


Fall Bloom & Fruit 2021


2021 Wild Blueberry Research and Extension Reports

January 2022

THE UNIVERSITY OF MAINE
Cooperative Extension


Mummy Berry Infection


Red Sorrel in Blueberry Rake


Slime Mold on Blueberry


Solar Panels Over Blueberry


SWD Trap


SWD Female Ovipositing


Fireworm Leaf Tie

The work presented here would not be possible without our farmer and processor collaborators and funding sources.

This document was compiled and edited by Dr. Lily Calderwood and her Research Assistants, Mara Scallon and Brogan Tooley.

Land Acknowledgement

The University of Maine recognizes that it is located on Marsh Island in the homeland of the Penobscot Nation, where issues of water and territorial rights, and encroachment upon sacred sites, are ongoing. Penobscot homeland is connected to the other Wabanaki Tribal Nations — the Passamaquoddy, Maliseet, and Mi'kmaq — through kinship, alliances and diplomacy. The University also recognizes that the Penobscot Nation and the other Wabanaki Tribal Nations are distinct, sovereign, legal and political entities with their own powers of self-governance and self-determination. The Wild Blueberry Cooperative Extension & Research team recognizes that the ongoing cultivation of wild blueberry in Maine is based on the knowledge and experience gained by generations of Passamaquoddy, Mi'kmaq, Maliseet, and Penobscot communities; these communities continue to cultivate and celebrate wild blueberry today.

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Lily Calderwood, Judy Collins, Phil Fanning, Becky Gumbrewicz,
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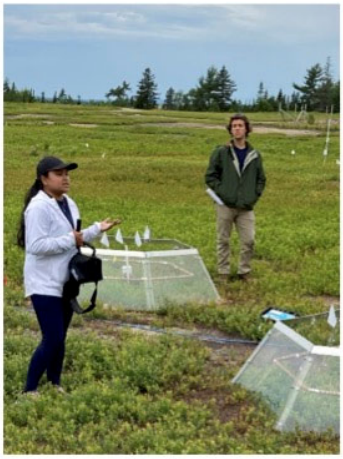
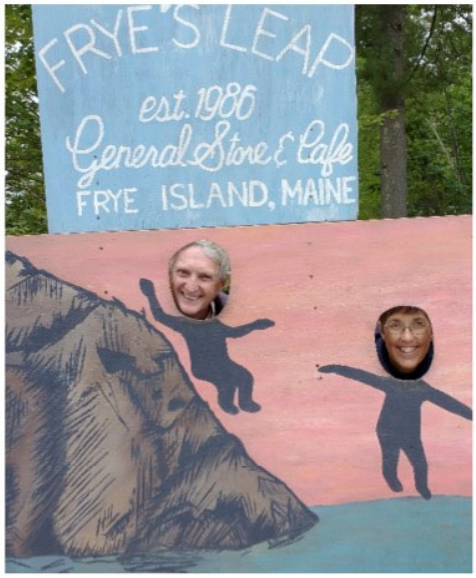
MAINE




FRYE'S LEAP
est. 1986
General Store & Cafe
FRYE ISLAND, MAINE

MAINE'S WILD BLUEBERRY EXTENSION PROGRAM 2021

THE UNIVERSITY OF MAINE Cooperative Extension

NEW 2021 Projects & Content:
Research: Dual-Use Solar in Wild Blueberry
Research: Harvest Timing and Berry Quality
Research: Water Retention, Ground Fertility
Factsheet: Farm Succession Planning for Wild Blueberry Growers
Factsheet: Growing and Establishing Wild Blueberries at Home
NEW 2022 Projects & Content to Look Forward to:
Education: Wild Blueberry Production Guide and Course
Research: Herbicide Trial (Express and Option)
Research: Foliar Fertility (Calcium application in the Crop Year)
Factsheet: Wild Blueberries & Climate Change
Factsheet: Wild Blueberries & Irrigation
Factsheet: Updated Nutrient Management Guidelines
Factsheet: Updated Mummy Berry Guidelines

2019 to 2021 Website Analytics: Website usage before and after the January 2020 website revision.

	Number of Page Views May to July			Average Time Spent Minutes		
	2019	2020	2021	2019	2020	2021
Total Page Views	41,557	57,678	55,814	2:27	2:36	2:31
Growing Blueberries from Seed	3,718	6,446	3,799	20:01	23:30	18:51
Wild Blueberry Homepage	2,879	2,344	1,945	1:44	1:12	1:02
Yellow Flowering Weeds	1,279	2,778	2,636	0:49	0:43	0:48
Blue/Purple Flowering Weeds	751	1,842	1,694	0:45	0:51	0:52
Grass, Sedge, &, Rush, Weeds	885	1,101	1,364	0:49	0:53	0:46
Improving Your WBB Yields	728	1,228	1,182	10:37	15:53	16:40

2021 Virtual Events (2021 Conference Series)	
29-Jan	Leveraging Technology for Wild Blueberry Sales
3-Feb	Insect Pest Management: Spotted Wing Drosophila
5-Feb	Weed Control Practices in New Jersey
10-Feb	Beginning Wild Blueberry Production
12-Feb	Specialty Food Industry and Market Trends
17-Feb	Acidic Podzol Soils
19-Feb	Disease IPM Update
24-Feb	Organic Wild Blueberry Pest and Crop Management
26-Feb	Wild Blue Wine
3-Mar	Fresh Pack Line Improvements
5-Mar	Pollinators and Pollination
10-Mar	Plant Stress and Climate Change
17-Mar	Wild Blueberry Commission of Maine Updates
19-Mar	Fertility and Water Management in Northern Highbush Blueberries
24-Mar	Crop Insurance, Food Safety and NRCS
2021 In-Person Events	
25-May	Climate Resilience at Ellsworth
15-Jun	Climate Resilience at Cherryfield
16-Jun	Organic Climate Resilience at Brunswick
22-Jun	Dual-use Solar over Blueberry at Rockport
23-Jun	Organic Climate Resilience at Stockton Springs
8-Jul	Field Day at BHF (100 growers attended)

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RESEARCH

INVESTIGATORS: P. Fanning and J. A. Collins

1. Validation of the spotted wing drosophila (SWD) male trap capture threshold, year six

OBJECTIVE
The purpose of this study was to further validate and fine-tune the current "risk-based" threshold recommendations for spotted wing drosophila.

LOCATION Aurora, Belfast, Columbia, Deblois, Jonesboro, T18 MD BPP, T19 MD BPP, and Union, ME
PROJECT TIMEFRAME June – August 2021

INTRODUCTION
Previous work has shown that captures of spotted wing drosophila males can be used as a reliable method for determining when fields are at risk from larval infestation. There was excellent validation of this risk-based threshold by Dr. Frank Drummond resulting from work in 2016 and 2017. However, the SWD threshold system appeared to have broken down in 2018. The 2018 growing season was atypically hot during the SWD buildup period of July and August in both the Downeast and Midcoast growing areas. Early harvest still avoided SWD infestation, but fields harvested starting in mid-August did not show a delay between the male SWD threshold and fruit infestation. In 2018, many threshold levels that in the past would suggest a low probability of fruit infestation actually became infested. Whether this was due to the extreme hot temperatures in mid- to late summer is unknown. If it was, and hot summers continue to characterize the Maine growing season, then the current threshold system for spotted wing drosophila may not perform adequately for determining if fields need to be immediately harvested or protected with an insecticide. Because of the unusual results observed in 2018, additional research was completed in 2019, 2020, and 2021.

METHODS
Cooperating growers allowed us to trap adult SWD and sample fruit for larval infestation on a weekly basis. Fields were maintained by the growers using typical wild blueberry production practices. Traps were placed in fifteen wild blueberry fields in Downeast and Midcoast Maine. Trapping for adult SWD began in late June and continued until larval infestation of fruit was detected or until fields were harvested. Traps were monitored at 6 to 8-day intervals. All traps were constructed from Solo®, 16 fl. oz, red polystyrene cups with light-blocking lids. Seven to ten, 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). Three traps were placed at each site and were hung 1-2 ft 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 and processed to determine the number of SWD males per trap. New traps were deployed weekly. Using these data, we calculated the number of males per trap captured from each site on each date and the mean cumulative number of SWD males captured over the collection period.

To compare adult abundance with larval infestation, weekly fruit samples were taken from the ten fields from mid-July until the fields were harvested. The samples were processed using the Salt Extraction Method (Van Timmeren et al., 2017). Each sample consisted of ca. 6 oz (by volume) of fruit collected from the vicinity of each of the three adult traps (three samples per field). Using these data, we calculated the mean number of larvae collected at each site on each sample date. These data were compared with the adult abundance data collected over the same time period.

RESULTS

The average date of the first trap capture of SWD in 2019 was 30 July (range: 22 July – 6 August); in 2020 the average date of the first trap capture of SWD was 18 July (range: 14 July – 3 August). In 2021, the average date of the first trap capture of SWD was 5 July (range: 14 June – 26 July). With the exception of one field, all fields tested had infestation before or at harvest (Table 1), which is comparable to 2020 results and indicative of the earlier onset of trap captures in both years compared to previous years' early dates in SWD's infestation of Maine. Seven of the fields evaluated in 2021 had fruit infestation despite having low numbers of male SWD detected prior to harvest (0.0 - 1.83 cumulative male SWD trap captures). However only low levels of infestation were detected: a sole maggot in the case of some fields. All other fields had extremely high cumulative male SWD trap captures (3.3 – 59.5) resulting in predicted chances of fruit infestation the following week of 9 – 99%. Seven of these fields had infestation before or at harvest. For the second year we experienced early trap captures of SWD and populations increased faster than previous years' observations. These years' data are currently being tested to see if their inclusion in the model increases its predictive power.

Table 1. Fruit Infestation, male SWD capture, and PREDICTED probability of fruit infestation based upon mean cumulative SWD captures.

Site #	Location	Date 1st SWD male detected	Status of fruit infestation before or at harvest	Date harvested or larval infestation detected	Mean Cumulative # of MALE SWD week **before** infestation or at last sample before harvest	Probability of fruit infestation the week after male SWD thresholds
1	Downeast	6-Jul	**Yes**	16-Aug	59.50	99%
2	Downeast	28-Jun	**Yes**	9-Aug	46.67	97%
3	Downeast	28-Jun	**Yes**	2-Aug	15.33	59%
4	Downeast	28-Jun	**Yes**	2-Aug	10.33	40%
5	Downeast	6-Jul	**No**	2-Aug	9.00	35%
6	Downeast	19-Jul	**Yes**	16-Aug	8.00	30%
7	Downeast	28-Jun	**Yes**	2-Aug	4.33	14%
8	Downeast	6-Jul	**Yes**	2-Aug	3.33	9%
9	Midcoast	14-Jul	**Yes**	4-Aug	1.83	4%
10	Midcoast	7-Jul	**Yes**	28-Jul	1.33	2%
11	Downeast	28-Jun	**Yes**	19-Jul	1.17	2%
12	Midcoast	14-Jul	**Yes**	28-Jul	1.00	1%
13	Midcoast	14-Jul	**Yes**	21-Jul	0.33	0%
14	Midcoast	14-Jun	**Yes**	28-Jul	0.33	0%
15	Downeast	26-Jul	**Yes**	13-Jul	0.00	0%

CONCLUSIONS AND RECOMMENDATIONS

This study completes six years (2016-2021) of validating the predictive model for SWD fruit infestation based upon the average cumulative male SWD trap capture. Over this time, we have monitored 71 wild blueberry fields in both the Midcoast and Downeast regions of Maine. Observations in five of the six years have resulted in model predictions that were mostly supported by what we found in the field. Predictions for 2018 were not well supported by fruit infestation observations in the field. Therefore, we have a fairly accurate model for predicting fruit infestation, but caution should be observed in using the model predictions.

Two recommendations should be followed when using this model for the management of SWD. First, a conservative approach is recommended. By this, we mean that, as a buffer from infestation, growers should use somewhat lower infestation likelihoods than their perceived risk level. This is particularly evident these last two years when the first trap capture of SWD was so early and populations increased faster than previous years' experience. Second, the predictive model should NOT be used WITHOUT sampling fruit and determining the status of infestation during the season, especially after SWD captures begin to climb.

NEXT STEPS

- We will continue to collect SWD trap and infestation data.
- Multiple new SWD projects will continue to refine and add data to this model.

ACKNOWLEDGEMENTS

We thank Ben Johnson, Abigail Fisher, and Hayley McGowan for their assistance with this study.

REFERENCES

Van Timmeren, S., L.M. Diepenbrock, M.A. Bertone, H.J. Burrack, and R. Isaacs. (2017). A filter method for improved monitoring of *Drosophila suzukii* (Diptera: Drosophilidae) larvae. Journal of Integrated Pest Management, 8(1), 23.

RESEARCH

INVESTIGATORS: P. Fanning and B. Johnson

2. Spatial distribution of spotted wing drosophila infestation in wild blueberry fields

OBJECTIVE

This trial builds upon work completed in 2019 and 2020 in which fruit infestation by larvae was concentrated at the edges of the field and decreased with distance into the field. This temporal-spatial pattern of infestation, if consistently true, suggests that perimeter sprays in wild blueberry for SWD might have the potential to be effective.

LOCATION Belfast, Columbia, T18 MD BPP, and Union, ME
PROJECT TIMEFRAME July - August 2021

INTRODUCTION

Spotted wing drosophila (SWD) is an invasive insect that lays its eggs in underripe and ripening fruit still in the field and is a pest of major concern for soft fruit growers. SWD was first detected in Maine in 2011 and since then has been found in all berry-producing regions of the state. Wooded edges that commonly border blueberry fields in Maine have the potential to act as refugia for SWD. Wild berries found in these woody edges provide early resources for SWD and allow pest populations to build up

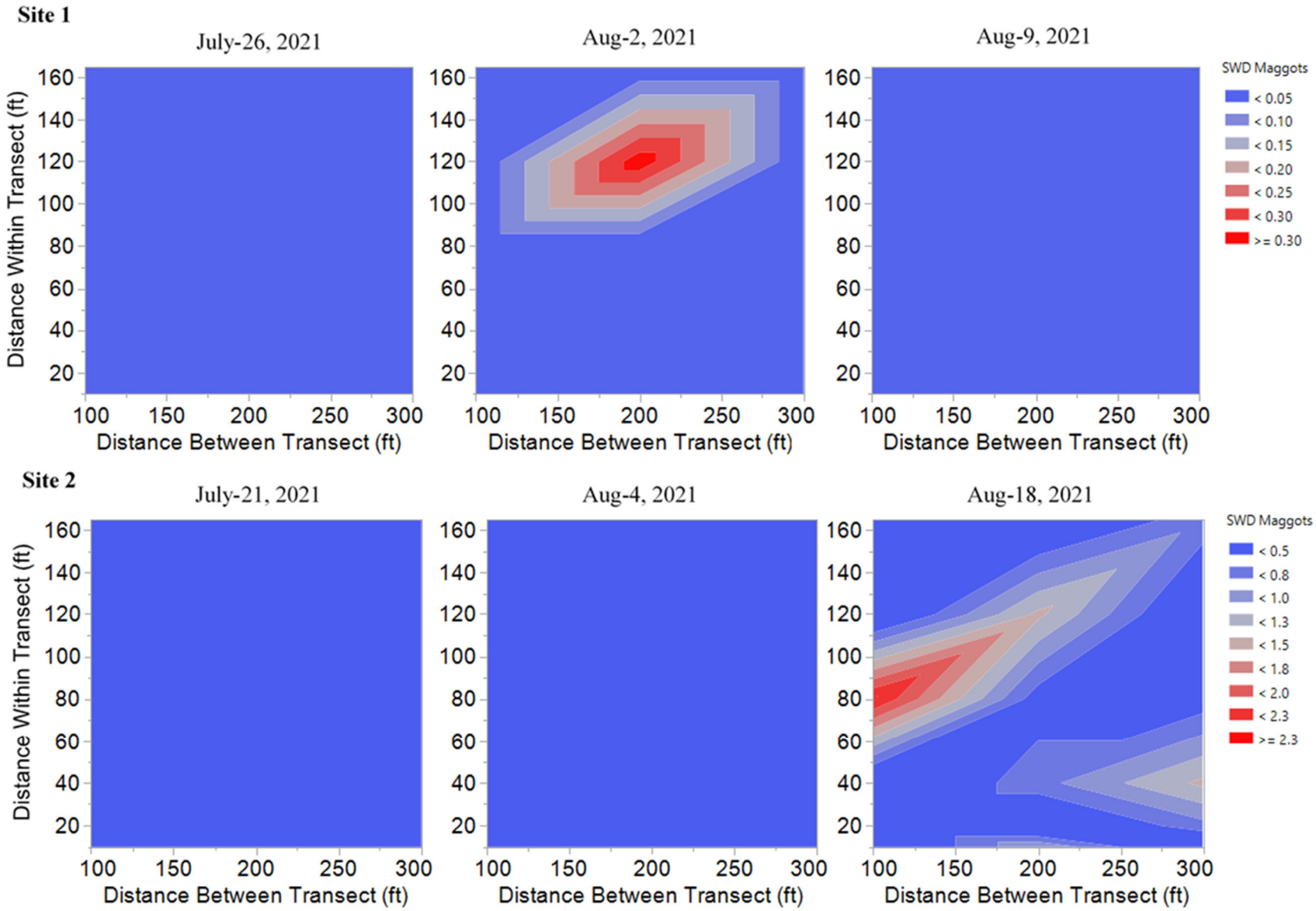
before wild blueberries are valid hosts. Previous work has shown that wild fruit abundance results in increased probability of SWD infestation (Drummond et al., 2018). Blueberry maggot fly has been found to invade commercial blueberry fields from unmaintained borders, and perimeter pesticide applications significantly reduced the number of blueberry maggot fly infestations in the field (Rodriguez-Sona et al., 2015; Collins and Drummond, 2008).

METHODS

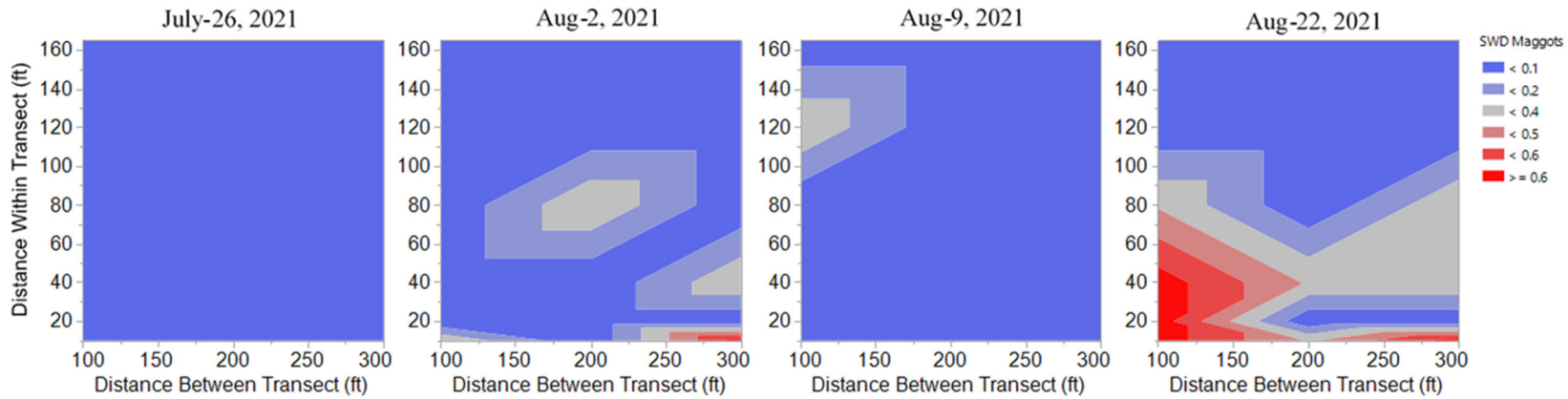
Six fields were monitored for this trial, sites 2, 5 & 6 were located in the Midcoast region, and 1, 3 & 4 in the Downeast region. Sites 5 and 6 were both 'holdover' fields, while sites 1-4 were all crop-year fields and received grower standard insecticide applications. Each site had three, 165ft long transects spaced approximately 100ft from one another running from the field edge towards the center of the field. Fruit samples (ca. 6 oz by volume) were collected at 10, 20, 40, 80, 120 and 165 ft from the field edge. Samples were taken either weekly or biweekly. The Salt Extraction Method (Van Timmeren et al., 2017) was used to assess larval infestation of the fruit samples. Spatial interpolation was used to generate the spatial pattern within fields and generate heat maps.

RESULTS

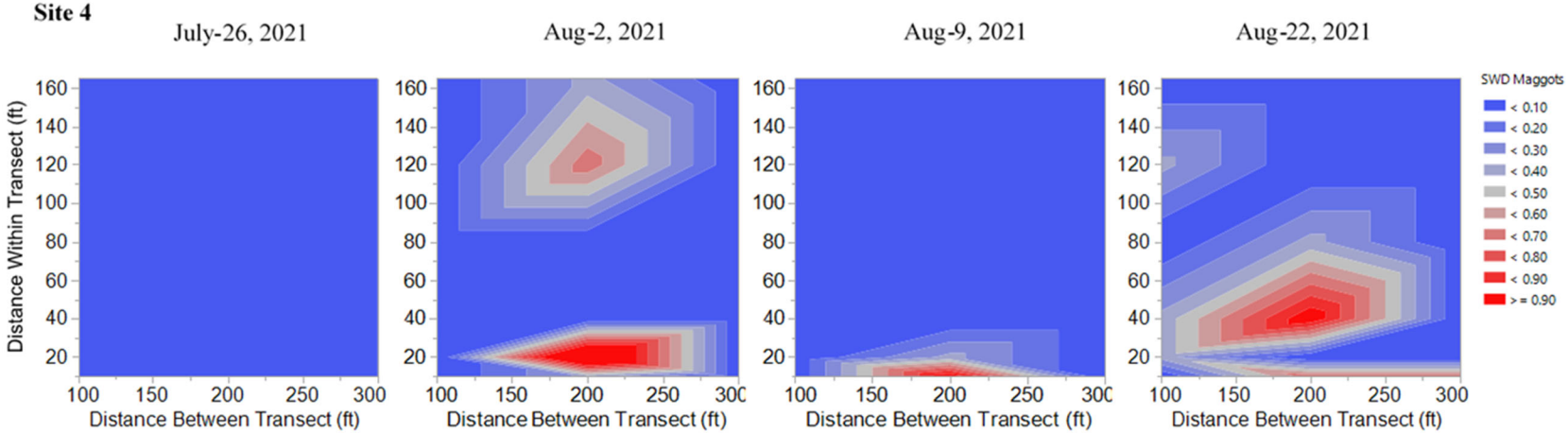
The six monitored fields varied greatly in their infestation levels, with the unmanaged holdover fields displaying much higher numbers of larvae per sample. Strong perimeter-driven behavior was observed in terms of SWD larval infestation, with highest levels of infestation typically being seen in the field edges (Fig. 1). Looking at the proportions of the infestation distributed from the field edge to the interior for all samplings where SWD was observed, and including all years (2019-2021) and sites (10+ sites), over 70% of the larval infestation occurred within the first 40ft of the field (Fig. 2).


Site 1
July-26, 2021
Aug-2, 2021
Aug-9, 2021
Distance Within Transect (ft)
Distance Between Transect (ft)
SWD Maggots
< 0.05
< 0.10
< 0.15
< 0.20
< 0.25
< 0.30
>= 0.30
Site 2
July-21, 2021
Aug-4, 2021
Aug-18, 2021
Distance Within Transect (ft)
Distance Between Transect (ft)
SWD Maggots
< 0.5
< 0.8
< 1.0
< 1.3
< 1.5
< 1.8
< 2.0
< 2.3
>= 2.3

Site 3


July-26, 2021
Aug-2, 2021
Aug-9, 2021
Aug-22, 2021
Distance Within Transect (ft)
Distance Between Transect (ft)
SWD Maggots
< 0.1
< 0.2
< 0.4
< 0.5
< 0.6
> = 0.6

Site 4


July-26, 2021
Aug-2, 2021
Aug-9, 2021
Aug-22, 2021
Distance Within Transect (ft)
Distance Between Transect (ft)
SWD Maggots
< 0.10
< 0.20
< 0.30
< 0.40
< 0.50
< 0.60
< 0.70
< 0.80
< 0.90
> = 0.90

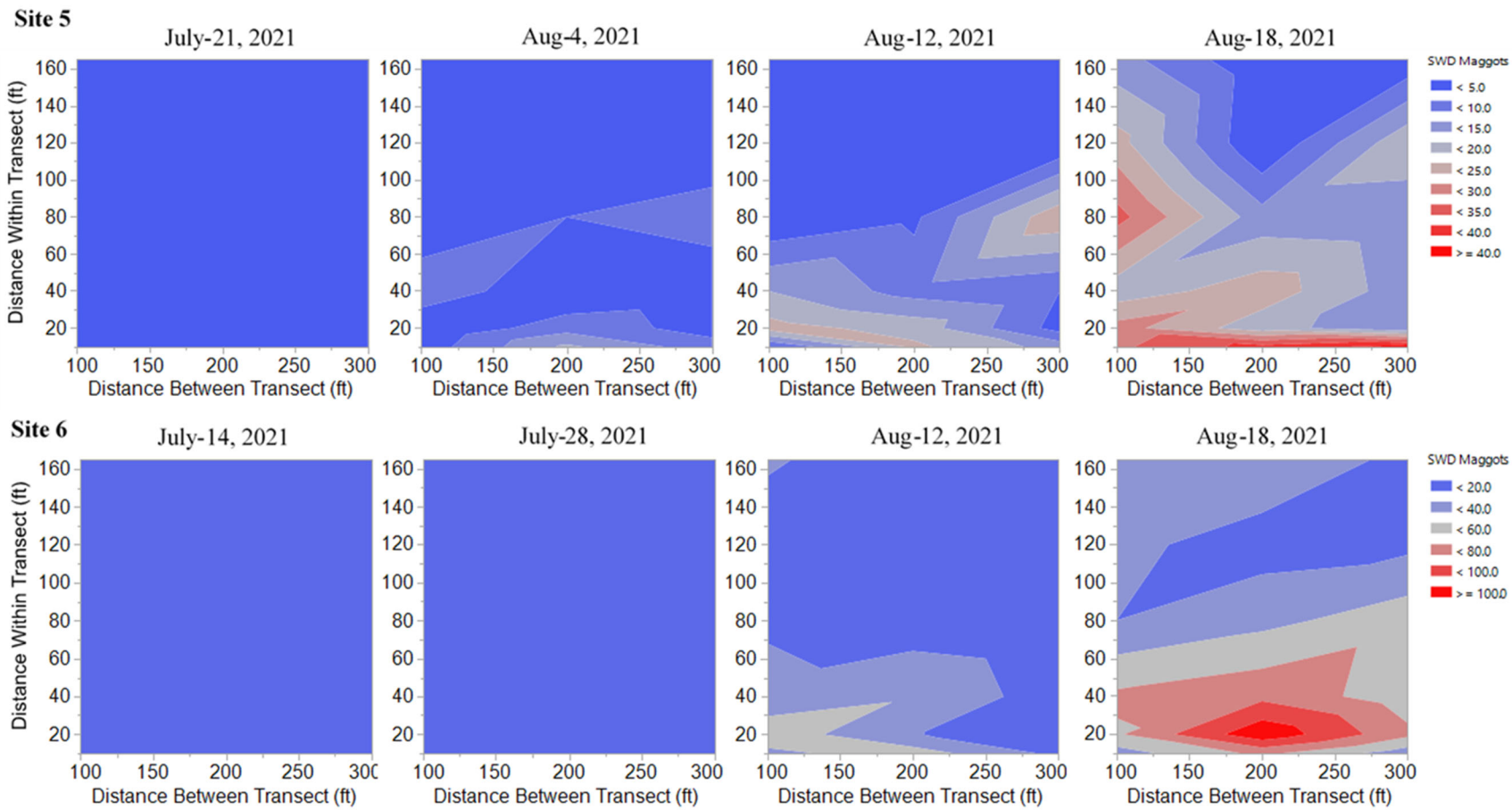


Figure 1. Heat maps of larval infestation within monitored fields. Dark blue is low larval density while red is high larval density.

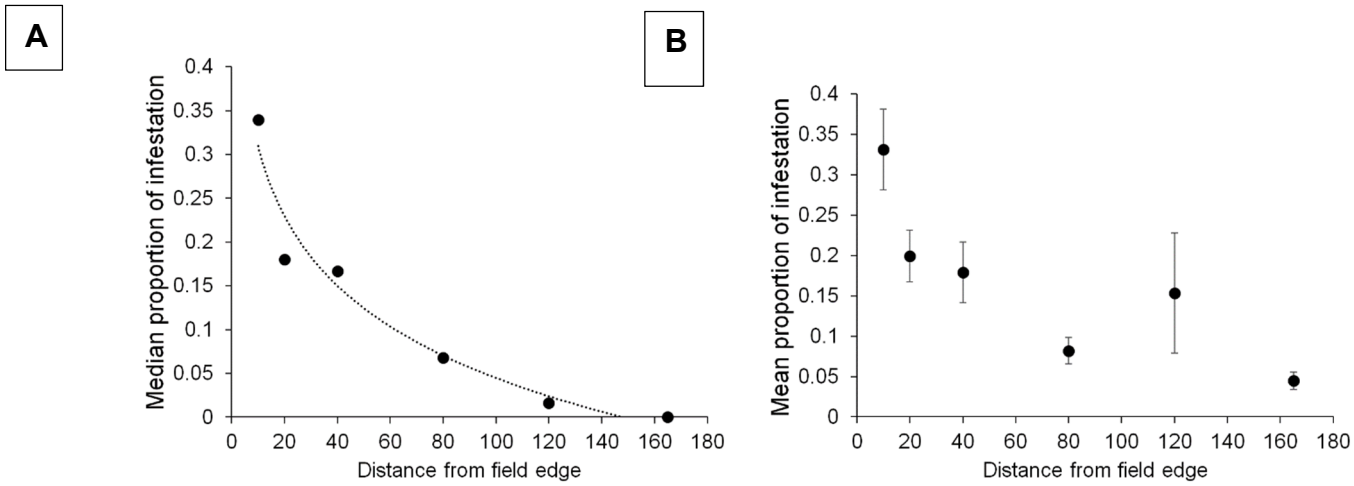


Figure 2. Graph showing the median (± S.E.) (A) and mean (± S.E.) (B) proportion of infestation from the edge to the interior of both treated and untreated fields.

CONCLUSIONS AND RECOMMENDATIONS:

This trial builds on and verifies trends from 2019 and 2020. In both holdover and crop-year fields, larval infestation is highest near the field edge and decreases as one moves inward, with over half of total infestation occurring in the first 40ft of the field. This behavior is similar to that of the blueberry maggot fly, where perimeter sprays have been shown to be an effective management strategy. In Florida blackberries, perimeter sprays resulted in a reduction of larval infestation in berries (Iglesias and Liburd, 2016). These data suggest perimeter applications might be a valid option for SWD management in wild blueberry. Larval infestation should be used in the implementation of perimeter sprays with monitoring for larval presence at the field edge as well as outside the perimeter treatment area.

NEXT STEPS

- This work will be continued next year, for validation of the perimeter spray with two larval monitoring locations.

ACKNOWLEDGEMENTS

We thank Judy Collins, Abigail Fisher, and Hayley McGowan for their assistance with this study.

REFERENCES

Collins, J.A., and F.A. Drummond. (2008). Field-edge based management tactics for blueberry maggot in wild blueberry. Small Fruits Review, 3(2-4), 285-293.

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RESEARCH

INVESTIGATORS: P. Fanning and B. Johnson

3. Comparison of behavioral control tactics for spotted wing drosophila

OBJECTIVE
The purpose of this trial was to compare the efficacy of different behavioral control tactics developed for spotted wing drosophila (SWD).

LOCATION Jonesboro, ME
PROJECT TIMEFRAME July – August 2021

INTRODUCTION
Behavioral control allows for the exploitation of insect pest responses to odors resulting in sustainable pest management methods to reduce insecticide use while maintaining pest control. This trial looked at different attract-and-kill strategies, specifically ACTTRA TD SWD® and Combi-Protec®. The attractive compounds lead to an increased oral absorption of the active ingredient, which in turn results in quicker fly death. This type of control should lead to a reduction in larval infestation, and a decreased risk of development of insecticide resistance due to lower levels of active ingredient being used.

METHODS
This trial followed a randomized block design with five treatments, each treatment being replicated four times. Treatments were as follows:

1. ACTTRA TD SWD + Delegate WG® (ACTTRA TD SWD mixed with Delegate WG at 0.25% a.i. (v/v), applied at a rate of 1.5 L of formulation/acre (6.90 mL formulation per cage of 200 sq. ft).
2. Combi-Protec + Delegate WG as a full cover spray, full rate (14 fl. oz Combi-Protec, 50 gal water and 6 oz Delegate WG® per acre).
3. Combi-Protec + Delegate WG as full cover spray, half rate (14 fl. oz. Combi-Protec, 50 gal water and 3 oz Delegate WG® per acre)
4. Delegate WG + Induce® spray at the highest field recommended rate for SWD control in blueberries (6 oz/acre of Delegate WG® in 50-gal water/acre + 0.12% Induce as surfactant)
5. Untreated control

Ten insect exclusion cages were used to keep out ambient SWD and retain introduced SWD. Cages were spaced at least 10m from each other to prevent any crossover effects. Treatment 1 was applied as three, 2-ml droplets equally spaced along the center of the cage on blueberry bush shoots. Treatments 2-4 were applied in 35 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. For fruit sampling purposes, each cage was split into quadrants, with fruit samples being collected in a 6 oz cup. All samples were then weighed to account for differences in sample weights. Pre-treatment samples were collected just before applying treatments to determine baseline infestation. One hundred SWD adults (50 male, 50 female) were added to each cage approximately 8 hours after application of treatments. Seven days after the addition of flies, post-treatment fruit samples were taken from the quadrants within each cage. All ten cages were then moved to new patches of wild blueberry and the trial was repeated for replicates 3 and 4. Replicates 1 and 2 were run from 27 July to 3 August and replicates 3 and 4 from 17 August to 23 August.

Larval infestation was determined through the Salt Extraction Method for all fruit samples (Van Timmeren et al., 2017).

RESULTS

Splitting the replicates between two different dates displayed no effect on larval infestation, so all replicates were grouped for analysis. Data were analyzed using a Kruskal-Wallis test. All treatments displayed a significant reduction in SWD larvae per gram of fruit sampled compared to the untreated control cages (P = 0.0005) (Fig. 1). Also, the Combi-Protec with full rate Delegate resulted in significantly lowered infestation than the full rate Delegate treatment (P = 0.0117) (Fig. 1).

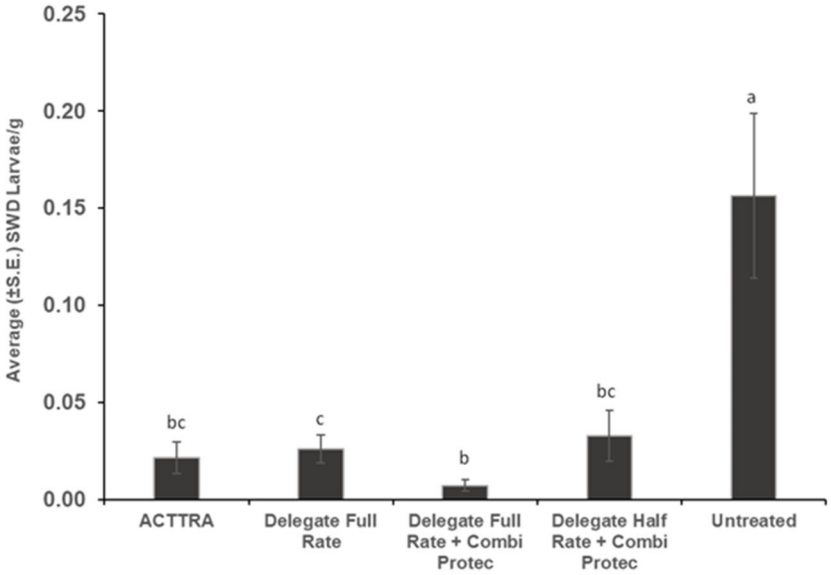

Figure 1. Average (± S.E.) number of SWD larvae per gram of blueberry. Columns topped with the same letter(s) did not display significant differences.

CONCLUSIONS AND RECOMMENDATIONS

All experimental treatments displayed a significant reduction in SWD larval infestation relative to the untreated control cage. In this trial both ACTTRA TD and Combi-Protec combined with reduced amounts of active ingredient provided the same control as a full-rate spray of Delegate.

NEXT STEPS

- Continue work next year to verify the results.

ACKNOWLEDGEMENTS

We thank Judy Collins, Abigail Fisher, and Hayley McGowan for their assistance with this study.

REFERENCES

Van Timmeren, S., L.M. Diepenbrock, M.A. Bertone, H.J. Burrack, and R. Isaacs. (2017). A filter method for improved monitoring of *Drosophila suzukii* (Diptera: Drosophilidae) larvae. Journal of Integrated Pest Management, 8(1), 23.

RESEARCH

INVESTIGATORS: P. Fanning

4. Assessment of susceptibility of spotted wing drosophila to insecticides

OBJECTIVE AND INTRODUCTION
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 spotted wing drosophila (SWD) to insecticides using a simple test that can be widely used by Extension staff without insect colonies and laboratories.

LOCATION Columbia and T18 MD BPP, ME
PROJECT TIMEFRAME November – December 2021

METHODS
Three materials were tested (Delegate® WG + Induce®, Mustang Max®, and Entrust® SC + Induce). Assays were completed on adult SWD collected from three locations in Washington County; Columbia (Site 1) and T18 MD BPP (Sites 2 & 3). Testing was also conducted on a laboratory population that was collected from multiple sites in 2020, and had not been exposed to insecticides for the prior 18 generations. The other populations were assumed to have high prior exposure to insecticides, and thus the highest chances of detecting if resistant genes were present. For each site to be assessed, SWD adults were collected from fruit (bananas) that had been left in the field and subsequently infested by wild SWD. Fruit was organic in order to avoid contamination with systemic insecticides and stored in the refrigerator prior to use to reduce the potential of background SWD infestation. Adult SWD were reared on a diet recipe adapted from the Standard Drosophila Recipe from the Drosophila Species Stock Center. The number of generations and age range of flies used in each assay are shown in Table 1.

For each assay, a 20-ml glass scintillation vial was treated with 1-ml of Delegate WG, Mustang Maxx, or Entrust SC. Once the solution was added, caps were tightly closed, and the vial was tipped gently upside down, and then turned it on its side and rotated to distribute the solution across all interior surfaces. The excess solution was then poured out into a waste container, vials were tapped on a piece of lab paper to remove any additional excess, and lids and vials were placed in a fume hood to dry overnight. On the following day, 10 female adult SWD flies from the sampled location were placed in each vial and held in a growth chamber at 23 ± 2°C and 74% RH to reduce control mortality. Vials were kept on their sides for the duration of the experiment. For each assay there were five replications and five non-treated controls. After 6 (Mustang Max and Delegate) or 8 (Entrust) hours in the vials, we evaluated mortality. Materials and populations tested are in Table 1. Flies were classified as either alive, dead, or moribund. $LC_{90 \times 8}$ (ppm) that were used for the Discriminating Dose assays are shown in Table 2.

Table 1. Type of assay and insecticide tested and generation and age of SWD.

Assay type	Location	Insecticides tested	SWD generation (F0 is collected in field)	SWD age
Discriminating dose	Lab colony Site 1 Site 2 Site 3	Entrust, Delegate, Mustang Maxx (all materials tested on SWD from each site)	Lab colony (F18) Site 1 (F3) Site 2 (F3) Site 3 (F3)	2-6 days

Table 2. $LC_{90 \times 8}$ discriminating dose used in assays.

Insecticide and formulation	$LC_{90 \times 8}$ (ppm)
Mustang Max 0.8EC	6.89
Delegate WG + Induce	861.92
Entrust SC + Induce	847.77

RESULTS

Discriminating dose bioassays revealed that there was some survival by flies exposed to residues of spinosad (Entrust) and spinetoram (Delegate) in populations in Washington County, but no other populations. No survival was observed in any population exposed to residues of Mustang Maxx (Fig. 1).

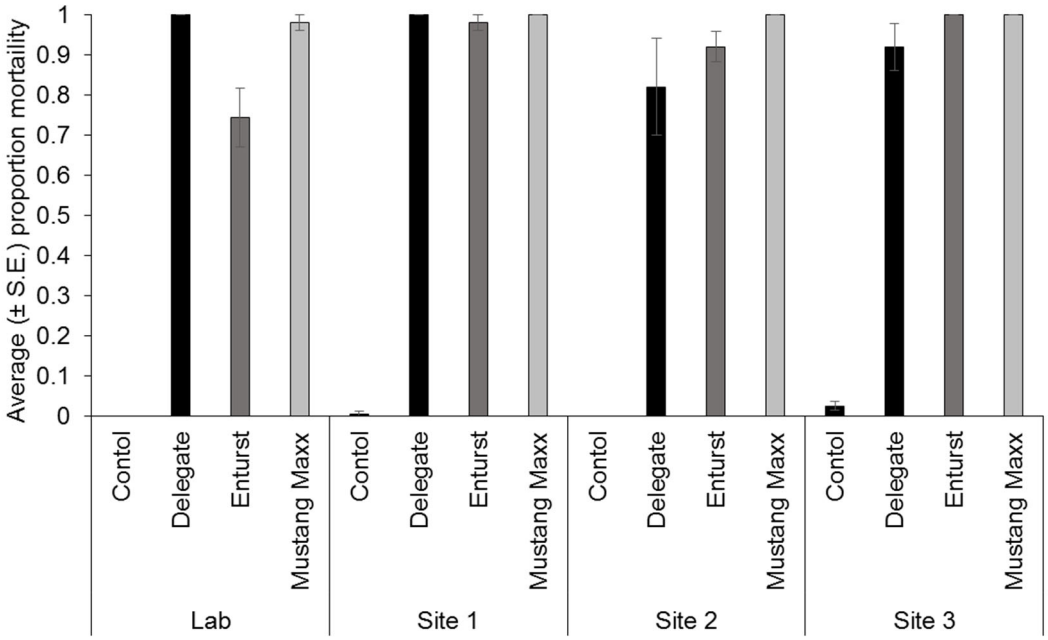

Figure 1. Discriminating dose bioassay results

RECOMMENDATIONS

This is the second year with indications there may be reduced sensitivity to spinosyns (Delegate and Entrust) late in the season. Rotation of insecticide classes is key to ensuring that populations do not develop resistance to insecticides used for its control.

NEXT STEPS

- This work is still in process and will continue with additional Discriminating Dose bioassays and Serial Dilutions of insecticides so that a mortality curve can be established and results verified.

ACKNOWLEDGEMENTS

We thank Ben Johnson, Abigail Fisher and Judy Collins for their assistance with this study.

RESEARCH

INVESTIGATORS: P. Fanning and B. Johnson

5. Efficacy of Combi-Protec® and insecticides in field and semi-field trials against spotted wing drosophila

OBJECTIVE

The purpose of this trial was to examine the efficacy of Combi-Protec®, a phagostimulant bait, combined with insecticides on controlling spotted wing drosophila (SWD) in both field and semi-field conditions.

LOCATION Jonesboro, ME
PROJECT TIMEFRAME August 2021

INTRODUCTION

Combi-Protec is an attractive mixture of plant extracts, proteins, and sugars. When combined with an insecticide, Combi-Protec leads to an increased oral absorption of the active ingredient, leading to a quicker fly death. Increased oral absorption also allows for a reduced amount of insecticide to be used.

METHODS

There were eight treatments:

1. Delegate WG®
2. Delegate WG + Combi-Protec (14 oz/50 gal)
3. Delegate WG (50% rate) + Combi-Protec (14 oz/50 gal)
4. Assail 70WP®
5. Assail 70WP + Combi-Protec (14 oz/50 gal)
6. Assail 70WP (50% rate) + Combi-Protec (14 oz/50 gal)
7. Combi-Protec only, control treatment (14 oz/50 gal)
8. Untreated control

There were four applications of each treatment: 3, 10, 17, and 25 August. Treatments were applied to 10 x 14-ft plots in a complete randomized block design. Each treatment was replicated four times. All materials were applied in 35 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 30 psi and at a slow walking speed. Walking speed for each application was regulated using a metronome.

Field Trial

For each plot, a ca. 6 oz (by volume) berry sample was taken weekly from 9 August through 31 August (9, 16, 24, and 31 August). Fruit samples were evaluated for larval infestation using the Salt Extraction Method (Van Timmeren et al., 2017). Each sample was weighed prior to being processed for larval infestation.

Semi-field Bioassay

A semi-field bioassay was conducted following the 17 August application using treated stems collected from the "Field Trial" plots. Each bioassay container consisted of a 32 oz deli cup, a full water pick, a wire-mesh container to hold loose berries, a fabric-mesh lid, and a small amount of fly diet (Fig 1). At one and three days after the treatment, leaf terminals with 3-4 leaves were clipped and placed in a water pick in a bioassay container. Thirteen berries were then collected from each plot and placed in the wire mesh cage. Ten, 5-7 day old SWD adults (5 male and 5 female) were then added to each bioassay container. Containers were placed in an environmental chamber (22°C; 70% RH) for 6 days. Adult fly mortality was assessed at 24 and 48 hours after the addition of adult SWD. On day 6, berries were removed and placed in a rearing cup in an environmental chamber (22°C; 70% RH) for 14 days to allow for adult emergence. Rearing cups were then frozen, and adult flies were counted at a later date.

Figure 1. Photo of a semi-field bioassay arena.

RESULTS

Field Trial

Due to no or low infestation the first two sampling dates (9 and 16 August) were not included in any analyses. Due to non-normality, data were analyzed using a Kruskal-Wallis test. Data were adjusted for sample weight prior to analysis. Of the fruit sampled on 24 August, the only difference was between the Combi-Protec (CP) only and Delegate WG + CP treatments (P = 0.0304) (Fig. 2). For fruit collected on 31 August there was a significant difference when comparing Assail 70WP to the untreated control, Delegate WG 50% + CP, and Delegate WG + CP (P = 0.0304) (Fig. 2).

Semi-field Bioassay

At the 1 day after treatment (D.A.T.) time point Delegate WG, Delegate WG mixed with Combi-Protec, and Delegate WG 50% mixed with Combi-Protec all had significantly higher mortality than the untreated control ($P < 0.05$) (Fig. 3). There was no significant difference in mortality between any of the treatments at the 3 D.A.T. time point (Fig. 3). When it came to emergence of adult flies from berries in the bioassay containers, none of the treatments were significantly different from the untreated control (Fig. 4).

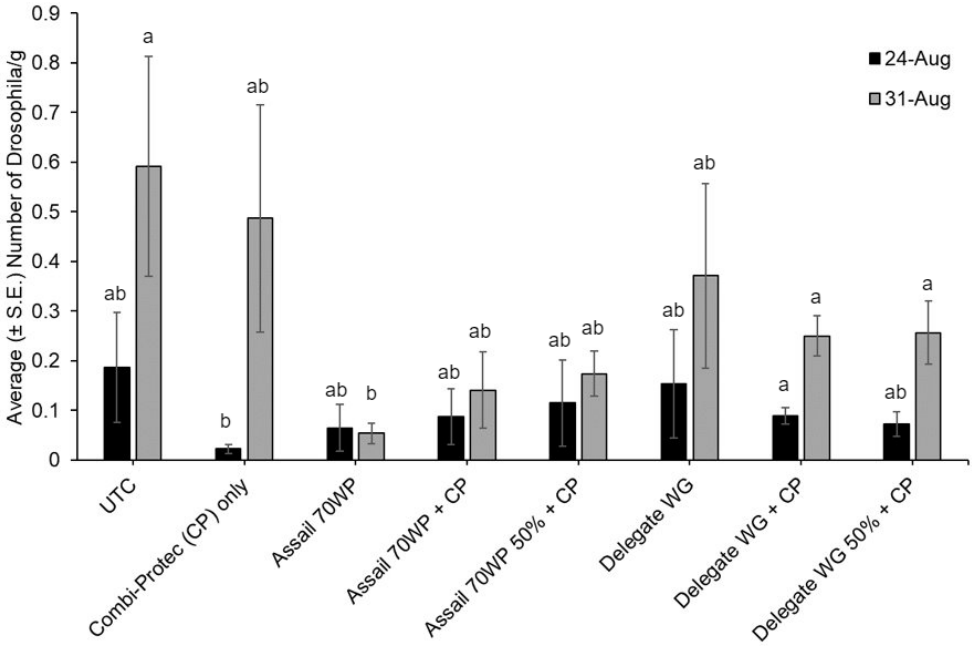

Figure 2. Average (±S.E.) number of SWD larvae per gram of fruit collected. Columns topped with the same letter(s) within the same sampling date did not display significant differences.

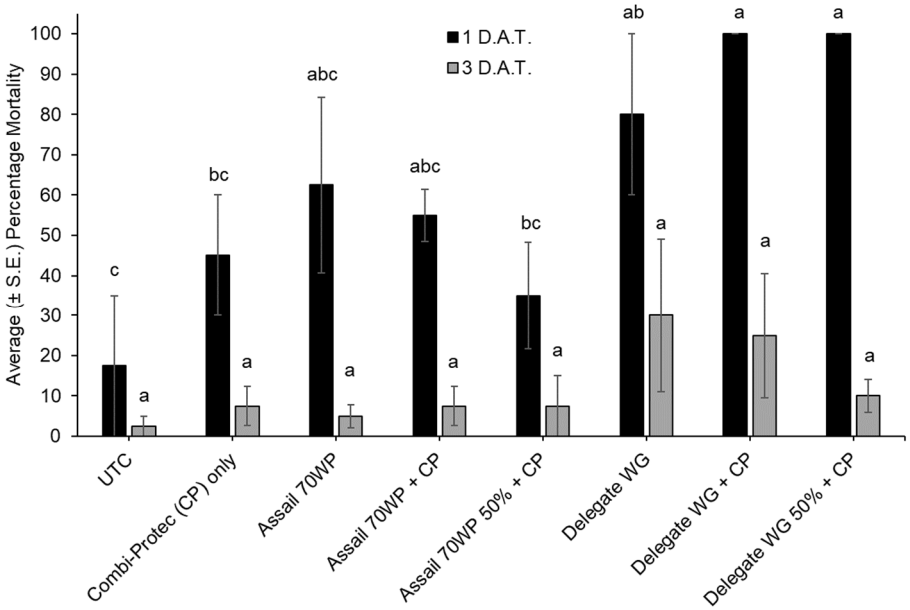

Figure 3. Average (±S.E.) percentage mortality in semi-field bioassays. Darker bars are for fruit and foliage collected 1 day after treatment application and lighter bars are for fruit and foliage collected 3 days after treatment. Columns topped with the same letter(s) within the same color bars did not display significant differences.

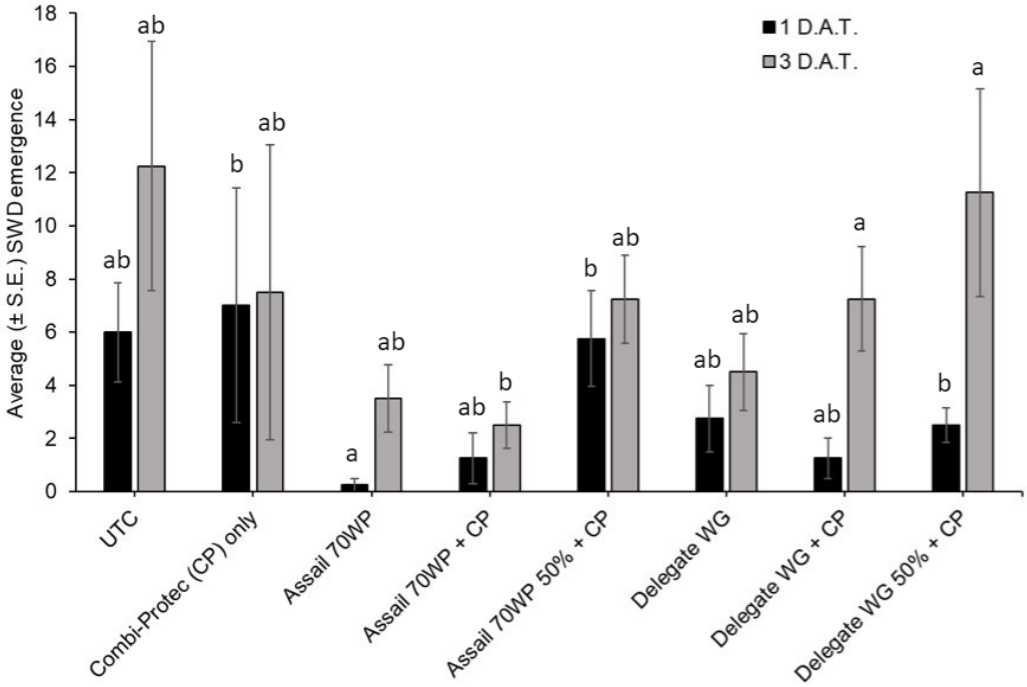

Figure 4. Average (±S.E.) adult SWD emergence from exposed fruit in the semi-field bioassays. Columns topped with the same letter(s) within the same color bars did not display significant differences.

CONCLUSIONS AND RECOMMENDATIONS

This was the first year of this trial. At the moment it appears that Combi-Protec mixed with a half-rate of some insecticides has the potential to provide adequate control for SWD. However, we did not include a treatment of just half-rate insecticide without Combi-Protec. This leaves open the possibility that differences seen might not be a result of the phagostimulant and would have been observed with just the half-rate insecticide.

NEXT STEPS

This work will be repeated in 2022.

ACKNOWLEDGEMENTS

We thank Judy Collins, Abigail Fisher, and Hayley McGowan for their assistance with this study.

REFERENCES

Van Timmeren, S., L.M. Diepenbrock, M.A. Bertone, H.J. Burrack, and R. Isaacs. (2017). A filter method for improved monitoring of *Drosophila suzukii* (Diptera: Drosophilidae) larvae. Journal of Integrated Pest Management, 8(1), 23.

INVESTIGATORS: P. Fanning and J. A. Collins

6. Field control of spotted wing drosophila on wild blueberry with the bioinsecticide, Spear®-T in rotation with Entrust® and Mustang Maxx®.

OBJECTIVE
To evaluate the bioinsecticide Spear-T® LC in rotation with the spinosad Entrust® SC and the pyrethroid Mustang Maxx® in management of spotted wing drosophila (SWD).

LOCATION Jonesboro, ME
PROJECT TIMEFRAME July 2021 - August 2021

INTRODUCTION
Spear®-T 2% Liquid Concentrate, GS-omega/kappa-Hxtx-Hv1a, is a bioinsecticide based on a peptide derived from spider venom. It is a bioinsecticide, but it is not organic due to restrictions on its manufacturing. It is labeled to control small, soft-bodied insects and mites in field and orchard crops. Spear-T works by disrupting the nicotinic acetylcholine receptor of the insect nervous system through contact activity, and is designed to be non-toxic to humans, other mammals, birds, and fish, and safe on beneficials. Spear-T, when used in a rotation with other materials and chemistries may be a tool in managing insecticide resistance when multiple applications are required for control of spotted wing drosophila (SWD). It has previously been shown to be effective against SWD in laboratory and field trials (Fanning et al., 2018).

METHODS
There were four replications of each treatment plus four untreated controls. Each plot measured 7 x 10-ft and was in a fruit-bearing wild blueberry field. There was a minimum 5-ft buffer around and between each plot.

There were four applications: 28 July, and 4, 10, and 17 August. All 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 30 psi and at slow walking speed. Walking speed for each application was regulated using a metronome. Materials, rates, and timing are in Table 1.

Efficacy of the insecticides was evaluated based on the number of SWD larvae collected from fruit samples one week after each application using the Salt Extraction Method (Van Timmeren et al., 2017). On each of four dates (4, 10, 17, and 24 August), a commercial blueberry rake was used to harvest one, ca. 6 oz sample (by volume) from each plot; an additional fruit sample was collected on 31 August, two weeks after the last (17 August) application. Each sample was weighed prior to being processed for larval infestation.

RESULTS
Data for SWD larvae were adjusted for sample weight and log(X+1) transformed to correct the normality and homoscedasticity and then differences were assessed using a one-way analysis of variance. Post-hoc tests were performed using an LSD test ($\alpha = 0.05$). The results are outlined in Table 1 and Figure 1.

The infestation data from this trial indicates that Spear-T in rotation with Mustang Maxx reduced infestation in comparison to the non-insecticidal control and slightly in comparison to the treatment

excluding Spear-T on the 24 August sample date. On the 31 August sample date, which was when SWD population increased significantly, the lowest infestation level was observed in the high rate of Spear-T/Mustang Maxx rotation (treatment 5), which had a mean infestation lower than all other treatments including the conventional rotation (treatment 2).

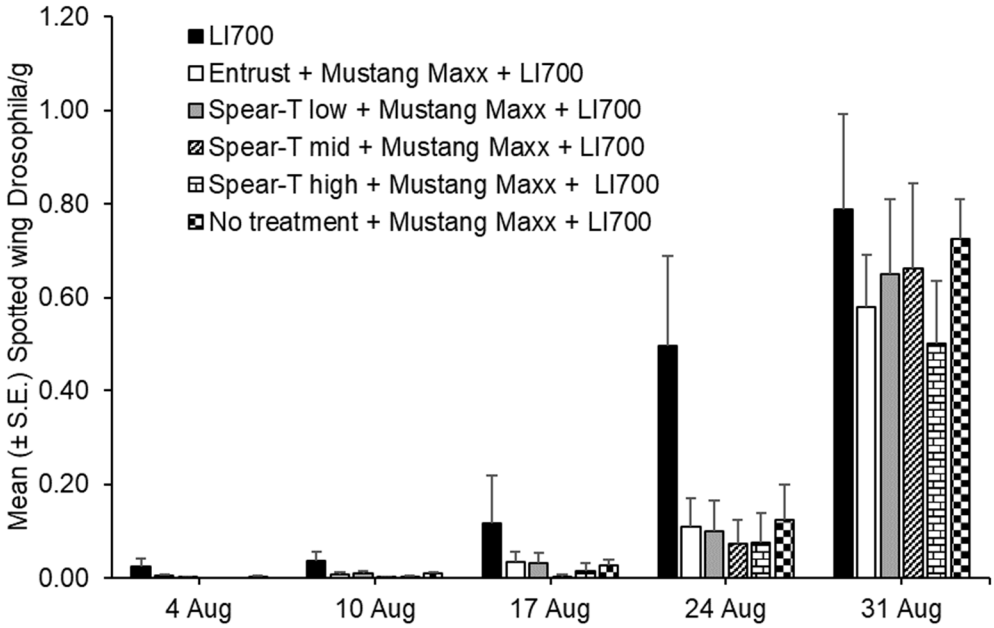

Figure 1. Mean (± S.E.) number of spotted wing drosophila larvae per gram of wild blueberry.

Table 1. Field control of spotted wing drosophila with insecticides, summary. Mean (± S.E.) number of larvae/gram extracted from blueberries treated with Spear-T and/or Entrust and Mustang Maxx.

				4-Aug				10-Aug				17-Aug				24-Aug				31-Aug			
Trt #	Material	Rate/acre	Timing[a]	Mean ± S.E.				Mean ± S.E.				Mean ± S.E.				Mean ± S.E.				Mean ± S.E.			
1	LI700		ABCD	0.025	±	0.017	a	0.038	±	0.020	a	0.117	±	0.103	a	0.496	±	0.19	a	0.787	±	0.20	a
2	Entrust	6	AC	0.006	±	0.004	ab	0.008	±	0.006	b	0.035	±	0.023	a	0.111	±	0.06	b	0.579	±	0.11	a
	Mustang Maxx	4	BD																				
	LI700		ABCD																				
3	Spear-T Low	64	AC	0.002	±	0.002	b	0.010	±	0.004	b	0.032	±	0.022	a	0.101	±	0.06	b	0.649	±	0.16	a
	Mustang Maxx	4	BD																				
	LI700		ABCD																				
4	Spear-T Mid	128	AC	0.000	±	0.000	b	0.002	±	0.002	b	0.004	±	0.004	a	0.073	±	0.05	b	0.663	±	0.18	a
	Mustang Maxx	4	BD																				
	LI700		ABCD																				
5	Spear-T High	256	AC	0.000	±	0.000	b	0.004	±	0.002	b	0.017	±	0.017	a	0.076	±	0.06	b	0.501	±	0.14	a
	Mustang Maxx	4	BD																				
	LI700		ABCD																				
6	No treatment		AC	0.004	±	0.002	ab	0.010	±	0.004	b	0.027	±	0.014	a	0.124	±	0.08	b	0.725	±	0.09	a
	Mustang Maxx	4	BD																				
	LI700		ABCD																				
Statistic				$F_{(5,18)} = 1.73$, $P=0.1778$				$F_{(5,18)} = 2.28$, $P=0.0897$				$F_{(5,18)} = 1.02$, $P=0.435$				$F_{(5,18)} = 2.59$, $P=0.062$				$F_{(5,18)} = 1.73$, $P=0.813$			

Means within columns followed by the same letter(s) are not significantly different; $P > 0.05$, LSD.
[a] Application number (date) A=1 (28 July), B=2 (4 Aug), C=3 (10 Aug), D=4 (17 Aug)
Data for SWD were adjusted for sample weight and Log(X+1) transformed for analysis; non-transformed means are shown in the table.

CONCLUSIONS AND RECOMMENDATIONS

This trial indicates that the Spear-T product could be a valuable rotational tool with products such as Mustang Maxx, especially as it is currently MRL exempt by the US EPA.

ACKNOWLEDGEMENTS

We thank Abigail Fisher and Hayley McGowan for their assistance with this study.

REFERENCES

Fanning, P. D., A. VanWoerkom, J.C. Wise, and R. Isaacs. (2018). Assessment of a commercial spider venom peptide against spotted-wing Drosophila and interaction with adjuvants. Journal of Pest Science, 91(4), 1279-1290.

Van Timmeren, S., L.M. Diepenbrock, M.A. Bertone, H.J. Burrack, and R. Isaacs. (2017). A filter method for improved monitoring of *Drosophila suzukii* (Diptera: Drosophilidae) larvae. Journal of Integrated Pest Management, 8(1), 23.

RESEARCH

INVESTIGATORS: P. Fanning and J. A. Collins

7. Field control of blueberry maggot fly and spotted wing drosophila on wild blueberry with insecticides

OBJECTIVE

The objective of this study was to evaluate the efficacy of three insecticides, Imidan® 70WP (phosmet), Assail® 70WP (acetamiprid), and NI-38 30.6SC (a new formulation of acetamiprid) against blueberry maggot fly and spotted wing drosophila.

LOCATION Jonesboro, ME
PROJECT TIMEFRAME July – August 2021

INTRODUCTION

Blueberry maggot fly (BMF) is the major insect pest of blueberries in Maine. And, the invasive spotted wing drosophila (SWD) has been a pest of berries and cherries across the United States for the past decade. Insecticides are an important management tool for both blueberry maggot fly and spotted wing drosophila. Annual field research trials continue to identify effective options for chemical control of these damaging insect pests.

METHODS

There were four replications of each treatment plus four untreated checks. Each plot measured 20 x 60-ft and was set along the edge of a fruit-bearing wild blueberry field. The first application was made when counts of BMF exceeded recommended treatment thresholds as determined by monitoring with baited, yellow, Pherocon® AM traps.

There were four applications: 23 July, and 3, 10, and 17 August. All 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 30 psi and at a slow walking speed. Walking speed for each application was regulated using a metronome.

Prespray populations of BMF were determined by distributing three baited, yellow, Pherocon AM traps within the study area**.** Traps were initially deployed on 23 June and checked at 3 to 8-day intervals. Adult BMF were counted and removed from the traps. Efficacy of the insecticides was evaluated based on the number of BMF and SWD larvae collected from fruit samples using the Salt Extraction Method (Van Timmeren et al., 2017). On each of five dates (2, 9, 16, 22, and 31 August), a commercial blueberry rake was used to harvest three, ca. 6 oz samples (by volume) from each plot.

RESULTS

Data for BMF and SWD larvae were log(X+1) transformed to correct the normality and homoscedasticity and then differences was assessed using a one-way analysis of variance. Post-hoc tests were performed using a Tukey HSD test (α = 0.05).

Weather experienced at the site can be seen in Figure 1.

Imidan 70WP provided excellent control of BMF across all sample dates (Table 1 and Fig. 2). Both Assail 70WP and NI-38 30.6SC formulations were also highly effective and comparable to each other in terms of their control. For SWD, pressure was low early in the trial, when pressure increased by the final sampling date (31 August), all treatments reduced the number of Drosophila in the fruit; although not significantly. On the 31 August sample date, Assail 70WP and NI-38 30.6SC formulations resulted in the lowest average infestation levels of the insecticides treatments and were comparable to each other (Table 2 and Fig. 3).

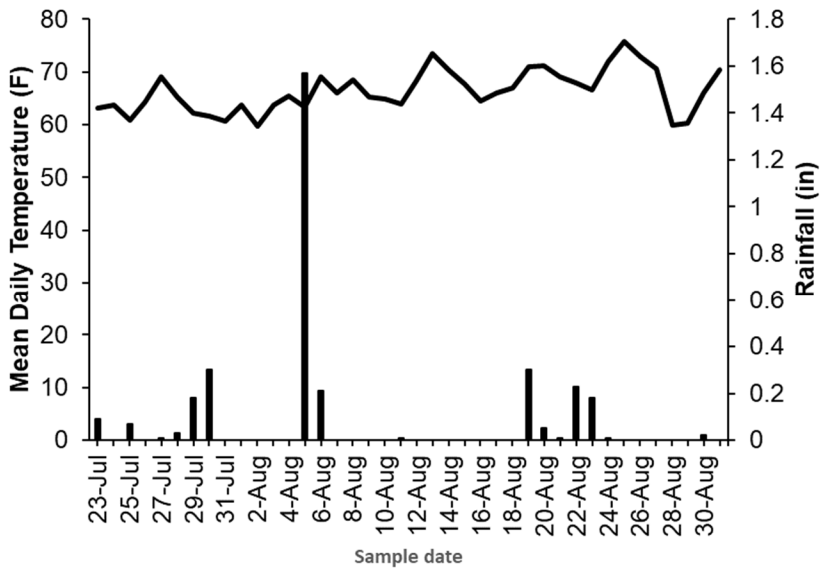

Figure 1. Weather conditions at Jonesboro during the trial. Bars indicate rainfall and the continuous line shows mean daily temperature.

Table 1. Field control of blueberry maggot fly with insecticides, summary. Average (± S.E.) number of larvae extracted from blueberries treated with three different insecticides.

Treatment	Rate/	2-Aug[c]				9-Aug				16-Aug				22-Aug				31-Aug			
Formulation	**acre**	**Avg**		**S.E**		**Avg**		**S.E**		**Avg**		**S.E**		**Avg**		**S.E**		**Avg**		**S.E**	
UTC		3.0	±	1.00	a	6.5	±	0.96	a	0.3	±	0.25	a	1.0	±	0.71	a	1.0	±	0.71	a
Imidan 70WP	22.3[a]	0.5	±	0.50	b	0.0	±	0.00	b	0.0	±	0.00	a	0.5	±	0.50	a	0.8	±	0.48	a
NI-38 30.6SC	2.3[b]	0.5	±	0.50	b	2.0	±	1.35	ab	0.5	±	0.50	a	0.3	±	0.25	a	0.0	±	0.00	a
Assail 70WP	4.5[a]	0.5	±	0.29	ab	2.8	±	2.14	ab	1.0	±	0.58	a	0.0	±	0.00	a	0.5	±	0.50	a
Statistic		$F_{(3,15)} = 4.361$, $P = 0.027$				$F_{(3,15)} = 5.492$, $P = 0.013$				$F_{(3,15)} = 1.026$, $P = 0.415$				$F_{(3,15)} = 0.875$, $P = 0.480$				$F_{(3,15)} = 0.825$, $P = 0.505$			

Table 2. Field control of spotted wing drosophila with insecticides, summary. Average (± S.E.) number of larvae extracted from blueberries treated with three different insecticides.

Treatment	Rate/	2-Aug[c]				9-Aug				16-Aug				22-Aug				31-Aug			
Formulation	**acre**	**Avg**		**S.E**		**Avg**		**S.E**		**Avg**		**S.E**		**Avg**		**S.E**		**Avg**		**S.E**	
UTC		0.0	±	0.00	a	0.8	±	0.48	a	1.0	±	0.41	a	7.8	±	2.69	a	139.8	±	43.65	a
Imidan 70WP	22.3[a]	0.0	±	0.00	a	0.3	±	0.25	a	1.0	±	0.71	a	1.0	±	0.41	a	66.3	±	41.21	a
NI-38 30.6SC	4.5[b]	0.0	±	0.00	a	2.5	±	1.85	a	0.8	±	0.48	a	3.8	±	2.59	a	34.5	±	7.96	a
Assail 70WP	2.3[a]	1.3	±	0.95	a	0.5	±	0.50	a	1.3	±	0.95	a	3.3	±	2.63	a	41.0	±	12.70	a
Statistic		$F_{(3,15)} = 2.278$, $P = 0.131$				$F_{(3,15)} = 1.035$, $P = 0.411$				$F_{(3,15)} = 0.058$, $P = 0.980$				$F_{(3,15)} = 1.358$, $P = 0.302$				$F_{(3,15)} = 1.503$, $P = 0.263$			

Means within columns followed by the same letter(s) are not significantly different; $P > 0.05$, Tukey HSD.
[a] oz (wt) product per acre.
[b] oz product per acre.
[c] Log(X+1) transformed data used for analysis, non-transformed means shown in the table.

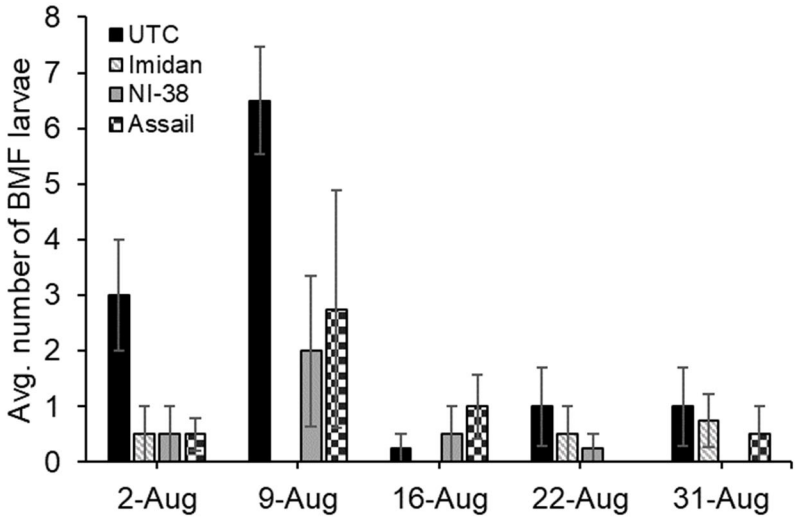

Figure 2. Average (± S.E.) number of blueberry maggot fly larvae extracted from blueberries treated with three different insecticides.

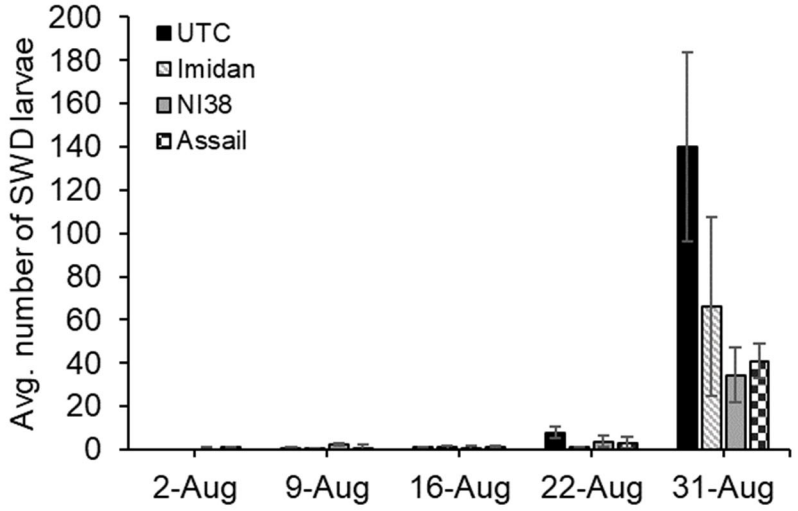

Figure 3. Average (± S.E.) number of spotted wing drosophila larvae extracted from blueberries treated with three different insecticides.

CONCLUSIONS AND RECOMMENDATIONS

The NI-38 30.6SC formulation of acetamiprid is not currently registered for use on wild blueberry. However, once registered it should provide another effective option for growers for control of blueberry maggot fly. Imidan 70WP and Assail 70WP continue to offer good alternatives.

NEXT STEPS

- Research into new control methods should continue in order to provide growers with the most safe, effective, and economical alternatives for insect control.

ACKNOWLEDGEMENTS

We thank Ben Johnson, Abigail Fisher, and Hayley McGowan for their assistance with this study.

REFERENCES
Van Timmeren, S., L.M. Diepenbrock, M.A. Bertone, H.J. Burrack, and R. Isaacs. (2017). A filter method for improved monitoring of *Drosophila suzukii* (Diptera: Drosophilidae) larvae. Journal of Integrated Pest Management, 8(1), 23.

RESEARCH

INVESTIGATORS: P. Fanning and J. A. Collins

8. Emergence of red-striped fireworm in Maine wild blueberry

OBJECTIVE
To develop an emergence model to predict the emergence of red-striped fireworm.

LOCATION T19 MD BPP, ME
PROJECT TIMEFRAME May - July 2021

METHODS
On 6 May, ten emergence cages (Fig. 1a) were deployed in a fruit-bearing, commercially managed wild blueberry field in Washington County, ME that had been heavily infested with red-striped fireworm (RSFW) as evidenced by the presence of leaf ties (Fig. 1b). The cages were checked periodically from 14 May to 19 June and adult RSFW were counted and removed. On each of two sample dates (13 and 29 July), larval infestation was assessed by counting the number of leaf ties in each of 20, m^2 quadrats.


Figure 1a (left). Emergence cage. **Figure 1b** (right). Example of leaf tie.

HOBO® TidbiT v2 data loggers (Onset Computer Corp, Bourne, MA, USA) were placed in four of the cages to monitor air temperature at ground level. An additional HOBO was deployed on 16 April to measure air temperatures at the site. Using these data, as well as information from a nearby weather station, 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]. Degree-days at base = 40ºF were accumulated from March 1. Cumulative percent adult emergence as a function of degree days with a threshold temperature of 40ºF was modeled with 3 parameter log-logistic model, the equation is below, where *a* is the growth rate, *b* is the inflection point and *c* is the asymptote.

$$Y = \frac{c}{((1 + \exp(-a \cdot (X - b)))}$$

RESULTS

First adult emergence in the cages was observed on 466.5 GDD (17 May) and adults continued to emerge until 1976.8 GDD (19 July); first larval infestation in the field (as evidenced by leaf ties) was noted on 1859.2 GDD (15 July) (Fig. 2). Delegate WG® was applied by the grower on 22 July; there were no applications of insecticides prior to that date. The fitted model had an $r^2 = 0.995$ (i.e., 99.5 % of the variance in cumulative adult emergence is described by air temperature degree-days (threshold of 40°F and degree-days accumulated from 1 March).

$$Cumulative.Adult\ Emergence = \frac{18.5}{((1 + \exp(-0.0066 \cdot (GDD - 1020)))}$$

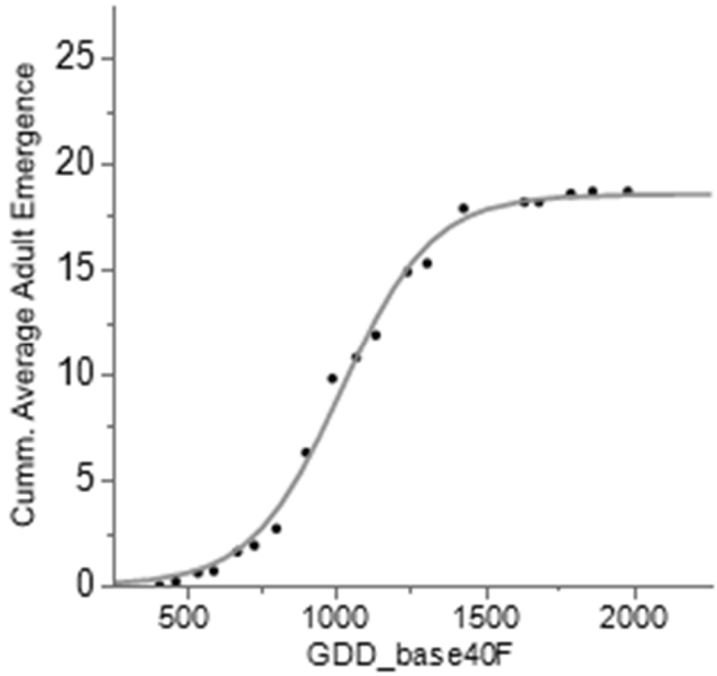

Figure 2. Cumulative emergence of red-striped fireworm adults.

CONCLUSIONS AND RECOMMENDATIONS

The emergence of RSFW adults was closely related to the ambient air temperature, and the fitted model could be used to predict the species emergence based on ambient air temperature. However, this is only based on a single year's data collection, and additional years' data will be needed to improve the model. Infestation by RSFW larvae did not closely follow adult emergence, with the first leaf ties recorded a full two months after the first adult emergence.

NEXT STEPS

- We will aim to collect additional years' data as infestation locations become available.

ACKNOWLEDGEMENTS

We thank Ben Johnson, Abigail Fisher, and Hayley McGowan for their assistance with this study.

RESEARCH

INVESTIGATOR(S): B. Gumbrewicz and L. Calderwood

9. Fertility Effects on Blueberry Gall Midge (*Dasineura oxycoccana* (Johnson) (Diptera: Cecidomyiidae)) in Wild Blueberry

OBJECTIVE(S)

- Determine effects of diammonium phosphate (DAP) application on gall midge density
- Determine combined effects of DAP application with galling on wild blueberry

LOCATION(S): Conventional fields in Washington, ME and UMaine Blueberry Hill Farm Experiment Station in Jonesboro, ME
PROJECT TIMEFRAME: May 2020 – September 2021

INTRODUCTION
Blueberry gall midge, *Dasineura oxycoccana* (Johnson) (Diptera: Cecidomyiidae), is a prevalent insect pest of both cranberry and wild blueberry. It was reported as one of the most important insect pests of blueberries grown in the southern US states (Dernisky et al., 2005) and has increased in population across wild blueberry fields in Maine since its discovery in this state in 2003 (Collins and Drummond, 2019). Blueberry gall midge reduces the number of buds and flowers per stem, which can amount to 50% yield loss on any one infested stem (Collins and Drummond, 2019; Yarborough et al., 2017).

Adults resemble small, mosquito-like flies and live up to two days in the blueberry canopy (Collins and Drummond, 2017). Females deposit their eggs on the tips of blueberry stems where the youngest vegetation grows. The larvae feed on this new growth and cause leaves to curl, forming leaf galls on the terminal ends of the stems (Figure 1). Colorless eggs develop through three instars marked by color changes from white to yellow to orange (Figure 2). Other symptoms that growers might identify as blueberry gall midge are blackened tips of stems and curled or dimpled leaves around unravelling galls (Figures 3). This is not to be confused with tip dieback or blueberry thrips.

Figure 1. New leaf gall in wild blueberry.

Figure 2. Late (orange) instar larva.

Figure 3. Uncurled gall with dead tip of stem

Climate change already impacts wild blueberry systems and the Northeast is warming faster than the rest of the United States (Fernandez et al., 2020) with the most significant temperature increases occurring in the Downeast region of Maine (Tasnim et al., 2021). Rising temperatures can significantly impact wild blueberry growth and development, resulting in major changes to the wild blueberry nutrient

economy and therefore grower fertilization regimes (Tasnim et al., 2020; 2021). These changes may in turn have impacts on pests responding to the wild blueberry nutrient environment.

Altering fertility regimes in the environment can significantly impact gall-inducing insect populations. Increasing fertility in wild blueberry is thought to increase pest presence by providing more habitat for the insect (Reekie et al., 2009). Yarborough et al. (2017) observed greater gall midge abundance in areas of higher boron and phosphorus levels. This suggests that leaf nutrient profiles impact gall midge levels. This pest is already known to respond to excess nitrogen in cranberry, with a preference for laying eggs on newer tissues (Leduc and Turcotte, 2004).

Diammonium phosphate (DAP [18-46-0]) is the traditional conventional fertilizer used by wild blueberry growers with the goal of adding nitrogen and phosphorus to the crop for better yields. Increasing nutrient inputs, primarily through nitrogen and phosphorus levels, may therefore increase gall midge infestations in Maine wild blueberry fields, but more research is needed to validate these hypotheses. It is valuable to know whether adding specific nutrients to this crop attracts blueberry gall midge and to what extent damage is caused if it does. This will allow growers to determine whether they want to budget for fertilizer costs or other expenses. This study aims to gain more insight on how nutrient management could be used to manage gall midge activity in wild blueberry systems as part of the wild blueberry integrated pest management plan.

METHODS

This study was located at two conventional field sites, one in Jonesboro and one in Washington, ME. Jonesboro was in the crop year and Washington was in the prune year when this study began in 2020. The experiment was a randomized complete block split-plot design with eight replicates or 'blocks' of each treatment (Figure 4). The whole plot factor was blueberry gall midge level, either present or absent. A "present" gall treatment only had stems <u>with galls</u> flagged for repeated measures. An "absent" gall treatment only had stems <u>without galls</u> flagged. The split-plot factor was fertility level, either fertilized with DAP or unfertilized. Therefore, flagged stems could be one of four treatments: not galled and not fertilized, not galled and fertilized, galled and not fertilized or galled and fertilized. Not galled and not fertilized stems represented the control treatment. Fertilized plots had 493.18 kg/ha DAP applied on June 10, 2020. Application rate was based on low nitrogen and phosphorus levels (1.17-1.25% N, 0.102-0.103% P) reported in a 2018 plant tissue report for the Jonesboro site containing UMaine Extension recommendations. This was the highest recommended rate for plant tissue with a leaf nitrogen level below 1.76% and a leaf phosphorus level below 0.111% (Yarborough and Smagula, 2013).

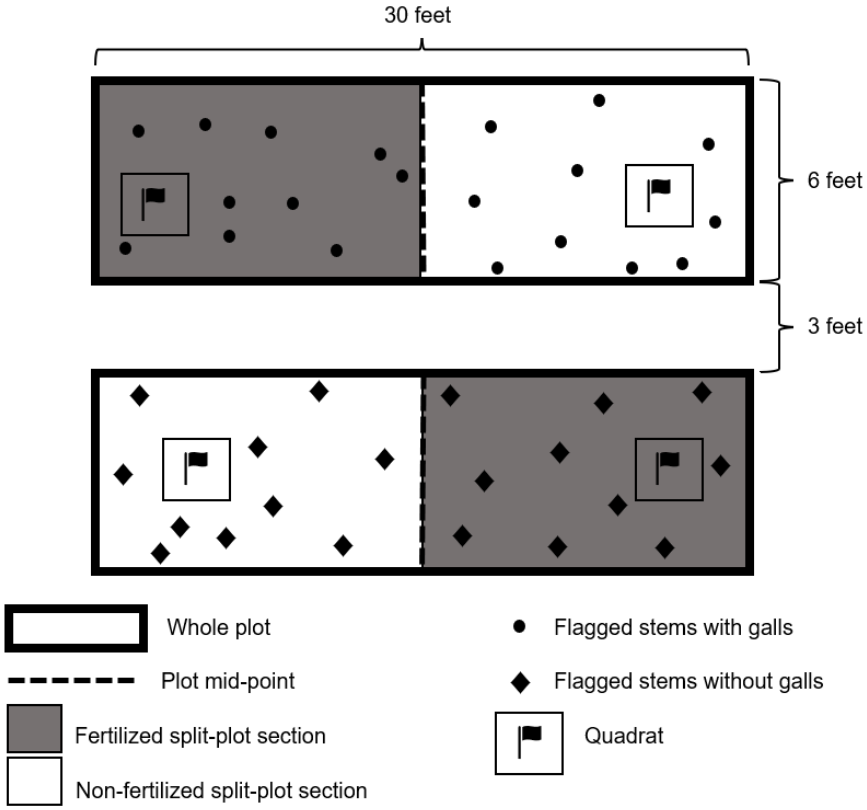

Figure 4. Example of one replicate for randomized complete block split-plot design.

Data Collection

Leaf samples were collected from each plot for foliar analysis on July 7, 2021, in Jonesboro and July 6, 2021, in Washington. A soil sample was collected from each split-plot on July 27, 2021, in Washington and on August 10, 2021, in Jonesboro. Soil and leaf samples were analyzed by the University of Maine Soil Testing Service in Orono, ME.

Prune & Crop Year Measures

Gall density was measured by counting the number of stems with leaf galls in two 0.37-m^2 permanent quadrats per plot, one in each split-plot section. Only newly formed galls were counted (Figure 1). Gall density was recorded at the Jonesboro site in 2020 on June 17; July 2, 7, 22, 28; and August 12. It was recorded in 2021 on June 2, 9, 16, 25, 30; July 7, 14, 21, and August 3. Gall midge density was recorded at the Washington site in 2020 on June 16; July 1, 10, 23, 30; and August 21. It was recorded in 2021 on June 1, 10, 17, 24, and July 1, 6, 13, 19, 27. Recording density stopped when no new galls were detected during the prune year or when a field was harvested during the crop year.

Blueberry cover was measured on the same dates as gall density based using a 0-6 scale ranking system representing even intervals of 100%, where: 0 = not present, 1 = ≤1%-16.67%, 2 = >16.67%-33.33%, 3 = >33.33%-50%, 4 = >50%-66.67%, 5 = >66.67%-83.33% and 6 = >83.33%-100%.

Ten stems per split-plot were flagged for repeated measures throughout the season. Leaf chlorophyll content was measured monthly as an indicator of leaf nitrogen using a SPAD Chlorophyll Meter (SPAD 502; Minolta Corp, Osaka, Japan) because SPAD measurements and leaf nitrogen content have been shown to be highly correlated (Xiong et al., 2015). One measurement was taken on each of two leaves per stem, one on the lowermost and one on the uppermost portion of the stem. SPAD measurements were taken at the Jonesboro site in 2020 on June 17, July 9, and August 12 and in 2021 on June 16 and July 23. SPAD measurements were taken at the Washington site in 2020 on June 18, July 10, and August 21 and in 2021 on June 17 and July 19.

Prune Year Measures

Stem heights, number of buds per stem, and stem density were recorded at both field sites during the prune year. Stem heights and bud numbers were recorded for each of the twenty flagged stems per plot. The number of stems in each existing quadrat was counted to record stem density. Stem heights and stem density were recorded in Jonesboro on August 3, 2021, and bud numbers were recorded on August 24, 2021. All three measurements were recorded in Washington on September 4, 2020.

Crop Year Measures

Berry count and weight data was collected at the end of the crop cycle in Jonesboro on August 12 and 13, 2020 and in Washington on July 27, 2021. The number of green, red, blue and diseased/overripe berries were counted on each stem. Only healthy blue berries were considered marketable, hand-picked, and weighed per stem. Berry weight per stem was used to extrapolate potential yield per hectare based on an approximate stem density of 500 stems/m^2. Berries in each quadrat were also hand raked and weighed. Because the Jonesboro site was in the crop year when the experiment started in 2020, new stems were flagged for prune year measures in 2021. Harvest data is not presented for the Jonesboro site due to severe leaf spot disease damage and dry weather conditions. Because of this, additional measures were included at the Washington site during its crop year 2021. The number of open flowers per stem was recorded in Washington on May 21, 2021. The numbers of green, red, and blue fruit per stem were recorded in Washington before and during fruit ripening on June 17 and July 6, 2021, respectively. The crop of 2021 was nearly two weeks ahead of the 2020 crop, resulting in early harvest and therefore any remaining August measures were not collected.

Data Analysis

The effects of fertilizer application on gall midge density, as well as gall midge presence on plant growth (chlorophyll content, foliar nutrients, blueberry cover, stem density, stem height, bud development, yield) were statistically analyzed using JMP (JMP® Pro, Version 15.2.0) at the 0.05 level of significance. One-way analysis of variance and two sample t-tests were used to compare treatments for data collected only once throughout the study, including foliar nutrients, end of prune year stem density, stem height, bud number per stem, flowers per stem, berries per stem, and soil nutrients. Data that did not follow a normal response distribution were analyzed through randomization tests to confirm the results of analyses of variance that did not meet standard assumptions of normality or equal variances.

All repeated measures (gall density, blueberry cover, chlorophyll content) were analyzed using a generalized linear mixed model (GLMM). In the GLMM, count data were modeled with a Poisson distribution and log link. Proportion (ranked) data were modeled with a binomial distribution and logit link. For model effects, 'block' was used as a random effect and all other variables were considered fixed. For analysis of gall density and blueberry cover, fixed effects included date and fertility level. For analysis of chlorophyll content, fixed effects included date, gall level, and fertility level. All fixed-by-fixed interactions were included in all models. Treatment effects were separated by Tukey's Highly Significant Differences test.

Linear regression was used to model the relationship between foliar percent nitrogen and phosphorus with mean gall density (number of galls/stem/m^2). This was used to test the hypothesis that gall density increases with increasing foliar nutrient levels. These two nutrients were chosen since they are the major nutrients in the fertilizer used in this study and because foliar nutrient levels are the primary method used to determine fertilizer needs in wild blueberry (Yarborough and Smagula, 2013) as opposed to soil nutrient levels. Both Jonesboro and Washington sites were pooled to determine the mean gall density. Only data from 2021 was used to perform the regression, the same year the foliar tests were taken.

RESULTS

There were no significant differences in soil nitrogen or phosphorus levels at the Jonesboro site. Both soil nitrogen and phosphorus levels were significantly greater in plots where DAP was applied (mean = 4.06 mg NH_4/kg, 21.34 kg P/ha) than in plots where DAP was not applied (3.38 mg NH_4/kg, 16.86 kg P/ha) at the Washington site. Fertilized stems with galls here had the highest mean foliar percent nitrogen (1.36%) compared to all other treatments.

In 2020, overall mean gall density was not significantly different in fertilized and unfertilized plots at the Jonesboro site (Figure 5). Gall density was significantly greater in fertilized plots (mean = 11.87 galls) than in unfertilized plots (mean = 4.62 galls) at the Washington site (Figure 6). There were 87.93% more galls in Washington across the 2020 season, on average, in plots where DAP was applied compared to those where it was not. In 2021, gall density was significantly greater in fertilized plots at both sites (Figures 5 and 6). There were approximately 25.79% more galls at the Jonesboro site and 121.71% more galls at the Washington site across the 2021 season in fertilized plots than in unfertilized plots.

