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Shade effect on phenology, fruit yield and phenolic content of two wild blueberry species of NW Ontario, Canada

Viktoriya Dyukaryeva and Azim U. Mallik *

Department of Biology, Lakehead University, Thunder Bay, Ontario, Canada; vdyukary@lakeheadu.ca (V.D.); amallik@lakeheadu.ca (A.U.M.)
* Correspondence: amallik@lakeheadu.ca

Abstract: Blueberries, rich in sugar, vitamins, amino acids, and enzymes that reduce oxidative cell damage are called superfood. In boreal forests wild blueberry plants grow vigorously after clear-cutting and fire, berry production remains high for 6 - 8 years, then declines with canopy closure. We studied the effect of shade on phenology, growth, berry yield and chemical content of two common blueberry species (*Vaccinium myrtilloides* and *V. angustifolium*) of NW Ontario. We hypothesized that high shade would delay vegetative and reproductive phenology, decrease berry yield by increased resource allocation to vegetative vs reproductive growth and moderate shade will increase berry phenolic content and antioxidant capacity. We subjected transplanted blueberry plants to 0, 30, 50, and 80% shade and recorded their phenological events from early May - September 2022. We measured leaf area, specific leaf area, leaf dry matter content, berry fresh weight, number, and size, phenolic content, and antioxidant capacity. High shade caused earlier leaf maturation in *V. myrtilloides*, delayed flowering in *V. angustifolium*, and prolonged fruit maturation in both. Berry yield of both species decreased with increasing shade. High shade reduced berry phenolic content and antioxidant capacity, especially in *V. myrtilloides*. We conclude that shade shifts species-specific vegetative and reproductive phenology leading to difference in resource acquisition causing lower berry yield and antioxidant activity.

Keywords: *Vaccinium angustifolium*; V. *myrtilloide;* shade treatment; vegetative buds; reproductive buds; vegetative growth; fruit chemistry; antioxidant capacity.

1. Introduction

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Light has a direct effect on photosynthesis, growth, morphological development, metabolism, and reproductive success in plants [1,2]. In combination with other environmental factors its effect on plant's resource metabolism and assimilation varies based on species [3]. Multiple environmental factors, such as soil and air temperature, soil moisture and fertility induce morphological and resource allocation plasticity in *Vaccinium* genus, but very few studies focused on its response to increasing shade [2,4-5]. Members of the *Vaccinium* genus include 25 species, such as cranberries and blueberries, have phenotypic plasticity that can be attributed to canopy covers (shade) of a typical boreal forest [4]. While valued for their high nutrient contents and secondary compounds, wild blueberries hold an important place in the diet of up to 50% of 200 forest-reliant communities in Northwestern Ontario [6].

Blueberries are well known for their high antioxidant capacity and are often considered a "superfood" by many [7]. It has been shown that increased UV radiation induces production of phenolic and anthocyanin compounds in *Vaccinium* species - both strongly associated with antioxidant activity, essential in reversing cellular oxidative damage [8-9]. Oxidative damage caused to DNA, protein, and lipids by increased production of reactive oxygen species (ROS) in human cell culture was linked to a multitude of chronic,

cardiovascular, and cancerous diseases [10]. Thus, investigating the effect of variability of shade-inducing morphological and resource allocation plasticity, berry yield and berry chemistry of *Vaccinium* species has a direct ecological, horticultural, and nutritional significance.

Plant phenology studies the timing of initiation and end of life cycle events, particularly the developmental stages, annual patterns, biotic and abiotic factors, and their interrelation [11]. Determining plant's response to biotic and abiotic factors has wide applications in agriculture, for example to determine optimal time for pruning, applications of pesticide and herbicide, and harvest, as well as be used to characterize carbon balance in terrestrial ecosystems, competition, and effects of climate change [4, 11-14]. In NW Ontario the two most common blueberry species are velvet leaf blueberry (*V. myrtilloides*) and lowbush blueberry (*V. angustifollium*), the former has been reported to be quite shade tolerant compared to the other [4,15]. However, no phenological studies have been reported comparing the effect of shade on these species.

Boreal forest, native habitat of Northwestern Ontario blueberries, is characterized by canopy shades that provide a range of light-related microenvironmental conditions [4]. Some of these factors such as temperature, precipitation, and humidity directly affect development of reproductive structures, where flower development, pollination fertilization, and fruit maturation are susceptible to microclimatic variation [16-17]. Direct and indirect effects of microclimate also affect vegetative phenology, plant height, and canopy cover [18]. Overall, accumulation and longevity of reproductive and vegetative phenological shifts play a vital role in local species distribution and their interaction with neighboring species while also increasing the risk of trophic asynchrony, population declines of higher-level consumers (pollinators and herbivores), and reduction of plant fitness [18-20].

