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Fertilizer and Fungicides: Effects on Wild Blueberry Growth, Insect Attack, and Leaf Spot Disease Incidence

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Abstract

The goal of our three-year field study (two replicated two year trials) was to evaluate the influence of fertility and disease management practices commonly implemented by Maine wild blueberry growers during the prune year. Our treatments reflected grower practices. We tested applications of chlorothalonil (formulated as Bravo[®]), pyraclostrobin and boscalid (formulated as Pristine[®]), pyraclostrobin and boscalid with diammonium phosphate fertilizer (formulated as DAP[®]), and a non-treated control. Measured responses included sap-feeding hemipteran insects, fungal leaf spot pathogens, premature flowering, stem growth characteristics, flower bud cluster production, leaf retention, foliar nutrients, and yield. Among plant growth measures that represent potential yield, only stem length responded consistently over both trials. Plots treated with Pristine + DAP had significantly greater stem length compared to the other three treatments. Plots treated with Pristine only were not different than Bravo treated plots, but stems were significantly longer than the non-treated control. The fungicide, Pristine, and the fertilizer, DAP applied in the prune year enhanced leaf spot disease in both years; however, the combination of Pristine and DAP did not consistently effect yields. We found a significant and diverse community of sap-feeding hemipteran incidence in the 2016 trial, but not in the 2015 trial. Only in the 2016 trial did the Pristine and DAP treatment enhance sap-feeding bugs. Multivariate analysis of variance provided evidence that leaf spot pathogens and sap feeding insect pests responded positively to nitrogen concentration in foliage. We also found that fungicides do not compensate for the enhanced disease levels brought about by fertilizer application.

Additional index words. *Vaccinium angustifolium*, arthropods, flower buds, yield, foliar disease, nutrients.

Introduction

Lowbush or wild blueberry (*Vaccinium angustifolium* Aiton) is an important crop in Maine (USA) with more than 44,000 acres in production (Yarborough, 2015). Maine currently produces 10 percent of all blueberries in North America, including wild and cultivated (highbush and rabbiteye) production (NASS, 2014). The Maine, Canada Maritimes, and Quebec wild blueberry crop production system is based upon wild plants that grow naturally as an understory perennial species in Acadian and Boreal forests. These plants are managed using practices to reduce weed competition and losses due to insect herbivores and plant pathogens (Jones et al., 2014). Because wild blueberry is native to the Northeast (Vander Kloet, 1988), many of its key pest species are also native (Phipps, 1930). Wild blueberry is typically managed in a two-year cycle (DeGomez, 1988); a given field will be pruned (mowed or burned) in year one, resulting in vegetative

growth and produce a crop in year two (Yarborough, 2015). Flower bud clusters are formed during the vegetative growth year (Bell et al., 2009). In May of the following year (the crop year), the flower buds open and come into bloom. Because of the pruning practices employed, only half of the acres are available to harvest every year (Yarborough, 2015). Although pest pressure is relatively low in this native crop, when there is an insect pest outbreak, pest management typically involves the use of organophosphate, carbamate, neonicotinoid, or biorational insecticides (Yarborough et al., 2018).

Commercial wild blueberry fields have traditionally been pruned by burning. It is believed that Native Americans periodically burned off trees and shrubs of the sand plains of eastern Maine to stimulate blueberry production (DeGomez, 1988). However, Trevett (1956) observed that repeated burning resulted in a decline in production associated with the destruction of the soil organic layer and exposure of the rhizomes. Research conducted at the University of Maine in the 1970's showed mowing stems to within one cm of the soil could give the same yields as burning (Ismail and Yarborough, 1979; Ismail et al., 1981). The change from burning to mowing for pruning wild blueberries has resulted in the loss of the sanitation benefit for pest management that burning provides (Yarborough, 2014). Along with the trend of longer, warmer growing seasons, there has been an increase in fungal leaf spot diseases. Heavy infection of leaf spot diseases, especially those resulting in early leaf drop, reduces wild blueberry yield (Yarborough, 2015; Yarborough et al., 2017).

Wild blueberries have responded well to fertilization, resulting in more rapid establishment, greater plant growth and higher yields (Smagula and Dunham, 1996; Yarborough, 2015). The current practice of regular applications of commercial fertilizers, especially nitrogen and phosphorus, to blueberry fields in the pruned year developed along with the use of selective herbicides (Yarborough et al., 1986). Fertilization recommendations were formerly based on noting stem height and leaf spotting and applying nitrogen from urea. Growers now use leaf tissue samples taken at the 90 to 100% tip dieback plant stage in the vegetative year, when the flush of spring growth ends, to determine if fertilizer is needed since standards of satisfactory levels of nutrients in leaf tissue have been developed (Yarborough and Smagula, 2013; 2017). Although nutrient deficiency symptoms are rarely observed, yield has been increased when leaf nitrogen (N) and phosphorus (P) concentrations have been raised above the N and P standards. Potassium does not appear to be deficient in Maine soils. Fertilizing with other nutrients such as iron, boron, zinc, and copper has not consistently improved growth or yield (Smagula, 1993; Bell et al., 2009).

Exposure of pest and beneficial insects to the various pesticides used in crop production has mostly focused on insecticides, especially the effects of insecticides on bees (Thompson and Wilkins, 2003; Scott-Dupree et al., 2009; Mullin et al., 2010; Johnson et al., 2013; Sprayberry et al., 2013; Laycock et al., 2014; Sanchez-Bayo and Goka, 2014; Zhu et al., 2017). In wild blueberry, similar studies on the effects of insecticides and fungicides have been conducted (Stubbs and Drummond, 1999; Choate et al., 2008; 2009; Drummond, 2012a; 2012b; 2012c; 2012d; Choate and Drummond, 2013). However, fungicides have also received increased attention. Iwasa et al. (2004) demonstrated that combining acetamiprid with fungicides increased toxicity to honey bees. Cage studies by Bernauer et al. (2015) showed that colonies of the bumble bee *Bombus impatiens* Cresson exposed to the fungicide chlorothalonil produced fewer workers and smaller queens compared to control colonies. Fenbuconazole, the active ingredient in several fungicides, when combined with the neonicotinoids acetamiprid and imidacloprid, was more toxic than either neonicotinoid product alone to *Apis mellifera* L. and to Japanese horn

faceted bees, *Osmia cornifrons* (Radoszkowski) (Biddinger et al., 2013). Recently Artz and Pitts-Singer (2015) looked at the effects of the fungicides Rovral® 4F (iprodione) and Pristine® (mixture of pyraclostrobin + boscalid) on nesting behavior in two managed solitary bees, *Osmia lignaria* Say and *Megachile rotundata* (F). They found evidence that these two commonly used fungicides can disrupt nest recognition.

Effects of fungicides on other beneficial arthropods have also been a research focus. One such study determined that three fungicides (myclobutanil, pyraclostrobin, and trifloxystrobin + tebuconazole), used in wild blueberry, caused no significant mortality to immature stages or pre-emergent adult *Trichogramma pretiosum* Riley, an important egg parasitoid and biological control agent of caterpillar pests (Khan and Ruberson, 2017). Benomyl, which was used occasionally in wild blueberry, but not currently, has been reported to significantly reduce predator populations, such as the lady beetle, *Coleomegilla maculata* (De Geer) in crop fields (Roger et al., 1994). These reductions resulted in resurgence of the resident pest populations such as *Pseudoplusia includens* (Walker) (Livingston et al., 1978), phytophagous mites (Bower et al., 1995) and aphids (Redcliffe et al., 1978). Yardim and Edwards (1998) studied the effects of selected pesticide application regimes (including the fungicide chlorothalonil, used in wild blueberry in the vegetative year, on predator populations as well as pests in tomato agroecosystems. They found that certain combinations of fungicides and herbicides resulted in increases in aphid populations.

