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Diversity in Stomata Morphology among Cultivated Blueberry Genotypes and Its Influence on Irradiance Response Dynamics

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Abstract. Stomata are pores on the leaf epidermis that control gas exchange between leaves and the atmosphere. Stomata differ in shape, size, and number among and within plant species. Stomata morphology has physiological consequences for some plant species. We investigated stomata morphology of different blueberry (*Vaccinium* spp.) genotypes and its influence on the speed of response to a change in irradiance. Understanding how these traits affect gas exchange can inform breeding programs and management practices to enhance agricultural productivity and resilience. Thirty-seven blueberry genotypes of southern highbush blueberry (SHB), northern highbush blueberry, and rabbiteye blueberry grown in seven different locations in North America were evaluated. Significant diversity in stomata area (S_a) and stomata density (S_d) were observed among the studied genotypes. Changes in S_a and S_d were not compensatory. During a follow-up study, the effects of S_a and S_d on the temporal response of stomatal conductance (g_s) to changes in irradiance were studied. The stomatal speed of response of the tested SHB genotypes was significantly affected by S_d, but not by S_a. Additionally, significant differences in minimum g_s were documented. Overall, this study provides foundational information about the stomatal biology of a globally important fruit crop.

Blueberry (*Vaccinium* spp.) production has expanded worldwide in the past two decades, increasing from approximately 0.4 million t in 2000 to over 1.5 million t in 2022 (US Department of Agriculture, National Agriculture Statistics Service 2023). The *Vaccinium* genus (family Ericaceae) includes over 400 blueberry species that occur naturally at different ploidy levels (Kloet 1988; Vander Kloet and Dickinson 2009). Most cultivated blueberries are from cultivars derived from tetraploid *V. corymbossum* L. (highbush blueberries) and *V. angustifolium* (lowbush) or hexaploid *V. ashei* Reade. (rabbiteye blueberry) (Retamales and Hancock 2018). This genetic diversity causes significant morphological, anatomical, and physiological diversity among blueberry plants. Previous studies have shown extensive genetic and phenotypic variability among blueberry populations, including genome size (Redpath et al. 2022), fruit firmness (Cappai et al. 2018), flower morphology (Lyrene 1994), and plant size (Retamales and Hancock 2018). However, to date, little is known about morphological traits of stomata in blueberry.

Stomata are pores on the surface of leaves and other plant organs that control plant transpiration and carbon uptake (Hetherington and Woodward 2003). Typically, the stomatal complex consists of two guard cells that encircle the stomatal pore (Nadeau and Sack 2002). Plants control the aperture of the stomatal pore by adjusting the turgor of the guard cells, thus regulating the exchange of gases between the atmosphere and interior of the leaf (Franks and Farquhar 2001; Zeiger 1983). Increases in guard cell turgor cause larger stomatal pore aperture, which allows for increased CO_2 intake and water vapor loss. Conversely, decreases in stomatal aperture limit CO_2 and water exchange. Turgor changes in guard cells are regulated by both internal and external factors (Mirasole et al. 2023; Zhang et al. 2024). Internally, the active transport of ions, such as potassium (K^+) and chloride (Cl^-), into and out of guard cells drives water influx or efflux (Roux and Leonhardt 2018). Externally, factors such as CO_2 concentration, light intensity, and air humidity also influence turgor pressure and stomatal movement (Ache et al. 2010; Jalakas et al. 2021; Yang et al. 2020; Zhang et al. 2024). Stomatal conductance (g_s) quantifies the efficiency of gas exchange through the stomata (Haworth et al. 2018; Hetherington and Woodward 2003; Woodward 1987).

Stomata area (S_a) and stomatal density (S_d) determine the potential surface available for regulated gas exchange (Franks and Beerling 2009). Stomata morphological traits are directly linked to plant gas exchange in several taxa (Franks and Beerling 2009; Hetherington and Woodward 2003; Wong et al. 1979). For example, *Banksia* spp. plants with high S_a had greater g_s compared with that of plants with low S_a (Drake et al. 2013). Similarly, *Arabidopsis* plants with high S_d had greater g_s rates than those of plants with low S_d (Franks et al. 2015). Consequently, stomata traits can significantly influence a plant's adaptability to environmental conditions (Chua and Lau 2024).

Morphological differences in stomata may arise because of genetic factors and/or in response to environmental conditions (Bertolino et al. 2019). Intraspecific and interspecific diversity in stomata morphology have been reported previously (Bertolino et al. 2019; Carlson et al. 2016; Hetherington and Woodward

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2003; Muchow and Sinclair 1989; Reich 1984). Genes that affect S_a and S_d, such as SPEECHLESS and MUTE, have been identified in *Arabidopsis thaliana* and in agricultural plants (Berger and Altmann, 2000; Falquetto-Gomes et al. 2024; Nir et al. 2023; Peterson et al. 2010; Wu et al. 2019). Environmental factors like light and CO_2 concentration can induce changes in S_d and S_a (Salisbury and Oliver 1928; Uprety et al. 2002; Vanhatalo et al. 2001; Woodward 1987). A consistent pattern has been observed across various species in which S_d decreases as CO_2 levels increase in geological time and in controlled environment conditions (Woodward 1987). Also, higher light intensities increase S_d (Lake et al. 2001; Schoch et al. 1984). This increase is likely caused by the enhanced demand for CO_2 diffusion during photosynthesis under high light conditions (Xiong et al. 2022). The response of stomata to these environmental factors is mediated by systemic signaling mechanisms because mature leaves sense the conditions and influence stomatal developmental in young leaves (Lake et al. 2001; Miyazawa et al. 2006). This regulatory process is likely to entail modifications to stomatal patterning pathways, integrating both environmental stimuli and long-distance pathways (Casson and Gray 2008).

