LEAF-LEVEL PHYSIOLOGICAL ACTIVITY OF *RHODODENDRON MAXIMUM* IN RESPONSE TO SEVERAL ENVIRONMENTAL FACTORS

by

EBONI E. HALL

(Under the Direction of Robert O. Teskey)

ABSTRACT

Leaf-level gas exchange was measured in response to temperature, vapor pressure deficit (VPD), photosynthetic active radiation, and daily dry-down periods from ten container-grown *Rhododendron maximum* plants. Diurnal patterns of net photosynthesis, stomatal conductance, and transpiration where also measured and showed a morning increase until it reached a peak during midday, followed by a decline during the evening in all age classes. For all measurements, the three different age classes varied significantly, with the one-year-old foliage exhibiting the highest rates of photosynthesis, stomatal conductance, and transpiration. Leaf water potential measurements during a dry-down period indicated *R. maximum* is sensitive to water stress. *Rhododendron maximum* leaves require mid-temperature ranges, low VPD levels, and low light intensities to reach maximum mean net photosynthetic rates, stomatal conductance, and transpiration rates. Stomatal conductance increased with increasing net photosynthesis, and transpiration increased linearly with stomatal conductance when induced stress was not a factor.

INDEX WORDS: *Rhododendron maximum*, Evergreen shrub, Understory species, *Tsuga canadensis*, Hemlock, Gas exchange, Southern Appalachians

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CHAPTER 1: INTRODUCTION

Several environmental and biological factors are modifying the structure, function, and composition of forest ecosystems throughout the southern Appalachian Mountain region. The introduction of an invasive insect is greatly affecting the forest overstory in the southern Appalachian Mountains. The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an exotic aphid species that was accidentally introduced from Asia to eastern parts of the United States in the 1950s and has spread as far south as northern Georgia (Cheah et al., 2004). These insects mainly affect eastern hemlock (*Tsuga canadensis* (L.) Carr.) and Carolina hemlock (*Tsuga caroliniana* Engelm.) by attaching to the needles and feeding directly on stored starches of the xylem ray parenchyma cells (Eschtruth et al., 2006). The decline of eastern hemlock caused by the HWA may lead to changes in the forest microclimate by increasing understory light intensity and temperature (Evans, 2004), and decreasing ecosystem water use. The impact of eastern hemlock mortality will be widespread and will remain a significant long-term disturbance because of its ecohydrological role in forests throughout its region (Ford and Vose, 2007).

HWA is affecting overstory canopy cover throughout its region, while *Rhododendron maximum* L. (Rosebay Rhododendron), a common understory shrub in hemlock-dominated forests, is altering species diversity throughout the understory. McGee and Smith (1967) concluded that fire suppression and the termination of traditional grazing patterns have contributed to the establishment and spread of *R. maximum*. McGinty (1972) argued that the death of American chestnut (*Castanea dentata* (Marshall) Borkhaussen) trees after the chestnut blight resulted in openings of the forest canopy and contributed to the establishment of *R. maximum*. All are likely, and have contributed to the dominance and persistence of *R. maximum*, which inhibits the growth of other native species.

Rhododendron maximum is an evergreen understory shrub native to North America. However, it is showing characteristics of an invasive species because of its persistence and recent dominance in the understory of riparian areas throughout the southern Appalachian Mountains (Dobbs and Parker, 2004). *Rhododendron maximum* shrubs have slowly decomposing leaves, a resulting thick litter layer, and canopies that foster dense shade (Lovett et al., 2004). In addition, *R. maximum* and its mycorrhizal association have been shown to alter nitrogen cycling in soils (Wurzburger and Hendrick, 2007). All of these characteristics likely contribute to the lack of recruitment and regeneration of tree seedlings beneath *R. maximum* canopies. In hemlock forests where *R. maximum* exists, future canopy gaps resulting from HWA-mediated hemlock mortality may expedite the establishment and spread of *R. maximum* as noted with the death of American chestnut after the chestnut blight (McGinty, 1972).

As thickets of *R. maximum* spread, dominating the understory, and as the hemlock overstory canopy species is being eliminated by the HWA, it is necessary to understand the physiological activity of *R. maximum* at both the whole-plant level in the near future and at the leaf-level to help estimate potential changes in forest ecosystem structure, function, and composition. A better understanding of the physiological activity of *R. maximum* will provide insight into how its performance will be affected in areas that will experience increased photosynthetic active radiation (PAR) levels due to overstory hemlock mortality.

Tying in leaf-level gas exchange data with whole-plant and microclimate data being collected at Coweeta Hydrologic Laboratory in Otto, North Carolina will give a better understanding of whole-plant physiological activity of *R. maximum* and its possible impacts on ecosystem water use and productivity. Seasonal dynamics of transpiration in *R. maximum*, eastern hemlock, *Acer saccharum* Marsh. (red maple), *Betula lenta* L. (black birch), and *Liriodendron tulipifera* L. (tulip poplar) are being determined by measuring sap flow using heat dissipation probes. Soil moisture is being measured with time domain reflectometry (TDR) sensors. Photosynthetic active radiation (PAR) is being measured in arrays using photodiodes calibrated against a quantum sensor at ten spatial locations above the *R. maximum* canopy along with the monitoring of relative humidity and air temperature.

The purpose of this study was to determine rates of net photosynthesis, leaf stomatal conductance, and transpiration of *R. maximum* and the main environmental factors affecting these processes. The results of this research will be used to assist in the modeling of possible hydrologic impacts of eastern hemlock mortality in areas with *R. maximum* understory cover. Specifically, the results will help determine the impact that the expansion of *R. maximum* cover will have on ecosystem water use, and provide insight into how *R. maximum* physiology will be affected in areas that will likely experience increased PAR levels, increased soil moisture, and increased temperature due to overstory hemlock mortality.

CHAPTER 2: LITERATURE REVIEW

Physiological processes of plants are mainly controlled by environmental factors and a plant's ability to acclimate to changes to avoid stress. Plants respond to environmental stress in many different ways, but the level of response is different across species and even within the same species. The type and severity of the environmental stress, such as temperature, light intensity, humidity, etc., determines a plant's ability to respond and the length of time to recover. From an ecological standpoint, a plant's survival depends on developing defense mechanisms to withstand extreme environmental conditions or a plant's ability to acclimate to possible physiological changes more efficiently than surrounding species.

Due to recent increases in hemlock mortality, overstory and understory species in the vicinity of the mortality will likely be exposed to several environmental changes. Changes in microclimate may cause *Rhododendron maximum* and other understory species to experience different levels of environmental stress. The persistence and spread of *R. maximum* in the understory over the years suggests that this species may adjust to changes in its surrounding environment after hemlock decline. This is likely due to its physiological responses to shifts in microclimate. Physiological processes in this species are not completely understood; however, evaluating the effects that environmental factors have on stomatal conductance, transpiration, and net photosynthesis will provide a better understanding of how *R. maximum* may respond physiologically to changes in microclimate following hemlock mortality.

Stomatal Conductance and Transpiration

Plants control water loss by regulating stomatal aperture because of the large water potential gradient between the inside of the leaf and the air (Fordham, 2001). Stomatal guard cell turgor controls the rate at which water vapor passes out of and CO_2 passes into the leaf. Guard cell turgor is affected by numerous variables, including the quality and quantity of light intercepted, moisture in the soil and air, time of day/night and time of year, intercellular CO_2 concentrations, photosynthetic activity, and abscisic acid concentration (ABA) (Farquhar and Sharkey, 1982). A small change in stomatal aperture creates a large change in the rate of transpiration, controlled through the response of stomatal guard cells on the surface of the leaf. Water vapor diffuses from the leaf to the atmosphere because of differences in the concentration of water vapor between the plant's intercellular spaces and the atmosphere (Larcher, 2003). Plants control guard cell turgor and in turn, stomatal aperture, in response to environmental conditions to manage physiological processes to support plant survival and prevent dying from desiccation (Schroeder et al., 2001; Brodribb and Holbrook, 2003).

Regardless of other environmental conditions, light is required for stomata to open. However, the amount and duration of light required to open stomata vary among species. For example, stomata of *Aegopodium podagraria* Variegatum (the ground elder), an understory species, require at least 15 minutes of high light intensity (>500 µmol m^{-2} s^{-1}) before opening (Kaiser and Kappen, 2000). The ability of understory leaves to quickly open stomata and increase conductance in response to short periods of intense light (e.g., sunflecks), may allow greater rates of carbon fixation to occur compared to leaves that do not respond to sunflecks (Hull, 2002). Under conditions of no incident PAR, stomata of shade-tolerant *Microstegium vimineum* (Trin.) Camus were basically closed (leaf stomatal conductance was at 0 mmol m^{-2} s^{-1}) but began to increase within one minute of being exposed to 550 µmol m^{-2} s^{-1} (Horton and Neufeld, 1998). Leaves of *Podophyllum peltatum* L., *Arisaema triphyllum* (L.) Schott, and *Smilacina racemosa* (L.) Desf. underwent periods of no irradiance over 1, 3, 10, and 30 minutes (Hull, 2002). Initiating 500 µmol m^{-2} s^{-1} PAR resulted in maximum values of net photosynthesis in all species after 40 seconds and after being in 1 minute of darkness, but a period of 100 seconds was needed to reach maximum values after being in 30 minutes of no light. The ability of stomata to respond to rapid changes in light intensity by commencing the uptake of CO_2 after a certain time period without light or a light level below the initiation of intense artificial light is called photosynthetic induction (Pearcy, 1990), but this has not be studied in leaves of *R. maximum*. Studying the ability of *R. maximum* to rapidly induce leaf responses to sunflecks may provide some insight as to how this species is persistent in understory habitats (Horton and Neufeld, 1998).

There is a linear relationship between leaf stomatal conductance and transpiration when other environmental conditions are not limiting. This relationship was evident in field grown understory hypostomatous herb (*Aegopodium podagraria* L. var. variegatum L. H. Bailey) in Kiel, Germany (Kaiser and Kappen, 2000). When irradiance was low (20 µmol m^{-2} s^{-1}) conductance and transpiration were slightly above 0 (<80 and < 1 mmol m^{-2} s^{-1}) respectively. When irradiance increased to 500 µmol m^{-2} s^{-1}, both leaf conductance and transpiration increased linearly.

The ability of a plant to respond to various levels of water deficits by controlling stomatal aperture to regulate the loss of water strongly contributes to survival. Water is the most limiting environmental factor for plant growth and survival, and measuring water potential is widely used to evaluate plant water status (Hsiao, 1973). Prolonged periods without water affect plant physiological processes such as nutrient uptake and plant growth (Ritchie, 1998), plant metabolism and photosynthesis (Tezara et al., 1999), and many other processes. Plants respond to declining leaf water potential (i.e., water stress) by decreasing stomatal conductance. The rate in which stomata respond to water deficits by decreasing conductance determines transpiration (Motzer et al., 2005), photosynthesis (Ni and Pallardy, 1992), carbon gain (Lipscomb and Nilsen, 1990) and other physiological processes within plants.

Stomata are important in regulating water movement to avoid water stress. Loss of water through the leaves via the stomata is necessary for carbon dioxide uptake and resulting carbon fixation. Water moves from the soil through the plant and out the leaf along a decreasing water potential gradient. Significant closure of *R. maximum* leaf stomata has been shown to occur at leaf water potentials of -1.50 MPa (Lipscomb and Nilsen, 1990). Another *Rhododendron* species, *R. periclymenoides* (Michx.) Shinners, experienced induced water stress and intercellular CO_2 concentrations within the leaf (Ci) remained high (>325 µmol ·mol) when stomatal conductance was less than 40 and greater than 250 mmol m^{-2} s^{-1} (Lipscomb and Nilsen, 1990). Leaf stomatal conductance of a riparian understory plant, *Arisaema heterophyllum* Blume, was also sensitive to water stress as seen in decreases of stomatal conductance by 74–84% over 7–10 days in water stressed plants (Muraoka et al., 2002). In diurnal measurements on leaves of *R. maximum*, stomatal conductance rapidly increased in the morning then declined throughout the day, resulting in transpiration increasing and water potential decreasing in the morning, and then transpiration decreasing and water potential increasing throughout the day (Lipp and Nilsen, 1997). In another shrub growing in a dry shrubland in Barcelona, *Erica multiflora,* transpiration rates were always highest in the morning, and ranged from 4-7 mmol m^{-2} s^{-1} in the summer to 2 mmol m^{-2} s^{-1} in the autumn months. The lowest diurnal values occurred in the afternoons (1-3 mmol m^{-2} s^{-1}) in all seasons (Llorens, 2003).

Leaf water potential varies seasonally and is dependent on environmental factors. Throughout the growing season, Nilsen (1985) showed that leaf water potential was lowest during winter months in which *R. maximum* leaves growing under an evergreen canopy had higher leaf water potentials than those growing under a deciduous canopy. During diurnal measurements from December-March, leaf water potential did not have an effect on leaf stomatal conductance because stomatal conductance remained low throughout the day. In May, gradual decreases in leaf water potential corresponded with gradual increases in leaf stomatal conductance and vice versa, probably because throughout the day plants had adequate water supply (Farquhar and Sharkey, 1982). Under evergreen canopies the lowest leaf water potential was -2.40 MPa in December and the highest, -0.70 MPa, was observed in May during midday measurements. Under deciduous canopies, -1.30 MPa was the lowest leaf water potential in May (Nilsen, 1985).

Under non-limiting soil moisture levels (i.e., high soil water potentials), stomatal conductance is tightly coupled to the water vapor content in the atmosphere surrounding the leaf. The air vapor pressure deficit (VPD) is the difference between the amount of moisture the air can hold at a given temperature and the actual amount of water in the air at a given temperature. Therefore, temperature and humidity also affect stomatal conductance because they directly affect VPD. The relationship between stomatal conductance and VPD is a negative exponential, with maximum conductance occurring near air saturation (low VPD) and rapidly declining conductance as VPD increases. While Bunce (1996) suggested that VPD is directly responsible for variations in stomatal conductance, Mott and Parkhurst (1991) showed that stomata do not respond directly to VPD but that stomata may be responding to transpiration rate instead.

Relative humidity plays a role in regulating VPD, which influences stomatal activity. Therefore, high relative humidity and low VPD result in high rates of stomatal conductance because adequate moisture around the leaf prevents stomatal closure. Leaves of understory species often decrease stomatal conductance in response to high levels of VPD (> 1.5 kPa), which is also observed in other plants (Bunce, 1996; Gonzalez-Rodriguez et al., 2002; and Motzer et al., 2005). For example, Mooney et al. (1983) showed that higher stomatal conductivities were observed in a humid tropic understory shrub (*Piper hispidum* Sw.) when humidity was high compared to when humidity was low. This response is observed because relative humidity decreases as VPD increases; therefore, the air surrounding the leaf is dry resulting in significant stomatal closure during this time to prevent water loss.

As stomatal closure is promoted because of high VPD and low relative humidity, transpiration rates are influenced in different ways, depending on the species and its environment. Stomata control transpiration but transpiration can continue to increase even if stomatal conductance decreases depending on the environmental factor, especially water availability. Transpiration rates initially increase linearly with decreasing stomatal conductance; upon stomatal closure, transpiration declines rapidly. For example, in leaves of *Abutilon theophrasti* Medik (an annual shrub-like herb found mainly in China), transpiration rates initially rapidly increased when stomatal conductance was high, but decreased as conductance began to decrease simultaneously with increasing VPD (Bunce, 1996). This type of response is observed because transpiration is controlled by stomatal conductance as VPD increases. Although leaf transpiration rates in *R. maximum* have not been reported, other understory species provide examples as to how these rates may be affected by environmental conditions. In *Persea indica* (L.) Spreng, the highest mean stomatal conductivity and transpiration (517 and 6.60 mmol m^{-2} s^{-1}, respectively) were observed in moist conditions with low VPD (below 0.5 kPa) (Gonzalez-Rodriguez et al., 2002). A combination of high conductance (~600 mmol m^{-2} s^{-1}) and high transpiration rates (~8 mmol m^{-2} s^{-1}) occurred in full sunlight with low VPD (below 0.5 kPa), and high VPD (> 1.5 kPa) resulted in lower stomatal conductance and transpiration rates (<100 mmol m^{-2} s^{-1} and <6 mmol m^{-2} s^{-1}, respectively) (Gonzalez-Rodriguez et al., 2002).