However, little is known about the impact of combined applications of fungicides and fertilizers during the prune cycle on insect pests or fungal plant pathogens of wild blueberry and its resulting productivity. The goal of our three-year study (two replicated two-year trials, 2015 Trial (2015 – 2016) and 2016 Trial (2016 – 2017) was to evaluate the influence of fertility and disease management practices primarily during the prune year on sap-feeding hemipteran insects, fungal leaf spot pathogens, premature flowering, stem growth characteristics, flower bud cluster production, leaf retention, foliar nutrients, and yield in wild blueberry. We studied the potential affects from late spring/summer applications of two fungicides, Pristine® (a mixture of pyraclostrobin + boscalid) and Bravo Weather Stik® (chlorothalonil). We also looked at a combination of Pristine and DAP® (diammonium phosphate) fertilizer applications.

Materials and Methods

Experimental design

In 2015 (Trial 1) and 2016 (Trial 2), a randomized block design was implemented with five replicated plots per treatment (6.1 x 6.1 m) in a pruned wild blueberry field at the University of Maine's Blueberry Hill Research Farm in Jonesboro, Maine (44° 38.532N, 67° 38.707W). Three different treatments were applied as follows: pyraclostrobin + boscalid formulated as Pristine (1.35 L/ha), Pristine (1.35 L/ha) + DAP (diammonium phosphate) fertilizer (204 kg/ha), and chlorothalonil formulated as Bravo Weather Stik (4.1 L/ha). All fungicides and DAP were applied on 24 June 2015 (Trial 1) or 23 June 2016 (Trial 2) at recommended label rates. All materials except DAP were applied in 94.6 L of water-mixture per acre with a CO₂-propelled, 203-cm boom sprayer (193-cm swath) equipped with four, flat-spray, 8002VS TeeJet® nozzles operating at 0.24 MPa and at a slow walking speed. Walking speed was regulated using a metronome. DAP was applied using a shaker can to spread the material evenly over the plot. A non-treated control (NTC) was also included in the experiment. In the spring of 2016 (Trial 1) and 2017 (Trial 2) (27 June and 2 June, respectively), a second application of Pristine and Bravo

were made to the same plots; the Pristine + DAP application was not repeated. Honey bee colonies were rented and deployed at the research farm to supplement native bee densities to ensure adequate pollination.

Yield

To evaluate potential yield in each plot, we collected 10 random stems per plot and counted the number of flower bud clusters and flowers on each stem. Stems were collected on 23 May and 25 or 30 May for Trials 1 and 2, respectively. We also determined actual plot yields. On 8 August 2016 (Trial 1) or 11 August 2017 (Trial 2), yields were determined by raking a diagonal swath across each plot with a commercial (Hubbard Rakes, Jonesboro, ME) blueberry rake (45 Tine, 34.3 cm) and weighing the harvested fruit; harvested area = 9.7 m².

Leaf spot rating and leaf retention

On 2 October 2015 (Trial 1) and 5 October 2016 (Trial 2), twenty stems were selected from each plot and rated for percent leaf spot fungi. For each sample, a rope with 20 evenly spaced markings was stretched diagonally through each plot and the stem closest to each marking was cut and bagged. The next day leaves were rated for leaf spot symptoms. Percent disease from powdery mildew (*Erysiphe vaccinii*), *Septoria* leaf spot (*Septoria* sp.) and leaf rust (*Thekopsora vaccinii*) were pooled and treated as a single entity, leaf-spot. We visually estimated the percent of leaf area that was diseased per stem; 100 stems per treatment (20 stems per plot) were rated. The same stems were used to evaluate leaf retention. Leaf retention was evaluated by counting the total number of leaves and nodes lacking leaves (leaves fallen) from each of the twenty stems.

Sweep samples

Sweep sampling for sap-feeding hemipterans was conducted on 2 July 2015 (Trial 1) and on 23 June, 11 and 25 July, and 1 and 9 August 2016 (Trial 2). Ten sweeps with a standard 30.5-cm diameter sweep net were taken systematically through the center area of each plot avoiding plot boundaries. Insects were shaken from the sweep net into a 4L Ziploc[®] bag, frozen, and then identified to family (with the exception of tarnished plant bug which was identified to species, *Lygus lineolaris* (Palisot de Beauvois)).

Premature flowering and stem measurements

Fall or premature flowering has been increasing over the past decade (Drummond, unpublished data). We decided to determine if applications of fertilizer and/or fungicides might be responsible. Premature flowering in the prune crop was assessed on 25 September 2015 (Trial 1) or 5 October 2016 (Trial 2) by rating plots as either with or without flowering stems. All stems within two, 30.48 cm quadrats per plot were cut and brought into the laboratory on 9 October 2015 (Trial 1) or 5 October 2016 (Trial 2) to measure stem density, length, number of branches, and number of flower bud clusters. To evaluate foliar nutrients, 10 stems were randomly collected from each plot on 9 October 2015 (Trial 1) or 5 July 2016 (Trial 2). Leaves were removed, dried at room temperature, and sent to the University of Maine, Maine Soil Testing Service for analysis.

Analysis of Variance (ANOVA) based upon a randomized complete block design (RCB) and post-hoc mean separation (Tukey Procedure) ($P \leq 0.05$) (JMP[®], 2015) were used to compare leaf retention, incidence of leaf spot, foliar nutrients, and stem measures including number of

flower bud clusters, length, branching, and stem density, as well as abundance of sap-feeding insects, and foliar nutrients among the treatments. Subplots were pooled within main plots and dependent variables that were measured more than once during the growing season were averaged such that the ANOVA was conducted on the mean seasonal response. Trial and its interaction with treatment were the replicated independent categorical variables considered. Data were transformed by the square root prior to analysis if necessary. Correlation analysis, general linear models (trial, nutrient, trial X nutrient), and multiple analysis of variance (repeated measures MANOVA) was conducted to determine if associations existed between foliar nutrients and nutrients and sap feeding insect pests and leaf-spot disease incidence (JMP®, 2015).

In addition, a series of general linear models were used to determine the effect of the continuous random independent variables not related to the treatment variables, such as, leaf nutrients on the following response variables: leaf retention, stem branching, percent leaf spot, and sap feeding insects per sweep. Trial was also included in these models, as well as, the interaction of trial and the independent variables.

Results

Despite the significant difference in number of flower bud clusters/stem noted in the fall of 2015 in Trial 1 ($F_{(3,12)} = 4.72$, $P = 0.0213$), there were no significant differences in the subsequent crop year in number of flowers/bud ($F_{(3,12)} = 1.11$, $P = 0.3823$)(Fig. 1A) and no significant differences were noted in Trial 2 ($P > 0.05$)(Fig 1B). There was also no significant interaction between trial and treatment for either flower bud clusters or flowers ($F_{(3,28)} = 1.17$, $P = 0.3385$ and $F_{(3,28)} = 0.46$, $P = 0.7096$; respectively). Treatment of plots with fungicides or the DAP + Pristine combination did not result in flowering during the autumn.

