The Effect of Light-limitation on Spinescent Structural Defence and its implications on Resistance to Herbivory in the Shade

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Abstract

Plants can resist herbivore pressure through structural or chemical defence or both. The ultimate goal of defence against herbivory is to reduce the amount of damage to biomass, but more specifically to protect against damage to meristematic tissue. The defences employed depend on the type of herbivory experienced, which is contingent on the herbivore and its mouthparts. This investigation was concerned with structural defence presented by spines. This type of defence protects against mammalian herbivores such as browsers. Spiny species do not dominate in low-light deep forest environments. Therefore the aim of this study was to determine the constraints on spines as a defence strategy under shaded conditions by assessing the effect of reduced light on spine efficiency. Spine efficiency was defined as the amount of defence afforded the plant given the resources available. Spines require carbon to be built and need to be arranged properly in order to present an adequate defence. Thus two non-mutually exclusive hypotheses were proposed: Light limitation reduces the ability of spines to present an adequate defence against browsers due to the architectural strategy employed and/or its influence on carbon gain. The spinescent plant chosen for study was *Carissa macrocarpa* (Ecklon) A.DC. Light condition of plants was determined using hemispherical photography. Spine efficiency of sun and shade plants was determined using a bite test and was evaluated using architectural and physiological analyses. Architectural analysis involved identifying levels of organisation within the plant across ontogeny and indentifying sun and shade growth strategies. Physiological analysis involved determining carbon gain of sun and shade individuals using gas-exchange measurements, as well as the measurement of biomass allocation by harvesting and oven drying different plant parts. Results showed that biomass allocation patterns of *C.macrocarpa* did not change in sun and shade but total biomass increased from shade to sunlit conditions. Architectural analysis revealed that in the sun the plant adopted a stout dense structure with high spine efficiency, while in the shade it was more elongated with lower spine efficiency. Therefore *C.macrocarpa* adapts to the light environment by adopting either the shade or the sun architectural strategy. The way in which this works is that light affects carbon gain, which either increases or decreases biomass and in turn leads the plant to adopt the sun or shade architectural strategy. The architectural strategy affects spine efficiency such that plants in the sun have higher spine efficiency than plants in the shade. Thus, spinescent plants do not do well in light limited environments because they are architecturally constrained to elongate in such conditions. This constraint would put them at higher risk of browser damage than plants in light-sufficient conditions, ultimately decreasing their fitness. If the patterns observed in *C.macrocarpa* prove to be general, then it helps to explain why spiny plants are more commonly found in open, sunlit environments than in deep shade.

Introduction

Plants are either structurally or chemically defended or both (Craine 2009). Chemical defence involves the production of chemical compounds that are either organic (e.g. phenolics and glycosides) or inorganic (e.g. heavy metals such as Nickel - Martens 1994) (Craine 2009). Structural defence involves the production of structures such as thorns, prickles and spines (Grubb 1992). Thorns are sharp, modified twigs or branches, while spines form from stipules, epidermis or leaves (Perez-Harguindeguy 2013). Prickles are outgrowths from the plant stem that are often irregularly arranged (Grubb 1992). These defences, both structural and chemical are an adaptation to the "disturbance" of herbivory (Craine 2009). Herbivory is defined as a disturbance because it serves to reduce the reproductive ability of the plant through the destruction of biomass (Craine 2009). The ultimate goal of defence against herbivory is to reduce the amount of damage to biomass (Craine 2009; Grubb 1992), but more specifically to protect against damage to meristematic tissue (Craine 2009, Grubb 1992, Gowda 1996). Defences employed depend on the type of herbivory experienced, which is contingent on the herbivore and its mouthparts (Craine 2009).

This investigation was concerned with structural defence presented by spines. This type of defence protects against mammalian herbivores such as browsers (Cooper and Owen-Smith 1986; Grubb 1992; Gowda 1996; Craine 2009), by reducing the amount of plant material browsers are able to remove while at the same time increasing time spent foraging (Cooper and Owen-Smith 1986; Bevlovsky et al. 1991; Milewski et al. 1991; Wilson and Kerley 2003; Shipley 2007). However, the presence of spines alone does not guarantee adequate defence. The spines need to be arranged in order to effectively prevent the mouthparts of browsers removing a substantial amount of tissue. Adopting a caged branching structure coupled with the presence of spines, a plant is able to deter browsers by preventing the snout getting close to leaves and stems (Archibald and Bond 2003; Staver et al. 2012). Therefore, the amount of protection afforded the plant by spines is dependent on both branching architectural strategy and the presence of spines.

The distribution of spiny species ranges from evergreen and deciduous forests to woodland, savannah, grassland and desert regions (Grubb 1992). Spiny species dominate the open thorny woodlands and semi-desert regions of Africa, dominated by *Acacia* or *Prosopis* species (Grubb 1992). It has, however, been suggested that spiny species do not dominate evergreen and deciduous forests (Grubb 1992).

Forest understories tend to be low-light environments (Niinemets 2001). This lack of light would restrict the plant's overall growth according to Liebig's "law of the minimum" (Craine 2009). Liebig's law of the minimum basically states that plant growth is limited by the resource that is least supplied to the plant (Craine 2009). Therefore, the comparative lack of spiny species in the forest environment would suggest that this is due to light-limitation. The restriction of growth would lead plants to adopt low-light growth strategies and physiological traits in order to compete both inter and intra specifically (Craine 2009).

According to the "carbon gain" hypothesis, plants in shaded environments adopt traits that will enable them to maximise their light use efficiency and thus also their carbon gain (Givnish 1988; reviewed by Valladares and Niinemets 2008). Plants that are shade-tolerant have lower maximum photosynthetic carbon assimilation rates (Givnish 1988), coupled lower respiratory costs and thus lower light compensation points (Craine 2009). In addition they also have leaves that are thinner and less dense than those of plants in sunny environments (Niinemets 2001); which are arranged such that there is minimal self-shading, thus maximising light use (Craine 2009). In order to adopt shade-tolerant traits, however, the plant has to first allocate its resources appropriately. Resources are allocated to defence, reproduction and growth (Bazzaz et al. 1987), such that biomass is partitioned to those parts of the plant that would increase the fitness of the plant in the face of light-limitation (Bloom et al. 1985; Mcconnaughay and Coleman 1999). When resources are limiting, allocation to growth seems to take precedence (Bazzaz et al. 1987) and when the limiting resource is light then more biomass is allocated to stem and leaf growth than to other vegetative organs (Bloom et al. 1985). This allocation pattern leads to stem elongation and other shade tolerant traits such as canopy densification (Valladares and Niinemets 2008). This ability of plants to adapt their morphology and physiology in response to their light environment is known as phenotypic plasticity (Valladares and Niinemets 2008). Favouring allocation to stems and leaves, however, reduces biomass allocation to defence functions. Therefore, light-limitation could cause a decrease in "spinyness" and thus spine efficiency. Consequently, a plant in the shade could be more vulnerable to damage by herbivory while it tries to optimise its light resource.

This study aims to determine the the constraints on spines as a defence strategy under shaded conditions by assessing the effect of reduced light on spine efficiency. According to the resource availability hypothesis (Coley et al. 1985), plants growing in light-sufficient conditions should invest less in defences. However, this is not the case as many species have been observed to be spinier in the sun than the shade (Grubb 1992). Thus it would seem that plants are able to defend themselves better in the sun than in the shade because their spine efficiency is higher. The "spine efficiency" can thus be defined as the amount of defence afforded the plant given the resources available.

Aside from biomass allocation, spine efficiency may also be affected by ontogeny (Gowda and Palo 2003; Boege and Marquis 2005). The ontogenetic stage of a plant has an effect on the growth rate of a plant, and thus the manifestation of a trait at an early ontogenetic stage would be different from that of a later stage (Wright and Mcconnaughay 2002). Thus is assessing the spine efficiency of plants in the sun and shade it is important to consider ontogeny as spine efficiency may fluctuate during plant development. The effect of ontogeny on the trajectory of a trait is known as an endogenous process (Barthelemy and Caraglio 2007), because the change in the trait is due to a process that comes from the plant itself (i.e. the plant's inherent growth rate). Phenotypic plasticity observed in relation to different environmental conditions is an exogenic constraint (Barthelemy and Caraglio 2007) that also causes a change in the trajectory of a trait. It is important to separate exogenic constraints from the endogenous processes of the plant when considering the effect that light condition has on spine efficiency. This is because in some cases the effect of ontogeny on a trait could be profound enough such that ignoring ontogenetic effects could lead to under or overestimation of the change in the trait (Wright and Mcconnaughay 2002). For example, if spine efficiency is to be compared in the sun and shade, the traits that are assessed need to be examined on plants that are at the same stage of development, as some traits may be more pronounced at some growth stages but not in others. Thus from a defence point of view the plant may be well defended at some stages and not at others. Therefore in order to get a full picture of the overall spine efficiency of the plant, traits should be examined at all growth stages (capturing change due to ontogeny) and across light conditions. Thus we have seen that both resource allocation and ontogeny are important factors when examining defence. Therefore the following two hypotheses regarding light condition and spine efficiency have been proposed:

Architectural Hypothesis:

Light limitation reduces the ability of spines to present an adequate defence against browsers due to the architectural strategy employed by the plant as well as the growth stage of the plant.

Prediction:

Ontogeny and light availability should have a significant effect on the parameters of spine efficiency, such that there is higher spine efficiency in the sun than the shade and that this trend is either more or less pronounced depending on the growth stage of the plant. Therefore the expectation is that plants should show phenotypic plasticity with regard to light condition, with shade plants being more elongated, less spiny and less cage-like than sun plants. These differences should be evident across all growth stages but differ in their degree between growth stages.

Physiological Hypothesis:

Light limitation reduces the ability of spines to present an adequate defence against browsers through its influence on carbon gain.

Prediction:

Plant growth stage has no effect on the parameters of spine efficiency. Thus phenotypic plasticity will be entirely due to the exogenic constraint of light condition driven by carbon gain and resource allocation. Therefore the expectation is that sun plants should have higher carbon gain than shade plants. More biomass should be allocated to stem and leaf growth in shade plants than in sun plants, and overall biomass allocation to defence should be higher in the sun than the shade plants. The architectural manifestation resulting from these differences in resource allocation and carbon gain will be entirely due to light condition and there will be no difference due to growth stage.

The mechanisms of the above hypotheses are not mutually exclusive, since spine efficiency depends on both the plants physiological response as well as its architectural strategy in order to present a suitable defence against herbivory. Therefore, separating spine efficiency into its architectural and physiological explanations (as with the hypotheses above) allows one to assess the effect of ontogeny as well as physiology on spine efficiency under different light-environments.

Methods

Study Area and Spiny Species

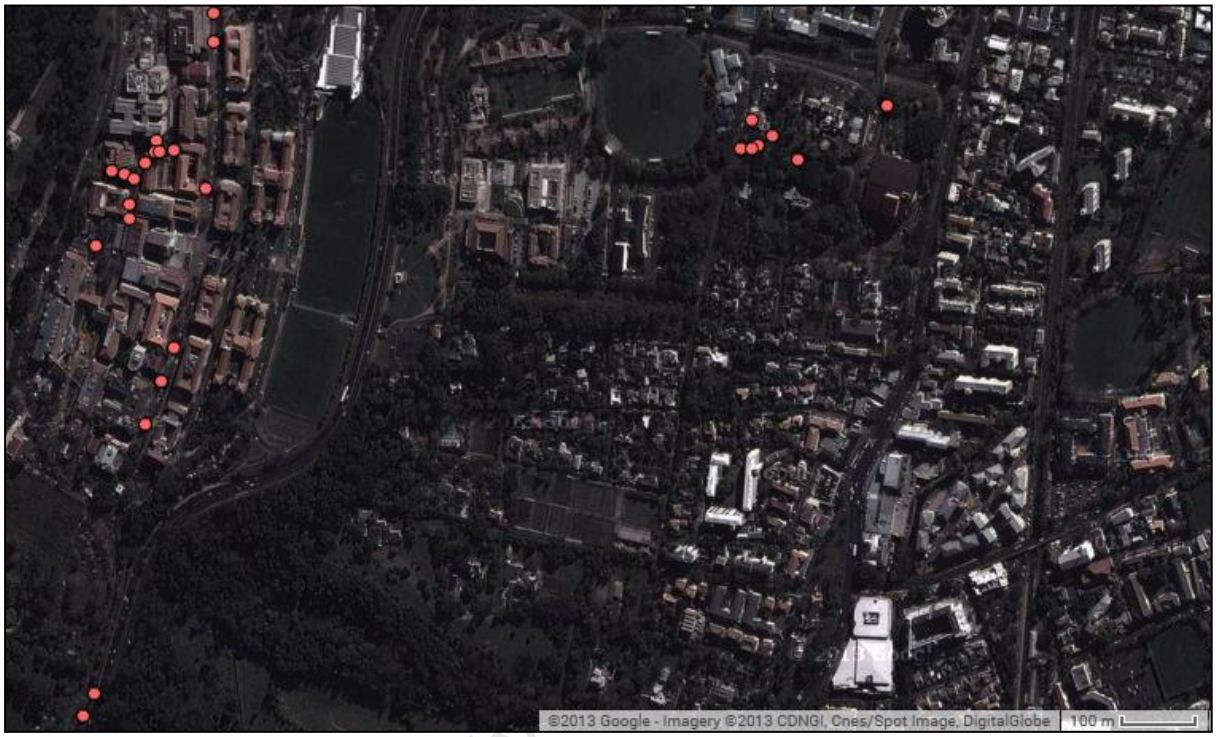

Fig.1. Study site – University of Cape Town (UCT) campus. The red dots indicate each individual plant sampled (n = 27).

The study was conducted on the campus of the University of Cape Town (UCT) (figure 1), situated in Cape Town, South Africa (33.9575° S, 18.4606° E). The spinescent plant chosen for study was *Carissa macrocarpa* (Ecklon) A.DC (Coates Palgrave et al. 2002). *C.macrocarpa* is a much branched spiny shrub or tree up to 4 meters in height in the family Apocynaceae (Coates Palgrave et al. 2002). It is defended by spines and milky latex (Coates Palgrave et al. 2002). *C.macrocarpa* occurs in the eastern coastal regions of South Africa and the southern parts of Mozambique (Coates Palgrave et al. 2002). The plants on UCT campus had not grown naturally but had been planted as a hedge at many locations (often alongside buildings) across UCT campus. The UCT plants undergo regular maintenance by an outsourced gardening service. As a result, all the *C.macrocarpa* on campus had undergone cutting and pruning in some way or another, as well as regular watering and application of fertiliser to their soils.

Overview and Experimental Design

The methods employed below ultimately sought to define spine efficiency in *C.macrocarpa*, and evaluate it in relation to light condition using an architectural and physiological analysis. The light condition of all individuals (n =27) was measured and all the individuals arranged on a light gradient based on percentage canopy openness. A "shade" group and "sun" group was formed from the individuals along the light gradient. This categorical grouping as well as the light gradient itself was used in the architectural and physiological analyses. The architectural analysis was conducted on all individuals (n =27), while physiological biomass allocation measurements were conducted on 24 individuals on the light gradient. Spine efficiency was determined using a bite test. Measured physiological and architectural parameters were then evaluated in relation to spine efficiency and light environment.

