The University of Maine [DigitalCommons@UMaine](https://digitalcommons.library.umaine.edu/)

[Wild Blueberry Research Reports](https://digitalcommons.library.umaine.edu/blueberry_resreports) **Wild Blueberry Research**

1-2018

2017 Wild Blueberry Project Reports

Frank A. Drummond

Judith A. Collins

E. Ballman

Alexander J. Chandler

Seanna Annis

See next page for additional authors

Follow this and additional works at: [https://digitalcommons.library.umaine.edu/blueberry_resreports](https://digitalcommons.library.umaine.edu/blueberry_resreports?utm_source=digitalcommons.library.umaine.edu%2Fblueberry_resreports%2F50&utm_medium=PDF&utm_campaign=PDFCoverPages) Part of the [Agriculture Commons](https://network.bepress.com/hgg/discipline/1076?utm_source=digitalcommons.library.umaine.edu%2Fblueberry_resreports%2F50&utm_medium=PDF&utm_campaign=PDFCoverPages), [Entomology Commons](https://network.bepress.com/hgg/discipline/83?utm_source=digitalcommons.library.umaine.edu%2Fblueberry_resreports%2F50&utm_medium=PDF&utm_campaign=PDFCoverPages), [Food Science Commons](https://network.bepress.com/hgg/discipline/84?utm_source=digitalcommons.library.umaine.edu%2Fblueberry_resreports%2F50&utm_medium=PDF&utm_campaign=PDFCoverPages), [Human and](https://network.bepress.com/hgg/discipline/97?utm_source=digitalcommons.library.umaine.edu%2Fblueberry_resreports%2F50&utm_medium=PDF&utm_campaign=PDFCoverPages) [Clinical Nutrition Commons](https://network.bepress.com/hgg/discipline/97?utm_source=digitalcommons.library.umaine.edu%2Fblueberry_resreports%2F50&utm_medium=PDF&utm_campaign=PDFCoverPages), and the [Plant Sciences Commons](https://network.bepress.com/hgg/discipline/102?utm_source=digitalcommons.library.umaine.edu%2Fblueberry_resreports%2F50&utm_medium=PDF&utm_campaign=PDFCoverPages)

This Report is brought to you for free and open access by DigitalCommons@UMaine. It has been accepted for inclusion in Wild Blueberry Research Reports by an authorized administrator of DigitalCommons@UMaine. For more information, please contact um.library.technical.services@maine.edu.

Authors

Frank A. Drummond, Judith A. Collins, E. Ballman, Alexander J. Chandler, Seanna Annis, Rachael Martin, Nghi Nguyen, Jennifer D'Appollonio, and David E. Yarborough

2017 Wild Blueberry Project Reports

January 2018

The University of Maine does not discriminate on the grounds of race, color, religion, sex, sexual orientation, including transgender status and gender expression, national origin, citizenship status, age, disability, genetic information, or veteran status in employment, education, and all other programs and activities. The following person has been designated to handle inquiries regarding nondiscrimination policies: Director, Office of Equal Opportunity, 101 North Stevens Hall, 581.1226, [eoinfo@umit.maine.edu.](mailto:eoinfo@umit.maine.edu)

TABLE OF CONTENTS

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

1. I. TITLE: Control tactics for blueberry pest insects, 2017.

*Study 1.**Field control of blueberry tip midge and associated crop loss on wild blueberry.*

METHODS:

Tip midge efficacy studies

Insecticide control trials were conducted in 2012-2015 and 2017. For all five trials, materials were applied in 25 gallons of water-mixture per acre with a CO_2 -propelled, 80-inch boom sprayer (76-inch swath) equipped with four, flat-spray, 8002VS TeeJet® nozzles operating at 35 psi and at a slow walking speed. Walking speed for each application was regulated using a metronome.

On various sample dates as indicated in the tables, damage was assessed by counting the number of blueberry stems with and without tip midge damage as evidenced by curled leaves from each of three, ft^2 or m^2 samples per treated plot. There were four (2012, 2014, 2015), five (2017), or six (2013) replications of each material. Plot size for all trials was 7 x 20 ft.

In 2012 (10 June) and 2013 (17 June) we made foliar applications of Assail[®] 30SG (acetamiprid) and Imidan® 70WP (phosmet) in pruned-year fields after tip midge damage was evident (curled leaves on stems).

In 2014 Rimon® 0.83EC (novaluron), Success® 480SC (spinosad), and Entrust® SC (spinosad) were applied on 11 June to a pruned-year field. A second application of each material plus a first application of Assail 30SG and Mustang Maxx® (zeta-cypermethrin) were made on 19 June.

In 2015 Rimon 0.83EC (novaluron), Movento® SC (spirotetramat), Exirel® SE (cyazypyr), Sivanto® 200SL (flupyradifurone), and Assail 30SG (acetamiprid) were applied on 27 May to a pruned-year field. A second application of each material was made on 5 June.

In 2017 Assail 30SG (acetamiprid), Mustang Maxx (zeta-cypermethrin), and Success 2SC (spinosad) were applied on 24 May, 30 May, and 8 June to a pruned-year field. Blueberry stems were just emerging on 24 May, scattered and ½ to 1 inch tall on 30 May, and scattered and 1 to 1½ inches on 8 June. On 2, 13, and 27 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:

Tip midge efficacy studies

Subplots were pooled within main plots. Analyses of Variance (ANOVA, RCB) and LSD $(P < 0.05)$ were used to compare mean number of curls among the treatment plots (2012, 2013, 2014, 2017) or mean percent stems with curls (2015). In 2013 we also used Multivariate Analysis of Variance (MANOVA, CRD). Data were transformed by the square root (2012, 2013, and 2014) or arcsine (2015) to stabilize variance prior to analysis.

2012

Assail and Imidan were both ineffective in suppressing tip midge as evidenced by leaf curls (Table 1 and Fig. 1). Post-spray populations in the treated plots were higher than the nontreated check.

Table 1. Field control of tip midge with insecticides – one application, data from 2012.

Means within columns followed by the same letter(s) are not significantly different (LSD, *P* < 0.05). Data were transformed by sqrt prior to analysis.

Fig. 1. Mean number of curls/ft² (lines are standard error of the mean), data from 2012.

2013

A similar result was observed in 2013. Assessment of treatments via ANOVA suggested no significant difference among the treatments on 17 June (Prespray). Assail and Imidan were both ineffective in suppressing tip midge as evidenced by leaf curls (Table 2 and Fig. 2). Postspray populations in the treated plots were either higher (1 July) or not significantly different (25 June and 8 July) than the non-treated checks. MANOVA also revealed no treatment

differences ($F_{(2,11)} = 1.589$, $P = 0.247$) and no time x treatment interaction ($F_{(6,18)} = 1.283$, $P =$ 0.313), but a significant time effect $(F_{(3,9)} = 31.134, P < 0.0001)$. This suggests that there was a continual decline of tip midge curls through the beginning of July and then resurgence by 8 July independent of treatment.

Table 2. Field control of tip midge with insecticides – one application, data from 2013.

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

Fig. 2. Mean number of curls/m² (lines are standard error of the mean), data from 2013.

2014

In 2014 two applications of Rimon, Success, and Entrust or one application of Assail and Mustang Maxx were all initially effective in suppressing tip midge infestation. Significantly fewer damaged stems were found in the treated plots compared with the non-treated checks on the first sample date on 26 June $(F_{(5,15)} = 7.50, P = 0.001)$. Although the number of damaged stems in all the plots was much lower by the second sample date on 18 July, it does appear that tip midge populations in the treated plots had rebounded somewhat. Plots treated with Entrust,

Success, Rimon, and Mustang Maxx all had significantly MORE damaged stems than the nontreated check plots $(F_{(5,15)} = 7.16, P = 0.0013)$ (Table 3 and Fig. 3).

Table 3. Field control of tip midge with insecticides – one or two applications, data from 2014.

Means within columns followed by the same letter are not significantly different (LSD, *P* < 0.05). Data were transformed by sqrt prior to analysis.

Fig. 3. Mean number of stems with curls/m² (lines are standard error of the mean), data from 2014.

2015

As in 2014, Rimon, Movento, Exirel, Sivanto, and Assail were all effective, at least initially, in suppressing tip midge populations. Significantly fewer damaged stems were found in the treated plots compared with the non-treated checks on the first sample date on 12 June (F _(5,15,)) $= 19.31, P = 0.0001$. By the second sample date on 25 June there appeared to be a trend towards increased numbers of damaged stems in treated plots compared with the non-treated controls; however, the difference was not significant ($F_{(5,15)} = 0.56$, $P = 0.7260$) (Table 4 and Fig. 4).

Table 4. Field control of tip midge with insecticides – two applications, data from 2015.

Means within columns followed by the same letter are not significantly different (Tukey's, *P* < 0.05). Data were transformed by arcsine prior to analysis.

Fig. 4. Mean percent stems with curls/ft² (lines are standard error of the mean), data from 2015.

2017

At the initial assessment on 2 June following two applications, there was no significant difference among the treatments $(F_{(3,12)} = 4.58, P = 0.8286)$ (Table 5 and Fig. 5). On 13 June, following three applications, significantly fewer curls were found in plots treated with Mustang Maxx, and by 27 June there were significantly fewer curls in all treated plots compared with the non-treated check plots.

Table 5. Field control of tip midge with insecticides – three applications, data from 2017.

Fig. 5. Mean number of curls/m² (lines are standard error of the mean), data from 2017.

METHODS:

Development of economic thresholds

Another focus of our investigations has been the development of economic thresholds based upon the amount of crop loss from tip midge infestations estimated in seven trials conducted between 2010 and 2017. For each trial, stems with and without tip midge damage as evidenced by the presence of leaf curls were selected and marked in the spring of the pruned year. In the fall, ½ of the stems were cut and brought into the laboratory where we counted the number of flower bud clusters per stem. In the spring of the following year, the remaining stems were cut and the number of individual flowers per stem was determined. 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 2017 report, but will be updated when the flower numbers per stem are estimated in the spring of 2018.

RESULTS:

Crop loss and development of economic thresholds

Previous studies demonstrated that blueberry plant response in flower-bud production can be quite variable. In our 2010-2011 trial we found NO difference in flower-bud clusters per stem due to blueberry tip midge $(F_{(1,48)} = 0.01, P = 0.9054)$ (Fig. 6); 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. 7).

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. 6). 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. 7).

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. 6). 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. 7).

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 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. 7).

The evaluation of the 2016-2017 trial showed no significant difference in the number of flower-bud clusters (ANOVA, CRD; $F_{(1,48)} = 0.43$, $P = 0.5172$) between infested compared to non-infested stems (Fig. 6). And, stems with blueberry tip midge infestation developed significantly MORE flowers then those without tip midge infestation (ANOVA, CRD; $F_{(1,48)} =$ 6.71, *P* = 0.0127)(Fig. 7). This was also observed in our 2011-2012 trial. The count of flower-bud clusters in our 2017-2018 trial once again showed there was a significant difference in the number of flower-bud clusters (ANOVA, CRD; $F_{(1,48)} = 12.39$, $P =$ 0.001). Stems without damage had significantly more flower-bud clusters than infested stems of the same clone (Fig. 6). The number of individual flowers that develop on infested vs noninfested stems will be evaluated in the spring of 2018.

Fig. 6. 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, 2016-2017, and 2017-2018.

Fig. 7. Bar graph comparing mean number of individual flowers per stem between stems with and without tip-midge damage. Data collected from six trials completed over a seven-year period from 2010 through 2017.

We have a very interesting relationship starting to emerge. It appears that when flower bud clusters are reduced by tip midge, subsequently, the number of flowers per stem is reduced to about 40% of the non-infested stems. But sometimes tip midge attack actually stimulates an increase in bud cluster production. When this happens there are more flowers per stem in infested stems than non-infested stems. This increase in the number of flowers per stem increases dramatically as tip midge infestation gets heavier (Fig. 8). Therefore, it appears that heavy attack by blueberry tip midge stimulates flower bud production compensation by the plant resulting in greater potential yield the following year.

In order to explain the shift in tip midge having a detrimental effect on flower bud development and flower production to a stimulatory effect on bud and flower production, we hypothesized that the cumulative air degree-days from June 15-August 30 during the bud production period might explain this relationship. We did not see any relationship among proportion flower bud viability and degree-days. We are planning on collecting another year of data to see if this relationship holds.

Fig. 8. Relationship between flower bud cluster ratio between infested and non-infested stems and the ratio of flowers / stem the following year. The dashed lines are when infested stems and non-infested stems have the same bud cluster or flower density per stem. Each data point is from a trial representing 6 trials over 5 years.

CONCLUSIONS AND RECOMMENDATIONS: Insecticide applications have not provided adequate control of blueberry tip midge. In fact, in all three of the first trials (2012, 2013, 2014), but especially in 2012 and 2014, the insecticide-treated plots ended up with more tip midge damage (stems with leaf curls or stem hooking) than the non-treated check plots. The same trend was observed in 2015. This has not occurred in any other insect pest spray trials in past years. Although the reason for increasing populations in the treated plots is unclear, it is possible that the applications had a depressing effect on native predators of the tip midge, thus insecticide treatment might exacerbate a tip midge outbreak.

The trial in 2017 was the first year that we made three applications and also the first time that any significant level of control was obtained. All three insecticides provided significant control relative to the non-treated check. Because of this we suspect that it may be the intensive frequency of application that determines control more than the selection of a specific insecticide. Therefore, we recommend that growers who need to control blueberry tip midge should apply an insecticide starting with the appearance of the first tip midge gall in the field and then continue to apply two more applications at 5-7 day intervals.

Damage resulting from blueberry tip midge attack is primarily a result of gall formation that appears to reduce flower bud cluster development. Figure 6 shows that in four of seven trials, bud cluster numbers per stem were less in stems attacked by blueberry tip midge. Figure 7 shows that when flower buds were examined, four of seven trials exhibited reduced flower numbers per stem on stems attacked by blueberry tip midge.

An economic threshold analysis was conducted for blueberry tip midge. For this analysis, we assumed that on average 44.3% flower loss results from blueberry tip midge attack (based upon data from this report). We used a grower price of \$0.60 and \$1.00 for the analysis. We did not use the current \$0.30 grower price because at this price we do not recommend controlling this localized insect pest, unless the infestation is a large scale one. The results of our economic threshold analysis are shown in Figure 9. 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% infestation (the level where the cost of control equals the cost of crop loss) when the price for blueberries is \$1.00 per pound. A threshold for the same average yield (3,000 lbs / acre) when the estimated price for berries is \$0.60 per pound is 10% infestation. Figure 9 also shows that higher expected yield for a field results in a threshold at a lower infestation level. This is because a given percent area in the field will have higher yield and thus higher value. The opposite relationship holds for fields with very low expected yields, a higher infested percent of the field will trigger control. A price of \$0.30 or less per pound was not modeled, but would be expected to show that blueberry tip midge would not be worth controlling except at very high levels of infestation, greater than 20% infestation of a field, levels of infestation that we have not seen in Maine wild blueberries at this time.

Study 2. Efficacy of boron as a low toxicity control tactic for spotted wing drosophila. Report from Troy Cloutier (undergraduate Biology student), Judith Collins, and Frank Drummond

OBJECTIVE: Since its introduction to the Continental United States from Asia in 2008, the invasive vinegar fly *Drosophila suzukii* (Matsumura) has been an economic concern among growers of soft fruits (Ballman and Drummond, 2017). *D. suzukii* is an especially effective pest due to its serrated ovipositors and polyphagous nature. Native drosophila lay their eggs in rotting fruits while *D. suzukii* use their serrated ovipositors to lay eggs in ripe soft-skinned fruits (Asplen et al., 2015). The damage caused by *D. suzukii* on the cultivation of soft-skinned fruits has made the pest a focus among researchers who are working to develop effective control measures. An important factor to consider when developing insect control measures for agricultural applications is the potential for toxicity. A low toxicity insecticide for *D. suzukii* would help minimize health risks to consumers as well as local wildlife. *D. suzukii* are commonly treated with synthetic insecticides, but researchers are focusing on developing less hazardous solutions. This includes the introduction of indigenous predatory insects, as well as the use of fungal pesticides, essential oils, and other low toxicity insecticides (Bohinc and Trdan, 2014).

Boric acid powder has been commonly used as a less toxicity alternative to other synthetic insecticides when controlling household pests such as cockroaches. Symptoms of boric acid poisoning in cockroaches indicate that boric acid has a neurotoxic effect on insects as well as causing starvation by altering the insect's midgut (Dayer and Karvandian, 2016) The concentration of boric acid used against insects has little effect on human health. Many household products including skin powder, ointments, and mouthwash contain boric acid. Evidence shows that boron can have a negative effect on drosophila as well. In a laboratory study, the fruit fly *Drosophila melanogaster* experienced increased mortality and decreased fecundity when fed diets containing sodium tetraborate (Erdem et al., 2016).

The objective of this research was to test the efficacy of boron, formulated as the commercial product Octabor®, as a low toxicity insecticide against *D. suzukii*. Two laboratory trials and a semi-field bioassay were conducted to test the insecticidal properties of boron. For the laboratory trials, *D. suzukii* were placed in containers with raspberries that were treated with various boric acid solutions. The laboratory trials also tested the effect of sugar as an attractant when mixed with boron. The semi-field bioassay tested the insecticidal properties of boron when sprayed on blueberry plants. The stems of treated blueberry plants were infested with *D. suzukii* within a laboratory container.

METHODS:

Laboratory control of spotted wing drosophila with Octabor. 2016/2017

Boron, formulated as Octabor® (disodium octaborate tetrahydrate) (U.S. Borax Inc.) was evaluated in the laboratory to assess its potential to control spotted wing drosophila (SWD). The results of two laboratory trials were pooled into a single statistical analysis. For the first trial, two rates were tested (0.6% v/w and 1.0% v/w with and without 16 oz/acre of sugar). For the second trial, a single rate was tested (0.6% v/w with and without 16 oz/acre of sugar). Laboratory-reared SWD adults (6-10 per cage) were placed in plastic cages (9 x 4.38 x 4.13in) with five raspberries. Between conducting the two trials, each plastic cage was sterilized with a 1:10 bleach solution. All fruit was washed and dried before each trial. Prior to introduction of

the SWD into the cages, the fruit was treated by mixing the various rates in 200ml water in a misting spray bottle set to the finest mist possible. Two sprays (enough to wet the surface) were applied to the fruit that was spread out in a single layer in an open petri dish. For the first trial there were four replications of each treatment and four non-treated checks. For the second trial there were five replications of each experiment and five non-treated checks. SWD were introduced into the cages after the material had dried on the fruit (1hr post application). Cages were monitored daily for one week and the number of live SWD was recorded. In the second trial the number of SWD on the fruit was also recorded. Data were transformed by the square root prior to analysis. Mean separation was by Least Square Means.

Control of spotted wing drosophila with Octabor, a semi-field bioassay. 2017

Octabor was evaluated in a semi-field bioassay to assess its potential to control spotted wing drosophila (SWD). Octabor (1.0% w/v) was applied to a crop-year blueberry field at Jonesboro, ME on 31 July. The 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. At 0 (material allowed to dry on foliage for 4 hours), 3, and 7 days after treatment (DAT), stems containing leaves and ripe berries were cut off the bushes and placed in water picks (10cm long single anchor water pick, AquaPic[®] brand) inside 32oz clear plastic deli cups. The water picks were inserted through a hole in the bottom of the container such that the lip of the water pick was even with the bottom of the cup. Berries were removed from the stems and placed in wire mesh containment boats to reduce loss of residues on the berries. Ten adult SWD (5 male, 5 female) that were between 2 and 5 days old were removed from a laboratory colony, anesthetized with $CO₂$, and added to the cups (4 replicates per treatment). To limit fly mortality, cotton balls moistened with water were placed in each cup, and a 1oz portion cup was filled with drosophila diet (Instant drosophila medium, Carolina Biological Supply company, Burlington, North Carolina) and placed in each cup to provide food for the flies. To minimize moisture build up, lids had a 5cm diameter hole cut in them and fine mesh affixed over the hole using hot glue. Cups with collected blueberry fruit and flies were placed in an environmental chamber at 25°C, 75% RH, and a 16:8 L:D cycle. Adult mortality was assessed daily for 8 to 10 days; fruit infestation was assessed after 1 week. Nominal logistic fit was used compare adult mortality and fruit infestation among the treatment

RESULTS: From the two laboratory experiments we found that the presence of boron increased SWD mortality. The results also show that sugar may increase the effectiveness of boron as an insecticide. Table 1 and Figure 1 show that the low 0.6% rate had significantly greater effect on SWD when combined with sugar in the first laboratory trial (Experiment 1, Trial 1); 87.5% mortality was observed by day six when sugar was added to the 0.6% rate compared to only 12.5% mortality in the 0.6% rate without sugar; mortality in the untreated check was 11.1% on day six without sugar.

Table 1. Laboratory control of SWD, summary (Experiment 1, Trial 1)

Means within columns followed by the same letter are not significantly different (LSD, $P \leq$ 0.05).

* Rate for Octabor is expressed as % volume with water.

** Observation made 4 hrs post treatment

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

Fig. 1. Percent mortality of SWD exposed to Octabor over time (Experiment 1, Trial 1).

This effect was evident in the second laboratory trial, but at a much lower level (Table 2 and Fig. 2). A 0.6% rate of Octabor was effective both with and without the addition of sugar.

Treatment	Rate*	Average % mortality	Average % SWD on fruit
Octabor	0.6%	10.0	73.9
$Octabor + Sugar$ Non-treated check	$0.6\% + 16.0$ oz/a -	8.0 2.2	73.3 68.2

Table 2. Laboratory control of SWD, summary (Experiment 1, Trial 2).

* Rate for Octabor is expressed as % volume with water.

Fig. 2. Percent mortality of SWD exposed to Octabor over time (Experiment 1, Trial 2).

From the semi-field bioassay we found that boron applied on the fruit in the form of Octabor did appear to reduce infestation by larvae at day 0 and day 3 after application (Fig. 3). All fruits were infested by Day 7. This effect was not strong, as there was high variation among replicates; therefore, the difference between the Octabor-treated fruit and the non-treated fruit

was significant only at $P = 0.10$ (X^2 ₍₁₎ = 2.721); although, when evaluating fruit infestation pooled over all the dates, there was a strong effect on infestation when comparing boron treated fruit to non-treated fruit (X^2 ₍₁₎ = 15.623, P < 0.0001). We do not know whether this response to boron is due to a repellent effect on adult ovipositing females such that they lay fewer eggs in treated fruit, or if boron causes mortality of the eggs or larvae inside the fruit. When we looked at adult survival when confined to arenas either with boron-treated fruit or control (non-treated fruit), we found a significant boron effect (Fig. 4). However, the pattern of mortality was not as expected. We observed no difference in mortality when flies were confined to fruit that had just been treated with boron compared to the control. We did find significantly higher mortality in the boron treatment relative to the control for day 3 and day 7 fruit. One explanation for this might be the chemical transformation that Octabor might experience under field conditions over time. We hypothesize that in the field on fruit over time Octabor, or disodium octaborate tetrahydrate, slowly transforms to boric acid, the toxic form of boron to the flies. If this is the case, then we might have observed repellency to ovipositing flies from disodium octaborate tetrahydrate and mortality to flies from boric acid.

Fig. 4. Percent mortality of SWD over time.

CONCLUSIONS AND RECOMMENDATIONS: The combined results of the laboratory studies and the semi-field bioassay show potential for boron as a SWD control mechanism; however, more research is required before applying boron in an agricultural context. The semifield bioassay showed that high concentrations of boron can deter SWD from fruit for short periods of time. Fruit that was treated with boron received less attention from SWD for up to seven days (Fig. 3). While this does not kill SWD, it does keep them from laying eggs in fruit. However, the potency of boron is short lived and would need to be extended before becoming a useful repellent. A better understanding of what boron's repellency is based on, as well as what causes it to lose its potency, may help generate methods that could increase the duration over which boron is repellent to SWD. This research may include testing how environmental conditions such as ultraviolet light alter boron and its repellent properties.

In addition to having repellent effects, boron had a negative effect on SWD mortality, especially when mixed with sugar. However, the toxic effect of boron on SWD is not powerful enough to use as an insecticide. Many synthetic insecticides can result in a mortality rate of close to 100 percent after a few days. The most lethal solution assessed in the laboratory trials contained 1.0 percent boron with 16 oz/acre of sugar attractant, resulting in 100 percent mortality after 6 days (Fig. 1). Increased mortality when mixed with sugar is most likely due to SWD imbibing boron after being attracted by sugar. However, 6 days is too long for boron to be competitive with synthetic insecticides. Further study to test whether this time period can be decreased would further our understanding of boron's potential as an insecticide. Increased sugar concentrations and decreased boron concentrations may reduce boron's repellent effects and attract more SWD to ingest it. Past experiments have tested the effect of boron-rich diets on *Drosophila melanogaster* mortality and fecundity (Erdem et al., 2016). Replicating this

experiment using *D. suzukii* may further our understanding of boron's effect on SWD. Testing the effect of boron on SWD fecundity may provide additional insight on boron's potential as a control mechanism. The relatively poor toxic effect of boron on SWD could be offset by a strong effect on fecundity that would dampen future generations. The short generational period of drosophila make fecundity an important factor when controlling pests. Until further experimentation has been conducted, boron should not be used as an insecticide in an agricultural setting, but it does have potential that warrants further study, especially at higher rates.