Along with environmental factors discussed above, temperature also plays an important role in regulating stomatal activity. Leaf stomatal conductance of *R. maximum* leaves measured at 25 °C reached maximum rates of 115 mmol m^{-2} s^{-1} (Lipscomb and Nilsen, 1990). Leakey et al. (2003) studied variations of leaf stomatal conductance in *Shorea leprosula* Miq. seedlings at 28 and 38 °C and similar VPD ($\leq$1.2 kPa). Higher temperature affected stomatal activity by decreasing maximum rates of conductance from 0.08 to 0.05 mol m^{-2} s^{-1}. Landhausser et al. (1997) observed that higher temperatures

(20°C) reduced stomatal conductance moreso than lower temperatures (7°C) in understory species (*Pyrola asarifolia* Michx.) growing in a boreal forest. Leaf stomata of understory species may show this type of response because VPD increases with temperature (Landhausser et al., 1997), and understory species stomata are also sensitive to high VPD levels (Motzer et al., 2005). Even though high temperatures decrease stomatal conductance, transpiration rates increase with increasing temperatures because of its effect on VPD (Mellander et al., 2004).

Change in stomatal conductance occurs seasonally because of variations in environmental conditions. Nilsen (1985) reported diurnal and seasonal field measurements of stomatal conductance in *R. maximum* leaves. Stomatal conductance was measured monthly in *R. maximum* thickets growing under a deciduous canopy and an evergreen canopy. Diurnal patterns of leaf stomatal conductance were measured during the months of February, March, May, and December. *Rhododendron maximum* had low leaf conductance during the winter months (<0.25 mm s^{-1}) at both sites even though irradiance levels were higher during this time compared to summer months. During February, March, and December, stomatal conductance remained less than 0.25 cm s^{-1} throughout the day under both canopy covers. May was the only month that showed a diurnal pattern of stomatal conductance. In May, temperature and VPD were higher, and a typical diurnal pattern was observed where leaf stomatal conductance reached maximums during midday and lowest conductance during morning and evening. In the deciduous stand, stomatal conductance gradually increased from close to 0 cm s^{-1} in the morning to maximum values of 0.14 cm s^{-1} during midday followed by a gradual decrease to values near 0 cm s^{-1}. *Rhododendron maximum* leaves growing in the evergreen stand during the same month reached maximum stomatal conductance (0.10 cm s^{-1}) two hours before leaves growing in the deciduous stand, leveled off for four hours, and then gradually declined to values near 0 cm s^{-1}. In that study, *R. maximum* leaves had little to no stomatal conductance during the winter months even when temperatures reached 14 °C in February. December and March received the lowest maximum amount of irradiance (<50 µmol m^{-2} s^{-1}) under the evergreen canopy. Under the deciduous canopy irradiance was less than 100 µmol m^{-2} s^{-1} throughout the day in December, but reached maximums greater than 350 µmol m^{-2} s^{-1} in March. In February, March, and May irradiance reached maximums greater than 350 µmol m^{-2} s^{-1} under the deciduous canopy but less than 180 µmol m^{-2} s^{-1} under the evergreen canopy.

Temperature and VPD are two environmental factors that influence leaf gas exchange seasonally. Nilsen (1985) recorded monthly maximum and minimum air temperatures and VPD in southern Appalachian evergreen and deciduous stands with *R. maximum* understories. Winter temperatures were the lowest in February (< -10 °C) and the highest temperatures were observed in summer (~33 °C). Diurnal air temperature was measured during December, February, March, and May. Temperature was most consistent throughout the day in December and May, and fluctuated the most during February and March. Vapor pressure deficit was less than 0.5 kPa during December and March, less than 1.0 kPa during February under both canopy types, and reached maximums greater than 2.0 kPa under the deciduous canopy and greater than 1.5 kPa under the evergreen canopy in May (Nilsen, 1985). Because warmer air holds more moisture than cooler air, relative humidity is higher in warmer conditions, causing VPD to increase with higher temperatures and decrease with lower temperatures; therefore, stomatal conductance typically decreases with increasing temperatures (Bunce, 1997).

Simultaneous decreases in leaf stomatal conductance and CO_2 fixation reduce physiological activity in plants (Ni and Pallardy, 1992). This is attributed to stomatal sensitivity to high levels of Ci, so stomata close to avoid water stress and CO_2 assimilation is not manipulated by stomatal activity (Farquhar and Sharkey, 1982). Intercellular CO_2 concentrations (Ci) have been reported to increase with increasing rates of net photosynthesis in favorable environmental conditions (Thomas and Eamus, 1999; Tezara et al., 2005; Bunce, 1996). Under water stress, leaf stomatal conductance and net photosynthesis of *R. maximum* decreased but Ci remained high and mostly stable (Lipscomb and Nilsen, 1990), suggesting non-stomatal limitations to photosynthesis were important factors under water stress. To understand if physiological processes within plant leaves are inhibited by stomatal or non-stomatal limitations, intercellular CO_2 concentrations must be taken into consideration.

Stomatal and non-stomatal limitations in addition to environmental factors assist in manipulating photosynthesis. Reductions in photosynthetic activity are considered to be limited by factors other than stomatal conductance when Ci remains high (Ramanjulu et al., 1998). In contrast, stomatal conductance can directly limit photosynthesis by reducing Ci if, for example, stomatal closure is promoted when leaf water potential decreases, ABA is produced, etc. Abscisic acid (ABA) is a chemical signal (stress hormone) that advocates the closure of stomata during severe water loss and drought. During periods of continued drought regimes, decreases in Ci confirm the ability that stomatal limitations have on constraining the process of photosynthesis (Flexas and Medrano, 2002).

Net Photosynthesis

Photosynthesis commonly decreases with increasing leaf age, which is probably an indirect effect of a successive translocation of leaf nitrogen from older to newer leaves, loss of electron transport capacity, a reduction in enzyme activity, a change in quantum yield and/or a reduction in stomatal conductance (Nilsen et al., 1988). The typical response of photosynthesis in plant leaves as light intensity increases is first a rapid linear increase followed by a less rapid increase in the rate of photosynthesis with each consecutive increase in irradiance until the point is reached where no further change in photosynthesis occurs with increasing light (i.e., light saturation) (Teskey et al., 1995; Nilsen et al., 1988).

Responses of net photosynthesis in *R. maximum* leaves varies with leaf age, and like other shade-tolerant species, it varies depending on the light environment in which it is growing. The only study that has measured rates of net photosynthesis of *R. maximum* leaf cohorts *in situ* in response to increasing light intensities is the study of Nilsen et al. (1988). Three different habitats experiencing low (dense hemlock canopy), intermediate (deciduous canopy), and high (open canopy) levels of PAR were used to develop relationships between gas exchange and PAR (i.e., light response curves) in *R. maximum* throughout the spring, summer, and fall seasons. Leaves were longer-lived in the low light sites (2–62 months) compared to the intermediate and high light intensity sites (2–38 months). Light saturated photosynthesis decreased with leaf age in all three microhabitats. Light saturation points of *R. maximum* leaves were highest in young foliage (4.40-5.80 µmol m^{-2} s^{-1}) under deciduous canopy and lower in older foliage (<2 µmol m^{-2} s^{-1}) in high, low, and intermediate light environments. In the open, the rate of light saturated photosynthesis was reached earlier in the day than that of low light environments for all leaves except the two month old foliage (Nilsen et al., 1988). This same pattern is not only observed in understory plants and shrubs, but also in understory tree seedlings. Ethier et al. (2006) studied CO_2 assimilation responses of one, three, and four year old foliage of *Pseudotsuga menziesii* (Mirb.) Franco seedlings in the understory. They found that net photosynthesis decreased as leaf age increased and that the amount of light required to saturate the photosynthetic response decreased with increasing leaf age (1 yr, 3 yr, and 4 yr old foliage 600, 400, and 100 µmol $m^{-2}s^{-1}$, respectively).

Differences in the responses of photosynthesis to light intensity are most noticeable between shade-tolerant and intolerant species. Typically, shade-intolerant species have higher maximum rates of net photosynthesis, light compensation points, and light saturation points than shade-tolerant species (Holmes and Cowling, 1993). For example, a shade-tolerant understory species in the Great Smokey Mountains (*Microsteguim vimineum* (Trin.) A. Camus) was grown under 25% and 50% ambient sunlight (Horton and Neufeld, 1998). In plants growing under 25% sunlight, maximum net CO_2 assimilation rates were 12.71 µmol m^{-2} s^{-1} and these rates were reached at 750 µmol m^{-2} s^{-1} irradiance. Plants growing under 50% sunlight reached maximum rates of 16.09 µmol m^{-2} s^{-1} at light levels of 815 µmol m^{-2} s^{-1}. Gas exchange of another shade-tolerant understory species was measured in different light environments. Rates of net photosynthesis where measured in low (50 µmol m^{-2} s^{-1}), intermediate (150 µmol m^{-2} s^{-1}), and high (550 µmol m^{-2} s^{-1}) light environments for well-watered *Arisaema heterophyllum* (Araceae) (Muraoka et al., 2002). Saturation points in the low, intermediate, and high light environments were 300, 400, and 800 µmol m^{-2} s^{-1} PAR respectively. Mean maximum net CO_2 assimilation ratesof *A. heterophyllum* in the low, intermediate and high light environments were 9.61µmol m^{-2} s^{-1}, 12.03 µmol m^{-2} s^{-1} and 13.30 µmol m^{-2} s^{-1}, respectively (Muraoka et al., 2002). In contrast to these shade-tolerant species, the photosynthetic responses of a shade-intolerant tree species, *Pollalesta discolor* (Kunth) Aristeg. showed maximum mean rates of net photosynthesis of 10.53 µmol m^{-2} s^{-1} growing in full light with a saturation point greater than 1000 µmol m^{-2} s^{-1} PAR (Davidson et al., 2002). When growing in partial shade (83% of ambient light intensity), maximum mean rates of net photosynthesis were reduced by half (4.66 µmol m^{-2} s^{-1}); seedlings growing in full shade died before the study was complete. These studies agree with that of Gonzalez-Rodriguez et al. (2002), and suggest that shade-intolerant species have higher photosynthetic responses than shade-tolerant species and require higher light intensities for saturation. Lipscomb and Nilsen (1990) concluded that *R. maximum* does not require high light intensities for growth and physiological processes, but requires an abundant amount of water to prevent significant reductions in rates of photosynthesis.

Constant variations in temperature, water availability, light intensity, and other environmental factors contribute to a plant's photosynthetic response. *Miconia ciliata* (Rich.) DC, an understory species growing in an eastern Amazonian forest, exhibited significant reductions in rates of CO_2 assimilation during dry periods, in which light saturation points were 3 to 4 times lower than irrigated plants (Fortini et al., 2003).

Similarly, *Arisaema heterophyllum* (Araceae) plants growing in dry soil conditions showed mean maximum net CO_2 assimilation rates of only 36% of that of well-watered plants (Muraoka et al., 2002). Cao (2000) observed that high VPD resulted in decreased maximum rates of photosynthesis in five understory saplings growing in a Bornean heath forest, due primarily to reductions in stomatal conductance. Rates of net photosynthesis of understory species similar to *R. maximum* decline in response to water stress and high levels of VPD, are inhibited in low and high temperatures depending on the plant's optimal temperature range, and vary among shade tolerant and intolerant species.

Although we know little about seasonal gas exchange in *R. maximum*, we do know the seasonal patterns of gas exchange in some other evergreen shrub species. For example, the light saturated rate of net photosynthesis in *Aucuba japonica* Thunb. varied seasonally, and with its growing environment (Muller et al., 2005). This species had its highest rates of photosynthesis (9.20 µmol m^{-2}s^{-1} to 6.50 µmol m^{-2}s^{-}) from August to January in plants growing in created canopy gaps. In plants growing under an evergreen canopy, the maximum rate of net photosynthesis was low and remained nearly constant throughout the year. Net photosynthesis reached a maximum of 4 µmol m^{-2}s^{-1} in August, and a minimum of 2.10 µmol m^{-2}s^{-1} in December. The daily average temperature was the highest in August, around 21 °C, and lowest in February, around 2 °C (Muller et al., 2005). *Pistacia lentiscus* L., an evergreen shrub, showed intra-annual variations in net CO_2 assimilation (Flexas et al., 2001). The timing of maximum values of net photosynthesis varied within the year depending on the location of the site; however maximum values ranges from 11.50–12.50 µmol m^{-2}s^{-1}.

Optimum temperature ranges permit maximum physiological responses within the leaf when other environmental conditions are not a factor. Leaf gas exchange measurements of *Persea indica* (L.) Spreng in response to leaf temperature showed that carbon assimilation reached maximums (>10 µmol $m^{-2}s^{-1}$) at 23 °C (Gonzalez-Rodriguez et al., 2002). Assimilation rates increased from minimums at 10 °C to maximums between 17 to 23 °C followed by gradual declines at higher temperatures until no assimilation occurred. The relationship between light intensity and carbon assimilation was linear, rapidly increasing until it reached the light saturation point at 700 µmol $m^{-2}s^{-1}$ and an assimilation rate of 10 µmol $m^{-2}s^{-1}$. As the rate of carbon assimilation increased, leaf stomatal conductance also increased. Once maximum rates of carbon assimilation were achieved (i.e., no further increase in carbon assimilation), stomatal conductance continued to increase (Gonzalez-Rodriguez et al., 2002). The relationship between leaf stomatal conductance and net photosynthesis was highly correlated in *Pseudotsuga menziesii* such that when net photosynthesis increased, leaf stomatal conductance increased (Ethier et al., 2006). This was also evident in *Pistacia lentiscus* L. leaves (Flexas et al., 2001).

All plants have an optimum temperature range for physiological responses. A remaining question is whether *R. maximum* photosynthesizes in the winter. It is an evergreen species and receives more incident light during the winter compared to other seasons (Nilsen, 1985), but it appears that they are not physiologically active during this time (Martinez, 1975). Nilsen (1985) only made measurements of stomatal conductance, but his results indicated that stomata open little (if at all) between December and May regardless of climatic conditions. Because *R. maximum* retains leaves all year long, a wide range of temperatures are experienced, and thus the potential for temperature to affect leaf physiological responses is great. For example, extreme winter temperatures may cause embolisms that can permanently damage water-carrying vessels (discussed below).

Additional aspects of the autecology of *Rhododendron maximum*

Water Availability

Adequate water supply is vital for plant function and may be the most important environmental factor contributing to the distribution and dominance of *Rhododendron maximum* Lipp and Nilsen (1997). In addition, the recent dominance and persistence of this species is noticeable in areas of high water availability and in areas of existing *R. maximum* thickets (Dobbs and Parker, 2004). A study conducted in the Dryman Fork and Coweeta basins of North Carolina showed that *R. maximum* dominance and amount of vegetation cover in existing thickets decreased with increasing distance from water sources. Using Geographical Information Systems (GIS) and logistic regression, Dobbs and Parker (2004) determined that *R. maximum* expansion occurred between 1976-1993 mainly along streams and within existing *R. maximum* thickets. *Rhododendron maximum* shrubs need adequate water availability and may need the presence of existing thickets and overstory canopy to continue their dominance in the understory because direct sunlight inhibits reproduction (Plocher and Carvell, 1987).

