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Investigating shade tolerance and phenotypic plasticity of Virginia spiraea (*Spiraea virginiana* Britton), a federally threatened shrub

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Abstract

Virginia spiraea (*Spiraea virginiana* Britton, Rosaceae) is a shade-intolerant, disturbance-adapted, riparian shrub species native to the southern Appalachian Mountains. This species was listed as threatened in 1990, and a recovery plan was developed in 1992. Current reassessment of the recovery plan includes proposals for propagation and outplanting to supplement and restore wild populations. Without flooding disturbance, competing vegetation needs to be actively managed to reduce shade for spiraea to thrive. Genotypes with greater shade acclimation would likely have higher survival in natural populations, would require less frequent shade-reduction management, and could be integrated into populations that need restoration. In summer 2022, we examined photosynthetic characteristics (maximum light-saturated photosynthetic, dark respiration rates, quantum yield, light compensation point, and pigment concentrations) and the ability to respond to sunflecks (photosynthetic induction and loss) of cloned propagules from five different source populations along an artificial light gradient (100%, 75%, 50%, and 20% of full sun) in a common garden. Data were compared among light treatments and source populations using ANOVA or non-parametric tests. Light treatment had significant effects of maximum photosynthesis, dark respiration, specific leaf mass, and light compensation, but not

quantum yield, pigment concentrations, or sunfleck utilization. Source population did not have a significant effect on any parameter. The five source populations were all from the same river drainage (New River, Ashe County, NC), and studies have shown little genetic difference among individuals within the same drainage. Higher genetic variability has been shown between drainages. Future studies should examine photosynthetic characteristics of individuals from genetically contrasting source populations. More work needs to be done to understand the species' plasticity and acclimation potential under a wider range of environmental conditions to help develop a plan for successful recovery of Virginia spiraea in wild populations.

1. Introduction

Virginia spiraea (*Spiraea virginiana* Britton) is a federally listed (threatened) species of rhizomatous shrub in the Rosaceae family. It is native to the southern Appalachian Mountains with populations in southern Blue Ridge or the Appalachian (Cumberland) Plateau physiographic provinces on streams that flow into the Ohio River drainage basin[1]. This species' range has been declining since environmental shifts associated with glacial retreat[1]. It is now found in riverine habitats including steep, south-facing slopes, open canopies, and sites with little herbaceous cover[2]. The U.S. Fish and Wildlife Service added this species to the U.S. Endangered Species list as threatened in 1990, and in 1992 a recovery plan for Virginia spiraea was proposed: preserve, understand, extend knowledge, manage, and monitor[1,3]. A reassessment of this species is currently being conducted in hopes of de-listing the species. Recovery methods may involve propagating and outplanting Virginia spiraea to supplement and/or restore natural populations.

Virginia spiraea grows alongside rivers on loose deposits in parts of scoured banks of high gradient streams[1]. Flooding can pose a significant challenge to plants in these riparian areas, whose establishment on banks is threatened by unpredictable surges of water scouring the waterway following substantial rainfall events[4]. However, Virginia spiraea is a clonal species, with root systems and vegetative characteristics that allow it to thrive under appropriate disturbance regimes[3]. Reproduction is primarily asexual through vegetative propagation from fragmentation during floods[5], and viable seeds seem to be rarely produced[3]. This results in a disadvantage for Virginia spiraea because its most serious competitors are plants that have similar ecological niches but with the added advantage of prolific sexual reproduction and dispersal (e.g., *Rosa multiflora* Thunb. and *Spiraea japonica* L. f)[3]. Virginia spiraea is a disturbance-dependent species that is mostly shade-intolerant and is susceptible to shading from competing vegetation. Many populations require the competing vegetation to be actively managed to prevent population declines. Due to its threatened status, more work needs to be done to

understand the species' physiological plasticity and acclimation potential under a wider range of environmental conditions. Phenotypic plasticity is a mechanism by which organisms respond to environmental shifts with beneficial phenotypic changes that allow for favorable reactions to environmental variability. Species that have adaptive phenotypic plasticity may become established, acclimate to novel environments, and outcompete other species in a variety of habitats[6]. However, plants that experience low genetic diversity (i.e., clonal species) usually show lower levels of phenotypic plasticity[7]. Brzyski and Culley[4] examined genetic variation in the context of natural and anthropogenic challenges imposed on the riparian environment, giving insight of the complex patterns of genetic variation that exist in natural populations of Virginia spiraea. They found that genetic variation was low within, but high among populations, and they found no relationship between genetic and geographic distances.