REFERENCES CITED:

- Asplen, M. K., Anfora, G., Biondi, A., Choi, D. S., Chu, D., Daane, K. M., and Desneux, N. (2015). Invasion biology of spotted wing drosophila (*Drosophila suzukii*): a global perspective and future priorities. J. of Pest Sciences 88(3): 469-494.
- Ballman, E. S. and Drummond, F. A. (2017). Infestation of wild fruit by *Drosophila suzukii* surrounding Maine wild blueberry fields. J. Agric. Urban Entomol. 33: 1–10.
- Bohinc, T. and Trdan, S. (2014). Control of spotted wing drosophila (*Drosophila suzukii* [Matsumura], Diptera, Drosophilidae) with the emphasis on environmentally acceptable methods. Acta Agriculturae Slovenica. 103: 323–329.
- Dayer, M. S. and Karvandian, K. (2016). Toxicity of *Metarhizium anisopliae* (Deuteromycota: Hyphomycetes) and boric acid against nosocomial cockroaches, *Blattella germanica*. Arthropods. 5(3): 114-124.
- Erdem, M., [Büyükgüzel,](http://www.bioone.org.prxy4.ursus.maine.edu/doi/abs/10.18474%2FJES16-11.1) E. and [Büyükgüzel,](http://www.bioone.org.prxy4.ursus.maine.edu/doi/abs/10.18474%2FJES16-11.1) K. (2016). Effect of dietary sodium tetraborate on adult longevity and fecundity of *Drosophila melanogaster* (Diptera: Drosophilidae). J. of Entomological Science. 51(4):305-313.

*Study 3. Assessing the effect of insecticides on immature life stages of spotted wing drosophila occurring in fruit, laboratory and field studies***.**

OBJECTIVE: To determine the efficacy of insecticides in controlling different life stages of spotted wing drosophila (SWD) that occur within the fruit, to include the impacts of successive insecticide applications in the SWD population model.

METHODS:

Laboratory trial

Wild blueberries were collected from the University of Maine's Wild Blueberry Research farm from a pesticide free area. The berries were divided into three size ranges: small $(0.21g \pm$ 0.06g), medium (0.33g \pm 0.05g), and large (0.55g \pm 0.10g). Fruit were grouped by size and placed onto 9cm Petri® dishes so that there was a single continuous layer of fruit. Water was added to each dish until the water covered half of each berry. A dish of each fruit size was placed into a cage containing 200 SWD for eight hours to allow oviposition; this was repeated across four cages. After the oviposition period, the dishes of fruit were removed from the cages and the number of eggs in each berry was counted under a stereomicroscope. Berries with zero or more than five eggs were discarded. Ten pieces of infested fruit of each size were placed onto

new Petri dishes and placed inside of 540ml plastic deli containers for a total of five dishes per fruit size. The deli containers were placed inside of a growth chamber set to 20°C with 70% RH and 16:8 L:D. The plates of fruit were assigned to either the control or pesticide treatment such that each treatment had equal numbers of eggs.

The oviposition process was conducted on days 1, 3, 5, and 7 so that those fruit would contain $3rd$ instars, $2nd$ instars, $1st$ instars, and eggs respectively. On day 8, all infested fruit was treated with either a water control or Malathion 8F (1.75 pts/acre). Fruit was treated by spraying two squirts from a water bottle (0.44ml of material) and then gently rolling the berries to ensure complete coverage. The berries were allowed to dry for one hour before being returned to their deli containers in the chamber. A yellow sticky card was attached to the top of the deli container to trap emerging SWD. On days 14 and 21, containers were checked for SWD. This experiment was run twice for a total of 10 replicates per fruit size and treatment. A logistic regression was run to look for differences among treatments, fruit size, instars, and blocks. All analyses were run using JMP® 13 statistical software.

Field trial

Individual wild blueberry stems with fruit were flagged and covered with mesh bags (6 x 14" nylon organza) on 7 August; fruit was removed to standardize the number of fruit across all clusters. The effects of two insecticides (Malathion 8F and Imidan 70WP) were tested on four SWD life-stages (eggs, $1st$, $2nd$, and $3rd$ instar). There were 10 bags per life stage per treatment plus 10 non-treated controls. Laboratory reared SWD adults (5 males + 5 females) were introduced into the bags for a period of 24 hours and then the bags were removed so the adults escaped. The clusters of fruit were then re-covered until insecticides were applied (see Table 1 for a schematic of the timeline for the experiment).

Immediately prior to the application of insecticides the fruit was uncovered to ensure bags did not hinder insecticide application. Malathion 8F (1.75 pts/acre) or Imidan 70 WP (1.3 lbs/acre) was applied at 50 GPA using 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. Following insecticide application, all clusters were re-covered as soon as safe to ensure fruit were not re-infested. Fruit remained covered and on the plants for a period of 9 days after application of insecticides after which the stem/clusters were removed and returned to the laboratory to assess for emergence. Fruit was placed clean deli cups each containing a small yellow sticky card stapled to the lid. Fruit was held at laboratory conditions and the yellow sticky card was checked twice per week for two weeks for emergence of adult SWD in the deli cups. The mean emergence for each insecticide and duration past infestation (life stage) was compared to non-treated plots.

Table 1*.* Timeline for field trial.

Day 1	3	5	7	8	17	18	19
Infest 10 clusters (3rd) instars in	Infest 10 clusters (2nd) instars	Infest 10 clusters (1 st) instars	Infest 10 clusters (eggs instars	Apply insecticides or leave untreated	Collect fruit into emergence	Start assessing emergence	Continue assessing emergence
7 days)	in 5 days)	in 3 days)	in ₁ day)	(control block)	containers		

RESULTS:

Laboratory trial

The Malathion treatment killed nearly all SWD at all life stages and fruit sizes within the fruit. There was a significant difference between treatments ($X^2 = 1137.91$, df = 1, *P* < 0.0001), and instars ($X^2 = 7.55$, df = 3, P = 0.56). There were no significant interactions among the treatments (insecticide vs fruit size, vs instar). On average, survival to the adult stage in the Malathion treatment was 0.3% while the control had a survival rate of 40.35%. Survival to the adult stage was not statistically different among fruit sizes; although, a trend is apparent with less emergence in larger fruit (Fig. 1). However, if this trend is real we have no likely explanation; we would actually hypothesize the opposite phenomenon, higher emergence in larger fruit. The second instar larvae had a significantly higher survival rate compared to the egg stage ($P =$ 0.054), while the rest of the instar stages were not significantly different from one another (Fig. 2). The reason that first instar survival is not different from second instar survival is the variation among replicates. However, there was a trend toward a difference between first and second instar survival ($P = 0.089$). If fruit size is considered a quantitative rank from small to large then a linear trend in reduced survival associated with larger fruit is seen at a marginally significant level ($P = 0.055$). When life stage was considered a rank quantitative variable, life stage survival was no longer significant $(P = 0.225)$. Therefore, because our conclusions vary depending upon the way we treat the variables (categorical vs ordinal), we put less faith in the conclusion that life stage affects the outcome of survival to adult.

Fig. 1. Average adult emergence by fruit size across treatments.

Fig. 2. Average adult emergence by instar across treatments. Different letters denote statistical differences at the 0.05 level.

Field trial

No adults were observed in any of the treatments. Pupae were noted in only four of a total of 120 deli cups; pupae were observed in three of 40 Malathion-treated cups (two 3rd instar and one $2nd$ instar); and in one non-treated control cup.

CONCLUSIONS: Malathion was extremely effective at killing SWD before they could complete their development in the fruit under laboratory conditions. Because almost no flies survived the Malathion treatment, it is difficult to determine how pesticide efficacy is impacted by fruit size or SWD age. A lower rate of pesticide application may be necessary to measure the impact of fruit size and SWD age. The important underlying result is that Malathion and possibly other insecticides used to control SWD adults might also kill the larvae within fruit. Whether this affects the marketability of the fruit is unknown. It is possible that dead immature SWD within fruit might cause fruit decay prior to processing.

*Study 4.**Rainfall-mediated loss of efficacy of Malathion against spotted wing drosophila.*

METHODS:

Field study

Malathion[®] 8F (28 oz/ac) with and without the addition of the adjuvant Nu-Film[®] (6 oz/ac) and with or without simulated rainfall was applied at weekly intervals for four weeks beginning on 7 August. All applications were made in 50 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. Speed was regulated using a metronome. There were four replications of each treatment. Plot size was 14 x 20ft. Figure 1 shows the treatments and experimental design.

All applications were made early in the morning, and residues were allowed to dry for a period of 4-6 hours, following which rainfall was simulated; plots received ¼ - ½ an inch of irrigation "rain" (6.35 – 12.7mm). The volume of "rain" delivered to each plot was measured using rain gauges, placed in the middle of rows to avoid the canopy interfering with the measurement.

Fig. 1. Experimental design.

During the field trial, larval infestation in the plots was assessed on day 7 of each week (before the next scheduled spray) by collecting 4-6 oz of ripe fruit. Collected berries were counted and weighed, and then each sample was assessed using the Salt Test to sample for the presence of larvae.

Semi-field bioassay

A semi-field bioassay was conducted during week 2 of the study. Larval infestation was assessed at 1 DAT in addition to the 7 DAT timing. At 0 (material allowed to dry on foliage for 4 hours), 3, and 7 days after treatment (DAT), stems containing leaves and ripe berries were cut off the bushes and placed in water picks (10cm long single anchor water pick, AquaPic® brand) held upright in a flask. Flasks were placed in clear plastic containers (Fig. 2). Berries were removed from the stems and placed in wire mesh containment boats to reduce loss of residues on the berries. Ten adult SWD (5 male, 5 female) that were between 2 and 5 days old were removed from a laboratory colony, anesthetized with $CO₂$, and added to the containers (4 replicates per treatment). A cotton ball soaked with sugar/yeast was placed in each container to provide food for the flies. To minimize moisture build up, containers had a 5cm diameter hole cut in them and fine mesh affixed over the hole using hot glue. Cups with collected blueberry fruit and flies were held at room temperature and adult mortality was assessed at 24 and 48 hours after exposure to the field collected fruit and foliage. Fruit was held in the laboratory for 7 additional days then assessed for the presence of larvae using the Modified Salt Test.

Fig. 2. Container used for semi-field bioassay.

RESULTS: 1.13 inches of natural rainfall fell over the course of the study between 7 August and 3 September; 0.15, 0.21, 0.10, 0.41, and 0.26 inches on 8, 12, 13, 18, and 19 August, respectively.

Field study

Analysis of Variance (ANOVA, RCB) was used to compare berry weight among treatments, while logistic regression was used to compare the number of maggot infested fruit among treatments. Berry weight data were transformed by the square root prior to analysis to stabilize variance. Mean separation of berry weights was by LSD ($P \le 0.05$).

For weight per berry, there was no significant difference in berry weight among treatments at the end of each week (Table 1). A full model with treatment, week, and treatment x week, provided evidence that block was significant.

Table 1. Average berry weight (g) for each week of the study and all samples combined. Means within each column followed by the same letter(s) are not significantly different.

Fig. 3. Weight per berry, all sample dates combined. Lines are standard error.

There was a significant difference in maggot infested berries among treatments $(X^2_{(5)} = 65.612, P)$ < 0.0001). Week and the week x treatment interaction were not significantly different among treatments. The two control treatments had more infested fruit than the four Malathion treatments $(X^2_{(1)} = 60.071, P < 0.0001)$. When only the Malathion treatments were considered, Nu-Film did not affect berry infestation ($P = 0.350$), nor did irrigation ($P = 0.626$).

Fig. 4. Percentage of plots from each treatment with infested fruit, all weekly samples combined.

Semi-field bioassay

In our semi-field bioassays we found that larval infestation of fruit was determined by treatment ($X^2_{(5)}$ = 29.977, *P* < 0.0001) and days after treatment (DAT, $X^2_{(1)}$ = 25.868, *P* < 0.0001). There was not a treatment by DAT interaction ($P > 0.05$). The overall pattern in infested fruit as a function of treatment and DAT is shown in figure 5. For each of the three DAT treatments we assessed if differences in infested fruit occurred between control non-treated berries vs Malathion treated berries, and then with the four Malathion treatments we compared irrigation vs no irrigation and Nu-Film vs no Nu-Film application. Results are listed in Table 2. The only significant differences were between control and Malathion treated berries, but only on 0 DAT and 3 DAT. By Day 6 after treatment, the protection from maggot infestation afforded by Malathion had disappeared. There was no evidence to suggest that irrigation or the application of Nu-Film affected fruit infestation.

Fig. 5. Percentage of plots from each treatment with infested fruit, for 0, 3, and 6 DAT.

Table 2. Fruit infestation comparisons between treatments for each of the three DAT (orthogonal binomial contrasts). Bold probabilities are significant ($P \leq 0.05$).

	0 DAT	3 DAT	6 DAT	
Control vs Malathion $P = 0.001$ $P < 0.0001$ $P = 0.362$ Irrigation vs no irrigation $P = 0.912$ $P = 0.365$ $P = 0.399$ Nu-Film vs no Nu-Film $P = 0.298$ $P = 0.537$ $P = 0.437$				

Adult fly mortality after exposure to fruit from the same treatments was assessed in the laboratory. Exposure was evaluated for 24h and 48h periods. Figure 6 shows the percent mortality of flies for both the 24h and 48h exposure periods. Treatment $(X^2_{(5)} = 203.852, P <$ 0.0001), DAT (X^2 ₍₁₎ = 116.309, *P* < 0.0001), and the treatment x DAT interaction (X^2 ₍₅₎ = 43.936, *P* < 0.0001) were all highly significant in determining fruit infestation. For each of the three DAT treatments we assessed if differences in percent fly mortality occurred between control non-treated berries vs Malathion treated berries, and then with the four Malathion treatments we compared irrigation vs no irrigation and Nu-Film vs no Nu-Film application. Results are listed in Table 3.

Table 3. Adult percent mortality comparisons between treatments for each of the three DAT (orthogonal binomial contrasts). Bold probabilities are significant ($P \le 0.05$).

In both the 24h and 48h exposure times, Malathion affected percent fly mortality compared to the control at all DAT, even at 6 DAT, although at a much less extent than at the earlier DATs. The effect of Nu-Film was less clear. In the 24hr sample, the addition of Nu-Film significantly affected percent fly mortality at 0 and 6 DAT, but not at 3 DAT (Table 3). Irrigation resulted in significantly lower fly mortality in Malathion treated plots than observed in non-irrigated plots (Table 3). However, the differences in percent mortality were minimal (Fig. 6). These differences are most likely due to irrigation washing off the Malathion residues from the fruit and leaves in the plots. Similar to what we observed in the Nu-Film treatment, at 3 DAT we did not find any evidence suggesting that differences in fly mortality were due to irrigation (Table 3).

CONCLUSIONS AND RECOMMENDATIONS: Nu-Film is often used by growers to enhance the longevity of insecticide residues on treated surfaces, leaves and fruit to insect pests like SWD. In the field we did not find evidence that Nu-Film increased adult SWD mortality resulting in decreased fruit infestation. In addition, in our semi-field experiment there was no evidence that Nu-Film reduced fruit infestation. The effect on SWD adults (flies) was unclear since there was less fly mortality at 0 DAT, when one would think that the effect of Nu-Film would be the greatest. There was no effect of Nu-Film at 3 DAT, and at 6 DAT Nu-Film resulted in significantly more mortality compared to plots that were not treated with Nu-Film.

In the field study we found that the effects of Malathion on fruit infestation might be reduced in irrigated plots (a non-significant trend). We did not see this in the semi-field experiment, but we did find evidence that irrigation resulted in less SWD adult mortality due to Malathion compared to non-irrigated plots. Therefore, with the evidence provided by both the field and semi-field studies we can conclude that irrigation of levels of $\frac{1}{4}$ - $\frac{1}{2}$ an inch of irrigation water can reduce the effectiveness of a previously applied insecticide. This is important information because previously, the rule of thumb was that insecticide residues will not be significantly washed off treated surfaces unless 1 inch or more of rain occurs within a day.

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

2. II. TITLE: Pest biology and IPM, 2017.

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

OBJECTIVE: 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 2016. The wintering cups of pupae were separated into eight 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.

Two line transects of 47 baited, yellow, Pherocon[®] AM traps were set in a pruned year blueberry field adjacent to a fruit-bearing field at Blueberry Hill Farm. Transects were set parallel with traps set at 300ft (91m) and 600ft (183m) from a release point. Within each transect traps were spaced 10ft apart. Ammonium acetate superchargers were attached to every other trap to enhance attractiveness to BMF. Approximately 1500 blueberry maggot fly adults were released on 27 June. Traps were checked daily for nine days. We calculated the distance each captured fly travelled per day from the release point.

RESULTS: In 2017 we recaptured a total of 148 flies (9.9%) (Table 1) over both transects. The first fly was recaptured 335.4ft from the release point on day 1 after release. Over both transects, the furthest distance travelled by any individual fly was 335.4ft/day (range 33.99 to 335.4ft) (Table 1 and Fig. 1).

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 328.2ft/day (range 56.6 to 328.2ft) (Table 1 and Fig. 1).

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 travelled per day by the seven recaptured flies was 288.6ft. The furthest distance travelled by any individual fly was 453.4ft/day (Table 1 and Fig. 1).

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 travelled was 336.0ft on day 1. On day 1, two BMF traveled 320.2ft. Mean travel distance per day was 113.3ft (Table 1 and Fig. 1).

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 travelled per day by the seven recaptured flies was 123.8ft. The furthest any BMF travelled 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. 1).

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

n = number of flies captured.

Fig. 1. Distance (meters) of daily travel of blueberry maggot fly (BMF) adults over pruned field landscapes.

CONCLUSIONS AND RECOMMENDATIONS: We have conducted the study of long-range movement of BMF for five years. Despite the low recovery in captures, we conclude that there is no relationship between capture rate out to 1312ft (400m) and likelihood of trap capture. This suggests that BMF can easily travel well over 400m to neighboring fields. However, we do not know if only a small percentage of the flies travel long distances (the initial assumption based upon our low recapture rates) or if the traps used to recapture the flies are too inefficient to provide a realistic estimate of the number of flies that migrate prior to fruit ripening. We suspect that only a small proportion of flies migrate long distances upon emergence. We believe this based upon the highly effective tactic of managing isolated fields on a single cropping cycle. These fields rarely have outbreaks of blueberry maggot fly. Therefore, this study supports the use of managing isolated wild blueberry fields whenever possible as a single unit in the same cropping cycle and we encourage growers to do this so that they can eliminate insecticide applications to prevent damage by the blueberry maggot fly.

Study 2. Long-term trends (20 years) in parasitism of the blueberry maggot fly.

OBJECTIVES: 2017 marks the final year of a 20-year effort (1998-2017) to study the population dynamics of blueberry maggot fly and its wasp parasitoid *Opius melleus* (Gahan).

METHODS: Diet cups containing blueberry maggot fly (BMF) pupae (118 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 blueberry maggot 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 (2017 data).

RESULTS: Figure 1 shows the time series of blueberry maggot percent parasitism from 1998 to 2017 (2016 parasitism is estimated one year later in 2017). There was a sharp increase in parasitism rates in 2016 (15.98%) (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 $(F_{(1,17)} = 10.043, P = 0.006)$. Figure 3 shows this relationship, suggesting 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 blueberry fly increase in numbers from one year to the next; although, only 37.1 % of the variation in fly increase is explained by parasitoid numbers. Figure 4 shows the relationship between the logarithm of fly abundance in year t versus the log rate of increase from year t to year $t+1$ (Log(Nt+1 /Nt)). The linear relationship suggests that a density dependent relationship exists between fly abundance and the next year's increase or decrease in the blueberry maggot fly
population $(F_{(1,17)} = 21.860, P = 0.0002)$. 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 blueberry maggot fly.

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 (11.8 flies / trap) over the period from 1998 – 2017.

Fig. 3. Relationship between fly population increase and parasitoid density the previous year (1998-2017).

Fig. 4. Relationship between fly population reproduction and fly density the previous year. Dotted line demarks point of zero population increase (1998-2017).

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- 2017).

Two other relationships have emerged from the long-term BMF data. First, the data documents that while an increase in the number of flies results 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 noisy and not significant at the 5% level, but is at the 10% level (*P* $= 0.075$).

CONCLUSIONS AND RECOMMENDATIONS: This study is the only long-term monitoring of the blueberry maggot fly in North America. It shows that in 20 years there have been roughly four explosive outbreaks of the blueberry maggot fly in Downeast Maine. The fourth outbreak began in 2013. Each outbreak has 2-3 years to peak and then fly densities fall precipitously after the peak. This study is not directly applicable to management of the blueberry fly, but it does help explain what regulates its densities. One such factor is the wasp parasitoid, *Opius melleus*. This wasp also appears, in part, to regulate the frequency of outbreaks. The relationship of threshold fly captures (10 flies / trap) and the population growth of this very important pest is significant. The threshold fly capture level appears to be the point at which population growth either increases (below the threshold) or decreases (above the threshold). This reflects our finding that as population numbers of flies increase from one year to the next, the parasitoid increases and brings down the fly population. The wasp is important in that it dampens the fluctuations of the fly, meaning that explosive outbreaks are not as high and do not last as long as would be predicted without the parasitoid. Therefore, whenever possible, insecticides should be used judiciously in order not to decimate the parasitoid populations.

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. 2015-16 and 2016-17.

METHODS: In 2016, 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 2016 at recommended rates. 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. DAP was applied using a shaker can to spread the material evenly over the plot. A non-treated control (UTC) was also included in the experiment. 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.

In the spring of 2017 (2 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 between 25 and 30 May 2017. We also determined actual yields. On 11 August 2017, yields were determined by raking a diagonal swath across each plot and weighing the harvested fruit.

RESULTS: This was the third year of a three-year study (two replicated trials) designed to evaluate potential affects from summer applications of fungicide. Our analysis of flower-bud clusters from our first trial 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) (Fig. 1) or in yields among the fungicide or nitrogen fertilizer treatments ($F_{(3,12)} = 1.86$, *P* = 0.1909) (Fig. 2). In our second trial initiated in 2016, there were no significant differences in the number of flower-bud clusters/stem due to fungicide or fertilizer application ($F_{(3,12)} = 0.1.86$, $P = 0.1864$) (Fig. 3), and there was no significant difference in the subsequent crop year in number of flowers/bud due to fungicide or fertilizer application ($F_{(3,12)} = 0.42$, $P = 0.7432$) (Fig. 3). There was a significant difference in yields $(F_{(3,12)} = 4.78, P = 0.0204)$ (Fig. 4). Plots treated with Bravo alone had significantly higher yields than those treated with Pristine only or the nontreated checks.

Fig. 1. Mean flower-bud clusters/stem and flowers/bud. Lines are standard error of the mean. Letters which are different denote significant differences (*P* < 0.05). Data from 2015-16 trial.

Fig. 2. Mean yield (kg). Lines are standard error of the mean. Data from 2015-16 trial.

Fig. 3. Mean flower-bud clusters/stem and flowers/bud. Lines are standard error of the mean. Data from 2016-17 trial.

Fig. 4. Mean yield (kg). Lines are standard error of the mean. Data from 2016-17 trial.

We have also evaluated various plant response measures in addition to flower density and yield. Analysis of Variance (ANOVA) and LSD ($P \le 0.05$) 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 the 2016-17 trial, 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 non-treated controls. Plants treated with Pristine only also had longer stems than those in the non-treated control plots (Fig. 5).

Fig. 5. Mean stem length (cm). Lines are standard error of the mean. Letters which are different denote significant differences (*P* < 0.05). Data from 2016-17 trial.

Similarly to the 2015-16 trial, we observed no fungicide effects on premature flowering; there was no evidence of fall flowering in any of the plots in the 2016-17 trial.

There were some other differences between the two replicated trials. In 2015-16, 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. 6).

Fig. 6. Mean percent leaf retention. Lines are standard error of the mean. Letters which are different denote significant differences (*P* < 0.05). Data from 2015-16 trial.

This was not the case in 2016-17 when we observed no significant differences in leaf retention among the treatments $(F_{(3,12)} = 1.11, P = 0.3838)$ (Fig. 7).

Fig. 7. Mean percent leaf retention. Lines are standard error of the mean. Data from 2016-17 trial.

As far as fungal induced leaf spot, in 2015-16 we did observe a treatment effect ($F_{(3,12)}$ = 3.32, $P = 0.0569$). Figure 8 shows that Pristine + DAP fertilizer resulted in more leaf spot than either Bravo or Pristine without fertilizer. A similar result was observed in 2016-17 when Pristine + DAP-treated plots had more leaf spot than Bravo-treated plots ($F_{(3,12)} = 2.90$, $P =$ 0.0787) (Fig. 9).

Fig. 8. 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 2015-16 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. 10) and tarnished plant bug ($F_{(3,12)}$ = 4.70, *P* = 0.0216) (Fig. 11) 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. 11). There was no significant difference in the number of weevils.

Fig. 10. Effect of fungicide treatment on leafhopper abundance. Letters which are different denote significant differences ($P < 0.05$). Data from 2016-17 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 non-treated control plots ($F_{(3,12)} = 5.16$, $P = 0.0161$) (Fig. 12).

Fig. 11. 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- 17 trial.

Fig. 12. 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 2016-17 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. 13). 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.

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 + DAPtreated 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 then 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 ($R = -0.485$ and -0.629 , respectively). Positive associations were observed between boron and aluminum, iron, and manganese ($R = +0.456, +0.521,$ and $+0.556$, 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 AND RECOMMENDATIONS: Pristine and DAP in both prune years appears to enhance leaf spot disease; however, the combination of Pristine and DAP does not appear to suppress yields. We found a significant and diverse community of sap-feeding bugs colonizing blueberry. Fungicide applications affected sap-feeding bug incidence in the 2016-17 trial, but not in 2015-16. In 2015, the Pristine and DAP treatment again enhanced sap-feeding bugs. Our data suggest that it is fertilizer applied in the prune year that is the mechanism behind increased disease levels and sap-feeding bug enhancement and not the fungicide applications.

We recommend that wild blueberry growers think carefully about applying nitrogen fertilizer. The increase in yields directly due to fertilization might be indirectly compensated by an increase in crop loss due to increased weed abundance and increased disease and insect sap feeding.

