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Functional Diversity in Blueberries and Their Responses to Extreme Drought

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FUNCTIONAL DIVERSITY IN BLUEBERRIES AND THEIR RESPONSES TO EXTREME DROUGHT

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An Abstract of the Thesis Presented

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Climate change is expected to lead to novel climate conditions with an increase in frequency and severity of drought across many places around the globe including the north-eastern (NE) United States. Therefore, experimental studies that test the impacts of changing environmental conditions over long time scales or experimental studies that mimic these conditions are crucial to understand the potential impact on crops in this region. Wild lowbush blueberries and highbush blueberries are two important crops in NE USA. In this study, the leaf functional, structural, nutrient traits across genotypes of wild blueberries (*Vaccinium angustifolium* and *V. myrtilloides*) at Blueberry Hill Farm, Jonesboro, Maine were monitored across two crop growth cycles for four years and were related to changing environmental conditions. Additionally, I investigated how four blueberry population- varieties (two *V. angustifolium* populations and

two *V. corymbosum* varieties) respond to extreme experimental drought conditions to reveal the physiological mechanisms regulating their drought responses.

The results showed that wild blueberries showed strong variation both within and across genotypes in leaf structure, physiology, and nutrient status. The variation could be explained more by intra-genotype variance than by inter-genotype variance. Comparing their leaf economic spectrum (LES) traits to the Glopnet (a global dataset of plant leaf traits), the blueberries fell within the domain of Glopnet species, but global LES relationships were not always found. Also, I found that these two species showed similar or higher values across most traits compared to *Vaccinium* species in the Glopnet. Further, a principal component analysis (PCA) with all leaf functional, nutrient, structural traits, soil properties, rainfall and temperature showed overlaps in the soil nutrient requirements but clear separation in leaf nutrient, structural traits, physiological traits, and rainfall. Therefore, there was a clear differentiation in water and nutrient use between these two species and temporal variation in environmental conditions also shifted the traits. These findings can help us to predict how these species will respond to future climate change, and how changes in environmental conditions will shape the trait development and coordination, as well as the community composition.

In the drought experiment, the two lowbush populations (Ang 1 and Ang 2) and two highbush varieties (Bluecrop and Patriot) showed a coordinated response of all the physiological processes including stomatal conductance, photosynthesis rate, transpiration rate, photochemistry, and plant hydraulic systems under declining stem water potential (Ψ_{stem}; a measure of water tension within the plant) and soil moisture conditions. Notably, there were quick declines in stomatal conductance, photosynthesis, and water loss before the turgor loss point (TLP) and the progressive decline of photochemistry, leaf browning, and leaf dropping

after the TLP as Ψ_{stem} and soil moisture declined across all population- varieties of blueberries and reached -4.0MPa to -4.5MPa Ψ_{stem} and less than 5% soil moisture at the end of the drought treatment. Importantly, physiological processes, for example, F_v/F_m in Ang 2 and Patriot declined more quickly compared to Ang 1 and Bluecrop during the drought treatment. Ang 1 and Patriot showed 100% loss of hydraulic conductivity (PLC), while the Ang 2 and Bluecrop reached 87% and 83% PLC at the end of the 4-week-long drought. Ang 1 and Ang 2 populations had high regrowth of new stems from underground rhizomes in the following season, indicating the resilience of wild lowbush blueberries. All groups showed high stem mortality when water potentials were as low as -4.0MPa to -4.5 MPa, indicating that these population- varieties are vulnerable to extreme drought. The results of this study not only allowed us to understand the drought responses of these population- varieties but also allowed us to understand the turgor loss point as a threshold beyond which damages in photochemistry, leaf shedding, hydraulic failure, and plant mortality occur. In the blueberry fields, blueberry population- varieties may respond to drought in different ways especially for *Angustifolium* populations. The wild blueberry populations in the field conditions might show higher resistance compared to potted plants because of their large rhizome systems in the field. Therefore, the findings from this study could be further tested at larger scales in the field.

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TABLE OF CONTENTS Page

LIST OF TABLES Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

MPa	Megapascal
NE US	Northeastern United States
PLC	Percent loss of conductivity
RCBD	Randomized complete block design
SMA	Standardized major axis
PCA	Principal Component Analysis
LS	Leaf Size
LMA	Leaf mass per area
A_a	Light-saturated CO2 assimilation per leaf area
g_s	Maximum stomatal conductance
Alg	Water use efficiency
A_m	Light-saturated CO2 assimilation per leaf dry mass
A_N	Light-saturated CO2 assimilation per nitrogen
A_P	Light-saturated CO2 assimilation per phosphate
Chl_m	Chlorophyll concentration per mass
PH	Plant height
SD	Stem diameter
F_v/F_m	Maximum photochemical efficiency of PSII
A_{midday}	Midday photosynthesis rate
Midday g_s	Midday stomatal conductance
E	Midday transpiration Rate
*K*plant	Plant hydraulic conductance
Predawn Ψ_{stem}	Predawn stem water potential
Midday Ψ_{stem}	Midday stem water potential
Ψstem	Stem water potential
TLP	Turgor Loss Point

CHAPTER 1: ECOLOGICAL NICHES AND LEAF ECONOMIC SPECTRUM ACROSS GENOTYPES OF WILD BLUEBERRIES IN A SEMI-NATURAL AGRICULTURAL SYSTEM

1.1 ABSTRACT

Wild blueberries are an important crop to the state of Maine commercially and culturally. They are characterized by high inter-genotypic variation seen both within and across two main species *Vaccinium angustifolium* and *Vaccinium myrtilloides,* which have been coevolving for thousands of years. These wild blueberries species spread by rhizomes underneath the soil but above ground appear as a mosaic of individual genotypes, which differ in biological traits, like height, color and hue, phenology, and yield. Despite the likely impact that genotypic variation has on structural and physiological traits, we know very little about the natural variation and range of these characteristics. It is also unknown whether their leaf trait development follows the global leaf economic spectrum (LES) principles, and how changing environmental conditions are shaping their variability and the coexistence of the two species. To address these questions, leaf functional traits across genotypes of *V. angustifolium* and *V. myrtilloides* were measured at Blueberry Hill Farm, Jonesboro, Maine for two crop growth cycles across four years. Wild blueberries showed strong variations both within and across genotypes in leaf structure, physiology, and nutrient status. The overall variation was explained more by intra-genotype variance rather than inter-genotype variance. I also found that the functional traits of blueberries fell within the domain of Glopnet (a global dataset of plant functional traits including mainly angiosperms), but global LES relationships were not always found. Similarly, I found that the two studied species showed similar or higher values in most traits compared to *Vaccinium* species in the Glopnet. Further, a principal component analysis (PCA) of all plant traits and environmental conditions showed that the two species showed overlaps in soil nutrient requirements but a clear separation in leaf nutrient, structural traits, physiological

traits. Also, changes in environmental conditions over the years were shifting the traits. Therefore, clear differentiation in water and nutrient use was found between these two species and the temporal variation in environmental conditions also shift the traits. These findings can help us to predict how these species will respond to future climate change, and how changes in environmental conditions will shape the trait development, and the community composition.

1.2 INTRODUCTION

Leaf economic spectrum or LES (Westoby et al., 2002; Garnier & Navas, 2013; Wright et al., 2004) is widely used to identify the manifold of strategies within and among communities (Falster et al., 2012). LES characterizes a strategy for a fast or a slow rate of return on a carbon investment in leaves (Reich et al. 1997; Wright et al. 2004) and reflects a trade-off between long leaf lifespan and high photosynthetic rate. Trait variations can be found at all spatiotemporal and organizational scales: within a single organism (Pigliucci, 2001), within a species (Valladares et al., 2000; Takahashi et al., 2005; McGill et al., 2006; Rozendaal et al. 2006), among species (Wright et al., 2001; Westoby et al., 2002), and among communities (Ackerly et al., 2002; Wright et al., 2004; Rozendaal et al., 2006; Ackerly & Cornwell, 2007). These trait variations also follow some well-known LES trait relationships at the global scale (Wright et al., 2004), in different plant lineages (Zhang et al., 2015; Mason et al., 2015; Edward et al., 2014; Bai et al., 2015; Martin & Issac, 2021), and certain geographical regions (Asner et al., 2016; Hu et al., 2015; Pan et al., 2020; Wright et al., 2005). However, the LES trait relationships in uniform agricultural systems and semi-natural agricultural systems are relatively not well-understood (Xiong & Flexas, 2018; Martin et al., 2018).

The wild blueberry agricultural system is a unique and semi-natural agricultural system with diverse genotypes naturally growing in the field and managed to promote high yield. The two common species of wild blueberry typically found in managed fields in North America are sweet blueberry *Vaccinium angustifolium* Aiton and the sour top or velvetleaf blueberry *Vaccinium myrtilloides* Michx (Hall et al., 1979). In this system, inter-genotype variation is highly pronounced of the two co-occurring species (Bolnick et al., 2003), which have been coevolving for thousands of years (Borns, 2004; Drummond et al., 2009). These two species have a unique growth habit and life cycle (Bell et al., 2009) i.e., they grow vegetatively by rhizome and have a two-year production cycle alternating between a crop year (reproductive

year) and a prune year (vegetative growth year). Also, these two species coexist in most of the managed fields in Maine, which I here refer to as a semi-natural agricultural system. I examined the leaf functional traits of these two species through two crop growth cycles over four years to understand the spatial and temporal variation of the highly diversified wild blueberry system.

Functional traits can indicate how an individual relates and responds to its environment, which offers a powerful approach to address ecological questions (McGill et al., 2006). Managed wild blueberry fields show highly pronounced inter-genotype and intra-genotype variations within each species (Vander Kloet, 1978; Smagula et al.,1997). Thus, this is a good system to study both different scale processes in shaping community composition and how ecosystem function responds to environmental conditions (Lavorel & Garnier, 2002; McGill et al., 2006; Westoby & Wright 2006). Although it is well-known that the wild blueberry fields have a high phenotypic diversity, the physiological diversity of these wild blueberry species are yet unknown. Nor do we know whether the variation in traits in this unique system would be constrained by similar LES principles as in other angiosperms (Wright et al., 2004). Studies of LES traits and different functional traits in this wild blueberry system may reveal unique patterns in trait correlations and how LES in a semi-natural agricultural system differs from the global LES.

A niche is defined by the set of conditions, resources, and interactions that a species needs to carry out its ecological role (Miller & Spoolman, 2009). Each species fits into an individualistic ecological community and has its own tolerable ranges for many environmental factors. Two co-occurring species in the same environment that often show substantial niche overlap (Berdugo et al., 2018; Mahdi, Law, & Willis, 1989) are also able to coexist if they are nearly equivalent in their average competitive abilities (Pastore et al., 2021), if they have close-to-equal performance in the same environment (Hubbell, 2001), and/or if they partition resources

as they grow (Chesson, 2000). However, the competition between the two co-occurring species can also be doubled if they have high niche overlap, limited resources, and/ or large competitive differences (Simberloff et al., 1991). A community characterized by low niche overlap and small competitive differences is more resilient to change than a community with high niche overlap or large competitive differences (Pastore et al., 2021). Unfortunately, these concepts of niche overlap, species coexistence, and competition have not been applied in wild blueberry systems. We also do not know how the two species of wild blueberry partition their resources, their relative competitive advantages, the extent of their resource overlaps and differences, and the mechanism for their coexistence in terms of convergence or divergence in functional traits and resource use.

Environmental filtering is also one of the key community assembling processes that constrain species establishment through the selection of functional traits (Diaz et al., 1998). During this process, habitats act as filters removing species lacking trait attributes for persisting under a given environment (Keddy, 1992). Precipitation and temperature also act as strong environmental filters by driving the differences in vegetation and between biomes (Grime et al., 2006; Ordonez et al., 2009) that constrain the number of successful trait combinations and lead to community-level trait convergence (Bruelheide et al., 2018). As a result, co-occurring species in a given habitat exhibit similar ecological strategies and share similar traits (Cornwell et al., 2006), leading to the trait convergence of co-occurring species and shaping community structure (Lavorel & Garnier, 2002; Lebrija-Trejos et al., 2010). Investigating trait environment linkages or consistent associations between sets of plant attributes and certain environmental conditions could provide insights into the mechanisms of species coexistence and species distribution (Keddy, 1992). The role of local-scale environmental filters in shaping wild blueberry community composition and structure and how wild blueberry respond to shifting environmental condition is not well-understood.

The wild blueberry is an important crop to the State of Maine culturally and economically. Therefore, how these species respond to changing environmental conditions is important to consider for future management of these wild blueberry fields and to understand species coexistence and competition behavior. Importantly, wild blueberries are a two-year cycle crop with a vegetative growth and a crop year, which is very unique among crop species aside from biennial bearing plants. Therefore, how wild blueberry species differ in their crop growth cycles is important for us to understand from the management point of view. Additionally, most studies have focused on studying LES at large or global spatial scales and with a large number of species, but very little attention has been given to LES studies at local scales in agricultural systems. Therefore, there is limited knowledge on how the LES relationships at local scales in a farm setting differ from those found globally. Here, I aimed to assess for blueberry species the traits associated with photosynthetic rate, nutrient composition, and leaf structure based on studies of angiosperms for four years on a farm. Our objectives were to 1) to test whether the LES principles hold in the unique blueberry system where there is high inter-genotype and intra-genotype variations, 2) to examine whether the two coexisting species show convergence in functional traits or divergence in niches in the field, 3) to test whether the leaf functional, nutrient and structural traits shifts over years due to changes in environmental conditions, and 4) to understand whether LES traits in crop year differ from those observed in prune year. This study can help us understand whether the two coexisting species show convergence in traits or have different sets of resource availability.

1.3 MATERIALS AND METHODS

1.3.1 STUDY SITES AND PLANT MATERIALS

This research was carried out on Blueberry Hill Farm in Jonesboro (latitude of 44.6439° N, longitude of 67.6465° W, elevation 257 meters), Maine, USA. The study site has a yearly average temperature of 6.3^{o}C and yearly average precipitation of 1297.94 mm (USCD, 2021)

typified by large seasonal temperature differences, with warm summers and cold winters. The two species of wild blueberries (*V. angustifolium* and *V. myrtilloides*) were used under our study for four years from 2017 to 2020 for the measurement of physiological and morphological traits. Both of these species were in the prune (vegetative growth) phase in 2017 and 2019, and the crop (reproductive) phase in 2018 and 2020. According to climatic conditions of the growing season from the month of May to August, 2017 was an extreme drought year with precipitation of 11.83 mm, 2018 was a moderate year with precipitation of 8.72 mm, 2019 was an extremely wet year with high precipitation of 16.6 4mm, and 2020 was a drought year with precipitation of only 7.95 mm (Table A1.6). These climatic condition data were obtained from a weather station that was installed in Blueberry Hill Farm Research Station, Jonesboro, Maine. All the measurements under our study were carried out from May to August in all four years.

The genotypes of *V. angustifolium* and *V. myrtilloides* studied were selected randomly in 2017 and 2019 and the same genotypes were studied for all the years. The way I selected these genotypes was by separating the farm into a number of grids and generating random numbers to pick the grids for sampling. In the 2017- 2018 crop growth cycle, 12 genotypes (6 for each species) were studied, while four more were added in the 2019- 2020 crop growth cycle and increased the sample size leading to a total of 16 genotypes (8 from each species). The 16 genotypes showed high variation in terms of phenological characteristics and morphological features such as leaf and fruit color, taste, and fruit yield. Variation in environmental conditions and soil nutrients among genotypes were also considered. Six stems were selected for measurements and marked with a ribbon for each genotype. The middle section of the leaves was used for physiological measurements such as gas exchange and chlorophyll concentrations and all other morphological, phenological, and physiological measurements were taken using the marked stems and their branches.

1.3.2 LMA, LEAF SIZE, TOTAL LEAF AREA, AND LEAF THICKNESS

Morphological data like plant height was recorded for the main stem from 2 cm above the ground to its top excluding the winter injury section and the stem diameter was recorded from a stem section where 2 cm was marked above the ground surface for six marked stems with a ribbon from each genotype. For leaf size measurement, all fully mature and healthy leaves from six different plant stems across all genotypes were collected from the stems that were not marked with a ribbon. The total area of leaves from each individual stem was determined by a leaf area meter (LI-3000A area meter, Li-Cor, Lincoln, NE, USA) and leaf size was calculated by dividing total leaf area by total leaf number. For Leaf Mass per Area (LMA) measurements, six matured healthy leaves from six individual stems from each genotype were measured for leaf area by the leaf area meter (LI-3000A area meter, Li-Cor, Lincoln, NE, USA) then the leaves were oven-dried at 70°C for 48 hours, and weighed, and LMA was determined as leaf dry mass divided by leaf area (gm^{-2}). Leaf thickness was measured using a digital micrometer (Mitutoyo, digital micrometer, 0.0001mm accuracy).

1.3.3 GAS EXCHANGE MEASUREMENTS

The light-saturated net CO_2 assimilation rate per area (A_a, µmol m^{-2} s^{-1}) and stomatal conductance (g_s; µmol m^{-2} s^{-1}) were measured using a portable photosynthetic system LI-6400 (Li- Cor, Lincoln, NE, USA) in the year 2017 and 2018 and LICOR-6800 (Li-Cor, Lincoln, NE, USA) in year 2019 and 2020. The leaves were measured on sunny days between 08:30 and 10:30 h solar time subjected to 10 minutes of a constant light intensity with the photosynthetic flux density (PPFD) at 1000 µmol m^{-2} s^{-1}, ambient CO_2 concentration, and temperatures. Relative humidity ranged from 40% to 63% during the measurements. All the photosynthetic parameters were expressed on a projected leaf area basis. Light-saturated net CO_2 assimilation rate per mass (A_m; µmol g^{-1} s^{-1}) was determined as A_a divided by LMA. Intrinsic water use efficiency was calculated by dividing the light-saturated CO_2 assimilation per leaf area by stomatal conductance (Hatfield & Dold, 2019).

1.3.4 NUTRIENT AND CHLOROPHYLL CONCENTRATIONS

For leaf nutrient concentrations, ~40-60 fully mature and healthy leaves from each genotype were collected during August, and oven-dried at 70°C for 48 hours. After oven drying leaves were ground into a fine powder and sent to the University of Maine Soil Testing Laboratory for Standard Plant Tissue Analysis. Standard Plant Tissue Analysis was done separately for each genotype in both prune year and crop year during our study. Photosynthetic N use efficiency (A_N) and P use efficiency (A_P) were determined as A_m divided by mass-based concentrations of N and P (N_m and P_m), respectively. Leaf chlorophyll concentration was measured with a chlorophyll meter (SPAD 502 DL meter, Konica-Minolta, Japan) for six sun-exposed mature leaves from six stems per genotype that were marked with a ribbon. Leaf C and N ratio was determined by dividing carbon per mass by nitrogen per mass.

1.3.5 SOIL NUTRIENT CONCENTRATIONS

For the soil nutrient concentration test, 8-10 samples (to fully represent the plot) from each genotype were extracted using a soil sampling probe at the depth of 10 cm for the analysis. All the 8- 10 samples collected from each genotype were mixed thoroughly and stems, leaves, stones, or any other bigger particles to prevent the contamination of testing were removed. The mixed soil sample was filled in the sample container box labeled with the genotype name and was sent to the University of Maine Soil Testing Laboratory for the measurements of and soil pH, levels of nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), sulphur (S), and organic matter level (OM).

1.3.6 STATISTICAL ANALYSIS

The leaf (structural, photosynthetic, and nutrient) traits were averaged within genotypes and genotypes within species for regression analysis. Statistical analyses were applied using R v.4.0.3 (R core team 2021). I analyzed the relationship between functional traits using linear or nonlinear regressions according to which best approximated the structure of the relationship. Equal variances of the variables were tested, and nested one-way ANOVA was used to determine the trait variation within and between two blueberry species since eight genotypes of *V. angustifolium* were different from the eight genotypes of *V. myrtilloides*. In the nested one-way ANOVA, species were assigned as the fixed factor in which random factors (eight genotypes each) were nested within the fixed factor (two species). And variance was calculated from the mean sum of squares of the nested one-way ANOVA results using samples within each genotype. Then, a stem plus error variance and genotype variance was extracted using 'varcomps' function to extract variance components using R v.4.0.3 (R core team 2021). The standardized major axis (SMA) tests were used to see the differences in slope and intercept of bivariate relationships (LMA, $A_{m,}$ and N_m) between blueberries and the global datasets (Wright et al., 2004) using the 'smatr' package in R (Warton et al., 2012). I calculated SMA regressions to determine whether there were significant differences in slope between the genotypes of two lowbush blueberry species (*V. angustifolium* and *V. myrtilloides*) versus the Glopnet leaf economics data set (Wright et al., 2004). A Principal Component Analysis (PCA) was performed to summarize the joint variation of the functional traits for the two species (Pearson, 1901), with mean trait values of each species used for the analyses.

1.4 RESULTS

1.4.1 VARIATION IN LEAF TRAITS AMONG *V. ANGUSTIFOLIUM* AND *V. MYRTILLOIDES*

The two species of lowbush blueberries varied greatly in leaf morphology and shape (Figure A1.2), significantly in photosynthetic traits, and leaf nutrient concentration as well as in other

leaf structural and functional traits (Figure 1.1; Table 1.1). In both crop years, A_a, A_m, and LMA were significantly higher in *V. angustifolium* than in *V. myrtilloides* but A_P and N_m were significantly higher in *V. angustifolium* than in *V. myrtilloides* in only one crop year, 2018. Similarly, C_m and Fe_m were significantly higher in *V. myrtilloides* than in *V. angustifolium* in both crop years, but C/N was significantly higher in *V. myrtilloides* than in *V. angustifolium* in only one crop year, 2018. In both prune years, C_m was significantly higher in *V. myrtilloides* than in *V. angustifolium*, but Fe_m was significantly higher in *V. myrtilloides* in only one prune year, 2017. Similarly, A_a, P_m and C/N were significantly higher in *V. angustifolium* than in *V. myrtilloides* in only one prune year, 2017 and LMA was significantly higher in *V. angustifolium* than in *V. myrtilloides* in another prune year, 2019.

In several trait variations, *V. angustifolium* and *V. myrtilloides* showed lower values than the Glopnet species but similar to higher values compared to the *Vaccinium* species in the Glopnet (Table 1.1, A1.2). However, the values were not always lower in *V. angustifolium* and *V. myrtilloides* compared to Glopnet species in all years of measurement. Instead, I found that A_a, A_m, A_N, A_P, P_m, LMA of both species showed higher mean values compared to Glopnet species and *Vaccinium* species in the Glopnet in 2019, a prune year (Table 1.1, A1.2). But N_m of both species showed lower values compared to Glopnet species in the same year but similar values compared to *Vaccinium* species in the Glopnet (Table 1.1, A1.2). In 2017, another prune year, all the traits of both the species had lower values than the Glopnet species and *Vaccinium* species in the Glopnet but LMA had higher values compared to *Vaccinium* species in the Glopnet (Table 1.1, A1.2). Similarly, all the traits of both the species had lower values than the Glopnet species in both crop years but A_a, A_m, A_N and A_P were higher compared to *Vaccinium* species in the Glopnet in both crop years (Table 1.1, A1.2). Further, the LMA of both species was lower compared to *Vaccinium* species in the Glopnet in both crop years.

The variance partition analysis revealed that the trait variation of these two species was generally explained more by intra-genotype (stem) variance plus error rather than inter-genotype (genotype) variance (Figure 1.1; Table A1.3). However, the source of variation did not always show a consistent pattern across years, across species, and across traits (Figure 1.1a, b, c, d, e, f). Instead, I found a consistent pattern in *V. myrtilloides* where stem plus error variance was always higher than genotype variance and was significantly different in all years (Figure 1.1b, d, f). Similarly, I found that *V. angustifolium* had significantly higher values of stem plus error variance than genotype variance in 2020, but not in 2019 and 2018 (Figure 1.1b, d, f).

Table 1.1: Comparison of two species of blueberries during four years of field study included in this study in the mean and range of light-saturated photosynthetic rate per area (A_a), light-saturated photosynthetic rate per mass (A_m), photosynthetic nitrogen (N) use efficiency (A_N), photosynthetic phosphorus (P) use efficiency (A_P), Nitrogen concentration per mass (N_m), Phosphorus concentration per mass (P_m), Chlorophyll concentration per mass (Chl_m), leaf mass per area (LMA), leaf size (LS), Carbon concentration per mass (C_m), Iron concentration per mass (Fe_m), Carbon/ Nitrogen ratio (C/N) values. Treatments with the different letters are significantly different and treatments with the same letters are not significantly different. Differences were testedwith the Nested ANOVA using species as a factor and genotypes as a factor for each year at alpha level 0.05 and 95% confidence interval.