Rhododendron maximum shrubs retain leaves throughout the year, so extreme temperatures during the winter may influence physiological responses during early spring due to recovery time. *Rhododendron maximum* winter physiology may be controlled by frequent freeze-thaw cycles and resulting embolisms (Lipp and Nilsen, 1997). Although annual rainfall regularly exceeds 1700 mm in some parts of the southern Appalachian Mountains (Swift et al., 1988), water stress can occur during the winter months if freezing events cause air bubbles to develop in the vessels and disrupt the water column (i.e., xylem embolism). During the winter, reduced hydraulic conductance from freezing-induced embolisms inhibit the growth of *R. maximum*, even more so in shrubs growing in canopy gaps than those in non-gap areas (Nilsen, 1992; Cordero and Nilsen, 2002). Embolized vessels in *R. maximum* may be difficult to repair due to their relatively large diameter.

Temperature

Extreme temperatures can cause stress and physiological dysfunction within plant parts resulting in dramatic decreases in net photosynthetic activity (Teskey et al., 1995). *Rhododendron maximum* and *Rhododendron catawbiensis* Michx. leaves change horizontal leaf orientation (thermonasty) to withstand cold temperatures because they retain their leaves throughout the year (Nilsen, 1990). Thermonastic leaf movements in this evergreen understory shrub (*R. maximum*) occur seasonally as temperatures vary (Nilsen, 1992). For example, the leaves of *R. maximum* are horizontal or partially vertical under low light conditions during warm summer conditions, but they hang vertically and are highly curled during cold winter days regardless of the light intensity (Nilsen, 1992). During winter months, thermonastic leaf movements occur but leaves of *R. maximum* are not photosynthetically active during this time even if surrounding climatic conditions are spring-like (Nilsen, 1992; Martinez, 1975).

While the exact causes of thermonastic leaf movements are unknown, because cold-hardy shrubs more readily exhibit this behavior, this behavior may prevent loss of turgor pressure, prevent leaf tissue impairment, and reduce incident leaf surface area (Nilsen, 1992; Nilsen, 1990). Leaf movements may have adaptive significance, further explained by Nilsen (1990) in the following theories: 1) Evolution relict theory: major changes in leaf angle may not require metabolic energy and the movements may occur only because turgor is lost. 2) Mechanical theory: the leaves change orientation as a defense mechanism during winter conditions. 3) Desiccation theory: in addition to changes in leaf angle, the leaves curl to reduce surface area, usually to prevent water loss, but Nilsen stated his previous data on leaf water potential did not supply evidence that water stress occurred in periods of cold temperatures. 4) Heat balance theory: the leaves of *R. maximum* respond conversely to that of desert plants because they move in response to cold, not hot temperatures. 5) Photoinhibition theory: the potential for photoinhibition is important if canopy gaps are created because of the high levels of light intensity intercepted during winter conditions. Changes in leaf angle relate directly to leaf water potential and light intensity (Nilsen, 1992), while temperature causes leaf curling. 6) Freezing damage theory: the effects of leaf freezing points can cause damage to internal parts of the leaf because of responses to a rapid warming period.

Light Intensity

The irradiance level reaching the forest floor is greatly affected when canopy cover is influenced by tree removal or mortality. Clinton (2003) created canopy gaps by girdling overstory trees in areas with and without *R. maximum* thickets. He examined the impacts of canopy gaps on the understory microclimate: temperature, soil moisture, and most importantly light intensity. Lower levels of PAR reached the forest understory in gaps dominated by *R. maximum* than that of non-Rhododendron gaps. *Rhododendron maximum* is sensitive to high levels of irradiance, and only requires low PAR levels for photosynthesis (Nilsen et al., 1988), and additional shading created by *R. maximum* thickets prevents the penetration of irradiance to the forest floor (Lei et al., 2006) for use by competing understory shrubs or tree seedlings. The successive thinning and opening of forest canopies may have contributed to the introduction of more Rhododendron individuals and the recent dominance of the species in riparian zones (Clinton, 2003).

Daily average PAR measurements in the Coweeta basin show that irradiance levels throughout the forest floor are highest in late October through early June, when the tree canopy is not at maximum leaf area (Ford and Hall, unpublished data). Correspondingly, Nilsen (1985) showed that the lowest PAR in *R. maximum* canopies was during June through October, and the highest incident PAR occurred in February through April. Roberts (2006) showed the lowest light conditions in a hemlock dominated stand with the highest canopy cover and a substantial amount of *R. maximum* in the understory. These data suggest that light is least available in the warmer months and most available in the colder months.

Baker and Van Lear (1998) observed the effects of *R. maximum* on other understory species and its ability to expand between Nantahala Lake and Wine Spring Bald in the southern Appalachian Mountain region. Species diversity was greater in plots where *R. maximum* had lower stem densities compared to plots with higher stem densities. Reasons for this response may have been due to: (1) dense *R. maximum* patches prohibited light from penetrating to the forest floor (Clinton et al., 1994), (2) the slow decomposing leaves of *R. maximum* can inhibit seedling regeneration (Boettcher and Kalisz 1990), and (3) the possible release of an allelopathic chemical that hinders the growth and development of other understory species (Stephenson et al., 2006). *Rhododendron maximum* is not only dominant in riparian areas of forest ecosystems, but also in areas where this species is already persistent because it requires minimal light for photosynthetic activity (Nilsen, 1988).

CHAPTER 3: MATERIALS AND METHODS

Ten containerized *Rhododendron maximum* plants, each with three cohorts of foliage, were obtained in June 2007 from the Gardens of the Blue Ridge nursery (Newland, NC). The plants were approximately 18-24 inches tall in 3 gallon pots, and had been grown outside under mature oak trees from the beginning of spring until the end of fall. Once transported, the plants were placed in Whitehall Forest greenhouse managed by the University of Georgia Warnell School of Forest Resource, surrounded all around by 70% shade cloth at all times, watered twice a week, and fertilized bi-weekly. The temperature in the greenhouse exceeded outside temperatures and reached maximums around 35 °C while the plants were stored there.

After nearly two weeks of growing in the greenhouse, the plant leaves began to desiccate brown and wilt. They were quickly transferred to an on campus growth chamber, in more favorable conditions, where they were grown at 20°C, 45% humidity, and an irradiance level of 250 μmol m^{-2} s^{-1}. Over the next two weeks the plants maintained sufficient, positive net photosynthetic responses to use for light response curves, but shortly after, the plants responded negatively to different levels of light and were replaced. Ten more plants were obtained from the Gardens of the Blue Ridge nursery and immediately placed in a growth chamber (Model 36, Environmental Growth Chambers, Chagrin Falls, OH) because of conditions unsuitable for the survival of this species in the greenhouse. The chamber environmental conditions were set to a temperature of 22°C, 45% humidity, and an irradiance level of 200 μmol m^{-2} s^{-1}. Four slits were cut into the bottoms of each container because *R. maximum* thrive in well drained soils. The plants were watered to capacity every three days.

Response Curves

Leaf-level gas exchange in *R. maximum* was measured and analyzed to further understand its responses to several environmental factors. A non-dispersive portable infrared gas analyzer (IRGA, LI COR 6400) was used to obtain environmental response curves for net photosynthesis and stomatal conductance in *R. maximum*. Important environmental factors contributing to certain physiological variation in plants were measured, including photosynthetically active radiation (PAR), temperature, vapor pressure deficit (VPD), and a period of induced water stress until no gas exchange was measured. For all chamber measurements, the plants were allowed at least three days to acclimate to chamber conditions.

Responses of net photosynthesis and stomatal conductance to light were measured at PAR levels ranging from 0-1600 µmol $m^{-2}s^{-1}$ in increments of 0, 50, 100, 200, 300, 400, 500, 700, 1100, 1500 and 1600 µmol $m^{-2}s^{-1}$. One leaf of each of the three foliage age classes (years 1, 2, and 3) was measured on each containerized plant. All leaves appeared healthy, so ten leaves were randomly selected from each cohort, measured, and tagged for future measurements. Ten leaves were measured during light response cures. For all other curves, five of the ten tagged leaves where measured.

Net photosynthesis and stomatal conductance were also measured at three VPD levels; 0.5, 1.0, and 1.5 kPa. Measurements were made on the same three foliage age class leaves that were tagged for PAR measurements. Humidity and temperature were controlled in the chamber to maintain the different levels of VPD. High levels of humidity were attained by placing two humidifiers in the chamber, lowering the chamber temperature to 15 °C, and controlling H_2O (Reference), H_2O (Sample), and scrubbing or bypassing the desiccant from the IRGA.

Temperature response curves of net photosynthesis and stomatal conductance were measured at 5, 15, 25, and 35°C on the same tagged leaves. Vapor pressure deficit, temperature, and induced water stress responses where measured at an irradiance level of 700 µmol m^{-2} s^{-1}. This PAR level was chosen because initial measurements indicated that this irradiance level was the point of light saturation of all foliage classes.

Diurnal Measurements

Diurnal curves for net photosynthesis were derived by randomly measuring five of the ten tagged leaves in the three different age classes of *R. maximum* foliage six times during the day under uncontrolled environmental conditions every two hours between 7 a.m. and 5 p.m. The measurements were obtained outside on three consecutive days (September 26-28, 2007) with similar climatic conditions and undisturbed by rain during the fall under a shaded environment of evergreen trees.

Water availability

The *R. maximum* plants where forced to undergo drought stress until no photosynthetic activity occurred and there was a complete loss of stomatal conductance. The first set of measurements during the dry-down period were taken 24 hours after watering and continued every 24 hours at 700 µmol m^{-2} s^{-1}. As in the previous measurements, net photosynthesis and stomatal conductance in response to the induced water stress were measured using a LICOR 6400 portable photosynthesis system. Measurements were made in the growth chamber. The chamber was set to 22 °C, 45%

humidity, an irradiance level of 200 μmol m^{-2} s^{-1}, and 12 hours of light and 12 hours of darkness.

The second set of measurements during the dry-down period was current leaf water potential, which was estimated by xylem pressure potential measurements using a portable plant pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, CA, USA). Two sets of water potential measurements were taken, one at “pre-dawn” conditions with no light availability for the previous 10 hours and the other at “midday” conditions after 10 hours of light (200 μmol m^{-2} s^{-1}) in the growth chamber. For both pre-dawn and midday measurements, two leaves per foliage class were obtained by randomly selecting leaves that were not being used for dry-down measurements.

Statistical Analysis

The completely randomized data were automatically plotted for the best-fit curve by either linear or non-linear regression equations and tested for significance ($P < 0.05$) using Sigma Plot version 10.0. The results are calculated as means, where $n = 10$ or $n = 5$ ± SE and are appropriately noted.

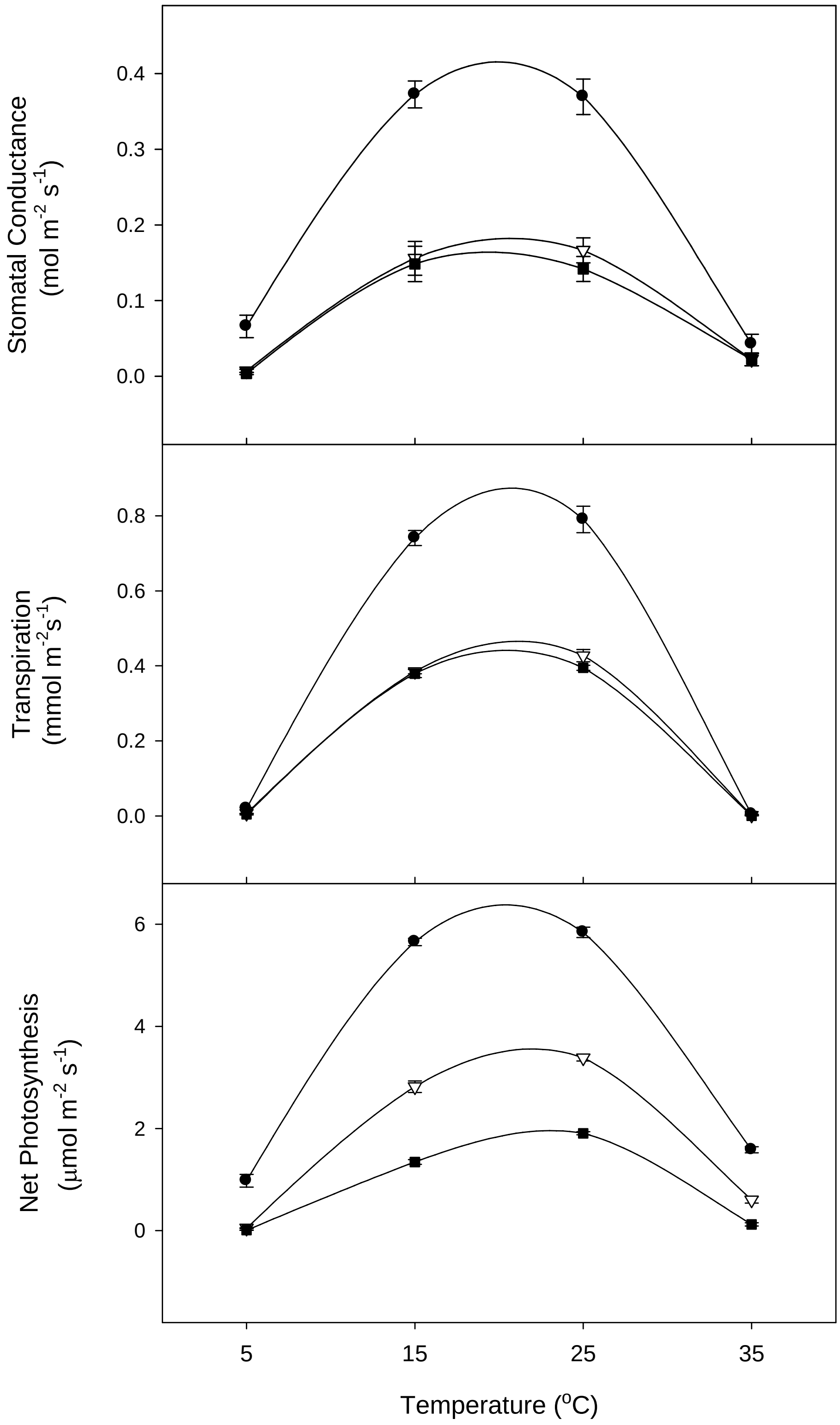
CHAPTER 4: RESULTS

Response Curves

Temperature Maximum rates of net photosynthesis of *R. maximum* were observed at 15 and 25 °C in all foliage classes, suggesting that *R. maximum* plants may have an optimum temperature for net photosynthesis between this temperature range (Figure 1). Net photosynthesis changed from a minimum of 0.66 to 5.84 µmol m^{-2} s^{-1} in one-year-old foliage as temperature changed from 5 to 15 °C. The maximum rate of net photosynthesis was observed in one-year-old foliage at 6.08 µmol m^{-2} s^{-1} at 25 °C. Two and three-year-old foliage showed the lowest rates of net photosynthesis at 5 °C (0.03 and 0.06 µmol m^{-2} s^{-1}, respectively). Net photosynthesis constantly increased until reaching maximum rates of 3.59 µmol m^{-2} s^{-1} in two-year-old foliage and 1.98 µmol m^{-2} s^{-1} in three-year-old foliage at 25 °C. Rates of net photosynthesis in younger foliage were similar between 15 and 25 °C, but net photosynthesis was slightly higher at 25 °C in the older foliage compared to 15 °C. One, two, and three-year-old foliage classes decreased to minimum rates of 1.42, 0.51, and 0.05 µmol m^{-2} s^{-1}, respectively at 35 °C.

Patterns of stomatal conductance and transpiration followed net photosynthesis; showing very low rates at 5 °C, increasing to maximums at 15 and 25 °C, then decreasing at 35 °C. Stomatal conductance changed from 0.0096 mol m^{-2} s^{-1} to 0.4391 mol m^{-2} s^{-1}in one-year-old foliage as temperature increased from 5 to 15 °C. Similar rates were recorded at 25 °C. Stomatal conductance then decreased to a minimum of 0.14 mol m^{-2} s^{-1} at 35 °C. Rates of stomatal conductance in two and three-year-old foliage were comparable. Both two and three-year-old foliage classes had minimum rates of conductance (< 0.0023 mol m^{-2} s^{-1}) at 5 °C and less than 0.0064 mol m^{-2} s^{-1} at 35 °C.