The temporal response of g_s to changing irradiance in different species has been investigated (Drake et al. 2013; McAusland et al. 2016). Stomatal opening is primarily regulated by guard cells responding to light (Zhang et al. 2024). Blue light activates photoreceptors in guard cells, stimulating proton pumps (H^+-ATPases) in the plasma membrane and creating an electrochemical gradient that enables the entrance of K^+ ions via potassium channels (Hayashi and Kinoshita 2011). Simultaneously, the accumulation of Cl^- and malate lowers the osmotic potential in the guard cells, facilitating water inflow via aquaporins (Ding and Chaumont 2020). Ultimately, the increasingly turgid guard cells form an opening for gas exchange. Various steady-state models have been used to describe g_s responses (Damour et al. 2010; Vialet-Chabrand et al. 2013). However, changes in g_s do not occur instantaneously with variations in environmental factors. Therefore, dynamic models are used to describe g_s responses with more precision (Damour et al. 2010; Vialet-Chabrand et al. 2013). Kirschbaum et al. (1988) proposed a model in which the temporal response of g_s to irradiance was the result of three subsequent dynamics with different time constants governing each step: first, irradiance induced a biochemical signal; second, an osmotic change was produced by fluxes of K^+ into or out of the guard cells; and, finally, water movement into or out of the guard cells occurred. These steps lead to a sigmoidal curve for g_s in response to a change in irradiance.

Previous studies suggested that stomata morphology can affect the temporal response of g_s in some species. For example, plants with smaller stomata exhibit faster movement in response to different light intensity across *Banksia* and cereal species (Drake et al. 2013; McAusland et al. 2016). The relationship between stomata morphology and temporal responses in six tree species was also observed in response to water deficit (Aasamaa et al. 2001). Additionally, plants with higher S_d may have faster opening (Lawson and Vialet-Chabrand 2019) and closing (Gerardin et al. 2018) responses. However, stomata morphology did not have an effect on the stomata closing speed of *Hordeum vulgare* and *Lepidozamia peroffskyana* (Elliott-Kingston et al. 2016).

The first objective of this study was to survey stomata morphology among different blueberry genotypes. The second objective of this study was to evaluate the effect of stomata morphology on the speed of response to the changing photosynthetic photon flux density (PPFD). We hypothesized that blueberry plants with larger S_a and lower S_d have slower responses to changes in PPFD.

Materials and Methods

Plant material. A collection of 37 clonally propagated blueberry genotypes, including southern highbush blueberry (SHB; *V. corymbosum* L. interspecific hybrids), northern highbush blueberry (NHB; *V. corymbosum* L.), and rabbiteye blueberry (RBE; *V. ashei* Reade), were used to investigate stomata morphological traits. Genotypes sampled included publicly released cultivars, proprietary cultivars, and breeding selections growing in seven different locations in North America, including Auburn, AL, USA (lat. 32°35′49.7″N, long. 85°29′17.3″W), Tallassee, FL, USA (lat. 32°49′54.0″N, long. 85°53′30.4″W), Gainesville, FL, USA (lat. 29°38′15.696″N, long. 82°21′51.026″W), Citra, FL, USA, (lat. 29°24′54.652″N, long. 82°8′42.986″W), Venus, FL, USA (lat. 27°10.1″N, long. 81°32′22.6″W), Mt. Horeb, WI, USA (lat. 42°57′23.0″N, long. 89°39′41.5″W), and Tangancicuaro, Michoacan, Mexico (lat. 19°51′55.1″N, long. 102°11′44.7″W). Plants were grown either in fields or in greenhouses and managed according to commercial practices (Table 1).

Stomata density and area. Stomata morphological traits of five plants per genotype were evaluated. Two fully developed leaves from the upper canopy and two fully developed leaves from the center of the canopy were collected from each plant. The S_d and S_a were determined using the imprint method (Rogiers et al. 2011). Stomata imprints were made using a modified version of the protocol described by Das and Santakumari (1977). Briefly, a thin coat of clear nail polish (Color 103; Sally Hansen, NY, USA) was applied on both the abaxial and adaxial sides of each leaf. After drying, the nail polish film, which had an imprint of the leaf epidermis, was removed and mounted on a microscope slide with clear adhesive tape. All imprints were made in the center of the leaf lamina while avoiding the leaf margin and midvein. Imprints were digitalized using light microscopes [Micromaster II (Fisher Scientific, Newington, NH, USA) or B490B (Amscope, Irvine, CA, USA)] and a mobile phone camera (iPhone 7, 28-mm 12-megapixel camera with optical image stabilization; Apple, Cupertino, CA, USA). The S_d was determined by counting the number of stomata present in 1 mm^2 of the imprint at 40× magnification using the multipoint counter of ImageJ (version 1.53r) (Rueden et al. 2017). Stomata length and width were manually measured in 30 imprints per genotype at 100× magnification. A stage micrometer was used to calibrate images. The S_a (μm^2) was modeled as an ellipsoid and calculated using measurements from three stomata per slide using the following equation: $S_a = \pi*A*B$, where A is length of major radius and B is length of minor radius. Leaf porosity (P_l) was calculated as $P_l = S_a*S_d$. This variable represents the allometric relationship between the stomata area and density in 1 mm^2 of leaf lamina.