Photosynthetic processes and carbon assimilation define successful plant performance [21]. Light availability directly affects photosynthesis [2]. Under increased shade the plants respond by shifting phenological events and morphological and physiological adjustments to specific leaf area (SLA), inter-node and petiole lengths, leaf size, leaf thickness, and leaf mass [22]. This allows it to maintain optimal performance under increased shade, a direct plastic response of active alleviation of environmental stress and reduced resource availability [22]. Shade intensity correlates with berry yield and quality of blueberry [2]. With prolonged exposure to low light blueberries experience delayed fruit development and maturation and decreased yield [2]. Moderate shade, either artificial through use of shading nets or natural canopy overstory, reduces light and temperature stress which may have a positive effect on yield and quality of blueberries [2,4]. These favourable environmental factors lead to increased nutritional value of blueberries, but also ensure a stable supply of berries for northern communities [4].

The numerous health benefits linked to blueberries come from the array of secondary metabolites contained in edible berries [7]. In general, genetic background determines the secondary metabolite profile of species, whereas environmental factors, such as light intensity and quality, nutritional status, water balance, can cause prominent qualitative and quantitative changes to the metabolite composition [24]. In *Vaccinium*, the phenolic and anthocyanin content is directly affected by genetic and physiological processes during fruit development and maturation, but their accumulation in stems, leaves, and berries is affected by light and temperature conditions in a species-specific manner [24].

The focus of this research was to determine the effects of shade on the phenology, beery yield, and berry chemistry of commonly occurring native northwestern Ontario blueberry species, V*accinium myrtilloides* and *V. angustifolium*. To achieve these objective transplanted plants were exposed to a range of experimental shade treatments and recorded plant phenological events, berry yield and berry chemistry.

Our specific objectives were to i) determine the effect of shade and near ground air temperature on phenological events such as vegetative and reproductive growth and ii) total anthocyanin and phenolic contents in berries. We hypothesized that i) deeper shade and lower soil and air temperature will delay vegetative and reproductive phenological events for both blueberry species, with milder effects in *V. myrtilloides* because of its shade tolerance, ii) increased shade will decrease berry yield and enhanced resource allocation to vegetative organs resulting increased leaf area, specific leaf area, and leaf dry matter content in both species, and iii) moderate shade and milder temperatures will result in the highest total anthocyanin and phenolic content, consequently increased antioxidant capacity.

2. Results

Figures 1 and 2 show vegetative and reproductive phenologies of *V. angustifolium* and *V. myrtilloides* respectively. The lines indicate periods during which the tagged vegetative and reproductive buds spent in indicated phenological stages. Vegetative buds, formed in 2021, were swollen by early-May 2022; emerging leaf buds were first observed on May 15; 50% leaves had unfolded by May 25 - 31, 2022. At the end of the growing season more leaves had reached maturity (full leaf unfolding) in both species. With an increasing shade the tagged vegetative buds spent more time in each phenological stage. Leaf development of both species peaked under 80% shade with 81% tagged buds reaching maturity for *V. angustifolium* and 87% for *V. myrtilloides*.

Both blueberry species flowered in late May and flowering stage was extended under 80% shade. Immature (green) fruit started emerging one week earlier (June 17) in *V. angustifolium* than *V. myrtilloides*, (June 24). For both species partial shade (30 and 50%) extends green fruit development period yielding more fruits than those in full sun and deep shade (Figs. 2**a**, **b**). Deep shade delayed flowering and produced less berries. For example, *V. angustiflium* under 80% shade started flowering on June 24; in open sun (0% shade) 87% of tagged flower buds produced mature fruits compared to only 33% buds producing fruits under 80% shade. For *V. myrtilloides* 86% tagged flower buds under 0% shade produced mature fruits compared to only 58% buds under 80% shade.