Acclimation to lower light environments comes through adjustments of leaf morphology and both steady-state and dynamic photosynthetic responses. One method of assessing steady-state photosynthetic characteristics is through constructing steady-state light response curves[6]. Steady-state photosynthetic capabilities can be determined from these curves. This includes light compensation point (LCP), dark respiration (Rd), maximum photosynthesis (PnMax), and quantum yield (QY) and how they acclimate to shade. LCP indicate the minimum light level required for survival by estimating when photosynthetic carbon gain offsets respiratory carbon costs of leaf metabolism. Rd estimates the respiratory carbon costs regarding leaf metabolism when leaves are not exposed to light. PnMax estimates the maximum photosynthetic rate when leaves are light saturated. QY estimates the efficiency for converting absorbed light into fixed carbon[6]. Plants acclimating to low light environments generally have lower PnMax and Rd rates, and higher QY. This results in lower LCP which allows shade acclimated plants to maintain positive carbon gain in limited light environments[6]. Genotypes that are more plastic and better acclimate to shade should exhibit lower LCP.

Plants in low light environments often rely heavily on sunflecks, brief periods of high light coming through plant canopies, for positive carbon gain[8,9]. Although sunflecks are present for a small fraction of the day, they often contribute a majority of the daily photosynthetic photon flux density (PPFD) available for photosynthesis[10]. Efficient use of sunflecks allow plants to maintain positive carbon gain in lower light environments. Plants that are better able to use sunflecks generally have more rapid photosynthetic induction, a process where leaves demonstrate a lag period with increases in light before maximum photosynthetic rates are achieved. This is due to both biochemical and stomatal transient responses to a change from low to high light[11]. The biochemical stage of induction involves the light activation enzymes and the activation of Rubisco (Ribulose 1, 5-biphosphate carboxylase/oxygenase) and the buildup of metabolite pools in the RuBP regeneration pathway, and the stomatal limitations occur when stomata open and photosynthesis rates move towards steady-state[12]. A rapid induction response

with a slow rate of induction loss may serve to ensure that sunflecks are efficiently utilized[8].

The recovery plan from the U.S. Fish and Wildlife Service stated that there may be as few as 20 different genotypes across the range of Virginia spiraea[3], and a more recent study from Brzyski and Culley[4] examining genetic variation of Virginia spiraea found only 39 genotypes and many clones across the range of Ohio, Kentucky, and Tennessee, indicating that there is likely greater genetic variation within this species across its natural range than previously thought. In shadier habitats with greater competition for light, individuals with greater physiological plasticity imparting greater shade tolerance would likely have higher survival in natural populations and would require less frequent management interventions to reduce shade from adjacent vegetation. Propagules from these individuals can then be integrated into populations that need restoration. The goal of this research was to examine plasticity in photosynthetic physiology from different source populations.

2. Methods

Photosynthetic parameters were measured in a common garden located at the University of North Carolina Asheville during summer 2022 with shrubs established from several wild populations of Virginia spiraea (Table 1). Four replicate clones of plants from five western North Carolina source occurrences (20 shrubs total) were exposed to different levels of light treatments (100%, 75%, 50%, and 20% of full sunlight). This was done with neutral-density shade cloth that was installed over PVC frames in late March 2022 before shrubs leafed out, allowing leaves to develop in their randomly assigned light environment with room for plants to grow under the shade cloth frames. Photosynthetic characteristics including maximum light-saturated photosynthetic rate (PnMax), light compensation point (LCP), quantum yield (QY), dark respiration (Rd) rates, and the ability of genotypes to respond to sunflecks were assessed. Photosynthetic capacity and adjustments along light gradients were measured to determine the daily carbon gain while under various light treatments.

Table 1. Establishment data and locations for sources of Virginia spiraea.

Source*	**Latitude**	**Longitude**	**Elevation (m)**	**County**	**River**
EO-2	36.4370	81.3457	787	Ashe	South Fork New
EO-16	36.1851	81.2623	787	Ashe	South Fork New
EO-17	36.1907	81.2526	787	Ashe	South Fork New
EO-23	36.1849	81.2755	787	Ashe	South Fork New
EO-46	36.1815	81.2932	787	Ashe	South Fork New

*EO refers to the Element of Occurrence number from the North Carolina Natural Heritage Program.

2.1. Physiological measurements

Beginning in June 2022, steady-state (light response curves) and dynamic (photosynthetic induction and induction loss) photosynthetic characteristics were examined using two portable photosynthesis systems equipped with a CO_2 injector and red and blue LED light source (Li-6400, LiCor Biosciences, Lincoln, NE). For all measurements, CO_2 concentration in the chamber was maintained at 420 µmol/mol and relative humidity (~70%) and air temperature (25°C) were maintained near ambient conditions. Newest fully expanded leaves were selected to complete measurements, and plants were measured in a random order. All measurements were taken between 8:00AM and 12:00PM because the afternoon atmospheric water stress could potentially result in stomatal closure. Leaves were dried with a paper towel before attaching the gas exchange chamber. If measured leaves did not completely fill the chamber (6 cm^2), the leaf was photographed and analyzed using Image-J (National Institutes of Health) to estimate leaf area, then measurements were recomputed.