Stomata speed. Plants of SHB genotypes 'Arcadia', 'Colossus', 'Meadowlark', and 'Optimus' (n = 6 per genotype) were planted into 11.35-L pots filled with a substrate mixture of 3:2:1 (v/v) of peat, pine bark, and perlite, respectively. These genotypes were selected for further testing based on the results from the stomata morphology survey. Plants were watered as needed

Table 1. List of genotypes, type, and sampling location of plants studied during this experiment.

Genotype	Type	Sampling location	Cultivation media	Genotype	Type	Cultivation media	Sampling location
Arcadia	SHB[i]	Gainesville, FL, USA	Substrate	FL16-194	SHB	Substrate	Gainesville, FL, USA
Avanti	SHB	Gainesville, FL, USA	Substrate	FL16-64	SHB	Soil	Citra, FL, USA
Baldwin	RBE[iii]	Tallassee, FL, USA	Soil	FL18-188	SHB	Soil	Citra, FL, USA
Bluecrop	NHB[ii]	Auburn, AL, USA	Soil	Indigocrisp	SHB	Soil	Tallassee, FL, USA
Bluegold	NHB	Auburn, AL, USA	Soil	Jewel	SHB	Soil	Tallassee, FL, USA
Blueray	NHB	Mt. Horeb, WI, USA	Soil	Legacy	NHB	Soil	Tallassee, FL, USA
Brightwell	RBE	Gainesville, FL, USA	Soil	Meadowlark	SHB	Soil	Gainesville, FL, USA
Climax	RBE	Gainesville, FL, USA	Soil	Northland	NHB	Soil	Mt Horeb, WI, USA
Colossus	SHB	Gainesville, FL, USA	Substrate	Optimus	SHB	Substrate	Gainesville, FL, USA
Driscolls1	SHB	Michoacan, Mexico	Soil	OzBlu1	SHB	Soil	Venus, FL, USA
Driscolls2	SHB	Michoacan, Mexico	Soil	OzBlu2	SHB	Soil	Venus, FL, USA
Duke	NHB	Auburn, AL, USA	Soil	Patriot	NHB	Soil	Mt Horeb, WI, USA
Echo	NHB	Gainesville, FL, USA	Substrate	Powderblue	RBE	Soil	Gainesville, FL, USA
Elliott	NHB	Gainesville, FL, USA	Substrate	Sentinel	SHB	Substrate	Gainesville, FL, USA
Emerald	SHB	Gainesville, FL, USA	Substrate	Sweetcrisp	SHB	Soil	Gainesville, FL, USA
Farthing	SHB	Gainesville, FL, USA	Soil	Toro	NHB	Soil	Auburn, AL, USA
FL06-19	SHB	Gainesville, FL, USA	Substrate	Victoria	SHB	Soil	Tallassee, AL, USA
FL09-311	SHB	Gainesville, FL, USA	Substrate	Vireo	SHB	Substrate	Gainesville, FL, USA
FL15-145	SHB	Citra, FL, USA	Soil	—	—		—

[i] SHB = southern highbush blueberry, *Vaccinium corymbosum* L. interspecific hybrids adapted to tropical and subtropical environments.
[ii] NHB = northern highbush blueberry, *Vaccinium corymbosum* L. interspecific hybrids adapted to temperate environments.
[iii] RBE = rabbiteye blueberry, *Vaccinium ashei* Reade.

for the length of the experiment and fertilized twice per week. The fertilizer solution was prepared with 100 mg/L of a soluble fertilizer (21N–3.05P–5.81K; Peters Professional Acid Special; ICL Specialty Fertilizers, Summerville, SC, USA) with 11.5% and 9.5% of the N provided as ammonium and urea, respectively. Plants were pruned and allowed to grow in a temperature-controlled greenhouse located in Gainesville, FL, USA, for a duration of 10 to 12 weeks. Stomata morphology of the same leaves used for gas exchange was determined as aforementioned. Leaf gas exchange was recorded with an infrared gas analyzer (model CIRAS-4; PP-systems, Amesbury, MA, USA) equipped with a leaf cuvette and an integrated light-emitting diode light unit. Air flow inside the cuvette during measurements was 300 $\mu mol \cdot min^{-1}$. The cuvette CO_2 concentration was 400 $\mu mol \cdot mol^{-1}$. Leaf temperature was maintained at a constant 25 °C, while the relative humidity level was controlled at 60%. Plants were subjected to dark acclimation during the night. Gas exchange measurements commenced at sunrise (between 6:30 AM and 6:50 AM in Nov and Dec 2023) following a custom-built script, with leaves kept in the dark for 15 min before being exposed to an instantaneous increase in irradiance (800 $\mu mol \cdot m^{-2} \cdot s^{-1}$) that lasted 150 min. Temporal responses of g_s of one young fully expanded leaf per plant were measured. Data were collected every 20 s after stabilization of conditions inside the chamber. Gas exchange measurements were collected inside the greenhouse.

