AN UNUSUAL SUCCULENT IN THE APPALACHIAN UNDERSTORY: LIGHT CAPTURE AND DROUGHT TOLERANCE IN *Sedum ternatum*

A Thesis
by
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Abstract

Sedum ternatum Michx., a prostrate succulent native to Appalachian deciduous forests, is exceptional in that it thrives in the understory, yet exhibits a leaf morphology more typical of species in dry, high-light environments. The thick leaves of *S. ternatum,* with their small surface area, would seem ill adapted to light harvesting in the shady forest, in contrast with the broader, thinner leaves generally associated with shade plants. However, because its leaves persist throughout the winter, I hypothesized that *S. ternatum* functions as a sun plant in early spring before canopy emergence, and in autumn after canopy senescence, accomplishing much of its carbon gain during these high-light seasons. Over a year of fieldwork, I demonstrated that *S. ternatum* shows plasticity in responding to seasonal light, adjusting pigment levels throughout the year and maximizing growth in spring and autumn. Its thick leaf is therefore well adapted for light harvest when the understory is brightest. I also hypothesized that because *S. ternatum* often colonizes dry microhabitats within an otherwise moist understory, and because leaf succulence often confers drought tolerance, it would exhibit high drought tolerance when subjected to water deprivation. In a greenhouse experiment, I showed that *S. ternatum* easily withstood five weeks of drought in part by engaging the photosynthetic pathway known as Crassulacean Acid Metabolism (CAM). Because CAM is becoming more important both in ecology and in human agriculture as climate change causes increasing drought around the world, the qualities that make *S. ternatum* an "unusual succulent in the Appalachian understory" today may well enhance its future survival.

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Foreword

References are in the style of the scientific journal *Plant Ecology*, in anticipation of submitting the project for publication. Figures are given at the end of the document, with one figure per page, as required by *Plant Ecology*.

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Introduction

Light Capture and Drought Tolerance in *S. ternatum*

Walking in a Southern Appalachian forest, it is easy to overlook the small, prostrate plant *Sedum ternatum* Michx, or simply "woodland stonecrop." *S. ternatum* grows at the very base of the herbaceous understory, rarely over 15 cm in height. In summer, taller herbaceous species hide *S. ternatum* from view, and in autumn leaf litter may partially bury it. Even *S. ternatum* growing on boulders—as its common name suggests—or climbing the base of tree trunks is often camouflaged by surrounding moss **(Figure 1a-c)**. Yet on close inspection, *S. ternatum* strikes the viewer as a very unusual understory species. Its leaves are thick and succulent, yet the trait of succulence is most often associated with plants growing in dry, high-light environments (Landrum 2002; Eggli and Nyffeler 2009; Ogburn and Edwards 2010), rather than temperate forests with abundant rainfall. The range of *S. ternatum* in the eastern U.S. is broad, starting just above Florida in the South and extending north to Maine. However, its range stops short of the drier Western states (USDA Plants Database 2017), suggesting it cannot compete in more arid and sunny locations**.** Why would a plant on the moist forest floor exhibit a thick, succulent leaf, yet prove uncompetitive in drier environments? *S. ternatum* is a strange understory plant in that it is a succulent—and a strange succulent in that it is an understory plant.

The thick *S. ternatum* leaf would seem to pose an impediment to light harvesting in the shady understory of a deciduous forest, where light levels can often be 5% or less of full sun (Neufeld and Young 2014). The stereotype of "shade leaves" as broad and thin, with a large surface area to harvest scarce photons (Boardman 1977; Smith and Hughes 2009; Taiz and Zeiger 2010; Neufeld and Young 2014), does not fit *S. ternatum*. Instead, its small surface area to mass ratio poses the apparent mystery of a "sun leaf in the shade." How does the thick *S. ternatum* leaf intercept sufficient sunlight and avoid self-shading of lower mesophyll cells? As background to investigating this question, the following Introduction first surveys existing knowledge of plant

growth, photosynthesis, and pigment production relative to seasonal light. I then return to the question of drought tolerance, specifically how the photosynthetic pathway known as Crassulacean acid metabolism (CAM) facilitates plant survival in the face of water scarcity.

Seasonal Light and Plant Phenology on the Forest Floor

Solar irradiance on the forest floor follows relatively predictable annual patterns (Neufeld and Young 2014). The understory is brightest in spring, when the solar angle is higher than in winter and the canopy is absent. As the canopy forms in late spring, light drops sharply, and continues to fall until the leaf area index (LAI) peaks in late summer. Plants still receive light in the form of sunflecks, defined as intermittent periods when shifting gaps in the canopy raise light levels above 50 or 100 μmol m^{-2} s^{-1}. After canopy leafout, sunflecks may contribute 10-80% of photosynthetically active radiation (PAR) available for understory photosynthesis in summer (Way and Pearcy 2012). In autumn as the canopy senesces, the understory again brightens but not as much as in spring, due to the declining solar angle (Neufeld and Young 2014). Slope aspect also impacts understory light, particularly in winter when the sun is at its lowest angle. In the Northern Hemisphere, south-facing slopes are oriented towards the sun and receive a higher intensity and duration of light than do north-facing slopes (Warren 2008, 2010).

As light changes with the seasons, plants balance their annual carbon budgets through distinct phenological strategies (Uemura 1994; Rothstein and Zak 2001; Neufeld and Young 2014). Spring ephemerals emerge before canopy closure, and benefit from abundant springtime light, water, and nutrients before falling dormant in summer. In contrast, “summer-greens” leaf out during or after canopy closure, and senesce before winter. “Winter-greens” form leaves in late summer or early autumn that overwinter, then senesce the following summer. This senescence distinguishes them from “true evergreens,” which have a leaf lifespan of one to several years. The

least common phenology is that of "heteroptics," which comprise as few as 1% of understory species (Neufeld and Young 2014). Heteroptics display leaves for the entire year, but produce them in two distinct flushes: spring-produced leaves specialized for warm weather, and fall-produced leaves specialized for cold. The advantages of a heteroptic phenology have been shown by comparing *Hexastylis arifolia,* a true evergreen, and *Heuchera americana,* a heteroptic (Skillman et al. 1996). The "generalist" *H. arifolia* leaf has less plasticity, for example showing more photoinhibition in cold, bright conditions. In contrast, the "specialist" winter leaves of *H. americana* exhibit less photoinhibition. As we shall see, *S. ternatum* is heteroptic—yet another unusual trait for its environment.

Plants differ in their capacity to alter pigment levels over the year (Harvey 1980; Neufeld and Young 2014). For plants that spend part of the year shaded and part in sun, the capacity to adjust chlorophyll permits acclimation to changing light. On the leaf level, plants can acclimate by altering total chlorophyll levels. Shaded leaves usually exhibit higher total chlorophyll by dry mass, while total chlorophyll by surface area in shade plants is often equal to or lower than that in sun plants, although this is variable (Boardman 1977; Murchie and Horton 1997; Taiz and Zeiger 2010; Neufeld and Young 2014). On the chloroplast level, shaded plants tend to exhibit lower chlorophyll a/b ratios than sun plants, as chlorophyll b is strongly associated with light harvesting (Boardman 1977; Murchie and Horton 1997; Taiz and Zeiger 2010). Summer-greens acclimate to canopy closure by increasing chlorophyll, whereas spring ephemerals do not (Neufeld and Young 2014). True evergreens also contrast with heteroptics: both the heteroptic *H. americana* and true evergreen *H. arifolia* have higher chlorophyll in summer than winter, but the difference is greater in *H. americana*—with its two flushes of seasonal specialist leaves—than in *H. arifolia*, with its one generalist leaf (Skillman et al. 1996).

The production of anthocyanin pigments is another response to changing light, especially in winter (Hughes et al. 2005; Hughes and Smith 2007a, 2007b; Hughes et al. 2010). When light is

high but temperature is low the Calvin cycle slows, while photosynthetic light reactions—which are not temperature-dependent—may produce excess energy that causes photoinhibition and/or damages the photosynthetic apparatus (Powles 1984, Adams et al. 2004, Verhoeven 2014). Anthocyanins—which are flavonoid pigments imparting a red color to leaves—serve an internal shading function by absorbing damaging light wavelengths (mainly green) before they reach the chloroplasts. Because winter reddening was noted during early field observations of *S. ternatum* **(Figure 2)**, I hypothesized that *S. ternatum* is among the overwintering species that produce anthocyanins for photoprotection.

Through a year of field measurements, I used the framework of phenology to test the hypothesis that *S. ternatum* benefits from its thick leaf for light harvest in the bright windows of early spring and late autumn. Specifically, I hypothesized that 1) the *S. ternatum* leaf would prove thicker and more succulent than leaves of congeners; 2) *S. ternatum* would exhibit high rates of photosynthesis and growth in spring and fall; 3) total leaf chlorophyll would be higher in summer, and lower in spring and fall; 4) anthocyanins would increase in winter; and 5) sunflecks would support limited summer photosynthesis. Because these hypotheses were largely supported by the data that I collected, this study will demonstrate that the leaf thickness enhancing drought tolerance in *S. ternatum* is also beneficial for light harvest, as *S. ternatum* functions as a "sun plant" in the bright windows of spring and fall.

Informal field observation provided further insight as to why a succulent leaf might be adaptive in a temperate understory with plentiful rainfall. Our site was in fact not uniformly moist, and *S. ternatum* frequently colonized the drier microhabitats within it. These xeric microhabitats with rapidly drying substrate include thin soil atop boulders, the base of tree trunks, and badly eroding south-facing slopes **(Figure 3a-d)**. One study of a Tennessee forest ecosystem featuring tight species packing found that *S. ternatum* competed successfully for a very shallow soil depth gradient that other species would not tolerate (Bratton 1976). By surviving suboptimal conditions

of lower soil moisture and nutrients, *S. ternatum* avoids competition with congeners that require a steadier water supply. It is also unusual for an understory evergreen in our region to grow on south-facing slopes, as most tend to colonize north-facing slopes (Warren 2008, 2010), but I have observed *S. ternatum* thriving on sunny slopes of southern aspect where soil dries quickly. Moreover, when these microhabitats are elevated (boulders, tree trunks, slopes) they also protect *S. ternatum* from trampling by mammals such as deer. Although *S. ternatum* does sometimes grow in deeper forest floor soil, this entails greater competition from congeners, along with the threat of trampling.

Even in the shallow substrate of these drier microhabitats, moisture fluctuations are tempered by the overall climate. In the southern Appalachians, drought, when it occurs, is generally a gradual process that develops over a period of several days to weeks. In spring, sunlight strikes plants and substrate directly, but rain tends to be abundant. In summer, canopy shade ensures that even in longer dry spells, soil moisture drops slowly. In autumn, fresh leaf litter buffers soil moisture. Although *S. ternatum* is more drought tolerant than most understory congeners, it may not require the degree of drought tolerance needed in more arid locations. The present study explores in greater depth the drought tolerance of *S. ternatum* by examining the extent to which it engages the photosynthetic pathway known as Crassulacean Acid Metabolism (CAM), and specifically the facultative, low-level CAM pathway variant well suited to short-term water fluctuations in the drier microhabitats that *S. ternatum* tends to colonize.

The Plasticity of CAM Photosynthesis

Of the three major photosynthetic pathways—C_3, C_4, and CAM—CAM has the greatest water use efficiency (WUE). The average WUE of CAM plants is at least six times greater than C_3 and three times greater than C_4 (Borland et al. 2011). CAM is currently known to exist in about

6% of the world's vascular plant species and CAM plants are currently increasing their ranges around the world as climate change increases aridity in many regions (Yu and D'Odorico 2015). Certain CAM species may also experience sustained growth increases under elevated CO_2 regimes, a potential advantage as atmospheric CO_2 rises (Drennan and Nobel 2000). Because of their promise in future climate scenarios, ongoing research is exploring the potential uses of CAM plants—both naturally occurring, and genetically engineered—for agricultural production (Borland et al. 2011; Borland et al. 2014; Yang et al. 2015; Brilhaus et al. 2016; Furbank and Sage 2016).

The best-known type of CAM is strong, obligate (or "constitutive") CAM, with a 24-hour cycle in four phases (Lüttge 2002, 2004; Borland et al. 2011). In phase I, stomata open at night and take in atmospheric CO_2, which is fixed by the enzyme phosphoenolpyruvate carboxylase (PEPC), and stored in cell vacuoles as malic acid. This process imposes extra energetic costs on CAM plants, but by opening stomata nocturnally—instead of during the day, as C_3 plants do—CAM plants lose less water through transpiration in the higher relative humidity of cool night air. In phase II, starting at dawn, PEPC is deactivated, setting the stage for Rubisco to catalyze the Calvin cycle. Phase III comprises the daytime hours in which obligate CAM plants close their stomata, decarboxylate malic acid, and refix the resulting CO_2 through the Calvin cycle. Because the CO_2 concentration around Rubisco is very high, the inefficiencies associated with photorespiration are avoided. Phase IV marks the transition from late afternoon to evening, when malic acid stores have been depleted and stomata reopen. In sum, strong obligate CAM is a single-cell photosynthetic mechanism that temporally separates C_4-style carbon fixation via PEPC at night from the C_3 pathway of the Calvin-Benson cycle during the day.

Due to these extra metabolic processes and more complex circadian rhythms, obligate CAM sometimes results in slower growth relative to the C_3 pathway (Lüttge 2004; Borland 1996). In this sense, obligate CAM favors survival in a harsh climate over biomass production. But where tradeoffs exist between CAM and rapid growth, "facultative" (or "inducible") CAM combines the

advantages of C_3 with those of CAM. Facultative CAM plants use C_3 photosynthesis (or, more rarely, C_4) when unstressed, but switch to CAM under circumstances such as water deprivation (Herrera 2009; Winter and Holtum 2014; Brilhaus et al. 2016). Facultative CAM plants benefit at most times from C_3 growth levels, but engage CAM when under drought, permitting survival until conditions improve (Borland 1996). By ensuring survival during harsh times, facultative CAM helps plants live long enough to reproduce (Herrera 2009), even though overall gas exchange in CAM mode is lower than C_3, partly because plants are drought stressed (Winter and Holtum 2014). A recent study of *Talinum triangulare* elucidates the transcriptional regulation of facultative CAM, analyzing mRNA to demonstrate the molecular events involved in C_3-CAM transitions (Brilhaus et al. 2016).

The flexibility of CAM is further seen in "CAM cycling" and "CAM idling" (Borland et al. 2011). In CAM cycling, plants open stomata during the day, at least partially, to perform C_3 photosynthesis, but also generate some malic acid—presumably from recycled respiratory CO_2 (Martin et al. 1988a)—overnight, when stomata are closed. CO_2 released from this malic acid is used in the Calvin cycle the following day, supplementing atmospheric CO_2 intake. CAM cycling may help plants conserve water by keeping stomata more closed during the day. For example, five CAM-cycling *Talinum* species showed a negative correlation between nocturnal acid accumulation and diurnal C_3 gas exchange (Harris and Martin 1991a). Moreover, the degree of CAM cycling correlated positively with the aridity of their field environments—though experimental drought produced the reverse result: species from *more* arid environments did *less* CAM cycling, presumably because they are more drought adapted (Harris and Martin 1991b).

CAM cycling may help keep plants primed to perform a more extreme CAM variant known as CAM idling (Harris and Martin 1991b). In CAM idling, stomata are closed both night and day, with nocturnal acid accumulation presumably derived from respiratory CO_2. By maintaining a capacity for nocturnal CO_2 recycling, CAM cycling may ease the transition into

CAM idling, although sometimes plants move directly from C_3 to CAM idling. CAM idling, in turn, may permit maintenance of metabolic activities such that regular gas exchange can quickly resume after even minimal rainfall (Harris and Martin 1991b). In dire drought, CAM idling can continue for years (Borland et al. 2011; Nobel 1988).

CAM may also be strongly or weakly expressed (Borland et al. 2011). Strong CAM plants fix most or all of their carbon via CAM, and tend to live in the most arid environments. Weak CAM plants express CAM at a lower level, and fix most of their carbon via C_3. Carbon isotope ratios ($\delta^{13}C$) indicate the degree of CAM performed by a given species. Because PEPC does not discriminate against the ^{13}C isotope as much as does Rubisco, $\delta^{13}C$ values for obligate CAM plants are greater (less negative) than for C_3 plants and weak CAM plants. A survey of $\delta^{13}C$ for over 500 species (Winter and Holtum 2002) showed that isotope values display a bimodal distribution with weak CAM species clustered around the more negative peak, and strong CAM species around the less negative peak. Plant fitness may therefore be best served by committing primarily to one photosynthetic pathway or the other. Some researchers adopt the term "C_3-CAM species" for weak CAM plants (Winter et al. 2015), a group that includes *S. ternatum*, with its $\delta^{13}C$ of around -30 (Martin et al. 1982).