		A_a	A_m	A_N	A_P	N_m	P_m	Chl_m	LMA	LS	C_m	Fe_m	C/N
2020													
V. angustifolium	Mean	9.49a	127.79a	0.08a	1.19a	1.51a	0.11a	0.39a	75.82a	1.05b	48.34b	42.14b	32.12a
	SE	0.40	6.11	0.01	0.16	0.02	<0.01	0.01	1.29	0.06	2.08	0.13	0.52
V. myrtilloides	Mean	6.91b	97.41b	0.06a	0.85a	1.49a	0.11a	0.37a	70.89b	1.41a	49.29a	65.91a	33.34a
	SE	0.31	4.09	0.01	0.13	0.04	< 0.01	0.01	1.43	0.13	8.23	0.11	0.92
2019													
V. angustifolium	Mean	18.21a	178.01a	0.11a	1.59a	1.51a	0.11a	0.35a	105.42a	2.09a	49.21b	31.3a	32.85a
	SE	0.37	5.62	0.01	0.14	0.06	0.01	0.01	2.55	0.09	1.78	0.14	1.19
V. myrtilloides	Mean	17.56a	183.2a	0.12a	1.76a	1.51a	0.1a	0.37a	98.69b	2.14a	50.05a	41.75a	33.27a
	SE	0.33	5.67	0.01	0.15	0.03	< 0.01	0.01	2.25	0.09	4.61	0.13	0.62
2018													
V. angustifolium	Mean	9.92a	117.58a	0.08a	1.14a	1.37a	0.1a	0.34a	87.13a	1.85b	50.72b	28.64b	37.2b
	SE	0.47	5.27	0.01	0.13	0.02	0.01	0.01	2.80	0.10	1.01	0.08	0.49
V. myrtilloides	Mean	6.97b	92.75b	0.07a	0.79b	1.21b	0.11a	0.35a	75.2b	2.21a	51.37a	34.74a	42.71a
	SE	0.37	6.48	0.01	0.07	0.02	< 0.01	0.01	1.50	0.08	1.00	0.1	0.81
2017													
V. angustifolium	Mean	7.57a	68.72a	0.05a	0.59a	1.4a	0.11a	0.39a	93.36a		50.92b	30.72b	36.83a
	SE	0.22	2.51	< 0.01	0.03	0.02	< 0.01	0.02	3.92		0.72	0.10	0.48
V. myrtilloides	Mean	6.34b	56.9a	0.04a	0.52a	1.5a	0.11b	0.37a	104.86a		51.64a	36.52a	34.64b
	SE	0.55	8.87	0.01	0.06	0.01	< 0.01	0.02	5.24		0.75	0.06	0.29

Table 1.1 Continued

Glopnet								
	Mean	11.50	128.00	6.31	1.18	1.94	0.11	128.0
	SD	5.93	103.13	0.02	0.60	0.98	0.09	118.35
	Minimum	1.00	4.80	0.63	1.74	0.25	0.01	14.00
	Maximum	42.00	662.00	25.50	0.25	6.36	0.60	1510
Vaccinium Glopnet								
	Mean	6.69	79.62	< 0.01	0.09	1.52	0.08	90.29
	Minimum	4.96	40.00	< 0.01	< 0.01	0.89	< 0.01	40.20
	Maximum	10.96	140.1	600.0	1.00	2.50	0.22	246.21
	SE	0.03	0.06	21.50	0.42	0.04	0.14	0.05

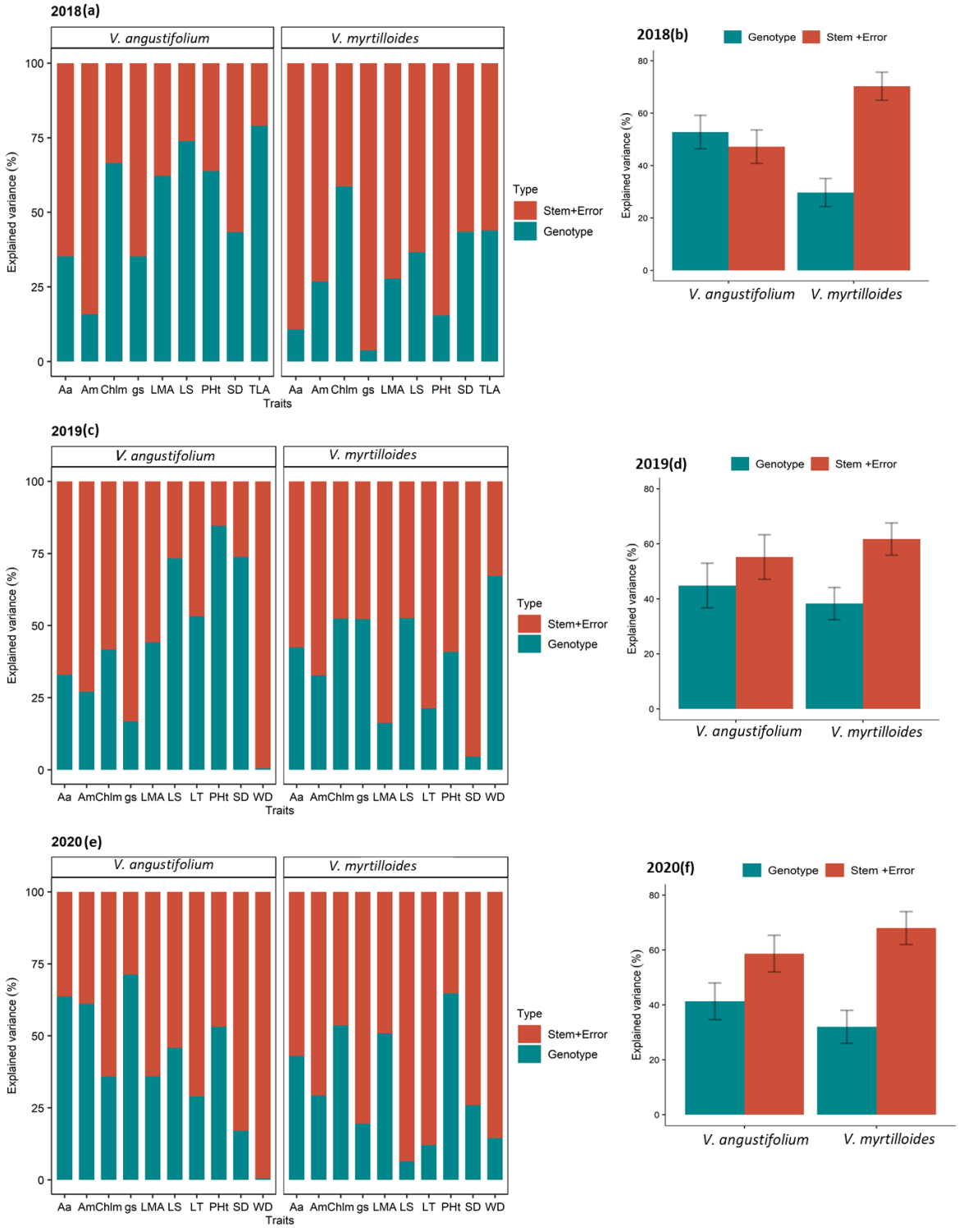
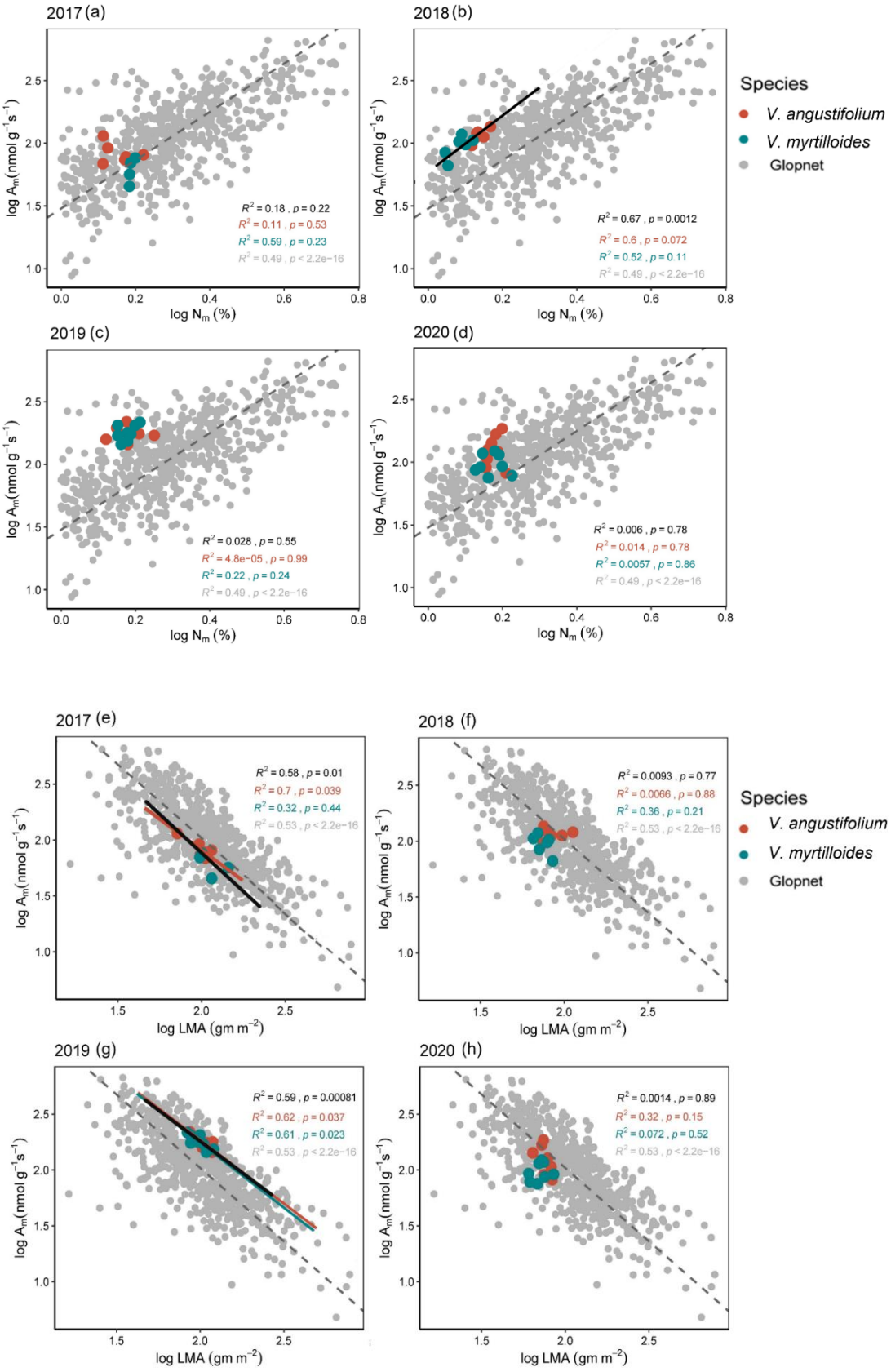


Figure 1.1 Source of variation of different traits for the two species studied (*V. angustifolium* and *V. myrtilloides*) across different years. A large fraction of the total variance in each trait is found within the stems plus error (red) vs between genotypes (green). Species names are at the top and trait names are at the bottom (a, c, e). Percentage contribution of genotype and stem plus error variance of both the species in different years when traits percentage variance was all averaged together (b, d, f). Explained variance percentages was calculated from the mean sum of square values obtained from the Nested ANOVA results. Data are means ± SE, n=total number of traits of each corresponding year on the left side. For trait abbreviations, refer to Table A1.3.

1.4.2 COMPARISON ON TRAIT RELATIONSHIPS IN *V. ANGUSTIFOLIUM* AND *V. MYRTILLOIDES* WITH THOSE OF GLOPNET SPECIES

Although all traits fell within the domain of the Glopnet species, the bivariate relationships among LMA, A_m, and N_m across genotypes of *V. angustifolium* and *V. myrtilloides* were not always consistent with the relationships found in the Glopnet (Figure 1.2a to 1.2l). The A_m and N_m relationship found in the Glopnet was also found here across species in one crop year (Figure 1.2b), but not found within species for both species in both crop and prune years (Figure 1.2a, b, c, d). I found a significant negative relationship between A_m and LMA within *V. angustifolium* and across both species in prune years (Figure 1.2e, g), but the relationships were absent in crop years (Figure 1.2f, h). Whereas in *Vaccinium myrtilloides*, the relationship was only present in one prune year (Figure 1.2g), but absent in the other prune year (Figure 1.2e) and both crop years (Figure 1.2f, h). Similarly, the negative relationship between N_m and LMA was only found in *V. myrtilloides* in one of the crop years (2020; Figure 1.2l), but the relationship was absent in the other crop year (Figure 1.2j), and the prune years (Figure 1.2i, k).


2017 (a)
2018 (b)
2019 (c)
2020 (d)
2017 (e)
2018 (f)
2019 (g)
2020 (h)
log A_m(nmol $g^{-1}s^{-1}$)
log N_m (%)
log LMA (gm m^{-2})
Species
V. angustifolium
V. myrtilloides
Glopnet
R^2 = 0.18 , p = 0.22
R^2 = 0.11 , p = 0.53
R^2 = 0.59 , p = 0.23
R^2 = 0.49 , p < 2.2e−16
R^2 = 0.67 , p = 0.0012
R^2 = 0.6 , p = 0.072
R^2 = 0.52 , p = 0.11
R^2 = 0.49 , p < 2.2e−16
R^2 = 0.028 , p = 0.55
R^2 = 4.8e−05 , p = 0.99
R^2 = 0.22 , p = 0.24
R^2 = 0.49 , p < 2.2e−16
R^2 = 0.006 , p = 0.78
R^2 = 0.014 , p = 0.78
R^2 = 0.0057 , p = 0.86
R^2 = 0.49 , p < 2.2e−16
R^2 = 0.58 , p = 0.01
R^2 = 0.7 , p = 0.039
R^2 = 0.32 , p = 0.44
R^2 = 0.53 , p < 2.2e−16
R^2 = 0.0093 , p = 0.77
R^2 = 0.0066 , p = 0.88
R^2 = 0.36 , p = 0.21
R^2 = 0.53 , p < 2.2e−16
R^2 = 0.59 , p = 0.00081
R^2 = 0.62 , p = 0.037
R^2 = 0.61 , p = 0.023
R^2 = 0.53 , p < 2.2e−16
R^2 = 0.0014 , p = 0.89
R^2 = 0.32 , p = 0.15
R^2 = 0.072 , p = 0.52
R^2 = 0.53 , p < 2.2e−16

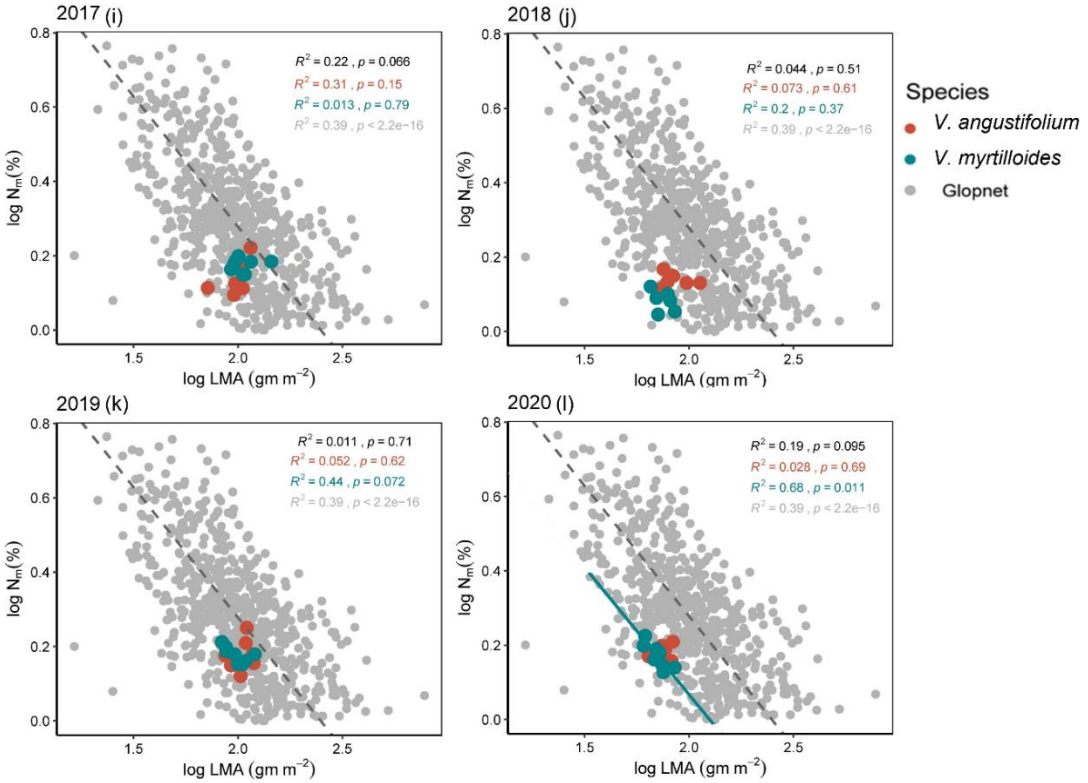


Figure 1.2 Mass-based photosynthetic rate (A_m) in relation to leaf nitrogen concentration (N_m; a, b, c, d), and leaf mass per area (LMA; e, f, g, h), and leaf nitrogen concentration (N_m) in relation to leaf mass per area (LMA; i, j, k, l) for 2017, 2018, 2019, and 2020 across and within two blueberry species *V. angustifolium* (VA, red circles) and *V. myrtilloides* (VM, green circles) and global dataset (Glopnet). The grey points are from a global dataset of Wright et al. (2004). All variables were log-transformed. Break lines are standardized major axis (SMA) lines fitted to the global dataset, whereas solid lines are SMA lines fitted across (black solid line) and within (red solid line, *V. angustifolium* and blue solid line, *V. myrtilloides*) to the blueberry species. P values of less than 0.05 are significant and are marked with a corresponding line color as described above.

1.4.3 COORDINATION OF LEAF TRAITS WITHIN AND ACROSS V. ANUSTIFOLIUM AND V. MYRTILLOIDES SPECIES

For other functional traits, *V. angustifolium* and *V. myrtilloides* species showed some trait relationships but the relationships were not always consistent within and across species across years (Figure 1.3a to 1.3l). There was a significant negative relationship between A_m and C_m in both crop years (Figure 1.3b, d) and a significant positive relationship between A_m and Chl_m across species in prune years (Figure 1.3e, g). Within species, *V. angustifolium* and *V. myrtilloides* showed a significant negative relationship between A_m and C_m in one of the crop

years, but not all other (Figure 1.3 a, c, b, d). *V. angustifolium* had significant positive relationship in one of the prune years between A_m and Chl_m (Figure 1.3e), but not in the other prune year (Figure 1.3g) and both crop years (Figure 1.3f, h). A relationship between A_m and C/N across species relationship was found in one of the crop years (Figure 1.3j) but not in all other years (Figure 1.3i, k, l).

Interestingly, leaf structural traits showed relationships with some photosynthetic traits, but the relationship was not always present and consistent across all years and both within and across species. I found a negative significant relationship of A_N and A_P with LMA across species in prune years (Figure 1.4a, c, e, g), but the relationship was absent in crop years (Figure 1.4b, d, f, h). The relationship between A_N and LMA was also found within species *V. angustifolium* in both prune years (Fig1.4a, c). The relationship between A_P and LMA was found within both *V. angustifolium* and *V. myrtilloides* in one of the prune years (Figure 1.4e, g). The relationship between Chl_m and LMA was found within both *V. angustifolium* and *V. myrtilloides* species in one of the prune years (Figure 1.4i, k), and across species in one prune year (Figure 1.4k). Chl_m was significantly and positively related to N_m across species in only one prune year (Figure 1.4o), and within *V. angustifolium* in one crop year (Figure 1.4p).

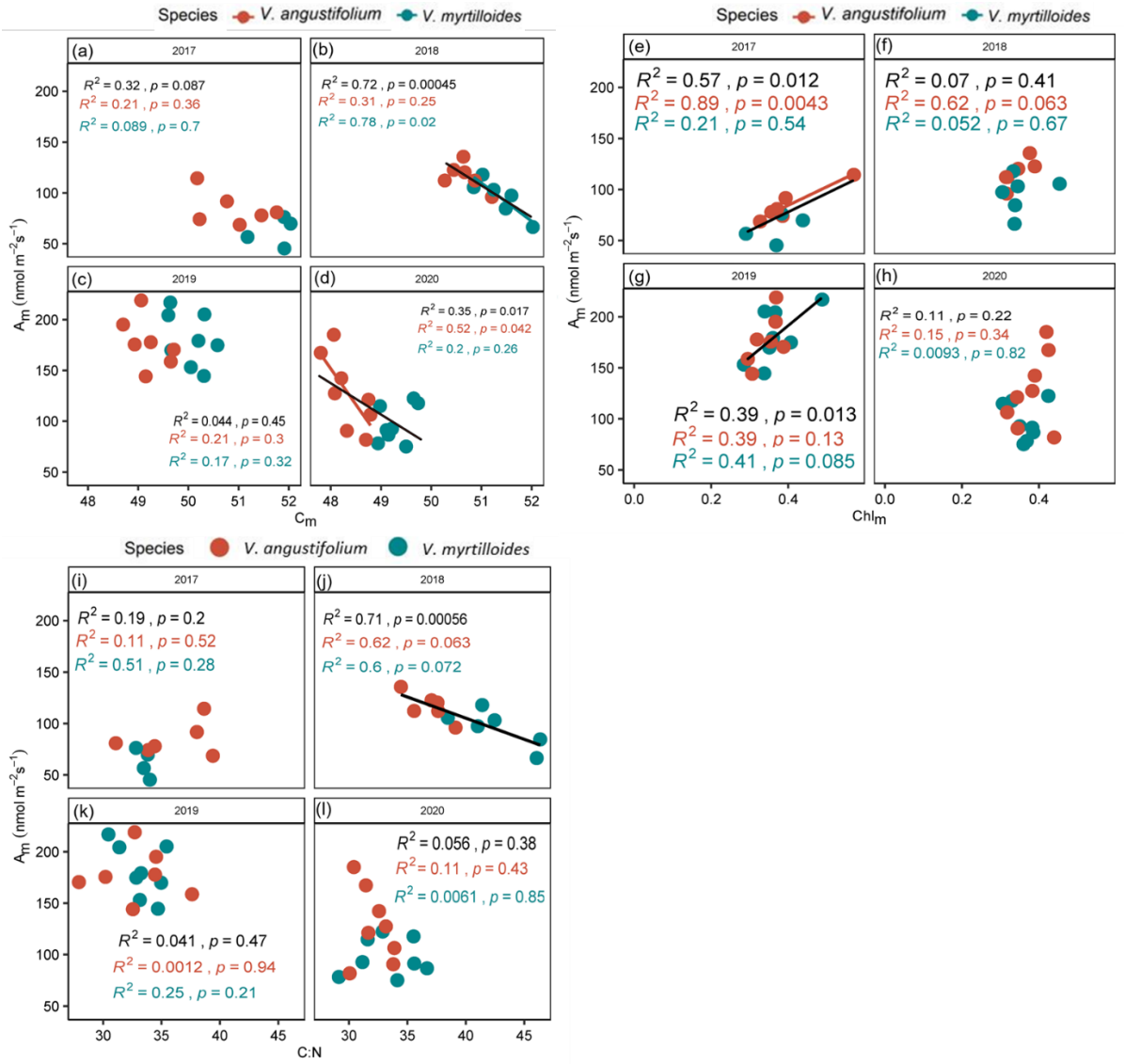


Figure 1.3 Mass based photosynthetic rate (A_m) in relation to carbon concentration per mass (C_m; a, b, c, d), chlorophyll concentration per mass (Chl_m; e, f, g, h) and carbon nitrogen ratio (C:N; i, j, k, l) for 2017, 2018, 2019, and 2020 across and within two blueberry species *V. angustifolium* (VA, red circles) and *V. myrtilloides* (VM, green circles). Points are means. Linear (a & c) regressions were fitted to the data across (black solid line) and within (red solid line, *Angustifolium* and green solid line, *Myrtilloides*) blueberry species. *p* values of less than 0.05 are significant and are marked with a corresponding line color as described above.

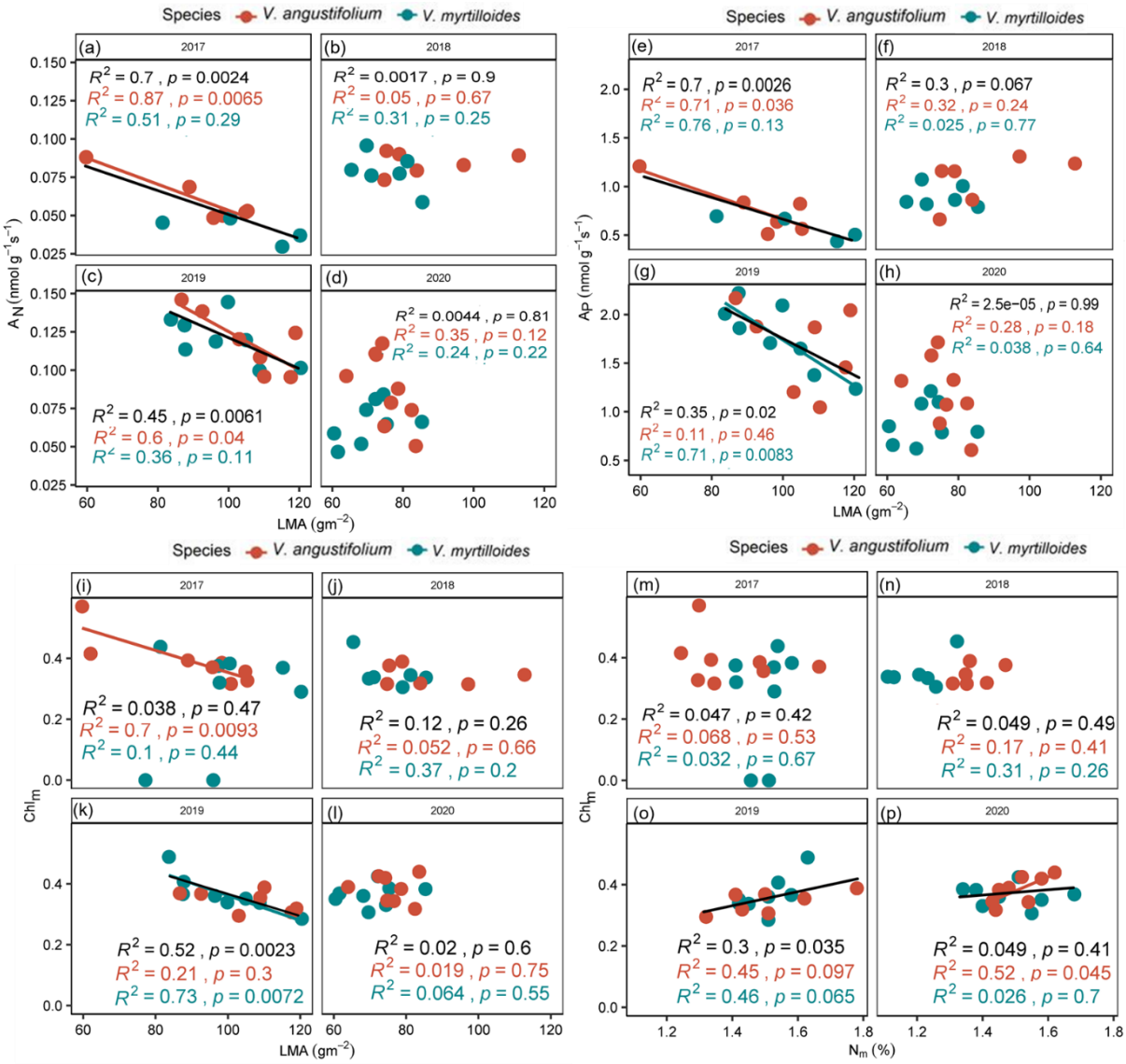


Figure 1.4 Photosynthetic nitrogen (N) use efficiency (A_N; a, b, c, d), photosynthetic phosphorus (P) use efficiency (A_P; e, f, g, h) and chlorophyll concentration per mass (Chl_m; i, j, k, l) in relation to Leaf Mass per Area (LMA) and chlorophyll concentration per mass (Chl_m) in relation to nitrogen concentration per mass (N_m; m, n, o, p) for 2017, 2018, 2019, and 2020 across and within two blueberry species, *V. angustifolium* (VM, red circles) and *V. myrtilloides* (VM, green circles). Points are means. Linear (a & b) regressions were fitted to the data across (black solid line) and within (red solid line, *Angustifolium* and green solid line, *Myrtilloides*) blueberry species. P values of less than 0.05 are significant and are marked with a corresponding line color as described above.

1.4.4 RELATIONSHIP OF LEAF TRAITS ACROSS YEARS

The same leaf traits of different years showed correlations only in some cases. LMA in 2019 was correlated to that of 2020 across species (Figure 1.5b). LMA in 2018 was also correlated with that of 2020 across species. There was no relationship between LMAs of different years

within species. There was no relationship between LS from different years within species and across genotypes of the two species (Figure 1.5e, f).

