The Consequences of Regulated Trimming and Hurricane Stressors on Secondary Growth, Wound Wood Production, and Chlorophyll Content of Black mangroves (*Avicennia germinans*).

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Abstract:

In the State of Florida, mangrove trees are protected by the *1996 Mangrove Trimming and Preservation Act*. The Act states that most trimming of mangroves must be permitted, must adhere to ANSI A300 pruning standards, and must be overseen by qualified mangrove trimmers. ANSI A300 are the generally accepted industry standards for tree care practices developed by the Tree Care Industry Association and written by a committee called the Accredited Standards Committee (ASC) A300. To date, a quantitative analysis of mangrove stress reactions to regulated trimming techniques has not been performed. The goal of this research was to monitor and assess health, structure, and growth of black mangroves (*Avicennia germinans*) when exposed to repeated trimming as described by Florida Administrative Code. Physiological indicators of tree stress, including chlorophyll content, leaf water pressure, and the formation of wound wood, were recorded for trees receiving one of two ANSI compliant trimming techniques: top trims and window trims. Indicators for trimmed trees were compared to measurements taken from unpruned trees, as well as abiotic stressors such as hurricane damage, and soil accretion. The data suggest proper cut location, trimming aspect ratio, and total biomass removal < 30% in a 12 month period resulted in no significant reduction in chlorophyll production under normal growing conditions. However, chlorophyll data analysis did indicate that trimmed trees may have experienced more stress from hurricanes than untrimmed trees. Soil accretion greater than mangrove pneumatophore level caused total plant necrosis. Regulated mangrove trimming does not appear to reduce the health of *A. germinans* during normal growing conditions but trimmed trees do experience more stress than untrimmed trees during hurricanes.

1. Introduction

Mangrove communities dominate approximately two thirds of the Florida coastal shoreline where they provide valuable habitat and ecosystem services. Benefits provided by healthy mangrove communities include shoreline stabilization, water purification, fisheries habitat, and storm buffering (Ewel, Twilley, and Ong 1998). Because mangroves exist on the boundary between terrestrial and marine environments, they play a critical role in wave energy mitigation, which enables upland habitats to establish stable soils and plant communities (Ewel, Twilley, and Ong 1998; Odum and McIvor 1990). Mangroves provide a first line of defense against storm surges. Research has shown that a well developed mangrove forest can attenuate up to 99% of normal wind generated wave energy (Massel, Furukawa, and Brinkman 1999). Even unhealthy, sparse mangrove forests can attenuate up 87% of normal wave energy (Massel, Furukawa, and Brinkman 1999). Studies have shown that coastal flooding from Hurricane Wilma in South Florida in 2005 would have penetrated 70% further inland without the storm surge mitigation provided by a 6-30 km area of established mangroves (Romañach et al. 2018). Loss of human life during the 1999 cyclone that targeted Orissa, India was significantly reduced because of mitigated flooding from mangroves (Barbier 2016). When viewed in context of all of the ecosystem benefits derived from mangrove communities it is clear that preservation of mangroves should be a priority for coastal urban areas.

In 1996, at the time of the ratification of the Mangrove Trimming and Preservation Act (MTPA), Florida had an estimated 550,000 acres of mangrove forests, but due to decades of coastal development, many areas have lost most of their functional mangrove communities (*Mangrove Trimming and Preservation Act* 1996). Lake Worth has lost more than 86% of historic mangrove populations (FLDEP 2017) while Tampa Bay, one of the ten largest ports in the United States, has lost more than 44% of its mangroves and coastal wetlands over the last hundred years (FLDEP 2017). In terms of monetary value, a study of stored carbon in the Everglades National Forest estimated the total value of its old growth mangrove forest at $2-$3.4 billion dollars (Jerath et al. 2016). This study focused on stored legacy carbon and used ecogeomorphic and socioeconomic attributes, among other metrics to generate its value (Jerath et al. 2016). This places the total carbon value of mangrove forests in Everglades National Park higher than that of a boreal, temperate, or tropical forest (Jerath et al. 2016).

