2023 Wild Blueberry Research and Extension Report

January 2024

1865 THE UNIVERSITY OF MAINE
Cooperative Extension

MAINE AGRICULTURAL AND FOREST
EXPERIMENT STATION

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 Assistant, Jordan Parks.

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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1. Impact of Augmentorium on Populations of the Parasitoid *Ganaspis brasiliensis* in Maine, Year One

INVESTIGATORS: S.M. Chowdhury and P. Fanning

OBJECTIVE

- To increase the population of *Ganaspis brasiliensis* in commercial wild blueberry fields using augmentorium.

LOCATIONS: Penobscot, Waldo, and Washington Counties, ME

PROJECT TIMEFRAME: May 2023-September 2023

INTRODUCTION

An augmentorium is a tent-like structure designed to receive pest-infested fruits. It is equipped with a screen net with a mesh size small enough to sequester the target pest and big enough to allow their parasitoids to escape (Deguine et al., 2011; Rossi-Stacconi et al., 2015, 2019). This way, pests emerging from the infested fruits inside the augmentorium are not released into the field to cause damage, but the parasitoids can escape and contribute to suppressing pest populations. The augmentorium is intended to be placed directly in the field, so pest-infested fruits can be harvested or placed inside on a regular basis. The low cost and low maintenance associated with this strategy, coupled with its ease of use and overall effectiveness, make it an emerging management strategy among growers (Rossi-Stacconi et al., 2019).

Screen selection is critical to ensure that an augmentorium is effective for a particular system (Desurmont et al., 2022). Among pest management strategies to enhance the action of natural enemies of insect pests, the use of augmentoria (singular: augmentorium) has been proposed to control pest tephritid flies (Jang et al., 2007; Deguine et al., 2015). It has been implemented particularly in the tropical islands of Hawaii and La Reunion. Augmentative releases should be timed carefully to optimize both temperature and host availability, allowing the parasitoid's survival and reproduction (Pfab et al., 2018; Rossi Stacconi et al., 2019). In conjunction with other control methods, properly planned augmentative biological control can provide an effective control mechanism for growers and may be an effective strategy to enhance populations of the parasitoid *Ganaspis brasiliensis* (Gb) as a biological control of spotted-wing drosophila (SWD).

METHODS

Laboratory Bioassay

A series of laboratory bioassays were conducted to determine the appropriate mesh size that should be used in an augmentorium to allow *G. brasiliensis* to disperse while preventing SWD from escaping into the field. In this experiment, we compared fabric and rigid wire meshes where SWD and *G. brasiliensis* pass-through and subsequent emergence was determined. Inside, the arenas consisted of 163 ml plastic soufflé cups with 5 cm circular holes cut into the lids. “Oval” mesh had ~0.8 x 1.2 mm oval openings (Casa Solid White #1474-1649, JoAnn Fabric, Hudson, OH, USA). “Square” mesh with slightly larger openings were tested for *G. brasiliensis* given that this species is larger and might have difficulty passing through the oval mesh. It has ~1.1 x 1.1 mm squarish openings (Shiny Tulle #1696-6277, JoAnn Fabrics, Hudson, OH, USA). Sizes of the wire mesh openings were 1.04 mm^2, 1.08 mm^2, 1.11 mm^2, 1.13 mm^2, 1.18 mm^2, and 1.53 mm^2 (Figure 1).

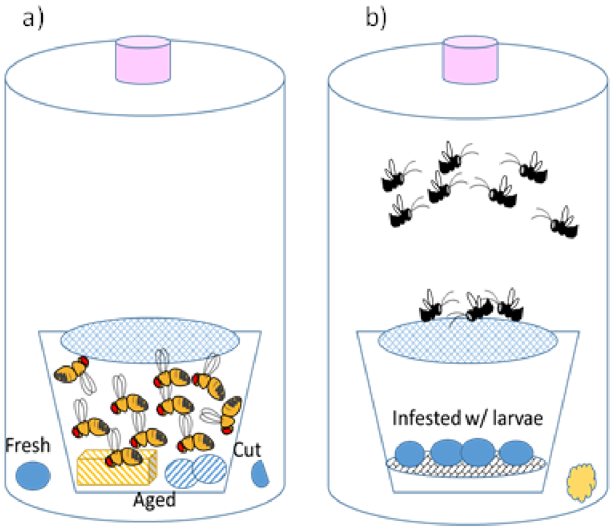

Figure. 1. Arena to test movement of SWD (a), and *G. brasiliensis* (b) through various fabric mesh or no mesh.

Field Study

A replicated field experiment was conducted to determine the impact of augmentorium in increasing the population of *G. brasiliensis* in commercial wild blueberry fields. Three separate sites were located in Penobscot, Waldo, and Washington Counties. At each site, three augmentoria were placed on the edge of the field a maximum of 150 ft apart. Untreated and uninfested wild blueberry were placed in the augmentorium and seeded with SWD and *G. brasiliensis*. Periodically, additional SWD was added to each Augmentoria. The augmentorium were fitted with the optimal mesh of 1.1 mm^2 fabric, which was determined from the laboratory bioassay. To test if SWD was successfully infesting fruit and *G. brasiliensis* was successfully parasitizing SWD within the cages, 8 oz fruit samples were collected are regular intervals throughout the season. Outside the augmentorium three transects were set up to assess the spread of *G. brasiliensis* away from the augmentorium and into the field (Figure 2).

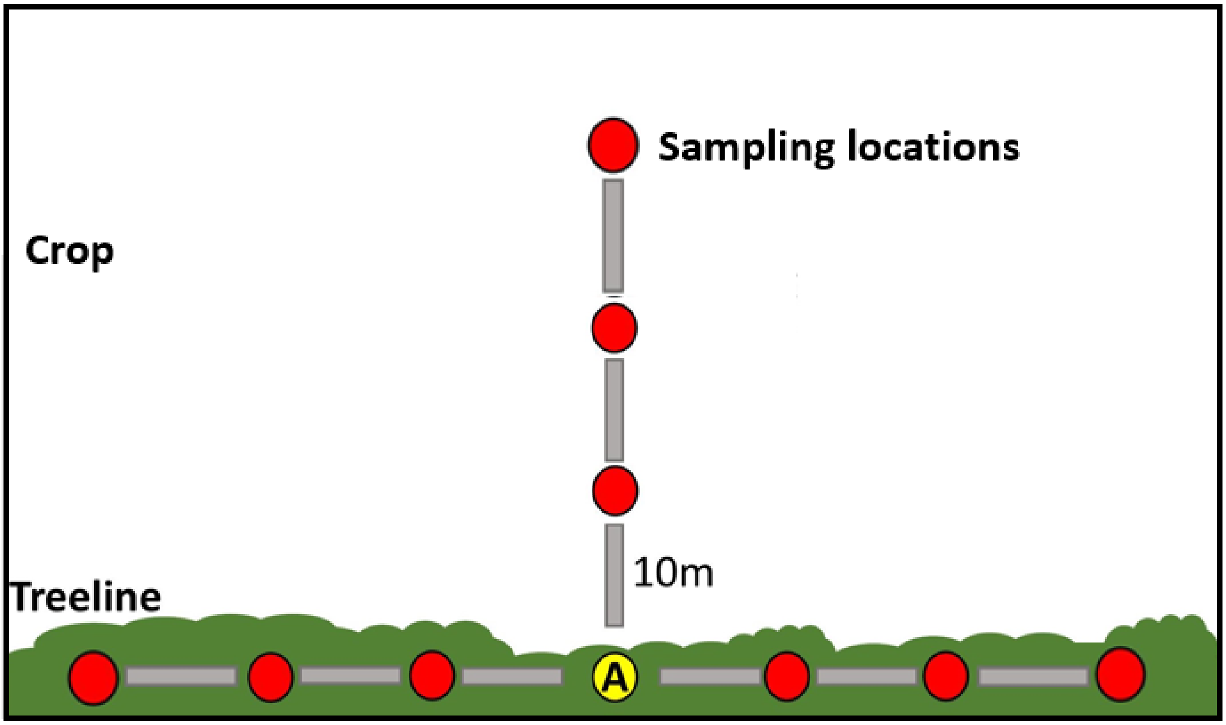

Figure 2. Schematic of sample transects and locations away from augmentorium.

At these sampling locations, assessment of fruit infestation was done using sentinel traps and when possible, fruit collections. The sentinel traps entailed the purchase of blueberries from supermarkets, infesting them in the lab with SWD for two days, and then placing them in plastic clamshells in the field. To prevent emergence of SWD, the sentinel traps were removed after seven days. Traps were then returned to the lab and the blueberries were placed in rearing containers and kept for at least 40 days, with all emerging insects being counted and identified. Sampling was done three times throughout the season; control locations away from the augmentorium were also sampled at each site.

RESULTS

Laboratory Bioassay

The 1.1 mm^2 fabric mesh was found to be the most suitable for restricting SWD while still allowing *G. brasiliensis* to escape (Figure 3); 62.5% of *G. brasiliensis* were able to pass through the mesh and no adult SWD were able to move through the mesh.

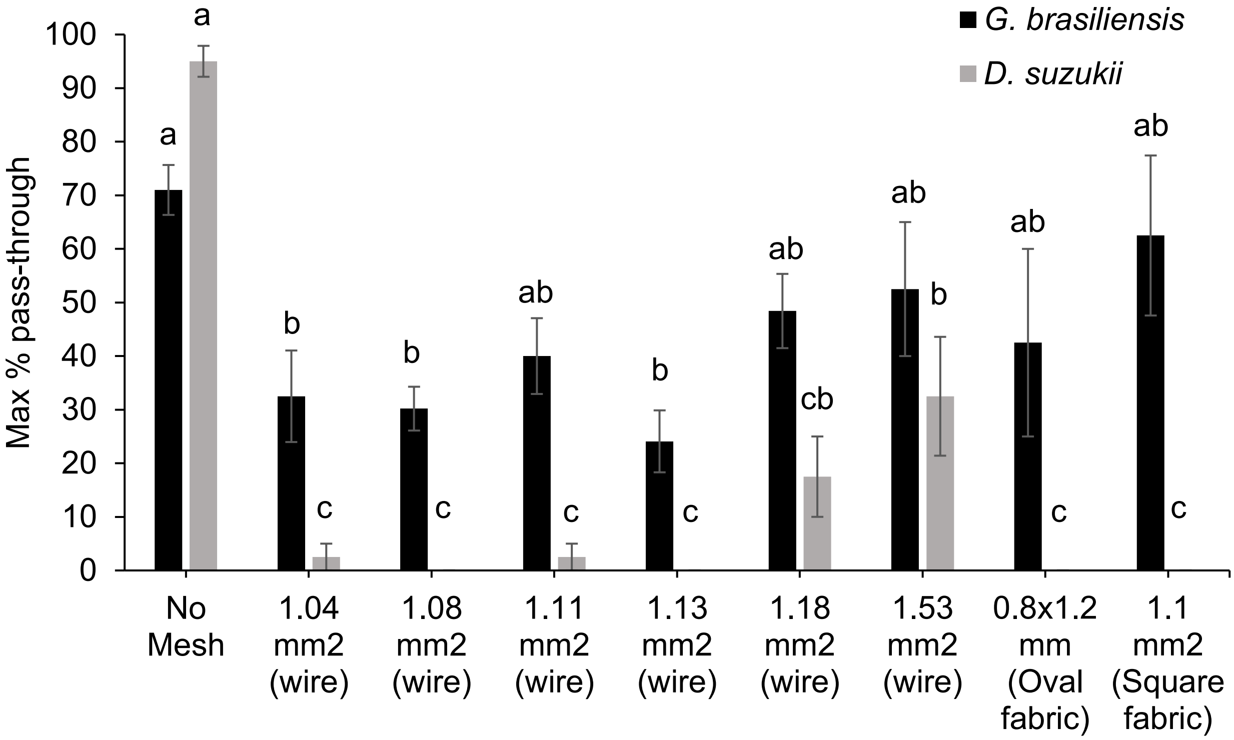

Figure 3. Percent (±SE) of SWD and *G. brasiliensis* passing through each size mesh in laboratory bioassays. Column with the same letter(s) were not significantly different from each other ($P < 0.05$, Tukey HSD test).

<u>Field Study</u>

Inside the augmentorium, *G. brasiliensis* successfully parasitized SWD in supplied fruits, with initial average parasitism rates as high as 33% (± 5.7% SE) recorded on week ending August 12th. The parasitism rate declined to as low as 4% (± 1.5% SE) on the week ending September 9th (Figure. 4). This decline is likely due to a limitation of suitable fruit inside the augmentorium, as there was only a sole addition of fruit at the start of the experiment. This data suggests that fruit will need to be added more regularly to maintain high rates of parasitism. Outside the augmentorium cages, sentinel traps did not collect any parasitoids across all the sites and locations. Parasitoids were retrieved from fruit samples outside the augmentorium at one site. However, the number of fruit samples were limited by the availability of wild fruit and wild blueberry once the fields were harvested.

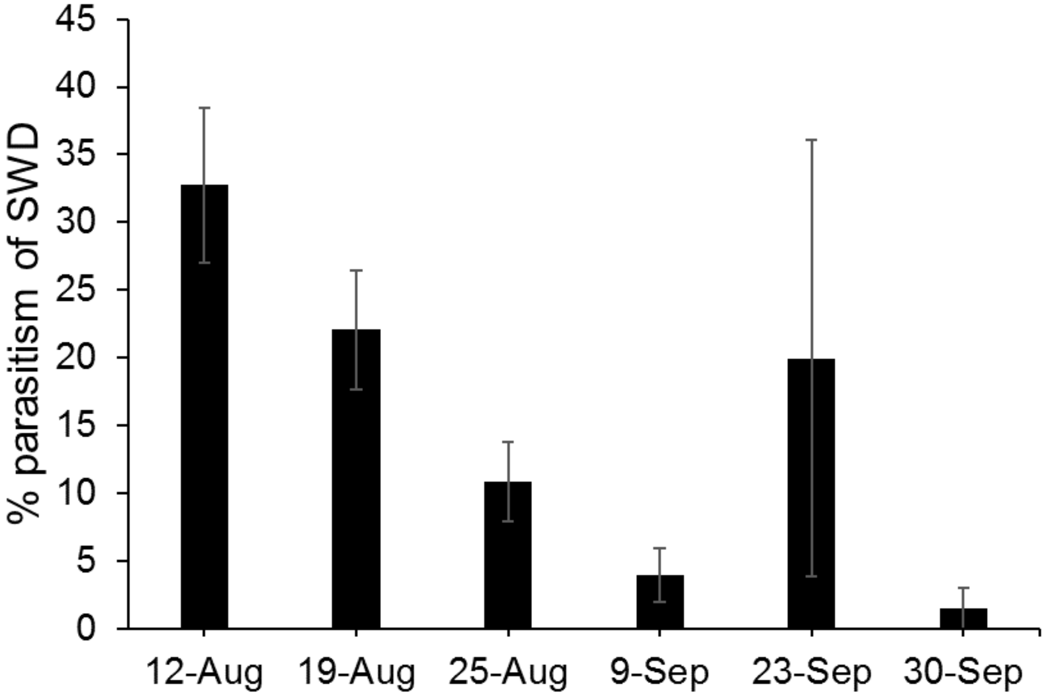

Figure 4. Increase in the average (± S.E.) parasitism of *G. brasiliensis* inside augmentorium adjacent to wild blueberry fields.

CONCLUSIONS AND RECOMMENDATIONS

In this study we were able to successfully rear *G. brasiliensis* inside augmentoria in the field. We could not conclude that there was a measurable increase in field populations of the parasitoid, primarily due to the limitations of the sentinel traps. In the future, augmentorium may be an effective strategy to enhance populations of the parasitoid *G. brasiliensis* as a biological control of spotted wing drosophila; however, additional research is needed.

NEXT STEPS

- This work will be continued in 2024. In the coming field season, we will regularly add additional fruit to the augmentorium so it is not a limiting factor in the percent observed parasitism.
- Additionally, we will deploy the augmentorium earlier in the 2024 field season to better assess parasitism outside the cage using wild blueberry before they are harvested.

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2. Behavioral Interactions between Spotted-Wing Drosophila and its Parasitoid *Ganaspis brasiliensis*

INVESTIGATORS: P. Fanning and D. Crowley

OBJECTIVE(S):

- To determine if there are differences in the behavior of adult spotted-wing drosophila (SWD) in the presence of *G. brasiliensis* and if there are impacts on egg-laying leading to a reduction in the number of larvae in fruit.

LOCATION(S): Penobscot Co., ME

PROJECT TIMEFRAME: November 2023

INTRODUCTION

Biological control has been studied intensively since *Drosophila suzukii* (SWD) was first discovered in the United States (Lee et al., 2019). Naturally occurring predators consume and remove a substantial portion of SWD (Woltz et al., 2015; Woltz & Lee, 2017). Carabid beetles, crickets, green lacewing larvae, and rove beetles are likely predators (Lee et al., 2019). Despite the activity of these native predators, SWD continues to cause major economic damage indicating that they are insufficient to keep populations below economic thresholds. In addition to predators, resident parasitoids have been surveyed across North America

In biological control, parasitoids and predators primarily impact their prey or host through direct computation. The primary impact of *G. brasiliensis* on SWD is through the parasitism of its larvae, which has been well-investigated; however, additionally there are non-lethal effects, risk effects, or trait-mediated interactions that result in changes in prey biology driven by the threat of predation. Reduced oviposition in the face of threat by parasitoid wasps has been documented in the fruit fly species *D. melanogaster* (Lefèvre et al., 2011), but has not been studied in SWD.

METHODS

Behavioral Assay

Adult SWD were placed in 20 mm diameter petri dishes alone or in the presence of *G. brasiliensis*. A five-minute acclimatization period followed the transfers to prevent impacts of the handling on behavior. High-definition video was recorded using a document camera positioned overhead. Individual arenas (petri dishes) were placed on a labelled cardboard grid to allow for future identification of specific replicates. Each observation period was limited to 60 min. Observations noted included physical interaction between *G. brasiliensis* and SWD (i.e. antennation), courtship behavior (between SWD), and mating. Courtship behavior was defined as any of the behaviors described in the literature: orienting, tapping, singing, and mounting (Bontonou & Wicker-Thomas, 2014).

Oviposition Assay

To assess the impact of *G. brasiliensis* on SWD oviposition, ten, 5-7 day old, adult SWD (5 male and 5 female) were placed in each of 20, 8 oz deli cups containing 15 conventionally grown blueberries obtained from a grocery store. Five female *G. brasiliensis* were added to each cup. A similar number of SWD-only cups were set up as controls (n = 18). In addition, there were three, fruit-only controls. Cups were maintained in a growth chamber at 25°C on a 16:8 photoperiod for 48 h to allow infestation to occur. After 48 h, all adult SWD and *G. brasiliensis* were removed. The cups (with fruit) were then returned to the incubator for 5 days to allow for larval growth. The salt flotation method described by Van Timmeren et al. (2017) was used to assess the presence of larvae in fruit.

JMP®, Version 16 (SAS Institute Inc., Cary, NC, 1989–2021) was used for all statistical analysis. All data were tested for normality and deviations from homoscedasticity using the Shapiro-Wilk test and Levene's test, respectively. All data were determined to be non-normal, and transformations were not successfully able to correct the normality. For the behavioral bioassays, Wilcoxon tests ($\alpha = 0.05$) were used to look at the difference in the record behaviors. Data for the Oviposition assays was assessed using a Kruskal Wallis test, with post-hoc being performed by Wilcoxon each pair test ($\alpha = 0.05$).

RESULTS

For the behavioral assays, the number of observed courtship (CS), significant movement (GR), cross species interaction (XI) and mating (MT) were determined and the percentage of each event type of the total number of recorded events was calculated (Figure 1). Overall, cross species interaction in the SWD + GB treatment was low, and there was no significant difference in the percentage of courtship (CS) ($X^2 = 1.20$, df = 1, $P < 0.2733$) or significant movement (GR) ($X^2 = 0.36$, df = 1, $P < 0.5475$). Despite observed courtship, no mating was observed in any of the assays.

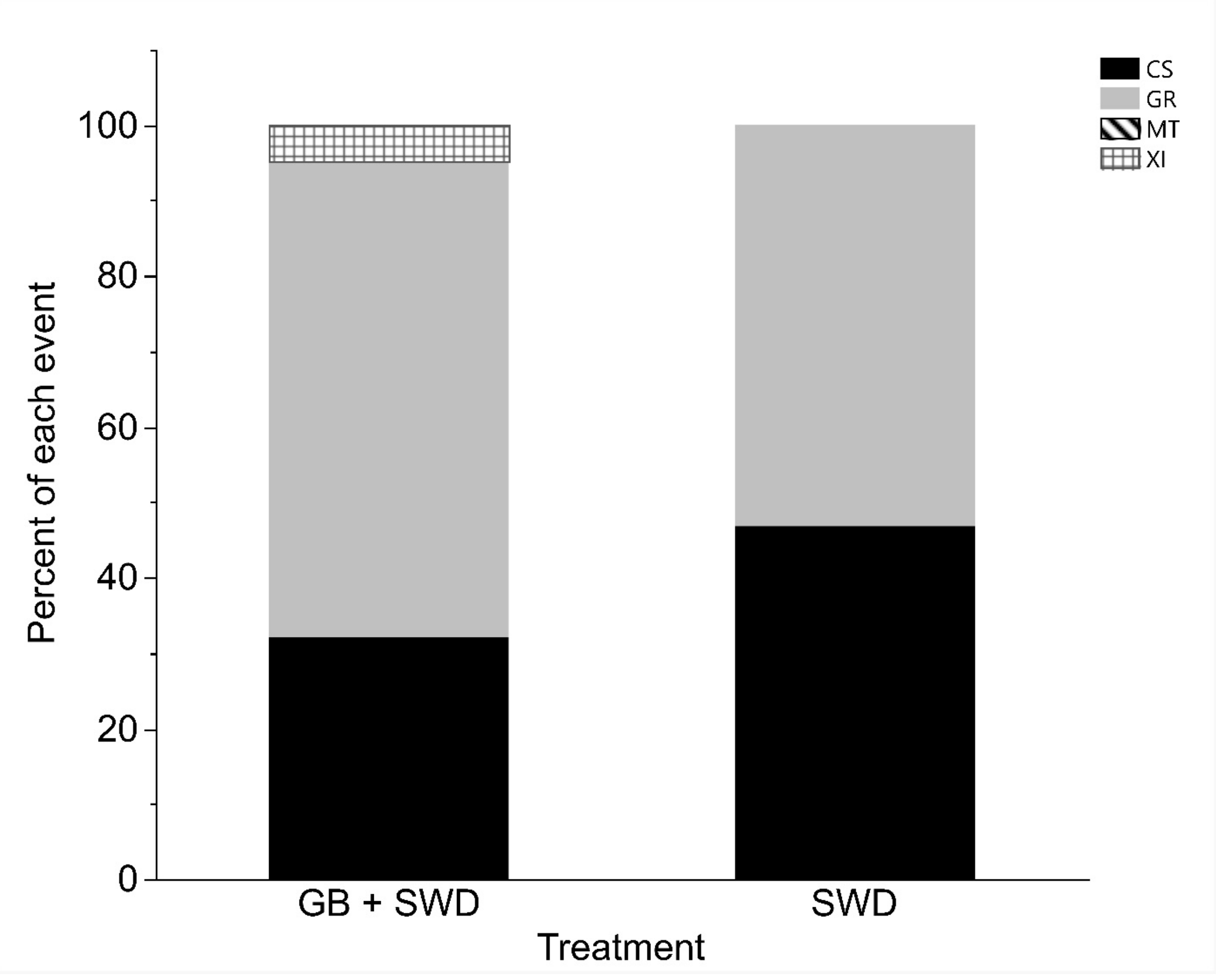

Figure 1. Average percentage of different behavioral events of observed courtship (CS), significant movement (GR), cross species interaction (XI) and mating (MT) in bioassay containers.

In the oviposition assay there was a significant difference among the ($X^2 = 28.48$, df = 2, $P < 0.0001$) (Figure 2). The highest infestation was seen in the SWD only group; the average (± S.E.) number of SWD larvae was 15.6 (± 1.75). This was significantly higher ($P < 0.05$, Wilcoxon test) than both the SWD + *G. brasiliensis* and the fruit-only groups that had an average (± S.E.) of 3.2 (± 0.73) and 0.6 (±

0.66) larvae; respectively. Some background infestation was seen in the fruit-only treatment, with two larvae in one of the three replicates. The larvae appeared to be of a non-drosophilid species.

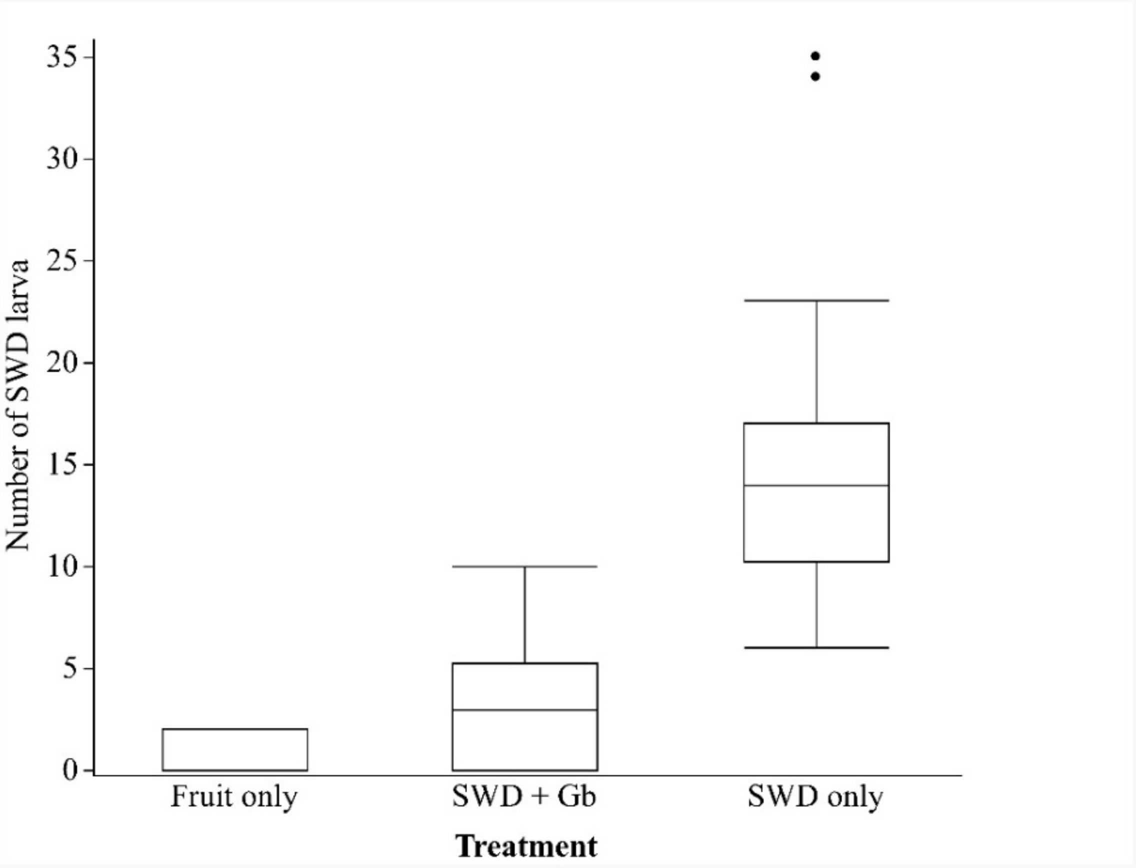

Figure 2. Average number of larvae infesting fruit exposed to SWD in the presence of *G. brasiliensis* (Gb), SWD only and a fruit only control.

CONCLUSIONS AND RECOMMENDATIONS

This experiment had mixed results, most notably a failure of SWD to mate in the laboratory environment. This resulted in a challenging behavioral assay that showed no impact among all measured behaviors (courtship and movement). However, the results of the oviposition assay were encouraging, demonstrating that female SWD display decreased oviposition in this presence of *G. brasiliensis*. This reduction in reproductive output is a notable finding. Although oviposition depression in predators has been described in response to other parasitoids, this finding has not been reported in *G. brasiliensis*. This is an encouraging finding for integrated pest management (IPM) researchers who are seeking to employ *G. brasiliensis* as a biocontrol agent, suggesting that its mere presence may assist in the suppression of SWD.

NEXT STEPS

- The significance of direct physical interactions between parasitoids and adult SWD has not been well documented and may warrant further investigation.
- Additional trials are planned to vary the density of the parasitoids and SWD to simulate different densities that are relevant to the phenology of parasitoid in the field.
- Additional trials will also be conducted on *Leptopilina japonica* another parasitoid now attacking SWD in Maine.

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3. Overwintering of *Ganaspis brasiliensis,* a Parasitoid of Spotted-wing Drosophila, in the Northern Temperate Conditions of Maine, Year One

INVESTIGATORS: S, Munnaf Chowdhury and P. Fanning

OBJECTIVE(S):

- To assess the ability of diapausing pre-pupae of *Ganaspis brasiliensis* to survive and overwinter in the northern temperate climate conditions of Maine.

LOCATION(S): Penobscot Co., ME

PROJECT TIMEFRAME: January - April 2023

INTRODUCTION

Classical biological control has been proposed as a promising option for the control of spotted-wing drosophila (SWD), particularly in unmanaged areas, such as wild habitats and public or private gardens. These areas act as reservoirs for SWD due to the current lack of local control methods (Daane et al., 2016; Girod et al., 2018; Lee et al., 2019). In foreign exploration for parasitoids in SWD's native range in Asia, three larval parasitoids, *Ganaspis brasiliensis* (Ihering), *Leptopilina j. japonica* Novković & Kimura, and *Asobara japonica* Belokobylskij, were chosen based on frequent occurrence. These species were imported into quarantine laboratories in the U.S. for evaluation. 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 laboratory colony, and large numbers of *G. brasiliensis* were reared for release in the summer of 2023.

Overwintering of *G. brasiliensis* is poorly understood. However, the species' ability to overwinter and understanding the year-to-year variation in survival is essential to predict how many years of releases will be needed to build stable parasitoid populations. This is especially important in north temperate regions in the United States, where the species will encounter extremely low temperatures and increasing variance in winter temperature.

METHODS

A field site was set up along a wooded edge at the University of Maine owned Rogers Farm in Old Town, ME. Thirteen holes were dug using a golf hole digger. The soil plug was placed into a 36 oz deli cup leaving 1-2 cm (ca. ½ inch) open at the top. This space was filled with organic matter from the site (old leaves, twigs, etc.). Fifty chilled diapausing SWD pre-pupae were put in a sachet made with fabric mesh. Then the sachets were added to the organic matter and covered in 2-3 cm (ca. 1 inch) of dead leaves. A double layer of 1 mm mesh was secured over the top of the deli cup and held in place with rubber bands. The deli cup was then placed into the hole so that the lip of the deli cup was level with the ground. The mesh was tucked into the ground around the cup and then covered with surrounding leaf litter. The cups were secured and marked with flags (Image 1).

Image 1. Overwintering experimental process.

Every two weeks, a set of four deli cups was dug up, transported back to the laboratory, and held in the growth chamber at 25°C, 16:8 D: L and 45% RH. The mesh cover was removed, and the SWD pupae from each sachet were placed in a petri dish. After two weeks the number of *G. brasiliensis* that emerged from the pupae was counted. The first set of cups were collected the day after the experiment was launched (day 1). This experiment ran for 8 weeks beginning on February 7th, 2023.

Temperature at the field site was recorded using Hobo® data loggers (Onset® Computer Corporation, Bourne, MA, USA). A set of loggers were suspended from a nearby tree branch to measure air temperature, and a set of loggers were placed between the soil and leaf litter in the deli cups to measure the temperature diapausing pre pupae were exposed to. Data loggers recorded temperatures every hour for as long as the cups remained in the field.

RESULTS

On day 1 (the next day after placement), an average (± S.E) of 23% (± 2.98) of SWD pupae appeared to have developing *G. brasiliensis*. A similar percentage of SWD appeared to have developing *G. brasiliensis* on day 61 (28% ± 2.14) (Figure 1). There was no significant difference in terms of *G. brasiliensis* development inside the puparium between day 1 and day 61. However, no adult *G. brasiliensis* were observed emerging.

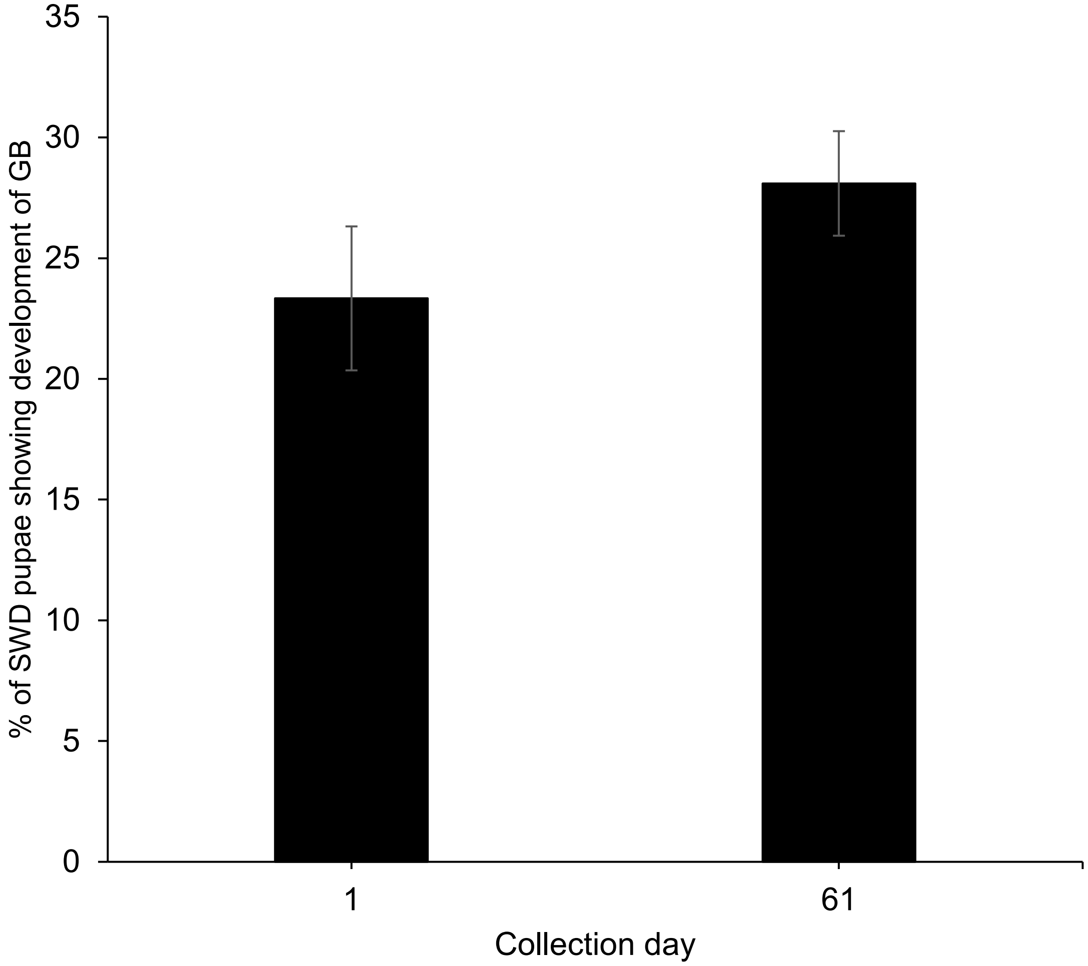

Figure 1. Percent SWD pupae with developing *G. brasiliensis*. N.S. indicates that there was no significant difference (α = 0.05, t-test)

CONCLUSIONS AND RECOMMENDATIONS

It is likely that no adult *G. brasiliensis* developed because parasitized SWD pupae were damaged during handling. Next year we will make a change to our overwintering protocol to reduce the risk of damaging pupae before placing them in the field. A new site for the experiment will be selected in 2024 to ensure that we can get enough temperature variations in our study.

NEXT STEPS

- This work will be continued in the winter of 2023-2024.

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4. Larval Versus Adult Monitoring of *Drosophila suzukii* to Inform Management Decisions in Wild Blueberry

INVESTIGATORS: P. Fanning and B. Johnson

OBJECTIVE:

- Compare the efficiency of larval vs adult monitoring of *D. suzukii* for informed decision making on management decisions.

LOCATION: Washington Co., ME

PROJECT TIMEFRAME: July 2022 – August 2023

INTRODUCTION

Drosophila suzukii, also known as Spotted-wing Drosophila or SWD, is an invasive insect pest attacking ripe and ripening fruit. Management decisions regarding SWD in Maine can be informed by a threshold model, in which adult SWD male trap captures have been correlated to probability of having fruit infestation the following week (Drummond et al., 2019).

While monitoring fields and conducting research, many labs collect both crop and wild fruit and use the salt test method (Van Timmeren et al., 2017) to assess levels of larval infestation. Direct sampling of fruit for larval infestation via the salt test answers whether or not infestation is present in a field, while adult monitoring only serves as a proxy for the potential for SWD infestation.

Making informed management decisions based on adult male trap captures is better than following calendar sprays. However, past research has shown that the threshold model is not always 100% accurate. This can lead to unnecessary application of pesticides due to the absence of infestation or sprays that are applied after SWD have already become established in the field. Direct monitoring of larvae in fruit is the most effective and accurate way to determine the presence or absence of larval infestation in the crop (Van Timmeren et al., 2021).

METHODS

Working with a grower cooperator, a crop-year field was split into North and South sections. The grower monitored adult presence of SWD at their standard locations around the entire field, while the Fanning Lab monitored for larval infestation in fruit at the edges and interior of the entire field. Grower SWD traps were made from red Solo® cups, baited with a yeast/sugar water mixture, and placed along the wooded perimeter of the field. Fruit collections were taken at the same locations as the cup traps along the edge of the field, as well as 120 ft into the field from the edge. For each sample, 6 oz by volume of fruit was collected. Fruit was returned to the lab and a subsample of 10 berries per collection location was assessed under a microscope for the presence of SWD eggs. The entire sample was then processed following the salt test procedure outlined by Van Timmeren et al. (2017). Adult monitoring traps were checked and fruit samples were taken weekly.

Management decisions for the North half the field were made based on adult male SWD captures observed by the grower in cup traps, while management decisions for the South half of the field were based on presence or absence of larval infestation found in fruit samples collected by the Fanning Lab. If any pesticide applications were required for SWD management, they would first be a perimeter application covering only 100 ft into the field; additional sprays would be full field applications.

Adult captures that would trigger a spray application were a cumulative average greater than 3 male SWD per 3 traps in the North field. A single larval occurrence or presence of an SWD egg would trigger a spray for the South field.

RESULTS

Both the North and South sections received full-field sprays on 14th and 28th of July for control of blueberry maggot fly. The North section received a perimeter spray on August 12th and was harvested August 20th-21st. The South section received a perimeter spray on August 14th and was also harvested August 20th-21st.

This year high populations of blueberry maggot fly overlapped with the beginning of active SWD populations, and the final two sprays for blueberry maggot fly impacted early season SWD. There was then a slow ramp up of SWD populations until they became active in the field, both as adults and larvae (Figures 1 and 2).

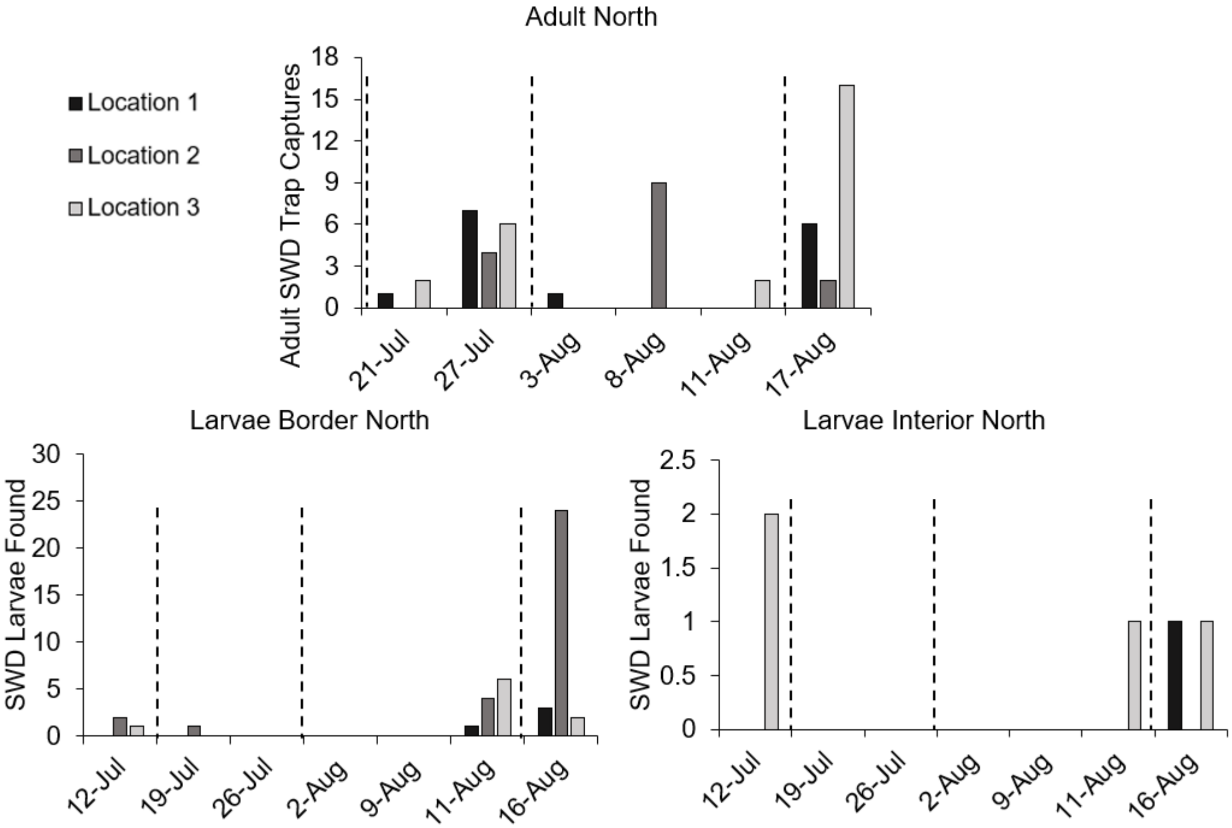

Figure 1. North section adult male SWD and larval SWD captures across the monitoring season. Dashed lines represent insecticide applications (July 14th and 28th, and August 12th).

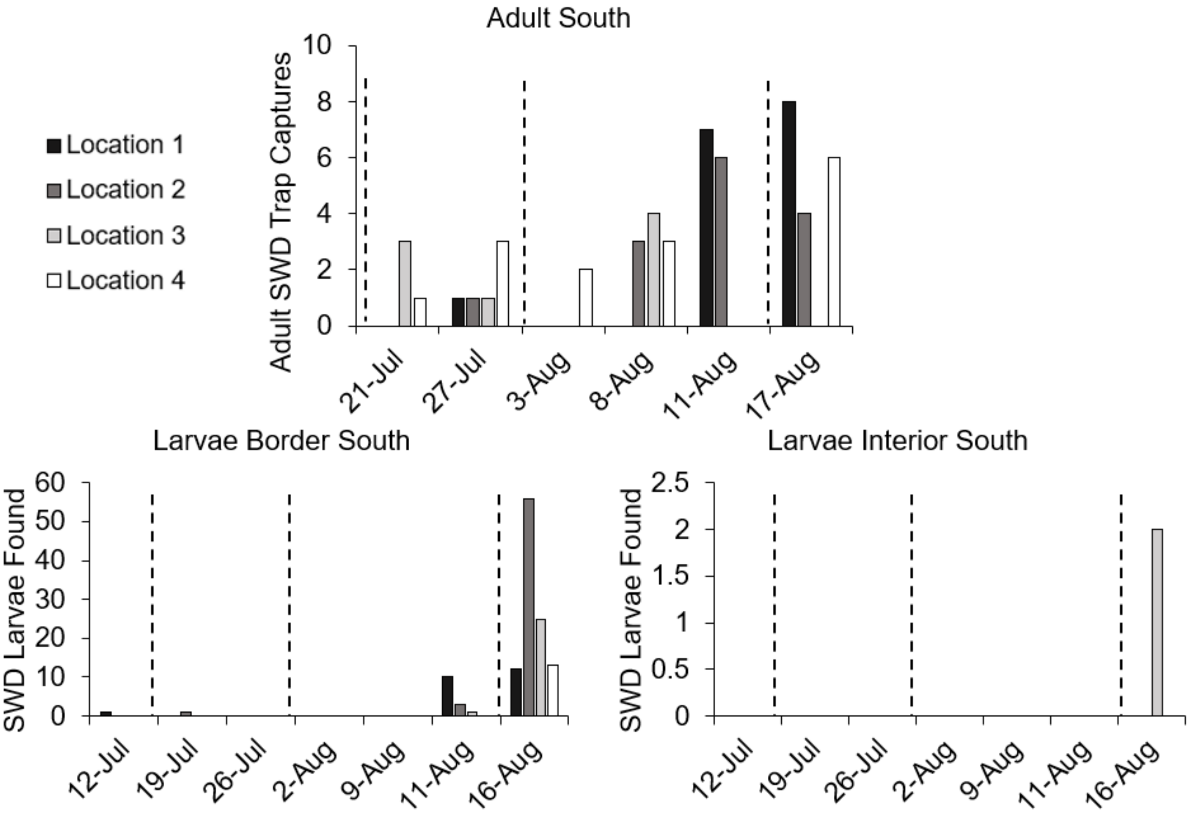

Figure 2. South section adult male SWD and larval SWD captures across the monitoring season. Dashed lines represent insecticide applications (July 14th and 28th, and August 14th).

CONCLUSIONS AND RECOMMENDATIONS

We have developed a framework for growers to use the salt test method to inform management decisions in addition to the established cumulative adult male threshold model. We recommend growers monitor their fields for the presence of larval SWD in the fruit as well as monitor adult presence to better inform management decisions.

NEXT STEPS

- We would like to repeat this trial in future years as further proof that direct monitoring for larval infestation is an adequate metric for management decision making and has the potential to reduce the number of insecticidal sprays required for the control of SWD.

ACKNOWLEDGEMENTS

We would like to thank Trevor Lowe and Lilit Mathieu for their assistance collecting fruit and counting SWD eggs and larvae for this study.

REFERENCES

Drummond, F., E. Ballman, and J. Collins. (2019). Population dynamics of spotted wing Drosophila (*Drosophila suzukii* (Matsumura)) in Maine wild blueberry (*Vaccinium angustifolium* Aiton). Insects 10, 205. doi.10.3390/insects10070205

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

Van Timmeren, S., A.R. Davis and R. Isaacs. (2021). Optimization of a larval sampling method for monitoring *Drosophila suzukii* (Diptera: Drosophilidae) in Blueberries. Journal of Economic Entomology 114(4), 1690-1700.

5. Spotted-wing Drosophila Threshold Monitoring with Cup and Sticky Red Panel Traps

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

OBJECTIVE:

- Correlate adult *D. suzukii* trap captures with the risk of larval infestation the following week using both red Solo cup traps and sticky red panel traps.

LOCATIONS: Knox, Waldo, and Washington Counties, ME.

PROJECT TIMEFRAME: June 2023 – August 2023

INTRODUCTION

The invasive *Drosophila suzukii*, also known as Spotted-wing Drosophila or SWD, is an insect pest attacking ripe and ripening fruit while it is still in the field. The most effective management option for controlling this pest is the use of targeted pesticide applications. Implementation of pesticide usage can be informed by the presence of the pest. Past research has shown the cumulative weekly average (from three traps) male trap captures of SWD can be used to predict risk of larval infestation the following week (Drummond et al., 2019). This work seeks to build upon this existing model with more data and an additional trap type.

METHODS

Working with grower cooperators we monitored adult SWD population numbers along field edges, and larval infestation numbers in crop fruit from the field edges. Adult SWD were monitored with two different traps: red Solo® cup traps and sticky red panel traps (Image 1). Red Solo cup traps were baited with a yeast/sugar water mixture (1 tsp active live yeast, 4 tsp sugar, and 120 ml [4 oz] distilled water per trap), and the sticky red panel traps were baited with a commercially available SWD lure from Scentry® Biological (purchased from Great Lakes IPM and linked at end of this report). Every field received three of each style trap, with each trap being spaced approximately 20 m (66 ft) apart.

Image 1. Traps used in this study.

Every week, old traps were collected, new traps were placed in the field, and a 6 oz by volume fruit sample was collected from the field edge nearest each trap. Red Solo traps received new yeast/sugar water bait every week, and red panel traps had lures changed every 4 weeks per manufacturer recommendations. Fruit samples were processed via the salt test method (Van Timmeren et al., 2017) to determine presence of SWD larvae. Traps were returned to the lab, and the number of adult male

SWD per trap was recorded. This process was repeated until the field was harvested or larval infestation was detected. We monitored in this fashion at seven different wild blueberry fields from June 15th to August 3rd.

RESULTS

All monitored fields had larval infestation prior to harvest. Monitoring with red Solo cup traps resulted in five of the seven fields having infestation occurring in the same week as the first male SWD capture (Tables 1 and 2), while monitoring with sticky panel traps only had two fields with infestation occurring the same week as first male detection (Table 3).

Over the past 11 years both first occurrence of male SWD (Figure 1) and date of larval infestation (Figure 2) have been happening earlier. Monitoring with baited red panel traps displays earlier first male captures than red Solo cup traps (Figure 3).

Table 1. 2023 threshold results based on data used in the original threshold model.

Site #	**Location**	**Date 1st SWD male detected**	**Status of fruit infestation before or at harvest**	**Date harvested or larval infestation detected**	**Mean Cumulative # of MALE SWD week before infestation**	**Probability of fruit infestation the week after male SWD thresholds**
1	Downeast	n/a	Infested	6-Jul	0	0
2	Midcoast	20-Jul	Infested	27-Jul	0.33333333	0.002375633
3	Midcoast	20-Jul	Infested	13-Jul	0	0
4	Midcoast	20-Jul	Infested	13-Jul	0	0
5	Midcoast	13-Jul	Infested	20-Jul	0.66666667	0.007428394
6	Midcoast	20-Jul	Infested	13-Jul	0	0
7	Midcoast	13-Jul	Infested	13-Jul	0	0

Table 2. 2023 threshold results based on the original model with six additional years of trap captures.

Site #	**Location**	**Date 1st SWD male detected**	**Status of fruit infestation before or at harvest**	**Date harvested or larval infestation detected**	**Mean Cumulative # of MALE SWD week before infestation**	**Probability of fruit infestation the week after male SWD thresholds**
1	Downeast	n/a	Infested	6-Jul	0	0
2	Midcoast	20-Jul	Infested	27-Jul	0.333333333	0.02794797
3	Midcoast	20-Jul	Infested	13-Jul	0	0
4	Midcoast	20-Jul	Infested	13-Jul	0	0
5	Midcoast	13-Jul	Infested	20-Jul	0.666666667	0.053527179
6	Midcoast	20-Jul	Infested	13-Jul	0	0
7	Midcoast	13-Jul	Infested	13-Jul	0	0

Table 3. 2023 threshold results based on a preliminary model using sticky red panel traps and data from 2022-2023.

Site#	**Location**	**Date 1st SWD male detected**	**Status of fruit infestation before or at harvest**	**Date harvested or larval infestation detected**	**Mean Cumulative # of MALE SWD week before infestation**	**Probability of fruit infestation the week after male SWD thresholds**
1	Downeast	n/a	Infested	6-Jul	0	0
2	Midcoast	20-Jul	Infested	27-Jul	28	0.88602357
3	Midcoast	20-Jul	Infested	13-Jul	1	0.14461240
4	Midcoast	20-Jul	Infested	13-Jul	0	0
5	Midcoast	13-Jul	Infested	20-Jul	1.666666667	0.20436893
6	Midcoast	20-Jul	Infested	13-Jul	1.5	0.19042904
7	Midcoast	13-Jul	Infested	13-Jul	1.666666667	0.20436893

Table 4. Comparison of risks from the original model (data from 2012-2017), an updated model (data from 2012-2023), and the red panel model (data from 2022-2023).

	Probability of Infested Fruit the Following Week		
Cumulative Males (average from 3 traps)	2012-2017	2012-2023	Red Panel
0.33	0.0023	0.0276	0.0670
0.66	0.0073	0.0530	0.1086
1	0.0143	0.0779	0.1446
1.3	0.0218	0.0990	0.1728
1.66	0.0321	0.1235	0.2038
2	0.0429	0.1459	0.2307
3	0.0794	0.2078	0.3002
3.5	0.0996	0.2368	0.3310
5	0.1647	0.3170	0.4115
10	0.3885	0.5258	0.6018

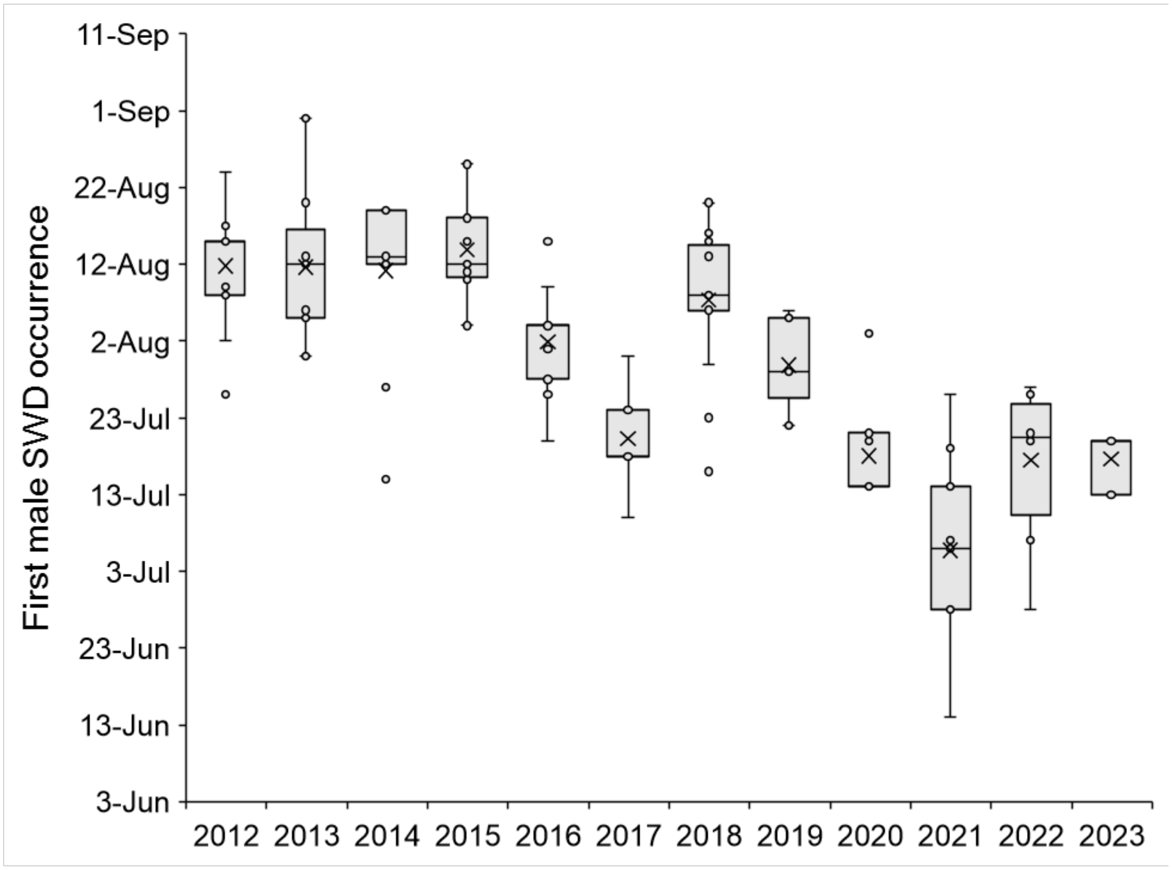

Figure 1. Average first encounters with male SWD in red Solo yeast/sugar cup traps from all fields 2012-2023.

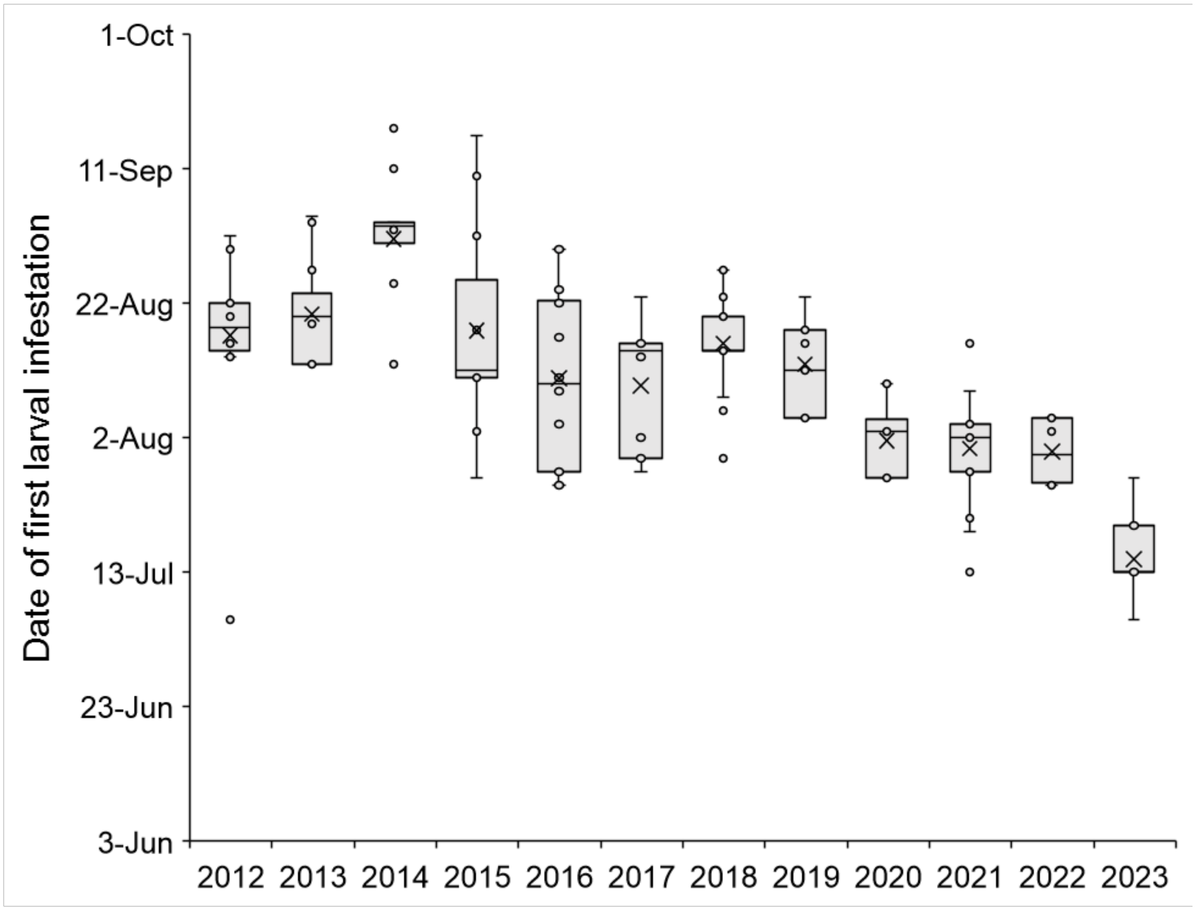

Figure 2. Average first occurrence of larval SWD infestation in fruit from all fields 2012-2023.

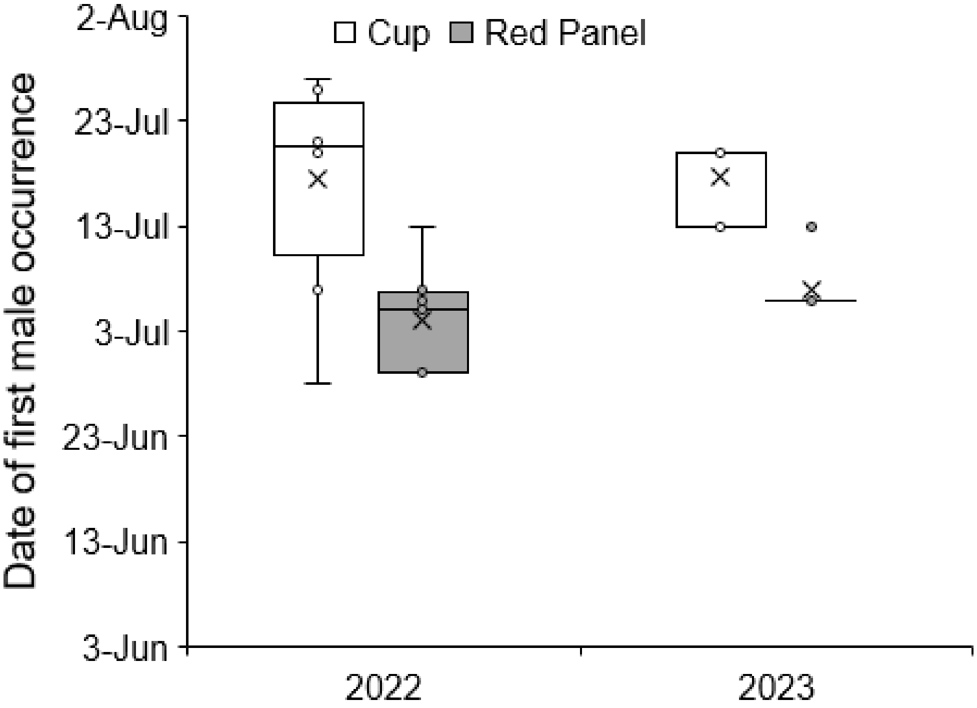

Figure 3. Average first encounters with males in cup traps and red sticky traps across the same sites.

CONCLUSIONS AND RECOMMENDATIONS

There were no male SWD captures in red Solo cup traps in five of the seven sites the week before larval infestation was found. In the remaining two sites, there were mean cumulative captures of 0.333 and 0.667 adult males, correlating to a 0.2% and 0.7% chance of infestation. Adding additional year's data to the threshold model caused an increase in the probability of having infested fruit with fewer cumulative male captures (Table 3). We now have two years' worth of data on the use of sticky red panel traps for predicting larval infestation but need additional years before recommending it for use to growers.

A potential reason for the inconsistency in the threshold might be explained by the continuing progression of earlier captures of SWD males (Figure 1), and the earlier dates of larval infestation (Figure 2). More work needs to be done to determine the cause of these earlier detections and what impacts they will have on SWD management practices.

We continue to recommend growers monitor their fields using red Solo cup traps baited with yeast/sugar water, and that growers assume an extra layer of caution when relying on the predictive capabilities of adult male captures. We also encourage growers to check their fields for larval infestation using the salt test method (Van Timmeren et al., 2017) in addition to monitoring for adult presence.

NEXT STEPS

- We would like to continue monitoring for adult and larval presence in and around wild blueberry fields using cup and panel traps to strengthen this threshold model, as well as add in additional variables such as winter climate conditions and early spring rates of warming.

ACKNOWLEDGEMENTS

We would like to thank Trevor Lowe and Lilit Mathieu for their assistance with this study – driving from site to site, swapping traps, and counting SWD adults and larvae.

REFERENCES

Drummond, F., E. Ballman, and J. Collins. (2019). Population dynamics of spotted wing Drosophila (*Drosophila suzukii* (Matsumura)) in Maine wild blueberry (*Vaccinium angustifolium* Aiton). Insects, 10, 205. doi.10.3390/insects10070205

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SOURCES FOR RED PANEL TRAPS AND LURES:

Red sticky trap: GL/TR-3324-10
https://www.greatlakesipm.com/monitoring/traps/trece-pherocon/gltr332410
trece-pherocon-swd-stky-adhesive-traps-10cs

Scentry Lure: GL/SC-5100-12
https://www.greatlakesipm.com/monitoring/lures/fruit-fly/glsc510012
Sentry-spotted-wing-drosophila-swd-lures-12cs

1. Herbicide Stacking Demonstration at Blueberry Hill Farm

INVESTIGATORS: L. Calderwood, J. Stubbs, and J. Parks

OBJECTIVES

- Demonstrate herbicide stacking to wild blueberry growers at field days
- Collect basic plant and phytotoxicity data for efficacy observations

LOCATION: Blueberry Hill Farm, Jonesboro, ME

PROJECT TIMEFRAME: April 2022 – August 2024

INTRODUCTION

Weed management is a continual challenge for wild blueberry growers across the state of Maine. Weeds compete with blueberry plants for water, nutrients, and sunlight as well as interfering with harvesting equipment and damaging fruit (McCully et al., 1991). Different species of weeds require different management techniques which include reducing the soil pH, applying mulch, cutting stems or trunks 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 the crop for sunlight, water, and nutrients. However, repeated use of the same product without rotating to different modes of action and group numbers will increase the chances for resistance. 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 (Johnson et al., 2005).