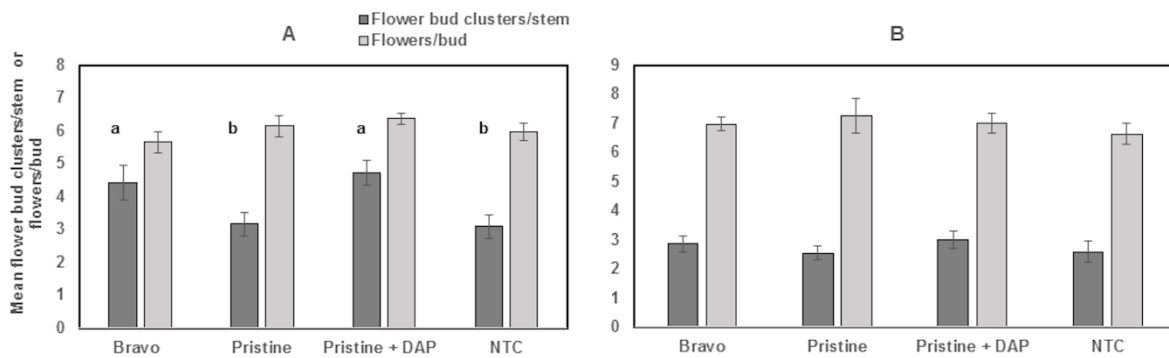


Figure 1. Effect of fungicide treatment on the mean flower bud clusters/stem and flowers/bud. Lines are standard error of the mean. Letters which are different denote significant differences ($P < 0.05$). Data from Trial 1 (A) and Trial 2 (B).

We did find significant differences in yield due to treatment, but this effect varied by trial ($F_{(3,28)} = 3.19$, $P = 0.039$, interaction of treatment x trial). When analyses were conducted by trial we found that in Trial 1 there were no treatment effects on yield ($F_{(3,12)} = 1.81$, $P = 0.1993$) (Fig. 2), while in Trial 2, there was a significant difference in yield ($F_{(3,12)} = 5.69$, $P = 0.0117$) (Fig. 2). In Trial 2, plots treated with Bravo alone had significantly higher yields than those treated with Pristine and DAP, and the non-treated controls.

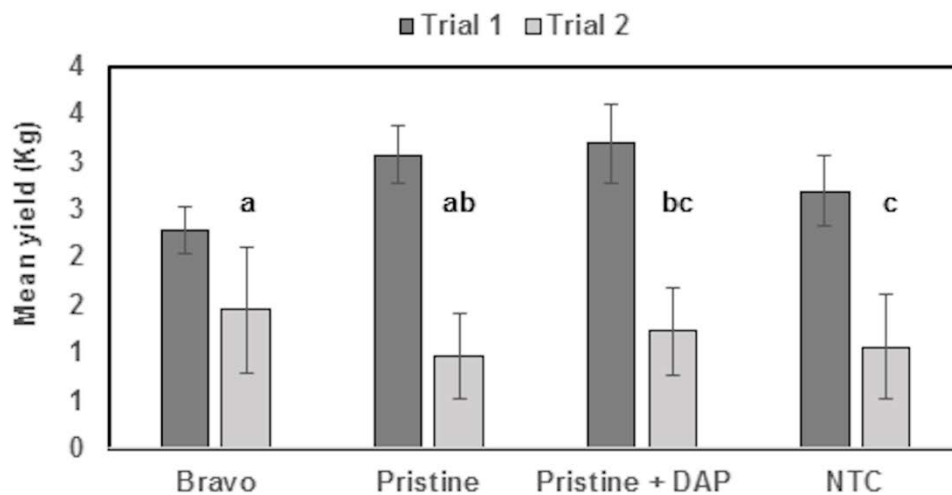


Figure 2. Effect of fungicide treatment on the mean yield (kg). Lines are standard error of the mean. Letters which are different denote significant differences ($P < 0.05$). Data from both trials, combined.

We evaluated various plant response measures in addition to number of flower bud clusters and flowers, and yield. There were no significant differences in stem density or stem branching in either trial ($P > 0.05$). The only effect was for trial; over all treatments, mean stem density was significantly greater in Trial 1 ($F_{(3,28)} = 16.52$, $P = 0.0004$). We did observe differences in stem length over both trials ($F_{(3,28)} = 6.51$, $P = 0.0018$). Plots treated with Pristine alone had significantly greater stem length compared to the other three treatments (Fig. 3). Pristine + DAP-treated plots were not different than Bravo treated plots, but stems were longer than the non-treated control.

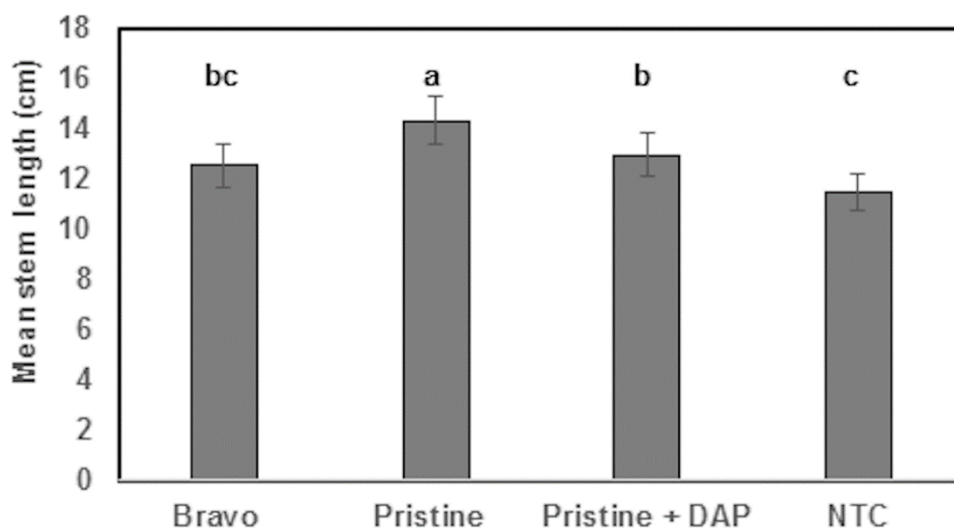


Figure 3. Effect of fungicide treatment on the mean stem length (cm). Lines are standard error of the mean. Letters which are different denote significant differences ($P < 0.05$). Data from both trials, combined.

We found a significant difference in leaf retention due to treatment which varied by trial ($F_{(3,28)} = 2.46, P = 0.083$, interaction of treatment x trial). In Trial 1, the plots treated with Bravo retained significantly more leaves in October than those treated with Pristine + DAP ($F_{(3,12)} = 6.5, P = 0.0074$)(Fig. 4). This was not the case in Trial 2 when we observed no significant differences in leaf retention among the treatments ($F_{(3,12)} = 1.09, P = 0.3919$) (Fig. 4).