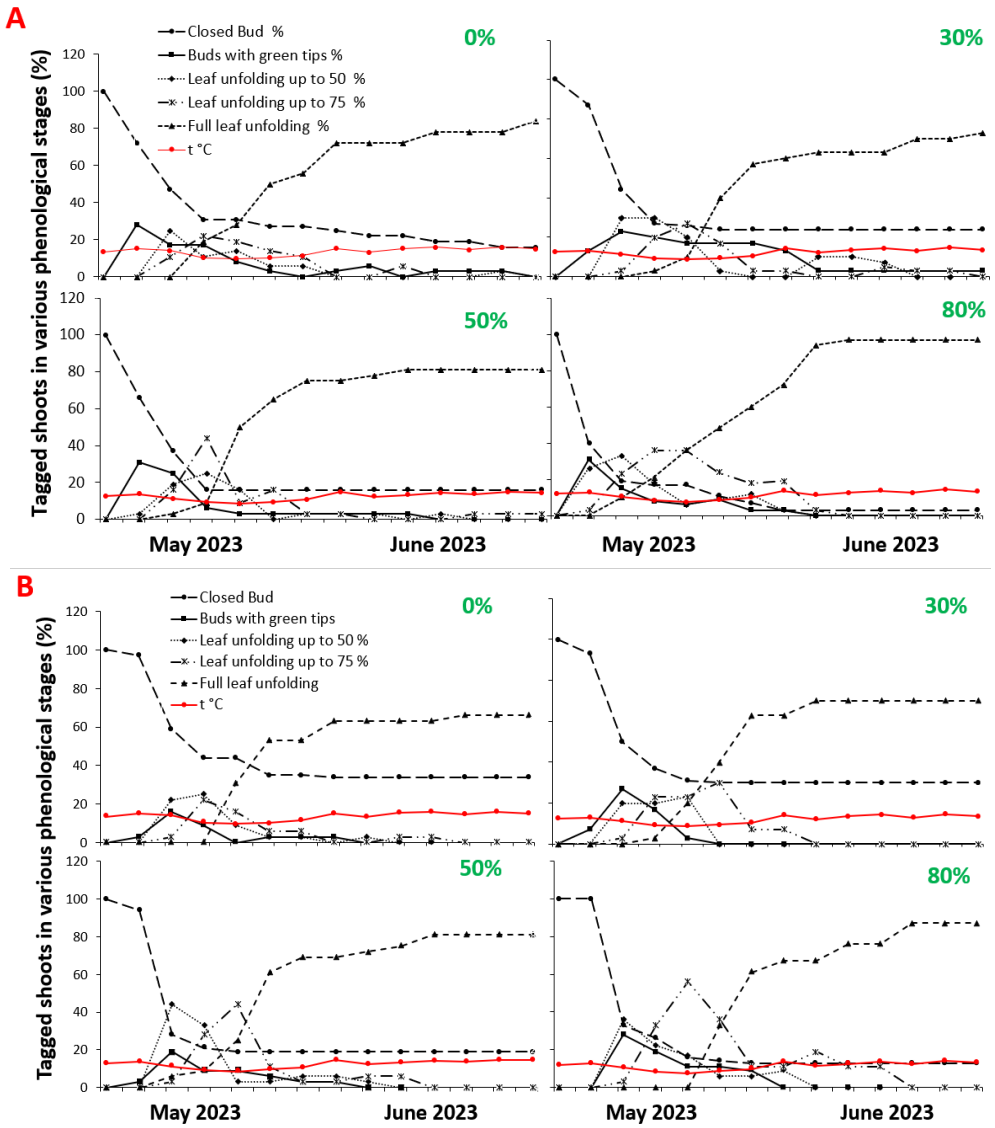

Figure 1. Phenological development of vegetative shoots of: (**a**) *V. angustifolium* and (**b**) *V. myrtilloides* under 0, 30, 50, and 80% shade treatments during the 2022 growing season.