Table 1. Foliage analysis.

*Study 4.**Influence of bees on the spread of mummy berry, a field study***.**

OBJECTIVE: Wild blueberries are an important crop to the Maine economy. *Monilinia vaccinii-corymbosi* (Reade), the causal agent of mummy berry disease, attacks blueberries during the flowering stage. Primary infection attacks and kills the leaves and flowers on the plants before producing new spores during the secondary stage of infection. Spores during secondary infection are transmitted to healthy flowers and turn them into shriveled pieces of fungal tissue known as mummies. This disease can greatly reduce yields. Bees are attracted to clusters of fungal spores and transmit them to healthy flowers during pollination. This study is a continuation of a 2016 study that measured mummy berry infection over time, the number of spores on individual bees in the local pollinator community, and a cage study that examined bumble bee transmission of fungal spores to healthy flowers.

METHODS:

Conidia on field bees

All field mummy berry experiments took place in a commercial wild blueberry field in Deblois on the blueberry barrens. The field was not treated with any fungicides during the course of this study. We collected the three wild blueberry pollinators: honey bees, bumble bees, and Andrenid bees to quantify conidia on them. We collected bees three times during bloom on 26 May, 1 June, and 7 June 2017. During each collection period, we collected 10 bees of each type, except for the final date where we were only able to collect seven Andrenid bees. We collected a total of 87 bees. We collected bees by catching them in a 50ml centrifuge vial and then immediately placing them in a cooler with ice. Bees were brought back to the lab and held in a refrigerator overnight, and were processed the following day for conidia.

Bees were processed using the same methods from 2016, reiterated below. We attempted to remove all conidia from the bees' bodies by dunking them into an aqueous solution and submitting them to a vortex. We added 4ml of tween to the tubes of refrigerated bees. The bee was vortexed for 30s and then 1.4ml of the vortexed solution was added to a 1.5ml centrifuge tube. The solution was centrifuged for 5m at 13,000 rpm. 1,260uL of the supernatant was discarded and 60uL of 100% glycerol was added to the solution. This final solution of glycerol and pelleted spores was vortexed for 30s and then placed into a -80°C freezer.

After all bees were processed, put into solution and frozen, we counted the number of conidia from each bee sample. The samples were defrosted, vortexed for 30s, and then two 10uL 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 to estimate 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. Logistic regression was used to assess bee taxon-specific differences in the likelihood that a bee will pick up spores vs not picking them up. We used ANOVA to determine if differences in spore per bee existed between bee taxa for bees, given that they had come in contact with lesions and picked up spores.

Bumble bee conidia transfer

Two 1.8 x 3.0m flight cages were set up on newly mowed grass behind the University of Maine's greenhouse. A single research hive of commercial bumble bees (*Bombus impatiens*) was placed inside each flight cage and given a sod of wild blueberry plants with open flowers for 24h. The day before the experiment, 120 stems with sporulating mummy berry conidiophores were collected from the blueberry field in Deblois, wrapped in damp paper towels, and transported back to the university. The stems were divided into six beakers of water and placed in the bumble bee cages (60 stems per cage). The sods of wild blueberries were removed and the cage was closed so that the bees were only able to forage on the infected stems for 24h.

Prior to the start of the experiment, two sods of wild blueberry plants were dug from the University of Maine's wild blueberry farm and transported back to the University. The sods were kept outside under a mesh cage to exclude pollinators. All open flowers from the wild blueberry sods were cut off the day before the experiment was conducted. The following day, any stems with flowers that had opened overnight were cut and placed in a 10 x 75mm vial of water. The flowers were removed so that each stem only contained a single flower.

The experiment was conducted during sunny days when bumble bees were observed foraging. During the experiment, vials containing stems of healthy blueberry flowers were set on a cinderblock in the cage. After a single bee visited a flower, the bee was caught in a 50ml centrifuge tube and put in a cooler with ice, and the flower was removed from the cage. The bees were processed for conidia as described above. The flower stem was placed in water and left inside a lab at room temperature for three days. After three days the flower was placed in 1M KOH and autoclaved for 15 minutes. Styles were carefully removed from tubes, placed on a slide and then stained with aniline blue (0.05% aniline blue in 0.067M K₂HPO₄, 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.

This experiment was conducted two times. During the first trial, 15 bees visited flowers, and during the second trial, 36 bees visited flowers. A total of 51 bees and flowers were processed for conidia.

RESULTS:

Conidia on field bees

Results in 2017 were different from 2016 as only 14 out of 87 bees had any detectable spores. Six Andrenid bees had spores, seven bumble bees carried spores, and only a single honey bee was collected with any detectable spores.

We found evidence for differences among the bee taxa for picking up spores vs not picking up spores ($X^2_{(2)}$ = 6.602, *P* = 0.037). Estimated odds ratios suggest that honey bees were more likely to pick up spores than bumble bees (9.441X, $P = 0.017$) and andrenids (8.295X, $P =$ 0.028). There was no difference between bumble bees and andrenids $(P = 0.847)$. We found that honey bees carried significantly more spores than bumble bees or andrenids, a pattern similar to what we found in 2016, except that we compared average spore loads of all bees, not just those that had spores on them. Figure 1 shows this bee taxon-specific relationship.

Bumble bee conidia transfer

A total of 51 wild blueberry flowers were collected and processed after a visit from a conidia exposed bumble bee. Of the 51 flowers, only eight had any spores on them (16%), and of those, only four had spores that were germinating (8%). One flower had seven spores, three of which were germinating. The other flowers with spores had only a single spore per flower. We did not observe any spore germ tubes growing down the style. As seen in our 2016 study, conidial transfer by bumble bees occurred infrequently and infection of the flower style was rare. However, even an 8% transmission rate with hundreds of thousands of flowers visited per day has the potential to result in considerable secondary infection and yield loss.

Fig. 1. The relationship between bee taxon and the number of spores per bee for those bees that had contacted lesions and picked up spores.

CONCLUSIONS: Our previous study in 2016 found that honey bees carry more conidia than bumble bees and andrenids. In 2017, most bees did not carry any spores, probably because disease sporulation was much lower compared to 2016. By examining only conidia carrying bees, we were able to see a similar pattern to our 2016 study. Once again, honey bees carried more conidia than native bees. Our spore transfer study also reconfirmed our previous results which suggest that spore transfer from bees to flowers is a rare and random event. Additional studies in 2018 will be useful to further verify our findings over the past two years.

Study 5. Seasonal biology of blueberry tip midge.

We have not conducted many studies on the general biology of blueberry tip midge. In 2010, in the course of collecting data from various thrips trials, tip midge was observed in curls having typical thrips curl characteristics (tight, red, cigar-shaped leaf galls). The gall caused by tip midge is typically loose, green, and bubble-shaped. A study was undertaken to begin to assess the potential for competition between tip midge and blueberry thrips and to document the phenological characteristics associated with blueberry leaf galls resulting from infestation by these insects. There appeared to be very little interaction between thrips and tip midge within the curls; 49 curls contained only thrips while 23 contained only tip midge. Only four curls were found to contain both species (Table 1). This first year of sampling suggests that gall form cannot be used accurately for distinguishing between pest species. The majority of "thrips type galls" did indeed have thrips in them, but 26.1% had tip midge. The "tip midge type galls" were even less definitive (33% with thrips); although, the sample size was very small ($n = 28$ total galls). Evidence for competition was lacking, out of the 76 galls that had either thrips or tip midge associated with them, only 5.3% of these galls had both pests. Although, another possibility is that one pest is predating on another and reducing their numbers.

Table 1. Abundance of blueberry tip midge and blueberry thrips; interaction within leaf curls. Data from 2010.

METHODS: In 2017, an effort was made to follow seasonal density of blueberry tip midge by collected curls and examining for larvae. At intervals beginning on 22 May when damage as evidenced by leaf curls first became apparent and continuing until 3 September, ten stems with leaf galls were collected, brought into the laboratory and examined for the presence tip midge and/or thrips.

RESULTS: A total of 89 stems were collected over 9 sample dates. Of those stems, 47.2% (n = 42) were found to have tip midge larvae; 22.5% (n = 20) contained thrips; and 13.5% (n = 12) contained both tip midge and thrips. Figure 1, depicting the percent of galls infested, illustrates the overlapping generations of continuously reproducing thrips during the growing season (dashed line). Figure 1 (percent galls infested with tip midge) and Figure 2 (the mean density of tip midge per gall) suggest that tip midge may complete three generations during the growing season. The first generation appeared to start in early May and peaked in late May. The second generation peaked 2-3 weeks later in mid-June and the third generation peaked in early August.

Fig. 1. Percent curls with tip midge (solid line) or thrips (dashed line).

CONCLUSIONS: We historically hypothesized that wild blueberry was a suboptimal host for the tip midge and that only one generation was completed in the spring. However, more intensive sampling has revealed that, at least in the prune year, blueberry tip midge may complete three generations on its wild blueberry host. This in part may explain the difficulty we have had in controlling this pest with one or two insecticide applications and why in 2017 three applications of insecticide resulted in good control (See Study 2 of Progress Report No. 1 – Control Tactics for Blueberry Pest Insects, 2017).

ENTOMOLOGY

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

E. Ballman, Research Associate in Invasive Species/Entomology

3. III. TITLE: Biology of spotted wing drosophila, 2017.

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

OBJECTIVE: Current sampling of fruit for the detection of 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. This study was repeated in 2017 to validate the results.

METHODS: In both 2016 and 2017 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/](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 mean infestation (# larvae per cup) from the sequential sampling effort conducted in 2016 and 2017. Infestation levels were higher in the 2017 sampled fields (4.83 larvae / cup) compared to the fields sampled in 2016 (2.21 larvae / cup). Figure 2 shows that independent of year, sampling precision reaches an acceptable level of 20% (standard error to mean ratio) by taking three cups in a field to estimate infestation and drops below a high level of sampling precision, 10%, after taking six samples in a field.

Fig. 1. Cumulative mean from sequential sampling of infested fruit. Data from 2016 and 2017, dashed lines are overall means for each year.

Fig. 2. Precision, calculated as the standard error to mean ratio from sequential sampling of infested fruit. Data from 2016 and 2017, dashed line represents a moderate level of precision at 20% standard error to mean ratio.

RECOMMENDATIONS: We have determined that growers wanting to sample their fields for larval infestation should take a minimum of three cups representatively scattered throughout the wild blueberry field. This intensity of sampling will provide a moderate level of precision, 20%.

Study 2. Early harvest and action thresholds for spotted wing drosophila.

OBJECTIVE: 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. In 2017 we repeated the 2016 study on an additional 11 farms.

METHODS: 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. Relevant dates and grower applied insecticides are shown in Table 1. These insecticides were applied for blueberry maggot fly control and SWD control. Beginning in mid-July before any SWD had been captured and continuing until fields were harvested, traps were placed in 11 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 $(1tbsp) + sugar(4tbsp) +$ 12oz water (makes enough for four traps). The traps were hung 1-2ft above the top of the canopy using 36 inch 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 these 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 11 wild blueberry fields on various dates from mid-July until the fields were harvested, and processed using the Salt Extraction Method described in Maine wild blueberry factsheet #210 [\(http://umaine.edu/blueberries/factsheets/insects/210-spotted-wing-drosophila/\)](http://umaine.edu/blueberries/factsheets/insects/210-spotted-wing-drosophila/).

Each sample consisted of a 1-cup sample collected from the vicinity of each of the three adult traps (three samples per field. Using these data we calculated the mean number of maggots collected from each site on each sample date. These data were compared with the adult abundance data collected over the same time period.

Utilizing data from the 11 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 a cumulative average of 3 males per trap is captured then harvest, and 4) wait until a cumulative average of 9 males per trap is captured then harvest.

RESULTS: We found that both year ($F_{(5,77)} = 2.886$, $P = 0.019$) and cropping system ($F_{(3,77)} =$ 14.179, $P < 0.0001$) were significant predictors of insecticide application frequency. This corroborates a similar finding reported by Yarborough et al. (2017). Figure 1 shows that as cropping system transitions from organic to low input conventional, to medium input, and then to high input, insecticide frequency increases. Year most likely reflects the insect pest pressure of blueberry maggot fly and spotted wing drosophila, the two pests overlapping in the late summer.

Factors that influence whether a field has infested fruit were mean cumulative male spotted wing drosophila trapped $(X^2_{(1)} = 65.713, P < 0.0001)$ and insecticide application frequency $(X^2_{(1)} = 6.051, P = 0.014)$. Models could be fit with cropping system or year, but only if insecticide application frequency was dropped, indicating co-linearity among these predictors.

The level of infestation in each field (percent fruit infestation) was best described with the independent variables year $(F_{(5,75)} = 3.222, P = 0.011)$ and mean cumulative male spotted wing drosophila trapped $(F_{(1,75)} = 94.735, P < 0.0001)$. Cropping system and frequency of insecticide applications were not significant ($P > 0.05$). The overall R² for this model was 0.645; although, the residuals are only marginally well behaved. The high number of zeroes in the percent infestation of fields makes it difficult to meet the assumptions of normality and homogeneity of variance. In order to minimize this problem we modeled only the infested fields to assess factors that might affect the level of infestation (% infestation). We found a similar pattern with year $(F_{(5,26)} = 3.680, P = 0.012)$ and mean cumulative male spotted wing drosophila trapped $(F_{(1,26)} = 29.007, P < 0.0001)$.

Table 1. Grower insecticide applications in 2017.

Fig. 1. The average number of late summer insecticide applications by wild blueberry cropping system (2012-2016).

We fit a probability density function to the mean cumulative male spotted wing drosophila trap captures in fields that had infestation prior to harvest. This was done in order to suggest action thresholds that could be associated with a risk of infestation the week following the trap capture numbers. This would provide growers time to protect their fields if their selected action threshold was reached. The Gamma distribution provided the best fit to the empirical data. Estimates for a two parameter Gamma distribution were: alpha (shape) = 1.6765 $(1.0949-2.4512, 95\% \text{ CI})$, and sigma (scale) = 9.43259 (6.1286 – 15.7678, 95% CI). The Cramer-von Mises W^2 test suggests that the empirical data are not significantly different from the theoretical frequency distribution ($W^2 = 0.153$, $P = 0.25$). Using the probability density function, we calculated a series of quantiles that represent a gradient of action thresholds, ranging from conservative to liberal risk, in terms of the likelihood that the following week will result in infestation of a field (Table 2).

Table 2. Grower action threshold sliding scale of increasing risk.

Validation of the thresholds estimated in 2015 was conducted in 2016 and 2017; although, we also used these data to develop the final action thresholds delineated in Table 2. In 2016, the growers (9 fields) that used the action thresholds of 1 or 3 male SWD had no damage by harvest; however, 40% of the fields that used the action threshold of 9 male SWD (5 fields) incurred damage prior to the growers being able to protect the fields with insecticides. This was not unexpected since the action threshold of 9 male SWD in 2016 was expected to result in 25% probability of infestation. In 2017, the action thresholds validated were 1.6, 2.8, and 6.0 cumulative male SWD per trap. The three fields using the action threshold of 1.6 and the three fields using the threshold of 2.8 did not have infested fruit following these thresholds. The fields following the threshold of 1.6 were all harvested prior to any detected infestation. The three fields using the threshold of 2.8 did not get infestation until the cumulative male SWD captures reached 8.0, 14.3, and 32.7. The last four fields in 2017 used a threshold of 6 cumulative male SWD per trap. One of these fields was infested at a male SWD capture level of 4.7. The three other fields did not become infested until male SWD numbers were 13.0, 16.3, and 55.3. Therefore, 25% of the fields (1 of 4) using the higher threshold became infested before reaching 6 male SWD. This is what was predicted.

These action thresholds based upon the likelihood of infection are probably more robust than thresholds based upon a certain amount of infection. For instance, the current blueberry maggot fly action thresholds are 10 cumulative flies per trap. This threshold is not arbitrary. It represents the level of fruit infestation that is detectable (4 maggots per quart which is approximately 0.8% infested fruit) through the boil test. The USDA sampling of fruit for assessing grade has the same lower detection limit because they also currently use the boil test. However, research into molecular detection methods will probably provide inspectors and the food processing industry with much more sensitive techniques. If this happens, a threshold that results in many fields not being sprayed or only sprayed 1-2 times might be abandoned with the result of a much more insecticide intensively produced crop. An action threshold such as developed here for SWD should be robust to better detection methods because the aim is to produce no infestation. However, the adoption of different or better monitoring methods (traps or baits) will require re-parameterizing these action threshold levels.

Early harvest is also a tactic that some growers use to avoid SWD infestation. Figure 2 shows the relationship between harvest date (expressed as Julian Day) and the percent of ripe or potentially marketable fruit harvested at that date. It can be seen that while early harvest may result in avoidance of SWD infestation, as it did in the first three fields harvested in this study, the crop loss that results from non-ripe berries can be considerable. However, non-ripe fruit can be easily sorted out in the processing line, while SWD infested fruit is much more difficult to eliminate unless it is heavily infested with late instar larvae and thus characterized by fruit with little integrity, i.e. soft, collapsed fruit.

Fig. 2. Relationship between date of harvest and the percentage of ripe (blue) berries harvested as a percent of the total berry yield. (% ripe = $100/[\overline{1} + e^{(30.903 - 0.159 \cdot 3) \cdot \text{Julian Date}}]$), pseudo r² = $0.795, P = 0.002$.

The incorporation of an early harvest tactic along with a tactic of action thresholds would be compatible and superior to either tactic by itself. Use of this integrated pest management strategy would allow a grower to plan on early harvest, but monitor for SWD males at the same time. If the field has not reached the action threshold, but has a high percent of non-ripe fruit, then the grower could hold off on harvest another week until either the threshold is reached or the fruit is more ripe (an economic level that does not cause too much revenue loss) and then the grower could harvest in the following week dependent upon either or both conditions being reached. In addition, our logistic regression analysis of factors affecting the likelihood of a field becoming infested suggests that growers should also incorporate their summer spray schedule into their decision-making. The estimated odds ratio for the effect of frequency of insecticide applications suggests that for every increase in the number of sprays, the likelihood of infestation will be reduced by 0.374. This means that growers can be more liberal in their action threshold selection if they have been spraying for blueberry maggot fly during the summer.

CONCLUSIONS AND RECOMMENDATIONS: In conclusion, we have been developing a database of male fly captures and the associated likelihood of infestation by SWD the week following the captures. These data have allowed us to estimate likely thresholds for varying levels of accepted risk. Our field trials in 2016 and 2017 suggest 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. Based upon our probability model, we are now planning on using 1, 2, and 3.5 cumulative male SWD for action thresholds; although, for those growers very averse to risk, a threshold of 0.5 cumulative male SWD is recommended. Caution is still advised in following

these thresholds and it is recommended that growers use these thresholds, but in addition sample for fruit infestation to verify the predictions.

LITERATURE CITED:

Yarborough, D., Drummond, F., Annis, S. and D'Appollonio, J. 2017. Maine wild blueberry systems analysis. Acta Horticulturae 1180:151-160. ISHS 2017. DOI: <https://doi.org/10.17660/ActaHortic.2017.1180.21>

*Study 3.**Winter survival of spotted wing drosophila***.**

OBJECTIVE: The severity of spotted wing drosophila (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 certain spots in the United States, it is not known whether they can overwinter in Maine's cold northern climate. This study will attempt to determine survival rate over the next several years. The reason why this is important to Extension scientists and growers is that it provides the basis for explaining predictions of the timing of SWD wild blueberry field colonization (see Study #7 of this report on degree-day prediction).

METHODS: We are currently testing the overwintering survival capability of spotted wing drosophila as part of a multi-state project. This is an ongoing project that was initiated in 2016 and will extend into March 2019.

Establishing SWD colony 2016

Adult SWD acquired from our laboratory colony were moved to new 0.3 X 2.0 cm Drosophila culture tubes that contained Instant Drosophila Medium formula 4-24 (Carolina Biological Supply Co., Burlington, N.C.). 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^oC, 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.

Establishing SWD colony 2017

Wild flies were live trapped in an organic wild blueberry field in Stockton Springs, ME. Live traps consisted of a commercial yellow jacket trap (Victor Model # M362) with a lab created fermentation lure snapped to the inside of the lid and with a Drosophila culture tube that contained Instant Drosophila Medium inside. Fifteen traps were deployed along the field edge and were changed daily for one week. Each day, flies in the containers were anesthetized with $CO₂$ and inspected to verify that they were SWD. All confirmed SWD were placed into Drosophila tubes with media as described above and placed in a 25°C chamber with a 18:6 L:D cycle. Flies were transferred to new media tubes every two weeks. Two weeks before placing flies in the field, flies were moved to 10°C within 48hr of eclosing and held at 10°C for a minimum of one week.

Design of field experiment 2016 and 2017

A field site was set up along a wooded edge at the University owned Rogers Farm in Old Town, ME. Twenty-four holes were dug using a fence post digger. The soil plug was placed into a 36oz deli cup 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 (old leaves, twigs, etc.). A 1/8 piece of apple was set on top of the organic matter and 50 chilled SWD males and females (total of 100 flies) were added to the organic matter and covered in 2-3cm of dead 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 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 covered with surrounding leaf litter.

Every two weeks, a set of four pots were dug up, transported back to the laboratory, 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 was brought back the same day the experiment was launched. This experiment ran for 10 weeks beginning on 15 November 2016. An identical experiment was set up in December 2017.

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 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 field 2016 and 2017

Flies were 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, 250ml water and one drop of unscented dish soap. The flies were strained out of solution and preserved in ETOH 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.

Live trapping adults 2016

Adults were live trapped during warm days above 10° C by using four red Solo[®] cups elevated 3ft above the ground and baited with 60% merlot wine and 40% apple cider vinegar mixture. The bait was in a one ounce portion cup covered with fine 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 Dr. Dalila Rendon at Oregon State University to analyze nutritional content of flies.

RESULTS:

2016

A total of six live flies were trapped during the weeks of 9 and 15 November. Drowning traps caught flies during the weeks of 29 November (93 SWD), and 13 December (8 SWD). No other wild SWD were caught during the remainder of the study.

Survival started at nearly 90% on the first day which means roughly 10% of flies died from handling and experimental setup. Survival dropped to 46% after two weeks outside, and continued to drop steadily during the study. No flies survived to the final week (Fig. 1). There was no significant differences in survival between sexes ($X^2 = 2.0$, df = 1, $P = 0.16$) (Fig. 2).

Fig. 2. 2016 survival of male and female SWD over time in cups.

2017

Results are forthcoming in 2018 for the 2017 deployment of flies and final results of the study will be available after the project is completed in 2019.

CONCLUSIONS AND RECOMMENDATIONS: Although survival declined rapidly during this experiment, there were a few flies alive after 56 days of winter. No flies survived to day 70, but it is possible that a larger sample would have yielded a few survivors. It is still unclear if *D. suzukii* are capable of surviving winter in Maine. Changes in temperature or snowpack may have a significant impact on adult survival. Repeating this study in 2017-2018 should yield additional insight.

*Study 4.**Seasonal biology of spotted wing drosophila***.**

OBJECTIVE: This study is part of a national effort to determine seasonal changes in the genetic (SNP signature, SNP = single nucleotide polymorphism) composition of SWD populations from locations with variable climates and to then use that information to determine the origin of spring populations in northern areas (flies that overwintered locally v. migrated from milder climates). We are working from the assumption that peak populations will have the highest genetic heterogeneity, and that the more cold stress a population experiences, those lessadapted flies will be killed, leaving behind a more genetically homogenous population in winter samples. Six geographic locations will be sampled at various time points over two years (total n $= 6$; 2017&2018); three cold climates (ME, NY, MI) and three warm climates (NC, GA, OR).

METHODS: Adults were trapped monthly beginning in early July using an attractive lure and salt water drowning solution. Each sample consisted of a minimum of 30 female flies. Drowning solution consisted of water, salt (144g NaCl in 400ml water) and a drop of unscented soap (we use Seventh Generation®). Flies were stored at 4° C and preserved in 95% ETOH prior to shipment to UC Davis for DNA extraction and SNP determination.

In order to identify SNPs that are important to cold stress tolerance in SWD, comparisons will be made between peak populations (LATE AUGUST) and cold-stressed populations (LATE NOVEMBER). We anticipate a range of cold stress from various sampling locations that will allow for good comparisons and can quantify the amount of cold stress each population experienced based on weather station data and the "hours below zero" metric we estimate from the field overwintering survival project (see Study 3 of this report).

In order to identify potential origin of SWD in northern locations (or higher elevations; (locations where SWD are not captured for long periods of time in the early spring), we will compare SNP signatures of SWD sampled from different locations as soon as flies are available (MAY&JUNE), at peak densities and late season with SNP signatures from locations further south (lower elevations). Since we do not know the date(s) when migration will occur and since northern movement may occur multiple times during the season, having repeated sampling through the year would be the most useful. Spring-time sampling in more northern latitudes is the most significant challenge for this project. Spring populations are likely very low (or possibly zero) and adult SWD are not typically captured in traps until early summer.

RESULTS: We will not know the results of this study until flies are processed and genetic markers are analyzed. The conclusions that are made from our fly collection should support the results that we obtain from our SWD overwintering study.

*Study 5.**Novel lure testing for spotted wing drosophila.*

OBJECTIVE: Spotted wing drosophila (SWD) is an invasive insect of concern in Maine that lays its eggs in soft fruit such as lowbush blueberries. In Maine, SWD first appears during the late summer in late July or early August. Fruit damage can be greatly reduced when using economic thresholds to time treatments or harvest. Currently, a home-made yeast-sugar bait is recommended to attract SWD to monitoring traps. Growers have expressed an interest in commercial products that are easier and less messy to use. 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 in 2016. This study is a continuation of the 2016 study and tests new lures as well as multiple lures in combination with one another. This study was conducted to determine which lure, or combination of lures, was the most effective at catching SWD while limiting other drosophila species.