Response curves were fitted using the asymmetric sigmoidal model described by Vialet-Chabrand et al. (2013) as implemented by Gerardin et al. (2018). The asymmetric model was formulated according to a standard Gompertz function with the following equation:

$$g_s = g_{min} + (g_{max} - g_{min}) * e^{-e^{\left(1-\frac{t}{\tau}\right)}} \quad [1]$$

where g_s is the fitted stomatal conductance, g_{min} is the minimal starting value of stomatal conductance, g_{max} is the maximum stomatal conductance, λ is the lag time of the stomatal response (the time needed to reach the inflection point of the curve from the moment of the irradiance change from 0 to 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and τ is the response time from the first response to change in irradiance until g_{max} is reached. The total response time (T) was calculated by summing τ and λ. The stomatal speed response (SL_{max}) was calculated as follows:

$$SL_{max} = \left(\frac{1}{t}\right) * \frac{(g_{max} - g_{min})}{e} \quad [2]$$

where ($g_{max} - g_{min}$) represents the amplitude of the stomatal response and *e* represents the Euler constant. This model was fitted in R (version RStudio 2021.09.2) using the NLIMB function with a large uniform grid of possible random starting values for τ and λ. The range of values selected was between 1 and 100 for both parameters, varying incrementally by 1. They were optimized through a grid search until stability and convergence were achieved.

Experimental design and data analysis. Experiments were performed as a completely randomized design, with each plant considered a replication. For S_d and S_a, leaves from the same plant were considered pseudo-replications. Data were analyzed via an analysis of variance using the *agricolae* package (Mendiburu 2021) in R (version RStudio 2021.09.2). Mean separation was performed using Fisher's protected least significant differences. Unpaired *t* tests were used to compare stomata density in leaves from the upper and central parts of the canopy. The Pearson correlation was calculated between stomata traits and model parameters in R. *P* values were considered significant at $\alpha \leq 0.05$.

The importance of genotype, leaf position, and species type and the estimate of their effect on leaf density and area were analyzed by fitting a linear mixed model. All model components were set as random terms except the intercept. The model was fitted using the *Asreml* R package (Butler et al. 2023) as follows:

$$y = \mu + Z_1 g + Z_2 p + Z_3 t + e$$

where y is the vector of the response variable, μ is the overall mean, g is the vector of the genotype effect, p is the vector of the leaf position effect, t is the vector of the species type effect, and e is the random error. The incidence matrices are denoted as Z_1, Z_2, and Z_3. All random effect terms are assumed to follow a multivariate normal distribution. Variance components were estimated using the restricted maximum likelihood. The significance of random terms was assessed using the likelihood ratio test, which compares the full model to a reduced model excluding the term being tested. Data visualization and graphing were performed using the *ggplot2* package (Wickham 2011) in R.

Results and Discussion

Stomata density and area. Our survey confirmed that *Vaccinium* spp. are hypostomatous plants because stomata were not observed on the adaxial side of leaves of all genotypes evaluated (1110 imprints). Blueberry stomata were composed by two symmetric dumbbell-shaped guard cells. Subsidiary cells were not discernible using light microscopy.

The S_d and S_a varied considerably among different blueberry genotypes (Fig. 1), and the genotype effect accounted for the highest variance explained compared with blueberry type and position of the leaves (Table 2). There was an approximately three-fold difference in S_a between the genotype with the smallest ('Optimus') and largest ('Northland') stomata. Overall, NHB genotypes exhibited more stomata than SHB and RBE genotypes studied in this experiment (Fig. 1A). There was an approximately four-fold difference between the genotype with lowest (FL16-194) and highest ('Brightwell') S_d. Additionally, variations in stomata traits were observed among genotypes of the same blueberry type (Supplemental Figure 1). In other species, increases in S_d co-occur with smaller stomata (Carlson et al. 2016; Franks and Farquhar 2001; Hetherington and Woodward, 2003). This allometric relationship was not observed in highbush blueberries during our survey (Fig. 1C). Correlations between S_a and S_d were not significant among the genotypes tested ($P = 0.18$).

The S_a has been used to predict ploidy levels of many plant species, including blueberry (Chavez and Lyrene 2009), wheat (Wang et al. 1989), alfalfa (Bingham 1968), coffee (Mishra 1997), *Crataegus* spp. (McGoey et al. 2014), and *Bromus inermis* (Teare et al. 1971). Blueberries can be found at different ploidy levels (Hummer et al. 2015; Lyrene 2006; Sakhanokho et al. 2018). We showed that S_a is not an ideal tool for assessing