The practice of herbicide stacking is not unique to wild blueberry and is also practiced in North American canola, rice, and soybean production (The Canola Council of Canada, 2021). This project was primarily a 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 have emerged 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 to protect pollinators. When flowers are out you can assume that bees are active.

METHODS

Two separate demonstration sites exist, one in the 2022 prune year field where herbicides were only applied in 2022 and a second in the 2023 prune year field where herbicides were only applied in 2023. The 2023 site was land-leveled in 2021 and therefore had more weeds and lower wild blueberry cover compared to the 2022 site. On April 12, 2023, demonstration plots were laid out in the 2023 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 were 45 ft x 120 ft and were divided by 10 ft buffer strips. Within each plot, six 0.37 m^2 quadrats were staked out to enable repeated data collection throughout the season. The products applied were: Matrix, Callisto, Arrow, Zeus, Zeus Prime, and Capreno (Table 1). Capreno is an herbicide that is **NOT** yet labeled for use in wild blueberry.

Table 1. Herbicide products, groups, and application rates used in the 2022 and 2023 herbicide stacking demonstrations in Jonesboro, ME.

Year Used (Prune)	Active Ingredient	Trade Name	Group	Application Rate
2022, 2023	Rimsulfuron	Matrix	9	4 oz/A
2022, 2023	Mesotrione	Callisto	27	3 oz/A
2022, 2023	Clethodim	Arrow	1	4 oz/A
2022	Sulfentrazone	Zeus	14	12 oz/A
2022, 2023	Carfentrazone & Sulfentrazone	Zeus Prime XC	14	15.2 oz/A
2022, 2023	Thiencarbazone-methyl & Tembotrione	Capreno	2 & 27	**EXPERIMENTAL. NOT YET LABELED FOR USE.**

Table 2. Herbicide stacking schedule for 2023. All dates were classified as pre-emergence ("PRE") or post-emergence ("POST") based on actual emergence that occurred the week of April 14, 2023, in Jonesboro, ME. In the "Target" column, GR = grasses, BL = broadleaves, and S = sedges. All treatments except the control had LI700 surfactant added.

Treatment	Date (2023)	PRE/POST	Product Trade Name	Target
A	20-May 19-Jun	POST POST	Capreno + Capreno	BL BL
B	18-Apr 20-May	PRE POST	Arrow Capreno	GR BL
C	14-Apr 20-May	PRE POST	Water (Untreated Control) Water (Untreated Control)	None None
D	14-Apr 20-May	PRE POST	Matrix Callisto	GR, BL BL
E	14-Apr	PRE	Matrix	GR, BL
F	18-Apr 20-May 19-Jun	PRE POST POST	Arrow Callisto Callisto	GR BL BL
G	18-Apr 18-Apr	PRE PRE	Zeus Prime Arrow	Gr, BL GR
H	20-May	POST	Capreno	BL

Table 3. Herbicide stacking schedule for 2022. All dates were classified as pre-emergence (“pre”) or post-emergence (“post”) based on actual emergence that occurred the week of April 25, 2022, in Jonesboro, ME. In the “Target” column, GR = grasses, BL = broadleaves, and S = sedges. All treatments except the control had LI700 surfactant added.

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 and Arrow	GR, BL
	5-May	POST	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

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. Post treatment applications were applied when wind speed was less than 10MPH and relatively dry conditions were present in 2023.

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

<u>Data Collection</u>

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 May 18th, June 13th, and July 7th in 2023 and June 1st, July 7th, and August 27th in 2022. Blueberry and weed presence in 2023 were quantified as percent cover using equal interval ranks between 0 and 5, where: 0 = not present, 1 = 1-10%, 2 = 20-40%, 3 = 40-60%, 4 = 60-80%, and 5 = 80-100%. In 2022, a 0 through 6 scale was used, 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 and weeds were counted and the number of plants displaying phytotoxicity symptoms were also recorded. All weed species within the sample were identified and recorded.

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, 1989-2023), followed by a Tukey's HSD Pairwise means comparison in, testing the effects of date, treatment, and any interaction between date and treatment ($\alpha \leq 0.05$). Additionally, a bivariate linear 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}). Data improved in qqplots with transformations but did not pass a Shapiro-Wilks test. As long as the homoscedasticity of the residuals was adequate and qqplots improved, a parametric test was used. F-tests have been found to be accurate without significantly affecting Type I error under non-normal conditions with homoscedastic variance (Blanca et al., 2017). Back-transformed means are presented in figures when transformations were used which is why some figures do not have standard error bars.

RESULTS

Prune Cycle

Wild blueberry cover was measured on May 18th, June 13th, and July 7th, 2023, at both the 2022 and 2023 sites. Blueberry coverage was low in the 2023 site but the CaprenoPOST and CaprenoPOST + CaprenoPOST had significantly greater coverage when compared to MatrixPRE ($P < 0.001$) and ArrowPRE + CaprenoPOST ($P < 0.001$) (Figure 1). In 2022, 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 2).

Blueberry stems in the prune cycle were evaluated for phytotoxicity after applications and sampling dates needed to be separated due to a treatment on July 7th being significantly greater ($P = 0.042$). On May 18th, ArrowPRE + CaprenoPOST (1.5 stems m^{-2}) significantly greater than all other treatments ($P = 0.001$) (Figure 3). There were no differences detected between treatments on June 13th, however on July 7th, the ZeusPrimePRE + ArrowPRE (39 stems m^{-2}) was significantly greater than all other treatments ($P < 0.001$) (Figure 3). In 2022, although there were no significant differences detected between treatments, the greatest levels of phytotoxicity damage were measured in the ArrowPRE + CallistoPOST treatment (85 stems m^{-2}), followed by the CaprenoPOST treatment (64 stems m^{-2}) (Figure 4).

The number of weeds in the prune field were greatest in the WaterPRE + WaterPOST control (130 stems m^{-2}) when compared to the CaprenoPOST treatment (29 stems m^{-2}) ($P = 0.009$) (Figure 5). All other treatments were similar to each other. The greatest number of weeds in 2022 were measured in the ZeusPrimePRE + ArrowPRE treatment (129 weeds m^{-2}) which was 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}) (Figure 6).

Phytotoxicity on weed species in 2023 was low compared to 2022. There were no significant differences detected between treatments in the 2023 prune cycle (Figure 7). In 2022, there were also no significant differences detected between treatments, but the CaprenoPOSTx2 treatment (48 weeds m^{-2}) resulted in greater phytotoxicity, followed by CallistoPOSTx2 (41 weeds m^{-2}), and ZeusPRE + ArrowPRE (38 weeds m^{-2}) (Figure 8).

The most abundant weed species in the 2023 prune cycle were, red sorrel (*Rumex acetosella*), toadflax *(Linaria vulgaris*)*,* grasses *(Poaceae* spp.)*,* and aronia (*Aronia melanocarpa),* which were primarily woody broadleaf perennials, broadleaf perennials, and annual or perennial grasses, which is characteristic of many blueberry fields across the state. The ZeusPrimePRE + ArrowPRE treatment had significantly lower numbers of red sorrel when compared to the WaterPRE + WaterPOST control ($P = 0.030$) (Figure 9). In terms of phytotoxicity, the ArrowPRE + CaprenoPOST had the greatest and CaprenoPOST had the second largest effect on red sorrel (Table 4). Red sorrel was the only weed to develop phytotoixcity from all applications.

In the 2022 prune cycle, the only annual broadleaf weeds observed in the trial location were orange St. John's-wort *(Hypericum gentianoides*)*,* observed in plots where CallistoPOSTx2, CaprenoPOSTx2, ZeusPrimePRE + ArrowPRE, and ZeusPRE + ArrowPRE were applied, and violet *(Viola* spp.)*,* observed where CallistoPOSTx2 and ZeusPRE + ArrowPRE were applied (Table 5). The top two weeds observed across almost all treatments included the broadleaf perennial red sorrel (present in all treatments except CaprenoPOST) and sedge (*Carex* spp.) (present in all treatments except ArrowPRE + CallistoPOST and MatrixPRE + CallistoPOST) (Table 5). 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). Phytotoxicity in red sorrel was 90% in the CaprenoPOSTx2 treatment and 7% in the ZeusPrimePRE+ArrowPRE treatment (Figure 10). The CaprenoPOST and ControlWaterPRE treatments did not exhibit any phytotoxicity within red sorrel.

A bivariate linear regression for the 2022 prune cycle, showed a significant negative linear relationship as blueberry cover decreases, weed cover increases ($P < 0.001$) (Figure 11). This relationship was not detected in 2023 for the prune or crop cycles (data not shown).

Crop Cycle

In the 2023 crop cycle that was previously treated in the prune year (2022), the ZeusPrimePRE + ArrowPRE had significantly greater blueberry cover than ZeusPRE + ArrowPRE ($P = 0.002$), ArrowPRE + CallistoPOST ($P = 0.008$), and the WaterPRE control ($P = 0.016$) (Figure 12).

The number of remaining weeds were measured on May 18th and June 13th. These dates had to be analyzed separately due to the number of weeds decreasing in some treatments from May to June ($P < 0.001$). On May 18th, weed presence was the lowest in the ZeusPrimePRE + ArrowPRE (Mean = 0.210) when compared to the WaterPRE control ($P = 0.036$) and CallistoPOST + CallistoPOST ($P = 0.045$) (Figure 13). By June 18th, there were no significant differences detected between treatments and the majority of weeds decreased (Figure 13).

Red sorrel presence was analyzed separately because it was a predominant weed from the prune year (2022). Both sample dates needed to be separated due to a significant interaction between sample date and treatment ($P < 0.001$). There were no significant differences detected between treatments on May 18th or June 13th (Figure 14).

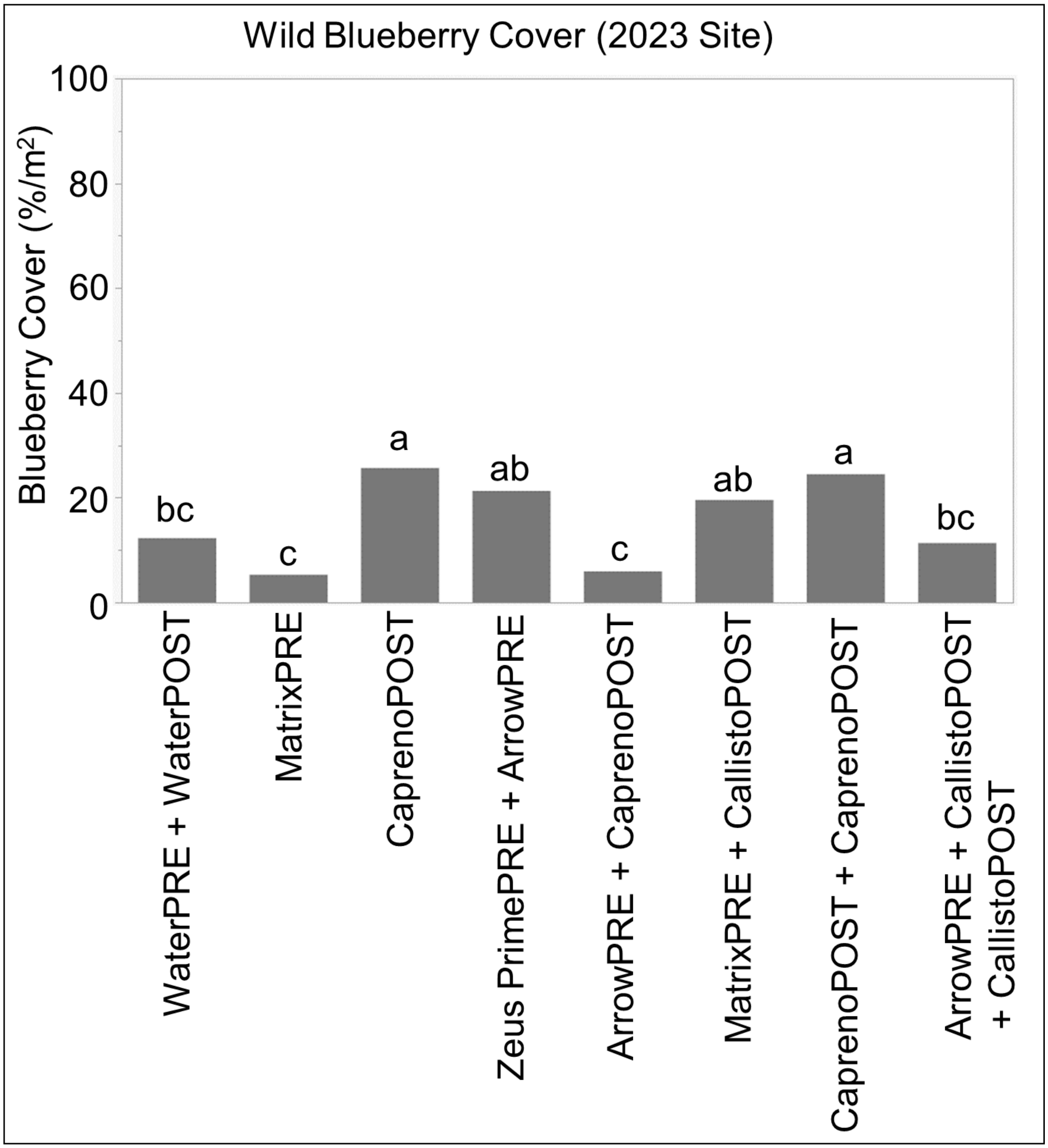

Figure 1. Average wild blueberry cover measured in herbicide treatments on May 18th, June 13th, and July 7th, 2023, at Blueberry Hill Research Station, Jonesboro, Maine. All treatments except the control had LI700 surfactant added to application. Back-transformed means are shown from a cube root transformation. Means sharing letters are not statistically different using a Tukey's HSD test ($\alpha \leq 0.05$).

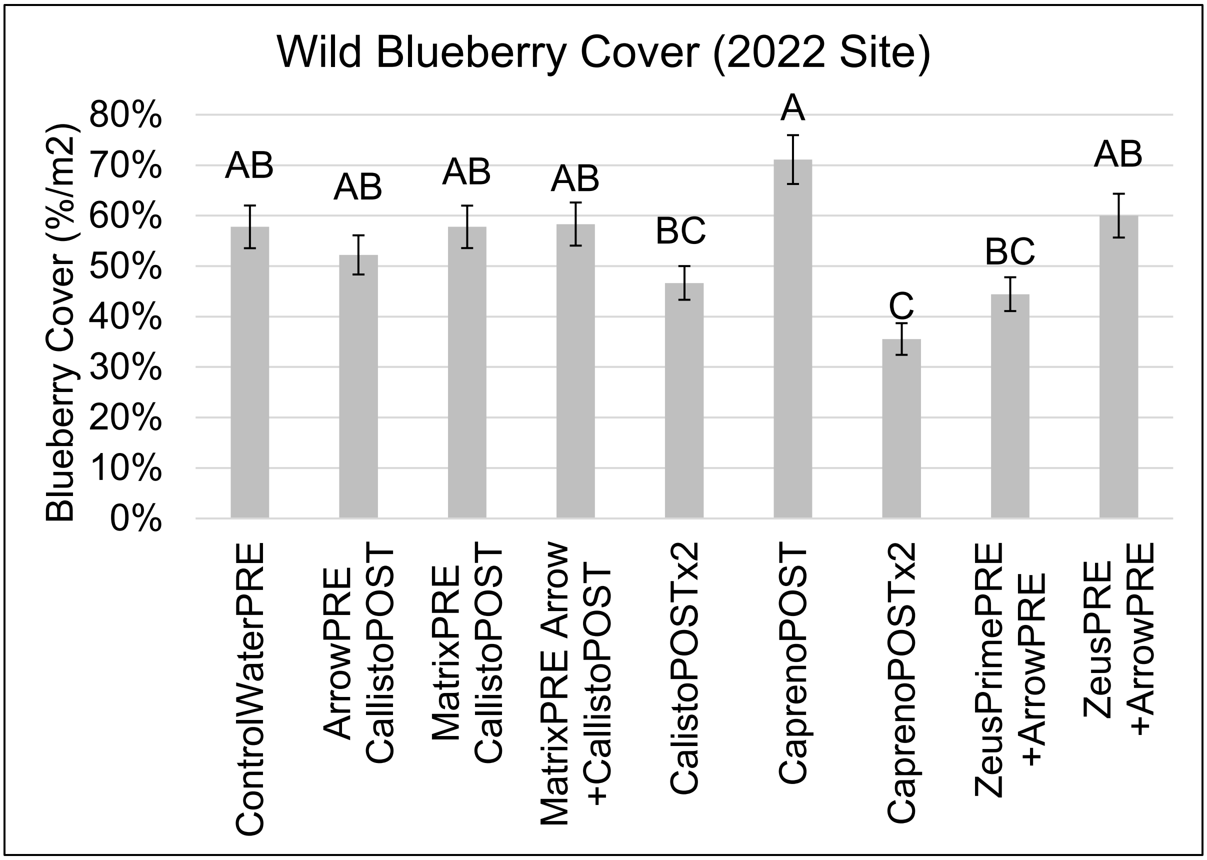

Figure 2. Average blueberry cover (%/m^2) measured in herbicide treatments on June 1st, July 7th and August 25th, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Means sharing letters are not statistically different using a Tukey's HSD test ($\alpha \leq 0.05$). Error bars represent the standard error of the mean. Data were transformed using a square root transformation for analysis, untransformed data is presented above for readability.

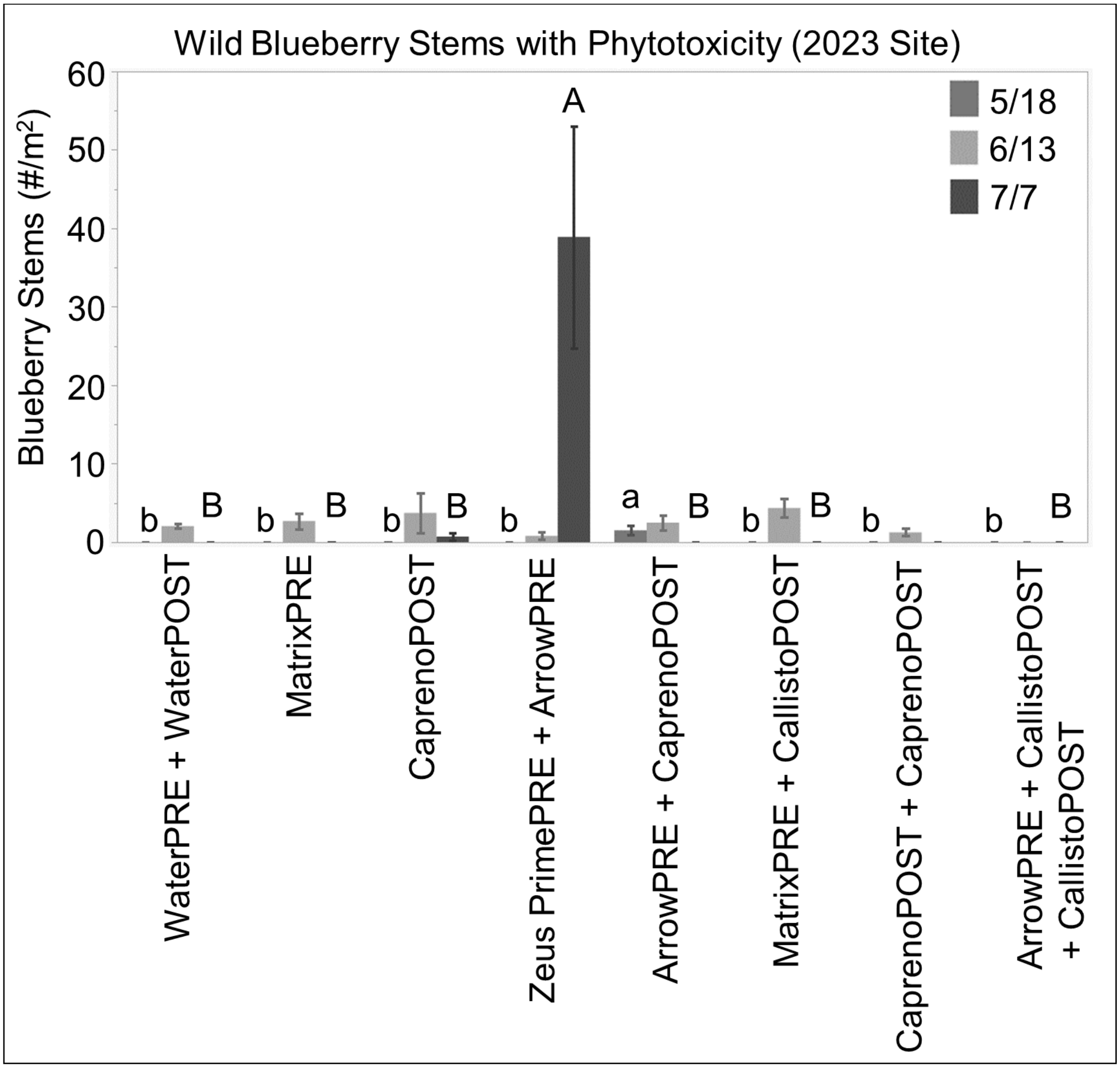

Figure 3. Average wild blueberry stems with phytotoxicity measured in herbicide treatments on May 18th, June 13th, and July 7th, 2023, at Blueberry Hill Research Station, Jonesboro, Maine. All treatments except the control had LI700 surfactant added to application. Means sharing letters are not statistically different using a Tukey's HSD test (α ≤ 0.05). Treatments were not significantly different on June 13th.

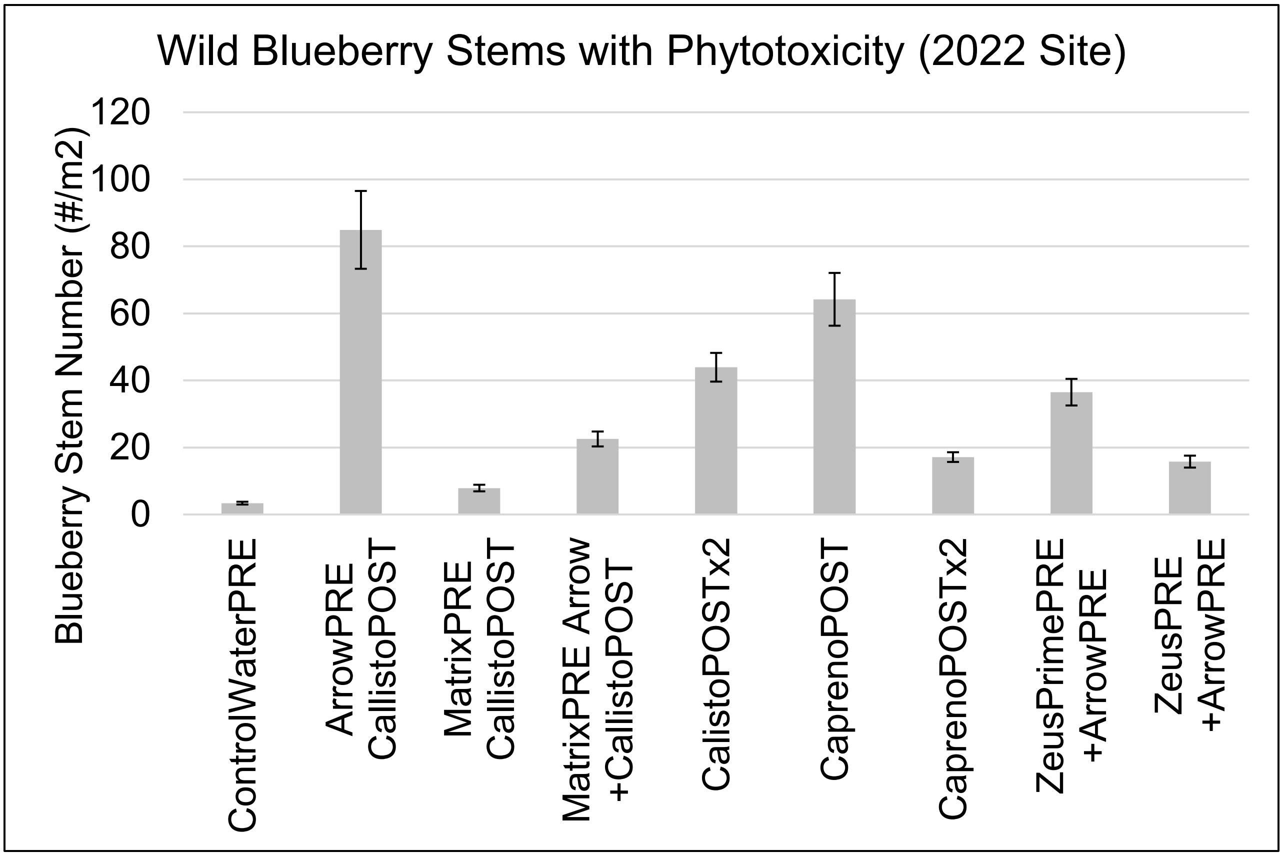

Figure 4. Average blueberry stems with phytotoxicity (#/m^2) measured in herbicide treatments on June 1st and July 7th, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Data collected on August 25th was not included because it was late in the season. Treatment differences were not significant. Error bars represent the standard error of the mean.

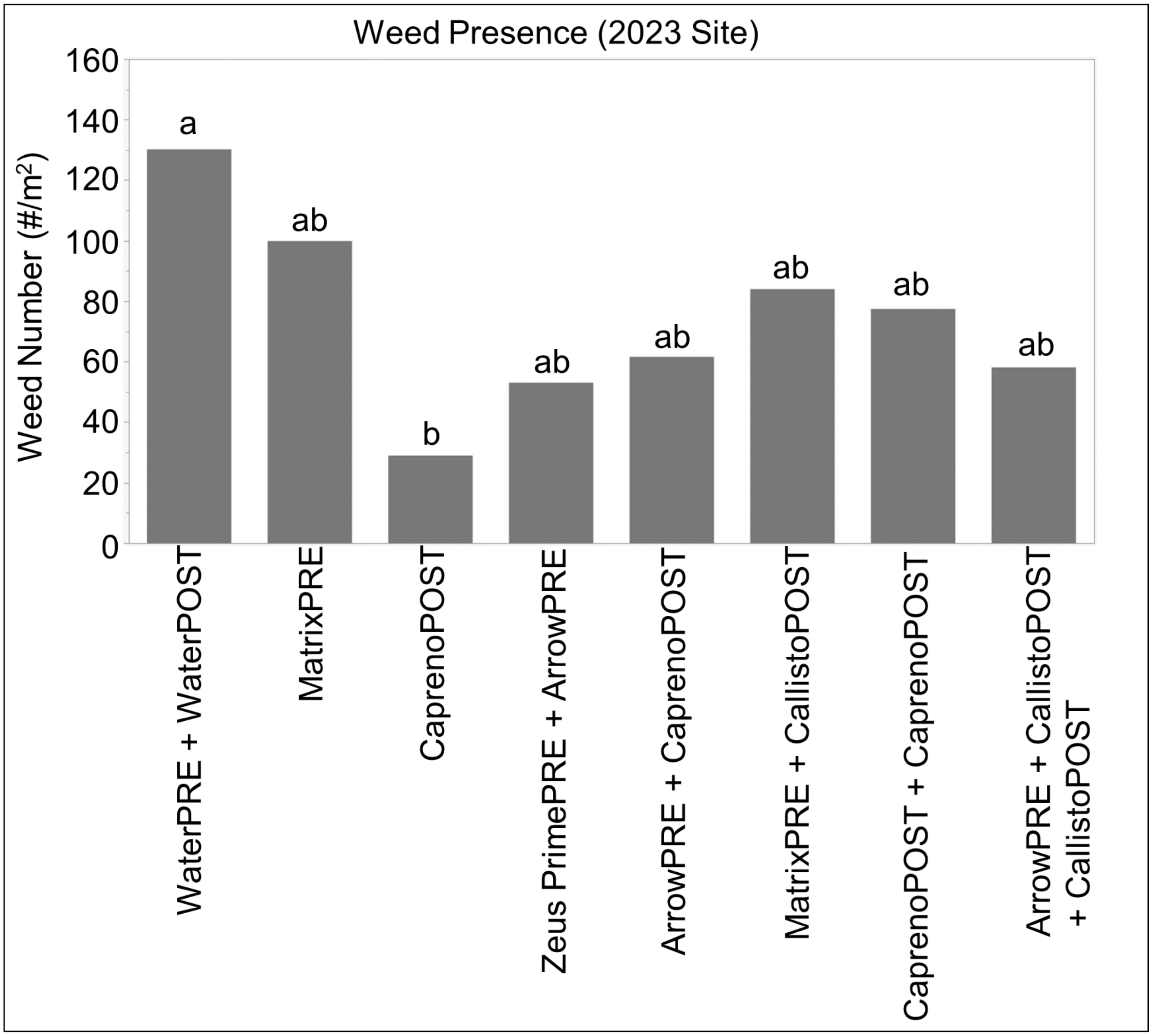

Figure 5. Average wild blueberry stems with phytotoxicity measured in herbicide treatments on May 18th, June 13th, and July 7th, 2023, at Blueberry Hill Research Station, Jonesboro, Maine. All treatments except the control had LI700 surfactant added to application. Back-transformed means from a cube root transformation are presented. Means sharing letters are not statistically different using a Tukey's HSD test (α ≤ 0.05).

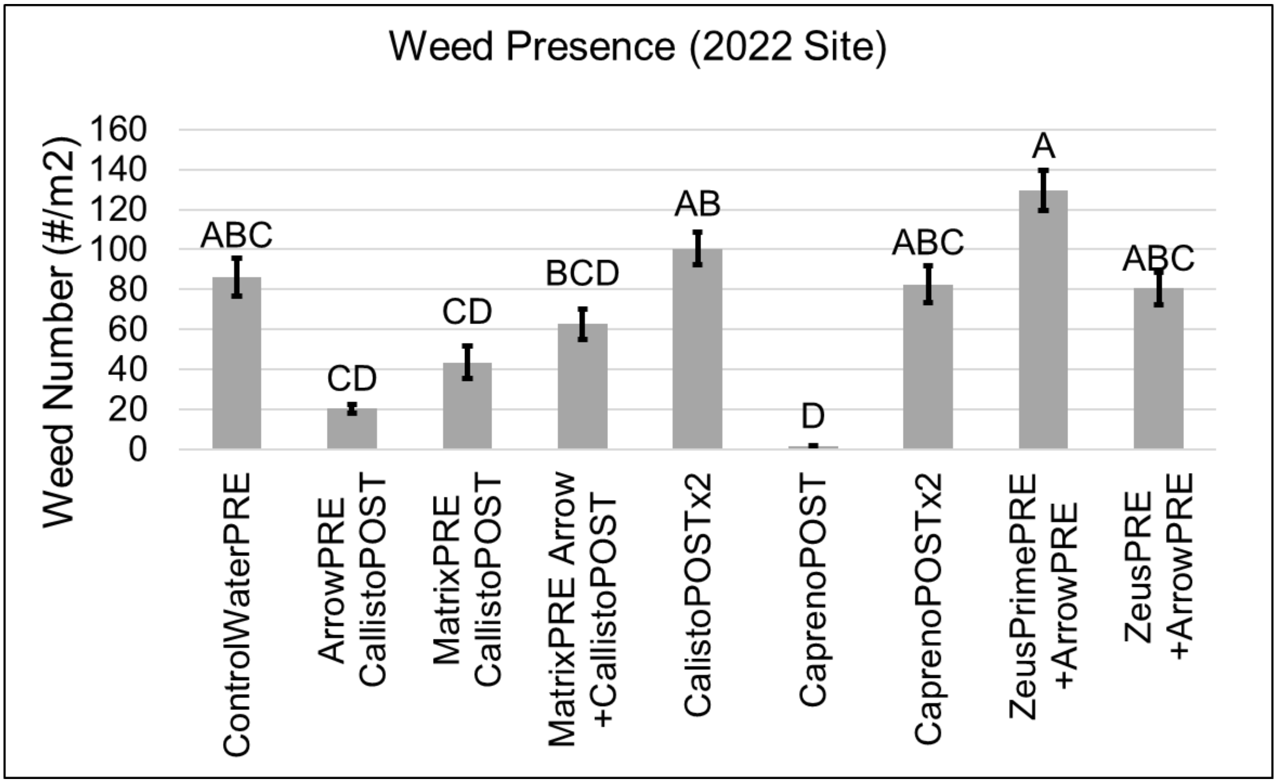

Figure 6. Average weed number (#/m^2) measured in herbicide treatments on June 1st, July 7th, and August 25th, 2022 at Blueberry Hill Farm, Jonesboro Maine. Means sharing letters are not statistically different using a Tukey's HSD test ($\alpha \leq 0.05$). Error bars represent the standard error of the mean. Data were transformed using a square root transformation for analysis, untransformed data is presented above for readability.

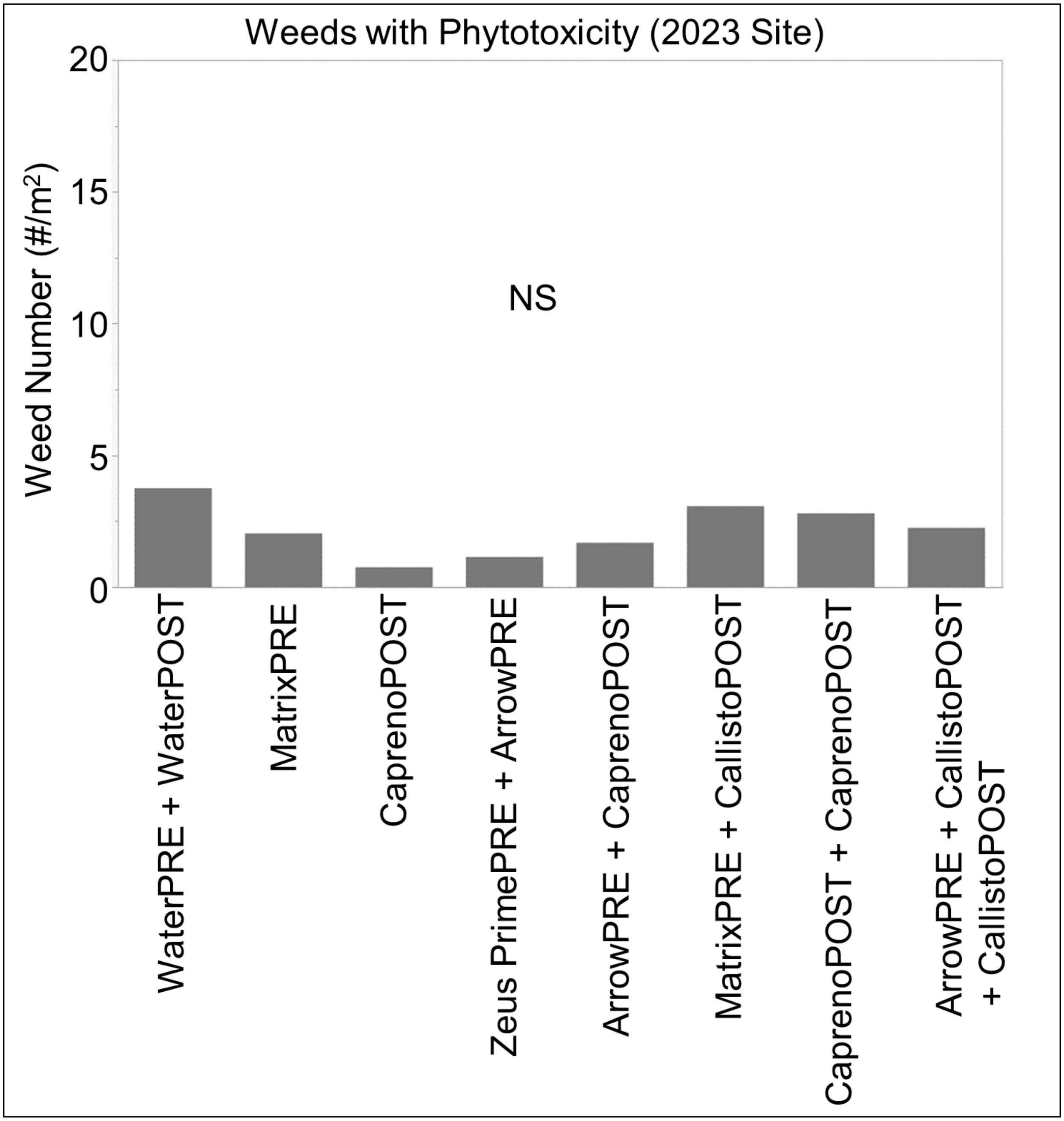

Figure 7. Number of weeds with phytotoxicity measured in herbicide treatments on May 18th, June 13th, and July 7th, 2023, at Blueberry Hill Research Station, Jonesboro, Maine. All treatments except the control had LI700 surfactant added to application. Back-transformed means from a cube root transformation are presented. No significant differences detected (NS).

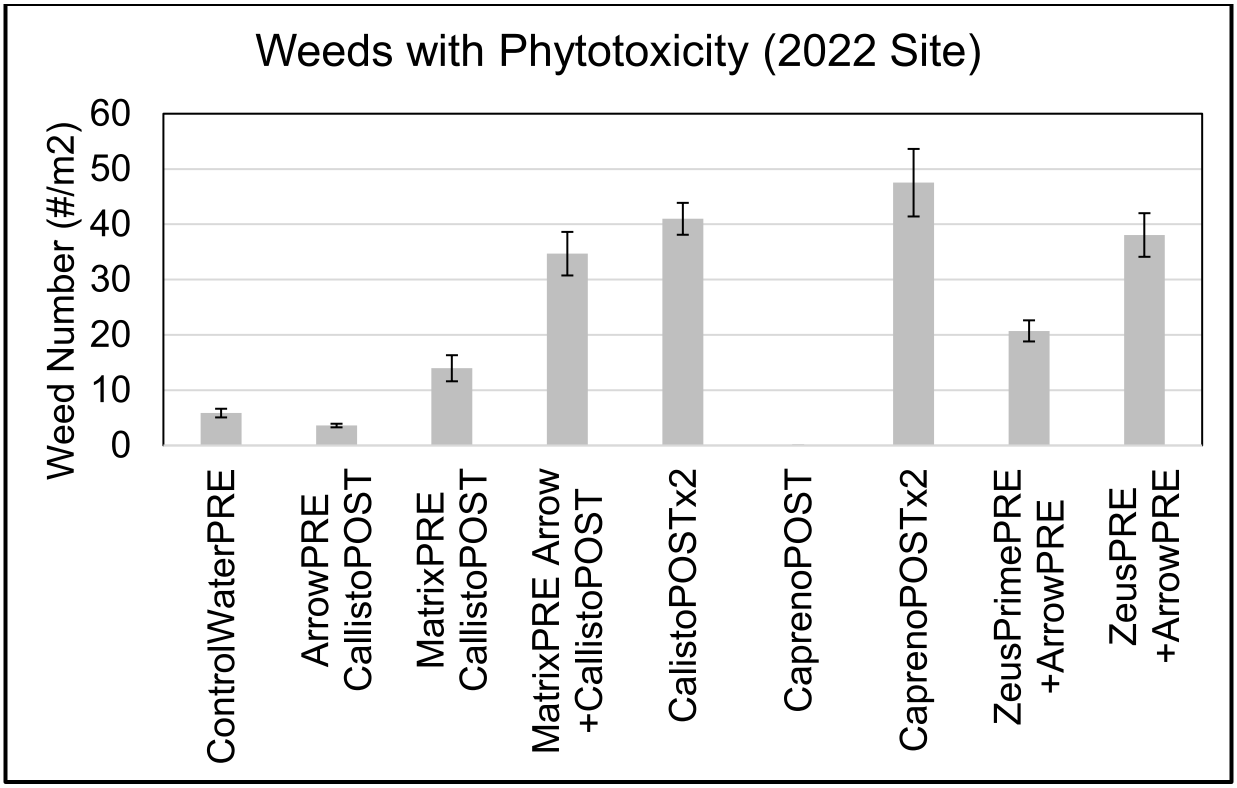

Figure 8. Average weeds with phytotoxicity (#/m^2) measured in herbicide treatments on June 1st and July 7th, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. Treatment differences were not significant. Error bars represent the standard error of the mean.

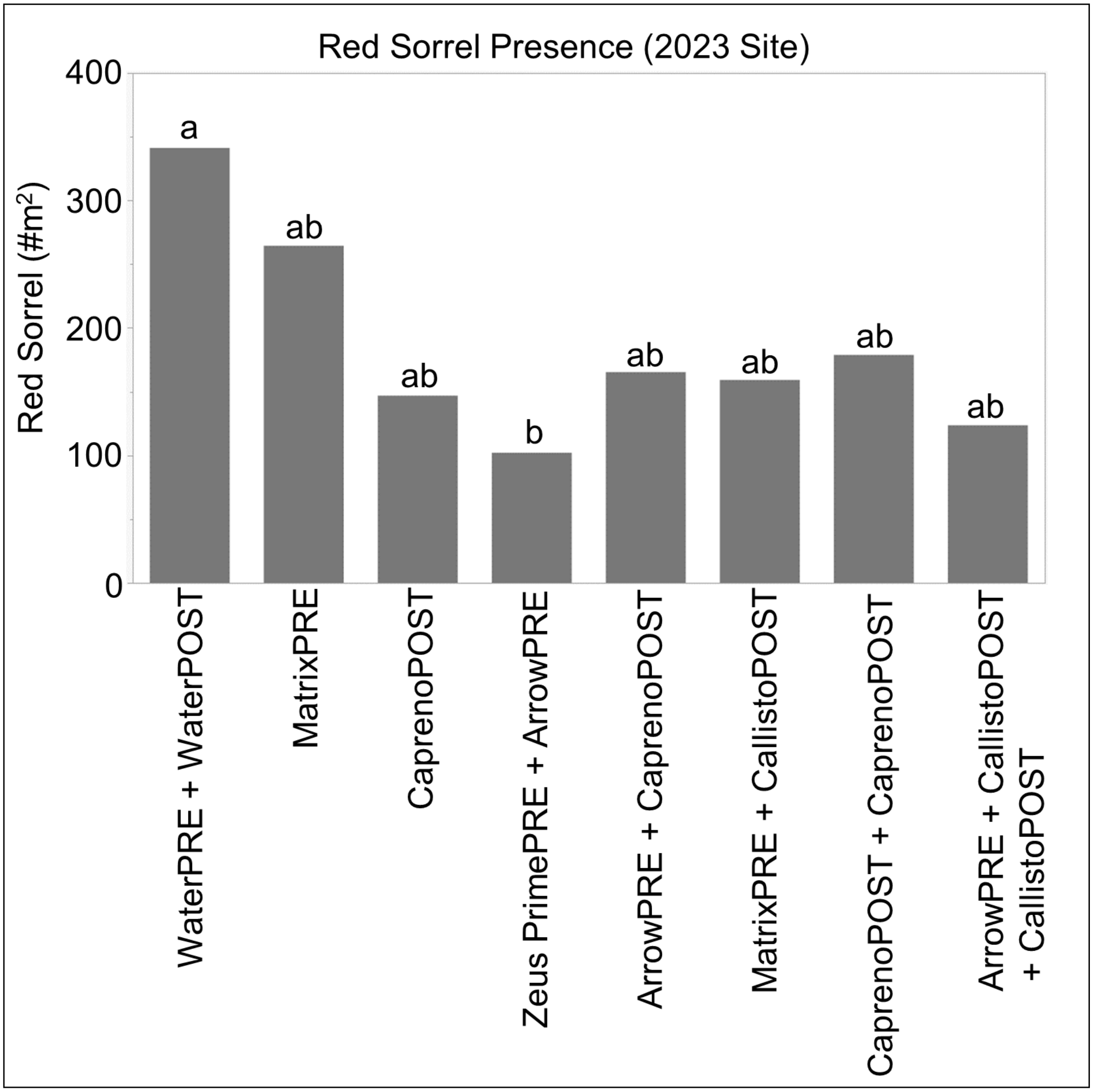

Figure 9. Number of remaining red sorrel (*Rumex acetosella*) measured in herbicide treatments on May 18th, June 13th, and July 7th, 2023, at Blueberry Hill Research Station, Jonesboro, Maine. Back-transformed means are shown from a cube root transformation. Means sharing letters are not statistically different using a Tukey's HSD test (α ≤ 0.05).

Table 4. 2023 average total weeds present (#/m^2) with the average percent of weeds with phytotoxicity in parentheses by herbicide treatment and weed species for May 18th, June 13th, and July 7th, 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 Number of Weeds and total with Phytotoxicity (%) per m^2		**WaterPRE + WaterPOST (Control) 297.5 (11%)**	**MatrixPRE 235.5 (6%)**	**CaprenoPOST 66.7 (14%)**	**Zeus PrimePRE + ArrowPRE 104.3 (11%)**	**ArrowPRE + CaprenoPOST 148.1 (16%)**	**MatrixPRE + CallistoPOST 176.6 (12%)**	**CaprenoPOST + CaprenoPOST 169.6 (11%)**	**ArrowPRE + CallistoPOST + CallistoPOST 119.4 (13%)**
Weed Common Name	Life Cycle								
Aronia	**W**	**0.0**	**34.2 (5%)**	**0.0**	**0.0**	**82.3 (0%)**	**0.0**	**0.0**	**180.2 (43%)**
Bladder Campion	P	2.7 (0%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Braken Fern	**P**	**0.0**	**0.0**	**31.5 (31%)**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Bramble	**W**	**0.0**	**0.0**	**0.0**	**2.7 (33%)**	**0.0**	**0.0**	**0.0**	**0.0**
Dogbane	**W**	**0.0**	**0.0**	**10.6 (9%)**	**2.7 (0%)**	**5.9 (15%)**	**0.0**	**11.8 (11%)**	**6.3 (29%)**
Downy Violet	P	0.0	2.7 (0%)	0.0	0.0	0.0	0.0	0.0	0.0
Goldenrod	**P**	**0.0**	**0.0**	**0.0**	**6.3 (0%)**	**12.2 (89%)**	**0.0**	**0.0**	**0.0**
Grass	**G**	**124.3 (15%)**	**549.5 (18%)**	**0.0**	**159.7 (16%)**	**106.3 (29%)**	**287.1 (6%)**	**38.7 (70%)**	**194.0 (5%)**
Grassleaf Goldenrod	P	0.0	2.7 (0%)	0.0	0.0	0.0	0.0	0.0	0.0
Hares Foot Clover	A	2.7 (0%)	0.0	0.0	0.0	89.2 (0%)	0.0	0.0	0.0
Honeysuckle	P	0.0	0.0	0.0	0.0	98.6 (0%)	0.0	0.0	0.0
Loosestrife	P	0.0	64.9 (0%)	0.0	0.0	27.0 (0%)	0.0	0.0	0.0
Moss	P	2.7 (0%)	2.7 (0%)	2.7 (0%)	2.7 (0%)	2.7 (0%)	0.0	2.7 (0%)	2.7 (0%)
Narrow Leaf Goldenrod	P	0.0	2.7 (0%)	0.0	0.0	0.0	0.0	0.0	0.0
Orangegrass	A	0.0	0.0	0.0	10.8 (0%)	0.0	0.0	0.0	0.0
Purple Violet	A	0.0	0.0	0.0	0.0	0.0	0.0	10.8 (0%)	0.0
Red Sorrel	**P**	**407.1 (18%)**	**383.3 (5%)**	**178.8 (14%)**	**130.3 (10%)**	**201.0 (29%)**	**222.8 (18%)**	**232.3 (14%)**	**179.8 (6%)**
Rush	G	2.7 (0%)	0.0	2.7 (0%)	4.5 (0%)	12.6 (0%)	0.0	2.7 (0%)	0.0
Sedge	**G**	**0.0**	**0.0**	**2.7 (0%)**	**8.1 (33%)**	**17.1 (0%)**	**0.0**	**0.0**	**17.6 (65%)**
St John's-wort	**P**	**2.7 (0%)**	**2.7 (0%)**	**0.0**	**0.0**	**8.1 (0%)**	**7.2 (0%)**	**5.4 (0%)**	**8.1 (33%)**
Toadflax	**P**	**464.9 (0%)**	**259.8 (1%)**	**9.0 (0%)**	**190.5 (9%)**	**455.1 (4%)**	**73.0 (0%)**	**444.7 (4%)**	**29.7 (5%)**
Whorled Loosestrife	**P**	**0.0**	**0.0**	**0.0**	**0.0**	**32.4 (100%)**	**0.0**	**67.6 (96%)**	**0.0**

Table 5. Average total weeds present (#/m^2) with the average percent of weeds with phytotoxicity in parentheses by herbicide treatment and weed species for June 1st and July 7th, 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	ArrowPRE CallistoPOST	MatrixPRE CallistoPOST	MatrixPRE Arrow+ CallistoPOST	Callisto POSTx2	Capreno POST	Capreno POSTx2	ZeusPrimePRE ArrowPRE	Zeus PRE ArrowPRE
		98.6 (6%)	**19.6 (18%)**	**45.0 (31%)**	**79.3 (44%)**	**121.2 (34%)**	**2.0 (0%)**	**73.2 (65%)**	**112.8 (18%)**	**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**

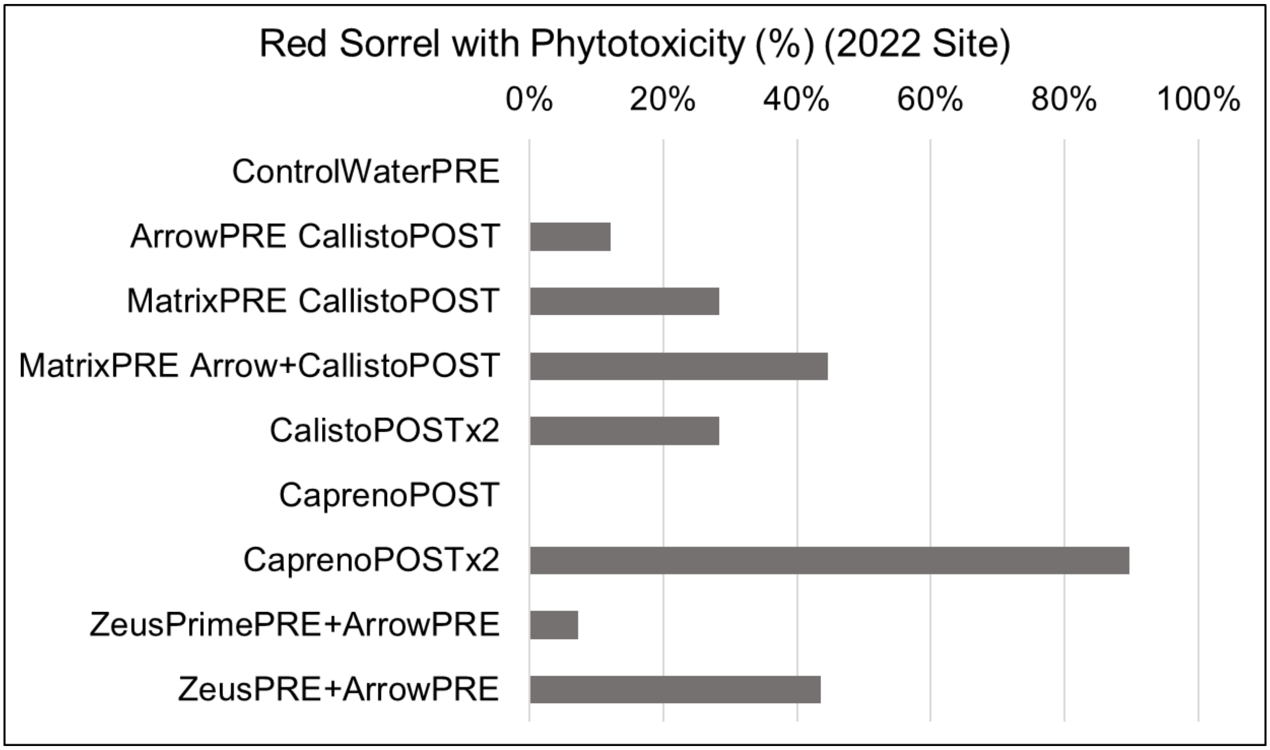

Figure 10. 2022 average percent of red sorrel (*Rumex acetosella*) with phytotoxicity (%/total red sorrel present) measured in herbicide treatments on June 1st and July 7th, 2022, at Blueberry Hill Research Station, Jonesboro, Maine.

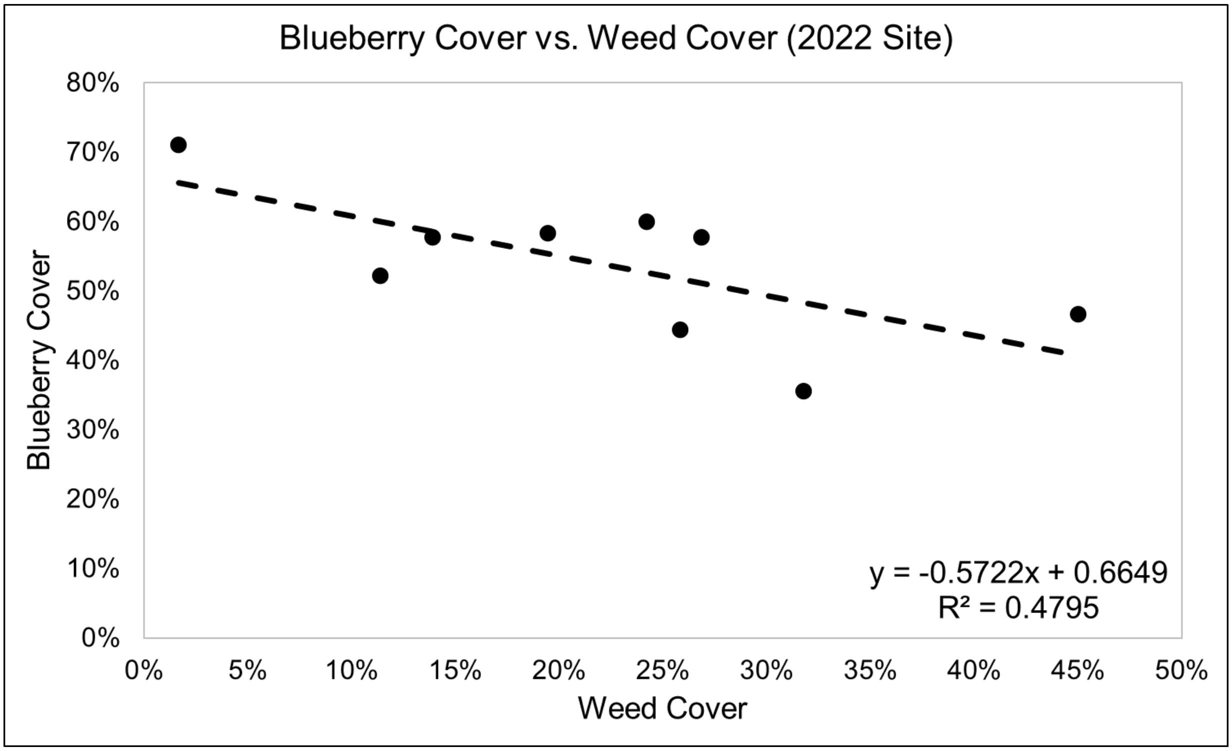

Figure 11. 2022 relationship between blueberry cover and weed cover in herbicide treatments on June 1st, July 7th, and August 25th, 2022, at Blueberry Hill Research Station, Jonesboro, Maine. The trendline exhibits the negative linear relationship between blueberry density and weed density where higher blueberry cover corresponds with lower weed cover and vice versa.

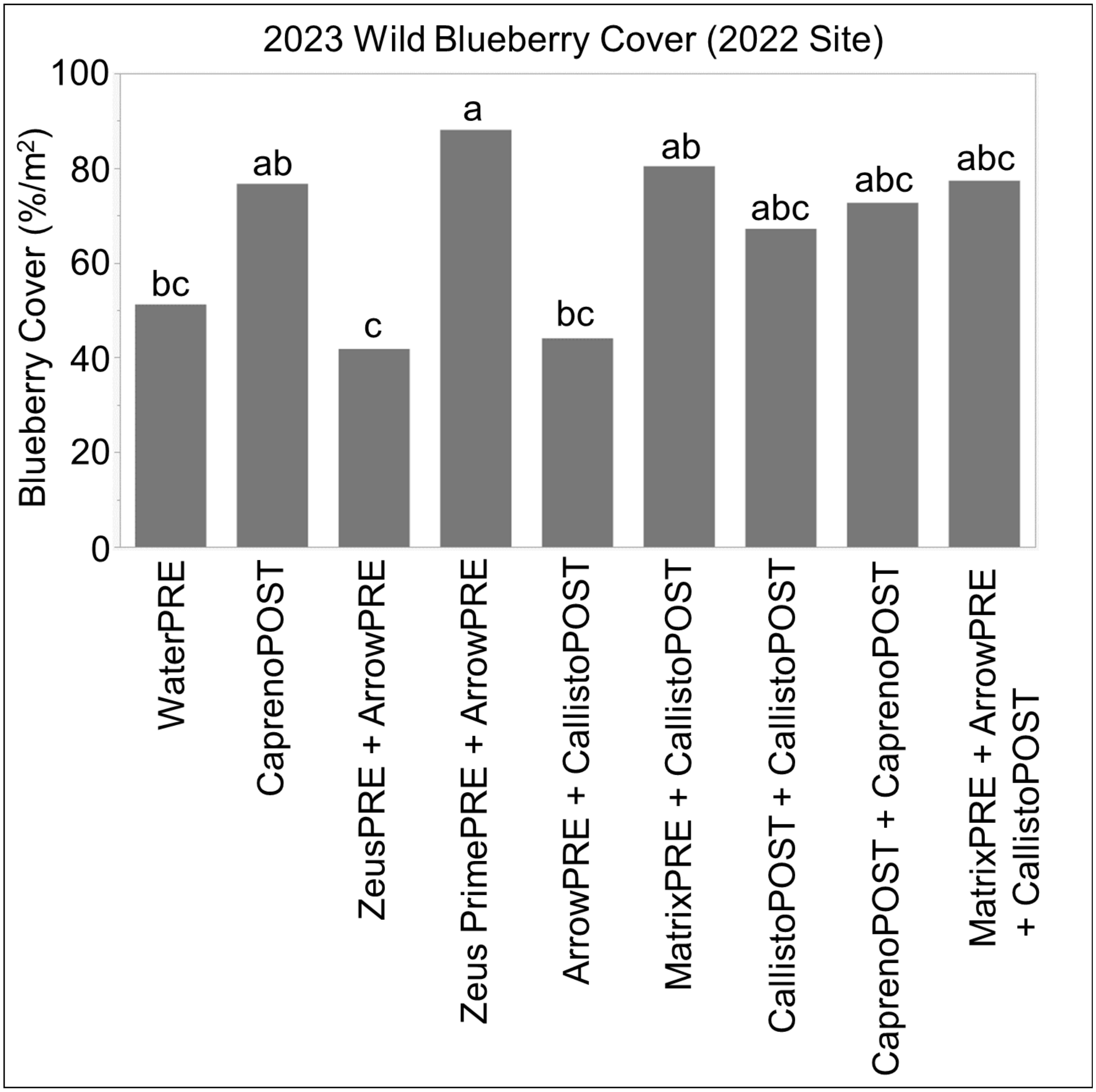

Figure 12. Average wild blueberry cover, in crop cycle, measured in herbicide treatments on May 18th and June 13th, 2023, at Blueberry Hill Research Station, Jonesboro, Maine. Back-transformed means are shown from a logit transformation. Means sharing letters are not statistically different using a Tukey's HSD test (α ≤ 0.05).

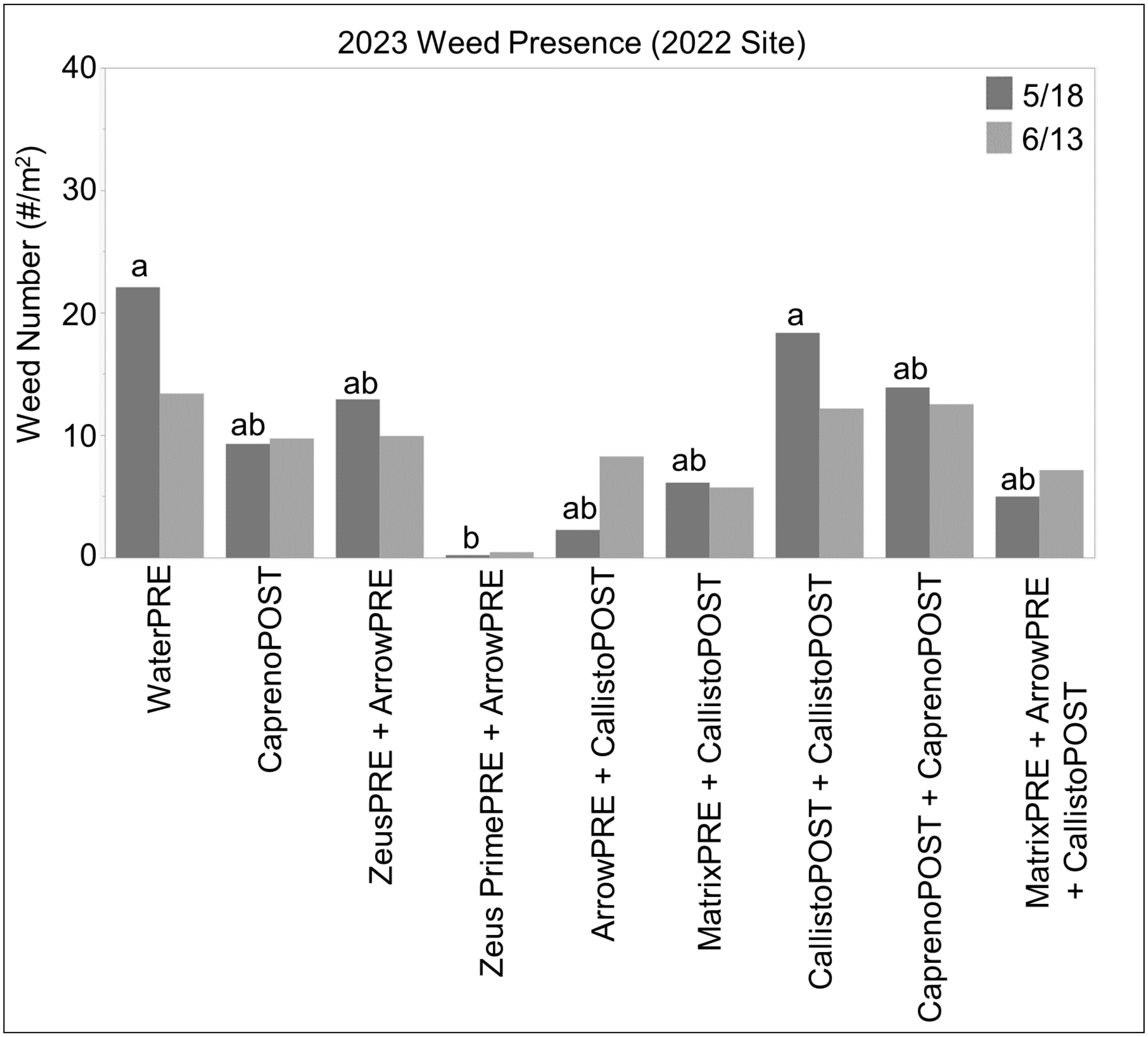

Figure 13. Number of remaining weeds measured in herbicide treatments on May 18th and June 13th, 2023, at Blueberry Hill Research Station, Jonesboro, Maine. Back-transformed means are presented from a cube root (5/18) and ln(x+1) (6/13) transformations. Means sharing letters are not statistically different using a Tukey's HSD test ($\alpha \leq 0.05$). No significance was detected on 6/13.

