

FACTORS AFFECTING THE ABILITY OF ROSA MULTIFLORA TO INVADE AND
PERSIST IN THE UNDERSTORY OF SOUTHERN APPALACHIAN FORESTS

A Thesis
by
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Abstract

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Rosa multiflora (multiflora rose; MR) is an invasive, perennial shrub that was originally brought to the United States from east Asia in the 1800s as an ornamental and later promoted for use by farmers. MR is known to be shade intolerant, preferring disturbed, riparian, open, and edge habitats. Recently, MR has invaded the forest understory in the southern Appalachians, raising the question of how it persists under such shady conditions. MR may succeed in the understory by growing in canopy gaps, but it also may have a wide tolerance to varying light conditions. Furthermore, MR has evergreen stems, which may augment CO₂ assimilation by leaves, but with less water loss, thereby raising the whole-plant water use efficiency. The ability MR stems to conduct photosynthesis may enable it to survive in xeric habitats, including that in the understory of forests. I made weekly phenological measurements on MR shrubs growing in the understory of the ASU Nature Preserve and showed that MR began leafing out by mid-February, approximately four-six weeks before native vegetation and kept some leaves until mid-November, nearly two weeks longer than

native species. Thus, MR gains a competitive advantage by using high light prior to canopy leaf out and after canopy senescence to gain carbon. Gas exchange measurements showed significantly higher total daily carbon gain in the spring before canopy leaf out ($190 \pm 21.5 \text{ mmol CO}_2 \text{ m}^{-2} \text{ day}^{-1}$) and much lower amounts when the canopy was fully leafed out ($-12 \pm 0.09 \text{ mmol CO}_2 \text{ m}^{-2} \text{ day}^{-1}$) and even after the canopy senesced ($5 \pm 1.2 \text{ mmol CO}_2 \text{ m}^{-2} \text{ day}^{-1}$), due in large part to the lack of leaves at this time of year. The photosynthesis (A) of MR leaves ($4.6 \pm 0.784 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$) and stems ($0.02 \pm 0.001 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$) differed significantly ($p < 0.001$), but there was no significant difference for water use efficiency ($p \geq 0.05$). MR appears to successfully persist in the forest understory because it gains most of its carbon in the early spring before canopy leaf out, due to its early spring phenology. The influence of stem photosynthesis may contribute to its ability to persist on xeric sites and may contribute to carbon gain in the winter when the canopy is leafless. However, there does not appear to be external gas exchange, which suggests that stems depend in respiratory CO_2 and dissolved CO_2 in xylem water for their substrate. However, this must be further investigated to assess its importance to the success of MR in the understory.

Acknowledgments

I thank my thesis advisor, Dr. Howard S. Neufeld, without whom this project could not have been completed. I thank my committee members, Dr. Annkatrin Rose and Dr. Mike Madritch. I also thank my lab mates Emily Riffe, Ariel Waldroop, Zane Sink, Rachel Jordan, Erica Pauer, and Morgan Ayers. I would also like to thank Mike Hughes and Dana Greene, the heads of the instrument shop and Dr. Guichuan Hou, the head of the microscopy lab. Finally, I would like to thank the Appalachian State University Office of Student Research for funding my project.

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Foreword

This thesis was completed with my labmate and fellow master's student, Emily Riffe, to understand the ability of invasive non-native woody species to invade and persist in the Southern Appalachian forest understory. The format and references follow the guidelines of the journal *New Phytologist*.

Introduction

Non-native invasive plants (NNIPs) are a fixture of ecological research because of their impacts on community composition and ecosystem processes (Driess and Volin, 2013; Smith et al., 2014; Moser et al., 2016; Esch et al., 2019). NNIPs are responsible for losses in biodiversity, second only to habitat destruction and fragmentation (Banasiak & Meiners, 2009; Yates et al., 2004; Bonebrake et al., 2011). Moser et al. (2016) proposed that competitive release, propagule pressure, resource availability, and disturbance are factors that influence the invasive success of NNIPs. Their success may depend on access to habitats with abundant available resources, frequent disturbance, and low environmental stress, which makes them conducive to invasion (Davis et al., 2000; Heberling & Fridley, 2016). However, the aforementioned factors do not exhaust all potential determinants in the susceptibility of a habitat to invasion, especially since a number of NNIPs invade environments with limited light, water, and nutrients, such as the understories of deciduous forests (Heberling & Fridley, 2016). The success of woody NNIPs in habitats with limiting resources can be attributed to their functional diversity, with extended phenologies, for example, influencing their presence and persistence, although this is not true in all cases (Zohner & Renner, 2017). In the case of NNIPs in a forest understory, many have extended growing seasons that can be up to four weeks longer than those experienced by native species. Many NNIPs may leaf out earlier in the spring and hold onto leaves later in the fall than native vegetation (Fridley, 2012; Heberling et al., 2018).

Functional traits and responses, such as an extended phenology, and physiological traits, such as high relative growth rate, are common among NNIPs, giving them a competitive edge over native species (Smith et al., 2014; Moser et al., 2016). A recent meta-

analysis suggests that the two most important features contributing to invasive success are vegetative reproduction and long-distance seed dispersal (Nunez-Mir et al., 2019). However, the invasibility of communities can fluctuate as disturbances and other events influence resource availability (Davis et al., 2000). Competitive traits in NNIPs can change over the course of an invasion, especially as these species spread out from the source population, where intraspecific competition is dominant, to an invasion front with dominant interspecific competition (Banasiak & Meiners, 2009; Iacarella et al., 2015).

An enigma in the field of invasive ecology involves the vast spread of invasive species in mid-to-late successional deciduous forests, especially those in the Eastern United States (EUS), as these environments are usually low in essential resources such as light, water, and nutrients (Heberling & Fridley, 2016; Neufeld & Young, 2014). NNIPs may be invading these habitats due to fragmentation of forests and the creation of edge habitats, which often facilitate their spread into the forest understory (Yates et al., 2004), especially if there are no barriers to encroachment (Dlugos et al., 2015). Disturbances within forests (e.g. canopy disturbances) may also facilitate invasion (Driscoll et al., 2016), along with the presence and availability of invasive species propagules (Davis et al., 2000). However, some NNIPs are simply adapted to the understory environment and take advantage of it, especially if they originate from similar habitats (Fridley, 2008).

Many of the in EUS shade-tolerant, invasive woody species in EUS deciduous forests are of Eurasian origin (Robertson et al., 1994; Fridley, 2008; Heberling & Fridley, 2016). The spread of these NNIPs could be due to climatic similarities between the two regions of the EUS and eastern Asia (Robertson et al., 1994; Fridley, 2008), with introduced plant species from areas of similar climate, soil, or disturbance conditions potentially being pre-

adapted to invade foreign areas (Fridley, 2008). However, the invasion of eastern Asiatic, woody forest flora in the EUS is more complex (Fridley, 2008). The exchange of species between the EUS and Asia started in the Neogene, with the connection of the regions by Beringia, which later was broken by sea level rise and continental drift, causing genus-level taxonomic disjunctions due to isolation and climate change over time (Fridley, 2008). Also, it is noted that most invasive plants in the EUS from eastern Asia have native congeners to EUS natives, including *Rosa multiflora* (many natives), *Berberis thunbergii* (native is *B. canadensis*), and *Elaeagnus umbellata* (*E. commutate* is the native species), and it has been suggested that recent invasions of EUS forests are the most serious over a long history of east Asian invasions in mesic temperate forests (Fridley, 2008).

Another potential reason for the successful invasion of woody NNIPs in the EUS could be that many outcompete natives by virtue of their having extended phenologies. The extended phenologies may provide a competitive advantage over native plant species advantageous to their growth (Smith, 2013). Fridley (2012) has observed NNIPs in EUS forests extending their fall growing season up to four weeks, with most carbon gained during this time as opposed to the spring.