Prior to 1996, Florida's mangroves did not have significant legislative protection, which ultimately allowed for significant mangrove habitat loss in areas of Florida (FDEP 2017). The MTPA set in place a state regulated series of permits and exemptions that allowed for certain trimming practices to take place. To minimize stress and maintain good plant health, the MTPA used the current American National Standards Institutes (ANSI) guidelines for arboricultural practices as a framework for trimming mangroves. Many Best Management Practices guidelines have been produced by state and local agencies detailing the processes and benefits of trimming mangroves per the MTPA guidelines but to date no experimental research has been conducted on actual mangroves quantifying the reactions of the regulated trimming. In short, it has been expected that adhering to ANSI standards and following the trimming requirements set forth by the MTPA would result in an acceptable level of stress to mangroves.

Mangroves are well adapted to grow in environments with both frequent changes in soil hydrology and high levels of soil dissolved salts, mostly sodium and chloride ions from ocean water (Baskin and Baskin 1998; Karim and Karim 1993). *A. germinans* is able to overcome persistent inundation by use of specialized adventitious aerial root structures known as *pneumatophores* which are able to grow and persist vertically at the mean local high-tide level(Baskin and Baskin 1998; Odum and McIvor 1990; Yabuki 2004). Pneumatophores, like the rest of the root structure, use lenticels for respiration that allows mangroves to maintain sufficient gas exchange during periods of inundation such as rising and high tide (Yabuki 2004). *A. germinans* regulate internal vascular ionic concentration by excreting salt through glands on individual leaflet surfaces (Baskin and Baskin 1998; Odum and McIvor 1990). *A. germinans* has been documented maintaining xylem sap concentration at 1/7 salt water (Odum and McIvor 1990), allowing plants to grow in soils that with salt concentrations >80 ppt (Baskin and Baskin 1998; Odum and McIvor 1990).

A. germinans reproduce through production of propagules (Baskin and Baskin 1998; Simpson et al. 2017). These structures are seeds which undergo embryonic development while attached to the parent plant(Baskin and Baskin 1998; Odum and McIvor 1990). This adaptation is a useful reproductive strategy for plants that grow in inundated soils with persistently high levels of dissolved salts(Baskin and Baskin 1998; Odum and McIvor 1990; Simpson et al. 2017). In north

Florida the propagules are also produced biannually (Spring and Fall) in response to spring tides, possibly for greater dispersal.

The goal of this research was to quantify the reaction of mangroves to repeated trimming stress as allowed by the MTPA, where it was hypothesized that mangroves trimmed within MTPA limits will remain healthy and vigorous during normal growing conditions.

2. Material and Methods

2.1. Study Site

A section of mangrove forest located on property managed by the University of Florida Whitney Laboratory for Marine Bioscience (Whitney Lab) was the chosen site for this study (29°40'16.32"N, 81°12'52.53"W) (Fig. 1). The Whitney Lab is situated on the Intracoastal Waterway in North East Florida, an area dominated mostly by *A. germinans* due to persistent cold temperatures during the winter months (Cavanaugh et al. 2014; Simpson et al. 2017). The mangrove study plots were located on the landward side of a barrier island, which is protected from surf activity and breaking waves. The regional tide was diurnal and had a range of o-4.5 feet above mean sea level (NOAA 2018).

2.2 Trimming Methodology

All trimming and measurements were made over an 18-month period beginning July 2016 and ending January 2018. During this time the research mangrove stand was divided into four adjacent plots consisting of six individual trees. Two plots were designated as untrimmed mangrove control plots, and two plots were trimmed per MTPA guidelines. One trimmed plot was reduced in vertical height via vertical reduction trimming or “top trimmed”, and the second trimmed plot was internally thinned or “window trimmed”. Window trimming is a method whereby the interior and lower foliage is systematically removed while retaining the upper and lower canopy, a method that is used mostly with taller trees where canopy reduction is not a viable option, still allowing for visibility through the canopy (FLDEP 2015). All trimming followed ANSI A300 methods for proper tree work. All reduction cuts were made back to appropriate aspect-ratio subordinate branches large enough to assume a new terminal branch lead. In order to preserve the branch collar intact, one stem of the opposite stem branch union was removed back to the node on herbaceous portions of the stem or branch collar on lignified portions of the stem (Fig 2). No more than 30% total canopy biomass was removed from any tree at one trimming event. Biomass was calculated by measuring the height and width of individual tree canopies to generate a total canopy area.