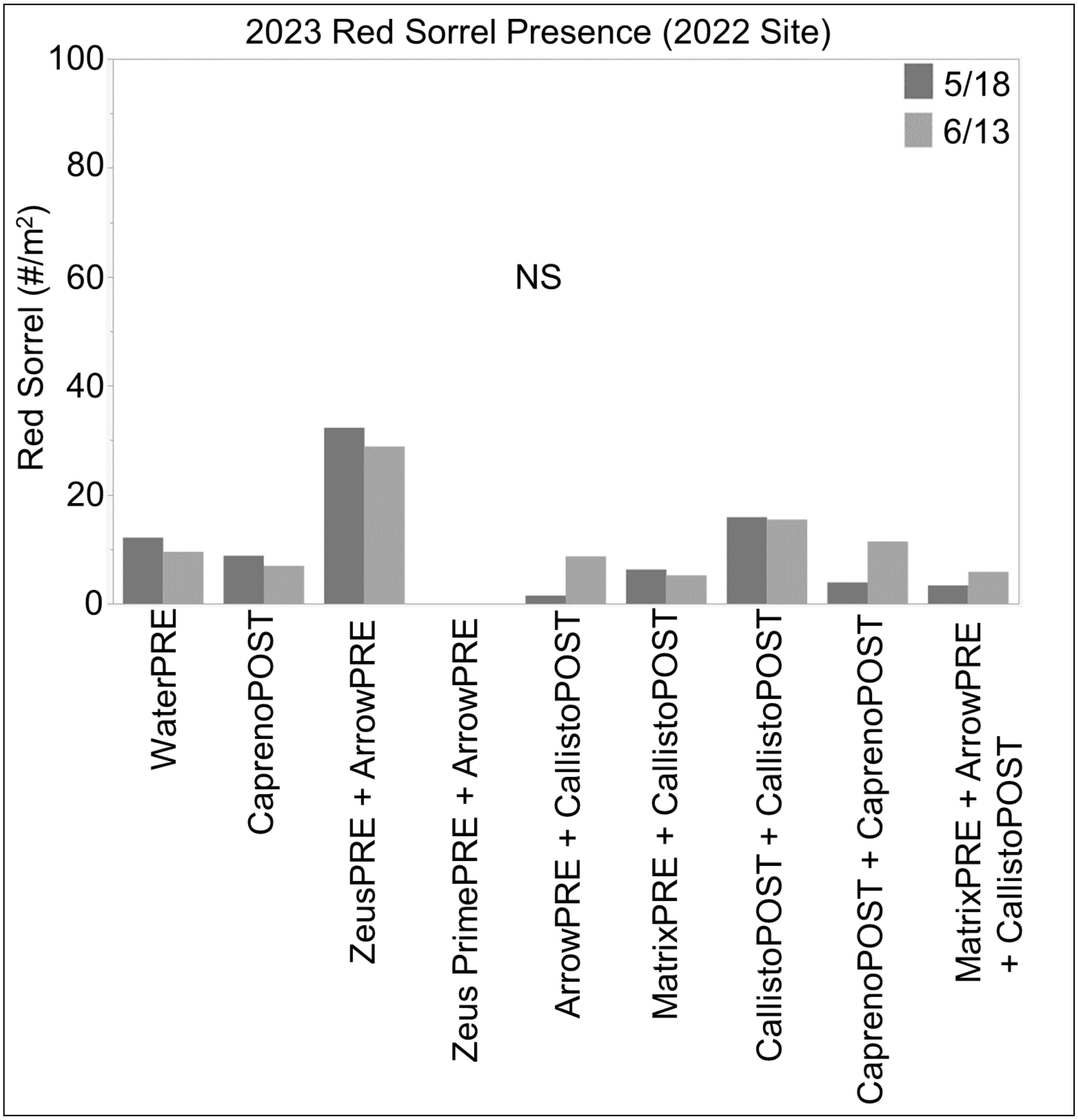

Figure 14. Number of remaining red sorrel (*Rumex acetosella*) measured in herbicide treatments on May 18th and June 13th, 2023, at Blueberry Hill Research Station, Jonesboro, Maine. Back-transformed means are shown for a cube root (5/18) and ln(x+1) (6/13) transformations. No significant differences were detected (NS).

DISCUSSION

Blueberry cover in at the 2023 site which had herbicide applied this year, ranged from 15 to 20%, which was much lower than the 2022 site with a range of 50 to 60% (Figure 1 and 2). These two sites were in different fields with varying spatial and plant variability due to the 2023 site being land-leveled in 2020/2021. Treatments CaprenoPOST and CaprenoPOST + CaprenoPOST, both had low numbers of remaining weeds, which lowers competition and increases the amount of water and nutrients available to the blueberry in this location for years to come (Figures 1 and 5). The CaprenoPOST treatment at the 2022 site, also had the greatest blueberry cover and the lowest number of remaining weeds (Figure 2 and 6). Capreno treatments also exhibited low phytotoxicity to the blueberry and therefore we will pursue a label for use of this product on lowbush blueberry.

Phytotoxicity at the 2023 site was low on blueberry plants for all treatments for May 18th and June 13th but on July 7th. The ZeusPrimePRE + ArrowPRE exhibited a large amount of damage (Figure 3) likely because ZeusPrime was applied too late this season when blueberry plants had come too close to the soil surface or already emerged in some locations. When using Zeus or Zeus Prime be sure to apply it as a true pre-emergent product. At the 2022 site, in the prune 2022 year, ArrowPRE + CallistoPOST had the greatest amount of phytotoxicity (85 stems m^{-2}) although it was not significantly different from other treatments (Figure 4). In both years phytotoxicity was seen in the control application (2 stems m^{-2} in 2023 and 3 stems m^{-2} in 2022). It is possible that treatments were subjected to herbicide drift or residual effects of herbicides applied in earlier field applications at the site or nearby (Figure 3 and 4).

The number of remaining weeds in the 2023 prune field was the greatest in the WaterPRE + WaterPOST control, which is expected since no weed control was implemented (Figure 5). There were no detectable differences between treatments, except for comparing the CaprenoPOST to the control (Figure 5). In the CaprenoPOST treatment, 7 out of 22 weed species were found in the treatment area and only 3 species exhibited phytotoxicity (Table 4). This can be due to the way weeds establish in an area, it is often patchy, with variable densities which can increase standard error in treatments. The treatment with the best broad-spectrum effects was the ArrowPRE + CallistoPOST + CallistoPOST which was able to cause phytotoxicity across 7 out of 8 weed species in the experimental area (Table 4). Another treatment with good broad-spectrum effects was the ArrowPRE + CaprenoPOST which also caused the highest percentage of phytotoxicity (Table 4).

At the 2022 site in 2022, CaprenoPOST had the lowest remaining weeds, but within the treatment area there were two sedge plants, neither of which showed phytotoxicity (Figure 6 and 8; Table 5). The location was chosen for easy access during field days, which meant that fewer weeds were present. Without a big enough sample size, it is difficult to draw conclusions from this application. Another factor contributing to the absence of weeds in particular treatments was likely due to the spatial variation in weed communities. The experimental CaprenoPOSTx2 treatment demonstrated substantial weed control, where 48 of the 72 weeds per m^{-2} (65%) demonstrated phytotoxicity (Table 5). The CallistoPOSTx2 demonstrated decent weed control as well, with 41 out of 121 weeds/m^{-2} (34%) exhibiting phytotoxicity (Table 5). Of the weeds controlled, red sorrel and bunchberry (*Cornus canadensis*) were among the most common to exhibit phytotoxicity. Weeds after treatment by CaprenoPOSTx2 exhibited the most phytotoxicity, particularly chokecherry *(Prunus virginiana*)*,* goldenrod *(Solidago* spp.)*,* meadowsweet *(Filipendula ulmaria*)*,* 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 more broad (one broadleaf annual, one woody broadleaf perennial, one annual/perennial grass, and three broadleaf perennials).

At the 2023 site in 2023, the most abundant weed was red sorrel. Large numbers of this weed in blueberry fields can decrease yields, attract pests, and increase incidences of *Botryis cinerea* infection if the red sorrel pollen gets into the flowers during bloom (Hughes et al., 2016). Red sorrel is also problematic due to its growth habit. It has been observed that when hexazinone effects decrease, the plant is able to grow back and produce many seeds (McCully et al., 1991). The treatment with the greatest phytotoxicity (29%) and least amount of red sorrel (102 weeds m^{-2}) was the ZeusPrimePRE + ArrowPRE (Figure 9; Table 4). The next best treatment for red sorrel was the MatrixPRE + CallistoPOST with a phytotoxicity of 18% (Table 4). In 2022, the best treatment for control of red sorrel was CaprenoPOSTx2 (90%) (Figure 10). Then second best with phytotoxicity was the MatrixPRE + ArrowPRE + CallistoPOST (45%) (Figure 10).

CURRENT RECOMMENDATIONS

- We recommend herbicide stacking with all products tested at appropriate timings as described **EXCEPT for Capreno which is not labeled for use in wild blueberry yet.**
- Be cautious to apply Zeus and ZeusPrime as truly pre-emergent products. Phytotoxicity to blueberry was observed.

NEXT STEPS

- Conduct a similar 2024 herbicide stacking demonstration on the lower field at Blueberry Hill Farm to control fine leaf sheep fescue (*Festuca filiformis*).
- Continue gathering data to determine if herbicide applications reduce shoot density in future seasons instead of only causing phytotoxicity damage.

ACKNOWLEDGEMENTS

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1. Research into the Management of Leaf Spot Diseases in Wild Blueberry in 2023

INVESTIGATORS: S. Annis, M. Roberts, Z. Colwell, and K. Bagale

OBJECTIVES

- Improve identification and control of leaf spots.
- 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*)
- Develop a DNA-based method for detecting *Sphaerulina vaccinii* infected leaves and spores
- Test fungicide and fertilizer interactions and impacts on plant physiology while managing *Sphaerulina vaccinii*

LOCATIONS: Waldo, Lincoln, Knox, Hancock and Washington Counties, Maine

PROJECT TIMEFRAME: January 2022 – August 2023

INTRODUCTION

Leaf spot diseases, Sphaerulina leaf spot, leaf rust, and powdery mildew, are present in the majority of wild blueberry fields surveyed, but the severity of effects of these diseases on yield is unclear. There is typically a complex of diseases affecting leaves that vary in their severity depending upon the year so it is difficult to determine which disease or combination of diseases is causing the most damage to the plants. More information on when initial inoculum is produced, what conditions are necessary for infection, and which disease are having a greater impact on the plants will allow for development of improved control measures for the most damaging of these diseases. A more precise timing of fungicide applications coordinating with initial spore release of key fungi, such as those causing Sphaerulina leaf spot and leaf rust, will improve control of these diseases. There is also little data on how fertilizers interact with fungicides on incidence and severity of leaf spots. Fertilizers may increase disease by producing more young or nutritious tissues for the fungus to attack and live on, or may improve plant health to ward off disease. The use of fungicides with fertilizers may help decrease any negative effects that the diseases may cause. Finally, new materials to control leaf spots need to be tested incase current materials are no longer available or become unacceptable in some markets.

METHODS

Survey of weather and levels of disease in wild blueberry fields

Thirteen fields with weather stations were rated for leaf spot diseases between October 3rd and 12th, 2023. Four plots of 0.25 m^2 were randomly selected and rated by one surveyor visually estimating percentages of disease coverage on blueberry leaves for the following diseases: *Sphaerulina* leaf spot, powdery mildew, and leaf rust. Disease ratings were averaged across the four sampling plots within the field. Rating data from 2021 and 2023 were compared using paired two-sample t-tests for means in Excel.

Identification of Sphaerulina leaf spot using molecular methods

Zoe Colwell, MS student. DNA was extracted from fungal isolates collected from leaf lesions. *Sphaerulina*-specific primers were identified and tested. Spore traps for water-dispersed spores were placed in six wild blueberry fields in late May to June. Traps were collected weekly and replaced until late-stage lesions were observed on the foliage. DNA was extracted from spore trap samples, and single leaves with Sphaerulina leaf spot symptoms and then tested with the *Sphaerulina*-specific primers and leaf rust fungus *Thekopsora minima* specific primers developed by grad student Nghi Nguyen in 2019.

Spore dispersal and leaf spot diseases

On May 8th, 2023, a vacuum spore trap was placed in a prune field at Blueberry Hill Research Farm (BHF) in Jonesboro, ME. The spore trap tapes were collected weekly until October 5th, 2023. Spore trap tapes were cut in half; half was frozen for future DNA work, and the other half was mounted on glass slides for examining spores. Tapes will be used to gather data on spore release timing for leaf spot diseases.

Fungicide and fertilizer trial

Keshav Bagale, PhD student. To study the interaction of fertilizers and fungicide applications on leaf spots, a randomized block design experiment was established in a vegetative lowbush blueberry field where high levels of leaf spots had been previously reported at BHF. Treatments were randomly assigned to 6 ft x 15 ft plots with a 3 ft buffer lane between each plot across 12 blocks (Table 1). Treatments consisted of applications of granular fertilizer (ammonium sulfate), and foliar fertilizer (Maximum N-pact K, Loveland Products, Loveland, CO, USA) in different combinations. Half of the blocks were treated with Proline fungicide (Bayer Corporation, Whippany, NJ, USA) to test fungicide effects on fertilizer treatments. Proline and the foliar fertilizer, Maximum N-pact K (Loveland Products, Loveland, CO, USA) were applied at 35 psi with a CO_2 backpack sprayer equipped with a 4-nozzle boom, 8002VS T Jet tips, and 50-mesh screens applied.

Disease symptoms and leaf loss were rated two times: August 21st and September 19th, 2023. 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. Rating occurred in the 3 to 5 days after harvest. Out of the 20 stems collected, 15 stems were rated for the proportion of leaf cover with disease symptoms of *Sphaerulina* leaf spot, leaf rust, or powdery mildew. Leaves and bare nodes were counted for leaf loss. The remaining 5 stems were photographed to be used for developing image-based disease quantification in the program ImageJ.

Table 1. Treatments employed in fungicide and fertilizer trial.

Treatment	Application Rate	Application Timing
Ammonium Sulfate	250 lb/acre	May 15, 2023
Proline	5.7 fl oz/acre	June 23, 2023
Maximum N-pact K	2 qt/acre	July 28, 2023

Fungicide efficacy trial to manage leaf spots

To test new materials for their efficacy in controlling leaf spots, a completely randomized design experiment was established in a vegetative lowbush blueberry field where high levels of leaf spots had been previously reported at the BHF. Fungicides were completely randomly assigned to 6 ft x 15 ft plots with a 3 ft buffer lane between each plot with eight repetitions (Table 2). Plots were treated with fungicides on June 23rd or 30th to test “Early” and “Late” applications, respectively. Proline, Luna Sensation, Luna Tranquility, and Propulse were all applied with the adjuvant DyneAmic (Helena Agri-Enterprises,LLC, Collierville, TN, USA) at 0.12% final concentration. Ecoswing was applied with the adjuvant Capsil (Aquatrols, Paulsboro, NJ, USA) at 0.06 fl oz/acre. 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. Negative control plots received no spray applications. Disease symptoms were rated two times: July 31st and September 12th, 2023. A rope with 20 evenly spaced markings was stretched on a transect through each plot, and stems were collected and rated as above.

The average proportion of leaf area affected and incidence of stems with symptoms was calculated for each leaf spot disease. Incidence of diseases were calculated by dividing the number of stems with specific disease symptoms by the total number of stems. Proportion data (incidence) was arcsin square-root transformed for analysis. Plot averages were used in further analysis using Proc Glimmix in SAS software (SAS Institute Inc., Cary, NC, USA) and Tukey's least square mean test to compare among treatments. All tests were conducted that $P < 0.05$ was considered significant.

Table 2. Materials used in fungicide efficacy trial for control of leaf spots.

Fungicide	**Materials**	**Company**	**Application Rate (fl oz per acre)**	**Application timing**
Proline + Oxidate 5.0	Prothioconazole + Hydrogen Peroxide and Peroxyacetic acid	Bayer + Biosafe Systems	5.7 + 1% v/v	One application June 23 (Early), or one application June 30 (Late)
Luna Flex	Fluopyram and Difenoconazole	Bayer	12.8	One application June 23 (Early), or one application June 30 (Late)
Propulse	Fluopyram and Prothioconazole	Bayer	10.0	One application June 23 (Early), or one application June 30 (Late)
Proline	Prothioconazole	Bayer	5.7	One application June 23 (Early), or one application June 30 (Late)
PerCarb	Sodium Carbonate Peroxyhydrate	Biosafe Systems	0.5 lb	Two applications on June 23 and 30

RESULTS AND DISCUSSION

Survey of leaf spot diseases in wild blueberry fields

In late September and early October, there were high levels of leaf rust in many fields, and Sphaerulina leaf spot symptoms were still being found in high levels (greater than 10%) (Figure 1). Powdery mildew was lower in Mid-coast fields than Downeast fields farther west. When the levels of disease from similar, or the same fields from 2021 is compared to all of the fields in 2023, there was no significant difference in average percent coverage of *Sphaerulina*. There were significantly more powdery mildew and rust in fields in 2023 compared to 2021. In 2023, fields were rated one to two weeks after the date that fields were rated in 2021. This difference in time could have allowed rust and powdery mildew to progress further in 2023. There was also a difference in people doing the rating that could add to variability in the data. Leaf loss was not rated in 2023 since the typical autumnal leaf loss had already begun when the stems were rated.

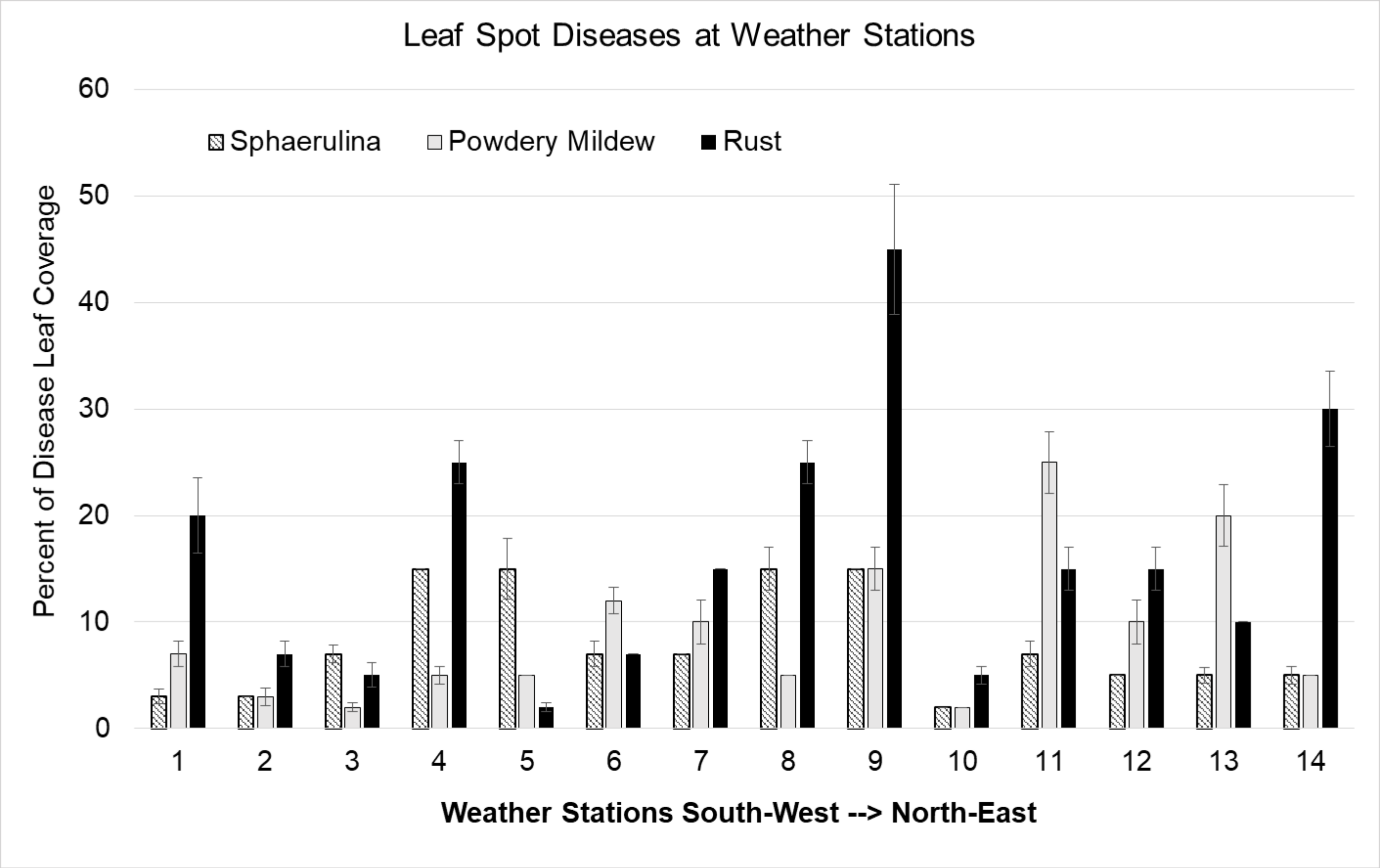

Figure 1. Estimated percentage of leaf area with *Sphaerulina* leaf spot (striped), powdery mildew (solid grey), and leaf rust (solid black) symptoms at fields with weather stations. Error bars indicate the standard error of the mean of four sample plots evaluated in each field.

Identification of Sphaerulina leaf spot using molecular methods

Twenty five isolates were identified as *Sphaerulina vaccinii* by sequencing a portion of their DNA and comparing it to known samples. The *Sphaerulina vaccinii* specific primers were able to detect Sphaerulina DNA, presumably from spores, on spore trap tapes from almost all fields sampled in 2022 and 2023 (twelve fields in total). *Sphaerulina vaccinii* spores were released every week during the bloom period of wild blueberry, and may have been produced earlier and later then bloom. Spores are suspected to be released earlier in the Downeast region than the Mid-coast. *Sphaerulina vaccinii* DNA was detected in almost all of the leaves with *S. vaccinii* symptoms and *T. minima* DNA was detected in two leaves. DNA from other spore trap tapes will be checked for *S. vaccinii* DNA and the number of spores will be estimated in early 2024.

Spore dispersal and leaf spot diseases

Tapes from the vacuum spore trap will be used to gather data on spore release timing for leaf spot diseases. Tapes are being extracted for spore DNA. The DNA will be tested for the presence of *Sphaerulina* and *Thekopsora* spores using the molecular methods developed to detect them.

Fungicide and Fertilizer Trial

Sphaerulina leaf spot, powdery mildew, and leaf rust were found in the treatments in both August and September. Physiological measurements were also taken in each treatment. The disease and plant physiological data is currently being analyzed.

Fungicide Efficacy Trial

No phytotoxicity was seen with any of the treatments. There were significant differences between fungicide treatments on leaf loss for each month of rating (Figure 2). In July, the early application of Oxidate and Proline, Luna Flex, and Propulse had significantly lower levels of leaf loss than the control. The late application of Proline and early application of Proline with Oxidate produced significantly less leaf loss in September. There were no significant differences between fungicide treatments on the incidence (percentage of stems with symptoms) (Figures 3, 4, and 5,) or the severity (estimate of leaf area affected) (Figures 6, 7, and 8) of *Sphaerulina* leaf spot, powdery mildew, or leaf rust symptoms, respectively. All of the incidence of the disease greatly increased from July to September (Figures 3, 4, and 5) with little effect of the different treatments on incidence levels.

In July, there were more stems with Sphaerulina leaf spots, than leaf rust. Both of those diseases had higher levels than powdery mildew in July. By September, all of the diseases had incidences ranging from 80 to 100%. Disease severity also increased from July to September (Figures 6, 7 and 8). In July, Sphaerulina leaf spots were lower with all of the fungicide treatments but were not significantly different from the control. By September, there was no significant differences among the fungicides or control for symptoms of all of the leaf spots (Figures 6, 7 and 8). The early application of Oxidate with Proline treatment appears to work better but is not significantly different than the other treatments with Proline. The Oxidate may remove spores and early growth of fungi on the surface the leaves with the Proline providing longer term protection. Propulse and Luna Flex also appear effective against leaf loss.

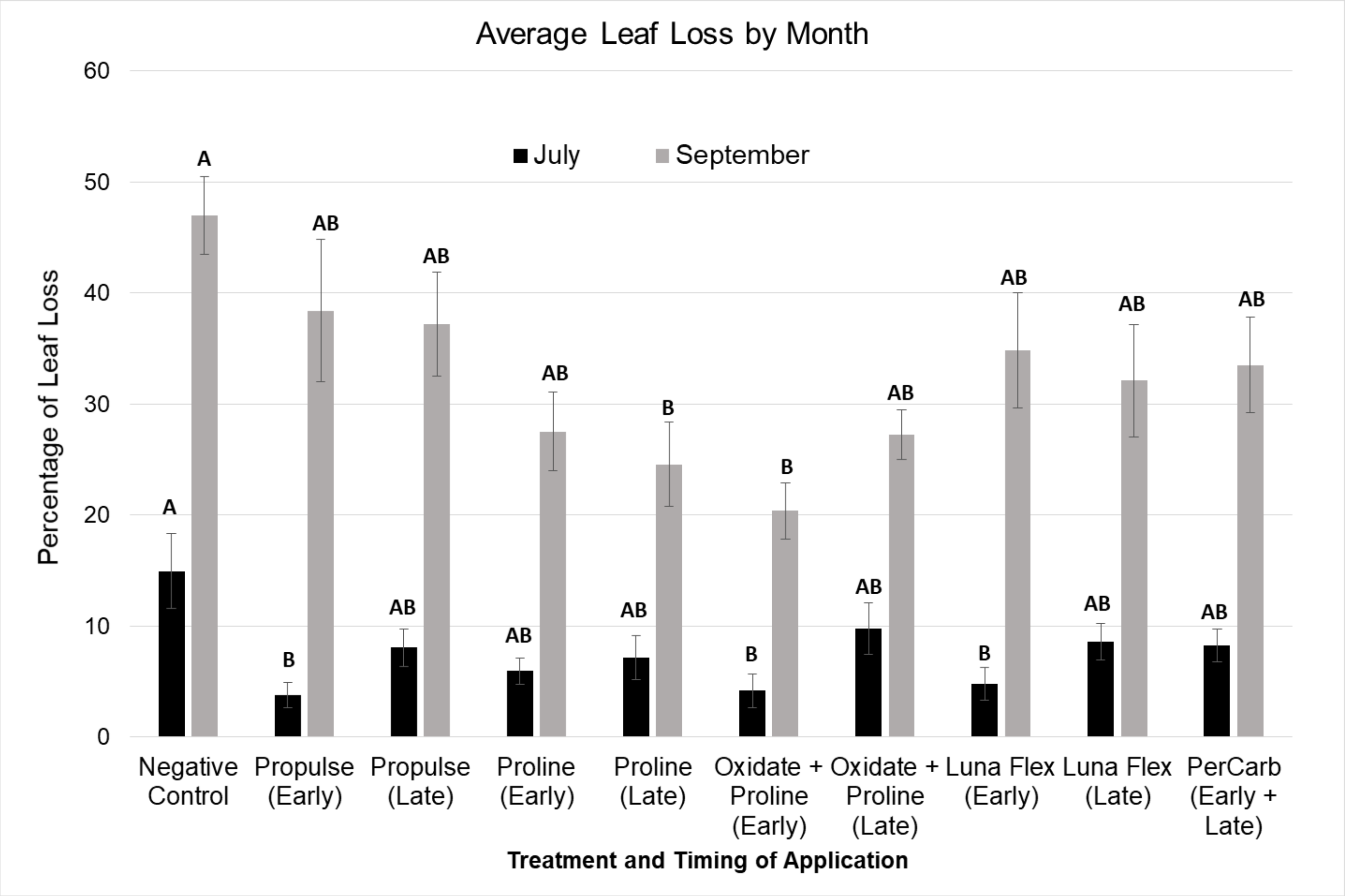

Figure 2. Percentage of leaf loss in July (black bars) and September (grey bars) for fungicide treatments applied at different dates. Error bars represent the standard error of the mean. Letters indicate significant differences between treatments at α = 0.05; comparisons are made among treatments in each month.

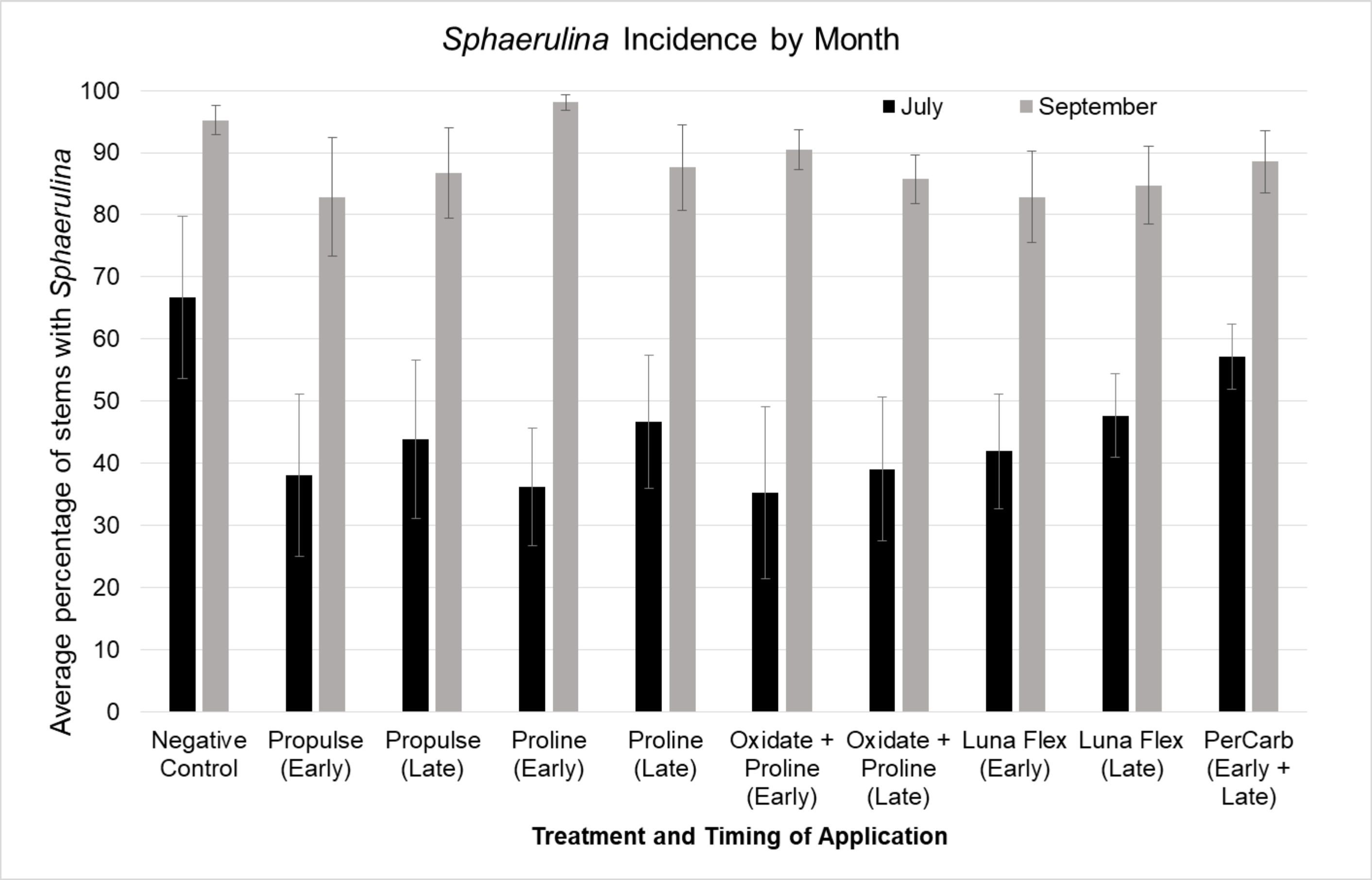

Figure 3. Average percentage of stems with *Sphaerulina* symptoms in July (black bars) and September (grey bars). Error bars indicate standard error of the means.

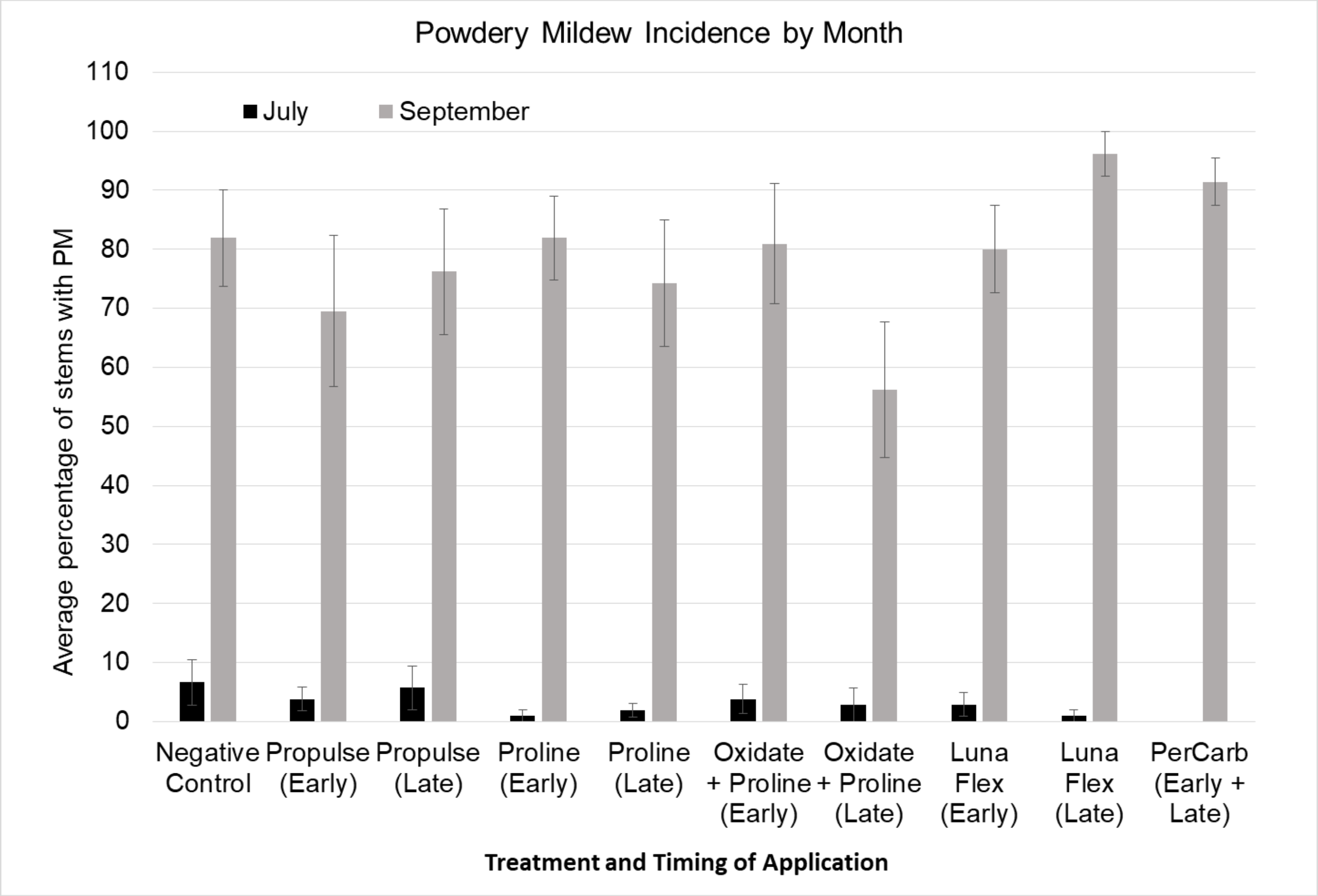

Figure 4. Average percentage of stems with powdery mildew symptoms in July (black bars) and September (grey bars). Error bars indicate standard error of the means.

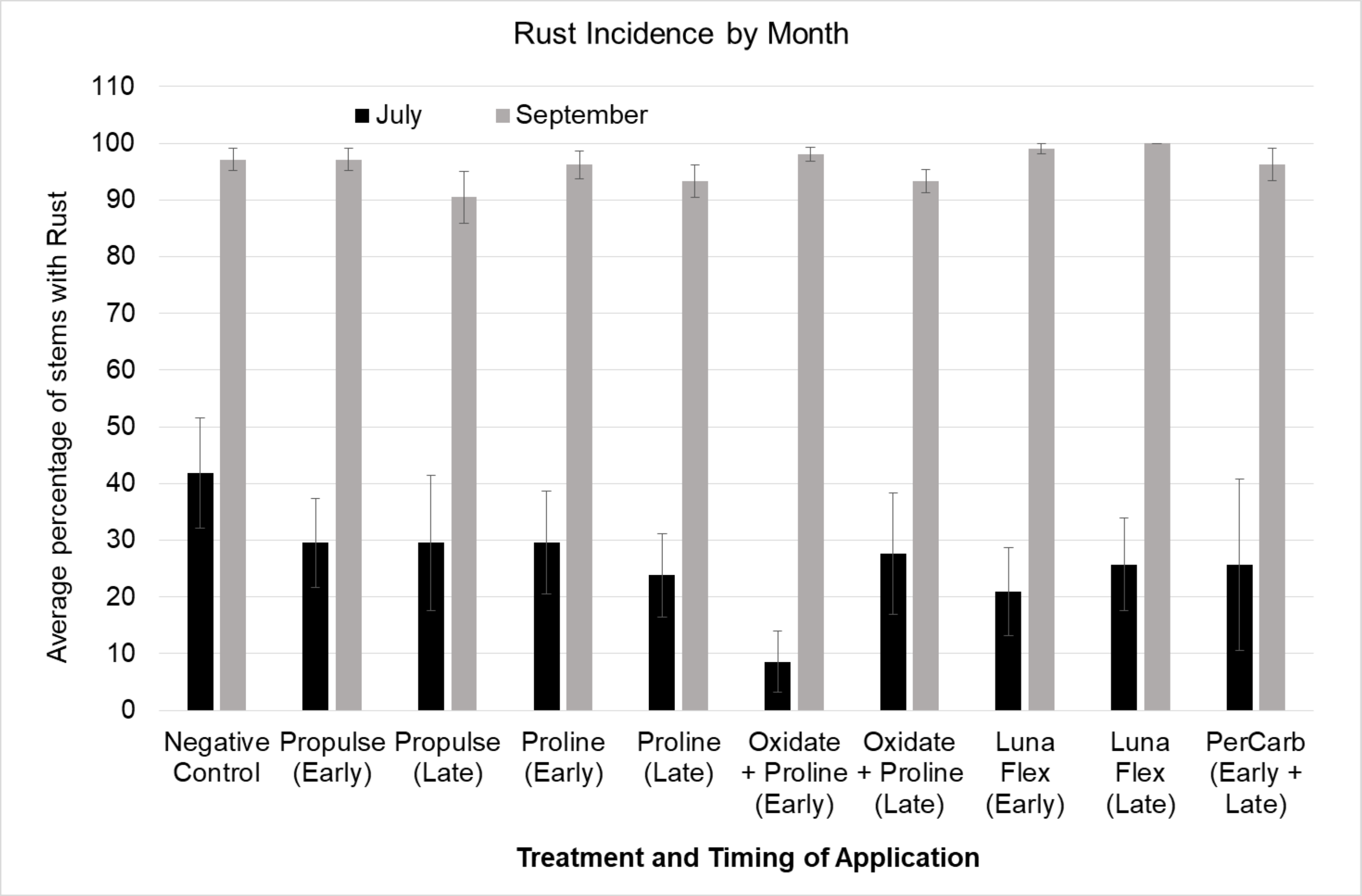

Figure 5. Average percentage of stems with rust symptoms in July (black bars) and September (grey bars). Error bars indicate standard error of the means.

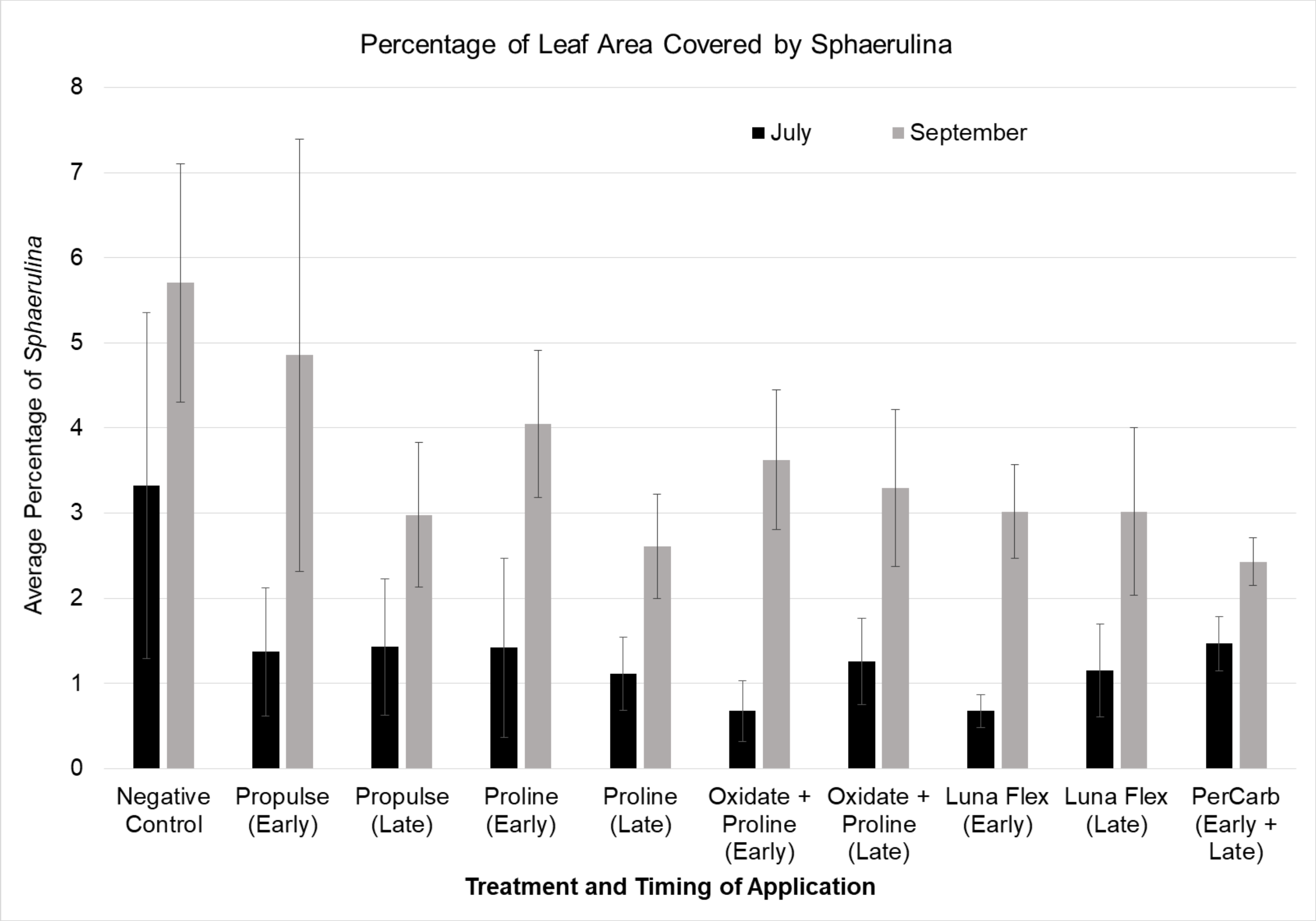

Figure 6. Average percentage of coverage of leaves with Sphaerulina leaf spot in July (black bars) and September (grey bars). Error bars indicate standard error of the means.

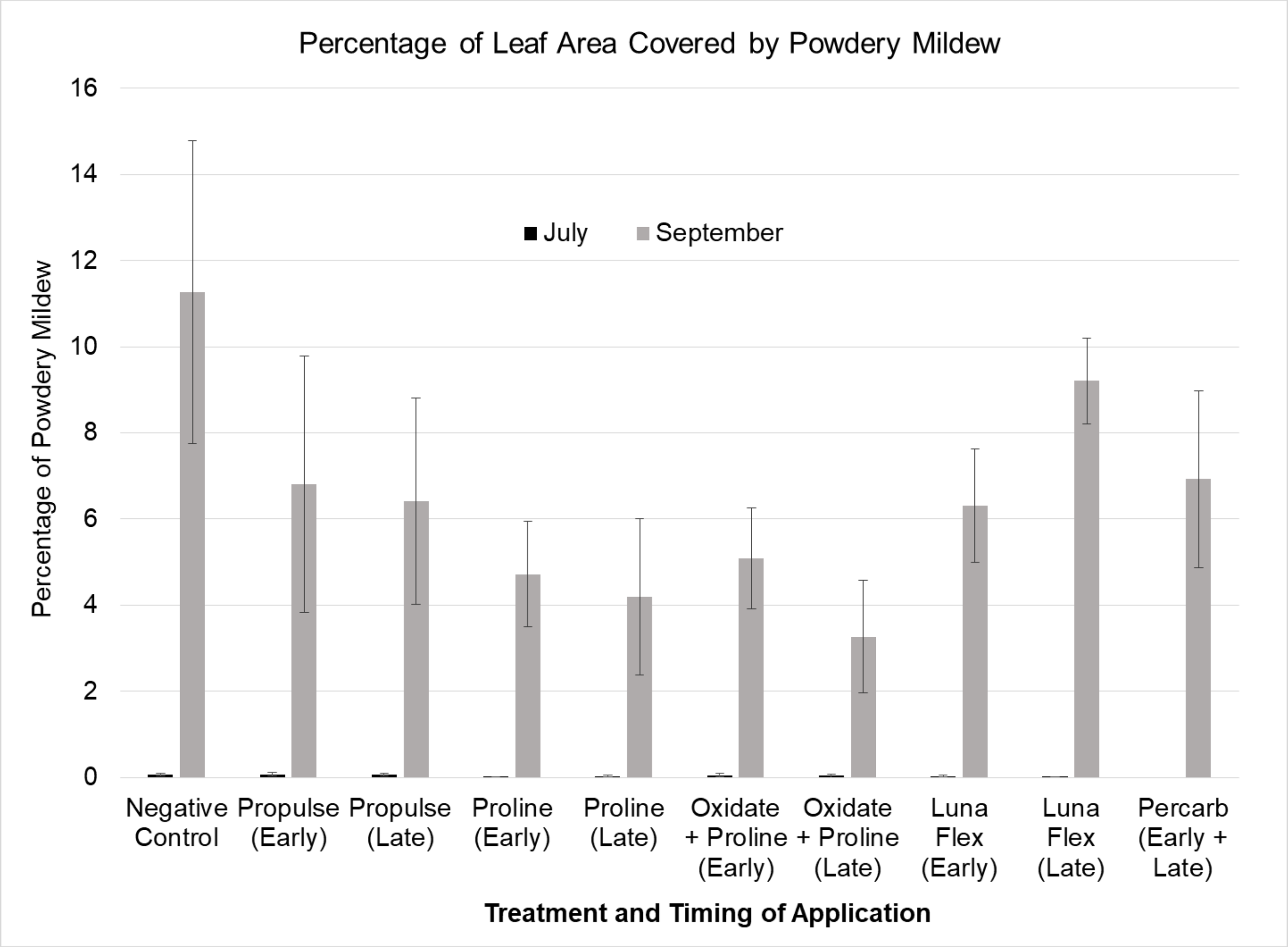

Figure 7. Average percentage of coverage of leaves with powdery mildew symptoms in July (black bars) and September (grey bars). Error bars indicate standard error of the means.

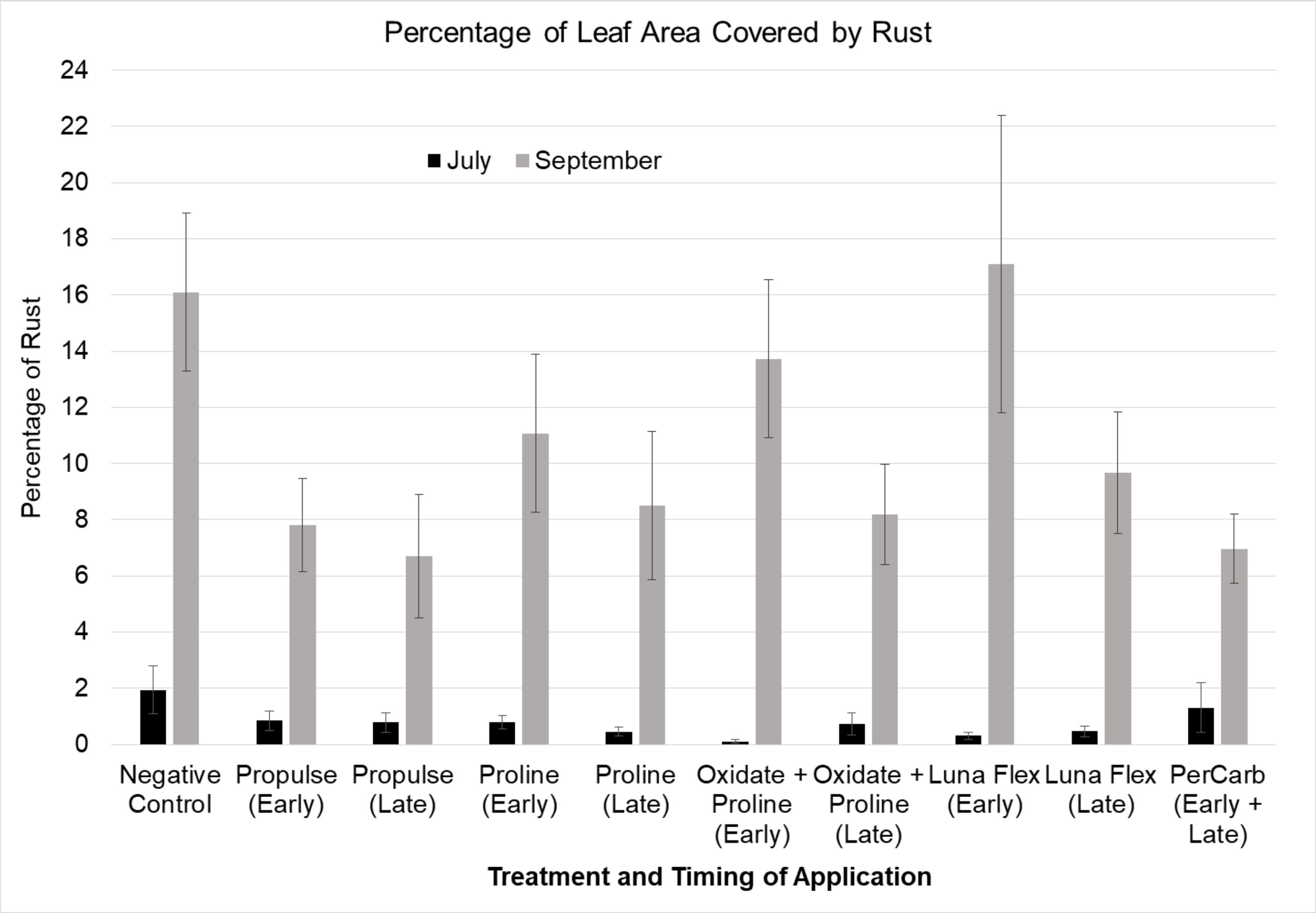

Figure 8. Average percentage of coverage of leaves with rust symptoms in July (black bars) and September (grey bars). Error bars indicate standard error of the means.

CURRENT RECOMMENDATIONS

Proline and Propulse were found to be effective materials to control leaf loss when applied earlier in June. These products and timing likely worked well because it reduced early infections and subsequent secondary leaf spot infections.

NEXT STEPS

Evaluations of the effects of fertilizer and fungicide treatments and their interactions on leaf loss and leaf spot will be continue in the crop year of the field experiment set up in 2023. The fungi found in leaves under the various treatments will be determined using DNA sequencing and identification of fungi by their sequences. We will also use the molecular methods to detect the fungi, *Sphaerulina* and *Thekopsora,* will be used to determine the timing and symptoms of the development of Sphaerulina leaf spot and leaf rust, respectively. Luna Flex and Luna Sensation will be retested for their efficacy to control leaf spots in 2024.

ACKNOWLEDGEMENTS

We would like to thank blueberry growers around the state for allowing us to work in their fields. Funding for this research was provided by the State of Maine Department of Agriculture, Conservation and Forestry through Specialty Block Grants and the Wild Blueberry Commission of Maine.

2. Research for Improved Control of Mummy Berry 2023

INVESTIGATORS: S. Annis, M. Roberts, and N. Kabir

OBJECTIVES

- Improve control of mummy berry caused by *Monilinia vaccinii-corymbosi* (MVC) through research and extension.
- Provide growers with forecast reports of MVC infection periods, Botrytis blight risk, and possible cold weather damage using 15 weather stations in blueberry fields.
- Test the efficacy of new materials for their control of mummy berry symptoms.
- Determine the effects of MVC on blueberry plants after infection, determine the effects of fertilizer on mummy berry disease, and what resistance compounds are found in wild blueberries.

LOCATIONS: Waldo, Lincoln, Knox, Hancock and Washington Counties, Maine

PROJECT TIMEFRAME: January 2023 to November 2023

INTRODUCTION

Mummy berry is potentially the most damaging disease on wild blueberries. While there is an IPM program in place providing effective control of mummy berry, there are some gaps in our knowledge of *Monilinia vaccinii-corymbosi* (MVC), causal agent of mummy berry, that affects its control. The timing of fungicide control is based on development of the plant and fungus, and suitable weather conditions for infection. We provide growers with information on the timing of possible MVC infection with blueberry weather stations. The majority of growers use Group 3 fungicides, mostly propiconazole, to control mummy berry symptoms. There are still only a few fungicides that are commonly used and fungicides with different modes of action and organically susceptible fungicides need to be identified.

METHODS

Survey of weather and disease in wild blueberry fields

In early April 2023, weather stations with cellular internet connections were deployed in 14 blueberry fields across the Mid-coast and Downeast regions in Maine (Image 1). These fields were located in Waldoboro, West Rockport, Liberty, Appleton, Searsport, Ellsworth, Sedgewick, Aurora, Eastbrook. Steuben, Deblois, Blueberry Hill Research Farm (BHF) in Jonesboro, Whiting, and Crawford. Each station was equipped to measure air temperature and leaf wetness in the canopy, soil temperature at 1in below the surface, and soil moisture in the blueberry rhizome and root zone. Three mummy berry (pseudosclerotia) plots were set up at many of the fields in fall 2022 and monitored by Annis lab personnel or growers (Image 1). Fields with weather stations were evaluated between May 23rd to June 5th, 2023 for symptoms of mummy berry disease. A field at Montegail pond was rated instead of the BHF in Jonesboro, and the field in Crawford was not rated. Twenty randomly chosen stems along each of four 20 ft transects were evaluated for mummy berry symptoms on leaves and flower buds, frost damage, and winter kill. Mummy berry 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. In addition, the number of markings at bare places (missing data) was recorded. Ratings were analyzed and compared to 2021 ratings using paired t-tests in Excel.

Image 1. A map of the locations of weather stations and mummy berry plots across the state. Weather station locations are marked by the square-in-drop symbol. Mummy berry plots are marked by the diamond symbol.

Fungicide efficacy trial

A trial was set up in a crop field at the BHF using a complete randomized block design with 12 treatments, 11 fungicide treatments plus one untreated control, replicated in 8 blocks (Table 1). Each plot measured 6 ft X 20 ft and was separated from the adjacent plot by a 3 ft alley. On April 27th, 2023, all treatments except Luna Sensation were applied to plots. Luna Sensation arrived in the lab on April 27th and was applied April 28th to its plots. Luna Flex, Luna Sensation, Luna Tranquility, Cevya, and Propulse were applied with the adjuvant DyneAmic at a rate of 0.12% final concentration, and Ecoswing was applied with the adjuvant Capsil at a rate of 0.6 fl oz / acre. Fungicide applications were timed to occur before an infection period was predicted using the mummy berry disease forecast method (Annis and Hildebrand, 2023; Delbridge et al. 1998). Disease ratings were made on May 25th, 2023. A transect with 20 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 August 9th, 2023. Plots treated with Luna Sensation, Luna Flex, Cevya 5.0, Propulse, and the negative control plots were harvested with a two-foot wide hand rake down the plot center and the fresh weight was measured. These samples were set aside to be tested for maximum residue levels (MRLs). All plots were harvested in a 2 ft wide strip down each plot center with a mechanical harvester, and the fresh weight was measured. For the MRL plots, the mechanically harvested weight was added to the hand raked weight. For all plots, the plot weight was used to calculate pounds per acre.

The data for flower rating, leaf rating, flower incidence (percentage of stems with flower symptoms), leaf incidence (percentage of stems with leaf symptoms), and disease incidence (percentage stems with flower or leaf symptoms) were not normally distributed. An Arcsin square root transformation was used on percentage data. Data were analyzed by plot averages in SAS (SAS Institute Inc., Cary, NC, USA) using mixed model procedures (PROC GLIMMIX). Tukey's Least Square means were used to determine specific differences among treatments and any difference with a P value of less than 0.05 was considered significant.

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

Fungicide	Active Ingredient	Rate / Acre (fl oz)	EPA Reg. #	Company	FRAC Group	Registered on lowbush blueberry	Registered for Mb
Luna Flex	Fluopyram / Difenoconazole	13.6	264-1218	Bayer	7 / 3	Yes	Yes
Luna Sensation	Fluopyram / Trifloxystrobin	7.6	264-1090	Bayer	7 / 11	No	No
Luna Tranquility	Fluopyram / Pyrimethanil	16.0	264-1085	Bayer	7 / 9	Yes	Yes
Miravis Prime	Pydiflumetofen / Fludioxonil	13.4	100-1603	Syngenta	7 / 12	Yes	Yes
Cevya	Mefentrifluconazole	5.0	7969-407	BASF	3	Yes	Yes
Propulse	Fluopyram / Prothioconazole	13.6	264-1084	Bayer	7 / 3	Yes	Yes
Inspire Super	Difenoconazole / Cyprodinil	16.0	100-1317	Syngenta	3 / 9	Yes	Yes
Omega 500F	Fluazinam	20	71512-1-100	Syngenta	29	Yes	No
Ecoswing	*Swinglea glutinosa*	32	10163-357	Gowan		Highbush	Yes
Tilt	Propiconazole	6 fl oz	100-617	Syngenta	3	Yes	Yes

RESULTS AND DISCUSSION

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. Downeast fields had 4 to 9 infection periods, while Mid-coast fields had 6 to 9 infection periods. The level of mummy berry disease was affected by the level of local inoculum, whether the field was mowed or burned, and if fungicides were applied. Apothecia at BHF were first observed approximately April 24th in the field, and dried up approximately May 11th. There were at least four possible infection periods for MVC within this time frame (Figure 1).

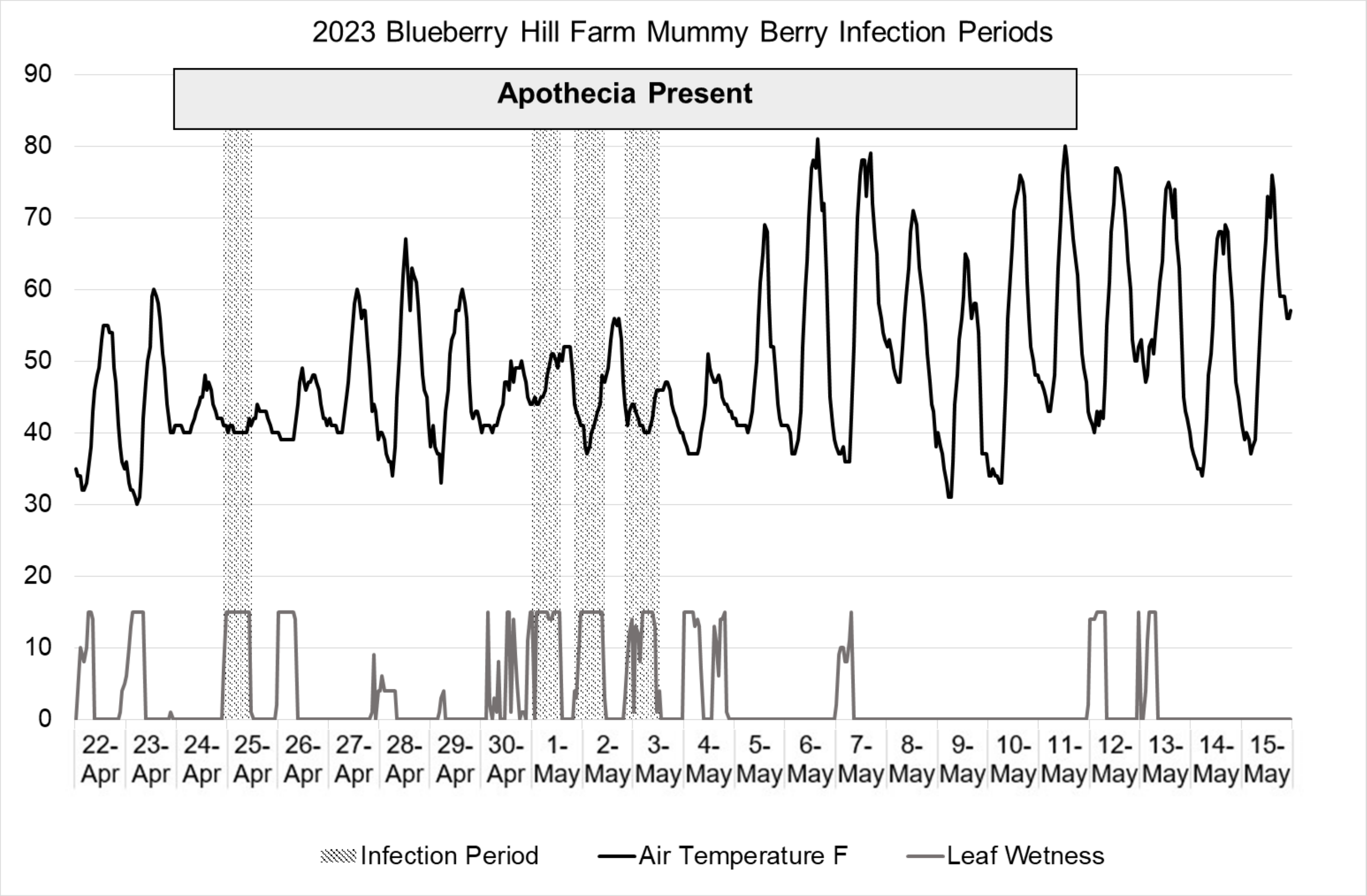

Figure 1. Infection periods at Blueberry Hill Research Farm in 2023. Four infection periods (dashed columns) occurred while apothecia were present, and air temperature (black line) and leaf wetness (gray line) conditions were appropriate for infection.

Across all weather stations rated for mummy berry blight in 2023, there was an average of 5.3% flower incidence and 8.4% leaf incidence (Figure 2). No Botrytis was detected in the weather station fields. In 2021, the same fields rated for mummy berry blight averaged 4.3% flower incidence and 11.3% leaf incidence. There was no statistically significant difference between the respective leaf and flower incidences from 2021 to 2023. In 2022, mummy berry blight was rated in fields near by the 2021 and 2023 fields, and disease levels averaged 4.4% flower incidence and 7.6% leaf incidence.

