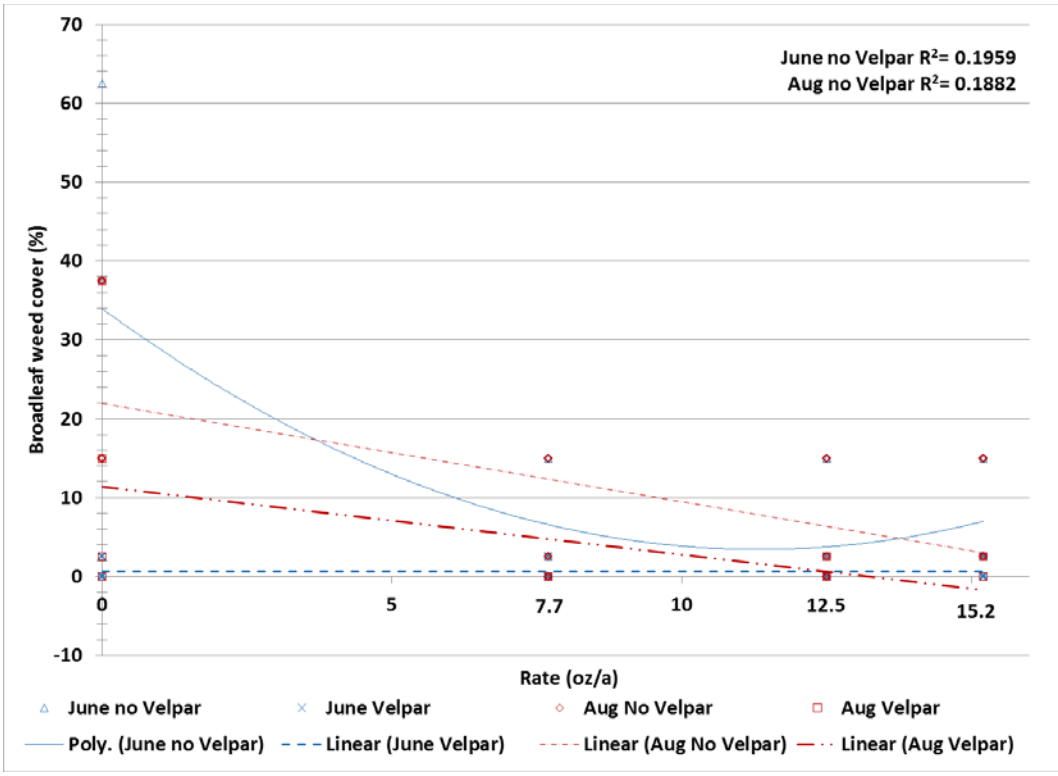


Figure 13. Regression analysis of broadleaf weed cover in Wesley for increasing rates of Zeus Prime XC with or without Velpar (0 oz/a, 7.7 oz/a, 12.5 oz/a and 15.2 oz/a; 0 oz/a with Velpar is Velpar alone).

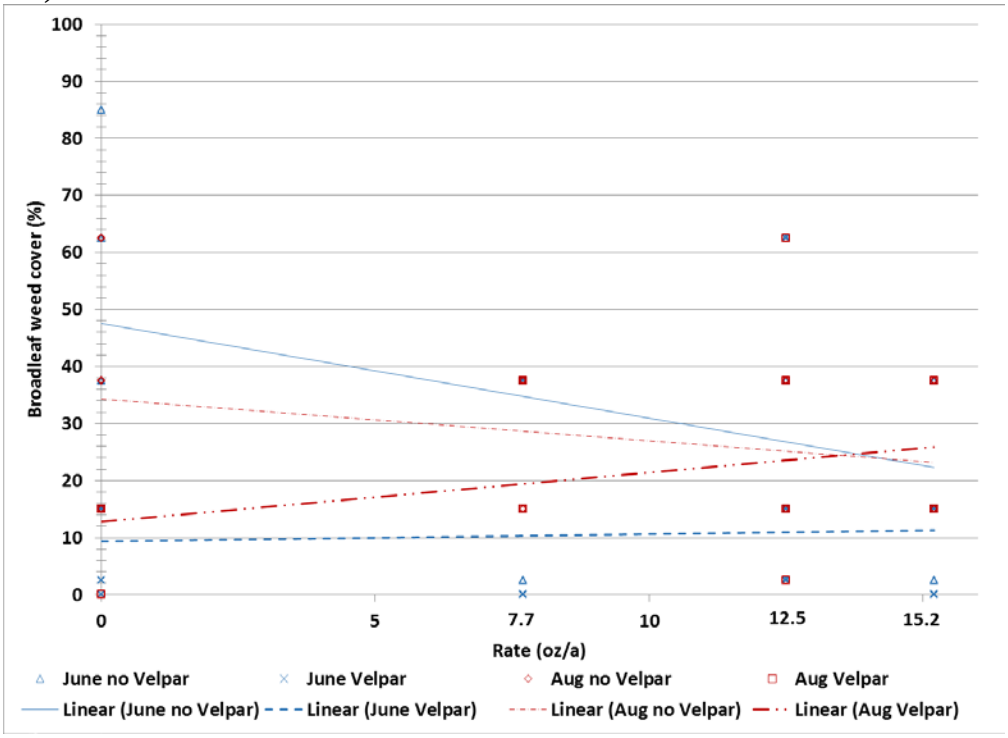


Figure 14. Regression analysis of grass cover in Jonesboro for increasing rates of Zeus Prime XC with or without Velpar (0 oz/a, 7.7 oz/a, 12.5 oz/a and 15.2 oz/a; 0 oz/a with Velpar is Velpar alone).

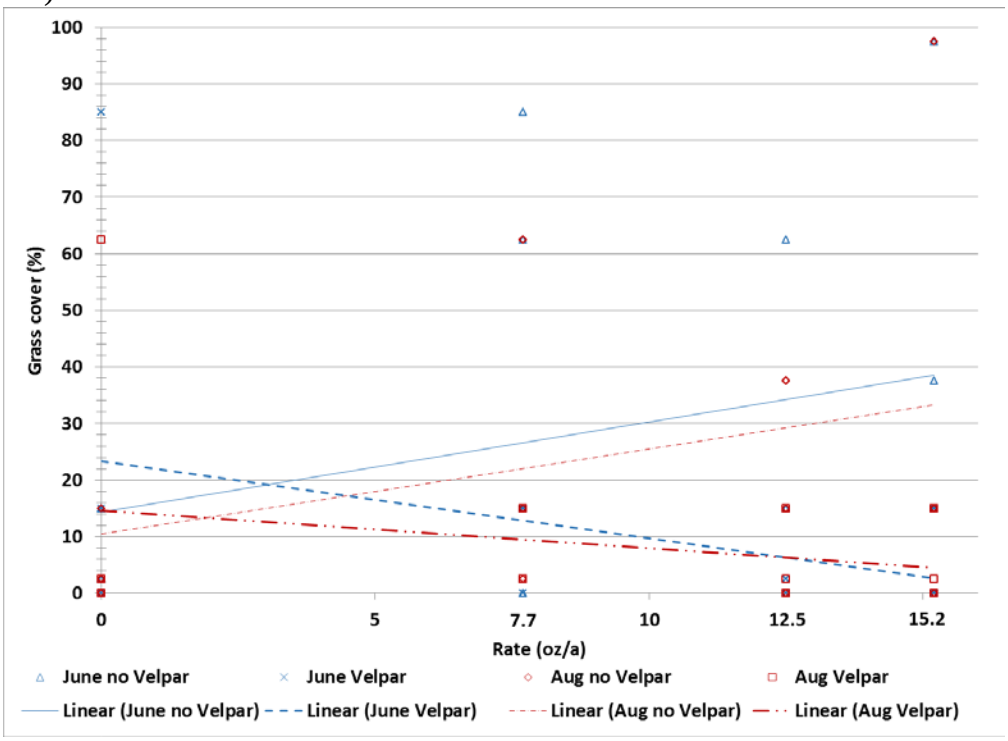
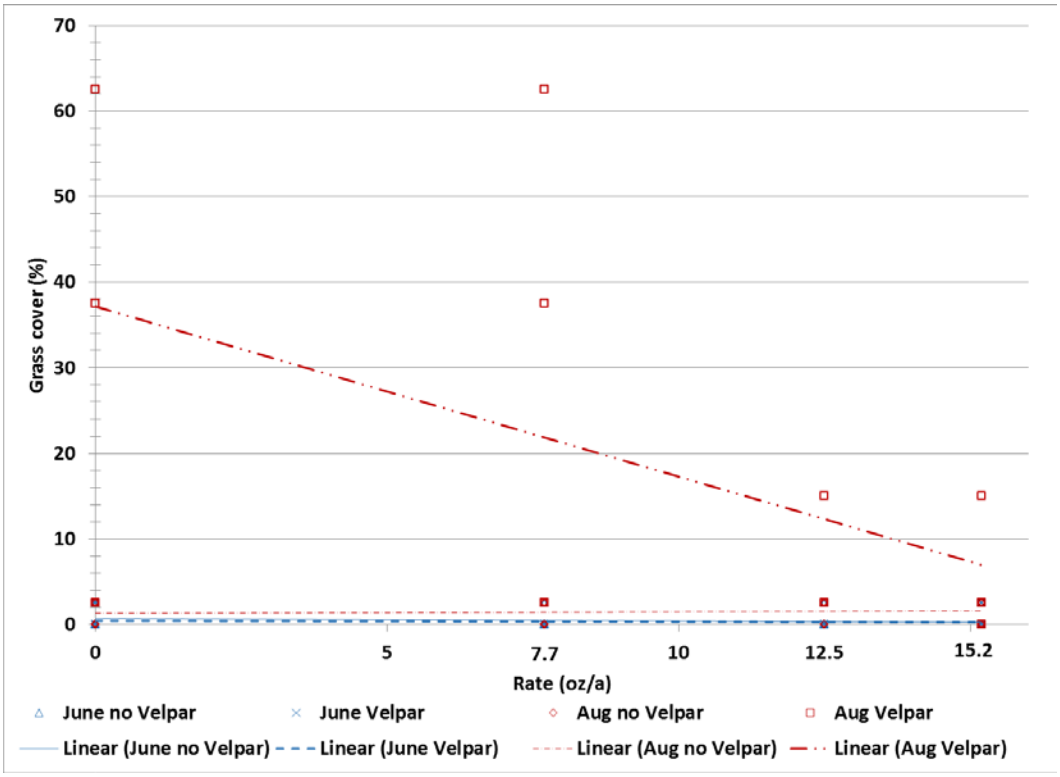


Figure 15. Regression analysis of grass cover in Wesley for increasing rates of Zeus Prime XC with or without Velpar (0 oz/a, 7.7 oz/a, 12.5 oz/a and 15.2 oz/a; 0 oz/a with Velpar is Velpar alone).



CONCLUSIONS:

All-treatment comparisons

Phytotoxicity to wild blueberry was observed mainly as stunting and was observed in the Solida applications, especially in combination with Aim. The Aim+Solida treatment was the only one with visible stunting in August and occurred in Wesley, which has heavier soil than Jonesboro (Photo 3). In June, it was noted that Aim+Solida controlled broadleaf weeds better than the other treatments, including problem weeds such as red sorrel and bladder campion, but there was also more injury to wild blueberry. The lack of treatment differences in broadleaf weed cover in Wesley was likely due to insufficient replication from separate site analysis.

It is unclear as to why there were more broadleaf weeds in Wesley in the Zeus pre+fall treatment compared to Zeus Low as of the time of this publication, regardless of Velpar or evaluation date, even though both were treated with the same rate of Zeus on the same date (see Figure 6). In Jonesboro in the no-Velpar treatments, the broadleaf weed control by Zeus and Zeus combinations released grasses, and the Zeus Low and combinations (which contained the low Zeus rate) did not control grasses well. The treatments with Velpar also slightly released grasses but not to the same extent (see Figures 5 and 7).

At the August evaluation in Wesley, grass cover was primarily witchgrass, a later germinating species, and cover appeared unrelated to broadleaf weed cover. It is more likely that the release of grasses was related to phytotoxicity in the treatment plots. Higher phytotoxicity in June as stunting was reflected as more grasses in August, as the blueberry plants did not fill in as quickly or grow as high, leaving more room and light for grasses (see Figures 2 and 4, Photo 4).

Photo 3. A. Phytotoxicity as stunting in the Aim+Solida treatment in Jonesboro in June (no Velpar, foreground); and B. in Wesley in August (with Velpar, foreground).

Photo 4. Aim+Solida treatment with Velpar in Wesley in August. Early season stunting of blueberry plants led to increased late season grass cover.

T-tests

In regard to the t-test on wild blueberry cover in Wesley for Zeus pre+Solida post no-Velpar versus Velpar, both treatments had the same amount of phytotoxicity in June and no phytotoxicity in August. Both had the same amount of broadleaf weeds in August, but no-Velpar had more broadleaf weeds in June (24% no-Velpar versus 4% Velpar; see Figure 6). Neither had grasses in June and the Velpar treatment had more grasses in August (29% Velpar versus 4% no-Velpar; see Figure 8). It appears that the early season broadleaf weeds outcompeted the blueberry plants, preventing or delaying filling in of the blueberry canopy.

In regard to the t-test on grass cover in Wesley for Zeus Mid and Zeus pre+Solida post no-Velpar versus Velpar, the difference does not appear due to broadleaf weed cover in August, as cover in the Velpar treatment and no-Velpar treatment was roughly equal (see Figure 6). It is more likely due to Velpar controlling broadleaf weeds early in the season, which resulted in more favorable conditions for germinating grasses.

Regression analyses

Finally, the regression analyses for the relationship between increasing rates of Zeus (with or without Velpar) and weed cover are not strong and should not be considered definitive. The quadratic relationship at Jonesboro for Zeus rate versus broadleaf weed cover suggests there is a diminishing return for the higher rates and there may be some injury to the blueberry plants or release of other weeds at this high rate (see Figure 12). Overall, we believe the lack of relationships may be due to small sample size. We expect that if we had been able to pool the sites, or if we had known about the site differences before starting the trial and had increased the sample size at each site, we would have seen more and/or stronger relationships.

RECOMMENDATIONS: Continue to evaluate the combinations on other sites with different weed complexes, and increase the replication on each site to eight in order to better account for the infield variability to determine if lack of effectiveness is real or because of insufficient replication.

WEED MANAGEMENT

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

10. TITLE: Comparisons of Matrix and Callisto in combination with Matrix or Sinbar for weed control in wild blueberry fields, 2016.

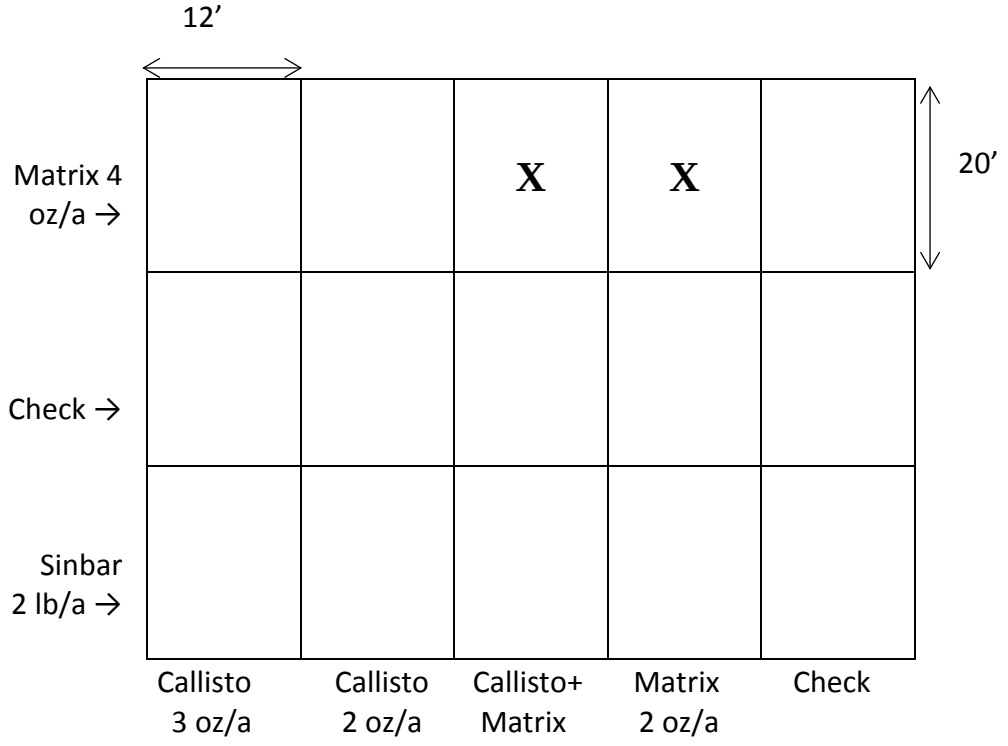
METHODS: We are continuing to test combinations of Matrix and Callisto in conjunction with each other and Sinbar WDG in order to refine application timings and tank mixes for weed control efficacy as well as wild blueberry phytotoxicity. A trial was initiated at Blueberry Hill Farm in Jonesboro, ME in spring 2016. A Randomized Complete Split Block Design was replicated four times with 12'x60' plots split in thirds lengthwise, with Matrix 4 oz/a or Sinbar 2 lb/a applied randomly to one 12'x20' section of each block pre-emergence on 19 May 2016. The main treatments were applied twice or thrice post-emergence as follows:

1. Untreated check;
2. Callisto 3 oz/a with NIS 0.25% v/v (2x);
3. Callisto 2 oz/a with NIS 0.25% v/v (3x);
4. Matrix 2 oz/a with NIS 0.25% v/v (2x); and
5. Callisto 2 oz/a + Matrix 2 oz/a with NIS 0.25% v/v (2x).

The post-emergence treatments were applied on 10 and 24 June 2016, and the third application for the Callisto 2 oz/a treatment occurred on 8 July 2016. It should be noted that Blueberry Hill Farm applied DAP fertilizer at 150 lb/a on 20 May 2016. Also note that the post-emergence Matrix applications were not applied to the block sections that had received pre-emergence Matrix (Figure 1), so as not to exceed the per year maximum per acre. Therefore, all of the Matrix treatments in the Matrix section received pre-emergence Matrix only, while the Matrix treatments in the check and Sinbar sections received post-emergence Matrix only.

The plots were evaluated for wild blueberry cover and phytotoxicity, broadleaf weed cover and grass cover on 8-9 June (immediately prior to the 1st post-emergence application) and 1 August (after the 3rd post-emergence application). Cover data were determined by using the Daubenmire Cover Class system converted to percent; phytotoxicity data were gathered using a scale of 0-10 (0=no damage, 10=100% damaged/dead) converted to percent. The treatments were compared to each other using Tukey's tests (α=0.05). T-tests were also performed for significant differences in main effects, i.e. all main treatments were pooled and analyzed for differences among the herbicides alone, with Matrix and with Sinbar (Bonferroni adjusted to α=0.0167).

Figure 1. Example layout of a trial block; Xs denote sections that did not receive post-emergence Matrix.



RESULTS:

Main effects

There were no differences among the herbicides alone, with Matrix or with Sinbar for June wild blueberry cover (Figure 2). In June, the Sinbar treatments had significantly more phytotoxicity compared to the other treatments alone, but at 2%, was not a concern. In August, the Sinbar treatments had significantly higher blueberry cover compared to the other treatments alone or with Matrix; the Sinbar treatments also had significantly higher phytotoxicity than those with Matrix but equal to the herbicides alone (Figure 2).

