VASCULAR EPIPHYTES AS BIOINDICATORS OF CLIMATE CHANGE AND THEIR OWN VULNERABILITY

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"When you learn through the crisis - if you are able! - ... You really learn from your heart…
and, this knowledge remains deeply rooted in you…"

Siouxsie Correa

"In the middle of difficulties lies the opportunity"

Albert Einstein

Claude Levi-Strauss ***said that the scientist is not a person who gives the right answer; he is one who asks the right questions. It may be partially true; however, I would rather complete this sentence saying: … that the scientist may have the, apparently, right answer today, but it may not be valid tomorrow.***

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CONTENTS

CHAPTER V

ABSTRACT

The global climate has been markedly changing since the pre-industrial era due to anthropogenic activities. The expected increase in the atmospheric concentrations of carbon dioxide (CO_2) by the end of this century causes an increased concern about the effects this may have on global biodiversity. Special attention has been given to the impact on tropical areas due to the high biodiversity in these ecosystems. Epiphytic plants are common dwellers of tropical forest canopies, rooting non-parasitically on other organism such as trees and, may comprise 50% of the total flora present in a single locality. Epiphytes take water and nutrients needed for their growth and survival directly from the atmosphere. Because of this tight-coupling to the atmosphere they are considered particularly vulnerable to global change. Especially some species from the family Bromeliaceae whose roots have been suggested to miss any possible functionality as absorbing organ may be critically threatened by the future climate change scenario. The aim of the present thesis was to evaluate the effects of elevated CO_2 concentration (e[CO_2]) together with changes in water availability (WA), temperature (T) and nutrient availability (NA) along different ontogenetic stages of epiphytic bromeliads, which comprises the most relevant group in the Neotropics. First, I studied the effect of WA and T on the germination (G) of 16 epiphytic bromeliads collected from natural populations in Panama. Germination trials were carried out in growth chambers that allowed for the control of different climatic factors. The main aim of these trials was to develop a standard protocol on G of epiphytic bromeliads that may allow the comparisons of results derived from different studies. Therefore, I also included a trial in which I tested the effect of seed maturity for which seeds were collected at different dates before natural dehiscence and compared their G to that of seeds collected close to natural fruits opening. I found that seeds collected before natural dehiscence are able to germinate, i.e. there is after-ripening. In a trial to test the effect of different T regimes on G, I found that apparently it is irrelevant whether species are subjected to either fluctuating or constant T since there were not consistent differences in any of the species tested. Nonetheless, fluctuating T may reflect natural conditions better. Setting different water availabilities using different water potentials or the use of wet-dry cycles of different intensity showed quantitative differences in the G of the species tested. Given that in nature species are subjected to rain events spread in a time-frame of hours, days or weeks, the simulation of varying water availability using different wet-dry cycle may produce more realistic results than the commonly used water potentials. Interestingly, this study showed that the duration of the water period was more important for these plants than its frequency, which emphasizes the stress-tolerance of this plant group. However, the effect of WA is modulated by T. Germination may be more restricted in response to drought when seeds are subjected to low T. Hence, it becomes imperative to acknowledge the actual T ranges to which species are subjected in natural conditions and include them in the *ex-situ* experiments to have a more realistic understanding of their actual situation.

Secondly, I studied the relative growth rate (RGR) of three epiphytic C_3 bromeliads in response to CO_2 and T over a year. I used four custom-built chambers of the Oldenburg University that allowed for the control of CO_2 and T. The chambers were set up inside the greenhouse and light was supplied by natural sunlight supplemented with artificial lights. I designed a multifactorial experiment with two [CO_2] (ambient= ca. 400ppm (a[CO_2]) and elevated= ca. 800ppm (e[CO_2])) and two T (low= 27°C/22° and high= 30°C/25°C, day/night). This experiment simulated the expected increase in 3°C in T estimated for tropical areas by the end of the current century with the doubling of the current CO_2 concentration. The main objective was to assess if doubled [CO_2] may counteract the negative effect of the 3°C increase in T on the RGR of epiphytic bromeliads. Additionally, I expected that RGR of the plants subjected to e[CO_2] would acclimate with time. I found that growth was negatively affected by an increased in 3°C in T at both [CO_2]. A stimulatory effect of e[CO_2] was only perceivable from the

six months on. Furthermore, survival of the species under high T was more negatively affected in species grown with a[CO_2] than with the e[CO_2]. Survival ranged from 10% to 90% in the a[CO_2] compared to 60% to 100% in the e[CO_2]. These results show that responses to e[CO_2] are rather species-specific and may vary from highly to barely advantageous. Leaf area (LA) and specific leaf (SLA)) were also negatively affected by increased T, which could partly explain the observed low RGR. Under high T evaporative demands increase which causes a reduction of the surface/volume ratio of leaves to reduce further water loss. Unexpectedly, nitrogen (N) concentration increased in plants subjected to high T and e[CO_2]. An increased N concentration in leaves may be the result of a continuous photosynthetic activity since enzymes involved in this process account for a large proportion of total N. Regarding the concentration of non-structural carbohydrates (NSC), unexpectedly, plants grown at the aCO_2 had the highest NSC by the end of the treatment than those grown at the e[CO_2]. Additionally, concentration of NSC was higher in leaves of individuals grown in the low T than in those grown in the high T treatments. Since a slight positive effect of e[CO_2] in plants subjected to 3°C increase in T was perceivable just from six months on, one may expect that epiphytes will be relatively unresponsive to increases in the concentration of this gas in a future time, or, at least, a conspicuous response could be detectable after a much longer time period than it may be expected.

Third, I studied the effect of [CO_2] and NA on reproduction of one C_3 and two CAM bromeliads. The aim was to assess whether an e[CO_2] together with high NA could increase the reproductive output of epiphytic bromeliads, measured as number of produced seeds. Additionally, I expected that plants grown under these conditions may profit from lower reproductive costs (RC) than plants grown at the ambient conditions. The experiment was carried out in climate chambers that allowed the control of light, T, humidity and CO_2. I used two [CO_2] (a[CO_2]= 350 ppm and e[CO_2]= 700 ppm) and three levels of nutrient (NPK1 > NPK2 > NPK3). From the three species tested only the C_3 species *C. nutans* produced fruits with mature seeds whereas the other two CAM species had only one single individual which developed flowers (*T. bulbosa*) or flowering display was substantial, but fruits did not reached maturity (*T. subulifera*). The two CAM-species belong to the atmospheric morphotype. Species with this feature usually mature at a much slower pace than species with the tank strategy such as *C. nutans*. Unexpectedly, I found that plants grown at e[CO_2] produced a total lower number of seeds (3150) than plants grown at the a[CO_2] (5648). However, this result is partly explained by the presence of a higher number of adult individuals in the latter treatment. Furthermore, in terms of mean seed number fruit capsules of plant individuals grown at the a[CO_2] contained between 100 and 150 seeds whereas at the e[CO_2] individuals produced fruits containing less than 100 seeds in average. Due to the clonal nature of epiphytes, different shoots still attached to a single rosette may belong to different generations each of them with their corresponding life history. This may influence the response of the different individuals to environmental changes, whereby any interpretation on the effect of external changes could be misleading. Plant growth after reproduction was enhanced by increased NA and e[CO_2]. However, most of the plant individuals present in that enriched environment did not survive until the end of the treatment. Survival was lower for the atmospheric species *T. bulbosa* (25%) and *T. subulifera* (50%) than for the tank species *C. nutans* (60%). Because *C. nutans* was the only species which successfully completely its reproductive cycle until seed production, I only ran analyses of biochemistry (NSC and nitrogen (N)) and morphological variables (LA and SLA) of leaves for this species. As a general trend, initial total NSC in leaves of *C. nutans* decreased significantly in all treatments after one year. However, decreases were slightly lower in leaves of plants grown in the e[CO_2] and high to medium NA. Similarly, concentrations of most nutrient elements decreased after one year of treatment; and again, most nutrient concentrations were slightly higher in leaves of plants grown under conditions of e[CO_2] and high NA. Only potassium (K) and nitrogen (N) were significantly (-73%) and slightly (-17%) lower in leaves of plants grown under this treatment. N could have been slightly diluted by increased carbon availability whereas; K could have influenced the general trend of NSC in the treatment because it plays an important role in the loading and long-distant transport of sugar in the phloem. Plants grown under e[CO_2] and high NA had higher LA and SLA than plants grown at the a[CO_2] and low or medium NA. This may explain the slightly higher growth rates observed in this treatment. However, since plants grown at the a[CO_2] and low NA had higher reproductive output, the slow RGR and LAs of plants grown under these conditions may indicate a higher RC. This may have important implications under a future limiting environment. Furthermore, it may indirectly indicate that an enriched resources atmosphere may slowdown the RC in these plant species.