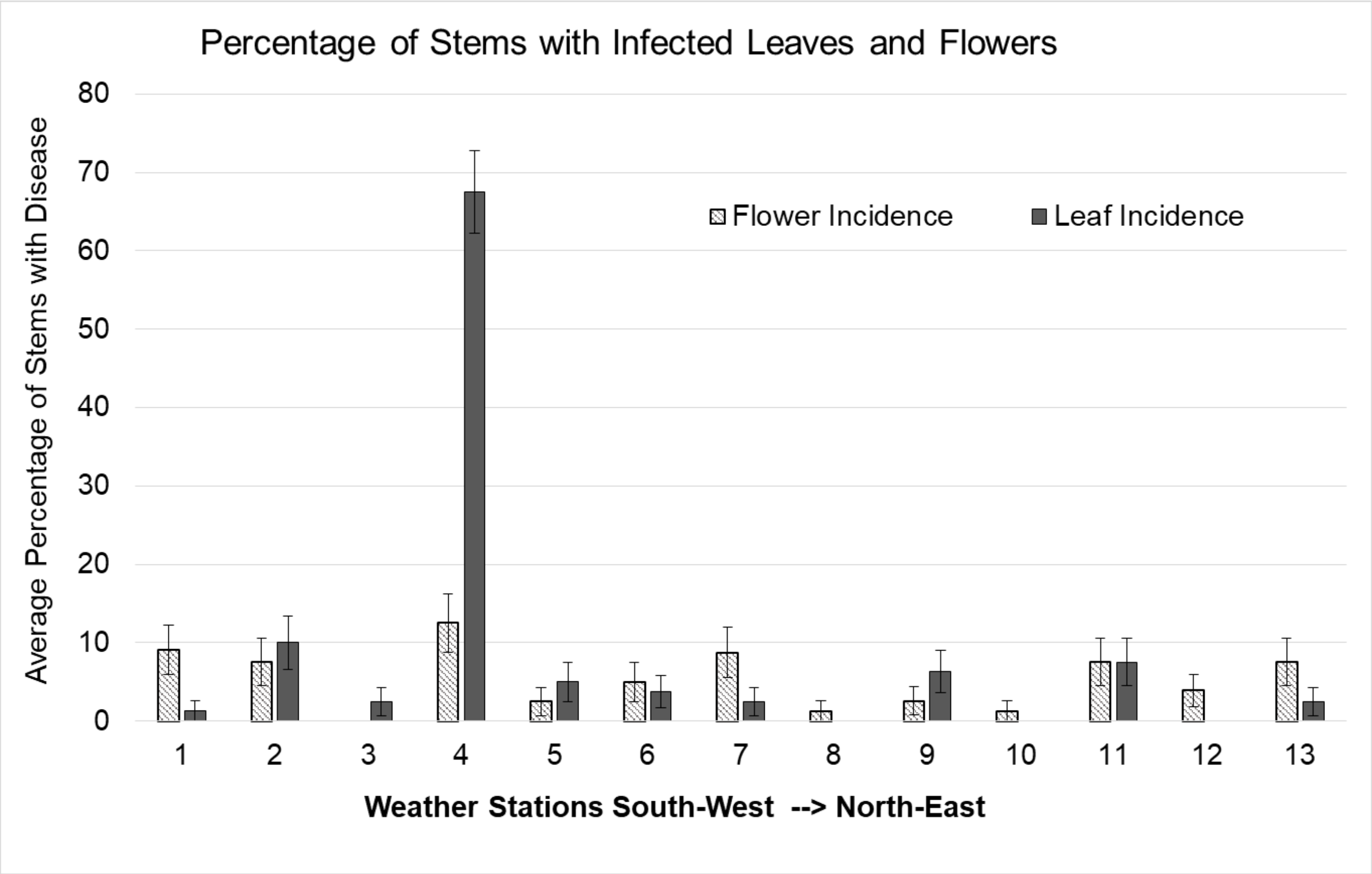

Figure 2. Average percentage of stems with mummy berry symptoms on flowers or leaves in the Midcoast (1-5) and Downeast (6-13) regions. Error bars represent the standard error of the mean.

Fungicide efficacy trial

There was high variability in the field experiment on the severity and incidence of mummy berry disease. Leaf ratings of disease were higher than flower ratings (Figure 3). For leaf ratings, only Propulse had significantly less leaf blight than the untreated control. The treatment with Ecoswing with Capsil as an adjuvant was at similar levels to the untreated control for both flower and leaf ratings and disease incidence (Figures 3 and 4). Omega 500F, Miravis Prime, both levels of Cevya, Propulse, and Tilt had significant lower leaf disease levels than Ecoswing but not the negative control due to the high variability. Luna Tranquility, Luna Sensation, both levels of Cevya, and Propulse had significantly lower level of disease than the Ecoswing treatment but no the negative control. The Capsil adjuvant may be the cause of the higher level of disease with Ecoswing. A lower level of Capsil (0.6 fl.oz/acre) was used this year than last year (1.2 fl. oz./acre) but both rates has similar disease results. In 2024, a small trial will be setup to test if Capsil or Ecoswing treatments are resulting in high levels of mummy berry disease.

There were no significant differences between treatments for the harvested fresh weight (lb/acre) (Figure 5). Luna Sensation and Miravis Prime treatments appeared to have higher yields. The field site had amounts of berries ranging from 0.7 lb to 9.5 lb harvested in a plot.

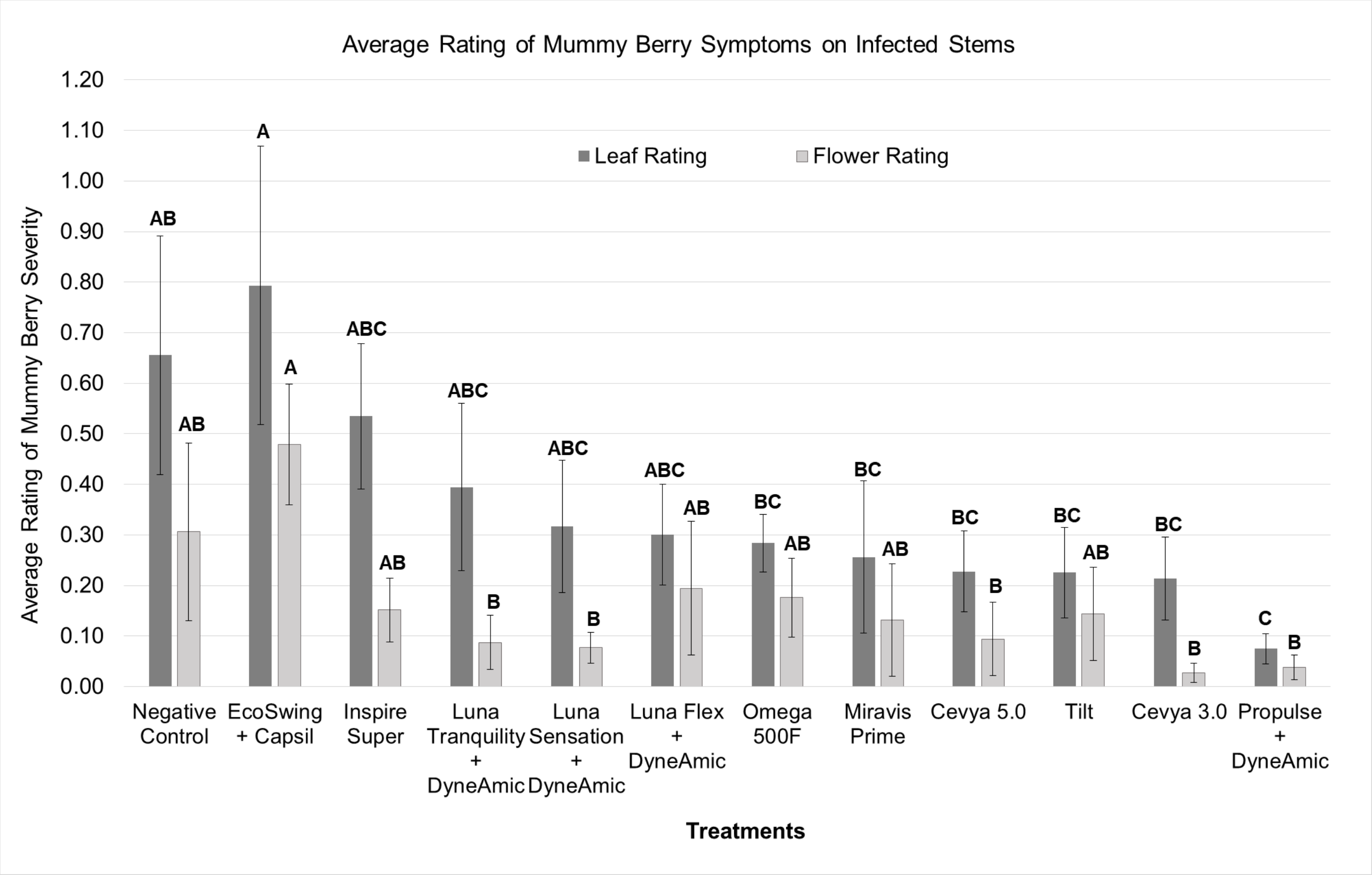

Figure 3. The average rating of mummy berry symptoms on infected flowers and leaves in the fungicide efficacy trial. Bars represent the standard error of the mean. Letters indicate significant differences within flower rating and leaf rating categories among treatments at α = 0.05.

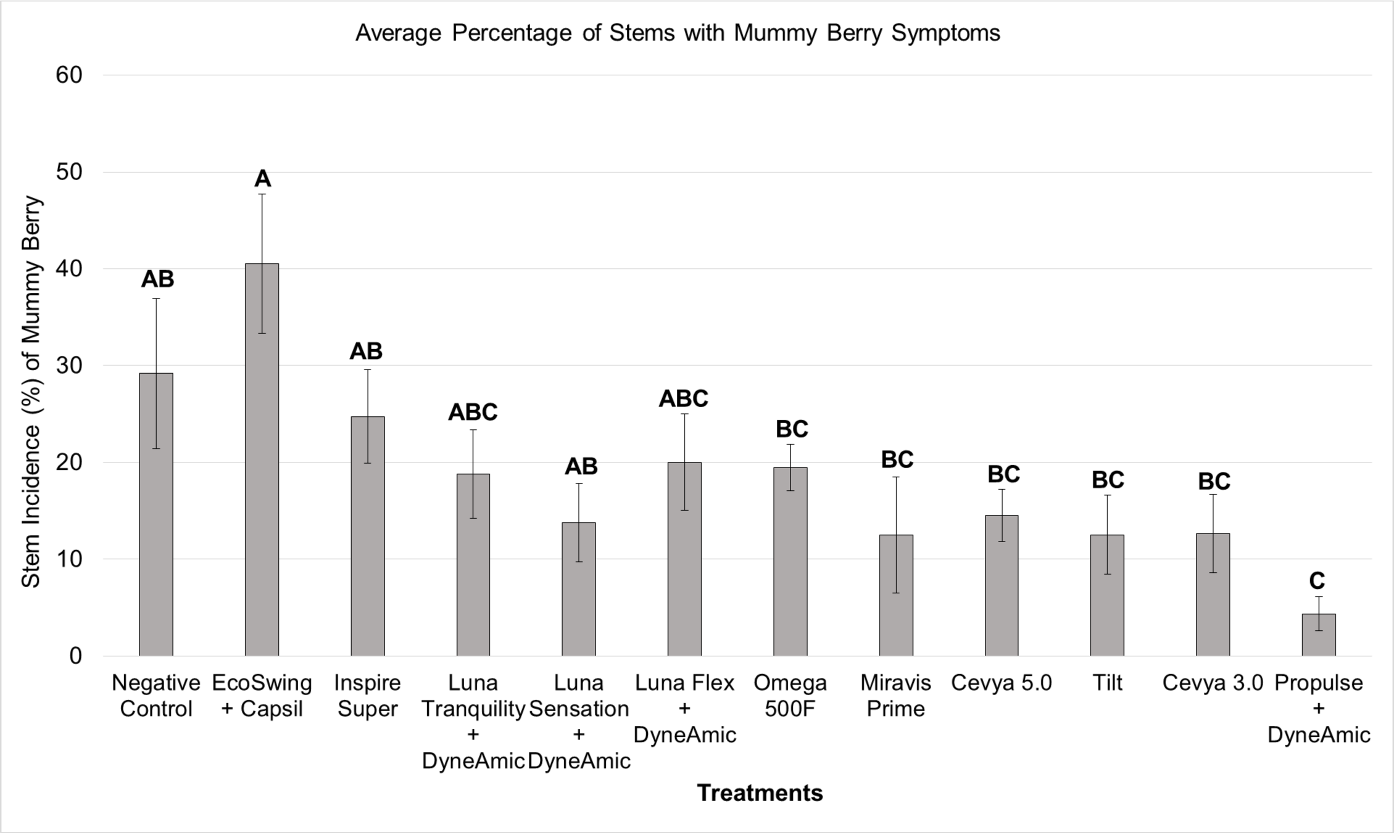

Figure 4. The average percentage of stems with mummy berry symptoms in the fungicide efficacy trial, 2023. Error bars represent standard error of the mean. Same letters indicate no significant differences among treatments at α = 0.05.

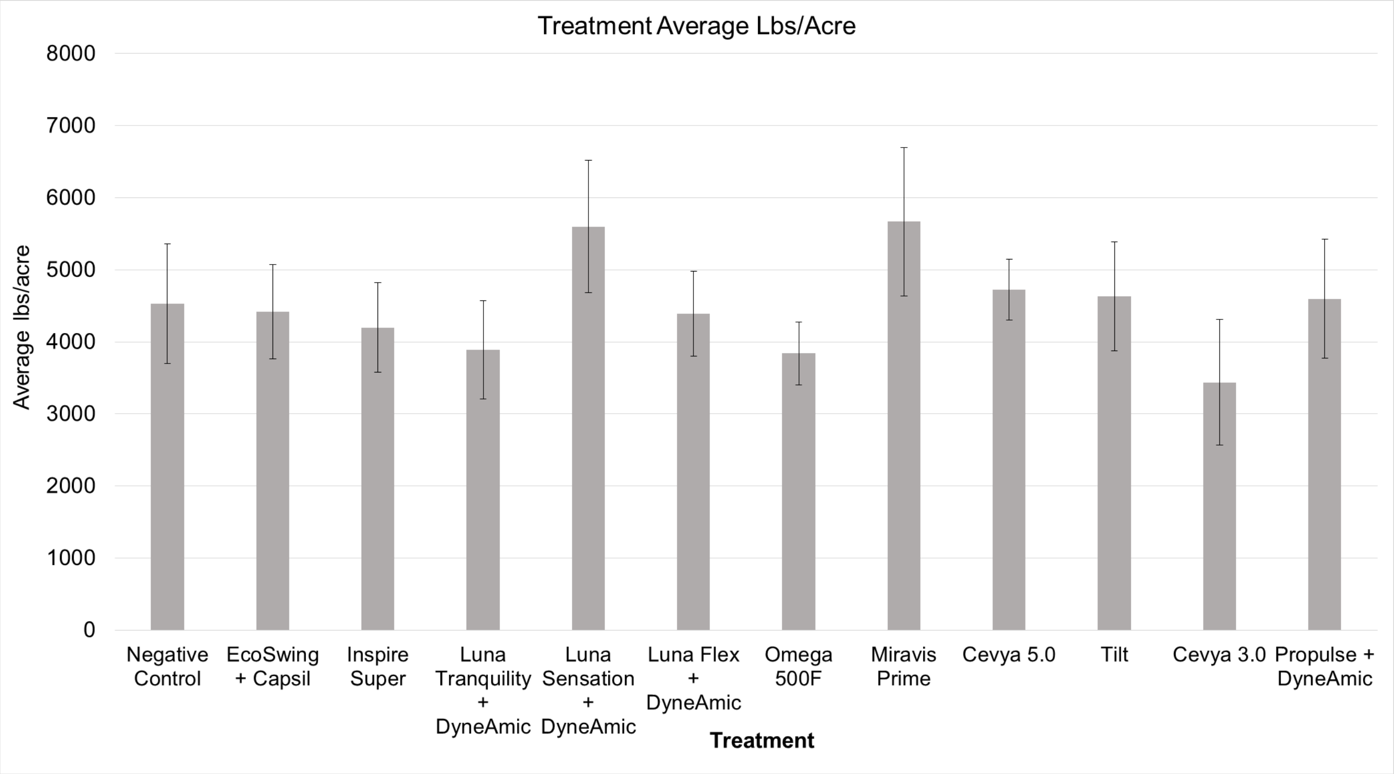

Figure 5. Average blueberry fresh fruit yield as pounds per acre. Error bars represent the standard error of the mean.

CURRENT RECOMMENDATIONS

We recommend Miravis Prime and Cevya at the 3 oz/acre rate in addition to Propulse and Omega 500F for mummy berry control in wild blueberry. We suggest not using Capsil as an adjuvant until we can determine if it is involved in causing higher levels of mummy berry disease. Luna Sensation will be retested next year.

NEXT STEPS

Impact of mummy berry blight on lowbush blueberry plant physiology

Nahida Kabir, a new Ph.D. student in Biological Science, with co-advisors of Drs Seanna Annis and Yongjiang Zhang will be developing her research on MVC. The main objectives of her research are to determine the effects of mummy berry disease on lowbush blueberries by monitoring their physiology during infection, and post-infection to harvest. Her project will also include evaluating whether blueberry genets have different susceptibility to other diseases after being infected by mummy berry blight, observing the effects of fertilization on the establishment of mummy berry blight, and finally, investigating the presence of secondary metabolites on the mummy berry blight-infected plant and their potential roles in plant recovery.

ACKNOWLEDGEMENTS

We would also like to thank blueberry growers around the state for allowing us to work in their fields. Funding for this research was provided by the State of Maine Department of Agriculture, Conservation and Forestry through a Specialty Block Grant and the Wild Blueberry Commission of Maine.

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

INVESTIGATORS: L. Calderwood and J. Parks

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: Mid-coast to Downeast (9 locations: Warren, Hope, Searsport, Sedgwick, Orland, Aurora, Jonesboro, Northfield, and Whiting)

PROJECT TIMEFRAME: April 2020 - Ongoing

INTRODUCTION

As climate change and technology use on farms progresses globally, local growers are facing increased uncertainty and variable conditions during the growing season. Online 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 tools, several years' worth of plant development data must be paired with air temperature and humidity in addition to soil temperature and moisture. This project began in 2020 with 2023 being the fourth consecutive year of weekly plant development and weather data collection. This data will be used to create wild blueberry crop development tools 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. Our ultimate goal is to merge all wild blueberry decision support tools into the Maine Climate Office Website managed by Dr. Sean Birkel.

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 (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). Longer seasons not only perpetuate wild blueberry development but also those of weeds, diseases, and insects. 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 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 (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. 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 limiting the positive impact on growth and development of wild blueberry plants (Tasnim et al., 2022).

Changes in climate result in more chaotic and extreme weather events and this pattern has been occurring in Maine over the past several years. Spring of 2023 included an April that was warm and wet in the southern half of Maine (NOAA/NIDIS, 2023a). On May 18th New England experienced a frost event with temperatures as low as –7°C (19°F). We measured a low temperature of 27°F (-2.8°C) on a crop field in the Mid-coast region. This freeze event zapped a majority of the southern Maine wild blueberry flowers and apple blossoms across the Northeast region. On the nights of May 23rd and May 30th two more frost events occurred with temperatures dipping down to 27°F (-2.8°C) impacting some fields along Rt.9 in Hancock and Washington counties. The effects on blueberry plants from low temperatures are shown (Table 1). As is typical, 2023 bloom occurred over the course of 3 weeks (5/15 to 6/6) with some plants flowering early, the majority flowering in the middle, and some flowering late. Because of the naturally staggered bloom and a major freeze event in the middle we saw what one could call an "early and late harvest" with a gap in the middle where flowers died from the freeze and therefore no fruit was produced. This made it difficult for some farmers to decide when to start their harvest.

Table 1. Temperature effects on wild blueberry flowers and leaves according to Hall et al. (1971), Hall and Hildebrand, (1988), and Hicklenton et al., (2000).

Temperature	**Damage**
30.5°F	Slight injury to flowers, little effect on fruit set, occasional necrosis at edges of leaves
28 °F	Greater than 2 hours, 40 to 60% loss of fruit set in open flowers and 20 to 30% in closed flowers, unclear if berry weight is affected
26°F	Open flowers, after 2 hours, 75% of flowers lost resulting in little fruit set. After 2 hours, 50% of closed flowers do not open, but if they do open, little effect on fruit set. With increased time, fewer flowers open.
Below 24°F	Flowers die, no fruit set, leaves are water soaked and may not recover

May 31st was unusually hot in New Brunswick, Canada and Maine with a temperature of 92°F (33.6°C), and Yarmouth, N.S. had its driest spring on record which contributed to the largest wildfire on record burning 17,000 ha (42,000 acres) reducing air quality as far away as Massachusetts (NOAA/NIDIS, 2023a). This was followed by up to 100 mm (4 in.) of rain in Maine with as much as 200 mm (8 in.) in some areas of Nova Scotia just in the first week of June. Portland, ME saw its coldest temperatures since records began in 1940 on June 3rd and 4th. July was the all-time warmest month on record for Caribou, ME and Maritime Canada and was also wetter than normal which contributed to flash flooding. August was the wettest on record for Maine and Maritime Canada. Wet conditions led to larger blueberries in Maine on our well-draining soils yet severe yield loss occurred for vegetable, grain, potato, corn, and hay crops in New England (NOAA/NIDIS, 2023b). Total precipitation for the state of

Maine was 21 inches from May to August (NOAA/NCEI, 2023). During this time, 10 out of the 16 weeks received an inch or greater of precipitation (NOAA/NCEI, 2023). Farmers started harvesting this year between 7/31 and 8/6. Because August was a wet and cool month, farmers were able to harvest long into August without losing overripe fruit. Overall, the wild blueberry harvest was an average yield. Official yield numbers have not been reported yet.

METHODS

Nine locations stretching from Washington to Knox counties were utilized for this study in 2023. By county, locations included: Washington (Whiting, Jonesboro, and Northfield), Hancock (Aurora, Orland, and Sedgwick), Waldo (Searsport), and Knox (Hope and Warren). Each location consisted of one prune field and one crop field, totaling eighteen study locations across nine municipalities. Whiting 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 as medium to high input conventional fields. Locations were grouped approximately based on location: Mid-coast (Hope and Warren), Ellsworth (Searsport, Orland, and Sedgwick), Downeast (Aurora and Jonesboro), Far Downeast (Whiting and Northfield) (Image 1).

Wild Blueberry Staging and GDD

In each location's crop field, a HOBO H21-USB Micro Weather Station (ONSET Computer Corporation, Bourne, MA, USA) was installed with air temperature, relative humidity, soil temperature, and soil moisture sensors. In each crop and prune field, six different plants were selected at random and within each plant, 10 individual stems were tagged and flagged for continuous monitoring. Crop fields were visited weekly for eighteen weeks (4/3-8/2/23). Wild blueberry crop-stages (T1-T5, bloom, pinhead, green, color change ("red"), and blue fruit) were visually identified for each tagged stem at each location for the duration of the project. Each week, the total number of buds/flowers/fruit were counted on all marked stems (n=180).

Image 1. Phenology locations across major blueberry growing regions: (left) Mid-coast (small square) & Ellsworth (star), and (right) Downeast (large circle) & Far Downeast (diamond).

The prune-cycle was monitored from leaf emergence (week 6, 5/8/23) until the end of bud development (week 29, 10/16/23). Six plants were randomly chosen and a sixteenth of a square meter area (25 cm x 25 cm) was marked with flags at each corner. Every week, the total number of stems emerged was documented within the quadrat from 5/8 to 6/26/23, then the number of stems with tip dieback was documented from 7/3 to 7/24/23. The small quadrat was then removed for bud development data collection. Ten healthy stems per plant were tagged and flagged at random for long-term data collection. The number of immature green buds and hardened brown buds were documented from 7/31 to 10/16/23.

Data gathered each week was averaged by location and crop cycle 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/) and through the Wild Blueberry Newsletter.

In November, prune fields were walked in transects to make preliminary observations about the extent of fall flower and fruit development. Nine individual prune fields were chosen across the state for observation. The data collector started on an edge of a field and would start walking diagonally to the other side of the field. While walking, any stem with flowers was recorded as one stem in bloom. Any stem with fruit was recorded as one stem in fruit. If a stem had flowers and fruit, it was counted as two to separate flowers and fruit. The data collector made a zigzag pattern across the prune field until they completed 5 transects.

Data analysis did not include statistics. Air temperature and relative humidity were downloaded from the HOBO weather station. 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 for 2022 and 2023. Fruit loss was estimated by subtracting the peak green fruit from peak blue fruit per stem. Fruit set percentage was calculated to evaluate yield and fruit loss from frost. This was calculated by dividing the number of peak green fruit by the peak number of open flowers per stem then multiplying by 100 (Drummond, 2020). When fruit set is 25% or below it is considered poor; 25-50% is average; 50-70% is good; and 70-100% is excellent fruit set (Drummond, 2020).

RESULTS

Cumulative growing degree days (GDD) showed a steady upward trend for Mid-coast, Ellsworth, and Far-Downeast (Figure 1). Downeast was a bit cooler and increased at the same rate but at a lower number of GDDs (Figure 1). Downeast weather stations had a malfunction and were fixed on May 2nd, which is why the plotted data for that region starts later in the timeline (Figure 1).

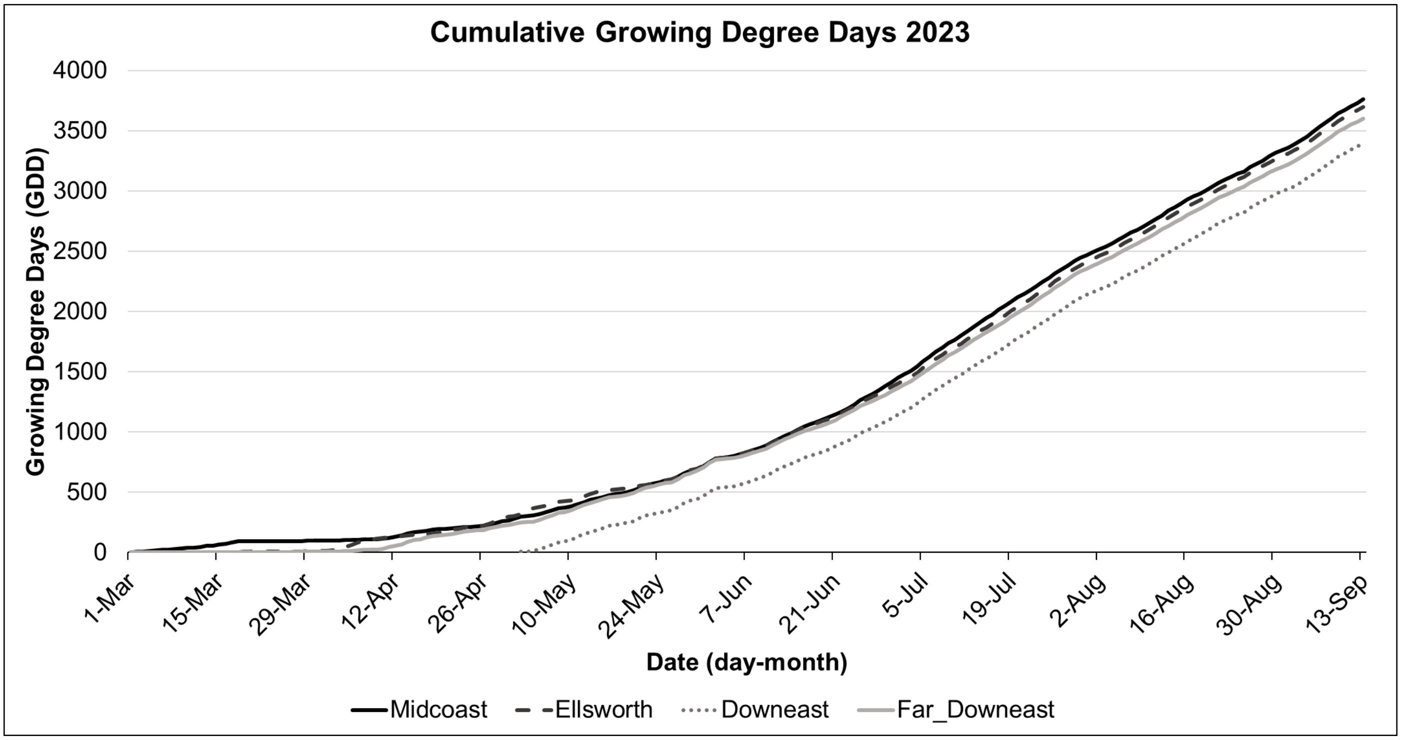

Figure 1. Average cumulative growing degree days (GDD) by region for the 2023 field season. Growing degree days were calculated with a base temperature of 40°F and started accumulation on March 1st. Downeast region weather data was not collected from March 1st to May 1st due to an equipment malfunction.

Crop Phenology by Region

In years 2021-2023, peak T1 stage occurred on 4/8/2021, 4/9/2022, and 4/15/2023 in the state of Maine (Figure 2). Peak bloom occurred on 5/24/2021, 5/21/2022, and 5/22/2023 in Maine (Figure 2). Peak blue fruit occurred on 7/26/2021, 8/1/2022, and 7/27/2023 in Maine (Figure 2).

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 2023 bud development in the T1 to T2 stage was five to seven days later than 2021 and 2022 (Figure 2). However, stages T3 to blue fruit were all within one to two days of past years (2021 and 2022). The GDDs were higher than previous years in the early stages (T1-T3) and then dropped off to lower than previous years until peak color change fruit (Figure 2).

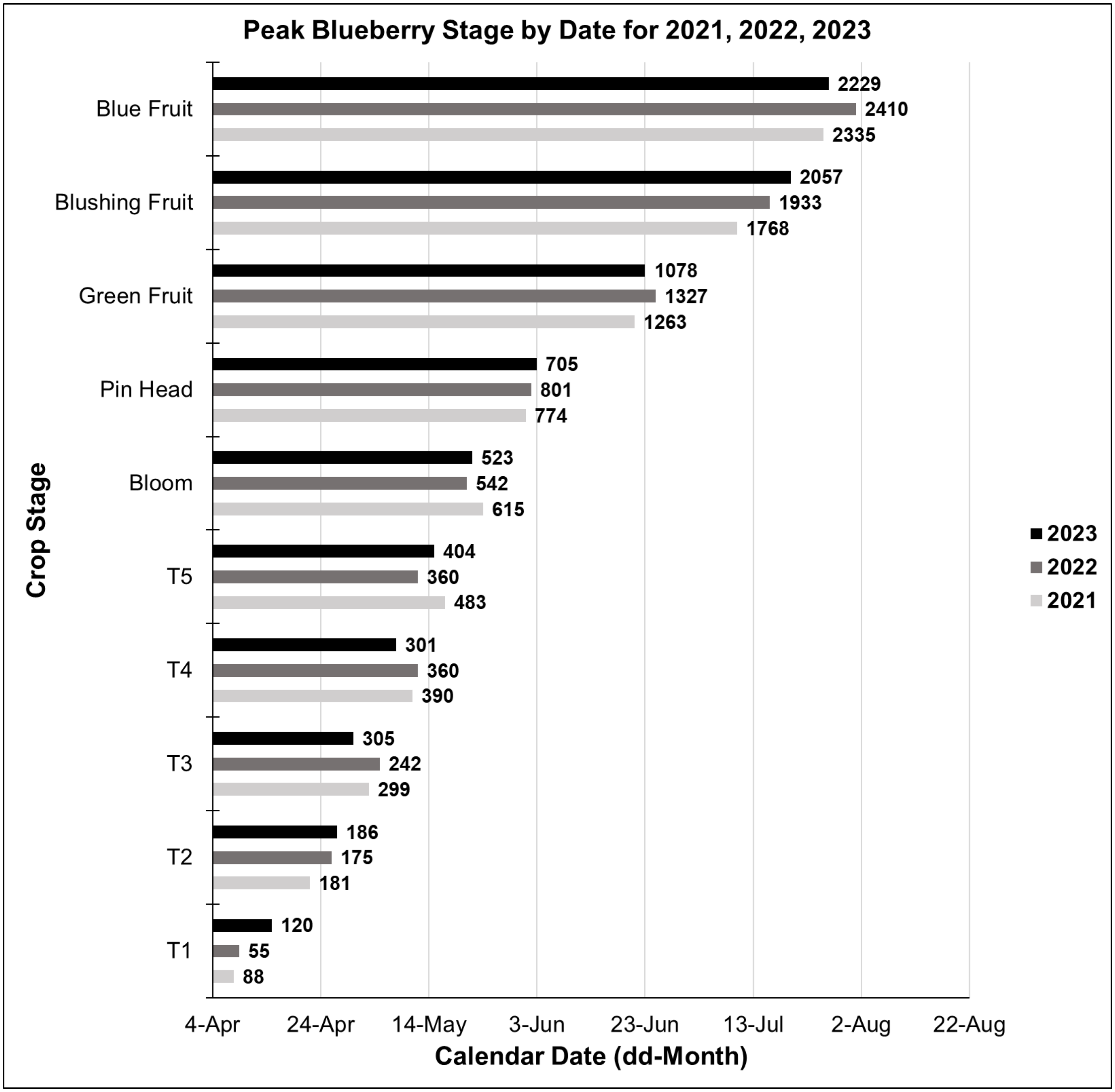

Figure 2. Peak blueberry crop stage by date for 2021, 2022 and 2023 with growing degree days (GDDs) as data labels. Bud stages are labeled T1-T5 with T1 being bud swell, T2 being bud break, and T5 being early bloom.

Fruit loss per stem was evaluated by subtracting peak green fruit from peak blue fruit. The Mid-coast region on average had fewer fruit per stem than the other regions and low fruit loss (Figure 3a). The Ellsworth and Downeast regions had a high number of green fruit per stem (14.5 and 16.1 fruits/stem, respectively), but the Ellsworth region had the highest fruit loss (8.1 fruits/stem) (Figure 3b and 3c). The decreasing green fruit line is due to that fruit turning blue. Percent fruit set ranged from poor to excellent this year depending on loss from frost and the number of bee hives distributed per acre with the majority of locations having good yields (Figure 4).

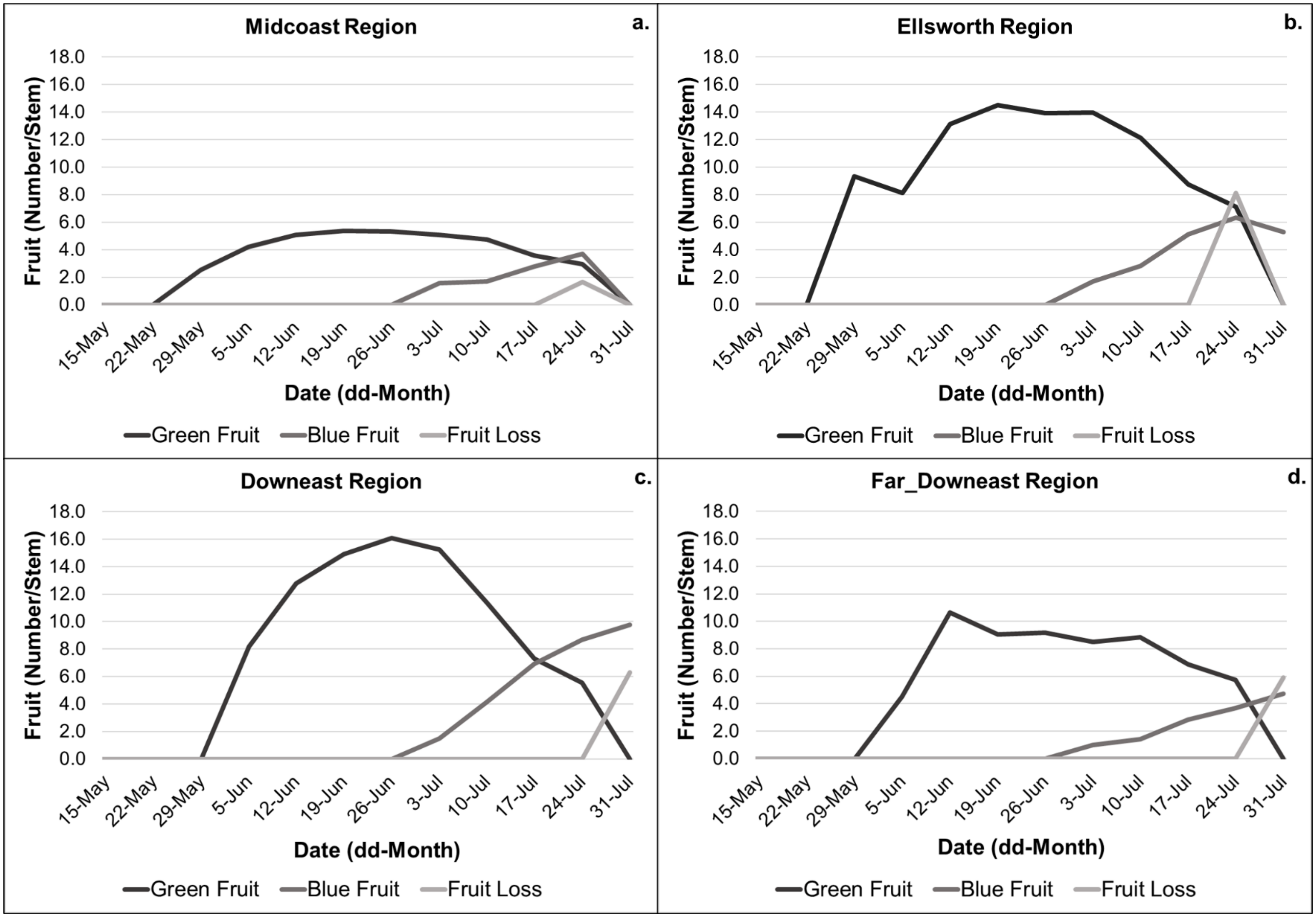

Figure 3. Average number of green and blue fruit per stem and estimated average fruit loss per stem by region from May 15th to July 31st, 2023. Average peak green fruit for Maine was June 20th and data collection stopped due to harvest on July 31st.

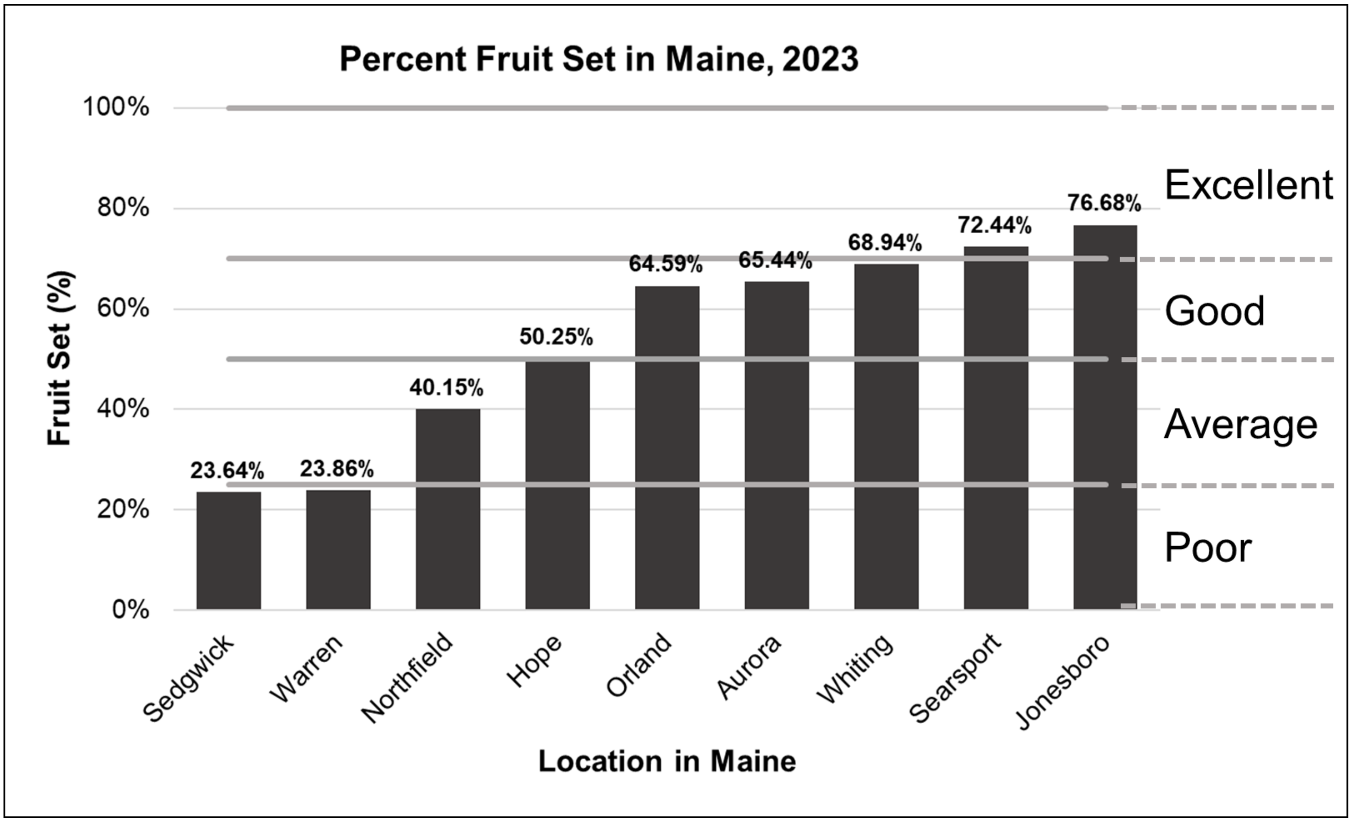

Figure 4. Percent fruit set in the phenology plots in the Mid-coast, Ellsworth, Downeast, and Far-Downeast regions of Maine. Fruit set ranges on the right are, poor 0-25%, average 25-50%, good 50-70%, and excellent 70-100% (Drummond, 2020).

Prune Phenology

Bud development is important to track and manage in order to have an adequate number of fruit for the following year. The overall number of buds per stem for all regions was much lower than 2022 (Figure 5). The Downeast and Far-Downeast regions were slower to develop buds (Figure 5).

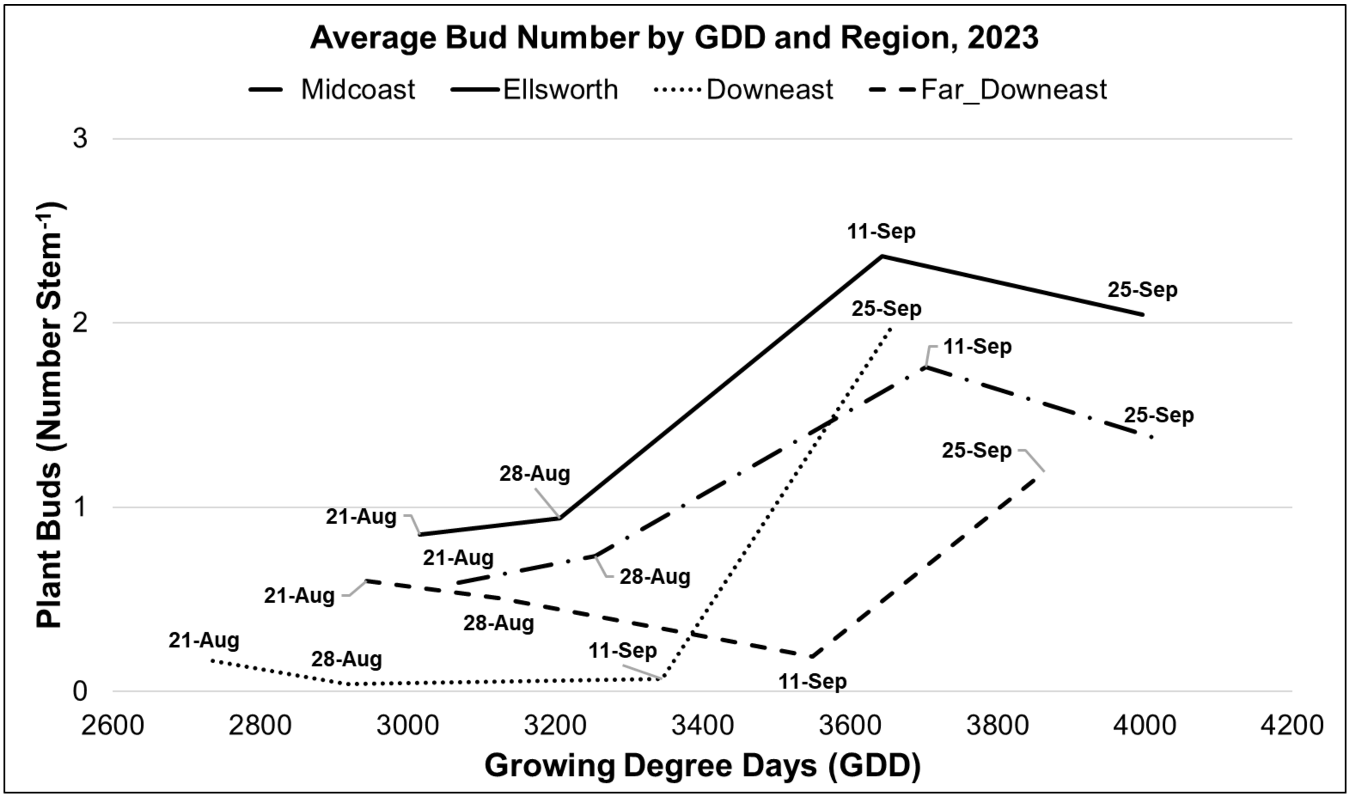

Figure 5. Average bud number (Number stem^{-1}) by region relative to growing degree days (GDD) with calendar dates as data labels for the 2023 growing season.

When fall bloom was evaluated in November, the locations with the highest number of stems with flowers were Orland, Union, and Northfield (Table 2). The number of fruit on stems was very low for all locations. According to data collected by Frank Drummond there are between 2,023,431 and 7,284,353 stems per acre with an average of 4,125,531.25 stems per acre. The amount of fall bloom on average was 0.0011% per acre for the state of Maine. The highest percentage was in the Orland area, which was 0.0017% per acre, which is no need for concern.

Table 2. Preliminary results of fall bloom in the state of Maine in 2023, during mid-November. Stems with flowers and/or fruit were counted while walking transects across fields.

			Fall Bloom in Maine, 2023	
Date	**Location**	**Region**	**Number of Stems with Flowers**	**Number of Stems with Fruit**
15-Nov	Warren	Mid-coast	3	2
15-Nov	Union	Mid-coast	276	2
15-Nov	Hope	Mid-coast	8	0
9-Nov	Searsport	Ellsworth	81	3
16-Nov	Orland	Ellsworth	404	3
16-Nov	Aurora	Downeast	0	0
16-Nov	Jonesboro	Downeast	16	0
16-Nov	Whiting	Far-Downeast	1	0
16-Nov	Northfield	Far-Downeast	236	3

DISCUSSION

Crop Phenology

In April, temperatures in the Maritime region and New Hampshire were warmer than usual and conditions were dry in Maine until later in April when a large precipitation event dropped three to seven inches of rain on the southern and western parts of the state (NOAA/NIDIS, 2023a). This jumpstarted the GDDs for the Mid-coast region allowing for buds to start swelling and bursting six days earlier than 2021 and 2022 (Figure 1 and 2). By peak T1, Maine had accumulated 120 GDD, which is 65 GDD units higher than 2022 (Figure 2). Unfortunately, plants were in peak bloom in the middle of May when three frost events happened. The Mid-coast and Ellsworth regions suffered heavy losses from this event and experienced temperatures as low as 19°F (–7°C). Flowers, whether closed or open, can lose fertility and have a decline in fruit set (Hicklenton et al., 2000) (Table 1). Fruit set declines can be >40% when exposed to 27.5°F (-2.5°C) regardless of temperature duration and once the temperature reaches 25.7°F (< -3.5°C), no fruit is set from flowers (Hicklenton et al., 2000) (Table 1). In the Downeast region, the last spring frost typically occurred within the month of March from 1980 to 2020, which shows how much of a surprise May freeze events still are to growers and plants (Tasnim et al., 2022).

Crop Phenology by Region

Fruit loss was calculated by subtracting peak green fruit from peak blue fruit to determine the amount lost from falling off the stem or loss from pests. The Mid-coast region had a low number of fruit on the stem compared to the other Maine Regions (Figure 3a). This can be attributed to the frost that happened in May during bloom. Many farmers do not have irrigation on their fields to help protect against frost events and frost fans are too expensive. An experienced farmer knows which areas on the farm are prone to frost and maybe those areas can be prioritized with frost protection instead of protecting the entire farm. The other regions had high peak green fruit counts and lost six to eight berries per stem once ripe. Berry loss could be due to several factors including plant stress, over ripeness, pest removal, other environmental damage or the person collecting data (ripe fruit can fall off easily).

Percent fruit set was greater than expected after multiple frost events this year. The increase in precipitation allowed the crop to receive adequate water almost every week. In the Mid-Coast region, Hope had an average fruit set and Warren had a poor fruit set (Figure 5). These farms have different pollination styles but are also located at different elevations. Hope is about 400 ft higher than Warren, which may have helped mitigate cold air pooling. The Ellsworth region had good to excellent fruit set apart from Sedgwick which was on the edge of average fruit set (Figure 5). The Downeast and Far-Downeast regions had good to excellent fruit set this year apart from Northfield which was average (Figure 5). Northfield had a change in management this year and therefore was only harvested and mowed.

Prune Phenology

This year prune fields in all regions produced only half of what was seen in 2022 (3-5 buds per stem) (Figure 4). Given the wet season and abundance of growth, we would expect high bud counts going into next season. However, low bud counts may be due to leaf spot diseases prominent in all regions due to moist conditions. Many of the sampled stems lost their leaves before buds could develop or the buds died while developing. This result means that in wet years with prominent leaf spot, it is important to manage this disease in the prune fields.

The bud count for the Mid-coast and Ellsworth regions started to decline on 09/25/23 (Figure 4). This is because some of the buds continued to develop into flowers. This was first documented by Collins and Drummond (2018) in the fall when studying fertilizer and fungicide effects on wild blueberries. They noticed an increase in fall bloom over the past two decades, but after evaluating clones in fields, less than 5% of the plants had flowers in Winterport, ME (Collins and Drummond, 2018). *Vaccinium myrtillus* (European blueberry) plants in pots were grown outside during the summer and then put into a greenhouse with a 20 hr photoperiod and a temperature of 64°F (18°C) on October 1st after plants had lost their leaves (Selas et al., 2015). It was found that the plants were not completely dormant and were able to produce flowers (Selas et al., 2015). Even when plants were subjected to different chilling periods in the dark (34°F (1°C)) they still flowered until they were chilled for four or more weeks then they went into dormancy (Selas et al., 2015). The growing season in New England is predicted to extend into the fall months with sporadic warm spells in the spring before the end of frost events (Wolfe et al., 2018). This year in Washington County, the average temperature in October was 52°F (11°C) with a max temperature of 78°F (26°C) on 10/28/2023 and a minimum of 27°F (-2.7°C) on 10/31/2023 (NOAA/NCEI, 2023). This high average temperature for the month could help explain why some of the plants were flowering in prune fields.

Prune fields were evaluated for fall bloom in the Mid-coast, Ellsworth, Downeast, and Far-Downeast regions. There was a high number of stems observed in Orland, Union, and Northfield (Table 2). However, these numbers are negligible and almost zero percent. This is good news for farmers and the objective of this survey was to determine how significant an issue fall bloom is and if a more refined testing protocol is needed since little research has been done on this topic. One way to prevent buds from developing further in the fall would be to apply a plant growth regulator which has not yet been studied in wild blueberry. So far, Collins and Drummond (2018) and our results have not found any detrimental losses from fall bloom across the state, but we will keep observing this during the fall of next year.

CURRENT RECOMMENDATIONS

- Manage leaf spot disease in prune years.
- 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 2024.

- Quantify and monitor fall bloom, fruit, and vegetative growth in 2024 and future years.

ACKNOWLEDGEMENTS

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2. Effects of Historical and Future Precipitation on Wild Blueberries

INVESTIGATORS: A. Bello and R. E. Schattman (University of Maine Agroecology Lab)

OBJECTIVES:

- Explore the morphological effects of wild blueberries under two current precipitation scenarios and one probable future precipitation scenario.
- Determine the effects of precipitation amount and seasonal distribution on crop development.

LOCATION: Roger Clapp Greenhouse, University of Maine, Orono

PROJECT TIMEFRAME: May 2022 - October 2023

INTRODUCTION

Wild blueberry growers in Maine (U.S.A.) face challenges imposed by changing climate conditions (Tasnim et al., 2021). Rainfall variability and episodic drought have already impacted agricultural production in the region, and are expected to present further challenges in coming decades. Over the last century, Maine's climate has warmed by an average of 3^{o}F (1.66^{o}C), with the highest temperature increases occurring along the coastal areas. These regions are also where the majority of wild blueberries are grown in the state. In addition to changes in temperature, the total annual precipitation has increased by 6 inches (15.24 cm) since 1895 (Birkel et al., 2018).

A warming climate has multiple effects on soil-water-atmospheric dynamics. Rising atmospheric temperatures increase saturated vapor pressure, which has the potential to increase both precipitation amount and evaporative demand (Ficklin & Novick, 2017). Increases in evaporative demand (ETo) is a key indicator of hydrological intensification, as conditions characterized by high ETo lead to faster water evaporation from soils (Huntington, 2006). At the same time, excessive precipitation in single rain events can lead to leaching of nutrients from root zones and contamination of ground and surface waters.

Changes in precipitation patterns are increasingly affecting plant growth, physiology and edaphic features of wild blueberries. However, the future influence of these changes on Maine wild blueberries is not yet fully understood. Considering this knowledge gap, we tested two historical and one probable future precipitation scenarios to determine the effects of changing rainfall patterns on the edaphic conditions and the morphological and functional traits of wild blueberries. The overall goal of this study was to determine the impact of changing precipitation patterns on wild blueberries by the end of the 21st century. Gaining a better understanding of how this crop will be affected by climate change can help wild blueberry growers develop climate change mitigation strategies for future food security and sustainable business development.

METHODS

This study was conducted during the wild blueberry growing season of 2022 and 2023 (between April and October). Plants were harvested from Deblois in Washington County, Maine, United States (Lat. 44^{o} 36’34.57” N, Long. 67^{o}55’38.40” W). Forty-five study plants (~10” x 10” in size) were harvested from six in situ plants, tagged and maintained for data collection and measurements for the duration of the experiment. Three of these six “parent plants” chosen (A, B, and C) were in "crop" year in 2022, while the remaining three (D, E, and F) were in "prune" year, which produced flowers and fruits in 2023. This determination was made based on the biennial nature of the crop, prior research showing the high degree of genetic diversity in wild blueberry fields (Beers et al., 2019; Barai et al., 2022), and visual observation of plant phenological differences. Plants in each group (ABC and DEF) received the same experimental treatments over two growing seasons. Three treatments were developed for this study. Two were developed from observed historical precipitation, allowing us to develop a day-by-day watering calendar that mimicked rainfall amounts during a typical wet and typical dry year in recent history. The final treatment simulated a plausible precipitation calendar that reflected climate forecasts relevant to Maine. Such forecasts estimate that Maine will receive more annual rainfall in coming decades, but that this rainfall will arrive in shorter periods punctuated by dry periods or drought.

All treatment schedules (see Figure 1) were built upon daily precipitation observations from the PRISM dataset for the warm season (May 1st - October 30th) for the years 2001 and 2006 from Jonesboro, Maine (Record period 1991-2020). The location has rocky shorelines with well-draining acidic sandy loam soils. It is characterized by a temperate climate with an average precipitation of 51 inches (1.29 meters) per year and snow cover of 65 inches (1.65 meters) per year, making it one of the wettest locations in Maine. The three treatments were as follows:

1. **HistDry:** 2001 was a dry year on record in the early 21st century in Maine. 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 was 1.38 inches (3.51 cm) on 18 May, and the total May – October rainfall was 12.53 inches (31.79 cm). Figure 4 shows precipitation accumulation across the growing season for this and the following treatments.
2. **HistWet:** Based on 2006 observations, there were frequent and well-distributed rainfall events throughout the growing season, without any prolonged period of dry days. Within the range of May - October, rainfall exceeding 1 inch (2.54cm) occurred on eleven separate days. The total May – October rainfall was 36.5 inches (92.62cm).
3. **Amp2.915:** Modified from the observed 2001 precipitation record (HistDry). Daily values were multiplied by a factor of 2.915 to increase the total growing season rainfall to match that observed in 2006 (HistWet). This plausible future scenario is intended to simulate Maine's growing season precipitation patterns as they may occur by the end of the 21st century.

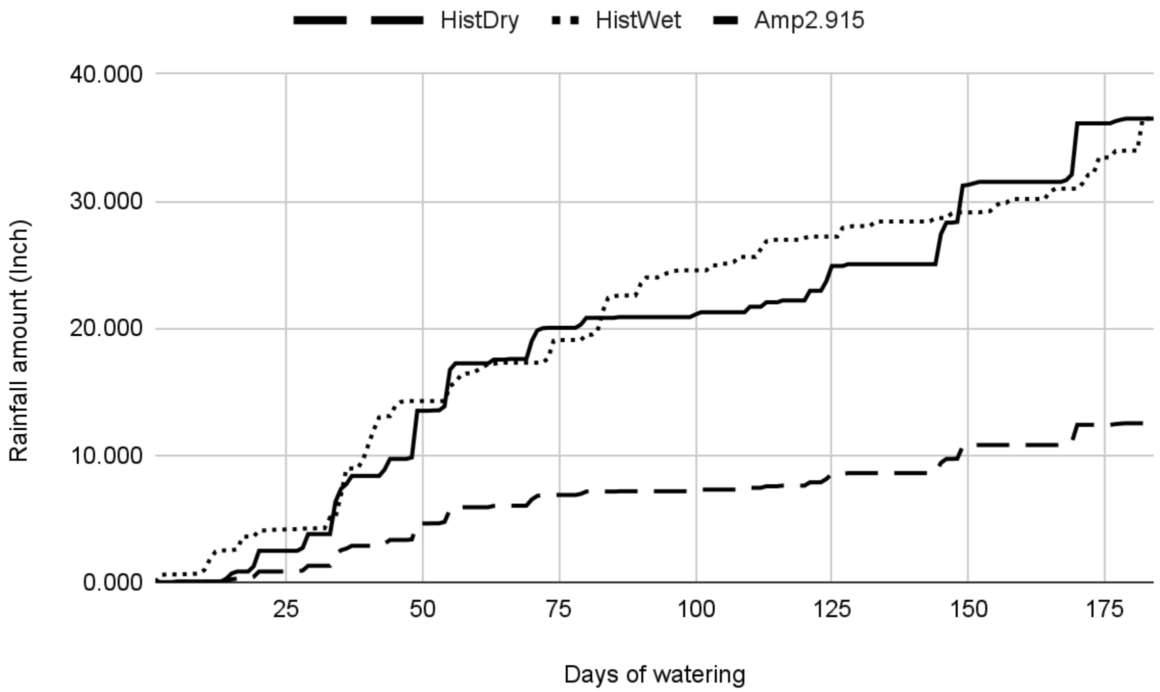

Figure 1. Cumulative water applications of the experimental treatments (HistDry, HistWet and Amp2.915). In 2022, day 0 = May 20. In 2023, day 0 = April 30.

Prior to analysis, data were checked for normality using the Shapiro-Wilk's test, Q-Q plots and histograms. Kruskal-Wallis and Analysis of Variance (ANOVA) tests were conducted using R statistical software (4.3.2, 2023) to test and compare the overall performance of several functional traits (leaf chlorophyll concentration, leaf fluorescence, and leaf temperature), morphological traits (stem length, number of leaves and branches per stem), and edaphic conditions (soil water content, soil temperature, and soil electrical conductivity). For assessing functional traits, three stems from the center of the pots were tagged and monitored using a SPAD Chlorophyll Meter (SPAD MC-100, Logan, Utah, USA), Portable Fluorometer (Prmyslová 470, 664 24 Drásov, Czech Republic), and Infrared Thermometer (Fluke Corporation, Everett, WA, USA) for leaf chlorophyll concentration, leaf chlorophyll fluorescence, and leaf temperature, respectively. Soil conditions were measured with a Fieldscout TDR 150 Soil Moisture Meter (Spectrum Technologies Inc., Aurora, IL, USA) for soil water content (SWC), soil temperature, and electrical conductivity.

RESULTS

Here, we present an abbreviated selection of findings. For an in-depth report, please contact the lead author of this study. Specifically, we present findings on the effect of precipitation scenarios on one morphological trait (average number of leaves per stem), one functional trait (leaf chlorophyll fluorescence), and one edaphic condition (soil electrical conductivity). Note that for reasons explained in our companion report, we will not reference yield data or fruit quality in our results.

First, Kruskal-Wallis H-tests were used to determine the relationship between treatments and plant morphological traits. It should be noted that this analysis was only completed during the fruit year (2022 for transplants in group ABC; 2023 for transplants in group DEF). In 2022, there was not a statistically significant relationship between the average number of leaves per stem (Figure 2A). However, our results revealed that plants watered according to Amp2.915 had significantly more leaves than HistDry in 2022 (P = 0.042). In 2023 (Figure 2B), the HistWet treatment led to plants with statistically more leaves than HistDry. This suggests that the amount of precipitation, especially early in the season, may have a greater influence on leaf development than distribution of water across the growing season. Both the Amp2.915 and HistWet treatments had a relatively high annual precipitation total, with heavy rainfall early in the growing season. Amp2.915 was also characterized by uneven distribution across the season, with a notably dry period between days 75 and 125 of the experiment. These results indicate that low rainfall early in the growing season reduces the leaf production of wild blueberries, but that dry periods later in the season (after leaf development stage) do not necessarily lead to reduced leaf retention.

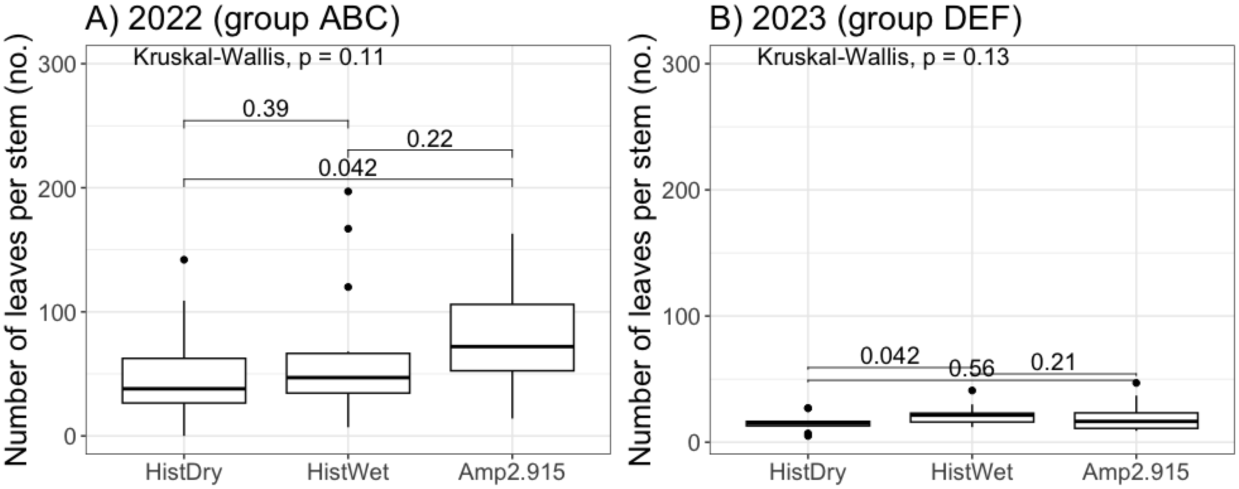

Figure 2. Kruskal-Wallis H-tests and post hoc results assessed the differences in the average number of leaves per stem of wild blueberries.

Second, we examined the effect of precipitation treatments on plant functional traits, including leaf chlorophyll fluorescence (measured in Fv/Fm) which is often used as a proxy for assessing crop stress. Chlorophyll fluorescence, or photosynthetic potential, is the ratio of variable to maximum fluorescence. In the present study, a statistically significant difference among treatments was observed in leaf chlorophyll fluorescence in both 2022 (P = 0.003) and 2023 (P = 0.010). This indicates the significant effects of rainfall amount and distribution on leaf water stress of wild blueberries. However, results suggest a lack of consistency in which treatment induced the most crop stress. For example, in 2022 the HistDry treatment induced the least amount of stress in the crop. In 2023, HistDry and HistWet induced similar degrees of crop stress. One possible explanation for this is variation in drought resistance of different wild blueberry genotypes. Our future work will examine both (a) the relationship between precipitation and Fv/Fm over time using linear models which will better describe inter-seasonal variation, and (b) genomic analysis of individual plants.