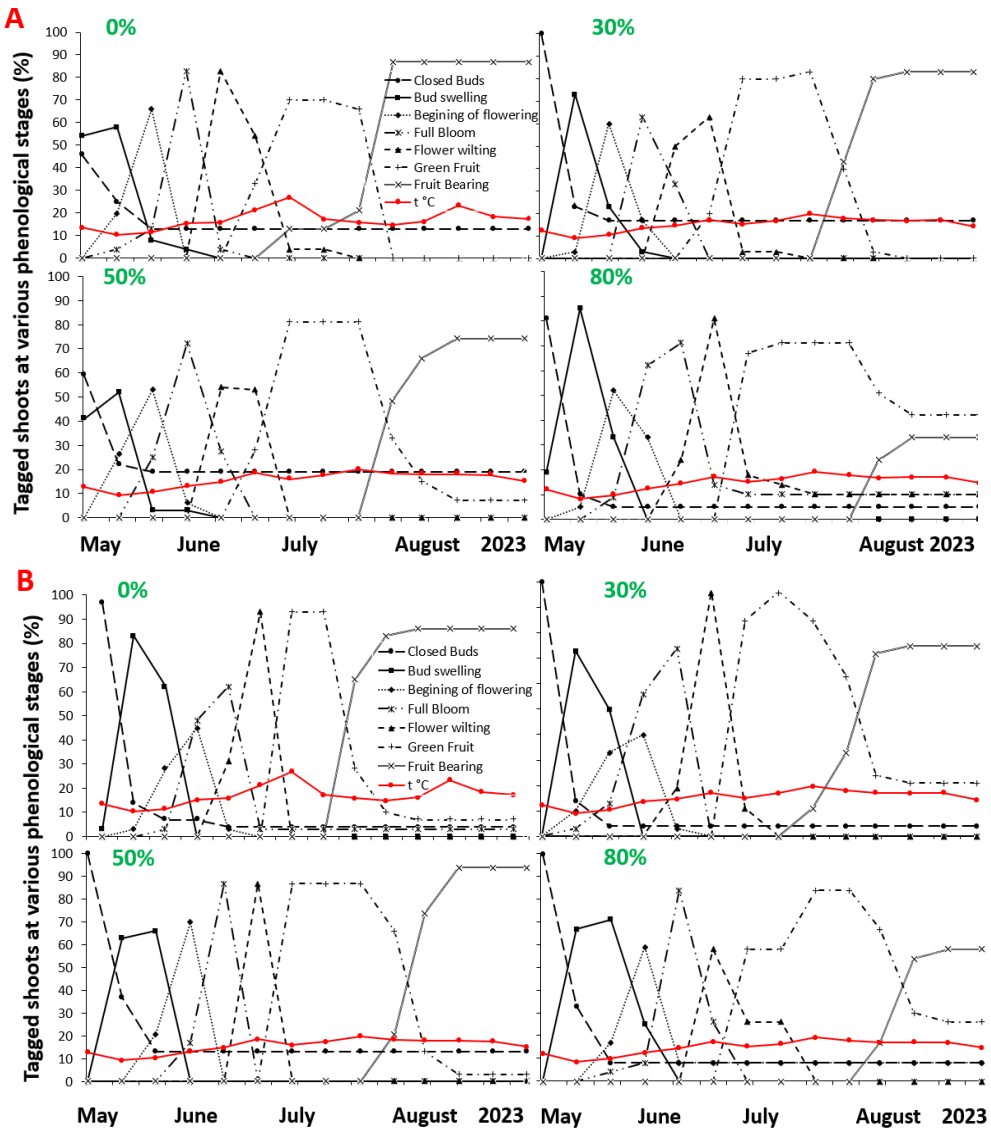

Figure 2. Phenological development of reproductive shoots of: (**a**) *V. angustifolium* and (**b**) *V. myrtilloides* under 0, 30, 50, and 80% shade treatments during the 2022 growing season.

An inverse relationship was observed between SLA and LDMC in both *Vaccinium* species, with increasing shade SLA steadily increased, while an opposite trend was observed for LDMC. Peak values for SLA occurred at 80% shade with 4.57 mm^2/mg in *V. angustifolium* and 5.48 mm^2/mg in *V. myrtilloides.* Conversely, peak values for LDMC occurred at 0% shade with 55.71 % in *V. angustifolium* and 57.07 % in *V. myrtilloides*. Across all shade levels *V. myrtilloides* had larger SLA (16.85%) and LDMC (9.12%) than *V. angustifolium*.

Both species showed an overall decrease in their reproductive index under increasing shade. *V. angustifolium* had a larger reproductive index than *V. myrtiloides*, peaking at 0 and 50% shade (Fig. 5).

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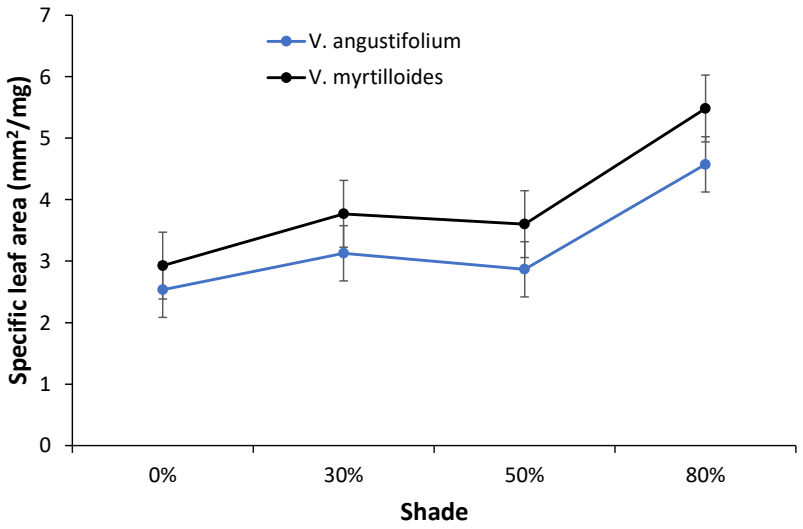

Figure 3. Change in specific leaf area of *V. angustifolium* and *V. myrtilloides* across shade treatments. The error bars represent the standard error in the data.

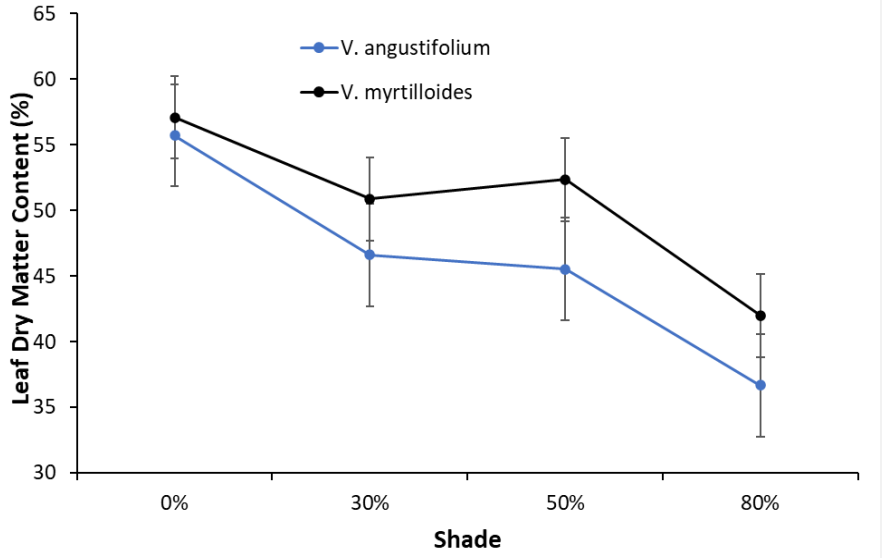

Figure 4. Leaf dry matter contents of *V. angustifolium* and *V. myrtilloides* across shade. The error bars represent the standard error in the data.