In addition to conserving water, CAM may also enhance photoprotection in high light and drought conditions (Adams and Osmond 1988; Castillo 1996; González-Salvatierra et al. 2010). Photoinhibition can occur when energy generated by the photosynthetic light reactions exceeds what can be absorbed by the Calvin-Benson cycle, reducing the efficiency of photosystem II. CAM releases CO_2 through diurnal decarboxylation of malic acid, with a photoprotective effect shown in an experiment on *Kalanchoë pinnata*, a plant with variable CAM expression (Adams and Osmond 1988). *K. pinnata* plants that had been raised in high light were divided into two groups, with one receiving regular air during the night, and the other 2% O_2 with no CO_2. Both groups were exposed to high light during the day, and while the controls showed no photoinhibition, the

group that had received no CO_2 the night before—and thus was unable to release CO_2 into the leaf via decarboxylation—did show photoinhibition. Adams and Osmond (1988) draw a parallel between this photoprotective effect and that of CO_2 released through photorespiration in C_3 plants. Because *S. ternatum* expresses only weak CAM, it generates less malic acid than *K. pinnata*, and might rely more heavily on photorespiration for diurnal photoprotection. Another *Sedum* species, *Sedum album*, uses both CAM cycling and the production of antioxidative enzymes for photoprotection (Castillo 1996).

CAM in the Sedum Genus

The large *Sedum* genus, with ~420 species (Nikulin et al. 2016) across a wide geographical range, illustrates the plasticity of CAM. Research into *Sedum* phylogeny is ongoing (Nikulin et al. 2016; Mort et al. 2001), such that the evolution of CAM within it cannot be reconstructed here. However, it is known that *Sedum* exhibits great variability in CAM expression. A study of 26 European, three Mexican, and two African *Sedum* species found that three perform little or no CAM; five exhibit obligate CAM; and most of the European species perform facultative CAM under water stress (Pilon-Smits et al. 1991). Regarding intraspecific variability, populations of *S. wrightii* from sites varying in moisture and elevation had different proportions of diurnal and nocturnal CO_2 uptake, and variable $\delta^{13}C$ ratios (Gurevitch et al. 1986; Kalisz and Teeri 1986). The C_3-CAM intermediate *Sedum telephium* displays no acid fluctuation when well-watered, but engages CAM cycling, low-level CAM, and CAM idling under water deficit (Conti and Smirnoff 1994). It switches rapidly between C_3 and CAM in response to water fluctuations in the rock crevices and stony soil where it grows, as can many Crassulaceae species (Borland and Griffiths 1990).

A drought experiment performed on five *Sedum* species (Gravatt and Martin 1992) was one inspiration for the present study. Testing gas exchange, $\delta^{13}C$ ratios, and nocturnal acid accumulation, the study found that *S. ternatum* and *S. integrifolium* displayed only C_3 photosynthesis when well-watered, and CAM cycling when water-deprived. *S. telephioides* and *S. nuttallianum* performed CAM cycling when well-watered, and CAM cycling combined with low-level CAM when water-stressed. *S. wrightii* performed CAM both when well-watered and under drought. Gravatt and Martin (1992) therefore identified *S. ternatum* as a C_3-CAM species that engages low-level facultative CAM under water stress. However, as their experiment only droughted plants for three days, it did not test CAM expression in *S. ternatum* throughout a more prolonged drought.

My study expanded on the results of Gravatt and Martin (1992) with a longer drought in larger containers to simulate a more gradual soil dry-down in the field, and to further test the limits of drought tolerance in *S. ternatum*. I sought to ascertain at what point in my drought experiment water stress became evident, and to quantify its effect on leaf succulence, photosynthesis, chlorophyll fluorescence, and nocturnal acid accumulation. For my greenhouse experiment, I hypothesized that 1) droughted plants would withstand water deprivation for a significant period of time, continuing photosynthesis and maintaining succulence; 2) plants would respond to drying with CAM cycling and idling, as photosynthetic rates and FW:DW began to decline; 3) droughted plants would exhibit photoinhibition; and 4) plants would experience a rapid recovery upon rewatering. Insofar as these hypotheses were largely supported by my results, this experiment demonstrates that low-level facultative CAM is an important trait allowing *S. ternatum* to tolerate prolonged water deprivation.

Methods: Fieldwork

Field Site

The field site was Appalachian State University's State Farm Trails Area in Boone, NC, elevation approximately 1016 m **(Figure 4a-b)**. The site is home to numerous native plant species and has a topography of low ridges and streambeds. Patches of *S. ternatum* are found on slopes of varying aspect, both on boulders and in deeper forest soil. Patches vary in size, sometimes occupying under a square meter but often carpeting several square meters. For sampling, I used larger patches where *S. ternatum* plants were abundant and healthy. I did not use patches close to wide footpaths or locations where my footsteps would cause significant soil erosion.

Light Levels

Understory light was measured above *S. ternatum* patches from Feb.-Dec. 2015. For each patch, I took five measurements at the corners and center of a fixed square meter area between 1130-1300, using an Li-250 light meter equipped with an Li-190R Quantum Sensor (Li-Cor, Inc., Lincoln, NE). The sensor was held ~20 cm above the ground. On Feb. 3, Mar. 4, and Apr. 11, nine patches were measured. On all other dates, 10 patches were measured.

Leaf Thickness and Succulence

In June 2014, three *S. ternatum* leaves were taken from each of five plants. Here, and for all sampling unless stated otherwise, plants were found over 10 m apart and leaves were taken from the bottom whorl of terminal rosettes, representing the youngest fully-formed leaves. Cross-sections were cut from the leaf center with a Leica VT 1000S Vibratome (Leica Biosystems, Nussloch, Germany), imaged with an Olympus SZX12 stereoscopic microscope and measured on

an Olympus IX-81 light microscope (Olympus Corporation, Tokyo, Japan) with Microsuite Biological Suite software. I also measured six congeners, including bloodroot (*Sanguinaria canadensis*), trout lily (*Erythronium americanum*), star chickweed (*Stellaria pubera*), mayapple (*Podophyllum peltatum*), umbrella leaf (*Diphylleia cymosa*), and wood-nettle (*Laportea canadensis*). For congeners, one of the youngest fully-expanded leaves (three leaves in the case of *L. canadensis*) was taken from each of 3-5 plants. Hand sections were made with razors, placed in wet mounts, imaged with the IX-81 light microscope, and measured with Microsuite Biological Suite software at the thickest part of the cross-section excepting the midvein **(Figure 5)**. For *S. ternatum*, because it does not have a midvein, I measured the thickest part of the cross-section. For all species, four measurements were averaged per cross-section. Leaf succulence was measured for *S. ternatum* and four congeners. After recent rain, leaf samples were taken from each of five plants per species. Leaves were weighed to obtain fresh weight (FW), oven-dried at 80° C, and reweighed to obtain dry weight (DW). Succulence was calculated as FW:DW (g:g) ratio.

Stomatal Characteristics

In June 2014, three leaves were taken from each of five *S. ternatum* plants. Abaxial and adaxial epidermal peels were placed in a wet mount and imaged with the IX-81 light microscope at 100X magnification (objective lens set at 10X). Stomatal density was analyzed using Microsuite Biological Suite software **(Figure 6)**. Three fields of view were averaged per leaf.

Spring Growth

In early March 2015, 10 *S. ternatum* patches were chosen for growth measurements. On five plants per patch, red thread was tied to the petiole of a leaf to be measured in early spring. Stem length was measured from the red tag to base of the terminal rosette as internodes above the

tag elongated. When red-tagged leaves senesced **(Figure 7)**, in May blue tags were placed on new leaves **(Figure 8)** known to have originated in early spring. From Mar. 26 - June 30, blue-tagged leaves were measured and new whorls at the stem apex were counted. From Mar. 10, rosette size was measured as leaf length on the lowest whorl. Only non-flowering stems were measured, but time of flowering was noted.

Fall Growth

In late August, 10 quadrats of 900 cm^2 were established **(Figure 9)**. Yellow threads were placed below the terminal whorl on five stems recognized as older by having leaf lengths greater than 1 cm, and often showing herbivore damage **(Figure 10)**. Red threads were placed below the terminal whorl on five newly emerged stems **(Figure 11)**, recognized as new by having leaf lengths below 0.5 cm, and little to no herbivore damage. Every two weeks, I counted total whorls above the tags, and measured leaf length of all stems in the quadrat with a ruler.

Light Curves

On June 26, light curves of photosynthesis were made using the Extended Reach Chamber on an Li-6400 gas exchange system (Li-Cor, Inc., Lincoln, NE). Because the Extended Reach Chamber does not possess its own light source, an LED flashlight was suspended above the cuvette and adjusted in height to vary the light level, which was measured using the Li-250 light meter and quantum sensor. Leaves still attached to their mother plant were selected for analysis and illuminated using a second LED flashlight at a PAR of 800 μmol m^{-2} s^{-1} for 30 mins prior to being harvested and inserted in the extended reach cuvette to do the light curves. Preliminary work had shown that leaves could maintain constant rates of gas exchange for up to 30 mins after being detached from the plant (data not shown). Because the leaves were so small, and had very short

petioles, they were not easily inserted in the cuvette while still attached to the mother plant. Furthermore, to obtain sufficient replicates under controlled conditions, it was better to bring the leaves to the gas exchange system, rather than vice-versa. Light values used for the curves were, in this order: 1600, 1200, 800, 500, 250, 100, 50, 25, and 0 μmol m^{-2} s^{-1} and conditions in the cuvette were: flow rate of air 200 μmol s^{-1}, fan set at fast speed, chamber temperature of 25ºC, and a reference CO_2 of 400 ppm. Linear regression of the first four points on the curve yielded an estimate of the apparent quantum efficiency and where the curve intercepted the Y-axis at a net photosynthetic rate of zero, the light compensation point. Light saturation was determined as the PAR at which no more increases in photosynthetic rate occurred as PAR was increased, and the rate at saturating light was the light saturated rate of photosynthesis. Dark respiration was determined as the rate of gas exchange at zero PAR.

Photosynthesis

On June 19, gas exchange was measured with the Extended Reach Chamber of the Li-6400 (flow 200, fan fast, chamber 25ºC, reference CO_2 400 ppm). For 10 leaves, photosynthetic rate was logged in ambient understory light after two minutes. Because the canopy was well established by this point, ambient light was very low (8 μmol, n = 10). Two leaves were exposed to a fleck of 1250 μmol and measurements were logged every 10 seconds for 30 minutes. Only the rate after five minutes is recorded in the Results, so as to match the later dates.

On Sept. 3, Oct. 7, and Nov. 24, 2015, then April 26 and May 25, 2016, photosynthesis was measured on 6-10 detached leaves or rosettes per date (rosettes were used on Nov. 24 because not enough large leaves remained on stems). On Sept. 3, reference CO_2 was set to 500 ppm to match measured ambient CO_2, but in all subsequent sessions 400 ppm was used. On all dates, flow speed was 200, fan fast, and block temperature set either to 20˚or 25˚C, to suit ambient

temperature. Gas exchange readings in ambient light were logged after two minutes. An 800 μmol fleck—just above the light saturation point as determined by light curve assays—was then generated by an LED flashlight suspended above the leaf chamber, and photosynthetic rate was logged every 10 seconds for five minutes **(Figure 12)**.

On each day that photosynthesis was measured, soil moisture levels were assessed using the Campbell Scientific HydroSense II soil moisture probe (Campbell Scientific Inc., Logan, Utah). Because this instrument cannot measure very shallow soil, these moisture levels simply confirmed that extended drought did not occur during our study and that moisture status was roughly equivalent on the different days of measurement (data not shown). They did not capture short-term moisture fluctuations in the shallow substrate underneath many *S. ternatum* patches.

Chlorophyll Concentration and Anthocyanin Amount

Samples of 3-4 leaves were taken from each of nine patches spaced at least 10 m apart, and carried to the lab on ice. Fresh weight (FW) was measured on a Sartorius Practum 224 1S Analytical Balance (Sartorius AG, Göttingen, Germany). Surface area was calculated by laying the leaves on a flatbed scanner (CanoScan 9000F Mark II scanner, Canon USA, Melville, NY), clarifying leaf outlines with Adobe Photoshop CC 2014 (Adobe Systems Inc., San Jose, CA), and then converting pixels from these jpg images to area in cm^2 using Black Spot, a shareware program (Varma and Osuri 2013). For all analyses, the sample of 3-4 leaves was first dipped in liquid nitrogen for 20 seconds prior to pigment extraction. For chlorophyll analyses, leaves were then placed in a vial containing 3.0 or 3.5 mL dimethylformamide, macerated with a glass rod, and left to extract in a refrigerator for seven days. Absorbances were then measured in a Shimadzu UV-1800 spectrophotometer (Shimadzu Mfg. Inc., Portland, Oregon) according to the procedure and equations of Porra (2002).

For anthocyanin extraction, leaves were placed in 2 mL of an extraction solution containing 6 M HCl, water, and methanol (MeOH) in a ratio of 7:23:70 v:v and extracted in the refrigerator for 3-4 days. Before measuring absorbances at 530 nm and 653 nm, an extra 1 mL of extraction solution was added for a total of 3 mL per sample. Absorbance due solely to anthocyanins was calculated as $A_{530} - 0.24*A_{653}$ (Murray et al. 1991), and expressed as abs g^{-1} FW.

Statistical Analyses

A one-way ANOVA was used to identify differences in leaf thickness and succulence among *S. ternatum* and congeners. For analysis of stomatal density, a paired two-tail *t*-test was used to compare abaxial and adaxial stomatal density. Differences were considered significant if $p \leq 0.05$.

Methods: Greenhouse

Plant Propagation

In June 2015, nine stem cuttings of *S. ternatum* were taken from the State Farm site. Cuttings were rooted in pots of 15 cm diameter and ~9 cm soil depth filled with Metro-Mix 360 soil mix (Sungro Horticulture, Agawam, MA) and raised outdoors at the author's home for about six weeks, at which point each had produced at least six shoots. In August, four stem cuttings from each of these parent plants were rooted in new pots, placed on a greenhouse bench in a 4 X 9 arrangement, and rotated (two rows lengthwise, two rows widthwise) every two weeks. After an initial regimen of more frequent watering, plants were watered twice a week beginning the third week after propagation. Plants were treated with Clearys 3336F Turf and Ornamental Systemic

Fungicide (Cleary Chemicals LLC, Alsip, Illinois) as needed until, but not after, imposition of drought. On Sept. 22, 1.23 mL Osmocote 14-14-14 slow-release fertilizer (The Scotts Company, Marysville, Ohio) was applied to each pot.

Experimental Setup

On Nov. 2, plants were separated into well-watered (control) and drought (experimental) groups, with two offspring from each original parent plant in each group for a total of 18 control plants and 18 experimental plants. Treatments were placed at opposite ends of a greenhouse bench **(Figure 13)**, and rotated weekly throughout the experiment. Control plants were watered biweekly. No fungicides, pesticides, or fertilizer were applied during the experimental period.

Timeframe and Imposition of Drought

Throughout the experiment, measurements were taken of pot weight, leaf fresh weight to dry weight ratio (FW:DW), chlorophyll fluorescence (F_v/F_m), photosynthetic rate (*A*), and nocturnal acid accumulation. For three days before drought imposition, baseline measurements were taken. Water was then withheld from the drought treatment for 35 days, beginning on Nov. 6 ("Day 1"), until rewatering on Dec. 11. Final measurements were taken thereafter.

Soil Moisture, Leaf Succulence, and Plant Water Potential

To ascertain rate of soil dry down, nine randomly selected pots per treatment were weighed on a balance before regular waterings of the controls. To ascertain plant water status throughout the experiment, leaf FW:DW was measured as follows: at dawn, samples of three leaves per plant were taken from three randomly selected plants per treatment. Samples were

weighed, oven-dried at 80º C for at least seven days, and re-weighed. Late in the experiment, plant water potential was also measured with a Scholander Pressure Chamber (PMS Inc., Corvallis, OR) on detached stems. Stems with leaves attached were used instead of just leaves because of the high leaf succulence. Water potentials were taken on four or five plants at dawn, before visible wilting (Day 28), during severe wilting (Day 35), and the day after rewatering.

Chlorophyll Fluorescence

Chlorophyll fluorescence (F_v/F_m) was recorded to assess quantum efficiency of photosystem II using a Handy PEA Chlorophyll Fluorescence Meter (Hansatech Instruments Ltd., Norfolk, UK). One leaf from each of nine plants per treatment was dark adapted using a clip for a minimum of 30 minutes, then exposed to a 1 sec saturating light flash (3500 μmol m^{-2} s^{-1}).

Photosynthesis

Net photosynthesis was measured using an open-flow gas exchange system, the Li-6400XT equipped with the Li-6400-15 Extended Reach Chamber, chosen because its small 1-cm diameter allowed a mature *S. ternatum* leaf to fill it completely. The cuvette was set to near ambient conditions (temperature 25ºC, reference CO_2 400 ppm). An LED flashlight was suspended above the leaf at an appropriate height to provide 600 μmol m^{-2} s^{-1} of light. Readings were taken on five randomly selected plants per treatment, using a single detached leaf. All measurements of leaves in this experiment were completed in less than five minutes. After a minimum of two minutes and a maximum of five minutes, steady-state photosynthetic rate was logged. All measurements were taken between 10:00 and 14:00 hrs. Light levels above each plant group were recorded with the Li-250 light meter equipped with an Li-190R Quantum Sensor (mean of six measurements per group).