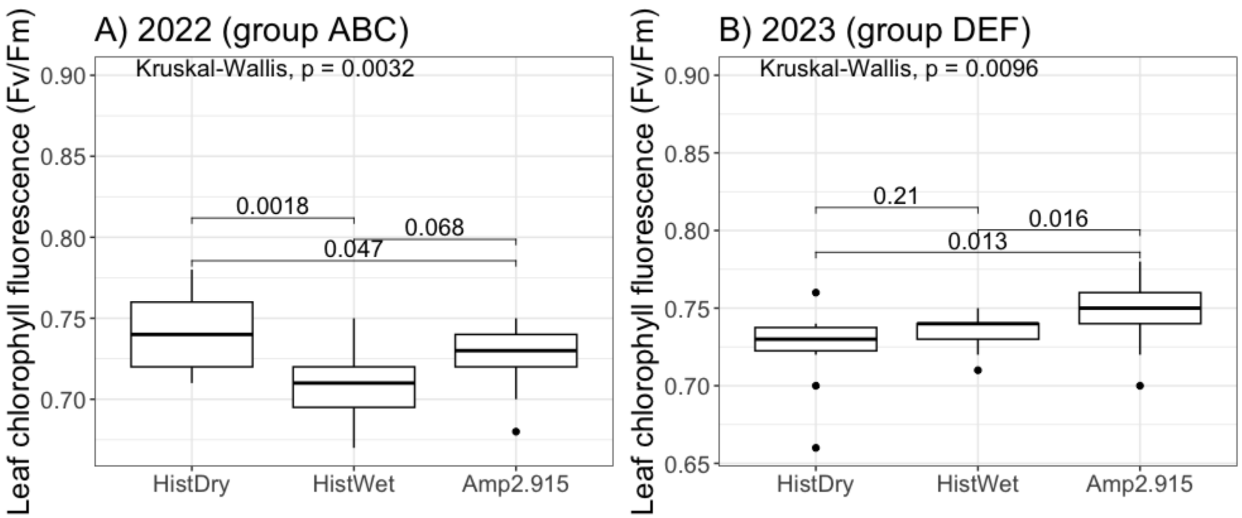

Figure 3. Kruskal-Wallis H-test and post hoc test results assessed the differences in leaf chlorophyll fluorescence (Fv/Fm) in wild blueberries. Note that lower Fv/Fm values indicate higher levels of plant stress. Conditions are only reported for the fruit year for each group.

Third, we assessed the effect of precipitation treatments on soil electrical conductivity, an example of an edaphic condition that affects crop nutrient access. Soil electrical conductivity (SEC) is driven by a collection of factors including the presence/absence of soluble salts, percentage clay content, present/absence of minerals, soil water content, bulk density, and more. Kruskal-Wallis tests were again used, along with post hoc tests to assess pairwise differences. A statistically significant difference was found among and between treatments in SEC in both 2022 and 2023 ($P < 0.001$, Figure 4). This suggests that evenly distributed rainfall across the growing season increases SEC in wild blueberry soils and by extension will enhance nutrient availability, while low rainfall amounts across the growing season will most likely reduce plant access to important nutrients.

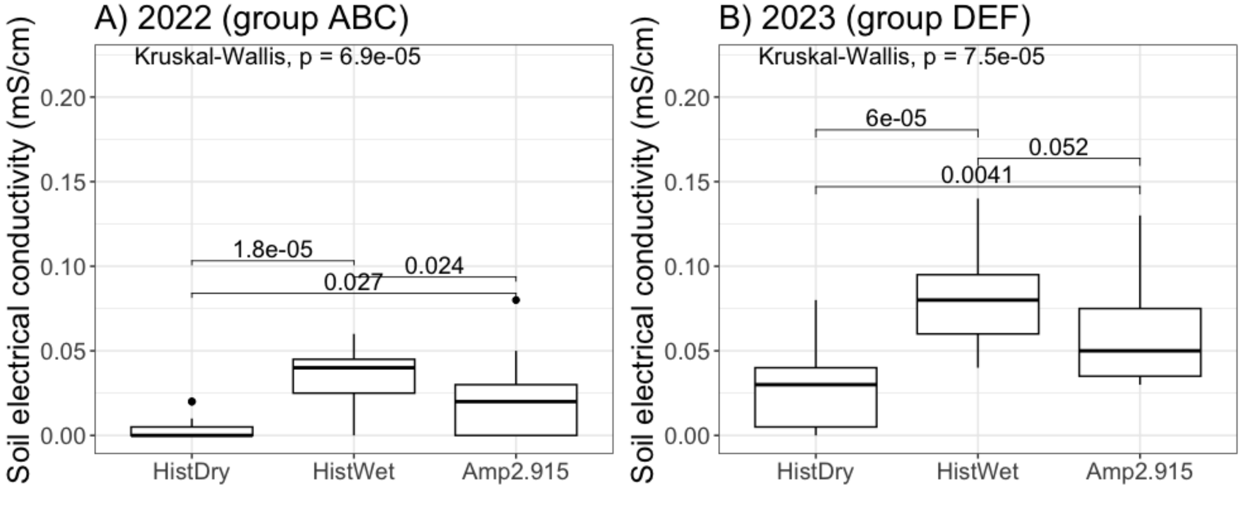

Figure 4. Kruskal-Wallis H-tests and post-hoc results assessed the differences in soil electrical conductivity (SEC) in wild blueberries. Conditions are only reported for the fruit year for each group.

DISCUSSION

This study demonstrates that total amount and seasonal distribution of precipitation has noticeable effects on wild blueberry morphology, functional traits, and soil edaphic conditions. While some results are straightforward, others are more difficult to parse and require further study. The most important conclusions described in this report are summarized, and grounded in the prior literature, below.

First, we found that precipitation that arrives early in the season, when wild blueberries are developing their leaves, has the greatest influence on the average number of leaves per stem. The number of leaves per stem increases the energy storage and survival rates of plants and is positively correlated with stomatal density (Dambroskie and Aarsen, 2012). Stomatal density modifies growth characteristics, photosynthetic capacity, and water use efficiency of wild blueberries. Therefore, higher numbers of leaves per stem can enhance the ability of wild blueberries to respond to the environmental stress and strike a balance between energy storage and fruit production. It has also been found, however, that greater leaf mass is negatively correlated with yield in wild blueberries (Barai et al. 2022). This raises an important question for growers to consider: Is the management goal healthier plants or higher yields? If the goal is healthier plants that can withstand an increasingly dramatic swing between wet and dry conditions, a higher number of leaves is indeed desirable. If the goal is higher yields, then an increased number of leaves may not be a satisfactory outcome.

Second, while we found that total annual precipitation amount *and* seasonal distribution affect chlorophyll fluorescence. However, our results do not clearly point to the direction in which this relationship runs. In 2022, the driest conditions led to the least amount of plant stress, while in 2023 there was no significant difference between the driest treatment (HistDry) and the historically representative wet treatment (HistWet). One possible explanation for this is variation in drought resistance of different wild blueberry genotypes. Wild blueberry is a highly genetically diverse crop where thousands of genetically distinct individuals can exist in a single field (Beers et al., 2019). Our future work will examine both (a) the relationship between precipitation and florescence, a proxy for plant stress often expressed as *Fv/Fm,* over time using linear models which will better describe inter-seasonal variation, and (b) genomic analysis of individual plants.

Third, we found that precipitation frequency is an important driver of soil electrical conductivity (SEC), which is a measure of how well soils transmit electrical currents. Wet soils are known to transmit electricity more effectively than dry soils. As wild blueberries often grow in soils that are gravelly and well drained, they are subject to fluctuations in SEC throughout the season. It is well known that soil water is needed to facilitate nutrient uptake by crops. Prior studies have shown that higher measures of SEC are associated with higher amounts of inorganic nitrogen (NO_3-N) in soils (Zhang and Wienhold, 2022). University of Maine Extension nutrient recommendations for wild blueberries prescribe applying nitrogen and phosphorus according to leaf tissue test results (Yarborough, 2013). Growers should be aware that applying fertilizers while SEC is low is likely to inhibit crop uptake of these important nutrients.

CURRENT RECOMMENDATIONS

Understanding the effects of climate change can be complex and difficult to respond to. This research seeks to simplify this complexity enough to make meaningful management recommendations. Specifically, we suggest that growers prepare themselves to supplement crop water needs with irrigation. Recent droughts in Maine have had a notable effect on wild blueberries. Our results and those from previous studies show that, while wild blueberries are robust and adaptable to an array of weather conditions, their adaptations may not always be advantageous for commercial production. For example, an increased number of leaves per stem may lead to greater drought resistance, but diminish yields. The best way to ensure crop health is to provide even amounts of water across the growing season, in both crop and vegetative years.

NEXT STEPS

This project is complete.

FUNDING

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3. Assessment of Water Needs in Wild Blueberries at Four Phenological Stages

INVESTIGATORS: A. Bello and R. E. Schattman (University of Maine Agroecology Lab)

OBJECTIVES

- Assess the effects of irrigation treatments on morphological traits, functional traits, and soil conditions.
- Segment findings by phenological stage to better understand when irrigation is more, or less, important for ensuring crop health and productivity.

LOCATION: Roger Clapp Greenhouse, University of Maine, Orono

PROJECT TIMEFRAME: May 2022 - October 2023

INTRODUCTION

In Maine, wild blueberries (mostly *Vaccinium angustifolium* Ait. and some *V. myrtilloides* Mitchx.) are often grown without irrigation. However, in recent years, variability of rainfall across the growing season has necessitated a broader use of irrigation. Although wild blueberries are considered drought resistant, several studies have indicated that the application of supplemental water during the dry periods can reduce crop failure, improving yield and quality of harvested berries (Struchtemeyer, 1956; Hunt, 2009). Considering this, it has been recommended that water be supplied evenly across the season (through rainfall or irrigation), or growers may suffer severe losses. For example, drought conditions experienced in Maine in 2020 were partially responsible for 43.7% mean crop loss among a subset of wild blueberry growers (Schattman et al., 2021). Drought is projected to have a significant influence on future agricultural water demand and supply (Birkel and Mayewski, 2018). It is expected that drought will present major challenges in wild blueberry production in Maine in the future.

Wild blueberries propagate through rhizomes that give rise to new roots and stems. Drought conditions impede the spread of rhizomatous roots, resulting in reduced growth and development of this crop. The crop has a shallow root system, which makes it susceptible to both drought and excess water conditions. Prior research suggests that, for optimal growth and development, wild blueberries require one inch (2.54 cm) of water per week in both crop and prune years (Hunt et al., 2008). The reliability of consistent precipitation at this level is variable across the season, however. Long-term climate projections for wild blueberry production regions in Maine and maritime Canada suggest that wild blueberries will receive enough water (in the form of rainfall) in only one out of every five years in August (Dalton and Yarborough, 2004; Hunt, 2009).

Rather than relying on rainfall alone, irrigating wild blueberries in both crop and prune years can significantly increase the vegetative growth and fruit yield (Hunt, 2009). Although a number of research projects on plant-water needs of wild blueberry have been previously conducted, a simple technique to determine plant-water demand at various stages of wild blueberry developmental and production cycles has yet to be explored (Percival et al., 2003). We hypothesize that plant-water demand at various growth and developmental stages is not well aligned with current irrigation scheduling recommendations. The purpose of this study is to establish recommendations for wild blueberry irrigation schedules that consider optimal amounts, frequency, and crop developmental stages. Results will help growers fine-tune their irrigation scheduling approach.

METHODS

This study was conducted between April and October in 2022 and 2023 at the unheated high tunnel located in the nursery section of Roger Clapp Greenhouse, University of Maine, Orono campus. Wild blueberry plants were harvested from Deblois in Washington County, Maine, United States (Lat. 44°36'34.57" N, Long. 67° 55'38.40" W). Forty-five study sub-plants (~10" x 10" in size) were harvested from six field plants (between 7-8 sub-plants from each field plant), tagged, and maintained for data collection and measurements for the duration of the experiment. Three of these six "parent plants" (labeled A, B, and C) were in "crop" year in 2022, while the remaining three (D, E, and F) were in "prune" year. The ABC group produced flowers and fruit in 2022; the DEF group produced flowers and fruits in 2023. In 2022, bee colonies were introduced into the tunnel on May 20th, to enhance pollination; however, they were later removed on July 14th. This study design was developed based on the biennial nature of the crop, prior research showing the high degree of genetic diversity in wild blueberry fields (Beers et al., 2019; Barai et al., 2022), and by visual observation of plant phenological differences.

The study used a factorial design (2 x 3) to assess effects of irrigation frequency, with crop cycle being the first factor. The treatment schedule consisted of three irrigation frequencies, including 1-inch of water per week (high irrigation frequency, HIF), 2-inches of water every other week (medium irrigation frequency, MIF), and 3-inches of water every third week (low irrigation frequency, LIF). In each of these three treatments, the total volume of water used over the simulated growing season was equal to 27 inches (68.58 cm). Individual plants were handled under the same treatment for both years of the study (2022, 2023).

Plant morphological traits assessed in this study included number of leaves, number of branches, number of flowers, and stem length. A single stem from the middle of the pot was selected, tagged and maintained for data collection on morphological traits. For the functional traits, three stems from the middle of the pots were selected, tagged, and maintained for assessment of leaf chlorophyll concentration, leaf chlorophyll fluorescence and leaf temperature, using a SPAD Chlorophyll Meter (SPAD MC-100, Logan, Utah, USA), Portable Fluorometer (Prmyslová 470, 664 24 Drásov, Czech Republic) and Infrared Thermometer (Fluke Corporation, Everett, WA, USA), respectively. The soil conditions assessed in this study included soil water content (SWC), soil temperature, and soil electrical conductivity, all of which were measured using a Fieldscout TDR 150 Soil Moisture Meter (Spectrum Technologies Inc., Aurora, IL, USA). SWC was measured a day before watering, in an effort to capture the driest part of the irrigation cycle.

A series of Kruskal-Wallis H-tests were used to determine trait variation (i.e., leaf, floral and fruit phenology) between treatments at four phenological stages. Wilcoxon post hoc tests were used to determine differences between treatments. An alpha level of 5% was used to establish significance. The effects of irrigation treatments on morphological traits, functional traits, and edaphic conditions were analyzed using R statistical software (Version 4.3.2, 2023) and the packages ggplot2 (V3.4.4; Wickham, 2016) to create box and bar plots, and ggpubr (Kassmbara, 2023) for visualizations of paired comparisons.

RESULTS

For the purpose of this report, we focus on a selected number of findings. A more complete report on our study will be made available upon request. Here, we focus on leaf development, leaf chlorophyll concentration, flower development, and soil water content. We do not include yield data in this summary. In 2022, squirrels invaded the experimental site and ate all the ripening fruit. In 2023, most plants did not produce sufficient numbers of berries for us to perform a reliable statistical analysis.

Our results indicated that irrigation frequency affected the average number of leaves per stem, though these effects differed depending on treatment group (ABC versus DEF, Figure 1). In 2022, there was no significant difference between treatments in the average number of leaves per stem at leaf development stage or green fruit stage. However, high irrigation frequency (HIF) was associated with a larger number of leaves per stem at the fruit ripening stage, compared to low irrigation frequency (LIF). This suggests that extended periods between waterings left plants vulnerable to senescence and leaf loss later in the growing season, especially when plants produced high numbers of leaves. In 2023, no significant difference was observed between treatments in either leaf development, green fruit, or fruit ripening stages.

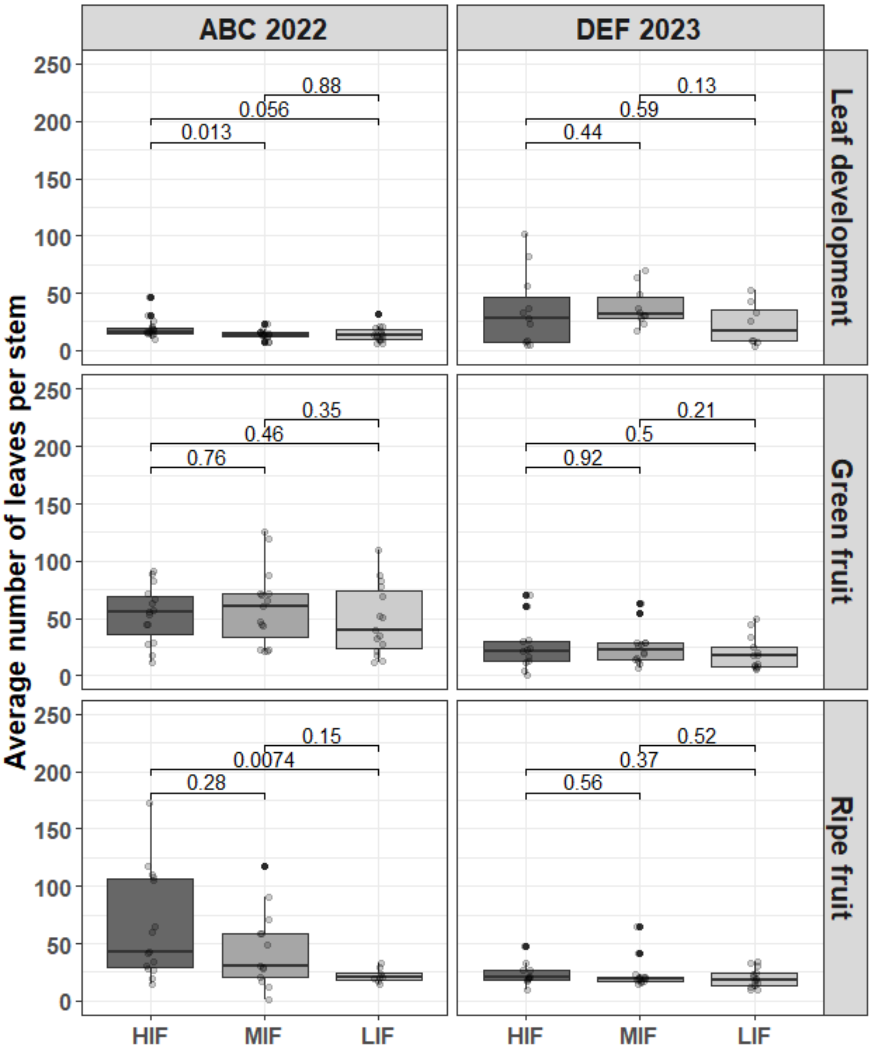

Figure 1. Average number of leaves by treatment at leaf development stage, green fruit, and fruit ripening stages. Error bars show standard deviation. HIF = high irrigation frequency, HIF; MIF = medium irrigation frequency; LIF = low irrigation frequency.

Leaf chlorophyll concentration largely determines the photosynthetic capacity of wild blueberry leaves. There was a noticeable and significant impact of irrigation frequency on the leaf chlorophyll concentration of the ABC group during the leaf development stage, but not other phenological stages (Figure 2). Our results show that plants in this group that were watered under the HIF treatment had significantly higher photosynthetic rates than plants watered under other treatments at the leaf development stage only, and only in 2022. In addition, our results suggest that irrigation spaced multiple weeks apart (LIF) can reduce the photosynthetic rate of wild blueberries at the leaf development stage. Additionally, chlorophyll concentration of plants in the DEF group was not significantly affected by irrigation frequency at any phenological stage, with the exception of the green fruit stage. At this stage, plants watered under the LIF treatment had leaves that had lower SPAD readings than plants watered under MIF treatments.

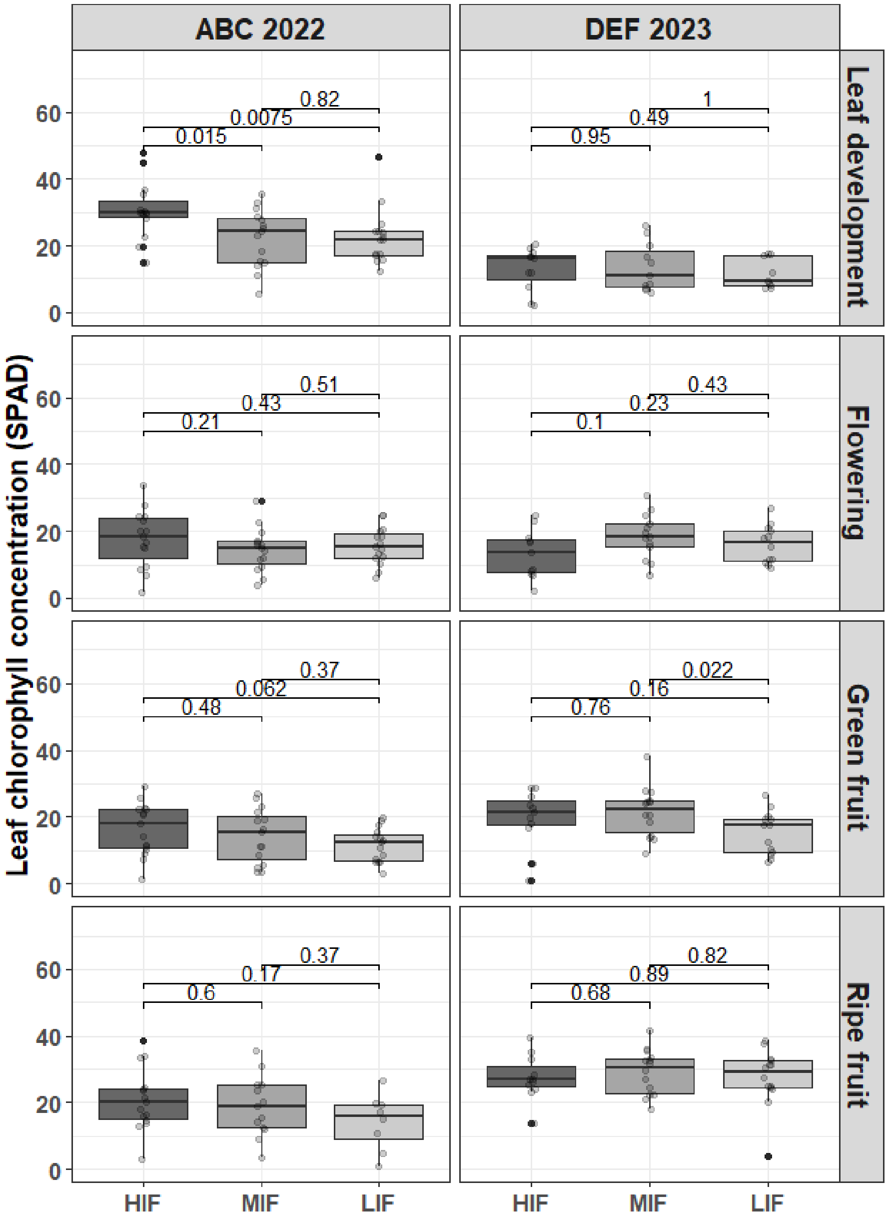

Figure 2. Leaf chlorophyll concentration (SPAD) by treatment at leaf development stage, flowering stage, green fruit, and fruit ripening stages. Error bars show standard deviation. HIF = high irrigation frequency, HIF; MIF = medium irrigation frequency; LIF = low irrigation frequency.

As shown in Figure 3, the irrigation frequency had a minimal effect on the number of flowers of wild blueberries. In 2022, the plants in group ABC that were watered under the MIF treatment had significantly more flowers than plants watered under the LIF and HIF treatments. There were no significant differences between treatments in 2023. This suggests that, at flowering, reduced frequency of watering may not have severe negative effects on crop potential.

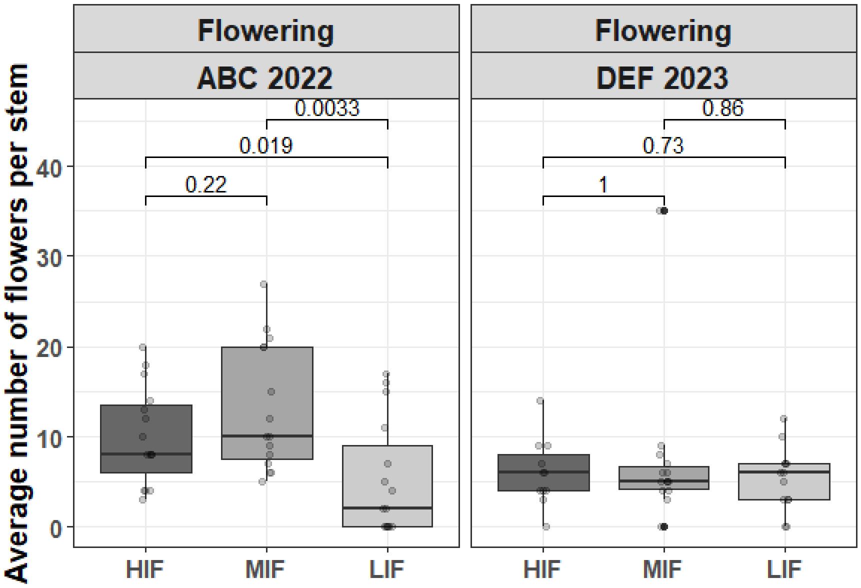

Figure 3. Number of flowers by treatment at flowering stage. Error bars show standard deviation. HIF = high irrigation frequency, HIF; MIF = medium irrigation frequency; LIF = low irrigation frequency.

Our results indicate that irrigation frequency has a considerable impact on the soil water content (SWC). In 2022 and 2023, the soils managed under HIF and MIF treatments plants had significantly higher soil water content ($P < 0.001$) when compared to soil managed under the LIF treatment, specifically at leaf development stage (Figure 4). As leaves are developing, the evapotranspiration rate of a plant is likely reduced. This likely makes the variation in our experimental water applications more obvious in soil conditions. This trend was also evident in the ripe fruit stage. Often, soil water content did not differ significantly between the HIF and MIF treatment. The exception to this was at flowering stage (in both 2022 and 2023), where the HIF treatment had significantly higher soil water content than MIF. This was also true at the fruit ripening stage in 2023. Our results indicate that the soil water content of wild blueberry soils increases with an increase in irrigation frequency, and that growers need to be attentive to when crop water needs are highest (flowering, green fruit, and ripe fruit stages) to ensure that crops receive adequate water during these periods.

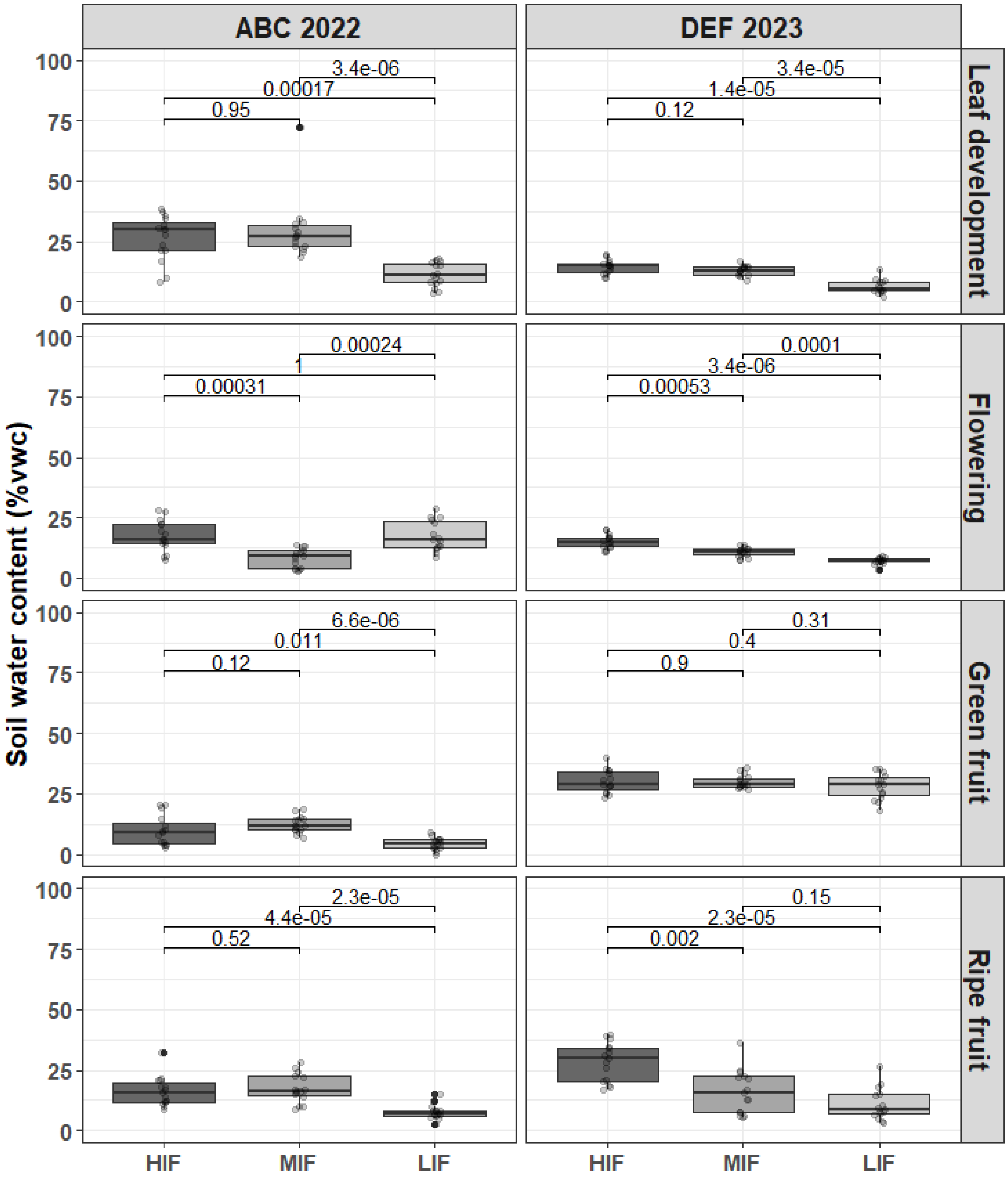

Figure 4. Soil water content (%VWC) by treatment at leaf development stage, flowering stage, green fruit, and fruit ripening stages. Error bars show standard deviation. HIF = high irrigation frequency; MIF = medium irrigation frequency; LIF = low irrigation frequency.

DISCUSSION

Leaf development stage

Leaves function to store water and produce chemical energy, thereby playing a significant role in the overall functions of plants (Dawson and Goldsmith, 2018). The number of leaves per plant is a visual key trait (phenotype) that determines the growth rate and health status of plants. The average number of leaves did not statistically differ between treatments at the leaf development stage. Plants watered under the HIF treatment had higher leaf chlorophyll concentration and soil water content compared to LIF and MIF, though this trend did not extend consistently beyond leaf development stage. This points to the importance of consistent rainfall or irrigation during the early stages of crop development (in spring). The even distribution of rainfall at this point in the season is critical for development of healthy and robust plants with good photosynthetic capacity.

Flowering stage

There was no significant difference observed in leaf chlorophyll concentration (SPAD) among the treatments at the flowering stage. However, the results of pairwise comparisons indicate that, in some years, plants watered under the MIF treatment are more likely to have higher mean number of flowers per stem than plants watered under the LIF treatment. Applying 2-inches (5 cm) of water every other week has the potential to produce as many flowers as applying 1-inch (2.5 cm) per week. In some years, reduced irrigation/precipitation frequency will lead to reduced flower counts. As flowers are the reproductive organ of flowering plants, stress-induced changes in structure can have severe consequences on the functions of both plants and pollinators (Lawson and Rands, 2018). Soil water content (%vwc) is likely to be lower during flowering in wild blueberry soils that receive low frequency precipitation/irrigation.

Green fruit stage

Our results revealed no significant difference between treatments in the average number of leaves per stem in the green fruit stage. This finding is consistent with results from the leaf development stage, suggesting that water stress does not lead to senescence or leaf drop at this stage of crop growth. No significant differences were observed between treatments in chlorophyll concentration (SPAD) or soil water content at this stage.

Fruit ripening stage

Data on the number of ripened berries per stem was not collected at the fruit ripening stage. Plant stress incurred by the experiment led to a low number of ripened berries across the experiment, making statistical analysis unfeasible. However, we were able to collect data on other plant development characteristics (e.g., number of leaves per stem) and soil characteristics at this state, which were robust enough to perform statistical analysis. Our results indicated that there was a significant difference in the number of average leaves per stem in one year, but not the other. In 2022, our results showed a significantly lower number of leaves in the LIF treatment, compared to the HIF treatment. This indicates that, by the time wild blueberries are ripening, plants under water stress may begin to lose leaves. Despite the decreased number of leaves in LIF treated plants, we found that there was no significant difference in leaf chlorophyll concentration (SPAD). Our results also indicate that HIF increased the soil water content (VWC) of wild blueberry soils, with HIF soils consistently higher in soil water than LIF soils. Consistently moist soils likely have a positive effect on soil electrical conductivity, which in turn facilitates nutrient uptake by crops.

CURRENT RECOMMENDATIONS

Based on our findings, we propose that low irrigation frequencies (high volume waterings spaced far apart) are undesirable and likely lead to plant stress. Whether wild blueberry growers are relying on ambient precipitation or supplemental irrigation, water applications should be evenly dispersed throughout the growing season. It is of great importance that the crop has sufficient water (at least 1 inch per week) early in the growing season when leaves and flower buds are developing. Our results suggest, however, that irrigation frequency may be extended to 2 inches applied every two weeks in some cases. Many of our measures were not statistically different between the HIF and MIF treatments, including leaf chlorophyll concentrations (SPAD) at flowering, green fruit, and fruit ripening stages.

Growers who have limited time or labor availability may find this to be useful information, as some growers may not have enough water to irrigate every week and irrigation often requires attention and effort during the busiest times of the growing season.

NEXT STEPS

Our final steps in this project is to conduct further statistical analysis to assess trends across the season. This study will also inform future research studies starting at the Wyman's Wild Blueberry Research and Innovation Center, located at the University of Maine in Orono.

FUNDING

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4. Using Mulch and Biochar to Improve Wild Blueberry Soil Moisture

INVESTIGATOR(S): L. Calderwood, Y. Zhang, J. Parks, and S. Karki

OBJECTIVE:

- Evaluate softwood sawdust and woodchip mulch and several biochar products as drought management tools.

LOCATION: Blueberry Hill Farm Lab, Jonesboro, ME

PROJECT TIMEFRAME: April 2023 – March 2025

INTRODUCTION

This project continues research on the impact of mulches and biochars on crop production that began in 2021. This report discusses the expanded, properly replicated, and controlled study now underway at Blueberry Hill Farm in Jonesboro.

Wild blueberry plants grow well in sandy, well-draining soils but these same soils have low water-holding capacity, which inhibits plant 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 at least 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).

In drier, warmer, and longer growing conditions wild blueberry plants require more water (Tasnim et al., 2022). Higher air temperatures increase evapotranspiration and dry out soils (Tasnim et al., 2020). Low soil moisture results in small and fewer berries when plants are stressed (Barai et al., 2022). Therefore, growers are keen to maintain or increase soil moisture 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, require irrigation, or other management techniques to improve soil water content and yield. Other research is exploring the economic and logistic feasibility of irrigation systems on wild blueberry farms. Irrigation infrastructure is expensive and farm specific, requiring a lot of technical assistance to determine the location of wells and/or ponds.

Increasingly, growers use mulch to increase soil water-holding capacity and improve field water retention. This study tests the effects of softwood mulch, hardwood mulch, and three biochar products on improving soil water retention and plant and soil health. Mulches are materials broadcast across fields in the fall or spring after pruning and before plants emerge. Mulch is not incorporated into the soil. We have found that mulches (sawdust, shavings, and wood chips) benefit wild blueberry by reducing water loss, moderating soil temperatures, suppressing weeds, reducing leaf spot disease, reducing tip midge presence, 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. This makes wood chips (not bark) the most feasible and commonly used particle size. A second study on softwood woodchip mulch is underway to explore the economics and plant response to whole field mulching (see CIG Mulch Report on page 105). We are frequently asked if sawdust can be applied to wild blueberry fields. Kinder and Eggert (1966) explored sawdust where researchers incorporated sawdust into a homogenized soil and transplanted wild blueberry cuttings into a reconstructed soil. Neither treatment gives an accurate representation of how sawdust would interact with our current production system. They found a decrease in rhizome development and yield when sawdust was incorporated into the soil (Kinder and Eggert 1966). Because we recently found sawdust to be the material that improved soil moisture content the most, we included sawdust in this study.

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 lbs of inorganic nitrogen (available for plant use) and two lbs 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 which is not a practice done in wild blueberry production (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. Biochar can also increase soils' abilities to sequester carbon which is of benefit to the environment. Nutrients can also be sequestered by biochar. The benefit to biochar sequestering nutrients in some systems is that it holds onto nitrogen and phosphorous reducing the amount of nutrients that run off into waterways. Using biochar in this way can reduce water quality issues (Woolf et al., 2010; Wang et al., 2021; Blanco-Canqui, 2019; Abas et al., 2022; Rahman et al., 2021). However, we do not yet know how wild blueberry soils or plants will respond to the change in nutrient availability when biochar is present.

The quality and characteristics of biochar depend on the source material and the conditions in which it was manufactured. Utilization of biochar is of interest in Maine because of the state's robust forestry industry, which includes thousands of forestry landowners and sawmills, 10 wood pellet manufacturers, and 19 industrial biomass-based combined heat and power (CHP) plants (Novak et al., 2022). A few biochar companies have intentions to make and sell biochar in Maine, however none are currently available, and the price of material is not yet clear. At the University of Maine, it is our role to investigate practices and materials, such as biochar, for growers so they do not have to take unnecessary risk. Therefore, available raw biochar was sourced from where possible. The cost and feasibility of getting biochar to Maine at this time was a challenge. The wood biochar received has a high pH from the pyrolysis process. Budget constraints prevented our ability to acid-wash the biochar to get it down to an appropriate wild blueberry soil pH. The UMaine Forest Bioproducts Research Institute (FBRI) makes an acidic biochar with a pH<2, yet this material is unsuitable for direct field use because the extreme acidic condition could cause plant toxicity and hamper microbial activity.

Many questions remain about how different biochar products interact with the wild blueberry cropping system. The three questions that this study aims to address include: 1) Can raw biochar or wood ash alone increase water holding capacity of the soil in the field?, 2) Does raw biochar or wood ash cause damage to plants?, and 3) Does raw biochar or wood ash lock up nutrients in the acidic soil that would otherwise be available to plants?

METHODS

In May 2023, the study was laid out on a prune field at Blueberry Hill Farm in Jonesboro, ME. The experimental design was of a randomized complete block design where 14 treatments (Table 1) are replicated six times in 6 ft by 30 ft plots, for a total of 84 plots. Within each plot, two permanent 0.37 m^2 (61 cm x 61 cm) quadrats were flagged, and five random stems were identified with metal numbered tags within each quadrat Mulch Softwood chips and sawdust (Dean Young Forestry, Franklin Maine, USA) were applied on 6/2/23. Three biochar products were tested in this study including two commercially available biochar products (Wakefield BioChar, Georgia,USA, and Highland Pellets, Arkansas, USA). A wood ash/biochar mixture was also applied (Maine Wood Pellets, Athens, Maine, USA) and is a material derived from a local biomass Combined Heat and Power (CHP). Wakefield Biochar produces biochar from Southern Yellow Pine, with a pH of 10.66. Similarly, Highland Pellets utilizes pyrolysis on Southern Yellow Pine, with a pH of 10.00. In contrast, Maine Wood Pellets processes low-quality forest biomass using a CHP plant, yielding products with a pH of 11.40. Mulch application rates were determined based on previous results (Gumbrewicz and Calderwood 2022). Biochar rates were determined based on previous results (Tasnim and Zhang 2022).

Table 1. Study treatments replicated six times at Blueberry Hill Farm in Jonesboro Maine.

Abbreviation	**Treatment Details**
Control	No material applied
HP	Highland Biochar 7.5 yd^3 per acre
HP+P	Highland Pellets 7.5 yd^3 per acre
W	Wakefield Biochar 7.5 yd^3 per acre
Wx2	Wakefield Biochar 15 yd^3 per acre
Wx4	Wakefield Biochar 30 yd^3 per acre
WA	Wood Ash 7.5 yd^3 per acre
WAx4	Wood Ash 15 yd^3 per acre
Comp	Compost 7.5 yd^3 per acre
Comp+W Bioch	Compost + Wakefield Bioch (1:1 ratio) 7.5 yd^3 per acre
Sawdust 1.0	Softwood Sawdust 144 yd^3 per acre
Sawdust 2.0	Softwood Sawdust 169 yd^3 per acre
Woodchips 1.0	Softwood Woodchips 144 yd^3 per acre
Woodchips 2.0	Softwood Woodchips 169 yd^3 per acre

Soil Moisture

Soil moisture content was measured using a FieldScout TDR 150 Soil Moisture Meter (Spectrum Technologies, Inc., Aurora, IL, USA) to a depth of 12 cm (4.8 inches). Biochar materials were applied from July to August 2023. Five random soil moisture readings were taken from each plot on August 31st, September 23rd, and October 23rd, 2023.

Blueberry Phenology

The number of developing green buds and hardened brown buds were counted from the five stems within each quadrat. This was conducted on August 16th, 2023. Total stem counts within each quadrat were recorded on July 7th and August 16th, 2023.

Pest Evaluation

Within each sample area, blueberry cover, weed, insect, and disease presence were recorded on August 16th, 2023. Pest severity (percent cover) for weeds, insect and disease were quantified using equal interval ranks between 0 and 5, where: 0 = not present, 1 = ≤1-20%, 2 = 20-40%, 3 = 40-60%, 4 = 60-80%, and 5 = 80-90%. 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.

Limitations

Due to labor and supply constraints, the woodchip and sawdust treatments were applied two weeks late on June 2nd, 2023.Owing to the unavailability of locally sourced biochar, logistical constraints necessitated the transportation of biochar from both Arizona and Georgia. This process incurred a delay in the application of biochar treatments which ultimately took place between July 20th to August 10th 2023. Unfortunately, due to the late application of biochar, data collection on crop physiological performance for this year was not feasible. As of now, we only have soil moisture data available for analysis. Despite these challenges, we remain committed to the thorough documentation and analysis of the biochar's impact on our study.

Data Analysis

Most of the data collected this year (2023) could not be statistically tested for significance due to the lack of repeated measures. Descriptive statistics including mean and standard error were used to look at the data observationally to give us ideas of what might be expected next year. The only data set that was subjected to statistical testing was stem counts. For that data, JMP® Pro, version 17 (SAS Institute Inc., Cary, NC, 1989-2023) software was used. An ANOVA was used to test for significance. To test differences between treatments, a Tukey's HSD means comparison test was used with an $\alpha \leq 0.05$. Residuals of the model were tested for normality using a Shapiro-Wilks test and homoscedasticity was visually checked.

RESULTS

Soil Moisture

Relative soil moisture content was greater than 30% in all plots on all days (Figure 1). This suggests that 2023 was a wet year, and no soil water deficits have been detected. There was a statistically significant difference across treatments on August 31st of 2023, with WAx4 showing significantly higher soil moisture content than that of control ($P < 0.001$) (Figure 1). However, on subsequent assessment dates in September and October, no significant variations in soil moisture were observed among the treatments (Figure 1). In wet conditions, the effects of biochar and mulch on soil moisture are neglectable.

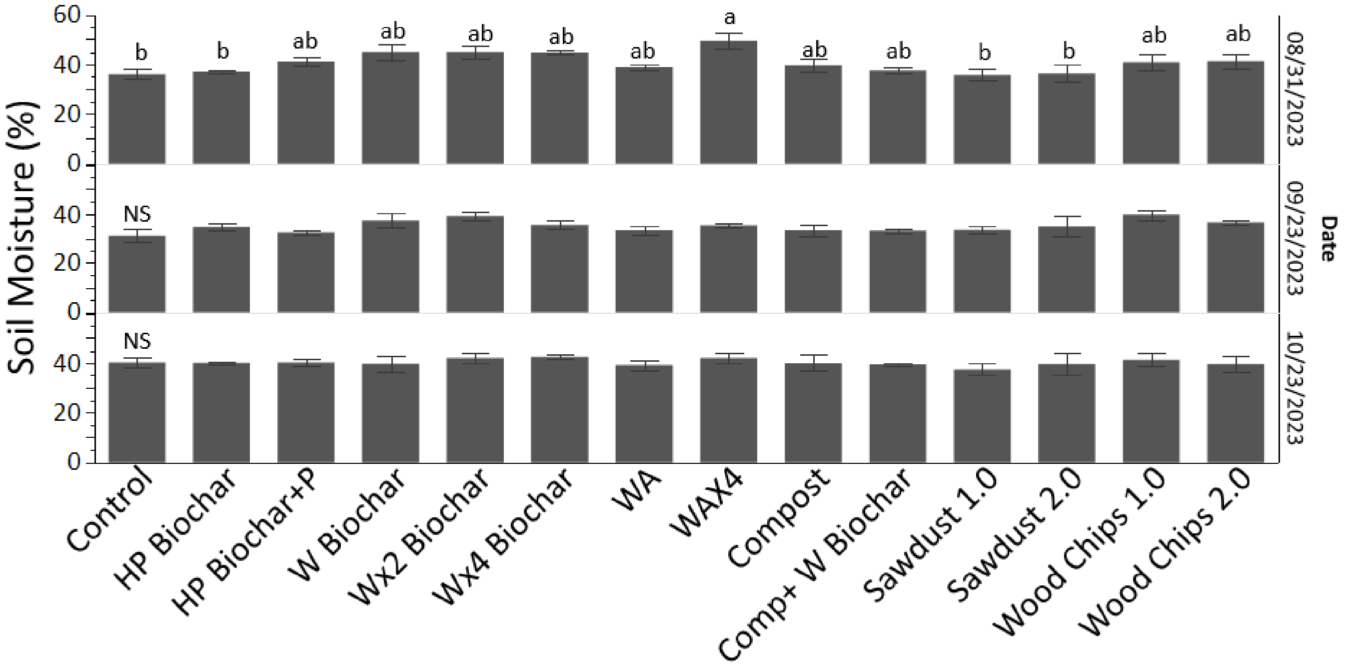

Figure 1. Volumetric soil water content for mulch and biochar treatments. Soil moisture was collected on August 31st, September 23rd, and October 23rd, 2023. Means with the same letters are not statistically different using the Tukey's HSD at an α ≤ 0.05. Error bars represent standard error of the mean. NS represents not significant treatment effects.

Blueberry Phenology

Because data was only collected once during the prune 2023 season, no statistical tests except descriptive statistics were conducted on the phenology data (Figure 2). Observationally, the other mulch treatments appear to have more buds than the control. Stem counts were also conducted on 7/7/23 and 8/16/23. The control had 35% more stems than 1.0 in sawdust mulch ($P = 0.007$) and 88% more than the 2.0 in wood chip mulch ($P < 0.001$) (Figure 3). All of the mulch treatments experienced lower stem counts than the mulch free control (Figure 3).

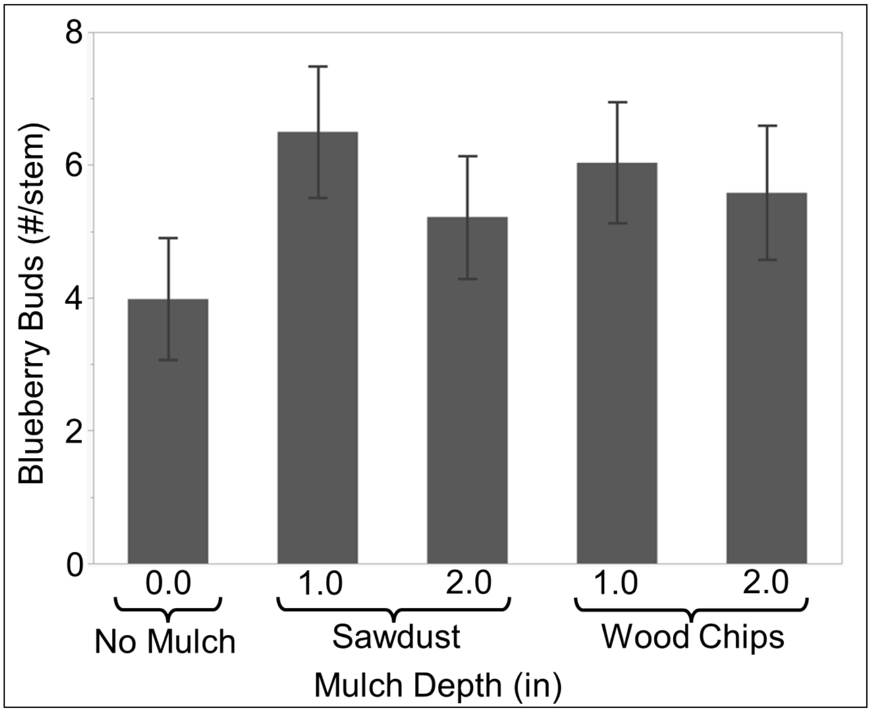

Figure 2. Number of blueberry buds (immature green buds and hardened brown buds) by mulch treatment in Jonesboro, ME Blueberry Hill Research Farm. Data collected 8/16/23. No statistical tests were conducted on data except for standard error due to lack of repeated measures. Error bars represent standard error of the mean.

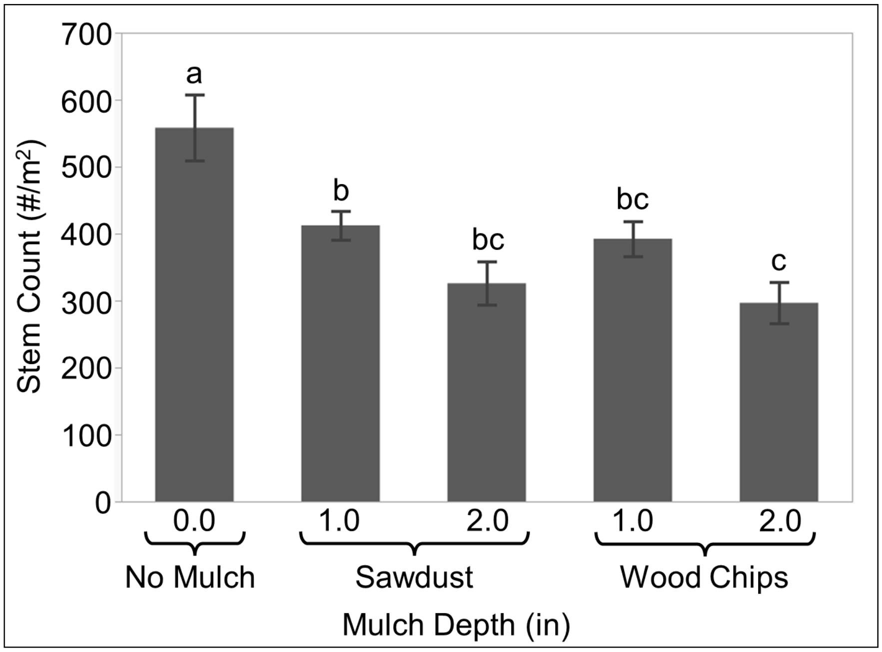

Figure 3. Blueberry stem count within different mulch types and depths. Data was recorded July 7th, and August 16th, 2023, in Jonesboro, ME at Blueberry Hill Farm. Means with the same letters are not statistically different using the Tukey's HSD at an α ≤ 0.05. Error bars represent standard error of the mean.

Pest Evaluation

The most prominent weed in the field was red sorrel (*Rumex acetosella*). Other weeds including dogbane (*Apocynum androsaemifolium)* and aronia (*Aronia melanocarpa)* were present as well but did not have a high enough density in all plots to show any trend. The amount of red sorrel in the control and sawdust 1.0 was preliminarily greater than the other mulches, but significance could not be accurately tested.

There were minimal insect pests in the prune field. The most abundant pest was tip midge (*Dasineura oxycoccana*), which caused the most damage to blueberry stems. There were also signs of red-striped fireworm (*Aroga trialbamaculella*), but the density in all plots were not great enough to display in a figure. Tip midge damage visually looked to be spread out across all treatments, but without more data, statistical differences cannot be made.

Due to the wet and cool growing season, leaf spot disease was in many fields throughout Maine. In Jonesboro, ME, leaf spot and powdery mildew (*Erysiphe*) were prominent at the research site. Data was collected 8/16/23 and because of the one collection, statistics except for descriptive statistic were not done on the data set. Viewing the data observationally, the 1.0in sawdust and wood chips and control plots had greater numbers of leaf spot than the other thicker mulch treatments. Powdery mildew visually looked to be evenly spread throughout treatments.

DISCUSSION

This report describes the baseline data collected after mulches were applied in the first prune year of this study. Due to labor and material constraints both mulches and biochar treatments were applied later than planned. It is best to apply mulch in the spring of the prune year before plant emergence.

Soil Moisture

The 2023 season was wetter than usual, which kept the soil moist. This was observed over the growing season where soil moisture was relatively constant between treatments (Figure 1). This was also observed in our CIG Whole Field Mulch trials in Hope and Orland page 105.

Soil pH

The high pH of the currently available biochar products could be a concern. However, there is currently no biochar on the market specifically designed with a low pH suitable for wild blueberries. Biochar application could increase soil pH due to its alkaline nature. Studies by Shokuhifar et al. (2021) and Smebye et al. (2016) have reported a considerable pH increase with biochar. In other agricultural systems, under higher soil moisture, no pH change was observed after biochar applications (Shokuhifar et al. 2021). In wet conditions, the impact of biochar and mulch on soil moisture appears to be minimal. This is likely due to the prevalence of anaerobic conditions when soil moisture content is high, leading to reduced oxygen levels. In anaerobic conditions, microbial processes become more active, potentially resulting in the release of protons (H+) and acidic compounds, contributing to a decrease in soil pH. On the other hand, under drier conditions, where oxygen is more available, aerobic microbial activities may have different effects on soil pH. Additionally, the influence of biochar on pH might vary depending on the moisture level and the specific chemical properties of the biochar. Another contributing factor could be the facilitation of leaching in high soil moisture conditions. Under these circumstances, certain compounds from the biochar might be more prone to leaching. If alkaline components are among those leached out, it could result in a diminished pH-raising effect of the biochar.

Blueberry Phenology

Stem counts were lower in all mulch treatments compared to the control. This is because mulch was applied June 2nd, about 2 weeks after blueberry emergence. The mulch buried some of the emerged stems, setting vegetative growth and stem density back.

Pest Evaluation

Leaf spot disease presence was high in this field which may impact plant health next season (2024). Many wild blueberry fields in Maine experienced leaf spot infections in their fields due to wet conditions this year. Leaf spot was highest in the control and 1.0in mulches compared to the 2.0in mulches (data not shown). The even spread of powdery mildew across the study area makes sense based on how this disease spreads. The small fruiting bodies (cleistothecia) of powdery mildew break open during periods of rainfall and expel spores (ascospores) which are carried by the wind to infect other leaves on blueberry plants (Hildebrand et al., 2016).

It is difficult to use insecticide on tip midge because the maggots are protected within the galled leaves. Collins (2017) determined that there is no difference in the population with sprayed and non-sprayed areas. If the issue is severe, a burn in mid to late June can be done to reduce populations, but this will negatively affect the blueberry plant's ability to produce flower buds in the prune year (Collins, 2017).

CURRENT RECOMMENDATIONS

Apply mulch and biochar in the spring of prune years before wild blueberry leaves emerge. When possible, give plenty of time between ordering and delivery of mulches and biochar.

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5. Whole Field Mulching for Wild Blueberry Drought Management

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

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 live in sandy, well-draining soils yet these soil characteristics 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.

Drought on Maine wild blueberry lands occurred in five of the past eight years. Years 2016, 2017, 2018, 2020, and 2022 were seasons where drought conditions occurred for at least part of the growing season while in 2019, 2021, and 2023 the crop received adequate rainfall (one inch per week for at least part of the season) (NOAA/NIDIS; Birkel, 2016 and 2019). Drought reduces wild blueberry crop yield. For example, 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). In 2020 total blueberry yield for the state of Maine was 47.4 million pounds and experienced one of the most severe droughts since 2000 (USDA/NASS, 2022; Birkel, 2020). In 2021, total blueberry yields increased to 103.8 million pounds with drought carrying over from 2020, but the growing season received adequate rainfall to mitigate the temperatures (USDA/NASS, 2022; Birkel, 2021).

Conditions in 2023 were lower temperatures and more precipitation compared to 2022. From May to August, 10 of 16 weeks had rainfall events over one inch (NOAA/NCEI, 2023). In May, two of the weeks received greater than one inch of rain; June received over one inch for three of the weeks; July received greater than one inch for two weeks; and August received over an inch of rain for three weeks, one of them receiving four inches (NOAA/NCEI, 2023). Total rainfall from May to August was 21 inches (NOAA/NCEI, 2023). The blueberry fields received adequate rainfall for most of the growing season which was beneficial.

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 the growing season or at other times of the year (Hunt et al., 2008).

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.

Many growers use wood chip mulch to build soil organic matter with the purpose of increasing wild blueberry rhizome spread into bare patches and to increase the 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 and 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 two 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 their available equipment 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 (40 quadrats total per site). Mulch was applied in early spring of the prune year before wild blueberry plants emerged. Wild blueberry stem stubble was about 1 in tall, making the stems in the 1.5 and 2 in 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 of stem height, stem density per quadrat, number of buds per stem, and collecting leaf chlorophyll content with a SPAD meter.

In the 2023 season, soil moisture, chlorophyll content, stem counts, and pest scouting were collected during the crop year to observe effects of wood chip mulch on fruit bearing plants (Table 1). Due to labor constraints, Orland stem counts were not able to be collected. In 2022, the same data that was stated previously was collected without the pest scouting measurements (Table 2). Two additional stem tags were randomly added within each quadrat and used for data collection bringing the total number of stem tags to five. Chlorophyll content was measured by sampling two leaves per tagged stem with a Soil Plant Analysis Development (SPAD) meter (SPAD 502; Minolta Corp., Osaka, Japan). High chlorophyll content values are correlated with a healthier plant. Soil moisture was measured using a Time Domain Reflectometry (TDR) meter (FieldScout TDR 150 Soil Moisture Meter, Spectrum Technologies, Inc., Aurora, IL, USA) to a depth of 12 cm (4.8 in), in two random areas within each quadrat.

Pest scouting in 2023 was conducted in each sample area for weeds, disease, and insect damage. This was done by classifying all weed species and counting them by group and counting damaged blueberry stems from disease and insects. The most prominent pests were used in analysis and displayed in figures.

Table 1. Data collection types and dates at both research sites in 2023.

Date	Location	Data collected
5/31/2023	Hope & Orland	TDR, Pest Scout
6/16/2023	Hope	TDR, SPAD
6/21/2023	Orland	TDR, SPAD
7/12/2023	Hope	TDR, Pest Scout
7/17/2023	Orland	Pest Scout
7/18/2023	Orland	TDR
8/15/2023	Hope	TDR, Stem Counts, Pest Scout

Table 2. Data collection types and dates at both research sites in 2022.

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

Yield and Berry Quality

Once the locations reached peak blue fruit, 07/25/2023 for Orland and 08/01/2023 for Hope, sample plots were hand raked. Once the fruit was collected it was weighed on a scale and then a random sample was taken for a lab analysis. In the lab, 100 count berry weights were recorded. These same samples were also pureed for brix measurements (Atago RX-5000i Refractometer, Atago Co, Ltd., Tokyo, Japan) to test the sugar content of the fruit. Yields were calculated and extrapolated from these eight sample sites for each treatment. Mulch debris collected in hand rakes was counted.

Data Analysis

The statistical software used for data analysis was JMP Pro 17 (SAS Institute Inc., Cary, NC, 1989–2023). Measures of chlorophyll content, soil moisture, and stem number were all analyzed using one-way ANOVA with an $\alpha = 0.05$. Time and Time*Treatment was used as a random effect in the model. Significant means were compared using a Tukey's honest significant difference (HSD) test. Normality of residuals from the model were checked using a Shapiro-Wilks test and homoscedasticity of residuals were visually checked. If the residuals failed to pass these checks the data was transformed. Once a successful transformation was performed on the data, the predicted formulas were saved, and a back-transformation was conducted to accurately display the data within a figure or table. If back-transformed data were significantly lower than original values, then the original data were used for clarity.

RESULTS

Analyzing precipitation and soil moisture can show how well mulch reduces water loss to the atmosphere or soil profile. The growing season for 2023 experienced 98% more precipitation than the 2022 season ($P < 0.001$) (Figures 1 and 3). In 2023 both locations were separated for analysis due to Hope having more frequent rainfall events than Orland ($P < 0.001$) and different crop histories. At the

Hope location, no statistical differences were detected in 2023 between treatments by date except for June 16th between the 0.5in and 1.5in mulch depths (Figure 1). In Orland, no significant differences were detected in 2023 (Figure 3). Precipitation in 2022 was low, which led to a significant decrease in soil moisture (Figures 2 and 4). On August 16, 2022, in Hope, soil moisture levels were measured at 4% for the control while the 1.5in and 2.0in mulch treatments retained 8% and 12%, respectively (Figure 2). Thicker mulch treatments were able to significantly retain soil moisture throughout the dry 2022 season (Figures 2 and 4). Orland in 2022, experienced similar drought effects but at a larger scale (Figure 4). On August 16, 2022, soil moisture levels reached 0.3% in the control yet the 2.0in treatment had significantly greater soil moisture (1.4%) (Figure 4). During the main part of the dry 2022 growing season, the thicker mulches retained significantly more soil moisture (Figure 4).

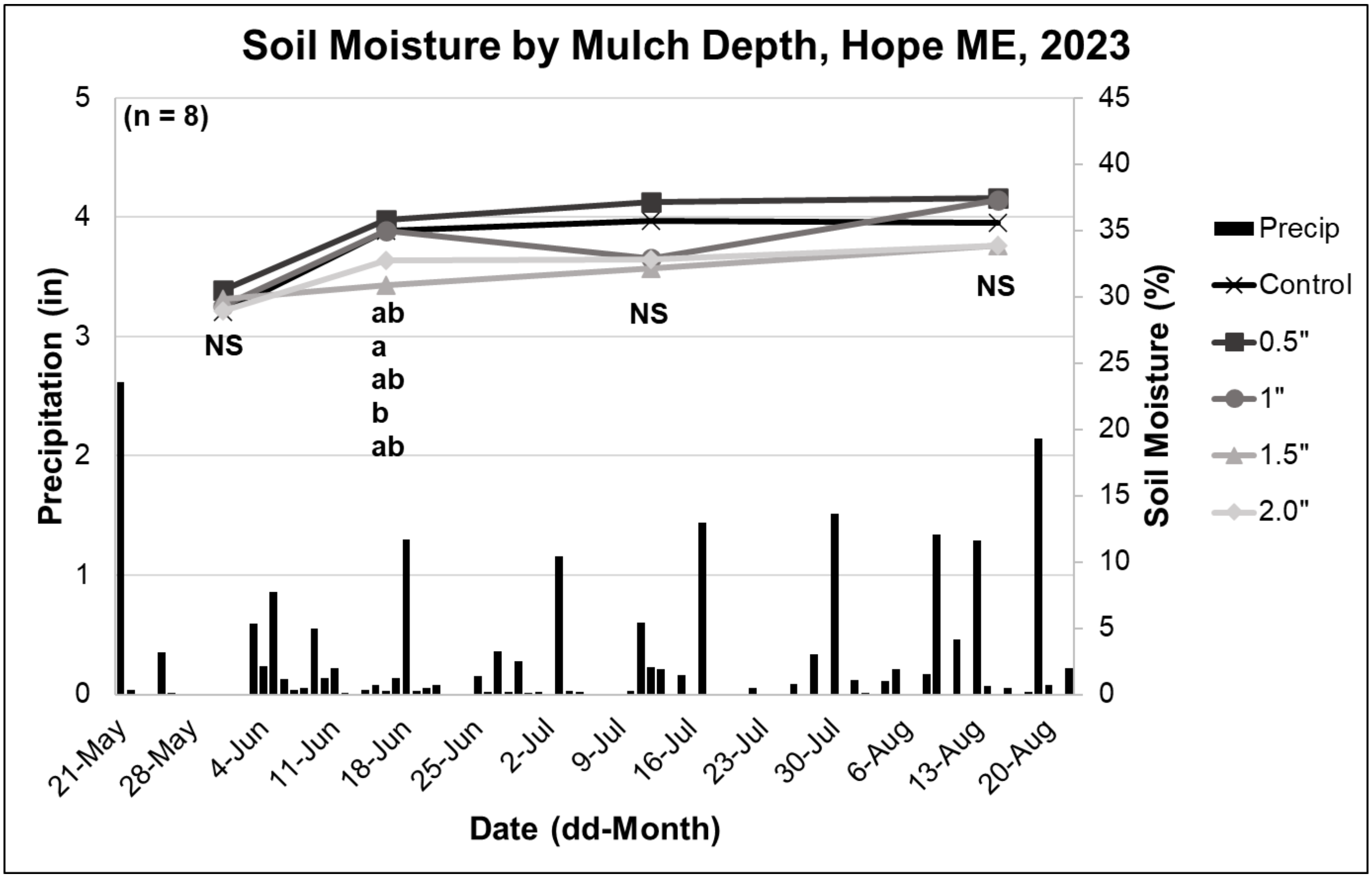

Figure 1. Average soil moisture by date and mulch depth for the wild blueberry crop year (2023) in Hope, Maine. Black bars represent daily local precipitation events (precipitation data is from NOAA, https://www.ncei.noaa.gov/cdo-web, Windsor Station). Lines correspond to mulch depth treatments. Means with the same letters are not statistically different using the Tukey's HSD at an α ≤ 0.05. Letters correspond to legend order (Control: top letter; 2.0": bottom letter). When there were no significant differences between treatments, data was labeled with a NS. Error bars were removed from the graph for better data visualization.

