2022 Wild Blueberry Research & Extension Reports

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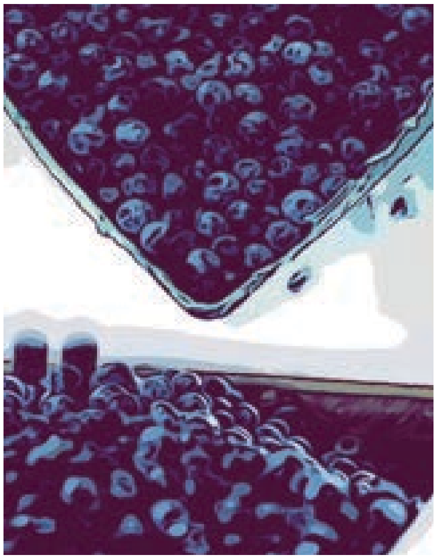

The work presented here would not be possible without
our farmer and processor collaborators and funding sources.

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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INVESTIGATORS: P. Fanning and B. Johnson

1. Baited trap comparison for monitoring of spotted-wing drosophila, Year 2

OBJECTIVES

This is year two of a study that began in 2021. The intent of this trial was to examine variations to the standard yeast/sugar baited red Solo® cups used in Maine to monitor for spotted wing drosophila (SWD) with the goal of increasing adult SWD capture and retention.

LOCATION: Jonesboro, ME
PROJECT TIMEFRAME: June 2022 – August 2022

INTRODUCTION

It has been shown that the current trap style has room for improvement when it comes to these two key metrics. Improved trapping methodology will result in a more accurate representation of SWD populations in and around wild blueberry fields.

METHODS

Six trap styles were tested:

1) Trécé® Pherocon PeelPak Broad Spectrum Lure + Cup trap
2) Trécé Pherocon PeelPak Broad Spectrum Lure + Red sticky trap
3) Scentry® Lure + Cup Trap
4) Scentry Lure + Red sticky trap
5) Yeast/Sugar + Red Solo cup
6) Aged diluted grape juice + Red Solo cup

There were four replicates. Individual trap placement was randomized within each replicate each week to prevent location biases. Traps were suspended 1-2 ft above the canopy along the field edge with approximately 10m (33ft) between neighboring traps. Trapping began on 14-June and ended on 9-August. Traps were checked weekly, with number of adult male and female SWD captures being recorded and new traps placed along the field edge. All Scentry and Trécé lures were replaced every 4 weeks, while the drowning liquid and liquid baits were changed every week.

RESULTS

The first trap capture occurred during the week of 14-June, and for the first two weeks only the red panel traps captured SWD. Due to extremely low trap captures in the first five weeks of this trial, only data from 26-July to 9-August were included for analysis. Overall trap capture data were log(x+1) transformed to correct for normality and analyzed using a one-way ANOVA with Tukey post-hoc tests ($\alpha=0.05$). The ANOVA revealed significant differences between the treatments ($F_{(5,66)} = 11.266$, $P = 0.0001$) (Figure 1).

A Kruskal-Wallis was run on cumulative male trap captures within each week, with there being no significance between trap styles until the last two weeks (Figure 2, Figure 3).

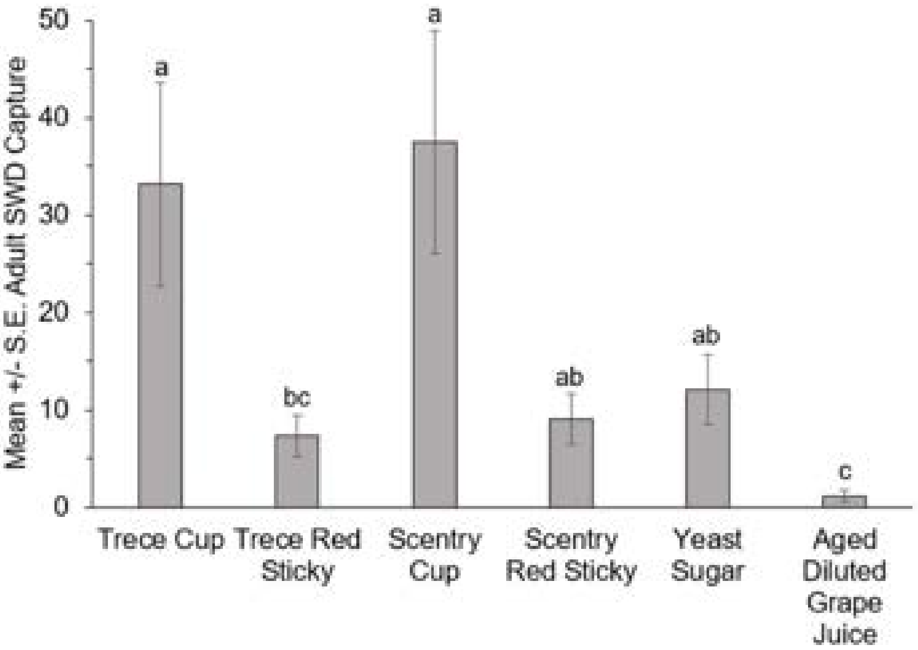

Figure 1. Average weekly adult capture from 26-July to 9-August. Columns topped by different letters are significantly different.

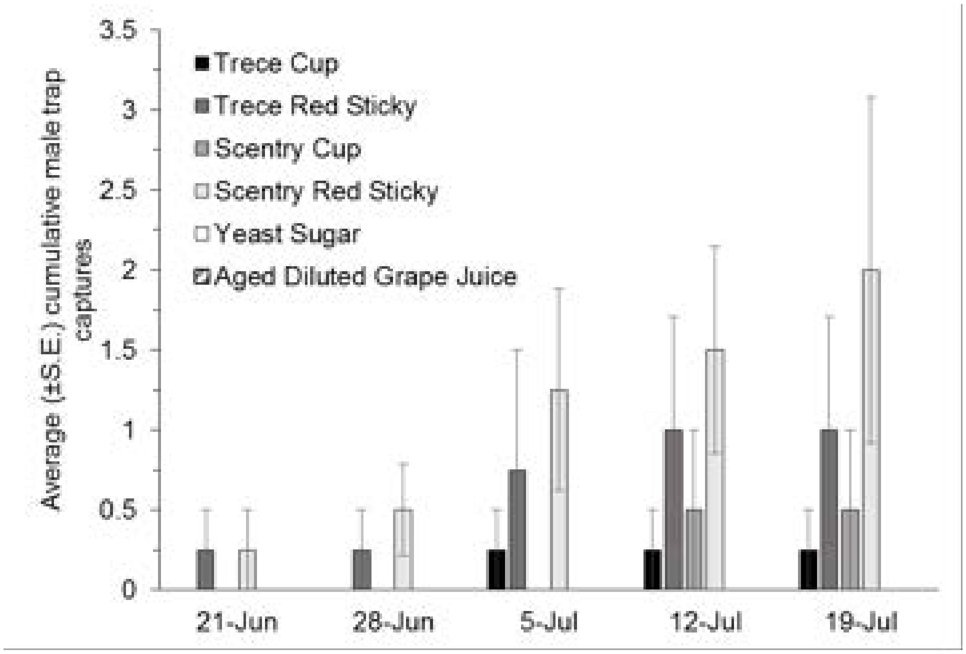

Figure 2. Average cumulative male trap captures for the first five weeks of trapping. There was no significant difference between trap styles during these weeks.

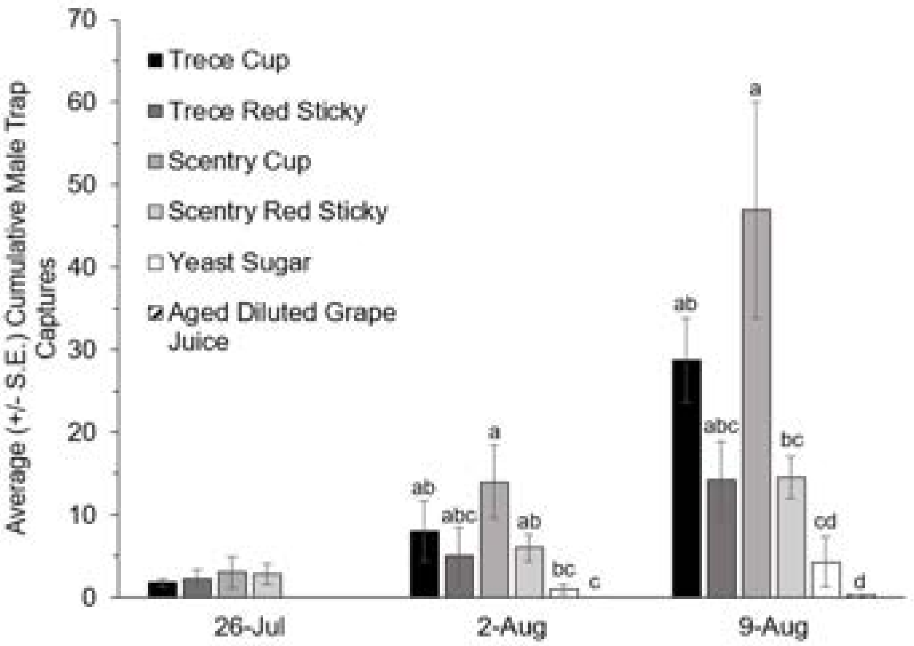

Figure 3. Graph displaying cumulative male trap captures for the last three weeks of trapping. Columns topped with different letters within each date display significantly different values.

CONCLUSIONS AND RECOMMENDATIONS

When it came to overall adult trap captures, all traps (except for the diluted aged grape juice) performed as well as the standard red solo cup baited with yeast and sugar water. This contrasts with last year where a red sticky card baited with the Trécé Pherocon PeelPak lure performed better than the other trapping styles. When looking at cumulative male trap captures, by the last week the standard red Solo yeast/sugar trap caught a cumulative total average of 4.25 males, significantly fewer than cup traps baited with commercial Scentry and Trécé lures. This year saw the first trap capture at the same time as in 2021 but captures remained low for several weeks after. This could be due to the unusually warm and dry weather.

Monitoring remains an integral component of managing for SWD, and consistent monitoring is key. A threshold model based on adult male SWD capture is being created for red sticky traps, to give growers more options for monitoring their fields for SWD.

NEXT STEPS

- This work will be continued in 2023.

ACKNOWLEDGEMENTS

We thank Abigail Fisher and Eric Desbois for their assistance with this study.

INVESTIGATORS: P. Fanning and B. Johnson

2. Efficacy of Combi-Protec® and insecticides in field and semi-field trials against spotted-wing drosophila, Year 2

OBJECTIVES

This is year 2 of a study initiated in 2021. The purpose of this trial was to further 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. In 2021, the only significant difference of note was between the Combi-Protec (CP) Only and Delegate WG + CP treatments (*P* = 0.0304). However, as 2021 did not include a treatment of just half-rate insecticide without Combi-Protec, this left 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. 2022 saw the addition of this treatment.

LOCATION: Jonesboro, ME
PROJECT TIMEFRAME: August – September 2022

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 (6 oz/acre; full rate)
2) Delegate WG (6 oz/acre) + Combi-Protec (14 oz/50 gal)
3) Delegate WG (3 oz/acre; 50% rate)
4) Delegate WG (3 oz/acre; 50% rate) + Combi-Protec (14 oz/50 gal)
5) Delegate WG (6 oz/acre) @ 50% coverage – 17.5 gal/acre
6) Delegate WG (6 oz/acre) + Combi-Protec (14 oz/50 gal) @ 50% coverage – 17.5 gal/acre
7) Combi-Protec only, control treatment (14 oz/50 gal)
8) Untreated control

There were four applications of each treatment, applied on 3, 10, 19, and 25 August. Treatments were applied to 10 x 14-ft plots in a complete randomized block design at Blueberry Hill Farm in Jonesboro, ME. Each treatment was replicated four times. All materials were applied in 17.5 (50% coverage) or 35 gallons (full rate) 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 6oz (by volume) berry sample was taken weekly from 9 August through 31 August (9, 16, 23, and 1 September). 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 3 August application using treated foliage and berries collected from the “Field Trial” plots. Each bioassay container consisted of a 32 oz deli cup, a 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 post treatment, leaf terminals with 3-4 leaves were clipped and placed in a filled water pick in a bioassay container. Thirteen berries were collected from each plot and placed in the wire mesh containers. Ten, 5-7 day old SWD adults (5 male and 5 female) were then added to each bioassay arena. 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 addition of adult SWD. On day 6, berries were removed from the bioassay containers and placed in a rearing cup in an environmental chamber (22°C; 70% RH) for 20 days to allow for adult emergence, which was then quantified.

Figure 1. Photo of semi-field bioassay arena.

RESULTS

Field Trial

Due to non-normality and heteroscedasticity, data were analyzed using a Kruskal-Wallis test. Infestation was extremely low over the first three sample dates; therefore, only the last day (1 September) was used in the analysis. No significance was found between the treatments ($H_{(7)}$=13.108, *P* = 0.0695). The results are outlined in Table 1 and Fig. 2.

Table 1. Field control of spotted-wing drosophila with insecticides, summary. Mean (± S.E.) number of larvae/gram extracted from blueberries.

		Rate	9-Aug			16-Aug			23-Aug			1-Sep		
Trt #	**Material**	**oz/acre**	**Mean ± S.E.**			**Mean ± S.E.**			**Mean ± S.E.**			**Mean ± S.E.**		
1	Delegate WG	6 oz	0.000	±	0.000	0.030	±	0.014	0.025	±	0.009	0.596	±	0.078
2	Delegate WG +	6 oz	0.002	±	0.002	0.000	±	0.000	0.013	±	0.013	0.722	±	0.229
	Combi-Protec	9.8 oz												
3	Delegate WG	3 oz	0.012	±	0.009	0.011	±	0.004	0.018	±	0.013	0.976	±	0.100
4	Delegate WG +	3 oz	0.011	±	0.011	0.000	±	0.000	0.023	±	0.012	0.813	±	0.116
	Combi-Protec	9.8 oz												
5	Delegate WG (50% coverage)	6 oz	0.002	±	0.002	0.000	±	0.000	0.007	±	0.005	0.472	±	0.096
6	Delegate WG (50% coverage)	6 oz	0.002	±	0.002	0.008	±	0.005	0.000	±	0.000	0.465	±	0.024
	Combi-Protec	9.8 oz												
7	Combi-Protec only	9.8 oz	0.000	±	0.000	0.018	±	0.012	0.035	±	0.016	1.132	±	0.150
8	UTC		0.005	±	0.003	0.034	±	0.013	0.007	±	0.005	0.919	±	0.304
		Statistic										*$H_{(7)}$ = 13.108, P=0.0695*		

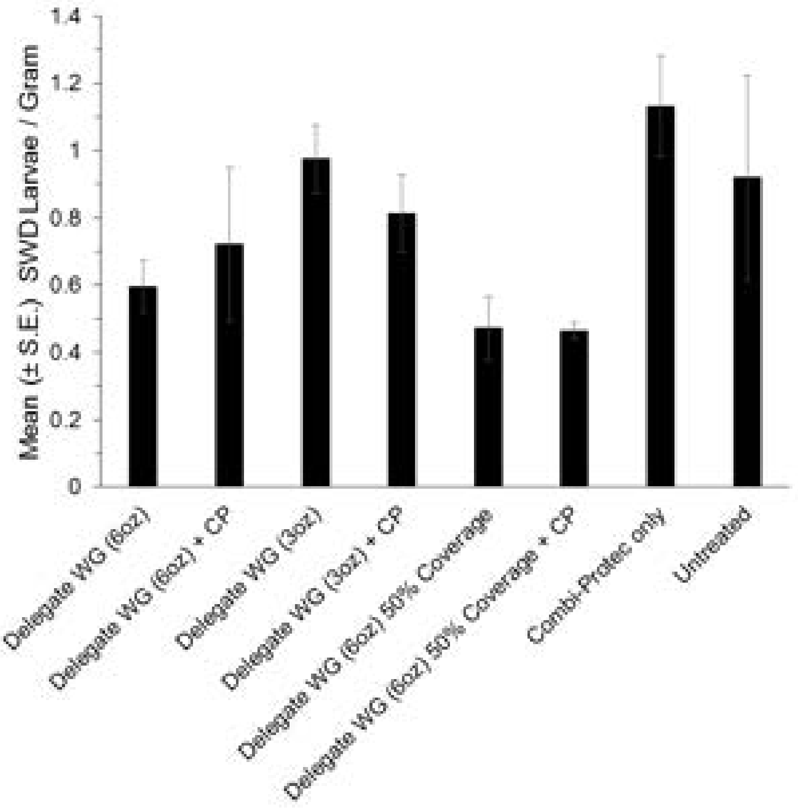

Figure 2. Mean (±S.E.) number of spotted-wing drosophila larva per gram of wild blueberry for the 1-Sept collection date. There were no significant differences seen between treatments at α=0.05.

Semi-field Bioassay

Due to non-normality and unequal variances, data were analyzed using a Kruskal-Wallis test. There was no significant difference in adult mortality between the treatments at the 1 D.A.T. ($H_{(7)} = 13.648$, $P = 0.058$) or 3 D.A.T. ($H_{(7)} = 3.318$, $P = 0.854$) time points (Fig. 3). Adult emergence at the 1 D.A.T. time point was non-normal and heteroscedastic and was therefore analyzed with a Kruskal-Wallis. There was no significant difference in emergence at the 1 D.A.T. ($H_{(7)} = 6.3818$, $P = 0.4959$). The 3 D.A.T. emergence data was analyzed using a one-way ANOVA with a Tukey HSD post-hoc ($\alpha=0.05$). The ANOVA showed significant differences between treatments ($F_{(7,24)} = 3.2416$, $P = 0.0145$) and the Tukey revealed that the Delegate half rate treatment had significantly higher emergence than both the Delegate full rate + Combi-Protec ($P = 0.0473$) and the Delegate half rate + Combi-Protec ($P = 0.0130$) treatments. The fact that Delegate half rate saw a significantly higher emergence than Delegate half rate + Combi-Protec indicates that the addition of Combi-Protec to a half rate spray provides greater protection than the half rate insecticide alone (Fig. 4).

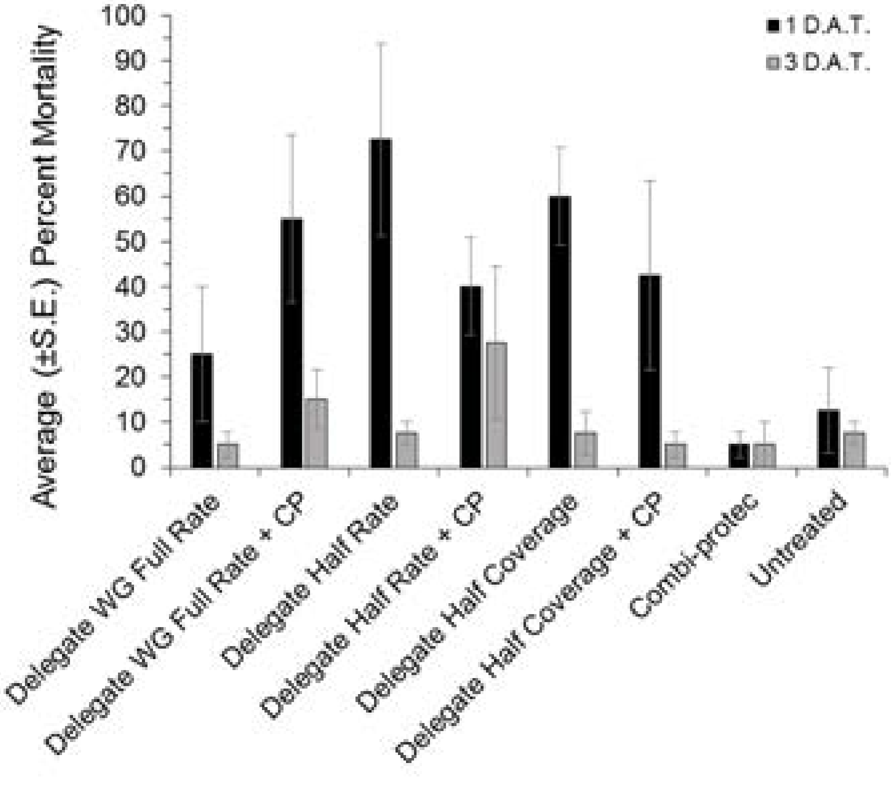

Figure 3. Average (±S.E.) percentage mortality in semi-field bioassays. Darker bars are for fruit and foliage collected 1 day after treatment application and lighter bars are for fruit and foliage collected 3 days after treatment. There were no significant differences in average percent mortality among treatments at the 1 or 3 D.A.T. time points.

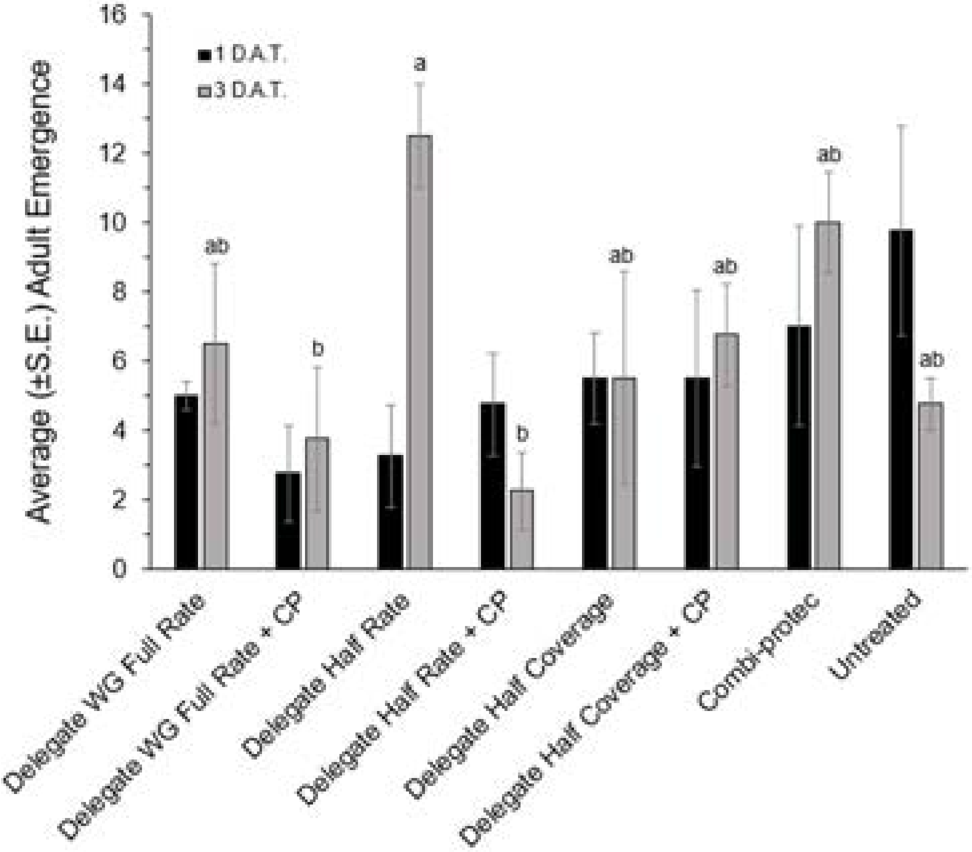

Figure 4. Average (±S.E.) adult SWD emergence from exposed fruit in the semi-field bioassays. Significance was only found in the 3 D.A.T. emergence samples. Different letters above bars indicate significance.

CONCLUSIONS AND RECOMMENDATIONS

Field trials in both 2021 and 2022 show promising results from the addition of the phagostimulant bait Combi-Protec to insecticides. This year we saw a significant reduction in adult emergence in the semi-field bioassays from a half rate spray mixed with Combi-Protec than when compared to the half rate spray alone. We will continue to investigate beneficial properties of Combi-Protec in the upcoming field season, and currently would only recommend using Combi-Protec as an adjuvant with a full rate insecticidal application.

NEXT STEPS

- This work will be continued in 2023.

ACKNOWLEDGEMENTS

We thank Judy Collins, Abigail Fisher, Eric Desbois, Serena Leonard, and Dominic Crowley 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. doi:10.1093/jipm/pmx019

INVESTIGATOR: P. Fanning

3. Evaluation of two insecticidal peptides VST-6350 4% SL and VST-6700 for the control of spotted-wing drosophila in wild blueberry

OBJECTIVES

The objective of this study was to evaluate two different formulations of Spear-T® (VST-6350 4% SL and VST-6700) compared to a conventional insecticide standard, for the control of spotted-wing drosophila (SWD) in wild blueberry. The latter peptide is a new formulation in development by Vestaron Corporation (Kalamazoo, MI, USA), which targets the sodium channel of insects similar to the pyrethroids.

LOCATION: Jonesboro, ME
PROJECT TIMEFRAME: August - September 2022

INTRODUCTION

Peptides derived from venoms have enormous potential as bioinsecticides. Spear-T 2% Liquid Concentrate, GS-omega/kappa-Hxtx-Hv1a, is a peptide derived from spider venom, which has previously been shown to be effective against spotted-wing drosophila.

METHODS

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

There were four applications of each treatment applied on 5, 12, 19, and 25 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. 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 (11, 19, and 25 August and 1 September), a commercial blueberry rake was used to harvest one, 6oz sample (by volume) from each plot. 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 ($P > 0.05$). The results are outlined in Table 1 and Figure 1. No significant differences were observed; however, on the third sample on 25 Aug, infestation of fruit was lowest in both the rotation of Entrust/Mustang Maxx (trt 2) and the 268.8 oz/acre rate of VST-6700 (trt 6). There was a significant rainfall event between the third and fourth sampling events, leading to a reduction in efficacy and treatment effect.

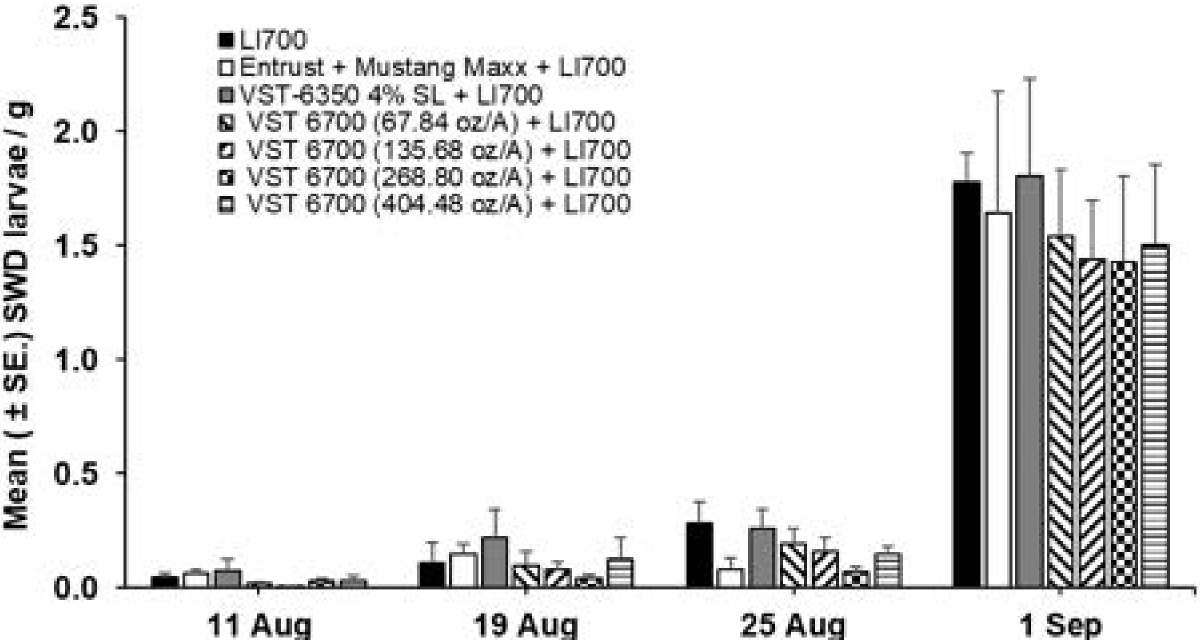

Figure 1. Mean (± S.E.) number of spotted-wing drosophila larva per gram of wild blueberry.

Table 1. Field control of spotted-wing drosophila with insecticides, summary. Mean (± S.E.) number of larvae/gram extracted from blueberries.

				11-Aug	19-Aug	25-Aug	1-Sep
Trt #	Material	Rate oz/acre	Timing[a]	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
1	LI700		ABCD	0.045 ± 0.016 a	0.106 ± 0.093 a	0.281 ± 0.099 a	1.777 ± 0.13 a
2	Entrust	6	AC	0.066 ± 0.016 a	0.147 ± 0.047 a	0.080 ± 0.052 a	1.645 ± 0.532 a
	Mustang Maxx	4	BD				
	LI700	0.125% v/v	ABCD				
3	VST-6350 4% SL	128	ABCD	0.075 ± 0.052 a	0.221 ± 0.123 a	0.261 ± 0.085 a	1.806 ± 0.4295 a
	LI700	0.125% v/v	ABCD				
4	VST-6700	67.84	ABCD	0.017 ± 0.010 a	0.096 ± 0.060 a	0.195 ± 0.065 a	1.541 ± 0.2938 a
	LI100	0.125% v/v	ABCD				
5	VST-6700	138.68	ABCD	0.005 ± 0.003 a	0.080 ± 0.033 a	0.161 ± 0.062 a	1.441 ± 0.2581 a
	LI700	0.125% v/v	ABCD				
6	VST-6700	268.8	ABCD	0.028 ± 0.014 a	0.043 ± 0.018 a	0.070 ± 0.021 a	1.427 ± 0.3797 a
	LI700	0.125% v/v	ABCD				
7	VST-6700	404.48	ABCD	0.031 ± 0.020 a	0.124 ± 0.095 a	0.146 ± 0.036 a	1.503 ± 0.3524 a
	LI700	0.125% v/v	ABCD				
Statistic				$F_{(6,21)}=1.19$, $P=0.35$	$F_{(6,21)}=0.55$, $P=0.7638$	$F_{(6,21)}=1.51$, $P=0.2223$	$F_{(6,21)}=1.19$, $P=0.35$

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

[a] Application number (date) A=1 (5 Aug), B=2 (12 Aug), C=3 (19 Aug), D=4 (25 Aug)

Data for SWD were adjusted for sample weight and Log(X+1) transformed for analysis; non-transformed means (larvae/gram) are shown in the table.

CONCLUSIONS AND RECOMMENDATIONS

This trial indicates that these peptide products could be a valuable rotational tool with standard conventional products, especially as they are currently MRL-exempt by the US EPA.

NEXT STEPS

- This work will be continued in 2023.

ACKNOWLEDGEMENTS

We thank Judy Collins, Ben Johnson, Abigail Fisher, Eric Desbois, Serena Leonard, and Dominic Crowley for their assistance with this study.

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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. doi:10.1093/jipm/pmx019

INVESTIGATORS: P. Fanning, J. Collins, and B. Johnson

4. Releases of *Ganaspis brasiliensis* as a biological control agent for Spotted-wing Drosophila, Year 1

OBJECTIVES

Determine baseline/pre-release parasitoid sampling of *Drosophila* parasitoids. Release and establish the parasitic wasp *Ganaspis brasiliensis* as a classical biological control agent for the control of Spotted-wing Drosophila.

LOCATIONS: Hancock, Knox, Waldo, and Washington Counties, ME
PROJECT TIMEFRAME: August – September 2022

INTRODUCTION

Spotted-wing Drosophila (SWD) continues to impact production practices here in Maine. As an invasive species, SWD's explosive annual population growth is due in part to a lack of natural enemies, such as those that commonly regulate the outbreak of our native pests, with some exceptions. Surveys of native *Drosophila* parasitoids in Maine have not revealed native parasitoids attacking SWD. Studies elsewhere have determined that the impact on SWD populations is limited, with less than 10% parasitism. Therefore, additional biological controls are needed.

In recent foreign exploration for parasitoids in SWD's native range, three larval parasitoids, *Ganaspis brasiliensis* (Ihering), *Leptopilina j. japonica* Novković & Kimura, and *Asobara japonica* Belokobylskij, were chosen based on frequent occurrence in the field in Asia. These species were imported into quarantine labs for evaluation, and it was found that *G. brasiliensis* was the most efficient and host-specific parasitoid of SWD (Daane et al., 2016; Giorgini et al., 2019). A petition to release *G. brasiliensis* from quarantine was reviewed with USDA APHIS, and a release permit was issued in the fall of 2021. The Fanning lab obtained and established a colony in the lab and reared up large numbers for release in the summer of 2022. A key benefit of biological control is that in addition to impacting SWD population within crops, a *G. brasiliensis* has excellent potential to significantly reduce populations in the landscape (such as in wild hosts outside the crop), thereby reducing pressure on susceptible crops such as wild blueberry.

METHODS

In the summer of 2022, four wild blueberry fields were identified in Hancock, Knox, Waldo, and Washington Counties. These sites were selected to include areas with abundant wild SWD hosts outside and around the crop. Sites were sampled before releases with sentinel SWD larvae and collections of fruit. Following these pre-release samples in late July, we began releases of adult *G. brasiliensis* in hedgerows adjacent to the four release sites. Over three weeks, we released 400 adults at each location (200 females, 200 males) for a total of 1200 adults. Following releases, we waited ~ 1 month before conducting post-release sampling to ensure we were sampling a new generation of *Ganaspis*. For post-release sampling, sentinel fruit and wild fruit collections were conducted where possible. Pre- and post-release samples were returned to the lab and reared out to determine the presence of *Drosophila* parasitoids.

RESULTS

In pre-release samples, wild blueberry (*Vaccinium angustifolium*), wild raspberry (*Rubus* sp.), and Canadian bunchberry (*Cornus canadensis*) were all sampled in addition to the sentinel fruit samples. No parasitoids were reared from pre-release samples confirming that native *Drosophila* parasitoids are not present in large numbers or providing control of SWD in wild blueberry fields here in Maine. In post-release samples *G. brasiliensis* was recovered at two of the four sites, in Knox and Hancock counties (Fig. 1). These sites had higher background SWD populations than the sites in Waldo and Washington counties.

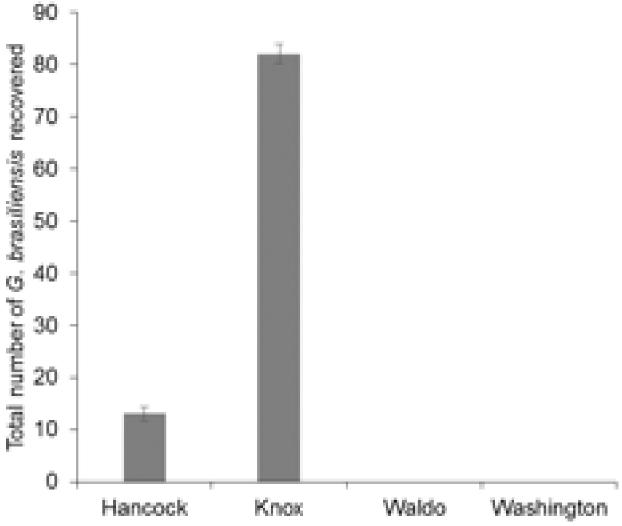

Figure 1. Total number of *G. brasiliensis* recovered.

CONCLUSIONS AND RECOMMENDATIONS

In 2022, low SWD populations might have impacted the establishment at some sites. We will continue to conduct releases in 2023. No changes in growers’ practices to manage SWD are recommended at this time.

NEXT STEPS

- This work will be continued in 2023; we will resample *G. brasiliensis* at these sites in the spring to determine if there was successful overwintering of the populations at these sites. Additional releases will be conducted at these and other sites

ACKNOWLEDGEMENTS

We thank Abigail Fisher, Eric Desbois, Serena Leonard, and Dominic Crowley for their assistance with this study.

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INVESTIGATORS: P. Fanning, and S. Bushmann

5. Resampling wild bee populations in Maine wild blueberry, Year 1.

OBJECTIVE: To resample wild bee populations in previously sampled wild blueberry: determining status and developing long-term trends in pollinator communities.

LOCATIONS: Surry, Ellsworth, Penobscot, Sedgwick, Orland, and Blue Hill ME.
PROJECT TIMEFRAME: April – June 2022

INTRODUCTION

In Maine, bees pollinating wild blueberry have been studied regularly due to their necessity for higher yields (Boulanger et al., 1967; Bushmann & Drummond, 2015; Drummond & Stubbs 1997a, 1997b, 2003; Stubbs et al., 1992). Regular sampling and resampling at the same locations using the same method can provide invaluable data on how factors such as changing management practices and weather patterns might impact wild bee populations and inform crop management and conservation practices. Bushmann and Drummond (2015) measured bumble bee and other wild-bee activity in 40 study fields. They found that, on average, the WB abundance made up 36% of the total bee abundance in fields; although, abundance is not consistent across fields. This study aims to continue our assessments of wild bees in wild blueberry fields to ensure that the populations remain healthy in light of documented reductions in other locations.

METHODS

In spring 2022, we sampled six fields. Sampling methods were the same as those used in the 2010-2012 survey and described in Bushmann and Drummond (2015). Sampling included bloom time surveys of bees using bee bowls and hand-collecting bees observed foraging on flowers. Each field was visited 4-6 times throughout bloom.

For bee bowl sampling, cup traps with the interior surface painted either fluorescent yellow or blue, or left an unpainted opaque white, were placed in fields for 24 h. When possible, cups were placed in all the study fields for the same 24 h. Cups were set 10m apart along a straight line in the fields. A total of

24 cups (8 per color) were placed at each site. Weather conditions were determined at the timing of the cup placement. Cups were set out when the 24 h collecting period was forecasted for daytime highs of at least 65°F, winds under 5mph, and minimal (0–30%) cloud cover. At the end of each 24 h sampling period, each cup was drained of water, and the bees were either stored in alcohol until pinned or immediately dried and pinned.

For hand collections, all hand collections and observed foraging on flowers were conducted by PI Bushmann, collecting bees for exactly 15 min. Collection sessions took place during ideal weather conditions described in the bowl sampling methods above. During these sessions, any non-Bombus bee found foraging on any flower in the field (including non-blueberry) was collected into a clean cup and the flower's common name was recorded. Hand collections were repeated three times during blueberry bloom.

Additionally, wild bee and honey bee abundance were measured using 1 meter square quadrats. Three times during blueberry bloom, workers placed 1 meter square quadrats in the field and counted the number of bumble bees and honeybees that visited the quadrat for one minute. Seven to thirteen measures were conducted at each visit. Only clones with open flowers and bee activity were chosen.

RESULTS

Confirmations of bee identifications are currently outstanding, primarily for the sweat bees (*Lasioglossum*) by Dr. Jason Gibbs of the University of Manitoba.

The bee abundance measurements at these sites were generally low this year, for the times we sampled, particularly of honeybees, despite most fields being stocked with honey bees. Average wild bee abundance was higher than honeybee abundance. This is likely reflective of the colder temperatures during bloom in 2022; however, the average temperature during the quadrat sampling was 65.7^{o}F. Data for stocking rates and yield for the sampled fields are currently being checked with the site owners.

CONCLUSIONS AND RECOMMENDATIONS

None currently.

NEXT STEPS

- This work will be continued in 2023; we will resample at these sites or locations nearby during bloom 2023.

ACKNOWLEDGEMENTS

We thank Judy Collins, Benjamin Johnson, Abigail Fisher, Eric Desbois, Serena Leonard, and Dominic Crowley for their assistance with this study.

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

1. Tribenuron-methyl (Express®) Herbicide Field Survey

OBJECTIVE

Survey weeds beyond bunchberry after Express herbicide application in two pre-production wild blueberry fields.

LOCATION: Deblois, Maine
PROJECT TIMEFRAME: April – August 2022

INTRODUCTION

Tribenuron-methyl is the active ingredient in Express® with TotalSol® herbicide. The product is sold as- in the form of soluble granules and is manufactured by DuPont. It is a selective postemergence broadleaf herbicide that has been granted Special Local Need 24(C) in Maine for bunchberry (*Cornus canadensis)* control in wild blueberries since 2008. Express is a group 2 herbicide and therefore fills an important rotational niche, reducing the risk of resistance development. The UMaine Extension herbicide chart, which contains twenty products, contains two Group 2 products (Express and Sandea).

The current 24(C) label expires 12/31/2025 and allows the use of Express with TotalSol (EPA Reg. No. 352-632) to control bunchberry in wild blueberry fields in Maine. In 2018, the 24(C) was renewed for one year to establish the safety of this product in ground water. Well water sampling was conducted during the growing season of 2019 after Express application upon request by the Maine Board of Pesticide Control. Residues were not detected in any of the three sampled wells over a 6-month period. Because no product was detected in the wells, the 24(C) label was extended for another 5 years through 12/31/2025.

The goal for this season was to start a survey of what other weeds beyond bunchberry may be controlled by Express. Bunchberry (*Cornus canadensis)* is a low-growing woody weed in wild blueberry fields that is difficult to manage because of its low-growing rhizomatous habit. Because this weed can grow underneath the blueberry canopy it competes with wild blueberry for space, nutrients and water. According to the label, other weeds that this product may control include prickly lettuce (*Lactuca serriola*), vetch species (Vicia spp), and thistle species (Cirsium spp).

METHODS

This survey was conducted in two locations in Deblois, ME, on fields owned and managed by Jasper Wyman and Son company. The fields are called Junior Grant and Wilson. Neither location was actively producing wild blueberries but both were being brought into production. The Junior Grant field was very grassy with a very wide strip of standing water running through the middle of the field. The Wilson field had a mixed broadleaf weed community. Both fields had sparse wild blueberry cover but stems were present.

In September 2021, research plots were established at both locations. Two transects were drawn at each location and along each transect, ten 0.37m^2 quadrats were placed. Wooden stakes were hammered in at the corner of each transect. At each location, there were ten quadrats along two transects, for a total of twenty quadrats at each site. One transect served as the untreated control and one transect was treated with Express with TotalSol. The product was applied by a tractor on May 19, 2022. Express was applied at a rate of 1.38 fl oz/acre within 40.3 gallons of water. The tank included water, LI 700 surfactant, and Express.

At the time of quadrat establishment on September 21, 2021, and on several dates throughout the following 2022 season, all quadrats were evaluated for weed presence and phytotoxicity damage to all plants within the quadrats. Weed presence was 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%. Weeds were identified and the number of stems of that weed was recorded. Phytotoxicity damage was identified and the number of stems per weed type showing phytotoxicity were counted.

Wild blueberry cover was quantified using the same equal interval ranks. Wild blueberry stems demonstrating phytotoxicity damage were also counted. No statistics were run on the data collected. All graphs were made using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

RESULTS

The time at which weed species emerge, flower, reproduce and senesce depends on the species, the plant life cycle (annual vs. perennial), and environmental conditions. Many of the perennial weeds observed, including poverty oat grass (POG; *Danthonia spicata*), cinquefoil (CINQ; Potentilla spp.), red sorrel (RS; *Rumex acetosella*), butter and eggs (B&E; *Linaria vulgaris*), and strawberry (STRAWB; Fragaria spp.) increased until the June 2, 2022 sample date, exactly 2 weeks following the May 19, 2022 Express application (Figure 1). From June 2, 2022 until July 7, 2022, strawberry showed the greatest decrease (100%), followed by poverty oat grass (93%) and red sorrel (84%). While red sorrel showed a large decrease from June to July, there was a 68% increase in overall weed number from July 7 to August 19, 2022. An additional six weed species exhibited a notable increase in number during the 2022 growing season (Figure 2). Here, bunchberry (BUN; *Cornus canadensis*) showed the greatest spike on July 7 following the May 19, 2022, application of Express, followed by goldenrod (GROD; Solidago spp.), brambles (BRAM; Rubus spp.), and violet (VIO; Viola spp.). From two (June 2, 2022) to seven weeks following the application (July 7, 2022), violet showed the greatest increase (100%; Table 1). After violet, bunchberry (46%), goldenrod (36%), brambles (30%) and whorled loosestrife (WLS; 30%) also decreased over weeks two through seven after the Express application.

Perennial woody weeds, perennial broadleaf weeds and sedge were most affected by the Express treatment. Weed species most impacted by the Express application, were aspen (ASP; Populus spp.), butter & eggs (yellow) toadflax (B&E; *Linaria vulgaris)* and strawberry (STRAWB; Fragaria spp.) which all exhibited 100% phytotoxicity of their total population. More than 50% of Canadian mayflower (MAY; *Maianthemum canadense*), meadowsweet (MEAD; *Filipendula ulmaria*), more than 60% of red sorrel (RS; *Rumex acetosella*) and wintergreen (WG; *Gaultheria procumbens*), and more than 70% of wild lettuce (WL; *Lactuca virosa*) exhibited phytotoxicity following treatment. Interestingly, only 45% of bunchberry (BUN; *Cornus canadensis*) exhibited phytotoxicity, which may be due in part to the large number of bramble weeds growing above bunchberry, which is not typical of wild blueberry fields and may have resulted in the reduced efficacy of Express.

Several weed species, including moss (MOSS; not identified further), poverty oat grass (POG; *Danthonia spicata*), and whorled loosestrife (WLS; *Lysimachia quadrifolia*) did not exhibit any phytotoxicity following Express application on the June 2, 2022 sample date. Poverty oat grass was the most prevalent weed identified in this study, and is often found in wild blueberry fields outside this study.

Table 1. Average weed counts in the Express herbicide application treatment at both sites with total observed weed counts (#/m^2), weed counts by weed type (#/m^2), weed counts with phytotoxicity (#/m^2) and the proportion of weeds with phytotoxicity relative to total weeds present (%/total weeds), measured on June 2, 2022. Weed species exhibiting greater than 30% phytotoxicity are in bold text in the table. Some weeds identified in the charts above (Figures 1 and 2) did not demonstrate any phytotoxicity and so are not found in this table.

Weed Counts in Express Treatment, Post-Spray			**Total Weeds Present**	**Weeds with Phytotoxicity**	
			# per m^2	# per m^2	% per total weeds
	Total Weed Counts	**ALL**	225.8	24.3	11%
Abbreviation	**Weed Name**	**Life cycle**	# per m^2	# per m^2	% per total weeds
ASP	**aspen**	**W**	**0.4**	**0.4**	**100%**
B&E	**butter & eggs (yellow toadflax)**	**P**	**6.4**	**6.4**	**100%**
BIR	birch	W	1.1	0.1	13%
BRAMB	bramble	W	11.9	1.1	9%
BUN	**bunchberry**	**P**	**22.4**	**10.0**	**45%**
CINQ	cinquefoil	P	39.7	8.9	22%
GR	grass	G	12.4	1.2	10%
GROD	goldenrod	P	12.7	2.3	18%
HS	heath speedwell	P	0.1	0.0	0%
LAU	**laurel**	**P**	**11.8**	**5.0**	**43%**
MAY	**Canadian mayflower**	**P**	**7.6**	**3.9**	**52%**
MEAD	**meadowsweet**	**P**	**0.9**	**0.5**	**57%**
MILK	milkweed	P	0.1	0.0	0%
MOSS	moss	P	1.9	0.0	0%
POG	poverty oat grass	G	48.5	0.0	0%
RB	rudbeckia	P	4.9	0.1	3%
RS	**red sorrel**	**P**	**23.2**	**14.7**	**63%**
SEG	**sedge**	**G**	**4.7**	**1.8**	**37%**
SF	sweet fern	W	0.1	0.0	0%
STRAWB	**strawberry**	**W**	**3.5**	**3.5**	**100%**
WG	**wintergreen**	**W**	**7.2**	**4.9**	**68%**
WL	**wild lettuce**	**A**	**0.9**	**0.7**	**71%**
WLS	whorled loosestrife	P	0.9	0.0	0%

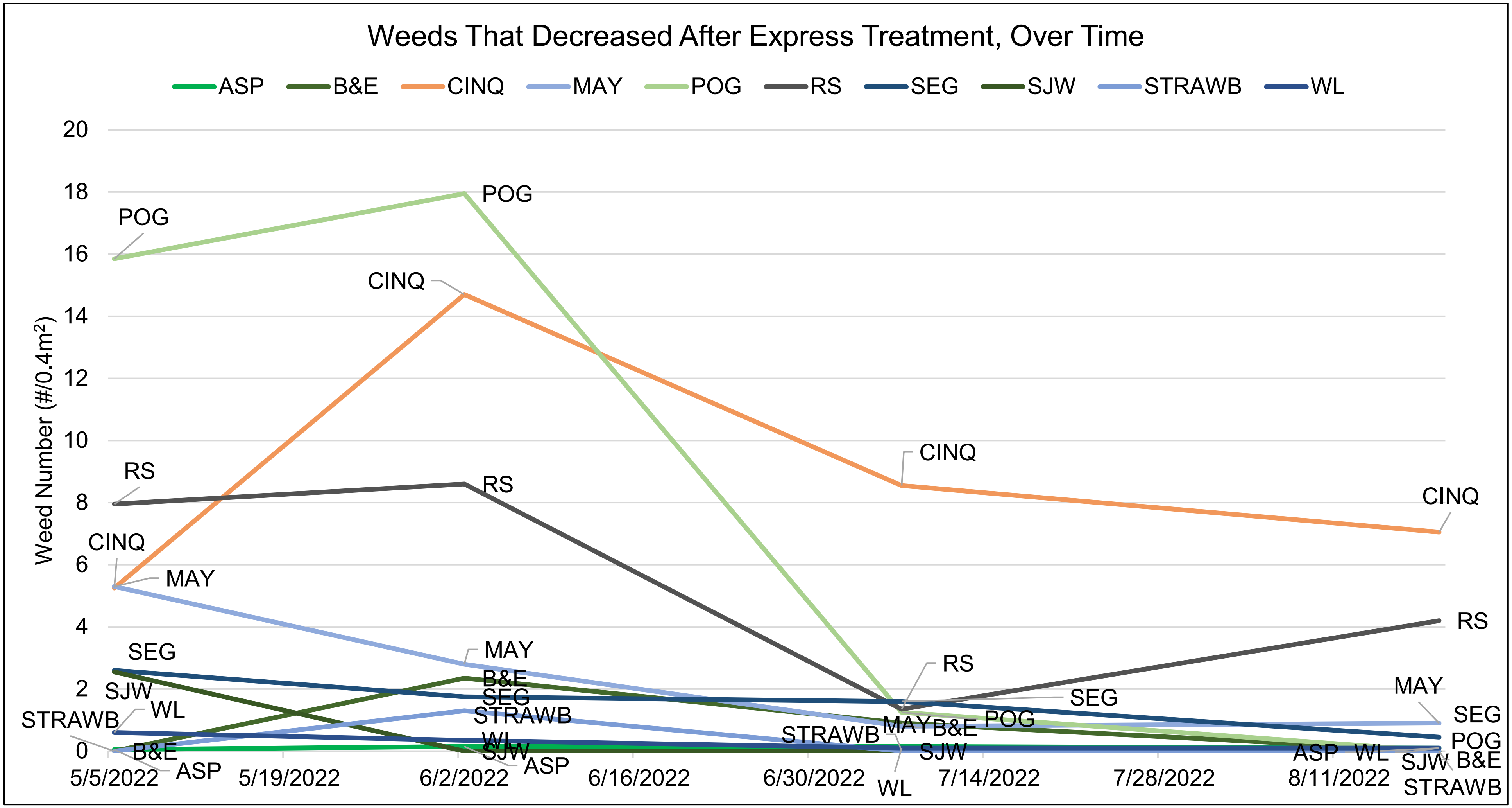

Figure 1. Average weed numbers (#/0.37m^2) of species that exhibited a decrease in density after the Express treatment at both sites on May 19, 2022.

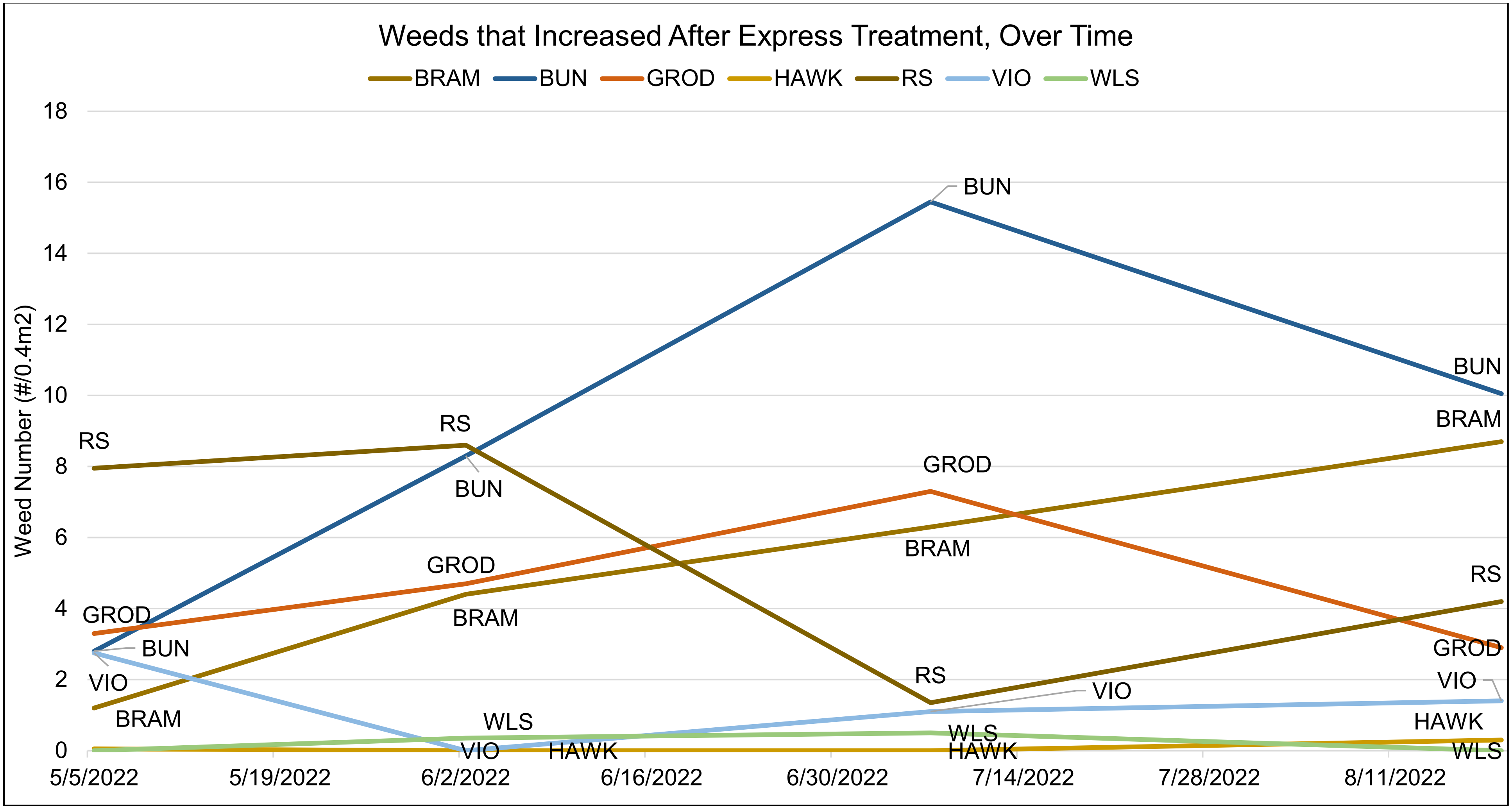

Figure 2. Average weed numbers (#/0.37m^2) of species that exhibited an increase in density after the Express treatment at both sites on May 19, 2022.

Table 2. Table of abbreviations used in Figures 1 and 2 and their common weed names.

Changes in Weed Presence from June 2 to July 7, 2022					
Weeds Decreasing			Weeds Increasing		
Abbreviation	**Common Name**	**% Decrease Between Dates**	**Abbreviation**	**Common Name**	**% Increase Between Dates**
ASP	aspen	0%	**BRAM**	brambles	30%
B&E	butter & eggs	62%	**BUN**	bunchberry	46%
CINQ	cinquefoil	42%	**GROD**	goldenrod	36%
MAY	Canadian mayflower	71%	**HAWK**	hawkweed	0%
POG	poverty oat grass	93%	**RS**	red sorrel	0%
RS	red sorrel	84%	**VIO**	violet	100%
SEG	sedge	9%	**WLS**	whorled loosestrife	30%
SJW	St. John’s-wort	0%			
STRAWB	strawberry	100%			
WL	wild lettuce	71%			

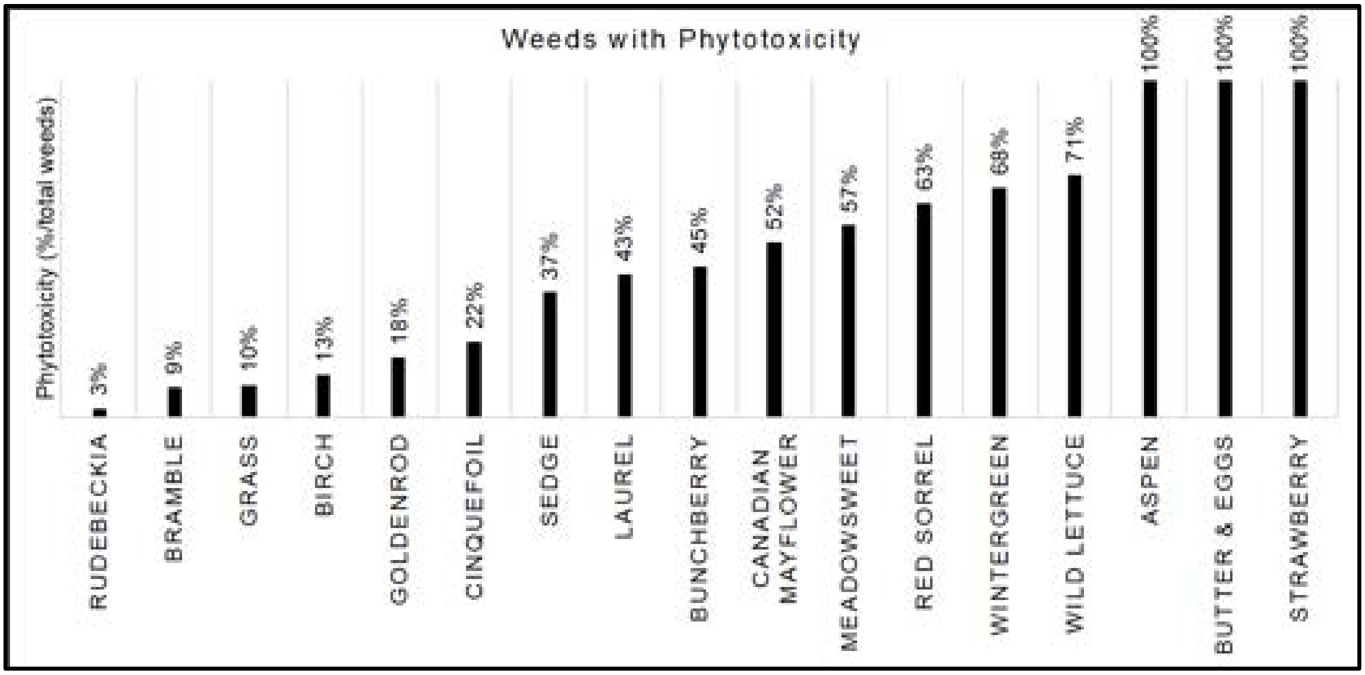

Figure 3. Percent of weeds exhibiting phytotoxicity of total weeds observed at both sites on June 2, 2022.

DISCUSSION

Identifying which weeds decreased due to their life cycle vs herbicide application is difficult to tease apart. Additionally, plants can demonstrate phytotoxicity, but this damage does not mean the weed has been killed. Phytotoxicity is damage to a plant caused by exposure to a substance, such as an herbicide spray, that can stunt plant growth, cause leaves to develop speckles, necrotic areas, yellow spots (chlorosis), cupped or twisted leaves, or eventual plant death (Getter, 2015). After phytotoxicity, plants can regrow around areas that experienced chlorosis (yellowing) but not necrosis (browning, indicating localized tissue death) (Getter, 2015).

A plant that survives phytotoxicity and demonstrates chlorosis may be living but with significantly reduced capacity to photosynthesize, since the lack of green color in the leaves indicates a lack of chlorophyll, the compound that facilitates photosynthesis. So, some plants that experience phytotoxicity and recover may be killed following another herbicide application.

Image 1. An example of narrow-leaved goldenrod demonstrating chlorosis, a symptom of phytotoxicity. The damaged weed is a bright yellow compared to the dark green and brown plants surrounding it.

The data above show that certain weeds may be susceptible to phytotoxicity damage from Express application, but that repeat applications are needed to meaningfully reduce the weed population. For instance, 45% of the bunchberry (*Cornus canadensis*) observed at both sites on June 2, 2022 demonstrated phytotoxicity damage, but this did not reduce the weed's population. In fact, on May 5, there were an average of 2.8 bunchberry/quadrat observed at both sites, but this increased to 8.3 on June 2 and 15.45 on July 7, 2022. These increases in the number of bunchberry observed align with the life cycle of the plant, which flowers in May to July and produces fruit from July through the fall (Gucker, 2012). As bunchberry is flowering and fruiting, the plant continues to grow from late spring until the fall, when it senesces, so an increase in plant numbers over the course of summer 2022 aligns with the plant's growth habits (Gucker, 2012). It is probable that other plants identified and tracked throughout this research also saw increases and/or decreases in their numbers as a natural part of their life cycles, and the influence of Express application on the plant health was negligible. Further study and better approximation of the impacts of Express-induced phytotoxicity on plant health is necessary.

Bunchberry was not the plant that was best controlled by Express, and in fact, there were nine plants that exhibited higher rates of phytotoxicity damage than bunchberry: aspen, butter & eggs (yellow) toadflax, strawberry, witch hazel, Canadian mayflower, meadowsweet, red sorrel, wintergreen, and wild lettuce. Additional data is needed to determine whether control of these other plants with Express is consistent and lasting.

CURRENT RECOMMENDATIONS

None at this time.

ACKNOWLEDGEMENTS

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

2. Herbicide Stacking Demonstration at Blueberry Hill Farm

OBJECTIVES

- Demonstrate herbicide stacking to wild blueberry growers at the BHF Field Day
- Collect basic plant and phytotoxicity data for efficacy observations

LOCATION: Blueberry Hill Farm, Jonesboro, ME
PROJECT TIMEFRAME: April – August 2022

INTRODUCTION

Weed management is a continual challenge for wild blueberry growers across the state of Maine. Different species of weeds require different management techniques which include reducing the soil pH, applying mulch, cutting stems several times in a growing season, cutting weeds above the wild blueberry canopy, and applying herbicides that target specific plants or classes of plants (broadleaves, grasses, and sedges).

In any cropping system, including wild blueberry, repeated application of herbicides will weaken and eventually kill weeds that compete with wild blueberry for sunlight, water, and nutrients. Hexazinone (Velpar/Velossa) is a kill-all broad-spectrum herbicide that has been registered for use in wild blueberry fields since 1983 (Yarborough, 2008). Because of its broad-spectrum nature, this herbicide does not discriminate between killing broadleaf or grassy weeds and has been used by many growers every other year regardless of weed monitoring. Therefore, resistance to this herbicide has developed and wild blueberry fields with repeated hexazinone application appear to be stressed. This and the availability of more narrow-spectrum herbicides has caused growers to move away from hexazinone for a new approach called herbicide stacking. Herbicide stacking is the layering of two or three products in one season with the goal of hitting the weeds with multiple modes of action at different growth stages.

The practice of herbicide stacking is not unique to wild blueberry and is also practiced in North American canola, rice, and soybean production. This demonstration with some data collection aimed to understand what combinations of herbicides applied pre- and post-emergence may reduce weed number and what impacts these combinations have on wild blueberry plants. In this demonstration we targeted a variety of grass, broadleaf, and sedge weed species present in the Jonesboro prune field.

In wild blueberry, the prune year is the most effective time to apply herbicides and the only time to apply pre-emergent products. Pre-emergent products such as rimsulfuron (Matrix) and clethodim (Arrow) are most effective when applied BEFORE wild blueberry and weeds emerge from the ground. If you see any green growth, it’s too late to apply a pre-emergent product. Post-emergent products are most effective after wild blueberry and weeds emerge but are not more than a few inches tall. Ideally, the post-emergent product of choice is applied shortly after emergence, which is typically when crop fields are in bloom. Care must be taken to avoid applying herbicides and other pesticides while flowers are in bloom. When flowers are out you can assume that bees are active.

METHODS

On April 12, 2022, demonstration plots were laid out in a prune field at Blueberry Hill Farm in Jonesboro, ME. Because this was truly a demonstration, treatments were not replicated and plots were large for tractor application. Each of the nine large plots was 45’ x 120’ and were divided by 10’ buffer strips. Within each plot, six 0.37 m^2 quadrats were staked out to enable repeated data collection through the season. The products applied were: Matrix, Callisto, Arrow, Zeus, Zeus Prime, and Capreno. Capreno is an herbicide that is **NOT** yet labeled for use in wild blueberry.

Treatment rates were calculated using the application information on the product labels. Treatments were applied using a John Deere 6330 tractor with a 45-ft boom sprayer, and products applied on the same day were applied simultaneously in a mixed tank.

Image 1. Product application occurring at Blueberry Hill Farm in Jonesboro, ME.

Table 1. Herbicide products, groups, and application rates used in the herbicide stacking demonstration.

Active Ingredient	Trade Name	Group	Application Rate
Rimsulfuron	Matrix	9	4 oz/a
Mesotrione	Callisto	27	3 oz/a
Clethodim	Arrow	1	4 oz/a
Sulfentrazone	Zeus	14	12 oz/a
Carfentrazone & Sulfentrazone	Zeus Prime XC	14	15.2 oz/a
Thiencarbazone-methyl & Tembotrione	Capreno	2 & 27	**EXPERIMENTAL. NOT YET LABELED FOR USE.**

Table 2. Herbicide stacking schedule. All dates were classified as pre-emergence ("pre") or post-emergence ("post") based on actual emergence that occurred the week of April 25 in Jonesboro. In the "Target" column, GR = grasses, BL = broadleaves, and S = sedges.

Treatment	Date	Pre/Post	Product Trade Name	Target
A	13-Apr	Pre	Matrix	GR, BL
	5-May	Post	Callisto	BL
B	13-Apr	Pre	Arrow	GR
	5-May	Post	Callisto	BL
C	13-Apr	Pre	Matrix	GR, BL
	5-May	Post	Arrow and Callisto	GR, BL
D	13-Apr	Pre	Zeus Prime and Arrow	GR, BL, S
E	13-Apr	Pre	Zeus and Arrow	GR, BL, S
F	5-May	Post	Callisto	BL
	23-May	Post	Callisto	BL
G	13-Apr	Pre	Water (untreated control)	
H	5-May	Post	Capreno	BL
I	5-May	Post	Capreno	BL
	23-May	Post	Capreno	BL

Data Collection

Blueberry health was quantified using percent cover, stem density, and the number of stems exhibiting phytotoxicity. These were measured in the six established 0.37 m^2 quadrats per treatment on June 1, July 7, and August 27, 2022. Blueberry presence was 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%. Blueberry stems were counted and the number of stems displaying phytotoxicity symptoms were also recorded. Weed presence was quantified on the same dates using the same 0-6 interval ranking system. Weeds were counted, identified by genera, and the number of stems displaying phytotoxicity were counted.

Data Analysis

All data including blueberry cover (%/m^2), weed cover (%/m^2), weed number (#/m^2), blueberry stems and weed stems with phytotoxicity (#/m^2), were analyzed using a full-factorial repeated-measures mixed model design in JMP (JMP®, Version 16.0, SAS, Cary, NC, USA), followed by a Tukey's Pairwise comparison in, testing the effects of date, treatment, and any interaction between date and treatment ($\alpha = 0.05$). Additionally, a bivariate regression between blueberry cover and weed cover was performed in JMP and plotted in a 1-to-1 relationship using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

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 included blueberry cover (%/m^2), weed cover (%/m^2), weed number (#/m^2), blueberry stems and weed stems with phytotoxicity (#/m^2). These data improved visually following transformation. Transformed data continued to statistically fail for normality, however, statistical tests were carried out despite non-normality after establishing there were no serious problems with the data.

RESULTS

The treatment CaprenoPOST had significantly greater blueberry cover (71%/m^2) than treatments: CallistoPostx2 (47%/m^2), ZeusPrimePRE+ArrowPRE (44%/m^2) and CaprenoPostx2 (36%/m^2; Figure 1). The ControlWaterPRE exhibited significantly higher blueberry cover (58%/m^2) than CaprenoPostx2 only.

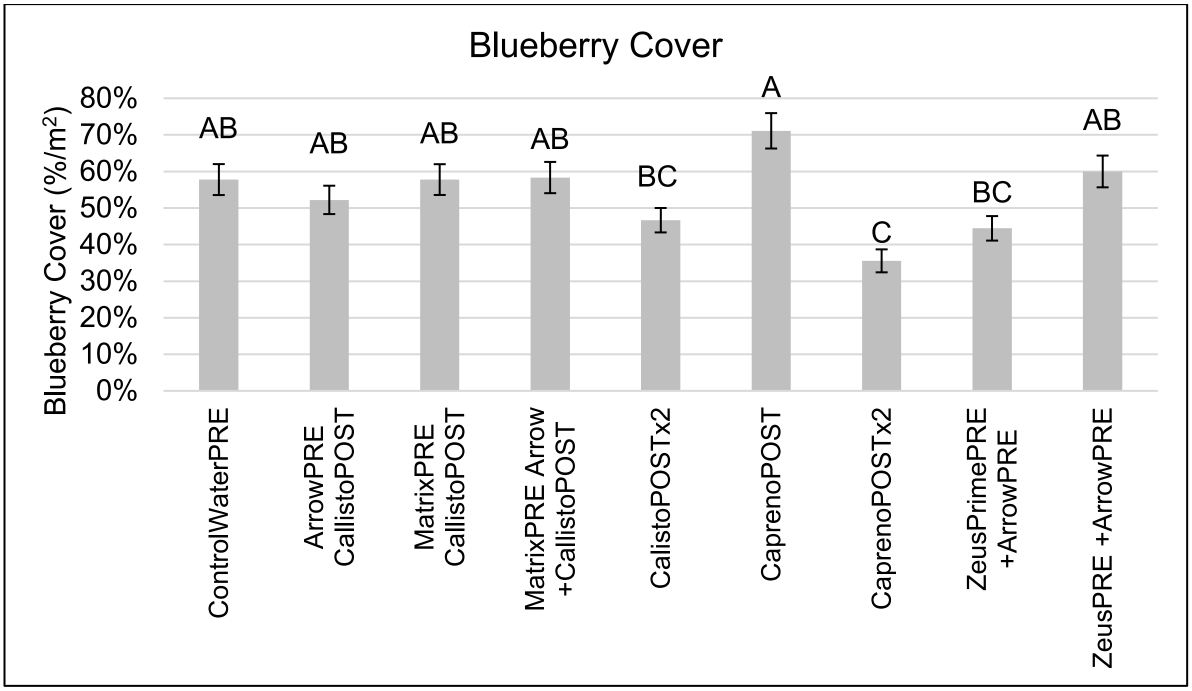

Figure 1. Average blueberry cover (%/m^2) measured in herbicide treatments on June 1, July 7 and August 25, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean. Data were transformed via a SQRT transformation prior to statistical evaluation, yet untransformed data is pesented above for readability.

The greatest levels of phytotoxicity damage to wild blueberry were measured in the ArrowPRE+CallistoPOST treatment (85 stems/m^2), followed by the CaprenoPOST treatment (64 stems/m^2; Figure 2). The ControlWaterPRE treatment exhibited a slight amount of phytotoxicity (3 stems/m^2) to the blueberry, this may be due to recent pesticide applications in the same field in the past twelve months, suggesting residual activity. Other than the control, treatments that exhibited relatively low phytotoxicity to blueberry included: MatrixPRE+CallistoPOST (8 stems/m^2), CaprenoPOSTx2 (17 stems/m^2), and ZeusPRE+ArrowPRE (16 stems/m^2).

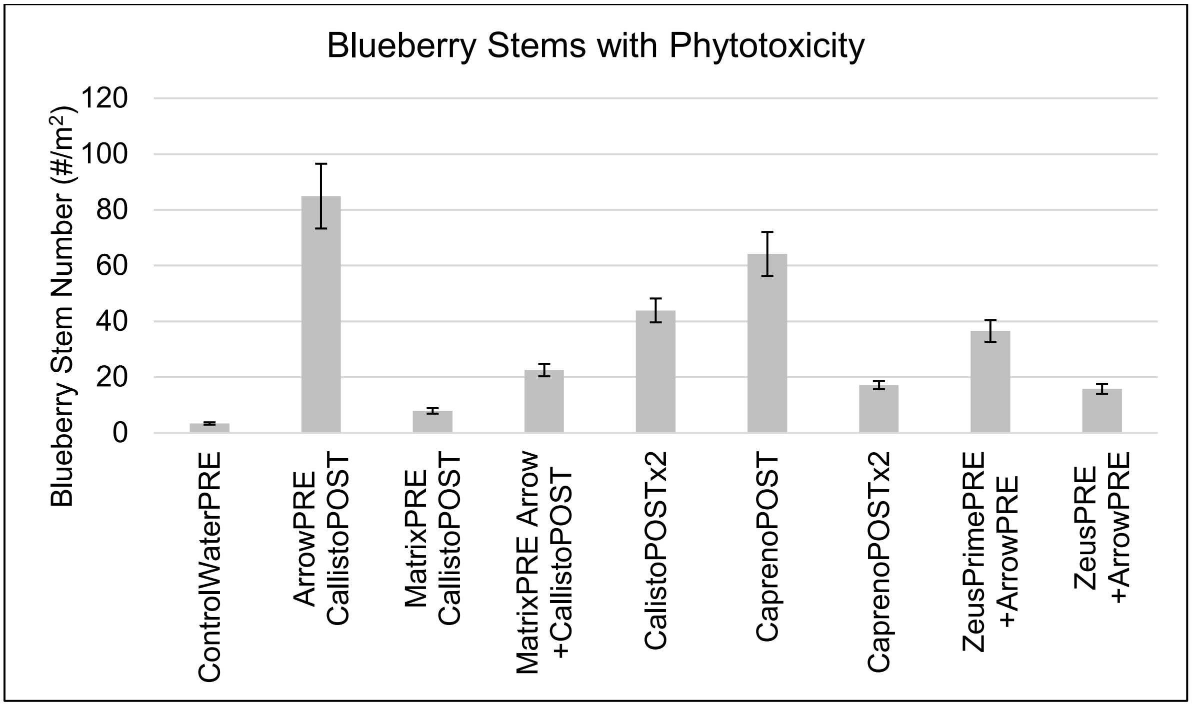

Figure 2. Average blueberry stems with phytotoxicity (#/m^2) measured in herbicide treatments on June 1 and July 7, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Data collected on August 25 was not included because it was so late in the season. Treatment differences were not significant. Error bars represent the standard error of the mean.

The greatest number of weeds were measured in the ZeusPrimePRE+ArrowPRE treatment (129 weeds/m^2; Figure 3). The ZeusPrimePRE+ArrowPRE had significantly greater weed presence than ArrowPRE+CallistoPOST (20 weeds/m^2), MatrixPRE+CallistoPOST (44 weeds/m^2), MatrixPRE Arrow+CallistoPOST (63 weeds/m^2), and CaprenoPOST (2 weeds/m^2). Only CaprenoPOST with 2 weeds/m^2 had significantly fewer weeds than the control (ControlWaterPRE, 86 weeds/m^2).

The greatest number of weeds displaying phytotoxicity were measured in the CaprenoPOSTx2 treatment (48 weeds/m^2), followed by CallistoPOSTx2 (41 weeds/m^2), and ZeusPRE+ArrowPRE (38 weeds/m^2; Figure 4). While the CaprenoPOST treatment had the fewest number of weeds present, zero weeds exhibited phytotoxicity in this treatment, likely due to the low number present. The ControlWaterPRE treatment had 5.9/m^2, suggesting residual effects from prior herbicide applications on that field.

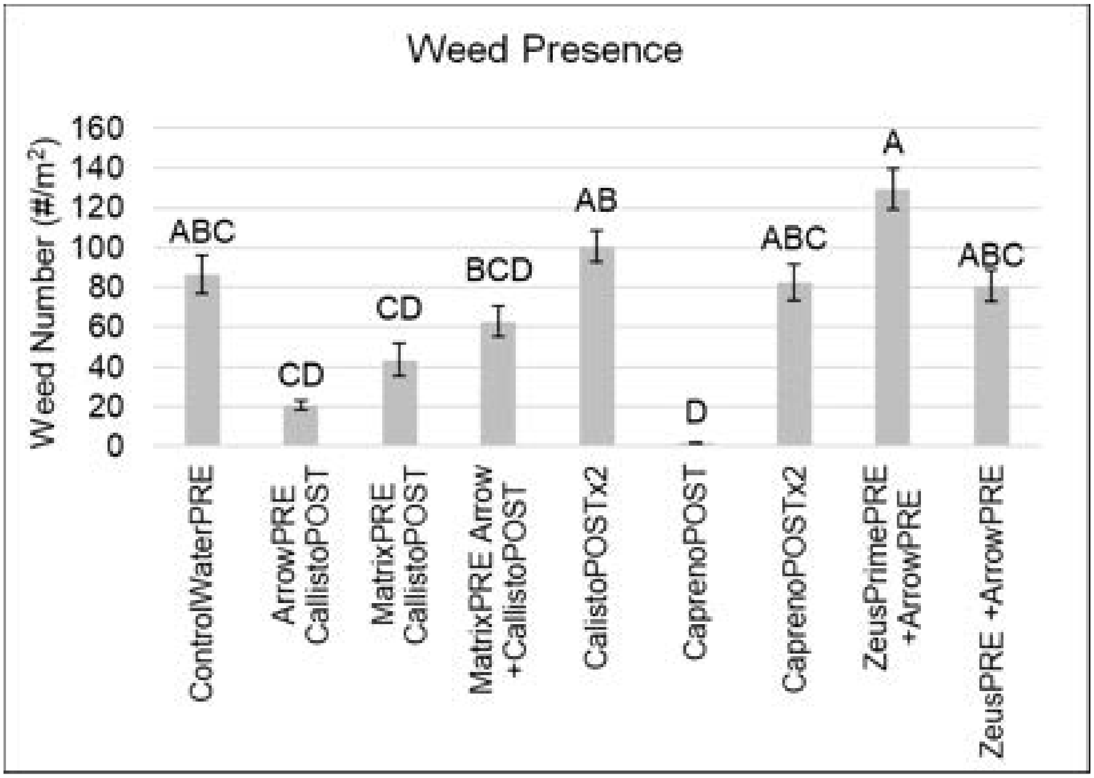

Figure 3. Average weed number (#/m^2) measured in herbicide treatments on June 1, July 7, and August 25, 2022 at Blueberry Hill Farm, Jonesboro Maine. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean. Data were transformed via a SQRT transformation prior to statistical evaluation, untransformed data is pesented above for readability.

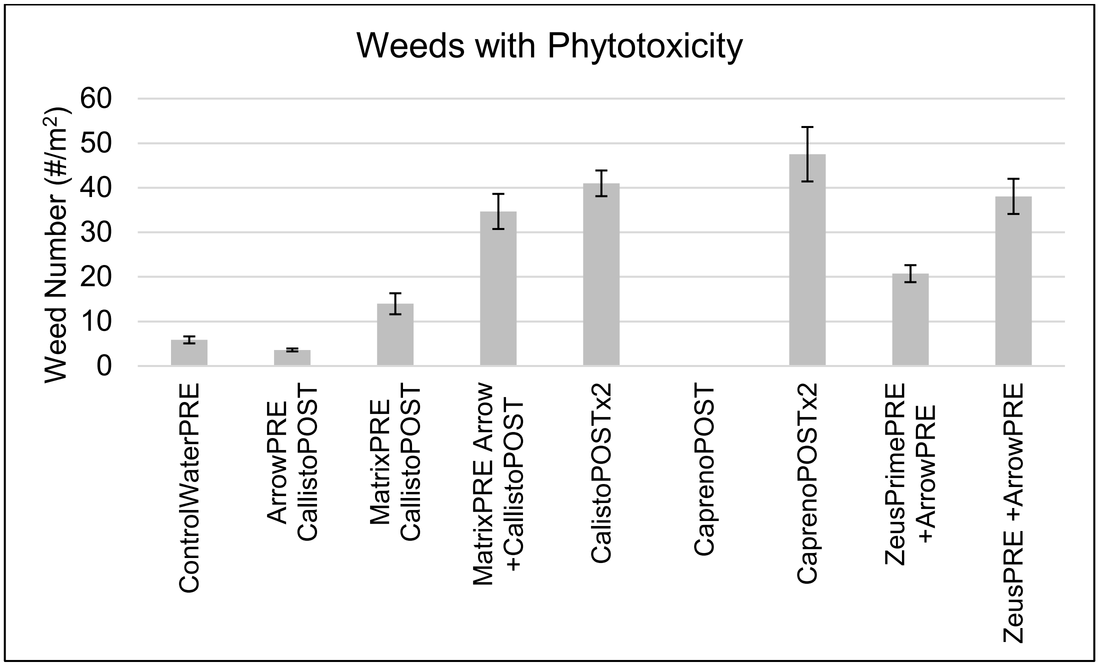

Figure 4. Average weeds with phytotoxicity (#/m^2) measured in herbicide treatments on June 1 and July 7, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Treatment differences were not significant. Error bars represent the standard error of the mean.

Weeds that were present in the trial location were primarily woody broadleaf perennials, broadleaf perennials, and annual or perennial grasses, which is characteristic of many blueberry fields across the state (Table 3). The only annual broadleaf weeds observed in the trial location were orange St. John's-wort, observed in plots where CallistoPOSTx2, CaprenoPOSTx2, ZeusPrimePRE+ArrowPRE, and ZeusPRE+ArrowPRE were applied, and violet, observed where CallistoPOSTx2 and ZeusPRE+ArrowPRE were applied. The top two weeds observed across almost all treatments included the broadleaf perennial red sorrel (present in all treatments except CaprenoPOST) and the perennial grass sedge (present in all treatments except ArrowPRE+CallistoPOST and MatrixPRE+CallistoPOST). The absence of weeds in particular treatments is likely due to the spatial variation in weed communities. The location was chosen for easy acess at field days, which meant that fewer weeds were present.

Weeds that experienced phytotoxity from the applied products included woody perennial weeds: chokecherry and pincherry; broadleaf perennial weeds: bunchberry, goldenrod, meadowsweet, milkweed, red sorrel, sarsparilla, and whorled loosestrife; annual and perennial grasses (identifed to the general category of grass, as well as poverty oat grass, sedge and witchgrass); and annual broadleaf weeds: orange St. John's-wort and violet. Here, red sorrel exhibited the greatest response to almost all products and treatments with the exception of CaprenoPOST where it was not present and the control (ControlWaterPRE).

The double post emergent herbicide applications of Callisto and Capreno exhibited the broadest sprectrum impacts assesed by the presence of phytotoxicity relative to all other products. CaprenoPOSTx2 exhibited the greatest percent of weeds with phytotoxicity damage (65% of 73 weeds/m^2). Affected weeds included chokecherry (100%), goldenrod (100%), meadowsweet (50%), orange St. John's-wort (5%), and red sorrel (90%). CallistoPOSTx2 only exhibited phytotoxicity on 34% of the 121 average total weeds/m^2, however, this treatment presented broader sprectrum effects with phytotoxocicty damage on bunchberry (75%), pin cherry (100%), poverty oat grass (33%), red sorrel (28%), sedge (19%), violet (100%) and whorled loosestrife (5%).

The double pre-emergent herbicide application of ZeusPrime+Arrow and Zeus+Arrow both impacted bunchberry with 40% and 55% phytotoxicity respectively. While the Zeus+Arrow exhibited a higher percentage of phytotoxicity of the total weeds present (40% of 95 weeds/m^2), only three weed species exhibited phytotoxicity including: bunchberry, red sorrel, and sarsaparilla. In contrast, the ZeusPrime+Arrow only had phytotoxicity on 18% of 113 weeds/m^2, but the phytotoxic damage was present on five weed species including: bunchberry, poverty oat grass, red sorrel, witchgrass, and whorled loosestrife.

Table 3. Average total weeds present (#/m^2) with the average percent of weeds with phytotoxicity in parentheses by herbicide treatment and weed species for June 1 and July 7, 2022 when peak weed populations were present at Blueberry Hill Farm, Jonesboro, Maine. In the life cycle column, 'W' indicates woody broadleaf perennial, 'P' indicates broadleaf perennial, 'G' indicates annual or perennial grass, and 'A' indicates broadleaf annual. Bold text highlighted with light grey indicates weed species with phytotoxicity.

Total Average Weeds (% with phytotoxicity)		**Control WaterPRE 98.6 (6%)**	**ArrowPRE CallistoPOST 19.6 (18%)**	**MatrixPRE CallistoPOST 45.0 (31%)**	**MatrixPRE Arrow+ CallistoPOST 79.3 (44%)**	**Callisto POSTx2 121.2 (34%)**	**Capreno POST 2.0 (0%)**	**Capreno POSTx2 73.2 (65%)**	**ZeusPrimePRE ArrowPRE 112.8 (18%)**	**Zeus PRE ArrowPRE 95.0 (40%)**
Weed Name	**Life Cycle**									
Bramble	W	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4
Bunchberry	**P**	**18.7 (29%)**	**0.0**	**0.0**	**0.0**	**28.2 (75%)**	**0.0**	**0.0**	**25.7 (40%)**	**22.7 (55%)**
Chokecherry	**W**	**0.0**	**0.0**	**1.8 (100%)**	**0.0**	**0.0**	**0.0**	**0.9 (100%)**	**5.9**	**0.0**
Cinquefoil	P	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7
Grass	G	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Goldenrod	**P**	**1.6**	**0.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.2 (100%)**	**0.0**	**1.1**
Maple	W	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Meadowsweet	**P**	**0.0**	**1.4 (67%)**	**0.2**	**0.0**	**0.0**	**0.0**	**0.5 (50%)**	**0.0**	**0.0**
Milkweed	**P**	**0.0**	**1.4 (83%)**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Moss	P	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0
Orange St. John's-wort	**A**	**8.6**	**0.9**	**0.0**	**0.0**	**22.7**	**0.0**	**17.1 (5%)**	**9.7**	**1.6**
Pin Cherry	**W**	**0.0**	**0.0**	**0.2**	**0.0**	**7.2 (100%)**	**0.0**	**0.0**	**0.0**	**0.0**
Poverty Oat Grass	**G**	**5.0**	**0.0**	**0.0**	**0.0**	**1.4 (33%)**	**0.0**	**0.0**	**0.9 (75%)**	**0.0**
Rose	W	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Red Sorrel	**P**	**58.8**	**13.1 (12%)**	**42.8 (28%)**	**75.7 (45%)**	**37.4 (28%)**	**0.0**	**50.0 (90%)**	**47.1 (7%)**	**57.0 (43%)**
Rush	G	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.4
Sarsaparilla	**P**	**0.9 (50%)**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**1.8 (38%)**
Sedge	**G**	**1.4**	**0.0**	**0.0**	**3.6 (25%)**	**4.7 (19%)**	**2.0**	**3.4**	**2.3**	**2.9**
St. John's-wort	P	0.2	0.9	0.0	0.0	0.0	0.0	0.0	0.5	0.0
Strawberry	W	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Toadflax	P	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
Violet	**A**	**0.0**	**0.0**	**0.0**	**0.0**	**0.2 (100%)**	**0.0**	**0.0**	**0.0**	**0.2**
Witchgrass	**G**	**0.0**	**0.0**	**0.0**	**0.0**	**11.0**	**0.0**	**0.0**	**12.2 (41%)**	**0.0**
Whorled Loosestrife	**P**	**0.0**	**0.0**	**0.0**	**0.0**	**8.3 (5%)**	**0.0**	**0.0**	**8.6 (16%)**	**0.0**

Different weeds saw different levels of control from the herbicides. Red sorrel (*Rumex acetosella*) is a persistent weed difficult to control. Almost all treatments with evidence of phytotoxicity on weeds or wild blueberry displayed phytotoxicity on red sorrel, with 90% of red sorrel in the CaprenoPOSTx2 treatment displaying phytotoxicity, and 7% in the ZeusPrimePRE+ArrowPRE treatment (Figure 5). The CaprenoPOST and ControlWaterPRE treatments did not exhibit any red sorrel with phytotoxicity.

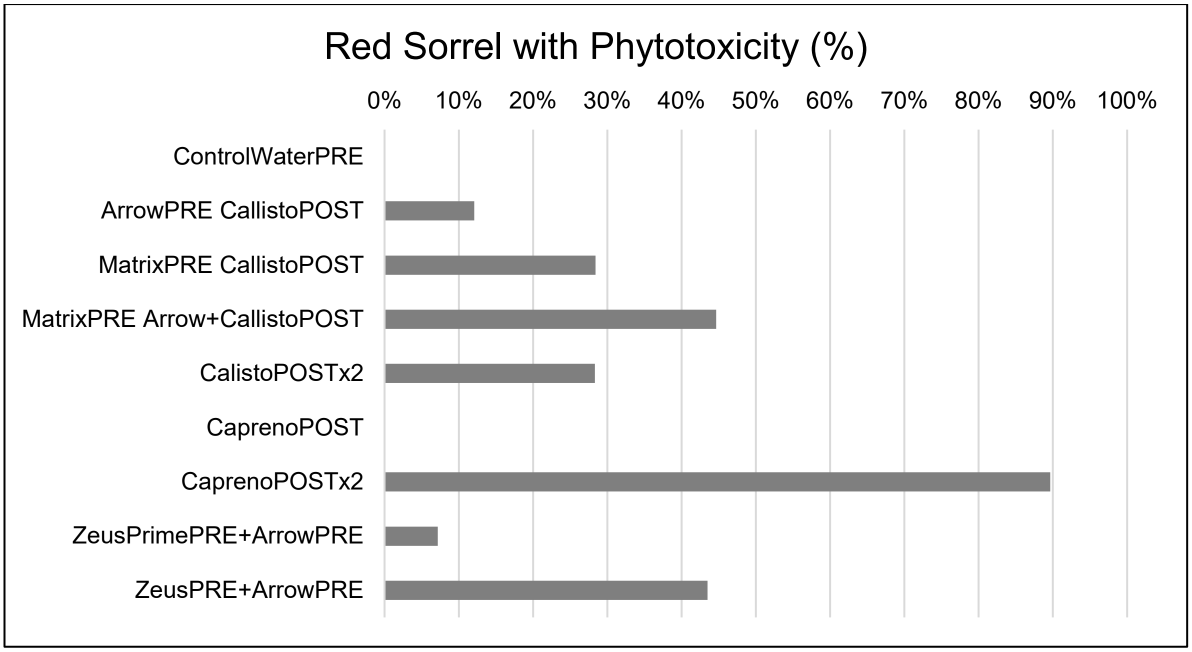

Figure 5. Average percent of red sorrel with phytotoxicity (%/total red sorrel present) measured in herbicide treatments on June 1 and July 7, 2022, at Blueberry Hill Research Station, Jonesboro, Maine.

A bivariate linear regression showed a signifcant negative linear relationship ($p < 0.001$) between weed cover and blueberry cover (Figure 6). Here, higher blueberry coverage corresponded to lower weed coverage and higher weed coverage corresponded to lower blueberry coverage.

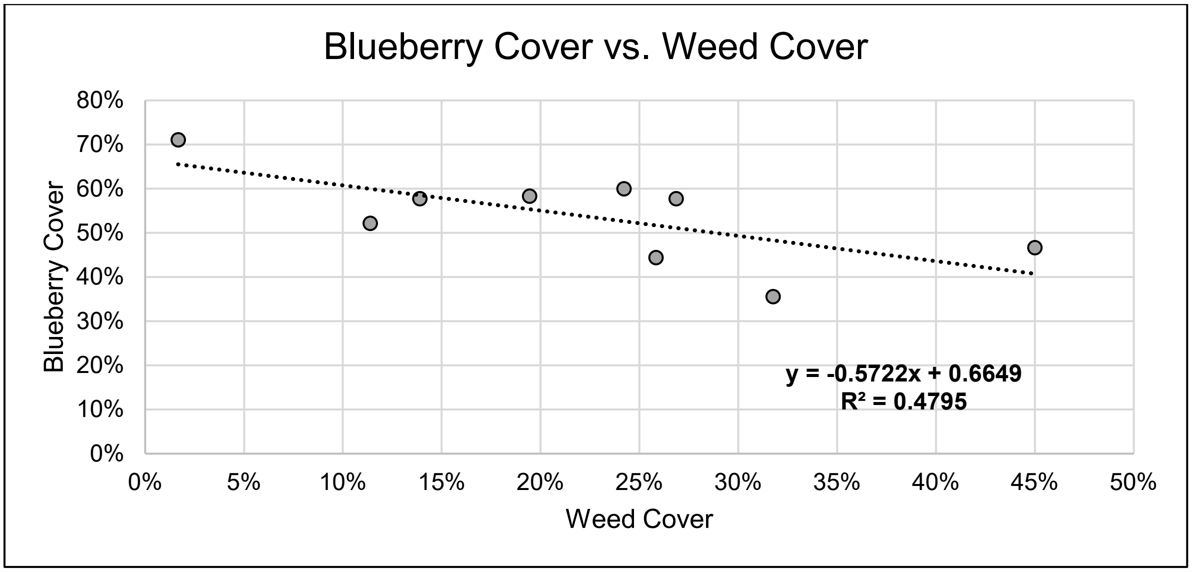

Figure 6. Relationship between blueberry cover and weed cover in herbicide treatments on June 1, July 7, and August 25, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. The trendline

exhibits the negative linear relationship between blueberry density and weed density where higher blueberry cover corresponds with lower weed cover and vice versa.

DISCUSSION

The most phytotoxicity to wild blueberry was caused by the ArrowPRE+CallistoPOST application, which saw 85 stems/m^2 with phytotoxicity. CaprenoPOST saw 64 stems/m^2 with phytotoxicity. Since the ControlWaterPRE treatment had a phytotoxicity level of 3 stems/m^2 in wild blueberry, it is possible that some or all treatment sites were subject to herbicide drift from this study or the residual effects of herbicides applied in earlier research at the site or nearby.

The experimental CaprenoPOSTx2 treatment demonstrated substantial weed control, where 48 of the 72 weeds/m^2 (65%) demonstrated phytotoxicity. CallistoPOSTx2 demonstrated decent weed control as well, with 41 out of 121 weeds/m^2 (34%) exhibiting phytotoxicity.

Of the weeds controlled, red sorrel and bunchberry were among the most common to exhibit phytotoxicity. Weeds after treatment by CaprenoPOSTx2 exhibited the most phytotoxicity, particularly in chokecherry, goldenrod, meadowsweet, orange St. John’s-wort, and red sorrel (three broadleaf perennials, one woody broadleaf perennial, and one annual/perennial grass). Weeds after treatment by CallistoPOSTx2 exhibited less phytotoxicity, but the spectrum of weeds damaged was broader (one broadleaf annual, 1 woody broadleaf perennial, one annual/perennial grass, and three broadleaf perennials).

The CaprenoPOST treatment area did not contain many weeds (just 2 weeds/m^2), and none of those weeds exhibited phytotoxicity so it is impossible to say whether the treatment is effective at controlling weeds. Further study into the effectiveness of CaprenoPOST in an area with more weeds of several species may answer this question.

CURRENT RECOMMENDATIONS

- We recommend herbicide staking with all products tested at appropriate timings as described **EXCEPT for Capreno which is not labeled for use in wild blueberry yet.**

NEXT STEPS

- Conduct a similar 2023 herbicide stacking demonstration on the upper field at Blueberry Hill Farm because it has a different and more dense weed community.
- Continue gathering data to determine if herbicide applications reduce shoot density in future seasons instead of only causing phytotoxicity damage.

ACKNOWLEDGEMENTS

This project was funded by the Maine Agriculture and Forestry Experiment Station and the Wild Blueberry Commission of Maine. We greatly appreciate their support. Thank you to Abby Cadorette, Charles Cooper, Julian LaScala, and Jordan Ramos for data collection and analysis assistance.

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INVESTIGATORS: Seanna Annis, Jacob Schwab, Zoe Colwell

1. Research into control of various leaf spot in wild blueberry in 2022

OBJECTIVES: Improve identification and control of leaf spots.