Acid Accumulation

At dawn and dusk, samples of six leaves were collected from each of four plants per treatment. Samples were immediately frozen in liquid N or on dry ice (those frozen on dry ice were submerged in liquid N before titration), then stored at -80ºC until extraction. For extraction, 10 mL 80% methanol was placed in test tubes, and weighed. Frozen leaf samples were placed in tubes, which were then weighed again to obtain leaf fresh weight. Leaves were macerated and then tubes were heated in a hot water bath at 70º C for 75 min. Deionized water was added to bring samples back to the original weight and then samples were cooled to room temperature. Titrations were subsequently performed using 2 mM NaOH. I titrated to an endpoint of pH 8.3, but used an endpoint of 7.0 for statistical analyses.

Statistical Analyses

Paired *t*-tests (pairing clonal offspring from the same parent plants across the two groups) were used to assess differences between control and treatment. This reduced intraspecific genetic variation as a confounding variable. Differences were considered significant if $p \leq 0.05$, and all *t*-tests were one-tailed unless stated otherwise.

Results: Fieldwork

Leaf Thickness and Succulence

S. ternatum had significantly thicker leaves than any congener **(Figure 14)**. At 881.0 ± 51.86 μm, the leaf thickness of *S. ternatum* was almost double that of the thickest congener, *E. americanum,* with a thickness of 455.4 ± 16.12 μm. The thinnest leaf belonged to the only species

that blooms in late summer, rather than spring—*L. canadensis*, with a leaf thickness of 89.9 ± 6.60 μm. A similar result was found for leaf succulence **(Figure 15)**. With a FW:DW ratio of 15.1 ± 0.58, *S. ternatum* was more succulent than any congener, and *E. americanum* was the most succulent among the congeners, with a FW:DW of 10.5 ± 0.57. The two were often found side-by-side in April, with their thick leaves harvesting the high light **(Figure 16)**.

Stomatal Characteristics

S. ternatum exhibits amphistomy, with 77% as many adaxial as abaxial stomata **(Figure 17)**. Stomatal density of the abaxial (38.0 ± 7.53 stomata mm^{-2}) surface was significantly larger ($p < 0.005$) than that for the adaxial (29.4 ± 5.63 stomata mm^{-2}) surface. Occasional stomatal clustering, in the form of paired stomata, was observed **(Figure 6)**.

Understory Light Levels

Photosynthetically active radiation (PAR) was highest in spring, lowest in summer, and intermediate in autumn **(Figure 18).** The highest instantaneous mean at 847 μmol m^{-2} s^{-1} occurred on April 27. The lowest PAR of 8 μmol m^{-2} s^{-1} occurred on June 21. Canopy closure occurred in mid-May and opening was in mid-October.

Leaf Chlorophyll and Anthocyanin Concentrations

Chlorophyll concentration peaked in summer and was lower in spring **(Figure 19)**. In April, relative to surrounding dates, total chl g^{-1} FW was unusually high, while total chl cm^{-2} was unusually low. The chl a/b ratio was higher in spring, and lower in summer **(Figure 20)**. *S.*

ternatum produced anthocyanins in late February and in March, coinciding with visible reddening **(Figure 21)**. Anthocyanins were absent in early February, and declined during April and May.

Spring Growth in the Field

In spring, overwintered leaves gradually senesced and were replaced by new leaf whorls at the apices of elongating stems **(Figure 7)**. Until canopy closure, stem tips continued to produce new whorls and to lengthen **(Figure 22)**, and new spring leaves also grew longer **(Figure 23).** This growth spurt continued until flowering occurred around the time of canopy closure in mid-May **(Figure 24)**. After canopy closure growth largely ceased and leaves showed increasing herbivore damage throughout the summer **(Figure 25)**. By early fall, most spring-produced leaves that remained were damaged and/or senescing, indicating that spring-produced leaves have a lifespan of about half a year.

Fall Growth in the Field

S. ternatum had a major fall growth spurt, producing both new leaf whorls and new stems, which generally originated from nodes of parent plant stems **(Figure 26)**. Across ten 900 cm^2 quadrats, a total of 245 stems was counted on August 21; this number increased to 388 stems by October 23. On Nov. 20, the number of stems had dropped to 361, suggesting that new stem production had ceased **(Figure 27)**. This slight decline probably represents background loss to trampling and herbivory. Regarding new leaves **(Figure 27)**, on August 21, across ten 900 cm^2 quadrats, 46 plants had a leaf length of 0.3 cm or less and were assumed to be new. By Nov. 20, across 10 quadrats 140 plants with leaves of this size were observed. Fall-formed leaves remained on average much smaller than those formed in spring, even after several weeks of growth. On Nov.

20, the average leaf length across all quadrats was 0.62 ± 0.028 cm, approximately one-third the length recorded on June 30 of 1.81 ± 0.066 cm.

Field Photosynthesis: Light Curves

Light curve results **(Figure 28)** clarify various traits of early summer photosynthesis in *S. ternatum*. The light saturation point, after which additional light made little difference in photosynthesis, was ~500 μmol photons m^{-2} s^{-1}. The apparent quantum efficiency of photosynthesis was 0.0286 μmol CO_2 μmol^{-1} photons. The light compensation point was 4 μmol photons m^{-2} s^{-1} and dark respiration was -0.1 μmol CO_2 m^{-2} s^{-1}. The maximum rate of photosynthesis at saturating light was 4.4 ± 0.59 μmol CO_2 m^{-2} s^{-1}.

Field Photosynthesis: Ambient and High Light

In ambient (low) light, photosynthetic rates were extremely low, and sometimes even negative, on all dates, with the exception of a slightly higher rate in October when ambient light itself was higher **(Figure 29).** The high-light flecks produced higher photosynthetic rates, especially in spring and fall. On October 7, rates rose under the high-light fleck to 6.6 ± 0.67 μmol m^{-2} s^{-1}; on April 26, to 4.9 ± 0.38 μmol m^{-2} s^{-1}; on November 24, to 4.5 ± 0.88 μmol m^{-2} s^{-1}; and on May 25, to 3.6 ± 0.53 μmol m^{-2} s^{-1}. In months when the canopy was present, a lesser rise in photosynthetic rate was observed under the high-light fleck. On June 19, the rate rose to 2.6 ± 0.33 μmol m^{-2} s^{-1}; and on September 3, to just 1.35 ± 0.305 μmol m^{-2} s^{-1}.

Results: Greenhouse

Pot Weight

The relatively large size of our experimental pots allowed for a gradual dry-down, with a steady rate of water loss for the first two weeks until pots had lost most of their water weight **(Figure 30)**. By Day 14 of the drought 56% of the original pot weight was lost, and a few days later daily rate of water loss was minimal: between Day 19-20 they only lost 13.3 g, an overnight loss of 3.4%. By Day 35, pots had lost 74% of their starting weight.

Succulence

On Day 20, there was no significant difference in the FW:DW ratio between control and droughted plants **(Figure 31)**. The first significant difference ($p = 0.005$) was found on Day 27, with FW:DW of controls at 15.0 ± 0.96, and droughted plants at 11.7 ± 0.63. Controls kept a FW:DW ratio around 15 throughout the experiment, while droughted plants declined to 9.4 ± 0.43 by Day 35. After rewatering at 10:50 AM on Dec. 10, droughted plants appeared well on the road to recovery later that day **(Figure 32)**, and recovered to pre-drought levels within one day of rewatering.

Water Potential

On Day 28, a significant difference ($p = 0.001$) was found between watered (-0.16 ± 0.025 MPa) and droughted (-0.85 ± 0.080 MPa) stems **(Figure 33)**. By Day 35, water potential had decreased to -0.97 ± 0.049 MPa for the droughted group. One day after rewatering, the droughted group (-0.15 ± 0.018 MPa) was statistically equivalent to the controls (-0.11 ± 0.026 MPa).

Photosynthesis

From the time of baseline measurements through Day 7, no difference in photosynthesis was found between watered and droughted plants **(Figure 34)**. On Day 12, a higher rate (p = 0.035) was found in watered plants (7.0 ± 0.45 μmol CO_2 m^{-2} s^{-1}) than droughted plants (5.1 ± 1.05 μmol CO_2 m^{-2} s^{-1}). Photosynthesis of droughted plants then declined until diurnal gas exchange ceased by Day 26, after which no further measurements were taken until rewatering. Three days after rewatering, a higher rate (two-tail paired t-test, p = 0.008) was found in previously droughted (6.7 ± 0.50 μmol CO_2 m^{-2} s^{-1}) than watered control plants (4.5 ± 0.62 μmol CO_2 m^{-2} s^{-1}).

Chlorophyll Fluorescence (F_v/F_m)

No difference in F_v/F_m was found between well-watered and droughted plants up to Day 15 **(Figure 35)**. On Day 19 F_v/F_m was greater (p = 0.018) for the watered (0.823 ± 0.0039) than the droughted (0.800 ± 0.0070) plants. Droughted plants remained at a lower F_v/F_m through Day 35 (p = 0.002), but recovered to pre-drought levels the day after rewatering.

Nocturnal Acid Accumulation

The day before imposing drought, I found greater nocturnal acid accumulation among control plants compared to the group about to be droughted **(Figure 36)**, if a one-tail t-test was used (p = 0.032), but not if a two-tail t-test was used (p = 0.064). Because no directional difference was hypothesized for baseline measurements, the two-tail t-test is accepted as evidence that neither group had a greater intrinsic tendency towards acid fluctuations. However, if the controls did tend toward greater acid accumulation, this would bias our results in a conservative direction, i.e. make it harder to demonstrate CAM expression in droughted plants.

On Days 16-17 a marginally significant difference ($p = 0.065$) was found, with a mean among droughted plants of 80.0 ± 7.38 μmol H^+ g^{-1} DW, and among control plants of 53.9 ± 13.93 μmol H^+ g^{-1} DW. On Days 19-20, although the droughted mean (104.3 ± 59.50 μmol H^+ g^{-1} DW) exceeded the watered (87.0 ± 16.95 μmol H^+ g^{-1} DW), the difference was insignificant. The high degree of variability among the droughted plants, as individuals shut down at different rates, was one factor making it impossible to achieve a significant result.

Late in the experiment, droughted plants unequivocally accumulated more nocturnal acid than controls. On Days 27-28, a difference emerged ($p = 0.016$) with the drought treatment accumulating 216.7 ± 40.68 μmol H^+ g^{-1} DW and controls accumulating 56.0 ± 31.26 μmol H^+ g^{-1} DW. On Days 34-5, this difference increased ($p = 0.011$), with well-watered plants at 6.8 ± 47.86 μmol H^+ g^{-1} DW, and droughted plants at 278.4 ± 42.70 μmol H^+ g^{-1} DW. This difference had disappeared by two days after rewatering.

Discussion

Leaf Anatomy

Measurements confirmed my initial hypothesis that *S. ternatum* has a far thicker and more succulent leaf than its congeners. The trout lily had leaves about half as thick as did *S. ternatum*, but no other species came closer than this. The low stomatal density, stomatal clustering, and amphistomy of *S. ternatum* are likewise advantageous in arid environments. Stomatal clustering reduces transpirational water loss through overlap of stomatal gaseous diffusion shells (Gan et al. 2010). Amphistomy conserves water by facilitating CO_2 mesophyll diffusion from both upper and lower leaf surfaces (Smith and Hughes 2009), and is not usually found in understory plants, most of which are hypostomatous (Neufeld and Young 2014), although it does tend to correlate positively with leaf thickness (Smith and Hughes 2009). *S. ternatum* leaf cross-sections also show

chlorophyll throughout the lower mesophyll, not just near the adaxial surface (**Figure 37**). This suggests that high light penetrates to lower chloroplasts, an anatomy which again better fits the stereotype of a "sun leaf" rather than a "shade leaf."

Light Capture in the Field

My study confirms that *S. ternatum* functions as a sun plant in seasons of high understory light, before canopy leaf out and after canopy senescence. With its heteroptic phenology, *S. ternatum* had growth spurts in each of these sunny windows, producing two leaf flushes that each lasted about half a year. The spring featured elongation of existing stems, rapid production of large leaves, and flowering, while autumn saw production of new stems and of leaves that were smaller than those formed in spring. Overwintered stems elongated continuously in spring, in contrast with overwintered leaves which senesced as new spring leaf whorls emerged at stem apices. Leaves grew to their maximum length in April and May, corresponding to the highest light measurement of 847 μmol photons m^{-2} s^{-1} on April 27.

Spring photosynthetic rates were high, total chlorophyll levels were generally lower in spring than in summer measured either by mass or surface area, and the chl a/b ratio was higher in spring than in summer. Therefore, *S. ternatum* plants in the springtime exhibited many traits associated with high-light plants. The inconsistent mid-April results for total chlorophyll—with chl g^{-1} FW appearing higher than surrounding dates, and chl cm^{-2} appearing lower **(Figure 19)**—may relate to shifting leaf proportions in this season of rapid growth. Flowering was observed during the first three weeks of May as the canopy closed, with understory light dropping from 670 μmol photons m^{-2} s^{-1} on May 6 to 172 μmol photons m^{-2} s^{-1} on May 13.

Understory light declined to a low of 8 μmol photons m^{-2} s^{-1} on June 21. Spring-formed leaves acclimated to this transition and persisted through the summer, albeit with scarce light for

photosynthesis. Light was minimal through August, with few sunflecks, especially given that *S. ternatum* is shaded both by the tree canopy and by taller herbaceous species. Total chlorophyll rose in summer, measured either by mass or surface area. This contradicts the generalization that total chlorophyll by surface area should be equal for sun and shade plants (Neufeld and Young 2014), but echoes observations of *Hoya carnosa* mentioned above (Martin et al. 2010). The chlorophyll a/b ratio decreased in summer, a characteristic response of many, if not all, plants to shading (Boardman 1977, Murchie and Horton 1997). *Sedum ternatum* responded to artificial light flecks with an increased photosynthetic rate, and may thus achieve modest carbon gain in summer, though leaves showed increasing herbivore damage over the summer and were often quite ragged by fall. In addition, the light fleck data showed only small increases in photosynthesis rates compared to what was possible in the spring or fall. The fall growth spurt began as early as August, perhaps powered by carbohydrates stored in spring. Future research could examine possible triggers of this growth spurt, for example the increasing damage by trampling and herbivory inflicted on the plants throughout the summer.

New stem production was the most striking feature of fall growth, and was only seen in this season. Prostrate *S. ternatum* stems are vulnerable to animal trampling and abrasion from jagged boulders, so abundant stem production is essential. Beginning in late summer and continuing through canopy senescence, a profusion of young stems arose from nodes of older stems, and the resulting tangle of old and new stems overwintered until stems resumed elongation in spring. Light increased with canopy senescence, but peaked at less than half the springtime high, consistent with the lower autumn solar angle. The high was 290 μmol photons m^{-2} s^{-1} on Oct. 30, dropping to 251 μmol photons m^{-2} s^{-1} by Dec. 4. Photosynthesis was nevertheless robust, and many new leaves appeared in fall.

In autumn, *S. ternatum* risks burial under falling leaves. Some plants did disappear beneath the leaf litter, but those elevated by boulders, tree trunks, or large tree roots still received

significant light, and growing stems sometimes pierced dead leaves **(Figures 1b, 26b).** Some light was likely transmitted through leaf litter to plants below. Fall-formed leaves never reached the size of spring-formed leaves, and probably contributed less to annual carbon budgets. Their primary role seemed to be surviving winter to jump-start spring photosynthesis until new leaves appeared. Larger spring-formed leaves then powered growth and flowering. Further research is needed to elucidate how *S. ternatum* benefits from producing two leaf flushes per year, given the energy expenditure required. Perhaps smaller fall leaves have a lower water content than larger spring leaves, helping them survive winter freezing and thawing cycles **(Figure 38)**.

This study did confirm that *S. ternatum* is among those plant species that produce winter anthocyanins. Reddening was observed on field plants in Feb. 2014 and Feb. 2015, and spectral analysis in 2015 confirmed that anthocyanin levels increased in February, then declined in late March and April. Anthocyanins presumably provide photoprotection in months when photosynthetic light reactions outpace the temperature-dependent Calvin cycle, sometimes producing excessive energy (Hughes et al. 2005; Hughes and Smith 2007a, 2007b; Hughes et al. 2010). Widespread reddening was not seen in winter 2016, the warmest winter of this study, supporting the hypothesis that anthocyanins protect against the deadly duo of high light and cold. Reddening of entire plants was uncommon in other seasons, although occasional redness appeared on leaf edges, undersides, and stems. Red leaf margins are frequently observed and may be related to protecting these portions of the leaf from photoinhibition (Hughes and Lev-Yadun 2015).

This discussion of seasonal light and plant phenology has shown that the thick *S. ternatum* leaf not only may provide drought tolerance, but also enhances understory light capture by functioning as a sun leaf, in spring and autumn when the canopy is absent. This allows it to assimilate enough carbon to persist on minimal photosynthesis under shady conditions in the summer. In these bright windows it captures enough energy to produce two leaf flushes per year, one adapted for winter and the other for rapid spring growth followed by summer idling, and both

sets of leaves show the capacity to adjust key pigment levels as light conditions change. The thick leaf is therefore adaptive for light harvest while still functioning for water storage that allows it to colonize drier understory microhabitats.