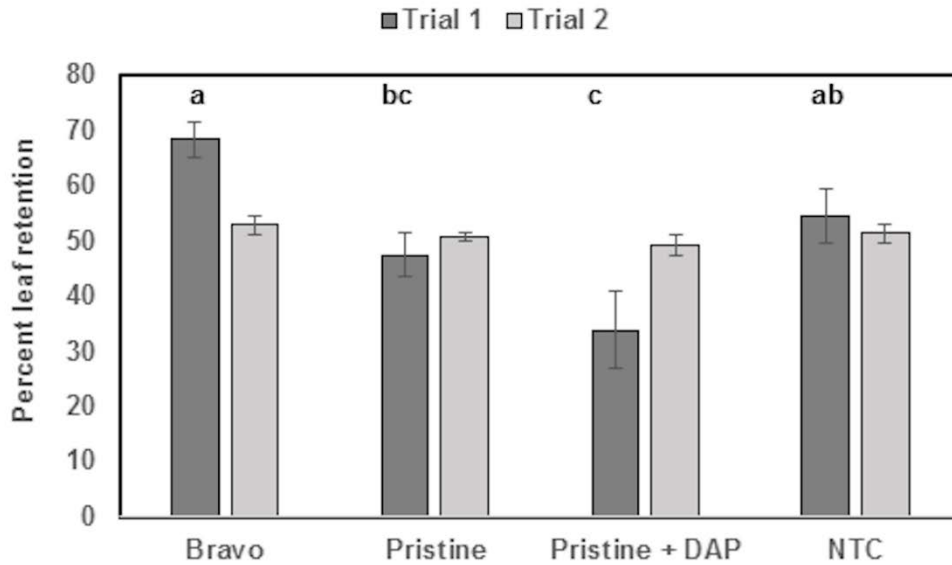


Figure 4. Effect of fungicide treatment on the mean percent leaf retention. Lines are standard error of the mean. Letters which are different denote significant differences ($P < 0.05$). Data from Trial 1 and Trial 2.

As far as fungal induced leaf spot, in Trial 1 we did observe a treatment effect; however, it was only significant at $P < 0.10$ ($F_{(3,12)} = 2.68, P = 0.0943$). Pristine + DAP fertilizer resulted in more leaf spot than either Bravo or Pristine without fertilizer. A similar trend was observed in Trial 2 when plots treated with Pristine only and Pristine + DAP-treated plots had more leaf spot than Bravo-treated plots ($F_{(3,12)} = 3.05, P = 0.07$). When both trials were combined, there were significant treatment effects ($F_{(3,28)} = 3.61, P = 0.0254$)(Fig. 5); plots treated with Pristine + DAP had more leaf spot than those treated with Bravo or the non-treated controls.

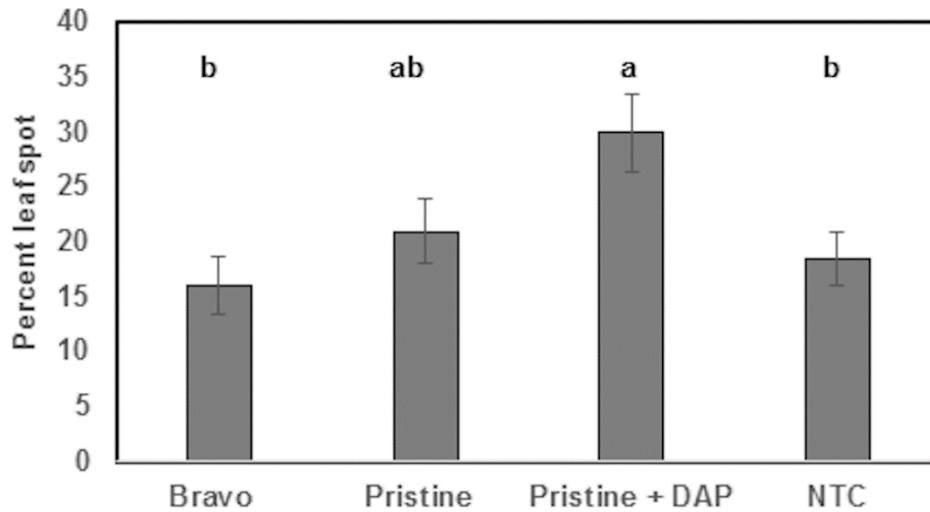


Figure 5. Effect of fungicide treatment on the mean percent leaf spot. Lines are standard error of the mean. Letters which are different denote significant differences ($P < 0.10$). Data from both trials, combined.

We also found that the percent leaf spot across plots was determined by stem branching ($F_{(1,36)} = 17.206$, $P = 0.0008$, Fig. 6), suggesting that an increased plant canopy and leaf biomass results in greater leaf spot infection during the prune year vegetative growth phase. Trial ($P = 0.206$) and trial x stem branching ($P = 0.161$) were not significant predictors of percent leaf spot. The level of stem branching, on the other hand, was determined by % foliar nitrogen ($F_{(1,38)} = 5.102$, $P = 0.029$, Fig. 7), but this was only significant when the non-significant terms trial was taken out of the model suggesting that this effect of foliar nitrogen was highly correlated with trial or the seasonal effect of the growing season.

When we assessed the effect of percent leaf spot in each plot and the level of leaf retention, we found a highly significant negative relationship ($F_{(1,36)} = 17.241$, $P = 0.0003$, Fig. 8), suggesting that increased leaf spot reduces leaf retention throughout the season. Trial ($P = 0.811$) and the trial x leaf spot interaction ($P = 0.185$) were not significant determinants of leaf retention.

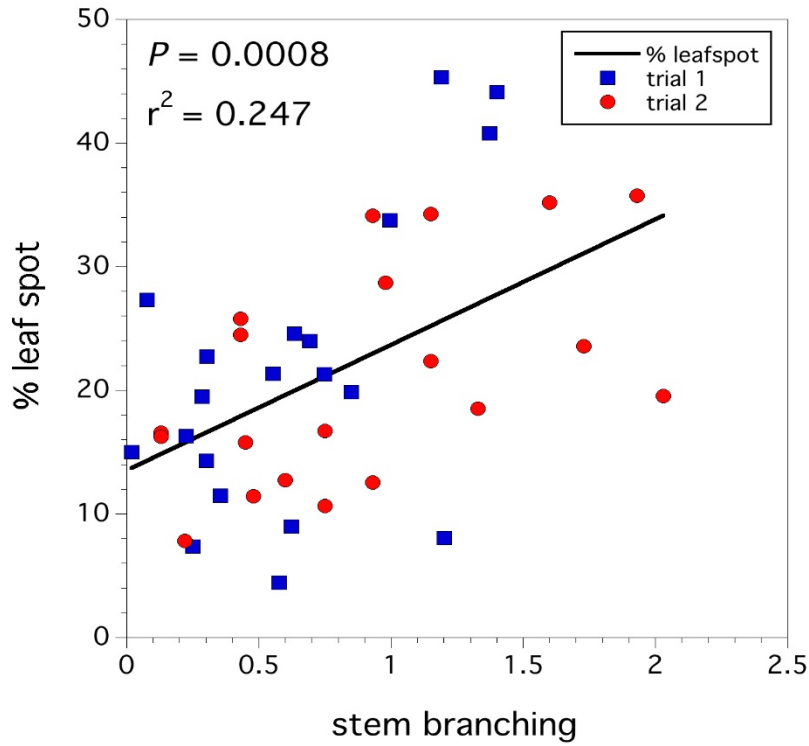


Figure 6. The relationship between percent leaf spot in plots and the number of lateral branching off of the main stem (0 = no lateral branches).