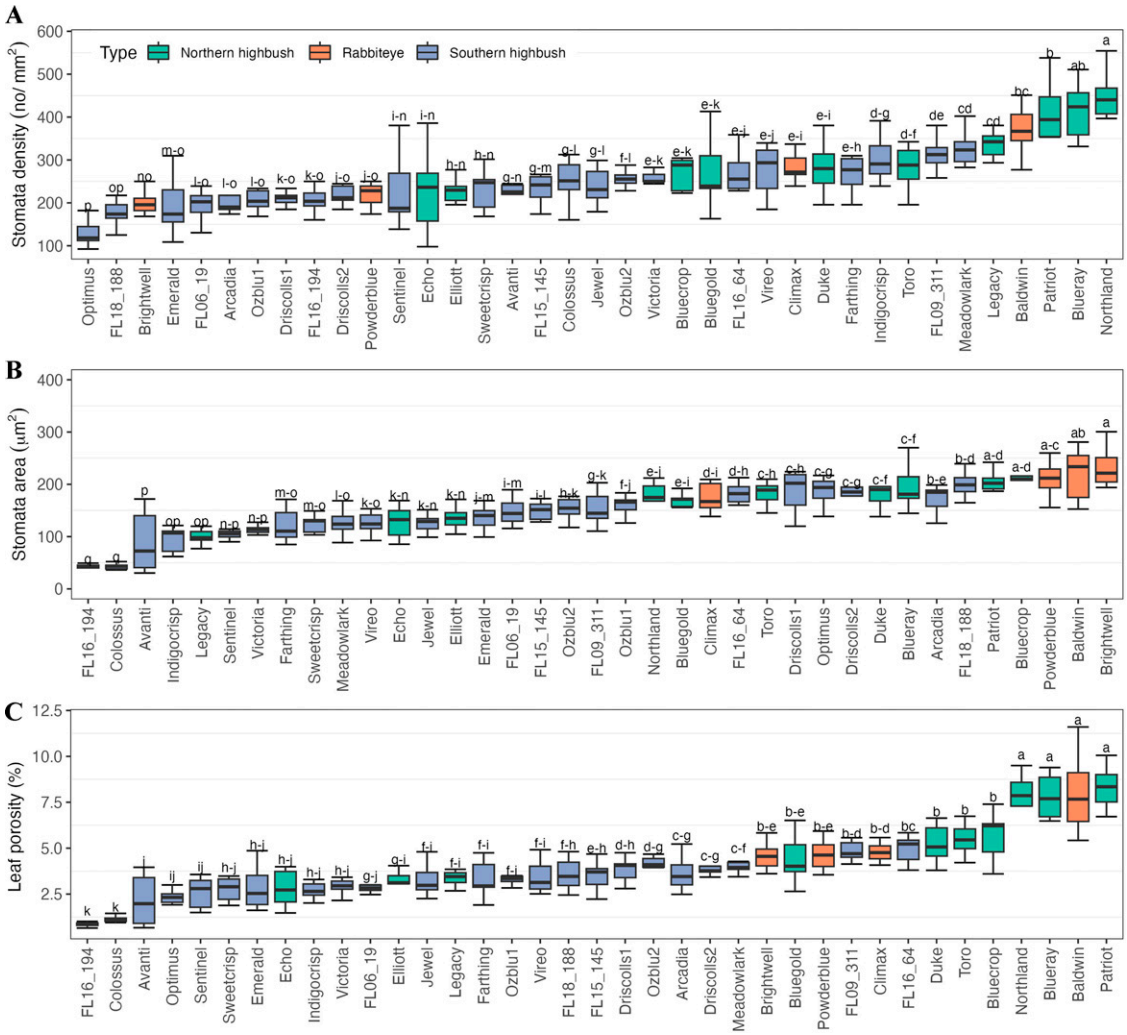

Fig. 1. Stomata density (**A**), stomata area (**B**), and leaf porosity (**C**) measured on the abaxial side of leaves of 37 blueberry genotypes grown across eight sites in North America. Boxes are colored according to blueberry type. Different letters indicate significant differences between genotypes ($P \leq 0.05$).

Table 2. Estimate of variance component for genotype, leaf position, and species type for leaf stomata density and leaf area in blueberry.

Source of variance	Genotype	Position	Type	Error
Density	0.07***	0.01^{ns}	0.01*	0.91
Area	0.38***	0.24*	0.00^{ns}	0.38

Significance testing ($P < 0.05$) for random effects was conducted using the likelihood ratio test to compare the full model with a reduced model excluding the term under examination. Model components represent the percentage of variance explained. ns, *, **, ***Nonsignificant or significant at $P < 0.05$, $P < 0.01$, or $P < 0.001$, respectively.

blueberry ploidy because the presumed ploidy level did not correlate with the stomata area ($r = 0.008$; $P = 0.87$).

Light intensity and quality can influence stomata traits. Kim et al. (2011) reported fewer and larger stomata in leaves of NHB 'Bluecrop' plants as shade levels increased. We considered leaf position in the canopy as a proxy for sun exposure during leaf development. In the present study, this factor affected S_d only in a subset of the genotypes tested. 'Sentinel', 'Sweetcrisp', 'Meadowlark', 'Victoria', 'Elliott', 'Duke', 'Patriot', 'Bueray', 'Northland', 'Bluegold', 'Climax', 'FL18-188' and 'Brightwell' had significantly higher ($P < 0.05$) S_d on leaves collected from the top of the canopy (full sun exposure) compared with that on leaves collected from the center of the canopy (Fig. 2). These differences are likely the result of differences in light exposure because leaves in the upper canopy receive higher light intensities, which are known to influence stomatal development (Idris et al. 2018). All genotypes sampled in Wisconsin exhibited differences in S_d on leaves collected from the top of plant canopy compared with that on leaves from the middle to lower canopy. These results could be related to pruning practices or environmental conditions in the northern United States. Pruning varies by region based on climate, blueberry genotype, and farming practices (Fang et al. 2020; Kovaleski et al. 2015; Retamales and Hancock 2018).