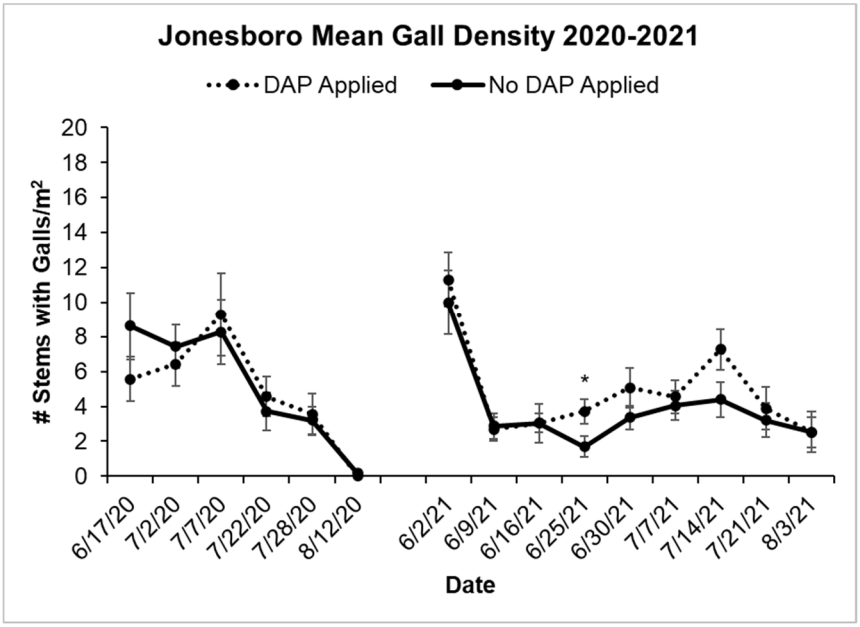

Figure 5. Mean gall density by date at the Jonesboro field sites during the 2020 wild blueberry crop cycle and 2021 wild blueberry prune cycle. Error bars represent the SEM (n = 32). *Significant at the 0.05 level of significance.

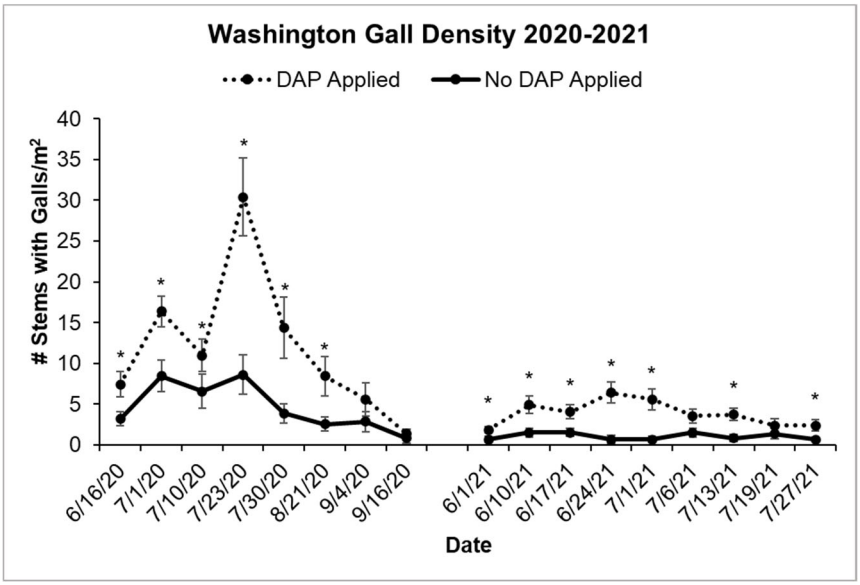

Figure 6. Mean gall density by date at the Washington field site during the 2020 wild blueberry prune cycle and 2021 wild blueberry crop cycle. Error bars represent the SEM (n = 32). *Significant at the 0.05 level of significance.

The effects of foliar percent nitrogen and phosphorus on gall density were significant in the regression analysis (Figure 7). We conclude that there was a significant trend showing the number of galled stems/m^2 increased with increasing foliar percent nitrogen and phosphorus.

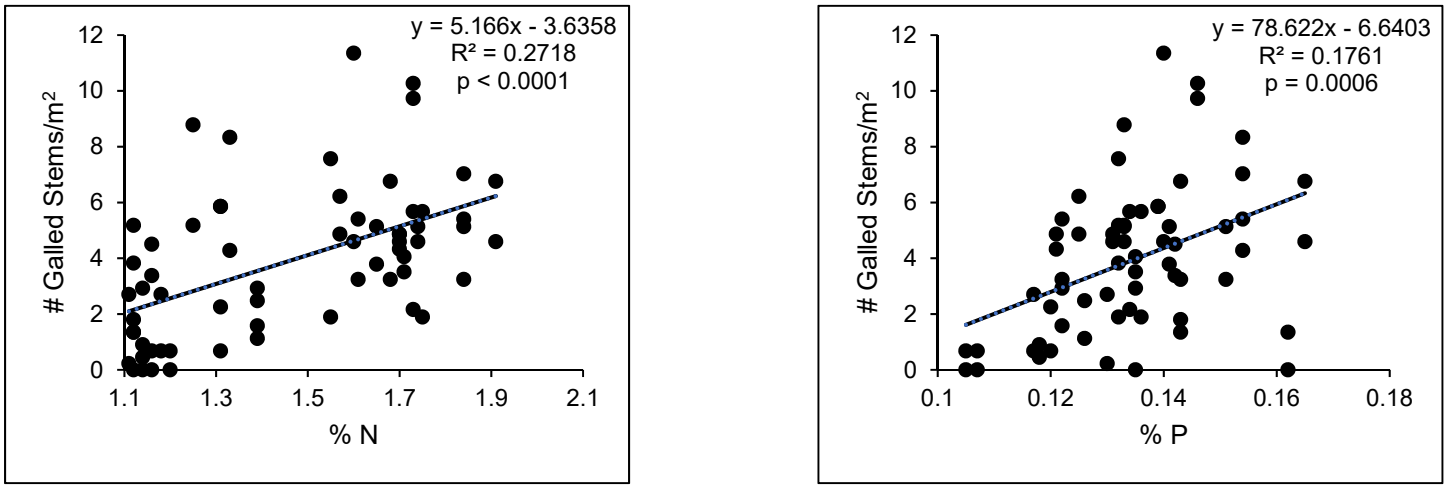

Figure 7. Gall density increase as it relates to percent foliar nitrogen (left) and percent foliar phosphorus (right). The solid lines represent the least square regression.

No significant differences in stem density were detected at either site between fertilized and unfertilized plots. In Jonesboro, stems without galls were 15.8% taller than stems with galls. Fertilized stems without galls had a greater mean number of buds per stem than unfertilized stems with galls. In Washington, fertilized stems without galls had the greatest mean stem height (mean = 19.35) and number of buds per stem (mean = 3.39 buds). Unfertilized stems with galls had significantly shorter stems and fewer buds per stem compared to fertilized stems without galls at both sites.

At the Washington site, fertilized stems without galls had the greatest mean number of flowers per stem. Before ripening and during ripening, fertilized stems without galls had significantly more green berries per stem than all other treatment combinations. During ripening, fertilized stems without galls also had significantly more ripe berries per stem than treatments without DAP applied. At harvest, galled stems had significantly fewer green berries (63.53%) and significantly fewer ripe berries (37.03%) in Washington. There was also significantly more blue fruit per fertilized stem compared to stems without DAP applied (59.98% more). Blue fruit weight per stem was significantly heavier by 71.54% for fertilized stems than unfertilized stems. No significant differences were detected for the number of red berries per stem at any stage of development recorded. All fertilized stems had significantly greater yields than stems without DAP applied, regardless of gall presence (Figure 8).

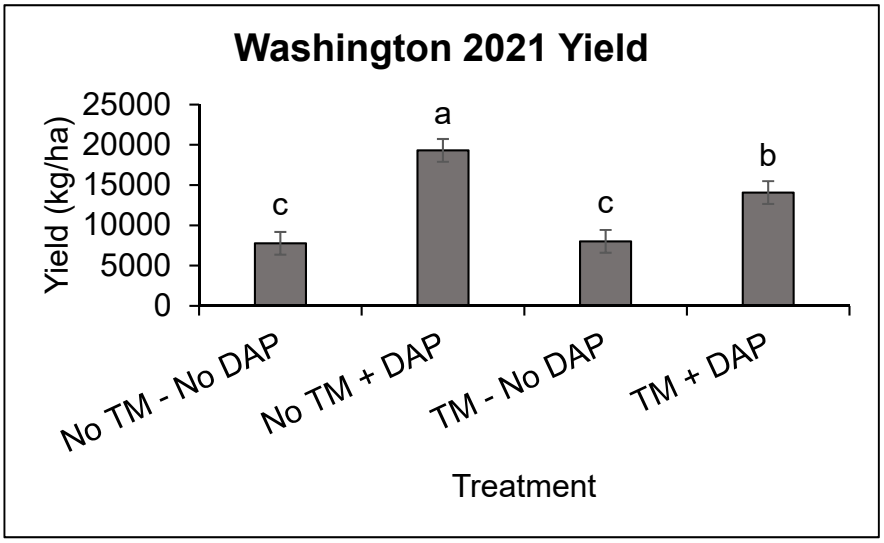

Figure 8. Mean total yield by treatment at the Washington field site during the 2021 wild blueberry crop cycle. Error bars represent the SEM (n = 320). Letters indicate pairwise differences.

Blueberry cover was significantly greater in fertilized plots at the Washington site from mid-July through August 2020. DAP application did not have a significant effect on blueberry cover at the Jonesboro site. Blueberry cover at this site greatly decreased by August 2020 due to leaf spot disease defoliation. In 2021, blueberry cover was significantly greater in plots where DAP was applied throughout June 2021 in Jonesboro and on July 19 and 27, 2021 in Washington. Significant differences were primarily detected during the prune year at both sites.

By July 2020 in Jonesboro and August 2020 in Washington, treatments with DAP applied had significantly greater chlorophyll content than treatments without DAP applied, regardless of gall presence or absence. In 2021, leaf chlorophyll content was significantly greater in stems without galls than stems with galls at the Jonesboro site. In Washington, treatments without DAP applied had significantly lower chlorophyll content than treatments with DAP applied in June. By July, stems without galls or DAP applied had significantly lower chlorophyll content than other treatment combinations.

DISCUSSION

Blueberry gall midge density was significantly greater where a high rate of DAP fertilizer was applied during both years at the Washington site and during the prune year at the Jonesboro site. This is consistent with previous findings where blueberry gall midge was detected in greater densities in prune than in crop fields, most likely due to the requirement that many gall-inducing insects utilize young leaf tissue for gall formation (Collins and Drummond, 2019; Rohfritsch,1992). The Jonesboro site was also greatly affected by leaf spot disease, which caused high rates of defoliation and may have also impacted the non-significance of our results at that location. Regression analysis indicated a clear trend that gall density increased with increasing foliar nitrogen and phosphorus. Galling reduced stem height and bud numbers at both sites as well as the number of flowers, green, and blue fruit per stem at the Washington site. These results agree with previous research by Collins and Drummond (2019).

DAP applications improved blueberry plant growth as expected (Percival et al., 2004; Yarborough and Smagula, 2013), resulting in more buds per stem at both sites, and taller stems and greater number and weight of ripe berries per stem at the Washington site, despite increases in habitat availability (blueberry cover) and gall density. At the Washington site, yields in treatments where DAP was applied

were significantly greater than in treatments without DAP applied even when galling was present. Applying DAP may therefore help compensate for the impact galls have on plant growth despite also providing more habitat and, in turn, more opportunity for gall midges residing in the blueberry canopy to feed and reproduce.

It is important for farmers to consider whether DAP is necessary for crop production based on the level of gall midge infestation in their fields and their production goals. Reducing the amount of DAP applied may prevent increasing pest presence and environmental damage, such as soil nutrient leaching from fertilizer overuse. Our study suggests that blueberry gall midge is more attracted to areas where a high rate of DAP fertilizer has been applied, however, yields were still greater where this rate of fertilizer was used.

CURRENT RECOMMENDATIONS

Growers should continue monitoring fields for gall midge infestations and only apply nutrient inputs based on recent foliar analysis. Growers might also consider maintaining budgets that include fertilizer costs if blueberry gall midge is present in their fields rather than forgoing nutrient inputs when market prices are low.

NEXT STEPS

- Measure blueberry gall midge preference for specific nutrients.
- Evaluate effect of fertilizer application on blueberry gall midge fitness.
- Monitor effect of galling on wild blueberry nutrient uptake and development rate.
- Determine threshold at which density of galls outweigh benefits of fertilizer application and vice versa using multiple fertilizer rates.

ACKNOWLEDGEMENTS

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PLANT DISEASE

EXTENSION

INVESTIGATORS: S. Annis and J. Schwab

1. Research into control of leaf spot diseases 2021

OBJECTIVE
Improve control of leaf spot diseases on lowbush blueberry including Sphaerulina leaf spot (*Sphaerulina vaccinii*), powdery mildew (*Erysiphe vaccinii*), and leaf rust (*Thekopsora minima*) using a field trial.

LOCATIONS: Multiple wild blueberry fields around Maine
PROJECT TIMEFRAME: June - October 2021

METHODS
Survey of weather and levels of disease in wild blueberry fields
Fifteen fields with weather stations were rated for leaf loss and leaf spot diseases between September 28 and 29, 2021. Four plots of 0.25 m^2 were randomly selected and rated by one surveyor visually estimating percentages of blueberry coverage, blueberry leaf loss, and blueberry leaf area with the following leaf spot diseases: Sphaerulina leaf spot (formerly Septoria leaf spot), powdery mildew, and leaf rust. Fall disease ratings were averaged across the four sampling plots within a field.

Spore dispersal and leaf spot diseases
On April 15, 2021, a spore trap was placed in a crop field at Blueberry Hill Research Farm (BBHF) in Jonesboro, ME and on April 19, another spore trap was placed in a prune field near Deblois (Long Pond). We collected spore trap tapes weekly until October 14, 2021. Spore trap tapes were cut in half; half was frozen for future DNA work, and the other half was mounted on glass slides for examining for spores.

Both fields with spore traps were assessed for leaf diseases starting on July 15, 2021, and assessed weekly, except on September 9 due to heavy rain, until October 14, 2021. Four sampling plots of 0.25 m^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: Sphaerulina leaf spot, powdery mildew, and leaf rust. Plots were averaged for each field

Fungicide efficacy trial
A randomized complete block experiment was established in a vegetative lowbush blueberry field where high levels of leaf spots had been previously reported at the Blueberry Hill Research Farm in Jonesboro, Maine. Fungicides (Table 1) were randomly assigned to 6ft x 30ft plots with a 3ft buffer lane between each plot and replicated in eight blocks. Plots were treated on June 9 and June 16, 2021. Some fungicides were only applied at one timing and others at both timings. Fungicides were applied at volumes equivalent to 20 gallons per acre at 35 psi with a CO_2 backpack sprayer equipped with a 4-nozzle boom, 8002VS T Jet tips, and 50-mesh screens applied. Control plots received no spray applications.

Table 1. Fungicides tested in 2021 for efficacy in control of leaf spots in lowbush blueberry.

Treatment	Material	Appl. timing	Appl. Rate	Company	EPA Reg. Number	Registered on Wild Blueberry
Theia (and Dyne-Amic 0.375%v/v)	*Bacillus subtilis* strain AFS032321	Both	3.0 lb/acre	AgBiome	None	Not yet
Howler (and Dyne-Amic 0.375%v/v)	*Pseudomonas chlororaphis* strain AFS009	Both	5.0 lb/acre	AgBiome	91197-3-92488	Yes
Ecoswing (and Capsil)	*Swinglea glutinosa*	Both	32.0 lb/acre	Gowan	10163-357	Yes
Fungicide 1 (FRAC 7/3)	Not available	Early or Late	10.0 fl.oz/acre	Bayer	Not available	Yes
Proline (FRAC 3)	Prothioconazole	Early or Late	6.0 fl.oz/acre	Bayer	264-825	Yes

Disease symptoms and leaf loss were rated three times: July 12, August 9, and September 13, 2021. A rope with 20 evenly spaced markings was stretched on a transect through each plot, and the stem closest to each marking was cut, bagged, and put on ice in a cooler for transport and stored in a refrigerator until rated. Each stem was rated for the leaf cover with disease symptoms of Sphaerulina leaf spot, leaf rust, or powdery mildew, and leaves and bare nodes were counted for leaf loss. Percent data were transformed with arcsine square root method. Data were analyzed by plot averages using mixed model procedures (PROC GLIMMIX, SAS, Statistical Analysis Software - SAS Cary, NC). Least square means were used to determine specific differences among treatments ($\alpha = 0.05$).

RESULTS
Survey of leaf spot diseases in wild blueberry fields
There were higher than expected levels of Sphaerulina leaf spot found in September in many wild blueberry fields (Figure 1). This may be due to warmer weather allowing the leaves infected with this disease to persist on the plant or may be due to the difficulty of distinguishing early leaf rust lesions

that are not sporulating with older Sphaerulina leaf spots. Fields with high levels of possible Sphaerulina were also fields with a higher presence of leaf rust, except for field 1 which had a high level of Sphaerulina-like leaf spots and little leaf rust present. A new graduate student, Zoe Colwell, who started in September 2021 will be examining the timing and persistence of Sphaerulina and leaf rust infections using molecular methods during her MS in the next two years.

There were low levels of powdery mildew and leaf rust in most fields. Overall levels of leaf loss at the end of September (Figure 2) were similar to levels found in 2020 though individual fields varied in their levels from the previous year. Leaf loss and disease levels will be compared to fungicide applications and weather conditions in individual fields.

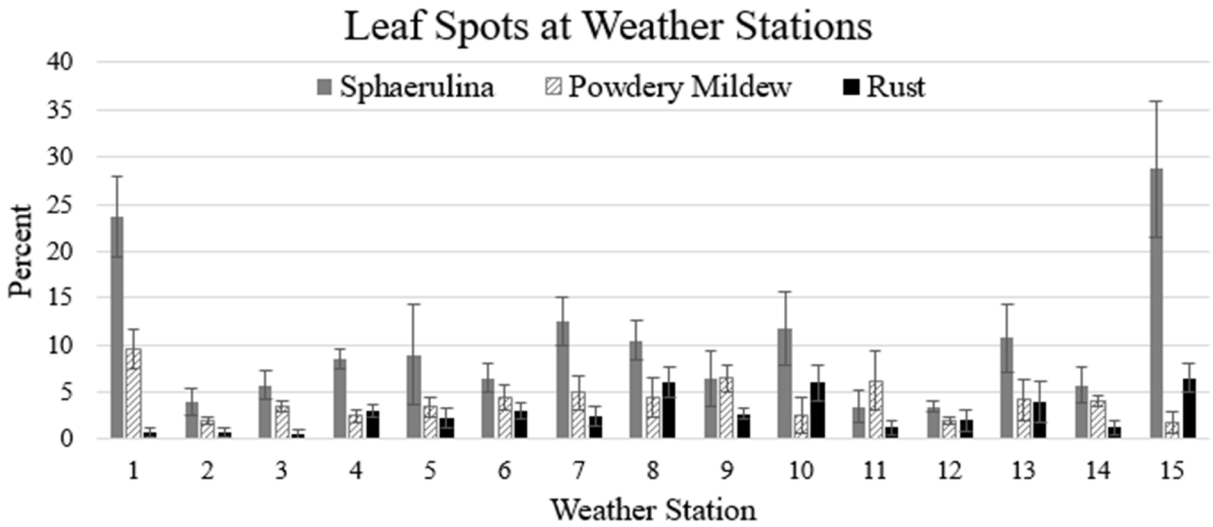

Figure 1. Estimated percentage of leaf area with Sphaerulina leaf spot (solid grey), powdery mildew (striped), and rust (solid black) symptoms at each weather station. Bars indicate the standard error of the mean of four samples at each station.

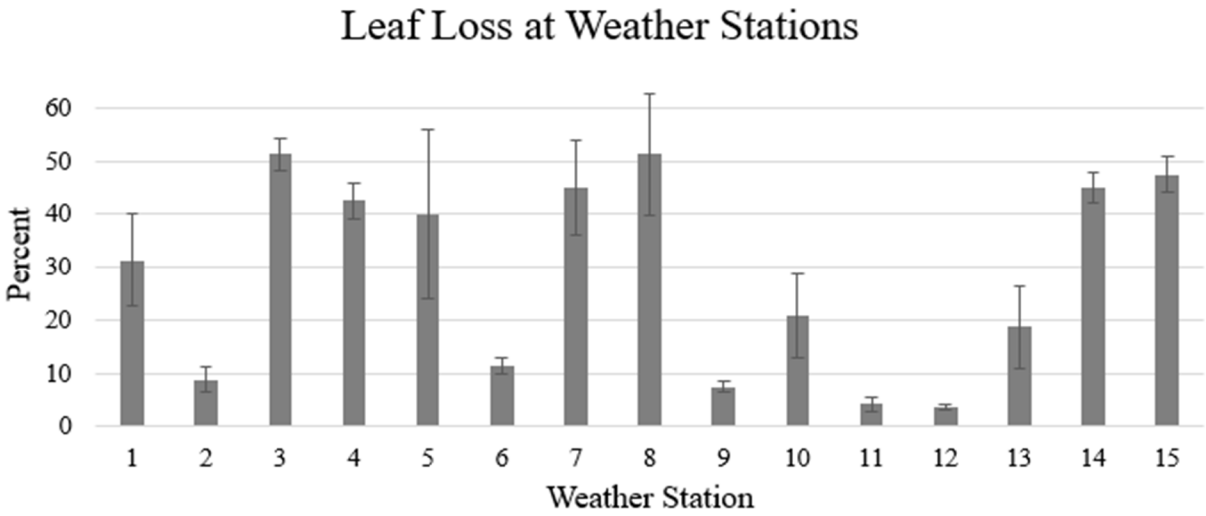

Figure 2. Leaf loss at weather stations. Bars indicate standard error of the mean of four samples at each station

Spore dispersal and leaf spot diseases

The level of leaf spot diseases and leaf loss at the Long Pond field was very low compared to the BBHF field (Figure 3). At BBHF, there was some leaf loss in the middle of July through August which may have been from normal leaf drop due to shading of lower leaves by upper leaves and Sphaerulina leaf spot infections. There was a spike in leaf loss in the middle of September in the BBHF field that was probably associated with leaf spot diseases. The level of leaf loss in the Long Pond field did not increase as drastically as in the BBHF field. By the middle of October, only 40% of leaves were lost in the Long Pond field but 60% of leaves had been lost in the BBHF field. Levels of leaf rust were low in both fields, but unsporulating leaf rust lesions may have been mistaken for Sphaerulina leaf spots. Spore trap tapes were collected and will have their DNA extracted for estimating the number of spores. Delays in obtaining molecular supplies have slowed this work.

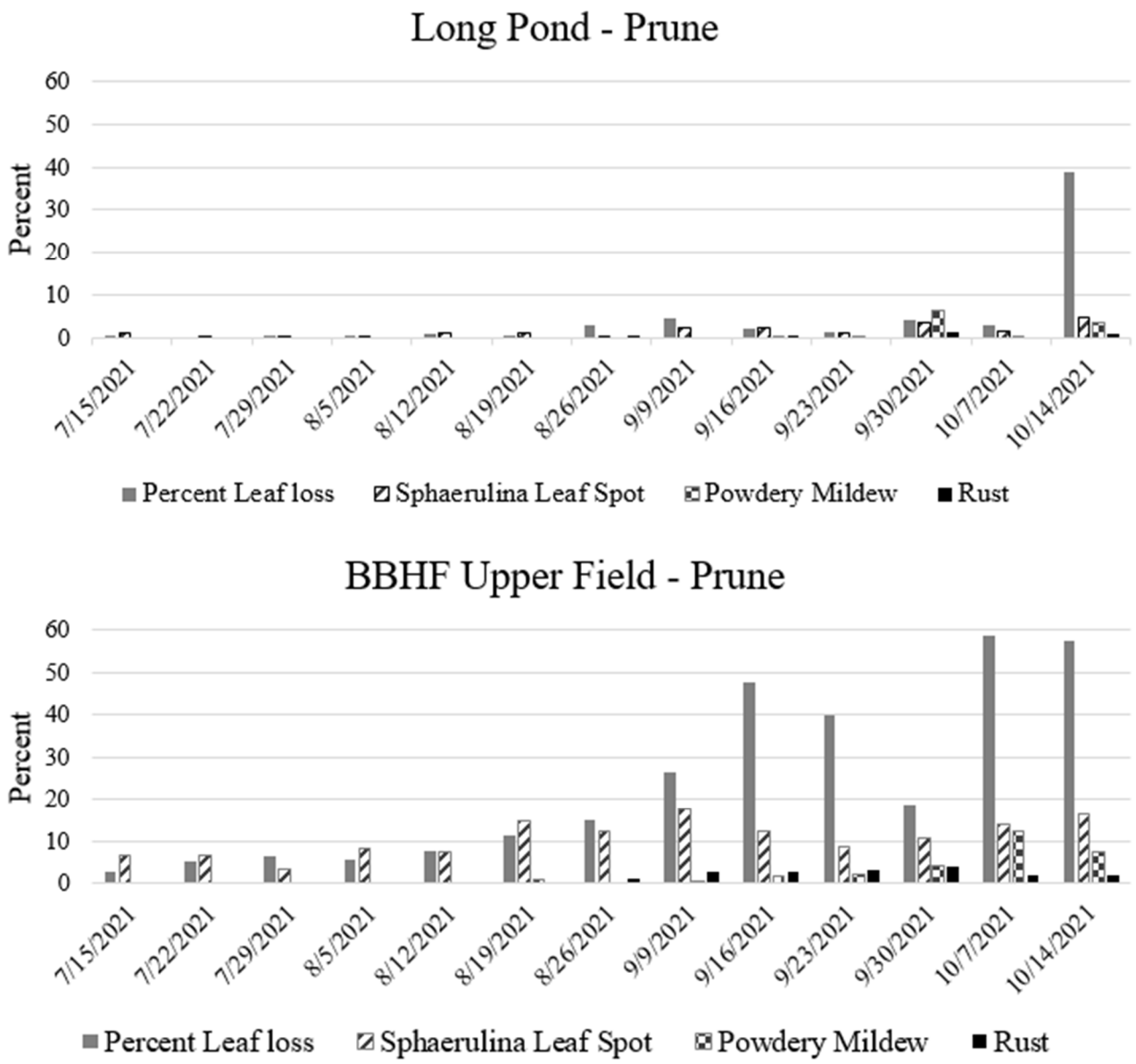

Figure 3. Leaf loss (solid grey) and symptoms of leaf spot including Sphaerulina leaf spot (stripe), powdery mildew (checker), and rust (solid black). Two fields rated weekly at Blueberry Hill Farm, Jonesboro, ME and Long Pond, near Deblois, ME.

Fungicide efficacy trial

No phytotoxicity was detected with any of the fungicide treatments. The leaf loss levels increased from less than 10% in July to less than 30% in August (Figure 4). Most leaf loss occurred from August to September where leaf loss was approximately 40 to 50%. There was no effect of the treatments on leaf loss in July but in August, Proline and Fungicide 1 as early and late treatments, had significantly less leaf loss than the control (Figure 4). In September, Fungicide 1 had significantly less leaf loss than the control for both early and late treatments and slightly less leaf loss than early and late applications of Proline. The treatments with two applications of Ecoswing, Howler, or Thiea had similar levels of leaf loss as the controls in each month of sampling.

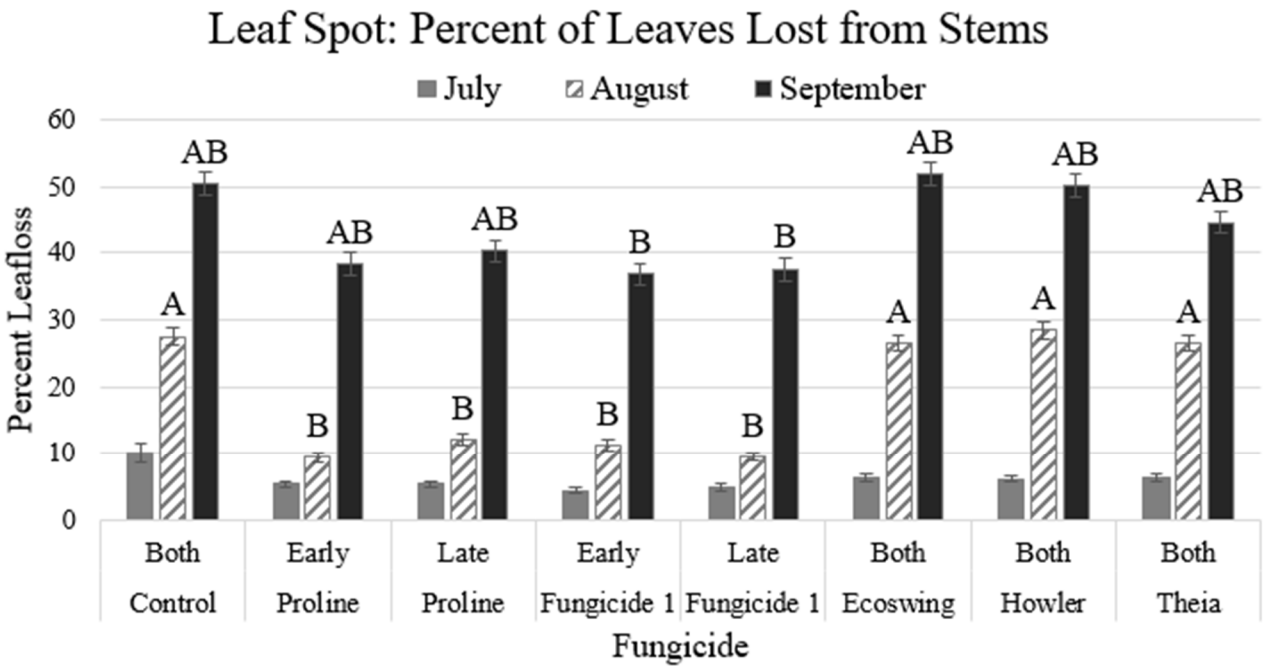

Figure 4. Percentage of leaves lost from stems. Bars represent the standard error of the mean. Letters indicate significant differences between treatments at α= 0.05; comparisons are made between treatments in each month.

Sphaerulina leaf spot levels also increased from July through September, but the Sphaerulina leaf spot reported in September may also reflect leaf rust spots that were not sporulating (Figure 5). The early and late applications of Proline and late application of Fungicide 1 had significantly less Sphaerulina leaf spot compared to the control in August but not in July or September. None of the other treatments significantly decreased Sphaerulina levels compared to the control. Powdery mildew and leaf rust did not appear in the plots until August and were at very low levels, below 2%, in September (data not shown). The levels of powdery mildew and leaf rust were too low to detect any effects of fungicide applications. Recommendations are to retest Fungicide 1 in 2022 for control of leaf spots and leaf loss.

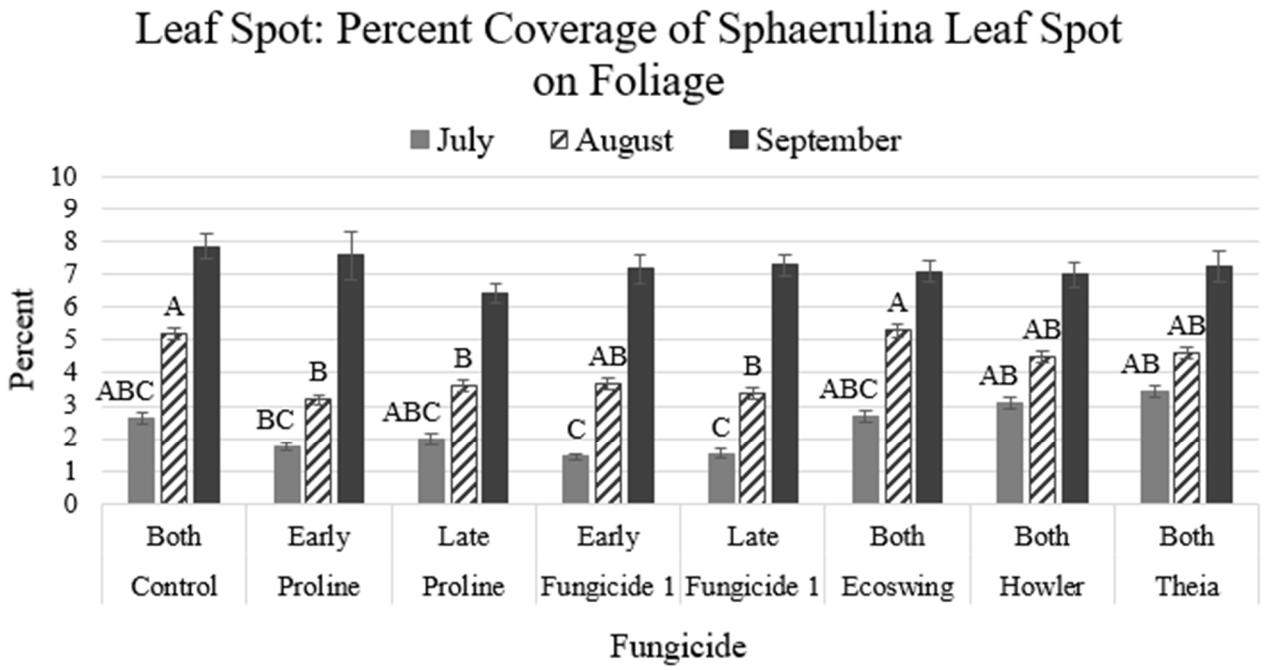

Figure 5. Percentage of Sphaerulina leaf spot coverage on stems. Bars represent the standard error of the mean. Letters indicate significant differences between treatments at α= 0.05; comparisons are made between treatments in each month.

ACKNOWLEDGEMENTS
We would like to thank Judith Collins for help with fungicide applications and the crew of Blueberry Hill Farm for setting up of experimental plots. Sophia Suriano and Erica Roche were our summer students who helped with all field experiments. We would also like to thank all of the growers who allowed us to conduct research on their land. Funding is from Specialty Crop Block grant from the Maine Department of Agriculture, Conservation and Forestry and the Wild Blueberry Commission of Maine.

RESEARCH & EXTENSION

INVESTIGATORS: S. Annis and J. Schwab

2. Research into control of Exobasidium leaf spot in 2021

OBJECTIVE Improve control of Exobasidium leaf spot on wild blueberry using a field trial and determine potential effect of high pH fungicides on soil chemistry.

LOCATIONS: Union, Maine
PROJECT TIMEFRAME: March - August 2021

METHODS
Fungicide efficacy trials (Exobasidium)
On March 23, 2021, a field trial was set up in a wild blueberry field in Union, Maine in plots where Exobasidium leaf spot had been identified in August 2020. The plots were set up with three treatments, Lime Sulfur Ultra at two rates: 25.6 fl oz/acre and 51.2 fl oz/acre, Sulforix at 25.6 fl oz/acre,

and an untreated check, with five replications for each treatment. The treatments were randomly assigned so that each treatment was present at least once in each disease patch. Plots were 6 x 5 ft and adjacent to each other. Fungicides were applied on March 24, 2021, at volumes equivalent to 20 gallons per acre at 35 psi with a CO_2 backpack sprayer equipped with a 4-nozzle boom, 8002VS T Jet tips, and 50-mesh screens.

Leaf spot disease ratings were made on June 11, 2021. Two separate 2.7 ft^2 subplots were randomly selected within each treatment plot and the quantity of Exobasidium leaf spots were recorded. Phytotoxicity was also rated during periodic field visits.

On July 23, 2021, all fruit within each treatment plot was harvested using blueberry rakes and weighed for total yield. A subsample of ~1 qt was taken from the total harvest, bagged, and placed on ice for later analysis. From the ~1 qt subsample all berries with Exobasidium fruit spots were counted. Finally, a random selection of 100 berries from the subsample were weighed to estimate berry weight.

On August 20, 2021, multiple samples of the top four to six inches of soil were collected using a soil corer in each plot to fill one 0.5 qt sample box per plot. Plot samples were analyzed for the "basic soil test" at the Maine Soil Testing Service, University of Maine, Orono, ME.

Counts of leaf spots, fruit spot, total yield and weight of berries and soil measures were checked for normality using PROC UNIVARIATE and then analyzed for effect of treatments using PROC GLIMMIX using SAS (Statistical Analysis Software - SAS Cary, NC).

Results

The Lime Sulfur Ultra and Sulforix treatments decreased the number of leaf spots and fruit spots compared to the control but due to high variability among plots there were no significant differences among treatments and the control (Figure 1). Subplots adjacent to each other varied from 9 to 30 leaf spots in June. There was typically one Exobasidium spot per berry or leaf with only one berry having two spots and very few leaves. The treatments did not have any significant effects upon overall yield or the weight of 100 berries (Table 1). No phytotoxicity was noted on the plants.

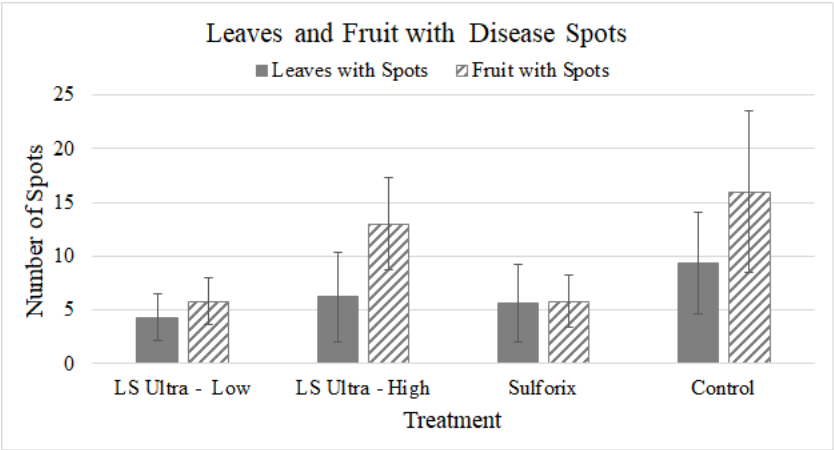

Figure 1. Number of Exobasidium spots on leaves (solid grey) and fruit (grey stripes) per treatment. Bars represent the standard error of the mean. There was no significant difference between treatments.

Table 1. Effect of fungicides on yield as measured by the total fruit yield per plot (lbs) and weight per 100 fruit (oz) harvested on July 23, 2021. Averages presented ± the standard error of the mean.

Treatment	Average fruit yield per plot (lbs)	Weight per 100 fruit (oz)
Lime Sulfur Ultra – Low (25.6 fl oz/A)	4.23 ± 0.38	1.69 ± 0.19
Lime Sulfur Ultra – High (51.2 fl oz/A)	3.20 ± 0.29	1.73 ± 0.20
Sulforix (25.6 fl oz/A)	4.21 ± 0.76	1.77 ± 0.11
Control	4.04 ± 0.40	1.71 ± 0.16

Soil was sampled from each treatment plot to check the effect of the lime sulfur treatments on soil characteristics. The treatments had no significant effect on soil pH, calcium, sulfur or other soil mineral nutrients measured (Table 2).

Table 2. Percent calcium, pH, and ppm sulfur measured in soil sampled for individual treatment plots on August 20, 2021. Averages presented ± the standard error of the mean.

Treatment	Calcium [%]	pH	Sulfur [ppm]
Lime Sulfur Ultra – Low (25.6 fl oz/A)	11.80 ± 1.55	4.40 ± 0.05	111.20 ± 8.81
Lime Sulfur Ultra – High (51.2 fl oz/A)	14.22 ± 1.53	4.52 ± 0.04	111.20 ± 5.41
Sulforix (25.6 fl oz/A)	12.30 ± 0.89	4.44 ± 0.08	117.00 ± 6.99
Control	11.30 ± 1.56	4.46 ± 0.07	126.00 ± 7.40

ACKNOWLEDGEMENTS
We would like to thank Bill Little for allowing us to use his land to set up this field experiment. Sophia Suriano and Erica Roche were our summer students who helped with all field experiments. Funding is from Specialty Crop Block grant from the Maine Department of Agriculture, Conservation and Forestry and the Wild Blueberry Commission of Maine.

EXTENSION

INVESTIGATORS: S. Annis and J. Schwab

3. Research for Improved Control of Mummy Berry 2021

OBJECTIVE
Improve control of mummy berry, caused by *Monilinia vaccinii-corymbosi* (MVC), through research and the deployment and operation of weather monitoring stations to advance a disease forecasting system.

1. Provide growers with forecast reports of MVC infection periods
2. Determine the timing of ascospore release for MVC and the effect of weather conditions on spore levels
3. Test the efficacy of new materials for their control of mummy berry symptoms

LOCATION(S): Multiple wild blueberry fields in Maine
PROJECT TIMEFRAME: March - October 2021

METHODS

Timing of apothecia development and susceptibility of wild blueberry to mummy berry

Ian Leonard, MS graduate student, has been conducting research of pseudosclerotia (mummy) germination and wild blueberry susceptibility to primary infection of MVC from September 2020. A common garden experiment in a complete random block design was established in October 2021. The experiment involved exposing pseudosclerotia (mummies) collected from 4 different wild blueberry fields throughout the Downeast and Midcoast regions of Maine to the same environmental conditions monitored by an on-site weather station. The weather station recorded air temperature, soil temperature, soil moisture and leaf wetness data. Germination of structures produced by mummies (stipes, pinheads, and apothecia also called cups) were tracked and recorded 3 to 4 times per week between April and May 2021. The data collected were combined with data collected in 2016 and 2017 to gain a better understanding of what weather factors affect each stage of development. The model of mummy berry development using weather factors is currently in development using binomial regression.

Wild blueberry susceptibility was monitored and observed between April and June 2021 in 4 fields throughout the Downeast region of Maine. Each field had a weather station established to monitor air temperature, soil temperature, soil moisture and leaf wetness. In each field, 5 different genets were selected for disease monitoring, and within each genet, 5 individual stems were selected for phenological development and disease rating. Phenological development was evaluated weekly using Hildebrand and Braun's (1991) stages of leaf and flower bud development. Once flowers had fully developed, phenological evaluation stopped and disease rating began. In addition to monitoring the individual stems, transects were used to collected data on 15 random stems per genet. Disease rating involved looking for floral and leaf symptoms of mummy berry and Botrytis, and frost damage, winterkill, or insect damage. In late July, each selected stem and 15 random stems within a genet were collected for determining healthy fruit weights and counts.

Survey of weather and disease in wild blueberry fields

In late March 2021, weather stations with cellular internet connections were deployed in growers' fields across the Midcoast and Downeast regions in Maine ranging from Waldoboro (Lincoln County) to Crawford (northern Washington County) (Figure 1). Each station was equipped to measure air temperature and leaf wetness at approximately 4 inches above the soil, soil temperature at 1 inch below the surface, and soil moisture between 1 and 5 inches below the surface. The depth of soil moisture sensor aligns with the predominant depth of blueberry roots. Plots of MVC-infected blueberry fruit (pseudosclerotia) were established at eleven of the sites. These were monitored by growers during anticipated apothecia (cup) development in April and May.

All fields with weather stations were evaluated for mummy berry disease and other problems between May 21 and 27, 2021. Thirty randomly chosen stems along a 30 ft transect were evaluated at four random locations in each field. Each stem was evaluated for MVC symptoms on leaves and flower buds, frost damage, and winter kill. MVC symptoms were recorded for leaves and flower buds on a 0 - 4 scale with 0 = no disease present, 1 = 1 bud infected, 2 = 2 buds infected, 3 = 3 buds infected and 4 = 4 or more buds infected.

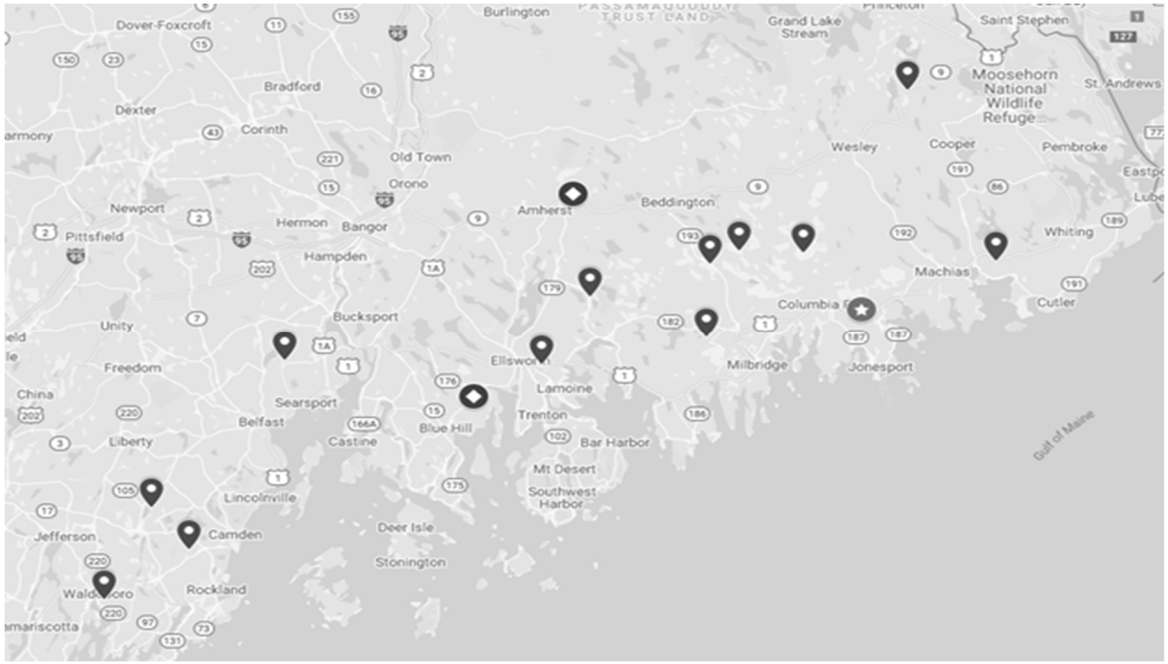

Figure 1. Map of weather station locations for 2021. The star is a Davis Station at BBHF, all other marks are WatchDog stations. Encircled diamond markers indicate the two locations where a weather station did not have an associated mummy berry plot.

Timing of MVC ascospores

On April 15, 2021, a spore trap was placed in a prune field at Blueberry Hill Research Farm (BBHF) in Jonesboro, ME, and another trap was placed on the border of a prune- and crop-field (Airport) in Deblois, ME. On April 19, 2021, a third trap was placed in a crop field (Long Pond) near Deblois, ME. Each location had a weather station nearby. Spore collection tapes were collected weekly and MVC ascospores were counted under a microscope in hourly intervals for the date ranges of April 13 to May 13 for BBHF and are being counted from April 17 to May 13 for Long Pond and Airport.

Fungicide efficacy trial

In April 2021, a field experiment was set up in a crop field (Long Pond) with a history of mummy berry disease. A randomized complete block design was used with 13 treatments, 12 fungicide treatments plus one untreated control, replicated in 8 blocks (Table 1). Each plot measured 6 ft X 30 ft and was separated from the adjacent plot by a 3 ft alley. Fungicides were applied on April 20, 2021 and May 3, 2021 at volumes equivalent to 20 gallons per acre at 35 psi with a CO_2 backpack sprayer equipped with a 4-nozzle boom, 8002VS T Jet tips, and 50-mesh screens.

Disease and frost ratings were made on June 2, 2021. Thirty stems were rated in the same manner as above for the weather stations along a 30 ft transect per plot. The percentage of stems with mummy berry symptoms was the number of stems with symptoms divided by the total number of rated stems (30 minus the number of bare locations) for each plot. Phytotoxicity was also rated at the same time disease assessments were made.

Table 1. Table of all treatments applied to fungicide trial in 2021.

Treatment	Material	App. Rate (per acre)	Company	FRAC Group	EPA Reg. Number	Registered on Wild Blueberry
Inspire Super	Difenoconazole / Cyprodinil	20 fl oz	Syngenta	3, 9	100-1317	Yes
Miravis Prime	Pydiflumetofen / Fludioxonil	13.4 fl oz	Syngenta	7, 12	100-1603	Yes
Tilt	Propiconazole	6.0 fl oz	Syngenta	3	100-607	Yes
Omega 500F	Fluazinam	20.0 fl oz	Syngenta	29	71512-1-100	Yes
Cevya (and LI700)	Mefentrifluconazole	5.0 fl oz	BASF	3	7969-XXX	No
Cevya (and LI700)	Mefentrifluconazole	4.0 fl oz	BASF	3	7969-XXX	No
Fungicide 1		13.7 fl oz	Bayer	N/A	N/A	No
Fungicide 2		16.0 fl oz	Bayer	7, 9	N/A	Yes
Fungicide 3		10.0 fl oz	Bayer	7, 3	N/A	Yes
Theia (and Dyne-Amic 0.375%v/v)	*Bacillus subtilis* strain AFS032321	3.0 lbs	AgBiome		none	No
Howler (and Dyne-Amic 0.375%v/v)	*Pseudomonas chlororaphis* strain AFS009	5.0 lbs	AgBiome		91197-3-92488	Yes
Ecoswing (and Capsil)	*Swinglea glutinosa*	32.0 fl oz	Gowan		10163-357	Yes

Blueberries were mechanically harvested on August 2, 2021, in a 2-foot-wide strip down each plot center. The fresh weight per plot was measured and used to calculate lb/acre.

The proportion of stems with disease were arcsine square root transformed to try to normalize the disease measurements. The yield data had a normal distribution and was not transformed. 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 ($\alpha = 0.05$).

RESULTS

Timing of apothecia development and susceptibility of wild blueberry to mummy berry

In the common garden, mummies germinated to produce a structure (stalk, pinhead or cup) between April 12 and May 6, 2021. The first cup was present on April 14, and the last cup and remaining structures all died by May 16. Of all of the mummies in all of the plots, approximately 9.0% germinated any structure. Of those that did germinate, 76.7% reached maturation with the development of cups while 14.0% only developed pinheads and 9.3% only developed stalks. Mummies collected from Airport field near Deblois, ME had the greatest amount of germination at 14.2%. Mummies collected from BBHF field, fields near Montegail pond, and Searsport, ME had 7.5%, 8.3% and 5.8% germination, respectively. Of the four plots for each field's mummies, Searsport did not germinate any structure in two plots. Searsport was the only location in the Midcoast region where mummies were collected for the common garden experiment.

An observation was made that in 2016 and 2017, was that mummies collected from the Midcoast region of Maine did not germinate well in the common garden using BBHF soil. Similar observations were made in 2021. An experiment was designed to expose mummies collected from the Downeast and Midcoast regions of Maine to soils in each region to compare how each would germinate. Three fields in each region were selected. Soil samples were collected from each field and analyzed by the Soil Analytical Lab on University of Maine, Orono campus (Table 1). The soil pH, CO_2 respiration, plant water availability, and sand, silt and clay content were compared between regions, textures (loam and silt loam) and interactions between regions and textures using ANOVA. The soil collected from BBHF, sandy loam, was omitted from ANOVA tests due to there being only one replicate. Significance was found between regions for sand and silt content. Midcoast (MC) soils were mostly made of silt loam, and loam was a minor soil in that region. Downeast (DE) soils were mostly loam, with the exception being the sandy loam located at BBHF in Jonesboro, ME. CO_2 respiration was generally greater in the MC region. Plant water availability (PAW) was relatively similar in both regions. There was more sand and less silt content in soils found in DE soils compared to MC soils. An incubation experiment will be conducted in 2022 comparing the germination of mummies in soil collected from Downeast to that collected from Midcoast.

Table 2: Partial results from the soil testing conducted by the Soil Analytical Lab.

Region	Location	Texture	pH	CO_2-C[1]	% PAW[1]	% Sand	% Silt	% Clay
MC	Appleton	silt loam	4.2	290	21.9	24	63	13
MC	Hope	silt loam	4.2	290	21.7	36	54	10
MC	West Rockport	loam	4.9	320	21.6	42	48	10
MC	Waldoboro	silt loam	4.9	210	24.1	37	51	12
MC	Warren	loam	4.6	320	19.0	47	43	10
DE	North Washington	loam	4.6	280	20.6	44	46	10
DE	Jonesboro	sandy loam	4.4	110	17.1	65	28	7
DE	Montegail	loam	4.6	300	19.8	47	43	10
DE	Hancock	loam	4.3	250	22.6	46	44	10

[1] CO_2 respiration rate – indicator of microbial activity; PAW – plant water availability

During the field season of 2021, mummy berry symptoms were found in all four fields used for phenology observations. Transect data from disease rating showed that the field located in Eastbrook, ME had the greatest amount of mummy berry infection (45.3%) followed by Searsport (37.3%). Fields in Steuben and Ellsworth, ME each had approximately 24% of stems infected. Eastbrook had the greatest amount of winterkill (12.8%), while the other three fields had less than 4%. Eastbrook also had the greatest amount of Red Leaf present among genets (8.4%), followed by Searsport (6.2%). Steuben and Ellsworth had less than 2% of Red Leaf. Searsport had the greatest amount of insect damage (28.4%), while the other three fields had less than 2%. Frost damage was observed in all fields at an occurrence of less than 5%. Steuben had the greatest average healthy fruit count and weight at an average of 15 berries and average weight of 5.7 grams per stem. Searsport and Ellsworth fields had an average of 13 and 9 berries and average weight of 5.4 and 4.0 grams per stem, respectively. Eastbrook field was harvested by the grower before it could be evaluated.

Survey of weather and disease in wild blueberry fields

We had low levels of mummy berry in most fields with weather stations in 2021 (Figure 2). Mummy berry ranged from 1 to 31% and an average of 11% of stems with symptoms in lowbush blueberry fields with weather stations around the state. Two fields with no fungicide applications had 14 or 31% mummy berry incidence while in the past some years have had 80% stems with symptoms with no fungicide applications. This level of disease is lower than in past years and is likely from cooler weather with shorter periods of rainfall that was experienced in many fields compared to prior years. The level of

mummy berry disease was affected by the level of local inoculum, whether the field was mowed or burned and if and when fungicides were applied. There was no detected Botrytis in the weather station fields and little frost damage.

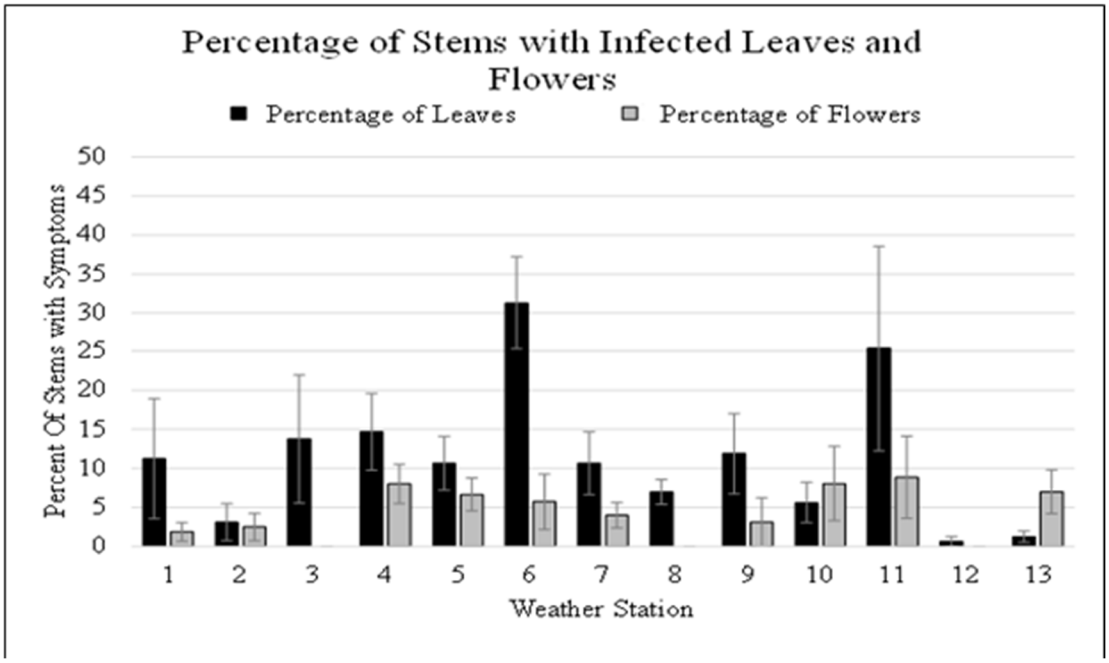

Figure 2. Percent of stems with mummy berry symptoms at weather stations in 2021.

Timing of MVC ascospores

In BBHF field, MVC ascospores were seen on spore trap tapes from April 19 to May 13 with the majority of the spores detected from April 28 to May 6 (Figure 3). Most ascospores were released overnight.

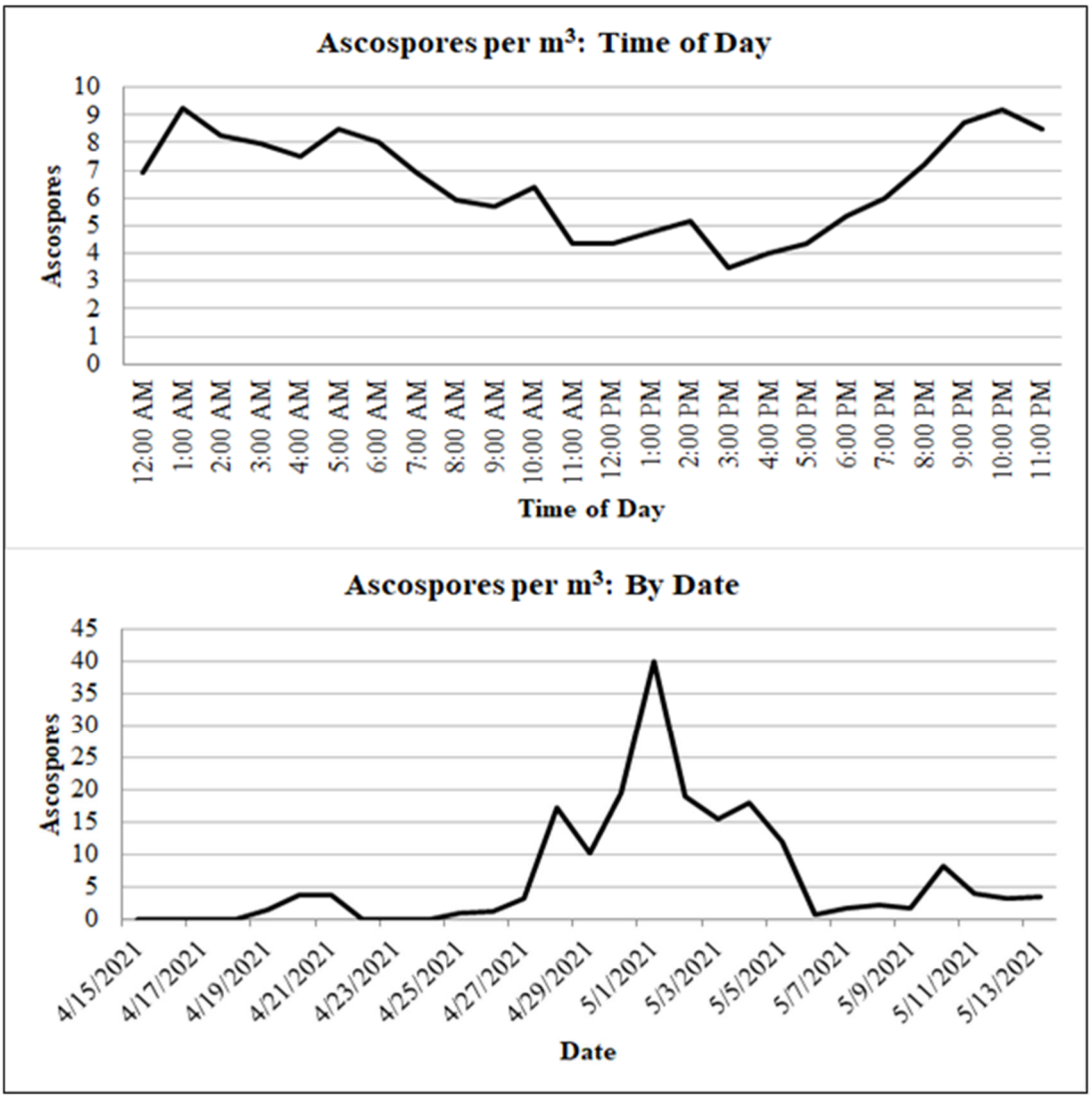

Figure 3. Top graph indicates average concentration of ascospores per m³ in one-hour intervals from April 15 to May 13, 2021. Bottom graph indicates total ascospores per m³ on each day.

Fungicide efficacy trial

It was estimated that there were four infection periods in fields near the fungicide efficacy trial and that these periods occurred approximately four to six days apart (Figure 4). There were fewer infection periods than have occurred in many years in the past. Fungicide applications were timed before infection periods and assumed activity of the fungicides for seven to ten days. At the mummy berry fungicide trial site, we had very low levels of disease with an average of 3.75% stems with symptoms in the control plots (Figure 5). We were concerned that our rating of mummy berry symptoms on June 2 had been either too early or too late to catch maximum disease levels. Three other fields in Hancock and Washington counties were rated once a week and had increasing levels of mummy berry symptoms from May 19 to June 3 and the highest levels of disease on June 3. This suggests that our mummy berry trial did have maximum levels of mummy berry when it was rated.

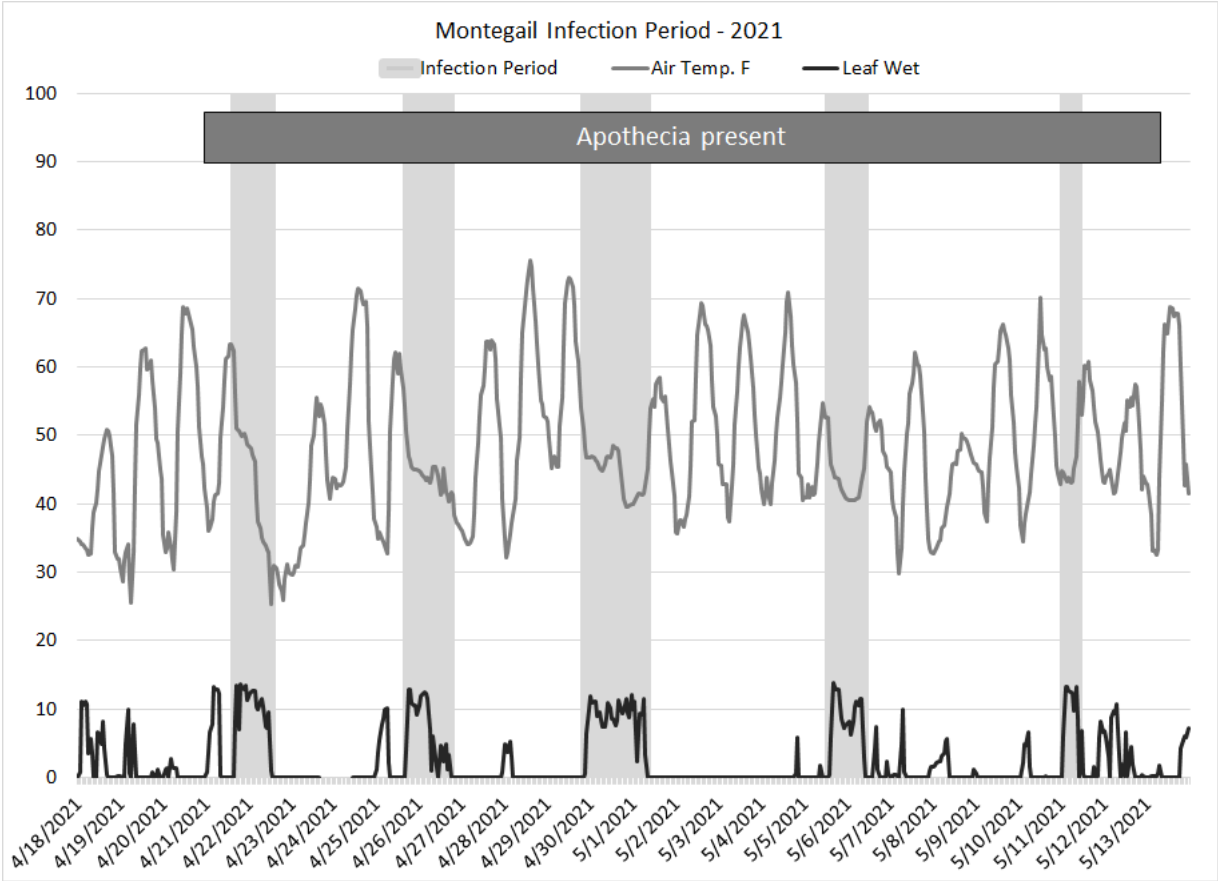

Figure 4. Infection periods (vertical bars) at field in Montegail. Infections based on air temperatures (upper line) during times of higher leaf wetness (lower line). Bar at top indicates when apothecia (cups) were present.

All of the fungicides tested had less disease (less than 1%) than the untreated control plots (3.9%) and some were comparable to the standard of propiconazole (data not shown). The disease levels were highly variable among blocks and so low that no significant differences were found between the treatments and controls. With the very low levels of disease, it is difficult to judge the efficacy of the treatments. There was no significant effect on yield of any of the treatments.

The recommendation is to test all materials next year at Blueberry Hill research farm. The research farm has had low levels of mummy berry in the past but over the last two years has had 25 to 30 percent of stems with disease symptoms.

ACKNOWLEDGEMENTS
We would like to thank Judith Collins for help with fungicide applications and the crew of Blueberry Hill Farm for setting up of experimental plots. Sophia Suriano and Erica Roche were our summer students who helped with all field experiments. We would also like to thank all of the growers who allowed us to conduct research on their land. Funding is from Specialty Crop Block grant from the Maine Department of Agriculture, Conservation and Forestry and the Wild Blueberry Commission of Maine.

REFERENCES
Hildebrand, P.D. and P.G. Braun. 1991. Factors affecting infection of lowbush blueberry by ascospores of *Monilinia vaccinii-corymbosi*. Canadian Journal of Plant Pathology. 13: 232-240.

WEED SCIENCE

RESEARCH & EXTENSION

INVESTIGATORS: L. Calderwood, M. Scallon, and B. Tooley

1. Organic Weed Management for Wild Blueberry Growers; Tine-Weeding in the Prune Year

OBJECTIVES

- Evaluate the effect of 2 and 4 passes with the tine weeder on a section of field with even weed and blueberry cover

LOCATIONS: UMaine Blueberry Hill Farm Experiment Station, Jonesboro ME
PROJECT TIMEFRAME: 2020 - 2022

INTRODUCTION

For an introduction on the need for mechanical weed management and the mechanics of tine weeding see the 2020 report 8b page 61, "Organic Weed Management for Wild Blueberry Growers".

In the tine weed trial initiated in 2019, the number of passes made with the tine weeder on a single date affected the efficacy of weed removal. The first pass appeared to only loosen the soil, which allowed the second pass to dislodge weeds. A flex-tine weeder performs best on level ground with dry, loose soil (Bowman, 2002) but wild blueberry fields are often bumpy and have never been tilled which changes the way a tine weeder works. Observationally, two passes with the tine weeder in 2019 pulled some weeds up entirely but left others hanging on by a few roots, allowing them to reestablish. Increasing the number of passes with the tine weeder would theoretically loosen the soil with every pass, allowing the tines to move deeper and hit different parts of the field, and sever any lingering thread roots, thereby increasing the effectiveness of using a tine weeder for weed control. However, more intensive mechanical weed removal may also be injurious to the blueberry because as the tines reach deeper into the soil, they are more likely to catch a blueberry rhizome and uproot it above the loosened soil.

We have not yet documented a decrease in blueberry yield due to tine weeding with two passes, and it is documented that wild blueberry responds well to mechanical stimulus such as fall pruning, burning, and cutting rhizomes (Libby, 2011). This trial continues investigation of whether multiple passes with the tine weeder might stimulate rather than injure wild blueberry by studying a conventional field at Blueberry Hill Farm in Jonesboro, ME, beginning in May 2020.

METHODS

The selected field at Blueberry Hill Farm is levelled, de-rocked, and planted, resulting in improved crop uniformity. At the beginning of this project the selected field also had even coverage of red sorrel and poverty oat grass. These factors make this location a good research site for studying the impact that prune year tine weeding has on wild blueberry growth and specific weed species reduction. This trial was set up in a randomized complete block design replicated six times with three basic treatments. Treatments included a control (no weeding), tine weeded with 2 passes, and tine weeded with 4 passes arranged in eighteen, 6 ft x 30 ft plots, with a 3 ft buffer between plots. Although the selected field is conventionally managed, no chemical weed control applications were made to this field in 2020. Tine weeding for both treatments occurred on May 11, 2020.

Data Collection

Measures of weed and blueberry crop growth were collected using two 0.37m^2 quadrats per plot. Two quadrats were placed in each plot and flagged for repeated measurements throughout the study. Weed and blueberry cover were ranked within each quadrat based on the Daubenmire Cover Scale, where 0 = not present, 1 = ≤1-5% coverage, 2 = 6-25% coverage, 3 = 26-50% coverage, 4 = 51-75% coverage, 5 = 76-95% coverage and 6 = 96-100% coverage (Daubenmire, 1959), and the total number of weeds with the top three weeds present were recorded on 5 dates throughout the 2020 season, and 3 dates throughout the 2021 season (May 7, June 16, and August 17, following the harvest).

Blueberry response was measured during the 2020 field season by counting the number of blueberry stems on two separate days, the number of emerging blueberry shoots on one day, and the number of stems within each quadrat on three separate days. In fall 2020, the heights of four random stems and the number of buds on each stem were recorded.

Blueberry response was measured during the 2021 field season by repeated observations of blueberry cover as well as physical measures of stem height, stem number, and bud number per stem. In 2021, six random stems were flagged per quadrat and monitored repeatedly for fruit-set and fruit-drop for the two tine weed treatments and the control. Fruit-set measures included bud counts in late spring (May 7), flower counts at peak bloom (June 3), green fruit counts prior to ripening (June 16), and blue fruit counts during ripening (July 29). Percent fruit-set was calculated from the number of green fruit and the number of flowers per stem, while fruit-drop was calculated from the number of blue fruit and the original number of green fruit observed for each stem.

Fruit was harvested on July 29 and August 3, 2021 by hand-raking the separate quadrats and then entire plots. Berry weight was measured from the individual quadrats (two measures per plot), and the entire plot (one measure, including the weight from the two quadrats). The weight of 100 random berries selected from the harvested sample was also weighed to assess berry size. A sample of berries from each plot was sampled to determine the berry sugar content (Brix).

Data Analysis

Data measurements with high numbers of zeros had skewed distributions, making them fail some of the assumptions necessary for parametric statistical testing. Here, these measurements included phenological development (bud, flower and fruit counts), the number of blueberry stems per quadrat, weed cover, blueberry cover and the total number of weeds per quadrat. Transforming the data via a square root transformation resulted in a normal distribution for blueberry stem number only. The remaining data improved visually with transformation but continued to statistically fail for normality. Non-normal data was transformed using a square root transformation prior to all statistical testing and statistical tests were carried out despite non-normality after establishing there were no serious problems with the data.

Single date measurements including: the counts of buds, flowers, green fruit and blue fruit, harvest yield, berry size and sugar content were evaluated using a generalized linear model (GLM), followed by a Tukey's Pairwise comparison in JMP (JMP®, Version 15.2) across all tine weed management treatments and the controls ($\alpha = 0.05$). Ranked weed and blueberry cover data were transformed to their corresponding percent mid-point and compared across both years (2020 and 2021) using a full-factorial repeated-measures mixed model design in JMP. Blueberry stem number and weed number were also compared across both years using the same method. Here, the full-factorial tested the effects of date, treatment and any interaction between date and treatment for the ranked response variables.

RESULTS

Effects of Tine Weeding on Blueberry Health

In 2020, four passes with the tine weeder resulted in a visual reduction in blueberry cover, blueberry development, and accumulated vegetative growth (Figure 1). The image below, taken over a month after tine weeding, shows the disturbance from four passes of the tine weeder with a dark strip consisting of large patches of bare ground, undeveloped stems, uprooted rhizomes, and less green vegetation than the surrounding treatments. In 2021 the tine weeded treatments had become less distinguishable apart from unhealed, uprooted rhizomes.

Figure 1. Tine weeded strip of blueberry that received four tine weeder passes on May 11, 2020, of the prune year at Blueberry Hill Research Farm, Jonesboro Maine. Photo taken June 18, 2020, approximately 5.5 weeks following tine weeding.

Post-tine weeding blueberry cover and blueberry stem number measured across 2020 and 2021 showed a reduction with increased passes with the tine weeder (Figure 2). Blueberry cover was significantly greater in the control than both tine weed treatments by as much as 10% and 17% compared to the tine with two passes and with four passes, respectively. Blueberry stem number showed significant reduction with four passes of the tine weeder relative to the control. Here, four passes with the tine weeder resulted in 20% fewer stems.

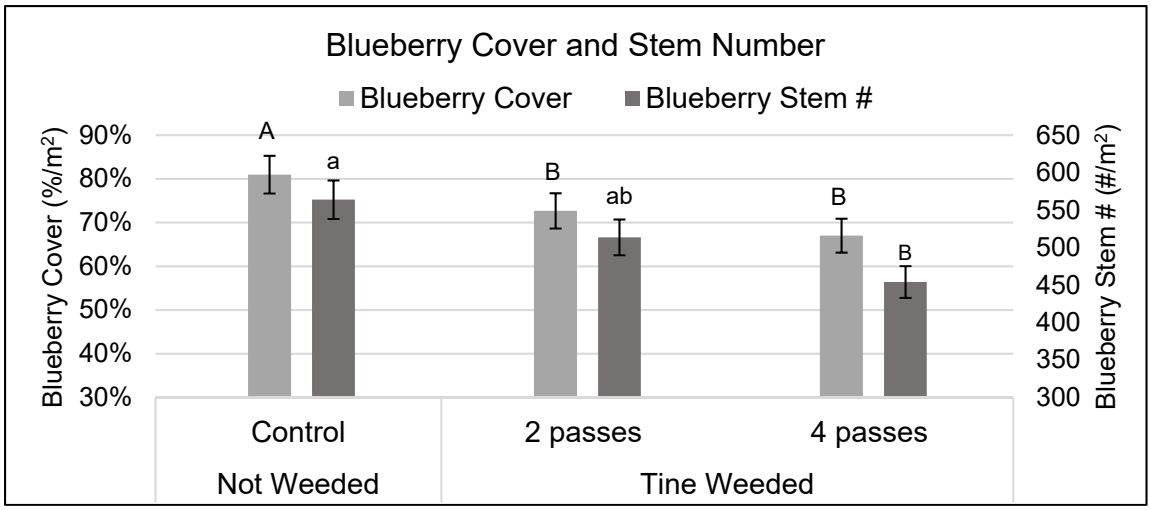

Figure 2. Blueberry cover (%/m^2) and blueberry stem number (#/m^2) by treatment following tine weeding, measured in 2020 and 2021. Letters indicate significance at the 0.05 level of significance for blueberry cover and blueberry stem number. Capital letters are to be compared separate from lowercase letters. Error bars indicate the standard error of the mean.

Comparing Before and After Tine Weeding in 2020

When comparing the single date pre-tine measures to the average post-tine measures collected throughout the season, a percent change can be generated to show the difference between the two time periods by treatment (Figure 3). Here, comparing pre- and post-tine measures for 2020, there was little difference in blueberry cover in the control (-2%), while the tine weed treatments exhibited a 17% and 21% decrease in blueberry cover for treatments that received two passes and four passes, respectively. Weed cover increased by 23% in the control treatment as weeds emerged throughout the season, but weed number decreased (see discussion). Both tine weed treatments experienced a decrease in weed cover and weed number with the greatest decrease in the tine treatment with four passes. Here, more passes with the tine weeder led to a 14% decrease in weed cover and a 65% decrease in weed number (#/m^2) following tine weeding.

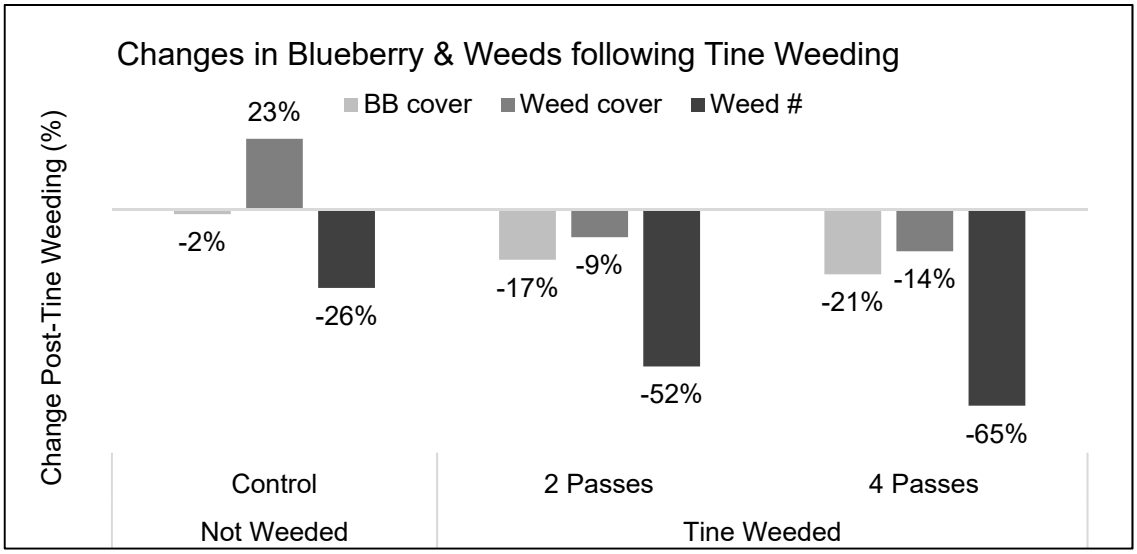

Figure 3. Changes in blueberry cover, weed cover and weed number following tine weeding is calculated by the difference (%) between pre-tine weed measures taken on May 6, 2020, and post-tine weed measures taken May 14 to September 1, 2020.

Effects of Tine Weeding on Weed Pressure

When comparing the types of weed species present across treatments, tine weeded with four passes had the lowest diversity of weed species present but the highest occurrence of grass (Table 1). Tine weeded treatments had a higher percentage of broadleaf weeds than the control. Overall, red sorrel was the top broadleaf species in all treatments (post-tine weeding), followed by St. John's wort and violet.

Table 1. Weed composition in the tine weed treatments and the controls in 2020 and 2021 observed on May 15 and 27, June 18, July 7 and September 1, 2020, and May 7, June 16 and August 17, 2021.

		Not Weeded	**Tine Weeded**	
2020 & 2021		Control	2 passes	4 passes
Weed Species Identified		9	10	7
Frequency	Broadleaf	32%	46%	43%
	Grass	8%	8%	9%
Top Broadleaf Species	1st	Red Sorrel	Red Sorrel	Red Sorrel
	2nd	St. John's Wort	St. John's Wort	St. John's Wort
	3rd	Violet	Violet	Violet

When evaluating weed presence by life cycle, treatment differences were non-significant, but an interesting trend occurred (Figure 4). Tine weeding resulted in a visible increase in annuals and grasses while exhibiting a decrease in perennial weeds relative to the control. This shows that tine weeding may impact weeds differently based on their growth and reproductive patterns (see discussion).

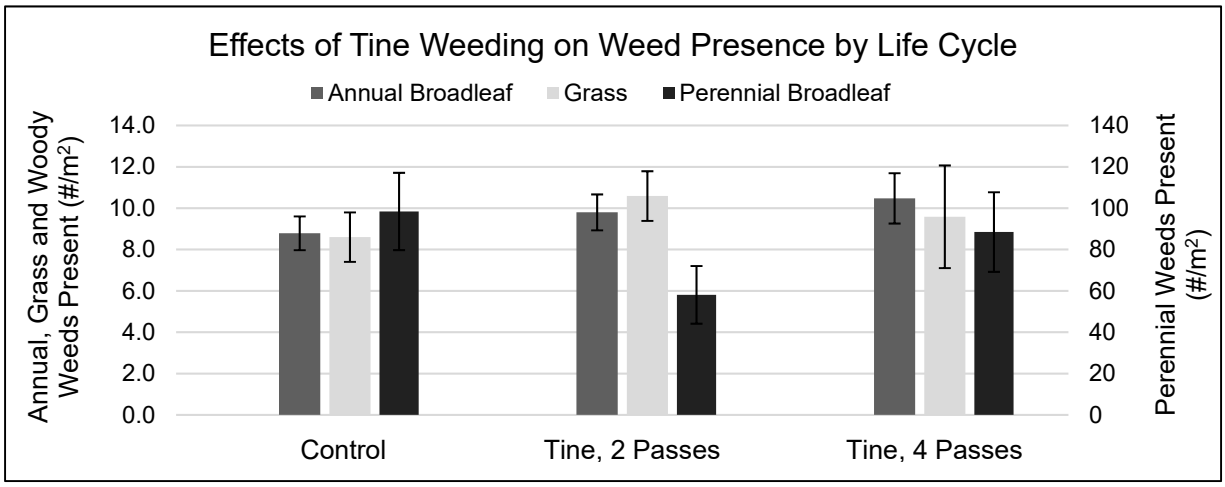

Figure 4. Weed presence by type and lifecycle relative to the 2020 tine weed treatments, measured May 7 and June 16, 2021. Treatment differences were nonsignificant for weed numbers by life cycle and type. Error bars indicate the standard error of the mean.

When evaluating the effect tine weeding on weed cover and weed number there is a visible reduction with the increasing number of passes when compared to the control (Figure 5). In 2020, weed numbers (#/m^2) were significantly less in the 2 and 4 pass treatments relative to the control. When evaluating 2020 and 2021 together, both weed cover and weed numbers are not significantly different between treatments suggesting the weed pressure may have rebounded slightly in 2021. Overall, weed presence was considerably less in the plots that were tine weeded relative to the control. Compared to the control, weed covers (%/m^2) were 25% and 31% less and weed numbers (#/m^2) were 36% and 37% less with 2 passes and 4 passes, respectively.

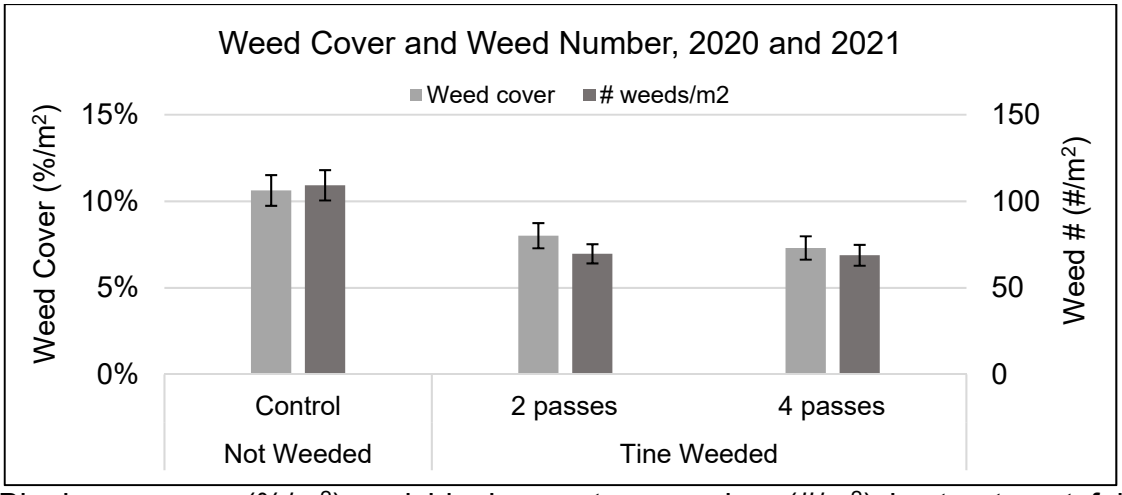

Figure 5. Blueberry cover (%/m^2) and blueberry stem number (#/m^2) by treatment following tine weeding, measured in 2020 and 2021. Treatment differences were nonsignificant for weed cover and weed number. Letters indicate significance at the 0.05 level of significance for blueberry stem number. Error bars indicate the standard error of the mean.

Effect of Tine Weeding on the Crop

Lowbush blueberry bud number and stem height were analyzed in the 2020 prune year by tine treatment where no significant differences were observed (see 2020 report). On average, however, stems were 1 cm shorter with 0.80 fewer buds in the tine treatments relative to the control. Phenological development of the blueberry in the 2021 crop year showed variable effects on reproductive stages relative to tine treatment (Figure 6), although treatment differences by phenological stage were non-significant.

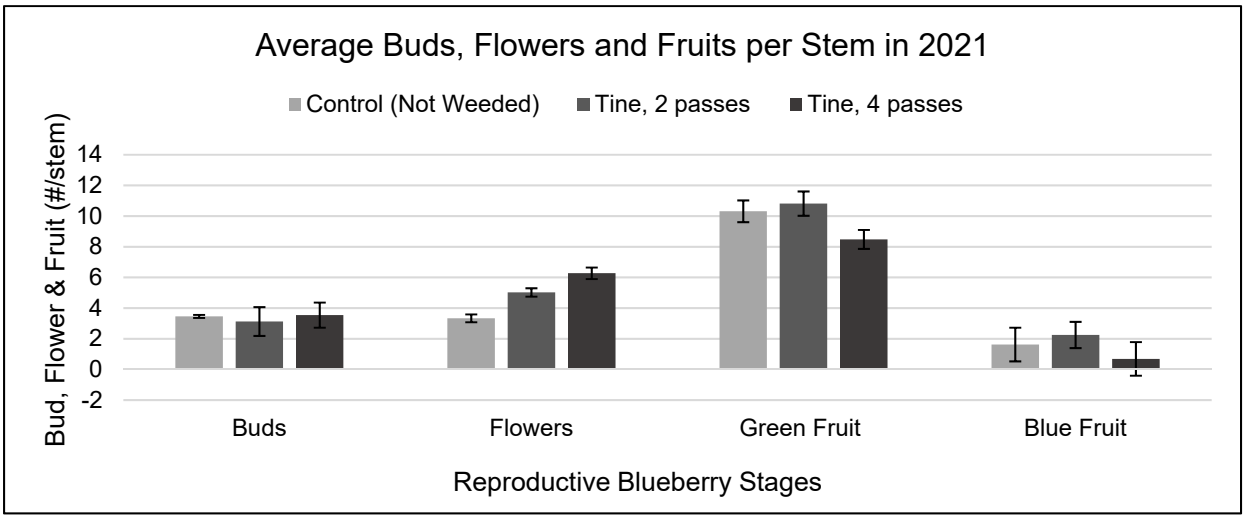

Figure 6. Average bud number, flower number, fruit numbers for green fruit and blue fruit measured on May 7, June 3, June 16, and July 29, 2021, for each peak stage respectively. Treatment differences were nonsignificant for all reproductive stages. Error bars indicate the standard error of the mean.

Average total bud number in the spring of 2021 presented little difference in bud number between treatments with a slightly larger standard error in the tine weeded treatments, meaning greater variation in bud numbers between stems. Other reproductive stages varied by treatment with the greatest average flower number and the lowest green fruit occurring in the tine treatment with four passes, perhaps suggesting poor pollination in this treatment or a more likely scenario being reduced nutrient

or water availability. Green fruit and blue fruit counts were highest on average in the tine treatment with two passes, possibly reflecting differences in development rate.

Fruit development (% stage by treatment), evaluated for July 29, 2021 (the day of harvest), suggests the fruit developed slower in plots tine weeded with two passes than plots tine weeded with four passes relative to the control (Figure 7). This is clear through the ratio of green fruit to blue fruit on the day of harvest.

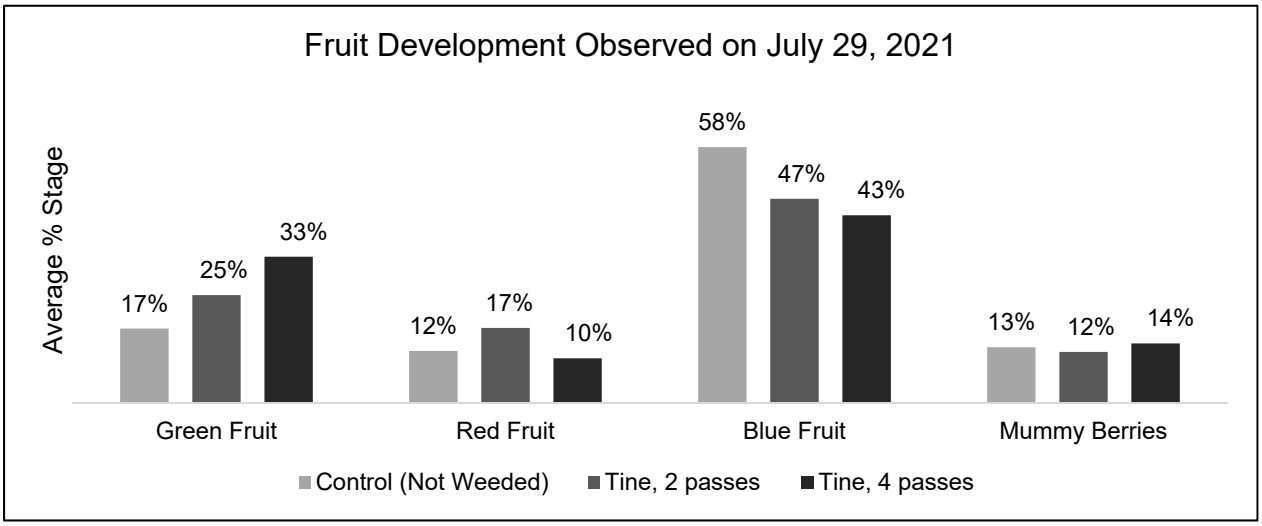

Figure 7. The average percent green fruit, red fruit, blue fruit, and mummy berry fruit measured on July 29, 2021, prior to harvest. Treatment differences were nonsignificant for all fruit development stages.

Harvest yield and berry sugar content showed significant treatment differences such that the four passes tine weed treatment had significantly lower yield and sugar content than the control (Figure 8), which is logical given that blueberry within this treatment had significantly lower stem number and exhibited a slower rate of development. Compared to the control, blueberry yields were 8% and 26% lower in the tine treatments with two passes and four passes, respectively. Similarly, berry sugar contents of the tine weed treatments compared to the control were 4% and 9% lower in the tine treatments with two passes and four passes, respectively.

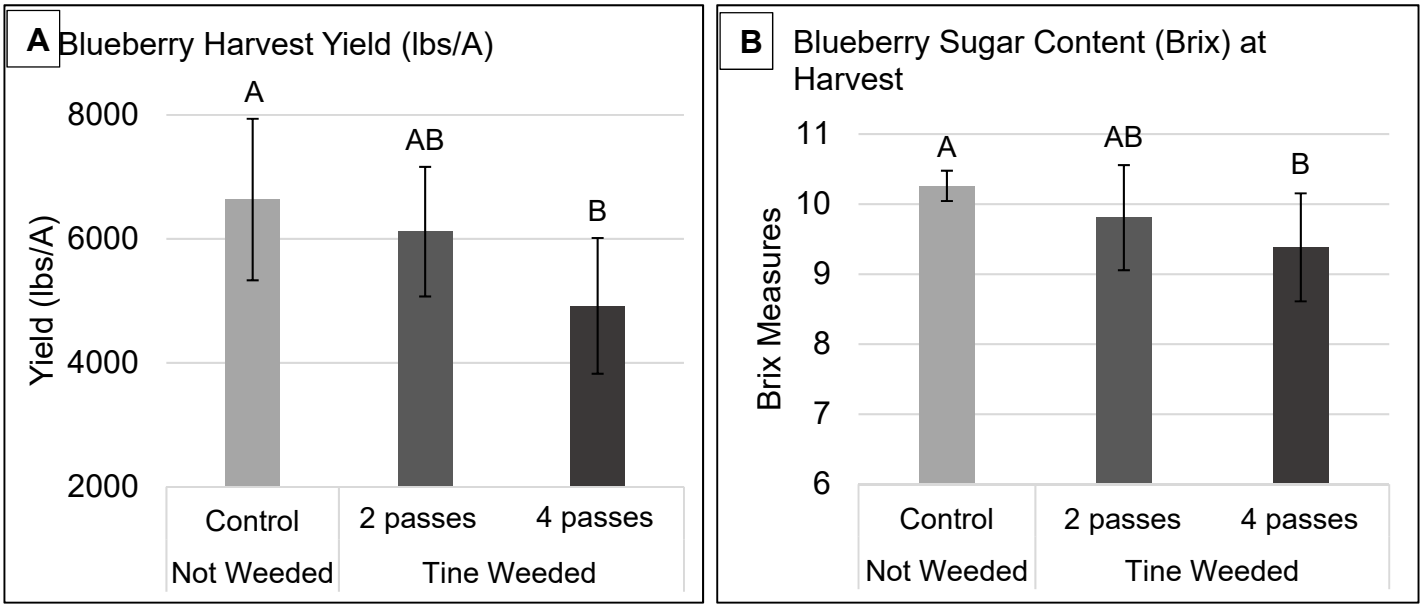

Figure 8. Lowbush blueberry harvest yield (lbs/acre) (8a) and blueberry sugar content (°Bx, degrees of Brix) (8b) by tine weed treatments, harvested on July 29, 2021. Letters indicate significance at the 0.05 level of significance for blueberry harvest yield and sugar content. Graphs are to be compared separately. Error bars indicate the standard error of the mean.