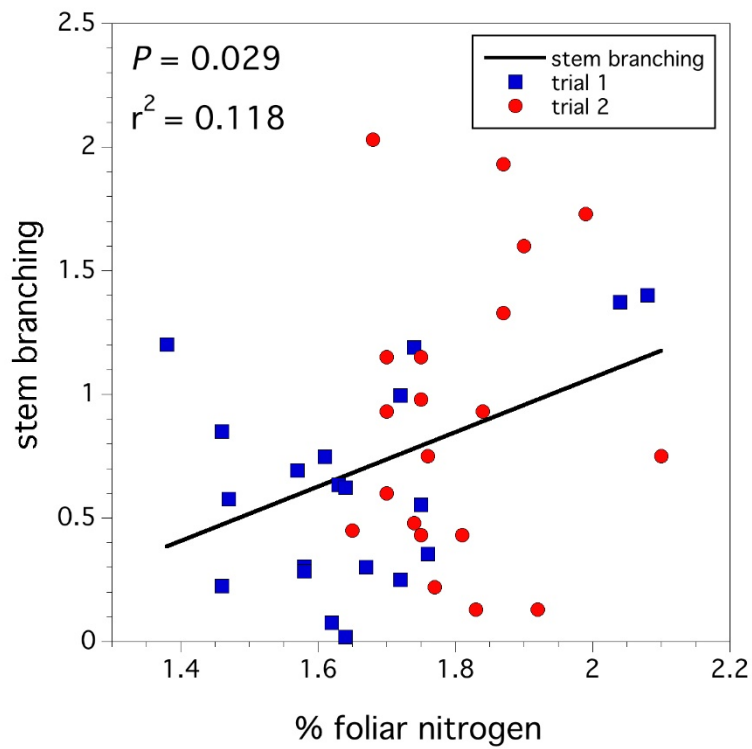


Figure 7. The relationship between % foliar nitrogen and stem branching.

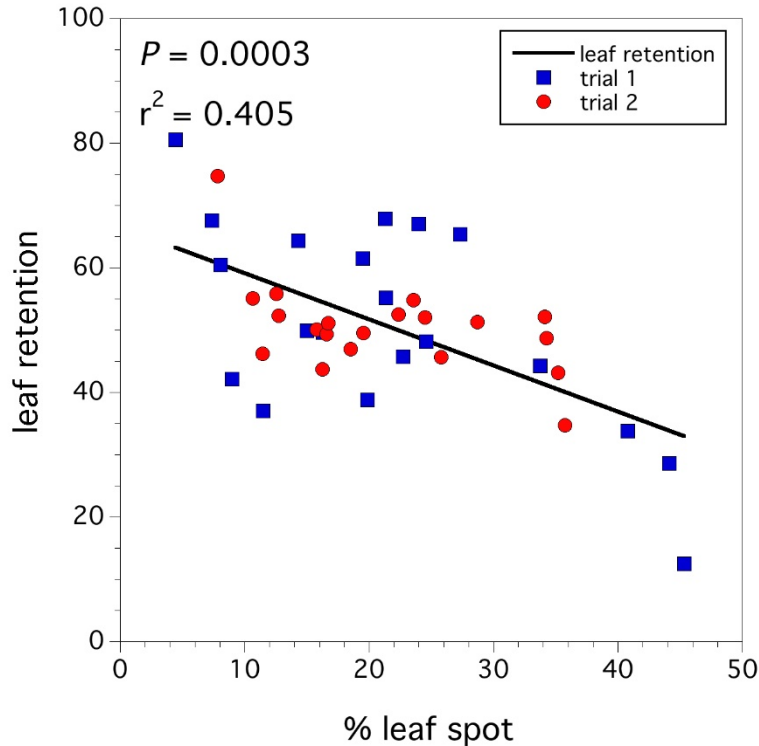


Figure 8. The level of leaf retention as a function of percent leaf spot infection.

Sap feeding insects were more diverse in Trial 2 than in Trial 1 where we only observed leafhoppers. In Trial 1, five morpho-species of leafhoppers were collected in sweep samples; we did not observe any treatment effects ($F_{(3,12)} = 1.36$, $P = 0.3004$) (Table 1). It is interesting, that, though not significant, there were more of the five morpho-species in the non-treated control plots than the fungicide treated plots. Five taxa of sap feeding insects were collected in sweep samples in Trial 2 (Table 1). The most abundant taxa was leafhoppers (five morpho-species), and we also collected tarnished plant bugs, aphids, lygaeids, and weevils. There were significant differences among the treatments. Plots treated with Pristine + DAP had significantly more leafhoppers ($F_{(3,12)} = 6.56$, $P = 0.0071$); a similar trend was observed for tarnished plant bug ($F_{(3,12)} = 3.19$, $P = 0.0628$). Aphids were most abundant in plots treated with Bravo ($F_{(3,12)} = 4.67$, $P = 0.022$). There was no significant difference in the number of weevils or lygaeids due to treatment ($P > 0.05$) (Table 1).

Table 1. Effect of fungicide treatment on relative abundance (per 10 sweeps) of tarnished plant bug, aphids, lygaeids, weevils and leafhoppers. Letters within columns which are different denote significant differences ($P < 0.05$).

Mean per 10 sweeps					
Treatment	Tarnished plant bugs	Lygaeids	Aphids	Weevils	Leafhoppers
<u>Trial 1</u>					
Bravo					3.80±0.73a
Pristine					5.60±1.57a
Pristine + DAP					5.40±2.04a
NTC					7.80±1.59a
<u>Trial 2</u>					
Bravo	0.05±0.05b	0.05±0.05a	0.20±0.09a	0.25±0.12a	6.55±1.60b
Pristine	0.05±0.05b	0.35±0.22a	0.00±0.05b	0.25±0.16a	6.40±1.24b
Pristine + DAP	0.35±0.15a	0.10±0.07a	0.10±0.07ab	0.65±0.22a	12.2±2.77a
NTC	0.05±0.05b	0.20±0.12a	0.00±0.00b	0.35±0.17a	4.15±1.16b

In regards to sap-feeding insect taxon richness (number of taxa), in Trial 2, plots treated with Pristine + DAP had a significantly greater number of taxa captured than Bravo, Pristine alone, or the non-treated control plots ($F_{(3,12)} = 4.53$, $P = 0.0241$)(Fig. 9). In addition, we found that there was a negative association between aphids and lygaeids ($r = -0.511$, $P = 0.021$), but a positive association between leafhoppers and tarnished plant bugs ($r = +0.540$, $P = 0.014$).

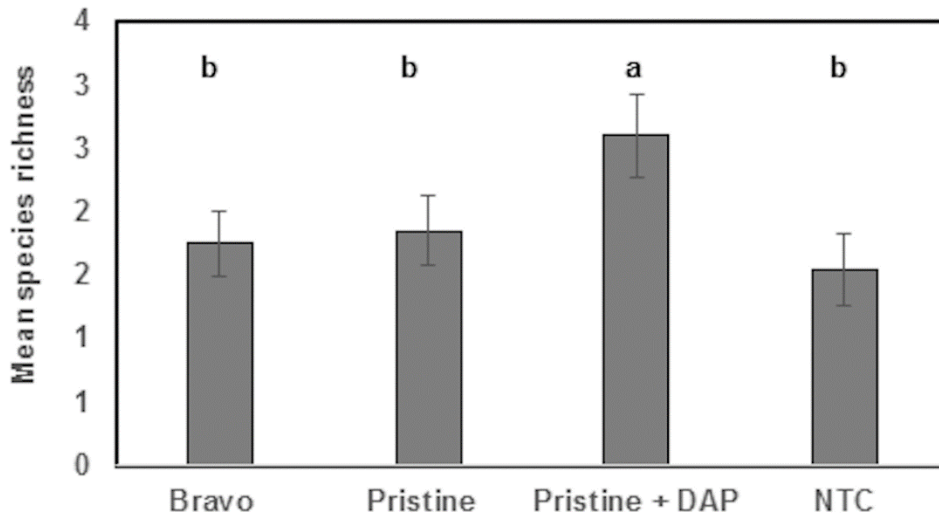


Figure 9. Effect of fungicide treatment on the number of sap-feeding insect taxa captured (richness). Lines are standard error of the mean. Letters which are different denote significant differences ($P < 0.05$). Data from Trial 2 only.