2.3 Chlorophyll Content

Leaf chlorophyll content was measured in field using the CCM 200-PLUS Chlorophyll Content Meter (Opti-Sciences, Hudson, NH). Chlorophyll content meters operate by transmitting infrared light at discrete wavelengths to determine light absorption by the chlorophyll pigments in plant tissues. These measurements are then used to calculate a chlorophyll content index (CCI) value that is proportional to the chlorophyll content value in the sample. CCI readings were taken six times over an 18-month period, with two samples taken from each tree: one from the outer canopy near the apical meristems and another from the interior, shaded canopy. Both readings were averaged to produce one overall chlorophyll reading per tree. All chlorophyll measurements were made during a window of 1.5 hours before and after solar noon in order to minimize the effect of temporal variations in chlorophyll levels.

2.4 Secondary Growth

Circumferential trunk expansion was also measured six times over the study period, using a standard forestry diameter caliper to determine trunk diameter. Due to the natural decurrent growth pattern of *A. germinans*, standard diameter at breast height (DBH), or 4.5 feet from substrate level, measurement was not always possible. When standard DBH measurements were not possible, modified DBH measurements were taken at the smallest trunk circumference below the lowest branch and above the basal trunk flare. Occurrence and development of wound wood formation was also monitored at each trimming cut site.

2.5 Statistical Data Analysis

All statistical analyses were performed using the program R (ver. 1.0.136). Chlorophyll content measurements were analyzed for differences in trimming treatments (top trim vs. window trim) and the control group. Two major stochastic events occurred during the study period, Hurricane Matthew in October 2016, and Hurricane Irma in September 2017. These storms destroyed one control plot and inflicted physical damage to most of the remaining trees in the study. Additional chlorophyll readings were taken within 30 days of each hurricane event, creating clear points at which additional tests on chlorophyll content could be performed (i.e. comparing readings taken before and after hurricane events), in an attempt to control for possible physiological effects of the hurricanes on remaining trees. Differences in trimming treatments were analyzed for statistical significance (α=0.05) using ANOVA tests, with Tukey Post-Hoc tests performed to determine pairwise differences between treatment groups. This same statistical methodology was applied to the circumferential data collected for treatment groups, using ANOVA to test for differences between treatments and t-tests to analyze trunk growth within each group (i.e. comparing values recorded at the end of the study to baseline measurements).

3. Results

3.1 Chlorophyll Analysis

The mean chlorophyll content for treatment groups over the entire study period was 69.02 CCI (st.dev.=14.89) for window trimmed trees, 73.19 CCI (st.dev.=17.84) for top trimmed trees, and 71.50 CCI (st.dev.=17.18) for control trees. Data for chlorophyll readings were subset by collection periods coinciding, with periods within the study pre-hurricane post-Matthew and post-Irma. Mean chlorophyll readings taken before the onset of hurricanes were 59.45 CCI (st.dev.=13.97) for window cut treatment, 65.54 CCI (st.dev.=15.22) for top cut treatment, and 55.38 CCI (st.dev.=12.46) for the control group (Fig 3). Mean chlorophyll readings taken after onset of Hurricane Matthew were 76.83 CCI (st.dev.=12.97) for window treatment, 81.28 CCI (st.dev.=18.34) for top cut treatment, and 78.08 CCI (st.dev.=12.69)for control treatment group (Fig 4). Mean chlorophyll readings taken after onset of Hurricane Irma were 64.74 CCI (st.dev.=9.57) for window cut treatment, 64.21 CCI (st.dev.=10.02) for top cut treatment, and 84.03 CCI (st.dev.=14.43) for the control group (Fig 5). ANOVA tests showed no significant differences between treatments in the period before (p-value=0.21, F-value=1.62, df=2) or after hurricane Matthew (p-value=0.65, F-value=0.43, df=2). Chlorophyll content between groups was significantly different in the period after hurricane Irma (p-value=0.01, F-value=5.74, df=2), and post-hoc analysis revealed significant differences in CCI between the top trim group versus the control group (p-value=0.02), and the window trim group versus the control group (p-value=0.03).