Berry sizes, measured by 100-berry weight followed a similar trend to the harvest yield where berry sizes were small in the tine with two passes and smallest in the tine with four passes relative to the control (data not shown). While no significant treatment differences were detected it is worth noting the berry sizes of the tine treatments were smaller suggesting delayed development, reduced water uptake due to dislodged rhizomes or other growth limiting factors were present.

DISCUSSION

Effects of Tine Weeding on Blueberry Health: Before and After Tine Weeding in 2020

Our comparison of blueberry cover, weed cover, and weed numbers before and after tine weeding showed that this method was an effective weed reduction technique. Two passes with the tine weeder controlled certain weeds, yet wild blueberry yield and blue fruit count was not significantly different than the control.

The control plots saw an increase in weed cover (but not number) as the weeds in these plots were not stressed by the tine weeding. Both two and four passes with the tine weeder reduced weed cover by 9-14% and weed number 52-65%. It also reduced blueberry cover by 17-21% for two and four passes, respectively. These reductions prove the hypothesis that multiple passes with the tine weeder effectively dislodge and remove more and more weed roots with each pass; reasonably, rhizomes of wild blueberry are also dislodged, particularly with multiple tine weeder passes.

Effect of Tine Weeding on the Crop

Disturbance stimulates wild blueberry growth, and this knowledge has been integrated into crop management in the form of mowing or burning to prune the plants and encourage future growth in addition to some more advanced methods of disturbance on some farms (DeGomez, 1988). One of our hypotheses was that tine weeding would stimulate wild blueberry plant production. Over the course of two tine weeding trials in both cycles over 3 years we can conclude that this type of disturbance does not increase plant production. We did see an increase in flower number when prune plants were tined

twice and four times yet this increase did not continue into green fruit and blue fruit at harvest. There were significantly more green fruit at harvest indicating that fruit development was delayed in tined plots. We saw a decreasing trend towards smaller berry size, lower Brix content, and lower yield from control to two and four passes. These delays may be due to a “late pruning” event or from dislodged rhizomes that reduced the plants’ ability to take up nutrients and water.

Effects of Tine Weeding on Weed Presence

Comparison of which weed species were present across treatments made clear that four passes with the tine weeder decreased diversity of weed species present. This may be due to some weed species being more susceptible to tine weeding than others. Interestingly, the most frequently identified top broadleaf species under each of the treatment conditions were exactly the same: red sorrel, St. John’s wort, and violet. The tine weed treatments presented a higher occurrence of broadleaf weeds relative to the control suggesting greater species diversity and more consistent presence across all plots and sample dates. The average occurrence of the annual violet species were three times higher in tine weeded plots relative to the control. Annual plants may have been planted by the tine weeder, which moved seeds from parent plants and dropped the seeds in the top layer of duff, where they lay dormant until the right conditions occurred. Grasses and broadleaf perennials may have grown more numerous as the tine weeder physically broke apart clusters of grasses and rhizomes, thereby stimulating more growth.

CURRENT RECOMMENDATIONS

Generally, we do not recommend tine weeding because our study has found a decrease in several wild blueberry health parameters even though yield itself did not significantly decrease the year after tine weeding. Growers seeking to manage weeds using tine weeding will find two passes with the tine weed sufficient for reducing perennial broadleaf weeds in their fields, IF the field is level, with minimal adverse impacts on fruit yield and quality. Tine weeding is not recommended for growers with grasses and annual weeds. The speed and pressure settings for the tine weeder will need to be adjusted based on localized conditions, such as field rockiness and levelness. Growers interested in tine weeding are encouraged to do an assessment of what weeds are present in their fields prior to tine weeding in order to make sure tine weeding is right for their field and to measure the impact of tine weeding at your location.

NEXT STEPS

- Continue monitoring weed and blueberry cover in the 2022 season to confirm that wild blueberry is not stimulated by tine weeding
- Collect more information on the presence of weed types by annual and perennial.

ACKNOWLEDGEMENTS

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RESEARCH

INVESTIGATOR(S): L. Calderwood, A. Ayers, and B. Tooley

2. Wild Blueberry Weed Survey

OBJECTIVE(S)

- Determine the current weed composition in lowbush blueberry fields
- Compare weed communities and wild blueberry cover between organically and conventionally managed fields
- Compare this weed survey's results to Yarborough and Bhowmik's 1980 survey
- Evaluate the perceptions that growers have about weeds in their fields and better understand how growers manage these weeds

LOCATION(S): Twenty wild blueberry fields located in Cumberland, Knox, Lincoln, Waldo, Hancock, and Washington counties of ME.
PROJECT TIMEFRAME: 2019 - 2021

INTRODUCTION
Weeds are one of the most challenging pests for wild blueberry growers since they compete directly with wild blueberry for sunlight, nutrients and water and can reduce yields by up to 4,000 pounds per acre (NBDAAF 2017; Yarborough 2012). Thus, weed management is a crucial component of wild blueberry farm management to reduce weed competition and the presence of pest insects and diseases. The risk for diseases such as mummy berry (*Monilinia vaccinii-corymbosi*) is elevated in fields with poor weed management since more moisture becomes trapped in the top canopy (Drummond et al. 2012). While weeds compete with wild blueberry and can increase the risk of disease and insect pests, weed species can serve as habitat for native and managed bees and natural enemies of known pests (Kremen and Miles, 2012; Drummond et al. 2017).

Prior to this survey, the most recent weed survey of Maine's wild blueberry fields was conducted in 1980 and the results were published in 1989. (This report refers to this paper as the 1980 survey, but citation for the actual paper contains its 1989 publication year.) This study evaluated the impact of the herbicide hexazinone on weed populations at 14 wild blueberry fields, and found several weed genera in each surveyed field, including goldenrod (*Solidago spp.*), wintergreen (*Gaultheria procumbens)* and willow (*Salix spp.)* (Yarborough and Bhowmik, 1989). Since 1980, invasive species' ranges have expanded, climate change has continued, and wild blueberry growers have additional tools for handling weeds; these factors necessitated an updated weed survey on Maine's wild blueberry farms.

METHODS
Twenty total fields were surveyed in 2019 and 2020 (ten per year) and fields were selected to represent multiple geographic regions and management practices. Within each geographic region (Midcoast, Ellsworth, Downeast, Way Downeast), at least one organic and one conventional field were surveyed (Table 1). Fields were surveyed during early summer (June – early July) and late summer (August – early September) during the prune year since this is the year most weed management occurs.

Table 1. Soil characteristics (soil type and pH) relative to location by region, county and sample year and field management type (conventional vs. organic).

Survey Locations		Year Sampled	Management Type	Soil		
Region	County			Type[a]	pH	OM[b]
Midcoast	Knox	2019	Conventional	Lyman-Rock outcrop Tunbridge complex	4.8	16.0
		2019	Conventional	Tunbridge-Lyman complex	4.9	13.1
		2019	Organic	Marlow fine sandy loam	3.9	12.0
		2020	Organic	Lyman-Rock outcrop Tunbridge complex	4.3	12.0
		2019	Organic	Lyman-Rock outcrop Tunbridge complex	4.4	15.2
	Lincoln	2020	Conventional	Lyman-Rock outcrop Tunbridge complex	4.4	18.1
Ellsworth	Hancock	2019	Conventional	Colton gravelly sandy loam	4.4	16.5
		2020	Conventional	Peru Fine Sandy Loam & Peru-Colonel complex	4.7	14.1
		2019	Organic	Hermon-Monadnock-Skerry complex	4.0	10.9
		2020	Organic	Peru-Colonel complex	4.2	16.5
Downeast	Washington	2019	Conventional	Colton gravelly sandy loam	4.5	14.5
		2020	Conventional	Colton gravelly sandy loam	4.7	19.3
		2020	Conventional	Colton gravelly sandy loam	5.0	5.8
		2020	Organic	Rawsonville-Lamoine-Hogback complex	4.4	16.4
		2020	Organic	Colton gravelly sandy loam	4.6	16.3
		2019	Organic	Lyman-Tunbridge-Abram complex	4.9	16.9
Far Downeast		2019	Conventional	Peru-Colonel complex	4.9	11.9
		2020	Conventional	Colton gravelly sandy loam	4.9	9.6
		2019	Organic	Rawsonville-Hogback complex	4.9	26.5
		2020	Organic	Hermon-Monadnock-Skerry complex	5.2	15.5

[a] Soil types were obtained from NRCS (NRCS 2021)
[b] OM, organic matter (%)

Data collection

One study site (up to 165 m^2 or approximately 0.04 acres in size) was set up in each field, more than 15.5 m from the field edge. This study site size was chosen since most fields are much larger than 165 m^2 but several are smaller and thus could accommodate the study size without modification to the study site dimensions. Within each study site, four transects were placed in the field in a M pattern; since block size was malleable, transect lengths varied site to site but averaged 70 m long. Three 1 m^2 quadrats were placed at the intersects of the lines comprising the M shape and 14 more quadrats were equally spaced along the lines; each M contained 17 quadrats (17 m^2), and an additional 3 quadrats (3 m^2) were randomly placed on the field edges outside of the M shape to identify what species overlap exists between the field edges and center. Quadrat placement at each site covered 20 m^2. The entire survey across both years included data from 400 total quadrats (400 m^2), of which 220 quadrats (220 m^2) were in conventionally managed fields and 180 quadrats (180 m^2) in organic fields.

Within each quadrat, broadleaf weed, grass, sedge, rush and wild blueberry cover was ranked using the Daubenmire Cover Scale, where 0 = not present, 1 = ≤1-5% coverage, 2 = 6-25% coverage, 3 = 26-50% coverage, 4 = 51-75% coverage, 5 = 76-95% coverage and 6 = 96-100% coverage (Daubenmire 1959). In addition, all weed species in each quadrat were identified to genus level (at minimum) and ranked using the Daubenmire Cover Scale.

Questionnaires were sent to participating growers via e-mail and snail mail in order to understand field management history and grower perceptions of weed severity. Responses were gathered between June and December 2019.

Data Analysis

Each Daubenmire Cover Scale rating was converted to the midpoint of the range; a Daubenmire rating of 1 (1-5%) was converted to 2.5%, 2 (6 – 25%) converted to 15%, 3 (26 – 50%) converted to 37.5%, 4 (51 – 75%) converted to 62.5%, 5 (76 – 95%) converted to 85%, and 6 (96 – 100%) converted to 97.5%. These percentages were then converted to area (m^2) and reported as surface area covered by that species. Surface area covered for each weed was summed to rank weed genera or species by area for each survey period (early or late summer weeds) and management practice (conventional or organic). In addition, a summary table for the entire survey was generated to detail weed species, frequency, and surface area covered. Field uniformity was calculated as the number of fields where a given weed occurred.

To compare results between this survey and the 1980 survey, weed species and genera were totaled and categorized by life cycle: annuals, biennials, herbaceous perennials, woody perennials, ferns, and grasses. These life cycles were confirmed using the USDA NRCS Plant Database (USDA 2020). Weeds identified only to genera were assumed to species represented in the University of Maine Cooperative Extension's Weed ID Tool (Calderwood 2020).

Species diversity was evaluated using the Shannon diversity index value (*H'*) calculated for each quadrat, where:

$$H' = -\Sigma_i \, p_i \, ln(p_i)$$

Here, p_i is the proportion of individuals within the *i*th species of each quadrat. The proportional abundance (evenness) of the species present is reflected in the calculated diversity indices. The data did not meet distributional assumptions for a parametric analysis and, hence non-parametric methods were used. The Wilcoxon-Mann Whitney test was used to compare species number, Shannon diversity index, blueberry cover and weed cover, similar to Hyvönen and Salonen (2002).

In the grower questionnaire, growers were asked to identify and rank weed species, prevalence, management techniques, and weed impact on growing wild blueberry. These responses were compared with the field survey results and matches identified.

RESULTS

In the 2019 and 2020 weed survey, 71 weed genera of 34 families were identified. When grouped by weed type (broadleaf, grass, woody) and life cycle (annual vs. perennial), conventionally managed fields were dominated by herbaceous broadleaf annuals (15%), perennials (62%) and grasses (13%) (Table 2). Organically managed fields also had a high occurrence of herbaceous broadleaf perennials (52%), followed by woody weeds (25%) and grasses (12%).

Table 2. Weed frequency and area grouped by life cycle, observed in organically managed (O) and conventionally managed (C) fields in Maine lowbush blueberry fields in 2019 and 2020.

		2019 & 2020			
		Frequency (%)†		Average Area (%/m^2)	
Weed Type and Life Cycle		**O**	**C**	**O**	**C**
Annual	**Herbaceous**	8%	15%	7%	7%
Perennial	**Broadleaf**	53%	62%	11%	9%
Perennial	**Fern**	2%	2%	37%	11%
	Woody	25%	8%	12%	10%
	Grass	12%	13%	12%	10%

† Here, weed frequency was calculated as a function of all weed observations by management type. Weed observations totaled 396 and 1087 for conventional and organic fields, respectively, across 200 quadrats per management strategy.

Weed abundance (number of genera), species diversity (Shannon *H'* index) and weed cover were significantly greater in organic fields than conventional fields (Table 3). The average number of species observed in organic fields (2.79 weeds/m^2) were 64% greater than that of conventional fields (0.99 weeds/m^2), when measured across all geographic regions. When organized by geographic region, the greatest species abundance in organic fields was observed Far Downeast (4.26 spp. per quadrat), while the lowest abundance occurred Downeast (2.11 spp. per quadrat). In conventional fields, the greatest species abundance also occurred Far Downeast (1.79 spp. per quadrat), while the lowest abundance was observed in Ellsworth (0.21 spp. per quadrat).

Table 3. The mean value and differences in the number of genera (# per quadrat), Shannon diversity indices (by quadrat), weed cover (%/m^2), and lowbush blueberry cover (%/m^2) between management strategies, evaluated by growing region (Wilcoxon Two-sample Test).

		Mean		**Wilcoxon Two-sample Test**			
Region	**Variable**	**O†**	**C**	***Z***	***n***	**df**	***p-value***
Midcoast	No. of Genera	2.55	0.86	6.29	120	118	<0.0001
	Shannon *H'*	0.21	0.11	4.77	120	118	<0.0001
	Weed Cover	25%	8%	5.35	120	118	<0.0001
	Blueberry Cover	52%	68%	-4.53	120	118	<0.0001
Ellsworth	No. of Genera	2.73	0.21	8.61	80	78	<0.0001
	Shannon *H'*	0.27	0.02	8.65	80	78	<0.0001
	Weed Cover	34%	2%	8.69	80	78	<0.0001
	Blueberry Cover	50%	72%	-5.37	80	78	<0.0001
Downeast	No. of Genera	2.11	1.11	5.94	120	118	<0.0001
	Shannon *H'*	0.29	0.14	6.38	120	118	<0.0001
	Weed Cover	32%	12%	-6.68	120	118	<0.0001
	Blueberry Cover	55%	61%	-1.36	120	118	0.1733
Far Downeast	No. of Genera	4.26	1.79	4.7	80	78	<0.0001
	Shannon *H'*	0.26	0.14	4.44	80	78	<0.0001
	Weed Cover	39%	14%	4.78	80	78	<0.0001
	Blueberry Cover	51%	75%	-5.67	80	78	<0.0001
All Locations	No. of Genera	2.795	0.99	12.23	400	398	<0.0001
	Shannon *H'*	0.26	0.11	11.92	400	398	<0.0001
	Weed Cover	32%	8%	-12.62	400	398	<0.0001
	Blueberry Cover	52%	68%	-8.04	400	398	<0.0001

† *Organic (O) and Conventional (C)*

Species diversity indices were significantly higher in organically managed fields compared to conventional fields. The observed regions with the greatest weed species diversity were Downeast (organic, *H'* = 0.29; conventional, *H'* = 0.14) and Far Downeast (conventional, *H'* = 0.14). The Far Downeast region presented the greatest average weed cover for both organic (39%/m^2) and conventional fields (14%/m^2). Observed blueberry covers were highest in the Downeast and Far Downeast regions for organic (55%/m^2) and conventional fields (75%/m^2), respectively.

The 71 total genera identified in 2019 and 2020 were more than double the number of weed genera identified in the 1980 weed survey of conventionally managed wild blueberry fields. In the 1980 weed survey conducted by Yarbrough and Bhowmik (published 1989), Maine lowbush blueberry fields were dominated by grass (52% frequency) followed by woody weeds (18%) and herbaceous broadleaf perennials (13%) (Table 4, Figures 1 and 2). In contrast, this most recent weed survey shows a shift in dominant weed species to herbaceous broadleaf perennials (62% frequency), followed by annuals (15%) and grasses (15%).

Table 4. Comparison of weed frequency observations grouped by life cycle from the 1980 weed survey of conventionally managed wild blueberry fields in Maine with the 2019 and 2020 weed survey of conventional fields.

Weed Type and Life Cycle		Frequency (%)		Change (%)
		1980	2019 & 2020	1980 to 2020
Annual	**Herbaceous**	0%	15%	15%
Perennial	**Broadleaf**	13%	62%	49%
	Ferns	6%	2%	-5%
Perennial	**Woody**	18%	8%	-10%
	Grass	52%	13%	-38%

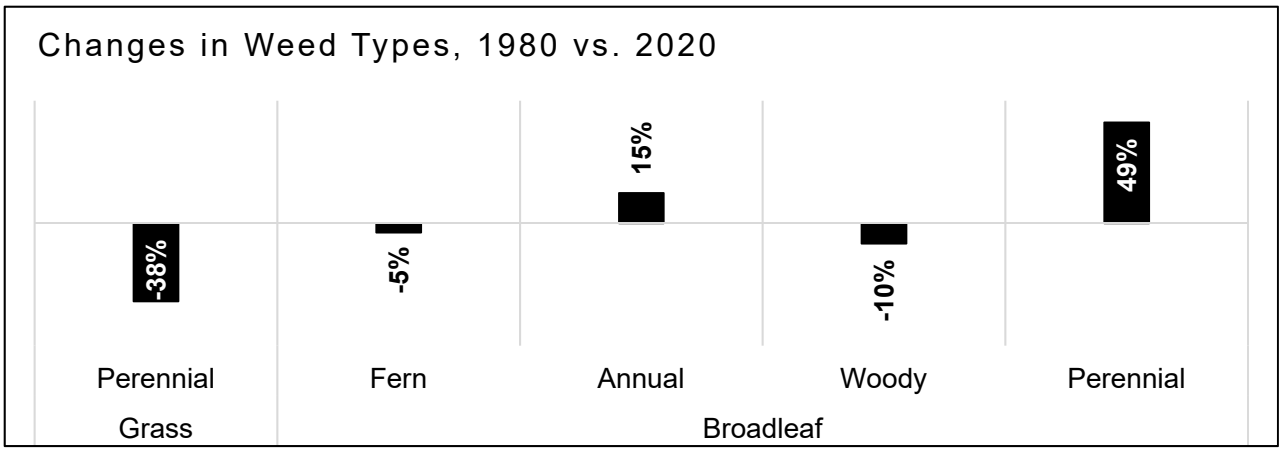

Figure 1. Changes in weed type and lifecycle (%) from the 1980 weed survey and the 2019/2020 weed survey.

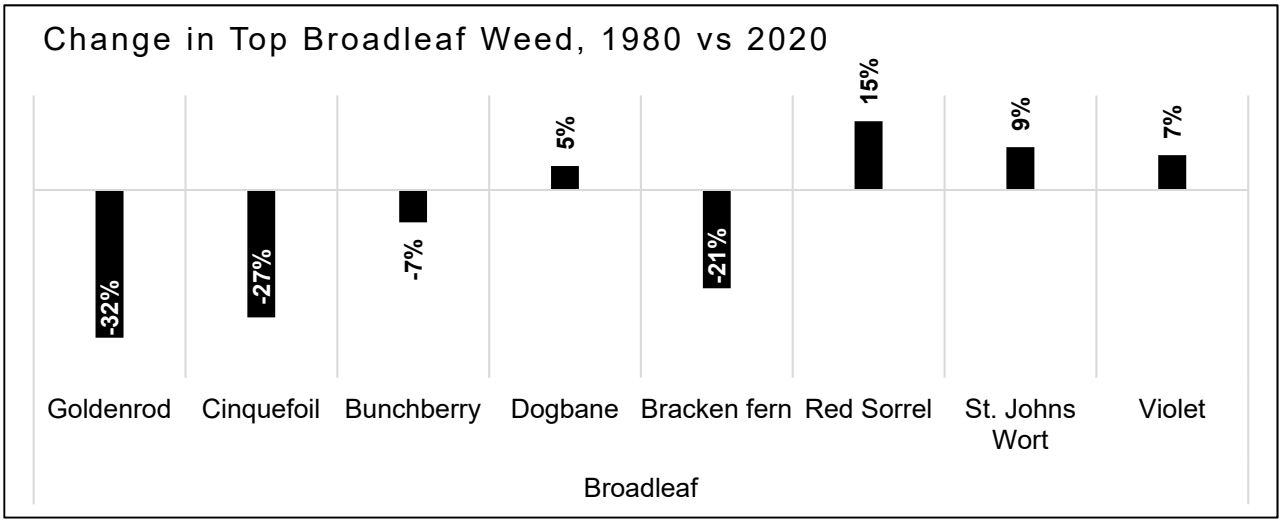

Figure 2. Changes in the top broadleaf weeds (%) from the 1980 weed survey and the 2020 weed survey.

In organically managed fields surveyed 2019 & 2020, goldenrod species had the highest field uniformity (34%) followed by grass (18.4%) and red sorrel (15%) (Table 5). In these same fields, species with the greatest area of cover were elm (0.39m^2), bracken fern (0.37m^2) and mountain laurel (0.20m^2). Weed species with the highest field uniformity in conventionally managed fields were red sorrel (14.5%), St. John's wort (12.5%) and grass (9%). While species with the greatest area of coverage were chokeberry (0.38m^2), followed by mountain laurel, black huckleberry and aster (each with an average of 0.16m^2 area of coverage). Due to genera sharing a common descent, 20-29% of all genera identified belonged to the Asteraceae (aster) family, while 12-13% of all genera identified belonged to the Rosaceae family.

Table 5. Observed weed species with associated coverage (m^2) and frequency sampled across 20 Maine lowbush blueberry prune fields with two types of management, organic (O) and conventional (C) over two years (2019 and 2020).

WEED GENERA PRESENT					2019 & 2020 Frequency†		Coverage	
Common Names of Species	**Family**	**Genus**	**Species EPPO**	**Life Cycle**	**O**	**C**	**O**	**C**
					------ % ------		----- m2 -----	
RED MAPLE	Sapindaceae	*Acer*	ACRSS	W	1.8	0.5	0.030	0.030
COMMON YARROW	Asteraceae	*Achillea*	ACHSS	P	0.5	0.0	0.030	0.000
ALDER SPP.	Betulaceae	*Alnus*	ALUSS	W	1.0	0.0	0.061	0.000
SPREADING DOGBANE	Apocynaceae	*Apocynum*	APCSS	P	13.8	6.5	0.144	0.073
SARSAPARILLA	Araliaceae	*Aralia*	ARLSS	P	0.5	0.5	0.030	0.093
CHOKEBERRY	Rosaceae	*Aronia*	ABOSS	W	10.8	0.5	0.148	0.380
SILK WEED	Apocynaceae	*Asclepias*	ASCSS	P	0.3	0.0	0.155	0.000
ASTERS SPP.	Asteraceae	*Aster*	ASTSS	P	1.5	0.3	0.109	0.155
BIRCH SPP.	Betulaceae	*Betula*	BETSS	W	5.3	1.5	0.155	0.072
SEDGE SPP.	Cyperaceae	*Carex*	CRXSS	G	2.3	0.0	0.141	0.000
FIREWEED	Onagraceae	*Chamerion*	CHASS	P	0.3	0.3	0.030	0.030
SWEET FERN	Myricaceae	*Comptonia*	COVSS	W	0.3	0.0	0.030	0.000
HORSEWEED	Asteraceae	*Conyza*	CNDSS	A	0.0	1.3	0.000	0.055
BUNCHBERRY	Cornaceae	*Cornus*	CRWSS	P	11.3	7.5	0.187	0.138
HAWTHORN	Rosaceae	*Crataegus*	CSCSS	P	0.3	0.0	0.030	0.000
POVERTY OATGRASS	Poaceae	*Danthonia*	DANSS	G	9.3	4.3	0.119	0.122
NORTHERN HONEY SUCKLE	Diervillaceae	*Diervilla*	DIVSS	W	3.0	0.0	0.080	0.000
BURNWEED	Asteraceae	*Erechtites*	ERESS	A	5.3	1.0	0.094	0.093
EASTERN DAISY FLEABANE	Asteraceae	*Erigeron*	ERISS	A	1.3	0.5	0.055	0.093
COMMON BONESET	Asteraceae	*Eupatorium*	EUPSS	P	1.0	0.0	0.061	0.000
CYPRESS SPURGE	Euphorbiaceae	*Euphorbia*	EPHSS	P	0.8	0.0	0.113	0.000
EYEBRIGHT	Orobanchaceae	*Euphrasia*	EPASS	A	2.0	0.0	0.093	0.000
WILD STRAWBERRY	Rosaceae	*Fragaria*	FRASS	P	1.8	0.0	0.066	0.000
WINTERGREEN	Ericaceae	*Gaultheria*	GAHSS	P	1.3	0.0	0.055	0.000
HUCKLEBERRY	Ericaceae	*Gaylussacia*	GAYSS	W	0.3	0.3	0.030	0.155
GROUND IVY	Lamiaceae	*Glechoma*	GLESS	P	0.0	0.3	0.000	0.030
HAWKWEED	Asteraceae	*Hieracium*	HIESS	P	8.3	1.0	0.091	0.061
AZURE BLUET	Rubiaceae	*Houstonia*	HOUSS	P	2.8	0.0	0.064	0.000
ST. JOHN'S WORT	Hypericaceae	*Hypericum*	HYPSS	P	6.0	12.5	0.056	0.084
RUSH SPP.	Juncaceae	*Juncus*	IUNSS	G	2.0	0.0	0.061	0.000
JUNIPER SPP.	Cupressaceae	*Juniperus*	IUPSS	W	0.0	0.3	0.000	0.030
LAUREL SPP.	Ericaceae	*Kalmia*	KAMSS	W	4.0	0.3	0.202	0.155
WILD LETTUCE	Asteraceae	*Lactuca*	LACSS	A (B)	0.0	2.0	0.000	0.048

OX-EYE DAISY	Asteraceae	*Leucanthemum*	LCASS	P	3.0	0.0	0.061	0.000
YELLOW TOADFLAX	Plantaginaceae	*Linaria*	LINSS	P	1.5	0.5	0.030	0.030
LOOSESTRIFE SPP.	Primulaceae	*Lysimachia*	LYSSS	P	4.8	2.8	0.157	0.053
CANADIAN MAYFLOWER	Asparagaceae	*Maianthemum*	MNHSS	P	6.8	1.0	0.049	0.061
COW-WHEAT	Orobanchaceae	*Melampyrum*	MEASS	A	5.5	0.8	0.041	0.072
SANDWORT	Caryophyllaceae	*Moehringia*	MGJSS	P	2.3	0.8	0.030	0.030
SWEET GALE	Myricaceae	*Myrica*	MYRSS	W	0.5	0.0	0.093	0.000
BLUE TOADFLAX	Plantaginaceae	*Nuttallanthus*	NUTSS	P	3.5	5.8	0.048	0.046
EVENING-PRIMROSE	Onagraceae	*Oenothera*	OEOSS	A (B)	0.0	0.3	0.000	0.030
PINE SPP.	Pinaceae	*Pinus*	PIUSS	W	0.0	0.5	0.000	0.030
PLANTAIN	Plantaginaceae	*Plantago*	PLASS	P	0.3	0.0	0.030	0.000
GRASS SPP.	Poaceae	*Poaceae*	POASS	G	18.3	9.0	0.122	0.095
MILKWORT	Polygalaceae	*Polygala*	POGSS	A	1.0	0.0	0.030	0.000
COTTONWOOD	Salicaceae	*Populus*	POPSS	W	1.8	1.0	0.119	0.030
CINQUEFOIL SPP.	Rosaceae	*Potentilla*	PTLSS	P	9.0	0.3	0.082	0.030
WHITE RATTLESNAKE ROOT	Asteraceae	*Prenanthes*	PNNSS	P (B)	0.5	0.3	0.030	0.030
CHERRY SPP.	Rosaceae	*Prunus*	PRNSS	W	10.3	1.5	0.087	0.134
BRACKEN FERN	Dennstaedtiaceae	*Pteridium*	PTESS	F	5.0	1.5	0.365	0.113
OAK SPP.	Fagaceae	*Quercus*	QUESS	W	0.3	0.0	0.030	0.000
ROSE SPP.	Rosaceae	*Rosa*	ROSSS	W	5.8	0.0	0.126	0.000
BRAMBLE SPP.	Rosaceae	*Rubus*	RUBSS	W	8.3	0.3	0.155	0.030
BLACK EYED SUSAN	Asteraceae	*Rudbeckia*	RUDSS	P	5.5	2.8	0.058	0.064
RED SORREL & CURLY DOCK	Polygonaceae	*Rumex*	RUMSS	P	15.0	14.5	0.125	0.099
WILLOW SPP.	Salicaceae	*Salix*	SAXSS	W	0.3	0.0	0.155	0.000
RAGWORT	Asteraceae	*Senecio*	SENSS	A	0.0	1.3	0.000	0.055
BLUE-EYED GRASS	Iridaceae	*Sisyrinchium*	SISSS	P	0.8	0.3	0.030	0.030
GOLDENROD SPP.	Asteraceae	*Solidago*	SOOSS	P	34.0	3.5	0.140	0.101
SPINY SOWTHISTLE	Asteraceae	*Sonchus*	SONSS	A	0.3	0.0	0.030	0.000
MEADOWSWEET & SPIREA	Rosaceae	*Spiraea*	SPVSS	W	11.8	0.8	0.093	0.072
COMMON DANDELION	Asteraceae	*Taraxacum*	TARSS	P	0.5	0.5	0.030	0.030
YELLOW SALSIFY	Asteraceae	*Tragopogon*	TROSS	A (B)	0.0	0.3	0.000	0.030
STARFLOWER	Primulaceae	*Trientalis*	TNTSS	P	0.5	0.0	0.030	0.000
TREFOIL (CLOVER)	Fabaceae	*Trifolium*	TRFSS	A & P	4.0	0.0	0.093	0.000
ELM SPP.	Ulmaceae	*Ulmus*	ULMSS	W	0.8	0.3	0.388	0.030
COMMON SPEEDWELL	Plantaginaceae	*Veronica*	VERSS	P	0.3	0.0	0.030	0.000
VIBURNUM	Adoxaceae	*Viburnum*	VIBSS	W	1.0	0.0	0.124	0.000
VETCH SPP.	Fabaceae	*Vicia*	VICSS	A & P	2.3	0.0	0.058	0.000
VIOLET SPP.	Violaceae	*Viola*	VIOSS	A	7.0	7.3	0.070	0.077

The 2-dimensional ordination plots generated by principal component analyses (PCA) show the magnitude of species correlation (species proximity or grouping on the plot) or species antagonism (polarity or positioning on opposite ends of the plot). How these species are positioned (grouped or un-grouped) in an ordination plot is typically due to species traits or environmental factors. In organically managed wild blueberry fields, soil pH appeared to be a clear indicator of species occurrence (Figure 3a-d). The three dominant woody weeds (bramble, meadowsweet and mountain laurel species) corresponded to the lowest soil pH value of 3.80 and were most correlated to the Midcoast region; while their counterparts (common sheep sorrel, and sedge species) corresponded to soil pH of 4.63 and the Downeast region.

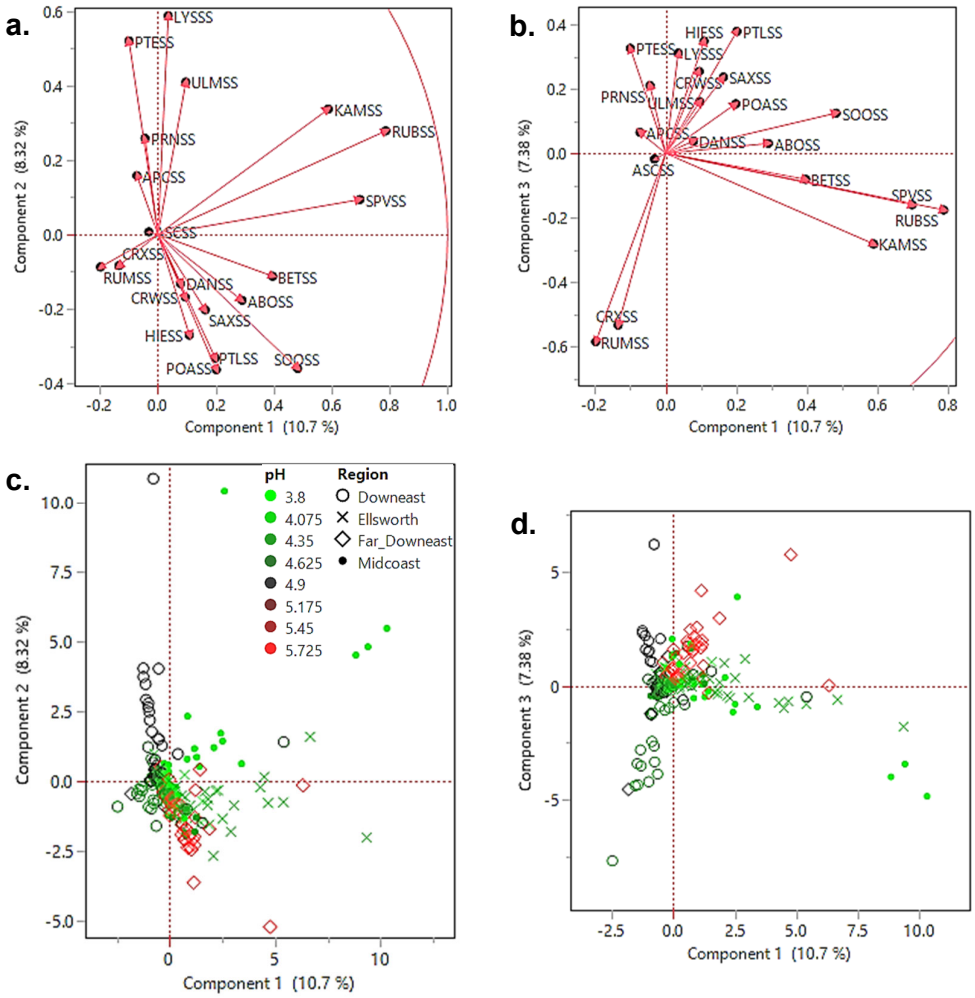

Figure 3a-d. Principal Component Analysis (PCA) ordination diagrams for organically managed Maine lowbush blueberry prune fields in 2019 and 2020. Top 20 most abundant weed genera observed are reported. The supplementary variable (soil pH) is displayed for principal components 1 & 2 (Fig. 3c) and 1 & 3 (Fig. 3d). The relative species distributions, abbreviated using EPPO codes (OEPP/EPPO, 2021) are displayed for principal components 1 & 2 (Fig. 3a) and 1 & 3 (Fig. 3b), see Table 1 for full species names.

The soil pH range of conventionally managed fields was much narrower (between 4.35 and 4.95) (Figure 4a-d). In these fields, grass, poverty oat grass, black eyed Susan, spreading dogbane, cherry species, aster, and red sorrel corresponded to pH values between 4.65 and 4.85. Many of the species listed in Table 5 did not correlate to specific regions except for bunchberry, chokecherry and goldenrod. These weeds appeared to correspond a slightly lower pH and the Midcoast region and the grasses were associated most with the Downeast region.

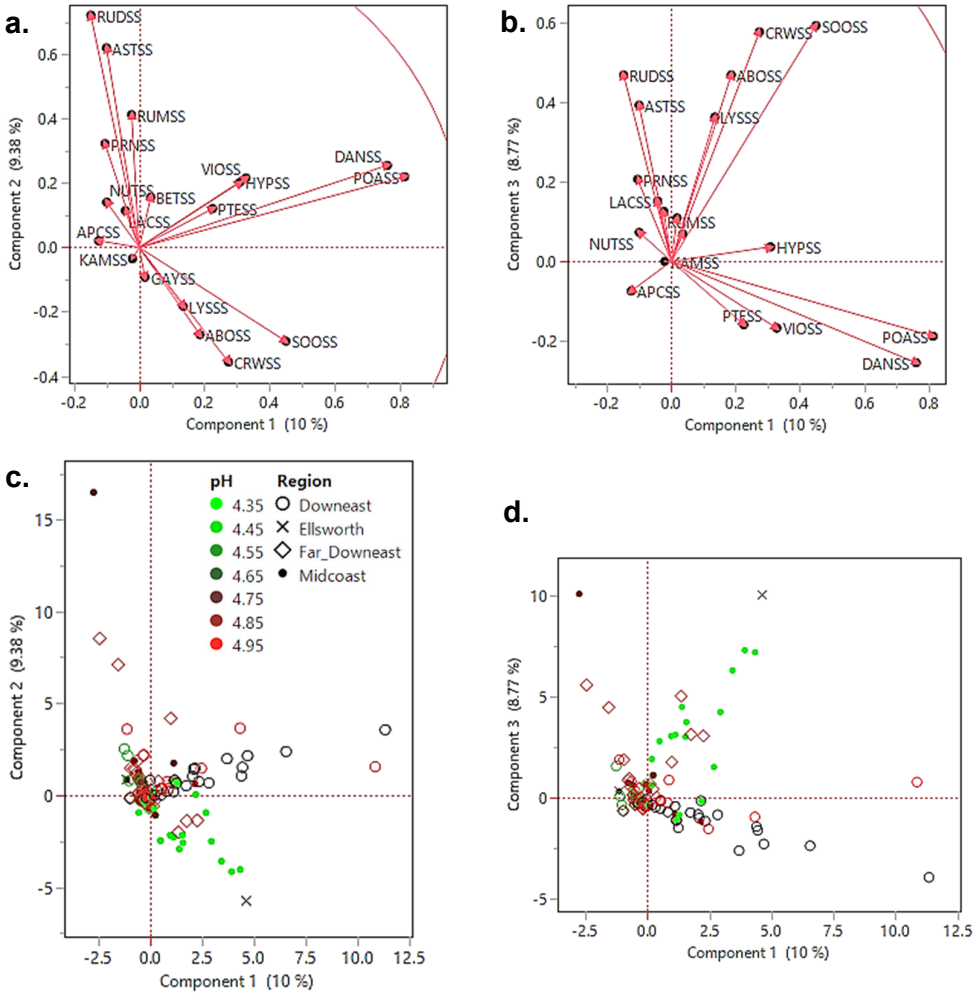

Figure 4a-d. Principal Component Analysis (PCA) ordination diagrams of conventionally managed Maine lowbush blueberry prune fields in 2019 and 2020. The top 19 weed genera observed are reported. The supplementary variable, soil pH is displayed for principal components 1 & 2 (Fig. 4c) and 1 & 3 (Fig. 4d). The relative species distributions, abbreviated using EPPO codes (OEPP/EPPO, 2021) are displayed for principal components 1 & 2 (Fig. 4a) and 1 & 3 (Fig. 4b), see Table 1 for full species name.

In 2019, twenty-one participating growers completed a survey about weed management practices and conditions in their fields; some survey responses required typing responses, rating options, and answering yes or no questions. Some results are listed below:

- "How important is weed management to wild blueberry yield and quality?"
  - 20 of 21: "very important"
  - 1 of 21: "somewhat important"
- "How weedy are your fields?"
  - 15 of 21: many weeds
  - 6 of 21: few weeds
- "What would you say is the most abundant weed(s) across all of your fields?"
  - Grasses (7)
  - Goldenrod (5)
  - Dogbane (6)
  - Bracken fern (3)
  - Huckleberry (2)
  - St. John's wort, vetch, bittersweet, sweet fern, poplar trees, sheep laurel (1 each)
- "Is the most abundant weed on your farm a top concern?"

    - 14 of 21: yes
    - 7 of 21: no
- “We surveyed one of your fields indicated on the map. Was this location representative of weed pressure in all of your fields?”
    - 5 of 21: “The surveyed field has fewer weeds than other fields”
    - 11 of 21: “The surveyed field has the same weeds than other fields”
    - 5 of 21: “The surveyed field has more weeds than other fields”
- “Over the past 2 cycles, how have you managed weeds? Check all that apply.”
    - 11 of 21: herbicide
    - 9 of 21: sulfur
    - 11 of 21: hand pulling
    - 9 of 21: burning
    - 13 of 21: weed whacking
    - 15 of 21: mowing
    - 1 of 21: wood chips
- “Over the past 2 cycles, have you applied fertilizer?”
    - 7 of 21: yes
    - 14 of 21: no
- “Do you mow or burn your fields?”
    - 10 of 21: mow
    - 1 of 21: burn
    - 10 of 21: both

DISCUSSION

This updated survey of wild blueberry fields showed that the weed communities varied greatly between regions and were dependent on field management style. Weed abundance, diversity, and cover were greater in organic fields than conventional, and the average number of species observed in organic fields were greater than in conventional fields. The Far Downeast region saw the greatest average weed cover for both organic and conventional fields, and average blueberry cover was highest in the Downeast (organic) and Far Downeast (conventional) regions possibly due to the high number of small grower fields that are now under managed in this region.

Seventy-one total genera were identified, and this number was more than double the number of genera identified in the survey published in 1989. The earlier survey identified grasses (52% frequency), woody weeds (18%), and herbaceous broadleaf perennials (13%) as the most frequent weeds, while the recent survey identified herbaceous broadleaf perennials (62%), annuals (15%), and grasses (15%) as the most dominant species. The shift in dominant weed species may be due to a combination of the increased interchange between Maine and Canadian wild blueberry fields, the development of herbicide resistant weeds, the shift from broad to narrow spectrum herbicides, an increase in organic production, and our more global society where foreign plant material, introduced and invasive, is more common.

Our PCA analysis allowed us to look at Maine wild blueberry weeds grouped by region, other weeds, and soil pH. The soil pH range of conventionally managed fields was much narrower (between 4.35 and 4.95). In these fields, grass, poverty oat grass, black eyed Susan, spreading dogbane, cherry species, aster, and red sorrel corresponded to pH values between 4.65 and 4.85. Bunchberry, chokecherry and goldenrod appeared to correspond a slightly lower pH and the Midcoast region. The grasses were associated most with the Downeast region. In organically managed wild blueberry fields, soil pH appeared to be a clear indicator of species occurrence. The three dominant woody weeds, bramble, meadowsweet and mountain laurel species corresponded to the lowest soil pH value of 3.80 and were most correlated to the Midcoast region. Their counterparts, common sheep sorrel, and sedge species corresponded to soil pH of 4.63 and the Downeast region.

The results from the growers surveyed reveal useful information. Nearly all growers (20 of 21; 95%) identified weed management as “very important" for wild blueberry yield and quantity and most growers (15 of 21; 71%) classified their fields as having “many weeds”. This is not new information, but it does indicate a willingness by growers to better understand and integrate new weed management techniques into their field management. Understanding which weeds are growing where is the first step in developing an effective weed control strategy. Growers listed grasses, goldenrod, and dogbane as the most prevalent weeds across all fields and 14 of 21 (66%) agreed that the most abundant weed in their fields were a top concern. While the weeds dominating the fields were varied, growers have used a range of techniques to manage them, including herbicides (11 of 21; 53%), sulfur to reduce soil pH (9 of 21; 43%), hand pulling (11 of 21; 53%), burning (9 of 21; 43%), weed whacking (13 of 21; 62%), and mowing (15 of 21; 71%). Most growers have not applied fertilizers to their fields in the last 2 cycles (14 of 21; 67%). Understanding which management techniques are currently used and favored by growers offers an indication of which types of weeds might become more easily established in wild blueberry fields. Based on the survey responses here, roughly half of growers employ each weed management technique making it difficult to make a broad statement about which methods are most predicative of certain weeds.

Growers are the first people to understand which weeds are growing well and moving spreading, and which weeds are no longer prevalent. Half of the growers indicated that the location surveyed in their fields were as weedy as their other fields (11 of 21), and the remaining half indicated that the surveyed location was more (5 of 21) or less (5 of 21) weedy than their other fields. This is a good measure to have since it confirms that the locations sampled were indicative of the individual fields, and by extrapolation, indicative of regional trends as well.

CURRENT RECOMMENDATIONS

Walking wild blueberry fields to produce a list of present weeds is the first step in creating a plan for managing weed pests. Identify the weeds using the University of Maine Cooperative Extension Weed ID Tool or contacting Dr Lily Calderwood (lily.calderwood@maine.edu) for extra assistance. Evaluating weed presence should be done every 10 years to maintain successful weed management plans.

ACKNOWLEDGEMENTS

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RESEARCH

INVESTIGATOR(S): L. Calderwood

3. Establishing economic thresholds for common weeds in wild blueberry fields

OBJECTIVE(S)
Gather preliminary field data to develop larger research project on weed economic thresholds for bunchberry and red sorrel.

LOCATION(S): Surry and Hope, ME
PROJECT TIMEFRAME: 2021 – 2023

INTRODUCTION
Wild blueberries are an economically and culturally important crop in Maine that brings millions of dollars into the state's economy each year. In 2008, the industry brought $54.8 million to the state, but the value of this industry has decreased annually, bringing in just $28.6 million in 2020 (Quick Stats). Approximately 485 growers produce wild lowbush blueberries on 41,000 acres, and in 2017, 76% of surveyed Maine growers did not make a profit (USDA, 2020; Calderwood 2018 Grower Survey). Weeds are one of the most challenging pests for wild blueberry growers because they compete directly with wild blueberry for sunlight, nutrients, and water and can reduce yield by up to 4,000 pounds per acre (Yarborough, 2012). Maine's blueberry growers are aware of this and 95% of 21 interviewed growers in 2020 ranked weed management as "very important" for wild blueberry yield, 71% classified their fields as having "many weeds", and 66% agreed that the most abundant weed in their field was a top management concern (Weed Survey Grower Interviews, 2020).

Growers utilize a range of integrated pest management (IPM) techniques to manage weeds including herbicides, sulfur to reduce soil pH, hand pulling and weed whacking, and burning and/or mowing fields. The method of control depends on the management style (organic or conventional), prevalence, and type of weed(s) being controlled. Tools missing from our IPM weed toolbox are economic thresholds. Economic thresholds are more commonly used to manage insect and disease pests, yet they can also be developed for weeds. A weed economic threshold would assist growers in their decision making around when to control a particular weed. Once a weed reaches a certain density or spread that is projected to reduce yield (income), then it would be time to control the weed with the grower's chosen method(s). As articulated by Coble and Mortensen (1992), "An economic threshold is the weed population at which the cost of control is equal to the crop value increase from control of the weeds present." Economic thresholds for bunchberry and red sorrel in relation to wild blueberry crop systems have not been established and this project begins establishment of such economic thresholds.

Bunchberry (*Cornus canadensis* L.; known by many names including Canadian dogwood) is a fast and low-growing plant very common especially along the edges of Maine wild blueberry fields. Bunchberry is most frequently found along the partially shaded forest edge of fields. Bunchberry spreads through rhizomes which emerge early in the spring, growing from dormant buds underground. This plant can be released from dormancy with the standard blueberry management techniques of burning and mowing. Bunchberry produces small red "fruits" around

the same time that blueberry fruits ripen, contaminating the blueberries harvested. Although cleaning lines are employed in wild blueberry processing, the bunchberry berry is a similar size to wild blueberries and can be missed, costing processor time (Sampson, 1995). Because the growth habit of bunchberry and wild blueberry are very similar, this weed is difficult to manage with methods other than chemical control. Therefore, it would help growers to understand when a bunchberry patch is reducing their wild blueberry yield economically so as to save a spray application until it is required. Bunchberry does not shade out wild blueberry but competes underground at the rhizome level for water, nutrients, and space.

Red sorrel or common sheep sorrel (*Rumex acetosella* L.) was found to have the highest and third highest field uniformity in conventional and organic fields, respectively, of all weeds identified in our 2019-2020 Wild Blueberry Weed Survey (2020 report, page 32). Red sorrel is thought to have arrived in Maine from Canadian wild blueberry production and has increased its prevalence over the last 10 years. Red sorrel is also now the most abundant weed in Nova Scotia (Lu et al., 2021) and has the ability to produce 124 to 247 seeds per stem in addition to reproducing through vegetative and underground shoots. Root fragments as small as 0.5 inches can grow into new plants and importantly seeds can be dispersed by agricultural equipment. The varied methods of spread employed by this weed have contributed to its widespread and increasing presence (Stopps et al., 2011).

Management of this weed is difficult and there is not currently a reliable chemical control option. Burning and mowing do not reduce its frequency and burning may in fact increase its abundance. Hand-pulling this weed by carefully removing the entire plant and its root system is the most effective control, though impractical on a large scale; chemical treatments can be more effective (DiTomaso, 2013). Red sorrel ramets emerge earlier and for longer than wild blueberry ramets, thereby acting as pollinator competition (White, 2015). Pollinators may also deposit red sorrel pollen in the blueberry flowers, thereby increasing the possibility of infection of the blueberry flowers with *Botrytis cinerea*, which can reduce fruit yield and quality (Hughes, 2015). Developing an economic threshold for this weed is important because it would help reduce herbicide applications thereby reducing the likelihood of herbicide resistance which has been seen by this weed (Li et al., 2014). Growers would also have a better idea of how much red sorrel is too much. Right now, this weed can seem visually over abundant in some fields, yet we do not know what percent coverage or plant density causes economic yield loss.

METHODS

Bunchberry – Surry, ME

Weed population baseline data was measured at one study location in Surry, ME on an organically managed farm in a prune field. On June 11, 2021, fifteen 1m^2 quadrats were situated along a 100 ft section of wild blueberry along the forest edge of the field. The number of bunchberry plants was counted within each quadrat and weed density targets were determined based on bunchberry density in this field. Of these 15 quadrats, three were established as controls that did not contain any bunchberry plants, just blueberry and three quadrats each were established as low bunchberry density (20 plants/m^2), medium density 1 (50 plants/m^2), medium density 2 (74 plants/m^2), and high density (105 plants/m^2). As necessary, quadrats were hand-weeded to achieve the desired plant density, and all quadrats were marked with flags to allow for repeated measurements throughout the season. When establishing quadrats, a quadrat was placed on the ground and the number of bunchberry were counted. The appropriate number of bunchberry plants were then removed in order to reach the desired weed density. At each visit, bunchberry plants were removed to maintain each density. This weed density must be maintained for at least two seasons before collecting blueberry density and yield. On June 3 and 11, 2021, the number of bunchberry and blueberry plants, and the number of bunchberry plants in bloom were counted in each quadrat. On July 22, 2021 the number of bunchberry plants per quadrat was counted again to maintain weed density.

Red sorrel trial – Hope, ME

Following the same method, red sorrel weed population baseline data was measured at the one study location in Hope, ME on a conventionally managed farm in a crop field. On June 10, 2021, fifteen 1m^2 quadrats were situated within an approximately 50x50ft patch of red sorrel chosen for the uniform density of the weed. The number of bunchberry plants was counted within 15 quadrats and weed density targets were determined based on bunchberry density in this field. Of these 15 quadrats, three were established controls that did not contain any red sorrel stems, and three each were established as low density (34 stems/m^2), medium density 1 (160 stems/m^2), medium density 2 (214 stems/m^2), and high density (280 stems/m^2). As necessary, quadrats were hand-weeded to achieve the desired plant density, and all quadrats were marked with flags to allow for repeated measurements throughout the season. On June 10, 2021, the number of blueberry and red sorrel stems were counted and the number of green fruit on 4 random blueberry stems were counted. On August 11, 2021, in each quadrat, the heights of 6 random blueberry stems and 6 random red sorrel stems were measured, the wild blueberry yield per quadrat was recorded, in addition to the stage of red sorrel development.

RESULTS

The data collected establishes the baseline for both studies to continue in the 2022 season and tested our methods in the field. Thus, no results were generated.

NEXT STEPS

- 2022 season: Continue measuring bunchberry, red sorrel, and wild blueberry health parameters
- Collect weed seed from quadrats to better understand regeneration of each weed population

ACKNOWLEDGEMENTS

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CROP GROWTH & PHYSIOLOGY

RESEARCH

INVESTIGATORS: P. Fanning and J. A. Collins

1. Predicting the bloom period in Maine wild blueberry

OBJECTIVE

An extensive data collection process was initiated in 2015 to develop a more appropriate model for Maine under conditions where wild blueberry plants traditionally 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, 2017, and 2020 by sampling additional sets of wild blueberry fields to estimate the progression of bloom. To continue fine-tuning our model, data collection continued in 2021, to test when degree-days start accumulating.

LOCATION Deblois, Hope, Searsport, T19 MD BPP, Jonesboro, and N. Ellsworth, ME
PROJECT TIMEFRAME May 2021

INTRODUCTION

A degree-day model for wild blueberry bloom in Maine has been developed based upon field sampling across the two major growing areas in Maine over four years (2015-2017, 2020). The 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. Data from 2020 suggested that the % cumulative bloom occurred at lower degree-days than indicated in the established model. Preliminarily, we theorized that the mild winter experienced in 2020 resulted in more degree-days accumulated prior to April 1 than was assumed in previous years that the model was developed.

METHODS

On various May dates, we visited six wild blueberry fields in the Mid-coast and Downeast growing regions of Maine (Deblois, Hope, Searsport, T19 MD BPP, Jonesboro, and N. Ellsworth). At each visit, we collected six stems from each of six clones, counted the number of closed and open or dropped flowers, and calculated the proportion of flowers in the field in bloom. To develop a degree-day model, daily maximum and minimum air temperatures were collected from weather stations in 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 for bloom estimates measured in the field in previous modeling conducted in 2015, 2016, and 2017. The model is:

$$\text{Percent bloom} = 100 / [1 + e^{(6.939 - 0.027^{*}\,DD)}]$$

To use this model, degree-days at base = 40ºF are accumulated from March 1.

RESULTS

Figure 1A shows the model for predicting bloom developed with data collected in 2015, 2016, and 2017, across a total of 33 wild blueberry fields to fit a final model. The final model was:

$$\text{Percent bloom} = 100 / [1 + e^{(6.939 - 0.027 * DD)}]$$

with an $R^2 = 0.953$ (i.e., 95.3 % of the variance in percent bloom is described by degree-days with a threshold of 40ºF). To use this model, degree-days at base = 40ºF are accumulated from April 1. This is a date at which, on average, frost has left the upper layers of the soil in most of Maine's production areas and plants begin to develop. The model and two additional years' data of % cumulative bloom compare to both a March 1 and April 1 start of GDD base 40ºF accumulation is observed in Figure 1B.

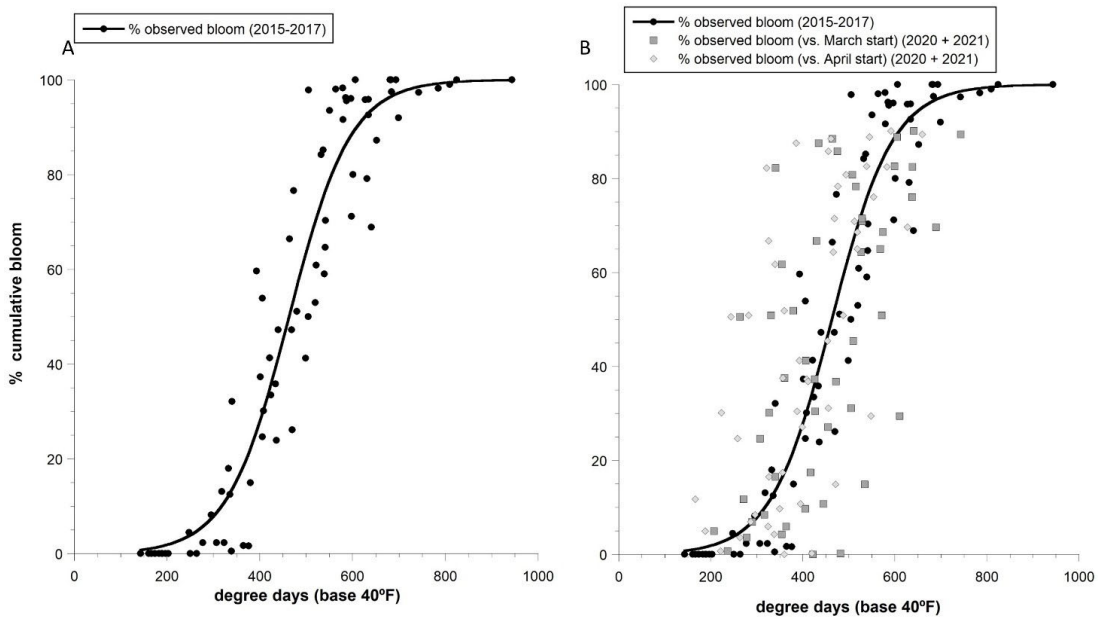

Figure 1. Predictive model for wild blueberry bloom in Maine (left), and predictive model with validation data (right). Model building data are solid round black data points, n = 33 fields; and overlaid validation data versus March 1 (grey squares) and April 1 (grey diamonds) start of GDD base 40ºF accumulation.

CONCLUSIONS

The data from the previous two years suggest that mild winters impact the accuracy of the existing model with neither 1 March nor 1 April start dates for degree-day accumulation aligning with expected bloom observations. This suggests that a more dynamic approach to predicting when growing degree-days are accumulated might be needed, particularly as Maine's climate changes; incorporating soil temperature could refine this model. This model still predicted with accuracy the onset of bloom and predicted the threat of frost damage, mummy berry infection, and timing for the importation of commercial pollinators.

NEXT STEPS

- Models to predict the onset of physiological development in wild blueberry will be investigated.

RESEARCH

INVESTIGATOR(S): B. Gumbrewicz and L. Calderwood

2. Comparison of Wood Mulch Particle Sizes for Wild Blueberry Management in a Changing Climate

OBJECTIVE(S):
Determine effects of mulch particle size on:

- Wild blueberry soil environment (soil moisture, temperature, nutrients, and organic matter)
- Wild blueberry plant growth (foliar nutrients, stem height, bud/flower/fruit development)
- Pest pressure (density and cover of weeds, insects and disease)

LOCATION(S): Stockton Springs, ME
PROJECT TIMEFRAME: May 2020 – September 2021

INTRODUCTION
The sandy, well-draining nature of wild blueberry soils lends to a low water holding capacity and therefore this crop is unable to maintain adequate levels of soil moisture for long periods of time. Increasing drought conditions during Maine summers with many growers without irrigation make it important to explore field management practices that increase soil water holding capacity (Birkel, 2020; 2021; Schattman et al., 2021). Water is critical for all aspects of plant growth including nutrient uptake and fruit expansion.

Wild blueberries require 2.54 cm (1 in.) of rain per week between April and October to sustain plant demand (Trevett, 1967; Hunt et al., 2008). The probability of reaching this rainfall requirement is estimated to be less than 50% during most of the growing season and less than 20% during the critical fruiting period (July – August) based on rainfall histories since 1959 (Dalton and Yarborough, 2004). Today's climate variability influences not only soil moisture, but also blueberry growth rhythms, plant nutrition, pollination patterns and introduces more extreme weather events such as drought and flooding in the same season (Fernandez et al., 2020).

Mulch is any material spread on top of the soil. A 5-8 cm (2-3 inch) layer of wood mulch applied to bare patches in wild blueberry fields has been the traditional recommendation to stimulate rhizome growth, prevent frost heaving, increase retention of nutrients, suppress weeds, moderate soil temperature, conserve soil moisture, and reduce soil erosion (Yarborough, 2012; Hunt et al., 2010; Drummond et al., 2009). Some wild blueberry growers have begun applying mulch across whole fields to increase blueberry coverage, water holding capacity, and yield. It is not economically feasible to make whole-field applications at the 5-8 cm rate. For this reason, it is important to identify the minimum mulch thickness required to improve soil water holding capacity. In addition, the particle size used may also impact soil moisture, among other factors influencing blueberry growth. This study evaluated four different particle sizes of softwood mulches spread 1.27 cm (½ inch) thick as a farm management strategy to improve blueberry productivity.

METHODS
The study took place at a MOFGA-(Maine Organic Farmers and Gardeners Association) certified organic field in Stockton Springs, ME. This field was in the prune year in 2020 and crop year 2021 (one production cycle). Softwood pine mulch was applied at a depth of 1.27 cm on May 18 and 19, 2020. Treatments of smallest to largest mean particle size were sawdust, shavings, bark mulch, and wood chips (Figure 1).

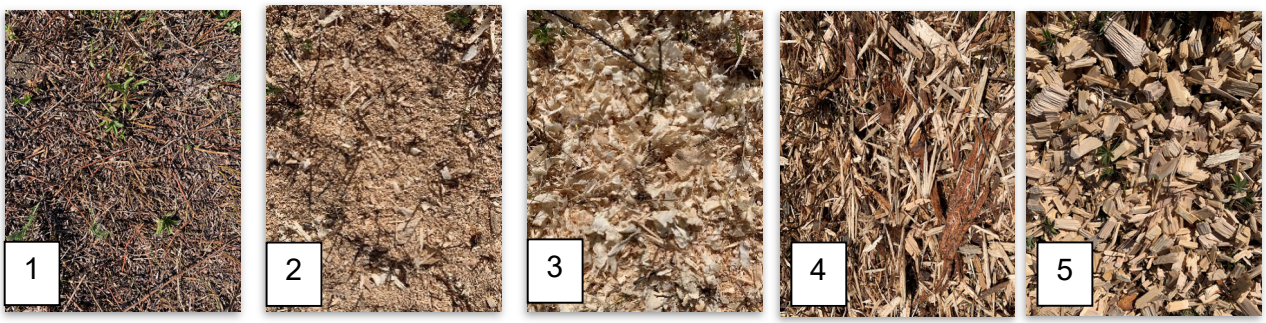

Figure 1. Mulch treatments after application in field and relative particle size: (1) control – no mulch, (2) sawdust – small, (3) wood shavings – medium, (4) bark mulch – large, and (5) wood chips – extra-large.

Particle size was determined using 2.0, 4.0, 6.3, 9.5, and 12.5 mm soil sieves to measure the fraction of material that could pass through each sieve. A control was included where no mulch was applied. Mulches were purchased as by-products with no inorganic additions from N.C. Hunt Lumber, a sawmill and manufacturing company in Maine (Table 1). The experimental design was a randomized complete block replicated six times. Each plot was 1.83 m by 9.14 m (16.72 m^2) separated by 0.91 m of wild blueberry, which functioned as a buffer.

Table 1. Mulch dry matter application rate and cost comparison for treatments purchased from N.C. Hunt Lumber. Cost is based on one application. Prices may vary based on quantity purchased, mulch type, and retailer.

Treatment	Dry Matter Rate (kg/ha)	Cost ($/$m^3$)	Cost ($/ha)
Sawdust	12,976	12.97	1,647.19
Shavings	4,363	12.97	1,647.19
Bark Mulch	11,005	37.28	4,734.56
Wood Chips	14,788	39.89	5,066.03

Data Collection

Soil

Each plot was sampled before treatment application on May 19, 2020, and again after harvest on July 30, 2021. Soil samples were analyzed by the University of Maine Soil Testing Service in Orono, ME.

Soil temperature (°C) and moisture (% volumetric water content [VWC]) were recorded using TDR-300 (time domain reflectometry) probes (FieldScout TDR 150 Soil Moisture Meter, Spectrum Technologies, Inc., Aurora, IL, USA) inserted 12 cm (4.8 in.) into the blueberry root zone soil. Eight random readings were recorded per plot between 10:00 and 2:00 pm on each collection date to achieve relatively consistent mid-day sampling weather conditions. Collection dates were performed weekly and, when possible, twice weekly before and after rain events. Collection dates for 2020 were June 2, 4, 8, 16; July 1, 10, 15, 24, 21; and August 10, 20, 31. Collection dates for 2021 were June 1, 10, 18, 24; and July 1, 6, 16, 22, 28.

Plant Growth

Blueberry cover was visually estimated in each of two 0.37-m^2 permanent quadrats in each plot. Blueberry cover was recorded in 2020 on June 19, July 15, and August 20 and in 2021 on June 18 and July 13. Blueberry cover was rated based on a 0-6 scale ranking system representing even intervals of 100%, where: 0 = not present, 1 = ≤1%-16.67%, 2 = >16.67%- 33.33%, 3 = >33.33%-50%, 4 = >50%-66.67%, 5 = >66.67%-83.33% and 6 = >83.33%-100%.

Stem density was measured in each quadrat at the beginning and end of the vegetative year on June 19 and August 31, 2020. Stem heights and bud numbers were recorded for each of five stems

per quadrat on April 23, 2021. These five stems were marked with metal tags to track fruit development. The number of open flowers per stem was recorded during peak bloom on May 21, 2021. The number of green, red, and blue fruit per stem was recorded three times: prior to ripening on June 18, 2021, during ripening on July 6, 2021, and during harvest on July 28, 2021.

Twenty stems were flagged in each plot and measured for chlorophyll content throughout each season. Leaf chlorophyll content was measured as an indicator of leaf nitrogen using a SPAD Chlorophyll Meter (SPAD 502; Minolta Corp, Osaka, Japan). A measurement was taken on each of two leaves per stem, one on the lowermost and one on the uppermost portion of the stem. Measurements were recorded in 2020 on June 19, July 15, and August 20 and in 2021 on June 18 and July 15.

Berries were harvested by hand raking plots on July 28, 2021. Quadrat yield was raked and weighed separately before adding to the whole plot yield. Measures of berry quality included 100-berry weights as an indication of berry size and °Brix as an indication of sugar content. For 100-berry weights, a representative sample of 100 berries were randomly counted and weighed. This sample was then blended using a Ninja Express Chop blender (SharkNinja Operating LLC., Newton, MA, USA). A subsample of the purée was deposited on a handheld PAL-BRIX/ACID F5 refractometer (Atago, Saitama, Japan) for the °Brix measurements taken on July 30, 2021.

Wild blueberry plant development during 2021 was at least two weeks ahead of what was observed in 2020, which was also several weeks ahead in development. Therefore, harvest took place much earlier in the season. Any measurements planned for August 2021 were terminated after harvest took place.

Pest Pressure

Pest pressure (insects, weeds, disease) was measured monthly (June, July, and August) in each quadrat on the same dates as and using the same ranking scale used for blueberry cover. The actual number of weed plants and the number of stems with insect or disease damage indicated as weed, insect, or disease density were also recorded in each quadrat on each date.

Data Analysis

The effects of the mulch treatments on soil (moisture, temperature, nutrients), pest pressure (insects, disease, weeds) and plant growth (chlorophyll content, stem height, stem density, bud/flower/berry development), yield and berry quality were statistically analyzed in JMP (JMP® Pro, Version 15.2.0). One-way analysis of variance was used to analyze data sets for only one date, including stem density, stem height, bud number per stem, flowers per stem, berries per stem, and soil nutrients. Randomization testing was used to analyze data that did not follow a normal distribution and confirm results of analyses of variance that did not meet standard parametric assumptions. All repeated measures were analyzed using a series of mixed models. Data that met assumptions of normality and equal variances were analyzed using a linear mixed model of standard least squares. Data that could not meet assumptions of normality and equal variances were analyzed using a generalized linear mixed model (GLMM) after concluding there were no other major problems with the data set. In the GLMM, count data were modeled with a Poisson distribution and log link. Proportion data (ranks) were modeled with a binomial distribution and logit link. To model rank data, ranks were first converted to the percent midpoint of the range that each rank represents. In each model, ‘block’ was used as a random effect and all other variables were considered fixed effects. All fixed-by-fixed effect interactions were included in each model for statistical analysis. Treatment effects were separated by Tukey’s Highly Significant Differences test at the 0.05 significance level.

RESULTS

Statewide, Maine's 2020 and 2021 summer (June – August) temperature and rainfall averages were warmer and drier than normal (Birkel, 2020). Average summer temperature in 2020 was 18.72°C, the third warmest since 1895. This was 0.89°C warmer than the 2019 average and 1.66°C warmer than the 1901-2000 historical mean (17.06°C) Average summer temperature in 2021 was 18.67°C, the fourth warmest since 1895. Total precipitation for the 2020 summer period was 22.35 cm, the 14th driest since 1895. Total precipitation for 2021 was 26.00 cm, 2.03 cm less than the historical mean (28.04 cm).

No treatment had significantly greater mean soil moisture compared to the control across each season. Mean soil moisture for the shaving treatment was significantly greater than the wood chip treatment during 50% of the 2020 sampling dates. Overall mean soil moisture was greater in the shaving and control treatments compared to the wood chip treatment during both years. Soil moisture ranged from 2.8% to 46.6% in 2020 and from 10.4% to 45.3% in 2021. No significant differences were detected in soil temperature among treatments throughout the 2020 and 2021 field seasons. Soil temperature ranged from 22.2°C to 37.6°C in 2020 and from 16.4°C to 43.7°C in 2021.

No significant differences were detected in stem density measured at the end of the prune year. There were significance differences in percent change between the beginning and end of the prune year. All mulch treatments increased in stem density (sawdust by 23.93%, shavings by 34.07%, bark by 11.47%, chips by 18.74%) while control plots had a decrease of 9.47%. Sawdust and shavings treatments had significantly taller mean stem height than control plots. All mulch treatments had significantly more mean number of buds per stem than control plots (Figure 2).

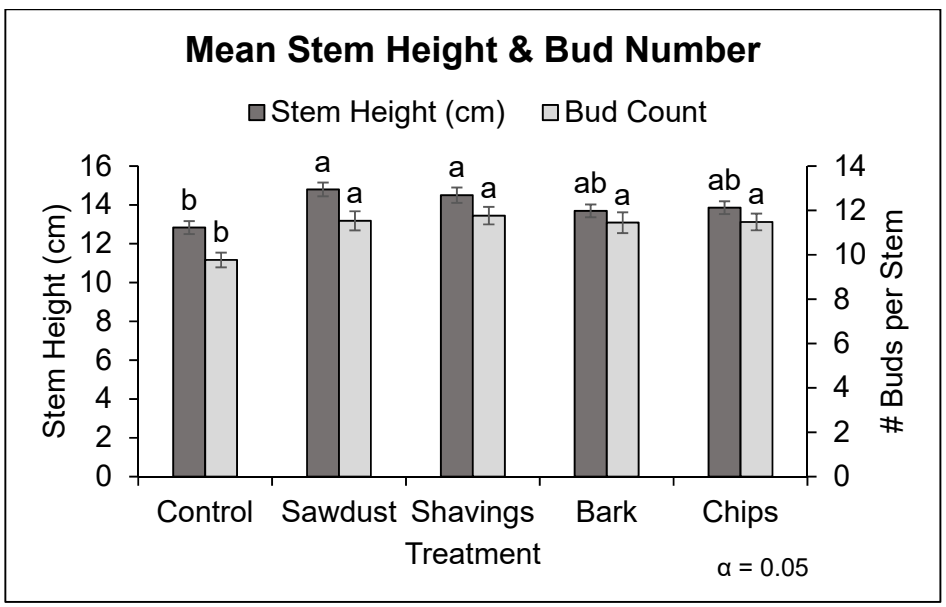

Figure 2. Mean stem height and number of buds per stem by treatment. Error bars indicate the standard error of the mean (SEM). Letters indicate pairwise differences.

The sawdust treatment had significantly more flowers per stem during bloom while bark mulch had significantly more blue fruit per stem at harvest compared to the control treatment (Table 2). Sawdust and shaving treatments had the greatest mean total yield compared to the control. No significant differences were detected among treatments for the number of green fruit per stem, 100-berry weights or sugar content as indicated by the °Brix measurements.

Table 2. Blueberry flower and fruit counts, yield, berry sample weights and °Brix sugar content as affected by mulch treatments during wild blueberry crop year (2021) growing season.

	Means					
Treatment	**# Flowers per stem**	**# Green fruit per stem**	**# Blue fruit per stem**	**Yield (kg/ha)**	**100-berry weights (g)**	**°Brix**
Mulches						
Sawdust	13.20 a	7.38	4.07 ab	2516 a	34.00	10.79
Shavings	10.32 ab	6.43	4.57 ab	2646 a	32.00	10.89
Bark	11.85 ab	7.33	5.57 a	2168 ab	31.00	10.80
Chips	11.37 ab	7.17	4.65 ab	2077 ab	34.17	11.11
Control	8.58 b	5.58	3.52 b	1680 b	32.33	11.28

Overall mean chlorophyll content in 2020 was significantly greater in sawdust (31.48 SPAD units), shavings (30.71 SPAD units), and wood chip (30.74 SPAD units) treatments compared to the control (29.48 SPAD units), which had the lowest mean. Control plots were significantly lower than sawdust, shaving, and wood chip treatments during July and significantly lower than the wood chip treatment during August 2020. In 2021, sawdust treatments had the greatest overall mean chlorophyll content (26.19 SPAD units).

In 2020, there were no significant differences detected among treatments in weed density, weed cover, or insect density. The most abundant broadleaf weeds observed in 2020 were common cinquefoil (*Potentilla simplex* Michaux.), goldenrod (*Solidago* spp.), and golden clover (*Trifolium aureum* Pollich.). Grasses also made up a large fraction of weed plants observed. The most abundant insects observed were blueberry gall midge (*Dasineura oxycoccana* Johnson), red-striped fireworm (*Aroga trialbamaculella* Chambers), and flea beetle (*Altica sylvia* Malloch). The most abundant disease observed was Sphaerulina leaf spot caused by Sphaerulina vacinii, which accounted for 99.7% of disease observations per stem and therefore accounted for significant differences among treatments. The control treatment had significantly more disease cover and density than all mulch treatments in July and August 2020. In July, the control had 141%, 157%, 141%, and 129% more disease cover than sawdust, shaving, bark, and wood chip treatments, respectively. In August, control plots had 75%, 118%, 91%, and 91% more disease cover than sawdust, shaving, bark, and wood chip treatments, respectively.

In 2021, there were no significant differences detected among treatments across density and cover of all weeds, insects, and disease. The most abundant broadleaf weeds observed in 2021 were common cinquefoil (*Potentilla simplex* Michaux.), goldenrod (*Solidago* spp.), and common vetch (*Vicia sativa* L.). Grasses again made up a large fraction of weed plants observed. The most abundant insects observed were blueberry gall midge (*Dasineura oxycoccana* Johnson) and blueberry thrips (*Frankliniella vaccinii* Morgan and *Catinathrips kainos* O'Neill). The most abundant diseases observed were again leaf spot diseases, which accounted for 97.6% of disease observations per stem.

DISCUSSION

Soft wood mulch is an effective tool for soil moisture conservation and improving plant health. Organic matter that covers the soil surface protects the soil from drying and sealing shut, improves soil porosity, and therefore enhances water infiltration and storage (Bot and Benites, 2005). Agricultural soils can hold an estimated 16,500 gallons of water per acre of soil at a depth of one foot for every 1% of organic matter content (Gould, 2015). Therefore, adding softwood mulch to blueberry fields can increase the soil organic matter fraction to aid in water holding capacity and promote a drought-resistant soil. Because no mulch treatment demonstrated significantly greater overall mean soil moisture compared to the control, a layer thicker than the 1.27 cm mulch layer used in this study is necessary to conserve significantly more soil moisture than the control

treatment. This thickness of mulch was chosen as an economically and physically feasible amount of material for growers to apply to entire fields, yet further research should investigate different thicknesses of these mulches.

All mulch treatments resulted in significantly less disease cover in the first year and more buds per stem by the second year compared to the control treatment. Mulch has previously been shown to reduce diseases like leaf spot and mummy berry by preventing dispersal of overwintering spores (Alfieri, 1991; Drummond et al., 2009). These results agree with previous research that showed leaf spot incidence in wild blueberry fields was inversely related to flower bud set and berry production (Annis and Stubbs, 2004; Ojambo et al., 2002). Sawdust and shaving treatments also had significantly taller stems, greater change in stem density, and greater yields compared to the control treatment.

Nitrogen immobilization or “locking” has been a concern when applying mulches to crops (Krewer et al., 2008; Sønsteby et al,. 2004). As soil microorganisms use nitrogen during decomposition of mulches, nitrogen may be “tied up” and unusable by plants, so a resulting shallow depletion zone may then form at the interface of the soil surface and mulch layer. According to our results, control plots had the lowest overall mean chlorophyll content in year one and were among the lowest in year two. Therefore, nitrogen locking was not observed in our mulch treatments.

According to these results growers should consider using finer particle sizes rather than wood chips. Over time, however, larger particle size mulches may perform better than finer particle size mulches if the finer sizes are not reapplied, due to their tendency to erode and the slower decomposition process of the larger wood chips (DeGomez and Smagula, 1990). That being stated, any mulch particle size will improve plant health.

CURRENT RECOMMENDATIONS

Growers should consider using finer particles rather than wood chips and use a thicker application than what was used in this study. Growers should focus this application toward lower-yielding portions of fields. A thicker application will increase the amount of material needed and therefore cost for material but can improve overall productivity. Growers might consider top-dressing finer particles with a larger particle size to weigh down the lightweight material, increase stability, and slow decomposition rate to reduce the need for reapplication. Applying a second material would be more expensive, however; the finest particle size treatments were the least expensive materials to purchase for this study.

NEXT STEPS

- Evaluate different thicknesses of mulch application for their decomposition rate and effect on wild blueberry growth and pest pressure.
- Compare effects of softwood and hardwood mulch types on wild blueberry growth and pest pressure.

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RESEARCH

INVESTIGATOR(S): R. Tasnim and YJ. Zhang

3. Investigation of soil amendments (biochar-compost and mulch) on soil water availability and resilience of wild blueberries to warming

LOCATION(S): Conventional fields in Deblois, ME and UMaine Blueberry Hill Farm Experiment Station in Jonesboro, ME
PROJECT TIMEFRAME: January – December 2021

OBJECTIVES: Test the effectiveness of mulching and biochar-compost mix application in mitigating the adverse effects of warming on soil water and nutrient availability.

INTRODUCTION
Wild blueberries have played an important role in Maine's economy for centuries, where the crops are grown commercially on 40,000 acres of land. Wild blueberry production is now under the threat of climate change including warming (Tasnim et al., 2021) and frequent drought (Barai et al., 2021). Recent preliminary investigation by Tasnim et al. (2020) using open-top chambers with heating to manipulate a warmer environment showed that the warming has changed the growth pattern of wild blueberries and has negative effects on their physiology due to increased crop water loss and decreased soil water and nutrient availability, which could further affect wild blueberry production. Therefore, to sustain wild blueberry production in a future with warmer and drier summers, management techniques need to be developed and tested to mitigate these negative effects. The negative effects of elevated temperatures can be mitigated with irrigation. However, irrigation systems are costly and wild blueberry fields have low water use efficiency due to the low water-holding capacity of the sandy soils. Therefore, soil amendment techniques such as mulching and biochar-compost mix application to enhance soil water and nutrient holding capacity could be better solutions. Biochar-compost mix application could be a potential environment-friendly technique to mitigate the negative effects of warming on soil water and nutrient availability. Biochar (carbon material produced from plant biomass) is highly recommended to use with compost for better crop production as biochar can hold soil moisture and nutrients enhancing soil fertility. Since Maine has vast resources of forest (wood) residuals, some companies are producing biofuel from wood pellets through pyrolysis. Biochar is produced as a waste-byproduct but can be a potentially available option to enhance wild blueberry productivity. Investigations are needed to test the potential benefits of using this biochar on improving wild blueberry production and mitigating warming effects because biochar reacts with different soil and plants in different ways. Therefore, the objective of this study is to test the effectiveness of mulching, and biochar-compost mix application in mitigating the adverse effects of warming on soil water and nutrient availability. Mulching is hypothesized to keep moderate soil temperature and retain soil water and nutrients while biochar-compost mix is hypothesized to improve soil water holding and nutrient availability (Liang et al., 2014; Mukherjee & Zimmerman ,2013).

METHODS

We selected three different genotypes in each of these two locations: Blueberry Hill Farm in Jonesboro, and Wyman's wild blueberry field in Deblois for this study. Within each genotype, we selected five plots (Fig. 1a) during the vegetative year. Here it is to be noted that the two studied fields are under different management regimes. Wyman's blueberry field has been frequently irrigated and fertilized, while Blueberry Hill Farm's field is neither irrigated nor fertilized. However, we closed the irrigation system before we started this experiment in the Wyman's field at Deblois to standardize conditions in both fields throughout this experiment.

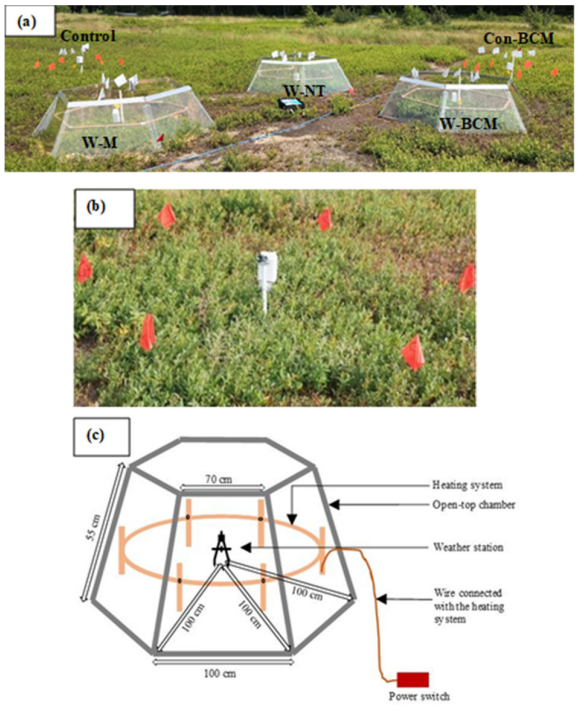

Figure 1. The open-top chambers (OTC) and control plots used for this study in the wild blueberry field: (a) Two control flagged plots with no warming chamber (one plot with no treatment named as "Control"; one plot with biochar-compost mix on the soil surface named as "Con-BCM") and three OTCs (warming chamber with no treatment named as "W-NT"; warming chamber with mulch on the soil surface named as "W-M" and warming chamber with biochar-compost mix on the soil surface named as "W-BCM". (b) Example of control flagged plot with no chamber. (c) Schematic diagram of OTC with heating (to supply additional heat) [Hexagon dimensions: 100 cm (ground); 55 x 70 cm (top); 100 cm (radius)].

After selecting those six genotypes, we collected soil samples from each of those genotypes separately and sent them for a comprehensive soil test to the Analytical Soil Testing Laboratory, University of Maine, Orono, ME. The soil pH and organic matter of the studied Deblois field soil were 3.7 - 4.2 and 9.3 - 20.5%, respectively, whereas the soil pH and organic matter of the studied Blueberry Hill Farm field soil were 4.6 - 5.2 and 4.5 - 9.5%, respectively. Within each genotype, we marked two open plots with flags (Fig. 1b) that had no warming chamber: one plot was not treated (referred to as "Control"), and the other open plot was treated with biochar compost mix (referred to as "Con-BCM"). Three other plots in each genet had open-top chambers (OTC) with a heating system inside (Figs. 1a & 1c). Based on the preliminary investigation on wild blueberry crops by Tasnim et al. (2020), this warming chamber would increase the ambient temperature by 3-5 °C. Out of these three chambers in Fig. 1a, the soils inside one chamber were not treated (referred to as "W-NT") and soils inside two other chambers were treated with softwood bark mulch (referred to as "W-M") and biochar-compost mix (referred to as "W-BCM"), respectively. We applied 0.5" deep softwood bark mulch on the soil surface and applied the biochar-compost mix (ratio of 1:1) at a rate of 7.5 yd3/A. Biochar was provided by Maine Wood Pellets Co. and contained ash. After receiving

the biochar at the University of Maine, we separated the ash from biochar by sifting the raw biochar-ash mix in a sieve shaker. Compost was provided by the University of Maine composting facility. At the time of compost application, a sample was sent for comprehensive testing to the Analytical Soil Testing Laboratory, University of Maine, Orono, ME; results have yet to come back.

Data Collection

We installed weather stations in the middle of each plot (Fig. 1) for real-time monitoring of the atmospheric temperature and relative humidity using Watchdog 1000 series micro stations (Spectrum Technologies, Inc, Aurora, IL, USA) and HOBO weather stations (ONSET Computer Corporation, Bourne, MA, USA). We marked six random stems in each plot to monitor stem length, leaf number, leaf temperature, chlorophyll and anthocyanin concentrations every two weeks during June – November 2021. During this time, we also measured soil moisture, soil temperature and electrical conductivity in each of the plots using a Fieldscout TDR 150 soil moisture meter (Fieldscout TDR 150, Spectrum Technologies Inc., Aurora, IL, USA) at 6 random locations throughout each plot. Chlorophyll concentration was measured by a CCM-200 plus chlorophyll content meter (Opti-Sciences, Inc., Hudson, NH, USA) and Anthocyanin concentration was measured by an ACM-200 anthocyanin meter (Opti-Sciences, Inc., Hudson, NH, USA). We also conducted gas-exchange measurements on three random plants from each plot using a portable photosynthetic measurement system (li-6800; Li-Cor Biosciences, Lincoln, NE, USA) on a sunny day in mid-July between 10:00 and 15:00 h solar time at a photosynthetic photon flux density of 1500 µmol m^{-2} s^{-1}. To quantify crop water status, we collected one wild blueberry stem from each plot at midday 12:00-12:30 h solar time and measured midday leaf water potential by a leaf pressure chamber (PMS Inc., Albany, OR, USA). We conducted this measurement twice: once in July and once in August 2021. Once on 18 July and once on 19 August, we collected six random stems from each plot to quantify leaf number, leaf area, leaf dry biomass, and leaf nutrition. We measured leaf area using an LI-3000A area meter (Li-Cor, Lincoln, NE, USA), then the leaves were oven-dried at 70ºC to constant mass and weighed. Leaf mass per area (LMA) was determined as leaf dry mass divided by leaf area (g m^{-2}). Then we ground up those dried samples and sent them to the University of Maine Soil and Plant Tissue Testing Laboratory in Orono, Maine for leaf tissue nutrient testing. We also sent dried, ground, and homogenized samples to UC Davis Stable Isotope Facility (Davis, CA, USA) for natural abundance carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) measurement to determine water use efficiency, and nitrogen uptake, respectively. We also collected soil samples from each plot on October 2 and sent them to the Analytical Soil Testing Laboratory, University of Maine, Orono, ME for full soil health test.

The effects of soil amendments and fertilizer treatments on soil moisture, physiology (chlorophyll concentration, photosynthetic rate), morphology (leaf size, number of leaves per stem and total leaf area per stem), and major leaf nutrients (N, P, K, Ca, C) of wild blueberry plants were statistically compared using a general linear model followed by LSD (least significant difference) post-hoc test ($\alpha = 0.05$) in SPSS software (IBM Corp., Armonk, NY, USA). In this model, main effects of treatments were considered as a fixed factor, genotypes were used as random factors, and a Bonferroni correction was also applied for confidence interval adjustment.

RESULTS

Over a 24-hour period, the air temperature in the warming chambers was ~3-5°C higher during the day and ~1-2°C higher during the night than in the control plots (Fig. 2a). The relative humidity in the warming chambers was ~6-10% lower than in the control plots (Fig. 2b).

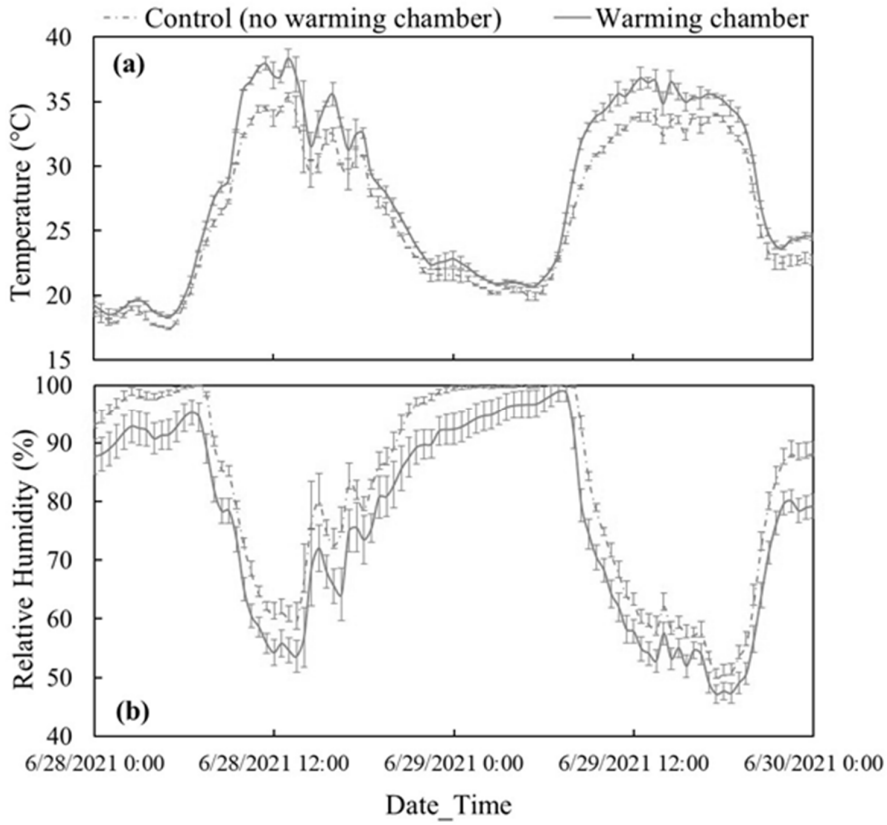

Figure 2. Diurnal changes in (a) atmospheric temperature (°C) and (b) relative humidity (%) during two typical sunny days (28 – 29 June 2021).

Soil moisture content was slightly lower (almost negligible) in warming chambers with no treatment and mulch (W-NT & W-M) than the control plot during mid-June to mid-August (Fig. 3a). However, after mid-August until mid-October, soil moisture content was distinctively (~5-8%) lower in the W-NT and W-M plots than the control plot. In contrast, during the whole season from mid-June to mid-October, plots treated with biochar-compost mix (Con-BCM & W-BCM) had consistently higher soil moisture than the control plot. The Con-BCM plot had the highest soil moisture content followed by the W-BCM plot and control plot. For the average soil moisture from the whole season (Fig. 3b), it is more distinctive that the Con-BCM had significantly higher soil moisture followed by W-BCM and control plots, with W-BCM plot showing the highest VWC among the 3 warming chambers (Fig. 3). The significantly lower soil moisture compared to the control plot was observed in W-NT and W-M plots.