1) Test fungicides for their efficacy in managing Exobasidium leaf and fruit spot and determine potential effect of lime sulfur fungicides on soil chemistry.
2) Test fungicides for their efficacy in managing leaf spot diseases on lowbush blueberry including Sphaerulina leaf spot (*Sphaerulina vaccinii*), powdery mildew (*Erysiphe vaccinii*), and leaf rust (*Thekopsora minima*)
3) Develop a DNA-based method for detecting *Sphaerulina vaccinii* infected leaves and spores

LOCATIONS: Multiple wild blueberry fields around Maine
PROJECT TIMEFRAME: January 2022 – August 2023

METHODS

Fungicide efficacy for management of Exobasidium leaf and fruit spots

On March 26, 2022, 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 2021. The plots were randomly assigned one of three treatments: Lime Sulfur Ultra at two rates: 1 and 2 gal/acre, Sulforix at 1 gal/acre, or an untreated control, with six replications for each treatment. 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 15, 2022. All Exobasidium leaf spots were counted in two randomly placed 2.7 ft^2 subplots per plot. Phytotoxicity was also rated during visits. On August 2, 2022, all fruit within each treatment plot was harvested using blueberry rakes and weighed for total yield. A subsample of ~1 lb was taken from the total harvest, bagged, and placed on ice. All of the berries with Exobasidium fruit spots were counted in the subsample. A random selection of 100 berries were weighed to estimate berry weight. In August, 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, USA).

Fungicide efficacy trial to manage leaf spots

A randomized complete block experiment was established in a vegetative lowbush blueberry field where high levels of leaf spot 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 2 and 9. 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. Disease symptoms and leaf loss were rated three times, on July 11, August 15 and September 13, 2022. 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 in July and August was rated for the leaf cover with disease symptoms of Sphaerulina leaf spot, leaf rust, or powdery mildew. In September, only incidence of the various leaf spots was recorded. For all ratings, leaves and bare nodes were counted for leaf loss.

Table 1. Materials used in fungicide efficacy trial for control of leaf spots

Fungicide	Application Rate (fl. oz./acre)	Application timing
Oxidate 5.0 (and CapSil)	1% v/v (1.2)	2 applications: June 2 and 9
Oxidate + Proline	1% v/v + 5.7	2 applications: June 2 and 9
Luna Flex - Early	11.2	One application: June 2
Luna Flex - Late	11.2	One application: June 9
Propulse - Early	10.0	One application: June 2
Propulse - Late	10.0	One application: June 9
Proline - Early	5.7	One application: June 2
Proline - Late	5.7	One application: June 9

Proportion 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, USA). Least square means were used to determine specific differences among treatments ($\alpha = 0.05$).

Identification of Sphaerulina leaf spot by DNA methods

Zoe Colwell, MS graduate student, collected leaves with the appearance of Sphaerulina leaf spot from multiple fields. The leaves were surface sterilized and plated out to isolate possible Sphaerulina cultures. DNA was extracted and a portion of the ITS regions amplified. Spore traps to collect wet spores were developed and placed in numerous wild blueberry fields from late April to June. Spore traps were collected and frozen until they can be used.

Results

Fungicide efficacy for management of Exobasidium leaf and fruit spots

The Lime Sulfur Ultra and Sulforix treatments decreased the number of leaf spots compared to the control but due to high variability among plots (subplots varied from 96 to no leaf spots), there were no significant differences among treatments and the control (Figure 1). At harvest, there were very low levels of fruit spots and there was no difference among the treatments and the untreated control (Figure 1). The treatments did not have any significant effects upon overall yield or the weight of 100 berries (Table 2). No phytotoxicity was noted on the plants. 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 3).

Table 2. Effect of fungicides on fruit spots and yield as measured by the total weight per plot (lb) and the weight per 100 fruit (g). Averages presented ± the standard error of the mean.

Treatment	Average total weight per plot (lb)	Weight per 100 fruit (g)
Lime Sulfur Ultra – Low (1gal/A)	1.6 ± 0.4	40.6 ± 3.2
Lime Sulfur Ultra – High (2 gal/A)	2.1 ± 0.4	37.5 ± 2.4
Sulforix (1 gal/A)	2.4 ± 0.3	42.5 ± 1.1
Control	1.7 ± 0.3	39.6 ± 2.3

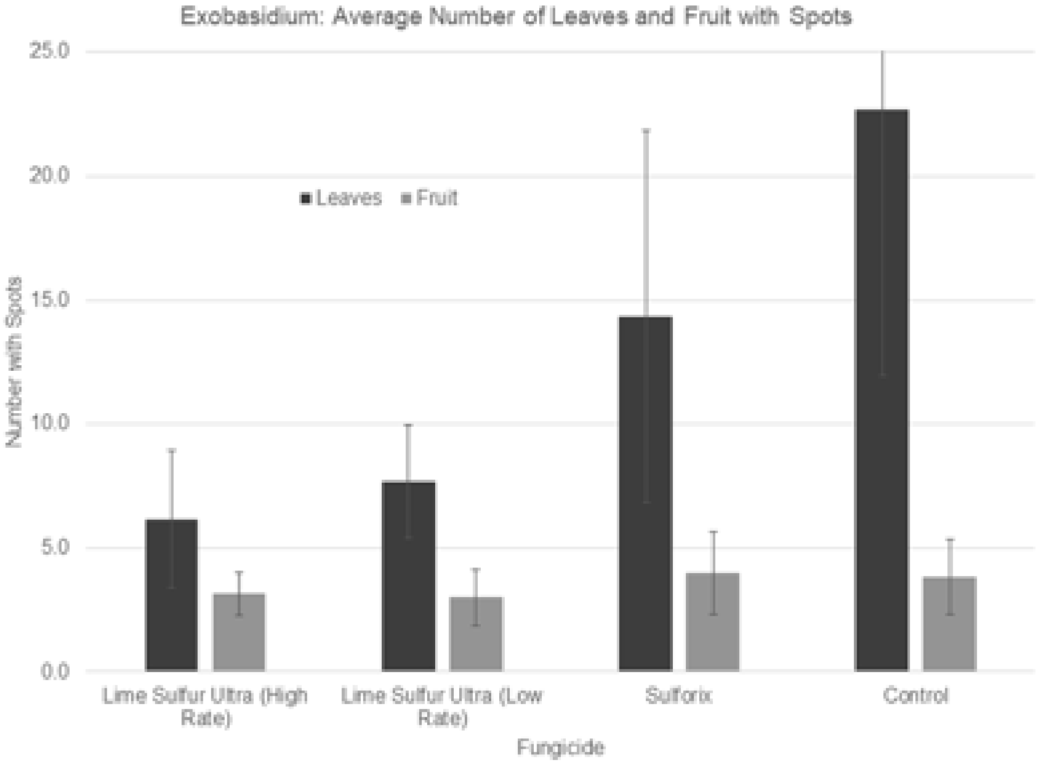

Figure 1. Number of Exobasidium spots on leaves and fruit per treatment. Bars represent the standard error of the mean. There was no significant difference between treatments.

Table 3. Percent calcium, pH, and ppm sulfur measured in soil sampled for individual treatment plots. Averages presented ± the standard error of the mean.

Treatment	Calcium (%)	pH	Sulfur (ppm)
Lime Sulfur Ultra – Low	16.5 ± 2.0	4.4 ± 0.02	101.7 ± 4.2
Lime Sulfur Ultra – High	16.1 ± 1.5	4.3 ± 0.04	104.0 ± 3.6
Sulforix	13.4 ± 1.0	4.35 ± 0.02	104.0 ± 1.4
Control	16.7 ± 2.6	4.4 ± 0.04	92.8 ± 4.3

Fungicide efficacy trial to manage leaf spots

No phytotoxicity was seen with any of the treatments. The level of leaf loss in the control increased from 4% in July to 32 to 33% in August and September, respectively. The treatment of Oxidate and Proline was the only treatment with significantly lower leaf loss in July compared to the control (Figure 2). In August (Figure 2), all of the fungicide treatments, except Oxidate alone, significantly decreased leaf loss by approximately half of the control. In September (Figure 2), only the late applications of Luna Flex, Proline, and the Oxidate with Proline treatments had significantly less leaf loss than the control. There was a high incidence of stems with Sphaerulina leaf spot in July but very low percent cover (Figure 3). Most stems had symptoms of Sphaerulina leaf spot in August and September but still there were low percentages of cover in August (Figure 3). In August, all of the fungicide treatments had significantly lower leaf cover with Sphaerulina than the control. In September, there were no effects of the treatments on Sphaerulina incidence. Only incidence of Sphaerulina on stems was rated since it is difficult at that stage to distinguish early symptoms of leaf rust and late symptoms of Sphaerulina. Powdery mildew had higher incidence in August than September and had low percent cover in August (Figure 4). The lower incidence of stems with Sphaerulina leaf spot and powdery mildew in September probably was due to loss of leaves with these diseases. Rust was only recorded when rust pustules were producing the characteristic uredinospores on the underside of the blueberry leaf, and was only identified in September (Figure 5). There were no significant effects of the treatments on the percent cover or incidence for any of other diseases in July, August or September. There is a high degree of variability among lowbush blueberry genotypes and stems on the symptoms which makes estimating percent cover difficult. The recommendation is to retest these materials in 2023.

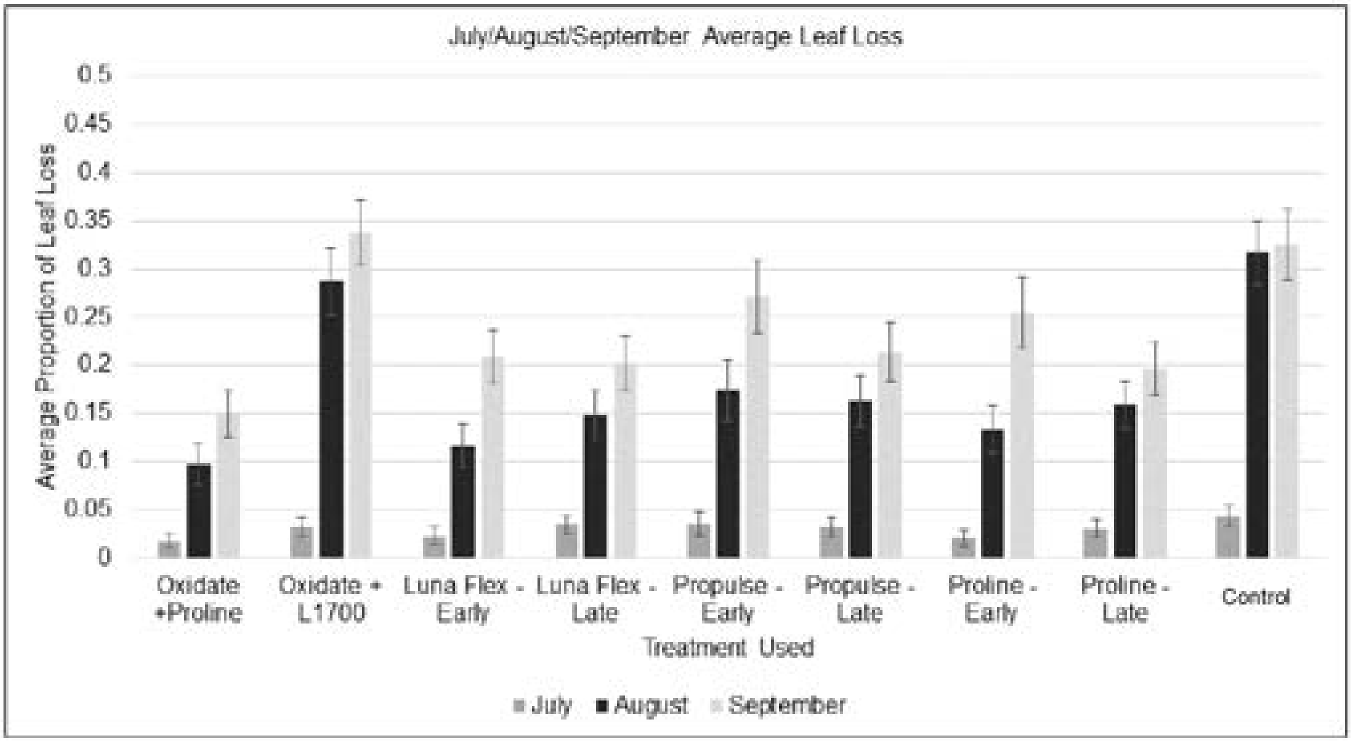

Figure 2. Leaf loss in July, August and September for plots treated with different materials and at different time periods. Bars represent the standard error of the mean.

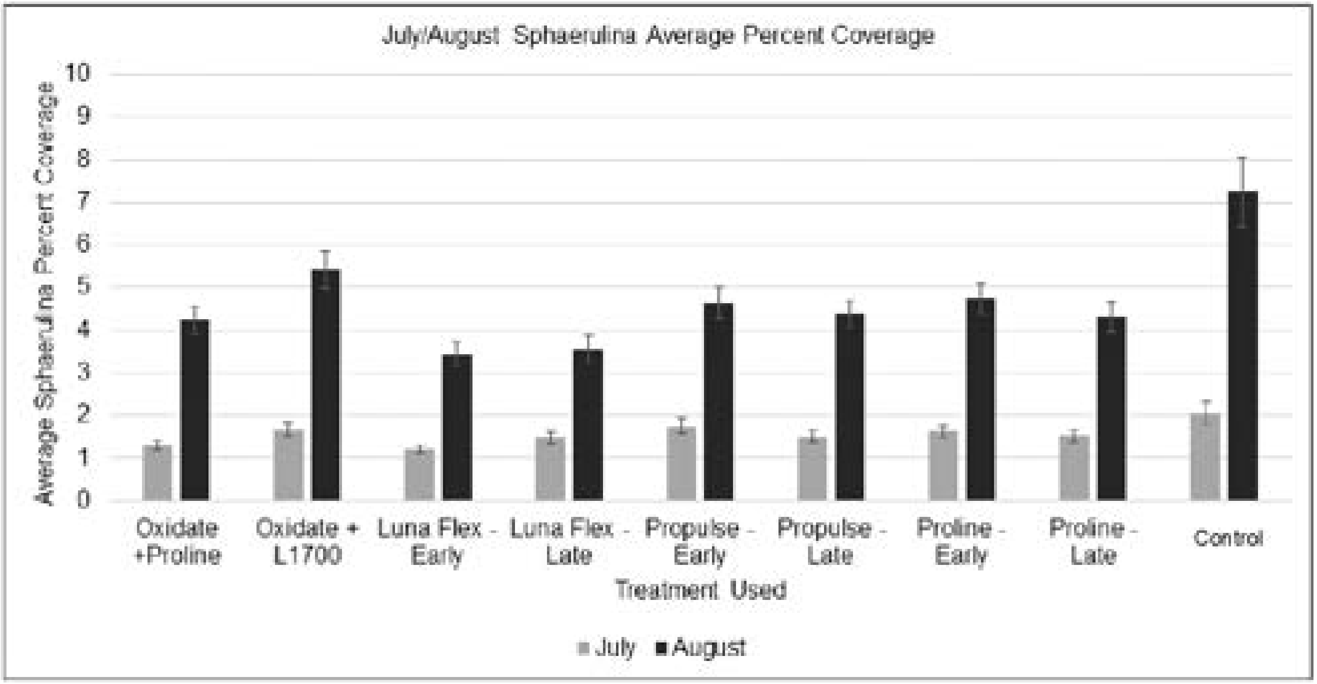

Figure 3. Average percent coverage of leaves with Sphaerulina leaf spot in July and August. Bars indicate standard error of the mean.

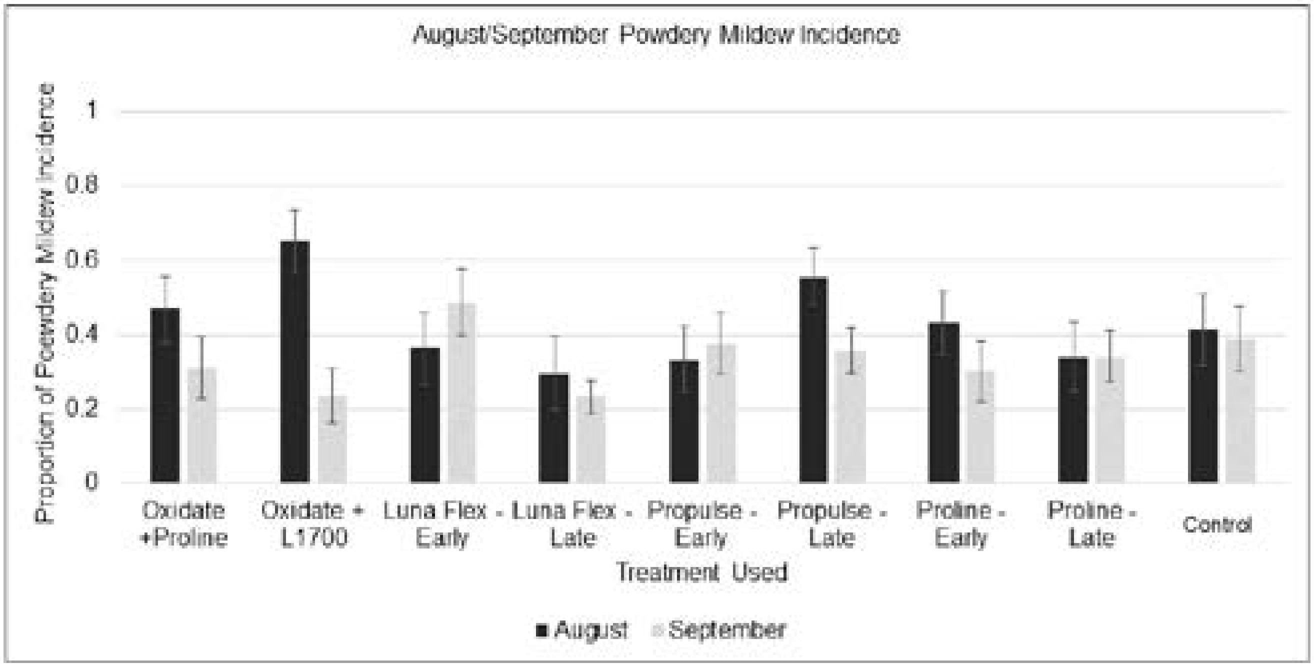

Figure 4. Average percent coverage of leaves with powdery mildew symptoms in July and August. Bars indicate standard error of the mean.

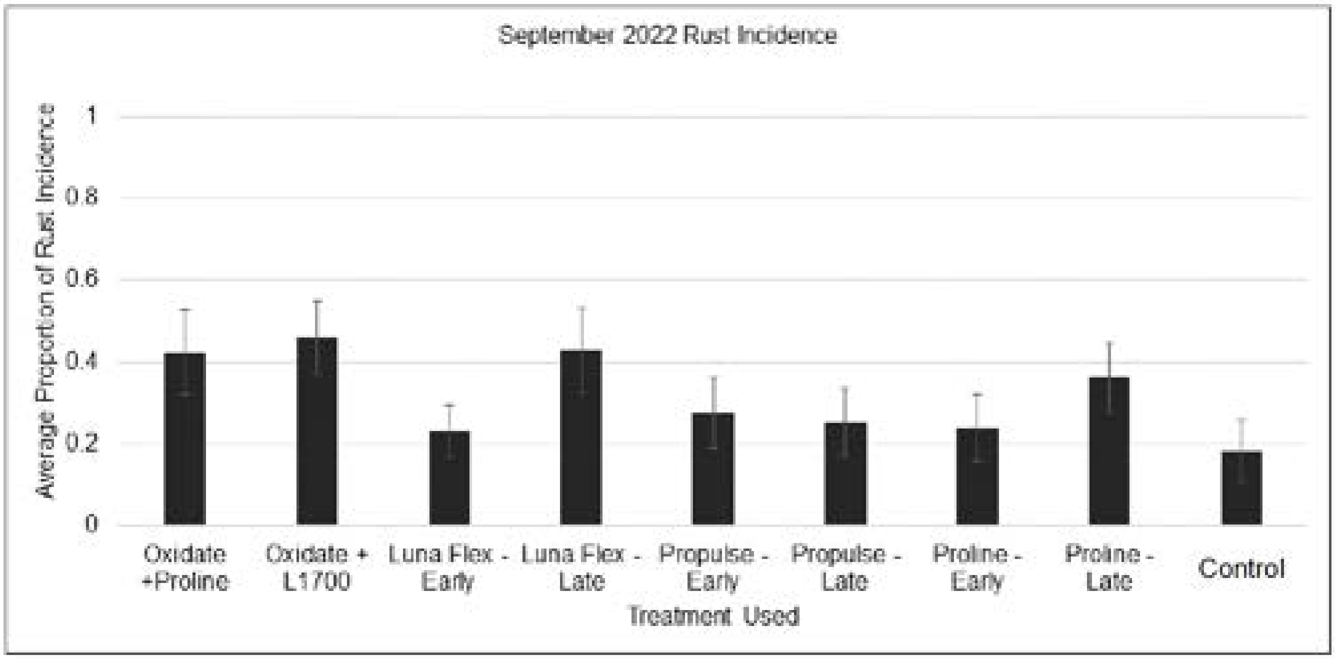

Figure 5. Average proportion of stems with leaf rust in September. Bars indicate standard error of the mean.

Identification of Sphaerulina leaf spot by DNA methods

Over fifty isolates have been collected from possible Sphaerulina infected leaves. The isolates were grouped by morphology and a subset of approximately 35 isolates were used to extract DNA and amplify the ITS region of the fungal genome. The fragments have been quantified and will be sequenced in January 2023. Spore traps tapes and slides are stored in the freezer until they can be extracted in spring of 2023. A DNA based method to identify the fungus will be developed and its efficacy will be tested on the spore traps and infected leaves from the field.

ACKNOWLEDGEMENTS
We would like to thank Bill Little and all of the other growers for allowing us to use their land in these experiments. Sophia Suriano was our summer student who helped with all field experiments. Funding was from a Specialty Crop Block grant from the Maine Department of Agriculture, Conservation and Forestry and the Wild Blueberry Commission of Maine.

INVESTIGATORS: Seanna Annis, Jacob Schwab, and Ian Leonard

2. Research for Improved Control of Mummy Berry 2022

OBJECTIVE

Improve control of mummy berry caused by *Monilinia vaccinii-corymbosi* (MVC).

1) Determine the factors affecting pseudosclerotia (mummified berries) germination and primary infection of wild blueberry
2) Provide growers with forecast reports of MVC infection periods
3) Test the efficacy of new materials for their control of mummy berry symptoms

LOCATIONS: Multiple wild blueberry fields in Maine
PROJECT TIMEFRAME: January 2022 to November 2022

METHODS:

Factors affecting apothecia development and susceptibility of wild blueberry to mummy berry
Ian Leonard, MS graduate student, has been conducting research on pseudosclerotia (mummy berries; PS) germination and wild blueberry susceptibility to primary infection of MVC. From results of the 2021 field season, the effect of soil type on PS development was tested. An incubator experiment used silt loam (Midcoast) and sandy loam (Downeast) soils and PS from four different blueberry fields. The PS germination was followed every two days and effects of soil and origin of PS analyzed. A second experiment was set up in fall of 2021 where PS from Downeast and Midcoast were both planted in six fields in the Midcoast and Downeast. These fields were used for monitoring fungal and plant development, disease levels and weather conditions. Fields were visited twice a week during April and May. The data collected in the field in 2022 and from common garden experiments in 2013, 2016, and 2021 were combined to try to develop a model of mummy berry germination using weather factors.

Wild blueberry bud development (Hildebrand & Braun, 1991) was monitored between April and June 2022 in eight fields throughout the Midcoast and Downeast regions of Maine. Each field had a weather station and five different genets were selected for disease monitoring, and within each genet, five individual stems were selected for rating phenology twice a week. Mummy berry disease on marked stems and a transect was rated once a week for three weeks overlapping bloom. The plant development data was compared to fungal development and weather conditions to determine if there were any correlations among these to disease level.

Survey of weather and disease in wild blueberry fields
In early April 2022, weather stations with cellular internet connections were deployed in growers' fields across the Midcoast and Downeast regions in Maine (Figure 1). Each station was equipped to measure air and soil temperature, leaf wetness and soil moisture. Mummy berry (pseudosclerotia) plots were set up at many of the sites and monitored by Annis lab personnel or growers. All fields with weather stations

were evaluated between May 23 to 26, 2022. Thirty randomly chosen stems along each of four 30 ft transects were 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.

Fungicide efficacy trial

A field trial was set up in a blueberry field at the Blueberry Hill Research Farm in Jonesboro, ME during a crop year. A randomized complete block design was used with 10 treatments, 9 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. On April 25, 2022 and May 3, 2022, fungicides were applied to the treatment plots. Fungicide applications were timed to occur before infection periods, and were predicted using the mummy berry disease forecast method (more information in UMaine Cooperative Extension Bulletin #217). Disease ratings were made on May 20, 2022. A transect with 30 evenly spaced markings was used in each plot to rate the stem closest to each marking for disease symptoms on flowers and leaves as above. Phytotoxicity was rated at the same time. Blueberries were harvested on July 28, 2022 in a 2-ft wide strip down each plot center with a mechanical harvester, and the fresh weight was measured. Proportion measurements were transformed using arcsin of the square root 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, USA) using mixed model procedures (PROC GLIMMIX). Least Square means were used to determine specific differences among treatments (α = 0.05).

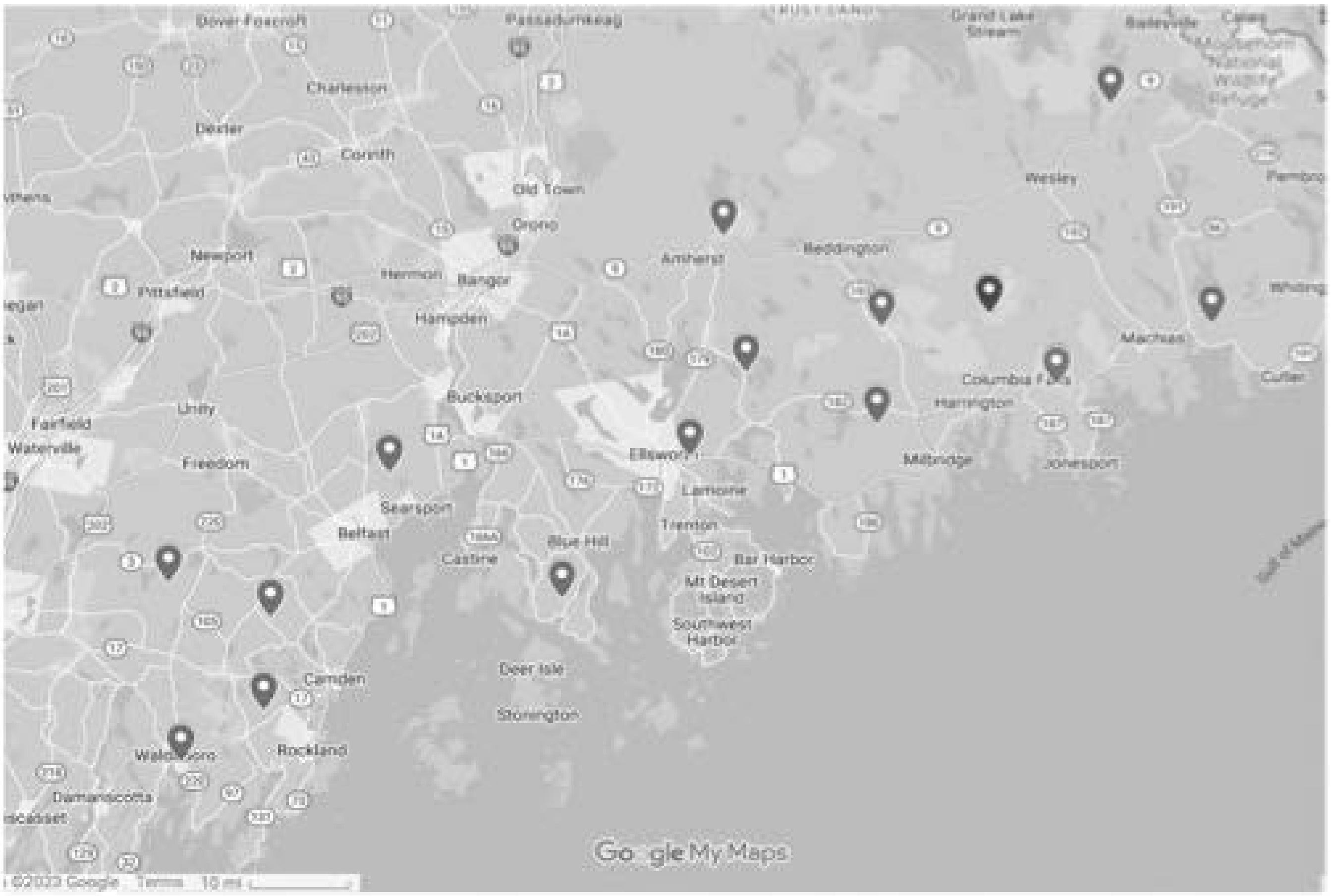

Figure 1. Location of weather stations in 2022.

Table 1. Materials tested for efficacy to control mummy berry in 2022.

Fungicide	Active Ingredient	Rate / Acre	EPA Reg. #	Manufacturer	FRAC Group	Registered on lowbush blueberry	Registered for Mb
Inspire Super	Difenoconazole / Cyprodinil	20 fl oz	100-1317	Syngenta	3 / 9	Yes	Only in highbush
Miravis Prime	Fludioxonyl/ SYN545984	13.4 fl oz	100-1603	Syngenta	7 / 12	Yes	Yes
Cevya (w/ LI700)	Mefentrifluconazole	5.0 & 3.0 fl oz	7969-407	BASF	3	No	No
Theia (w/ Dyne-Amic)	*Bacillus subtilis* strain AFS032321	3 lb	X	AgBiome	X	X	X
Howler (w/ Dyne-Amic)	*Pseudomonas chloroaphis* strain AFS009	5 lb	91197-3-92488	AgBiome	BM02	Yes	Yes
Omega 500F	Fluazinam	20 fl oz	71512-1-100	Syngenta	29	Yes	No
Ecoswing (w/ CapSil)	*Swinglea glutinosa*	32 fl oz	10163-357	Gowan		Highbush	*Monilinia* spp.
Tilt	Propiconazole	6 fl oz	100-617	Syngenta	3	Yes	Yes

RESULTS

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

In the incubator experiments, the Downeast mummies germinated more than the Midcoast mummies but soil type had no effect. In the field experiments, there was no difference in the germination of mummies from two different origins and in any of the fields. The high variability in weather, elevation, and other field conditions may have affected their germination. Soil moisture, soil temperature, solar radiation, chill hours and growing degree days (with a base of 32°F) all affect mummy berry germination. Unfortunately, none of the models that were developed so far were able to accurately predict timing of apothecia (cup) production. Further analysis will be conducted to try to develop an accurate model. The fields with the most mummy berry disease were in the Midcoast in 2022. The wild blueberry plants were typically increasing in their proportion of susceptible buds while apothecia were present. However, there was no pattern in 2022 of the number of possible infection periods correlating with disease levels. There also was no clear pattern between development stages of the plant and resulting disease levels.

Survey of weather and disease in wild blueberry fields

Mummy berry forecast reports were sent out via email, blog, and recorded phone message from April through June. There was little Botrytis pressure this year so no reports were sent out. Most field had low levels of disease but some fields with no fungicide applications or wetter soil had up to 25% disease (Figure 2). More stems had infected leaves than flowers. Many fields had only two to three possible infection periods which decreased the overall risk of infection. 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 Botrytis or frost detected in the weather station fields.

Fungicide efficacy trial
Apothecia were first observed approximately April 20 in the field and were finished approximately May 11. There were at least three to four possible infection periods for *Monilinia* within those weeks. There were no significant differences between the untreated control and the treatments, possibly due to variability among the blocks (Figures 3 and 4). The incidence and severity of disease were lower with Miravis Prime and similar with Omega 500F, Cevya (both rates), and Inspire Super to those treated with the standard Tilt (propiconazole), and lower by at least 50% compared to the untreated control. Cevya seemed to particularly work well in decreasing leaf infection. Cevya 3.0 fl oz/acre worked as well as the 5.0 fl oz/acre rate. There was no visible phytotoxicity seen in the treatments. The incidence and severity of disease of plots treated with Howler were similar to those of the untreated control. The Ecoswing treatment had higher disease levels than the untreated control which requires further experimentation to figure out this effect. Plots treated with Miravis Prime had significantly higher yield (lb/acre) than the Tilt, Cevya, Omega 500F or Inspire Super treated plots or the untreated control (Figure 5). The recommendation is to retest promising materials for mummy berry management in 2023. We have one year of data with a relatively lower level of disease pressure.

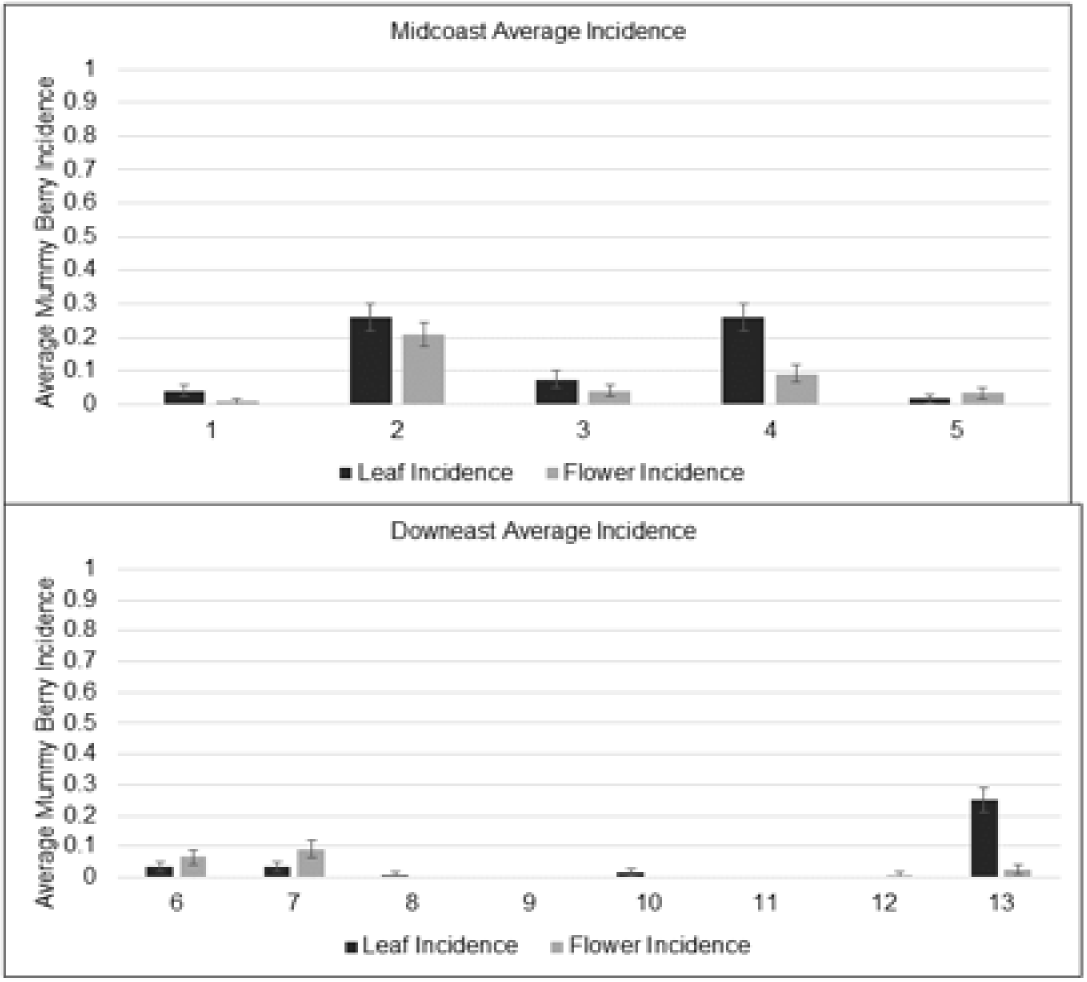

Figure 2. Average proportion of stems with mummy berry symptoms on flowers or leaves (branches)

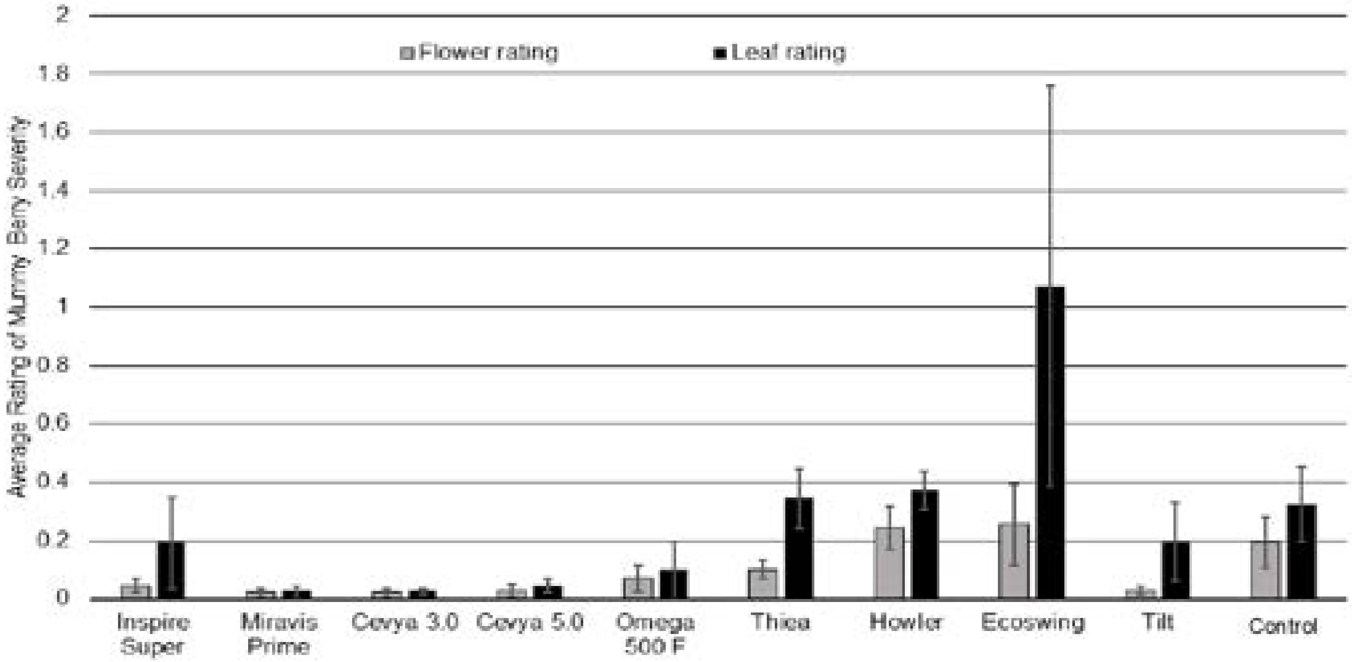

Figure 3. Average rating of mummy berry symptoms on infected flower and leaves (branches) in fungicide efficacy trial, 2022. Bars represent the standard error of the mean.

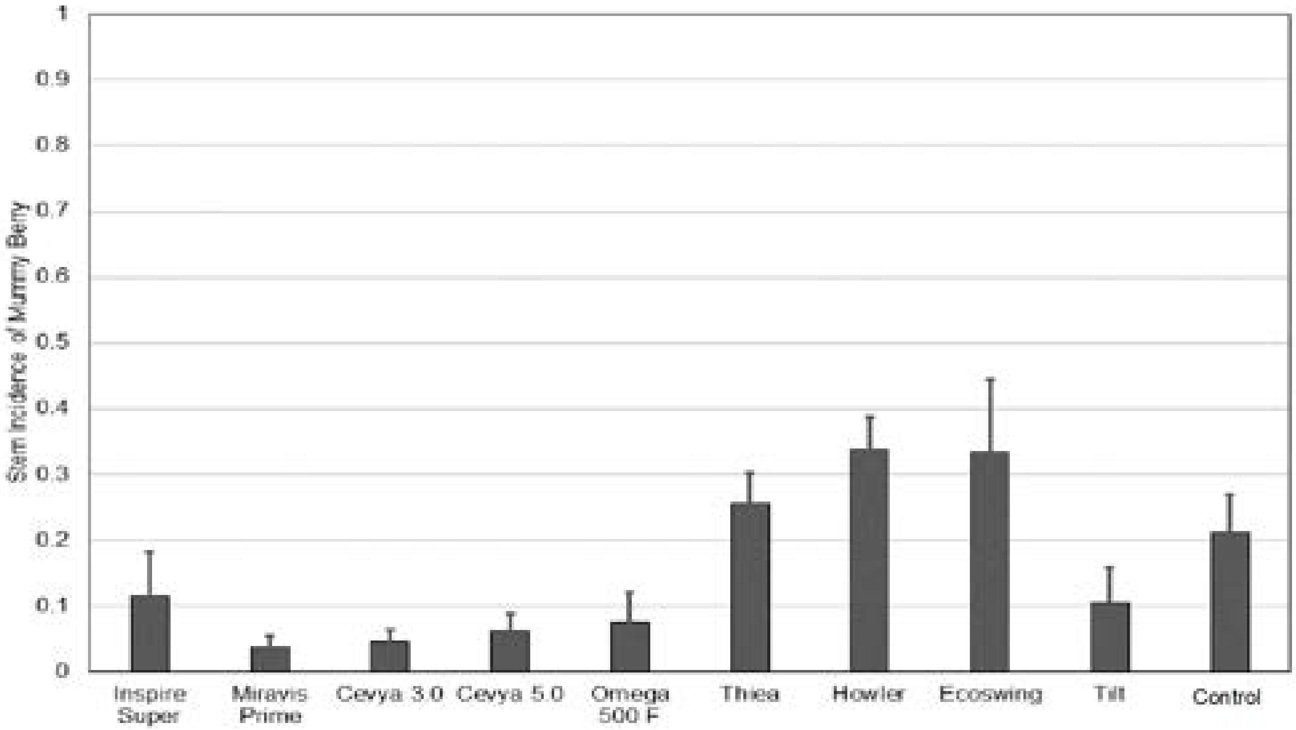

Figure 4. Average proportion of stems with mummy berry symptoms in fungicide efficacy trial, 2022. Bars represent the standard error of the mean.

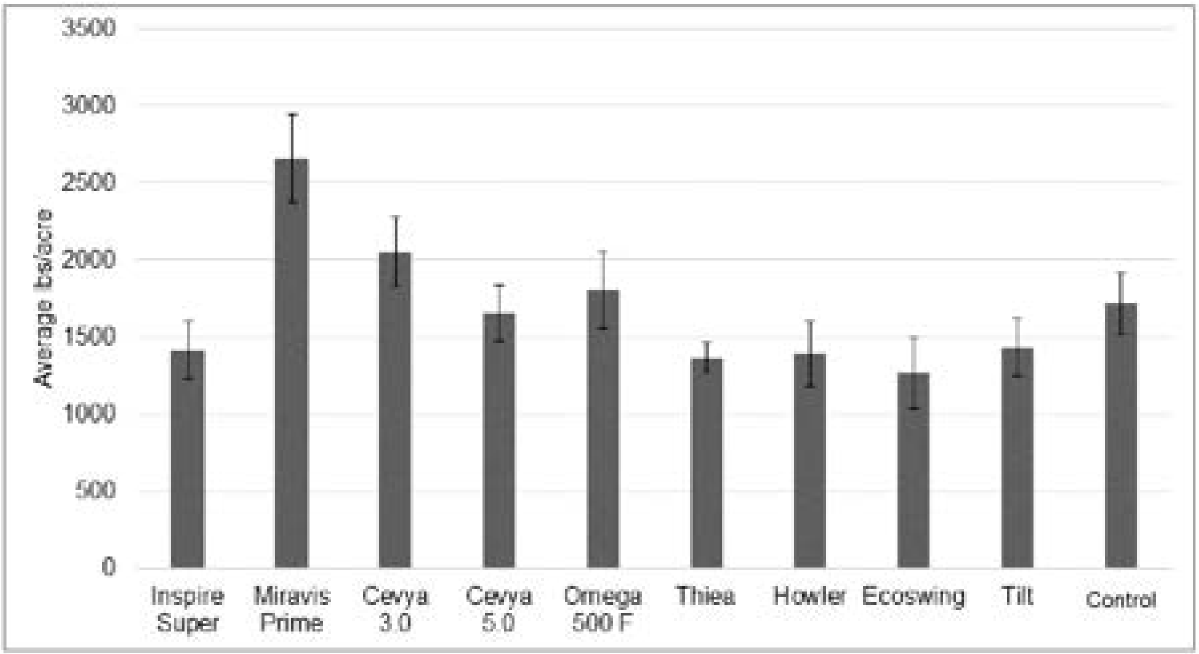

Figure 5. Average blueberry fruit yield as lb per acre. Bars represent standard error of the mean.

No fungicide residues (analyzed thanks to Wyman's) were found in berries harvested from plots treated with Omega 500F, Miravis Prime, Cevya (either rate), or Inspire Super.

ACKNOWLEDGEMENTS

We would like to thank the crew of Blueberry Hill Farm for helping to set up of experimental plots. Sophia Suriano was our summer student 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.

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INVESTIGATORS: K. Barai and YJ. Zhang

1. Using UAV and thermal-based remote sensing to detect spatial variation in water stress of wild blueberries

OBJECTIVES

- Validate the use of the thermal-based crop water stress index (CWSI) in predicting crop water status (Leaf Water Potential, LWP) in large and heterogenous wild blueberry fields for precision agriculture.
- Establish the CWSI-LWP model for wild blueberries across growth stages and quantify the spatial variation of water status within wild blueberry fields using the established model.

LOCATION: Wyman's Farm, Deblois, Maine.
PROJECT TIMEFRAME: July 2019 – August 2021

INTRODUCTION

Water availability is a major factor driving crop growth, development, health, and yield. Soil water deficits can cause stomatal closure and decreases transpiration and photosynthetic rate (Limpus, 2009). Consequently, water deficits can adversely affect crop physiology, resulting in reduced growth and overall production (Rossini et al., 2013).

Wild blueberries are considered relatively adapted to drought stress. However, one inch of water per week during the growing season is recommended, and a total of 43% increase in yield can be obtained with effective irrigation (Murray et al., 2002; Yarborough, 2004). Insufficient irrigation can lead to reduced plant growth and yield, while over-irrigation can increase fungal diseases and drain pesticides and nutrients through surface water runoff or out of the root zone into the ground.

Also, due to genetic diversity among the genotypes and spatial variability of soil properties (e.g., soil water retention capability), crop water use, and crop water status can be drastically different within a field (Farooque et al., 2012). But current conventional irrigation practices provide irrigation uniformly without considering the spatial variability of soil properties, genotype-specific water needs, and crop developmental stages. These uniform irrigation practices may result in over or under-application of water resources. These uniform irrigation practices may result in the over- or under-application of water resources. So, to increase yield and profitability, we need an efficient irrigation system, which requires continuous measurement of plant water status to effectively quantify crop water needs in real-time and allow the possibility of providing just enough water to meet those needs at the right time (Osroosh et al., 2015).

Crop water status and canopy temperature are directly correlated, and canopy temperatures can indicate plant water stress (Gonzalez-Dugo et al., 2014). Plant canopy temperature increases when plants absorb solar radiation as energy, but the canopy temperature cools down when the process of transpiration uses the energy. When water is scarce, a plant slows its transpiration by stomatal regulation, which raises the temperature of its leaves relative to unstressed, well-watered plants (Gonzalez-Dugo et al., 2014). Based on this idea canopy temperature-based crop water stress index (CWSI), developed by Idso et al. (1981), has been widely used. However, no previous studies have yet been done to validate the usefulness of CWSI in temperate crop systems like wild blueberries.

UAVs (Unmanned Aerial Vehicle) with thermal sensors are unique in offering high-quality remote sensing data at the required spatial and temporal scale (Maes & Steppe, 2019). By detecting the temporal-spatial heterogeneity of crop water status using UAV-thermal sensor-based CWSI, irrigation could be intelligently controlled in wild blueberry fields.

METHODS

Study Site

The study was conducted on two commercial blueberry fields of Wyman's in Deblois, Maine (Longitude: -68.0001° N, Latitude: 44.7350° W). These commercial crop fields contain many different genotypes of wild blueberry plants growing within a particular field. The study site had two adjacent fields (Figure 1), with one irrigated (Airport) and one non-irrigated (Baxter). These two fields were selected to understand the effectiveness of current irrigation practices in maintaining good water status in a single area.

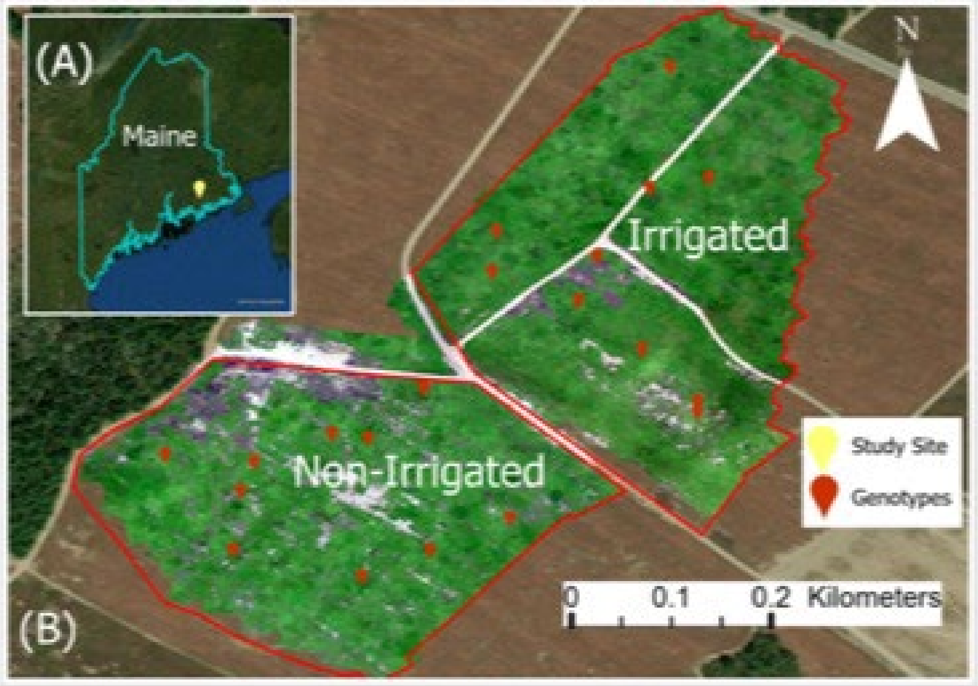


Figure 1. Location of the study site. (A) The yellow dot indicates the study site location in Maine (B) Red dots indicate genotypes at the irrigated Airport field and non-irrigated Baxter wild blueberry field (red boundary line) for on-ground and drone data collection.

UAV Data Collection

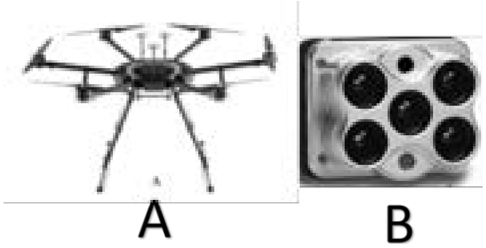


Figure 2. UAV platform and sensors used in acquiring thermal images. (A) UAV platform; (B) Altum MicaSense sensor. The images of the UAV platform and Altum sensor were acquired from micasense.com.

The thermal image acquisitions and ground measurements were conducted three times on three major crop developmental phases in the summer of 2019, 2020, and 2021 (Table 1). Image acquisition and field ground data collection dates were carried out on sunny days. The UAV images were processed using the Agisoft Metashape software 1.6.2 (Agisoft LLC., St. Petersburg, Russia). The final thermal images (Orthomosaic tiff) files were then transferred to ArcGIS Pro (Version 2.7, ESRI, Redlands, CA, USA) for further analysis (see below).

Ground Sampling

A total of 30 genotypes (15 in each field) in 2019 (crop year), and 20 genotypes (10 in each field) were systematically selected in 2020 (vegetative growth year) and 2021 (crop year) to cover the entire field and a wide range of genotypes based on morphological differences. One stem was randomly selected at midday to measure midday leaf water potential (LWP) using a leaf pressure chamber (PMS Inc., Albany, OR, USA). LWP data was used for validating CWSI for detecting crop water stress. Canopy leaf temperature was measured using a Fluke 62 Max+ hand-held infrared thermometer (Fluke Corporation, Everett, WA, USA) from randomly selected four leaves of each genotype. Soil water content was measured by a FieldScout TDR 150 Soil Moisture Meter (Spectrum Technologies Inc., Aurora, IL, USA) from four random places within each of the genotypes.

Climatic Data Acquisition

Variables such as standard precipitation and evapotranspiration index (SPEI), the mean precipitation, and mean air temperature of the last 14 consecutive days before the flight dates were acquired and used to understand the water and climatic conditions. SPEI data was collected from (spei.csic.es); accessed on 10 March 2022. Total precipitation and mean temperature were acquired from the Prism database using (clim-engine.appspot.com/climateEngine); accessed on 10 March 2022.

CWSI-LWP Regression Models

To examine the relationship between CWSI and Leaf water potential (LWP), the mean canopy temperature values of individual clones were extracted from the UAV-based thermal imagery. Calculations of the CWSI were done according to Equation 1 (Jackson et al., 1981), where T_{canopy} is the thermal image-based canopy temperature, T_{wet} is the lower boundary temperature corresponding to a fully transpiring leaf with open stomata, and T_{dry} is the upper boundary temperature corresponding to a non-transpiring leaf with closed stomata, respectively (Equation 1).

$$\mathrm{CWSI} = \frac{T_{canopy} - T_{wet}}{T_{dry} - T_{wet}} \quad \text{..(1)}$$

The canopy temperature pixels in the thermal image were obtained using two assumptions (Meron et al., 2010). The first assumption is that canopy pixels are different from soil pixels, which were separated in ArcGIS Pro by higher and lower limits related to the temperature of air using Equation 2:

$$(T_{air} - 10) < T_{cr} < (T_{air} + 7) \quad \text{.....................................(2)}$$

In Equation 2, T_{air} represents air temperature (°C), and T_{cr} represents thermal canopy pixels in thermal imagery. The second assumption is that the temperature of the canopy is associated with the mean temperature of the coldest 33% of canopy pixels. T_{canopy} was calculated using Equation 3 in ArcGIS Pro:

$$T_{canopy} = \frac{\sum_{i=1}^{0.33n} T_{cr_i} * f}{\sum_{i=1}^{0.33n} f_i} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

Where the total number of pixels in canopy temperature 33% class histogram is denoted by f, and n is the total number of pixels after discarding non-crop related pixels such as soils (Equation 2).

Various T_{wet} and T_{dry} baselines have been developed and applied for calculating CWSI and water stress mapping. Recently, two approaches, the statistical and measured bio-indicator approach, were suggested for determining the T_{wet} and T_{dry} baselines for precision irrigation of large fields (Cohen et al., 2017). In this study, the statistical T_{wet} and empirical T_{dry} reference approaches were taken in 2019, 2020, and 2021. The statistical approach, in general, considers the average canopy temperature of the cooler 5-10% of pixels as the T_{wet} reference, suggested by Clawson et al. (1989). The empirical approach was taken for calculating T_{dry} by adding 5°C with air temperature: $T_{dry} = T_{air} + 5°C$. Air temperature data was taken from a field-based meteorological station. Bio-indicator based T_{wet} and T_{dry} reference approach was introduced in 2021, which is based on the actual canopy temperature measurements. Bio-indicator based T_{wet} and T_{dry} were measured by an infrared thermometer from three leaves from three arbitrarily selected stems from three arbitrarily selected genotypes. For T_{wet} measurements, leaves were wetted with a detergent solution on both sides using a sprayer, and for T_{dry}, leaves were treated with a thin layer of petroleum jelly (Vaseline, Unilever, London, England) on both upper and lower surfaces to prevent transpiration (Jones, 1999).

Statistical Analysis

RStudio software (RStudio, PBC, Vienna, Austria) was used for statistical analysis. We determined the statistical significance of the linear regression relationship using the coefficient of determination and its significance (α) at $p < 0.05$. We tested the effect of different flights (growth stages) on the LWP-CWSI models using a linear mixed model with flight as a factor. The effect of different flights and fields on water condition-related variables SWC, LWP, and CWSI was tested using a Generalized Linear Model in R using Field and Flight as factors.

Table 1: Dates of data collection in different crop developmental stages in the years 2019, 2020, and 2021 along with the mean precipitation, mean air temperature and standard precipitation and evapotranspiration index (SPEI) of the last consecutive 14 days before the flight dates. A positive SPEI value represents a wet condition, whereas a negative SPEI value indicates a dry condition.

2019 **Crop Year**	Flight 1 07/03/2019	Flight 2 07/25/2019	Flight 3 08/14/2019
Developmental Stage	Green Fruit	Color Break	Mature Fruit
Precipitation	10.16	2.93	2.54
Temperature	15.45	18.15	19.68
SPEI	1.45	0.17	-0.44
Conditions	(Very Wet)	(Slightly Wet)	(Moderate Dry)

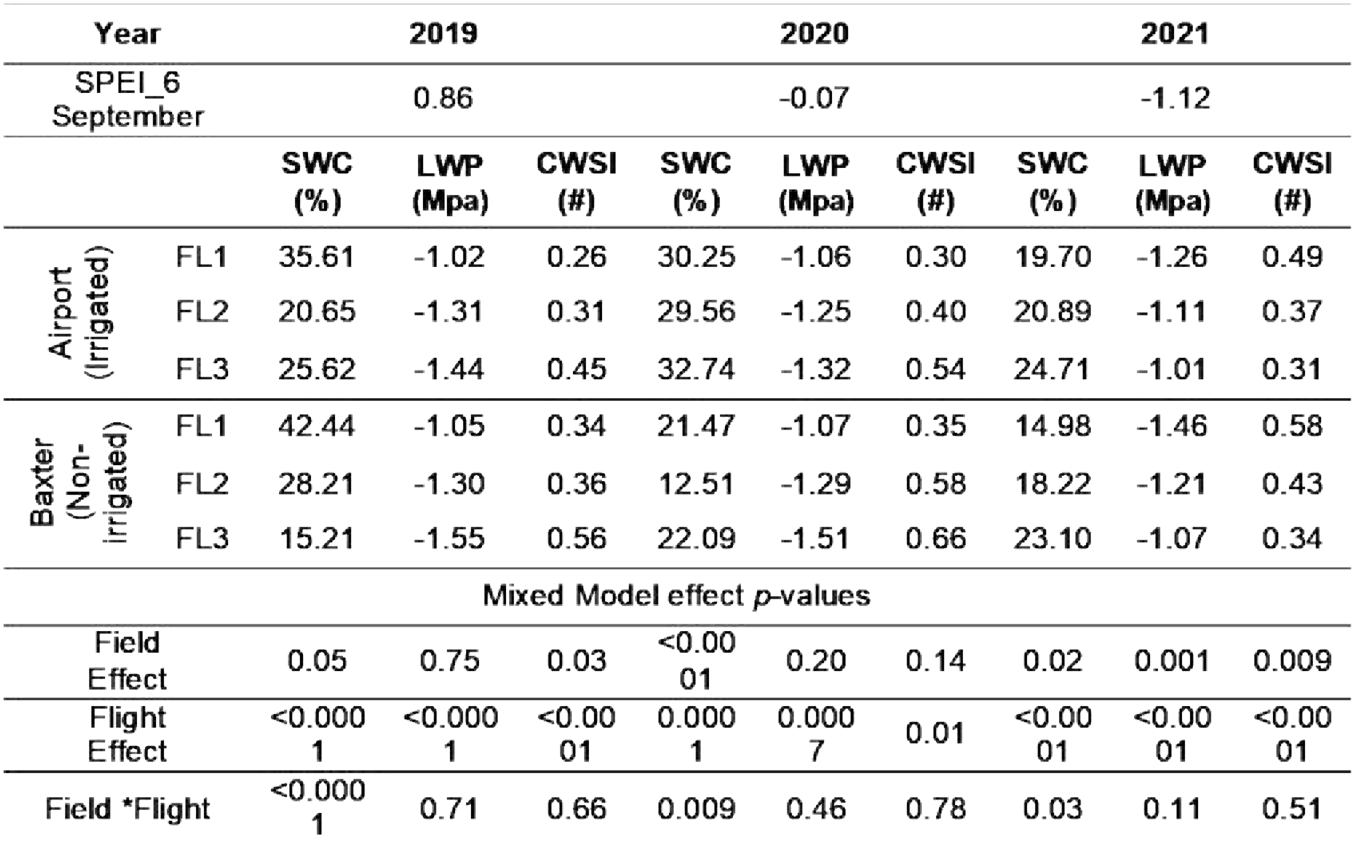
2020 **Vegetative Year**	Flight 1 07/09/2020	Flight 2 08/04/2020	Flight 3 08/26/2020
Developmental Stage	Leaf Development	Full Mature Leaves	Leaf Senescence
Precipitation	3.96	2.60	0.69
Temperature	19.51	21.7	12.37
SPEI	0.17	-0.32	-0.54
Conditions	(Slightly Wet)	(Moderate Dry)	(Moderate Dry)

2021 **Crop Year**	Flight 1 06/18/2021	Flight 2 07/01/2021	Flight 3 07/15/2021
Developmental Stage	Green Fruit	Color Break	Early Mature Fruit
Precipitation	1.14	2.93	7.02
Temperature	19.25	20.7	17.79
SPEI	-1.45	-0.24	1.69
Conditions	(Very Dry)	(Slightly Dry)	(Wet)

RESULTS

Variation In Soil and Crop Water Conditions

Table 2: Comparison in water conditions between irrigated (Airport) and non-irrigated (Baxter) wild blueberry fields in the mean of soil water content (SWC), leaf water potential (LWP), crop water stress index (CWSI) in three different flights during the 2019, 2020, and 2021 seasons and the effects of field and flight differences on these variables. Statistical significance (*p*-value) was tested with the linear mixed model with 5% confidence level.



Year		2019			2020			2021		
SPEI_6 September		0.86			-0.07			-1.12		
		SWC (%)	LWP (Mpa)	CWSI (#)	SWC (%)	LWP (Mpa)	CWSI (#)	SWC (%)	LWP (Mpa)	CWSI (#)
Airport (Irrigated)	FL1	35.61	-1.02	0.26	30.25	-1.06	0.30	19.70	-1.26	0.49
	FL2	20.65	-1.31	0.31	29.56	-1.25	0.40	20.89	-1.11	0.37
	FL3	25.62	-1.44	0.45	32.74	-1.32	0.54	24.71	-1.01	0.31
Baxter (Non-irrigated)	FL1	42.44	-1.05	0.34	21.47	-1.07	0.35	14.98	-1.46	0.58
	FL2	28.21	-1.30	0.36	12.51	-1.29	0.58	18.22	-1.21	0.43
	FL3	15.21	-1.55	0.56	22.09	-1.51	0.66	23.10	-1.07	0.34
Mixed Model effect *p*-values										
Field Effect		0.05	0.75	0.03	<0.0001	0.20	0.14	0.02	0.001	0.009
Flight Effect		<0.0001	<0.0001	<0.0001	0.0001	0.0007	0.01	<0.0001	<0.0001	<0.0001
Field *Flight		<0.0001	0.71	0.66	0.009	0.46	0.78	0.03	0.11	0.51

There was a significant effect of field type (irrigation/non-irrigated), flight date (developmental stages), and the interaction of field by flight on SWC in 2019, 2020, and 2021 (Table 2). For LWP, the effect of flight date was significant in 2019, 2020, and 2021 but the effect of the field was not significant in 2019 and 2020. Interaction between field and flight was also not significant for any years. For CWSI the effect of flight date was significant in 2019, 2020, and 2021 but the effect of the field was not significant only in 2020. Interaction between field and flight was also not significant for all years.

Variations in the field and plant water status-related traits (SWC, LWP, and CWSI) were determined mainly by the differences in flight date rather than the differences in field conditions (irrigated/non-irrigated) in 2019 which was a relatively wet year (Table 1). In 2020, a dry year, variation in water condition-related traits was also determined mainly by the differences in flight date rather than the differences in field conditions (irrigated/non-irrigated). 2021 was a dry year, and the variation in water condition-related traits was determined by both the differences in flight dates and the differences in field conditions.

Relationships Between UAV Thermal Sensor-Based Crop Water Stress Index and Midday Leaf Water Potential

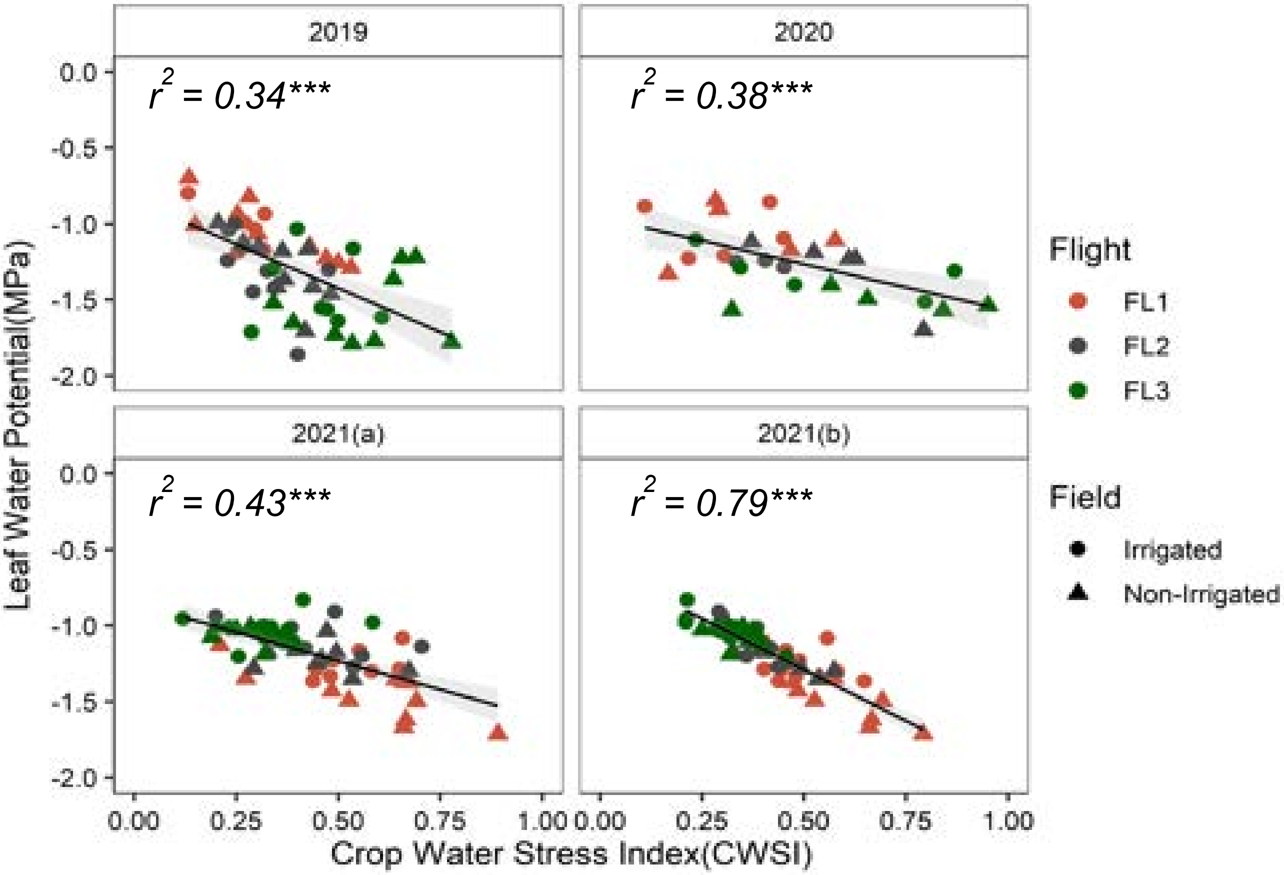


Figure 3: Field-measured leaf water potential (LWP) in relation to crop water stress index (CWSI) in 2019, 2020, and 2021. Statistical T_{wet} and empirical T_{dry} reference-based approach was taken to calculate CWSI in 2019 and 2020, whereas in 2021, both statistical (2021a) and bio-indicator based (2021b) T_{wet} and T_{dry} reference approach were used. CWSI values are in the range of 0–1 with a higher value of CWSI indicating high water stress.

We found significant positive linear relationships between UAV-based CWSI and LWP. But the performance of the CWSI calculated based on the statistical T_{wet} and empirical T_{dry} reference was inferior to bio-indicator reference (T_{wet} and T_{dry}) based CWSI (Figure 3).

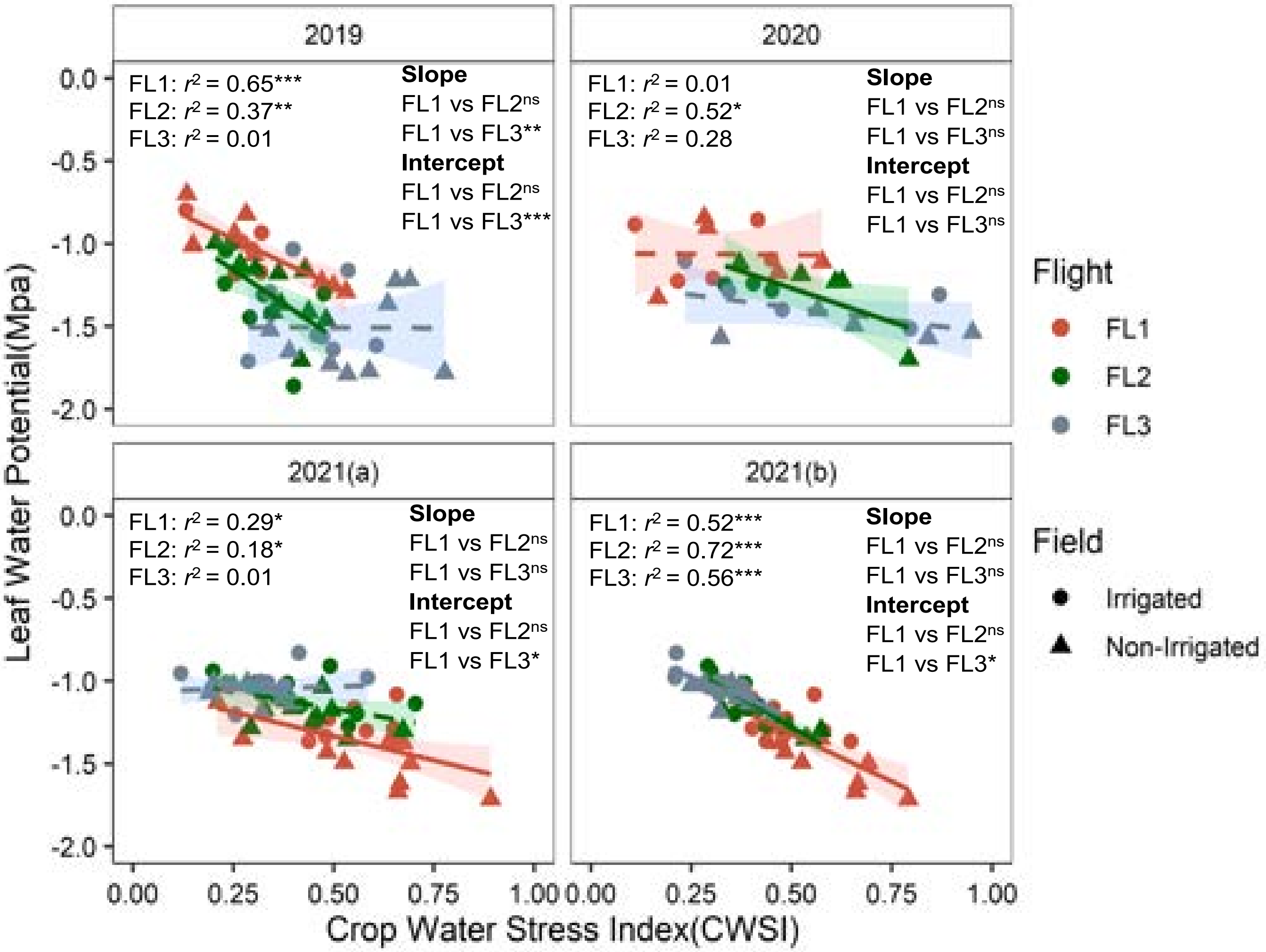


Figure 4: LWP in relation to CWSI in 2019, 2020, and 2021. Differences in slope and intercept between flights were determined using a linear mixed model with flight/crop developmental stage as a factor. ns, no significant difference ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

When we used flight/crop developmental stage as a factor for the fitted linear mixed model, we also found that the slopes and intercepts of CWSI-LWP models differ significantly ($p < 0.05$) across different flights in years 2019, 2021 but not in 2020 for the statistical T_{wet} and empirical T_{dry} based CWSI (Figure 4). For the bio-indicator reference-based CWSI, there was no significant difference ($p < 0.05$) in the slope and intercept of CWSI-LWP models across different flights were significantly similar across different flights in 2021 except for the intercept between FL1 and FL3.

Leaf Water Potential Variability Maps

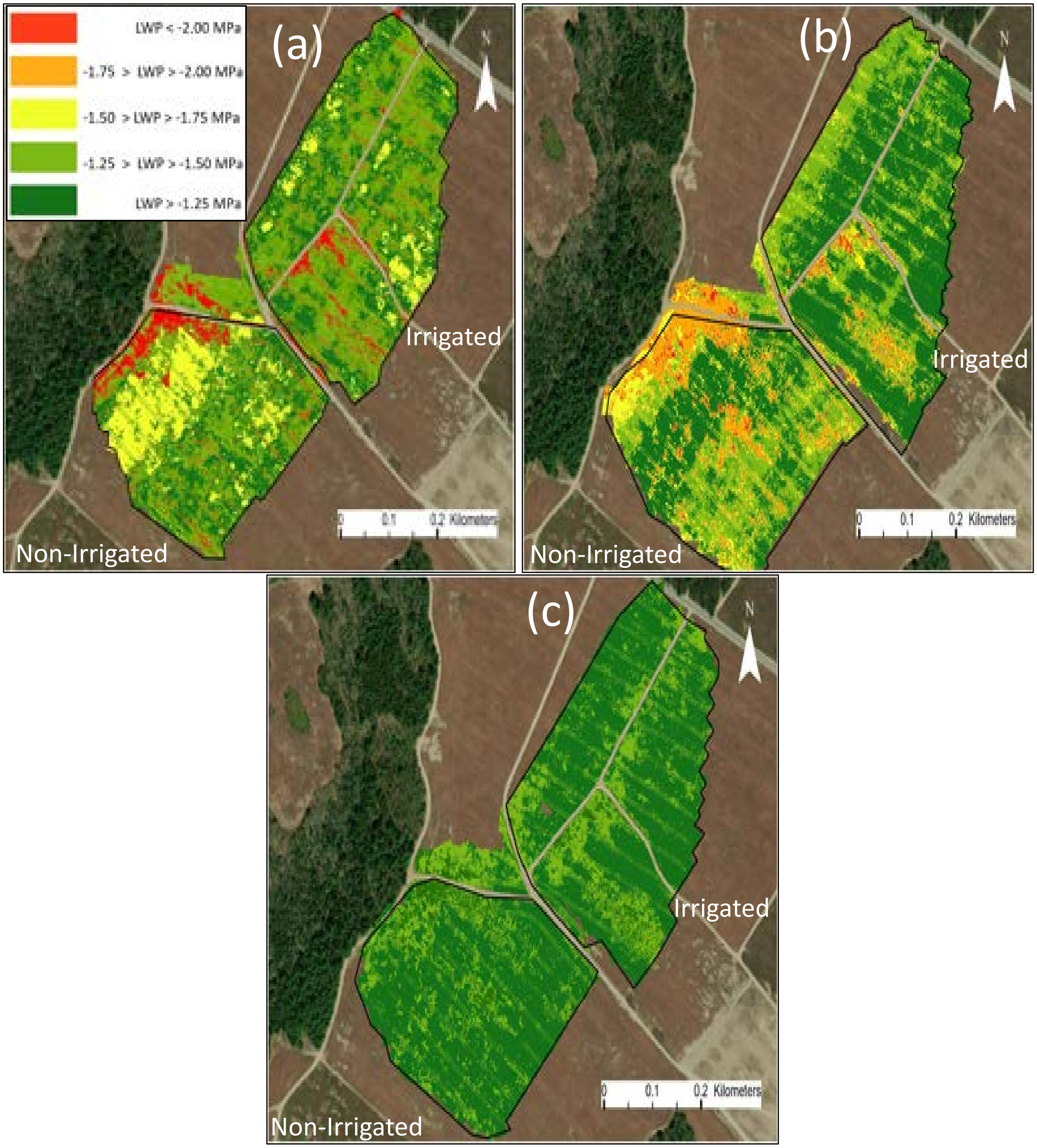


Figure 5: Predicted leaf water potential (LWP) variability map of adjacent irrigated (Airport) and non-irrigated (Baxter) fields in (a) flight 1, (b) flight 2, and (c) flight 3 of 2021. Calculated LWP was derived from the LWP-CWSI (bio-indicator) model.

In 2021, the water condition before the last 14 days of the first flight was very dry (SPEI 14D: -1.45), and in the predicted LWP variability map, we found high variability in LWP within the irrigated and non-irrigated fields as well as between the irrigated and non-irrigated fields (Figure 5A). Though most of the part of the irrigated and non-irrigated field was without any water stress (Light Green: -1.25 > LWP > -1.50 MPa), there is a huge portion of the non-irrigated field, and some part of the irrigated field in low water stress (Yellow: -1.50 > LWP > -1.75 MPa). We also found that some small parts of the irrigated and non-irrigated fields were under severe water stress (Red: -2.00 < LWP), which might be a result of the unavailability of natural precipitation for a long time.

The water condition before the last 14 days of the second flight was slightly dry (SPEI 14D: -0.24), and in the LWP variability map, we can also see variability in LWP within the irrigated and non-irrigated field as well as between the irrigated and non-irrigated fields (Figure 5B). We found that plants in most parts of the irrigated field were in good water status (Light Green: -1.25 > LWP > -1.50 MPa; or Dark green: LWP > -1.25 MPa). Very few portions of the irrigated field were in low water stress (Yellow: -1.50 > LWP > -1.75 MPa) and in moderate water stress (Orange: -1.75> LWP > -2.00 MPa). In the non-irrigated field, we found most parts of the field were in good water conditions (Light Green: -1.25 >LWP > -1.50 MPa), along with some low water stressed (Yellow: -1.50 > LWP > -1.75 MPa) area and moderately water stressed area (Orange: -1.75 > LWP > -2.00MPa).

In flight 3 of 2021, the water condition before the last 14 days was Wet (SPEI 14d: 1.69). Plants in most parts of the irrigated and non-irrigated field were in good water status (Dark green: LWP > -1.25 MPa; Light Green: -1.25 > LWP > -1.50 MPa) (Figure 5C).

DISCUSSION

UAV Thermal Sensor-Based Crop Water Stress Index Predicts Midday Leaf Water Potential

We found that UAV thermal sensor based CWSI can successfully predict crop water status for wild blueberries. Also, we found that the statistical T_{wet} and empirical T_{dry} based CWSI was inferior to the bio-based calculated CWSI in predicting leaf water potential in wild blueberries. The performance of the CWSI calculated from the bio-indicator based T_{wet} and T_{dry} was also better in differentiating the difference in water stress between irrigated and non-irrigated fields (Figure 3). CWSI calculated based on statistical T_{wet} and empirical T_{dry} reference has been found effective in indicating crop water stress in arid or semi-arid conditions (Alchanatis et al., 2010; Gonzalez-Dugo et al., 2013; Rud et al., 2014), but we find it less suitable for predicting water stress of wild blueberries in Maine's humid summer weather with significant variability of weather conditions. The variability of the environment, including changes in temperature and vapor pressure deficit, can also affect the canopy temperature of fully transpiring leaves (T_{wet}) as well as fully non-transpiring leaves (T_{dry}) (Jones, 1999). Successful measurement of the leaf temperature of complete non-transpiring leaves as T_{dry} and full-transpiring wet leaves as T_{wet} is very important for the effective calculation of CWSI (Cohen et al., 2017). The use of the canopy temperature of non-transpiring dry leaves as T_{dry} base and the canopy temperature of real wet leaves as T_{wet} base was found effective for temperate humid regions (Jones, 1999), which aligns with our findings. Although CWSI was found to be a good index for water stress detection in our study, this index has some limitations. We also found that the intercepts of the bio-indicator based CWSI-LWP models vary for different flights, suggesting the need for a model adjustment based on flight dates/development stages. CWSI also has some difficulty in its application for large areas with varying topography (Rahimzadeh-Bajgiran & Berg, 2016).

Spatial Variation in Leaf Water Potential

We found spatial variability in LWP within irrigated and non-irrigated fields, between irrigated and non-irrigated fields, as well as among three different flights of 2021, based on the predicted LWP maps constructed from the CWSI-LWP model (Figure 5). The variation between irrigated and non-irrigated fields was higher when natural precipitation was limited. Overall higher LWP and lower CWSI in the irrigated field compared to the non-irrigated field also suggests that irrigation can provide water needs effectively. However, the variability of soil water content, LWP and CWSI within the irrigated field suggests that despite uniform irrigation management, the current irrigation system (Nelson Full-Circle Impact sprinklers) cannot provide uniform soil water content due to the variation in soil retention capability, which might be due to the spatial variability of soil properties in wild blueberry fields. A recent study found a significant reduction in stomatal conductance, photosynthesis, and transpiration rate before or at the turgor loss point (-2.00 MPa) (Pahadi, 2021). In mild drought conditions, the majority of plants may adjust their stomatal conductance to prevent low water potentials (Sperry et al., 2016). Turgor loss and xylem cavitation occur beyond a certain threshold of drought stress when plants are incapable of maintaining the balance between water loss and uptake (Mingeau et al., 2001). This can result in damage to the photosynthetic apparatus and xylem embolism, resulting in mortality (Pahadi, 2021). For pressure chamber measured LWPs, we did not find any genotypes reaching the suggested turgor loss point of -2.00 MPa. However, we found a large portion of the non-irrigated field and a small portion of the irrigated field showed LWP over -2.00 MPa (Flight 1: 2021) and in the range of -1.75>LWP> -2.00 MP (Flight 2: 2021) in the predicted LWP variability maps, which could potentially impact the major physiological processes of wild blueberries like stomatal conductance, transpiration, and photosynthesis. Declined photosynthetic carbon assimilation can also result in reduced biomass production and crop yield. Moreover, the drought frequency in Maine is predicted to increase (Fernandez et al., 2020), which might affect the physiological processes of non-irrigated wild blueberry fields in the future, ultimately reducing yield and impacting the wild blueberry industry.

CURRENT RECOMMENDATIONS

UAV-based thermal sensors and calculated Crop Water Stress Index (CWSI) can effectively monitor crop water status remotely. By detecting the temporal-spatial heterogeneity of crop water status, irrigation can be intelligently controlled by adopting precision irrigation management systems to minimize crop water stress, maximize overall production, and save water use. Due to the high spatial heterogeneity in the wild blueberry field, precise management of water conditions (applying water at the right location with the right amount and at the right time) can significantly improve water use efficiency.

NEXT STEPS

Further study should be conducted to determine the effectiveness of:

- UAV-based hyperspectral sensor to detect leaf N status for precision fertilization.
- UAV-based hyperspectral sensor for early detection and monitoring of diseases.

ACKNOWLEDGEMENTS

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

OBJECTIVE

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

LOCATIONS: Conventional fields in Deblois, ME and UMaine Blueberry Hill Farm Experiment Station in Jonesboro, ME
PROJECT TIMEFRAME: January – December 2022

INTRODUCTION

Wild blueberries have played an important role in Maine's economy for centuries, where the crop is grown commercially on 40,000 acres of land. Wild blueberry production is now threatened by climate change's effects including warmer temperatures (Tasnim et al., 2021) and frequent drought (Barai et al., 2021). A recent preliminary investigation by Tasnim et al. (2020) using open-top chambers to create a warmer environment showed that warming changes 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, such 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 amendments such as wood mulch and biochar-compost mix can enhance soils' water- and nutrient-holding capacities and could be long-term economic solutions than irrigating. Biochar-compost mix application could be an effective amendment because biochar (a carbon material produced from plant biomass) can increase soils' water-holding capacity while the compost can increase soils' nutrients. Maine has vast resources of forest (wood) residuals, and some companies are producing biofuel from wood pellets through pyrolysis where biochar is produced as a waste byproduct, but could be diverted from landfills to be used as a soil amendment by wild blueberry growers.

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 (Figure 1A) during the vegetative year. Here, it is to be noted that the studied two fields are under different management regimes. Wyman's blueberry field has been frequently irrigated and fertilized, while the Blueberry Hill Farm field is neither irrigated nor fertilized. For the entirety of this experiment, the irrigation system at Deblois was closed and unused to create more similar conditions at the two sites.

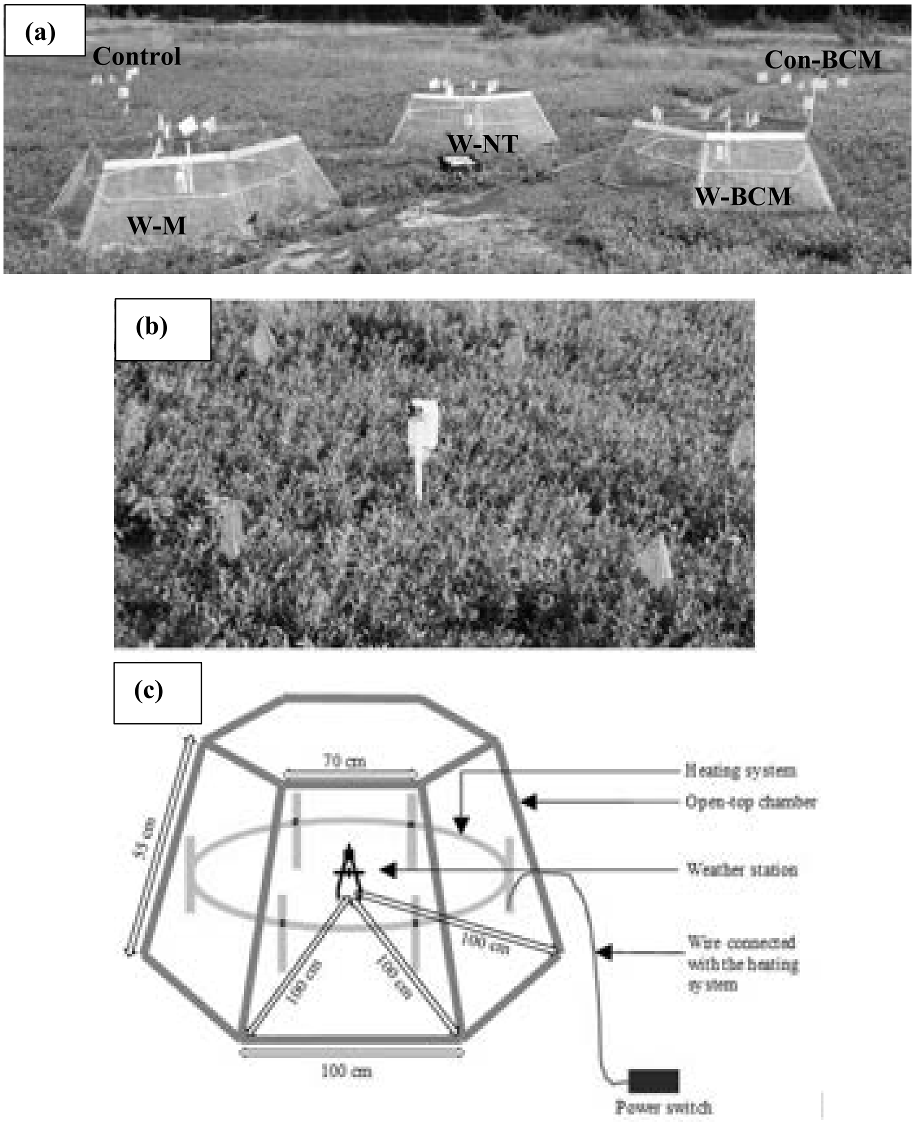

Figure 1. The open-top chambers (OTC) and control plots used for this study in the wild blueberry field: (A) Two control flagged plots with no warming chamber (one plot with no treatment named as "Control"; one plot with biochar-compost mix on the soil surface named as "Con-BCM") and three OTCs (warming chamber with no treatment named "W-NT"; warming chamber with mulch on the soil surface named "W-M" and warming chamber with biochar-compost mix on the soil surface named "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 side length) x 55 cm (height) x 70 cm (top side length); with a 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. 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 (Figure 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 genotype had open-top chambers (OTC) with a heating system inside (Figures 1A & 1C). Based on the preliminary investigation on wild blueberry crops by Tasnim et al. (2020), this warming chamber increased the ambient temperature by 3-5°C. Out of these three chambers in Figure 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 softwood bark mulch on the soil surface to a depth of 0.5” and applied the biochar-compost mix (ratio of 1:1) at a rate of 7.5 yd^3/A. We spread the biochar-compost mix on the soil surface manually and as uniformly as possible, though application depth was too shallow and negligible. Biochar was provided by Maine Woods Pellets Company and contained ash. After receiving the biochar at the University of Maine, we separated the ash from biochar. 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 to the Analytical Soil Testing Laboratory for comprehensive testing.

Data Collection

We installed weather stations in the middle of each plot (Figure 1) for real-time monitoring of 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) in June of the vegetative year (2021). We started this experiment in June 2021 (vegetative year) and continued until August 2022 (crop year). During the growing season (May – August) in the crop year (2022), we measured soil moisture, soil temperature, and electrical conductivity in each of the plots using a FieldScout TDR 150 soil moisture meter (Spectrum Technologies, Inc., Aurora, IL, USA) at 6 random locations throughout each plot.

During June – August 2022, we also measured chlorophyll concentrations on the marked six stems in each plot by a CCM-200 plus chlorophyll content meter (Opti-Sciences Inc., Hudson, NH, USA). We conducted gas-exchange measurements on three randomly-selected stems from each plot using the portable photosynthetic measurement system (LI-6800; Li-Cor Biosciences, Lincoln, NE, USA) on a sunny 15 June 2022 between 10AM and 3PM 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 12:00-12:30PM on 15 June 2022 and measured midday leaf water potential by a leaf pressure chamber (PMS Instrument Company, Albany, OR, USA).

We harvested and measured yield from the warming chambers on the 13 and 18 July, and from the control plots on 5 August when fruit maturation reached 90-95%. We used a 4sqft quadrat at the center of each plot and counted as well as cut all the stems from that quadrat to quantify the stem density and measured the fresh weight (actual yield) of mature blue fruit after hand-picking them from those stems. We also separated 100 random berries from each of those samples to measure their fresh weight and then we oven-dried the berries at 70ºC to constant mass and weighed. Then we ground up those dried berry samples and sent them to the University of Maine Soil and Plant Tissue Testing Laboratory in Orono, Maine for nutrient testing.

From the fresh harvest, we also subsampled 80g of berries and smashed them to make purée before measuring three subsamples with a handheld PAL-BRIX/ACID F5 refractometer (Atago, Saitama, Japan) to measure berry sugar content as Brix (%). When we harvested the fruit, we also sampled eight random stems in each plot to quantify leaf number, leaf area, leaf dry biomass, and leaf nutrition. We measured the leaf area, 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 leaf 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 in August after the harvesting and sent them to the Analytical Soil Testing Laboratory in Orono, Maine for comprehensive soil testing and measurement of the total carbon and total nitrogen in those samples.

Data Analysis

The effects of soil amendments on soil moisture, plant physiology (chlorophyll concentration and photosynthetic rate), morphology (leaf size, number of leaves per stem, and leaf mass per area), berry quantity, and fruit quality were statistically compared using a general linear model followed by LSD (least significant difference) post-hoc test in JMP Pro 16.2 software ($\alpha = 0.05$). 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

During the experimental period of this study from June 2021 to August 2022, monthly average temperature in the warming chambers was approximately 1 to 3°C higher than the ambient temperature in the control plots (Figure 2A). Also, monthly average relative humidity in the warming chambers was ~5 to10% lower than the ambient relative humidity in the control plots (Figure 2B).

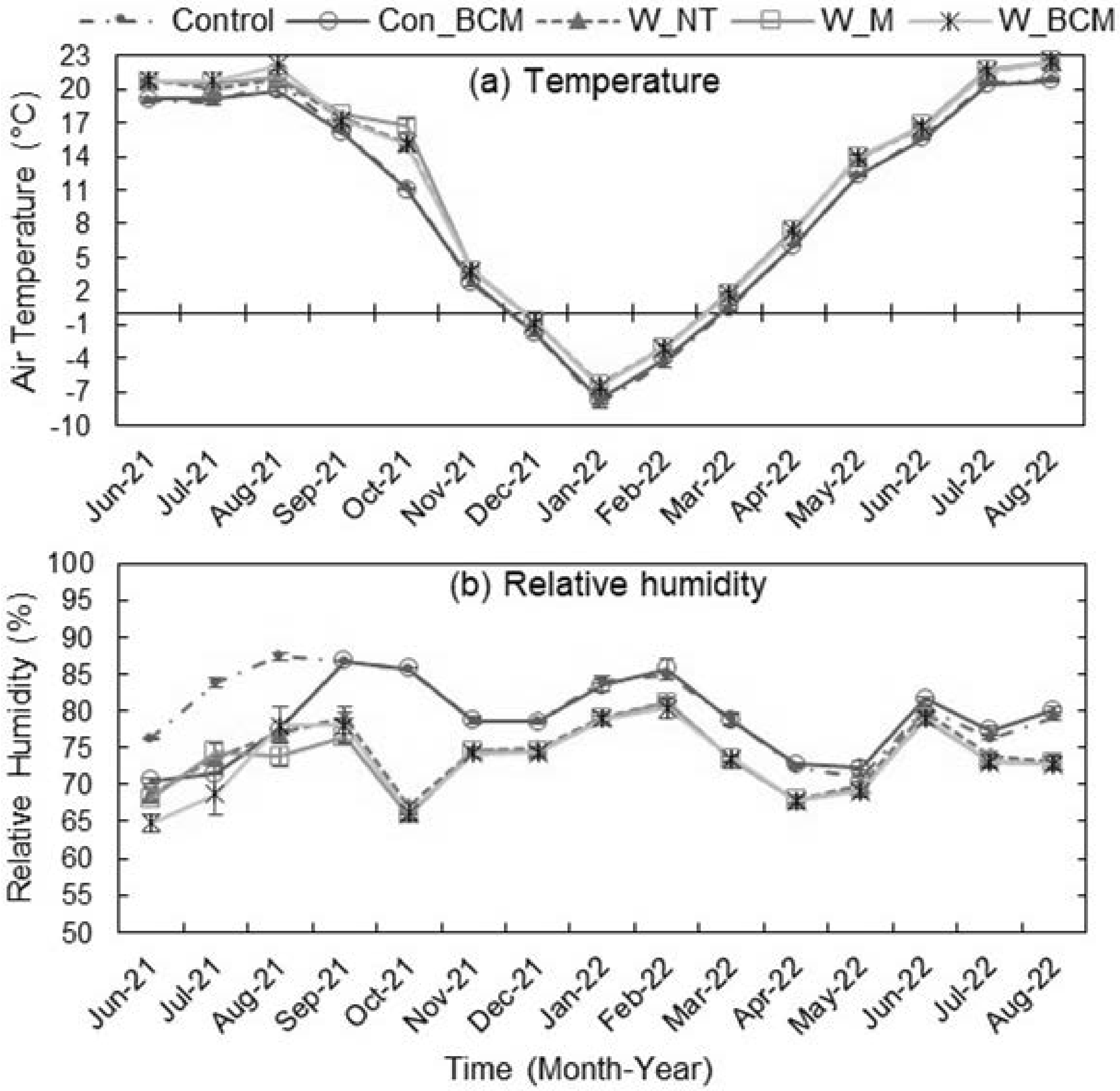

Figure 2. Monthly changes in average (A) atmospheric temperature (°C) and (B) relative humidity (%) from June 2021 (vegetative year) to August 2022 (crop year). Error bars represent the standard error of the mean.