3.2 Circumferential Trunk Expansion

All three treatment groups had measureable gains in DBH during the study period, with mean increases in diameter measuring 1.88cm (st.dev.=0.48) in the window cut group, 0.81cm (st.dev.=0.53) in the top cut group, and 1.09cm (st.dev.=0.54) in the control group (Fig 6). Baseline DBH measurements for treatment plots were statistically significant at the start of the study, precluding further comparison of trunk circumference between treatment groups. Mean increases in DBH within groups, indicating trunk growth, were not statistically significant.

3.3 Effects of Trimming

On trimming cuts made to younger, more herbaceous portions of the stem, axillary buds at the base of the node developed to reestablish the original opposite branch formation (Figure 2). Axillary bud development was not observed on trimming cuts made to older, more lignified portions of the stems. Development of external wound wood on cuts made to older, lignified wood was not detected on any cut site during the study period.

3.4 Leaf Water Pressure

The initial study design of this project called for leaf water potential analysis through the use of a Pump Up Chamber Leaf Water Potential Meter (PMS Instrument, Albany, OR). Collecting leaf water potential measurements on *A. germinans* proved ineffective due to the high soluble salt content present in the xylem and possibly phloem vascular tissue. Because *L. racemosa* employ the same method of processing soluble salt within their vascular tissue, water potential readings may also prove ineffective as a metric for health for this species. However, it is expected that due to the ability to exclude salt through osmotic pressure, use of a leaf water potential meter would be appropriate for use on *R. mangle*.

4. Discussion

Due to its relative tolerance to cold *A. germinans,* rather than *Rhizophora mangle* or *Laguncularia racemosa*, was the most abundant mangrove species in North Florida (Cavanaugh et al. 2014). The observations made during this study were limited to *A. germinans* and similarly, conclusions are limited to this single species. Living on fringe of their subtropical range, North Florida mangroves are on average smaller than the mangroves growing in South Florida (Saenger and Snedaker 1993). Smaller mangroves may have less insulating capabilities around the critical meristems and may also exhibit different physiologic responses to storms due to the variations in wind loading and canopy buffering capacity. As mangroves in North Florida become more established and increase in size, their reaction and tolerance to stressors may change as well.

Deposited materials from storm movement interfered with root soil and gas exchange resulting in eventual death of the trees. One of the two designated control plots was destroyed by hurricane induced soil accretion thus reducing the number of control trees by 50%, five months after the commencement of the study.

Carotenoids, which comprise part of the chemical composition of chlorophyll, are part a known chemical stress response mechanism in plants (Havaux 1998; Zhang et al. 2012). As a group they are thought to play at least 5 roles in plant chemical stress responses. At a cellular level carotenoids harvest light, aid in photoprotection during periods of intense solar radiation, scavenge singlet oxygen which photodegrades cells, dissipate excess metabolic energy, and provide structural cellular support (Frank and Cogdell 1996; Frank 1999).When plants are exposed to environmental stressors such as changes in light, temperature, or physical stress, certain classes of carotenoids help stabilize oxidative membrane damage within plant cells (Havaux 1998; Zhang et al. 2012). Changes in chlorophyll levels found in mangroves may be attributed to fluctuated carotenoid levels as a result of hurricane stress. This theory may be explored through the use of mass spectrometry isolation of specific leaf chemical constituents. Unfortunately the unpredictable and stochastic nature of hurricanes presents a realistic limitation to such research. Regardless, the chlorophyll content results of this research project suggest a link between the chemical responses to physical and environmental stressors and the need for further investigation.