To conclude, epiphytic bromeliads show a very conservative response to environmental changes. They seem to benefit little from high resource availability, which emphasizes their rather stress-tolerant nature. Additionally, the species-specific responses indicate that future epiphyte assemblages may be missing some of the species currently present. Some species may tolerate the effects of climate change well, e.g. increased temperature or drought intensity, whereas other species may be more vulnerable. However, the present study included only a few species. The expansion of the species spectrum will be necessary to broaden our understanding of the impact of future climate change on epiphyte communities. Due to the clonal nature of epiphytic bromeliads it may be important for future studies to ensure that all individuals belong to the same generation. In the field, it may be recommendable to interpret the observed patterns cautiously, and to include at the populations dynamics present. In this way, one may avoid drawing conclusions about perceived impact of specific environmental changes that may rather be a consequence of the intrinsic nature of the observed populations.

ZUSAMMENFASSUNG

Das globale Klima hat seit der vorindustriellen Zeit allmählich geändert aufgrund menschlicher Tätigkeiten und es gibt eine erhöhte Besorgnis über die Auswirkungen, die der erwartete Anstieg der atmosphärischen Konzentrationen von Kohlendioxid (CO_2) bis Ende dieses Jahrhunderts auf die globale Biodiversität haben könnte. Besondere Aufmerksamkeit gilt den tropischen Gebieten wegen der in diesen Ökosystemen besonders hohen Artenvielfalt. Epiphytische Pflanzen sind Bewohner von Baumkronen. Diese nicht-parasitisch auf Baumrinde wachsenden Pflanzen können 50% des gesamten Flora in einem einzigen Ort umfassen. Sie nehmen direkt aus der Atmosphäre Wasser und Nährstoffe auf, die sie für ihr Wachstum und Überleben benötigen. Aufgrund der engen Kopplung mit der Atmosphäre werden sie oft als besonders anfällig für Klimaveränderungen angesehen. Das Ziel der vorliegenden Arbeit war, die Effekte der erhöhten CO_2 Konzentrationen (e[CO_2]) in Verbindung mit der Verfügbarkeit von Wasser (WA), Temperatur (T) und Nährstoffversorgung (NA) über verschiedene ontogenetische Stadien epiphytischer Bromelien, einer in den Tropen der Neuen Welt besonders wichtigen Gruppe, zu bewerten. Zunächst studierte ich die Wirkung von WA und T auf die Keimung (K) epiphytischer Bromelien. Keimversuche mit 16 Arten, die in natürlichen Populationen in Panama gesammelt wurden, wurden in Wachstumskammern durchgeführt. Das Hauptziel dieser Studien war es, ein Standardprotokoll für Keimversuche mit epiphytischen Bromelien zu entwickeln, welches Vergleiche der Ergebnisse verschiedener Studien erleichtern würde. Ich untersuchte die Bedeutung der Samenreife, wobei zu verschiedenen Entwicklungszeitpunkten Früchte gesammelt wurden. Es zeigte sich dass Samen schon lange vor der natürlichen Freisetzung keimfähig sind, d.h. es gibt Nachreifung. In einer Studie zur Untersuchung der Wirkung unterschiedlicher Temperaturbedingungen auf die Keimung (K) zeigte sich, dass diese sich bei konstanten oder oszillierenden T nicht unterscheiden. Dennoch sind schwankende T unter natürlichen Bedingungen natürlich eher die Regel. Ein weiterer Aspekt beschäftigte sich damit, wie sich die Keimung (K) bei unterschiedlicher Wasserverfügbarkeit (WA) unterscheidet, einmal via unterschiedliche Wasserpotentiale, einmal über unterschiedlich lange Nass-Trocken-Zyklen. Die Ergebnisse waren qualitativ vergleichbar. Interessanterweise zeigte sich, dass die Dauer der Feuchtperiode wichtiger war als die Frequenz, was dass Stress-Toleranz der Epiphyten betont. Ich empfehle die Verwendung von Nass-Trocken-Zyklen, um unterschiedliche WA zu simulieren, womit realistischer die natürlichen Verhältnisse simuliert werden. Die Wirkung der WA wird durch die T moduliert; Daher ist es zwingend notwendig, die tatsächlichen Temperaturbereiche zu erfassen, welche Arten in der Natur erfahren und diese in den *ex-situ* Experimenten zu verwenden.

Zweitens habe ich die relative Wachstumsrate (RGR) von drei C_3 epiphytischen Bromelien als Reaktion auf CO_2 und T über ein Jahr untersucht. Ich benutzte vier von der Elektronikwerkstatt gebaute Miniklimaschränke, die die Kontrolle von CO_2 und T erlaubten. Die Schränke befanden sich im Gewächshaus, neben natürlichem Sonnenlicht wurde zusätzliches künstliches Licht verwendet. Ich habe ein multifaktorielles Experiment mit zwei [CO_2] (Umgebung = ca. 400ppm (a[CO_2]) und erhöht = ca. 800ppm (e[CO_2])) und zwei T (niedrig = 27°C/22° und hoch = 30°C /25 °C, Tag/Nacht) entworfen. Hier wollte ich den erwarteten Anstieg der T um 3°C in tropischen Gebieten bis zum Ende des jetzigen Jahrhunderts mit der Verdoppelung der [CO_2] simulieren. Das Hauptziel bestand darin, zu beurteilen, ob die verdoppelte [CO_2] dem negativen Effekt von 3°C der T auf dem RGR der epiphytischen Bromelien entgegenwirken könnte. Darüber hinaus erwartete ich, dass sich Pflanzen, die e[CO_2] ausgesetzt waren, mit der Zeit akklimatisieren würden. Ich fand, dass das RGR bei um 3°C erhöhter T bei beiden [CO_2] negativ beeinflusst wurde. Die stimulierende Wirkung der e[CO_2] war unmittelbar nach sechsmonatiger Behandlung wahrnehmbar. Darüber hinaus wurde das Überleben der Spezies in Reaktion auf die hohe T bei Spezies, die unter a[CO_2] wuchsen, negativer beeinflusst als

bei der e[CO_2]. Die Überlebensbereiche betrugen 10% bis 90% im a[CO_2] im Vergleich zu 60% bis 100% im e[CO_2]-Umfeld. Diese Ergebnisse zeigen, dass die Reaktionen auf e[CO_2] eher artspezifisch sind und von stark bis kaum vorteilhaft variieren können. Blattfläche (LA) und spezifische Blattfläsche (SLA) wurden auch durch erhöhte T negativ beeinflusst, was teilweise die beobachteten niedrigen RGR erklären könnte. Bei Hoch-T-Verdunstungsanforderungen steigen die Erhöhung, die eine Verringerung des Flächen/Volumen-Verhältnisses der Blätter bewirkt, um weitere Wasserverluste zu reduzieren. Unerwartet erhöhte sich die Stickstoffkonzentration (N) in Pflanzen, die hohen T und e[CO_2] ausgesetzt waren. Eine erhöhte N-Konzentration in Blättern kann das Ergebnis der Photosynthese-Kontinuität sein, da die an diesem Prozess beteiligten Enzyme einen großen Anteil an Gesamt-N ausmachen. Die nicht-strukturellen Kohlenhydrat- (NSC-) Konzentration waren unerwarteterweise in den a[CO_2] Pflanzen höher als unter e[CO_2]. Zusätzlich war die Konzentration dieser organischen Komponenten bei Blättern von Individuen, die bei der niedrigen T gewachsen waren, höher als bei den bei den Hochtemperaturbehandlungen gewachsenen Pflanzen. Anscheinend war eine e[CO_2] nicht so ein starker Vorteil für Epiphyten vor erhöhten T, was eine große Herausforderung für sie im zukünftigen Veränderungsszenario darstellen kann. Wahrscheinlich kann eine Wirkung der e[CO_2] länger dauern, um aufgrund der konservativen Natur dieser Pflanzenarten wahrgenommen zu werden.