Broadleaf weed cover decreased slightly in June with the addition of Matrix or Sinbar, but cover did not change much by the second evaluation, and there were no significant differences (Figure 3). In contrast, the addition of Sinbar significantly reduced grasses compared to the herbicides alone or with Matrix. The addition of pre-emergence Matrix released grasses so that it had significantly higher grass cover compared to the other two by August.

Figure 2. Wild blueberry cover and phytotoxicity immediately prior to and following post-emergence applications of Callisto and Matrix (August evaluation), both by themselves (main treatments alone) and with pre-emergence applications of Matrix and Sinbar (June evaluation) (letters denote significance at α=0.0167 only).

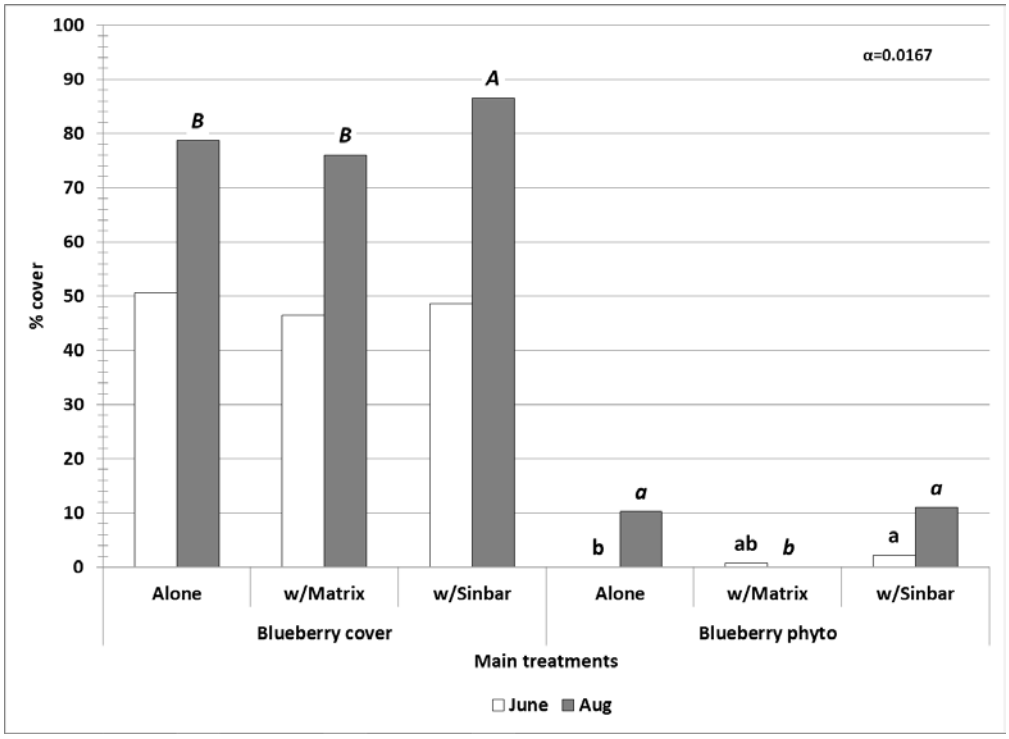
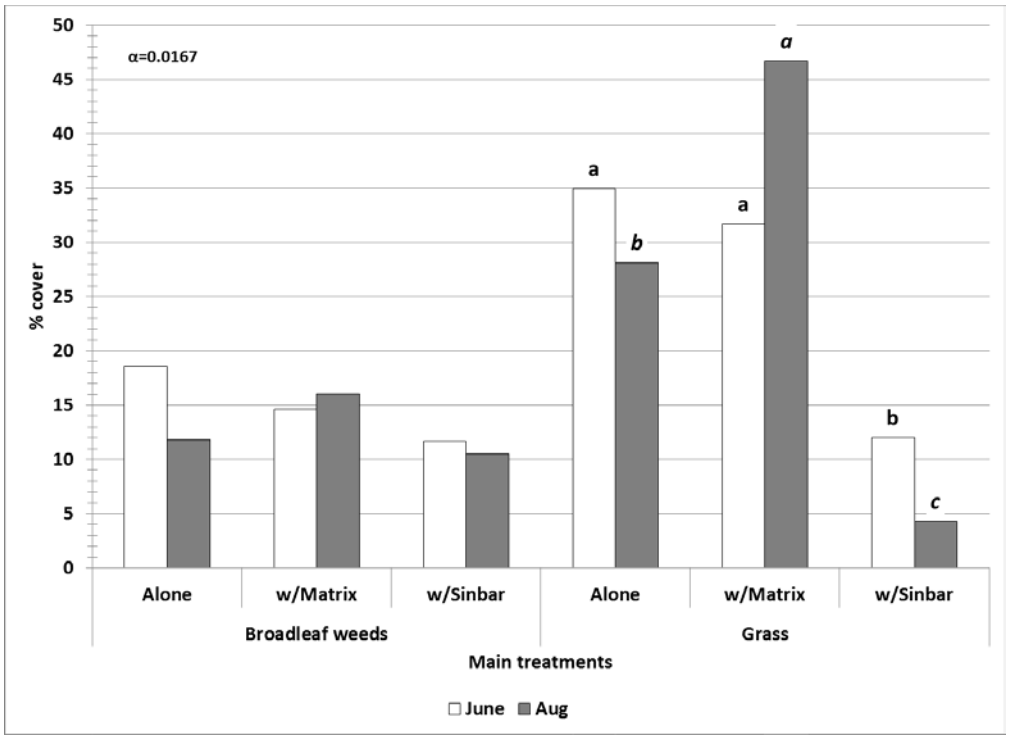


Figure 3. Broadleaf weed and grass cover immediately prior to and following post-emergence applications of Callisto and Matrix (August evaluation), both by themselves (main treatments alone) and with pre-emergence applications of Matrix and Sinbar (June evaluation) (letters denote significance at α=0.0167 only).



All-treatment comparisons

There were no significant differences among treatments for wild blueberry cover at either evaluation (Figure 4). There were no significant differences in phytotoxicity at the June evaluation, and injury was minimal at 4% and below (Figure 5). In August, Matrix 2 oz/a and Callisto+Matrix alone and with Sinbar had significantly more injury to blueberry compared to the other treatments. The plants in these plots were visibly stunted or delayed compared to the same clones outside the plots (Photos 1-2).

Figure 4. Wild blueberry cover immediately prior to and following post-emergence applications of Callisto and Matrix (August evaluation), both by themselves (main treatments alone) and with pre-emergence applications of Matrix and Sinbar (June evaluation) (only significant differences at α=0.05 denoted by different letters).

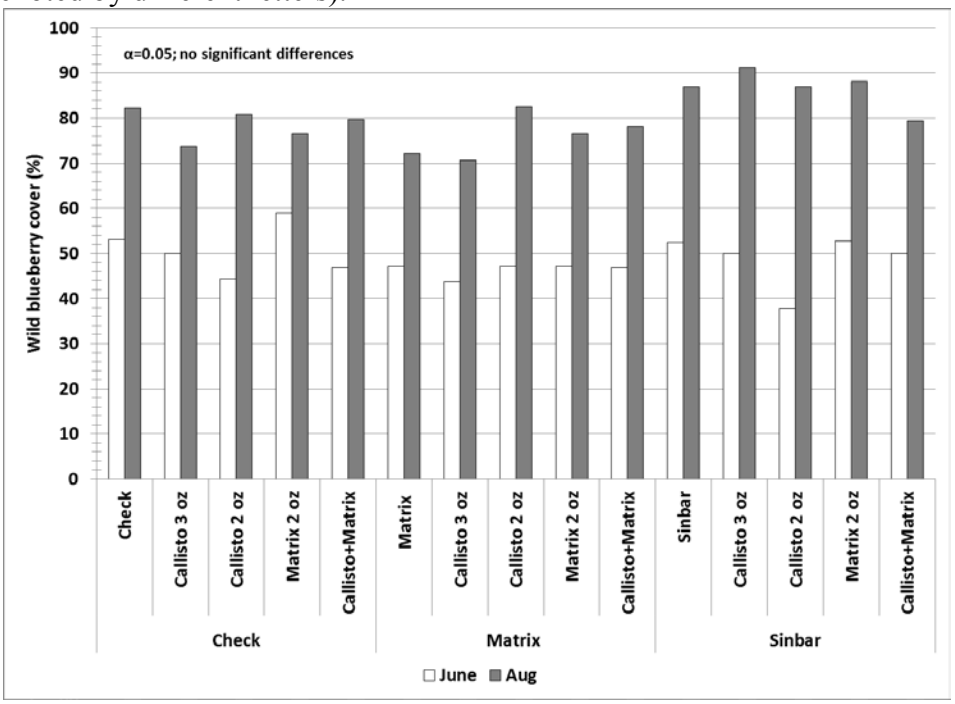


Figure 5. Wild blueberry phytotoxicity immediately prior to and following post-emergence applications of Callisto and Matrix (August evaluation), both by themselves (main treatments alone) and with pre-emergence applications of Matrix and Sinbar (June evaluation) (only significant differences at α=0.05 denoted by different letters).

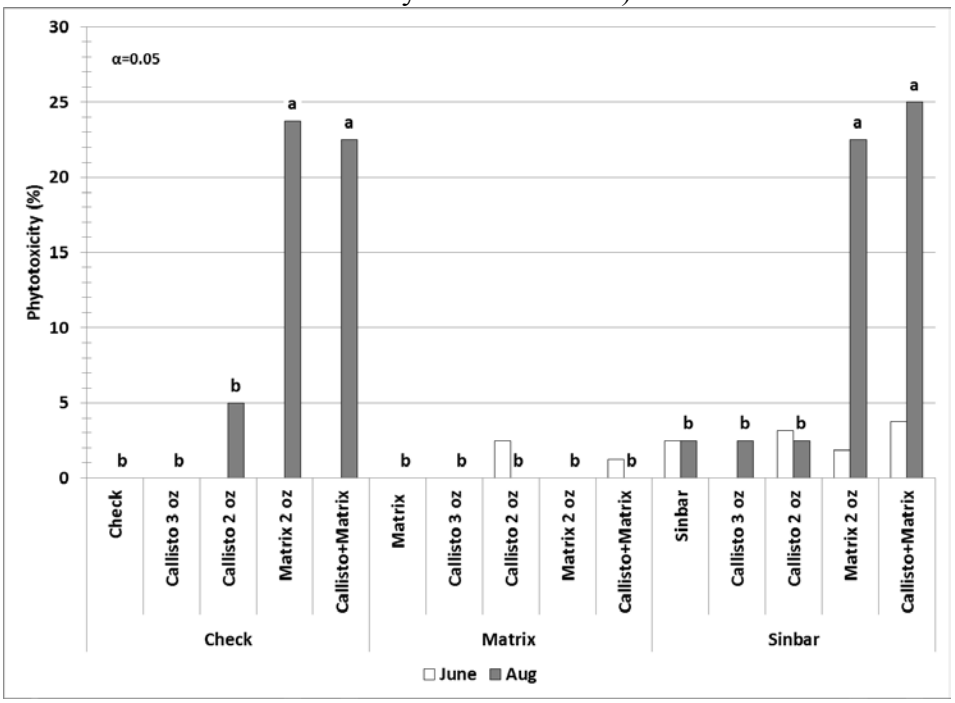
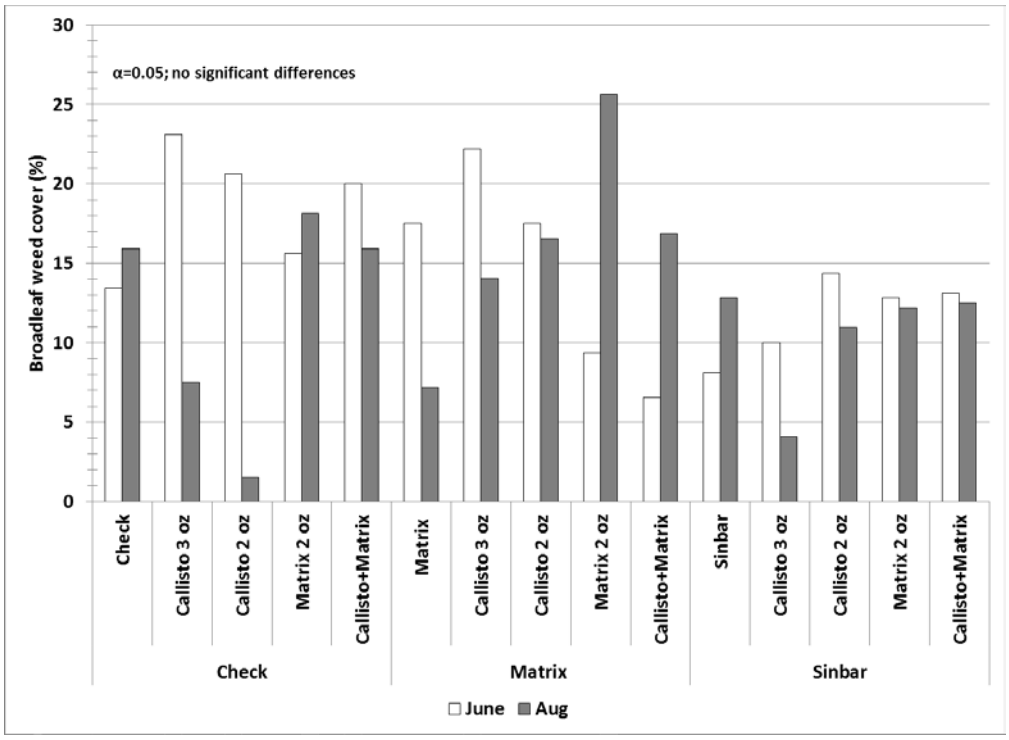


Photo 1. August phytotoxicity as stunting in the Matrix 2 oz/a treatment. Post-emergence Matrix is in foreground; mid weedy section is pre-emergence Matrix; background section is post-emergence Matrix with Sinbar.

There were no significant differences among treatments for broadleaf weed cover in June or August (Figure 6). Some trends emerged, however. In the post-emergence main treatments alone (Check section), both Callisto treatments reduced weeds by a factor of 3 to 10+ from June to August, but the Callisto+Matrix treatment only slightly reduced broadleaf weeds and the post-emergence Matrix only saw a slight increase. The Matrix and Callisto+Matrix plots in the Matrix section, which only received pre-emergence Matrix (see Photo 3), saw a marked increase in broadleaf weeds, while the Callisto 3 oz/a and 2 oz/a plots had a slight reduction. The "Matrix" plots (aka check with pre-emergence Matrix) in the Matrix section received identical applications as the "Matrix 2 oz" plots in the Matrix section; it is unclear as to why the "Matrix" plots reduced broadleaf weeds but the "Matrix 2 oz" plots increased them. Finally, Callisto 3 oz/a with Sinbar performed better than Callisto 3 oz/a alone in August, but Callisto 2 oz/a alone performed better than with Sinbar. Combining post-emergence Matrix with Sinbar slightly improved broadleaf weed control over time compared to Matrix alone or with Callisto either pre- or post-emergence.

Figure 6. Broadleaf weed cover immediately prior to and following post-emergence applications of Callisto and Matrix (August evaluation), both by themselves (main treatments alone) and with pre-emergence applications of Matrix and Sinbar (June evaluation) (α=0.05, no significant differences).



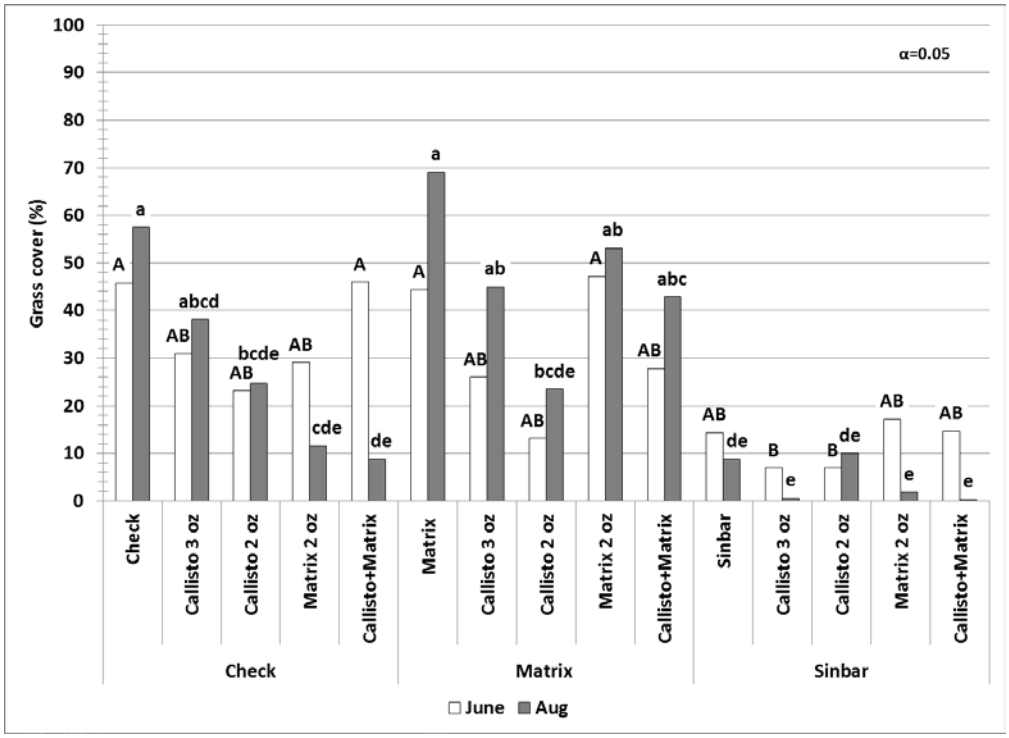
There were many grasses in the trial area, and several treatment effects were observed. In June (just prior to the post-emergence applications), higher overall grass cover occurred in the Check and the Matrix sections as compared to the Sinbar section (Figure 7; see Conclusions section for a discussion of significance in the June evaluation). In August, the untreated check and pre-emergence Matrix treatments (both as "Matrix" and "Matrix 2 oz") had the most grass cover, and were not different from Callisto 3 oz/a alone or with Matrix, or from pre- Matrix + Callisto. Alone or with Matrix, Callisto 2 oz/a applied 3x performed better than Callisto 3 oz/a applied 2x, but not significantly so. Post-emergence Matrix, either alone (Check section, Photo 2) or with Sinbar, significantly reduced grasses compared to pre-emergence Matrix alone (Photo 3) but not Sinbar alone.

Photo 2. Example of weed control and phytotoxicity in a post-emergence Matrix plot.

Photo 3. Example of poor weed control in a pre-emergence Matrix only plot.

Photo 4. Example of weed control in a Sinbar only plot.

Figure 7. Grass cover immediately prior to and following post-emergence applications of Callisto and Matrix (August), both by themselves and in combination with each other and pre-emergence applications of Matrix and Sinbar (June) (only significant differences at α=0.05 denoted by different letters).