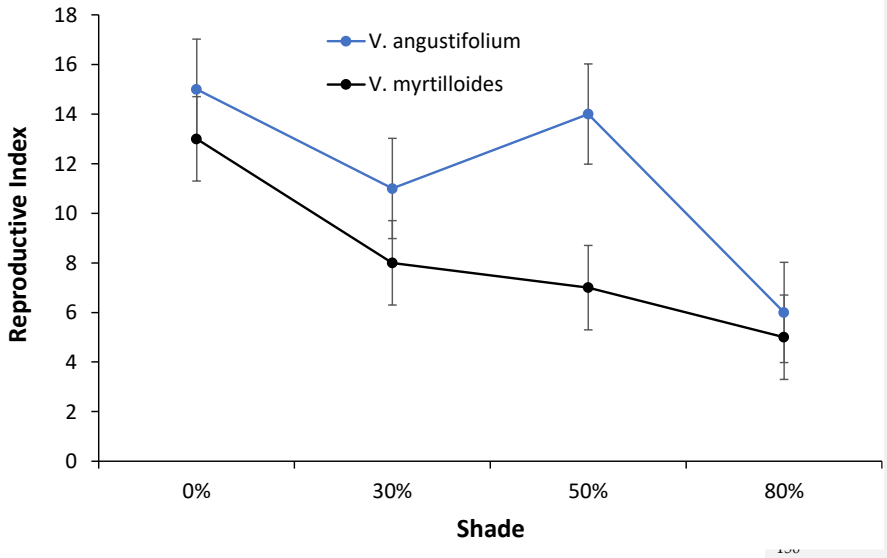

Figure 5. Berry yield index (berry fresh weigh, number, and size) of *V. angustifolium* and *V. myrtilloides* across shade treatments. The error bars represent the standard error of mean.

Phenolic content of *V. myrtilloides* peaked at 0% shade (594.87 mg), while *V. angustifolium* peaked at 30% (310.07 mg), both peaks falling within 12-14°C average seasonal air temperature (Table 1). *V. mytrilloides* berries had higher phenolic content across all shade levels. *V. myrtilloides* had a 59% decrease in total phenolic content under 80% shade compared to its peak under 0% shade (control). *V. angustifolium* showed a 20% decrease between its peak (30% shade) and 80% shade treatment. A similar trend was observed with air temperature, which decreased by 15% from 0% to 80% shade treatments.

Antioxidant activity of *V. myrtilloides* peaked at 0% shade (107.1 μmol Fe^{2+}/g FW), while that for *V. angustifolium* peaked at 30% (82.4 μmol Fe^{2+}/g FW). Both peaks fall within 12-14°C average seasonal air temperature (Table 1). *V. mytrilloides* berries had higher antioxidant activity (FRAP index) across all shade levels. Overall, with an increasing shade both species experienced a decrease in antioxidant activity. Antioxidant activity in *V. myrtilloides* decreased by 29% from no shade, where it peaked, to 80% shade, while *V. angustifolium* experienced a 19% decrease between 30% (its peak) and 80% shade treatments.

Table 1. Total phenolic content and antioxidant capacity (as FRAP assay index) of *V. angustifolium* (Va) and *V. myrtilloides* (Vm) with daily average air temperature (*t*) under four shade treatments.

Shade intensity %	Ground temp. (°C)	Total phenol content (mg GAE/100 g FW)		FRAP index (μmol Fe^{2+}/g FW)	
		Va	Vm	Va	Vm
0	13.5	221.4 ± 20.3[a]	594.9 ± 47.2[a]	21.1 ± 3.2[a,A]	107.1 ± 2.2[a]
30	12.4	310.1 ± 29.2	447.2 ± 8.0	82.4 ± 5.5[A]	80.8 ± 5.5
50	12.2	305.1 ± 54.8	403.3 ± 94.2	74.5 ± 15.3	75.8 ± 9.2
80	11.5	247.6 ± 36.4	331.9 ± 2.6	67.1 ± 15.7	75.9 ± 0.9

[1] Data presented as mean ± SD (n=3) per 100 gram of fresh weight

[2] The superscripts [a & A] denote significant difference ($p < 0.05$) between species and shade treatments respectively.

3. Discussion

This experiment provides a novel insight into the effects of shade treatments on two most common blueberry species in the absence of competition by revealing species-specific phenological response and fruit chemistry. The long-term implications for the vegetative and reproductive phenological delays (Figs. 1-2) and growth effects reported can lead to reproductive bottlenecks and overall decrease in blueberry plant's reproductive fitness. Delayed flowering mediated decreased reproductive growth and phenological mismatch between blueberries and their pollinators can have broader negative ecological impacts causing lower supply of wild blueberries for wildlife species and indigenous peoples in NW Ontario [4].