METHODS: The trial was run at the University of Maine's Blueberry Hill Farm in Jonesboro, ME and at an organic commercial blueberry farm in Stockton Springs, ME. Six different lures were used in this study in addition to a water control (Table 1). Two replicates of each trap type were deployed in two separate blocks at each location for a total of 28 traps. Traps were a commercial yellow jacket trap (Victor Model # M362) (1,100mL capacity) (Fig. 1). Traps with a lure had the lure snapped into the inside of the trap lid. For traps that had only one lure, a blank tube was snapped into the opposite cap so that all traps had two tubes attached to the lid. Mesh with 2mm openings was glued to the two openings on top of the trap to exclude large insects. A single row of traps were set up along a wooded edge adjacent to a wild blueberry field. Traps were hung 1m high (either from tree limbs or fence panels), were separated by 5m, and the two blocks at each site were 12m apart from one another.

Lures were changed in the traps every two weeks and the drowning solution was collected and changed on a weekly basis. The order of traps within each block was rerandomized every week. Insects were drained out of the drowning solution and stored in 70% ETOH in a refrigerator until they could be sorted and counted at the end of the season. Traps were deployed in Stockton Springs on 2 August and on were deployed in Jonesboro on 8 August, and ran for six weeks at each location.

Fig. 1. Lure trap with lure snapped in place.

For each trap catch, the number of male and female SWD, non-SWD drosophila, and non-drosophila Diptera were counted. Traps that appeared to contain more than 100 SWD were subsampled using a 10 x 10cm gridded Petri dish. To subsample, trap catches were spread across as many dishes necessary to make for easy counting. Flies were counted in ¼ of the grids in the dish and the number of flies was multiples by four to estimate the total fly capture.

Data were transformed using a log transformation. A one-way ANOVA was conducted to measure differences between sites and treatments. Tukey's test was run to measure differences between individual treatments across both sites combined. All analyses were conducted using JMP® 13 statistical software.

Table 1. Trap lure treatments.

RESULTS: The fermentation lure and yeast sugar bait caught the largest amount of SWD and other drosophila (Table 2, Fig. 2 and 3). In 2016, the fermentation lure caught significantly more non-SWD drosophila compared to the yeast sugar bait, but in 2017 they caught roughly the same amount. The addition of a yeast, leaf, or yeast-leaf combination lure to the fermentation lure reduced the number of SWD caught as compared to the fermentation lure alone. Non-drosophila Diptera represented a small number of the total Diptera catch. The number of SWD caught in traps increased during the season at Stockton Springs, but in Jonesboro, fly densities peaked in early September and then declined (Figs. 4 and 5).

Table 2. Average trap catches across all dates and sites, letters designate statistical differences within each column. All analyses were run on log-transformed data.

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

Fig. 3. The average number of SWD, non-SWD drosophila, and non-drosophila Diptera caught over the season in Stockton Springs.

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

Average SWD Trap Catches Per Week Jonesboro

Fig. 5. The average number of SWD (male and female combined) caught over the season in Stockton Springs.

Average SWD Trap Catches Per Week Stockton Springs

CONCLUSIONS AND RECOMMENDATIONS: The fermentation lure appears to be as effective at trapping SWD, and did not capture significantly more non-SWD drosophila, compared to the traditional yeast sugar bait. However, adding additional lures to the fermentation lure appeared to act as a deterrent. The fermentation lure may be a promising choice for growers looking for an easier trapping method than the traditional home-made yeast sugar mixture.

Study 6. Rapid resistance monitoring for spotted wing drosophila.

OBJECTIVE: This protocol was developed based on dose-response assays conducted by Michigan State University and the University of Georgia as part of the SCRI-SWD project. It is intended to provide a rapid assessment of the susceptibility of SWD to insecticides using a simple test that can be widely used by extension staff without insect colonies and laboratories. The objective of the study is to 1) compare response of SWD across the United States to LC_{50} levels of insecticides and 2) to determine whether any SWD populations exhibit survival at concentrations that will kill 100% of susceptible SWD.

METHODS: Five assays were completed on adult SWD collected from three locations. Two sites were located in Washington Co. (Jonesboro - BBH and Deblois - BC) and one site in Waldo Co. (Stockton Springs - HF). For each site to be assessed, SWD adults were collected from live traps placed at that site. For Assay #1 (BBH) we used flies collected directly from the field (designated as F0). For the remaining four assays, field collected flies were reared on drosophila diet (Instant drosophila medium, Carolina Biological Supply company, Burlington, North Carolina) in the laboratory for one generation (designated as F1).

For each assay, a 20ml scintillation vial was treated with 1ml of a Malathion 8F solution prepared as outlined below. Flies were assayed against the LC_{50} (4.98 ppm) and the LC_{99x2} (32.5 ppm) levels. Once the solutions were added to vials, caps were tightly closed and the vial was shaken a few times to distribute the solution, the excess solution was then poured out and the vials and lids were placed in a fume hood to dry. The next morning, 5 female and 5 male adult SWD flies from the sampled location were placed in each vial and left in a growth chamber and held at 24.4 °C and 73.9% RH to reduce control mortality. For each assay there were three (Assay 1) or four (Assays 2-5) replications for each LC level + three or four non-treated controls. After 6 hours in the vials, we counted the number of flies that were alive (i.e. not moribund or dead).

Guide to making stock solutions of Malathion 8F for testing SWD against LC₅₀ and LC_{99x2}

Step 1 – Make the stock solution 52.16 microliters Malathion $8F + 100$ ml acetone (500 ppm) Step 2 – Make the LC_{50} solution – 1 ml stock solution + 99 ml acetone (5 ppm) Step 3 – Make the LC_{99x2} solution – 6.6 ml stock solution + 93.4 ml acetone (33 ppm)

RESULTS: We found that there was a difference in the response between flies collected in the field live and assayed immediately and those collected live from the same location and reared through one generation in the lab and then assayed (field vs lab, concentration x source interaction: $\overline{X}^2_{(1)} = 43.317$, $P < 0.0001$). Figure 1 suggests that the flies reared through one generation may have become more susceptible to the LC_{50} concentration of Malathion. Figure 2 shows the percent mortality in response to concentrations of Malathion for three sites. Two of the assays were for the same site, HF. One can see that the assay for HF1 had a high level of control mortality. Because of this we only compared assays that had low levels of control mortality. When we compared the response of flies collected at the three sites we found that there was no site effect ($\overline{P} = 0.481$), only a concentration effect ($\overline{X^2}_{(1)} = 352.141$, $P < 0.0001$). It can be seen all the sites were characterized by a very high level of mortality to the hypothesized LC_{50} rate, the rate at which it is expected that 50% of the flies would die. Therefore, there is no evidence that SWD is developing resistance to Malathion in Maine wild blueberry fields.

Fig. 1. Percent mortality for SWD adults collected in the field and immediately assayed compared to those reared through a generation in the laboratory.

Fig. 2. Percent mortality of flies collected live at three sites, reared in the laboratory, and then assayed at the Malathion LC_{50} and LC_{99x2} doses.

CONCLUSIONS AND RECOMMENDATIONS: We found no evidence to suggest that SWD is developing resistance to Malathion in Maine wild blueberry fields. We cannot be sure that this is also true for other commonly used insecticides. However, independent of our imperfect knowledge, the best recommendation to follow is to rotate insecticides with different modes of action (classes) within a year or between years at the same site in order to retard the development of resistance. This is challenging due to foreign MRL levels that vary among countries. This should be considered a priority as this pest has the potential to develop resistance quickly.
*Study 7.**Assessing Oregon State University's spotted wing drosophila phenology model as a predictor of spotted wing drosophila colonization of wild blueberry fields in Maine 2017***.**

OBJECTIVE: To evaluate Oregon State's spotted wing drosophila phenology computer model to see if its results could be used to predict timing of control in Maine wild blueberry fields.

METHODS: Oregon State University developed a spotted wing drosophila (SWD) phenology model based on degree-days (http://uspest.org/wea). This program is based on data collected in the Pacific Northwest and assumes SWD are able to overwinter successfully in the region of prediction. The model allows the user to select the nearest weather station as well as the date from which to begin the degree-day model. The program then outputs the total accumulated degree-days, as well as dates that correspond to SWD generations that are laying eggs and reaching peak emergence. The following categories and their corresponding dates are given in the output:

- 1) 1st EGG LAYING BY OW [over wintered] FEMALES
- 2) PEAK (ca. 50%) EGG LAYING BY OW FEMALES; 1st ADULT EMERGE 1st GEN
- 3) 1st EGG LAYING BY 1st GEN FEMALES
- 4) PEAK ADULT EMERGE 1st GEN
- 5) PEAK EGG LAYING BY 1st GEN FEMALES; MAX 2+ GENS.
- 6) PEAK ADULT EMERGE 2nd GEN; MAX 3+ GENS.
- 7) PEAK EGG LAYING BY 2nd GEN FEMALES; MAX 4+ GENS.
- 8) PEAK ADULT EMERGE 3rd GEN; MAX 5 GENS.
- 9) PEAK EGG LAYING BY 3rd GEN FEMALES; MAX 6+ GENS.
- 10) PEAK ADULT EMERGE 4th GEN; MAX 6+ GENS.
- 11) PEAK EGG LAYING BY 4th GEN FEMALES; MAX 7+ GENS.
- 12) PEAK ADULT EMERGE 5th GEN; MAX 8+ GENS.
- 13) PEAK EGG LAYING BY 5th GEN FEMALES; MAX 9+ GENS.

It would be the first generation predictions that would be useful for Maine wild blueberry growers (lines 1-5).

Since 2012, we have monitored SWD infestation weekly across multiple fields in Maine's blueberry growing region. We used this program to see if we could find a correlation between our trap captures, and this phenology model. We used the dates of the first SWD male captures as that is considered the most conservative minimum action threshold in our state. Growers who base their actions on a one male threshold have a 99% chance of no infestation until at least a week after this first capture date (see Maine wild blueberry factsheet #210, [http://umaine.edu/blueberries/factsheets/insects/210-spotted-wing-drosophila/\)](http://umaine.edu/blueberries/factsheets/insects/210-spotted-wing-drosophila/).

To test the usefulness of the Oregon predictive model for Maine, we selected two groups of fields across multiple years. The first group was made up of four locations that had three to six years' worth of data per field. The second group was comprised of the earliest and latest SWD capture from 2012 to 2016 for a total of 10 site and year combinations. We chose late and early infestation fields to see if the phenology model could accurately predict SWD infestation in either extreme. We looked at the difference between model outputs and our trap captures. This program does not offer suggestions as to when to time treatments, or harvest, so we compared a number of their program outputs to our trap capture dates to see if there was any pattern.

RESULTS: Regardless of which of the phenology outputs we assessed, there does not appear to be an accurate prediction of our trap captures by the phenology model. We specifically compared the dates of first male SWD capture and also the first SWD captured of any sex to the predicted dates of when the first generation began to lay eggs, when the first generation reached peak emergence, when the first generation reached peak egg laying, and peak emergence of the second generation. Categories after those generally fell beyond the date of our first SWD trap capture and so were not considered in our analysis as fields could potentially already be infested at this point. Time between phenology output events and SWD captures had considerable variation, and this variation increased in early and late field cases (Tables 1 & 2). For example, when we compared the timing of peak egg laying of first generation SWD in the model to actual first SWD trap captures, the predicted phenology event was anywhere between 13 days before our SWD trap captures, to 29 days after the first trap captures. If someone used this model instead of monitoring their field, they could potentially begin spraying two weeks early or a month late.

	Average days before first SWD male	Range of days before first SWD male	Average days before first SWD capture	Range of days before first SWD capture
Days between $1st$ egg	captures	captures		
laying by $1st$ generation and first SWD trap capture	40.70 ± 12.52	$15 - 64$	35.70 ± 13.08	$15 - 64$
Days between peak emergence of 1 st generation and first SWD trap capture	29.60 ± 12.59	$5 - 53$	24.60 ± 13.48	$5 - 41$
Days between peak egg laying by $1st$ generation and first SWD trap capture	17.20 ± 12.70	$-7 - 40$	12.20 ± 13.59	$-7 - 40$
Days between peak emergence of $2nd$ generation and first SWD trap capture	2.90 ± 12.93	$-22 - 27$	-2.10 ± 13.58	$-22 - 27$

Table 2. Time difference in days between predicted SWD phenology model events and the first male and first SWD captures in early and late infestation Maine wild blueberry fields.

CONCLUSIONS AND RECOMMENDATIONS: Oregon State's predictive phenology model does not appear to accurately predict SWD first trap captures in Maine wild blueberry fields. Therefore, it should not be recommended to growers to use in order to determine when to start trapping or treat a wild blueberry field for SWD in Maine. Basing insecticide application decisions on this model could potentially result in unnecessary pesticide applications or fruit infestation depending upon the timeliness of the prediction. The phenology model assumes that SWD can successfully overwinter. While this may be the case in Oregon, we do not know with certainty that SWD can overwinter in Maine. If they can overwinter here, it is likely that fewer of them survive than what is accounted for in the phenology model and this would greatly delay the probability of capturing SWD compared to the prediction based upon degree-days. Our recommendation is to continue monitoring fields with SWD traps (see wild blueberry SWD trap fact sheet) and use the action thresholds that have been developed for Maine [\(http://umaine.edu/blueberries/factsheets/insects/210-spotted-wing-drosophila/\)](http://umaine.edu/blueberries/factsheets/insects/210-spotted-wing-drosophila/). The combination of field level monitoring and action thresholds will give growers a more accurate idea of how best to manage their fields.

ENTOMOLOGY

INVESTIGATORS: F. A. Drummond, Professor of Insect Ecology/Entomology

J. A. Collins, Assistant Scientist of Insect Pest Management

E. Ballman, Research Associate in Invasive Species/Entomology

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

Study 1. Assessment of synergism between acetamiprid and propiconazole on the commercial bumblebee, Bombus impatiens, in wild blueberry.

Report from Alexander J. Chandler (U. Maine undergraduate), Frank Drummond, and Judith Collins

OBJECTIVE: Recently there has been concern about the decline of pollinators, especially honey bees and bumblebees. Since the decline there has been an increase in research to determine the reason. Potential causes of this decline have been identified, by some previous works, as neonicotinoid pesticides and non-insecticidal agrochemicals commonly used in farming (Iwasa et al. 2004, Laycock et al. 2014, Sanchez-Bayo and Goka 2014, Scott-Dupree et al. 2009, Sprayberry et al. 2013). Generally, honey bees (*Apis mellifera*) are used to determine how these compounds affect pollinators, but rarely has there been research done to find how bumblebees or other bee species are affected (Malone et al. 2007).

There have been a few studies that have found there may be synergy between certain insecticides and fungicides. Synergy being, "the toxicity of a mixture is greater than the sum of the toxicity of the mixtures components" (Thompson and Wilkins 2003). A lab study by Iwasa et al. (2004) found that some combinations have potential to increase the toxicity to *A. mellifera,* indicating synergism. In that experiment, they found acetamiprid had a much stronger effect than imidacloprid when applied with a synergist. Neonicotinoids, like acetamiprid, have been used because of their reduced toxicity to honey bees, while still being effective against Heteroptera, Coleoptera, and Lepidoptera. There has been some research indicating that certain non-insecticidal agrochemicals and insecticides have an effect on bumblebee (*Bombus impatiens*) and honey bee foraging behaviors and impair long-term memory, making it more difficult for the bumblebees to locate food and, given the choice, feed on non-contaminated food sources (Blacquière et al. 2012, Kindemba 2009, Sprayberry et al. 2013, Thompson et al. 2014). In particular, the combination of acetamiprid and propiconazole was found to have an increase in toxicity by 599-fold on *A. mellifera* when applied topically (Iwasa et al. 2004).

These two compounds are commonly used in Maine wild blueberry production. This is due to the fact that acetamiprid (formulated as Assail®) is an effective control tactic for blueberry flea beetles and blueberry spanworm, and moderately effective against blueberry maggot flies and spotted wing drosophila; and propiconazole (formulated as $\overline{Orbit}^{\circledast}$ or Tilt[®]) is effective against mummy berry. This study tests the synergism between these two compounds on the bumblebee *B. impatiens* at agriculturally realistic levels on blueberry fields*.*

METHODS: Nine 6 x 2 x 2m field cages were erected over wild blueberry in bloom at the University of Maine Blueberry Hill Experimental Farm, Jonesboro, ME. The crop inside the cages was sprayed at full bloom with either water (control), acetamiprid (formulated as Assail® at 4 oz/acre), or a combination of acetamiprid and propiconazole (formulated as Tilt[®] at 6

oz/acre). The 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 for each application was regulated using a metronome. Immediately after pesticide application, commercial bumble bee colonies (*B. impatiens* quads obtained from Koppert®) were placed separately in each of the 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 (Orono, ME). At the university the colonies treated with Assail® or Assail® and Tilt®, and non-treated hives were kept separate from each other to prevent drift between hives. The hives were monitored monthly for the rest of spring, summer, and early fall. The colonies were then moved into a walk-in freezer on the University of Maine campus for storage and to prevent changes in the colonies while data was being collected.

Data were collected by sorting the bumblebees in each hive into three categories: small workers (<1cm), medium workers (1 to 2cm), and potential queens (>2cm, also called gynes). Each hive was separated into eggs, larvae, pupae, and wax honey pots. The bees and pupae were counted, and the eggs, larvae, pupae, and honey pots were all weighed using an electronic scale. Total wax mass/hive was determined by summing larval pot mass/hive, egg mass/hive, and honey pot mass/hive. Wax was measured in grams. Waxworms (*Galleria grisella* (Fab.)) were counted and ranked on a scale from 0 to 3 based on the number of waxworms found in and on the hive (0=0 waxworms, $1 = 5$ waxworms, 2=5 to 25 waxworms, 3= >25 waxworms). Dysentery levels were determined by using a grid of 99 squares (11 squares x 9 squares) with a total area of 14.6cm x 11.8cm. The grid was placed over the area with the highest amount of dysentery and the number of squares more than half filled by dysentery were counted. This was converted into a percentage of squares that contained dysentery.

Analyses on the data were done using JMP® statistical software. All statistics were computed on a per hive basis. There were some bees lost whenever the hives were moved; hives were analyzed including and excluding these bees. There appeared to be no significant difference between the analyses of certain and uncertain number of bees, so hives were analyzed using only certain number of bees. To determine if there was a treatment effect, a nested ANOVA was used on each of the variables and a one-way ANOVA (pooled over hives within a rep) was performed on total bees by treatment to verify whether there was or was not a treatment effect. Linear correlations were used on any variables that may have had importance. A ranked logistic fit was performed on waxworms by total bees and waxworms by wax. Significance was determined using Student's *t*-tests (*P* < 0.05) if an ANOVA was found to be significant prior to the use of the Student's t test.

RESULTS:

Treatment effect

The only significant treatment effect was on the small workers ($F_{(2,24)} = 106.12$, $P <$ 0.0001, Fig. 1, Table. 1). Otherwise there did not appear to be any treatment effects on abundance of individuals: total bees ($F_{(2,24)} = 1.108$, $P = 0.372$), gynes ($F_{(2,24)} = 1.321$, $P =$ 0.314), medium workers ($F_{(2,24)} = 0.907$, $P = 0.4377$), or pupae ($F_{(2,24)} = 0.432$, $P = 0.662$).

Fig. 1. Effect of treatment on small workers per hive. Treatments with the same letters are not significantly different. Error bars are standard error.

There were also no treatment effects on mass of pupae $(F_{(2,24)} = 1.794, P = 0.221)$, larval pot mass ($F_{(2,24)} = 0.238$, $P = 0.765$), nor honey pot mass ($F_{(2,24)} = 1.761$, $P = 0.226$). A one-way analysis of various was also conducted and suggested that there was no treatment effect on total bees ($F_{(2,33)} = 0.587$, $P = 0.562$). There were marginal treatment effects on the egg mass ($F_{(2,24)} =$ 3.18, $P = 0.090$, Fig. 2) and the ranked levels of dysentery ($F_{(2,24)} = 4.08$, $P = 0.055$, Fig. 3) (Table 1).

Fig. 2. Effect of treatment on egg mass per hive. Treatments with the same letters are not significantly different. Error bars are standard error.

Fig. 3. Effect of treatment on ranked levels of dysentery. Treatments with the same letters are not significantly different. Error bars are standard error.

Linear correlation

Using linear correlation analysis, a few relationships were supported by the data, most of which were expected. Nearly all linear correlations found were resultant of total bees being an associated variable, they include: gynes ($R = 0.140$, $P = 0.025$), medium workers ($R = 0.996$, $P <$ 0.0001), small workers (R = 0.112, P = 0.046, Fig. 4), larval pot mass (R = 0.523, P<0.0001), egg mass (R = 0.650, *P* < 0.0001), wax (R = 0.653, *P* < 0.0001, Fig. 5), and honey pot mass (R = 0.568, $P < 0.0001$). There were no correlations found between number of total bees and number of pupae ($R = 0.001$, $P = 0.875$), number of total bees and pupae mass ($R = 0.016$, $P = 0.456$), or number of gynes and egg mass $(R = 0.029, P = 0.321)$.

Waxworm infestation

Waxworms were found in 20 of 36 hives, five of which were treated with Assail[®], nine of which were treated with Assail® and Tilt®, and six which were non-treated control hives. A ranked logistic fit was performed on waxworms as the dependent variable, with total bees as the independent variable $(X^2_{(1)}=5.59, P=0.018, Fig. 6)$. Another ranked logistic fit was performed on waxworms as the dependent variable, with wax mass as the independent variable $(X^2_{(1)} = 7.19,$ $P = 0.007$, Fig. 7). Both of the logistic fits showed correlations.

There were some instances where waxworms had eaten through larvae, pupae, and eggs. There were nine hives that contained dead pupae, four treated with Assail[®], four treated with Assail® and Tilt®, and one was a non-treated control. Waxworms were present in five of the hives containing dead pupae.

Fig. 4. Linear fit of total bees vs small workers.

Fig. 5. Linear fit of total bees vs wax.

Fig. 6. Probability of waxworm infestation as a function of total number of bees per hive for four regions of wax moth rank abundance.

Fig. 7. Probability of waxworm infestation as a function of wax area in hive for four regions of wax moth rank abundance.

Table 1. Analyses of data using: Nested ANOVA, One-way ANOVA, Linear Correlation, and Logistic Fit; *indicates trend; **indicates correlation; ***indicates significant correlation.

DISCUSSION AND CONCLUSIONS: The decline in pollinators has far reaching ramifications. An estimated 60 species of regularly cultivated crop plants would not produce fruit without bees (Sanchez-Bayo and Goka 2014). This could have economic consequences, as well as potentially causing food shortages. Pesticides and fungicides are used to prevent the loss of crops, but they may inadvertently be causing the loss of crops indirectly, through the decline of pollinators. This study tries to determine the safety of using a more common neonicotinoid insecticide (Assail®) that is generally considered safe for bees and the fungicide (Tilt®) on *B. impatiens* at high exposure levels during bloom.

The results of this research highlighted a few areas of interest. The first was that there did not appear to be any significant treatment effects on most components of the colony, except the average number of small workers hives treated with only acetamiprid tended to have the

highest number of small workers, followed by hives treated with both acetamiprid and propiconazole, and lastly the control hives. Bumblebee size is related to the amount of food given to the larvae by adult workers; larvae fed less during development tend to be smaller and larvae fed more tend to be larger. Bumblebees exhibit alloethism, which is the division of tasks based on size; small workers generally take care of hive maintenance and large workers forage. This is partly due to the fact that large workers are more effective at foraging (Peat et al. 2005). There was also a potential trend in average egg mass per hive. Colonies treated with acetamiprid had the highest egg mass, followed by those treated with acetamiprid and propiconazole, and lastly non-treated colonies. These two results together could indicate that the treated colonies were not foraging as effectively as the control colonies, leading to a reduction in adult bee size. Some research has found that bumblebees will alter their foraging behaviors in the presence of certain non-insecticidal agrochemicals and pesticides (Sprayberry et al. 2013, Thompson et al. 2014). This does not explain why the colonies treated with only acetamiprid had higher numbers of small workers and a higher egg mass than the colonies treated with both acetamiprid and propiconazole. Another factor arguing to the contrary, is that there was no treatment effect on honey pot mass. However, if the honey was contaminated, the bees may have had an aversion to feeding (Sprayberry et al. 2013) and this might result in stunted growth of workers.

There was a weak trend found in ranked levels of dysentery. Colonies treated with acetamiprid and propiconazole had the highest levels of dysentery, control having the second highest, and colonies treated with acetamiprid alone had the lowest. Dysentery in bumblebees is commonly caused by *Nosema bombi* and is usually a sign of a stressed colony. Fungicides and pesticides have been correlated to impaired immune responses in bees, making them more suseptable to infection from parasites like *Nosema* spp*.* (Goulson et al. 2015, McArt et al. 2017, Pettis et al. 2013). Most *Nosema* spp. are transferred from bee to bee through defecation, followed by the ingestion of contaminated wax, water, or honey. It has been correlated to an increase in the number of reproductive bees, especially males. However, *N. bombi* has not been found to be detrimental to bumblebee colony health (Whittington and Winston 2003).

It has been found that waxworms can cause large amounts of damage to bumblebee colonies. Once in the hive, it is difficult for the bumblebees to remove this pest because they hide in their silk domiciles and within the wax comb of the hive. At low densities they will normally only eat old wax, but at higher densities and later instars, they willingly feed on brood (Pelletier and McNeil 2003). Similar observations were made in this study. Over half of the colonies were infested with waxworms and some of those had dead pupae. It was found that the amount of wax present in the hive was a strong indicator of the intensity of the waxworm infestation. There was also a strong correlation between the total number of bees in a hive and the amount of wax. This would indicate that larger *B. impatiens* hives have a higher risk of a waxworm infestation. This was again indicated by the fact that there was a correlation between waxworms and total bees.