CONCLUSIONS:

Main effects

Wild blueberry cover was increased over time by the use of Sinbar combinations (Figure 2), likely because Sinbar controlled grasses significantly better than Matrix or the other herbicides alone (Figure 3) and allowed wild blueberry to take advantage of more resources. The August phytotoxicity observed in the herbicide treatments alone and with Sinbar was driven by the post-emergence Matrix applications, as the treatments with Matrix received the pre-emergence Matrix application only (Figure 2). Broadleaf weeds were not significantly affected positively or negatively by the addition of pre-emergence Matrix or Sinbar (Figure 3) or by the main treatments themselves, as is illustrated by percent cover in June before the post-emergence applications versus August after the post-emergence applications. See the section below for a more in-depth discussion.

All-treatment comparisons

Although there were no significant differences in June except in grass cover, the comparisons for the June evaluation should be looked at more as a group, aka Check vs Matrix vs Sinbar, as this evaluation occurred after the pre-emergence split applications but before the post-emergence main applications. Therefore, each treatment within each section had received identical applications at the time of the June evaluation. In accordance, we see that Sinbar improved early season grass control compared to no treatment or pre-emergence Matrix.

Although wild blueberry cover was not significantly reduced in August due to phytotoxicity from post-emergence Matrix applications, the four treatments that received post-emergence Matrix had almost 25% injury to blueberry as delay/stunting in growth and was visible six weeks after treatment. Applying Matrix post-emergence did control both broadleaf weeds and grasses better than pre-emergence Matrix, but post-emergence Callisto applications were comparable and were not significantly improved by adding either pre- or post- emergence Matrix. Pre-emergence Matrix applications appeared to release certain broadleaf weeds and grasses; this has also been observed in previous trials. The combination of Sinbar and post-emergence Callisto and/or Matrix improved both broadleaf weed and grass control, but not significantly over Sinbar alone. Finally, applying Callisto 2oz/a three times as opposed to 3 oz/a two times improved weed control when used alone, but not significantly so, and the trend did not consistently hold when combined with pre-emergence Matrix or Sinbar.

RECOMMENDATIONS: We observed that post-emergence Matrix application resulted in too much phytotoxicity and the pre-emergence Matrix treatment did not control weeds well enough alone or with Callisto. We expect you would need to add Velpar for control of broadleaf weeds released by Matrix, and Sinbar for grass control. Callisto 3x improved weed control somewhat compared to 2x but we don't believe the improvement was enough to warrant the cost of going out a 3rd time unless the grower is already going out to apply some post-emergence grass control treatments.

WEED MANAGEMENT

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

11. TITLE: Herbicide combinations with Sinbar WDG to assess efficacy on weed control in wild blueberry.

METHODS: In spring 2016 we set up a trial in two Maine prune fields, at Blueberry Hill Farm in Jonesboro and GM Allen in Orland, to continue assessing the WDG formulation of Sinbar in combination with other products registered for wild blueberry. We applied the main treatments pre-emergence in combination with Sinbar WDG 2 lb/a to examine efficacy on weeds when combined with other herbicides and with a post-emergence Callisto treatment. A Randomized Complete Block Design was replicated three times per site with 18' x 36' plots; main treatments were an untreated check, Sinbar 2 lb/a+Velpar 2 lb/a+ diuron 2 lb/a ("Trimix"), and Sinbar 2 lb/a+Velpar 2 lb/a+ Matrix 4 oz/a ("Matrix mix"). Each 36' plot length was split in half, with 18' receiving Callisto 3 oz/a with COC 1% v/v applied twice post-emergence. The main treatments were sprayed late pre-emergence on 19-20 May 2016, and the Callisto split treatment was applied on 10 and 24 June (Jonesboro) and 15 and 27 June (Orland). The sites were evaluated on 3/6 June just prior to the first post-emergence application, and again on 7-8 July after the second post-emergence application. Cover data were determined by using the Daubenmire Cover Class system converted to percent; phytotoxicity data were gathered using a scale of 0-10 (0=no damage, 10=100% damaged/dead) converted to percent. We expected that the sites were to be pooled for analysis, but preliminary analysis revealed significant site differences in weed cover, so the sites were analyzed separately. Main effects of the pooled main treatments by site with or without Callisto on wild blueberry cover and phytotoxicity, and broadleaf weed and grass cover, were compared using t-tests (α=0.05). All treatments by site were also compared to each other using Tukey's tests (α=0.05).

RESULTS:

Main effects

When the main treatments were pooled, there were no significant differences at either site between the plots with Callisto and without Callisto (Figure 1). There was a significant difference in blueberry phytotoxicity at both sites, between the no-Callisto plots and Callisto plots in July (Figure 2). The June evaluation was conducted just prior to the first Callisto application, and no differences were recorded at that time. In July, the plots with Callisto had significantly higher phytotoxicity compared to the no-Callisto plots.

Broadleaf weed cover (Figure 3) and grass cover (Figure 4) had no significant differences at either site for the no-Callisto plots versus the Callisto plots. The Jonesboro site was weedier in general, with at least 6 times the average broadleaf weed cover and grass cover compared to the Orland site.

Figure 1. Main effects by site on wild blueberry cover for all main treatments with or without post-emergence Callisto (α=0.05, no significant differences).

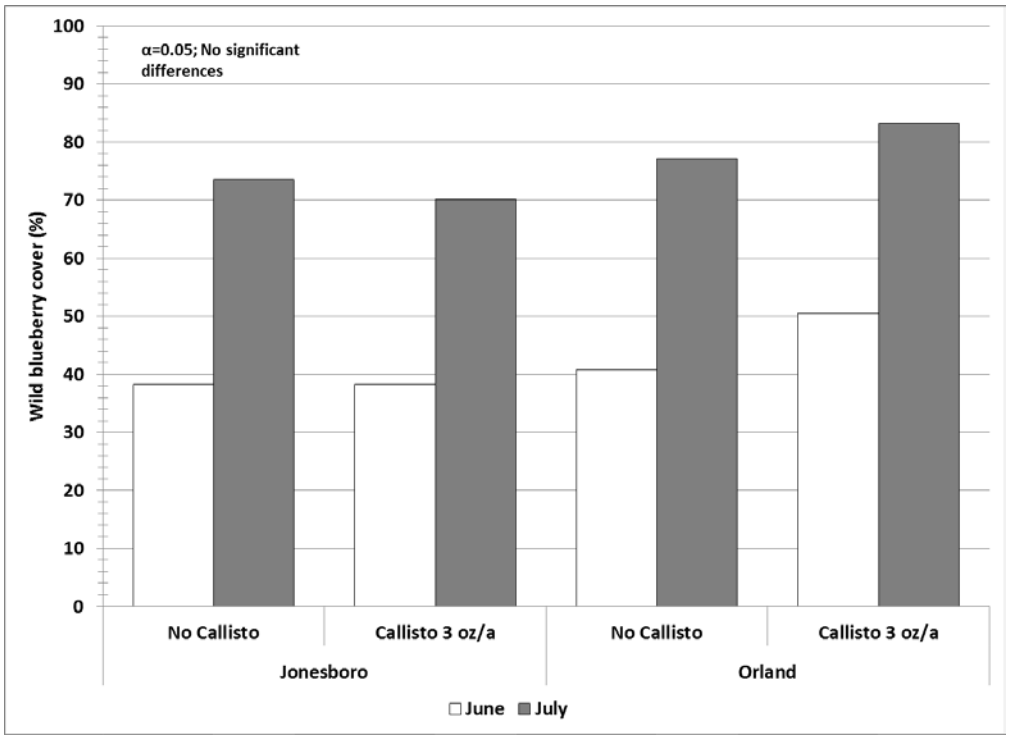


Figure 2. Main effects by site on wild blueberry phytotoxicity for all main treatments with or without post-emergence Callisto (α=0.05).

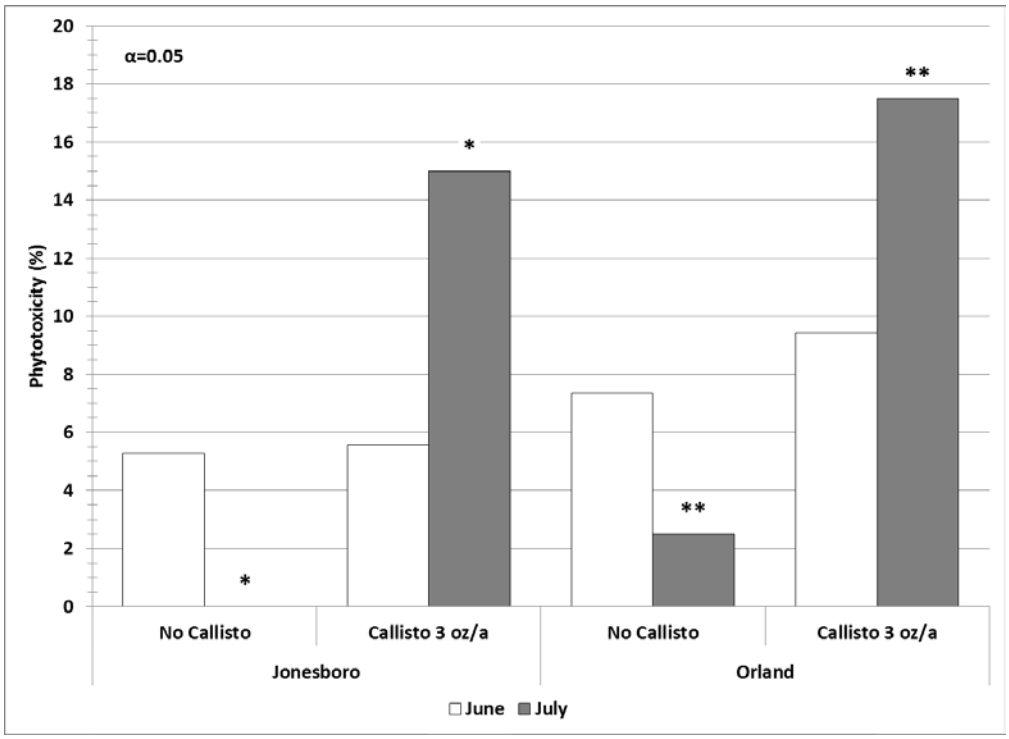


Figure 3. Main effects by site on broadleaf weed cover for all main treatments with or without post-emergence Callisto (α=0.05, no significant differences).

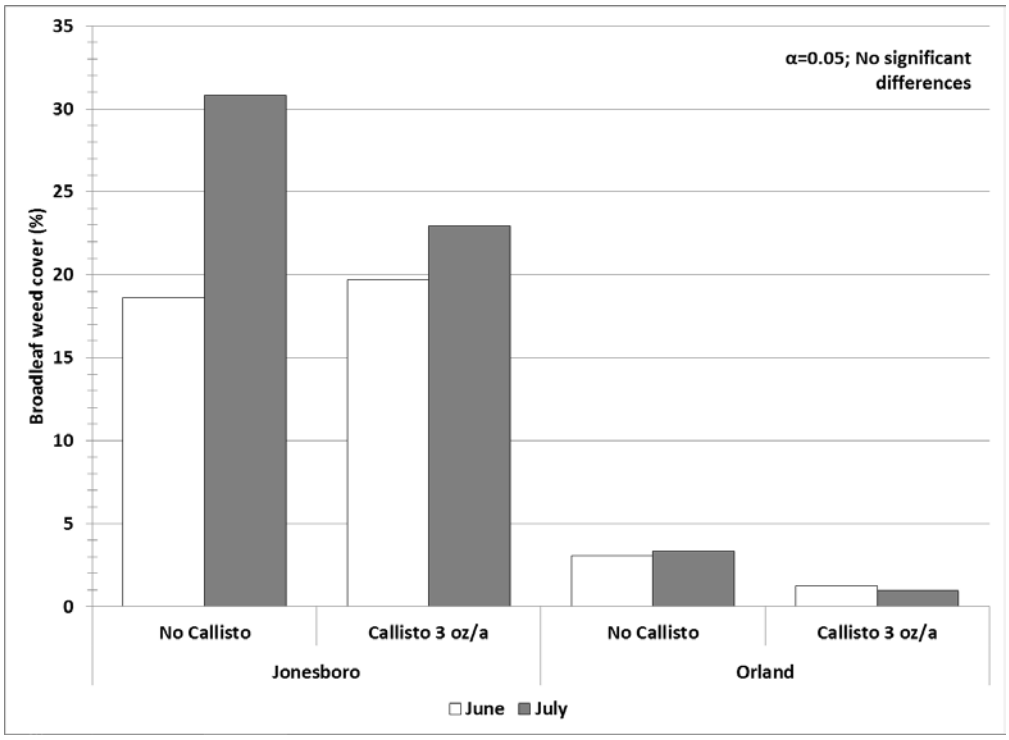
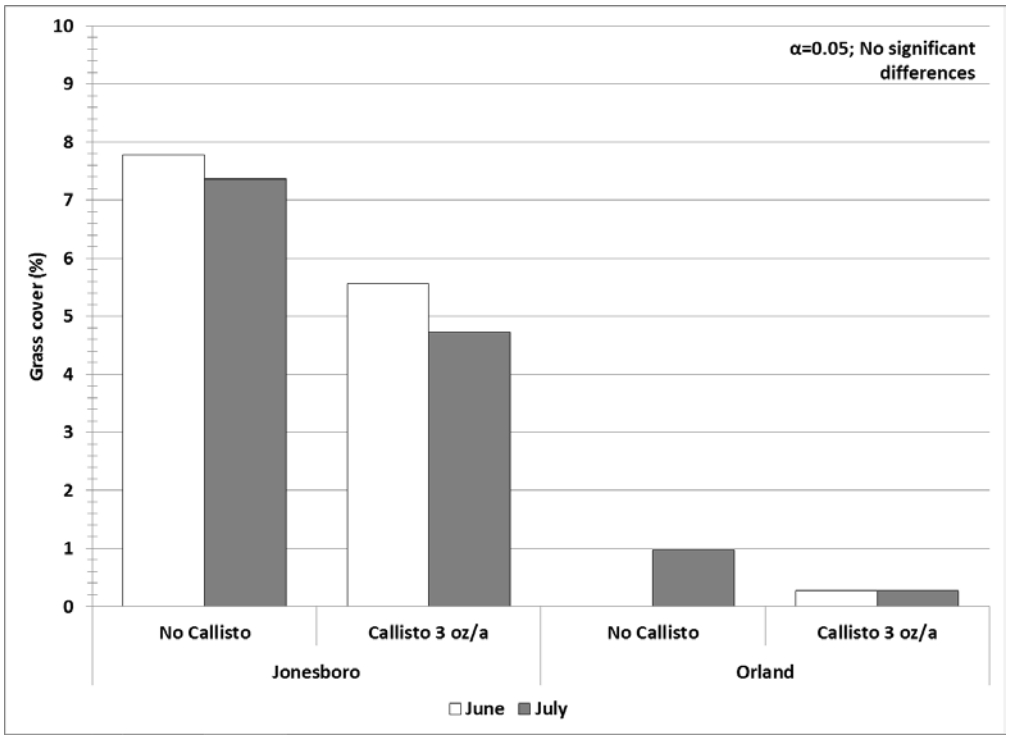


Figure 4. Main effects by site on grass cover for all main treatments with or without post-emergence Callisto (α=0.05, no significant differences).



All-treatment comparisons

There were no significant differences among treatments for wild blueberry cover at the Jonesboro site (Figure 5). The Matrix mix initially had the lowest cover, whether with or without Callisto, but the difference was not significant and the trend had essentially disappeared by July. In Orland, there were also no significant differences at either evaluation, and the Matrix mix was comparable to the other treatments (Figure 6).

At both sites, there was initially injury observed at the June evaluation, which was due solely to the Trimix and Matrix mix treatments, as the Callisto had not yet been applied. In Jonesboro, both the Trimix and Matrix mix averaged approximately 8% injury, but there were no significant differences (Figure 7). In July, the three Callisto treatments had injury to blueberry as chlorosis from the Callisto applications, and the injury was significantly greater than the three no-Callisto treatments. In Orland, there was also phytotoxicity observed at the June evaluation, including in the check and Callisto only treatment (another "check" at that time, as the Callisto had not yet been applied) (Figure 8). The background injury presented as minor chlorosis, but the origin was not apparent. The Trimix and Matrix mix treatments, with and without Callisto, were not significantly different from each other; the only differences were between Trimix and Matrix mix with Callisto versus the check and Callisto only, and between Trimix without Callisto and the check. In July, more phytotoxicity was observed in the treatments with Callisto and injury was significantly higher compared to the Matrix mix without Callisto and the check. The Trimix-Callisto treatment also had significantly more injury than the Trimix only treatment. Again, the injury observed in July appeared to be from the Callisto application. It should be noted that the second Callisto application at both sites occurred when the air temperatures were 74°F (Jonesboro) and 78°F (Orland); the Callisto label warns that injury may occur when air temperature exceeds 85°F.

Figure 5. Wild blueberry cover at the Jonesboro site, following pre-emergence applications of Sinbar-herbicide combinations with and without post-emergence Callisto (α=0.05, no SD).

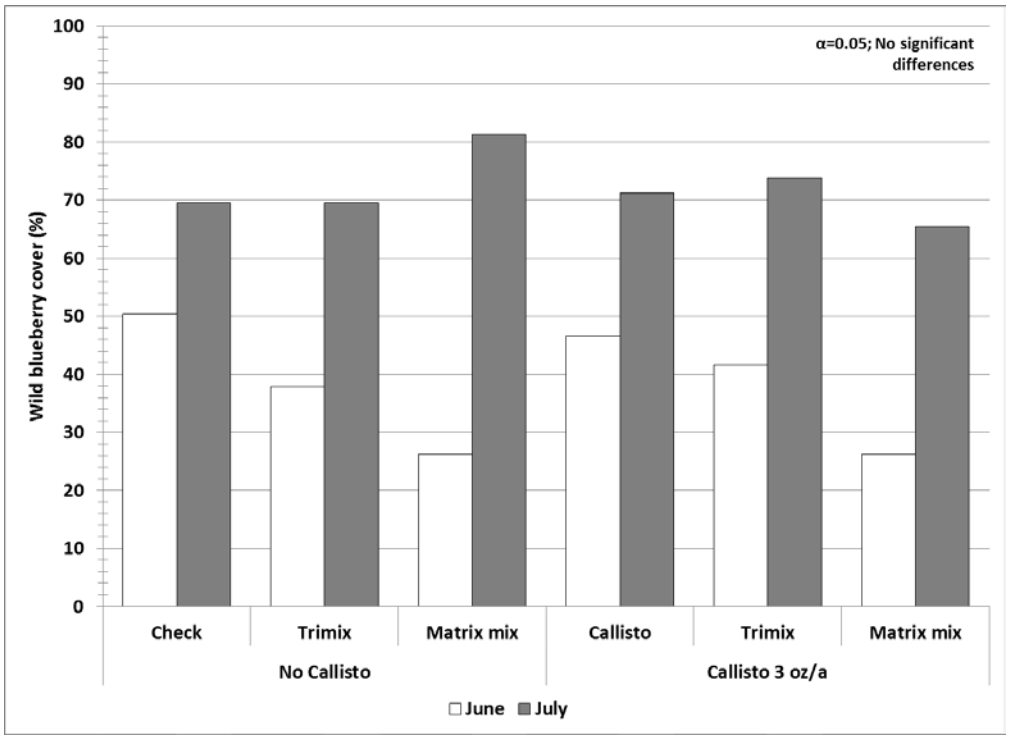


Figure 6. Wild blueberry cover at the Orland site, following pre-emergence applications of Sinbar-herbicide combinations with and without post-emergence Callisto (α=0.05, no SD).