Our findings of increased shade and decreased air temperature effect on vegetative and reproductive phenology and growth are consistent with previous results [24]. Increased shade led to more leaf buds reaching maturity across all shade levels, accompanied by larger leaf growth (LDMC and SLA), where *V. myrtilloides* experienced more positive effects across all shade levels. This difference could be attributed to species-specific phenotypic plasticity with some genetic effect as an adaptation to varied light and temperature conditions enabling it to be more shade tolerant [25]. It is generally believed that the plastic response of SLA enables plants to maintain higher performance under shade which often results in thinner leaves with lower LDMC [21,26]. This ensures sufficient light capture per gram of leaf tissue and maximizes mass-based photosynthesis [21]. In turn, the delayed flowering, observed in both species with effects generally more prominent in *V. myrtilloides*, can be attributed to differential carbon partitioning by the species,

partially driven by their genetic differences [24]. When faced with limiting environmental conditions (low temperature and light) plants employ resource partitioning between reproductive and vegetative meristems, where a higher vegetative growth (number of leaf buds) tends to delay flowering [24]. It appears that lower ground temperature under increased shade directly led to prolonged fruit maturation in both blueberry species, with *V. myrtilloides* experiencing more dramatic effects. These effects may be indicative of *V. myrtilloides*'s ability to yield berries for a longer period upon canopy closure compared to *V. angustifolium* [24]. Fruit development and maturation are also sensitive to microclimatic conditions. Fruit growth is primarily driven by temperature, which controls cell division and cell elongation [16]. Increased shading of blueberry results delayed fruit development and above 60% shade hinders photosynthetic ability and growth [27]. Considering that timing of reproductive events implies a trade-off between vegetative and reproductive growth, it suggests that increased shade also prompts both to respond to unfavorable conditions by partitioning resources between vegetative and reproductive growth [28].

Both blueberry species had a general decrease in seasonal reproductive growth (fresh berry weight, number, and size) with increasing shade, with *V. myrtilloides* having reduced berry yield across all shade levels (Fig. 5) Due to an increase in shade and decrease in air temperature late flowering blueberry plants may have decreased fruit development due to a shortened flowering and fruiting period affecting their reproductive growth [28]. In addition to a shortened available fruit development period this decrease can be attributed in part to phenological mismatch and pollination time between the blueberry species and their primary pollinators. Blueberry plants require insects, predominantly bees, to cross-pollinate flowers. Greater bee visitation to flowers forms larger fruits that ripen earlier and more evenly [29].

In a recent study Khan (2014), reported a similar trade-off between vegetative and reproductive growth at 50% shade [41]. The reversal of energy allocation in both species observed in both experiments can be attributed to both lower temperatures with increasing shade but also the absence of inter-species competition. In the same study, in the common garden experiment, *V. myrtilloides* was observed to yield more berries with increasing shade as compared to *V. angustifolium*. This directly contradicts the results of this study, where an opposite trend was observed (Fig. 5). The enclosed design of the shade structures used in this experiment prohibited pollinators easy access to the plants, moreover it created lower air and temperature conditions. Considering that the flowering was observed earlier in *V.angustifollium* (Figs. 1-2), coincided with peak activity of *Vaccinium*'s primary pollinators, the bees, more of its flowers were pollinated than *V. myrtilloides* leading to larger yield [42]. These two factors could have contributed to the reversal of reproductive growth trend observed in Khan's experiment [41].

Members of the *Vaccinium* genus are known for their high content of phenolics, which are highly dependent on light intensity, temperature, developmental stage, and species genetic profile [23,30]. In this experiment, both species showed decreased total phenolic content and antioxidant capacity with increasing shade, with *V. myrtilloides* peaking at 0% and *V. angustifolium* peaking at 30%. Literature reports optimal shade levels of 30-50%, with average seasonal temperatures between 12-16 °C for maximum antioxidant compounds production and accumulation in high bush blueberries (*V. corymbosum* L.) [2,31]. Both *V. corymbosum* and *V. angustifolium* are tetraploid with 48 chromosomes, while *V. myrtilloides* is a diploid with 24 chromosomes [24,32]. Thus, the decreased phenolic content and antioxidant capacity of *V. angustifolium* under 0% shade could be attributed to species genetic trait (ploidy-level).

Mallik and Hamilton (2017) reported that between the two genotypes of blueberries *V. myrtilloides* have higher total phenolics, as was found in this experiment [4]. The overall higher content of total phenolic and antioxidant activity in *V. myrtilloides* may be a result of genotypic difference in their ability to synthesize phenolic compounds [33]. This could also be attributed to the difference in average berry size between the two species. Delayed flowering phenology and decreased pollination may lead to production of smaller berries

in *V. myrtilloides* than *V. angustifolium* [24]. Smaller berries have a higher average surface area per gram of fresh weight. Considering that antioxidant compounds are largely stored in the berry skin, smaller *V. myrtilloides* berries would yield larger amounts of these compounds [34].

To maximize horticultural yield and health benefits these blueberry species may be grown in high light, temperate conditions, where 30% shade is optimal for *V. angustifolium* and 0% shade for *V. myrtilloides*, both within 12-16 °C air temperature. These results contradict the trends shown by Khan (2014) in their common garden experiment [41]. In the boreal forest, post-fire and post-harvest competition-free time would allow for optimal growing conditions for these species. For the reproductive benefit of the two blueberry species as well as their primary consumers (forest wildlife and recreational berry pickers) it is imperative that forest fires are not completely suppressed to continue the natural regeneration of blueberries and nutritious food supply. In absence of fire forest harvesting by clear cuts can benefit blueberry production.