Bees were lost whenever the colonies were moved, which may have skewed our results. While being monitored at the University of Maine, some of the hives had increased in number of bees to the point where they no longer fit in the hive and formed clusters of bees outside of the hive. An attempt was made to collect them all, but some were lost and there was no way of telling which hive the bees that were collected came from. Including more hives in the study may have made some potential trends more significant by reducing experimental error and increasing the power of the design.

In conclusion, this study found that there appeared to be no significant difference between the colony health of commercial *B. impatiens* hives when treated with agriculturally realistic levels of acetamiprid or acetamiprid and propiconazole vs. untreated colonies. There were differences in certain aspects of the colonies, such as small worker abundance, egg mass, and ranked levels of dysentery. There was some evidence that suggested that treated colonies may not feed as effectively or as much as untreated hives, causing a decrease in average worker size. This has a indirect affect on colony health because smaller workers are not as effective at foraging, which combined with the aversion to eating contaminated pollen and honey, has potential to decrease the overall ability of a hive to function properly.

So, as we found in a previous study with bumblebees and a study with honey bees, we found no evidence in this study for detrimental effects from acetamiprid or propiconazole exposure. There was also no evidence in all three studies that suggested synergism between acetamiprid and propiconazole was occuring.

More research should be conducted on the aversion of eating contaminated pollen and honey and if that has an affect on the average size of adult bumblebees or the amount of eggs laid. Gender of the bee should be taken into account because an overabundance of males would result in competition with workers for available food. If possible, wild colonies should be studied in this same manner to see if there is a difference between the effects of acetamiprid and propiconazole on commercial colonies vs. wild colonies.

RECOMMENDATIONS: Based upon our research (this study, one additional study with bumblebees (2015 report) and two studies with honey bees (2015 & 2016 reports)) we believe that wild blueberry growers should not hesitate in using acetamiprid (Assail[®]) to manage blueberry flea beetle or blueberry spanworm prior to bloom when propiconazole (Tilt[®]) is being used for mummy berry management. Our previous "caution note" on the Maine Wild Blueberry Extension Insect Control Recommendations will be removed for 2018. Although, growers should still NOT apply ANY pesticide in a crop field during bloom. This management protocol will minimize exposure of bees to pesticide residues on the flowers.

LITERATURE CITED:

- Blacquière, T., Smagghe, G. and van Gestel, C.A.M. (2012). Neonicotinoids in Bees: a Review *on* Concentrations, Side-Effects and Risk Assessment. Ecotoxicology, 21(4): 973–992. doi:10.1007/s10646-012-0863-x.
- Goulson, D., Nicholls, E., Botías, C. and Rotheray, E.L. (2015). Bee Declines Driven by Combined Stress from Parasites, Pesticides, and Lack of Flowers. Science, American Association for the Advancement of Science, 27 Mar. 2015. science.sciencemag.org/content/347/6229/1255957.
- Iwasa, T., Motoyama, N., Ambrose, J. T. and Roe, M. (2004). Mechanism for the Differential Toxicity of Neonicotinoid Insecticides in the Honey Bee, *Apis mellifera*. Crop Protection, 23(5): 371–378. doi:10.1016/s0261-2194(03)00230-8.
- Kindemba, Vicky. (2009). The Impact of Neonicotinoid Insecticides on Bumblebees, Honey Bees and Other Non-Target Invertebrates, Sep. 2009, [www.beyondpesticides.org/assets/media/documents/pollinators/Neonicotinoid%20insectic](http://www.beyondpesticides.org/assets/media/documents/pollinators/Neonicotinoid%20insecticides%20report-1.pdf) [ides%20report-1.pdf.](http://www.beyondpesticides.org/assets/media/documents/pollinators/Neonicotinoid%20insecticides%20report-1.pdf)
- Laycock, I., [Cotterell,](https://www.sciencedirect.com/science/article/pii/S0147651313004703#!) K. C[., O'Shea-Wheller,](https://www.sciencedirect.com/science/article/pii/S0147651313004703#!) T. A. and [Cresswell,](https://www.sciencedirect.com/science/article/pii/S0147651313004703#!) J. E. (2014). Effects of the Neonicotinoid Pesticide Thiamethoxam at Field-Realistic Levels on Microcolonies of *Bombus terrestris* Worker Bumble Bees. Ecotoxicology and Environmental Safety, 100: 153–158. doi:10.1016/j.ecoenv.2013.10.027.
- [Malone, L. A.,](http://www.tandfonline.com/author/Malone%2C+L+A) Scott-Dupree, C. D., [Todd, J. H. and](http://www.tandfonline.com/author/Todd%2C+J+H) [Ramankutty, P.](http://www.tandfonline.com/author/Ramankutty%2C+P) (2007). No Sub-Lethal Toxicity to Bumblebees, *Bombus terrestris*, Exposed to Bt-Corn Pollen, Captan and Novaluron. New Zealand Journal of Crop and Horticultural Science, 35(4): 435–439. doi:10.1080/01140670709510211.
- McArt, S. H., Urbanowicz, C., McCoshum, S., Irwin, R. E. and Adler, R. S. (2017). Landscape Predictors of Pathogen Prevalence and Range Contractions in US Bumblebees. Proceedings of the Royal Society B: Biological Sciences, 284(1867). doi:10.1098/rspb.2017.2181.
- Peat, J., Darvill, B., Ellis, J. and Goulson, D. (2005). Effects of Climate on Intra- and Interspecific Size Variation in Bumble-Bees. Functional Ecology, 19(1): 145–151. doi:10.1111/j.0269-8463.2005.00946.x.
- Pelletier, L. and McNeil, J. N. (2003). The Effect of Food Supplementation on Reproductive Success in Bumblebee Field Colonies. Oikos, 103(3): 688–694. doi:10.1034/j.1600- 0706.2003.12592.x.
- Pettis, J. S., Lichtenberg, E. M., Andree, M., Stitzinger, J., Rose, R. and van Engelsdorp, D. (2013). Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen *Nosema ceranae*. PLOS ONE, 8(7) doi:10.1371/journal.pone.0070182.
- Sanchez-Bayo, F. and Goka, K. (2014). Pesticide Residues and Bees A Risk Assessment. PLOS ONE, Public Library of Science, 9 Apr. 2014. journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0094482.
- Scott-Dupree, C. D., Conroy, L. and Harris, C. R. (2009). Impact of Currently Used or Potentially Useful Insecticides for Canola Agroecosystems on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymentoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae). Journal of Economic Entomology, 102(1): 177– 182. doi:10.1603/029.102.0125.
- Sprayberry, J. D. H., Ritter, K. A. and Riffell, J. A. (2013). The Effect of Olfactory Exposure to Non-Insecticidal Agrochemicals on Bumblebee Foraging Behavior. PLOS ONE, 8(10), doi:10.1371/journal.pone.0076273.
- Thompson, H. and Wilkins, S. (2003). Assessment of the Synergy and Repellency of Pyrethroid/Fungicide Mixtures. Bulletin of Insectology, 56: 131–134.
- Thompson, H. M., Wilkins, S., Harkin, S., Milner, S. and Walters, K. F. (2014). Neonicotinoids and bumblebees (*Bombus terrestris*): effects on nectar consumption in individual workers. Pest. Manag. Sci., 71: 946–950. doi:10.1002/ps.386.
- Whittington, R. and Winston, M. L. (2003). Effects of *Nosema bombi* and Its Treatment Fumagillin on Bumble Bee (*Bombus occidentalis*) Colonies. Journal of Invertebrate Pathology, 84(1): 54–58. doi:10.1016/s0022-2011(03)00123-x.

Study 2. Predicting the bloom period in Maine wild blueberry: 2015-2017.

OBJECTIVE: We initiated an extensive data collection process in 2015 to develop a more appropriate model for Maine under conditions where wild blueberry plants do not begin to physiologically develop toward bloom until at least March due to cold temperatures and frozen soil around the roots. Validation of this model was conducted in 2016 and 2017 by sampling additional sets of wild blueberry fields to estimate the progression of bloom. The use of this model will mainly be for simulation modeling in order to assess mummy berry dynamics and climate change effects on pollination.

METHODS: In 2017 we visited seven wild blueberry fields in the mid-coast and Downeast 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 these data, the number of degree-days was calculated for the threshold base temperature of 40ºF using the formula: degree-days = (average daily air temperature – threshold base temperature), where average temperature is: $[(maximum air temperature + minimum air temperature) / 2]$. The threshold of 40ºF was estimated as the best threshold in 2016 for bloom estimates measured in the field in both 2015 and 2016. In 2015 we visited 18 wild blueberry fields in the mid-coast (11 fields) and Downeast (7 fields) growing regions of Maine. In 2016 we visited eight wild blueberry fields and repeated the calculations. 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 fit a model to both years of data to develop the model for estimating percent bloom as a function of degree-days with a threshold temperature of 40°F. The model is: Percent bloom = $100/11 + e$ $(6.939 - 0.016 * DD)$]. To use this model, degree-days at base = 40°F are accumulated from April 1.

In 2017 we visited an additional seven sites to validate the model developed in 2016.

RESULTS: Figure 1 shows the model for predicting bloom developed in 2016 with data collected in 2015 and 2016, as well as the overlay of the validation data collected in 2017. It can be seen that the validation data are well described by the model. Because the 2017 validation data was well described by the predictive model, the parameters of the model were fine tuned by using the data from all three years and 33 wild blueberry fields to fit a final model. The final parameter values are shown in Figure 2. There was hardly any change to the new model parameters since the validation data was close to the predicted bloom. The final model now is: Percent bloom = $100 / [1 + e^{(6.939 - 0.015 * DD)}]$, with an R² = 0.953 (i.e. 95.3 % of the variance in percent bloom is described by degree-days with a threshold of 40ºF). To use this model, degreedays at base $= 40^{\circ}$ F are accumulated from April 1.

Fig. 1. Degree-day predictive model for Maine wild blueberry. Model building data are solid round black data points, $n = 26$ fields; and overlaid validation data are non-filled round data points, $n = 7$ fields.

Fig. 2. Final predictive model for wild blueberry bloom in Maine.

CONCLUSIONS: A degree-day model for wild blueberry bloom in Maine has been developed and validated based upon field sampling across the two major growing areas in Maine over three years (2015-2017). This model is different than a previous model developed in Nova Scotia

since air temperatures in Maine are not accumulated until April 1. In addition, a 40ºF base is used instead of the 32ºF base used for Nova Scotia. The precision for two years and two regions is higher than the typical 10% precision often seen in field based predictive models (see 2016 report). This model can be used to predict threat of frost damage, mummy berry infection and timing for importation of commercial pollinators.

DISEASE MANAGEMENT

INVESTIGATORS: Seanna Annis, Associate Professor and Associate Extension Professor, School of Biology and Ecology Rachael Martin, Research Assistant, School of Biology and Ecology Nghi Nguyen, MS Graduate Student, School of Biology and Ecology Jennifer D'Appollonio, Assistant Scientist, School of Food & Agriculture

5. 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

Twelve fields with weather stations were rated for leaf spot diseases, powdery mildew (*Erysiphe vaccinii*), Septoria (*Septoria* sp.), and leaf rust (*Thekopsora vaccinii*) between September 27 and October 9, 2017. Two fields were not rated, one due to early pruning and the other due to early weather station removal. One weather station was sent back to the manufacturer for repair and was not operational during the field season this year. Five sampling plots of 0.25m^2 were rated by one surveyor 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 five sampling plots within a field.

Spore dispersal measured by Burkard spore traps

In June 2017, spore traps were placed in a prune field 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 November 6, 2017. 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.

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.

Leaf rust and powdery mildew samples were collected for DNA extraction and sequencing. The spores or spore producing structures were collected from infected stems using a vacuum and extracted for DNA.

Fungicide trial

Split-plot complete randomized block experiments were established in a lowbush blueberry field near Deblois, Maine where high levels of leaf spots had been previously reported. Fungicides (Table 1) were randomly assigned to 6' x 30' plots with a 3' buffer lane between each plot and replicated in six blocks. Plots were divided in half and treatments were randomly assigned to one of two application timings (June 15 or June 27). Fungicides were applied at volumes equivalent to 20 gallons per acre at 35 psi with a $CO₂$ backpack sprayer equipped with a 4 nozzle boom, 8002VS T Jet tips and 50 mesh screens applied. Control plots received no spray applications.

Disease symptoms and leaf loss were rated three times; in July, August and September. 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. In several plots the blueberry cover was too sparse to cover a transect so 20 stems were selected randomly throughout the plot. The next day leaves were rated for disease symptoms. The total number of leaves, nodes lacking leaves (leaves fallen) and the estimated percent coverage of each disease on remaining leaves was noted per stem. One hundred twenty stems per treatment (20 stems per plot) were rated. Phytotoxicity was also rated at the same time disease assessments were made. In the September rating, the numbers of flower bud clusters were also counted. The number of opening flower buds will be counted in the spring of 2018. The first stems were collected July 25 and rated July 26 and 27. Stems were collected again on August 21 and rated August 22-24. A third collection of stems occurred on September 18, and was rated September 19-21.

Data were analyzed by plot averages in SAS (Statistical Analysis Software - SAS Cary, NC) using mixed model procedures (PROC GLIMMIX). Proportional data were transformed with arcsin square root method. Least Square means were used to determine specific differences among treatments (α = 0.05).

RESULTS:

Weather station fields

As in previous years, there were low levels of Septoria (average 1%) by the end of September when the ratings were performed (Fig. 1). Higher levels of powdery mildew (average 11%) and rust (average 8%) than Septoria were observed (Fig.1). These levels are very similar to what we observed in 2016 which was also a dry year. Rain is believed to wash many powdery mildew spores off of the leaves before they can be dispersed. The lack of rain during July through September may have allowed an increase in spread and infection in some fields.

Spore trap project

Septoria symptoms were detected from mid-July to mid-August (Fig. 2). From mid-August through September, symptoms of dark brown to black lesions with some chlorosis were observed which are consistent with both Septoria and leaf rust. By early October, some lesions were producing leaf rust spores and could be clearly identified as such. Lesions without spores were counted as Septoria but may also have been young rust lesions. Powdery mildew symptoms increased from early August through October and higher levels of powdery mildew

were found in BBHF than the Deblois field (Fig. 2). Leaf loss started in early August with less than 5% loss and increased slowly until October when leaf senescence occurred (Fig. 3). Since it is thought that lower leaves are typically infected with Septoria, some of the leaf loss in September may have also dropped Septoria infected leaves. We hope in the future to clarify which fungi are causing leaf lesions through the season using DNA identification techniques.

We are currently finishing up our rust spore counts for the 2017 season. Specific regions of the DNA were sequenced and compared to sequence of other fungi known to occur on blueberry. Regions of DNA unique to the fungus causing blueberry leaf rust were identified and are being tested for their usefulness as specific primers to leaf rust.

Fungicide trial

Seven different materials were tested at different rates or combinations at two different application times for their efficacy in controlling leaf spots (Table 1). None of the treatments caused visible phytotoxicity to the plants during any of the ratings.

Leaf loss increased on average from 5 to 10 to 16% over the three rating times (Fig. 4, 5, 6). The levels of Septoria leaf spot were low in all three ratings and ranged from 1 to 2% (Fig. 7, 8, 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 6% (Fig. 12). No rust was found in the July and August ratings. Rust ranged from 1 to 8% in the September ratings (Fig. 13).

July rating: There were no significant effects of the treatments or timing of fungicide applications in the first rating (July) on leaf loss or disease levels of Septoria or powdery mildew (Fig. 4, 7, and 10).

August ratings: There was a significant interaction between treatment and timing of application for their effects upon leaf loss so each application timing was examined separately. There was no significant differences among the treatments for the late timing (June 27, Fig. 5). With the early timing on June 15, Bravo and Proline had significantly less leaf loss compared to the check (Fig. 5). The other treatments were not significantly different from the check. There were no significant differences in the treatments on the levels of Septoria or powdery mildew in August (Fig. 8 and 11).

September ratings: There was no significant effect of timing or interaction between timing and treatment for leaf loss in the September rating (Fig. 6). The levels of leaf loss in early Bravo and Proline treatments were lower than the check but not significant. 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 (Fig. 14).

CONCLUSIONS AND RECOMMENDATIONS: We saw an effect of timing on leaf loss levels in August but not at other rating times. We did not see an effect of the treatments on disease levels at any of the rating times. It was a very wet spring and then very dry conditions from June through to October. The very dry conditions likely would depress levels of disease due to lack of moisture for infection but also put the plants under drought stress. Septoria and leaf loss levels were lower than in 2016. Chlorothalonil is the most commonly used material to control leaf spots and was effective in decreasing leaf loss in August. Proline (prothioconazole) was equally effect at decreasing leaf loss in August. None of the treatments decreased leaf loss in September. None of the other treatments significantly decreased leaf spots or 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 look at early timing and multiple applications of materials.

Treatment (Trade Names)	Material	Applicatio n Rate (per acre)	Manufactu rer	FRAC group	EPA Reg. Number	Reg. on Wild Blueberry
Bravo					50534-201-	
Ultrex	Chlorothalonil	3.6 lbs/a	Syngenta	M ₅	100	yes
	Bacillus					Biocontrol
Double	amyloliquefaciens					/ Not
Nickel	strain D747		Certis		70051-107	applicable
	Bacillus					Biocontrol
Double	amyloliquefaciens					$/$ Not
Nickel	strain D747	1.06 qt/acre	Certis		70051-107	applicable
Luna	fluopyram and					
Tranquility	pyrimethanil	16 fl oz/a	Bayer	79	264-1085	yes
			Bayer Crop			
Proline	Prothioconazole	5.7 fl oz/a	Science	3	264-825	yes
Evito 480	Fluoxastrobin,					
SC,	Laminarin,					
Vacciplant	Alcohol	4 fl oz/a				
and Surf AC	ethoxylate,	14 fl oz/a		11	66330-64	
820	alkylphenol	3 fl oz/100	Arysta Life-		83941-2-	
(surfactant)	ethoxylate	gallons	Science	P4	66330	yes
					66330-56	
Ph-D and	Polyoxin D and	6.2 oz/a	Arysta Life-	19	83941-2-	
Vacciplant	Laminarin	14 fl oz/a	Science	P4	66330	yes

Table 1. Fungicides tested in 2017 for their efficacy to control leafspots.

Fall Leaf Spot Rating at Weather Station Sites

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 at prune fields near Deblois, ME (A) and Blueberry Hill Farm in Jonesboro (B) where spore traps were placed. Error bars indicate standard error of the mean.

Leaf loss 2017

Figure 3. Leaf loss in spore trap fields rated weekly from June 26-October 31, 2017. Error bars indicate standard error of the mean.

Figure 4. Fungicide efficacy trial; percentage of leaf loss in July. There were no significant differences in the timing of treatments. There were no significant differences among treatments and no significant interactions.

Figure 5. Fungicide efficacy trial; percentage of leaf loss in August. There were significant interactions between timing and treatment. There were significant differences in treatments for the June 15 application. Bars with different letters indicate statistically significant differences at α =0.05. There were no significant differences in treatments for the June 27 treatments.

Figure 6. Fungicide efficacy trial; percentage of leaf loss in September. There were no significant differences in the timing of treatments. There were no significant differences among treatments.

Septoria July

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

Figure 8. Fungicide efficacy trial. Percentage of leaf area with Septoria by treatment and application timing in August. There were no significant differences in treatments or treatment timing and no significant interactions.

Septoria September

Figure 9. Fungicide efficacy trial. 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. Fungicide efficacy trial. 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.

Powdery Mildew August

Figure 11. Fungicide efficacy trial. 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.

Powdery Mildew September

Figure 12. Fungicide efficacy trial. 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. Fungicide efficacy trial. 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.

Figure 14. Fungicide efficacy trial. Average number of flower buds 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, School of Biology and Ecology Rachael Martin, Research Assistant, School of Biology and Ecology Jennifer D'Appollonio, Assistant Scientist, School of Food & Agriculture

6. 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 13 to April 30, 2017, 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). Eleven locations also had MVC mummy berry (pseudosclerotia) plots that growers monitored through May. Thirteen fields with weather stations were rated for mummy berry between May 24 and June 1, 2017. 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. 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 eight blocks per field. Fungicide applications were timed using the Mummy Berry disease forecast according to locally monitored conditions of fungal and plant development and weather conditions favoring disease development (Fig.1). More information about the Mummy Berry forecast method can be found in UMaine Cooperative Extension Bulletin #217 and the forecasts for prior years including 2017 are available at https://extension.umaine.edu/blueberries/blog/. Prior to April $30th$, not enough buds were open to have enough plant tissue susceptible to infection. Fungicides were applied on May 4 and May 10 in the Deblois and Township 19 fields as protectant applications before infection periods were expected to occur from monitoring plant conditions, fungal inoculum and forecasted weather conditions. Due to applicator error, plots 16, 31 and 39 did not receive the first application of Bumper on May 4 in the Township 19 field. Fungicides were applied at volumes equivalent to 20 gallons per acre at 35 psi with a $CO₂$ backpack sprayer equipped with a 4 nozzle boom, 8002VS T Jet tips and 50 mesh screens. 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 three 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 25. 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 3, 2017. Harvesting occurred in a 2 foot strip down each plot center with a mechanical harvester and fresh weight was measured.

Percent data was converted to proportion and then an arcsin transformation of the square root performed to normalize the disease measurements. The yield data had a normal distribution. Data were analyzed by plot averages in SAS (Statistical Analysis Software - SAS Cary, NC) using mixed model procedures (PROC GLIMMIX). Least Square means were used to determine specific differences among treatments (α = 0.05).

Timing of ascospore release

Two spore traps were placed in crop fields from April 24 to June 19, 2017. One field was near Deblois and the other at Blueberry Hill Research Farm (BBHF) in Jonesboro, ME. Both fields had weather stations. The number of *Monilinia* ascospores and conidia were counted under a microscope at hourly intervals for each day the spore traps were in the field.

Effects of field edges on mummy berry incidence

Eleven fields, which had mummy berry symptoms, were rated for disease incidence and the effects of abutting to a prune field or the forest edge. Four transects were placed perpendicular to the field edge 1m, 6m, 12m and 24m from the edge. The transects were 30m 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 20ft) were rated. Percent data were 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.).

RESULTS:

Weather stations and disease forecasting

Mummy berry infection at weather stations sites ranged from no disease detected in one field to 93% infection of stems (Fig 3). The average percent of stems infected in 2017, 32%, was much higher than the 5.5% of 2016. Wet weather in April and through May resulted in moist soils suitable for *Monilinia* apothecia formation and many infection periods (Fig. 2). Many fields were too wet for tractors to get in and apply fungicides. The worst hit fields in 2017 did not apply fungicides and probably had high levels of inoculum, but this was not measured in all fields.

Fungicide Trial

We found higher levels of mummy berry than we have seen in the past few years. Checks (untreated control plots) had averages of 40 to 50% of stems infected.

None of the treatments showed phytotoxicity on the plants. All of the fungicide treatments except Regalia significantly decreased the levels of mummy berry blight in both fields (Fig. 4). Regalia treatments had similar levels of disease as the check. In the Deblois field, Luna Tranquility worked as well as the standard propiconazole (Bumper) and significantly decreased disease in the Township 19 field. Proline worked as well as propiconazole in both fields.

Surprisingly, there was no effect of the high levels of mummy berry on yield (Fig. 5). Conditions from middle of June through harvest were very dry and many berries were reported to have fallen prematurely. Yields were lower by about a third in 2017 compared to 2016.

Ascospore release

In the Deblois area, *Monilinia* apothecia were observed from about April 26th to May 19th and in the BBHF area from about April 21^{st} to May 15^{th} . Low levels of ascospores were detected from April 24th to May 16th in both fields (Fig. 6 and 7). After May 16th, we found a large number of what looked like *Monilinia* ascospores. On further observation, we think most of these spores are not *Monilinia* but from another fungus producing spores of similar size and shape but with minor differences (shape of spore tip, ornamentation on spores). The production of spores from a fungus possibly other than *Monilinia* occurred at the end of the time when *Monilinia* apothecia were observed and continued for many days after that. Other than the possibly invalid peaks at the end of the season, we observed other peaks of *Monilinia* ascospores on April 29 and May 4 at both sites (Fig. 6 and 7).

Most *Monilinia* ascospores were produced between wet periods from April 24th to May 16th, and often had peaks right after the leaf wetness when high humidity levels were still present. Spores were rarely detected during leaf wetness events since rain typically pulls spores out of the air. More spores were released in the morning between midnight and noon at both sites during the season, and low levels of spores were released throughout the day (Fig. 8).

Mummy berry edge ratings

There was no significant difference in the level of mummy berry infection at different distances from the prune field/crop field edge or from the forest edge (Fig. 9). This is in contrast to last year, where there was significantly more infection was found 1m from the crop/prune edge than 12, 24 or 36 m from the edge. The level of mummy berry in 2017 was approximately twice as high as 2016 which may have obscured any edge effects. In both years, mummy berry was found at all distances from the field edges, indicating apothecia within the field are the most important inoculum.

RECOMMENDATIONS: Proline and Luna Tranquility will be recommended for use in controlling mummy berry if suitable MRL can be obtained. Regalia does not appear to be an option for mummy berry control, probably due to the rapidly expanding leaf material that needs to be protected. It may be effective if used with an organic protectant since it is reported to initiate resistance mechanisms in plants.

Figure 1. Locations of weather stations and mummy berry plots for 2017. Sites with black markers had a mummy berry plot; sites with gray markers did not.