Steady-state light response curves were constructed by placing a leaf in the chamber and allowing it to acclimate to a constant photosynthetic rate at a PPFD of 1500 µmol m^{-2} s^{-1}. Steady state gas exchange rates were measured over a decreasing range of photosynthetic photon flux density (PPFD: 1500, 1250, 1000, 800, 600, 400, 300, 200, 100, 75, 50, 25, 10, and 0 µmol m^{-2} s^{-1}), and at each light level leaves were allowed to equilibrate to a steady state rate before measurements were taken (Fig 1). Light response curves were fit to equations from Marshall and Biscoe[13] and Thornley and Johnson[14] using a macro in MS Excel. Steady-state photosynthetic parameters (PnMax, Rd, QY, LCP) were calculated from these fit curves.

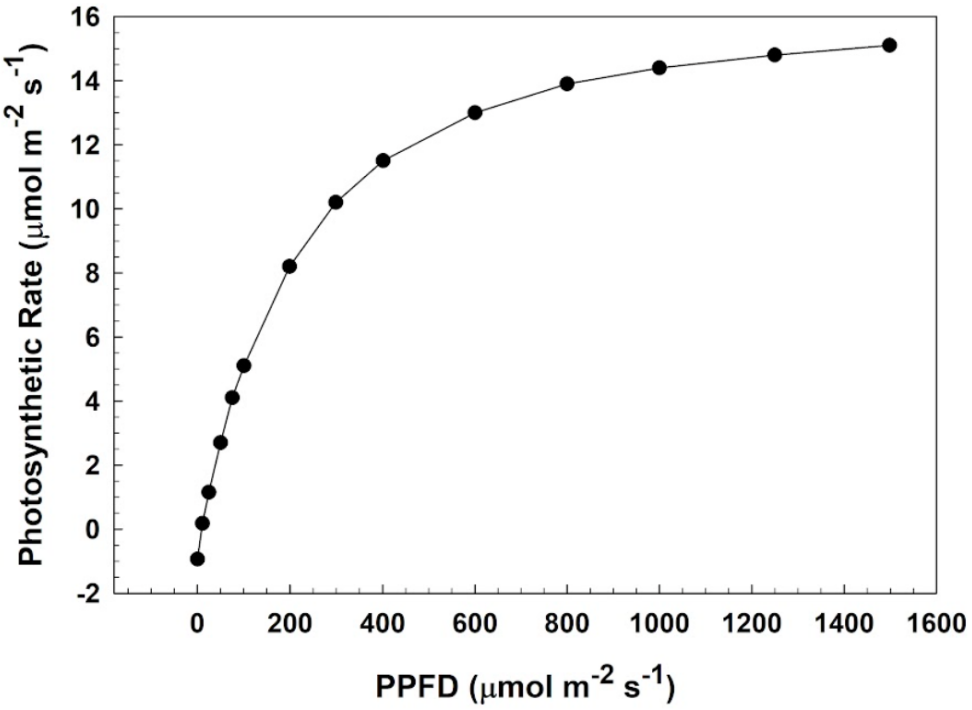

Figure 1. Example photosynthetic light response curve. Leaves acclimated to a constant photosynthetic rate at a photosynthetic photon flux density (PPFD) of 1500 μmol m^{-2} s^{-1}.

After gas exchange measurements, leaves were collected and chlorophyll content was estimated. A known leaf area (1.5 cm^2) was extracted in 5 mL of N, N-dimethylformamide, wrapped in foil, and stored at 5°C for 48 h. Absorbance of extracts was measured at two wavelengths (647 and 664 nm) using a spectrophotometer. Chlorophyll concentrations were estimated using the following equations[15] (1-3):

$$\text{Chlorophyll a} = 12.64 * A_{664} - 2.99 * A_{647} \quad (1)$$

$$\text{Chlorophyll b} = -5.60 * A_{664} + 23.26 * A_{647} \quad (2)$$

$$\text{Total Chlorophyll} = 7.04 * 20.27 * A_{647} \quad (3)$$

Specific leaf mass (SLM) was measured by drying a known leaf area (1.5 cm^2) at 60°C for 48 h and then weighing it with an analytical balance.

Photosynthetic induction was measured by first equilibrating leaves to 25 μmol m^{-2} s^{-1} PPFD until gas exchanged rates stabilized. The value of 25 μmol m^{-2} s^{-1} PPFD represented low light environments where Virginia spiraea is often found in natural habitats[2]. Leaves were then immediately exposed to a high PPFD of 1500 μmol m^{-2} s^{-1} then left to acclimate for 20 min, or until steady-state maximum rates were achieved.