The results of the analysis of foliar nutrients varied between years (Table 2). In Trial 1 there were significant differences in levels of nitrogen, calcium, potassium, phosphorus, and aluminum among the treatments. Pristine + DAP treatments had significantly more nitrogen ($F_{(3,12)} = 8.44$, $P = 0.0028$) and phosphorus ($F_{(3,12)} = 8.08$, $P = 0.0033$) than the other treatments. There was significantly more potassium in Bravo-treated plots compared with plots treated with Pristine ($F_{(3,12)} = 4.719$, $P = 0.0304$), and mean separation indicated more aluminum in Bravo-treated plots compared with plots treated with Pristine + DAP ($F_{(3,12)} = 2.00$, $P = 0.1675$) and higher levels of calcium in the Pristine + DAP treatment than Pristine alone ($F_{(3,12)} = 2.67$, $P = 0.0951$) (Table 2). In Trial 2, there were no significant differences between treatments in levels of nitrogen, calcium, potassium, or phosphorus. There were significant differences in levels of aluminum ($F_{(3,12)} = 5.82$, $P = 0.0108$) and iron ($F_{(3,12)} = 10.35$, $P = 0.0012$). Plots treated with Pristine alone had significantly more foliar aluminum and iron than Bravo or Pristine + DAP-treated plots.

Correlation analysis was conducted to determine if associations exist between leaf nutrients. We found significant ($P \leq 0.05$) negative correlations between boron and magnesium and phosphorus ($r = -0.485$ and -0.629 ; respectively), and negative associations were observed between manganese and phosphorus ($r = -0.477$). Positive associations were observed between boron and aluminum, iron, and manganese ($r = +0.456$, $+0.521$, and $+0.556$; respectively) and positive associations were observed between zinc and copper ($r = +0.631$) and between manganese and aluminum ($r = +0.494$).

We assessed all of the foliar nutrients independently using general linear models (trial, nutrient, trial x nutrient) to determine if individual nutrients had effects on total hemipteran sap feeding insects in Trial 1 and Trial 2. None of the nutrients were consistent significant predictors of sap feeding insect or disease levels over both trials. In Trial 2, where we observed greater insect pest abundance, a positive association was observed between leaf spot intensity and boron ($r = +0.628$, $P = 0.008$) and a negative association between leaf spot intensity and calcium ($r = -0.572$, $P = 0.022$). A negative relationship was observed between manganese and weevil

incidence ($r = -0.448$, $P = 0.036$), and between tarnished plant bug and magnesium ($r = -0.468$, $P = 0.030$).

We also conducted a repeated measures MANOVA using both leaf-spot incidence and total hemipteran sap feeding insects and three of the more promising nutrients from the univariate analyses (nitrogen, calcium, and magnesium). We found that when considering both leaf-spot disease incidence and sap feeding hemipterans, foliar nitrogen was a significant predictor of these pest incidences ($F_{(1,35)} = 5.244$, $P = 0.028$). We used a general linear model to determine if sap feeding insect herbivores and/or percent leaf spot, along with stem density, and percent foliar nitrogen determined yield. We found that year ($P < 0.0001$) and percent foliar nitrogen ($P = 0.051$) were significant predictors of yield, as determined by step-wise regression. Seventy-four percent (74.9%) of the variation in yield was determined by these two factors.

Discussion

This study evaluated the impact of two fungicides chlorothalonil (Bravo) and pyraclostrobin + boscalid (Pristine) and a fertilizer, diammonium phosphate (DAP), on sap-feeding hemipterans, premature flowering, stem characteristics, leaf spot, leaf retention, foliar nutrients, and yield in wild blueberry.

Chlorothalonil is an organic compound mainly used as a broad spectrum, non-systemic fungicide (Cox, 1997). Pyraclostrobin + boscalid deprives fungal cells of their energy source and eliminates the availability of chemical building blocks for synthesis of essential cellular components. It also interferes with cell respiration and the production of energy. Monoammonium phosphate or diammonium phosphate are the most commonly applied fertilizers to correct N and P deficiency in wild blueberry (Bell et al., 2009). When applied, it temporarily increases the soil pH, but over the long-term the treated soil becomes more acidic than before, due to nitrification of the ammonium (IPNI, 2018).

The analysis of flower-bud clusters from our first trial in 2015 suggested that chlorothalonil might increase potential yield, but that pyraclostrobin + boscalid will only increase potential yield when nitrogen fertilizer is also applied. This was not the case in Trial 2. The addition of fertilizer may increase the number of flower buds which are a measure of yield potential (Jeliazkova and Percival, 2003); however, Bajcz and Drummond (2017a; 2017b) found that wild blueberry is highly compensatory to reductions in flower density. A reduction of up to 60% in flower density will be compensated for by larger fruit and thereby yield will not be affected. However, despite the plant's compensatory ability, weed competition can negatively affect flower bud development. Adding fertilizer will have little effect on yield unless weeds are controlled (Penny and McRae, 2000). The increase in yields directly due to fertilization might be indirectly compensated by an increase in crop loss due to increased weed abundance and increased disease and insect sap feeding. In wild blueberry, Kennedy et al. (2010) found that fertilizer increased weed density in the absence of herbicides, had no effect on weed density in the presence of herbicides, and had no impact on the number of flower buds, and did not increase yields.

Other growth characteristics of wild blueberry that affect potential yield are stem density, stem branching, and stem length. All of these factors act by increasing flower bud density on a per unit area (Percival and Sanderson, 2004; Yarborough et al., 2017). We did not find that any of the fungicide or fertilizer treatments that we applied increased stem density or branching. This can be common if soil fertility is already high (Yarborough, 2004). We did find a Pristine + DAP

effect on stem length in both trials which was due to the fertilizer effect since Pristine alone did not show such an increase. Therefore, there are some positive benefits directly to plant growth and yield that are realized by growers who follow the current recommendations to apply both fertilizer and fungicide during the prune year (Yarborough and Smagula, 2013). However, we did find that as foliar nitrogen increased, the amount of stem branching increased. This suggests that a spatially heterogeneous soil nitrogen pattern may result in a spatially heterogeneous pattern in branching and subsequently yield and in addition, increased branching appears to increase the incidence of leaf spot. This is probably due to increased relative humidity within a larger and denser plant canopy, thereby, providing a more conducive environment for leaf spot pathogens to become established.