Treatment (Trade Names)	Application Rate (oz _{per}) acre)	Material	Manufacturer	FRAC group	EPA Reg. Number	Reg. on Blueberry for mummy berry
Regalia 12 (with Nu-Film P)	32	Reynoutria sachalinensis extract	Marrone Bio	P ₅	84059-21	Yes
Proline	5.7	Prothioconazole	Bayer Crop Science	3	264-825	Yes
Luna Tranquility	16	fluopyram and pyrimethanil	Bayer	79	264-1085	Yes
Positive Control - Bumper	6	propiconazole	Adama	3	66222-42	Yes

Table 1. Fungicides tested in 2017 for control of mummy berry.

Deblois 2017

Figure 2. Infection periods at the Deblois site. Top chart shows air temperature and leaf wetness which were used to determine infection periods (black vertical bars) for *Monilinia vacciniicorymbosi.* Horizontal bar on the top of the chart indicates when apothecia were present in the field. Bottom chart shows soil temperature and soil moisture during the same time period. For the soil moisture graph, lower numbers indicate higher levels of moisture in the soil.

Mummy Berry Infection Rating 2017

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

Figure 4. Average percentage of stems with symptoms of mummy berry in fungicide trials at A) Deblois and B) Township 19 fields. Error bars represent standard error of the mean of eight replicates. Bars with different letters were significantly different at p<0.05 within the fields.

A

Average Plot Yields-Deblois

B

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 eight replicates. There were no significant differences among the treatments within the Deblois and Township 19 fields.

Deblois Infection Periods with Spore Counts

Figure 6. *Monilinia* ascospore counts in a crop field near Deblois from April 24 to May 18, 2017. Air temperature (dark grey line) and leaf wetness (light gray line) are in the upper graph, and ascospore count (dark grey bars) is in the lower graph. The dotted black box indicates spores counted that may be *Monilinia* ascospores or of a different fungus.

BBHF Infection Periods with Spore Counts

Figure 7. *Monilinia* ascospore counts in a crop field at Blueberry Hill Research Farm in Jonesboro from April 24 to May 18, 2017. Air temperature (dark grey line) and leaf wetness (light gray line) are in the upper graph, and ascospore count (dark grey bars) is in the lower graph. The dotted black box indicates spores counted that may be *Monilinia* ascospores or of a different fungus.
\overline{A}

Figure 8. Average number of ascospores released per hour from April 24 to May 18, 2017 in crop fields near Deblois (A) and at Blueberry Hill Farm in Jonesboro (B). Error bars indicate standard error of the mean.

Figure 9. Proportion of stems infected with mummy berry along transects placed at 1, 6, 12, and 36m from the crop field edge A) bordering a prune field and B) bordering the forest. There was no significant difference among the transects in the crop/prune edge (A) or forest edge (B).

WEED MANAGEMENT

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

7. TITLE: Comparisons of Matrix and Callisto in combination with Matrix or Sinbar for weed control in wild blueberry fields, 2016 – 2017 crop year results.

METHODS: In spring 2016, we initiated a trial 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 and evaluate wild blueberry phytotoxicity. A Randomized Complete Split Block Design was replicated four times with 12'x 60' plots split in thirds lengthwise, with Matrix 4 oz/a or Sinbar 2 lb/a applied randomly to one 12'x 20' section of each block pre-emergence on 19 May 2016. The main treatments were applied twice or thrice postemergence 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 postemergence Matrix applications were not applied to the block sections that had received preemergence 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.

In the crop year, wild blueberry, broadleaf weed and grass covers were evaluated on 24 July 2017. The plots were harvested on 7 August by hand raking two 1 m^2 quadrats per 12'x 20' section of each main plot. Cover data were determined by using the Daubenmire Cover Scale converted to percent; yield weights were converted to lbs/a. The treatments were compared to each other using Tukey's tests (α =0.05). T-tests were also performed for significant differences in cover by 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 $\alpha=0.0167$).

Figure 1. Example layout of a trial block; Xs denote sections that did not receive postemergence Matrix, but did receive pre-emergence Matrix at 4 oz/a.

RESULTS:

Main effects

In August 2016, the Sinbar treatments had significantly higher wild blueberry cover compared to the other treatments alone or with Matrix; in 2017, the Sinbar treatments' cover was still significantly higher than the Matrix treatments but not the herbicides alone (Figure 2). Broadleaf weed cover in 2016 was less than 20% overall and there were no significant differences; the same trend was observed in 2017 but with less than 25% cover (Figure 2). Grass cover was significantly reduced by the addition of Sinbar compared to the herbicides alone but was significantly increased by Matrix compared to Sinbar. In 2017, the Matrix treatments continued to have the greatest grass cover, and both the Matrix treatments and herbicides alone were significantly higher than the Sinbar treatments (Figure 2, Photos 1-2).

Figure 2. Main effects on wild blueberry, broadleaf weed and grass cover in the crop year by main treatments alone, with pre-emergence Matrix or pre-emergence Sinbar (letters denote significance at α =0.0167 only).

Photo 1. Grass control in the check split (L) versus the Sinbar split (R).

Photo 2. Grass control in the Sinbar split (L) versus the Matrix split (R).

All-treatment comparisons

Crop year wild blueberry cover was comparable among treatments, with the exception of the Callisto 3 oz treatment with pre-emergence Matrix, which was significantly lower than Callisto 2 oz/a alone, Sinbar alone, and Callisto 3 oz/a with Sinbar (Figure 3).

Dominant broadleaf weeds in the trial area included bunchberry (*Cornus canadensis*), wild lettuces (*Lactuca biennis* and *L. canadensis*), butter-and-eggs (*Linaria vulgaris*), red sorrel (*Rumex acetosella*), downy goldenrod (*Solidago puberula*), and rough goldenrod (*Solidago rugosa*). There were no significant differences in broadleaf weed cover in the crop year, but whether alone, with Matrix or Sinbar, the Callisto 2 oz/a rate reduced weeds more-so than the 3 oz/a rate (Figure 4). However, Callisto at 2 oz/a in the Callisto+Matrix treatment resulted in more broadleaf weeds than Callisto 2 oz/a alone, except for in the Matrix split. Callisto 3 oz/a resulted in fewer broadleaf weeds than Callisto+Matrix when alone or with Sinbar, but postemergence Matrix 2 oz/a resulted in more weeds. Combining Sinbar with the main treatments did not improve broadleaf weed control compared to the herbicides alone (Photos 3+8).

Dominant grasses included wild oatgrass (*Danthonia spicata*), fine-leaf sheep fescue (*Festuca filiformis*), Canada bluegrass (*Poa compressa*) and Kentucky bluegrass (*Poa pratensis*). Grass pressure was high in 2017, and cover ranged from 38-85% in the check and Matrix splits (Figure 5, Photos 1-2). The addition of Sinbar significantly reduced grasses in all Sinbar treatments compared to Matrix and Matrix 2 oz (both pre-emergence Matrix only), and all but Callisto 2 oz/a with Sinbar were significantly lower than the untreated check. Pre-emergence Matrix grass cover was significantly greater than Callisto 2 oz/a or post-Matrix 2 oz/a alone, but pre-emergence "Matrix 2 oz" was not, even though it was exactly the same application as "Matrix".

Photo 3. Example of lack of broadleaf weed control, but good grass control, in the plots containing post-emergence Matrix with Sinbar.

Figure 3. All-treatment comparison of wild blueberry cover in the crop year (letters denote significance at $\alpha=0.05$; *did not receive post-emergence Matrix).

Figure 4. All-treatment comparison of broadleaf weed cover in the crop year (no significant differences; *did not receive post-emergence Matrix).

Figure 5. All-treatment comparison of grass cover in the crop year (letters denote significance at α =0.05; *did not receive post-emergence Matrix).

Yield

There were no significant differences in yield for main effects (Figure 6) or treatment comparisons (Figure 7). In general, however, the treatments in the Matrix split had the highest yield. Patterns of yield differences among main treatments were inconsistent among the splits. For example, Callisto 3 oz/a had the second lowest yield of all treatments in the check split, but the highest yield of all in the Sinbar split. By contrast, Callisto 2 oz/a had the third highest yield in the check split, but the lowest yield in the Sinbar split.

Figure 6. Comparison of main effects of herbicides alone, with Matrix or Sinbar on yield in the crop year (no significant differences; α =0.05).

Figure 7. All-treatment comparison of yield in the crop year (letters denote significance at $\alpha=0.05$; *did not receive post-emergence Matrix).

CONCLUSIONS:

Main effects

The significant reduction in crop year wild blueberry cover in the Matrix treatments is unclear, considering that all five treatments received pre-emergence Matrix only. In previous research, post-emergence Matrix application resulted in greater injury and a corresponding reduction of wild blueberry cover more so than pre-emergence Matrix. The crop year reduction in wild blueberry cover seen here may be because the pre-emergence rate was 4 oz/a while the post-emergence rate was 2 oz/a applied twice, but wild blueberry cover in the prune year was comparable for pre- versus post-Matrix, and post-Matrix had much more injury in August 2016. Additional research may be needed, focusing on Matrix alone at varying rates and timings at multiple locations. Although not significant, the greater amount of grasses in the Matrix treatments compared to the herbicides alone supports trends seen in other trials. In previous trials, depending on the suite of weed species present, Matrix was observed to sometimes release weeds because it controlled only certain weed species which may or may not have complemented the control range of tank mix partners, which then allowed weeds not controlled by Matrix and/or the partners to be released. In contrast, a product like Sinbar appears to control a wider range of weeds, in this case most grasses and some broadleaf weeds, so is not only more effective in grass control but tends not to release weeds when used with broadleaf herbicide tank mix partners.

All-treatment comparisons

It is unclear as to why pre-emergence Matrix with post-emergence Callisto at 3 oz/a resulted in the lowest wild blueberry cover in the crop year, considering that there were no significant differences among treatments in cover or phytotoxicity in the prune year, and that overall initial phytotoxicity (of pre-emergence Matrix alone, as phyto was assessed just before the post-emergence applications) was $\leq 4\%$. There was delayed phytotoxicity observed in 2016 in the four treatments with post-emergence Matrix, where at two weeks after the last postemergence spray they had almost 25% injury in the form of stunting, while post-emergence Callisto 3 oz/a with pre-emergence Matrix had no phytotoxicity observed at either evaluation. Previous trials showed that when applied at the timings used in this treatment, neither pre-Matrix nor post-Callisto exhibited unacceptable phytotoxicity. However, in the crop year all four postemergence Matrix treatments were comparable to each and the untreated check, while preemergence Matrix with post-emergence Callisto 3 oz/a resulted in the lowest cover of all treatments. By contrast, the Callisto 2 oz/a and Callisto+Matrix treatments in the Matrix split consisted of pre-emergence Matrix combined with Callisto 2 oz/a sprayed 3 times (instead of Callisto 3 oz/a sprayed 2 times), and neither exhibited a reduction in crop year wild blueberry cover.

Although the results were not significant, some general conclusions may be inferred regarding broadleaf weed cover. Broadleaf weed cover was reduced more by the Callisto 2 oz/a rate than by the 3 oz/a rate. Callisto at 2 oz/a was applied thrice instead of twice, so the late application on 8 July 2016 likely controlled later germinating weeds not controlled by the second application on 24 June. The Callisto rate in the Callisto+Matrix treatment was also 2 oz/a but did not control weeds as well as Callisto 2 oz/a (Photos 4-5), with the exception of the Matrix split. This is because Callisto 2 oz/a was only applied twice in the Callisto+Matrix treatment, and because the post-emergence Matrix was omitted in the Matrix split since it had already received pre-emergence Matrix, so that treatment only received two post-emergence applications of Callisto 2 oz/a. The fewer broadleaf weeds in Callisto 3 oz/a and more in Matrix 2 oz/a compared to Callisto+Matrix, when alone or with Sinbar, may be due to a combination of factors. The higher Callisto rate applied twice controlled weeds better than the lower Callisto rate applied twice, and pre-emergence Matrix may have released certain species of broadleaf weeds that were not controlled well by Callisto. This is supported by the results in the Matrix split, where Callisto 3 oz/a resulted in the greatest broadleaf weed cover and Callisto 2 oz/a was second highest (Photo 6). In both cases, the max amount of Callisto per acre per year (6 oz/a) was applied, and Matrix was applied pre-emergence. Both were higher than Callisto+Matrix in the check and Sinbar splits, which received a total of 4 oz/a Callisto and post-emergence Matrix. The pre-emergence Matrix (1 application of 4 oz/a) appears to have released more weeds than post-emergence Matrix (2 applications of 2 oz/a) (Photos 7-8), and perhaps those specific weed species had poor control with Callisto alone, as seen when comparing the same treatments in the check and Matrix splits (Figure 3). This is further supported by the pre-emergence Matrix only treatments ("Matrix" and "Matrix 2 oz" in the Matrix split) both resulting in greater broadleaf weed cover than the untreated check (Photo 9).

The grass cover results support the trends in broadleaf weed cover discussed above. In the Matrix split, Callisto 3 oz/a and 2 oz/a had the greatest broadleaf weed cover, and correspondingly they had the lowest grass cover in the split. In the two identical pre-emergence Matrix only treatments, "Matrix" and "Matrix 2 oz", "Matrix" broadleaf weed cover was lower and grass cover was higher, although neither comparison was significant at α =0.05. The control of the grass species in those plots may have provided better conditions for broadleaf weed growth, and/or vice versa.

The lack of weed control in the Matrix split may be a synergy of lack of control of a suite of weed species, coupled with control of certain species leading to improved growing conditions for other species. We can assume that most weed species on-site were present in most plots, but the same pattern of weed response seen in the Matrix split was not observed in the check or Sinbar splits. In the Matrix split, broadleaf weeds and grasses appeared to be negatively correlated, but when looking at Figures 4 and 5 a similar correlation is not seen in the check or Sinbar splits. Therefore, we can conclude that split application of post-emergence Matrix is generally more effective in controlling a wider range of weeds, but it also depends on the specific suite of weeds present; and concomitantly, the suite of weeds present should be identified prior to Matrix application to be sure that Matrix will be effective, whether applied pre- or post-emergence. In this trial, we were unable to determine which specific weeds were controlled by Matrix and how well they were controlled, because the cover data were collected in broad categories and weed phytotoxicity was not recorded (see Recommendations).

Photo 4. Broadleaf weed control by Callisto + Matrix in the check split.

Photo 5. Broadleaf weed control by Callisto 2 oz/a in the check split.

Photo 6. Lack of broadleaf weed control by Callisto 2 oz/a in the Matrix split (bunchberry).

Photo 7. Example of weed control in the plots receiving pre-emergence Matrix only.

Photo 8. Example of weed control in the plots receiving post-emergence Matrix only.

Photo 9. Weed cover in the untreated check.

Yield

When all treatments were compared to each other, there was variability in the yields both within and among the splits. However, the main effects showed that the Matrix split had higher yield overall, because in the Matrix split there was less variability among treatments within the split compared to within the check or Sinbar splits (see Figures 6-7). It is unclear why there was so much variability among treatments, especially the Callisto treatments; in numerous previous trials, Callisto was not shown to result in reduced yields, whether used alone or with other products. Wild blueberry injury and weed cover from the prune year were examined, and no clear cause could be determined for any specific factor in the prune year leading to reduced yield in the crop year. For example, in 2016 Matrix 2 oz/a and Callisto+Matrix in the check and Sinbar splits had significantly more injury compared to all other treatments (22-25% compared to $\langle 3\%$), but they had intermediate yields in 2017. Also, pre-emergence "Matrix" had the $5th$ greatest broadleaf weed cover in June 2016 while "Matrix 2 oz" had low initial broadleaf weed cover but the greatest cover in August 2016, but both had roughly the same yield. Matrix 2 oz/a, Callisto 2 oz/a and Callisto 3 oz/a with Sinbar had the highest yields. The two Callisto treatments had the lowest broadleaf cover in August 2016, but Matrix had the second greatest; grass covers in the three treatments were relatively low. By contrast, Matrix 2 oz/a with Sinbar had lower broadleaf cover than Matrix 2 oz/a alone and the $3rd$ lowest grass cover in August 2016, but the $3rd$ lowest yield in 2017. A possible contributor may be the species of weeds present in/near the quadrats harvested. A tall large-leaved weed such as spreading dogbane, goldenrod or raspberry (see Photo 7) could shade surrounding plants, which would reduce flower buds and therefore yield; whereas a low growing weed such as bunchberry or small-leaved weed such as butter-and-eggs would have little shading effect.

RECOMMENDATIONS: In 2016 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 expected that 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 believed the improvement may not be enough to warrant the cost of going out a $3rd$ time unless the grower was already going out to apply some post-emergence grass control treatments.

When carryover results were examined, we found that in the crop year, pre-emergence Matrix alone or with Callisto did result in more weeds than the herbicides alone or with Sinbar, but not significantly so (8 and 3% difference, respectively, see Figure 2). Our expectation that Sinbar would be needed for control of grasses released by Matrix was confirmed, as seen in Figure 2. Also, applying Callisto 3x resulted in better carryover control of both broadleaf weeds and grasses compared to Callisto 2x, as evidenced in Figures 4-5. Callisto applied 3x also resulted in slightly higher yields than Callisto 2x in the check and Matrix splits, but less than half the yield in the Sinbar split (see Figure 7), so applying Matrix thrice may not be a good strategy in fields with high grass pressure.

Matrix did not substitute for Sinbar for grass control, and so if grasses are a concern then it should be used pre-emergence or other grass herbicides should be applied post-emergence in combination with Callisto for broadleaf weeds, to address any weeds not controlled by the preemergence herbicide applications. We recommend that Matrix be tested alone, with preemergence versus post-emergence and single versus split applications, on multiple sites to determine efficacy on specific weed species. Soil samples should be collected to identify if there are differences in weed responses dependent on soil texture, pH and/or % organic matter.

WEED MANAGEMENT

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

8. TITLE: Pre-emergence and post-emergence applications of Zeus Prime XC for weed control in wild blueberry fields, 2016 – 2017 crop year results.

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 Wyman's 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 **preemergence** *(Zeus+Solida pre)*;
- 7. Zeus Prime XC 7.7 oz/a with COC 1% v/v **pre-emergence** plus Solida 4 oz/a **postemergence** *(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 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 crop year wild blueberry cover, broadleaf weed cover and grass cover on 17 and 25 July 2017, and the plots were harvested on 2 August. Cover data were determined by using the Daubenmire Cover Scale 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 $(\alpha=0.05)$.

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

Wesley | 5.0 | 15.3 | 48 | 42 | 10 |Ioam

RESULTS:

All-treatment comparisons

In 2016, we noted 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. The site differences persisted into the crop year, so we analyzed them separately again and found that some treatment differences were not captured again.

There were no significant differences in wild blueberry in both Jonesboro (Figure 1) and Wesley (Figure 2). Wild blueberry cover was lower overall at the Wesley site, and some large reductions in cover were seen in some treatments in Wesley which were not observed in Jonesboro. For example, in Wesley, wild blueberry cover in the Aim+Solida treatment was 20%, but in Jonesboro it was 94%. Furthermore, all herbicide treatments without Velpar at Wesley had at least 15% less blueberry cover than the check, but in Jonesboro all were within +6% of the check. The treatments with Velpar had slightly higher wild blueberry cover overall compared to without Velpar, but was still lower in Wesley than in Jonesboro. The one treatment that could not be assessed in 2016 because application wasn't completed until November 2016, Zeus pre+fall, had the lowest cover among the no-Velpar treatments at Jonesboro, and the third lowest with Velpar, but the differences were minimal. In Wesley, Zeus pre+fall had the third greatest cover without Velpar, and the greatest cover with Velpar. Wild blueberry cover by treatment at both sites in 2017 was very similar to August 2016 (see 2016 WBC year-end report no. 9).

Figure 1. Wild blueberry cover in Jonesboro in the crop year, following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida in the prune year $(\alpha=0.05)$; no significant differences).

Figure 2. Wild blueberry cover in Wesley in the crop year, following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida in the prune year $(\alpha=0.05)$; no significant differences).

As in 2016, the Wesley site had many more broadleaf weeds than Jonesboro (Figures 3- 4). Dominant species included red sorrel (*Rumex acetosella*), bladder campion (*Silene vulgaris*), ragweed (*Ambrosia artemisiifolia*), lance-leaved goldenrod (*Euthamia graminifolia*) and St. John's wort (*Hypericum perforatum*). The Jonesboro site was dominated by red sorrel, with rough goldenrod (*Solidago rugosa*) subdominant and tall blue lettuce (*Lactuca biennis*) present to a lesser extent.

There were no significant differences among treatments at either site. In Wesley, Zeus low, Zeus hi and Zeus+Solida pre were equal to the check; the other main treatments alone had greater broadleaf weed cover than the check (Photo 1A). Zeus hi and Zeus pre+fall had the fewest weeds with Velpar (Photo 1B); adding Velpar generally decreased the amount of broadleaf weeds compared to the main treatments alone. In Jonesboro, the addition of Velpar also generally reduced broadleaf weeds more-so than the main treatments alone (except for Zeus mid). Zeus+Solida pre resulted in the most broadleaf weeds of the herbicide treatments with or without Velpar, Zeus mid had the least without Velpar, and Zeus pre+Solida post had the least with Velpar.

Photo 1. Broadleaf weed cover at Wesley was greatest in the Zeus pre+fall treatment without Velpar (A), and lowest with Velpar (B).

Figure 3. Broadleaf weed cover in Jonesboro in the crop year, following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida in the prune year (α =0.05; no significant differences).

Figure 4. Broadleaf weed cover in Wesley in the crop year, following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida in the prune year (α =0.05; no significant differences).

As in 2016, 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 (*Festuca filiformis*) and colonial bentgrass (*Agrostis capillaris*), compared to Wesley which had colonial bentgrass almost exclusively with a small amount of yellow foxtail (*Setaria pumila*) and bluegrasses.

There were no significant differences at the Jonesboro site (Figure 5). All of the main treatments alone appeared to release grasses compared to the untreated check (Photo 2A), while the addition of Velpar reduced grass cover in all of the treatments (Photo 3B). The three tank mix treatments combined with Velpar resulted in the lowest grass covers of all treatments (Photo 2B). Although Wesley had fewer grasses overall, there were significant differences, likely due to the absence of grasses in some treatments at this site (Figure 6). All of the treatments, with and without Velpar, resulted in less grass cover than the check. However, the only significant differences were in the three treatments with no grasses compared to the check: Zeus+Solida pre with and without Velpar, and Zeus pre+Solida post with Velpar.

Photo 2. Grass cover at Jonesboro was greatest in the Zeus low treatment without Velpar (A), and lowest in Zeus+Solida pre with Velpar (B).

Figure 5. Grass cover in Jonesboro in the crop year, following pre-emergence and/or postemergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida in the prune year (α =0.05; no significant differences).

Figure 6. Grass cover in Wesley in the crop year, following pre-emergence and/or postemergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida in the prune year (α =0.05).

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 Wesley, wild blueberry cover in the Zeus pre+Solida post treatment significantly increased with the addition of Velpar (Figure 7). This significant difference was also seen at the late season evaluation during the prune year. In Jonesboro, adding Velpar to Aim+Solida significantly reduced broadleaf weed cover (Figure 8). Although not significant, it should be noted that Velpar reduced broadleaf weeds in general at this site but increased weeds in the Zeus mid treatment.

Figure 7. Wild blueberry cover in Wesley; a comparison of no Velpar versus Velpar within each main treatment (α =0.05, shaded "check" bar is Velpar only).

Figure 8. Broadleaf weed cover in Jonesboro; a comparison of no Velpar versus Velpar within each main treatment (α =0.05; shaded "check" bar is Velpar only).

Regression analyses

In Jonesboro, there was a linear trend of decreasing broadleaf weeds with an increase of the rate of Zeus alone (Figure 9), and rate accounted for 46.26% of the variation in broadleaf weed cover. To a lesser extent, the same relationship was seen in August of the prune year. In Wesley there were no significant relationships between Zeus rate and broadleaf weed cover with or without Velpar, and trends were both positive and negative (Figure 10). Trends in August 2106 also varied, but in the opposite directions. There were no significant relationships, and trends varied, between Zeus rate and grass cover at the Jonesboro site (Figure 11); trends were similar as in August 2016. In Wesley, there was a linear trend of decreasing grasses with increasing rate of Zeus alone, which explained 23.62% of variation in grass cover (Figure 12).

Figure 9. 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; *no Velpar significant).

Figure 10. 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).

129

Figure 11. 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 12. 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; *no Velpar significant).

Yields

The results of the yield measurements collected in Wesley will not be discussed here because of issues during data collection. Many plots had barely enough wild blueberry cover to harvest two 1 m^2 quadrats of berries, and most were under dense weeds, so that the plots had to be examined thoroughly by moving aside weeds. Other plots did not have enough blueberry plants present to harvest, or had no blueberry plants at all. University personnel were used to hand rake the quadrats, and because they were unfamiliar with the plots they often did not find the plants and reported "no blueberry present" instead of "blueberry present but no berries", etc.

At the Jonesboro site, yields were relatively low and some treatment differences were obscured due to small sample size and loss of Degrees of Freedom from separating the sites. Velpar alone (2181 lbs/a) and Zeus mid with Velpar (1960 lbs/a) resulted in the highest yield, but were only significantly higher than Aim+Solida, which had 443 lbs/a (Figure 13). Zeus hi, Zeus pre+fall with and without Velpar, and Zeus pre+Solida post with and without Velpar also had yields of less than 1000 lbs/a but were not different from the check or the other treatments within the splits. In general, adding Velpar to the main treatments slightly improved yield, with the exception of Zeus low; the only significant improvement was in the Aim+Solida treatment (Figure 14).

Figure 13. Yields in Jonesboro by treatment following pre-emergence and/or post-emergence applications of Zeus Prime XC alone and in combination with Solida, as well as Aim with Solida, in the prune year (α =0.05).