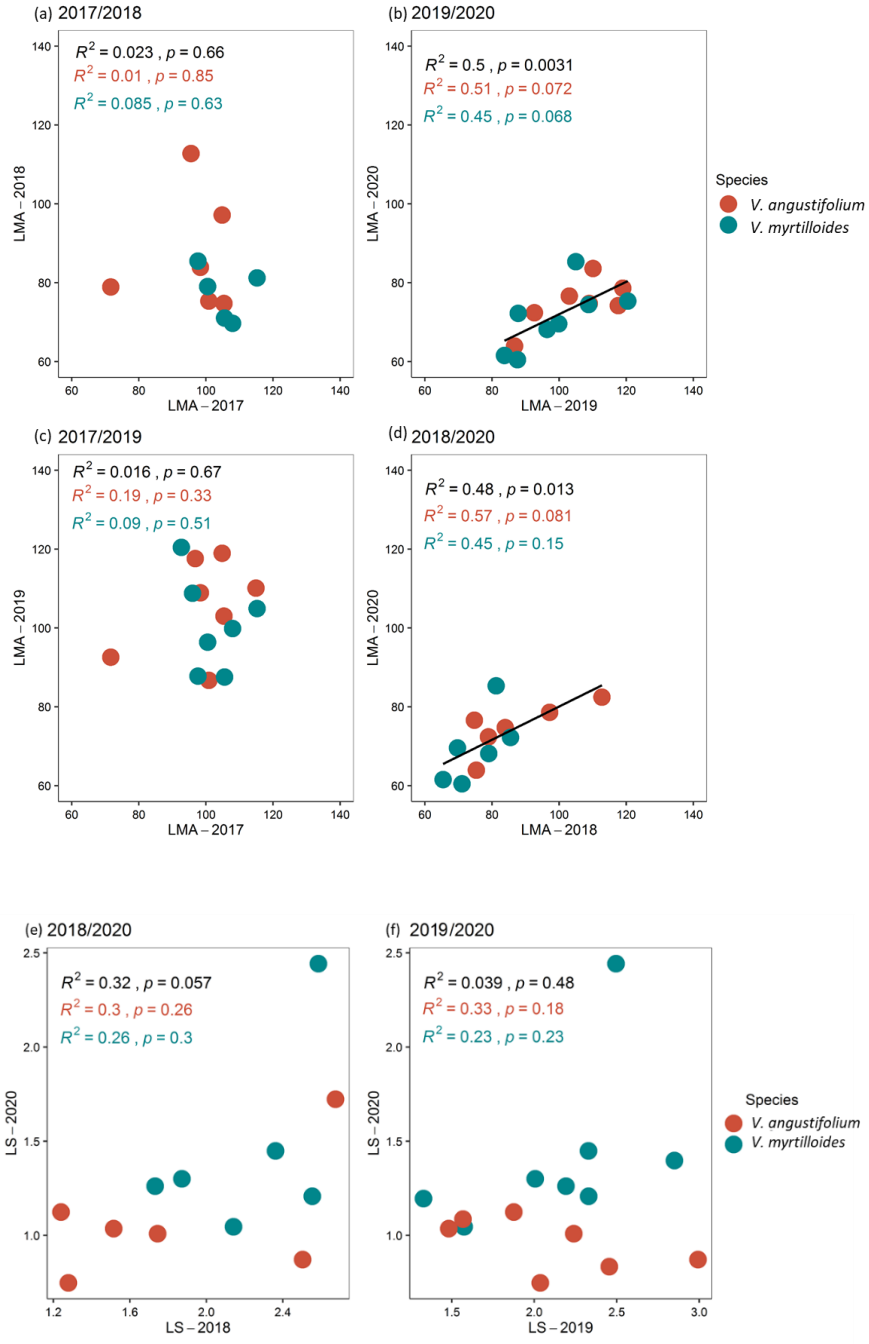


Figure 1.5 Relationship of leaf mass per area (LMA) of 2017 with LMA of 2018 (a), LMA of 2019 with LMA of 2020 (b), LMA of 2017 with LMA of 2019 (c) LMA of 2018 with LMA of 2020 (d), Leaf size (LS) of 2018 with LS of 2020 (e), and LS of 2019 with LS of 2020 across and within two blueberry species *V. angustifolium* (VA, red circles) and *V. myrtilloides* (VM, green circles). Points are means. Linear (i) regressions were fitted to the data across (black solid line) and within (red solid line, *Angustifolium* and green solid line, *Myrtilloides*) blueberry species. P values of less than 0.05 are significant and are marked with a corresponding line color as described above.

1.4.5 OVERALL TRAIT VARIATION ACROSS YEARS AND THE INFLUENCE OF ENVIRONMENTAL FACTORS

Changes in environmental conditions over the years strongly shape leaf functional traits, leaf structural traits, and leaf nutrient traits variation in *V. angustifolium* and *V. myrtilloides* species. In the PCA analysis of all leaf traits, soil properties, rainfall and temperature, PC1 explained 21.6% while PC2 explained 20.1% of the total variance (Figure 1.6a). The PCA1 was mainly positively associated with SD and C_m, and negatively with A_a, A_m, and Chl. The PC2 was mainly positively associated with A_a, A_m, and Chl, and negatively mainly with C: N ration. The PC1 represented a trade-off between productivity and carbon, while the PCA represented a trade-off between productivity and leaf toughness. An an extremely wet year, 2019 was clearly separated from 2018 and 2020 (Fig, 1.6a). Importantly, rainfall was positively related to leaf photosynthetic traits, leaf nitrogen, LMA, LS, water use efficiency whereas temperature was negatively related to these traits. Meanwhile, the temperature was positively related to leaf carbon traits C_m, C/N but rainfall was negatively related to these traits (Figure 1.6a). The species and genotypes loading of different years (Figure 1.6b, c, d) in the principal component analysis showed overlaps in the functional, structural, nutrient availability of both the species in 2019 (Figure 1.6c) and traits in this year were completely different from those of other years. However, there was a clear separation of species and genotypes in 2018 and 2020. In 2018 and 2020, *Vaccinium angustifolium* was located more towards the centre and *V. myrtilloides* was located away from the centre but lied on the same side of the PCA (Figure, 1.6b, d). However, the pattern was not distinct in 2020 compared to 2018 as genotypes 2 and 7 in 2020 showed some overlaps near the centre.

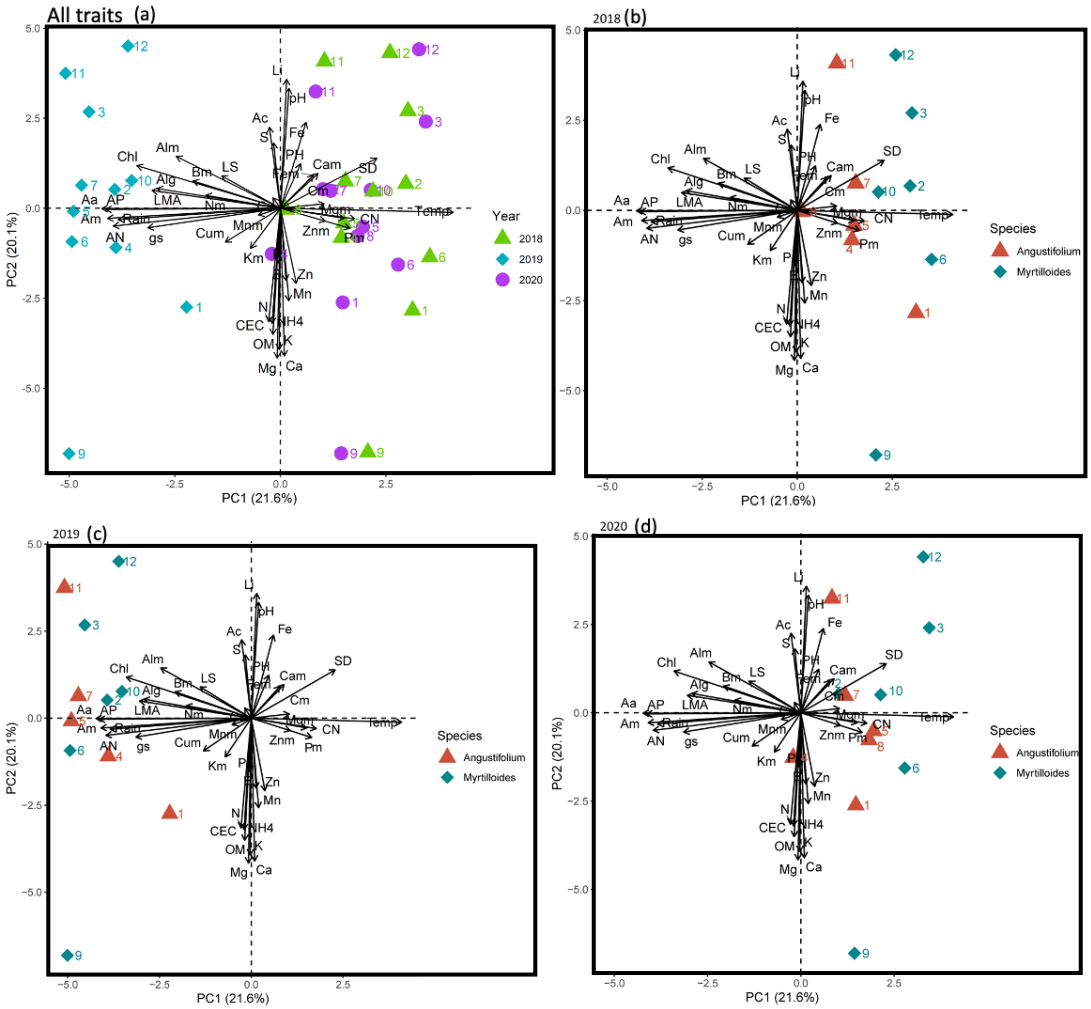
In the PCA analysis of leaf nutrient traits and other functional traits, rainfall, and temperature, PC1 explained 36.3% while PC2 explained 16.4% of the total variance (Figure 1.6e). The PC1 was mainly positively associated with C/N and C_m, and negatively mainly with N_m. The PC2

was mainly positively associated with A_a, A_m, and Chl, and negatively mainly with Fe_m and P_m. The loadings of different years leaf nutrient and functional traits in the same principal component clearly separated all three years; 2018 was associated with C_m, C/N, and several other traits, 2019 was associated with leaf photosynthetic traits, water use efficiency, rainfall, and several other leaf nutrient traits, whereas 2020 was associated with Fe_m, P_m and temperature showing distinct species trait availability in each year (Figure, 1.6e). The two species in 2019 showed more overlaps compared to 2018 and 2020. Also, *V. angustifolium* genotypes were located more towards the centre and *V. myrtilloides* were located away from the centre in 2018 and 2020 (Figure, 1.6f).

In the PCA analysis of leaf structural, functional traits, rainfall, and temperature, PC1 explained 57.8% while PC2 explained 15.3% of the total variance (Figure 1.6g). The PC1 was mainly positively associated with SD and negatively mainly with A_a, A_m, A/g. The PC2 was mainly positively associated with LMA and LS, and negatively mainly with temperature. The loadings of leaf structural, functional traits, rainfall, and temperature clearly separated 2019, a wet year from 2018 and 2020. Species and genotypes loadings of different years in the principal component also clearly separated genotype and species trait availability in each year where 2018 was scattered mostly in the first quadrant between the structural trait, 2019 was scattered mostly in the second and third quadrants between the leaf photosynthetic traits, LMA and rainfall whereas, 2020 was mostly scattered in fourth quadrants and seems to be associated with temperature (Figure 1.6h, i, j). The species and genotypes in 2018 and 2020 showed no overlaps, but in 2019 there were more overlaps between the genotypes of the two species (Figure 1.6h, i, j).

In the PCA analysis of soil nutrients traits, the first axis explained 45.9% of the total variation and the second axis explained 13.3% of total variation (Figure 1.7). The PC1 was mainly

positively associated with soil N and P, and negatively mainly with soil Fe, soil acidity. The PC2 was mainly positively associated with soil P^H, and S, and negatively mainly with B, CEC, OM. Interestingly, the PCA analysis of species loadings showed overlaps in the soil nutrient availability as both species were scattered between the first and second axis in the PCA (Figure 1.7).



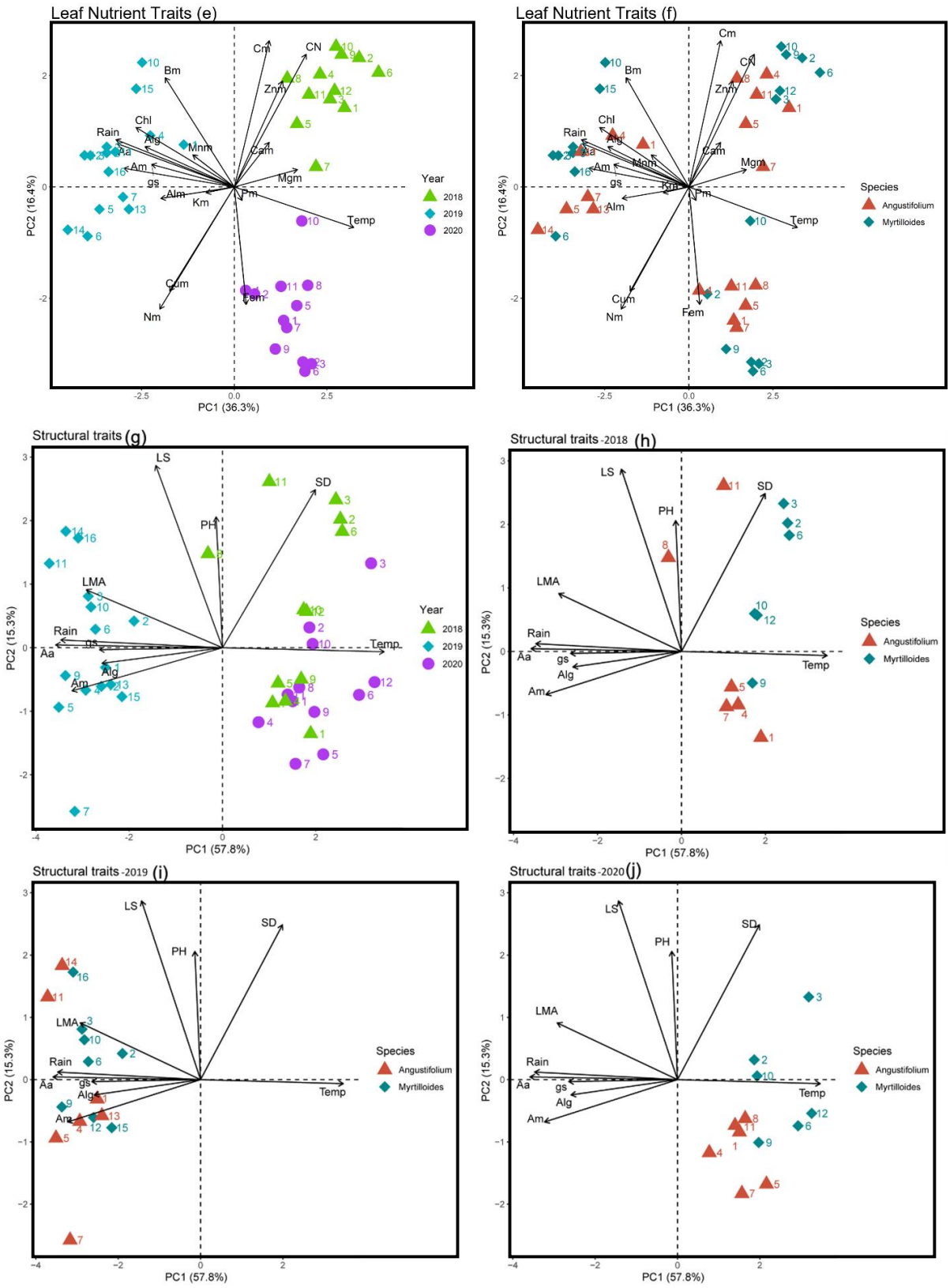


Figure 1.6 Principal Component Analysis (PCA) of mean values of the combination of leaf functional traits, leaf structural traits, leaf nutrient traits, and soil nutrient traits combined for 2018, 2019, 2020 with years loading on the background (a) and with genotypes and species loadings on the background (b, c, d). PCA of leaf nutrients and leaf functional traits combined for 2018, 2019, 2020 with years

loading on the background (e) and species loadings on the background (f). PCA of leaf structural and leaf functional traits combined for 2018, 2019, 2020 with years loading on the background (g) and species loadings on the background (h, i, j). PCA of soil nutrients for 2018 with species and genotypes loadings on the background (k). Trait symbols are listed in supporting information in the table A1.4. Green triangles represent 2018, blue squares represent 2019, and purple circles represent 2020 on the background PCA. Red triangles represent *V. angustifolium* (*Angustifolium*), and blue square represents *V. myrtilloides* (*Myrtilloides*) where each species is labelled with their corresponding genotypes number (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12).

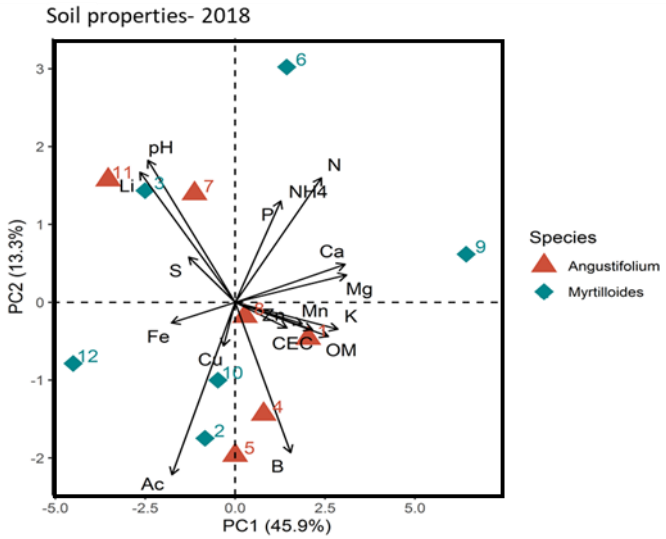


Figure 1.7 Principal Component Analysis (PCA) of mean values of the soil properties of the year 2018 with species and genotypes loadings on the background (a). Red triangles represent *V. angustifolium* (*Angustifolium*), and blue squares represents *V. myrtilloides* (*Myrtilloides*) where each species is labelled with their corresponding genotypes number (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12).

1.5 DISCUSSION

Blueberries have previously been characterized by high inter-genotype variations in many biological traits, e.g., age, height, color and hue, phenology, and yield (Vander Kloet, 1978; Smagula et al., 1997). Here I found a high variation in leaf structure and physiology. Most impressively, the blueberries showed higher intra-genotype variance plus error as compared to inter-genotype variance in the majority of leaf physiological, nutrient, and structural traits. *Vaccinium angustifolium* and *V. myrtilloides* species showed lower mean nutrient concentrations and mean gas exchange values than the Glopnet mean values but were similar

to that of *Vaccinium* species in Glopnet. Also, the LES relationships found in the Glopnet were not always present across genotypes in *V. angustifolium* and *V. myrtilloides*. The PCA analysis revealed the influence of environmental conditions in shifting functional traits across years, confirming the vital role of environmental conditions in shaping plant structure and function (Ballejo et al., 2018; Grassel et al., 2015; Wright 2002) over time.

Trait variations exist at all spatiotemporal and organizational scales: within a species (Valladares et al., 2000; Takahashi et al., 2005; McGill et al., 2006; Rozendaal et al., 2006), and across species (Wright et al., 2001; Westoby et al., 2002). Our study revealed high intra-genotype variations, within and across blueberry species. The LES relationships found in the Glopnet were not always present in our study across genotypes in two species in a farm setting, suggesting global LES relationships might not always be found at smaller scales such as a single field location, and with fewer species. Messier et al. (2016) suggested that global LES correlation might be absent or showing the opposite at local scales but is consistent across global scales (Wright et al., 2004; Albert et al., 2010a; Asner et al., 2014), which is similar to our findings.

1.5.1 VARIATION IN LEAF FUNCTIONAL TRAITS AND COMPARISONS WITH GLOPNET SPECIES

The large variations found in leaf structure, physiology, and nutrient content within and across blueberry species when compared to Glopnet species mean values suggest high physiological diversity among the blueberry species. Blueberry genotypes grow vegetatively by a slowly expanding underground network “rhizomes”, the distribution and genetic variation evident in millions of different lowbush wild blueberries may be because of the natural selection process that took place over a period perhaps millennia (Borns 2004; Drummond et al., 2009) or may be due to genetic drift over evolutionary timescales (Smith et al., 1985; Roff, 2000; McGuigan, 2006; Gardner & Latta, 2007). The two species of wild blueberry, *V. angustifolium* and *V.*

myrtilloides examined at a single research farm varied strongly across years especially in traits known to shift with the change in environmental conditions like LMA, photosynthetic rate (A_m), and nitrogen (N_m) concentration. Notably, *V. angustifolium* possessed higher and statistically different mean values of photosynthetic traits (A_a, A_m, A_P), LMA, N_m, and lower mean values of leaf size (LS), carbon (C_m), iron (Fe_m) and carbon nitrogen ratio (C/N) compared to *V. myrtilloides* species, suggesting that *V. angustifolium* invested larger proportion of its energy in photosynthetic machinery, and less in structural and stress tolerance traits. This strategy can result in a faster growth rate (Mathan et al., 2016), greater above-ground biomass (Thompson et al., 2017), sugar accumulation and yield increase (Ainsworth and Long, 2005; Kooi et al., 2016), and increased abundance (Li et al., 2015). This could be the reason for the higher yield and abundance of *V. angustifolium* compared to *V. myrtilloides* in the field according to Drummond. (2019) and Drummond & Rowland (2020). In contrast, *V. myrtilloides* possessed significantly higher leaf size, C_m, C/N, and Fe_m (traits related to toughness and construction) and lower mean values of photosynthetic traits, suggesting a conservative strategy that a relatively larger part of the energy is invested in structural and stress tolerance. This could be the reason for having low productivity in *V. myrtilloides* as compared to *V. angustifolium* species (Minore et al., 1972; USDA Forest Service, 2020). This pattern suggests differentiated resource use between these two species, which may minimize competition between them and promote co-existence in the community (Pastore et al., 2021). However, the mean value of C/N was greater in *V. angustifolium* than in *V. myrtilloides* in 2017, a drought year, which could be the effect of drought in trait development and the high plasticity of *V. angustifolium*. Environmental conditions are an important factor in the trait development (Tardieu et al., 2013; Jung et al., 2010; Paine et al., 2011; Enquist et al., 2015).

Interestingly, *V. myrtilloides* tend to show significantly higher intra-genotype variation, which was higher than inter-genotype variations in all years. Although *V. angustifolium* had higher

intra- genotype or inter stem variation in most of the years, this species only showed significantly higher intra-genotype variation than inter-genotype variations in one of the years. According to Albert et al. (2010b), intra-genotype variations reflect environmental heterogeneity rather than being driven by genetic factors. This pattern of higher intra-genotype or inter stem plus error variation than inter-genotype variations in both the studied species suggests that species are affected by the environment in idiosyncratic ways (Hultine & Marshall 2000), meaning that each species is uniquely affected by environmental conditions. Some of the micro-environmental factors like soil (pH, organic matter content, texture), disturbance (fire, and mowing), and competition (weeds) (Albert et al., 2010) might have also played a significant role in shaping traits at the individual level in this semi-natural agricultural system. Also, ecologists increasingly appreciate that within-species variation can have greater consequences for community dynamics and structure (Bolnick et al. 2003; Clark et al. 2004; Clark, 2005) than the interspecies variation.

1.5.2 TRAIT RELATIONSHIPS IN WILD BLUEBERRIES WITH RESPECT TO THE GLOBAL LEAF ECONOMIC SPECTRUM

The relationships of fundamental leaf functional traits previously reported in the global leaf economic spectrum (Field & Mooney, 1986; Reich et al., 1997; Wright et al., 2004) do not always hold in wild blueberries. A large variation in LES associated traits at local scales have been found in studies of several grass, herb, shrub, and tree species including those of *V. myrtilloides* (Albert et al. 2010), and tropical forest species (Hulshof and Swenson 2010). Another study on trees by Messier et al. (2016) revealed that LES relationships at smaller scales (site, species, individual) might not be present compared to the LES at the global scale. This difference in LES trait relationships between local scales and the global scales could be because of a shift in the dominant drivers of phenotypic integration, and that the locally dominant driver of phenotypic integration (biophysical constraints, genetic constraints, environmental filtering)

favors distinct traits and relationships from those observed globally (Messier et al., 2017). Also, different selective pressures might dominate at different scales, particularly within species versus across species (Albert et al., 2010b; Messier et al., 2010; Kichenin et al., 2013), and different traits have different sensitivities to such pressures (Messier et al., 2016) leading to distinct trait relationships at local scales.

Notably, the relationship of N_m and LMA was only found within *V. myrtilloides* in one of the crop years but was absent within *V. angustifolium* and across species. The relationship between A_m and N_m was also absent within both species in all the years but was found across species in one crop year. Reich et al. (1999) analyzed the data from six study sites and found out that the relationships between A_m and N_m could be absent within species, similar to our findings in which A_m and N_m relationships were absent within species. The absence of A_m and N_m could also be due to significantly lower nitrogen of blueberry species than the Glopnet species mean values (Table 1.1), resulting in a relatively narrow range in N_m. However, the presence of A_m and N_m relationship across genotypes of both species supports its universal application and the direct causal relationship between N_m and A_m (Field & Mooney, 1986). Interestingly, the relationship between A_m and LMA was present across species and within *V. angustifolium* in both prune years but was absent in crop years. Also, within the *V. myrtilloides*, the A_m *vs* LMA relationship was only present in one year. The presence of A_m and LMA relationships across species and within *V. angustifolium* species in both the prune years could be because of the energy allocation pattern in the vegetative growth phase. *V. angustifolium* and *V. myrtilloides* had higher LMA values in both the prune years compared to crop years thus it is evident that blueberry species spent more energy in building leaf structure traits (LMA). The absence of the A_m and LMA relationship in crop years (reproductive phase) could be due to the confounding effect of investment in reproduction. The variable environmental conditions across years may also affect the LES trait relationships (Ordonez et al., 2009; Messier, McGill

& Lechowicz, 2010; Sandel et al., 2010). Gerdol (2005) showed that two related species from the same growth form (deciduous dwarf shrubs, *V. myrtillus* and *V. uliginosum*) can have very different growth performance and nutrient concentrations along environmental gradients. From variance partition analysis I have observed significantly higher intra-genotype - variance plus error compared to inter-genotype variance in all of the years in *V. myrtilloides* and in one of the years in *V. angustifolium*.

In addition to the relationships among LMA, A_m, and N_m, I found relationships of A_m with C_m, Chl_m, C/N, and LMA with Chl_m, Fe_m, and photosynthetic nutrient use efficiency (A_N and A_P), but the relationships were not always found across all years. Some relationships, e.g., between photosynthetic traits (A_m, A_N, A_P) and LMA across species were found in both prune years but absent in both crop years. In contrast, I found a strong relationship between A_m and N_m as well as between C_m, and C/N across species in one of the crop years (2018) and the relationship of Chl_m with N_m within *V. angustifolium* in one of the crop years (2020). This shows how the difference in dominant factors shape functional traits in crop and prune years. It is likely that blueberries species spend less energy on leaf toughness, structure, and construction traits like C_m and C/N. Overall, several trait relationships studied in blueberry species showed differences among years.