Drittens habe ich die Wirkung der [CO_2] und der NA bei der Reproduktion eines C_3 und zwei CAM epiphytischen Bromelien untersucht. Ziel war es zu beurteilen, ob eine e[CO_2] zusammen mit hoher NA die Fortpflanzungsleistung von epiphytischen Bromelien erhöhen könnte, gemessen als Anzahl der Samen. Darüber hinaus erwartete ich, dass Pflanzen, die unter diesen Bedingungen gezüchtet werden, eine niedrigere Fortpflanzungskosten (RC) erleben können als Pflanzen, die bei den Umgebungsbedingungen gewachsen sind. Das Experiment wurde in Klimakammern durchgeführt, wo es möglich war, Licht, T, Feuchtigkeit und CO_2 zu kontrollieren. Ich habe zwei [CO_2] (a[CO_2]= 350ppm und e[CO_2]= 700ppm) und drei NA (NPK1> NPK2> NPK3) verwendet. Von den drei untersuchten Arten entwickelte nur die C_3-Spezies *C. nutans* Früchte mit reifen Samen, während bei den beiden anderen CAM-Spezies nur eine einzige Pflanze Blüten (*T. bulbosa*) ausbildete bzw es trotz vieler Blüten zu keiner Fruchtbildung kam (*T. subulifera*). Die beiden letzteren Arten gehören zum atmosphärischen Morphotypen, die Arten mit dieser Eigenschaft reifen in der Regel langsamer als die Spezies mit der Tankstrategie wie *C. nutans*. Unerwarteterweise fand ich, dass Pflanzen, die an der e[CO_2] gewachsen waren, eine geringere Anzahl von Samen (3150) erzeugten als Pflanzen, die unter a[CO_2] (5648) wuchsen. Dieses Ergebnis wird jedoch teilweise durch die Anwesenheit einer höheren Anzahl von erwachsenen Individuen in der letzteren Behandlung erklärt. Wegen der klonalen Natur der Epiphyten können Individuen, die in einer Kolonie wachsen, zu verschiedenen Generationen gehören, jede von ihnen mit ihrer entsprechenden Lebensgeschichte. Dies kann die Reaktion der verschiedenen Individuen vor Umweltveränderungen beeinflussen, wobei jede Interpretation über die Wirkung von externen Veränderungen irreführend sein könnte. Pflanzenwachstum nach Reproduktion wurde durch erhöhte NA und e[CO_2] erhöht. Allerdings haben die meisten der in dieser bereicherten Umgebung anwesenden Pflanzenindividuen nicht bis zum Ende des Experiments überlebt. Die Überlebensrate war für die atmosphärischen Arten (*T. bulbosa* (25%) und *T. subulifera* (50%)) niedriger als für die Tankspezies (*C. nutans* (60%)). Da *C. nutans* die einzige Art war, die ihren Reproduktionszyklus bis zur Saatgutproduktion erfolgreich absolvierte, führte ich nur eine Analyse der Inhaltsstoffe (NSC und N) und morphologische Variablen (LA und SLA) von Blättern dieser Art durch. Als allgemeiner Trend sank der anfängliche Gesamt-NSC in Blättern von *C. nutans* in allen Behandlungen nach einem Jahr deutlich ab. Allerdings war der Rückgang niedriger bei Blättern von Pflanzen, die bei e[CO_2] angebaut wurden, und eine hohe bis mittlere NA hatten. Ähnlich sanken die Konzentrationen der meisten Nährstoffe nach einem Jahr der Behandlung; Allerdings waren die meisten Elemente in der etwas höheren Konzentration bei e[CO_2] und hoher NA. Nur Kalium (K) und

Stickstoff (N) waren signifikant (-73%) und geringfügig (-17%) niedriger bei Blättern von Pflanzen, die bei dieser Behandlung gezüchtet wurden. Insbesondere könnte N durch eine erhöhte Kohlenstoffverfügbarkeit leicht verdünnt worden sein, während im Gegenteil K die allgemeine Tendenz der NSC im Experiment beeinflusst haben könnte, da sie eine wichtige Rolle bei der Beladung und dem weit Transport von Zuckern im Phloem spielt. Schließlich hatten Pflanzen, die unter e[CO_2] und hoher NA wuchsen, ein höheres LA und SLA als Pflanzen, die bei aCO_2 und niedrigem oder mittlerem Nährstoff wuchsen. Dies kann teilweise für die leicht höheren RGR verantwortlich sein, die bei der in dieser Behandlung angebauten Spezies beobachtet wurden.

Zusammengenommen zeigen die epiphytischen Bromelien eine ganz konservative Reaktion auf Umweltveränderungen. Sie scheinen wenig von hoher Ressourcenverfügbarkeit zu profitieren, was ihre eher stresstolerante Natur hervorhebt. Darüber hinaus zeigen die artspezifischen Reaktionen, dass Epiphytengemeinschaften in einem zukünftigen Klimawandelszenario einige der derzeit vorhandenen Arten fehlen könnten. Manche Arten sind toleranter, z.B. auf erhöhte T oder Dürreintensität, während andere Arten weniger effizient sein können. Dennoch enthielt die vorliegende Studie nur einige Arten. In einem nächsten Schritt sollte deswegen das Artenspektrum erweitert werden. Schließlich kann es aufgrund der klonalen Natur der epiphytischen Bromelien wichtig sein, sicherzustellen, dass alle Individuen der gleichen Generation angehören. Bei oder bei Reproduktionsexperimente wäre es empfehlenswert, Pflanzen aus Samen zu ziehen. Auf diese Weise hat man eine bessere Kontrolle über alle möglichen Ereignisse haben, die von Pflanzen über der verschiedenen Stufen bis zur Reifung erlebt werden.

RESUMEN

El clima global ha venido cambiando marcadamente desde la era pre-industrial debido a actividades antropogénicas. Esto ha aumentado la preocupación sobre los efectos que el esperado incremento en la concentración de dióxido de carbono ([CO_2]) para finales del presente siglo podría tener sobre la biodiversidad global. Se ha prestado especial atención al impacto que podría acarrear esto para las áreas tropicales, ya que éstas representan los ecosistemas más diversos. Las plantas epifitas son moradores habituales del dosel de los bosques tropicales, las cuales se enraízan de manera no parasítica sobre otros organismos tales como árboles y, podrían llegar a representar hasta el 50% de la flora total presente en una localidad individual. Éstas toman agua y nutrientes para su crecimiento y sobrevivencia directamente de la atmósfera. Debido a este estrecho acoplamiento con la atmósfera, ellas son consideradas particularmente vulnerables al cambio global. El objetivo de esta tesis fue evaluar los efectos de la concentración elevada de CO_2 (e[CO_2]) junto con cambios en la temperatura (T), disponibilidad de agua (WA) y nutrientes (NA) a lo largo de diferentes estadios ontogenéticos de las bromelias epifíticas, las cuales constituyen uno de los grupos de plantas más prominentes en el Neotrópico. Primero, yo estudié el efecto de WA y T en la germinación (G) de 16 bromelias epifitas colectadas en poblaciones naturales en Panamá mediante el uso de cámaras de crecimiento. El objetivo principal de estos ensayos fue desarrollar un protocolo estandarizado sobre la G de bromelias epifíticas que podría permitir la comparación de resultados derivados de diferentes estudios. Por lo tanto, yo también incluí un ensayo en el cual he evaluado el efecto de la madurez de las semillas para el cual las semillas fueron colectadas en diferentes tiempos de desarrollo. Este estudio mostró que las semillas colectadas antes de la dehiscencia natural eran capaces de germinar, i.e., si existe "post-maduración". En un ensayo para probar el efecto de diferentes regimenes de T en la G, yo observé que las especies no discriminaban entre T constante o fluctuante. No obstante, T fluctuantes son más la regla en condiciones naturales. Al establecer diferentes niveles de WA usando diferentes potenciales hídricos o el uso de ciclos de sequía y humedad de diferente intensidad reveló similitudes cualitativas en la G de las especies evaluadas. Interesantemente, este estudio mostró que la duración del período hídrico era más importante para las plantas que su frecuencia, lo cual enfatiza la naturaleza stress-tolerante de este grupo de plantas. Yo recomiendo el uso de diferentes ciclos de humedad y sequía para simular la variabilidad en la WA, ya que esto podría simular de manera más realista el comportamiento en la naturaleza. El efecto de la WA es modulado por la T. Debido a esto, se hace imperativo obtener conocimiento sobre los rangos de T reales a los cuales están sujetos estás especies corrientemente en condiciones naturales, e incluirlos en experimentos *ex-situ*.