Figure 1. Temperature Response: Relationship of transpiration, stomatal conductance, and net photosynthesis of *Rhododendron maximum* leaves in response to four different temperatures of one-year-old foliage (●), two-year-old foliage (▽), and three-year-old foliage (■). Error bars depict standard error (n=5 for each measurement).

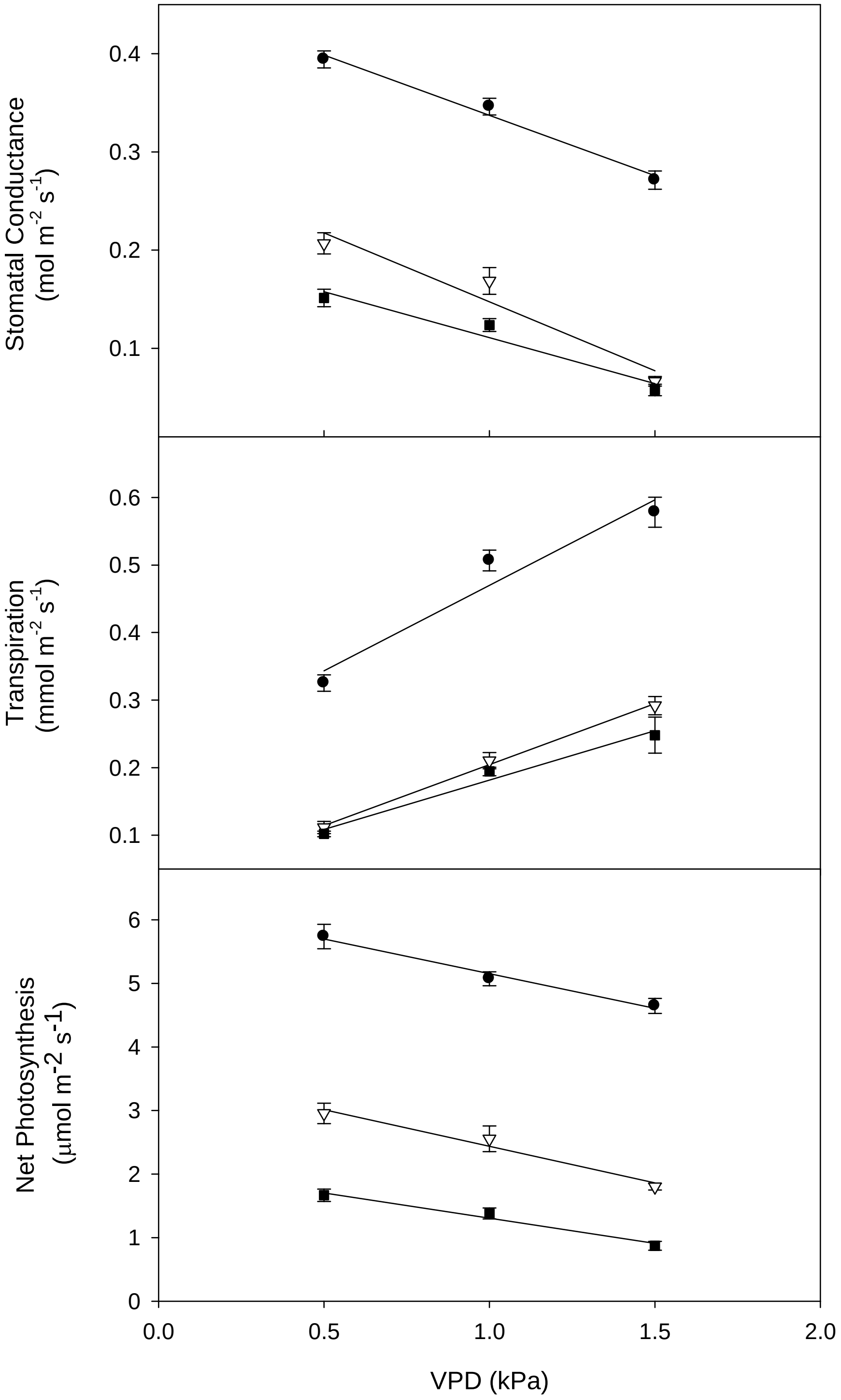

Stomatal Conductance
(mol m^{-2} s^{-1})
0.4
0.3
0.2
0.1
0.0
Transpiration
(mmol m^{-2}s^{-1})
0.8
0.6
0.4
0.2
0.0
Net Photosynthesis
(μmol m^{-2} s^{-1})
6
4
2
0
5
15
25
35
Temperature (^{o}C)

Maximum rates of leaf conductance were 0.22 mol m^{-2} s^{-1} in two-year-old foliage and 0.23 mol m^{-2} s^{-1} in three-year old foliage at 15 °C (Figure 1). Transpiration of all the foliage classes was very low (<0.03 mmol m^{-2} s^{-1}) at the lowest and highest temperatures (5 and 35 °C). The pattern of transpiration in two and three-year-old foliage was almost indistinguishable because of similar responses. One-year-old foliage reached maximum transpiration rates of 0.80 mmol m^{-2} s^{-1} at 15 °C, two-year-old (0.50 mmol m^{-2} s^{-1}) at 25 °C, and three-year-old foliage (0.42 mmol m^{-2} s^{-1}) at 15 °C.

In response to temperature, net photosynthesis, stomatal conductance, and transpiration of *R. maximum* leaves followed comparable patterns where maximum rates were observed between 15 and 25 °C and minimum rates were observed at 5 and 35 °C, with younger foliage having the highest rates. During temperature response measurements, *R. maximum* leaves slightly rolled up in response to high and low temperatures (5 and 35 °C). At these temperatures, transpiration rates approached zero and stomatal conductance and net photosynthesis were lowest in all foliage classes.

Vapor Pressure Deficit Net photosynthesis and stomatal conductance decreased with increasing vapor pressure deficit (VPD); conversely, transpiration increased with increasing VPD in all foliage classes (Figure 2). One year-old-foliage decreased from a maximum rate of net photosynthesis of 6.20 μmol m^{-2} s^{-1} at 0.5 kPa to a minimum rate of 4.36 μmol m^{-2} s^{-1} at 1.5 kPa and had the greatest change in maximum and minimum response values. Two and three-year-old foliage followed the same decreasing pattern with increasing VPD. Two-year-old foliage dropped from maximum rates of net photosynthesis of 3.38 μmol m^{-2} s^{-1} at 0.5 kPa to 1.66 μmol m^{-2} s^{-1} at 1.5 kPa. At the

Figure 2. VPD Response: Relationship of net photosynthesis, stomatal conductance, and transpiration of *Rhododendron maximum* leaves in response to increasing vapor pressure deficits of one-year-old foliage (●), two-year-old foliage (▽), and three-year-old foliage (■). Error bars depict standard error (n=5 for each measurement).


Stomatal Conductance
(mol m^{-2} s^{-1})
0.1
0.2
0.3
0.4
Transpiration
(mmol m^{-2} s^{-1})
0.1
0.2
0.3
0.4
0.5
0.6
Net Photosynthesis
(µmol m^{-2} s^{-1})
0
1
2
3
4
5
6
0.0
0.5
1.0
1.5
2.0
VPD (kPa)

lowest VPD (0.5 kPa), three-year-old foliage reached a maximum rate of net photosynthesis, 1.91 µmol m^{-2} s^{-1}, and decreased to the lowest rate, 0.63 µmol m^{-2} s^{-1}, at the highest VPD (1.5 kPa) (Figure 2). Leaf stomatal conductance, like net photosynthesis, also decreased with increasing VPD.

Rhododendron maximum stomata, in all three foliage classes, were sensitive to changes in VPD (Figure 2). The decreasing rate of stomatal conductance with increasing VPD was most evident during the transition from 1.0 to 1.5 kPa in two-year-old foliage. Leaf conductance decreased from a maximum of 0.42 mol m^{-2} s^{-1} at 0.5 kPa to 0.25 mol m^{-2} s^{-1} at 1.5 kPa in one-year-old foliage. Two-year-old foliage reached a maximum conductance of 0.24 mol m^{-2} s^{-1}, then dropped to 0.06 mol m^{-2} s^{-1} at 1.5 kPa. Three-year-old foliage also decreased to the lowest rate of 0.04 mol m^{-2} s^{-1} at high VPD but reached a maximum value of leaf conductance (0.17 mol m^{-2} s^{-1}) at 0.5 kPa. Even though a small range of VPD was used, stomatal closure was promoted and excess water loss through transpiration occurred in response to increasing VPD.

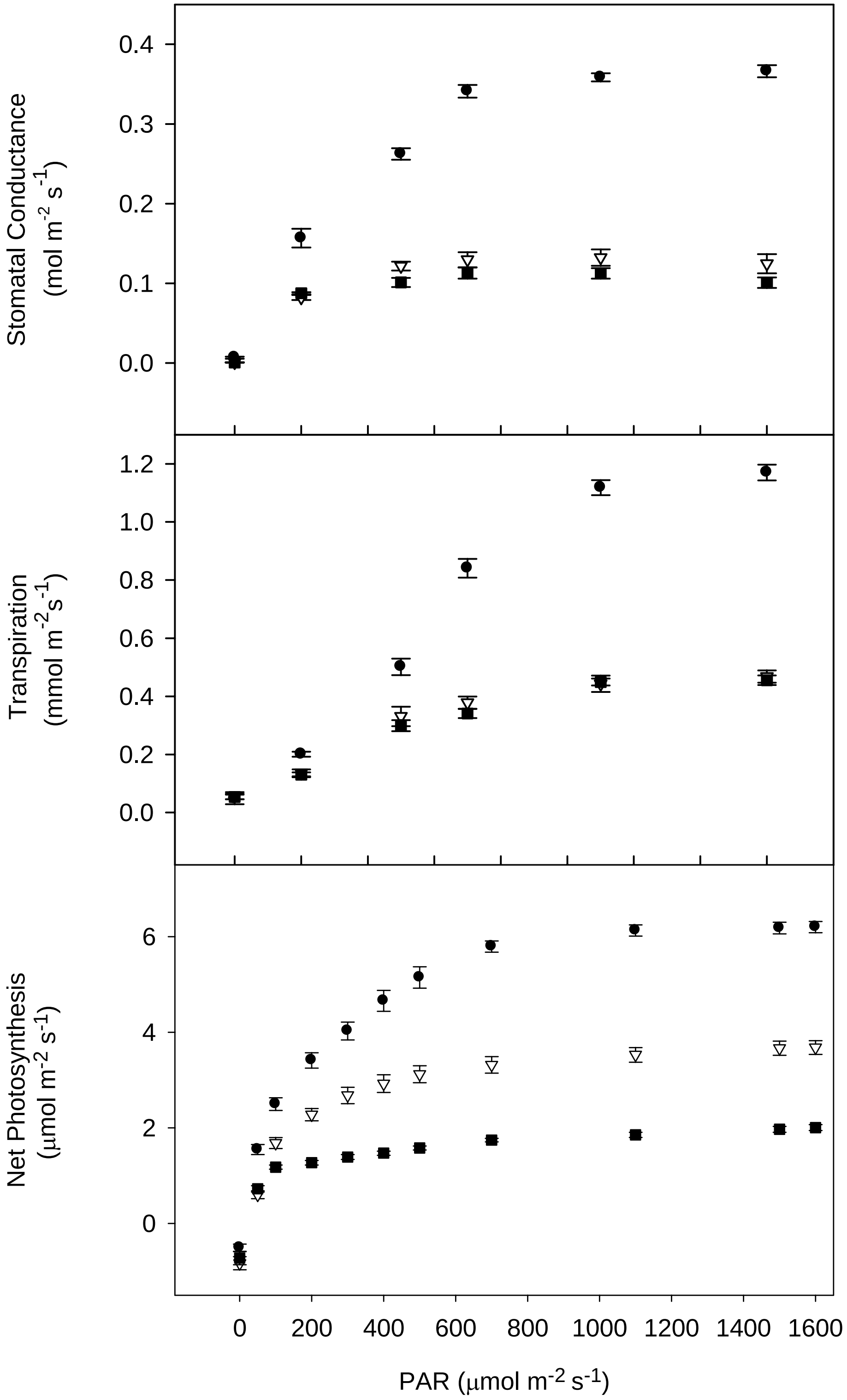
The response of transpiration to increasing VPD was contrary to that of leaf conductance and the net photosynthetic response to increasing VPD, (i.e., when stomatal conductance and net photosynthesis decreased, transpiration increased). The highest transpiration rate (0.71 mmol m^{-2} s^{-1}) was observed in the one-year-old foliage at the highest VPD level (1.5 kPa), conversely its lowest rate (0.29 mmol m^{-2} s^{-1}) was at 0.5 kPa (Figure 2). Two and three-year-old foliage also had low rates of conductance at 0.0820 and 0.0864 mmol m^{-2} s^{-1}, respectively at 0.5 kPa followed by maximum increases to 0.3680 and 0.3240 mmol m^{-2} s^{-1}, respectively at 1.5 VPD.

Light Intensity Photosynthetic active radiation influenced rates of net photosynthesis in different age classes of *Rhododendron maximum* leaves. Net photosynthetic responses to increasing irradiance followed a typical light response pattern where net photosynthesis increased with increasing light intensity until maximum rates were reached and then net photosynthesis did not continue to rise with increasing PAR. Maximum rates of net photosynthesis varied among leaf cohorts with one-year-old foliage exhibiting the highest rates and three-year old foliage demonstrating the lowest rates.

As expected, rates of photosynthesis, stomatal conductance, and transpiration decreased with increasing leaf age in response to increasing light intensity (Figure 3). One and two-year-old foliage showed similar patterns where net photosynthesis gradually increased in response to increasing irradiance, but one-year-old foliage's most rapid response occurred at low irradiance levels (0-100 μmol m^{-2} s^{-1}). By this rate of PAR, three-year-old foliage reached approximately 75% of its maximum rate of net photosynthesis. Net photosynthesis increased with increasing light intensity from zero irradiance at a very low light compensation point at 10, 29, and 23 μmol m^{-2} s^{-1} PAR in one, two, and three-year-old foliage classes (mean values of 0.06, 0.04, and 0.05 μmol m^{-2} s^{-1} respectively).

Net photosynthesis of all foliage classes continued to rise after the light compensation point until saturating. Maximum rate of net photosynthesis in one-year-old foliage was 6.68 μmol m^{-2} s^{-1} at the highest PAR level (1600 μmol m^{-2} s^{-1}), but 95% of its light saturation was attained at 700 μmol m^{-2} s^{-1} PAR where the maximum rate of

Figure 3. Light Response: Relationship of stomatal conductance, transpiration, and net photosynthesis of *Rhododendron maximum* leaves in response to increasing light intensity of one-year-old foliage (●), two-year-old foliage (▽), and three-year-old foliage (■). Error bars depict standard error (n=10 for each measurement).


Stomatal Conductance
(mol m^{-2} s^{-1})
0.4
0.3
0.2
0.1
0.0
Transpiration
(mmol m^{-2}s^{-1})
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Net Photosynthesis
(µmol m^{-2} s^{-1})
6
4
2
0
0
200
400
600
800
1000
1200
1400
1600
PAR (µmol m^{-2} s^{-1})

photosynthesis was 6.51 µmol m^{-2} s^{-1} (Figure 3). Two-year-old foliage reached a maximum rate of net photosynthesis (4.11 µmol m^{-2} s^{-1}) at 1500 µmol m^{-2} s^{-1} PAR, but reached 95% of its saturation at a maximum rate of 3.91 µmol m^{-2} s^{-1} at 500 µmol m^{-2} s^{-1} PAR. The lowest rate of net photosynthesis and the lowest light saturation point was observed in three-year-old foliage. The maximum rate of net photosynthesis of 1.92 µmol m^{-2} s^{-1} was reached at 1500 µmol m^{-2} s^{-1} PAR. But 95% of the light saturation point was reached at a very low PAR level of 100 µmol m^{-2} s^{-1} where the maximum rate of net photosynthesis was 1.71 µmol m^{-2} s^{-1}. Quantum yield of *R. maximum* foliage was determined from the slope response of net photosynthesis to PAR and was higher in three-year-old foliage than two-year-old foliage (Figure3, Table 1). Quantum yield was determined from the slope of the first three light intensity measurements (0, 10, and 50 µmol m^{-2} s^{-1} PAR), and is the percent of light used for photosynthesis.