Drought Tolerance and CAM Expression

Besides anatomical leaf thickness and succulence, this study demonstrates that low-level CAM expression enhances drought tolerance in *S. ternatum.* Although I did not measure CAM expression in field plants, my greenhouse experiment confirmed that *S. ternatum* engages low-level CAM under water stress, which would enhance survival in the face of rapid water fluctuations in shallow substrate. Our experimental results reveal a closely synchronized suite of drought responses in *S. ternatum,* culminating in CAM idling. The first physiological reaction was a decline in photosynthetic rates that became significant 11 days after drought onset, when droughted pots had lost almost half of their starting soil water weight. Photosynthesis continued to decline from Day 12-18. The next measured response was declining efficiency of photosystem II, first observed on Day 19 in a reduced F_v/F_m. By Day 26, with 70% of starting pot weight lost, diurnal gas exchange had shut down completely, and although F_v/F_m briefly rose again, it then plunged to its lowest point by Day 35. Droughted plants did not lose succulence until Day 27, and after this point FW:DW dropped until day 35. Water potential likewise changed nearly synchronously with succulence, declining from Days 28-35. The ability to maintain succulence so late into the experiment testifies to the very high drought tolerance of *S. ternatum.*

CAM expression as measured by nocturnal acid accumulation cannot be unambiguously assessed for the middle days of this experiment. CAM cycling may have begun around Days 16-17, when droughted plants showed a higher mean than controls for acid accumulation with a near-significant *p*-value of 0.065. However, high variability among droughted plants on Days 19-20

makes it impossible to identify a significant trend. Once diurnal gas exchange shut down completely, plants unambiguously moved into CAM idling, with peak acid accumulation on Days 28 and 35, and the lowest FW:DW and F_v/F_m of the experiment on Day 35. I did not measure nocturnal gas exchange, but presume from earlier research (Gravatt and Martin 1992) that stomata remained closed at night and all acid fluctuations came from recycled respiratory CO_2.

CAM idling may help plants maintain metabolic functioning for rapid recovery after drought (Harris and Martin 1991b), and droughted plants recovered almost immediately after I rewatered them on the morning of Day 35. One day later, drought and control treatments had statistically equivalent FW:DW ratios, water potential, and chlorophyll fluorescence. Two days later, no difference in nocturnal acid accumulation was found between the groups, indicating that CAM expression ceased quickly upon rewatering. Four days after rewatering, the drought treatment displayed a statistically higher photosynthetic rate than did controls. This quick recovery from severe water stress demonstrates strong resilience of *S. ternatum* in the face of drought.

These results tend to confirm those of Gravatt and Martin (1992). Because they grew their experimental plants in smaller containers and only imposed three days of drought, their timescale for soil dry down differed from my longer experiment. However, they too found that well-watered *S. ternatum* displayed only C_3 photosynthesis, with no acid fluctuations. After three days' drought, they found no significant decline in FW:DW, corresponding to my observation that leaf succulence does not diminish until water stress is more severe. After three days of drought, they report that the carbon assimilation rate of *S. ternatum* dropped by 51%, roughly echoing our finding that around Day 12-13 the diurnal photosynthetic rate of droughted plants dropped to about half that of controls, bearing in mind that soil dried more slowly in my larger pots. Finally, they found that *S. ternatum* initiated low-level CAM as gas exchange declined, and although they confirmed CAM cycling they did not drought plants long enough to induce CAM idling. At the

end of my experiment, acid accumulation from CAM idling was approximately twice that reported by Gravatt and Martin from CAM cycling after three days of drought.

Although my study lasted over a month, this did not test the full limits of drought tolerance in *S. ternatum,* as I rewatered ~10 days after onset of wilting. Given the plants' rapid and full recovery, they might well have survived a longer drought. Yet considering that *S. ternatum* thrives in temperate forests, its full drought tolerance is probably well below that of *Sedum* species native to hotter, brighter environments. For example, an experiment on the CAM plant *Sedum rubrotinctum* (Teeri et al. 1986)—whose common name of "Jelly Bean Plant" evokes its rounded, water-storage-enhancing leaf form—found that after *two years* of drought, apical leaves were still alive and had some turgor. In my study, given that *S. ternatum* wilted fairly quickly once pots became very dry, it seems unlikely that it would have survived that long without water.

The need to prevent water loss by closing stomata raises the threat of photoinhibition after atmospheric CO_2 intake declines. CAM may protect against photoinhibition by internally generating CO_2 in these circumstances (Adams and Osmond 1988). This question is posed in a study (Castillo 1996) of *Sedum album,* a northern temperate succulent often growing in shallow soil that performs CAM cycling when well watered. Under experimental drought, CAM cycling in *S. album* increased, and diurnal gas exchange decreased, as a sharp decline in F_v/F_m indicated photoinhibition. As with *S. ternatum,* recovery after rewatering was rapid. However, Castillo concludes that although CAM cycling may provide modest diurnal photoprotection, the concomitant production of antioxidative enzymes may be more essential. Although I did not measure antioxidative enzymes, my results suggest that low-level CAM alleviated photoinhibition in drought-stressed *S. ternatum.* Photosynthesis in droughted plants first declined on Day 11, and photoinhibition (as evidenced by declines in F_v/F_m) was first detected on Day 19. As CAM idling began around Day 28, F_v/F_m remained lower in the drought group, with the difference peaking on Day 35. Although this indicates photoinhibition, the efficiency of photosystem II might have

declined even more without this engagement of low-level facultative CAM. Furthermore, the rapid recovery of F_v/F_m upon rewatering suggests that the damaging effects of photoinhibition were able to be quickly repaired, something that might have taken longer had there not been internal recycling of respired CO_2 via CAM idling.

Conclusion

In sum, my experiment identified physiological traits that enhance the fitness of *S. ternatum* in drier understory microhabitats. Faced with a fluctuating water supply, *S. ternatum* is able to continue some photosynthesis with minimal water, without wilting, for a significant amount of time, and recover quickly upon rewatering. Its succulent leaf and CAM expression enhance survival in habitats inaccessible to congeners with thinner leaves and C_3 photosynthesis alone. Although the thick leaf of *S. ternatum* does not possess the "shade leaf" characteristics that would allow significant growth in the summer understory, the plant is able to accomplish most of its carbon gain in the sunny windows of spring and autumn. The thick, succulent leaf does not appear to be simply a byproduct, now irrelevant, of traits once needed by a hypothetical high-light ancestral species. In the present, they allow *S. ternatum* to occupy an unusual dry micro-habitat alongside understory congeners with very different leaf characteristics and which grow in deeper soils less prone to drought.

Parenthetically, other species may symbiotically benefit from co-occurring with a drought-tolerant *Sedum*. A study of *S. album* growing with moss on limestone pavements in Sweden (Sand-Jensen et al. 2015) showed that while *S. album* benefits from the water-holding capacity of moss when they co-occur on rocks, *S. album* in turn benefits the moss through shading and wind protection. *S. album* has such low transpiration and poses so little competition for water that when under water stress the mosses derive a net benefit from *S. album,* which provides shade, and

deflects sunlight through its reflective leaf surface. Future research could examine whether *S. ternatum* likewise benefits the mosses that usually surround it.

While shedding light on how *S. ternatum* survives in the present, our study also raises intriguing questions about its past and future. The large *Sedum* genus poses ongoing phylogenetic challenges (Mort et al. 2001, Nikulin et al. 2016) and until further research is completed, the history of CAM evolution within it will remain speculative. One hypothesis posits that weak CAM such as that found in *S. ternatum* constitutes "incipient" CAM, i.e., a first step towards development of strong and obligate CAM (Borland and Griffiths 1990). Weak CAM species would then constitute a genetic reserve for radiations of new CAM lineages (Silvera et al. 2010). In this hypothesis, weak CAM plants harbor genetic keys to the origins of C_3-CAM transitions in many plant lineages—and by the same token keys to genetically engineering CAM genes into C_3 agricultural crops. Alternatively, *S. ternatum* could descend from high-light adapted ancestors expressing strong CAM, with these adaptations becoming lost or latent after understory colonization. As a C_3-CAM intermediate, *S. ternatum* could also be a hybrid of C_3 and CAM parents, as seen in *Yucca gloriosa*, a C_3-CAM intermediate resulting from hybridization between the C_3 species *Yucca filamentosa* and the CAM species *Yucca aloifolia* (Heyduk et al. 2016).

Ongoing genomics and transcriptomics research is now revealing which genes distinguish C_3 from CAM species, and which genes are turned on when CAM is engaged (Hartwell et al. 2016; Heyduk et al. 2016; Zhang et al. 2016). This research sheds light onto both the phylogenetic origins of CAM, and strategies for engineering CAM genes into C_3 agricultural crops, an attractive strategy to improve crop drought tolerance as climate change leaves our planet hotter and drier. The recent publication of the pineapple genome is a major landmark (Ming et al. 2015, 2016). Concerning facultative CAM, a recent study of *Talinum triangulare*—which performs weak CAM during drought—examined mRNA encoding a variety of CAM metabolites (Brilhaus et al. 2016).

As more such studies appear, the molecular mechanisms underpinning the diverse CAM variants will become more evident, complementing physiological studies such as the present thesis.

In the future, as climate change causes increasing heat and drought on many parts of the planet, CAM plants and genes are poised to play greater roles in both agriculture and forest ecology due to their high level of drought tolerance. This study has emphasized the unusual nature of *Sedum ternatum* in the Southern Appalachian understory at present, but if we could return to the forests of this region in the distant future, perhaps we would find succulent CAM species in greater abundance, especially if future climate change leads to less precipitation in this region. From its inconspicuous position so low in the understory, *S. ternatum* may well model traits that will enhance both its own survival, and the survival of similar species, under rapidly changing future conditions.

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Appendix I: Effects of High and Low Light on Greenhouse-Grown *S. ternatum*

Introduction

Sun- and shade-adapted leaves often exhibit anatomical and biochemical differences (Boardman 1977; Murchie and Horton 1997; Taiz and Zeiger 2010; Neufeld and Young 2014). Sun leaves are generally thicker, with less surface area, lower chlorophyll content by dry mass, and higher chlorophyll a/b ratio. Shade leaves are thinner, with greater surface area, higher chlorophyll content by dry mass, and lower chlorophyll a/b ratio (Boardman 1977). Sun and shade leaves typically have comparable total chlorophyll per unit surface area. Often, shade plants even have lower total chlorophyll per unit surface area (Boardman 1977). The greater leaf thickness of sun plants comes from a thicker palisade (Neufeld and Young 2014). These sun and shade leaf stereotypes often apply not only among different species native to high- or low-light environments, but also to individuals within a species grown in high or low light—although case-by-case variability exists. For example, *Hoya carnosa,* a succulent CAM plant, is an epiphytic vine that receives more or less sunlight depending on its height in the forest canopy. Leaves situated in higher light exhibit greater CAM expression, lower total chlorophyll on either mass or surface area basis, and thicker leaves than plants growing in shadier spots. Both groups of leaves have similar chlorophyll a/b ratios (Martin et al. 2010).

My greenhouse experiment compared *S. ternatum* plants grown in ambient greenhouse light (43% of full sun) or under 90% shadecloth (6% of full sun). As shown above, *S. ternatum* in the field spends the summer under canopy shade and the rest of the year in higher light. As a heteroptic, it produces two leaf flushes per year. Spring-produced leaves harvest high light before canopy leafout, then become largely dormant in summer. Fall-produced leaves appear before canopy senescence, then persist through the higher light of autumn and winter. *S. ternatum* clearly

acclimates well to changing seasonal light, and in this greenhouse experiment I hypothesized that plants grown under high and low light would exhibit similar acclimation. Specifically, I hypothesized that 1) shade-grown plants would have greater total chlorophyll by mass than sun-grown plants, but equal chlorophyll by surface area; 2) shaded plants would have a lower chlorophyll a/b ratio than high-light plants; 3) leaf succulence would be greater for high-light plants; 4) shaded plants would have longer stems, as height tends to be selected for in shady environments to enhance light interception (Neufeld and Young 2014).

Methods

In February 2014, 50 *S. ternatum* cuttings from the State Farm site were propagated in pots of 15 cm diameter and ~9 cm soil depth using 1 part Metro-Mix 360 soil mix (Sungro Horticulture, Agawam, MA), 1 part vermiculite, ½ part sand, and ⅓ part perlite. Plants were watered biweekly or weekly, given Osmocote 14-14-14 slow release fertilizer (The Scotts Company, Marysville, Ohio) periodically, and treated with Clearys 3336F Turf and Ornamental Systemic Fungicide (Cleary Chemicals LLC, Alsip, Illinois) and Ban-Rot 40 WP (The Scotts Company LLP, Marysville, Ohio) as needed. In April, five cuttings were taken from each of these plants to propagate 250 new plants. In June, 60 plants were placed into each of two treatments: 1) ambient greenhouse light (747 ± 72 μmol photons m^{-2} s^{-1}, mean ± SE); or 2) 90% shadecloth treatments (104 ± 12 μmol photons m^{-2} s^{-1}, mean ± SE, realized shading of 86%). Midday full sun outdoors was 1726 ± 8 μmol photons m^{-2} s^{-1} (mean ± SE; all measurements are from Aug. 26), so ambient greenhouse light was 43% of full sun, and the shadecloth treatment 6% of full sun. Plants were left to grow until they produced enough new leaves for measurements. Treatments were rotated on the bench every 2 weeks.

Leaf thickness and succulence were measured on five pairs of clones, each with one plant from the high-light and one from the low-light treatment. I used the most mature leaves formed under experimental conditions, usually the 5th whorl from the stem apex for shade plants, and the 7th whorl down for high-light plants. To measure leaf thickness, cross-sections were cut with a Leica VT 1000S vibratome (Leica Biosystems, Nussloch, Germany), imaged with an Olympus SZX12 stereoscopic microscope (Olympus Corporation, Tokyo, Japan), and measured on an Olympus IX-81 light microscope (Olympus Corporation, Tokyo, Japan) with Microsuite Biological Suite software. Four measurements were averaged per leaf cross section. To calculate succulence, on Dec. 7 samples consisting of six fresh leaves per plant were weighed on a Sartorius Practum 224 1S Analytical Balance (Sartorius AG, Göttingen, Germany), then oven-dried at 80˚ for four days and reweighed to obtain FW:DW ratio. For stem length, the longest stem of 14 plants per treatment was measured with a ruler. Primary and secondary branching were counted for the same 14 plants on the top 15 cm of stem.

To measure chlorophyll content, samples of six leaves were taken from each of five plants per treatment, and carried to the lab on ice. Fresh weight (FW) was measured on a Sartorius Practum 224 1S Analytical Balance. Surface area was calculated by laying the leaves on a flatbed scanner (CanoScan 9000F Mark II scanner, Canon USA, Melville, NY), clarifying leaf outlines with Adobe Photoshop CC 2014 (Adobe Systems Inc., San Jose, CA), and then converting pixels from these jpg images to area in cm^2 using Black Spot, a shareware program (Varma and Osuri 2013). All leaves were dipped in liquid nitrogen for 20 seconds prior to pigment extraction, then placed in a vial (6 leaves per sample) containing 3.0 or 3.5 mL dimethylformamide, macerated with a glass rod, and left to extract in a refrigerator for seven days. Absorbances were then measured in a Shimadzu UV-1800 spectrophotometer according to the procedure and equations of Porra (2002).

Results

Clones propagated from the same parent developed very different morphologies under high and low light treatments **(Figure 39a-d)**. High-light leaves, with a thickness of 1044.4 ± 42.51 μm, were 63% thicker than shaded leaves, with a thickness of 681.0 ± 45.72 μm **(Figure 40)**. High-light leaves were also more strongly curved, while low-light leaves appeared flatter, and high-light leaves had a more pronounced palisade layer **(Figure 37)**. Shaded leaves were significantly ($p < 0.001$) more succulent (16.2 $\pm$ 0.40) than high-light leaves (9.7 ± 0.56, **Figure 41)**.

Compared to high-light plants, plants grown under 90% shade cloth had longer stems with less branching. High light produced dense, tightly appressed rosettes. Shade treatment produced widely spaced leaves **(Figure 39)**. Shaded plants had a stem length of 22.4 ± 0.69 cm, significantly ($p < 0.001$) longer than high-light plants, whose stem length was 16.3 ± 0.46 cm **(Figure 42)**. High-light plants had primary branching of 20.2 ± 1.08 per 15 cm stem length, significantly ($p < 0.001$) greater than shaded plants, with only 7.9 ± 0.71 branches per 15 cm stem **(Figure 43)**. High-light plants had secondary branching of 12.0 ± 2.65 per 15 cm stem, significantly ($p < 0.001$) greater than shaded plants, which showed almost no secondary branching, only 0.6 ± 0.29 per 15 cm stem length **(Figure 44)**.

Shaded plants had higher ($p < 0.001$) total chlorophyll than high-light plants by mass, with 485.1 ± 26.58 μg chl g^{-1} FW for high light and 840.9 ± 11.28 μg chl g^{-1} FW for low light **(Figure 45)**. By surface area, the groups had equivalent total chlorophyll, with 33.8 ± 1.76 μg chl cm^{-2} leaf surface for high-light plants and 38.3 ± 1.34 μg chl cm^{-2} for shaded plants **(Figure 46)**. Shaded plants, at 2.3 ± 0.08, had a significantly higher chlorophyll a/b ratio ($p = 0.001$) than the high-light group, at 1.6 ± 0.09.