Segundo, yo estudié el crecimiento relativo (RGR) de tres bromelias epifitas C_3 en respuesta al CO_2 y la T a lo largo de un año. Yo usé cuatro mini-cámaras de crecimiento construidas en la Universidad de Oldenburg, las cuales permitían controlar el CO_2 y la T. Las cámaras fueron instaladas en el invernadero y la luz fue suministrada por la radiación solar incidente suplementada con luces artificiales. Yo diseñe un experimento multifactorial con dos concentraciones de CO_2 (ambiente = ca. 400ppm (a[CO_2]) y elevada = ca. 800ppm (e[CO_2])) y dos t (baja = 27°C/22°C y alta = 30°C/25°C, día/noche). En este experimento se simuló el esperado incremento de T en 3°C para las áreas tropicales hacia finales del presente siglo con

la duplicación de la concentración de CO_2. El objetivo principal fue evaluar si el doble de la concentración de CO_2 podría contrarrestar el efecto negativo de un incremento de 3°C en T sobre el RGR de bromelias epifíticas. Adicionalmente, yo esperaba que el crecimiento de las plantas sujetas a e[CO_2] podrían aclimatarse con el tiempo. Yo encontré que el crecimiento fue negativamente afectado por un incremento de 3°C en la T a ambas [CO_2]. Un efecto estimulante de la e[CO_2] fue sólo perceptible a partir de los seis meses de tratamiento. Además, la sobrevivencia de las especies bajo condiciones de alta T fue afectada más negativamente en especies creciendo a concentración de CO_2 ambiente (a[CO_2]) que a e[CO_2]. La sobrevivencia osciló entre 10% y 90% a a[CO_2] comparado con 60% y 100% a e[CO_2]. Estos resultados muestran que la respuesta al e[CO_2] es especie-especifica y, podría variar de altamente a vanamente ventajoso. El área foliar (LA) y el área foliar específica (SLA) también fueron afectadas negativamente por el incremento en la T, lo cual podría, en parte, explicar las tasas de crecimiento observadas. A alta T la demanda evaporativa incrementa, lo cual causa una reducción en la relación superficie/volumen de las hojas con el objeto de reducir pérdidas de agua ulteriores. Inesperadamente, la concentración de nitrógeno (N) incrementó en plantas sujetas a alta T y e[CO_2]. Un incremento en la concentración de N en hojas podría ser la el resultado de una actividad fotosintética continuada, ya que las enzimas involucradas en este proceso dan cuenta de una gran proporción del N total. En relación a la concentración de carbohidratos no-estructurales (NSC), inesperadamente, las plantas crecidas a a[CO_2] tuvieron una concentración más alta al final del tratamiento que aquellas crecidas en la atmósfera enriquecida con CO_2. Adicionalmente, la concentración de NSC fue más alta en las hojas de individuos crecidos a baja T que en las hojas de aquellos crecidos a altas T. Una atmósfera enriquecida con CO_2 parece no representar una ventaja para las plantas epifitas frente a altas T, lo cual podría representar un gran reto para ellas en un escenario futuro de cambio climático. Debido a la naturaleza conservativa de este grupo de plantas, un efecto conspicuo ante e[CO_2] podría no ser detectable hasta transcurrido un largo período de tiempo.

Tercero, yo estudié el efecto de la concentración de CO_2 y disponibilidad de nutrientes en la reproducción de una bromelia C_3 y dos bromelias CAM. El objetivo era evaluar si e[CO_2] junto con una alta disponibilidad de nutrientes podría incrementar el rendimiento reproductivo de las bromelias epifíticas, medido como número de semillas. Adicionalmente, yo esperaba que las plantas crecidas bajo esas condiciones pudieran experimentar un coste reproductivo más bajo que las plantas crecidas bajo condiciones ambientales. El experimento fue llevado a cabo en cámaras climáticas que permitían el control de la luz, la T, la humedad y, el CO_2. Yo usé dos concentraciones de CO_2 (a[CO_2]) = 350ppm y e[CO_2]) = 700ppm) y tres NA (NPK1 > NPK2 > NPK3). De las tres especies evaluadas, sólo la especie C_3 *C. nutans* produjo frutos con semillas maduras mientras que las otras dos especies CAM sólo tuvieron un único individuo que produjo flores (*T. bulbosa*) ó el despliegue de flores fue sustancial, pero los frutos no alcanzaron la madurez (*T. subulifera*). Las dos especies CAM pertenecen al morfotipo atmosférico. Usualmente, las especies con este rasgo maduran a un ritmo más lento que las especies con estrategia tanque como *C. nutans*. Inesperadamente, las plantas crecidas en la atmósfera enriquecida con CO_2 produjeron un número total de semillas más bajo (3150) que las plantas crecidas a a[CO_2] (5648). Sin embargo, este resultado es, en parte, explicado por la presencia de un número más alto de individuos adultos en el tratamiento ambiente. Además, los individuos sujetos a a[CO_2] produjeron capsulas que contenían entre 100 y 150 semillas mientras que en la atmósfera enriquecida los individuos produjeron frutos que contenían menos de 100 semillas en promedio. Debido a la naturaleza clonal de las epifitas, individuos dentro de una colonia, podrían pertenecer a diferentes generaciones, cada una de ellas con su correspondiente historia de vida. Esto podría influenciar la respuesta de los individuos ante los cambios ambientales, por lo cual, cualquier interpretación de los efectos de factores externos podría ser engañosa. El crecimiento de las plantas luego de la reproducción fue potenciado por un incremento en NA y e[CO_2]. Sin embargo, la mayoría de los individuos presentes en ese ambiente enriquecido no sobrevivieron hasta el final del tratamiento. La sobrevivencia fue más baja para las especies atmosféricas *T. bulbosa* (25%) y *T. subulifera* (50%) que para la especie tanque *C. nutans* (60%). Como *C. nutans* fue la única especie que completó exitosamente su ciclo reproductivo hasta la producción de semillas, yo sólo realicé análisis de variables bioquímicas (NSC y N) y morfológicas (LA y SLA) en hojas de esta especie. Como tendencia general, la concentración inicial de NSC en hojas de *C. nutans* disminuyó significativamente en todos los tratamientos después de un año. Sin embargo, las disminuciones fueron ligeramente menores en hojas de plantas crecidas a e[CO_2] y alta a media NA. Similarmente, las concentraciones de la mayoría de los nutrientes esenciales disminuyeron a lo largo de un año de tratamiento; y una vez más, las concentraciones de nutrientes fueron ligeramente más altas en hojas de plantas crecidas bajo condiciones de e[CO_2] y alta NA. Únicamente potasio (K) y N resultaron significativamente (-73%) y ligeramente (-17%) más bajos en hojas de plantas crecidas en este tratamiento. Nitrógeno pudo haber sido ligeramente diluido por el incremento en la disponibilidad de carbono mientras que el K podría haber influenciado la tendencia general de NSC en el tratamiento, ya que éste juega un papel importante en la carga y transporte a larga distancia de azúcares en el floema. Finalmente, las plantas crecidas bajo e[CO_2] y alta NA tuvieron un LA y SLA más altas que plantas crecidas bajo a[CO_2] baja ó media NA. Esto podría explicar las tasas de crecimiento ligeramente más altas observadas en este tratamiento.