Light Condition and Selection of Individuals

Individuals selected for analysis (n = 27) were at least ten meters apart, their GPS location and elevation was recorded with an accuracy of three to four meters using a "Garmin eTrex 10" GPS system. They were tagged using a tag made of a clear plastic bag and a piece of string. The light condition of each individual was determined using hemispherical photography analysed using the software programme Gap Light Analyser (GLA) version 2.0 (Frazer et al. 1999). Hemispherical photographs were taken using the C-110 Digital Plant Canopy Imager (CID Bio-Science Inc., Camas, WA), during July of 2013. Picture brightness, contrast and gamma were set to 50%, while the orientation setting was set to indicate magnetic north. The images were captured above the plant of interest. Thus the height at which images were captured differed between individuals.

Capturing images entailed holding the arm (and camera) above the plant and moving the camera until the north indicator was pointing to magnetic north. Once the camera was stable and orientated to magnetic north, the image was captured. The geographic orientation of images was important for later analysis in GLA. The images were captured during late afternoon as the sun was setting in order to ensure that there was a good contrast between canopy and sky and also to avoid any interference by direct sunlight on estimates obtained during image analysis (Leblanc et al. 2005).

Images were processed in GLA to obtain estimates of canopy openness (shading) as well as total direct and diffuse radiation transmitted through the canopy. GLA estimates canopy openness during image processing by dividing pixel intensities into sky and non-sky classes and then determining the percentage of pixel values that are sky (Frazer et al. 1999). Transmitted radiation is estimated using a solar radiation model. Essentially, the path of the sun across the sky is plotted and an estimate of the average amount of radiation the plant receives for a particular growing period is calculated (Frazer et al. 1999).

Images were prepared before running calculations by first registering each image, viewing it in a blue colour plane and then manually adjusting its threshold as per Frazer et al. (1999). During image registration, the user defines the geographical orientation of the image. Geographical orientation is important as it affects the modelled path of the sun across the sky, thus affecting canopy openness and radiation estimates. The north point on the images was determined using the north indicator of the C-110 plant canopy imager. Thresholding classifies the image into black (non-sky) and white (sky) pixels based on the bitmap intensity of the pixels of the image (Frazer et al. 1999). The amount of thresholding of each image was determined manually. Changing the threshold value would change a pixel to black or white based on the original pixel intensity (Frazer et al. 1999). Therefore reflective objects in an image that would show as sky could be darkened and classified as non-sky.

Once prepared, estimates for canopy openness and transmitted radiation could be computed. Certain configuration settings had to be set in order for the calculations to be run with sufficient accuracy. Each of the images was orientated to magnetic north. This was corrected to true north in the configuration settings by setting the angle of magnetic declination for Cape Town (25° W). The latitude (in degrees, minutes, seconds) and elevation (meters above mean sea level) was entered for each individual. Projection distortion settings were set to polar. The projection distortion defines how objects in the hemispherical field of view are projected onto an image plane (Frazer et al. 1999). The polar projection function ensures that distances in degrees in the hemispherical field of view are proportional to the radial distances in pixels on the image plane (Weiss and Baret 2010).

The overall orientation of all sites was set as horizontal and no topographical shading mask was not used. A topographical shading mask takes into account the shading effect of surrounding topography that is not canopy (Frazer et al. 1999). However, this was not implemented because angular coordinate data (acquired from a clinometer or digital elevation model) of the position of the surrounding topography was not available (Frazer et al. 1999). Therefore the shading effect of buildings was taken as canopy cover during calculations.

C. macrocarpa is an evergreen plant, therefore the growing season over which estimates were to be calculated was set from 1 January to 31 December (one full year). Solar radiation data was calculated using the "modelled" option, which would compute estimates based on a built in GLA solar radiation model. Estimates were computed using the default parameter values set by GLA, except for "Sky-Region-Brightness" which was set to SOC ("Standard Overcast Sky"). Sky-Region-Brightness describes the intensity of the diffuse sky and SOC assumes that the zenith is three times as bright as the rest of the sky region (Frazer et al. 1999). Data on the percentage canopy openness was used to rank individuals on a light gradient and group them into "sun" and "shade" groups. Individuals that had up to 45% openness were regarded as shaded, while all those above this were regarded as sunlit.

Architectural Analysis

The architectural analysis of *C.macrocarpa* was performed using the modified methods of Charles – Dominique (2010, 2012). The analysis and methodology employed was based on the architectural concepts of Hallé et al. (1978), revised by Barthelemy and Caraglio (2007). Plant form is ultimately the result of the repetition of elementary botanical units (Barthelemy and Caraglio 2007). The repetition of elementary botanical units organises the plant such that its architecture is a hierarchical branched system with distinctive axes that can be grouped according to their morphological, functional or anatomical features (Barthelemy and Caraglio 2007). The grouping of distinctive axes in this manner is at the centre of architectural analysis. The axes identified as such are known collectively as the "architectural unit" of the plant (Barthelemy and Caraglio 2007).

The different axes of the architectural unit are identified using morphological traits based on the growth process, branching process, general axis spatial orientation and position of reproductive structures on the axis (if any) (Barthelemy and Caraglio 2007). Thus a plant is organised by elementary botanical entities, which make up the architectural unit of the plant. The architectural unit is then reiterated over ontogeny to give rise to the whole plant organism (Barthelemy and Caraglio 2007). The morphogenetic change from elementary entity, to architectural unit and finally to whole organism is facilitated by growth, branching process and reiteration at all levels of organisation (Barthelemy and Caraglio 2007). Many morphological traits are, however, subject to change over ontogeny (Barthelemy and Caraglio 2007). Therefore the crux of architectural analysis is to identify plants throughout their ontogeny and compare the architectural unit of the plant across the same ontogenetic stage in order to separate environmental effects on the architectural unit from that of ontogenetic effects. In so doing one is able to gain insight into the architectural strategy of a plant in relation to a given environmental condition.

Determination of Growth Stage

The general growth form of *C.macrocarpa* consisted of many branch complexes that were essentially derived from one another. The periodic cutting of *C.macrocarpa* caused "traumatic" reiteration of these branch complexes - repetition of the architectural unit of the plant in response to physical damage (Barthelemy and Caraglio 2007). Thus the new branch complex that formed after cutting was essentially a "fresh" shoot sprouting from an older trunk. The branch complexes chosen for analysis were the fresh shoots that essentially formed the last and highest order branch complex in the plant. The branch complexes of *C.macrocarpa* are derived from one another such that more branch complexes are produced over time and throughout the plant's ontogeny. Thus the rank of the last branch complex in the plant was used as a proxy for the developmental stage of the plant. The rank of this last branch complex was determined by counting the number of subsequent branch complexes. This was done by locating the first order trunk that the branch complex of interest was attached to. The first order trunk was usually one of the main trunks that grew from the rootstock. This main trunk was then designated as rank one and successive branch complexes that were attached to this trunk were counted consecutively until the final branch complex (the one chosen for analysis) was reached. Plants were designated as being in an early, middle or late stage of development depending on the rank of the branch complex that was examined. Plants with branch complexes ranked from one to five were regarded as being in the early stage of ontogeny, while those whose branch complexes ranked from six to nine and from ten upwards were regarded as being in the middle and late stages of ontogeny respectively.

Determination of Architectural Unit

Representative modules and axis categories of *C.macrocarpa* were identified using qualitative morphological criteria as explained previously. Modules form the axis categories which essentially form the architectural unit of the plant (Barthelemy and Caraglio 2007). Therefore the identification of modules was essential in the identification of axis categories and thus the architectural unit of the plant. Ten individuals were chosen at random, irrespective of position along the light gradient, and their qualitative characteristics examined. The qualitative characteristics examined were growth direction, degree of secondary growth and conicity (whether cone-shaped or cylindrical), branching, phyllotaxy, as well as flowering and qualitative spinescence characteristics.

Four modules were identified based on these qualitative characteristics. They were primarily distinguished based on their growth direction and their point of attachment to the main trunk of the plant or to other modules. The grouping of module types was used to distinguish between different axis categories of *C.macrocarpa*. Quantitative data was collected from the modules of each branch complex. This was done to capture the change in growth development in successive modules from the base to the periphery of the branch complex. The following quantitative characteristics were measured:

Module length (cm) (figure 4 and 5) - the length from the absolute base of the module attachment, up to and including the spine base of attachment.

Module diameter (cm) (figure 4 and 5) - the overall diameter of the centre of the most distal internode within a module.

Number of internodes in the module (figure 4) – the number of internodes between nodes. Note that there is another smaller internode between the spine and the distal large internode in a module. This internode was not counted, so strictly speaking the number of internodes in the module would be n+1, where n is the number of large internodes in the module.

Number of node and apex leaves (figure 4 and 5) - count of the node and apex leaves, based on direct observation or scars present.

Node and apex leaf lengths (cm) (figure 6)-Measurement taken along the main vein on the abaxial side of the leaf from just after the petiole, up to but not including the tip of the leaf.

Spine forking (once/twice/both) (figure 7 and 8) – spines could fork once (one pair of prongs), twice (one pair of prongs that further divaricates at their tips) or both (one prong divaricated, the other remains single).

Spine length (mm) (figure 7) - the length from the start of growth of the spine, up to and including the perpendicular prong distance.

Spine prong distance (mm) (figure 7) - once forked- the distance up to and including the prong tips; twice forked – distance (up to and including the prong tips) between the last two prongs of the spine.

Spine Diameter (mm) (figure 7) - measured at centre of spine primary axis.

Method Protocol (per individual)

Quantitative measurements were taken on each module per branch complex per individual using a vernier calliper and a ruler or tape measure where appropriate. Sampling took place during March, April and May 2013.

Physiological Analysis

The methodology centred on identifying trends in resource allocation in the sun and shade groups. The net carbon gain in the shade and sun was estimated and supplemented with data on biomass allocation.

Gas-Exchange measurements and net carbon gain

To determine the photosynthetic response of *C.macrocarpa* to variable light availability, light response curves were gathered for two sun and shade plants using LI-6400XT portable infrared gas analyser (IRGA) (Li-Cor, Inc., Lincoln, NE). A LI6400-02B cuvette was used, with leaf temperature set to 25°C, reference CO2 was set at 400 ppm, and flow was maintained at 300µmol.s^{-1}. Photosynthetic rates were recorded at the following light intensities: 25, 50, 100, 300, 500, 1000, 1500 and 2000µmol.s^{-1}. A non-rectangular hyperbola was fitted to the light response curves using the following equation:

$$A+R_d = \frac{\left\{I\Phi+Amax-\left[(I\Phi+Amax)^2-4\Phi Amax\theta\right]^{0.5}\right\}}{2\theta} \qquad \text{Eqn. (1)}$$

where A is the photosynthetic rate (µmol CO_2.m^{-2}.s^{-1}), R_d is the dark respiration rate (µmol CO_2.m^{-2}.s^{-1}), Amax is light-saturated photosynthetic rate (µmol CO_2.m^{-2}.s^{-1}), I is the amount of incident radiation at the time of measurement (µmol photons.m^{-2}.s^{-1}), Φ is the quantum yield efficiency, the initial slope of *A* in response to light availability (Beaudet et al. 2000) and θ is the curvature factor of the curve (dimensionless). The "solver" function in Microsoft Excel 2007 was used to estimate the above parameters, given the data on photosynthetic rate and light intensity (light response curve data). The photosynthetic rate where light intensity was zero was used as an initial value for the dark respiration.

The initial value for quantum yield was calculated as the slope of photosynthetic rate (A) versus I at light intensities of 0 to 50 µmol photons.$m^{-2}.s^{-1}$ (Herrick and Thomas 1999), and an arbitrary initial curvature factor was set at 0.5. Solver was then used to estimate the parameters of equation 1 using the initial values set. By this process, each individual that was selected for gas exchange measurement had a fitted model curve. The parameters of an average shade and sun curve was calculated by taking the mean of the parameters of separate sun and shade curves.

The total transmitted radiation data (mol photons.$m^{-2}.d^{-1}$) collected from the sites of all the individuals (n = 27) was then converted to µmol photons.$m^{-2}.s^{-1}$ using equation 2 below. This was then used to calculate net carbon gain in the sun and shade using equation 1 and either the shade or sun model parameters calculated above (depending on the group to which the plant belonged). The net carbon gain (µmol CO_2.$m^{-2}.s^{-1}$) was converted to kilograms carbon per meter squared per year using equation 3 below. Net carbon gain for the year was obtained by assuming constant photosynthetic rates as measured in May, varying only with daily irradiation as estimated using gap light analyser.

µmol photons.$m^{-2}.s^{-1}$ = 0.0864 x mol photons.$m^{-2}.d^{-1}$ Eqn. (2)

kg.C.m^{-2}.yr = Net carbon gain (µmol CO_2.$m^{-2}.s^{-1}$) x 1.39 Eqn. (3)

The constant 0.0864 (equation 2) is derived from the conversion of seconds into days (86400 seconds in a day), which is then divided by 1000000 to convert micromoles to moles. The constant 1.39 (equation 3) is derived from the conversion of mol CO_2 .$m^{-2}.d^{-1}$ to mol CO_2 .$m^{-2}.yr^{-1}$ by multiplying 0.0864 by 365 days (there are 365 days in a year). Carbon dioxide is composed of one carbon atom and two oxygen atoms. Therefore one mol of carbon dioxide contains one mol of carbon. Thus mol CO_2 .$m^{-2}.yr^{-1}$ was then converted to g C .$m^{-2}.yr^{-1}$ (using equation 4 below), which was then converted to kilograms carbon by dividing by 1000.

Number of mols = $\frac{Mass(g)}{Molecular\ Mass}$ Eqn. (4)

Biomass Allocation

The effect of resource allocation with regard to light condition was of interest to this investigation. Therefore the vertically growing axis category (selected during architectural analysis) was harvested in order to get an indication of biomass allocation to leaves, stems and spines as per Perez-Harguindeguy et al. (2013). Biomass allocation was determined for 24 individuals along the light gradient. Leaves, spines and stems were oven dried to constant weight at 70°C for five days. On removal they were placed in a dessicator for one hour after which they were weighed. The large amount of stem material was weighed on a "Mettler PE-11" scale. The leaf and spine material was weighed on an analytical balance (Shimadzu ATX 224). Before drying, total leaf area of the vertical axis category was determined for 6 shade and 4 sun individuals. Leaf area was measured using a leaf area meter (LI-3100 Area Meter; Li-Cor, Inc., Lincoln, Nebraska, USA).

This methodology is based on optimality theory, which suggests that plants partition biomass to different vegetative parts in response to a limiting resource (Mcconnaughay and Coleman 1999). In this case the limiting resource was assumed to be light condition. The regular maintenance of *C.macrocarpa* on UCT campus would have ensured that the plants were well looked after in terms of soil conditions and the provision of water. The only variable that could not be directly influenced by human action was light condition. Therefore, the assumption that it could be a limiting resource was deemed appropriate.