This study provides novel insights into shade and near ground temperature effects of the northwestern Ontario blueberry species under controlled shade treatment. The uniform shade provided by the shade structures does not accurately imitate the variable and dynamic shade created by the boreal forest canopy under natural conditions. Furthermore, separation of the individual plants removed the effects of inter and intra species competition and possible niche differentiation observed in nature [43]. Future studies should focus on assessing the effects of shade on *V. angustifolium* and *V. myrtilloides* under field conditions [15]. It would be worthwhile to further investigate the phenological responses of blueberries to humidity, pollinators, and spectral characteristics of light in controlled and field conditions. Measuring photosynthetic activity under different shade treatments would help determine the adaptive plasticity traits, such as SLA and LDMC, to variable shade.

4. Materials and Methods

4.1 Shade treatment

Three wooden shade structures were constructed during late April 2022, consisting of a wooden base (244 cm x 244 cm x 152 cm) and dome-shaped canopy made of PVC pipe. Horticultural shade cloths (Black Greenhouse Shade Cloth 30%-80%, Greek-tek, Inc., Janesville, WI, USA) of select fabric density were used to create 30, 50 and 80% shade treatments. Unshaded space near the shaded domes was used to place control blueberry plant pots. The structures were placed strategically, away from buildings or trees. The horticultural cloth was placed to avoid overlapping or gaps.

4.1.1 Blueberry transplants in pots

In September 2021, 50 ramets (bushes) of *Vaccinium angustifolium* and *V. myrtilloides* were selected based on uniformity of size and transplanted into 5-gallon black plastic pots with 5 cm native mineral soil at the bottom and mulched with *Pluerozium schreberi* moss from a six-year-old clearcut site in Nipigon area (49°15'38.8" N 88°22'41.3" W). The transplanted plants were once every three days to field capacity until late autumn freezing.

4.1.2 Phenology and temperature logging

In April 2022, ten blueberry containing pots of each species were placed under 0, 30, 50 and 80% shade. Ground temperature was measured hourly from May 10 to September 28, 2022, using TEROS 11 + TEROS 12 (METER Group Inc., Pullman, WA, USA) temperature probes on soil surface connected to Z6L Basic and Em50 data loggers. Soil moisture was maintained at 70% by watering the pots to field capacity every three days.

The phenology of each plant was recorded following the method of Fournier *et al.* (2020) [24]. Before the growing season began, 6 shoots per plant, 3 vegetative and 3 reproductive, were randomly selected and marked with flagging. The shoots were selected based on their viability and their distance from the surface of the soil (> 5 cm). The phenology of all marked shoots was recorded every 3 days from May 10 to September 28, 2022, according to a 6-stage development protocol.

4.1.3 Blueberry seasonal growth and berry yield

Five randomly selected, mature leaves with petioles were collected from each plant on August 24, 2022, and scanned by HP Smart app in 1200 DPI and their average leaf area quantified using WinFOLIA Basic software (Regent Instruments Inc.). Fresh and dry weight of the leaves were measured using an analytical scale. The leaves were dried at 35°C for 72 hours using VWR® Signature™ Forced Air Safety Oven. Leaf dry matter content (LDMC) was calculated as a ratio of leaf dry mass and leaf fresh mass. Specific leaf area was calculated as a ratio of leaf area to leaf dry mass.

Throughout the season, blueberries from each pot were collected individually as they fully ripened, and their fresh weight determined. Mean fruit size was determined as a function of the total number of berries per pot divided by their fresh weight. Fruits were stored in a freezer at -18°C until chemical analysis. The reproductive (berry) yield of both *V. angustifolium* and *V. myrtilloides* during the growing season were expressed as a reproductive index calculated from berry fresh weight, number, and size per pot. It was created by assigning a score to each of three parameters. Each point indicated ten units of increase, either fresh weight (g), # of berries, or g/berry respectively. The points for three parameters were summed up to yield the "Reproductive Index" score.

4.2 Chemical analysis

4.2.1 Blueberry extraction

Solvent extraction was done using methanol, distilled water, hydrochloric acid at a ratio of 70:30:1 (organic solvent:water:acid, v/v/v) as an extraction solvent [35]. Blueberry samples (700 mg) was lyophilized and mechanically grinded, followed by adding 7mL extraction solvent. The mixture was homogenized on ice for 4 min [36], then centrifuged at 20,000g for 20 minutes at 4°C [9,35]. The supernatant was collected, and the extraction repeated with the remaining pellet.