Discussion

In many respects, experimental high- and low-light treatments produced *S. ternatum* leaves conforming to stereotypes of shade and sun leaves. Shaded plants developed higher chlorophyll g^{-1} FW than high-light plants, but the groups had equivalent chlorophyll cm^{-2} surface area. Shaded plants developed longer stems, enhancing light harvest. The dense packing of high-light leaves along shorter stems protects against intense light, while the widely spaced leaf arrangement along stems of low-light plants minimizes shading among leaves, an important adaptation for plants growing in a light limited environment. My results did not support the initial hypothesis regarding chlorophyll a/b ratios, as shaded plants showed a higher a/b ratio than high-light plants, but on occasion, this has been found in other plants (Murchie and Horton 1997). Also, succulence was lower for high-light plants, perhaps reflecting greater dry mass in these leaves, e.g., denser cell packing and/or thicker cell walls.

This experiment confirms that *S. ternatum* shows morphological and biochemical plasticity in acclimating to variable light. Many of the differences between treatments were striking to the eye, such as the deeper green color and more elongated stems of the shade treatment. However, my results also confirm that stereotypes of "sun" and "shade" leaves do not always hold true in all details. It is important to test the responses of individual species on a case-by-case basis. To further complicate matters, both my low- and high-light treatments looked very different from any plants observed at my field site **(Figure 39)**. Field plants have the most clearly defined terminal rosettes of the three groups, consistently exhibiting three or four leaf whorls distinctly separated from the lower whorls. Although the lower whorls of field plants are not tightly packed together on the stem, as seen in our high-light treatment, neither do stems of field plants become greatly elongated, as seen in our low-light treatment. Unlike both greenhouse treatments, field plants rarely exhibit any above-ground branching; instead, new stems emerge from nodes of parent plants around or just below ground level. Finally, the very deep green color of plants grown under the

shadecloth was much darker than the color of any leaves observed in the field. This corresponds to the fact that the shadecloth-grown plants had a higher total chlorophyll level (840.9 ± 11.28 μg chl g^{-1} FW) than field plants, for which total chlorophyll peaked around 600 μg chl g^{-1} FW **(Figure 19).**

This brief assay also shows that *S. ternatum* achieves significant growth at the low light level of ~100 μmol m^{-2} s^{-1} found under the shade cloth. After placing plants under the cloth, they produced new leaves for experimental measurements in a reasonable time. Some of this growth may have been fueled by stored carbohydrates, but our fieldwork demonstrated that field plants ceased production of new leaves as light levels dropped after canopy closure. The steady growth under our cloth shows that *S. ternatum* makes good use of relatively low light. On the other end of the spectrum, high-light plants easily tolerated ~750 μmol m^{-2} s^{-1}, growing profusely with no sign of photoinhibition. The extremely different phenotypes of the two groups were well adapted to light harvest in very different levels of irradiance.

Figures

Figure 1a. In the summer understory, *S. ternatum* is hidden by taller herbaceous plants. Here, a rosette (bottom center) is partially illuminated by a sunfleck. June 2015, Blue Ridge Parkway.

Figure 1b. In autumn, *S. ternatum* is often buried by fresh leaf litter. Here, a stone elevates plants above the dead leaves. Oct. 2015, State Farm.

Figure 1c. *S. ternatum* is seen interspersed with moss and a few taller perennials on a large boulder. June 2015, Blue Ridge Parkway.

Figure 2. Winter leaf reddening of *S. ternatum* was seen in 2014 and 2015. The color change was found to be caused by the production of anthocyanin pigments. February 2014, State Farm.

Figure 3a. Large boulders are a dry microhabitat colonized by *S. ternatum.* Here, plants grow on the shaded left-hand portion of this boulder. June 2015, Blue Ridge Parkway.

Figure 3b. Eroding slopes are another dry microhabitat in which *S. ternatum* outcompetes less drought-tolerant plants. Here, most other vegetation has washed away on this south-facing, nearly vertical slope. May 2014, State Farm.

Figure 3c. *S. ternatum* growing in dry, shallow substrate on top of a rock. The surrounding moss appears dessicated, but the *S. ternatum* is not wilted. April 2015, State Farm.

Figure 3d. *S. ternatum* sometimes climbs up the base of a tree trunk, where moisture availability is limited. April 2015, State Farm.

Figure 4a. My field site was Appalachian State University's State Farm Trails Area in Boone, NC. Sunfleck. Summer 2014.

Figure 4b. Below, the author looks for plants at the field site. Winter 2014.

Figure 5. This cross-section of *Stellaria pubera* shows measurements of leaf thickness. Measurements were taken at the thickest point excepting the midvein (which begins to bulge in the upper left-hand corner). Four measurements (blue lines) were averaged per cross-section. The scale bar is 200 μm.

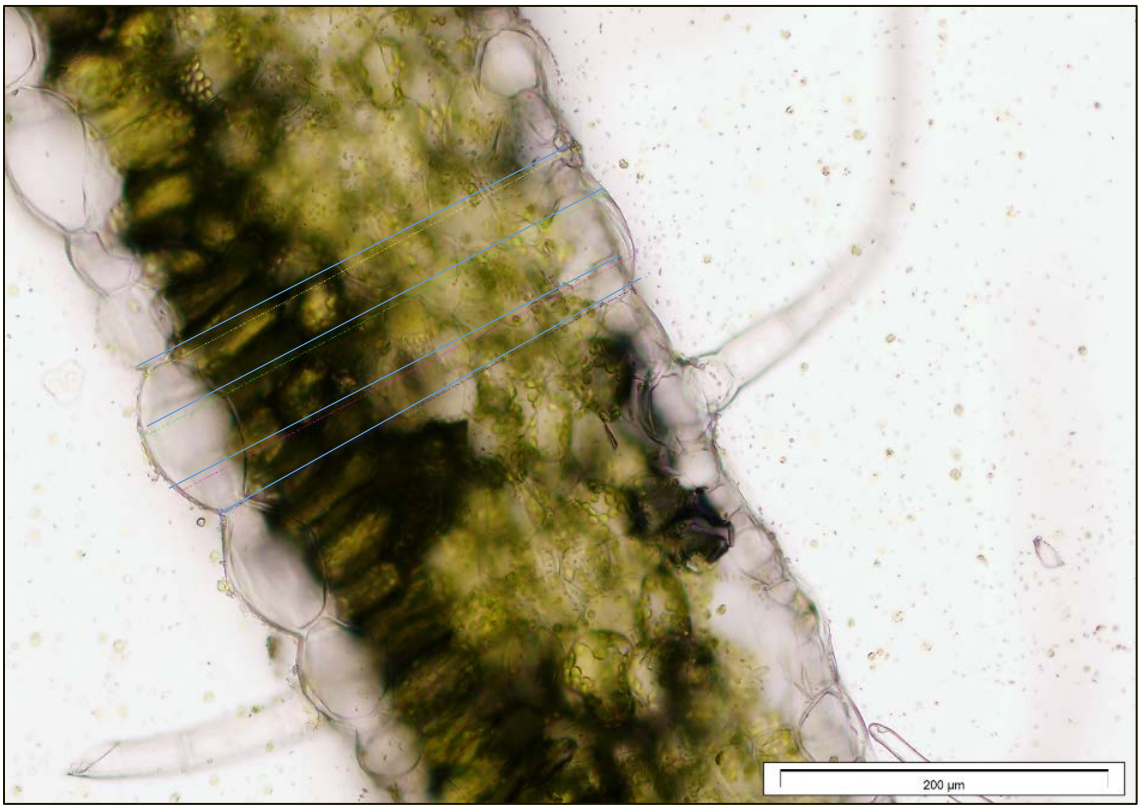


Figure 6. This micrograph of an epidermal peel shows stomatal density (here, of the abaxial surface). Arrows point to paired stomata, indicating stomatal clustering, a trait that enhances drought tolerance.

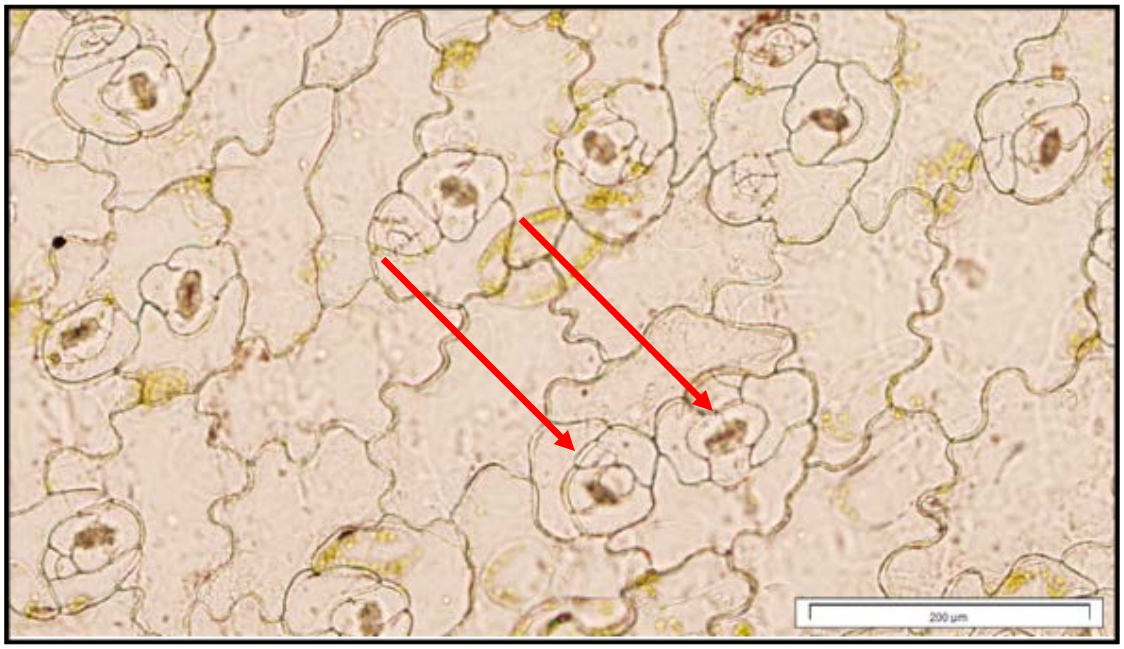


Figure 7. Overwintered field leaves senesced in the spring. In March 2015, red tags were placed on leaves formed the previous fall. By May, red-tagged leaves were senescing, and new leaves had emerged at the stem apex. May 2015, State Farm.

Figure 8. Blue-tagged field leaves were used for spring growth measurements. These large, spring-formed leaves harvest the high sunlight of early spring and support flowering just as the canopy closes. May 2015, State Farm.

Figure 9. 900 cm^2 quadrats were established to measure autumn leaf and stem production. Leaf litter often had to be carefully pulled back to find the plants. Oct. 2015, State Farm.

Figure 10. Yellow threads were placed beneath the terminal rosette of older stems to measure autumn leaf production at the stem apex. Nov. 2015, State Farm.

Figure 11. Red threads were placed beneath the terminal rosette of new stems to measure autumn leaf production at the stem apex. Nov. 2015, State Farm.

Figure 12. An LED flashlight was used to create lightflecks for photosynthesis measurements.

Figure 13. Pots were arranged on a greenhouse bench for the drought experiment. The drought treatment is at one end of the bench, and controls at the other end. Pots were rotated on a weekly basis. November 2015, Appalachian State University Greenhouse.

Figure 14. Leaf thickness of *S. ternatum* was compared to that of understory congeners. Bars that do not share the same letter indicate a significant difference ($p \leq 0.05$) in leaf thickness. Bars are mean ± SE of leaf samples from 3-5 plants.

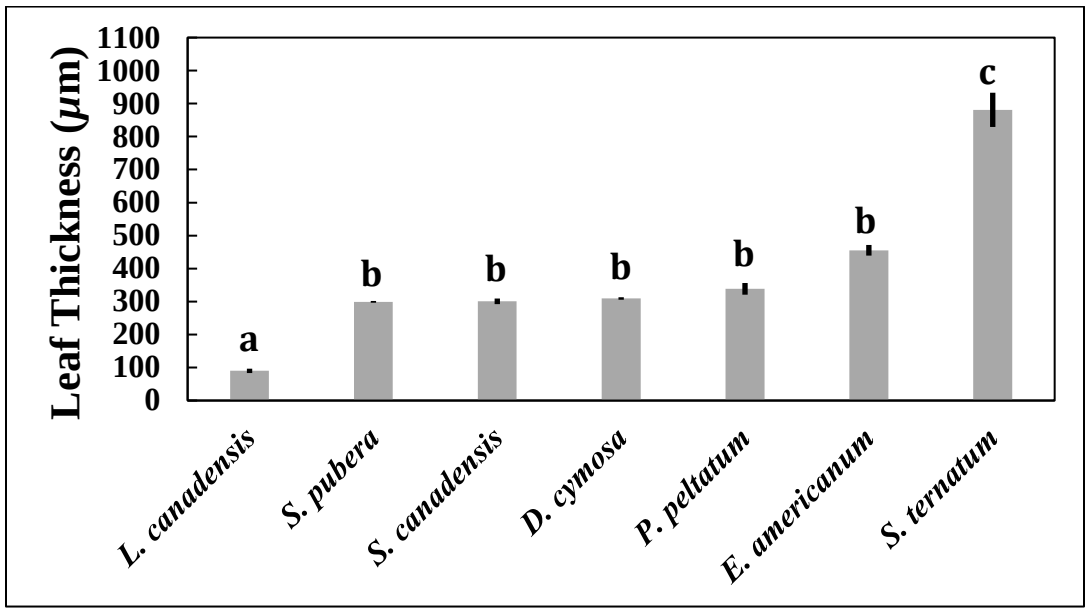


Figure 15. Leaf succulence of *S. ternatum* was compared to that of understory congeners. Bars that do not share the same letter indicate a significant difference ($p \leq 0.05$) in FW:DW. Bars are mean ± SE of leaf samples from five plants.

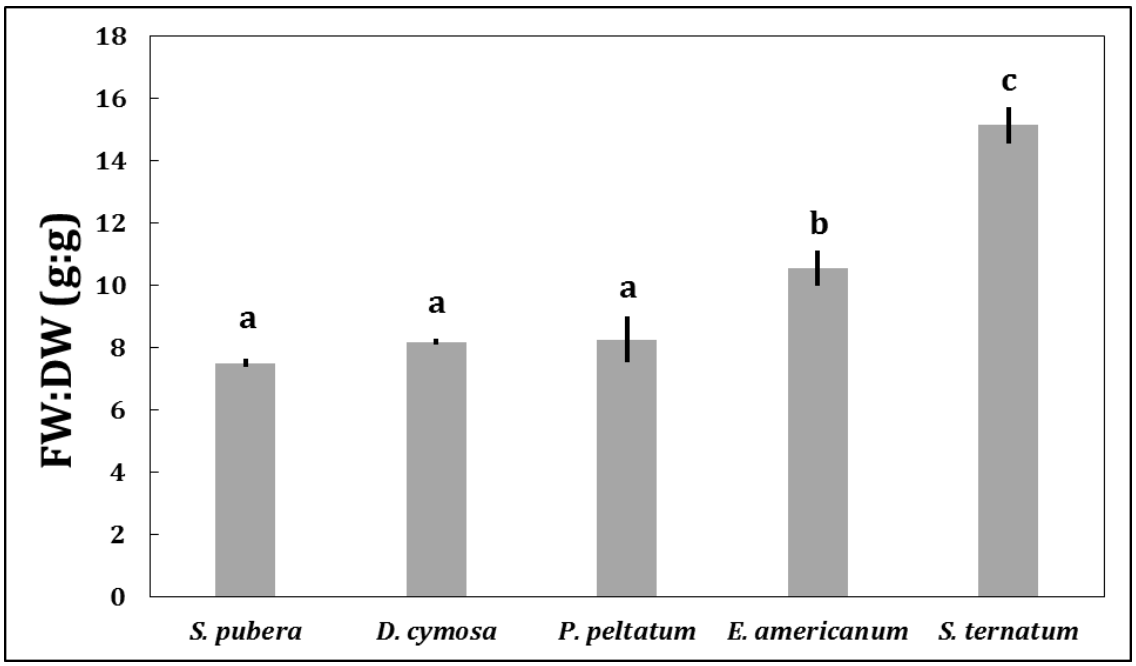


Figure 16. *S. ternatum* and *E. americanum* often grew side-by-side in the springtime. Both benefit from thick leaves to harvest spring light, but the leaf of *S. ternatum* is approximately twice as thick as that of *E. americanum*. April 2015, State Farm.

Figure 17. *S. ternatum* exhibits amphistomy. The asterisk indicates that the two leaf surfaces have significantly different ($p \leq 0.05$) stomatal density. Bars are mean ± SE of stomata counted on three epidermal peels from each of five plants.