There is very little literature on the effect of fungicides on premature flowering or leaf retention in blueberry. Work that has been conducted focuses on plant diseases. Ojiambo et al. (2006) found that *Septoria* leaf spot reduces flower bud set and yield potential of rabbiteye and southern highbush blueberries. Cline (2002) reported on highbush blueberry bud set and yield following the use of fungicides for leaf spot control in North Carolina. However, the literature on evolution of plant defense suggests that leaf drop in deciduous woody perennial plants, apart from minimizing effects of harsh climatic conditions, might be a mechanism for reducing herbivory (Owen, 1978; Simberloff and Stiling, 1987; Karban, 2007). Leaf drop has been shown to reduce herbivore insect oviposition, early spring colonization, and overwintering success when grass and leaf curls support insect overwintering stages. If this is the case, then leaf retention could have negative effects on wild blueberry plant productivity. We found that in one of the two trials, leaf retention was enhanced by an application of Bravo. Bravo may reduce percent leaf spot infection that in turn might increase leaf retention. This hypothesis is supported by our finding that increased percent leaf spot infection directly reduces leaf retention.

The direct effects of pesticides including fungicides on beneficial predatory arthropods have been widely studied in crops as diverse as barley (Sotherton et al., 1987), wheat (Sotherton and Moreby, 1988), tomatoes (Yardim and Edwards, 1998), and apples (Hardman et al., 2006). Much less is known about the effect of fungicides alone and in conjunction with fertilizers on pest insects. Application of fertilizer with Pristine influenced sap-feeding insects in our trials, but not consistently. We found no effects on sap-feeding insect pests in Trial 1. In Trial 2 we found that Pristine + DAP resulted in higher abundance of leafhoppers and tarnished plant bug, and Bravo resulted in higher numbers of aphids. The positive response to aphids in plots treated with Bravo may be due to an indirect negative effect on fungal pathogens of aphids. In potato production in Maine, it was found that that aphid populations are partly regulated by pathogens (Alyokhin et al., 2011). We suggest that the higher abundance of leafhoppers and tarnished plant bug may be due to an increased nutritional quality of wild blueberry. In Trial 1 but not in Trial 2, percent foliar nitrogen was highest in Pristine + DAP treated plots.

The effects of plant nutrition on the life history of sap-sucking insects can be dramatic, although not consistent across all taxa (Price et al., 2011). Increases in nitrogen in plants have been shown to increase the population growth rate of citrus mealybug, *Planococcus citri* Risso (Hogendorp et al., 2006) and *Aphis gossypii* Glover (Nevo and Coll, 2001). England et al. (2011) studied how whitefly life history characteristics are affected by both the presence and formulation of fertilizer added to poinsettia plants in the greenhouse; all fertilizers tested increased whitefly survivorship. It has also been found that elevated concentrations of N in willows and cottonwoods increase abundance and biomass of Homoptera (Wiesenborn, 2011). Previous studies have found populations of homopterans respond to increases in plant N due to

fertilization on salt marsh cordgrass (Bowdish and Stiling, 1998) and cotton (Cisneros and Godfrey, 2001). Rates of survival and fecundity by aphids on rice increase when host plants are supplemented with N (Jahn et al., 2005). Drummond et al. (2009) studied insect and disease dynamics of an organic wild blueberry system. Our work suggested that pests respond to horticultural management practices not designed specifically for insect or disease suppression. Fertilization was only seen to directly affect grasshopper density, resulting in higher densities in plots receiving the higher fertilizer rates. However, in general, it was shown that plant nutrition has an effect on the insect pest community with more similar species inhabiting plants with more similar nutritional signatures. Although nitrogen has been found to be the most significant plant nutrient affecting insect growth and development (Price et al., 2011) other nutrients can also positively or negatively affect insect pest abundance. In a previous study in wild blueberry, we found that over the course of three production cycles, foliar concentrations of boron and phosphorous positively affected densities of blueberry tip midge (*Dasineura oxycoccana* Johnson), while aluminum was found to positively affect blueberry thrips complex (*Frankliniella vaccinii* Morgan and *Catinathrips kainos* O'Neill) abundance (Yarborough et al., 2017). These minor nutrients may also have negative impacts. Our study did show negative correlations, but only in Trial 2, with magnesium and tarnished plant bug abundance and manganese and weevil abundance. Although due to the correlations among nutrients in wild blueberry plant tissue it is not possible to know if a relationship between the insect abundances that we recorded and single nutrient levels are causal phenomena.

Fertility has also been demonstrated to influence the development of numerous plant diseases. In both replicated trials, we observed consistent treatment effects on the incidence of fungal induced leaf spot. Pyraclostrobin + boscalid + DAP fertilizer resulted in more leaf spot. Therefore, it appears that fungicides reduction of leaf-spot disease incidence in wild blueberry is overridden by a potential increase in pathogens with high fertility. Yarborough et al. (2017) showed that at the field level, fungicides reduce leaf spot incidence in wild blueberry but fertilizer indirectly affects phomopsis disease incidence by changing soil pH and that phomopsis incidence is highly positively correlated with leaf-spot disease incidence. Therefore, the effects of simultaneous applications of fertilizer and fungicides might be complicated by the resulting infection from wild blueberry pathogens.

Diseases have been found to be less responsive to management practices in wild blueberry (Drummond et al., 2009) compared to many other crops. Much of this might be due to the genotypic and phenological diversity in the crop. However, fertilization does appear to increase mummy berry disease levels. Higher incidence of disease was found associated with plots that received higher rates of fertilizer. They hypothesized that this effect might be due to the response of increased weed growth in fertilized plots that in turn protect this pathogenic fungus from harsh environments. Fungicides, in general, have a strong negative effect on mummy berry primary infection levels. However, confounding this relationship is the positive effect that bees have on secondary infection on mummy berry disease levels (Yarborough et al., 2017). We also observed in Trial 2 only, a positive association between leaf spot intensity and boron, and a negative association between leaf spot intensity and calcium. We do not feel confident in these results from a single trial, especially since Yarborough et al. (2017) found a positive relationship between foliar boron levels (ppm) and fungal leaf-spot disease incidence.

We were only able to find consistent foliar nutrient effects on sap feeding insect pest abundance and leaf-spot disease incidence in our study when we combined sap feeding insect pest abundance and leaf-spot incidence as an overall measure of plant attack. The effect was

strong and was consistent over both years, suggesting that nitrogen fertilization during the prune year might increase wild blueberry yield due to enhanced plant productivity, but that in order to realize this increased plant physiological productivity, fungicides and herbicides might need to be applied to cancel out negative effects of plant competition from weeds (Yarborough et al. 2017) and negative effects of leaf-spot disease and sap feeding insects (when they occur simultaneously) on yield. At this point we have no evidence that sap-feeding insects alone in the absence of leaf spot, negatively affect wild blueberry yield.

In conclusion, in this study, applications of fertilizer during the prune year did not consistently affect yield; although, increased stem length, a characteristic that is an indicator of potential yield increase, was a consistent consequence of fertilizer application when combined with Pristine. The best model for predicting yield included trial, a variable that represents the growing seasons over the two-year cropping cycle, and percent foliar nitrogen in the prune year. This model explained 75% of the variation in yield over the two cropping cycles. It is likely, that percent foliar nitrogen increased stem branching, another plant attribute that has been associated with increased yield (Yarborough et al. 2017). Our results also suggest that it is fertilizer applied in the prune year that is the mechanism behind increased disease levels and sap-feeding insect enhancement and not the application of fungicides. This appears to be due to increased nitrogen; although, we only saw an increase in foliar nitrogen concentration in one of the two trials. We also found that fungicides might not compensate for the enhanced disease levels brought about by fertilizer application.