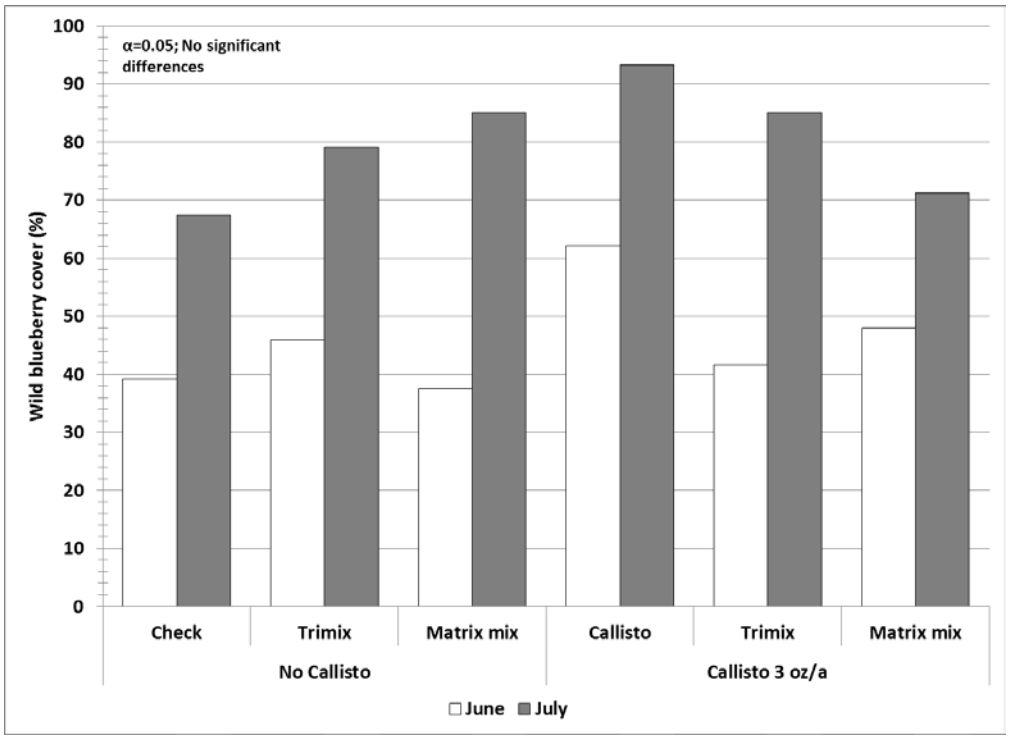


Figure 7. Wild blueberry phytotoxicity at the Jonesboro site, following pre-emergence applications of Sinbar-herbicide combinations with and without post-emergence Callisto (different letters denote significance only, at α=0.05).

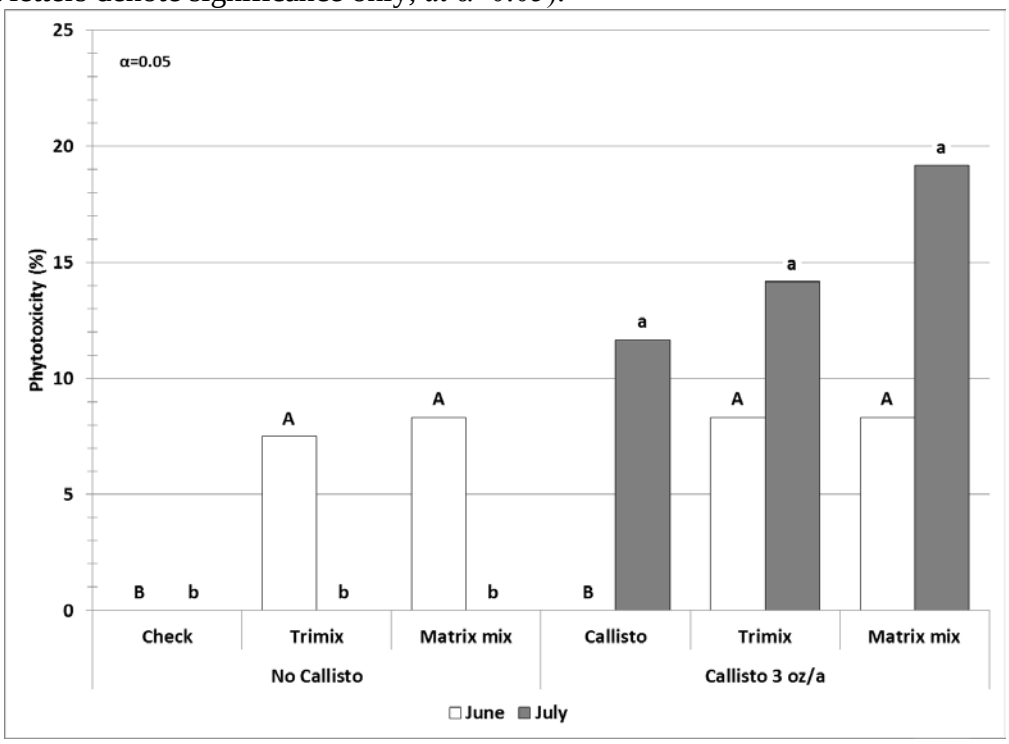
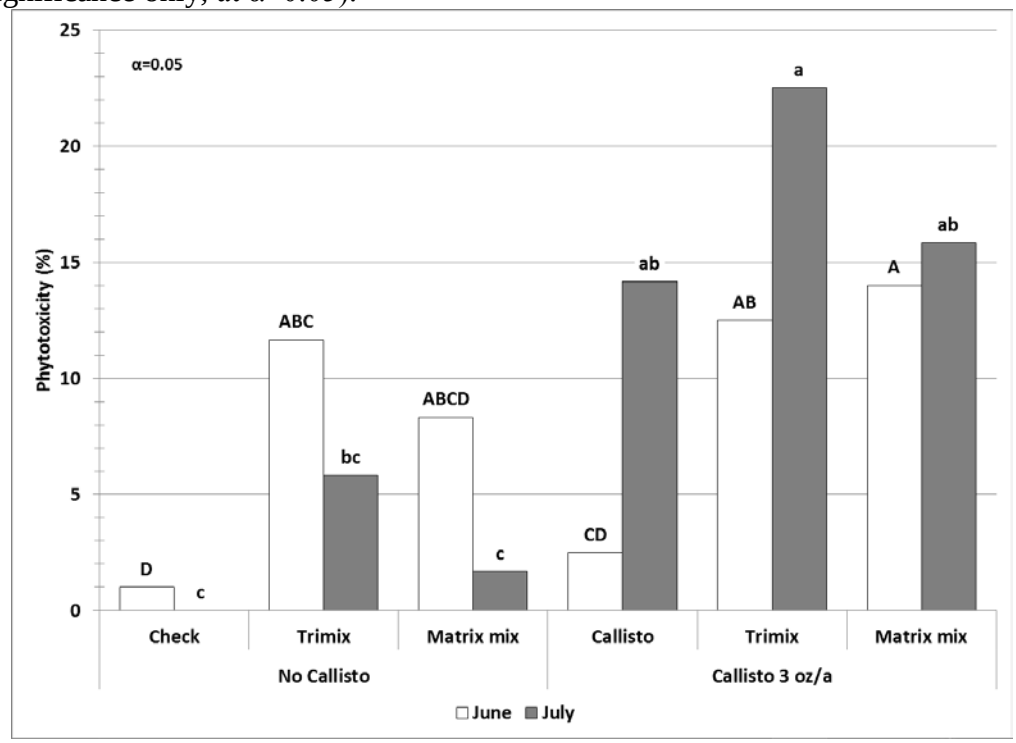


Figure 8. Wild blueberry phytotoxicity at the Orland site, following pre-emergence applications of Sinbar-herbicide combinations with and without post-emergence Callisto (different letters denote significance only, at α=0.05).



There were no significant differences in broadleaf weed cover in Jonesboro at either evaluation (Figure 9). In general, the check and Callisto treatment (a second check in June) had the most broadleaf weed cover while the Matrix mix had the least. In July, the trend held true for the no-Callisto treatments; when Callisto was added broadleaf weed cover was reduced slightly in the check and Trimix but not in the Matrix mix. A visual observation was made at the July evaluation, that the Trimix had killed bunchberry (*Cornus canadensis*). Injury to bunchberry was also observed in the plots with Callisto.

The Orland site did have significant differences in broadleaf weed cover at the June evaluation, but the differences were no longer significant by July (Figure 10). Initially, the check, Callisto (aka 2nd check), and Matrix mix both with and without Callisto had significantly more broadleaf weeds compared to the Trimix with or without Callisto at 0-0.5% cover. By July, broadleaf weeds were absent in the Matrix mix alone treatment, Callisto alone and Trimix with Callisto; weeds increased in the Trimix treatment and remained steady in the Matrix with Callisto treatment. Note that in June, dead sessileleaf bellwort (*Uvularia sessilifolia*) was observed in the Matrix mix treatment; dead St. Johnswort (*Hypericum perforatum*) was observed in the Matrix mix with Callisto treatment in June, and dead dewberry (*Rubus flagellaris*) was observed in July.

Figure 9. Broadleaf weed cover at the Jonesboro site, following pre-emergence applications of Sinbar-herbicide combinations with and without post-emergence Callisto (α=0.05, no SD).

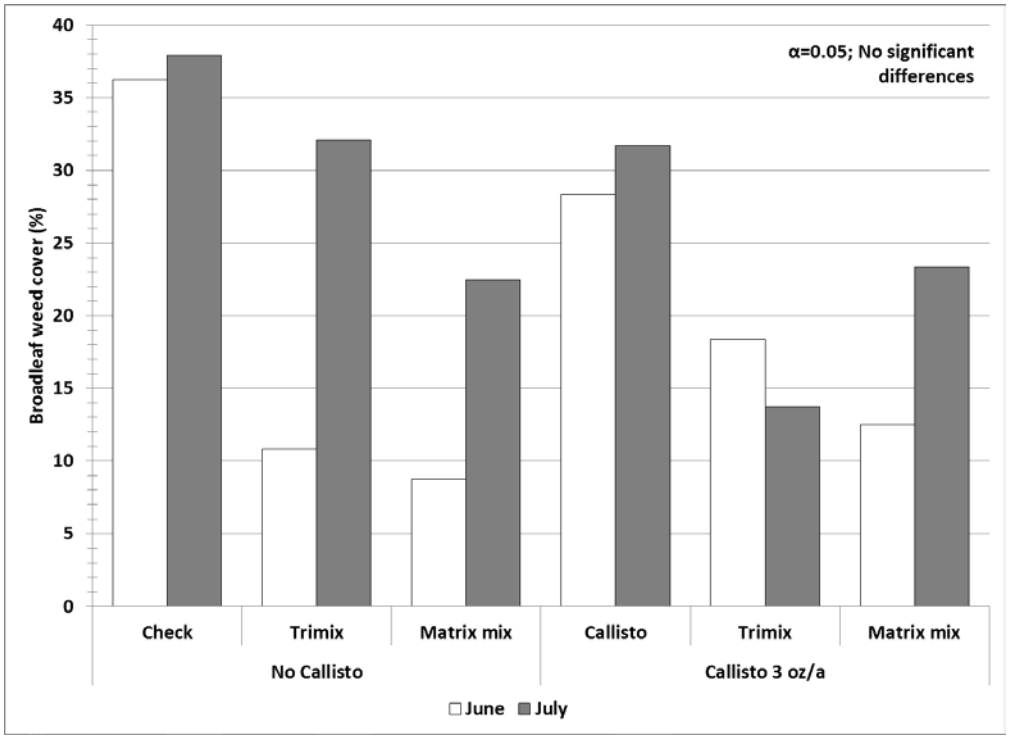
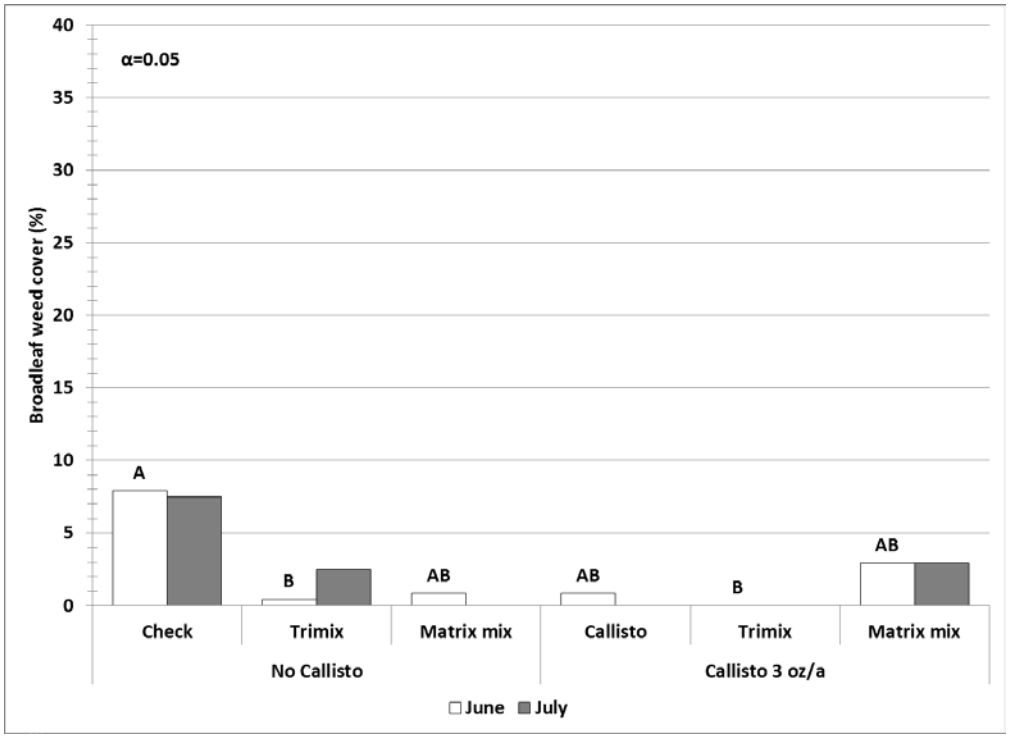


Figure 10. Broadleaf weed cover at the Orland site, following pre-emergence applications of Sinbar-herbicide combinations with and without post-emergence Callisto (different letters denote significance only, at α=0.05).



Grass cover was higher overall at the Jonesboro site. In June there were no significant differences among treatments, and the check/Callisto (2nd check) had the highest grass cover (Figure 11). It should be noted that visual observation showed that the Matrix mix and Trimix killed Kentucky bluegrass (*Poa pratensis*) and fineleaf sheep fescue (*Festuca filiformis*). By July, grasses had been eliminated in all treatments except the check and Callisto only.

There were almost no grasses at the Orland site; in June only Matrix mix with Callisto had any grass (<1% cover) and in July only the check and Matrix mix with Callisto had grasses (3% and <1%, respectively; Figure 12). There were no significant differences at either evaluation.

Figure 11. Grass cover at the Jonesboro site, following pre-emergence applications of Sinbar-herbicide combinations with and without post-emergence Callisto (different letters denote significance only, at α=0.05).

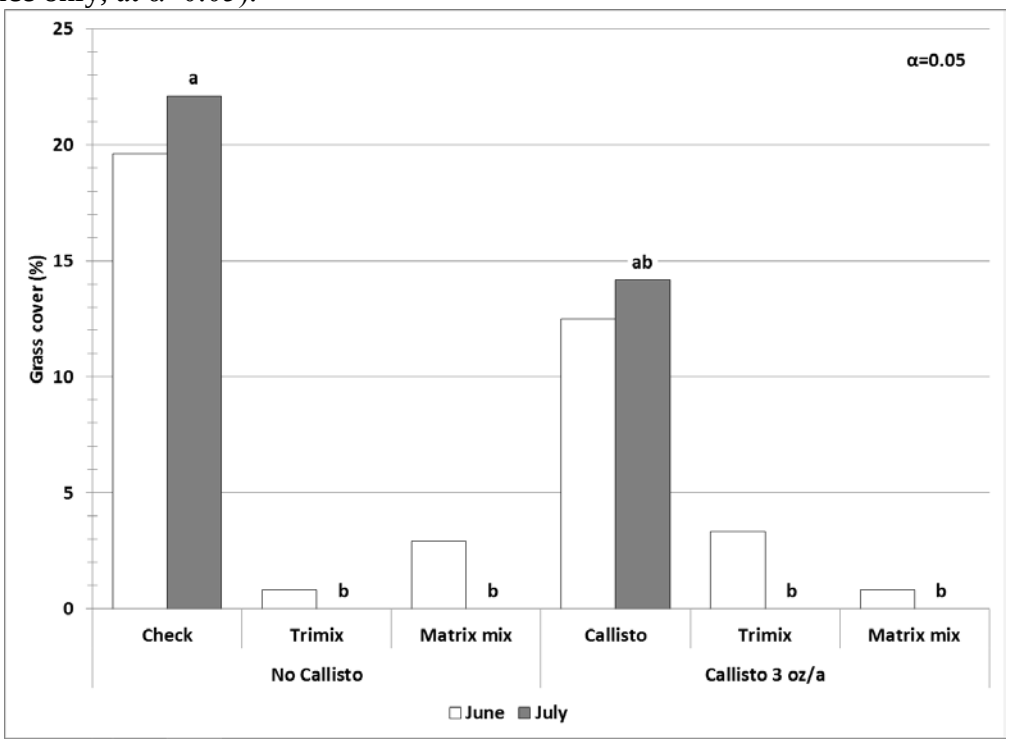
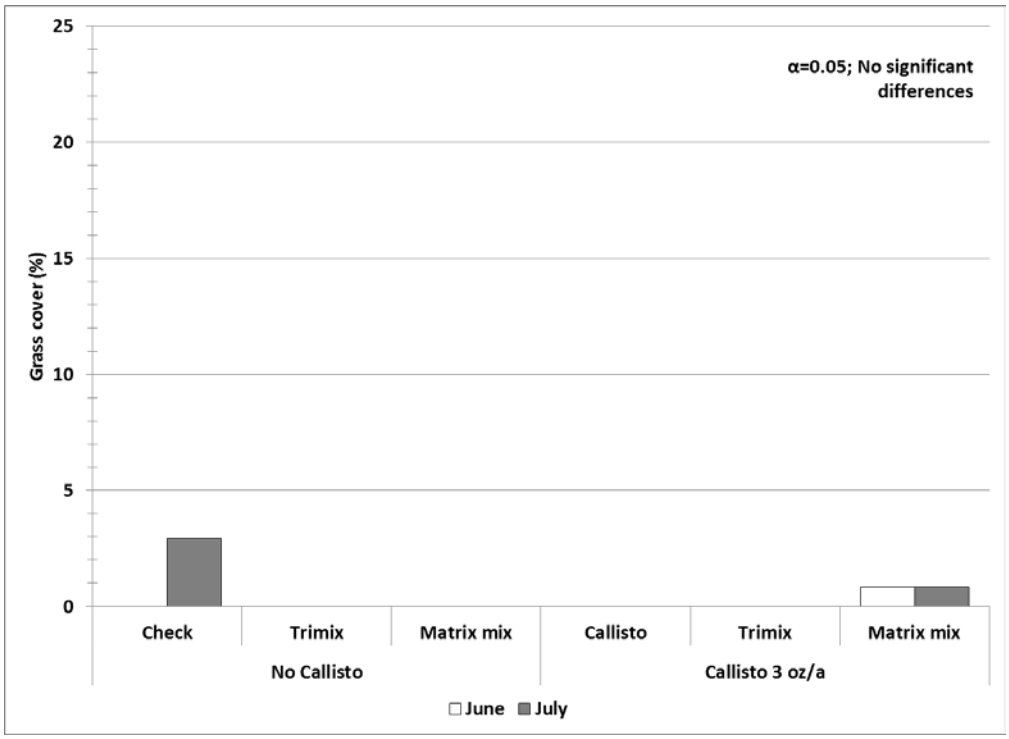


Figure 12. Grass cover at the Orland site, following pre-emergence applications of Sinbar-herbicide combinations with and without post-emergence Callisto (α=0.05, no SD).