The percentage biomass allocation to leaves, stems and spines was calculated using the equations below and the effect of light condition on percentage biomass allocation was evaluated.

Total Biomass (g) = Leaf biomass (g) + Stem biomass (g) + Spine biomass (g) Eqn. (5)

Biomass Allocation (%) = $\frac{\overline{Total\ Biomass\ (g)}^{x}}{Total\ Biomass\ (g)}$ x 100, where x =leaf, stem or spine biomass Eqn. (6)

Spine efficiency

Spine efficiency was defined from the perspective of the payoff to the plant. The magnitude of the payoff depends on the effectiveness of spines to mitigate damage to the plant by browsers (Perez-Harguindeguy 2013). The key to the efficiency of spines at deterring herbivores lies in the way the spines are arranged in space. Spine dimensions such as spine length and prong distance are only effective if they are arranged in a particular fashion such that their lengths and prongs are able to shield leaf material from browsers. Thus physiological measures such as relative spine mass in the sun and shade cannot give an indication of how efficient those spines are at protecting the plant. A plant that has a high mass of spines could have a few large spines that fail to protect the leaf material at all, or they could have many small spines that adequately protect leaf matter and are thus more efficient. Therefore, a test of the effect of light condition on spine efficiency would have to incorporate the effect that spines (on plants in different light conditions) have on the ability of browsers to remove leaf material from the plant.

A bite test was used in this investigation using the human face as a proxy for the snout of a browser. To simulate the amount of space a browser has to stick its head into the plant, a 15 x 15 x 7 cm frame was made. The dimensions for this frame were decided using the diameter of the author's face, from outside one cheekbone to just outside the other (15 cm). The depth dimension was determined by observation and measurement of the longest spine present on an axis category of a random plant (7 cm). Thus the browser would have a space of 1575 cm^3 in which to remove leaf material.

Therefore, the amount of space available to the browser should be a function of spine efficiency such that the more spines present within the given volume; the higher the spine efficiency as they occupy more space and are able to cause more pain to different areas of the browser's snout simultaneously and thus reduce ability of the browser to remove leaf material. For each individual (n = 24), the cardboard frame was laid flat against the branches either at head height (when the plant was tall) or directly onto the centre of the plant (if the plant was lower than chest height).

The cardboard frame was laid down with the branches of the plant running perpendicular to the height dimension of the frame. In this way leaves and spines (if any) were made available within the frame. Once the frame was in place an attempt was made to remove leaf material in a single bite. Only one bite attempt was taken per individual because *C.macrocarpa* contains a toxic milky white latex containing cardiac glycosides (Wink and Van Wyk 2008). Leaf material (if any) that was removed during the bite procedure was stored in brown paper bags.

Any spines within the framed volume that stabbed the face and caused enough pain to inhibit the removal of leaf material was also removed and bagged. The leaf and spine material was oven dried (along with the biomass material above) to constant weight for five days at 70°C. Once dry they were weighed and spine efficiency defined as the index below:

$$\text{Spine Efficiency} = \frac{Spine\ Mass\ Protecting\ (g)}{Leaf\ Mass\ Removed\ (g)} \qquad \text{Eqn. (7)}$$

Therefore, according to equation 7, spine efficiency increases with an increase in spine mass but is also affected by the amount of leaf mass removed. Spine efficiency was then evaluated for the sun and shade groups. However, architectural stem parameters such as module length and diameter as well as architectural spine parameters such as spine length and prong distance also play a part in spine efficiency. Spines are attached to modules, therefore module lengths and diameters determine how far apart spines are and how they are arranged in space. This in turn affects the impact their prongs and lengths have on protecting leaf matter. Therefore the effect of these parameters on spine efficiency was also examined. The effect of canopy openness (as both a gradient and categorical grouping) on these parameters was also assessed, in order to evaluate the relationship of canopy openness to the architectural parameters of spine efficiency.

Statistical Analysis

Data analysis was conducted using the statistical package "Statistica" (Statistica 12, StatSoft Inc., Tulsa, USA), as well as the "ggplot2" package in "R" (R Core Team 2013). Significance testing was done to determine if there were statistically significant differences between quantitative architectural parameters as well as biomass parameters in the sun and shade. The choice of statistical test (parametric or non-parametric) was based on whether the data to be tested satisfied assumptions of normality and homoscedasticity. Non- parametric tests were used where data was not normally distributed.

Correlation analyses and non-parametric local regressions were used to assess the relationship between light condition and architectural parameters as well as biomass parameters. These correlation analyses and local regressions were carried out on data for 24 individuals along the light gradient. For each individual the average of the architectural parameter was calculated over all its modules. Therefore, for a particular individual, an architectural parameter such as module length was averaged over all the modules of that individual, to give one value for module length as opposed to separate module values. Correlation analyses were also used to assess the relationship of certain architectural and biomass parameters against spine efficiency.

A general linear model (GLM), coded and run in "R", was used to determine the change in architectural quantitative variables in modules from the base to the periphery of an axis category, as well as to quantify the change in these variables across growth stages and light condition. The GLM was run on the data of all individuals (n =27), regarding each of the modules as separate entities. Significant differences were reported by reporting the sign of the estimated slope of the GLM. Thus, if there was a significant difference and the estimate was negative then it was concluded that the particular architectural parameter being assessed decreased across the levels of module type, light condition or growth stage. GLMs of parameters that were normally distributed were run with a Gaussian error structure, while slope estimates of non-normal parameters were calculated using quasi-likelihood (Crawley 2007).

Results

Light Environment

The site of each individual was ranked according to percentage canopy openness (figure 2). Site is used here instead of individual because the light environment estimated was not a point light estimate but was instead an overall estimate of light coming in based on the field of view of the hemispherical lens. An arbitrary cutoff of about 45% was used to distinguish between "sun" and "shade" groups. Those sites up to and including 45% canopy openness (indicated by black bar– figure 2) were regarded as shaded, while all those above this cutoff were regarded as being sunlit. There was a significant difference in light availability between the sun and shade groups (U = 23; Z = -3.28; p = 0.000528; r = 0.63). Sites 26 and 27 have the highest canopy openness because they had no canopy cover at all. They also have equal canopy openness because the same photograph was used to determine the openness of both these sites. Both the grouping and the light gradient (figure 2) were used in the analysis of architectural and physiological parameters in relation to light condition.

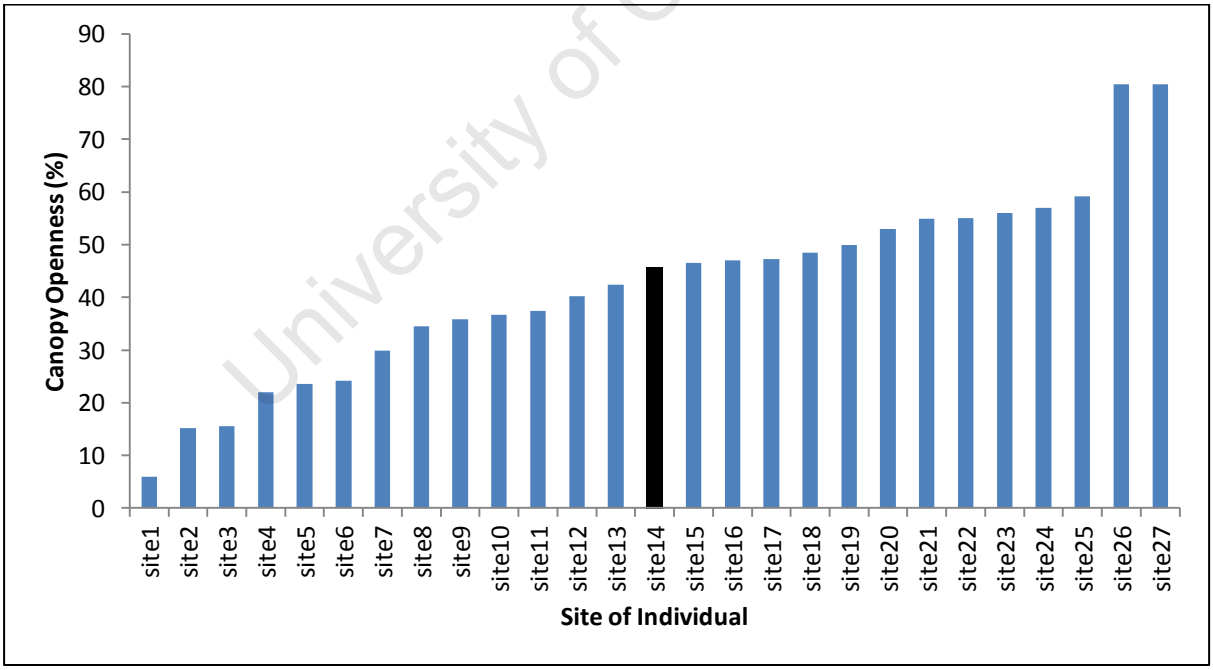

Fig.2. Canopy openness at each site of *C.macrocarpa* individual. Each site has been ranked according to percentage canopy openness. An arbitrary cutoff percentage of about 45% distinguishes between "sun" and "shade" groups (indicated by black bar).

Architecture

Qualitative Analysis

C.macrocarpa is clonal (figure 3), with each ramet being organised into vertical and horizontally growing axis categories ultimately formed from pseudomonopodial growth (figure 10). The vertical axis category is constructed by three consecutive module types, while the horizontal axis category is constructed by one module type (figure 10). The classification of these modules was based on their primary growth direction; since different growth directions restrict an axis to a specific syndrome of morphological features (Edelin 1984; reviewed by Barthelemy and Caraglio 2007).

Fig.3. Clonal growth of *C.macrocarpa*. The red box indicates the stolon connecting two ramets indicated by blue lines.

The four modules identified were classified as category 1 (C1), category 2 (C2), Intermediate (INT) and category 3 (C3) (Table 1a). The general structure of all modules consisted of a distal apex, nodal leaves and internodes. The apex consisted of two spines, each accompanied by a leaf and its two subtending buds either developed into shoots or not (figure 4). All modules had a cylindrical, dichasial sympodial structure that displayed rhythmic branched growth (figures 4 and 5) (Table1a).

Branching was immediate (except in the case of C3, which was both immediate and delayed) and followed an acrotonic gradient with bilateral amphitonic symmetry (Table1a). Leaves were ovate and arranged opposite to each other (figures 5, 6 and 8c). During growth the module axis rotated such that nodal and apex leaves were arranged in perpendicular planes to each other (figures 4, 5 and 6).

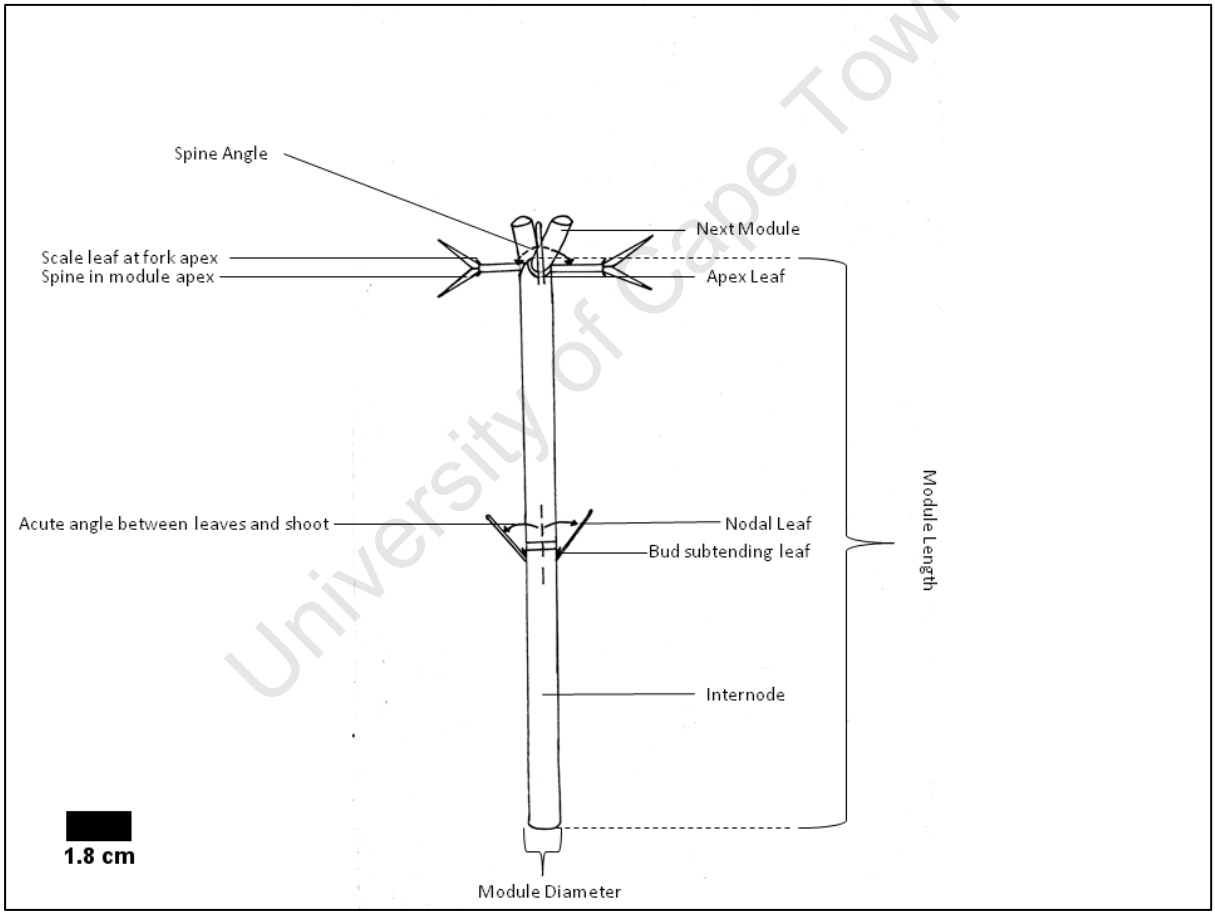

Fig.4. General structure of a typical module of *C.macrocarpa*. The spines shown here are once forked and are 180° apart (or at a 90° to the module primary axis). This will vary between module types. There is rotation during the growth of the module such that apex leaves lie in a perpendicular plane to that of nodal leaves.

Fig.5. Modules of *C.macrocarpa*. (a) C1 module, (b) C2 module, (c) C3 module, (d) Intermediate module. The number of internodes is constant (2 internodes per module) with the exception of the C2 module which has an extra internode. The lack of apex leaves in the C1 module (a) is due to the leaves having fallen off. Notice the rotation of leaves from apex to nodal leaves in the C3 and intermediate module (c and d), such that they lie perpendicular to each other. There is less rotation in the C2 module (b). The C3 (c) and C1 (a) module both have a greyish brown bark while this is absent on the intermediate (d) or C2 (b) modules.