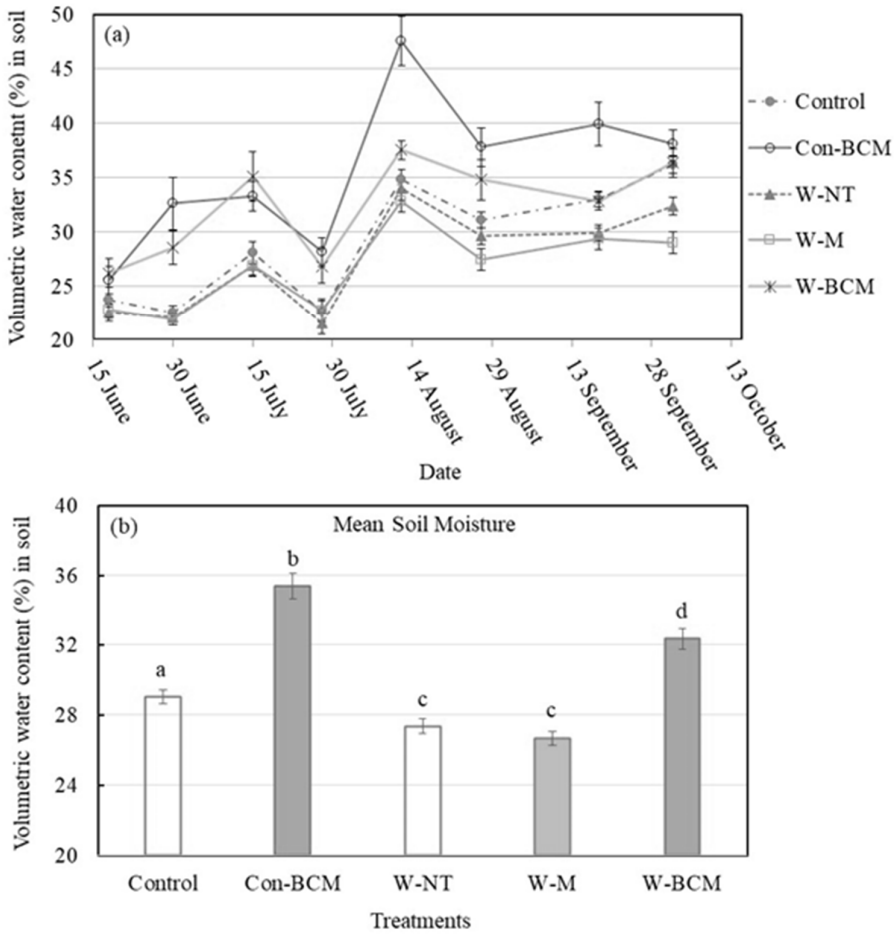

Figure 3. (a) Seasonal changes in volumetric water content in soil across five different treatments from mid-June to mid-October 2021. (b) Comparison in mean soil moisture content by treatment type over all field season data collection dates (form mid-June to mid-October 2021). Error bars indicate the standard error of the mean. Different letters indicate significant differences at the significance level of $p < 0.05$.

Despite the significant differences in soil moisture content, there were no significant differences in leaf water potential of wild blueberry plants growing under different treatments (Fig. 4) indicating that those plants did not differ in water deficits. On average in July (although not significant), plants in the Con-BCM plot were the least stressed (~-1.1 MPa) compared to the Control, W-NT, W-M and W-BCM plots (~-1.2 to -1.3 MPa). This trend was also observed in August, but the differences were smaller among the treatments than the observed differences in July.

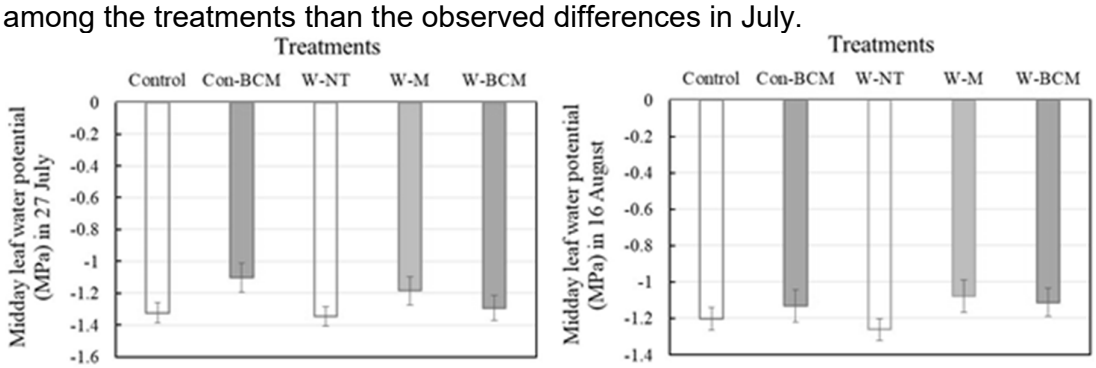

Figure 4. Comparison in midday leaf water potential of wild blueberry plants among five different treatments on two typical sunny days (27 July and 16 August 2021); Error bars indicate the standard error of the mean. Here, no significant differences were observed among the treatments at the significance level of $p < 0.05$.

Overall, from the gas-exchange measurements, we observed that plants in the warming treatments had higher photosynthetic rates (Fig. 5a), stomatal conductance (Fig. 5b), and transpiration rates (Fig. 5c) compared to the control plots. Plants growing in the biochar-compost treated soil inside warming chambers had significantly higher photosynthetic rates, stomatal conductance, and transpiration rates compared to the other treatments. Consequently, plants growing in the warming chambers (W-NT, W-M and W-BCM) showed significantly lower water use efficiency than the plants in control plots where plants in Con-BCM plot showed the highest water use efficiency.

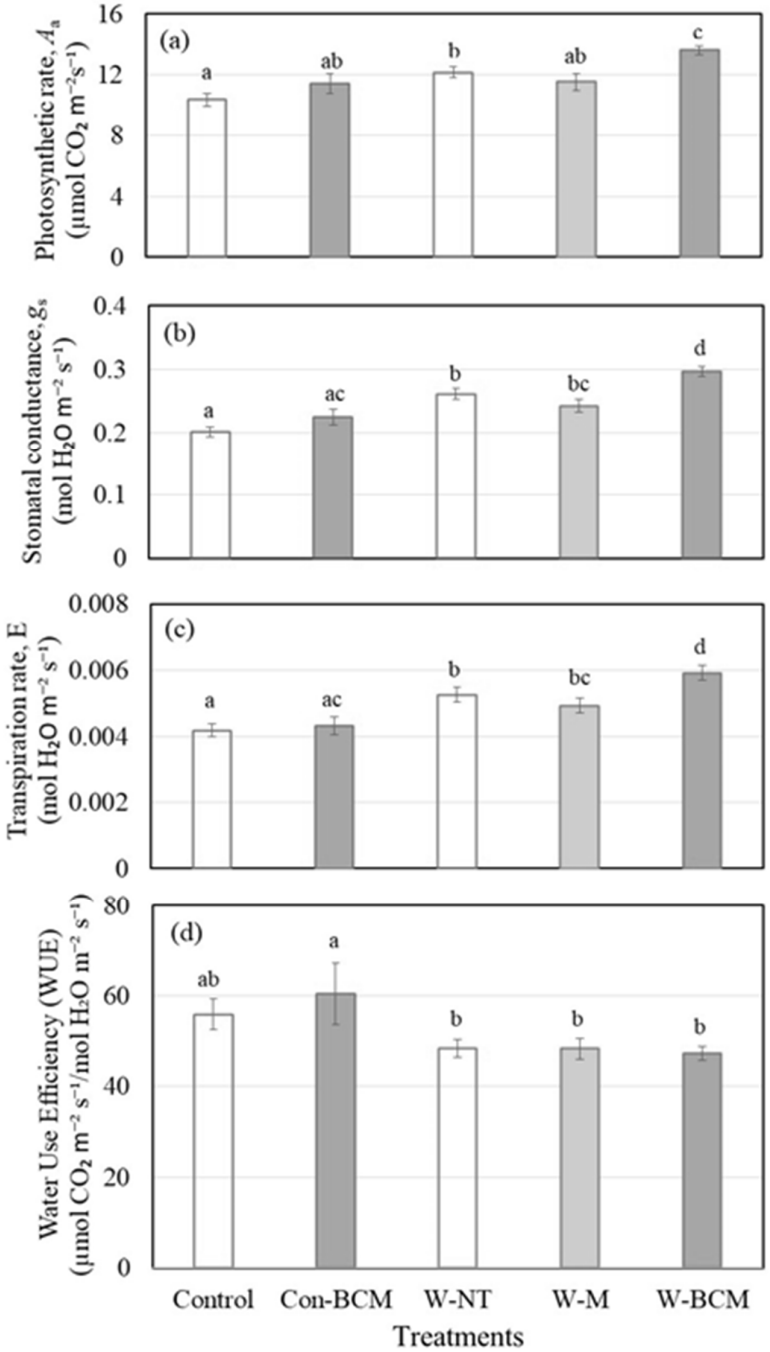

Figure 5. Comparison in (a) Photosynthetic rate, (b) Stomatal conductance, (c) Transpiration rate, and (d) Water use efficiency of wild blueberry plants among five different treatments on two typical sunny days in July 2021 (11 July in Deblois field and 12 July in Blueberry hill farm field). Error bars indicate the standard error of the mean. Different letters indicate significant differences at the significance level of $p < 0.05$.

Based on the seasonal changes in chlorophyll concentration in wild blueberry leaves, plants in warming chambers had lower chlorophyll concentration than the control plots from June to July. Plants in the control and Con-BCM plots reached peak chlorophyll concentration (35-38 SPAD) in mid-July and then declined gradually over the season down to 20-23 SPAD by mid-October. In contrast, plants in the warming chambers (W-NT, W-M and W-BCM) maintained consistently higher chlorophyll concentrations (34 -37 SPAD) from mid-July until September and then declined down to 26-32 SPAD in mid-October.

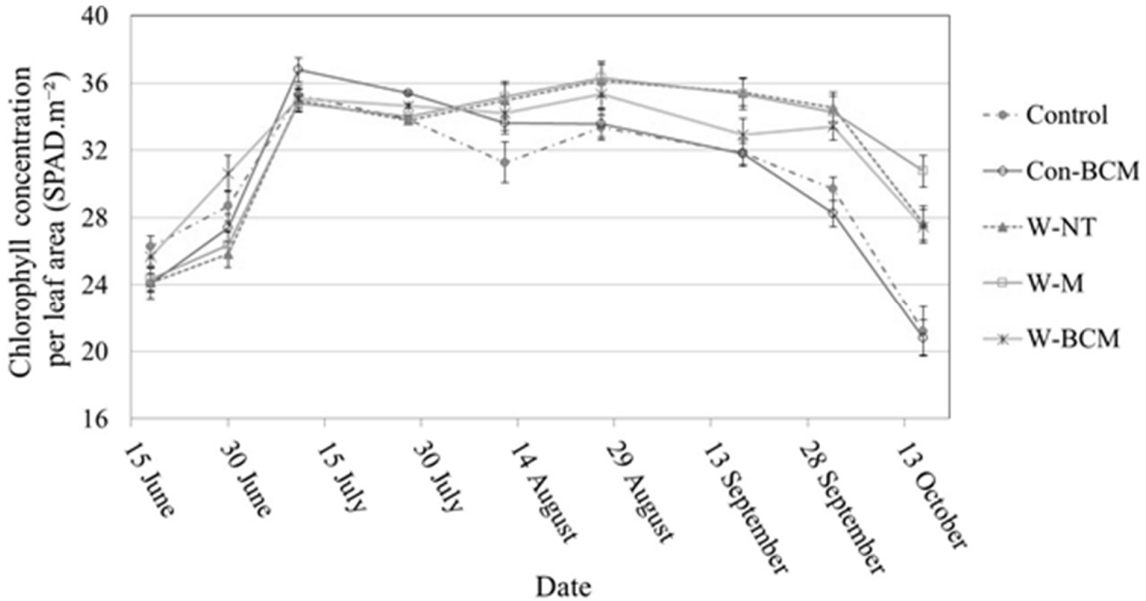

Figure 6. Seasonal changes in chlorophyll content per leaf area in the wild blueberry leaves across five different treatments from mid-June to mid-October 2021. Error bars indicate the standard error of the mean.

Wild blueberry stems were consistently the tallest in the W-M plots, followed by the W-NT, Con-BCM, and W-BCM plots whereas control plots had the shortest stems (Fig. 7a). Similar to the stem heights, leaf numbers (Fig. 7b) from mid-June to November were consistently the highest in the W-M and W-BCM plots followed by the Con-BCM, and W-NT plots while the control plots consistently had the fewest leaves per stem. For leaf numbers (Fig. 7b), the control plot reached peak leaf numbers in mid-July then gradually declined until all leaves were dropped by the end of October. In contrast, the plants in biochar-compost treated control plots as well as in the warming chambers had their highest number of leaves on 30 July and then started to drop gradually. While plants from several genotypes in the W-M treatment kept their leaves until late November, plants in other treatments mostly dropped all their leaves by the end of October to early November.

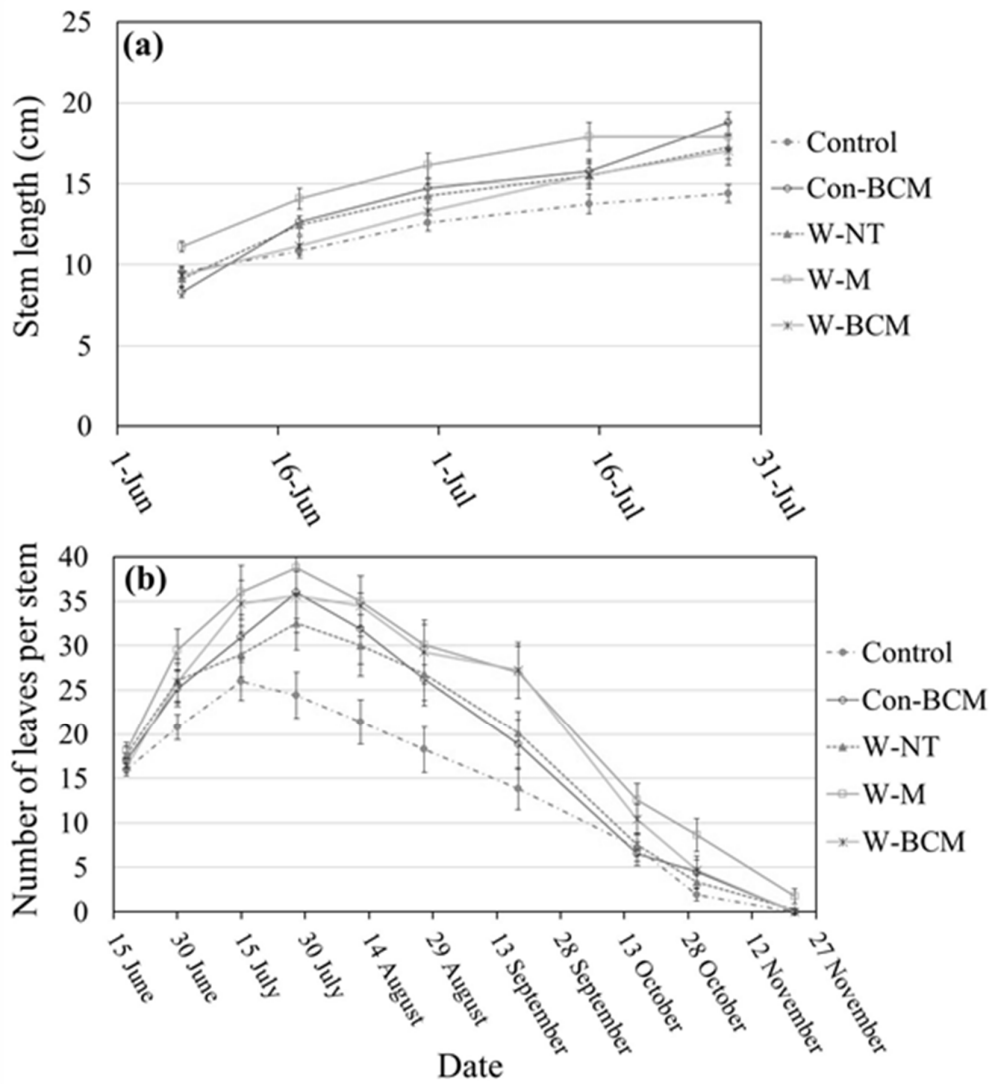

Figure 7. Seasonal changes in (a) Stem length and (b) Number of leaves per stem of the wild blueberry plants across five different treatments from mid-June to November 2021. Error bars indicate the standard error of the mean.

At the end of July and August, plants in the warming chambers (W-NT, W-M, W-BCM) had more leaves per stem than the control plots (Control, Con-BCM), and the W-BCM plot had of the most leaves among all the treatments (Fig. 8a); this difference was significant. Similarly, leaf size (Fig. 8b) was larger on plants in the warming chambers (W-NT, W-M, W-BCM) than the control plots (Control and Con-BCM) but differences among the treatments were not significant. In contrast, plants in the control plots (Control and Con-BCM) had higher leaf mass per area (LMA) than the warming chambers (W-NT, W-M, W-BCM). LMA was especially significantly higher in the control plots in July (Fig. 8c).

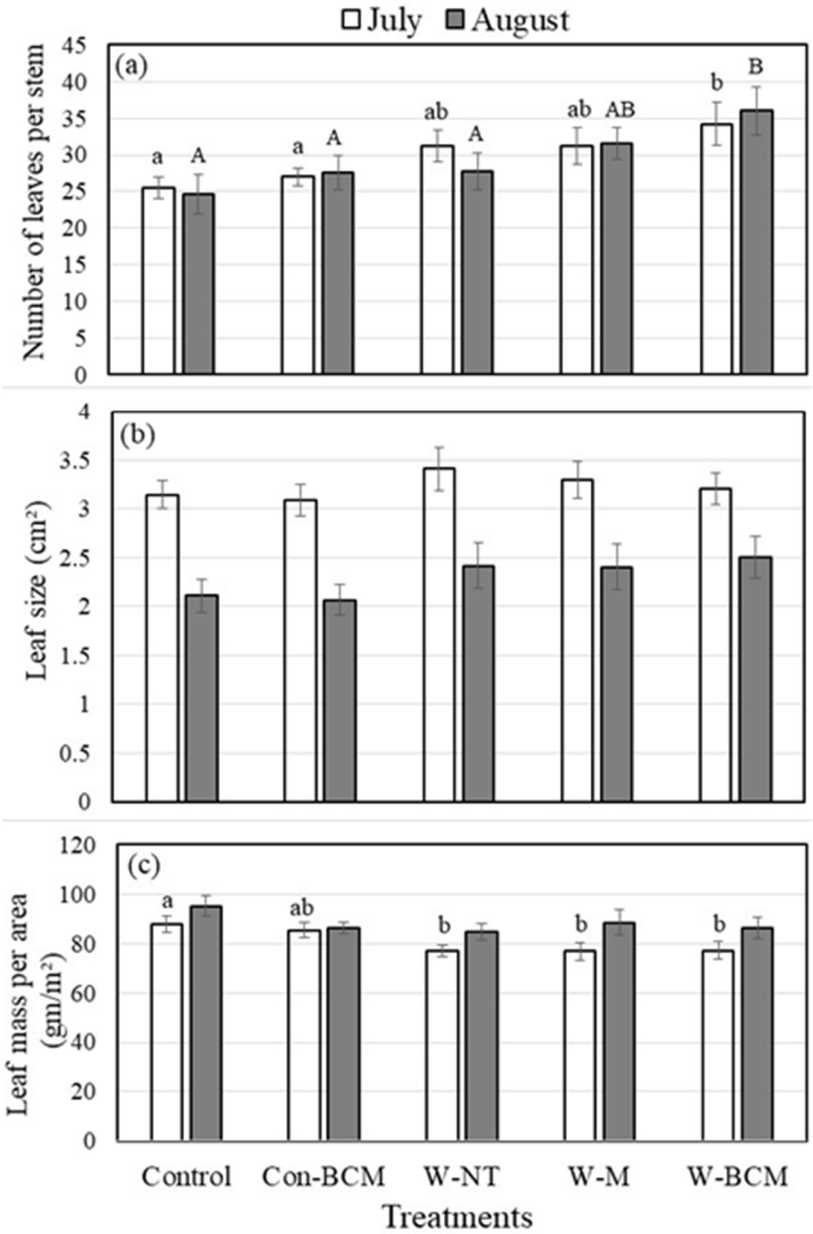

Figure 8. Comparison in (a) Number of leaves per stem, (b) Leaf size, and (c) Leaf mass per area of the wild blueberry plants among five different treatments in July and August 2021. Error bars indicate the standard error of the mean. Bars with no letters above indicate no significant differences among the treatments, and different letters indicate significant differences at the significance level of $p < 0.05$.

At the end of August, plants in the control plots (Control, Con-BCM) had higher % of $\delta^{13}C$ (higher water use efficiency) than the plants in the warming chambers (W-NT, W-M, W-BCM) but the differences were not significant (Fig. 9a). Although not significant, this trend agrees with the water use efficiency trend calculated from the gas-exchange measurements in Fig. 5d where plants in the control plots had significantly higher water use efficiency. Also, not significant but on average, biochar-compost treated plots (Con-BCM, W-BCM) had higher % of $\delta^{15}N$ (higher nitrogen uptake and allocation to the leaves) than other treatments, Con-BCM in particular had the highest leaf nitrogen accumulation (Fig. 9b).

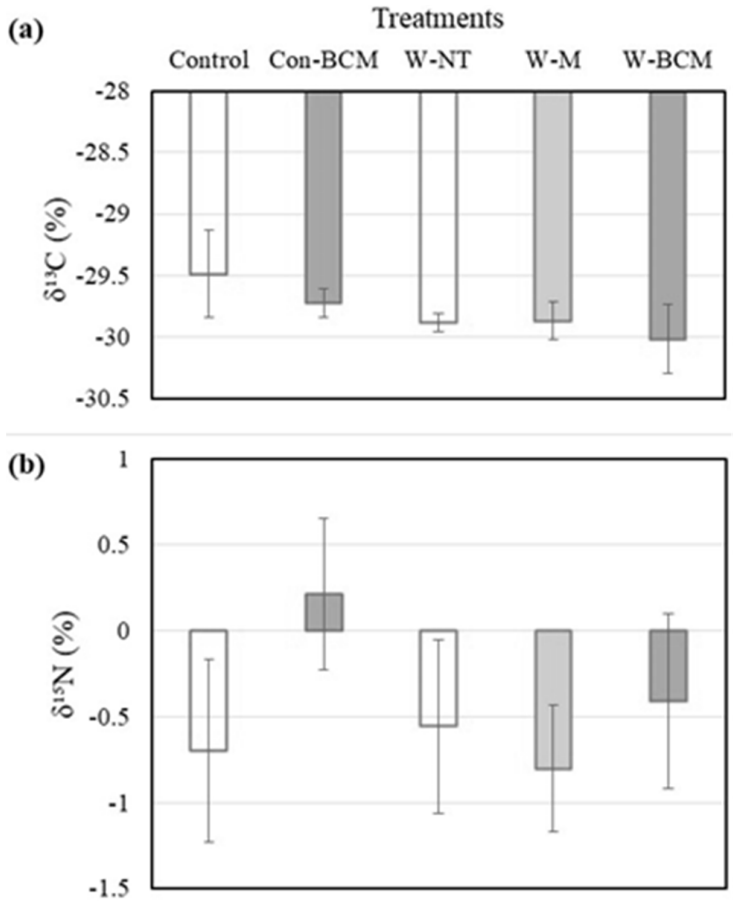

Figure 9. Comparison in (a) $\delta^{13}C$ isotope and (b) $\delta^{15}N$ isotope of the wild blueberry plants among five different treatments in August 2021. Error bars indicate the standard error of the mean. Bars with no letters above indicate no significant differences among the treatments at the significance level of $p < 0.05$.

At the end of July, there were no significant differences in major leaf nutrients (N, P, K, Ca, C) among the treatments (Fig. 10). Leaf nitrogen (Fig. 10a) and phosphorous (Fig. 10b) were lower than the optimum recommended levels whereas leaf potassium (Fig. 10c) was higher, and leaf calcium (Fig. 10d) was the same as the recommended optimum level.

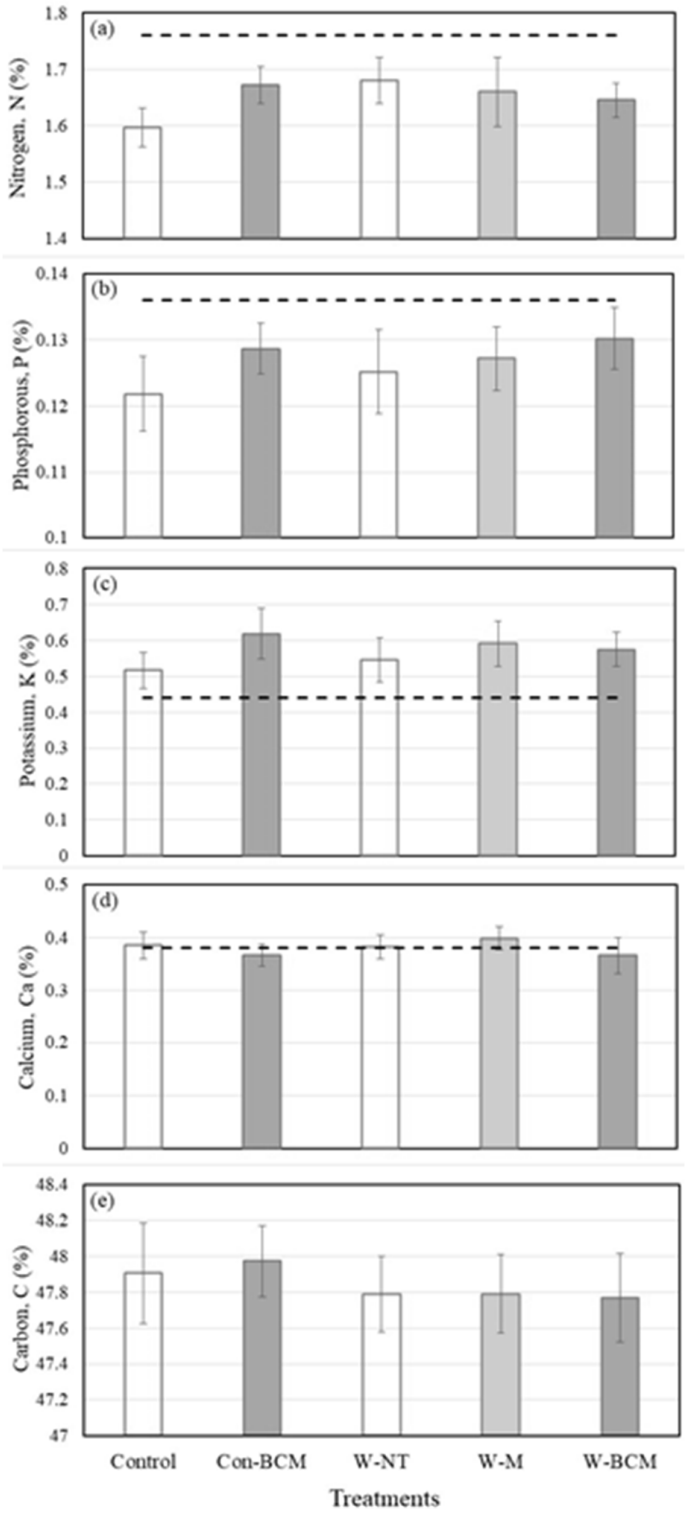

Figure 10. Comparison in leaf nutrients: (a) Nitrogen, (b) Phosphorous, (c) Potassium, (d) Calcium, and (e) Carbon of the wild blueberry plants among five different treatments in July 2021. Error bars indicate the standard error of the mean. Bars with no letters above indicate no significant differences among the treatments at the significance level of $p < 0.05$. The dashed lines represent the recommended optimum nutrient levels in wild blueberry leaves (Santiago 2011).

DISCUSSION

In the warming environment (1 to 5 °C higher than ambient), the average soil moisture of the growing season (June-October) was significantly lower than that in the ambient environment. However, soil moisture in warming chambers (W-NT) was barely lower than the control before mid-August possibly because of a higher amount (9-20% which is 2-3 times higher than the optimum) of organic

matter (Vallete et al., 1994) on the studied field soils. It is possible that the soil organic matter was decomposing and mineralizing during summer (June to August) and as a result, the organic matter content reduced and hence, soil moisture was much lower in the warming chamber than in the control from mid-August to October. This phenomenon can only be confirmed from the soil test results which we sampled at the end of September. Further, we used softwood bark mulch and biochar-compost mix to test whether they can retain more moisture in the wild blueberry field soil especially under a warmer climate. Our results showed that 0.5" layer of mulch was not sufficient as the soil moisture level was lower than the control plots and it was the same as the soil moisture in the warming chamber with no treatments. Previous researchers also recommended applying a thicker layer (at least 2-3") of wood mulch to wild blueberry fields to conserve soil moisture (Hunt et al., 2010). On the other hand, the use of biochar-compost mix resulted in significantly higher soil moisture content in both ambient and warming environments throughout the whole season. It could be because biochar helps sandy soil hold more water (Li et al., 2021). Therefore, biochar material applications on crops in sandy soils could reduce irrigation costs by saving water (Kroeger et al., 2021).

Wild blueberry plants growing in the warmer environment had higher stomatal conductance, transpiration rate, and photosynthetic rate meaning they were transpiring more water and photosynthesizing more than the plants growing in ambient environment. Plants growing in biochar-compost-treated soil in warmer environments showed significantly higher rates of photosynthesis than all other treatments. However, plants growing in the ambient environment had higher water use efficiency, especially in plants in the biochar-compost treated soil compared to the plants growing in the warmer environment. Interestingly, wild blueberry plants in a warmer environment seemed to be performing better as indicated by their higher photosynthetic capacity, stomatal conductance, chlorophyll content, and growth (stem length, leaf numbers). These observations make sense since the wild blueberry crop is growing in a temperate climate and many temperate crops are expected to benefit from 1 to 3ºC warming of ambient temperature (Easterling et al. 2007; Hatfield et al., 2011). Also, in agreement with the soil moisture results, wild blueberry plants growing on the biochar-compost treated soil had better physiological performance, growth and better leaf nitrogen uptake and accumulation among all the treatments; this is possibly due to more soil moisture availability (Agegnehu et al., 2017; Li et al., 2021, Ariz et al., 2015).

Complete soil health test reports and leaf nutrient results sampled at the end of the season will provide more information and explanation of our observations from this experiment. So far, our observations indicate that wild blueberry plants might grow better in warmer temperatures with sufficient soil moisture. Higher organic matter and/or other soil amendments like biochar-compost application on the soil surface can be beneficial for wild blueberries in drier summers. Since we started this experiment on the first year (vegetative year, prune stage) of wild blueberry crops, continuing this experiment on their crop year (when the plants will bear fruits) will provide us a full picture of how warming interacted with soil amendments would affect crop growth and yield.

CURRENT RECOMMENDATIONS

These preliminary results show biochar can be used to improve soil water holding capacity for wild blueberries.

NEXT STEPS

- Soil moisture, plant physiological performance and growth will be monitored in the crop year.
- Soil and leaves will be resampled for testing soil health, leaf nutrients, sugar and starch. Yield and berry quality will also be measured in 2022.
- The entomology research team will assess bee pollination as well for this experiment in 2022.

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We thank Mr. Joshua Stubbs and Christopher McManus from the Blueberry Hill Farm as well as staff from the HVAC shop at the University of Maine for constructing the warming chambers on very short notice. We thank Maine Woods Pellet Co, and Dr. Ling Li from the University of Maine for providing biochar and the University of Maine Composting Facility for providing compost for this experiment. We also thank Samuel Roberts and Atharv Desai for setting up the experiment and carrying out the measurements. This research is supported by the Wild Blueberry Commission of Maine, Maine Food and Agriculture Center, Maine Department of Agriculture, Conservation and Forestry, Maine Agricultural and Forest Experiment Station, USDA National Institute of Food and Agriculture Hatch (ME0-21832 and ME0-22021), and Jasper Wyman & Son blueberry company.

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RESEARCH

INVESTIGATOR(S): P. Pahadi, and YJ. Zhang

4. Responses of lowbush and highbush blueberries to extreme drought: threshold of coordinated declines in physiological processes and branch die back

OBJECTIVE(S)

- To understand how extreme droughts would impact different population-varieties of blueberry
- To quantify the stem water potentials that would cause severe declines in the physiological processes of blueberry population-varieties.

LOCATION(S): Nursery at University of Maine, Orono, Maine
PROJECT TIMEFRAME: July 2020 – August 2020

INTRODUCTION

Global climate change is increasing the likelihood of heatwaves, warmer temperatures, and drought that can have negative consequences on plants (Eisenach, 2019). Drought is one of the most prevalent environmental factors that can lead to decreased gross primary productivity, carbon storage (Allen et al., 2010; McDowell and Allen, 2015; D'Orangeville et al., 2018), and even plant death (Allen et al., 2010) under extreme conditions. Maine has already experienced several incidences of drought between 1900 and 2000, during 2002 and 2003 (Fernandez et al., 2020) and recently in 2016 and 2020 (NOAA, 2020) which caused substantial impact to the lowbush blueberry industry (Maine Agricultural Water Management Advisory Committee, 2003; Schattman et al., 2021). As the climate warms, future droughts and periods of limited moisture are likely to increase, because higher temperatures increase soil and crop water loss. the average annual atmospheric temperature is expected to increase by 2 to 6°C in Maine by 2100 (Jacobson et al., 2009). Importantly, many plant species, including blueberries, may lack the adaptations necessary to withstand expected future drought conditions (Liénard et al., 2016).

Identifying the threshold for declines in a physiological process during dehydration is critical for understanding and predicting plant response to drought (Anderegg et al., 2017). Under mild drought conditions, some plants adjust stomatal conductance to avoid low water potentials (Sperry at al., 2016). As the drought progresses, plants are no longer able to maintain the balance between water loss and uptake, and as a result, turgor loss and xylem cavitation (water column breakage) take place (Mingeau et al., 2000). Dehydration can result in substantial damage to the photochemical apparatus, and high levels of xylem embolism (Hoffmann et al., 2011), and almost complete canopy loss, which often leaves the plants damaged beyond recovery (Gauthey et al., 2021). Most studies on drought in blueberries have focused on the exposure of these blueberry plants to medium-level drought and studied effects on limited physiological processes (Ameglio et al., 2000; Percival et al., 2003; Glass et al., 2003; Glass et al., 2005). However, there is less knowledge on how blueberries respond to extreme drought conditions and how it impacts other aspects of physiological processes, such as stomatal conductance, photosynthesis rate, transpiration rate, plant hydraulic conductance, turgor loss point, chlorophyll fluorescence, chlorophyll content, and leaf browning. To investigate the response of different physiological processes to extreme drought conditions, a drought experiment was conducted to understand how extreme droughts would impact different population-varieties of blueberry and to estimate the stem water potentials that would cause severe declines in the physiological processes of high- and lowbush blueberries. Understanding how different population-varieties of blueberries will respond to future extreme drought conditions is important for the protection and management of the lowbush blueberry industry, which carries huge cultural significance and economic importance for the state of Maine.

METHODS

Plant materials and experimental design

To study the drought response of blueberry species native to NE US, four blueberry population-varieties (Table 1) expected to have differences in drought response were studied. The experiment was carried out in a 5.5m long, 3.0m wide, and 2.1m high rectangular-shaped rainfall exclusion house constructed at the nursery of University of Maine campus in Orono, Maine in July 2020. For our study, 40 individually irrigated plants from two populations of the lowbush blueberry species *Vaccinium angustifolium* (hereafter referred to as Ang 1 and Ang 2) and two varieties of cultivated highbush blueberry species *Vaccinium corymbosum* (hereafter referred to as Bluecrop and Patriot) were used. Out of 40 plants from the above-mentioned population-varieties, 30 (10 each of Ang 1, Bluecrop, and Patriot) were bought from a local nursery that imported them from New Jersey and the other 10 (Ang 2) were field-grown and collected from Blueberry Hill Farm in Jonesboro, Maine; these included soils to 10 cm depth and were planted in 2-gallon buckets.

To understand the drought response of four blueberry population-varieties, water was withheld from them for four weeks. The plants were arranged in a randomized complete block design with 5 experimental blocks and two treatments (control and drought), where four population-varieties were assigned to each block with each treatment combination (Figure 1a). Blocking was done by size class to reduce the impact of natural variation in the initial sizes of plants. The experiment consisted of control plants, which were watered thrice per week. Drought-treated plants were allowed to dehydrate gradually by withholding water.

The variation in temperature and relative humidity of the experimental site was recorded with two weather station sensors ATMOS 14 (METER Group Inc. Pullman, WA, USA) connected to ZL6 data loggers (METER Group Inc. Pullman, WA, USA). Soil moisture (volumetric water content; VWC) was measured using a soil moisture meter with 10 cm probes (TDR 150 Soil Moisture Sensor, Spectrum Technologies, Inc., Aurora, IL, USA) inserted into the soil to 10 cm depth.

Table 1. Study populations or variety, the species they belong to, their origin, and the plants' categorization as lowbush or highbush.

Population-varieties	Species Name	Origin	Lowbush/ Highbush Category
Ang 1	*Vaccinium angustifolium*	New Jersey	Lowbush
Ang 2	*Vaccinium angustifolium*	Blueberry Hill Farm, Jonesboro	Lowbush
Bluecrop	*Vaccinium corymbosum*	New Jersey	Highbush
Patriot	*Vaccinium corymbosum*	New Jersey	Highbush

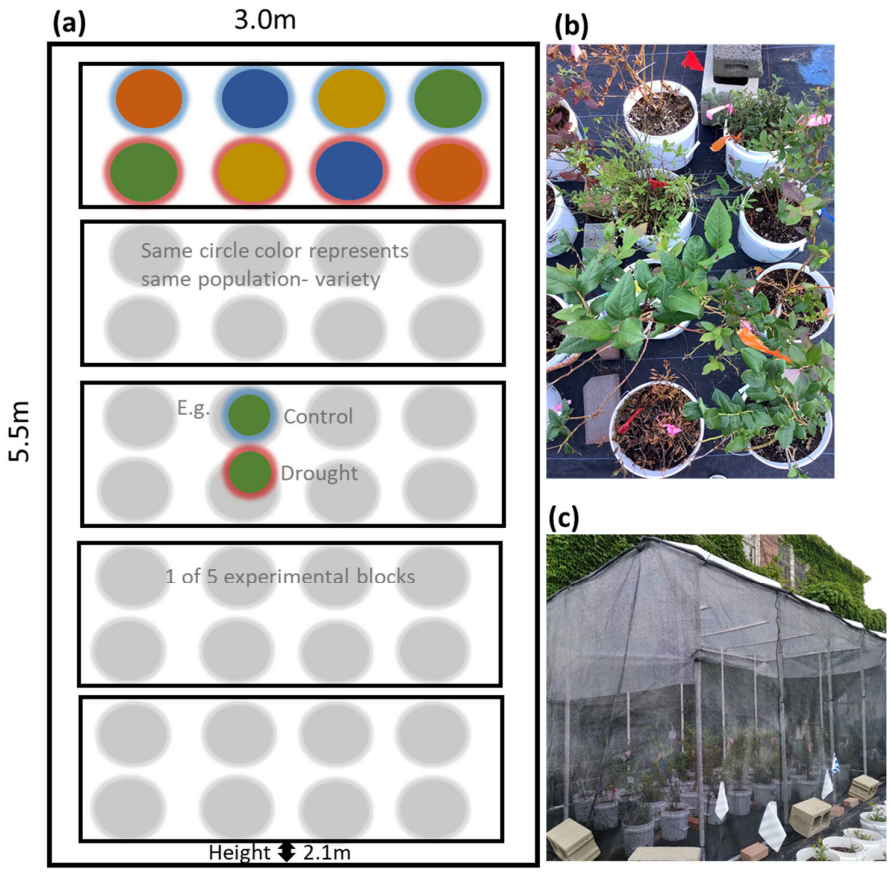

Figure 1. a) Experimental layout of 5.5m × 3.0m × 2.1m rainfall exclusion house. 40 plants were arranged in 5 blocks in a randomized complete block design (RCBD), with each population-variety and treatment combination appearing once per block, for a total of 5 replicates per population-variety and treatment combination. b) One experimental block of plants in August 2020. c) Rainfall exclusion house in July 2020 after construction.

Physiological measurements

Stem water potential (Ψ_{stem}) was measured at predawn (predawn Ψ_{stem}) and at midday (midday Ψ_{stem}) using a pressure chamber (Model 1505D; PMS Instrument Company, Corvallis, OR, USA) with non-transpiring leaves. Chlorophyll content was measured using a SPAD-502 chlorophyll meter (Konica-Minolta, Japan). Daily maximum stomatal conductance (Max g_s) was measured by an SC-1 leaf porometer (METER Group Inc. Pullman, WA, USA) and midday stomatal conductance (Midday g_s) and midday photosynthetic rate (A_{midday}) was measured using LICOR-6800 (Li-Cor, Lincoln, NE, USA). All the photosynthetic parameters were expressed on a projected leaf area basis and were later converted to mass-based dividing by leaf mass per area (LMA). The maximum quantum yield of PSII (F_v/F_m) was measured using a portable leaf fluorescence meter FluorPen (FP 110, Drásov, Czech Republic). Percentage loss of hydraulic conductivity (PLC) was measured for all the samples at the end of the drought experiment using single unbranched stem segments (Sperry et al., 1988; Lo Gullo & Salleo, 1991). Native flow rate and the maximum flow rate was calculated from the slope of initial flow rate and background flow rate and maximum flow rate and background flow rate.

Percentage loss of conductivity (PLC) was calculated by:

$$PLC = \frac{Kmax - Knat}{Kmax} \times 100\%$$

Plant hydraulic conductance (K_{plant}) was measured by the evaporative flux method (EF method) (Tsuda & Tyree, 2000), involving the measurement of steady state evaporative flux densities (E) and water potential of soil Ψ (Predawn Ψ_{stem}) and midday Ψ_{stem} (Midday Ψ_{stem}). E is assumed to be proportional to water potential difference:

$$E = K_{plant} (\Psi_{soil} - \Psi_{stem})$$

Where K_{plant} is the stem and root hydraulic conductance, and Ψ_{soil} and Ψ_{stem} are water potential of soil and root boundary, respectively.

Pressure–volume ("pv") curves were established by plotting the inverse of leaf water potential (−1/Ψ) of each sample vs relative water content. From the pressure–volume curve, leaf water potential at turgor loss point (Ψtlp), and modulus of elasticity (ε) were calculated according to methods described by Bartlett et al. (2012). The water potential during pv analysis of the leaf was measured using a pressure chamber (Model 1505D; PMS Instrument Company, Corvallis, OR, USA) and weight was measured using a high precision analytical balance (RADWAG X2 PLUS, Miami, FL, USA).

Canopy leaf death, mortality and resprouting

Leaf browning and canopy leaf shedding were recorded during the drought experiment where the rating was based on the whole plant. On each date, plants were given a rating of 0 (all green) to 6 (no leaves) based on the proportion of leaf canopy that was dead or brown; 0= all green, 1= 0-24% brown, 2= 25-49% brown, 3= 50-74% brown, 4= 75-99% brown, 5= 100% brown, and 6= no leaves (modified from Blackman et al., 2019).

After the drought experiment was completed in 2020, plants were rehydrated and left outside to experience winter dormancy. Then the evaluation for the mortality rate and resprouting of new stems were done based on individual and stem basis in June 2021, which was nine months after the termination of drought. Pots with completely dead plants and pots with living plants were counted to calculate the individual mortality rate. Plants with a living basal part that had growth of new leaf buds were also counted as living although the apical part was dead. On a stem basis, completely dead stems, completely new stems' growth (new sprouts), and new branches' growth in each pot were counted and calculated for both stem mortality rate, completely new stem regrowth rate, and new branches' regrowth rate.

Statistical analysis

To determine the rate of change in soil moisture, predawn and midday Ψ_{stem}, A_{midday}, maximum and midday g_s, K_{plant}, E, F_v/F_m, leaf browning, and leaf drop among population-varieties and treatments, the data was plotted using the R package 'ggplot 2', 'lubridate '(Spinu et al., 2021), 'dplyr '(Wickham et al., 2018) with species and day of drought as covariates. The relationship between soil moisture and stem water potentials (midday Ψ_{stem} or predawn Ψ_{stem}) was fitted using negative exponential models. To determine the pattern of A_{midday}, maximum and midday g_s, K_{plant}, E, F_v/F_m, leaf browning and leaf drop among population-varieties and treatment as a function of predawn and midday Ψ_{stem} was plotted using the R package 'ggplot 2', 'lubridate '(Spinu et al., 2021), 'dplyr '(Wickham et al., 2018), 'multcomp '(Hothorn et al., 2021). Also, the turgor loss point line was fitted in the above-mentioned relationships using R v.4.0.3 (R core team, 2021). To compare the difference in PLC, stem-level mortality rate, individual-level mortality rate, stem-level resprouting rate, individual-level resprouting rate, branch-level regrowth rate, and individual-level branch regrowth rate among the different population-varieties and treatments were plotted on a bar graph by taking the mean of five samples and fitting error bars using R package 'ggplot 2' (Spinu et al., 2021).

RESULTS

Soil moisture and Ψ_{stem} decline during dehydration

Drought caused decreases in soil moisture and midday Ψ_{stem} to a much lower level compared to control plants. During the first day of the drought (day 0), all the population-varieties had similar soil moisture content (30-40%) and midday stem water potentials (midday Ψ_{stem}; ~-1.0MPa) in both the

control and drought treatments (Figure 2a, 2b). As the drought progressed, different population-varieties under the drought treatment appeared to drop both their soil moisture and midday Ψ_{stem} and reached ~0% and ~4.5MPa respectively by day 31 of drought treatment (Figure 2a, 2b). Before the turgor loss point, all population-varieties maintained their midday Ψ_{stem} at ~1.9 MPa, but after the turgor loss point there was a sharp drop in midday Ψ_{stem} of all population-varieties.

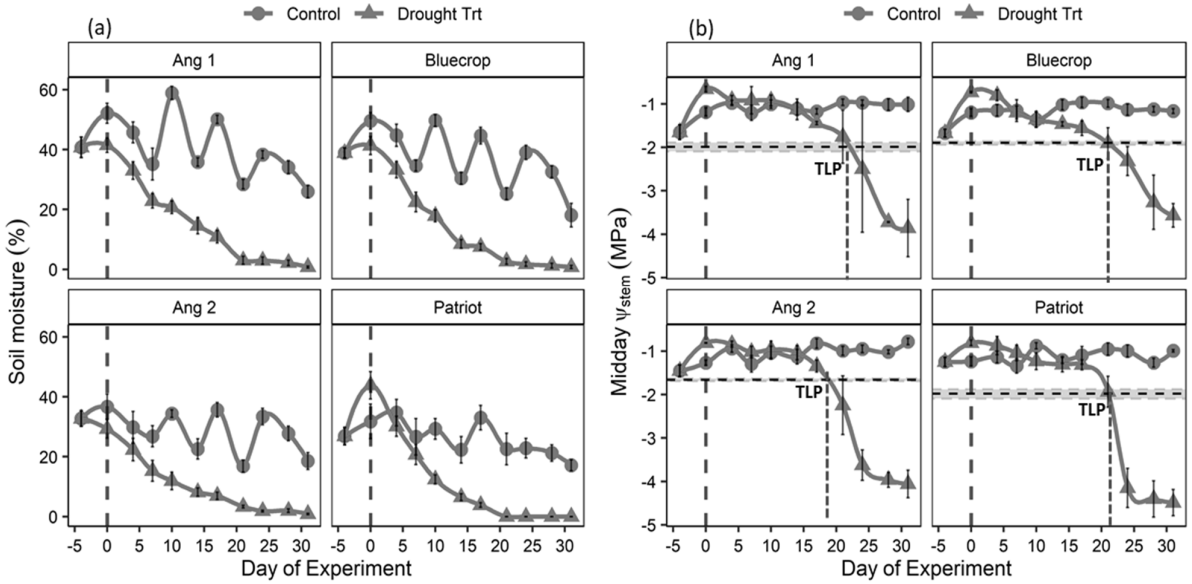

Figure 2. (a) Changes in soil moisture (%). (b) Changes in midday stem water potential, midday Ψ_{stem} (MPa) as a function of the day of the experiment (DOE) recorded in the control treatment (circles) and the drought treatment (triangles) of each population-variety: Ang 1, Ang 2, Bluecrop, and Patriot, during the course of the drought experiment. The thicker, vertical long dashed line is day 0 of the drought period, the intersection of the slender dashed lines with the drought treatment line are the Turgor Loss Points ("TLP"), and light grey background behind the dashed lines are the mean TLP ±SE values. Values are means ±SE for stem water potential (n = 3) and soil moisture (n = 5).

Stem water potentials (Ψ_{stem}) response to soil moisture during dehydration

Blueberry population-varieties under high soil moisture (30-40%) maintained high predawn Ψ_{stem} and midday Ψ_{stem} for all population-varieties (Figure 3a, 3b). As the soil moisture declined to 5%, the midday Ψ_{stem} and predawn Ψ_{stem} of blueberries population-varieties also started to drop sharply and reached -2.0 MPa and -1.0 MPa respectively. The turgor loss points were reached when soil moisture was around 5% for all population-varieties and that is when the sudden dropping of both midday Ψ_{stem} and predawn Ψ_{stem} occurred.

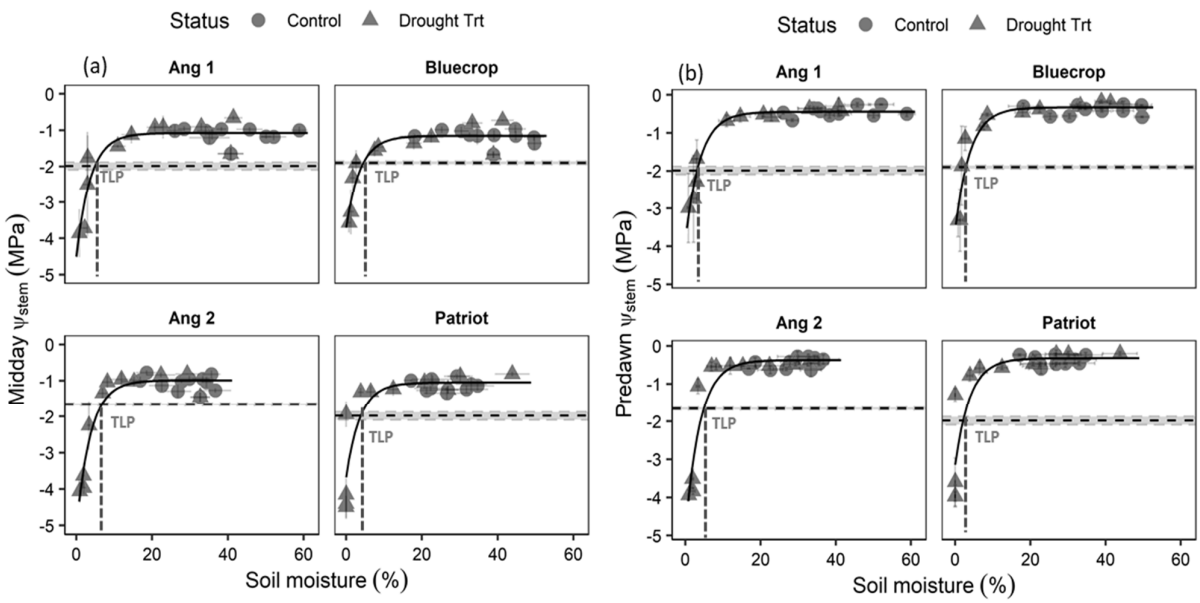

Figure 3. (a) The midday stem water potential, midday Ψ_{stem} (MPa). (b) The predawn stem water potential, predawn Ψ_{stem} (MPa) as a function of soil moisture in the control treatment (circles) and drought treatment (triangles) of each population-variety: Ang 1, Ang 2, Bluecrop, and Patriot. Negative exponential models (solid line) were fit for each population-variety. The intersection of the dashed lines are the Turgor Loss Points ("TLP"), and light grey background behind the dashed lines are the mean TLP ±SE values. Values are means ±SE for midday and predawn Ψ_{stem} (n=3) and soil moisture (n = 5).

Declines in some physiological processes before TLP, and progressive decline in some physiological processes after TLP, and the associated water potentials and soil moisture

Midday and maximum stomatal conductance (midday g_s, max g_s), midday photosynthesis rate (A_{midday}), transpiration rate (E), and plant hydraulic conductance (K_{plant}) dropped to their minimum values before or during the occurrence of turgor loss point (TLP) in all population-varieties of blueberries (Figures 4 and 5). After TLP, F_v/F_m started to decline rapidly and leaf browning and leaf dropping also started to increase rapidly.

Table 2. The minimum decline of midday stomatal conductance (midday g_s), maximum stomatal conductance (max g_s), midday photosynthesis rate (A_{midday}), transpiration rate (E), plant hydraulic conductance (K_{plant}), leaf browning (leaf br), photosynthetic efficiency of PS II (m) before the occurrence of Turgor Loss Point (TLP), TLP values and TLP ±SE values, midday Ψ_{stem} of minimum loss of midday g_s, A_{midday}, E, F_v/F_m, leaf browning before TLP, soil moisture and day of the drought treatment of minimum loss of midday g_s, A_{midday}, E, F_v/F_m, leaf browning before TLP in each population-variety: Ang 1, Ang 2, Bluecrop, and Patriot.

Population-variety	Midday g_s before TLP	Max g_s before TLP	A_{midday} before TLP	E before TLP	F_v/F_m before TLP	K_{plant} before TLP	Leaf Br before TLP	ΨTLP	ΨTLP ±SE	Ψ_{stem} of minimum midday g_s, A_{midday}, E, F_v/F_m, leaf browning before TLP	Soil moisture	Drought Day
Unit	mmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	µmol CO_2 m^{-2} sec^{-1}	Mol m^{-2} sec^{-1}		mmol H_2O m^2 MPa^{-1}	%	MPa	MPa	MPa	%	day
Ang 1	0.04	0.19	2.66	0.002	0.70	0.005	60%	-1.99	-1.99± 0.099	-1.76	3.02%	21
Ang 2	0.07	0.21	2.40	0.003	0.81	0.004	20%	-1.66	-1.66± 0.031	-1.34	6.96%	17-18
Bluecrop	0.03	0.33	1.26	0.001	0.82	0.001	0%	-1.90	-1.9± 0.038	-1.91	2.66%	21
Patriot	0.03	0.26	2.35	0.001	0.80	0.003	20%	-1.98	-1.98± 0.105	-1.94	0%	21

Stomata response to water potentials and soil moisture during dehydration

Maximum and midday g_s were highly sensitive to declining predawn and midday Ψ_{stem} and soil moisture. At high soil moisture (30-40%) and the midday Ψ_{stem} (-1.0MPa), midday g_s was at the high values for all populations or variety (Figure 4a) but appeared to decline linearly with the decline in midday Ψ_{stem}. By the time midday Ψ_{stem} had reached TLP (< 5% soil moisture), midday g_s declined to the minimum values (Figure 4a, 4c).

Maximum g_s also declined linearly with the decline in predawn Ψ_{stem} and soil moisture. At high soil moisture (30-40%) and the predawn Ψ_{stem} (-0.5MPa), maximum g_s was at the high values but appeared to decline linearly with the decline in midday Ψ_{stem}. When predawn Ψ_{stem} reached the turgor loss point (< 5% soil moisture), maximum g_s also declined to the minimum values (Figure 4b, 4d) across all population-varieties.

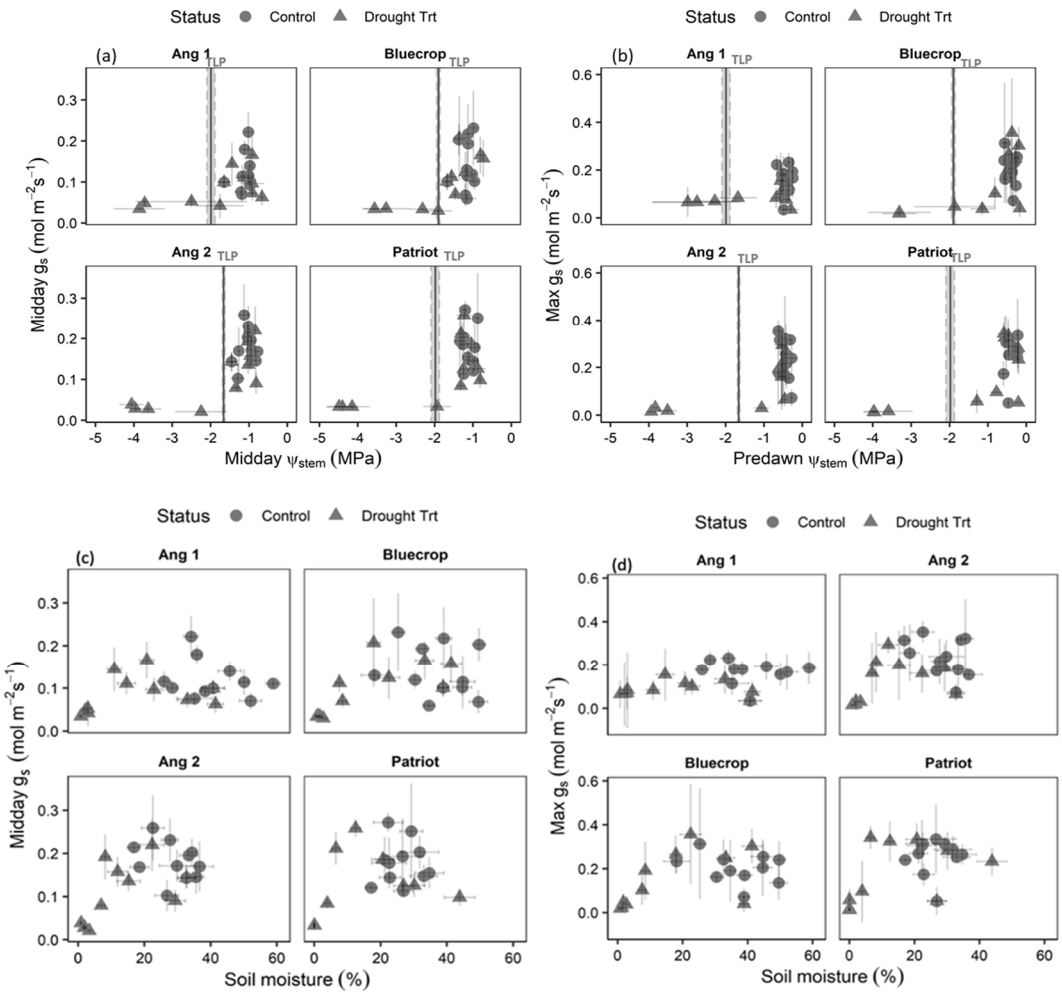

Figure 4. (a) The midday stomatal conductance, midday g_s (mol m^{-2} sec^{-1}) as a function of midday stem water potential, midday Ψ_{stem} (MPa). (b) Maximum stomatal conductance, max g_s (mol m^{-2} sec^{-1}) as a function of predawn water potential, Predawn Ψ_{stem} (MPa). (c) Midday stomatal conductance, midday g_s (mol m^{-2} sec^{-1}; c). (d) Maximum stomatal conductance, Max g_s (mol m^{-2} sec^{-1}) as a function of soil moisture (%) in the control treatment (blue dots) and drought treatment (red triangle) of each population-variety: Ang 1, Ang 2, Bluecrop, and Patriot. In (a) and (b), light grey background bounded with dashed lines represents mean TLP ±SE values and the solid lines in the middle are TLP for the corresponding population-varieties. Values are means ±SE for midday and predawn g_s, midday and predawn Ψ_{stem} (n=3) and soil moisture (n = 5).

Response of plant hydraulic conductance to dehydration

K_{plant} was also very sensitive to the decline in midday Ψ_{stem}. With the decrease in midday Ψ_{stem}, K_{plant} declined sharply. By the time midday Ψ_{stem} had reached TLP (< 5% soil moisture), K_{plant} also declined to the minimum values (Figure 5).

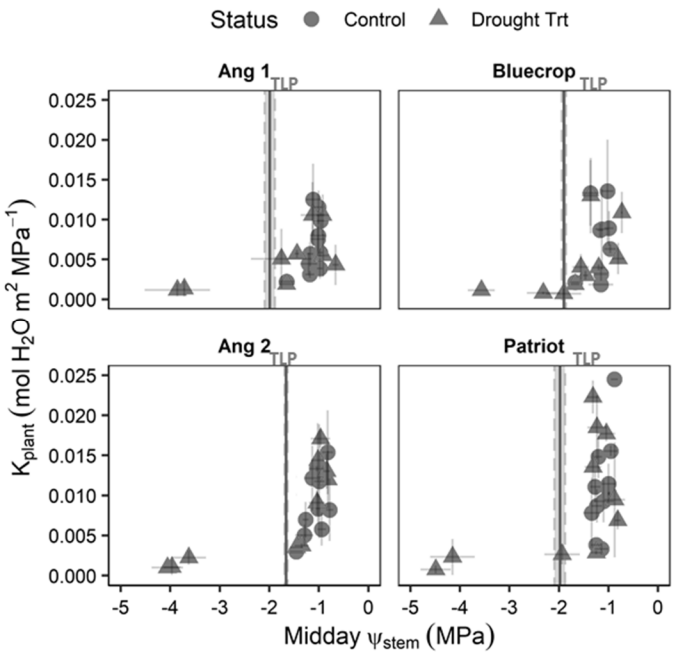

Figure 5. Plant hydraulic conductance, K_{plant} (mmol H_2O m^2 MPa^{-1}) as a function of midday stem water potential, midday Ψ_{stem} (MPa) in the control treatment (circles) and drought treatment (triangles) of each population variety: Ang 1, Ang 2, Bluecrop, and Patriot. Light grey background bounded with dashed lines represents mean TLP ±SE values and the solid lines in the middle are TLP for the corresponding population-varieties. Values are means ±SE (n=3) for midday Ψ_{stem} and K_{plant}.

Response of PSII to water potential and soil moisture during dehydration

F_v/F_m was not sensitive to declining midday Ψ_{stem} and soil moisture at the initial stage before TLP. At high soil moisture (30-40%) and before TLP, F_v/F_m was at the high values of 0.8 for all populations or variety (Figure 6a, 6b). At TLP, these populations still seemed to maintain high F_v/F_m values (0.8). After TLP or the range of TLP, there was a progressive decline of F_v/F_m values and when soil moisture was less than 5% across all population-varieties.

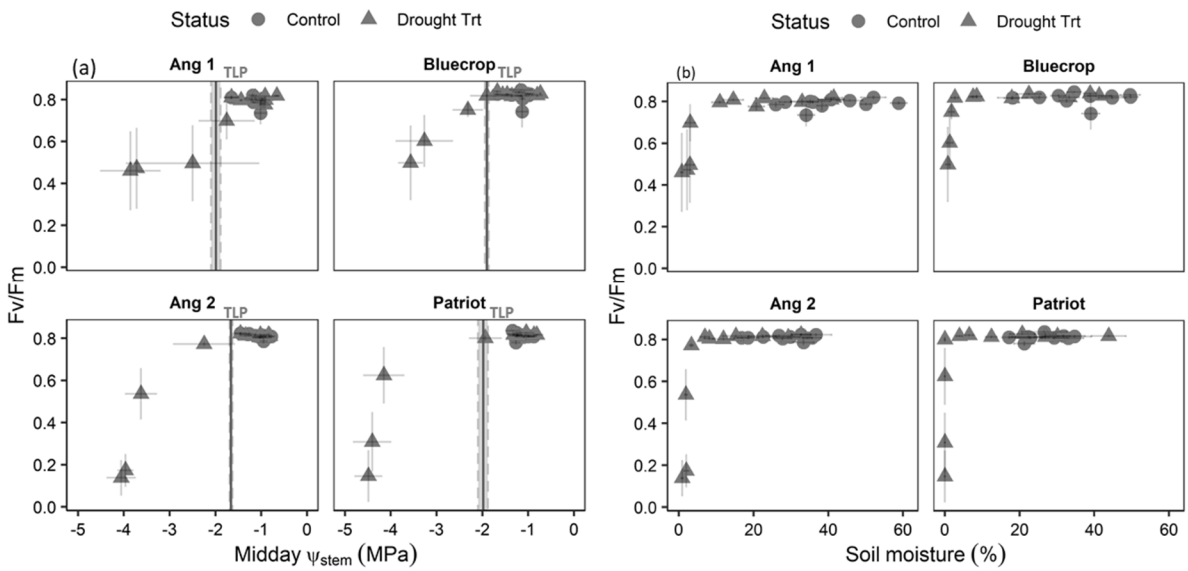

Figure 6. (a) Maximum photochemical efficiency of PSII (F_v/F_m) as a function of midday stem water potential, midday Ψ_{stem} (MPa). (b) Maximum photochemical efficiency of PSII (F_v/F_m) as a function of soil moisture (%) in the control treatment (circles) and drought treatment (triangles) of each population-variety; Ang 1, Ang 2, Bluecrop, and Patriot. Light grey background bounded with dashed lines represents mean TLP ±SE values and the solid lines in the middle are TLP for the corresponding population-varieties. Values are means ±SE for F_v/F_m (n = 5) and soil moisture and for midday Ψ_{stem} (n = 3).

Leaf photosynthetic rate in stomatal conductance during dehydrations

A_{midday} was significantly and positively related with midday g_s in all the population-varieties (Figure 7). Thus, the decline in A_{midday} during the drought was closely related to declines in midday g_s.

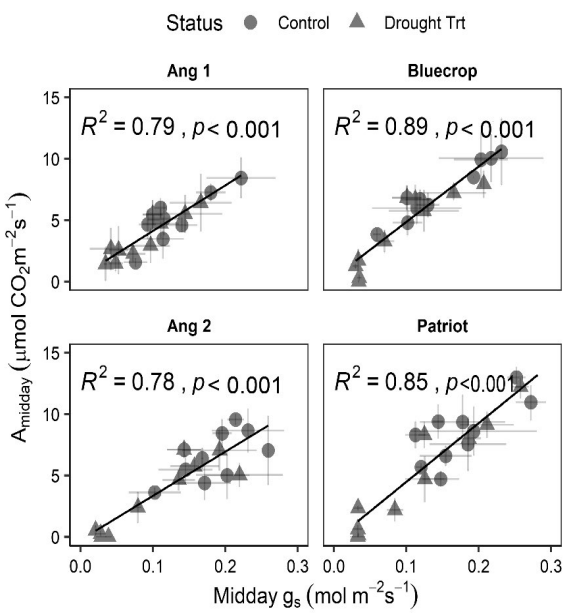

Figure 7 Midday photosynthesis rate, A_{midday} (µmol CO_2 m^{-2} sec^{-1}) as a function of midday stomatal conductance, Midday g_s (mol m^{-2} sec^{-1}) in the control treatment (circles) and drought treatment (triangles) of each population-variety: Ang 1, Ang 2, Bluecrop, and Patriot. Data are means ±SE (n=3).

Response of leaf browning to water potentials during dehydration
Leaf browning and leaf dropping in blueberry population-varieties were not very sensitive to the declining midday Ψ_{stem} and soil moisture at the initial stage before TLP (Figure 8a) except for the Ang 1 population. However, after TLP, leaf browning seemed to increase rapidly in all the population-varieties and reached almost 100% in Ang 1, 75% in Ang 2 and Patriot, and 40% in Bluecrop at the end of the drought treatment (Figure 8a).

Similarly, leaf dropping was not very sensitive to the declining midday Ψ_{stem} and soil moisture at the initial stage before TLP in all populations or varieties (Figure 8b). However, after the TLP, there was a progressive increase in the leaf dropping across all population-varieties except for the Bluecrop population which did not drop leaves even till the end of the drought treatment.

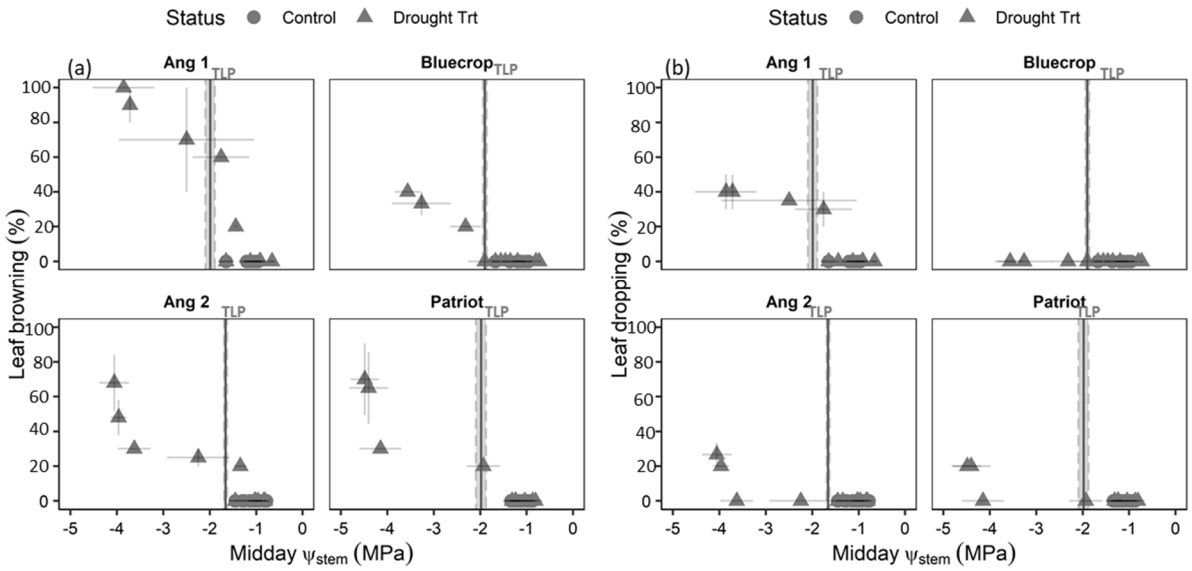

Figure 8. (a) Leaf browning as a function of midday stem water potential, midday Ψ_{stem} (MPa). (b) Leaf dropping as a function of midday stem water potential, midday Ψ_{stem} (MPa), in the drought treatment (triangles) of each population-variety: Ang 1, Ang 2, Bluecrop, and Patriot. Light grey background bounded with dashed lines represents mean TLP ±SE values and the solid lines in the middle are TLP for the corresponding population-varieties. Values are means ±SE for midday Ψ_{stem} (n=3) and n= variable number for leaf browning and leaf dropping depending upon the color change and leaf dropping pattern. The percentages for leaf browning and leaf dropping were rated as a percentage from 0% to 100% where 0= all green and 100= all brown leaves.

PLC, plant mortality, and regrowth during dehydration and after recovery
At the end of the drought treatment, PLC in Ang 1 and Patriot was close to 100%, which is higher than Ang 2 and Bluecrop that maintained PLC at 86.7% and 83.27%, respectively (Figure 9). Although Ang 1 and Patriot had high PLC, only Ang 1 had high individual mortality (40%) and stem mortality rate (60.1%). In contrast, Patriot had a high stem mortality rate (84.3%) and a low individual mortality rate (20%; Figure 10a, 10b). Bluecrop and Ang 2 showed an individual mortality rate of 20%. The stem mortality rate was 81% in Ang 2 and 68.3% in Bluecrop (Figure 10a, 10b).

Regrowth of new stem and branches in blueberry population-varieties was detected. Regrowth of new stems from roots underneath the soil was found in Ang 1 and Ang 2, and regrowth of new branches in Patriot and Bluecrop occurred from the living parts of the stems. Ang 1 and Ang 2 showed 40% and

80% individual regrowth rate, and 10% and 11% stem regrowth rate (Figure 10c, 10d). Aside from the growth of completely new stems, Patriot and Bluecrop showed regrowth of completely new branches and showed 80% and 40% individual level branch regrowth rate and 15.5% and 10.2% branches regrowth rate respectively (Figure 10e, 10f).

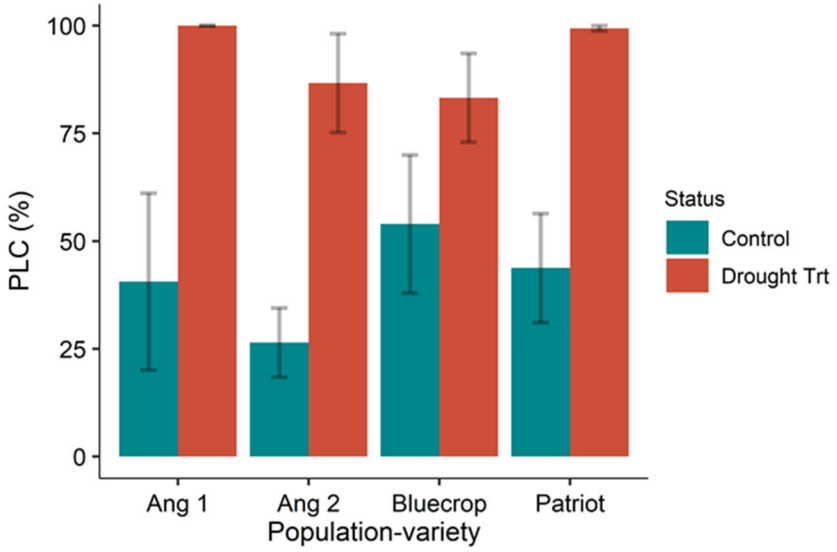

Figure 9. Percentage loss of hydraulic conductivity (PLC) of each population-variety (Ang 1, Ang 2, Bluecrop and Patriot) at the end of the drought experiment. The blue bars represent control treatment and red bars represent drought treatment. Values are means ±SE (n=5) for PLC in both control and drought treatment.

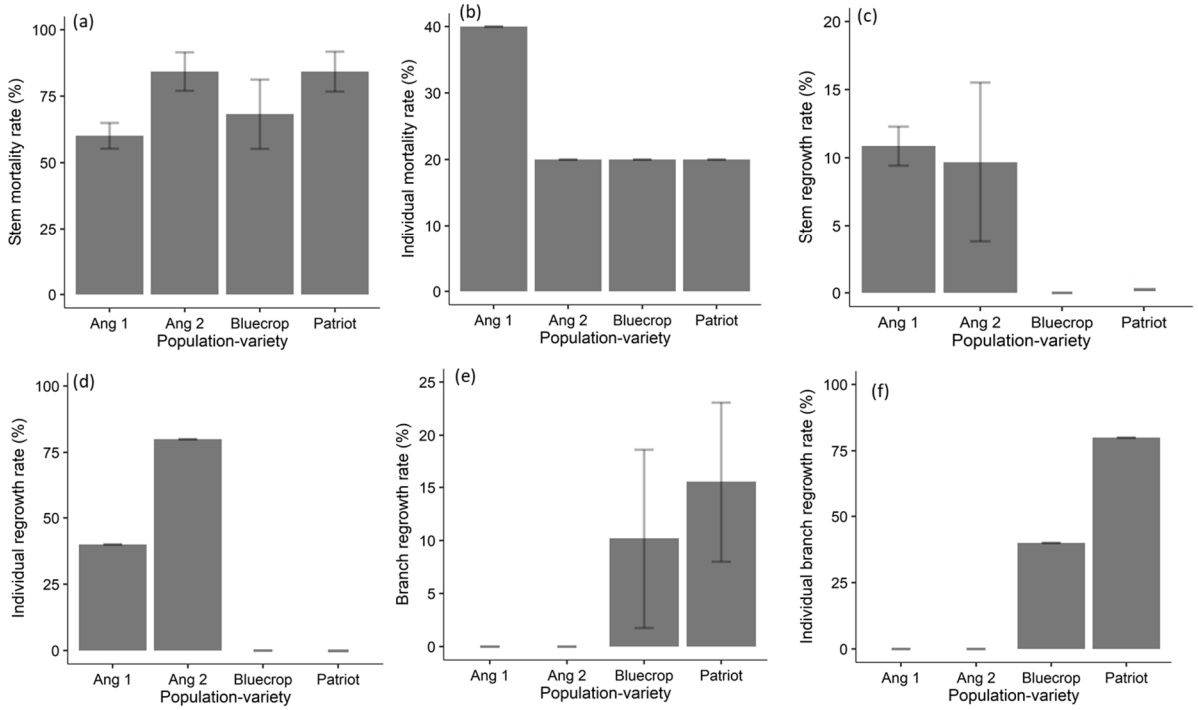

Figure 10. Stem mortality rate (a), individual mortality rate (b), stem regrowth rate (c), individual level stem regrowth rate (d), branch regrowth rate (e), individual level branch regrowth rate (f) of each population-variety (Ang 1, Ang 2, Bluecrop and Patriot) at the end of the drought experiment. Bars represent drought treatment across all graphs. The controls of all population-varieties are not plotted as they never reported mortality rate and regrowth is taking place as a continuous process. Values are means ±SE (n = 5) traits.

DISCUSSION

Our study revealed quick declines in stomatal conductance, photosynthesis, and water loss before the turgor loss point (TLP), and the progressive decline of photochemistry, leaf browning, and leaf dropping after the TLP as Ψ_{stem} and soil moisture declined across all population-varieties of blueberries. Thus, TLP is a threshold for all population-varieties after which permanent damages started to happen. The stomata of all population-varieties were very sensitive to declining Ψ_{stem} and soil moisture. Interestingly, the leaf browning increased after TLP, which coincided with declines in F_v/F_m showing a coordinated response. Blueberry population-varieties showed 83 to 100% loss of hydraulic conductivity (PLC) and high mortality rates when Ψ_{stem} reached -3.6 to -4.5 MPa. However, these population-varieties showed high regrowth of new stems and branches. In addition, Ang 1 experienced 100% PLC, and the highest mortality rate but demonstrated a high regrowth of new stems from the rhizomes post-drought. Our results provide important insight regarding blueberry response to extreme drought and drought-induced damages before and after TLP. This information is critical for understanding the response of blueberries to extreme drought conditions, especially in preparation for a future in which drought events are expected to increase in frequency all over the northeastern US (Wake et al., 2014).

Turgor loss point as a proxy of drought stress and thresholds for the decline of different physiological processes

Maximum and midday g_s, A_{midday}, E, and K_{plant} in the studied blueberry population-varieties were all sensitive to declines in Ψ_{stem} and reached minimum values before or at TLP. This demonstrates that all of them adopted a more drought-avoidance strategy to avoid water stress using sensitive stomatal control. Therefore, the downregulation of stomatal conductance and several other physiological processes as well as turgor loss in blueberry population-varieties might serve as a protective mechanism against xylem cavitation or xylem embolism (Hochberg et al., 2017). Davies and Johnson (1982) reported a critical water potential of -2.2 MPa in *Vaccinium ashei* Reade (Rabbiteye blueberry) for stomatal closure, which is close to the TLP in our study.

In contrast, leaf biochemistry indicated by F_v/F_m was not sensitive to drought at the initial stages or before the occurrence of TLP at -2.0MPa in Bluecrop, Patriot and Ang 2. Ang 1 dropped its F_v/F_m slightly and reached 0.7, along with an increase of leaf browning to 60% at TLP. In Patriot and Ang 2, leaf browning reached 20% at TLP, while Bluecrop did not initiate leaf browning at TLP. Thus, although a Ψ_{stem} of -2.0MPa indicates a lethal dose of water stress in many perennial crops such as grapes, citrus, apples (Smart, 1974; Kaufmann and Levy, 1976; West and Gaff, 1976), and some trees (Barigah et al., 2013), it is not the case in studied blueberry population-varieties. However, following TLP, there was a progressive and relatively fast decline in F_v/F_m, and a progressive increase of leaf browning and leaf dropping across all population-varieties. This shows the close coordination between F_v/F_m, leaf browning, and leaf shedding. The decline of these physiological processes after TLP could be due to the disconnection of the stem from the soil and the formation of significant xylem embolism across blueberry population-varieties. This suggests that TLP is an important indicator of water stress in blueberries, beyond which can cause branch dieback and plant mortality.

Further, the coordinated decline of physiological processes along with midday Ψ_{stem} after the TLP across all population-varieties could be due to higher vulnerability to embolism which could be the reason for 100% PLC across all population-varieties under extreme drought stress conditions. In research conducted by Ameglio et al. (2000) on the Bluecrop variety, they found that embolism increased rapidly below -1.2 MPa midday Ψ_{stem}, and that below -2.1 MPa, embolism was total. The findings of Ameglio et al. (2000) relate to ours in that most physiological processes dropped to lower values before the TLP of nearly -2.0MPa midday Ψ_{stem}, and after TLP there was a progressive decline

in photochemistry. It is likely that embolism might have already occurred at TLP across all the blueberry population-varieties in our studies based on the findings of Ameglio et al. (2000).

Hydraulic safety and productivity

There could be a tradeoff between maximum productivity and hydraulic safety. Patriot showed higher plant hydraulic conductance to support higher maximum and midday g_s, higher A_{midday}, and higher E, meanwhile it also showed higher PLC (100%). In contrast, Bluecrop, with intermediate maximum and midday g_s, A_{midday}, E, and K_{plant} values, experienced 83% loss of hydraulic conductivity, which was less than all others irrespective of its larger plant and stem size. However, Ang 1 with relatively low maximum and midday g_s, E, A_{midday} and K_{plant} had 100% loss of hydraulic conductivity. High PLC in Ang 1 could be a strategy to protect the rhizomes and roots by abandoning the aboveground parts, and leaf browning and shedding could be mechanisms to avoid further water loss and to protect the rhizomes and roots.

Mortality and regrowth

High PLC and branch dieback were detected at the end of the drought treatment, but some population-varieties were able to regrow new stems from their surviving parts, showing high recovery capacity. Patriot experienced 100% PLC in terminal stems and had the highest stem mortality rate (84%), coupled with a low individual mortality rate (20%) and high regrowth of new branches (15.54% at branch level; 80% at the individual level). Regrowth and resprouting occurred from the living stems and rhizomes, whereas terminal branches remained dead. Ang 2 with 87% PLC showed the highest stem mortality rate but had a lower individual mortality rate. Also, Ang 2 had the highest rate of resprouting of new stems at the individual level (80%) and had a high rate of regrowth of new stems (10%) at the stem level. Ang 1 and Ang 2 populations' rapid leaf browning, leaf shedding, and high stem dieback could potentially protect them from further water loss and prevent the depletion of carbohydrates in the rhizomes and roots which could be used for regrowth and resprouting in the following year, as found in other shrubs and some tree seedlings (Galvez et al., 2011; Barigah et al., 2013; Vilagrosa et al., 2014). Ang 1 also showed rapid browning and leaf shedding, which could protect its stem by minimizing water loss. As the drought progressed to – 3.6 MPa of midday Ψstem, Ang 1 reached 100% PLC with high stem and individual mortality, suggesting high vulnerability to embolism. However, it also displayed a high individual and stem regrowth rate (40% and 11%). In contrast, Bluecrop had very low browning percentages, did not show leaf drop, and maintained the lowest PLC (83%) with the lowest individual and stem mortality, suggesting that this variety could be highly resistant to drought-induced embolism. In contrast to Patriot, Bluecrop did not show high regrowth of new branches, due to a low mortality rate and the regrowth of new branches that was observed only in those stems whose basal stems parts were alive; the terminal branches remained dead. Despite high PLC and high mortality rate, high resprouting and regrowth in the following year could be a mechanism in shrub drought response (Zeppel et al., 2014). In *Angustifolium* populations, almost 85% of the lowbush blueberry biomass exists as a shallow underground rhizome (Hall, 1957) providing an ability to recover through resprouting. Notably, the pots used limit the size of the rhizomes (Kramer, 1983), which could lead to lower resistance in studied Ang 1 and Ang 2 populations compared to plants in the field.

CONCLUSION

This study provides an examination of how stomatal, transpiration, photosynthetic, photochemistry, and plant hydraulic systems coordinated in the response of blueberries to extreme drought. The results showed that the turgor loss point (TLP) is an important threshold for different physiological processes. Stomatal conductance, photosynthesis, and transpiration all reached the minimum before or at TLP. After TLP, the decline in stem water potential accelerated likely because of xylem embolism, which limited the supply of available water to stem and leaves. This resulted in declines in photochemistry (indicated by F_v/F_m), as well as accelerated leaf browning and leaf shedding after TLP. This study concludes that blueberry plants could be resistant to the level of TLP at -2.0MPa, while lower levels of

drought can result in significant damages, high leaf browning, and leaf shedding. When exposed to extreme drought of -4.5 MPa, they showed high PLC of 83 to 100% and high branch dieback. However, the blueberries had a high regrowth rate, and high resprouting rates of new stems from rhizomes were observed in *Angustifolium* populations. Replicating this experiment in the field and further study of the recovery processes would provide more insights into the drought response strategies of blueberries.

CURRENT RECOMMENDATIONS

It is recommended that growers use the leaf turgor loss point (-2.0MPa) as the critical water potential threshold, which should be avoided in the field to minimize tissue damage, branch mortality, and ultimately the death of the whole plant. Soil relative moisture content of 5% is also critical soil moisture threshold below which plant cannot tolerate drought by affecting all the physiological processes leading to branch mortality and plant death. By regular monitoring of soil water content, it can help farmers to decide when drought mitigation practices like irrigation are needed.

NEXT STEPS

Conduct drought stress studies in field conditions since plant stands in the field conditions may respond differently to drought conditions depending upon the genotypes present.

- Test soil amendments in mitigating warming-induced water deficits.
- Explore factors contributing to the positive effects of warming on crop yield.

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RESEARCH

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5. Effects of Biochar on Improving Sandy Soil Water Retention and Wild Blueberry Plants' Resilience to Drought

OBJECTIVE(S)

- To characterize the morphology and physical properties of forestry biomass-derived biochar.
- To evaluate how the presence of biochar in sandy soils can impact the resilience to drought of wild blueberry crops.

LOCATION(S) University of Maine, Rogers Farm, Orono, Maine
PROJECT TIMEFRAME September 2020 - December 2022

INTRODUCTION

The projected increase in climate variability imposes an enormous threat to agricultural systems (Brooks & Levinsohn, 2011; Cohn et al., 2017; Field et al., 2012; Masson-Delmotte et al., 2018; Ren et al., 2018). For agricultural systems with sandy soils, the impacts will be exacerbated due to their low water retention and capacity to buffer increasing variability in rainfall. Although certain types of crops can thrive and remain productive in sandy soils, such as lowbush (or wild) blueberries

(*Vaccinium angustifolium* Ait.), these crops consume considerable water and fertilizer because the coarse texture of sandy soils has quick water drainage. Also, the sandy soils are easy to warm up in spring and tend to dry out in summer and suffer from low nutrients that are washed away by rain. The severity, duration, and frequency of drought are predicted to increase in many regions of the world, including New England, USA (Dai, 2013; Field et al., 2012; Masson-Delmotte et al., 2018). Further, the elevation of the annual average global temperature would lengthen the warm season (defined as when the average daily temperature is above freezing). For example, it is reported that the warm season in Maine, USA, extended by two weeks in the past 100 years and will continuously increase by approximately two more weeks over the next 50 years in Maine (Fernandez et al., 2015). The extended warm season may result in increased water use and enhanced drought effects on unirrigated farmland in dry seasons or excessive amounts of nitrate leaching from the root zone in wet seasons.

Biochar as a soil amendment has been extensively studied over the past decades (De Melo Carvalho et al., 2014; Labbe et al., 2013; Pyoungchung et al., 2014; Rehrah et al., 2014; Song & Guo, 2012; Suliman et al., 2017). It has a porous structure, a relatively large surface area, multiple functional groups (e.g., H/C-ratio), and abundant mineral elements, etc. Such favorable attributes make biochar an excellent soil addition, in general, to increase the crop yield by mixing with fertilizers or organic compost, which can retain water in the soil, delay nutrients leaching from the soil, favorably modify the pH, improve ventilation and permeability of the soil, and immobilize heavy metals. The effects of biochar additions on the improvement of water and nutrient retention capacity of sandy soil are significantly greater than other soil types, such as clay soil. Based on the classifications of soil pore sizes by the Soil Science Society of America (Soil Science Glossary Terms Committee, 2008), macropores (>75 μm in diameter) contribute to the rapid flow of water (including nutrient-rich water) through soil by gravity, and mesopores (30 μm to 75 μm in diameter) allow water to move in response to matric potential differences (i.e., from wetter to drier areas). It is micropores (5 μm to 30 μm in diameter) that hold water in place by capillarity to maximize water and nutrient retention in soils. The smallest pore sizes are ultra-micropores (0.1 μm to 5 μm in diameter) and cryptopores (<0.1μm in diameter). Sandy soils usually comprise a large portion of coarse sands with grain sizes ranging from 250 μm to 2,000 μm (Petersen et al., 2016). Adding biochar with a large portion of micropores to the sandy soils should be able to modify the pore size distribution of the soils and help hold large volumes of water even at elevated matric potentials. Nutrient retention can be realized by the trapping of nutrient-rich water held by capillary forces generated in the micropores (5μm to 30μm in diameter) of biochar (Kameyama et al., 2019). Thus, dissolved nutrients would be retained near the soil surface if the water is immobile or moves slowly. Plants can access part of the nutrients in this retained soil solution as they transpire and elevate soil matric potential (Lehmann & Joseph, 2009).

Wild (or wild) blueberry (*Vaccinium angustifolium* Ait.) crops in Maine have played an essential role in the economy of the state, making a significant economic contribution of approximately $250 million in 2007 (Planning Decisions, Inc., 2009). Being a leading source of antioxidant phytonutrients, wild blueberries take a considerably large market share as a health food supplement in North America. There are about 485 wild blueberry farmers and growers in Maine. Unfortunately, more than 70% of the wild blueberry farmlands are not covered with irrigation systems (Yarborough, 2004), which limits the implementation of certain management practices, such as irrigation in drought years. There is an urgent need for improving on-farm water and fertilizer use efficiencies, lowering the cost of management practices, and securing the growth of wild blueberries crops and berry yield.

This study aimed to provide a cost-effective solution to improve the sandy soil water retention and wild blueberry plants' resilience to drought. Woody biochar produced using local forestry biomass as a byproduct of uncompleted biomass combustion was studied due to the abundance of forest resources in Maine. A drought experiment was carried out on wild blueberry plants in a greenhouse in the past summer to investigate how biochar would alter the sandy soil's properties including volumetric

water content, electrical conductivity, and pH, and how biochar can help crops in extreme weather conditions by examining the crop physiological performances including leaf water potential, stomatal conductance, leaf chlorophyll content, and leaf photosynthetic rate. As an on-going project, we have completed the biochar characterization and greenhouse experiment. Only a part of the experimental results is given in this progress report.

METHODS

Data collection

Characterization of residual biochar sourced from Maine

The biochar material used as a soil amendment was collected from the biomass combined heat and power plant of Maine Woods Pellet Co. located in Athens, ME. The biochar was produced as a byproduct of biomass combustion. The biomass feedstock, i.e., logging residues, came from the local forests. The raw biochar was mixed with ash when delivered to UMaine's biomass energy testing laboratory. Pure biochar was obtained by sifting the mixture to remove ash using a biomass screener. The physiochemical properties of pure biochar (two replicates for each test) were tested at UMaine collaborative laboratories, which are listed below:

- Biochar's surface area was measured using a Micromeritics ASAP® 2020 Accelerated Surface Area and Porosimetry Analyzer;
- The pore volume (individual, medium, and cumulative), pore size distribution, and bulk density of biochar was measured by a Micromeritics Auto Pore V Mercury Intrusion Porosimetry;
- Biochar's pH was measured by testing the water after soaking the biochar into water using FisherScience pH strip (Fisher Scientific, Pittsburgh, PA, USA);
- The moisture content of biochar, defined as the ratio of the mass of water to mass of oven-dried biochar, was tested by drying the biochar sample in an oven until the mass of biochar reached a constant value following ASTM D4442-20 Standard Test Methods for Direct Moisture Content Measurement of Wood and Wood-Based Materials (ASTM, 2020); and
- The morphology of biochar was observed under an AMRay 1820 Scanning Electron Microscope (SEM).