The lack of statistically significant growth within each group is indicative of the environmental stressors mangroves encounter in the northern extent of their natural range. Persistent cold encountered during the winter months, compounded with extreme weather events such as hurricanes would reduce a plant’s ability to produce statistically significant secondary growth in a twelve month growing season. Due to the high metabolic requirement of wound wood production, this would also explain the lack of observable wound wood on the trimmed mangroves. The temporal parameters for development of reaction wood, and wound wood are not currently known for North American mangroves. A study design with a longer observation period may yield positive results for wound wood manifestation of trimmed North American mangroves.

Reduction trimming on larger mangroves may help create a more compact structure which has been shown to create more wind resistant trees (Gilman, Masters, and Grabosky 2008; Gilman, Jason Miesbauer, and Masters 2015) but most mangroves do not reach the large size that would be benefit from reduction trimming. Therefore trimming smaller mangroves is structurally unnecessary and reduces the overall biomass to a degree that may allow the trees to be more affected by hurricane level stressors without positively affecting structure. Allowing mangroves to remain untrimmed, thus maintaining their natural balance of photosynthetic biomass, is ultimately the most beneficial method of mangrove management. This research suggests that allowing a longer period of recovery time between trimming sessions (24 mos vs. 12 mos) would allow for mangroves with more dense canopies that would be more resistant to hurricane stressors (Gilman, Masters, and Grabosky 2008; Gilman, Jason Miesbauer, and Masters 2015). Any method of plant management that reduces stress will ultimately ensure a plant that is more resistant to pests, trimming, disease, and the environment.

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Figures

Figure 1. Study site location at University of Florida Whitney Laboratory (Wh) in St Johns County, Florida, United States.

Figure 2. Axillary bud reestablished opposite leaf structure of the cut stem within the study period. A fresh reduction cut is visible above the new stem.

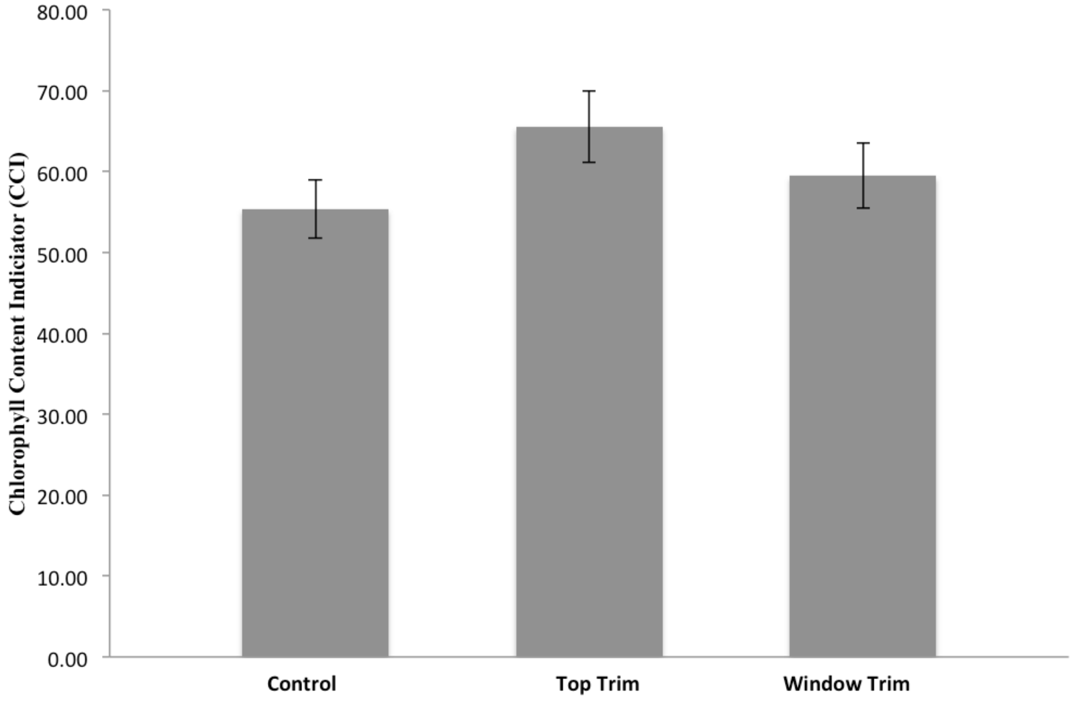

Figure 3. Chlorophyll levels pre-hurricanes.