Rosa multiflora Thunb. (Multiflora rose; MR) is a branching perennial shrub native to Korea, Japan, and other parts of eastern Asia (Steavenson, 1946; Banasiak & Meiners, 2009; Murphy et al., 2016). MR is a mid-successional species of disturbed grasslands in its native habitat and possibly prefers mesic soils (Huebner et al., 2014; Banasiak & Meiners, 2009), although there are no controlled studies confirming this. A single MR shrub can produce up to one-half million seeds annually that are bird dispersed. It is pollinated by generalist insect

pollinators (Jesse, 2006) and has an extensive capability for vegetative reproduction (Banasiak & Meiners, 2009).

MR was first introduced into the United States in the early 1800s as an ornamental and was later promoted for use by farmers as a natural fence in the 1900s, which aided in its widespread distribution (Steavenson, 1946; Jesse, 2006; Huebner et al 2014; Dlugos et al., 2015). MR is distributed in 42 states to-date (<http://www.eddmaps.org>) and is considered invasive in 31 states (Banasiak & Meiners, 2009). Although MR is found commonly in open, disturbed, riparian, and edge habitats (Dlugos et al., 2015; Murphy et al., 2016), more recently, it has infiltrated interior forest habitats and is increasing in abundance in EUS deciduous forests (Banasiak & Meiners, 2009; Dlugos et al., 2015). This is of interest because the growth of MR is seemingly limited in low-light habitats (Huebner et al., 2014).

The ability of MR to invade and persist in the low-light environment of forest understories suggests that it possesses either shade-avoidance mechanisms, such as leafing out before the canopy closes, and maintaining leaves after the canopy opens, or it can adjust its foliar physiology to tolerate the low-light conditions in summer when the canopy is fully leafed out (Fridley, 2012; Driess & Volin, 2013; Dlugos et al., 2015). Furthermore, MR may rely on gaps in the forest canopy for sufficient light, with greater growth and vegetative reproduction of MR observed in conditions of increased light under the canopy compared to plants covered by the canopy (Huebner et al., 2014; Dlugos et al., 2015). In low-light closed-canopy conditions of the forest understory, seed production and colonization are influenced primarily by propagule rain and fertile soils, and less so by light availability (Banasiak & Meiners, 2009; Huebner et al., 2014).

Observed traits of MR in the understory, such as an extended leaf phenology and year-round green stems, have not been extensively studied, with the latter receiving little to no attention (Robertson et al., 1994; Dlugos et al., 2015; Murphy et al., 2016). Some research has been done on the phenological characteristics and gas exchange of MR in the understory, with results showing MR individuals leaf out in the spring before native vegetation and leaf drop in autumn after native plants, with higher photosynthetic rates in the spring (before canopy leaf out) and in the fall (after canopy senescence) (Dlugos et al., 2015). The lengthened leaf span of MR could account for a sizable portion of its annual carbon gain when growing in the understory.

Since MR stems are present year round, including those months when the canopy is absent, they may be able to take advantage of the high light conditions by continuing carbon assimilation via gas exchange across the epidermis or sub dermally from respired and xylem-dissolved CO₂, both in the summer when it is warm (Banasiak & Meiners, 2009; Cernusak & Cheesman, 2015; Dlugos et al., 2015) and during the colder months in the winter. The presence of anthocyanins in the winter on the side of the stem receiving the most radiation (per observation) suggests they may serve to prevent photoinhibition which would further enhance carbon uptake at this time of year (Smillie & Hetherington, 1999; Gould et al., 2010). However, there do not appear to be any published studies of stem photosynthesis in MR.

Stem photosynthesis more frequently contributes a significant portion to total plant carbon gain than any other means of non-foliar photosynthesis, such as floral, fruit, or root (Aschan & Pfanz, 2003). As proposed by Ávila et al. (2014), there are two types of stem photosynthesis: stem net photosynthesis (SNP) and stem recycling photosynthesis (SRP).

Both SNP and SRP benefit plants by maintaining carbon gain during times of drought, when leaves may or may not be present, and this improves water use efficiency (WUE) over that of leaves due to the occurrence of carbon gain without concomitant water loss (Ávila et al., 2014; Cernusak & Cheesman, 2015; Ávila-Lovera et al., 2017).

With light availability being the dominant limiting resource of understory plants in deciduous forests (Neufeld & Young, 2014; Heberling et al., 2018), the seasonal changes in leaf and stem photosynthesis of MR could give it an advantage over native plants. The improvement of WUE with stem photosynthesis may allow MR to persist on drier sites with limited water availability.

The purpose of this study was to investigate and understand the success of MR in the deciduous forest understory, specifically regarding its foliar and stem photosynthesis. I investigated ecophysiological traits of MR in the forest understory, including photosynthetic carbon assimilation and stomatal conductance of its leaves and stems in response to controlled light conditions. The results of this study will add to the literature on how and why invasive shrubs, such as MR, can persist in an understory environment and perhaps elucidate the ecophysiological traits that make them superior competitors in this environment.

Methods

Study Site

The study site containing multiflora rose (MR) is located within the Appalachian State University Nature Preserve (36.2130°, -81.6910°, 1053 m), a 27 ha parcel of protected land adjacent to the west side of the campus. It is comprised of successional forest dominated by native tree species: red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), black locust (*Robinia pseudoacacia*), tulip poplar (*Liriodendron tulipifera*), hemlock (*Tsuga canadensis*), and mockernut hickory (*Carya tomentosa*) and a shrub understory, consisting mostly of great laurel (*Rhododendron maximum*), with several invasive shrubs/trees including multiflora rose (*Rosa multiflora*), autumn olive (*Elaeagnus umbellata*), and Japanese barberry (*Berberis thunbergii*). Native wildflowers also grow abundantly in the Nature Preserve (e.g. wild geranium (*Geranium maculatum*), violets (*Viola* spp.), snowy orchis (*Galearis spectabilis*), and mayapple (*Podophyllum peltatum*), to name a few).

Multiflora rose is found on upper to mid slopes along the contours of the northern portion of the nature preserve (~1053 m elevation; ~24 m below peak elevation). Twenty MR were randomly chosen for field measurements during the 2017 growing season. On each individual, five branches were randomly selected and marked for phenology measurements, and five of the 20 plants were randomly selected for diurnal gas exchange measurements.

Phenological Methodologies

Weekly measurements of MR phenology were taken during the 2017 growing season. In early February, I began counting the number of swollen and breaking leaf buds and the number of buds beginning to leaf out, with measurements restricted to the first distal 15 cm of five branches on each plant. Once all branches had begun leaf out, I counted the number of

leaves on each branch only (mid-April 2017). Additionally, the ambient light, measured as photosynthetically active radiation (PAR), was measured weekly throughout the 2017 growing season using a PAR meter (Li-650, Li-Cor, Inc., Lincoln, NE) equipped with a LI-190R Quantum Sensor positioned horizontally in the center of each MR. These measurements were then compared to the incident radiation with the sensor oriented directly towards the sun. The weekly leaf counts and ambient light measurements allowed me to calculate the leaf survivorship and to compare that to light availability throughout the growing season.

Leaf Gas Exchange Measurements: Diurnals

Gas exchange measurements were made using the Li-6800 portable gas exchange system (Li-Cor, Inc., Lincoln, NE) equipped with the 6 cm² chamber with LED lighting. Diurnal patterns of photosynthesis (A) and stomatal conductance (g_s) were made at approximately three-hour intervals during the day from 8:00 am to 8:00 pm. These measurements were taken on three leaves of five MR plants at each three-hour interval, once a month (weather permitting) from April to October. The five plants used were randomly chosen at the beginning of spring 2017, and the same five plants were used throughout the season. Leaves were chosen randomly on each plant, while avoiding using the same leaf multiple times in one day. Cuvette parameters were set to match ambient light and temperature and adjusted throughout the day as these changed, while CO₂ was kept constant at 400 $\mu\text{mol mol}^{-1}$. However, if there was a change in ambient light of over 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (due to shading or prolonged sunflecks), then I would adjust the light. The relative humidity was set to match the ambient humidity but adjusted to avoid excess moisture in the system

and ranged from 10% in October to 70% in June; summer months typically had higher humidity, except for July which was unusually dry at 15%.