Measurements were taken at 5 s intervals throughout each induction period. Induction times to 50% and 80% maximum photosynthetic rates were calculated (Figure 2).

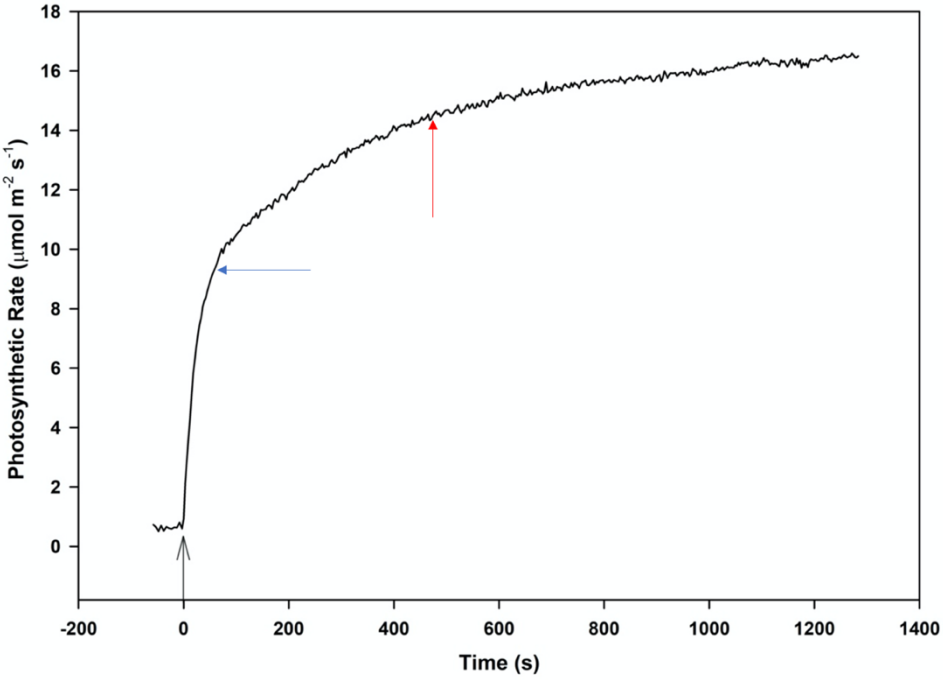

Figure 2. Example of a photosynthetic induction curve. The black arrow indicates when light increased to 1500 μmol m^{-2} s^{-1}, the blue arrow represents the time it took to reach 50% induction state, and the red arrow represents the time it took to reach 80% induction state.

Induction loss rates were measured by exposing fully induced leaves (1500 μmol m^{-2} s^{-1}) to "shade flecks" (25 μmol m^{-2} s^{-1} PPFD) for a random sequence including 1, 2, 3, 5, 10, 15, and 20 min. Plants were allowed to fully acclimate to a PPFD of 1500 μmol m^{-2} s^{-1} before the next shade fleck (Figure 3). Induction state (IS%) was determined by measuring gas exchange during a 5 second flash following low light exposure using this equation from Chazdon and Pearcy[8] (4):

$$IS\% = (P_{LF} - P_L)/ (P_H - P_L) * 100\% \quad (4)$$

Where P_{LF} is the rate of CO_2 assimilation at the end of the 5 s flash, P_L is the steady-state CO_2 assimilation at the low light level (25 μmol m^{-2} s^{-1}), and P_H is the steady-state CO_2 assimilation rate during high light (1500 μmol m^{-2} s^{-1}). After each low light period, plants were exposed to a high light of 1500 μmol m^{-2} s^{-1} to fully induce the plant before the next low light period[6].