1.5.3 TRAIT RELATIONSHIP OF LEAF MASS PER AREA AND LEAF SIZE ACROSS YEARS.

The functional traits of one year cannot be used to predict the traits in other years. The relationship of LMA and LS across years showed variation in crop or prune year which could be related to differences in energy allocation between crop and prune years, as well as changes in environmental conditions across years. There was a significant and positive relationship of LMA between crop years, but the relationship was absent between the prune years. The absence of the relationship in the prune years could be due to the completely different environmental

conditions prevalent in prune years as one being a dry year and another being an extremely wet year. Similarly, there were LMA trait relationships in one crop cycle (between 2019 and 2020), that were absent in another crop cycle (between 2017 and 2018). This also could be an effect of variable environmental conditions prevalent in a given year, as each year was unique in terms of variable environmental conditions. I also did not find LS trait relationships in crop cycles and prune years. Thus, the trait values measured in one year cannot be used to predict the performance of these species in the other years. These species might not necessarily have the same energy allocation patterns, and different genotypes may show different responses to environmental changes.

1.5.4 ENVIRONMENTAL INFLUENCE, COEXISTENCE OF TWO BLUEBERRY SPECIES

The PCA analysis overall demonstrated a strong influence of environmental conditions on trait variations, and the differentiated response of two co-occurring species to changing environmental conditions. The PCA of all traits for all the years showed that traits in the extremely wet year 2019 were completely different and were located on a different side of the PCA compared to species in 2018 and 2020, which were located on the same side of the PCA (Figure 1.6a). The PCA for leaf structure and physiological traits was consistent with the above pattern but the PCA for leaf nutrient and physiological traits separated all three years from one another on completely different sides of PCA (Figure 1.6e). Changing environmental conditions may be acting as a filtering effect in shaping the community composition of these two species, and different species have advantages in different years and environmental conditions. High rainfall resulted in high leaf physiological, nutrient, and structural traits in an extremely wet year (2019), whereas the relatively dry conditions of 2018 and 2020 resulted in traits related to high leaf toughness, low leaf nutrients and plant morphological traits (Figure 1.6a). The PCA analysis of leaf structural and physiological traits (Figure 1.6g) was consistent

with the above pattern and showed traits and year 2019 on the completely different side of the PCA as compared to traits and year 2018 and 2020 position on the PCA.

The selection of different functional traits under changing environmental conditions became clearer when PCA was plotted for the leaf physiological and nutrient traits (Figure 1.6e). The PCA analysis showed that, every year was unique in that species occupied the first quadrant in 2018, fourth quadrant in 2020 and second and third quadrants in 2019. The adaptations observed in our study could be due to phenotypic plasticity, as it has been shown that species can respond to changing environmental conditions through phenotypic plasticity (Pelletier et al., 2018; Hendry et al., 2016). This similarly relates Shi et al. (2018), who mentioned that changing environmental conditions act as a strong environmental filter that constrains the number of successful trait combinations and leads to community-level trait convergence (Bruelheide et al., 2018). As a result, co-occurring species in a given habitat exhibit similar ecological strategies and share similar traits (Cornwell et al., 2006), leading to trait convergence in co-occurring species and shaping community structure (Lavorel & Garnier, 2002; Lebrija-Trejos et al., 2010).

Importantly, I also revealed some mechanisms for the coexistence of these two species. There were few or no overlaps between the two species (Figure 1.6b, d, f, j, l) in 2018 and 2020 across all PCA, whereas there were clear overlaps between the species (Figure 1.6c, f, k) in the extremely wet year 2019. According to the modern coexistence theory species may coexist if they have differentiated resource use and thus might not significantly interact, or they can coexist with partially overlapping resource use if they are nearly equivalent in their average competitive abilities (Pastore et al., 2021). These two species exhibited significant differences in mean leaf physiological, nutrient, and structural traits in 2018 and 2020, but they were not significantly different in 2019. Thus, each species has its differentiated traits and resource use

in the community. In contrast, the PCA for 2019 revealed notable overlap suggesting wet conditions may weaken the advantages of *V. angustifolium*. Thus, the separation of niches could be in the temporal scales. Hubbell (2021) suggested that if species have close-to-equal performance in the same environment, species can coexist for extended periods of time.

The overlaps in the genotypes of both the species in 2019 suggest that the particularly wet conditions allowed for the equal performance of both species resulting in increased competition. Positive environmental effects can increase species competition (Chesson et al., 2000), which could explain the partial overlaps in the genotypes of different species in the PCA for 2019. High rainfall can increase species competition by creating opportunities for species by improving soil moisture availability or creating nutrient diversity and contributing to species fitness to shape community composition (Kimball et al., 2012). In contrast, negative environmental effects like higher temperatures decrease species competition by limiting opportunities for species through increased evapotranspiration, disrupting photosynthesis, decreasing soil moisture (Berry & Bjorkman 1980; Goyal 2004), jeopardizing the species fitness in the community. Therefore, the pattern of species overlaps or lack of overlaps according to changing environmental conditions in the PCA reflects the species coexistence and competition of blueberry species. Overall, the temporal dynamics of environmental variation play an important role in shaping species coexistence and competition as well as trait convergence and community composition.

Interestingly, I also observed mixed patterns in the soil properties as both species were scattered along both the axis in the PCA. From the soil nutrients PCA, both species seem to be more abundant in locations with high soil acidity, low pH, and high boron content, which are typical characteristics of the blueberry plants that thrive in acidic soil conditions (Bell et al., 2009; Smagula, 1993). Fujita et al. (2013) mentioned that the availability of soil nutrients is one of

the main factors in determining the species composition of plant communities, because each species is evolved to adapt to certain environments and therefore has contrasting availability for nutrients (Fujita et al., 2013). Overall, these two species shared soil nutrient preferences, indicated by overlaps in the PCA. *V. myrtiloides* tend to occupy locations with more extreme soil conditions, and both species showed high variation among genotypes. Thus, spatial heterogeneity in soil properties might not be the driving force for species coexistence. Rather, differentiation in water and nutrient use, and variable environmental conditions across temporal scales could be more important factors in shaping the pattern of coexistence.

1.6 CONCLUSION

This study quantified the structural and physiological diversity of wild blueberries in a semi-natural agricultural system, which helped us understand both inter-genotype and intra-genotype variations. Further, the study provides evidence that general global leaf economic spectrum relationships are not always consistent with those observed globally at local scales within a farm across genotypes within the species. Importantly, changing environmental conditions act as a strong filter for shaping trait combinations. Additionally, I found overlaps in the soil nutrient traits of both species but a clear differentiation in water and nutrient use, which play vital roles in shaping coexistence and community composition. Our findings provide insights on wild blueberry diversity, coexistence, and how changing environmental conditions shape functional traits. This information can be important for the prediction of the community composition of these species especially in the face of rapidly changing environmental conditions.

CHAPTER 2: RESPONSES OF WILD AND HIGHBUSH BLUEBERRIES TO EXTREME DROUGHT: THRESHOLD OF COORDINATED DECLINES IN PHYSIOLOGICAL PROCESSES AND BRANCH DIEBACK

2.1 ABSTRACT

Although previous studies suggested that blueberry plants can resist drought, we lack a mechanistic understanding of the thresholds for irreversible declines in physiological function and plant dieback. How different population- varieties of blueberries respond to extreme drought is also not well-studied. As the frequency and severity of drought are increasing at an alarming rate in many places including North-eastern (NE) US, there is an increasing need to understand how different population- varieties of blueberries respond, and how different physiological processes respond to extreme drought conditions. Therefore, in this study, I investigated different physiological processes including turgor loss, stomatal conductance, photosynthesis rate, transpiration rate, photochemistry, and plant hydraulics in four different population- varieties of blueberries (two lowbush populations and two highbush varieties) native to NE US. The two lowbush populations (Ang 1 and Ang 2) and two highbush varieties (Bluecrop and Patriot) showed a coordinated response of all the physiological processes including stomatal conductance, photosynthesis rate, transpiration rate, photochemistry, and plant hydraulic systems under declining stem water potential (Ψ_{stem}; a measure of water status and xylem tension within the plant) and soil moisture conditions. Notably, all the studied population- varieties reduced their stomatal conductance to the minimum levels before the turgor loss point of nearly -2.0MPa and after turgor loss point there was a progressive decline of F_v/F_m along with the declining midday Ψ_{stem} and soil moisture conditions until plants experienced extreme drought at midday Ψ_{stem} of -4.0MPa to -4.5MPa and soil moisture of less than 5%. Importantly, F_v/F_m was unaffected before the turgor loss point but as soon as all the population- varieties reached turgor loss point of -2.0MPa, there was a quicker decline of the

maximum quantum efficiency of PSII (F_v/F_m) in Ang 2 and Patriot compared to Ang 1 and Bluecrop. Ang 1 and Patriot showed 100% loss of hydraulic conductivity (PLC), while the Ang 2 and Bluecrop reached 87% and 83% at the end of the 4-week-long drought. Ang 1 and Ang 2 populations had high regrowth of new stems from their underground rhizomes in the following season, indicating the resilience of these populations. Interestingly, all of the plant groups showed high branch mortality under Ψ_{stem} as low as -4.0MPa to -4.5 MPa indicating that these population- varieties were vulnerable to extreme drought. The results of this study not only allowed us to understand the drought response of these population- varieties but also allowed us to understand that the turgor loss point is a threshold beyond which declines in photochemistry, leaf shedding, hydraulic failure and plant mortality occurred, leaving some plants beyond recovery.

2.2 INTRODUCTION

Global climate change is increasing the likelihood of heatwaves, warmer temperatures, and drought that can have negative consequences to the plants (Eisenach, 2019). Drought is one of the most prevalent environmental factors that can lead to decreased gross primary productivity, carbon storage (Allen et al., 2010, McDowell and Allen 2015, D'Orangeville et al., 2018), and even lead to plant death (Allen et al., 2010) under extreme conditions. Maine has already experienced several incidences of drought between 1900 and 2000, but the occurrence during 2002 and 2003 was notably severe and had a substantial impact on the state of Maine (Maine's Climate Future, 2020). During this period, approximately 17000 private wells dried, most major surface-water reservoirs released water at levels below their regulatory minimum flows, instream flows for aquatic life were reduced, critical summer irrigation was limited and farmers in Maine lost more than 32 million dollars in crops (Lombard, 2004). Some wild blueberry growers recorded crop losses of 80 to 100 percent according to a Maine Department of Agriculture water-use survey to which 28 percent of Maine farmers responded (Maine Agricultural Water Management Advisory Committee, 2003). More recently, Maine experienced moderate to severe drought recently in both 2016 and 2020 (NOAA, 2020). As the climate warms, future droughts and periods of limited moisture are likely to worsen, with higher temperatures favoring increased drying as the average annual atmospheric temperature is expected to increase by 2 to 6^{o}C in Maine by 2100 (Jacobson et al., 2009). Climate models have predicted increased severity of both short- and long-term drought and extreme heatwave events that are expected to increase in frequency by two to three times (Wake et al., 2014) under the ongoing changes in global climate scenarios (IPCC 2007; Walter et al., 2011; Coumou & Rahmstorf 2012; Coumou et al., 2013; IPCC, 2013; Perkins-Kirkpatrick et al., 2017). Importantly, many plant species, including blueberries, may lack the adaptations necessary to withstand expected future drought conditions (Lienard et al., 2016). As the likelihood of extreme drought events is

expected to increase in frequency, there is a need to study the drought response in blueberries and the threshold for causing severe damage to them. The information derived from studying drought response in blueberries can help predict their responses to future drought, and lead to the development of more informed mitigation strategies for farmers.

Identifying the threshold for declines in a physiological process during dehydration is critical for understanding and predicting plant response to drought (Anderegg et al., 2017). Under mild drought conditions, some plants adjust stomatal conductance to avoid low water potentials (Sperry at al., 2016). As drought stress becomes more severe, plants are no longer able to maintain the balance between water loss and uptake, and as a result, turgor loss and xylem cavitation take place (Mingeau et al., 2000). As the drought stress becomes more severe, it is increasingly difficult for the plant to avoid dehydration. This results in substantial damage to the photochemical apparatus, and high levels of xylem embolism (Hoffmann et al., 2011) , and almost complete canopy loss, which often leaves the plants beyond recovery (Gauthey et al., 2021). Notably, a field study by Glass et al. (2005) using rainfall protectant shelters suggested that blueberry plants were able to maintain turgor and other physiological processes without affecting photosynthesis rate when they were exposed to a stem water potential of as low as -2.5 MPa. Although -2.5MPa stem water potential causes drought stress and turgor loss in many other crop species (Kaufmann and Levy, 1976; Smart 1974; West and Gaff 1976), blueberries in the study by Glass et al. (2005) were unaffected at this stem water potential. However, studies conducted by Ameglio et al. (2000) and Mingeau et al. (2000) on the most popularly cultivated highbush blueberry cultivar 'Bluecrop' found that under drought stress the plants reacted quickly by decreasing transpiration and stomatal conductance. In their study, as the drought progressed to severe conditions it resulted in stem cavitation or embolisms in the xylem vessels with cavitation threshold or P12 (xylem water potential causing 12% loss of conductivity) reaching only at -1.2 MPa. Ameglio et al. (2000) also found that the efficiency of their stomatal

regulation protected the plant from both runaway embolism and shrub drying. Bluecrop plants in the drought experiment by Ameglio et al. (2000) also showed a good aptitude at recovery after rehydration. According to Xu et al. (2010), depending upon the duration and the intensity of drought, application of a watering pulse could acclimatize plants to episodic drought or watering pulses by abandoning older plant parts and renewing their younger plant parts, and by promoting re-allocation of biomass especially starch into roots. However, whether or not the recovery process occurs in blueberry species and at what water potential values the drop-down of different physiological processes occur is not yet well understood.

Plants may respond differently under declining soil moisture conditions. When plants experience low water availability in the soil, they exhibit different stomatal responses with variable strategies; some tend to close their stomata earlier than others under increasingly negative water potential conditions and some keep their stomata open. Some species that maintain plant water status at relatively safe levels via stringent stomatal control (Bartlett et al., 2016, Fu & Meinzer, 2018) may be vulnerable to carbon depletion, especially under chronic drought conditions, while species that maintain open stomata and allow water potentials to drop close to critical thresholds of xylem cavitation may be more vulnerable to desiccation and catastrophic hydraulic failure (Blackman et al., 2019). Similarly, a low (more negative) turgor loss point (TLP) can allow the leaf to remain turgid despite decreasing leaf water potentials and thereby maintain photosynthesis, water transport, transpiration, and growth, conferring high drought resistance as a mechanism of desiccation tolerance (Larcher et al., 2003., Tyree et al., 2003). A high (less negative) TLP may also promote drought resistance by leading to early stomatal closure, enabling plants to maintain high water potentials and hydration even under declining soil water status, reflecting a mechanism of desiccation avoidance (Tyree et al., 2003; Sun et al., 2020). Additionally, species or cultivars with higher hydraulic conductivity can have higher transpiration and photosynthesis but tend to be susceptible to drought-induced

hydraulic failure in accordance with the widely reported trade-off between hydraulic efficiency and cavitation resistance (Martínez-Vilalta et al., 2002; Tombesi et al., 2014). How blueberries respond to much lower water potential conditions and the strategies they use to respond to extreme drought conditions are not yet well understood.

Blueberries are an important part of the agricultural industry in Maine which faces challenges such as increasing temperature anomalies and drought effects. Therefore, understanding how different population- varieties of blueberries will respond to future extreme drought conditions is an important consideration for the protection and management of an industry that carries a huge cultural significance. Importantly, most studies on drought in blueberries have focused on the exposure of these blueberry plants to medium level drought and studied effects on limited physiological processes (Ameglio et al., 2000; Percival et al., 2003; Glass et al., 2003; Glass et al., 2005). There is less knowledge on how extreme drought impacts other aspects of physiological processes, such as stomatal conductance, photosynthesis rate, transpiration rate, plant hydraulic conductance, turgor loss point, chlorophyll fluorescence, chlorophyll content, and leaf browning. Additionally, highbush blueberries may be more vulnerable to extreme drought conditions since they lack large rhizomatous growth and have larger vessels size and larger leaves to lose water through transpiration compared to lowbush blueberries. To investigate the response of different physiological processes to extreme drought conditions, I conducted a drought experiment to estimate the threshold of stem water potentials that would cause severe declines in high- and lowbush blueberries, in order to identify the potential differences in response to extreme drought. Our objectives were to 1) understand how extreme droughts would impact different population-varieties, and 2) quantify the threshold of stem water potential for declines in different physiological processes.

2.3 MATERIALS AND METHODS

2.3.1 STUDY DESIGN AND PLANT MATERIALS

To study the drought response of blueberry species native to NE US, I studied four blueberry population- varieties (Table 2.1), which I expected to have differences in drought response. A 5.5m length, 3.0m width and 2.1m high rectangular-shaped rainfall exclusion house was constructed on the University of Maine campus in Orono, Maine in July 2019, in which 40 individually irrigated plants were planted in individual 2-gallon buckets with seven 1-inch holes. For our study two populations of the wild blueberry species *Vaccinium angustifolium* (hereafter referred to as Ang 1 and Ang 2) and two varieties of cultivated highbush blueberry species *Vaccinium corymbosum* (hereafter referred to as Bluecrop and Patriot) were used. The plants were arranged in a randomized complete block design with five experimental blocks of eight plants each (40 plants in total; Figure 1.1A). Out of 40 plants from above mentioned population- varieties, 30 (10 each of Ang 1, Bluecrop, Patriot) of them were bought from the local nursery. The plants were imported from New Jersey by the nursery. The plants were then transplanted to a 2-gallon bucket that contained a potting mix of 4:2:1 ratio of peat: vermiculite: perlite (modification of Smagula, 1983). And 10 (Ang 2) of them were the field-grown population, which was dug to a 10cm depth from Blueberry Hill Farm, Jonesboro, Maine filled with soils and plants in a 2-gallon bucket. For the first year, these plants were allowed to grow in the open area without rainfall exclusion structure and experienced ambient rainfall and climatic conditions, and were each hand irrigated three times per week. I observed that some of our plants experienced winter damage at the tip of the stems due to extreme cold, but the rest of the plant parts had a good regrowth of new shoots, branches for our experiment. Plants that had high percentages of dead stems (approximately 90%) and few branches were excluded from our experiment (2 individually potted plants across all species).

Table 2.1: Study populations or variety, the species they belong to, their origin and the plants category as lowbush or highbush.

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

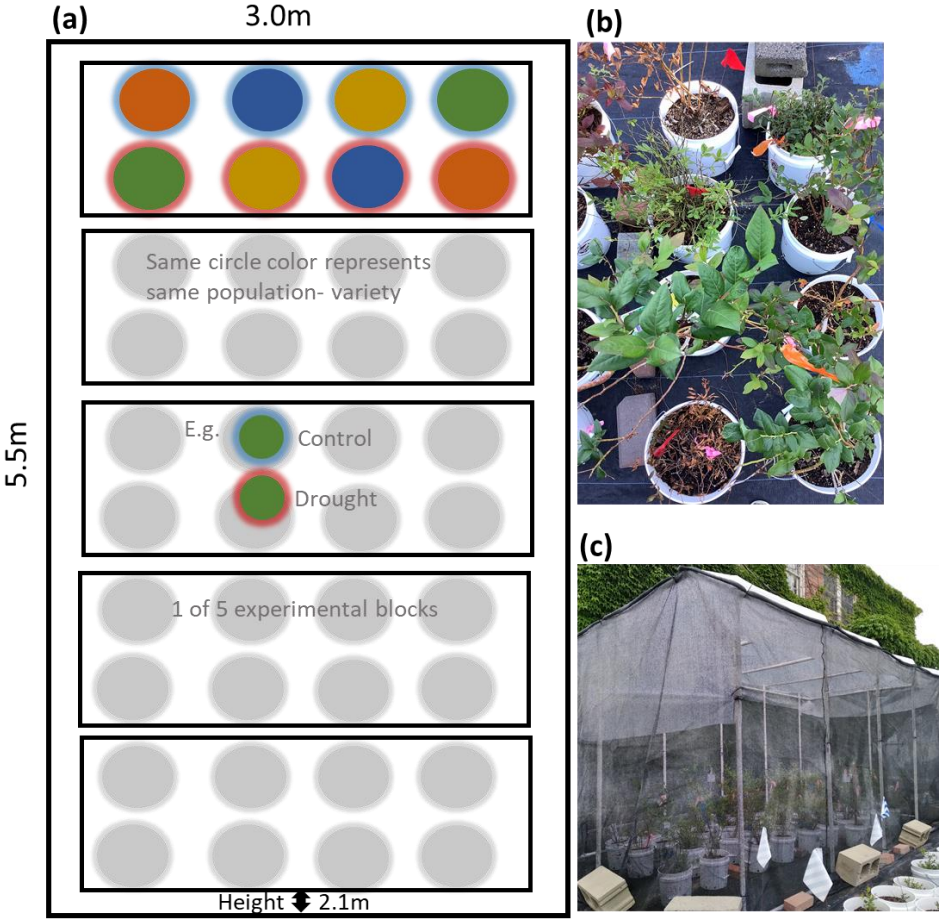


Figure 2.1 a) Experimental layout of 5.5m × 3.0m × 2.1m rainfall exclusion house. 40 plants were arranged in 5 blocks in a randomized complete block design (RCBD), with each population- varieties and treatment combination appearing once per block, for a total of 5 replicates per population- varieties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

2.3.2 EXPERIMENTAL TREATMENT

To understand the drought response of four blueberry population- varieties, I withheld water from them for one month (four weeks). The plants were arranged in a randomized complete block design with 5 experimental blocks, two treatments (control and drought) where four population- varieties were assigned in each block with each treatment combinations (Figure 2.1a). Each experimental block (Figure 2.1b) contained all four population- varieties and two individuals of each species, such that each block only had one individual replicate of each unique population- varieties and treatment combination. Population- varieties and treatment combinations were randomly arranged within each block. Blocking was done by size class to reduce the impact of natural variation in the initial sizes of plants. The experiment consisted of control plants, which were watered thrice per week. Drought-treated plants were allowed to dehydrate gradually by withholding water. To block the rainfall and impose the drought, a polyvinyl greenhouse covering was installed over the structure in 2020, and sides were covered by a black muslin cloth throughout the experiment that facilitated air circulation, and limited heating (Figure 2.1c). Weeds growing in containers with plants were removed weekly by cutting at the soil surface. The variation in temperature and relative humidity of the experimental site was recorded every 10 minutes with two weather station sensors ATMOS 14 (METER Group Inc. USA) connected to ZL6 data loggers (METER Group Inc. USA) that were placed on two different sides inside the rainfall exclusion structure throughout the experiment. Soil moisture (volumetric water content; VWC) was measured at every two days in each container using a soil moisture meter with 10 cm probes (TDR 150 Soil Moisture Sensor, Spectrum Technologies Inc., IL, USA) inserted at the soil surface.

In this study, I took measurements before, during, and after the drought treatment. Before the drought phase, data were recorded before 21 July at the peak of the growing season (hereafter

referred to as day -5), during the drought phase, data were recorded from 22 July (hereafter referred to as day 0) and after drought or at rehydration phase, data were recorded for mortality rate, regrowth of new stems and branches. Physiological changes were recorded at every one- or two-days interval starting from the first day of water withholding (day 0) to until plants experienced extreme drought conditions at day 31. Chlorophyll content was measured once per week for all the plant samples throughout the experiment using a SPAD- 502 chlorophyll meter (Konica-Minolta, Japan). All other destructive (predawn water potential; predawn Ψ_{stem}, midday stem water potential; midday Ψ_{stem}) and non-destructive (midday photosynthesis rate; A_{midday}, maximum and midday stomatal conductance (g_s); max g_s and midday g_s, F_v/F_m, soil moisture, phenology; leaf browning and leaf drop) measurements were measured at every one- or two-days throughout the experiment.

2.3.3 PRESSURE-VOLUME ANALYSIS

Pressure volume analysis was carried out before the start of the drought experiment for 6 samples per population- varieties. A small section of the terminal branch was enclosed inside a zip bag covered with aluminium foils the evening before the measurement day. Later the enclosed samples were cut in the early morning and the samples were taken 30-45 minutes after watering to obtain less negative leaf water potentials for initial measurements. Samples were transported to the laboratory within 10 minutes for measurement. The water potential of the leaf was measured using a pressure chamber (Model 1505D; PMS Instrument Company, Corvallis, OR USA) and weight was measured using a high precision analytical balance (RADWAG X2 PLUS, NE, USA). This process was repeated over time until at least eight points were obtained beyond the point at which zero turgor was detected. The leaf area of the sample was measured by a leaf area meter (LI-3100; Li-Cor Biosciences, Lincoln, NE, USA) and samples were oven-dried at 65 °C for 72 h. Pressure–volume curves were established by

plotting the inverse of leaf water potential (−1/Ψ) of each sample vs relative water content. From the pressure–volume curve, leaf water potential at turgor loss point (Ψtlp), and modulus of elasticity (ε) were calculated according to methods described by Bartlett et al., 2012.

2.3.4 SOIL MOISTURE CONTENT (%)

Soil moisture content was measured at midday for all the samples at the same time when plants were measured for leaf gas exchange and midday Ψ_{stem}. A TDR 150 (Spectrum Technologies Inc., IL, USA) soil moisture sensor was used for the measurement of soil relative water content and soil temperature.

2.3.5 Ψ_{STEM} AND CANOPY LEAF DEATH

Stem water potential (Ψ_{stem}) was measured at predawn (predawn Ψ_{stem}) and at midday (midday Ψ_{stem}) by cutting three leaves of each population- varieties selected randomly from each control and drought treated plants totaling 24 samples from five blocks at each sampling effort. Samples for the predawn Ψ_{stem} were collected at least one hour before the first light (3:30 AM - 5:30 AM) using a fresh razor blade, placed inside a cooler, and were immediately transported to the laboratory for water potential measurements using a pressure chamber (Model 1505D; PMS Instrument Company, Corvallis, OR USA). The samples for the midday Ψ_{stem} were measured using a non-transpiring leaf covered with a Ziplock bag that was wrapped with an aluminium foil and allowed to stabilize for 45 minutes to produce a non-transpiring leaf before being collected from 12:30 pm to 2:30 pm (Begg & Turner 1970; Nardini, Tyree & Salleo 2001; Sack, Cowan & Holbrook 2003; Bucci et al. 2004). Samples for the midday Ψ_{stem} were again cut using fresh razor blades, placed inside a cooler, and were immediately transported to the laboratory and then measured for water potential using the pressure chamber (Model 1505D; PMS Instrument Company, Corvallis, OR USA).

I also monitored browning and canopy leaf shedding for all the plants during the drought experiment where the rating was based on the whole plant. On each date, we gave each plant a rating of 0 (All green) to 6 (No leaves) based on the proportion of leaf canopy that was dead or brown; 0= All green, 1= 0-24 %, 2= 25-49 %, 3= 50-74 %, 4= 75-99 %, 5= 100 % brown, and 6= No leaves (Modified from Blackman et al., 2019).

2.3.6 LEAF GAS EXCHANGE AND QUANTUM YIELD OF PSII (F_V/F_M)

Daily maximum stomatal conductance (Max g_s) was measured by an SC-1 leaf porometer (METER Group Inc. USA), starting from 10:00 am to 11:00 am whereas midday stomatal conductance (Midday g_s) and midday photosynthetic rate (A_{midday}) was measured at 12:05 pm to 1:05 pm using LICOR-6800 (Li-Cor, Lincoln, NE, USA). The sampled plant used for the measurement of maximum g_s was later used for the measurement of A_{midday}, midday g_s and midday Ψ_{stem}. During the measurement of midday g_s and A_{midday}, leaves were subjected to 10 min of a constant light intensity with PPFD (photosynthetic flux density) at 1000 µmol m^{-2} s^{-1}. The air flow was turned on but temperature, CO_2 and H_2O control was turned off as I used ambient conditions for these measurements. A buffer bottle was used to avoid any CO_2 contamination from our breathing while taking the measurement. All the photosynthetic parameters were expressed on a projected leaf area basis and were later converted to mass-based dividing by Leaf mass per area (LMA).