To calculate the daily carbon assimilation for individual plants, I used linear extrapolation between points and geometry to find the area under each diurnal curve. When rates were negative, e.g., early in the morning and sometimes late in the afternoon, those integrated carbon values were subtracted from the other totals where A was positive. These values were averaged for the plants measured on each day. Daily assimilation totals were relativized by the percent of leaves on the branches used to measure leaf out to gain a relative value of whole plant carbon gain. For example, in April 67% of the leaves were present, so the daily totals were multiplied by 0.67, whereas in October, only 6% of the leaves were present, so the total was multiplied by 0.06. This enabled an estimate of carbon assimilation based on both leaf number and individual leaf assimilation rates.

Leaf Gas Exchange Measurements: Response Curves

Light and vapor pressure deficit (VPD) curves were assessed three and two times throughout the season respectively: (1) before the canopy closed (May) – light curve only, (2) when the canopy was fully closed (July) – light and VPD curves, and (3) after canopy leaves had fallen (October) – light and VPD curves. For all curves, cuvette CO_2 was kept constant at $400 \mu\text{mol mol}^{-1}$. Measurements were usually completed before 2:00 pm to avoid diurnal influences.

Light Response Curves – Leaves

Light response curves were measured on three-four plants per period. The light levels and the order in which they were used were: 2000, 1500, 1250, 1000, 750, 500, 300, 150, 50

and 0 ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Leaves were first acclimated to ambient light for that particular day, before being brought up to full sunlight ($2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) and completing the curve. Temperature was set to reflect the ambient temperature of that day. VPD varied depending on the ambient humidity of that day but ranged between 1.22 and 2.77 kPa.

A three-parameter exponential rise to maximum equation was used to fit each light response curve using SigmaPlot Ver. 14 (Systat Software Inc., San Jose, CA):

$$[y = y_0 + a(1 - e^{-bx})] \quad (\text{eq.1})$$

where y is the rate of net photosynthesis (A_{net}), y_0 is the respiration rate at 0 PAR, a and b describe the curvature and dependence on PAR, and x is the level of PAR. From this, I extracted the dark respiration rate (at 0 PAR), light compensation point (where $A_{\text{net}} = 0$), apparent quantum efficiency (slope derived from linear regression of first three points), A_{max} (average of four highest rates of A_{net}), and saturation light intensity (where $A_{\text{net}} = 97\%$ of A_{max}).

VPD Response Curves - Leaves

I generated VPD response curves on three randomly selected plants. VPD response curves covered a range from 1 to 3 kPa. Measurements began at ~ 1.5 kPa before dropping to 1 kPa and then being raised in 0.5 intervals to the highest VPD possible. For October, the lowest VPD I was able to measure was 1.7 kPa, and for July, the lowest VPD was 1.0 kPa. Light was kept constant at $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, temperature was set to 25°C , and CO_2 at 400 ppm.

For the VPD measurements, I fit 2nd degree polynomial functions to the response curves using SigmaPlot Ver. 14:

$$[y = y_0 + ax + bx^2] \quad (\text{eq. 2})$$

where y is A_{net} , x is VPD , and y_0 is a constant.

Stem Gas Exchange Measurements

Special gas exchange chambers for stems were built for use with the Li-6400 portable gas exchange system (Li-Cor, Inc., Lincoln, NE). These chambers were constructed of clear polycarbonate tubing (McMaster-Carr, Atlanta, GA) sized to 14.8 cm in length and 1.6 cm in inner diameter (See Figure 1). The chamber was connected to Bev-A-Line tubing using brass quick coupler attachments (Li-Cor, Inc., Lincoln, NE) and inserted upstream from the leaf cuvette on the sample line. Differences between incoming and outgoing CO_2 and water vapor were used to calculate gas exchange by the stem segment in the stem chamber. The diameter of each stem was measured with a caliper from two directions at the distal, middle, and basal portions of the stem where the chamber was to be placed over it. After this was done, the surface area of each stem was calculated and used as a basis for reporting the measured gas exchange rates. The chamber was placed on the portion of a stem near the maximum of the arch (i.e., where a tangent to the stem would be approximately horizontal) and placed between two leaves. Thorns were removed the day before to allow the stem to recover from any potential wound responses.

An Omega HH12C digital thermometer (Omega Engineering, Inc., Norwalk, CT) was used to monitor the temperature of the air in the stem chamber and the stem itself. One thermocouple was inserted into the outer cortex of the stem and the other left in the air in the cuvette. Then, the stem chamber was sealed on each end using PRIMA Plastilina modeling clay (Sculpture House, Fort Pierce, FL). Gas exchange was measured on two stems on each of three MR shrubs. Measurements were not taken until the stem chamber reached a CO_2 level of 400 ppm. Measurements were recorded every 10 seconds and the IRGAs were

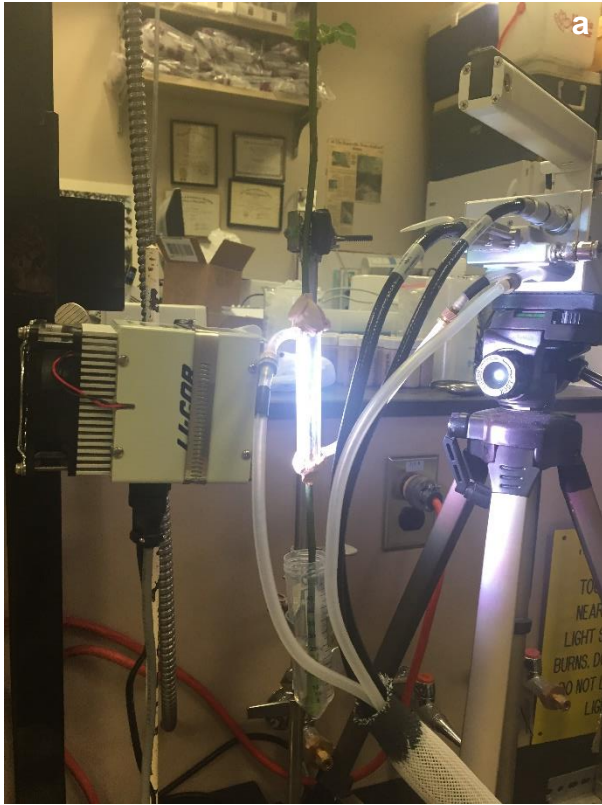


Figure 1. A demonstration of the (a) stem chamber set up with the Li-6400 portable photosynthesis system, and (b) a close up view of the stem chamber connected to Bev-A-Line tubing using brass quick coupler attachments.

matched every five minutes. Light for the stem was provided by the Li-6400 18A RGB Light Source (Li-Cor, Inc., Lincoln, NE) which was set at $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and kept at a close distance to the stem chamber ($\sim 3.5 \text{ cm}$) to cancel out any influence of ambient light. Stems were usually in the shade and ambient light levels were generally very low.

Leaf and Stem Pigments

Chlorophyll content ($\mu\text{g cm}^{-2}$) was measured three times during the growing season: (1) before the canopy closed (April 16, 2017), (2) when the canopy was fully closed (July 11, 2017), and (3) after canopy leaves had fallen (October 20, 2017). I also measured the chlorophyll content of stems (October 23, 2018), so they could be compared to the amounts found in leaves.

I measured chlorophyll from three leaf punches per plant (0.84 cm² total leaf area) of ten MR shrubs in 3 mL of DMF (*N,N*-Dimethylformamide) in the dark, in a refrigerator at 5°C for a minimum of 24 hours. For stems, I extracted chlorophyll from a 1 cm long segment from ten shrubs. Stem area was calculated as length times circumference. Because extraction from stems was difficult, they were first cut into small pieces and placed into liquid nitrogen to lyse the cells and ground up before being added to the DMF. Each frozen and ground stem sample was extracted in 5 mL of DMF in the dark at 5°C for a minimum of 24 hours. Absorbances for both stem and leaf extractions were measured using a Shimadzu UV-1800 spectrophotometer (Shimadzu Corp., Kyoto, Japan). Chlorophyll concentrations were calculated according to equations in Porra (2002).