Stomatal conductance also increased with increasing irradiance levels until maximum values were reached and the response to increased light intensity did not result in an increase of leaf conductance (Figure 3). Maximum rates of leaf stomatal conductance were measured in the youngest foliage. Older foliage showed similar patterns of low conductance. At the lowest light level (0 µmol m^{-2} s^{-1}), stomatal conductance was at or slightly above zero in all foliage classes. Conductance of one-year-old foliage gradually increased until little change occurred after 700 µmol m^{-2} s^{-1} and the maximum rate was 0.48 mol m^{-2} s^{-1}. In older foliage, stomatal conductance gradually increased until reaching 500 µmol m^{-2} s^{-1}, and maximum rates of 0.20 mol m^{-2} s^{-1} in two-year-old foliage and 0.15 mol m^{-2} s^{-1} in three-year-old foliage at the highest light level (1600 µmol m^{-2} s^{-1} PAR).

Table 1. Significance of the relationship of slopes in physiological responses of *R. maximum* among three foliage age classes to environmental factors. (PAR = photosynthetic active radiation; SC = stomatal conductance; Pn = net photosynthesis; E = transpiration; Ci = intercellur CO_2 concentration; LWP = leaf water potential)

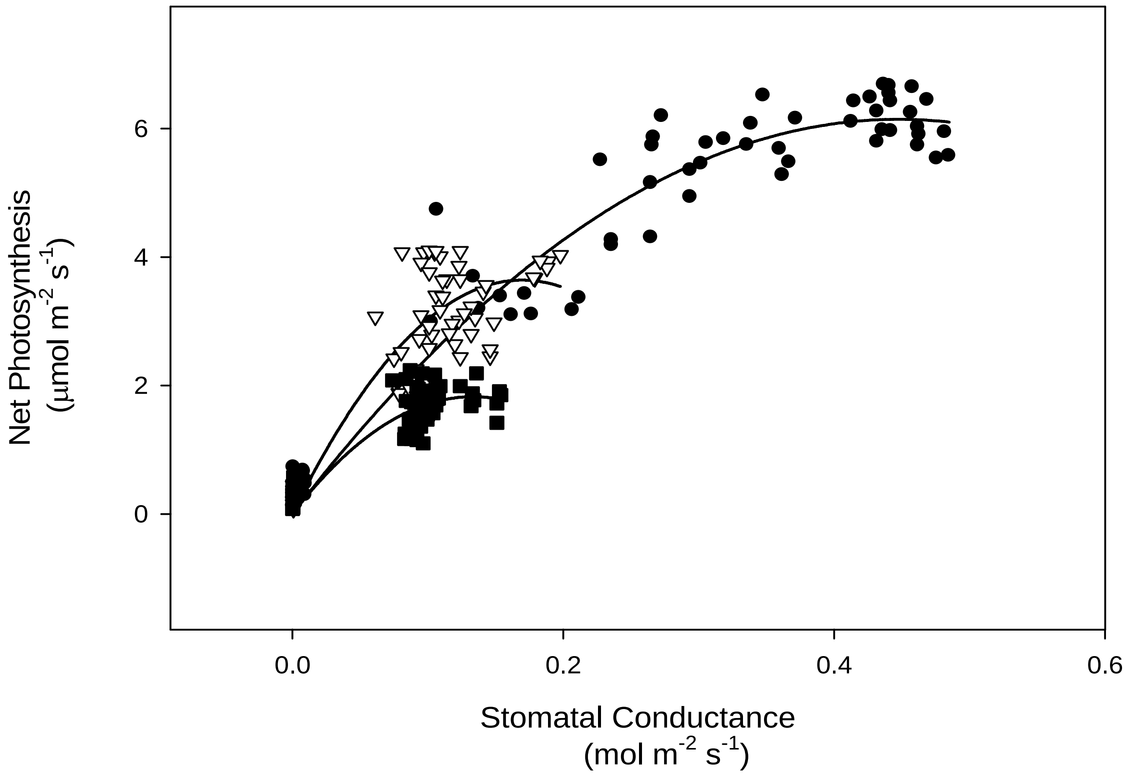
Figure	Foliage class	R^2	Regression equation	F	P
3	one-year-old	0.9331	$y = -0.5105 + 0.0411PAR$	251.1558	< 0.0001
	two-year-old	0.8185	$y = -0.8321 + 0.0286PAR$	81.1987	< 0.0001
	three-year-old	0.8336	$y = -0.7262 + 0.0290PAR$	90.1485	< 0.0001
4					
	one-year-old	0.9355	$y = 0.3779 + 24.7989SC + -26.5627(SC/Pn)^2$	413.0394	< 0.0001
	two-year-old	0.7880	$y = 0.2760 + 38.5204SC + -110.1930(SC/Pn)^2$	105.9495	< 0.0001
	three-year-old	0.7984	$y = 0.2930 + 22.0932SC + -79.8128(SC/Pn)^2$	112.8352	< 0.0001
5					
	one-year-old	0.8946	$y = -0.0251 + 2.4988SC$	492.5455	< 0.0001
	two-year-old	0.4798	$y = 0.0654 + 2.3268SC$	53.4862	< 0.0001
	three-year-old	0.5639	$y = 0.0747 + 2.1987SC$	75.0062	< 0.0001
8					
	one-year-old	0.9091	$y = -0.5290 + 0.0167Ci$	629.8799	< 0.0001
	two-year-old	0.8913	$y = -0.2286 + 0.0114Ci$	393.6539	< 0.0001
	three-year-old	0.7842	$y = 0.1277 + 0.0059Ci$	156.2709	< 0.0001
9					
	midday				
	one-year-old	0.8774	$y = 6.9628 + -2.5946LWP + -0.7409(LWP/Pn)^2$	221.8649	0.0245
	two-year-old	0.7796	$y = 3.4738 + -0.3974LWP + -1.1521(LWP/Pn)^2$	74.2698	0.7406
	three-year-old	0.7340	$y = 2.1340 + -0.5869LWP + -0.1869(LWP/Pn)^2$	51.0415	0.4076
	predawn				
	one-year-old	0.9312	$y = 6.4651 + -34.6768LWP + 48.7144(LWP/Pn)^2$	419.3958	< 0.0001
	two-year-old	0.8687	$y = 3.8371 + -11.9676LWP + 8.5661(LWP/Pn)^2$	138.9982	< 0.0001
	three-year-old	0.7614	$y = 1.9877 + -6.3069LWP + 4.9425(LWP/Pn)^2$	59.0288	< 0.0001

Figure 3: $y = Pn$; **Figure 4**: $y = Pn$; **Figure 5**: $y = E$; **Figure 8**: $y = Pn$; **Figure 9**: $y = Pn$

Mean transpiration rates of all foliage classes were below 0.10 mmol m^{-2} s^{-1} at 0 µmol m^{-2} s^{-1} with one-year-old foliage showing the highest rate (0.09 mmol m^{-2} s^{-1}) and three-year-old foliage showing the lowest rate (0.07 mmol m^{-2} s^{-1}) (Figure 3). Transpiration rates continued to increase with increasing PAR until reaching 1000 µmol m^{-2} s^{-1} irradiance level in all foliage classes. Younger foliage reached maximum rates of transpiration at 1.26 mmol m^{-1} s^{-1} at the highest PAR level (Figure 3). Older foliage reached maximum rates of transpiration (0.52 mmol m^{-1} s^{-1} and 0.50 mmol m^{-1} s^{-1}) in two and three-year-old foliage, respectively, as it slowly increased with PAR until maximum rates were observed at 1100 µmol m^{-2} s^{-1}.

When net photosynthesis was plotted against stomatal conductance, a strong curvi-linear positive relationship was observed in all foliage classes (Figure 4, Table 1). This suggests that leaf stomatal conductance increased with increasing rates of net photosynthesis in *Rhododendron maximum* leaves until maximum rates of photosynthesis were reached. At this point, only stomatal conductance continued to increase in each foliage class. At low rates of net photosynthesis (< 1 µmol m^{-2} s^{-1}), stomatal conductance remained at or slightly above zero in all foliage classes. Once the response of net photosynthesis exceeded 1 µmol m^{-2} s^{-1}, variation among age classes in the response of stomatal conductance was observed. One-year-old foliage demonstrated the widest range of conductance, changing from 0.10 to 0.48 mol m^{-2} s^{-1} at net photosynthesis rates of 2.98 to 6.68 µmol m^{-2} s^{-1}. Rates of net photosynthesis and conductance in two-year-old foliage increased from minimums of 1.83 µmol m^{-2} s^{-1} and 0.61 mol m^{-2} s^{-1}to maximums at 4.10 µmol m^{-2} s^{-1} and 0.20 mol m^{-2} s^{-1} (Figure 4). Conductance of three-year-old foliage ranged

Figure 4. Stomatal Conductance: Relationship of net photosynthesis of *Rhododendron maximum* leaves to leaf stomatal conductance of one-year-old foliage (●), two-year-old foliage (▽), and three-year-old foliage (■). Each point represents an individual measurement.


Net Photosynthesis
(μmol m^{-2} s^{-1})
6
4
2
0
0.0
0.2
0.4
0.6
Stomatal Conductance
(mol m^{-2} s^{-1})

When transpiration was plotted against leaf stomatal conductance, each foliage class showed a linear response, such that when transpiration increased stomatal conductance did also (Figure 5, Table 1). Even when stomata of *R. maximum* leaves had very low conductance, transpiration occurred in all foliage classes (probably due to the LI-COR 6400 not accurately measuring stomatal conductance at very low rates) (Figure 5).

When conductance was slightly positive (barely > 0 mol m^{-2} s^{-1}), the transpiration rate of one-year-old foliage was 0.18 mmol m^{-2} s^{-1}, 0.08 mmol m^{-2} s^{-1} in two-year-old foliage, and 0.07 mmol m^{-2} s^{-1}in three-year-old foliage. Maximum rates of transpiration were reached at 1.32 mmol m^{-2} s^{-1} in one-year-old leaves when conductance was 0.43 mol m^{-2} s^{-1}. In two-year-old foliage, 0.52 mmol m^{-2} s^{-1} was the maximum transpiration rate at a conductance of 0.18 mol m^{-2} s^{-1}. Three-year-old foliage had the highest transpiration rate of 0.50 mmol m^{-2} s^{-1} at a conductance of 0.11 mol m^{-2} s^{-1}. Maximum rates of stomatal conductance were 0.49 mol m^{-2} s^{-1}in one-year-old foliage, 0.20 mol m^{-2} s^{-1}in two-year-old foliage, and 0.15 mol m^{-2} s^{-1}in three-year-old foliage.

Induced Water Stress

As with temperature responses, during the time of induced water stress *Rhododendron maximum* leaves curled to prevent water loss. However, leaves that were not being measured showed other signs of desiccation including browning and wilting before induced water stress measurements were complete. They also changed leaf orientation/angle in response to drought and decreasing water potential rates by drooping. There were considerable differences in the relationship between water potential and net photosynthesis among age classes (Figure 6), with a more rapid decrease in one-year-old

Figure 5. Stomatal Conductance: Relationship of transpiration of *Rhododendron maximum* leaves to leaf stomatal conductance of one-year-old foliage (●), two-year-old foliage (▽), and three-year-old foliage (■). Each point represents an individual measurement.

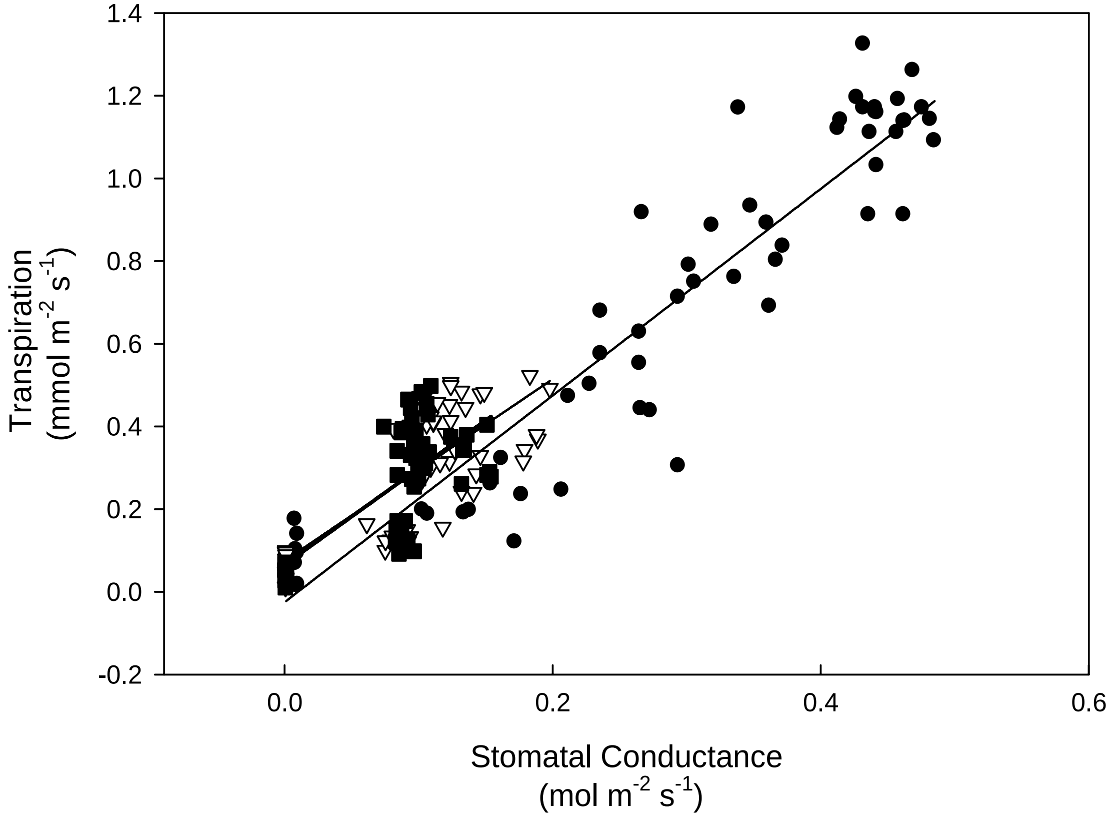
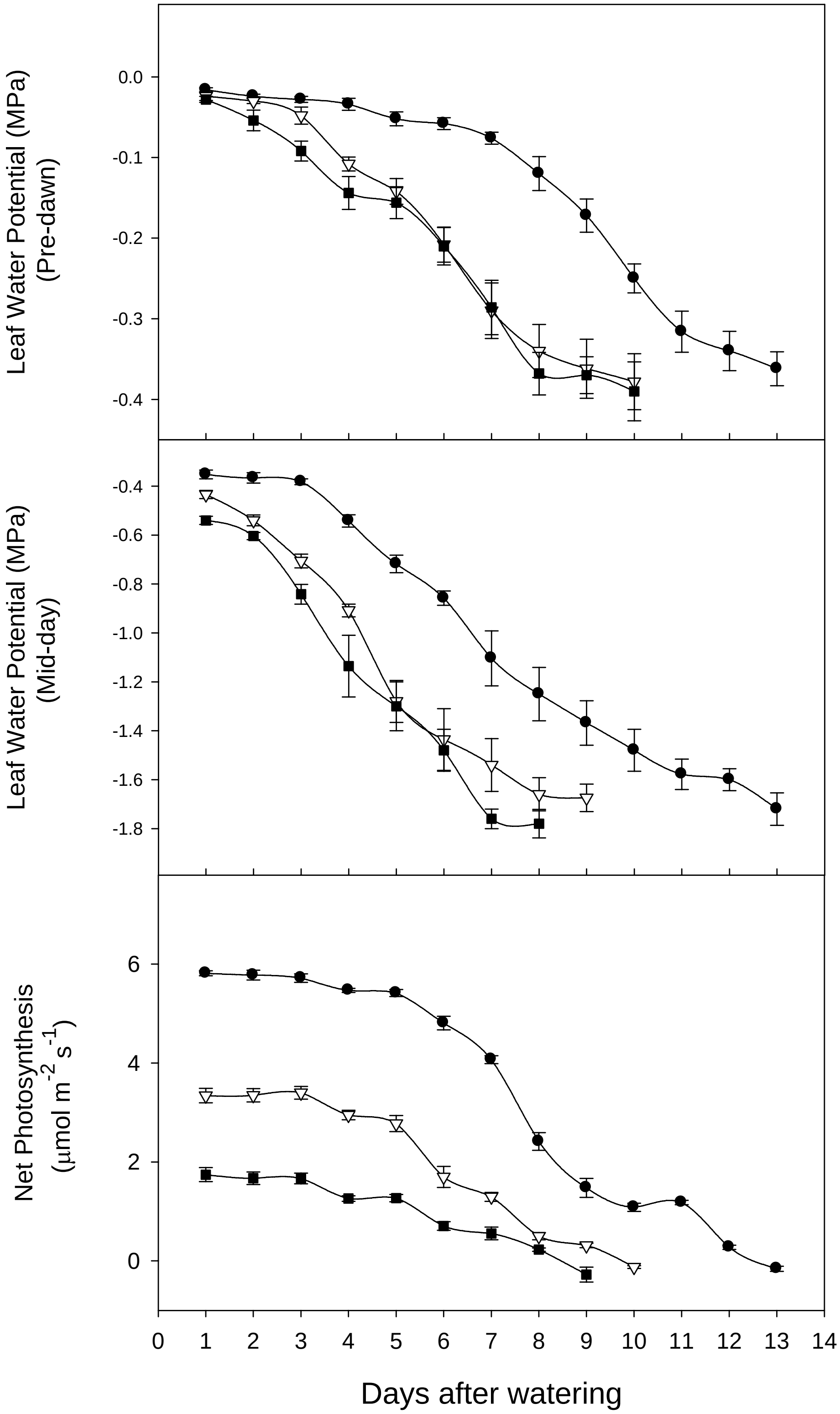