CONCLUSIONS:

Main effects

Figure 2 shows that injury to blueberry in this trial was driven mainly by the addition of Callisto, and was evidenced by chlorosis in July consistent with Callisto injury symptoms (Photo 1). The injury is probably due to the Callisto being applied on warm days in the 70s, although previous work and the label indicate that injury is more likely when applied at temperatures exceeding 85°F.

Figures 3 and 4 indicate that the addition of post-emergence Callisto aided in controlling broadleaf weeds and grasses, but was not the main driver of weed control on these sites. Therefore, we can conclude that post-emergence Callisto is an effective component of a multi-herbicide, multi-Mode of Action approach to weed control but should not be relied upon as the sole source of weed control.

Photo 1. Phytotoxicity in July in the Matrix mix treatment with Callisto, Orland. Note chlorosis consistent with Callisto injury.

All-treatment comparisons

When injury to blueberry is examined without the effect of Callisto factored in (see Figures 7-8), we see that pre-emergence Trimix and Matrix mix resulted in minor phytotoxicity initially, but the plants had recovered by mid-season (Photos 2-3). The addition of post-emergence Callisto resulted in a higher level of injury that persisted further into the season.

Broadleaf weed control did not show a consistent trend over time or between sites. In Jonesboro, Callisto resulted in fewer broadleaf weeds when added to the check or Trimix, but did not affect weed cover in the Matrix mix (see Figure 9). The Matrix mix controlled early season weeds better than Trimix but not necessarily by mid-season, and the differences were not significant. In Orland, Trimix resulted in the best early season weed control, but not necessarily

mid-season control (see Figure 10). The weed species at the two sites were almost completely different, and the efficacy of weed control likely depended on which species were present and susceptible to the herbicides used.

Grass control at the Jonesboro site was driven by the components of the Matrix mix and Trimix, most likely Sinbar, not by the addition of Callisto (see Figure 11 and Photos 2-3). No conclusions may be made about control of grasses at the Orland site, as there were almost no grasses onsite to begin with (see Figure 12). However, there were many grasses along the access road and rock wall immediately adjacent to the plots; the difference was that the trial area had been pruned by burning, whereas the adjacent areas had not been burned (Photo 4). Therefore, it is likely that on the Orland site grasses were controlled by burning, because the Jonesboro site was pruned by mowing and had several grass species. The burning at the Orland site was also likely why the site had fewer broadleaf weeds overall compared to Jonesboro.

Photo 2. The Matrix mix treatment in July, Block 3 in Jonesboro. Note weed control in the foreground (no Callisto), which opened up bare ground between blueberry stems.

Photo 3. The Trimix treatment in July, Block 3 in Jonesboro. Note weed control in the foreground (no Callisto), which opened up bare ground between blueberry stems.

Photo 4. The Orland site in July, looking across blocks from Block 1 towards Block 3. Note the weeds to left along rock wall and in foreground, compared to lack of weeds inside trial area.

RECOMMENDATIONS: The Matrix mix controlled early season weeds better than Trimix but not necessarily by mid-season, and the differences were not significant; so Matrix could be a substitute for diuron in the Trimix combination, but the efficacy of weed control will likely depend on which weed species were present and if they are susceptible to the herbicides used.

WEED MANAGEMENT

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

12. TITLE: Single vs split applications of post-emergent herbicides for spreading dogbane (*Apocynum androsaemifolium*) control in wild blueberry fields – crop year results.

METHODS: Spreading dogbane (*Apocynum androsaemifolium*) is a major weed pest in wild blueberry fields, and is not being controlled with the registered herbicides. In spring 2015 we initiated a trial at the University of Maine's Blueberry Hill Experiment Station Farm, to examine the effect of Callisto and Matrix combinations on dogbane control. Dogbane was sprayed post-emergence to four 1 x 2 m plots in the following combinations, all with COC 1% v/v: Callisto 6 oz/a, Matrix 4 oz/a, Callisto 6 oz/a + Matrix 4 oz/a, Callisto 3 oz/a (2x), Matrix 2 oz/a (2x) and Callisto 3 oz/a + Matrix 2 oz/a (2x), on 3 June 2015, and the split treatments were sprayed a second time on 17 June 2015. In June 2016, wild blueberry cover, dogbane cover and other weed cover were compared among treatments, to an untreated check and to the Blueberry Hill Farm's 5/13/15 pre-emergence application of Velpar 2 lb/a, Sinbar 2 lb/a and diuron 1.6 qt/a with Grounded additive at 2 pt/a combined with a 5/27/15 post-emergence application of Callisto 6 oz/a and Select 6 oz/a. Cover data were determined by using the Daubenmire Cover Class system converted to percent. As in 2015, the treatments were compared using Tukey's tests (α=0.05) to determine significant differences.

RESULTS: There were no significant differences among treatments for wild blueberry cover, dogbane cover (Figure 1) or broadleaf weed or grass cover (Figure 2). Although Blueberry Hill Farm's 2015 applications eliminated other broadleaf weeds and grasses, it did not eliminate dogbane. Of the trial treatments, the best carryover dogbane control was observed in the Callisto 6 oz/a and split Callisto 3 oz/a treatments, although the effect was not significant (Figure 1). For other broadleaf weeds, most carryover control was seen in the full rate Callisto 6 oz/a plus Matrix 4 oz/a combination treatment, followed by the split rate Callisto 3 oz/a and full rate Matrix 4 oz/a treatments; again, the effect was not significant (Figure 2). One application of Callisto did not result in as good carryover control as the split application, regardless of whether it was combined with Matrix. Finally, grass cover was very low, most likely because of 2016's dry hot summer; no treatment had over 5% grass cover, but carryover grass control was best in the Callisto treatments, as the Matrix only treatments had slightly more grass cover.

Figure 1. Wild blueberry and dogbane cover in 2016 following 2015 post-emergence applications of Callisto and Matrix (α=0.05, no significant differences).

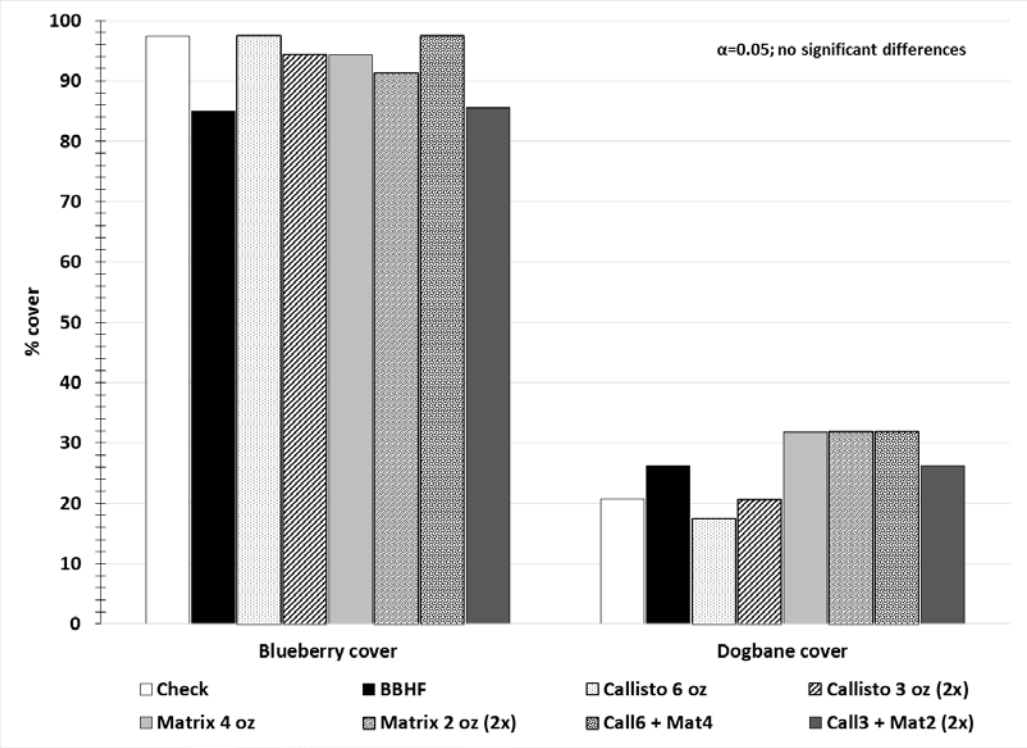
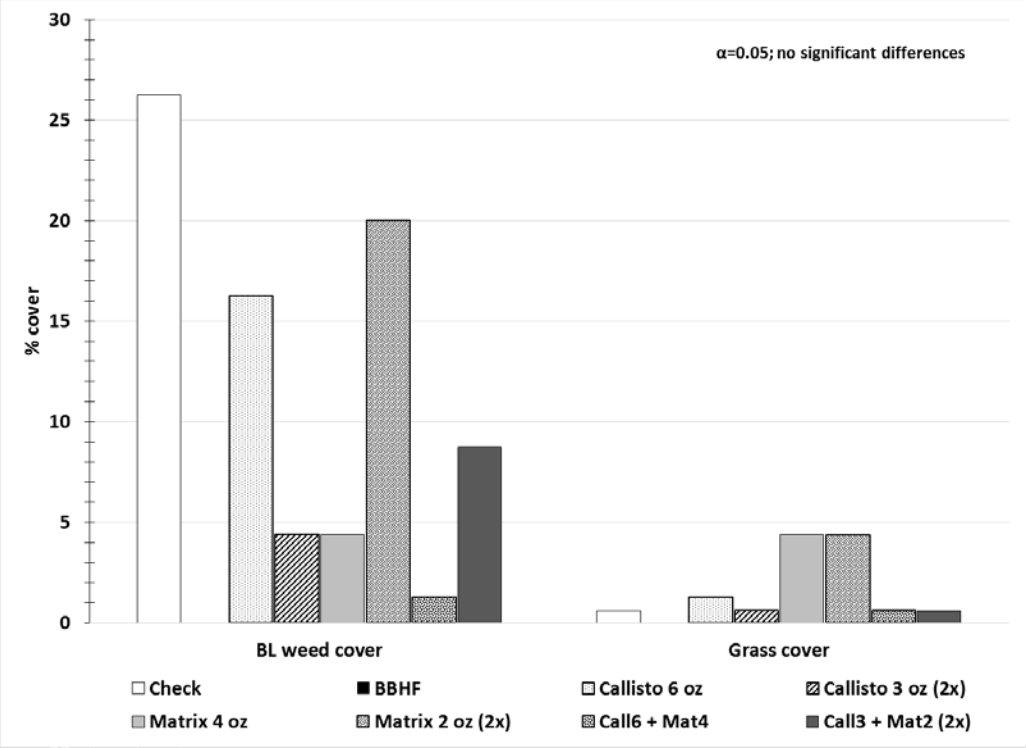


Figure 2. Broadleaf weed and grass cover in 2016 following 2015 post-emergence applications of Callisto and Matrix (α=0.05, no significant differences).



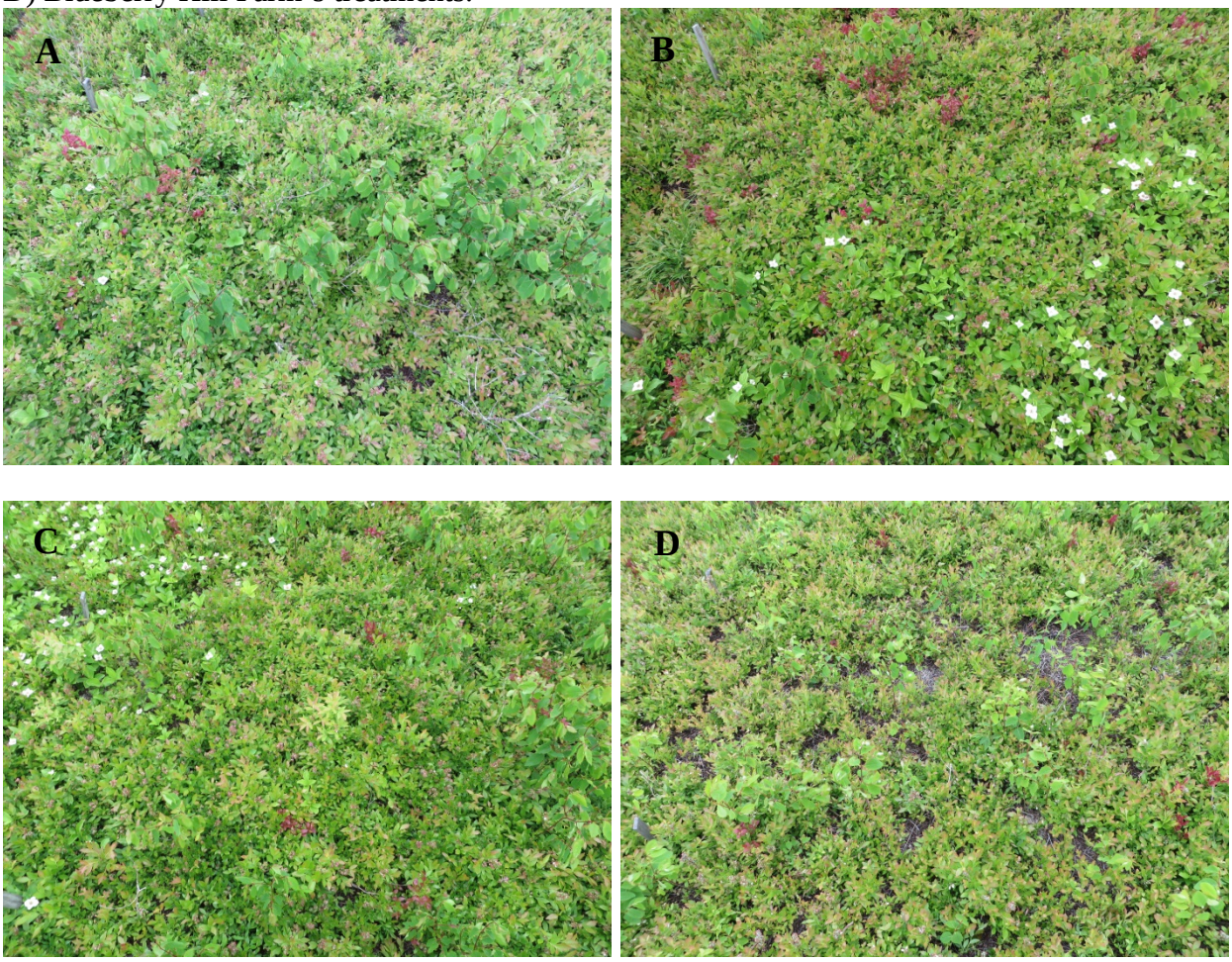
CONCLUSIONS: In 2015, the Callisto and Callisto + Matrix tank mixes resulted in higher dogbane injury and lower cover overall, indicating that Callisto was the most effective treatment for controlling this weed. Although there were no significant differences in 2016, the Callisto and split Callisto + Matrix tank mix treatments also resulted in the most carryover control of dogbane. The blueberry phytotoxicity observed in 2015 in the Matrix 4 oz/a and Callisto + Matrix split treatments did not significantly reduce blueberry cover in the crop year.

Although BBHF's 2015 treatments of pre-emergence Velpar 2 lb/a + Sinbar 2 lb/a + Diuron 1.6 qt/a with one post-emergence application of Callisto 6 oz/a + Select 6 oz/a effectively eliminated other weeds in the crop year, it not only did not eliminate dogbane, but appeared to release it slightly compared to the check or to Callisto only treatments, as seen in Figure 1. It is unclear as to why the Farm's Callisto treatment did not control dogbane as well as the trial treatment did, but it may be at least partly due to the Farm's treatment eliminating other weeds thereby reducing competition.

In 2015, we concluded that the split Callisto 3 oz/a treatment was the most effective in controlling spreading dogbane, that the addition of Matrix did not improve the control and was unnecessary, and that additional applications of Callisto later in the season would be needed to get complete control of dogbane. Although the latter two statements were supported in crop year 2016, the post-emergence Callisto 6 oz/a treatment continued to suppress dogbane in the crop year at least as well as the split treatment (Figure 1), indicating that if time and money to apply herbicides are issues, then applying Callisto once will reasonably suppress dogbane as long as other weeds aren't a significant problem (see Figure 2 for other broadleaf weed and grass cover, Callisto 6 oz/a versus 3 oz/a)

In conclusion, the split Callisto 3 oz/a treatment was the most effective in controlling dogbane and other weeds in both the prune and crop years, and Matrix is unnecessary to control this weed. However, additional Callisto applications do appear necessary to better control this weed; see the 2016 Dogbane Trial report for results and discussion of the effects of four Callisto applications on dogbane.

Photo 1. Dogbane in 2016 in A) the untreated check, B) Callisto 6 oz/a, C) Callisto 3 oz/a and D) Blueberry Hill Farm's treatments.



RECOMMENDATIONS: Pursue a 24-C label change to increase the number of applications of Callisto.

WEED MANAGEMENT

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

13. TITLE: Comparison of multiple post-emergence Callisto applications for spreading dogbane (*Apocynum androsaemifolium*) control in wild blueberry fields.

METHODS: Spreading dogbane (*Apocynum androsaemifolium*) continues to be a major weed pest in wild blueberry fields. In spring 2015 we initiated a trial at the University of Maine's Blueberry Hill Experiment Station Farm, to examine the effect of Callisto and Matrix on dogbane control. Dogbane was sprayed post-emergence either once at 6 oz/a or twice at 3 oz/a, but neither rate fully controlled dogbane in either the prune or crop year.

In 2016, a follow-up trial was initiated at Cherryfield Foods' Pike Brook 3 Lot, which has had a large dogbane population. The trial was set up as a Completely Randomized Design with each plot split in half; the main treatments consisted of an untreated check, Callisto 2 oz/a + COC 1% v/v and Callisto 3 oz/a + COC 1% v/v. Six replications of 4-m^2 plots per main treatment were staked pre-emergence and half of each plot was treated pre-emergence with Velpar 2 lb/a on 10 May 2016. Once wild blueberry emerged, dogbane emergence and growth were tracked on a weekly basis and the plots were sprayed in entirety at approximately two week intervals for a total of three post-emergence Callisto applications on 26 May, 8 June and 22 June. Prior to each Callisto application, wild blueberry cover and phytotoxicity, dogbane cover and phytotoxicity, broadleaf weed cover and grass cover were assessed, and at 2.5 weeks after the last application, on 11 July. Cover data were determined by using the Daubenmire Cover Class system converted to percent, and phytotoxicity using a scale of 0-10 (0=no damage, 10=100% damaged/dead) which was converted to percent. The treatments were compared using Tukey's tests (α=0.05) to determine significant differences among all treatments, and t-tests (α=0.05) to compare Velpar versus no Velpar for each main treatment.