The maximum quantum yield of PSII (F_v/F_m) was measured using a portable leaf fluorescence meter FluorPen (FP 110, Drásov, Czech Republic) with a modulated light source of 0.2 µmol m^{-2} s^{-1} at 660 nm and a saturation pulse from a white light-emitting diode with an intensity of 7700 µmol m^{-2} s^{-1} for a duration of 1.5 sec for all the control and drought-treated plants of all population- varieties. Measurements were performed on the adaxial surface at the middle part of the leaf blade, avoiding main veins for about 1.5 sec. Leaves were dark-adapted all night

and the measurement was done from 3:00 am to 3:45 am using a leaf clip to avoid the effects of nonphotochemical acute photoinhibition during measurements. The (F_v/F_m) was estimated as the ratio of variable to maximum fluorescence. F_v/F_m indicates the maximum efficiency at which light is absorbed by the PSII for reduction of the primary electron acceptor quinone molecule of PSII (Genty et al., 1989) and is used as an important indicator of drought resistance in plants.

2.3.7 PERCENTAGE LOSS OF HYDRAULIC CONDUCTIVITY (PLC) AND PLANT HYDRAULIC CONDUCTANCE (K_{PLANT})

Percentage loss of hydraulic conductivity (PLC) was measured for all the samples at the end of the drought experiment using single unbranched stem segments (Sperry et al., 1988; Lo Gullo & Salleo, 1991). Before cutting the samples for the hydraulic measurement, plants were watered early in the morning for 45 minutes, allowed them to uptake water and relax their water potential as the plants were under stress. To avoid possible artifacts of cutting under tensions I took a petri dish filled with water close to the ~3cm to 4.5cm long stem section and the stem was cut under water using fresh razor blades. The cut samples were then placed inside the cooler filled with water and were transported to the laboratory in submerged condition. During the measurement in the lab, the excised stem segment was submerged underwater, and 1 cm sections were trimmed from both segment ends with a fresh razor to eliminate potentially air-filled conduit elements (Zimmerman, 1983). While still submerged, the proximal end of the stem section (~0.5 cm) was securely attached to a tubing and the water in the tubing was replaced by a 2 mmol KCL solution prepared with deionized degassed distilled water filtered at 0.22 μm. The stem section with the tubing was then attached to the hydraulic apparatus where the deionized distilled water was flowing from the pressure head or height of ~13 cm or 14 cm via stem section and to the balance for recording the flow rate. The flow rate was recorded using a BC Wedge software (V. 1.0, TAL Tech Inc. USA) that communicates with a high

precision (accurate to 0.01 mg) analytical balance (RADWAG X2 PLUS, NE, USA) and records the flow rate at every 2 sec intervals. Then, any embolism was removed by flushing the stem for 10 min at constant pressure (1.5 bar). Native and the maximum flow rate was calculated from the slope of initial and background and maximum and background. Finally, Percentage loss of conductivity (PLC) was calculated by:

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

Where, K_{max} is maximum hydraulic conductivity, Knat is native hydraulic conductivity and PLC is the percentage loss of hydraulic conductivity. All the plants from both the control and drought treatment were measured for PLC after which the plants were allowed to rehydrate.

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

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

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

2.3.8 MORTALITY AND RESPROUTING

After the drought experiment was completed in 2020, plants were rehydrated and left outside to experience winter dormancy. Then I evaluated the mortality rate and resprouting of new stems based on individual and stem basis in June 2021, nine months after the termination of drought. On an individual basis, I first counted the number of pots with completely dead plants and pots with living plants to calculate the individual mortality rate. Plants with the living basal part that had growth of new leaf buds were also counted as living although the apical part was

dead. On a stem basis, I counted completely dead stems, completely new stems growth (new sprouts), and new branches growth in each pot and calculated both stem mortality rate, completely new stem regrowth rate, and new branches regrowth rate.

2.3.9 STATISTICAL ANALYSIS

To test the significant difference between plant height, stem diameter, leaf size, leaf mass per area of different population- varieties, I used one-way ANOVA. To determine the rate of change in soil moisture, predawn and midday $\Psi_{stem,}$ A_{midday}, maximum and midday g_s, K_{plant}, E, F_v/F_m, leaf browning, and leaf drop among population- varieties and treatments over the course of the experiment, the graph was plotted using the R package 'ggplot 2', 'lubridate' (Spinu et al. 2021), 'dplyr' (Wickham et al. 2018) with species and day of drought as covariates. I then fitted the relationship between soil moisture and stem water potentials (midday Ψ_{stem} or predawn Ψ_{stem}) using negative exponential models. To determine the pattern of A_{midday}, maximum and midday g_s, K_{plant}, E, F_v/F_m, leaf browning and leaf drop among population-varieties and treatment as a function of predawn and midday Ψ_{stem} was plotted using the R package 'ggplot 2', 'lubridate' (Spinu et al. 2021), 'dplyr' (Wickham et al. 2018), 'multcomp' (Hothorn et al. 2021). Also, the turgor loss point line was fitted in the above-mentioned relationships using R v.4.0.3 (R core team 2021). To determine the difference in PLC, stem level mortality rate, individual-level mortality rate, stem level resprouting rate, individual-level resprouting rate, branch level regrowth rate, individual-level branch regrowth rate among the different population- varieties and treatment, a bar graph was plotted taking the mean of five samples and fitting error bars using R package 'ggplot 2'. The graph of temperature and VPD with time was plotted in Excel taking the averages of an hourly record of the parameters. Data for all traits were taken averages from at least three data points for the standard error values that are in each graph.

2.4 RESULTS

2.4.1 SOIL MOISTURE AND Ψ_{STEM} DECLINE DURING DEHYDRATION

During the first day of the drought (day 0) all the population- varieties had similar soil moisture content (30-40%) and midday stem water potentials (midday Ψ_{stem}; ~-1.0MPa) in both control and drought treatment (Figure 2.2a, 2.2b). As the drought progressed, population- varieties under the drought treatment appeared to drop both its soil moisture and midday Ψ_{stem} and reached ~0% and ~4.5MPa respectively by the day 31 of drought treatment (Figure 2.2a, 2.2b). From the soil moisture and day of experiment graph, Patriot and Ang 2 dropped their soil moisture quickly, while Bluecrop and Ang 1 lost their soil moisture relatively slowly. Although Patriot and Bluecrop are taller and thicker stem diameter variety as compared to smaller and thinner Ang 1 and Ang 2 populations (Table A2.3), Bluecrop lost its soil moisture later and Ang 2 lost earlier irrespective of their size difference. Also, Patriot reached ~0% soil moisture on day 21, when all others were still maintaining higher soil moisture (~3%) on this date (Figure 2.2a). At the end of the drought experiment on day 31, Ang 1 maintained the soil moisture at 0.8%, Ang 2 maintained at 0.78%, Bluecrop maintained at 0.84%, while Patriot was at 0%.

Similarly, at the starting of the drought, all the population- varieties maintained high $_{midday}\Psi_{stem}$ at the range of -0.6MPa to -1.0MPa. On the last day of the drought treatment (day 31), midday Ψ_{stem} of Patriot and Ang 2 dropped to -4.5MPa and -4.06MPa, respectively, whereas Ang 1 and Bluecrop appeared to drop their midday Ψ_{stem} to -3.9MPa and -3.6MPa, respectively (Figure 2.1a, 2.2b). Although Patriot and Ang 2 appear to drop their midday Ψ_{stem} quickly together with soil moisture compared to Bluecrop and Ang 1, Patriot lost its turgor together with Ang 2 and Bluecrop on day 21, respectively. And Ang 2 lost its turgor on day 17- 18, which was earlier than all others (Figure 2.2b). After the turgor loss, there were sharp drops in midday Ψ_{stem} across all population- varieties and reached midday Ψ_{stem} of nearly -4.5MPa at the end of the drought treatment.

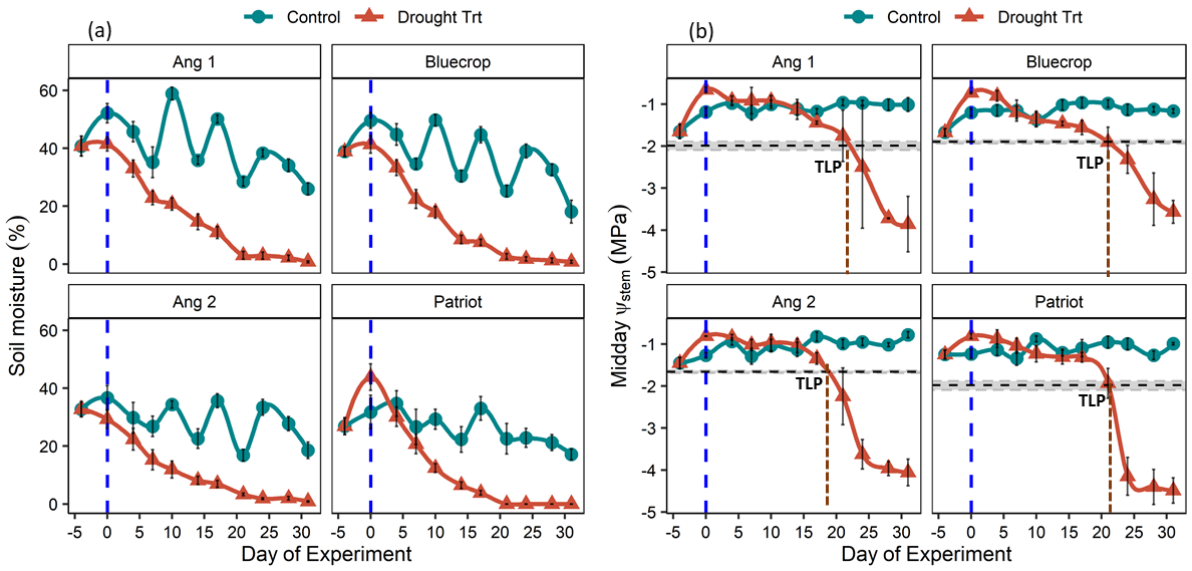


Figure 2.2 Changes in soil moisture (%; a) midday stem water potential, midday Ψ_{stem} (MPa; b) as a function of the day of the experiment (DOE) recorded in the control treatment (blue dots and line) and the drought treatment (red triangle and line) of each population- varieties: Ang 1, Ang 2, Bluecrop, and Patriot, during the course of the drought experiment. The blue dotted lines mentioned are the first day of the start of the drought experiment, represented by day 0, the intersection of black and brown dotted lines mentioned are the Turgor Loss Point, represented by TLP and light grey background bounded with dashed grey lines are the mean TLP ± SE values. Values are means ± SE for stem water potential (n = 3) and soil moisture (n = 5).

2.4.2 STEM WATER POTENTIALS (Ψ_{STEM}) RESPONSE TO SOIL MOISTURE DURING DEHYDRATION

Blueberry population- varieties under high soil moisture (30-40%) maintained high predawn Ψ_{stem} at the range of -0.19 and -0.5 MPa and midday Ψ_{stem} at the range of -0.6 and -1.0 MPa for all population- varieties (Figure 2.3a, 2.2b). As the soil moisture declined to 20%, the midday Ψ_{stem} and predawn Ψ_{stem} of blueberries population- varieties also started to drop to below -1.5 MPa and -1.0 MPa, respectively. Predawn and midday Ψ_{stem} of Ang 2 started to drop earlier and reached turgor loss point at only -1.66 MPa and at 7% soil moisture on day 17-18 followed by Bluecrop, Patriot, and Ang 1 which reached turgor loss point at -1.90MPa, -1.98MPa and -1.99MPa respectively at less than 5% soil moisture on day 21 (Figure 2.3a, 2.3b). Patriot lost its turgor later than Ang 2, and both recached -4.5MPa and -4.06MPa

midday Ψ_{stem} when soil moisture was 0% in Patriot and 0.08% in Ang 2 on day 31. The midday Ψ_{stem} of Bluecrop and Ang 1 declined relatively slower after turgor loss point compared to Patriot and Ang 2 and reached -3.6MPa and -3.9MPa at 0.8% soil moisture on day 31. The turgor loss points were reached when soil moisture was around 5% for all population-varieties.

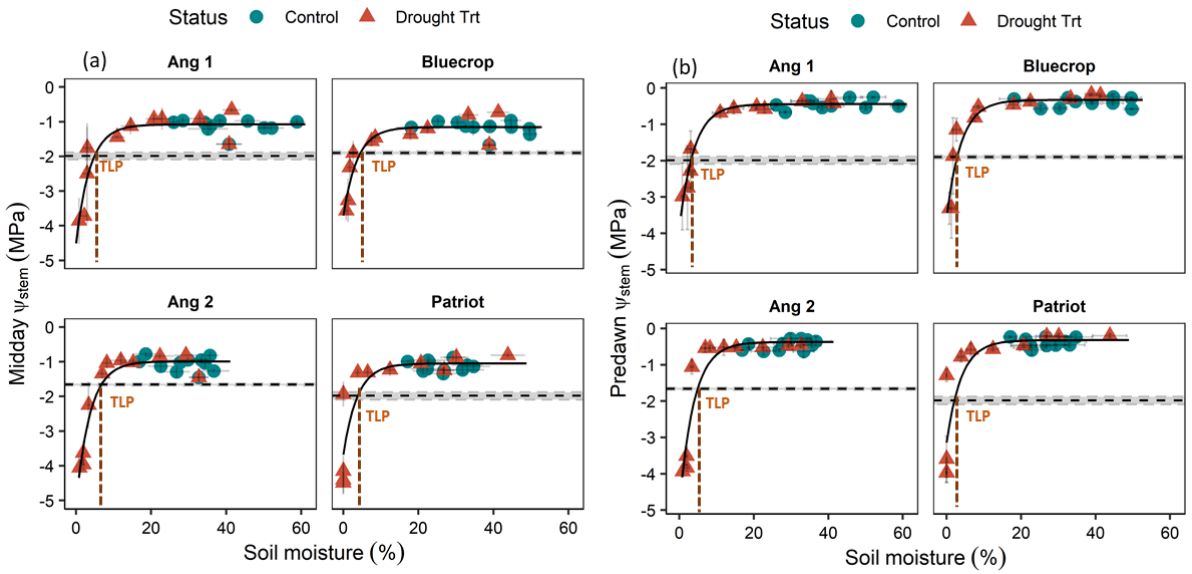


Figure 2.3 The midday stem water potential, midday Ψ_{stem} (MPa; a) and the predawn stem water potential, predawn Ψ_{stem} (MPa; b) as a function of soil moisture in the control treatment (blue dots) and drought treatment (red triangle) of each population- varieties: Ang 1, Ang 2, Bluecrop, and Patriot. Negative exponential models (solid black line) were fit for each study population- varieties mentioned above. The intersection of black and brown dotted lines mentioned are the Turgor Loss Point, represented by TLP and light grey background bounded with dashed grey lines are the mean TLP ± SE values. Values are means ± SE for midday and predawn Ψ_{stem} (n=3) and soil moisture (n = 5).

2.4.3 MINIMUM LOSS OF PHYSIOLOGICAL PROCESSES BEFORE TLP, THEIR PROGRESSIVE DECLINE AFTER TLP, AND THE ASSOCIATED WATER POTENTIALS AND SOIL MOISTURE

Midday and maximum stomatal conductance (Midday $g_{s,}$ Max g_s), midday photosynthesis rate (A_{midday}), transpiration rate (E), plant hydraulic conductance (K_{plant}) dropped to their minimum values before or during the occurrence of turgor loss (TLP) in all population- varieties of blueberries (Table 2.2). However, the photosynthetic efficiency of PS II (F_v/F_m) did not show declines at TLP for Ang 2, Bluecrop, Patriot but had started to decline for Ang 1 and reached 0.7. Leaf browning in Ang 1 had already reached 60% at TLP (-1.99MPa). The decline in F_v/F_m and leaf browning of Ang 1, occurred on day 21 of drought treatment when midday Ψ_{stem} and soil moisture were -1.76MPa and 3.02%. Ang 2 lost its turgor earlier (day 17-18) than all other at -1.66MPa; leaf browning had reached 20%, soil moisture was 7%, midday Ψ_{stem} was only -1.34MPa. After the turgor loss point, both Ang 1 and Ang 2 increased their leaf browning and leaf dropping progressively.

Bluecrop and Patriot lost their turgor at -1.90MPa and -1.98MPa. When they lost their turgor on day 21, soil moisture had already reached 0% in Patriot and 2.66% in Bluecrop. However, F_v/F_m does not seem to be affected in both the varieties at TLP, but leaf browning had reached 20% in Patriot and in Bluecrop browning had not started. Bluecrop and Patriot, after the TLP, increased their leaf browning and leaf dropping progressively. Thus, in all the blueberries population- varieties, after turgor loss point, F_v/F_m started to decline, while leaf browning and leaf dropping also started to increase rapidly.

Table 2.2 The minimum loss of midday stomatal conductance (Midday g_s), maximum stomatal conductance (Max g_s), midday photosynthesis rate (A_{midday}), transpiration rate (E), plant hydraulic conductance (K_{plant}), leaf browning (Leaf Br), photosynthetic efficiency of PS II (F_v/F_m) before the occurrence of Turgor Loss Point (TLP), TLP values and TLP± SE values, midday Ψ_{stem} of minimum loss of midday g_s, A_{midday}, E, F_v/F_m, leaf browning before TLP, soil moisture and day of the drought treatment of minimum loss of midday g_s, A_{midday}, E, F_v/F_m, leaf browning before TLP in each population- varieties: Ang 1, Ang 2, Bluecrop, and Patriot.

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

2.4.4 STOMATA RESPONSE TO WATER POTENTIALS AND SOIL MOISTURE DURING DEHYDRATION

Maximum and midday g_s were highly sensitive to declining predawn and midday Ψ_{stem} and soil moisture. At high soil moisture (30-40%) and the midday Ψ_{stem} of nearly -1.0MPa, midday g_s was at the high values of ~0.27 mmol m^{-2} s^{-1} for all populations or variety (Figure 2.4a). Midday g_s appeared to decline linearly with the decline in midday $\Psi_{stem.}$ By the time midday Ψ_{stem} had reached TLP (< 5% soil moisture), midday g_s declined to the minimum values; 0.03 mmol m^{-2} s^{-1} in Bluecrop and Patriot, 0.04 mmol m^{-2} s^{-1} and 0.07 mmol m^{-2} s^{-1} in Ang 1 and Ang 2 respectively (Figure 2.4a, c).

Maximum g_s also declined linearly with the decline in predawn Ψ_{stem} and soil moisture. At high soil moisture (30-40%) under the predawn Ψ_{stem} of nearly -0.5MPa, maximum g_s was at the high values of ~0.37 mmol m^{-2} s^{-1} for Bluecrop, Patriot, and Ang 2 while maximum g_s was ~0.23 mmol m^{-2} s^{-1} for Ang 1 (Figure 2.4b). When predawn Ψ_{stem} reached the turgor loss point (< 5% soil moisture), maximum g_s declined to the minimum values (0.33 mmol m^{-2} s^{-1} in Bluecrop and Patriot, 0.19 mmol m^{-2} s^{-1} and 0.26 mmol m^{-2} s^{-1} in Ang 1 and Ang 2 respectively; Figure 2.4b, d).

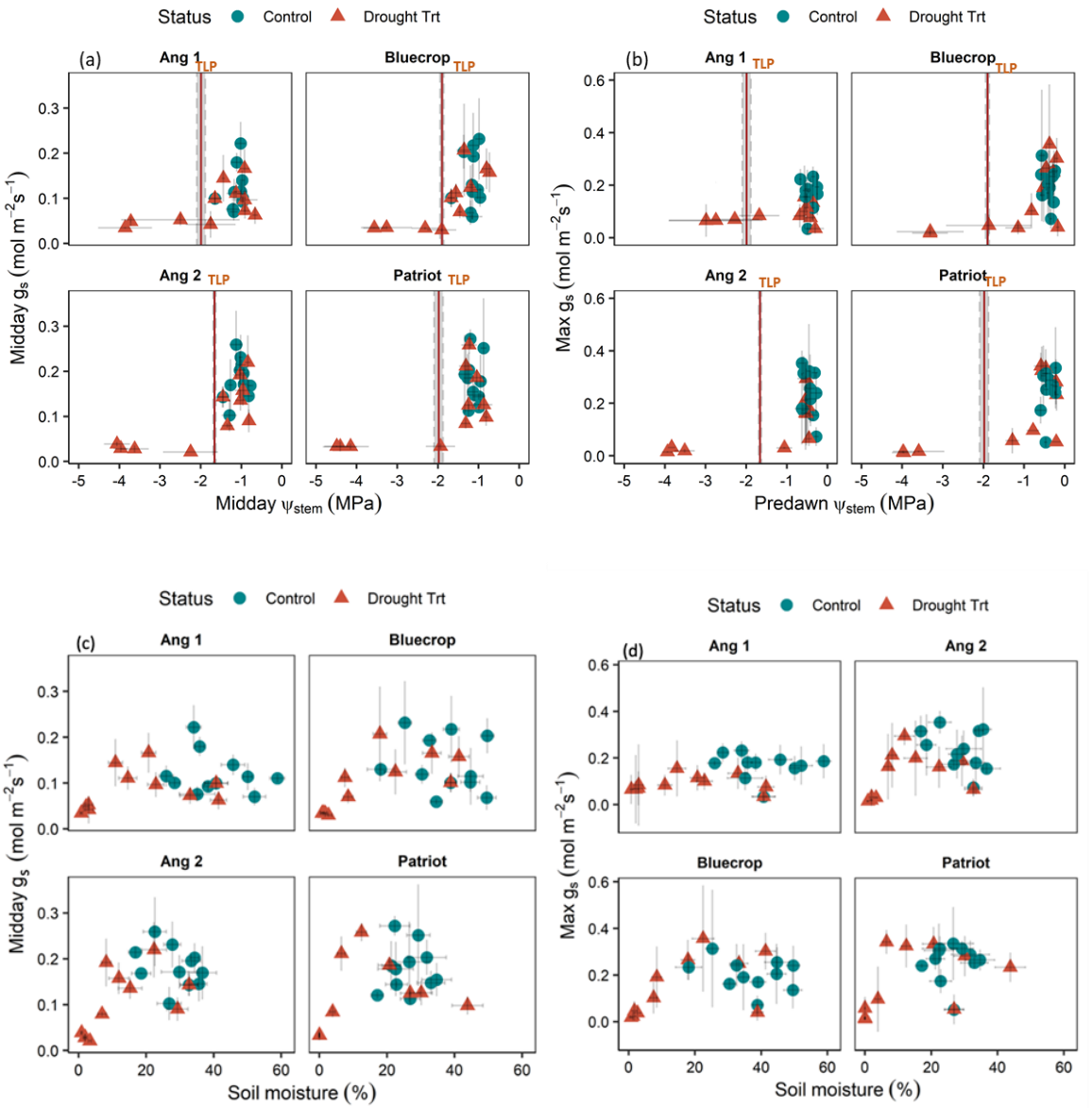


Figure 2.4 The midday stomatal conductance, Midday g_s (mol m^{-2} sec^{-1}) as a function of midday stem water potential, midday Ψ_{stem} (MPa; a), maximum stomatal conductance, Max g_s (mol m^{-2} sec^{-1})as a function of predawn water potential, Predawn Ψ_{stem} (MPa; b), and midday stomatal conductance, Midday g_s (mol m^{-2} sec^{-1}; c) and maximum stomatal conductance, Max g_s (mol m^{-2} sec^{-1}; d) as a function of soil moisture (%) in the control treatment (blue dots) and drought treatment (red triangle) of each population- varieties: Ang 1, Ang 2, Bluecrop, and Patriot. In (a) and (b), light grey background bounded with dashed grey lines represents mean TLP ± SE values and the brown solid lines in the middle are Turgor Loss Point, represented by TLP for the corresponding population- varieties. Values are means ± SE for midday and predawn g_s, midday and predawn Ψ_{stem} (n=3) and soil moisture (n = 5).

2.4.5 RESPONSE OF PLANT HYDRAULIC CONDUCTANCE TO DEHYDRATION

K_{plant} was also very sensitive to the decline in midday Ψ_{stem}. With the decrease in midday Ψ_{stem}, K_{plant} reached minimum when midday Ψ_{stem} reached -1.3MPa for Ang 2, -1.76MPa for Ang 1,

and -1.9MPa for Bluecrop and Patriot, which were before the occurrence of TLP (Figure 2.5; A2.1d).

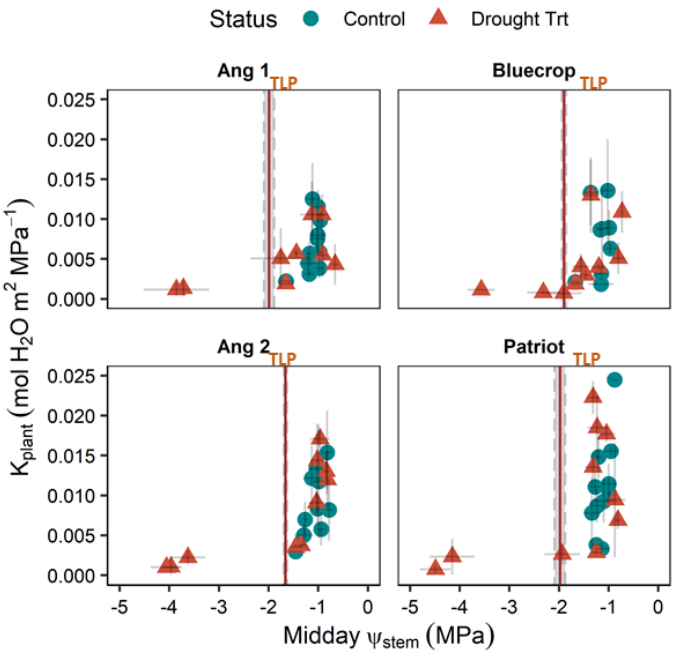


Figure 2.5 Plant hydraulic conductance, K_{plant} (mmol H_2O m^2 MPa^{-1}) as a function of midday stem water potential, midday Ψ_{stem} (MPa) in the control treatment (blue dots) and drought treatment (red triangle) of each population variety: Ang 1, Ang 2, Bluecrop, and Patriot. Light grey background bounded with dashed grey lines represents mean TLP± SE values and the brown solid lines in the middle are Turgor Loss Point, represented by TLP for the corresponding population- varieties. Values are means ± SE (n=3) for midday Ψ_{stem} and K_{plant}.