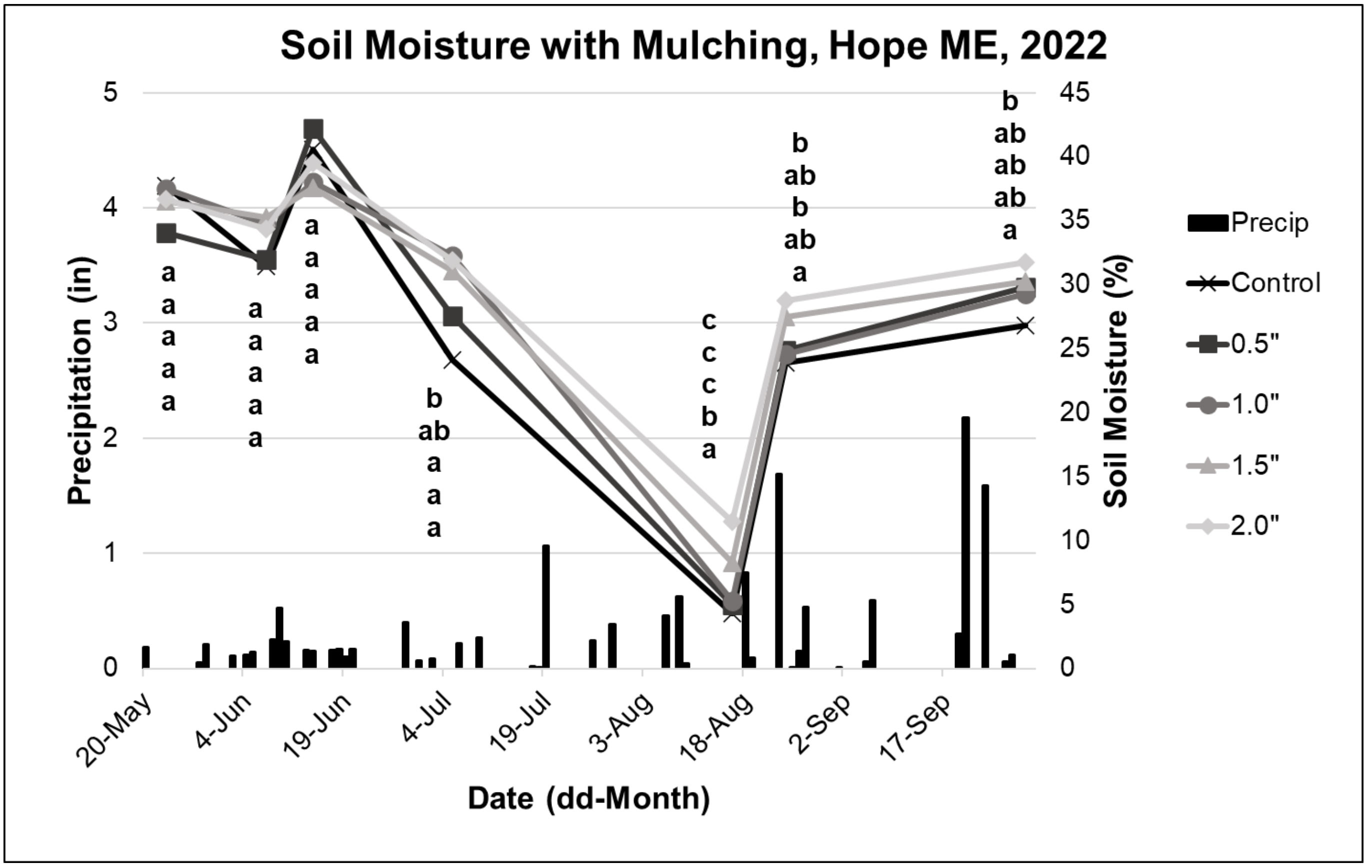

Figure 2. Average soil moisture by date and mulch depth for the wild blueberry crop year (2022) in Hope, Maine. Black bars represent daily local precipitation events (precipitation data is from NOAA, https://www.ncei.noaa.gov/cdo-web, West Rockport Station). Lines correspond to mulch depth treatments. Means with the same letters are not statistically different using the Tukey's HSD at an α ≤ 0.05. Letters correspond to legend order (Control: top letter; 2.0": bottom letter). Error bars were removed from the graph for better data visualization.

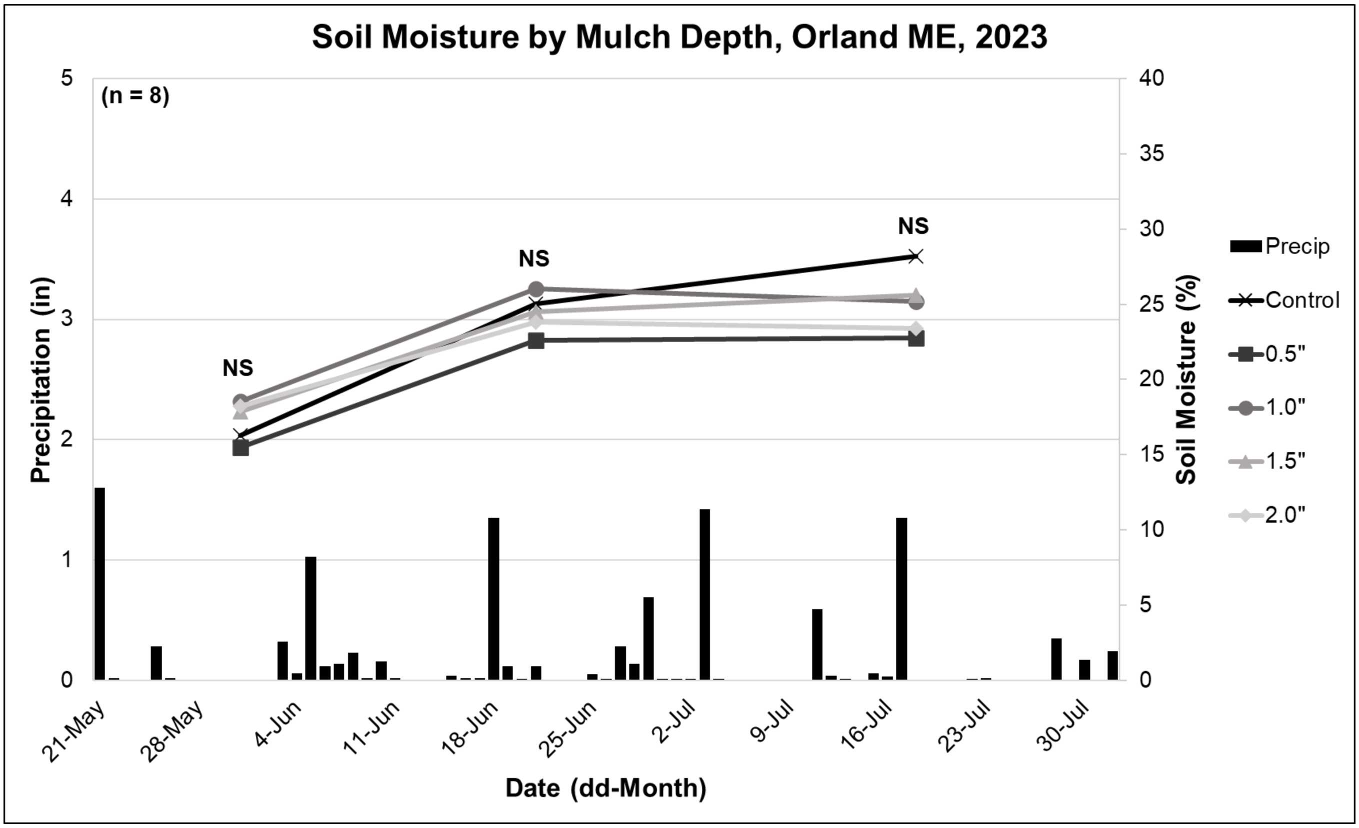

Figure 3. Average soil moisture by date and mulch depth for the wild blueberry crop year (2023) in Orland, Maine. Black bars represent daily local precipitation events (precipitation data is from NOAA, https://www.ncei.noaa.gov/cdo-web, Bucksport Station). Lines correspond to mulch depth treatments. An ANOVA was used to test significant differences between treatments (α ≤ 0.05). No significant differences were found between treatments and are labeled with NS. Error bars were removed from the graph for better data visualization.

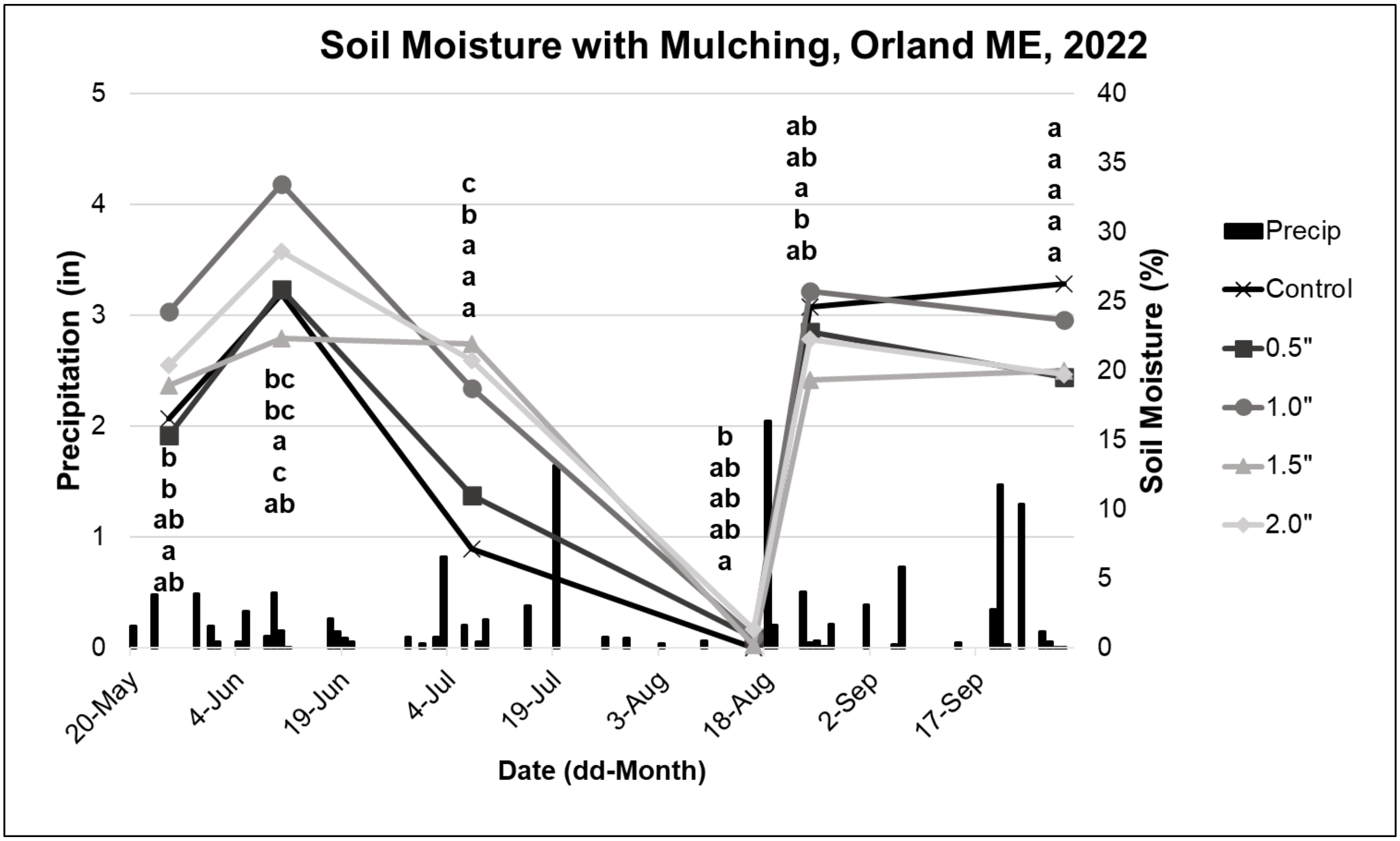

Figure 4. Average soil moisture by date and mulch depth for the wild blueberry crop year (2023) in Orland, Maine. Black bars represent daily local precipitation events (precipitation data is from NOAA, https://www.ncei.noaa.gov/cdo-web, Bucksport Station). Lines correspond to mulch depth treatments. An ANOVA was used to test significant differences between treatments. Means with the same letters are not statistically different using the Tukey's HSD at an α ≤ 0.05. Error bars were removed from the graph for better data visualization.

Leaf chlorophyll content can be used as a way to measure plant stress, with higher values indicating a healthier plant with more chlorophyll. At the Hope site in 2023, chlorophyll content was significantly greater than the Orland site and they were separated for analysis (P = 0.011). Chlorophyll content was not significant between mulch treatments in Hope (Figure 5). However, in Orland, 1.0 and 1.5in treatments had significantly greater chlorophyll (27.3 and 25.9 nm respectively) than the 0.0in control (20.5 nm) (P < 0.001) (Figure 5). No significant effects were detected in the Hope location in 2022 (Figure 6). All mulch treatments were significantly greater than the control in Orland for 2022 (Figure 6). Overall, chlorophyll levels were higher in 2022 compared to 2023 (Figures 5 and 6).

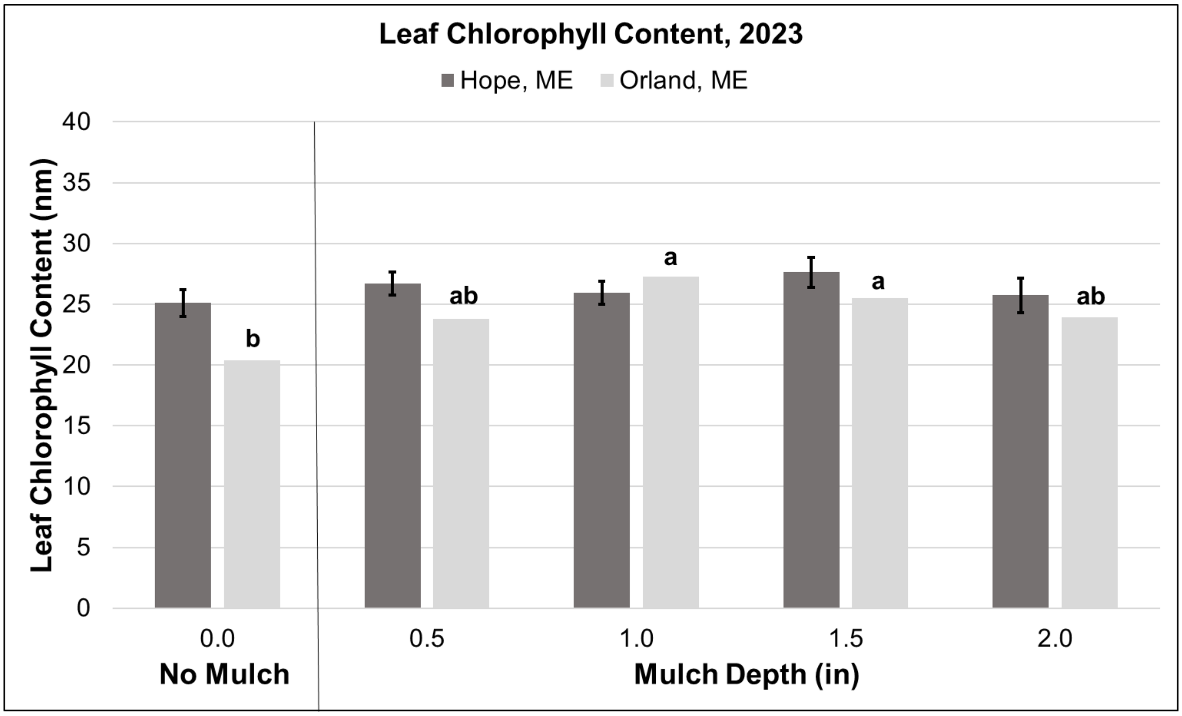

Figure 5. Average leaf chlorophyll content (SPAD (nm)) by mulch depth for the wild blueberry crop year (2023) in Hope and Orland, Maine. Leaf chlorophyll content was collected on 06/16/23 for Hope and 6/21/23 for Orland. Means with the same letters are not statistically different using the Tukey's HSD at an α ≤ 0.05. Error bars represent standard error for Hope. Back-transformed means from a cube root transformation are presented for Orland.

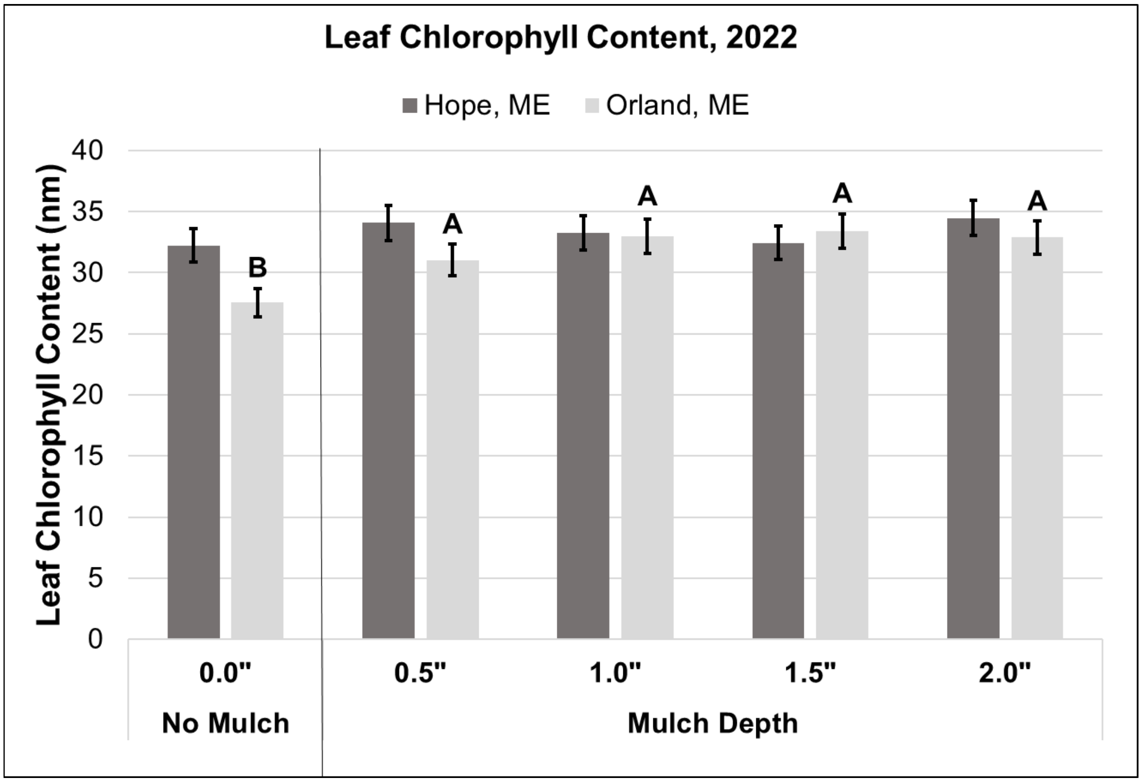

Figure 6. Average leaf chlorophyll content (SPAD (nm)) by mulch depth (in) for the wild blueberry prune year (2022) in Hope and Orland, Maine. Leaf chlorophyll content was collected on four separate dates for each location from June 10 to August 24, 2022. Means with the same letters are not statistically different using the Tukey's HSD at an α ≤ 0.05. There were no significant differences between treatments in Hope. Error bars represent standard error.

Stem density can help determine the health of the wild blueberry stand and yield potential after the mulch application in 2022. This was collected in 2023 for the Hope site and treatments were not significantly different from each other (Figure 7). The number of blueberry stems were not significantly different between where the mulch treatments were placed (Figure 8). At the Orland location, stem count was lower than the Hope site, likely due to de-rocking the field in 2021. However, no differences were detected between treatments (Figure 8).

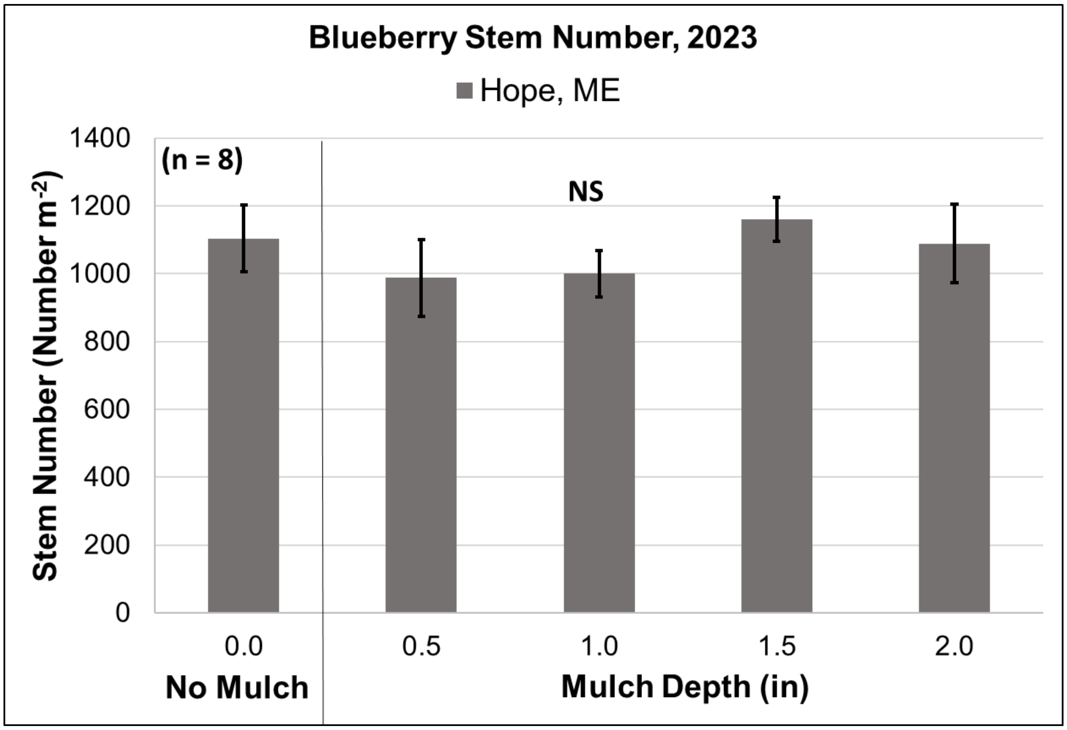

Figure 7. Average stem number by mulch depth for the wild blueberry crop year (2023) in Hope, Maine collected on August 15, 2023. Treatment means were not significantly different (NS). Error bars represent standard error.

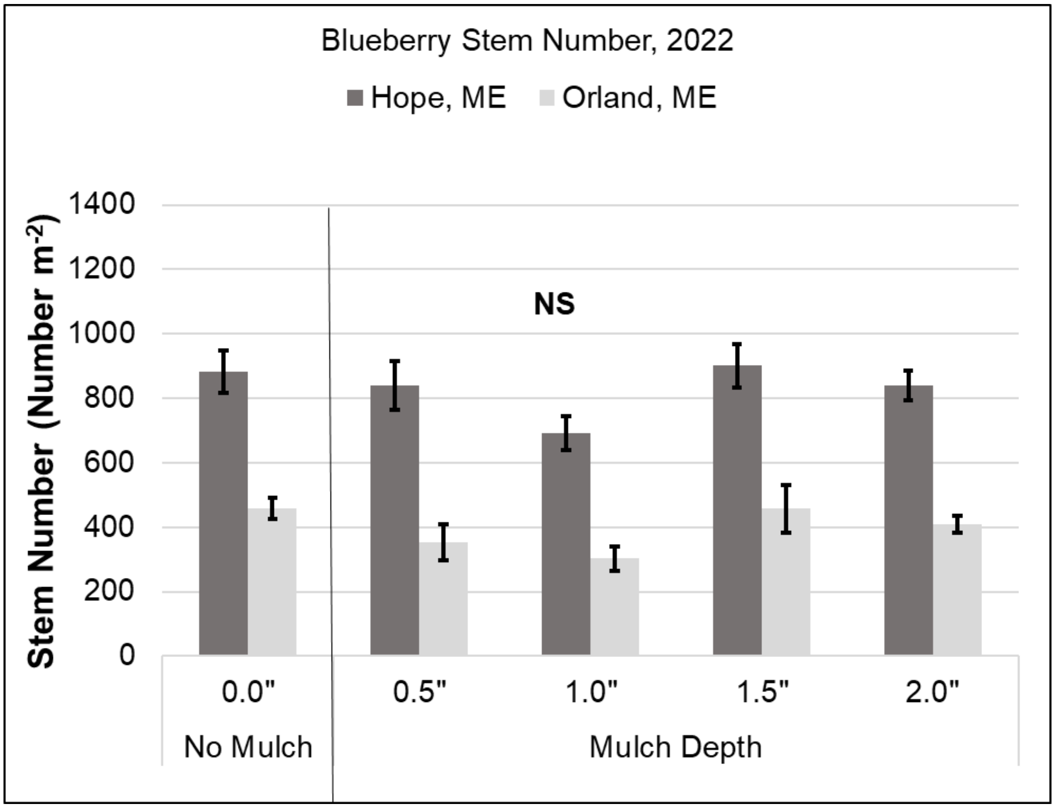

Figure 8. Average stem number by mulch depth for the prune blueberry prune year (2022) in Hope and Orland, Maine collected on September 29, 2022. Treatment means were not significantly different (NS). Error bars represent standard error.

Applying mulch to whole fields is an expensive practice and therefore NRCS has a cost-share program that pays for most if not all mulch material, equipment, and labor costs associated with application. The price remained the same in 2023 at $333 per 1000sqft of coverage which is approximately $14,500 per acre at a 2in depth (Table 3). 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

Weed species within sample areas were recorded to observe if mulch depth had a positive effect on weed suppression. In the Hope location, the 2.0in mulch suppressed 81% and the 1.5in suppressed 83% more weeds when compared to the 1.0in thickness ($P = 0.017$ and $P = 0.029$, respectively) (Figure 9). The Orland location experienced higher weed counts and the 2.0in suppressed 85% more weeds when compared to the 0.0in no mulch control ($P = 0.006$) (Figure 9). However, we were not able to detect a difference between other treatments (Figure 9). Broadleaves and grasses were separated and tested for differences, but no significant effects were found (data not shown).

Disease in the field was recorded to see if the mulch treatments could suppress pathogens like mummy berry and leaf spot species, especially during this rainy growing season. In Hope, leaf spot diseases in the 2.0in, decreased 49% when compared to the 0.5in treatment ($P = 0.035$) (Figure 10). Leaf spot diseases in Orland were less prevalent, but mulch depths had the same treatment effect with less leaf spot in the 2.0in when compared to the 0.0in mulch ($P = 0.023$) (Figure 10). Hope had a significantly greater amount of mummy berry in the 2.0in mulch (156 stems) than the 0.0in (9 stems) ($P = 0.042$) (Figure 11). In Orland, mummy berry was greatest in the 1.5in mulch (54 stems) when compared to all other treatments ($P < 0.001$) (Figure 11).

Insect damage on blueberry plants was observed for positive or negative effects of mulch depths. Flea beetle, tip midge, red stripped fire worm, and span worm were the insect pest species documented. We did not test fruit for spotted wing drosophila or blueberry maggot fly. In 2023, flea beetle damage was the most prominent insect pest in Hope and Orland. Flea beetle damage was significantly greater in the 1.0in (76 stems) compared to the 2.0in (25 stems) ($P = 0.006$) (Figure 12). Orland experienced a significantly high number of flea beetle damage in the 2.0in (178 stems) when compared to the other treatments ($P < 0.001$) (Figure 12). In Hope, tip midge was more prominent than in Orland. The 2.0in mulch treatment in Hope had significantly less tip midge damage (1 stem) than the 0.0in control treatment (35 stems) ($P = 0.007$) (Figure 13).

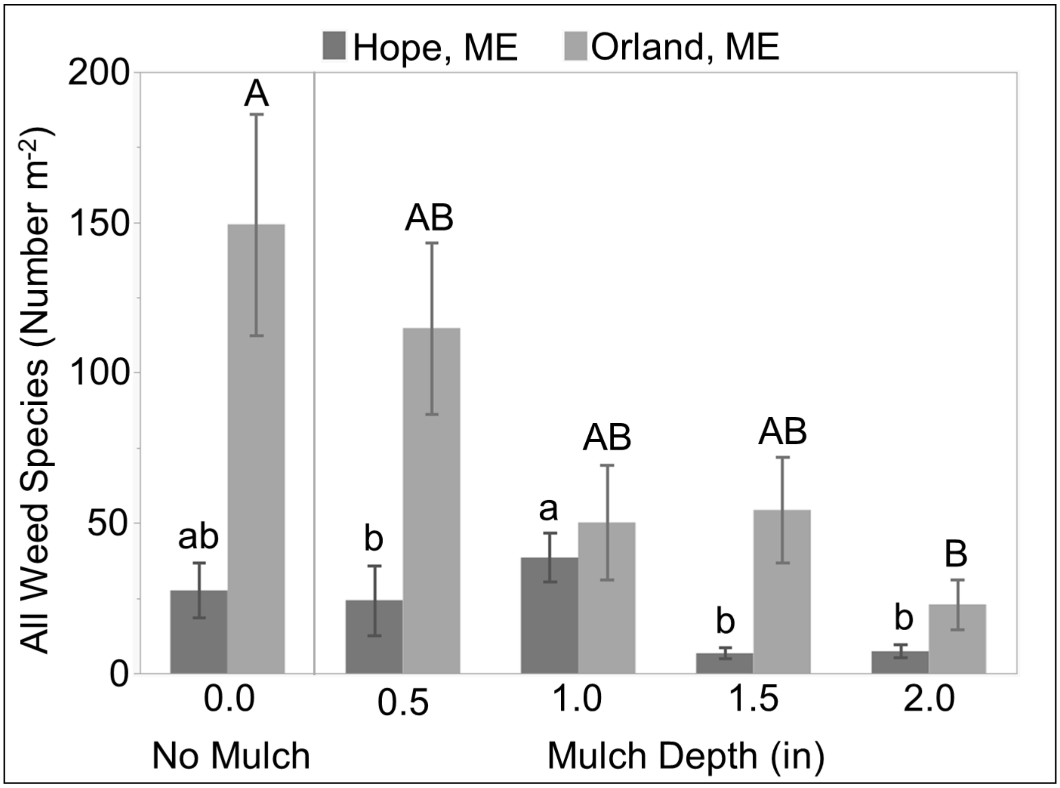

Figure 9. Grass, broadleaf, and sedge counts by mulch depth. Means with the same letters are not statistically different using the Tukey's HSD at an $\alpha \leq 0.05$. Error bars represent standard error. A cube root transformation was used for analysis of both locations. Back-transformed data is not shown in the figure due to misleading low values.

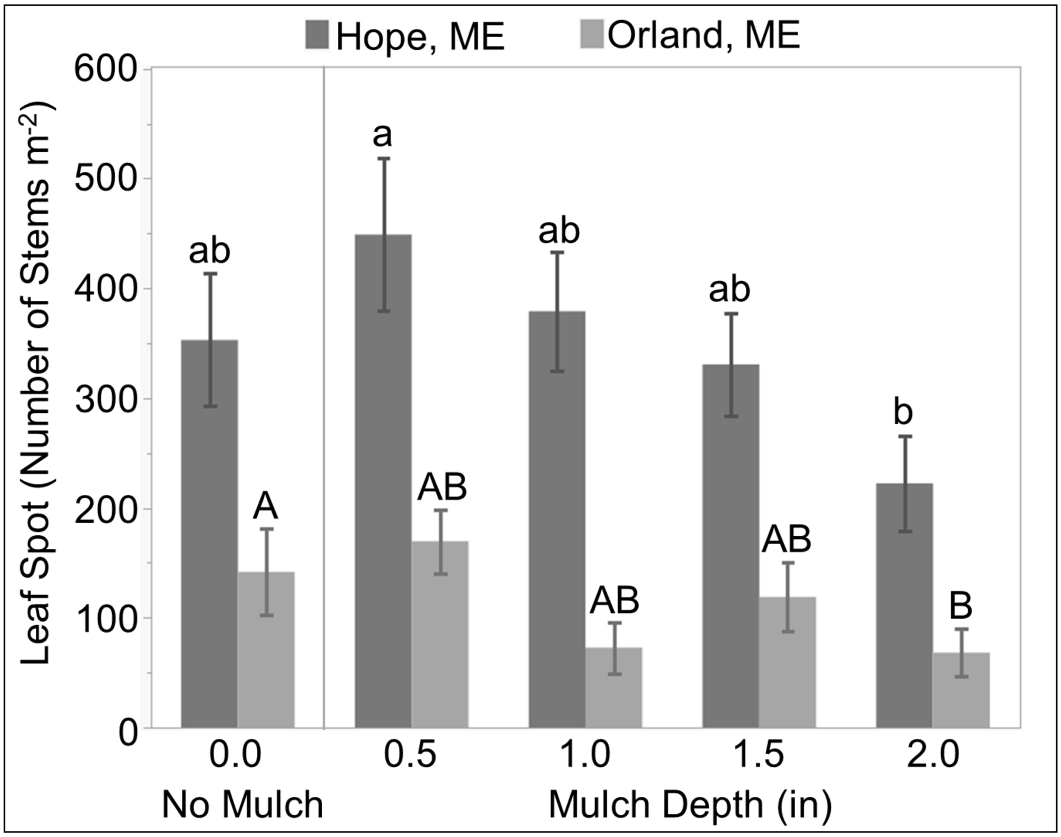

Figure 10. Infected blueberry stems with leaf spot by mulch depth. Means with the same letters are not statistically different using the Tukey's HSD at an α ≤ 0.05. Error bars represent standard error. A cube root transformation was used for analysis in the Hope data. Back-transformed data is not shown in the figure due to misleading low values but the decreasing trend with increasing mulch depth is clear at Hope.

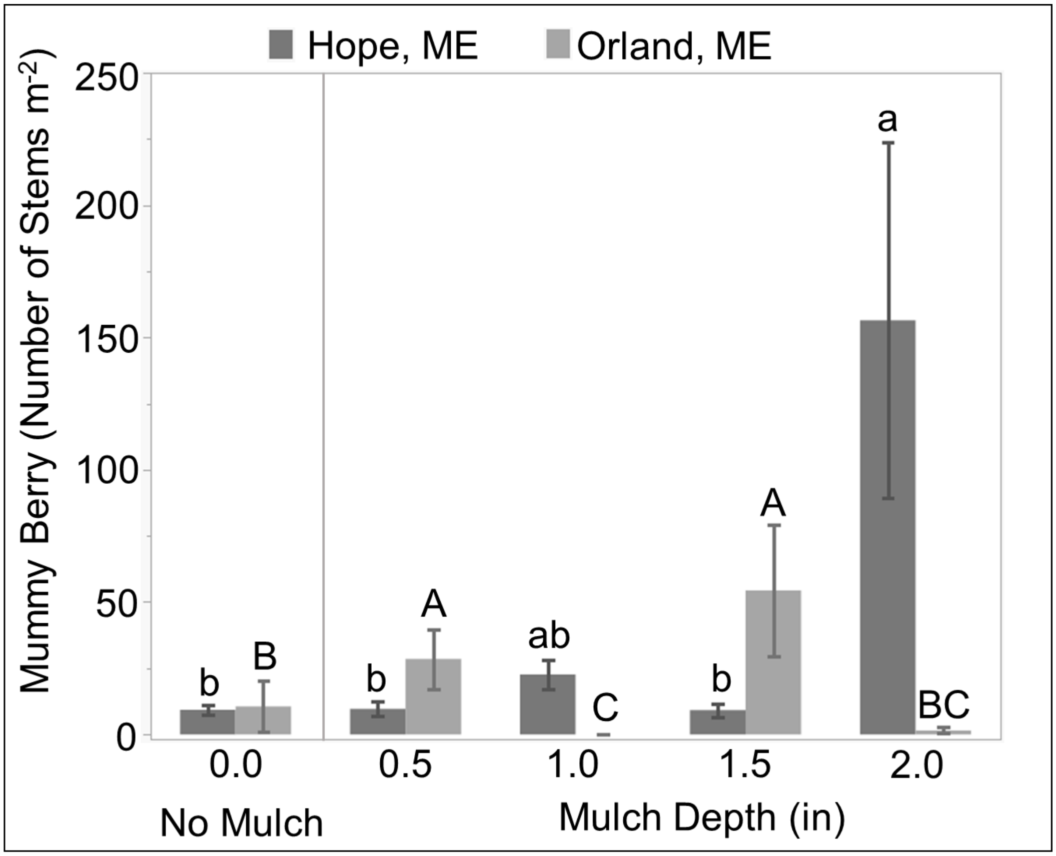

Figure 11. Shows number of blueberry stems infected with mummy berry at both locations by mulch depth at the 0.05 LOS. Means with the same letters are not statistically different. Error bars represent standard error.

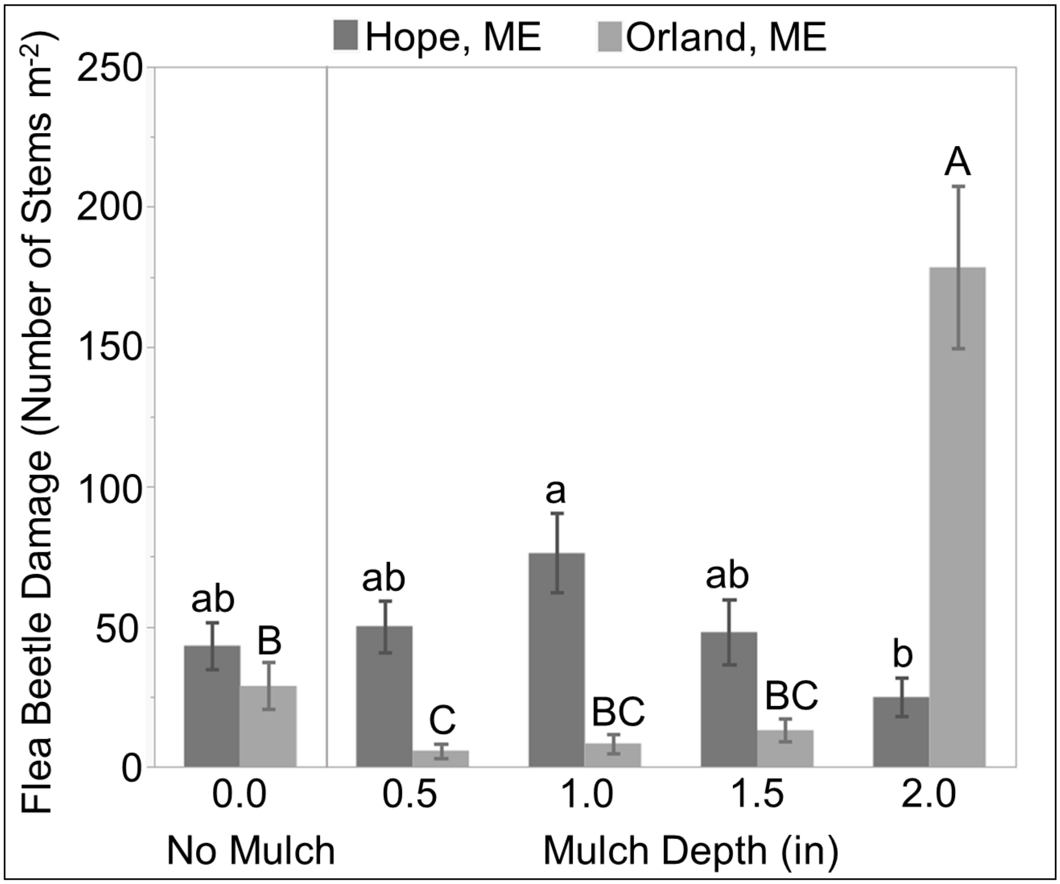

Figure 12. Flea beetle damage per stem in both locations by mulch depth. Means with the same letters are not statistically different at the 0.05 LOS. Error bars represent standard error. A square root transformation was used to normalize residuals for means comparison in both locations.

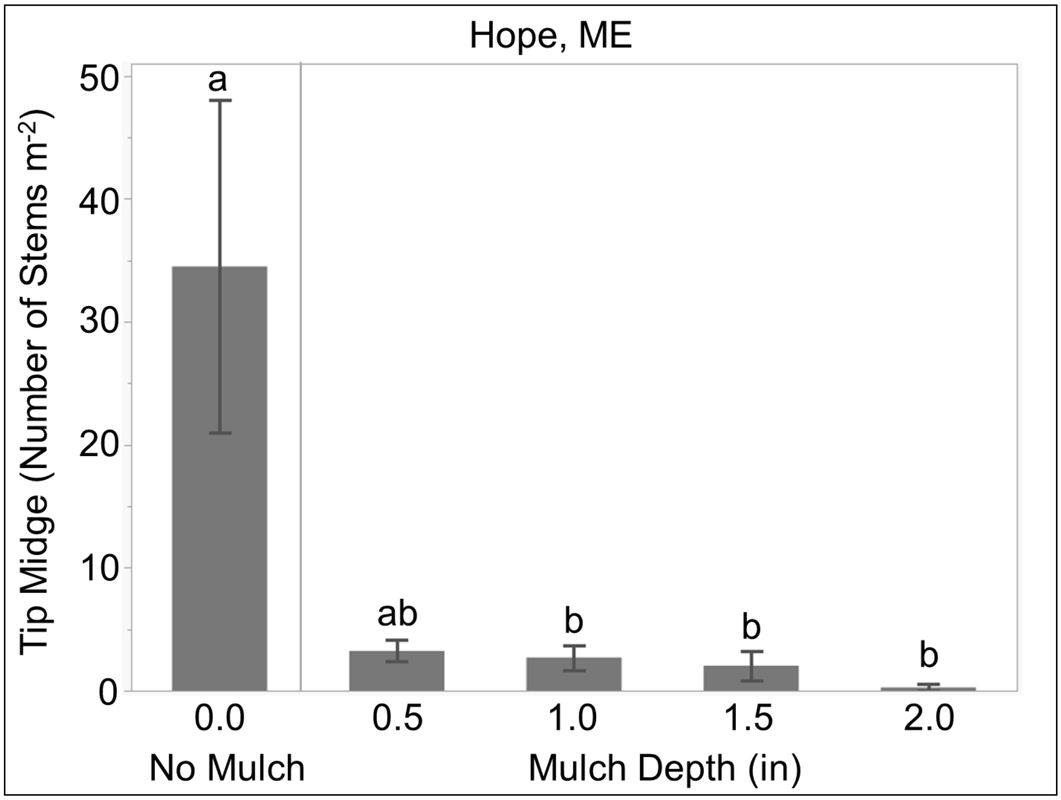

Figure 13. Tip midge damage to blueberry stems in Hope by mulch depth. Means with the same letters are not statistically different using the Tukey's HSD at an α ≤ 0.05. Error bars represent standard error. A cube root transformation was used for analysis. Back-transformed data is not shown in the figure due to misleading low values.

Yield and Berry Quality

There were no significant differences detected between treatments for yield in Hope or Orland (Figure 14). Hope produced more berries than the Orland location. Analyzed yield was collected by hand rake. Only in Hope whole plot yields were recorded by the farmer (Table 4). The control and the 2.0in treatment produced the most compared to the other treatments. This trend can also be seen in the sample plot data, although they are not significantly different from other treatments (Figure 14). The amount of mulch debris collected had no statistical difference between treatments (Figure 15). Data from Hope and Orland were combined for 100 count berry weights due to insignificance; no treatment effects were detected between treatments. Brix content data from Hope and Orland was combined due to not being significantly different. Brix content was not significant between treatments.

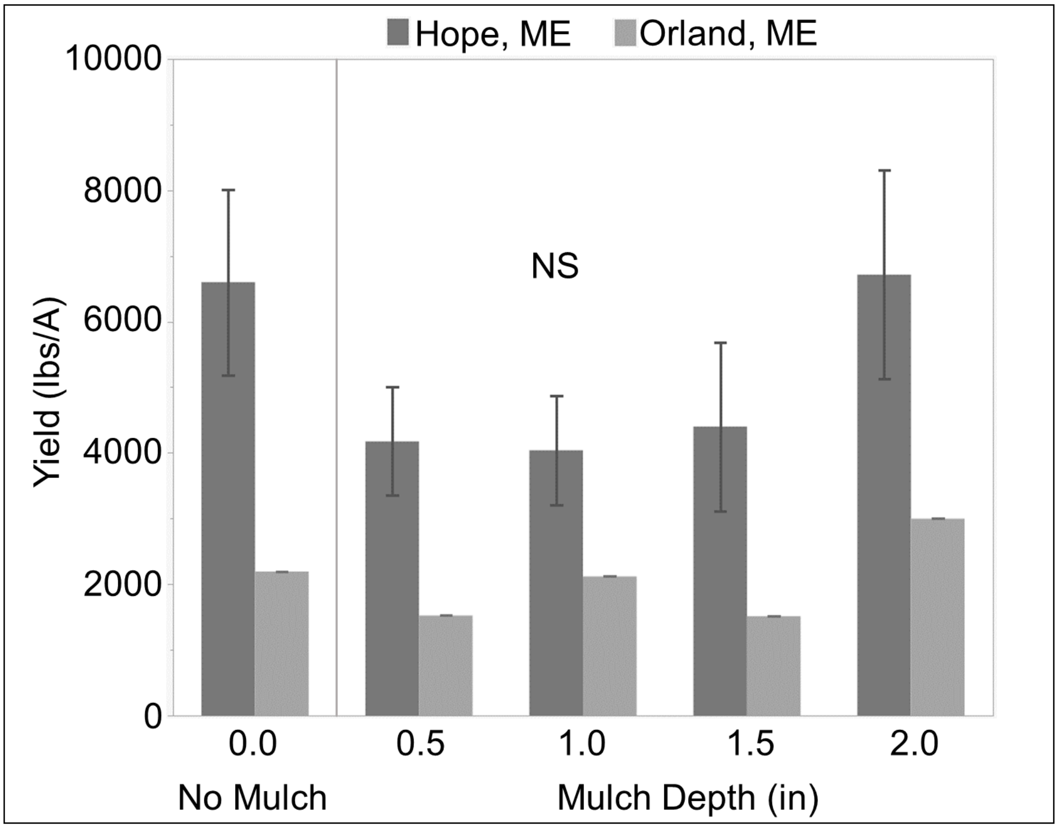

Figure 14. Wild blueberry raked yield by mulch depth treatment in 2023. Results of a one-way ANOVA using an α ≤ 0.05 show treatments were not significantly different in Hope or Orland, ME (NS). Error bars represent standard error. A square root transformation was used, and back-transformed means are shown for Orland. These data are not whole field yield values.

Table 4. Total weight of fruit harvested from each mulch treatment plot with a DH machine at Hope, Maine on August 1, 2023.

Blueberry Yield, 2023	
	Hope, ME
Mulch Treatment (in)	**Pounds per Plot (50 ft x 200 ft)**
Control	1360.0
0.5	960.0
1.0	992.0
1.5	812.0
2.0	1311.0

DISCUSSION

In 2023 Maine received more rain than the first year of this study (2022) changing from drought conditions in 2022 when mulch was applied to saturated conditions in the second year. In 2022, 1.0, 1.5, and 2.0 inches of mulch increased soil moisture content when compared to the no mulch control in July due to reduced evapotranspiration (Figures 2 and 4). In 2023, soil moisture levels did not significantly change between treatments due to frequent rain events at both locations (Figures 1 and 3). In terms of soil moisture, we conclude that recently de-rocked and land leveled sites benefit more greatly from whole field mulching while more subtle improvements can be seen on well-established fields in the first and second years after mulch application. Fields also receive more benefits from whole field mulching in drought years than in wet years. However, mulching benefits the crop as a pest management tool in all years.

Disease and Insect Presence

In 2023, disease pressure from leaf spot species was high because of the wet conditions. In Hope, leaf spot diseases reduced incrementally with increasing mulch depth (Figure 10). Tip midge presence was significantly lower in all mulch treatments compared to the control (Figure 13). In both cases, the mulch creates a barrier between the pest organism that overwinters in the soil or on leaf litter and the plant above. The spores and larvae that emerge from the ground simply can't reach the plant as well.

Mummy berry disease was significantly higher in the Hope 2.0in treatment most likely because the more moist conditions allowed for better survival and germination of existing mummy berries below the mulch (Figure 11). The number of stems with flea beetle damage was significantly higher in the Orland 2.0in treatment most likely due to random chance, however if poor pruning occurs due to the thickness of mulch, increased populations of flea beetle could be observed. Both of these observations only occurred in one location and one year so further investigation is required.

Weed Presence

Weed density at the Orland location was greater than at the Hope location, but the effects of the thicker mulch lowered the number of weeds significantly (Figure 9). The 2.0in mulch was able to shade out and inhibit weed growth when compared to the 0.0in mulch (Figure 9). This effect was also seen in Hope but less dramatically. The density and location of weeds impacted these results where the 1.5 and 2.0in mulch suppressed more weeds, but the treatments with the greatest weeds were the 0.0 and 1.0in (Figure 9). Sedge had the greatest abundance, out of all the weed species in Hope, which can grow through a thicker mulch like the 1.0in, but then was significantly decreased in the 1.5 and 2.0in mulch applications. These patches of sedge were found evenly spread throughout the control, 0.5in, and 1in treatments.

Plant Health

In 2022, Orland had significantly higher chlorophyll content in all mulch depths compared to the control while Hope showed no significant differences in chlorophyll content (Figure 6). We still conclude that this indicates a greater benefit from mulch on sites that have been de-rocked, and land leveled recently. In 2023, Hope received more precipitation events than Orland which means more clouds blocked quality sunlight from reaching wild blueberry leaves below (Figure 1). This may be why we did not see significant leaf chlorophyll content differences in the Hope location (Figure 5). In Orland, 1.0 and 1.5in treatments showed significantly higher chlorophyll content compared to the control (Figure 5) and overall, those same treatments had fewer weeds, leaf spot diseases, and flea beetle damage (Figures 9, 10, and 12). Perhaps these treatments that had higher quality leaves were more able to withstand and outgrow pest damage.

Stem density was not affected by any thickness of mulch which indicates that the plants can maintain stand thickness regardless of mulch application (Figure 7). This is a promising result. If the spreader or application of mulch is not perfectly applied to the desired depth, the plants will still be able to establish between 0.5 and 2.0in. In 2022, stem counts in Orland were a magnitude lower than Hope, most likely due to de-rocking the field in 2021 and the plants are still recovering (Figure 8). There were no differences in stem density between treatments at either location when the study started in 2022 and in Hope there remained no significant difference in stem density showing that mulch did not smother productive plants (Figures 7 and 8). Further years of study will reveal any increase in stem density depending on the mulch depth.

Yield and Quality

According to the IQF processor who partnered with us on this project, the degree to which mulch chips get caught in the processing line depends on how dry or wet the berries are coming in. In 2022 and other drought years, most mulch floats off in the float tank. In 2023, mulch chips didn't float as easily but most were removed by the laser sorter. The harvester used to harvest the Hope mulch treatments in 2023 was the DH which allowed for very little mulch debris to be caught. There was very little debris found in the fruit and on the fresh pack line. Pieces that did get through were removed with little effort. The 100 count berry weight did not vary between mulch treatments which means the fruit was not inhibited by the mulch for weight and brix content was also not affected by the mulch, which is also good news for fruit quality.

This study, having occurred in the first and second years of mulch application, is young. Mulch takes years to decompose and reveal the entire soil and plant health story. We will continue to monitor these sites for long-term results.

CURRENT RECOMMENDATIONS

- After the two years of this study, we recommend either the 1.0 or 1.5 inch thicknesses of softwood mulch wood chips. Mulch has many benefits to wild blueberry plant health and pest suppression. Given that mummy berry and flea beetle increased at the 2.0 inch thickness, some growers may choose to reduce the risk of these two pests, opting for a 1-1.5 inch application across whole fields. Two inches of mulch remains a good practice for filling in bare spots which 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 pest presence, soil moisture and soil characteristics in the 2024 season (prune year).

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6. Airborne Spectral Detection of Leaf Chlorophyll Concentration in Wild Blueberries

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OBJECTIVE(S)

- To test the possibility of using airborne spectral sensors to detect leaf chlorophyll concentration for wild blueberries.
- To test the performance of remote leaf chlorophyll concentration detection models in wild blueberries using machine learning.

LOCATION(S): Wyman’s Farm, Deblois, Maine.

PROJECT TIMEFRAME: July 2019 – August 2023

INTRODUCTION

The quantification of leaf chlorophyll concentration (LCC) holds significant implications for agricultural practices, as it enables the continuous assessment of crop physiological status, determination of overall crop health, and estimation of photosynthetic potential (Gitelson et al., 2003). This information is particularly valuable for monitoring large commercial fields, where rapid, non-destructive, and spatially extensive techniques are essential for accurate diagnosis and assessment.

Although traditional methods of wet extraction analysis through field sampling provide accurate estimations of LCC, these methods are not always practical for estimating LCC over large areas of the farm. Alternatively, non-destructive measurement of leaf spectral reflectance offers an instantaneous and alternative method for assessing the LCC of plants over a large spatial scale (Lu et al., 2018). This method involves measuring the light reflected by leaves, which varies according to the chlorophyll concentrations (Lu et al., 2018; Zhang et al., 2014). By analyzing the spectral reflectance of the leaves, we can determine the LCC of the plants. This non-destructive approach can be particularly useful for quick, accurate, large-scale vegetation health evaluations.

Airborne or UAV (Unmanned aerial vehicle)- based hyperspectral remote sensing has emerged as a promising avenue for monitoring various plant parameters, including LCC, due to its non-destructive nature and ability to cover large spatial extents (Hu et al., 2023). However, the challenge lies in developing LCC prediction models that can accommodate the inherent variability in structural and physiological properties across different crops.

The wild blueberry crop has diverse genotypes grown in semi-natural systems (Barai et al., 2022), so implementing precision management becomes challenging. To address this challenge, we have investigated the application of machine learning (ML) techniques for UAV-based remote LCC detection in wild blueberries. The objective is to evaluate the performance of ML models in accurately predicting LCC, considering the unique characteristics and variability inherent in wild blueberry fields. This research endeavors to contribute to advancing remote sensing applications in agriculture, specifically in the context of wild blueberries, by enhancing the robustness and adaptability of LCC prediction models by utilizing machine learning methodologies.

METHODS

Study Site

The study was conducted on commercial blueberry fields of Wyman’s in Deblois, Maine

(Latitude: 44.7350° N, Longitude: 68.0001° W). These commercial crop fields contain many different genotypes of wild blueberry plants growing within a particular field.

UAV-based Hyperspectral Data Collection and Ground Sampling

The hyperspectral image acquisitions and ground measurements were conducted one time in the summer of 2019 and three times in 2022 and 2023, covering different developmental stages within large commercial blueberry fields. Image acquisition and field ground data collection dates were carried out on sunny days. A total of 30 genotypes in 2019 (crop year), and 40 genotypes were systematically selected in 2022 (vegetative growth year) and 2023 (crop year) to cover the entire field and a wide range of genotypes based on morphological differences. Six wild blueberry stems were randomly selected from each genotype area to measure chlorophyll content. LCC indicated by SPAD values were collected using the SPAD-502 chlorophyll meter (Konica Minota Inc., Japan) at the field site and then converted to LCC ($\mu g/cm^2$) values using the SPAD to LCC conversion model following Zhu et al. (2012).

We used a Headwall Photonics Micro A-Series Sensor (Bolton, MA, USA) hyperspectral imaging spectrometer attached to a DJI Matrice 600 Pro UAV for data collection. Data collection was conducted between 12:00 PM ± 2 hours local time. The sensor captured 324 spectral bands between 400 to 1000 nm in the visible and near-infrared electromagnetic spectrum. After processing the imagery with the Headwall Spectral View application, we used ENVI software (version 5.6) to identify and extract pixels of ground-sampled genotypes. These delineations were used as samples in both training and validation data sets. All downstream analyses were performed in Python.

Model Development

In the preliminary data analysis of the UAV-borne hyperspectral imagery for LCC prediction, different ML techniques were used, using Scikit-Learn in Python. Splitting the data into 80/20 for training and testing, we utilized various machine-learning methods such as kernel ridge regression (KRR), ElasticNet, and XGBoost. We additionally used partial least square regression (PLSR). PLSR is a form of regularized linear regression where the number of components controls the strength of the regularization, which is suitable for predictors with high collinearity. Kernel ridge regression (KRR) combines ridge regression (linear least squares with 12-norm regularization) by learning a linear/ non-linear function in the space induced by the respective kernel and the data. Elastic net regression aims to select the predictor variables most important for predicting the target variable while using regularization to avoid overfitting the model. XGBoost is a decision-tree-based ensemble algorithm that uses gradient boosting and hardware optimizations to produce quick, accurate results for regression, classification, ranking, and time series tasks.

While previous research has used ML and ensembles for similar tasks, this research focused on using various preprocessing techniques to attempt to create more learnable features from the data that could be used in ensemble structures. A general challenge of working with hyperspectral data is the collinearity of bands. The dimensionality of the dataset was reduced using Non-negative Matrix Factorization (NMF) and Gaussian Mixture Model (GMM) methods. Thirty-four different chlorophyll vegetation indices were calculated to create an additional dataset for the ensemble structure. To tune utilized hyperparameters, Optuna, an automatic hyperparameter optimization software framework, was used to construct the search spaces for the hyperparameters dynamically. Finally, this study incorporated PyTorch to calculate the optimal combination of base learners. We first developed a rough estimate for a base learner coefficient and then used PyTorch and gradient descent to tune the optimal coefficients. We also explored the optimal wavelength bands for widely used indices such as simple differences (R_{Y1}-R_{Y2}), simple ratios (R_{Y1}/R_{Y2}), and normalized differences ($(|R_{Y1}-R_{Y2}|/(R_{Y1}-R_{Y2}))$).

RESULTS

When examining the mean reflectance properties (400-1000 nm) of different genotypes, it was observed that the visible wavelengths (400-700 nm) had much lower reflectance compared to the near-infrared wavelengths (700-1000 nm) (Figure 1). Within the visible spectrum, the green wavelength (~540 nm) had high reflectance, while the blue (~450 nm) and red (~670 nm) regions had comparatively low reflectance. On the other hand, the near-infrared region (720-1000 nm) had continuous high reflectance properties.

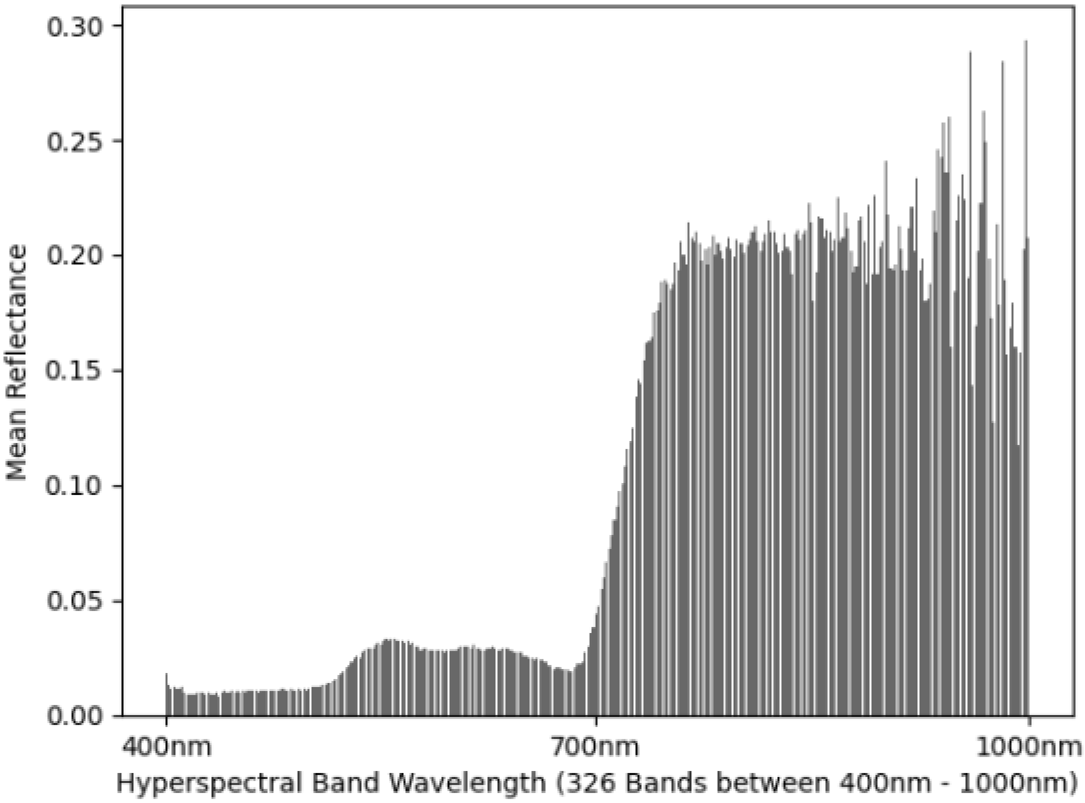


Figure 1. Mean reflectance of Genotypes in different wavelength bands ranging from 400 nm to 1000 nm.

When investigating the correlation coefficients of the wavelengths to the LCC, it was found that there were various sensitive regions (Figure 2). The green wavelength region had high positive correlations, while the blue and red regions of the visible spectrum had low or negative correlations.

Typically, green vegetation exhibits high reflectance in the visible green and near-infrared regions (400-1000 nm) of the electromagnetic spectrum. In contrast, the visible blue and red regions have high absorbance (Figure 2).