Para concluir, las bromelias epifitas mostraron una respuesta conservativa a los cambios ambientales. Ellas parecen beneficiarse poco de una alta disponibilidad de recursos, lo cual enfatiza su naturaleza stress-tolerante. Adicionalmente, la existencia de respuestas especie-específica indica que algunas de las especies actualmente presentes en los ensamblajes de plantas epifitas podrían estar ausentes en un escenario futuro de cambio climático. Algunas especies podrían tolerar bien los incrementos de temperatura o intensidad de la sequía, mientras que otras podrían ser menos eficientes. No obstante, el presente estudio incluyó sólo unas pocas especies. El siguiente paso sería extender el espectro de especies. Finalmente, debido a la naturaleza clonal de las bromelias epifitas podría ser importante asegurar en estudios futuros que todos los individuos pertenezcan a la misma generación.

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ABBREVIATIONS AND ACRONYMS

%	percent
&	´et´ (Lat.); ´and´
>	more than
Δt	difference of time
°	arc degree
°C	degree Celsius
AIC	Akkaika criterion, statistics
C_3	basic type of photosynthesis
ca.	“circa” (Lat.), ´about´
Ca	calcium
CAM	crassulacean acid metabolism
cm	centimeters, unit of length
CO_2	carbon dioxide
$a[CO_2]$	ambient carbon dioxide concentration
$e[CO_2]$	elevated carbon dioxide concentration
d	days
d.f.	degrees of freedom, statistics
e.g.	“exempli gratia” (Lat.; ´example given´ or ´for example´)
et al.	“et alli” (Lat.); ´and others´
F	test statistic, named in honour of Sir Ronald A. Fisher
Fv/Fm	maximum quantum efficiency (FV= variable fluorescence, Fm= maximal fluorescence)
g	grams
glm	general/generalized lineal model
i.e.	“id est” (Lat.); ´that is´
K	potassium

LA	leaf area
LL	longest leaf
Lm	linear model
Ln	logaritmo neperiano
m	meter, unit of leangth
M	biomass
Mg	magnessium
mM	milimolar
mg	miligramos, unit of weight
mg g^{-1} d^{-1}	unit for relative growth rate
min	minutes, unit of time
N	nitrogen
Na	sodium
NA	nutrient availability
NSC	non-structural carbohydrates
P	phosphorus
PFD	photosynthetic photon flux density
PSII	photochemical efficiency
ppm	parts per mill
RGR	relative growth rate
n	sample size
P	p-value, statistics
r	correlation coefficient, statistics
r^2	coefficient of determination, statistics
s	second, unit of time
se	standard error

SLA	specific leaf area
T	temperature
vs.	“versus” (Lat.); ´against´ or ´in opposition to´

CHAPTER I

GENERAL INTRODUCTION

Global climate has been rapidly changing during the last two centuries primarily due to anthropogenic activities (Solomon *et al.* 2007) and there is an expectation that changes will be more pronounced by the end of the current century as a consequence of a doubling of carbon dioxide (CO_2) concentration (IPCC 2014; Dlugokencky & Tans 2015). Changes in the concentration of CO_2 ($[CO_2]$) are projected to bring about changes in the global precipitation and temperature patterns (Solomon *et al.* 2007). Due to the relatively constant climatic conditions along the year in the tropical areas (Janzen 1967), predicted climatic changes for these zones such as an increase of just 3°C in annual temperatures, or rainfall variations (Solomon *et al.* 2007) and, reduced cloud water in the montane areas (Still, Foster & Schneider 1999) can still have important consequences for the vegetation, particularly in such diverse ecosystems such as tropical rain forests (Zotz & Bader 2009). These ecosystems harbor a unique and astonishing plant group called epiphytes, which germinate and root non-parasitically on other plants at all stages of life (Zotz 2016).

Relevance of plant material

Epiphytes constitute a significant proportion of the total flora found in these ecosystems and in terms of global biodiversity, a thorough compilation of epiphytic diversity found that this plant group account for 9% of global biodiversity (Zotz 2013). Regardless of their high diversity and abundance epiphytes have been found to be inherently slow-growing species, time to reach maturity has been estimated to be a decade or more (Benzing 1981; Larson 1992; Zotz 1998; Hietz, Ausserer & Schindler 2002; Schmidt & Zotz 2002a). Both, an intensification of the selective pressure imposed by rapid climatic change and parallel habitat fragmentation restrict genetic flow, particularly in slow-growing species (Jump & Peñuelas 2005). Among the most sensitive plant groups due to their long generational time may be trees (Parmesan 2006; Valladares 2008) as well as epiphytes. Consequences on epiphytes could be even more pronounced since they depend on other organisms such as trees as structural supports. In addition to it, epiphytes are also almost entirely dependent on atmospheric inputs in order to fulfill their water and nutrient requirements for growth and survival. Due to this tight coupling to the atmosphere epiphytes have been called particularly vulnerable to global change (Lugo & Scatena 1992; Benzing & Stiles 1998).

The families with higher abundance in epiphytic flora, in decreasing order, are Orchidaceae, and Bromeliaceae. Nonetheless, if we consider all climatic zones where epiphytes can be found (Neotropics, Palaeotropics and the few? temperate areas) Bromeliaceae figure as the second in abundance, but in the Neotropics this plant family takes greater ecological importance since its contribution to the local epiphytic flora abundance and diversity have been observed to override that of orchids in several occasions (Zotz 2013). Hence, its importance in these ecosystems apart from the easier experimental manipulation**,** have raised a series of studies addressed to understand the biology and ecology of these plants, particularly over the past three decades (Benzing 1990; Zotz & Winter 1994; Zotz & Hietz 2001; Zotz, Hietz & Schmidt 2001; Mondragón, Calvo-Irabien & Benzing 2004; Manzano & Briones 2010; Wester & Zotz 2010; Zotz *et al.* 2010). However, the number of studies is still few and we are far from understanding the whole biology of these plant group compared to the number and completeness of studies carry out with other life forms. Even fewer are studies focused on the impact of climate change on epiphytic flora, research on the impact of climate change involves in higher proportion temperate ecosystems (Parmesan 2006) and those addressed to tropical areas mainly deals with the tree component (Malhi & Phillips 2005). Just about two decades ago, some authors draw attention to the susceptibility of epiphytic plants in the face of the imminent climate change and, consequent impact on the whole ecosystem if e.g. epiphytic flora would suffer a dieback, which could alter the efficiency of cloud forests in the use of mist as a moisture source (Benzing 1998; Still, Foster & Schneider 1999). Particularly, the expected changes in altitude of the cloud formations may set the limit of epiphytes distributional range (Bader & Zotz 2009). Adult individuals count on a series of strategies to growth and survive through the dry periods. However, knowledge on the ability of the early stages such seeds and seedlings to overcome the intensification of the drought periods is still rather sketchy (Bader et al. 2009). In the present study, I designed two experiments in order to assess the germination response of epiphytic bromeliads to different water potentials and wet-dry-cycles of specific intensity. Since in nature plants are usually encountered with rain periods of different intensity, the latter trial may give a better insight on what we could expect in field conditions. Additionally, it could be identify whether frequency or duration of water availability is more important for epiphytes (Chapter II).