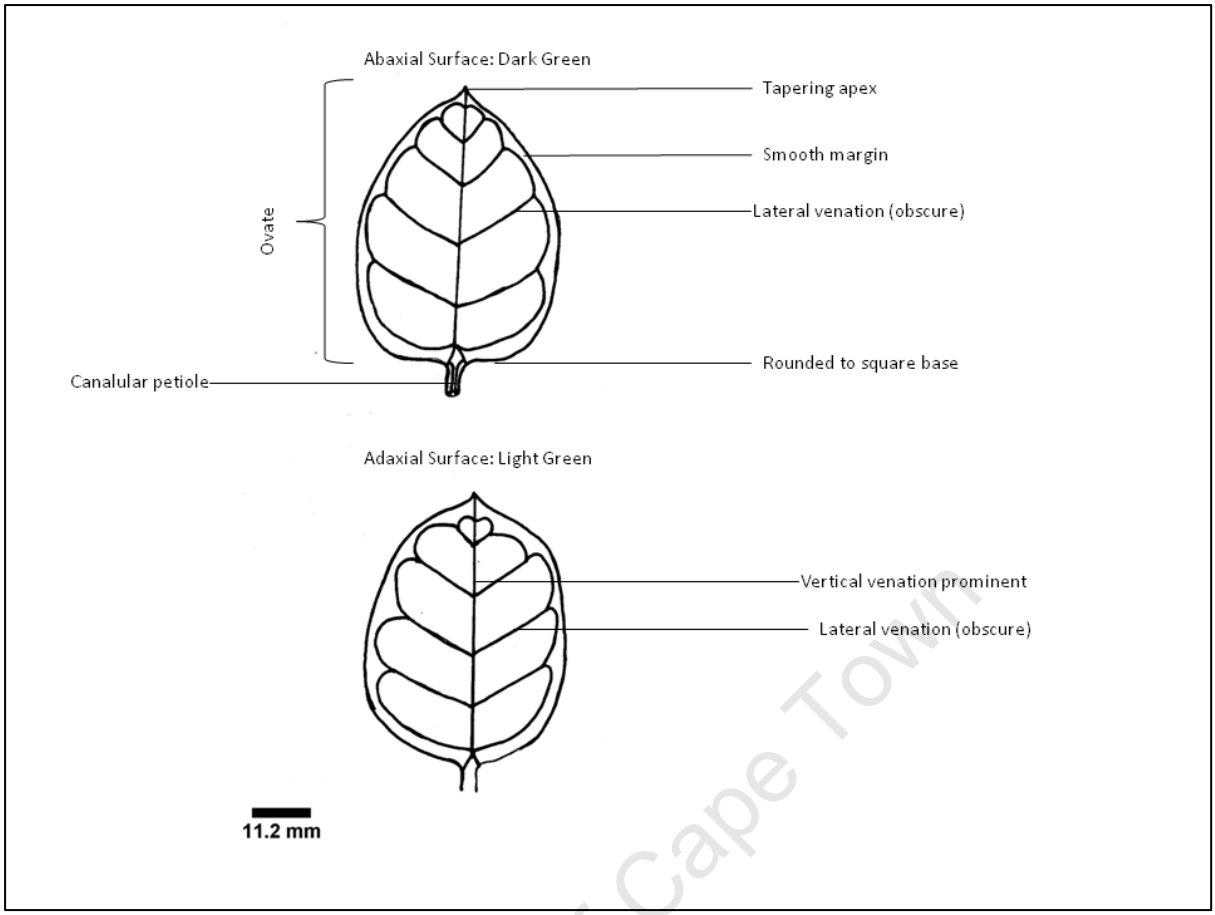

Fig.6. Leaf morphology of *C.macrocarpa*. Leaves are generally ovate with a canalular petiole and are a dark green on their abaxial surface and light green on their adaxial surface.

The primary axis of spines was cylindrical, with conical prongs (figure 7). Spines could either be forked once, twice or both (figure 7 and 8a and b). Thus spines either had only two prongs (forked once) or had an extra pair of prongs upon those prongs (forked twice), or they had one singular prong and a double forked prong (both) (figure 7 and 8a and b). C1 modules and intermediate modules could be forked according to any of these forking categories (Table1a), while C2 and C3 modules were restricted to being forked either once or twice (figure 8a and b). Also, spines on C1 and intermediate modules were angled at about 90° as opposed to those of C2 and C3 modules which were angled at about 60° (Table 1a). The spines of the C1, C2 and intermediate modules were much larger and more rigid than those of the C3 modules. Spines of the C3 modules were quite small and could be easily broken off (figure 8b). Modules differed in their primary and secondary growth properties, flowering, bark colour and texture and certain spine properties. C1 modules displayed orthotropic primary growth with strong vertical secondary growth (Table 1a, figure 9a).

C2 modules displayed plagiotropic growth with strong horizontal secondary growth, while intermediate modules grew ageotropically and displayed light oblique secondary growth (figure 9a). C3 modules also grew plagiotropically, but displayed medium horizontal secondary growth (Table 1a). Flowering was observed only on C2 and C3 modules with flowers occurring apically. The basal branch complexes of *C.macrocarpa* were covered with a greyish brown bark (figure 9). The branch complexes that were measured in this investigation were of "fresh" growth as mentioned earlier. Therefore, there was little bark on the trunks of these branch complexes. However, in cases where bark was present, it was mostly C1 modules that had bark with a striated texture (figure 5a). C3 modules, where barked, were heavily striated (figure 5c). No bark was observed in C2 or intermediate modules (figure 5b and d).

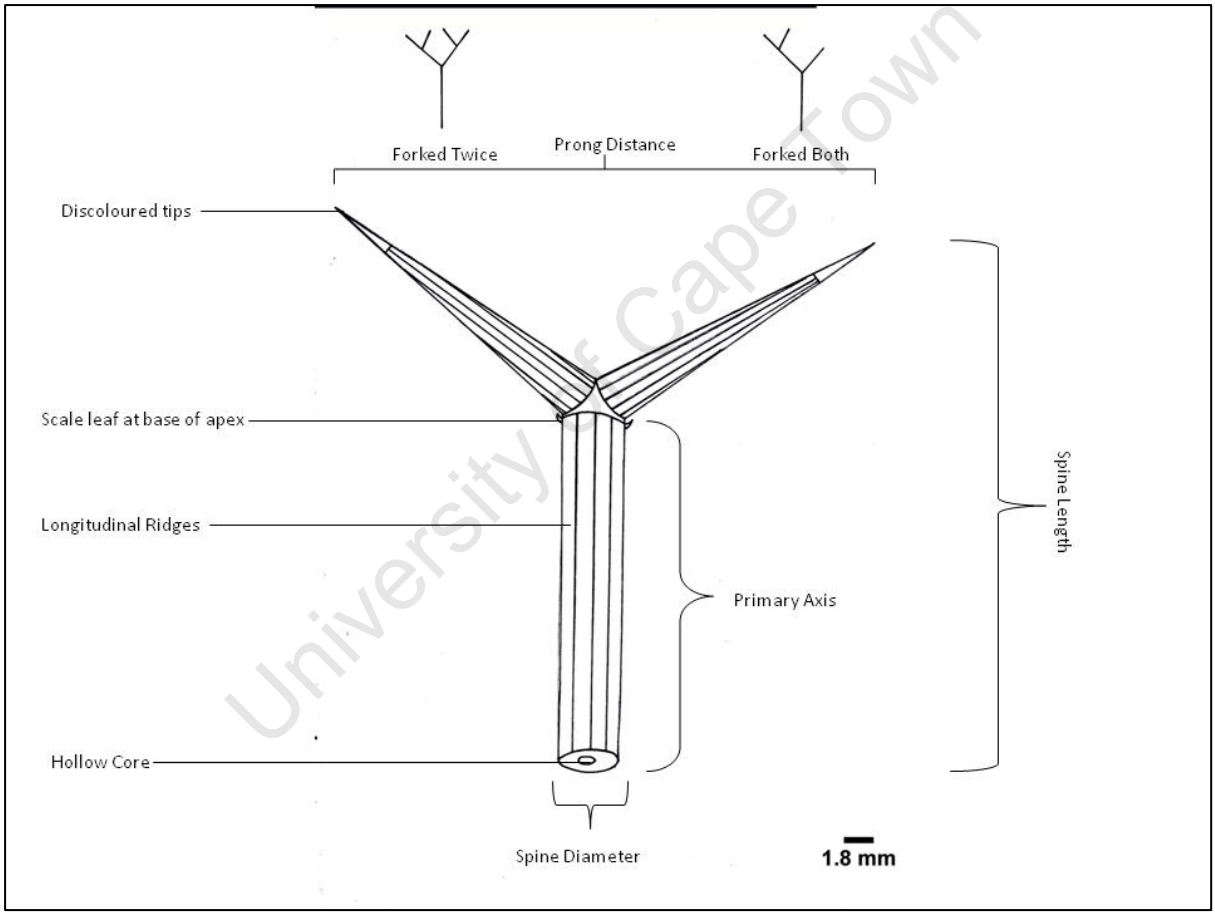

Fig.7. Structure of a typical spine of *C.macrocarpa*. The spine shown here is once forked; however there is variation in forking as indicated at the top of the picture.

Fig.8. Spines and leaves of *C.macrocarpa*. (a) Spines of C1, intermediate and C2 modules, (b) Spines of C3 modules, (c) Abaxial and adaxial surface of leaves. There is a decrease in spine length from C1 to C2 modules (a) as well as some twice forking spines in the C1 and intermediate spines (a). The spines of C3 modules are forked once with some variation in lengh (b). The adaxial surface of C.macrocarpa leaves is lighter than the abaxial surface; also the vertical venation on the adaxial surface is more prominent (c).

Fig.9. Module attachment of *C.macrocarpa*. (a) Sequence of module attachment, (b) Attachment of C3 growth relative to C1 growth, (c) Attachment of delayed C3 growth to the base of the plant, (d) Branching of trunks. An orthotropic C1 module bears an ageotropic intermediate module, which in turn either repeats itself or produces a plagiotropic C2 module (a). C3 growth (red rectangle) extends laterally from the same base of attachment as C1 growth (blue triangle) (b). Growth starts with the generation of one or more C1 modules (d) which then repeat the sequence seen in (a).

Ontogenesis

The development of *C.macrocarpa* starts with the growth of one or more C1 modules from the rootstock that grow to be unbranched monopodial primary axes (trunks) (figure 9d, figure 10). These C1 modules eventually fork into two intermediate modules of similar size and orientation at the apex of the previous trunk (figure 10). The meristems of these intermediate modules then give rise to another intermediate module and one C1 module (figure 10). The subsequent intermediate modules give rise to further intermediate modules until C2 modules are produced (figure 10, figure 9a). The transition from intermediate to C2 occurs when a newly formed module growing from an intermediate module ends up growing in a horizontal plane such that its orientation is also altered.

The change in orientation of C2 modules ensures that new modules produced at each C2 apex are also produced in a horizontal plane. Module length decreases from C1 to C2 modules (table 2), giving the impression that C2 horizontal growth produces more leaves per unit area.

The C1 module produced at the apex of the original intermediate module (figure 10) repeats the above sequence: C1 formation, intermediate module formation, more intermediate module formation followed by C2 formation (figure 10, figure 9a). These modules ultimately make up the next level of organisation in *C.macrocarpa* – that of the axis category. The C1 , intermediate and C2 modules form a vertical axis category, while the C3 modules form a horizontal axis category. Together these vertical and horizontal axis categories form the branch complexes of *C.macrocarpa* (figure 10). Over time the structure of *C.macrocarpa* is edified by the reiteration of branch complexes made up of C1, C2 and intermediate modules in sequence (figure 10). Thus, older plants would have more branch complexes than younger individuals (figure 10).

The lateral growth of C2 modules in each successive branch complex gives the plant a leafy shrub-like form with a laterally growing leafy "dress" (figure 10, figure 11). This dress is supplemented by C3 growth. C3 growth is essentially delayed C2 growth (figure 9c, figure 10). Thus they are newly formed modules from a much older module and appear anywhere on the older trunks, even at the point of attachment of first order trunks (figure 9c). They are usually attached to an older module by a delayed intermediate module or they may grow straight out of the older module (figure 9b). Therefore, the ontogenesis of *C.macrocarpa* occurs through the formation of a modulated branch complex and its reiteration over time to produce a plant with a leafy "dress" that extends laterally, in addition to shoots concerned with vertical growth (figure 11).

Environment-Induced Variations

Two behaviours were observed in *C.macrocarpa* in sun and shade conditions. In the shade the vertical branch complex seemed to be more elongated than in the sun (figure 11). Thus, C1 and intermediate modules were essentially longer in the shade than in the sun. The spines produced in the shade seemed to be shorter and looked like they had larger diameters than in the sun. There was also a reduction in the amount of horizontal C3 growth in the shade than in the sun (figure 11b and d).