RESULTS:

All treatment comparisons

There were no significant differences in wild blueberry cover (Figure 1) or phytotoxicity (Figure 2). As expected, blueberry cover increased over time, with the Callisto 2 oz/a treatment ultimately having the highest cover regardless of Velpar application. There was initially some phytotoxicity observed at the May evaluation in all treatments; it was determined that this was due to Cherryfield Foods' driving through the trial area while spraying the rest of the field with Callisto 3 oz/a + NIS + Request on 18 May. Although they turned off the tractor's spray boom, residual pressure in the boom caused spray solution leakage from the nozzles onto plants in the trial area, and therefore was assessed as background injury because it could not be separated from injury due to our trial applications (Figures 2, 4). The wild blueberry recovered by the second evaluation and from thereon out, all blueberry and dogbane phytotoxicity was assumed to be from trial treatment effects.

Dogbane cover was not significantly different among treatments at the first three evaluations, but by the fourth evaluation Callisto at 2 oz/a with Velpar, and Callisto at 3 oz/a both with and without Velpar, controlled dogbane significantly better than the check, Velpar alone or Callisto 2 oz/a (Figure 3, Photo 1A-C). In fact, the former three treatments reduced

dogbane cover to less than 10% by July, and no new seedlings were observed (new seedlings had been observed in May and at both June evaluations). The two June evaluations and July evaluation had significant differences in dogbane phytotoxicity (Figure 4). The treatments with Velpar tended to have slightly more injury to dogbane than those without, but at all evaluations there were no differences between the Callisto treatments with Velpar compared to without Velpar. There was also no difference between the check and Callisto only treatments on 8 June, but on 22 June and 11 July the Callisto treatments resulted in significantly more dogbane injury compared to the check or Velpar alone. The greatest dogbane injury was ultimately in the Callisto 3oz/a + Velpar treatment, which correspondingly resulted in the lowest dogbane cover (2%).

There were no significant differences among treatments for broadleaf weed cover (Figure 5) or grass cover (Figure 6). Grass cover was extremely low in 2016, likely due to the hot dry summer; even the check had <1% grass cover at all evaluations and therefore, treatment differences or lack thereof could not be determined with certainty. Although there were no differences in broadleaf weed cover, the Callisto 2 oz/a treatment had the lowest cover overall at each evaluation, regardless of Velpar, while the 3 oz/a treatment had the highest regardless of Velpar.

T-tests

T-tests for examining the effects of Velpar addition to the main treatments yielded no significant differences for any of the variables assessed, for any of the treatments at any evaluation date. Therefore, the results are not presented here.

Figure 1. Wild blueberry cover following pre-emergence application of Velpar and post-emergence applications of Callisto (α=0.05, no significant differences).

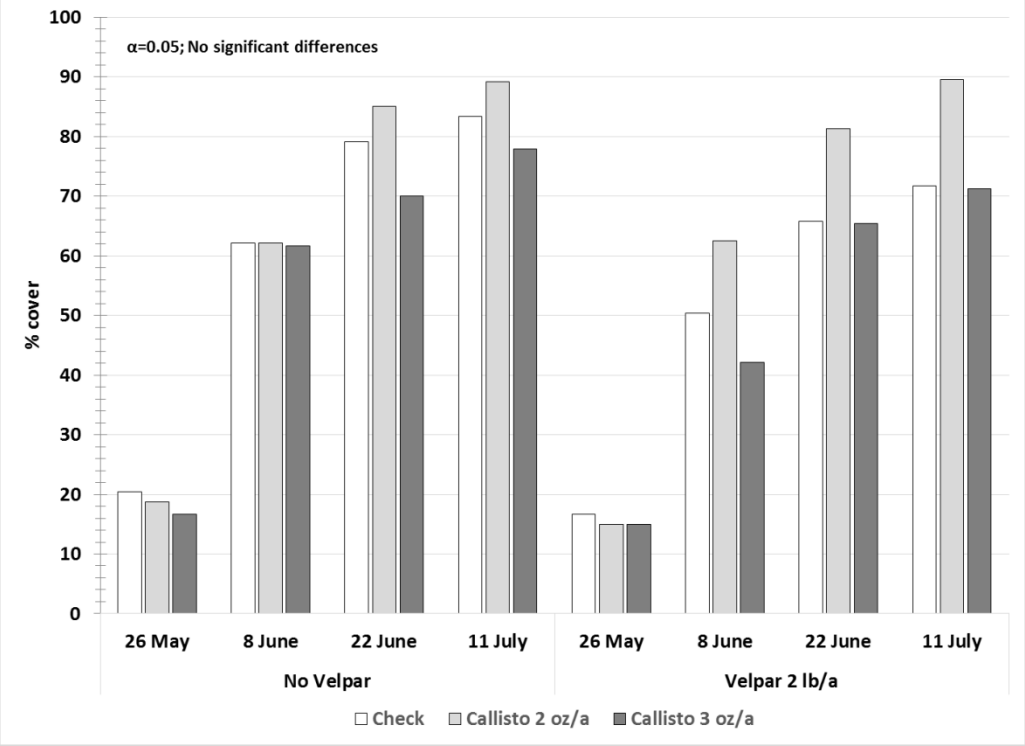


Figure 2. Wild blueberry phytotoxicity following pre-emergence application of Velpar and post-emergence applications of Callisto (α=0.05, no significant differences).

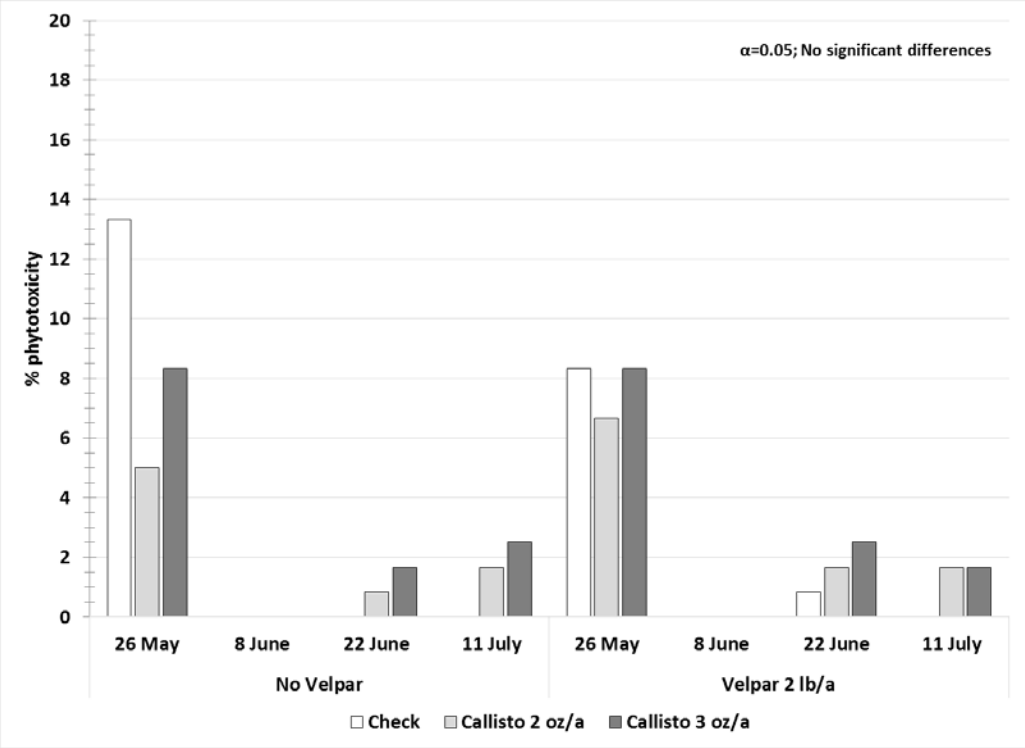


Figure 3. Dogbane cover following pre-emergence application of Velpar and post-emergence applications of Callisto (letters denote significant results only, at α=0.05).

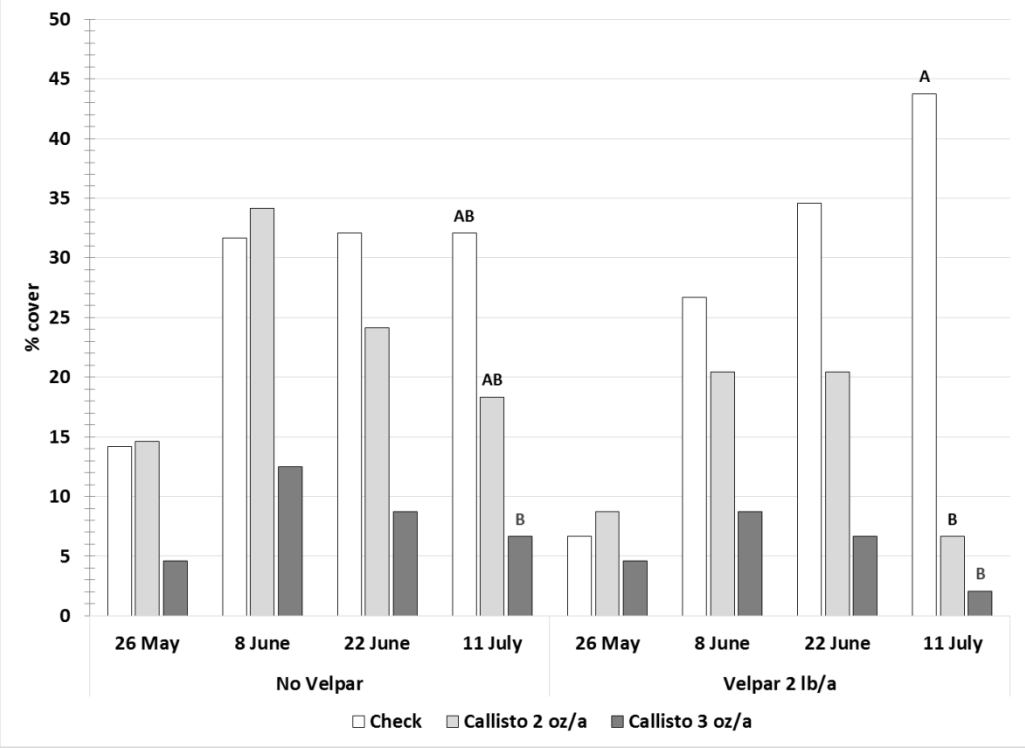


Figure 4. Dogbane phytotoxicity following pre-emergence application of Velpar and post-emergence applications of Callisto (α=0.05).

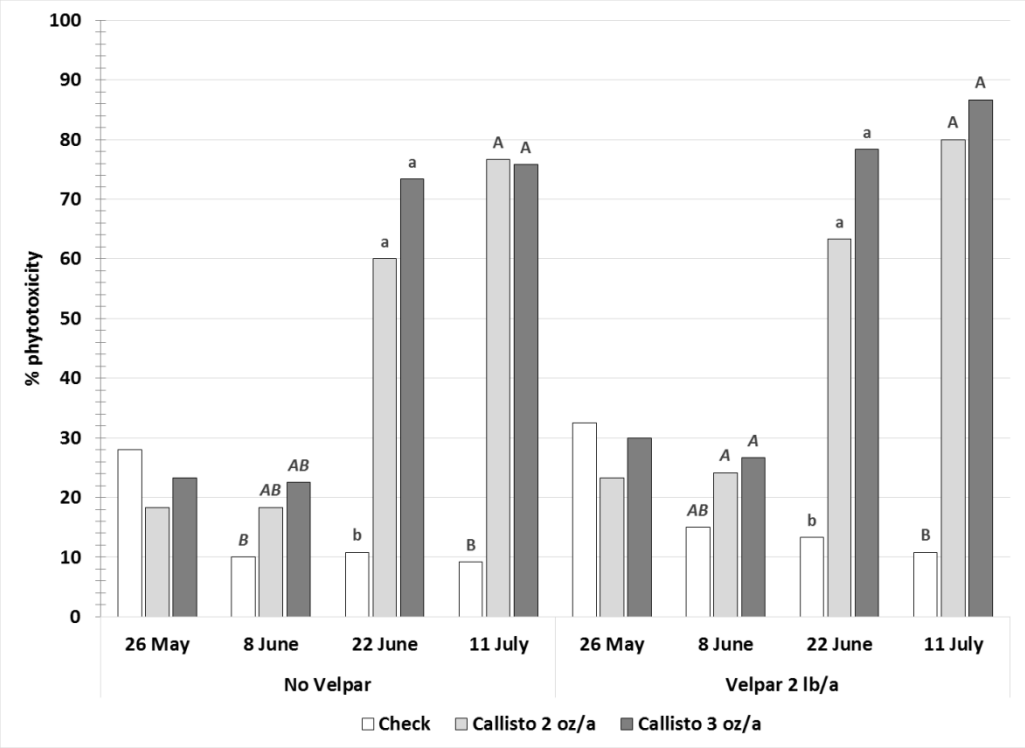


Figure 5. Broadleaf weed cover following pre-emergence application of Velpar and post-emergence applications of Callisto (α=0.05, no significant differences).

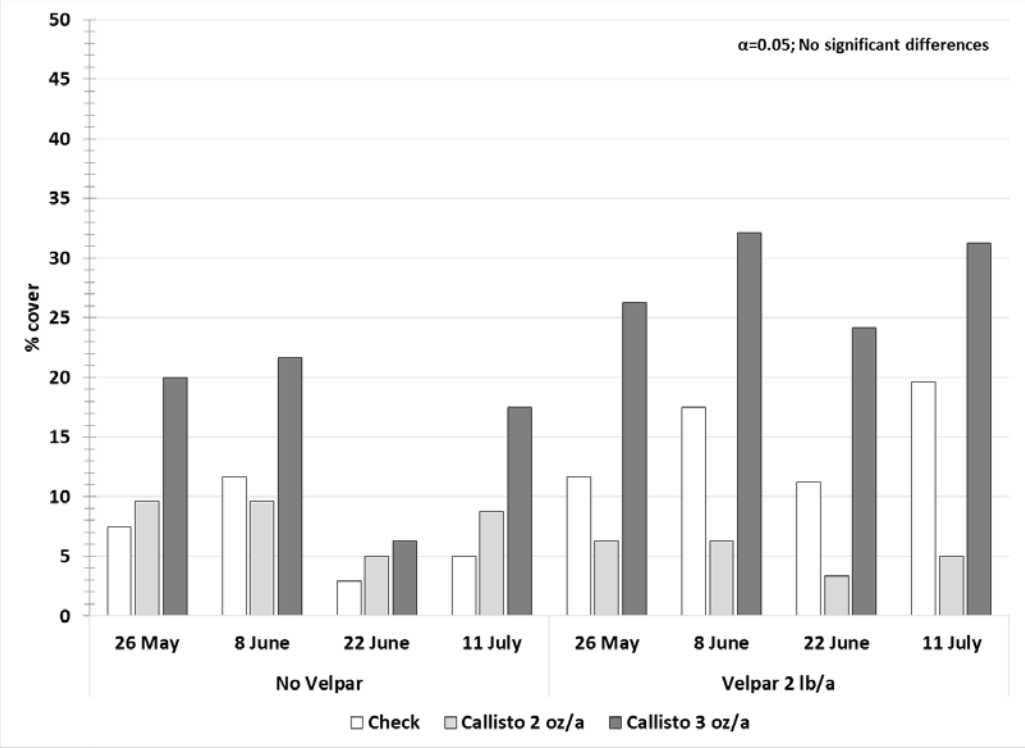
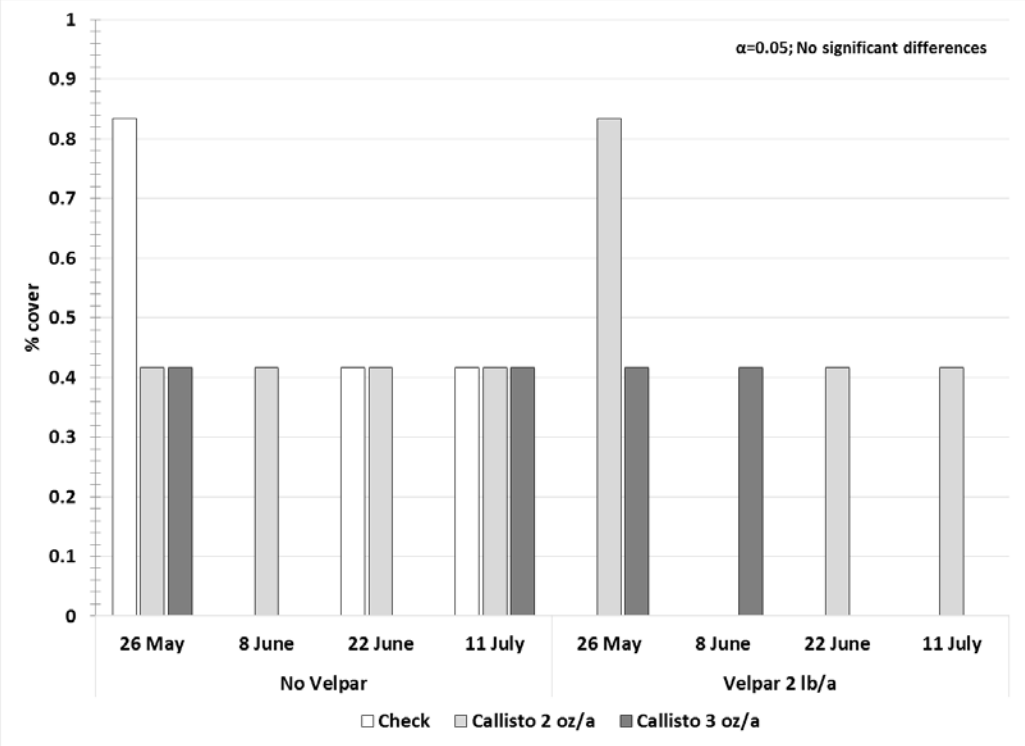


Figure 6. Grass cover following pre-emergence application of Velpar and post-emergence applications of Callisto (α=0.05, no significant differences).



CONCLUSIONS: Although the Velpar combinations and Callisto 3 oz/a alone almost eliminated dogbane and no new seedlings were observed in July, dogbane was not completely controlled by any treatment. Some stems which appeared dead on 22 June showed slight regrowth of lateral leaves in July (Photo 2). Because dogbane is perennial, it is uncertain whether the reduction in root reserves from leafing out again would reduce or prevent emergence or reproduction the next year.

In contrast to the effects on dogbane cover, in which the Callisto 3 oz/a treatment was most effective in reducing dogbane and more so when combined with Velpar, the effect on other broadleaf weeds was the opposite. The higher rate of Callisto resulted in higher broadleaf weed cover, more so when combined with Velpar. The principal weed in this category was red sorrel (*Rumex acetosella*), another problem weed which is hard to control with Velpar or Callisto and is the subject of several University of Maine trials. We posit that the increase in broadleaf weeds with the higher rate of Callisto, namely red sorrel, is because the reduction of dogbane opened up the over story and increased the amount of light available which increased the growth of red sorrel, while at the same time any dogbane over story still present intercepted some spray solution so the red sorrel did not receive as much herbicide (Photo 1C).