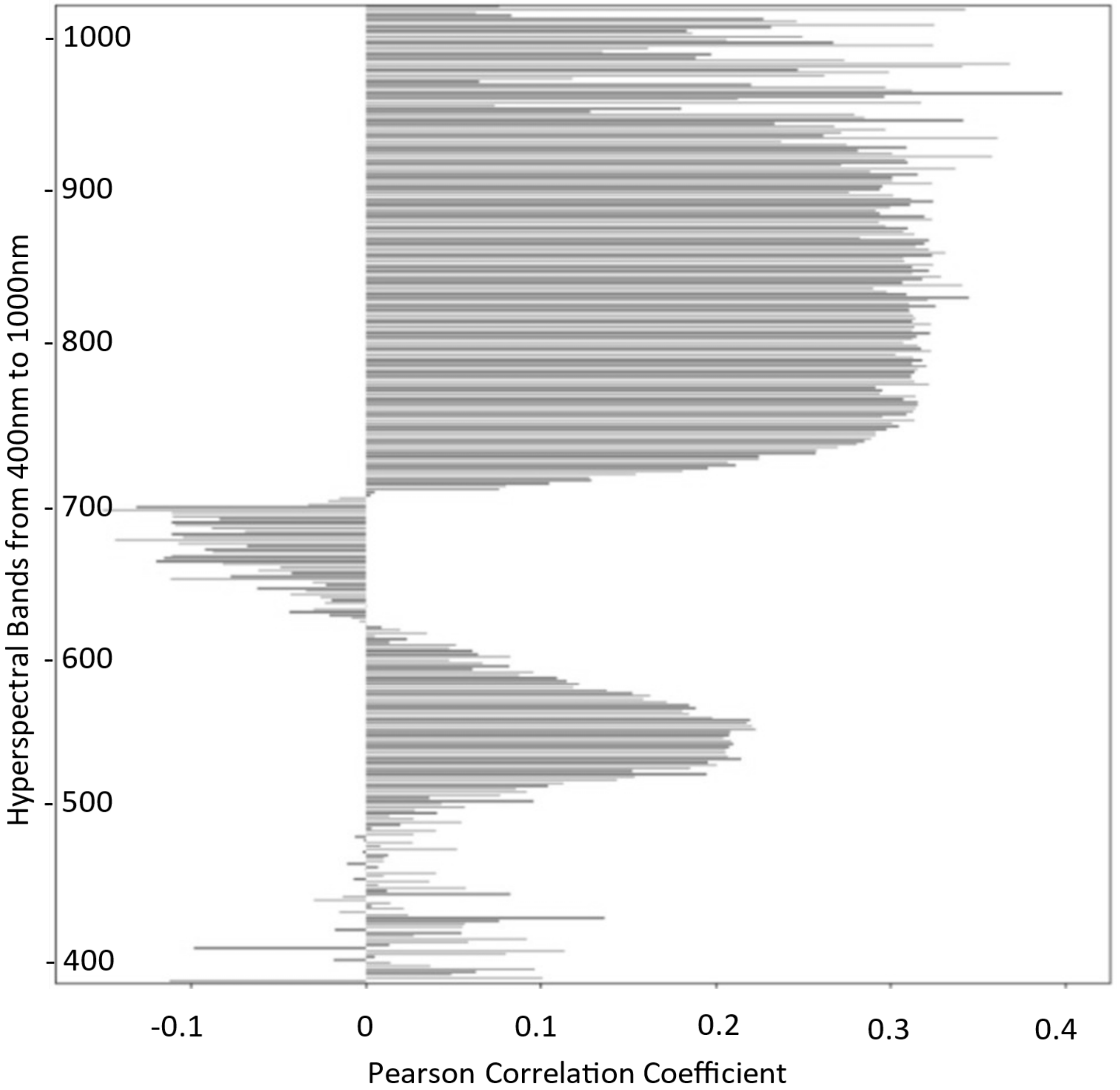


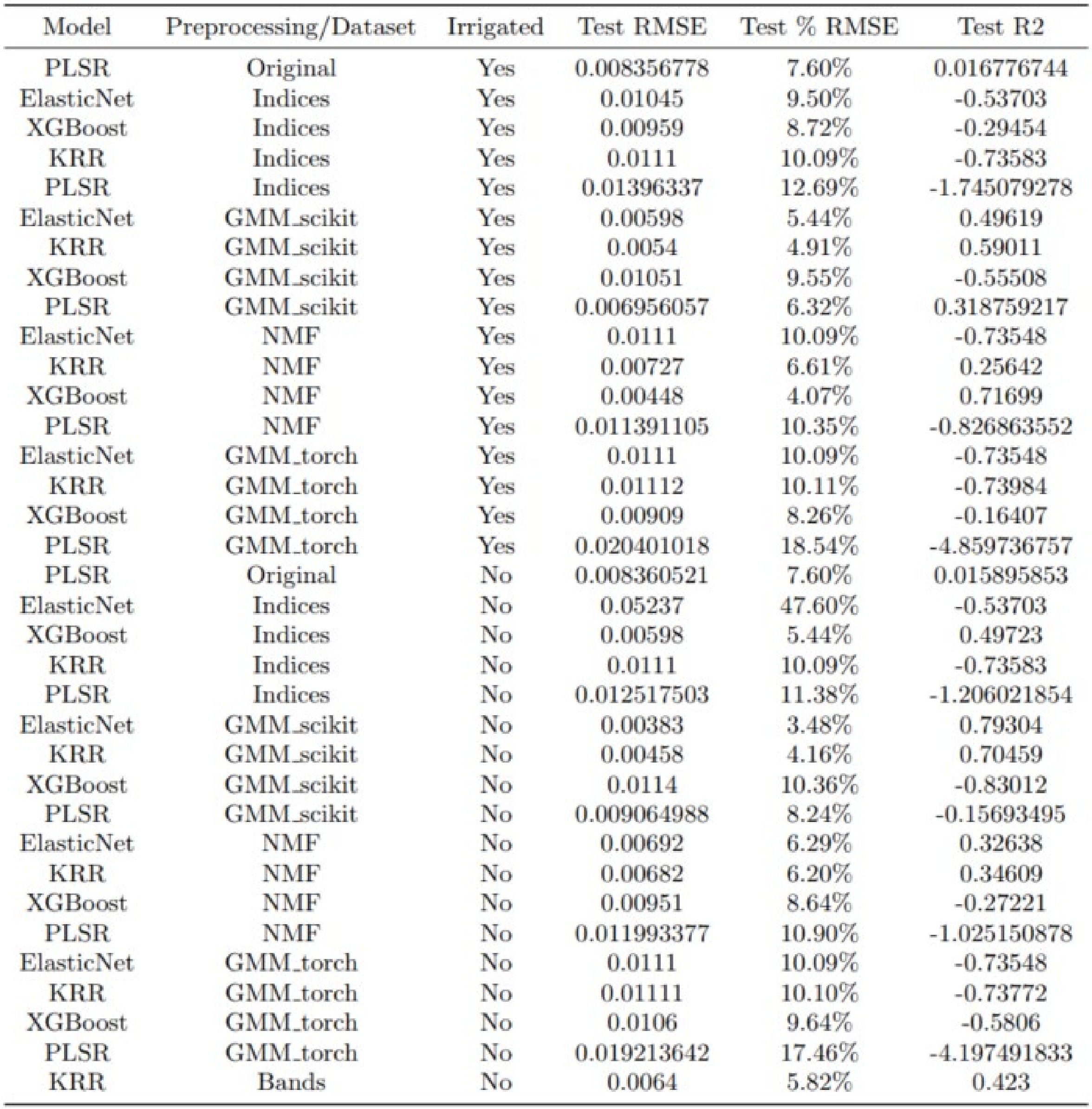
Figure 2. Pearson correlation coefficients between bands ranging from 400 nm to 1000 nm and LCC.

We explored the optimal wavelength bands for simple differences (R_{Y1}-R_{Y2}), simple ratios (R_{Y1}/R_{Y2}), normalized differences (|R_{Y1}-R_{Y2}|/ (R_{Y1}-R_{Y2})), and MDATT index (|R_{Y3}-R_{Y1}|/ (R_{Y3}-R_{Y2})) and the most robust wavelength combination for these indices mostly located in the near-infrared and red region for predicting the LCC in wild blueberry (Table 1).

Table 1. Optimal spectral bands for predicting LCC in several indices.

Vegetation Index	**Formula Equation**	**Optimal Bands**
SD	$SD = \mathrm{R}_{\lambda_1} - R_{\lambda_2}$	R_{919}, R_{921}
SR	$SR = \mathrm{R}_{\lambda_1}/R_{\lambda_2}$	R_{828}, R_{858}
ND	$ND = \frac{\lvert R_{\lambda_1} - R_{\lambda_2} \rvert}{R_{\lambda_1} + R_{\lambda_2}}$	R_{701}, R_{946}
MDATT Index	$MDATTIndex = \frac{R_{\lambda_3} - R_{\lambda_1}}{R_{\lambda_3} - R_{\lambda_2}}$	$R_{647}, R_{668}, R_{821}$

Table 2. Results of Machine Learning Model Performance



Model	Preprocessing/Dataset	Irrigated	Test RMSE	Test % RMSE	Test R2
PLSR	Original	Yes	0.008356778	7.60%	0.016776744
ElasticNet	Indices	Yes	0.01045	9.50%	-0.53703
XGBoost	Indices	Yes	0.00959	8.72%	-0.29454
KRR	Indices	Yes	0.0111	10.09%	-0.73583
PLSR	Indices	Yes	0.01396337	12.69%	-1.745079278
ElasticNet	GMM_scikit	Yes	0.00598	5.44%	0.49619
KRR	GMM_scikit	Yes	0.0054	4.91%	0.59011
XGBoost	GMM_scikit	Yes	0.01051	9.55%	-0.55508
PLSR	GMM_scikit	Yes	0.006956057	6.32%	0.318759217
ElasticNet	NMF	Yes	0.0111	10.09%	-0.73548
KRR	NMF	Yes	0.00727	6.61%	0.25642
XGBoost	NMF	Yes	0.00448	4.07%	0.71699
PLSR	NMF	Yes	0.011391105	10.35%	-0.826863552
ElasticNet	GMM_torch	Yes	0.0111	10.09%	-0.73548
KRR	GMM_torch	Yes	0.01112	10.11%	-0.73984
XGBoost	GMM_torch	Yes	0.00909	8.26%	-0.16407
PLSR	GMM_torch	Yes	0.020401018	18.54%	-4.859736757
PLSR	Original	No	0.008360521	7.60%	0.015895853
ElasticNet	Indices	No	0.05237	47.60%	-0.53703
XGBoost	Indices	No	0.00598	5.44%	0.49723
KRR	Indices	No	0.0111	10.09%	-0.73583
PLSR	Indices	No	0.012517503	11.38%	-1.206021854
ElasticNet	GMM_scikit	No	0.00383	3.48%	0.79304
KRR	GMM_scikit	No	0.00458	4.16%	0.70459
XGBoost	GMM_scikit	No	0.0114	10.36%	-0.83012
PLSR	GMM_scikit	No	0.009064988	8.24%	-0.15693495
ElasticNet	NMF	No	0.00692	6.29%	0.32638
KRR	NMF	No	0.00682	6.20%	0.34609
XGBoost	NMF	No	0.00951	8.64%	-0.27221
PLSR	NMF	No	0.011993377	10.90%	-1.025150878
ElasticNet	GMM_torch	No	0.0111	10.09%	-0.73548
KRR	GMM_torch	No	0.01111	10.10%	-0.73772
XGBoost	GMM_torch	No	0.0106	9.64%	-0.5806
PLSR	GMM_torch	No	0.019213642	17.46%	-4.197491833
KRR	Bands	No	0.0064	5.82%	0.423

Various ML models were implemented, such as PLSR, ElasticNet, XGBoost, and KRR on the original dataset (without any preprocessing), vegetation indices (ensemble structure), and two-dimensionality reduction methods (GMM and NMF). The overall performance of the PLSR models was found unsatisfactory in all the datasets. The model performance was poor when utilizing the original unprocessed and ensemble indices datasets. The performance of the ML models on the GMM and NMF processed dataset (dimensionality reduced) was improved. The best single learning model was an ElasticNet regression with a coefficient of determination (R^2) of 0.79 and a normalized root mean square error (nRMSE) of 3.48%, which was trained on the dataset reduced with the GMM dimensionality reduction method (Table 2).

PyTorch was used to combine six base models with differently preprocessed datasets into an optimal weights meta-learner architecture (Table 3) that achieved a better performance of an R^2 of 0.89 and an nRMSE of 2.53%

Table 3. Base models used in the meta-learning model and their individual performance.

Model	Preprocessing/Dataset	Irrigated	Test RMSE	Test % RMSE	Test R2	Meta Learner Coefficient
XGBoost	NMF	Yes	0.0045	4.07%	0.717	0.036
ElasticNet	GMM-Scikit	No	0.0038	3.48%	0.793	0.671
KRR	GMM-Scikit	No	0.0046	4.16%	0.705	0.001
XGBoost	Indices	No	0.0060	5.44%	0.497	0.285
Linear Regression	NA	Yes	0.0167	15.21%	-2.941	0.017
KRR	Bands	No	0.0064	5.82%	0.423	-0.007
Meta Learner	All	Yes	0.0028	2.53%	0.891	NA

DISCUSSION

In this study, advanced technologies such as machine learning and remote imaging spectroscopy were utilized to accurately predict the leaf chlorophyll concentration of genotypes in wild blueberry fields. We tested three different approaches: using all available spectral bands, selected vegetation indices, and dimensionality-reduced datasets as predictor variables. The findings showed that machine learning approaches outperformed PLSR (partial least square regression) when all spectral bands were used as input predictors. Moreover, using dimensionality reduction preprocessed data as input predictors further enhanced the ML model performance. Ultimately, the meta-learning model that combined the best-performing models achieved the highest level of predictive performance.

Upon analysis of the mean reflectance properties across various wavelengths, it was observed that the visible green and near-infrared regions of the electromagnetic spectrum tend to exhibit high reflectance. In contrast, the visible blue and red regions demonstrate high absorbance, which can be attributed to the absorption of light by chlorophyll in the visible range (Huete, 2004). Additionally, cellular structures such as cell walls and internal components are responsible for the strong reflectance in the NIR region (Huete, 2004). This also explains the high correlation pattern between LCC and wavelengths at different regions.

Partial Least Squares Regression (PLSR) has gained popularity as a statistical technique to establish relationships between hyperspectral reflectance and various biochemical factors in plants. However, it is important to note that its performance may significantly vary across different plant species, regions, and growth environments. In some cases, PLSR may yield highly accurate predictions, while in others, its performance may be suboptimal due to factors such as spectral interference, signal noise, and variations in plant physiology (Fu et al., 2019). This might be the reason for the low performance of PLSR that we see in our study.

The results of the machine learning models suggest the importance of dimensionality reduction to improve the high collinear hyperspectral data. Studies have found that selecting important bands for modeling through dimensionality reduction algorithms can lead to better model performance compared to using full-spectrum models (Wang et al., 2022). To enhance the performance of our model, we employed advanced techniques known as ensemble or meta-learning. This involved combining multiple weaker learners to create a stronger one. By doing so, we were able to leverage the strengths of each learner and compensate for their weaknesses, resulting in a more accurate and reliable model. This process allowed us to achieve superior results compared to using a single strong learner, as the ensemble technique reduces the risk of overfitting and increases the stability of the model. Overall, the use of ensemble/meta-learning proved to be a valuable strategy in improving our model's performance, which is parallel to some recent studies (Fu et al., 2019; Sterling & Di Rienzo, 2022).

Though we found a satisfactory performance from our meta-learner model, its real-life application to monitor the field-level spatial heterogeneity of LCC for precision agriculture could be complex due to high computational need. To develop a simplified but robust LCC prediction model, work is ongoing on developing a neural vegetation index (NVI) that searches for wavelengths in the space ratio of linear functions of reflectance to automate the process of index development. During training, a neural network searches the space of functions that minimizes a given loss function. The general framework of NVI will also be tested across species and different nutrients.

Photosynthetically active radiation (400-770nm) absorption by leaves depends on photosynthetic pigment concentrations such as chlorophyll, which can impact the efficiency of CO_2 assimilation and primary production (Richardson et al., 2002). Accurate estimation of leaf chlorophyll content is required for monitoring vegetation stress, physiological conditions, and response to environmental factors (Croft et al., 2015). Integration of UAV-based remote sensing of hyperspectral data with machine learning shows promising results for estimating the spatial variability of leaf chlorophyll status in wild blueberries.

CURRENT RECOMMENDATIONS

UAV-based hyperspectral sensors and machine learning models can effectively monitor crop LCC remotely. By detecting the temporal-spatial heterogeneity of LCC, crop health and stresses can be intelligently monitored for precision management to improve crop health.

NEXT STEPS

Further study should be conducted to determine the effectiveness of:

- Using more data (2022 and 2023 data) to improve the model.
- Test the use of UAV-based hyperspectral sensor to detect leaf N status for precision fertilization.
- Test the use of UAV-based hyperspectral sensor for early detection and monitoring of diseases.

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7. Investigating Dual-Use Solar in Wild Blueberry

INVESTIGATORS: L. Calderwood and J. Parks

OBJECTIVES

- Connect with regional leaders on the topic of agrivoltaics
- Document the costs of managing wild blueberry under solar panels

LOCATION: Rockport, ME

PROJECT TIMEFRAME: 2020 – 2024

INTRODUCTION

In 2021 and 2022 we collected plant and environmental data to understand the impact of installing a solar array on wild blueberry land. We concluded that installation did not significantly reduce wild blueberry cover. This array was not built as a true dual-use array because it has spacing very similar to a standard array with eight-foot rows and panels eight feet off the ground. Shading exceeded 90% reduction in sunlight, thereby reducing wild blueberry yield dramatically, as expected. In 2023 we did not collect further plant or environmental data but focused on bringing experts from the New England region together for knowledge sharing on dual-use agriculture and began understanding more about the costs associated with managing wild blueberries under a solar array.

Dual-use solar is the installation of solar panels in such a way that agricultural activities such as crop production and animal grazing can still occur profitably. Such arrays include higher panel heights and increased row spacing to allow enough sunlight to reach the crop underneath and for equipment and workers to maneuver.

METHODS

Agrivoltaics Knowledge Sharing

On November 14, 2023 we convened a group of major players in the Northeast dual-use agriculture sphere for a knowledge sharing session. Participants included those from UMaine Extension, UMass Extension, State of Maine Department of Agriculture, Conservation, and Forestry (DACF), State of Massachusetts Department of Agricultural Resources (MDAR), Maine Governor's Energy Office (GOE), Maine Farmland Trust, American Farmland Trust, ReVision Energy, and BlueWave Solar. A summary of relevant information that we learned can be found in the Results section.

Cost Comparison

The Rockport array was installed in 2021, making the 2022 crop year in this field still a recovery season and therefore the crop was not harvested. The revenue made from the solar panels is not yet available. This field will be harvested in 2024 which will allow us to complete our budget analysis. A cost comparison before and after solar was begun given the limited data available thus far. A one-on-one interview with the farmer who manages the Rockport solar-blueberry site was completed by going through costs with the farmer. We used a calculator made in Microsoft Excel to determine annual return, revenue per acre, and total cost per acre.

RESULTS

Agrivoltaics Knowledge Sharing

From the UMaine Extension organized knowledge sharing meeting held on November 14, 2023, we learned from the States of Maine and Massachusetts. The State of Massachusetts MDAR receives applications for dual-use solar projects and approves them through a six-step process. UMass Clean

Energy Extension receives dual-use project proposals and provides a Pre-Determination of the project based on their agronomic and energy expertise with suggestions for a successful project. There have been 35 dual-use solar projects on 435 acres approved by MDAR on commercial cranberry, mixed vegetable, hay, and livestock farms since 2018. In Massachusetts, 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 does not exceed 50% during the growing season (Clean Energy Extension, 2022). This tool was created by the Solar Massachusetts Renewable Target (SMART) program, the Massachusetts Department of Energy and Resources (DOER) and BlueWave Solar for Massachusetts at their longitude and latitude and is therefore not entirely transferrable to Maine sites. The user can input solar panel dimensions, number of rows, the tilt of panels, region in the state, and the output generates a sun or shade map of the area and design. The University of Massachusetts Amherst uses this for research and education while solar developers use it to evaluate potential sites. A webinar on the Shading Analysis Tool can be found on the BlueWave Solar website. Challenges to the Massachusetts dual-use project approval process are that it takes longer to get a project approved, more research is needed, and it is hard to optimize both the crop and energy.

Dual-use arrays with commercial crops below are possible with solar companies that are learning how to work with farmers to address their needs with modified spacing and panels such as elevated single-axis tracking panels. Research is underway in Massachusetts in a collaboration between UMass and American Farmland Trust to explore broccoli, squash, lettuce, and hay production in a dual-use solar array with 28ft wide rows. As of 2018, the State of Massachusetts has had an incentive program for dual-use solar. Rate payers (residents receiving energy from the grid) pay $0.06 cents per kW/h to solar developers for dual-use solar projects.

The challenge with establishing dual-use projects that work in Maine is that our power grid infrastructure is outdated. Three phase power is required at the road to install a solar array. Until our grid has been updated companies like BlueWave that have worked with farmers to design innovative dual-use projects will not be able to do so. In 2022, with the passing of LD 1881, the state of Maine DACF requires a compensation fee to be paid if solar is sited on prime agricultural soils. This fee can be waived if an equivalent square foot area of land is put into conservation. The Department is currently developing definitions of “prime agricultural soils” and “dual-use solar” which aim to protect the environment and Maine’s farmland. They are hiring someone to manage a new permitting scheme. Before agreeing to any contracts with solar companies, land owners need to contact their municipal assessor to investigate what types of tax consequences will be put in place with the addition of solar if their land is considered “high value farmland” (LD 1881). The U.S. Department of Energy Technologies Office (SETO) is researching agricultural benefits and setbacks to agrivoltaics that are added to farm operations (DOE, n.d.).

Cost Comparison

The Rockport dual-use wild blueberry site is a leased field managed by an IQF processor grower. We interviewed the farm manager about their crop management costs before and after this particular solar array was installed in 2021. The costs from the 2019 crop year in Table 1 are from the same area that solar now stands. The solar array was built in 2021 (crop year) and was held over for a crop year again in 2022 to get all sections of the field into the same cycle. The grower did not harvest when the solar array was built in 2021 due to construction. In 2022, the grower estimated the cost of harvest was not worth the return from the low yield while the field was still recovering. Weed management was done using herbicide wiping and spot mowing, along with some disease control to prepare the crop for 2024. There were no fertilizer applications, insect management, pollinating costs, or harvest costs in 2023 (Table 1).

Table 1. Itemized revenue comparison of before and after building a solar array in wild blueberry located in Rockland, ME. Costs are from 2019 before the array, and 2022 after construction in 2021 Parentheses represent a loss. The field has not yet been commercially harvested after construction.

Category	Description	Before Array	After Array
Pruning	Bush hog mowing	$ 400.00	$ 367.50
	Mowing	$ 560.00	$ 630.00
Weed Control	Application of Callisto	$ 160.00	$ -
	24 oz of Callisto	$ 100.08	$ -
	LI700 Surfactant	$ 9.12	$ 7.85
	Choice water conditioner	$ 7.22	$ -
	Application of Poast	$ -	$ 100.08
	Poast	$ -	$ 225.20
	Brush wiping	$ -	$ 1,500.00
Disease Control	Application Fitness and Radiate	$ 96.00	$ -
	Fitness	$ 96.00	$ -
	Fitness	$ 28.20	$ -
	Radiate	$ 38.40	$ 48.40
Pollination	Bee hives (12)	$ 1,184.16	$ -
Insect Control	Application of Imidan	$ 96.00	$ -
	10 lbs of Imidan	$ 112.80	$ -
	LI700 Surfactant	$ 7.49	$ -
Harvest	Cost of harvest (approximate)	$ 3,259.64	$ -
Yield	Blueberry yield ($0.40 per lb)	$ 6,862.40	$ -
Tax	Blueberry tax ($0.0075 per lb)	$ 128.67	$ -
Pounds Harvested	Total Yield (lbs)	17,156.00	0.00
	Total Costs and Revenues		
	Total fixed costs	$ 16.08	$ -
	Total variable costs	$ 769.39	$ 372.96
	Total costs ($ per acre)	$ 785.47	$ 372.96
	Returns ($ per acre)	$ 72.33	$ (372.96)
	Average total annual return to management	$ 578.62	$(2,983.70)
	Yield (lb per acre)	$ 857.80	$ -

DISCUSSION

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 and to determine if you have three-phase power at the road or not. Finally, make sure you have excellent communication between yourself, the solar developer, and the construction company.

In 2024, we plan on building mock solar panels and spacing them 28ft apart and 10-15ft high to evaluate shading over wild blueberry. In Rockport, we will also evaluate wild blueberry plant density and yield again to conclude the project. We will then finalize the cost of wild blueberry management under the Rockport solar array.

CURRENT RECOMMENDATIONS

- When considering a solar array, decide if it the main goal is crop or energy production.
- Ensure excellent communication between you, the solar developer, and the construction company.

ACKNOWLEDGEMENTS

Thank you to Northeast SARE grant LNE22-448R for funding this three-year project (2022-2024).

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1. Impact of Wild Blueberry Plant Nutrients and Phenology on Berry Quality

INVESTIGATORS: L. Calderwood, B. Calder, K. Davis-Dentici, B. Perkins, J. Perry (University of Maine); and T. Esau (Dalhousie University)

OBJECTIVES

- Measure the impact of sunlight 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, Sedgwick, and Columbia Falls, Maine; Kemptown and Highland Village, Nova Scotia

PROJECT TIMEFRAME: April 2021 – March 2024

INTRODUCTION

Several studies have expanded our knowledge of wild blueberry ripening (Mallick and Hamilton, 2017; Gibson et al., 2013; Forney et al., 2012) yet few have looked at regional patterns in ripening in connection to plant nutrition. This report explains results from the final year of a three-year project exploring the impact of local weather conditions and nutrient availability on berry ripening through the season. At three Maine (ME) and two Nova Scotia (NS) locations, we continued to research how plant phenology and nutrient availability were related to berry quality measures including Brix, titratable acidity, color, and organic acids at green, color change, and blue fruit stages. This work has implications for fertility programs in wild blueberry fields and value-added product creation.

METHODS

Field Data Collection

This was the final year of a three-year project and data collection continued largely unchanged from the previous year. The project encompassed six on-farm trial locations: three conventional fields in ME 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.

Phenology and Plant Nutrient Content (Calderwood of UMaine)

At all three locations in ME, one foliar sample and one flower/fruit sample was taken from each 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 30 g 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 fluctuated; then ground to a fine texture and analyzed by the Soil Testing Lab in Orono. 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.

For phenology, the total number of green, color change, and blue fruit were counted on 10 stems per plant (6 plants per location) at peak green, color change, and blue fruit. This was done in ME and NS in both 2022 and 2023.

Data Analysis
Phenology data was analyzed using Microsoft Excel. Stem data was averaged by growth stage and location.

Data from percent nitrogen, phosphorus, calcium, and potassium were separated by leaf and fruit nutrients and analyzed using JMP (JMP® Pro Version 17.0.0. SAS Institute Inc., Cary, NC 1989-2023). Data from 2022 was combined with 2023 and was analyzed with the follow model for ME; Random variables: site location nested within Year, Year, Year*Growth Stage, and Fixed Variable: Growth Stage. If Year or Year-by-treatment was significant the data was separated and analyzed separately. Non-significant random variable interactions of the highest order were removed first until the fixed variable reached a p-value of ≤ 0.05 or site location nested within Year and Year were the remaining random variables. The model used for NS data was, Random variables: Year and Year*Growth Stage; Fixed variables: Growth Stage. The same process was used for the model as described previously for the ME location.

Model residuals for nutrient analysis were checked for normality using a Shapiro-Wilks test and studentized residuals were visually checked for homoscedasticity. If the model satisfied assumptions, significant results were further explained using a Tukey's Honest Significant Differences test for mean comparisons. If the model failed normality, transformations were used. Successful transformations were used for analysis and the back-transformed means are shown in figures. If transformations did not work, a non-parametric Kruskal-Wallis test was used. Significant results were analyzed using Dunn's post hoc test for mean comparisons among ranks and p-values were provided instead of a connecting letters report. Figures without connecting letters were subjected to the Dunn's test and significant p-values are shown in the results.

Multivariate linear regression models were used to analyze dependent variables of wild blueberry yield and Brix (separated by leaf and fruit samples) by macro- and micro-nutrients. Normality of residuals were checked with a Shapiro-Wilks test and homoscedasticity of studentized residuals were visually checked. To increase the usability of models, non-significant predictor variables were removed to increase the adjusted R^2 value until the value started to decrease, then removal was stopped. Prediction equations of models showing an adjusted R^2 of ≥ 0.50 were displayed in the results. If a model did not satisfy assumptions, data transformations were used on the dependent variable. Significant effects were shown in bivariate linear regression models where the same assumptions were met.

<u>Berry Quality Field Collection and Lab Analysis (Calderwood and Calder of UMaine)</u>
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 110-850 g of wild blueberries was handpicked from just outside each plot within a 0.5 m x 0.5 m quadrat but within the same plant. Harvests occurred during the green fruit phase (June 23, 29, and 30), color change phase (July 13, 17, and 21), and blue fruit phase (August 1, 2, and 8). 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 (TA) (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 50 g sample from each quadrat was counted as well. Researchers weighed a 50 g sample from a quadrat, counted the number of fruits comprising that sample, and recorded the number. The 50 g sample was then dumped back into the bag, gently shaken, and another 50 g 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. All 13-415 g NS wild blueberry samples were handpicked during the green fruit phase (June 29 and July 12), color change phase (July 21 and 27), and blue fruit phase (August 4 and 8). All NS samples were delivered to Acadia University's Laboratory for Agri-Food and Beverage. The lab evaluated color, pH, TA, fructose and glucose, organic acids, and TA for green berries.

Imaging for Ripeness (Esau of Dalhousie University)

On each phenological and harvest collection date, digital pictures were taken of a 0.5 x 0.5 m quadrat (including all fruit and stems visible) from a 1 m height within the same plant. Each of the six plots were oriented to have one of the sides facing north. Using this approach, the same corner of each plot was harvested, weighed and imaged on each date. The order of selected corners follows the dates in (Image 1). After the photo was taken, all fruit was hand-harvested from within the 0.5 x 0.5 m quadrat, and the fruit weighed.

Following data 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. 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. All regression equations were developed using Minitab® version 19 (Minitab LLC, State College, PA, USA). For each regression, significance of higher order interactions was assessed and adjusted R^2 and Root Mean Square Error (RMSE) were used for comparing between models.

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) 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 (2022) data was analyzed solely using YOLOv4. Further, as with year one 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.

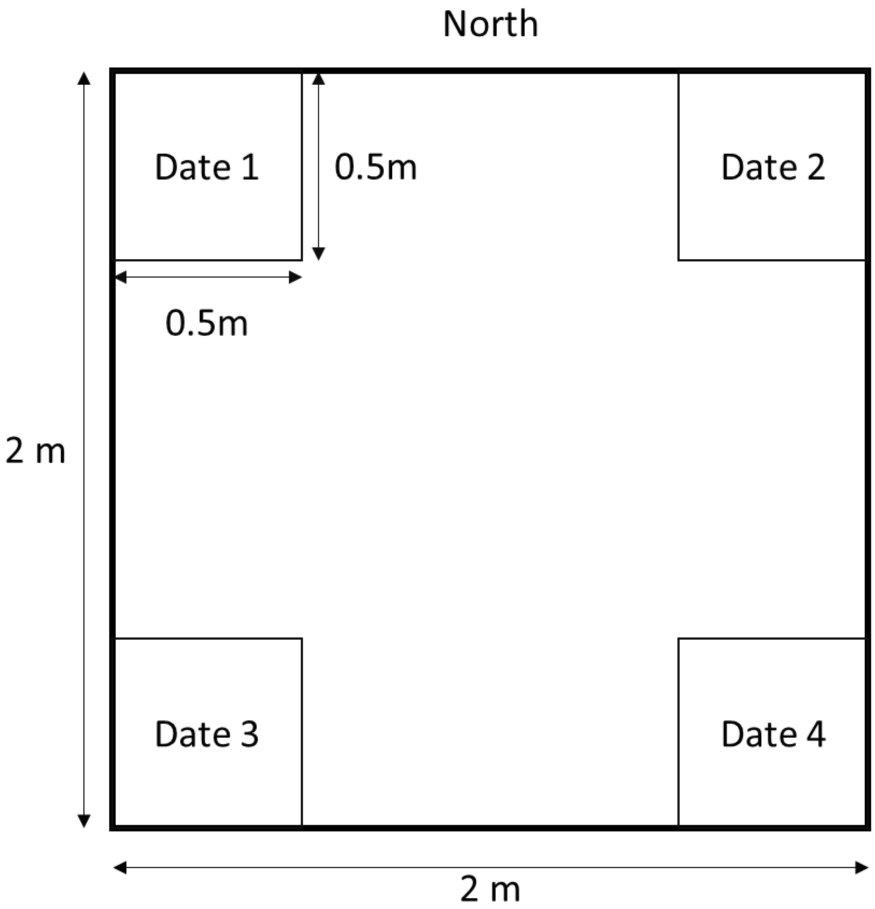

Image 1. Figure outlining the order in which corners were selected for imaging and harvesting

RESULTS

Phenology and Plant Nutrients (Calderwood of UMaine)

Phenology Development

Nova Scotia had an increase in the number of fruit per stem from 2022 to 2023 at both green and blue fruit stages. In ME there was an increase in the number of green fruit from 2022 to 2023 yet blue fruit decreased from 2022 to 2023. In both locations the number of green fruit per stem was higher than the number of blue fruit per stem in both years (Figure 1).

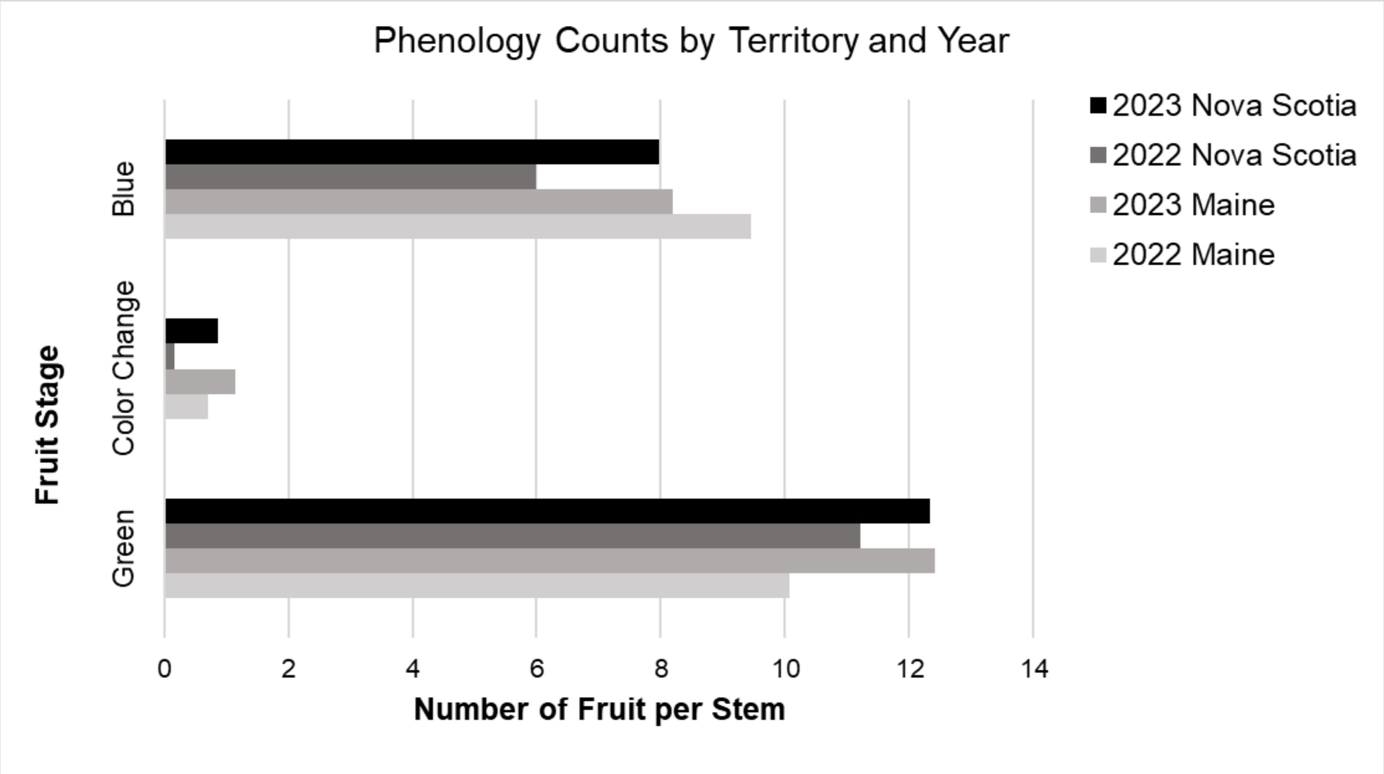

Figure 1. Development of wild blueberry fruit from green to blue in 2022 and 2023. Peak green, color change, and blue fruit were calculated for each stem and then averaged by location. Locations included Maine (Hope, Sedgwick, and Columbia Falls) and Nova Scotia (Webb and Kemptown). Peak sampling times varied by location.

Plant Nutrient Contents

Prune year optimal leaf nutrients were compared to the crop leaf samples collected in 2022 and 2023 (Tables 1 and 2). There are no crop year established standards for what the optimal ranges are for percent nitrogen, phosphorus, calcium, or potassium within the fruit. We are currently using the prune year nutrient standards to evaluate crop year leaf samples. Nitrogen content in the leaves (standard range 1.55-1.85%) was significantly greater at the flower stage than the fruit stages in ME and in NS ($P < 0.001$ and $P < 0.001$, respectively) (Figure 2). Nitrogen content in the fruit significantly decreased from the flower stage to the blue fruit stage in ME and NS ($P = 0.001$ and $P < 0.001$, respectively) (Figure 2). Phosphorus content in the leaves (standard range 0.11-1.43%) dropped 52% from the flower stage to the green fruit stage in NS ($P = 0.002$) (Figure 3). In ME, leaf phosphorus content was significantly greater in the flower stage when compared to the other stages ($P < 0.001$) (Figure 3). Phosphorus content in the fruit for ME and NS followed similar trends and significantly decreased from the flower stage to the blue fruit stage ($P = 0.001$ and $P = 0.003$, respectively) (Figure 3). Potassium content in leaves (standard range 0.31-0.56%) significantly decreased from flower stage to the fruiting stages in ME and NS ($P = 0.032$ and $P = 0.003$, respectively) (Figure 4). The potassium content in the fruit significantly decreased from the flower stage to the blue fruit stage in ME and NS ($P = 0.002$ and $P = 0.009$, respectively) (Figure 4). Calcium content in the leaves (standard range 0.31-0.40%) significantly increased from the flower stage (0.28) to the blue fruit stage (0.66) in ME ($P = 0.002$) (Figure 5). Calcium content in NS did not change significantly except that the flower stage (0.25) was significantly lower than the color change stage (0.49) ($P = 0.001$) (Figure 5). Calcium content in the fruit significantly decreased from the flower stage to blue fruit stage in ME ($P = 0.007$), however, the only significant difference in NS was between color change (0.17) and blue fruit (0.27) ($P = 0.037$) (Figure 5).

Table 1. Optimum leaf nutrient levels for prune year compared to those sampled over the crop year growing season in Maine, 2023. N = 18 for mean nutrient sample values.

Maine, 2023		**Mean Sample Levels by Stage in Leaves**			
Nutrient	**Optimum**	**Flower**	**Green**	**Color Change**	**Blue**
N (%)	1.76	1.88	1.40	1.45	1.44
P (%)	0.14	0.22	0.12	0.11	0.12
K (%)	0.44	0.62	0.38	0.34	0.37
Ca (%)	0.38	0.27	0.43	0.61	0.69
Mg (%)	0.17	0.14	0.18	0.23	0.24
Fe (ppm)	33.60	31.26	28.32	32.08	36.92
Mn (ppm)	521.00	1010.33	1128.28	1630.89	1721.06
Zn (ppm)	6.30	22.54	12.83	16.40	13.10

Table 2. Optimum leaf nutrient levels for prune year compared to those sampled over the crop year growing season in Maine, 2022. N = 30 for mean nutrient sample values.

Maine, 2022		**Mean Sample Levels by Stage in Leaves**			
Nutrient	**Optimum**	**Flower**	**Green**	**Color Change**	**Blue**
N (%)	1.76	1.94	1.53	1.27	1.40
P (%)	0.14	0.21	0.16	0.12	0.11
K (%)	0.44	0.54	0.53	0.45	0.38
Ca (%)	0.38	0.34	0.33	0.43	0.59
Mg (%)	0.17	0.15	0.15	0.17	0.23
Fe (ppm)	33.60	42.55	30.06	29.58	42.28
Mn (ppm)	521.00	1142.88	991.66	1100.27	1505.09
Zn (ppm)	6.30	20.77	19.76	11.44	15.69

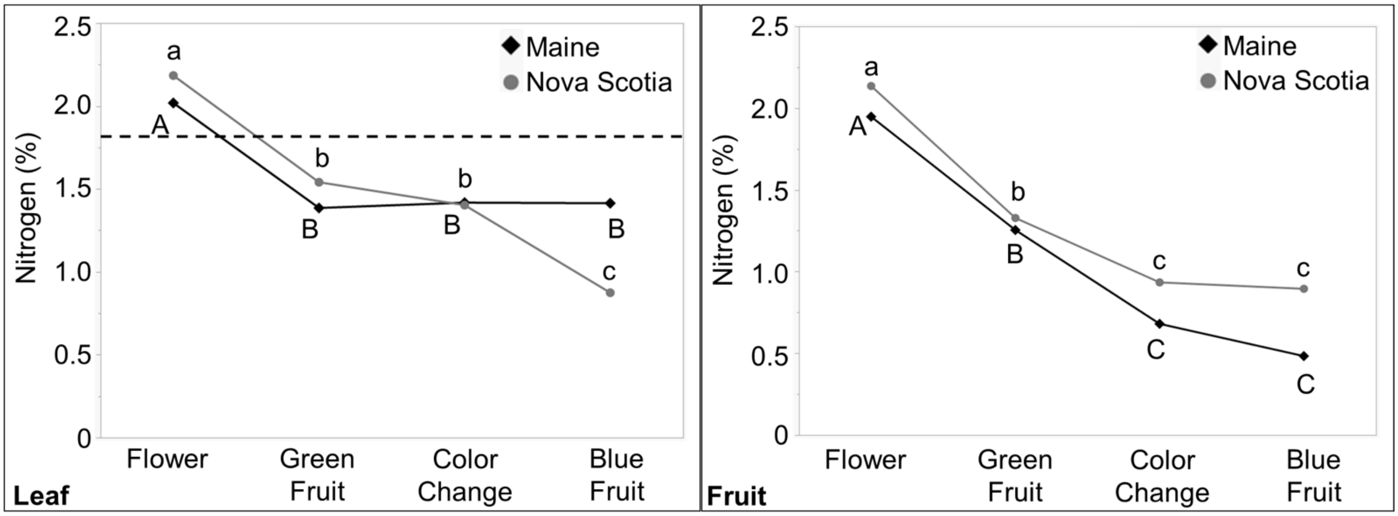

Figure 2. Nitrogen content in leaves and fruit throughout wild blueberry growth stages. The horizontal dashed line indicates optimum foliar nutrient level in the *prune* year. Locations included Maine (Hope Sedgwick, and Columbia Falls) and Nova Scotia (Webb) with 2022 and 2023 combined. Data was subjected to a Tukey's HSD means comparison, means sharing letters are not significantly different α ≤ 0.05.

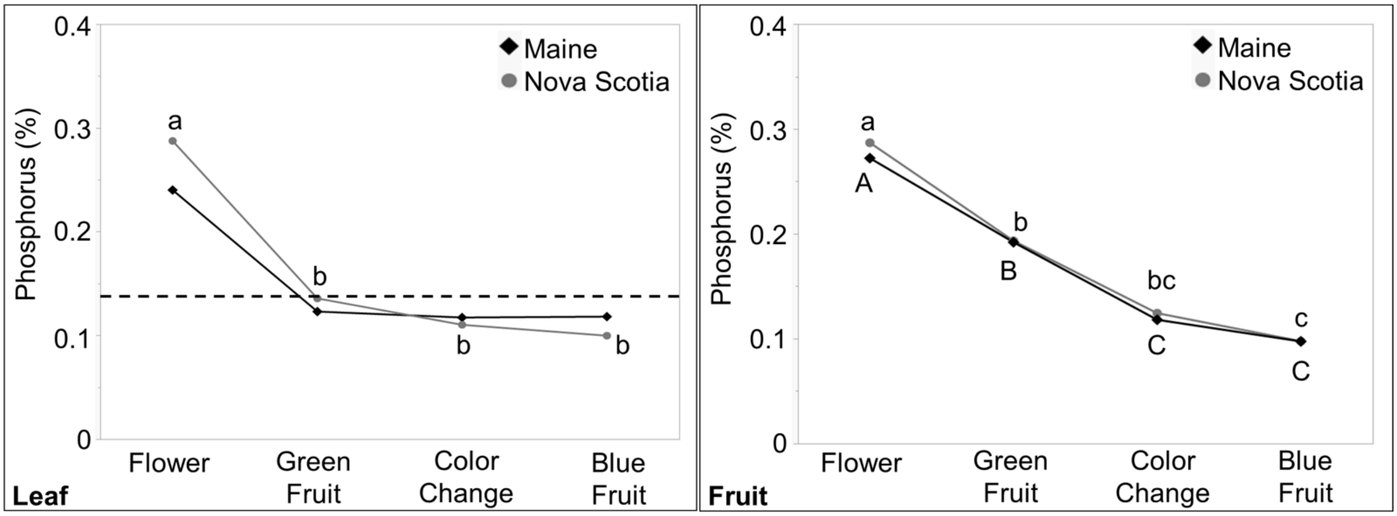

Figure 3. Phosphorus content in leaves and fruit throughout wild blueberry growth stages. The horizontal dashed line indicates optimum foliar nutrient level in the *prune* year. Locations included Maine (Hope Sedgwick, and Columbia Falls) and Nova Scotia (Webb) with 2022 and 2023 combined. Data was subjected to a Tukey's HSD means comparison, means sharing letters are not significantly different α ≤ 0.05.

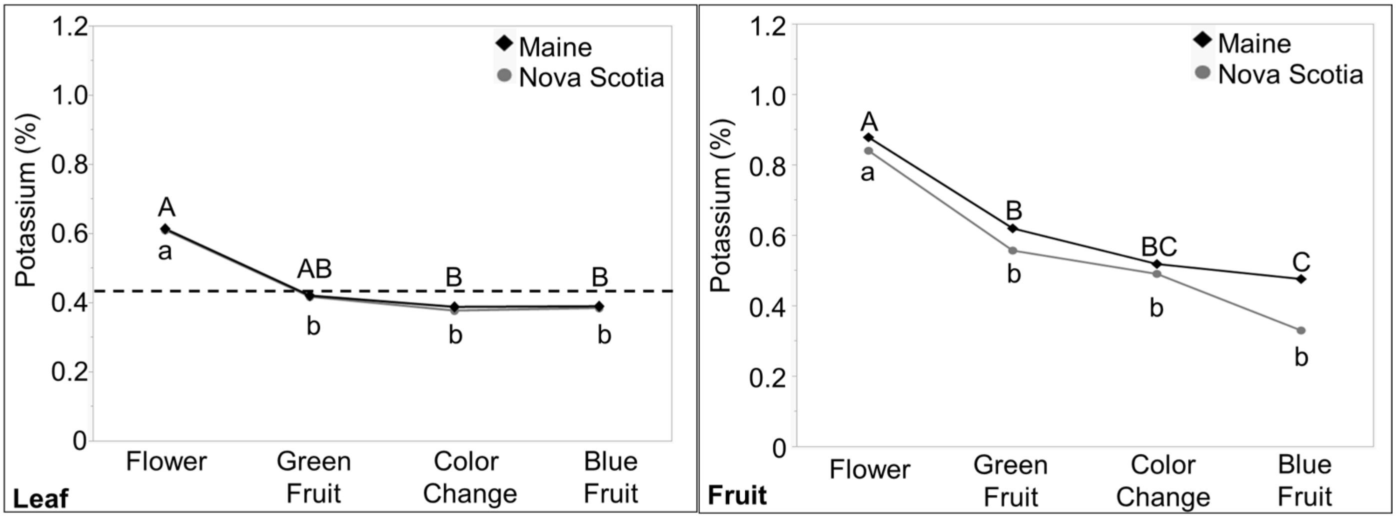

Figure 4. Potassium content in leaves and fruit throughout wild blueberry growth stages. The horizontal dashed line indicates optimum foliar nutrient level in the *prune* year. Locations included Maine (Hope Sedgwick, and Columbia Falls) and Nova Scotia (Webb) with 2022 and 2023 combined. Data was subjected to a Tukey's HSD means comparison, means sharing letters are not significantly different α ≤ 0.05.

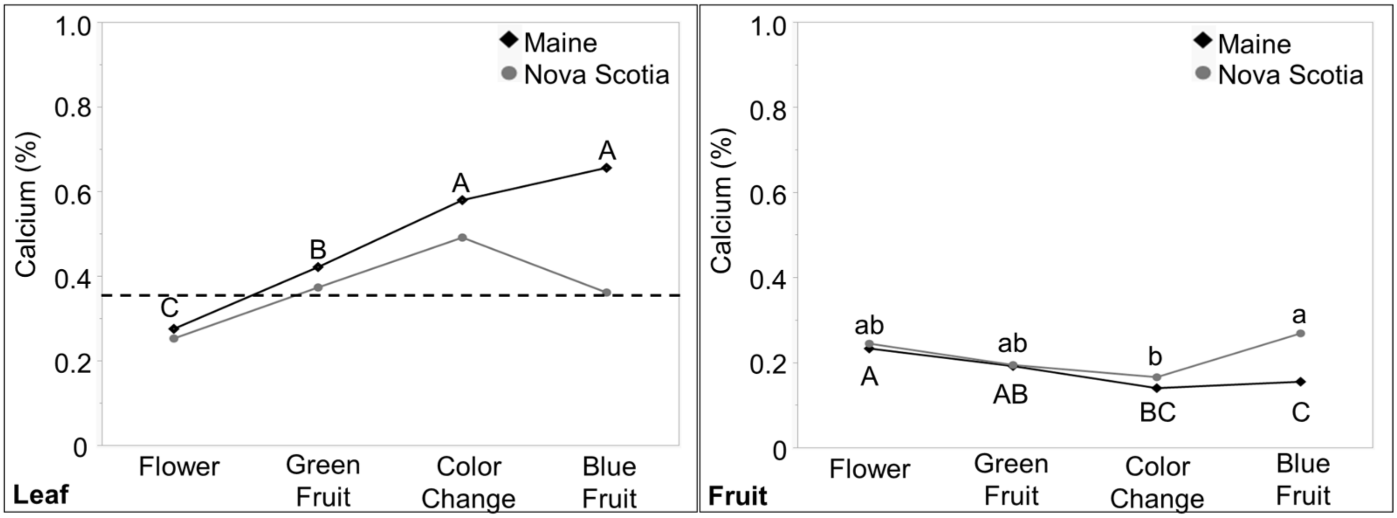

Figure 5. Calcium content in leaves and fruit throughout wild blueberry growth stages. The horizontal dashed line indicates optimum foliar nutrient level in the *prune* year. Locations included Maine (Hope Sedgwick, and Columbia Falls) and Nova Scotia (Webb) with 2022 and 2023 combined. Data was subjected to a Tukey's HSD means comparison, means sharing letters are not significantly different α ≤ 0.05.

Yield, Berry Quality and Plant Nutrients

Multivariate linear regression was used to evaluate if any of the leaf or fruit nutrients could be used to predict yield or berry quality. Wild blueberry yield for ME and NS was used as the dependent variable and the predictors or independent variables used were Brix, fruit pH, percent TA, nitrogen, phosphorus, potassium, magnesium, and calcium measured from fruit samples (Table 3). Once insignificant predictors were removed from the model, Brix was the only significant variable (P = 0.003), but it depended on sampling percent phosphorus, potassium, calcium, and TA (Table 3). The model equation is not shown due to the low adjusted R^2 of 0.23 (Table 4). A bivariate linear regression of wild blueberry yield and fruit Brix content showed that high Brix (fruit sugar content) correlated to lower yield ($P < 0.001$) (Figure 6).

Evaluating wild blueberry yield from ME and NS with leaf nutrients nitrogen, potassium, magnesium, and calcium, resulted in different predictor variables (Table 4). Once non-significant predictors were removed from the model, it was found that predicting yield depends on sampling leaf potassium, calcium, and magnesium content (Table 4). Bivariate linear regression of yield and leaf potassium content showed a decreasing trend in yield as leaf potassium increased ($P = 0.005$) (Figure 7). Lastly, a bivariate linear regression of yield and leaf calcium content showed an increase in yield with increased leaf calcium content ($P = 0.047$) (Figure 8).

Brix from ME and NS was then evaluated as a dependent variable in a multivariate linear regression with fruit macro- and micro-nutrients from fruit as predictor variables since Brix was a significant predictor in yield for the previous model (Table 5). Once insignificant predictors were removed, nitrogen, phosphorus, calcium, and magnesium were all significant when predicting Brix content (Table 5). The model equation is not shown due to a low adjusted R^2 value of 0.20 (Table 5). Bivariate linear regressions were conducted for significant predictors in the model, but they depended on each other for the multivariate model, so they were not significant in a bivariate model (data not shown).

A multivariate linear regression was used to evaluate Brix from ME and NS as a dependent variable with leaf nutrients as predictors (Table 6). All macronutrients and manganese were significant, and calcium, magnesium, boron, and iron were dependent on the model (Table 6). The equation for the model is shown with an adjusted R^2 value of 0.57 (Equation 1). The only bivariate linear regression shown is fruit Brix by leaf potassium since the other nutrients depended on each other and potassium showed a stronger correlation. As potassium in the leaf increases, Brix content significantly increases ($P < 0.001$) (Figure 9).

Table 3. Multivariate linear regression table that shows the predicted influence of the independent variables %TA (titratable acidity), Brix (fruit sugar content), fruit pH, fruit macronutrients (%N, %P, %K, %Mg, and %Ca) on the dependent variable Yield (lbs/A). Only blue fruit was included for Maine (Hope, Sedgwick, and Columbia Falls) and Nova Scotia (Webb) for the years 2022 and 2023. The Bold text indicates a significant linear relationship at $\alpha \leq 0.05$. Yield data residuals were normalized with a Box Cox transformation (lambda = 0.053).

	Dependent Variable: Yield (lbs/A)		
	Adjusted R^2	**F Ratio**	***p***
	0.23	2.45	**0.043**
Independent Variables		**t-value**	***p***
Fruit	**P**	1.12	0.274
	K	-1.49	0.148
	Ca	-0.55	0.584
	Brix	**-3.20**	**0.003**
	TA	-0.17	0.862

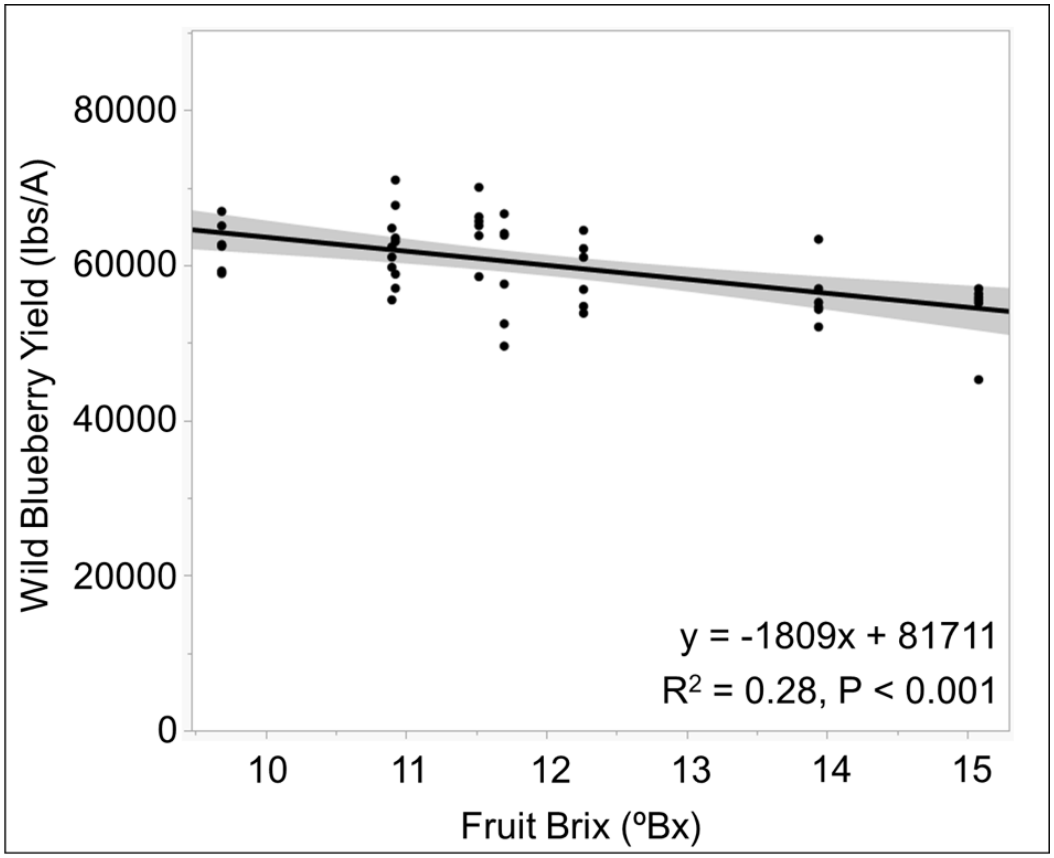

Figure 6. Significant relationship ($\alpha \leq 0.05$) of wild blueberry yield by fruit Brix for locations, Maine (Hope, Sedgwick, and Columbia Falls) and Nova Scotia (Webb) for years 2022 and 2023. Blue fruit data is shown and was extrapolated from 0.25 m^2 plots handpicked in the field; yields of 10,000 +lbs/A are only representative of small plots not entire fields.

Table 4. Multivariate linear regression table that shows the predicted influence of the independent variables, leaf macronutrients (%N, %P, %K, %Mg, and %Ca) on the dependent variable Yield (lbs/A). Only blue fruit was included for Maine (Hope, Sedgwick, and Columbia Falls) and Nova Scotia (Webb) for the years 2022 and 2023. The Bold text indicates a significant linear relationship at $\alpha \leq 0.05$. Yield data residuals were normalized with a Box Cox transformation (lambda = 0.231).

Dependent Variable: Yield (lbs/A)			
	Adjusted R^2	**F Ratio**	***p***
	0.21	5.11	**0.004**
Independent Variables		**t-value**	***p***
Leaf	**K**	**-2.94**	**0.005**
	Ca	**2.20**	**0.033**
	Mg	-1.37	0.179

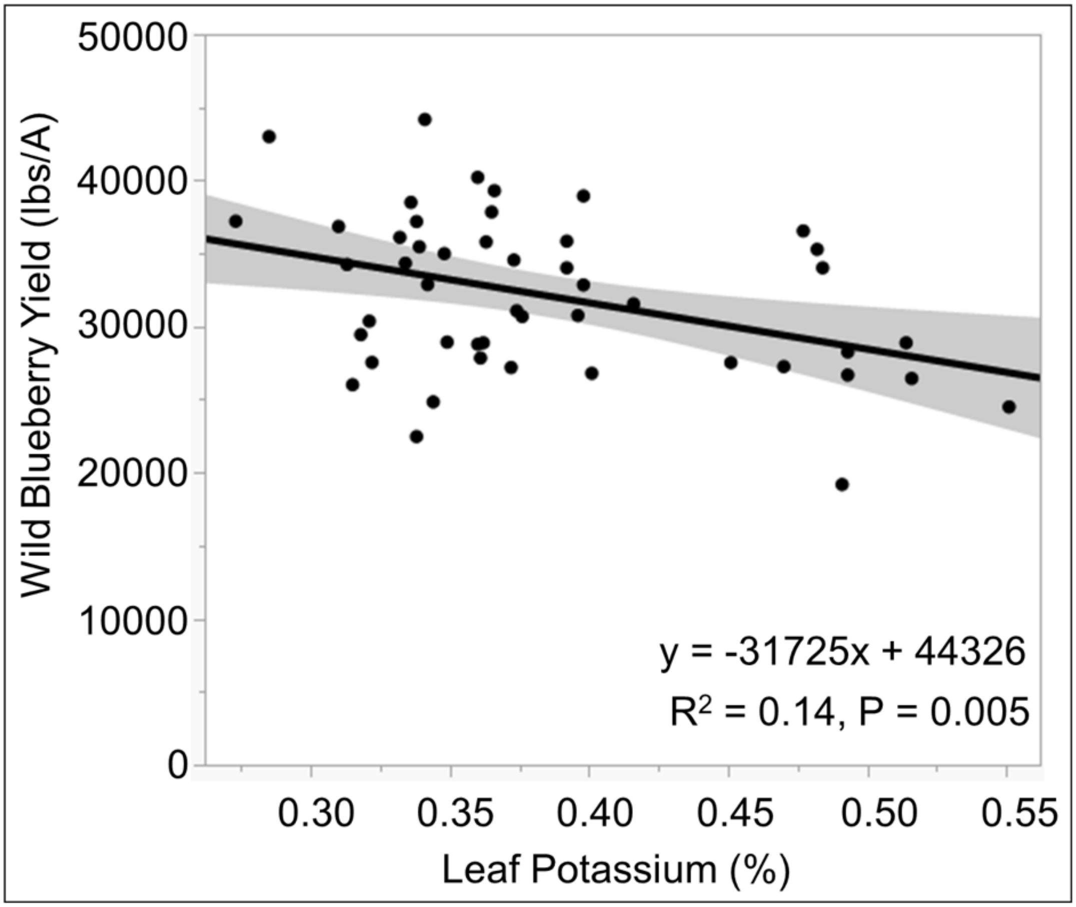

Figure 7. Significant relationship ($\alpha \leq 0.05$) of wild blueberry yield by leaf potassium for locations, Maine (Hope, Sedgwick, and Columbia Falls) and Nova Scotia (Webb) for years 2022 and 2023. The yield data shown was extrapolated from 0.25 m^2 plots handpicked in the field; yields of 10,000 +lbs/A are only representative of small plots not entire fields.

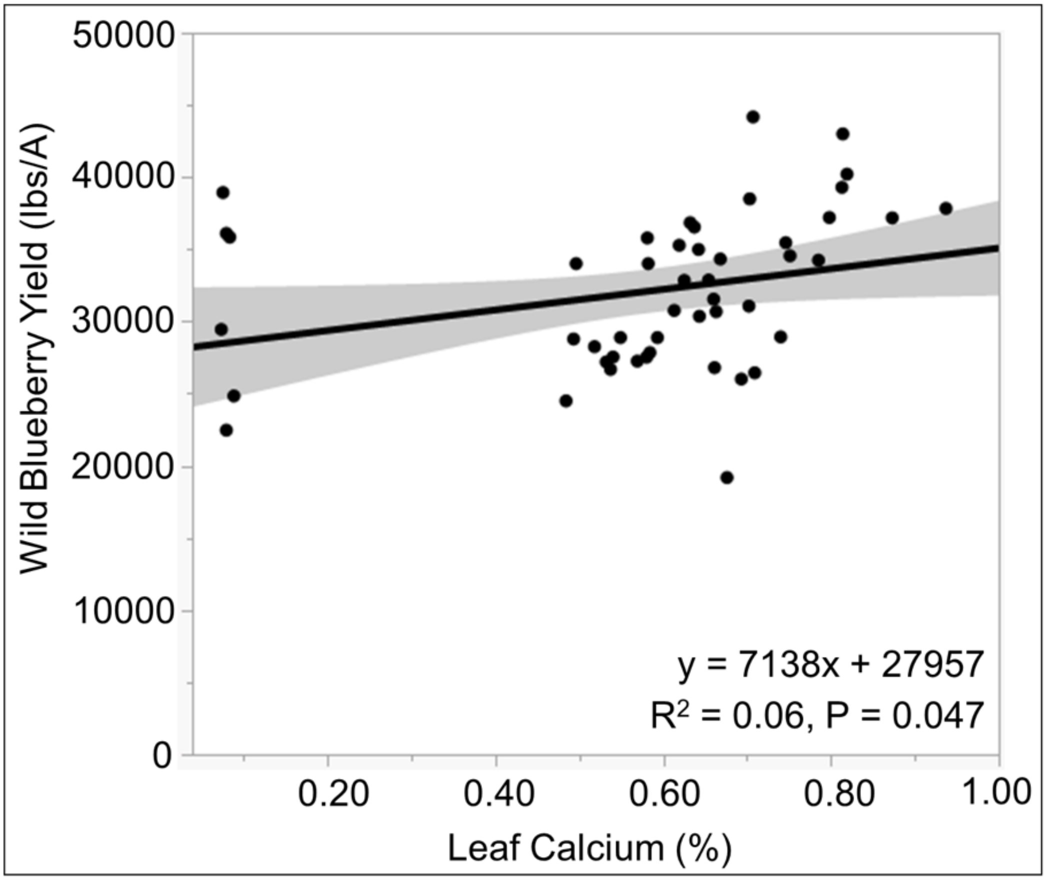

Figure 8. Significant relationship (α ≤ 0.05) of wild blueberry yield by leaf calcium for locations, Maine (Hope, Sedgwick, and Columbia Falls) and Nova Scotia (Webb) for years 2022 and 2023. The yield data shown and was extrapolated from 0.25 m^2 plots handpicked in the field; yields of 10,000 +lbs/A are only representative of small plots not entire fields.

Table 5. Multivariate linear regression table that shows the predicted influence of the independent variables, Fruit macronutrients (%N, %P, and %K) and fruit macronutrients (%Ca, %Mg, Fe ppm, Mn ppm, Zn ppm) on the dependent variable Brix (°Bx). Only blue fruit was included for Maine (Hope, Sedgwick, and Columbia Falls) and Nova Scotia (Webb) for the years 2022 and 2023. The Bold text indicates a significant linear relationship at α ≤ 0.05.

	Dependent Variable: Brix (ºBx)		
	Adjusted R^2	**F Ratio**	**p**
	0.20	3.17	**0.027**
Independent Variables		**t-value**	***p***
Fruit Macronutrients	**N**	**2.91**	**0.007**
	P	**-2.31**	**0.028**
Fruit Micronutrients	**Ca**	**-3.35**	**0.002**
	Mg	**2.76**	**0.010**

Table 6. Multivariate linear regression table that shows the predicted influence of the independent variables, Leaf macronutrients (%N, %P, and %K) and Leaf macronutrients (%Ca, %Mg, Fe ppm, Mn ppm, Zn ppm, and B ppm) on the dependent variable Brix (°Bx). Only blue fruit leaf samples were included for Maine (Hope, Sedgwick, and Columbia Falls) and Nova Scotia (Webb) for the years 2022 and 2023. The Bold text indicates a significant linear relationship at α ≤ 0.05. The Brix data was transformed with a square root transformation for analysis.