Greenhouse experiment of wild blueberry plants

Research production location, site location, and species

A greenhouse experiment was conducted at Rogers Farm in a controlled setting with frequent monitoring. Approximately 80 wild wild blueberry (*Vaccinium angustifolium*) plants were transplanted from Wyman's gravel pits in July 2021. A total of five genotypes of blueberry plants were selected. They were placed in five blocks, respectively (Figure 1). Four treatment types were designed for the five blocks, which are listed as follows:

1. Drought, sandy soil & biochar mixture at a ratio of 1:1 by volume (abbreviation: D-B)
2. Drought, sandy soil without biochar (abbreviation: D-NB)
3. No drought, sandy soil & biochar mixture at a ratio of 1:1 by volume (abbreviation: ND-B)
4. No drought, sandy soil without biochar (abbreviation: ND-NB)

Note: There were four replicates (i.e., four plants) for each treatment and for each genotype.

Figure 1. One block of wild blueberry plants in the Rogers Farm Greenhouse.

Implementing extreme drought onto wild blueberries in a controlled setting

Before doing the greenhouse experiment, there was a three-week establishment period to ensure that the plants were in healthy condition. During this time, the plants were watered twice a day using an irrigation system for uniform watering. Additionally, biochar with 5 grams of nitrogen-based fertilizer (i.e., ammonium sulfate) was added to each container in the last week of the establishment in preparation for the drought experiment. At this stage, weekly measurements of physical traits of plants (such as stem width and height), basic physiological measurements of plants (such as chlorophyll and anthocyanin content), and soil properties (such as pH and soil moisture) were taken. The soil volumetric water content (VWC) of soils at four treatments was measured periodically, as shown in Figure 3. During the establishment period, weeks 1 to 3, the soil volumetric water content (VWC) was measured once a week. During the drought period, weeks 4 to 13, soil volumetric water content was measured coinciding with stomatal conductance, water potential and photosynthesis measurements. This was based on the dehydration rate and could vary from every other day to once a week. Treatments with biochar, D-B and ND-B were the only treatments measured during week 13. A weather station was placed in the greenhouse and outside to record the temperature and relative humidity of the air. It was found that the temperatures in the green house were usually 2 to 3 °C (3.6 to 5.4 °F) higher than the outside ambient temperature.

After the establishment period, a drought treatment for half of the plants was applied by withholding irrigation. The drought treatment was based on monitoring the wild blueberry plants until their water potentials reached -4MPa, when the stem water transport system was severely damaged (air bubbles blocked the pathway of transportation, called xylem embolism). As controls, other plants were watered regularly.

Since this study aimed to evaluate the effects of biochar as a soil amendment on the soil water retention and plants' resilience to drought, some key properties related to water were measured, including soil volumetric water content, soil electrical conductivity, soil pH, and plant physiological parameters including leaf water potential, stomatal conductance, leaf chlorophyll content, and leaf photosynthetic rate.

The instruments used for measuring the soil properties and plant physiological parameters are listed as follows:

- Soil volumetric water content (VWC) and electricity conductivity (EC) were measured using a Fieldscout TDR 150 Soil Moisture Meter (Spectrum Technologies, Inc, Aurora, IL, USA). Soil water potentials were measured with the Teros 21 soil moisture sensors with ZL6 logger (Teros 21; Metergroup Inc., Pullman, WA, USA).

- The soil pH was measured with the Fisher brand pH strips (Fisher Scientific, Pittsburgh, PA, USA).
- Plant stem width was taken with a caliper (General Tools & Instruments 3-in Digital Caliper; Lowe's, Bangor, ME)
- Plant height was taken in centimeters (cm) with a measuring tape (Lowe's, Bangor, ME)
- Leaf water potential was measured by a leaf pressure chamber (Model 1505D; PMS Instrument Company, Corvallis, OR USA).
- Leaf chlorophyll content was measured by a chlorophyll meter (SPAD 502; Minolta Corp., Osaka, Japan).
- Anthocyanin content was measured by the ACM-200 anthocyanin meter (Opti-Sciences, Inc., Hudson, NH, USA).
- Stomatal conductance was measured by the LI-600 portable porometer (600; Li-Cor Biosciences, Lincoln, NE, USA).
- The photosynthetic rate was measured using the Y(II) Meter (Opti-Sciences, Inc., Hudson, NH, USA).

Data Analysis

The mean and standard error of soil properties and plant physiological properties were calculated and reported. The effects of biochar as a soil amendment on soil properties and plant physiological properties will be carried out by doing two-way ANOVA analysis via R software.

RESULTS

Characteristics of biochar

Our results indicate that the biochar sourced from Maine Combined Heat and Power Plant was generally comparable to the biochar reported in literature (Table 1). The porosity of the biochar was about 52%, which is less than the reported value by 20%. The medium pore diameter was 14.50 μm, which is close to the reported value. The surface area was about 300 m^2/g, which is about 20% less than the reported value. The pH of the water in which biochar was soaked was around 9 to 10, indicating biochar amended into the soil is prone to create a basic environment. In our greenhouse test, the soil pH was monitored, and the results are discussed in the following section. The difference in these properties between our biochar and literature might be caused by the different wood species and manufacturing processes.

Table 1. Summary of the biochar compared to other studies.

Property	Value	References
Moisture Content (MC)	3 %	-
Bulk density	0.061 g/cm^3	-
Porosity	52 %	71.80 % (Lu & Zong, 2018)
Medium pore diameter	14.50 μm	11.43 μm (Lu & Zong, 2018)
Surface area	301.87 m^2/g	371.24 m^2/g (Li et al., 2018)
pH	9 to 10	6 to 12 (Zornoza et al., 2016)

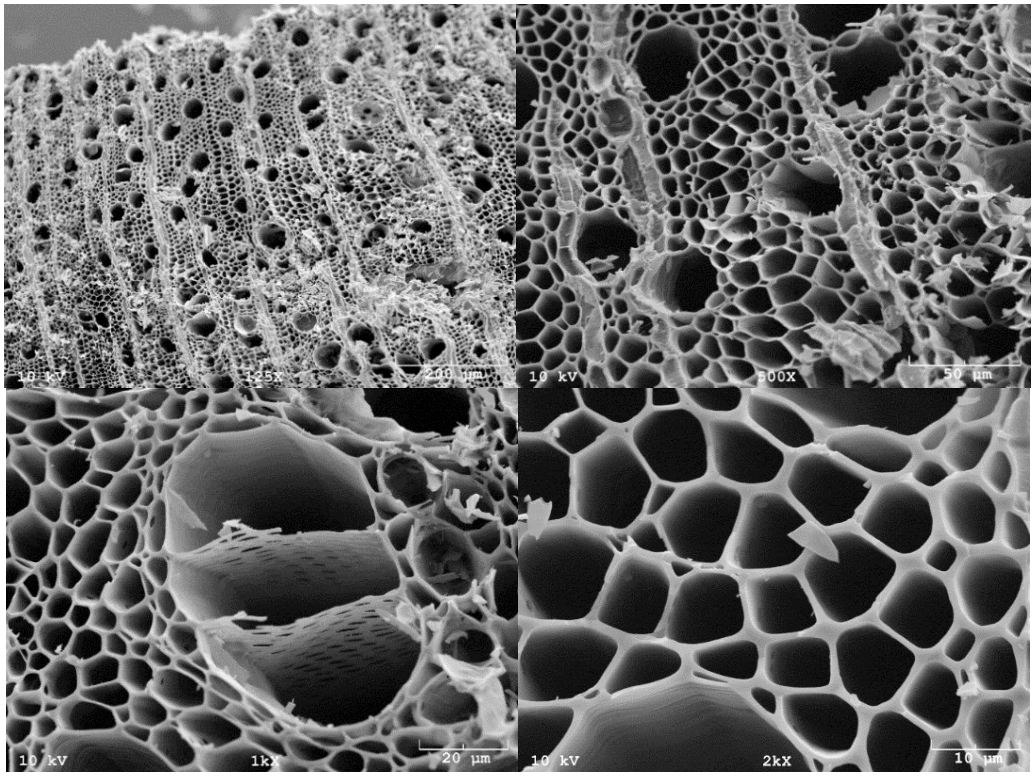

Figure 2. SEM images of a biochar sample taken at four amplification ratios (top left: 125 x; top right: 500 x; bottom left: 1000 x; bottom right: 2000 x). The images show the cross section of a hardwood-derived biochar.

Fig. 2 shows the morphology of a biochar sample taken by an SEM at four amplification ratios of 125x, 500x, 1000x, and 2000x. It can be seen that the biochar maintains the hierarchical structure of wood, i.e., the porous structure of wood.

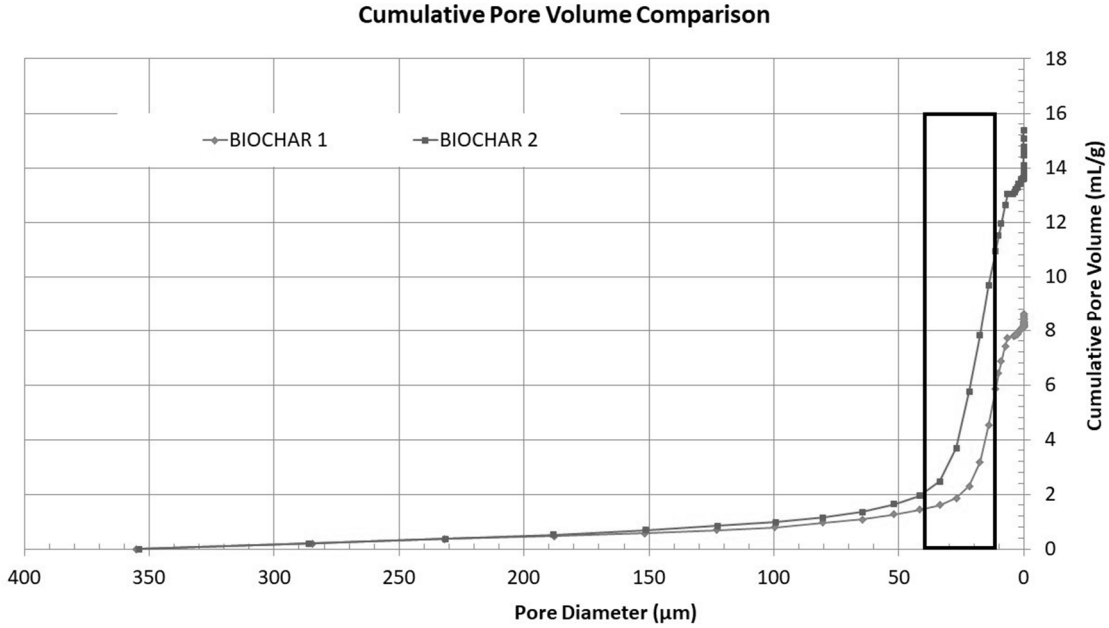

Figure 3. Cumulative pore volume of biochar samples measured with biochar pore size decreasing from 350 µm to 0 µm. Diamonds represent Biochar 1 and squares represent Biochar 2.

The cumulative pore volume of two biochar samples tested shows that a significant increase in the pore volume began at the pore size of 35 µm and ended at the pore size of 5 µm (Fig. 3, outlined by box). The

percentage of subtotal pore volume with pore sizes ranging from 5 to 35 μm took about 66.7% (biochar 1) and 62.5% (biochar 2) of the total pore volume, respectively.

Results of greenhouse experiment of wild blueberry plants

Properties of soil

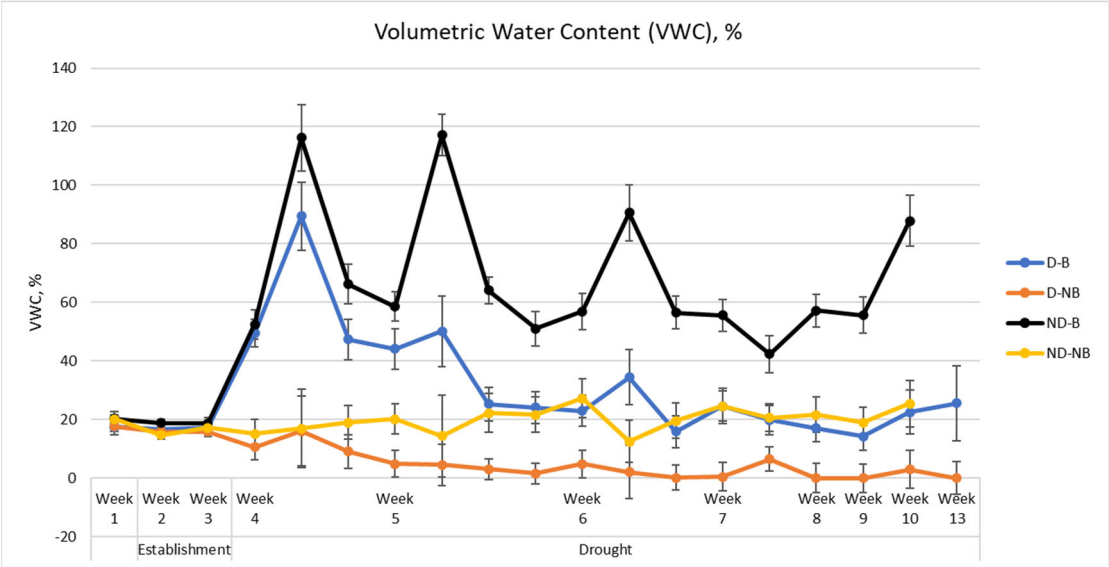

Figure 4. Soil volumetric water content (VWC) measured by TDR. Weeks 1 to 3 are during the plant's establishment period, where VWC was measured once a week. Weeks 4 to 13 represent the time when the plants were under drought conditions, where VWC was measured in coordination with stomatal conductance, water potential measurements, and photosynthetic rate varying between twice a day to once a week.

The addition of biochar in sandy soils resulted in higher VWC for both drought and no drought groups compared to the ones without biochar addition (Fig. 4). In particular, throughout the drought period, the VWC of D-B treatment remained higher than the D-NB treatment. Starting from week 6, the VWC of D-B was remained at about 20%, while that of D-NB decreased to about 4%.

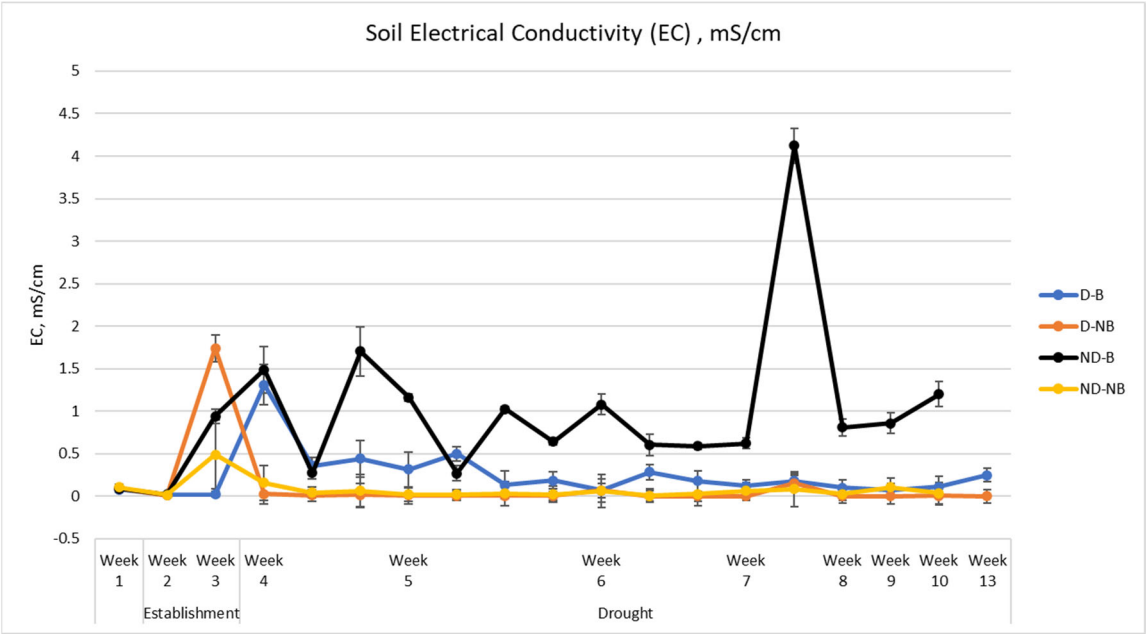

Figure 5. Soil electrical conductivity (EC) measured by TDR. The amount of salts in the water shows throughout the experiment. EC was measured once a week. Weeks 4 to 13 represent the time period when the plants were under drought conditions, where EC was measured in coordination with stomatal conductance, water potential measurements, and photosynthetic rate varying between twice a day to once a week. *Note: There is an abnormal measurement in week 7, which could be due to the environmental conditions during that week.

In general, the trend of soil EC (Fig. 5) shares a similar trend with the soil VWC (Fig. 4). Throughout the establishment and drought periods, the EC of ND-B was much higher than that of other treatments due to regular irrigation, which made fertilizer dissolve in the water and the water was retained in biochar. The EC of D-B was slightly higher than that of ND-NB and D-NB. During the drought period, the soil amended with biochar still retained a bit of fertility. It is important to note that in week 7, there was an abnormal measurement, which could be due to the weather conditions, such as humidity or rainfall.

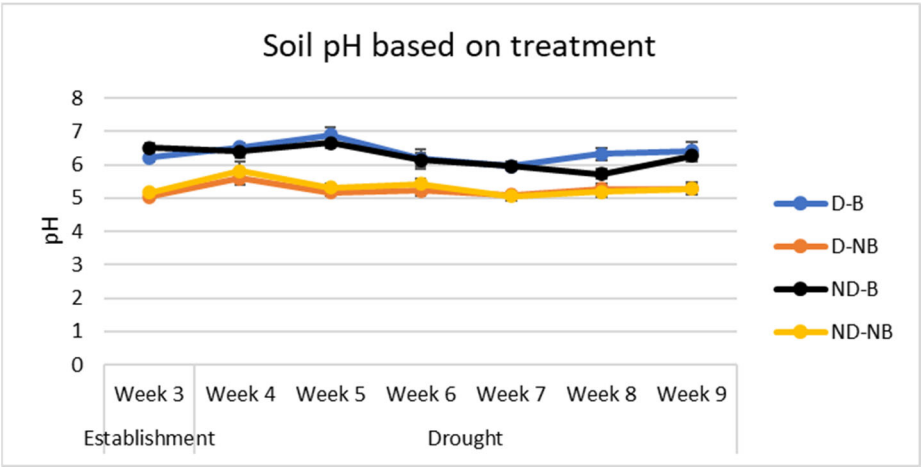

Figure 6. Soil pH dynamics during the experiment. During week 3, the establishment period an initial measurement was taken of the plants. After that the pH was measured once a week during the drought period, weeks 4 to 9.

The two treatments with biochar (D-B and ND-B) had a majority of pH values in the range of 6 to 7 (Fig. 6). The pH values of treatments without biochar (D-NB and ND-NB) fell in the range of 5 to 6. The increase of pH value of soil and biochar mixture was caused by the basic biochar.

Physiological properties of wild blueberry plants

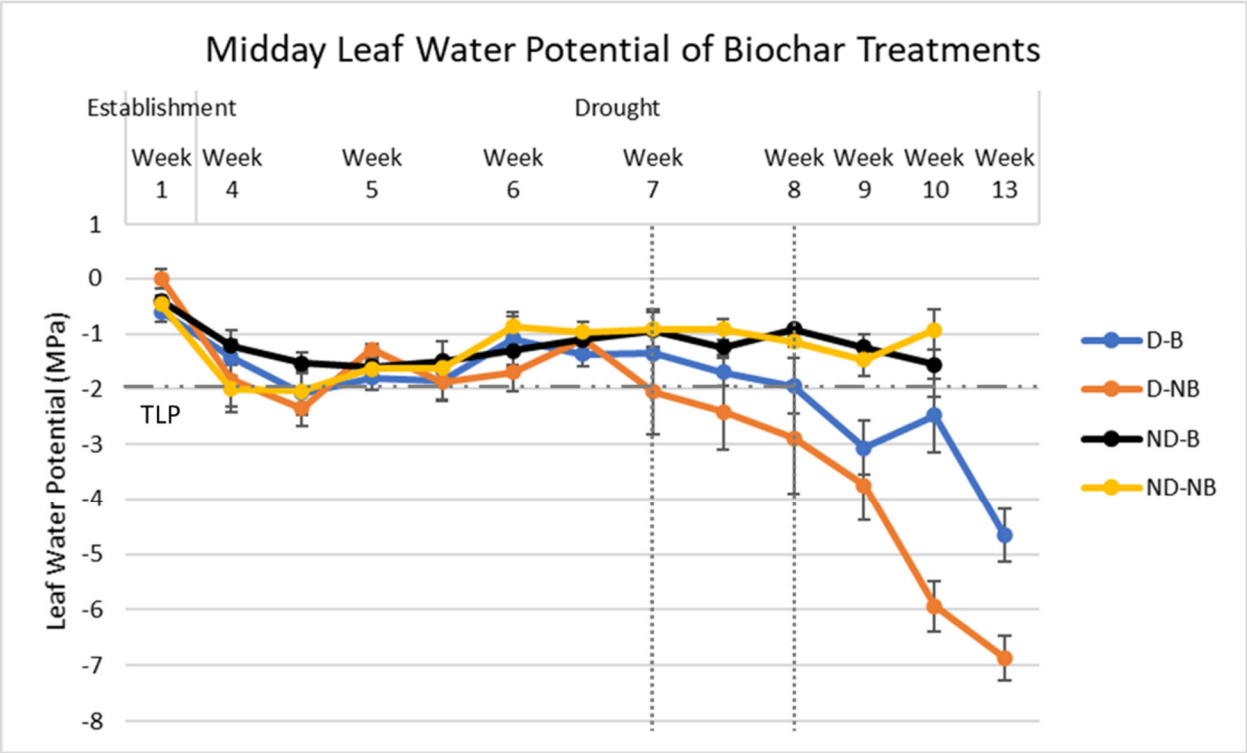

Figure 7. Midday leaf water potential measured by the leaf pressure chamber throughout the drought period. Frequency of measurements was based on the status of the plants and varied from every two days to once per week.

The midday leaf water potential had a slow decline in the first four weeks of the drought treatment, weeks four to seven (Fig. 7). Starting from week 4 when the drought began, the midday leaf water potential of the plants (D-B and D-NB) started dropping to the wild blueberry crop's turgor loss point (TLP) of approximately -2 MPa and then fluctuated at this level for a few weeks (Pahadi 2021). Biochar showed a positive impact on reducing the decline time and rate of leaf water potential. The leaf water potential of D-NB group had a sharp decrease starting from week 7, reached approximately -4 MPa in week 9 and -7 MPa at the end of the drought period (week 13). Comparatively, the sharp decline of leaf water potential of D-B group was delayed for one week and stopped at about -4.8 MPa in week 13. For the two no drought treatments, biochar had little influence in the variation of leaf water potential of plants. The leaf water potential of the ND-B and ND-NB groups was higher than or around TLP.

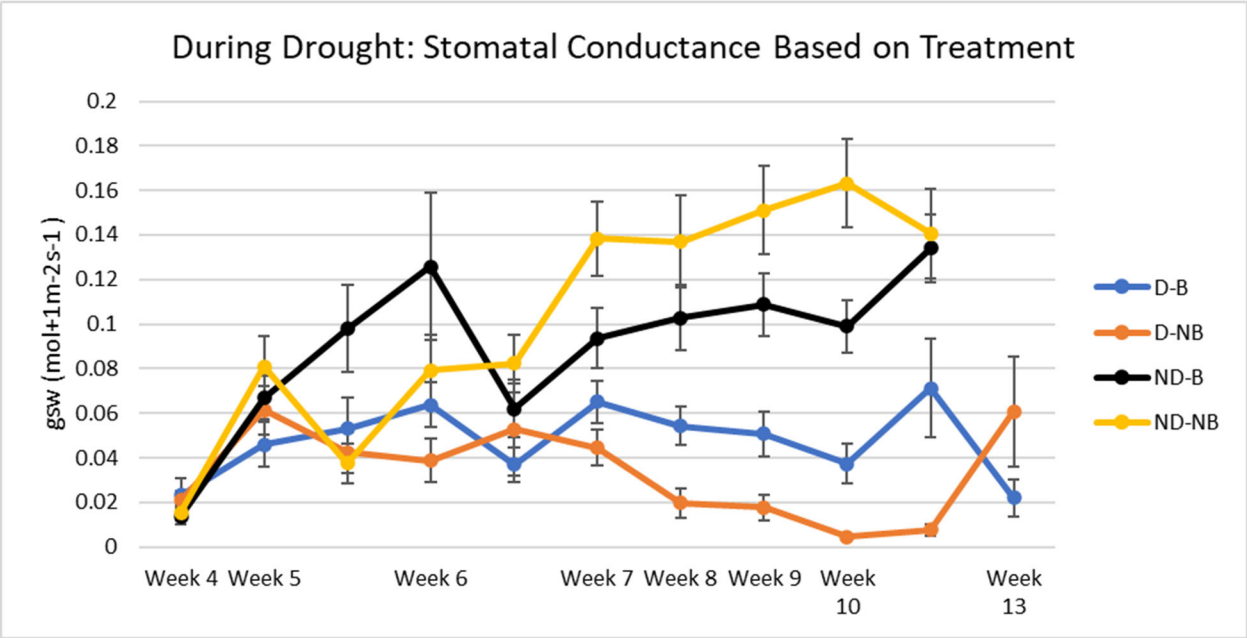

Figure 8. Stomatal conductance measured by the LICOR 600 during the drought period, weeks 4 to 13 to account for the physiological interaction the plants would have to their environmental conditions. This measurement was taken based on the status of the plants, varying from every two days to once per week.

In general, the stomatal conductance of the plants under the drought treatment (D-B and D-NB) was lower than that of the plants without a drought treatment (ND-B and ND-NB; Fig. 8). The lowered stomatal conductance reveals that the plants have less access to water and therefore they partly closed their stomata to conserve water. In the two drought treatment groups, biochar had a positive impact on stomatal conductance. The stomatal conductance of plants in the sandy soil amended with biochar was stabilized in the range of 0.04 to 0.06 $molm^{-2}s^{-1}$; however, that in sandy soil only dropped to zero after a few weeks of drought treatment (Fig. 7). In the no drought treatment groups, the stomatal conductance of both ND-B and ND-NB under regular irrigation maintained up to 0.14 $molm^{-2}s^{-1}$.

DISCUSSION

The role of biochar derived from local forestry logging residues in improving the water retention of sandy soils was validated in this study. The addition of biochar particles with different sizes and shapes in sandy soils reduced the volume of large space between soil particles (i.e., interpore) and increase the portion of micropores (5 µm to 30 µm in diameter) contributed by the intrapores of biochar. Due to the hierarchical structure of woody biomass and pyrolysis process, the woody biochar used in this study contained pore size mainly in the range of 5 µm to 35 µm (Fig. 3), the majority of which fall in the range of micropores. This can maximize water and nutrient retention in the sandy soils by increasing the pathways to extend the time of water penetration and holding water due to capillary action.

The greenhouse experiment results reveal that, when biochar was added to wild blueberries it helped mitigate the effects of drought to an extent. The soils that were treated with biochar were able to maintain a much higher soil moisture (soil volumetric water content) and electrical conductivity than the untreated during drought treatment. This shows that biochar can aid in increasing the available water to the plants, whether it be from the ground or the atmosphere. Although our biochar increased the soil pH from around 5 to 6 raised to 6 to 7, it did not significantly impact the acidity of the crop's soil during this short observation period. The long-term effects of using basic biochar need to be better studied to gain more insights. The performance of the stomata of the wild blueberries shows that the D-B plants were able to maintain stomata open through a longer period of time, with a delayed response

to when the drought started. This indicates that the plants experienced less drought stress in soils with biochar treatment. Wild blueberries in the drought treatments are seen to have a lower midday leaf water potential remaining consistently low for a longer period of time compared to no biochar treatments. Based on the midday water potential, plants in soils with the addition of biochar reached the leaf wilting (TLP) a week later compared to those without biochar. Further, the D-B plants took an extra three weeks after the D-NB plants reached their -4MPa, showing that biochar helped retain water in the soil. Therefore, biochar seems to be a promising management practice for wild blueberries facing drought conditions.

NEXT STEPS

- Complete statistical analysis to further interpret the effects of biochar as a soil amendment to improve the resilience to drought.
- Modify biochar's pH for acidic soil types
- Improve the greenhouse experimental plan to add more variables, such as different mixing ratios of biochar and soil, for investigating the plant response to increasing climate variability.

ACKNOWLEDGEMENTS

We thank Maine Wood Pellets Co. for providing raw biochar materials, Rogers Farm's Josh Hatley and Joe Cannon for helping build the greenhouse and providing us with a space for our research, Jessica Hutchinson (undergraduate graduate assistant) for assisting Abigayl Novak in carrying out the lab and greenhouse experiments in summer 2021, and Bruce Hall for providing expert advice and accommodating our team when on site at Wyman's. This research is supported by the Maine Wild Blueberry Commission grant; Maine Department of Agriculture; Conservation and Forestry (ADG and SCBG); & 2021 UMS Reinvestment Fund; USDA National Institution of Food and Agriculture (NIFA) through the Maine Agricultural & Forest Experiment Station: McIntire-Stennis (ME042205) and Hatch Fund (ME022021); 2020 Maine Agricultural Development Grant.

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RESEARCH & EXTENSION

INVESTIGATOR(S): L. Calderwood, M. Scallon, and B. Tooley

6. Wild Blueberry Phenology

OBJECTIVE(S)

- Document wild blueberry phenology (plant development stages) with growing degree days
- Provide growers access to live wild blueberry development data throughout the whole season through the Phenology Tracker on the UMaine wild blueberry website.

LOCATION(S): Midcoast to Downeast (10 locations: Searsport, Hope, Warren, Sedgwick, Ellsworth, Deblois, Montegail, Jonesboro, Wesley, Marion)
PROJECT TIMEFRAME: April – September 2020 and 2021

INTRODUCTION

Maine's wild blueberry growers have long depended on localized knowledge of climactic and weather conditions to inform their management decisions, including the timing of applications of fertilizers and pesticides. Nationwide the development of decision support tools for growers of all crops has increased, providing localized and effective predictions of when pests and crops will develop to different stages. Maine wild blueberry growers have utilized Dr. Seanna Annis' mummy berry and botrytis disease forecasting tools on the AgriNet website to determine when to apply fungicide, resulting in a reduced number of spray applications, in turn reducing unnecessary or poorly-timed chemical applications. The purpose for collecting weekly weather and plant development data in this project is to eventually be able to create crop development tools for wild blueberry to calculate and predict emergence date, bloom date, ripening stages, and harvest date.

Use of such tools will become more necessary as the climate continues to change. Through calculations, these electronic resources on phone apps and websites can identify changes more quickly and accurately than people who are comfortable with long-known timelines for management. Maine's wild blueberry growers are already experiencing the effects of climate change on their crop; these changes will continue into the foreseeable future, in unforeseeable ways. The growing season has lengthened by more than one month over the last 50 years (Drummond, 2014) and a warmer, drier climate is impacting the phenology of wild blueberry and the quality of its fruit. Summer 2021 was a climactically chaotic season for the northeastern US and the Maritimes: a hot, dry June that broke heat records across the region but also saw a frost event in the Maritimes; a cooler and very wet July that ranked among the region's wettest Julys but that had a stretch of humid, hazy days at the end of the month caused by wildfire smoke from the western US and Canada; a hot, dry August that continued to see drought conditions throughout much of the region and more heat-related records broken (NOAA, 2021).

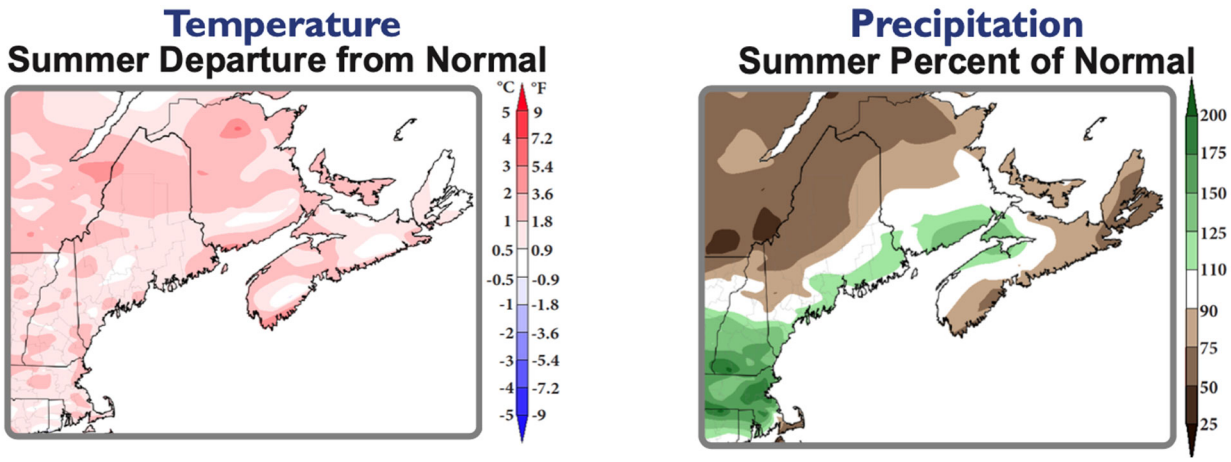


Figure 1. Left: temperature map showing deviations from normal temperatures for summer 2021 in the Gulf of Maine region, with darker red locations seeing temperatures significantly higher than usual. US temperature normals based on 1991 – 2020 data and Canadian temperature normals based on 1981 – 2020 data (NOAA, 2021). Right: precipitation map showing deviations from normal precipitation rates for summer 2021 in the Gulf of Maine region, with darker green locations seeing precipitation significantly above normal, and darker brown locations seeing significantly less than normal; white areas show where precipitation levels were at their normal levels. US precipitation normals based on 1991 – 2020 data and Canadian precipitation normals based on 2002 – 2020 data (NOAA, 2021).

Farmers will need to adjust their timeline for management techniques as blueberry stage development deviates from colloquial timing knowledge. Harvestable fruit arrived almost two weeks early in 2021, and there was widespread development of buds, blooms, and fruits in the fall. Climate has become more volatile, with hotter, drier conditions requiring careful management of water resources but sudden precipitation and frost events wreaking havoc on fruit development and set. As one farmer put it, “The plants are confused.” It is safe to say the farmers are confused as well, and phenological data gathered in the 2021 season serves to refine the Maine-specific growing degree day (GDD) and peak bloom predictive models developed in 2020 to assist growers in making management decisions based on localized conditions. Just as growers have seen a reduction in expenses associated with fungicide application following adoption of Dr. Annis’s mummy berry and botrytis prediction tools, so it is anticipated that growers will realize economic benefits from honed GDDs and crop predictive tools.

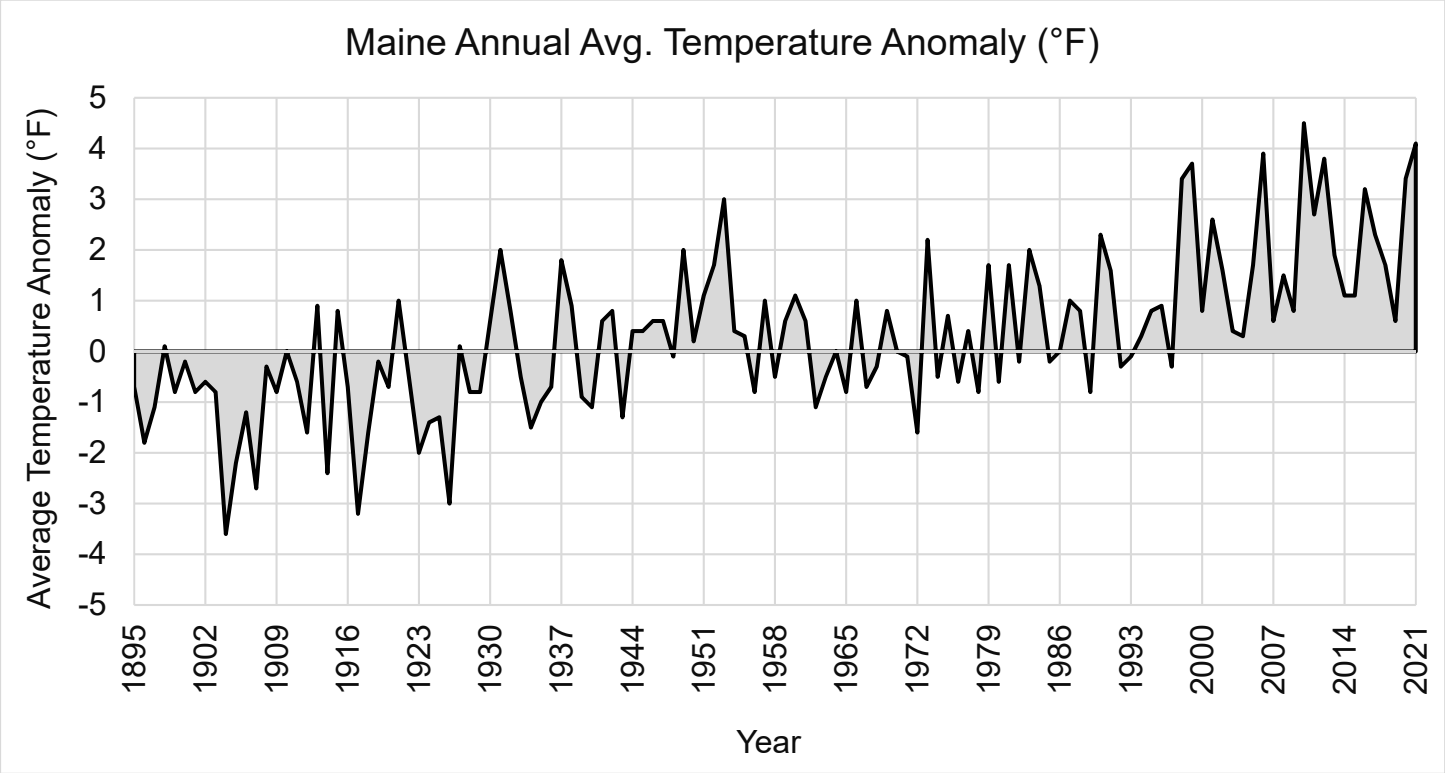


Figure 2. Chart showing Maine's statewide temperature anomalies from 1895 – 2021 with a 1901-2000 climate baseline. The vertical axis shows how many degrees Fahrenheit the annual average temperature has been above or below the baseline. The horizontal axis shows calendar years. Shapes above the 0ºF line indicate the annual temperatures have been above baseline (warmer) and shapes below the 0ºF line indicate temperatures have been below baseline (cooler). There is a clear increase in warmer conditions beginning in 1937 and accelerating dramatically in 1969 through the present. The last cooler temperature anomaly was recorded in 1997 (Maine Climate Office, 2021). This data is from NOAA Climate Divisional Database, the UMaine Climate Change Institute and the Maine Climate Office: https://mco.umaine.edu/data_monthly/.

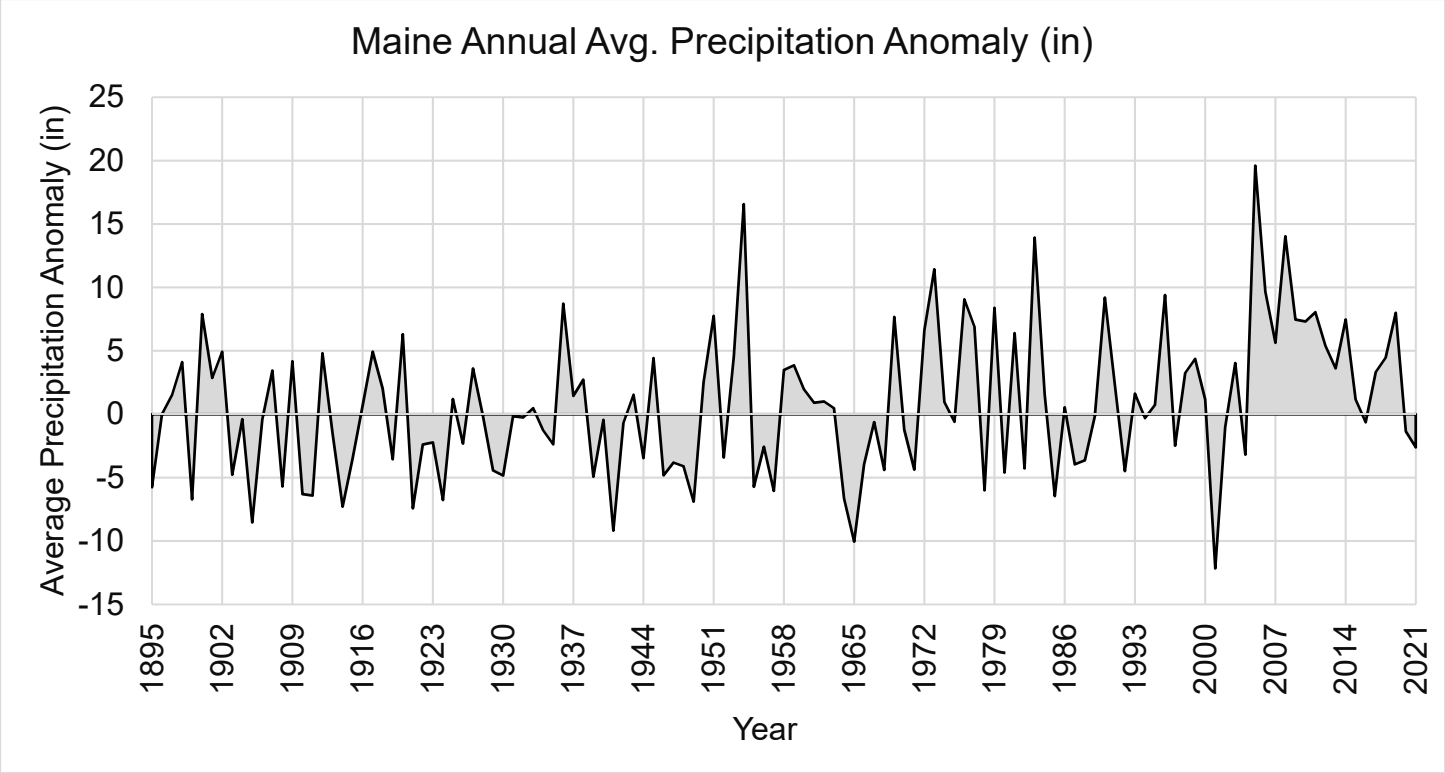


Figure 3. Chart showing Maine's statewide precipitation anomalies from 1895 – 2021 with a 1901-2000 climate baseline. The vertical axis shows how many degrees Fahrenheit the annual average temperature has been above or below the baseline. The horizontal axis shows calendar years. Shapes above the 0ºF line indicate the annual precipitation has been above baseline (wetter) and shapes below the 0ºF line indicate precipitation has been below baseline (drier). Though the values alternate between cooler and drier at similar rates from 1895 to 1965, from 1965 onwards there is a shift towards more frequent, and wetter years through to 2021 (Maine Climate Office, 2021). This data is from NOAA Climate Divisional Database, the UMaine Climate Change Institute and the Maine Climate Office: https://mco.umaine.edu/data_monthly/.

The tip-dieback (TDB) stage in wild blueberry traditionally marks the transition from vegetative growth to reproductive growth during the prune year in wild blueberry (Figure 4a). Following TDB the stem buds develop from a soft green tissue at the upper joints between leaves and stem; these buds then swell, develop scales and turn brown with the essential process of 'bud hardening' prior to winter. Adequate bud hardening prior to plant dormancy is essential in bud survival through the winter. Low snow coverage and harsh winter conditions can harm hardened buds in what is identified as 'winterkill' the following spring when the bud fails to 'burst' (Figure 4b). In recent years, especially in 2020, new growth was observed in the form of branching, leaf emergence from new buds, and flower and fruit emergence from new buds immediately after TDB such that a second TDB occurred later in the season. The extent and potential for bud hardening following new growth so late in the season may be reflected in the 2022 spring winterkill and even yields. Further research on this phenomenon is necessary.

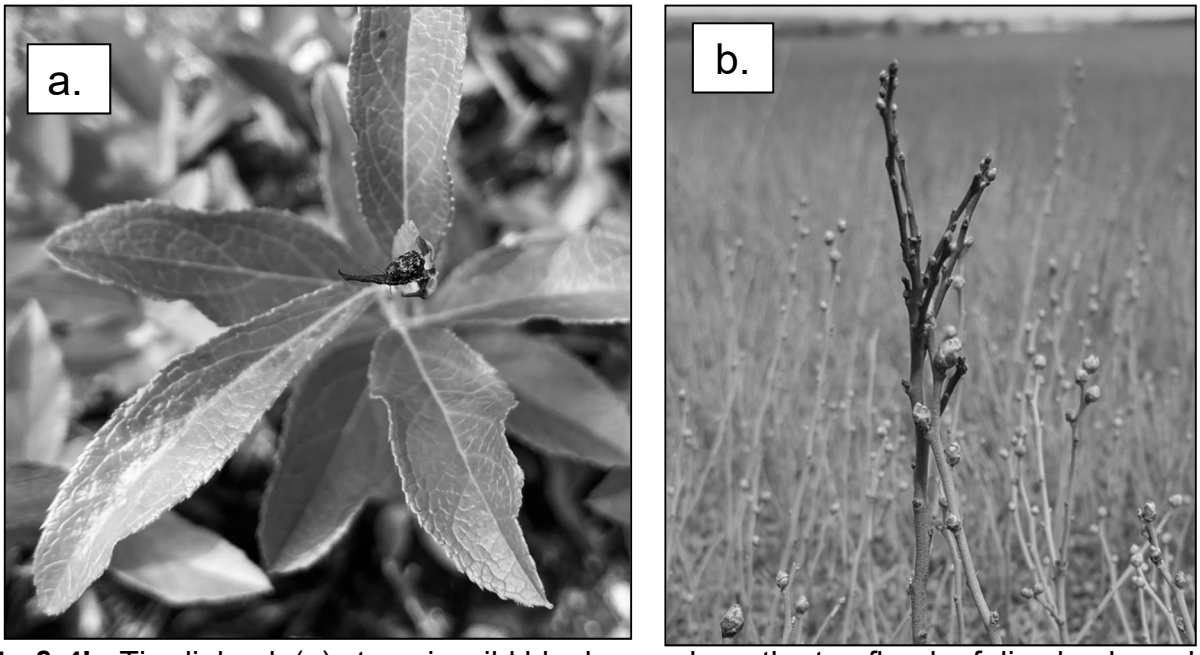


Figure 4a & 4b. Tip-dieback (a) stage in wild blueberry where the top flag-leaf dies back, and dimpling may occur in top surrounding leaves. Winterkill (b) in wild blueberry, a phenomenon where harsh winter conditions or inadequate bud hardening leads to a ‘dead’ or inactive bud in the spring.

Wild Blueberry Phenological Development in the Crop Year

Leaf stages coincide with bud and early flower stages. Stages not shown include dormant leaf and flower buds (V0 and T0).

Leaf Stages

Early Green Tip (V1): Spikey green tissue emerges from tightly closed buds along stem (spikes < 2mm).

Green Tip (V2 & V3): Green tissue continues to emerge (V2; spikes 2-5mm), and tips begin to sperate (V3; spikes >5mm)

Shoot Expansion (V4): Leaf shoots expand, leaves unfurl, and enlarge in a whorled pattern.

Bud Stages

Bud Swell (T1): Buds continue to swell with scales separating. Flower parts start to round inside bud.

Early Bud Burst (T2): Buds continue to swell with scales separating. Scale tips are pointed.

Bud Burst (T3): Bud scales separate fully with to show rounding flower parts.

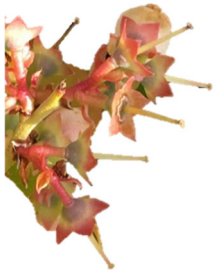
Tight Cluster (T4): Petals (corolla) in flower buds are visible, elongated, but remain closed.

Bloom

Early Flower (T5): Pre-bloom; petals (corolla) extend beyond the green base of the bud (calyx) but remain closed.

Open Flower (Bloom): Flowers may be various shades of white to pink. Flower petals are open for pollination.

Fruit Development



Petal Fall (Pin Head): Petals drop but the calyx and stamen remain.

Early Green Fruit: Fruit swell begins, but fruit remains hard, small, and green.

Late Green Fruit (Blushing): Green fruit begins to blush around the top but remains green where connected to the stem.

Red Fruit: Fruit is formed and turns various shades of pink and red prior to turning blue. Acid content is still high.

Blue Fruit: Fruit is ripe, sugar content is high.

Stages correlate with Hildebrand, P.D., and P.G. Braun. 1991. Factors Affecting Infection of Lowbush Blueberry by Ascospores of Monilinia Vaccinii-Corymbosi. Canadian Journal of Plant Pathology 13(3): 232–240. Images taken by UMaine Extension.

METHODS

Wild Blueberry Staging and GDD

In addition to 10 crop fields monitored for bloom date, a nearby prune field was also selected for each location and a HOBO air temperature and humidity sensor (ONSET Computer Corporation, Bourne, MA, USA) was installed (Figure 5). All 20 fields (both prune and crop) had a soil moisture meter installed as well for later use. In each field, 6 different clones were selected at random and flagged for continuous monitoring. All fields were visited weekly for 22 weeks (4/12-9/14/21). With each visit, pictures were taken of each of the six clones (using a white binder as the background; Figure 1). Initial wild blueberry crop-stages (T1-T5, bloom, pinhead, green fruit and pink fruit; previous page) were visually identified for each tagged stem (6 per plant, 5 plants measured) at each location (60 clones total between the crop and prune fields) for the duration of the project. Each week, at each stem, each type of crop-stage was counted.

Figure 5. Phenology locations across major blueberry growing regions: (left) Midcoast & Ellsworth, and (right) Downeast, and Far Downeast.

The prune-cycle was monitored from leaf emergence (week 4, 5/4/21) until the end of bud development (week 25, 9/28/21). Six flags were placed, one each in a different plant, and these flagged plants were tracked for the entirety of the season. For the first 17 weeks following leaf emergence, vegetative growth was quantified by counting the number of stems growing per one foot transect, as bisected by the flag. Beginning week 13 (7/6/21), the number of buds growing on those counted stems was also recorded. Beginning week 17 (7/27/21), six stems per plant (or per one flag) were tagged with numbered tags for the remainder of the season (through week 25, 9/28/21). These six stems were visited weekly to measure the number of buds growing on the tagged stems. Stem number was no longer counted after the numbered tags were placed.

Data gathered each week was consolidated and shared with the public on the University of Maine Cooperative Extension webpage entitled “Wild Blueberry Phenology Tracker” (https://extension.umaine.edu/blueberries/real-time-wild-blueberry-phenology/).

Temperature and humidity were downloaded from the HOBO Manager program. Growing degree days (GDD) were calculated in Microsoft Excel with a base temperature of 40°F. In 2020, April 1 was used as the start date in calculating cumulative GDD. However, in 2021, 50-100 GDD had already accumulated by April 1 and so all GDD calculations were adjusted for a March 1 start date.

RESULTS

Cumulative growing degree days (GDD) had a steeper slope between March 1 and August 30 (Figure 6c and d) in 2021 relative to the same fields monitored in 2020 (Figure 6a and b). The steeper slope (across all 4 regions) shows a greater accumulation of GDD over the same time period. This increase in GDD accumulation is a direct result of more frequent warm (optimal growing) days and higher overall air temperature. Interestingly, greater regional differences occurred in 2021 relative to 2020, especially in the Midcoast region relative to Ellsworth, Downeast, and Far Downeast, suggesting that observed warming trends are not uniform across the state.

Field Conditions 2021 & 2021: Cumulative GDD

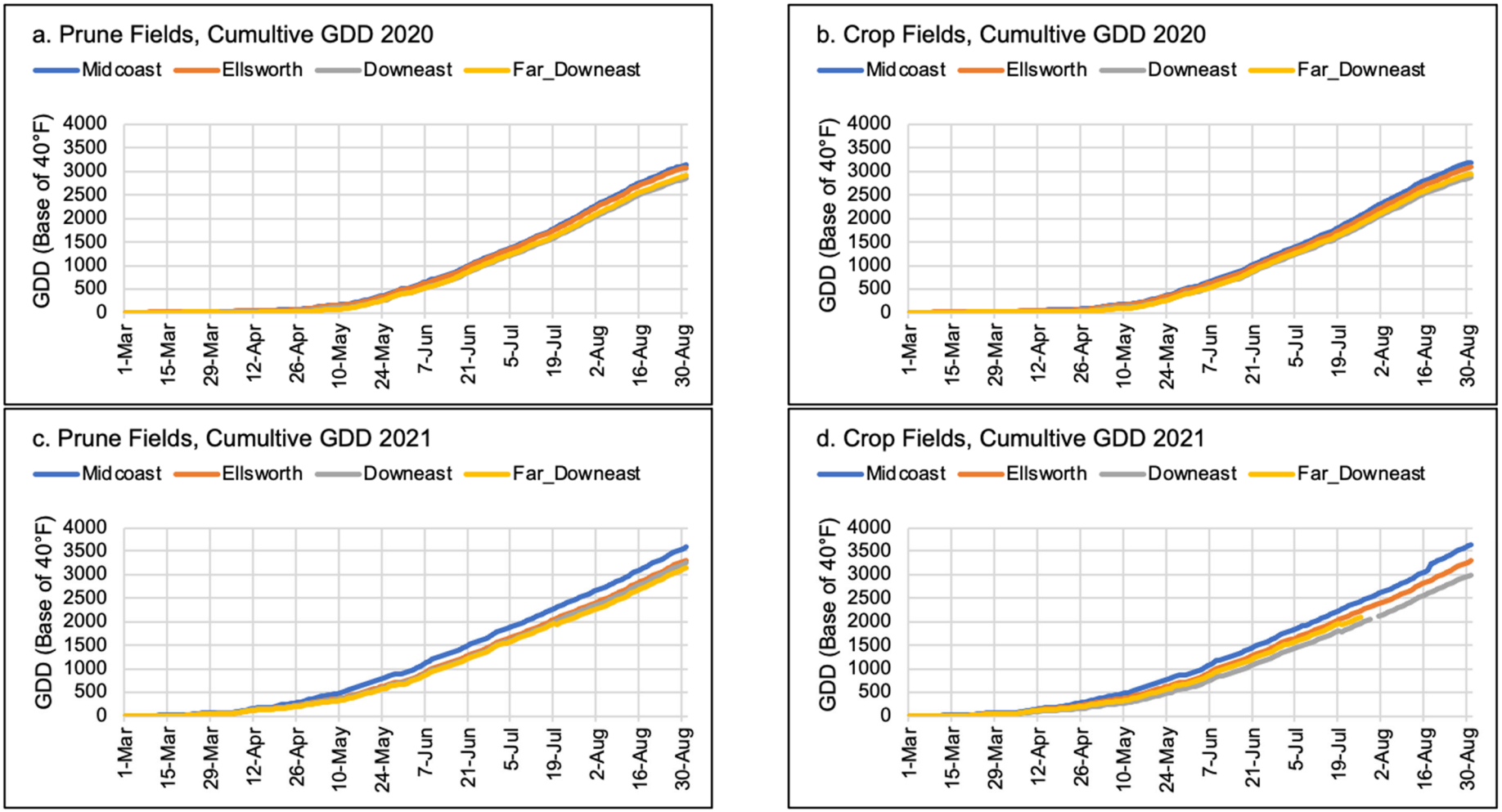


Figure 6. Average cumulative growing degree days (GDD) by field cycle and year during the peak growing season. Growing degree days were calculated with a base temperature of 40°F and started accumulation on March 1 of each year.

Soil moisture showed greater regional differences across both years (2020 and 2021) than air temperature, soil temperature and GDD (Figure 7). Here, the regional differences are likely a function of regional precipitation, irrigation, and soil type. In 2020 there is a clear decline in soil moisture, even in irrigated fields (Downeast), which corresponds with the drought experienced in 2020. In 2021, soil moisture is low at the start of the season (April 1) and remained fairly consistent in the Ellsworth and Downeast regions with greater seasonal change in the Midcoast and Far Downeast regions. Here, in 2021, the Midcoast region had notably higher soil moisture, despite no supplemental irrigation in these fields. The Far Downeast region increased in soil moisture throughout the season, particularly in the crop fields.

Field Conditions 2020 & 2021: Soil Moisture

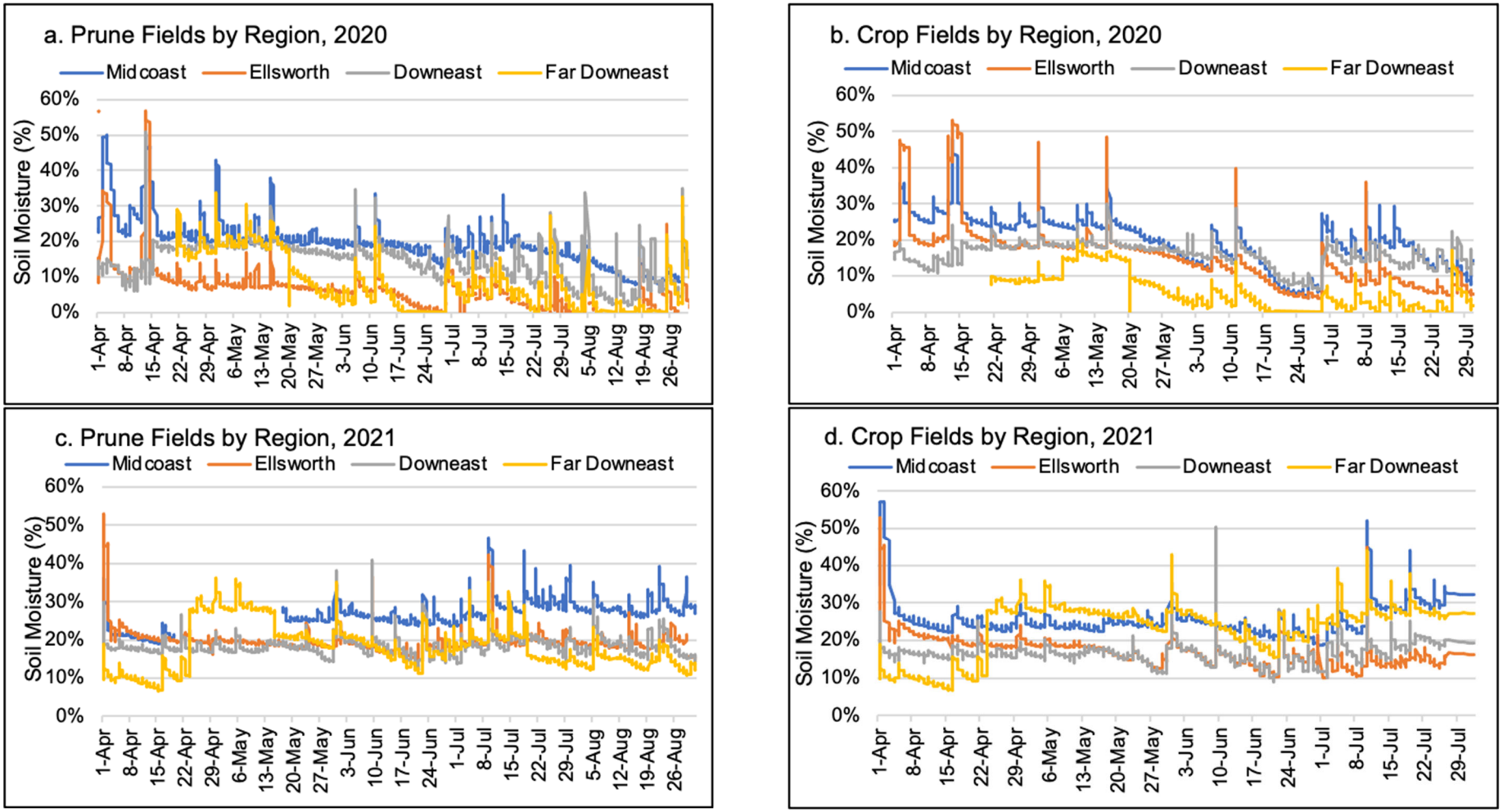


Figure 7. Average soil moisture (%) by field cycle, region, and year during the peak growing season.

Crop Phenology at All Locations: Fast Development

In 2020, it was observed that peak blueberry stages appeared earlier than in past years. This was especially apparent to growers who know the approximate calendar timing of stage-based management methods by heart. Because growers were not prepared to harvest early (labor, equipment, COVID-19) yield was lost that could have been harvested one to three weeks earlier. Accelerated development in 2020 was attributed to environmental stress from the statewide drought throughout the season and heat during blue fruit. In 2021, however, temperatures were warmer and growing degree days (GDD) accumulated faster leading to an even earlier accumulation of GDD and another early ripening season (Figure 7d, above), except this time growers were prepared to harvest earlier. While the statewide drought ended in 2021 with a succession of high-volume rain events, the achievement of peak stages occurred on average 0.8 calendar days earlier for bud stages and 4 calendar days earlier for flower and fruit stages (Figure 8). Furthermore, the faster accumulation of GDD in 2021 resulted in peak bud stages having (on average) 160 more GDD than in 2020, and flower and fruit stages having (on average) 284 more GDD than in 2020.

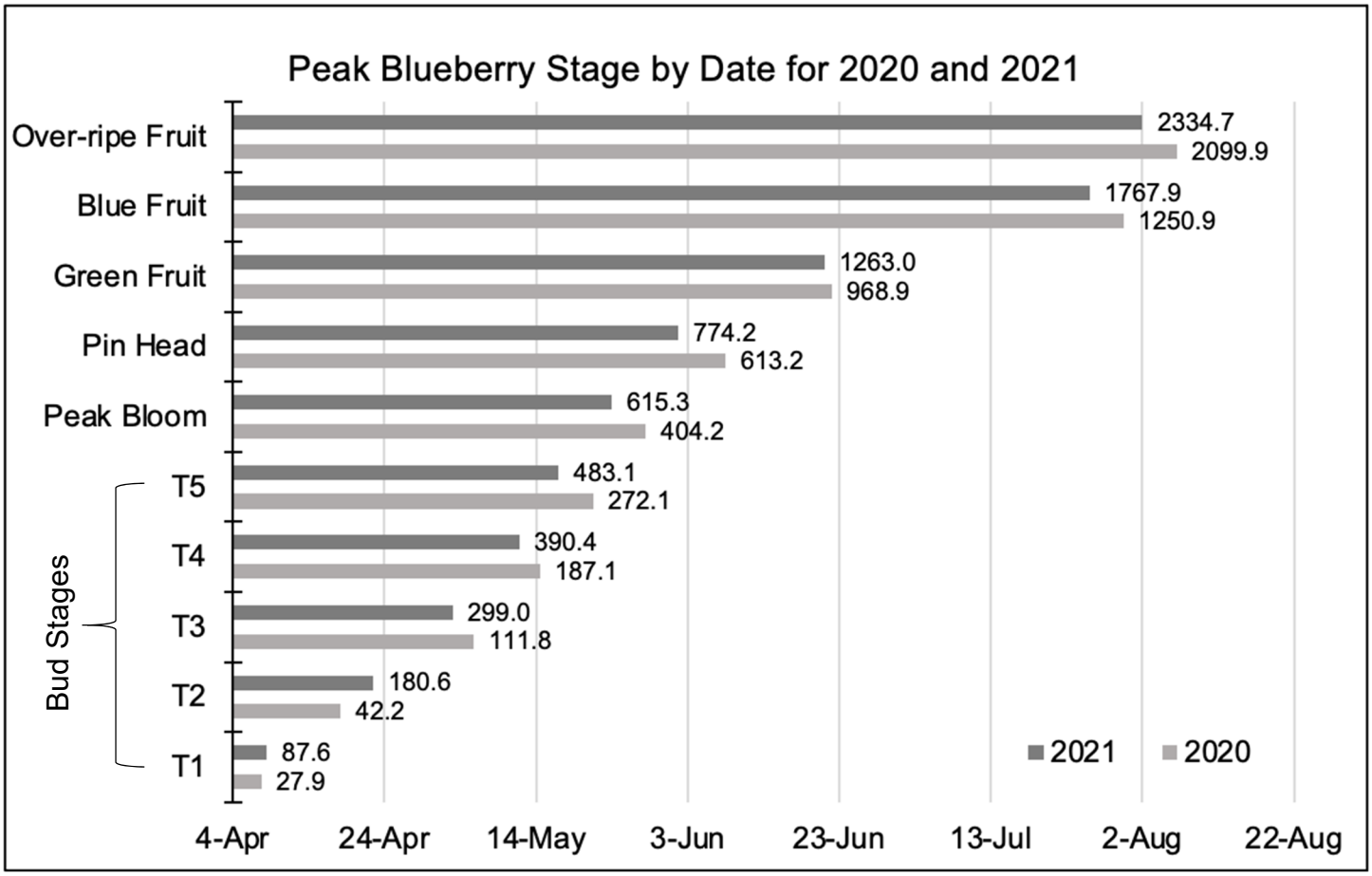


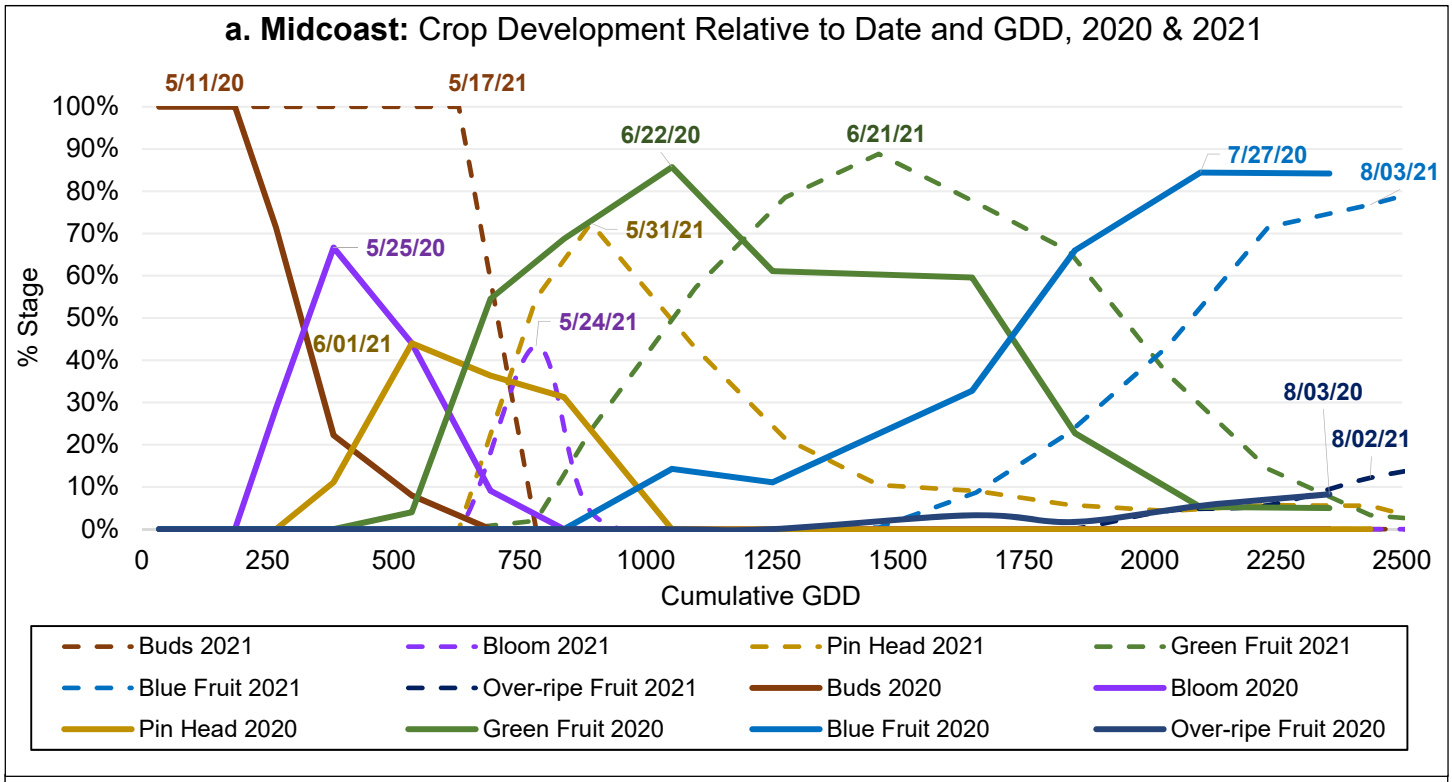
Figure 8. Peak blueberry stages by date for 2020 and 2021 with GDD totals as data labels at the end of each bar. Comparing peak stage by year shows stages occurred at earlier dates and with more GDD in 2021.

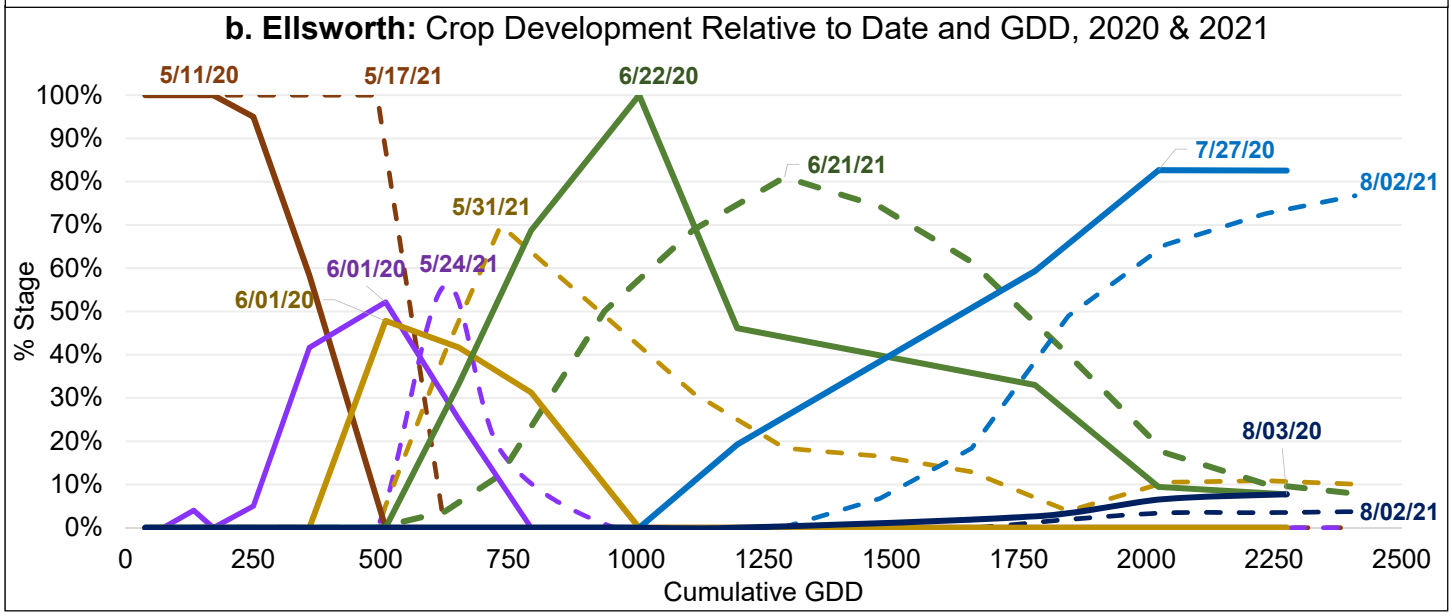
Crop Phenology by Region

Statewide phenology data clearly show (Figure 8, above) that as GDD accumulate faster, peak stages shift differently where some stages occurred earlier on the calendar in 2021 and others around the same calendar date as 2020. It is also clear that development rates and timing vary by region, as Midcoast and Ellsworth blueberry growing regions accumulate growing degree days faster than Downeast and Far Downeast ("Far DE") regions, which are geographically northeast of Midcoast (Figure 9a to 9d). The transition from bud to flower development in 2020 occurred in the weeks of May

11 and 18 in the Midcoast/Ellsworth and Downeast/Far DE regions, respectively. In 2021 this transition occurred the week of May 17 at all locations with 382 and 228 more GDD (than in 2020 at the transition point) in Midcoast/Ellsworth and Downeast/Far DE, respectively.

In 2020, peak bloom occurred the week of May 25 in the Midcoast, Downeast, and Far DE regions at an average of 318 GDD, and Ellsworth the week of June 1 at 508 GDD. In 2021, peak bloom occurred the week of May 24 at all locations at an average 615 GDD. Peak green fruit in 2020 occurred the week of June 22 in all regions at an average of 969 GDD, while in 2021, peak green fruit occurred the week of June 21 across all locations at an average of 1263 growing degree days. In 2020, crop harvest occurred around the first (August 3) and second (August 10) weeks of August depending on the region. In 2021, crop harvest began as early as the third (July 19) and fourth (July 26) weeks of July at some Midcoast and Downeast fields, while other fields did not start harvest until the first (August 2) week of August, depending on the desired berry sugar content and seasonal labor availability. In 2020, there was an average accumulation of 2220 GDD reached by the week of August 3, and in 2021, there were 2320 accumulated by the week of August 2. However, for those locations that started harvesting 1 week earlier in 2021, the average cumulative GDD at harvest was closer to that of 2020 at 2180 GDD.


a. Midcoast: Crop Development Relative to Date and GDD, 2020 & 2021
% Stage
Cumulative GDD
5/11/20
5/17/21
6/22/20
6/21/21
7/27/20
8/03/21
5/25/20
5/31/21
5/24/21
6/01/21
8/03/20
8/02/21
Buds 2021
Bloom 2021
Pin Head 2021
Green Fruit 2021
Blue Fruit 2021
Over-ripe Fruit 2021
Buds 2020
Bloom 2020
Pin Head 2020
Green Fruit 2020
Blue Fruit 2020
Over-ripe Fruit 2020


b. Ellsworth: Crop Development Relative to Date and GDD, 2020 & 2021
% Stage
Cumulative GDD
5/11/20
5/17/21
6/22/20
6/21/21
7/27/20
8/02/21
5/31/21
6/01/20
5/24/21
6/01/20
8/03/20
8/02/21

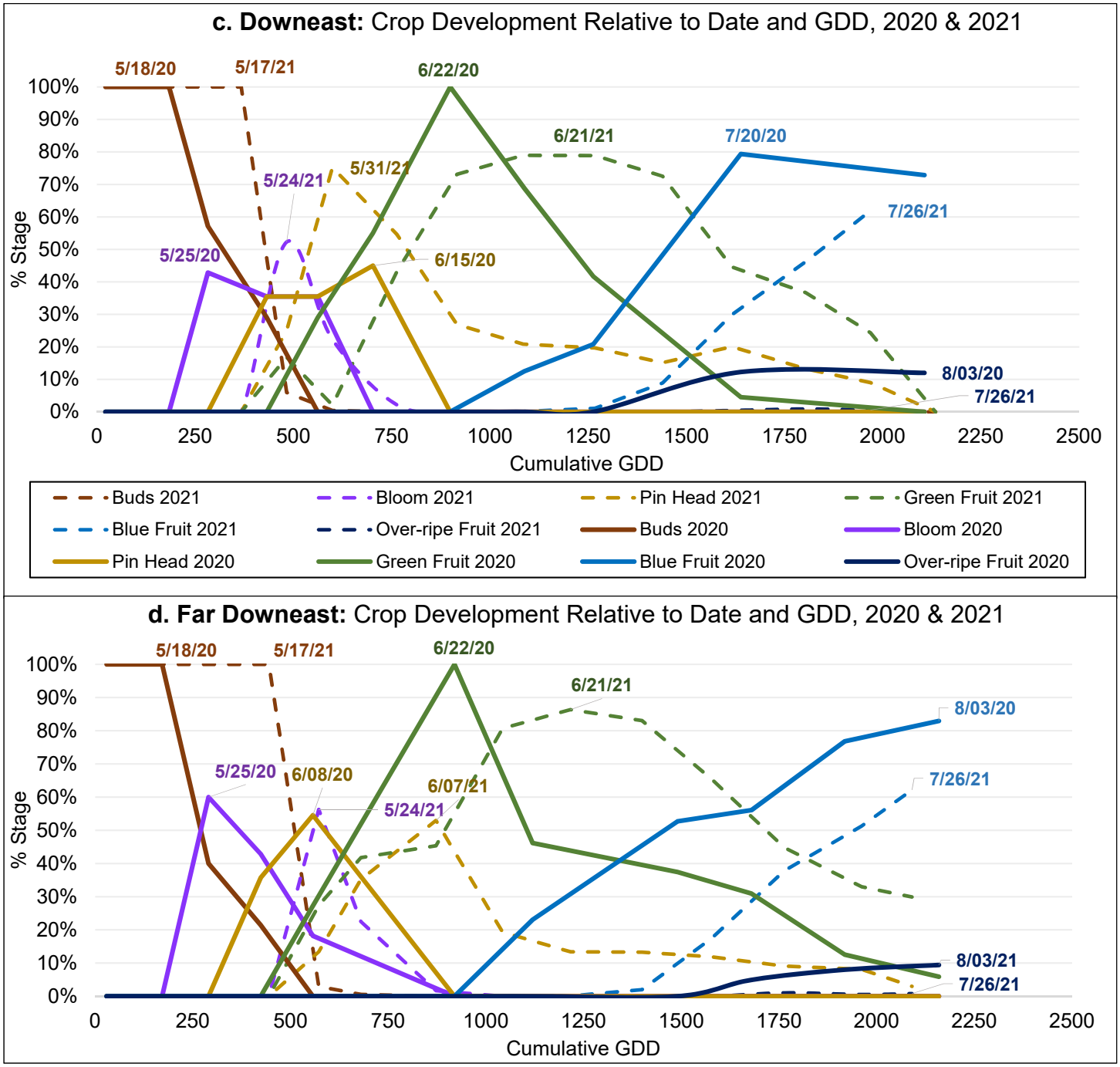


Figure 9. Crop development by region: Midcoast (a), Ellsworth (b), Downeast (c) and Far Downeast (d) and year (2020 and 2021), plotted against GDD with calendar dates as data labels indicating the peak of the respective stages. Solid lines indicate 2020 and dashed lines indicate 2021.

<u>Prune Phenology</u>

The Ellsworth region reached tip dieback (TDB) quickly with the cumulative peak (94% of all stems) occurring the week of July 12 at 1850 GDD. The Midcoast region was more gradual, reaching 66% the week of July 5 (1881 GDD) and 89% TDB the week of July 26 at 2478 GDD. Cumulative tip dieback was difficult to quantify in 2021 as new vegetative growth was observed in many plants after TDB, an unusual phenomenon. New growth following TDB in the prune fields was attributed to unseasonably warm temperatures and abundant precipitation.

The cumulative bud development following TDB varied by region and GDD (Figure 10, below). The Downeast region experienced rapid bud development from August 16 to September 13, 2021 (2775 to 3533 GDD), while the Midcoast, Downeast, and Far DE regions experienced slower bud development over the same time period. In 2020, Downeast bud development showed a similar trend to that of 2021 with rapid bud accumulation between August 24 and September 14 (2711 to 3153 GDD). All locations across both years show a strong positive relationship between bud development and growing degree days, however, the stop-and-go progression (on the chart, the lack of a straight line) suggests the influence of other environmental and/or management variables (i.e. precipitation, irrigation, fertility programs, heat stress). In 2020, accumulated buds were nearly double that of 2021. Reduced bud numbers in 2021 are likely a result of drought conditions in 2020 prune fields. This phenomenon was observed in high frequency across all locations in 2021 (Figure 11).

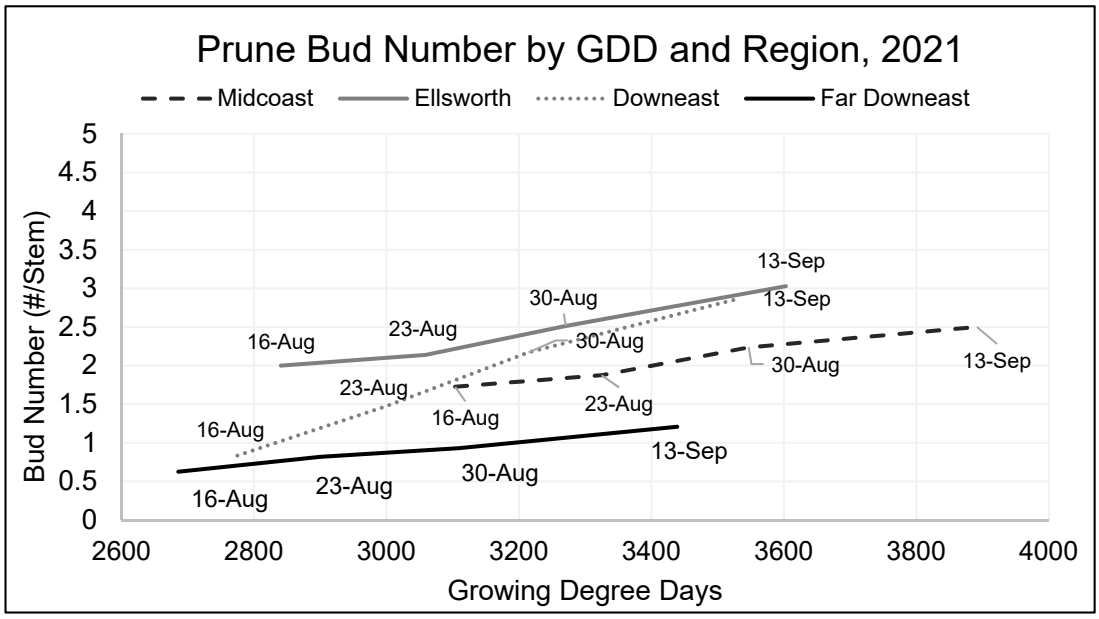


Figure 10. Bud number in prune fields by GDD and region with 2021 calendar dates as data labels.

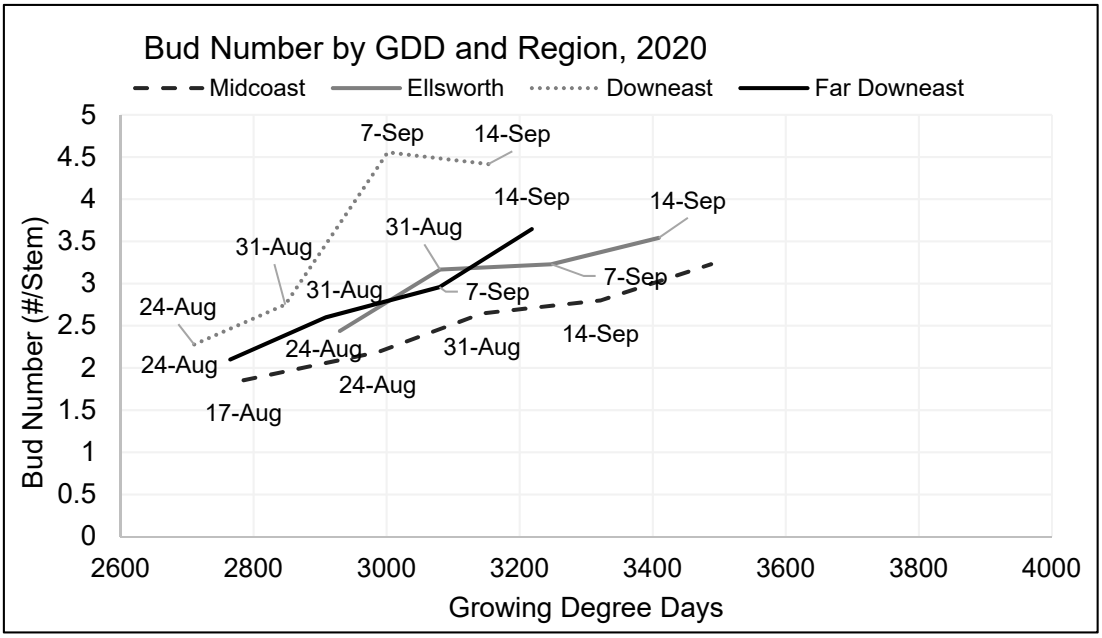


Figure 11. Bud number by GDD and region with 2020 calendar dates as data labels.

Figure 12. September vegetative growth and green fruit development in a conventionally managed prune Ellsworth-region field on the blueberry species *Vaccinium myrtilloides*. Photos taken September 14, 2021.

DISCUSSION

Crop Phenology Across the State

Since the peak of all development stages in 2021 appeared earlier than in years past, farmers who know the approximate calendar timing of when certain stages are achieved found their knowledge did not match the actual development happening in their fields. The early arrival of peak bud (0.8 calendar days early), flower, and fruit stage (both 4 calendar days early) complicated farmers' efforts to time management activities appropriately. With 2021's faster GDD accumulation across all stages, the length of each development stage was shortened, resulting in earlier ripe fruit: the earliest 2021 harvest began 7-14 days earlier than in 2020 (varied by location). This was an improvement over the too-late 2020 harvest. Harvesting early as an in-season decision is not easy and caused significant harvesting challenges for some growers related to labor shortages and supply chain disruptions associated with COVID-19.

Interestingly, all 2021 stages were ahead of 2020 by an average of 300 GDD until blue fruit indicating that ripening of wild blueberries is not only temperature (GDD) driven. This phenomenon has also been seen in highbush blueberry grown at different altitudes concluding that temperature has only a "fine tuning" role in anthocyanin (red and blue pigment) accumulation (Spinardi et al., 2019). This means we will not be able to predict harvest date with GDD (air temperature) alone. July precipitation saved the 2021 crop by bulking up berries and giving them the ability to take up the nutrients they needed to complete the ripening process.

In 2021, accumulated buds were nearly half that of 2020, a result of drought conditions in 2020 prune fields. This phenomenon was observed frequently at all locations.

Crop Phenology by Region

As GDD accumulate faster, peak stages are affected differently, but the field's location (within the regions of Midcoast, Ellsworth, Downeast, and Far DE) may also influence the timing of stage

development. Midcoast fields accumulated GDD fastest. In 2021, the transition from bud to flower development occurred at approximately the same time as in 2020 but with significantly more GDD accumulated (382 more GDD at Midcoast & Ellsworth; 228 more GDD at Downeast & Far DE). This early GDD accumulation shortened the bud to flower transition time period and then shortened the time from first bloom to peak full bloom. In 2021, bud to peak bloom (615 GDD) occurred in just one week, but in 2020 it occurred over 2 – 3 weeks. This shortened timeframe has the biggest impact on mummy berry spray timing prior to pollinator arrival. Early bloom dates make it more likely that bloom will overlap with a late season frost or freeze event. Despite an accelerated timeframe for 2021's transition from bud to peak bloom, all regions reached peak green fruit at approximately the same time in 2020 and 2021, albeit with many more GDD accumulated by that point in 2021.

Prune Phenology

The tip dieback (TDB) stage approximates the transition point when prune cycle blueberry plants transition from vegetative to reproductive growth: stems stop elongating, the growing tip turns from green to black, and the plant begins to grow reproductive features such as buds. TDB traditionally occurs just after the Fourth of July, depending on regional variation in weather. In 2021, Ellsworth achieved TDB quickly and steadily, peaking by July 12, but Midcoast achieved it more gradually, peaking as late as July 26. The Midcoast likely peaked sooner, on July 5, however rapid vegetative regrowth following TDB made this difficult to quantify. Vegetative regrowth following TDB is likely a result of unseasonably warm temperatures and abundant precipitation later in the season causing the blueberry to potentially undergo TDB twice (not physically observed, but suspected) or to delay TDB with the opportunity for more growth. UMaine researchers and growers have never before witnessed late season growth to this degree and crop season implications are unknown.

With so much growth occurring after TDB and so many GDD accumulated during a warm fall, there was significant bud development in some fields with some buds starting out as vegetative but becoming reproductive. This phenomenon has been observed in highbush blueberry and can be due to day length, temperature, and/or plant health condition (Williamson et al., 2018). It will be interesting to see the impact that lush vegetative growth has on 2022 crop fields. For example, it is possible that we will see more winterkill in crop 2022 fields if the late formed buds were not able to harden off before winter. In both 2020 and 2021, the Downeast region saw rapid bud development over approximately the same period of time (the second and third weeks of August). The rapidity of this region's bud development exceeded that of the other regions and this may be due in part to the use of supplemental irrigation and fertilizers by the larger producers included in this study.

There is a strong positive relationship between bud development and a high accumulation of GDD, but the uneven progression through development stages in several regions indicates there may be additional variables influencing the buds' development as has been observed in highbush blueberry (Williamson et al., 2018). Shifts in plant phenology have already begun in many parts of the world due to our changing climate (Rosbakah et al., 2021). The data gathered in just two seasons have shown that this change is already occurring. Obviously, fall bloom and fruiting is a worrying development because fall fruiting means no bud, bloom, or fruit development will occur on that stem the following crop year. Furthermore, it is possible that the autumnal appearance of flowers and fruit could provide additional food and habitat resources for pests. Fall fruiting also has implications for nutrient management if the plant is using energy stored for the crop year too early in the prune year. At this time the amount of fall bloom and fruit observed is not enough to produce a second crop.

Soil Moisture

Each of our growing regions experienced the 2020 drought differently, which will have impacts on the 2021 crop. Colleague, Dr. Zhang, has now found that soil moisture above 10% is adequate for wild blueberry plant production, however once soil moisture drops to 5% permanent damage can occur.

The soil moisture data presented here indicates that the Ellsworth and Far DE regions were hit harder by the 2020 drought than other regions. Most of the DE region fields that we monitor are irrigated and those that are not would likely fall into the Ellsworth and Far DE category of severely impacted by drought in 2020. In 2020 prune Ellsworth and Far DE fields dropped below 10% volumetric water content from June 17 – harvest. DE soil moisture was slower to drop due to irrigation but readings were still below 10% from July 22 through harvest. In 2020 crop fields only the Far DE region dropped below 10% severely from May 20 – harvest. Again in 2021, soil moisture dropped below 10% in the Far DE region but only in April.

CURRENT RECOMMENDATIONS

Use our Phenology Tracker to help manage your crop this season. Reach out if you have any questions. https://extension.umaine.edu/blueberries/real-time-wild-blueberry-phenology/

NEXT STEPS

- Seek funding to repeat this project in 2022, as many more years of this research are necessary.
- Quantify and monitor fall bloom, fruit, and vegetative growth in 2022 and future years.

ACKNOWLEDGEMENTS

Thank you to the Wild Blueberry Commission of Maine for funding this project. Thank you to Judy Collins and Phil Fanning for assisting with data collection. Their bloom date report (#1 in this section) is a sister study.

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INVESTIGATOR(S): L. Calderwood, M. Scallon and B. Tooley

7. Investigating the Impact of Solar Installation Methods on Wild Blueberry Production

OBJECTIVE(S)

- Identify whether use of distinct construction and solar installation methods can minimize the impact of construction on existing wild blueberry fields.

LOCATION(S): Rockport, ME
PROJECT TIMEFRAME: August 2020 – August 2022

INTRODUCTION

Agrivoltaics (co-locating solar and agricultural operations on the same land) is a growing industry, which has successful installations on cranberry bogs in Massachusetts (Mupambi 2020). The solar and agriculture industries in Maine have increasing interest in developing similar projects on wild blueberry fields. Like cranberry, wild blueberry is grown low to the ground, tolerant of shade and moderate physical disturbance. Solar development firms are interested in installing on agricultural land because these lands have low tax rates and are already cleared. Wild blueberry farmers are interested in responsibly stewarding their farmland and in diversifying revenue streams. To our knowledge, farmers have been offered approximately $2,500/acre within solar contracts, which usually last 15-20 years. On average, a wild blueberry farmer in Maine produces 3,000 lbs/acre at $0.40 cents/lb for an income of $1,200/acre (Personal Communications and NASS 2020). A solar installation is thus attractive financially, especially as growers continue to face financial challenges with late frosts (50% of the crop lost in 2019) and drought conditions (45% of the crop lost in 2020) (Schattman et al. 2021), which will continue to occur as climate change progresses.