Stem Cross-Sections and Imaging

A young stem and an old stem of the same length from the same MR shrub were cut in the field and brought in for imaging. A vibratome was used to slice thin sections of each stem, and was set at a feed of 50 µm, a speed of six, and a frequency of seven for each stem sample. After sections were sliced, three were placed on a slide for imaging. Images were used to locate chlorophyll and anthocyanins on the stem. This process was completed four separate times in June and August 2017. An Olympus Ix81 motorized inverted research microscope with cellSens software (Olympus Corp., Waltham, MA) was used for imaging.

Statistical Analysis

Statistical analyses and figures were completed using Sigmaplot Ver. 14 (Systat Software, Inc., San Jose, CA) and Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA). Calculation of means and standard errors were used to compare trends for the phenology data, weekly PAR, diurnal measurements, and chlorophyll content of leaves and

stems. I performed a repeated measures ANOVA in SAS using each plant as a block and time as the factor to compare DR, LCP, AQE, A_{\max} , and saturation light intensity of light curves. For all analyses, $p \leq 0.05$ was used for significance.

Results

Phenological Methodologies (2017)

Multiflora rose (MR) started budding in mid-February before any native plants, and its leaves were fully flushed by the second week of March, with some remaining until mid-November, when native plants were no longer leafed out, resulting in a nine-month (252 days) leaf out period. Approximately two weeks before the canopy began leafing out in March, 20% of MR leaves were fully flushed, with 100% leaf flush by April. Light levels peaked in March, with an average PAR of $879 \pm 52 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($n = 80$) and declined to $35 \pm 7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($n = 80$) by June, when the canopy was fully leafed out. Light levels increased as the canopy senesced in the fall, averaging $247 \pm 29 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($n = 80$) in the month of October. Approximately three weeks after the canopy senesced, which was in October, MR had only 6% of its leaves left. Light levels increased in November, with an average PAR of $445 \pm 27 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($n = 60$), but survivorship of leaves was at virtually 0% (Fig. 1). Oscillations of surviving leaf percentages were due to weather and mid-season senescence, with leaves increasing in number until mid-May and steadily decreasing until complete senescence in late November (Fig. 2).

I also observed mid-season yellowing of MR leaves and mid-season senescence and regrowth of leaves that contributed to oscillations of surviving leaf percentages. The cause of the yellowing is unknown, but may be due to light stress, as the yellowing leaves were towards the crown of MR shrubs and often over-shadowed by overarching MR canes and leaves (See Figure 3).

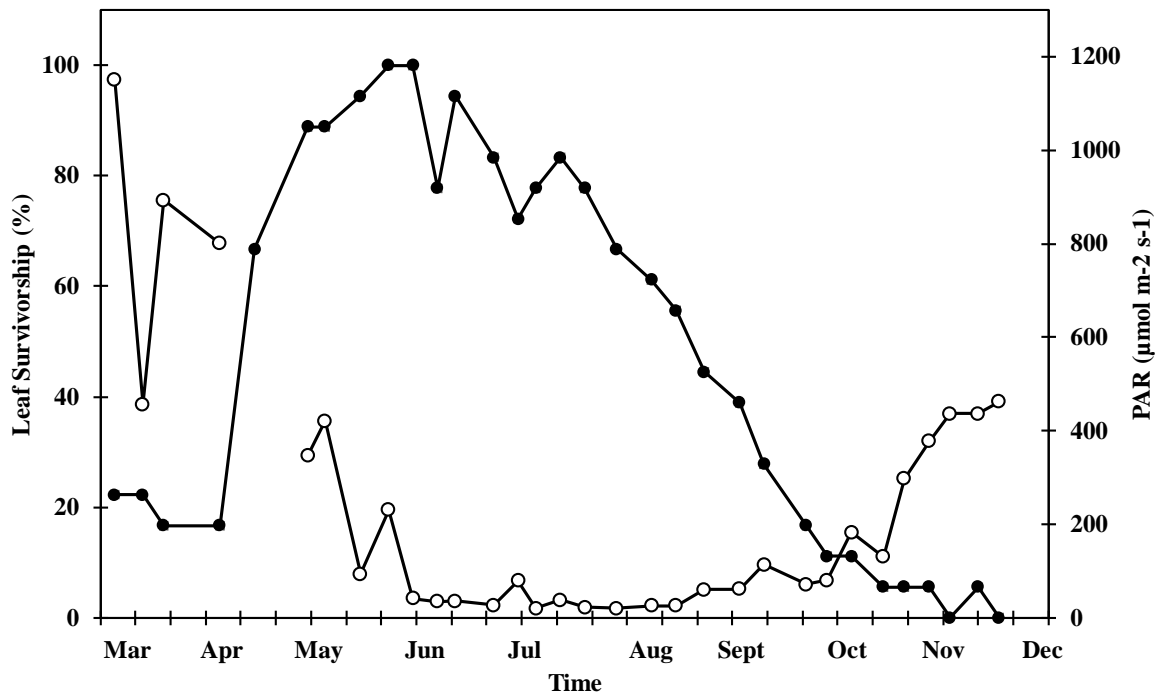


Figure 2. Seasonal survivorship of *R. multiflora* leaves and ambient light availability (2017). Oscillations of leaf percentages are due to weather and mid-season senescence. The missing light measurement in mid-April was due to poor weather conditions.



Figure 3. Mid-summer leaf yellowing of multiflora rose.

Leaf Gas Exchange Measurements (2017): Diurnals

Prior to canopy leaf out in April, stomatal conductance (g_s) was high early in the day before dropping throughout the rest of the day, whereas photosynthesis (A) peaked mid-to late afternoon (Fig. 4a). In July, when the canopy was closed, g_s was lower in the morning, with peak A still around mid-day (Fig. 4c). In October, after the canopy senesced, g_s and A came back to a similar pattern to that of April, but the maximum stomatal g_s was lower than that of April (Fig. 4e).

In April and July, PAR peaked around late morning and dropped throughout the day, with PAR being much higher in the former month than the latter (Fig. 4b, d). Leaf temperature and VPD increased and reached a peak around mid-day in April (Fig. 4b) and increased and reached a peak around dusk in July (Fig. 4d). In October, PAR increased from the morning until mid-day, where it reached its peak before decreasing throughout the remainder of the day (Fig. 4e). Leaf temperature and VPD followed a similar pattern, with both increasing from morning until about dusk, and then decreasing into the evening (Fig. 4f).