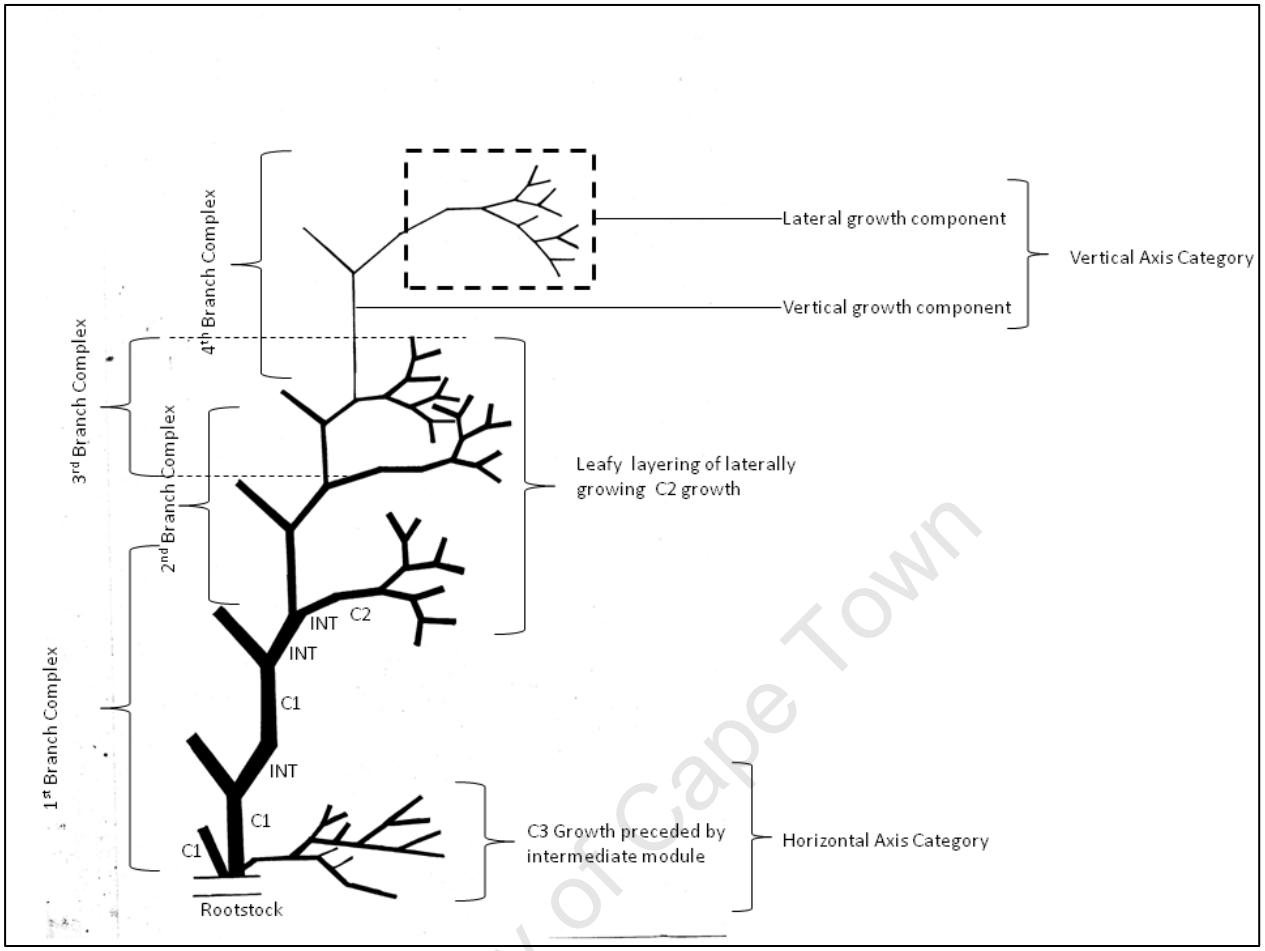

Fig.10. Ontogenetic programme of *C.macrocarpa*. The plant is ultimately constructed by modules, forming vertical and horizontal axis categories, which eventually form branch complexes. One or more C1 modules grow from the rootstock. These modules then split into intermediate modules which give rise to another C1 coupled with another intermediate module. The C1 module will grow to form the next vertical axis category of the plant while the intermediate module will eventually form the lateral C2 growth of the previous axis category. This sequence repeats itself generating many branch complexes over time. Thus the more mature the plant is the more branch complexes it forms. Each branch complex formed by a vertical axis category consists of a vertical and lateral component. The vertical component originates from C1 growth, while the lateral component originates from intermediate growth. The growth of successive branch complexes generates a layered formation of leafy lateral growth. This lateral growth is supplemented by delayed C3 growth which occurs anywhere on older trunks.

Fig.11. Growth stages of *C.macrocarpa*. (a) Early growth stage in the shade, (b) Late growth stage in the shade, (c) Early growth stage in the sun, (d) Late growth stage in the sun. The vertical axis category in the shade is longer than in the sun. The vertical axis category in the sun is more condensed. The horizontal axis category seems to be less pronounces in the shade than in the sun.

Quantitative Analysis

Changes in module parameters in Sun and Shade

C1 modules showed a significant decrease in spine length (U= 53; Z = -1.82; p = 0.034; r = 0.35), prong distance (U = 43; Z = -2.01; p = 0.0215; r = 0.39) and spine diameter (U = 40.5; Z = -2.43; p = 0.0062; r = 0.47) from open to closed canopy conditions (Table 1b). Canopy conditions did not have a significant effect on module length, diameter, nodal and apex leaf lengths of C1 modules (Table 1b). Canopy conditions did not affect the number of internodes or the number of apex and node leaves on C1 modules (Table 1b). C2 modules showed a significant increase (U = 49.5; Z = 1.99; p = 0.0213; r = 0.38) in module length and node leaf length (U = 50; Z = 1.97; p = 0.0024; r = 0.38) from open to closed canopy conditions (Table 1b). There was, however, a significant decrease in module diameter (U = 42; Z = -2.35; p = 0.0008; r = 0.45), spine length (U = 47.5; Z = -2.09; p = 0.0165; r = 0.4) and prong distance (U = 48; Z = -2.08; p = 0.019; r = 0.4) from open to closed canopy conditions (Table 1b). Canopy conditions had no significant effect on apex leaf lengths, the number of apex leaves or nodal leaves in the module as well as the number of internodes in C2 modules. Intermediate modules only showed a significant increase in module length (U = 45; Z = 2.21; p = 0.0125; r = 0.43) and nodal leaf length (U= 47; Z= 2.11; p= 0.0165; r = 0.41) from open to closed canopy conditions (Table 1b). Light conditions seemed to have no effect on the parameters of C3 modules (Table 1b).

In summary only the spines of C1 modules were affected by light condition. The spines of C1 modules are longer, thicker and have more pronounced prongs in the sun than in the shade. C2 modules are shorter and thicker in the sun and have longer spines with more pronounced forks than in the shade. The leaves of C2 modules are also shorter in the sun than they are in the shade. Only the leaves and module length of intermediate modules was affected by light condition. Intermediate modules were shorter and had shorter leaves in the sun than in the shade. C3 modules were not affected by light condition.

Table 1: Qualitative and quantitative architectural properties of *C.macrocarpa* modules in open and closed canopy conditions. *C.macrocarpa* is constructed of four modules – category 1 (C1), category 2 (C2), category 3 (C3) and intermediate. Qualitative module differences occur in primary growth direction, the strength of secondary growth, flowering ability, spine forking and angle, as well as bark colour and texture. There were significant differences in spine length, diameter and prong distance for C1 modules. C2 modules showed significant differences in module length and diameter, spine length and diameter and nodal leaf lengths. Intermediate modules showed significant differences in module length and diameter as well as significant differences in nodal leaf lengths. C3 modules showed no change in parameters in open or closed canopy. All significance testing was done using a non-parametric Mann Whitney U-test.

(A)		C1		C2		C3		Intermediate	
Qualitative Properties		**Open**	**Closed**	**Open**	**Closed**	**Open**	**Closed**	**Open**	**Closed**
Primary growth	**Structure**	Dichasial Sympodial		Dichasial Sympodial		Dichasial Sympodial		Dichasial Sympodial	
	Primary growth direction	Orthotropic		Plagiotropic		Plagiotropic		AgeoTropic	
Secondary growth	**Secondary growth direction**	Vertical		Horizontal		Horizontal		Oblique	
	Type	Branched		Branched		Branched		Branched	
	Strength	Strong		Strong		Medium		Light	
	Conicity	Cylindrical		Mostly cylindrical		Mostly cylindrical		Cylindrical	
Branching	**Type**	Rhythmic		Rhythmic		Rhythmic		Rhythmic	
	Chronology	Immediate		Immediate		Immediate/delayed		Immediate	
	Location	Acrotonic		Acrotonic		Acrotonic		Acrotonic	
Symmetry		Bilateral Amphitony		Bilateral Amphitony		Bilateral Amphitony		Bilateral Amphitony	
Leaves	**Phyllotaxy**	Opposite		Opposite		Opposite		Opposite	
	Form	Ovate		Ovate		Ovate		Ovate	

(A) Qualitative Properties		C1		C2		C3		Intermediate	
		Open	Closed	Open	Closed	Open	Closed	Open	Closed
Flowering	Ability	No		Yes		Yes		No	
	Location	none		Apical		Apical		none	
Spinescence	Forking	Once/Twice/Both		once/both		once/both		once/twice/both	
	Conicity	cylindrical		cylindrical		cylindrical		cylindrical	
	Conicity	conical		conical		conical		conical	
	Location	Apex		Apex		Apex		Apex	
	Angle	90		60		60		90	
Other	Bark colour	greyish brown		none		greyish brown		none	
	Texture	Striated		none		heavily scaled/striated		none	

(B) Quantitative Properties	C1		C2		C3		Intermediate	
	Open	Closed	Open	Closed	Open	Closed	Open	Closed
Module Length (cm)	25.56 ± 4.04	20.57 ± 2.26	7.55 ± 0.60*	10.12 ± 0.89*	8.43 ± 1.09	6.73 ± 0.53	8.9 ± 0.64*	11.92 ± 1.06*
Module Diameter (cm)	1.39 ± 0.13	1.12 ± 0.10	0.56 ± 0.05***	0.41 ± 0.03***	0.83 ± 0.09	0.59 ± 0.09	0.98 ± 7.71	0.56 ± 0.04
No. Internodes in module	4 ± 0.45	4 ± 0.23	2 ± 0.14	2 ± 0.17	2 ± 0.17	2 ± 0.11	2 ± 0.21	2 ± 0
Spine Length (mm)	42.15 ± 3.51*	37.42 ± 6.11*	36.61 ± 3.92*	26.5 ± 2.74*	23.3 ± 2.86	16.96 ± 1.75	34.92 ± 2.91	33 ± 3.02
Prong Distance (mm)	42.25 ± 3.31*	36.91 ± 5.86*	31.42 ± 4.99*	19.64 ± 2.07*	20.65 ± 1.98	15.13 ± 2.16	31.19 ± 3.2	31.21 ± 4.11
Spine Diameter (mm)	4.13 ± 0.22*	3.26 ± 6.99*	2.82 ± 0.29	2.41 ± 0.31	2.36 ± 0.37	1.54 ± 0.17	3.4 ± 0.36	2.73 ± 0.21
No. Node leaves	6 ± 1	5 ± 0.46	3 ± 0.28	3 ± 0.34	3 ± 0.35	3 ± 0.39	3 ± 0.36	2 ± 0
No. Apex leaves	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0
Node leaf lengths (cm)	2.22 ±0.25	2.56 ± 0.31	2.84 ± 0.17***	3.52 ± 0.28***	2.20 ± 0.04	2.59 ± 0.05	2.74 ± 0.11*	3.66 ± 0.33*
Apex leaf lengths (cm)	3.56 ± 0.29	3.96 ± 0.43	3.68 ± 0.25	3.91 ± 0.31	2.94 ± 0.04	3.58 ± 0.07	3.35 ± 0.12	4.02 ± 0.3

Note: Significant differences are indicated as follows: *p<0.05; **p<0.005; ***p<0.0005

Ontogenic Change Within Branch Complexes, across Growth Stages and Canopy Openness

The aim of this investigation was to evaluate spine efficiency in *C.macrocarpa*, therefore only parameters that were deemed important for spine efficiency were analysed below. Both the vertical and horizontal axis categories of *C.macrocarpa* showed a significant decrease in module parameters moving from the base to the periphery of the axis category (Table 2). This was true for all parameters considered, except for spine length in the vertical axis category. Spine length does not change from the base to the periphery of the vertical axis category (Table 2). This indicates that there was no change in spine length between C1, C2 and intermediate modules moving from the base to the periphery of the vertical axis category. The number of internodes in a module also did not change with successive C3 modules in the horizontal axis category (Table 2).

Growth stage had no effect on any of the parameters considered in the horizontal axis category (Table 2). In the vertical axis category there was only a significant increase in module diameter and spine prong distance with growth stage (Table 2). This indicates that only module diameter and prong distance increased over successive growth stages. Canopy openness had no overall effect on the parameters of C3 modules (Table 2). Module diameter, spine length, prong distance and spine diameter, however, increased with canopy openness (Table 2).

The architectural parameters of spine efficiency respond to the level of canopy openness when considering canopy openness as a continuous gradient and not by categorical grouping (figure 12). Therefore the non-significant effect of canopy openness on module length in Table 2 and Table 1b for C1 modules, could be due to the categorical grouping imposed or due to lack of statistical power in the case where the Mann-Whitney U-test was used (Table 1b). Module length decreases while module diameter increases with canopy openness (figure 12a and b). Spearman's rank correlation analyses (with $\alpha = 0.1$) of these variables revealed a significant negative relationship between module length and canopy openness ($\rho(22) = -0.39$; $p = 0.06$) and a significant positive relationship between module diameter and canopy openness ($\rho(22) = 0.63$; $p=0.000956$). Prong distance and spine length did not show any significant relationship with canopy openness according to a Spearman's rank correlation analysis (figure 19c and d). However, this could be because spine length and prong distance do not show any monotonic trend with canopy openness, thus violating one of the assumptions of Spearman's rank correlation analysis (Crawley 2007).

In summary, the modules of the vertical axis category become shorter and thinner with thinner spines that have less pronounced forks as one moves from the base to the periphery of the axis category. The spine length of successive modules in the vertical axis category stays the same from the base to the periphery of the axis category. The effect of growth stage is minimal and only affects module diameter and spine prong distance in the vertical axis category. Modules become thicker and have more pronounced spines with successive growth stages. Canopy openness affects more parameters than growth stage in the vertical axis category. In the sun modules become thicker and shorter with longer, thicker spines that have more pronounced forks. The horizontal axis category was not affected by canopy openness or growth stage.

Table 2: Change in parameters of spine efficiency at different spatial scales. Canopy openness shows no effect on module length. However there is a significant increase in module diameter and quantitative spine properties such as spine diameter, length and prong distance from closed to open canopy. The number of internodes in a module does not change with canopy openness. Only module diameter and prong distance increase in successive growth stages. There is a decreasing sequence of change from the base to the periphery of a branching complex (from C1 through to C2 modules), under all growth stages and canopy conditions, with the exception of spine length, which does not change from the base to the periphery.