The results of the t-tests indicate that the addition of Velpar does not significantly change the effects of Callisto on dogbane, although as stated above, there was a non-significant effect of slightly increased dogbane control and injury. Cherryfield Foods' herbicide regime for the same field resulted in less lateral regrowth of dogbane compared to the plants in the trial area. They applied Callisto 3 oz/a on 18 and 31 May with NIS and Request adjuvant, but they also hand wiped the plants with Roundup + Request on 18-20 June (Photo 1D).

Photo 1. Dogbane cover at the July 2016 evaluation in A) the untreated check, B) Callisto 2 oz/a no Velpar, C) Callisto 3 oz/a with Velpar, and D) Cherryfield Foods' treatments.

Photo 2. Example of lateral regrowth from nodes, in the trial area.

RECOMMENDATIONS: Pursue a 24-C label change to allow for more applications of Callisto.

WEED MANAGEMENT

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

14. TITLE: Evaluation of spring applications of herbicides targeting red sorrel in wild blueberry fields – crop year 2016 *(final report, SCRI ancillary study)*.

METHODS: Several herbicides, both registered and unregistered, are currently under review for use on wild blueberry. Alion (6.5 oz/a), Chateau (12 oz/a), Sandea (1 oz/a) and Trellis (1.33 lb/a) are pre-emergence herbicides, while Matrix (4 oz/a) may be used pre- or post-emergence. We treated red sorrel (*Rumex acetosella*) in spring 2015 to evaluate these herbicides on this established problem weed. Experimental design was a completely randomized plot. The treatments were applied to red sorrel on ten 1-m^2 plots on 14 May 2015 and five of the plots were treated with Velossa (0.4 gal/a) on the same day by the grower. In the crop year, wild blueberry, red sorrel, broadleaf weed and grass percent cover were evaluated on 14 June 2016. Cover data were determined by using the Daubenmire Cover Class system converted to percent. All treatments were compared to each other using Tukey's tests (α=0.05) to determine significant differences. Velossa versus no Velossa for each main treatment were also compared using t-tests (α=0.05) to determine significant differences. The plots were hand raked on 10 August 2016 and were weighed on-site with an analog scale. The weights were converted to lbs/a, all treatments were compared using a Tukey's test, and Velossa versus no-Velossa within each main treatment was compared using t-tests (α=0.05).

RESULTS:

Cover

There were no significant differences in wild blueberry cover, either among treatments or between Velossa and no-Velossa for each main treatment (Figure 1). Red sorrel cover was lower overall in the treatment plots with Velossa. There were no differences among treatments, but the plots without Velossa had significantly higher red sorrel cover compared to the plots with Velossa in the Alion, Chateau and Matrix treatments (Figure 1, Photos 1-3). Other broadleaf weed cover and grass cover were both low in the crop year (<12% overall), and there were no differences among all treatments or between Velossa and no-Velossa (Figure 2).

Figure 1. Wild blueberry cover and red sorrel cover in 2016 following spring 2015 treatments for red sorrel control (α=0.05. All treatments compared with Tukey's test; no Velossa vs Velossa compared with t-tests – symbols indicate significance within treatment).

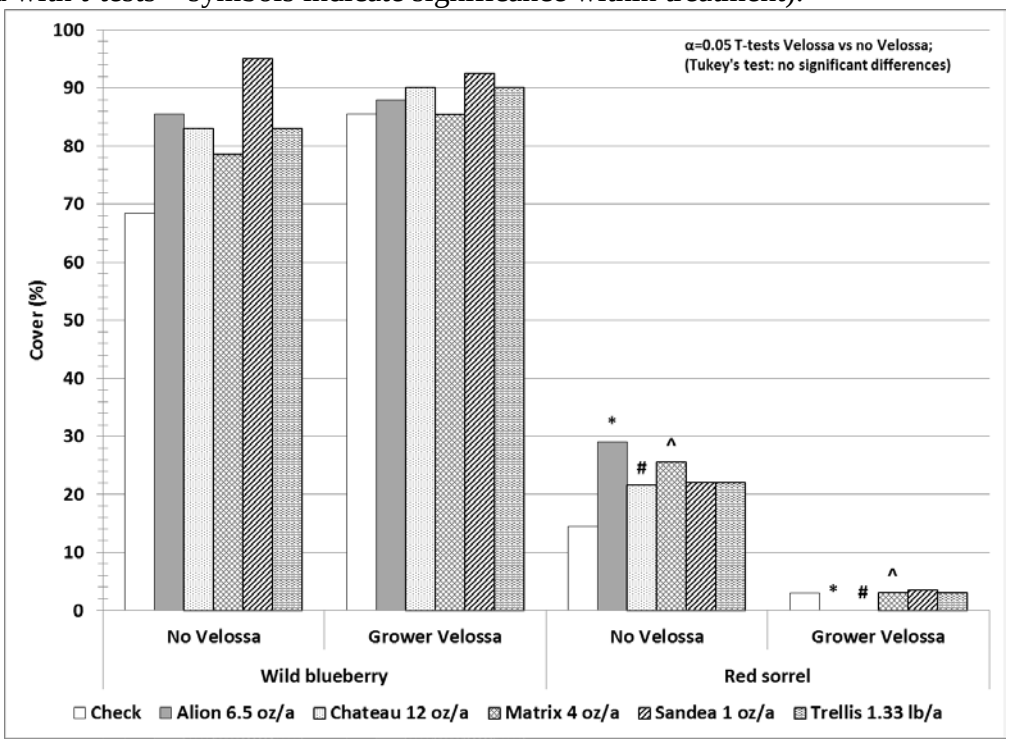


Figure 2. Broadleaf weed and grass cover in 2016 following spring 2015 applications of herbicides for red sorrel control (α=0.05. All treatments compared with Tukey's test; no Velossa vs Velossa compared with t-tests – no significant differences).

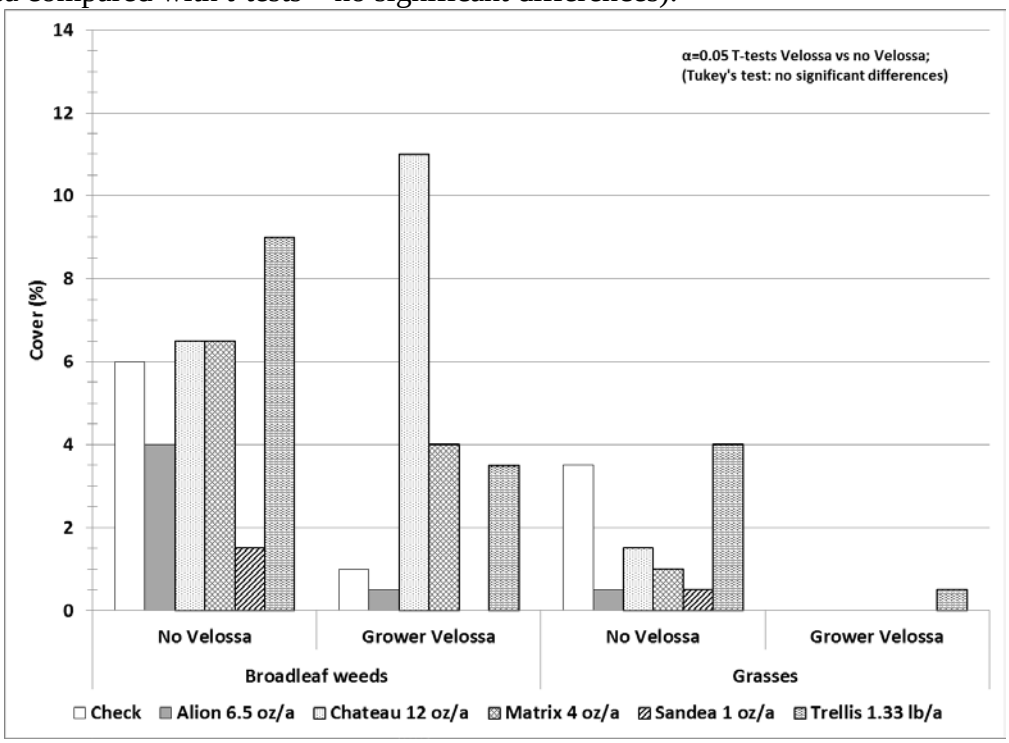


Photo 1. The Alion treatment in the crop year. L: no Velossa, R: with Velossa.

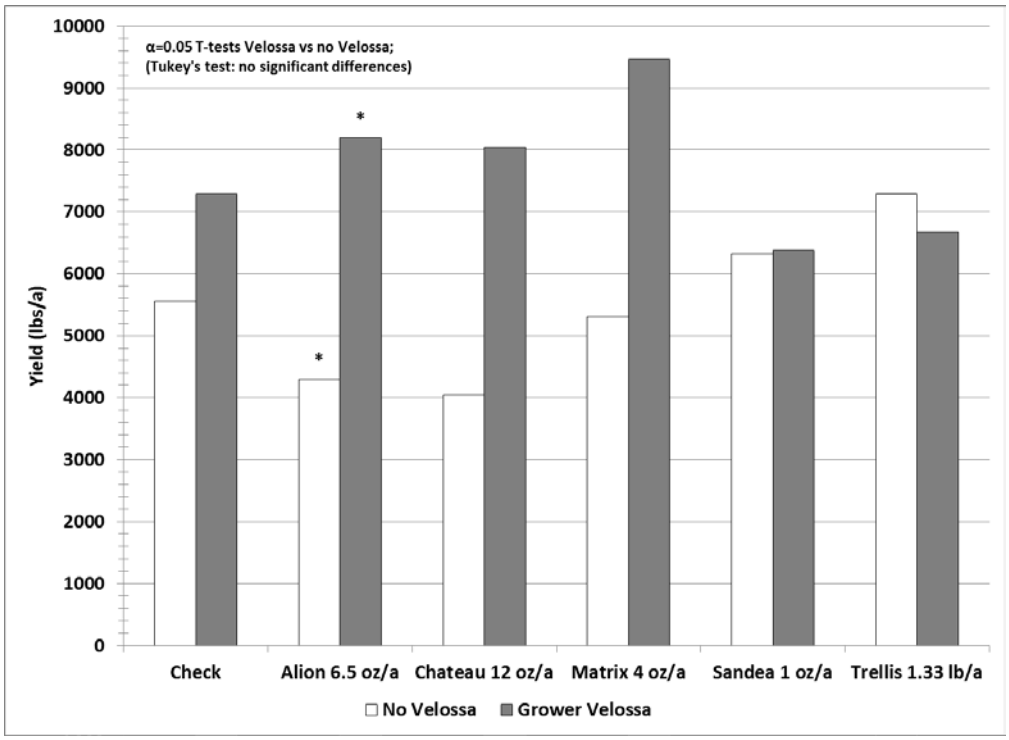
Photo 2. The Chateau treatment in the crop year. L: no Velossa, R: with Velossa.

Photo 3. The Matrix treatment in the crop year. L: no Velossa, R: with Velossa.

Yield

Yield was generally higher in the plots with Velossa compared to those without, with the exception of Sandea and Trellis (Figure 3). However, the only significant difference was found in the Alion treatment, which had significantly higher cover in the Velossa plots. When all treatments were compared, there were no significant differences. Matrix+Velossa resulted in the highest yield (9,460 lbs/a), while Chateau alone resulted in the lowest yield (4,047 lbs/a).

Figure 3. 2016 yield following spring 2015 herbicide applications for red sorrel control (α=0.05. All treatments compared with Tukey's test; no Velossa vs Velossa compared with t-tests – symbols indicate significance within treatment).



CONCLUSIONS/RECOMMENDATIONS: The plots without Velossa had higher red sorrel cover overall, which translated to having lower yields overall. Alion, Chateau and Matrix were the only treatments to have significantly lower red sorrel cover in the Velossa plots compared to no-Velossa, and the Velossa plots correspondingly had the three highest yields. However, red sorrel cover in the check (3%), Sandea (3.5%) and Trellis (3%) Velossa treatments were comparable to the Matrix Velossa treatment (3%), yet had lower yields. The yield reduction does not appear to be correlated with broadleaf weed cover or grasses, because these covers were very low in the crop year as well as the prune year. Phytotoxicity to wild blueberry in the prune year was highest in the Sandea treatment in June 2015, which may have contributed to the lack of yield response in the Velossa plots, but it was less than 16% in June and there was no phytotoxicity evident in September 2015. Phytotoxicity to wild blueberry was below 20% for all treatments in 2015, so it is unlikely that blueberry injury caused the lower yields in the no-Velossa plots or in the Sandea and Trellis Velossa treatments. It is more likely that early season phytotoxicity to red sorrel influenced yield in the crop year. Overall phytotoxicity was higher in the Velossa plots for all treatments at both prune year evaluations, except for no-Velossa Matrix

in June 2015. In September 2015, phytotoxicity in the Velossa plots was 90-100% for all treatments. In June 2015 red sorrel injury in the Velossa plots was highest in the check, Alion and Chateau treatments. The check, Alion and Chateau Velossa treatments correspondingly had the 4th, 2nd and 3rd highest yields in 2016, respectively. However, it is unclear as to why the Matrix Velossa treatment had the highest yield, considering that it had 39% red sorrel injury in June 2015 compared to Chateau at 88%, Alion at 62% and Velossa only at 53% as well as 12% June cover as opposed to Chateau at 0.5% and Alion at 1.5%.

In 2015 we stated that the addition of Alion and Chateau improved the effectiveness of red sorrel control when combined with Velossa, particularly early in the growing season when the blueberry plants are small and more easily outcompeted, and should be evaluated further. In 2016 we found that the addition of Velossa to any of the products tested reduced red sorrel cover in the crop year and increased yields, even if the differences weren't necessarily significant. In 2015 we also stated that Trellis was dropped from further trials as it wasn't more effective than other products alone or with Velossa. The crop year yield bears this out to an extent, as the addition of Velossa did not increase yields as seen with Alion, Chateau or Matrix, although Trellis did have higher yields than the other products alone. Finally, in 2015 we stated that injury to blueberries was observed on spring treatments from past experiments with both Alion and Chateau, so these treatments should be applied in the Fall. The 2016 wild blueberry cover carryover and yield with Velossa do not support this supposition, although the yield for the herbicides without Velossa were the lowest of all treatments. In contrast, Matrix with or without Velossa had the lowest crop year blueberry cover and 2nd highest red sorrel cover, and had the 3rd lowest yield without Velossa but highest yield with Velossa. These inconsistencies lead us to believe that there may be another unknown confounding factor influencing yields.

WEED MANAGEMENT

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

15. TITLE: Evaluation of spring applications of herbicides targeting red sorrel in wild blueberry fields, 2016-17.

METHODS: Several herbicides, both registered and unregistered, are currently under review for use on wild blueberry. Alion, Chateau and Sandea are pre-emergence herbicides, while Matrix may be used pre- or post-emergence. We have been treating red sorrel (*Rumex acetosella*) since spring 2015 to evaluate these herbicides on this problem weed. This trial was set up pursuant to the results from the 2015-16 red sorrel trial, of which the crop year report is presented this year. In fall 2015 we located an area of red sorrel on Wyman's E.O. Morse lot. Ten $1m^2$ plots per treatment were set out, five which would be in prune in 2016 and five which would be in crop. The treatments were applied on 4 November 2015 to the dormant blueberry plants going into crop, or to the previously mowed field going into prune. Treatments were as follows:

1. untreated check;
2. Alion 6.5 oz/a;
3. Chateau 12 oz/a;
4. Matrix 4 oz/a; and
5. Sandea 1 oz/a.

Wild blueberry cover, red sorrel cover, broadleaf weed cover and grass cover were evaluated in July 2016. Cover data were determined by using the Daubenmire Cover Class system converted to percent. The treatments were compared individually to the check using t-tests (α=0.05) to determine significant differences. Yield data were gathered from the crop plots on 10 August 2016. The plots were hand raked and weighed onsite with an analog scale. The weights were then converted to lbs/a and t-tests (α=0.05) were conducted to compare each treatment to the check.

RESULTS:

Cover

There were no significant differences between any treatment and the check in the prune plots or crop plots for wild blueberry cover or red sorrel cover (Figure 1). The Chateau treatment resulted in the lowest red sorrel cover in both the prune and crop plots. In the prune field, all of the treatments had higher red sorrel cover than the check, but in the crop field only the Alion treatment was higher than the check.

Broadleaf weed and grass cover was less than 20% overall in the prune plots, and there were no grasses or broadleaf weeds other than red sorrel in the crop plots; there were no significant differences between any treatment and the check (Figure 2).

Because of the small sample size (n=5), we thought that variability in the data may have obscured treatment differences; therefore, we also plotted the Standard Error of the Mean (SEM) to confirm lack of significant differences. The SEMs did not show any differences in broadleaf weed or grass cover, but did show some differences in wild blueberry and red sorrel cover. In the prune field, Chateau had higher blueberry cover compared to the check (as well as Alion), and Sandea had higher red sorrel cover than the check (as well as Chateau). In the crop field, the check had higher blueberry cover compared to Alion (which was also lower than Chateau), Matrix and Sandea, while red sorrel cover in the check was higher than Chateau (which was also lower than Alion).

Figure 1. Wild blueberry and red sorrel cover in summer 2016 following fall 2015 applications for red sorrel control (α=0.05. No significant differences for t-tests; error bars are SEMs).