Maine lacks regulatory and/or financial incentives to encourage development of such dual-use solar projects. Massachusetts has these incentives and the accompanying rapid growth of these projects. Developing agrivoltaics is more expensive because the array's design must be angled and elevated 8-10 feet off the ground to allow sunlight to reach the plants and for growers to maneuver underneath. This project, situated in Rockport, is a case study for Maine to understand how such incentives could be established. Studying this installation will also identify whether using deliberate construction methods mitigates damage to wild blueberry and what management changes and costs growers can expect when transitioning to agrivoltaics. This solar array utilizes two different types of solar panels: monofacial and bifacial. Monofacial panels are standard panels that have solar receptors only on the sun-facing side of the panel and generate energy just from that side. Bifacial panels are a newer technology with solar receptors on both sides of the panel that can generate energy from both sides. Bifacial panels allow more sunlight through the panel and generate energy from solar energy reflected off the surface underneath the panels. This was the first year of several tracking the wild blueberry to determine how much damage the plants suffered and how quickly they recovered. Data collection in future years will improve our understanding of wild blueberry production under this array and sunlight penetration to wild blueberry plants in shade, partial-shade, and full sun.

(Information on how farmers can start discussing options for solar development on their land can be found under the "Current Recommendations" header.)

METHODS

Panel installation (completed by construction contractors)

A south-facing 12-acre portion of the 40-acre installation was allocated for this study and divided into three categories: Standard, Mindful, and Careful construction methods. Rows of panels are separated by a drive-row which is wide enough for a vehicle to pass, if needed.

- In Standard (rows 27-31): construction and installation methods were unaltered from industry and company standards; equipment could drive and operate anywhere, was not restricted from turning or rotating, and foot traffic was not limited.
- In Mindful (rows 14-18): equipment could only enter and exit the site along one path; equipment could only rotate 90°; and foot traffic was limited to as few paths as possible.
- In Careful (rows 2-6): poly mats (see Figure 1, below) were placed on top of the blueberry plants to work and drive equipment on; poly mats could remain in place for only 4 weeks at a time in spring and as summer progressed the mats could only be in place one workday at a time; equipment could only turn 90° if the equipment was situated fully on plywood (otherwise, equipment could only drive straight in and straight out); and foot traffic was allowed only along one path.

Figure 1 Dr. Lily Calderwood discusses impacts on blueberry plants with members of the construction and panel installation team. All are standing on the poly mats used to minimize disruption to existing blueberry plants in the Careful treatment. These mats were driven on by construction and installation equipment and served as pathways for workers. Photo credit: Brogan Tooley.

Detailed construction methods and restrictions were designed by the planning team, which included Dr. Calderwood, members of BlueWave Solar, Solar Agricultural Services, and CS Energy. CS Energy, the construction firm, took it upon themselves to write down the protocol and train their workers in the field on how to implement the three construction methods. Employees took a blueberry protection training prior to entering the site.

Figure 2. Aerial view of the solar array under installation. Photo credit: BlueWave Solar.

Data collection (completed by University of Maine team)
Pre-construction baseline data was collected on November 20, 2020 and included quadrat data taken along 4 randomly selected field transects. Within each of 4 quadrats per transect, 6 stems were selected for stem heights and bud counts. Stem density and soil compaction were also collected in each quadrat and a single soil sample was taken across the whole field.

After construction and panel installation was complete, multiple data were collected in each of the construction categories and at a control site situated outside the solar array, which was not impacted by construction or installation (referred to as “external control”). Measures quantifying the immediate impacts of installation included soil compaction and blueberry cover. Long-term impacts may include increased weed pressure, and changes in soil moisture, organic matter, and nutrient availability due to soil disturbance and compaction. The direct impacts of reduced light availability were quantified through observations of phenological growth and development, leaf chlorophyll content and soil moisture. 2021 data collection is described below. Fruit yield and berry quality will be collected in 2022.

Multiple Photosynthetically Active Radiation (PAR) sensors with data loggers (ZL6 from METER group, Pullman, WA) were installed on July 9, 2021 to measure, in 15-minute intervals, the amount of sunlight penetrating through the solar panel array. This sun was assumed to be available to the wild blueberry plants below and was measured in the following locations: directly under panels, called “under-panel”, partial shade (in drive rows behind panels, called “drive-row-shade"), and full sunlight conditions (in drive rows between solar arrays, called “drive-row-sun"). There were 4 sensors (full shade, partial shade, full sun, and a localized control, called “array control”) installed in each construction category (Standard, Mindful, Careful), for a total of 12 PAR sensors. No PAR sensors were installed in the control plots situated outside of the array perimeter (called “external control”) for pest and plant data collection. PAR sensors in full sunlight provided the localized control value, or “light quantities,” for comparison with the partial and full shade conditions.

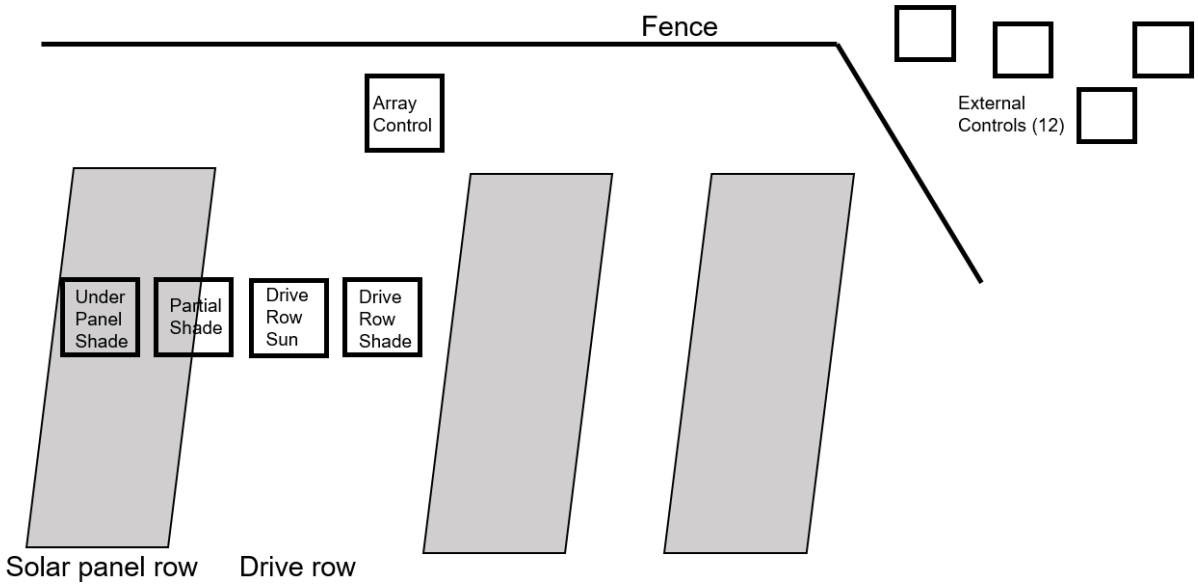

Figure 3. Example of one replication plot layout in the field where black squares represent quadrats where data collection occurred.

A 0.37 m^2 quadrat was used at each PAR sensor location for four panel rows; this totaled 16 quadrat samples per construction method for a total of 48 quadrats within the array. In addition, there were 12 external control quadrat plots, for a total of 60 quadrat plots across the entire project. All 60 quadrats were flagged for repeated measurements in the same locations throughout the season.

Wild blueberry health was evaluated within each quadrat by ranking overall blueberry cover using the Daubenmire Cover Scale of 0-6, where 0 = not present, 1 = ≤1-5% coverage, 2 = 6-25% coverage, 3 = 26-50% coverage, 4 = 51-75% coverage, 5 = 76-95% coverage and 6 = 96-100% coverage (Daubenmire 1959). Weed pressure was evaluated using the Daubenmire rank, totaling the number of weeds present, and listing the top three weed species present. Disease pressure was evaluated by counting the number of blueberry stems showing signs of disease, listing the top three diseases present, and ranking the severity of the disease observed. These blueberry coverage, weed pressure, and disease pressure measurements were all taken three times in 2021, on August 6, August 13, and September 27. In addition, blueberry stem heights and stem number per quadrat were recorded on August 13, 2021.

Wild blueberry plant health was further evaluated by gathering SPAD and TDR data. SPAD (Soil Plant Analysis Development) is a measure of how much chlorophyll is present in the leaves of the plant and was measured using a handheld chlorophyll meter (SPAD 502; Minolta Corp., Osaka, Japan). The higher the value calculated by the meter, the healthier the plant. SPAD values were taken in pairs on the same stem, reading the value on a lower and upper leaf. These pairs were taken on 4 randomly selected stems within each quadrat. TDR (Time Domain Reflectometry) is a measure of soil moisture content and temperature and was measured using a FieldScout TDR 150 Soil Moisture Meter (Spectrum Technologies, Inc., Aurora, IL, USA) to measure soil conditions to a depth of 12 cm. TDR samples were taken twice in each quadrat. SPAD and TDR samples were gathered thrice in the 2021 season, on August 13, August 27, and September 27.

Soil compaction was measured in each quadrat using an AgraTronix penetrometer (AgraTronix; Streetsboro, OH, USA) thrice in each quadrat. This compaction data was collected on August 6, 2021.

A soil sample in each of the three construction categories was collected on August 6, 2021 and analyzed at the University of Maine Soil Analysis Lab, Orono, ME, USA. This sample provided information about the nutrient profiles and organic matter present at each location. The Soil Analysis Lab also analyzed dried leaf samples to determine leaf nutrient content. These leaf samples were gathered on August 13 and 27, 2021.

Data analysis

Due to locational changes of the PAR sensors in the first month of the trial, the quadrat data collected around the PAR sensors was removed from analysis. All quadrat related data presented below (soil compaction and moisture, leaf chlorophyll, blueberry stem density and height) are from the 36 plots within the array and the 12 'external' control plots outside of the array. The 12 external control plots were randomly assigned to a section (careful, mindful, standard) to maintain equal sample size in statistical comparison. Computations were carried out using JMP Version 15.2 (SAS, Carry, NC) statistical software. PAR data and pest pressure data did not meet statistical assumptions of a normal distribution and statistics were not performed but will be revisited with more data in 2022. When data met statistical assumptions (i.e soil compaction, soil moisture, plant chlorophyll, stem density and height), evaluations were completed using a one-way ANOVA and a Tukey's Pairwise comparison.

RESULTS

Environment

The greatest levels of soil compaction occurred under-panel (relative to the control) and in the Standard and Mindful construction methods where greater disturbance likely occurred (Figure 4). Here, the Standard drive-row-shade and the Mindful under-panel had significantly more compacted soils than the external control. The Mindful under-panel also had significantly more compacted soils than the Careful drive-row-sun, suggesting the more careful construction methods may have mitigated disturbance.

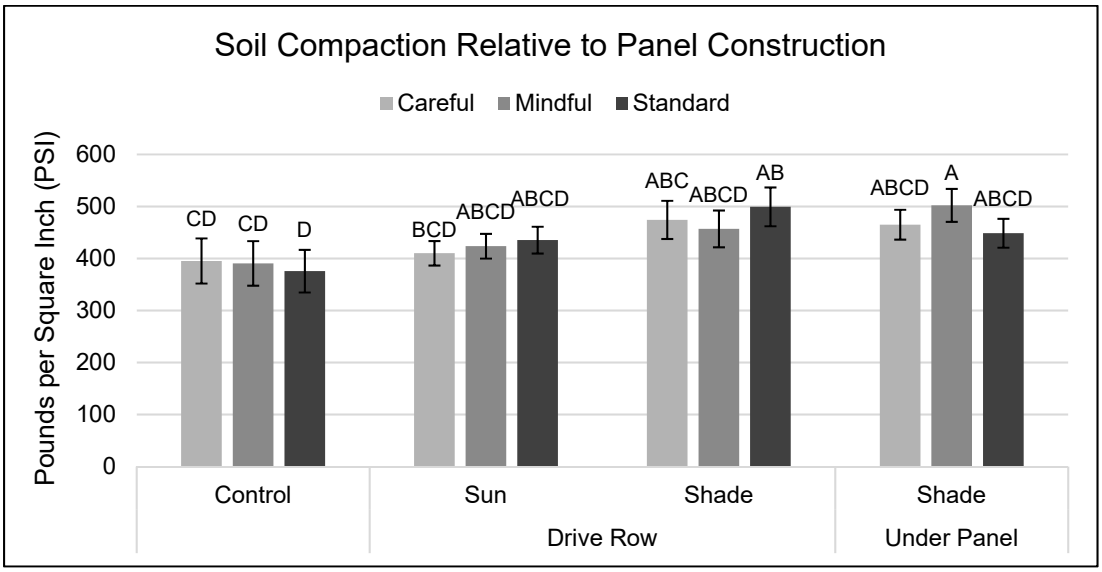

Figure 4. Soil compaction relative to construction mode and amount of shading from solar panels measured on August 6, 2021. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean.

All under-panel locations across all construction methods had significantly higher soil moisture relative to the external control with the exception of the Mindful drive-row-sun and Mindful under-panel (Figure 5). Of the treatments exhibiting significant differences, the volumetric water content of the external control soil ranged from 23-25% while the under-panel soil moisture ranged from 32-38%. The under-panel soil moisture measures were 35-54% greater than the external control soil moisture measures.

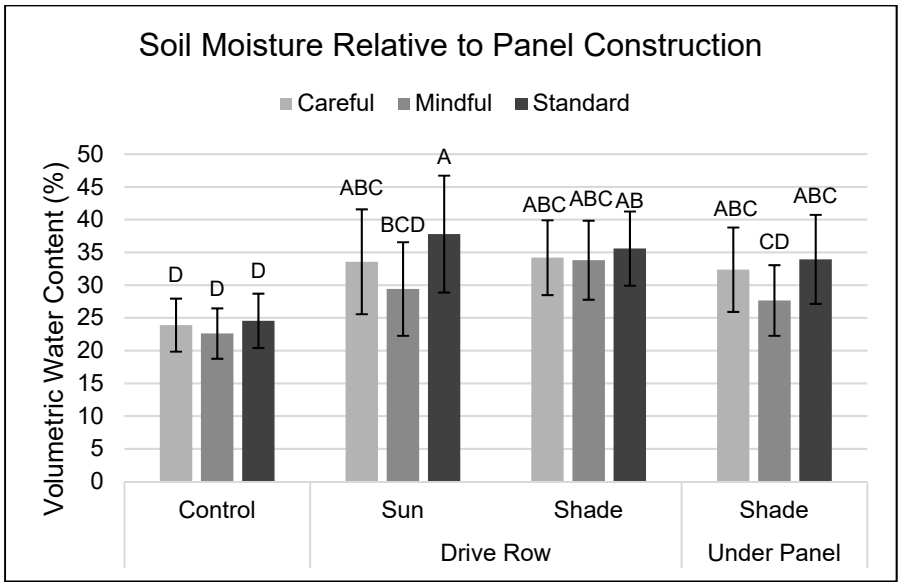

Figure 5. Soil moisture (% VWC) relative to the mode of construction and amount of shading from solar panels, measured August 13, 2021. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean.

A clear difference existed in PAR measures between the panels (drive-row-sun and array control) and underneath the panels (drive-row-shade and under-panel) (Figure 6). Although not yet statistically analyzed due to the failure of the data to meet parametric assumptions, the drive-row-sun plots received on average 18% less PAR than the full sun array control. The drive-row-shade and under-panel locations received as much as 83% and 88% less PAR (sunlight) than the control, respectively.

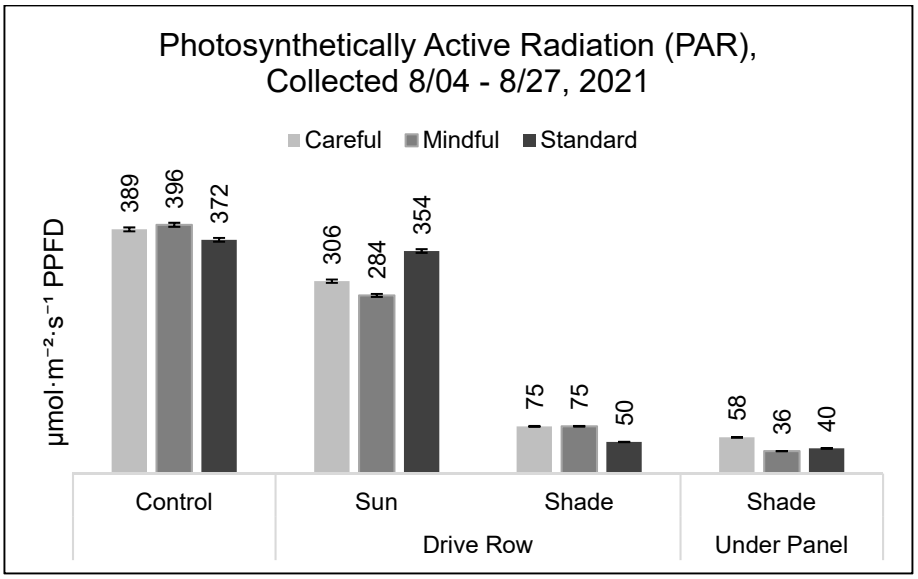

Figure 6. PAR relative to the mode of construction and amount of shading from solar panels, measured August 4 - 27, 2021. Significant differences were not tested due to non-normality in the data. Error bars indicate the standard error of the mean.

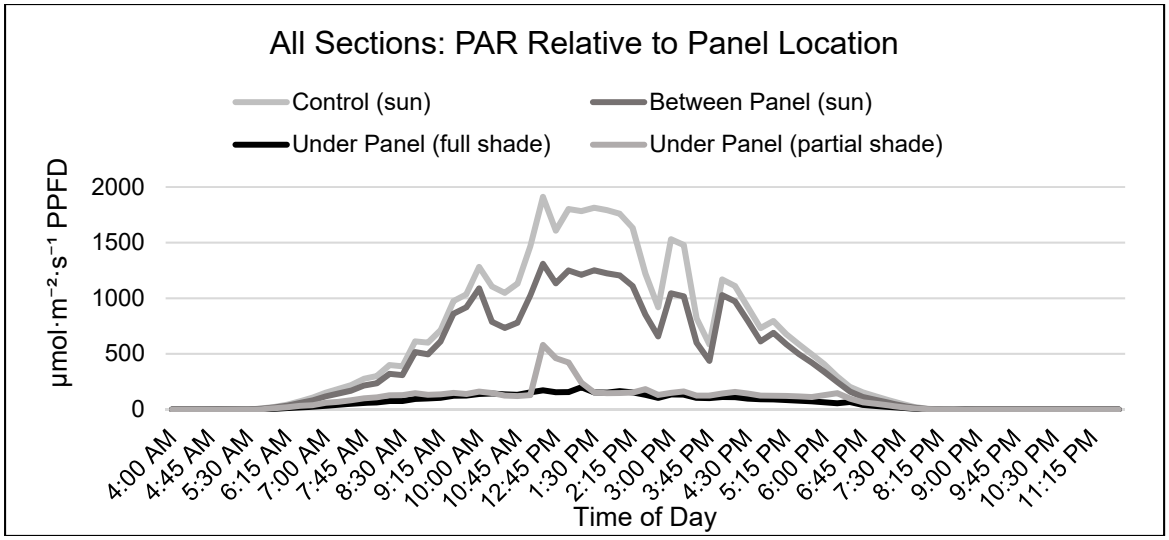

Figure 7. Hourly PAR for a sunny day (August 8, 2021) relative to panel location.

Plant Health

In response to reduced PAR within the array, leaf chlorophyll concentrations (as measured with SPAD) in drive-row-sun and under-panel were significantly higher than the external full sun control except for Standard drive-row-sun and Careful and Standard drive-row-shade (Figure 8). The leaf chlorophyll content of external control ranged from 23.4 to 27.2, while the under-panel had the highest leaf chlorophyll ranging from 38.3 to 40.2. Overall, the leaf chlorophyll content of the plants within the array (under-panel and drive-row-shade) were 10-51% higher than the external control plants.

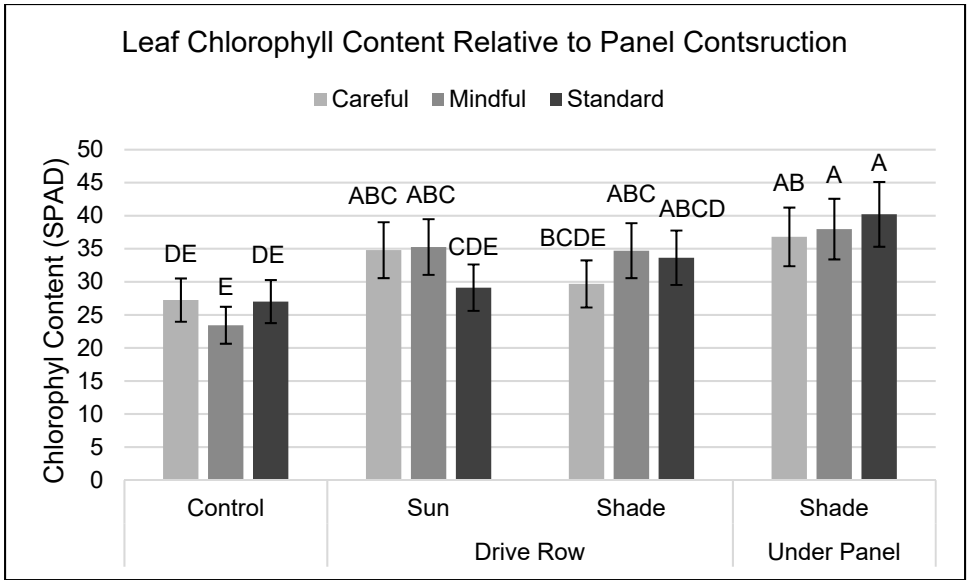

Figure 8. Leaf chlorophyll content (SPAD) relative to the mode of construction and amount of shading from solar panels, measured August 13, 2021. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean.

Lowbush blueberry stem density and stem height did not present statistically significant differences between treatments, but still exhibited some interesting trends (Figure 9). Stem density appeared to reduce with the mode of construction such that Careful maintained the highest stem counts (even in drive-row) compared to Mindful, Standard, and the external control (Figure 9a); Mindful and Standard saw reduced stem numbers within their drive-rows as well. Blueberry stem height reflected greater differences in sample locations within the panels (i.e., drive-row vs. under-panel) rather than with mode

of construction (Figure 9b). The external control had the tallest stems followed by Mindful under-panel. Careful drive-row had the tallest stems, followed by Mindful and Standard.

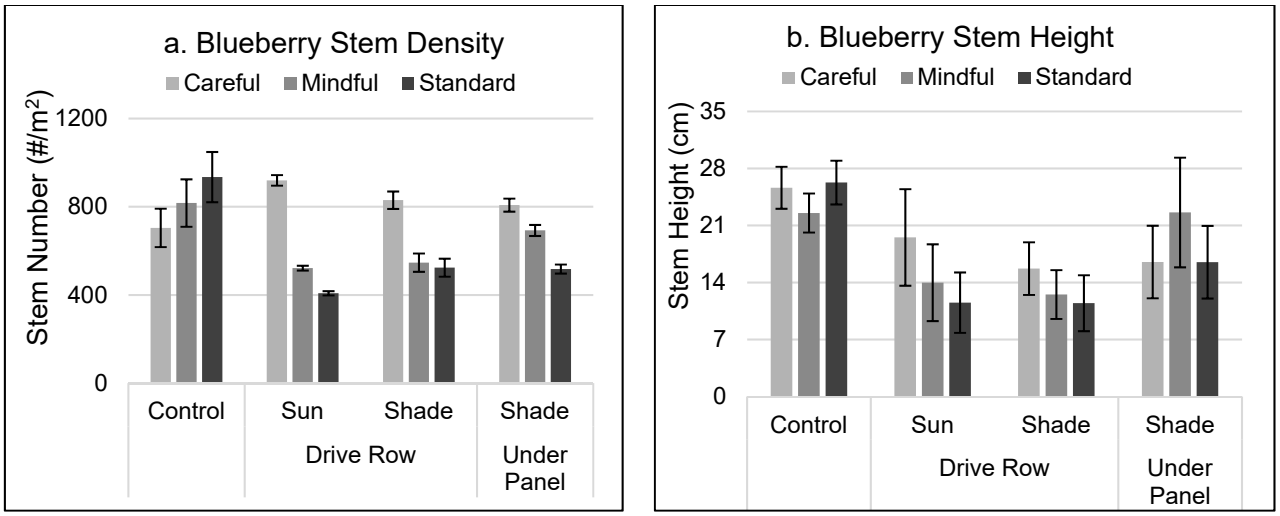

Figure 9. Lowbush blueberry stem density (9a) and stem height (9b) relative to the mode of construction and amount of shading from solar panels. Differences in blueberry stem number and stem heights were non-significant at the 0.05 level of significance. Error bars indicate the standard error of the mean.

Pest Pressure

Weed, insect and disease pressures in the blueberry were non-significant between treatments (within the array vs. outside the array; data not shown). Observationally, insect and weed pressures were variable in this initial year. Disease presented a visual trend, where shaded plots within the array exhibited less disease than the external control, likely due to the higher soil moisture and reduced heat stress. However, higher disease presence and early leaf drop was observed within the array in the fall where rain hit panels and fell to the ground at breaks between the panels.

DISCUSSION

Observationally, the wild blueberry crop is recovering faster than expected from construction disruptions, with darker than usual, healthy-looking leaves in the shaded areas (under-panel and drive-row-shade). Parts of this field were in prune and crop cycles in 2021 and are in the process of being transitioned to one unified cycle across the whole area. Wild blueberry plants in the crop year bloomed during construction and produced some fruit under the solar panels after construction finished yet harvest did not take place in this first year.

The soil was most compacted directly underneath the panels for all construction methods, and particularly under Standard and Mindful methods where more disturbance occurred compared to the Careful section.

Preliminarily, shaded locations within the array exhibited higher soil moisture content than what was measured in the external full sun control. Under-panel soil moisture levels were 35-54% greater than those in the external control. This indicates that the solar panels improve soil moisture retention, perhaps by preventing the evapotranspiration of moisture from the soil and plants. Drought conditions have caused crop loss in wild blueberry with 45% of the crop lost in 2020 (Schattman et al., 2021) making these findings important if the crop can maintain berry production under the shade produced from solar panels. Even more intriguing is the trend towards higher soil moisture in the partial shade

(drive-row-shade) and partial sun (drive-row-sun) in the drive row where it will be much easier to continue farming. Wild blueberries require 1 inch of rain per week between April and October to sustain plant demand (Trevett, 1967; Hunt et al., 2008). The probability of reaching this rainfall requirement is estimated to be less than 50% during most of the growing season and less than 20% during the critical fruiting period (July – August) based on rainfall histories since 1959 (Dalton and Yarborough, 2004). Recent research by UMaine colleague, Yongjiang Zhang, suggests that volumetric water content of 10% or higher is adequate for wild blueberry production but should not drop to 5% or lower. All soil moisture readings during this first year of study were 20% or higher. Continued observation and measurement will indicate whether or not these solar panels serve to shield soils from drying out as quickly as uncovered soils do.

Mindful plots consistently saw lower soil moisture and higher compaction levels, indicating these measures could be correlated and indicative of preconstruction landscape conditions. The entire solar array is mostly situated atop rocky outcroppings with exposed patches of bedrock with the most exposed bedrock in the Mindful section. This area therefore has less soil and is more vulnerable to compaction and reduced soil moisture levels.

Though PAR data did not meet parametric assumptions, comparing PAR measures directly shows a stark difference in the levels of PAR reaching the plants under each sunlight condition. Intuitively, control plots received more sunlight and therefore measured the greatest amount of PAR. Compared to the control, the drive-row-sun plots measured 18% less PAR, the drive-row-shade plots measured 83% less PAR, and the under-panel-shade plots measured 88% less PAR. Wild blueberry are tolerant of shade, but a near-total reduction in received PAR will likely prove more limiting for the wild blueberry than the plant can handle. Further study in future growing seasons will increase our understanding of how the wild blueberry handles reductions in PAR availability.

Variations in PAR availability for the plants also impacted the level of leaf chlorophyll concentrations, as measured by SPAD. Drive-row-sun (less the Standard plot) and under-panel plots were significantly higher than the control. This is the plant's response to shade allowing them to absorb more light. With less PAR reaching the plant, it is advantageous for the plant to produce more chlorophyll to better utilize what limited PAR does reach the plant. Under-panel leaves were visually observed as being consistently darker green than those with higher light quantity.

Lowbush blueberry stem density and height did not exhibit statistically significant differences between treatments, although they did display some other interesting trends. Careful saw the greatest stem density in all plots (even the drive-rows) compared with the other construction methods and the external control, which aligns with the knowledge that blueberry plants respond well to slight disturbance. Meanwhile, blueberry stem heights were more varied based on their location (drive-row vs. under-panel) instead of construction method. Generally, stem heights were shorter in drive-row than under-panel. Taken in combination, the stem density and height information indicate that blueberry plants were either successfully protected by the Careful precautions taken or the plants responded to disturbance by producing more stems which has been seen in other cases (Libby 2011).

While weed, insect and disease pressures in the wild blueberry were nonsignificant between treatments and varied in their occurrence and cover. Plots within the array displayed less disease than the external control, likely due to the increased shading and soil moisture which reduced drought or heat stress to the plant. The greatest disease pressure was measured in Standard drive-row-shade, which aligns with knowledge that leaf spot can spread through physical disturbance. In general plant diseases will increase where more moisture is present, this was visually apparent along the drip edge of the panels. The lack of treatment differences for weed, insect and disease pressure may have been due to late season sampling, due to late construction completion in July 2021. Measuring the vernal emergence

of pests and their impact on wild blueberry plants during the 2022 season will be more indicative of the long-term impacts of construction methods and a solar array on pest presence.

CURRENT RECOMMENDATIONS

None at this time.

NEXT STEPS

- Continue data collection (dependent on funding)
- During and after the 2022 growing season, quantify costs associated with managing fields that now host solar arrays and identify costs and management changes needed to transition to hosting solar while continuing to harvest wild blueberry commercially
- Form group of wild blueberry farmers with interest in agrivoltaics to advise research, education, and adoption efforts
- Present results and recommendations at UMaine Wild Blueberry Conference, summer blueberry field meetings, and UMaine Blueberry Hill Field Day

ACKNOWLEDGEMENTS

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RESEARCH

INVESTIGATOR(S): YJ. Zhang, L. Calderwood, and R. Tasnim

8. Using Foliar Fertilizers and Soil Amendments to Improve Wild Blueberry Production and Resilience to Warming

OBJECTIVE(S)

- Test mulch and biochar as a drought and pest management tools.
- Test a lower rate of Cheep Cheep and the efficacy of ammonium sulfate as crop fertilizer.
- Evaluate the impacts on crop and pests of promising foliar fertilizer products identified in 2019 – 2020 research.

LOCATION(S): Blueberry Hill Farm Lab, Jonesboro, ME
PROJECT TIMEFRAME: April 2021 – March 2023

INTRODUCTION

This project expands on the results of a previous project (see the 2020 report, page 82, "Effects of Foliar Fertilizers on Wild Blueberry Physiology and Pest Presence") which measured the impacts of several foliar fertilizers on blueberry health and separately simulated warming conditions to measure physiological changes in the blueberry that may occur with climate change, during one prune and crop cycle. This study continues these measures with different products and materials.

The earlier study's results identified a calcium product as a promising foliar fertilizer and outlined the relationship between foliar fertilizers, pest pressure, and plant growth. The earlier study also quantified the negative impacts of warming on the blueberry plant. This study continues the study of foliar-applied calcium, which will be applied in the crop year and begins an exploration of ammonium sulfate and a lower rate of Cheep Cheep (chicken manure). Separately, in our effort to adapt to drier seasons, especially in the Downeast region, we began investigating the effects of three materials on the water- holding capacity of the soil: softwood mulch, hard wood mulch, and biochar-compost. Biochar-compost was evaluated because biochar can increase water-holding capacity of sandy soils (Li et al., 2021) such as those found in wild blueberry fields. In fact, both the biochar and compost tested in this study were separately evaluated in the laboratory for their water-holding capacity in wild blueberry field soil before field testing it. From laboratory testing, we found that a biochar-compost mix can increase water-holding capacity as well as provide nutrients. Compost can provide soils with nutrients but not increase water-holding capacity. Biochar can increase soils' water-holding capacity but not nutrient levels. Thus, compost or any other nutrient or nitrogen-rich granular fertilizer is recommended to use with biochar.

These seemingly disparate avenues of experimentation are in fact interdependent. Maine wild blueberry farmers are aware that the growing season for wild blueberries has shifted, with a 2014 study by Drummond and Yarborough showing that the wild blueberry growing season had extended by two weeks on both ends of the season compared to the average season length 50 years earlier. This year's "Wild Blueberry Phenology" report on page 123 shows clear shifts in crop development stages. As the wild blueberry crop adapts to a warmer, drier world, growers must understand new timing and rates of fertilizers that will assist the crop in reaching high productivity in the face of a changed environment. Growers are aware that the season is shifting and are interested in understanding how and when to apply different fertilizers and moisture management products (like mulch) to improve the robustness of their wild blueberry plants and better manage pests and

disease. This study intends to provide relevant, contemporary information for farmers to better manage the timing and quantity of inputs on their fields.

METHODS

In May 2021, the study was laid out at Blueberry Hill Farm in Jonesboro, ME in a randomized complete block design with each treatment replicated multiple times in 6' by 30' plots, for a total of 78 plots, with 24 plots dedicated to ground fertility trials, 30 to foliar fertility trials, and 24 to moisture materials' trials.

Moisture materials trial (mulch)

Twenty-four plots were laid out for this portion of the trial and each treatment was replicated 6 times. Baseline soil samples were taken at these plots in May 2021 and were differentiated by treatment type. Foliar samples were collected on June 21 and July 20 and sent to Nova Crop Control for plant sap analysis, to track how plants use nutrients. The foliar and soil samples will be compared with future samples taken after applying varying the different treatments. Product names, ingredients and rates and application details are listed in Table 1.

Table 1. Moisture materials treatment summary

Applied in the prune year (2021)		
Treatment	**Rate**	**Date Applied**
Control	N/A	N/A
Biochar-compost	7.5 yd^3/A (each)	5/24/21
Hardwood mulch	135 yd^3/A (1 inch)	5/19/21
Softwood mulch	135 yd^3/A (1 inch)	5/19/21

Moisture materials trial (biochar-compost mix)

To be effective, biochar had to be applied with a soil material. We applied the biochar-compost mix (ratio of 1:1) at a rate of 7.5 yd^3/A. Biochar was provided by Maine Wood Pellets Co. and contained ash. After receiving the biochar at the University of Maine, we separated the ash from biochar by sifting them in sieving equipment. Compost was provided by the University of Maine composting facility. At the time of collecting and using that compost, we also sent a sample of compost for comprehensive testing to the Analytical Soil Testing Laboratory, University of Maine, Orono, ME.

The characteristics of the used compost were as follows: 8.0 pH; 79.7% organic matter; 849 ppm nitrate-nitrogen; 15 ppm ammonium nitrogen; 6335 lb/A phosphorus; 57.2% potassium; 19.7% calcium; 23.1% magnesium; 518 ppm sulfur; 8.7 ppm boron; 0.7 ppm copper; 24 ppm iron; 97 ppm manganese; 3.3 ppm zinc. The biochar had a known pH of 7.0-7.5 and it contained no nutrients which is why we added the nutrient-rich compost with this biochar. Furthermore, the texture of biochar is very fine. Applied alone, biochar would likely blow away.

Product assessment trial (ground fertility)

Twenty-four plots were laid out for this portion of the trial, and each treatment was replicated 6 times. Baseline soil samples were taken in these plots in May 2021 and were differentiated by treatment type. Foliar samples were analyzed on June 21 and July 20 by Nova Crop Control for plant sap analysis, to track how plants use nutrients. The foliar and soil samples will be compared with future samples taken after applying varying the different treatments. Products were applied to the plots at the manufacturer-recommended rates on May 26, 2021. Product names, ingredients and rates and application details are listed in Table 2.

Table 2. Ground fertility treatment summary

Applied in the prune year (2021)					
Treatment		**Material Rate**	**Nitrogen (N) Content**	**Sulfur (S) Content**	**Date Applied**
Control		N/A	N/A	N/A	N/A
Cheep Cheep		700 lbs/A	lbs N/A	N/A	5/26/21
Ammonium sulfate	Low ($NHSO_4$-L)	214lbs/A	45 lbs N/A	51 lbs S/A	5/26/21
	High ($NHSO_4$-H)	381 lbs/A	80 lbs N/A	91 lbs S/A	5/26/21

Product assessment trial (foliar fertility)

Thirty plots were laid out for this portion of the trial, and each treatment was replicated 5 times. Baseline soil samples were taken in these plots in May 2021 and were differentiated by treatment type. Product names, ingredients and rates and application details are listed in Table 3.

Table 3. Foliar fertility treatment summary

To be applied in the crop year (2022)			
Treatment	**Active ingredient rate**	**Mixing rate per application**	**Application frequency**
Control	Just water	N/A	Twice
Calcium (Poma) 0.5x2	0.5 gal/A x 2	242 gal H_20/A	Twice
Calcium (Poma) 1x1	1.0 gal/A x 1	484 gal H_20/A	Twice (1 water)
Calcium (Poma) 1x2	1.0 gal/A x 2	484 gal H_20/A	Twice
Calcium (Poma) 2	2.0 gal/A x 1	986 gal H_20/A	Twice (1 water)

Data Collection

Soil moisture

Soil temperature (°C), volumetric water content (%) and electrical conductivity were recorded using a FieldScout TDR 150 soil moisture meter (FieldScout TDR 150, Spectrum Technologies Inc., Aurora, IL, USA) probe inserted 12 cm (4.8 inch) into the blueberry root zone soil. Six random readings were recorded per plot on June 9, July 8, August 2 and 19.

Blueberry physiology

Eight stems from each plot were randomly selected to measure chlorophyll concentration and anthocyanin concentration from May to August. Chlorophyll concentration was measured by a CCM-200 plus chlorophyll content meter (Opti-Sciences Inc., Hudson, NH, USA) and anthocyanin concentration was measured by ACM-200 (Opti-Sciences Inc, Hudson, NH, USA). Photosynthetic rates (CO^2 assimilation) were also measured on one random plant from each plot using a portable photosynthetic measurement system (li-6800; Li-Cor Biosciences, Lincoln, NE, USA) on a sunny day in mid-July between 10:00 and 15:00 h solar time at a photosynthetic photon flux density of 1500 µmol m^{-2} s^{-1}.

Blueberry morphology

On July 22 and August 24, eight random stems from each treatment plot were collected to quantify the number of leaves on each stem, leaf size, dry biomass, and nutrients. Leaf area of three leaves at three different positions (top, middle, and bottom) on each of those stems was determined using a LI-3000A area meter (Li-Cor, Lincoln, NE, USA). All the leaves from those eight stems were oven-dried at 70 °C to constant mass and weighed, then ground and sent to the University of Maine Soil and Plant Tissue Testing Laboratory in Orono, Maine for leaf nutrient testing. On June 18 and July 18, a minimum of 80 grams of fresh leaves from each treatment (leaves were pooled from 6 replicated plots in one sample) were collected and sent to the Nova Crop Control Lab in Ohio for sap nutrients testing. On October 2,

soil samples were collected from each plot and sent to the University of Maine Analytical Soil Testing Laboratory in Orono, Maine for a comprehensive soil testing. The soil sampling results are reported in Table 4.

Table 4. Recommended optimum ranges and comparisons of wild blueberry soil characteristics among different soil amendments and fertilizer treatments on October 2, 2021, in the trial's conventional wild blueberry field (Blueberry Hill Research Farm, Jonesboro, ME). Soil characteristics for different treatments are represented as mean of six replicated soil samples ± standard error of the mean.

Soil Characteristics	Optimum range	No treat-ment	Moisture Retention Trial			Ground Fertility Trial		
		Control	Hardwood mulch	Soft-wood mulch	Biochar-Compost mix	Chicken manure	Low ammonium sulfate	High rate of ammonium sulfate
pH	4.0-4.5	4.6±0.12	4.8±0.13	4.8±0.09	4.92±0.04	4.7±0.05	4.7±0.13	4.6±0.09
Organic matter (%)	5-8	9.0±0.9	6.5±0.8	9.92±1.6	10.1±1.5	9.3±2.1	7.5±0.95	8.3±1.1
CEC (me/100 g)	>5	5.8±0.5	4.98±0.66	5.87±0.87	5.85±0.61	6.8±0.81	5.25±0.65	5.5±0.64
Nitrate-N (ppm)	20-30	1.3±0.2	1	1.3±0.2	1.7±0.2	2±0.5	1.4±0.3	7.2±2.8
Ammonium-N (ppm)	<10	2.2±0.37	2.5±0.66	3.5±1.43	2.7±0.84	2.8±0.6	2.2±0.3	5.8±1.9
Phosphorous (lb/A)	10-40	9.3±1	7.6±1.32	9.3±0.89	9.05±1.15	10.9±2.15	7.7±0.85	9±1.6
Potassium (% saturation)	2.1 - 3.0	2.2±0.17	2.7±0.34	2.8±0.25	4.8±0.7	2.2±0.2	2.25±0.2	2.2±0.2
Calcium (% saturation)	20 - 30	27.3±2.3	27.7±2.4	28.9±3.2	33±2.9	33.9±4.6	29.5±1.8	28±1.9
Magnesium (% saturation)	5-10	9.5±0.5	9.45±0.46	9.7±0.8	9.95±0.6	9.3±0.5	8.6±0.66	6.7±0.57
Sulfur (ppm)	>50	45.8±8.5	36.7±9.96	31.3±2.57	62.7±7.6	32±6.16	40.8±6.85	75.2±14.98
Copper (ppm)	0.25 - 0.6	0.2±0.03	0.15±0.03	0.2±0.05	0.2±0.05	0.19±0.03	0.25±0.06	0.48±0.3
Iron (ppm)	6-10	55.5±10.6	43.82±12.98	47.2±6.7	50.8±7.5	51.1±8.3	46.6±9.42	49.4±6.84
Manganese (ppm)	4-8	10.8±2.5	8.12±1.2	14.2±4.6	10.4±1.7	18.3±7.12	14.32±1.6	25.5±11.3
Zinc (ppm)	1-2	2.8±0.3	2.87±0.43	4.3±0.75	3.4±0.5	4.8±0.99	3.32±0.75	3.1±0.42
Boron (ppm)	0.5 - 1.2	0.3±0.02	0.27±0.02	0.28±0.03	0.32±0.03	0.3±0.02	0.27±0.02	0.32±0.015

Crop Productivity
Repeated stem number counts were taken throughout 2021 using a 0.37 m^2 quadrat in the same flagged locations, twice per plot. On June 3 and August 20, 2021, these measurements were recorded for the foliar treatment plots. On August 20, 2021, there were stem height measurements and bud counts measured on 8 random stems within each of those quadrats, for a total of 16 random stems per treatment plot. Blueberry cover was quantified using the same equal interval ranking at the time of each pest scouting.

Pest Scouting
Repeated pest and plant growth measurements were taken throughout 2021 using a 0.37 m^2 quadrat in the same flagged locations, twice per plot. Blueberry cover, weed, insect, and disease pressure were recorded on June 16, July 21, August 20, and September 20, 2021. Pest severity (percent cover) for weeds, insect and disease were quantified using equal interval ranks between 0 and 6, where: 0 = not present, 1 = ≤1%-17%, 2 = 17%-33%, 3 = 33%-50%, 4 = 50%-67%, 5 = 67%-83% and 6 = 83%-100%. Weeds were identified by species and counted to obtain weed number per quadrat. The number of wild blueberry stems with insect or disease damage were also counted in addition to ranking percent cover.

Data Analysis
Soil moisture
The effects of soil amendments on soil moisture were statistically compared using a general linear model followed by LSD (least significant difference) post-hoc test in SPSS software ($\alpha = 0.05$). In this model, the main effects of soil amendments were considered as a fixed factor, experimental blocks as random factors and a Bonferroni correction was also applied for confidence interval adjustment.

Blueberry physiology
The effects of soil amendments and fertilizer treatments on physiology (leaf chlorophyll concentration and photosynthetic rate) of wild blueberry plants were statistically compared using a general linear model followed by LSD (least significant difference) post-hoc test in SPSS software ($\alpha = 0.05$). In this model, the main effects of treatments (soil amendments and fertilizers) were considered as a fixed factor, experimental blocks as random factors and a Bonferroni correction was also applied for confidence interval adjustment.

Blueberry Morphology
The effects of soil amendments and fertilizer treatments on morphology (leaf size, number of leaves per stem and total leaf area per stem), and major leaf nutrients (N, P, K, Ca) of wild blueberry plants were statistically compared using a general linear model followed by LSD (least significant difference) post-hoc test in SPSS software ($\alpha = 0.05$). In this model, the main effects of treatments (soil amendments and fertilizers) were considered as a fixed factor, experimental blocks as random factors and a Bonferroni correction was also applied for confidence interval adjustment.

Crop and Pest Data
Due to the nature of count data collected in the field (which often has a high number of zeros creating a skewed distribution) much of our data failed the assumptions of normality and equal variance often required to run parametric statistical tests. All non-normal data was transformed with a square root transformation prior to any statistical testing. All ranked data, pest density, and crop productivity data visually improved following transformation, but the data continued to statistically fail for normality. Statistical tests were carried out despite non-normality after establishing there were no serious problems with the data.

Single date measurements including bud counts, stem heights and leaf nutrients were evaluated using a generalized linear model (GLM), followed by a Tukey's Pairwise comparison in JMP (JMP®, Version 14.3) across all treatments (α = 0.05). All ranked blueberry cover and pest data were transformed to their corresponding percent mid-point. Ranked blueberry cover, blueberry stem number, weed number and stems with pest pressure (insect and disease) were sampled on multiple occasions throughout the season. These were analyzed using a full-factorial repeated-measures mixed model design, followed by a Tukey's Pairwise comparison in JMP, testing the effects of date, treatment, and any interaction between date and treatment.

Foliar Fertility data analysis will take place following the foliar fertilizer application in 2022.

RESULTS

Moisture Retention

Soil Moisture

During the growing season (June-August), biochar-compost-treated soil maintained consistently higher moisture content on average among all the treatments (Fig. 1a). While comparing seasonal average soil moisture (Fig. 1b) among all treatments, it is clear that soils treated with the biochar-compost mix had significantly higher moisture content than the control and mulch treatments. All treatments and control remained above 10% volumetric soil content, the threshold for healthy crop production.

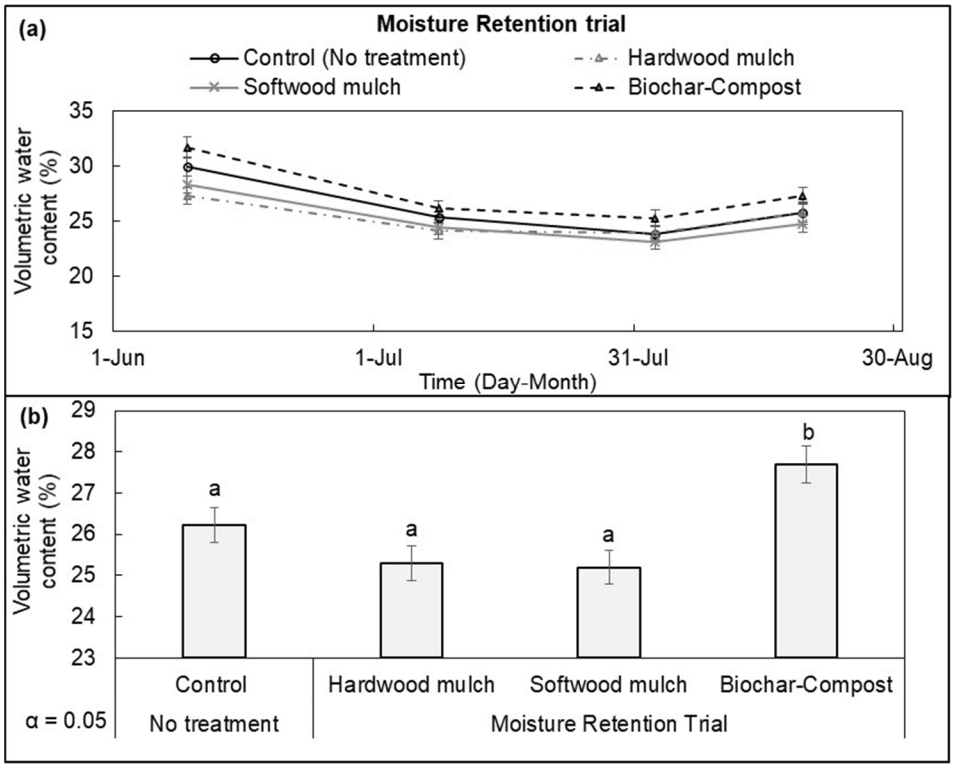

Figure 1. Comparison of (a) soil moisture during June to August 2021 among soil amendment treatments, and (b) mean soil moisture content by treatment type over all field season data collection dates (June 9, July 8, August 2 and 19) in 2021 in a conventional wild blueberry field (Blueberry Hill Research Farm, Jonesboro, ME). Error bars indicate the standard error of the mean. Different letters indicate significant differences at the significance level of $p < 0.05$.

Blueberry Physiology

For chlorophyll concentrations during the growing season (June-August), all the treatments in the moisture retention trial (Fig. 2) had similar leaf chlorophyll concentrations. On average during the peak

season in July, mulch treatments showed higher leaf chlorophyll concentrations compared to others in the moisture retention trial (Fig. 2a). While comparing average leaf chlorophyll concentrations throughout the season (Fig. 2b) among the treatments, no significant differences were found.

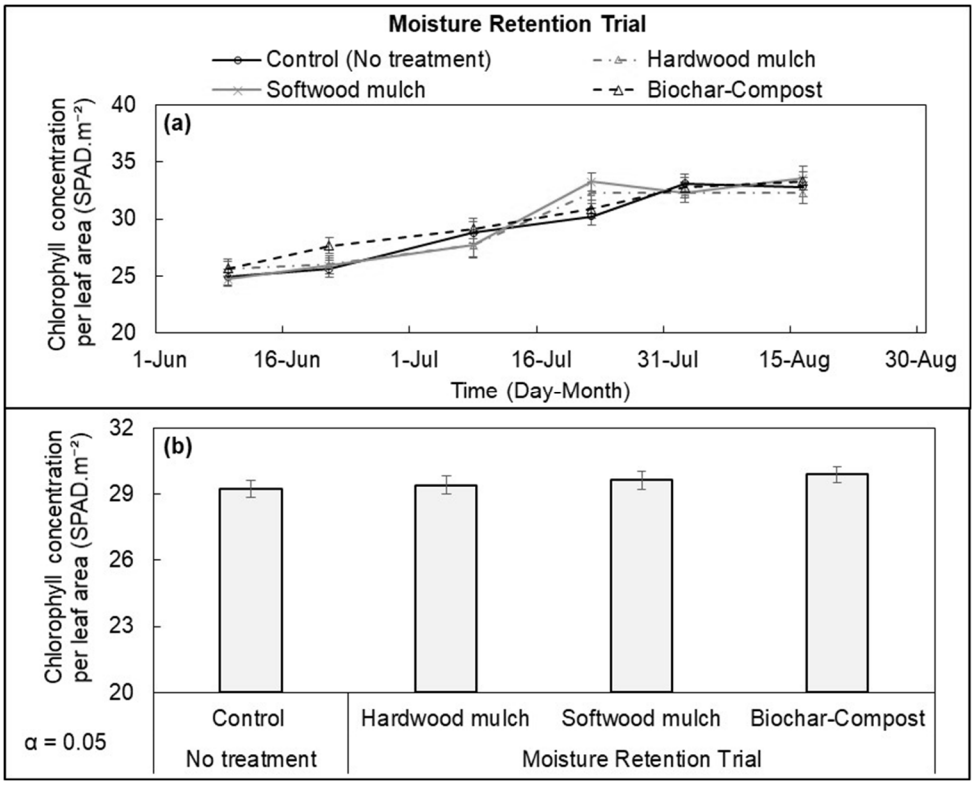

Figure 2. Comparison of (a) leaf chlorophyll concentration during June to August 2021 among soil amendment treatments, and (b) mean leaf chlorophyll concentration by treatment type over all field season data collection dates in 2021 in a conventional wild blueberry field (Blueberry Hill Research Farm, Jonesboro, ME). Error bars indicate the standard error of the mean. No letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

For leaf photosynthetic rate (Fig. 3) among the treatments, plants treated with softwood mulch had significantly higher leaf photosynthetic rate compared to control and biochar-compost treatments.

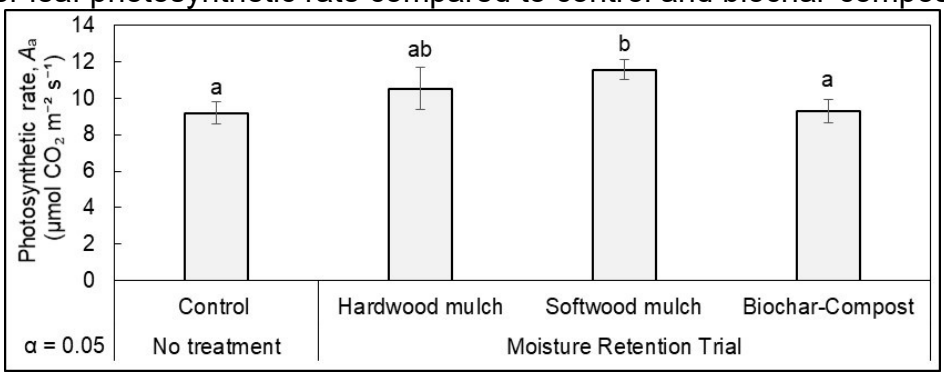

Figure 3. Comparison of photosynthetic rate of wild blueberry leaves in mid-July among different soil amendment treatments in a conventional wild blueberry field (Blueberry Hill Research Farm, Jonesboro, ME). Error bars indicate the standard error of the mean. Different letters indicate significant differences at the significance level of $p < 0.05$.

Blueberry Morphology

In July 2021, leaf size (Fig. 4a), number of leaves per stem (Fig. 4b), and total leaf area per stem (Fig. 4c) were not significantly different among the treatments. In August 2021, leaf size (Fig. 4a) was found to be significantly lower but the number of leaves per stem (Fig. 4b) was found to be significantly higher in the softwood mulch-treated plot compared to the control. However, total leaf area per stem (Fig. 4c) was still similar across all treatments and no significant differences were found.

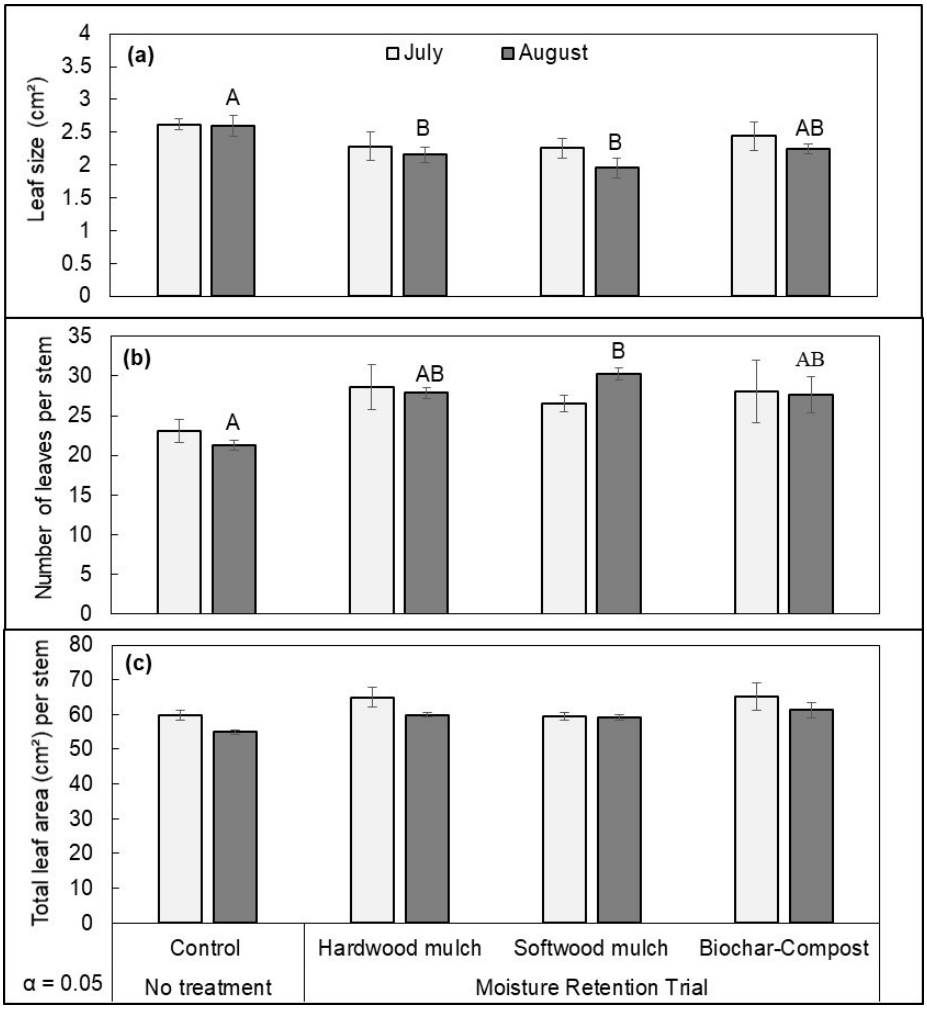

Figure 4. Comparison of (a) leaf size, (b) number of leaves per stem, and (c) total leaf area per stem of wild blueberry plants among different soil amendments on July 22 and August 24, 2021, in a conventional wild blueberry field (Blueberry Hill Research Farm, Jonesboro, ME). Error bars indicate the standard error of the mean. Different letters indicate significant differences at the significance level of $p < 0.05$. No letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

Wild Blueberry Leaf Nutrition

Leaf nitrogen (N), phosphorus (P), and potassium (K) were found to be lower than their optimum recommended levels (i.e., 1.76% N, 0.136% P, 0.44% K) in all treatments (Figs. 5a-c) (Santiago, 2011) and also, they were not significantly different among the treatments in the moisture retention trial. In contrast, leaf calcium (Ca) was found to be higher than the optimum recommended level (0.38% Ca) in all treatments (Fig. 5d) (Santiago, 2011) and significantly lower leaf Ca was found in mulch treatments.

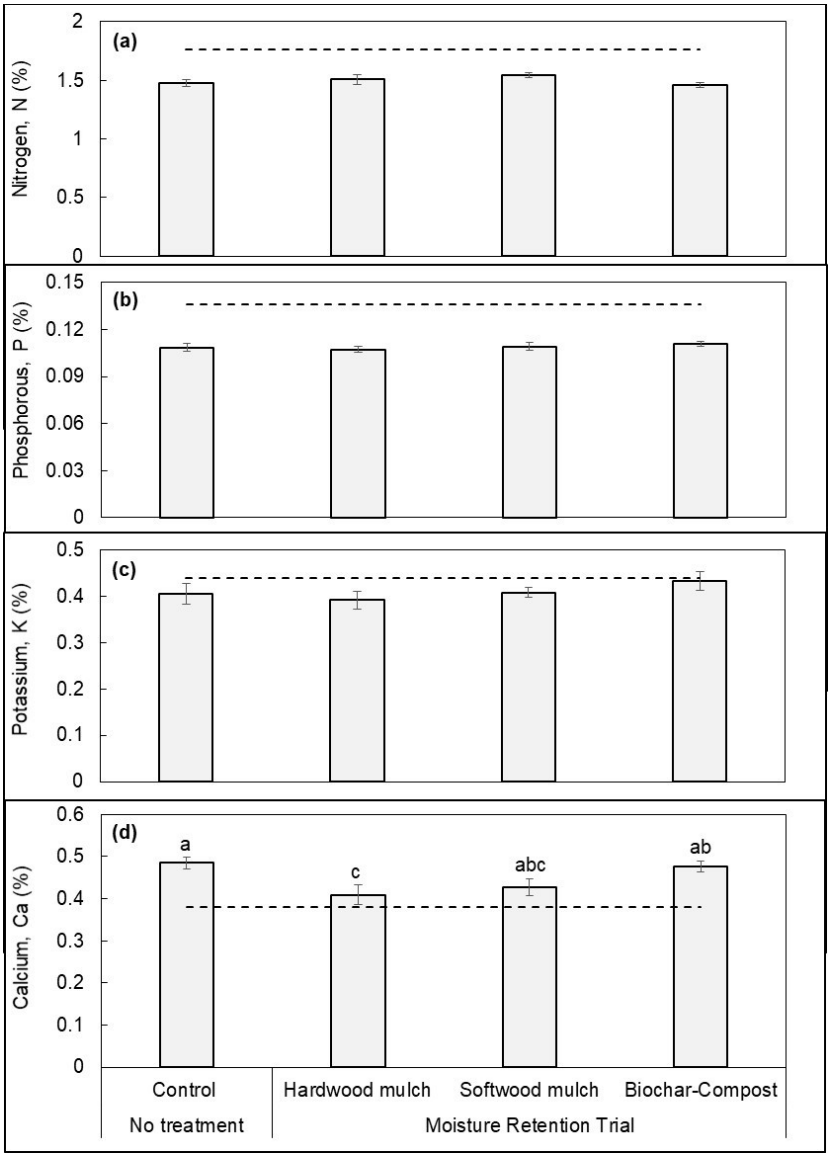

Figure 5. Comparison of wild blueberry leaf (a) nitrogen, (b) phosphorus, (c) potassium, and (d) calcium levels under different soil amendments on July 22 in a conventional wild blueberry field (Blueberry Hill Research Farm, Jonesboro, ME). Error bars indicate the standard error of the mean. The dashed lines represent the recommended optimum nutrient levels in wild blueberry leaves (Santiago, 2011). Different letters indicate significant differences and no letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

Plant Development & Productivity

Blueberry cover and blueberry stem number as indicators of plant health and productivity both exhibited significant differences (Figure 6). Interestingly, both parameters were significantly higher in the control and biochar-compost treatments relative to the hard- and softwood mulch treatments. Here, the application of mulches occurred in May 2021, at initial blueberry shoot emergence following fall 2020 flail mower. It was observed in the field that the mulch covered and weighed down the new shoots. The timing of the mulch application may have hindered the potential emergence of the blueberry stems. Blueberry stem heights and bud numbers did not exhibit significant treatment differences across moisture retention treatments (Figure 6). It is worth noting that the number of buds per stem were greater across all moisture retention amendments, especially the hardwood mulch, with 48% more buds than the control.

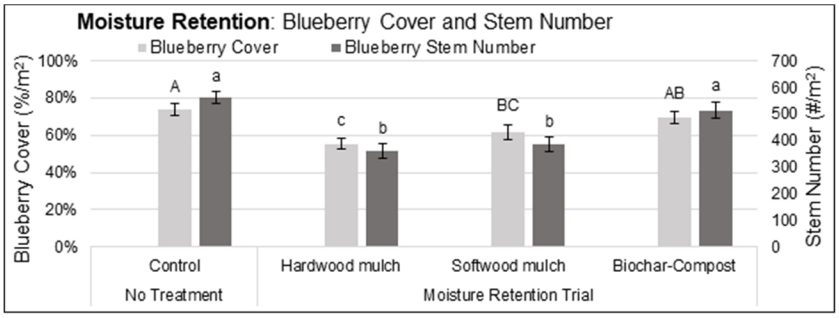

Figure 6. Blueberry cover (%/m²) and number of stems (#/m²) measured on June 9, July 21, August 20, and September 20, 2021, for moisture retention treatments applied in 2021. Letters indicate significance at the 0.05 level of significance for blueberry cover and stem number. Capital letters are to be compared separate from lowercase letters. Error bars indicate the standard error of the mean.

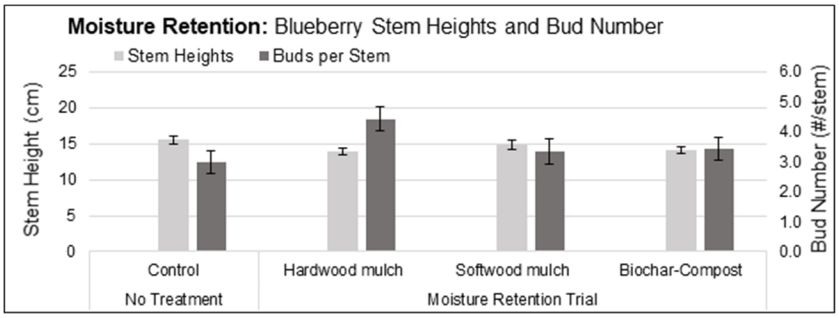

Figure 7. Blueberry stem height and bud number per stem measured on August 20, 2021, for moisture retention treatments applied in 2021. Treatment differences for blueberry stem heights and buds per stem were not significant. Error bars indicate the standard error of the mean.

Pest Pressure

Pest pressures, including weeds, insects, and disease in the moisture retention trial did not present significant treatment differences in the initial year of this study (Figures 8 and 9). There are trends worth noting in regard to the potential underlying pest responses to applied treatments. Weed and insect pressures were all higher than the control with notably high weed concentrations in the biochar-compost treatment and high insect pressure in the softwood mulch treatment. The number of blueberry stems with disease were relatively consistent across moisture retention treatments except for the hardwood mulch treatment which was relatively higher. Premature leaf drop was very apparent in August and early September. Here, leaf drop was less than the control in all moisture retention treatments by as much as 9% to 19%, with softwood mulch exhibiting the lowest levels of leaf drop.

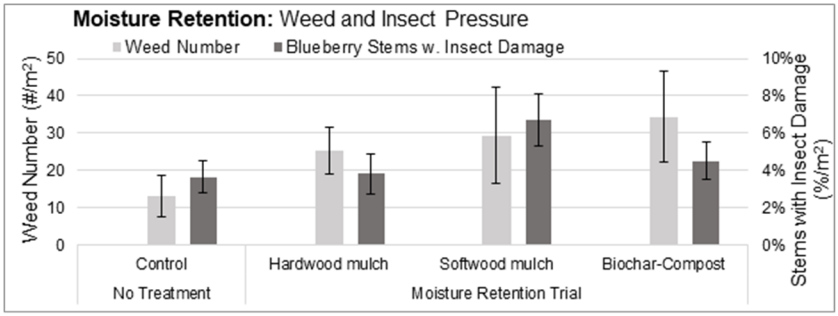

Figure 8. Weed number (#/m^2) and the number of stems with insect damage (#/m^2) measured on June 9, July 21, August 20, and September 20, 2021, for moisture retention treatments applied in 2021. Treatment differences for weed number and insect damage were not significant. Error bars indicate the standard error of the mean.

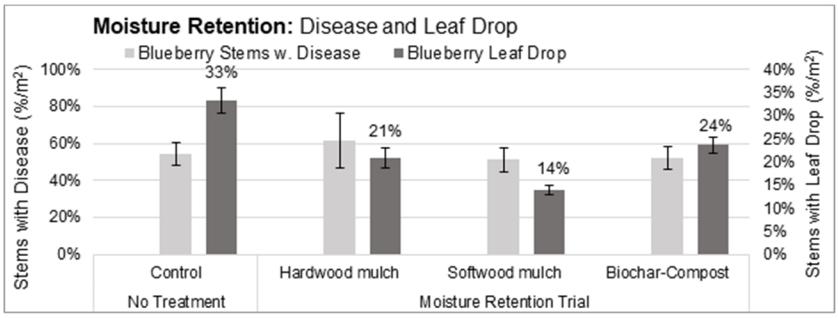

Figure 9. The number of stems with disease damage (#/m^2) measured on June 9, July 21, August 20, and September 20, 2021, and leaf drop measured on September 20, 2021, for moisture retention treatments applied in 2021. Treatment differences for blueberry stems with disease and leaf drop were not significant. Error bars indicate the standard error of the mean.

<u>Ground Fertility</u>

Blueberry Physiology

For chlorophyll concentrations during the growing season (June-August), all the treatments in the ground fertility trial (Fig. 10) had similar leaf chlorophyll concentrations. On average during the peak season in July, ammonium sulfate ($(NH_4)_2SO_4$) treatments showed higher leaf chlorophyll concentrations compared to other treatments (Fig. 10a). While comparing average leaf chlorophyll concentration across the season (Fig. 10b) among the treatments, the low rate of ammonium sulfate treatment showed significantly higher leaf chlorophyll concentrations than that of the control and Cheep Cheep.

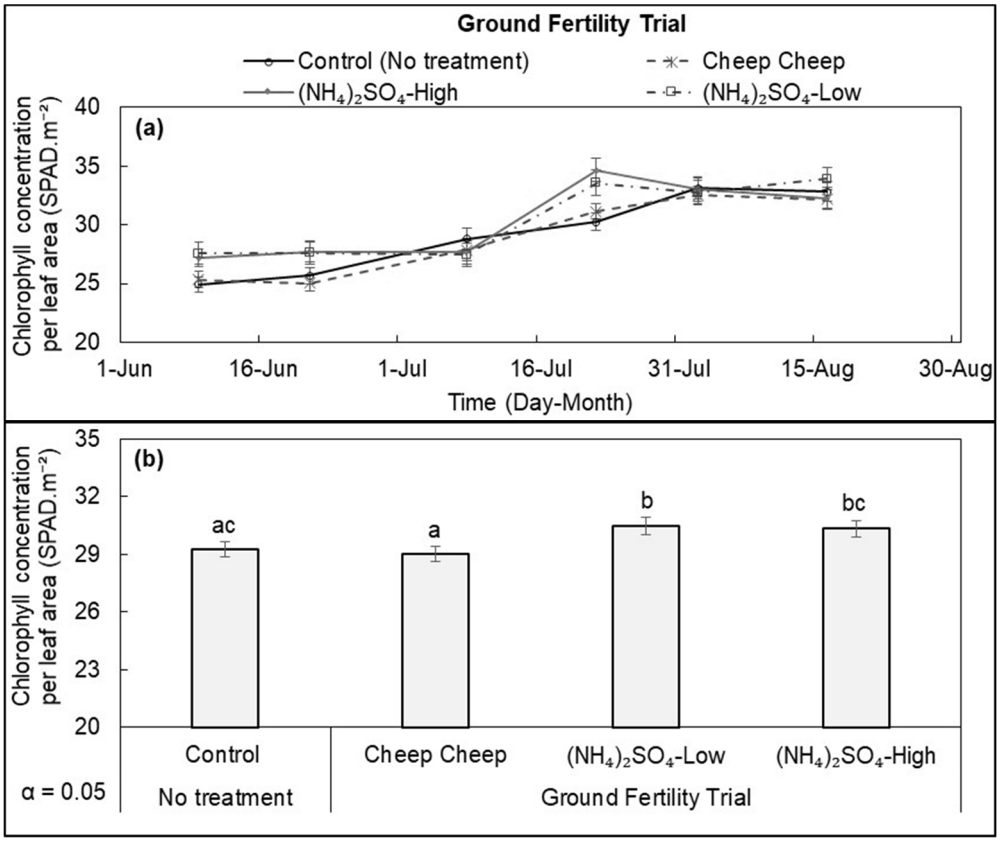

Figure 10. Comparison in (a) leaf chlorophyll concentration during June to August 2021 among soil fertilizer treatments, and (b) mean leaf chlorophyll concentration by treatment type over all field season data collection dates in 2021 in a conventional wild blueberry field (Blueberry Hill Research Farm, Jonesboro, ME). Error bars indicate the standard error of the mean. Different letters indicate significant differences at the significance level of $p < 0.05$.

For leaf photosynthetic rate (Fig. 11), all the treatments showed similar leaf photosynthetic rates and no significant differences were found among them.

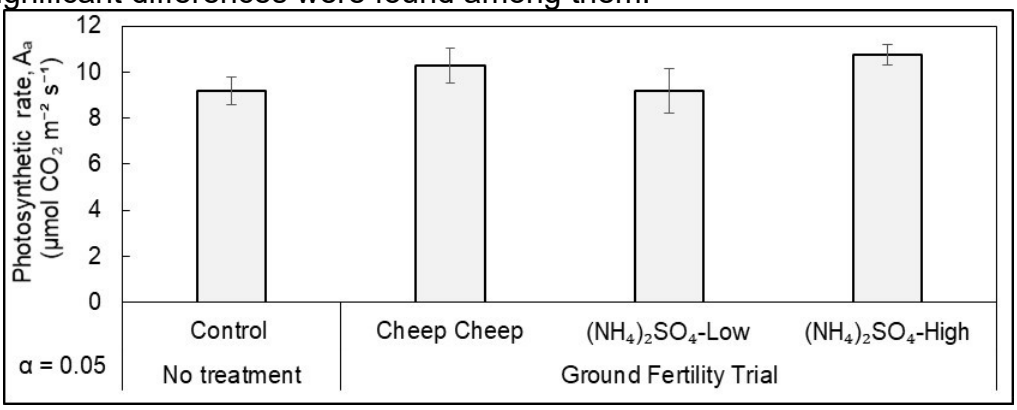

Figure 11. Comparison in photosynthetic rate of wild blueberry leaves in mid-July among different fertilizer in a conventional wild blueberry field (Blueberry Hill Research Farm, Jonesboro, ME). Error bars indicate the standard error of the mean. No letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

Blueberry Morphology

In both July and August 2021, leaf size (Fig. 12a) was not significantly different among the treatments. However, in both July and August 2021, the highest number of leaves per stem (Fig. 12b) and total leaf

area per stem (Fig. 12c) were observed in the high rate of ammonium sulfate treatment among the treatments.

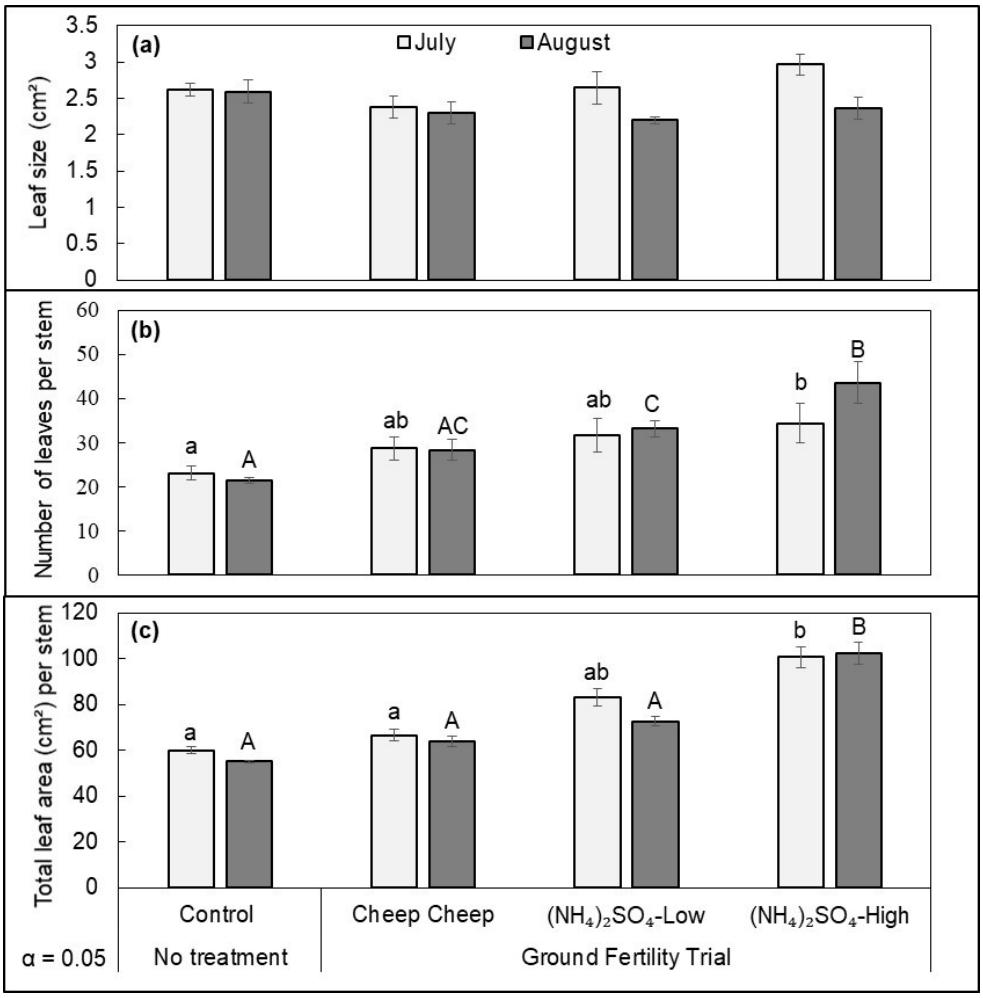

Figure 12. Comparison in (a) leaf size, (b) number of leaves per stem, and (c) total leaf area per stem of wild blueberry plants among different fertilizer treatments on July 22 and August 24 in a conventional wild blueberry field (Blueberry Hill Research Farm, Jonesboro, ME). Error bars indicate the standard error of the mean. Different letters indicate significant differences at the significance level of $p < 0.05$. No letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

Wild Blueberry Leaf Nutrition

Leaf nitrogen (N), phosphorus (P), and potassium (K) were found to be lower than their optimum recommended levels (i.e., 1.76% N, 0.136% P, 0.44% K) or closer to the optimum levels in some treatments (Figs. 13a-c) (Santiago, 2011). In contrast, leaf calcium (Ca) was found to be higher than the optimum recommended level (0.38% Ca) across all treatments (Fig. 13d) (Santiago, 2011). Leaf N and P were almost at the optimum level and were significantly higher in the high rate of ammonium sulfate treatment than other treatments (Figs. 13a-b). There were no significant differences in leaf K among the treatments (Fig. 13c). In contrast to the other major nutrients, leaf Ca was significantly lower in the high rate of ammonium sulfate treatment than control (Fig. 13d).

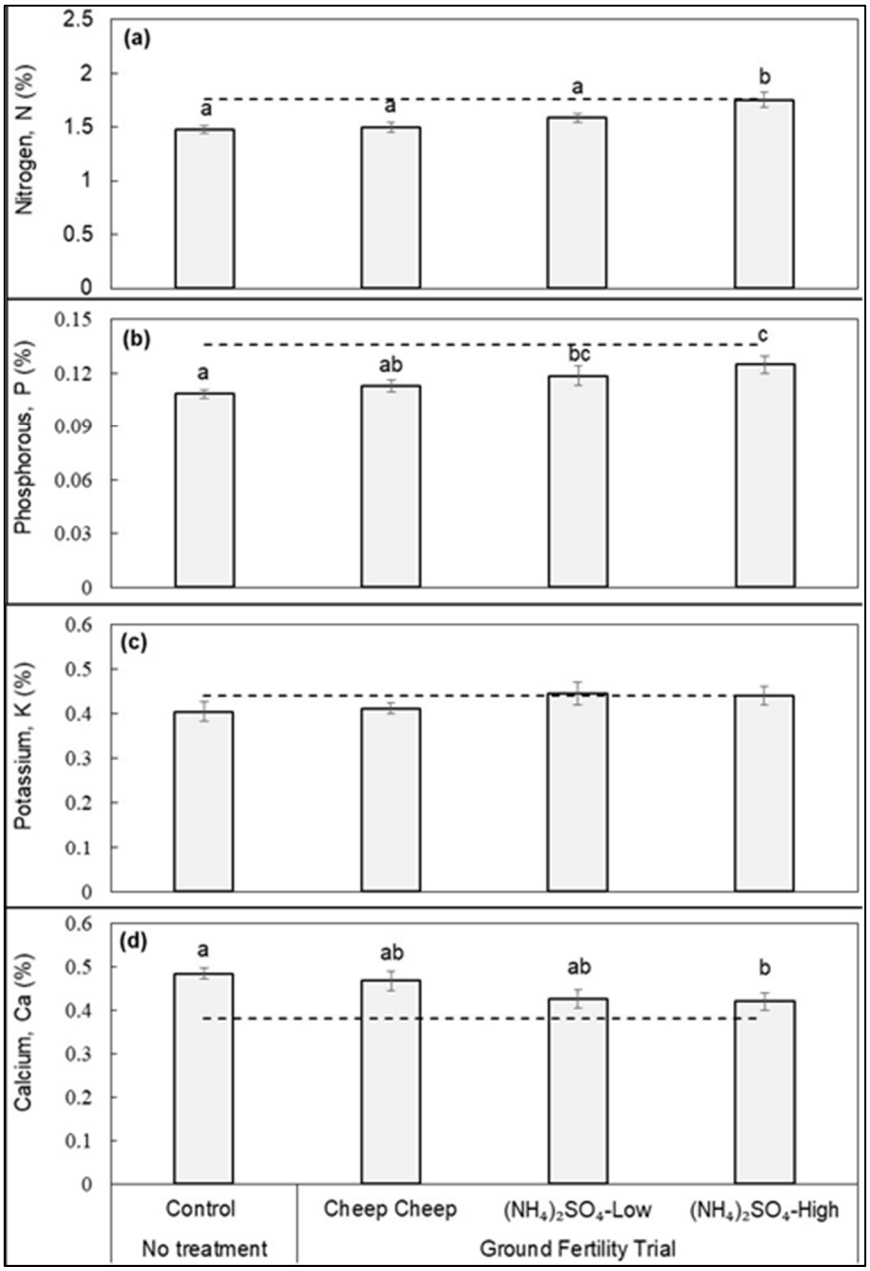

Figure 13. Comparison in leaf (a) nitrogen, (b) phosphorus, (c) potassium, and (d) calcium of wild blueberry plants among different fertilizer treatments on July 22 in a conventional wild blueberry field (Blueberry Hill Research Farm, Jonesboro, ME). Error bars indicate the standard error of the mean. The dashed lines represent the recommended optimum nutrient levels in wild blueberry leaves (Santiago, 2011). Different letters indicate significant differences and no letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

Plant Development & Productivity

Observationally, within months of the May 26, 2021, application of ammonium sulfate, the blueberry plants were visibly taller and greener with greater vegetative accumulation than the surrounding untreated plants (including the Cheep Cheep treatment; Figure 14).

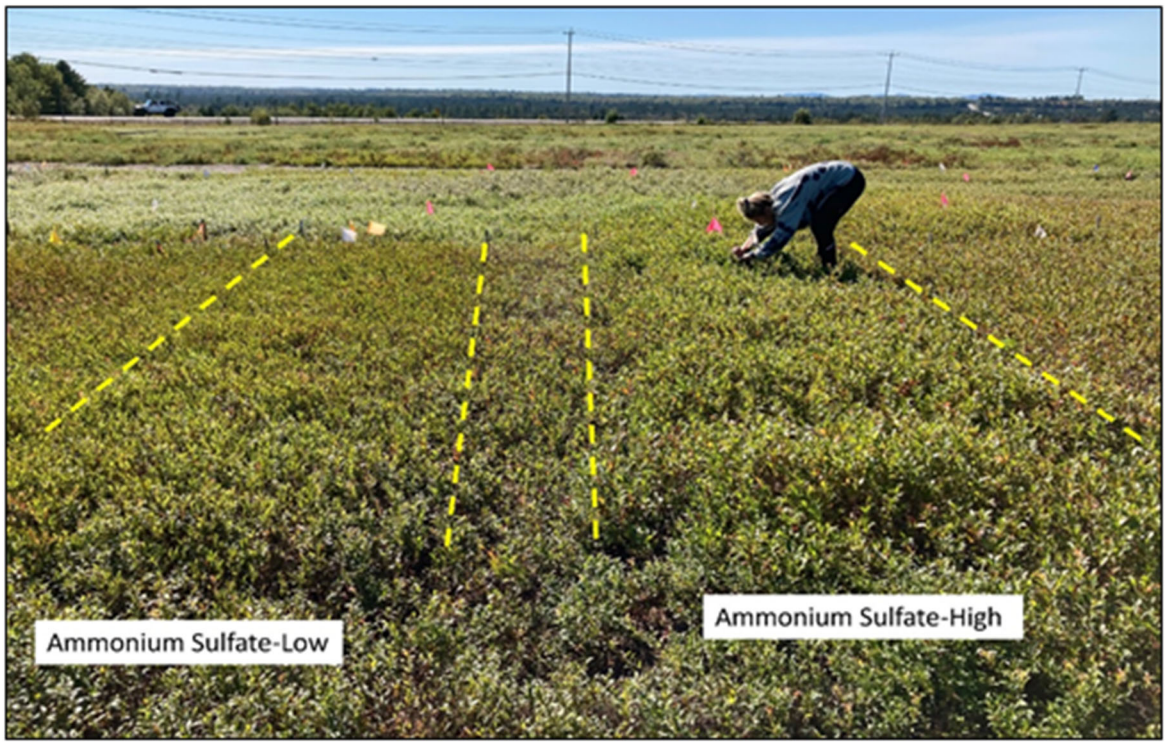

Figure 14. The higher rate of application of $(NH_4)_2SO_4$ resulted in taller blueberry stems with more leaves and greener plants. Photo taken September 20, 2021.

Blueberry stems of the plants treated with the high rate of ammonium sulfate were significantly taller than the control, Cheep Cheep and the low rate of ammonium sulfate (Figure 15). The number of buds per stem were not significantly different between treatments, however, all amended treatments exhibited greater bud numbers than the control, with the highest average bud numbers occurring in the high rate of ammonium sulfate treatment. Lower bud numbers observed in the ground fertility trial relative to the moisture retention trial (despite the trials being side-by-side) suggests there was delayed maturation in the ground fertility trial likely due to prolonged growth with fertilizer.

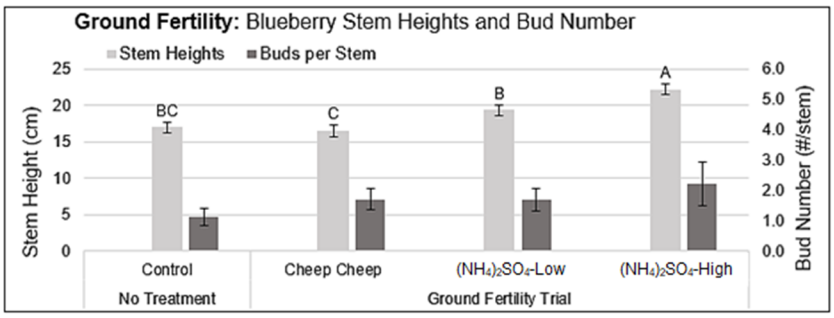

Figure 15. Blueberry stem height and bud number per stem measured on August 20, 2021, for the ground fertility treatments applied in 2021. Treatment differences were nonsignificant for bud number per stem. Letters indicate significance at the 0.05 level of significance for stem heights. Error bars indicate the standard error of the mean.

Blueberry cover was significantly higher when plots were treated with high rate of ammonium sulfate and Cheep Cheep when compared to the control. Blueberry stem number did not present significant differences but did follow a similar trend to the blueberry cover where plots treated with the high rate of ammonium sulfate and Cheep Cheep had on average 60 more stems per m^2.

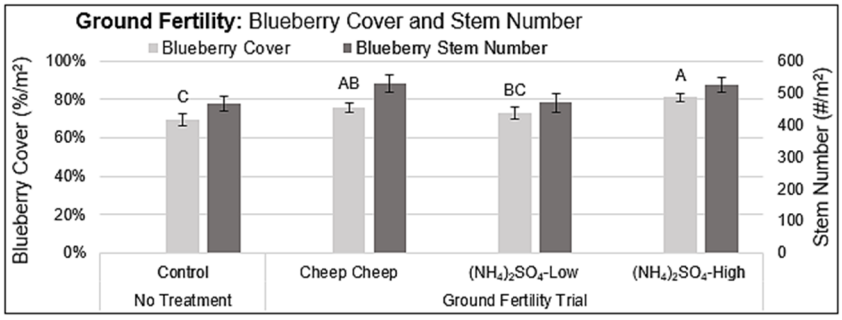

Figure 16. Blueberry cover (%/m^2) and blueberry stem number of stems (#/m^2) measured on June 9, July 21, August 20, and September 20, 2021, for the ground fertility treatments applied in 2021. Treatment differences were nonsignificant for blueberry stem number. Letters indicate significance at the 0.05 level of significance for blueberry cover. Error bars indicate the standard error of the mean.

Pest Pressure

Pest pressures including weed, insect, and disease, exhibited dynamic and variable responses to the ground fertility treatments (Figures 18 and 19). Here, weed pressures were significantly higher where the low rate of ammonium sulfate had been applied compared to the high rate of ammonium sulfate and the control. Insect pressure did not present significant differences between treatments, however, the Cheep Cheep and the high rate of ammonium sulfate had lower insect damage relative to the control. Blueberry stems where the high rate of ammonium sulfate was applied had significantly lower disease incidence when compared to the control (Figure 18). Reduced disease with higher fertilization rates suggests increased crop resilience to environmental stressors such as heat, drought, and precipitation. Leaf drop followed a similar trend to disease, decreasing with fertilization; however, the low rate of ammonium sulfate had slightly less drop than the Cheep Cheep and high rate of ammonium sulfate. Blueberry leaf drop did not present significant treatment differences.

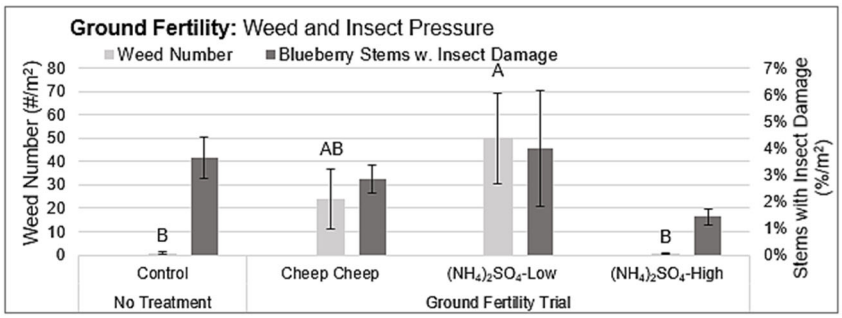

Figure 17. Weed number (#/m^2) and the number of stems with insect damage (#/m^2) measured on June 9, July 21, August 20, and September 20, 2021, for the ground fertility treatments applied in 2021. Treatment differences for stems with insect damage were nonsignificant. Letters indicate significance at the 0.05 level of significance for weed number. Error bars indicate the standard error of the mean.

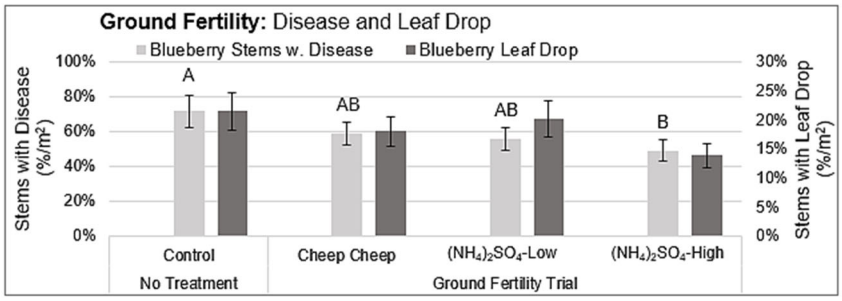

Figure 18. The number of stems with disease damage (#/m^2) measured on June 9, July 21, August 20, and September 20, 2021, and leaf drop measured on September 20, 2021, for the ground fertility treatments applied in 2021. Treatment differences for blueberry leaf drop were nonsignificant. Letters indicate significance at the 0.05 level of significance for stems with disease. Error bars indicate the standard error of the mean.