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
Transpiration
(mmol m-2 s-1)
0.0
0.2
0.4
0.6
Stomatal Conductance
(mol m-2 s-1)

Figure 6. Induced Water Stress: Relationship of predawn and midday leaf water potential and net photosynthesis of *Rhododendron maximum* leaves in response to induced water stress of one-year-old foliage (●), two-year-old foliage (▽), and three-year-old foliage (■). Error bars depict standard error (n=5 for net photosynthesis, n=2 for leaf water potential).


Leaf Water Potential (MPa)
(Pre-dawn)
0.0
-0.1
-0.2
-0.3
-0.4
Leaf Water Potential (MPa)
(Mid-day)
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
Net Photosynthesis
(µmol m-2 s-1)
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days after watering

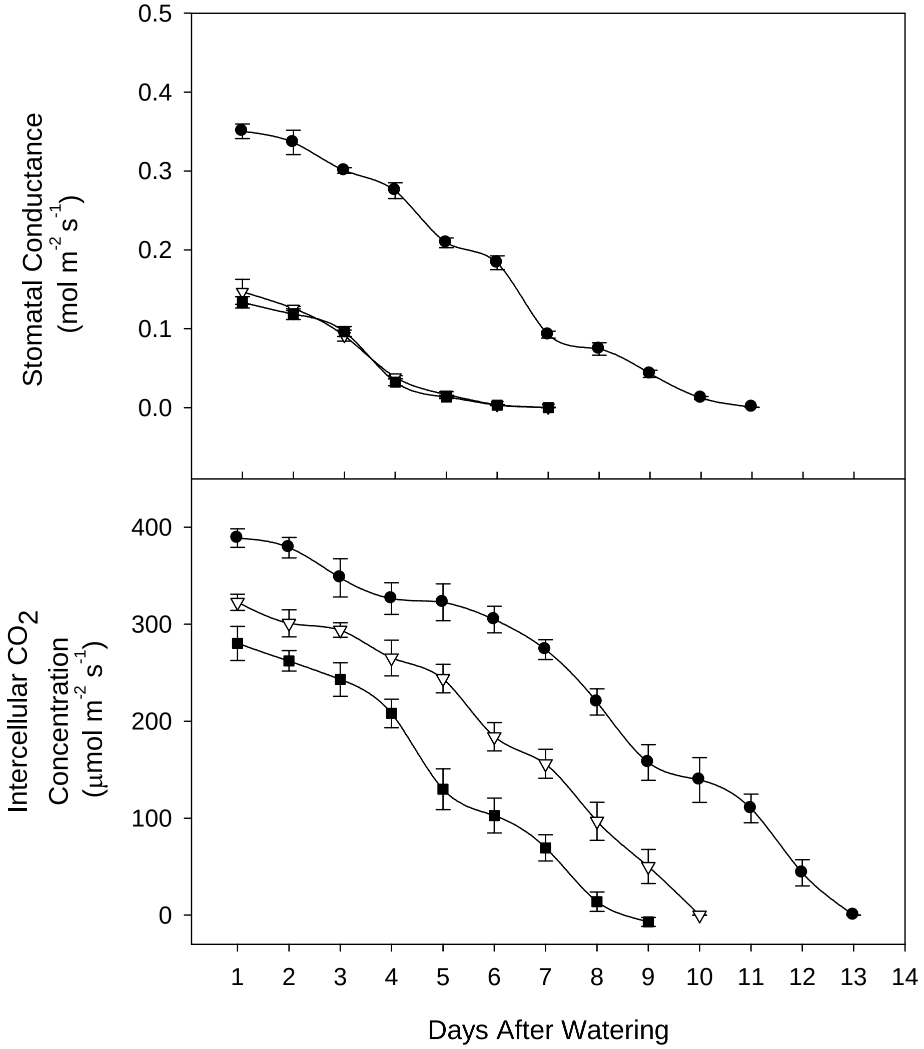
response to drought and decreasing water potential rates by drooping. There were considerable differences in the relationship between water potential and net photosynthesis among age classes (Figure 6), with a more rapid decrease in one-year-old foliage rates of net photosynthesis after five days. Two-year-old foliage was steady over the first three days and then rapidly declined with continued water stress. Three-year-old foliage gradually decreased throughout the dry-down period. Rapid decreases in midday leaf water potential occurred in all foliage classes while predawn water potential gradually decreased in one-year-old foliage. Two-year-old foliage gradually decreased the first three days followed by rapid decreases thereafter and three-year-old foliage constantly decreased at rapid rates (Figure 6).

Mean predawn leaf water potential was very close in all foliage classes on the first day of measurements ranging from -0.01 to -0.03 MPa in one to three-year-old leaves. When net photosynthesis reached zero, mean predawn leaf water potential reached minimums of -0.40 MPa in one-year old foliage after 13 days and after 10 days (-0.40 MPa) in two and three-year-old foliage. Midday water potentials declined more rapidly than predawn potentials over the course of the dry-down period (Figure 6), and midday water potential was significant in one-year-old foliage (Table 1). On the first day after watering, mean midday water potentials were at maximums of -0.30 MPa in one-year-old foliage, -0.40 MPa in two-year-old foliage, and -0.50 MPa in three-year-old foliage. Minimum mean midday values were observed at -1.80 MPa in one-year-old leaves, -1.80 MPa in two-year-old leaves, and -1.90 in three-year-old leaves.

As water stress continued stomatal conductance and Ci decreased (Figure 7). Along with decreasing rates of net photosynthesis, Ci also reached zero after thirteen days in one-year-old foliage, after ten days in two-year-old foliage, and after nine days in three-year-old foliage. When under constant induced drought conditions and as rates of net photosynthesis declined, leaves of *R. maximum* had a steady reduction in leaf stomatal conductance in younger leaves and rapid decreases in older leaves. Complete leaf stomatal closure occurred after eleven days in younger leaves and after seven days in older foliage. The reduction of net photosynthesis was at least partially due to water stress-induced stomatal closure because there was a simultaneous decrease in Ci.

Maximum rates of net photosynthesis were 6.18 μmol m^{-2} s^{-1} in on-year-old leaves, 3.95 μmol m^{-2} s^{-1} in two-year-old leaves, and 2.29 μmol m^{-2} s^{-1} in three-year-old on the first day after watering and decreased throughout the dry-down period until no net photosynthesis was observed (Figure 7). One-year-old foliage had maximum leaf conductance on the first day of measurements at 0.38 mol m^{-2} s^{-1} in one-year-old leaves, 0.19 mol m^{-2} s^{-1} in two-year-old leaves, and 0.14 mol m^{-2} s^{-1} in three-year-old leaves. Intercellular CO_2 concentrations gradually decreased as leaf stomatal conductance and net photosynthesis decreased with increasing water stress. Maximum rates of Ci were also observed on the first day of measurements at 411 μmol m^{-2} s^{-1} in one-year-old foliage, 340 μmol m^{-2} s^{-1} in two-year-old foliage, and 321 μmol m^{-2} s^{-1} in three-year-old foliage. Even when stomatal conductance reached zero, there were still positive but minimum photosynthetic rates and Ci observed in all age classes, which may be a result of the LI-COR 6400 not accurately measuring conductance when it is very low.

Figure 7. Induced Water Stress: Relationship of stomatal conductance and intercellular CO_2 of *Rhododendron maximum* leaves in response to induced water stress of one-year-old-foliage (●), two-year-old foliage (▽), and three-year-old foliage (■). Error bars depict standard error (n=5 for each measurement).


Stomatal Conductance
(mol m^{-2} s^{-1})
0.5
0.4
0.3
0.2
0.1
0.0
Intercellular CO$_2$
Concentration
(μmol m^{-2} s^{-1})
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Days After Watering

To examine further how the drought regime influenced gas exchange, net photosynthesis was plotted against Ci (Figure 8). In all leaf age classes, maximum rates of Ci were observed when maximum rates of net photosynthesis occurred and there was a linear decrease in the relationship with continuous drought. Maximum Ci decreased with increasing leaf age. Maximum rates of net photosynthesis were observed when Ci levels ranged between 411-268 μmol m^{-2} s^{-1} in one-year-old leaves, 340-226 μmol m^{-2} s^{-1}in two-year-old leaves, and 321-116 μmol m^{-2} s^{-1}. The rate at which net photosynthesis declined with decreasing Ci was not only significant but also significantly different in all foliage classes (Figure 8, Table 1).

When net photosynthesis was plotted against predawn and midday leaf water potential, net photosynthesis declined as leaf water potential decreased during continuous drought regimes (Figure 9, Table 1). Rates of net photosynthesis of *R. maximum* leaves declined rapidly in response to predawn leaf water potentials in younger foliage, while older foliage gradually declined with three-year-old foliage showing a linear decrease. Predawn leaf water potential declined at faster rates than midday water potential. When rates of net photosynthesis reached 0 μmol m^{-2} s^{-1}, minimum predawn water potential was -0.40 MPa in one-year-old leaves, -0.47 MPa in two-year-old leaves, and -0.45 MPa in three-year-old leaves.

Midday leaf water potential of younger leaves decreased faster than older leaves of *R. maximum*. One and two-year old leaves had a curvilinear relationship and three-year-old leaves had a linear relationship. The regression equation for the relationship between net photosynthesis and midday water potential was significant for the youngest

Figure 8. Induced Water Stress: Relationship of intercellular CO_2 and net photosynthesis of *Rhododendron maximum* leaves in response to induced water stress of one-year-old foliage (●), two-year-old foliage (▽), and three-year-old foliage (■). Each point represents an individual measurement.

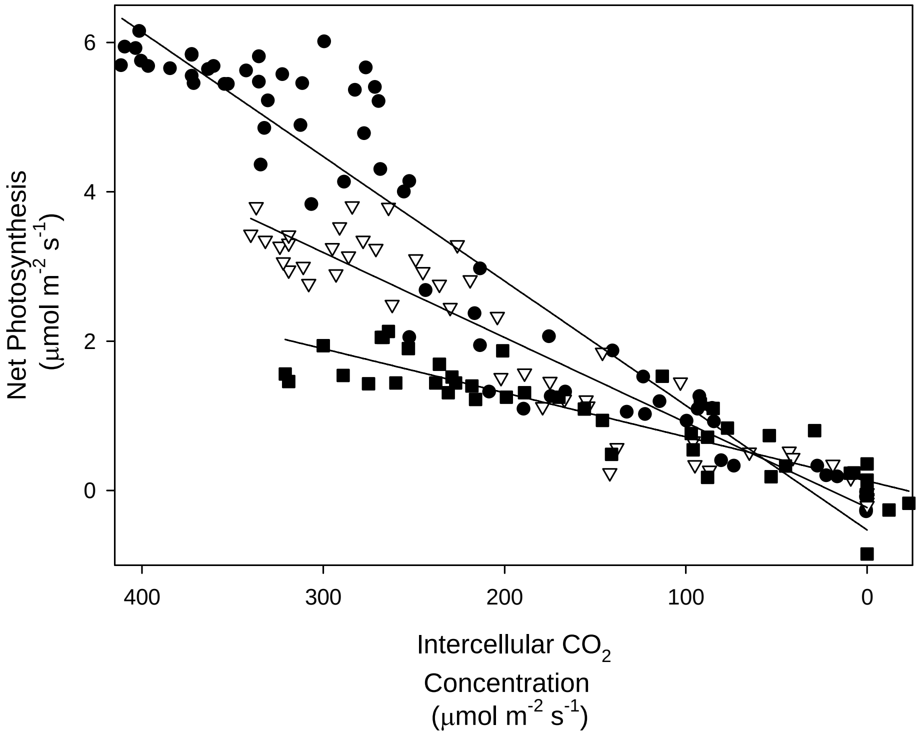
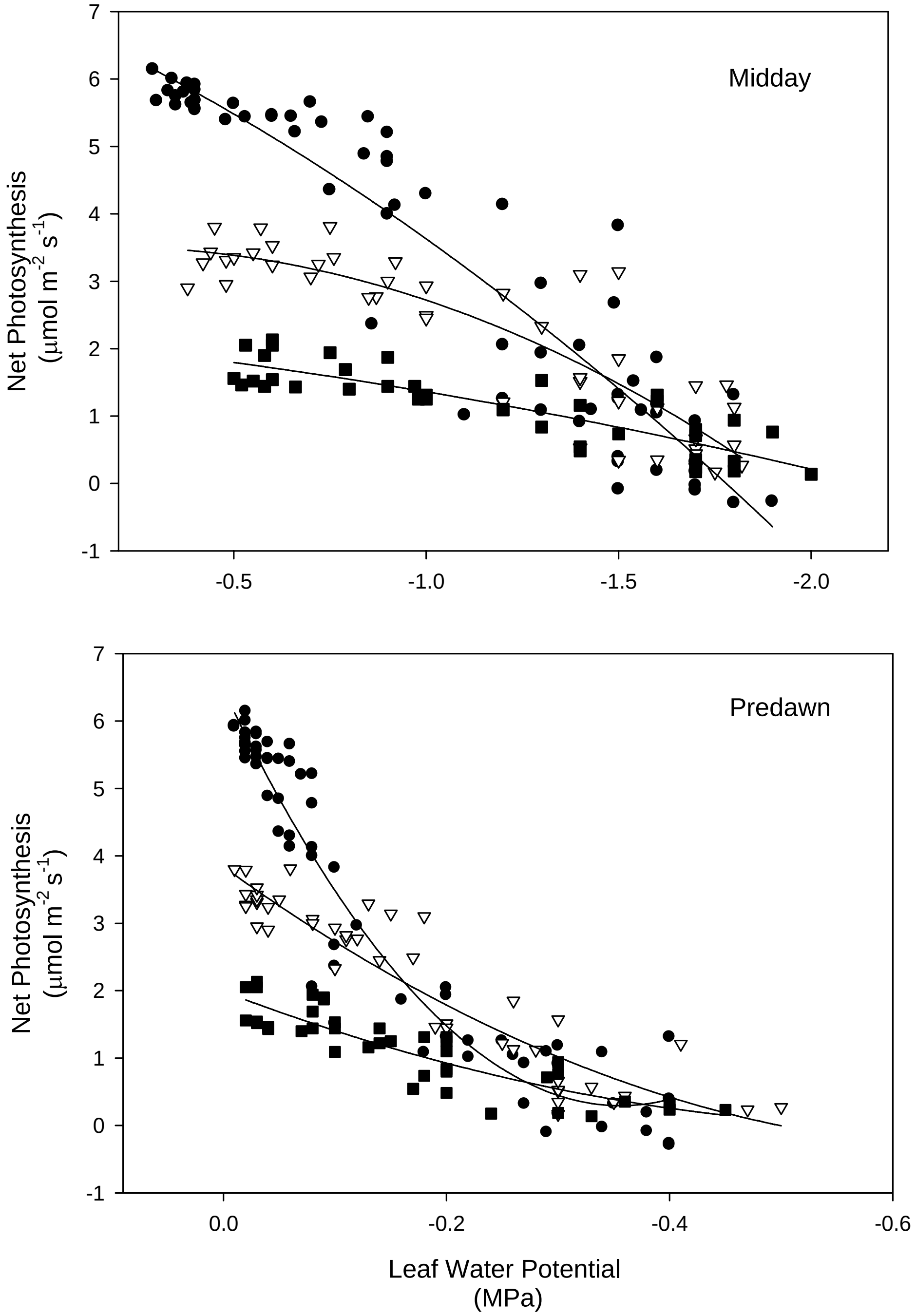