2.4.6 RESPONSE OF PSII TO WATER POTENTIAL AND SOIL MOISTURE DURING DEHYDRATION

F_v/F_m was not sensitive to declining midday Ψ_{stem} and soil moisture at the initial stage before TLP. At high soil moisture (30-40%) and before TLP, F_v/F_m was at the high values of 0.8 for all populations or variety (Figure 2.6a, b). At TLP, these populations still seemed to maintain high F_v/F_m values i.e., Ang 1 had 0.7 F_v/F_m and Bluecrop, Ang 2, Patriot had 0.8 F_v/F_m values (7% soil moisture on day 17-18). After TLP or the range of TLP, there was a progressive decline of F_v/F_m values across all population- varieties i.e., Patriot and Ang 2 reached 0.14 F_v/F_m values at -4.5MPa midday Ψ_{stem} when soil moisture was less than 5%. But Blucerop and

Ang 1 reached F_v/F_m values of 0.4 at -4.0MPa midday Ψ_{stem} when soil moisture was less than 5%.

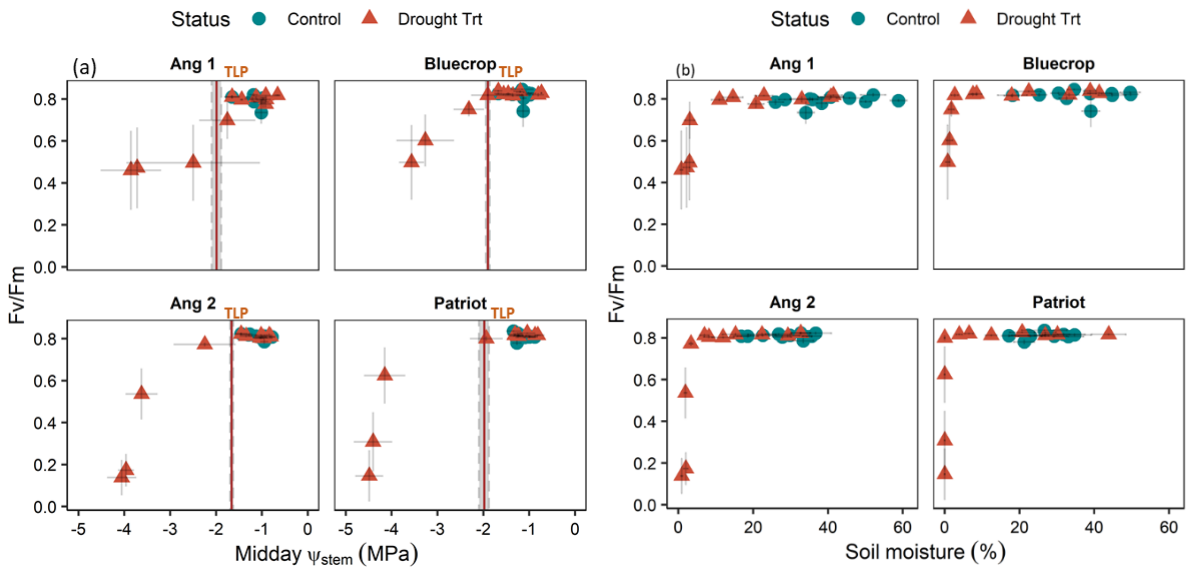


Figure 2.6 Maximum photochemical efficiency of PSII (F_v/F_m) as a function of midday stem water potential, midday Ψ_{stem} (MPa; a), and soil moisture (%; b) in the control treatment (blue dots) and drought treatment (red triangle) of each population- varieties; Ang 1, Ang 2, Bluecrop, and Patriot. Light grey background bounded with dashed grey lines represents mean TLP± SE values and the brown solid lines in the middle are Turgor Loss Point, represented by TLP for the corresponding population-varieties. Values are means ± SE for F_v/F_m (n = 5) and soil moisture and for midday Ψ_{stem} (n = 3).

2.4.7 LEAF PHOTOSYNTHETIC RATE IN RELATION TO STOMATAL CONDUCTANCE DURING DEHYDRATION

A_{midday} was significantly and positively related with midday g_s in all the population- varieties (Figure 2.7). Thus, the decline in A_{midday} during the drought was closely related to declines in g_s.

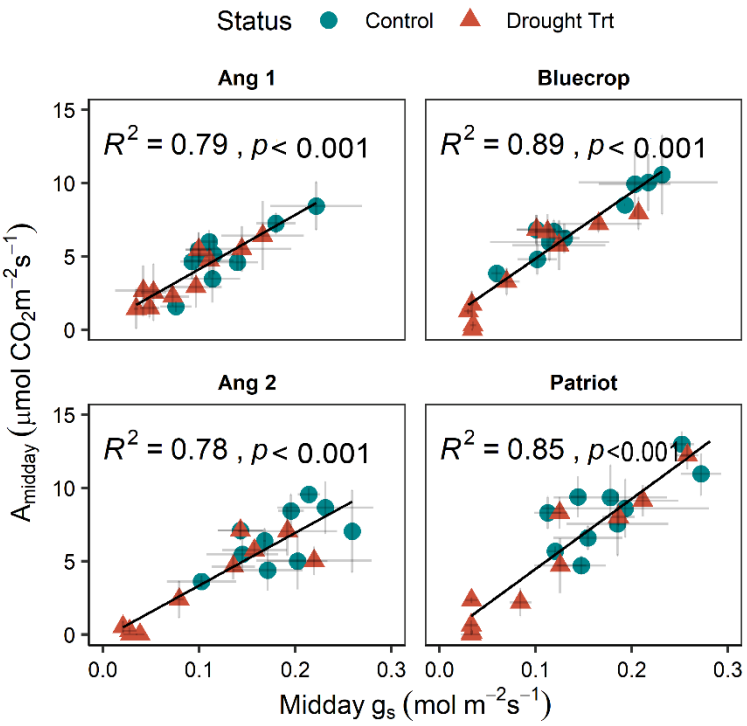


Figure 2.7 Midday photosynthesis rate, A_{midday} (µmol CO_2 m^{-2} sec^{-1}) as a function of midday stomatal conductance, Midday g_s (mol m^{-2} sec^{-1}) in the control treatment (blue dots) and drought treatment (red triangle) of each population- varieties: Ang 1, Ang 2, Bluecrop, and Patriot. Data are means ± SE (n=3).

2.4.8 RESPONSE OF LEAF BROWNING TO WATER POTENTIALS DURING DEHYDRATION

Leaf browning in Ang 1 and Ang 2 started early (day17- 18) at -1.5MPa midday Ψ_{stem} and reached 60% and 20% before TLP (Figure 2.8a, A2.3a). Leaf browning in Patriot appeared to start at -2.0 MPa midday Ψ_{stem} (day 21) which is close to TLP (-1.98 MPa). In Bluecrop, leaf browning did not occur before TLP until day 25 of the drought experiment (Figure 2.8a, A2.3a). However, after TLP, leaf browning seemed to increase rapidly in all the population- varieties and reached almost 100% in Ang 1, 75% in Ang 2 and Patriot, and 40% in Bluecrop at the end of the drought treatment (Figure 2.8a).

Similarly, leaf dropping was also detected as midday Ψ_{stem} declined in all blueberry population-varieties. Ang 1 appeared to start leaf dropping at -1.7MPa midday Ψ_{stem}, which was before the

TLP. For Ang 2 and Patriot, leaf dropping did not occur before TLP until day 27 of the drought treatment and only occurred when midday Ψ_{stem} was -4.0MPa and -4.5MPa, respectively on day 30 of the drought treatment (Figure 2.8b, A2.3b). Bluecrop did not drop leaves even at the end of the drought treatment.

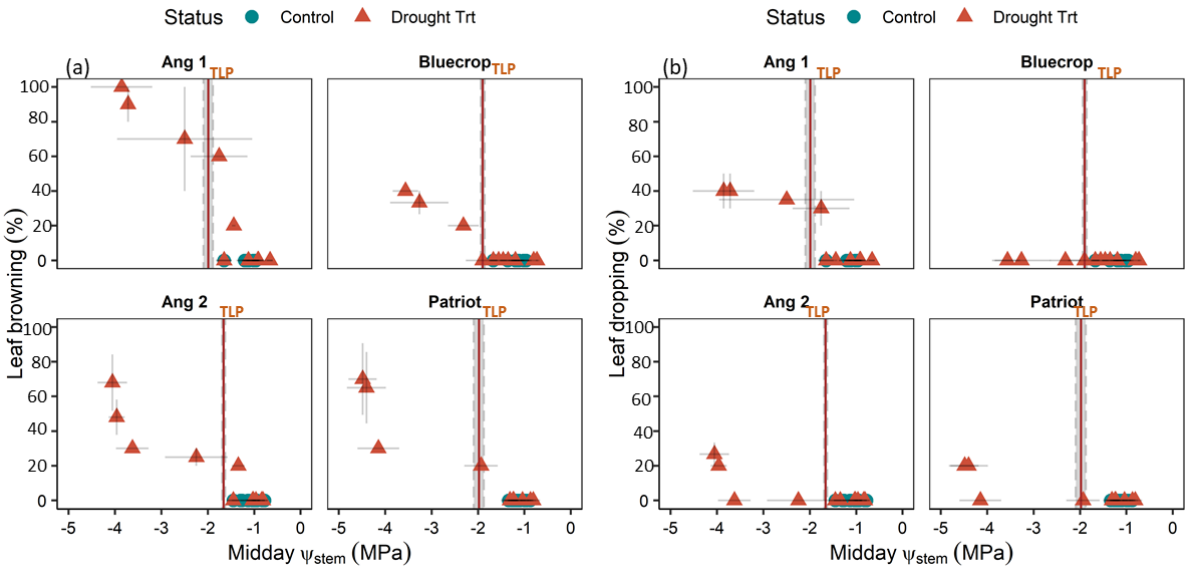


Figure 2.8 Leaf browning as a function of midday stem water potential, midday Ψ_{stem} (MPa; a), and leaf dropping as a function of midday stem water potential, midday Ψ_{stem} (MPa; b), in the drought treatment (red triangle) of each population- varieties: Ang 1, Ang 2, Bluecrop, and Patriot. Light grey background bounded with dashed grey lines represents mean TLP ± SE values and the brown solid lines in the middle are Turgor Loss Point, represented by TLP for the corresponding population- varieties. Values are means ± SE for midday Ψ_{stem} (n=3) and n= variable number for leaf browning and leaf dropping depending upon the color change and leaf dropping pattern. Numbers on Y axis are the percentages for leaf browning and leaf dropping from 0% to 100% where 0= All green and 100% is all brown leaves.

2.4.9 PLC, PLANT MORTALITY, AND REGROWTH DURING DEHYDRATION AND AFTER RECOVERY

At the end of the drought treatment, PLC in Ang 1 and Patriot was close to 100%, which is higher than Ang 2 and Bluecrop that maintained PLC at 86.7% and 83.27%, respectively (Figure 2.9). Although Ang 1 and Patriot had high PLC, only Ang 1 had high individual mortality (40%) and stem mortality rate (60.1%). In contrast, Patriot had a high stem mortality rate (84.3%) and a low individual mortality rate (20%; Figure 2.10a, b). Bluecrop and Ang 2

showed an individual mortality rate of 20%. The stem mortality rate was 81% in Ang 2 and 68.3% in Bluecrop (Figure 2.10a, b).

Regrowth of new stem and branches in blueberries population- varieties were detected. Regrowth of new stems from rhizomes underneath the soil was found in Ang 1 and Ang 2, and regrowth of new branches in Patriot and Bluecrop occurred from the living parts of the stems. Ang 1 and Ang 2 showed 40% and 80% individual regrowth rate, and 10% and 11% stem regrowth rate (Figure 2.10c, d). Aside from the growth of completely new stems, Patriot and Bluecrop showed regrowth of completely new branches and showed 80% and 40% individual level branch regrowth rate and 15.5% and 10.2% branches regrowth rate respectively (Figure 2.10e, f).

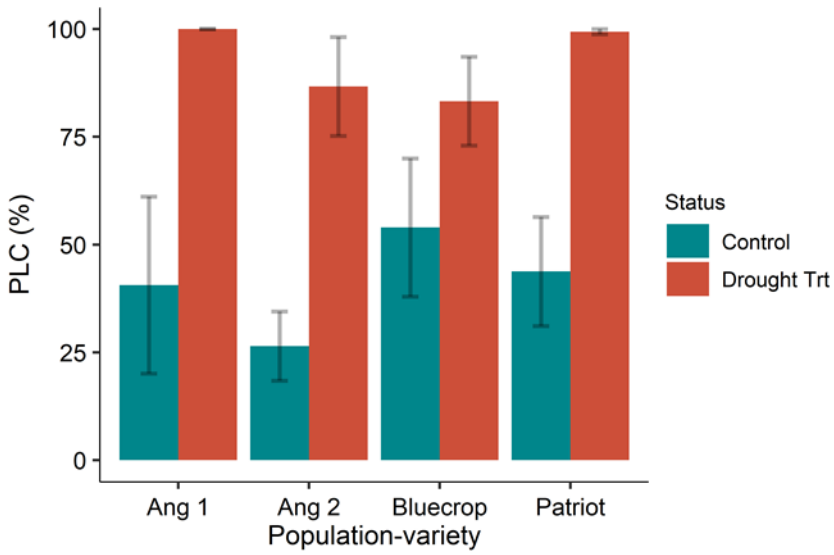


Figure 2.9 Percentage loss of hydraulic conductivity (PLC) of each population- varieties: Ang 1, Ang 2, Bluecrop and Patriot at the end of the drought experiment. The blue bars represent control treatment and red bars represent drought treatment. Values are means ± SE (n=5) for PLC in both control and drought treatment.

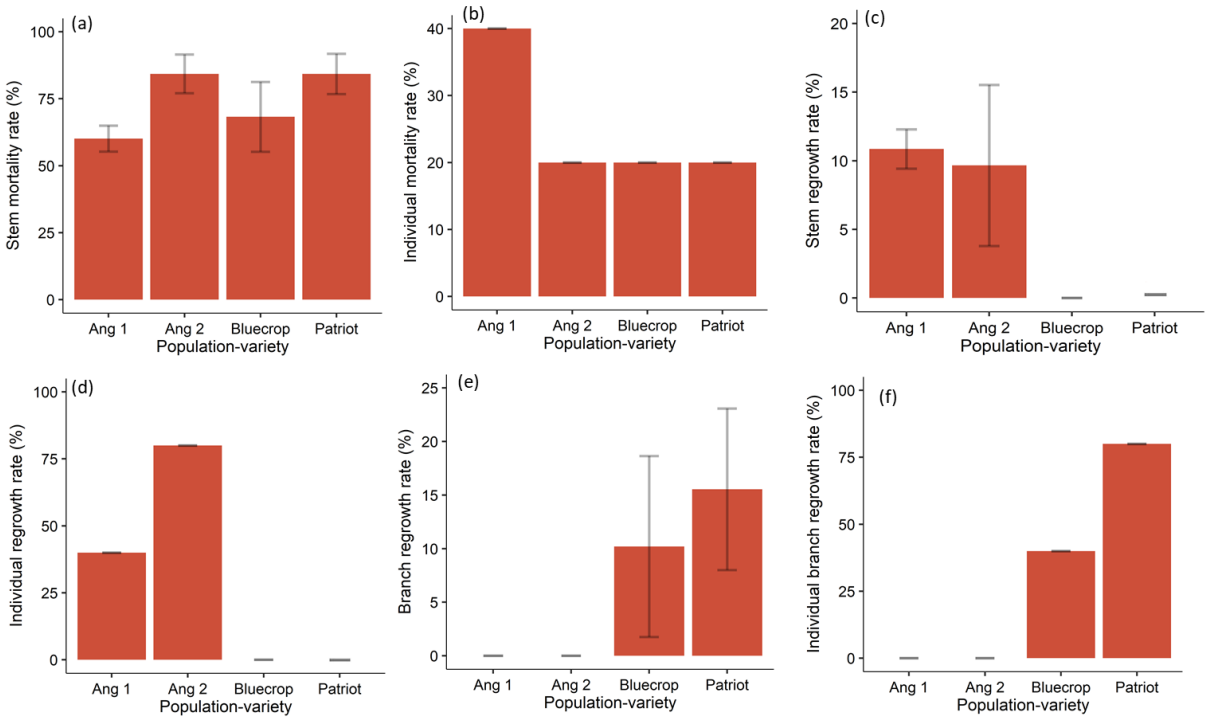


Figure 2.10 Stem mortality rate (a), individual mortality rate (b), stem regrowth rate (c), individual level stem regrowth rate (d), branch regrowth rate (e), individual level branch regrowth rate (f) of each population- varieties: Ang 1, Ang 2, Bluecrop and Patriot at the end of the drought experiment. Red bars represent drought treatment across all graphs. The controls of all population- varieties are not plotted from as they never reported mortality rate and regrowth is taking place as a continuous process. Values are means ± SE (n = 5) traits.

2.5 DISCUSSION

Our study revealed quick declines in stomatal conductance, photosynthesis, and water loss before the turgor loss point (TLP), and the progressive decline of photochemistry, leaf browning, and leaf dropping after the TLP as Ψ_{stem} and soil moisture declined across all population- varieties of blueberries. Thus, TLP is a threshold for all population- varieties after which there was a progressive decline. The stomata of all population- varieties were very sensitive to declining Ψ_{stem} and soil moisture. Interestingly, the leaf browning increased after TLP, which coincided with declines in F_v/F_m showing a coordinated response. Blueberry population- varieties showed 83 to 100% loss of hydraulic conductivity (PLC) and high mortality rates when Ψ_{stem} reached -3.6 to -4.5 MPa. However, these population- varieties showed high regrowth of new stems and branches. In addition, Ang 1 experienced 100% PLC,

and the highest mortality rate but demonstrated a high regrowth of new stems from the rhizomes post- drought. Our results provide important insight regarding blueberry response to extreme drought and drought-induced damages before and after TLP. This information is critical for understanding the response of blueberries to extreme drought conditions especially in preparation for a future in which drought events are expected to increase in frequency all over North-eastern US (Wake et al. 2014).

2.5.1 TURGOR LOSS POINT AS A PROXY OF DROUGHT STRESS AND THRESHOLD FOR THE DECLINE OF DIFFERENT PHYSIOLOGICAL PROCESSES

Maximum and midday g_s, A_{midday}, E, and K_{plant} in the studied blueberry population- varieties were all sensitive to declines in Ψ_{stem} and reached minimum values before or at TLP. This demonstrates that all of them adopted a more drought-avoidance strategy to avoid water stress using sensitive stomatal control. The loss of leaf turgor pressure is recognized as the initial stage of leaf wilting, and that the loss of guard cell turgor is related to stomatal closure (Cowan, 1977). The downregulation of stomatal conductance and several other physiological processes as well as turgor loss in blueberry population- varieties might serve as a protective mechanism against xylem cavitation or xylem embolism (Hochberg et al., 2017). Davies and Johnson (1982) reported a critical water potential of -2.2 MPa in *Vaccinium ashei* Reade (Rabbiteye blueberry) for stomatal closure, which is close to the TLP in our study. Also, leaf browning had already started in Ang 1 and Ang 2 populations at or around the range of TLP, suggesting high sensitivity of leaf tissues in *Vaccinium angustifolium* species to declines in water potentials.

In contrast, leaf biochemistry indicated by F_v/F_m was not sensitive to drought at the initial stages or before the occurrence of TLP at -2.0MPa in Bluecrop, Patriot and Ang 2. Ang 1 dropped its F_v/F_m slightly and reached 0.7, along with an increase of leaf browning to 60% at TLP. In Patriot and Ang 2, leaf browning reached 20% at TLP, while Bluecrop did not initiate

leaf browning at TLP. Thus, although a Ψ_{stem} of -2.0MPa indicates a lethal dose of water stress in many perennial crops such as grapes, citrus, apples (Smart, 1974; Kaufmann and Levy, 1976; West and Gaff, 1976), and some trees (Barigah et al., 2013), it is not the case in studied blueberry population- varieties. However, following TLP, there was a progressive and relatively fast decline in F_v/F_m, and a progressive increase of leaf browning and leaf dropping across all population- varieties. This shows the close coordination between F_v/F_m, leaf browning, and leaf shedding. The decline of physiological processes after TLP could be due to the disconnection of the stem from the soil and the formation of significant xylem embolism across blueberry population- varieties. This suggests that TLP is an important indicator of water stress in blueberries, beyond which can cause branch dieback and plant mortality. By the time midday Ψ_{stem} had reached -4.5MPa and soil moisture was less than 5%, all the physiological processes dropped to zero and all blueberry population- varieties showed high PLC (83% to 100%) and mortality rates (20% to 40%). Thus, these midday Ψ_{stem} and soil moisture values seemed enough to create extreme drought stress in blueberry plants.

In previous research conducted by Glass et al. (2005), it was determined that wild blueberries exposed to midday Ψ_{stem} as low as -2.5 MPa were unaffected by this water potential and were able to maintain turgor and physiological process. They also found that the relationship between the midday Ψ_{stem} and the photosynthesis rate was absent, indicating that the photosynthesis rate was not limited by moisture supply. Considering the nature of the project, Glass et al., 2005 exposed the field-grown plants of size 3m × 3m to drought, while in this study potted plants (2.5gallon size and 15 cm deep) were exposed to drought. Differences between potted and field-growing plants could be part of the reason for observed differences. In the Glass et al. (2005) experiment, blueberries were able to maintain all the physiological processes at -2.5MPa, which could be due to overnight recharging of plant water and recharging through rainfall events of ≤50%. But blueberries in our experiment had no

probability of overnight recharging. Glass et al. (2005) also concluded that blueberries are drought resistant plants when exposed to lower midday Ψ_{stem} of -2.5MPa. Based on my finding's blueberries can resist drought conditions up to the turgor loss point of -2.0MPa after which photochemistry showed a progressive decline. Our findings also revealed that when the midday Ψ_{stem} reached -4.5MPa and soil moisture was less than 5%, they showed a high percentage loss of conductivity and high mortality rates.

Among the differences in drought response observed among population- varieties was that decline in predawn Ψ_{stem} and midday Ψ_{stem} was slower in Bluecrop and Ang 1, and occurred when soil moisture was less than 5%. But in Bluecrop, the decline of both Ψ_{stem} 3 days later compared to others as well as a decline of lower loss of hydraulic conductivity compared to all others, suggesting Bluecrop could be more drought resilient than other varieties. Further, the coordinated decline of physiological processes along with midday Ψ_{stem} after the TLP was quicker in Patriot and Ang 2 than in Ang 1 and Bluecrop. This pattern could be because Patriot and Ang 2 could be more vulnerable to embolism compared to Ang 1 and Bluecrop, supported by 100% PLC under extreme drought stress conditions in these two population-varieties. Patriot has a larger vessel size than all other varieties whose xylem might be more vulnerable to embolism. In research conducted by Ameglio et al. (2000) on the Bluecrop variety, they found that embolism increased rapidly below -1.2 MPa midday Ψ_{stem}, and that below -2.1 MPa, embolism was total. The findings of Ameglio et al. (2000) relate to ours in that most physiological processes dropped to lower values before the turgor loss point of nearly -2.0MPa midday Ψ_{stem}, and after turgor loss point there was a progressive decline in photochemistry. It is likely that embolism might have already occurred at TLP across all the blueberry population-varieties in our studies based on the findings of Ameglio et al. (2000).

2.5.2 HYDRAULIC SAFETY AND PRODUCTIVITY

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

2.5.3 MORTALITY AND REGROWTH

High PLC and branch dieback were detected at the end of the drought treatment, but some population- varieties were able to regrow new stems from their surviving parts showing high recovery capacity. Patriot experienced 100% PLC in terminal stems and had the highest stem mortality rate (84%), coupled with a low individual mortality rate (20%) and high regrowth of new branches (15.54% at branch level; 80% at the individual level). Regrowth and resprouting occurred from the living stems and rhizomes, whereas terminal branches remained dead. Aug 2 with 87% PLC showed the highest stem mortality rate but had a lower individual mortality rate. Also, Ang 2 had the highest rate of resprouting of new stems at the individual level (80%) and had a high rate of regrowth of new stems (10%) at the stem level. Its rapid leaf browning, leaf shedding, and high stem dieback could potentially protect them from further water loss and prevent the depletion of carbohydrates in the rhizomes and roots (Moreira et al.,2012), which could be used for regrowth and resprouting in the following year, as found in other shrubs and some tree seedlings (Galvez et al.,2011; Barigah et al.,2013; Vilagrosa et al., 2014).

Ang 1 also showed rapid browning and leaf shedding, which could protect its stem by minimizing water loss. As the drought progressed to – 3.6 MPa of midday Ψstem, Ang 1 reached 100% PLC with high stem and individual mortality, suggesting high vulnerability to embolism. However, it also displayed a high individual and stem regrowth rate (40% and 11%). In contrast, Bluecrop had very low browning percentages, did not show leaf drop, and maintained the lowest PLC (83%) with the lowest individual and stem mortality, suggesting that this variety could be highly resistant to drought-induced embolism. In contrast to Patriot, Bluecrop did not show high regrowth of new branches, due to a low mortality rate and the regrowth of new branches that was observed only in those stems whose basal stems parts were alive; the terminal branches remained dead. Despite high PLC and high mortality rate, high resprouting and regrowth in the following year could be a mechanism in shrub drought response (Zeppel et al., 2014). In *angustifolium* populations, almost 85% of the lowbush blueberry biomass exists as a shallow underground rhizome (Hall, 1957) providing an ability to recover through resprouting. The pots used limit the size of the rhizomes (Kramer, 1983), which could lead to lower resistance in studied Ang 1 and Ang 2 populations compared to plants in the field.

2.6 CONCLUSIONS

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

plants could be resistant to the level of TLP at -2.0MPa, while lower levels of drought can result in significant damages, high leaf browning, and leaf shedding. When exposed to extreme drought of -4.5 MPa, they showed high PLC of 83 to 100% and high branch dieback. However, the blueberries had a high regrowth rate, and high resprouting rates of new stems from rhizomes were observed in *Angustifolium* populations. Replicating this experiment in the field and further study of the recovery processes would provide more insights into the drought response strategies of blueberries. I recommend that growers use the leaf turgor loss point as the critical water potential threshold, which should be avoided in the field to minimize tissue damage and branch mortality.

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APPENDICES
APPENDIX 1.

Table A1.1 Blueberry species studied in Blueberry Hill Farm, Jonesboro, Maine (BBHF), the blueberry research station of the University of Maine. Nomenclature, distribution and native habitats were taken from Native Plant Trust Go Botany (https://gobotany.nativeplanttrust.org/species/vaccinium/angustifolium/ and https://gobotany.nativeplanttrust.org/species/vaccinium/myrtilloides/), The USDA Forest Service (https://www.fs.fed.us/database/feis/plants/shrub/vacang/all.html) and (Rogers, 1974; Pritts and Hancock, 1984) and (Smith and William, 1966; Smith, D. 1969; Uttal and Leonard, 1987; Kloet et al., 1981)

Species	Family	Original distribution	Native habitat
V.angustifolium	Ericaceae	Northern Canadian tundra to the New England states, westward to Minnesota and southward to Virginia	Alpine or subalpine zones, cliffs, balds, or ledges, grassland, meadows and fields, mountain summits and plateaus, ridges or ledges, woodlands
V. myrtilloides	Ericaceae	Canada to British Columbia and the Northwest Territories, In eastern North America, it extends southward through the mountains of New England, New York, and Pennsylvania to West Virginia and Virginia	Bogs, cliffs, balds, or ledges, fens, forests, meadows and fields, mountain summits and plateaus, ridges or ledges, woodlands

Table A1.2 Percentile distribution of mean trait values of *Vaccinium angustifolium* and *Vaccinium myrtilloides* compared to the mean trait values of species in the Glopnet.