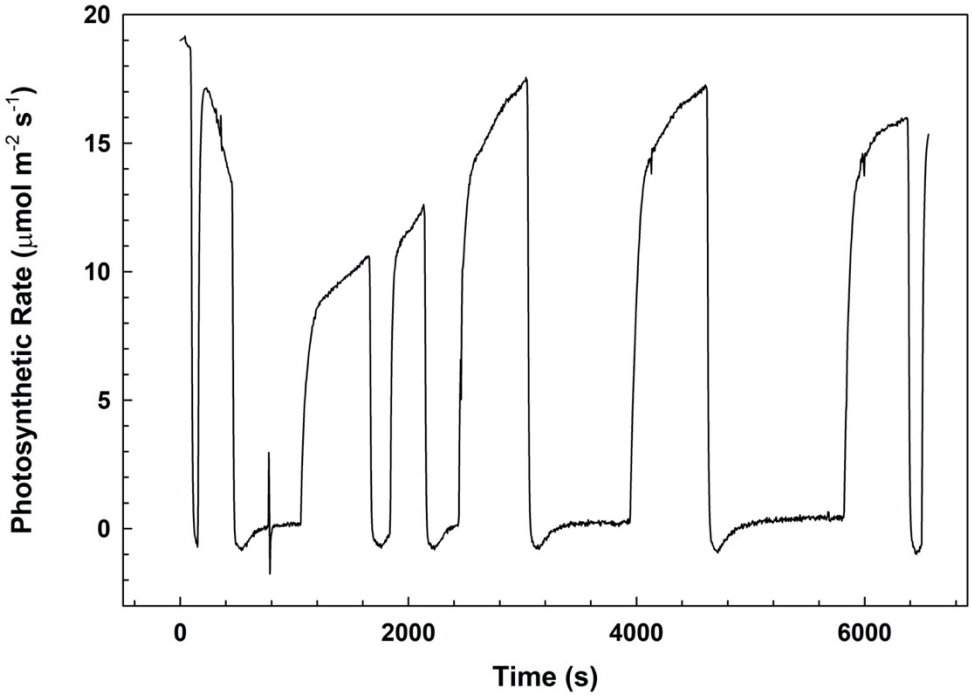

Figure 3. Example induction loss showing photosynthetic rate by time in seconds. Induction loss rates were measured exposing fully induced leaves (1500 μmol m^{-2} s^{-1}) to periods of a low light of 25 μmol m^{-2} s^{-1} PPFD for a random sequence of 1, 2, 3, 5, 10, 15, and 20 minutes.

Induction loss was modeled using a two-parameter exponential decay function from Horton and Neufeld[16] (5):

$$y = 100 * \exp^{\ln(1-p)*(D+X_p)*[X_p*(D+X_p)]} \quad (5)$$

where *y* equals induction state, *p* is the relative amount of induction loss, X_p is the time to either 50% or 80% loss of initial induction, and *D* is a parameter to be estimated[6] (Figure 4).

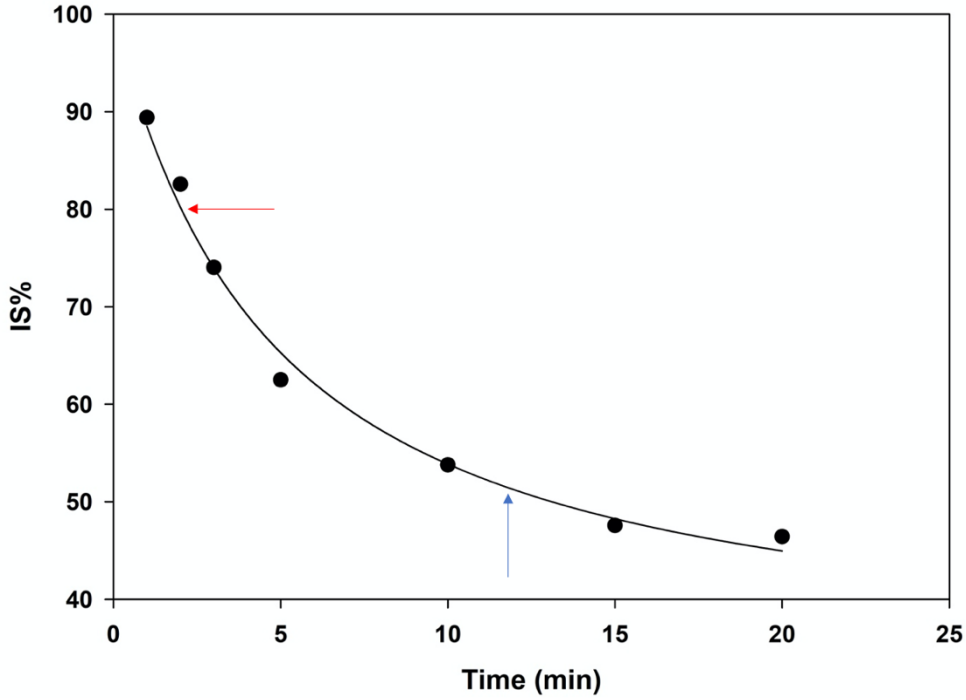

Figure 4. Example induction loss showing induction stage (IS%) versus time in minutes. The red arrow indicates the time of 80% induction (T80), and the blue arrow indicates the time of 50% induction (T50).

2.2. Data Analysis

All data were tested for normality (Kolmogorov-Smirnov). Parameters meeting the assumptions of normality (PnMax, Rd, QY, LCP, chlorophyll concentrations, SLM) were analyzed using analysis of variance (ANOVA), with source population and light treatment analyzed individually as independent variables. Induction and induction loss times were analyzed with the non-parametric one-way test (Kruskal-Wallis). All analyses were done in SAS software v 9.4 (SAS Institute 2012).