Figure 14. Yields in Jonesboro; a comparison of no Velpar versus Velpar within each main treatment (t-tests; α =0.05. Shaded "check" bar is Velpar only).

CONCLUSIONS:

All-treatment comparisons

Although prune year herbicide treatment did not result in significant reduction of wild blueberry cover in the crop year at Jonesboro, severely depressed wild blueberry cover was observed in Wesley. The large differences at Wesley were not significant due to the amount of variability in the data at this site. We believe that the large amount of variability was because of heavy weed pressure at this site due to previous agricultural practices and high soil fertility. There were large patches of weeds at this site that usually would not occur in such densities in wild blueberry fields, such as ragweed (*Ambrosia artemiisifolia*), corn spurry (*Spergula arvensis*) and bladder campion (*Silene vulgaris*). Other weed species common in wild blueberry fields were present in the trial area in dense patches and were tall and vigorous, such as St John's wort, goldenrods and colonial bentgrass (Photo 3A); and the effects of Velpar could not be seen visually in Wesley as in Jonesboro (Photo 3B). We believe that in most treatment plots, the heavy weed cover and large replacement seedbank overwhelmed treatment effects by shading out blueberry plants; by individuals germinating later in the season, thereby receiving reduced residual action; and/or by tall weeds intercepting post-emergence spray before it contacted short weeds or the ground. This is supported by examples of plots where herbicidal action did control weeds, such as plot 18 in the Zeus mid treatment with Velpar. In this plot, there were relatively few weeds but even fewer blueberry plants available to fill in bare ground (Photo 4). The heavier soil in Wesley also tied up the Zeus, as was evidenced by the lack of weed control in the Zeus only treatments. The Zeus pre+fall treatment would have been expected to have the most action on broadleaf weeds; it received the maximum rate per acre per year and the latest application timing relative to crop year weed growth. However, when applied without Velpar it

resulted in more weeds than the check and the second highest weed cover of all treatments; by contrast, with Velpar is resulted in the lowest broadleaf cover among treatments, tied with Zeus hi. A similar reaction of Zeus to heavier soils is discussed in the 2017 Zeus Rely trial (Report no. 10).

The remainder of this discussion will focus on Jonesboro, which had less weed pressure and more clearly defined treatment differences. However, no definitive conclusions can be made from this trial, because there were only four blocks per site and the sites had to be analyzed separately thus obscuring true treatment effects. There was no pattern of broadleaf weed control with increasing rate of Zeus with or without Velpar, and tank mixing with Solida did not improve control. Aim, which is carfentrazone alone, was less effective on broadleaf weeds when applied with Solida (rimsulfuron) post-emergence compared to Zeus pre+Solida post, but the effect was relatively small and not significant. Adding Velpar appeared to slightly improve control by the main treatments, but the effect was minimal. Grass cover declined with increasing Zeus rate alone, but the split treatment of the maximum Zeus rate (Zeus pre+fall) performed no better than the lowest rate of pre-emergence Zeus (Zeus low). The same was observed for broadleaf weed cover, which suggests that the fall application of Zeus had little effect on weeds in the crop year. Grass control was improved by Velpar, and the three tank mix treatments performed better than Zeus alone. The three tank mixes had Solida in common, so grass control appeared to be driven by rimsulfuron, not carfentrazone, sulfentrazone or hexazinone.

Photo 3. A. Several weed species in the Wesley trial area were observed to be tall and vigorous. The effect of Velpar could not be seen visually (no Velpar left, with Velpar right); B. Visual effect of Velpar on weed control in Jonesboro (with Velpar left, no Velpar right).

Photo 4. Zeus mid treatment with Velpar in Wesley. The treatment controlled weeds, but revealed an underlying lack of blueberry plants available to fill in bare spots.

T-tests

Although at least one difference was significant, no definitive conclusions can be made regarding the effect of adding Velpar to the main treatments on wild blueberry cover in Wesley. There were too many other factors affecting wild blueberry cover, like large weeds shading out blueberry plants, dense stands of weeds out competing the plants, tall weeds intercepting herbicides before they contacted the soil, etc.

In Jonesboro, we can conclude that adding Velpar to the treatments improved broadleaf weed control, but the effect was only significant for Aim+Solida. Again, this was because of the loss of four Degrees of Freedom from analyzing the sites separately and an insufficient number of blocks.

Regression analyses

In 2016, the regression analyses for the relationship between increasing rates of Zeus (with or without Velpar) and weed cover were not strong and were not considered definitive. Last year in Jonesboro, we suggested that the quadratic early season relationship for Zeus rate versus broadleaf weed cover $(\overline{R^2} = 19.59\%)$ indicated 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. The late season relationship was linear but not strong (R^2 =18.82%). In 2017, the relationship was linear and much stronger (R^2 =46.26%), indicating that Zeus rate and crop year weeds have an inverse relationship. It is unclear why the relationship between Zeus rate with Velpar and broadleaf weeds was not significant, because the slopes were almost identical.

Zeus rate and grasses at Wesley did not have significant relationships in 2016, but in 2017 increasing rate of Zeus alone was negatively linearly correlated with crop year grass cover $(R^2=23.62)$. Again, because of the small sample size as well as low grass cover at Wesley, definitive conclusions cannot be made.

Yields

The reduced yield in several treatments in Jonesboro may have been correlated with prune year wild blueberry injury or weed cover, but a consistent pattern with herbicide type and/or rate could not be determined. As seen in Figure 14, the addition of Velpar did improve yield slightly overall, with the exception of Zeus low. We believe the results were confounded because the University Farm Crew did not apply fungicides in spring 2017 for mummy berry control in the trial area. During crop year evaluation, several clones with mummy berry damage were observed, which would have led to a reduction in yield in the plots regardless of herbicide treatment. Therefore, no definitive conclusions can be made regarding yield.

RECOMMENDATIONS: Pursuant to the prune year results of this trial, a follow-up trial assessing Zeus was initiated on three sites in spring 2017, with six blocks per site and a range of soil conditions. See Report no. 10 for a discussion of this trial.

WEED MANAGEMENT

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

9. TITLE: Fall versus spring application of Zeus Prime XC for weed control in wild blueberry fields, 2016-17.

METHODS: Starting in spring 2016, we have been testing the Group 14 herbicide Zeus Prime XC (carfentrazone + sulfentrazone) for weed control efficacy in wild blueberry. In fall 2016 we set up a trial on Wyman's EO Morse field to compare fall post-pruning versus spring preemergence application of Zeus. A Completely Randomized Design was replicated ten times per treatment with 1 m² plots containing Zeus plus COC 1% v/v applied on 1 November 2016 or 3 May 2017; the plots were compared to each other and an untreated check. The maximum rate of Zeus, 15.2 oz/a, was supposed to be applied to the treated plots, but due to a calculation error the actual fall 2016 applied rate was 9.12 oz/a so the same rate was applied to the spring 2017 plots. This rate is between the "low" (7.7 oz/a) and "mid" (12.5 oz/a) rates set by the manufacturer in the 2016 Zeus Prime XC Trial. Wild blueberry cover and phytotoxicity, broadleaf weed cover and grass cover were assessed on 6 June and 5 July 2017. The dominant weed in the trial area was red sorrel (*Rumex acetosella*), so red sorrel cover was also assessed separately. Cover data were determined by using the Daubenmire Cover Scale converted to percent; phytotoxicity data were gathered using a scale of 0-10 (0=no damage, 10=100% damaged/dead) converted to percent. T-tests were performed for significant treatment differences, Bonferroni adjusted to $\alpha = 0.0167$.

RESULTS: There were no significant differences in wild blueberry cover or phytotoxicity at either evaluation (Figure 1). Both Zeus treatments had slightly greater cover than the check; phytotoxicity was minimal and noted mainly as chlorosis. It should be noted that at the June evaluation, the check also had some injury. This injury appeared to be due to mummy berry symptoms but could not be separated from herbicide injury in the treated plots, and so was recorded as a baseline injury level.

There were also no significant differences among treatments for broadleaf weed cover, grass cover, or red sorrel cover (Figure 2). Red sorrel was the only broadleaf weed of note in the trial area at both evaluations; wild oatgrass (*Danthonia spicata*) was the only grass. Regardless of timing, Zeus application reduced both broadleaf weeds and grasses compared to the check, but weed suppression continued into July only on the wild oatgrass. Spring Zeus application resulted in the least red sorrel at both evaluations (Photo 1), while fall Zeus application resulted in more red sorrel than the check by July (Photos 2-3).

Figure 2. Broadleaf weed, grass and red sorrel covers for fall versus spring applied Zeus $(\alpha=0.0167)$.

Photo 1. Red sorrel and other weeds present in the spring Zeus treatment in July.

Photo 2. Red sorrel and other weeds present in the fall Zeus treatment in July.

Photo 3. Red sorrel and other weeds present in the untreated check in July.

CONCLUSIONS: In this trial, Zeus application did not result in unacceptable reduction in wild blueberry cover or injury to wild blueberry. Although conclusions cannot be made regarding broadleaf weed control because of a lack of broadleaf weeds other than red sorrel, we can conclude that Zeus does have some action on wild oatgrass, the only grass observed in the trial area. In regard to red sorrel, the pre-emergence spring application was more effective than the post-pruning fall application. Because of the error in calculating the amount of product the treated plots received, we do not know whether the maximum Zeus rate would have controlled red sorrel better overall.

RECOMMENDATIONS: Evaluate carryover weed control and harvest. Repeat using maximum Zeus rate on red sorrel with a fall and spring application.

WEED MANAGEMENT

10. TITLE: Comparisons of pre-emergence applications of Zeus Prime XC and Rely 280 for weed control in wild blueberry fields.

METHODS: Zeus Prime XC is a product which has a general blueberry label in the U.S., but has not been evaluated for weed control in wild blueberry. It is a Group 14 herbicide with carfentrazone and sulfentrazone as the active ingredients; we currently have only one other Group 14 herbicide registered for wild blueberry which must be applied the year before to prevent injury to the 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 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). We also began testing Rely 280, a glufosinate product in Group 10, for its effects on red sorrel and wild blueberry. Unlike glyphosate (Group 9), glufosinate is only a contact herbicide, but we currently do not use any Group 10 herbicides so it also has the potential to be a tool for resistance management.

In 2017 we initiated a trial examining the effects of Zeus and Rely on weeds in general, both alone and in various tank mixes. Part of the trial was to evaluate a new formulation of Velpar DF CU to determine if there were any differences with the new product. Three sites were chosen to encompass a range in weeds and soil conditions: Clary Hill, Union; Blueberry Hill Farm, Jonesboro; and Joan's Lot, in Wesley. A partially Randomized Complete Split Block Design was replicated six times on each site with 12'x 60' plots and 5' alleys, some of which were split in half with Velpar L 1 lb/a applied to one half of each block (Figure 1). All treatments were applied pre-emergence on 12, 17 and 18 May 2017 as follows:

- 1. Untreated check (split with Velpar L);
- 2. Zeus Prime XC 12.5 oz/a (*Zeus,* split with Velpar L);
- 3. Zeus Prime XC 12.5 oz/a + Rely 280 29 oz/a + ReQuest AMS 2 pt/a (*Zeus+Rely,* split with Velpar L);
- 4. Sinbar WDG 2 lb/a + Velpar DF 1 lb/a + diuron 2 lb/a (*Trimix,* no split);
- 5. Sinbar WDG 2 lb/a + Velpar DF 1 lb/a (*Sinbar+Velpar,* no split);
- 6. Velpar DF 1 lb/a + Matrix 4 oz/a + diuron 2 lb/a (*Matrix mix,* no split); and
- 7. Velpar DF 1 lb/a + Rely 29 oz/a + diuron 2 lb/a + ReQuest AMS 2 pt/a (*Rely mix,* no split).

Figure 1. Example of a block layout.

The plots were evaluated for wild blueberry cover and phytotoxicity, broadleaf weed cover and grass cover on 6-7/12-13 June and 6, 12 and 14 August. Cover data were determined by using the Daubenmire Cover Scale 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. All treatments (the Velpar and no-Velpar split main treatments were considered separate treatments for analysis) were compared to each other with using Tukey's tests (α =0.05). T-tests were also performed for significant differences between no-Velpar and Velpar for each main split treatment (α =0.05).

Table 1. Differences in soil conditions among the three trial areas: Union, Jonesboro and Wesley.

RESULTS:

All-treatment comparisons

Wild blueberry cover and phytotoxicity

Wild blueberry cover at the first evaluation followed the same overall trends at all three sites (Figures 2-4). The Zeus and Zeus+Rely treatments resulted in the lowest initial wild blueberry cover, both with and without Velpar, with significant differences that varied by site. At all three sites, by the second evaluation in July all significant differences disappeared, and the wild blueberry plants in the Zeus and Zeus+Rely treatments had recovered to where cover was comparable with the other treatments.

The Zeus and Zeus+Rely treatments, both with and without Velpar, also resulted in the highest initial phytotoxicity at all sites. Blueberry injury manifested as both a delay in emergence and stunting of plants. The greatest injury occurred on the Union site (Figure 5, Photo 1), which had the highest soil pH and lowest % organic matter (Table 1). The treatments that were not split, and the check/Velpar alone, had significantly lower phytotoxicity compared to the split treatments, except the Rely mix where phytotoxicity was significantly greater than the other no-split tank mixes. The Wesley site followed the same pattern of injury, but phytotoxicity in the Zeus, Zeus+Rely and Rely mix treatments were approximately half that of Union (Figure 7, Photo 3); Wesley had the highest % organic matter and so had the heaviest soil (Table 1). The Jonesboro site was intermediate in soil pH and organic matter (Table 1), but soil texture was almost identical to Union and so levels of injury were also very similar (Photo 2), with the exception of the Rely mix which was lower (Figure 6).

The symptoms of phytotoxicity persisted to the July evaluation in almost all treatments at the Union site, mainly as residual stunting and delay in emergence. The Trimix phytotoxicity was significantly higher than the Matrix mix, Rely mix, the check and Velpar alone, but all treatments were < 6% and so had effectively recovered. The second phytotoxicity evaluation was lower in Jonesboro and lowest in Union, and there were no significant differences at either site in July.

Figure 2. Wild blueberry cover in Union following pre-emergence applications of Zeus Prime XC alone and in combination with Rely, split with Velpar L; as well as tank mixes with no split (only significant differences at α =0.05 denoted by different letters).

Figure 3. Wild blueberry cover in Jonesboro following pre-emergence applications of Zeus Prime XC alone and in combination with Rely, split with Velpar L; as well as tank mixes with no split (only significant differences at α =0.05 denoted by different letters).

Figure 4. Wild blueberry cover in Wesley following pre-emergence applications of Zeus Prime XC alone and in combination with Rely, split with Velpar L; as well as tank mixes with no split (only significant differences at α =0.05 denoted by different letters).

142

Figure 5. Wild blueberry phytotoxicity in Union following pre-emergence applications of Zeus Prime XC alone and in combination with Rely, split with Velpar L; as well as tank mixes with no split (only significant differences at α =0.05 denoted by different letters).

Figure 6. Wild blueberry phytotoxicity in Jonesboro following pre-emergence applications of Zeus Prime XC alone and in combination with Rely, split with Velpar L; as well as tank mixes with no split (only significant differences at α =0.05 denoted by different letters).

Figure 7. Wild blueberry phytotoxicity in Wesley following pre-emergence applications of Zeus Prime XC alone and in combination with Rely, split with Velpar L; as well as tank mixes with no split (only significant differences at α =0.05 denoted by different letters).

Photo 1. June phytotoxicity as stunting and delay of emergence in the Zeus+Rely treatment in Union (with Velpar, foreground).

Photo 2. June phytotoxicity as stunting and delay of emergence in the Zeus+Rely treatment in Jonesboro (inside plot border on left).

Photo 3. June phytotoxicity as stunting and delay of emergence in the Zeus+Rely treatment in Wesley (no Velpar, foreground).

Broadleaf weed and grass cover

Dominant early season weeds recorded at the Union site in June included early goldenrod (*Solidago juncea*), rough goldenrod (*Solidago rugosa*), Canada goldenrod (*Solidago canadensis*), black sedge (*Carex nigra*), red clover (*Trifolium pratense*) and bluegrass (*Poa* spp.). In Jonesboro, dominant early season weeds included black chokeberry (*Aronia melanocarpa*), spreading dogbane (*Apocynum androsaemifolium*), red sorrel (*Rumex acetosella*), blue toadflax (*Nuttallanthus canadensis*), wild oatgrass (*Danthonia spicata*) and colonial bentgrass (*Agrostis capillaris*). In Wesley, early dominant weeds included redtop (*Agrostis gigantea*), St. John's wort (*Hypericum perforatum*), violet (*Viola* spp.), cow vetch (*Vicia cracca*), hedge bedstraw (*Galium mollugo*) and red sorrel.

In Union in July, later dominant weeds included lance-leaved goldenrod (*Euthamia graminifolia*), Canada goldenrod, rough goldenrod, black sedge and white clover (*Trifolium repens*). In Jonesboro, dominant later season weeds included black chokeberry, spreading dogbane, bunchberry (*Cornus canadensis*), wild oatgrass, colonial bentgrass and witchgrass (*Panicum capillare*). Dominant later weeds in Wesley included redtop, St. John's wort, violet, cow vetch, hedge bedstraw and red sorrel. It should be noted that in Wesley, a large patch of Virginia creeper (*Parthenocissus quinquefolia*) had spread out from an old cellar hole near Blocks 3 and 6, so several plots on the end of the trial area near the cellar hole were dominated by this species, although it is not found frequently in wild blueberry fields.

Union initially had the lowest background level of broadleaf weeds, and there were no significant differences among treatments in June (Figure 8). Jonesboro had significantly reduced broadleaf weeds in the Zeus, Zeus+Rely and Zeus+Rely with Velpar treatments, as well as a significant reduction in weeds for the four non-split treatments in June (Figure 9). At Wesley, the Zeus and Zeus+Rely treatments resulted in the fewest broadleaf weeds in June, but the effect was only significant for Zeus with Velpar compared to the Rely mix treatment (Figure 10). In addition, broadleaf weeds in the non-split treatments responded differently to herbicide application compared to the other sites; at Union and Jonesboro treatment reduced broadleaf weeds compared to the check, but in Wesley treatments released weeds except for the Matrix mix.

By the July evaluation at Union, broadleaf weeds in all treatments were significantly reduced compared to the check and were less than 10% cover overall (Figure 8). Broadleaf weeds were also reduced compared to the check in Jonesboro, but in the Zeus+Rely and Matrix mix treatments, the effects were not significant (Figure 9). Weed response at Wesley continued to differ from the other sites; Zeus with Velpar and Zeus+Rely with Velpar continued to have the lowest cover but were only different from the Rely mix, and broadleaf weeds in the non-split treatments were greater than the check (Figure 10).

Figure 8. Broadleaf weed cover in Union following pre-emergence applications of Zeus Prime XC alone and in combination with Rely, split with Velpar L; as well as tank mixes with no split (only significant differences at α =0.05 denoted by different letters).

Figure 9. Broadleaf weed cover in Jonesboro following pre-emergence applications of Zeus Prime XC alone and in combination with Rely, split with Velpar L; as well as tank mixes with no split (only significant differences at α =0.05 denoted by different letters).

Figure 10. Broadleaf weed cover in Wesley following pre-emergence applications of Zeus Prime XC alone and in combination with Rely, split with Velpar L; as well as tank mixes with no split (only significant differences at α =0.05 denoted by different letters).

The only grass of note at the Union site was in the genus *Poa*, and it could not be identified to species due to lack of distinguishing reproductive characteristics; grass cover was very low initially and was reduced further by July (Figure 11). Jonesboro had the greatest grass cover but the overall cover was below 11% for the check (Figure 12). Grasses in almost all treatments increased from June to July, with the exception being the two treatments containing Sinbar (Trimix and Sinbar+Velpar). Wesley also had very little grass cover, and the only grass of note was redtop; in this case, grasses increased from June to July in all treatments, including the two containing Sinbar (Figure 13). There were no significant differences at either evaluation for any site.

Figure 11. Grass cover in Union following pre-emergence applications of Zeus Prime XC alone and in combination with Rely, split with Velpar L; as well as tank mixes with no split (only significant differences at α =0.05 denoted by different letters).

Figure 12. Grass cover in Jonesboro following pre-emergence applications of Zeus Prime XC alone and in combination with Rely, split with Velpar L; as well as tank mixes with no split (only significant differences at α =0.05 denoted by different letters).

Figure 13. Grass cover in Wesley following pre-emergence applications of Zeus Prime XC alone and in combination with Rely, split with Velpar L; as well as tank mixes with no split (only significant differences at α =0.05 denoted by different letters).

Visual observations were also recorded at each evaluation. The treatments containing Zeus and/or Rely are highlighted here. In June, Zeus had the greatest reduction of yarrow (*Achillea millefolium*), black sedge, hedge bedstraw, bluet (*Houstonia caerulea*), bladder campion (*Silene vulgaris*) and blue-eyed grass (*Sisyrinchium* spp.). However, there were several species it did not control at all, including: colonial bentgrass, redtop, St. John's wort, fall dandelion (*Leontodon autumnalis*), goldenrods, red clover and violets. Bluegrass, wild oatgrass, spreading dogbane and cow vetch had varying responses (Photo 4). When Rely was added to Zeus, it did not appear to improve control of the species not controlled by Zeus alone; but did increase control of bunchberry, blue toadflax, red sorrel and wild oatgrass. In July, many more weed species were present and most were not controlled by Zeus or Zeus+Rely; in addition, several species controlled in June were not controlled as well by July (Photo 5). In both cases, adding Velpar appeared to weaken weeds, especially at the July evaluation for Zeus alone and June evaluation for Zeus+Rely (Photo 6-7).

When Rely was applied tank mixed with Velpar and diuron, several species were killed or almost killed in June, including: colonial bentgrass, black sedge (Photo 8), field chickweed (*Cerastium arvense*), wild oatgrass, quackgrass (*Elymus repens*) (Photo 9), buckwheat (*Fagopyrum esculentum*), hedge bedstraw, St. John's wort, plantain (*Plantago major*), goldenrods and red clover. Several were not controlled, including: black chokeberry, milkweed (*Asclepias syriaca*), fall dandelion, witchgrass, red sorrel, bladder campion and sessile bellwort (*Uvularia sessilifolia*). Spreading dogbane, bluegrass and cow vetch had varying responses. Again, several weed species weakened at the June evaluation recovered in July; continued

control was observed in black sedge, wild oatgrass, quackgrass, blue toadflax and Canada goldenrod (*Solidago canadensis*), but all except black sedge also had individuals that were not affected.

Photo 4. Example of variability in control in June (Jonesboro). Bluegrass and wild oatgrass are poorly controlled in the Zeus treatment without Velpar (no Velpar in the foreground, with Velpar beyond lone stake on right).

Photo 5. Several species exhibited a reduction in control or continued lack of control by Zeus alone, such as hedge bedstraw, cow vetch and redtop (Wesley, July).

Photo 6. Example of colonial bentgrass control in June by Zeus+Rely treatment, Jonesboro (inside plot to left).

Photo 7. When Velpar was added to Zeus+Rely, control of bluegrass and quackgrass improved (Union, July).

Photo 8. Control of black sedge by the Rely mix treatment in Union (June).

Photo 9. Rely mix treatment in Wesley (June) controlled quackgrass and redtop, but not Virginia creeper (stakes are at corner of outer two edges of plot).

T-tests

T-tests were performed for no Velpar versus Velpar within each split treatment; only the significant results are presented here. There were no significant differences in grass cover for any site at either evaluation.

In July, there was a significant difference in wild blueberry cover at Jonesboro for the Zeus treatment with and without Velpar (Figure 14); however, the difference was relatively small (~8%) and cover was comparable with the check. All three sites had significant differences in wild blueberry phytotoxicity in June, but in all three cases it was the difference between the untreated check and Velpar alone (Figures 15-17); this was not surprising, considering that the untreated check had 0% injury and so would be expected to differ from even a minor level of injury.

All three sites had significant differences in broadleaf weed cover. In both Union and Jonesboro, there were significantly fewer broadleaf weeds in the Velpar treatment versus the untreated check at the July evaluation (Figures 18-19); again, this was expected because the untreated check would have no species controlled. The Jonesboro site also had a significant difference in June for the check versus Velpar. In Wesley, not only was the check versus Velpar significantly different at both evaluations, but Zeus alone also had significantly more weeds compared to Zeus with Velpar (Figure 20).

Figure 14. Comparison of wild blueberry cover for no Velpar versus with Velpar within the split treatments at Jonesboro (α =0.05).

Figure 15. Comparison of wild blueberry phytotoxicity for no Velpar versus with Velpar within the split treatments at Jonesboro $(\alpha=0.05)$.

Figure 16. Comparison of wild blueberry phytotoxicity for no Velpar versus with Velpar within the split treatments at Union (α =0.05).

155

Figure 17. Comparison of wild blueberry phytotoxicity for no Velpar versus with Velpar within the split treatments at Wesley (α =0.05).

Figure 18. Comparison of broadleaf weed cover for no Velpar versus with Velpar within the split treatments at Union (α =0.05).

156

Figure 19. Comparison of broadleaf weed cover for no Velpar versus with Velpar within the split treatments at Jonesboro (α =0.05).

Figure 20. Comparison of broadleaf weed cover for no Velpar versus with Velpar within the split treatments at Wesley (α =0.05).