Stomata responses to irradiance. The SHB genotypes grown under common environmental conditions were used to confirm stomata survey results and study the effects of stomatal traits on stomatal responses (Fig. 3). Genotypes 'Colossus' and 'Optimus' exhibited lower stomata area ($P < 0.05$) and higher stomatal density ($P < 0.01$) than those of 'Meadowlark' and 'Arcadia'. In this subset of genotypes, cultivars 'Arcadia' and 'Colossus' maintained their stomata characteristics between the survey and follow-up experiment, but 'Optimus' and 'Meadowlark' did not. Dynamic models with acceptable fit metrics (g_{min}, g_{max}, λ, t, and SL_{max}) were fit for most of the studied leaves ($n = 23$). Model parameters from each genotype were compared (Supplemental Table 1). The stepwise increase in irradiance led to stomatal responses in all genotypes (Fig. 4). 'Colossus' exhibited the fastest stomata response to changing irradiance, which was significantly different ($P = 0.001$) from that of 'Meadowlark'. The amplitude of the stomatal response ranged from 19.48 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 164.47 $\mu mol \cdot m^{-2} \cdot s^{-1}$ across genotypes. Notably, λ and τ exhibited different relations with stomatal morphology. Additionally, λ differed significantly ($P < 0.01$) among the genotypes, such that the inflection point of the curve from the moment of irradiance changed rapidly for all genotypes except 'Optimus'. Stalfelt (1927) introduced the term "Spannumgsphase" to describe a phenomenon characterized by anticipatory reactions in the stomata that are not accompanied by movement. While it has been suggested that the speed of signal transduction is unlikely to cause significant delays in stomatal opening or closing relative to the

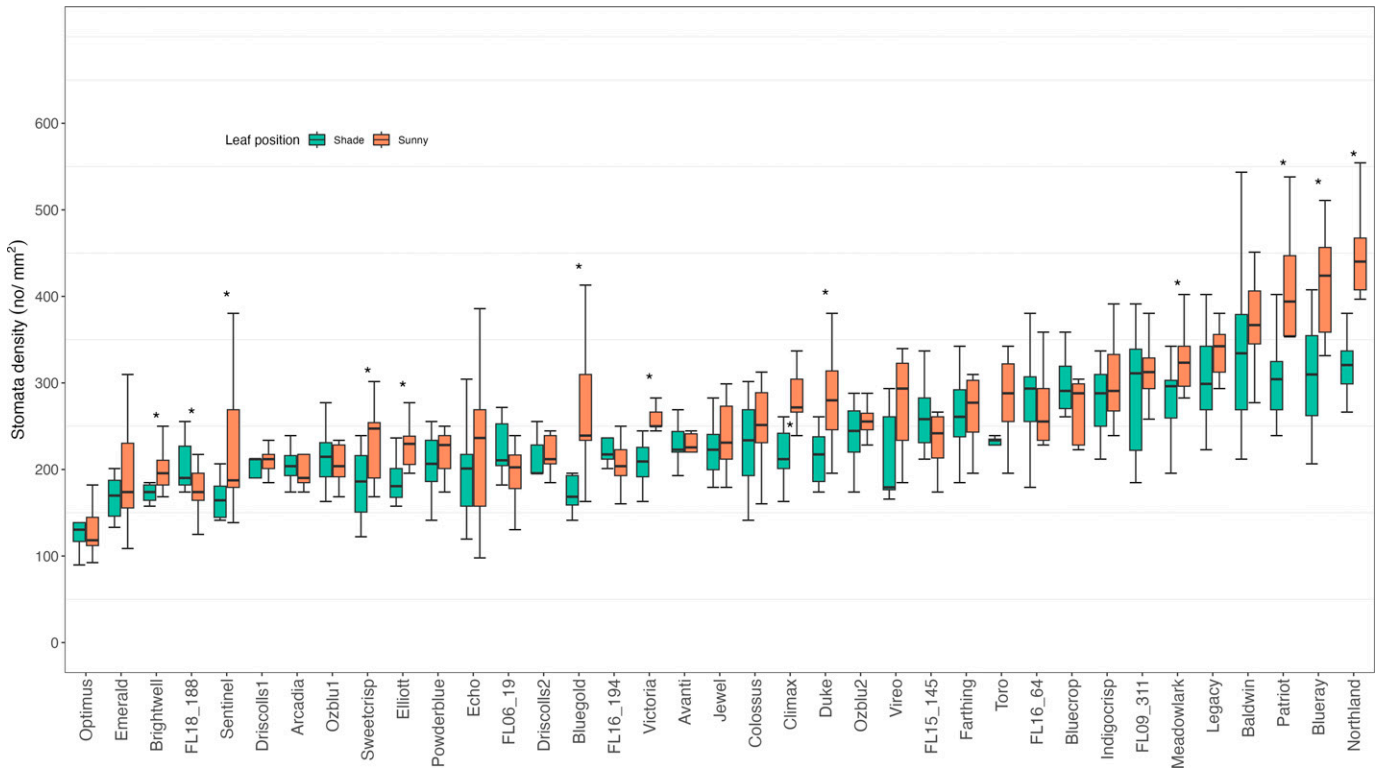

Fig. 2. Stomata density measured on the abaxial side of leaves from the upper (full sun exposure) and central (shaded area) canopy of 37 blueberry genotypes grown in five locations. Boxes are colored according to the leaf position. *Significant differences between leaf positions ($P \leq 0.05$).