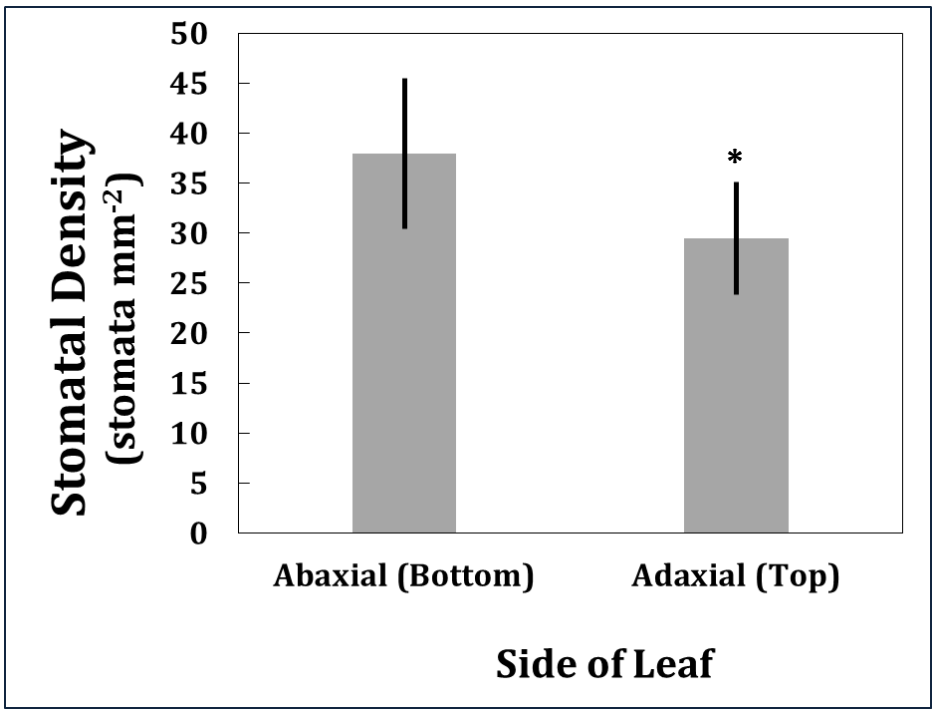


Figure 18. Photosynthetically active radiation (PAR) in the understory changes by season. Each data point represents mean ± SE of light measured above ten *S. ternatum* patches.

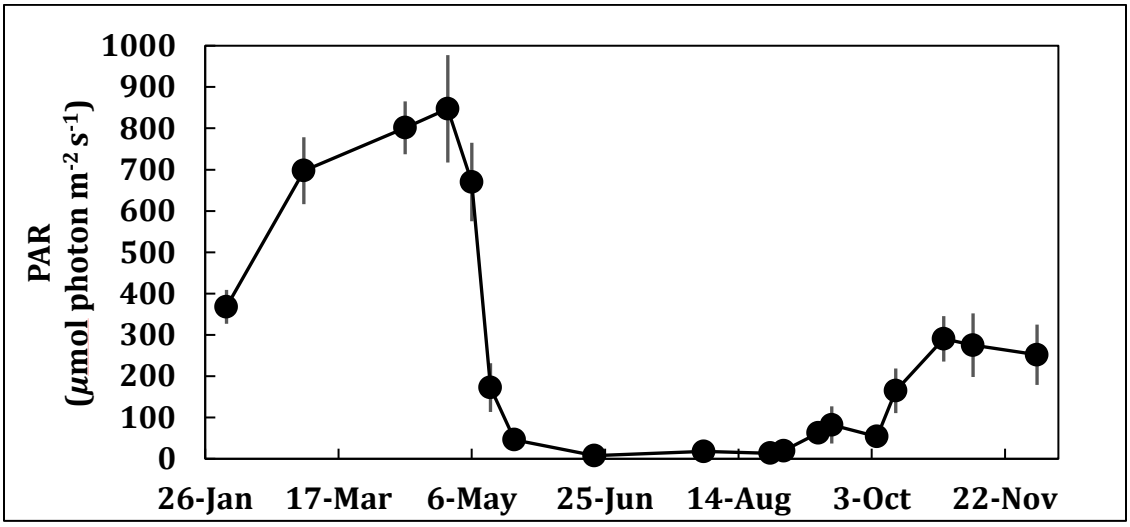


Figure 19. Total chlorophyll of field-grown *S. ternatum* was compared by season. Each data point represents mean ± SE in leaf samples from nine *S. ternatum* patches, either per unit FW (left-hand axis) or per unit surface area (right-hand axis).

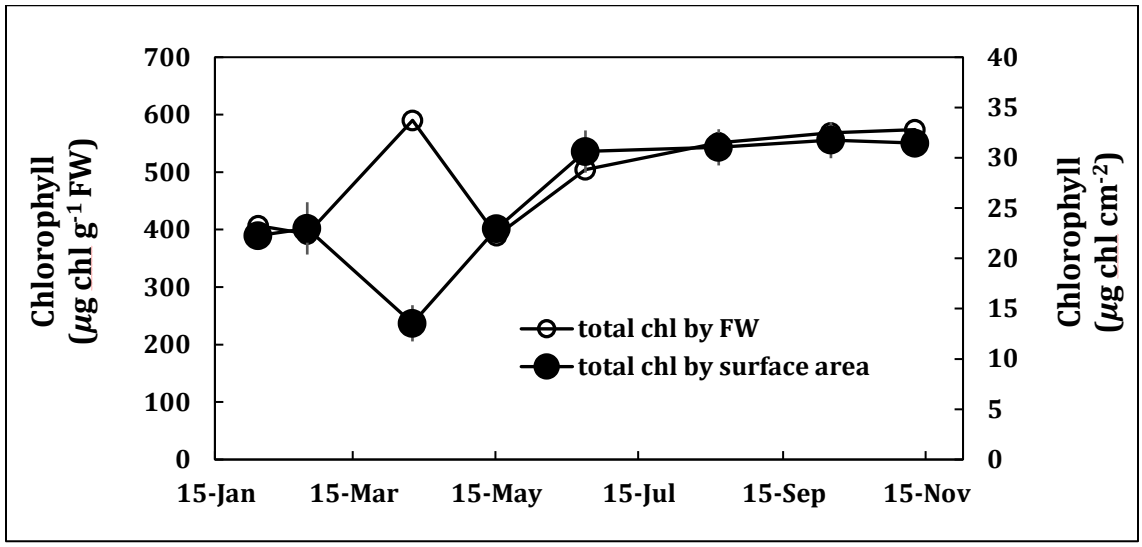


Figure 20. The chlorophyll a/b ratio of field-grown *S. ternatum* was compared by season. Each data point represents mean ± SE in leaf samples from nine *S. ternatum* patches. Error bars are smaller than data points.

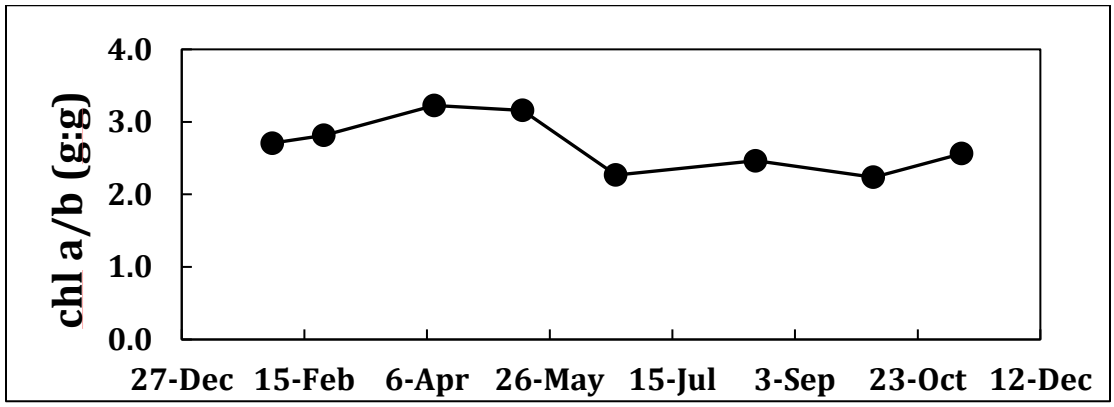


Figure 21. The anthocyanin content of field-grown *S. ternatum* was measured in winter and spring. It is shown here in comparison with total chlorophyll. Each data point represents mean ± SE measured in leaf samples from nine *S. ternatum* patches.

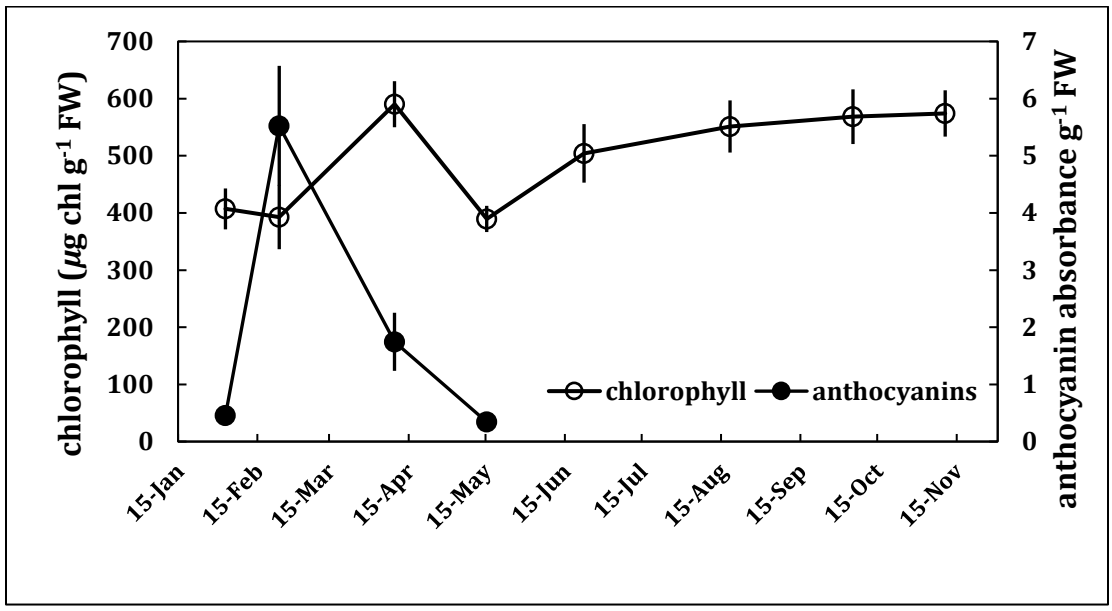


Figure 22. *S. ternatum* showed stem elongation and production of new leaf whorls in spring. Each data point represents mean ± SE of five plants from each of 10 *S. ternatum* patches. Data points represent cumulative totals beginning in early April.

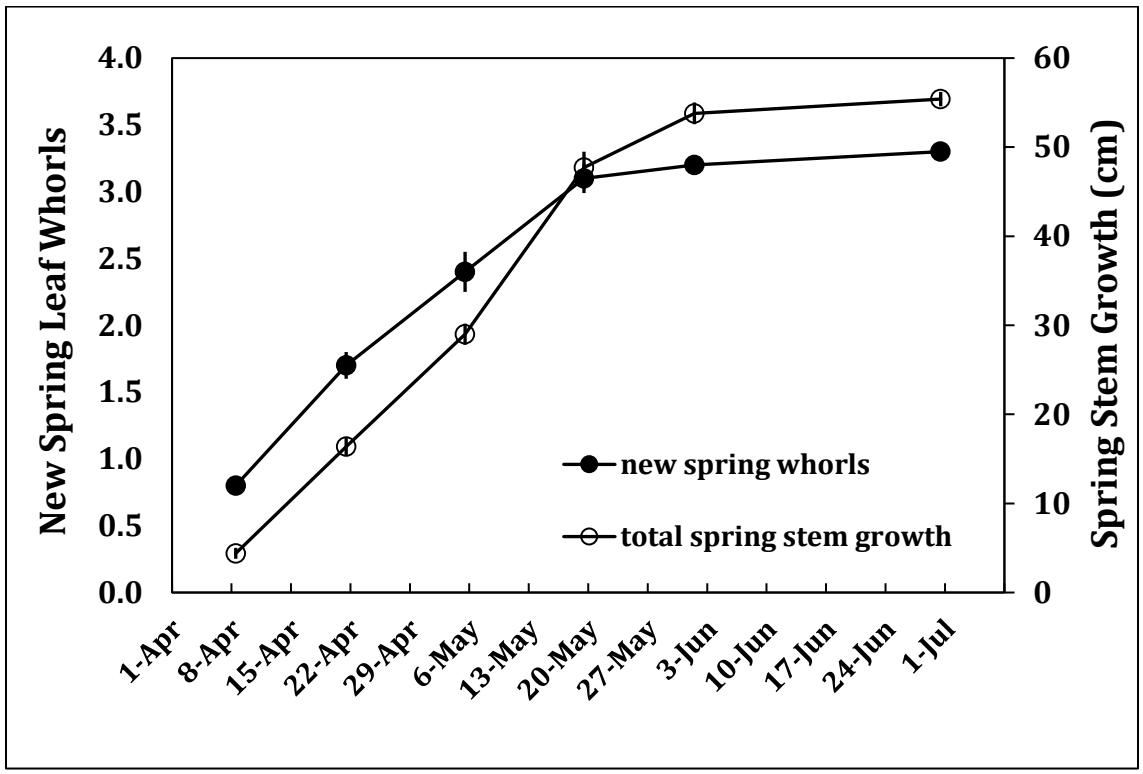


Figure 23. *S. ternatum* showed a springtime increase in leaf length. Leaves that had overwintered grew slightly but then senesced, while spring-formed leaves grew rapidly before canopy closure. Data points represent mean ± SE for five plants from each of ten patches.

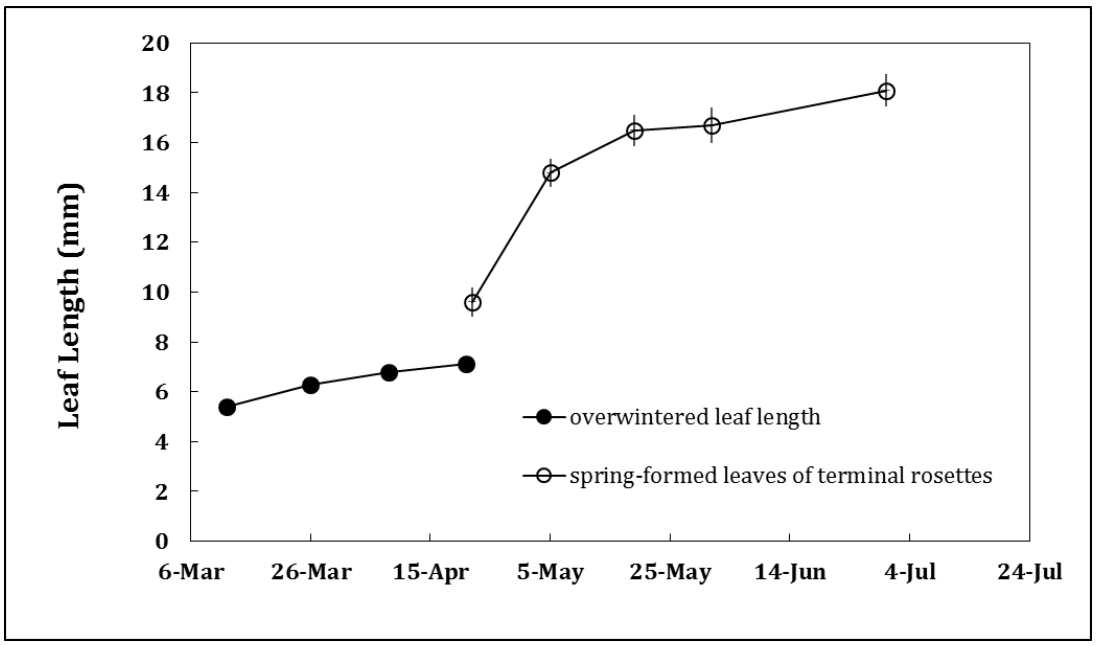


Figure 24a. *S. ternatum* flowered in the spring, seen here in a close-up of the inflorescence. State Farm, May 2015.

Figure 24b. *S. ternatum* flowered in the spring, seen here with a flowering stem in the foreground, and non-flowering stems carpeting the forest floor behind it. State Farm, May 2015.

Figure 25. *S. ternatum* showed herbivore damage in the late summer of 2015. Further research could explore whether such damage helps to trigger onset of the “fall” growth spurt, which in fact begins in late summer. July 2015, Blue Ridge Parkway.

Figure 26a. New *S. ternatum* stems arose from nodes of parent plant stems in autumn. October 2015, State Farm.

Figure 26b. Growing *S. ternatum* shoots sometimes pierced leaf litter. November 2015, State Farm.

Figure 27. *S. ternatum* produced new stems and leaf whorls in autumn. Data points represent total sums of stems (left axis) and new leaf whorls (right axis) over ten 900cm^2 quadrats. There are no error bars because these are totals, not means.