Carbon dioxide concentration ([CO_2])

Impact of elevated atmospheric CO_2 concentration (e[CO_2]) and simultaneous changes in global climate on vegetation are diverse and do not lie just on variations in the general availability of water along the ecosystem. For instances, an e[CO_2] has been reported to cause changes in plant metabolism and physiology such as increased photosynthetic rates (Habash *et al.* 1995), a lower stomatal frequency (Thomas & Harvey 1983; Rogers *et al.* 1994) and increase in water use efficiency (Eamus 1991). An increased photosynthetic rate has been suggested to possibly lead to higher dry matter production, particularly in terrestrial C_3 species (Tuba & Lichtenthaler 2007). In fact, earlier reviews showed that a doubled CO_2 caused an average of 33% increase in the productivity of C_3 species (Kimball 1983; Cure & Acock 1986). This is usually explained by a higher availability of carbon substrate that enhances the carboxylating activity of Rubisco (the catalytic enzyme of the photosynthetic reaction) instead of the respiratory one (). Many epiphytes perform crassulacean acid metabolism (CAM), which mainly consists of nocturnal carbon fixation by phosphoenolpiruvate carboxylaxe (PEP-Case). Because of this feature, plants with CAM metabolism are expected to benefit little from an enriched CO_2 atmosphere (Bowes 1991). Nonetheless, findings during the last two decades in CAM terrestrial and epiphytic species suggest the contrary. For instances, growth under e[CO_2] have been found to enhance CO_2 uptake in CAM terrestrial plants (Nobel, Israel & Wang 1996; Zhu & Goldstein 1997; Zhu, Goldstein & Bartholomew 1999), and cause increase thickness of the photosynthetic organs (Nobel & Israel 1994; North, Moore & Nobel 1995; Zhu & Goldstein 1997). In epiphytic bromeliad and orchid species growth rates were similarly stimulated in C_3 and CAM species under an enriched CO_2 atmosphere (Monteiro, Zotz & Körner 2009). In the present study, I included both C_3 and CAM epiphytic species in order to assess the possible stimulatory effect of e[CO_2]on relative growth rates (RGR) and had the expectancy that both groups were going to experience a positive effect of an enriched CO_2 atmosphere (Chapter III and Chapter IV).

One of the most consistent changes after long-term exposure of plant individuals to e[CO_2] is a decrease in specific leaf area (SLA) (Lambers, Chapin III & Pons 2008). Changes in plant biochemistry could come along with these changes, for example, an accumulation of carbohydrate in leaves in response to increased CO_2 level has been suggested to be responsible for the observed increases in leaves thickness (Lambers, Chapin III & Pons 2008). Reductions in leaves nitrogen concentration have also been found to be positively correlated with reductions in SLA (Campbell, Allen & Bowes 1990; Rowland-Bamford *et al.* 1991).

Decreased nitrogen (N) concentration after long-term exposure to e[CO_2] has been attributed to different reasons, but especial emphasis has been addressed to dilution by increased non-structural carbohydrates (NSC) or allocation of nitrogen to non-photosynthetic organs (Tuba & Lichtenthaler 2007). This negative effect on tissue N concentration has been taken as a limiting mechanism for continuous enhancement of plant growth (Loehle 1995), which could be translated into acclimation of growth response in plant individuals. This argument is derived from modeling the response of plants to ambient and e[CO_2] during a long time period, which showed that growth of plants under e[CO_2] approached that of plants grown under an ambient CO_2 concentration (a[CO_2]) by the end of the growing period. More evidence of such a response can be found in previous studies by other authors (Pinter *et al.* 1993; Poorter 1993; Callaway *et al.* 1994). Growth under an enriched atmosphere may also increase nutrient demands (Rogers, Runion & Prior 1999; Tuba & Lichtenthaler 2007). Many grassland species has shown an increased allocation of carbon to root construction under an enriched atmosphere, which has been considered as a mechanism to increase nutrient acquisition (e.g. nitrogen, Jarvis 1995). This, may, in turn, compensate for increased nutrient demands. However, this finding may also indicate that stressful levels of other environmental factors such a nutrient availability may shadow the positive effects of e[CO_2] (Tuba & Lichtenthaler 2007).

In epiphytic plants, morphological and biochemical changes in individuals subjected to e[CO_2] has not been studied so far. The increases in growth rates in bromeliad and orchids species observed by Monteiro, Zotz and Körner (2009) were not supported by information on the internal or external changes undergone by plant individuals, which could have caused this positive effect. At least in the case of epiphytic bromeliads, which are mostly conformed by a mass of leaves and, functionality of **its** roots may be questionable (Zotz 2016), the reported increases of growth rates under e[CO_2] could be partly due to an increase in the area or mass of leaves, similar to reports for other plants. Hence, growth in a future CO_2 enriched scenario could be also expected to cause morphological changes in epiphytic plants as well as the biochemical variations that have been reported to take place in parallel to these changes. With regards to changes in allocation dynamics of carbon and nutrients, reports are diverse (Benzing & Davidson 1979b; Fenner 1986; Reekie & Bazzaz 1987; Zotz 1999b; Zotz 1999a) but they focus in the natural dynamics during reproduction without the added influence of external factors such as increases in CO_2 concentration. Zotz & Richter (2006), for instance, studied the allocation dynamics in reproductive individuals of the epiphytic bromeliad *Vriesea sanguinolenta* under natural conditions and found that a high proportion of non-structural carbohydrates were contained in the stem. Many epiphytic species are polycarpic which refers to the continuous production of vegetative offshoots once reproduction have taken place or, before the individual dries out completely or die after becoming senescent. Few other epiphytic species are monocarpic which comprises a single shoot, each of which die once fruits production and seeds released have taken place, without further vegetative replacement. Particularly, in the polycarpic types an increase allocation of carbon to the stem during a reproductive phase could be related to the posterior formation of vegetative shoots, which could contribute to growth maintenance of the individual.

In some *Tillandsia* colonies, it has also been suggested that after fruits have matured and seeds have been released individual plants seems to recycle the remaining resources (e.g. N, P & K) contained in floral stalks and reinvest them in production of new buds (Benzing & Renfrow 1971). Benzing (1990) stated that fitness could possibly be favored if some resources that could actually be destined to sexual investment are re-directed for successive growth. It has often been suggested that vegetative growth and fruit development compete for resources once reproduction has taken place (Nitsch 1971; Larcher 1980). This competition causes a reduction in future growth and survival, which has been referred as ´reproductive cost´ (Obeso 2002). However, the previous findings on epiphytic plants seem not to follow this pattern and a highly efficient compensation mechanism of resource allocation occurs instead. Zotz & Richter (2006) indicated that, at least, carbon demands could possibly be covered by actual leaf photosynthesis and by CO_2 assimilation of the reproductive structures *per se* such as it has been reported for other plants species (Bazzaz, Carlson & Harper 1979; Ashman 1994; Aschan & Pfanz 2003). Elevated [CO_2] has been observed to alter strength and direction of allocation dynamics (Tyree & Alexander 1993; Rusterholz & Erhardt 2014). For instances, tropical species with clonal comportment such as guava (*Psidium guayava*) and avocado (*Persea americana*) trees grown under an enriched CO_2 atmosphere experienced an stimulation in axillary shoots development (Kriedemann, Sward & Downton 1976), which had direct positive effect on relative growth rates. Also some studies on reproduction found that an enriched CO_2 atmosphere increased flowers production and commencement (Badeck et al. 2004; Rusterholz & Erhardt 2014). Hence, it seems reasonable to suppose that epiphytes could greatly benefit from a CO_2 enriched environment since a higher availability of this substrate could reduce even more a possible competition between vegetative development and sexual reproduction due to a higher availability of the substrate. In the present thesis, I take into account the ability of individuals to develop vegetative offshoots after reproduction as a measure of survival together with maintenance of mother plants *per se* with no further development of leaf offshoots. This give a good insight on how efficient are epiphytes in reducing the cost of the high reproductive investment they are known for.

Timing of flowering is referred as one of the critical stages of plant life cycle since it could lead to changes in fruit maturation and seed dispersal patterns (Bloor *et al.* 2010), which, in turn, could alter ecosystem dynamics at different levels (Arft *et al.* 1999; Walther *et al.* 2002; Badeck *et al.* 2004; Cleland *et al.* 2007). Several studies on crop species have documented the effects of e[CO_2] on seed size such as in soybean (Baker *et al.* 1989; Pan 1996; Thomas 2001b) and dry bean (Prasad *et al.* 2002), though, none of these studies found a significant influence of CO_2 on seed size. Meanwhile, some other studies did find either a negative (Jolliffe & Ehret 1985) or a positive effect (Bhattacharya *et al.* 1985; Ahmed, Hall & Madore 1993) of e[CO_2] on number of seeds. But there are two other important dimensions of seed quality, apart from seed size that have been badly addressed under an enriched CO_2 atmosphere, i.e., composition or nutritional quality and the ability to germinate and grow (Vara Prasad, Allen & Boote 2005). In respect to seed composition results has been diverse (Allen *et al.* 1988; Wu *et al.* 1997; Jablonski, Wang & Curtis 2002; Thomas *et al.* 2003), but in terms of germinability Thomas (2001a) suggested that seeds are well-buffered which could determine the low or even null response they have in front of increases in CO_2 concentration. In epiphyte plants, effects of CO_2 on reproductive output have not been addressed so far. The most recent study on reproductive phenology of epiphytes only focused on the possible effect of increase nutrient availability (Lasso & Ackerman 2013). In this thesis, I tested how an e[CO_2] may influence the final reproductive output (number of seeds) and biochemical composition, i.e., carbohydrates and nutrients of the seeds, whereas the monitoring of germinability of these recently produced seeds was left out (Chapter IV).