Foliar fertility results will be presented in 2022 following 2022 application in the crop year.

DISCUSSION

Soil Moisture, Blueberry Physiology, Morphology & Leaf Nutrition

Moisture Retention

Our results showed that the 1" layer of mulch was not sufficient to increase soil moisture in the year of application, yet biochar-compost treated plots did show an increased soil moisture. Previous researchers recommended applying at least a 2-3" layer of wood mulch in wild blueberry fields to conserve soil moisture (Hunt et al., 2010). Here, we show that the use of biochar-compost mix helped the soils to maintain comparatively higher soil moisture content throughout the whole season. This is because biochar particles can increase the soil water holding capacity in sandy soil (Li et al., 2021) which we have verified in laboratory testing. In our laboratory investigation, we observed that soil water holding capacity increases with the increasing biochar amount in the wild blueberry field soil rather than the compost alone. Therefore, growers with crops in sandy soils could reduce irrigation costs by using biochar materials (Kroeger et al., 2021). Also, we observed from the field soil sample testing at the end of season that the biochar-compost mix added more organic matter to the soil (Table 4) which also helps soils to retain more moisture. Agricultural soils can hold 16,500 gallons of water per acre of soil at a depth of one foot for every 1% of organic matter content (Gould, 2015). Organic matter protects the soil from drying and improves soil porosity, thus enhancing water infiltration and water storage (Bot and Benites, 2005).

Ground Fertility

Comparing all the treatments, high rate of ammonium sulfate performed the best in terms of overall observed parameters as it had higher leaf chlorophyll concentration, photosynthetic rates, and highest number of leaves and total leaf area in the plants. This could be because the higher rate of ammonium sulfate-treated plots had the highest amount of soil nitrogen (Table 4), leaf nitrogen, and phosphorus among all the treatments. Their better physiological and morphological responses might be because of the higher leaf nitrogen and phosphorus (Taiz et al., 2015; Zhang et al., 2015) which were also at the optimum level required for wild blueberries. However, due to the slow-growing nature of wild blueberry plants and the time needed for the breakdown of organic matter, this study requires more than one field season to make any conclusive recommendations for a specific treatment.

Plant Development & Productivity

Moisture Retention

The unexpected and significant reductions in blueberry cover and stem number were observed where mulch materials (hard- and softwood) were applied. This could be a direct result of the timing of mulch application, which occurred during blueberry shoot emergence. Here, a relatively thick layer of mulch (1") was applied, which may have suppressed or inhibited the growth of green tissue shoots that were just emerging. Stem height did not respond strongly to moisture retention treatments in this initial year; however, bud numbers (although nonsignificant) were relatively high where hardwood mulch had been applied.

Ground Fertility

The ground fertility treatments yielded a fast and significant response from the blueberry such that the high rate of ammonium nitrate (381 lbs/A) had significantly greater blueberry cover and taller stems than the control and the low rate of ammonium sulfate. The Cheep Cheep treatment had significantly greater blueberry cover than the control but no difference in stem height. Taller stems are not always desired by growers if winterkill is a problem in their location. Sometimes too much vegetation can make harvest difficult, making a low rate of Cheep Cheep a possible contender for commercial use. Chicken manure (Cheep Cheep) is a slow-release organic product and its effects may not yet be fully visible while ammonium sulfate is a quick-release conventional product. Conclusions can be made after foliar nutrients and soil pH are compared from before (2021) and after material applications have had time to react (2023).

Pest Pressure

Moisture Retention

Pest pressures in response to moisture retention treatments were nonsignificant, however, there are interesting trends worth noting. Weed presence was higher in all moisture retention treatments relative to the control, suggesting the weeds are benefiting from the amendments, especially the biochar-compost treatment which had higher and more diverse nutrient concentrations. Insect presence was higher where softwood mulch and biochar-compost had been applied. Leaf nutrient analysis data is forthcoming. Disease presence was slightly higher where the hardwood mulch had been applied relative to all other treatments and the control. Interestingly, leaf drop was lower across all treatments by as much as 9% to 19% relative to the control suggesting that the moisture retention treatments reduced plant stress and leaf spot disease, extending the potential development period prior to dormancy.

Ground Fertility

Under the ground fertility treatments, weed concentrations were significantly higher with the low rate of ammonium sulfate relative to the high rate of ammonium sulfate and the control. Here, the significant differences in the two rates of ammonium sulfate could have a few different root causes: the nutrients in the low rate of ammonium sulfate likely fed the weeds while the sulfur component of the ammonium sulfate may have been slow-acting or too low to discourage weed growth. The low weed presence exhibited with the high rate of ammonium sulfate may be a result of too much nitrogen burning the weeds, or the corresponding higher rate of sulfur may have discouraged weed growth.

Blueberry stems with insect pressure did not present significant treatment differences, although insect presence was lowest with the high rate of ammonium sulfate. Disease pressure in the ground fertility treatments was also lowest with the high rate of ammonium sulfate being significantly lower than the control. This suggests that the high rate of ammonium sulfate supplemented the plants' nutrient needs such that environmental stress was reduced, and natural pest resilience occurred relative to insects and disease.

Costs

Material costs play a large role in grower consideration and use. While the blueberry that received the high rate of ammonium sulfate performed well, the cost of application is $2.20 per acre, which is over $800 per acre for the higher application rate (80lbs N per acre). Blueberry cover and stem height were higher with the low rate of application of Cheep Cheep (45lbs N per acre) relative to the control and slightly higher than the low rate of ammonium sulfate. The low rate of Cheep Cheep is considerably less than the ammonium sulfate in cost. Long-term efficacy of these products and rates will need to be observed prior to making recommendations related to effectiveness and cost.

Table 5. Material costs relative to the rates tested in this trial. Prices may vary based on quantity purchased, grower size, retailer, and year. Prices do not include labor.

Trial	Treatment	Target	Rate/A	Cost/Unit	Cost/A
Moisture Retention	Softwood Woodchips	1.0" thickness	134.0 yd^3	$18.00 yd^3	$2,420
	Hardwood Woodchips	1.0" thickness	134.0 yd^3	$25.00 yd^3	$3,361
	Biochar + Compost*		7.5 yd^3	-	-
Ground Fertility	Cheep Cheep	45 lbs N/A	700.0 lbs	$21.00 per 50 lbs	$294
	Ammonium Sulfate	45 lbs N/A, 51 lbs S/A	214.0 lbs	$110.00 per 50lbs	$470
		80 lbs N/A, 91 lbs S/A	381.0 lbs		$838
Foliar Fertility	Poma (calcium)	N/A	1 gal/A	$18.00 per Gal	$18
	Poma (calcium)	N/A	2 gal/A		$36

**Biochar mixed with compost is not yet commercially available; a price could not be obtained for this cost analysis.*

CURRENT RECOMMENDATIONS

None at this time.

NEXT STEPS

- Apply 2022 foliar fertilizer products.
- The 2022 season is the crop year and therefore flower count, blueberry fruit yield (weight) and fruit quality (Brix sugar content) will be measured
- Soil moisture, plant physiological performance, and growth will be monitored in 2022, a crop year.
- Soil and leaves will be resampled for testing soil health, leaf nutrients, and sap nutrients in 2022, a crop year.

ACKNOWLEDGEMENTS

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RESEARCH

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9. Effects of Organic Soil Amendments on Physiology and Pest Pressure

OBJECTIVES

Nutrient Management:

- Evaluate approved organic amendments applied to wild blueberry soil and leaves at different times and rates on three organic wild blueberry farms in Maine.
- Quantify the effects of different organic amendments on wild blueberry physiology and morphology.

Pest Management:

- Evaluate disease, insect, and weed severity under organic amendments

LOCATIONS: Appleton, Surry, and Columbia Falls, ME
PROJECT TIMEFRAME: May 2019 – September 2022

INTRODUCTION

This study is a continuation of the project discussed in the 2020 season report, page 98, "Effects of Organic Soil Amendments on Physiology and Pest Pressure".

More organic soil amendments for improving plant health and yield in organic wild blueberry fields are needed. This study continues research into the rates, timing of applications, cost, physiological benefits and potential impacts on pest pressure with such additions. Weeds challenge all blueberry growers, but particularly organic growers who do not have effective herbicides to control weeds and know that adding fertilizers to the crop can "feed the weeds". Weed presence is one of the factors impacting nutrient uptake in wild blueberry, alongside soil pH, water availability, and nutrient availability (Drummond et al., 2009). Observationally, organic wild blueberry fields have shorter stems, less dense blueberry cover, and overall what appear to be "stressed" plants in comparison to conventional fields.

Organic soil amendments in other crops have improved crop resilience and production by increasing soil moisture retention, soil water holding capacity and increased nutrient availability (Barber, 1995; Jungk, 1996; Williams et al., 2016), depending on the material

applied. Improved crop resiliency is needed as Maine shifts towards a warmer climate with more sporadic weather events (Maine Climate Office, 2020). This study in its third year aims to evaluate the efficacy of four organic soil amendments and one foliar spray treatment and their impact on wild blueberry growth and corresponding pest presence.

METHODS

This project is replicated at three farm locations selected to represent three farm sizes (small, medium, large) and the three major Maine wild blueberry growing regions (Midcoast, Ellsworth, and Downeast). The experimental design per location is a randomized complete block replicated six times with nine treatments applied to 6' by 30' plots (Table 1). Soil was sampled at each location in 2019 and will be resampled in 2022. The foliar fertilizer and Cheep Cheep (chicken manure) were applied at the recommended time and rate according to the label's and company representative's instructions. The Coast of Maine Cobscook blend, mulch, and compost were applied according to recommendations from University of Maine Extension Educator Mark Hutchinson (personal communication, 2019). All products were applied one time except for foliar fertilizer which was applied three times as recommended by the manufacturer.

All products were applied during the 2019 prune-cycle except for one foliar fertilizer treatment applied in the 2020 crop year (Table 1). The foliar fertilizer (SeaCrop16) was applied three times per site at key growth stages throughout the season. Cheep Cheep was applied in Surry and Appleton the week of June 3 and in Columbia Falls the week of June 12. The Coast of Maine Cobscook blend was applied in Appleton the week of June 17, and in Columbia Falls and Surry the week of June 24. University compost was applied in Appleton (only) the week of June 17 and mulch was applied in Columbia Falls and Surry the week of July 22.

Table 1. Products tested at each of three organic farms in a randomized complete block design with six replicates.

Product	Location	Material	Rate	Rate Type	Crop Cycle	%N-P-K*
Control	ALL	None	N/A	N/A	N/A	N/A
NorthAmericanKelpCo. SeaCrop16 Foliar Fertilizer	ALL	Liquid Foliar Spray	1.2 L/242 gal. H_2O/A	N/A	Prune	0.18% N 6.37% P 4.89% K
				N/A	Crop	
NorthCountryOrganics Cheep Cheep 4-3-3	ALL	Granular Soil Applied	1089 lbs./A	Low	Prune	4% N 3% P 3% K
			2178 lbs. /A	High	Prune	
CoastofMaineCobscook Blend Garden Soil	ALL	Loose material Soil Applied	7.5 yd^3/A	Low	Prune	0.4% N 0.14% P 0.12% K
			15 yd^3/A	High	Prune	
MarkWrightDisposal Dark Brown Mulch	Columbia Falls & Surry	Loose material Soil Applied	7.5 yd^3/A	Low	Prune	N/A
			15 yd^3/A	High	Prune	
UniversityofMaine Compost	Appleton Only	Loose material Soil Applied	7.5 yd^3/A	Low	Prune	0.41% N 0.11% P 0.10% K
			15 yd^3/A	High	Prune	

*N-P-K represented as total nitrogen, phosphorus as P_2O_5, and potassium as K_2O

Data Collection

Soil Moisture

Soil temperature (°C), volumetric water content (%), and electrical conductivity were recorded using a FieldScout TDR 150 soil moisture meter (FieldScout TDR 150, Spectrum Technologies Inc., Aurora, IL, USA) probe inserted 12 cm (4.8 inches) into the blueberry root zone soil. Six random readings were recorded per plot on 21-25 May, 21-23 June, 15-16 July, and 18-19 August in the prune year 2021.

Physiology and Morphology

At each site, eight stems from each plot were randomly selected to measure their heights, chlorophyll and anthocyanin concentrations from June to August. Chlorophyll concentration was measured by a CCM-200 plus chlorophyll content meter (Opti-Sciences, Inc., Hudson, NH, USA). Photosynthetic electron transport rates were measured in leaves from six stems in each plot by a Y(II) meter (Opti-Sciences, Inc., Hudson, NH, USA) on 28-31 July between 10:00 and 14:00 h solar time. In August, eight random stems from each treatment plot were collected to quantify the number of leaves on each stem, leaf size, dry biomass, and nutrients. Leaf area of three leaves at three different positions (top, middle, and bottom) from each of those stems was determined using LI-3000A area meter (Li-Cor, Lincoln, NE, USA). All the leaves from those eight stems were oven-dried at 70°C to constant mass and weighed, then were ground and sent to the University of Maine Soil and Plant Tissue Testing Laboratory in Orono, Maine for nutrition analysis.

Pest Pressure

Insects, weeds, and disease were monitored in the same 0.37 m^2 quadrats (twice per plot) throughout each field season. In the 2019 prune year, pest scouting occurred once each in July, August, and September at each of the three locations. In the 2020 crop year, pest scouting occurred once each in May, June, and July at each of the three locations. In 2021, pest scouting occurred once each in June, August, and September at each of the three locations. Pest severity for weeds, insects, and disease were quantified as percent cover using equal interval ranks between 0 and 6, where: 0 = not present, 1 = ≤1%-17%, 2 = 17%-33%, 3 = 33%-50%, 4 = 50%-67%, 5 = 67%-83% and 6 = 83%-100%. In 2019, weeds were classified into two categories (grass or broadleaf) and in 2020 and 2021, weeds were identified by genera and counted to obtain weed number per quadrat. In 2020 and 2021, the number of wild blueberry stems with insect or disease damage were also identified and counted in addition to ranking severity using the same equal interval ranks.

Crop Productivity

Blueberry cover was quantified at the same time as each pest scouting by using the same 0-6 equal interval ranking. In the 2019 prune year, stem heights and the number of buds per stem were recorded for eight random stems per plot at all locations late August to early September; this was done again in the 2021 prune year, with measurements recorded in late September. In the 2020 crop year, fruit-set and fruit-drop were monitored with repeated measures on the same four stems per plot.

The 2020 harvest took place on August 3, 6, and 11 in Appleton, Surry and Columbia Falls, respectively, and yield weights were recorded and Brix measures and 100 berry count conducted to ascertain berry quality. The second harvest of these plots will occur in 2022.

Data Analysis

The effects of the applied organic treatments on soil moisture, physiology (chlorophyll concentration and photosynthetic electron transport rate), and morphology (total leaf surface area per stem) of wild blueberry plants were statistically compared using a general linear model followed by LSD (least significant difference) post-hoc test in SPSS software ($\alpha = 0.05$). In this model, the main effects of applied treatments were considered as a fixed factor, experimental blocks as a random factor, and a

Bonferroni correction was also applied for confidence interval adjustment. Each site (Appleton, Surry, and Columbia Falls) was analyzed individually for the measurements taken in the prune year 2021.

Due to the nature of count data collected in the field (which often has a high number of zeros creating a skewed distribution) much of our data failed the assumptions of normality and equal variance often required to run parametric statistical tests. All data was transformed with a square root transformation prior to any statistical testing. Ranked data and pest count data visually improved following transformation, but the data continued to statistically fail for normality. Statistical tests were carried out despite non-normality after establishing there were no serious problems with the data.

Ranked blueberry cover and pest cover data were transformed to their corresponding percent mid-point. Ranked blueberry cover, pest cover and pest counts (#/m^2) were compared across all years (2019-2021) using a full-factorial repeated-measures mixed model design in JMP (JMP®, Version 15.2). Here, the full factorial tested the effects of date, treatment, and any interaction between date and treatment for the ranked response variables.

RESULTS

Effects of Organic Amendments on Soil Moisture

Overall, no significant differences were found in seasonal average soil moisture among the treatments in any of the studied locations (Fig. 1). However, on average in the Appleton field (Fig. 1a), all the treated plots except the high rate of the Coast of Maine Cobscook blend treatment showed a trend towards higher soil moisture compared to the control plot. In contrast, in the Surry field (Fig. 1b), only the low rate of mulch-treated plots showed comparatively higher soil moisture on average than control plots whereas other treated plots had similar soil moisture as the control. In Columbia Falls field (Fig. 1c), all treatments showed comparatively higher soil moisture content than the control where the highest soil moisture was found in the high rate of mulch followed by Cheep Cheep-treated plots.

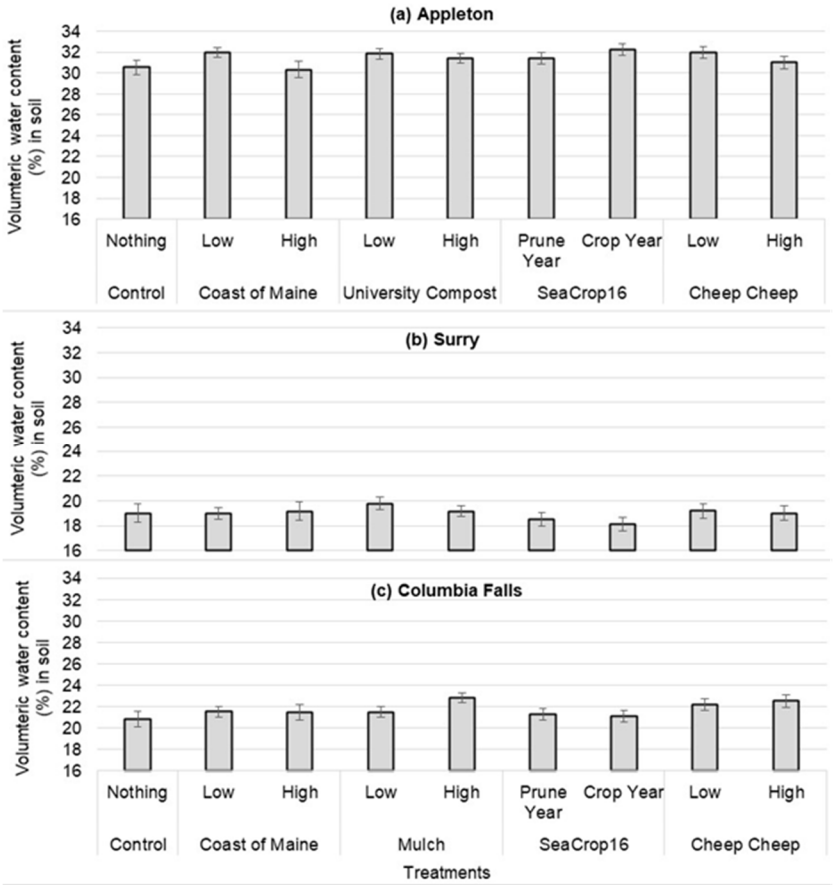

Figure 1. Comparison in mean soil moisture measured in May to August in the prune year 2021 among different treatments applied in the organic wild blueberry fields at: (a) Appleton, (b) Surry, and (c) Columbia Falls. Error bars indicate the standard error of the mean. No significant differences were observed in soil moisture at any of the studied fields at the significance level of $P < 0.05$

<u>Effects of Organic Amendments on Wild Blueberry Physiology</u>

Overall, no significant differences were found in peak leaf chlorophyll concentration (average of the replicated plots) among the treatments applied in any of the studied locations (Fig. 2). However, in the Appleton field (Fig. 2a), the average leaf chlorophyll concentration of wild blueberry plants was the highest in the low rates of Cheep Cheep and the Coast of Maine Cobscook blend treatments among all treatments, but this was not significant. In contrast, in the Surry (Fig. 2b) and Columbia Falls (Fig. 2c) fields, average leaf chlorophyll concentration was the highest in the low rate of Cheep Cheep, but not significantly different from other treatments.

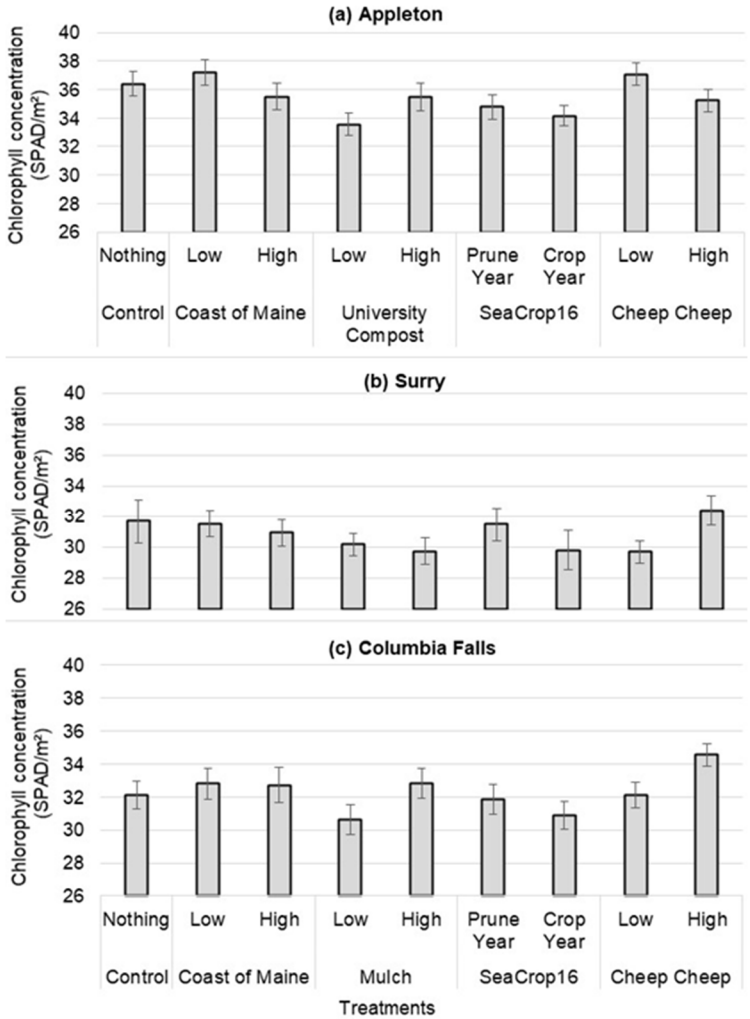

Figure 2. Comparison in chlorophyll concentration of wild blueberry leaves at the end of July in the prune year 2021 among different treatments applied in the organic wild blueberry fields at: (a) Appleton, (b) Surry, and (c) Columbia Falls. Error bars indicate the standard error of the mean (averaged over replicated plots). No significant differences were observed in leaf chlorophyll concentration at any of the studied fields at the significance level of $p < 0.05$.

Overall, no significant differences were found in leaf photosynthetic electron transport rates (averaged over the replicated plots) among the treatments applied in any of the studied locations (Fig. 3). However, in the Appleton field (Fig. 3a), average leaf electron transport rate was higher in the SeaCrop16, Cheep Cheep, and the Coast of Maine Cobscook blend treatments compared to the control. In the Surry field (Fig. 3b), average leaf electron transport rate was higher only in the low rate of Cobscook blend and high rate of mulch treatments compared to the control. On the contrary, in Columbia Falls (Fig. 3c), the average leaf electron transport rate was higher in the Cobscook blend and low rate of mulch treatments compared to the control, among all treatments.

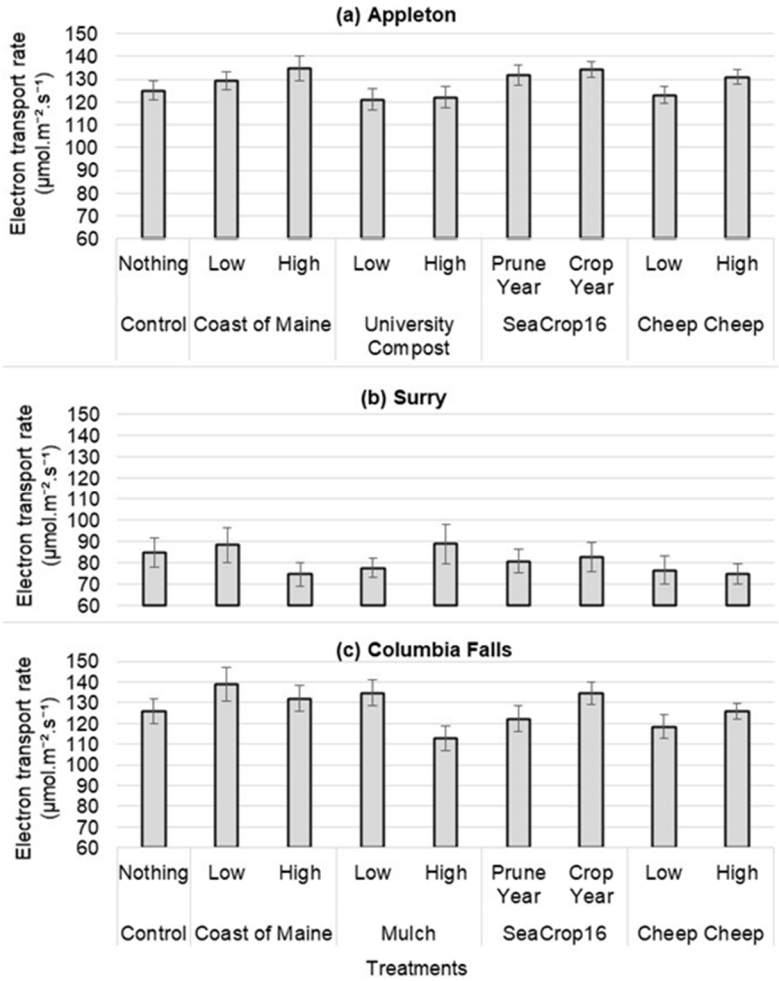

Figure 3. Comparison of photosynthetic electron transport rates of wild blueberry leaves in peak summer (July) of the prune year 2021 among different treatments applied in the organic wild blueberry fields at: (a) Appleton, (b) Surry, and (c) Columbia Falls. Error bars indicate the standard error of the mean (averaged over replicated plots). No significant differences were observed in leaf electron transport rate at any of the studied fields at the significance level of $p < 0.05$.

<u>Effects of Organic Amendments on Wild Blueberry Morphology</u>

Overall, no significant differences were found in leaf surface area per stem (when averaged by plot) among the applied treatments in any of the organic wild blueberry fields (Fig. 4). Although not significant, the average leaf area per stem in both the Appleton (Fig. 4a) and Surry (Fig. 4b) fields, the low rate of Cheep Cheep treated plots had comparatively higher total leaf area per stem than the control. On the contrary, in the Columbia Falls field (Fig. 4c), all treatments except the Cheep Cheep-treated plots had a higher total leaf area per stem on average compared to the control (not significant).

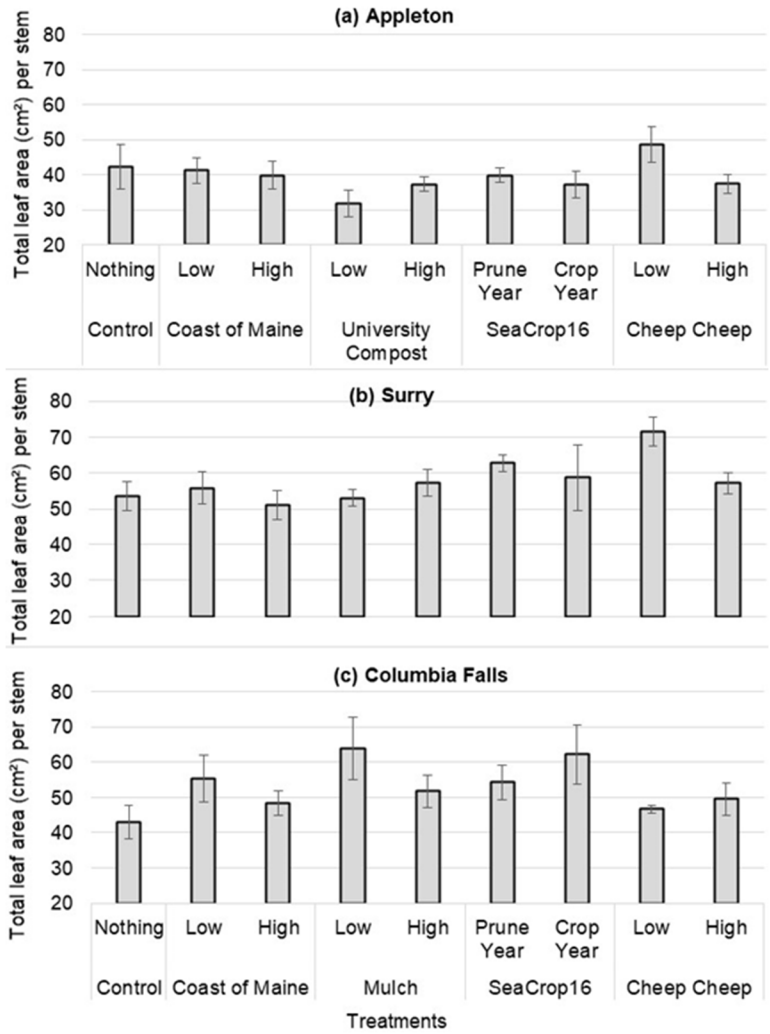

Figure 4. Comparison in total leaf area per stem of wild blueberry plants in August 2021 among different treatments applied in the organic wild blueberry fields at: (a) Appleton, (b) Surry, and (c) Columbia Falls. Error bars indicate the standard error of the mean (averaged over replicated plots). No significant differences were observed in leaf electron transport rate at any of the studied fields at the significance level of $p < 0.05$.

<u>Effect of Organic Amendments on Blueberry Cover and Pest Incidence, 2019-2021</u>

Blueberry cover responded well to all organic amendments with 2% to 4% greater coverage than the control, when observing all three locations over three years (Figure 5). While the blueberry exhibited a positive response to all amendments, only the SeaCrop16 foliar spray applied in the prune year (2019) exhibited significantly greater blueberry cover relative to the control. Other strong responses, although not significant, include SeaCrop16 applied in the crop year, Cheep Cheep, and mulch.

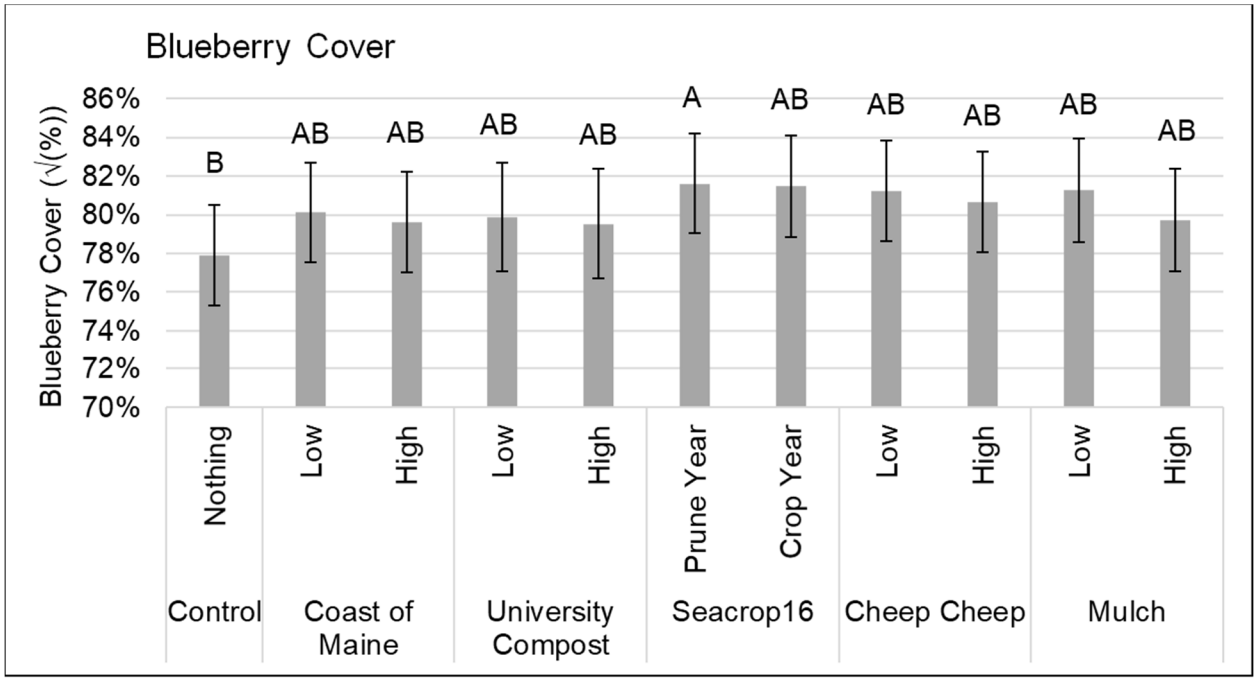

Figure 5. Blueberry cover measured across all three locations (Appleton, Surry and Columbia Falls) over three years (2019-2021) under organic amendment treatments. The least squares mean estimates are presented following a square root transformation. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean.

Weed cover observed across all three locations over the three-year period was significantly higher where Cheep Cheep was applied at the higher rate (Figure 6). Here, the high rate of Cheep Cheep had on average 6% more weeds than the control and 19% more weeds than the treatment with the lowest weed pressure, University Compost. The treatments that exhibited fewer weeds were the University Compost (both rates), the SeaCrop16 applied in the crop year, and the high rate of mulch. All four treatments listed had significantly less weed coverage than the control by as much as 7% to 13%. Weed number (#/m^2) showed similar results to that of the weed cover above where Cheep Cheep had significantly more weeds per area compared to the control (Figure 7). Looking at weed number, only SeaCrop16 applied in the crop year had significantly fewer weeds than the control.

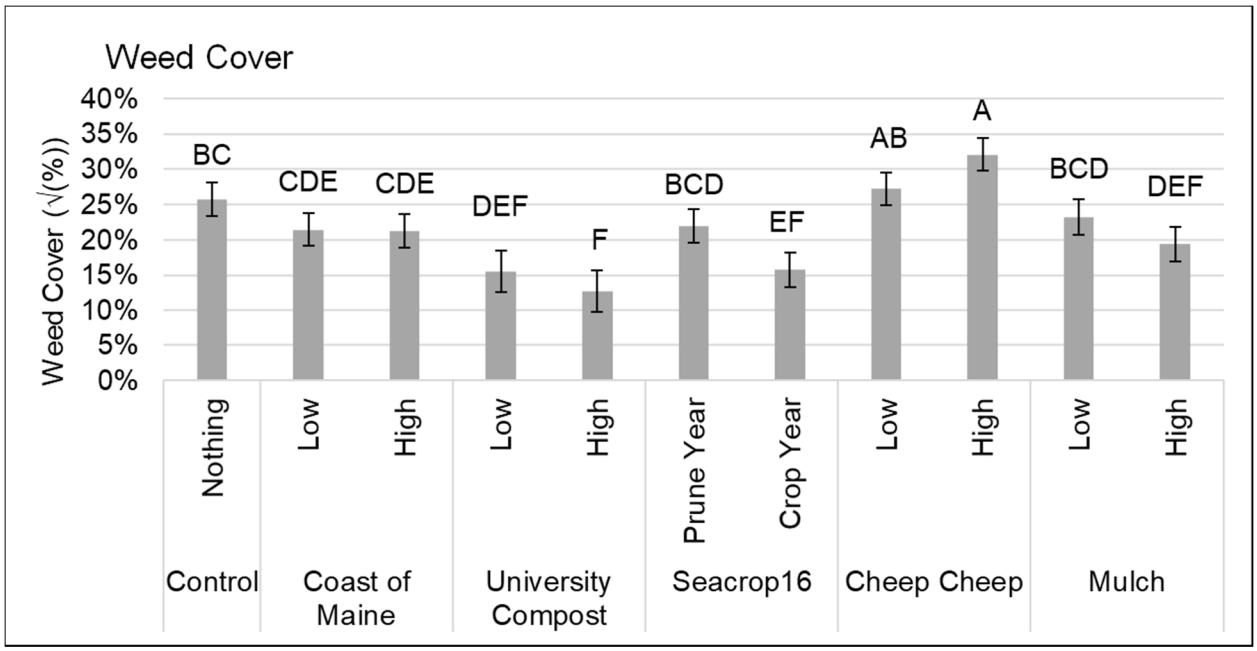

Figure 6. Weed cover representing the area of weed pressure measured across all three locations (Appleton, Surry and Columbia Falls) over three years (2019-2021) under organic amendment treatments. The least squares mean estimates are presented following a square root transformation. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean.

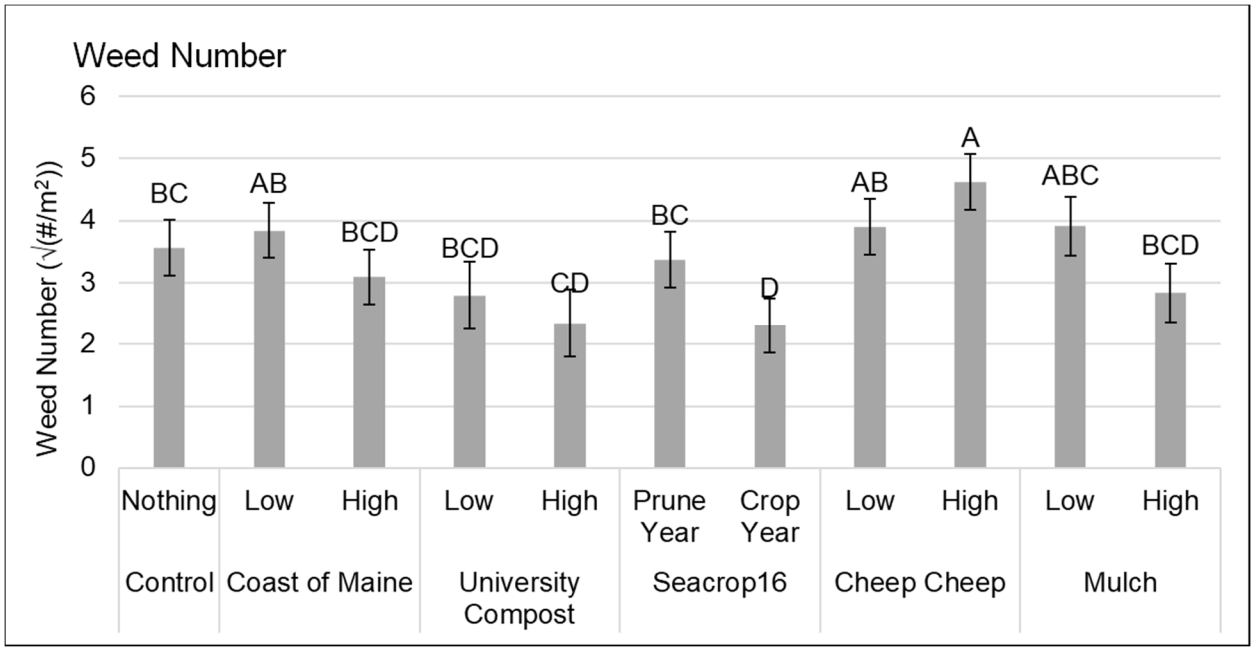

Figure 7. Weed number (#/m^2) measured across all three locations (Appleton, Surry and Columbia Falls) over two years (2020-2021) under organic amendment treatments. The least squares mean estimates are presented following a square root transformation. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean.

When evaluating the weed population by the general categories of grass and broadleaf, there was a consistent trend with higher frequencies of grass species relative to broadleaf species (Table 2). The control, Coast of Maine Cobscook blend, and University compost-treated plots showed an expected trend where greater weed frequency were present in the prune years (2019 and 2021) and reduced weed frequency was observed in the crop year (2021), due to greater blueberry leaf coverage

in the crop year. The Cheep Cheep- and mulch-treated plots, however, showed a steady increase across all three years with an increase in weed frequency in the crop year despite competition from the blueberry. SeaCrop16 did not follow either trend. Across treatments and all years grass species consisted primarily of: poverty oat grass, witchgrass, barnyard grass, rushes, sedges, and other unidentified grasses. Broadleaf species' frequency varied by year. Top broadleaf species in 2020 consisted of: cow wheat, Canada mayflower, aronia, bracken fern, and red sorrel. In 2021 top broadleaf species were primarily: red sorrel, aronia, toadflax, wintergreen, and dogbane. Weeds were not identified to genera in 2019.

Table 2. Broadleaf and grass frequency by treatment measured across all three trial locations and three years (2019-2021). Frequency is the occurrence of each weed type relative to the total number of samples, including samples where no weeds were present.

		2019 Prune year		**2020 Crop year**		**2021 Prune year**	
		Broadleaf	**Grass**	**Broadleaf**	**Grass**	**Broadleaf**	**Grass**
		---------- % ----------		---------- % ----------		---------- % ----------	
Control	Nothing	6%	16%	2%	17%	15%	21%
CoastofMaine Cobscook Blend	Low	13%	21%	4%	15%	13%	22%
	High	10%	21%	5%	17%	11%	19%
University Compost	Low	9%	14%	4%	8%	7%	14%
	High	5%	10%	0%	6%	5%	15%
SeaCrop16	Prune	8%	15%	8%	15%	12%	23%
	Crop	-	-	3%	10%	7%	17%
Cheep Cheep	Low	11%	15%	7%	19%	11%	20%
	High	6%	14%	9%	20%	14%	22%
Mulch	Low	4%	15%	8%	15%	15%	20%
	High	2%	9%	7%	13%	14%	20%

Insect coverage, a spatial measure of insect pressure generally indicated by pest damage to leaves or observation of the actual culprit, was significantly higher in the Cheep Cheep plots (both rates) and in the mulch (high rate), relative to the control (Figure 8). Here, the mulch (high rate) amendment had the greatest insect pressure, 6% greater than the control; followed by the Cheep Cheep high rate and low rate, 5% and 4% greater than the control, respectively. The University compost (both rates) presented relatively low insect pressure, although, no treatments were significantly less than the control (Figure 9). Insect numbers, however, did show the highest concentrations in the mulch treatments at both rates relative to all other treatments and the control. The top three insects across all three years (2019 to 2021) were tip midge (54% frequency), red striped fireworm (26% frequency) and flea beetle (22% frequency).

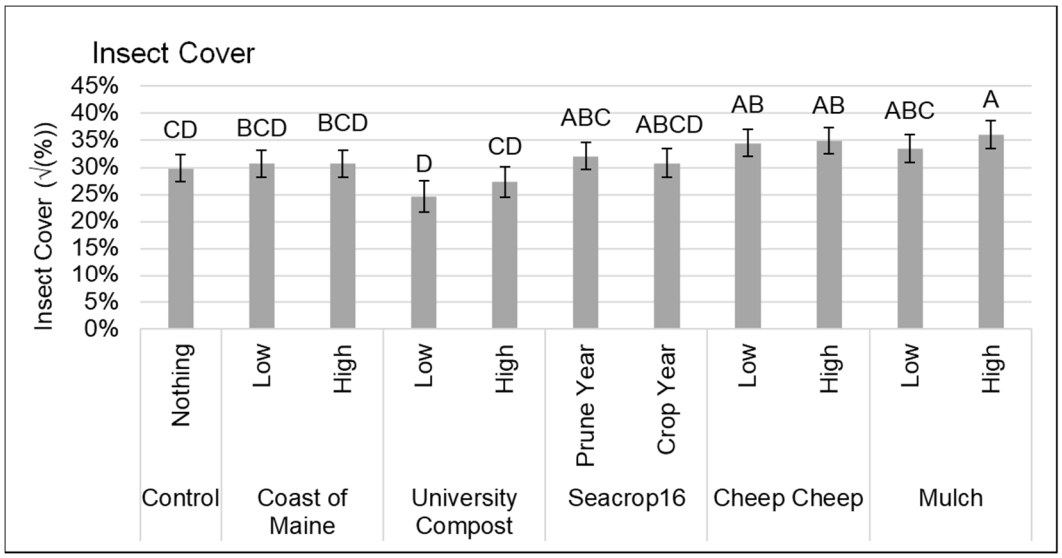

Figure 8. Insect cover representing the area of insect pressure measured across all three locations (Appleton, Surry and Columbia Falls) over three years (2019-2021) under organic amendment treatments. The least squares mean estimates are presented following a square root transformation. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean.

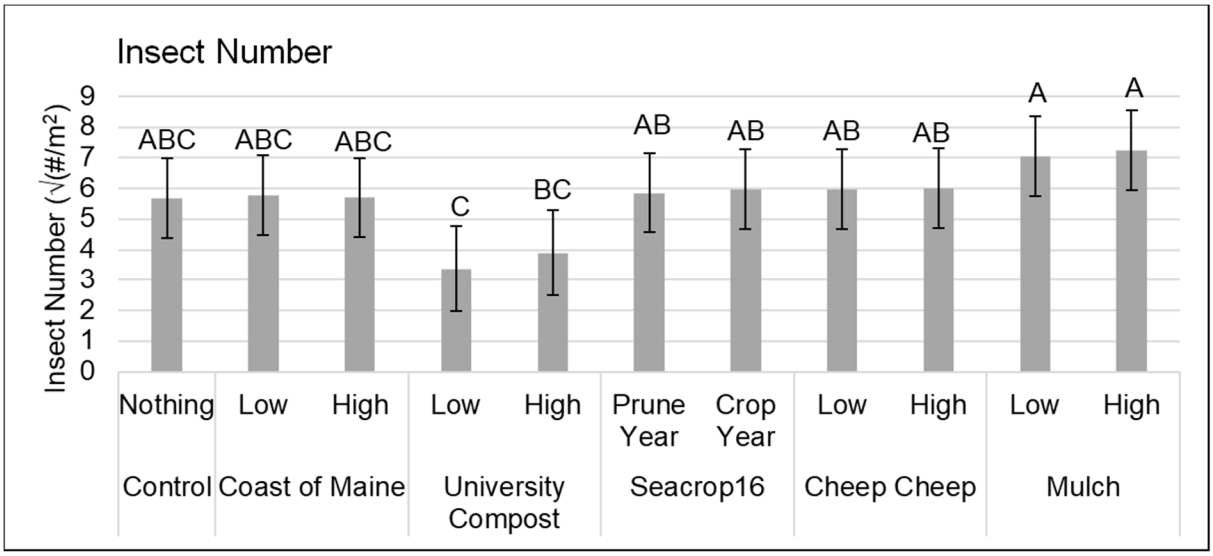

Figure 9. Insect number (#/m^2) measured across all three locations (Appleton, Surry and Columbia Falls) over two years (2020-2021) under organic amendment treatments. The least squares mean estimates are presented following a square root transformation. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean.

Disease coverage, including leaf spot species, mummy berry, and phomopsis, as indicated by a spatial measure of disease pressure, was relatively similar across all treatments with the exception of Cheep Cheep (high rate), which exhibited 5% less disease than the control (Figure 10). The number of stems with disease showed fewer trends than the ranked coverage, however it is worth noting that the SeaCrop16 applied in the crop year was the only treatment with significantly greater disease (number) with this measure (Figure 11). High disease with SeaCrop16 applied in the 2020 crop year may be a function of applying foliar fertilizer in peak drought conditions experienced in 2020. Reduced disease with the higher application of Cheep Cheep suggests increased crop resilience with added nutrients.

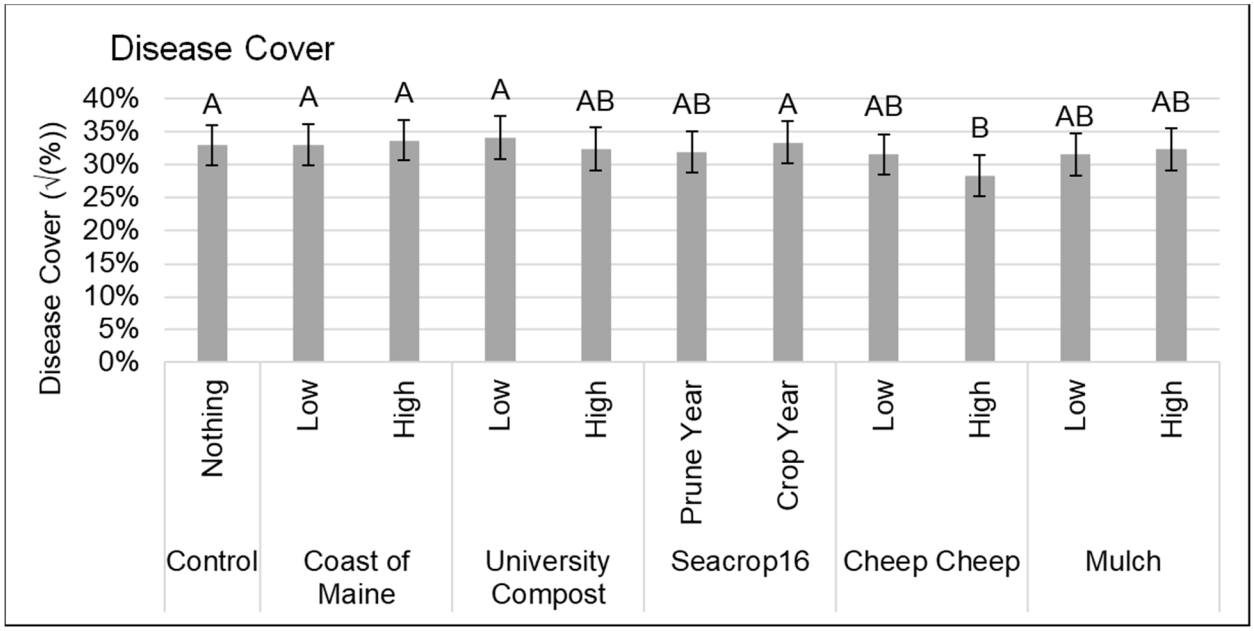

Figure 10. Disease cover representing the area of disease pressure measured across all three locations (Appleton, Surry and Columbia Falls) over three years (2019-2021) under organic amendment treatments. The least squares mean estimates are presented following a square root transformation. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean.

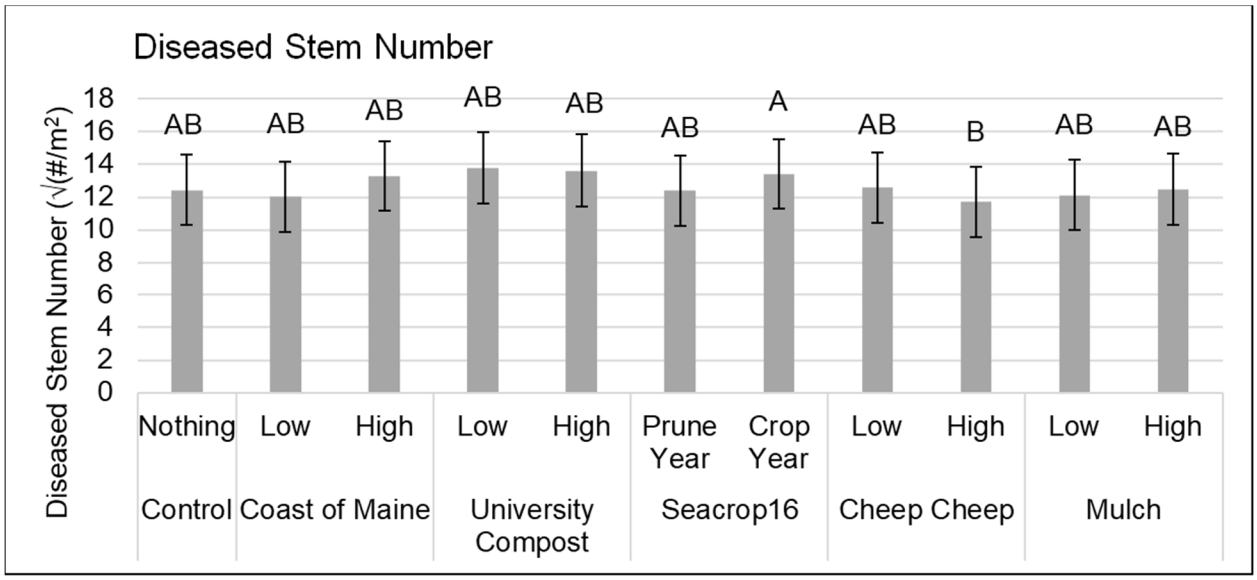

Figure 11. Diseased stem number (#/m^2) measured across all three locations (Appleton, Surry and Columbia Falls) over two years (2020-2021) under organic amendment treatments. The least squares mean estimates are presented following a square root transformation. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean.

DISCUSSION

From the three years of data collected so far from this four year study, some soil amendments may be able to improve the condition of organic wild blueberry plants. In the third year, treatment effects seemed to decline in wild blueberry physiology and morphology since no significant differences in those parameters were found among the treatments from the second year. This may mean that treatments should be applied every few years to achieve consistent improvements in physiological

and morphological performance, and thus yield. This observation requires another year's observation and data collection to support or refute it.

However, blueberry cover and pest data has begun to show patterns in the third year indicating that plant cover and pest response to soil amendments is delayed until the next cycle. In the first three years of this trial, blueberry cover was significantly higher in all treatments excepting Coast of Maine (high rate), mulch (high rate), and University Compost (both rates); the greatest blueberry cover was observed after the application of Cheep Cheep, resulting in visibly taller, fuller, and greener plants. Cheep Cheep-treated plots also had the highest weed cover (predominantly grasses) and there was a significant correlation between weed pressure and reduced yield in the Cheep Cheep (high rate) plots. Cheep Cheep's high macro- and micro-nutrient concentration (N-P-K is 4-3-3 & Fe, Cu, S, Ca, Mg, Zn, Mn are present) benefits weeds as much as wild blueberry. The 2021 – 2022 Fertility Trial ("Using Foliar Fertilizers and Soil Amendments to Improve Wild Blueberry Production and Resilience to Warming", page 150 of this report) evaluated the impact on weed growth of a lower Cheep Cheep application rate.

Applications of Cheep Cheep appear to have increased the soils' water-holding capacity as well (albeit not significantly), since this fertilizer added organic matter to the soils (Gould, 2015; Bot & Benites, 2005). Cheep Cheep treatments resulted in higher leaf chlorophyll concentrations and greater leaf area than other treatments (though not significant) which may be due to the higher nitrogen content in chicken manure (Cheep Cheep's N-P-K is 4-3-3) than in other amendments studied; nitrogen is the single most important nutrient for building leaf chlorophyll, which improves photosynthetic performance and results in improved crop production (Taiz et al., 2015). A better understanding of the leaf nutrient concentrations will better explain the contradictory responses of photosynthetic electron transport rates when compared to applied treatments; these results will be explained in next year's report.

When including the third year of data (2021) in the repeated measures' statistical analyses, blueberry cover waned in all organic amendment treatments except for SeaCrop16 applied in the 2019 prune year. SeaCrop16 contains cytokinin, a plant growth hormone that can potentially protect plants from drought and frost damage and promote photosynthesis (Novakova et al., 2007). In 2020, wild blueberry fields experienced warmer temperatures and drought conditions making plants stressed in these fields that are not irrigated. The SeaCrop16's mode of action may have led to the high performance of the blueberry with this treatment.

Insect presence was greater where mulch had been applied when compared to all treatments, this effect was especially prominent with insect 'cover' in response to the high rate of mulch. While future research into this trend is necessary, the mulch may be improving the structural habitat for insect pests, especially over winter when insects take refuge in the duff below the plants. This 'effect' of mulch on insect pressure may also be due to site-specific tendencies, since mulch was not applied at all locations.

Disease cover across all three years was significantly lower where the high rate of Cheep Cheep had been applied relative to the control, and a number of other treatments. Here, lower disease occurrence in response to a high application of both macro- and micronutrients is likely a result of reduced plant stress.

<u>Product Costs</u>

The cost of products used plays a critical role in implementation by wild blueberry growers (Table 3). The Coast of Maine Cobscook Blend was the most expensive product, followed by North Country Organics Cheep Cheep. Both the North American Kelp SeaCrop16 foliar fertilizer and Mark Wright Disposal mulch had lower costs per unit and were also applied at lower rates compared to

the Cheep Cheep, thus resulting in overall lower costs compared to all other treatments. No cost was given for compost because it was donated by the University of Maine for this study.

Table 3. Cost of a single application of the organic amendments used in this trial. Prices may vary based on quantity purchased, grower size, retailer and year. Prices do not include labor.

Product	Rate Type	Rate Applied	Rate Unit	Cost ($/acre)	Unit Cost
Control	N/A	N/A	N/A	N/A	N/A
North American Kelp Co. SeaCrop16 Foliar Fertilizer	Prune or Crop	1.2 /242	L /gal H_2O/A	$14.70	$49/gal
*North Country Organics Cheep Cheep Cheep Cheep 4-3-3	Low	1000.0	lbs/A	$814	$0.74/lb
	High	2000.0	lbs/A	$1628	
Coast of Maine Cobscook Blend Garden Soil	Low	7.5	yd^3/A	$2025	$270/$yd^3$
	High	15.0	yd^3/A	$4050	
Mark Wright Disposal Dark Brown Mulch	Low	7.5	yd^3/A	$240	$32/$yd^3$
	High	15.0	yd^3/A	$480	
**University of Maine Compost	Low	7.5	yd^3/A	N/A	N/A
	High	15.0	yd^3/A	N/A	

**Rate applied is total amount of material per acre to achieve the target 'low' rate of 40 lbs. N/acre and the target 'high' rate of 80 lbs. N/acre.*
**Cost unknown, provided by university for study*

CURRENT RECOMMENDATIONS

Organic growers should consider applying a lower rate of chicken manure (Cheep Cheep) IF they have excellent weed management practices already in place. SeaCrop16 or other liquid kelp product can be applied during the prune year to increase blueberry cover. Compost or mulch may be used to build organic matter and suppress weeds. Importantly, all composts have slightly different nutrient and organic matter content based on the materials used. Apply compost in a small area of the field to test impacts in your field.

NEXT STEPS

- Monitor plant growth in the 2022 crop year: blueberry cover, flower and fruit development and harvest yield.
- Monitor pest pressure in the 2022 crop year: weed, insect, and disease pressure.
- Monitor soil moisture as well as leaf chlorophyll content, leaf anthocyanin content, and leaf photosynthetic electron transport rate once every month (May to August) in the 2022 crop year.
- Measure the leaf size, their dry biomass, stem height, leaf nutrients, yield at the end of the season.
- Resample leaf nutrient concentrations and soil composition in 2022.

ACKNOWLEDGEMENTS

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NEEDS ASSESSMENT

RESEARCH & EXTENSION

INVESTIGATOR(S): J. Malacarne, P. Fanning, L. Calderwood

1. State of the Maine Wild Blueberry Survey

OBJECTIVE(S)

This project is organized under the following three objectives:

1. Conduct a comprehensive survey of Maine's Wild Blueberry growers to update information on areas under cultivation, production practices, marketing channels, and perceptions of future risks and opportunities.
2. Undertake a wide-reaching survey of current and potential end users of Maine Wild Blueberries to better understand market demand, potential areas of expansion, and perceived barriers to obtaining and using Maine Wild Blueberries.
3. Integrate the information received from the grower and end user surveys into a new "Maine Wild Blueberry Dashboard" that will provide up-to-date, locally relevant, and rapidly accessible information on both the production and demand sides of the Maine Wild Blueberry Market.

LOCATION(S): Statewide
PROJECT TIMEFRAME: 2020-2021

INTRODUCTION

Supporting a robust and resilient market for Maine Wild Blueberries requires high quality, up-to-date information on the issues facing both producers and end users. This project began as the first step in an ongoing effort to improve data collection and dissemination regarding the Maine Wild Blueberry Industry. Existing data, provided primarily through the USDA National Agricultural Statistics Survey (NASS), is updated infrequently and aggregated at a level that is not conducive for its use by growers across the state. The needs, concerns, and risks that face participants in the Maine Wild Blueberry industry are dependent on an individual's location and the nature of their operation. As highlighted in this report, considerable variation in activities, management style, and market focus exists across wild blueberry operations in the state. Different operations face different challenges and rely on different data to inform their decisions. While this is true generally, it is particularly salient at the moment as many agricultural markets – wild blueberries among them – are experiencing a period of transition due to aging grower populations, increasing climate risk, supply chain disruptions, and the increased prominence of online and direct-to-consumer marketing.

In this environment, it is important for Extension and research faculty to have current data and reliable methods of quickly sharing that data with industry participants. Ideally, this effort is viewed as a partnership between researchers and industry participants, with industry participants contributing their experiences in return for researchers aggregating and sharing information that can be used by everyone as they make decisions.

METHODS AND PARTICIPATION

The survey was organized around four topic areas:

- Production (area, yields, management practices, field location)
- Marketing (marketing channels, products, prices)
- Expectations and Concerns (prices, pests, production, market access)
- Future Plans (innovation, adaptation, succession)

These topic areas were chosen to provide additional, operation-level detail to complement the aggregate data provided by USDA-NASS, as well to update the stylized facts reported in Rose et al (2013) pertaining to grower management style, market channels, and plans for the future.

Information was collected via web survey using the *SurveyCTO* mobile data collection platform. A list of industry participants was then compiled using the UMaine Cooperative Extension database. Additional industry participants were identified via a web search. Postcards were then sent to the updated contact list. The postcards contained a link to the web survey and a personalized code. This strategy was intended to provide respondents with the flexibility to answer the survey at a time of their own convenience, as well as to remove the need for paper copies to be mailed back to the researcher for manual data entry.

A few weeks after the initial mailing, email reminders were sent to those contacts in the list for which email addresses were available. Undergraduate and graduate student researchers in the School of Economics then made two rounds of phone calls to respondents that had not yet completed the survey. In these phone calls, the researchers offered assistance with the web form or the option to complete the survey over the phone.

In total, 571 individuals – including growers, processors, landowners, agricultural service providers, wild blueberry researchers, and other parties with an interest in the wild blueberry industry – were invited to take the survey. Around fifteen percent (14.5%) of invitations resulted in completed surveys and nine percent (8.8%) of respondents declined participation. The remaining invitations and phone calls (76.7%) were never answered.

Table 1. Survey participation

	NUMBER	PERCENTAGE
COMPLETED	83	14.5 %
REJECTED	50	8.8 %
NO RESPONSE	438	76.7 %
TOTAL	571	100%

While not an exceptionally low response rate for a phone/internet survey, fifteen percent was well below the hoped-for response rate for a number of reasons. First, the goal of the survey was to take a snapshot of practices and perceptions in Maine's Wild Blueberry industry as a whole. Second, the web-first format was chosen for the ease with which it could be replicated year-after-year. The low level of response makes it unlikely that the data fully reflect the state of the industry and also suggests that the web-first approach might not be the solution to maintaining up-to-date information on Maine's wild blueberry growers.

Nevertheless, the information collected provides a useful look at various aspects of the industry. The distribution of responses across counties closely reflects the geographic distribution of the initial contact list, further increasing its usability. As the initial contact list does not contain information on contact roles or operation characteristics, it is not possible to speak to the extent to which the sample is representative of the industry in these dimensions.

Table 2. Respondent roles in the industry

ROLE	NUMBER	% OF RESPONDENTS
GROWERS	48	57%
PROCESSOR	14	17%
LANDOWNER	38	46%
SERVICE PROVIDER	12	14%

(RESPONDENTS COULD SELECT MULTIPLE ROLES)

As intended, the survey collected responses from individuals occupying various roles in the wild blueberry industry. Table 2 summarizes respondents' own description of their roles in the industry. Respondents could select multiple roles. The roles selected affected the question the respondent was presented with later in the survey. The majority (57%) of respondents include “wild blueberry grower” in their industry role. Additional roles represented included landowner, wild blueberry processors, and a variety of agricultural service providers. Each respondent was only asked questions relevant to their self-identified roles.

Table 3. Location of grower respondents (% of respondents)

Washington	46%	**Oxford**	2%
Hancock	23%	**Penobscot**	2%
Lincoln	17%	**Piscataquis**	2%
Knox	10%	**Androscoggin**	-
Kennebec	6%	**Aroostook**	-
Waldo	6%	**Sagadahoc**	-
Cumberland	4%	**Somerset**	-
Franklin	2%	**York**	-

While the number of growers responding to the survey was relatively small, the area of land managed for wild blueberries by respondents was significant. Respondents report managing just under 14,000 acres of land in a typical year and harvesting just over half that acreage. To put this in perspective, USDA-NASS reports that between 2018-2020, an average of 20,000 acres of wild blueberries were harvested in Maine (20,700 acres in 2020). The respondents in this year's survey, then, reflect thirty-five percent (35%) of the wild blueberry acreage harvested in the State.

In what follows, we will call attention to the heterogeneous nature of wild blueberry operations. The area managed for wild blueberry production by respondents is a good place to begin this discussion. While the median grower respondent reports managing 32 acres, the mean of acres managed is 292, with the full range of areas managed spanning from fewer than five acres to over 5,000 acres. Throughout this report, we will highlight the different insight gained by weighting survey questions by the number of respondents and by area managed. The first provides insight into the actions and concerns of individual operations. The second gives a voice to each acre of wild blueberries managed.

Both perspectives are important for understanding the activities and concerns of the industry. To illustrate, Table 3 reports the location of grower respondents across Maine counties. If we were to reweight Table 3 by area-managed, however, it would further highlight the outsized influence of Washington county, which accounts for 66% of the acres managed among grower respondents. Note that the purpose of such a weighting is not to suggest that the concerns of an individual respondent in Washington county should count any more than those of an individual respondent elsewhere, only that drought in Washington county will affect more acres of blueberries than drought in another part of the State.

The heterogeneity observed in area managed also extends to the role of wild blueberries in respondents' income-generating portfolio. Figure 1 depicts the contribution of wild blueberries to household income among grower respondents. While the median respondent earns 20% of their income from the sale of wild blueberries, a significant set of respondents earns little income from blueberries while another set of respondents earns all or most of its income from blueberries. In fact, only 30% of grower respondents report growing blueberries as a full-time occupation and 57% of grower respondents report being paid for off-farm work in the past year.

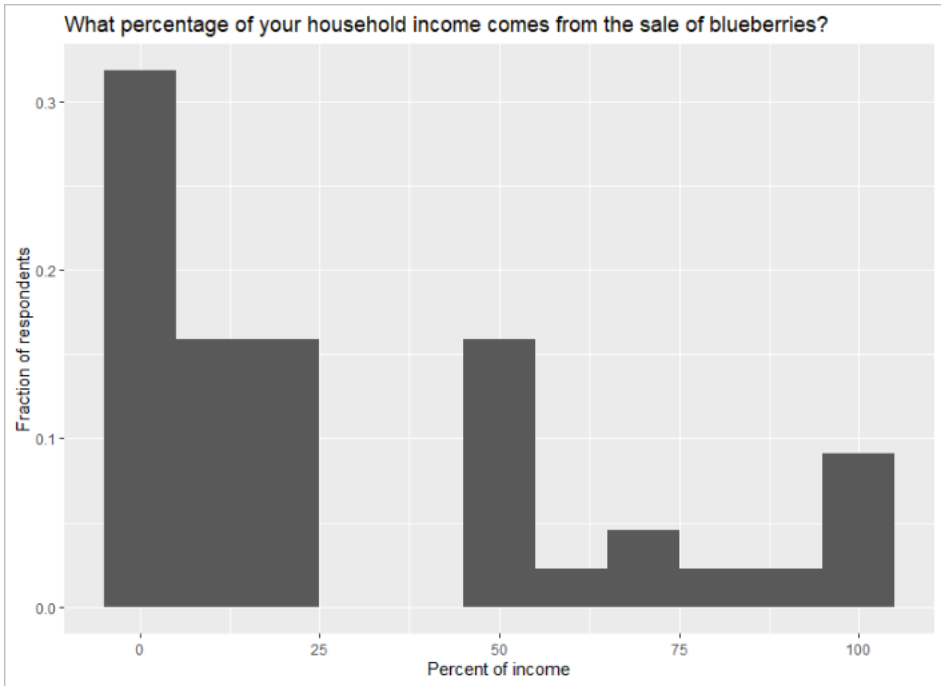

Figure 1. Wild blueberries' contribution of household income

Weighted by area, the split is even more stark, with nearly all acres managed either by an operation that derives less than 25% of its income from the sale of blueberries or an operation deriving almost all of its income from wild blueberries.

RESULTS

In the remainder of this report, we will discuss the management practices, market channels, and concerns for the future reported by survey respondents. In much of this reporting, both respondent-weighted and area-weighted results will be reported. In doing so, we hope to highlight areas in which operations of different sizes have similar and divergent needs.

Acknowledging this difference highlights the need for disaggregated data that allows farm operators to draw conclusions and compare their experience to similar operations in the state. It also allows agricultural service providers and Extension to better understand the number of individuals and the number of wild blueberry acres that share practices and concerns.

Management Practices

The Tables and Figures below show the prevalence of various management styles and practices – both in terms of the share of respondents and the share of wild blueberry acres. Table 4 highlights the added utility of this approach well. In both sets of columns, "integrated pest management" emerges as the most common pesticide management strategy. Considered by number of respondents, however, "certified organic" is close behind at 33% of respondents. In terms of wild blueberry acres, however, only 7% are managed as certified organic while 85% are managed according to IPM.

Table 4. Pesticide management style

How would you describe your pesticide management style?

	# of Respondents	% of Respondents	# of Acres	% of Acres
Certified Organic	16	33	1039	7
No Spray	4	8	231	2
IPM	19	40	11906	85
Conventional/Traditional	8	17	785	6
No Response	1	2	35	<1
Total	48	100	13996	100

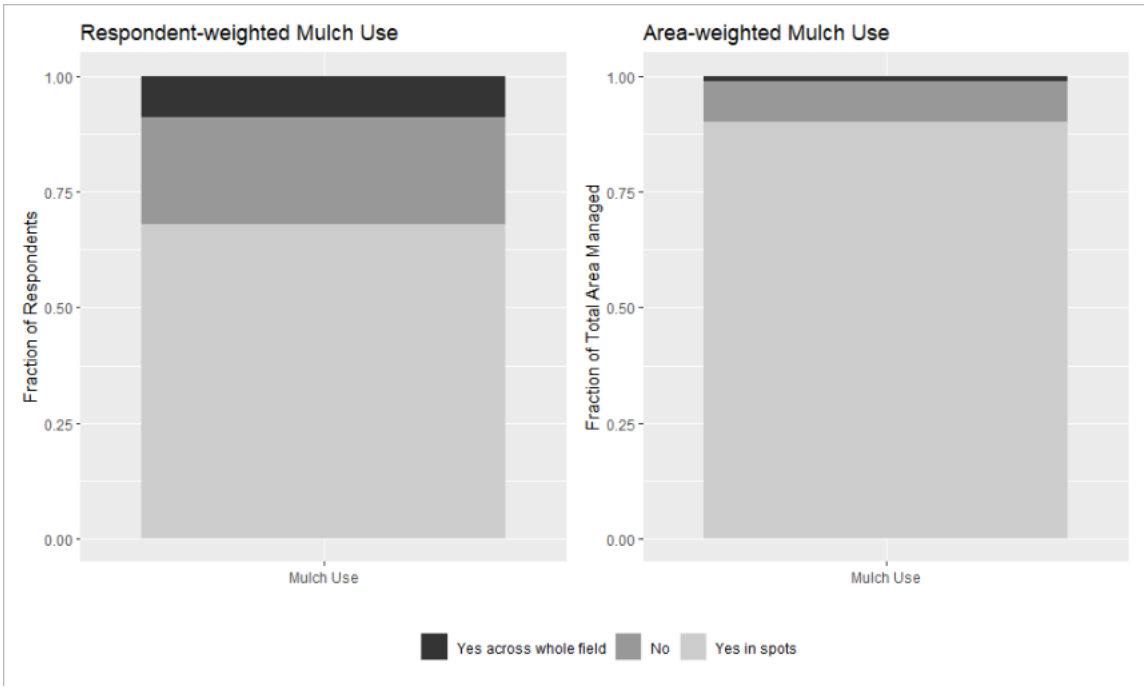

Figure 2. Mulch use by wild blueberry growers.

Such a difference, however, is not uniform across management questions. Figure 2, for example depicts similar approaches to mulching when considered by either the respondent or area-based formulation. Similar to Table 5, which reports prune methods and burn frequencies, respondent-based tabulation gives more weight approaches that may be more feasible over smaller areas – such as whole field mulching.

Table 5. Prune methods and burn frequency

Prune Method	# of Respondents	% of Respondents	# of Acres	% of Acres
Burn (Straw) Only	2	4	34	<1
Burn (Oil) Only	1	2	600	4
Mow Only	19	41	1858	13
Mow and Burn (Straw)	11	24	2396	17
Mow and Burn (Oil)	13	28	8933	65
Total	46	99	13821	100

Burn Frequency	# of Respondents	% of Respondents	# of Acres	% of Acres
Every year	3	11	674	6
Every other	9	33	10567	88
Every 3	2	7	95	1
Every 4+	13	48	627	5
Total	27	99	11963	100

Fertilization is common among grower respondents (Figure 3), with DAP and MAP (Table 6) being the most common fertilizer products. Here, the difference among respondent- and area-weighted tabulations is one of degree rather than behavior: the top product categories are used on the vast majority of acres, but their relative importance is the same. A slightly different picture is visible in Table 7, which reports how respondents determine when to apply fertilizer. Respondent-weighting increases the prevalence of testing (both soil and foliar) while area-weighting increases the prevalence of fertilizing every prune year. Note, however, that many respondents that report fertilizing every prune year still rely on testing in order to determine the quantity of fertilizer to apply.

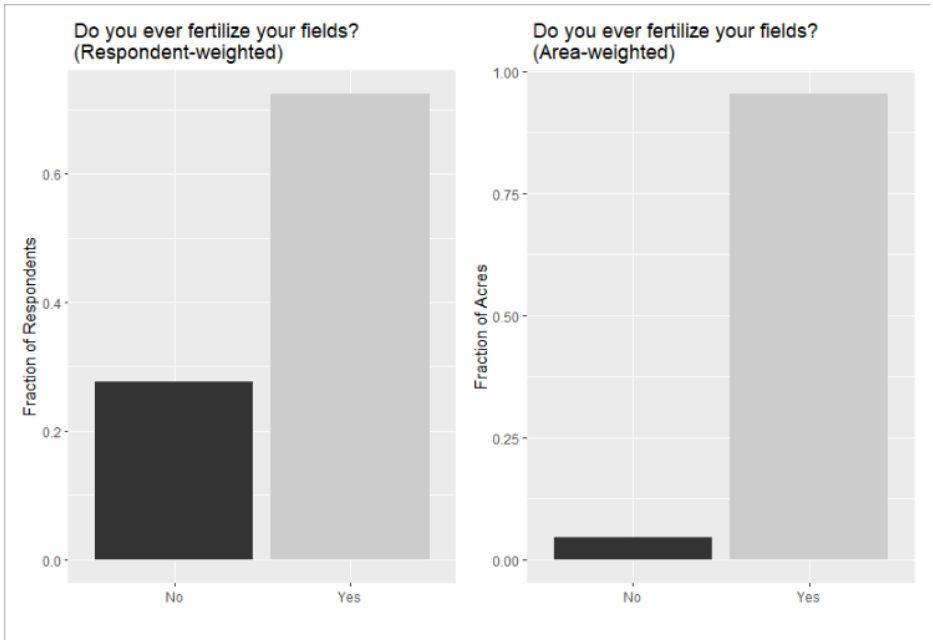

Figure 3. Fertilizer use by wild blueberry growers.

Table 6. What fertilizer products do you use?

	Respondents		Acres	
	#	%	#	%
DAP	20	0.59	12792	0.96
MAP	12	0.35	9671	0.73
Foliar Products	9	0.26	9014	0.68
Fish Products	2	0.06	72	0.01
Plant Growth Regulators	2	0.06	890	0.07

Note: Respondents could select multiple. Conditional on fertilizing fields.

Table 7. How do you determine when to apply fertilizer?

	Respondents		Acres	
	#	%	#	%
Soil Test	10	0.29	955.77	0.07
Foliar Test	15	0.44	4231.77	0.32
Fertilize Every Year	5	0.15	2085	0.16
Fertilize Every Prune Year	14	0.41	8987	0.68
Other	5	0.15	1010	0.08

Note: Respondents could select multiple. Conditional on fertilizing fields.

Similar to fertilizer, grower respondents uniformly rely heavily on renting bees to pollinate their blueberries. While 25% of respondents (15% of acres) report renting both honeybees and bumblebees, rental of honeybees is dominant.

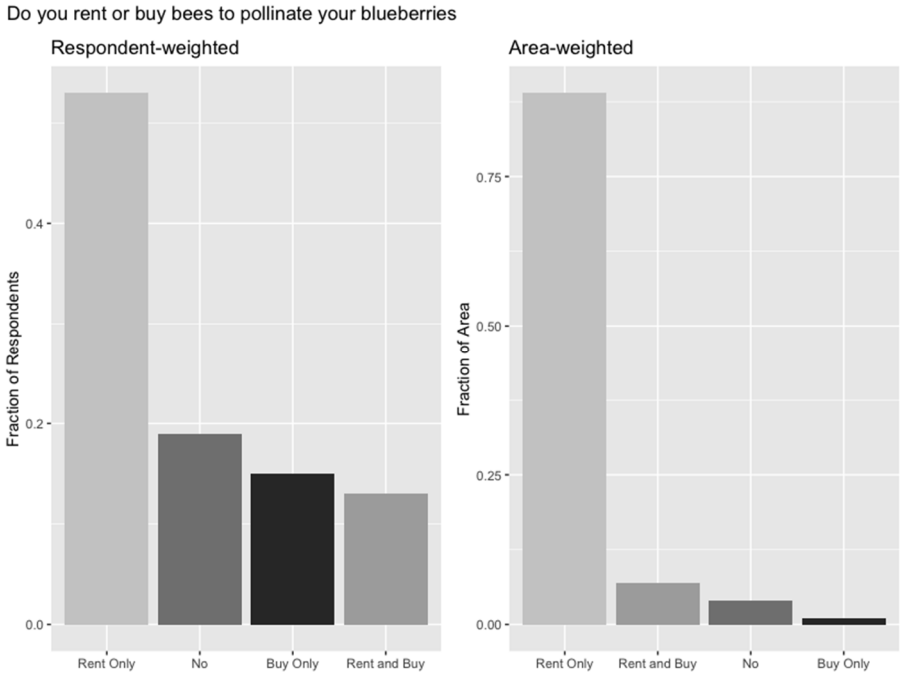

Figure 4. Use of bees by wild blueberry growers.

Among the biggest differences in management practices that emerge between respondent-weighting and area-weighting is with respect to irrigation (Figure 4). While only 32% of respondents report using irrigation, 79% of acres are managed by a respondent that irrigates at least some of their acreage.

Note, however, that the survey did not collect specific data on the number of acres irrigated. Still, given the challenges and concerns facing growers, which will be discussed below, the differences visible in the ability to manage water are notable.

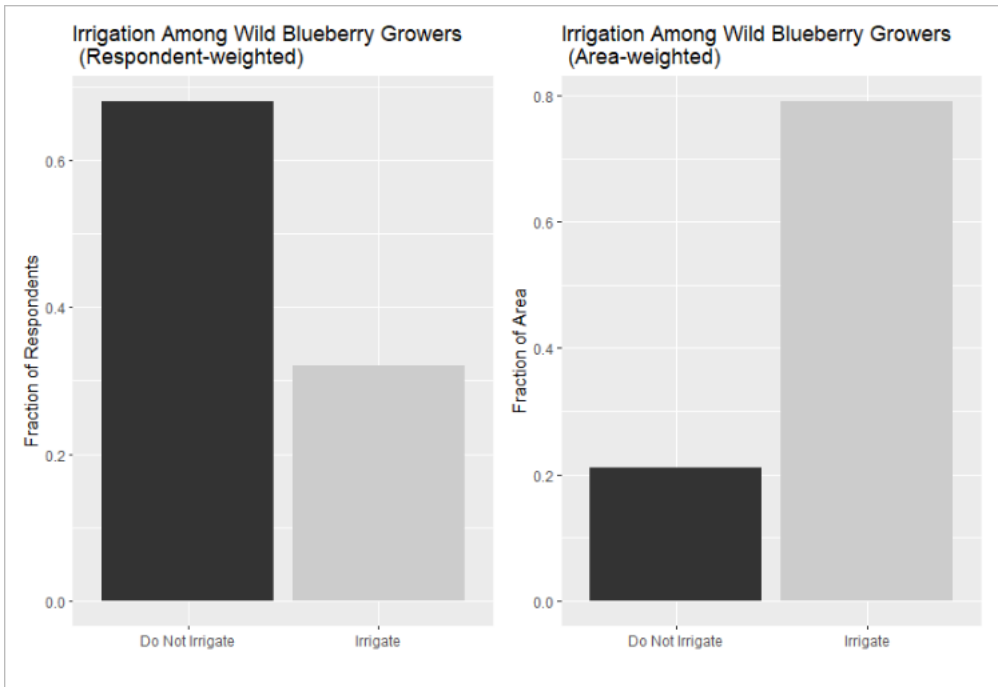

Figure 5. Use of irrigation by wild blueberry growers.

Market Channels

Similar to management practices, it is useful for both farm operators and agricultural service providers to be informed about the market channels through which wild blueberries are being sold. Once again, there exists significant variation in the products and sales channels used by growers across the state. While the majority of blueberries are still sold as frozen. As Table 8 shows, weighted by area, 81% of blueberries are sold in this form versus 60% of blueberries with responses weighted by respondents. A significant share of blueberries are also sold fresh, nearly 40% when weighting by respondents, with a small amount going to value-added products as well.

Table 8. Percent of wild blueberries sold as:

Product	Respondent-weighted	Area-weighted
Frozen	60	81.2
Fresh	37	18.4
Other/Value Added	3	0.4
Not Sold	<.01	<0.01

With regard to how blueberries are sold, it is unsurprising then that 69% of respondents report selling blueberries to processors. The 42% and 40%, however, that sell direct to consumers and direct to businesses is non-trivial. Respondents reported that the relative importance of the market channels presented by Figure 6 had remained mostly unchanged over the past five years.

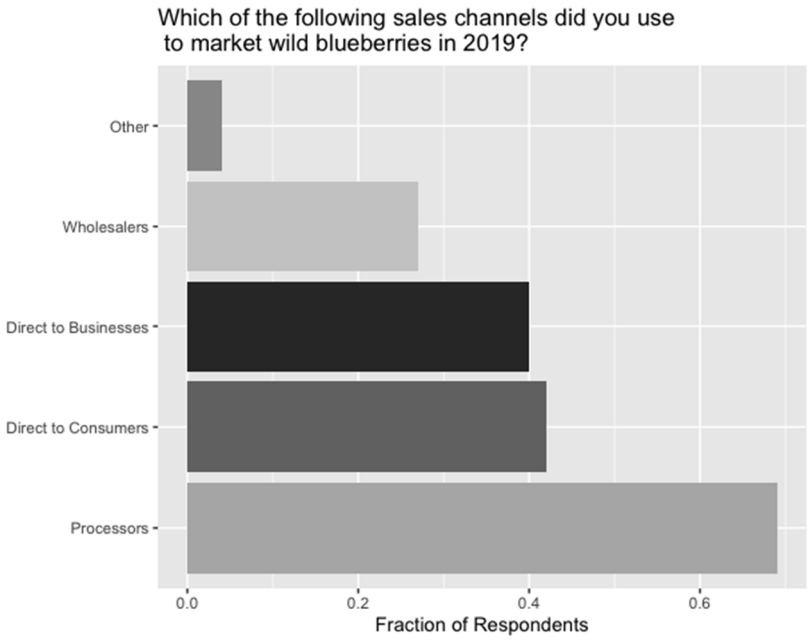

Figure 6. Market channels used.

Within the direct-to-consumer sales channel, wild blueberry growers reported making use of a wide variety of marketing tools – including farmers markets (40%), farm stands (45%), and websites (35%). Respondents also mentioned using local media, social media, home delivery, you-pick, and word-of-mouth marketing to sell blueberries directly to consumers.

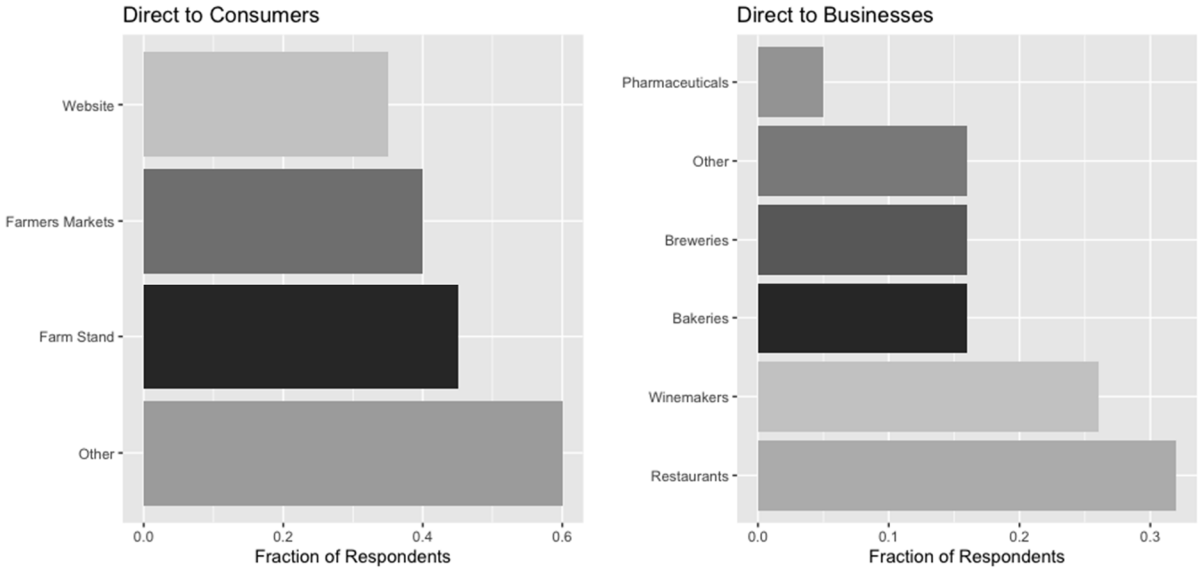

Figure 7. Direct-to-consumer and direct-to-business marketing methods.

Respondents selling directly to businesses were the most likely to mention selling to restaurants (32%) and winemakers (26%), while lesser numbers of respondents also mentioned selling to bakeries (16%) and breweries (16%).

The past few years have highlighted the increasing need for flexibility in marketing. In particular, they have highlighted the role that the ability to market and sell product through the internet can play in ensuring sufficient access to robust markets – both direct to consumers and to businesses and

institutions through a growing number of internet-based wholesale platforms. Despite this, wild blueberry marketing is still a predominantly offline activity. Only 32% of respondents report having a website. Amount those that do have a website, only 33% sell directly through that website. While this may partially be a technical issue, it also appears to be a deliberate business preference. When asked if they would like to receive support in using technology to market wild blueberries, 63% of respondents replied that, no, they were not interested in receiving this type of support.

Table 9. Web-based marketing

	Do you have a website?			Do you sell directly through your website?		
	#	%			#	%
No	32	0.68		No	10	0.67
Yes	15	0.32		Yes	5	0.33
Total	47	1		Total	15	1

Risks and Challenges

The management and marketing strategies discussed thus far are, in part, chosen to deal with the many risks and challenges faced by blueberry growers. The growing season preceding the survey highlighted these risks, particularly those associated with drought, to full effect (Table 10, Figure 8).

Table 10. Which of the following events affected your harvest this past season?

	Respondents		Acres	
	%	#	#	%
Heavy Spring Rainfall	0.23	11	740	0.04
Drought	0.85	41	13497	0.66
Extreme Temperatures	0.56	27	5456	0.27
Fall Bloom	0.08	4	804	0.04
None	0.04	2	34	0.00

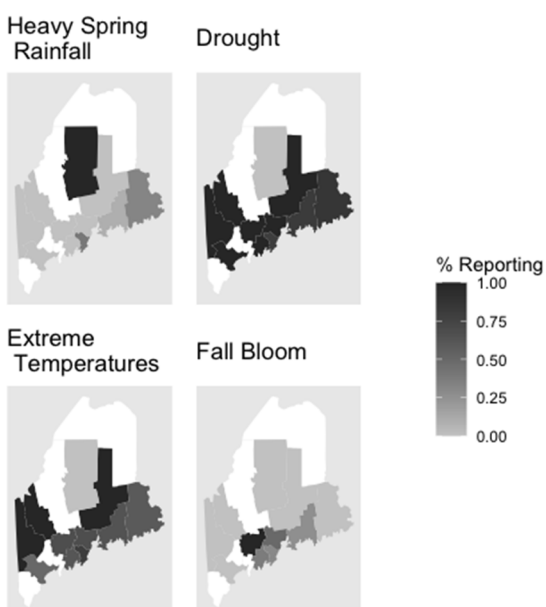

Figure 8. Adverse weather events across the state by county.

Note that the risks associated with weather events affect the incentives that growers face to invest in irrigation and consider mulching and other costly management strategies to mitigate the effects of adverse weather events on the blueberry harvest. Similarly, the challenges posed by insect pests, disease, and weed pressure discussed below necessitate action on the part of growers and incentivize innovation by researchers, agricultural service providers, and growers themselves as the industry works together to limit the effect of these factors on grower livelihoods.

For each of the categories of pressure discussed below – insect pests, disease, and weeds – respondents were asked to identify the most prevalent element, the most impactful element in the past year, and the element that represents the biggest future concern. Once again, the data is presented in both a respondent-weighted and area-weighted format in order to better capture the diverse experiences of the industry.

Figure 9 presents current and future industry concerns related to insect pests. Blueberry maggot fly appears as the most common and, presently, the most impactful insect pest in both versions of the figure. In both versions, spotted wing drosophila is also currently common and impactful, while the area-weighted data additionally highlight the importance of spanworm. Looking forward, however, it becomes clear that spotted wing drosophila is the insect pest is most cited as a future concern.

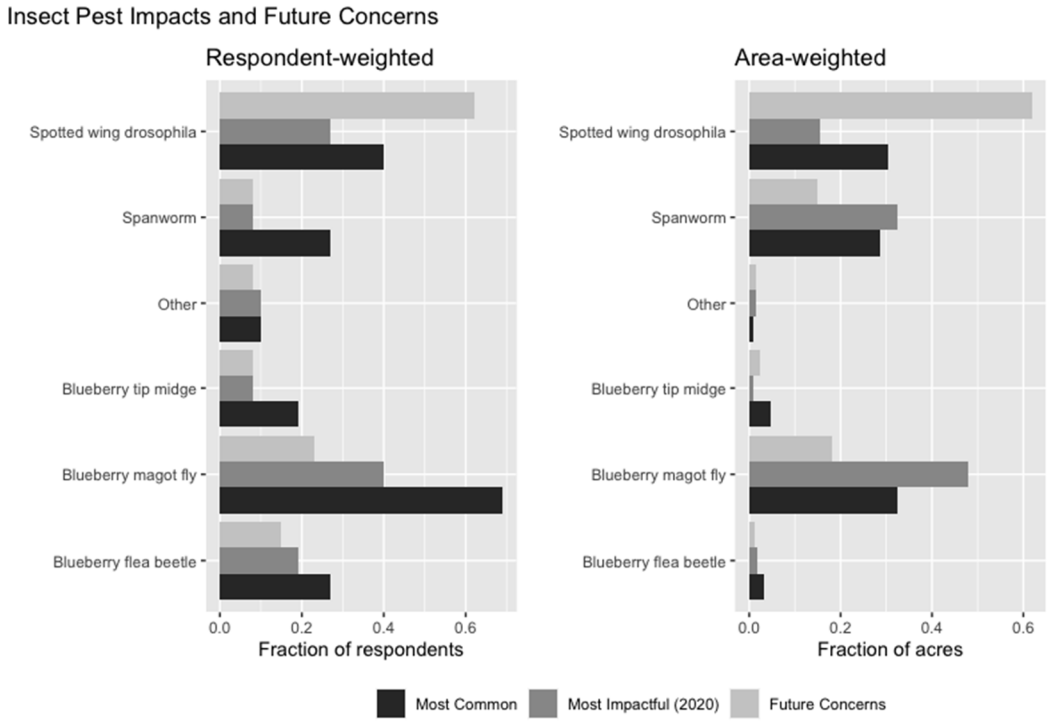

Figure 9. Insect pests of concern by wild blueberry growers.