4.2.2 Total phenolic content

Singleton and Rossi (1965) method were used to determine the total phenolic content of blueberry samples [37]. Gallic acid (3,4,5-trihydroxybenzoic acid) was used as standard. The calibration curve consisted of 100, 250, 500, 750 and 1000 μg/ml Gallic acid solutions. Each standard and blueberry extract (diluted tenfold) were reconstituted with 5.0 ml 2 N Folin Ciocalteu reagent (diluted tenfold). After 5 min 4.0 ml sodium carbonate solution (75 g/l) was added, the solution was shaken, closed, and stored in dark at room temperature for 30 minutes. Absorbance was measured at 765 nm at 25 °C using BioTek Synergy H1 reader.

4.2.3 Total monomeric anthocyanin

The pH differential method as described by Mallik & Hamilton (2017) was used to determine the total anthocyanin content of blueberries [4]. Using the extinction coefficient of cyanidin-3-glucoside (29,600g) the total anthocyanin content of each blueberry sample was expressed as Cy-3-glu equivalents mg/100g fresh weight [38]. DDW was used as instrument zero (Mallik & Hamilton, 2017) [4]. Absorption of equally divided blueberry extracted reconstituted with 1) pH 1.0 buffer (0.025 M potassium chloride) and 2) a pH 4.5 buffer (0.4 M sodium acetate) at a 1:12 ratio was read within 5 minutes after preparation at 520 and 700 nm using BioTek Synergy H1 reader. Turbidity (haze) was corrected for using absorbance at 700 and 510 nm [39].

4.2.4 Ferric reducing antioxidant power assay (FRAP)

Direct measure of antioxidant activity in blueberry samples was done using microplate ferric reducing antioxidant power assay (FRAP) described by Bolanos de la Torre *et al.* (2015) [40]. Calibration curve consisted of 1000, 2000, 3000, 4000, 5000 μmol/L Fe^{2+} standards prepared daily. DDW was used as instrument zero. For microplate FRAP assay sample solution (10 μl), DDW (10 μl), and working FRAP solution (280 μl) were added directly to the 96-well microplate. The plate was shaken, incubated at 37 °C in the dark for 30 min after which the absorbance was read at 593 nm [40].

4.3 Data analysis

Data analysis was performed using a combination of R (version 4.2.0), Paleontological statistics software for analysis and education (version 4.11), and Microsoft Excel (version 2212). Ground temperature readings were averaged in Excel using the '=AVERAGE' function, and daily maximum and minimum temperatures were calculated using '=MAX(array)' and '=MIN(array)' functions respectively.

The phenological data analysis was performed manually. For vegetative phenology the phenological changes were summed every 3 days from May 11 - June 18, 2022. Reproductive phenology changes were summed on a weekly basis from May 11- September 3, 2022.

Statistical analysis using two-way ANOVA was performed on blueberry fresh weight (g), berry size (g/berry), berry number per pot, leaf area, SLA, and LDCM in the four shade treatments to determine if there was a significant difference in the vegetative growth or yield parameters between *V. myrtilloides* and *V.angustifolium*.

Data on shade effect on blueberry chemistry (total phenol, anthocyanin, antioxidant activity) were analyzed using two-way ANOVA. The differences between means were evaluated by Kruskal-Wallis test ($p < 0.05$) using the R Studio software version 4.2.0. The results of all determinations were reported as the means ± standard deviations.

5. Conclusions

Increased shade conditions lead to changes in microclimate, evidenced by decrease in average air temperature. Together they contributed to shifts in reproductive and vegetative phenology of *V. angustifolium* and *V. myrtilloides* leading to difference in resources causing decreased berry yield and berry antioxidant properties; deeper shade producing pronounced. These plastic responses are a direct consequence of the plant's effort to maintain optimal activity under decreased light conditions. To maximize horticultural productivity, berry nutritional value, and ecological benefit *V. myrtlloides* may be grown in 0-30% shade and *V. angustifolium* in 30-50% shade in competition-free environments. To fully understand the scope of species-specific phenological responses and adaptive plasticity to changing light and light-related conditions further investigation simulating field conditions should be conducted.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: Vegetative phenological stages of *Vaccinium* sp. (Fournier *et al*., 2020); Figure S2: Floral phenological stages of *Vaccinium* sp. (Fournier *et al*., 2020); Figure S3: Fruit phenological stages of *Vaccinium* sp. (Fournier *et al*., 2020); Table S1: Phenological data for tagged vegetative and reproductive shoots of *V. myrtilloides*; Table S2: Phenological data for tagged vegetative and reproductive shoots of *V. angustifolium*; Table S3: Hourly air temperature under 0% shade treatment from May 10, 2023 to June 22, 2023; Table S4: Hourly air temperature under 30% shade treatment from May 10, 2023 to June 22, 2023; Table S5: Hourly air temperature under 50% shade treatment from May 10, 2023 to June 22, 2023; Table S6: Hourly air temperature under 80% shade treatment from May 10, 2023 to June 22, 2023.

Author Contributions: Conceptualization, AUM; methodology, AUM, V.D; validation, AUM; formal analysis, V.D.; investigation, V.D., A.U.M.; resources, A.U.M.; data curation, V.D.; writing—original draft preparation, V.D.; review and editing, V.D., A.U.M.; supervision, A.U.M.; project administration, A.U.M.; funding acquisition, A.U.M.; Both authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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