3. Results

Maximum photosynthesis (PnMax) was significantly higher in the 75% light treatment compared to the other treatments, which did not differ ($p = 0.0006$; Figure 5A). Dark respiration (Rd) was significantly lower in the 20% light treatment and significantly higher in the 100% light treatment compared to the other treatments, which did not differ ($p = 0.0037$; Figure 5B). Light compensation point (LCP) was significantly lower in the 20% light treatment and significantly higher in the 100% light treatment compared to the other treatments, which did not differ ($p = 0.0035$; Figure 5C). Quantum yield (QY) did

not show any significant differences by light treatment (p = 0.538; Figure 5D). Source population did not show any significant differences for any parameter (PnMax p = 0.85, Rd p = 0.88, LCP p = 0.84, QY p = 0.33).

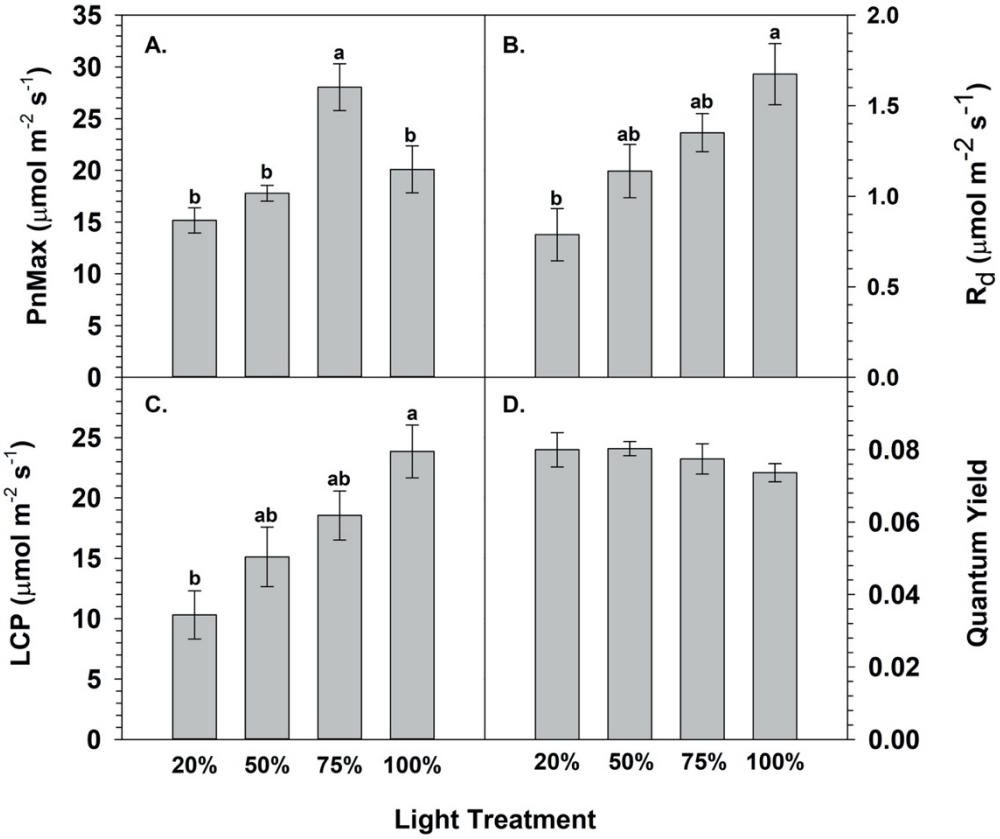

Figure 5. Mean (± 1 SE) maximum photosynthesis (A. PnMax), dark respiration (B. Rd), light compensation point (C. LCP), and quantum yield (D. QY) of Virginia spiraea grown in light treatments of 20%, 50%, 75%, and 100% of full sunlight. Tukey's post hoc test was used for bars with different letters representing significant differences ($p < 0.05$).

Time required for plants to reach 50% (T50 $\bar{X}$ = 1.3 ± 0.3 min) and 80% induction (T80 $\bar{X}$ = 5.5 ± 0.8 min) did not differ significantly among light treatments (T50 p = 0.44, T80 p = 0.21). Times required for plants to reach 50% (T50 $\bar{X}$ = 2.3 ± 0.2 min) and 80% induction loss (T80 $\bar{X}$ = 13.8 ± 1.3 min) did not differ significantly among light treatments (T50 p = 0.85, T80 p = 0.21). Induction was relatively quick and induction loss was slower but also relatively quick, although both were non-significant among source population and light treatment.

Specific leaf mass (SLM) increased significantly at each light level, with the 20% light treatment being the lowest and 100% light treatment being the highest (p = 0.0001; Table 2). No significant differences by source population were shown (p = 0.86; Table 2). Chlorophyll a, chlorophyll b, and total chlorophyll did not show any significant

differences by light treatment (CHL a p = 0.37, CHL b p = 0.49, CHL tot p = 0.42; Table 2) or source population (CHL a p = 0.62, CHL b p = 0.81, CHL tot p = 0.69; Table 2).