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

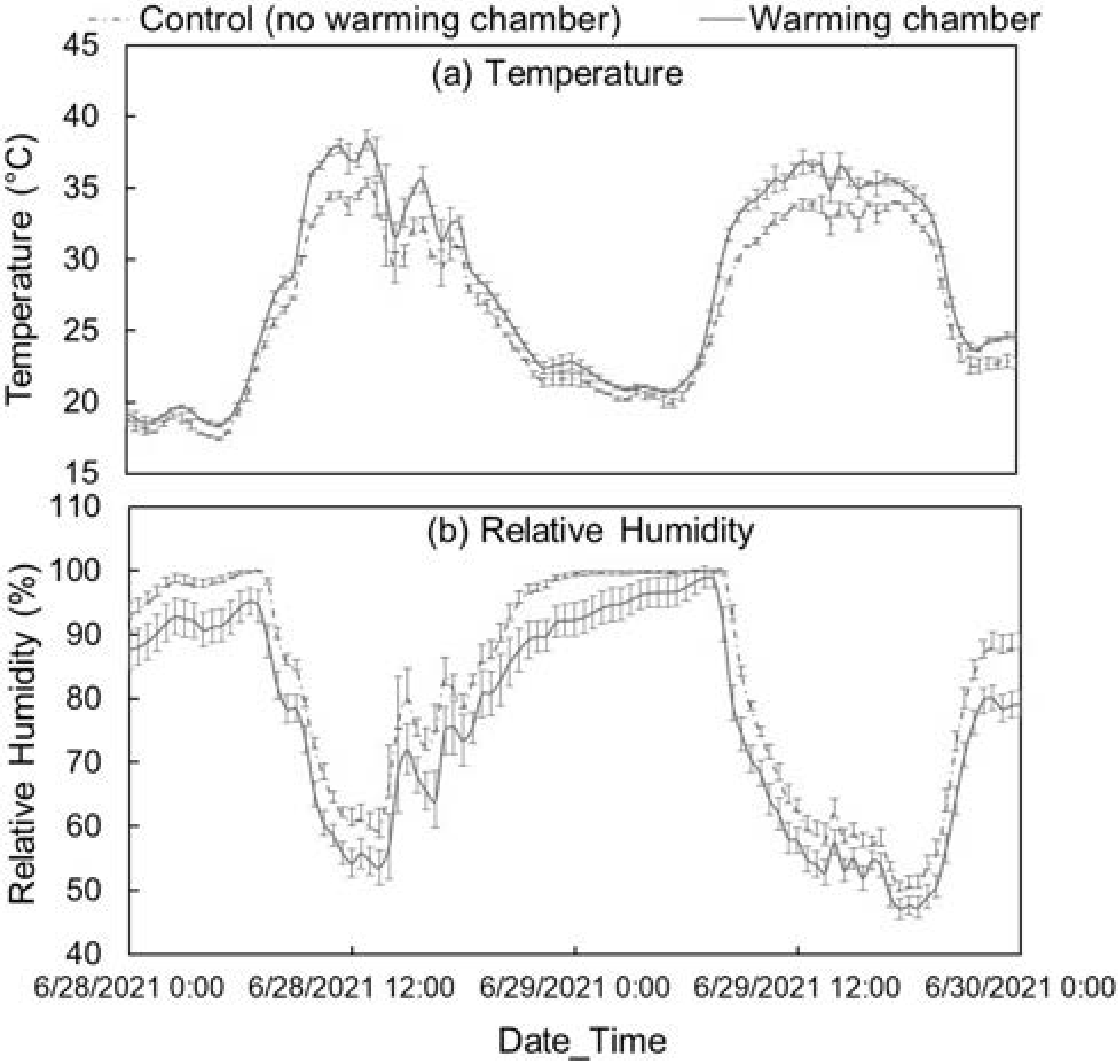

Figure 3. Diurnal changes in (A) atmospheric temperature (°C) and (B) relative humidity (%) during two sunny days (28 – 29 June 2021). Error bars represent the standard error of the mean.

Volumetric water content in soil was significantly lower in warming chambers with no treatment and mulch (W-NT and W-M) than the control plots during the growing period (May – July) (Figure 4). However, soil water content was significantly higher in the warming chamber with biochar-compost treatment (W-BCM) compared to other warming chambers (W-NT and W-M). In fact, the soil moisture content in the W-BCM plot was similar to the Control plots (Control and Con-BCM) (Figure 4).

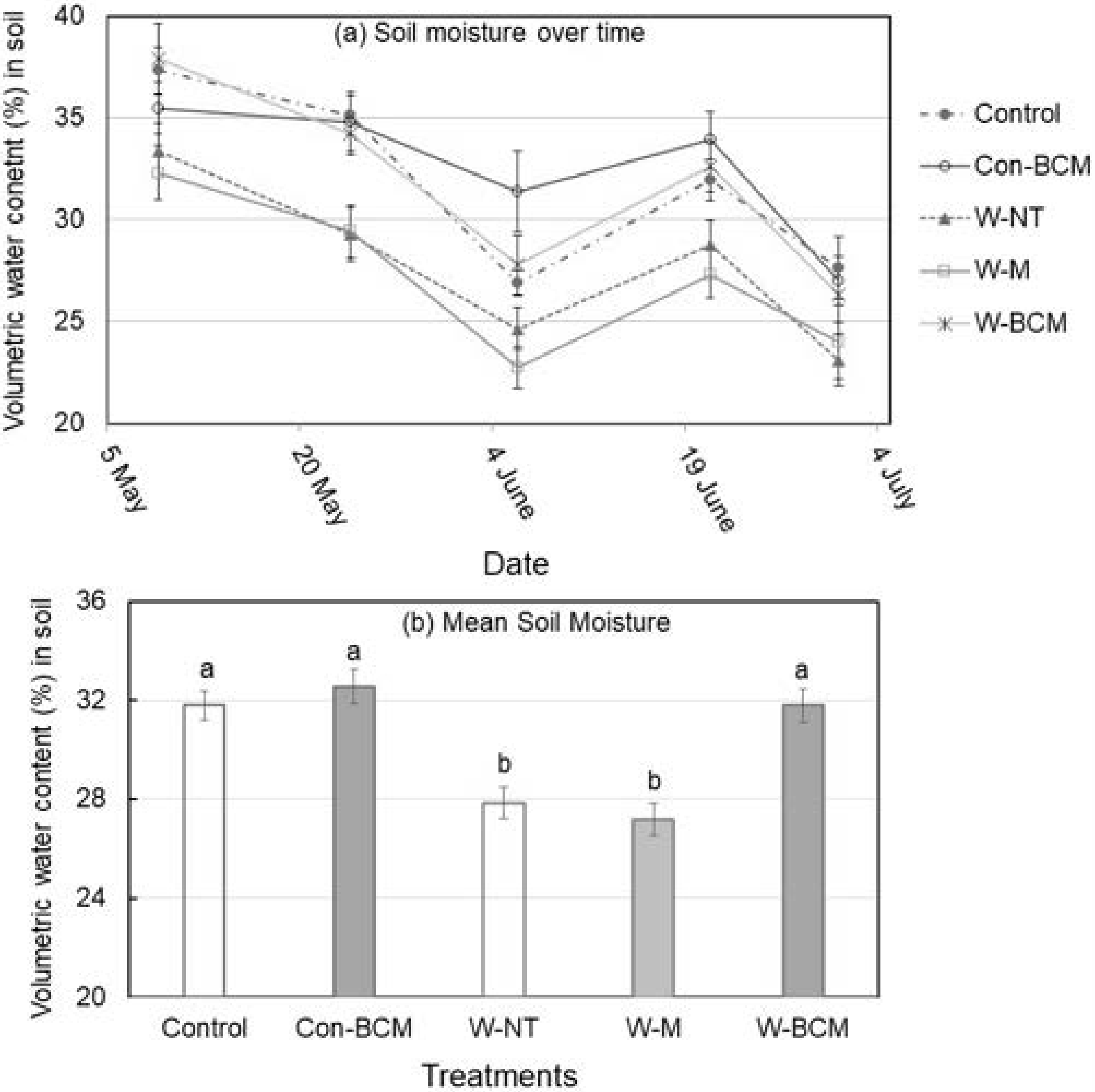

Figure 4. (A) Seasonal changes in volumetric water content in soil across five different treatments, as measured from May to August 2022. (B) Comparison in mean soil moisture content by treatment type over all field season data collection dates (from May to August 2022). Error bars indicate the standard 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 midday leaf water potential of wild blueberry plants growing under different treatments (Figure 5) indicating that those plants did not differ in water deficits.

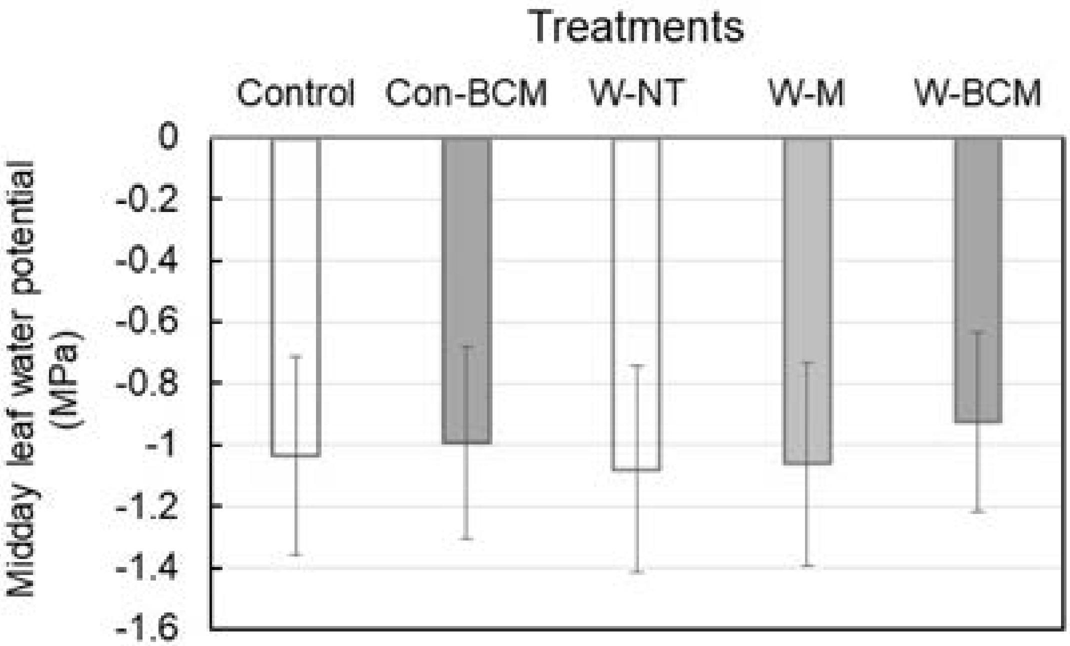

Figure 5. Comparison in midday leaf water potential of wild blueberry plants among five different treatments on a sunny 15 June 2022). Error bars indicate the standard error of the mean. Here, no significant differences were observed among the treatments at the significance level of $p < 0.05$.

Overall, from the gas-exchange measurements, plants in the warming treatments (W-NT, W-M, and W-BCM) had higher photosynthetic rates (Figure 6A), stomatal conductance (Figure 6B), and transpiration rates (Figure 6C) compared to the control plots. However, the addition of biochar-compost and mulch treatments did not affect the photosynthetic rates, stomatal conductance, and transpiration rates of the plants growing in the warming chambers. Although the plants in the control plots with the biochar-compost mix had slightly higher average photosynthetic rates, stomatal conductance, and transpiration rates compared to the control plot with no soil amendment, the difference was not significant (Figure 6A-C). On the other hand, the water use efficiency of the wild blueberry plants growing under different treatments showed no significant difference (Figure 6D).

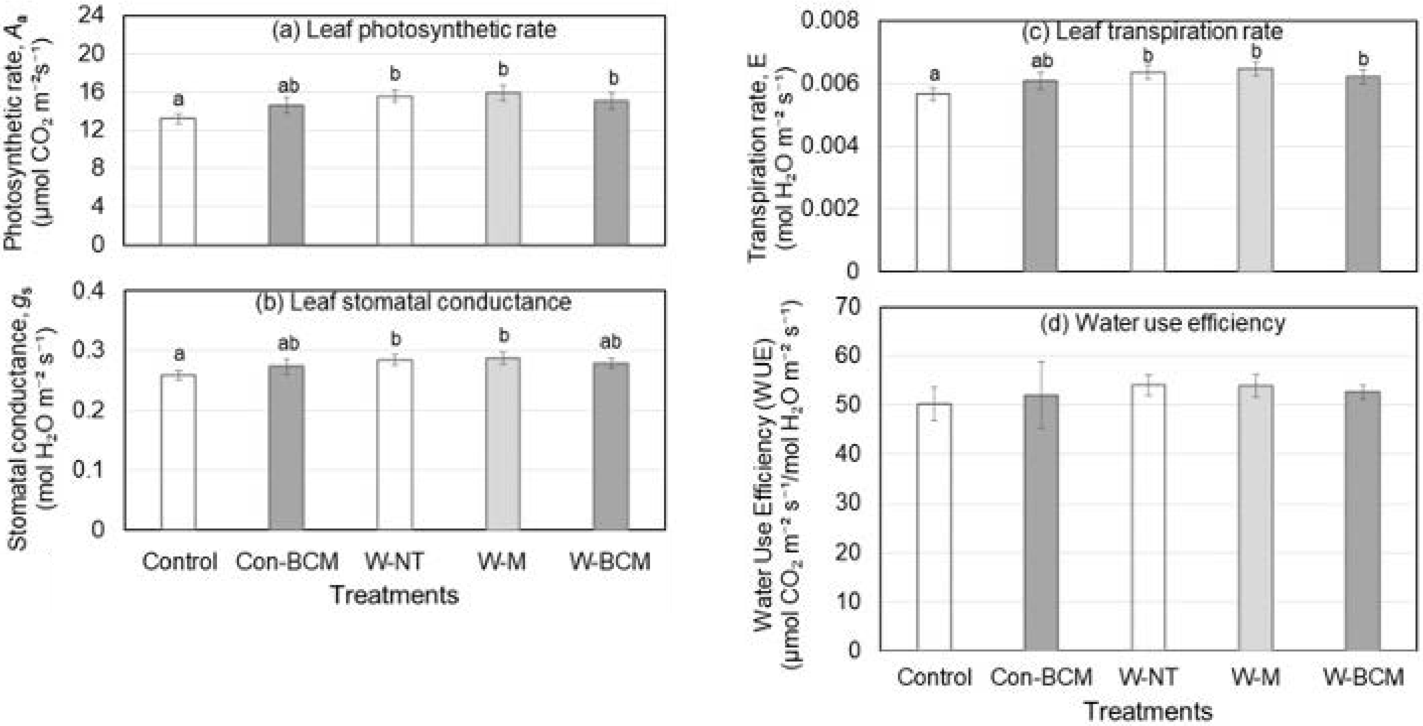

Figure 6. Comparison in (A) photosynthetic rate, (B) stomatal conductance, (C) transpiration rate, and (D) water use efficiency of wild blueberry plants among five different treatments on a sunny 15 June 2022. Error bars indicate the standard error of the mean. Different letters indicate significant differences whereas no letters indicate no significant differences among the treatments at the significance level of $p < 0.05$.

Plants in the warming chambers had significantly higher chlorophyll concentration than the control plots during the growing season (May – early August; Figure 7). Plants in the Control and Con-BCM plots showed similar chlorophyll concentration trends while the plants in the warming chambers with and without soil amendments (W-NT, W-M, and W-BCM) showed similar chlorophyll concentration trends. Plants in the control plot with biochar-compost mix had slightly higher average chlorophyll concentration than the control plot during mid-June to early August. Similarly, during that period, plants in the warming chamber with biochar-compost treatment had slightly higher average chlorophyll concentration than the warming chambers with mulch and no soil amendment where the difference was significant in early June and early August.

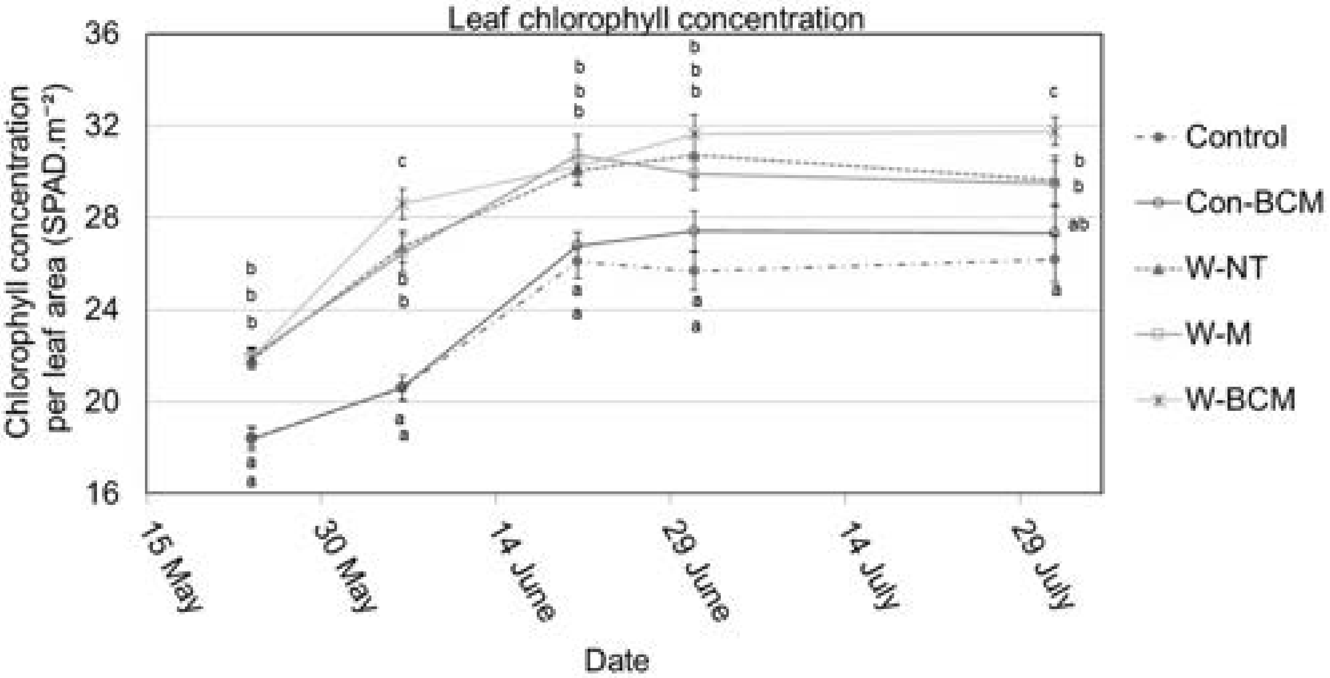

Figure 7. Seasonal changes in chlorophyll concentration per leaf area in wild blueberry leaves across five different treatments, as measured from May to early August 2022. Error bars indicate the standard error of the mean. Different letters indicate significant differences among the treatments on different measurement dates at a significance level of $p < 0.05$.

At the end of the season, plants in the warming chambers (W-NT, W-M, and W-BCM) had a significantly higher number of leaves per stem than the control plots (Control and Con-BCM) (Figure 8A). There were no significant differences in leaf size (Figure 8B) and LMA (Figure 8C) among different treatments.

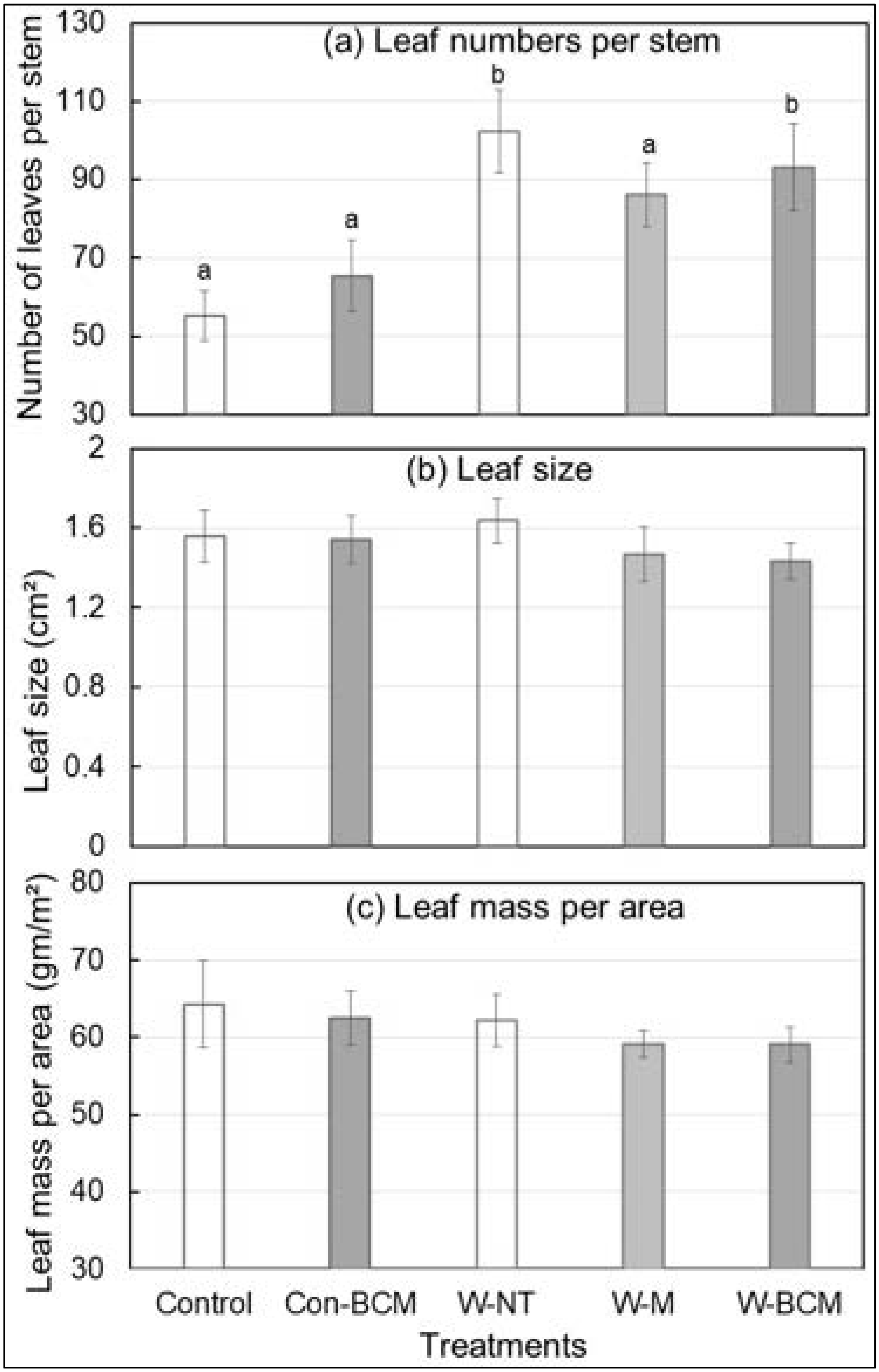

Figure 8. Comparison in (a) number of leaves per stem, (b) leaf size, and (c) leaf mass per area of wild blueberry plants among five different treatments, as measured in July and August 2021. Error bars indicate the standard error of the mean. Bars with no letters above indicate no significant differences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

At the end of season, fruit yield (Figure 9A), weight of fruits per stem (Figure 9B) and weight of 100 berries (Figure 9C) were significantly higher in the warming chambers (W-NT, W-M, and W-BCM) than the control plots (Control and Con-BCM). Control and Con-BCM plots had similar fruit production (Figure 9A-C) whereas W-BCM had the highest average yield (Figure 9A-B) among the warming chambers, but the difference was not significant compared to the W-NT and W-M plots. In contrast, wild blueberry sugar content did not differ among all the treatments (Figure 9D).

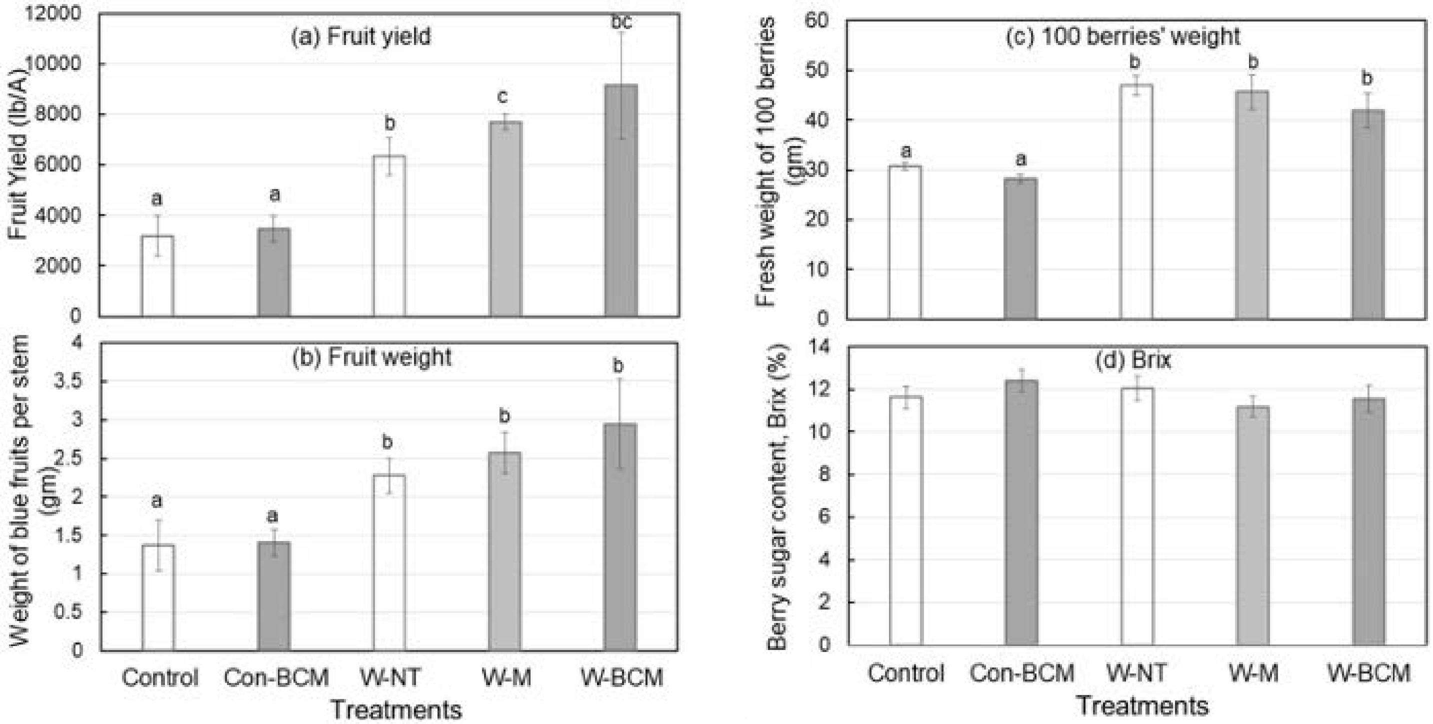

Figure 9. Comparison in (a) fruit yield, (b) weight of blue fruits per stem, (c) fresh weight of 100 berries, and (d) berry sugar content of the wild blueberry plants among five different treatments in 2022. Error bars indicate the standard error of the mean. Bars with no letters above indicate no significant differences among the treatments whereas different letters indicate significant differences at the significance level of $p < 0.05$.

DISCUSSION

Under the warmer environment (1 to 5 °C higher than ambient), the average soil moisture of the growing season (May – July) in the crop year (2022) was significantly lower than in the ambient environment. In fact, soil moisture in warming chambers with no treatment and with mulch (W-NT and W-M) was consistently lower than the control plots throughout the growing season. Our results showed that a 0.5” layer of mulch was not sufficient as the soil moisture level was lower than the control plots and it was same as the soil moisture in the warming chamber with no treatments. 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). On the other hand, the use of biochar-compost mix resulted in significantly higher soil moisture under both ambient and warmer environments throughout the whole season. It could be because biochar helps sandy soil, like the studied fields’ soil, hold more water (Li et al., 2021). Therefore, applying biochar materials to sandy soils could reduce irrigation costs by increasing soils’ water retention, thereby saving water (Kroeger et al., 2021).

Wild blueberry plants growing in the warmer environments had higher stomatal conductance, transpiration rates, and photosynthetic rates, suggesting that they were transpiring more water and photosynthesizing more than the plants growing in the ambient environment. However, plants growing in the ambient environment had similar water use efficiency to the plants growing in the warmer environment. Also, the plants growing in the warmer environment had consistently higher leaf chlorophyll concentrations than in the ambient environment, especially in the warming chamber with biochar-compost mix treatment. Moreover, the final fruit yield and fresh weight of berries were significantly higher in the warming chambers compared to the control plots. Interestingly, wild blueberry plants growing in the warmer environment seemed to be performing better as indicated by their higher photosynthetic capacity, stomatal conductance, chlorophyll concentration, and berry production.

These findings make sense since wild blueberries grow in a temperate climate and many temperate crops are expected to benefit from 1 to 3ºC warming of ambient temperature predicted under most climate change scenarios (Easterling et al., 2007; Hatfield et al., 2011). Also, in agreement with the soil moisture condition, wild blueberry plants growing on the biochar-compost treated soil had better physiological performance and better fruit production among all the treatments, possibly due to more soil moisture availability (Agegnehu et al., 2017; Li et al., 2021; Ariz et al., 2015). Further, complete soil and leaf nutrient test results (in progress) from this season will provide more information and explanations of our observations from this experiment. So far, our results conclude that wild blueberry plants might grow better in warmer temperatures with sufficient soil moisture. Furthermore, soil moisture and organic matter conserving soil amendments like biochar-compost application on the soil surface can be beneficial for wild blueberries in drier summers.

NEXT STEPS

- Test the effect of different rates of mulch and biochar treatment in mitigating the impact of warming and water deficits.

CURRENT RECOMMENDATIONS

- Based on our preliminary investigations of biochar-compost application, biochar can be used to improve soils' water-holding capacity for wild blueberries.

ACKNOWLEDGEMENTS

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3. Using Soil Amendments to Improve Wild Blueberry Soil Moisture

OBJECTIVE

Evaluate hard vs. soft wood mulch and biochar as drought management tools.

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

INTRODUCTION

This project continues the research begun in 2021 (see the 2021 report, page 150, “Using Foliar Fertilizers and Soil Amendments to Improve Wild Blueberry Production and Resilience to Warming”). This report discusses the aspect of the research regarding soil amendments and their impacts on soil moisture levels.

Wild blueberry plants grow well in sandy, well-draining soils but these same soils have low water-retention capacity, which inhibits the plants’ growth when drought conditions develop, oftentimes during the critical growing months of June, July, and August. Temperatures in Maine continue to increase, with the average annual temperature rising 3.2°F since 1895, increasing 0.05°F annually since 1960, and recording six of the warmest years on record since 1998 (Fernandez et al., 2020). Due in part to these temperature changes, the growing season has lengthened by one month over the last fifty years

(Drummond & Yarborough, 2014), with an increase of fourteen days in the last twenty years alone (Tasnim et al., 2022).

A longer growing season means more water resources are needed for the crops, and elevated air temperatures lead to increased rates of evapotranspiration, causing plants to utilize more of these valuable water resources (Tasnim et al., 2022). Higher air temperatures simultaneously dry out soils and cause plants to use more water from the soil (Tasnim et al., 2020; Tasnim & Zhang, 2022). Low soil moisture results in smaller, lower-quality berries, since berry size is largely due to water content (Barai et al., 2022). Therefore, growers are keen to maintain or increase soil moisture at all times, but especially during drought conditions. Long-term water conditions (more than four years) in a wild blueberry field have a greater impact on plant health and yield than do the current season's water conditions (Barai et al., 2021). Thus, growers in regions suffering several years of drought will require several very wet years to rehabilitate their fields or will require irrigation or other management techniques to improve their soils' water content and their yield. Other research is exploring the economic and logistic feasibility of irrigation systems on wild blueberry farms, and growers have tried wells, ponds, and trucking in water to increase the water available to their fields.

Increasingly, growers use mulch to increase soil water-holding capacity and improve field water retention. This study tests the effects that softwood mulch, hardwood mulch, and biochar + compost have on improving soil water retention. Mulches are materials spread atop soil instead of being incorporated into it. Mulches benefit wild blueberry by reducing water loss, moderating soil temperatures, suppressing weeds, reducing leaf spot disease, and promoting rhizome growth (Gumbrewicz & Calderwood, 2022; Broschat, 2007). Research by Gumbrewicz and Calderwood (2022) explored different softwood mulch particle sizes (sawdust, shavings, bark, and woodchips) and found that smaller particle sizes (sawdust and shavings) promoted the most growth in wild blueberry plants, however growers would likely need to apply a larger-particle mulch on top to prevent the smaller particles from eroding by wind or water, making wood chips (not bark) the most feasible particle size option.

Mulches made of wood provide the most benefit because the breakdown of these mulches will also increase the soil organic matter content while providing nutrients such as carbon, nitrogen, and phosphorous for use by plants. Soil tests show soil organic matter ("SOM") as a percentage, and for each percentage of SOM (where SOM = 1.0%), the soils contain approximately 20 pounds of inorganic nitrogen (available for plant use) and two pounds each of phosphorous, potassium, and sulfur (Fernandez & Kaiser, 2021; McLean et al., 2021). While growers may mulch just to increase soil water-holding capacity, organic growers should especially consider taking a soil test before mulch application to track SOM build up over time to understand the nutrients available from SOM.

Biochar is another material with the potential to increase soil water-holding capacity and promote plant growth. Biochar is created when wood products are burned in oxygen-poor conditions (a process called "pyrolysis"). The wood materials become carbonized while retaining their original woody cellular structure (IBI, 2015). The small crevasses that remain serve as reservoirs for water, thereby increasing the water- and nutrient-holding capacity of the soil when biochar is incorporated into the soil (Li et al., 2021). While it does increase soil porosity and thus water content, biochar does not directly increase nutrient content or organic matter of the soil (as wood mulches do). Thus, this experiment studies the effect of applying a combination biochar + compost product to improve soil organic matter and water-holding capacity.

Utilization of biochar is of particular interest in Maine because of the state's robust forestry industry, which includes thousands of forestry landowners and sawmills, ten wood pellet manufacturers, and 19 industrial CHP (biomass-based combined heat and power) plants, has the capacity to produce tons of biochar product per year (Novak et al., 2022), making this a potentially affordable, local, and reliable resource for wild blueberry growers in the state.

Recent research has established that using biochar as a soil amendment in a range of agricultural settings can achieve increases in the water- and nutrient-holding capacity of soil, increases in soils' abilities to sequester carbon, and filtration and sequestration of nutrients that can harm water quality (e.g. nitrogen and phosphorous) before rainfall events cause leaching or runoff from dairy manure storage lagoons or herbicide-treated fields (Woolf et al., 2010; Wang et al., 2021; Blanco-Canqui, 2019; Abas et al., 2022; Rahman et al., 2021).

This study began in 2021, when softwood mulch, hardwood mulch, and biochar + compost products were applied to research plots, to be compared with plots that did not receive any material. In 2021 and 2022, plants within these plots were measured and evaluated to track plant health and fruit yield. Soil and leaf samples from the different treatments were also taken and evaluated.

METHODS

In May 2021, the study was laid out at Blueberry Hill Farm in Jonesboro, ME in a randomized complete block design with each soil amendment treatment (softwood mulch, hardwood mulch, and biochar + compost) replicated six times in 6' by 30' plots, for a total of 24 plots. Biochar was combined with compost so that the compost would provide nutrients to the soil and prevent biochar from blowing away. Baseline soil samples were taken at these plots in May 2021 and the final soil samples were taken from this trial on July 30, 2022. Foliar samples were collected on July 26, 2022 for leaf tissue nutrient testing. Both soil samples and foliar samples were sent to the Analytical Soil Testing Lab at the University of Maine. The treatments were applied in late May 2021.

Data Collection

Soil Moisture

TDR (Time Domain Reflectometry) was used to measure 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 (4.8 inches). Six random readings were recorded per plot on May 11, June 6, and July 1, 2022.

Blueberry Phenology

Repeated plant phenology measures were taken on the same four stems in each treatment plot. Plants were tagged with numbered tags and were evaluated on May 19, June 15, and July 22, 2022. The number of buds, flowers, green, and blue fruit were recorded during each sampling. Stem heights were also measured using a meterstick and were recorded in centimeters.

Blueberry Physiology

Eight stems from each plot were randomly selected to measure chlorophyll concentration by a CCM-200 plus Chlorophyll Content Meter (Opti-Sciences, Inc., Hudson, NH, USA) on June 6 and July 1, 2022. 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 June 30, 2022, between 10 am to 2 pm.

Blueberry Morphology

On July 26, 2022, 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 Leaf Area Meter (LI-COR Biosciences, Lincoln, NE, USA). All the leaves from those eight stems were oven-dried at 70°C to constant mass and weighed, and then the dried leaf samples were ground and sent to the University of Maine Soil and Plant Tissue Testing Laboratory in Orono, Maine for leaf nutrient testing. On July 30, 2022, 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.

Plant Phenology

Repeated plant phenology measures were taken on the same four stems in each treatment plot. Plants were tagged with numbered tags and were evaluated on May 19, June 15, and July 22, 2022. The number of buds, flowers, green, and blue fruit were recorded during each sampling. Stem heights were also measured using a meter stick and were recorded in centimeters.

Table 1. Optimum soil characteristic ranges and comparisons of wild blueberry soil characteristics among different soil amendments as sampled on October 2, 2021 and July 30, 2022 at Blueberry Hill Research Station, Jonesboro, ME. Soil characteristics for different treatments are represented as mean of six replicated soil samples ± standard error of the mean.

Soil Characteristics	Year	Optimum range	No treatment	Treatments		
			Control	Hardwood mulch	Softwood mulch	Biochar + compost
pH	2021	4.0-4.5	4.6 (±0.12)	4.8 (±0.13)	4.8 (±0.09)	4.92 (±0.04)
	2022		4.2 (±0.1)	4.6 (±0.1)	4.6 (±0.1)	4.5 (±0.2)
Organic matter (%)	2021	5-8	7.5	8.9	8.2	7.8
	2022		11.7 (±2.5)	12.1 (±4.9)	9.5 (±1.7)	7.3 (±1.1)
Nitrate-N (ppm)	2021	20-30	1.3 (±0.2)	1	1.3 (±0.2)	1.7 (±0.2)
	2022		0.8 (±0.2)	1	1	1
Ammonium-N (ppm)	2021	<10	2.2 (±0.37)	2.5 (±0.66)	3.5 (±1.43)	2.7 (±0.84)
	2022		6.0 (±1.4)	6.0 (±2.4)	5.3 (±1)	4.8 (±0.8)
Phosphorous (lb/A)	2021	10-40	8.2	11.6	17.6	9.4
	2022		9.5 (±1)	11.9 (±3.7)	12.4 (±0.5)	11.4 (±1.3)

Pest Scouting

Repeated pest presence and plant growth measurements were taken throughout 2021 (four times in the season) and once in 2022 (June 15) using a 0.37 m^2 quadrat in the same flagged locations, twice per plot. Blueberry cover, weed, insect, and disease presence were recorded. 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.

Fruit Yield
Fruit was hand-raked on August 2, 2022. Within each treatment plot, two 0.37m^2 quadrats were placed at the same flagged locations used for pest scouting and all the fruit was harvested within the quadrat and the yield recorded. The entire plot was then also raked, and the yield recorded, so each plot generated three yield numbers: quadrat one, quadrat two, and total plot outside the quadrats. The fruit from each plot were then combined to enable fruit quality measures.

Fruit Quality
The harvested fruit was sampled in several ways to determine fruit quality. The weight of 100 berries was measured and recorded, allowing researchers to determine which treatments produced larger fruit, since the 100 berry weight had a higher mass. A sample of fruit from each treatment was also puréed for use in a handheld PAL-BRIX/ACID F5 refractometer (Atago, Saitama, Japan) to measure the samples' sugar content.

<u>Data Analysis</u>

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 (α = 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 leaf photosynthetic electron transport 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 (α = 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) of wild blueberry plants were statistically compared using a general linear model followed by LSD (least significant difference) post-hoc test in SPSS software (α = 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
Single date measurements including yield, Brix, 100 berry counts and phenology measures (by key stage) were evaluated using a generalized linear model (GLM), followed by a Tukey's Pairwise comparison in JMP (JMP®, Version 16.0, SAS, Cary, NC, USA) 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 height, weed number, and stems with pest presence (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.

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 included blueberry phenology, stem height and cover, pest presence (# or # of stems/m^2; weeds, insects and diseases), and 100 berry counts. These data improved following a square root transformation except for blueberry cover (which was left untransformed). Transformed data continued to statistically fail for normality, however, statistical tests were carried out despite non-normality after establishing there were no serious problems with the data. Blueberry yield and Brix measures were normally distributed; therefore, no transformation was needed prior to statistical testing.

RESULTS

Soil Moisture

During the 2022 crop year growing season (May - July), biochar + compost treated soil contained consistently higher moisture content on average among all treatments (Figure 1A). Unfortunately, this result is confounded by the fact that biochar was applied with compost which is known to contain high levels of organic matter that holds water very effectively. While comparing seasonal average soil moisture among all treatments, the soil moisture content was significantly higher in the biochar + compost treatment compared to the softwood mulch treatment but not compared to the control and hardwood mulch treatment (Figure 1B). All treatments and control remained above 10% volumetric soil content, the threshold for healthy crop production.

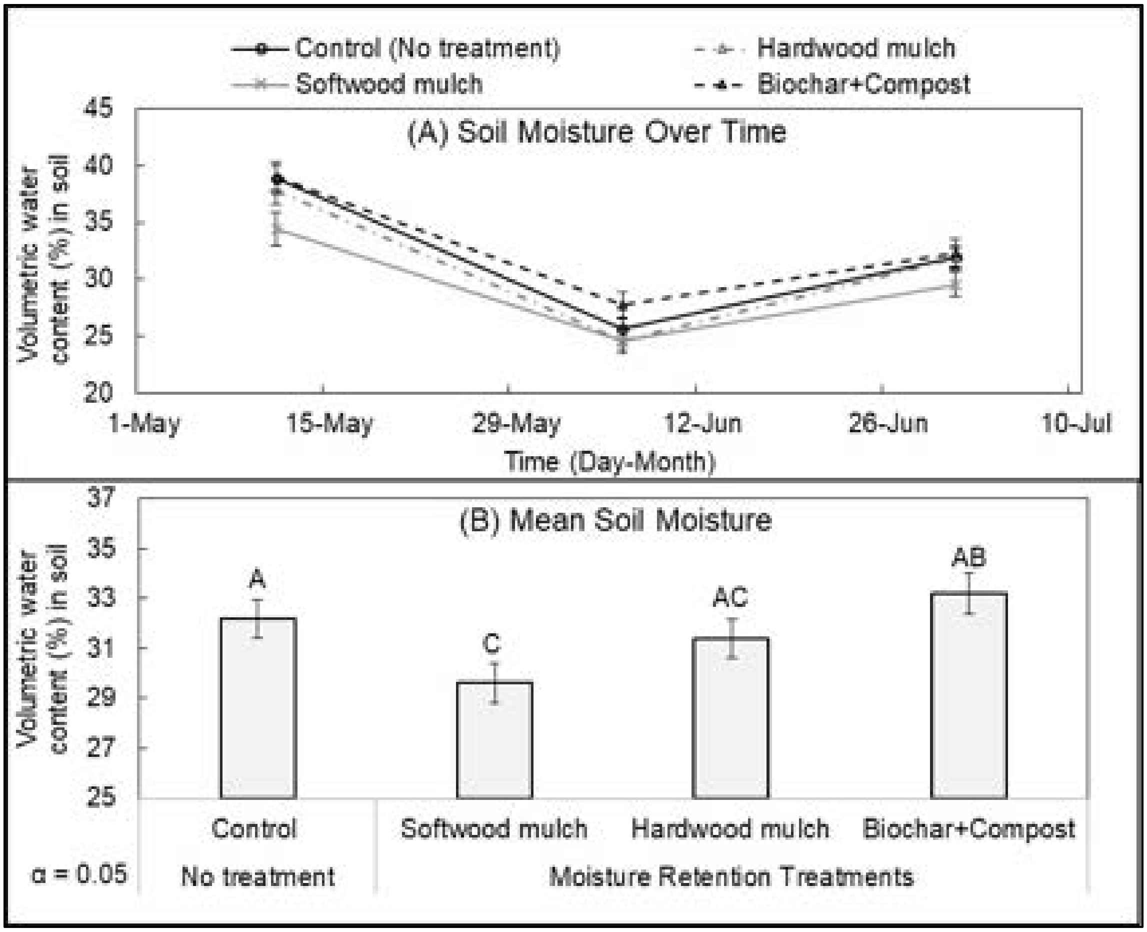

Figure 1. Comparison in (A) soil moisture during the 2022 growing season among soil amendment treatments, and (B) mean soil moisture content by treatment type as measured on May 11, June 6, and

July 1, 2022, at Blueberry Hill Research Station, 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, all treatments had similar leaf chlorophyll concentration in both June and July where no significant differences were found (Figure 2). The leaf chlorophyll concentration in all treatments ranged from 18 to 22 SPAD and 25 to 28 SPAD in June and July, respectively.

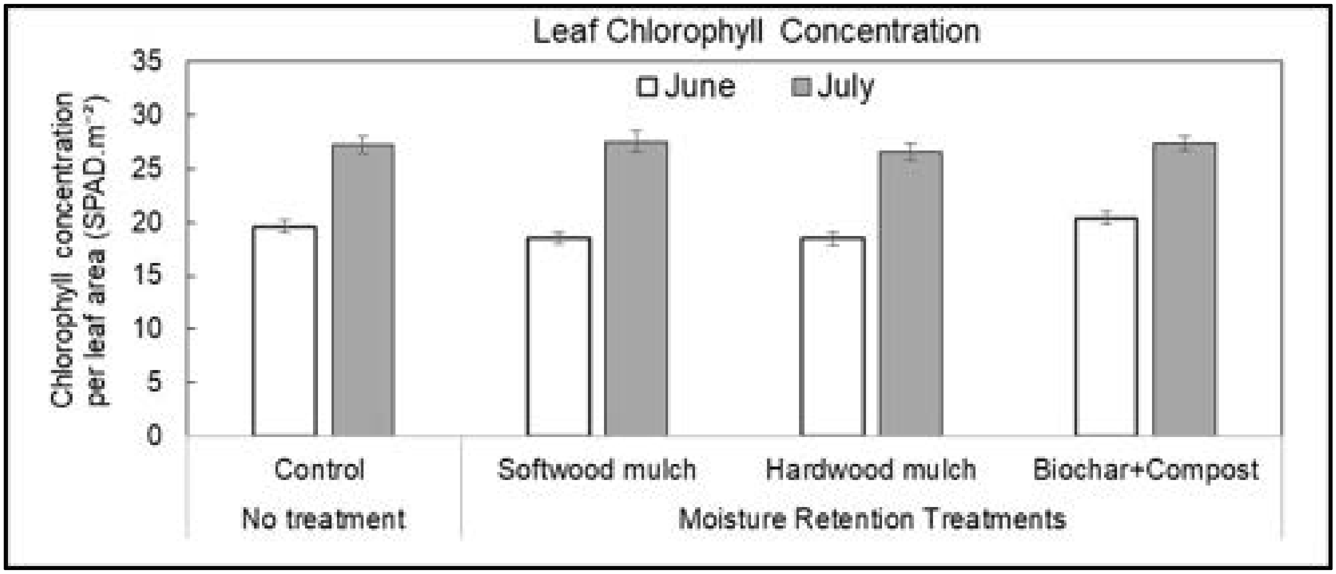

Figure 2. Comparison in chlorophyll concentration of wild blueberry leaves on June 6 and July 1, 2022, among different soil amendment treatments at Blueberry Hill Research Station, Jonesboro, ME. Error bars indicate the standard error of the mean. No letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

For leaf photosynthetic electron transport rate among the treatments, plants treated with hardwood mulch had significantly lower electron transport rates compared to the control (Figure 3). In contrast, electron transport rates in plants treated with softwood mulch and biochar + compost did not significantly differ from the control. Moreover, plants treated with softwood mulch had higher electron transport rates on average compared to the control, but this was not significant. However, the electron transport rate in softwood mulch treated plants were significantly higher than in hardwood mulch and biochar + compost treated plants.

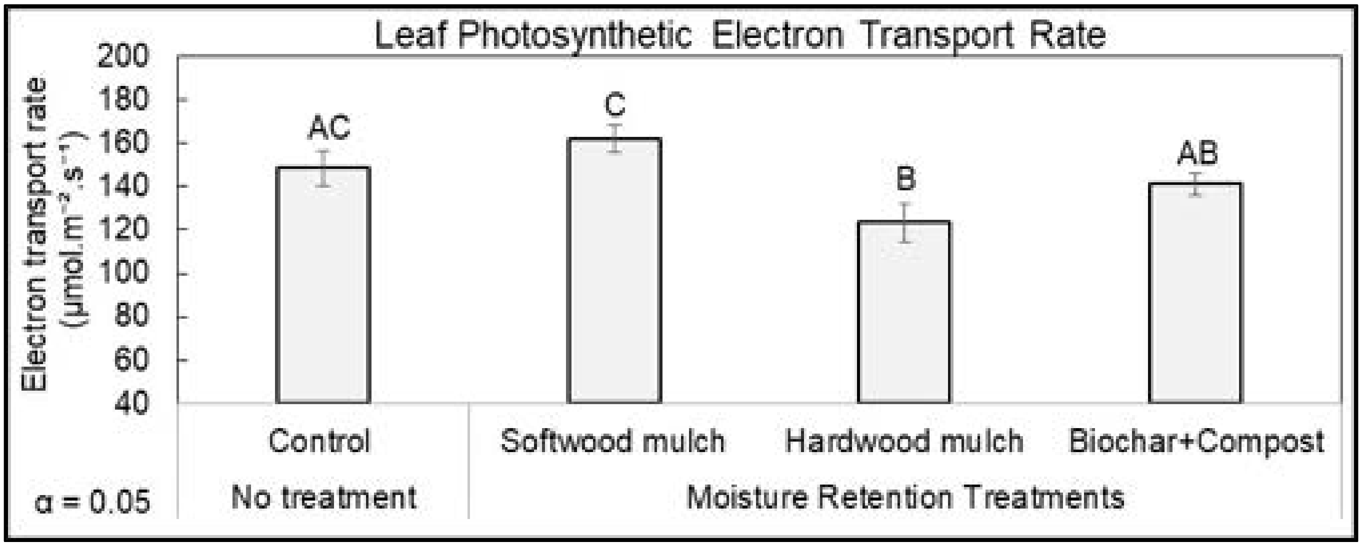

Figure 3. Comparison in photosynthetic electron transport rate of wild blueberry leaves on June 30, 2022 across different treatments at Blueberry Hill Research Station, Jonesboro, ME. Error bars indicate the standard error of the mean. Different letters indicate significant differences at the significance level of $p < 0.05$.

Blueberry Morphology

In July 2022, leaf size was the largest in the control, followed by the softwood mulch, biochar + compost, and hardwood mulch treatments (Figure 4A). Leaf size was significantly smaller in the hardwood mulch treatments compared to the control and softwood mulch treatments. In contrast, no significant differences were found in the number of leaves per stem across treatments (Figure 4B). Consequently, total leaf area per stem was significantly smaller in the hardwood mulch treatments compared to the control and softwood mulch treatments (Figure 4C). However, it did not significantly differ in biochar + compost treatments compared to other treatments and control.

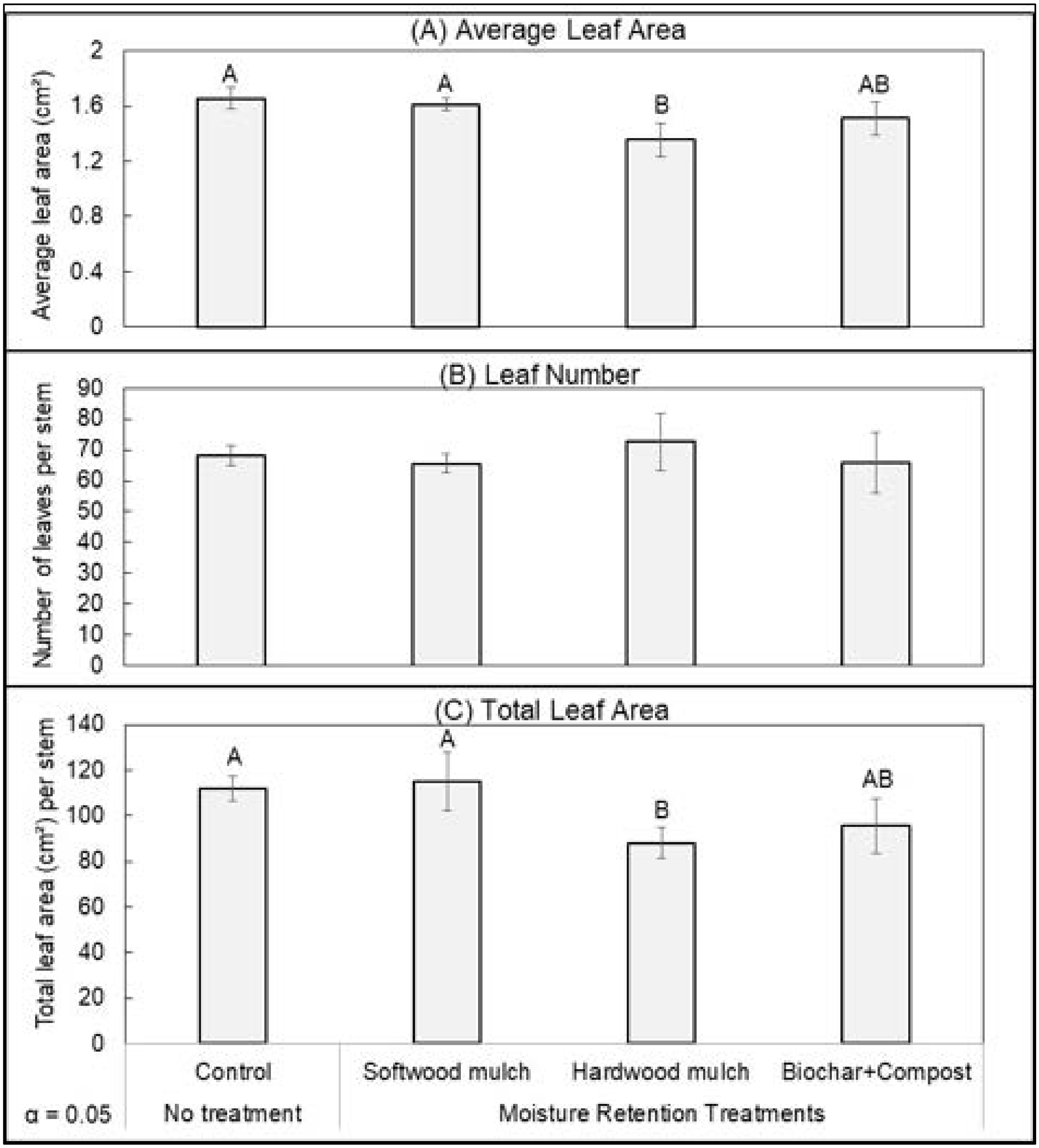

Figure 4. Comparison in (A) average leaf area, (B) number of leaves per stem, and (C) total leaf area per stem of wild blueberry plants across different treatments on July 26, 2022, at Blueberry Hill Research Station, Jonesboro, ME. Error bars indicate the standard error of the mean. Different letters indicate significant differences and no letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

Plant Phenology

The phenological development in the wild blueberry in response to the treatments exhibited no significant treatment differences (Figure 5). However, there are some visible differences that suggest the treatments may have influenced development potential or development timing relative to the control and when phenology counts were sampled. All treatments resulted in more buds, flowers and blue fruits per stem than the control. Stems in softwood mulch produced the greatest number of green fruit per stem (15.78 fruits/stem), though this did not result in a similarly large number of blue fruit on the stem (5.94 fruits/stem), suggesting the plant could not support the amount fruit successfully pollinated. All treatments produced more blue fruit than the control, but the treatment with the highest average blue fruit per stem (6.53 fruits/stem) was the hardwood mulch, which also had the lowest percent loss of 38% (along with biochar + compost), between June 15 and July 22, 2022 (Table 2).

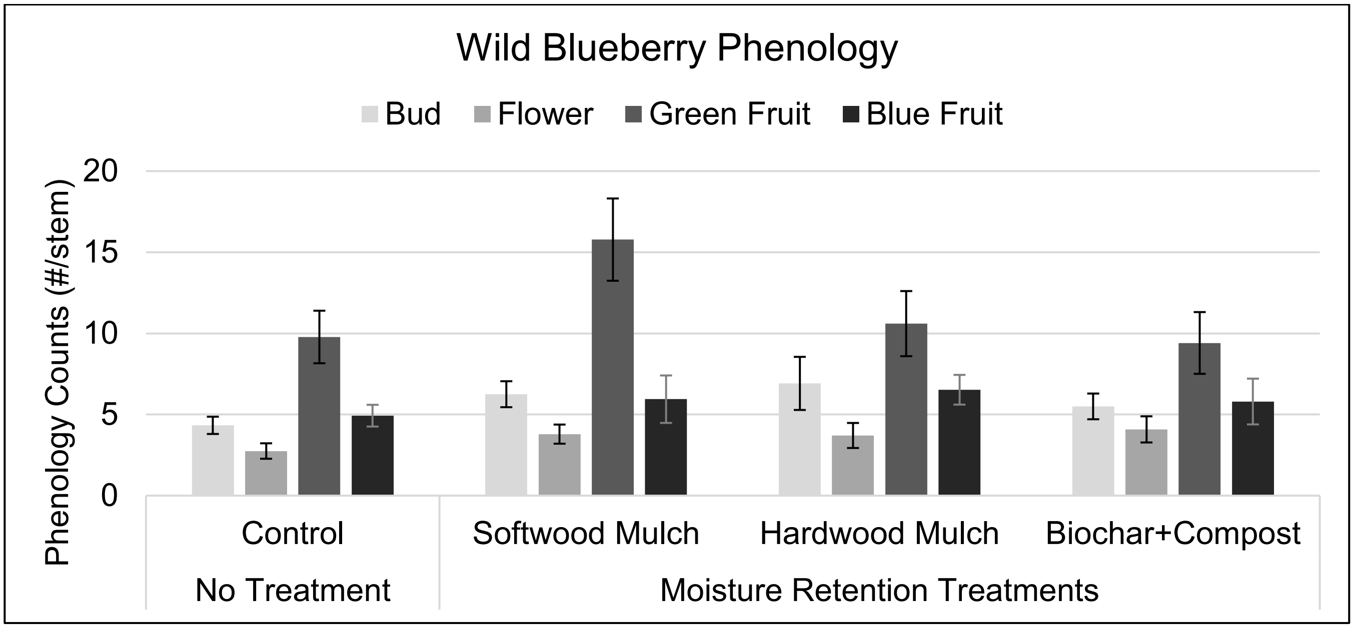

Figure 5. Average bud, flower and fruit counts per stem by treatment at Blueberry Hill Research Station, Jonesboro, Maine. Bud, flower, green fruit, and blue fruit counts were observed on May 19 (bud + flower), June 15, and July 22, 2022, respectively. Treatment differences in phenology counts were not significant. Error bars represent the standard error of the mean.

Table 2. Percent loss from green fruit counts sampled on June 15, 2022, to blue fruit counts sampled on July 22, 2022, by treatment type at Blueberry Hill Research Station, Jonesboro, Maine. Note that samples were not measured at the peak of either green fruit or blue fruit stage, so the percentage loss can only provide an approximation of the trend. Variation is likely due to genetic differences.

Treatment	Green fruit	Blue fruit	% loss
Control	9.78	4.93	49%
Softwood mulch	15.78	5.94	62%
Hardwood mulch	10.60	6.53	38%
Biochar + compost	9.41	5.80	38%

Stem Height

Blueberry stem height was used as an indication of plant health, whereby taller stems were produced by healthier plants. Softwood mulch produced the tallest plants (18.4 cm), followed by biochar + compost (17.5 cm), control (17.0 cm), and finally hardwood mulch (16.1 cm). There was no significant difference between treatments and the control (Figure 6).

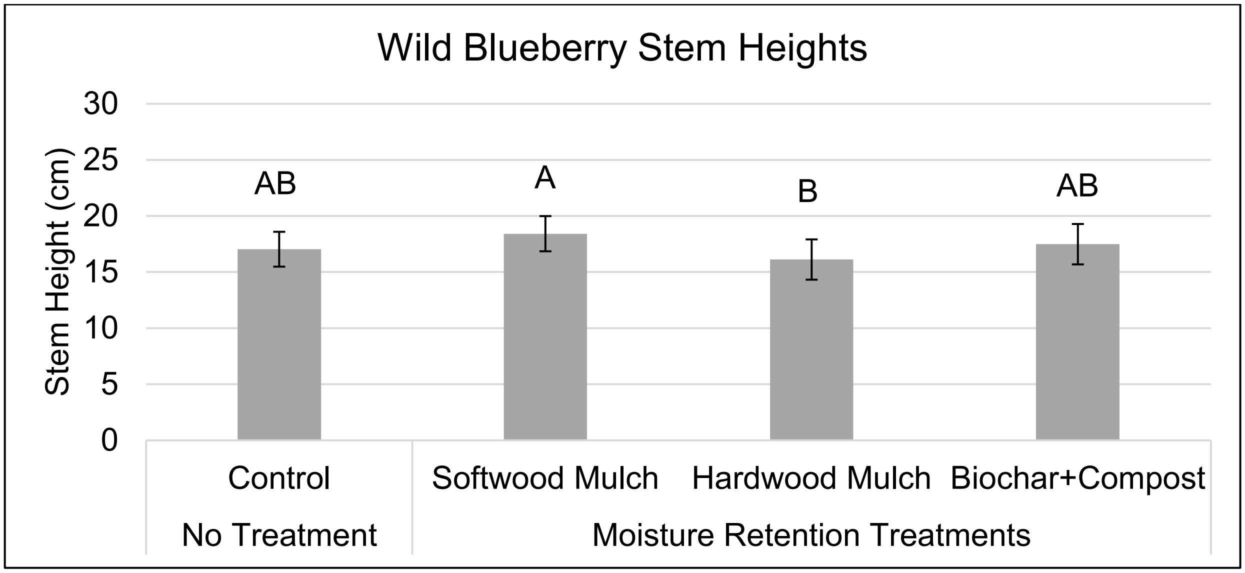

Figure 6. Average stem heights (cm) by treatment measured on 3 dates (May 19, June 15, and July 22, 2022) at Blueberry Hill Research Station, Jonesboro, Maine. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean.

Pest Presence

Weed presence was greater after all treatments compared to the untreated control (Figure 7). The soft- and hardwood mulches contained significantly more weeds (44.7 weeds/m^2 and 38.7 weeds/m^2, respectively) than the control (24.0 weeds/m^2). More weeds were observed after the biochar + compost treatment (41.0/m^2 weeds) than in the control as well, but this difference was not significant.

While treatment differences in insect damage were not significant, the softwood mulch treatment experienced the highest level of insect damage in both number and cover (18 stems/m^2, 5%/m^2) relative to the control and all other treatments across one entire prune/crop cycle (2021 and 2022; Figure 8). In contrast to the softwood mulch treatment, the hardwood mulch treatment had the lowest level of insect damage in number (8 stems/m^2). The number of blueberry stems with disease damage were also lowest in the hardwood mulch treatment (Figure 9). Disease numbers in the hardwood mulch treatment (159 stems/m^2) were significantly lower than the control (237 stems/m^2) by an average of 78 fewer diseased stems per m^2. Interestingly, the hardwood mulch treatment exhibited the highest percent coverage of disease (42%/m^2), 5% higher in rank than the average disease cover in the control (37%/m^2). This disparity suggests that while there were significantly fewer stems in the hardwood mulch treatment, the stems that were infected had a greater level of infection.

Disease presence in softwood mulch and biochar + compost treatments did not significantly differ from the control or hardwood mulch treatments.

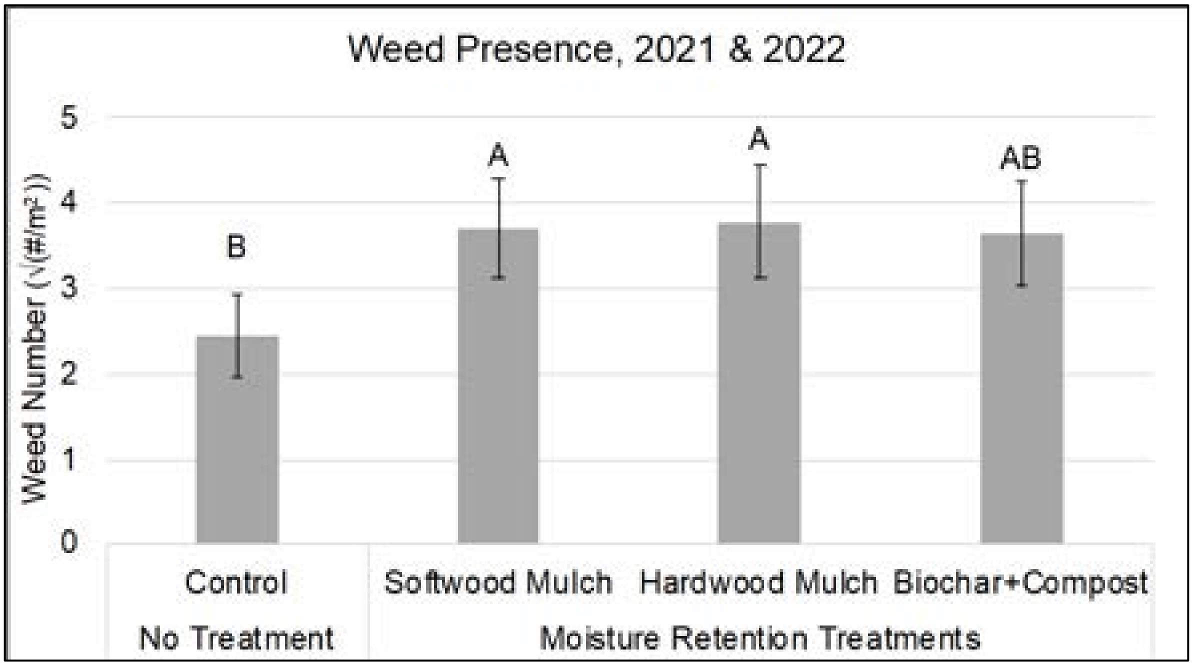

Figure 7. Average weed number (√#/m^2; see "Methods" section for explanation of unit) by treatment measured on 2 dates (May 19 and June 15, 2022) at Blueberry Hill Research Station, Jonesboro, Maine. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean.

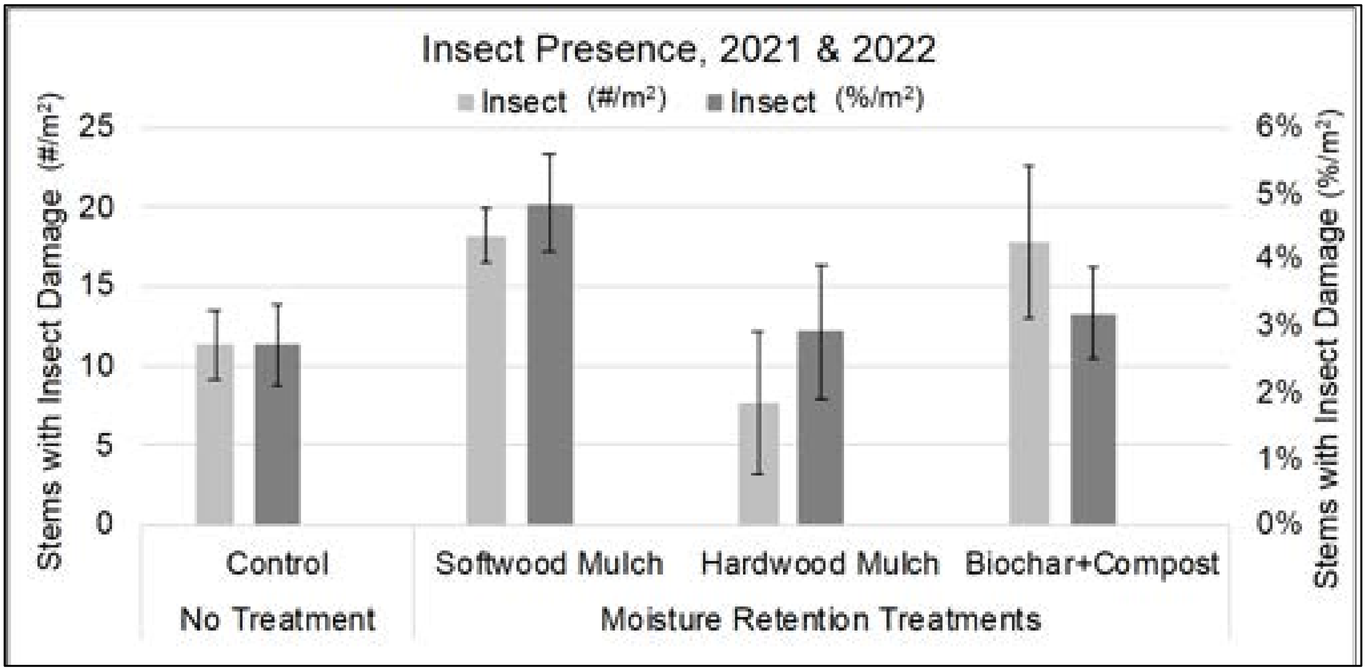

Figure 8. Average number and percent of blueberry stems with flea beetle and tip midge insect damage (#/m^2 and %/m^2) by treatment measured on 2 dates (May 19 and June 15, 2022) at Blueberry Hill Research Station, Jonesboro, Maine. Treatment differences were not significant for the number and percent of blueberry stems with insect damage. Error bars represent the standard error of the mean.

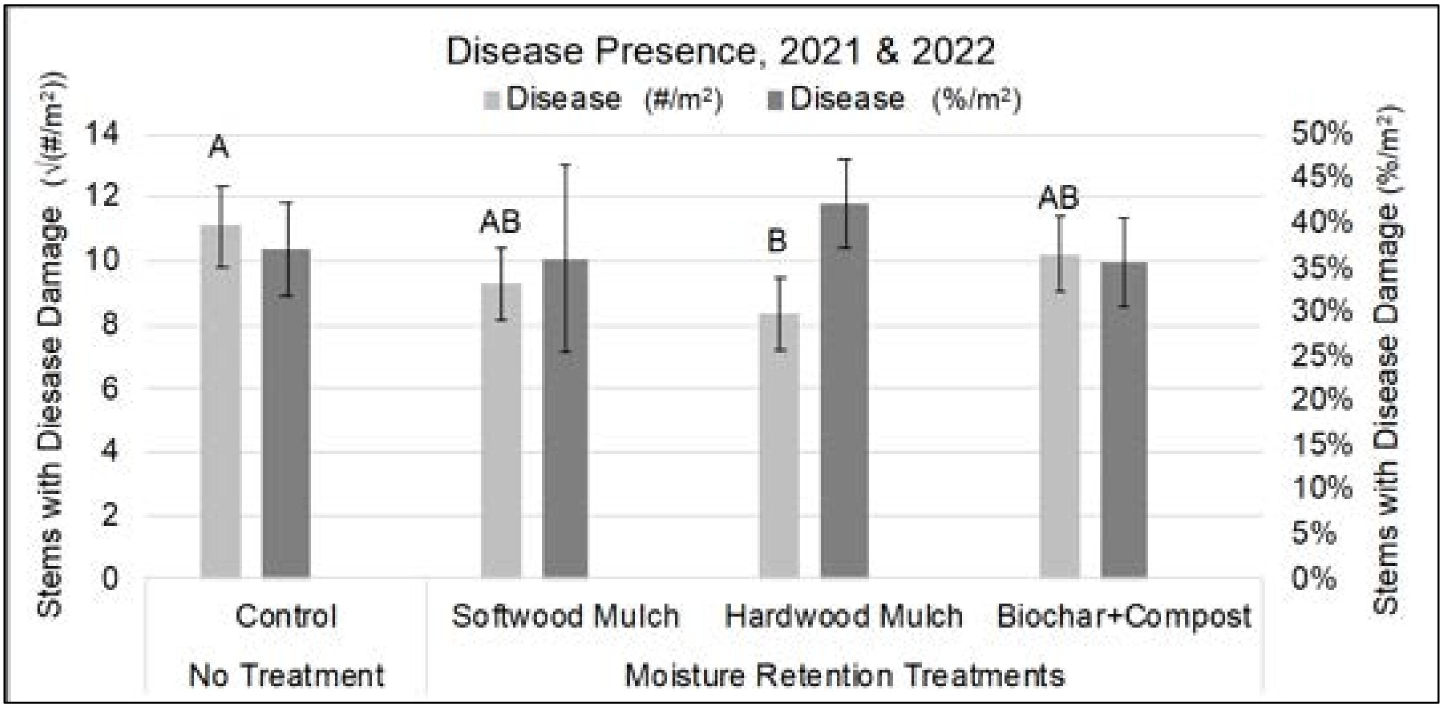

Figure 9. Average number and percent of blueberry stems with mummy berry and leaf spot disease damage (#/m^2 and %/m^2) by treatment measured on 2 dates (May 19 and June 15, 2022) at Blueberry Hill Research Station, Jonesboro, Maine. Treatment differences were not significant for the percent of blueberry stems with disease damage (%/m^2). Letters indicate significant differences at the 0.05 level of significance for the number of blueberry stems with disease damage (#/m^2). Error bars represent the standard error of the mean.

Fruit Yield and Quality

Harvest occurred on August 2, 2022 and varied by treatment relative to the control and was not significant (Figure 10). The highest yields were observed in the biochar + compost treatment with 1815 lbs/A, which was 219 lbs/A greater than the control (1596 lbs/A). The lowest yields observed were in the hardwood mulch with 1298 lbs/A, followed by the softwood mulch 1526 lbs/A; 298 lbs/A and 70 lbs/A less than the control, respectively.

While not significant, the largest berries were produced in the hardwood mulch treatment (42.1 g/100 berries). Similar-sized berries were produced in the control, softwood mulch, and biochar + compost treatment: 34.3 g/100 berries, 32.9 g/100 berries, and 33.5 g/100 berries, respectively (Figure 11). While also not significant, the control treatment produced berries with a slightly higher sugar content (10.7 Brix) than in the soft- (9.8 Brix) and hardwood mulches (9.5 Brix), but that was approximately the same as the biochar + compost (10.8 Brix) (Figure 12).

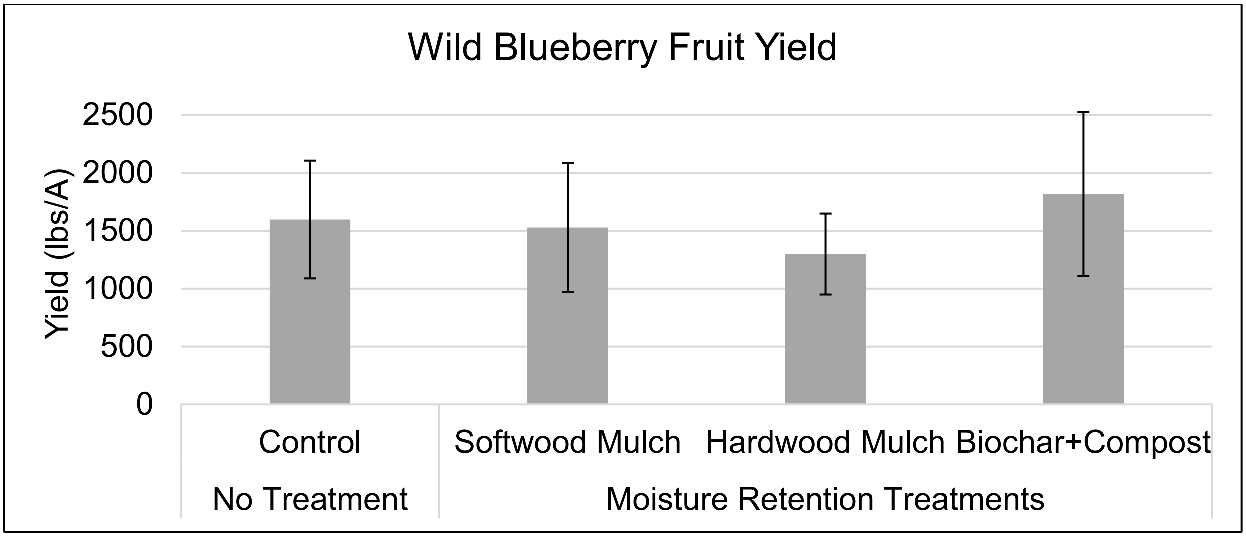

Figure 10. Average yield (lbs/A) by treatment harvested on August 2, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Treatment differences in crop yield were not significant throughout. Error bars represent the standard error of the mean.

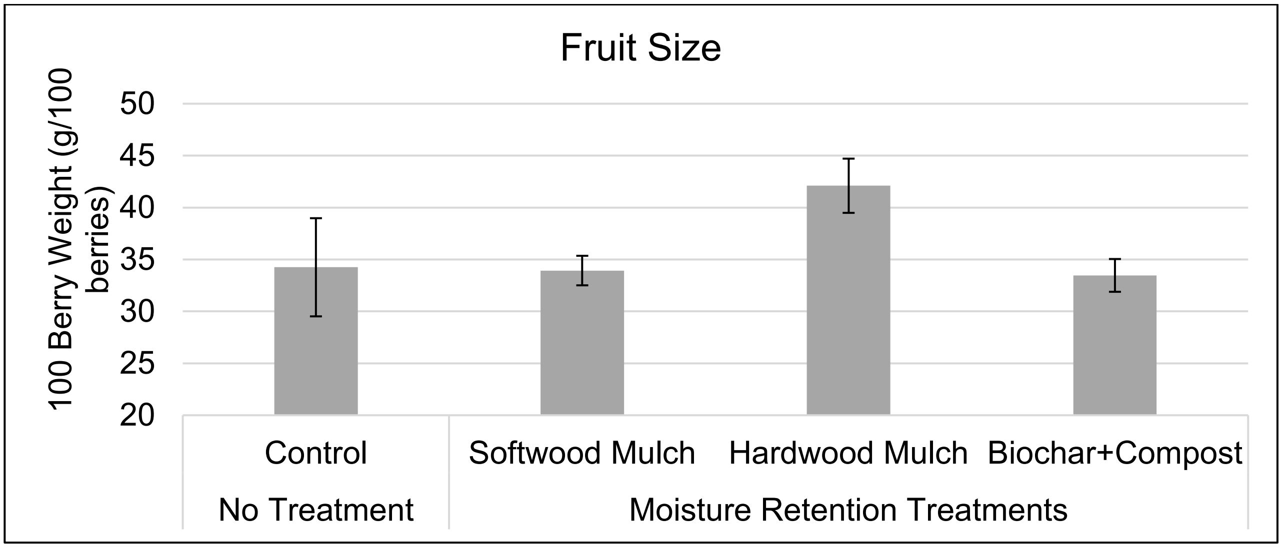

Figure 11. Average berry size (100 berry weight; gram/ 100 berries) by treatment harvested on August 2, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Treatment differences in berry size were not significant. Error bars represent the standard error of the mean.

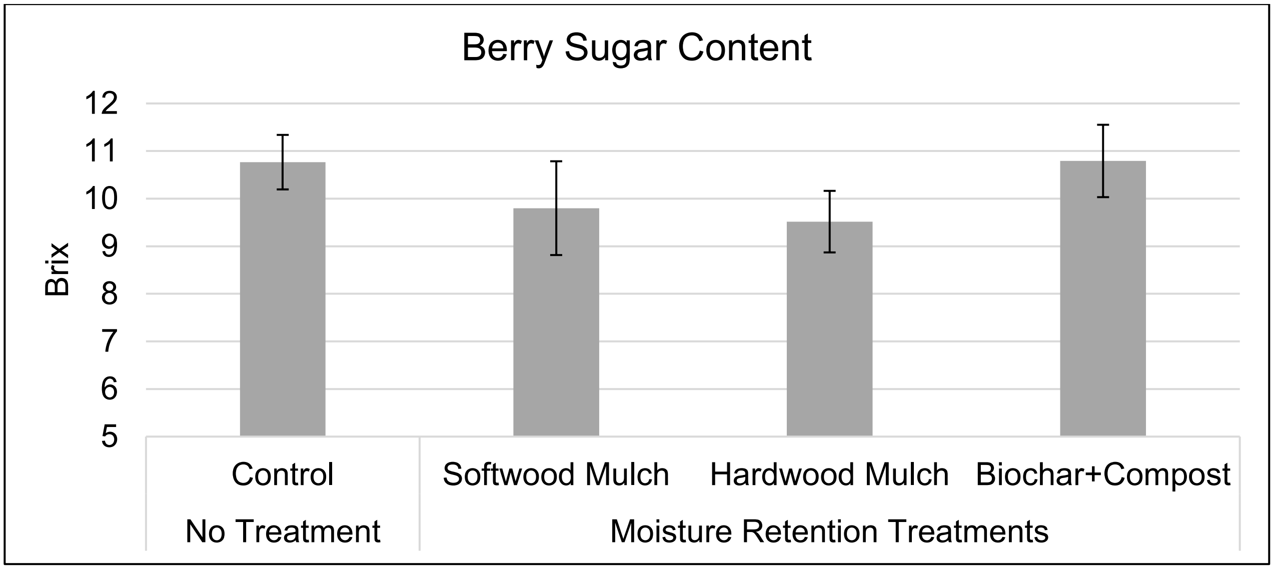

Figure 12. Average berry sugar content (Brix) by treatment harvested on August 2, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Treatment differences in berry sugar content were not significant. Error bars represent the standard error of the mean.

DISCUSSION

Unfortunately, biochar was applied with compost which confounds any results determining the effectiveness of biochar at increasing soil water-holding capacity. Compost is known to contain high levels of organic matter, a material that holds water very effectively, and it is impossible to determine whether the higher soil moisture levels in the biochar + compost treatments are from the biochar or the organic matter provided by the compost. Looking at the soft vs. hardwood mulch results, each performed well under different measurements (not only related to soil moisture) making results at this time inconclusive.

More years of data collection are required to tease out changes in soil characteristics due to soft and hardwood mulch application. Percent organic matter increased from 6.5 to 11.7% where hardwood mulch was applied yet remained at about 9% where softwood mulch was applied. To date, the soil pH did not change significantly in either soft or hardwood mulch treatments, and other studies have shown mixed results on whether hardwood and softwood mulches diverge in their impacts on soil pH (Maggard et al., 2012). Maggard et al. (2012) posits that changes in pH following mulch application may be attributable to the relative difference between mulch pH and soil pH. Rates of decomposition differ across mulch materials, with hardwood mulch decomposing more rapidly than softwood mulch (Green, 1978). Differences in mulch decomposition speed between the two materials result in differences in nutrient availability over time (Green, 1978).

Plant Physiology & Morphology

Similar physiological performance and leaf characteristics in the treatments compared to the control indicate that the application rate of mulch and application frequency of biochar-compost might need to increase to consistently have better performance over the years.

Pest Presence

Pest presence in response to treatments were not significant and likely due to past pest presence and wild blueberry genetic diversity. However, there are interesting trends worth noting. As first observed in 2021, weed presence continued to be higher in all treatments relative to the control, suggesting the weeds are benefiting from mulches and biochar + compost or that weed seeds were brought in with the materials. The spray window for mummy berry was missed. Disease presence was slightly higher where the softwood mulch had been applied relative to all other treatments and the control, and softwood mulch saw the only increase in disease presence from 2021 to 2022. Taken together, pest presence appears to have increased between the 2021 and 2022 seasons after the application of softwood mulch and reduced slightly after hardwood and biochar + compost applications, although not significant. Pest presence will continue to be monitored over time. Mulch can suppress weed growth when applied to depths of several inches, however, as 1” of mulch was applied in this study, it may not have been enough to adequately suppress the weeds.

Fruit Yield and Quality

Yields were not significantly different among treatments. Most likely due to plant genetic diversity, there was a trend towards greater fruit yield following the biochar + compost applications, but this trend was not significant. While the yields in the hardwood and softwood mulch treatments were lower, these treatments also had higher blue fruit counts eleven days before harvest, on July 22, 2022, suggesting fruit may have matured and dropped earlier than the other treatments. This maturation and fruit drop before harvest would impact overall fruit yield, 100 berry weight, and Brix measures.

Interestingly, the lowest yields were observed in the hardwood mulch treatments, yet these same treatments produced the largest individual fruit. As large berry size is an indication of plant health (healthier plants produce larger berries), the large berry size in the hardwood treatment indicates the plants perhaps had more nutrient and water resources than plants experiencing the same environmental conditions under different treatments.

ACKNOWLEDGEMENTS

This project was funded by the State of Maine Specialty Crop Block Grant Program and we greatly appreciate their support. Thank you to Ava Ardito, Abby Cadorette, Charles Cooper, Julian LaScala, Jordan Ramos, Mara Scallon, and Brogan Tooley for data collection and analysis assistance.

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4. The impact of glycine betaine applications on drought response in wild blueberries

OBJECTIVE

In this study, we studied the effect of foliar-applied glycine betaine applications on lowbush blueberries in both field and greenhouse drought experiments. The product being tested was Bluestim®, a foliar-applied product containing >96% pure glycine betaine. We measured the impact of this product on water potential, chlorophyll concentration, stomatal conductance, evapotranspiration, stem length, leaf buds, fruiting buds, soil moisture, leaf and fruit drop, and weight change.

LOCATIONS: Jonesboro and Orono, ME
PROJECT TIMEFRAME: June 2021 – July 2022

INTRODUCTION

In order to combat increasing drought conditions, new approaches to mitigate drought stress will be needed. Irrigation has proven to be a good option, but this form of control is expensive and not always accessible to smaller farmers. Mulching with wood chips has been shown to improve soil water retention during wet and dry periods (Hunt et al., 2010), improve growth (Gumbrewicz & Calderwood, 2022), and increase yields (Sanderson & Cutcliffe, 1991). However, mulching can be costly over larger areas and can impede harvesting.

One potential approach is glycine betaine, a naturally occurring compound derived from glycine that is water-soluble and non-toxic. This compound has been shown to increase resistance to abiotic stresses in both exogenous applications and when introduced through transgenes (Chen & Murata, 2008). Glycine betaine has a low molecular weight and water-soluble properties, making it a compatible solute. In the case of glycine betaine, it works as an osmoprotectant allowing the plant to maintain turgor pressure and protect enzymes and macromolecules from oxidation (Osman, 2015). Glycine betaine occurs naturally in some plants, referred to as accumulators, under different abiotic stresses such as high salinity and drought (Annunziata et al., 2019). Vaccinium plants have not been found to be accumulators; most accumulators fall into the Chenopodiaceae and Gramineae families (Weretilnyk et al., 1989). Studies have shown that foliar applications of glycine betaine have successfully reduced the effects of stress in both accumulating and non-accumulating plants, but it is not successful in all crops (Escalante-Magaña et al., 2019). In a study looking at pea plants, researchers found that applications of glycine betaine increase leaf count and the number of pods per plant (Osman, 2015).

Wild blueberries are economically important for Maine, and with temperatures in these fields increasing faster than in any other region of Maine, it is important to find new ways to combat the effects of drought (Barai et al., 2021; Tasnim et al., 2021).

METHODS

A two-year study was initiated in 2021 to investigate the effect of foliar-applied glycine betaine on drought stress. Plants in the prune year were used. Field plots were laid out in a randomized block design. There were 12 plots, 7 x 10ft, with a 5ft buffer between plots. Within these 12 plots, there were three treatments: control (no treatment), low rate, and high rate. The low rate was applied at 3047.3 g/ha, and the high rate was applied at 4035.0 g/ha. Applications were made on 24 June, 13 July, and 18 August 2021.

To assess the impact of treatments on plant physiology, we recorded chlorophyll concentration (SPAD), stem length, and water potential every two weeks, starting on 29 July and ending on 13 September 2021. After leaves had dropped, leaf and fruit buds were counted to assess potential productivity for the following crop year.

In year 2 (2022), we conducted two greenhouse experiments. Wild blueberry sods were collected on 6 May and placed in 2-gallon buckets. The bottom of the buckets had holes drilled into them, and gravel was placed in the bottom. Four genotypes were collected in order to account for different responses to drought and glycine betaine treatments. There were four treatments: control, control + glycine betaine, drought, and drought + glycine betaine.

For the first experiment, there were 48 plants total, 12 in every block, with three from each genotype. Two applications of glycine betaine were completed at a rate of 57.6 oz/acre. These applications were made on the glycine betaine and drought + glycine betaine plants; one on the day drought was initiated, (9 June), and the second two weeks later (23 June). The blocks not undergoing drought were watered three times in the first week, then watered every day for the following weeks. To assess the impact of treatments on plant physiology we recorded chlorophyll concentration (SPAD), stomatal conductance, water potential, leaf count, fruit count, and soil moisture (TDR). Measurements were taken weekly, and weight change was taken once in the second week. This trial lasted two weeks.

For the second experiment, there were 24 plants with two treatments: drought and drought + glycine betaine. The second round of measurements lasted three weeks. All measurements listed above were taken weekly, with the exception of fruit count which was not assessed during this round.

Statistical Analysis

All data were tested for normality and deviations from homoscedasticity using the Shapiro-Wilk test and Levene's test, respectively. For non-normal data, log X+1 transformations were carried out and normality and homoscedasticity rechecked. If assumptions were met, data were analyzed using analysis of variance (ANOVA) and where appropriate post-hoc tests were performed using a Tukey HSD test (α = 0.05). When assumptions were not met non-parametric tested were used, primarily the Kruskal-Wallis test, with post-hoc being performed by Wilcoxon each pair test.

RESULTS

In the year 1 field trial, there was a significant difference in the chlorophyll concentration of different treatments over time ($F_{(5,474)} = 3.217$, $P = 0.0072$), but there was no significant difference between treatments ($F_{(2,474)} = 2.92$, $P = 0.054$). Plants receiving the high rate did have significantly higher chlorophyll concentrations compared to control and low rate treatments (Figure 1). Similarly, there were no significant differences in glycine betaine on the water potential ($F_{(2,114)} = 0.03$, $P = 0.963$) of treated stems. However, the water potential of stems in all treatments did vary significantly over time ($F_{(3,114)} = 9.87$, $P = 0.002$), likely due to ambient environmental conditions (Figure 2).

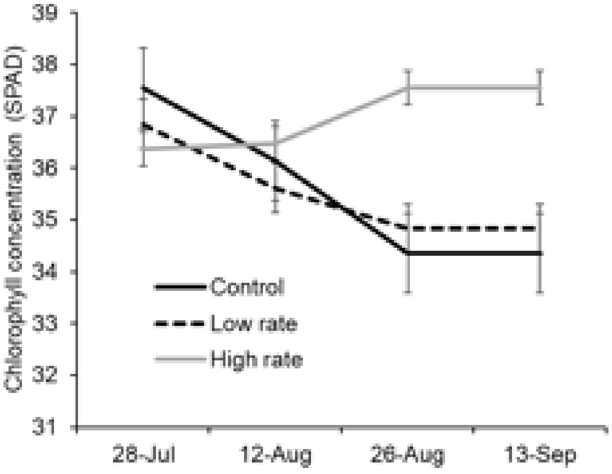

Figure 1. Average chlorophyll concentration (SPAD) of leaves in prune year plots of wild blueberry at no, low, or a high rate of glycine betaine in the summer of 2021.

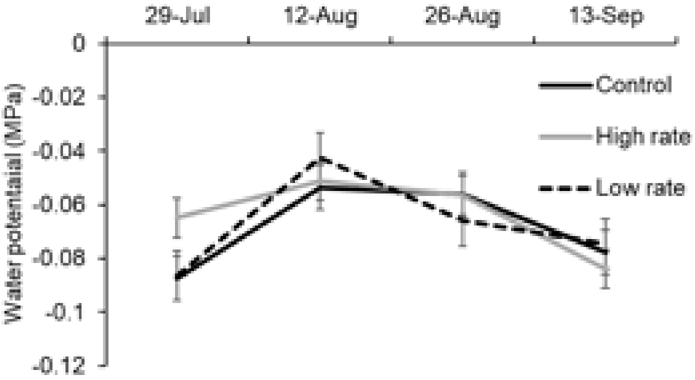

Figure 2. Average water potential measurements (MPa) of stems in prune year plots of wild blueberry at no, low, or a high rate of glycine betaine in the summer of 2021.

Looking at the impact of glycine betaine treatments on stem morphological characteristics, stem height was significantly different between different treatments ($F_{(2,470)}$ = 3.41, P = 0.031), with the low rate having significantly taller stems (P < 0.05, Tukey HSD) than the high rate (Figure 3); however, there was no significant difference between the low rate and control or the control and high rate (P >0.05, Tukey HSD). And, there was no significant difference in the number of leaf buds among different treatments ($F_{(2,175)}$ = 2.04, P = 0.132) and (X^2 = 5.30, df = 2, P = 0.070)

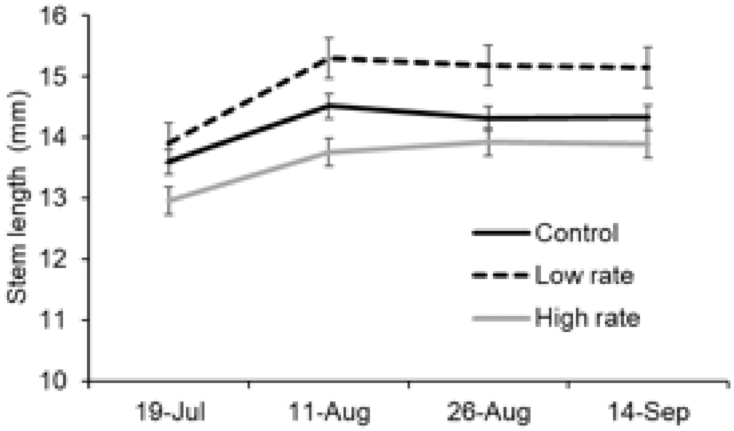

Figure 3. Average length (mm) of stems in prune year plots of wild blueberry at no, low, or a high rate of glycine betaine in the summer of 2021.

In the first greenhouse experiment in year 2, there were significant differences in chlorophyll data taken on 16 June, one week after drought was initiated ($F_{(3,44)}$ = 6.9185, P = 0.006) and 23 June, two weeks after drought was initiated (X^2 = 9.50, df = 3, P = 0.0233) (Figure 4). However, no significant differences were observed in the second round of experiments ($F_{(3,68)}$ = 0.4499, P = 0.5046).

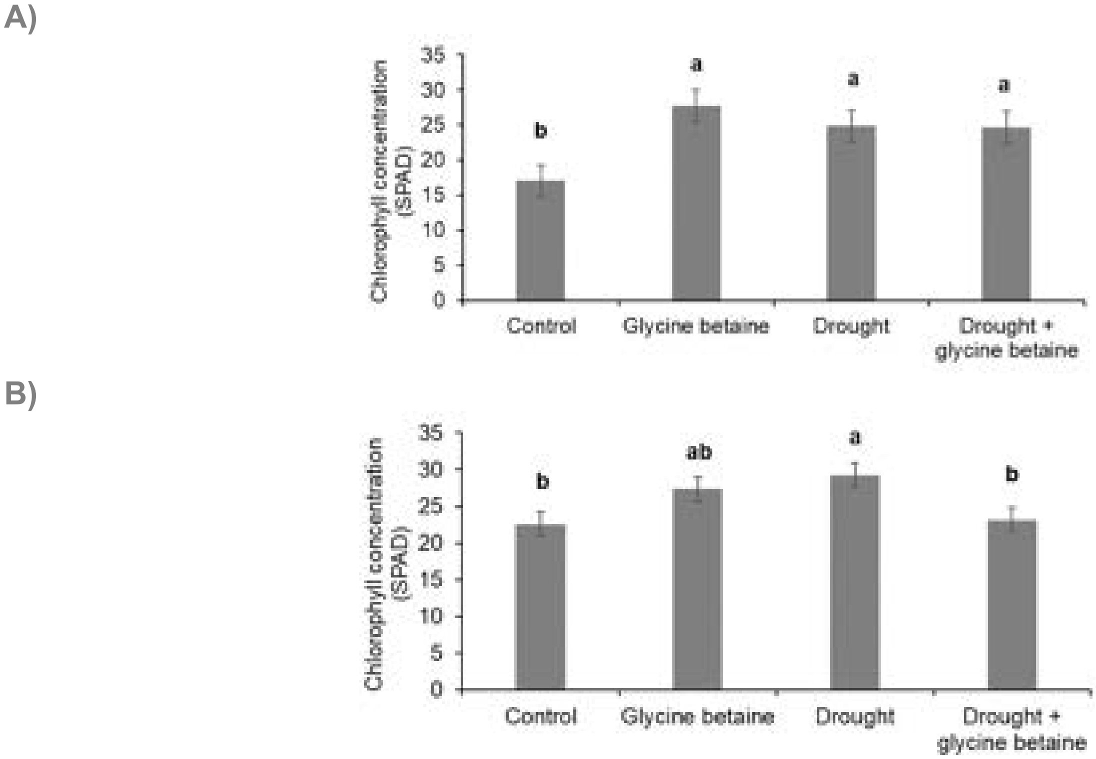

Figure 4. Average chlorophyll concentration (SPAD) of four genotypes of *Vaccinium angustifolium* subjected to different treatments of drought and glycine betaine. Summer 2022 round one measurements were taken on (A) 16 June, one week after drought, and (B) 23 June, two weeks after drought. The columns headed with the same letter are not significantly different (P < 0.05, Wilcoxon test).