Leaf Gas Exchange Measurements (2017): Light Response Curves – Leaves

Light curves for April, July, and October (Fig. 5) and all light response parameters (Table 1) showed varying responses, with photosynthetic rates being significantly higher in April than in July and October ($p < 0.001$), and with no significant difference between July and October ($p = 0.386$). A_{\max} (A at saturating light, $\sim 2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) of MR leaves was higher in April ($19.2 \pm 0.40 \mu\text{mol m}^{-2} \text{s}^{-1}$) when the canopy was open and at lower in July ($7.21 \pm 0.34 \mu\text{mol m}^{-2} \text{s}^{-1}$) when the canopy was closed, even though they were also exposed to saturating light during the light response curve procedure. A_{\max} rebounded slightly in

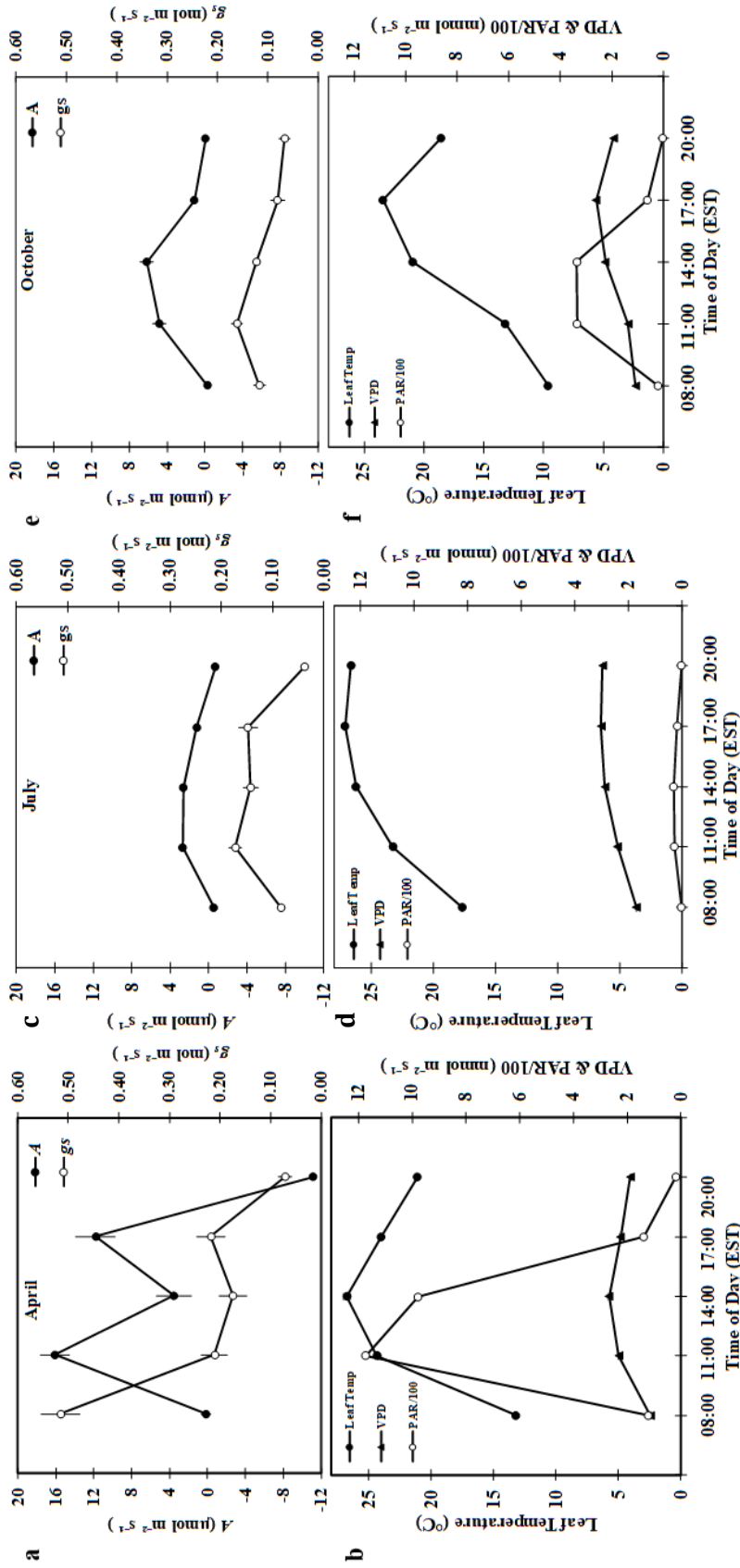


Figure 4. *R. multiflora* gas exchange responses to varying microclimatic conditions during the 2017 growing season: (a-f). Diurnal measurements of photosynthesis (A), stomatal conductance (g_s), leaf temperature, vapor pressure deficit (VPD), and ambient light levels.

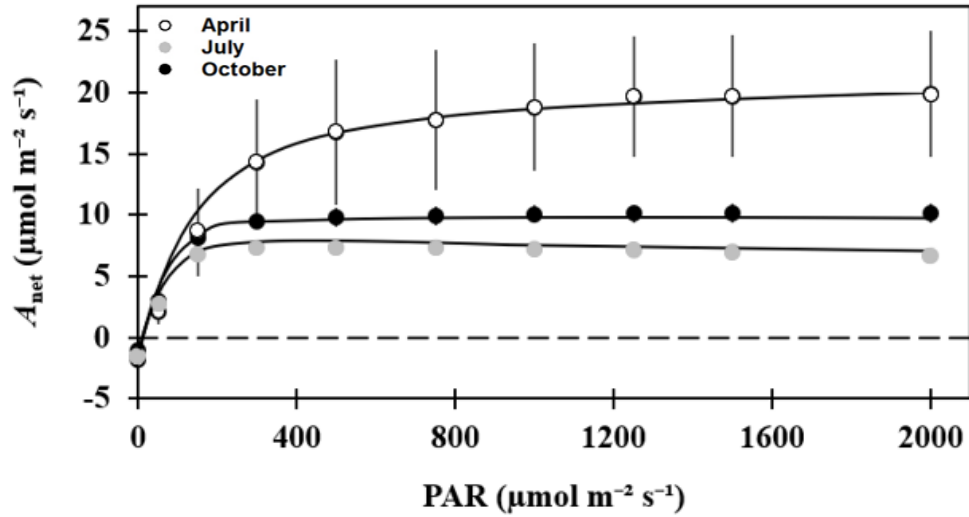


Figure 5. Photosynthetic responses of *R. multiflora* leaves to photosynthetically active radiation (PAR) at different times during the 2017 growing season. Means for A_{max} are statistically significantly different between April ($24.5 \pm 0.71 \mu\text{mol m}^{-2} \text{s}^{-1}$) and October ($10.1 \pm 0.77 \mu\text{mol m}^{-2} \text{s}^{-1}$), April and July ($7.21 \pm 0.34 \mu\text{mol m}^{-2} \text{s}^{-1}$), and July and October. Symbols are mean \pm se, $n = 3$.

Table 1. Light response parameters of MR leaves in the 2017 growing season, where A_{max} is photosynthesis at saturating light, LCP is light compensation point, AQE is apparent quantum efficiency, and DR is dark respiration rate. Numbers are mean \pm SE, $n = 3$.

	April	July	October
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	19.2 ± 0.40	7.21 ± 0.34	10.1 ± 0.77
LCP ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	25 ± 5	14 ± 3	11 ± 1
AQE ($\mu\text{mol CO}_2/\mu\text{mol photons}$)	0.0985 ± 0.00050	0.04963 ± 0.00160	0.06367 ± 0.00907
DR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	-2.1 ± 0.02	-1.6 ± 0.18	-1.2 ± 0.13
PAR when A saturates ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	1039 ± 80	304 ± 73	643 ± 19

October ($10.1 \pm 0.77 \mu\text{mol m}^{-2} \text{s}^{-1}$) after the canopy senesced. Month (i.e. April, July, October) had a marginally significant effect on A_{max} ($p = 0.07$) but becomes significant when one aberrant individual (Plant C4 in the month of April) is removed from the analysis ($p = 0.0002$). Light compensation points (LCPs) also varied, being highest in April ($25 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$), followed by lower values in July ($14 \pm 3 \mu\text{mol m}^{-2} \text{s}^{-1}$) and October ($11 \pm 1 \mu\text{mol m}^{-2} \text{s}^{-1}$), and were marginally significant with respect to time ($p = 0.08$). The LCP is not significant when the previously mentioned outlier is taken out ($p = 0.30$), and for all subsequent analyses, I only report the statistical significance with the outlier removed. Apparent quantum efficiency (AQE) varied also, with a higher value of $0.0985 \pm 0.00050 \mu\text{mol CO}_2/\mu\text{mol photons}$ in April and lower values of 0.04963 ± 0.00160 , and $0.06367 \pm 0.00907 \mu\text{mol CO}_2/\mu\text{mol photons}$ in July and October respectively. AQE was significantly higher in April than in the other months, which did not differ ($p < 0.001$). Finally, dark respiration rate (DR) did not differ among the months (Table 1; $p = 0.10$).