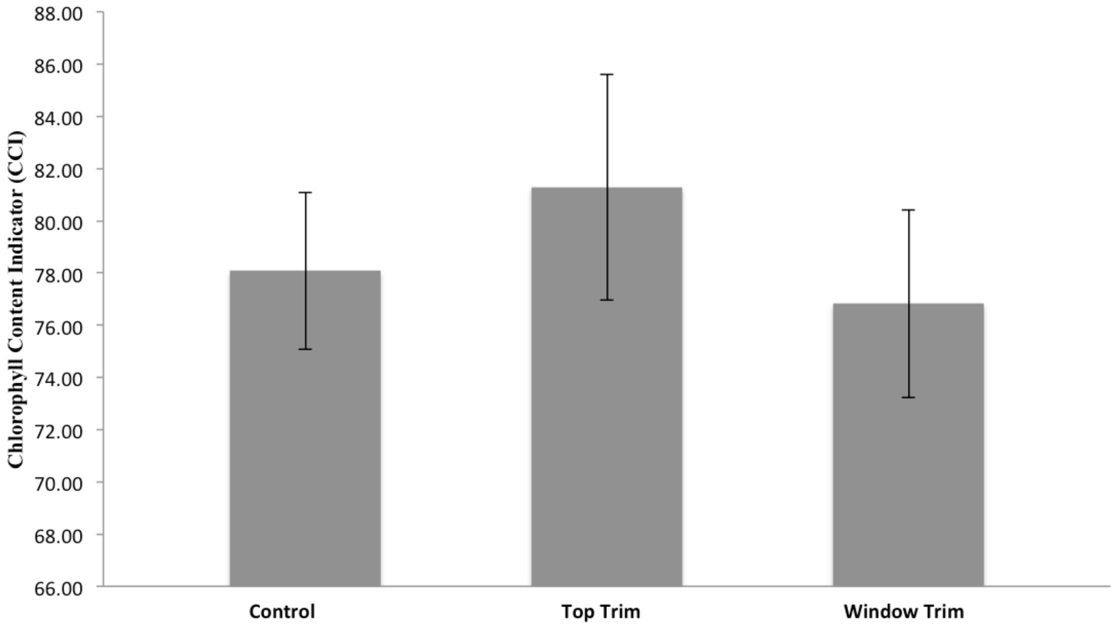

Figure 4. Chlorophyll levels post Hurricane Matthew (2016).