With respect to disease pressure (Figure 10), mummy berry is the most common, most impactful, and most pressing future concern among grower respondents. Botrytis blight was also mentioned by 30% of both respondents and acres as a pressing future concern. The area-weighted data also break with the respondent-weighted data and highlight Septoria Leaf Spot as common and impactful during the year preceding the survey.

Table 11 reports respondent concerns with respect to weeds. Weighted by respondents, grasses, bracken fern, and dogbane were the top three responses in each prompt – most common, most

impactful, and biggest concern for the future. Responses were more varied when the data were weighted by area managed.

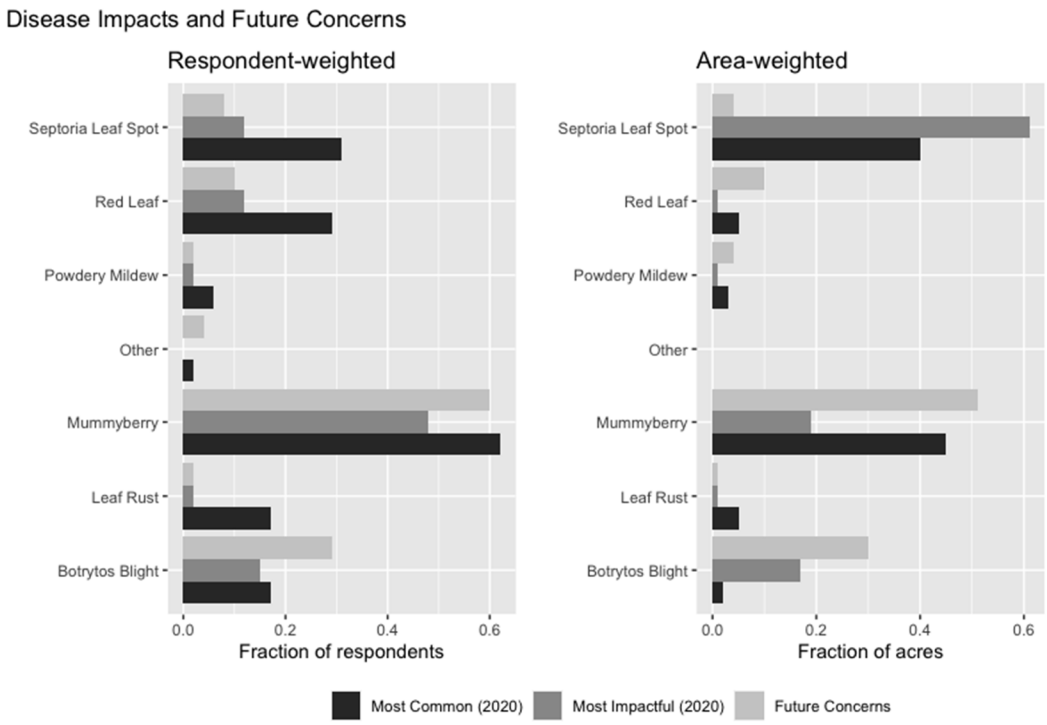

Figure 10. Disease concerns from wild blueberry growers.

Table 11. Weed concerns

Most Abundant		Most Difficult to Manage		Most Concerning for the Future	
By Resp.	By Acres	By Resp.	By Acres	By Resp.	By Acres
Grasses	Sedge	Grasses	Sedge	Grasses	Red sorrel
Bracken fern	Dogbane	Dogbane	Dogbane	Dogbane	Sedge
Dogbane	Bracken fern	Bracken fern	Bunchberry	Bracken fern	Dogbane

Looking forward

To conclude, we want to look forward and consider the plans and concerns that respondents expressed for the future. Both the variety of risks discussed above and the fact that, as shown in Table 12, the industry is aging, require explicit plans for the future in order to ensure a healthy wild blueberry industry for years to come. In addition to the average age of grower respondents, Table 13 suggests that a significant fraction of respondents plan to leave the industry in five to ten years. The second set of columns further highlights that these growers manage a sizable fraction of wild blueberry acres in the state.

Table 12. Age distribution of grower respondents

Age Distribution of Grower Respondents			
Mean	Percentiles		
	25th	50th	75th
64	58	65	72

Table 13. Do you expect to be growing wild blueberries in the future?

	Respondents 5 Years		Respondents 10 Years	
	Freq	Percent	Freq	Percent
Definitely Not	1	0.02	5	0.11
Maybe Not	14	0.31	17	0.38
Probably	17	0.38	13	0.29
Definitely	13	0.29	10	0.22

	Acre-weighted 5 Years		Acre-weighted 10 Years	
	Acres	Fraction	Acres	Fraction
Definitely Not	25	0	1025	0.17
Maybe Not	1447	0.25	815.77	0.14
Probably	3350.77	0.57	3133	0.53
Definitely	1063	0.18	912	0.15

While a number of respondents believe that they might not be managing blueberries in the near future, most hope that their land will stay in family production and the vast majority hope to see their land stay in production (Table 14). At the same time, only half of respondents report having an explicit plan for their land when they retire.

Table 14. Which best describes the plan for your land when you retire?

Response	Freq	Percent
Stay in family production	13	0.62
Sell to another grower	3	0.14
Sell land, out of prod.	1	0.05
Stay on land, other management	2	0.1
Other	2	0.1

The challenges facing growers – both those that plan to stay and those that might be considering an exit—are varied. More importantly, perhaps, is the fact that growers feel prepared to deal with these challenges to varying degrees. Tables 15 reports on the extent to which respondents are concerned about the profitability of their farming operation, the impact of climate change on their farming operation, and their mental and physical health. Table 16 follows up by reporting on the extent to which respondents feel like they have access to adequate resources to address these challenges.

Table 15. Please indicate the extent to which each of the following is a concern for you:

	Respondent-weighted		
	Not Concerned	Somewhat Concerned	Very Concerned
Profitability of my farming operation	0.09	0.27	0.64
The impact of climate change on my farming operation	0.18	0.36	0.47
My physical health	0.23	0.5	0.27
My mental health	0.52	0.39	0.09

	Acre-weighted		
	Not Concerned	Somewhat Concerned	Very Concerned
Profitability of my farming operation	0.05	0.12	0.83
The impact of climate change on my farming operation	0.21	0.23	0.56
My physical health	0.15	0.75	0.1
My mental health	0.36	0.61	0.03

Table 16. Do you feel that you have access to adequate resources to address your concerns about:

	Respondent-weighted		Acre-weighted	
	No	Yes	No	Yes
Profitability of my farming operation	0.21	0.79	0.1	0.9
The impact of climate change on my farming operation	0.49	0.51	0.69	0.31
My physical health	0.21	0.79	0.19	0.81
My mental health	0.1	0.9	0.16	0.84

The profitability of farming operations is the most significant concern among respondents, followed by the impact of climate change on their farming operation. Significant numbers of respondents (similarly when the data is weighted by area) also express some concern for their mental and physical health. As highlighted in Table 16, however, respondents feel equipped to deal with these challenges to very different extents. Despite 91% of respondents indicating that they are somewhat or very concerned about the profitability of their farming operation, 79% feel that they have access to adequate resources to deal with these concerns. On the other side, 79% of respondents report being somewhat or very concerned about the impacts of climate change on their farming operation, but only 51% feel that they have access to adequate resources to address those concerns.

DISCUSSION AND CURRENT RECOMMENDATIONS

While participation rates were lower than desired, the data collected through this first survey still manages to provide a glimpse at the management practices and marketing channels being used by blueberry growers across the state. It also highlights the many challenges facing the industry. While this report focused primarily on growers, it is worth noting that processors and agricultural service providers also view the industry as facing significant challenges. Table 17 reflects the responses of all industry participants and suggests that there is a wide belief that the industry is less healthy now than it was five years ago.

Table 17. Relative to five years ago, how healthy do you think the wild blueberry industry is?

Response	#	%
Healthier	3	0.04
Equally healthy	11	0.14
Less healthy	53	0.66
I do not know	13	0.16
Total	80	1

These challenges only further the need for more frequent, more detailed, data collection and the rapid dissemination of this data to decision-makers in the industry. Toward this goal, we are planning a second round of grower/processor surveys, as well as an end user/institutional buyer survey, to be fielded in early 2022. Drawing on the lessons learned in the first survey, the second round will be significantly shorter and the research team will take advantage of in-person events in order to avoid putting any extra burden on respondents. We are also eagerly soliciting feedback on what content industry participants are most eager to receive updates on and format that would make the information the most useful and accessible. Current plans include both factsheets and an online dashboard that would allow an interested participant to view a summary of the data specific to their county, their preferred market channel, or their management practice.

ACKNOWLEDGEMENTS

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BERRY QUALITY

RESEARCH & EXTENSION

INVESTIGATOR(S): L. Calderwood and M. Peronto

1. Improving Shelf Life of Fresh Pack Maine Wild Blueberries

OBJECTIVE(S)
This project aims to improve post-harvest handling of fresh pack wild blueberries to extend the berries' shelf life by:

- Identifying optimal cold storage temperatures for wild blueberries that cannot be kept cold throughout the entire cold chain
- Surveying current temperature and relative humidity of fresh pack buildings across Maine

LOCATION(S): Blueberry Hill Research Farm, Jonesboro, ME & Welch Farm, Roque Bluffs, ME
PROJECT TIMEFRAME: April 2021 – February 2023

INTRODUCTION
Many family wild blueberry farms in Maine (20-200 acres) do not have the capital to invest in the development of complete cold chain infrastructure that would extend the shelf life of their berries. A complete cold chain keeps berries at a consistent cold temperature from field to market and requires investment in on-farm cold storage and cold transportation. It also means ensuring that the end market has a cold place to store the berries until they are sold. Significant physiological differences between wild (lowbush) and highbush blueberries have led Maine producers of wild blueberries to develop a suite of innovative methods for maintaining berry quality, but many growers are hesitant to cool berries because they do not have a complete cold chain (Callahan, 2018; Boyette et al., 1993). The concern is that when berries are cooled down and then moved into a warmer space for transport, storage, or sale, condensation builds up on the fruit causing a severe decrease in quality. Growers have indicated that fresh wild blueberry storage temperatures range from 40°F to 70°F, and airflow and humidity within the storage unit are not often considered. Cold storage units were constructed in 2020 and 2021 on two farms to test berry freshness over time when stored at three different temperatures.

For an explanation of how the cold storage unit at Blueberry Hill Farm was constructed and for an approximate cost, see the 2020 report summary, entitled "Coolbot Cold Storage Room Construction and Costs" (page 148). A brief summary follows here.

Eight by eight foot insulated rooms were constructed at Blueberry Hill Farm in summer 2020 and 2021 (Fig. 1). Instructions from All One Farm and the University of Vermont were followed: https://www.uvm.edu/extension/produceportal/case-studies/coolbot-shoestring/

Materials were purchased, cold storage units were constructed of plywood (4 walls + ceiling), the box was insulated (with two stacked insulation boards and by using foam in the corners), and CoolBot and air conditioning units were installed. Tips and tricks about materials, purchasing, and construction methodology can be found in the University of Vermont resources and the 2020 report summary. When constructing the additional units at BHF in 2021, the walls and ceiling were insulated by two stacked pieces of R-10 instead of the planned single board of R-20 value, because of limited inventory at Home Depot. Higher R-values are ideal since this ensures the unit will be better insulated. The air conditioning unit in the 34° F unit was 16,000 BTU and the units in the 40°F and 50° F units were 12,000 BTU.

Figure 1. Left: finishing installation of the plywood roof. Right: view inside the cold storage unit while installing the air conditioning unit and CoolBot device.

METHODS

This two-year study is being carried out at Blueberry Hill Research Farm (BHF) in Jonesboro, Maine, at Welch Farm (RB) in Roque Bluffs, Maine. Twenty additional fresh pack facilities across Maine will be included in a survey of current fresh pack processing methods, which will be included in next year's report.

At RB there is an existing, homemade walk-in cooler (8ft x 24ft) made of a refrigerated truck trailer that is kept at 46-50°F by cooling with a 12,000 BTU air conditioning unit. At RB, an additional 8ft x 8ft cold storage unit was constructed using plywood, R-10 insulation panels doubled up, spray foam insulation, an industrial fan for airflow, a 12,000 BTU air conditioning unit, and a temperature and relative humidity (RH) sensor. This new cold storage unit was kept at 40°F with 95% RH and was installed in the same building as RB's existing cold storage unit, enabling the farm to pack and process berries as usual while integrating the new unit into their production process. The existing cold storage unit at RB will provide a valuable comparison between a homemade cold storage unit and a planned unit. At BHF, there is an existing cold storage unit, constructed in 2020. An additional two cold storage units were constructed there so all three units are 8ft x 8ft, with the same R-value 10 insulation panels doubled up, spray foam insulation, industrial fans for airflow, 12,000 BTU air conditioning units, and temperature and relative humidity sensors. These three units are kept at 34°F, 40°F, and 50°F.

Table 1. Summary of cold storage unit specifications.

	Cold storage units			Target relative humidity (RH)	Fruit storage size	Harvest date
	1	2	3			
Roque Bluffs (RB)	N/A	40°F	46-50°F	95%	Pints	8/9/21
		12,000 BTU	12,000 BTU			
		R-10 (doubled)	R-10 (doubled)			
Blueberry Hill Farm (BHF)	34°F	40°F	50°F	95%	Quarts	7/29/21
	16,000 BTU	12,000 BTU	12,000 BTU			
	R-10 (doubled)	R-10 (doubled)	R-10 (doubled)			

Berries at both RB and BHF were hand-raked. At BHF, berries were raked on July 29, 2021, then winnowed and transferred into industry-standard quart-size molded pulp produce baskets and immediately stored in the cold storage unit with baskets directly abutting one another. At RB, berries were hand-raked on August 9, 2021, then winnowed, put through a cleaning line, and transferred into industry-standard pint-size molded pulp produce baskets before immediate storage in the cold storage unit. At RB, berry quality was measured every 3 days for 26 days (total: 6 collection dates) because RB typically sells fresh pints within 7 – 14 days of harvest. At BHF, berry quality was measured every 3 days for 37 days (total: 11 collection dates) to mimic longer-term storage conditions for some growers. Berry quality measures taken at each site and during each sampling time included cold storage unit temperature and RH, internal temperatures of individual berries, internal pint temperature, berry firmness, and pint moisture level.

Continued berry sampling in the 2022 season (and hopefully beyond) is necessary to understand how outside weather conditions can impact the cold storage units' internal temperatures and RH.

In addition to directly sampling berry quality, surveys of conditions and practices within fresh pack facilities were conducted and will continue in the 2022 season. These surveys occurred on a packing day during harvest. Investigators asked growers and processors about their current post-harvest handling and storage practices, facilities' temperature and RH levels, timing for harvesting and processing, equipment providers and costs, customers, and markets. In addition, a portable temperature and RH sensor measured conditions in the processing and storage rooms. These surveys were conducted to foster discussion of current post-harvest handling and storage practices, and possibilities for improving these processes.

Data collection

Berries harvested at BHF were sampled two times per week over the course of 37 days from July 29 - September 3 while berries harvested at RB were also sampled two times per week over the course of 26 days from August 9 - September 3. The BHF units were constructed solely for this trial and therefore the doors of units were hardly opened unless berries were actively being sampled for this trial. The RB units were actively functioning on a farm and therefore integrated into the harvest, processing, and sales operations so they were opened as many as 30 times per day.

Temperature and RH data was gathered from a mounted sensor in the newly constructed cold storage units. A handheld DigiSense sensor or a separate, non-digital thermometer was used to verify the data on the mounted sensors. Berry and pint temperature were measured using a food-grade electronic thermometer (ThermPro Ultra-Fast Digital Food Thermometer TP-03B). Three separate berries were randomly selected and penetrated by the thermometer to obtain a temperature reading; these berries were then discarded from the quart or pint. Pint temperature was obtained by sticking the thermometer

into the center of each pint three times, taking care not to place the probe right next to the edges or bottom of the pint container.

Berry firmness was measured by blindly selecting 3 separate berries and rating the firmness on a scale of 1 to 3, with 1 being a firm, salable berry and 3 being a mushy or nonsalable berry; these berries were then discarded from the pint or quart. Pint moisture was documented by visual inspection of the exposed top of the pints (as a customer would) and rating the moisture on a scale of 1 to 3, with 1 being dry and 3 being wet.

Surveys of post-harvest handling and storage practices were conducted verbally by the investigators. Facility temperature and RH levels were measured using a handheld, portable DigiSense sensor.

Data analysis

Due to the nature of the data collected, especially the ranked data, much of the data failed the assumptions of normality and equal variance required to run parametric statistical tests. Transforming the data via a square root transformation visually improved the distribution, but the data continued to statistically fail the test of normality. All the data, including berry temperature, berry moisture and berry firmness, were transformed using a square root transformation prior to all statistical testing and statistical tests were carried out despite non-normality after establishing there were no serious problems with the data.

The effects of long-term storage on berry quality (firmness) were analyzed using a multivariate correlation to generate an R^2 in Microsoft Excel (Excel® Version 2110) to observe the level of change over time. Overall treatment differences were tested using a full-factorial repeated-measures mixed model design in JMP (JMP®, Version 15.2) for berry temperature only. Here, the full-factorial model tested the effects of date, treatment and any interaction between date and treatment.

RESULTS

Maximum daily air temperature and relative humidity (RH) were collected at a field-based weather station 180 meters from the Jonesboro cold storage units and 9 miles from the RB cold storage units. Maximum daily outside air temperature ranged from 71°F to 88°F and showed the greatest correlation with the two colder storage treatments (34°F and 40°F) for the Jonesboro location (Fig. 2). This suggests that the cold unit likely declined in efficiency when cooling to colder temperatures. This increased the temperature variability inside cold storage units. The warmer cold storage treatment (50°F) showed less temporal variability in air temperature fluctuations compared to the other units and outside temperatures. High variation in unit temperatures in the first week of storage may correspond to manual adjustments made by our team to obtain target temperatures. Outside RH ranged from 69% to 99% and correlated with all three cold storage units (Fig. 3).

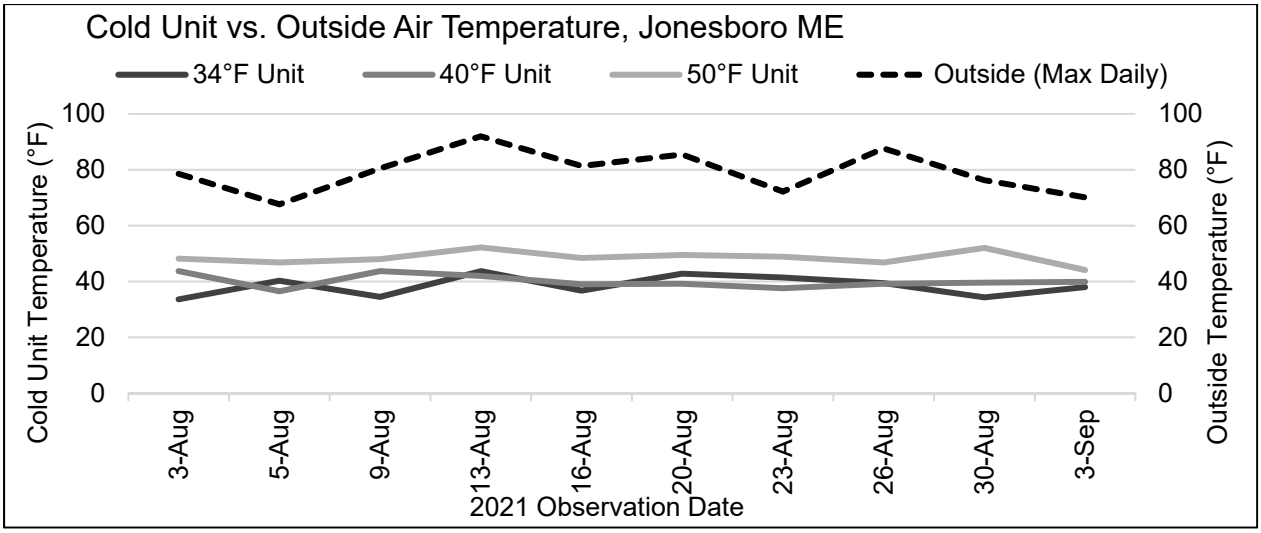

Figure 2. Cold storage air temperature of the Jonesboro units compared with outside maximum daily air temperature for BHF (Batch 1), on the dates that sampling occurred.

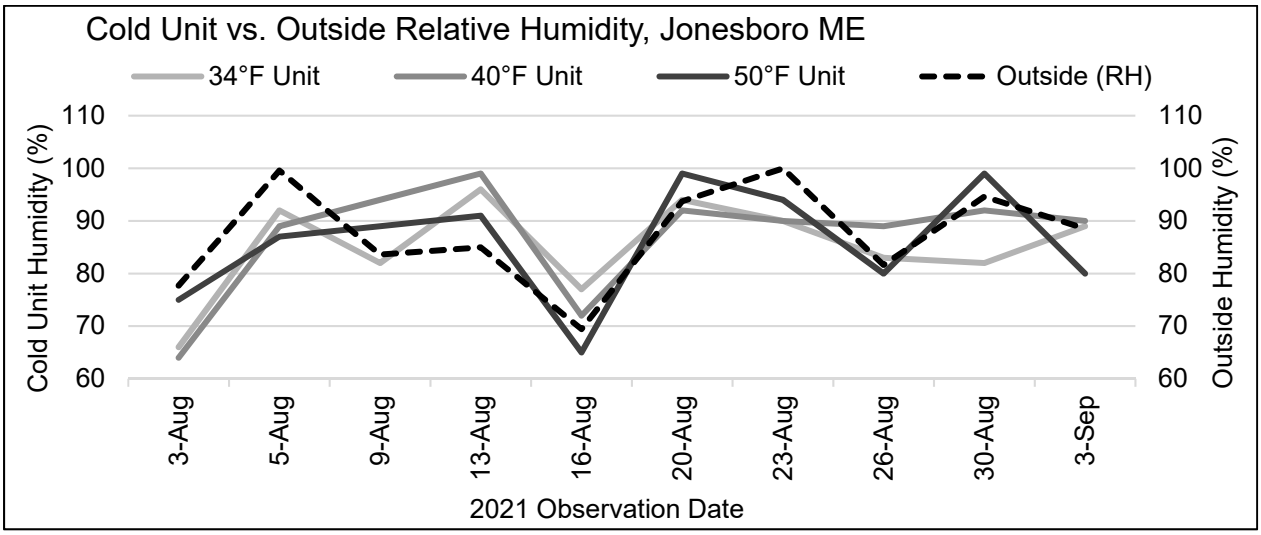

Figure 3. Cold storage unit relative humidity (bars) of the Jonesboro units compared with outside relative humidity (line) for BHF (Batch 1), on the dates that sampling occurred.

At BHF, when Batch 1 of berries first came out of the field following hand raking on July 29, 2021 (a full sun, 78°F day), internal individual berry temperatures averaged 75°F. The berries from this batch had cooled by the next sample date of August 3 (Fig. 4). Berry temperature fluctuations between 1 and 9°F were observed within the three temperature treatments during the 4 to 5 weeks after harvest. Units' air temperature at time of sampling also fluctuated despite having preset target temperatures. The 50°F cold storage unit exhibited the most stable trend over time. The 34°F and 40°F degree units require some troubleshooting.

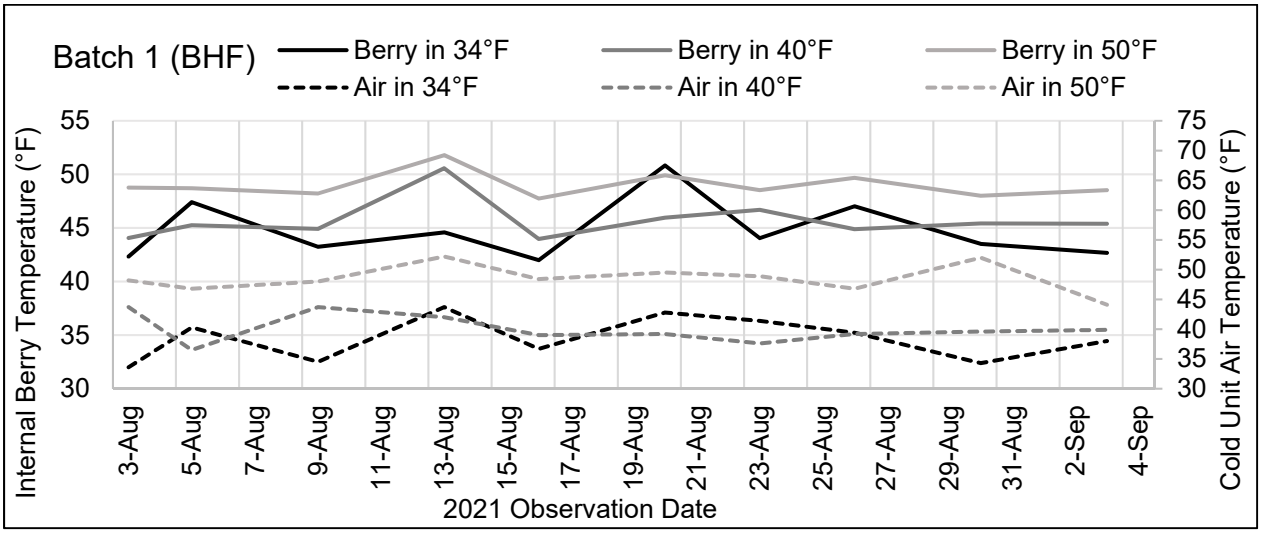

Figure 4. Berry temperature (°F; lines) and cold storage unit air temperature (°F; bars) by temperature treatment for Batch 1 at BHF (harvested and cooled 07/29/2021), monitored for 5 weeks following harvest.

Individual berry temperatures were not taken after harvest and before cooling for the second batch of berries ("Batch 2"), harvested on August 9, 2021, in RB due to logistics. Similar berry temperature fluctuations occurred often in response to fluctuations in cold storage unit air temperatures. The 40°F unit, maintained a relatively stable berry temperature trend over time. The higher temperature treatment, 50°F showed a steady decline in berry temperature over time (Fig. 5). The shift in berry temperature mimics a shift in cold storage unit air temperature in the 50°F temperature treatment as air temperature dropped from 55°F on August 9 to 45°F on September 3.

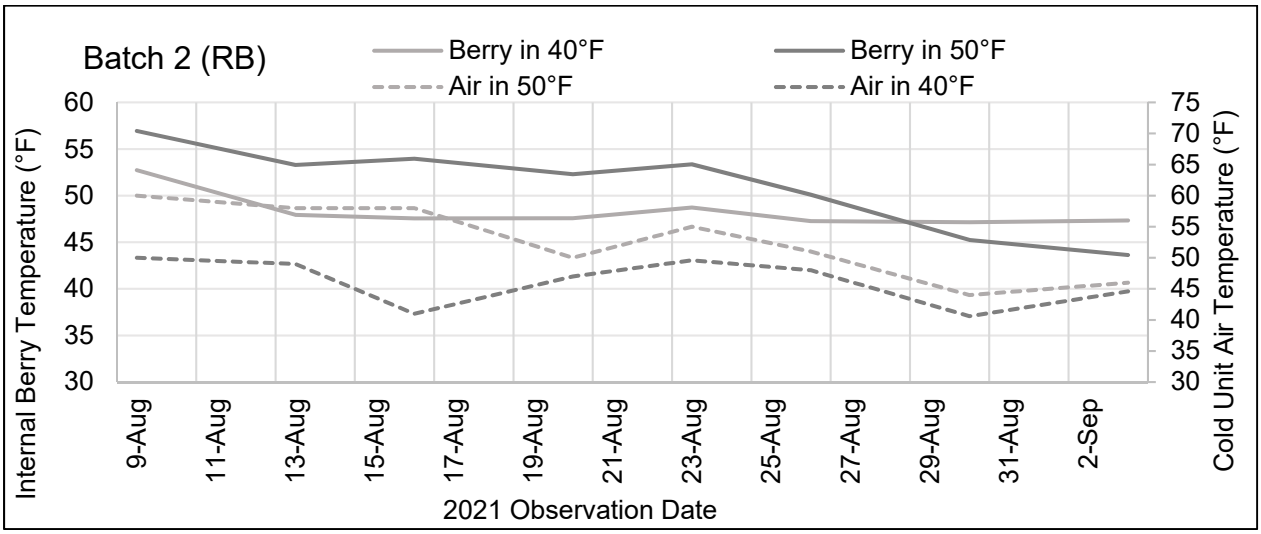

Figure 5. Berry temperature (°F; lines) and cold storage unit air temperature (°F; bars) by temperature treatment for Batch 2 (RB harvested 8/9/21 and stored 8/10/21), monitored for 4 weeks following harvest.

Overall, average berry temperatures were significantly different across all temperature treatments for both Batches (Fig. 6), with lower temperature treatments yielding significantly colder berries than warmer temperature treatments within the cold storage units. For Batch 1, the lowest temperature treatment (34°F) had an average internal berry temperature of 44.5°, while the middle treatment (40°F) and the warmer treatment (50°F) had internal berry temperatures of 45.5°F and 48.4°F, respectively.

Batch 2 berries, stored in pints, had warmer internal temperatures compared to Batch 1 that were stored in quarts by an average of 7°F in the first 5 weeks. As a result, Batch 2 at RB internal berry temperatures were warmer than Batch 1 (although not statistically compared) with internal berry temperatures averaging 48°F and 51°F for the 40°F and 50°F treatments, respectively. The warmer internal berry temperatures in Batch 2 further exhibits the direct relationship between cold unit air temperature and internal berry temperatures.

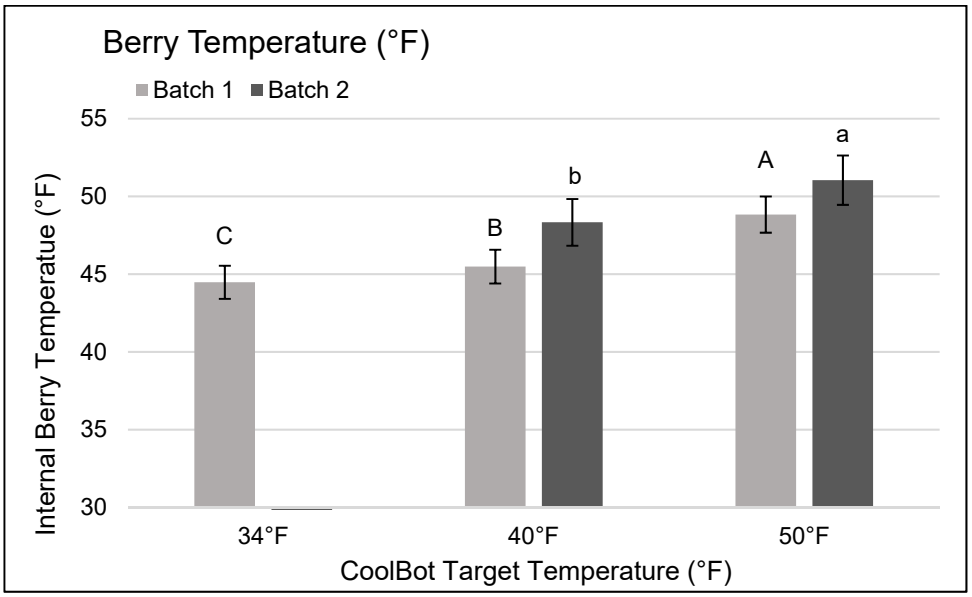

Figure 6. Berry temperature (°F), for Batch 1 (BHF harvested and stored on 7/29/21) and Batch 2 (RB harvested 8/9/21 and stored 8/10/21), monitored for 4 to 5 weeks following harvest, all measures averaged. Letters indicate significance at the 0.05 level of significance for berry temperature. Capital letters are to be compared separately from lowercase letters. Error bars indicate the standard error of the mean.

Ranked berry moisture data was variable and therefore decided to not be robust enough in this first year to present. Overall Batch 1 berries were very wet and not marketable due to the fact they were not cleaned after harvest. Berry moisture was clearly correlated to unit relative humidity. Cleaning removed most burst and damaged berries in Batch 2 leading to drier berries. Fluctuations in berry moisture greatly affect berry quality and berry firmness. As another indicator of berry quality, berry firmness declined drastically in Batch 1 over time with the greatest decline occurring under the warmest temperature treatments 40°F and 50°F. The coldest temperature treatment (34°F) saw declines in berry firmness over time, however, these reductions were not as dramatic as the warmer cooling treatments (data not shown).

By August 30, approximately 4-5 weeks after entering the cold storage unit, both batches of berries had clearly lost their volume and form at the 50°F cooling treatment and were classified as “unsaleable”. Mold growth was first observed on Batch 1 berries on August 26, approximately four weeks after entering the cold storage unit, across all three cooling treatments. Mold growth was first observed on Batch 2 berries on August 30 at the 50°F cooling treatment and on September 3 at the 34°F cooling treatment. Mold growth was not as prominent in Batch 2 at the 40°F cooling treatment. Mold growth was also observed on the walls of some cold storage units toward the end of sampling due to condensation.

DISCUSSION

Growers currently store their fresh pack berries for 24 – 48 hours on average. The length of this trial (4 – 5 weeks) greatly exceeds the current storage time because our aim is to extend fresh pack shelf life. Our short-term goal is for growers to be able to deliver higher quality fresh pack wild blueberries to consumers in Maine with a long-term goal for growers to be able to ship high quality fresh pack berries to markets outside of Maine.

This trial's year one results clearly showed the importance of relative humidity and outdoor weather conditions in wild blueberry storage. Fluctuations within the units' air temperature and humidity combined with the natural respiration and ripening processes contributed to the decline in berry firmness and losses of berry shape and volume and the mold that was observed. These changes were particularly evident in the 50°F temperature condition. Under the 34°F temperature condition, these changes and losses were less pronounced. Relative humidity and temperature variation within the units may be related to berry respiration, whereby berries continue to convert glucose into carbon dioxide, water vapor, and heat, even after being harvested. Like most produce, blueberries produce ethylene gas as they ripen, which accelerates their ripening as the rate of respiration increases. Storing berries at colder temperatures reduces the rate of respiration and can thus slow the ripening process. For every 10°F reduction in temperature, the respiration rate decreases by 50% (Callahan, 2018). Slowing the rate of respiration would also reduce the amount of heat and water vapor being produced by the berries. The berries in this study did not leave the cold storage unit and therefore only represent the first part of the fresh wild blueberry journey from the field to consumer. If the 34°F berries were removed and transported or stored at a temperature warmer than 34°F, condensation would occur, reducing berry quality dramatically.

Overall, RB's Batch 2 berries were less moist and of better quality than berries harvested at BHF due to better raking techniques and cleaning. Berries in pints (Batch 2) showed a trend towards having a warmer internal temperature than berries in quarts (Batch 1), yet this must be confirmed in 2022 by removing confounding variables. Possible reasoning for this is that the smaller pints are more vulnerable to temperature fluctuation within cold storage temperatures whereas quarts were able to hold their cooler temperature until the unit's temp dropped back down. Berries from both batches stored at 50°F had significantly warmer internal temperatures than those stored at 34°F and 40°F. Additionally, when relative humidity was low, our measure of berry moisture was also low indicating that our subjective method of monitoring berry moisture is a good indicator of relative humidity. Berry moisture presented significant treatment differences in Batch 2, such that the 40°F cooling treatment had significantly higher moisture than the 50°F treatment. This indicates that more condensation may develop on the berries at this temperature or the rate of cooling was a factor in moisture buildup within the system. The coldest temperature treatment (34°F) saw declines in berry firmness over time, however, these data were very variable and need to be repeated in 2022.

Some Maine wild blueberry growers have cold storage rooms, yet their berries remain warm and wet. Observationally, the harvest and cleaning techniques used greatly impacted berry quality, even more so than the temperature at which they are stored.

When constructing a cold storage unit, it is important to have a well-insulated and tightly sealed cold storage unit. Our units exhibited condensation, which caused mold buildup, which we plan to troubleshoot to eliminate this mold in 2022. Tilting the air conditioner back slightly so that condensation water drips out and away from the berries stored inside is critical. Managing relative humidity and air movement inside cold storage rooms is necessary to achieve high-quality berries. Constructing a unit akin to the cold storage units studied in this trial can cost a few thousand dollars per unit (including all materials and labor, building one unit in 2020 at BHF cost $2,600), and operational costs for the system

are similar to that of a refrigerator or walk in cooler (Callahan, 2013). Cooling to lower temperatures (such as 34°F and 40°F) will cost more than cooling to higher temperatures (such as 50°F) but the improved length of shelf life and resulting high-quality berries will likely justify the additional expense.

CURRENT RECOMMENDATIONS

- Field conditions at the time of harvest and the method of harvest impact berry quality and cannot be fixed with cooling measures.
- Consistently cool temperature is best.
- Temperature fluctuations cause wet berries.
- The earlier harvested berries can enter a cold storage unit, the better.
- For more information on post-harvest storage of wild blueberries please visit the Quality and Food Safety page of our website: https://extension.umaine.edu/blueberries/factsheets/quality/.

NEXT STEPS

- Adjust insulation, AC units, and CoolBot devices to maintain more stable temperatures within cold storage units.
- Make adjustments to make relative humidity stable within the cold storage units.
- Conduct surveys of 13 additional fresh pack facilities in summer 2022 season.
- Replicate study in summer 2022 season.
- Seek funding to test whether improving airflow and reducing humidity can improve berry quality.

ACKNOWLEDGEMENTS

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INVESTIGATOR(S): L. Calderwood, B. Calder, K. Davis-Dentici, B. Perkins, J. Perry (University of Maine), T. Esau (Dalhousie University), and J. Meyers (Cornell University)

2. Impact of Wild Blueberry Plant Architecture, Nutrients, and Phenology on Berry Quality

OBJECTIVE(S)

- Measure the impact of sunlight and temperature on berry development
- Conduct food science quality analyses on berries and grapes harvested on 4 harvest dates
- Collect leaf and berry samples for nutrient content throughout the season to understand when the plant uses certain nutrients

LOCATION(S): Appleton, Hope, Sedgwick, Columbia Falls, Maine; New York State; Nova Scotia, Canada

PROJECT TIMEFRAME: April 2021 – March 2024

INTRODUCTION

As more blueberries have been planted worldwide, the value of Maine's frozen wild blueberries has dropped, making it more attractive to diversify into value-added markets. Despite being grown commercially by 485 farmers on 41,000 acres, 76% of Maine (ME) growers surveyed did not make a profit in 2017 (Grower Survey 2018). Similar economic challenges are impacting wild blueberry growers in Nova Scotia (NS), New Brunswick (NB), and Prince Edward Island (PEI). This study includes NS locations through our collegial partnership with Dalhousie University.

Wild blueberry farmers are interested in expanding into value-added markets, which include the need to fresh pack, dry, juice, and freeze wild blueberries. Once the wild blueberries are in these different forms they can be sold by the farmer or processor to be made into tea, wine, beer, puree, juice, fruit leather, powder, and other value-added products. Several studies have expanded our knowledge of wild blueberry ripening (Mallick and Hamilton, 2017; Gibson et al., 2013; Forney et al., 2012) yet few have looked at regional patterns in ripening. In this study, we are particularly interested in the impact that local weather conditions and nutrient availability have on the crop throughout the season. In four ME locations and two NS locations, we are attempting to connect plant architecture, phenology, and nutrient availability with berry quality measures such as Brix, titratable acidity, color, organic acids, and fruit antioxidant content. Green, color change, and overripe berries are included in our study because finding uses for harvested waste berries is an important step in reducing "shrink" and the cost of production.

METHODS

Field Data Collection

This was the first year of a three year project and with so many PIs and moving parts, some data is still in the analysis stage as of report publication. Aspects not included in this year's report are berry size, antioxidant content, organic acid content, sugar content, color data and imaging analysis to predict yield. Additionally, an unofficial aspect of this project is to compare field and food science results with Bluet-brand wine made from fruit harvested from the exact same fields used in this study. This comparison has not yet occurred for 2021. The project encompassed six on-farm trial locations: four fields in ME (two organic, two conventional) and two fields in NS (one low- and one high-input farm).

Each wild blueberry farm contained six replicates with randomly selected plant diversity. Each plot was located within one distinct wild blueberry plant so that six plants were studied on each of the six farms. Each trial location had a weather station situated within 10 miles and each farm was managed according to standard grower practices.

Plant Architecture and Phenology (Calderwood of UMaine; Esau of Dalhousie)

Phenological data collection began at flowering stage 2 (F2). Measurements occurred within a 1 m^2 quadrat per plot and ten stems per quadrat were labeled for repeated measures throughout the season. Blueberry stem measurements included the numbers of leaf buds, flower buds, flowers formed, fruit set, green berries, pink berries, and green, pink, and blue/red berries on each of 4 harvest dates in all plots. To develop an understanding of the wild blueberry plant canopy, multiple photosynthetically active radiation (PAR) measurements were taken on each phenological and harvest date. PAR measurements were taken using an AccuPar LP80 (Meter Group, Pullman, WA, USA) within the 1x1m plot, creating a grid by taking measurements along the X-axis at 0, 20, 40, 60, 80, and 100cm and along the Y-axis at the same intervals (Figure 1). At each point on the grid where the LP80 readings met, a ruler stick was placed vertically alongside the closest stem to that grid vertex and the presence of fruit or leaves were manually recorded. Berry temperature was taken on three sunny and three shaded fruit clusters per plot using an Etekcity 1080 infrared digital temperature gun (VeSync Co. Ltd., Shenzen, China).

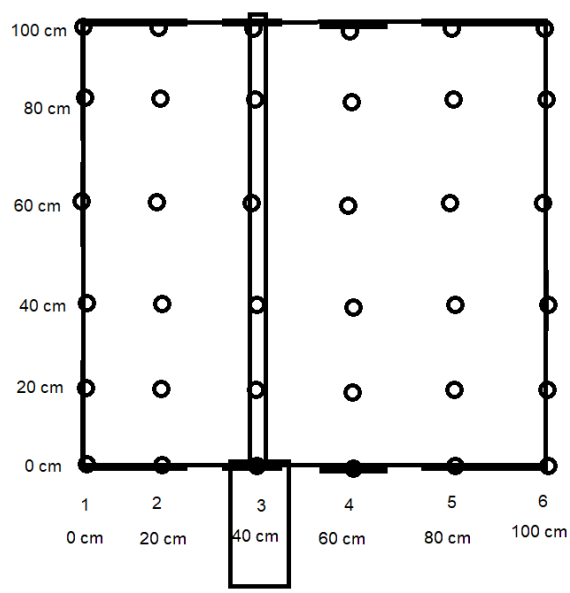

Figure 1. This figure shows where the PAR measurements and plant architecture data were measured. The X- and Y-axes are 1m long and PAR measurements (PAR meter indicated by the conjoined rectangles along the 40cm vertical line) were taken every 20cm along the X-axis. Canopy measurements were taken at the intersection of those 20cm divisions, marked in this image by circles.

The PAR readings, canopy data, and fruit temperature data were used to construct a canopy profile for the wild blueberry plants using Enhanced Point Quadrat Analysis (EPQA) (Meyers and Vanden Heuvel, 2018). This model approximates the amount of sunlight reaching each fruit cluster. The calculated exposure of fruit to sunlight was statistically analyzed for multivariate relationships among phenological data, fruit exposure to sunlight, and fruit chemistry variables in a similar manner to previous work with wine grapes (Meyers et al., 2013).

Canopy Architecture and Berry Quality (Meyers of Cornell)

Enhanced point quadrat analysis (EPQA) of wild blueberry was performed using an Excel spreadsheet and program originally designed to process point quadrat data from grapevines (Meyers and Vanden Heuvel, 2018). Some program modifications were made to account for the modified measurement methods needed for wild blueberries, specifically, the need to measure blueberry stems top-to-bottom versus side-to-side in grapevines.

Among the various metrics that the spreadsheet calculated, two were of greatest interest: Cluster Exposure Layer (CEL) and Cluster Exposure Flux Availability (CEFA). CEL calculates the average number of bio-mass contacts (i.e., leaves or fruits) that are providing shade to fruit where a higher number indicates more shading. CEFA incorporates photo flux measurements with CEL to calculate the average percentage of ambient sunlight (0% - 100%) that reaches fruit in the canopy. Regressions of fruit exposure, as measured by CEL and CEFA, with soluble solids (Brix), titratable acidity (TA), and pH were performed.

Plant Nutrients (Calderwood of UMaine)

At two locations in ME (Columbia Falls and Hope) and one location in NS (Kemptown), one foliar sample and one flower/fruit sample was taken from each plot/plant for nutrient analysis by the UMaine Analytical Lab and Soil Testing Service at full bloom, green fruit, color change, and blue fruit stages. Flowers and fruit were analyzed for nutrients using the same methods as foliar samples. Approximately 200g of leaves and flowers/fruit were collected and transported to Orono in paper bags. These samples were dried at 70°C until dry weights no longer dropped, ground to a fine texture, and then analyzed by the Soil Testing Lab. The Standard Analysis suite measured the levels of nitrogen, phosphorous, potassium, calcium, magnesium, aluminum, boron, copper, iron, manganese, and zinc present in each sample. Plant tissue nutrient data failed the normality assumption required for parametric testing for leaf tissue nutrients K, P, B, Cu, Fe, Mn, Zn and all fruit tissue nutrients except for K. Nutrients that failed the normality assumption were transformed using a square root transformation prior to all statistical testing. The transformation statistically improved leaf nutrients B and Mn and flower/fruit nutrients Ca, Fe, Mn and B. All other leaf and flower/fruit nutrients visually improved in their distribution following the square root transformation and subsequent statistics were carried out despite non-normality after establishing there were no serious problems with the data. Analyses were conducted in JMP (JMP® Pro, Version 15.2.0) to compare nutrient differences by location, stage and location by stage using a generalized linear model (GLM) followed by a Tukey's Pairwise comparison. Presented are the results of the interaction of stage by location relative to select nutrients.

Berry Quality Field Collection and Lab Analysis (Calderwood, Calder, and Perkins of UMaine)

Wild blueberries were harvested on 4 different dates throughout the season, during different phenological stages for food science analysis to determine changes in the fruits throughout the development and ripening process. Each harvest of approximately 150-300g of ME wild blueberries was handpicked from just outside each plot but within the same plant. Harvests occurred during the green fruit phase (June 14, 21, 23, & 29), color change phase (July 12 &14), blue fruit phase (July 20, 22, & 29), and overripe fruit phase (August 5). All ME wild blueberry samples were delivered to the University of Maine Food Science Labs in paper bags inside a cooler the day of harvest for food science analysis the next day. Samples were stored in a refrigerator overnight. The University of Maine Food Science Lab evaluated for color (HunterLab LabScan XE, Hunter Associates Laboratory Inc., Reston, VA, USA), pH (edge® meter, Hanna Instruments, Woonsocket, RI, USA), Brix (Atago RX-5000i Refractometer, Atago Co, Ltd., Tokyo, Japan), and titratable acidity (Orion Model Star A211 pH meter with glass ATC tip probe, Thermo Fisher Scientific, Waltham, MA, USA). The number of ME blueberry fruits present in a 50g sample from each quadrat was counted as well. Researchers weighed a 50g sample from a quadrat, counted the number of fruits comprising that sample, and recorded the number.

The 50g sample was then dumped back into the bag, gently shaken, and another 50g sample was drawn from the bag and counted. This counting was done after measuring color to prevent color alteration by handling.

The same harvest methods were used in NS excepting that NS's plants had more fruit and therefore it was possible to harvest more berries from each plant. All 200-400g NS wild blueberry samples were handpicked during the green fruit phase (June 24 & 30), pink phase (July 6 & 16), red phase (July 21 & 29), and blue fruit phase (August 4 & 17). In NS, ripening was spread out enough in time to allow for both pink and red harvests. In 2022 only one "color change" stage harvest will be collected. All NS samples were split and half per site were delivered to Dalhousie University's Health and Bio-products Lab and the other half to Acadia University's Acadia Laboratory for Agri-Food and Beverage. The Health and Bio-products Lab evaluated for color, pH, and titratable acidity. The Acadia Laboratory for Agri-Food and Beverage evaluated fructose & glucose, organic acids, and titratable acidity for green berries.

We experienced challenges with fruit harvesting and sampling, including the unexpected discovery that the green fruits were too dense and dry to homogenize, resulting in an inability to measure titratable acidity of green fruit at any site. In addition, the fruit ripened faster than usual this season, so the red stage at Sedgwick was missed as the ripe blue fruit was harvested nearly two weeks early.

Wine Spoilage Organisms (Perry of UMaine)

A subsample at each ME wild blueberry plot was analyzed for wine spoilage microorganisms using culture-based microbial analysis to see whether *Acetobacter* and *Gluconobacter* and *Pediococcus* organisms were present on the surface of the berries. Berries were aseptically portioned into sterile bags (in duplicate), diluted with sterile 0.1% peptone water, and homogenized by hand for one minute. The resulting homogenate was serially diluted as appropriate with 0.1% peptone water and spread plated (in duplicate) onto various agar media, including De Man, Rogosa & Sharpe (MRS, for enumeration of lactic acid bacteria), acidified potato dextrose (APDA, for enumeration of fungi), and malt extract yeast extract glucose peptone agar with copper (MYGP, for enumeration of "wild"/non-*Saccharomyces* yeasts). Inoculated plates were incubated for up to five days at appropriate temperatures (ranging from 25 - 35°C, dependent on target microbial population). Plates were counted and data were log transformed for normality before analysis.

RESULTS

Field Phenology and Nutrients (Calderwood of UMaine)

Phenological Development

Evaluating Nova Scotia's (NS) and Maine's (ME) weather and phenology averaged across both separate territories shows a clear difference in the timing of phenological stages and the accumulation of Growing Degree Days (GDD, see "Wild Blueberry Phenology" report on page 123 of this report) between the two territories (Figure 3). The NS field locations are 330 to 500 kilometers (205-310 miles) east and northeast of ME's eastern-most and western-most fields of study. In this first year, ME's growing season began earlier with faster/greater accumulation of GDD by calendar date forcing ME to conduct 3 harvests (green, color change, and blue fruit) while it was still possible for NS to conduct 4 harvests (green, pink, red, and blue fruit). In quantifying and establishing the growth and development of wild blueberry in response to geographic location and localized weather, these differences by territory are essential.

In ME, flower number per stem averaged across all sample locations peaked the week of May 17, around 590 GDD with 12.8 active flowers per stem. In Nova Scotia, peak bloom occurred the week of May 31, at 420 GDD with 27 active flowers per stem. Higher flower counts in NS have been attributed to increased branching and different fertility regimes which will be further investigated in 2022. In ME, peak green fruit occurred the week of June 21 at 1370 GDD, with an average of 8 green fruits per stem.

In NS, green fruit peaked the same week of June 21, but with 820 GDD and 16 green fruits per stem. Finally, peak blue fruit in ME occurred the week of July 26 at 2360 GDD and an average of 6 blue fruits per stem. In NS, peak blue fruit occurred the week of August 2 at 1700 GDD and an average of 7 blue fruits per stem.

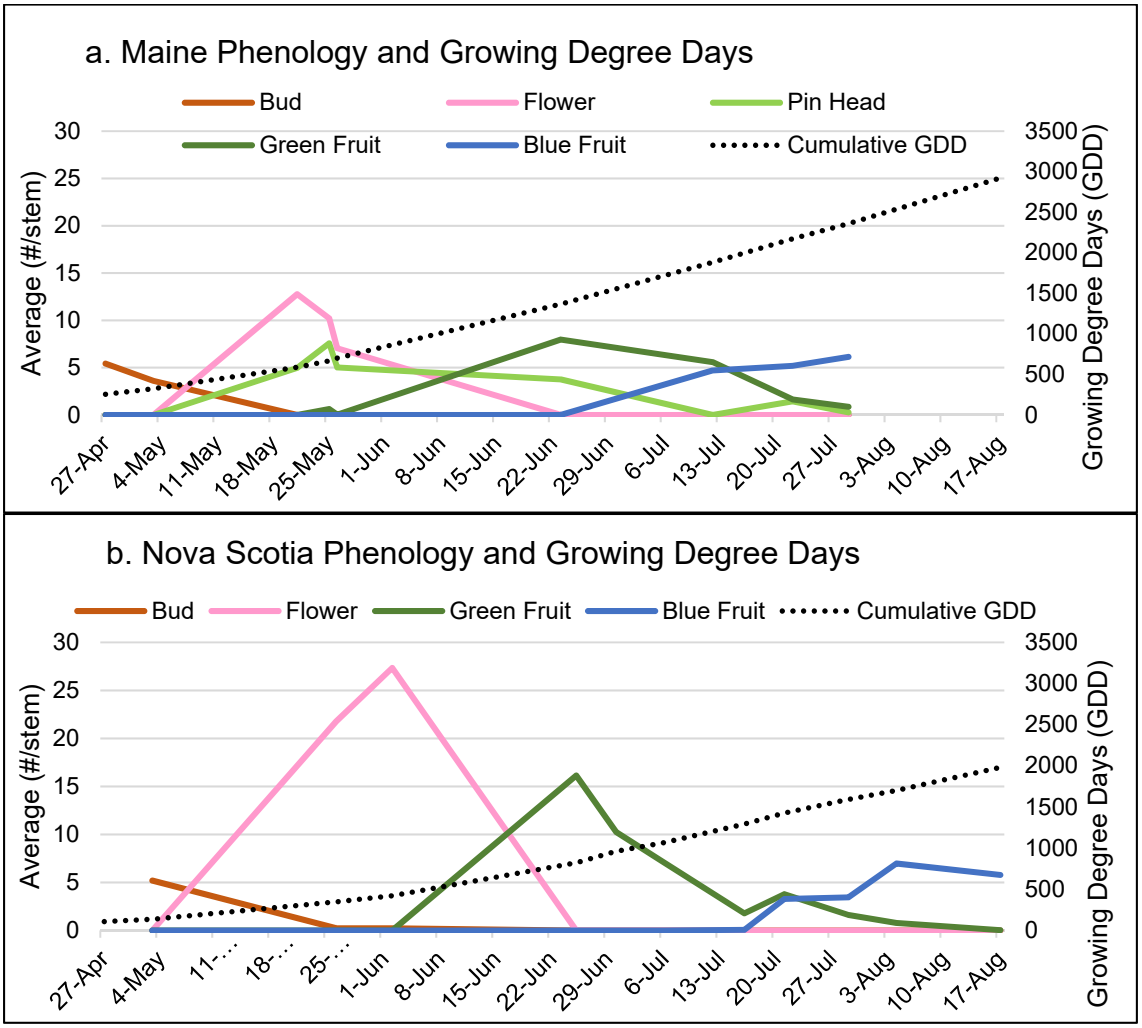

Figure 3. The phenological development of the blueberry paired with regional growing degree days (GDD) at all locations averaged by territory for Maine (3a) and for Nova Scotia (3b) from April 27 through August 17, 2021.

Plant Nutrient Contents

Plant nutrient contents of leaves in ME and NS decreased as the season progressed, despite differences in fertility management between farms (Figure 4). The standard recommendation for %N in leaves ranges from 1.55-1.85% indicating that leaves at full bloom had a high N content (all values above 1.85%) that dropped down to the standard range in leaves once the plant had green, color change, and blue fruit to support. The same pattern occurred for %P (standard range 0.111-1.43%; values started greater than 0.23%) and %K (standard range 0.31-0.56%; values started greater than 0.56%). Leaves at all stages had sufficient Ca falling within or above the standard recommendation of 0.31-0.40% Ca. Kemptown showed a trend toward lower %K in leaves at green, color change, and blue fruit stages. Fruit nutrient contents of wild blueberry do not have established standards but N, P, K, and Ca content declined from flower to blue fruit (Figure 5).

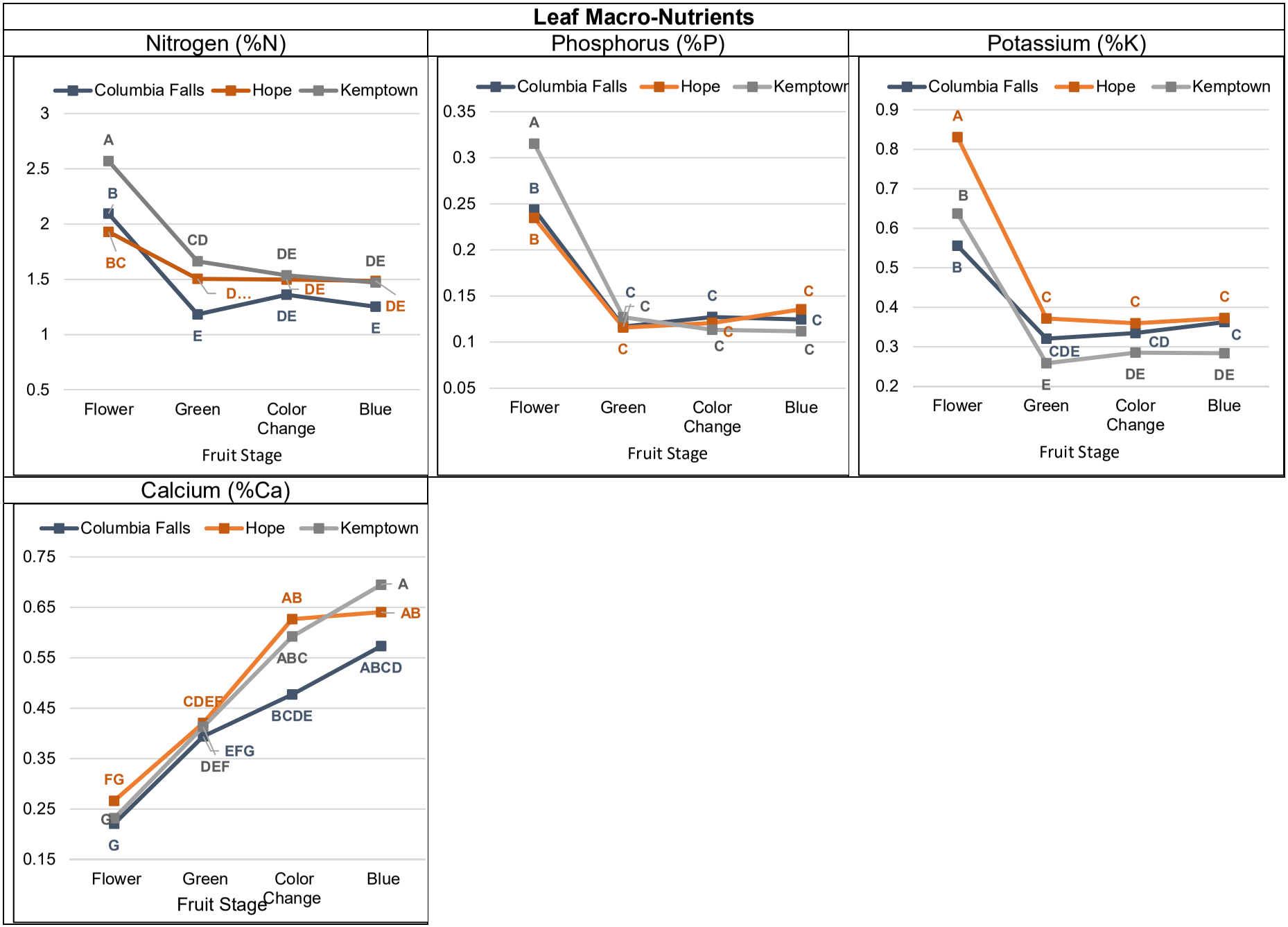

Figure 4. Leaf nutrients through the season in ME (Columbia Falls and Hope) and NS (Kemptown) taken at full bloom, peak green fruit, color change, and blue fruit. Percent N, P, K, and Ca are shown by crop fruiting stage.

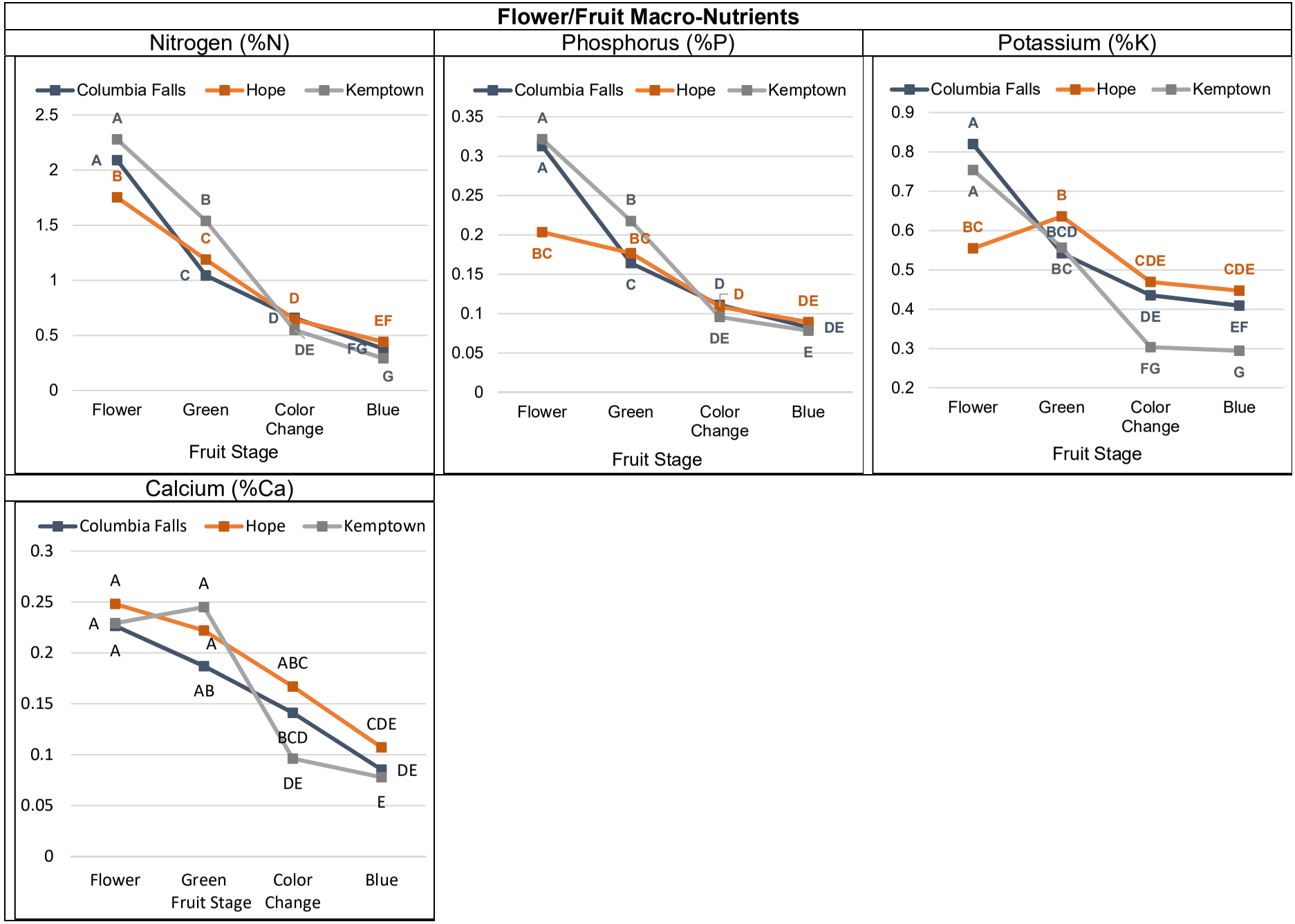

Figure 5. Flower and fruit nutrients through the season in ME (Columbia Falls and Hope) and NS (Kemptown) taken at full bloom, peak green fruit, color change, and blue fruit. Percent N, P, K, and Ca are shown by crop fruiting stage.

Berry pH, Titratable Acidity, and Brix (Calder of UMaine; Esau of Dalhousie)

Average blueberry pH shown in Figure 6 indicated that fruits' pH increased with maturation. As expected, the blueberries were more acidic during the early stages, and the pH levels increased as the fruits matured, which indicates a decrease in acidity over time. At the blue fruit stage, pH levels ranged from 3.22-3.38.

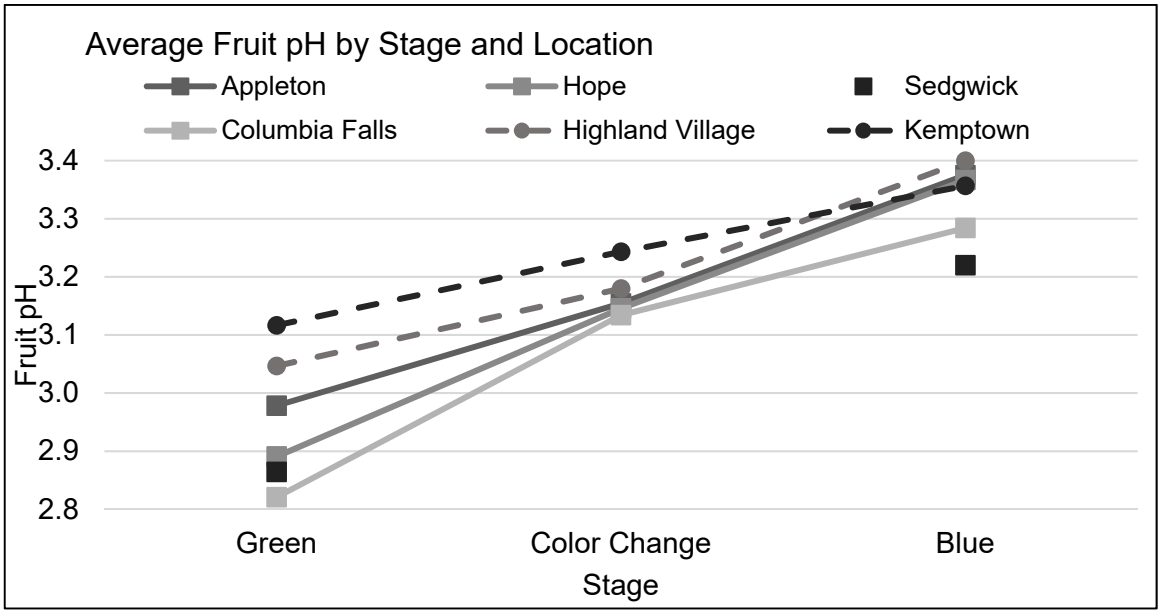

Figure 6. The average fruit pH by location and stage.

The percent titratable acidity (%TA) was measured to determine total acidity changes over time. The TA averages for the red and blue fruit stages are shown in Figure 7. The TA is reported as % citric acid, which is the predominant acid in blueberries. As the fruits matured, the TA decreased over time making berries sweeter. At the blue fruit harvest stage, TA ranged from 0.36-0.39, on a fresh weight basis. Green fruit TA was completed only at the NS lab where site locations ranged from 1.2 to 1.4.

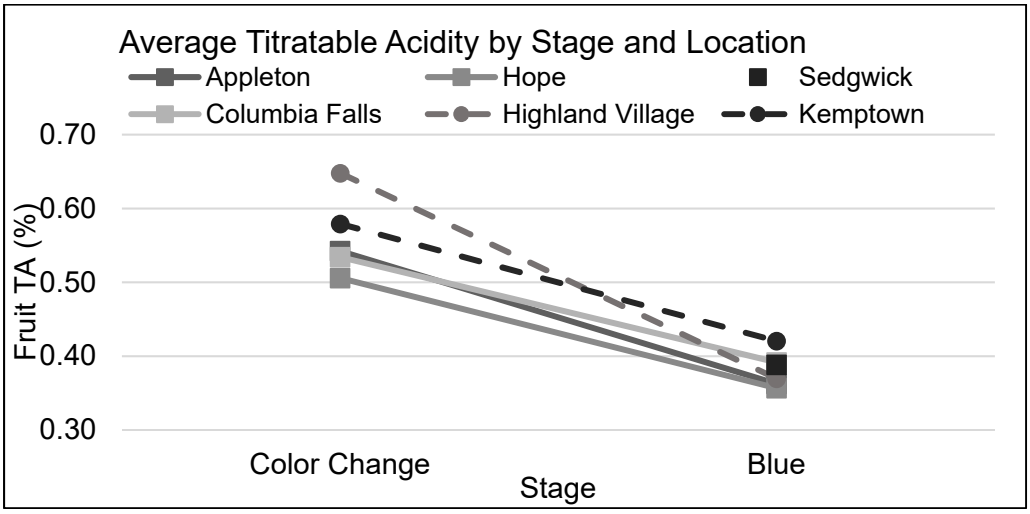

Figure 7. The average titratable acidity by location and stage. Green fruit is not included due to titratable acidity not being performed at this stage in ME.

The %Brix, or percent soluble solids is shown in Figure 8. %Brix is a way to quantify or measure changes in sucrose levels or sweetness changes as fruits mature. As expected, the %Brix level increased, especially between the green and red harvest stages. However, the %Brix levels either did not change or slightly decreased between the red and blue harvest stages, and the %Brix level ranged

from 10.98-12.22 at the blue fruit stage among the different sites. Overripe fruit at Columbia Falls was collected and expected to have higher %Brix content for use in wild blueberry wine but data is not shown here as it was the only site sampled for overripe fruit.

The relationship between %Brix and TA averages at red and blue fruit stages is shown in Figure 9. At the red fruit stage, the %Brix and TA followed a similar trend. However, during the blue fruit stage, the graph shows the decrease in TA, while the %Brix levels were maintained, with a slight decrease in the %Brix for blueberry samples at Columbia Falls (ME) and Kemptown (NS).

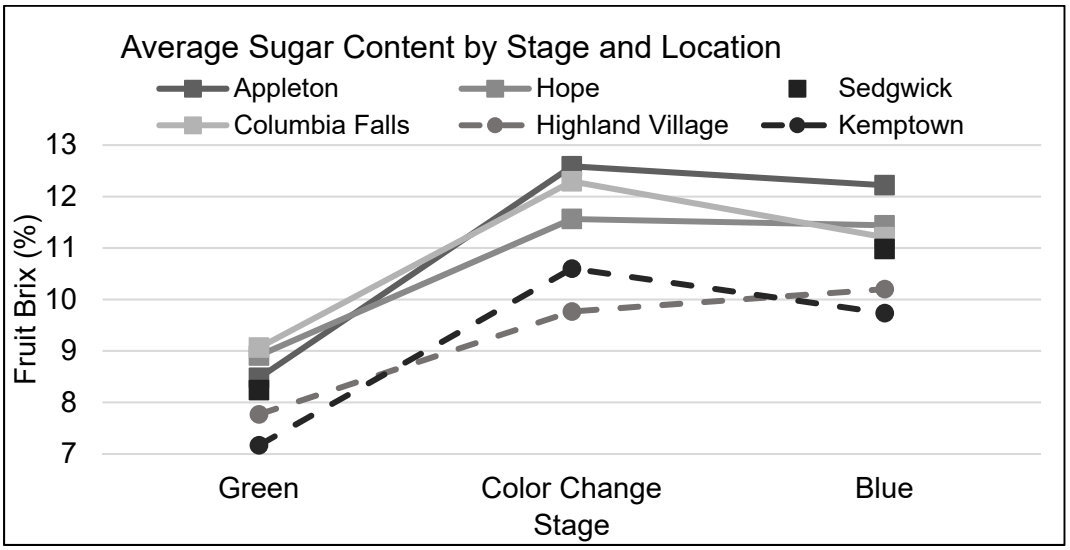

Figure 8. The average sugar content (% Brix) by location and stage.

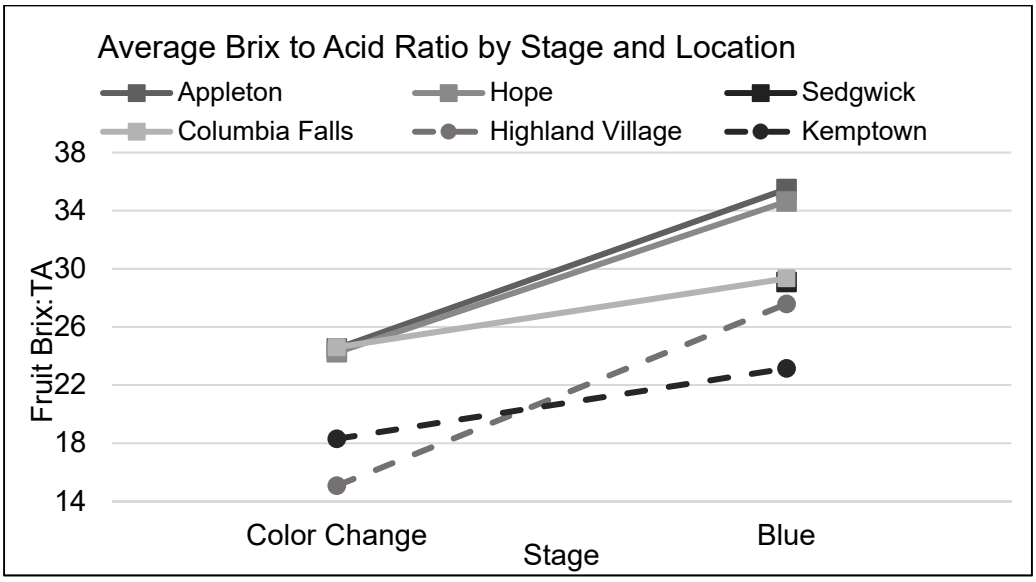

Figure 9. The average Brix (%) to acid (%) ratio by location and stage. Green fruit is not included due to titratable acidity not being performed at this stage.

<u>Wine Spoilage Organisms (Perry of UMaine)</u>

Sample sizes were small for wine spoilage analysis of ME fruit due to limited funds but included green fruit (n=18; samples from Hope, Appleton, and Columbia Falls), red fruit (n=12; samples from Hope, Appleton, and Sedgwick), and blue fruit (n=6; samples only from Columbia Falls). Figure 10 outlines the bacteria, yeasts, and mold cultured from green, color change, and blue fruit respectively. The

quantity of yeast detected was low compared to previous levels documented on wild blueberry fruit. No mold was detected on color change fruit or blue fruit.

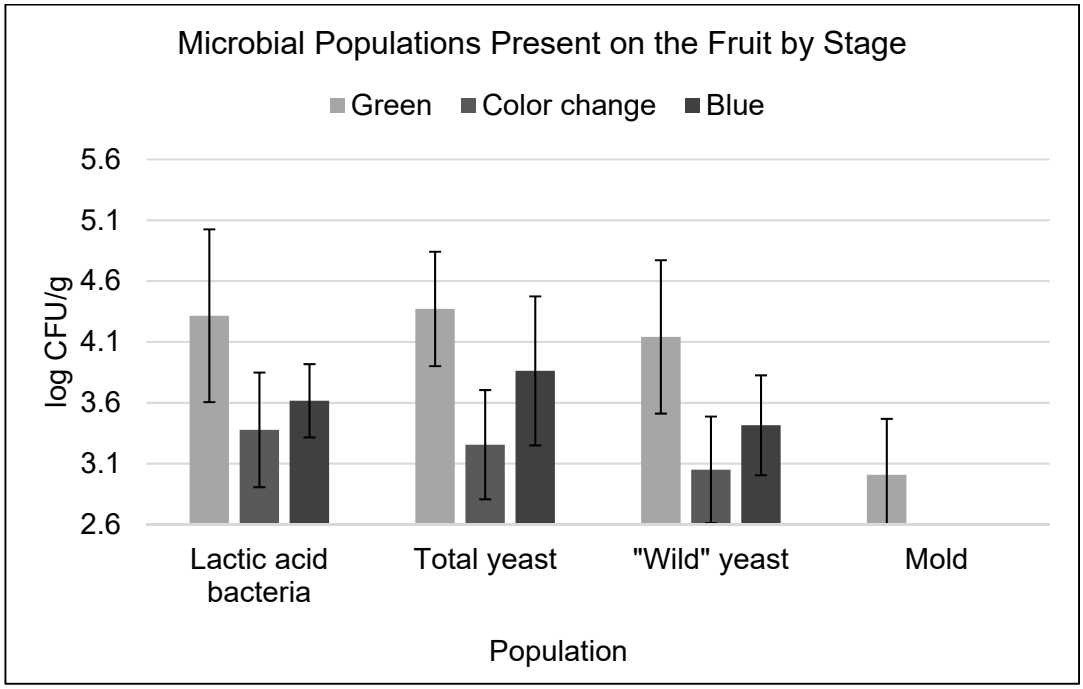

Figure 10. Microbial quality indicator populations on wild blueberry fruits during ripening. Error bars denote standard deviation. Minimum detection limit is 2.6 log CFU/g. Note that no mold was detected in red or blue fruit samples.

<u>Canopy Architecture Impacts on Food Science (Meyers of Cornell)</u>

Regressions of fruit exposure, as measured by CEL and CEFA, with soluble solids (Brix), titratable acidity (TA), and pH were performed. Figure 11 suggests that fruit exposure, as quantified with CEL and CEFA, does not correlate with Brix or pH in the green fruit stage (TA was not measured in this stage) with all R^2 values below 0.06. At red fruit, Figure 12 suggests that Brix is positively correlated with fruit exposure (lower CEL = higher sunlight exposure and higher CEFA = higher sunlight exposure) when quantified as either CEL (R2 = 0.57) or CEFA (R2 = 0.50). Figure 13 suggests that there are no correlations among fruit exposure metrics and berry chemistry in the blue fruit stage.

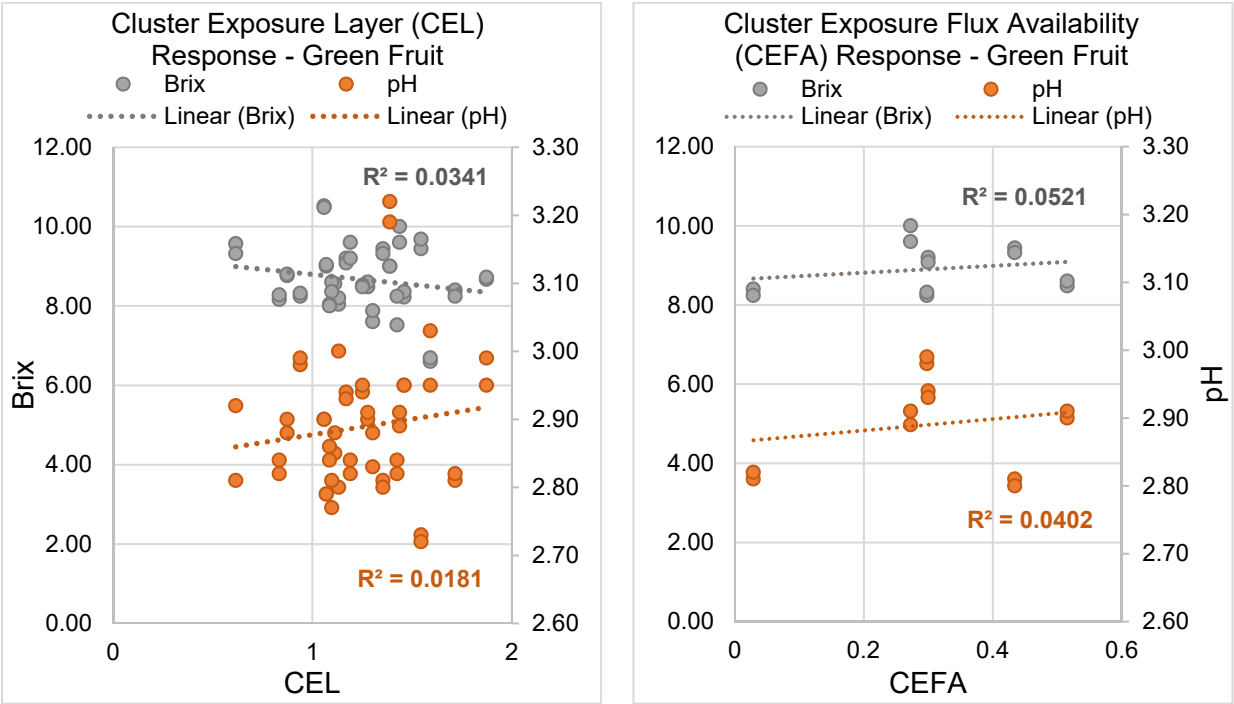

Figure 11. Correlations among Brix and pH with fruit sunlight exposure in green stage wild blueberries. Sunlight exposure is quantified as Cluster Exposure Layer (CEL) and Cluster Exposure Flux Availability (CEFA).

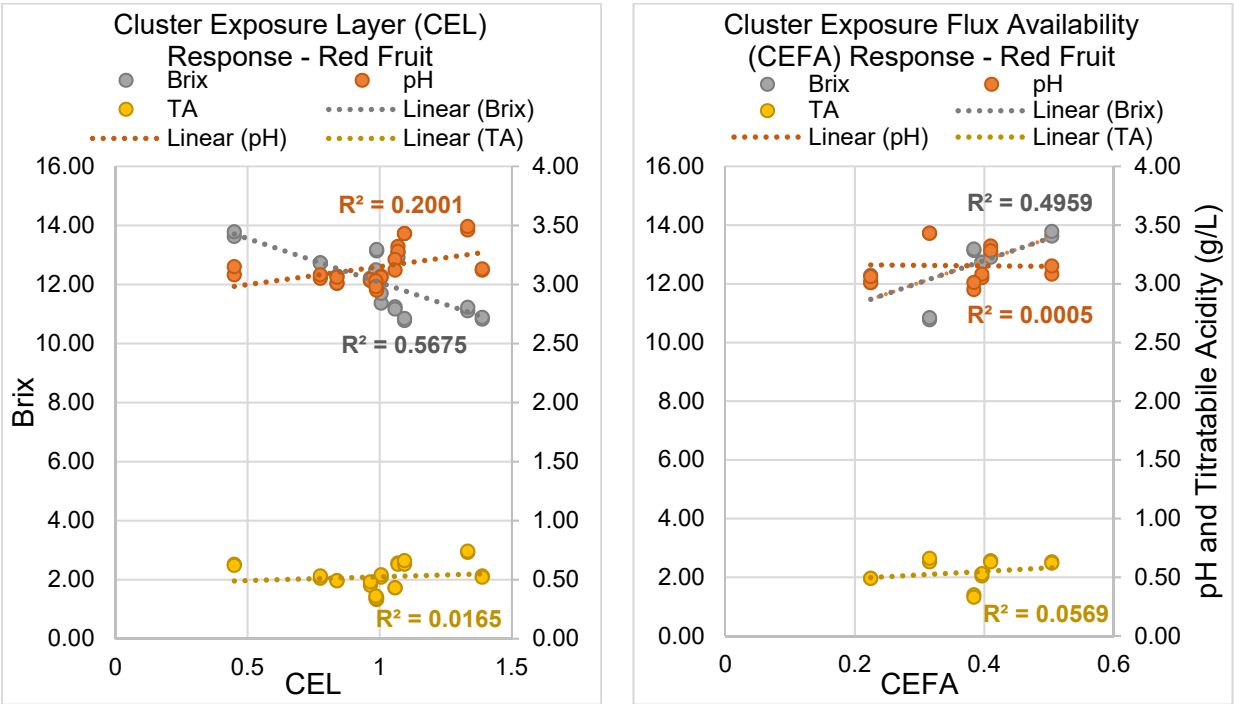

Figure 12. Correlations among Brix and pH with fruit sunlight exposure in red stage wild blueberries. Sunlight exposure is quantified as Cluster Exposure Layer (CEL) and Cluster Exposure Flux Availability (CEFA).

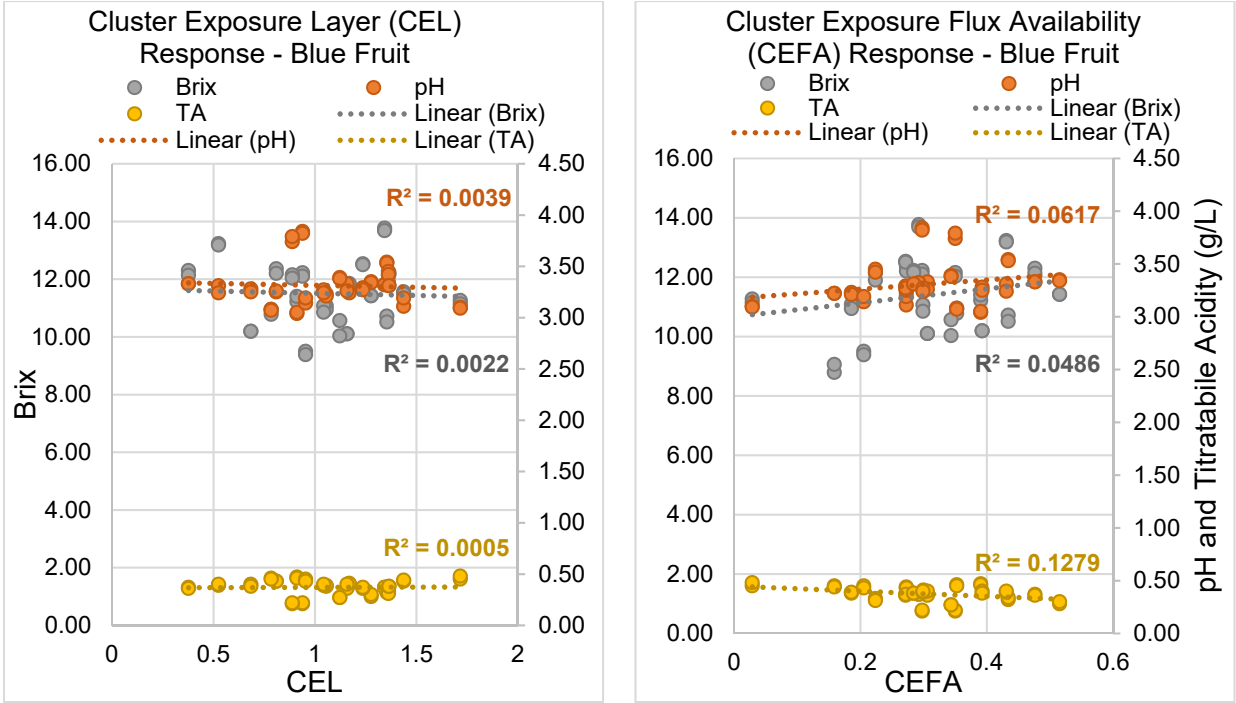

Figure 13. Correlations among Brix and pH with fruit sunlight exposure in blue stage wild blueberries. Sunlight exposure is quantified as Cluster Exposure Layer (CEL) and Cluster Exposure Flux Availability (CEFA).

DISCUSSION

Phenological Development

As expected due to latitude differences, ME accumulated GDD faster than NS with 660 more GDD than NS by peak blue fruit (ME: July 28, NS: Aug 3). ME crop stages were ahead of NS until green fruit which occurred the week of June 22 in both locations, indicating that temperature alone cannot predict berry development in wild blueberry (Spinardi et al., 2019). (For a more in-depth explanation, see "Wild Blueberry Phenology" on page 123 of this report). NS exhibited many more buds, flowers and green fruit per stem than ME but upon reaching the blue fruit stage, both territories had very similar numbers of blue fruit per stem. This requires further investigation, however, it is possible that NS's more sophisticated fertility management program (including foliar sprays) increased bud, flower, and green fruit production, but the plants could not carry such a high load of berries to full maturity. Fruit drop was not measured but it can be assumed that the fruit decline from green to blue fruit was fruit abortion (drop) by plants. It is very difficult to identify why fruit drop occurs because many factors can impact ultimate yield in wild blueberry (Drummond, 2019).

Plant Nutrients

Percent N, P, and K in leaves indicated that leaves during full bloom had a high nutrient content across locations which dropped down to the UMaine Analytical Lab and Soil Testing Service's standard ranges for each nutrient in leaves at the green, color change, and blue fruit stages. This shows the "source-sink" relationship we expected to see where nutrients from leaves were shifted to fruit or other parts of the plant once fruit began to form (Doyle et al., 2021). Interestingly, however, the fruit nutrient content data did not support the "source-sink" concept: over the course of fruit development, N, P, K, and Ca levels decreased. The standard leaf recommendations do not translate to flower or fruit recommendations, but it is interesting to note that N and Ca dropped well below the leaf standard recommendations. Further investigation and literature review is required.

Plant Architecture and Berry Quality
A correlation between pH, Brix, and TA was only observed with color change fruit. A possible explanation for the difference in responses across the three fruit ripening stages is that green fruit is still largely undifferentiated early in the season, meaning that green fruit is hidden behind leaves. The lack of correlations in blue fruit could suggest that the effect of sunlight exposure on Brix is short lived and only impacts fruit during color change, but future data is needed to draw any conclusions.

Quality
Blueberry flavor is complex and difficult to quantify. The %Brix/%TA ratio (Bx/TA; or sugar-to-acid ratio) was calculated to measure overall sweetness and tartness changes among samples, which is illustrated in Figure 6. The Bx/TA ratio increased during fruit development from red to blue fruit and ranged from 24.25 to 35.48. The highbush blueberry industry uses a suggested fruit quality standard of 10.0 to 33.0 Bx/TA ratio as an acceptable range for blueberries (Beaudry, 1992). However, this Bx/TA is only an estimate and would need to be validated with a consumer acceptability (sensory) test.

Wine Spoilage
Wine spoilage organisms were quantified on blueberry fruit from multiple study plots to aid in understanding how to make better wines from wild blueberries. The presence and health of microbiota in wine is partially dependent on the levels of acid, sugar and oxygen in the fermentation.

Lactic acid bacteria are overwhelmingly considered detrimental to wine quality because they acidify the product. Depending on which genera are present, these bacteria also produce various defects including buttery odor and flavor and ropiness (a texture that can be slimy or viscous).
The impact of yeast on wine quality is more complex. Yeast can significantly impact the volatile organic compound (VOC) composition of the finished wine. Generally, any yeast found would be expected to ferment juice and yield something that could be considered a wine. However, yeasts also have the capability to 1) produce different alcohols (as opposed to ethyl alcohol produced by brewing yeasts) and esters (usually beneficial, contributing fruity/floral aromas); 2) differentially reduce aldehydes or not; 3) most notably produce volatile phenols, which cause defects such as a "band-aid smell." The most common of the wild yeasts include genera such as *Brettanomyces* and *Pichia,* both of which usually produce a lot of phenols. Wild yeasts are also known to produce acetate, which has been observed by wild blueberry wine makers in Maine. Overall, the level of yeast observed was low. Levels of yeast in wild blueberry have been observed greater than 6 log CFU/g.

The levels of yeast represented here would be sufficient to drive a spontaneous fermentation. Further investigation is required to determine what species are present and how stable the community is because these yeasts could either produce a very variable product if unstable or if stable, could potentially lead to the reproducible production of unpleasant characteristics (in smell, texture, or taste). Another aspect of wine spoilage organisms that should be investigated is the impact that field management has on the microbiota community documented. For example, fungicides would be expected to have a variable effect on the different genera, allowing the farmer to select for "better" or "worse" yeasts on fruit without knowing it.

Although not shown in Figure 10, acetic acid bacteria were also included in the analysis. These bacteria are generally seen as having a negative influence on wine but are difficult to culture. No acetic bacteria were found until blue fruit when a high amount was detected. Considering the lack of any trend and the fact that the blue fruit samples were only from one site, we are not confident enough to draw any conclusions.

CURRENT RECOMMENDATIONS

None at this time.

NEXT STEPS

- Complete analysis of 2021 data
- Review 2021 challenges and modify methods as necessary
- 2022-2024 collect two more years of data

ACKNOWLEDGEMENTS

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