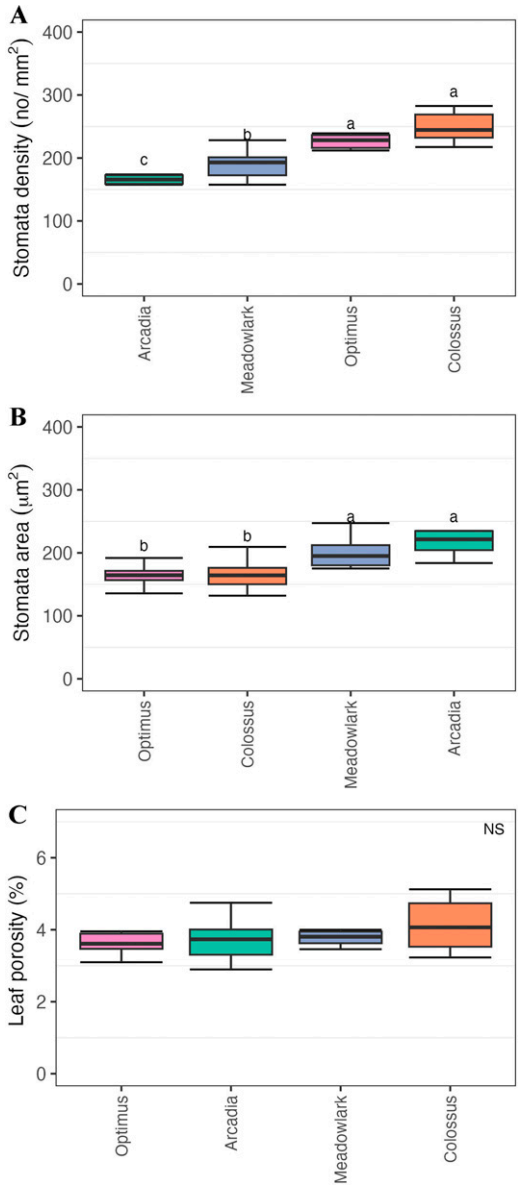

Fig. 3. Stomata density (**A**), stomata area (**B**), and leaf porosity (**C**) measured on the abaxial side of leaves of 'Meadowlark', 'Arcadia', 'Optimus', and 'Colossus' southern highbush blueberry grown in greenhouse in Gainesville, FL, USA. Different letters indicate significant differences between genotypes ($P \leq 0.05$).

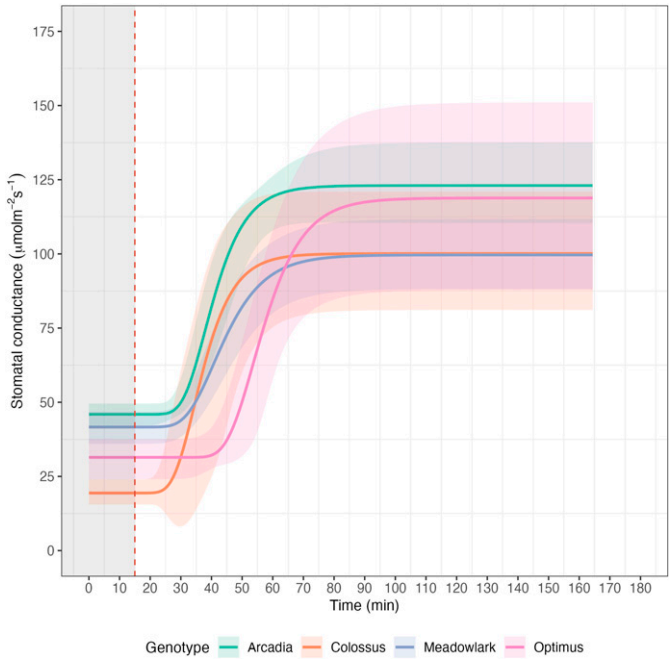

Fig. 4. Modeled temporal response of stomatal conductance (g_s; mol·m^{-2}·s^{-1}) submitted to an instantaneous increase in irradiance from 0 to 800 µmol·m^{-2}·s^{-1}. Each curve represents the average g_s of six different plants per genotype. Data were recorded every 20 s and represented with a time step of 15 min (900 s).

mechanisms of movement (Franks and Farquhar 2007), our results indicate that λ can significantly affect the total time from the moment of irradiance change until g_{max} is achieved ($P < 0.005$). The larger λ of 'Optimus' suggests a prolonged Spannugsphase, which may delay the osmotic adjustments required for guard cell turgor changes. This delay could be caused by limitations in the rate of osmolarity generation, such as K^+ influx and the production of organic solutes (Talbott and Zeiger 1996). However, our data suggest that neither S_a nor S_d affects λ.

No correlation was observed between S_a and SL_{max} ($r = 0.02$; $P = 0.91$), suggesting that the speed of the stomatal response is not directly influenced by S_a in SHB (Table 3). These results are in agreement with those of previous research of a large spectrum of species, including ferns, cycads, conifers, and angiosperms (Elliott-Kingston et al. 2016). However, they differ from those of previous reports that suggest that a smaller S_a leads to a faster stomatal response caused by a higher surface-to-volume ratio and, consequently, the lesser solute transport needed to drive stomatal movements (Drake et al. 2013; Lawson and Blatt 2014). It is possible that parameters other than stomatal morphology, such as variations in ion and water transport within guard cells (Kübarsepp et al. 2020; Lawson and Blatt 2014), impacted the speed of the stomatal response in the surveyed SHB genotypes.