Vertical Axis Category (Categorical Level)	Module Length	Module Diameter	Spine Length	Prong Distance	Spine Diameter	Number of internodes in Module
Canopy Openness (Closed to Open)	NoChange	Increase***	Increase**	Increase**	Increase**	NoChange
Growth Stage (young to developed)	No Change	Increase*	No Change	Increase*	No Change	NoChange
Module (C1/INT/C2)	Decrease***	Decrease***	NoChange	Decrease**	Decrease***	Decrease***
Horizontal Axis Category (Categorical Level)						
Canopy Openness (Closed to Open)	No Change	No Change	No Change	No Change	No Change	No Change
Growth Stage (young to developed)	No Change	No Change	No Change	No Change	No Change	No Change
Module (C3 – Proximal to Distal module)	Decrease***	Decrease***	Decrease***	Decrease***	Decrease***	No change

Note: Significant differences are indicated as follows: * p<0.05; **p<0.01; ***p<0.001

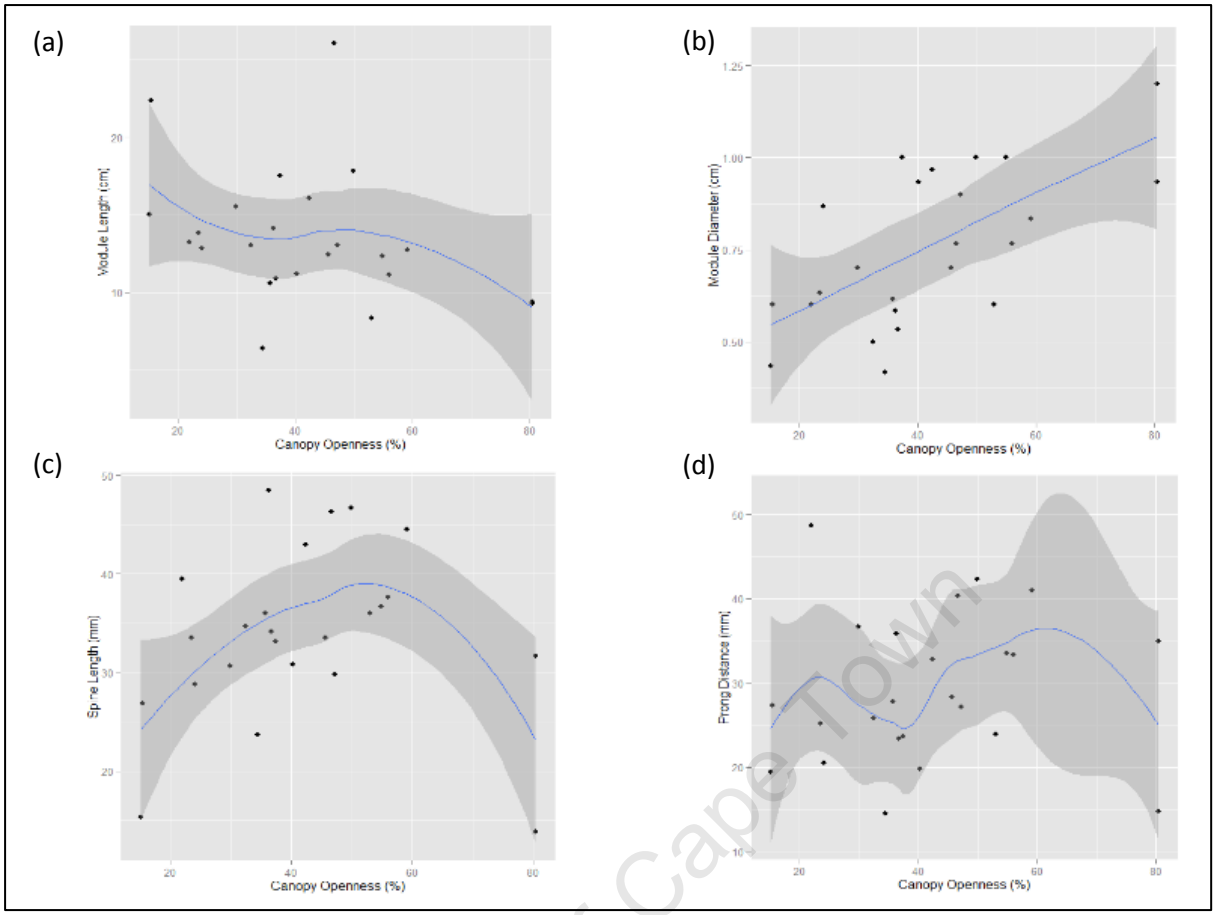

Fig.12. Architectural parameters of spine efficiency in relation to canopy openness. Module length (a) decreases with canopy openness while module diameter (b) increases. Spine length (c) increases with canopy openness, but only up to a certain point after which it decreases. Prong distance (d) also shows a general increase, after which it decreases at a certain level of canopy openness. Local regression lines have been fitted to each scatterplot. Dark bands indicate 95% confidence bands for these local regression lines. All local regression lines have been fitted with a span of 1.

Physiology

Biomass Allocation

There was no significant difference in biomass allocation to stems, leaves and spines between sun and shade individuals (figure 13). Thus average biomass allocation to stems, spines and leaves is 63%, 7% and 30% respectively (figure 14). Stems always receive the most resources, followed by leaves and then spines (figure 14). However, there is a strong increase in the mass of leaves, spines and stems across a gradient of canopy openness (figure 15). Spearman's rank correlation analyses revealed that there was a significant positive relationship between log transformed spine mass ($\rho(22)=0.41$; $p = 0.046$), leaf mass ($\rho(22)=0.58$; $p = 0.0031$) and stem mass ($\rho(22)= 0.47$; $p = 0.02$) with canopy openness. These variables, however, showed collinearity. Log transformed spine mass showed a significant positive correlation with that of leaf mass ($\rho(22)=0.77$; $p = 0.00001$) and stem mass ($\rho(22)=0.69$; $p = 0.00019$). Log transformed stem mass and leaf mass were also significantly positively correlated ($\rho(22)= 0.84$; $p = 0.000001$).

The collinearity between log transformed leaf mass, stem mass and spine mass led to the investigation of the relationship between total biomass and canopy openness. Total biomass showed a significant positive relationship to canopy openness ($\rho(22)= 0.53$; $p = 0.007$).There were also apparent differences in total leaf area and total specific leaf area in the sun and shade. Individuals in the sun group had a higher total leaf area (834 $cm^2 \pm 233$ cm^2) than the shade group (622 $cm^2 \pm 121$ cm^2) , while individuals in the shade group had a higher specific leaf area (50.22 $cm^2.g^{-1} \pm 3.69$) than individuals in the sun group (40 $cm^2.g^{-1} \pm 3.91$). However, these differences were non-significant as per a non-parametric Mann-Whitney U-test. This non-significant result could be due to a lack of statistical power considering that the sample size of sun individuals was only 4 (sites 16,22,25,19) and that of shade was only 6 (sites 1,2,5,7,9,13).

In summary the proportion of biomass allocation to stems, spines and leaves does not change in the sun and shade. Stems always receive the most resources followed by leaves and then spines. The mass of leaves, stems and spines increases with canopy openness. However they are collinear and it has been shown that total biomass is positively correlated with canopy openness. Shade plants had higher specific leaf area but lower total leaf area than sun plants.

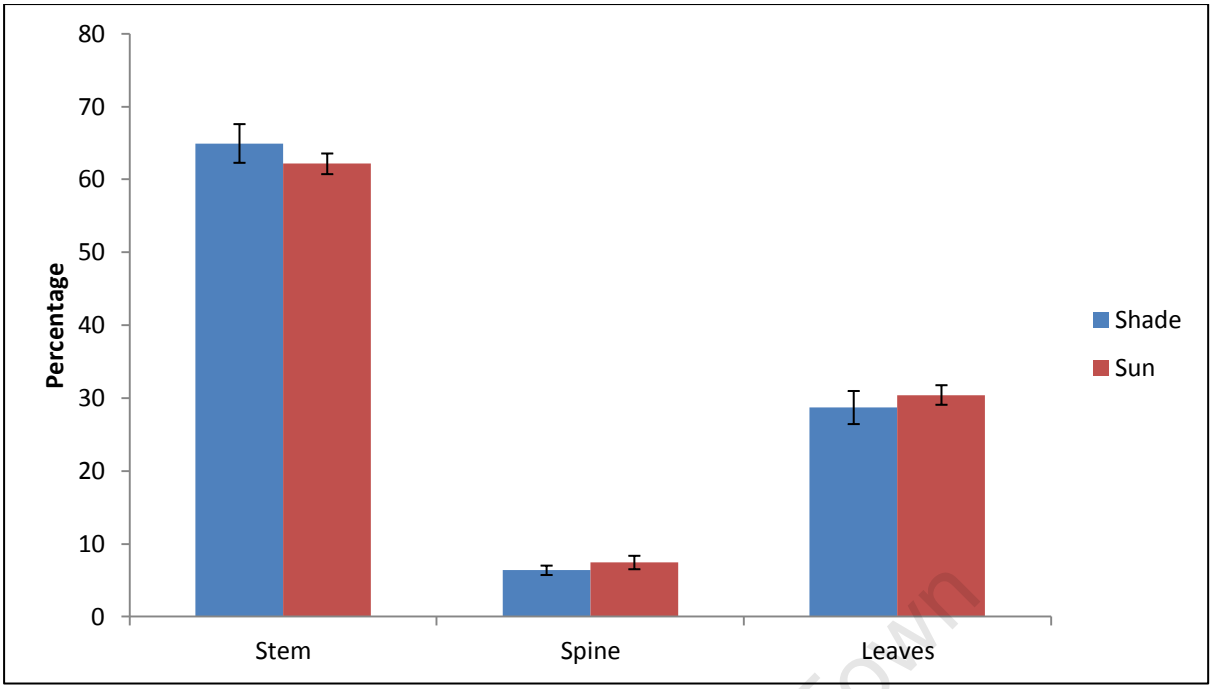

Fig. 13. Percentage biomass allocation to stems, leaves and spines for shade and sunlit conditions. There is no difference in allocation to different vegetative parts in both the sun and shade. Stems receive the most biomass followed by leaves and then spines.

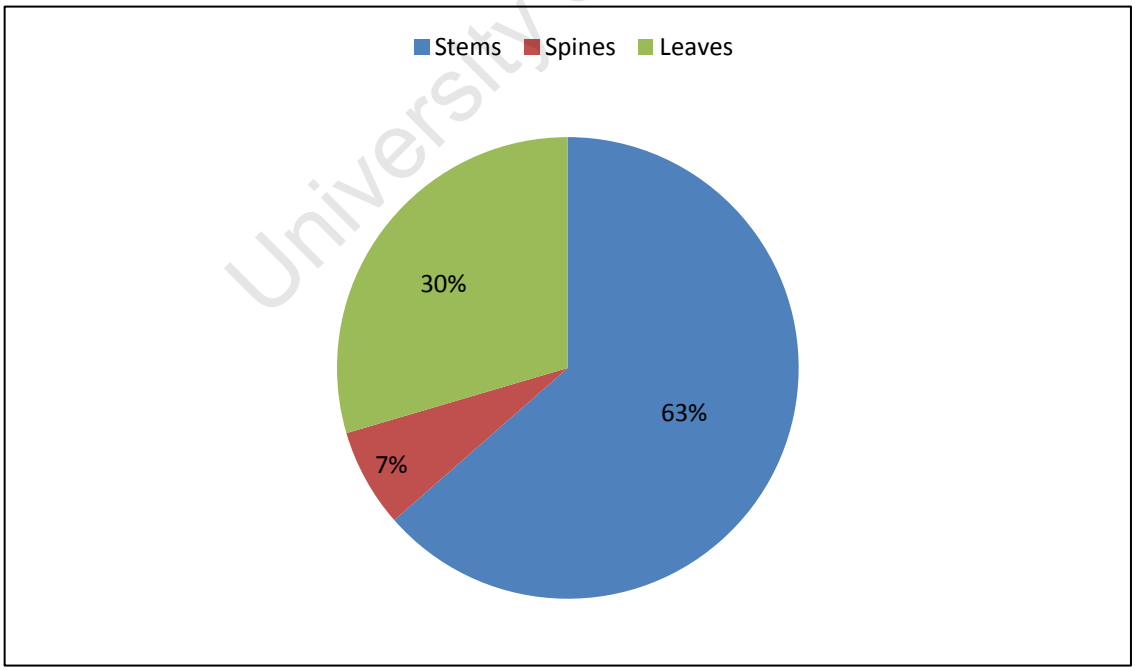

Fig.14. Average percentage allocation to leaves, stems and spines for shade and sun group plants. Stems receive the most biomass, followed by leaves and then spines.

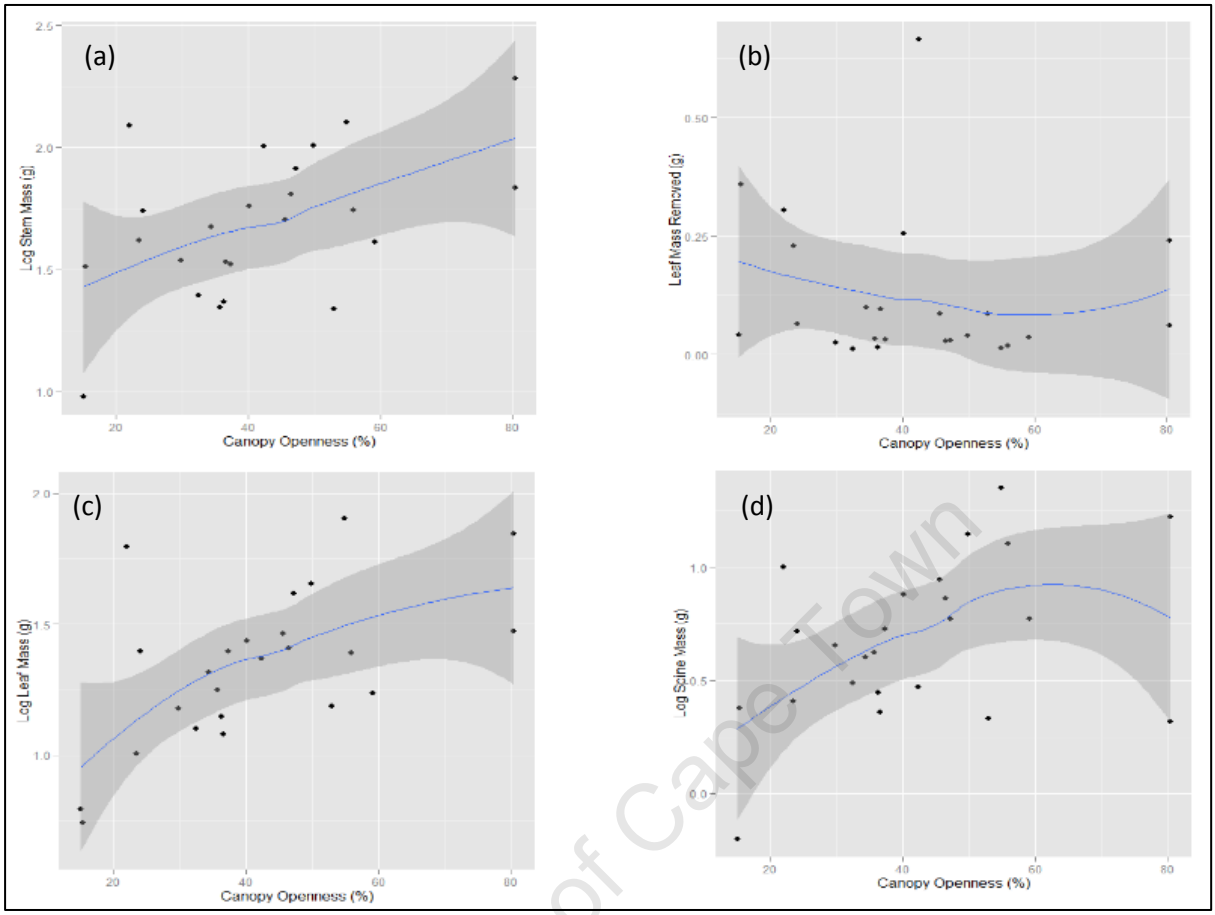

Fig.15. Biomass (log transformed) of stems (a), leaf mass removed (b) , leaves (c) as well as spines (d), along a gradient of canopy openness. Stems, leaves and spine biomass seem to increase over the gradient of canopy openness, while leaf mass removed decreases. Each scatterplot has been fitted with a non-parametric local regression line, each with a span of 1. The dark bands indicate the 95% confidence bands for each local regression line.