In the first greenhouse experiment, treatments of glycine betaine and drought had no significant effect on the water potential of stems on 16 June, one week after drought (X^2 = 5.87, df= 3, P = 0.117). However, significant differences were observed on 23 June, two weeks after drought (X^2 = 14.59, df= 3, P = 0.002), with the drought + glycine betaine treatment having a significantly lower water potential than all other treatments (P > 0.05, (Figure 5). In the second experiment, while there was a significant difference in water potential between dates ($F_{(2,68)}$ = 46.752, P < 0.001), as plants became more drought-stressed, there were no significant differences between stems undergoing drought with or without glycine betaine ($F_{(3,68)}$ = 0.9719, P = 0.327) (Figure 6). In round one, glycine betaine-treated plants were the only plants to not drop below the turgor loss point (TLP) for both dates. For round two, drought + glycine betaine started above the TLP but dropped below on 19 July; drought-only plants remained under the TLP during the whole round.

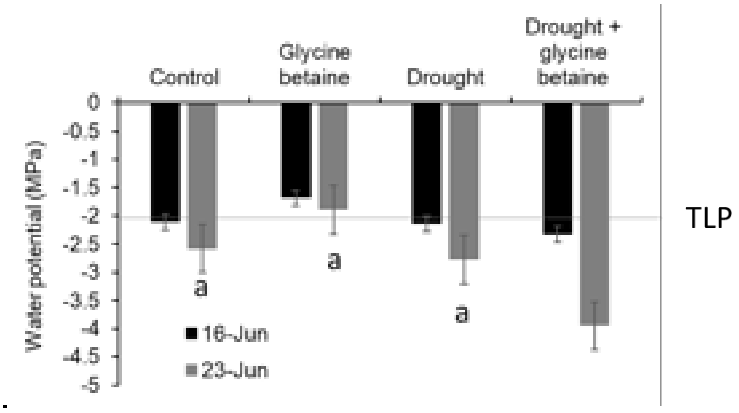

Figure 5. Average water potential (MPa) of four genotypes of *Vaccinium angustifolium* in crop year subjected to different treatments of drought and glycine betaine; summer 2022 round one. Columns headed with the same letter are not significantly different (*P* < 0.05, Wilcoxon test). The solid gray line represents the turgor loss point for lowbush blueberries (-2.0 MPa).

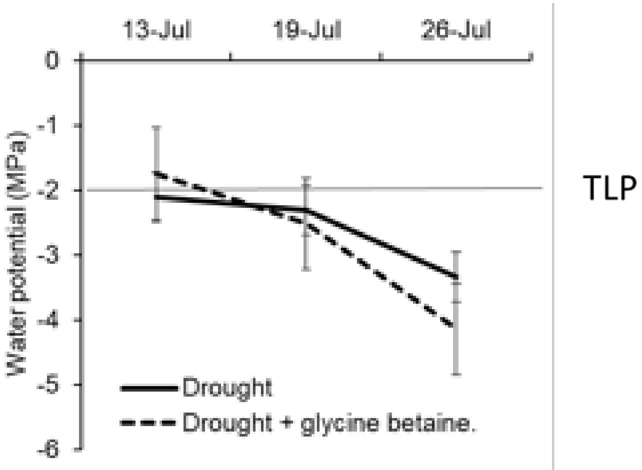

Figure 6. Average water potential measurements (MPa) of four genotypes of *Vaccinium angustifolium* in crop year subjected to different treatments of drought and glycine betaine; summer 2022 round two. The solid gray line represents the turgor loss point for lowbush blueberries (-2.0 MPa).

Table 1. Average (± S.E.) stomatal conductance for maximum and midday stomatal conductance (*gs*); max *gs* and midday *gs* in stems treated with different treatments of glycine betaine and drought in round one experiments. Columns with the same letter were not significantly different from each other (*P* < 0.05, Wilcoxon test).

	16-Jun								**23-Jun**							
Treatment	**Max *gs***				**Midday *gs***				**Max *gs***				**Midday *gs***			
Control	0.0342	±	0.005	a	0.0262	±	0.005	a	0.0214	±	0.004	ab	0.0089	±	0.002	ab
Glycine Betaine	0.0485	±	0.009	a	0.0501	±	0.015	a	0.0339	±	0.008	a	0.0153	±	0.004	a
Drought	0.0222	±	0.005	a	0.0239	±	0.007	a	0.0052	±	0.001	b	0.0040	±	0.001	b
Drought and Glycine Betaine	0.0354	±	0.012	a	0.0399	±	0.013	a	0.0085	±	0.003	b	0.0083	±	0.004	ab
Statistic	χ^2= 4.99, df= 3, *P* = 0.172				χ^2= 3.94, df= 3, *P* = 0.2679				χ^2= 18.75, df= 3, *P* = 0.0003				χ^2= 9.40, df= 3, *P* = 0.024			

Soil volumetric water content measurements were also conducted to determine if there was an impact of treatments on soil moisture and, thus, the uptake of water by plants. In the first experiment, there was a significant difference in soil moisture (X^2 = 9.73, df= 3, P = 0.020), with plants treated with glycine betaine having significantly higher soil volumetric water content than the drought and drought + glycine betaine treatment. However, it was not significantly different from the control treatment ($P < 0.05$, Wilcoxon test) (Figure 7). In the round two experiment, soil volumetric water content decreased significantly over time ($F_{(1,88)}$ = 26.20, $P < 0.001$). However, there was no impact of treatment on soil moisture ($F_{(2,88)}$ = 0.84, P = 0.3602) (Figure 8).

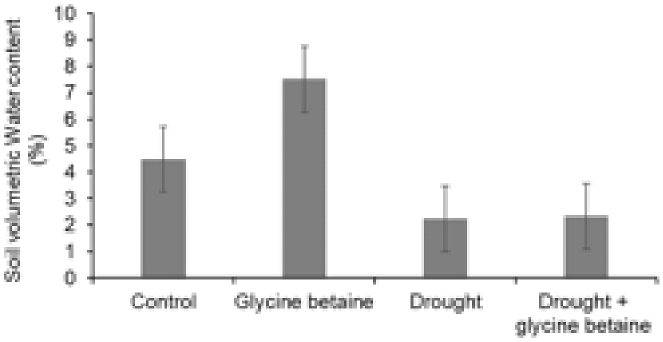

Figure 7. Average soil volumetric water content (%) of four genotypes of *Vaccinium angustifolium* in crop year subjected to different treatments of drought and glycine betaine; summer 2022 round one measurements taken on 23 June, two weeks after treatment. Columns headed with the same letter are not significantly different ($P < 0.05$, Wilcoxon test).

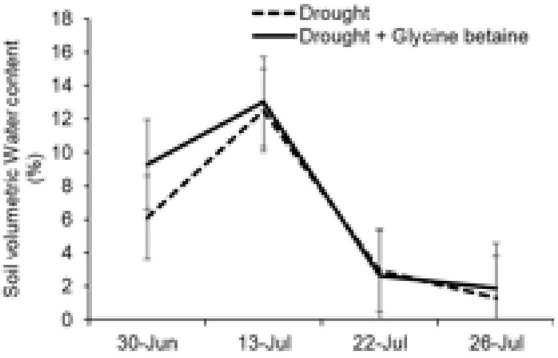

Figure 1. Average soil volumetric water content (%) of four genotypes of *Vaccinium angustifolium* in crop year subjected to different treatments of drought and glycine betaine; summer 2022 round two.

Looking at the daily evapotranspiration (change in weight), there were significant differences (X^2 = 31.15, df= 3, $P < 0.001$) among treatments in the first experiment, where the glycine betaine only plants had a significantly ($P < 0.05$, Wilcoxon test) higher transpiration rate than any other treatment (Figure 9). In the second experiment, there was a significant reduction in transpiration rate over time as plants became drought-stressed (X^2 = 4.24, df= 2, P = 0.039). However, there was no significant difference in transpiration between treatments (X^2 = 0.004, df= 1, P = 0.945) (Figure 10).

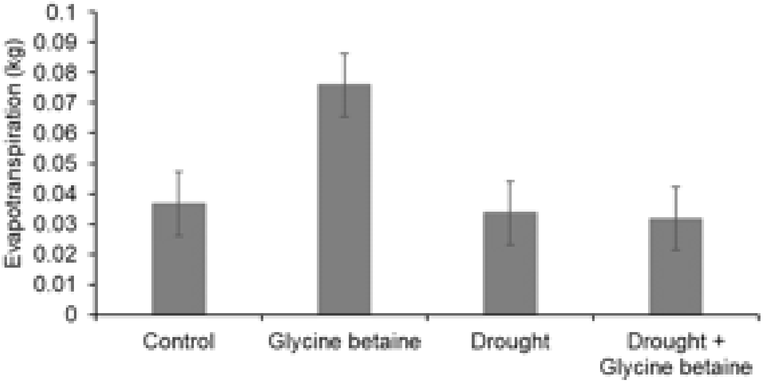

Figure 9. Average evapotranspiration (kg) of four genotypes of *Vaccinium angustifolium* in crop year subjected to different treatments of drought and glycine betaine; summer 2022 round one measurements were taken on 23 June, two weeks after drought was initiated.

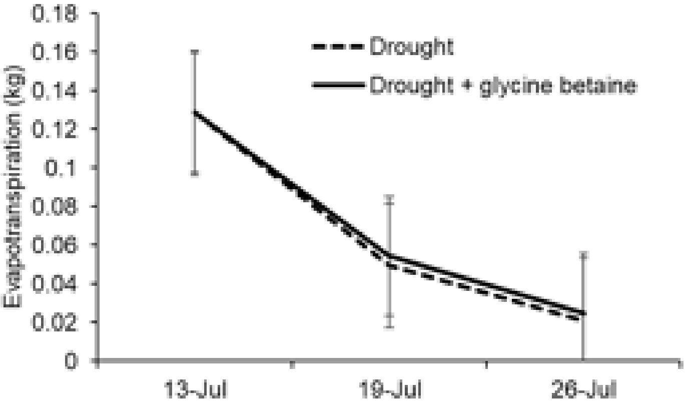

Figure 2. Average evapotranspiration (kg) of four genotypes of *Vaccinium angustifolium* in crop year subjected to different treatments of drought and glycine betaine; summer 2022 round two.

Looking at the impact of glycine betaine treatments on stem morphological characteristics, in the first experiment, the number of leaves was significantly different over time ($F_{(2,132)}$ = 11.65, P = 0.0008) and treatment ($F_{(3,132)}$ = 11.65, P = 0.0011) (Figure 11). Overall, stems from sods treated with glycine betaine had the highest mean number of leaves, which was significantly higher than stems that were drought treated; no significant differences were observed between the glycine betaine, control, and drought treatments. Similarly, there was a significant difference over time ($F_{(2,132)}$ = 17.02, P < 0.0001) and treatment ($F_{(3,132)}$ = 9.31, P < 0.0001) in the number of fruit (Figure 12). Overall, stems from sods treated with glycine betaine had the highest mean number of mature blue fruit, which was significantly higher than control stems. No significant differences were observed between the glycine betaine, drought, and drought + glycine betaine-treated stems.

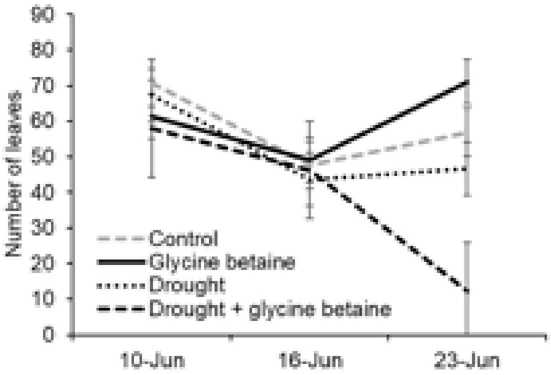

Figure 11. Average number of leaves of four genotypes of *Vaccinium angustifolium* in crop year subjected to different treatments of drought and glycine betaine; summer 2022 round one.

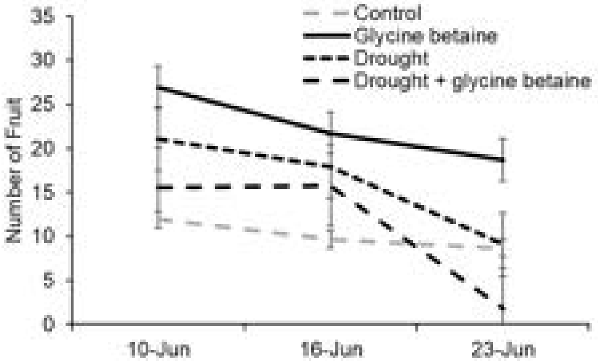

Figure 3. Average number of fruit for four genotypes of *Vaccinium angustifolium* in crop year subjected to different treatments of drought and glycine betaine; summer 2022 round one.

Table 2. Least square mean values for the number of leaves and fruit on stems treated with different treatments of glycine betaine and drought. Rows within columns with the same letter indicate no significant difference (Tukey HSD, α = 0.05)

Treatment	**No. of leaves**		**No. of fruit**	
Control	60.5	a	10.0	b
GB	58.4	a	22.4	a
Drought	52.5	ab	16.0	ab
Drought + GB	38.8	b	11.0	b

In the second experiment, the percent of defoliated stems was quantified; there was some background defoliation in buckets, but this was not significantly different across treatments. Once the drought treatment was initiated, the percent defoliated stems increased significantly over time ($F_{(3,88)}$ = 52.266, P < 0.0001); however, there was no significant difference in treatments ($F_{(1,88)}$ = 0.6733, P = 0.4140) (Figure 13).

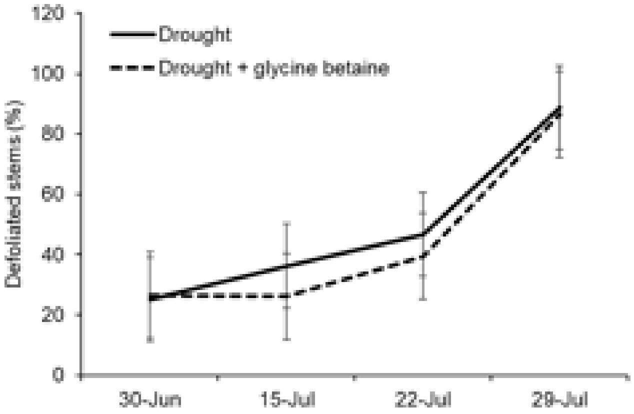

Figure 4. Average defoliated stems (%) of four genotypes of *Vaccinium angustifolium* in crop year subjected to different treatments of drought and glycine betaine; summer 2022 round two.

DISCUSSION

Though this study did not find glycine betaine applications to work well under drought conditions, it provided interesting data suggesting it could work well under well-watered conditions. This study did not match the results of other studies that found glycine betaine worked at alleviating abiotic stresses like drought. The difference between these studies, though, is that the plants used in other studies did not fall under the *Vaccinium* genus (Chen & Murata, 2008; Giri, 2011). Another reason glycine betaine might not have worked as well under drought conditions for *Vaccinium angustifolium* is that *Vaccinium* is not a natural accumulator of glycine betaine; it may have different physiological structures to combat drought that are not compatible with glycine betaine. Glycine betaine did, however, perform significantly better in multiple measurements like chlorophyll concentration and transpiration rates, when irrigated compared to other treatments. This could be due to the enhanced capacity to maintain turgor pressure seen in previous studies. For wild blueberry plants with conservative water use and using stomatal closure to save water during drought, lowering turgor loss point and keeping plants transpiring could cause damage to plants.

CURRENT RECOMMENDATIONS

Further studies should be done examining the impact of glycine betaine applications on wild blueberry growth and yield under well-watered conditions. A field study completed over multiple years comparing applications in both irrigated and non-irrigated fields should be done. The year one study indicated that there were differences between application rates, so understanding the optimal application rate would be of value to the grower. It would also be beneficial to account for genotypic differences to know if this product would work well for a majority of the field. This study provided information suggesting that glycine betaine applications do not help wild blueberries during drought but could have other potential benefits. This study, along with future studies, could improve the marketing of the product, and better inform growers of the potential use of the product.

ACKNOWLEDGEMENTS

We thank Judy Collins, Eric Desbois, Serena Leonard, and Dominic Crowley for their assistance with this study.

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INVESTIGATORS: Ali Bello & Rachel Schattman (University of Maine Agroecology Lab)

5. Past, present and future dynamics of wild blueberry production in Maine under precipitation scenarios

LOCATION: The study was conducted at an unheated high tunnel located in the nursery section at Roger Clapp Greenhouse, University of Maine (44.8975° N, 68.6689° W).
PROJECT TIMEFRAME: May 2022 - October 2023

OBJECTIVE

The major objective is to determine the effects of interannual and long-term precipitation variability in Maine wild blueberries by the end of 21st century.

INTRODUCTION

Changes in the distribution, duration, and amount of rainfall have been observed over the past several decades, and are expected to continue in the future, potentially having a considerable impact on agricultural crop yields and quality (IPCC, 2021). Historical precipitation observations from the Parameter-elevation Regression on Independent Slope Model (PRISM) have shown a change in daily precipitation observations from the wild blueberry (*Vaccinium angustifolium*) production regions of Maine over the past 40 years, which will likely intensify under a warming climate as we progress through the 21st century (Tasnim et al., 2021). Additionally, the region is projected to experience a 3 to 5^{o}C increase in average annual temperature by the end of the 21st century (Wolfe et al., 2018; Tasnim et al., 2020). Therefore, we designed this project to use precipitation modeling and simulations to predict the impact of changing precipitation patterns on Maine wild blueberries. The results of this study will highlight whether changes in irrigation management practices are necessary, and the consequences (if any) of conducting business as usual in the coming decades.

METHODS

Wild blueberry plants were harvested from Jasper Wyman & Sons in Cherryfield, Maine on May 6, 2022, and June 10, 2022. Plants were selected based on their physiological, morphological, and phenotypic differences, as observed in the field. Ten sections (transplants) measuring approximately 10 inches by 10 inches were harvested from each "parent plant" and transported to the University of Maine, Orono campus. Six parent plants were selected for this study, with transplants randomly assigned to the experimental treatments described in the following paragraph. These transplants were placed upon ~8" of gravel in 2-gallon buckets, and then watered daily for 2 weeks prior to the start of the study, in order to minimize transplant shock and ensure adequate establishment. We credit the success of the transplant process with this regular watering prior to the beginning of the experiment, as well as the amount of O horizon ("duff") harvested with the transplants (~3-5 inches for each plant). The study utilized a complete random design (CRD) approach and consists of three plausible precipitation scenarios. These scenarios are designed to be representative of two past and one probable future growing season precipitation patterns in Maine (Schattman et al., 2022). The three precipitation scenarios were used to develop treatment schedules, which dictated the amount of daily watering on wild blueberry plants via irrigation system. These schedules were created based on precipitation observations for the wild blueberry growing season (May 1st - October 30th) for the years 2001 and 2006, from Jonesboro, Maine (44.6454°N, 67.6495°W; elevation 194 ft; record period 1991-2020) obtained from the PRISM dataset. Figure 1 shows the cumulative simulated rainfall in the three treatments.

The precipitation scenarios were as follows:

1. **HistDry (PRISM 2001):** 2001 was a dry year on record in the early 21st century. It was characterized by very low and infrequent rainfall separated by prolonged periods of dryness. Within the range of May – October, 132 consecutive dry days (no rain) were recorded. The maximum amount of daily rainfall recorded was 3.5052 cm (1.38 inches) on 18 May, and the total May – October rainfall = 31.7944 cm (12.53 inches).

2. **HistWet (PRISM 2006):** Based on 2006 observations, there were frequent and well-distributed rainfall events throughout the growing season, without any prolonged period of dryness. Within the range of May - October, rainfall exceeding 2.54 cm (1 inch) threshold occurred on eleven (11) separate days. The total May – October rainfall = 92.6174 cm (36.5 inches).

3. **AmpDry2.915:** Modified from observed 2001 precipitation record – daily values were multiplied by a factor of 2.915 to increase the total growing season rainfall to match that observed in 2006. This plausible future scenario is intended to simulate Maine's growing season precipitation patterns as they may occur by the middle or end of 21st century.

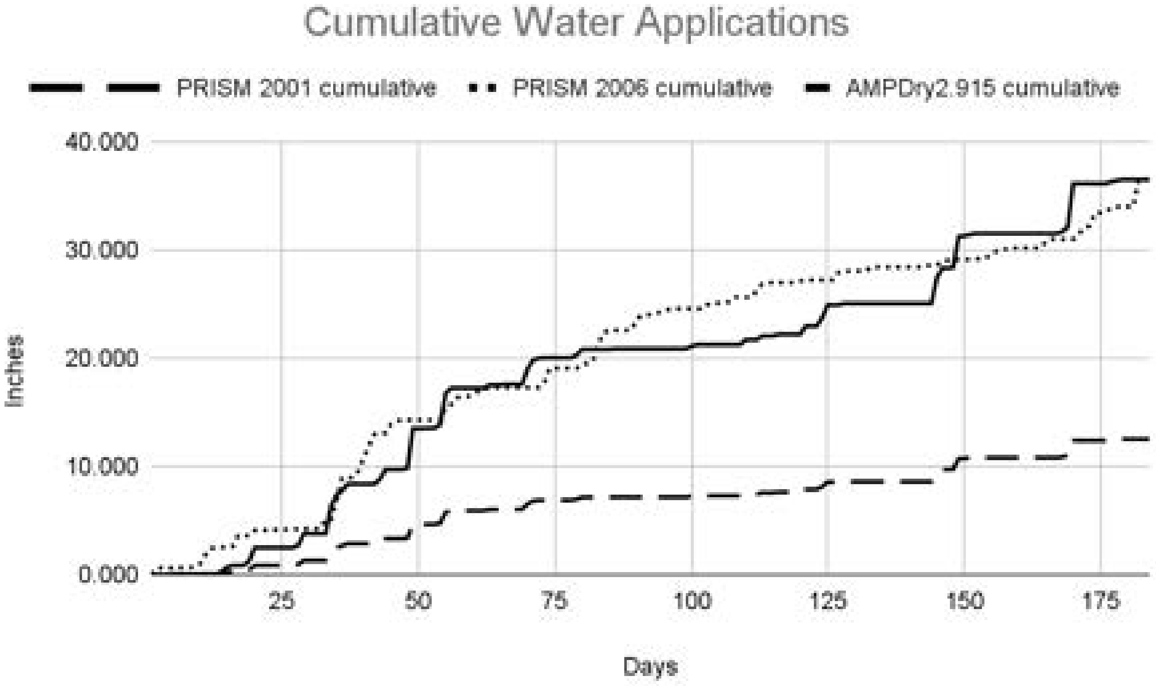

Figure. 1. Cumulative water applications across three experimental treatments (PRISM 2001, PRISM 2006 and AmpDry2.915)

Data Analysis

Kruskal-Wallis and Analysis of Variance (ANOVA) tests were conducted using R statistical software (R Foundation for Statistical Computing, Vienna, Austria) to test and compare the overall performance of several functional traits (leaf chlorophyll concentration, leaf chlorophyll fluorescence and leaf temperature), structural traits (stem length, number of branches and number of leaves per stem) and soil conditions (soil moisture content, soil temperature and soil electrical conductivity). Correlation coefficients were also used to determine the relationship between some dependent variables.

RESULTS AND DISCUSSION

Structural traits

Number of leaves per stem, number of branches per stem and stem length

Leaves are the most important organs of photosynthesis in wild blueberries, while stem length and stem diameter are important drought stress indicators and can also be used to determine the susceptibility of wild blueberries to frost damage and mechanical injury. A series of Kruskal-Wallis tests were used to compare the differences between independent variables on continuous dependent variables. The treatment designed to simulate future conditions (Amp2.915) had significantly more leaves per stem and greater stem diameter than HistDry and HistWet treatments. This suggests that the changes in seasonal total amount of precipitation may increase the stem length and leaf production of wild blueberries in future, as long as seasonal precipitation remains evenly distributed across the growing season.

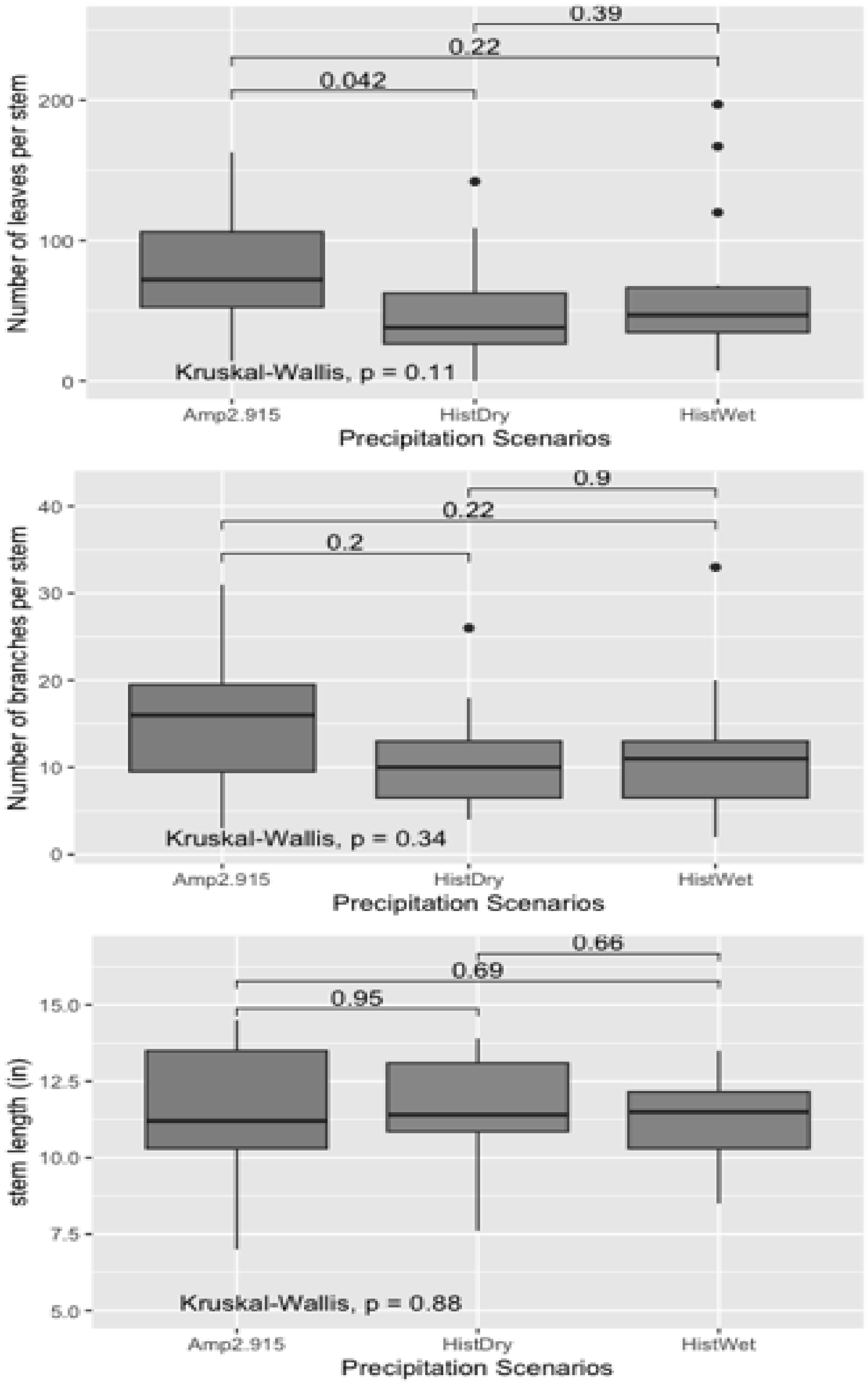

Figure 2. Structural variations in stem length, number of leaves, and number of branches over the course of the experiment.

Functional traits

Leaf chlorophyll concentration and leaf chlorophyll fluorescence

Leaf chlorophyll concentration, which is a measure of the photosynthetic rate of mature leaves of wild blueberries was assessed in the SPAD unit. ANOVA results show a statistically significant difference ($p = 0.031$) between Amp2.915 and HistDry treatments, suggesting that drought periods reduce photosynthetic rates of wild blueberries. Leaf chlorophyll fluorescence, which is measured in Fv/Fm, is used to determine the level of stress in wild blueberry plants. Although there was no significant difference observed between Amp2.195 and other treatments, a statistically significant difference was observed between HistDry and HistWet treatments, which also indicates that the seasonal drought leads to more plant stress.

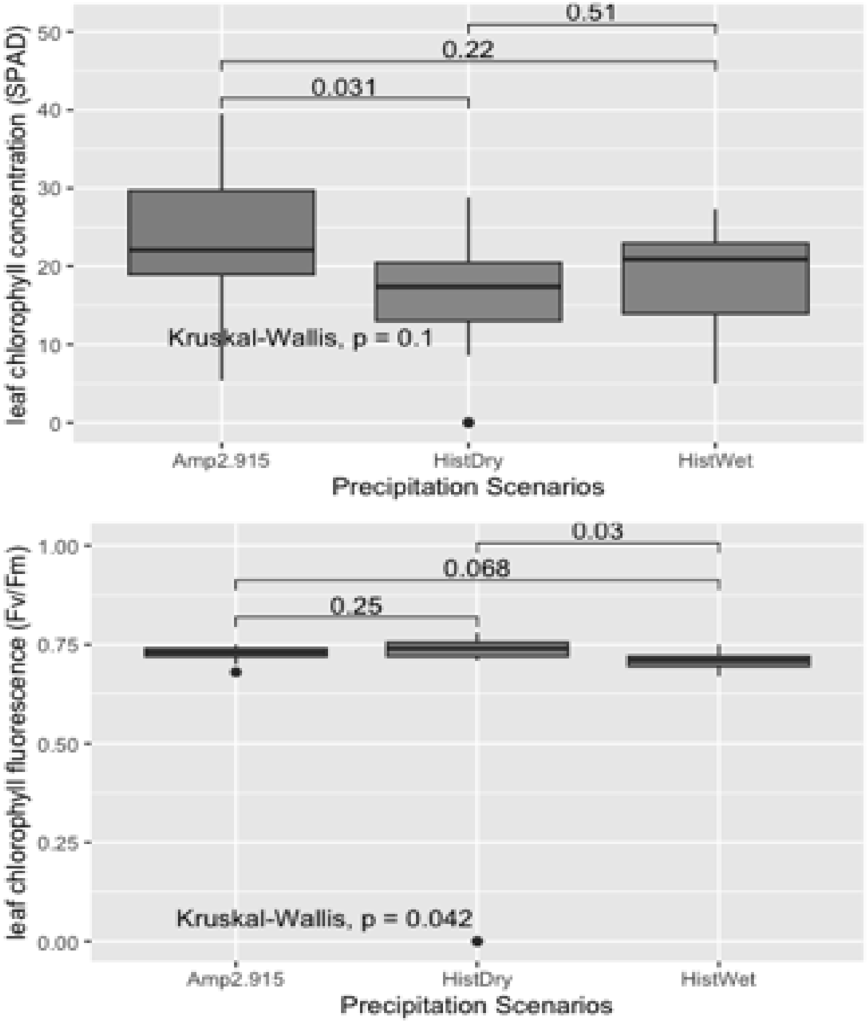

Figure 3. Differences in leaf chlorophyll concentration and leaf chlorophyll fluorescence (Fv/Fm) among experimental treatments

Edaphic components

Electrical conductivity, soil moisture content and soil temperature

Kruskal-Wallis tests were also used to determine the differences in treatment effects on soil electrical conductivity (EC), soil moisture content (SMC) and soil temperature. Soil EC measures the amount of nutrients available for plant uptake, while SMC measures the total amount of water available for plants to use in the soil. Soil temperature regulates the activities of beneficial soil microorganisms in the soils, with temperatures ranging from 77 to 86 degrees Fahrenheit (25 to 30 degrees Celsius) considered to be optimal for microbial abundance and diversity. (Pietikäinen et al., 2005). Statistical differences were found in both EC and SMC measurements, and HistWet was significantly higher. A Pearson's Rank Correlation Test confirmed that EC was strongly correlated with SMC (r = 0.716). These results suggest that higher annual rainfall that is evenly distributed across the growing season is the most conducive to high nutrient availability. Additionally, we observed a statistical difference in soil temperature, where

HistDry was significantly higher. This suggests that dry soils are more easily heated than wet or moist soils.

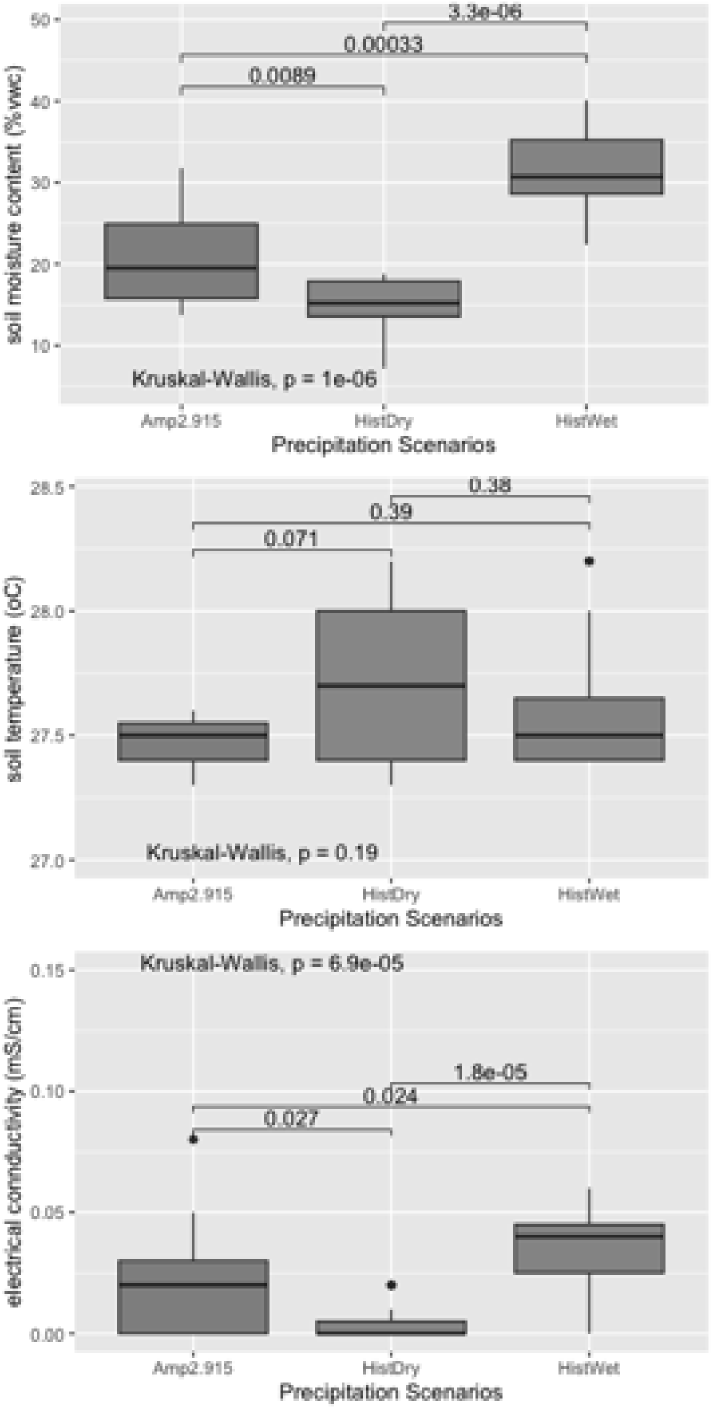

Figure 4. Variation in soil conditions among experimental treatments, including soil moisture content, soil temperature, and soil electrical conductivity

CURRENT RECOMMENDATIONS

In summary, an excess and evenly distributed rainfall across the growing season (as simulated through the HistWet treatment) led to reduced number of leaves and branches, with a relatively low chlorophyll concentration in the leaves. Lower average precipitation punctuated by seasonal drought leads to very low soil moisture content and electrical conductivity. High annual rainfall with uneven seasonal distribution, as simulated in the AMP2.915 treatment, however, leads to relatively low soil moisture content, along with very low soil temperature that may inhibit the water and nutrient uptake in wild blueberries. Therefore, growers will need to consider irrigation strategies that will ensure plants receive sufficient water, especially during droughts and dry periods.

NEXT STEPS

The results reported here are preliminary. The research will be repeated in the 2023 growing season, and extended to a field study in 2024, funding pending.

FUNDING

This work was supported by a research grant from the Wild Blueberry Advisory Committee.

ACKNOWLEDGEMENTS

We would like to thank Bruce Hall, Yongjiang Zhang, and Sean Birkel for their help and support. Special thanks to Jasper Wyman’s & Sons for helping us with wild blueberry transplants to conduct this research.

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INVESTIGATORS: L. Calderwood, S. Annis, YJ. Zhang, and R. Tasnim

6. Effects of Organic Soil Amendments on Physiology and Pests

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 the final report of the project discussed in the 2020 report, page 98, and the 2021 report, page 169, both entitled, "Effects of Organic Soil Amendments on Physiology and Pest Pressure".

Soil organic matter ("SOM") is of critical importance especially to organic growers of many crops, because SOM increases soil moisture, nutrient levels, provides habitat for micro- and macro-organisms, improving overall crop health. In organic wild blueberry production, growers should consider SOM to be a source of nutrients. Soil tests show SOM as a percentage, and for each 1% SOM the soil contains approximately 20 pounds of inorganic nitrogen and two pounds each of phosphorous, potassium, and sulfur available for plant use annually (Fernandez & Kaiser, 2021; McLean et al., 2021).

Wild blueberry growers prune the plant either by flail mowing or by burning, and both methods have advantages and disadvantages. Burning is an organic pest management tool for weeds, diseases and insects, yet can burn off organic matter in a hot, prolonged oil burn. Flail mowing allows for fallen material to accumulate SOM located in the organic pad layer, also known as the O horizon, yet mowing can spread pests (Warman, 1987; Ismail and Yarborough, 1981).

Currently, fertilizer is not typically applied to organic wild blueberry fields because fertilizer feeds weeds as well as the crop which leads to weed management issues. Weed presence is one of the factors impacting nutrient uptake in wild blueberry, along with soil pH, water availability, and nutrient availability (Drummond et al., 2009). Applying organic matter however, may boost crop productivity. Softwood mulch is now being applied across whole fields to increase SOM for water holding capacity. Mulching should be practiced by all organic growers to increase SOM for nutrient availability, soil water holding capacity, and for pest suppression (weeds and leaf spot disease). Until recently growers have applied mulch to suppress pests or aid wild blueberry rhizomes in colonizing bare patches caused by de-rocking or other disturbance (DeGomez & Smagula, 1990; Drummond et al., 2009) but ongoing research is exploring the benefits and practicality of mulching entire fields to improve soil moisture improvements and pest control (see page B26 in this report, "Using Soil Amendments to Improve Wild Blueberry Soil Moisture"). Research by Kender and Eggert (1966) demonstrates that some of the greatest benefits to mulched lowbush blueberry are not seen in the year following the application of mulch but five years after application. However, the 2022 mulching study indicates that you can see improved soil moisture the year that mulch is applied (see page B128 in this report, "Whole Field Mulching for Wild Blueberry Drought Management" for more information). Mulches and soil amendments must decompose somewhat before their constituent nutrients and materials can become available to plants, and this decomposition process requires time.

This report summarizes four years of organic research into the rates and timing of applications, cost, physiological benefits to wild blueberry, and potential impacts on pest presence under four different organic soil amendments and one organic foliar spray.

METHODS

This project was replicated at three farm locations selected to represent three organic 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 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
North American Kelp Co. 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	
North Country Organics Cheep Cheep 4-3-3	ALL	Granular Soil Applied	1089 lb/A	Low	Prune	4% N 3% P 3% K
			2178 lb/A	High	Prune	
Coast of Maine Cobscook 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	
Mark Wright Disposal 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	
University of Maine 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 June 10, 2022.

Physiology and Morphology

At each site, six stems from each plot were randomly selected to measure their leaf chlorophyll concentrations and photosynthetic electron transport rates on June 10 – 12, 2022. 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 June 10 – 12, 2022 between 10:00am and 2:00pm.

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 a LI-3000A area meter (Li-Cor, Lincoln, NE, USA). All the leaves from those eight stems were combined with other leaves from those eight stems, 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. Leaf mass per area (LMA) was calculated by dividing leaf dry mass by leaf area (g/m^{-2}).

Pest Presence

Insects, weeds, and disease were monitored in the same 0.37 m^2 quadrats (two 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. In 2022, pest scouting occurred in Appleton and Surry in May and June and at Columbia Falls in May and July.

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 2020 - 2022, 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.

In 2019, weeds were classified into two categories (grass or broadleaf) and in 2020 - 2022, weeds were identified by genera and counted to obtain weed number per quadrat. Insects were counted when an individual or their distinctive damage was observed. Diseases were similarly identified by distinctive characteristics. Fruit flies (BMF and SWD) were not quantified.

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. In the 2022 crop year, bud development, fruit-set, and fruit-drop were monitored with repeated measures on the same three stems per plot. The stems monitored in 2022 were not the same stems as were monitored in 2020.

The 2020 harvest took place on August 3, 6, and 11 in Appleton, Surry and Columbia Falls, respectively. The 2022 harvest took place on July 26, 28, and August 3 in Appleton, Surry, and Columbia Falls, respectively. In both 2020 and 2022, yield weights, Brix measures, and 100 berry counts were collected.

Data Analysis

The effects of the applied organic treatments on soil moisture, physiology (chlorophyll concentration and photosynthetic electron transport rate), and morphology (leaf size and leaf mass per area) 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 over 2 crop years (2020 and 2022).

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 – 2022) using a full-factorial repeated-measures mixed model design, followed by a Tukey's Pairwise Comparison in JMP (JMP®, Version 15.2, SAS, Cary, NC, USA). Here, the full factorial tested the effects of date, treatment, and any interaction between date and treatment for the ranked response variables. Additionally, crop phenology, harvest yield and berry quality measures were compared across the two crop years (2021 and 2022), full-factorial repeated-measures mixed model design.

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 were transformed with a square root transformation prior to any statistical testing. Ranked data and pest count data, as well as harvest yield and phenology 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. Quality measures of sugar content (Brix) and 100 berry counts were normally distributed and did not require transformation prior to analysis.

RESULTS

Soil Moisture

Overall, no significant differences were found in soil moisture among the treatments in any location (Figure 1). At Appleton, soil moisture was higher in the high rate of Cheep Cheep treatment than the control and other treatments. At Surry, average soil moisture was higher in the mulch treatments compared to the control and other treatments. At Columbia Falls, average soil moisture was higher in the mulch and both Cheep Cheep treatments compared to the control and other treatments.

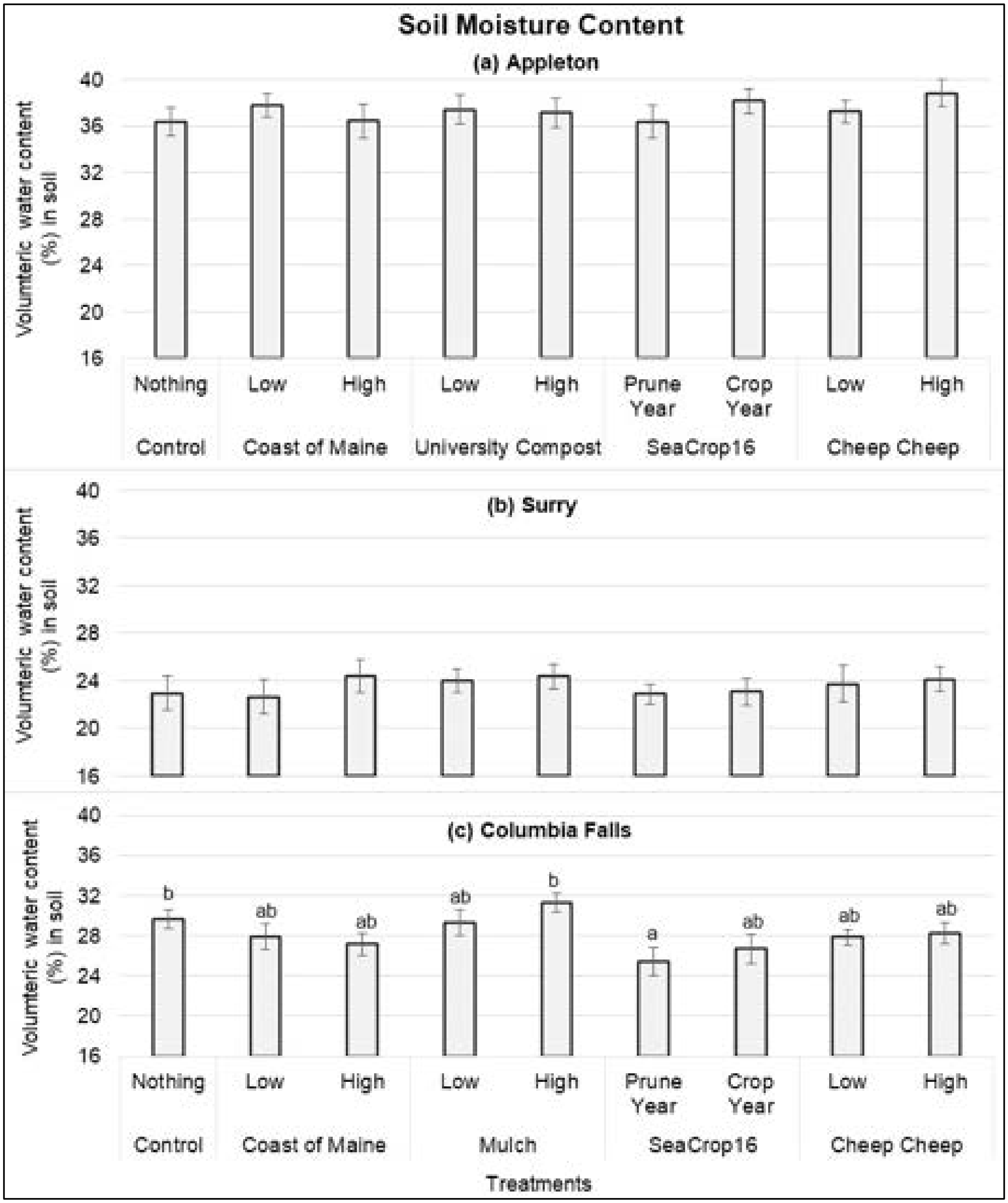

Figure 1. Comparison in soil moisture in June over two crop years (2020 and 2022) by treatments applied at: (A) Appleton, (B) Surry, and (C) Columbia Falls, Maine. Error bars indicate the standard error of the mean. No significant differences were observed at a significance level of *p* < 0.05.

Physiology and Morphology

Overall, no significant differences were found in leaf chlorophyll concentration among the treatments applied in any location (Figure 2). At Appleton and Surry, the average leaf chlorophyll concentration was higher in the high rate of Cheep Cheep compared to the control and other treatments. At Surry, average leaf chlorophyll concentration was lower in all treatments compared to the control. At Columbia Falls, average leaf chlorophyll concentration was higher in both rates of Cheep Cheep and the low rate of Coast of Maine compared to the control and other treatments.

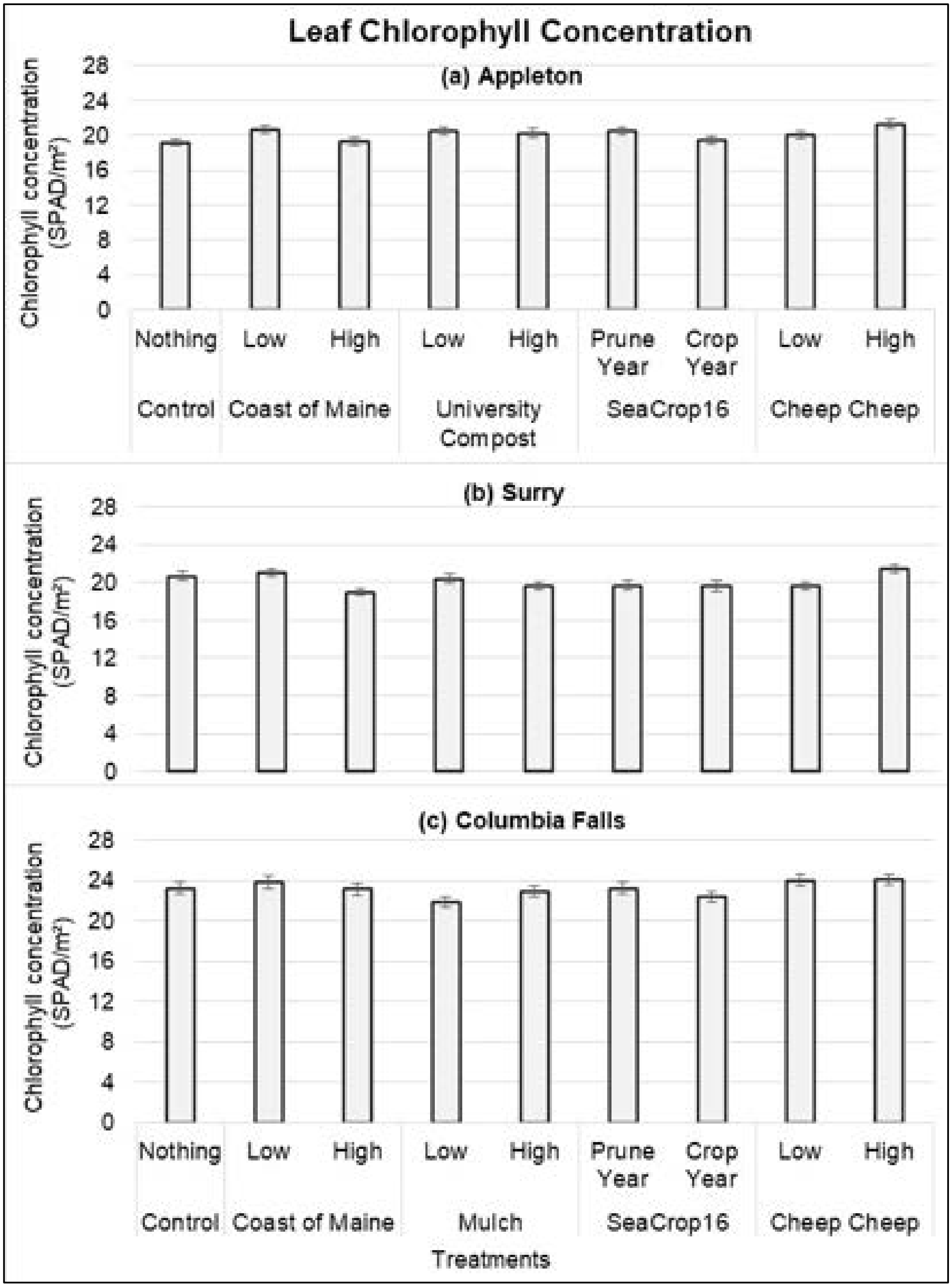

Figure 2. Comparison in chlorophyll concentration of leaves in June over two crop years (2020 and 2022) by treatments applied at: (A) Appleton, (B) Surry, and (C) Columbia Falls, Maine. Error bars

indicate the standard error of the mean (averaged over replicated plots). No significant differences were observed at a significance level of $p < 0.05$.

Overall, no significant differences were found in leaf photosynthetic electron transport rates among the treatments applied in any location (Figure 3). At Appleton, average leaf electron transport rate was higher only in the low rate of Coast of Maine treatment compared to the control and other treatments. By contrast, at Surry, average leaf electron transport rate was higher in all treatments except SeaCrop16 applied in the crop year. At Columbia Falls, average leaf electron transport rate was lower in all the treatments compared to the control.

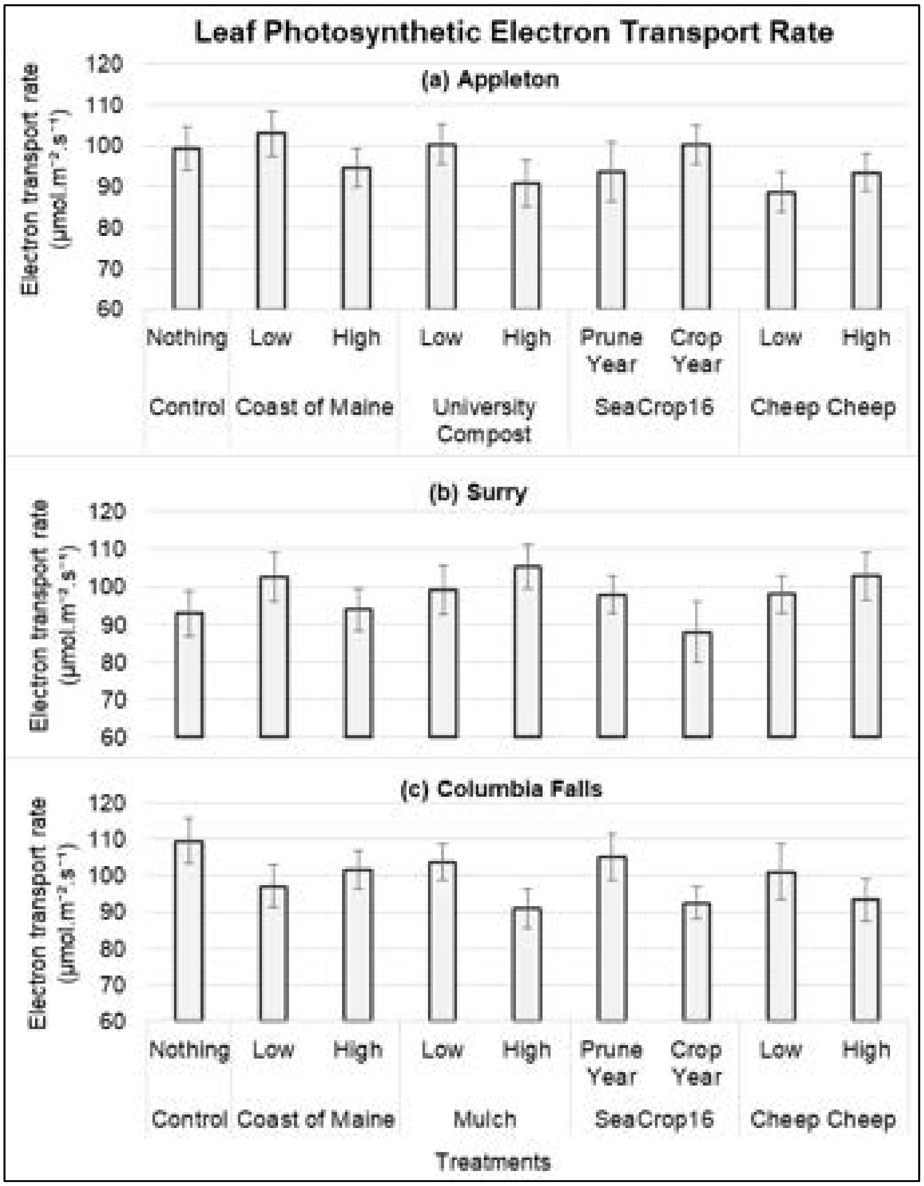

Figure 3. Comparison in photosynthetic electron transport rate of leaves in June over two crop years (2020 and 2022) by treatments applied at: (A) Appleton, (B) Surry, and (C) Columbia Falls, Maine. Error bars indicate the standard error of the mean (averaged over replicated plots). No significant differences were observed at a significance level of $p < 0.05$.

Overall, no significant differences were found in the wild blueberry leaf sizes among the applied treatments in any location (Figure 4). At Appleton, average leaf size was smaller in all treatments compared to the control. At Surry, both Cheep Cheep treatments had larger leaf sizes than the control and other treatments. At Columbia Falls, all treatments averaged larger leaf sizes than the control.

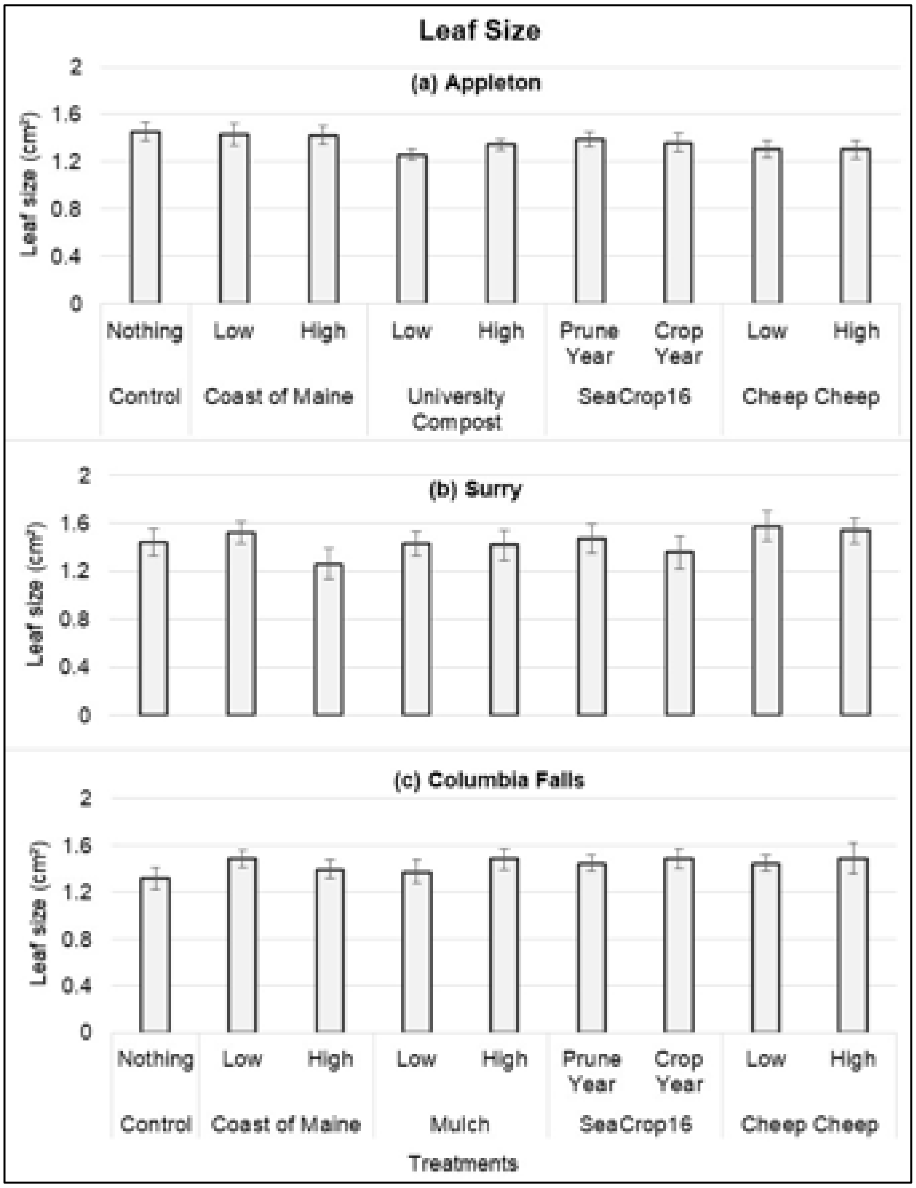

Figure 4. Comparison in leaf size in July over two crop years (2020 and 2022) by treatments applied at: (A) Appleton, (B) Surry, and (C) Columbia Falls, Maine. Error bars indicate the standard error of the mean (averaged over replicated plots). No significant differences were observed at a significance level of $p < 0.05$.

Overall, no significant differences were found in leaf mass per area (LMA) of the wild blueberry plants among the applied treatments in any location (Figure 5). At Appleton, average LMA was higher in the high rate of University Compost and high rate of Cheep Cheep compared to the control and other treatments. However, at Surry, average LMA was lower in all treatments compared to the control. At Columbia Falls, average LMA was higher in all treatments compared to the control.

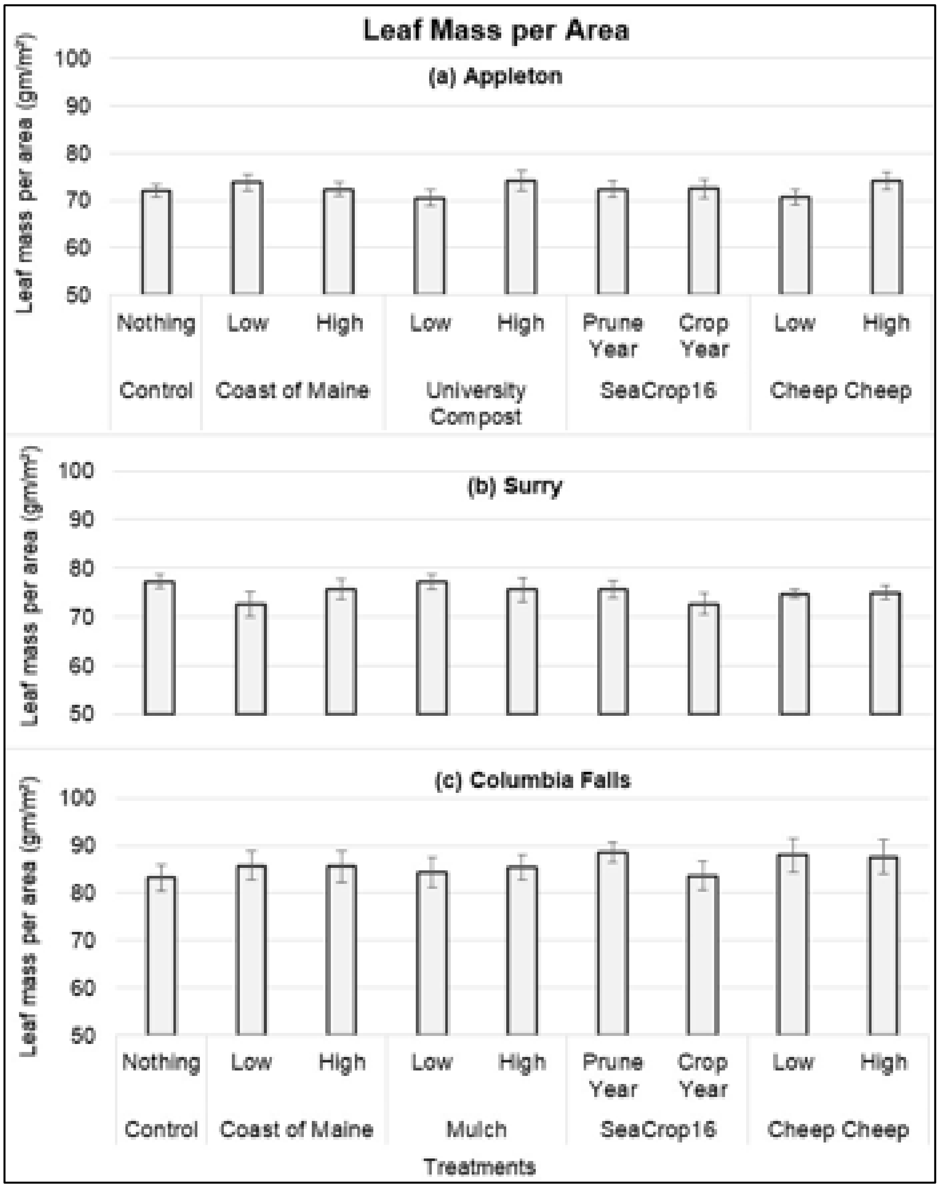

Figure 5. Comparison in leaf mass per area in July over two crop years (2020 and 2022) by treatments applied at: (A) Appleton, (B) Surry, and (C) Columbia Falls, Maine. Error bars indicate the standard error of the mean (averaged over replicated plots). No significant differences were observed at a significance level of $p < 0.05$.

Tables 2A-C. Soil characteristics among different soil amendment treatments compared to the optimum range in August 2022 at (A) Appleton, (B) Surry, and (C) Columbia Falls in Maine.

Table 2A. Appleton soil characteristics by treatment, as sampled on August 10, 2022.

Soil Characteristics	Optimum range	Control (No treatment)	Coast of Maine (Cobscook blend)		University compost		SeaCrop16		Cheep Cheep (Chicken manure)	
			Low	High	Low	High	Prune year	Crop year	Low	High
pH	4.0-4.5	4.8	4.8	4.7	4.7	4.8	4.3	4.3	4.5	4.3
Organic matter (%)	5-8	8.4	7	8.7	10	9	22.7	22.9	15	24.2
CEC (me/100 g)	>5	3	2.9	3.4	3.8	2.6	6.9	7	5.6	7.5
Nitrate-N (ppm)	20-30	1	1	1	1	1	1	1	1	1
Ammonium-N (ppm)	<10	2	3	2	2	2	6	6	4	6
Phosphorous (lb/A)	10-40	10.3	6.3	6.1	8.7	7	29.3	26.8	20	32.1
Potassium (% saturation)	2.1-3.0	3.9	4.4	2.6	4.1	5.2	4.8	5	3.9	4.6
Calcium (% saturation)	20-30	16.4	10.5	15.5	14	10.4	21.4	25.5	18.4	26
Magnesium (% saturation)	5-10	5.3	5.4	4.3	5.9	5.6	8.2	7.4	6.7	8.6
Sulfur (ppm)	>50	187	134	165	168	136	51	53	67	62
Copper (ppm)	0.25-0.6	0.2	0.13	0.18	0.20	0.18	0.4	0.27	0.31	0.59
Iron (ppm)	6-10	39	28	32	37	26	73	57	66	67
Manganese (ppm)	4-8	15	19	20	28	15	88	78	42	93
Zinc (ppm)	1-2	1	0.9	1.2	1.3	1.1	3.2	3.3	2.2	3.8
Boron (ppm)	0.5-1.2	0.6	0.5	0.5	0.6	0.5	0.8	0.7	0.5	0.8

Table 2B. Surry soil characteristics by treatment as sampled on August 4, 2022.

Soil Characteristics	Optimum range	Control (No treatment)	Coast of Maine (Cobscook blend)		Mulch		SeaCrop16		Cheep Cheep (Chicken manure)	
			Low	High	Low	High	Prune year	Crop year	Low	High
pH	4.0-4.5	5.1	4.6	4.8	4	4.3	4.6	5	3.8	4.3
Organic matter (%)	5-8	3.9	4.1	4.7	13.9	16	3	5.4	16.7	19.5
CEC (me/100 g)	>5	2.3	3.1	3.5	7	7.2	3.7	2.4	7.4	10.6
Nitrate-N (ppm)	20-30	1	1	1	1	1	1	1	1	1
Ammonium-N (ppm)	<10	1	1	1	2	4	1	1	3	5
Phosphorous (lb/A)	10-40	4.7	6.8	5.5	13	18.9	7.9	6.1	15.1	18.5
Potassium (% saturation)	2.1-3.0	2.5	1.9	2	3.1	3	1.9	2.4	2.9	2.5
Calcium (% saturation)	20-30	27.6	8.7	19.1	24.5	30.3	17.8	10.1	21	43.3
Magnesium (% saturation)	5-10	4.6	2.3	4.2	5	5.2	3.2	2.8	3.6	7.5
Sulfur (ppm)	>50	80	57	78	43	59	23	165	32	27
Copper (ppm)	0.25-0.6	0.04	0.09	0.09	0.21	0.2	0.09	0.09	0.13	0.22
Iron (ppm)	6-10	13	21	26	28	25	33	20	19	28
Manganese (ppm)	4-8	5.3	5	7.7	34	39	4.6	3.1	19	59
Zinc (ppm)	1-2	1.1	1.2	2.2	3.5	6.6	1.7	1	2.6	7.2
Boron (ppm)	0.5-1.2	0.4	0.4	0.4	0.6	0.6	0.4	0.4	0.6	0.6

Table 2C. Columbia Falls soil characteristics by treatment as sampled on August 4, 2022.

Soil Characteristics	Optimum range	Control (No treatment)	Coast of Maine (Cobscook blend)		Mulch		SeaCrop16		Cheep Cheep (Chicken manure)	
			Low	High	Low	High	Prune year	Crop year	Low	High
pH	4.0-4.5	4.8	4.7	4.9	4.9	4.7	5	4.8	5.1	4.7
Organic matter (%)	5-8	5.4	12.4	7.5	13.2	5.1	4.4	7.5	5	11.1
CEC (me/100 g)	>5	3.7	6.2	3.7	5.8	3.7	2.3	2.6	2.6	6
Nitrate-N (ppm)	20-30	1	<0.5	<0.5	1	<0.5	<0.5	1	1	<0.5
Ammonium-N (ppm)	<10	1	3	3	3	2	2	2	2	3
Phosphorous (lb/A)	10-40	8.5	12.1	13.7	7.9	6.3	6.9	8.8	9.4	12.2
Potassium (% saturation)	2.1-3.0	2.9	2.5	4.2	3.1	2.7	3.4	3	3.7	3
Calcium (% saturation)	20-30	14.4	27.7	19.1	33.1	13.3	5.6	5.6	8.6	33.6
Magnesium (% saturation)	5-10	5.9	10.4	12.5	9	6.1	3.4	3.7	5.2	8.1
Sulfur (ppm)	>50	84	67	88	45	91	74	80	112	51
Copper (ppm)	0.25-0.6	0.11	0.14	0.13	0.14	0.28	0.10	0.12	0.11	0.13
Iron (ppm)	6-10	30	27	31	42	26	22	39	21	28
Manganese (ppm)	4-8	4.2	9	4.6	22	4.7	1.8	1.8	2.9	11
Zinc (ppm)	1-2	1	3.7	1.5	2.9	1.3	0.6	0.8	0.6	3.2
Boron (ppm)	0.5-1.2	0.4	0.5	0.5	0.5	0.4	0.4	0.4	0.3	0.4

Treatments including all rates and timings of Cheep Cheep, Coast of Maine Compost, and SeaCrop16 improved blueberry cover more than did the control (Figure 6). Blueberry cover ranged from 64% in the control to 68% in the treatments where SeaCrop16 had been applied (both prune and crop). Blueberry cover in treatments where Cheep Cheep had been applied (67% at both rates) was close to the cover under SeaCrop16, 68%.

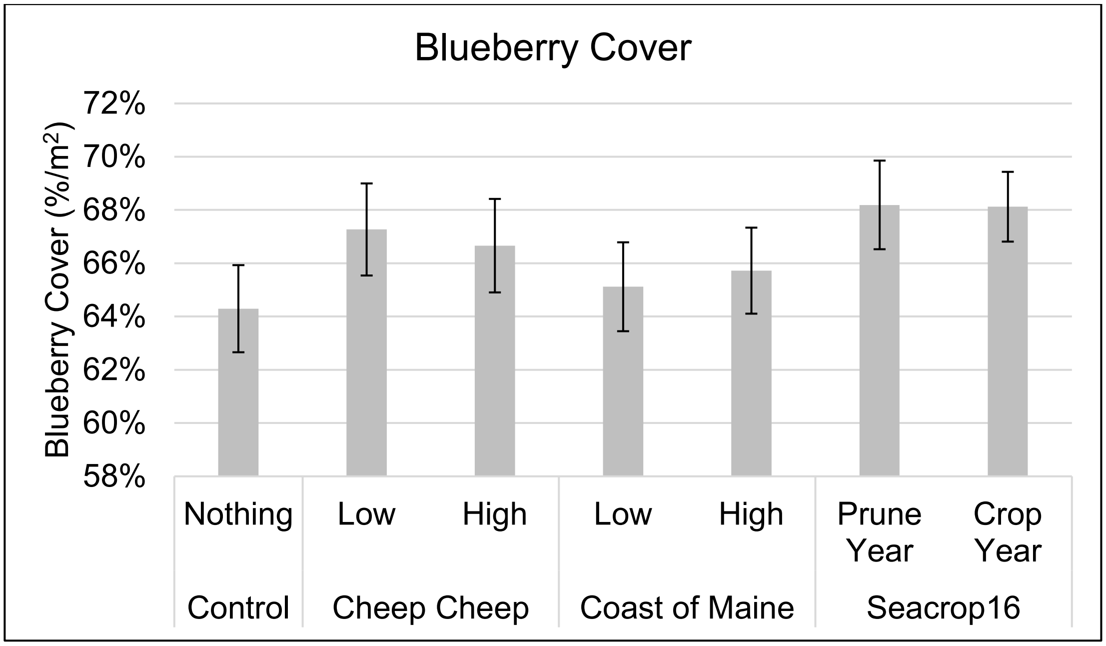

Figure 6. Average blueberry cover (%/m^2) measured across all three locations (Appleton, Surry and Columbia Falls) over four years (2019 – 2022) by treatments. Treatment differences were not significant. Error bars indicate the standard error of the mean.

The average number of green fruit per stem was averaged across both crop years, 2020 and 2022. The most green fruit were observed in the high rate of Cheep Cheep (8.13 green fruit/stem), and the fewest green fruit were observed in the high rate of Coast of Maine (7.00 green fruit/stem). The control averaged 7.62 green fruit/stem, which was only slightly higher than the crop year application of SeaCrop16, the low rate of Cheep Cheep, and the high rate of Coast of Maine.

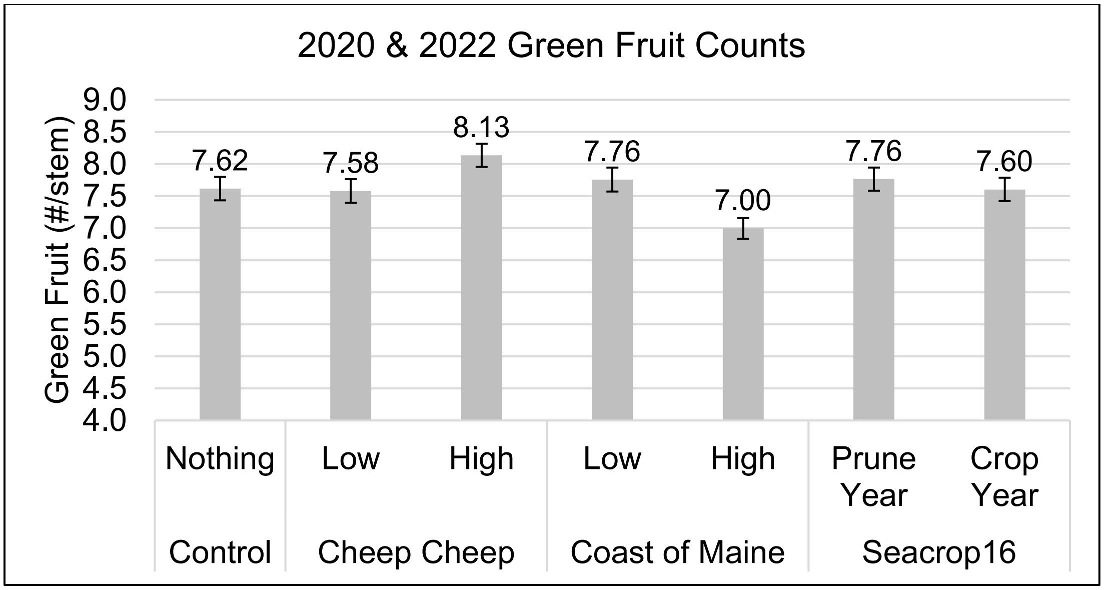

Figure 7. Average green fruit number (#/stem) measured across all three locations (Appleton, Surry, and Columbia Falls) over both crop years (2020 and 2022) by treatments. Treatment differences were not significant. Error bars indicate the standard error of the mean.

Pest Presence
Treatment differences in weed presence (#/m^2) over three years of this study (2020 – 2022) were not significant, however, interesting trends were present (Figure 8). Weed presence was highest under high rate of Cheep Cheep (#/m^2), followed by low rate of Cheep Cheep (#/m^2), and low rate of Coast of Maine (#/m^2). Weed presence was lowest where SeaCrop16 was applied during the crop year.

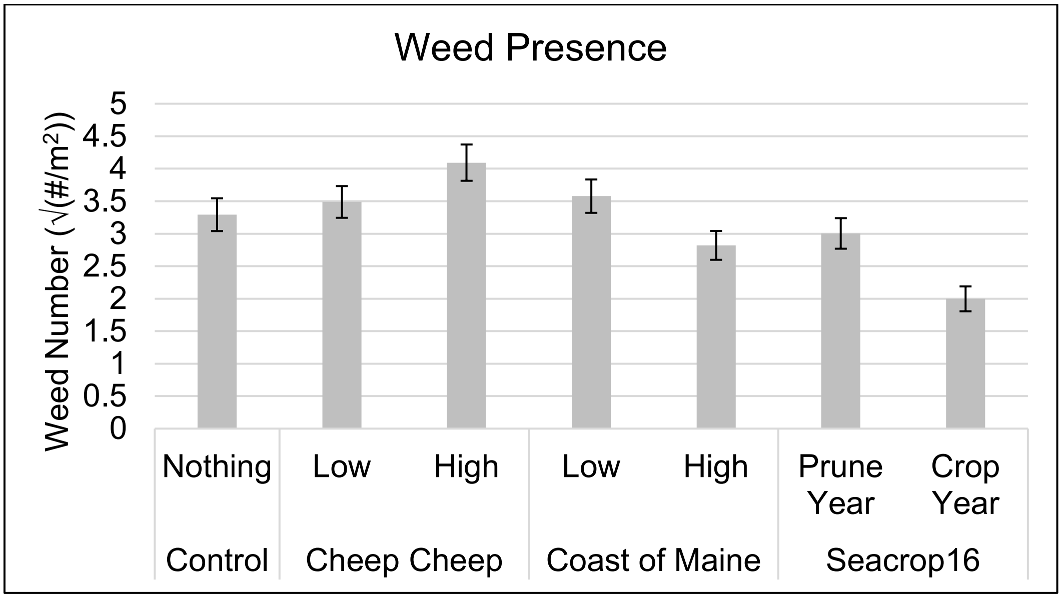

Figure 8. Average weed number (transformed; √(#/m^2)) measured across all three locations (Appleton, Surry, and Columbia Falls) over three years (2020 – 2022) by treatments. Treatment differences were not significant. Error bars indicate the standard error of the mean.

Site-specific differences were analyzed to compare the efficacies of University Compost (only applied at Hope) and mulch (applied at Columbia Falls and Surry; Figure 9). At Hope, the high rates of Coast of Maine and University Compost both had significantly lower weed cover than the control. Interestingly, at the Columbia Falls and Surry locations, the SeaCrop16 applied in the crop year and high rate of mulch had significantly lower weed cover than the control.

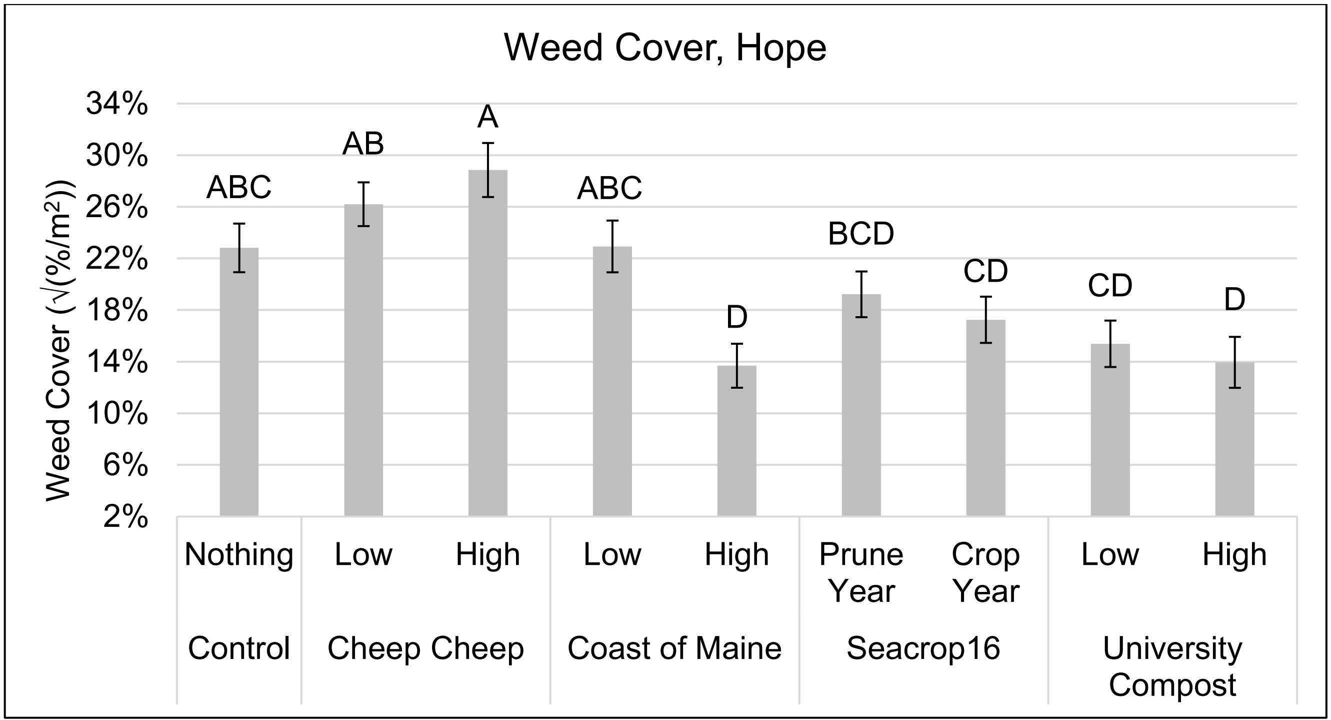

Figure 9. Average weed cover (transformed; √(%/m²)) measured in Hope, ME over four years (2019 – 2022) by treatment. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean. University Compost treatment included for comparison to all treatments per location.

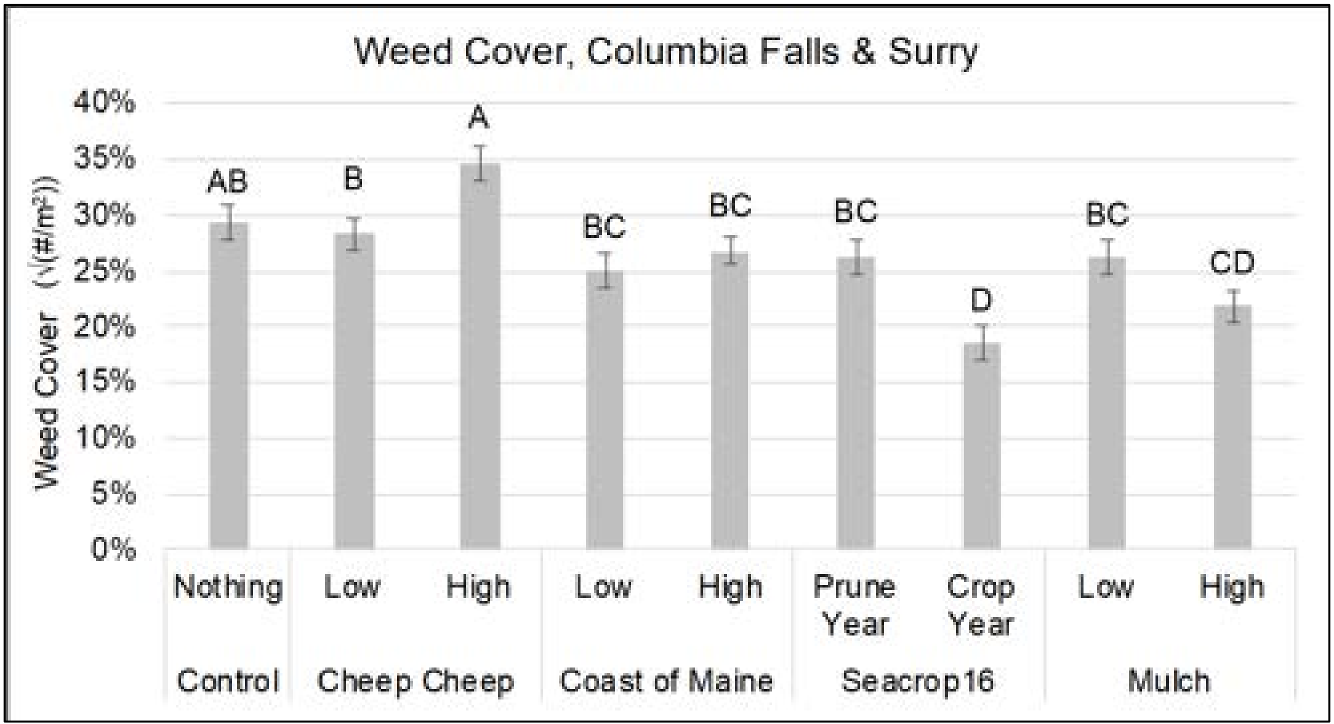

Figure 9. Average weed cover (transformed; √(%/m²)) measured in Columbia Falls and Surry, ME, over four years (2019-2022) by treatment. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean. Mulch treatment included for comparison to all treatments per location.

Insect coverage, a spatial measure of insect presence generally indicated by pest damage to leaves or observation of the actual culprit, was significantly higher in the low rate of Cheep Cheep (13%/m^2) relative to the control (10%/m^2; Figure 10). Over the four years of study, all other treatments were not significantly different from one another. Top insects included: tip midge, red striped fireworm, and flea beetle. Disease coverage, including leaf spot species, mummy berry, and phomopsis, as indicated by a spatial measure of disease presence, was relatively similar across all treatments except for the high rate of Cheep Cheep (12%/m^2), which exhibited significantly less disease presence than the control (14%/m^2; Figure 11).

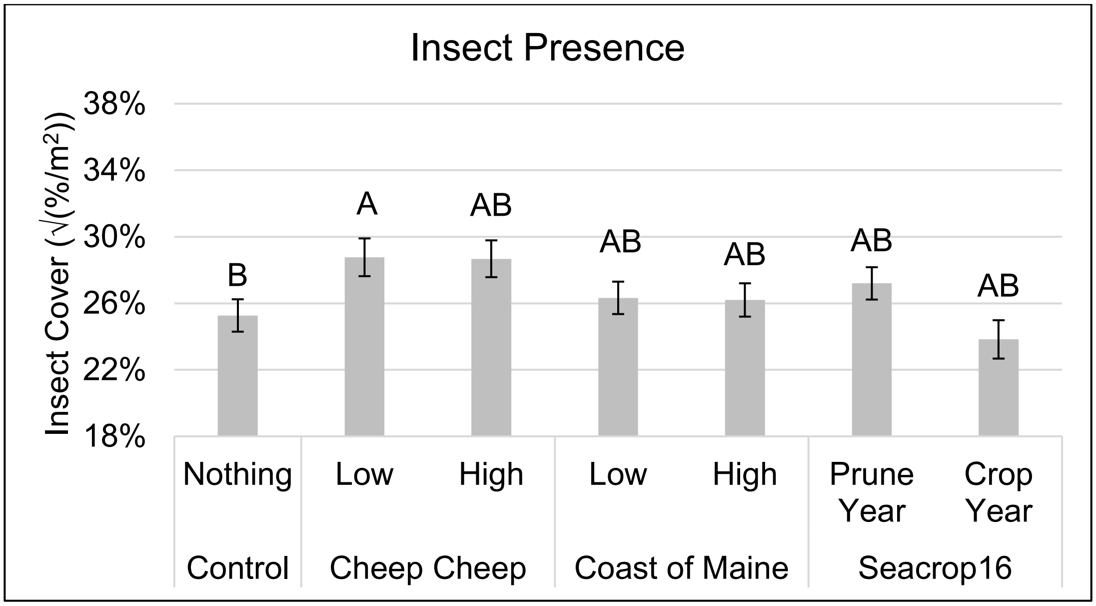

Figure 10. Average flea beetle, red striped fireworm, and tip midge insect pest coverage (transformed; √(%/m^2)) measured across all three locations (Appleton, Surry, and Columbia Falls) over four years (2019 – 2022) by treatment. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean.

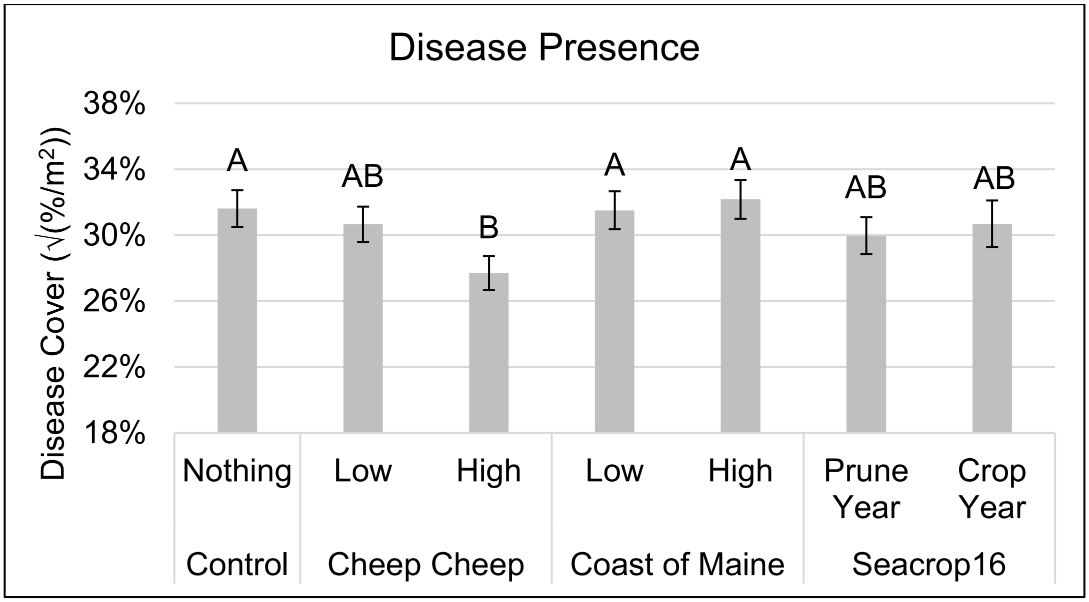

Figure 11. Average mummy berry and leaf spot disease cover (transformed; √(%/m^2)) measured across all three locations (Appleton, Surry, and Columbia Falls) over four years (2019 – 2022) by treatment. Letters indicate significance at the 0.05 level of significance. Error bars indicate the standard error of the mean.

Crop Productivity

Treatment differences in harvest yield were not significant across both crop years (2020 and 2022) and all locations, however, there are interesting trends worth noting (Figure 12). Blueberry yields were higher in the quadrat subsamples compared to whole plot yields. Whole plot yields are more susceptible to variation between clones and the physical loss that occurs when harvesting a larger area (16.7 m^2). The quadrat yields are more representative of the crop potential by thoroughly capturing a small area (0.37m^2).

In addition to the yield differences by sampling methods (quadrat vs. whole plot), there were notable yield differences by treatment. Both whole plot and quadrat yields were highest for the SeaCrop16 applied in the crop year (2552 lbs/A and 3988 lbs/acre), followed by the SeaCrop16 applied in the prune year (2391 lbs/A and 3762 lbs/A), with the third highest yield occurring with high rate of Cheep Cheep (2283 lbs/A and 3585 lbs/A). Quadrat yields from plots treated with SeaCrop16 crop year, SeaCrop16 prune year and the high rate of Cheep Cheep were 24%, 17% and 12% greater than the quadrat yields in the control (3214 lbs/A).

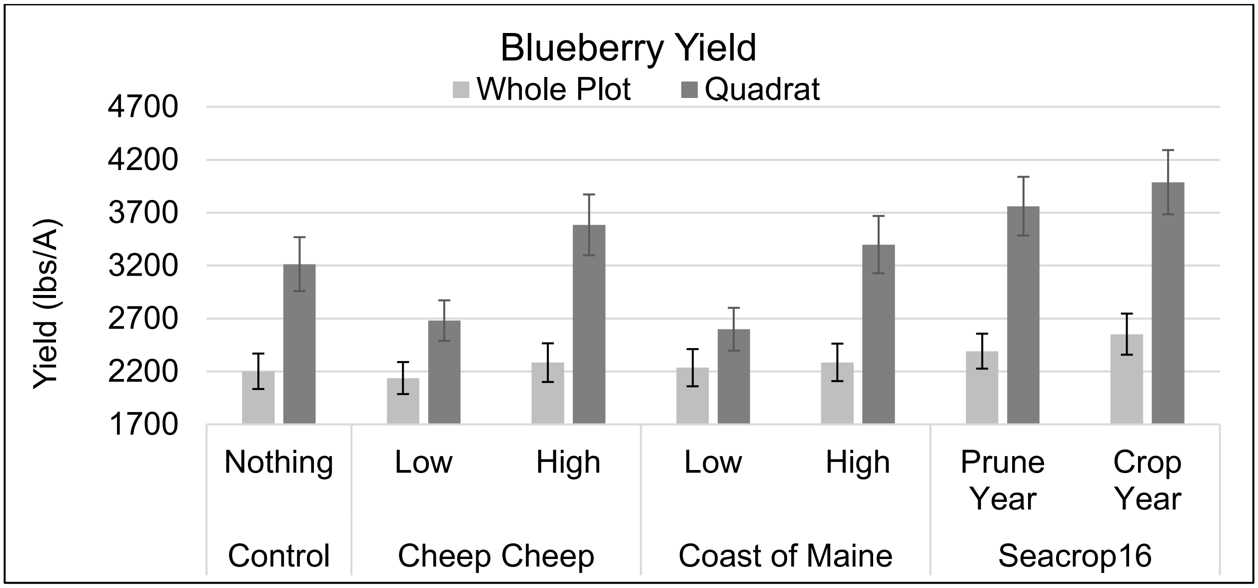

Figure 12. Average blueberry yield (lbs/A) of whole plot and quadrat subsample, measured across all three locations (Appleton, Surry and Columbia Falls) over two crop years (2020 and 2022) by treatment. Treatment differences were not significant. Error bars indicate the standard error of the mean.

DISCUSSION

While not significant, all treatment rates and timings of Cheep Cheep, Coast of Maine, and SeaCrop16 had greater blueberry coverage than the untreated control, indicating that the additional nutrients provided by each of the treatments was successful in increasing plant health and vigor. The treatments did not indiscriminately "feed the weeds”, as the weed coverage varied by location and treatment. Low weed coverage at Surry and Columbia Falls under high rates of mulch reinforce the knowledge that mulch applications confer many benefits, particularly the suppression of weeds (Gumbrewicz & Calderwood, 2022).

While all treatments saw a trend towards increased blueberry cover, not all treatments saw an increase in green fruit numbers. The high rate of Cheep Cheep produced the greatest number of green fruit across both crop years, indicating there may be a relationship between the high rate of nitrogen in this treatment (4%, the highest of all treatments) and the resulting green fruit produced. The forthcoming foliar nutrient analysis may prove or disprove this.

Higher insect presence in both rates of the Cheep Cheep and Coast of Maine treatments compared to the control indicates the wild blueberry plants were appealing to the insects, indicating a fairly healthy plant. The slightly lower rates of disease in the treated plots compared to the control indicate that the soil or mulch treatments provided a barrier to spores splashing from the ground to the plants. The rates of both insect and disease as observed in this study may be limited by identification methods. Structures such as tip midge galls on the tips of stems and mummy berry spores allowed for easy identification of the perpetrators. More general damage, such as chewed segments of leaves, was likely not attributable to a specific pest and so that pest's presence may not be appropriately recorded.

Based on the four years of data collected during this study, some soil amendments such as Cheep Cheep and mulch may be able to improve soil moisture availability and physiological performance of wild blueberry plants. Cheep Cheep and mulch treatments accumulated comparatively higher levels of soil organic matter ("SOM") and major nutrients (N, P, K, Ca) in soil at the studied sites as found from the soil testing results in the fourth and final year of this study (Tables 2A-C). Hence, Cheep Cheep and mulch applications appear to have increased water holding capacity in the soil by adding and protecting SOM (Gould, 2015; Bot and Benites, 2005).

This increase in water-holding capacity and SOM might have contributed to the slightly higher leaf chlorophyll concentrations that were observed in Cheep Cheep treatments. The higher nitrogen content in Cheep Cheep products may also have increased soil nitrogen levels, thereby benefitting the wild blueberry plants (Tables 2A-C). As nitrogen is the most important nutrient for building leaf chlorophyll, increasing the availability of this nutrient improves plants' photosynthetic performance and improves crop production (Taiz et al., 2015). Cheep Cheep had the highest macro- and micronutrient concentrations where N-P-K is 4-3-3 and Fe, Cu, S, Ca, Mg, Zn, and Mn are present. These ten nutrients comprise 40% of the product and the remaining 60% is organic matter. Despite the available nutrients from the applied treatments, photosynthetic electron transport rates showed rather contradictory responses from leaf chlorophyll concentration responses to the applied treatments. The reasons behind such contradictory results can be better explained with the leaf nutrient concentration information from this season which are still being tested (data forthcoming).

In 2022, the final year of this study, the effects of treatments seemed to wane somewhat as there were declines in soil moisture and there were no significant differences in wild blueberry physiology and morphology across treatments. This could mean that treatments applied at the rates described here should be applied every few years to achieve consistent improvements in plant physiology, morphology, and yield.

Product Costs

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. 2021 costs 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 ($/acre)	Unit Cost
Control	N/A	N/A	N/A	N/A	N/A
North American Kelp Co. SeaCrop16 Foliar Fertilizer	Prune or Crop	1.2 /242	L /gal H$_2$O/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 the University of Maine for this study*

CURRENT RECOMMENDATIONS

- Organic growers should apply any affordable and available source of organic matter as this benefited wild blueberry.
- Chicken manure can be applied at a rate of 700lbs/A (see page B138 in this report, "Using Ground Applied Fertilizers to Improve Wild Blueberry Production and Resilience to Warming" for more information) **IF** good weed management practices are already in place. 2,000 lbs/A increased weed presence.
- The effects of fertilizer waned in year four. Consider this when scheduling applications.