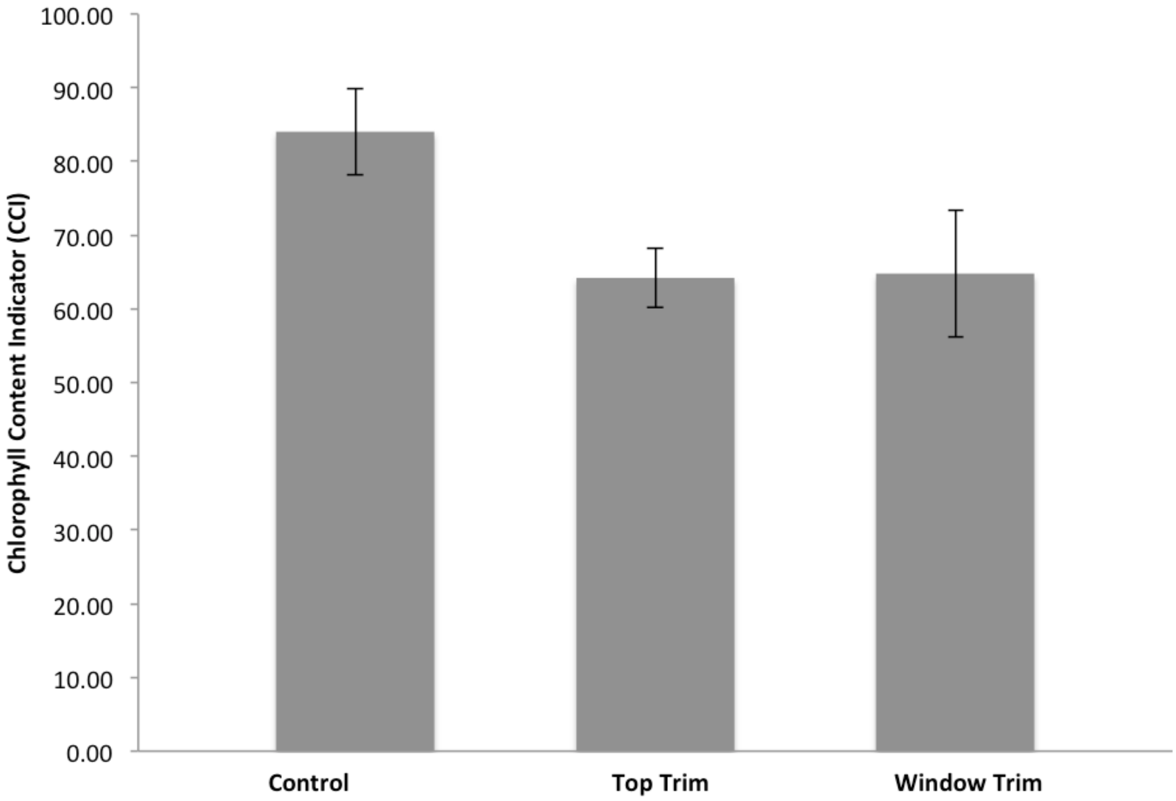

Figure 5. Chlorophyll levels post Hurricane Irma (2017).

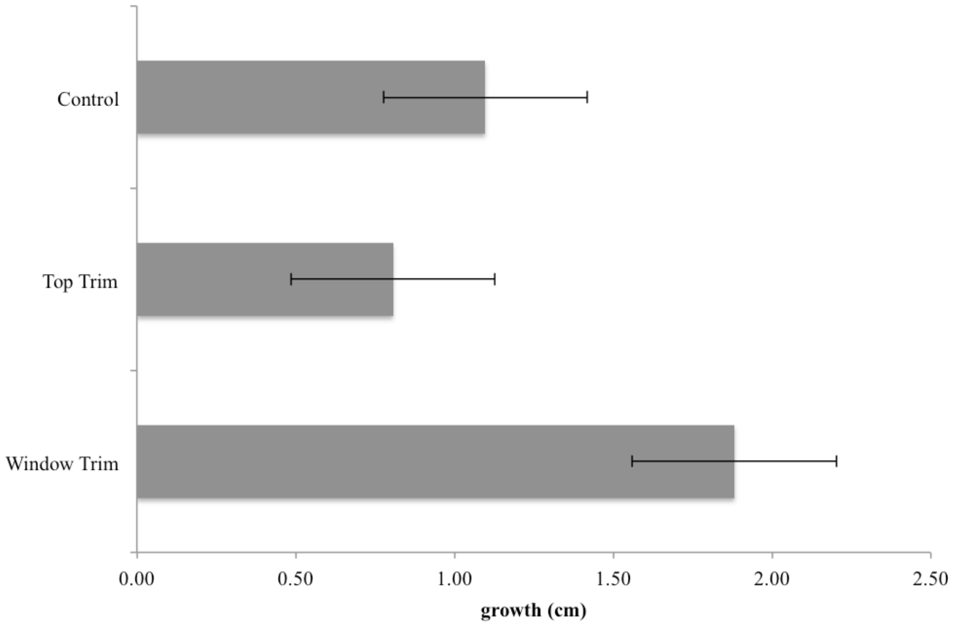

Figure 6. Average circumferential expansion during study period.