Carbon Gain

The light response curves of sun and shade plants show a higher net carbon assimilation rate in the sun than in the shade (figure 16). This is accompanied by higher response curve parameters in the sun than the shade (table 3).Individuals in the sun group had a significantly greater net carbon gain per meter squared per year (11.55kg.m^{-2}.yr ± 0.54) than those in the shade group (4.77 kg.m^{-2}.yr ± 0.64) (U = 2; Z = -4.29; p = 0.000001; r = 0.83) (figure 17). The total leaf area in the sun and shade has been estimated as 834 cm^2 and 622 cm^2 respectively (in previous section). This converts to 0.062 m^2 and 0.083 m^2 in the shade and sun respectively. Thus, multiplying by the net carbon gain of sun and shade groups gives 0.96kg and 0.3kg carbon gained for each group respectively over a year.

Therefore, given the biomass allocation in figure 2 and the differences in net carbon assimilation rate in figure 6, the modelled carbon allocation for individuals in the sun group would be 605g carbon to stems, 67g to spines and 288g to leaves. Individuals in the shade group would be able to devote only 189g of carbon to stems, 21g to spines and 90g to leaves.

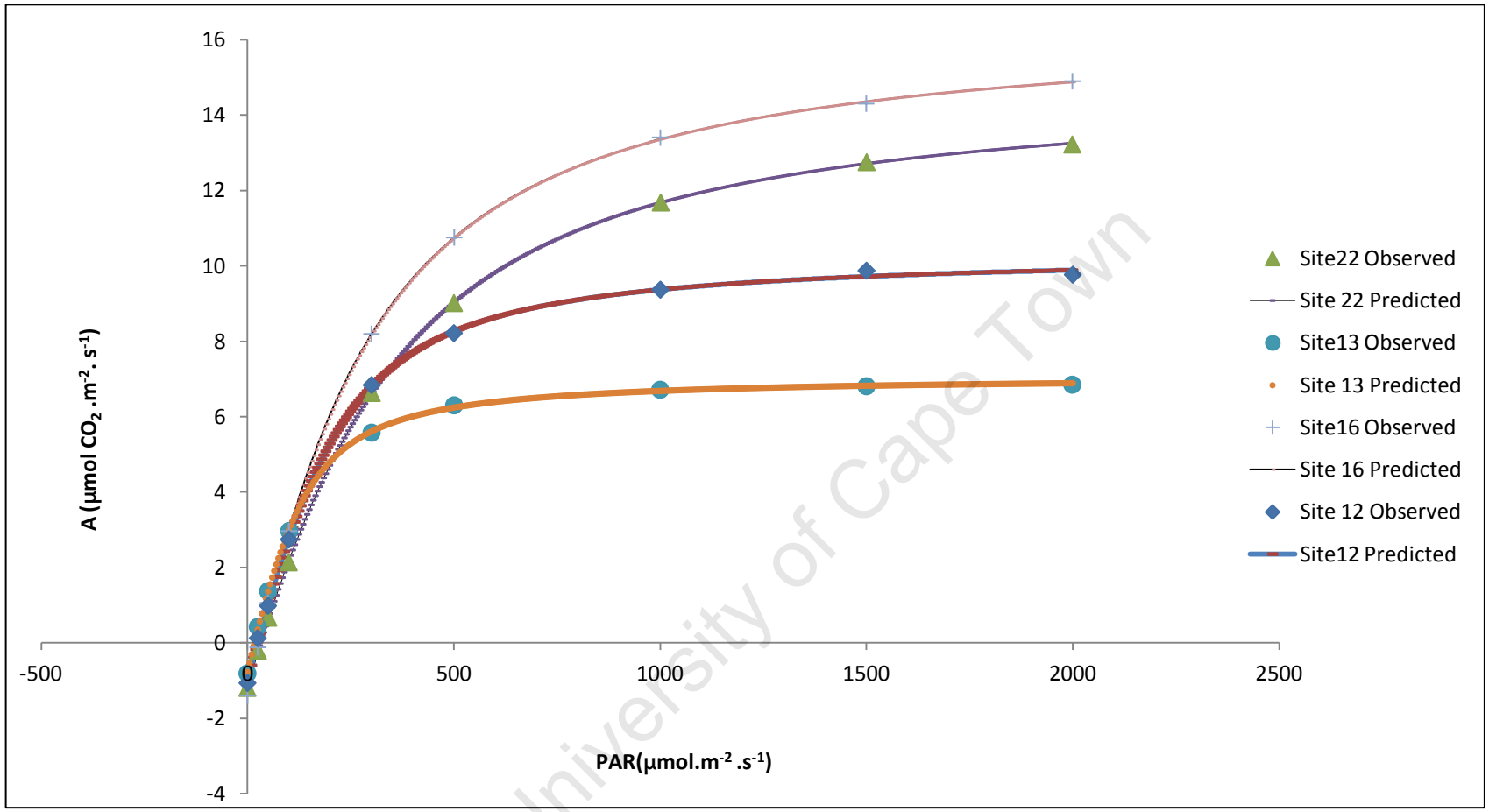

Fig.16. Light response curves of two shade (site 12 and 13) and two sun (site 22 and site 16) individuals of *C.macrocarpa*. The response curves of the sun individuals have higher net carbon assimilation rates (Amax) than those in the shade. The quantum yield efficiency (the initial slope of the curve) is initially similar but separates out at about 500 PAR, when the shade individuals start to saturate but the sun individuals carry on increasing their photosynthetic rate.

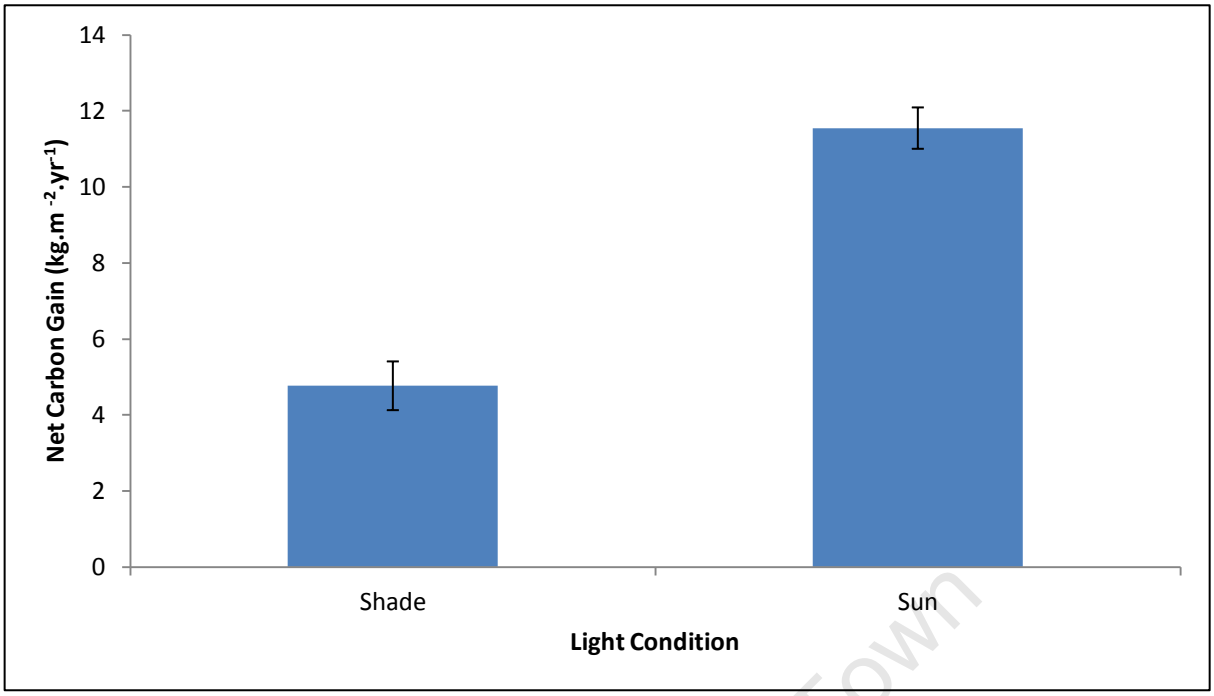

Fig.17. Net carbon gain in sun vs. shade (Means ± SE)

Table 3: Light response curve parameter values for shade and sun plants.

Light Condition	Dark Respiration rate (μmol $CO_2.m^{-2}.s^{-1}$)	ϕ (Quantum Yield)	Amax (μmol $CO_2.m^{-2}.s^{-1}$)	θ (Curvature Factor)	Light Compensation Point
Sun	1.26 ±0.10	0.044±0.006	16.97±0.889	0.49±0.017	31.12±2.10
Shade	0.90±0.12	0.045±0.001	9.62±1.76	0.70±0.009	22.01±3.88

Spine Efficiency

The index of spine efficiency was significantly higher in the sun group than the shade group (U = 32; Z = -2.26; p = 0.0011; r = 0.45) (figure 18). The relationship of certain parameters such as spine mass, spine length, prong distance, module length and module diameter to spine efficiency were examined (figure 19). Spearman's rank correlation analyses revealed a significant positive relationship between spine mass (ρ(22) = 0.55; p = 0.005), spine length (ρ(22) = 0.43; p =0.035) and prong distance (ρ(22) = 0.49; p = 0.016) with spine efficiency. Module length and diameter, however, did not show any significant relationship with spine efficiency.

In summary spine efficiency is higher in the sun than the shade. Spine mass, length and prong distance increase spine efficiency. This increase in architectural spine parameters is accompanied by the shortening and thickening of modules in the sun than in the shade.

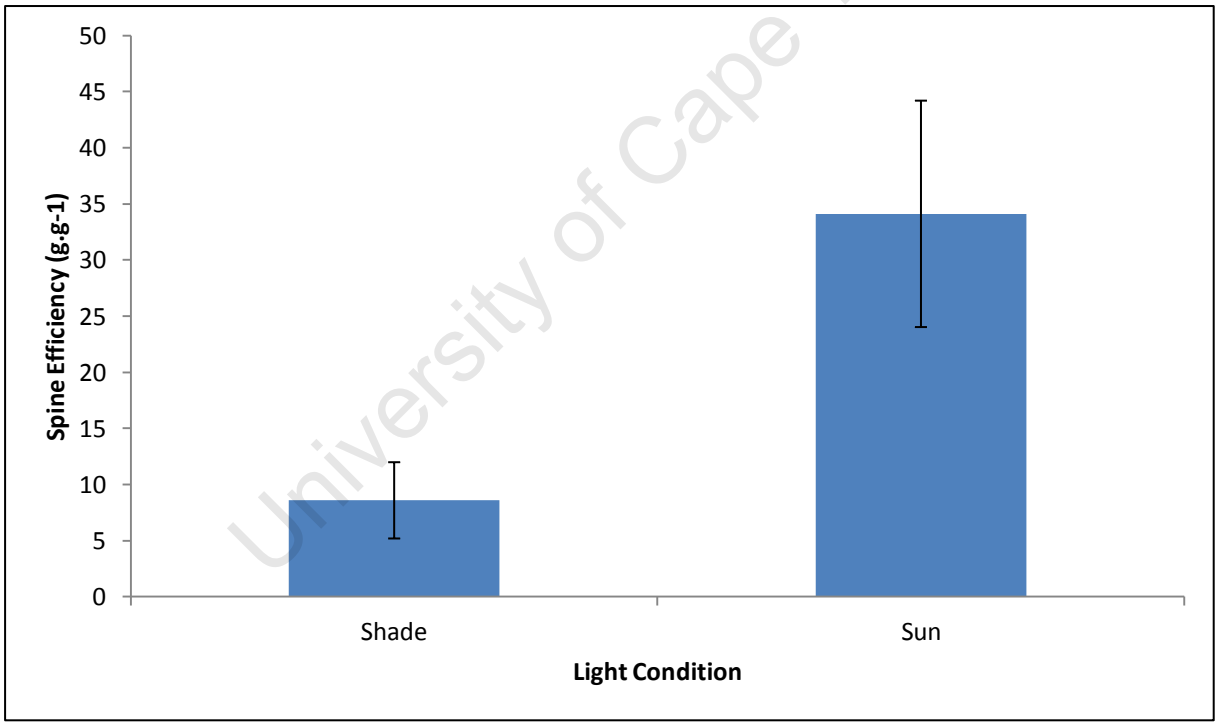

Fig.18. Spine efficiency for sun and shade groups. The sun group clearly has much higher spine efficiency than the shade group. Means ± SE are shown here.

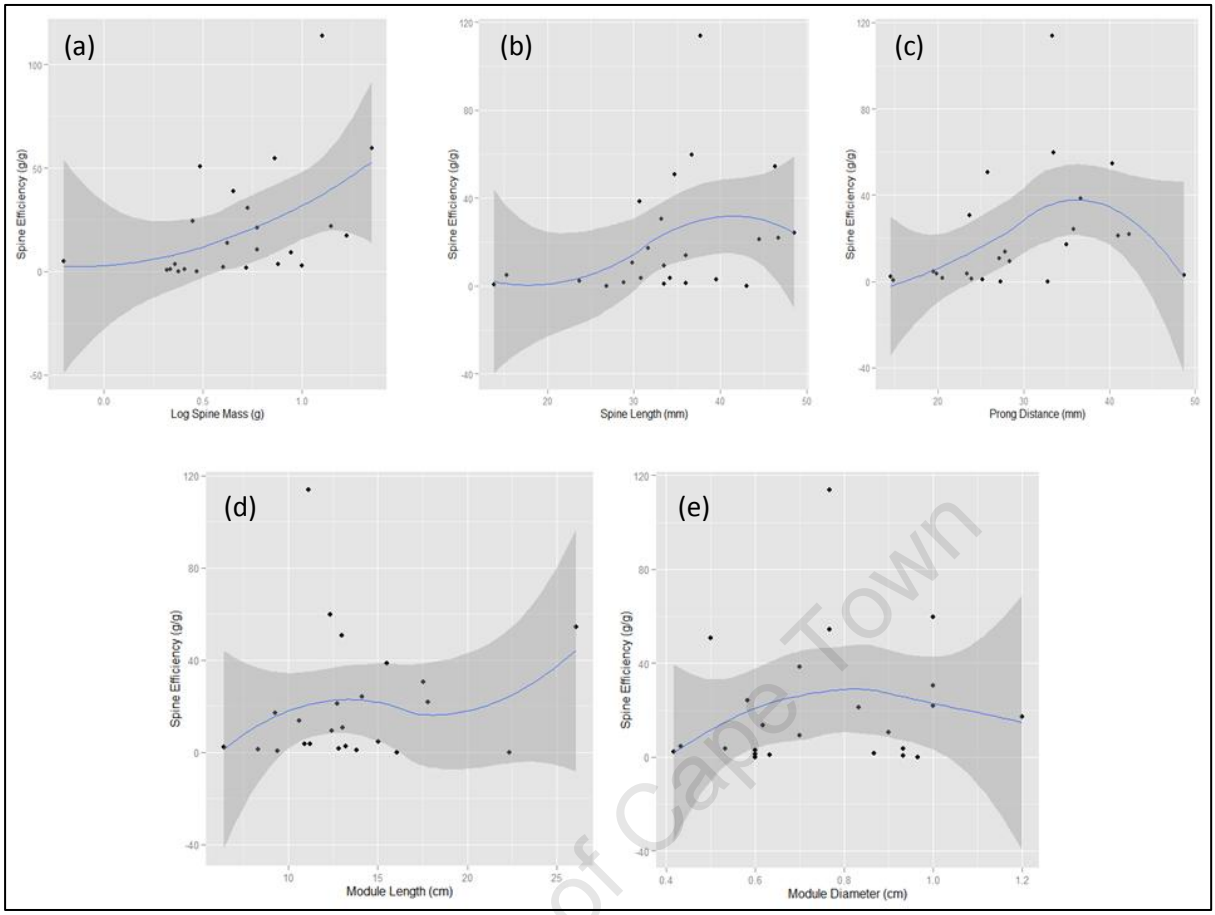

Fig.19. Spine efficiency vs. architectural parameters and spine mass. Spine efficiency is the measure of spine mass protecting divided by leaf mass removed in a single bite within a volume of 1575 cm^3. There is a general increase in spine efficiency with an increase in spine mass (a), spine length (b) and prong distance (c). Module length (d) does not have much of an effect on spine efficiency and neither does module diameter (e). Local regression lines have been fitted to each scatterplot. Dark bands indicate 95% confidence bands for these regression lines. All local regression lines have been fitted with a span of 1.