ACKNOWLEDGEMENTS

Thanks to Ava Ardito, Abby Cadorette, Charles Cooper, Julian LaScala, Jordan Ramos, Mara Scallon, and Brogan Tooley for data collection and analysis assistance.

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

7. Wild Blueberry Phenology: Tracking Prune and Crop Plant Development through the Season

OBJECTIVES

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

LOCATIONS: Midcoast to Downeast (10 locations: Warren, Hope, Searsport, Sedgwick, Ellsworth, Deblois, Jonesboro, Northfield, Marion, Aurora)
PROJECT TIMEFRAME: April 2020 - ongoing

INTRODUCTION

For a full explanation of the need for localized phenological prediction models, please see the 2021 report, page 123, "Wild Blueberry Phenology".

As climate change continues progressing globally, local growers are facing increased uncertainty and variable conditions during the growing season. Tools and applications have been developed and are constantly being revised to assist growers in predicting the timing of plant and pest development and subsequent management practices. In 2019 and 2020, a weather tool needs assessment was led by Dr. Calderwood and included in-person discussions with Maine industry members and farmers from wild blueberry, apple, and mixed vegetable operations. Only 34% of all growers surveyed indicated that they currently use weather-based decision support tools, but 86% expressed interest in future use (n=134) (Calderwood et al., 2022).

To develop such a tool, this project was begun in 2020 and continued in the 2021 and 2022 growing seasons by gathering weekly weather and plant development data. This data is being used to create a wild blueberry crop development tool to predict emergence date, bloom date, ripening stages, and harvest date. Dr. Annis' AgriNet currently alerts wild blueberry growers about mummy berry conditions and calculated cumulative growing degree days (GDD). The data collected could also support the development of other pest decision support tools.

Experienced wild blueberry growers have relied on general "rules of thumb" tied to specific calendar dates to determine the timing of various management practices and for decades this system worked fairly well. However, the growing season has lengthened by one month over the last fifty years (Drummond & Yarborough, 2014), with an increase of fourteen days in the last twenty years alone (Tasnim et al., 2022) and weather patterns are now unpredictable. Over the past forty years, as Maine's annual temperatures have increased, the greatest increases have come in the fall (+0.9-2.9°C) and winter seasons (+0.4-2.1°C), so the growing season has persisted for longer in the year rather than beginning earlier (Tasnim et al., 2022). Warmer fall temperatures delay leaf drop, encourage fall bloom, and render the plants more susceptible to frost events due to insufficient hardening of buds and stems (Tasnim et al., 2022). All of these delays in phenological development directly reduce the plants' fruit yield. Spring temperatures have fluctuated more than fall temperatures, limiting an earlier start to the growing season. However, sudden warm temperatures in the spring can jump-start plant development and recovery from winter dormancy, rendering the plants more vulnerable to late-season frost events (Tasnim et al., 2022). Late-season frost events have occurred more frequently in the past decade and will likely continue as climate change continues to cause chaos (Tasnim et al., 2022).

Importantly, minimum nighttime temperatures have increased faster than maximum daytime temperatures over the past forty years, and this may harm plants' ability to respire and participate in the carbon cycle, thereby diminishing the net carbon assimilated by wild blueberry and impacting the health and development of the plants (Tasnim et al., 2022). The precise impacts of warmer nighttime temperatures require additional research. Annual increases in precipitation from the past forty years have primarily occurred during the fall and winter seasons (Tasnim et al., 2022), limiting the positive impact on growth and development of wild blueberry plants.

Changes in climate result in more chaotic and extreme weather events and this pattern has been occurring in Maine over the past few years. Summer 2022 included: a cool June with a late-season frost warning in northern Maine, worsening dryness in New England but slow storms that soaked the Maritimes; one of the ten hottest Julys in Halifax, NS, and one of the five hottest months ever in Portland, ME, with long stretches of hot days across the region; and record heat and stretches of high temperatures at multiple sites in the region (NOAA/NIDIS, 2022). Summer precipitation across New England and the Maritimes varied greatly, with some areas in drought severe enough to run wells dry and require water be trucked in, while some areas, including Aroostook County in Maine and much of the Maritimes, had some of their wettest summers (NOAA/NIDIS, 2022).

Data from the U.S. Drought Monitor for the summer 2022 season show, on a week-by-week and county-by-county basis, how much of Maine experienced differing levels of drought at any given time (U.S. Drought Monitor, 2022). Descriptions of the different categories of drought conditions are described in Table 1. Moderate drought set in to Waldo, Knox, and Hancock counties during the week of June 14, and worsened to severe drought by the week of August 2. In one week in Waldo County (June 21 to 28), moderate drought conditions grew from 17% to cover 100% of the county.

In Knox County, conditions changed from 99% of the county being moderate drought conditions the week of July 26 to 99% experiencing severe drought conditions one week later, by the week of August 2 (Table 1). Hancock County saw just 0.07% of the county reach severe drought conditions by the week of August 2, and the bulk of the county experienced moderate drought conditions throughout the summer. As autumn progressed, the county quickly improved, from 60% moderate drought conditions the week of August 23 to 98% abnormally dry just a week later. Washington County experienced abnormally dry conditions from May 17 through September 20, beginning with 19% of the county classified as abnormally dry and climbing to a maximum of 99% of the county by the week of July 5, and persisting at that high percentage until dropping down to 66% of the county by the week of September 20.

Table 1. Percentage of each county in each drought level, by week during the 2022 growing season. Drought levels (D0 = abnormally dry, D1 = moderate drought, and D2 = severe drought) are explained in the subsequent table (Table 2).

County	Drought level	5/3	5/10	5/17	5/24	5/31	6/7	6/14	6/21	6/28	7/5	7/12	7/19	7/26	8/2	8/9	8/16	8/23	8/30	9/6	9/13	9/20	9/27
Washington	D0			19	19	19	57	57	57	68	99	99	99	94	94	79	79	71	71	71	66	66	
	D1																						
	D2																						
Hancock	D0			56	56	56	87	28	28	68	68	84	84	84									11
	D1																		3	3	3	3	
	D2														.1	.1	.1	.1					
Waldo	D0			23	23	23	96																
	D1							17	17	99	100	100	100	100									11
	D2														18	18	18	18	6	6	6	6	
Knox	D0			98	98	98	100																
	D1							25	25	99	99	99	99	99									75
	D2														99	99	99	99	67	67	67	67	

Table 2. Categories used by the U.S. Drought Monitor, edited for relevance to wild blueberry growing operations in Maine.

Category	Description	Possible Impacts
D0	Abnormally dry	• Going into drought: short-term dryness slowing growth of crops • Coming out of drought: lingering water deficits and crops not fully recovered
D1	Moderate drought	• Some damage to crops • Water shortages developing or imminent • Voluntary water-use restrictions requested
D2	Severe drought	• Crop losses likely • Water shortages common • Water restrictions imposed
D3	Extreme drought	• Major crop losses • Widespread water shortages or restrictions
D4	Exceptional drought	• Exceptional crop losses • Shortages of water creating water emergencies

The U.S. Drought Monitor does not predict drought conditions nor is it a statistical model. Instead, the Drought Monitor is developed by experts who synthesize data from a range of sources to classify drought conditions and severity. The Drought Monitor uses data generated by the National Drought Mitigation Center at the University of Nebraska-Lincoln, the National Oceanic and Atmospheric Administration (NOAA), and the U.S. Department of Agriculture (USDA) (U.S. Drought Monitor, 2022). Data produced by the Drought Monitor is used by growers to make management decisions, government entities to declare disasters and allocate relief programs, and local decision-makers to allocate water resource use (U.S. Drought Monitor, 2022). The Drought Monitor is an effective tool, when used in conjunction with temperature and precipitation data, for wild blueberry growers to understand how climactic conditions are impacting their crop.

METHODS

Ten locations stretching from Washington to Knox counties were utilized for this study (Image 1). By county, locations included: Washington (Marion, Deblois, Jonesboro, and Northfield), Hancock (Aurora, Sedgwick, and Ellsworth), Waldo (Searsport), and Knox (Hope and Warren). Each location consisted of one prune field and one crop field, totaling twenty study locations across ten municipalities. Marion, Hope and Warren are MOFGA-certified organic operations. Jonesboro and Northfield are managed as low-input fields, and Northfield is in the process of transitioning to organic operations. All other locations are managed conventionally. Deblois was the only irrigated field. Locations were grouped approximately based on location: Midcoast (Hope and Warren), Ellsworth (Ellsworth, Sedgwick, and Searsport), Downeast (Aurora, Jonesboro, and Deblois), Far Downeast (Marion and Northfield).

Wild Blueberry Staging and GDD

In each location's prune field, a HOBO air temperature and humidity sensor (ONSET Computer Corporation, Bourne, MA, USA) was installed (Figure 5). All twenty fields (both prune and crop) had a soil moisture meter installed as well for later use. In each field, six different clones were selected at random and flagged for continuous monitoring. All fields were visited weekly for nineteen weeks (3/28-8/1/22). Initial wild blueberry crop-stages (T1-T5, bloom, pinhead, green, color change ("red"), and blue fruit) were visually identified for each tagged stem (six per plant, six plants measured) at each location (180 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.

The prune-cycle was monitored from leaf emergence (week 8, 5/17/22) until the end of bud development (week 28, 10/12/22). 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 ten 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 18 (7/25/22), six stems per plant (or per one flag) were tagged with numbered tags for the remainder of the season (through week 28, 10/12/22) and the number of buds growing on those numbered stems was recorded. These six stems were visited weekly to measure the number of buds growing on the tagged stems. The number of stems were 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/).

Image 1. Phenology locations across major blueberry growing regions: (left) Midcoast (small circle) & Ellsworth (diamonds), and (right) Downeast (large circle) & Far Downeast (check mark).

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 and 2022, 50-100 GDD had already accumulated by April 1 and so all GDD calculations were adjusted for a March 1 start date.

Data analysis did not include statistics. Calculated estimates of unripened fruit and fruit drop were carried out in Microsoft Excel. Unripened fruit per stem were estimated by subtracting the peak green fruit for each stem from the total green fruits from the sum of blue fruit, red fruit and dropped fruit. The number of fruits dropped per stem were estimated by subtracting the total fruit observed per stem from the maximum number of green fruits observed per stem each week starting the week of peak green fruit, June 27, 2022.

RESULTS

Soil moisture showed dynamic regional differences starting in May and lasting until harvest in early August (Figure 1). Here, the regional differences were likely a function of regional precipitation, irrigation, and soil type. In the Ellsworth region, there was a clear decline in soil moisture, while, in the Downeast region some of the fields receive supplemental irrigation and are therefore more stable and even increasing in soil moisture by late July (peak ripening).

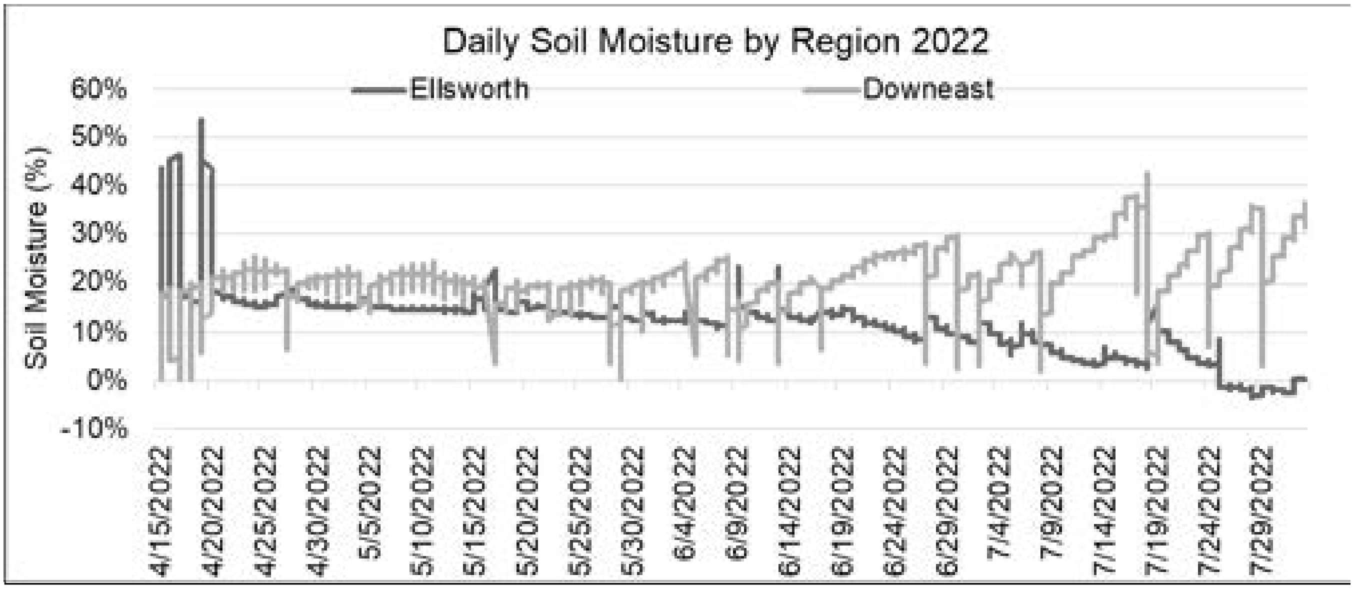

Figure 1. Daily soil moisture (%) in blueberry crop fields for the Ellsworth and Downeast regions in the 2022 growing season in the top 2 inches of soil. Soil moisture data from the Midcoast and Far Downeast regions were not used due to sensor malfunction.

Cumulative growing degree days (GDD) showed interesting regional differences in 2022 (Figure 2). The steep slope (across all 4 regions) shows a greater accumulation of GDD over time starting in early May. The Midcoast region accumulated noticeably higher GDD relative to Ellsworth, Downeast, and Far Downeast, suggesting that observed warming trends may not be uniform across the state. This increase in GDD accumulation is directly associated with more frequent warm (optimal growing) days and higher overall air temperature.

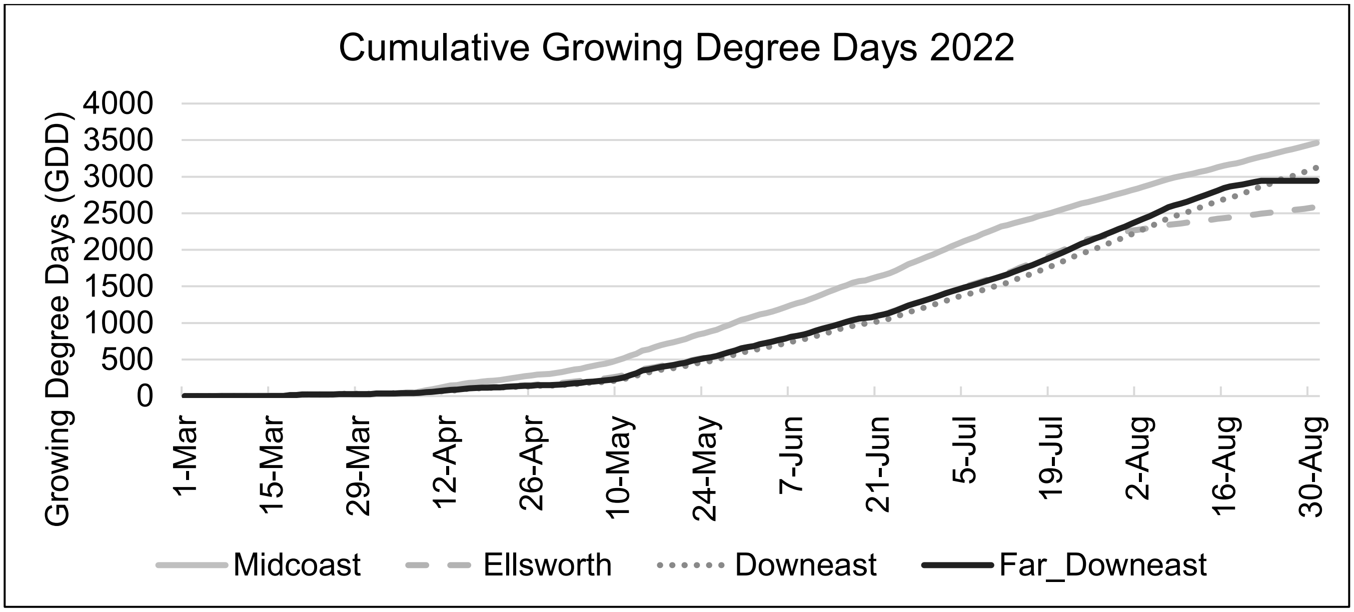

Figure 2. Average cumulative growing degree days (GDD) by region for the 2022 field season. Growing degree days were calculated with a base temperature of 40°F and started accumulation on March 1 of each year.

Crop Phenology by Region

Over the last 3 years (2020-2022), it’s been observed that peak blueberry stages appeared earlier than in past years (prior to 2020). This is especially apparent to growers who know the approximate calendar timing of stage-based management methods by heart. Accelerated phenological development in 2020 was attributed to environmental stress from the drought throughout the season and heat during the blue fruit stage. 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 (harvest) season (Figure 3). While the statewide drought ended in 2021 with a series 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 3).

In 2022, drought conditions became present in mid-June and worsened into early August. Peak bud and fruit stages appeared to occur later by calendar date in 2022 than in 2021 with growing degree days also accumulating slower until the peak pin head stage (801 GDD), where growing degree days began to accumulate faster compared to both 2020 and 2021. In 2022, peak bud stages occurred on average 1 day later than peak bud in 2021 and had an average 0 day difference from 2020. Peak bloom occurred notably earlier in 2022 (occurring the week of May 21), which was on average 2.8 days earlier than 2021 and 7.3 days earlier than 2020 (weeks of May 24 and 28, respectively). Peak fruit development in 2022 (representing peak green, red and blue fruit) occurred on average 5.4 days later than in 2021 and 6.2 days later than in 2020. Later achievement of peak stages starting in early June aligns with the increasing drought conditions, suggesting the plants’ development slowed in response to a lack of water resources, which also inhibits nutrient uptake from the soil.

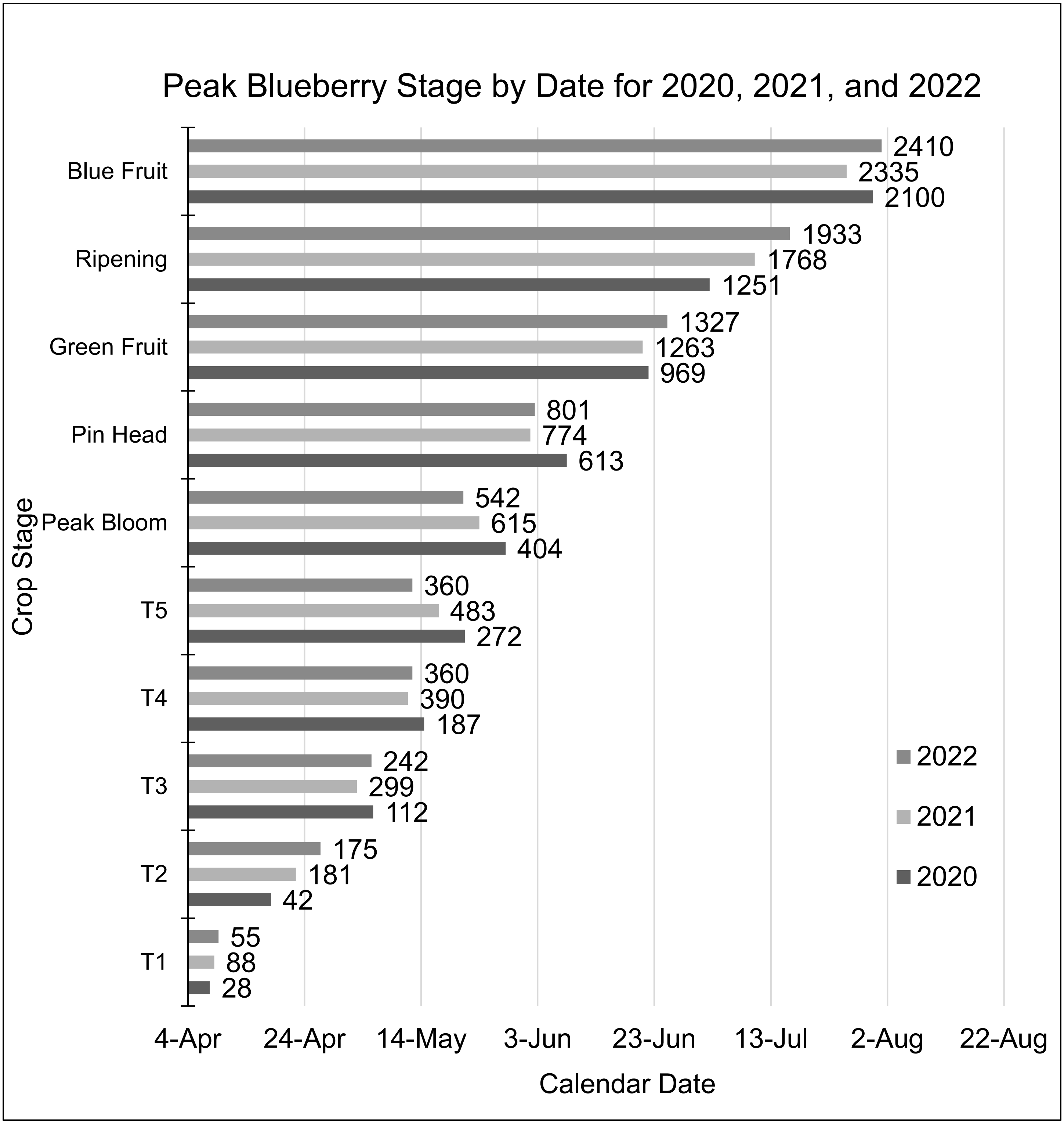

Figure 3. Peak blueberry crop stage by date for 2020, 2021 and 2022 with growing degree days (GDD) as data labels. Bud stages are labeled T1-T5 with T1 being bud swell, T2 being bud break, and T5 being early bloom.

In 2022, the greatest percentage of unripe (green) fruits remaining on the stem ranged from 29% in the Midcoast region on July 4, 2022, to 36% in the Downeast region on July 18, 2022 (Figure 4). By the week of July 25, 2022, the percentage of unripe fruit in the Midcoast region dropped to 4% and plateaued from that date forward suggesting peak ripeness had occurred. The Downeast and Far Downeast regions, however, continued to ripen until the week of August 1, 2022 with an average of 8% and 6% unripe fruit, respectively.

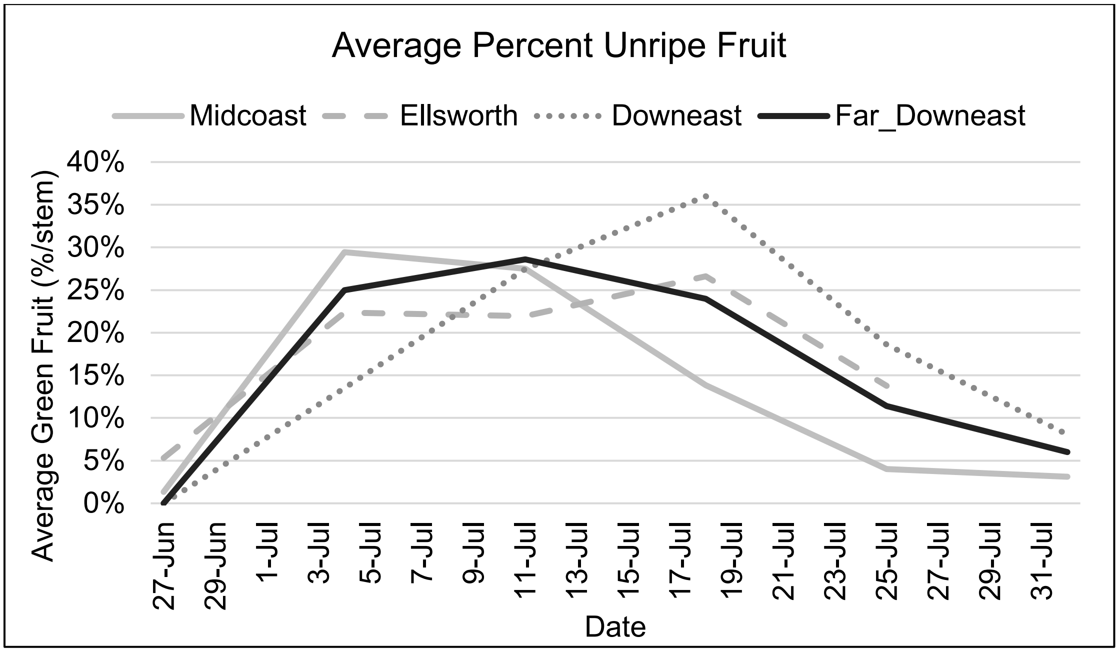

Figure 4. Estimated average unripe green fruits (%/stem) still on the stem by date and region for the 2022 growing season. Unripe fruits per stem were estimated by subtracting the peak green fruit for each stem from the total green fruits from the sum of blue fruit, red fruit and dropped fruit. Data collection stopped once fields were harvested.

Interestingly, when looking at an estimate of fruits dropped per week, the results are not linear, since some weeks experienced higher fruit drops than others, and there were differences by region as well (Figure 5). While the Downeast region exhibited higher fruit drop (up to 31% by the week of August 1, 2022), the Downeast region also had the greatest number of green fruits per stem (11.8 fruits/stem the week of July 4, 2022; Figure 5C, Table 3). The Ellsworth and Far Downeast regions plateaued in the average number of blue fruits per stem the week of July 25, 2022, with only a 3% increase in blue fruits per stem between the week of July 25 and August 1, 2022. The Downeast and Midcoast regions continued to increase in ripeness following the week of July 25, with a respective 15% and 12% increase in the number of blue fruits per stem between the week of July 25 and August 1, 2022. The fields sampled in the Midcoast region were all organic, suggesting these fields may have been relatively slow in development with no supplemental inputs like fertilizer. Whereas, some of the fields in the Downeast region were irrigated, suggesting the berries at these locations had the adequate resources to continue to ripen. Fields that ripened earlier in the Ellsworth and Far Downeast regions may have been affected by drought conditions.

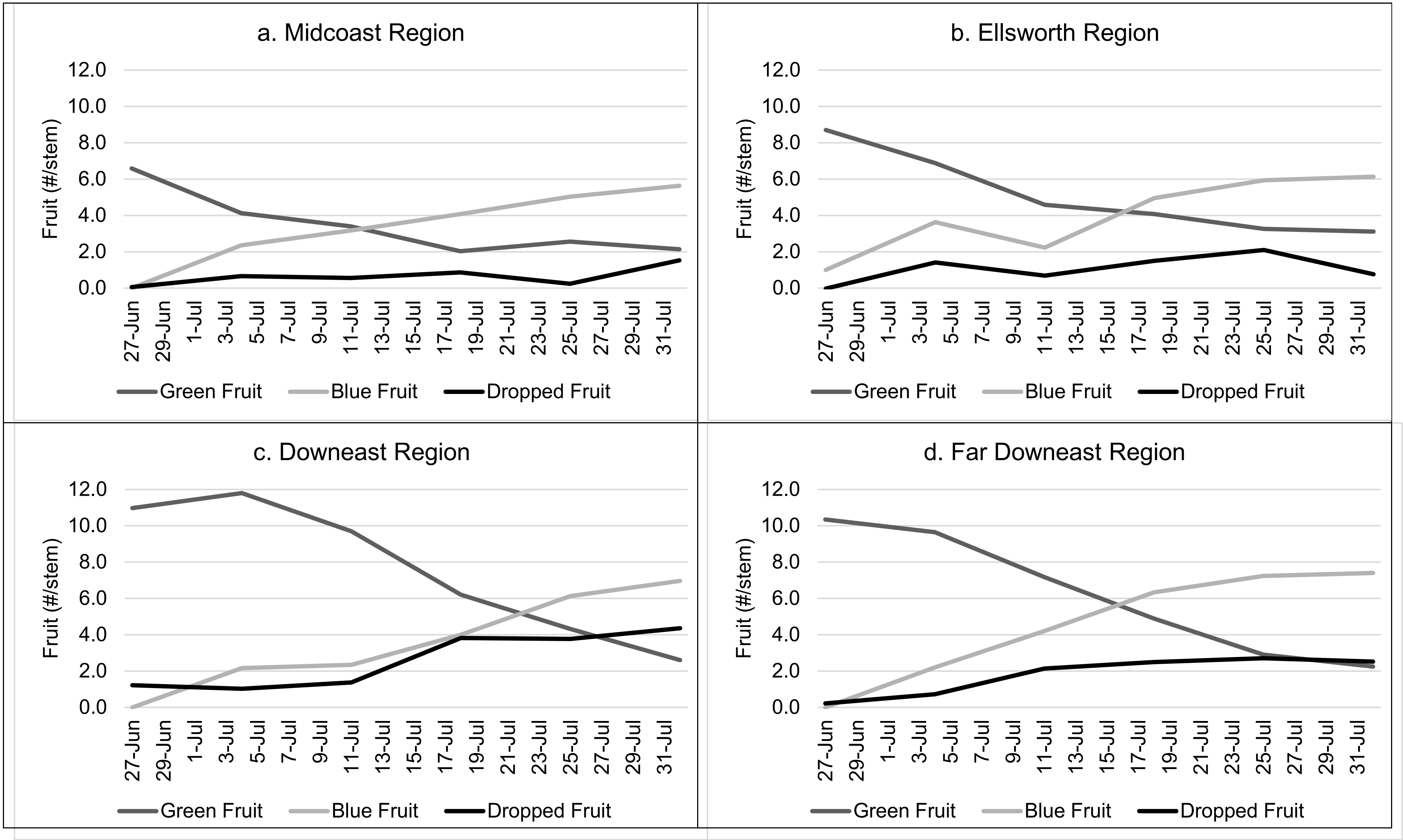

Figure 5. Average green fruit (#/stem), blue fruit (#/stem), and estimated average fruit drop (#/stem) by region, over time, from the week of peak green fruit (June 27, 2022) until harvest (August 1, 2022).

Table 3. Average weekly number and percentage of green, blue, and estimated dropped fruits per stem in the 2022 crop fields by region from the week of May 30 to harvest during the week of August 1, 2022. Fruit drop was estimated on a per stem basis by subtracting the peak green fruit from the total fruit each week. Weeks where peak blue fruit was reached relative to green fruit and estimated dropped fruits are in bold.

		Average Total Fruit (#/stem)			Average Composition (%/stem)		
Region	**Week of**	**Green**	**Blue**	**Dropped**	**Green**	**Blue**	**Dropped**
Midcoast	30-May	3.6	0.0		100%	0%	
	6-Jun	5.1	0.0		100%	0%	
	13-Jun	5.7	0.0		100%	0%	
	20-Jun	4.9	0.0		100%	0%	
	27-Jun	6.6	0.0	0.1	99%	0%	1%
	4-Jul	4.1	2.4	0.7	58%	33%	9%
	11-Jul	3.4	3.2	0.6	48%	44%	8%
	18-Jul	2.0	4.1	0.9	29%	59%	12%
	25-Jul	**2.6**	**5.0**	0.2	**33%**	**64%**	**3%**
	1-Aug	2.1	5.6	1.5	23%	61%	16%
Ellsworth	30-May	2.9	0.0		100%	0%	
	6-Jun	6.7	0.0		100%	0%	
	13-Jun	9.1	0.0		100%	0%	
	20-Jun	7.1	0.0		100%	0%	
	27-Jun	8.7	1.0	0.0	90%	10%	0%
	4-Jul	6.9	3.6	1.4	58%	30%	12%
	11-Jul	4.6	2.2	0.7	61%	30%	9%
	18-Jul	4.1	5.0	1.5	39%	47%	14%
	25-Jul	**3.3**	**5.9**	**2.1**	**29%**	**53%**	**19%**
	1-Aug	3.1	6.1	0.8	31%	61%	8%
Downeast	30-May	4.0	0.0		100%	0%	
	6-Jun	4.5	0.0		100%	0%	
	13-Jun	9.6	0.0		100%	0%	
	20-Jun	9.7	0.0		100%	0%	
	27-Jun	11.0	0.0	1.2	90%	0%	10%
	4-Jul	11.8	2.2	1.0	79%	14%	7%
	11-Jul	9.7	2.3	1.4	72%	17%	10%
	18-Jul	6.2	4.0	3.8	44%	29%	27%
	25-Jul	4.3	6.1	3.8	30%	43%	27%
	1-Aug	**2.6**	**7.0**	**4.4**	**19%**	**50%**	**31%**
Far Downeast	30-May	-	-		-	-	
	6-Jun	3.9	0.0		100%	0%	
	13-Jun	-	-		-	-	
	20-Jun	10.4	0.0		100%	0%	
	27-Jun	10.3	0.0	0.2	98%	0%	2%
	4-Jul	9.7	2.2	0.7	77%	18%	6%
	11-Jul	7.2	4.2	2.1	53%	31%	16%
	18-Jul	4.9	6.3	2.5	36%	46%	18%
	25-Jul	**2.9**	**7.2**	**2.7**	**23%**	**56%**	**21%**
	1-Aug	2.3	7.4	2.5	18%	61%	21%

Prune Phenology

The cumulative bud development varied by region and GDD (Figure 6). The Ellsworth region experienced rapid bud development relative to cumulative growing degree days from August 1 (2261 GDD) to September 12, 2022 (2696 GDD), while the Midcoast and Downeast experienced a higher GDD relative to the same date. Far Downeast data were excluded because it was very anomalous. Tip dieback was not considered alongside bud development because the sample size was too small.

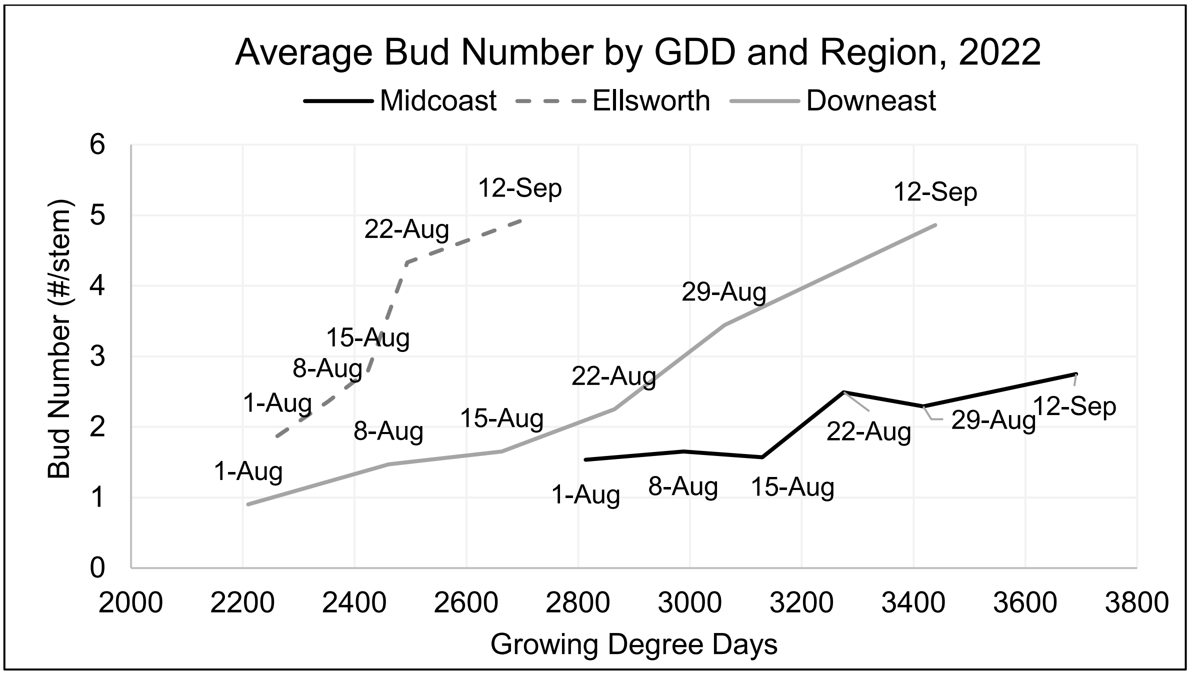

Figure 6. Average bud number (#/stem) by region relative to growing degree days (GDD) with calendar dates as data labels for the 2022 growing season.

DISCUSSION

Crop Phenology

Determining the optimal harvest day requires growers to visually inspect their fields to approximate the ratio of unripe fruit to ripe blue fruit. A visual inspection of fruit still on the stem is easy enough to conduct, and growers have historically utilized historical data to help them approximate when harvest ought to begin (e.g., the second week of August). As explained in depth earlier in this report, climate change is altering farmer calendar date notions, and the earlier accumulation of growing degree days (GDD) brought about by warmer temperatures can accelerate the rate of fruit maturation, especially when drought conditions further accelerate the rate of fruit ripening. Growers are harvesting fruit earlier in the season. Continuing to rely only on a calendar date to determine harvest date will cause growers to miss the number of unripe or overripe fruit that dropped from the stem due to unripe fruit abortion, complete fruit maturation, or physical removal due to heavy rains or physical disturbance.

Crop Phenology by Region

Phenological development timelines are advancing forward in the calendar and these changes are occurring on a broader scale than just season-to-season. In 2020, the accelerated fruit development was attributed to statewide drought conditions and heat during the blue fruit stage. In 2021, warmer temperatures accelerated GDD accumulation and again caused early ripening, on average 4 days earlier than 2020 in the flower and fruit stages. In 2022, drought conditions began in June and worsened by the beginning of August, yet the phenological development was slightly (1 day) behind 2021 development, with peak fruit development being reached 5.4 days later than in 2021.

From 2020 - 2022, it has been observed that peak blueberry stages appeared earlier than in past years (prior to 2020). This was especially apparent to growers who know the approximate calendar timing of stage-based management methods by heart. Fruit ripened more quickly in 2020 due to statewide drought and heat during blue fruit, and fruit ripened even more quickly in 2021 due to warmer temperatures and accelerated accumulation of GDD. In 2022, fruit ripened more slowly than in 2021 and 2020 despite achieving some peak phenological stages earlier (such as peak bloom) than the previous two years. Peak fruit development stages in 2022 were attained later in the season than in the previous two years, despite the 2022 season accumulating more GDD at every stage from pin head onward. Contradictorily, the later date of peak blue fruit ripening may be due to drought conditions: some fruit ripening patterns indicate that drought conditions can cause the fruit to ripen more quickly so as to allow the plants to conserve some resources (as observed in 2020 and 2021), while other fruit ripening patterns indicate that drought conditions may cause the fruit to ripen more slowly since the plant does not have sufficient water resources to increase fruit volume and enable the uptake of nutrients vital for ripening (as observed in 2022). More data is needed to ascertain the true relationship between drought conditions and fruit ripening. GDD is not the only variable that impacts plant development and fruit ripening. Soil type, fertilizer applied, pest management practices, and water availability at key development points play key roles in plant development.

Prune Phenology

Midcoast accrued the most GDD by September 12, but this did not result in a large number of buds (2.75), perhaps due to persistent drought conditions through the fall. Ellsworth accrued GDD very slowly, and accrued less GDD overall, but developed an average of 4.92 buds per stem, whereas Downeast accrued GDD more steadily, eventually producing 4.88 buds. Ellsworth also experienced persistent drought conditions (but high bud formation) whereas Downeast had milder drought conditions and includes one high input and irrigated field, which may have enabled a more consistent development of buds. GDD is not the only variable that impacts bud development. Soil type, fertilizer applied, pest management practices, and water availability at key development points play key roles in plant development.

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 continue data collection in 2023.
- Quantify and monitor fall bloom, fruit, and vegetative growth in 2023 and future years.

ACKNOWLEDGEMENTS

Thank you to the Wild Blueberry Commission of Maine for funding this project. Thank you to Dr. Phil Fanning for assisting with weather station troubleshooting and to Dr. Seanna Annis for filling in data gaps.

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INVESTIGATORS: YJ. Zhang, L. Calderwood, and R. Tasnim

8. Foliar Calcium Study

OBJECTIVES

Evaluate the effects of three foliar calcium products applied at T5 and green fruit stages on wild blueberry quality.

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

INTRODUCTION

Wild blueberry is well adapted to growing in nutrient poor soils yet farmers have long sought to increase nutrient availability to improve yield and quality. Calcium is a nutrient that is the building block of plant cell walls, making it a critical micronutrient. Applying calcium to plants through sprays has been observed to increase fruit quality in highbush blueberries, apples, tomatoes, and lychee, though some of the deficiencies corrected were not related to fruit firmness (Arrington & DeVetter, 2017; Agrinova et al., n.d.; Yang et al., 2019). Research by the highbush blueberry industry has researched calcium application timing and frequency (bloom through post-harvest), application method (foliar sprays on leaves and/or fruit, ground application, soaking harvested fruit), and product formulations (including high or low levels of nitrogen) (Ochmian & Kozos, 2014; Lobos et al., 2020). The varied results do not uniformly show that increased rates of calcium improve fruit firmness, improve fruit quality as indicated by color or resistance to squishing, or reduce fruit drop after fruit set. Instead, the range of results indicates that local climatic conditions, specific highbush cultivar, and timing of calcium applications may be the greater influences on fruit quality (Yang et al., 2019).

In highbush blueberry, soil application of calcium increased soil pH and calcium levels in soil yet the same calcium increases were not observed in leaves or fruit (Hanson & Berkheimer, 2004). This is because calcium moves from the roots to aboveground plant parts through the xylem and this movement is dependent on transpiration (water leaving the plant through stomata). The more transpiration there is (higher rates occur when leaves have many active stomata), the more calcium (and other nutrients) will move into the leaves and plants. Calcium transport into fruit is lower than to leaves (Gerbrandt et al., 2019).

Research into the effectiveness of foliar calcium sprays does show higher rates of calcium in leaves and fruit when compared to no treatment or ground treatment. In an experiment with the highbush blueberry “Liberty” cultivar, Lobos et al. (2020) sprayed low and high rates (0.36 lb/A and 0.71 lb/A, respectively) of calcium solutions (16% calcium) at or after fruit set (“early” treatment applied at 0, 8, and 16 days after fruit set, “late” treatment applied at 16, 24, and 32 days after fruit set), in two consecutive seasons. Results from this study showed that the untreated plants produced the softest fruit, and the plants treated early with both high and low rates produced the firmest fruit. Younger fruit has a thinner cuticle, which likely allows for increased calcium product absorption through stomata (Ochmian, 2012) and as fruit ripens, the stomatal conductance of berries decreases rapidly (Yang et al., 2019). Given this, targeted applications of calcium-rich products earlier in the fruit’s development will be absorbed more effectively by the fruit and will therefore better improve fruit firmness.

Wild blueberry plants and fruit differ substantially from their highbush counterparts, but general conclusions about benefits of calcium application timing and quantity can reasonably be expected to be appropriate for lowbush. Both frozen and fresh wild blueberry producers are interested in the use of calcium as a tool to improve fruit quality.

Earlier study (2019-2020) of foliar fertilizers on wild blueberry showed no significant results yet fruit firmness was not measured. Of the products tested, a calcium product (Poma) showed the most promise as a foliar fertilizer. This study expands our look at foliar-applied calcium by comparing three different calcium-containing foliar fertilizers applied in a 2022 crop field at bud stage T5, green fruit and at both T5 and green fruit.

METHODS

In May 2022, the study was laid out at Blueberry Hill Farm in Jonesboro, ME in a randomized complete block design with each foliar fertilizer treatment (Poma, Ele-Max CalciumFL, and water as the untreated control) replicated 6 times in 6' by 30' plots, for a total of 60 plots. The products applied in this season were: Poma, Ele-Max Calcium FL, and Biomin® Calcium. Poma was included due to our past research, Ele-Max is a conventional product available and used by growers through Helena, and Biomin was the most available certified organic calcium fertilizer. We recently learned that another calcium product currently used by growers in Maine is Nutrisync Ca (4-0-0 +10%Ca) but this was not included in this year's study.

Table 1. Summary of product characteristics.

Product	Company	Organic or Conventional	N-P-K	Calcium content (%)
Biomin Calcium	JH Biotech, Inc.	Organic	1-0-0	5
Poma	Agro100	Conventional	0-0-0	6
Ele-Max Calcium FL	Helena	Conventional	4-0-0	23.8

Each separate foliar fertilizer treatment was applied to six plots at the F5 phenology stage, six plots at green fruit, and six plots at both F5 and green fruit. Rates were chosen from the products' labels.

Table 2. Timing, rates, and frequency of treatments. Poma, Ele-Max, and Biomin were all flowable liquids (FL).

Phenology	Date	Treatment	Treatment rate
F5	5/11/22	Water (control)	121 gal water/A
		Poma	3.2 qt/A in 121 gal water/A
		Ele-Max Calcium	1 qt/A in 121 gal water/A
		Biomin Calcium	3.2 qt/A in 121 gal water/A
Green fruit	6/21/22	Water (control)	121 gal water/A
		Poma	3.2 qt/A in 121 gal water/A
		Ele-Max Calcium	1 qt/A in 121 gal water/A
		Biomin Calcium	3.2 qt/A in 121 gal water/A
F5 & green fruit	5/11/22 & 6/21/22	Water (control)	121 gal water/A
		Poma	3.2 qt/A in 121 gal water/A
		Ele-Max Calcium	1 qt/A in 121 gal water/A
		Biomin Calcium	3.2 qt/A in 121 gal water/A

Treatment rates were calculated using the application information on the product labels and through discussions with company representatives. The treatments were mixed before being placed into a Solo brand backpack sprayer. Technicians then used the hand pump and nozzle to apply the treatments to the leaves of the wild blueberry plants. Technicians administered the spray in an arc just a few inches above the leaves' surface to maximize uptake by the leaves and reduce the risk of drifting into other plots. Backpack sprayers were rinsed with water between treatment types. Water applied to control plots was sprayed through the backpack sprayers before any treatments were administered to reduce risk of contamination; approximately 0.5 gallons of water was administered, so uniform volumes of all treatments were applied to the plots.

Image 1. Technician wearing rubber gloves and goggles while using a backpack sprayer to apply a treatment.

<u>Data collection</u>

Blueberry Foliar Samples, Physiology and Morphology

Six stems from each plot were randomly selected to measure chlorophyll concentration by a CCM-200 plus Chlorophyll Content Meter (Opti-Sciences, Inc., Hudson, NH, USA) on June 21, 2022. 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 June 30, 2022 between 10AM and 2PM.

Right before harvesting, on 2 August, 2022, six random stems from each treatment plot were collected to quantify the number of leaves per 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 Leaf Area Meter (LI-COR Biosciences, Lincoln, NE, USA). All the leaves from those six stems were oven-dried at 70°C to constant mass and weighed, and then the dried leaf samples were ground and sent to the University of Maine Soil and Plant Tissue Testing Laboratory in Orono, Maine for leaf nutrient testing. We are still waiting on these results as of January 2023.

Soil Characteristics

Soil samples were taken on August 2, 2022 by sampling twice in each treatment plot, combining the samples, and sending one amalgamated sample by treatment from 2 replicated blocks to the University of Maine Soil Testing Service. In total, 3 separate samples for each treatment (one sample from block 1 and 2, one sample from block 3 and 4, and one sample from block 5 and 6) were sent for standard soil testing.

Plant Phenology

Repeated plant phenology measures were taken on the same four stems in each treatment plot. Plants were tagged with numbered tags and were evaluated on June 15, July 22, August 10, 2022. The number of buds, flowers, green, and blue fruit were recorded during each sampling. Stem heights were also measured on these same days using a meter stick and were recorded in centimeters.

Fruit Yield

Fruit was hand-raked on August 10, 2022. Within each treatment plot, three $0.37m^2$ quadrats were placed, and all the fruit was harvested within the quadrat and the yield recorded. Each plot generated three yield numbers: quadrat one, quadrat two, and quadrat three. The fruit from each plot were then combined to enable fruit quality measures.

Fruit Quality

The harvested fruit was sampled in several ways to determine fruit quality. The weight of 100 berries was measured and recorded, allowing researchers to determine which treatments produced larger fruit, since the 100 berry weight had a higher mass. A sample of fruit from each treatment was also puréed for use in a handheld PAL-BRIX/ACID F5 refractometer (Atago, Saitama, Japan) to measure the samples' sugar content.

Dr. Beth Calder of the UMaine Food Testing Services analyzed whole fruit to measure fruit firmness. For each treatment, twenty-five blueberries of sound quality and with good fruit turgor were randomly selected from the hand-raked harvest in each treatment. The berries were harvested late in the season and had maintained turgor but some fruit showed signs of softening. One at a time, berries were analyzed using the TA.XTPlus Texture Analyzer (Texture Technologies, Hamilton, MA) texture analyzer. The berries were placed on a stainless-steel base so the probe would puncture berries on their sagittal side, and a 2mm stainless steel probe (TA-52) was used to puncture the berry's skin. The probe traveled 6mm in distance to penetrate through the blueberry and a trigger force of 15g was used. The pre-test speed was set at 2.0mm/s, a test speed of 1.0mm/s and a post-test speed of 5.0mm/s. The force values were reported in g as the peak force it took to puncture the berry skin.

Data Analysis

Blueberry Physiology and Morphology

The effects of calcium foliar treatments on physiology (leaf chlorophyll concentration and photosynthetic electron transport rate) and morphology (leaf size, number of leaves per stem and total leaf 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 foliar treatments 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

Single date measurements including yield, Brix, 100 berry counts and peak force (berry firmness) phenology measures (by key stage) were evaluated using a generalized linear model (GLM), followed by a Tukey's Pairwise comparison in JMP (JMP®, Version 16.0, SAS, Cary, NC, USA) across all treatments ($\alpha = 0.05$). All ranked blueberry cover were transformed to their corresponding percent mid-point. Ranked blueberry cover and blueberry stem height 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.

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 included blueberry phenology, stem height and cover, and peak force (berry firmness). These data all improved following a square root transformation. Phenology, and blueberry cover data continued to statistically fail for normality following transformation. Statistical tests were carried out despite non-normality after establishing there were no serious problems with the data. Blueberry yield and Brix measures were normally distributed; therefore, no transformation was needed prior to statistical testing.

RESULTS

Blueberry Physiology and Morphology

For chlorophyll concentrations during the growing season, all treatments including the control, had similar leaf chlorophyll concentrations of 24 – 28 SPAD (Figure 1).

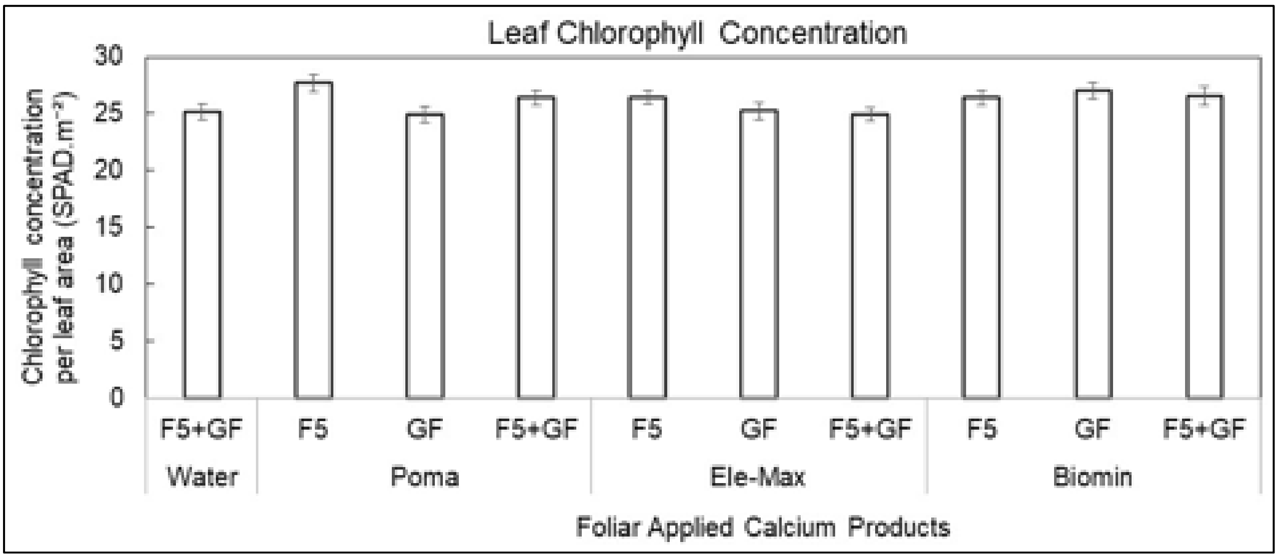

Figure 1. Comparison in chlorophyll concentration in leaves by treatments on June 21, 2022 at Blueberry Hill Research Station, Jonesboro, Maine. Foliar applied calcium products were applied at stages: F5 bud (F5), green fruit (GF), and at F5 + green fruit (F5 + GF). Error bars represent the standard error of the mean. No letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

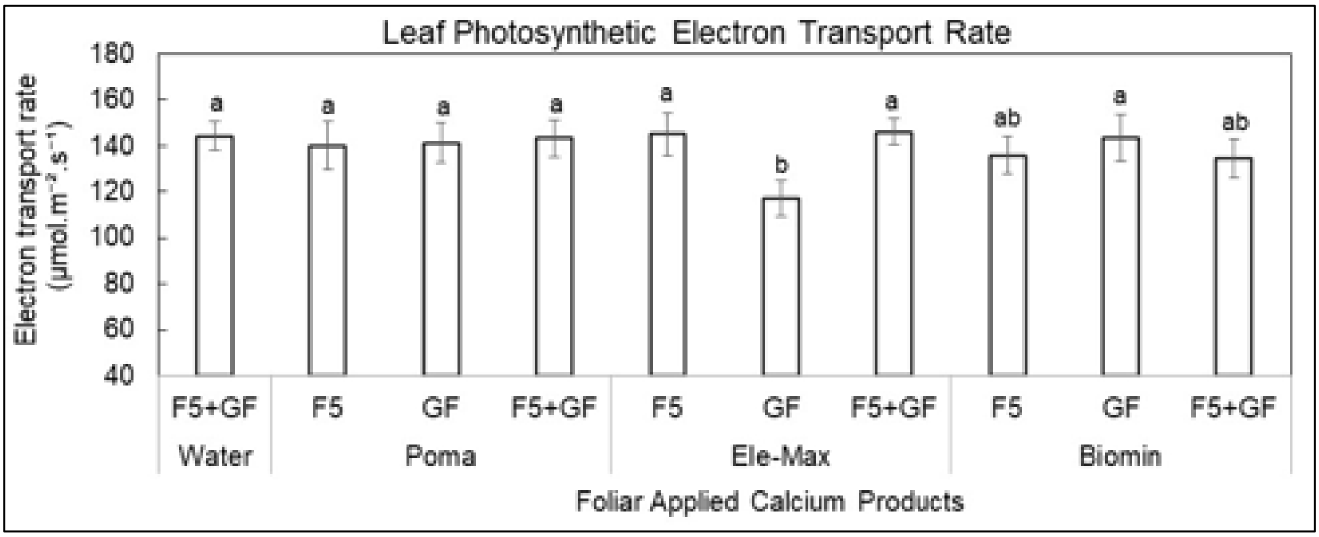

Figure 2. Comparison of photosynthetic electron transport rate of leaves by treatment on June 30, 2022 at Blueberry Hill Research Station, Jonesboro, Maine. Foliar applied calcium products were applied at stages: F5 bud (F5), green fruit (GF), and at F5 + green fruit (F5 + GF). Error bars represent the standard error of the mean. Different letters indicate significant differences at the significance level of $p < 0.05$.

Blueberry Morphology

No significant differences in leaf size, number, or total leaf area were found among the treatments compared to the control (Figure 3). However, on average, all the Biomin treatments showed consistently fewer leaves and total leaf area per stem compared to the control.

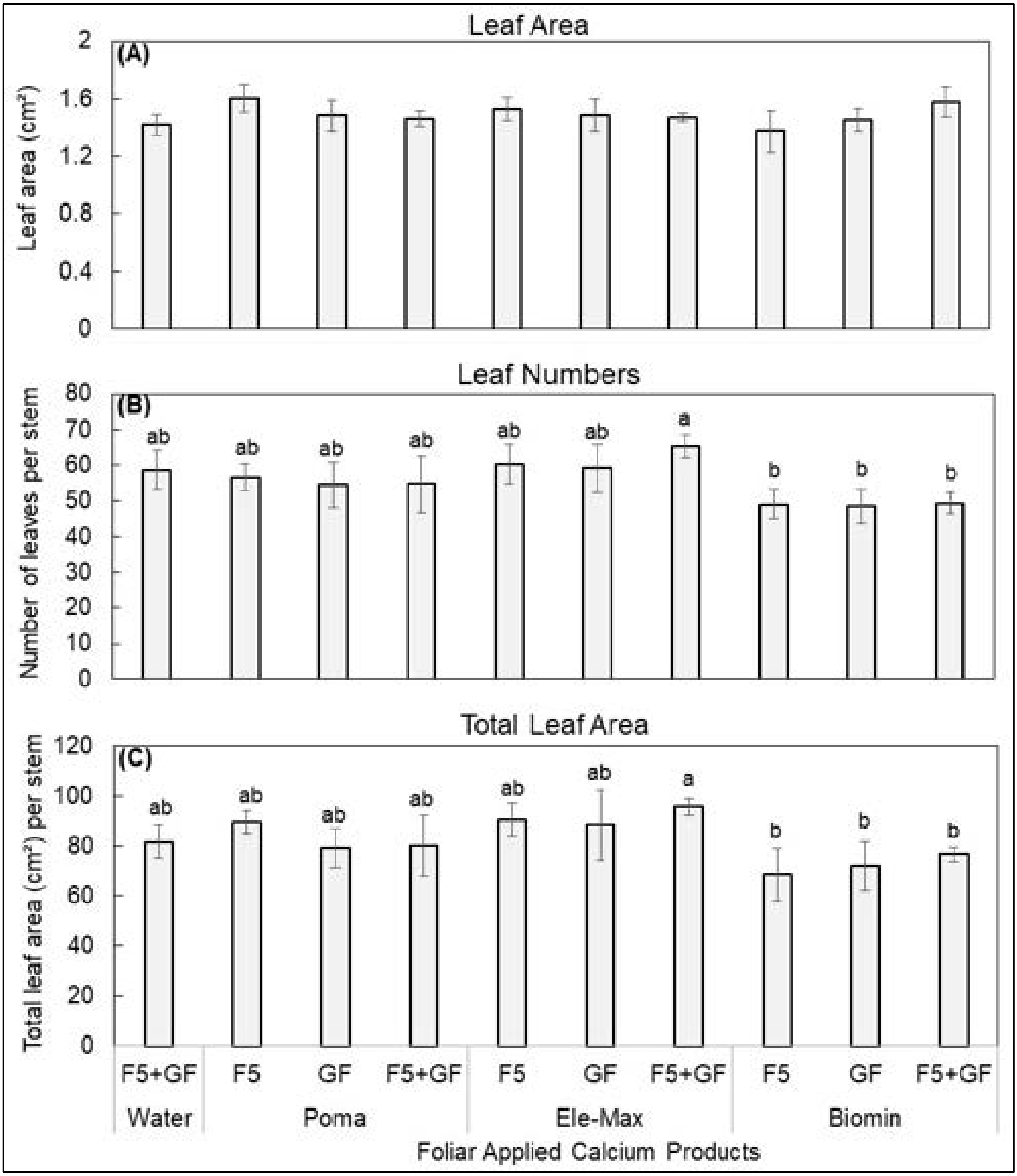

Figure 3. Comparison in (A) average leaf area, (B) number of leaves per stem, and (C) total leaf area per stem by treatments on August 2, 2022 at Blueberry Hill Research Station, Jonesboro, Maine. Foliar applied calcium products were applied at stages: F5 bud (F5), green fruit (GF), and at F5 + green fruit (F5 + GF). Error bars represent the standard error of the mean. Different letters indicate significant differences and no letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

Plant and Soil Characteristics

Unfortunately, foliar results are not yet available and will be reported in 2024. Soil pH at the research site ranged between 4.4 and 4.8 among research plots. Organic matter ranged from 5.5 to 9.7%.

The wide range of values for both pH and %OM may be due to sampling error. Soil characteristic data is not shared here because it is more relevant with foliar data, which is still forthcoming as of January 2023.

Plant Phenology

Applying treatments at different phenological stages was marketed to increase berry firmness and reduce fruit drop. Applying Biomin at the F5 stage yielded the highest number of green fruit per stem (6.9), which was significantly greater than the stems receiving Ele-Max at F5 (4.6 fruit/stem; Figure 4 and Table 3). Despite producing more green fruit after F5 application, Biomin did not also produce the greatest number of green fruit after application at the green fruit phenological stage or the F5 and green fruit phenological stages.

Applying Poma at the F5 stage yielded the greatest number of blue fruit per stem (8.5), which was significantly greater than treatments receiving Ele-Max at green fruit (6.1) and at F5 + green fruit (5.8), as well as Biomin at F5 (5.7) and at green fruit (5.1). Despite producing the most blue fruit after application at F5, Poma did not also produce significantly greater numbers of blue fruit per stem following the green fruit or F5 + green fruit applications.

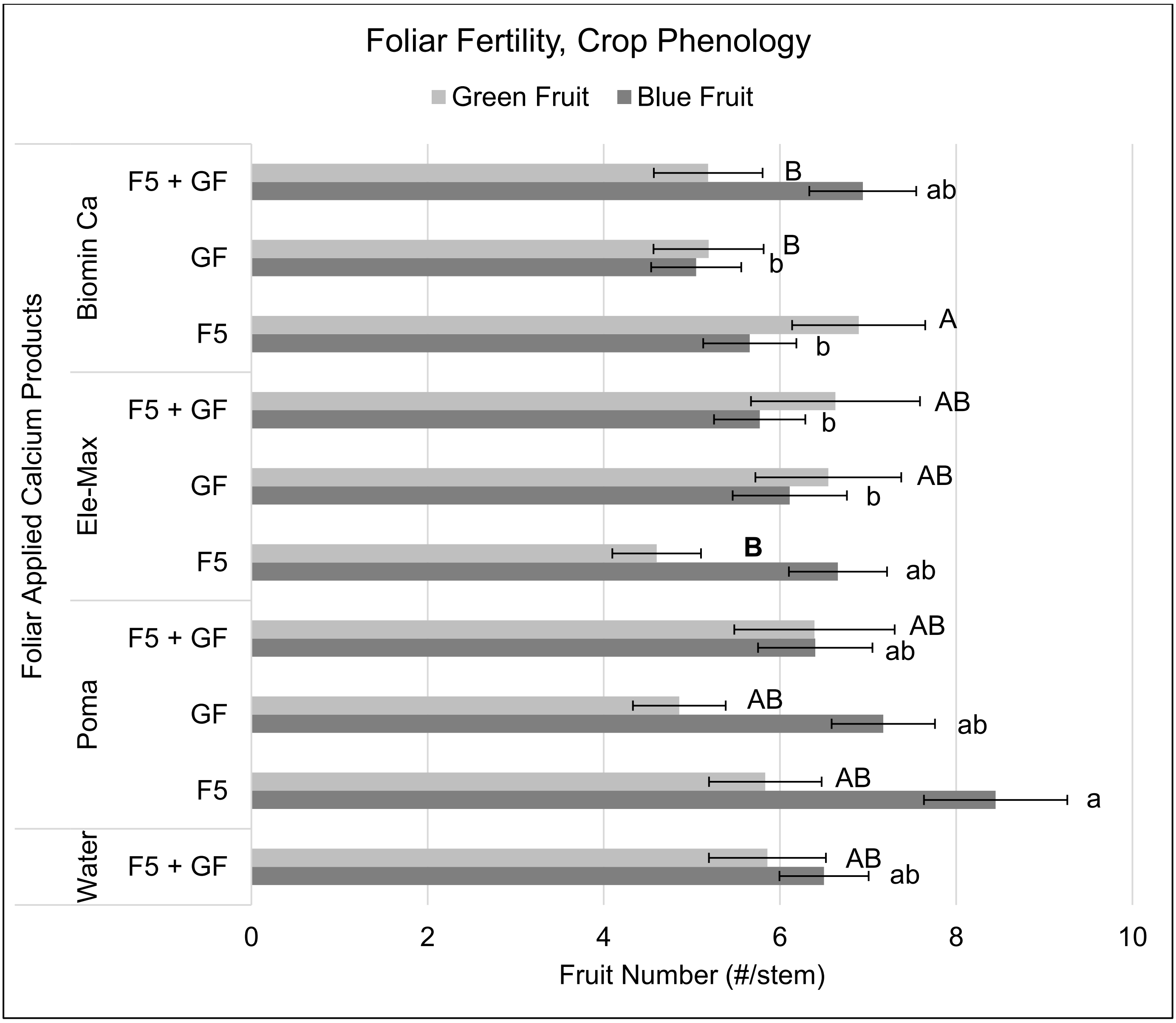

Figure 4. Average fruit counts per stem by treatment at Blueberry Hill Research Station, Jonesboro, Maine. Green fruit counts were observed on June 15 and July 22, 2022. Blue fruit counts were

observed on July 22 and August 10, 2022. Foliar applied calcium products were applied at stages: F5 bud (F5), green fruit (GF), and at F5 bud and green fruit (F5 + GF). Letters indicate significant differences at the 0.05 level of significance. Capital letters are to be compared separate from lowercase letters. Error bars represent the standard error of the mean.

Table 3. Average fruit counts per stem by foliar fertility treatment at Blueberry Hill Research Station, Jonesboro, Maine. Green fruit counts were observed on June 15 and July 22, 2022. Blue fruit counts were observed on July 22 and August 10, 2022. Foliar applied calcium products were applied at stages: F5 bud (F5), green fruit (GF), and at F5 bud and green fruit (F5 + GF). Data is the same as above chart (Figure 4).

Product	Stage of Application	Green fruit (#/stem)	Blue fruit (#/stem)
Water (control)	F5 & GF	5.9	6.5
Poma	F5	5.8	8.5
	GF	4.9	7.2
	F5 & GF	6.4	6.4
Ele-Max	F5	4.6	6.7
	GF	6.6	6.1
	F5 & GF	6.6	5.8
Biomin	F5	6.9	5.7
	GF	5.2	5.1
	F5 & GF	5.2	6.9

Wild blueberry stem heights were measured on June 15 and July 22, 2022 (Figure 5). While treatment differences in stem heights were not significant, there were some visual differences worth noting. The stem heights in the Biomin treatments were slightly taller than all others ranging from 20.4 cm (Biomin GF) to 22.3 cm (Biomin F5), with the exception of Poma applied at F5 where average stem heights were 20.4 cm. Stems in the Ele-Max treatment applied at green fruit were the shortest (17.8 cm).

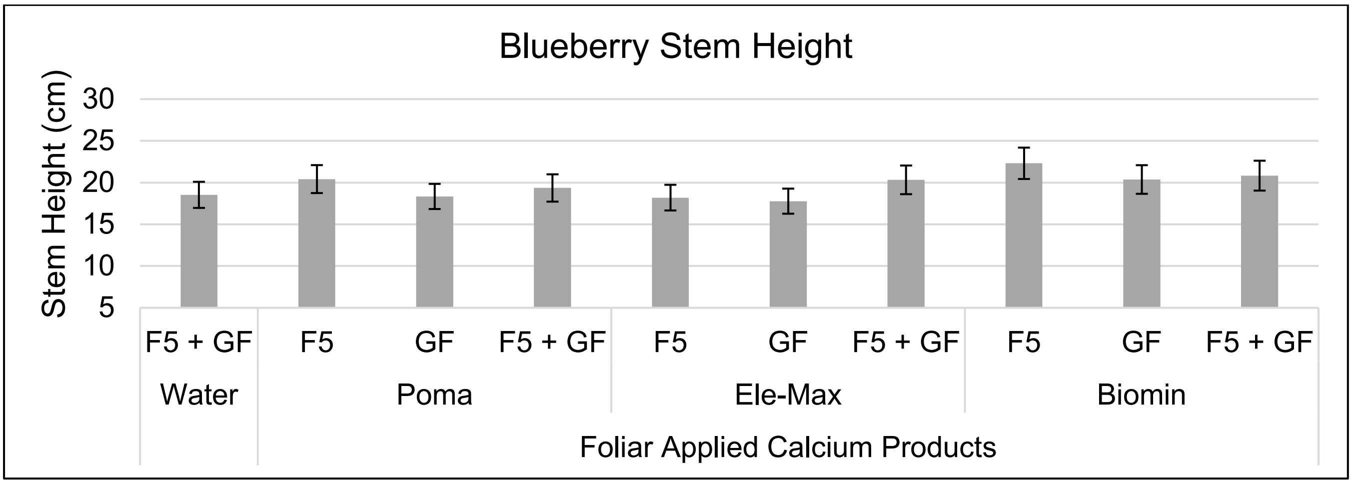

Figure 5. Average stem heights (cm) by treatment measured on June 15 and July 22, 2022 at Blueberry Hill Research Station, Jonesboro, Maine. Foliar applied calcium products were applied at stages: F5 bud (F5), green fruit (GF), and at F5 bud and green fruit (F5 + GF). Treatment differences in stem height were not significant. Error bars represent the standard error of the mean.

Fruit Yield and Quality

Blueberry yields were not significantly different among treatments, but interesting trends were present. Yields were highest where Ele-Max was applied at the F5 stage (1629 lbs/A), followed by Poma applied at the F5 stage (1444 lbs/A). These two highest yielding treatments Ele-Max at F5 and Poma at F5 were 405 lbs/A and 220 lbs/A greater than the control (1224 lbs/A), respectively.

Applying Poma, Ele-Max, and Biomin at the F5 + green fruit phenological stages produced the lowest yields (958, 1019, and 985 lbs/A, respectively).

Fruit quality was measured in several ways, and the effect of treatments on fruit quality was again not significant across all conditions. Using 100 berry size to calculate fruit size, the largest berries were produced after application of Poma at green fruit (33.9 g/100 berries) and the smallest berries were produced after application of Ele-Max at green fruit (27.3 g/100 berries) (Figure 6). These small berries were smaller even than those produced in the control (30.4 g/100 berries).

Brix sugar content of berries were also not significantly impacted by treatment type or timing. The highest berry sugar contents (13.3 Brix) were measured in fruit from the Poma at the F5 and green fruit phenological stages treatment. The lowest berry sugar contents (8.9 Brix) were measured in fruit from treatments of Ele-Max at the F5 and green fruit phenological stages. Interestingly, treatments of Poma and with Biomin experienced greater sugar content in those treated at the green fruit or F5 and green fruit stages, suggesting those with a green fruit application may have developed/ripened faster leading to less fruit on the stem by the August 10, 2022 harvest date.

Peak force or skin-breaking force as an indicator of berry firmness, was measured on the date of harvest (August 10, 2022), and results showed that the Biomin application at F5 and green fruit resulted in significantly firmer fruit (148g) when compared to Poma (118g) and Ele-Max (114g) at the same F5 and green fruit application timing (Figure 6).

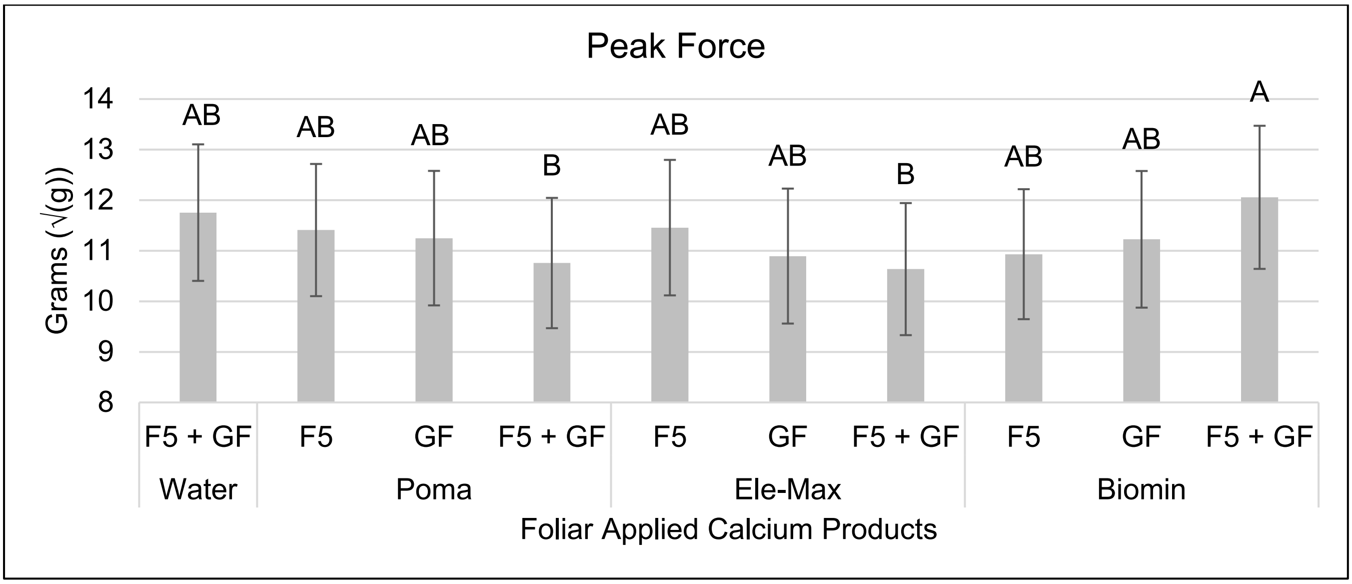

Figure 6. Peak force or skin-breaking force (transformed; √(g)) as an indicator of berry firmness by treatments at Blueberry Hill Farm in Jonesboro, Maine, harvested August 10, 2022. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean.

DISCUSSION

Blueberry Physiology and Morphology

Contradictory physiological and morphological performances were observed where most treatments did not have significant effects compared to the control. This may be because the soil characteristics and soil nutrient concentrations were similar to one another and close to the optimum ranges recommended for wild blueberry (data not included in this report). Another possible explanation could be that the wild blueberry leaves already contained the recommended calcium levels and then these levels increased beyond optimum ranges following the treatments (Santiago, 2011; Calderwood et al., 2020; Tasnim et al., 2022). Forthcoming foliar nutrient data will clarify possible causes.

Fruit Yield and Quality
Lower berry firmness in the two treatments with lower yields, lower berry size, and higher Brix levels suggests the fruit in these treatments were more mature at the time of sampling and natural fruit drop from overripening had already begun. Yields were lower in treatments applied at the green fruit or F5 and green fruit stages, possibly a result of increased researcher foot traffic but also possibly due to the accelerated rates of fruit development and ripening, as the high Brix rates and low berry firmness indicates.

Biomin applied at F5 + GF had significantly firmer skin than the other two treatments applied at the same intervals, suggesting that Biomin may have increased the berry firmness despite rapid development and overripening across all three treatments. This experiment was harvested on August 10, 2022 and was one of the last experiments to be harvested, with individual plants at all levels of fruit ripening and fruit drop. Therefore, yields may be artificially low due to fruit dropping prior to harvest, so more research is needed to fully evaluate the effects of treatment on yield. Another key difference that may set products apart are the types of calcium used. The Biomin active ingredients are calcium carbonate and soy protein while Ele-Max contains calcium carbonate and urea and Poma contains calcium acetate.

CURRENT RECOMMENDATIONS

- Repeat in 2023.

NEXT STEPS

This trial will be repeated in 2023, a prune year.

ACKNOWLEDGEMENTS

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

9. Investigating Dual-Use Solar on a Wild Blueberry Field in Rockport

OBJECTIVES

- Document the impact of solar array construction on wild blueberry.
- Determine the impact that array shading has on wild blueberry productivity.

LOCATION: Rockport, ME
PROJECT TIMEFRAME: 2020 – 2024

INTRODUCTION

For a full explanation of the interest in developing solar photovoltaic projects on agricultural land, please see the 2021 report, page 123, "Wild Blueberry Phenology".

Dual-use solar is the installation of solar panels installed in such a way that agricultural activities such as crop production and animal grazing can occur simultaneously. Such arrays may include higher panel heights and increased row spacing to allow enough sunlight to reach the crop underneath and for equipment and workers to maneuver. While farming wild blueberries under solar panels is not yet economical, perhaps a future incentive program would make it so. Solar companies continue to approach wild blueberry growers with development offers, and this research explores the impact of shade and array construction on wild blueberry productivity and evaluates the costs associated with continuing to farm under the panels.

There are three commercial cranberry bogs in Massachusetts that have dual-use solar systems installed (Shemkus, 2022; Mupambi, 2020), and these solar installations were installed with the lowest panel edge more than 8 – 10 feet off the ground, to best enable continued crop growth and management (Clean Energy Extension, 2022). No specific regulations guide the spacing of panels, but developers must use a Shading Analysis Tool to ensure that the maximum reduction in sunlight due to shading from panels on any square foot of land underneath the dual-use system may not exceed 50% during the growing season (Clean Energy Extension, 2022).

Agrivoltaic installations are not unique to the United States. An exciting project was developed in France, where rotating solar panels were installed above rows of wine grapes and the system was designed to prioritize grape production over energy production (Crellin, 2021). Much like wild blueberry, wine grapes are cultivated in well-suited local climates and these slow-growing plants cannot easily adapt to sudden changes in climatic conditions. The panels benefit the grapevines by mediating ground temperatures during times of extreme heat or cold, and some of this benefit is due to direct shading by the panels (Crellin, 2021). Another French agrivoltaic system was co-located in a lettuce field and delays in plant growth were observed in areas with more shade coverage (Marrou et al., 2013). Lettuce differs greatly from wild blueberry, but it is probable that any disruption in plants' ability to utilize sunlight to photosynthesize will impact plant health and productivity. Research into the impact of varied shade amounts and types of ultraviolet light allowed through shade (by controlling both factors using shade cloths) on the health of highbush blueberry has shown that reducing available sunlight by approximately 50% promotes plant photosynthetic characteristics and leaf traits, by increasing leaf chlorophyll levels and reducing leaf temperature (thereby improving photosynthetic efficiency) (Lobos et al., 2011). Taken together, the studies on shading in lettuce, grape, and highbush blueberry make it clear that research on the impact of shade on specific crops is necessary to understanding the impact that agrivoltaic shade may have on each cropping system.

Construction of this solar array caused obvious disturbance to the blueberry cover and soil, as mechanical equipment was used to de-rock, level, and drill post driven ground mounts across the site. These direct impacts, which include death of above ground plant stems, compaction of soil, possible weed seed bank mixing, cooler soil and above ground temperatures in the shade, and water falling to the ground along drip lines are some examples of possible impacts to the future health and yield of wild blueberry plants. Indirect impacts are more difficult to quantify and may take several seasons to fully understand. This solar array was completed in 2021 and continued data collection will articulate what harms or benefits the wild blueberry are subject to as the blueberry underneath the panels continues to recover from installation and be commercially managed.

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

METHODS

Installation (completed by the construction team)

During construction of the 40-acre site, 12 acres were set aside for this research and panel installation was done in one of three ways within the research section. The three construction methods were named Standard, Mindful, and Careful. In Standard: construction and installation methods were unaltered from industry and company standards. Equipment could drive and operate anywhere and was not restricted from turning or rotating, and foot traffic was not limited. In Mindful: 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: poly mats (Figure 1) were placed on top of the blueberry plants to work and drive equipment on. Poly mats could remain in place for only four 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.

Monofacial and bifacial panels were installed in this array. 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. Monofacial panels were installed in the area constructed with the Standard treatment. Bifacial panels were installed in the areas constructed with Mindful and Careful treatments. Additionally, it's worth noting there are terrain differences between steep slope where the bifacial panels are located and the shallow slope at the bottom of a hill, where the monofacial panels are located.

Data collection (completed by University of Maine Team)

Onset HOBO (Onset Computer Corporation, Bourne, MA, USA) temperature and relative humidity sensors (MX2300) were installed on wooden stakes driven into the ground to continuously track these metrics over time. One sensor was installed under monofacial panels, one under bifacial panels, and one in the array control underneath no panels.

Multiple Photosynthetically Active Radiation (PAR) sensors with data loggers (ZL6 from METER group, Pullman, WA) were installed on wooden stakes on June 27, 2022. These wooden stakes also supported the temperature and humidity sensors described above. These sensors measured, in 15-minute intervals, the amount of sunlight penetrating the solar panel array; this light was assumed to be available to wild blueberry plants growing underneath the panels. Sunlight penetrating the panels was measured directly under panels, called “shade under panel”, partial shade (in drive rows behind panels, called “shade drive row"), and full sunlight conditions (in drive rows between solar arrays, called “sun drive row"). 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 (Figure 1, below). No PAR sensors were installed in the control plots situated outside of the array perimeter (called “external control”). PAR sensors in full sunlight provided the localized control value, or “light quantities,” for comparison with the partial and full shade conditions.

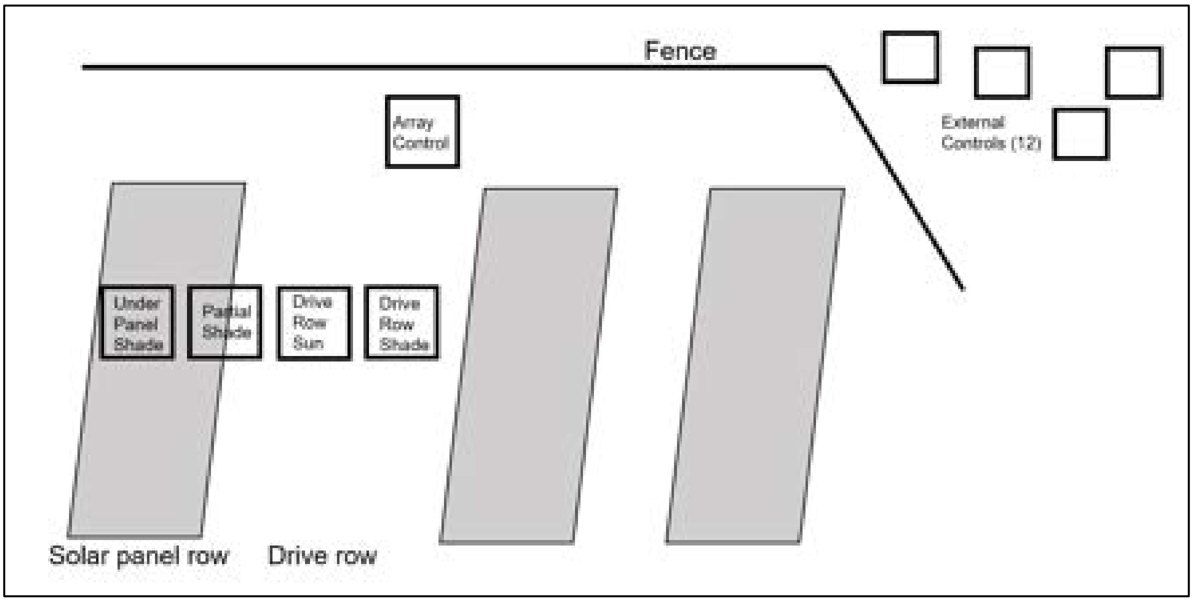


Figure 1. Example of one replication plot layout in the field where black squares represent quadrats where data collection occurred.

A 0.37 m^2 quadrat was used at each of the three PAR sensor locations totaling 16 quadrat sample locations 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 presence was evaluated using the Daubenmire rank, totaling the number of weeds present, and listing the top three weed species present. Disease presence 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. Numbered stem tags were placed on three stems within each quadrat and these three stems were visited repeatedly to count the number of buds and fruit that developed. These blueberry coverage, bud/fruit count, weed presence, and disease presence measurements were all taken three times in 2022, on May 17, June 14, and July 20.

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 samples were gathered on June 14 and July 20, 2022. TDR samples were gathered on May 17 and July 20, 2022.

Fruit was harvested July 20, 2022 by hand-raking within each quadrat and measuring the weight of the harvested fruit. The fruit samples were then combined by treatment type (sun, full shade, partial shade) and 100 random berries were selected, combined and weighed to calculate the "100 berry weight"; this information can give a sense of whether fruits were large or small in size. After calculating the 100 berry weight, a sample of fruit was removed from each combined treatment sample and macerated before being measured for Brix, a measure of sugar content.

Data analysis

Computations were carried out using JMP Version 16.0 (SAS, Cary, NC, USA) statistical software. All data collected via multiple samplings throughout the field season (soil moisture, soil temperature, SPAD, pest measures and blueberry cover) were analyzed using a repeated measures full factorial, followed by a Tukey's Pairwise comparison. Larger data sets of environmental conditions collected on a 24hr basis, including PAR, air temperature, humidity and dewpoint were shortened to 10 AM – 2 PM (peak growing hours) and analyzed using an REML mixed model with date and treatment. PAR was converted from units of $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD to %PAR relative to the average PAR received by the control outside of the array (1380 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD). Single date measures (blueberry phenology, yield and Brix) were analyzed using a one-way ANOVA and a Tukey's Pairwise comparison (following any necessary transformations). Multivariate and bivariate regressions were run to assess the degree of relation between the dependent variable yield and independent variables: air temperature, relative humidity, PAR, soil moisture, soil temperature and SPAD. Linear regression plots are presented below for variables with significant linear relationships following the bivariate regression analysis.

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. Data that did not meet the statistical assumption of normal distribution included: soil moisture, soil temperature, relative humidity, pest data, Brix, blueberry phenology and cover. These data were transformed using a square root transformation. The transformed data improved visually in their distribution but continued to statistically fail for normality. Statistical tests were carried out despite non-normality after establishing there were no serious problems with the data. When data met statistical assumptions (including air temperature, dewpoint, and yield), data were not transformed prior to statistical testing. Regressions were performed on untransformed data.

RESULTS

Environmental Conditions (Soil, Air, Sunlight)

While not significant, there were trends in soil moisture where the control experienced the lowest average soil moisture (29.5%), the shade under panel was slightly greater (30.3%), and greater still in the sun drive row (31.9%) and shade drive row (32.2%) (Figure 1). This may be due to the location of the drive row (being between the panels) where regular precipitation and panel run off is concentrated but the panels still shade and trap moisture from the sun.

The shade under panel experienced the lowest average soil temperature (81.7°F), close to the temperature of the shade drive row (81.9°F) (Figure 2). Soil temperatures in the sun drive row were 82.3°F and significantly higher at 84.9°F in the control. The average control temperatures were significantly warmer when compared to the treatments, which makes sense as the ground in the control experiences full sun.

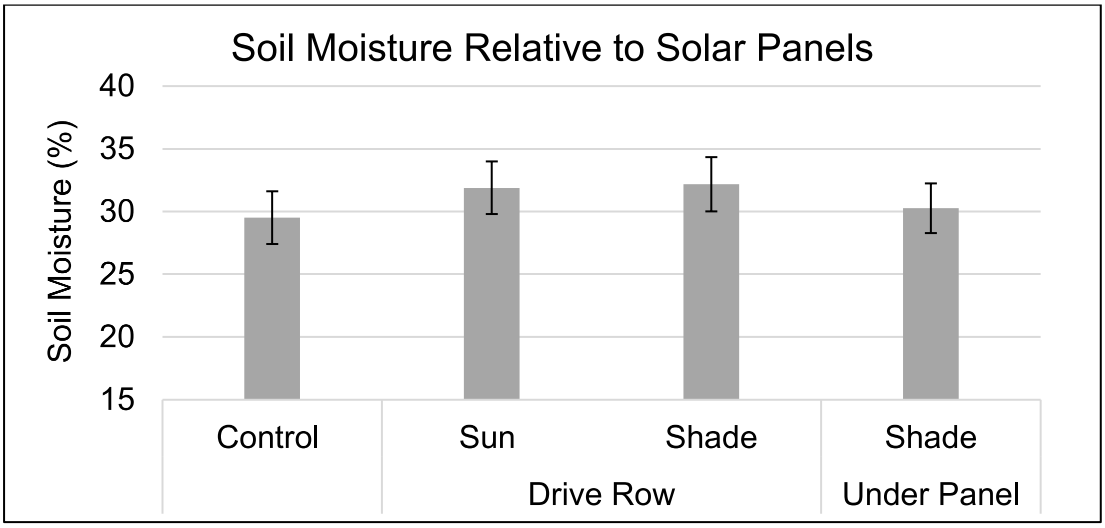


Figure 1. Average soil moisture relative to the amount of shading from the panels, measured on May 17 and July 20, 2022. The 'shade drive row' location received partial sun exposure depending on the time of day. Treatment differences (shading from panels) were not significant. Error bars represent the standard error of the mean.

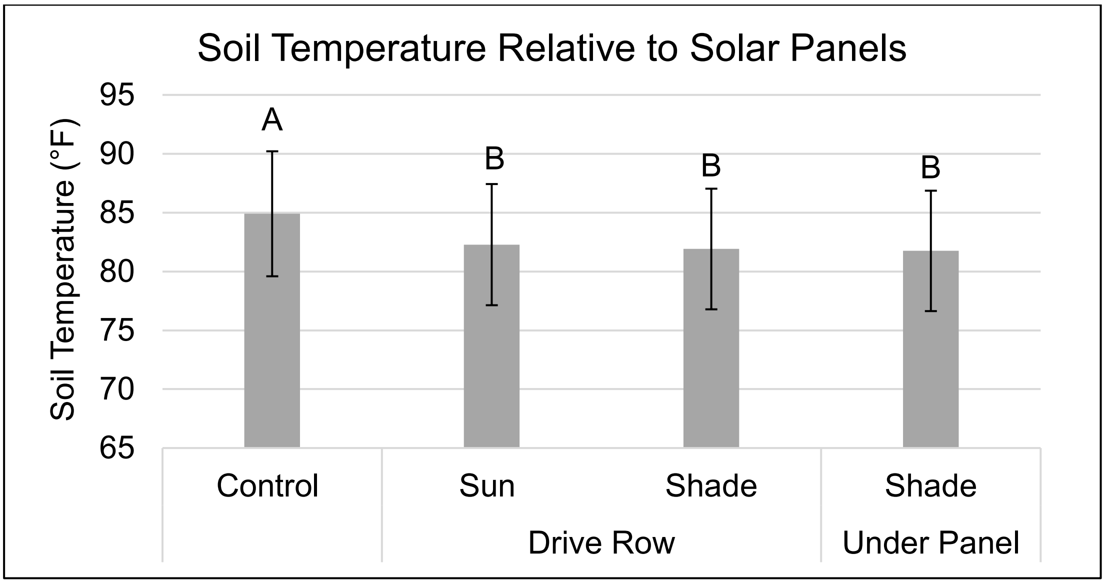


Figure 2. Average soil temperature relative to the amount of shading from the panels, measured on May 17 and July 20, 2022. The 'shade drive row' location received partial sun exposure depending on the time of day. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean.

Air temperature, dew point, and humidity were measured from 10 AM – 2 PM daily (most direct sun exposure) for the control and both panel types: monofacial and bifacial (Figure 3). Air temperatures were significantly higher outside of the panels (80.3°F), and lower underneath the bifacial panels (78.3°F) and monofacial panels (78.8°F). Dew point exhibited no significant treatment differences, with average dewpoint values of 64.9°F, 64.7°F, 63.8°F, in the control, bifacial and monofacial treatments, respectively. The bifacial panels experienced significantly greater average humidity (63.9%) than the average humidity observed under the control (60.4%) and monofacial panels (61.1%). The bifacial panels allow greater light penetration than the monofacial panels. The higher humidity under the bifacial panels suggests that the light penetration encourages evapotranspiration from the leaves and soil but also acted as a physical barrier to trap that humidity.

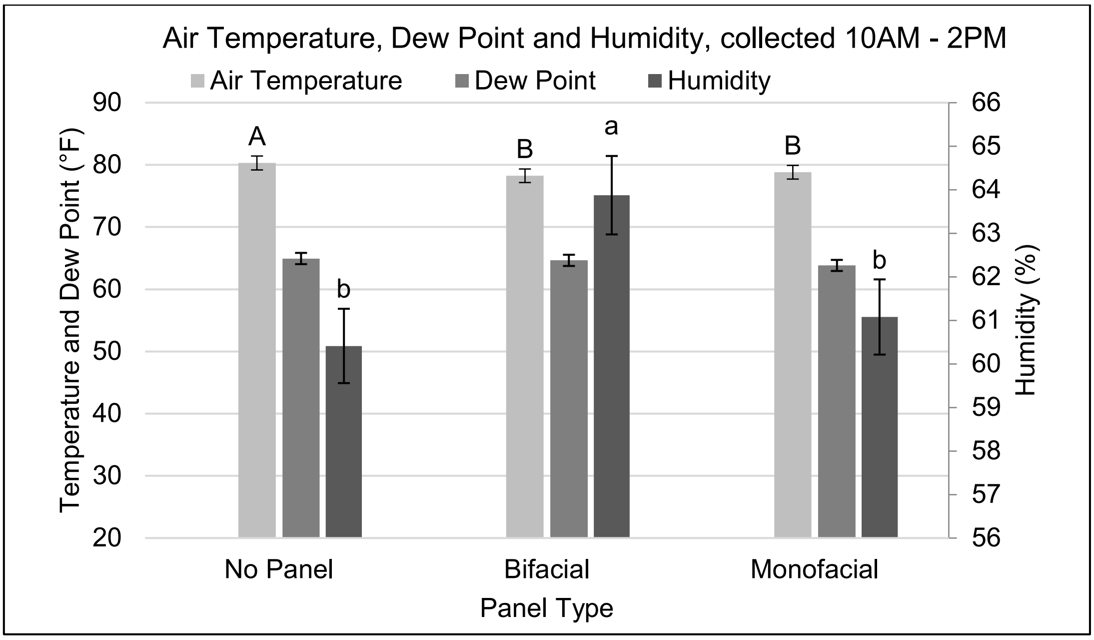


Figure 3. Average temperature (°F), dewpoint (°F), and humidity (%) relative to the amount of shading from the panels, measured from June 27 to August 31, 2022. Letters indicate significant differences at the 0.05 level of significance. Capital letters are to be compared separate from lowercase letters. Error bars represent the standard error of the mean.

Air temperature, dew point, and humidity were measured from 9 PM – 5 AM (overnight) for the control and both panel types: monofacial and bifacial (Figure 4). Air temperatures were highest outside of the panels (63.6°F), and slightly lower underneath the bifacial panels (63.1°F) and monofacial panels (63.3°F), although not significantly different. Average dew points were relatively similar across treatments, being 59.6°F, 59.2°F, and 59.6°F in the control, bifacial and monofacial treatments, respectively. Average humidity observed in the control was 86.9%, 87.4% under the bifacial panels, and 87.9% under the monofacial panels, although not significantly different. A lack of significant treatment differences in the nighttime hours when compared to the daytime hours suggests sunlight is the driving factor in the significant differences observed in Figure 3.

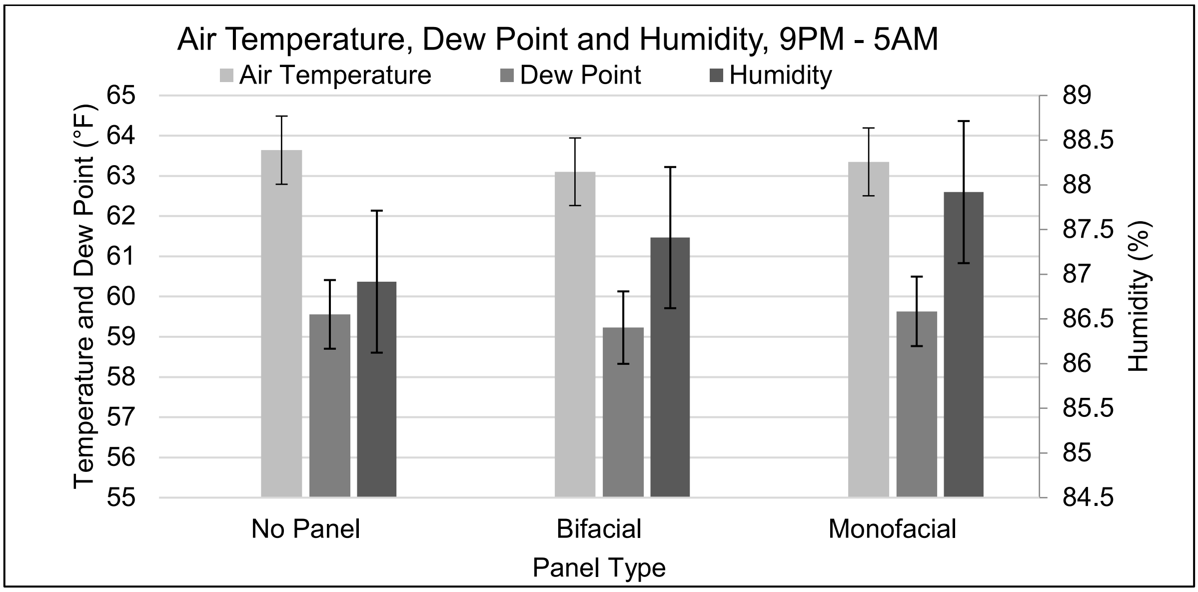


Figure 4. Average temperature (°F), dewpoint (°F), and humidity (%) relative to the amount of shading from the panels, measured from June 27 to August 31, 2022. Treatment differences (shading from panels) were not significant. Error bars represent the standard error of the mean.