157

CONCLUSIONS:

All-treatment comparisons

Zeus application resulted in high initial injury to wild blueberry and therefore the lowest initial wild blueberry cover among treatments, but the addition of Rely and/or Velpar did not increase the damage compared to Zeus alone. Rely, when used with diuron and Velpar, did not significantly reduce cover or injure blueberries more so than the other non-Zeus treatments, with the exception of the Union site. However, initial blueberry phytotoxicity from the Rely treatment was under 20% at this site, and so is not considered a major concern since phytotoxicity at the second evaluation was negligible. At all three sites, wild blueberry cover was comparable among treatments by the second evaluation and overall phytotoxicity was <10%, but any residual effects of the initial Zeus phytotoxicity and delay in emergence won't be determined until the sites are harvested in 2018. In previous trials, certain products such as Sandea exhibited the same pattern of high initial phytotoxicity followed by vegetative recovery, but yield was also significantly reduced due to a lack of flowers in the crop year. Also of interest are the phytotoxicity levels at the Wesley site compared to the other sites. Phytotoxicity in all non-Zeus treatments was comparable, but Zeus phytotoxicity at Wesley was half that of the other sites (see Photos 1-3). Wesley had the heaviest soil and highest organic matter (see Table 1), so it appears that on this site the Zeus was tied up and so less active.

Wesley also differed from the other sites in the response of broadleaf weeds to the treatments. Initial overall broadleaf weed cover in Union was low $(\leq 11\%)$, and the treatments kept it under 10% over time, as is evidenced in Figure 8 wherein the check $(-25\% \text{ cover})$ was significantly higher than all treatments by July. Jonesboro had more broadleaf weeds initially (~21% cover) and weed cover in the check was also about 25% in July, and the amount of weed suppression followed a similar pattern to Union. In Wesley, although percent weed cover in the check was similar to Jonesboro in June and July, and the Zeus treatments responded similarly to Union and Jonesboro over time, weed response in the non-Zeus treatments differed in both June and July resulting in broadleaf weed covers roughly equal to or higher than the check (see Figure 10).

It is difficult to draw conclusions regarding grass control, as grass cover at all sites was low overall and there were no significant differences. However, we can see from the Jonesboro site (Figure 12) and Wesley site (Figure 13) that Zeus did not control grasses well on its own, but did slightly better with Rely and/or Velpar. As expected, the two treatments containing Sinbar controlled grasses best, but the Matrix in the Matrix mix also appeared to control grasses to an extent.

The differences in phytotoxicity and broadleaf weed response in Wesley compared to Union and Jonesboro leads us to two conclusions. First, the Zeus rate used in this trial, 12.5 oz/a, may be higher than necessary to control weeds on sandier sites. Phytotoxicity was significantly lower in Wesley, indicating that some of the Zeus was being tied up in the heavier soil, but broadleaf weed control remained comparable to the other sites. Second, this trial confirms that soil composition can result in marked differences in weed responses when using soil applied herbicides. The heavier soil in Wesley prompted a release of broadleaf weeds in the four non-Zeus treatments instead of a reduction, with the exception of the Matrix mix. All four treatments contained Velpar DF, three contained diuron, and two contained Sinbar WDG. Only the Matrix mix reduced the grasses, which indicates that on sites with heavier soil, using Sinbar, Velpar and diuron could increase broadleaf weed cover when combined with a contact herbicide such as Rely which would only control those weeds that were emerged early in the season.

The visual observations reveal that a follow-up post-emergence herbicide or mid- to lateseason contact herbicide application may be needed for continued weed control when applying Zeus and/or Rely pre-emergence. Many weed species had varying levels of injury within the same treatment and even plot, and many individual weeds weakened at the first evaluation had recovered by the second. In addition, Rely did not have any effect on weeds that emerged after the herbicide application.

T-tests

The t-tests did not reveal much that wasn't expected; we know from many trials over the years that Velpar will significantly reduce broadleaf weeds compared to no treatment. Wesley was the only site to have a significant difference in broadleaf weed control for Zeus alone compared to Zeus with Velpar (see Figure 20). It may be that on sites with heavier soils like Wesley, which tend to tie up herbicides such as Zeus, that applying a combination of the two is synergistic or that each controls complementary species.

RECOMMENDATIONS: Since there is phytotoxicity and delay in emergence of wild blueberries with Zeus, evaluations of fall applications should be made to determine if they are as effective in controlling weeds with less or no injury to the wild blueberry plants. Also look at lower rates on sandy sites, both fall and spring on the same site.

WEED MANAGEMENT

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

11. TITLE: Evaluation of spring applications of herbicides targeting red sorrel in wild blueberry fields, $2016-17 - 2017$ crop year results.

METHODS: This trial was set up pursuant to the results from the 2015-16 red sorrel (*Rumex acetosella*) trial. In fall 2015 we located an area of red sorrel on Wyman's E.O. Morse lot. Ten $1m²$ 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.

The plots which were going into the crop cycle were harvested in August 2016, and the results were presented in the 2016 WBAC year-end reports. For the plots in crop this year, wild blueberry cover, red sorrel cover, broadleaf weed cover and grass cover were evaluated on 5 July 2017. Cover data were determined by using the Daubenmire Cover Scale 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 1 August 2017. 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

As in the prune year, there were no significant differences between any treatment and the check in the crop year for wild blueberry cover or red sorrel cover (Figure 1), so the Standard Errors of the Means (SEMs) were also plotted to examine treatment variability and differences. In the prune year, Chateau had the lowest red sorrel cover of the treated plots (the check had the lowest red sorrel cover overall) and highest wild blueberry cover; Matrix had the lowest blueberry cover, and Sandea had the greatest red sorrel cover. In the crop year, Alion had the lowest blueberry cover and the greatest red sorrel cover. Chateau continued to have the highest wild blueberry cover as well as having the lowest red sorrel cover. When the SEMs were compared, Chateau red sorrel cover was different from Alion, Matrix, Sandea and the check. Alion, Matrix and Sandea red sorrel covers were not different from each other, but Alion and Sandea were different from the check. The SEMs also showed Alion wild blueberry cover as different from the check and Alion, but no other treatment differed from the check.

Although there were no grasses and no broadleaf weeds other than red sorrel in the 2016 crop plots, there were both broadleaf weeds and grasses in the 2017 crop plots (Figure 2). In both cases, there were no significant differences between any treatment and the check, so the SEMs were plotted to examine trends. Sandea resulted in the lowest broadleaf weed cover, followed by Chateau (the check was highest); and Chateau had the lowest grass cover, followed by Sandea (Alion had the greatest).

Figure 1. Wild blueberry and red sorrel cover in July 2017 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 July 2017 following fall 2015 applications for red sorrel control (α =0.05. No significant differences for t-tests; error bars are SEMs).

Yield

Overall yields were lower in 2017 compared to 2016, and there were no significant differences, but the pattern was the same. As in 2016, Chateau resulted in the highest yield and Sandea the lowest (Figure 3). Matrix was slightly lower than the check, and Alion was slightly higher than Sandea. When the SEMs were plotted, Chateau was higher than Alion and Sandea, and the check was higher than Sandea.

Figure 3. Yield for the treatment plots in crop in 2017 (α =0.05. No significant differences for ttests; error bars are SEMs).

CONCLUSIONS: The lack of differences between treatments was due to the amount of variability in the plots, especially in broadleaf weed and grass cover, as shown by the SEMs. However, some general conclusions may still be inferred.

Chateau continued to perform best in this trial. Last year, the Chateau treatment had the highest blueberry cover in both the prune and crop plots, the lowest red sorrel cover in the crop plots, and second lowest cover in the prune plots. This year in the crop cycle, Chateau had the greatest wild blueberry cover and lowest red sorrel cover in last year's prune plots. Chateau also resulted in the highest yield of all treatments in both 2016 and 2017 (Photo 1). Chateau is a product that has specific environmental restrictions for application, namely soil which is not frozen, snow-covered or poorly drained; receiving moisture after application; and avoiding application during cool, wet conditions. The conditions on the application date were good: 52°F, partially sunny and dry with a soil temp of 48°F, and the site received rainfall within 24-48 hours. The consistently high performance in both the 2016 and 2017 crop years leads us to conclude that when applied in the fall under good conditions, Chateau is very effective in controlling red sorrel and thereby improving wild blueberry cover and yield.

Sandea and Alion continued to perform poorly in this trial. Sandea wild blueberry cover was average, and red sorrel control was mediocre. As in the 2016 crop plots, Sandea controlled other broadleaf weeds and grasses well, but did not control red sorrel well; Sandea had the lowest yield of all treatments again in 2017, almost half that of the check and over two times lower than Chateau (Photo 2). As in 2016, Alion had the greatest grass cover in 2017; this year Alion had the most red sorrel as well, and yield was almost as low as in the Sandea treatment

(Photo 3). Broadleaf weed control was mediocre and less effective than the other herbicide treatments.

Matrix had mixed results in 2017 as it had in 2016. Wild blueberry cover was relatively low and red sorrel control was relatively poor compared to the untreated check (Photo 4). Matrix exhibited carryover weed control when compared to the check but did not perform as well as Chateau or Sandea. As in 2016, yield was slightly lower but comparable to the untreated check.

Photo 1. Example of low weed and red sorrel cover, high blueberry cover and good yield in the Chateau treatment.

Photo 2. Example of high weed and red sorrel cover, low blueberry cover and low yield in the Sandea treatment.

Photo 3. Example of a plot in the Alion treatment, which had the most red sorrel of all treatments.

Photo 5. Example of a Matrix plot, showing poor red sorrel control and mediocre yield.

RECOMMENDATIONS: Last year, we stated that 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.

Pursuant to the results of this year's evaluation and harvest, we also recommend that Alion and Sandea not be applied in the fall post-pruning for control of red sorrel as the yields were poor. In the case of Alion, carryover control of other broadleaf weeds and grasses was also poor, and wild blueberry cover was reduced. Matrix could be used but; blueberry cover, red sorrel control, other weed control and yield were mediocre and not a significant improvement over no treatment at all so it is not recommended for sorrel control.

Pursuant to the results of this year's evaluation and harvest, we recommend that Chateau may be used as an effective component of a red sorrel control strategy. It should be stressed, however, that care should be taken to apply Chateau under the conditions stated on the label, as efficacy can be reduced and wild blueberry injured by incorrect application. We will continue to evaluate fall treatments of Chateau for red sorrel control using larger plots and more replications.

WEED MANAGEMENT

12. TITLE: Application timings of Rely 280 and Chateau for red sorrel control in wild blueberry fields, 2017-18.

METHODS: For the past several years, we have been investigating different rates and timings of various herbicides for efficacy in controlling red sorrel (*Rumex acetosella*) in wild blueberry. Red sorrel competes with wild blueberry, and current control methods often are not able to eliminate or effectively control this weed. In the most recent trial targeting this weed (see Report no. 11), Chateau (flumioxazin) was most effective of the herbicides tested in controlling red sorrel over the entire crop cycle. In this follow-up study, Chateau and Rely 280 (glufosinate), a burn-down contact herbicide, were applied at the same rate but different timings and crop cycles to further determine efficacy on red sorrel. The treatments were as follows:

2017 Prune cycle

- 1. Untreated check (*prune check*);
- 2. Rely 280 29 oz/a **spring pre-emergence** (*spring Rely*);
- 3. Rely 280 29 oz/a **fall going into crop** (*fall Rely*);
- 4. Rely 280 29 oz/a + Velpar 1 lb/a **spring pre-emergence** (*Rely+Velpar*);
- 5. Chateau 12 oz/a **fall going into crop** (*Chateau*);

2017 Crop cycle

- 6. Untreated check (*crop check*);
- 7. Rely 280 29 oz/a **fall post-pruning** (*crop Rely*); and
- 8. Chateau 12 oz/a **fall post-pruning** (*crop Chateau*).

ReQuest liquid ammonium sulfate (AMS) at 2 pt/a was added to all treatments. The spring treatments were sprayed on 3 May 2017, and the fall treatments were sprayed on 15 November 2017. Wild blueberry cover and phytotoxicity, red sorrel cover and phytotoxicity, broadleaf weed cover and grass cover were assessed on 6 June and 5 July 2017. Cover data were determined by using the Daubenmire Cover Scale converted to percent; phytotoxicity data were gathered using a scale of 0-10 (0=no damage, 10=100% damaged/dead) converted to percent.

Because of the treatment timing, the fall treatments will not be evaluated until 2018; therefore, the results presented in this report pertain to the spring treatments in the 2017 prune cycle. Ttests were performed for pairwise comparisons of spring Rely or Rely+Velpar versus the check in the prune cycle, and Rely no Velpar versus with Velpar within the prune cycle. In 2018 once crop cycle data are collected, treatments will also be compared to each other for spring versus fall in the prune cycle (spring vs fall Rely), for fall prune cycle versus fall crop cycle (fall Rely vs crop Rely; Chateau vs crop Chateau), and fall post-pruning versus spring pre-emergence (crop Rely vs spring Rely).

RESULTS: There were no significant differences between spring Rely and the check, Rely+Velpar and the check, or for Rely with versus without Velpar for wild blueberry cover or phytotoxicity at either evaluation (Figure 1). The only wild blueberry injury was noted in June in the spring Rely treatment, and it was under 1%.

Figure 1. Wild blueberry cover and phytotoxicity in the 2017 prune year for pre-emergence application of Rely 280 with and without Velpar (α =0.05; no significant differences).

There were also no significant differences between spring Rely or Rely+Velpar and the check for other broadleaf weed cover or grass cover at either evaluation (Figure 2). Broadleaf weed cover was low overall, as red sorrel was the dominant weed in the trial area, and consisted almost exclusively of cow vetch (*Vicia cracca*). The spring Rely treatment resulted in the lowest broadleaf weed cover, but it was not significantly lower than Rely+Velpar. Grasses were almost nonexistent in the check and absent in the spring treatments.

Figure 2. Broadleaf weed cover and grass cover in the 2017 prune year for pre-emergence application of Rely 280 with and without Velpar (α =0.05; no significant differences).

There were no differences in red sorrel cover at the June evaluation. At the July evaluation, red sorrel cover in the spring Rely treatment was significantly lower than the check (Figure 3). However, it was not different from Rely+Velpar, which was also not different from Rely. Red sorrel cover remained relatively high from June to July, but did not greatly increase even in the check. There was significantly more red sorrel injury in both spring treatments compared to the check, but injury levels were low and observed mostly as stunting and delay of flowering, as well as reddening of leaf margins, with only a few dead basal rosettes (Photo 1). The residual phytotoxicity observed in July was from a residual stunting effect.

Figure 3. Red sorrel cover and phytotoxicity in the 2017 prune year for pre-emergence application of Rely 280 with and without Velpar (α =0.05; t-tests).

Photo 1. Red sorrel in the spring Rely treatment. Rosettes were shorter and flowering delayed compared to those outside the plot; some damage along leaf margins was also observed as reddening.

CONCLUSIONS: Spring pre-emergence application of Rely, whether with or without Velpar, was not effective in significantly reducing or eliminating red sorrel. Some basal rosettes were killed (Photo 2), indicating that Rely did have an effect on red sorrel, but this weed has multiple generations in one growing season and Rely did not control individuals that germinated after application. Because Rely is a contact herbicide, it is unclear as to why stunting was observed in the Rely only treatment, and why the stunting was still apparent at the July evaluation. It may be that these individuals weren't truly stunted, but were juvenile rosettes that had just germinated. The plants inside the treated plots were noticeably shorter than those outside the plots and were shorter than the plants in the check plots, but the check plots may have also contained the juvenile individuals layered under the older ones (Photo 3). The leaf margin reddening did not appear to be due to insect damage; evidence of insect herbivory was noted but the "bites" did not exhibit the same reddening (Photo 4A-B). These treatments, and the fall prune treatments, will be carried over into 2018 for evaluation of carryover weed control and yield effects.

Photo 2. Rely+Velpar plot, showing dead red sorrel rosettes as well as small rosettes with leaf margin reddening.

Photo 3. Untreated check plot, showing tall and short individual red sorrel rosettes.

Photo 4. Red sorrel rosette with insect herbivory damage; note lack of leaf reddening (A) versus intact reddened leaves (B).

RECOMMENDATIONS: This trial will be carried over until the 2017 Crop cycle plots are harvested in 2019; recommendations will be made upon trial completion.

EXTENSION

INVESTIGATOR: David E. Yarborough, Extension Blueberry Specialist

13. TITLE: Wild Blueberry Extension Education Program in 2017.

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 survey, and from individual client contacts. The advisory committee gave priority to grower outreach, IPM, pesticide recommendations for weeds, insects and diseases, food safety and groundwater. Needs identified by the survey include weed management, economics/marketing, pest management, general information and fertilization. Needs identified by individual grower contact reinforce those previously identified, but also added was the need to evaluate cost effectiveness of crop inputs to manage fields with the current low prices.

RESULTS:

Meetings attended:

Northeastern Plant Pest and Soils Conference. Philadelphia, PA. January 3-6, 2017.

Augusta Agricultural Trade Show. Augusta, ME. January 12, 2017.

Journee de Information Bleuet. Dolbeau-Mistassini, Quebec. March 15, 2017.

Bleuet New Brunswick Blueberry Annual General Meeting. St. Andrews, New Brunswick. March 31 - April 1, 2017.

Organic Field Day. Whitefield, ME. June 22, 2017.

Wild Blueberry Producers of Nova Scotia Summer Meeting. Collingwood, NS. July 15, 2017.

Wild Blueberry Summer Field Day & Meeting. Jonesboro, ME. July 19, 2017.

Ethos Eating on the Wild Side. Portland and Dresden, ME. July 24-15, 2017.

WBANA blogger immersion tour. Bar Harbor, ME. August 13-15, 2017.

Legislative Tour. Machias and Jonesboro, ME. August 24-25, 2017.

American Marketing Service Tour. Jonesboro, ME. September 25, 2017.

20th Wild Blueberry Health Summit. Bar Harbor, ME. September 27-30, 2017.

Wild Blueberry Research and Extension Workers. Bar Harbor, ME. October 25-27, 2017.

Annual Meeting of the Wild Blueberry Producers Association of Nova Scotia. Truro, NS. November 17-18, 2017.

Presentations:

- Comparisons of single versus split postemergence mesotrione applications for spreading dogbane control in wild blueberry fields. Proceedings of The Northeastern, Plant, Pest, and Soils Conference. Philadelphia, PA. January 3-6, 2017.
- Preemergence and postemergence applications of sulfentrazone and carfentrazone for weed control in wild blueberry (*Vaccinium angustifolium*) fields. Proceedings of The Northeastern, Plant, Pest, and Soils Conference. Philadelphia, PA. January 3-6, 2017.
- Wild Blueberry Pest Management Update, Augusta Agricultural Trade Show. Augusta, ME. January 12, 2017.
- World Production Numbers. PEI Wild Blueberry Growers Association Marketing and Production Workshop, Charlottetown, PEI. January 18, 2017.
- Analyse comparative des différentes régies de production au Maine. Journee de Information Bleuet. Dolbeau-Mistassini, Quebec. March 15, 2017.
- L'effet des changements climatiques sur le bleuet sauvage. Journee de Information Bleuet. Dolbeau-Mistassini, Quebec. March 15, 2017.
- World Blueberry Production Numbers: Today/Tomorrow and Weed and Fertilizer Management Strategies for Reduced Inputs. Wild Blueberry Spring Meeting, Ellsworth, Waldoboro, Machias. March 22, 24, 26, 2017.
- World Crop & Market Outlook and the effects of climate change on Wild Blueberries. Bleuet New Brunswick Blueberry Annual General Meeting, St. Andrews New Brunswick. March 31 - April 1, 2017.
- Maine Wild Blueberry Systems Analysis. PEI Blueberry Information Day & Annual General Meeting of the PEI Wild Blueberry Growers' Association. Charlottetown, PEI. April 4, 2017.
- Calibration Workshop for Boom and Airblast Sprayers. Union, ME. April 14, 2017.
- Wild Blueberry ICM Scouting Sessions, Warren, Orland and Machias, ME. May 2, 3, 4, 30, 31 and June 1, 27, 28, 29, 2017.
- MaineAg in the Classroom Kids Day 2017. Windsor, ME. May 19, 2017.

Maine's Wild Blueberry Industry. Nobleboro Historical Society, Nobleboro, ME. May 20, 2017.

- Organic Field Day Presentation. Whitefield, ME. June 22, 2017.
- Maine's Wild Blueberry Industry. Acadia Senior College, College of the Atlantic, Bar Harbor, ME. July 12, 2017.
- Maine Wild Blueberry Crop. Wild Blueberry Producers of Nova Scotia Summer Meeting, Collingwood, NS. July 15, 2017.
- Open crop discussion and 2017 Weed Management Research Plots and Low Input Demonstration Plot. Wild Blueberry Hill Farm Field Day, Jonesboro, ME. July 19, 2017.
- 21st Century After School Program blueberry talk. Blueberry Hill Farm, Jonesboro, ME. July 20, 2017.
- Wild Blueberry Production and IPM Practices, Legislative Tour. Machias and Jonesboro, ME. August 24-25, 2017.
- Wild Blueberries in Maine. Big E, Springfield, MA. September 14 -16, 2017.
- Wild Blueberry Production, American Marketing Service Tour. Jonesboro, ME. September 25, 2017.
- Maine's Wild Blueberry Industry, Go Away Tours. Bar Harbor, ME. October 16, 2017.
- Comparisons of pre-emergence applications of Zeus Prime XC and Rely 280 for weed control in wild blueberry fields and Maine Wild Blueberry Crop Report. Annual Meeting of the Wild Blueberry Research and Extension Workers, Bar Harbor, ME. October 25-27, 2017.
- Strategies for optimizing yields. MOFGA Farmer to Farmer meeting, Northport, ME. November 5, 2017.
- Wild Blueberry Production PSE 215 lecture. Orono, ME. November 8, 2017.
- Blueberry Crop Trends 1996 -2017. Annual Meeting of the Wild Blueberry Producers Association of Nova Scotia, Truro, NS. November 17-18, 2017.

Publications:

- Yarborough, D., F. Drummond, S. Annis and J. D'Appollonio. 2017. Maine Wild blueberry systems analysis. Acta Horticulturae 1180:151-160. ISHS 2017. DOI: https://doi.org/10.17660/ActaHortic.2017.1180.21
- Chen, X., D. Yarborough and J. D'Appollonio. 2017. Wild blueberry systems approach economic and risk analysis. Acta Horticulturae 1180:143-150. ISHS 2017. DOI: https://doi.org/10.17660/ActaHortic.2017.1180.20
- Wu, V.C.H., F. A. Drummond, S. Tadepalli, M.E. Camire, K. Davis-Dentici, A. Bushway, and D.E. Yarborough. 2017. *Salmonella* spp. dynamics in wild blueberry, *Vaccinium angustifolium* Aiton. World Microbiol. 4(1): 64-71.
- Yarborough, D.E. and J.L. D'Appollonio. 2017. Comparisons of single versus split postemergence mesotrione applications for spreading dogbane control in wild blueberry fields. Proceeding of The Northeastern, Plant, Pest, and Soils Conference. Philadelphia, PA; January 3-6, 2017. No. 31.
- Yarborough, D.E. and J.L. D'Appollonio. 2017. Preemergence and postemergence applications of sulfentrazone and carfentrazone for weed control in wild blueberry (*Vaccinium angustifolium*) fields. Proceeding of The Northeastern, Plant, Pest, and Soils Conference. Philadelphia, PA; January 3-6, 2017. No 113.

Extension publications:

Revised: Fact Sheet #209 2017 Insect Control Guide for Wild Blueberries Fact Sheet #239 2017 Weed Control Guide for Wild Blueberries Fact Sheet #219 2017 Disease Control Guide for Wild Blueberries Fact Sheet #224 Commercial Pollinators Fact Sheet #260 Blueberry Enterprise Budget Wild Blueberry Crop Statistics web page 2017 Maine Wild Blueberry Pesticide Chart 1 of 3 Insecticides 2017 Maine Wild Blueberry Pesticide Chart 2 of 3 Fungicides 2017 Maine Wild Blueberry Pesticide Chart 3 of 3 Herbicides

New:

Weed Resistance Prevention Practices for Wild Blueberries, Wild Blueberry Fact Sheet No. 257

Wild Blueberry Management Tool (T. Esau, Dalhousie University, Truro, NS) version adapted for Maine growers by Cooperative Extension

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 94,700 page views in 2017 and so it 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/

*Other program activities***:**

I am the principal investigator for the SCBG project: *Preventing Weed and Disease Resistance: Maine Wild Blueberry Integrated Pest Management (IPM) (2016-2017).* I am responsible for compiling the reports for the Wild Blueberry Advisory Committee and HATCH and NRSP4 and Extension MPRS Project reports for the REEport on-line database and the Extension MPRS logic model reporting system. 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 serve on the peer review committee for Extension 2017-2019 and served on School of Biology and Ecology Peer Committee for Seanna Annis and Frank Drummond. I serve on the graduate committees of: Tyler Case M.S. student, Major advisor Seanna Annis 2015 - present; Nghi Nguyen M.S. student, Major advisor Seanna Annis 2017 – present; and Venessa Scurci, M.S. student, Major advisor John Zhang 2017 - present.

CONCLUSIONS: Growers are participating in IPM programs in the four primary wild blueberry growing counties: Washington, Hancock, Knox and Lincoln. The skills survey results indicate that growers are learning new skills and making positive changes in their management practices. A high percentage of participating growers indicated they had learned new skills and changed their practices by rotating herbicides, thereby reducing weed resistance in wild blueberry fields. Growers are using the blight forecast provided on the Wild Blueberry Website 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. In the short term, strategies to minimize costs with the current lower returns are being made. Two resources, the *Wild Blueberry Enterprise Budget* https://extension.umaine.edu/blueberries/factsheets/marketing-and-business-management/260 blueberry-enterprise-budget/ and the *Wild Blueberry Management Tool* https://extension.umaine.edu/blueberries/wp-content/uploads/sites/56/2010/06/Wild-Blueberry-Management-Tool-.xlsx provide the information needed to make informed decisions.

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.