	Dependent Variable: Brix (ºBx)		
	Adjusted R^2	**F Ratio**	**p**
	0.57	6.56	**<0.001**
Independent Variables		**t-value**	***p***
Leaf Macronutrients	**N**	**2.33**	**0.028**
	P	**-3.11**	**0.005**
	K	**4.84**	**<0.001**
Leaf Micronutrients	**Ca**	-1.07	0.294
	Mg	1.90	0.068
	B	-1.60	0.122
	Fe	1.44	0.162
	Mn	**-3.07**	**0.005**

$$\textit{Brix Content} = 3.741(\%N) - 3.108(\%Ca) + 18.029(\%K) + 9.845(\%Mg) - 54.554(\%P) - 0.042(B\ ppm) + 0.021(Fe\ ppm) - 0.001(Mn\ ppm) + 8.253 \qquad \textbf{Eq (1)}$$

$R^2 = 0.67$
$Adj.\ R^2 = 0.57$
$RMSE = 1.05\ °Bx$

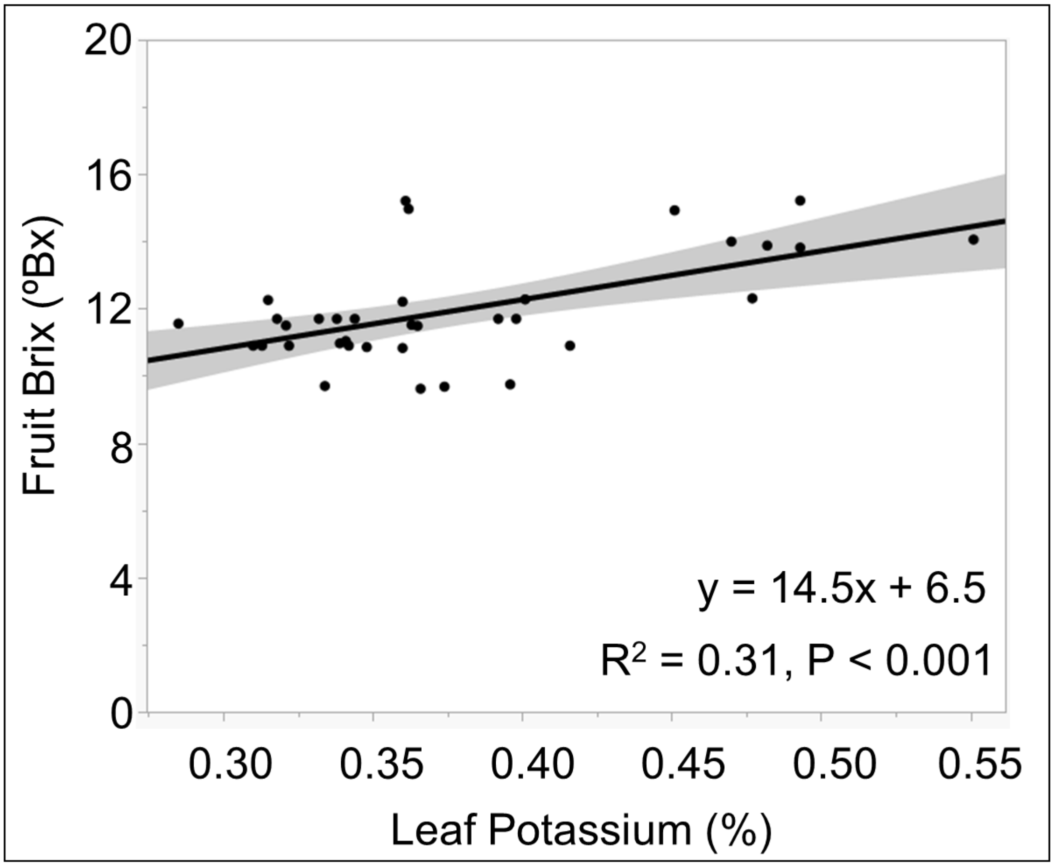

Figure 9. Significant relationship ($\alpha \leq 0.05$) of blue fruit Brix by leaf potassium for locations, Maine (Hope, Sedgwick, and Columbia Falls) and Nova Scotia (Webb) for years 2022 and 2023.

Imaging for Ripeness & Quality (Esau of Dalhousie University)

Across each of the four models (Tables 7-10), the 2-class, YOLOv4-Tiny performed the best in terms of adjusted R^2 and RMSE (Figure 10). 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 (data not shown).

Table 7. 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 8. 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 9. 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 10. 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%

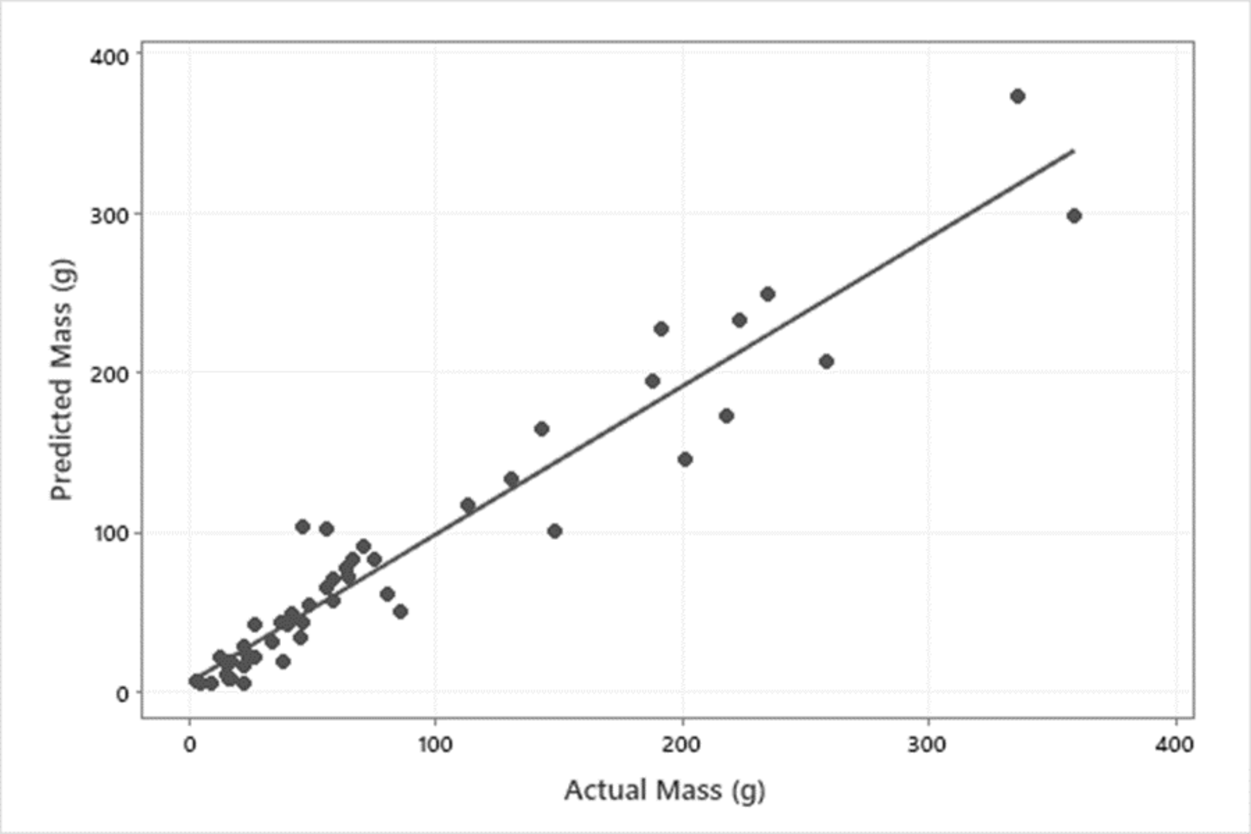

Figure 10. Plot of actual vs predicted yield using equation 2 to predict mass from plot images (R^2 = 92.6%) using the 2-class, YOLOv4-Tiny model.

*Predicted Mass = -4.78 + 0.9422RipeCount + 0.1512UnripeCount – 0.001244Ripecount*Unripecount* **Eq (2)**
R^2(adj) = 92.05%
RMSE = 24.89 g
The interaction between ripe and unripe count was significant in the model.

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 (α = 0.15) using detection data from the 2-class, YOLOv4 models including all images (Eq. 3 and 4; Figures 11 and 12).

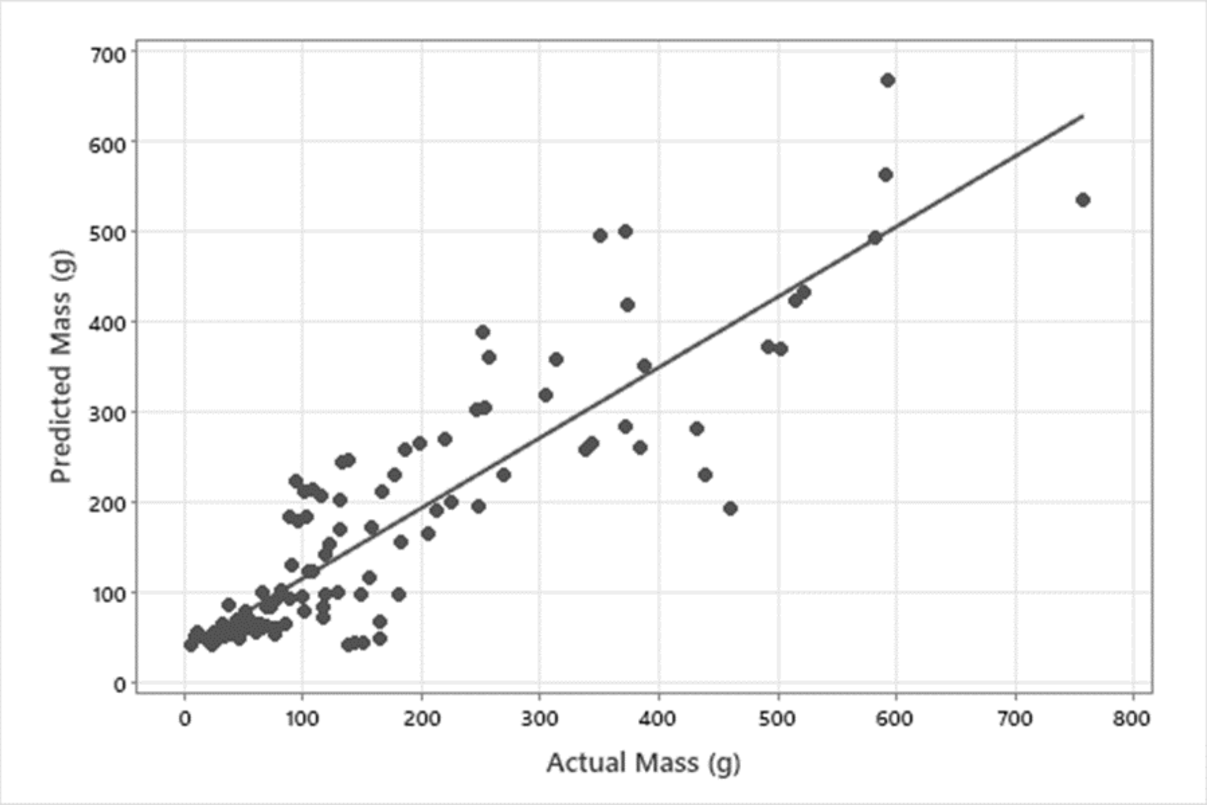

Figure 11. Plot of actual vs predicted yield with Maine and Nova Scotia combined using Equation 3 to predict mass from plot images (R^2 = 77.8%).

Predicted Mass = 40.8 + 0.6767RipeCount + 0.0671UnripeCount **Eq (3)**
R^2(adj) = 77.32%
RMSE = 75.00 g

After removing the data collected in Maine, model performance was drastically improved. Results of this analysis can be seen (Figure 12; Eq. 4).

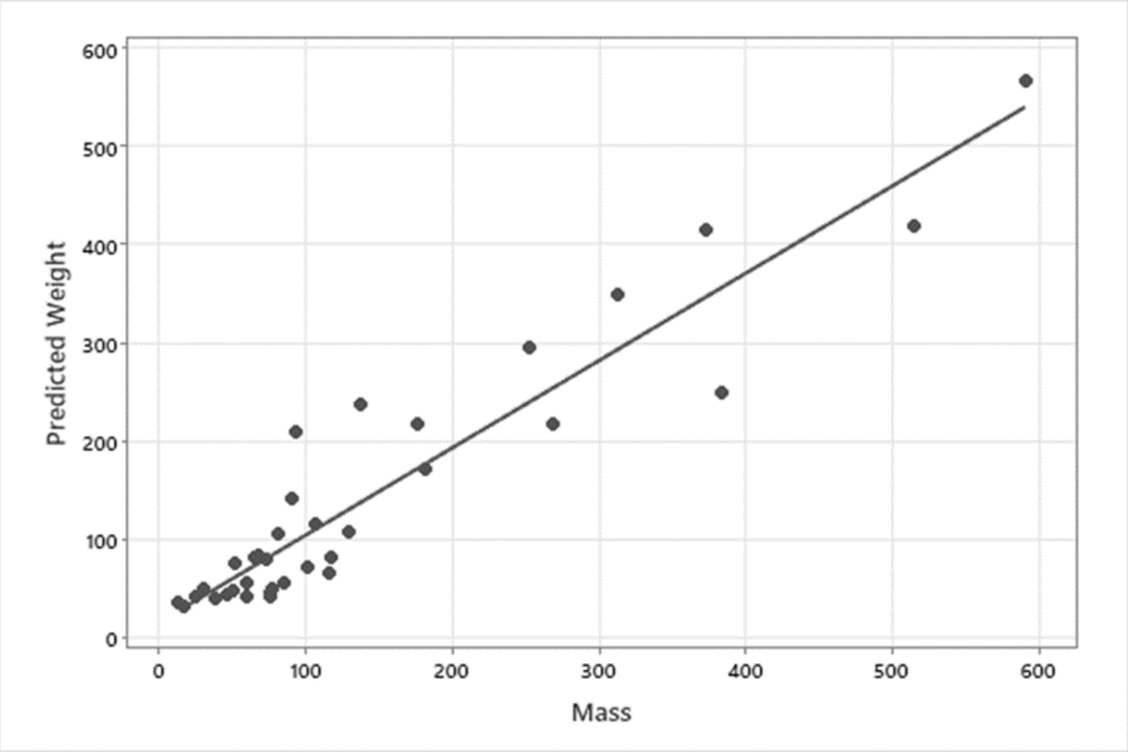

Figure 12. Plot of actual vs predicted yield using Nova Scotia sites using Equation 4 to predict mass from plot images (R^2 = 88.7%).

$Predicted\ Mass = 18.8 + 0.7021RipeCount + 0.1019UnripeCount$ **Eq (4)**
$R^2(adj) = 87.98\%$
$RMSE = 48.48\ g$

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 12).

Table 12. Percent ripeness on each sampling date as determined by the developed 2-class YOLOv4 model.

Field	Date	% Ripe	% Unripe
Columbia Falls	30-Jun-22	6.64%	93.36%
	21-Jul-22	75.62%	24.38%
	29-Jul-22	87.07%	12.93%
Hope	23-Jun-22	2.96%	97.04%
	11-Jul-22	37.53%	62.47%
	27-Jul-22	88.25%	11.75%
Sedgwick	29-Jun-22	0.85%	99.15%
	18-Jul-22	62.14%	37.86%
	25-Jul-22	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%
Highland Village	29-Jun-22	0.02%	99.98%
	15-Jul-22	7.82%	92.18%
	9-Aug-22	83.83%	16.17%

A third year of data collection was included as a means of confirming the results from year 2. Collected images were analyzed using the YOLOv4 models developed by (MacEachern et al., 2023). These models identify both ripe and unripe wild blueberries within images and provide counts of each class. These counts were then referenced versus the ground truthed weights of berries within each image using multiple linear regression. Data from 2022 and 2023 in Nova Scotia and Maine were then combined in several combinations with the aim of determining the optimal model. The results of these models can be seen in (Table 13).

Table 13. Results of the multiple linear regression for predicting total berry mass from ripe and unripe detections

Dataset	**Regression Equation**	**R^2 (adj.)**
2023 Combined	Mass = 37.5 + 0.6431 Ripe + 0.0742 Unripe	78.68%
2023 Nova Scotia	Mass = 36.8 + 0.7508 Ripe + 0.0558 Unripe	85.67%
2023 Maine	Mass = 32.9 + 0.6326 Ripe + 0.0889 Unripe	74.06%
2022/2023 Combined	Mass = 39.81 + 0.6601 Ripe + 0.0691 Unripe	78.03%
2022/2023 Nova Scotia	Mass = 29.83 + 0.7028 Ripe + 0.0776 Unripe	86.42%
2022/2023 Maine	Mass = 45.7 + 0.6434 Ripe + 0.0665 Unripe	73.76%

Plots comparing measured and model predicted berry masses for Maine and Nova Scotia combined and analyzed separately can be seen below (Figures 13-15).

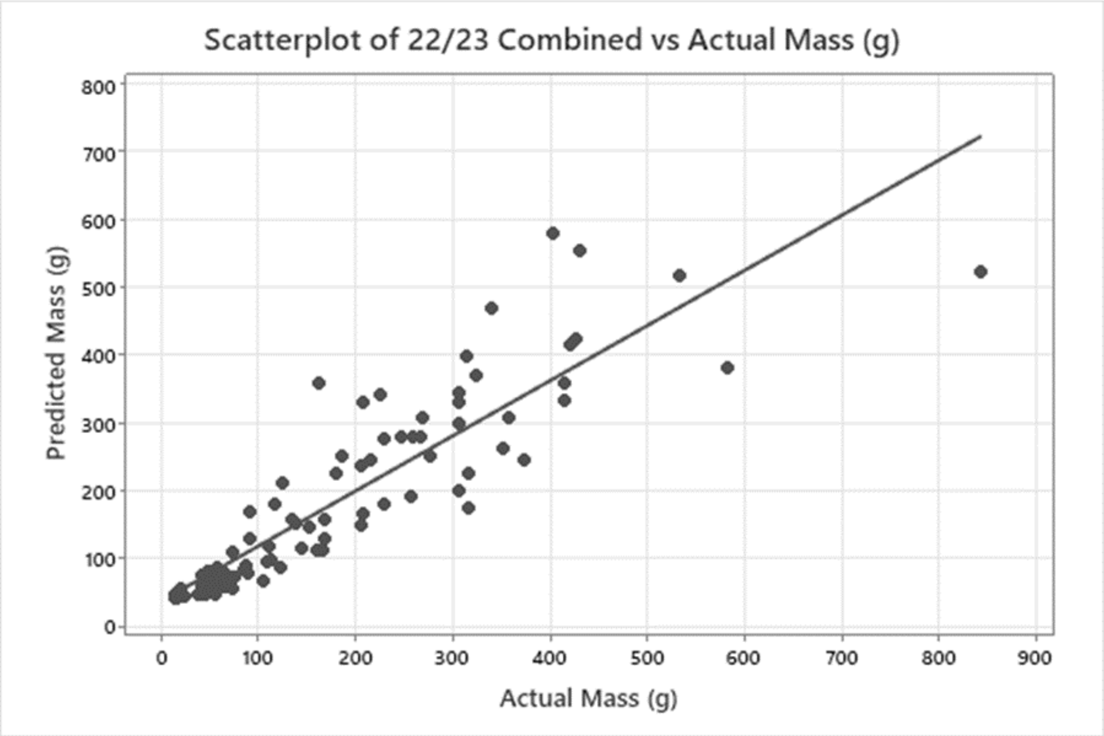

Figure 13. The model to predicted actual yield 78.03% of the time from images taken in 2022 and 2023 at both Maine and Nova Scotia sites. Predicted = 37.51 + 0.8131Actual and RMSE = 72.44 g.

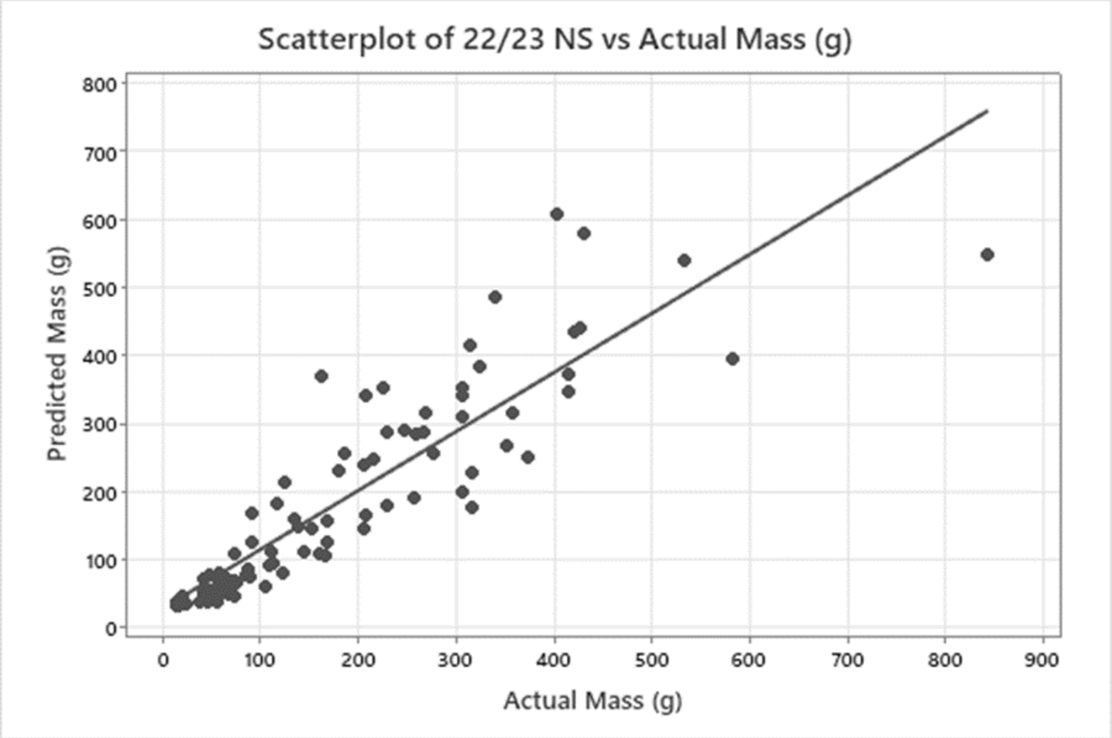

Figure 14. The model to predicted actual yield 86.42% of the time from images taken in 2022 and 2023 only at Nova Scotia sites. Predicted = 28.59 + 0.8653Actual and RMSE = 45.65 g.

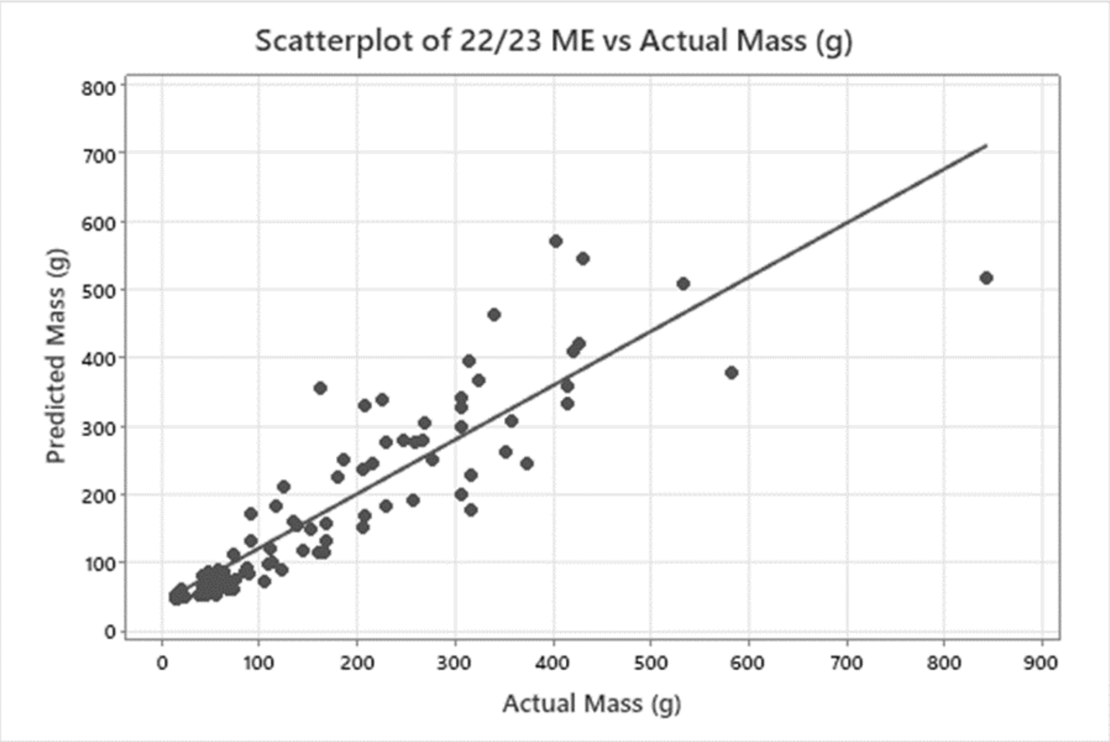

Figure 15. The model to predicted actual yield 73.76% of the time from images taken in 2022 and 2023 only at Maine sites. Predicted = 43.21 + 0.7926Actual and RMSE = 84.37 g.

DISCUSSION

Phenological Development (Calderwood of UMaine)

Fruit per stem for ME and NS in 2023 were similar in each fruit growth stage (Figure 1). The number of color change fruit was so low in ME and NS because the fruit change from green to blue almost overnight, and data is not collected every day, thus it appears to be low in the data (Figure 1). The number of green fruit per stem is greater than blue fruit per stem. Some of this loss can be correlated to what farmers call the “June Drop” (Drummond, 2002). This is when the plant drops fruits early because it cannot support the fruit load due to under fertilization, or the plant is sterile leading to fruit abortion (Drummond, 2002). Other sources of loss may be from disturbances such as sampling or over-ripe fruits. This season there was very little overripe fruit given the cooler wet conditions of the harvest.

Plant Nutrient Content (Calderwood of UMaine)

Nitrogen content of crop leaves is known to improve plant photosynthesis and therefore yield. Recently, it has been found to increase blueberry fruit polyphenols and soluble protein (Zhang et al., 2023). Applying a 21% N ammonium sulfate or a 37% N sulfur coated urea was found to increase fruit per stem and stem growth with a spring application on wild blueberry (Precival and Privé, 2000). Results found in our study contradict the notion that N increases yield. We did not find yield to be significantly affected by N (Tables 4 and 5). Our crop year fruit and leaf samples showed that nitrogen content started at an optimal level and dropped below optimum levels at green fruit (Figure 2). This is the same seasonal trend observed in multiple varieties of highbush blueberry (Strick and Vance, 2015). In wild blueberry, N started out at 2.0%N and dropped to 1.5% while in highbush N started at 3.5% and dropped to 1.5-2.0% (Strick and Vance 2015). Highbush blueberry growers apply a split application of ammonium sulfate at pre-bloom (T5) and at green fruit. Our wild blueberries are using N during the crop year.

In the 1990s it was observed that wild blueberry fields were lacking in phosphorous and therefore growers were encouraged to apply DAP and MAP. At the time, an increase in phosphorus led to an increase in wild blueberry yield (Smagula and Dunham, 1995). However, 2022 and 2023 data from ME and NS locations suggest that phosphorus does not directly increase yield but is needed to make a more robust model for predicting yield (Table 4 and 5). In addition, we observed a decline of phosphorus within the fruit and leaves which suggests the plant is using this nutrient over the season. This is the same seasonal trend observed in multiple varieties of highbush blueberry (Strick and Vance, 2015). In wild blueberry P started out at 0.25-0.30%P and dropped to 0.10% while in highbush, P started at 0.40% and dropped to 1.5-2.0% (Strick and Vance 2015). Wild blueberry starts and ends a little lower in P compared to highbush.

Potassium in the leaves started at 0.6% slightly above optimum and dropped to 0.4% by harvest (Figure 4). According to Strick and Vance (2015), highbush blueberry varieties started out with 0.80-1.1%K and dropped to 0.50%. Making sure sandy soils have adequate potassium for plant uptake is important and needs to be balanced with correct application rates or it can leach out of the soil. Percival and Sanderson (2004) found the soil application of potassium to directly increase stem density and fruit set. Having adequate potassium levels was found to increase soluble sugar and decrease titratable acidity in highbush blueberry (Zhang et al., 2023). Zhang et al. (2023) results follow the same trend we found with an increase of potassium in leaves leading to an increase in fruit brix (Figure 9; Table 7).

Research on calcium applications to wild and 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 calcium 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 the 2022 report). In this study we found that increased calcium content in the leaves correlated to an increase in yield (Figure 8). However, the relationship is stronger in the multivariate linear regression when potassium and magnesium are paired with calcium in the model for predicting yield (Table 5). Several years in a row we have seen that calcium increases through the season in the same manner that highbush blueberry varieties do. In this study, wild blueberry leaves started out at 0.3% and increased to 0.6% while according to Strick and Vance (2015), highbush varieties started out at 0.3-0.4% and increased to 0.8%.

Brix was negatively correlated with yield (Figure 6; Table 4). This suggests that as fruit ripens (brix increases), fruit falls off, reducing the yield that we measure. Cooler temperatures and increased precipitation in 2023 allowed farmers to harvest for a longer period of time reducing the amount of over-ripe fruit. The model for predicting brix found that fruit sugar content depends on macro- and micro-nutrients, not just on nitrogen, phosphorus, and potassium.

Imaging for Ripeness and Quality (Esau of Dalhousie)

In the first year of the study, after testing four different models the 2-class, YOLOv4-Tiny performed the best out of the four which 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 (Figure 10; Table 9). The success of the 2-class models, while providing somewhat less information, do a better job at accounting for all pictured berries (Tables 8 and 9). All four of the models do a good job of predicting yield with root mean square errors less than 28.87 g.

Considering the results of the models from all second year (2022) images, a significant reduction in model performance can be seen. This is likely the result of inconsistent image quality observed in the Maine images. Key contributors are likely to be the inconsistent angle and height of images along with the relatively lower image quality. Maine images were collected at various resolutions whereas all Nova Scotia images were collected at 5184 x 3456 pixels. In all cases, the resolution of Maine images was lower than Nova Scotian images. While it is not imperative to have identical resolutions for publication, it would smooth out the process especially where we are not trying to prove the methodology. It is advisable that Maine images be collected at the same resolution moving forward. This does not necessarily mean they need to be collected at the same resolution as Nova Scotia, but they should be the same across all Maine fields if possible, in order to reduce image variability. Further, images should be collected in the manner outlined by the provided methodology. It is imperative that the borders of the quadrats be at the extent of the photos in order to maximize the ground truthed area within the image. It should be noted that analysis of the Nova Scotian images alone confirms that one of, or some combination of the above is occurring as this data was not significantly different from year one data which again, performed much better than year two. As a reminder, in year one we also omitted Maine data for many of the same above listed reasons (Figure 12). Finally, please note that when analyzing the Maine images alone we are likewise observing poor model performance (adj R^2 = 73.17%). This confirms that there is a significant amount of variation among the Maine images themselves and not just when they are compared to Nova Scotian images. Consistency in image and ground truthing data collection is paramount between fields. Every time something is done differently, it will detriment model performance.

The optimal regression came from the 2022/2023 Nova Scotian data (Figure 14; Table 13). This is likely because the Nova Scotian images more closely resembled the training images used to develop the YOLOv4 model. Images collected in Maine were less consistent in terms of their collection height, field of view, angle, and lighting. A combination of these factors is the most likely cause for the poorer performance of models developed with this dataset. That said, the combined dataset from 2022/2023 will still provide the best model for all fields and imaging qualities. While the adjusted R^2 of 78.03% is a little discouraging, it still provides a suitable estimate of yield for a single image (Figure 13; Table 13). For yield prediction purposes, growers should be encouraged to collect as many images as possible from across a field and average the data for interpretation. While the results will not be definitive, the results can provide growers some indication of expected yields within a given field or section of field.

After plotting the actual versus predicted values for each of the datasets the Nova Scotia 2023 data can be confirmed as the best model by the RMSE (Figure 14). Despite this, it is still possible that the combined datasets better account for the variation across a variety of image qualities and spatiotemporal parameters (Figure 13; Table 13). The recommendation of the researchers would be to employ the Nova Scotia 2023 model if one can assure that the captured image quality and parameters follow the methodology exactly (Figure 14). Otherwise, it is advisable that the combined 2023 model be used to account for image variability (Figure 13).

Considering the results of the models from all third year (2023) images, yield can be well predicted using quality images taken at a standard height and position. Nova Scotia’s model for predicting yield was less accurate with the images taken in Maine due to collection technique (Figure 13). However, the model can be quite useful for wild blueberry growers and researchers.

CURRENT RECOMMENDATIONS

- Take foliar leaf samples to determine the fertilizer needs of your fields.
- If only N is required, consider using Ammonium sulfate instead of DAP or MAP.

ACKNOWLEDGEMENTS

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2. Baseline Water Loss of Wild Blueberry

INVESTIGATOR(S): J. Collins, B. Tooley, B. Hall, and P. Fanning

OBJECTIVE:

- To determine baseline values for water loss and dehydration of wild blueberry fruit.

LOCATIONS: Penobscot and Washington Counties, ME

TIMEFRAME: August 2023

INTRODUCTION

Softening and dehydration are two of the major factors that can limit the marketability of blueberries (Ehlenfeldt and Martin, 2002). Water loss is a major contributor to softening in highbush blueberries and plays a substantial role in softening during storage (Paniagua et al. 2003). Moggia et al, 2017 studied the impact of the stem scar and the cuticle on water loss in highbush blueberry cultivars. They found that water was lost through both after the fruit was harvested. More specifically the stem scar accounted for approximately 40% of the moisture loss when stored at 20°C, but there was considerable variation. No work has been done on postharvest water loss and dehydration in wild blueberries. This work aims to begin studying factors impacting water loss with implications related to fresh pack harvest procedures and duration until processing.

METHODS

Twelve wild blueberry genotypes (n=36) were selected on each of three dates (9, 11, and 14 August). The genotypes selected were part of a cooperative study with Wyman's who provided information on the foliar nutrients of the different genotypes and access to the firmness meter used in the experiments.

On each date, 5-10 stems with fruit were collected from each of 12 genotypes. The stems were wrapped with damp paper toweling to maintain the turgidity and transported in a cooler to the University of Maine at Orono for processing that same day. In the laboratory, 20 berries were selected randomly from each genotype. Berries were placed individually in preweighed,1 oz polystyrene portion cups (Figure 1a). Each cup was labeled with genotype # (1-36), and fruit # (1-20). Care was taken both in the lab and during transport to reduce the potential for mechanical damage to the berry impacting the rate of water loss.

The following measurements were collected for each individual fruit:

- Fruit diameter at the widest part and length between the stem scar and calyx (mm) – digital calipers (Figure 1b)
- Stem scar diameter – micrometer mounted on a dissecting microscope.
- Berry weight was determined at ca. 0, 24, 48, and 72 hours (Ohaus Explorer- Ohaus Corporation, Parsippany, NJ) (Figure 1c).
- Fruit firmness was assessed initially (0 hrs) and after 72 hrs (Note: the same fruit was not used for both measurements to reduce mechanical damage) using a Baxlo Fruit Firmness Tester HT6000/F0 (Figure 1d). This machine measures using a Shore scale that ranges from 0-100.
- All fruit was held at room temperature for the duration of the trial (Figure 1e)

- Mean storage temperature (25°C or 77°F, RH 60%) was monitored with a HOBO© MX2303 Ext. Temp and RH monitor (Onset Computer Corp., Bourne, MA)

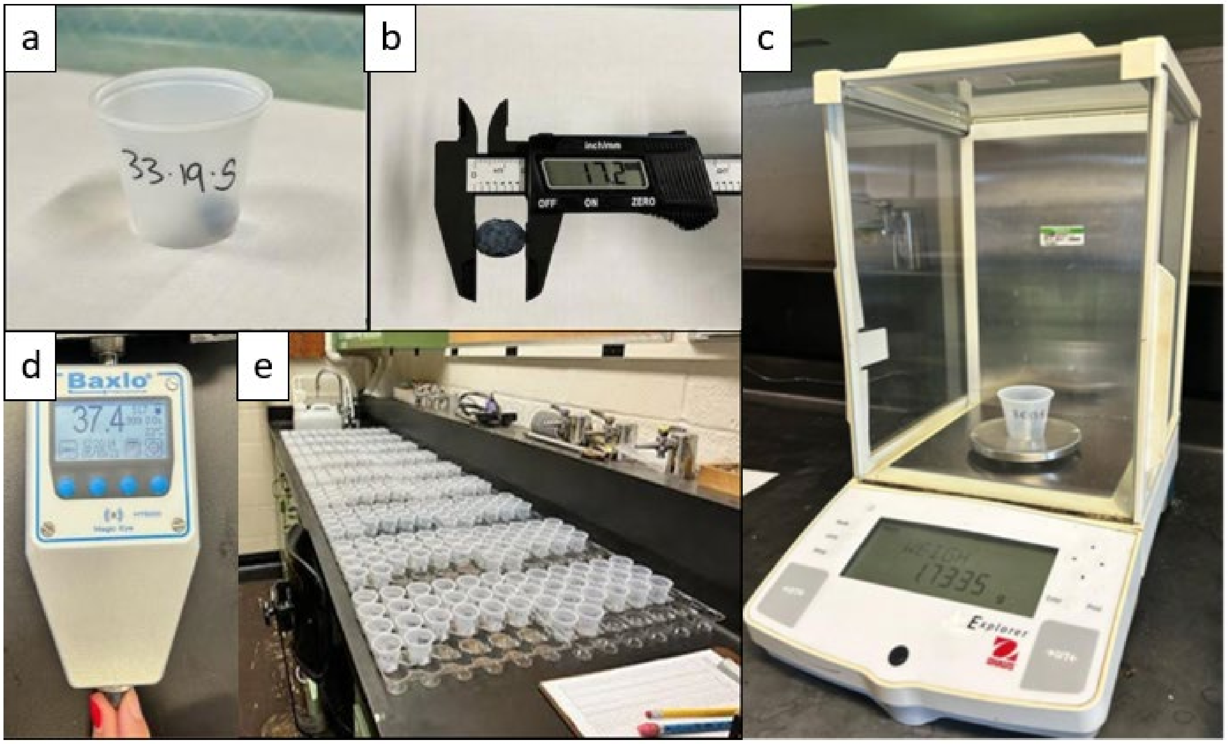

Figure 1. A) Fruit in individual portion cup, B) fruit measured with digital calipers, C) determining fruit weight, D) fruit firmness, and E) fruit held at room temperature.

RESULTS

Baseline Water Loss

In these experiments, a reduction in weight was assumed to be due to the loss of water. The only other potential loss of weight would be due to insect infestation; however, no infestation by any insects was found in the fruit. In total, 720 fruit were measured four times each for a total of 2,880 observations, across 36 genotypes.

There was a significant water loss from fruit over the 72-hour period in which fruit was measured ($F_{(1,2852)} = 5980.74$, $P < 0.0001$) calculated as the percentage of water lost compared to the initial or fresh weight of the fruit (Figure 2). The average (± S.E.) percentage reduction weight (water loss) compared to the starting weight increased over time and was recorded as follows: 9.0 (± 0.15), 18.1 (± 0.28) and 28.6 (± 0.44) at 24, 48 and 72 hours, respectively. Performing a regression on these data indicates a strong linear relationship between water loss and time with an R^2 value of 0.677 indicating that time explained 67.7% of the loss of water since harvest. Using the estimates of this regression, the expected % reduction in weight of fruit can be calculated using the following formula:

Expected % reduction in weight = -0.2868 + 0.3951*hours since harvest

For example, if 1000 lbs. of fruit was held for 36 hours the expected % reduction in weight of fruit, based on these data, would be as follows:

$$-0.2868 + 0.3951*36 = 13.9\%$$

Thus, the expected reduction in weight after 36 hours would be 13.9% or 139 lbs. of the fresh weight leaving a total of 861 lbs. of the fresh weight.

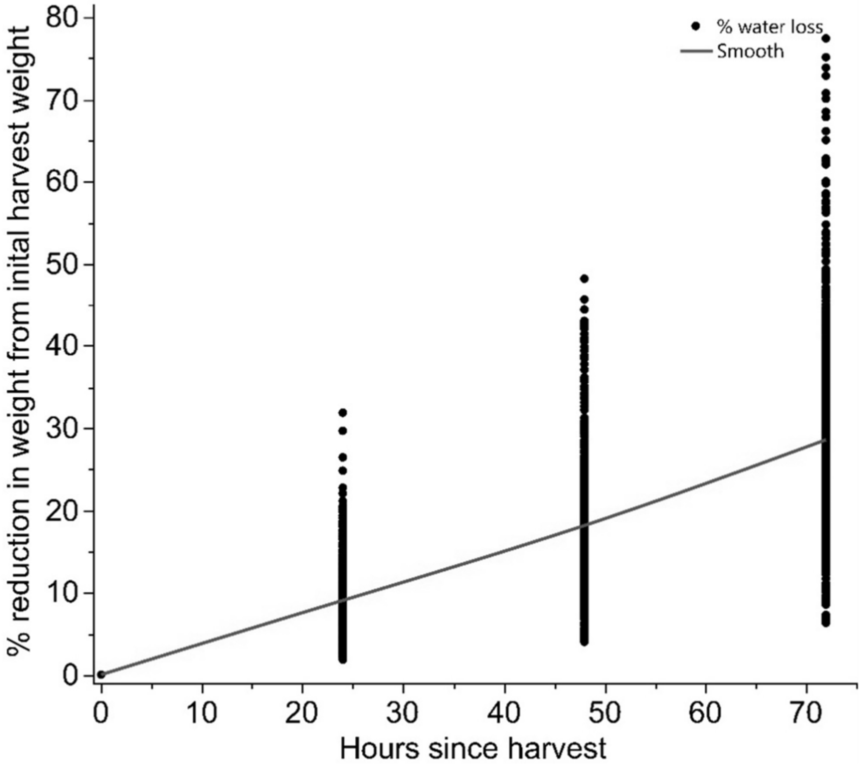

Figure 2. The percentage reduction in weight of fruit from harvest weight from 36 genotypes of lowbush blueberry at 24, 48 and 72 hours.

IT IS IMPORTANT TO NOTE that the actual reduction in weight will likely differ based on many factors. This data is based on 720 fruit from 36 different genotypes from a single field. The rate of water loss could likely be lower than observed in this experiment if our ambient temperature was lower and relative humidity was higher. The average temperature and relative humidity in the area where fruit was stored was 25°C (77°F) and RH 60%. At this temperature and relative humidity, the water vapor pressure deficit was likely high, leading to higher water loss in the fruit tested. The average relative humidity was expected to be higher, and if the experiment was repeated at lower temperatures the water vapor pressure deficit and the respiration rate of the fruit would be reduced.

Another important factor impacting the rate of water loss in this experiment was the handling of the fruit. The goal of this experiment was to estimate a baseline water loss from fruit, thus the fruit was handled and stored in ideal conditions reducing the damage that the fruit would experience by traditional harvesting (mechanical or raking) and compaction in storage bins used before fruit is packed into clamshells or frozen. Damage from harvest and compaction have been shown to impact the marketability and water loss in other crops (Brondino et al., 2022).

Fruit Factors Impacting Water Loss

The stem scar of the fruit will impact the rate of water loss. In some highbush cultivars, this accounted for approximately 40% of the moisture lost, with the rest being through the cuticle (Moggia et al, 2017). In these experiments, there were significant differences in the average area of the stem scar ($F_{(35,645)} = 21.986$, $P < 0.0001$), ranging from an average (± S.E.) of 0.73 mm^2 (± 0.21) to 4.7 mm^2 (± 0.21). In addition to the stem scar, water loss is expected through the cuticle. The surface area of the cuticle was calculated for 20 random fruit. Similar to the stem scar there were significant differences in the average area of the cuticle ($F_{(35,677)} = 10.023$, $P < 0.0001$), ranging from an average (± S.E.) of 1.05 cm^2 (± 0.11) to 2.8 cm^2 (± 0.11). Both the stem scar area ($R^2 = 0.112$) (Figure 3a) and the cuticle area ($R^2 = 0.05$) (Figure 3b) were positively correlated with the rate of water loss (ug s-1) after 24 hours; for both, large surface areas led to higher rates of water loss. When we looked at the correlation between both factors and the percentage of initial/fresh weight lost after 24 hours, we saw that there was not a strong correlation with stem scar area (Figure 3c), but there was a strong negative correlation between the area of the cuticle and the percentage of water lost (Figure 3d). Thus, as the surface area of fruit increases the percentage of the initial weight lost decreases. This highlights the problem of the surface area to volume ratio where smaller berries will lose a higher percentage of their mass at a higher rate. This is important to consider for post-harvest management of fruit in years or fields where environmental conditions (lack of rain/irrigation) or poor pollination result in smaller fruit overall. While it hasn't been tested this surface-to-volume ratio likely impacts fruit most before harvest, where plants with smaller fruit are likely less resilient to periods of drought or the impact of diseases that cause premature senescence of leaves impacting the ability of water to transpire.

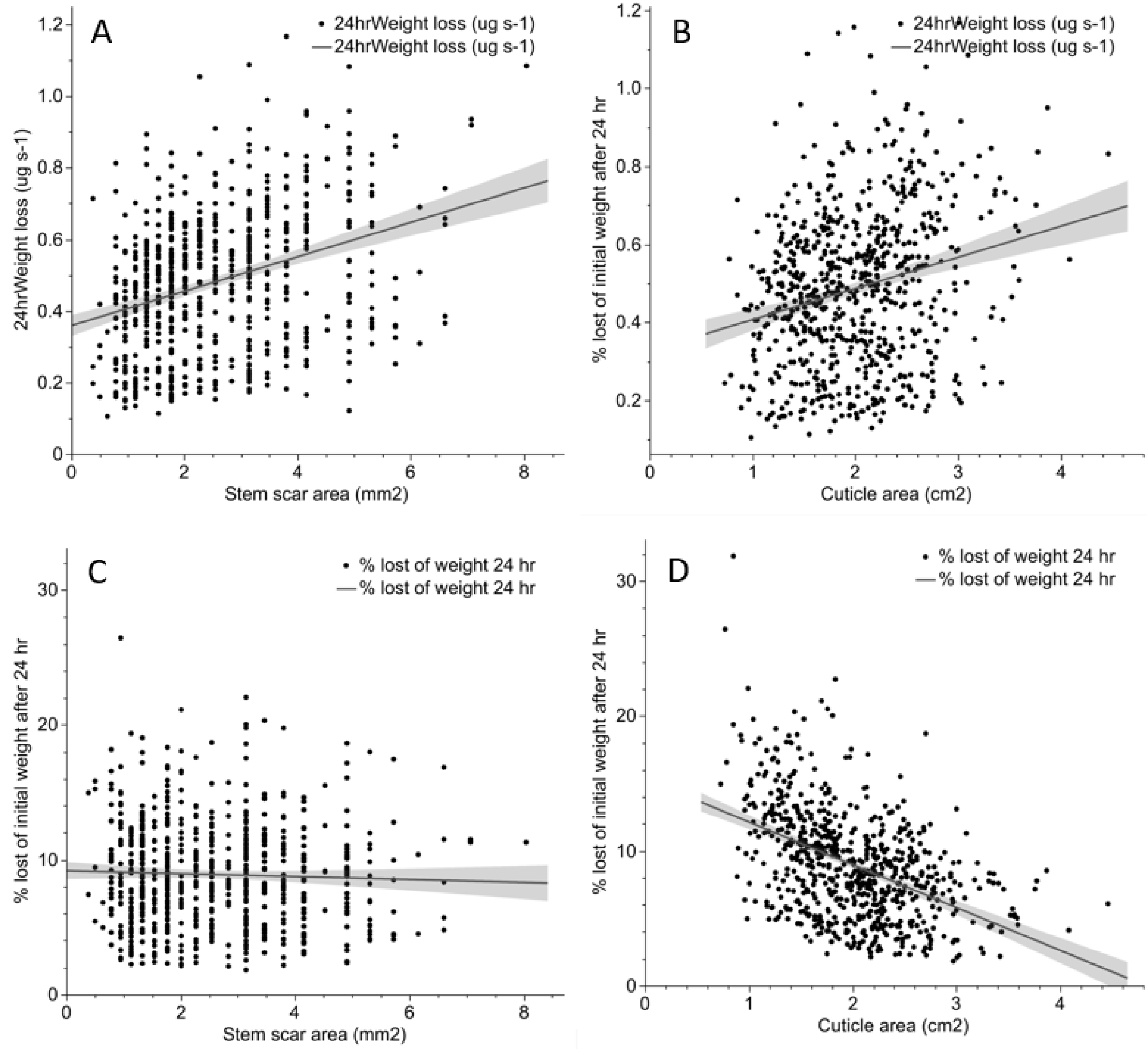

Figure 3. Relationship between the area of the stem scar (A), the area of the cuticle (B), and the rate of water loss and relationship between the area of the stem scar (C), the area of the cuticle (D) and the percentage weight of the initial harvest weight lost after 24 hours.

Fruit firmness is often a metric used to determine how fruit will be impacted by damage associated with harvesting and the length of time that fruit can be held before sale. In these experiments, we assessed fruit firmness in each genotype at the time of harvest. We saw no strong correlation between the starting firmness and the % of initial weight lost after 24 hours (Figure 4). This could be due to the narrow range of the starting firmness of the fruit used in these experiments. Starting firmness ranged from as low as 56 to 69 on the scale of 0 to 100. This narrow range was possibly due to the fruit all being harvested early in the morning (~7-8 am) before the ambient temperature began to rise and the fact that the fruit was harvested delicately.

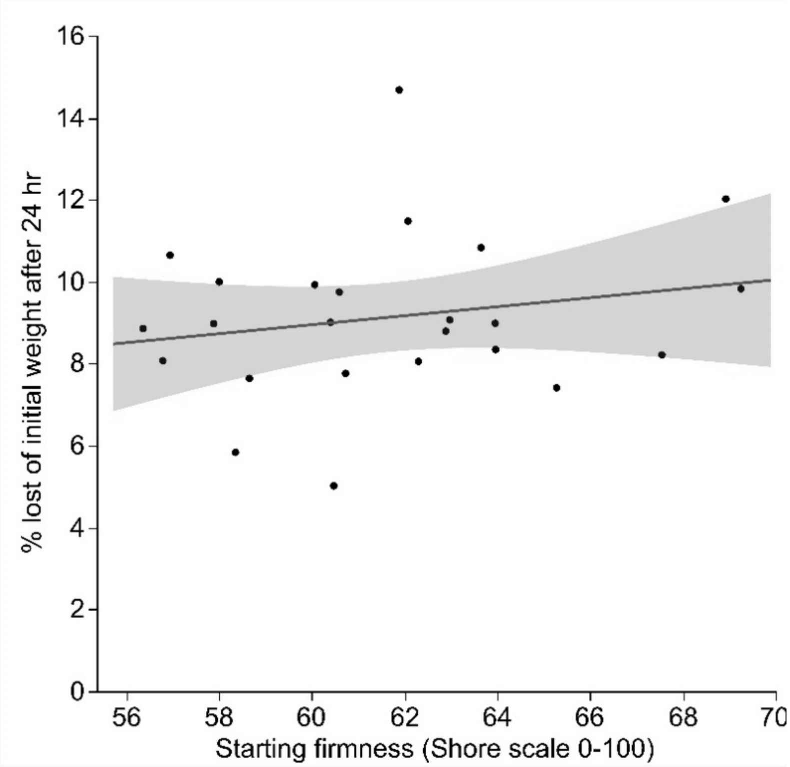

Figure 4. Relationship between the average starting firmness (Shore scale) and the percentage of initial weight lost after 24 hours of 24 genotypes of wild blueberry.

Finally, we assessed if the plants' nutrition had any impact on water loss. Studies have shown that calcium concentrations are important in the cuticle cell wall development and thus could impact the rate of water loss from fruit (Olmedo et al., 2021). Foliar samples were taken on August 10th, a day after the fruit collection for genotypes 1-12, and near the remaining 24 genotypes. Samples were analyzed for % nitrogen, % sulfur, % phosphorus, % potassium, % magnesium, % calcium, % sodium, Boron (ppm), Zinc (ppm), Magnesium (ppm), Iron (ppm), and Copper (ppm). We used regression analysis to correlate these leaf nutrients to the rate of water loss (ug s^{-1}) after 24 hours. Of these, Zinc concentration was the only leaf nutrient to significantly correlate with water loss, for which there was a significant negative correlation (R^2 = 0.150) ($F_{(1,34)}$ = 5.99, *P* = 0.0197). There was reduced water loss in fruit from the plants with higher concentrations of Zinc (Figure 5); however, we cannot explain this and do not recommend any changes in nutrition to include increases in Zinc. Similarly, it's important to note that the genotypes used in the experiment were from a single field and there was not a large range in the leaf nutrients in the tested genotypes.

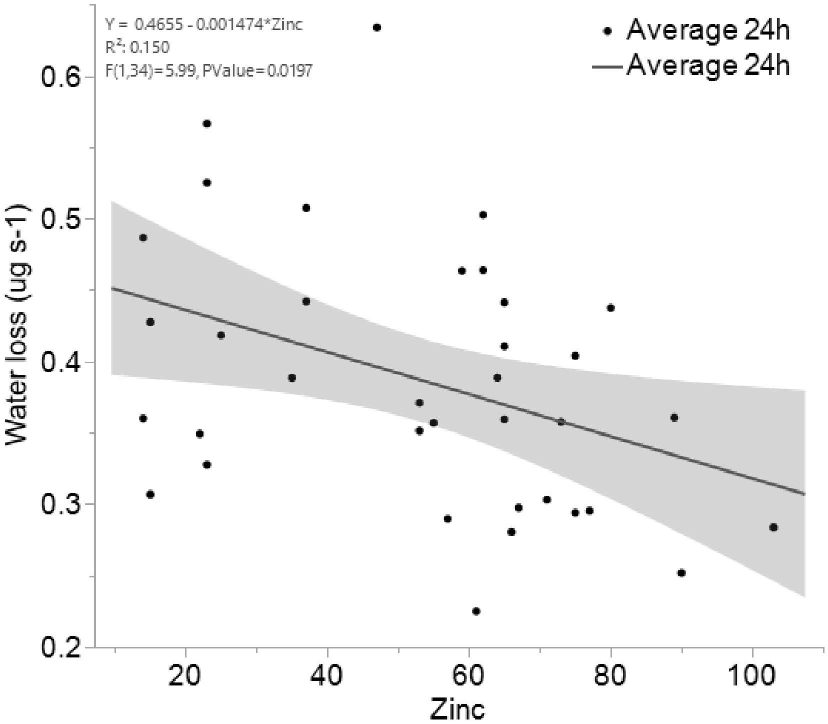

Figure 5. Relationship between Zinc concentration (ppm) and the rate of water loss (ug s^{-1}) after 24 hours.

CONCLUSIONS AND RECOMMENDATIONS

This study presents data on the baseline postharvest water loss from wild blueberry fruit at room temperature which was the goal. As stated, we had a slight water vapor pressure deficit in the room where the fruit was held during this experiment, but for the most part, the rate of water loss would likely be higher in fruit that is harvested and stored in other containers where fruit are compacted together.

This data can be used to estimate the baseline/ideal fresh weight lost after the fruit is harvested until it's frozen or until it is sold if not chilled and/or stored in a condition where there isn't as high a water vapor pressure deficit, which is the difference between the actual water vapor pressure in the air and the saturation water vapor pressure at the same temperature, indicating the drying potential of the atmosphere. A further interesting study would be to assess if that water vapor pressure deficit is lower when the fruit is packed tighter together.

Our results highlight that postharvest water loss is compounded in smaller fruit by the surface area to volume ratio, and that smaller fruit will have higher water loss compared to larger fruit that has a higher volume. Water loss occurred through both the stem scar and cuticle. In total, we observed 9% weight loss after 24 hours and 28% weight loss after 72 hours.

NEXT STEPS

- This data and the approaches used can form a baseline as research continues to examine harvesting and postharvest storage practices that optimize the marketable weight of wild blueberry.

ACKNOWLEDGEMENTS

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3. Wild Blueberry Harvester Innovations

INVESTIGATORS: L. Calderwood, B. Koda, J. Belding, E. Venturini
F. Wentworth, J. Parks, N. Mehta, and F. Ishraq

OBJECTIVES

- Assess current harvesting technology and practices with a focus group of industry experts
- Innovate data-driven improvements to harvesting technology and develop "best harvesting practices" to improve efficiencies of existing technology
- Drive adoption by carrying out a robust outreach program to deliver data-driven best harvesting practices and demo harvesting innovations
- Evaluate this project as a model to catalyze and deliver innovation to the Maine wild blueberry industry

LOCATION: Maine

PROJECT TIMEFRAME: 2023-2026

INTRODUCTION

The goal of this project is to reduce harvest loss in the field by harvesters of all scales. 2023 was the first year of this four year project which is a collaboration between UMaine Extension, UMaine Advanced Manufacturing Center, UMaine School of Mechanical Engineering, and the Wild Blueberry Commission of Maine. This effort is funded by the Maine Technology Institute (MTI).

Wild blueberry plants live low to the ground on rocky and uneven terrain. This means that harvest technologies developed for the larger cultivated blueberry industry are not suitable for wild blueberry applications. Harvest is the largest expense for wild blueberry producers. Predominate harvest technologies in Maine are increasingly outdated, are labor intensive, lack precision harvest capabilities, and result in high loss rates during harvest. Based on 2021 production statistics, the average producer harvests 38 acres and industry experts estimate shrink rates of 10-20%, equating to losses of $14,440-28,880 annually to berries grown but lost to the ground during harvest. Industry wide, we left an estimated 10.5 to 21 million pounds of fruit in the field in 2021, a loss of $8-$16 million in farm gate income (Wild Blueberry Commission of Maine 2022). Older harvesters, still used today, can leave a staggering 25-32% of the crop in the field (Hall et al. 1983). Of the portion harvested, machines can damage fruit, leading to additional shrink during processing.

Despite the clear need, there has been little to no *in-state* research to improve wild blueberry harvesters in over 40 years. In the 1950's innovation was spurred to mechanically harvest fruits and vegetables across the US due to increased field size, higher yields, and less reliable labor (Dale et al. 1994). Wild blueberry harvesters have evolved from manual labor to the mechanical technology characterizing today's machines.

In 1883, the industry witnessed its initial foray into mechanization with the introduction of the Tabbut hand rake in Columbia Falls, Maine. This rudimentary yet groundbreaking tool, featuring a comb-like structure, marked the pioneering steps toward automating the harvesting process, offering a more efficient alternative to manual labor.

However, it wasn't until the 1940s and 1950s that a seismic shift occurred in the industry, as walk-behind mechanical harvesters made their debut (Combine Harvester, Patent US1863691A). These machines, though basic in design, represented a significant departure from manual labor and brought about newfound efficiency. In 1957 a vacuum was attached to a modified Tabbut hand rake to pull berries from the rake to collection bins. This was modified until 1966 but ultimately not adopted because the modified rake teeth dug into the soil causing too much plant damage and too much debris accumulated in the rake. A vacuum and positive air assist system were attached to a hydraulic motor yet projected sand into the berries making them unsalable. In the 1970s the rotating picking head continued to develop into the pre-curser of the Bragg harvesters. At this time the picking head was considered a hollow reel raking system with a brush on the front and conveyor belts to carry berries to bins. Chisholm-Ryder Company adopted this design where tractor mounting modifications were made allowing the machine to pick 3.7 acres (1.5 ha) per day (Dale et al. 1994). Fast-forward to the 1990s, the industry experienced another wave of technological advancement with the integration of hydraulic picking reels (Lowbush Berry Harvester Patent US5375403A). This innovation allowed for enhanced control and precision in harvesting, reducing damage to the delicate berries in an attempt to increase overall efficiency.

Harvesting technology in Maine today includes manual harvesting with hand rakes (Hubbard Rake Company), non-mechanized wheeled-rakes (J.M. Bouchard), ride-on grasshopper mower with rake head attachment (DH Equipment Inc.), walk-behind harvesters (Acadian Machine Works and Maine Wild Blueberry Equipment Company), and tractor mounted harvesting heads (Doug Bragg Enterprises, D&D Manufacturing Inc). While Hubbard Rake Company is located in Maine, farmers have to buy any mechanized harvesting equipment from Canada. The recent retirement of the only Maine wild blueberry equipment producer, Zane Emerson of Maine Wild Blueberry Equipment Company, leaves Maine producers without a local supplier of wild blueberry equipment.

In the present era, harvesters used in other crops such as small grains (wheat and barley), have become sensor-driven and AI-powered with advanced sensors providing real-time data to adjust harvest speed, height, and grain quality measures according to the crop in real-time (Gray 2023). Several harvester studies have been conducted in Nova Scotia to improve the Bragg harvesting heads and tractor operation. Recent advancements in wild blueberry harvesting technology have incorporated a range of sensors to enhance the efficiency and precision of the harvesting process. Notable improvements include the integration of speed sensors, GPS technology, rake height sensors, and the ongoing testing of sensors for plant height and yield detection (Farooque et al. 2014, Esau et al. 2020). In the state of Maine, these sensors are currently employed by the Bragg and D&D harvesters except for plant height and yield sensors, which are still in the testing phase. These sensors play a crucial role in optimizing the harvesting process by providing real-time data on the speed of the harvester and rake head, ensuring accurate navigation through the fields. Additionally, rake height sensors contribute to minimizing berry damage by adjusting the height of the rake, and GPS technology aids in precise field mapping. It's noteworthy that while these harvesters utilize these advanced sensors, the DH (DH Equipment Inc.) and walk-behind harvesters rely on basic hydraulic-mechanical systems for rake height without incorporating AI-based sensors. The DH grasshopper ride-on mower harvester produced in Quebec is a very significant improvement for medium and large-scale growers and several Maine growers have more than one DH harvester now. These machines remain difficult to acquire and the number available for sale each year is limited.

Despite significant strides, a noteworthy aspect of wild blueberry harvesting technology has seen limited evolution over the last 60 years—the picking mechanism. The core method of detaching berries from plants has remained relatively unchanged, posing challenges to both berry quality and overall yield. The persistence of traditional methods in this crucial aspect has led to concerns about berry bruising, damage, and suboptimal harvesting efficiency.

While there has been an increased interest in enhancing harvesting processes through research, as exemplified by Dalhousie University's work on improving the Bragg harvester, the integration of cutting-edge technologies like sensors and AI into commercial harvesters is not yet widespread. Current applications of AI sensors are more commonly implemented in auxiliary equipment, such as GPS systems, harvester speed, and processing lines. Moreover, small farmers have not fully adopted these advanced technologies, due to factors such as cost, access, or the scale of their operations. The need for innovation in the picking mechanism persists, and ongoing research endeavors aim to address the longstanding challenges associated with wild blueberry harvesting.

In addition to a lack of innovation in harvesting equipment, the Maine wild blueberry industry lacks a reliable workforce, supply chain, and profitability. Over the past 20 years, the number of Maine wild blueberry farms has steadily decreased. In 1997, 741 Maine wild blueberry operations reported harvests. In 2017, only 435 farms reported harvests, a decrease of over 300 farms in 20 years (USDA NASS 2017). Today's farms continue to struggle to achieve profitability in the post pandemic economy. The Wild Blueberry Commission of Maine recently identified that a "A lack of profitability discourages the next generation from growing wild blueberries and contributes to the loss of farms and acreage." Low profitability hampers recruitment of new farmers; the average age of the Maine wild blueberry farmer is 64.5 (Malacarne personal comm. 2021). The broader industry, as represented during a series of three grower listening sessions the Commission held in 2022, identified their number one challenge as access to affordable and reliable labor. They recognized their second most significant shared challenge as profitability and the impacts of profitability on grower retention and recruitment.

METHODS

During the 2023 harvest season we visited eight wild blueberry farms for in-person semi-structured interviews with the farmers and employees who use harvesters. The visits occurred from July 17th to August 15th while wild blueberries were actively being harvested. Conversations were recorded and high-resolution videos were taken of harvesters in action for later analysis. We observed 5 Bragg tractor mounted harvesters, 2 D&D harvesters, 2 DH Equipment Company ride on harvesters, 2 Maine Wild Blueberry Equipment Company walk behinds, 1 Acadian Machine Works walk-behind, and 2 farms with hand rakers.

RESULTS

None at this time.

DISCUSSION

None at this time.

RECOMMENDATIONS

None at this time.

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Thank you to Sophia Cameron for organizing on-farm interviews.

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