PAR received by plants in each of the treatments was dramatically different. Areas underneath bifacial panels had slightly higher PAR levels than those with monofacial panels. All units for PAR are micromoles per meter per second (μmol/m⁻²/s⁻¹ PPFD) and indicate how much usable light energy reaches an area. The base level for full sunlight was 2000 μmol/m⁻²/s⁻¹ PPFD with sensors maxing out at 2325 μmol/m⁻²/s⁻¹ PPFD. The control received an average of 1380 μmol/m⁻²/s⁻¹ PPFD from 10a-2p each day May 18 to August 31, 2022, including both sunny and cloudy days. The control PAR level was used as the upper threshold for establishing %PAR in all other treatments. Relative to the average PAR observed in the control, bifacial areas received 90%PAR, 19%PAR, and 9%PAR in the sun drive row, shade drive row, and shade under panel, locations, respectively (Figure 5). The monofacial areas received slightly less than the bifacial with average levels of 83%PAR, 18%PAR and 7%PAR in the respective sun drive row, shade drive row, and shade under panel locations.

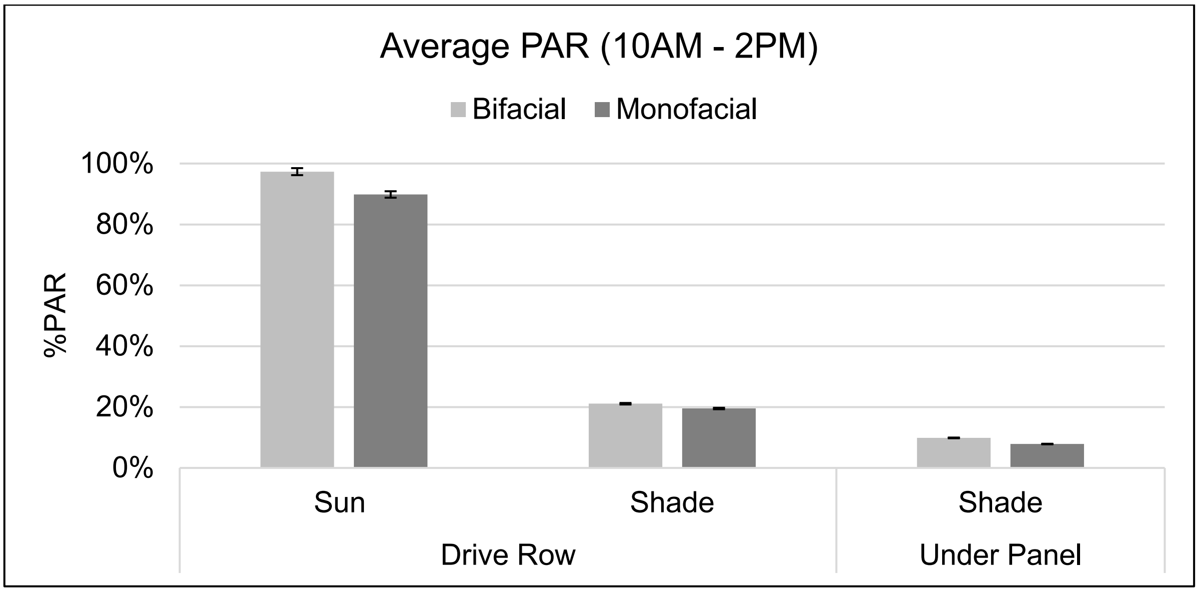


Figure 5. Average photosynthetically active radiation (PAR) presented as %PAR (of the control; 1380 μmol·m⁻²·s⁻¹ PPFD) observed in wild blueberry relative to the amount of shading from the panels, measured from May 18 to August 31, 2022. The ‘shade drive row’ location received partial sun exposure depending on the time of day. Error bars represent the standard error of the mean.

Plant Health

Leaf chlorophyll content in the shade drive row treatments were greatest in both the upper and lower leaves (upper 30.7 SPAD, lower 29.8 SPAD), while the lowest leaf chlorophyll content occurred in the control (upper 26.3 SPAD, lower 26.8 SPAD) (Figure 6). Although not significant, all treatments within the array exhibited greater leaf chlorophyll content in both the upper and lower leaves when compared to the control.

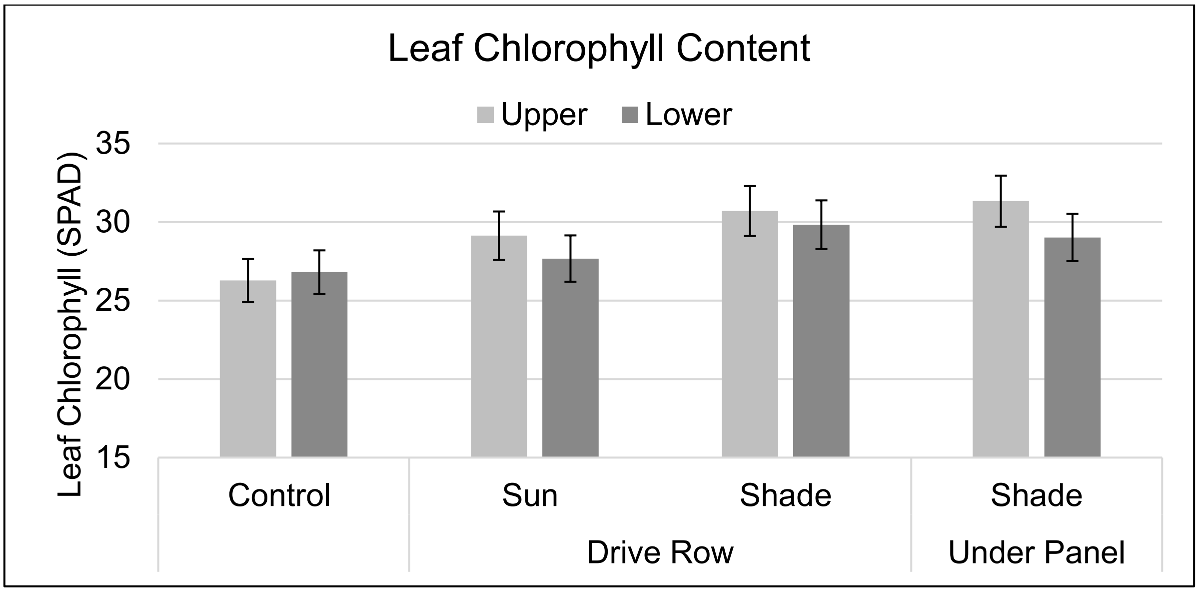


Figure 6. Average upper leaf and lower leaf chlorophyll content (SPAD) relative to the amount of shading from the panels, measured on June 14 and July 20, 2022. The 'shade-drive row' location received partial sun exposure depending on the time of day. Treatment differences (shading from panels) were not significant. Error bars represent the standard error of the mean.

Weed presence was greater within the array relative to the control (Figure 7). The shade drive row treatment (43 weeds/m^2) and the shade under panel treatment (28 weeds/m^2) had significantly greater weed numbers than the control (14 weeds/m^2). Although not significant, the treatments with the greatest number of blueberry stems with insect damage were the control (6.2 stems /m^2), followed by the sun drive row (4.1 stems /m^2), the shade drive row (2.5 stems /m^2), and the shade under panel (1.9 stems /m^2) (Figure 8). Disease presence also did not demonstrate significant treatment differences (Figure 9). However, the control exhibited the highest number of blueberry stems with disease (56.4 stems/m^2), followed by the shade drive row (41.6 stems /m^2), the sun drive row (26.7 stems /m^2), and the shade under panel (23.4 stems /m^2).

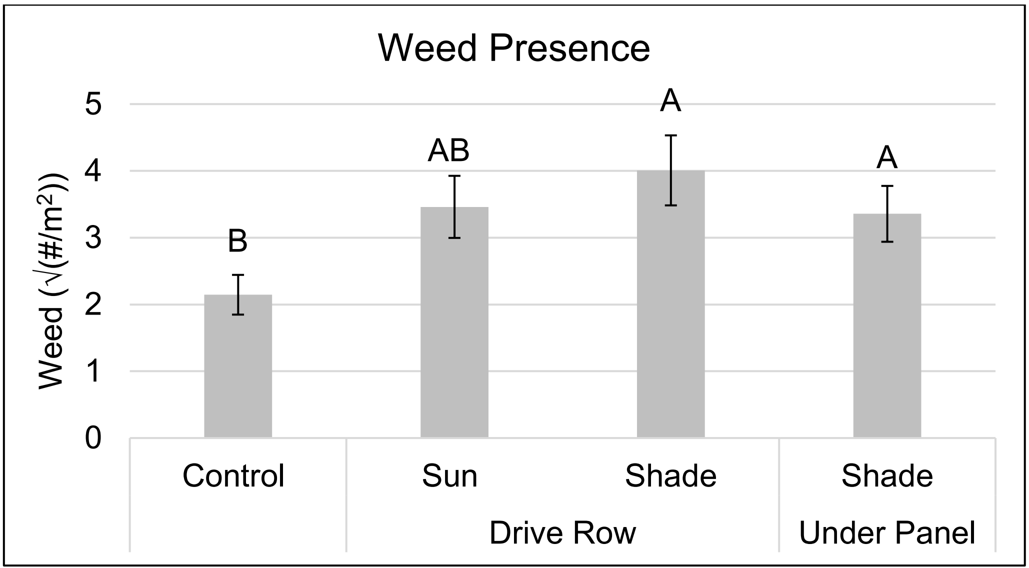


Figure 7. Average weed presence (transformed: √(#/m^2)) relative to the amount of shading from the panels, measured on May 17, June 14 and July 20, 2022. The 'shade drive row' location received partial sun exposure depending on the time of day. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean.

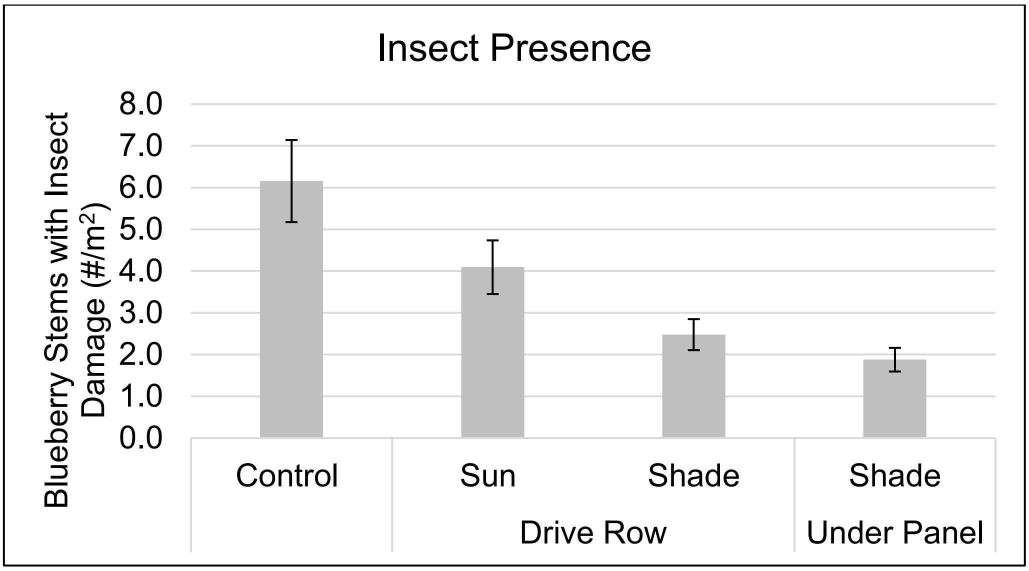


Figure 8. Average blueberry stems with insect damage (#/m^2) relative to the amount of shading from the panels, measured on May 17, June 14 and July 20, 2022. The 'shade drive row' location received partial sun exposure depending on the time of day. Treatment differences (shading from panels) were not significant. Error bars represent the standard error of the mean.

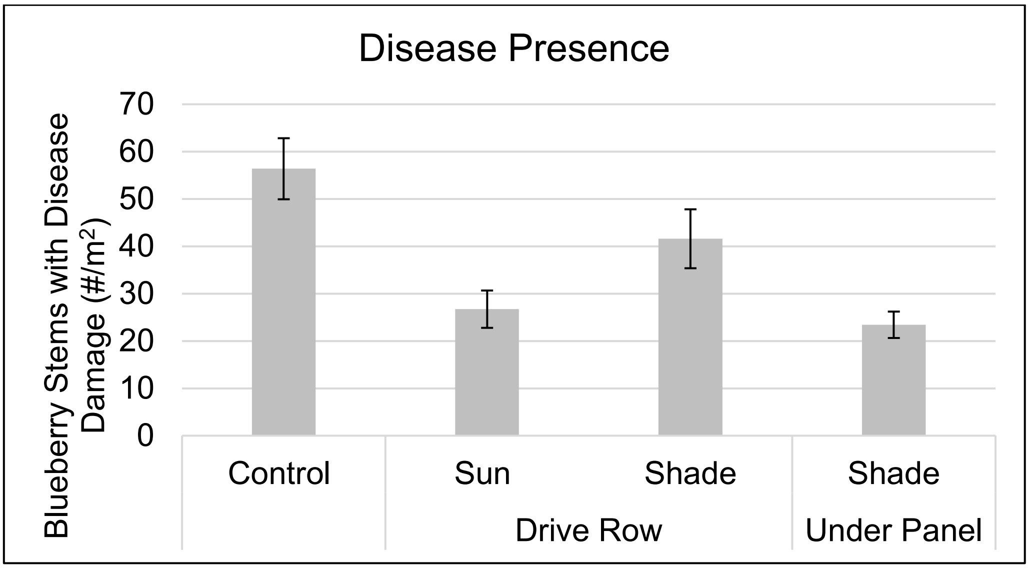


Figure 9. Average blueberry stems with disease damage (#/m^2) relative to the amount of shading from the panels, measured on May 17, June 14 and July 20, 2022. The 'shade drive row' location received partial sun exposure depending on the time of day. Treatment differences (shading from panels) were not significant. Error bars represent the standard error of the mean.

Phenology

On May 17, 2022, the blueberry stems in the control exhibited significantly higher bud numbers (2.8 buds/stem) compared to all other treatments (Figure 10). The stems in the shade drive row treatment exhibited the lowest average bud numbers (0.7 buds/stem). On June 14, 2022, treatment differences in phenological development were not significant (Figure 11). However, it is worth noting that blueberry stems in the control exhibited the highest number of pin heads (2.6 pin heads/stem) and green fruit (4.0 green fruit/stem). Similar to bud number, the lowest counts of pin heads (0.9 pin heads/stem), and green fruits (1.4 green fruit/stem).

At harvest on July 20, 2022, the greatest number of blue fruits occurred in the control (76%) and the sun drive row (82%) treatments (Figure 12). In contrast, the shade drive row and shade under panel were primarily dominated by the green fruit stage, with green fruit comprising 49% and 85% of the total fruit, respectively. These differences in fruit ripeness ratios by treatment suggest that the shaded treatments were behind in phenological development relative to the treatments receiving more sun. Additionally, the control may have been developmentally ahead of the treatments within the array, with 6% of the fruit counted as overripe (unmarketable) fruit.

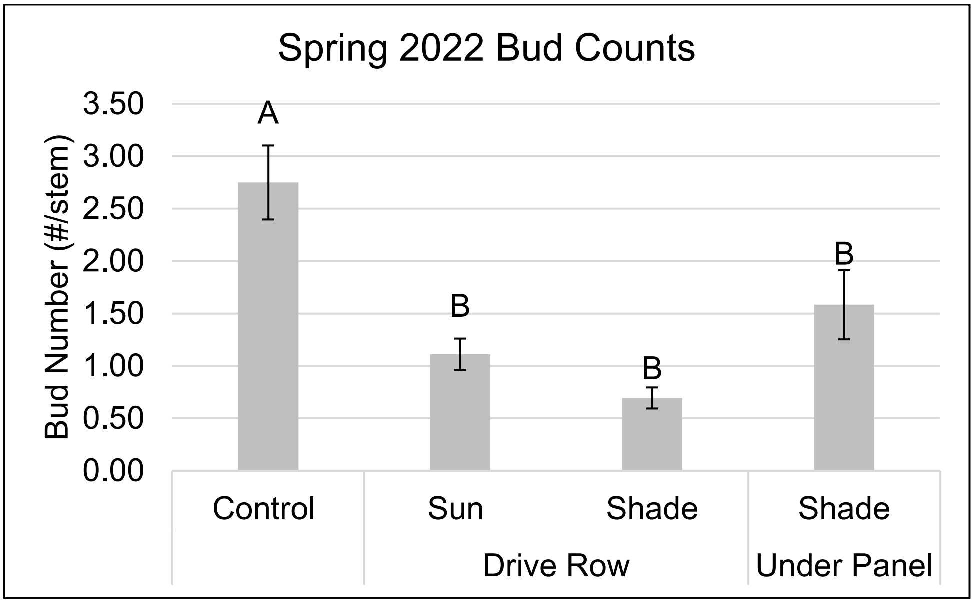


Figure 10. Average bud numbers (#/stem) relative to the amount of shading from the panels, measured on May 17, 2022. The ‘shade drive row’ location received partial sun exposure depending on the time of day. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean.

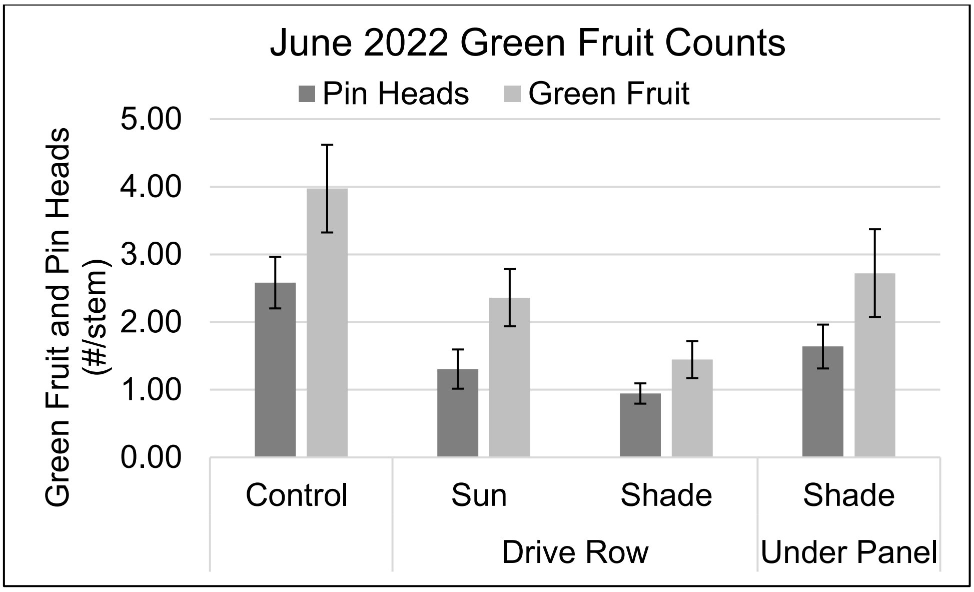


Figure 11. Average green fruit and pin head numbers (#/stem) relative to the amount of shading from the panels, measured on June 14, 2022. The ‘shade drive row’ location received partial sun exposure depending on the time of day. Treatment differences (shading from panels) were not significant. Error bars represent the standard error of the mean.

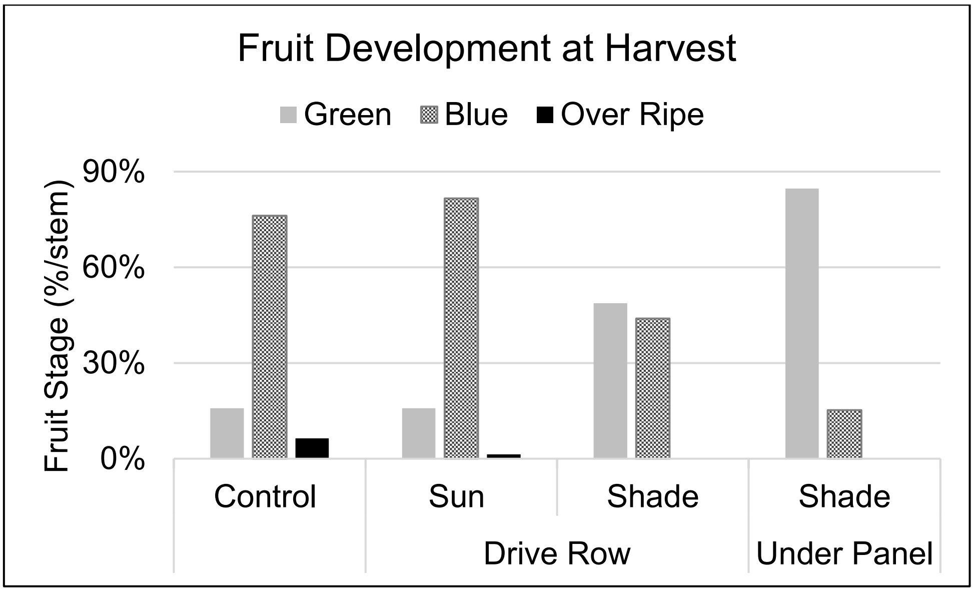


Figure 12. Average fruit development (%/stem) relative to the amount of shading from the panels at the time of harvest measured on July 20, 2022. The 'shade drive row' location received partial sun exposure depending on the time of day.

Fruit Yield & Quality

Fruit yield dramatically varied by treatment, with the greatest average yield harvested from the sun drive row treatment (1451 lbs/A), exceeding that harvested in the control (990 lbs/A) by 461 lbs/A (Figure 13). The yields of shade drive row (176 lbs/A), and the shade under panel (156 lbs/A) were significantly lower than that of the control and sun drive row treatments. The shade drive row and shade under panel yields were 87% and 89% less than the higher yielding treatment (sun driver row), respectively. While the higher percentage of green fruit in the shaded treatments suggests these treatments were developmentally behind, the low blueberry yield suggests lack of productivity in general.

When comparing blueberry yield by panel construction, the greatest yields were harvested in the standard-construction monofacial treatment (1112 lbs/A), exceeding that harvested in the control (990 lbs/A), the careful-construction bifacial treatment (686 lbs/A), and the mindful-construction bifacial treatment (528 lbs/A) (Figure 14). Treatment differences were not significant. Higher yields were hypothesized to occur under the bifacial panels due to the greater potential for light infiltration, however, the standard construction that occurred under the monofacial panels had a higher level of disturbance which the blueberry may have responded well to.

The highest berry sugar content, measured in Brix, was measured in the control (13.6 Brix), compared to the sun drive row (11.1 Brix), shade under panel (10.3 Brix), and shade drive row (9.9 Brix) (Figure 15). This corresponds to the ratios of phenological development, where a higher blue fruit ratio would inherently have higher berry sugar content.

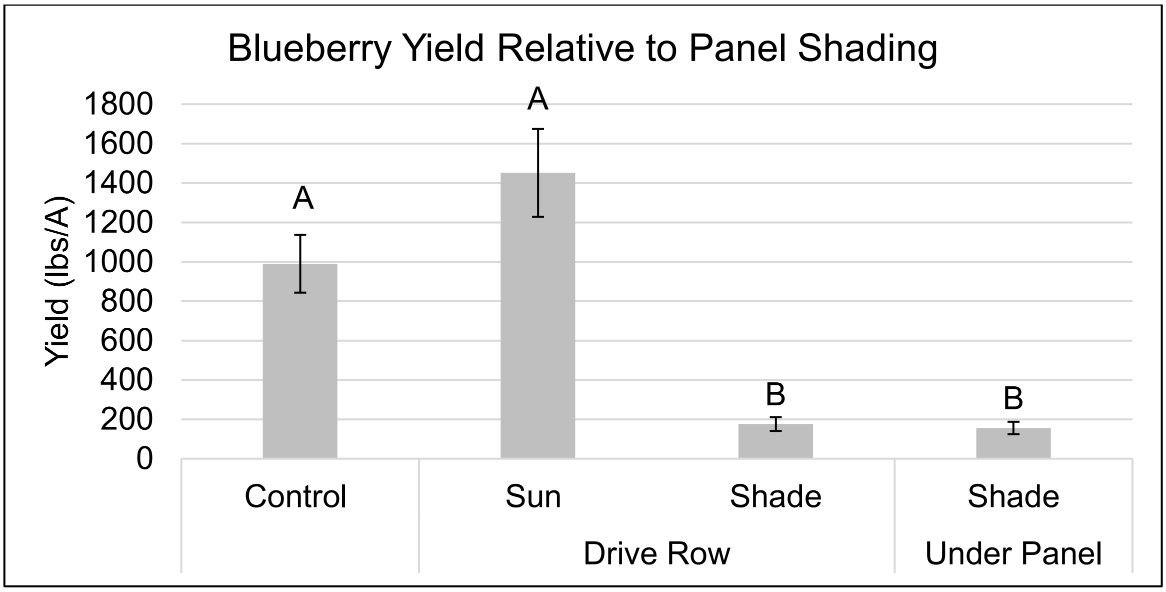


Figure 13. Average yield (lbs/A) relative to the amount of shading from the panels, harvested on July 20, 2022. The ‘shade drive row’ location received partial sun exposure depending on the time of day. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean.

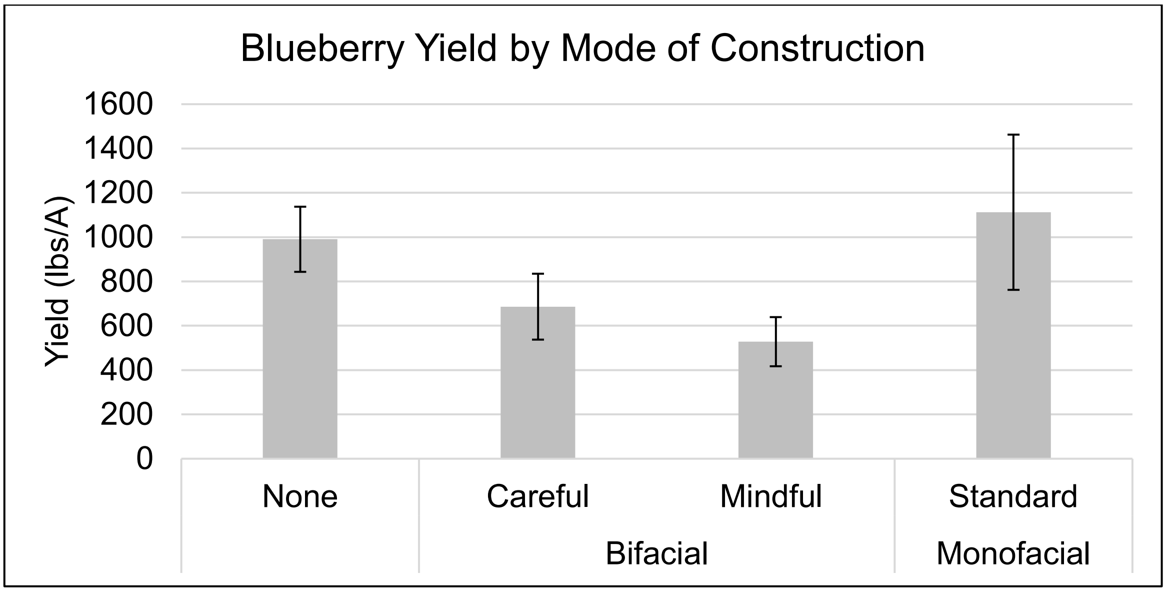


Figure 14. Average yield (lbs/A) relative to the mode of construction, harvested on July 20, 2022. The ‘shade drive row’ location received partial sun exposure depending on the time of day. Treatment differences by mode of construction and panel type were not significant. Error bars represent the standard error of the mean.

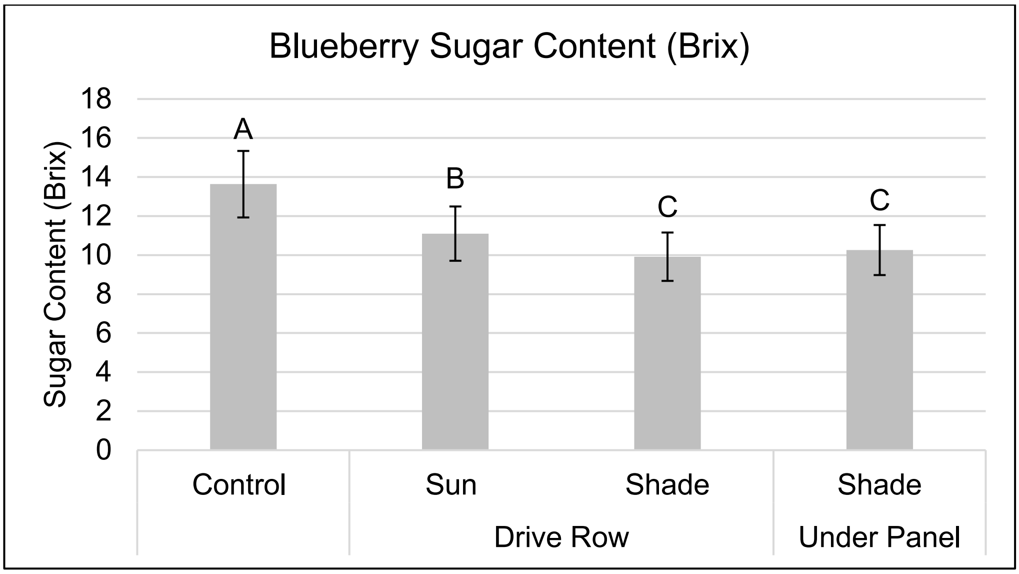


Figure 15. Average berry sugar content (Brix) relative to the amount of shading from the panels, harvested on July 20, 2022. The 'shade drive row' location received partial sun exposure depending on the time of day. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean.

A multivariate analysis evaluating the influence of independent variables (air temperature, relative humidity, PAR, soil moisture, soil temperature and SPAD) on the dependent variable of yield, exhibited a significant relationship for the model ($p < 0.0001$) with an R2 of 0.56 (1.0 would be a perfect 1:1 relationship; Table 1). Within the model, PAR had the greatest influence on yield ($p < 0.0001$; $t = 5.89$). A series of bivariate regressions provided a more in-depth view of each independent variable relative to yield (tested on an individual basis; Table 2). Here, PAR exhibited the most significant relationship ($p < 0.0001$; R2 = 0.44), followed by soil temperature ($p < 0.0104$; R2 = 0.14) and leaf chlorophyll content (SPAD; $p < 0.0298$; R2= 0.10).

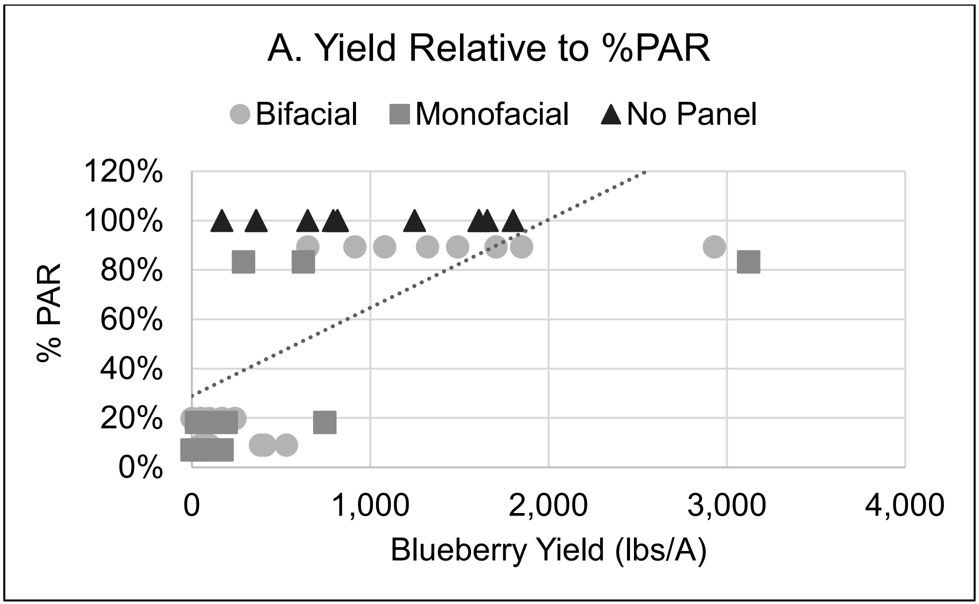
The linear regressions show the degree and the direction of the independent variables which had a significant relationship in the bivariate regressions (Figure 16). These variables included PAR, soil temperature and SPAD relative to yield (across all treatments).

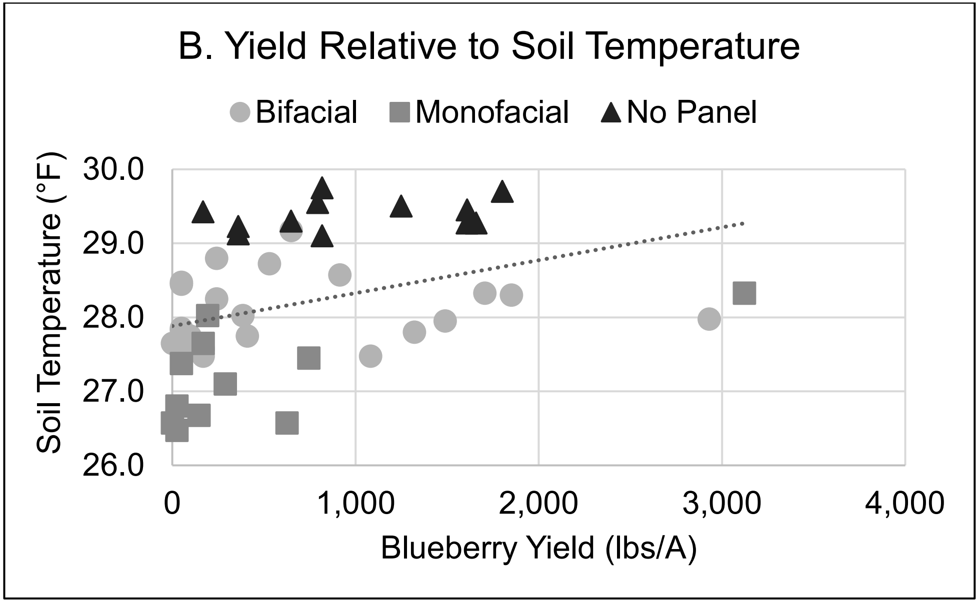
Table 1. Predicted influence of the independent variables (air temperature, relative humidity, PAR, soil moisture, soil temperature and SPAD), evaluate using a multivariate linear regression. Bold text indicates a significant linear relationship at the 0.05 level of significance.

Multivariate	**Dependent Variable: Yield**		
	R2	**F-value**	***p***
ALL	**0.56**	**8.6**	**< 0.0001**
Independent Variables:	**t-value**	***p***	
Air Temperature	-1.97	0.0559	
Relative Humidity	-1.15	0.2577	
PAR	5.89	**<0.0001**	
Soil Moisture	0.19	0.8528	
Soil Temperature	0.98	0.3318	
SPAD	-1	0.3224	

Table 2. Predicted influence of the independent variables (air temperature, relative humidity, PAR, soil moisture, soil temperature and SPAD), evaluate using a bivariate linear regression. Bold text indicates a significant linear relationship at the 0.05 level of significance.

Bivariate Independent Variables:	**Dependent Variable: Yield R2**	***p***
Air Temperature	4.70E-02	0.1426
Relative Humidity	0.01741	0.3766
PAR	0.44132	**< 0.0001**
Soil Moisture	5.86E-05	0.9593
Soil Temperature	**0.13727**	**0.0104**
SPAD	**0.10069**	**0.0298**





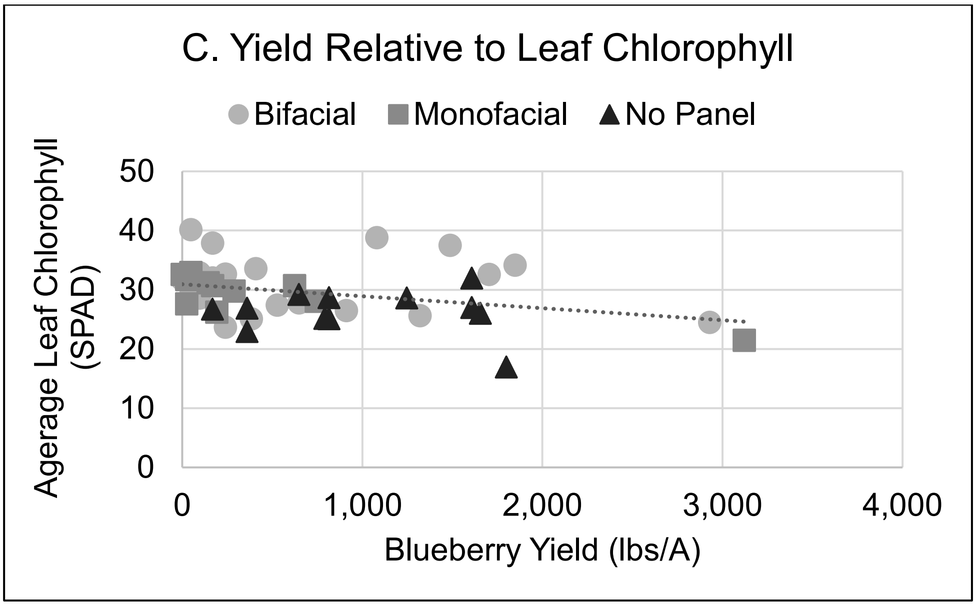


Figure 16A-C. Average blueberry yield (lbs/A) relative to average %PAR (Figure 16A; observed 10 AM - 2 PM), soil temperature (Figure 16B; °F) and average leaf chlorophyll (Figure 16C; SPAD). The dashed line represents the significant linear relationship between the two parameters. Datapoints are coded by panel type vs. control for additional descriptive detail.

DISCUSSION

This particular solar array in Rockport, ME was designed for energy output with farming as a secondary priority. Because of this, the rows between panels and height of panels were only slightly extended from standard array spacing. Our biggest take-home message for landowners considering a solar array is to determine whether the project is primarily for energy production or crop production.

Environmental Conditions (Soil, Air, Sunlight)

This array contains both monofacial and bifacial panels. Higher humidity levels under the bifacial panels during the time of maximum direct sun exposure could be an indication that more moisture from active plant respiration is trapped underneath the panels and this extra moisture is then heated further by the sunlight. This slight greenhouse effect was more pronounced in the 10 AM – 2 PM (maximum direct sun exposure) window under the bifacial panels and was more pronounced under the monofacial panels during the 9 PM – 5 AM (overnight) window. This suggests that there is more of a greenhouse effect on plants under monofacial compared to bifacial panels.

Research on highbush blueberries has identified the minimum threshold for good fruit yield from the plants as being 60% of full sunlight (2,000 PPFD) (Kim et al., 2011), or approximately 1,200 $\mu mol/m^{-2}/s^{-1}$ PPFD. These highbush blueberries grew under a range of sunlight conditions and there were significant differences in physical plant structure depending on the amount of sunlight received. Shaded plants produced fewer but longer shoots, more and larger leaves, thinner leaves with fewer stomata (which lowers the rate of photosynthesis), leaves with more chlorophyll, fewer flowers and thus fewer fruits. Some shading of the plant can prove beneficial since shade during hours of direct sunlight (10 AM – 2 PM) can improve plants' photosynthetic efficiency (Lobos et al., 2011), however, continual shade (of high shade intensity, so low sunlight intensity) may fail to produce these same benefits as we saw in Rockport this year.

The most sunlight reached the control since the only shading received there was from passing clouds. Sun drive row saw much more sunlight than the shade drive row and shade under panel rows, as expected. That the sun drive row received less sunlight than the control indicate there is still some shading from panels in sunny parts of the array. This experiment was not designed to approximate the amount of sunlight that was reaching the plants but the results show that the shading present was enough to reduce fruit yield significantly. The percent reduction from the control to shade was 91% and 93% for the bifacial and mono respectively much more than the functional 50% reduction in shade that allowed highbush blueberry to continue productivity (Lobos et al., 2011).

Plant Health

SPAD levels were highest where plants received the least amount of sunlight. Plants produce more chlorophyll in low-light conditions to maximize their potential to produce energy from the limited light. Generally, plants have higher SPAD levels on their lower leaves for this reason, but this trend was only observed in control this year. It is possible that the limited indirect light received by the plants underneath panels did not stimulate plants to produce more chlorophyll in those lower leaves. Perhaps producing more chlorophyll in low-light conditions was too costly for the plants.

Pests

There were significantly more weeds in treatments compared to the control, possibly because treatments were disturbed during construction, so weed seeds from the weed seed bank may have sprouted. Since the controls were situated outside of the area that experienced any construction and disturbance in the past few years, the wild blueberry is better-established and weeds are reduced in those control areas, possibly from chemical weed management techniques which have not yet been applied to areas cultivated within the solar array. The most weeds were measured in the shade drive row. The most insect damage was observed in the control and sun drive row, which are areas that received the most sunlight. Reasons for this need to be explored further, however some insects target healthy plants that were present in sunnier locations.

Phenology & Yield

The control produced the most buds (when measured in May), which correspondingly produced the most pin heads and green fruit a month later. The control then had high rates of blue fruit (76%) and the highest rate of overripe fruit (6%) of all treatments. However, the highest yield was harvested in the sun drive row, which contradicts the expectation that the undisturbed, unshaded control would have the highest yield. Therefore, it is possible the control did not have the highest yield because the fruit in the full sun, undisturbed conditions caused the fruit to overripen and drop from the stem before harvest.

Shade drive row produced the fewest buds in May and thus the fewest pin heads and green fruit a month later; at harvest, the fruit was nearly evenly split between green fruit and blue fruit. The shaded conditions prevented the plants from producing many buds and then delayed fruit development significantly. The final fruit yield was 9% of the fruit harvested in the drive sun row (the greatest yield).

Areas under the panels produced the second most buds and, correspondingly, the second most pinheads and green fruit a month later, but at harvest, nearly all the fruits were green fruit. Despite producing so many buds early in the season, the final fruit yield was so low that it was only 8% of the fruit harvested in the drive sun row (the greatest yield).

Wild blueberry is a plant that thrives best in full sun conditions. Introducing consistent shade over the plants impacted plant development and success in producing fruit. It was clearly observed that full and partial shade delayed plant development and reduced fruit yield to a fraction of the yield collected in full sun. Plants in the shade underneath the panels had fruit ripening profiles inverse to those of the sun drive row: full shade plants were 85% green and 15% blue at harvest but shade drive row was 16% green and 82% blue fruit at harvest. Therefore, the amount of sunlight received by plants in partial shade is more likely to result in ripe fruit at harvest than in full shade conditions.

The most fruit was harvested within standard monofacial areas and the control, far exceeding the yield in the careful and mindful bifacial areas. Though the bifacial panels, by design, allow more sunlight to penetrate the panels, the bifacial panels are situated on a steeper slope than monofacial panels. The inconsistent slope of the ground beneath the panels confounds the results and makes it impossible to clearly understand why yields varied by panel type.

Fruit Quality

Brix levels were significantly higher in the control compared to treatments, likely because the control plots contained more overripe fruit. As fruit ripens, sugar content within the fruit increases, and as the control was the only treatment to observe overripe fruit, the Brix levels from the sampled fruit were higher. The other treatments had more green fruit (low Brix level) and no overripe (high Brix level) fruit. As most wild blueberry growers harvest entire fields simultaneously, rather than harvesting one plant at a time during peak ripeness, the variability in fruit ripeness and corresponding Brix levels is important for growers regardless of the form in which berries are sold.

CURRENT RECOMMENDATIONS

- When considering a solar array, decide if it the main goal is food or energy production.
- If managing the crop underneath, plan to manage for weeds and pests underneath the panels.
- If managing the crop underneath, plan to manage for harvest in the sun drive row between panels. Additional complexities and labor associated with harvesting directly underneath the panels will make the extra effort unprofitable.

NEXT STEPS

- Continue data collection through 2024
- Compile cost-benefit analysis in 2023 and 2024

ACKNOWLEDGEMENTS

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INVESTIGATORS: Abigayl Novak, Jay Wason, Ling Li, & Yong-Jiang Zhang

10. Biochar application mitigated the effect of drought on wild blueberries

OBJECTIVES

1) Test the effect of biochar in mitigating the effect of rainfall shortages and drought on wild blueberry plants.
2) Quantify the physiological response of plants in biochar-amended soils and soils without biochar to drought.
3) Assess the influence on soil pH by untreated biochar and pH-modified biochar.

LOCATION: Rogers Farm, University of Maine, Orono, ME
PROJECT TIMEFRAME: January 2021 to December 2022

INTRODUCTION

Drought is a major limiting factor on agricultural production, and is predicted to intensify in many regions of the world (Fernandez et al., 2020a; Pörtner et al., 2022). In recent years, the Northeast region of the United States has experienced an increase in drought conditions with predictions for continued impacts on crop productivity (Kang et al., 2009). Further, heatwaves (hot days; temperatures above 32°C (90°F) for two or more consecutive days (NOAA, 2022)) are expected to increase in many regions. For instance, heatwaves in Maine are expected to increase by two- to four-fold by the 2050s (Fernandez et al., 2020b). The most recent heatwave in Maine occurred in the summer of 2022 (WMTW 2022). Partly as a result of the challenges imposed by drought and warming, and other uncertainties such as spring frosts, on the wild blueberry plants, the harvested acreage of wild blueberries declined by 1,400 acres, as 22,400 acres were harvested in 2015 and 21,000 acres in 2021 (Calderwood et al., 2022). Total yields also declined from around 100 million lbs in regular years to 47 to 68 million lbs in dry years such as 2017 and 2020 (Calderwood et al., 2022). Therefore, there is an urgent need for the development of solutions to address drought mitigation.

Compared with other drought management practices, such as irrigation systems, mulching, and adopting drought-resistanct varieties, maximizing the water-holding capacity using soil amendments like biochar is a promising solution due to the additional benefits, like providing nutrients, immobilizing heavy metals, and carbon storage. Many studies revealed that the effects of biochar additions on the improvement of water retention capacity of sandy soils are significantly greater than other soil types, such as clay soil (Gumbrewicz & Calderwood, 2022; Basso et al., 2013; Yu et al., 2013; 2017;).

There has been little research on the drought response of wild blueberries and how biochar applications can improve their performance under drought conditions (Glass et al., 2005; Pahadi, 2021). Wild lowbush blueberries (*Vaccinium angustifolium Aiton*) residing in sandy soils, are sensitive to drought because of the low water retention of sandy soils. Biochar has the potential to be an environmentally-friendly soil amendment to overcome this challenge. This study aimed to test whether locally-produced biochar could mitigate the effects of rainfall shortage and drought on the physiological performance of wild blueberry plants with minimum disturbances to the soil pH.

METHODS

Plant and biochar materials

The wild blueberry (*Vaccinium angustifolium*) plants were transplanted from Wyman's farm. Plants from five (year 1, 2021) or six (year 2, 2022) different genotypes were used. The plants were transported to a greenhouse located at Rogers Farm at the University of Maine. During both years, new plants were used, where year 1 plants were transplanted in July and year 2 plants were transplanted in May.

The biochar material was obtained from a local combined heat and power plant, Maine Woods Pellet Co. (Athens, ME, USA). Biochar was produced using low-quality forestry biomass, such as bark, branches, leaves, and wood chips. Untreated biochar with a pH of ~11.40 was used in 2021, while pH-modified biochar (pH of ~6.5) using a 2% acetic acid solution was used in 2022.

Ammonium sulfate (nitrogen-based) fertilizer and Sulfate of Potash (potassium-based) fertilizer were purchased from Northeast Agriculture Sales Inc. (Detroit, ME, USA) and used as the fertilizer mixture.

Experimental Design

A randomized block design of two factors (biochar and drought treatment; two by two) and five (year 1) or six genotypes (year 2) were implemented. The pots for growing the individual wild blueberry plants were also randomly assigned. Genotype was used as a random factor. In each block for each year, there was one replicate of each unique treatment combination of drought (2 levels), biochar (2 levels), and genotype (4-6 levels). There were 16 (year 1) and 24 (year 2) pots of wild blueberries for a total of 80 pots (year 1) and 144 pots (year 2). In the greenhouse, wild blueberries had an establishment period of 21 days, with a regular watering schedule at 8:00 and 16:00 for 10 minutes at a rate of 1.3L per hour.

The four treatments included: biochar with drought treatment, no biochar with drought treatment, biochar regularly irrigated, and no biochar regularly irrigated. For the biochar amended soils (BA soils), the soils were mixed, with 50% sandy soil from Wyman's farm (the source of the plants) and 50% biochar with an addition of ammonium sulfate and sulfate of potash at a 1:1 ratio by volume. For the drought treatment, watering stopped when the drought treatment began and the plants were allowed to dry out naturally. The drought treatment started on August 10, 2021, and July 4, 2022. When the mean value of the wild blueberry plants reached a midday leaf water potential (ψleaf) of -7MPa the drought was terminated. The control plants were regularly irrigated twice a day using irrigation lines, in the morning and early evening, through the end of the experiment period.

The relative humidity and temperature changes inside and outside the greenhouse were recorded using a ZL6 weather station (METER Group Inc., Pullman, WA, USA) for year 1 (2021) and an Onset HOBO weather station for year 2 (2022) (Onset, Bourne, MA, USA). The soil water potentials of the top 5 cm (1.96 inches) soil layer (n = 3 for biochar in drought and no biochar in drought treatments) were measured using TEROS 21 soil water potential sensors connected to ZL6 data loggers (METER Group Inc., Pullman, WA, USA). Soil temperatures were measured using a FieldScout TDR 150 Soil Moisture Meter (Spectrum Technologies, Inc., Aurora, IL, USA). Temperatures inside the greenhouse were typically 1 to 3°C higher than the daily maximum ambient temperatures outside of the greenhouse, which ranged from 2.8 to 24°C.

Measurements of Plant Physiological Properties

Midday leaf water potential (ψ_{leaf}) was measured using a leaf pressure chamber (Model 1505D; PMS Instrument Company, Corvallis, OR, USA). At least 12 samples were taken on a three day interval or longer depending on the previous leaf water potential (ψ_{leaf}) measurement and how fast the plants were drying. Midday stomatal conductance (g_s) was measured using the LI-600 portable porometer

(LI-600; Li-Cor Biosciences, Lincoln, NE, USA). Electron transport rate (ETR) for two leaves per plant was measured using a Y(II) Meter (Opti-Sciences Inc., Hudson, NH, USA) under ambient conditions in the greenhouse (Stratoulias et al., 2015). Leaf chlorophyll concentration was measured using a chlorophyll meter (SPAD 502, Minolta Corp., Osaka, Japan), and the anthocyanin concentration was measured by an ACM-200 anthocyanin meter (Opti-Sciences Inc., Hudson, NH, USA). These were taken once per week on all plants from establishment through the drought period on a representative leaf of each plant.

Measurement of Soil pH

The pH of soil samples with biochar and without biochar was measured by making a soil slurry and tested using Fisher brand pH strips (Fisher Scientific, Pittsburgh, PA, USA). This was done once per week in year 1 (2021) and before, during, and after the drought in year 2 (2022).

Statistical analysis

To visually show how different treatments impacted the physiological properties and soil pH, time series plots of averages across genotypes and blocks were made using RStudio (RStudio Team, 2020). To determine whether there were significant effects of different treatments, day, and block, a linear model (lm) was used to conduct an analysis of variance (ANOVA) to test the effects of biochar treatment (biochar or no biochar), drought treatment (yes or no), block, and day.

RESULTS

Changes in leaf water potential during the drought treatment

During drought treatment, midday leaf water potential (ψ_{leaf}) of drought-treated plants in biochar-amended (BA) soils and no biochar amended (NA) soils started to decline on day 21 in 2021 and day 20 in 2022. Irrigated plants grown in BA soils showed no difference in midday ψ_{leaf} compared to plants in NA soils in 2021 and 2022 (Figure 1). There was a significant effect of biochar treatment on midday ψ_{leaf} during the drought treatment in year 1 ($p < 0.001$). Plants grown in soils without biochar reached the turgor loss point (Ψ_{tlp}; -2.45 ± 0.08 MPa) on day 20, while plants grown in BA soils reached Ψ_{tlp} on day 33 in 2021 (Figure 1a). At the end of the drought treatment (day 64 in 2021), the plants in NA soils had a mean value of -6.81 ± 0.33 MPa, and those in BA soils in drought at a mean value of -4.50 ± 0.49 MPa (Figure 1a).

During year 2 (2022), there was a sharp decline in midday ψ_{leaf} of plants in BA and NA soils under drought treatment from day 18 to day 20 that may have been driven by a heatwave (Figure 1b). Midday ψ_{leaf} reached a mean value of -3.91 ± 0.93 MPa on day 19. Therefore, there is no difference in year 2 (2022) in time reaching the Ψ_{tlp}. The heatwaves resulted in massive leaf shedding, and both plants in BA and NA soils experienced extreme canopy dieback. On day 48 of year 2 (2022), at the end of the drought, the plants grown in BA soils in drought had a mean value of -7.30 ± 0.54 MPa.

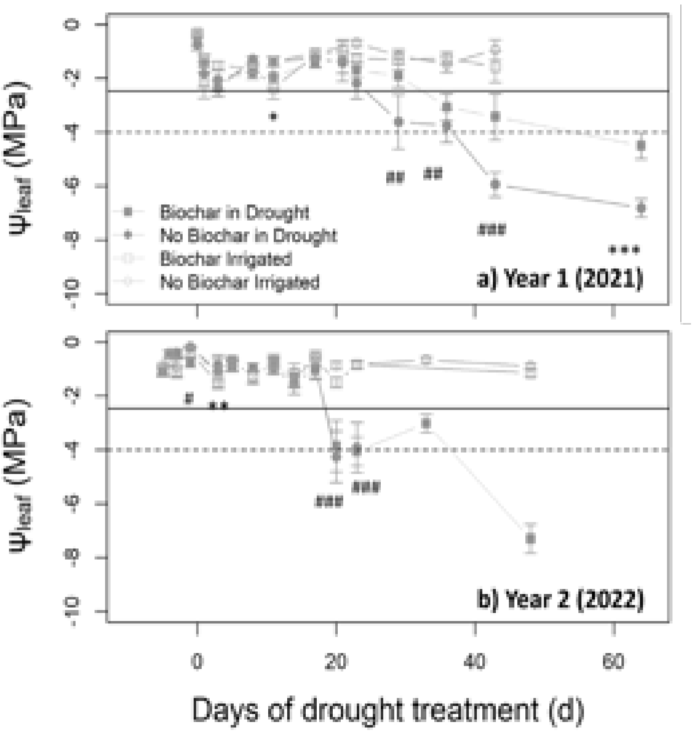

Figure 1. The midday (ψ_{leaf}) of year one (a) and year two (b) over time. Treatments are as follows: biochar in drought (closed squares), no biochar in drought (closed circles), biochar irrigated (open squares), and no biochar irrigated (open circles). The horizontal solid line indicates the turgor loss point (Ψ_{tlp}) at -2.45 ± 0.08 MPa of all genotypes, and the horizontal dotted line at -4.00 MPa represents the period of extreme drought for wild blueberry plants (Pahadi, 2021). Values are mean midday (ψ_{leaf}) ± SE values with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment. Drought significance indicated by # and biochar treatment differences indicated by *. ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$ (applies to * and # significance).

Changes in leaf stomatal conductance and ETR during the drought

In 2021, stomatal conductance (g_s) of irrigated plants in both BA and NA soils ranged from 0.04 to 0.25 mol $m^{-2}s^{-1}$ (Figure 2a). In 2022, g_s of irrigated plants ranged from 0.05 to 0.15 mol $m^{-2}s^{-1}$ (Figure 2b). Overall, drought treatment resulted in significant declines in stomatal conductance (g_s) in both years (Figure 2). The declines in g_s of plants in NA soils started on day 19 in 2021 and day 16 in 2022. In 2021, plants in NA soils reached the observed minimum level g_s (< 0.03 mol $m^{-2}s^{-1}$) on day 22, while plants in BA soils did on day 44 (Figure 2a). In 2022, plants in NA soils reached minimum g_s on day 18, while plants in BA soils did on day 21 (Figure 2b).

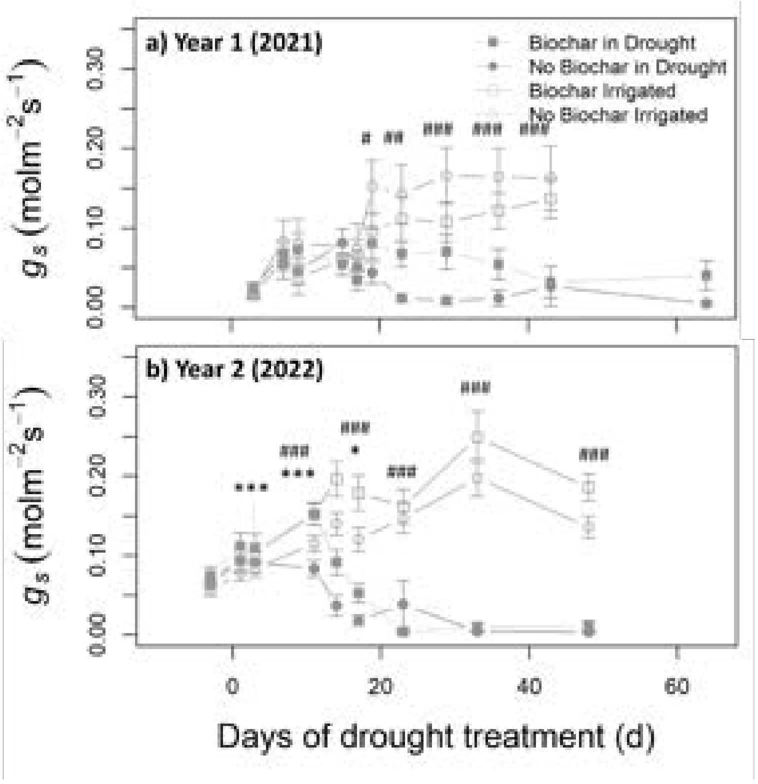

Figure 2. The midday g_s of year one (a) and year two (b) over time. For day 64 of Year 1 (2021), values of irrigated treatments are missing due to limited resource availability. Treatments are as follows: biochar in drought (closed squares), no biochar in drought (closed circles), biochar irrigated (open squares), and no biochar irrigated (open circles). Values are mean stomatal conductance (g_s) ± SE values with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment. Drought significance indicated by # and biochar treatment differences indicated by *. ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$ applies to * and # significance).

In 2021, no significant differences in ETR were found between irrigated plants and plants under drought treatment (Figure 3a). The slight declining trend over time could be because of leaf senescence. Drought-treated plants amended with and without biochar maintained mean values of ETR of 40.74 ± 16.98 to 91.40 ± 8.23 μmol $m^{-2}s^{-1}$ and 21.86 ± 5.87 to 73.10 ± 7.55 μmol $m^{-2}s^{-1}$, ranges derived from each day of the drought treatment (Figure 3a). In 2022, declines in ETR of drought-treated plants in NA soils started on day 14; drought-treated plants in NA soils showed significantly lower ETR compared to irrigated plants and drought-treated plants in BA soils on day 14 (Figure 3b). Declines in ETR of drought-treated plants in BA soils started on day 17. On day 23 and days after, no differences in ETR were found between drought-treated plants in BA and NA soils, while both showed significantly lower values compared to the irrigated controls (Figure 3b). There was a significant interaction between biochar treatment and drought ($p < 0.05$).

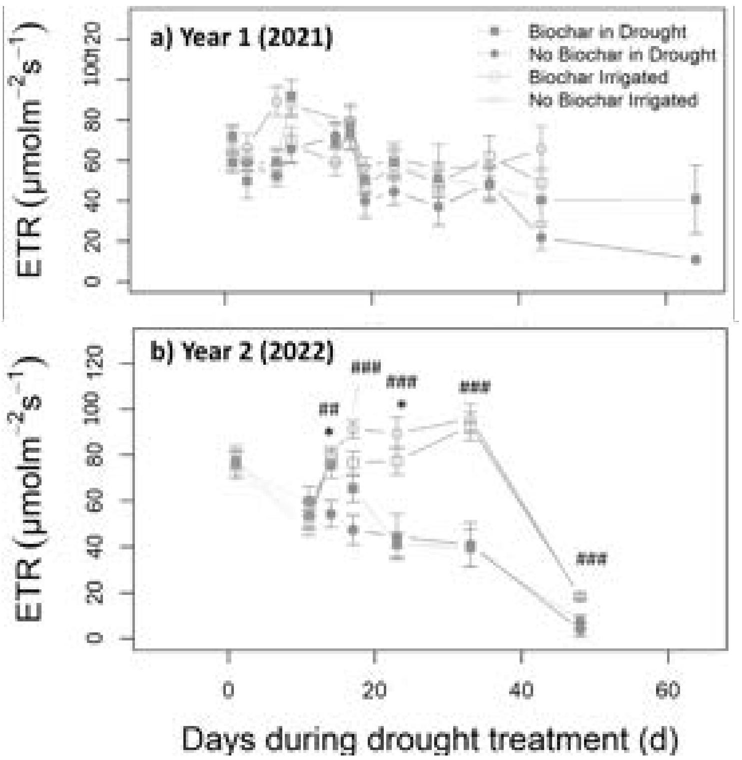

Figure 3. Photosynthetic electron transport rate (ETR) over time during the drought treatment of year 1 (a) and year 2 (b). Treatments are as follows: biochar in drought (closed squares), no biochar in drought (closed circles), biochar irrigated (open squares), and no biochar irrigated (open circles). Values are mean ETR ± SE values with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment. Drought significance indicated by # and biochar treatment differences indicated by *. ****p* < 0.001, ***p* < 0.01, and **p* < 0.05 (applies to * and # significance).

Changes in leaf chlorophyll and anthocyanin concentrations during the drought treatment

Overall, drought treatment showed no significant impact on anthocyanin concentration in both 2021 and 2022 (Figures 4a and 4c). However, in 2021, anthocyanin concentration of drought-treated plants started to increase on day 9, while it did so in irrigated plants on day 14. In 2022, biochar treatment showed a significant impact on anthocyanin concentration before the drought treatment and during the early stage of drought treatment (days 2 and 9). In 2022, anthocyanin concentration (Figure 4c) remained relatively low throughout the drought experiment, compared to a steady increase in 2021.

In 2021, when drought treatment occurred during late summer, drought treatment showed no significant impact on chlorophyll concentration, while biochar showed a significant impact on three days (days 0, 9, and 23; Figure 4c). Chlorophyll concentrations of plants in NA soils started to decline on day 9, while that of plants in BA soils (both irrigated and under drought treatment) did so on day 14 (Figure 4c). In 2022, when drought treatment occurred during early summer, no declines in chlorophyll concentrations were found in irrigated plants and drought-treated plants in BA soils (Figure 4d). In contrast, drought-treated plants in NA soils showed a distinct decline on day 22 (Figure 4d).

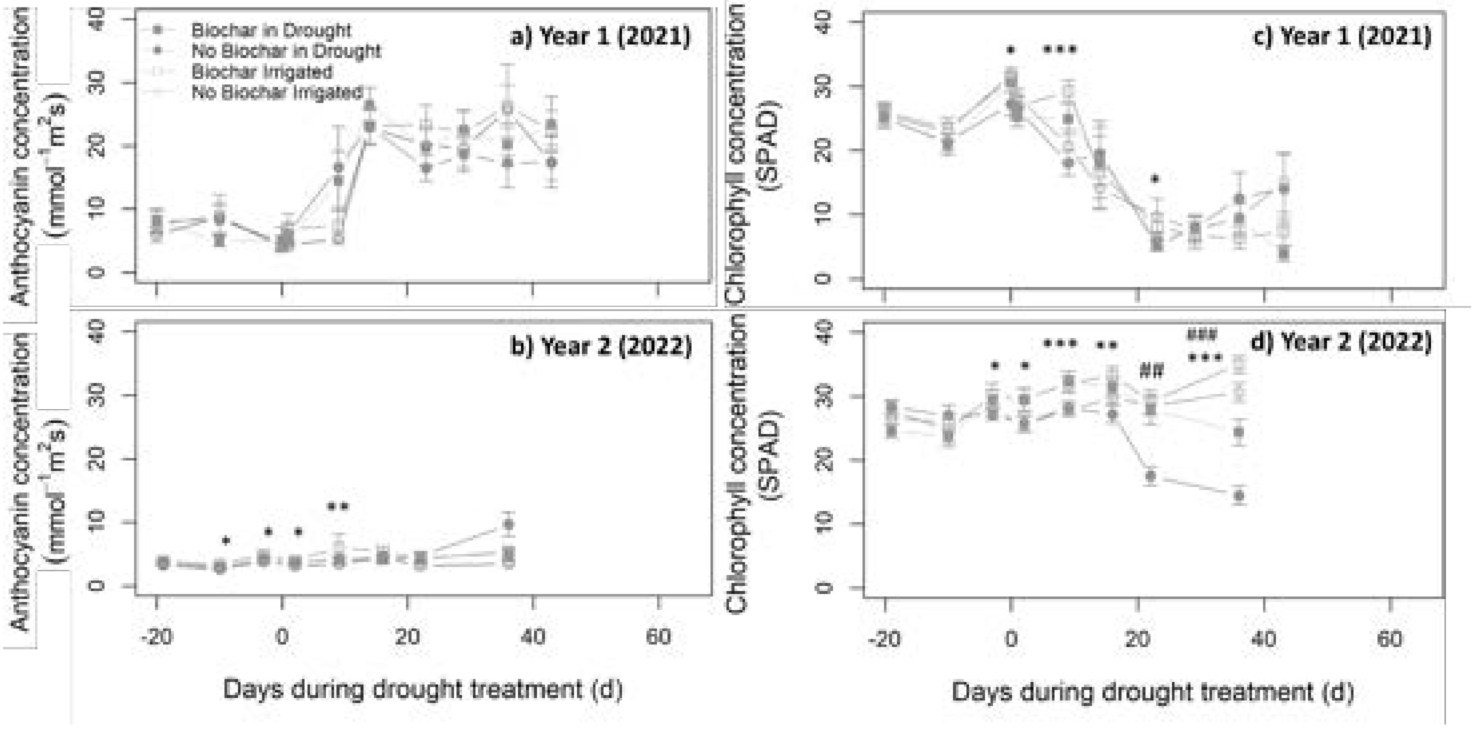

Figure 4. The anthocyanin (ACM) concentration and chlorophyll (SPAD) concentration during year 1 (a,c) and year 2 (b,d) of the drought experiment. Treatments are as follows: biochar in drought (closed squares), no biochar in drought (closed circles), biochar irrigated (open squares), and no biochar irrigated (open circles). Values are mean anthocyanin ± SE and chlorophyll ± SE with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment. Drought significance indicated by # and biochar treatment differences indicated by *. ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$ (applies to * and # significance).

Soil pH

There was no significant change in soil pH over time during the experiment period in both 2021 and 2022 (Figures 5a and 5b). In 2021, the soils amended with biochar (pH not modified) showed significantly higher ($p < 0.001$) pH (6.30 ± 0.07), compared to NA soils (pH 5.33 ± 0.06). In 2022, when pH-modified biochar was used, there was no significant difference in pH among all treatments (NA and BA soils).

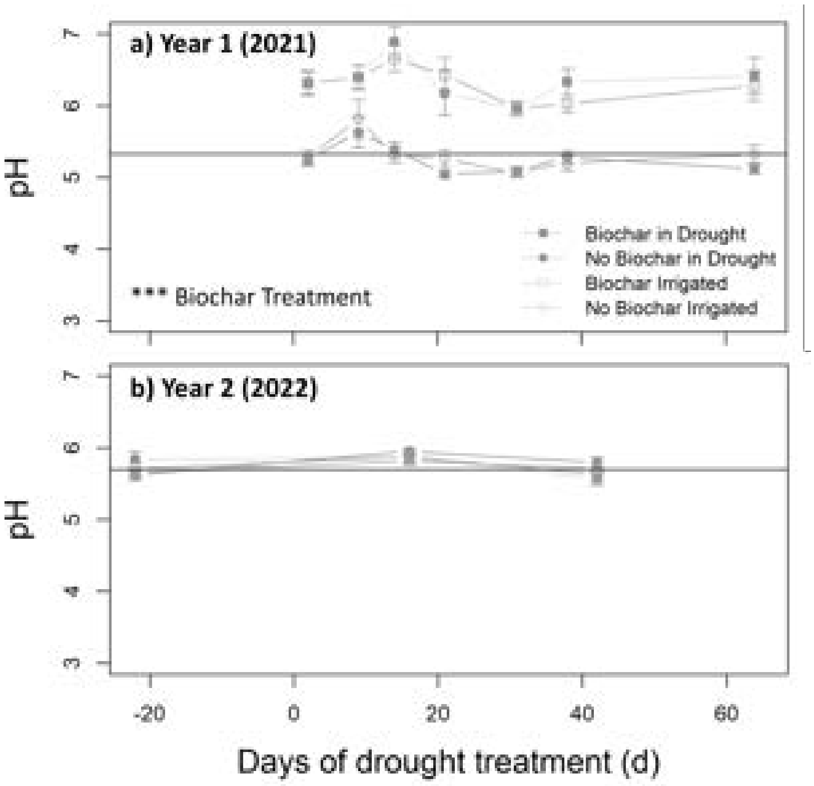

Figure 5. The soil pH of raw biochar in year 1 (a) and pH-modified biochar in year 2 (b) during the drought experiment. Treatments are as follows: biochar in drought (closed squares), no biochar in drought (closed circles), biochar irrigated (open squares), and no biochar irrigated (open circles). The line at approximately 5.33 (2021) and 5.70 (2022) shows the pH in the control with no biochar, indicating the optimal soil pH for wild blueberries. Values are mean soil pH ± SE values with at least 5 replicate measurements and are shown as standard error bars of that corresponding treatment. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ (applies to * and # significance).

DISCUSSION

Our study reveals the positive effects of biochar application in delaying the onset of soil water deficit, and consequently, biochar is effective in delaying the influence of rainfall shortage on plant physiological performance of wild blueberries. In a normal year of 2021 without heatwaves, the delayed decline of midday leaf water potential (ψ_{leaf}) could be mainly due to the effect of biochar in enhancing the water-holding capacity of sandy soils. Interestingly, when plants underwent an unexpected heatwave (2022; days 18 to 20), a delay in the decline of midday leaf water potential (ψ_{leaf}) was not found despite higher soil water potentials in BA soils.

Biochar delayed the decline of leaf water potential during the drought treatment

We found that biochar applications delayed the declines in midday ψ_{leaf} when the water supply stopped. Year 1 (2021) results showed that plants in BA soils reached leaf turgor loss point **13** days later, and reached the water potential of hydraulic impairment (-4 MPa; Pahadi, 2021) **16** days later, compared to plants in NA soils. From the perspective of management, **this > two-week delay of drought impact** will allow growers to finish harvesting or take mitigation actions such as temporary irrigation. However, during an unexpected heatwave (days 18 to 20) in 2022, biochar applications did not result in delayed declines in ψ_{leaf}, and plants growing in BA soils were also impacted heavily by the heatwaves. Indeed, plants growing in BA soils showed higher canopy dieback compared to those in NA soils. Under heatwaves, our plants experienced high water loss due to high evaporative demands (high vapor pressure deficits, VPD). Even though BA soils have a higher amount of available water compared to NA soils, the plants could reach the same low water potentials as those in NA soils. The impact of heatwaves on wild blueberry fields has not been well studied, but recent studies suggest that warming will negatively impact wild blueberries (Tasnim et al., 2020). The potential effect of biochar applications in wild blueberry plant response to heatwave asks for further studies.

Biochar helped maintain g_s, ETR, and chlorophyll during the drought

Our results show that wild blueberry plants growing in BA soils under drought conditions maintained higher stomatal conductance (g_s) values compared to plants in NA soils. However, under the heatwaves, biochar applications only showed an effect on g_s before and during the heatwave (days 18 to 20), and g_s declined to the observed minimum level after the heatwave. Higher g_s of plants grown in soils during the heatwave could be because of higher soil water potentials compared to plants in NA soils, which did not result in abscisic acid (ABA, a plant hormone) synthesis in roots to simulate stomatal closure (Ali et al., 2017; Mahmoud et al., 2022). This finding suggests that when crops are experiencing rainfall shortages, biochar amendment in soils can delay the plants from experiencing soil water deficits and drought effects. However, when they are experiencing heatwaves, high soil water content and high g_s of plants in BA soils could result in large transient plant water loss and leaf dieback. The electron transport rate (ETR) values agree with the g_s that plants in BA soils were able to maintain higher photosynthesis under drought due to the delay of soil water deficits. This was also seen in year 1, and in year 2 before the heatwaves. There were declines in chlorophyll concentration in plants under all treatments (including irrigated plants) toward the end of the drought treatment in year 1. This could be driven by leaf senescence in the late summer and fall. However, limited water availability can also induce declines in chlorophyll concentration of wild blueberries, as found in year 2 (NA soils under drought), and in other studies (Percival et al., 2012). The increase in anthocyanin concentration in year 1 is also probably related to leaf senescence (Zhou, 2015), as no increase in year 2 (early summer) was found. However, drought treatment did accelerate anthocyanin synthesis and leaf sentences in year 1.

Biochar effects on soil pH

Biochar did change soil pH. During year 1, the pH of BA soils ranged from 6 to 7 when alkaline biochar (pH of ~11.4) was used, significantly higher than the average pH of 5.4 in NA soils. This introduced a problem because wild blueberries naturally live in soils with a pH less than 5 (Drummond et al., 2009). Although no effects of changed pH on plant performance were found in greenhouse tests, long-term impact in the fields needs to be considered. Higher soil pH could alter the existing environment and weed presence, which might require mitigation management such as soil application of sulfur. For year 2, the soil pH was ~5.7 when pH-modified biochar was used. Considering the low concentration (2%) of acetic acid solution used, this method would be accessible and affordable for growers when they have to deal with alkaline biochars.

CURRENT RECOMMENDATIONS

Adding biochar as a soil amendment aids the wild blueberry plants by delaying the onset of soil water deficits and leaf water stress in wild blueberries during rainfall shortages. During heatwaves, biochar application and improved soil water conditions could help maintain high stomatal conductance and photosynthesis of wild blueberries, but this can result in high water loss and leaf shedding. However, improved soil water conditions can help plants to maintain better water status and physiological performance after the heatwave. As drought conditions continue to intensify and impact the agricultural systems, amending proper biochar to sandy soils would be an effective method to mitigate the effect of rainfall shortages or climate drought.

NEXT STEPS

This greenhouse project is complete. Further research on mid-term and long-term field studies will be considered to confirm the findings of our greenhouse study in the field. The best rates of field biochar application also need to be determined.

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11. Whole Field Mulching for Wild Blueberry Drought Management

OBJECTIVES

- Identify the thickness at which wood chip mulch increases soil moisture when applied across whole fields.
- Monitor the impacts of mulch thicknesses on plant productivity.
- Document the time and costs associated with applying mulch to whole fields using two application methods.
- Document harvesting after mulch application in the field and at a processor facility in 2023.

LOCATIONS: Orland and Hope, Maine
PROJECT TIMEFRAME: August 2021 – February 2024

INTRODUCTION

Wild blueberry plants thrive in sandy, well-draining soils yet these same soils pose challenges to plant growth during times of drought due to their low water-holding capacity. Maine’s seasonal droughts often occur during the summer months of June, July, and August which are critical growing months in both prune and crop years for wild blueberry. While drought conditions are not uncommon across Maine, moderate to severe drought conditions negatively impact fruit yields across the state.

Much of the state of Maine was in drought for most of 2016 (Birkel, 2016), and moderate drought persisted across coastal Maine through summer 2017, with much of the Downeast and Midcoast regions receiving less precipitation than normal (NOAA/NIDIS et al., 2017; Birkel, n.d.). Moderate drought conditions continued through 2018, with several high-volume precipitation events in July (NOAA/NIDIS, 2018). Summer 2019 saw late snow cover across most of the state yield to a cool, wet June which quickly became a hot and dry July before becoming a warm and wet August (Birkel, 2019). Hail fell in Maine and heavy rain events were not uncommon (NOAA/NIDIS, 2019).

Summer 2020 was the third warmest and fourteenth driest since 1895 (Birkel, 2020), with extremely low rainfall from May – September 2020 producing extreme drought conditions across much of New England (Birkel, 2021). Late frosts in June reduced flower numbers (NOAA/NIDIS, 2020), and in conjunction with drought conditions during the summer, there were large wild blueberry yield losses across the state (NOAA/NIDIS, 2020; Calderwood et al., 2022). The 2020 growing season saw an average of 44% yield loss due to the combination of drought and high temperatures, with individual growers losing anywhere from 0.5% to 97.0% of their typical yield (Schattman et al., 2021). Precipitation in October and December that year recharged groundwater resources (Gordon, 2021; Birkel, 2021). Drought conditions then developed again in April 2021 and worsened through June, before being ameliorated by high-volume rainfall in July and August (Birkel, 2021; NOAA/NIDIS, 2021). Fruit yield in the 2021 season was above average with a total of 105 million pounds (USDA/NASS, 2022).

This past summer of 2022 was the tenth warmest and thirty-fifth wettest summer season since 1895 (Birkel, 2022), with temperatures in July breaking records across the region (NOAA/NIDIS, 2022). The distribution of precipitation in 2022 was uneven across the state, and drought developed for the third consecutive summer, most significantly impacting the Midcoast region (Birkel, 2022). High air temperatures and low soil moisture levels significantly impacted wild blueberry production across the state, but particularly in the Midcoast region, with some growers seeing crop losses in excess of 50% (personal communications), however official total yield has yet to be reported for 2022.

Data from the U.S. Drought Monitor for the summer 2022 season show, on a week-by-week and county-by-county basis, how much of Maine experienced differing levels of drought at any given time (U.S. Drought Monitor, 2022). Moderate drought set in to Waldo, Knox, and Hancock counties during the week of June 14, and worsened to severe drought by the week of August 2. In one week in Waldo County (June 21 to 28), moderate drought conditions grew from 17% to cover 100% of the county. In Knox County, conditions changed from 99% of the county being moderate drought conditions the week of July 26 to 99% experiencing severe drought conditions one week later, by the week of August 2. Hancock County saw just 0.07% of the county reach severe drought conditions by the week of August 2, and the bulk of the county experienced moderate drought conditions throughout the summer. As autumn progressed, the county quickly improved, from 60% moderate drought conditions the week of August 23 to 98% abnormally dry just a week later. Washington County experienced abnormally dry conditions from May 17 through September 20, beginning with 19% of the county classified as abnormally dry and climbing to a maximum of 99% of the county by the week of July 5, and persisting at that high percentage until dropping down to 66% of the county by the week of September 20. (For more information on the challenges associated with drought, please see page B78 in this report, “Wild Blueberry Phenology: Tracking Prune and Crop Plant Development through the Season”.)

Both too little and too much water are predicted and current concerns for the northeastern United States under the current climate change reality. Since 1960, annual precipitation levels in Maine's fall, winter, and spring seasons have increased by 0.107 inches, more than double the long-term rate of increase of 0.048 inches (Fernandez et al., 2020). These increases in precipitation result in higher stream flows, higher soil saturation, and higher groundwater levels (Gordon, 2022). Highly saturated soils result in larger and more frequent runoff events, increasing the likelihood that organic material and chemical inputs will not remain at the blueberry plant, thereby wasting growers' resources and increasing off-target impacts. During spring and summer drought conditions, wild blueberry plants become stressed by the lack of water and also by their reduced ability to take up nutrients without water. Wild blueberry plants require one inch of water per week during the growing seasons of prune and crop years, and the plants do not benefit from sporadic, high-volume precipitation events during growing season or at other times of the year (Hunt et al., 2008). Wild blueberry researchers, NRCS professionals, and growers all agree that additional research is needed to determine whether the one inch of water per week "rule of thumb" is still appropriate given today's climate and technologies. Irrigation timing will be explored in 2023.

Research indicates that higher soil moisture results in larger, higher-quality berries, since berry size is largely due to water content (Barai et al., 2022) and so growers are keen to maintain or increase soil moisture at all times, but especially during drought conditions. Long-term water conditions (more than four years) have a greater impact on plant health and yield than the current season's water conditions (Barai et al., 2021). Thus, growers in regions that suffer from several years of drought will require several very wet years to rehabilitate their fields or will require irrigation or other management techniques to improve their soils' water content, and in turn, their yield. Ongoing research continues to explore the economic, logistic, and labor feasibility of irrigation systems on wild blueberry farms (not addressed here). Different growers have tried wells, ponds, and trucking in water to increase the water available to their fields.

Manipulating the timing and quantity of precipitation through irrigation is out of reach for most growers. Therefore, many growers use wood chip mulch to build soil organic matter with the purpose of increasing soils' water-holding capacity. Organic matter acts like a sponge, holding more water in the soil and enabling the plants to better function by taking up nutrients consistently and more easily. The current NRCS cost-share program through EQIP allows two to four inches of mulch to be applied to bare spots for erosion management, a recommendation based on work conducted at the University of Maine in the 1980s (DeGomez & Smagula, 1990).

Today, growers are interested in applying mulch to whole fields or large sections of fields to increase soil moisture in established wild blueberry stands or to encourage wild blueberry regrowth after de-rocking a field. Two inches is a lot of mulch to apply to entire fields so we are interested in understanding if less mulch could be applied while still benefiting the crop. The research described in this report seeks to provide knowledge for the possible expansion of the NRCS mulching practice beyond 2 inches only applied in bare spots.

METHODS

Mulch was applied in the spring of 2022 in two prune fields before wild blueberry emergence. At two participating wild blueberry farms in Hope and Orland, large plots to mimic "whole field mulching" were created. The mulch depths compared were 0.5, 1.0, 1.5, and 2.0 inches of softwood pine wood chips and one control that received no mulch. At the Hope site on April 29, 2022, mulch was applied to five total plots of 10,000 ft^2 each were staked out in 50' x 200' rectangles. This site had good wild blueberry coverage. In Orland on May 10, 2022, mulch was applied yet the uneven ledge topography required the five staked plots be smaller at 50' x 100' in size (5,000ft^2 each). The Orland site was recently de-rocked and land leveled in the fall of 2021. This site was rockier and more sloped than the Hope site.

The farmers of each field used the equipment available to apply mulch. In Hope, a 5.5 cubic yard manure spreader was used to spread mulch and one person followed behind to rake out larger piles to the planned thicknesses. In Orland two tractors with 1.3 cubic yard buckets were used to bring mulch from the pile to the field. Tractor operators feathered the mulch to the ground and five people spread out the mulch with rakes and shovels to an even layer at the planned thicknesses. The amount of mulch to be spread was calculated by multiplying the surface area to be covered by the desired depth, then dividing by the equipment's known capacity to determine the number of equipment loads needed.

Image 1. Left, mulch being spread by a manure spreader in Hope. Right, mulch being spread by rake after being deposited by a tractor in Orland.

Eight 0.37 m^2 quadrats (subplots) within each treatment plot were established for data collection throughout the season and the corners were marked using wooden stakes (total: 40 quadrats per site). Mulch was applied in early spring of the prune year before wild blueberry plants emerged. Wild blueberry stem stubble was about 1 inch tall, making the stems in the 1.5 and 2 inch depths completely covered by mulch. The placement of random subplots was therefore largely unaffected by stem density. Within each subplot, three random stems were chosen and tagged with a numbered metal tag to allow repeated measurements.

Throughout the season, plant health was quantified by measuring stem height, stem density per quadrat, counting buds per stem, and collecting leaf chlorophyll content with a SPAD meter. 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 a value on one lower and one upper leaf. SPAD measures were taken on three randomly selected stems within each quadrat.

A TDR (Time Domain Reflectometry) meter was used to measure soil moisture content and temperature (FieldScout TDR 150 Soil Moisture Meter, Spectrum Technologies, Inc., Aurora, IL, USA). Soil conditions were measured to a depth of 12 cm (4.8 inches).

Table 1. Data collection types and dates at both research sites.

Date	Location	Data collected
5/23/22	Hope	TDR
5/25/22	Orland	TDR
6/7/22	Hope	TDR, SPAD
6/10/22	Orland	TDR, SPAD
6/14/22	Hope	TDR, SPAD
7/5/22	Hope	TDR, SPAD
7/7/22	Orland	TDR, SPAD
8/16/22	Hope & Orland	TDR, SPAD
8/24/22	Hope & Orland	TDR, SPAD
9/29/22	Hope & Orland	TDR, bud #
10/13/22	Hope & Orland	Stem #/quadrat

Data Analysis

Measures of SPAD, bud number and stem height were all analyzed using a basic one-way ANOVA followed by a Tukey's Pairwise comparison when significant treatment differences were present. Soil moisture data were analyzed using a Standard Least Squares Analysis of Variance by date followed by a Tukey's Pairwise comparison.

Due to the nature of the data collected, the data for soil moisture and bud numbers failed the assumptions of normality 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. Statistical tests were carried out on the transformed data despite non-normality after establishing there were no serious problems with the data. Measure of SPAD and stem number had a normal distribution.

All data analysis were carried out using JMP Version 16.0 (SAS, Cary, NC, USA) statistical software.

RESULTS

Using both the 5.5 cubic yard manure spreader and the two 1.3 cubuc yard buckets it took about 1 hour to spread 1 inch of mulch wood chips across the 10,000 square foot plots. This means it would take 4.5 hours to mulch 1 acre with 1 inch of mulch using the smaller scale equipment on these farms.

Soil moisture varied drastically at both locations across all treatments from May 23 to September 29, 2022. Over those four months, which encompassed a majority of the peak growing season, soil moisture at the Hope location ranged from 42% to 4% and at the Orland location from 33% to 0% (Figures 1 and 2). The dramatic difference between the two fields led to the two fields being evaluated separately.

The Hope location exhibited the greatest treatment differences around two months following the mulch application date of April 29, 2022. The initial divergence between treatment moistures (statistically speaking) occurred in Hope on July 5, 2022, where soil moisture in the control was 24% and all other treatments ranged from 28% in the 0.5" treatment (not statistically different from the control) to 31% - 32% in the 1.0", 1.5" and 2.0" treatments, which were statistically higher than the control. Over the summer, soil moisture at both sites and across all treatments continued to drop due to the lack of precipitation as there was also an increase in air temperature. The lowest moisture was measured across all treatments on the August 16, 2022 sample date. Under these stressed conditions, soil moisture in the control was down to 4% while the 1.5" and 2.0" mulch treatments remained significantly higher than the control, with soil moisture levels of 8% and 12%. With increased precipitation toward the end of August and September, the 2.0” mulch treatment remained the only treatment with significantly higher soil moisture than the control.

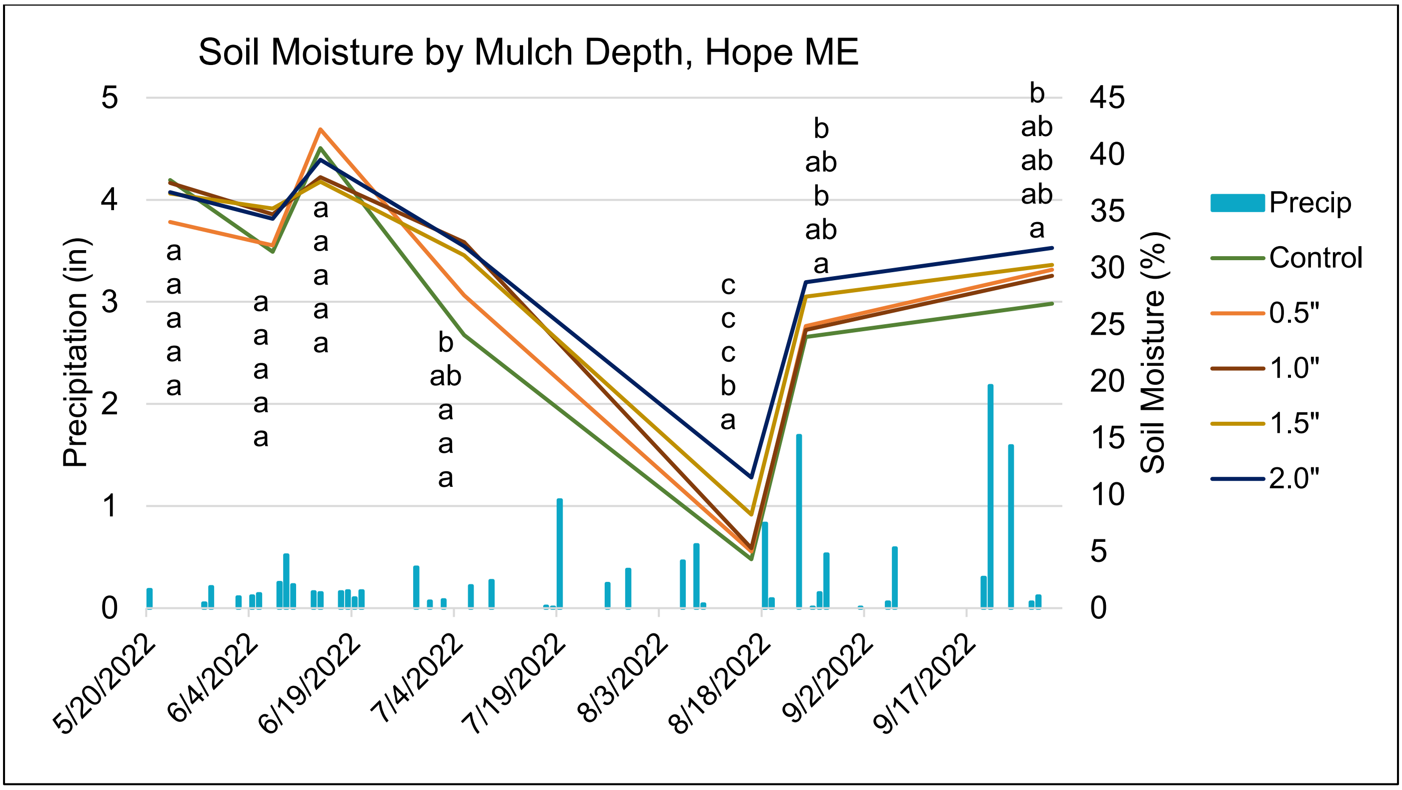

Figure 1. Average soil moisture by date and mulch depth for the wild blueberry prune year (2022) in Hope, Maine. Light blue bars represent daily local precipitation events (precipitation data is from NOAA, https://www.ncei.noaa.gov/cdo-web, West Rockport Station). Lines correspond to mulch depth treatments. Letters indicate significant differences at the 0.05 level of significance and are to be compared across mulch treatments by date (dates are to be compared separately). Letters correspond to legend order (control: top letter, 2.0”: bottom letter). Due to lack of normality, data were transformed using a sqrt transformation prior to statistical testing. Error bars were removed from the graph for better data visualization.

The Orland location exhibited treatment differences immediately following the mulch application on May 10, 2022. The 1.0” mulch treatment had significantly higher soil moisture than the control over the first two sample dates (May 25 and June 10). This may have been influenced by the terrain and spacing between the treatments due to lack of uniformity in the field, as the field had been de-rocked in fall 2021. Almost two months following the mulch application, all treatments had significantly higher soil moisture than the control, with the soil moisture levels in the 1.0” (19%), 1.5” (22%), and 2.0” (21%) treatments. During the driest drought conditions experienced in early August, only the 2.0” mulch treatment (1.4%) sampled on August 16, 2022 maintained significantly higher soil moisture than the control (0.3%). Treatment differences lacked significance and were variable following precipitation events from late August to early September.

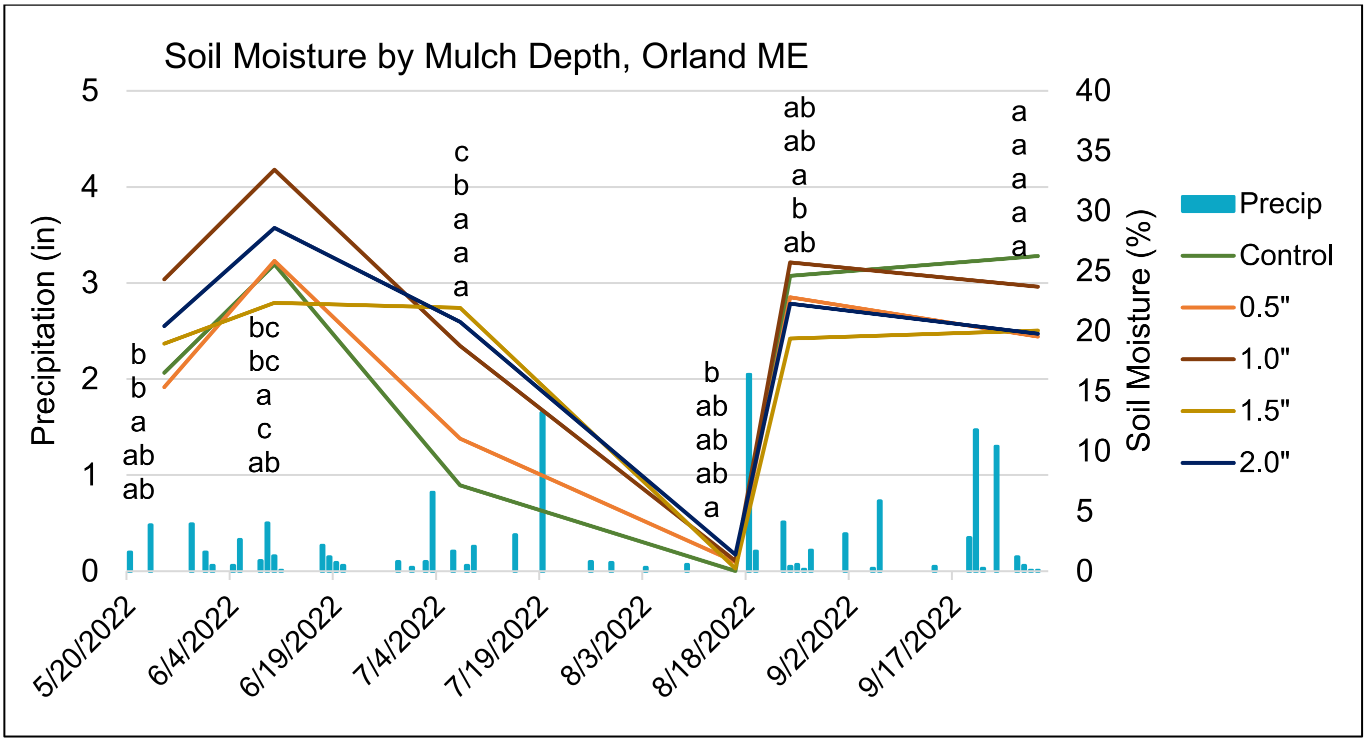

Figure 2. Average soil moisture by date and mulch depth for the wild blueberry prune year (2022) in Orland, Maine. Light blue bars represent daily local precipitation events (precipitation data is from NOAA, https://www.ncei.noaa.gov/cdo-web, Bucksport Station). Lines correspond to mulch depth treatments. Letters indicate significant differences at the 0.05 level of significance and are to be compared across mulch treatments by date (dates are to be compared separately). Letters correspond to legend order (control: top letter, 2.0”: bottom letter). Due to lack of normality, data were transformed using a sqrt transformation prior to statistical testing. Error bars were removed from the graph for better data visualization.

Table 2. Percent soil moisture difference between each treatment and the control on July 7 and August 16, 2022 in Hope and Orland, Maine.

Location	Mulch Depth	Sample Date	
		July 7, 2022	August 16, 2022
Hope	0.5”	4%	1%
	1.0”	8%	1%
	1.5”	7%	4%
	2.0”	8%	7%
Orland	0.5”	4%	1%
	1.0”	12%	1%
	1.5”	15%	0%
	2.0”	14%	1%

Leaf chlorophyll content, as an indicator of plant health and productivity, trended up in all treatments with the application of mulch at both locations when compared to the control (Figure 3, below). The Orland location presented significantly higher leaf chlorophyll content in 0.5” (31.0 SPAD), 1.0” (33.0), 1.5” (33.4), and 2.0” (32.9) treatments, relative to the control (27.6). Chlorophyll values at the Hope location were not significant and were variable relative to mulch depth. However, the 0.5” (34.1) and the 2.0” (34.5) treatments were notably higher than the control (32.2).

Average leaf chlorophyll content in the control at Hope was 4.7 SPAD units higher than the control at Orland. Taken with the results presented, this indicates that the Orland site saw greater improvements in leaf chlorophyll content under all mulch treatments, likely due to the less established and more stressed nature of the site.