	A_a	A_m	A_N	A_P	N_m	P_m	LMA
2020	%	%	%	%	%	%	%
V. angustifolium	41.94	63.90	42.21	66.22	35.80	54.26	36.8
V. myrtilloides	22.30	48.44	5.06	1.20	34.98	56.65	33.0
2019							
V. angustifolium	87.27	79.09	67.27	87.23	36.09	56.65	57.70
V. myrtilloides	85.70	79.87	73.12	100.0	35.81	53.06	53.75
2018							
V. angustifolium	46.30	58.83	40.52	56.65	30.62	53.32	46.54
V. myrtilloides	22.42	46.75	17.53	0.80	24.84	53.59	36.33
2017							
V. angustifolium	26.91	33.64	1.43	0.00	31.78	57.71	50.34
V. myrtilloides	17.45	25.32	0.65	0.00	35.27	54.26	57.05

Table A1.3 Variance explained by interspecific variation (Genotype), intraspecific variation (Stem+ Error) of functional traits and morphological traits of 2 blueberry species in Blueberry Hill Farm in Jonesboro, Maine. Trait observations are as listed below.

2020	Source	A_a	A_m	Chl	LMA	PHt	g_s	SD	LS	TLA	LT	WD
V. angustifolium	Genotype	63.64	61.15	35.83	35.89	53.14	71.25	17.05	45.85		28.92	0.50
	Stem+ Error	36.36	38.85	64.17	64.11	46.86	28.75	82.95	54.15		71.08	99.50
V. myrtilloides	Genotype	43.04	29.29	53.66	50.88	64.77	19.51	25.92	6.44		12.07	14.49
	Stem+ Error	56.96	70.71	46.34	49.12	35.23	80.49	74.08	93.56		87.93	85.51
2019												
V. angustifolium	Genotype	32.98	27.09	41.65	44.20	84.57	16.80	73.82	73.32		53.23	0.50
	Stem+ Error	67.02	72.91	58.35	55.79	15.43	83.19	26.18	26.68		46.77	99.50
V. myrtilloides	Genotype	42.42	32.73	52.42	16.26	40.85	52.24	4.59	52.58		21.37	67.24
	Stem+ Error	57.58	67.27	47.58	83.74	59.15	47.76	95.40	47.42		78.61	32.76
2018												
V. angustifolium	Genotype	35.22	15.86	66.5411	62.25	63.79	35.23	43.36	73.88	79.01		
	Stem+ Error	64.78	84.14	33.4589	37.75	36.20	64.77	56.64	26.12	20.99		
V. myrtilloides	Genotype	10.81	26.85	58.7	27.79	15.48	3.73	43.46	36.65	43.92		
	Stem+ Error	89.19	73.15	41.3	72.21	84.52	96.27	56.54	63.35	56.08		

Table A1.4 Traits, symbols, and units

Trait	Symbols	Units
Leaf Size	LS	
Leaf mass per area	LMA	g m-2
Total leaf area	TLA	g m-2
Stem height/ Plant height	SH/PH	cm
Light-saturated CO2 assimilation per leaf area	A_a	µmol m-2 s-1
Maximum stomatal conductance	g_s	mol m-2 s-1
Water use efficiency	Alg	µmol mol-1
Light-saturated CO2 assimilation per leaf dry mass	A_m	nmol g-1 s-1
Light-saturated CO2 assimilation per nitrogen	A_N	µmol g-1 s-1
Light-saturated CO2 assimilation per phosphate	A_P	µmol g-1 s-1
Chlorophyll concentration per area	Chl	spad
Nitrogen per mass	N_m	%
Carbon per mass	C_m	%
Phosphorus per mass	P_m	%
Iron per mass	Fe_m	mg kg-1
Potassium per mass	K_m	%
Calcium per mass	Ca_m	%
Zinc per mass	Zn_m	mg kg-1
Sulphate per mass	S_m	%
Manganese per mass	Mn_m	%
Magnesium per mass	Mg_m	%
Aluminium per mass	Al_m	%
Boron per mass	B_m	%
Soil P^H	P^H	
Soil phosphorus	P	ppm
Soil potassium	K	ppm
Soil magnesium	Mg	ppm
Soil calcium	Ca	ppm
Cation exchange capacity	CEC	me/100gm
Soil Acidity	Ac	
Soil organic matter	OM	%
Soil sulphur	S	ppm
Soil copper	Cu	ppm
Soil iron	Fe	ppm
Soil manganese	Mg	ppm
Soil zinc	Zn	ppm
Soil nitrogen	N	ppm
Soil Ammonium nitrate	NH4	ppm

Table A1.5 Means and standard errors for each trait by species. Trait symbols are in Table S2.

Year/ Species	A_a	A_m	A_N	A_P	N_m	P_m
2020						
V. angustifolium	9.49± 0.69	127.79±12.72	0.085±0.01	1.19±0.13	1.51±0.02	0.11±0.004
V. myrtilloides	6.91±0.67	97.41±6.52	0.066±0.01	0.89±0.08	1.49±0.04	0.11±0.002
2019						
V. angustifolium	18.21±0.77	177.27±9.19	0.12±0.01	1.667±0.16	1.51±0.06	0.11±0.01
V. myrtilloides	17.51 ±0.7	181.01±9.15	0.12±0.01	1.77±0.12	1.51±0.03	0.1±0.004
2018						
V. angustifolium	10.16±0.85	116.47±5.4	0.08±0	1.06±0.1	1.38±0.02	0.11±0.01
V. myrtilloides	7.15±0.47	95.83±7.38	0.08±0	0.9±0.05	1.21±0.03	0.11±0.01
2017						
V. angustifolium	7.57 ± 0.22	84.60 ± 6.74	0.06 ± 0.01	0.76 ± 0.10	1.40 ± 0.05	0.11±0.01
V. myrtilloides	6.34 ± 0.55	61.98 ± 6.89	0.04 ± 0.00	0.58 ± 0.06	1.50 ± 0.02	0.11±0.01

Year/ Species	Chl_m	LMA	C_m	Fe_m	C/N	g_s
2020						
V. angustifolium	28.98±1.81	75.82±2.85	48.34±0.13	42.14±2.08	32.12±0.52	0.16±0.01
V. myrtilloides	25.83±1.53	70.89±2.69	49.29±0.11	65.91±8.23	33.34±0.92	0.11±0.01
2019						
V. angustifolium	35.94±1.67	105.42±4.56	49.21±0.14	41.75±4.61	32.85±1.19	0.21± 0.02
V. myrtilloides	35.63±1.7	98.69±3.96	50.05±0.13	31.3±1.78	33.27±0.62	0.2± 0.02
2018						
V. angustifolium	29.83±2.13	87.13±6.13	50.68±0.13	28.9±1.11	36.9±0.68	0.17±0.02
V. myrtilloides	26.3±1.15	75.31±3.17	51.37±0.17	34.74±1.56	42.63±1.25	0.11±0.01
2017						
V. angustifolium	33.98 ±1.35	89.47 ± 6.51	50.92 ±0.19	30.72 ±1.63	36.79 ± 1.17	
V. myrtilloides	36.55 ±1.54	98.18 ± 5.20	51.64 ±0.12	36.56 ±1.13	34.57 ± 0.48	

Year/ Species	*Alg*	LS	LT	WD	PH	SD
2020						
V. angustifolium	65.33±5.28	1.05±0.43	0.15±0.01	0.57±0.04	21.88±1.13	2.12±0.11
V. myrtilloides	65.92±3.8	1.41±0.58	0.15±0.01	0.57±0.01	20.14±1.29	2.76±0.17
2019						
V. angustifolium	91.14±5.39	2.09±0.12	0.22±0.01	1.39±0.13	21.75±1.57	1.89±0.1
V. myrtilloides	92.19±6.59	2.14±0.17	0.19±0.01	1.42±0.04	22.61±1.91	2.34±0.12
2018						
V. angustifolium	62.08±3.68	1.83±0.25			22.78±	2.37±0.2
V. myrtilloides	66.43±3.81	2.21±0.14			23.47±1.06	2.98±0.19
2017						
V. angustifolium						
V. myrtilloides						

Table A1.6 Mean and standard errors of Temperature, Precipitation, and Relative Humidity of Blueberry Hill Farm, Jonesboro, Maine from May to August for the years 2017, 2018, 2019, and 2020.

Temperature (°C)	May	June	July	August
FY-2017	10.59±0.74	15.94±0.66	18.01±0.41	17.8±0.5
FY-2018	12.08±0.57	14.62±0.72	19.71±0.44	20.18±0.53
FY-2019	9.14±0.41	15.38±0.34	20.03±0.40	18.38±0.33
FY-2020	10.62±0.94	17.0±0.71	20.09±0.47	19.02±0.6
Precipitation (mm)				
FY-2017	6.81±2.30	2.44±0.85	1.18±0.50	1.4±0.60
FY-2018	1.36±0.65	4.45±1.89	0.91±0.46	2.0±0.73
FY-2019	3.66±0.91	5.41±1.8	2.79±0.98	4.78±1.96
FY-2020	2.56±0.98	1.84±10	1.55±0.58	2.0±1.19

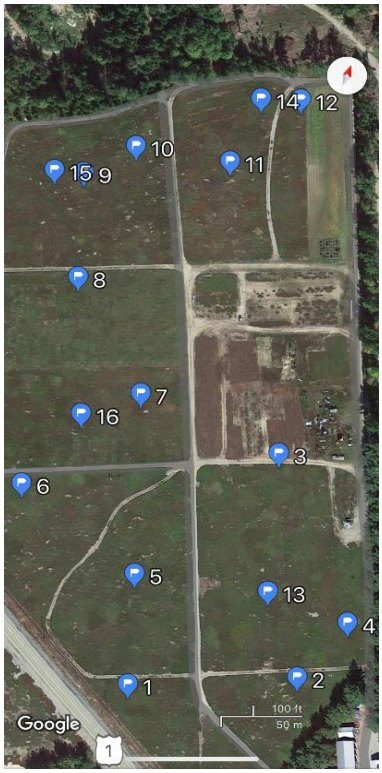


Figure A1.1: Map showing the location of *Vaccinium angustifolium* and *Vaccinium myrtilloides* species used in our study in the Blueberry Hill Farm, Jonesboro, Maine.

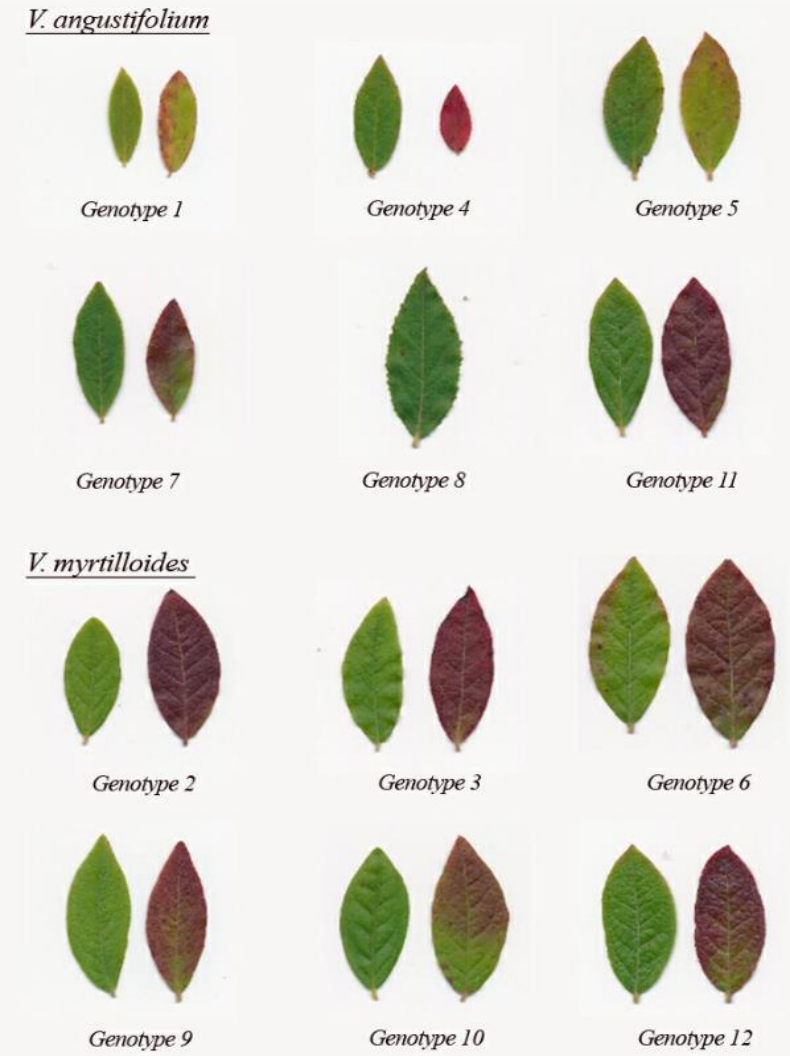


Figure A1.2: Diversity in leaf form, color, size, and morphology of *V. angustifolium* and *V. myrtilloides* species of lowbush blueberry. Genotype 8 does not have red leaves.

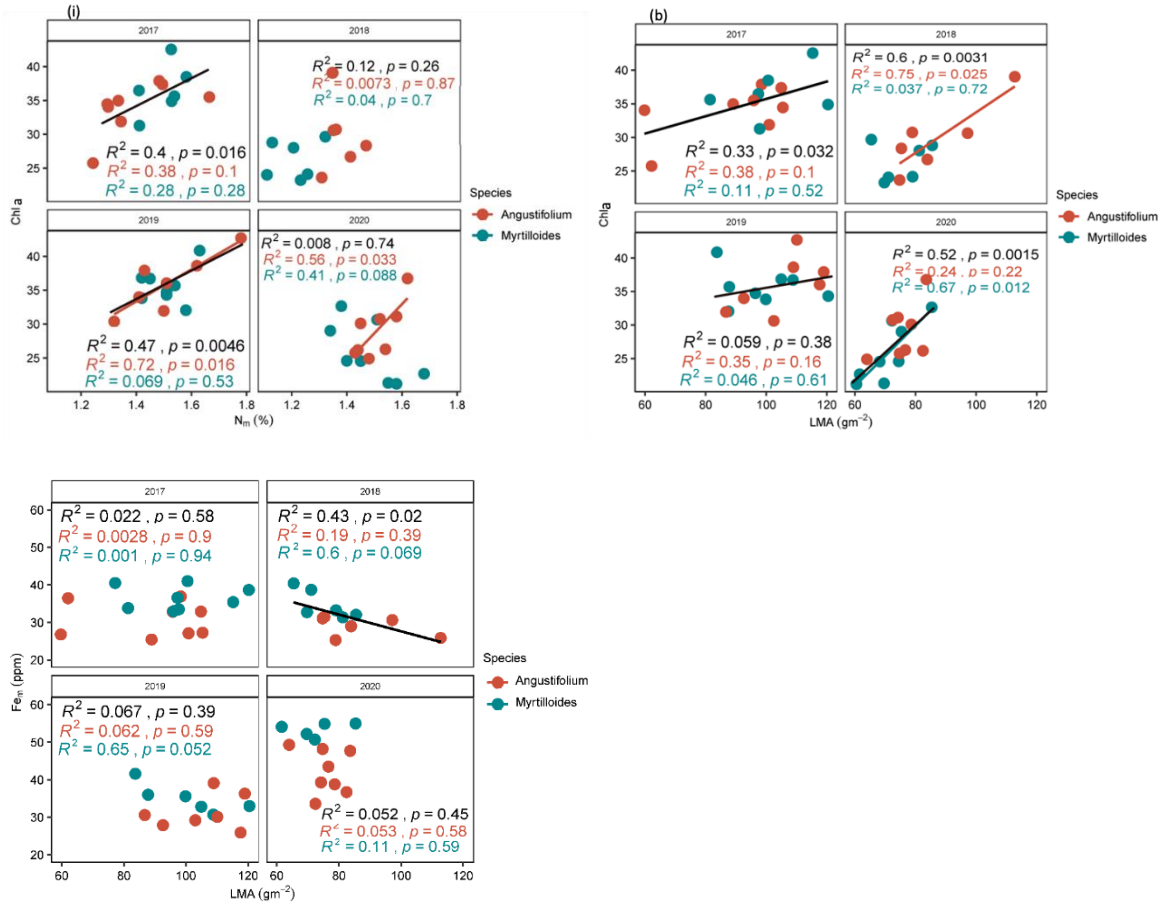
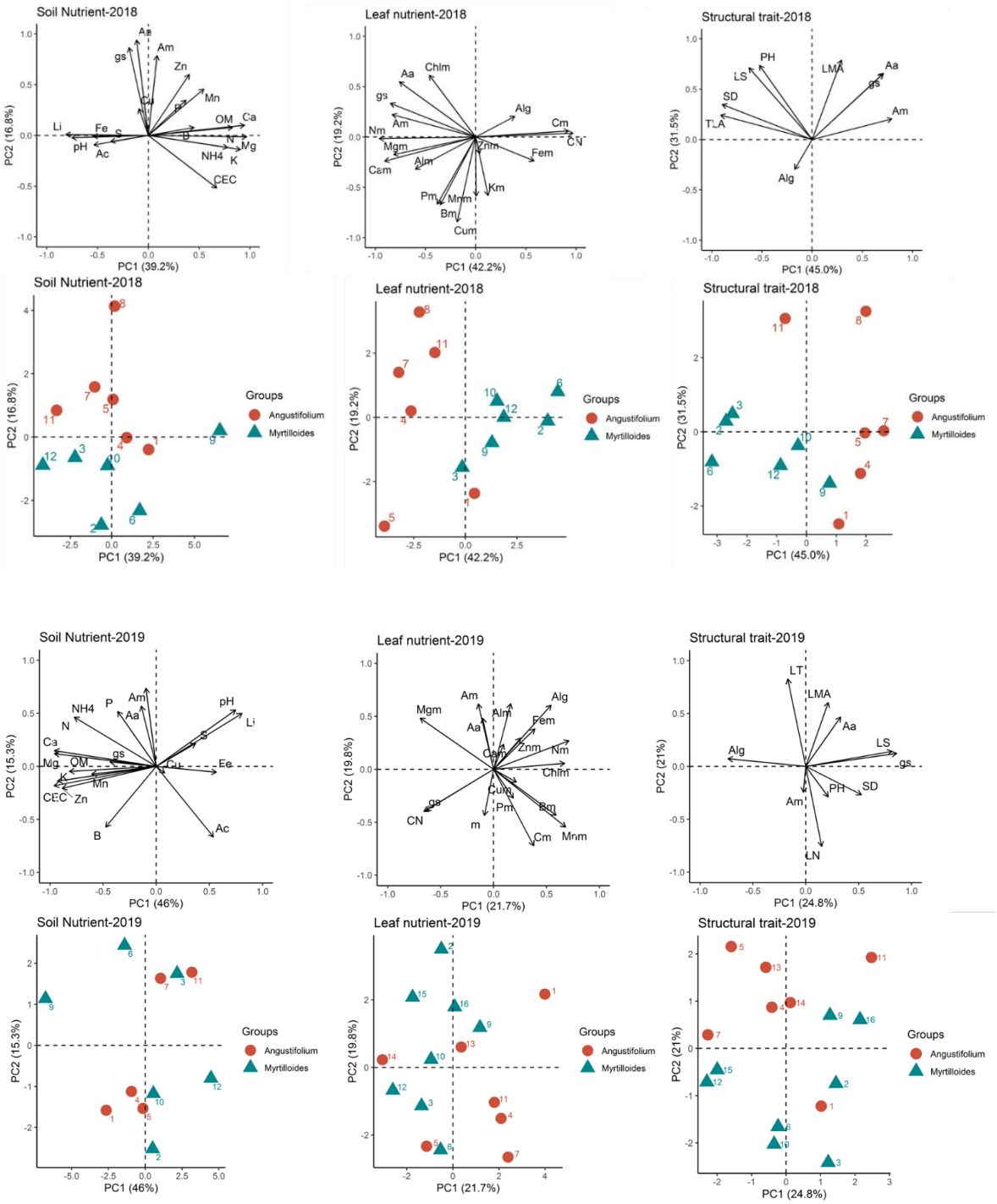


Figure A1.3: Relationship of area-based chlorophyll concentration (Chl_a) with mass-based nitrogen (N_m), and leaf mass per area (LMA), and mass-based iron (Fe_m) with LMA for 2017, 2018, 2019, and 2020 across and within two blueberry species, *V. angustifolium* (VM, red circles) and *V. myrtilloides* (VM, green circles). Points are means. Linear (a & b) regressions were fitted to the data across (black solid line) and within (red solid line, *Angustifolium* and green solid line, *Myrtilloides*) blueberry species. P values of less than 0.05 are significant and are marked with a corresponding line color as described above.


Soil Nutrient-2018
Leaf nutrient-2018
Structural trait-2018
Soil Nutrient-2019
Leaf nutrient-2019
Structural trait-2019
Groups
Angustifolium
Myrtilloides

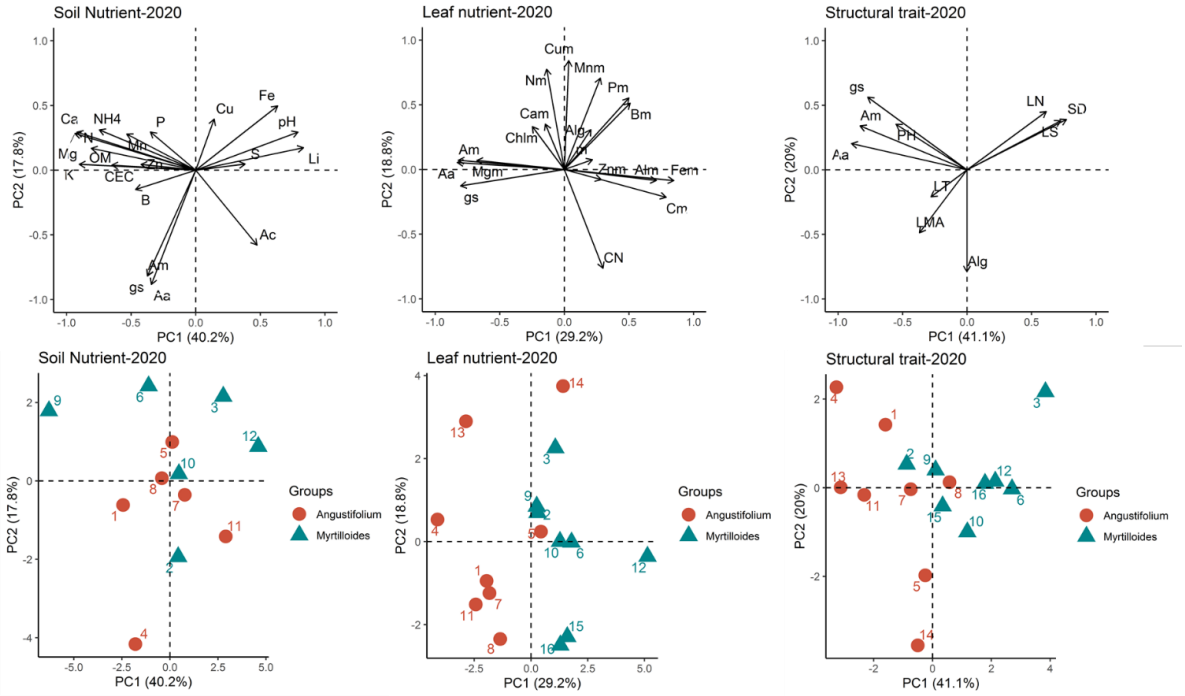


Figure A1.4: Principal Component Analysis of mean values of soil nutrient, leaf nutrient, and structural traits of *V. angustifolium* and *V. myrtilloides* species along with their genotypes for year 2018, 2019 and 2020.

APPENDIX 2.

Table A2.1 Blueberry species studied in the nursery at The University of Maine, in Orono, Maine. Nomenclature, distribution and native habitats were taken from Native Plant Trust Go Botany (https://gobotany.nativeplanttrust.org/species/vaccinium/angustifolium/, https://gobotany.nativeplanttrust.org/species/vaccinium/corymbosum/, The USDA Forest Service (https://www.fs.fed.us/database/feis/plants/shrub/vacang/all.htmland https://www.fs.fed.us/database/feis/plants/shrub/vaccor/all.html) and (Rogers, 1974; Pritts and Hancock, 1984) and (Kloet et al., 1980).

Species	Family	Original distribution	Native habitat
V. angustifolium	Ericaceae	Northern Canadian tundra to New England states, westward to Minnesota and southward to Virginia	Alpine or subalpine zones, cliffs, balds, or ledges, grassland, meadows and fields, mountain summits and plateaus, ridges or ledges, woodlands
V. corymbosum	Ericaceae	Northeastern Illinois and northern Indiana northeastward to southwestern Nova Scotia, south to Florida, and west to north-eastern Texas and adjacent Oklahoma	Bogs, fens, forests, shores of rivers or lakes, swamps, woodlands

Table A2.2 Traits, symbols, and units

Trait	Symbols	Units
Leaf Size	LS	
Leaf mass per area	LMA	g m-2
Leaf thickness	LT	mm
Plant height	PH	cm
Stem diameter	SD	mm
Maximum photochemical efficiency of PSII	F_v/F_m	
Midday photosynthesis rate	A_{midday}	µmol CO2 m-2 s-1
Midday stomatal conductance	Midday g_s	mol m-2 s-1
Midday transpiration Rate	E	mol m-2 s-1
Plant hydraulic conductance	K_{plant}	mmol H2O m2 MPa−1
Predawn stem water potential	Predawn Ψ_{stem}	MPa
Midday stem water potential	Midday Ψ_{stem}	MPa
Turgor Loss Point	TLP	
Soil moisture		%
Chlorophyll concentration	Chl	spad

Table A2.3 Comparisons of plant height, stem diameter, leaf mass per area (LMA), and leaf size of each population- varieties: Ang 1, Ang 2, Bluecrop, and Patriot. Values are means ± SE (n = 10).