A significant positive correlation was found between S_d and SL_{max} ($P = 0.04$), associating more stomata with faster responses. Similar results were found in *A. thaliana* (Vialet-Chabrand et al. 2016). Morphological traits related to the regulation of water movement within and out of the leaf such as higher vein density (Westbrook and McAdam 2021) and a more favorable boundary layer (Defraeye et al. 2013) have been related to this response. Morphological phenes that create a more efficient hydraulic network in the leaf–atmosphere continuum might allow speedy turgor pressure adjustments in the guard cells, facilitating stomata opening.

In this study, g_{max} was not different among the tested genotypes ($P = 0.18$), despite their differences in S_d. While in other species an increase in S_d can lead to higher g_s (Maruyama and Tajima 1990; Ohsumi et al. 2007; Schlüter et al. 2003), our results indicate no correlation between these variables ($P = 0.06$). This is consistent with the findings in the literature (Jones 1977; Kawamitsu et al. 1996). Additionally, we observed no correlation between g_s and S_a ($P = 0.41$). Therefore, our hypothesis was not supported by the data.

In this study, g_{min} differed significantly among the tested genotypes ($P < 0.001$). The g_{min}, which is often referred as to $g_{cuticular}$ or $g_{residual}$, reflects the conductance measured at maximal stomatal closure and is not directly regulated by guard cells (Caird et al. 2007). Previous studies have reported g_{min} estimates ranging from 0.004 to 0.020 µmol·m^{-2}·s^{-1} for genotypes of

Table 3. Correlation table of model parameters and the stomata morphological traits. Correlations and *P* values are provided in parentheses between stomata density (S_d), stomata area (S_a), porosity (P_l), minimal stomatal conductance (g_{min}), lag time (λ), response time to changes in irradiance (t), maximum stomatal conductance (g_{max}), SL_{max}, total response time to changes in irradiance (T), and amplitude of the stomatal response (SA). Data are from four southern highbush blueberry genotypes 'Arcadia', 'Colossus', 'Meadowlark', and 'Optimus' grown under the same conditions in a greenhouse.

	S_d	S_a	P_l	g_{min}	λ	t	g_{max}	SL_{max}	SA	T
S_d	1	−0.278	0.501	−0.638	−0.145	−0.207	−0.089	0.416	0.233	−0.179
	(-)	($P = 0.187$)	($P = 0.012$)	($P = 0.001$)	($P = 0.507$)	($P = 0.343$)	($P = 0.685$)	($P = 0.047$)	($P = 0.283$)	($P = 0.413$)
S_a		1	0.680	0.127	−0.007	0.051	0.145	0.022	0.083	0.006
		(-)	($P = 0.000$)	($P = 0.563$)	($P = 0.972$)	($P = 0.814$)	($P = 0.508$)	($P = 0.918$)	($P = 0.705$)	($P = 0.976$)
P_l			1	−0.376	−0.142	−0.175	0.068	0.398	0.261	−0.168
			(-)	($P = 0.076$)	($P = 0.516$)	($P = 0.422$)	($P = 0.755$)	($P = 0.059$)	($P = 0.228$)	($P = 0.441$)
g_{min}				1	−0.100	0.280	0.283	−0.416	−0.219	−0.016
				(-)	($P = 0.648$)	($P = 0.194$)	($P = 0.190$)	($P = 0.048$)	($P = 0.313$)	($P = 0.940$)
λ					1	0.406	−0.101	−0.378	−0.052	0.972
					(-)	($P = 0.054$)	($P = 0.643$)	($P = 0.0751$)	($P = 0.811$)	($P < 0.000$)
t						1	0.218	−0.730	0.079	0.606
						(-)	($P = 0.317$)	($P < 0.000$)	($P = 0.719$)	($P = 0.002$)
g_{max}							1	0.330	0.873	−0.033
							(-)	($P = 0.123$)	($P < 0.000$)	($P = 0.879$)
SL_{max}								1	0.547	−0.513
								(-)	($P = 0.006$)	($P = 0.012$)
SA									1	−0.025
									(-)	($P = 0.907$)
T										1
										(-)

wheat, grape, helianthus, and European trees and shrubs (Boyer et al. 1997; Burghardt and Riederer 2003; Howard and Donovan 2007; Kerstiens 1995; Rawson and Clarke 1988). Blueberry g_{min} ranged from 14 to 52 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at predawn. Genotypes 'Arcadia' and 'Meadowlark' had almost two-fold higher g_{min} values compared with those of 'Optimus' and 'Colossus' (Fig. 3). This could be a consequence of incomplete stomatal closure at night. However, it also may be influenced by factors such as the presence of dust or other materials preventing complete stomatal closure (Caird et al. 2007). Alternatively, differences in cuticle thickness, composition, and development among leaves could have caused the high g_{min} (Bi et al. 2017; Buschhaus and Jetter 2011; Pollard et al. 2008; Qiao et al. 2020). Finally, like other plants, SHB might exhibit predawn stomatal opening (Caird et al. 2007; Dodd et al. 2005; Howard and Donovan 2007; Schwabe 1952).

In conclusion, our study provides insights into the stomatal traits and responses of various blueberry (*Vaccinium* spp.) genotypes. We confirmed that blueberries are hypostomatous plants and documented significant diversity in stomatal morphology. This diversity extended to stomatal responses to irradiance, where S_d, but not S_a, affected the speed of stomatal response. Additionally, we documented differences in g_{min} among SHB genotypes. These results provide foundational information for the study of stomatal biology of a globally important food crop such as blueberry.

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