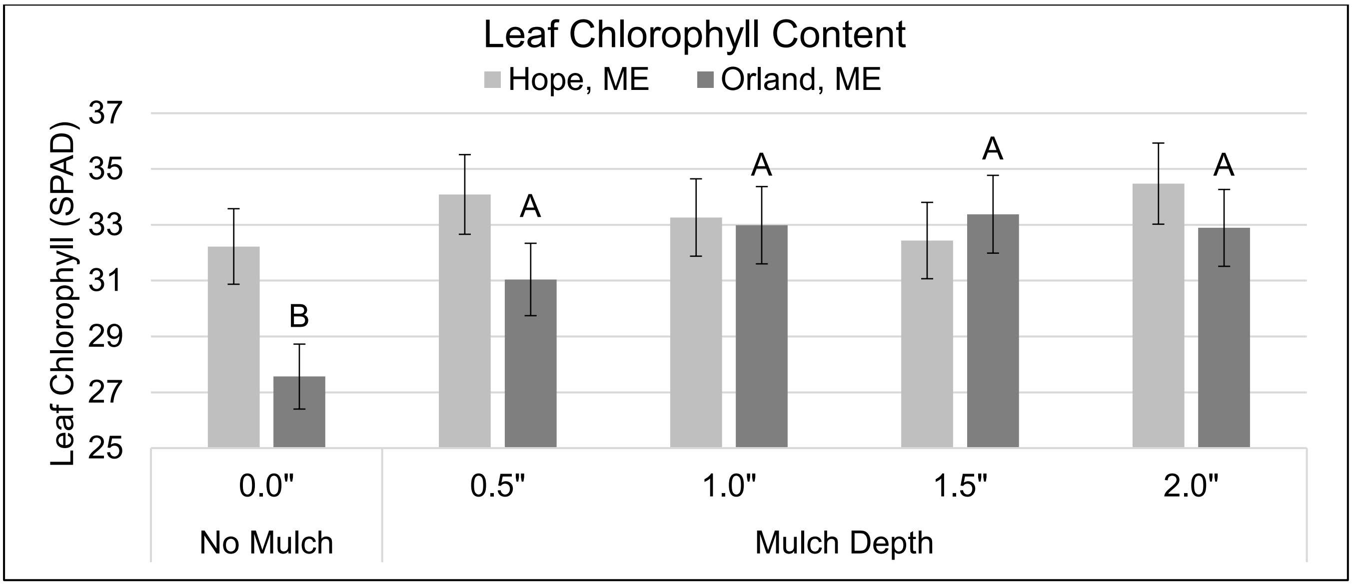

Figure 3. Average leaf chlorophyll content (SPAD) by mulch depth for the wild blueberry prune year (2022) in Hope and Orland, Maine. Leaf chlorophyll content was collected on 4 separate dates for each location from June 10 to August 24, 2022. Letters indicate significant differences at the 0.05 level of significance at the Orland location. Treatment differences at the Hope location were not significant. Error bars represent the standard error of the mean.

At both locations, treatments that received mulch exhibited higher average blueberry bud number (#/stem) than the controls where no mulch was applied (Figure 4, below). The Orland location presented a trend where higher bud numbers were observed with increasing mulch depth. However, at this location, only the 1.5” (6.6 buds/stem) and 2.0” (6.7 buds/stem) treatments had significantly higher bud numbers than the control (3.8 buds/stem). The bud numbers observed at the Hope location were variable relative to mulch depth. Here, only the 1.0” (7.0 buds/stem) mulch treatment had significantly higher bud numbers than the control (4.3 buds/stem). These location differences reiterate the hypothesis that stressed fields may benefit more from whole field mulching more than an unstressed field.

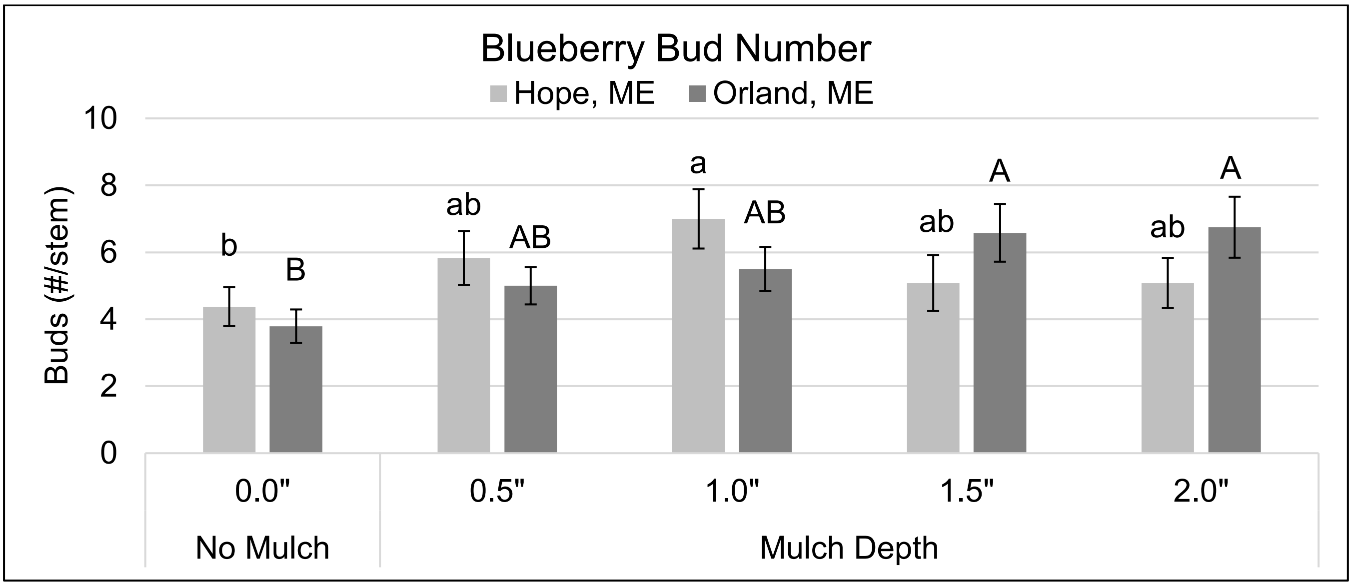

Figure 4. Average bud number by mulch depth for the wild blueberry prune year (2022) in Hope and Orland, Maine collected on October 13, 2022. Letters indicate significant differences at the 0.05

level of significance. Capital letters are to be compared separate from lowercase letters. Error bars represent the standard error of the mean.

The average blueberry stem densities varied by location and treatment (Figure 5, below). Stem densities at the Hope location ranged from 691 stems/m^2 (in the 1.0” treatment) to 901 stems/m^2 (in the 1.5” treatment). Stem densities at the Orland location were lower (likely due to fall 2021 de-rocking of the field), with a range of 303 stems/m^2 (in the 1.0” treatment) to 459 stems per/m^2 (in the control). Lower stem densities in the treatments where mulch was applied may be in response to the timing of the mulch applications, especially at the Orland site, where much was applied on May 10, 2022, coinciding with blueberry shoot emergence.

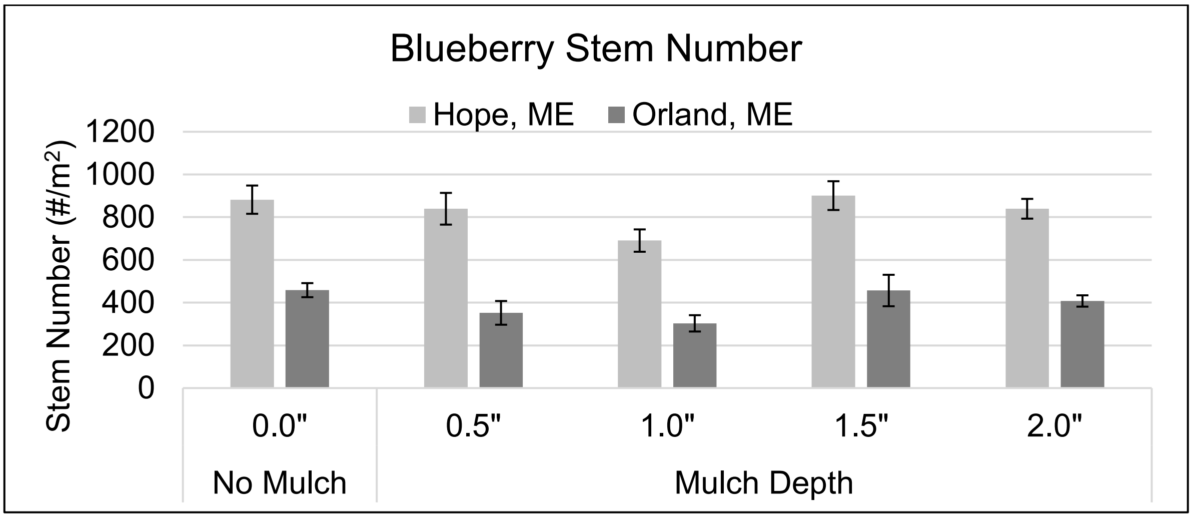

Figure 5. Average stem number by mulch depth for the wild blueberry prune year (2022) in Hope and Orland, Maine collected on September 29, 2022. Treatment differences were not significant at either location. Error bars represent the standard error of the mean.

In 2022, NRCS paid $14,500/acre for whole field mulching at a 2 inch depth (different than bare spot mulching). Table 3 describes the amount of mulch required to reach each mulch depth and the NRCS payment estimate where the cost of mulch was estimated to be $18-$25 per cubic yard.

Table 3. The estimated cost of mulch and material application that was covered by NRCS to mulch whole fields of wild blueberry land in 2022. The volume of mulch required to reach each depth studied is also listed. Mulch woodchip payment/acre was estimated using the high-end price of $25/cubic yard. NRCS payments vary every year. Data from NRCS.

Mulch depth (inches)	Cubic yards/acre	Woodchip payment/acre ($)	Remaining funds for material application/acre ($)	Total 2022 NRCS payment/acre ($)
0.50	67.50	1687.50	1937.25	3624.75
1.00	135.00	3375.00	3874.50	7249.50
1.50	202.50	5062.50	5811.75	10874.25
2.00	270.00	6750.00	7749.00	14499.00

DISCUSSION

The Orland field was de-rocked in the fall of 2021 and the Hope field was a stand of established wild blueberry with very few bare patches, making the two sites very different. These results show that in the year of mulch application, soil moisture increased by 4-14% in early drought conditions (July 7) and by 1-7% during severe drought (August 16) depending on the mulch depth.

In this first year we saw that in the year of mulch application, improvements in soil moisture and wild blueberry plant health across all mulch depths and at both locations. Greater improvement was observed in the more stressed field than the less stressed, more established field. Stress in this context is likely due to recent de-rocking, ledge, and lower SOM content. The plants in the less stressed field in Hope still saw improvements in soil moisture and plant health, but the gains were more subtle.

At both locations, the controls which received no mulch had lower soil moisture content than any of the mulch treatments, indicating that any amount of mulch can increase soil moisture content. At Orland, the drought period of mid-June to mid-August saw higher soil moisture content in 1.0", 1.5", and 2.0" treatment plots compared to the control. This large difference in Orland was likely influenced by the lower density of wild blueberry stems at this location. The same depths of mulch at Hope, which had a higher density of stems, similarly saw elevated soil moisture levels when compared to the control plot, but the gains were not as significant.

These findings and the site differences between Hope and Orland suggest that preexisting variability in soil conditions and terrain may influence the degree to which a field can benefit from whole field mulching. Additionally, drought severity may have varied geographically, impacting both sites differently.

Until now, mulch has been considered a long-term commitment in hopes of future water holding capacity. However, this year's data suggests that even in the year of mulch application, a significant increase in soil moisture can be achieved at 1.0, 1.5, and 2.0" thicknesses of pine woodchips with gains to plant health and soil moisture depending on the location and field history. The cost of mulching is not currently a major barrier at this time because NRCS (Natural Resource Conservation Service) provides a cost-share program.

CURRENT RECOMMENDATIONS

- After the first year of this study, it is clear that 1.0" or more of softwood mulch wood chips can increase soil moisture in both established and recently de-rocked wild blueberry fields.
- The manure spreader method of application worked well but larger and newer manure spreaders would speed up application from 4.5 hours per acre at the 1 inch thickness.

NEXT STEPS

- Continue measuring plant health and soil moisture in the 2023 season (crop year).
- Track mulch contamination and berry quality at harvest and through a freezer processing line.

ACKNOWLEDGEMENTS

Thank you to NRCS for funding this project through a Conservation Innovation Grant. Thank you to Ron Howard and Simeon Allen for hosting this research and to Abby Cadorette, Charles Cooper, Julian LaScala, Jordan Ramos, Mara Scallon, and Brogan Tooley for data collection and analysis.

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INVESTIGATORS: YJ. Zhang, L. Calderwood, and R. Tasnim

12. Using Ground Applied Fertilizers to Improve Wild Blueberry Production and Resilience to Warming

OBJECTIVE

Evaluate chicken manure and ammonium sulfate as crop fertilizer for wild blueberry.

LOCATION: Blueberry Hill Farm Lab, Jonesboro, ME

PROJECT TIMEFRAME: April 2021 – March 2023

INTRODUCTION

This project continues research that began in 2021 (see the 2021 report, page 150, "Using Foliar Fertilizers and Soil Amendments to Improve Wild Blueberry Production and Resilience to Warming") which measured the impacts of several foliar fertilizers and separately simulated warming conditions to measure physiological changes in the blueberry that may occur with climate change. This report discusses the aspect of the research regarding fertilizers and their impacts on plant physiology, soil conditions, and overall production.

This year's research continued studying the impacts of pelletized Cheep Cheep chicken manure, a high rate of ammonium sulfate ($(NH_4)_2SO_4$), and a low rate of ammonium sulfate ($(NH_4)_2SO_4$). These products were initially chosen because organic Canadian wild blueberry growers have success using pelleted chicken manure at the rates studied in this experiment. Cheep Cheep is a North Country Organics product that is available in the US in 50lb bags but may not be a practical source for growers applying to several acres (no brand loyalty or endorsement intended or implied). Ammonium sulfate is a standard fertilizer used by highbush blueberry growers (Hart et al., 2006) and was the recommended nitrogen source for wild blueberry in the 1970s (Trevett, 1970). In a 1987/1988 foliar nutrient survey of 76 fields, N levels were higher than the recommended 1.6% at the time and P levels were below the 0.125% standard at the time. Therefore, in the 1990s, DAP (diammonium phosphate, 18-46-0) and MAP (monoammonium phosphate, 11-52-0) were studied to deliver both N and P to plants (Smagula & Dunham, 1995). This was successful and DAP and MAP have been the standard fertilizer since that time (Yarborough & Smagula, 2013), yet they contain high levels of phosphorous. Fields that have applied DAP or MAP for 20-25 years now may have P levels that are high and additional P application may be lost to the environment, a waste of time and money to apply. Decisions on when to apply DAP, MAP, urea, ammonium sulfate, chicken manure, or any other fertilizer should be based on foliar sample results, not a calendar date. (For information on how to complete foliar nutrient sampling, see UMaine Fact Sheet 222, "Leaf and Soil Sampling Procedures", linked in References below.) Money, material, and time can be saved by knowing exactly how much of each nutrient is needed by plants.

Nitrogen plays an important role in building plant structures and compounds that enable plants to live, and is the most limiting nutrient (DeFelice et al., 2022; Prasad & Chakraborty, 2019). Nitrogen is a gas molecule that makes up most of the Earth's atmosphere yet cannot be used by plants or animals in this gaseous form: plants uptake nitrogen in the form of nitrate (NO_3^-) and ammonium (NH_4^+) which are produced by soil microorganisms that transform the gaseous nitrogen into the form usable by plants (a process called "nitrogen fixation") (Killpack & Buchholz, 2022A; Killback & Buchholz, 2022B). Nitrogen can also be produced as a fertilizer and applied to fields lacking the nutrient. Nitrogen is a major component of chlorophyll, which facilitates photosynthesis that regulates plant growth, and builds proteins and enzymes, which ultimately helps the plant take up nutrients and water (DeFelice et al., 2022).

Phosphorous is a nutrient that binds very tightly to soil particles in acidic soil conditions (<6.0 pH) making it difficult for plants in acidic soils to access the phosphorous (Prasad & Chakraborty, 2019). Phosphorous is a vital nutrient for plants where it is used to store and carry energy within cells and build plant structures (Prasad & Chakraborty, 2019). Unlike nitrogen, phosphorous does not exist in a gaseous form but is found in several solid forms in the soil, though the form that can be taken up by plants comprises the smallest portion (<20%) of the phosphorous in the soil (Prasad & Chakraborty, 2019). Phosphorous enters soils through fertilizers, manure, and/or decaying plant debris, and the process of weathering breaks phosphorous down before it can be taken up by the plant. Adding phosphorous in excess of plant needs does not increase plant growth or yield, but can cause runoff into surface waters, where it can degrade water quality by promoting algal growth

(Prasad & Chakraborty, 2019). Other production systems have seen so much phosphorous applied (through fertilizers or manure application) that phosphorous runoff occurs and causes water quality issues (Lory, 2018). Though this has not been studied nor observed in wild blueberry systems, it is imperative that money not be wasted on nutrients unused by the plants that might cause a water pollution issue (Killbeck & Buchholz, 2022B).

Soil organic matter (SOM) is another source of nutrients. Soil tests show SOM as a percentage, and for each 1% OM the soil contains approximately 20 pounds of inorganic nitrogen and two pounds each of phosphorous, potassium, and sulfur available for plant use (Fernandez & Kaiser, 2021; McLean et al., 2021).

The focus of this research is to continue studying fertilizer for both conventional and organic growers. We hope to better understand how different materials and rates impact plant physiology and growth. In this study, all products were applied on May 26, 2021, so data gathered during the 2022 growing season (including fruit yield) reflected one entire year's growth after receiving the fertilizer treatment.

METHODS

In May 2021, the study was laid out at Blueberry Hill Farm in Jonesboro, ME in a randomized complete block design with each soil fertilizer treatment (untreated control, Cheep Cheep, and ammonium sulfate and low and high rates) replicated six times in 6' by 30' plots, for a total of 24 plots. At Blueberry Hill Farm Research Station, the foliar nutrients in the research plots (tested in July 2020) contained 1.4% nitrogen where the optimum nitrogen level is 1.76% and therefore it is recommended that 45 lb/A nitrogen (2011) be applied (Calderwood et al., 2020).

Table 1. Ground fertility treatment summary

Applied in the Prune Year (5/26/2021)				
Treatment		**Total lbs of Material/acre Applied**	**lbs N, S, and OM/acre Applied**	**N-P-K +S (%) Content of Material**
Control		none	N/A	N/A
Cheep Cheep		700 lbs/acre	28 lbs N, 0 lbs S, ukn lbs N from OM	4-3-3
Ammonium sulfate	Low $(NH_4)_2SO_4$-L	214 lbs/acre	45 lbs N, 51 lbs S, 0 lbs N from OM	21-0-0 + 24
	High $(NH_4)_2SO_4$-H	381 lbs/acre	80 lbs N, 91 lbs S, 0 lbs N from OM	21-0-0 + 24

Table 2. Cost comparison table for different rates of different products, both studied here. All prices quoted on January 12, 2023 and should be considered among the lowest prices of the year.

Product	**Rate of Application**	**Cost per unit**	**Cost per application**
DAP (diammonium phosphate) Low rate to get 45lbs N	220lb/A	$950/ton $23.75/50 lb bag	$94.73/acre
DAP(diammonium phosphate) High rate to get 80lbs N	440lb/A		$168.42/acre
Cheep Cheep To get 28lbs N	700 lb/A	$650/ton $16.25/50 lb bag	$227.50/acre
Ammonium sulfate Low rate to get 45lbs N	214 lb/A	$895/ton $22.37/50 lb bag	$95.74/acre
Ammonium sulfate High rate to get 80lbs N	381 lb/A		$170.46/acre

Data Collection

Soil Moisture

TDR (Time Domain Reflectometry) was used to measure 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 (4.8 inches). Six random readings were recorded per plot on May 11, June 6, and July 1, 2022.

Blueberry Phenology

Repeated plant phenology measures were taken on the same four stems in each treatment plot. Plants were tagged with numbered tags and were evaluated on May 19, June 15, and July 22, 2022. The number of buds, flowers, green, and blue fruit were recorded during each sampling. Stem heights were also measured using a meterstick and were recorded in centimeters.

Blueberry Physiology

Eight stems from each plot were randomly selected to measure chlorophyll concentration by a CCM-200 plus Chlorophyll Content Meter (Opti-Sciences, Inc., Hudson, NH, USA) on June 6 and July 1, 2022. 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 June 30, 2022, between 10 am to 2 pm.

Blueberry Morphology and Foliar Nutrient Sampling

Right before harvesting, on July 26, 2022, 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 Leaf Area Meter (LI-COR Biosciences, Lincoln, NE, USA). In 2022, all the leaves from those eight stems were oven-dried at 70°C to constant mass and weighed, and then the dried leaf samples were ground and sent to the University of Maine Soil and Plant Tissue Testing Laboratory in Orono, Maine for leaf nutrient testing and we are still waiting on the results. At the same time on July 26, 2022, 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. Baseline soil samples in these plots were taken in May 2021 and differentiated by treatment. Additional soil samples were taken on July 30, 2022, and again differentiated by treatment to allow for adequate comparison. Foliar samples were analyzed in June and July 2021 by Nova Crop Control for plant sap analysis, to track how plants use nutrients. The treatments were applied May 26, 2021.

Pest Presence

Repeated pest presence and plant growth measurements were taken on May 19 and June 15, 2022. using a 0.37 m^2 quadrat in the same flagged locations, twice per plot. Weed, insect, and disease presence were recorded. 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.

Blueberry Cover

Blueberry cover was evaluated using the same equal interval ranks as pest presence.

Fruit Yield

Fruit was hand-raked on August 11, 2022. Within each treatment plot, two 0.37m^2 quadrats were placed at the same flagged locations used for pest scouting and all the fruit was harvested within the quadrat and the yield recorded. The entire plot was then also raked, and the yield recorded, so each plot generated three yield numbers: quadrat one, quadrat two, and total plot outside the quadrats. The fruit from each plot were then combined to enable fruit quality measures.

Fruit Quality

The harvested fruit was sampled in several ways to determine fruit quality. The weight of 100 berries was measured and recorded, allowing researchers to determine which treatments produced larger fruit, since the 100 berry weight had a higher mass. A sample of fruit from each treatment was also puréed for use in a handheld PAL-BRIX/ACID F5 refractometer (Atago, Saitama, Japan) to measure the samples' sugar content.

Data Analysis

Crop and Pest Data

Single date measurements including yield, Brix and 100 berry counts were evaluated using a generalized linear model (GLM), followed by a Tukey's Pairwise comparison in JMP (JMP®, Version 16.0, SAS, Cary, NC, USA) across all treatments ($\alpha = 0.05$). All ranked blueberry cover and pest data were transformed to their corresponding percent mid-point. Ranked blueberry cover, blueberry stem height, weed number and stems with pest presence (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.

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 included blueberry phenology, stem height and cover, pest presence (# or # of stems/m^2; weeds, insects and diseases), and one hundred berry counts. These data improved following transformation except for blueberry cover (which was left untransformed). Transformed data continued to statistically fail for normality, however, statistical tests were carried out despite non-normality after establishing there were no serious problems with the data. Blueberry yield and Brix measures were normally distributed; therefore, no transformation was needed prior to statistical testing.

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 leaf photosynthetic electron transport 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) 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.

RESULTS

Plant Phenology

While no significant treatment differences were observed in fruit counts as a measure of phenological development, all treatments produced more green and blue fruit per stem relative to the control (Figure 1). The high rate of ammonium sulfate treatment yielded the highest numbers of buds (6.4 buds/stem), flowers (5.1 flowers/stem), and green fruit (21.0 green fruits/stem), compared to all other treatments including the low-rate ammonium sulfate, Cheep Cheep, and the control.

The highest number of blue fruit per stem occurred after treatment with Cheep Cheep (7.9 blue fruits/stem) which were 83% greater than the control (4.3 blue fruits/stem), followed by the low-rate ammonium sulfate (6.3 blue fruits/stem) and high rate of ammonium sulfate (5.5 blue fruits/stem), which were 47% and 28% greater than the control, respectively.

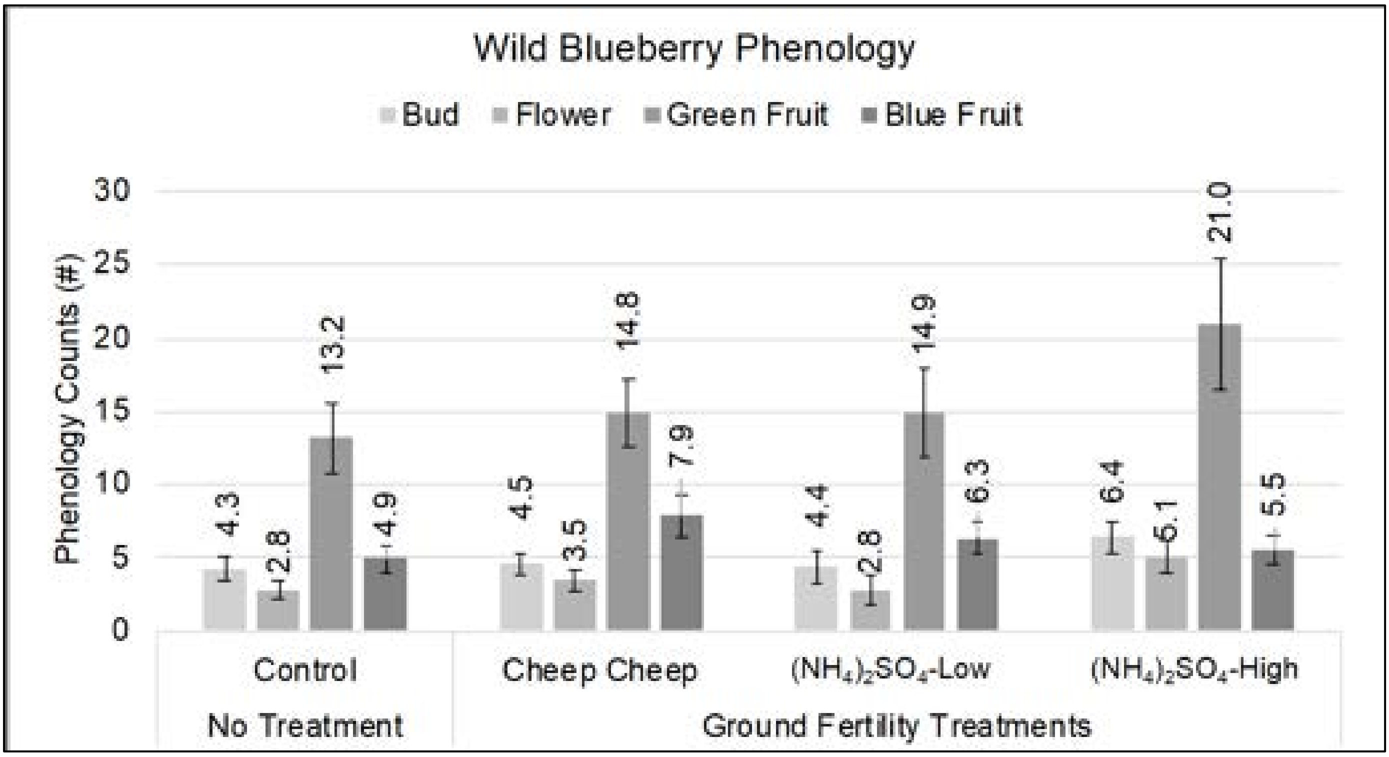

Figure 1. Average bud, flower and fruit counts per stem by treatment at Blueberry Hill Research Station, Jonesboro, Maine. Bud, flower, green fruit, and blue fruit counts were observed on May 19 (bud & flower), June 15, July 22, 2022, respectively. Treatment differences in phenology counts were not significant. Error bars represent the standard error of the mean.

The average blueberry stem height was significantly taller in the high ammonium sulfate treatment (24.8 cm) when compared to Cheep Cheep (20.5 cm) and the control (20.1 cm), but not the low-rate ammonium sulfate treatment (22.7 cm) which was not significantly different in height relative to any treatment (Figure 2).

The blueberry plants exhibited the highest coverage rank in the high rate of ammonium sulfate treatment (78%/m^2), followed by Cheep Cheep (72%/ m^2) which were greater than the control by 14% and 8%, respectively (Figure 3). The low-rate ammonium sulfate treatment had significantly lower blueberry cover (68%/m^2) when compared to the high rate of ammonium sulfate treatment only.

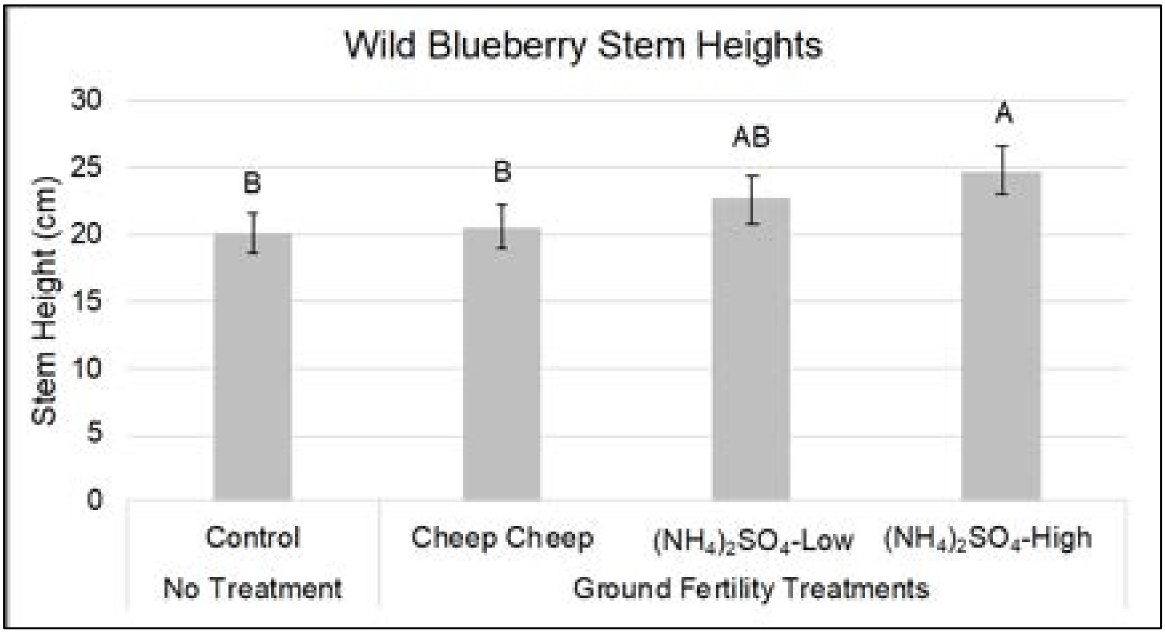

Figure 2. Average stem heights (cm) by treatment measured on two dates (June 15 and July 22, 2022) at Blueberry Hill Research Station, Jonesboro, Maine. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean.

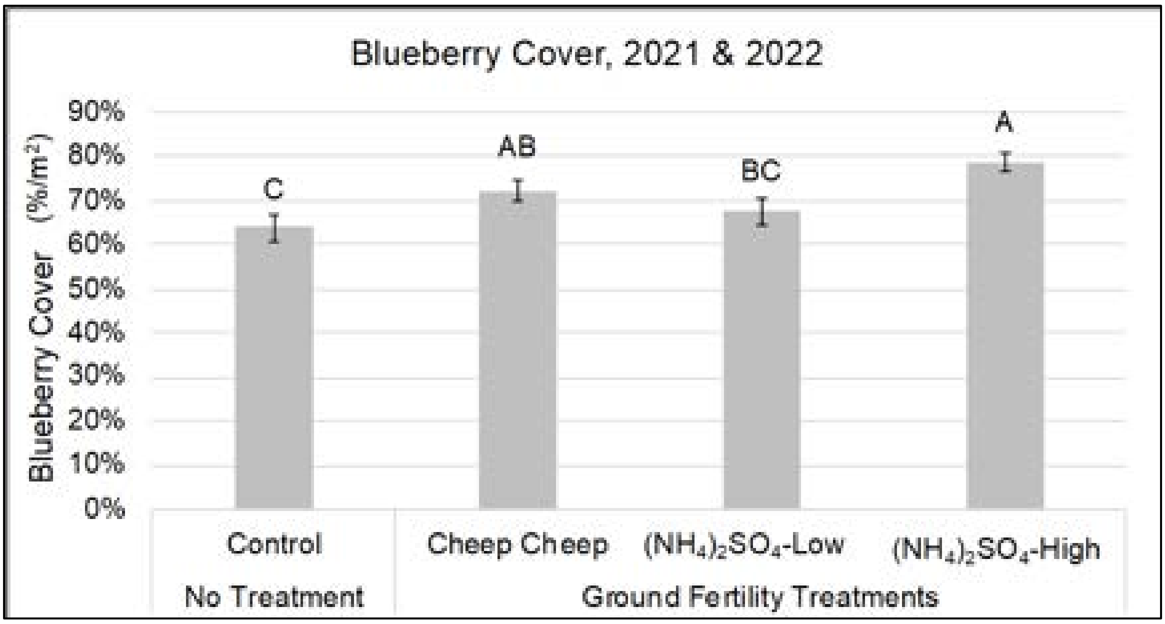

Figure 3. Average blueberry cover (%/m^2) by treatment measured on four dates in 2021 (June 9, July 21, August 20, and September 20), and two dates in 2022 (May 19 and June 15) at Blueberry Hill Research Station, Jonesboro, Maine. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean.

Blueberry Physiology and Morphology

For chlorophyll concentrations during the growing season, all treatments (Figure 4) had similar leaf chlorophyll concentration on June 6, 2022. On July 1, 2022, high rate of ammonium sulfate treatment showed the highest leaf chlorophyll concentration (28-30 SPAD) followed by the control (26-28 SPAD), low rate of ammonium sulfate (26-29 SPAD), and Cheep Cheep (23-25 SPAD) treatments. In July, the only significant difference in leaf chlorophyll concentration was found between the high rate of ammonium sulfate and Cheep Cheep treatments.

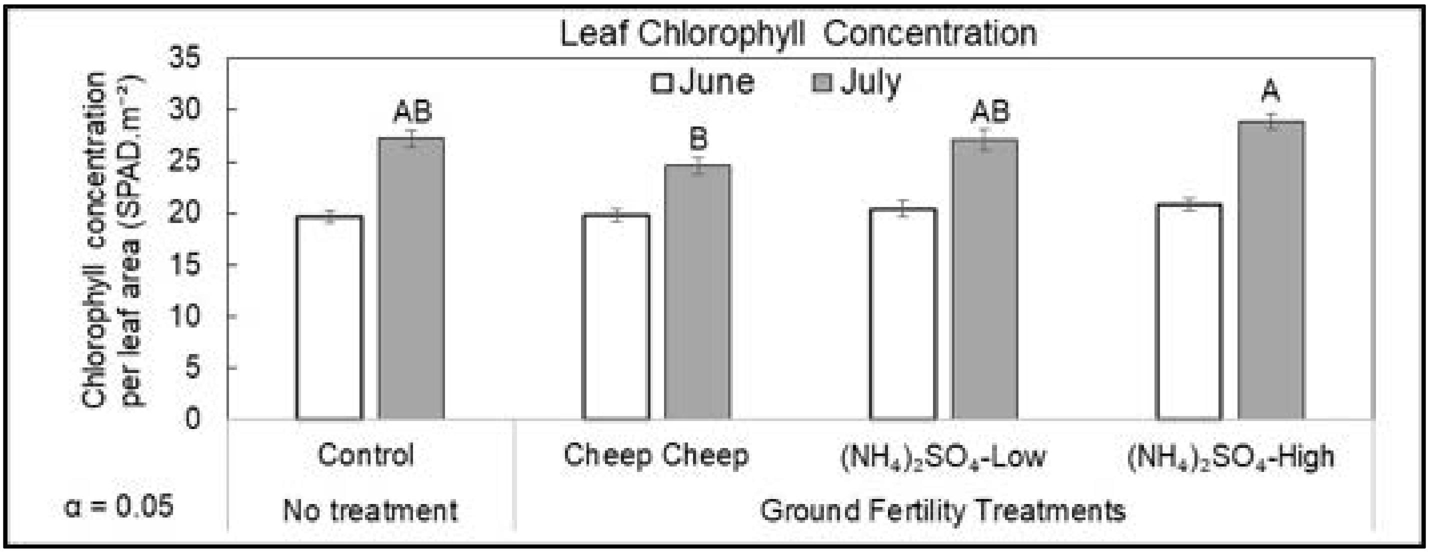

Figure 4. Comparison in chlorophyll concentration of wild blueberry leaves on June 6 and July 1, 2022, among different treatments at Blueberry Hill Research Station, Jonesboro, Maine. Error bars indicate the standard error of the mean. Different letters indicate significant differences and no letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

For leaf photosynthetic electron transport rate (Figure 5), measured June 30, 2022, all treatments showed similar electron transport rates, and no significant differences were found among them. However, on average, the low rate of ammonium sulfate treated plants showed higher leaf electron transport rates (160-178 ETR) compared to other treatments including control (140-162 ETR).

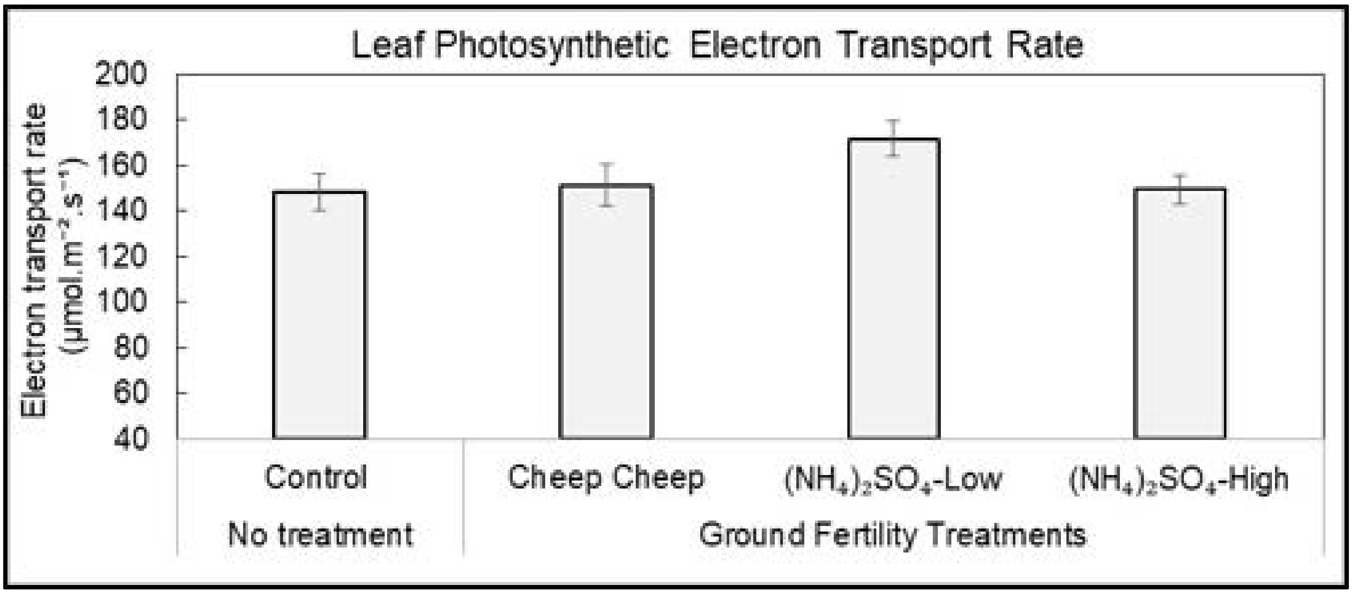

Figure 5. Comparison in photosynthetic electron transport rate of wild blueberry leaves on June 30, 2022, among different treatments at Blueberry Hill Research Station, Jonesboro, Maine. Error bars indicate the standard error of the mean. No letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

Table 3. Recommended optimum ranges and comparisons of wild blueberry soil characteristics among different soil amendments and fertilizer treatments on July 30, 2022, at Blueberry Hill Research Station, 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 treatment**	**Treatments**		
		Control	**Cheep Cheep**	**Ammonium sulfate**	
				Low	**High**
pH	4.0-4.5	4.2±0.1	4.1±0.1	4.3±0.1	4.6±0.1
Organic matter (%)	5-8	11.7±2.5	12.7±2.5	7.6±1	6.2±0.6
CEC (me/100 g)	>5	7.6±0.9	8.9±0.9	6.2±0.8	5.5±0.6

In July 2022, average leaf area (Figure 6A) was significantly smaller in the low rate of ammonium sulfate treatment compared to other treatments including the control. In contrast, there was no significant difference in the number of leaves per stem (Figure 6B) and total leaf area per stem (Figure 6C) among the treatments in ground fertility trial. On average, the number of leaves per stem and total leaf area per stem was the highest in the high rate of ammonium sulfate treatment compared to other treatments and control, although these differences were not significant.

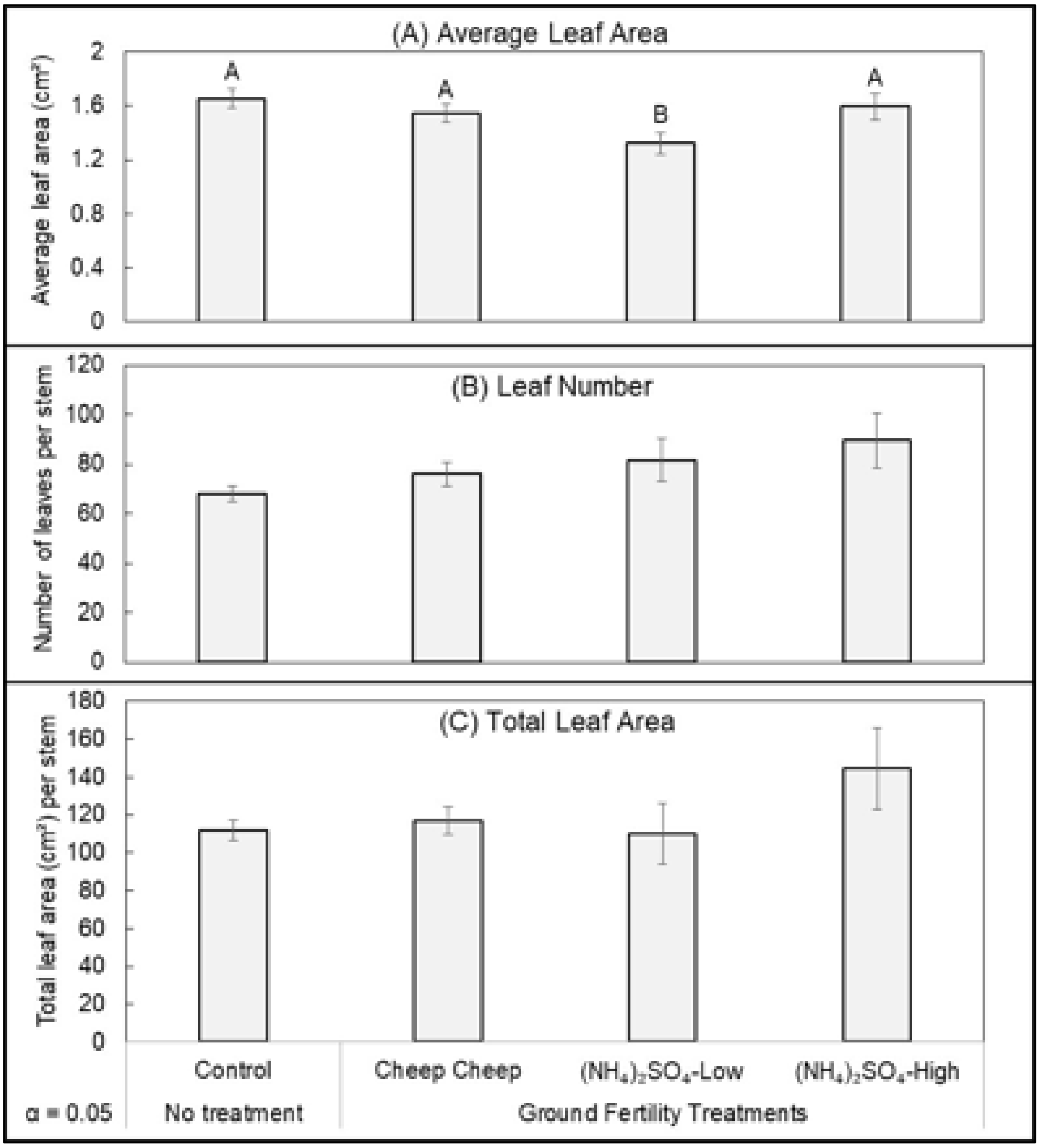

Figure 6. Comparison in (A) average leaf area, (B) number of leaves per stem, and (C) total leaf area per stem of wild blueberry plants by treatment as measured on July 26, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Error bars indicate the standard error of the mean. Different letters indicate significant differences and no letters on the bars indicate no significant differences at the significance level of $p < 0.05$.

Pest Presence

The number of weeds measured (Figure 7) on multiple sample dates in 2021 and 2022 were highest in the low ammonium sulfate treatment (43.1 weeds/m^2), followed by Cheep Cheep (35.9 weeds/m^2), the high rate of ammonium sulfate treatment (9.4 weeds/m^2), and the control (4.8 weeds/m^2), which exhibited the lowest weed densities. This is most likely due to perennial weed presence before product application yet more years of study are needed to confirm any weed number change due to fertilizer applications.

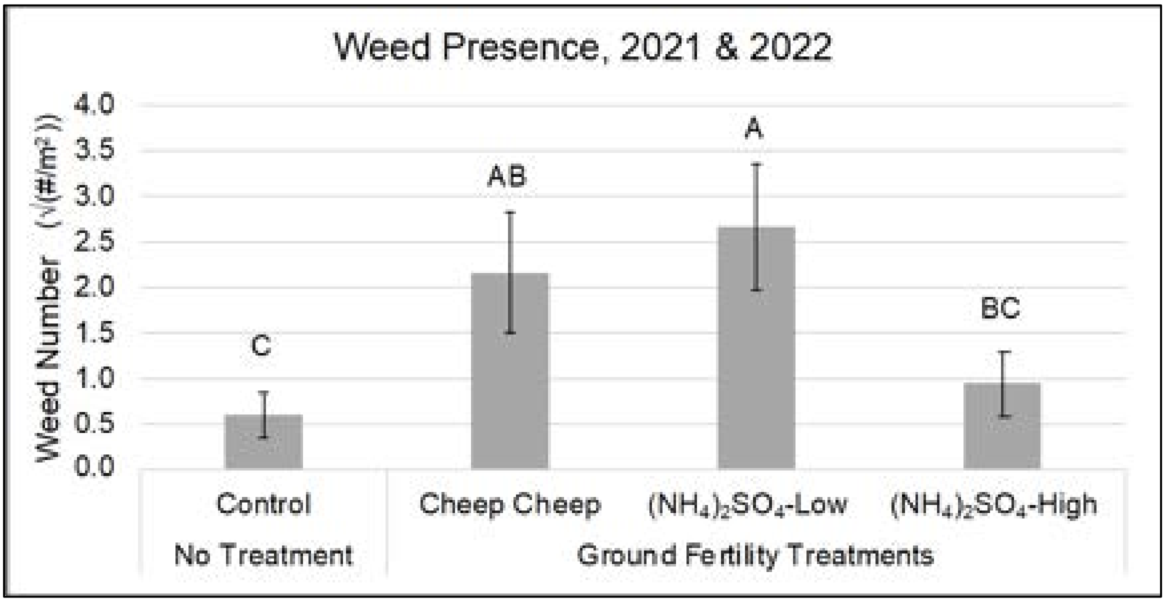

Figure 7. Average weed number (√#/m^2) by treatment measured on four dates in 2021 (June 9, July 21, August 20, and September 20), and two dates in 2022 (May 19 and June 15) at Blueberry Hill Research Station, Jonesboro, Maine. Letters indicate significant differences at the 0.05 level of significance. Error bars represent the standard error of the mean.

Blueberry stem damage attributable to flea beetle and tip midge insects (Figure 8) measured across 2021 and 2022 were highest in number in the control (9.16 stems/m^2) and highest in rank in the low-rate ammonium sulfate treatment (3%/m^2). Interestingly, the number of stems with insect damage and the coverage rank of insect damage were lowest in the high rate of ammonium sulfate treatment (4.39 stems/m^2, 1%).

Blueberry stem damage resulting from mummy berry and leaf spot disease was not significant when comparing the counts (#/m^2), however, the ranks (%/m^2) exhibited significantly higher disease coverage in the control (48%/m^2) when compared to the high rate of ammonium sulfate treatment (33%/m^2; Figure 9). The counts (#/m^2) are a measure of how many stems per area are infected, while the ranks (%/m^2) capture the degree of infection on the infected stems.

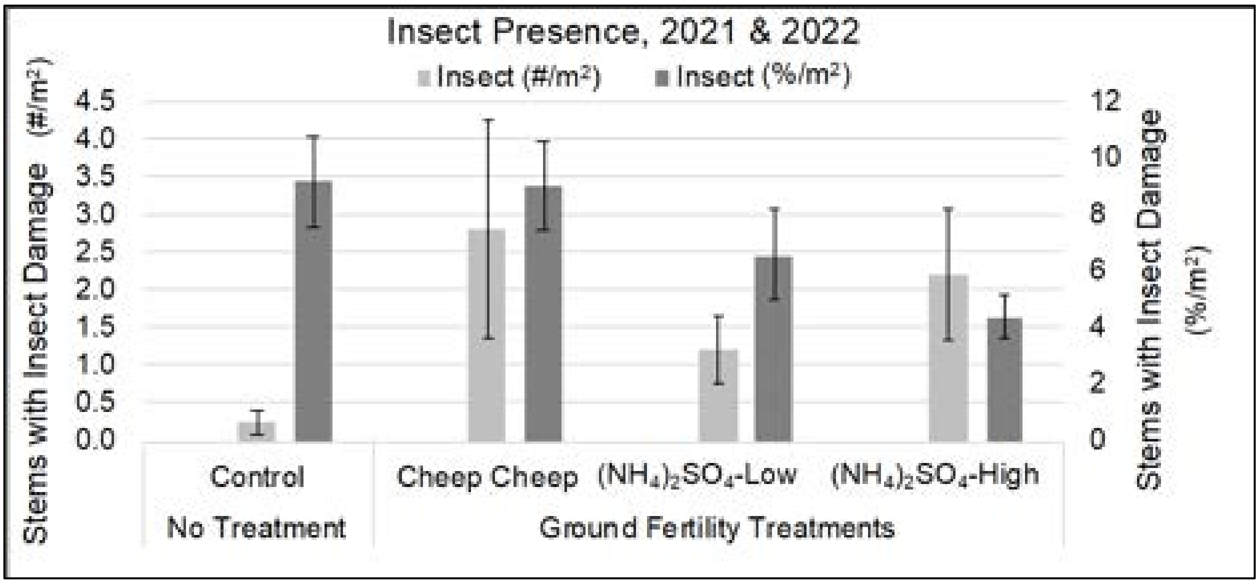

Figure 8. Average number and percent of blueberry stems with flea beetle and tip midge insect presence (#/m^2 and %/m^2) by treatment measured on four dates in 2021 (June 9, July 21, August 20, and September 20), and two dates in 2022 (May 19 and June 15) at Blueberry Hill Research

Station, Jonesboro, Maine. Treatment differences were not significant for the number and percent of blueberry stems with insect damage. Error bars represent the standard error of the mean.

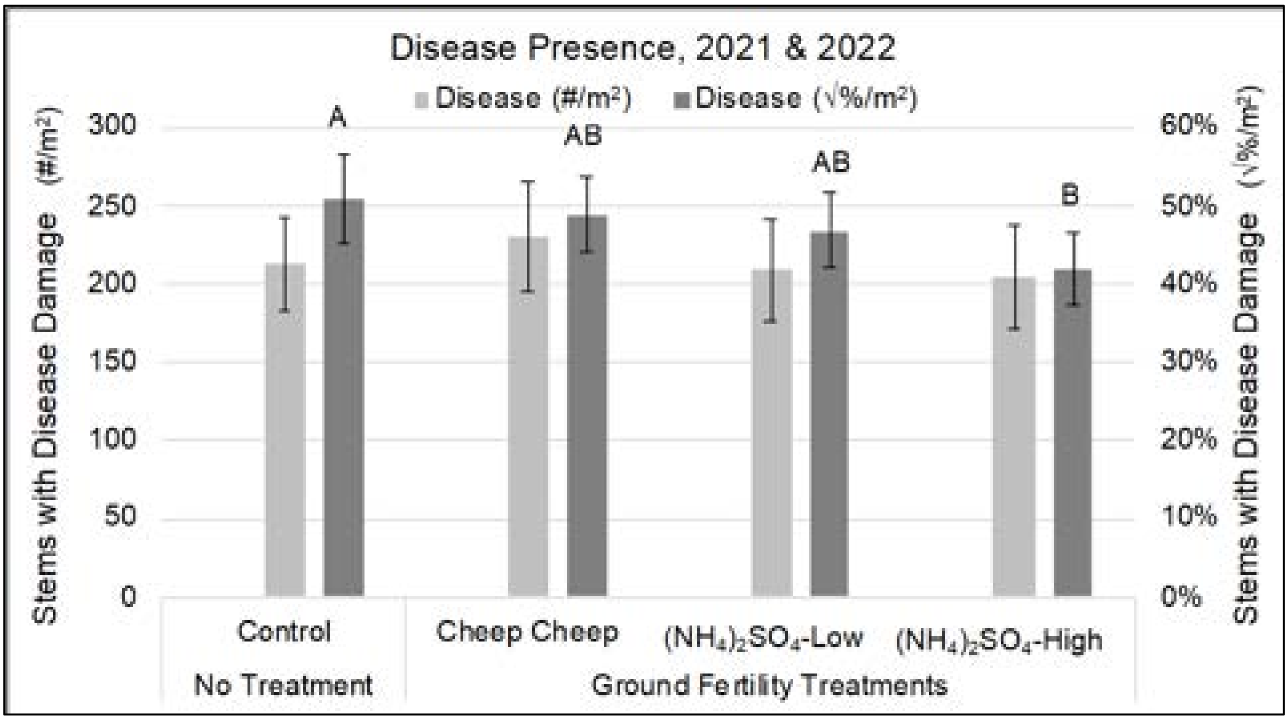

Figure 9. Average number and percent of blueberry stems with mummy berry and leaf spot disease presence (#/m^2 and %/m^2) by treatment measured on four dates in 2021 (June 9, July 21, August 20, and September 20), and two dates in 2022 (May 19 and June 15) at Blueberry Hill Research Station, Jonesboro, Maine. Letters indicate significant differences at the 0.05 level of significance for the percent of blueberry stems with disease damage (%/m^2). Error bars represent the standard error of the mean.

Fruit Yield and Quality

Treatment differences in yield varied relative to the control were not significant (Figure 10). The high rate of ammonium sulfate treatment exhibited the highest yield (2244 lbs/A) which was 29% greater than the control (1736 lbs/A). The yields harvested from the Cheep Cheep (1571 lbs/A) and low-rate ammonium sulfate (1244 lbs/A) treatments were 10% and 28% less than the control, respectively. Treatment differences in yield were not significant.

The highest berry weights (Figure 11) were measured in the low-rate ammonium sulfate treatment (44.4 g/100 berries). All other treatments including the control (31.2 g/100 berries), Cheep Cheep (32.9 g/100 berries) and the high rate of ammonium sulfate (31.9 g/100 berries) were similar in berry weight.

Brix, as a measure of berry sugar content, were similar across all treatments (Figure 12), with the highest Brix content in the low-rate ammonium sulfate treatment (12.9 Brix), followed by the control (12.2 Brix), Cheep Cheep (11.70 Brix), and the high rate of ammonium sulfate treatment (11.38 Brix).

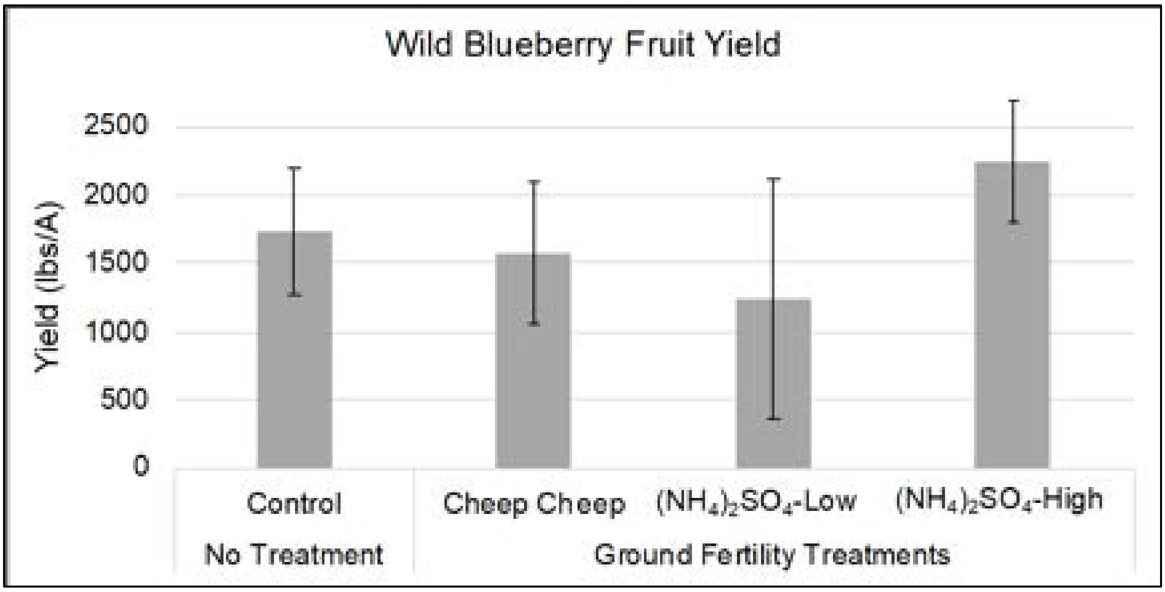

Figure 10. Average yield (lbs/A) by treatment harvested on August 11, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Treatment differences in crop yield were not significant. Error bars represent the standard error of the mean.

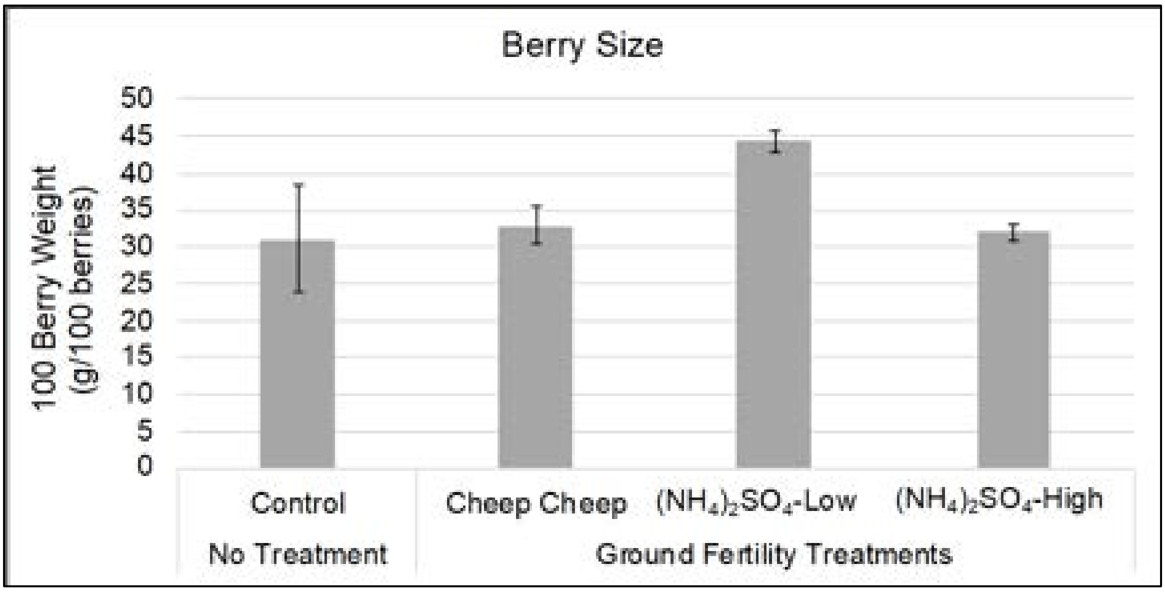

Figure 11. Average berry size (100 berry weight; g/100 berries) by treatment harvested on August 11, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Treatment differences in berry size were not significant. Error bars represent the standard error of the mean.

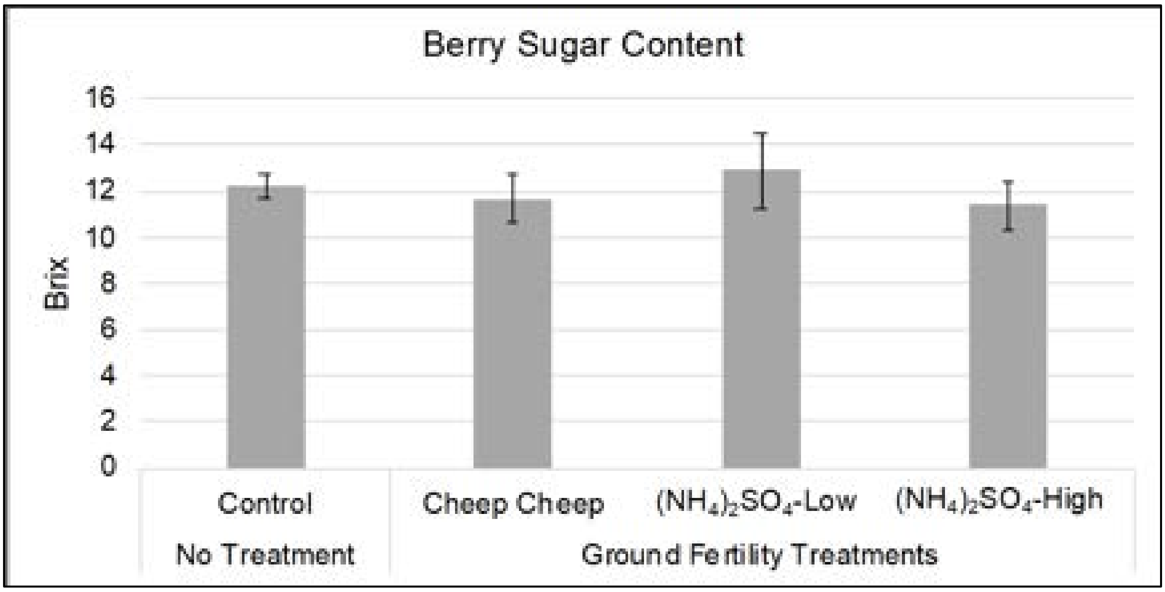

Figure 12. Average berry sugar content (Brix) by treatment harvested on August 11, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Treatment differences in berry sugar content were not significant. Error bars represent the standard error of the mean.

DISCUSSION

Plant Phenology

While the high ammonium sulfate treatment produced generally high numbers of buds, flowers, green fruit, and blue fruit, the difference between green fruit observed and blue fruit observed was very large (21.0 green vs. 5.5 blue), and treatments of low ammonium sulfate (14.9 green vs. 6.3 blue), Cheep Cheep (14.8 green vs. 7.9 blue) and the control (13.2 green vs. 4.9 blue) saw smaller differences in fruit number from green to blue fruit. Growers are more interested in lots of green fruit if it results in lots of blue fruit, and that trend was not necessarily observed in the high ammonium sulfate treatment.

The higher bud, flower and green fruit numbers in the high rate of ammonium sulfate treatment suggests this ground fertility amendment aided bud development in the prune year and fruit set in the crop year. Fewer blue fruits per stem in this treatment suggest higher green fruit abortion/drop because the plants could not support the number of green fruits formed, or these plants developed faster phenologically, leading to natural fruit maturation and drop prior to harvest on August 11, 2022.

As observed in 2021, the high rate of ammonium sulfate again produced the tallest stems and greatest blueberry cover, demonstrating that the product is a quick-release conventional product. Taller blueberry stems indicate more robust plant health and development likely obtained through greater usable resources at key development stages.

Pest Presence

The fertilizer treatments did not indiscriminately “feed the weeds.” If they had, we would expect to see that the high rate of ammonium would contain the most weeds, instead it contained the second-fewest weeds. The sulfur in ammonium sulfate did not reduce weed presence because ammonium sulfate alone does not reduce soil pH. This product can help maintain a low soil pH by mitigating the increase in pH that is always expected to occur with fertilizer application.

The decrease in insect and disease presence after amendments were applied relative to the control indicates that the amendments increased plant health, and so increased plant resilience to flea beetle, tip midge, mummy berry, and leaf spot. Some insect pests prefer to feed on plant tissue that is high in N and P yet that was not observed here.

Blueberry Cover

All the treatments had greater blueberry cover than did the control, indicating the treatments did encourage plant growth. Cheep Cheep and the high rate of ammonium sulfate treatments saw significantly greater blueberry cover compared to the controls.

Fruit Yield & Quality

Fruit produced after application of low-rate ammonium sulfate was larger in size and higher in Brix than fruit in any other treatment. Despite producing larger, higher-quality fruit, this treatment produced the lowest yield of all the treatments, including the control which may be due to genetic diversity of plants or trampling of the plots. On August 11 when this trial was harvested, the fruit was dropping and past peak ripeness. Though the differences in fruit Brix content was small, growers selling fruit for value-added products may be interested in these values.

Plant Physiology & Morphology

Plants that received low- and high rate of ammonium sulfate applications performed the best in terms of overall observed physiological and morphological parameters. This could be because of the available nitrogen (Taiz et al., 2015; Zhang et al., 2015) from the ammonium sulfate fertilizer. However, the differences in plant responses were not significant among the treatments, possibly because of the similar soil characteristics and available nutrients in the soil (Table 2). Their responses can be further explained based on the leaf nutrient concentrations from this season (results forthcoming). The decline in response differences among the treatments applied could also indicate that the promising treatments should be applied every few years to achieve consistent improvements in plant physiology, morphology, and fruit yields.

CURRENT RECOMMENDATIONS

- Conduct foliar tests before any fertilizer application. These foliar tests will tell you what nutrients are needed. If foliar test results show phosphorous is needed, applying MAP and DAP is appropriate. If phosphorous is not needed, apply ammonium sulfate.
    - See the following fact sheet for information on conducting foliar tests: Fact Sheet 222- Leaf and Soil Sampling Procedures: https://extension.umaine.edu/blueberries/factsheets/production/leaf-soil-sampling-procedures/
- For organic growers who have good control over weeds, apply pelleted chicken manure. If you do not have good weed control, control the weeds before applying pelleted chicken manure.

ACKNOWLEDGEMENTS

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1. Efficacy of a Homemade Forced Air Cooling (FAC) System on Wild Blueberry Quality

OBJECTIVE

This project aimed to prolong berry quality post-harvest by demonstrating the use of forced-air cooling to improve air flow in cold storage units for high-quality fresh pack.

LOCATION: Blueberry Hill Research Farm, Jonesboro, ME
PROJECT TIMEFRAME: July and August 2022

INTRODUCTION

Many family-run 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. When berries are cooled and then moved into a warmer space for transport, storage, or sale, this temperature fluctuation causes condensation to form on the fruit, and the combination of warmer temperatures and increased fruit surface moisture decreases fruit 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.

Wild blueberry is harvested at the peak of ripeness leaving growers and processors with a short amount of time to get fresh wild blueberries to consumers. Wild blueberry continues to respire after being harvested, and this respiration increases the temperature of the fruit and contributes to its eventual, inevitable decay in quality. Thus, slowing the rate of respiration is critical to maintaining higher-quality fruit for longer. The easiest way to reduce fruit respiration (and associated decay) is by lowering the temperature of the fruit: highbush blueberries stored at 80.6°F respire at a rate twenty times higher than that of fruit stored at 40°F (Boyette et al, 1993). Sanford et al. (1991) demonstrated that the ideal storage temperature for wild blueberries is close to 32°F, taking extra care to prevent the fruit from freezing, since that would ruin the fruit destined for the fresh market. Postharvest wild blueberries decay at a slower rate than do highbush blueberries (Sanford et al., 1991), but any loss of saleable product harms small growers.

One method to reduce fruit temperature is by removing harvested fruit from the field and placing it in a cold or refrigerated room. This ambient cooling does not cool the fruit quickly enough, so use of a forced air cooling system ("FAC system") can cool the berries by several degrees in just several hours, as opposed to several days. FAC systems increase airflow by using a blower or fan to pull cooled air over the fruit, thereby "effect[ing] rapid heat transfer" through the "close contact" of the warm fruit and cool air (Boyette & Rohrbach, 1993). FAC systems do not cool the berries by cooling air that is directed at the fruit but instead pulls already-cooled air over the fruit. By positioning a fan or blower at one end of the system, an air pressure gradient is created and so the air moves from the high-pressure side (where the focused air of the blower or fan is pointed) to the low-pressure side (further from the blower or fan) (Boyette et al., 1989). As air moves from high- to low-pressure, the cooled air is forced between the packaging and the individual fruit and the contact of that cooler air with the warm fruit effects a heat transfer from the high-energy object (the packaging or the fruit) to the low-energy object (passing air molecules). Thus, passing cooled air cools down the individual berries. The constant movement of air passing from high-pressure to low-pressure can also accelerate the rate of evaporation of moisture found on the surface of the fruit, thereby drying the fruit surface. Rates of fruit cooling and moisture reduction are thus dependent on fruit temperature, air temperature, rate of airflow, and type of fruit being cooled (Boyette et al., 1989). FAC systems cool berries ten times quicker than simply placing the fruit in a refrigerated room (Boyette, 1996).

By design, refrigeration and/or air conditioning units remove moisture from the air as the air is cooled, but very low air humidity is associated with a decline in fruit quality, so the humidity levels of the storage areas cannot drop too low (Boyette & Rohrbach, 1993). Removing moisture from the fruit surface is ideal because moisture can decrease quality and increase risk of postharvest disease (Boyette, 1996), but removing all moisture from the storage area will decrease quality of all the fruit, not just the wet fruit (Boyette & Rohrbach, 1993).

FAC systems can be found in shipping containers and refrigerated trucks all over the world. Since FAC systems do not directly cool the fruit, they operate in refrigerated spaces and pulled that cooled air through the system. Commercial systems can be prohibitively expensive for small and family farm operations but fortunately there are a range of smaller, homemade systems that achieve the same effect at much lower cost. Using resources from the University of Vermont Extension Ag Engineering website (https://blog.uvm.edu/cwcallah/2018/10/09/forced-air-cooling-on-the-farm/), a small version of a forced air cooling system was constructed at Blueberry Hill Farm in Jonesboro, ME. UVM's team constructed two versions of the FAC systems, the "countertop" version sized for one to three cartons (bulb crate or 1/9th bushel box) and a larger one sized for a partially- or fully-loaded pallet.

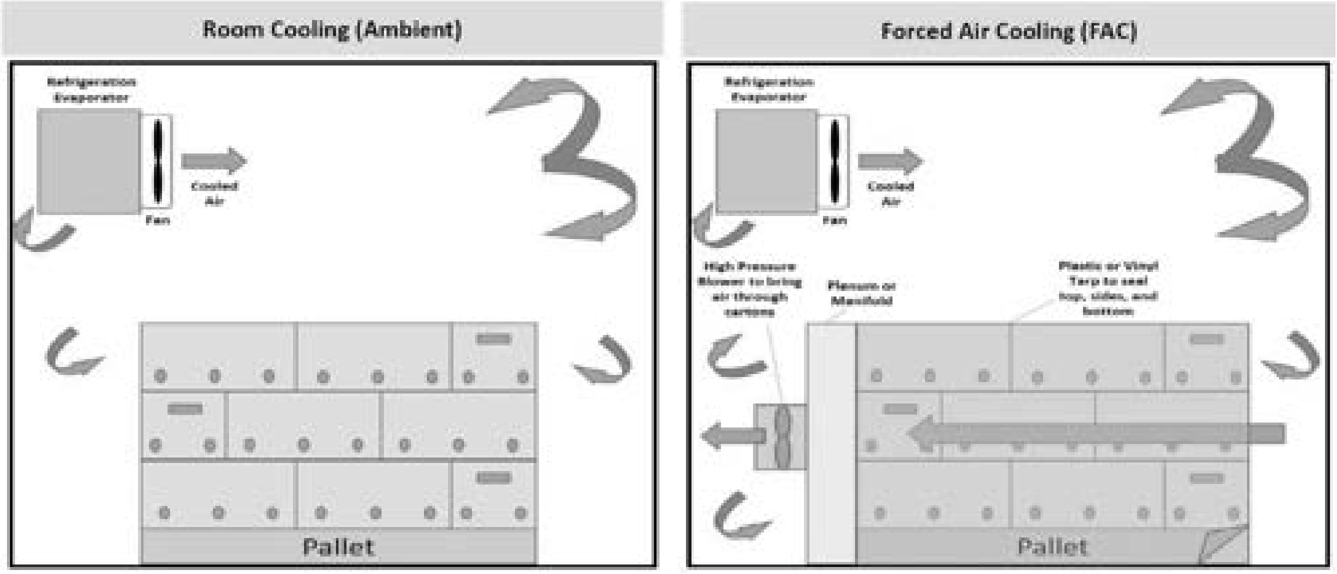

Image 1. Left, a diagram showing how cooling the entire room or space does not generate sufficient pressure or velocity to pass through the stacked produce crates to quickly cool the harvested produce. Right, a diagram showing how using a forced air cooling system to pull air over stacked produce crates completely sealed in plastic generates enough pressure and velocity quickly cool and dry the harvested produce. Images taken from the UVM Extension Ag Engineering site (Callahan, 2020).

Homemade forced air cooling systems can be constructed in an afternoon and require just a short list of easily accessible materials. In Table 1, the materials used to make the small FAC in Jonesboro are listed. This small unit was built in one day in July 2022 and cost roughly $250. Full building plans are described and should be followed from the University of Vermont Extension site listed above.

This project builds on earlier research into the optimal wild blueberry storage temperature within homemade cold storage units. Earlier research measured fruit quality for 30 days post-harvest in cold storage units and found that cooler berries maintained good quality for longer, but berry surface moisture was not studied. The question explored in 2022 was, does forced air cooling inside a cold storage unit reduce moisture on the berries?

Table 1. Table showing materials to construct a FAC system.

Framing lumber	Fender washers
Plywood	Plastic (4 mil poly)
Decking screws	Blower (12” portable blower fan)
Decking screws	

For a more thorough explanation of the need for, construction, and costs of the cool temperature storage facilities used at Blueberry Hill Farm, please see the 2020 report summary, entitled “Coolbot Cold Storage Room Construction and Costs” (page 148) and the 2021 report summary entitled, “Improving Shelf Life of Fresh Pack Maine Wild Blueberries” (page 200).

METHODS

Fruit quality was measured through photographs and visual inspection in conjunction with long-term storage unit temperature and relative humidity measurements. This study was conducted at Blueberry Hill Research Farm (BHF) in Jonesboro, Maine. At BHF, there are three 8ft x 8ft cold storage units, constructed in 2020 and 2021 (Table 2).

Table 2. Summary of cold storage unit specifications.

Cold storage units at Blueberry Hill Farm		
1	2	3
34°F	40°F	50°F
16,000 BTU	12,000 BTU	12,000 BTU
R-10 (doubled)	R-10 (doubled)	R-10 (doubled)

Fruit was harvested at BHF on August 2 and 10 into BerryMate plastic bins with aeration slits called “fresh pack bins”; the fruit was not winnowed and so contained some leaf debris and other organic matter. Two separate tests were completed to test the effectiveness of forced air cooling on berry surface moisture. The first test occurred from August 2-3 in the 50°F cold storage unit and the other occurred August 10-13 in the 40°F cold storage unit (Table 3).

On August 2, berries were hand raked into the fresh pack bins, kept in the shade when in the field, then brought to the cold storage units to have photos taken. Photos were again taken of these bins on August 3 and were later used to count total, wet, and shriveled fruit. On August 2, ten bins were photographed before being placed in any cold storage unit. On August 3, ten bins were photographed after 24 hours in the FAC system in the cold storage unit set to 50°F, ten bins were photographed after 24 hours in the cold storage unit set to 50°F (not inside the FAC system), and two bins were photographed after 24 hours being left in ambient temperatures outside any cold storage units; all photos were later used to count total, wet, and shriveled fruit.

On August 10, berries were hand raked into the fresh pack bins, kept in the shade when in the field, then brought to the cold storage units to have photos taken. When still in the field, iButtons (small, quarter-sized buttons that continuously measure temperature; Maxim Integrated, San Jose, CA) were placed in the centers of each bin, approximately one inch below the surface of the harvested fruit, and then covered up again by the fruit. Photos were again taken on August 13 and were later used to count total, wet, and shriveled fruit. On August 10, ten bins were photographed before being placed in any cold storage unit. On August 13, five bins were photographed after 72 hours in the FAC system in the cold storage system set to 40°F, and five bins were photographed after 72 hours in the cold storage unit set to 40°F (not inside the FAC system). iButtons continuously measured internal bin temperature and remained buried in the harvested fruit in the bins until the end of the study.

Table 3. Summary of bin samples.

Sample	**Harvest date**	**Dates sampled**
Bins	August 2	August 2 & 3
Bins plus iButtons	August 10	August 10 & 13

Image 2. Left, unwinnowed berries from BHF stored in a fresh pack bin with slats for aeration. Right, the countertop FAC system before being loaded with fresh pack bins and/or turned on.

Photographs of each bin during each sampling event were processed using FIJI/ImageJ's cell counter mode (FIJI software version 2.9.0, Madison, WI). Images were first scaled and a 4" x 4" square was drawn on the image, to approximate the area that would be visible if fruit were stored in a pint container. The berries in the images were then counted using the cell counter mode, which had three counter options: total fruit, wet fruit, shriveled fruit. Every single piece of fruit was hand-counted using the program's total fruit mode, and when appropriate, the fruit was also counted using the wet or shriveled berry counters. Each photo then generated three numbers, which could be compared across time and treatment: total fruit, wet fruit, and shriveled fruit.

<u>Data analysis</u>

Berry wetness data collected from the two storage units (40°F and 50°F), were analyzed using a One-way ANOVA, followed by a Tukey's Pairwise comparison in JMP (JMP®, Version 16.0, SAS, Cary, NC, USA). Due to the nature of the data collected, the berry wetness data collected Aug 10-13, 2022, in the 40°F storage unit failed the assumptions of normality and equal variance required to run parametric statistical tests. Transforming the data via a square root transformation resolved these issues. Berry wetness data collected August 2 – 3, 2022, passed assumptions for parametric statistical testing and a transformation was not required. All graphs were designed using Microsoft Excel (Excel® Version 2110, Microsoft Corporation, Redmond, WA, USA).

RESULTS

iButtons were placed within the bins while the bins and berries were still in the field but stored in the shade, and internal bin temperatures dropped from 77.4°F to 74.5°F after two hours post-harvest (Figure 1). The bins were then placed into the 40°F cold storage unit and the bin temperature dropped down to 65.9°F after one hour, and then 57.6°F after another hour in the unit. The internal bin temperatures then continued to cool for the next seventeen hours, at which point temperatures reached as low as 43.6°F and hovered between there and 44.2°F for another fourteen hours (Figures 1 and 2). Bin temperature then spiked at one hourly reading, without any accompanying cold storage unit temperature spike, to 45.7°F, before dropping down to 43.9°F the next hour, and remaining between 43.6°F and 43.9°F for eight more hours, until temperatures increased again above 44.0°F. These slight increases mimicked the slight increases in cold storage unit temperature.

Temperatures in the cold storage unit increased when the unit's door was opened to place the bins, from 45.1°F to 50.1°F (Figures 1 and 2, below). Unit temperatures then steadily dropped over the next three hours, to 44.6°F, and continued to drop to 43.6°F over the next ten hours. Temperatures did then begin a steady increase over the next twelve hours, peaking at 45.5°F and steadying there for three hours before decreasing again. Temperatures reached a low of 44.2°F overnight before beginning a steady increase up to 46.4°F, hovering there for about four hours, and dropping down again to lows of 43.7°F.

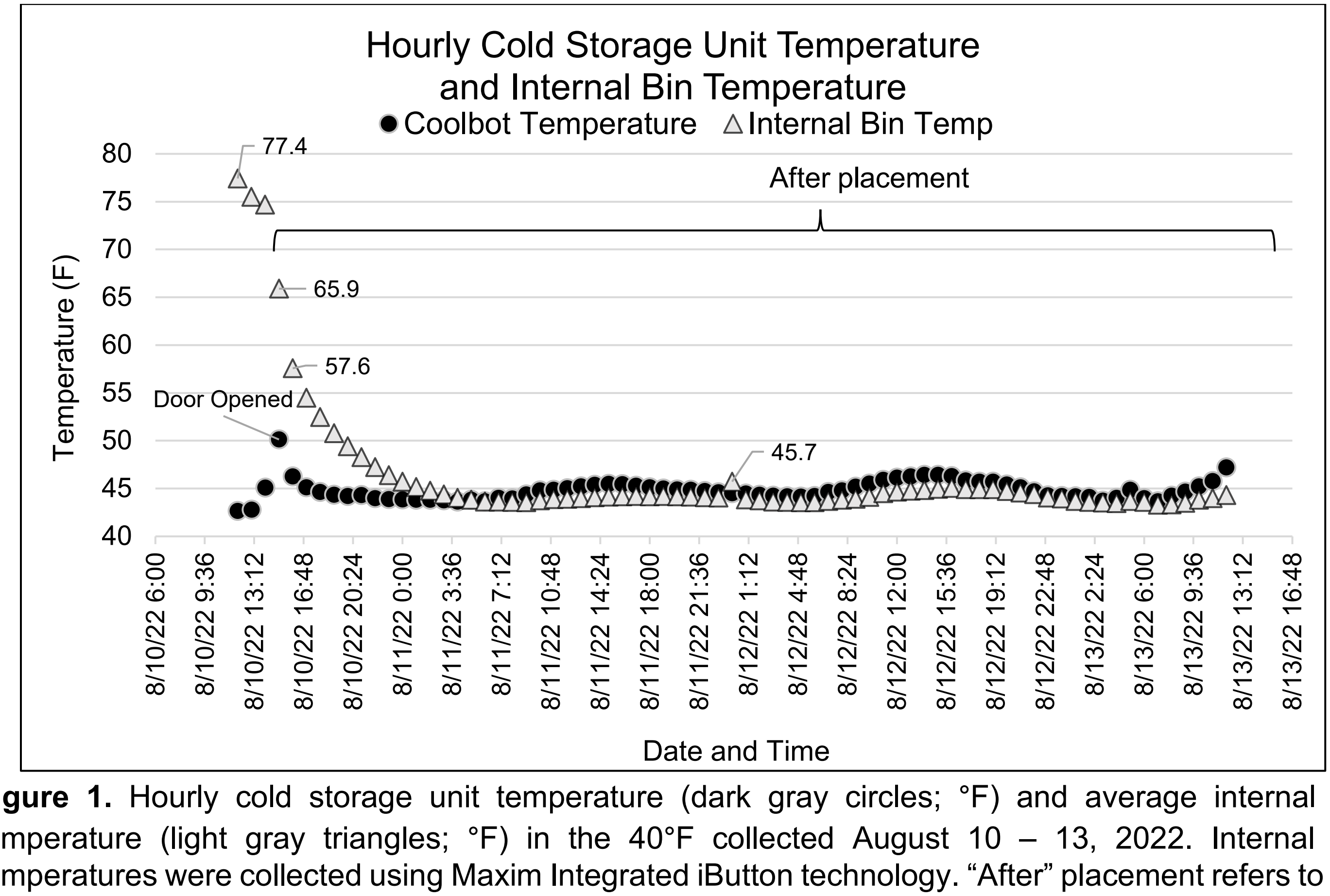

Figure 1. Hourly cold storage unit temperature (dark gray circles; °F) and average internal bin temperature (light gray triangles; °F) in the 40°F collected August 10 – 13, 2022. Internal bin temperatures were collected using Maxim Integrated iButton technology. "After" placement refers to the moment the bins were physically placed into the cold storage unit. See Figure 2 for more detail.

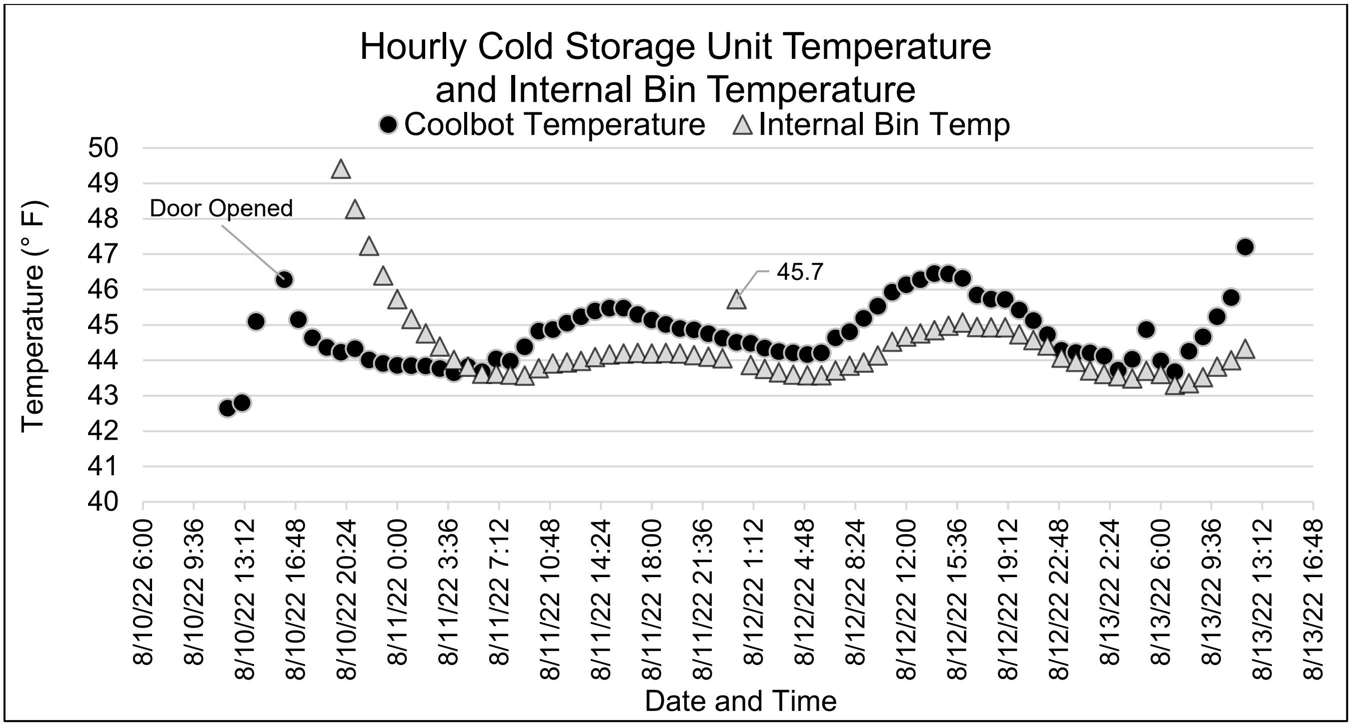

Figure 2. Hourly cold storage unit temperature (dark gray circles; °F) and average internal bin temperature (light gray triangles; °F) in the 40°F collected August 10 – 13, 2022. Internal bin temperatures were collected using Maxim Integrated iButton technology. This graph is a zoomed-in version of Figure 1 to facilitate better understanding of temperature variations.

Berries were harvested into bins and placed in the 50°F cold storage unit from August 2 - 3, 2022. In the 50°F cold storage unit, the wetness and shriveling of berries varied over time (Figure 3). Before being placed in the cold storage unit, 24% of the berries were wet and 8% were shriveled. After one day of cooling in the FAC system, 36% of berries were wet and 56% were shriveled (a statistically significant increase). After one day of cooling outside of the FAC system, 27% of berries were wet and 10% were shriveled (a slight increase).

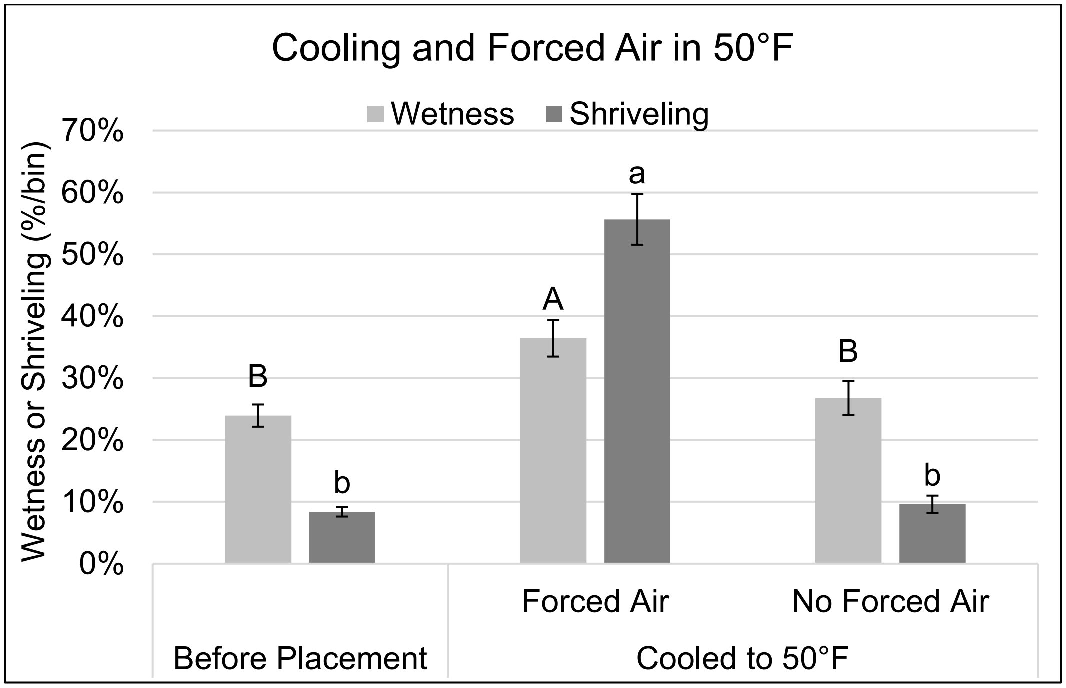

Figure 3. Average berry wetness (%/bin) and shriveling (%/bin) in 50°F cooling unit measured 24 hours after cooling and forced air treatments which took place from August 2 – 3, 2022. Letters indicate significant differences at the 0.05 level of significance. Treatment differences in berry wetness were not significant. Error bars represent the standard error of the mean.

Berries were placed in bins and placed in the 40°F cold storage unit from August 10 - 13, 2022. In the 40°F cold storage unit, the wetness and shriveling of berries increased over time (Figure 4). Before being placed in the cold storage unit, 12% of the berries were wet and 20% were shriveled. After 3 days of cooling in the FAC system, 18% of berries were wet (a slight increase) and 40% were shriveled (a statistically significant increase). After 3 days of cooling outside of the FAC system, 19% of berries were wet (a slight increase) and 40% were shriveled (a statistically significant increase).

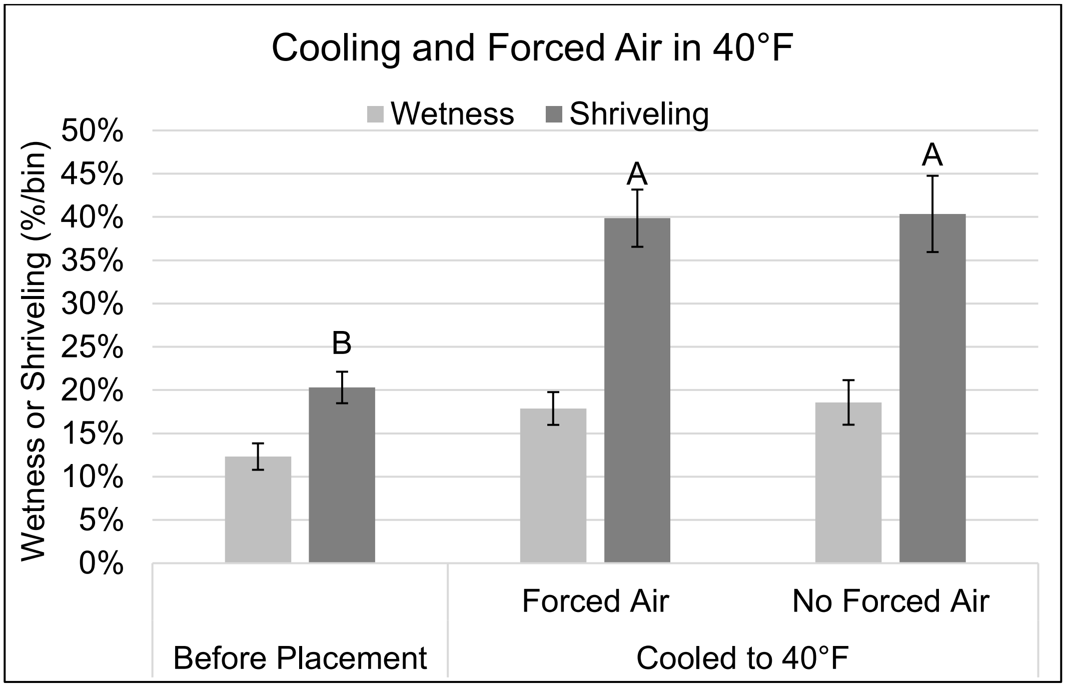

Figure 4. Average berry wetness (%/bin) and shriveling (%/bin) in 40°F cooling unit measured immediately after cooling and forced air treatments which took place from August 10 – 13, 2022. Letters indicate significant differences at the 0.05 level of significance. Treatment differences in berry wetness were not significant. Error bars represent the standard error of the mean.

DISCUSSION

Berries placed in the 50°F cold storage unit for 1 day grew significantly wetter and more shriveled over the storage time, regardless of whether the bin was situated in the FAC system or not. Berries placed in the 40°F cold storage unit for 3 days grew slightly wetter and significantly more shriveled over the storage time, regardless of whether the bin was situated in the FAC system or not.

These results are the opposite of what was expected indicating that we did not use the FAC unit correctly. Improvements that will be made for next year's trial of this include: not purposefully wetting berries before entering storage, placing more bins of berries into the FAC unit for it to run at full capacity, and not including any other bins of berries in the cold storage unit while the FAC unit is running. Several confounding factors influenced this first attempt at forced air cooling.

Fluctuations in the temperatures of the cold storage unit coincided with daily temperature changes associated with the time of day. At dawn (generally the coldest point of any day), unit temperatures were the lowest, unit temperatures increased as the sun came up and significantly increased during the hottest point of the day (late afternoon, early evening), before gradually cooling during the night. Accordingly, internal bin temperature mimicked the changes in unit temperature. The relationship between atmospheric temperature and unit temperature can likely be attributed to three things: the ambient temperature of the garage housing the unit, the airtightness of the unit, and the ability of the air conditioning unit, particularly in the 34°F unit (data in other Cold Storage Report, see page C9), to maintain temperatures at the programmed temperature. As outdoor temperatures increased, the ambient temperature of the garage also increased and would eventually increase the temperature of the cold storage unit if the air conditioning unit did not kick on and cool the air.

CURRENT RECOMMENDATIONS

- Place the cold storage units under some shelter (e.g., in a garage bay or barn) and ensure the unit is well-insulated and leak-free so the cold storage unit is not releasing dry, cold air or pulling in warm, moist air.

NEXT STEPS

- Modify methods and repeat in 2023.

ACKNOWLEDGEMENTS

Thank you to Northeast SARE for funding this project and to Karl Zukauskas for building the FAC unit. Thank you to Mara Scallon and Brogan Tooley for assistance with data collection and analysis.

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

2. Evaluating Cold Storage Temperatures on Fresh Pack Berry Quality

OBJECTIVE

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.

LOCATIONS: Blueberry Hill Research Farm, Jonesboro, ME and Welch Farm, Roque Bluffs, ME
PROJECT TIMEFRAME: July and August 2021 & 2022

INTRODUCTION

Many family-run 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. When berries are cooled and then moved into a warmer space for transport, storage, or sale, this temperature fluctuation accelerates the decline in fruit quality. Growers have indicated that fresh wild blueberry storage temperatures range from 40°F to 70°F, so many growers are hesitant to cool berries because they lack a complete cold chain or on-farm cooling infrastructure.