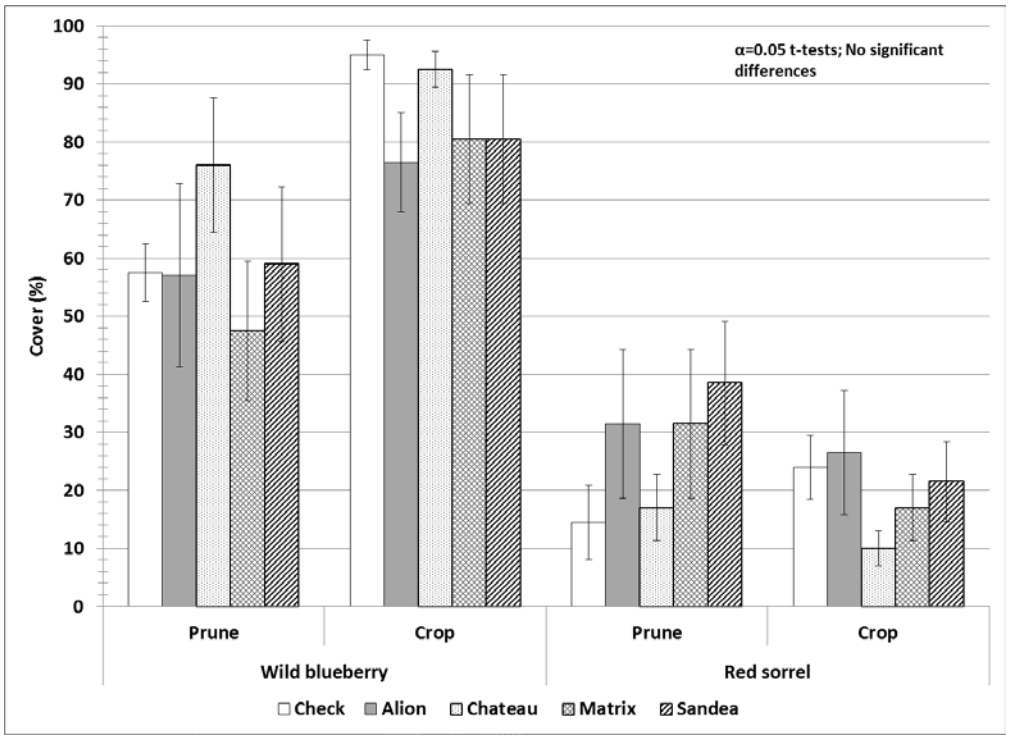
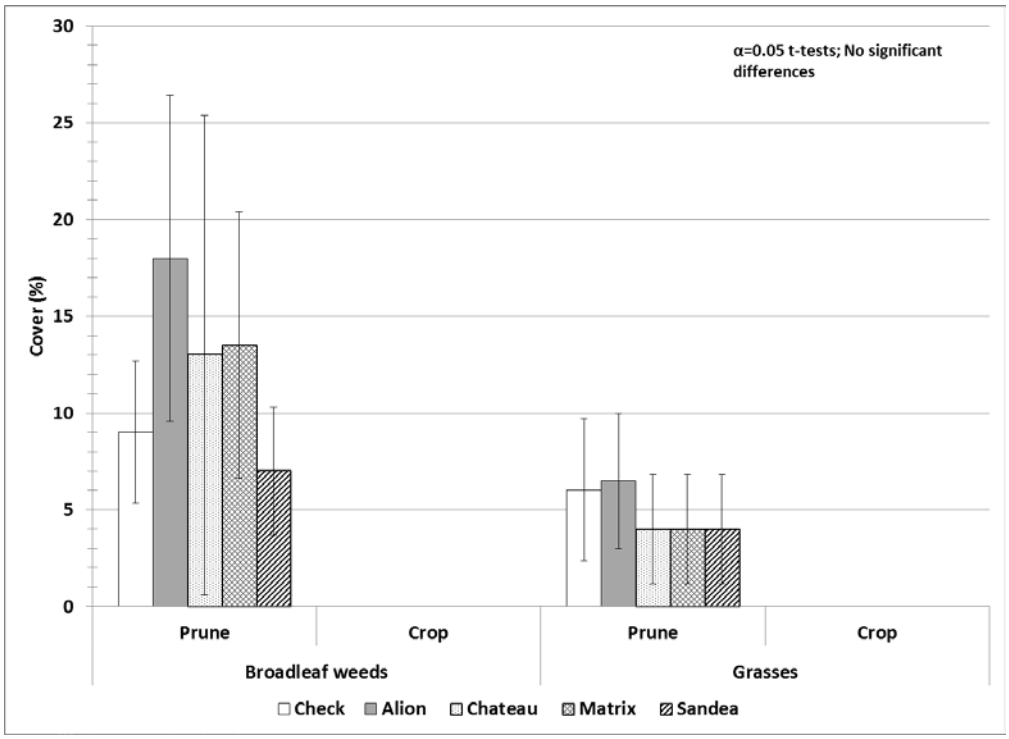


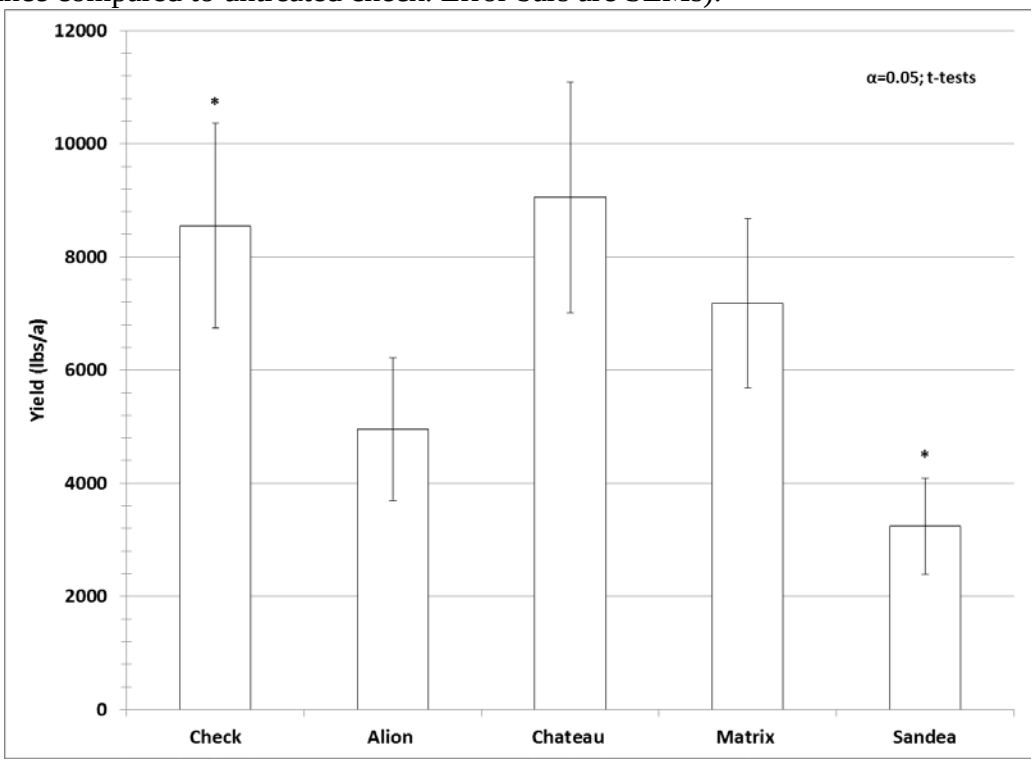
Figure 2. Broadleaf weed and grass cover in summer 2016 following fall 2015 applications for red sorrel control (α=0.05. No significant differences for t-tests; error bars are SEMs).



Yield

T-tests showed a significant difference in yield between the check and Sandea (Figure 3). When the SEMs were plotted, the check yield was also higher than the Alion treatment. In addition, Chateau yield was higher than Alion or Sandea, and Matrix yield was higher than Sandea as well.

Figure 3. Yield for the treatment plots in crop in 2016 (α=0.05 for t-tests - symbols indicate significance compared to untreated check. Error bars are SEMs).



CONCLUSIONS: Sandea did not perform well in this trial. Although Sandea showed good control of other broadleaf weeds and grasses, it did not control red sorrel well in either the prune or crop plots, and resulted in the lowest yield of any treatment. Several of the Sandea plots were situated in productive clones. At the July evaluation there were many berries on the plants outside the plots, but almost no berries on the plants inside the plots (Photo 1). In the prune plots, some stunting of blueberry plants was observed (Photo 2), which has been noted in other trials, but in a previous trial a fall application had resolved this issue.

Alion also did not perform well; in both the prune and crop plots blueberry cover was lower than the check and red sorrel cover was higher than the check (Photo 3). The Alion treatment also had the highest broadleaf weed and grass cover in the prune plots. In addition, yield from the crop plots was lower than the check as well as for Chateau and Matrix.

In this trial, the Chateau treatment performed very well. It had the highest blueberry cover of all treatments in both the prune and crop plots, and the lowest red sorrel cover except for the check in the prune plots (17% Chateau versus 14.5% check) (Photo 4).

Matrix had mixed results. Although not significant, prune year blueberry cover was the lowest of any treatment (Photo 5), and crop year cover was lower than the check. Prune year red sorrel control was mediocre (Photo 5) but somewhat better in the crop year. Prune year control

of other broadleaf weeds was also poor in the prune year application but was good on grasses. Crop year yield was average; it was lower than the check but not significantly so. This was the first experiment that we applied these herbicides in a pruned field that would be in crop the following year. All of the treatments except Chateau resulted in a reduction in yield from this treatment timing.

Photo 1. Crop year plot in the Sandea treatment. Note berries outside the plot and lack of berries inside the plot.

Photo 2. Prune year plot in the Sandea treatment, showing stunting of blueberry plants.

Photo 3. Example of a crop year plot in the Alion treatment. Note red sorrel and lack of berries.

Photo 4. Example of a crop year Chateau plot showing good yield and red sorrel control.

Photo 5. Example of a prune year Matrix plot; note low blueberry cover and mediocre red sorrel control.

RECOMMENDATIONS: A fall application of Alion, Sandea, or Matrix should not be applied in the fall of the prune year as it will reduce yields in the following crop year. A Chateau application with this timing did result in better sorrel control and a slightly higher but not significantly higher yield and so could be used. Continue to evaluate the carry over control and take yield data in 2017 on the plots treated after cropping. Continue to evaluate fall treatments of Chateau for red sorrel control using larger plots and more replications.

EXTENSION

INVESTIGATOR: David E. Yarborough, Extension Blueberry Specialist

16. TITLE: Wild Blueberry Extension Education Program in 2016.

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

METHODS: Conduct an educational program that will stress the use of best management practices in an integrated crop management program, which will improve the efficiency of culture and minimize the use of unnecessary pesticides and fertilizers. Conduct spring grower meetings and field days to introduce and reinforce the use of best management practices, integrated crop management and sound business management principles. Provide management information through the Wild blueberry newsletters, fact sheets in the wild blueberry grower's guide both in print form and on the web at http://extension.umaine.edu/blueberries/, through telephone and correspondence, and conduct field visits as appropriate. Cooperate with the Wild Blueberry Advisory Committee Research, 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, Conservation and Forestry, the Board of Pesticides Control) and federal agencies (USDA, IR-4) on wild blueberry related matters. Needs are determined from the Wild Blueberry Advisory Committee Research and Extension priorities, Wild Blueberry Newsletter surveys, 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 to address resistance management for diseases and weeds.

RESULTS:

Meetings attended:

First Annual Meeting of the Northeastern Plant, Pest, and Soils Conference, Philadelphia, PA, January 3-7, 2016.
Agricultural Trade Show, Augusta, ME, January 14, 2016.
PEI Blueberry Information Day & Annual General Meeting of the PEI Wild Blueberry Growers' Association Inc., Charlottetown, PEI, April 5, 2016.
XI International Vaccinium Symposium, Orlando, FL, April 10-14, 2016.
ICM Scouting Sessions, Warren, Jonesboro, Orland on April 26,27,29; May 24,25,26; June 28,29,30, 2016.
Organic Field Day, Montville, ME, July 6, 2016.
Wild Blueberry Producers of Nova Scotia Summer Meeting, Deburt, NS, July 16, 2016.
Wild Blueberry Summer Field Day & Meeting, Jonesboro, ME July 20, 2016.
Quebec Growers Maine Wild Blueberry Tour, Orland to T-16, July 21, 2016.
Davidson Nature Preserve in Vassalboro, ME, July 30, 2016.

Northeast Agricultural and Biological Engineers Conference, Orono, ME, August 2, 2016.
Agriculture in the Classroom Downeast Teachers, Blueberry Hill Farm, Jonesboro, ME, August 3, 2016.
WBANA blogger immersion tour, Bar Harbor, ME, August 7-10, 2016.
19th Wild Blueberry Health Summit, Bar Harbor, ME, August 10-13, 2016.
Legislative Tour, Machias and Jonesboro, ME, August 25-26, 2016.
Wild Blueberry Research and Extension Workers Fredericton, New Brunswick, October 20-21, 2016.
Wabanaki Climate Workshop, Wabanaki Cultural Center and Museum, Calais, ME, November 4, 2016.
Wild Blueberry Association of North America Annual Meeting, Ellsworth, ME, December 14, 2016.

Presentations:

2016 Wild Blueberry Pest Management Update. Agricultural Trade Show, Augusta, ME, January 14, 2016.

Evaluation of fall and spring applications of herbicides targeting resistant weeds in wild blueberry fields. First Annual Meeting of the Northeastern Plant, Pest, and Soils Conference, Philadelphia, PA. January 3-7, 2016.

Single vs split applications of post-emergence herbicides for spreading dogbane (*Apocynum androsaefolium*) control in wild blueberry fields. First Annual Meeting of the Northeastern Plant, Pest, and Soils Conference, Philadelphia, PA. January 3-7, 2016.

Maine's Wild Blueberry Industry. Windsor Elementary School, Windsor, ME. February 9, 2016.

Climate Change Effects on Wild Blueberry in Maine. Maine Legislative Breakfast, Augusta, ME. March 23, 2016.

World Blueberry Production Update. PEI Blueberry Information Day & Annual General Meeting of the PEI Wild Blueberry Growers' Association Inc., Charlottetown, PEI. April 5, 2016.

Maine wild blueberry systems analysis and Wild blueberry systems approach economic and risk analysis. XI International Vaccinium Symposium, Orlando, FL. April 10-14, 2016.

Wild Blueberry ICM Field Scouting Sessions. April, 28, 29, 30, May 26, 27, and June 30, July 1, 2, in Warren, Jonesboro and Orland, ME.

Wild Blueberry Production Field Talk. The National Association of State Departments of Agriculture, Rockport, ME. June 13, 2016.

Review of Organic Management Practices. Organic Field Day Presentation, Montville, ME. July 6, 2016.

Maine Wild Blueberry Crop Estimate. Wild Blueberry Producers of Nova Scotia Summer Meeting, Deburt, NS. July 16, 2016.

2016 Weed Management Demonstration Plots. Wild Blueberry Summer Field Day & Meeting, Jonesboro, ME. July 20, 2016.

Maine Wild Blueberry Production Practices. Quebec Growers Maine Wild Blueberry Tour, Orland to T-16. July 21, 2016.

Techniques for maintaining wild blueberry fields and the history of wild blueberries in Maine. Davidson Nature Preserve in Vassalboro, ME. July 30, 2016.

Technologies for sustainable food and fiber industries in Maine - Challenges and Future Strategies- Panel Discussion. Northeast Agricultural and Biological Engineers Conference, Orono, ME. August 2, 2016.

Wild Blueberry Production in Maine. Agriculture in the Classroom Downeast Teachers, Blueberry Hill Farm, Jonesboro, ME. August 3, 2016.

Wild blueberry production. University of Maine Alumni Association, Falmouth, ME. August 4, 2016.

Wild Blueberry Production in Maine and Overview of Integrated Crop Management. Legislative Tour, Machias and Jonesboro, ME. August 25-26, 2016.

Wild Blueberries in Maine. Big E, Springfield, MA. September 30 - October 2, 2016.

Maine's Wild Blueberry Industry. Go Away Tours, Bar Harbor, ME. October 17, 2016.

World Production Numbers Today/Tomorrow and Maine Wild Blueberry crop report and Maine Wild Blueberry systems analysis. Meeting of the Wild Blueberry Research and Extension Workers Fredericton, New Brunswick. October 20-21, 2016.

Climate Change Effects on Wild Blueberry in Maine. Wabanaki Cultural Center and Museum, Calais, ME. November 4, 2016.

World Production Numbers Today/Tomorrow and Maine Wild Blueberry crop report and Maine Wild Blueberry systems analysis. Annual Meeting of the Wild Blueberry Producers Association of Nova Scotia, Truro, NS. November 18-19, 2016.

Blueberry Crop Trends 1996 -2016. Wild Blueberry Association of North America Annual Meeting, Ellsworth, ME. December, 14, 2016.

Publications:

Yarborough, D.E. and J.L. Cote. 2016. Evaluation of fall and spring applications of herbicides targeting resistant weeds in wild blueberry fields. Proceedings of the First Annual Meeting of the Northeastern Plant, Pest, and Soils Conference. Pg 125.

Cote, J.L. and D.E. Yarborough. 2016. Single vs split applications of post-emergence herbicides for spreading dogbane (*Apocynum androsaefolium*) control in wild blueberry fields. Proceedings of the First Annual Meeting of the Northeastern Plant, Pest, and Soils Conference. Pg 84.

Yarborough, D., F. Drummond, S. Annis, and J. D'Appollonio. 2017. Maine wild blueberry systems analysis. Acta Horticultureae. *In Press.*

Chen, X., D. Yarborough, and J. D'Appollonio. 2017. Wild blueberry systems approach economic and risk analysis. Acta Horticultureae. *In Press.*

Extension Publications:

Revised:

Fact Sheet #209, 2016 Insect Control Guide for Wild Blueberries
Fact Sheet #239, 2016 Weed Control Guide for Wild Blueberries
Fact Sheet #219, 2016 Disease Control Guide for Wild Blueberries
Fact Sheet #224, Commercial Pollinators
Wild Blueberry Crop Statistics web page
2016 Maine Wild Blueberry Pesticide Chart 1 of 3 Insecticides
2016 Maine Wild Blueberry Pesticide Chart 2 of 3 Fungicides
2016 Maine Wild Blueberry Pesticide Chart 3 of 3 Herbicides

Wild Blueberry Website:

The Wild Blueberry website found at http://www.wildblueberries.maine.edu continues to be updated and has been revised to comply with the University of Maine content management system. It received 106,038 page views in 2016 and so is well used world-wide. The wild blueberry blog is being used to update growers on current activities including insect (both pollinator and SWD), and disease (mummyberry monitoring) posts at: http://mainewildblueberries.blogspot.com/

Awards:

NEWSS Fellow Award. Northeastern weed Science Society. January 5, 2016.
University of Maine Cooperative Extension Faculty Award: Applied Research. April 27, 2016.
Order of the Wild Blueberry, Wild Blueberry Association of North America, October 21, 2016.
Outstanding Service and Long Term Commitment Award, Wild Blueberry Producers of Nova Scotia, November 19, 2016.

***Other program activities*:**

I am the principal investigator for the SCBG project: *Improving Integrated Pest Management (IPM) Practices to Prevent Weed Resistance for Maine Wild Blueberry Growers* (2015-2016). I am responsible for compiling the reports for the Wild Blueberry Advisory Committee and HATCH and NRSP4 Project reports for the REEport on-line database. I serve as the liaison for Maine in the IR-4, Minor Use Registration Program and convey project needs for all crops, as well as conduct trials for residue analysis. The objective of the program is to register least toxic alternative pesticides to replace materials that have been canceled so that our growers will be able to keep the minor crop production practices viable in Maine.

I served on the peer review committee for the School of Food and Agriculture and serve as chair for all full professor promotions. I also served on the graduate committee of: Alex Bajcz PhD student, Major advisor F. Drummond 2013 to 2016; and for Tyler Case M.S. student, Major advisor Seanna Annis 2015-2016. I served as Chair of the AVS committee to hire a new faculty in Animal and Veterinary Science in 2016.

CONCLUSIONS: Growers are participating in IPM programs in the four primary wild blueberry growing counties: Washington, Hancock, Knox and Lincoln. The skills survey results indicate that growers are learning new skills and making positive changes in their management practices. A high percentage of participating growers indicated they had learned new skills and changed their practices by rotating herbicides, thereby reducing weed resistance in wild

blueberry fields. Growers are using the blight forecast provided on the Wild Blueberry Web site Blog to being able to control blight more effectively with less applications, use the proper traps to detect and control insects such as the blueberry maggot fly and spotted wing drosophila, and using leaf samples to determine fertilizer needs. Adoption of these management practices will enable growers to improve the efficiency of blueberry culture by reducing unnecessary pesticides and fertilizers. Developing alternative strategies for control of resistant weeds is necessary to prevent future losses in yield from weed competition. The introduction of the new pest, the spotted wing drosophila, presents an additional challenge in monitoring, identification and control to prevent losses from this pest.

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

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