Leaf Gas Exchange Measurements (2017): VPD Response Curves – Leaves

The leaf response to vapor pressure deficit (VPD) was higher in October (1.8 ± 0.033 kPa), than in July (1.0 ± 0.262 kPa). The sensitivity of g_s to VPD varied depending on the month. In July, it decreased slightly as VPD increased, while in October, it increased before peaking at ~ 2.25 kPa, after which it decreased. Photosynthesis did not exhibit much change in either month as VPD changed (Fig. 6).

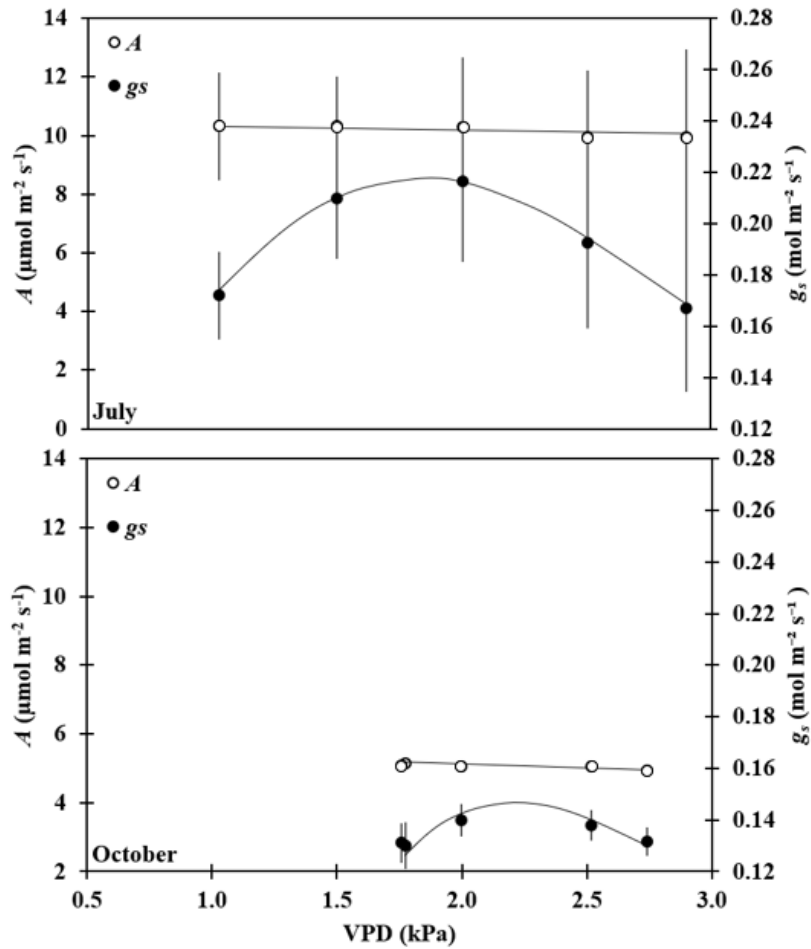


Figure 6. Vapor pressure deficit response of *R. multiflora* leaves during the 2017 growing season. Symbols are mean \pm se, n = 3.

Daily Carbon Assimilation (2017) – Leaves

Daily carbon uptake was high in April prior to canopy leaf out (190 ± 21.5 mmol CO₂ m⁻² day⁻¹) and negative in August (-12 ± 0.09 mmol CO₂ m⁻² day⁻¹). In general, daily carbon uptake was low in the summer when the canopy was fully leafed out, averaging 20 ± 9.3 mmol CO₂ m⁻² day⁻¹. Carbon uptake did not recover to spring levels in the fall even after the canopy senesced, with no significant difference ($p = 0.276$) between August and October (a mean of 5 ± 1.2 mmol CO₂ m⁻² day⁻¹; Fig. 7). This was due, in part, to lower light levels in

the fall than the spring due to asynchronous leaf fall and also because MR had many fewer leaves at this time of the year as a result of senescence.

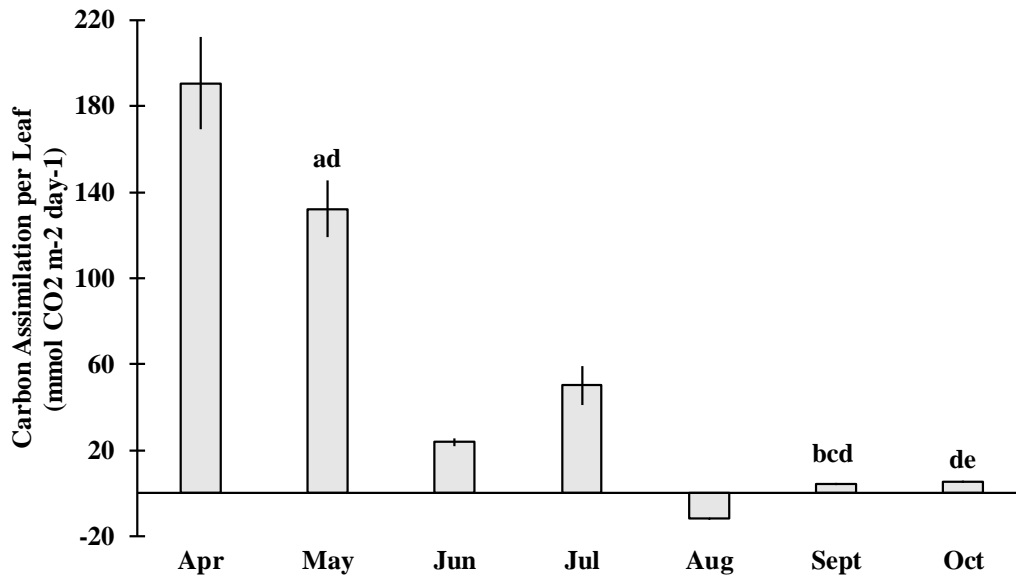


Figure 7. Daily carbon assimilation for individual *R. multiflora* shrubs in the 2017 growing season over an 8-hour period from 8:00 am to 8:00 pm. Bars are mean \pm se, $n = 3$. Means not followed by the same letter are different at $p < 0.05$.

Leaf and Stem Pigments

Mean chlorophyll contents of MR leaves in the field were significantly higher in October 2017 ($31 \pm 1.9 \mu\text{g}/\text{cm}^2$) than July 2017 ($23 \pm 2.2 \mu\text{g}/\text{cm}^2$; $p = 0.009$), with no significant differences among any other months (Fig. 8a). The mean chlorophyll content of stems ($32 \pm 5.7 \mu\text{g}/\text{cm}^2$) and leaves ($28 \pm 2.0 \mu\text{g}/\text{cm}^2$) in October 2018 did not differ ($p = 0.940$; Fig. 8b).