Table 2: Light treatment (% of full sunlight) and source by mean SLM, chlorophyll a, chlorophyll b, and total chlorophyll concentrations. Values with different lettered superscripts denote significant differences at $p < 0.05$.

Light Treatment	**SLM (g/m^2)**	**CHL a (g/m^2)**	**CHL b (g/m^2)**	**Total CHL (g/m^2)**
20%	51.342^{c}	0.234	0.205	0.434
50%	66.247^{bc}	0.236	0.210	0.441
75%	81.153^{ab}	0.257	0.222	0.474
100%	97.714^{a}	0.222	0.202	0.419
Source	**SLM (g/m^2)**	**CHL a (g/m^2)**	**CHL b (g/m^2)**	**Total CHL (g/m^2)**
EO-2	57.966	0.197	0.182	0.375
EO-16	54.654	0.187	0.158	0.341
EO-17	59.622	0.213	0.178	0.386
EO-23	67.903	0.186	0.170	0.352
EO-46	56.310	0.166	0.151	0.314

4. Discussion

Light treatment had significant effects of maximum photosynthesis (PnMax), dark respiration (Rd), specific leaf mass (SLM), and light compensation points (LCP), but not quantum yield (QY), pigment concentrations, or sunfleck utilization. Plants growing in 75% sunlight had significantly higher light saturated PnMax rates, which is unexpected because 100% sunlight typically yields the highest PnMax rates. A possible explanation for this could be photoinhibition in full sunlight. Powles[17] describes photoinhibition as a reduction in photosynthetic capacity induced by exposure to excess visible light. Plants that live in shaded environments may not have the ability to acclimate and grow under full sun conditions; therefore, they are susceptible to photoinhibition. However, a study investigating photoinhibition in field-grown plants found that leaves grown in and acclimated to full sunlight resulted in a light-induced reduction of the photochemical capacity[18], indicating that plants acclimated to high light can still undergo photoinhibiting effects. Two types of photoinhibition occur; dynamic (reversable) photoinhibition when quantum yield decreases but maximum photosynthetic rates are unaffected. Chronic (irreversible) photoinhibition is when both quantum yield and maximum photosynthetic rates decrease, and it is not readily reversible because it requires protein repair[19]. In this study, plants growing in 100% full sunlight exhibited dynamic photoinhibition with significantly lower PnMax and lower quantum yield[17,20]. This photoinhibition is likely photoprotection via the xanthophyll cycle, where carotenoid pigments are used to dissipate excess electron excitation energy from the light harvesting reactions through

thermal dissipation[22]. Without effective photoprotection, plants may experience chronic photoinhibition or damage to Photosystem II, particularly the D1 protein involved in the hydrolysis of water[22].

Photoprotective photoinhibition is not uncommon in plants exposed to full sunlight. A study investigating tropical rainforest plants divided their observed species into three groups: shade-tolerant, shade-tolerant but benefiting from gaps, and shade-intolerant gap specialists and had these acclimated plants exposed to full sun. It was found that all groups of plants experienced photoinhibition over various light levels. In addition, shade-intolerant plants were shown to orient their leaves vertically in order to avoid light induced damage[23]. This adaptive leaf movement suggests that even shade-intolerant plants can still undergo photoinhibition when exposed to very high solar radiation. No vertical orientation in leaves of Virginia spiraea was observed in this study, and it was not a parameter we were observing.

Dark respiration estimates the maintenance respiratory costs of leaves[6]. Plants growing in 100% sunlight resulted in a significantly higher rate of Rd. Higher light leaves tend to be thicker[6], with multiple palisade layers. Our leaves had significantly higher SLM, showing greater leaf thickness at 100% relative to other light levels. This pattern with Rd rates was not observed in Chinese silver grass (*Miscanthus sinensis* Andersson), an invasive perennial grass in eastern United States, when grown under a light gradient ranging from 5% to 100% sunlight[6]. However, this pattern of increasing Rd with increasing light exposure was observed with Japanese stiltgrass (*Microstegium vimineum* (Trin.) A Camus), a common invader of southeastern United States forest understories, when grown at 5% and 100% sunlight[24]. These studies focus invasive grass with C^4 photosynthesis unlike Virginia spiraea, but they do show a similar pattern in the parameters discussed. Reich et al.[25] found a similar pattern of increasing Rd with increasing specific leaf mass across many plant functional groups, including shrubs like Virginia spiraea.