Discussion

The aim of this study was to determine the reason spiny plants have an aversion to shaded conditions, by assessing light condition as a possible stressor reducing the spine efficiency and fitness of shaded spiny plants. Thus we examined the effect of light on the developmental programme (architecture) and physiology of plants in sun and shade environments. The developmental programme was discerned using architectural analysis that took into account the ontogeny of the plant, while physiological differences were examined by assessing biomass allocation patterns and net carbon gain in sun and shade plants.

Architectural levels of organisation in C.macrocarpa

C.macrocarpa has three levels of organisation nested within each other: the module, the axis category and the whole organism. The different module types (C1, C2, intermediate and C3) construct the axis categories which in turn construct the plant (figure 10). The vertical axis category functions primarily to set up the framework of the plant and explore vertical space. The horizontal axis category is constructed by delayed growth and is restricted to lateral growth. The module lengths decrease from the base to the periphery of the horizontal axis category, essentially arranging the leaves of successive modules closer together. Thus the horizontal axis category has more leaves per unit area in the lateral plane than the vertical axis category. Therefore this delayed axis category serves to increase the photosynthetic surface of the plant.

The effect of ontogeny and light condition on modules

During the construction of an axis category (a trunk or branch), the dimensions of successive modules decrease. However, this decreasing trend is modified by both light condition and growth stage. Therefore even though the sequence of modules constructing an axis category stays the same, the overall size of modules can either be larger or smaller depending on the growth stage or light condition the plant experiences. This suggests that *C.macrocarpa* exhibits complex plasticity (Wright and Mcconnaughay 2002). Complex plasticity occurs when both ontogeny (ontogenetic plasticity) and environmental conditions (passive plasticity) cause a change in a trait (Wright and Mcconnaughay 2002). In *C.macrocarpa* overall modification of modules is caused by environmental conditions and to a lesser degree by ontogeny.

Architectural strategies of C.macrocarpa and spine efficiency

Only the vertical axis category of *C.macrocarpa* was concerned with the plastic response of the plant with regard to light condition. This is evidence of hierarchical plasticity. Hierarchical plasticity simply means that the plastic response of the plant to a certain resource is dependent on certain units (such as modules or axis categories) that construct the plant rather than others (Navas and Garnier 2002). Hierarchical plasticity has also been observed in other architectural studies that have evaluated the effect of light condition on plant architectural strategy. In *Cornus sericea* (Charles-Dominique et al. 2010) and *Rhamnus cathartica* (Charles-Dominique et al. 2012) only the trunks responded to light condition. These trends of hierarchical plasticity in response to light condition highlight the importance of determining the levels of organisation within a plant, in order to properly assess its phenotypic plasticity. In *C.macrocarpa* the vertical axis category is responsible for phenotypic plasticity in relation to light condition. Thus it is this axis category that is responsible for the differences in spine efficiency observed in sun and shade.

Differences in spine efficiency become evident when one considers the architectural strategies adopted by the plant in the sun and shade. In the sun *C.macrocarpa* adopts a stout, dense well-defended form. This is due to the modules of the vertical axis category being shorter and thicker and producing spines that are longer and thicker with more pronounced forks. Modules that are shorter and thicker would increase the protective effect that spines have on leaf material by arranging them closer together such that the tips of their forks occupy more of the area above leaves. This arrangement ensures that as many prongs as possible stick into the snout of a potential browser, reducing the amount of leaf or stem material removed.

The decreased vertical growth in the sun due to decreased module length would allow for more C2 modules (horizontal modules) to grow per unit area, which in turn would increase the photosynthetic surface that the plant is able to utilise. This is supported by the fact that sun plants have a higher total leaf area than shade plants – higher total leaf area means more photosynthetic area. Plants in sunlit environments usually have smaller leaf areas than those in the shade (Givnish 1988). However, the fact that there is higher leaf area in the sun than the shade suggests that there are more leaves present per unit area in the sun. In addition to this, more horizontal module growth would also mean that the reproductive ability of the plant is higher in the sun, since flowering only occurs on horizontal modules.

The shade architectural strategy is the opposite of the sun strategy, being more elongated and less well defended. The elongation of modules in the shade positions spines too far apart, leaving leaf and stem mass open to attack by browsers. The emphasis on vertical growth reduces the amount of horizontal C2 growth, ultimately reducing the reproductive ability of the plant in the shade. Therefore, the growth strategy in the sun is geared towards increasing the photosynthetic surface and the protection of this surface (i.e. protection of the increased leaf mass), while growth in the shade is geared towards exploratory vertical growth. Thus, *C.macrocarpa* exhibits plasticity in relation to light condition. However, the most important point here is that spine efficiency – the ability of the plant to effectively reduce the amount of leaf material removed by browsers – is dependent on the architectural strategy employed, with increased spine efficiency exhibited in the sun architectural strategy. This increased spine efficiency together with the increased reproductive ability afforded by the sun architectural strategy ultimately increases the fitness of the plant.

Interaction between biomass allocation and architecture

Rules governing allocation are the same for both sun and shade plants. This was unexpected as optimality theory would suggest that plants in the shade would allocate more resources to stem and leaf growth in order to maximise carbon gain (Strauss et al. 2002). Thus the fact that there is no change in allocation suggests that there is some other mechanism allowing the plant to adapt to light-limitation. Another important observation is that as light becomes increasingly available so the biomass to stems, leaves and spines also increases. This is explained by higher net carbon gain for sun plants than for shade plants. Therefore sun plants produce more biomass than shade plants. This together with the fact that allocation rules stay constant suggests that *C.macrocarpa* uses its architectural plasticity to adapt to light condition: producing the same relative amount of stems but changing the quality of the stems in relation to light condition.

C.macrocarpa takes advantage of increased light conditions by shunting the increased biomass it produces in the sun into the construction of the sun architectural strategy. The increased biomass goes into constructing shorter thicker modules with longer more pronounced spines, arranged close together with a large photosynthetic surface area. Therefore, in the sun *C.macrocarpa* presents a dense heavily defended form that is able to perpetuate itself using its large photosynthetic surface area that increases carbon gain.

The sun architectural strategy provides positive feedback to the plant by maintaining high net carbon gain, which in turn produces more biomass which is then fed back into the construction of the sun architecture. Ultimately, light condition affects biomass production which affects the architectural strategy the plant uses. This in turn affects spine efficiency.

So far we have seen that a higher net carbon gain in the sun produces more biomass, which ultimately affects spine efficiency (as explained in the previous paragraph). Taking a carbon-costing perspective, however, also reveals that shade plants cannot produce enough carbon to produce efficient spines. The reasoning behind this is based on the fact that maximum spine efficiency is not proportional to maximum canopy openness. This is because spine length only increases up to 60% canopy openness, after which it decreases.

However, spine length is also positively related to spine efficiency which would suggest that spine efficiency reaches its maximum at 60 % canopy openness as well. The amount of carbon (taking biomass as a proxy for carbon) needed to build spines that aid in maximising spine efficiency is about 800mg (figure 19a). Given that sun plants allocate 67g carbon to spines in a year, they would be able to produce 184mg in a day. Therefore at a rate of 184mg/day sun plants could produce the required amount of carbon within 4 days.

Shade plants only allocate 21g carbon to spines in a year. Therefore they could only produce 57mg carbon in a day and would take 14 days (3 times as long as sun plants) to produce the amount of carbon needed for efficient spines. If shade and sun plants were exposed to the same browsing pressure, the sun plants that would be able to respond better by being able to produce efficient spines faster than the shade plants. However, in the end it is the architectural strategy that determines how efficient these spines are at deterring browsers. Thus shade plants are at a loss both architecturally and due to a lack of resources.

Scope regarding other studies on structural defence

This study has ultimately examined the effect of resources (in this case light) on the ability of spines to effectively protect the plant from browser attack. There has been a bias toward chemical defences when proposing hypotheses on the effect of resources on defence (Grubb 1992; Hanley et al. 2007). The resource availability hypothesis (Coley et al. 1985) and the Growth-Differentiation hypothesis (Herms and Mattson 1992) both propose that plants would be more defended in resource limited environments. However, it has been shown here and in other studies (e.g. Gowda et al. 2003 – reviewed by Hanley et al. 2007; Grubb 1992) that in the face of light as a limiting resource, the amount of structural defence actually increases with an increase in resources.

Other studies have shown that structural defences work by reducing the amount of plant material browsers are able to remove while at the same time increasing time spent foraging (Cooper and Owen-Smith 1986; Bevlovsky et al. 1991; Milewski et al. 1991; Gowda 1996; Shipley 2007, Wilson and Kerley 2003). However, they did not consider the resource implications of structural defence for the plant. Furthermore, other studies have shown that structural defences can be induced by herbivore pressure (Milewski et al. 1991; Karban and Myers 1989; Young 1987; Young et al. 2003). However, these studies also overlook the mechanism by which structural defences work and did not consider the effect of resources. In this study we have shown that when light is the limiting resource, the plant is not able to produce the carbon needed to construct the spines and their supporting stems necessary for maximum spine efficiency. Therefore, even if herbivory does induce the production of more structural defences (as suggested by the studies above); these defences might not be able to adequately defend the plant if light is limiting.

Grubb (1992) proposed that there are ultimately six variables that need to be considered to adequately examine plant defence - resource availability, architecture, type of herbivore, plant distribution over the landscape, phenology relative to neighbours and nutrient content relative to neighbours. The studies above have only considered the type of herbivore feeding on the plant in an effort to quantify structural defence. This study examined defence in terms of resource availability, architecture and the type of herbivore feeding on the plant (ungulate browsers). Certain studies that have addressed ungulate herbivory and architecture agree with the findings of this investigation. For example Staver et al. (2012) showed that *Acacias* adopted a "cage-like" densely ramified architecture in response to herbivore pressure. This cage-like architecture has been observed to be accompanied by larger spines when light is sufficient (Archibald and Bond 2003). This example illustrates that the effect of light on the spine efficiency of *C.macrocarpa* might be a general trend.

Unfortunately *C.macrocarpa* and its mammal herbivores were not observed in their natural setting. However, the methods and results reported here represent a significant advance on the environmental conditions and plant traits conducive to structural defences against ungulate herbivory.

Conclusions

Both ontogeny and light condition affect the architectural response of *C.macrocarpa*. However, light condition has the greatest overall effect on the architecture. In the sun *C.macrocarpa* adopts a dense well defended form constructed by short thick modules (stems) that bear long pronounced spines. The shortening of these modules arranges the spines in such a way that the leaves and stems of the plant are well protected by the prongs of the spines. The shade architectural strategy of *C.macrocarpa* is to adopt a more elongated form that is not as well defended as plants in the sun. Elongation is due to the construction of modules (stems) that are longer and thinner that bear spines that are shorter, thicker and less pronounced than those in the sun. The elongation of modules causes spines to be arranged further apart leaving more leaf and stem material open to attack by browsers. Thus the spine efficiency of *C.macrocarpa* in the sun is higher than in the shade.

The biomass allocation rules do not change for sun and shade plants. Most biomass is allocated to stems then leaves and lastly to spines. However, biomass increases with increasing canopy openness. Therefore, light condition controls biomass, which controls the architectural strategy the plant adopts, which in turn affects the spine efficiency of the plant. The higher amounts of biomass produced in the sun goes into constructing the sun architectural strategy of the plant.

Therefore, light limitation reduces the ability of spines to present an adequate defence against browsers due to the architectural strategy employed by the plant and is not due to resource allocation. This supports the initial architectural hypothesis proposed. Thus, spinescent plants do not do well in light limited environments because they are architecturally constrained to elongate in such conditions. This constraint would put them at higher risk of browser damage than plants in light-sufficient conditions. As far as we are aware, this is the first study of how resource constraints influence the development of structural defences. If the patterns observed in *C.macrocarpa* prove to be general, then it helps to explain why spiny plants are more commonly found in open, sunlit environments than in deep shade.

Shortcomings and Further Recommendations

The periodic cutting of *C.macrocarpa* made it difficult to determine the growth stage of the plant even by the methods employed above. When a stem was cut it would produce a new fresh C1 shoot from a lateral bud. Many of the main trunks of the plants had been cut, even at the base. This essentially produced a plant that was structured almost entirely by vertical C1 growth. Therefore, the determination of growth stage by choosing the highest fresh shoot and counting previous axis categories up to this shoot, could have given a distorted estimate of the actual growth stage of the plant. This is because subsequent axis categories might have grown at the same time as the axis category being measured. Thus by counting these axis categories one would conclude that the plant is at a more developed growth stage than it actually is. Therefore, it would have been better to assess the ontogeny of *C.macrocarpa* in its natural environment, free of any cutting. The production of many C1 shoots as a result of cutting also suggests that it could be the same behaviour that would be seen as an induced response of *C.macrocarp*a to heavy browsing.

The hemispherical photographs used to analyse light condition were taken during winter months. Trees shading *C.macrocarpa* were deciduous and had all lost their leaves when photographs were taken. Therefore, the estimates of light condition could be overestimates. Thus it would have been better to take these photographs in the summer months. However, this is only the case for some individuals, as most individuals were shaded by buildings.

The bite test to determine spine efficiency was only carried out on the vertical axis category of *C.macrocarpa* and not on C3 growth. Layered C3 growth had small spines and well exposed leaves that could be easily bitten. However, C3 growth was delayed and often appeared near to the base of the plant. Thus it was essentially protected from attack from above by the umbrella-like growth formed by the vertical axis category. Furthermore, the bite test was conducted on areas of the vertical axis category that were at head height (if the plant was tall) or from directly overhead (if the plant was shorter). The leaves at the periphery of the axis category were not considered in the test. Therefore a better quantification of spine efficiency should take the C3 growth into account and be able to incorporate bites taken from anywhere on the plant.

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