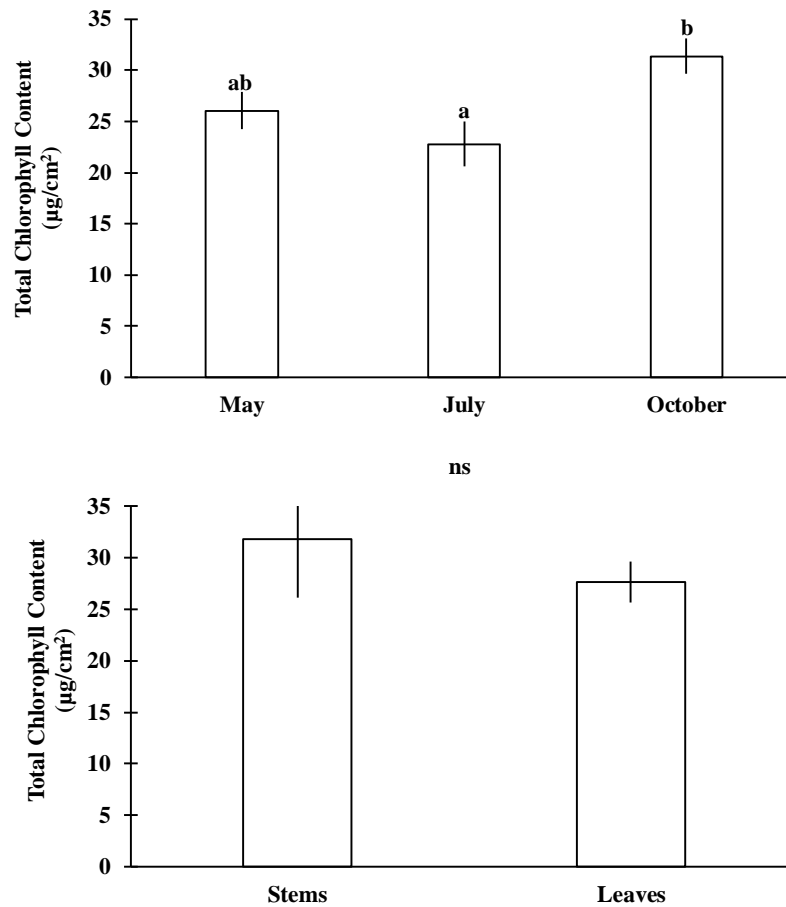


Figure 8. (a) Seasonal comparison of mean chlorophyll content of *R. multiflora* leaves during the 2017 growing season. Different letters denote significantly different means ($p < 0.05$, $n = 10$). (b) Mean chlorophyll content of *R. multiflora* stems and leaves during the 2018 growing season.

Stem Cross Sections and Imaging (2017)

Images of MR stems in cross-sectional view showed the location of chloroplasts as well as anthocyanins. Chloroplasts are found throughout the cross section, in parenchyma, epidermal, and cortical cells of mature stems (Fig. 10a, b), and in epidermal and cortical cells of immature stems (Fig. 10c, d). Stems also produce anthocyanins during the winter (Fig.

10e), which are located in the epidermis on the surface facing the sun. These are produced as a result of exposure to high light in the understory when there is no overstory canopy. An image of the epidermis shows what appear to be stomata with guard cells, but they are infrequent in number (Fig. 9), and it is not known if they are functional.

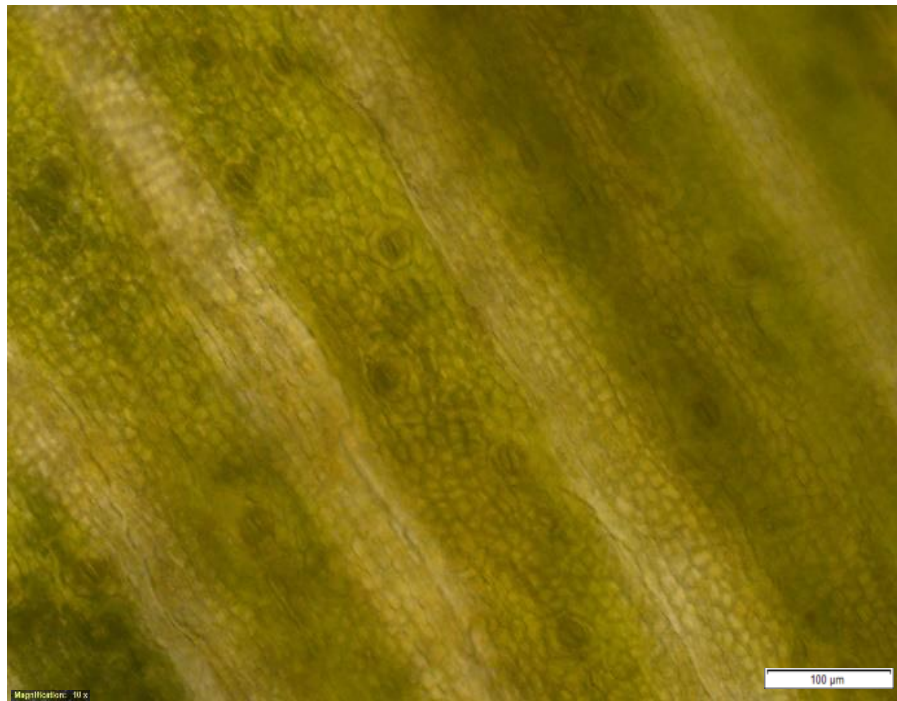


Figure 9. Stomata-like structures appear to be on the epidermis of the *R. multiflora* stem, as pictured, a 10x magnification with a 100 μm long ruler.

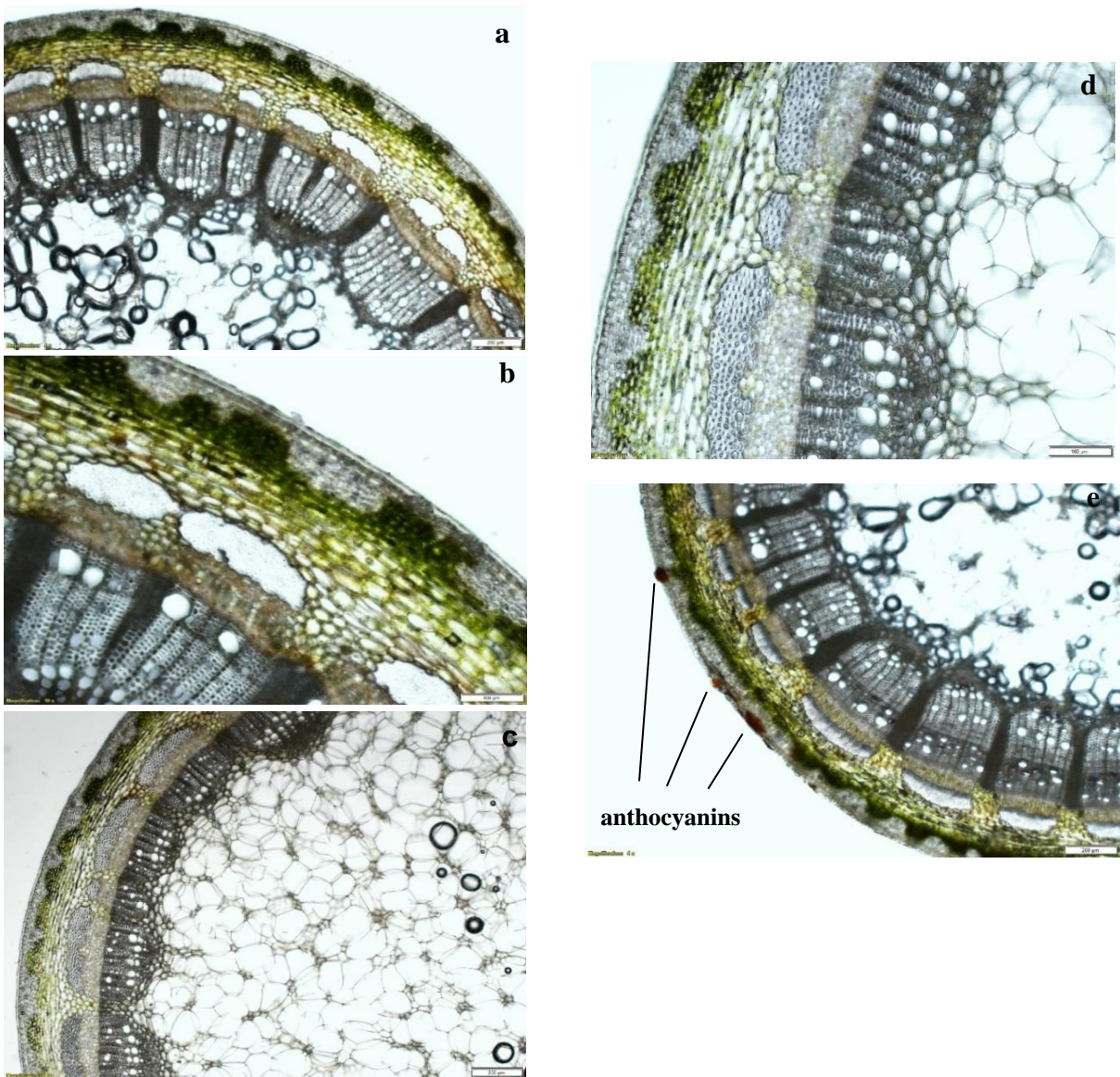


Figure 10. Chlorophyll is seen in the epidermis, cortex, and parenchyma of a mature MR stem at 4x (a) and 10x (b) magnification. Chlorophyll is seen in the epidermis and cortex of an immature MR stem at 4x (c) and 10x (d) magnification. Anthocyanins are seen in the epidermis at a 4x (e) magnification.