Quantum yield often increases in leaves grown in lower light[6]. Smith and Martin[26] found an increase in QY in rock muhly grass (*Muhlenbergia sobolifera* (Muhl. ex Willd.) Trin.) at a low PPFD, and stated that this was associated with an increase in chlorophyll a, chlorophyll b, and total chlorophyll concentrations which indicates a greater investment in light harvesting components at low PPFD. Studies of Japanese stiltgrass also showed non-significant shifts[16]. Zai et al.[27] conducted a study focusing on the relationships between chlorophyll content and chlorophyll fluorescence parameters to the quantum yield of Photosystem II of the beach plum shrub (*Prunus maritima* Marshall) when under salt stress. The results showed a significant increase in the maximum quantum yield when the chlorophyll a/chlorophyll b ratio also significantly increased[27]. There were no significant shifts in chlorophyll a, chlorophyll b, total chlorophyll or QY regarding light treatments in this study, but there were trends in QY by

decreasing when exposed to higher light levels and non-significant increases in chlorophyll content.

Light compensation points estimate the minimum light level for survival where photosynthetic carbon gain offsets respiratory carbon losses with increased QY, decreased SLM, and decreased Rd contributing to decreased LCP[6]. In our study, plants in 100% sunlight had significantly higher LCP and most likely due to SLM and Rd being significantly higher in plants in 100% sunlight, despite no significant shifts in QY. Plants in 20% sunlight had significantly lower LCP resulting from lower SLM and Rd, despite no significant shifts in QY. A decrease of LCP when in decreasing light suggests acclimation to shade[28]. A study observing Chinese silver grass (*Miscanthus sinensis* Andersson) showed decreasing LCP during low light. However, this decrease was attributed to the likely result of more efficient light harvesting in non-photoinhibiting environments because Chinese silver grass (*Miscanthus sinensis* Andersson) experienced a significantly higher QY at low light with no significant shifts in SLM or Rd[6].

Times required for plants to reach 50% and 80% induction did not differ significantly among light treatments. In order to fully understand carbon gain of Virginia spiraea in an environment that relies on utilizing sunflecks, future studies should be conducted with lower light intensities levels to emulate this environment[6]. Soil or plant water status was not monitored in this study, although there was likely little difference in these in our common garden setting. These properties should be monitored in future studies because differential water stress from competition with other plants or small-scale heterogeneity in soil composition could have impacts on leaf gas exchange properties[6]. If water stressed, plants might experience a faster time to 50% induction because of biochemical and stomatal activity and a slower time to 80% induction because of stomatal limitation.

Times required for plants to reach 50% and 80% induction loss did not differ significantly among light treatments. Future studies should include biochemical analysis and measurement of metabolite pools during various stages of photosynthetic induction[6]. Stomatal activity should also be better monitored in future studies that observe the photosynthetic induction loss of Virginia spiraea. Rapid stomatal responses save water during shade periods, although they can potentially decrease the daily net carbon gain[17]. A study observing Chinese silver grass found slow stomatal closing during periods of low light and relatively rapid stomatal opening during induction, which may be evidence of efficient use of sunflecks in shaded environments[6].

Source population did not have a significant effect on any parameter. The five source populations were all from the same river drainage (New River, Ashe County, NC), and subsequent studies have shown little genetic difference among individuals within the same drainage[4]. The clonal spread in Virginia spiraea is thought to be very high[3], and results in the idea that each wild population could be a single individual genotype[29]. Brzyski's[29] work found a lack of polymorphism in populations in Ohio, North Tennessee,

and South Tennessee which resulted in a low number of genotypes for these drainages (3, 6, and 3 respectively). Observations from Ogle[30] observed seedlings in wild populations to be non-viable. However, common garden settings have shown that seed viability to possible if individuals from different drainages are grown together if separated by an appropriate distance to exclude cross pollination[5,30]. Future studies should use a common garden setting when observing Virginia spiraea for a higher chance of getting potential propagule sources. Higher genetic variability has been shown between drainages. Anders and Murrell[5] examined the patterns and variation of gene flow and past migration of Virginia spiraea. They found genetic isolation in populations along the Cumberland Plateau drainage because of their phenotypic identity.

Future studies should examine photosynthetic characteristics of individuals from different river drainages in order to attain measurements from genetically contrasting source populations in an appropriate common garden setting. More work needs to be done to understand the species' plasticity and acclimation potential under a wider range of environmental conditions to help develop a plan for successful recovery of Virginia spiraea in wild populations. Nonnative species make up 6% of both herbaceous and vine cover in wild Virginia spiraea plots[2], so management should include removing nearby nonnative vegetation that could pose a threat to Virginia spiraea's sunlight availability. Another way to increase sunlight in wild populations that lack an appropriate amount would be management for open canopies, allowing light in the form of sunflecks can protrude to the forest floor. High rates of PnMax in plants growing in 75% sunlight could possibly indicate that wild populations grown in forest understories can effectively utilize increases in ambient light that could be caused from disturbance to the plant canopy.

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