Nutrient availability

Higher availability of nutrients in the ecosystems is expected to be promoted by the rise in temperature coming along with climate change (Nadelhoffer *et al.* 1991; Silver 1998; Lewis, Malhi & Phillips 2004; Conant *et al.* 2011). Since epiphytes live in nutrient and water poor habitats (Benzing 1990; Zotz & Hietz 2001), the higher availability of nutrients could be highly beneficial for them as some studies have already shown (Castro-Hernández *et al.* 1999; Zotz *et al.* 2010). For instances, fertilization of plants in their natural habitats stimulated growth of the different epiphytic species tested, which has been pointed out as an indication of nutrient limitation in their natural habitats (Castro-Hernández *et al.* 1999; Cardelús &

Mack 2010; Zotz & Asshoff 2010). However, growth enhancement by higher nutrient inputs is still low compared to that of water and, the effects showed to be size-dependent (Schmidt & Zotz 2002b; Laube & Zotz 2003a). In those same studies, particularly small-sized plants showed higher stimulation of their growth rates in response to e.g. increased nutrient availability whereas larger plants were relatively unresponsive. Nonetheless, a significant positive effect of increase nutrient availability has also been observed to favor a higher reproductive output in a tank bromeliad dwelling the canopy of tropical mountain forests (Lasso & Ackerman 2013). Both number of fruits and seeds were higher in plants receiving a higher nutrient supply, which, in turn, could play an important role in population growth as long as colonization spots are not meager (Ackerman, Sabat & Zimmerman 1996). Individuals grown under increased nutrient availability also showed higher growth rates and survival after reproduction than their conspecifics in control conditions; hence a reduction in the cost of reproduction due to higher nutrient inputs is deducible in future nutrient-enriched environments, which have been suggested to have positive consequences on individuals' fitness (Lasso & Ackerman 2013) and could even modulate changes in populations dynamics. Followed by these findings, I included in this work a study on the effects of different nutrient availabilities in combination to e[CO_2] on reproductive output of epiphytic bromeliads in order to figure out if the combination of these two factors would enhance even more the outcome already observed in other species (Chapter IV).

Epiphyte plants also count on a series of strategies that allows them to survive in the nutrient poor environment they inhabit. In the previous lines, I mentioned the existence of polycarpic and monocarpic species and the manner each type have to ensure the maintenance of their genes in the population. These strategies also constitute in certain degree a way of nutrient recycling, which is of great help in the impoverished habitats offered by the canopies of tropical forests. Nonetheless, epiphytes count on other strategies to ensure their growth and survival in these environments which comprise alteration of their morphological and anatomical architecture. Bromeliads, for instances, lay on water-nutrient impoundments called ´tanks ´ conformed by the overlap of the leaves in the rosette-type specimens that allows them to have a continuous water and nutrient supply by the accumulation of organic matter and moisture catched from leachates, litterfall and other sources (Benzing 1990). Species of the *Tillandsioideae* subfamily usually present the atmospheric strategy defined by a reduction of leaf surface with presence of succulence and a dense layer of trichomes, which act as water- and nutrient-absorbing structures, apart from serving for light reflectance. If nutrient availability would increase in a future climate change scenario both types would be expected to benefit from it. However, the atmospheric types usually take longer time to get mature that the tank types (Benzing 1973) and, time to take up the nutrients needed for reaching maturity is also reported to be more extended in time (Benzing 1990). Finally, in spite of the expectancy of presumably higher developmental performance of plant individuals under higher nutrient availability, effects of nutrient concentration on SLA of some epiphytic species was not significantly affected by nutrient availability (Zotz & Asshoff 2010). However, based on the commonly observed effects of both nutrient concentration and e[CO_2] on leaf traits of numerous species, I decided to include in this study the monitoring of the possible changes in leaves morphological traits such as LA and SLA of epiphytic bromeliads that may occur in respond to the interactive effect of these two factors with the expectation that the combined effect would have important impact on these leaves traits (Chapter IV).

Temperature

Beyond CO_2 and nutrient availability effects on diverse plant traits at different ontogenetic stages variations in different climatic factors are also reported to influence plant responses. Temperature, for instances, has been always mentioned to play an influential role on plant developmental processes such as growth, germination and flowering (Garcia-Huidobro, Monteith & Squire 1982; Kakani *et al.* 2005). The relevance of temperature on plants distributional patterns is also globally accepted (Archibold 1995) and it can also cause important effects on plants physiological processes such as photosynthesis, respiration, and the biochemistry related to them (for photosynthesis: (Ehleringer & Björkman 1977b; Jordan & Ogren 1984; Ehleringer, Cerling & Helliker 1997), for respiration: (Ehleringer & Björkman 1977a; Tjoelker, Oleksyn & Reich 1998), for biochemistry: (Ku & Edwards 1978; Jordan & Ogren 1984). Temperature has also been found to enhance growth rate of some plants´ forms such tomato but up to an optimum temperature value (Gent 1986). Gifford (1989) suggested that increased yields in plants subjected to e[CO_2] seems to be further promoted at warm environments rather than at cool ones. An enhancement of growth rates by temperature under an enriched CO_2 atmosphere has also been reported (Rawson 1992). Nonetheless, increases of temperature above the optimal level can represent an important stress for plants, for instances, increase photorespiration can cause carbon losses (Polley 2002) and decrease yield instead, as reported for crop species (Baker & Allen 1993). Simultaneously, e[CO_2] have also been said to ameliorate heat stress of plant individuals (Hogan, Smith & Ziska 1991), though strong negative effects are hardly offset (Polley 2002).

In epiphyte plants effects of temperature on adult plants have been addressed by few studies but these are mainly focused on carbon uptake and photosynthesis (Medina *et al.* 1977; Griffiths *et al.* 1986; Martin 1994a; Martin 1994b). Growth rate on an epiphytic orchid was negatively affected by an increase of just 3°C in temperature (Vaz, Figueiredo-Ribeiro & Kerbauy 2004; Vaz. A. P., Figuereido-Ribeiro & Kerbauy 2004), which is the predicted variation for the tropics in a future CO_2-enriched atmosphere. Temperature has also been reported to affect growth through variations of specific leaf area (SLA), particularly at high temperature Loveys *et al.* (2002). Loveys *et al.* (2002) found that SLA increased as growth temperature increased in a total of 14 out of 16 species tested. Nonetheless, high temperatures can cause higher vapor pressure deficit which could set plants under a higher evaporative stress and, consequently produced water stress. Hence, a reduction in the photosynthetic organ or lower allocation to transpiring leaves could be more advantageous in those conditions as has been found in other studies (Sims & Pearcy 1994; Poorter & Nagel 2000). A future CO_2 enriched atmosphere could, in part, counteract the evaporative losses through reduced stomatal conductance (Urban 2003) which consequently lead to an increased water-use-efficiency (Carlson & Bazzaz 1980; Morrison 1985). Plants subjected to temperatures above the optimum have also been reported to experience increased carbohydrate concentration in leaves (Tjoelker, Reich & Oleksyn 1999). These increased carbohydrate concentration could contribute to osmotic adjustment (Wullschleger *et al.* 2002) and reduce evaporative-stress as well. However, due to the rather stochastic nature of water availability in the canopies dwelled by epiphytic plants osmotic adjustment may not be the common mechanism under water stress. In this study, I addressed the question of whether a presumably negative effect of 3°C increase in temperature on growth of epiphytic bromeliads would be counteracted in a future enriched atmosphere. In addition to this, I included the monitoring of leaves morphological changes experienced by these plants that make contribute to the resulted trends (Chapter III).