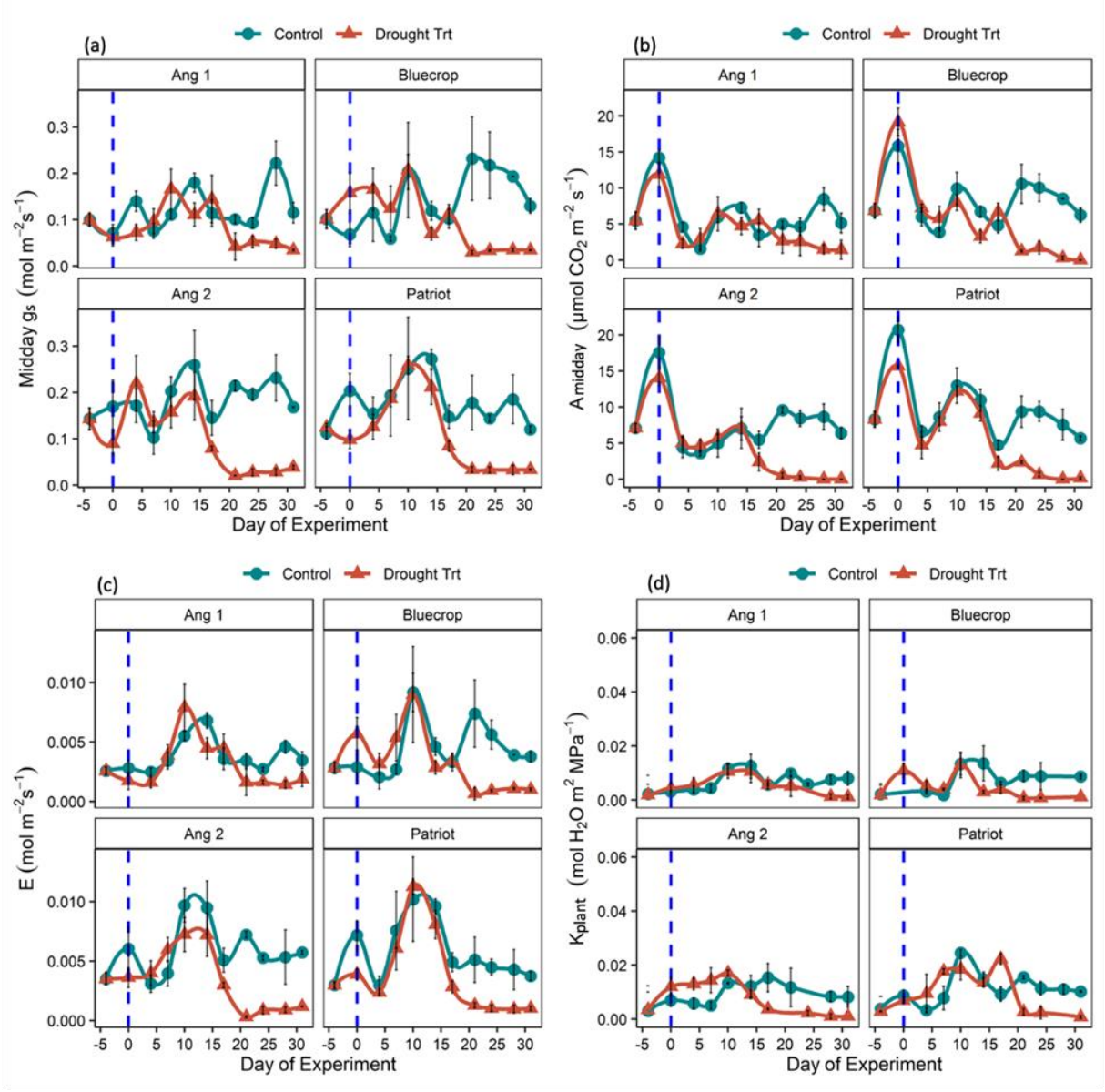
Species	Population- varieties	Plant height ± SE	Stem Diameter ± SE	LMA ± SE	Leaf Size ± SE
Unit		cm	cm	gm^{-2}	m^{-2}
V. angustifolium	Ang 1	30.21 ± 2.56b	3.55 ± 0.33b	0.0068 ± 0.0003a	1.40 ± 0.17a
V. angustifolium	Ang 2	18.21 ± 1.45c	2.25 ± 0.10b	0.0054 ± 0.0002b	1.39 ± 0.11a
V. corymbosum	Bluecrop	68.62 ± 5.40a	8.95 ± 0.82a	0.0073 ± 0.0004a	5.19 ± 0.27b
V. corymbosum	Patriot	60.92 ± 3.37a	9.556 ± 0.32a	0.0075 ± 0.0001a	5.49 ± 0.35b

Table A2.4 Physiological traits measured for each population- varieties (Ang 1, Ang 2, Bluecrop, and Patriot) using pressure- volume analysis**.**

Parameters	Ang 1	Ang 2	Bluecrop	Patriot
SWC	29.28	39.89	25.69	31.87
Po (MPa)	-1.61	-1.39	-1.59	-1.65
YTLP (MPa)	**-1.99**	**-1.66**	**-1.90**	**-1.98**
RWCTLP	98.33	99.34	99.13	99.22
e (MPa)	181.07	209.14	213.86	237.17
CFT (MPa-1)	0.008	0.004	0.005	0.004
CTLP (MPa-1)	0.020	0.014	0.017	0.013
CFT* (mol m-2 MPa-1)	0.64	0.55	0.309866	0.38

Table A2.5 Survival, mortality and regrowth of each population- varieties (Ang 1, Ang 2, Bluecrop, and Patriot) at individual level after the drought experiment.

Varieties/population	Survival	Mortality	New stew regrowth	New branch regrowth
Ang 1	3/5	2/5	2/5	0
Ang 2	4/5	1/5	4/5	0
Bleucrop	4/5	1/5	0	2/5
Patriot	4/5	1/5	0	4/5


(a)
Control
Drought Trt
Ang 1
Bluecrop
Ang 2
Patriot
Midday g_s (mol $m^{-2}s^{-1}$)
Day of Experiment
(b)
Control
Drought Trt
Ang 1
Bluecrop
Ang 2
Patriot
A_{midday} (µmol CO_2 m^{-2} s^{-1})
Day of Experiment
(c)
Control
Drought Trt
Ang 1
Bluecrop
Ang 2
Patriot
E (mol $m^{-2}s^{-1}$)
Day of Experiment
(d)
Control
Drought Trt
Ang 1
Bluecrop
Ang 2
Patriot
K_{plant} (mol H_2O m^2 MPa^{-1})
Day of Experiment

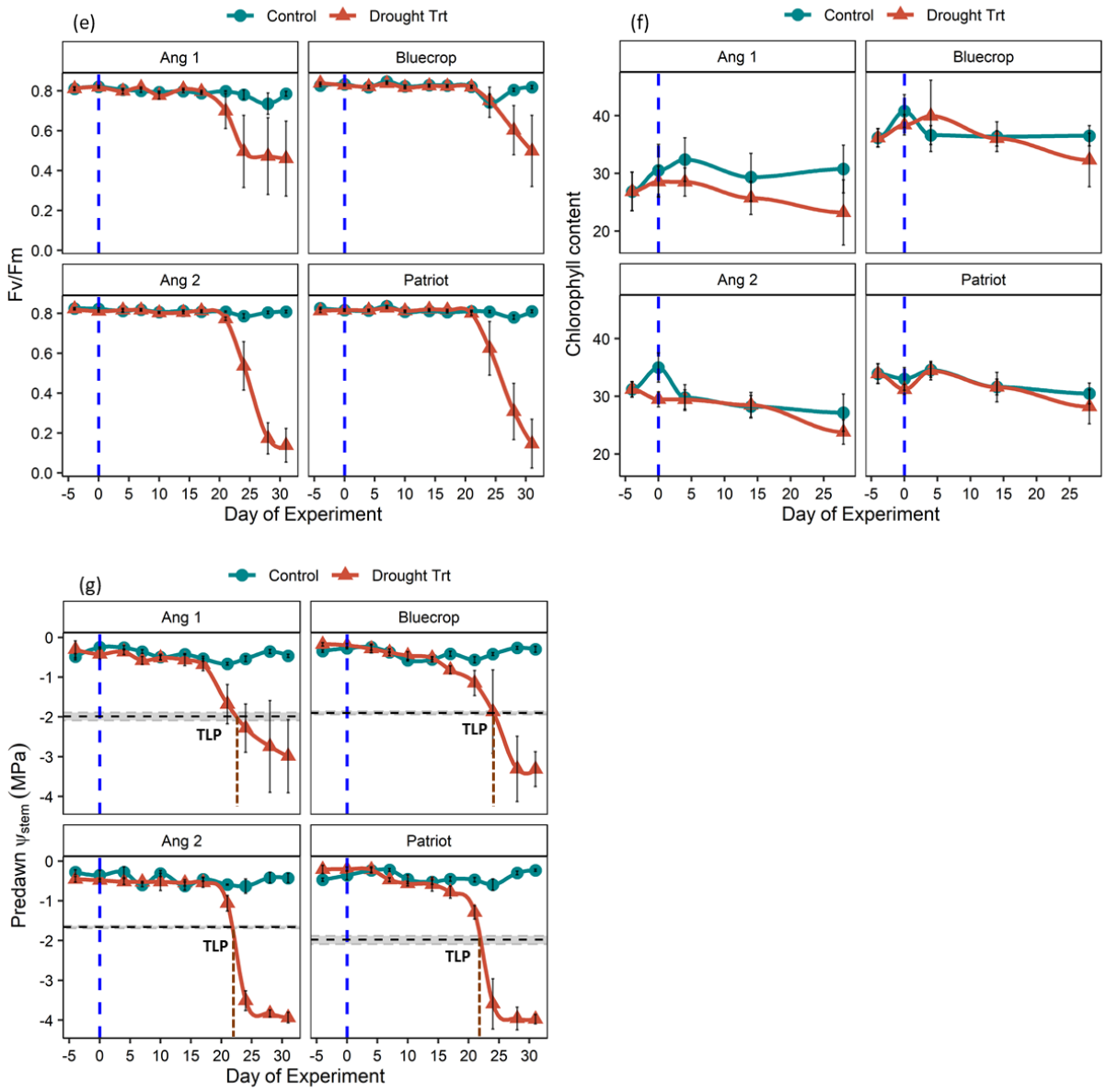


Figure A2.1 Changes in midday stomatal conductance, Midday g_s (mol m^{-2} sec^{-1}; a), midday photosynthesis rate, A_{midday} (µmol CO_2 m^{-2} sec^{-1}, b), transpiration rate, E (mol m^{-2} sec^{-1}; c), plant hydraulic conductance, K_{plant} (mmol H_2O m^2 MPa^{-1}; d), F_v/F_m (e), and chlorophyll content (f) as a function of the day of experiment (DOE) recorded in the control treatment (blue dots and line) and the drought treatment (red triangle and line) of each population- varieties: Ang 1, Ang 2, Bluecrop, and Patriot, during the course of the experiment. The blue dotted lines mentioned are the first day of the start of drought experiment and is represented by day 0, light grey background bounded with dashed grey lines in figure (g) represents mean TLP ± SE values and the intersection of black and brown dotted lines in the middle are Turgor Loss Point, represented by TLP for corresponding population- varieties. Values are means ± SE for midday stomatal conductance, midday photosynthesis rate, transpiration rate, plant hydraulic conductance, predawn Ψ_{stem} (n = 3), and F_v/F_m and chlorophyll content (n = 5).

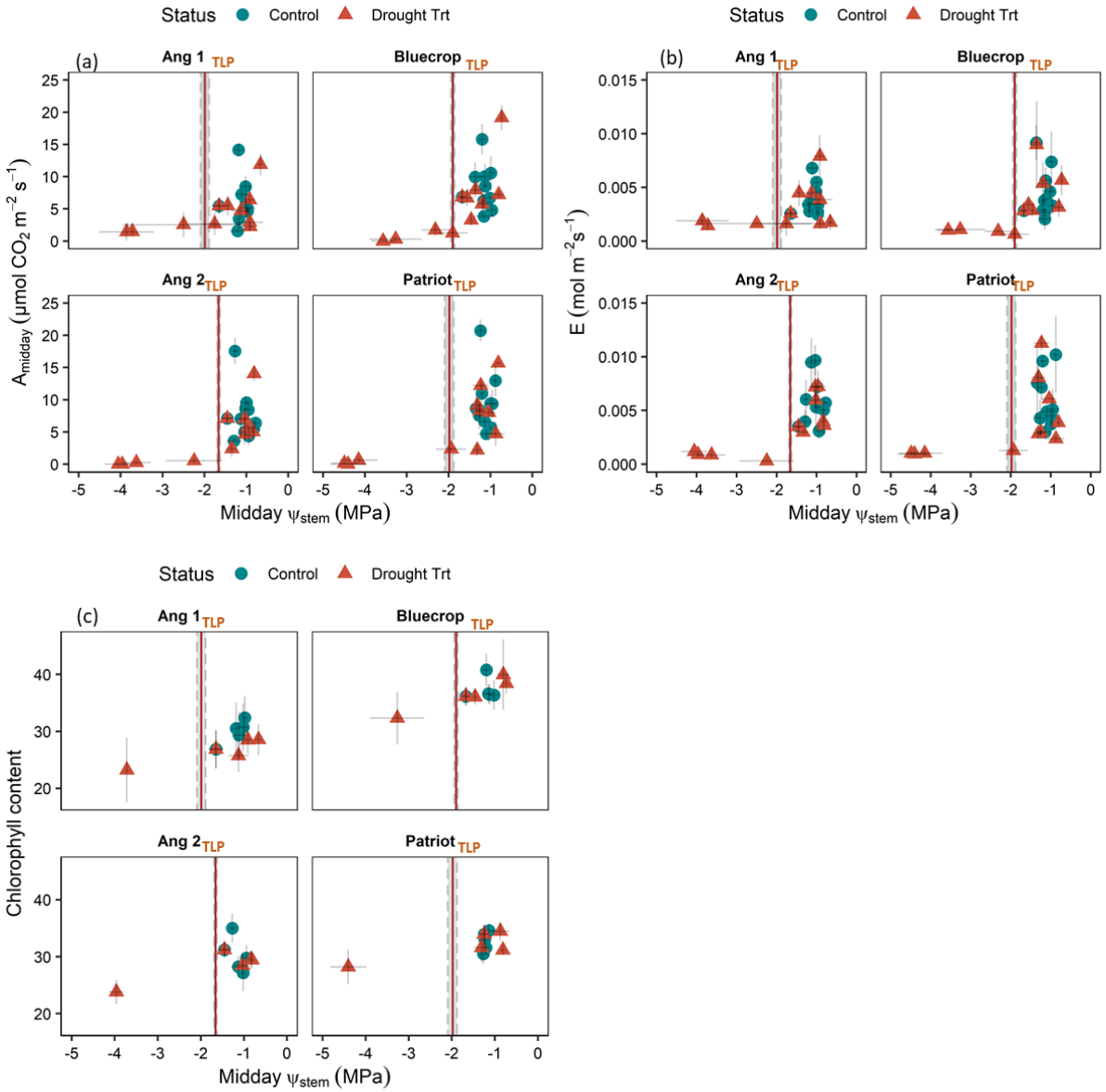


Figure A2.2 The midday photosynthesis rate, A_{midday} (µmol CO_2 m^{-2} sec^{-1}; a), and transpiration rate, E (molm^{-2} sec^{-1}; b), and chlorophyll content (c) as a function of stem water potential (stem Ψ; MPa) in the control (blue dots) and drought treatment (red triangle) of each population- varieties: Ang 1, Ang 2, Bluecrop, and Patriot. Light grey background bounded with dashed grey lines represents mean TLP ± SE values and the brown solid lines in the middle are Turgor Loss Point, represented by TLP for corresponding population- varieties. Values are means ± SE for midday Ψ_{stem}, A_{midday}, E (n = 3) and chlorophyll content (n = 5).

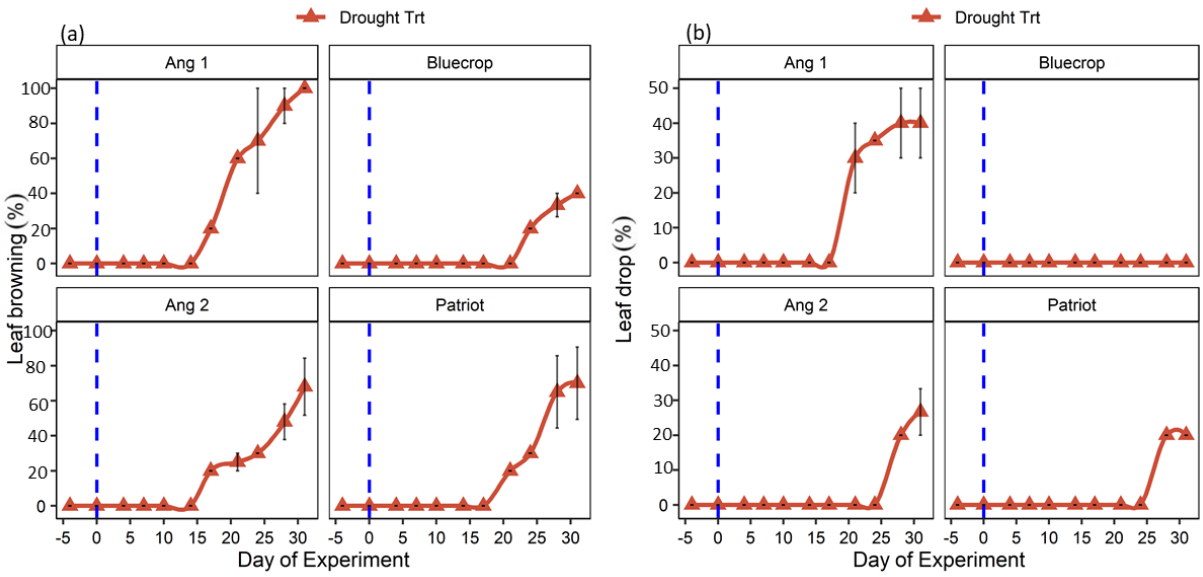


Figure A2.3 Changes in leaf browning, (a) and leaf dropping (b) as a function of the day of experiment (DOE) recorded in the control treatment (blue dots and line) and the drought treatment (red triangle and line) of each population- varieties: Ang 1, Ang 2, Bluecrop, and Patriot, during the course of the experiment. The blue dotted lines mentioned are the first day of the start of drought experiment and is represented by day 0. Values are means ± SE (n = 5).

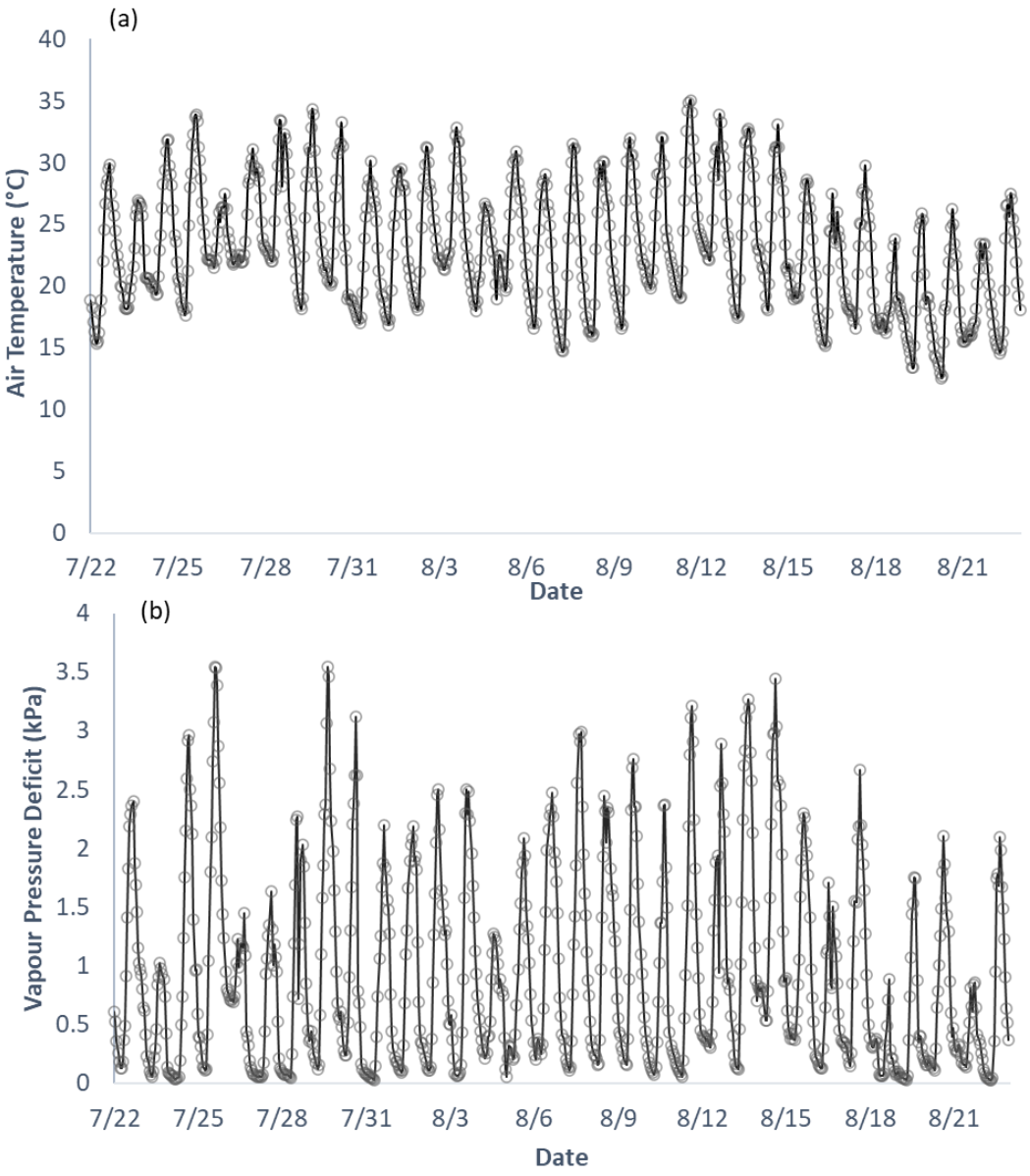


Figure A2.4 Hourly changes in air temperature (ºC; a), and vapor pressure deficit (kPa; b) over the course of the experiment (7/22 to 8/22) inside the rainfall exclusion house. Hourly values are means ± SE (n = 6); 6 is the change in air temperature and vapor pressure deficit recorded every 10 minutes interval.

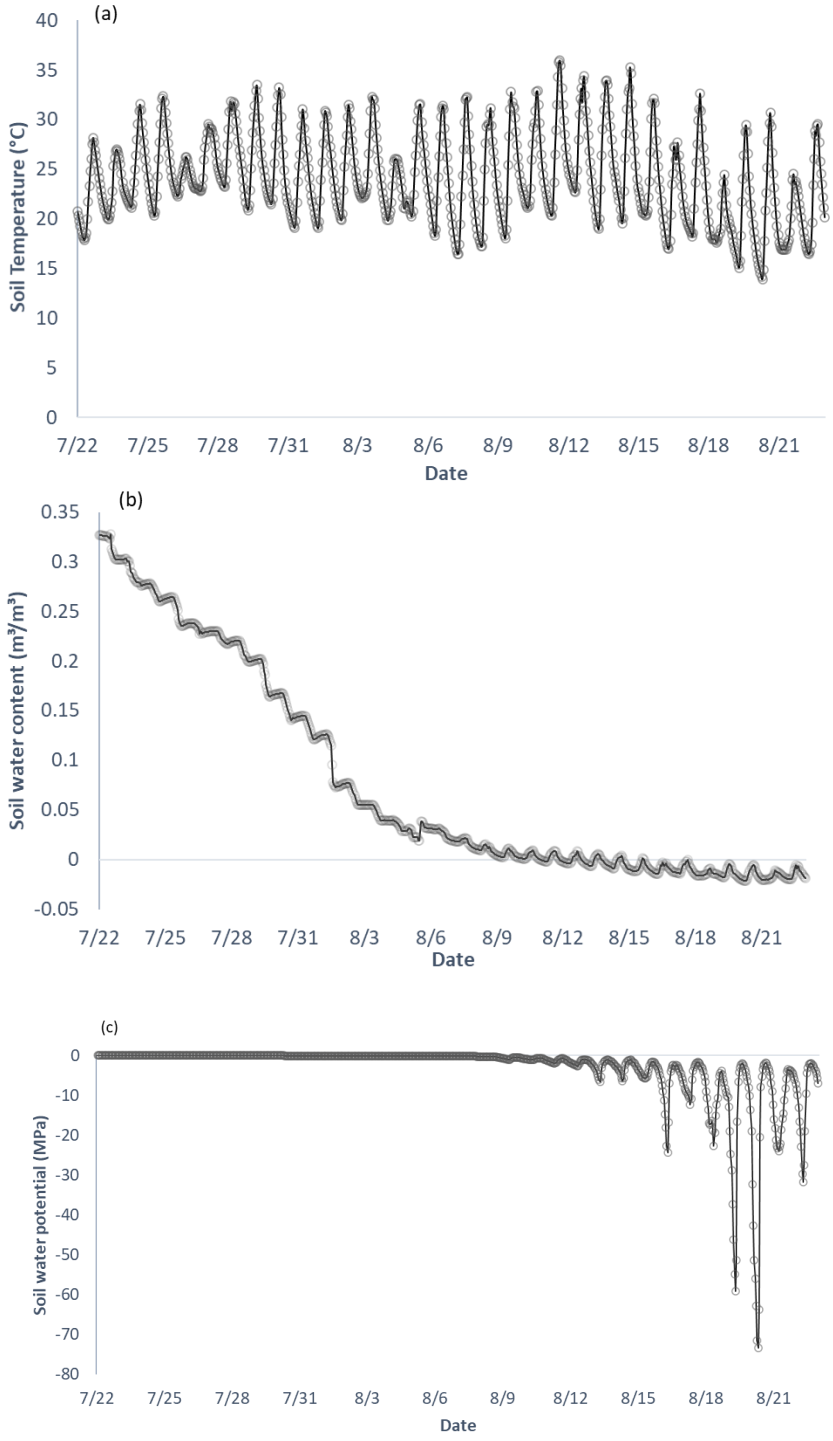


Figure A2.5 Hourly changes in soil temperature (°C; a), soil water content (m^3/m^3; b), and soil water potential (kPa; c) over the course of the experiment (7/22 to 8/22) inside the rainfall exclusion house. Hourly values are means ± SE (n = 6); 6 is the change in soil temperature, soil water content and soil water potential recorded every 10 minutes interval.

BIOGRAPHY OF AUTHOR

Pratima Pahadi was born in Sindhuli, Nepal where she grew up with her father, mother and her two siblings, Pradip and Pranita. She attended Caspian Valley College in Kathmandu, Nepal for her high school, where she graduated in 2010. Pratima obtained a Bachelor of Science degree in Agriculture with the major in Plant Breeding and Genetics from Tribhuvan University, Nepal in 2016. After graduation, she worked as a Research Assistant at Nepal Agriculture Research Council in the Wheat Breeding and Genetics Unit where she learned research trial conduction in field and laboratory conditions, crossing of wheat genotypes, selection of superior lines, data collection, analysis and interpretation. Pratima also worked as Livelihood Coordinator in a humanitarian organisation, World Vision International Nepal (WVIN) in the remote mountains of Karnali, Nepal where she trained farmers on a variety of crop production techniques and alternative farming techniques to best deal with drought in mountains. Later she also worked as Livelihood Coordinator in WVIN Earthquake Response Programme in Gorkha, Nepal, an epicentre of disastrous earthquake that took place in 2015 and killed lives of approximately 9000 people. There she was actively involved in the rehabilitation phase of Earthquake Response Programme and helped farmers with trainings, farm equipment and supplies, subsidies for those who lost their home and focused on child health, education and wellbeing. However, the worst climate change effects for example, snow melting, drought, dry water reservoirs that the people of mountains were already facing when working in Karnali inspired her to study further with an aim of helping people in the future. Pratima is passionate about research related to drought, heat, and temperature effects and how these effects together with changing climatic conditions impact plants in the future. She is a candidate for the Master of Science degree in Botany and Plant Pathology from the University of Maine in August 2021.