Wild blueberries are harvested at the peak of ripeness and growers and processors have a short amount of time to get fresh wild blueberries to consumers. Wild blueberries continue to respire after being harvested, and this respiration increases the temperature of the fruit and contributes to its eventual, inevitable decay in quality, where the fruit loses mass and firmness (Tetteh et al., 2004). Thus, slowing the rate of respiration is critical to maintaining higher-quality fruit for longer. The easiest way to reduce fruit respiration (and associated decay) is by lowering the temperature of the fruit: highbush blueberries stored at 80.6°F respire at a rate twenty times higher than that of fruit stored at 40°F (Boyette et al, 1993). Sanford et al. (1991) demonstrated that the ideal storage temperature for wild blueberries is close to 32°F, taking extra care to prevent the fruit from freezing, since that would ruin the fruit destined for the fresh market. Postharvest wild blueberries decay at a slower rate than do highbush blueberries (Sanford et al., 1991), but any loss of saleable product harms small growers. Consumers generally purchase fresh blueberries impulsively and are guided by the visual appearance of the fruit in deciding whether to purchase (Tetteh et al., 2004), so individual growers, local cooperatives, and Maine's entire industry benefit from delivering high-quality fruit to consumers (Wild Blueberry Commission of Maine, n.d.).

This project builds on earlier research into the optimal wild blueberry storage temperature within homemade cold storage units. Earlier research measured fruit quality for 30 days post-harvest in cold storage units and the 2022 season's research focused on the following two questions: A) Which of the cold storage temperatures of 34°F, 40°F, and 50°F best maintains fresh pack pint berry quality over 30 days? and B) Is there a relationship between outdoor/ambient temperatures and the temperatures within the internal storage units? To answer these questions, berry moisture, shriveling, and temperature were measured along with the temperature and relative humidity of five cold storage units throughout the fresh pack harvest season.

For a more thorough explanation of the need for, construction, and costs of the cool temperature storage facilities used at Blueberry Hill Farm, please see the 2020 report summary, entitled "Coolbot Cold Storage Room Construction and Costs" (page 148) and the 2021 report summary entitled, "Improving Shelf Life of Fresh Pack Maine Wild Blueberries" (page 200).

Image 1. View inside the cold storage unit with fruit stored in molded paper pulp pint containers.

METHODS

Part 1. 36-Day Storage of Fresh Pack Pints

Photographs and visual inspection were used in conjunction with long-term storage unit temperature and relative humidity measurements to quantify fruit quality. This study was conducted at the Blueberry Hill Farm Experiment Station (BHF) in Jonesboro, Maine and at Welch Farm in Roque Bluffs, Maine. At BHF, there are three 8ft x 8ft cold storage units, constructed in 2020 and 2021. At Roque Bluffs, there is one refrigerated truck trailer (8ft x 24ft; unit 2, see below) and one 8ft x 8ft cold storage unit, constructed in 2021.

Table 1. Summary of cold storage unit specifications.

Cold storage units at Blueberry Hill Farm		
1	2	3
34°F	40°F	50°F
16,000 BTU	12,000 BTU	12,000 BTU
R-10 (doubled)	R-10 (doubled)	R-10 (doubled)
Cold storage units at Welch Farm		
N/A	1	2
	40°F	46-56°F
	12,000 BTU	12,000 BTU
	R-10 (doubled)	R-10 (doubled)

Pints were picked up from Welch Farm on August 2 after being hand raked on August 1, stored overnight in a cold storage room at 50°F in wooden bins (lacking slats for ventilation), and finally were run through the fresh pack line on August 2, when the berries were transferred to pint-size molded pulp produce baskets. These pints were then transferred to BHF, where they were photographed and then thirty pints were stored uncovered on one shelf with pints directly abutting each other. A handheld digital thermometer with moveable humidity and temperature probes was placed in each unit; the temperature probe was buried in the berries of one pint and remained there for the duration of the study. The humidity probe remained exposed to the atmosphere.

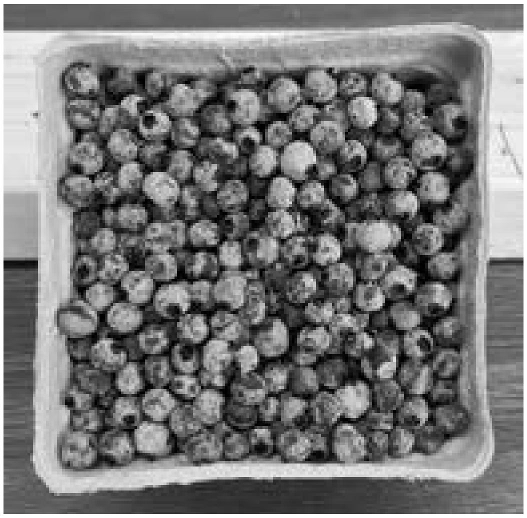

Image 2. Winnowed and cleaned berries stored in a pint-size molded paper pulp produce basket from Welch Farm.

Pints were sampled six times between August 2 and September 6 on August 2, 5, 15, 25, 30 and September 6 for a total of 36 storage days. Measures taken during each visit included cold storage unit air temperature as displayed on the air conditioning units, cold storage unit air temperature as displayed on a portable digital thermometer, and photos of 15 random pints from each cold storage unit for later measurement of berry moisture and shrinkage.

Table 2. Summary of pint samples.

Fruit storage size	Harvest date	Dates sampled	Date removed
Pints	August 2	August 2, 5, 15, 25, 30 & September 6	September 6

Photographs of each pint during each sampling event were processed using FIJI/ImageJ's cell counter mode (FIJI software version 2.9.0, Madison, WI). The berries in the images were counted using the cell counter mode, which had three counter options: total fruit, wet fruit, and shriveled fruit. Every fruit was hand-counted using the program's total fruit mode, and when appropriate, the fruit was also counted using the wet or shriveled berry counters. Each photo then generated three numbers, which could be compared across time and treatment: total fruit, wet fruit, and shriveled fruit.

Part 2. Ambient Temperature & Cold Storage Unit Temperature

Onset HOBO (Onset Computer Corporation, Bourne, MA, USA) temperature and relative humidity sensors (MX2300) were placed in each cold storage unit to continuously track these metrics over time. Three total sensors were placed in the Welch Farm fresh pack processing area and both cold storage units in late morning August 2. These sensors were retrieved from Welch Farm at midday on September 15. Four total sensors were placed in the BHF garage and all three cold storage units in late morning August 2. Sensors were retrieved from BHF on the morning of September 6.

Data analysis

Treatment differences in berry wetness across all dates were evaluated using a full-factorial repeated-measures mixed model design in JMP (JMP®, Version 16.0, SAS, Cary, NC, USA), followed by a Tukey's pairwise comparison (Figure 1). Here, the full-factorial model tested the effects of date, treatment and any interaction between date and treatment. Berry wetness data passed assumptions for parametric statistical testing and a transformation was not required. Treatment differences were established by date (Figure 2) using a Standard Least Squares Analysis of Variance followed by a Tukey's Pairwise comparison.

Due to the nature of the data collected, the berry shriveling data failed the assumption of a normal distribution required to run parametric statistical tests. Transforming the data via a square root transformation did not improve the distribution. Statistical tests were carried out on the untransformed data despite non-normality after establishing there were no serious problems with the data. Treatment differences were established by date (Figure 3) using a Standard Least Squares Analysis of Variance followed by a Tukey's Pairwise comparison.

RESULTS

Part 1. 30-Day Storage of Fresh Pack Pints

The wetness and shriveling of the berries increased as cold storage temperatures increased (Figure 1). The wetness of berries was 47% at 34°F, 51% at 40°F, and 53% at 50°F. The shriveling of berries was 52% at 34°F, 53% at 40°F, and 59% at 50°F.

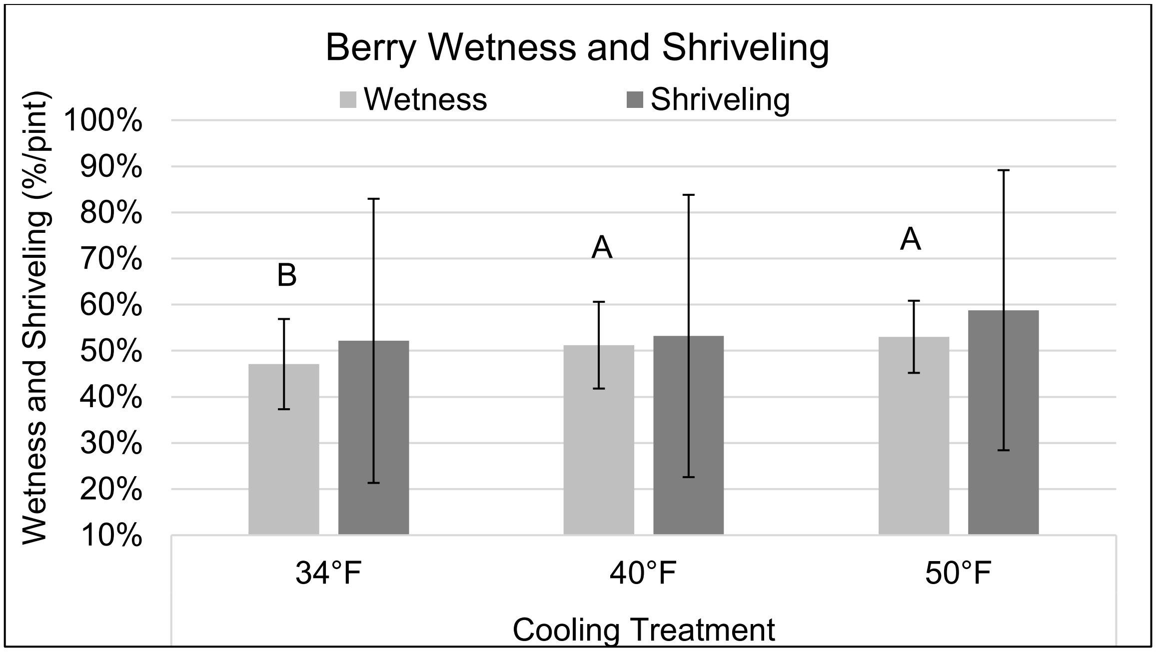

Figure 1. Average berry wetness (%/pint) and berry shriveling (%/pint) by cold storage unit temperature treatment, measured August 2 to September 5, 2022. Letters indicate significant differences at the 0.05 level of significance. Treatment differences in berry shriveling were not significant. Error bars represent the standard error of the mean.

The wetness of berries changed over time, decreasing after initial placement before climbing back up to levels near the initial wetness (Figure 2). When placed on August 2, 2022, pints in all temperatures had berry wetness levels of 59%. Berries in the 34°F unit (lightest gray data) dropped from 59% wetness on August 2 to 41% on August 5 before climbing to 42% on August 15, 49% on August 25, 51% on August 30, and peaking at 53% on September 6. Berries in the 40°F unit (lightest gray data) dropped from 59% wetness on August 2 to 44% on August 5 before climbing to 43% on August 15, 53% on August 25, peaking at 61% on August 30, and dropping to 57% on September 6. Berries in the 50°F unit (medium gray data) dropped from 59% wetness on August 2 to 49% on August 5 before climbing to 45% on August 15, 54% on August 25, peaking at 61% on August 30, and dropping to 57% on September 6.

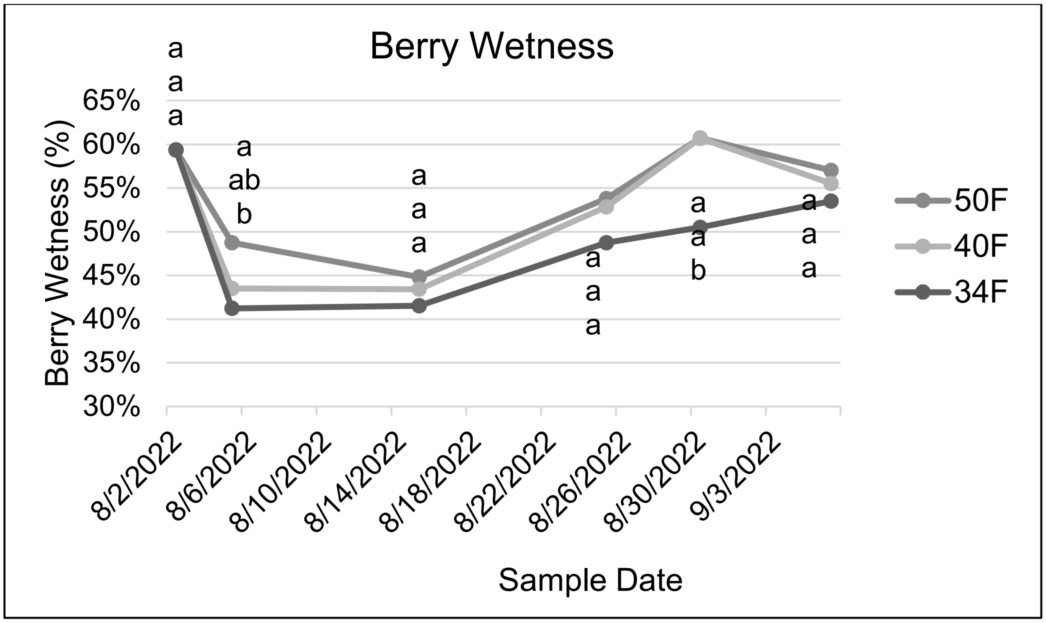

Figure 2. Average berry wetness (%/pint) by date and cold storage unit temperature. Letters indicate significant differences at the 0.05 level of significance and are to be compared across treatments by date (dates are to be compared separately). Letters correspond to legend order (50°F: top letter, 34°F: bottom letter).

The shriveling of berries steadily increased over time (Figure 3). When placed on August 2, 2022, pints in all temperatures had no shriveling at all. Berries in the 34°F unit (darkest gray data) increased from 0% shriveled on August 2 to 1% on August 5 before jumping to 37% on August 15, 66% on August 25, 72% on August 30, and peaking at 85% on September 6. Berries in the 40°F unit (lightest gray data) increased from 0% shriveled on August 2 to 1% on August 5 before jumping to 40% on August 15, 64% on August 25, 76% on August 30, and peaking at 85% on September 6. Berries in the 50°F unit (medium gray data) increased from 0% shriveled on August 2 to 1% on August 5 before jumping to 59% on August 15, 71% on August 25, peaking at 82% on August 30, and dropping slightly to 81% on September 6.

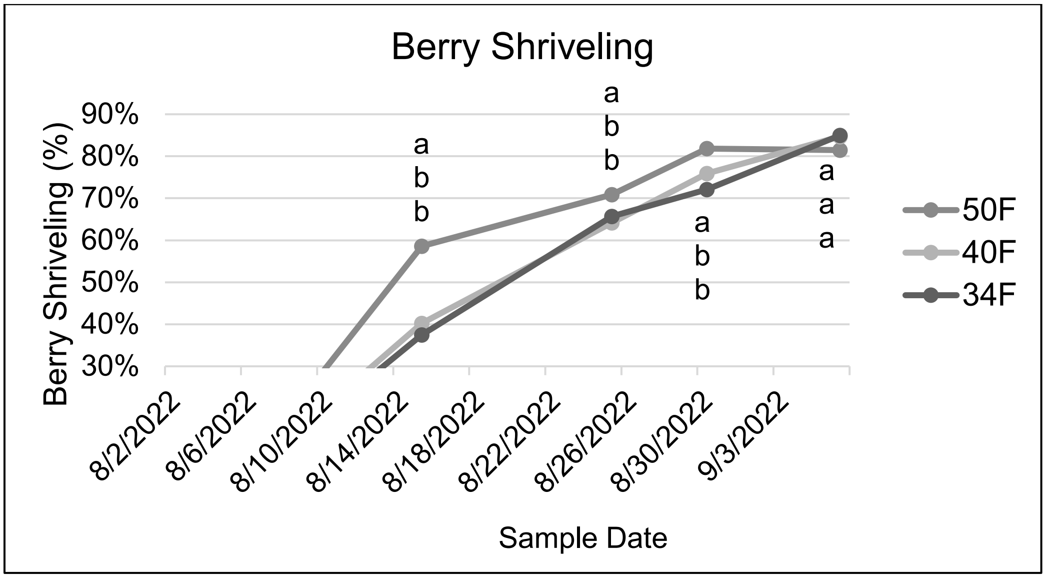

Figure 3. Average berry shriveling (%/pint) by date and cold storage unit temperature. Letters indicate significant differences at the 0.05 level of significance and are to be compared across mulch treatments by date (dates are to be compared separately). Letters correspond to legend order (50°F: top letter,

34°F: bottom letter). Shriveling values from the August 2 and August 5, 2022 sample dates were between 0 and 1%.

Part 2. Ambient Temperature & Cold Storage Unit Temperature

There are clear, sharp changes in the ambient temperature that coincide with sudden changes in the internal temperatures of the cold units (Figure 4). When the ambient temperature (darkest gray dots on graph below, top series of data) spiked at temperatures higher than 81°F from 11 AM to 4 PM on August 16, 2022, the temperatures in the cold units also increased during that same timeframe: 34°F (lighter medium gray dots on graph below, bottom series of data) increased to 40.39°F at 11 AM before peaking at 41.35°F at 3 PM; 40°F (lightest dots on graph below, third series of data from top) increased to 42.85°F at 11 AM before peaking at 43.33°F at 3 PM; 50°F (darker medium gray dots on graph below, second series of data from the top) increased to 49.22°F at 11 AM before peaking at 50.36°F at 3 PM.

Changes in the ambient temperature yielded similar changes in the temperatures recorded in the cold storage units, such as the decrease in ambient temperature in the morning of August 18 which occurred at the same time there was a decrease in the 40°F unit's temperatures, and the large increase in ambient temperature during the afternoon of August 20 which caused increases in all cold storage units.

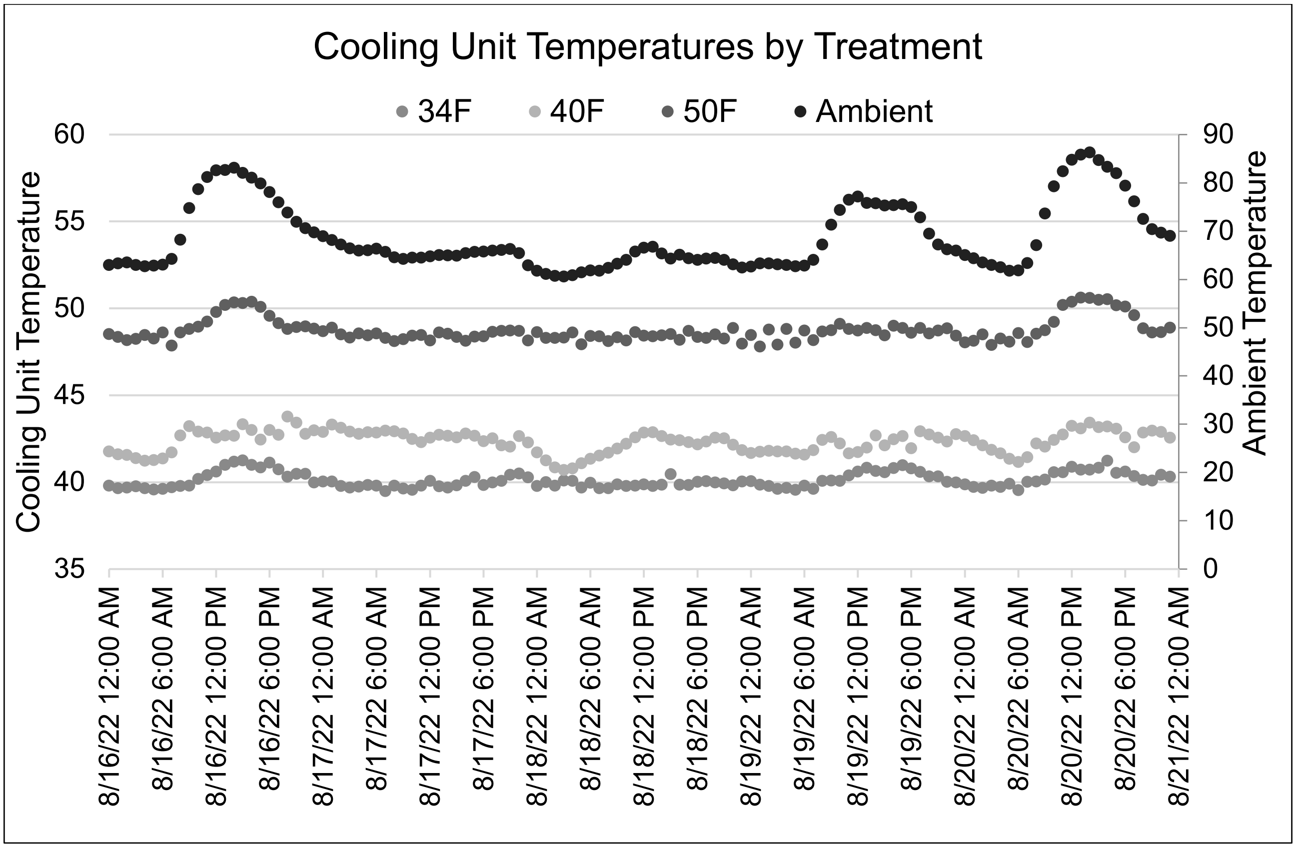

Figure 4. Hourly cooling unit temperatures (°F) by treatment collected from August 16 to August 21, 2022. Ambient temperature was collected outside the cold units reflecting the temperature of the open-air barn where the cooling units are installed at Blueberry Hill Farm, Jonesboro, ME.

DISCUSSION

Surface moisture on fruit was significantly lower from day 4 through 36 in the 34°F cold storage unit. Across storage temperatures, surface moisture started high most likely due to the break in the cold chain while being run through the fresh pack line and transported from Roque Bluffs to Jonesboro. The transport car had air conditioning on but temperature fluctuation still occurred. Day time outdoor temperatures on August 1 and 2 peaked at 91°F and 83°F, respectively. As storage in the cold storage units at BHF began, the surface moisture trend dropped and then increased from August 26 – September 6 at all storage temperatures. Surface moisture may have increased as fruit respiration reached a certain point or ambient humidity which was an average of 81% from August 26 to September 6, may have impacted fruit inside cold storage units. During the last 12 days of the experiment, average relative humidity was 82% in the 34F cold storage unit and 83% in both the 40F and 50F cold storage units.

As expected, shriveling increased consistently over time across all storage temperatures. Berries continue to respire after being harvested contributing to the fruit drying out. Respiration is the breakdown of sugars into CO2 and water which leaves the fruit through stomata. Thus, the more time fruit is stored, the more shriveled it will become. The lack of any significant shriveling from August 2 to 5 across all storage temperatures indicates growers may have a window of a few days before shriveling becomes visible on the top layer of fruit. The jump in shriveling across all storage temperatures observed on August 15 indicates the decay of the fruit accelerated. The largest gains in shriveling were observed in the 50°F unit, where the warmer temperatures did not slow down shriveling/respiration rates as much as the cooler units did.

Substantial and sudden changes in ambient temperature often yielded similar changes in the temperatures inside the cold storage units. Some units seemed to have more of a relationship to the ambient temperatures than did others, the two colder units (34°F and 40°F) seemed more likely to fluctuate with ambient temperature than did the 50°F unit.

CURRENT RECOMMENDATIONS

- In order to maintain quality for the longest time, wild blueberries should be stored at 34°F where the least surface moisture will develop.
- In this situation, wild blueberry quality was highest during the first 5 days of storage and quality began to really decline on day 14.

NEXT STEPS

- Conduct engineering research to improve rake and harvester technology to reduce damage to berries in the field.
- Study reducing the that time berries spend in the field and in process before cooling to maintain quality longer.
- Tweak fresh pack lines using new and old lines for fresh pack line efficiency.

ACKNOWLEDGEMENTS

Thank you to Lisa and Wayne Hanscom of Welch Farm for hosting portions of this project and for contributing their berries in 2022. Thank you to Northeast SARE for funding this project.

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3. Impact of Wild Blueberry Plant Architecture, Nutrients, and Phenology on Berry Quality

INVESTIGATORS: L. Calderwood, B. Calder, K. Davis-Dentici, B. Perkins, J. Perry (University of Maine); T. Esau (Dalhousie University); and J. Meyers (Cornell University)

OBJECTIVES

- Measure the impact of sunlight and temperature on berry development
- Conduct food science quality analyses on berries harvested on three harvest dates
- Collect leaf and berry samples for nutrient analysis throughout the season to understand when the plant uses certain nutrients

LOCATIONS: Hope (2 locations), Sedgwick, and Columbia Falls, Maine; Kemptown and Highland Village (Webb field), Nova Scotia

PROJECT TIMEFRAME: April 2021 – March 2024

INTRODUCTION

For a full explanation of the interest in developing solar photovoltaic projects on agricultural land, please see the 2021 report, page 209, "Impact of Wild Blueberry Plant Architecture, Nutrients, and Phenology on Berry Quality".

As more blueberries have been planted worldwide, the value of Maine's frozen wild blueberries continues to drop, making it more attractive to diversify into value-added markets, including tea, wine, beer, purée, juice, fruit leather, powder, and other products. Despite being grown commercially by 485 farmers on 42,000 acres, 99% of the crop is sold frozen, leaving many value-added opportunities untouched. A similar need to diversify exists in Nova Scotia (NS), New Brunswick (NB), and Prince Edward Island (PEI). This study includes NS locations through our collegial partnership with Dalhousie University.

Though the processes of wild blueberry fruit ripening have been studied, little research has examined regional patterns in ripening. This report explains results from the second of three years exploring the impact of local weather conditions and nutrient availability on crop production through the season. At four ME and two NS locations, we continue to research how plant architecture, phenology, and nutrient availability are related to berry quality measures such as Brix, titratable acidity, color, organic acids, and fruit antioxidant content at green, color change, and blue fruit stages.

METHODS

Field Data Collection

This was the second of a three-year project and data collection continued largely unchanged from the previous year. 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 research site 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 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 four 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 yardstick 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 a single input thermocouple thermometer (HH-25U, Omega Engineering, Inc., Norwalk, CT, USA).

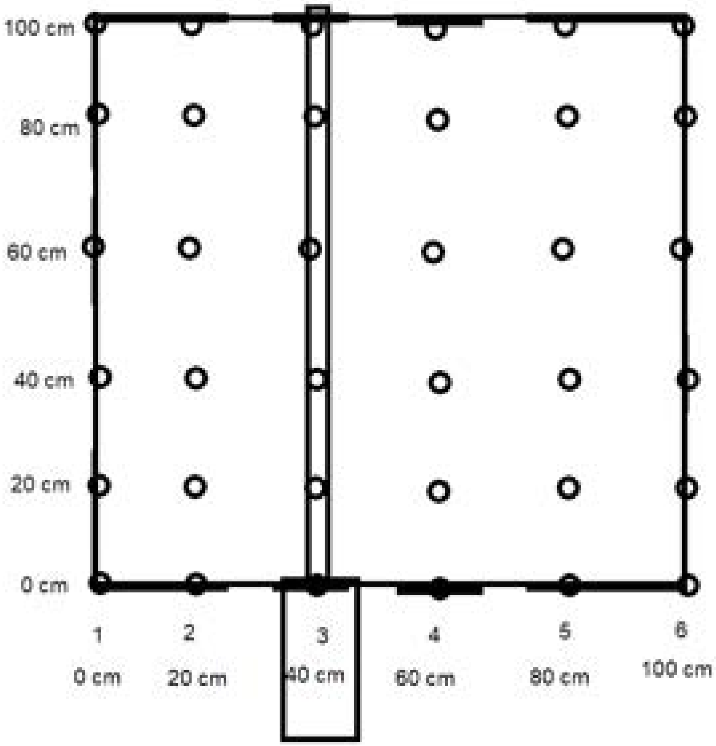


Image 1. This figure shows where the PAR measurements and plant architecture data were measured. The X- and Y-axes are 1m long and PAR measurements (PAR meter indicated by the conjoined rectangles along the 40cm vertical line) were taken every 20cm along the X-axis. Canopy measurements (indicating fruit, leaf, or unadorned stem) 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 again used to construct a canopy profile for the wild blueberry plants using Enhanced Point Quadrat Analysis (EPQA) (Meyers & 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 the data collected, entered into an Excel spreadsheet and modeled using a program designed by Dr. Meyers originally designed to process point quadrat data from grapevines (Meyers & 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 all four locations in ME, 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 30g 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. The same procedure was completed for samples from Highland Village in NS, whose tissue samples at the same sampling schedule were sent to the Nova Scotia Department of Agriculture Analytical Lab for analysis.

All fruit macronutrient data failed the normality assumption required for parametric testing. Nutrients that failed the normality assumption were transformed using a square root transformation prior to all statistical testing. 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. Leaf macronutrients were normally distributed and did not require transformation prior to statistical testing. Analyses were conducted in JMP (JMP® Pro, Version 15.2.0, SAS, Cary, NC, USA) 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 territory (Maine and Nova Scotia) relative to select macronutrients.

Multivariate and bivariate regressions were carried out in JMP to establish any relation between the dependent variable yield and independent variables berry quality measures (%TA, Brix, and berry pH), fruit and leaf macronutrients (N, P, and K). Fruit quality measures (Brix and pH) were also analyzed as dependent variables relative to the independent fruit and leaf macronutrients (N, P, and K) and micronutrients (B, Fe, Mn, and Zn), also using the multivariate (muti-variable) and bivariate (two-variable, 1:1), regressions. Significant relationships in the bivariate analysis were plotted using a scatterplot with a trendline (intersect not set to zero).

Berry Quality Field Collection and Lab Analysis (Calderwood and Calder of UMaine)

Wild blueberries were harvested on four 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 wild blueberries was handpicked from just outside each plot but within the same plant. Harvests occurred during the green fruit phase (June 23, 24, 29, and 30), color change phase (July 11, 13, 18, and 21), and blue fruit phase (July 25, 27, and 29). All ME wild blueberry samples were frozen in plastic bags the same day of harvest and were analyzed several weeks after harvest. 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 29 and 30), color change phase (July 15), and blue fruit phase (August 10 and 11). All NS samples were delivered to Acadia University's Laboratory for Agri-Food and Beverage. The lab evaluated for color, pH, titratable acidity, fructose and glucose, organic acids, and titratable acidity for green berries.

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 tryptic soy agar (TSA, for total bacterial count), Hsu's *Lactobacillus* and *Pediococcus* Medium (HLP), Lee's Multi-Differential Agar (LMDA, for enumeration of acetic acid bacteria), and Lin's Cupric Sulfate Medium (LCSM, 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.

Imaging for Ripeness & Quality (Esau of Dalhousie University)

On each phenological and harvest collection date, digital pictures were taken of a 0.5x0.5m smaller quadrat (including all fruit and stems visible) within the larger 1x1m quadrat at each site. After the photo was taken, all fruit was hand-harvested from within the 0.5x0.5m quadrat, and the fruit weighed. The images were processed using a custom image processing software developed in C++ using Visual Studio 2018 (Microsoft Corporation, Redmond, WA, USA) to estimate the percentage of blue pixels representing ripe fruit in the field of view.

Following data and image collection, the images were used in combination with two already-developed neural network models for assessing wild blueberry ripeness (MacEachern et al., 2021). When these images were run through the models, the number of detections in each image were counted and used to develop regression equations for predicting berry yield from still images. The employed neural networks were developed using the YOLOv4 and YOLOv4-Tiny models. Both 2-class (ripe vs. unripe) and 3-class (blue vs. red vs. green) networks were employed when developing the regression equations in order to observe any benefits to either. All regression equations were developed using Minitab 19 (Minitab, LLC, State College, PA, USA). For each regression, significance of higher order interactions was assessed and adjusted R2 and Root Mean Square Error (RMSE) were used for comparing between models.

For the 3-class models, a stepwise regression ($\alpha = 0.15$) was used for developing the regression models. In both cases, the red class was deemed to be an insignificant contributor to the model and was therefore removed.

RESULTS

Field Phenology and Nutrients (Calderwood of UMaine)

Phenological Development

There are clear differences between the number of reproductive appendages produced in Maine and Nova Scotia (Figure 1). NS produced more buds in 2022 than 2021, but many fewer flowers in 2022 than 2021 (25 vs. 15). NS produced more green fruit than ME in both years, though not as many blue fruit in either year. ME saw large increases in the number of reproductive appendages from 2021 to 2022, with 20 buds in 2022, up from 10 in 2021, and 10 green fruit in 2022, up from 7. ME produced more blue fruit per stem in 2022 than in 2021, 8 vs. 6.

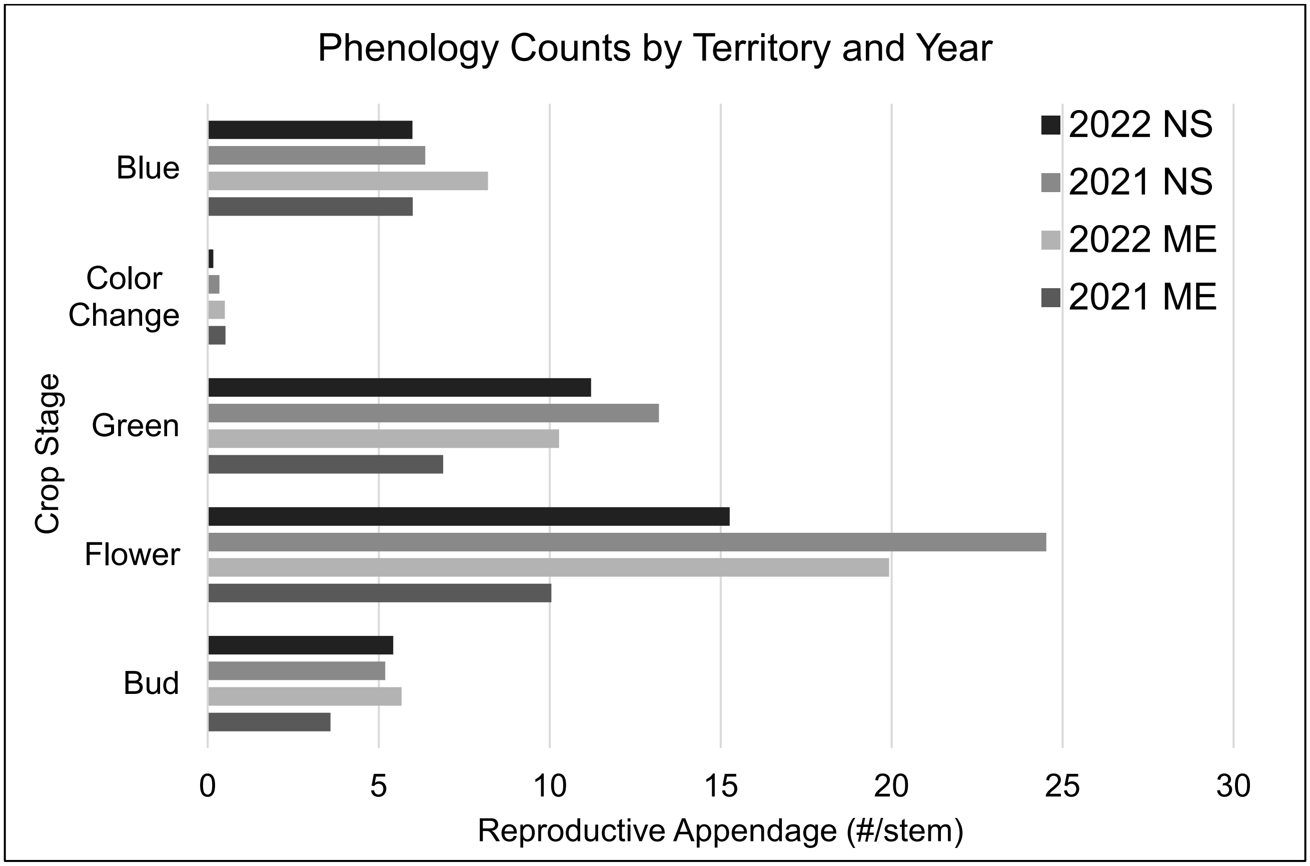


Figure 1. Phenological development, quantified through the numbers of reproductive appendages (bud, flower, green fruit, color change fruit, and blue fruit) compared by territory for 2021 and 2022, for each peak stage sampling (sample dates varied by location).

Plant Nutrient Contents

Plant nutrient contents, excepting calcium, of leaves in ME and NS decreased as the season progressed (Figure 2). The standard recommendation for %N in leaves ranges from 1.55-1.85% indicating that leaves at full bloom again had high N content that then dropped below the standard range for the green, color change, and blue fruit stages. A similar pattern occurred for %P (standard range 0.111-1.43%) and %K (standard range 0.31-0.56%), but the NS levels of K decreased well below the standard for the green, color change, and blue fruit stages. Leaves in both NS and ME saw increases in %Ca (standard range 0.31-0.40%) over the course of the season, with all locations surpassing standard ranges for the color change and blue fruit stages. These trends generally align with trends observed in 2021.

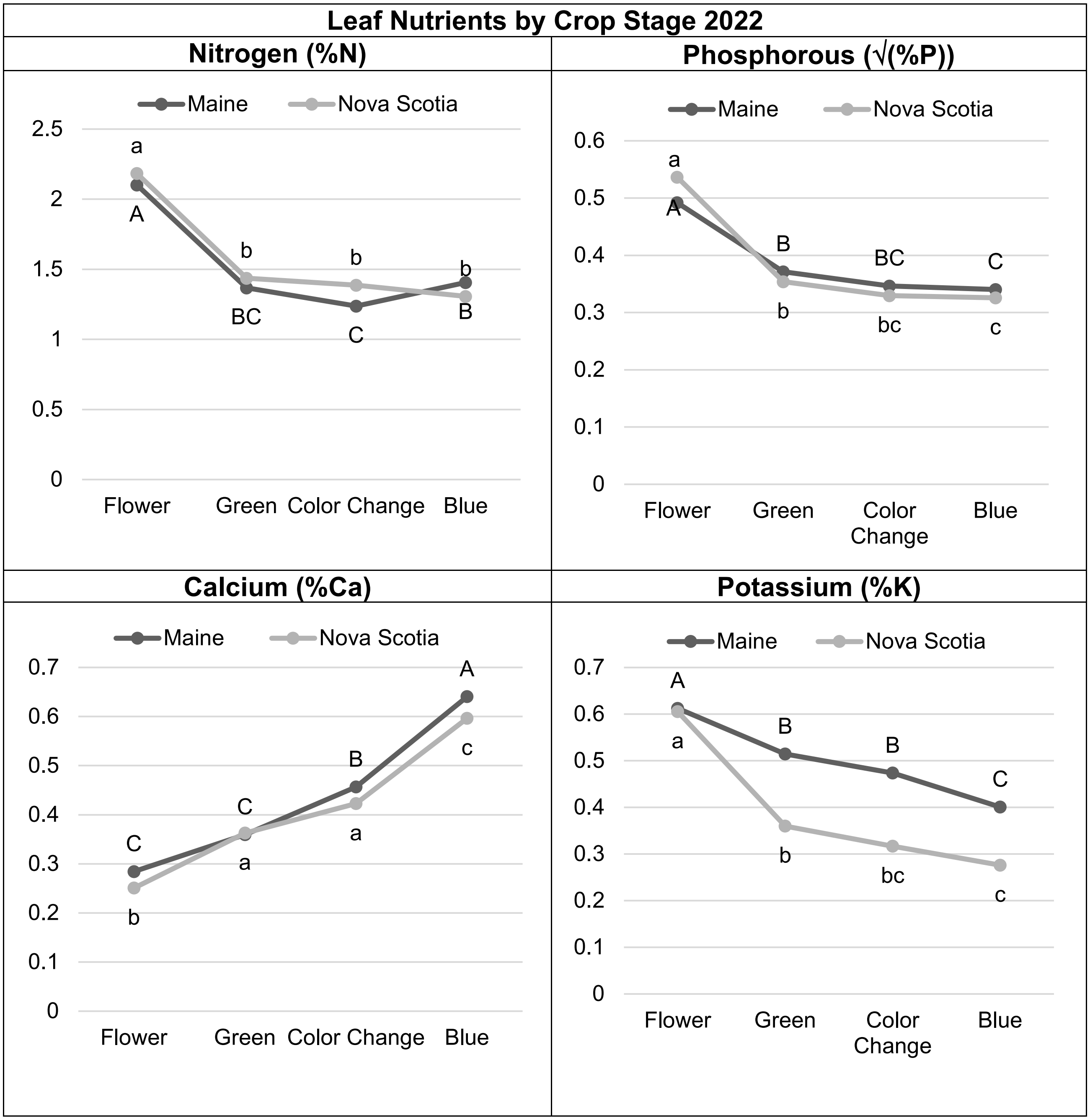


Figure 2. Leaf nutrients through the season in ME (Columbia Falls, Sedgwick, and Hope) and NS (Webb) taken at full bloom, peak green, color change, and blue fruit. Nutrient percentages are shown by crop fruiting stage. These data were transformed using a square root transformation.

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 3). %N in fruit/flower samples in ME saw a steady decline from flower to blue fruit, while NS samples saw a shallow decline from green to color change fruit and a sharper decline from color change to blue fruit. ME generally saw lower %Ca and %P levels than in NS, and both territories had similar decline patterns throughout the season. %K levels in both territories appeared to level off after an initial large decline from flower to green fruit.

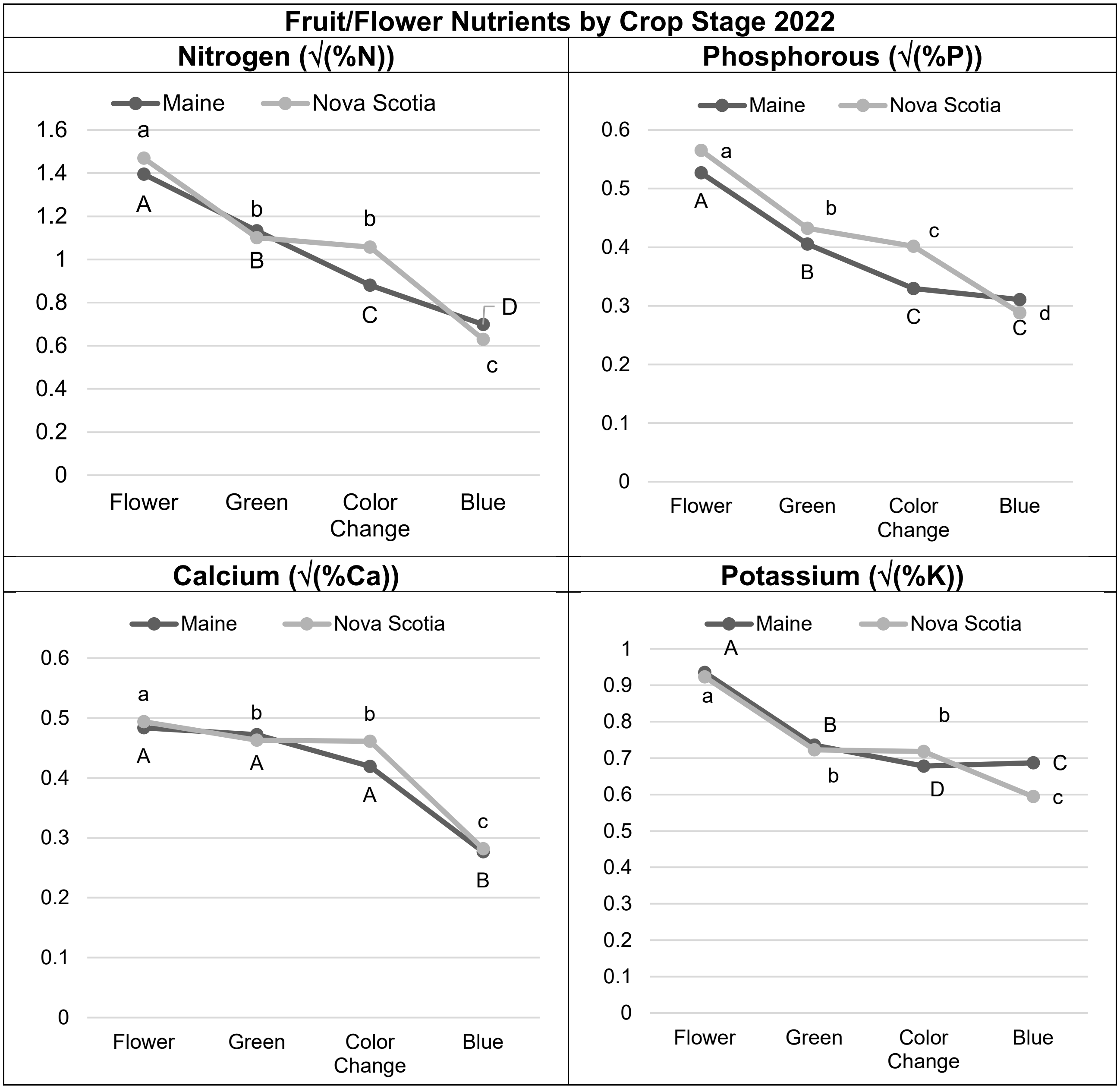


Figure 3. Flower and fruit nutrients through the season in ME (Columbia Falls, Sedgwick, and Hope) and NS (Webb) taken at full bloom, peak green, color change, and blue fruit. Nutrient percentages are shown by crop fruiting stage. These data were transformed using a square root transformation.

Yield vs. Quality, Fruit and Leaf Nutrients

A multivariate linear regression looking at the effects of independent variables (including %TA (titratable acidity), Brix (berry sugar content), berry pH, fruit macronutrients (N, P, K, Mg, and Ca) and nutrients (N, P, K, Mg, and Ca)), on the dependent variable (yield) had an R2 of 0.68, suggesting that 68% of the variation in yield is explained by these independent variables that were put into the model. Certainly, precipitation and pollination, for example, also impact yield. Leaf nutrients, Ca and P, exhibited a significant linear relationship with yield (Table 1). When evaluating relationships on a one-to-one basis using a bivariate regression, berry sugar content, leaf Ca and K showed a significant linear relationship with yield (Table 2, Figure 4B and C). Here, the most significant relationship occurred between leaf potassium and harvest yield (p = 0.002; Table 2). Using this second analysis method, yield exhibited a negative relationship with berry sugar content and leaf potassium, such that higher yields corresponded with lower potassium and lower berry sugar content. With higher yield we expect more fruit drop, reducing the average Brix value (lower sugar content). In contrast, yield exhibited a positive relationship with leaf calcium, where higher yields corresponded with higher calcium concentrations in the leaves (p = 0.007; Figure 4 A-C). Yield per acre was very high because producing plants were selected and fruit was hand-picked from small plots yields of 10,000+lb/A are only representative of the yield of the small plots and not entire fields.

Table 1. Multivariate linear regression table that shows the predicted influence of the independent variables %TA (titratable acidity), Brix (berry sugar content), berry pH, fruit macronutrients (N, P, K, Mg, and Ca) and leaf nutrients (N, P, K, Mg, and Ca)), on the dependent variable (yield). Only blue fruit data was included across all four locations in Maine and one location in Nova Scotia. Bold text indicates a significant linear relationship at the 0.05 level of significance.

		Dependent Variable: Yield		
		R2	**F-Value**	***p***
ALL		0.68	2.66	0.0332
Independent Variables		**t-value**	**p**	
Fruit	**%TA**	-0.39	0.7007	
	Brix	-0.52	0.6124	
	pH	0.06	0.953	
	N	-0.73	0.4734	
	P	-0.46	0.6528	
	K	0.38	0.7083	
	Ca	-1.16	0.2635	
	Mg	1.45	0.1672	
Leaf	**N**	1.47	0.1603	
	P	**-2.19**	**0.0438**	
	K	-1.29	0.2153	
	Ca	**2.31**	**0.0344**	
	Mg	-1.26	0.2255	

Table 2. Bivariate linear regression (comparisons were made on a 1:1 basis) showing the predicted influence of the independent variables %TA (titratable acidity), Brix (berry sugar content), berry pH, fruit macronutrients (N, P, K, Mg, and Ca) and leaf macronutrients (N, P, K, Mg, and Ca), on the dependent variable (yield). Only blue fruit data was included across all four locations in Maine and one location in Nova Scotia. Bold text indicates a significant linear relationship at the 0.05 level of significance.

		Dependent Variable: Yield	
	Independent Variables:	**R2**	***p***
Fruit	**%TA**	0.03	0.3332
	Brix	**0.29**	**0.0022**
	pH	0.64	0.177
	N	0.03	0.3411
	P	0	0.9536
	K	0.02	0.4519
	Ca	0.05	0.2347
	Mg	0.02	0.502
Leaf	**N**	0	0.913
	P	0	0.7791
	K	**0.29**	**0.002**
	Ca	**0.24**	**0.0065**
	Mg	0.09	0.1146

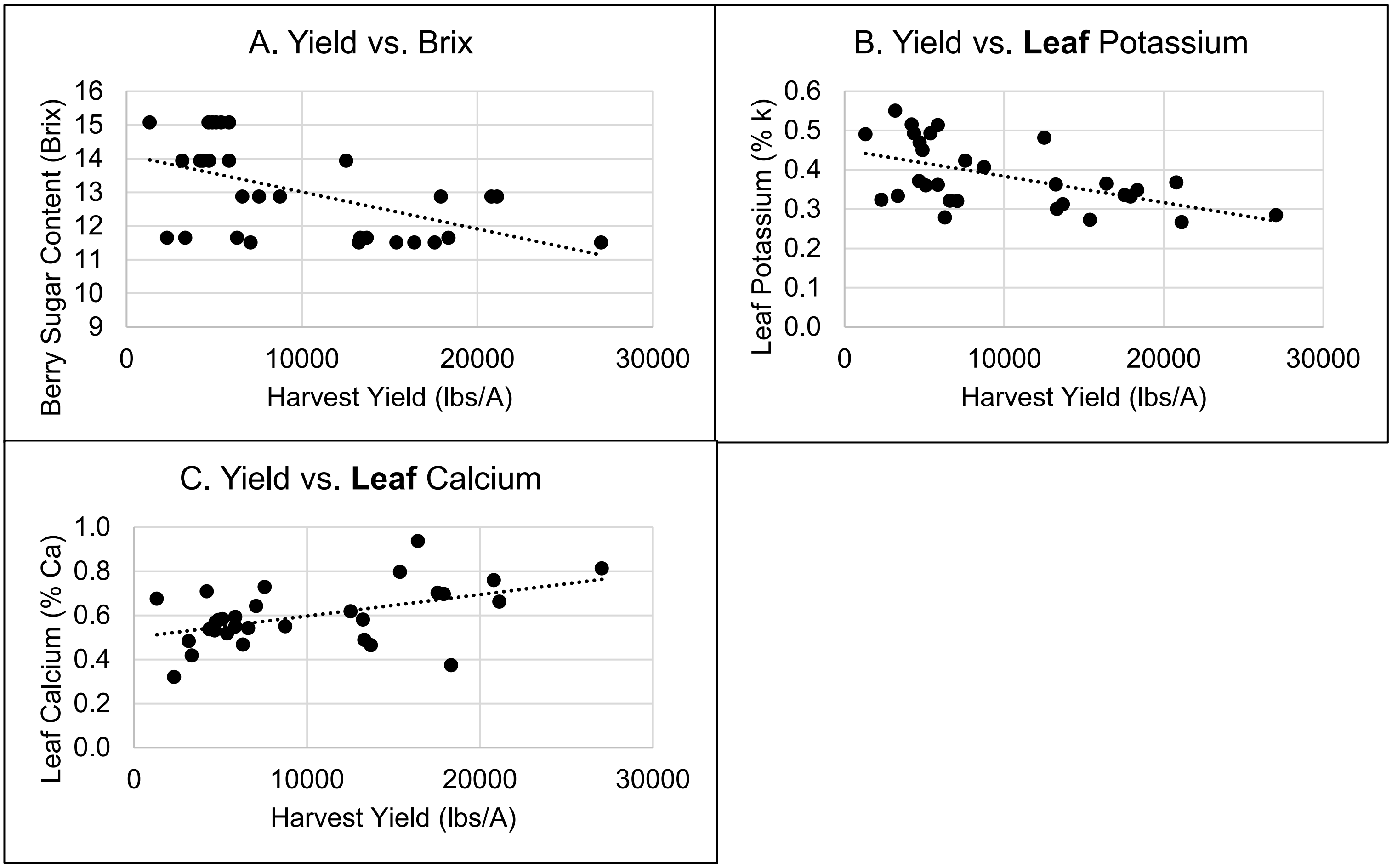


Figure 4 A-C. Significant linear relationships between the dependent variable yield and independent variables: berry sugar content (Brix; Figure 4A), leaf potassium (K; Figure 4B), and leaf calcium (Ca; Figure 4C), sampled at the blue fruit stage, across all four locations in Maine and one location in Nova Scotia. The dotted line represents the linear trend between the two variables on a one-to-one basis. Yield per acre is very high because producing plants were selected and fruit was hand-picked from small plots; yields of 10,000+lb/A are only representative of small plot yield and not entire fields.

Berry Quality vs. Fruit Nutrients in Maine

When using a multivariate linear regression analysis, the relationship between all fruit and leaf nutrients against berry sugar content (Brix), no significant relationships were present. The multivariate linear regression between all fruit and leaf nutrients against berry pH, showed only Mg (magnesium) to have a significant influence on berry pH ($p = 0.0461$). Overall, the Brix model R2 was 0.87 and the berry pH model R2 was 0.90, indicating that most nutrients contributed to some variation in berry Brix and pH (data not shown).

When one-to-one bivariate linear regressions were used to look at fruit and leaf nutrients against berry Brix, fruit nitrogen ($p = 0.0009$), potassium ($p = 0.0037$), and iron ($p = 0.0196$), as well as leaf potassium ($p < 0.0001$) and iron ($p = 0.0005$) exhibited significant linear relationships (Table 3, Figure 5A-E). Here, fruit nitrogen, fruit potassium and leaf potassium exhibited a positive relationship with Brix, such that higher berry sugar content was significantly related to higher levels of the nutrients listed (Figures 5A, B, and D). In contrast, iron in both the fruit and leaves exhibited a negative linear relationship with berry sugar content such that higher berry sugar contents were associated with lower levels of iron (Figures

5C and E). Higher iron concentration was observed primarily at the Columbia Falls location which may have skewed the data.

Berry pH also showed a significant positive linear relationship to fruit nitrogen (p = 0.0054) and potassium (p < 0.0001), where higher berry pH corresponded with higher nitrogen and potassium content in the fruit (Table 4, Figure 6A-F). Micronutrients of the fruit did not exhibit a significant linear relationship with berry pH. Leaf macronutrient potassium (p < 0.0001) and leaf micronutrients calcium (p = 0.044), magnesium (p = 0.0125), and manganese (p = 0.0018) all exhibited a significant positive linear relationship with berry pH (Figures 6C to 6F).

Table 3. Brix bivariate linear regression table showing the predicted influence of the independent variables (macronutrients of the leaves and fruit (N, P, K), micronutrients of the fruit (Ca, Mg, Fe, Mn, and Zn) and micronutrients of the leaves (Ca, Mg, B, Fe, Mn, and Zn)) on the dependent variable (Brix sugar content). The regression included nutrient and quality measures collected at the blue fruit stage only across all 4 locations in Maine and one location in Nova Scotia. Bold text indicates a significant linear relationship at the 0.05 level of significance.

		Dependent Variable: Brix	
Independent Variables:		**R2**	***p***
Fruit Macronutrients	**N**	**0.33**	**0.0009**
	P	0.02	0.4587
	K	**0.26**	**0.0037**
Fruit Micronutrients	**Ca**	0.01	0.6526
	Mg	0.02	0.4581
	Fe	**0.18**	**0.0196**
	Mn	0.06	0.205
	Zn	0	0.921
Leaf Macronutrients	**N**	0.03	0.3799
	P	0.01	0.5382
	K	**0.45**	**<0.0001**
Leaf Micronutrients	**Ca**	0	0.7528
	Mg	0.03	0.4019
	B	0.01	0.6678
	Fe	**0.36**	**0.0005**
	Mn	0.09	0.0993
	Zn	0.03	0.3471

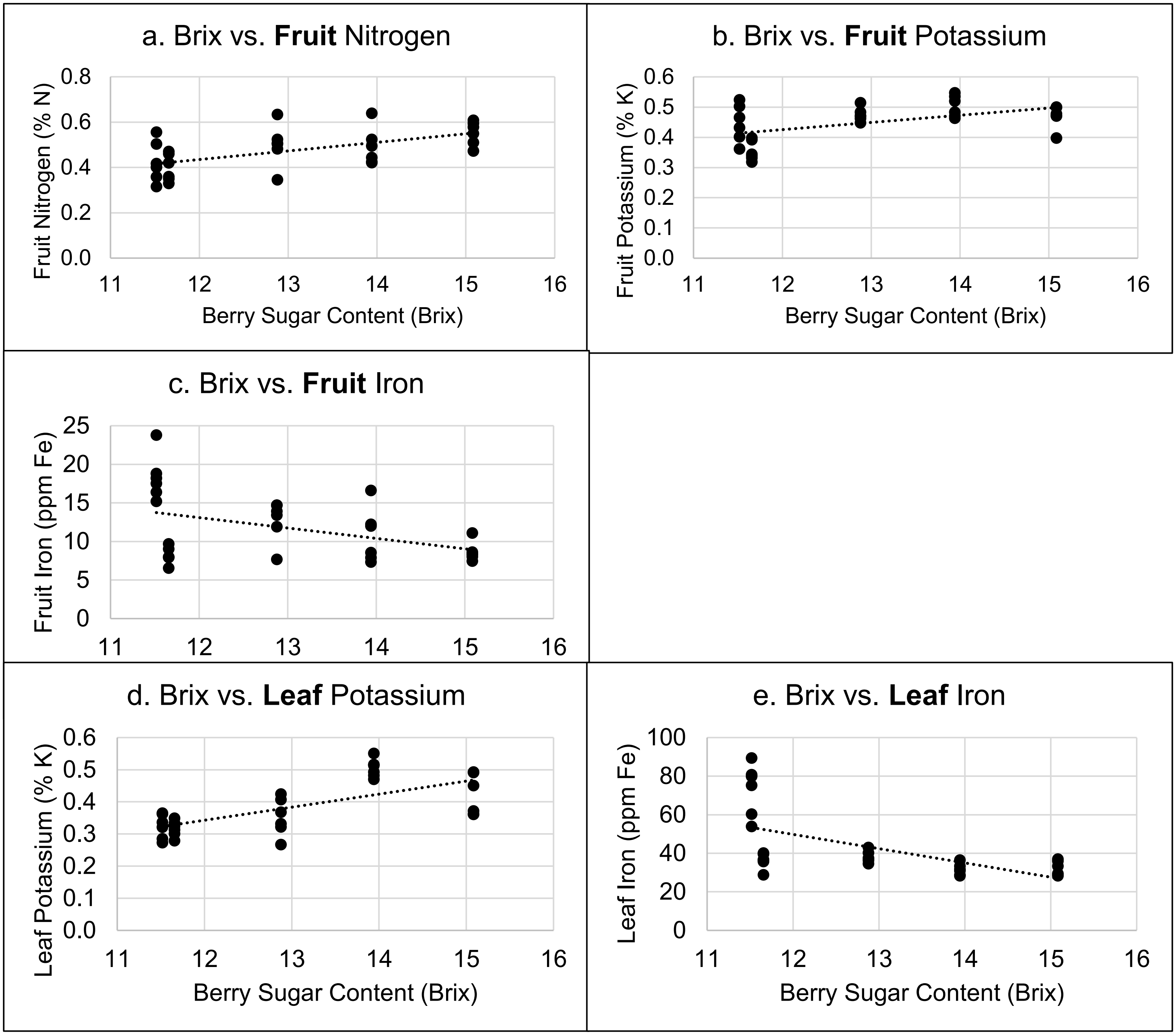


Figure 5 A-E. The direction of significant linear relationships from bivariate regression analysis. The dependent variable was berry sugar content (Brix) and independent variables were: fruit nitrogen (N; 5A), fruit potassium (K; 5B), fruit iron (Fe; 5C), leaf potassium (K; 5D), and leaf iron (Fe; 5E) sampled at the blue fruit stage, across all four locations in Maine and one location in Nova Scotia. The dotted line represents the linear trend between the two variables on a one-to-one basis.

Table 4. Berry pH bivariate linear regression table showing the predicted influence of the independent variables (macronutrients of the leaves and fruit (N, P, K), micronutrients of the fruit (Ca, Mg, Fe, Mn, and Zn) and micronutrients of the leaves (Ca, Mg, B, Fe, Mn, and Zn)) on the dependent variable (berry pH). The regression included nutrients and quality measures collected at the blue fruit stage only across all 4 locations in Maine and one location in Nova Scotia. Bold text indicates a significant linear relationship at the 0.05 level of significance.

		Dependent Variable: pH	
Independent Variables:		**R2**	***p***
Fruit Macronutrients	**N**	**0.25**	**0.0054**
	P	0.1	0.0866
	K	**0.63**	**<0.0001**
Fruit Micronutrients	**Ca**	0.05	0.2519
	Mg	0.08	0.1262
	Fe	0.01	0.7285
	Mn	0.07	0.1542
	Zn	0	0.7526
Leaf Macronutrients	**N**	0.01	0.6539
	P	0.04	0.3183
	K	**0.51**	**<0.0001**
Leaf Micronutrients	**Ca**	**0.14**	**0.044**
	Mg	**0.2**	**0.0125**
	B	0.1	0.0913
	Fe	0.05	0.2417
	Mn	**0.3**	**0.0018**
	Zn	0.01	0.6654

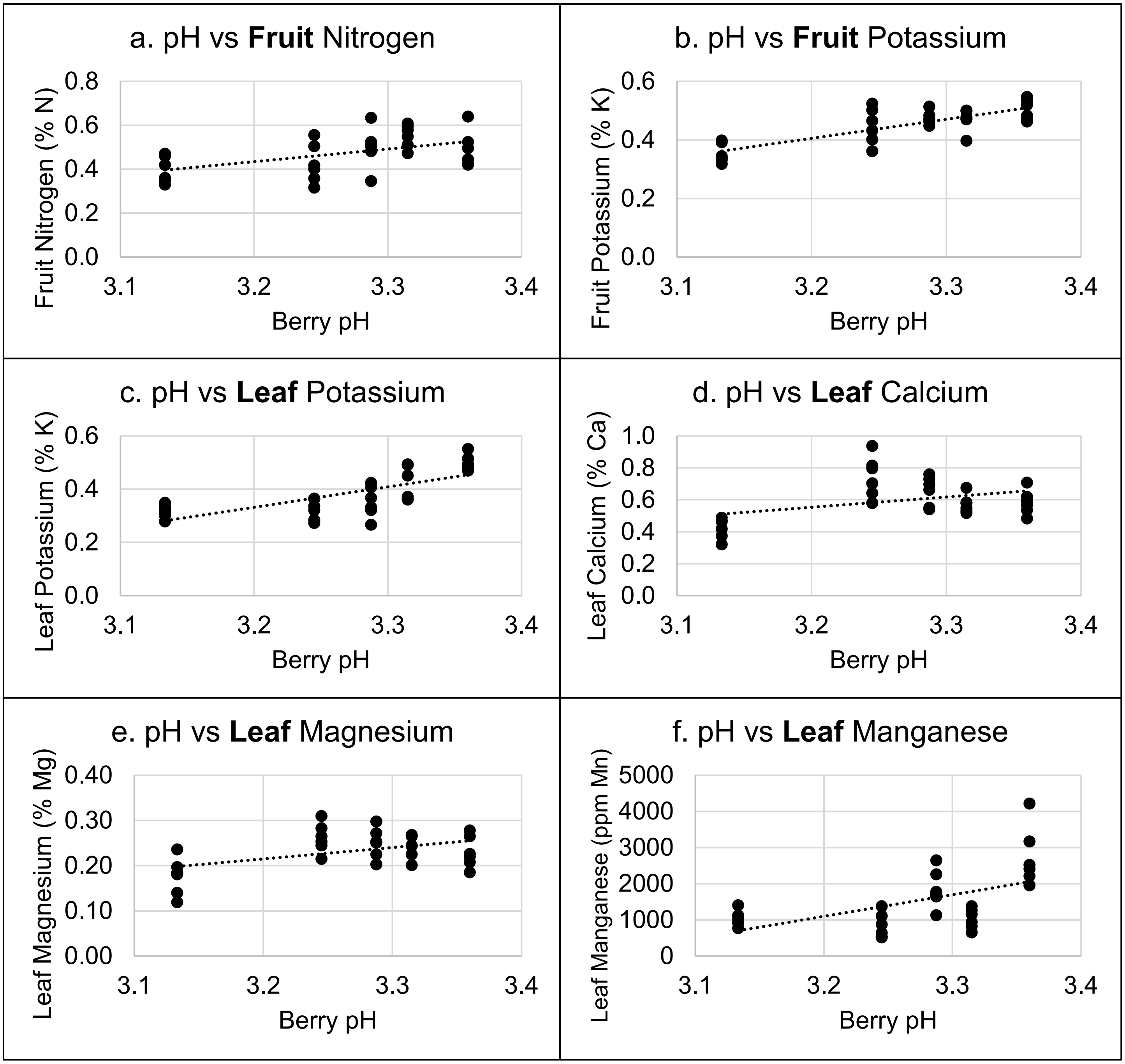


Figure 6A-F. The direction of significant linear relationships from bivariate regression analysis. Independent variables were fruit nitrogen (N; 6A), fruit potassium (K; 6B), leaf potassium (K; 6C), leaf calcium (Ca; 6D), leaf magnesium (Mg; 6E), and leaf manganese (Mn, 6F) and the dependent variable was berry pH, sampled at the blue fruit stage, across all four locations in Maine and one location in Nova Scotia. The dotted line represents the linear trend between the two variables on a one-to-one basis.

Wine Spoilage Organisms (Perry of UMaine)

Sample sizes were small for wine spoilage analysis of ME fruit due to limited funds. Only blue fruit (n=4; samples from all 4 locations in ME only). Figure 7 outlines the microbial organisms cultured from blue fruit samples in 2022. The quantity of yeast detected was low compared to previous levels documented on wild blueberry fruit. No mold was detected on blue fruit.

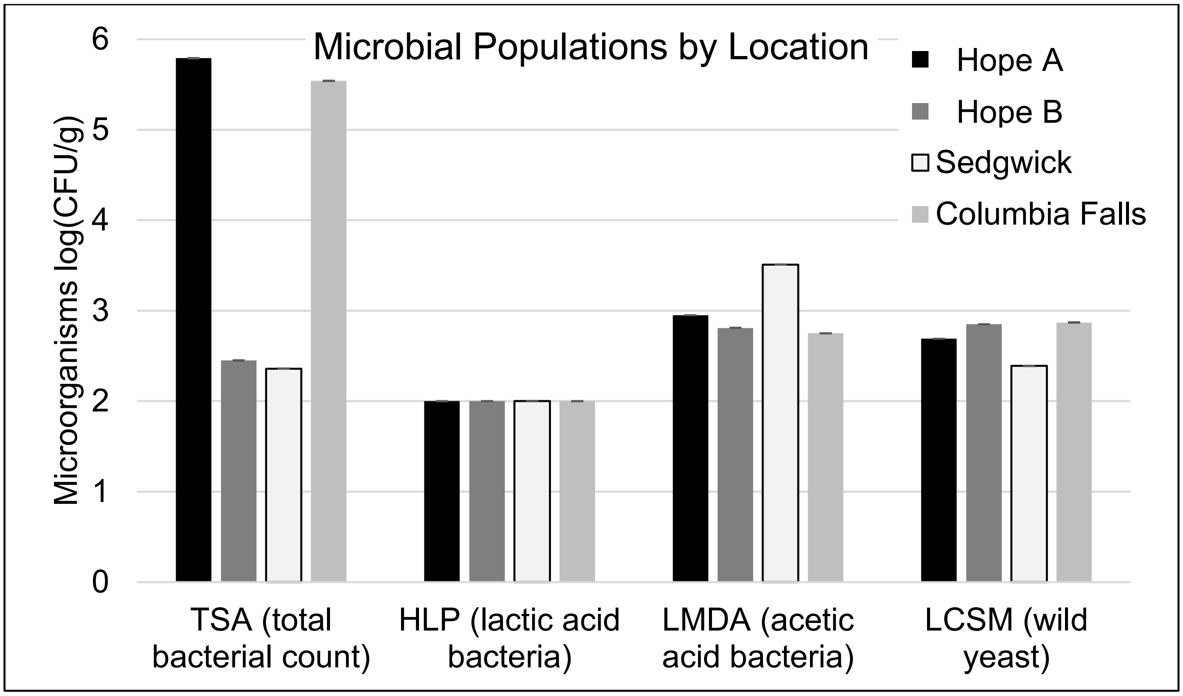


Figure 7. Microbial quality indicator populations on ripe wild blueberry fruit. Minimum detection limit is 2.0 log CFU/g and error bars denote standard deviation.

Imaging for Ripeness & Quality (Esau of Dalhousie University)

Across each of the four models (Tables 4A-D), the 2-class, YOLOv4-Tiny performed the best in terms of adjusted R^2 and RMSE (Figure 8). This is encouraging as YOLOv4-Tiny is computationally much cheaper than YOLOv4 (MacEachern et al., 2021) and as these models are transitioned into real time implementation, YOLOv4-Tiny is likely to be the choice of network for this reason. The success of the 2-class models, while providing somewhat less information, do a better job at accounting for all pictured berries. The stepwise approach, used in the 3-class models demonstrated that detected red berries did not have a significant effect on the models however, when combined with the green class they were deemed to be significant. Nonetheless, all of the models do a good job of predicting yield with root mean square errors less than 28.87g.

Following the results of MacEachern et al. 2023 where it was determined that YOLOv4 was the optimal model for identifying and quantifying berry ripeness, year two (2022 season) of data analysis focused solely on this model. In the first year's dataset, it had been discussed how YOLOv4-Tiny produced the optimal result however, that was a limited dataset and the work of MacEachern et al. 2023 utilizes a much larger one in order to gain a better understanding of model performance. For these reasons, year two data was analyzed solely using YOLOv4. Further, as with year one (2021 season) and in alignment with MacEachern et al. 2023, it was determined that red berry detection had no significant effect on model performance. For this reason, they were combined with green berry detections to get two classes, ripe and unripe detections. This approach allows for a more holistic picture of the target area to be captured and analyzed.

Year two data was analyzed in two different manners. The first analysis analyzed images across all six sites. The second analysis looked solely at images collected from the two Nova Scotian sites. This was done as the quality and manner of image collection from the Maine images was inconsistent enough to cause significant issues with model performance at the validation stage.

2-class YOLOv4-Tiny

Equation 2: Predicted Mass = -4.78 + 0.9422RipeCount + 0.1512UnripeCount – 0.001244Ripecount*Unripecount
R^2(adj) = 92.05%
RMSE = 24.89 g
Note: Interaction between ripe and unripe count was significant.

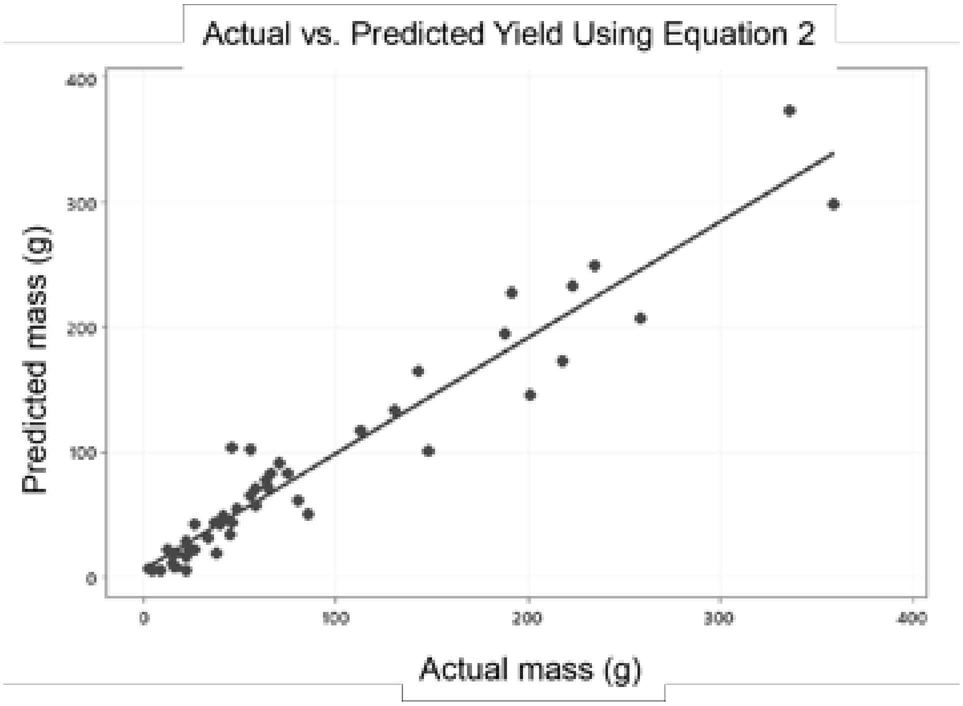


Figure 8. Plot of actual vs. predicted yield using Equation 2 to predict mass from plot images (R^2=92.6%).

3-class YOLOv4

Equation 3: Predicted Mass = 8.01 + 0.6817BlueCount + 0.0790GreenCount
R^2(adj) = 91.57%
RMSE = 25.63 g

Tables 4A-D. show the percent ripeness on each sampling date as determined by each of the trained models.

Table 4A. Percent ripeness on each sampling date as determined by the developed 2-class YOLOv4 model.

	Field	Date	%Ripe Berries	%Unripe Berries
2-class YOLOv4	Highland Village	25-Jun-21	1.37%	98.63%
		07-Jul-21	0.25%	99.75%
		21-Jul-21	30.53%	69.47%
		02-Aug-21	75.10%	24.90%
	Kemptown	30-Jun-21	0.00%	100.00%
		16-Jul-21	21.00%	79.00%
		27-Jul-21	35.80%	64.20%
		17-Aug-21	79.55%	20.45%

Table 4B. Percent ripeness on each sampling date as determined by the developed 2-class YOLOv4-Tiny model.

	Field	Date	%Ripe Berries	%Unripe Berries
2-class YOLOv4-Tiny	Highland Village	25-Jun-21	0.49%	99.51%
		07-Jul-21	0.17%	99.83%
		21-Jul-21	34.78%	65.22%
		02-Aug-21	77.68%	22.32%
	Kemptown	30-Jun-21	1.13%	98.87%
		16-Jul-21	23.25%	76.75%
		27-Jul-21	40.87%	59.13%
		17-Aug-21	82.87%	17.13%

Table 4C. Percent ripeness on each sampling date as determined by the developed 3-class YOLOv4 model.

	Field	Date	%Blue Berries	%Green Berries	%Red Berries
3-class YOLOv4-Tiny	Highland Village	25-Jun-21	0.15%	85.65%	14.20%
		07-Jul-21	0.13%	94.02%	5.85%
		21-Jul-21	28.88%	53.77%	17.35%
		02-Aug-21	72.79%	12.65%	14.57%
	Kemptown	30-Jun-21	0.05%	99.95%	0.00%
		16-Jul-21	20.70%	70.24%	9.06%
		27-Jul-21	34.07%	51.39%	14.54%
		17-Aug-21	78.87%	14.22%	6.91%

Table 4D. Percent ripeness on each sampling date as determined by the developed 3-class YOLOv4-Tiny model.

	Field	Date	%Blue Berries	%Green Berries	%Red Berries
3-class YOLOv4-Tiny	Highland Village	25-Jun-21	0.41%	81.35%	18.24%
		07-Jul-21	0.06%	95.13%	4.81%
		21-Jul-21	34.52%	50.36%	15.12%
		02-Aug-21	80.01%	9.19%	10.80%
	Kemptown	30-Jun-21	0.82%	99.03%	0.15%
		16-Jul-21	23.02%	68.90%	8.08%
		27-Jul-21	40.81%	47.26%	11.93%
		17-Aug-21	83.08%	11.06%	5.87%

Across each of the four models, the 2-class, YOLOv4-Tiny performed the best in terms of adjusted R^2 and RMSE. This is encouraging as YOLOv4-Tiny is computationally much cheaper than YOLOv4 (MacEachern et al., 2021) and as these models are transitioned into real time implementation, YOLOv4-Tiny is likely to be the choice of network for this reason. The success of the 2-class models, while providing somewhat less information, do a better job at accounting for all pictured berries. The stepwise approach, used in the 3-class models demonstrated that detected red berries did not have a significant effect on the models however, when combined with the green class they were deemed to be significant. Nonetheless, all of the models do a good job of predicting yield with root mean square errors less than 28.87g.

Following the results of MacEachern et al. 2023 where it was determined that YOLOv4 was the optimal model for identifying and quantifying berry ripeness, year two (2022 season) of data analysis focused solely on this model. In the first year's dataset, it had been discussed how YOLOv4-Tiny produced the optimal result however, that was a limited dataset and the work of MacEachern et al. 2023 utilizes a much larger one in order to gain a better understanding of model performance. For these reasons, year two data was analyzed solely using YOLOv4. Further, as with year one (2021 season) and in alignment with MacEachern et al. 2023, it was determined that red berry detection had no significant effect on model performance. For this reason, they were combined with green berry detections to get two classes, ripe and unripe detections. This approach allows for a more holistic picture of the target area to be captured and analyzed.

Year two data was analyzed in two different manners. The first analysis analyzed images across all six sites. The second analysis looked solely at images collected from the two Nova Scotian sites. This was done as the quality and manner of image collection from the Maine images was inconsistent enough to cause significant issues with model performance at the validation stage.

Results of the stepwise regression ($\alpha = 0.15$) using detection data from all models including all images is as follows (Figure 9):
Equation 5: Predicted Mass = 40.8 + 0.6767RipeCount + 0.0671UnripeCount
R^2(adj) = 77.32%
RMSE = 75.00 g

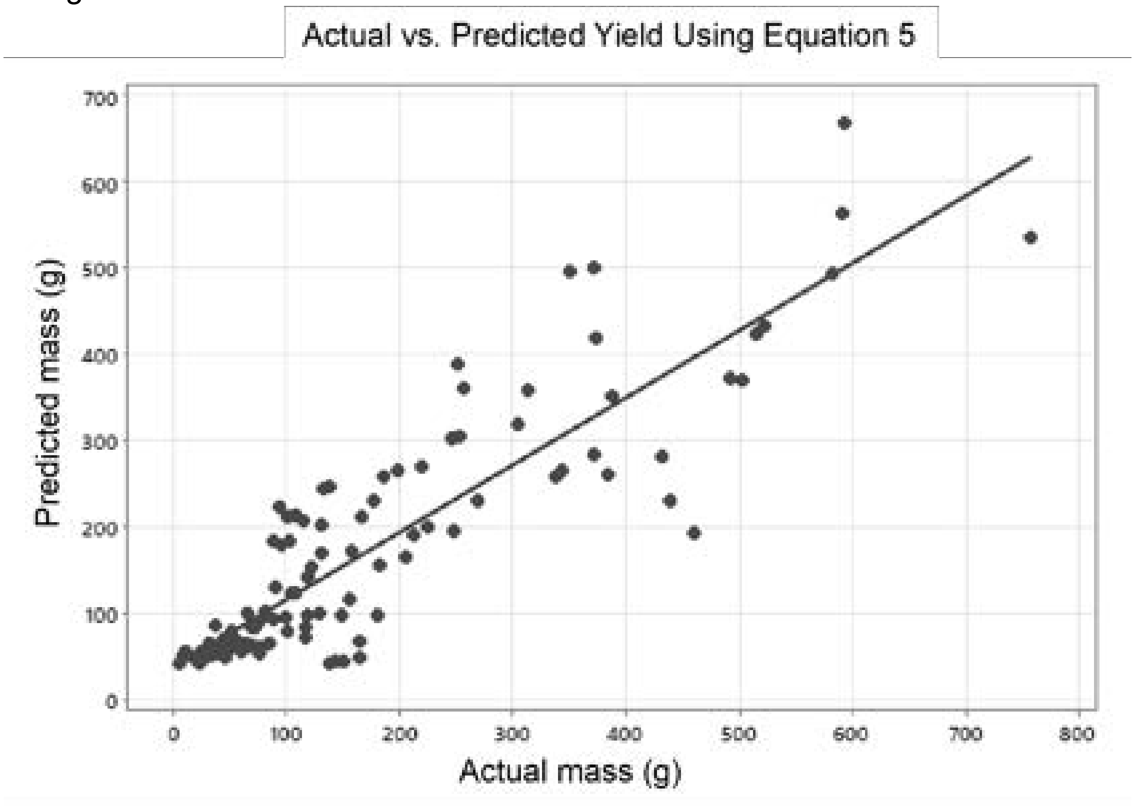


Figure 9. Plot of actual vs. predicted yield using Equation 5 to predict mass from plot images (R^2=77.8%).

After removing the data collected in Maine, model performance was drastically improved. Results of this analysis can be seen below (Figure 10):

Equation 6: Predicted Mass = 18.8 + 0.7021RipeCount + 0.1019UnripeCount

R^2(adj) = 87.98%

RMSE = 48.48 g

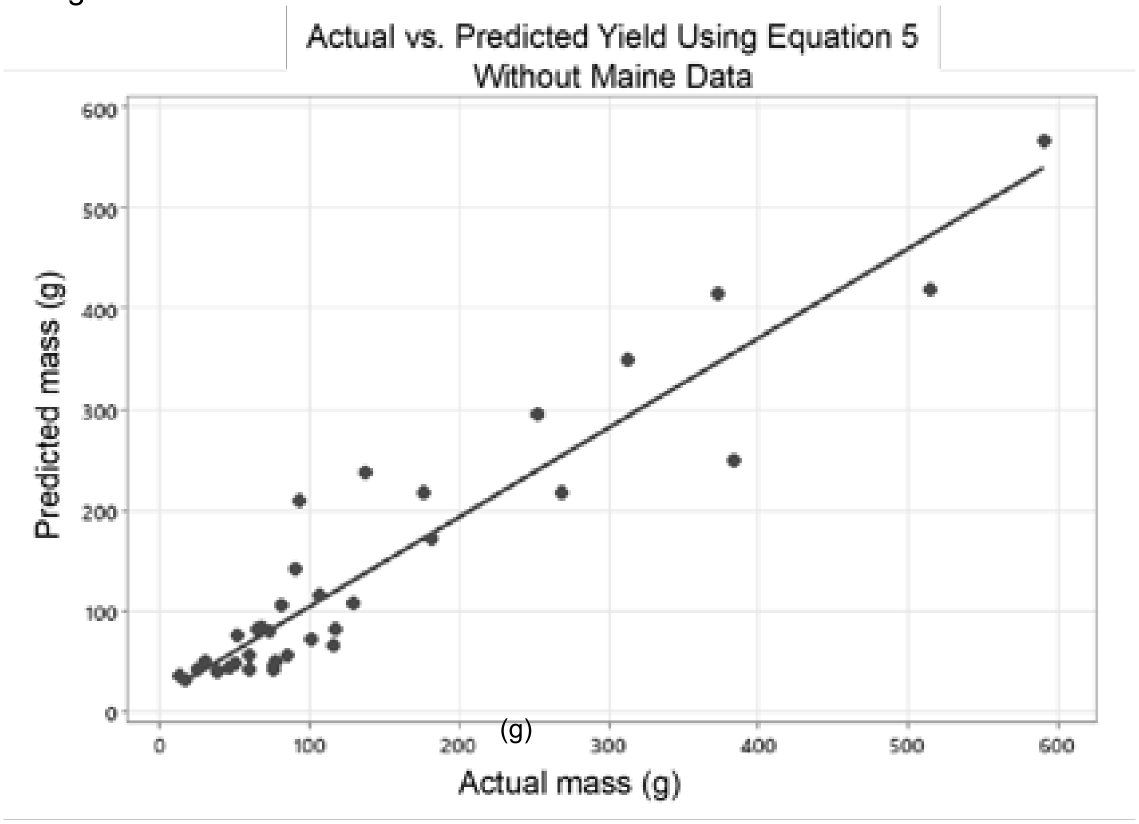


Figure 10. Plot of actual vs. predicted yield using Equation 5 to predict mass from plot images (R^2=88.7%).

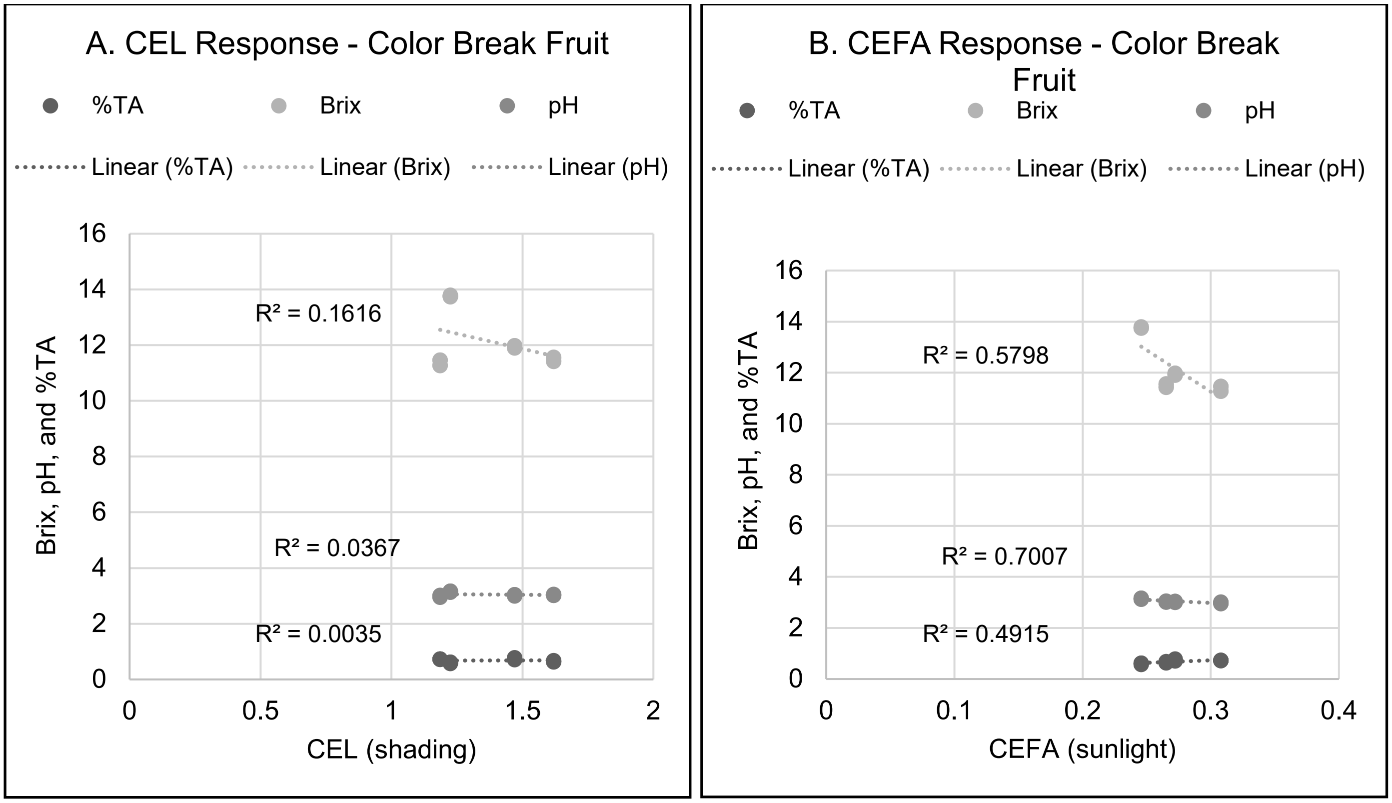
Predicted mass distribution between ripe and unripe classes determined by the YOLOv4 models for each of the field and date combinations can be observed in Table 5.

Table 5. Percent ripeness on each sampling date as determined by the developed 2-class YOLOv4 model.

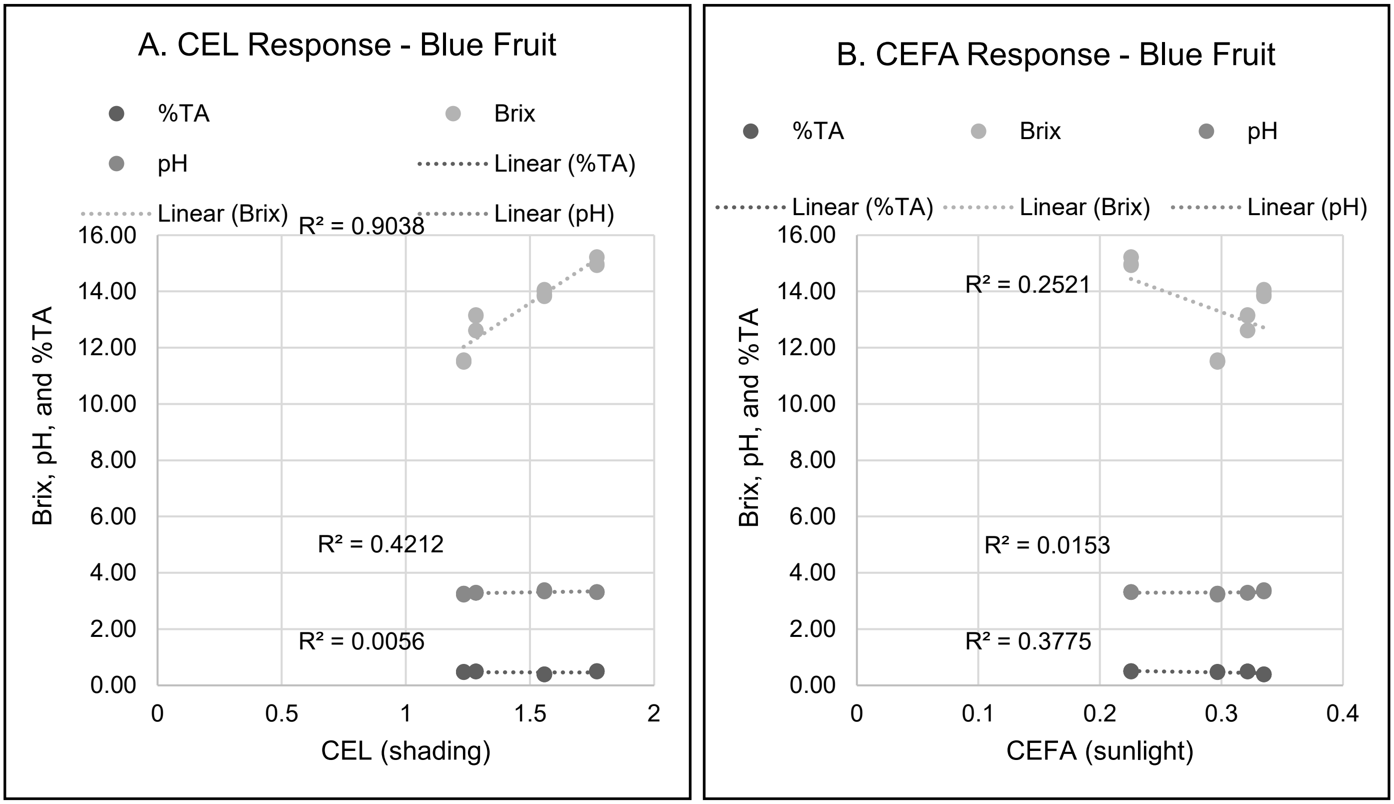
Field	Date	% Ripe	% Unripe
Hope Jackson	1st Date	1.64%	98.36%
	2nd Date	57.40%	42.60%
	3rd Date	63.77%	36.23%
Columbia Falls	1st Date	6.64%	93.36%
	2nd Date	75.62%	24.38%
	3rd Date	87.07%	12.93%
Hope Jones	1st Date	2.96%	97.04%
	2nd Date	37.53%	62.47%
	3rd Date	88.25%	11.75%
Sedgwick	1st Date	0.85%	99.15%
	2nd Date	62.14%	37.86%
	3rd Date	62.61%	37.39%
Kemptown	30-Jun-22	0.37%	99.63%
	15-Jul-22	2.98%	97.02%
	10-Aug-22	80.94%	19.06%
Webb	29-Jun-22	0.02%	99.98%
	15-Jul-22	7.82%	92.18%
	09-Aug-22	83.83%	16.17%

Canopy Architecture Impacts on Fruit Quality (Meyers of Cornell)

Regressions of fruit exposure, as measured by CEL (Cluster Exposure to Sun) and CEFA, with soluble solids (Brix), titratable acidity (%TA), and pH were performed. Figures 11 and 12 suggest that in 2022 fruit exposure, as quantified by CEL (shading) and CEFA (sunlight), correlated with Brix but not with pH or %TA at both color break and blue fruit stages. The strongest correlations were observed between Brix and CEFA (sunlight) at color break (R2 = 0.57) and between Brix and CEL (shading) at blue fruit (R2 = 0.90). At color break Brix declined as sunlight that reached the fruit increased (Figure 11B). At blue fruit, the data suggests that Brix increased as shading increased (Figure 12A). TA and pH did not show significant correlations to light availability through CEL or CEFA.



Figures 11A and B. Maine correlations among Brix, pH, and %TA (titratable acidity) with fruit sunlight exposure in color break wild blueberries. Sunlight exposure is quantified as Cluster Exposure Layer (CEL), a measure of shading within the canopy and Cluster Exposure Flux Availability (CEFA), a measure of how much sunlight reaches the fruit.



Figures 12A and B. Maine correlations among Brix, pH, and %TA with fruit sunlight exposure in blue stage wild blueberries. Sunlight exposure is quantified as Cluster Exposure Layer (CEL), a measure of shading within the canopy and Cluster Exposure Flux Availability (CEFA), a measure of how much sunlight reaches the fruit.

DISCUSSION

Phenological Development (Calderwood of UMaine)

Evaluating Nova Scotia's and Maine's phenology averaged across both territories separately shows a clear difference in the number of reproductive appendages at each crop stage (Figure 1). This may be at least partially due to the observation that NS fields have more stem branching than ME fields. NS produced more buds in 2022 than 2021, though the number of flowers produced in 2021 far exceeded those produced in 2022 (25 vs. 15), likely the result of studying a different crop field with slightly different management practices. NS produced more green fruit than ME, though not as many blue fruit. ME saw large increases in the number of reproductive appendages from 2021 to 2022, likely due to more precipitation during the 2021 prune year which led to more buds in 2022. Again, different fields were used in 2022 because crop fields were required for food science measures and contributed to variation.

Plant Nutrients (Calderwood of UMaine)

Leaf concentrations of Ca and K showed significant linear relationships with yield. This year's results showed that yield increased as Ca leaf concentrations increased and yield decreased as K leaf concentrations increased. P also showed a significant relationship to yield which has been documented and observed in the field (Smagula & Dunham, 1995). The significant positive relationship between Ca and yield was not expected yet was present in both the multi and bi-variate analyses. Research on Ca applications to highbush blueberry have varied and do not uniformly show that increased rates of calcium improve fruit quality (firmness or reduce fruit drop). Studies do not show an increase in yield from Ca applications. Instead, the range of results indicates that local climatic conditions, specific highbush cultivar, and timing of calcium applications may be the greater influences on fruit quality (Yang et al., 2019; see "Foliar Calcium Study" on page B91 in this report for more information).

Brix exhibited a negative relationship with yield most likely because as Brix reach their peak, fruit is also becoming over-ripe and more likely to fall off the stem. There appears to be a “sweet spot” where yield and Brix are both high before reaching an over-ripe state. High temperatures and drought during the 2022 harvest season caused over-ripeness to occur more abruptly. Average Brix levels observed varied by location. Columbia Falls averaged 11.5, Hope A averaged 12.8, Hope B averaged 13.9, Sedgwick averaged 15.0, and NS averaged 11.6. Fruit N, K, and leaf K showed significant positive relationships with Brix, meaning that as these nutrients went up so did berry sugar content. In contrast, Brix declined as Fe concentration in both fruit and leaves went up. The next step in analysis is to document the fertilizers that farmers applied in each year of the study. All foliar samples in this study were taken in the crop year yet all UMaine leaf nutrient thresholds and recommendations to this point have been for the prune year. Future research must develop crop year leaf nutrient thresholds.

Plant Architecture and Berry Quality (Meyers of Cornell)

At color break Brix declined as sunlight that reached the fruit increased while at blue fruit Brix increased as shading increased. One possible explanation is that there is more sunlight that penetrates a less dense canopy. A more thin canopy has lower photosynthesis due to fewer leaves and plant parts. Taken together this supports the overall notion that more dense wild blueberry cover (more leaves) across the field leads to higher sugar content in the fruit. With full canopy cover we can assume that plants simply have more energy because more photosynthesis occurs.

Wine Spoilage (Perry of UMaine)

Results of aerobic plate count analysis indicate that overall microbial profile likely varies by site. However, variations in counts of relevant wine spoilage organisms appears minimal across locations. Of particular note with regard to quality were the counts for acetic acid bacteria, particularly the relatively high level observed in samples from Sedgwick. These organisms are likely to contribute to quality deterioration by accumulation of acetate in fruit before fermentation, with increasing impacts tied to the level of fruit damage/rupture during harvest and transport.

Imaging for Ripeness and Quality (Esau of Dalhousie)

Considering the results of the models from all second-year images, a significant reduction in model performance can be seen. This is likely the result of inconsistent image quality observed in the Maine images.

CURRENT RECOMMENDATIONS

None at this time.

NEXT STEPS

- Review 2022 challenges and modify methods as necessary
- Collect field nutrient management histories
- Collect final year of data in 2023

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