A worrying phenomenon that has been occurring in the past two decades with increasing occurrence is fall flowering. We did not find evidence that stimulation of late season growth by fertilizer application, or enhanced leaf retention due to fungicide application (reduction in percent leaf spot infection) had any effect on fall flowering. Fall flowering is of major concern to many growers. An example of this is depicted in a photograph taken in Winterport, Maine in the fall of 2017, shown in Figure 10A. It can be seen that hundreds of flowers are in fall bloom in this clone. Figure 10B illustrates the rapid occurrence of fall flowering in a 2017 holdover (two crop cycles in a row) field in Winterport, Maine. While this progression is alarming the actual number of flowers that occurred across more than half of the clones was estimated to be less than 5%. We suspect that this fall bloom phenomenon might be more of a response to climate change. Climate change is already affecting the number of days that bees have available for wild blueberry pollination (Drummond et al. 2017) and so it might be that the warmer autumn periods are resulting in an early termination of dormancy (Wolfe et al. 2017). We are challenging the new generation of researchers¹ to address this relatively recent phenomenon. If it is linked to climate change, mitigation strategies need to be developed or Maine as a major producer of wild blueberries may be a historical footnote in the future agricultural landscape.

¹ At the time of writing this manuscript, Dr. Frank Drummond had just attended his last scientific meeting in his 30-year career, NABREW (North American Blueberry Researchers and Extension Workers Meeting). Good luck to the young Turks who will carry on!

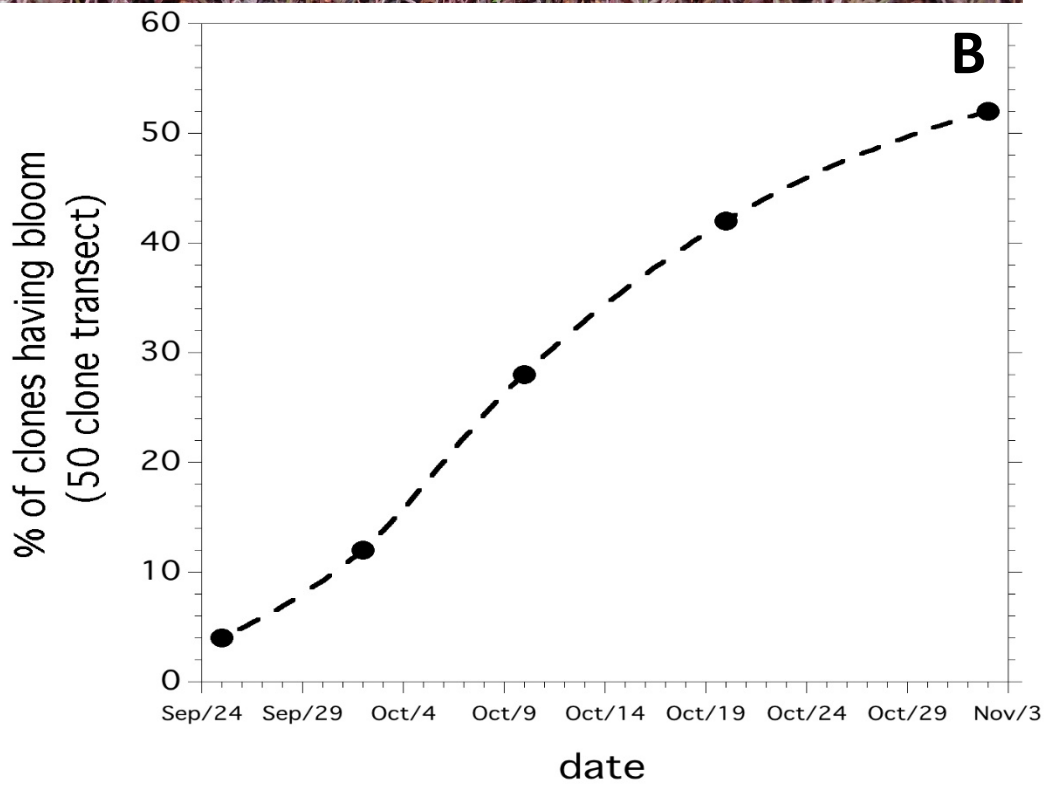


Figure 10. A wild blueberry clone in bloom on 27 October, 2017 (A), the rate of fall bloom progression (B) in Winterport, Maine.

Table 2. Wild blueberry nutrient foliage analysis. Letters within columns that are different denote significant differences ($P < 0.05$).

Treatment	(%) N	(%) Ca	(%) K	(%) Mg	(%) P	(ppm) Al	(ppm) B	(ppm) Cu	(ppm) Fe	(ppm) Mn	(ppm) Zn
<u>Trial 1</u>											
Pristine	1.54±0.06b	0.40±0.01b	0.46±0.01b	0.20±0.01a	0.12±0.003b	80.46a±6.97b	25.40±1.99a	4.04±0.37a	36.70±3.00a	917.40±115.88a	12.60±0.49a
Pristine + DAP	1.87±0.08a	0.49±0.03a	0.49±0.01ab	0.19±0.01a	0.16±0.01a	61.42±2.36b	23.54±1.14a	3.72±0.14a	40.84±0.97a	1040.20±135.25a	14.02±0.89
Bravo	1.62±0.05b	0.43±0.04ab	0.52±0.01a	0.18±0.01a	0.13±0.01b	91.52±13.79a	25.72±3.61a	4.30±0.46a	44.24±5.48a	1197.20±135.77a	15.10±1.33a
NTC	1.59±0.03b	0.46±0.02ab	0.48±0.004b	0.21±0.01a	0.12±0.002b	75.82±3.00ab	25.26±1.56a	4.18±0.33a	38.12±2.46a	947.60±59.74a	14.56±0.61a
<u>Trial 2</u>											
Bravo	1.73±0.01a	0.36±0.02a	0.49±0.01a	0.17±0.01ab	0.13±0.01a	75.76±6.71b	20.76±0.96a	5.42±0.45a	29.16±0.99b	369.00±48.69a	12.76±0.77a
Pristine	1.83±0.05a	0.36±0.01a	0.51±0.01a	0.18±0.01a	0.14±0.002a	93.28±4.54a	22.24±1.24a	6.29±0.29a	35.26±1.61a	380.80±22.35a	17.86±4.10a
Pristine + DAP	1.82±0.05a	0.33±0.02a	0.50±0.004a	0.16±0.004a	0.14±0.01a	73.62±4.24b	20.68±1.79a	16.16±10.71a	30.86±1.44b	361.80±53.00a	16.12±3.78a
NTC	1.82±0.06a	0.36±0.01a	0.50±0.01a	0.16±0.004a	0.14±0.01a	70.72±4.07b	19.86±1.90a	5.60±0.33a	31.20±1.38b	296.00±18.10a	13.68±0.91a

Acknowledgements

We would like to thank Dr. Dave Yarborough and Ms. Elissa Ballman for reviewing a draft of this manuscript and Ms. Rachael Martin for assistance with leaf spot ratings. Thanks also to University of Maine students Jade Christensen, Hunter Merchant, and Elesha Young for help with data collection. This is Maine Agricultural and Forestry Experiment Station journal article number 3632. Partial funding for this project was provided by the National Institute of Food and Agriculture, under the U.S. Department of Agriculture Specialty Crops Research Initiative, under Agreement No. 2015-51181-24252.

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