Net Photosynthesis
(µmol m-2 s-1)
6
4
2
0
400
300
200
100
0
Intercellular CO2
Concentration
(µmol m-2 s-1)

Figure 9. Leaf Water Potential: Relationship of net photosynthesis of *Rhododendron maximum* leaves in response to midday and predawn leaf water potential of one-year-old foliage (●), two- year-old foliage (▽), and three-year-old foliage (■). Each point represents an individual measurement.


Midday
Net Photosynthesis
(μmol m-2 s-1)
7
6
5
4
3
2
1
0
-1
-0.5
-1.0
-1.5
-2.0
Predawn
Net Photosynthesis
(μmol m-2 s-1)
7
6
5
4
3
2
1
0
-1
0.0
-0.2
-0.4
-0.6
Leaf Water Potential
(MPa)

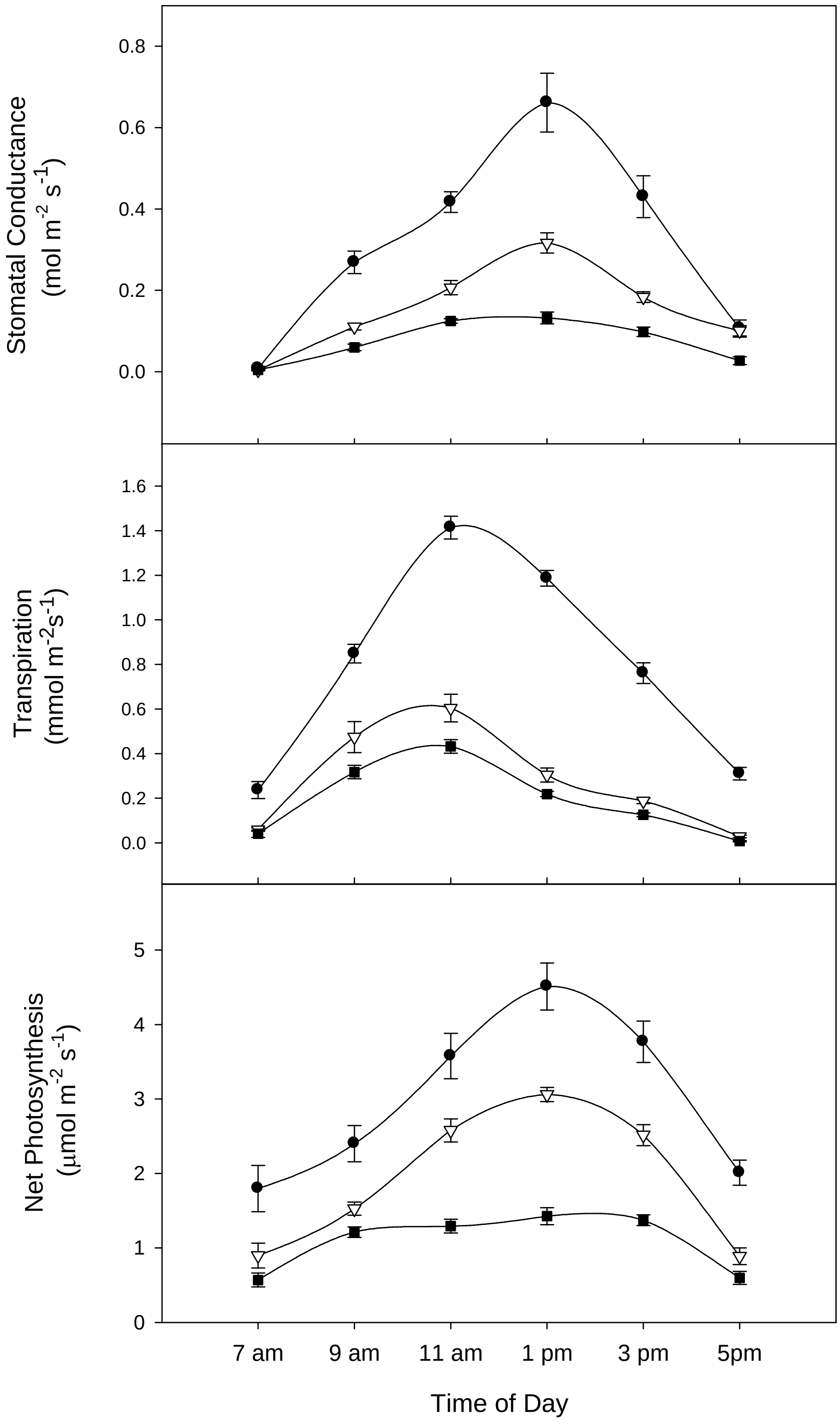
foliage, but not for the other two age classes (Figure 9, Table 1). Rates of net photosynthesis were highest when midday water potential were highest and gradually decreased as photosynthetic activity and the stomata became impaired by water stress. When rates of net photosynthesis reached 0 μmol m^{-2} s^{-1}, minimum midday water potential was about the same in the three age classes; -1.90 MPa in one-year-old foliage, -1.82 MPa in two-year-old foliage, and -2 MPa in three-year-old foliage.

Diurnal Measurements

Diurnal measurements were taken outside under shaded, uncontrolled field conditions and net photosynthesis increased throughout the morning, until it reached a peak during midday, followed by a continuous decline during the afternoon in all age classes (Figure 10). Maximum early morning incident light was 90 μmol m^{-2} s^{-1}, and it reached a maximum of about 435 μmol m^{-2} s^{-1} (due to sunflecks) during midday and gradually decreased throughout the rest of the day. Early morning (7 am, 39-90 μmol m^{-2} s^{-1} PAR) measurements of net photosynthesis showed one-year-old leaves reached rates of 2.60 μmol m^{-2} s^{-1}, 1.26 μmol m^{-2} s^{-1} in three-year-old leaves, and 0.82 μmol m^{-2} s^{-1} in three-year-old foliage.

Maximum rates of net photosynthesis were reached during midday (1 pm, 318-436 μmol m^{-2} s^{-1} PAR) in all foliage classes; 5.44 μmol m^{-2} s^{-1} in one-year-old foliage, 3.24 μmol m^{-2} s^{-1} in two-year-old foliage, and 1.77 μmol m^{-2} s^{-1} in three-year-old foliage. After reaching maximum values of net photosynthesis during midday, rates began to decline throughout the late afternoon. During the last set of diurnal measurements (5 pm, 20-71 μmol m^{-2} s^{-1}PAR), rates of net photosynthesis dropped to 2.56 μmol m^{-2} s^{-1} in one-

Figure 10. Diurnal Patterns: Diurnal response of stomatal conductance, transpiration, and net photosynthesis of *Rhododendron maximum* leaves in one-year-old foliage (●), two–year-old foliage (▽), and three-year-old foliage (■). Error bars depict standard error (n=5 for each measurement).


Stomatal Conductance
(mol m^{-2} s^{-1})
0.8
0.6
0.4
0.2
0.0
Transpiration
(mmol m^{-2}s^{-1})
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Net Photosynthesis
(µmol m^{-2} s^{-1})
5
4
3
2
1
0
7 am
9 am
11 am
1 pm
3 pm
5pm
Time of Day

year-old foliage, 1.09 μmol m^{-2} s^{-1} in two-year-old foliage, and 0.79 μmol m^{-2} s^{-1} in three-year-old foliage (Figure 10).

Midday maximum net photosynthesis, stomatal conductance, and transpiration during diurnal measurements can be compared to chamber measurements where maximum PAR levels during this time were less than saturation points from response curves (Figure 10 and Figure 3). Diurnal measurements confirmed that even when conductance or transpiration were low, there was some CO_2 assimilation. Maximum midday rates of net photosynthesis were 5.44 μmol m^{-2} s^{-1} in one-year-old foliage, 3.24 μmol m^{-2} s^{-1} in two-year-old foliage, and 1.77 μmol m^{-2} s^{-1} in three-year-old foliage, and were very similar to rates measured at 400 μmol m^{-2} s^{-1} PAR during response curve measurements in the laboratory.

Rates of net photosynthesis in the response curves were 5.95 μmol m^{-2} s^{-1} in one-year-old foliage, 3.78 μmol m^{-2} s^{-1} in two-year-old foliage, and 1.71 μmol m^{-2} s^{-1} in three-year-old foliage.Rates of net photosynthesis during both controlled (growth chamber) and uncontrolled (diurnal field) measurements were similar, but more variation was observed in diurnal measurements, probably because of sunflecks (Figure 10 and Figure 3). Maximum rates of net photosynthesis in three-year-old foliage at midday were very similar to that observed in controlled conditions near 95% of light saturation (1.92 μmol m^{-2} s^{-1}), suggesting that three-year-old foliage required very low light intensities for photosynthesis.

Minimum values for stomatal conductance of *R. maximum* did not vary between leaf age classes and ranged from 0.02-0.03 mol m^{-2} s^{-1} at 7 am. During midday measurements large differences in rates of stomatal conductance were observed at 0.79 mol m^{-2} s^{-1} in one-year-old foliage, 0.38 mol m^{-2} s^{-1} in two-year-old foliage, and 0.20 mol m^{-2} s^{-1} in three-year-old foliage compared to growth chamber measurements (Figure 10). Maximum rates of conductance observed during late afternoon hours (5 pm) were similar in one and two-year old foliage (0.1634 and 0.1482 mol m^{-2} s^{-1}, respectively) and were at 0.06 mol m^{-2} s^{-1} in three-year-old foliage. Maximum rates of stomatal conductance were observed at 400 µmol m^{-2} s^{-1} PAR during controlled measurements and were 0.25 mol m^{-2} s^{-1} in one-year-old foliage, 0.19 mol m^{-2} s^{-1} in two-year-old foliage, and 0.16 mol m^{-2} s^{-1} in three-year-old foliage. The difference between control and uncontrolled stomatal conductance may have been because of environmental conditions promoting stomatal activity. (Figure 10 and Figure 3)

The diurnal pattern of transpiration in younger leaves of *R. maximum* followed a bell shaped response while older foliage followed a comparable pattern to one another (Figure 10). During the first set of diurnal measurements (7 am), *R. maximum* leaves were transpiring even when leaf stomatal conductance was slightly above 0 mol m^{-2} s^{-1}. At 7 am, mean transpiration rates of one-year-old leaves were at 0.24 mol m^{-2} s^{-1} and at 0.0875 mol m^{-2} s^{-1} and 0.0810 mol m^{-2} s^{-1} in two and three-year old foliage, respectively. Maximum rates of transpiration were reached at0.92 mmol m^{-2} s^{-1} in one-year-old foliage, 0.64 mmol m^{-2} s^{-1} in two-year-old foliage, and 0.42 mmol m^{-2} s^{-1} in three-year-old foliage at 11 am.

During evening measurements (5pm), transpiration rates declined to mean values of 0.3131 mmol m^{-2} s^{-1} in one-year-old foliage, 0.0380 mmol m^{-2} s^{-1} in two-year-old foliage, and 0.02 mmol m^{-2} s^{-1} in three-year-old foliage (Figure 10). Maximum midday transpiration rates can be compared to controlled measurements at 300 µmol m^{-2} s^{-1} PAR where one-year-old foliage reached 0.34 mmol m^{-2} s^{-1}, 0.20 mmol m^{-2} s^{-1} in two-year-old foliage, and 0.19 mmol m^{-2} s^{-1} in three-year-old foliage (Figure 10 and Figure 3). There was a large difference in maximum rates of transpiration between control and uncontrolled measurements, which may result from different environmental conditions (VPD, air temperature) enhancing stomatal activity and therefore promoting transpiration.

CHAPTER 5: DISCUSSION AND CONCLUSIONS

Discussion

In this study net photosynthesis, leaf stomatal conductance, and transpiration were measured among three leaf cohorts in response to variations in temperature, VPD, light intensity, water availability, and over a diurnal period. *Rhododendron maximum* leaves appear to have an optimum temperature range for gas exchange between 15-25 °C because maximum rates of net photosynthesis, stomatal conductance, and transpiration were observed in this temperature range during this study. Similar understory species have shown comparable responses. Maximum rates of net photosynthesis were observed at mid-range temperatures and low rates of net photosynthesis were observed at both low (< 10°C) and high (> 35°C) temperatures in *Aralia nudicaulis* L. and *Pyrola asarifolia* ssp. asarifolia in a boreal mixed forest (Landhausser et al., 1997) and in Mediterranean shrubs *Erica multiflora* L. and *Globularia alypum* L. (Llorens et al., 2003).

Nilsen (1992) stated that *R. maximum* leaves are not photosynthetic during winter, but the temperature response of *R. maximum* in the current study indicated that some gas exchange could occur in similar conditions because we observed positive photosynthetic activity at 5°C. In the Nilsen (1985) study there was no photosynthetic activity and stomata were closed at similar temperatures. Responses in the present study may have been observed because the plants were measured under chamber conditions that were not held constant over a long period of time.

Temperature responses of gas exchange in *R. maximum* followed the same pattern as gas exchange of dense growing shrubs *Persea indica* (Gonzalez-Rodriguez et al.,

2002) *Erica arborea* L., *Erica multiflora*, and *Rosmarinus officinalis* L. growing in the Mediterranean (Gratani and Varone, 2004). These species exhibited minimum net photosynthetic and conductance responses in high (35 °C) and low (5 °C) temperatures, and maximum values were observed in the mid-temperature ranges (15 and 25 °C).