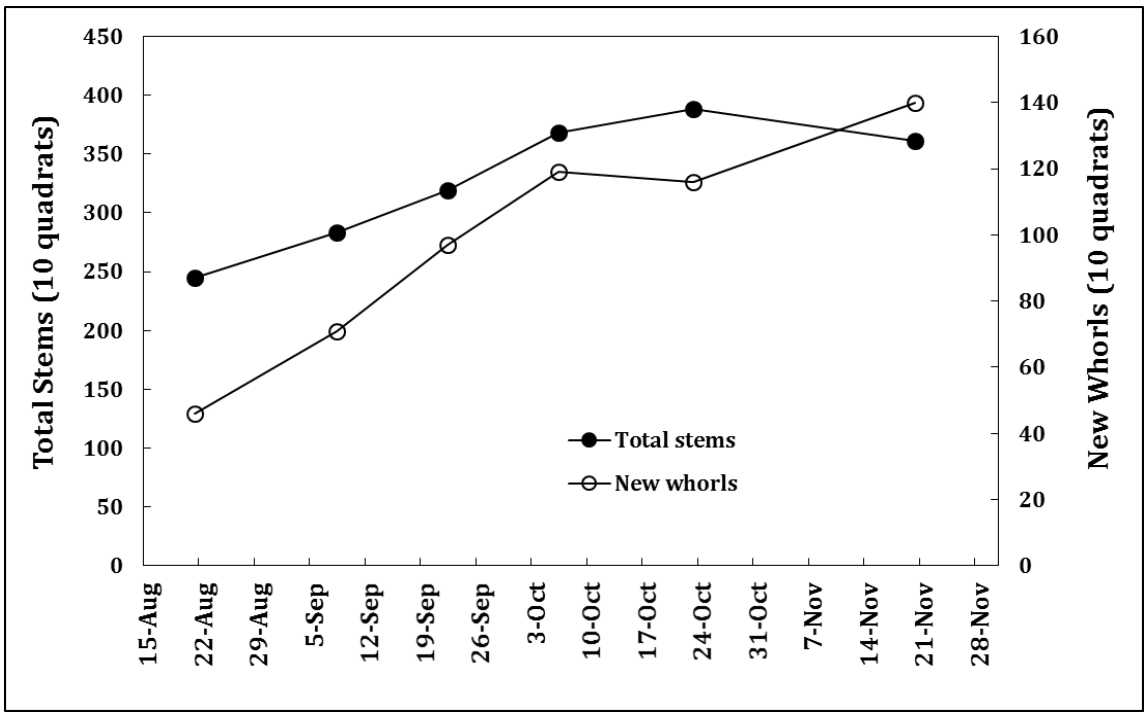


Figure 28. Photosynthetic light curves were made on field plants in 2015. June 2015, State Farm.

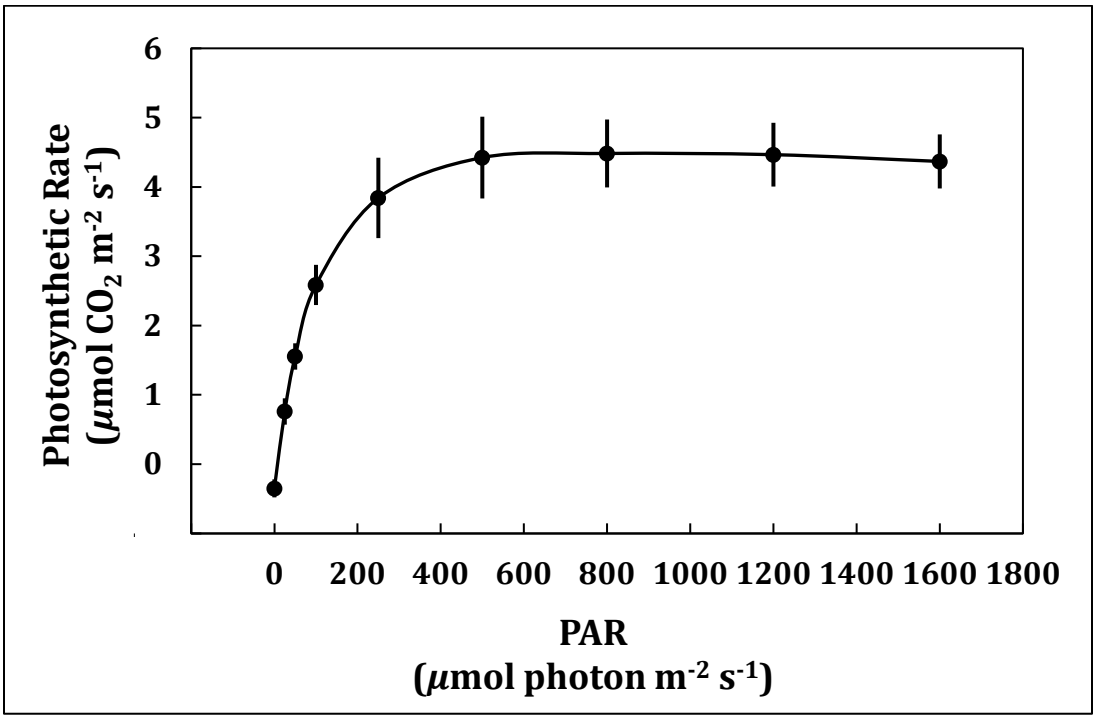


Figure 29. Photosynthetic rates were measured in ambient (low) light and under high-light flecks. Bars represent mean ± SE. On June 19, n = 2 for the high-light fleck, and n = 10 for ambient. For all other dates, n = 6-10 for both ambient and high light.

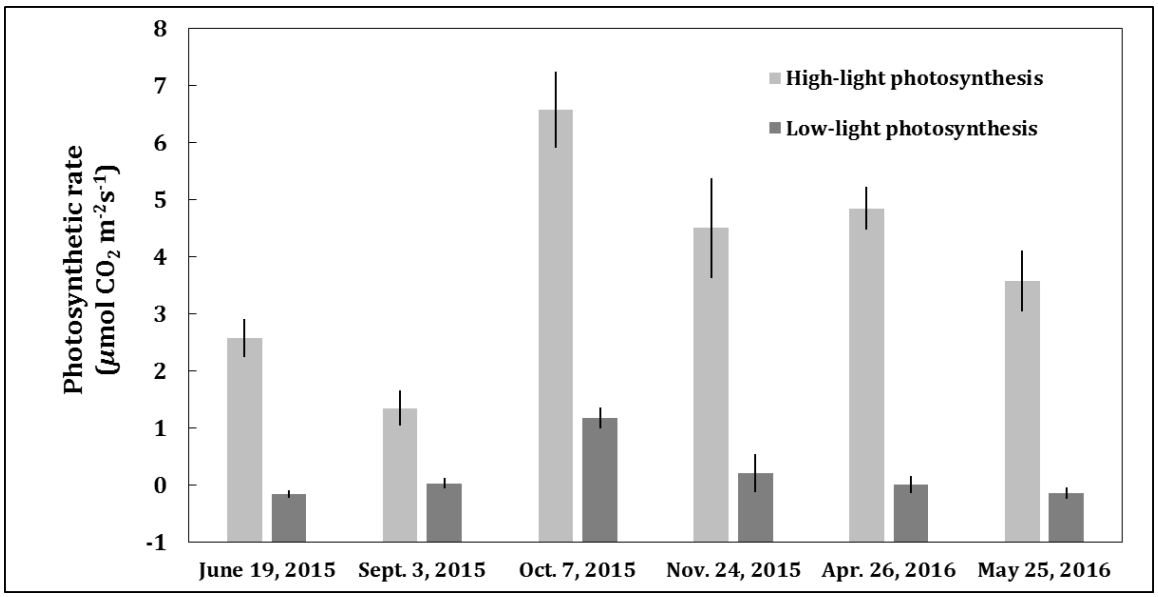


Figure 30. Rate of soil dry-down was measured by weighing experimental pots. Error bars are smaller than data point markers. Markers represent mean ± SE for nine pots, and an asterisk indicates a significant ($p \leq 0.05$) difference between treatments.

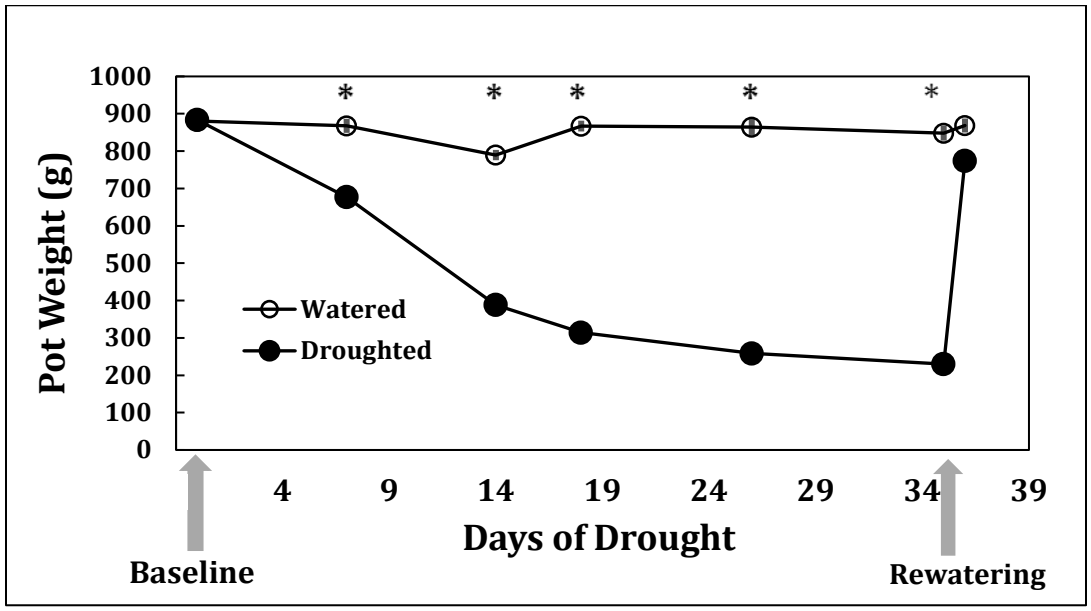


Figure 31. Succulence was compared in droughted and control plants. Data points represent mean ± SE for samples from three plants, and an asterisk indicates a significant ($p \leq 0.05$) difference between treatments.

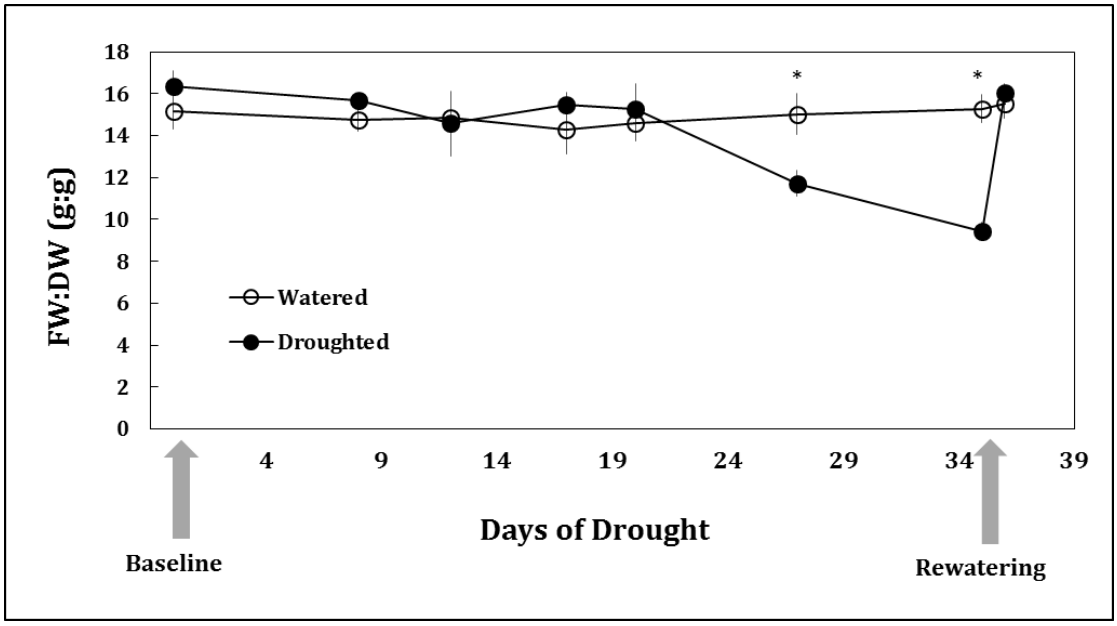


Figure 32. Plants recovered rapidly upon rewatering. In less than 24 hours after rewatering, a severely wilted plant (top left) appears completely recovered (bottom right). December 10, 2015, Appalachian State University Greenhouse.

Figure 33. Water potentials were compared in droughted and control plants. Bars represent mean ± SE, and an asterisk indicates a significant ($p \leq 0.05$) difference between treatments.

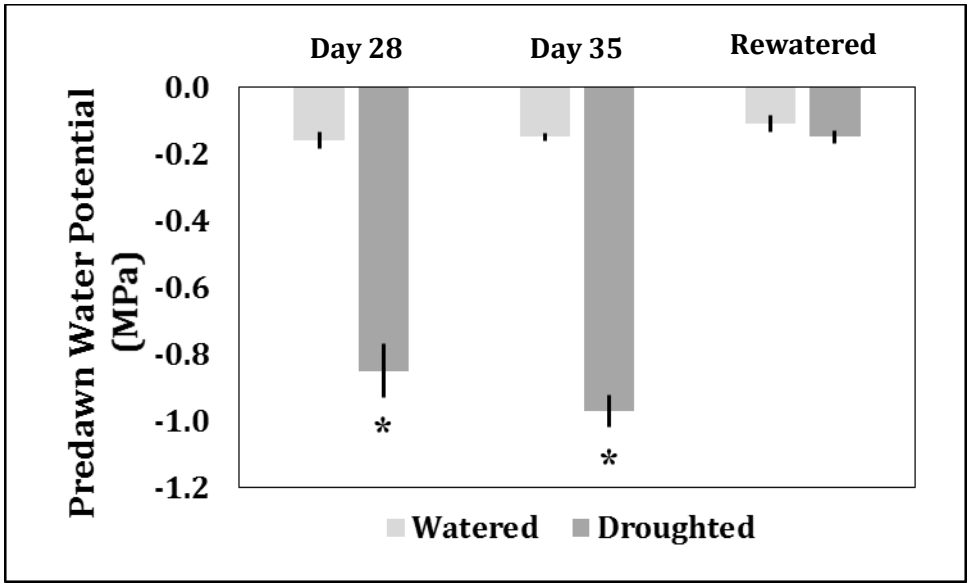


Figure 34. Photosynthesis was compared in droughted and control plants. Data points represent mean ± SE of rates from five plants, and an asterisk indicates a significant ($p \leq 0.05$) difference between treatments. Because no measurements were taken after droughted plants completely shut down photosynthesis, the final measurement after rewatering appears as a single data point (far right).

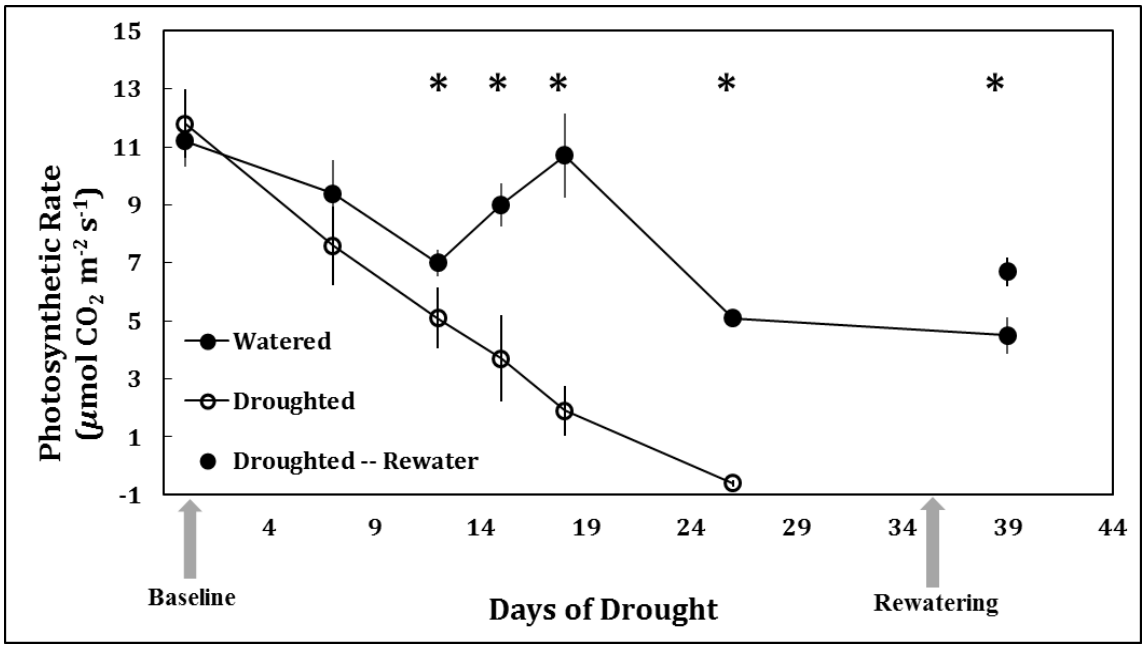


Figure 35. Fluorescence was compared in droughted and control plants. Data points represent mean ± SE for nine plants. An asterisk indicates a significant ($p \leq 0.05$) difference between treatments.