Stem Gas Exchange Measurements (2018)

Stem photosynthesis and transpiration, measured as the exchange of gases in the stem cuvette, were essentially non-existent, with rates so low as to be within the error of measurement for the Li-6400, and thus, they probably represent just instrument noise more so than the exchange of gases across the epidermis of the stems. *A* for leaves in 2017 at the same time of year ranges from $2.8 \pm 0.59 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $6.2 \pm 0.87 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($n = 3$). *E* of MR leaves at the same time of year in 2017 ranged from a low of $0.9 \pm 0.15 \text{mmol m}^{-2} \text{s}^{-1}$ to a high of $1.8 \pm 0.22 \text{mmol m}^{-2} \text{s}^{-1}$. Water use efficiency, measured as the ratio of *A/E*, ranged from a low of 0.01 ± 0.033 to a high of 0.09 ± 0.119 (data not shown).

Discussion

The extended phenology of MR found in this study is likely to contribute significantly to its ability to invade and maintain itself in the understory of southern Appalachian forests. This species leafs out early in spring and gains most of its carbon at this time. These phenological and physiological results are similar to the other two most important invasive woody plants in my study site, i.e., Japanese Barberry (*Berberis thunbergii*) and Autumn olive (*Elaeagnus umbellata*), both of which exhibit similar early leaf out and high daily carbon gain in the spring (Xu et al., 2007, Riffe, 2018). However, unlike these other woody invasives, MR can continually produce new leaves throughout the growing season, although, again, unlike its congener invasives, it was also the only one to undergo significant mid-season leaf senescence, possibly resulting from shading once the overstory canopy fully leafed out.

Another potential key to the invasibility of MR in the understory could be due to their branched architecture and WUE, both of which are found to be drivers of success for invasive roses in varying light environments (Murphy et al., 2016). The branched architecture of MR aids in its invasiveness by allowing it to overshadow neighboring plants, outcompeting them for sunlight (Murphy et al., 2016). WUE gives MR a competitive edge in times of drought, with MR not having as much water loss and potentially surviving in a water limited environment where native species with low WUE do not survive.

Interestingly, Dlugos et al. (2015) suggest that MR is shade intolerant and, therefore, potentially dependent on light gaps to persist in the understory. They found that photosynthetic rates of MR were higher along forest edges compared to interior habitats, and they also showed increased survivorship and biomass for MR grown in high light versus

shade in greenhouse experiments. I observed more MR shrubs in areas of my study site where gaps in the canopy were prevalent compared to areas where there were no gaps in the canopy, corroborating the conclusions of Dlugos et al. (2015) that MR fares better in high light conditions. Despite MR's preference for high light environments, it appears that it has successfully established itself throughout the understory in the forest understory of my study site and has become an invasive that can alter the composition and functioning of the lower strata of southern Appalachian forests.

MR allocates most of its resources to vegetative growth to maximize light interception in the understory and produces few flowers and berries (Dlugos et al., 2015). I observed new canes being produced by MR throughout the growing season.

One strategy used by plants to decrease carbon loss in the understory is to decrease their LCP and DR (Murphy et al., 2016). In my study, neither LCP or DR changed much throughout the growing season, suggesting a minimal ability to adapt to lower light conditions after canopy leaf out. The fact that LCP or DR did not change for MR differs from the findings of Dlugos et al. (2015), in which MR in simulated habitats lowered its LCP in response to shade. Nonetheless, MR seems to be successful in the forest understory because it can take advantage of an extended phenology to gain most of its carbon prior to canopy closure, can take advantage of canopy gaps to raise its carbon gain, and because its architecture may allow it to suppress competitors in its immediate vicinity.

Another reason MR might be successful in the forest understory is because it may require less chilling in the winter to initiate leaf out as an eastern Asiatic species, whereas North American species require more chilling (Zohner et al., 2017; Zohner & Renner, 2019). In fact, the impact of early leaf out of eastern Asiatic species could become even more

significant if winters become warmer and shorter, as a result of increased nutrient uptake and greater carbon gain (Zohner et al., 2017). It is important to note that while winter warming is predicted to benefit some understory species, particularly by promoting extended phenologies for native and non-native species (Zohner & Renner, 2019), it could have detrimental effects on vernal herbs because of lower light levels and shorter days compared to what they experience now (Heberling et al., 2018). Further, as suggested by Ladwig et al. (2019), phylogeny is the strongest factor in the association of early bud burst and warming, with similar responses among related species. Specifically, species within the Rosaceae family are found to have earlier phenologies (Ladwig et al., 2019), suggesting that other species of Rosaceae, both native and non-native, could respond similarly to winter warming as does MR in the forest understory.

Another potential advantage that may spur MR's invasive success in the forest understory is the photosynthetic capacity of its stems. In this study, low photosynthetic and transpiration rates of stems indicate they perform little to no carbon assimilation via stem net photosynthesis. Therefore, stem fixation of CO₂ in MR may derive from recycling CO₂ via internal cellular respiration and possibly from dissolved CO₂ in xylem water (stem recycling photosynthesis). Stem photosynthesis may be particularly important during times of drought stress because fixing CO₂ without appreciable water loss would result in a large increase in water use efficiency (Ávila et al., 2014). Carbohydrates produced by stem fixation could enable these plants to maintain their carbohydrate levels between rainfall events and avoid metabolic starvation during times of drought (Duan et al., 2018). Stem recycling photosynthesis may enable MR to colonize and persist in habitats subject to drought stress, as excessive drought can lead to mortality via carbohydrate starvation and/or cavitation (Duan

et al., 2018). It may also allow MR to colonize more xeric habitats, including mid-slopes in forests, where competition with overstory trees for water could be intense. I did notice that the main distribution of MR in my site was upslope from the Autumn olive, which occupied lower, more moist slopes, and this may be due to its greater WUE and competitive ability on those drier sites. In addition, stem photosynthesis may contribute to carbon gain in the winter when it would not be possible to have leaves due to the cold and wind at this time of year. However, there are no published studies of winter stem photosynthesis in MR.

Conclusion

Ultimately, the success of MR in the EUS forest understory seems to be reliant on its extended phenology and, during the summer, on its ability to take advantages of light gaps in the canopy. MR gains most of its carbon in the spring and only small amounts during the summer when the canopy is fully leafed out. Its ability to take advantage of the high light and moderate temperature conditions in the fall is tempered by the high rate of leaf senescence, which constrains its carbon gain at this time of the year. Carbon assimilation by external gas exchange in stems in the winter is negligible, but one cannot rule out internal fixation of CO₂ derived from respiratory or dissolved xylem (Teskey & McGuire, 2007). Because internal stem photosynthesis in this species occurs without measurable water loss, it would contribute to an overall higher water use efficiency on a whole-plant basis, possibly enabling this species to occupy more xeric habitats, and, as mentioned above, this species was more prevalent higher upslope than its congener, Autumn olive, which predominated in the lower, wetter portions of the Nature Preserve.

Future studies on MR in the forest understory should focus on measuring the contribution of stem photosynthesis throughout the various growing seasons to better understand how this mechanism may facilitate the ability of this species to compete with native woody species.

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Vita

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