Rates of net photosynthesis and leaf conductance of ~4 µmol m^{-2} s^{-1} and ~100 mmol m^{-2} s^{-1}, respectively, were observed above 35 °C in *Persea indica*, but in contrast, *R. maximum* net photosynthesis fell below 2 µmol m^{-2} s^{-1} in younger foliage and below 1 µmol m^{-2} s^{-1} in older foliage at 35 °C. No photosynthetic activity was reported in *Persea indica* leaves under 10 °C, but one-year-old foliage of *R. maximum* leaves still showed positive values (~1 µmol m^{-2} s^{-1}) at 5°C. Stomatal conductance response to temperature appears to have the same bell shaped response as net photosynthesis in *R. maximum* and other evergreen species (Leakey et al., 2003 and Gonzalez-Rodriguez et al., 2002). Like net photosynthesis, stomatal conductance was observed at 5°C during the current study but this may have been because plants were not measured and growing in the natural environment. Nilsen (1985) reported the stomata of *R. maximum* leaves were closed at similar temperatures during winter months. Transpiration has not been previously reported in *R. maximum*, but in the current study it also had a bell-shaped response to increasing temperatures.

Rates of net photosynthesis in *R. maximum* leaves decreased with increasing VPD in several understory species studied by Cao (2000). The decrease in net photosynthesis was not drastic which may have been because the VPD range was not wide but did result in noticeable changes in other gas exchange measurements. Intercellular CO_2

concentrations increased with increasing VPD, suggesting that stomatal limitations affected the response of net photosynthesis.

Stomatal conductance of *R. maximum* followed the same pattern of the evergreen *Persea indica* studied by Gonzalez-Rodriguez et al. (2002) and understory species by Motzer et al. (2005), where conductance decreased with increasing VPD. The low relative humidity and high temperatures that were required to maintain high VPD may have both enhanced stomatal closure. Previous studies on *R. maximum* have reported little about the response of stomatal conductance to VPD. Transpiration rates in *R. maximum* of the current study increased when VPD increased even when stomatal conductance decreased.

Rates of transpiration in response to VPD of understory species have rarely been reported, but effects that diurnal patterns of VPD have on transpiration rates has been observed. Diurnally, transpiration rates of *Persea indica* followed the same pattern of net photosynthesis in sun and shade leaves, but decreasing stomatal conductance did not result in decreasing transpiration rates in sun leaves (Gonzalez-Rodriguez et al., 2002). An explanation for the increase of transpiration with decreased stomatal conductance in *R. maximum* in this controlled environment study and in this similar evergreen understory species is that an abundance of soil water prevented severe water stress so stomata did not drastically close to avoid water loss with increasing VPD levels (Munne-Bosch et al., 1999).

Gas exchange of *R. maximum* increased with increasing light intensity until reaching the saturation point as PAR continued to rise. Maximum rates of net photosynthesis observed in one and three-year-old foliage (5.74 and 1.73 µmol m^{-2} s^{-1}, respectively) of *R. maximum* leaves were comparable to those measured at 700 µmol m^{-2} s^{-1} PAR in *R. maximum* leaves growing under a deciduous canopy in the Nilsen et al. (1988) study (~5.8 µmol m^{-2} s^{-1} in14-month-old leaves and ~1.6 µmol m^{-2} s^{-1} in 38-month-old leaves). Rates of net photosynthesis in two-year-old foliage (4.11 µmol m^{-2} s^{-1}) of the current study were slightly higher than rates observed by Nilsen et al. (1988) (~3 µmol m^{-2} s^{-1} in 26-moth-old leaves). Maximum rates of net photosynthesis and light saturation points decreased with increasing leaf age in this study.

Similar responses were observed in *R. maximum* by Nilsen et al. (1988), and in understory species studied by Holmes and Cowling (1993). Three-year-old foliage required the lowest light levels to achieve maximum rates of net photosynthesis, suggesting that increased levels of light intensity due to hemlock decline might cause damage to the photosystems of older foliage. *Rhododendron maximum* shrubs appear to perform best photosynthetically r under canopies that receive moderate light levels (Nilsen et al., 1988). The light response of *R. maximum* is comparable to that of the shade tolerant subcanopy trees *Cornus florida* L.and *Acer pensylvanicum* L. studied by Sullivan et al. (1996) in the Coweeta basin. The maximum rate of net photosynthesis in low light ($\leq$ 500 µmol m^{-2} s^{-1} PAR) was 2.66 µmol m^{-2} s^{-1} in *A. pensylvanicum* and 3.72 µmol m^{-2} s^{-1} in *C. florida*. In high light t ($\geq$ 1000 µmol m^{-2} s^{-1} PAR), *C. florida* had a maximum rate of net photosynthesis of 4.24 µmol m^{-2} s^{-1} and in *A. pensylvanicum* it was 2.34 µmol m^{-2} s^{-1}. Like these species, *R. maximum* is a shade-tolerant because of its ability to have positive net photosynthesis at relatively low light levels and because its leaves have low light saturation points. This was especially seen in the three-year-old foliage. During periods of low light intensities (< 400 µmol m^{-2} s^{-1}) *R. maximum* leaves had net photosynthetic rates that were more than 75% of their maximum photosynthetic rate at low light levels (<500 µmol m^{-2} s^{-1}). Other understory shrubs reached their saturation points by 200 µmol m^{-2} s^{-1}, but maximum rates of net photosynthesis were comparable (Holmes and Cowling, 1993 and Fortini et al., 2003). The light compensation point of *R. maximum* measured in this study was similar to those shrubs where positive net photosynthesis response was observed at very low light intensities (<10 µmol m^{-2} s^{-1} PAR) (Horton and Neufeld, 1998 and Nuraoka et al., 2002). This indicates that *R. maximum* is suited to a low-to-medium light environments. In addition, the shade created by *R. maximum* thickets reduce light penetrating to the forest floor to levels below light compensation points for photosynthesis in competing understory shrubs making it a very effective competitor in the understory (Lei et al., 2006).

Stomatal conductance has not been reported in *R. maximum* in response to increasing light intensity but has been reported in other understory species (Hogan et al., 1994 and Gonzalez-Rodriguez et al., 2002). In this study *R. maximum* leaves had responses similar to *Psychotria horizontalis* Sw. (Hogan et al., 1994) and *Persea Indica* (Gonzalez-Rodriguez et al., 2002) where stomatal conductance increased with increasing light intensity and did not decline at higher intensities once maximum rates were reached. Hogan et al. (1994) observed that stomatal conductance increased with increasing light intensity until leaf conductance no longer increased with irradiance, but remained constant. However, in the Gonzalez-Rodriguez et al. (2002) study, maximum rates of stomatal conductance increased with increasing light intensity from 0-1000 µmol m^{-2} s^{-1} PAR and rapidly declined to 0 mmol m^{-2} s^{-1} and negative values thereafter. Previous studies, in agreement with the current study, have also reported strong positive curvilinear relationships between net photosynthesis and stomatal conductance in *R. maximum* and other understory species (Lipscomb and Nilsen, 1990; Flexas et al., 2001; Kaiser and Kappen, 2000).

Comparisons of the rates of net assimilation and stomatal conductance are estimates of how PAR affects stomatal activity. From previous studies we know that rates of net photosynthesis increase with increasing irradiance until maximum values (light saturation point) are reached, stomatal conductance increases with increasing net photosynthesis, and high rates of stomatal conductance occur in low (<500 µmol m^{-2} s^{-1}) and high (>1100 µmol m^{-2} s^{-1}) light levels during induction (Flexas et al., 2001, Kaiser and Kappen, 2000, Horton and Neufeld, 1997). These studies suggest that high light intensity may have little direct effect on stomatal conductance since high rates of stomatal conductance occurred at low light intensity also. But, simultaneously with exposure to light, other environmental factors and/or plant physiological responses may influence stomatal activity. For example, Horton and Neufeld (1997) observed that conductance rates were minutely lower and sometimes the same at low light levels than high light levels in the and concluded that other environmental factors such as water availability, temperature, humidity, etc., and plant stress play a more important role in stomatal activity than light intensity.

Transpiration rates have rarely been reported for *R. maximum*, but in this study, like stomatal conductance, it increased with increasing light intensity. A relationship between transpiration and stomatal conductance was observed because transpiration rates were enhanced by increases in stomatal aperture. In agreement, transpiration rates in *R. maximum* leaves increased with increasing leaf temperature and stomatal conductance in the Lipp and Nilsen (1997) study. Like this study, when responses of stomatal conductance to increasing irradiance levels in the current study were plotted against transpiration rates, transpiration increased with increasing conductance. The same response of transpiration to increasing conductance was observed in similar shrubs: *Ruagea* cf. *pubescens*, *Trichilia guianensis*, *Naucleaopsis* sp., and *Hedysmum anisodorum* (Motzer et al., 2005).

My observations of leaf water potential of *R. maximum* suggest that this evergreen understory species is sensitive to water stress and may explain why thickets are more abundant in riparian areas (Baker and Van Lear, 1998). Net photosynthesis in *R. maximum* leaves during continued drought regimes gradually decreased daily as predawn and midday water potentials also decreased. This type of response was also observed in *R. maximum* leaves growing in Brush Mountain and in an understory shrub (*Miconia ciliate*) growing in the understory of an eastern Amazonian regrowth forest (Lipscomb and Nilsen, 1990 and Fortini et al., 2003). Gas exchange of a drought avoiding shrub, *Anthyllis cytisoides* L., growing in semi-arid southeastern Spain was highest in plants that were watered than in controls that experienced drought regimes (Haase et al., 2000).

Maximum rates of net photosynthesis and stomatal conductance were ~14.00 μmol m^{-2} s^{-1} and 0.28-0.35 mol m^{-2} s^{-1}, respectively, in watered plants but dramatically dropped to minimal levels of 1.60 μmol m^{-2} s^{-1} and 0 mol m^{-2} s^{-1} as water stress continued. The same response was seen in the current study of *R. maximum* where highest rates of net photosynthesis and leaf conductance was observed when plants had adequate water supply but stomatal conductance reached zero more rapidly during prolonged drought conditions. Cameron et al. (1999) studied another *Rhododendron* species and found that growth and flowering were inhibited because of prolonged water stress, suggesting that *R. maximum* shrub thickets may not expand or become established at rapid rates as distance from water sources increases.

As induced water stress continued, leaf stomatal conductance was the first factor that decreased to zero but at a slower rate in one-year-old foliage, implying that one-year-old foliage is less sensitive to water deficits than the older foliage. Cameron et al. (1999) measured the effects of induced water stress in container grown *Rhododendron* cv. Hoppy and reported that leaves acclimated to drought regimes by partially closing stomata, seen in rapid reductions of stomatal conductance. Predawn leaf water potentials of R. maximum during the dry down were similar to values reported for some other shade tolerant understory species, growing in a Neotropical moist forest. *Swartzia simplex* (Sw.) Spreng.and *Ouratea lucens* (Kunth) Engl.had predawn leaf water potentials that remained greater than -1.3 MPa while *Hybanthus prunifolius* (Violaceae) and *Psychotria horizontalis* Sw. reached -2.8 to -3.6 MPa then dropped to lows of -3.4 and -4.5 MPa respectively during midday measurements (Tobin et al., 1999).

Rhododendron maximum leaf water potentials did not drop as low as these field plants but did reach midday minimums around -1.7 MPa during the dry period, suggesting that they are quite drought sensitive. Lipscomb and Nilsen (1990) observed similar water potentials in *R. maximum* (-1.5 MPa) with significant stomatal closure during continued water stress, and *R. maximum* was more sensitive to low water potentials than *Kalmia latifolia* and *Rhododendron periclymenoides*. *Rhododendron maximum's* sensitivity to drought regimes might indicate why Dobbs and Parker (2004) found the expansion of this species occurred mainly along streams and water sources.

When water becomes a limited resource for plants, noticeable physical and measurable physiological changes occur throughout the plant. Results of this experiment indicate that the suppression of leaf photosynthetic rates in *R. maximum* has both stomatal and non-stomatal components. Intercellular CO_2 concentrations decreased with decreasing water potential, indicating that stomatal limitations affected the response of net photosynthesis and transpiration. Lipscomb and Nilsen (1990) observed that Ci remained constant when leaves were not water stressed, but as water became limited, Ci increased as stomatal conductance decreased. The opposite was also observed in *R. maximum* leaves of the present study, where Ci decreased with stomatal closure, indicating in those instances that stomatal limitations were more important than non-stomatal limitations (Ni and Pallardy, 1992). Net photosynthesis plotted against Ci measured during the dry-down period showed that there were significant stomatal limitations during this time period. Ci decreased dramatically as net photosynthesis decreased indicating that CO_2 diffusion into the leaf was limited by stomatal closure. Intercellular CO_2 concentrations remained fairly constant during other the measurements of the controlled environmental factors, suggesting in these instances non-stomatal limitations were the main limitations to net photosynthesis.

At time it appeared that stomatal conductance was zero even when positive net photosynthesis was measured. This likely was an error in measurement of stomatal conductance by the IRGA. Continued water stress did cause browning of *R. maximum* leaves and may have caused death in leaf tissues, resulting in a reduction of gas exchange. It would have been interesting to take measurements during the recovery period to see if *R. maximum* leaves could recuperate from the water stress that the plants were subjected to, and if so, how rapid and to what extent would they recover. Although this was not measured the present study, Galmes et al. (2007) observed recovery responses of gas exchange in several Mediterranean plants. Semi-deciduous shrubs recovered to net photosynthesis rates at or above rates observed in moderately water stressed plants during recovery measurements. Evergreen shrubs showed slight increases in net photosynthesis responses than those exposed to severe water stress after rewatering. The same pattern was also observed in stomatal conductance in the different shrub types. Gas exchange of evergreen shrubs may not recover from periods of water stress as quickly as semi-deciduous shrubs, but they show a sign of recovering.

A typical diurnal response curve was observed in *R. maximum* leaves, commencing at low rates in the morning, increasing to maximum values at midday, then decreasing back to low response rates throughout the afternoon and evening. Maximum rates of net photosynthesis, leaf stomatal conductance, and transpiration where observed at midday. Large variations during diurnal measurements were probably the result of short periods of high light intensity causing more variation in gas exchange rates. However, Nilsen et al. (1988) and other studies on similar understory shrubs (Flexas et al., 2001; Gonzalez-Rodriquez et al., 2002; Kaiser and Kappen, 2000) reported that diurnal patterns of gas exchange varied seasonally because of changes in climatic conditions.

Diurnally, *R. maximum* leaf water potential decreased to midday minimums (>-1.2 MPa) then increased nearly to zero in the late afternoon. Stomatal conductance and transpiration increased as water potentials decreased, then decreased as water potentials increased until both conductance and transpiration reached values close to or at zero (Lipp and Nilsen, 1997). Field grown *Rosmarinus officinalis* is another evergreen shrub in the Mediterranean that showed the same diurnal pattern of net photosynthesis in irrigated plants in which stomatal conductance peaked before midday and declined to minimums thereafter (Munne-Bosch et al., 1999).

Conclusions

Each environmental factor measured in this study affected gas exchange rates of *R. maximum* shrubs in different ways. High rates of net photosynthesis were reached at low light levels. Conversely, high light levels resulted in maximum rates of stomatal conductance and transpiration, suggesting that *R. maximum* leaves have lower water use efficiency in high light environments. From the results of this study it appears that *R. maximum* is very well adapted to moist understory environments. This shrub may not be capable of achieving maximum physiological performance in sites with high light intensities, low and high temperatures, high VPD levels, or low water availability. This suggests that as hemlock continues to decline in the southern Appalachian Mountain region, existing *R. maximum* plants may undergo a higher degree of stress.

Other species may even be more competitive with *R. maximum* in high light, warmer temperatures, and higher VPD conditions that will exist after the hemlock trees die. However, the evergreen foliage of *R. maximum* may enable it to gain more carbon in the spring and autumn when conditions may be more favorable for high rates of net photosynthesis, thus allowing it to keep its competitive advantage over other species even after hemlock death. Forest ecosystem water use, canopy transpiration and photosynthesis, and microclimatic conditions are factors that may shift with changes in understory species diversity and in overstory canopy cover. These modifications will influence physiological performance of *R. maximum*, altering forest structure, function, and composition. Tying in these data with whole-plant and microclimate data being collected at Coweeta Hydrologic Lab, Otto, North Carolina will give a better understanding of physiological activity of *R. maximum* and its possible impacts on hemlock mortality, ecosystem water use, and understory species diversity.

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