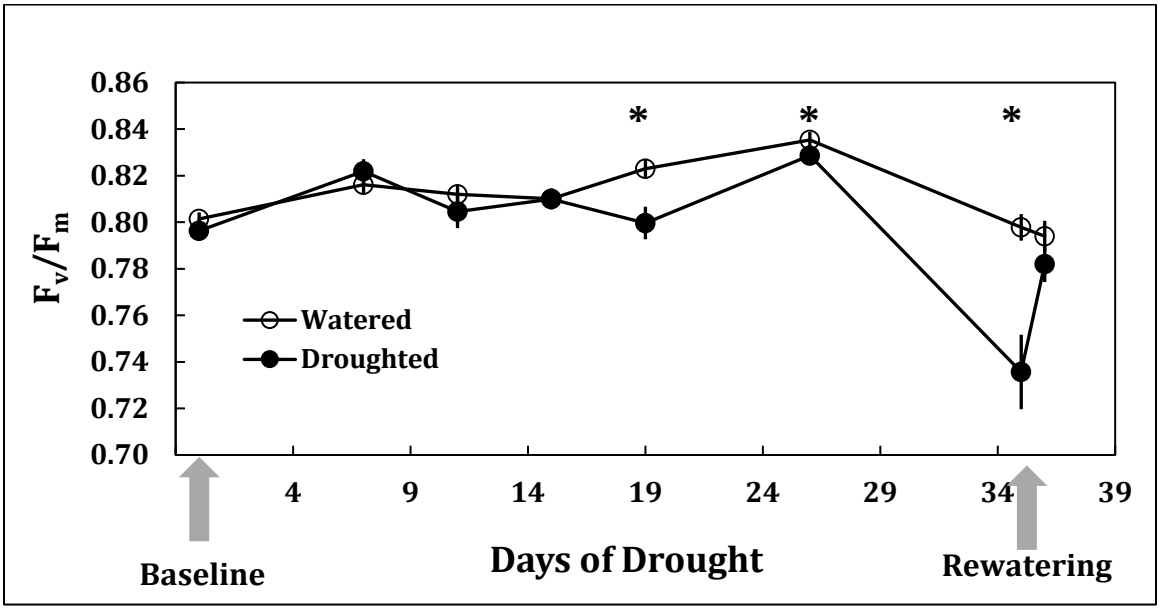


Figure 36. Nocturnal acid accumulation was compared in droughted and control plants.

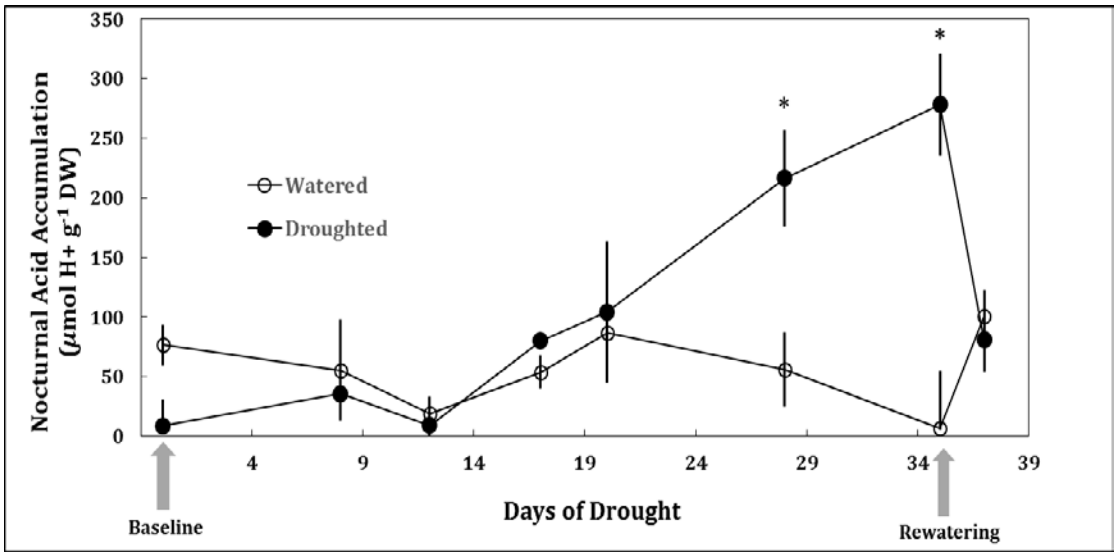


Figure 37. Leaf cross-sections of *S. ternatum* grown in high light (above) and low light (below) differ in morphology. The high-light plant has a thicker and more curved leaf than the shaded plant. Both leaves have chlorophyll distributed throughout the entire mesophyll.

Figure 38. *S. ternatum* must withstand repeated freezing and thawing cycles in the winter. Feb. 2016, State Farm.

Figure 39a-d. Plants grown in high light (a, left) and low light (a, right) in the greenhouse produced very different leaf morphologies. High-light plants produced dense, tightly appressed rosettes (b). Low-light plants had elongated stems with more widely spaced leaves (c). Field plants have a morphology that differs from either greenhouse treatment (d).

a)

b)

c)

d)

Figure 40. Leaf thickness was compared for high- and low-light treatments. Bars represent mean ± SE of five plants per treatment, and an asterisk indicates a significant difference between treatments.

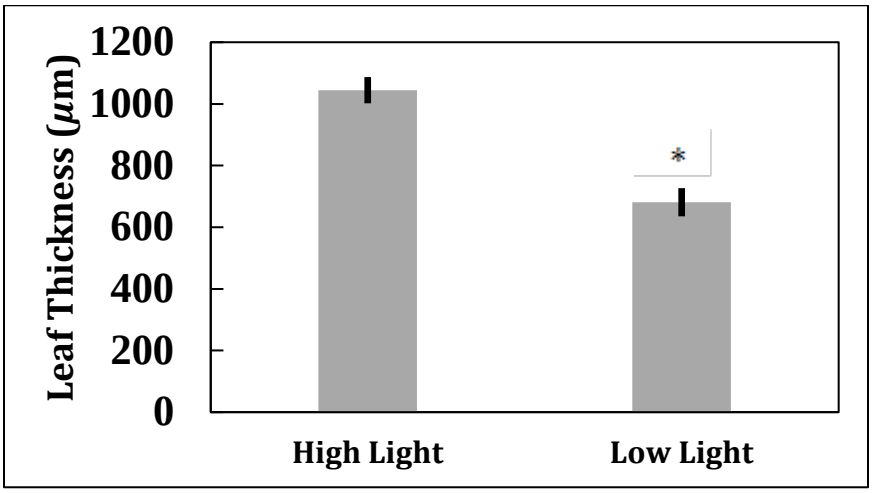


Figure 41. Leaf succulence was compared for high- and low-light treatments. Bars represent mean ± SE of five plants per treatment, and an asterisk indicates a significant difference between treatments.

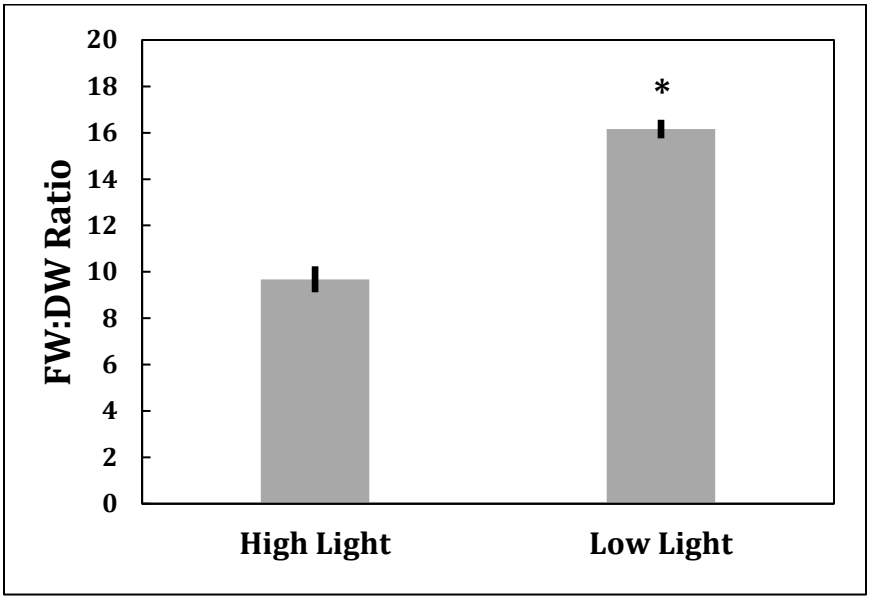


Figure 42. Stem length was compared for high- and low-light treatments. Bars represent mean ± SE of five plants per treatment, and an asterisk indicates a significant difference between treatments.

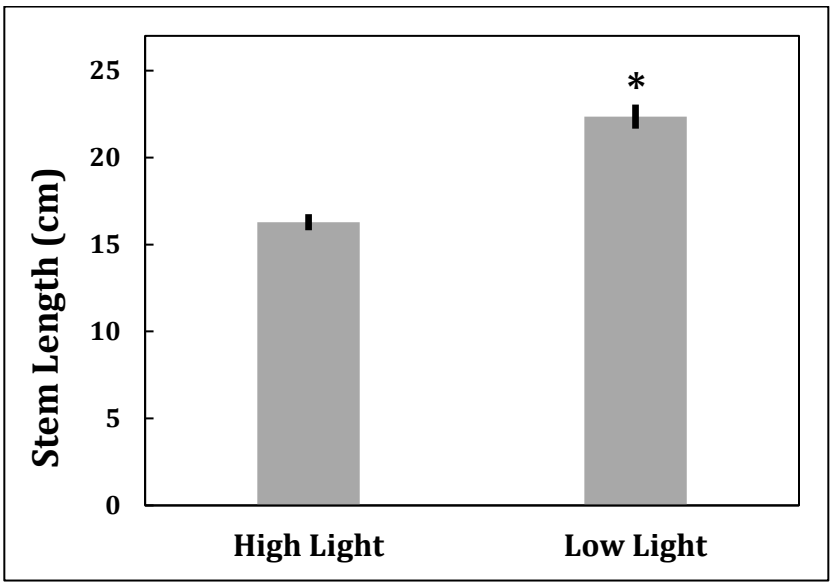


Figure 43. Primary branching was compared between high- and low-light treatments. Bars represent mean ± SE of five plants per treatment, and an asterisk indicates a significant difference between treatments.

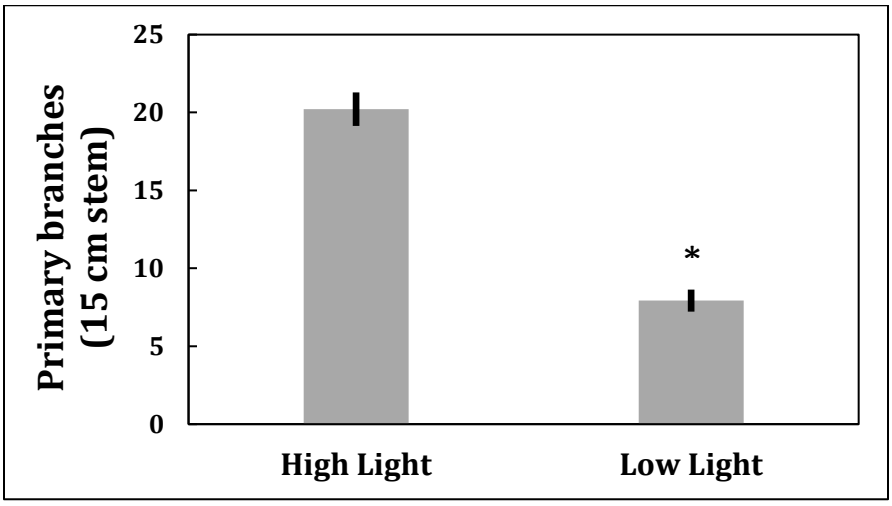


Figure 44. Secondary branching was compared between high- and low-light treatments. Bars represent mean ± SE of five plants per treatment, and an asterisk indicates a significant difference between treatments.

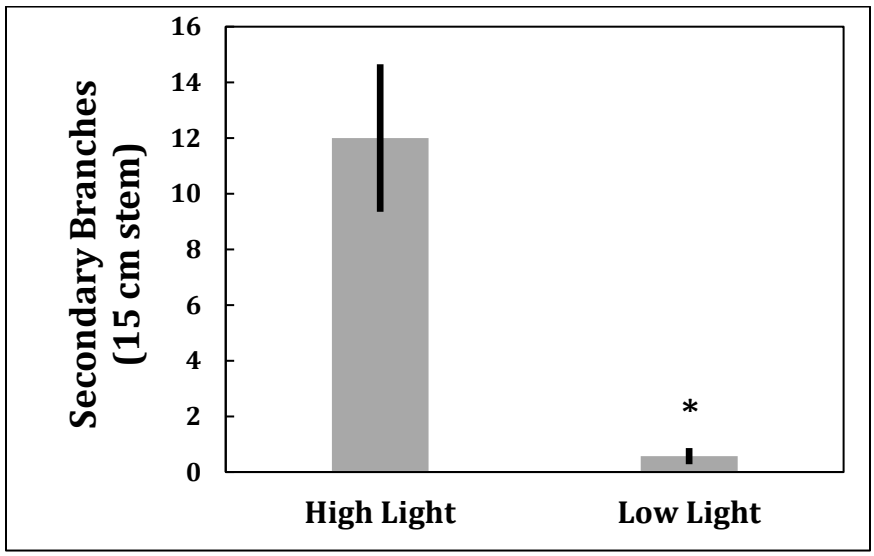


Figure 45. Leaf chlorophyll per unit mass was compared between high- and low-light treatments. Bars represent mean ± SE of samples from five plants, and an asterisk indicates a significant difference between treatments.

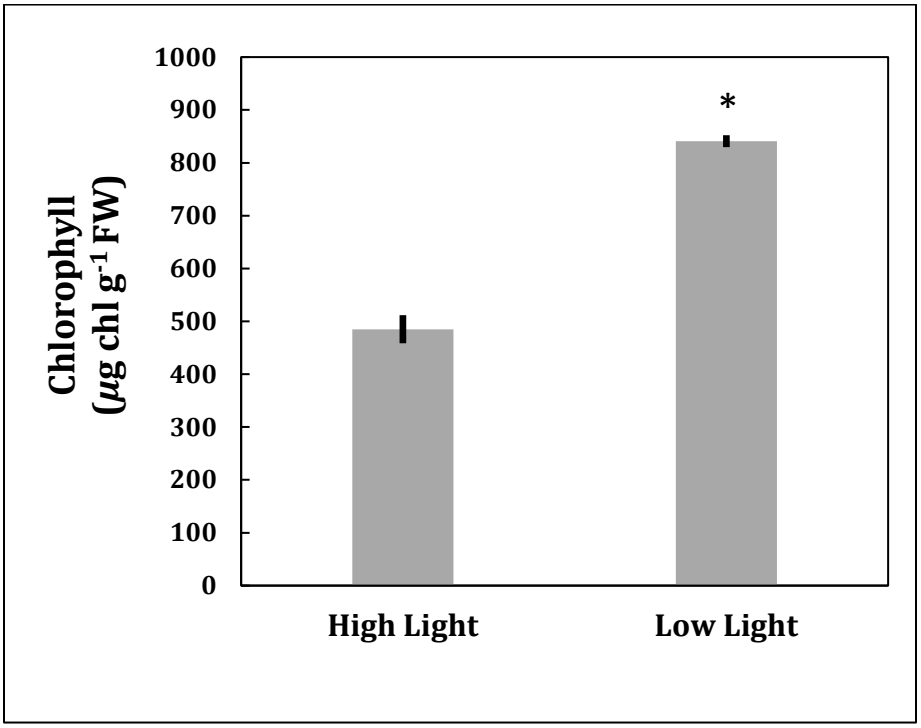
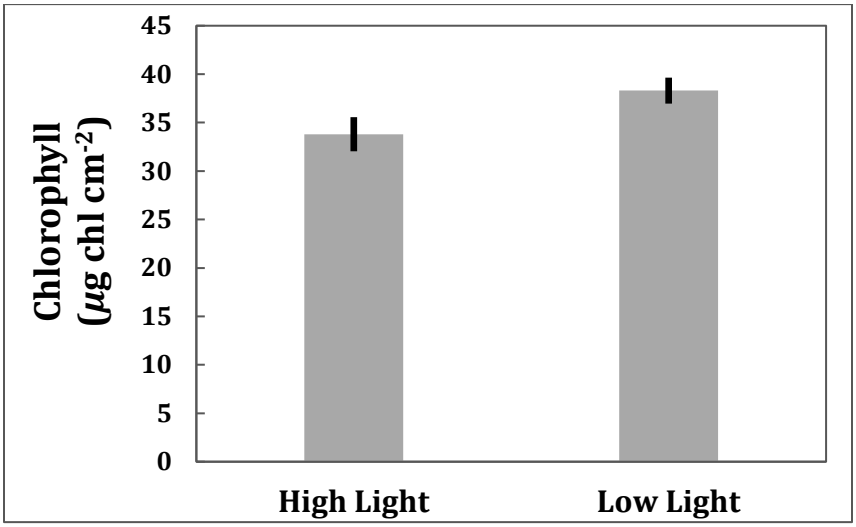


Figure 46. Leaf chlorophyll per unit surface area was compared between high- and low-light treatments. Bars represent mean ± SE of samples from five plants. No difference was found.



Vita

Catherine Jean Cole received a B.A. from Brown University (Providence, RI) in 1992, with a major in music, and a Ph.D. in musicology from the University in Chicago (Chicago, IL) in 2003. She then taught music history at the University of Iowa (2002-4) and Cleveland State University (2004-7). After moving to Boone, NC in 2008, she became interested in native plant communities of the Southern Appalachians and went back to school to earn this M.S. in Ecology and Environmental Biology, which was completed in May 2017.