Reduced nitrogen (N) concentration in leaves under elevated temperature has also been reported in many plant species (Coleman & Bazzaz 1992; Tjoelker, Reich & Oleksyn 1999). In terrestrial species, this reduction could be partly due to investment in root mass that enables plants to cover a more extensive area for water uptake. However, in epiphytic species such as bromeliads, if reductions in N concentration should occur, it could more likely obeyed to increase respiration of leaves or, translocation to other plant organs such as stems since roots has been suggested to have a more structural than a physiological role (Benzing 2000) and Zotz (2016) even pointed as questionable their functional role in natural conditions. Some species have experienced increases in nitrogen concentration instead after exposure to high temperature, without any subsequent enhancement of photosynthetic or growth rates. This increased N concentration has been suggested to possibly obey to build-up of heat-shock proteins in order to prevent damage to cells or photoxydation (Ougham & Howarth 1988; Huang & Xu 2008). An elevated CO_2 atmosphere could intensify either of these responses, in any case the result would be the same, plant tolerance to higher temperature would increase, which could have important consequences in plant population dynamics. For instance, proportion of C_3 to C_4 has been suggested to change since generally C_4 are more common in warmer environments (Ehleringer & Björkman 1977a; Ehleringer, Cerling & Helliker 1997). A simulation of climate change impact on the epiphytic flora in a subtropical forest predicted that most epiphytes would lose about 50% of their actual distribution range which could be mostly dependent on temperature variations (Hsu *et al.* 2012). In the present study, nitrogen concentration and content of three C_3 epiphytic bromeliads in response to 3°C in temperature both at ambient and e[CO_2] are also monitored (Chapter III).

Effects of temperature on germination of epiphyte species are also documented in the existing literature but these are still few and results are ambiguous. For instances, studies on the importance of constant versus fluctuating temperature with epiphytic bromeliads have shown both better performance under constant (Rosa & Ferreira 1998; Pereira, Cuquel & Panobianco 2010) and fluctuating temperature (Rosa & Ferreira 1998; Pompelli, Fernandes & Guerra 2006). Nonetheless, daily and annual oscillations of temperature are more the rule in natural conditions (Baskin, Thompson & M. Baskin 2006). Similarly, the effect of extreme temperatures have also different effects on several plant species, for example, lower temperatures has led to higher germination rates (Ortega-Baes & Rojas-Aréchiga 2007; Mantovani & Iglesias 2008; Ramos & de Andrade 2010; Simão, Takaki & Cardoso 2010) whereas few studies have reported a higher germination success at warm temperatures instead (Qu *et al.* 2008; Veiga-Barbosa & Pérez-García 2014). In epiphytic species, extremes temperatures had been found to restrict germination in an epiphytic bromeliad (Downs 1964). Zotz and Bader (2009) also suggested that such responses could cause a significant shift in the altitudinal ranges in response to future changes as long as germination would represent the bottleneck in plants life history. A more recent study by Müller, Albach and Zotz (2017) with 41 epiphytic bromeliads showed that current mean annual temperature across the distribution range of the different species tested is rather below their thermal optima estimates, which indicate that, at least at the seed level, the expected increase in 3°C in temperature by the end of the current century may not be negative for these species; contrary, it may stimulate further responses. However, they still refer to the high conservatism of these family members, which may not be so plastic in front of the future changes. Here, the assessment of temperature effect is mainly carry out to understand, at first, the nature of the interactive effect between optimal, suboptimal and supraoptimal temperature in combination with different availabilities of water on germination of epiphytic bromeliads. Apart from dilapidating if there is an actual relevance of different temperature regimes such as fluctuating vs. constant required by these species for germination to take place. The evaluation and understanding of these aspects of epiphytic bromeliad seeds was considered a *sine qua non* prior step before any further research on the actual response to the imminent climatic change could be addressed. This decision was mainly based on the ample gamma of reports showing not so clear patterns in regard to these features (see more in Chapter II).

What is to be found in the present thesis?

As previously exposed in the present chapter, temperature can also modulate the behavior of other environmental factors or the availability of resources such as water and nutrient. Hence, at least an indirect effect of temperature on plants responses can be found on studies testing the effect of different factors such as the ones mentioned above. Plant water relationships can be influenced by higher evapotranspiration rates under higher temperatures (Anjum *et al.* 2011). The effect of water stress on a series of features of all plants life forms have possibly been more thoroughly addressed than the effect of any other factor. The important role of water availability in plant biology and the relevance of this factor have been usually placed on top of the requirements for the different plant processes to be carried out effectively. Brown (1997) reported that the availability of water was the most important environmental factor limiting growth and survival of range plants. Shao *et al.* (2009) also recognized the relevance of water as a limiting resource which could severely impair plant growth and development, apart from production and crops performance over any other factor. Even in epiphyte plants, the effect of water has frequently been assessed (Zotz & Tyree 1996; Zotz & Andrade 1998; Schmidt & Zotz 2001; Martin *et al.* 2004; Chilpa-Galván *et al.* 2013; Zhang *et al.* 2015) and responses are more consistent among species than that to any other factor (Zotz *et al.* 2010). This particular interest to understand the relevance of water in the biology of epiphytes is not surprising, since epiphytic flora dwell in the canopies of the forest which are rather water limited environment, particularly due to the intermittent water supply (Benzing 1978; Zotz & Hietz 2001). Nonetheless, in this study I just assessed the effect of water availability on the germination response of different epiphytic bromeliads since this may represent the possible bottleneck for the observed distributional patterns (Chapter II).

Low water availability was found to cause decreases of up to 80% to 90% in the germination percentage of the moisture-demanding epiphytic bromeliad *Tillandsia fasciculata* (Bader, Menke & Zotz 2009). Germination of these species had also a longer lag phase, though the more drought-tolerant congener *Tillandsia flexuosa* did not experience such pronounced reductions in its response. In that study with the two epiphytic *Tillandsia* species the authors simulated the drought environment by applying dry periods of specific time duration (hours-days) instead of using the most common approach such different osmotic potentials (Jordan & Nobel 1979; Swagel, Bernhard & Ellmore 1997). Bader, Menke and Zotz (2009) presented the use of dry periods instead of water potential since it confers higher relatedness to the real situation in nature where specific periods of drought are more the rule. I decided to follow this same method in order to study the effect of this important factor on the germination of the selected bromeliad species and compare it to the commonly use establishment of different water potentials in order to show the informative nature and advantage of each of these methods.

In order to carry out germination studies, the maturity of capsules has often been referred to as a "*Sine qua non*" prior-step to be considered before starting germination studies. Though, Fernandez, Beltramo and Caldiz (1989) collected capsules of the species *Tillandsia recurvata* three months before natural dehiscence and found that seeds contained in these capsules were able to germinate, though at lower percentage than seeds from naturally mature capsules. Responsiveness of "apparently" immature seeds indicates that these seeds could be already equipped in case a sudden environmental signal activates the germination response before natural timing. Additionally, to figure out if the capacity of immature seeds to germinate is a widespread phenomenon among epiphytes could result of great benefit since it would facilitate field collection of different species at the same moment and, it would not be necessary to wait for the natural fruit opening time of each of the species in order to start an experimental procedure. Seeds inside the capsules are also in sterile conditions; therefore, a sterilization protocol – which could possibly influence the germination response – could be skipped by the use of seeds from immature capsules. In a future climate change scenario, in particular the concomitant changes in temperature patterns could favor earlier ripening of fruits and seeds (Arft *et al.* 1999; Webb *et al.* 2012). However, the assessment of the importance of maturity of seeds for germination trials has been addressed in this study with a merely methodological interest. In fact, regardless that the other two abiotic factors mentioned above, water and temperature, play a more relevant role in species biology and could be critical in germination and establishment of the species, which could be the possible bottlenecks of these plants´ life history, in the second chapter of the present study dedicated to germination responses of epiphytic bromeliads is almost entirely oriented from a methodological perspective, though, without forgetting a mention of the ecological meaning of the encountered responses.

In fact, regardless that water and temperature play a more relevant role in species biology and could be critical for their germination and establishment, in the present study the chapter on germination is mainly oriented from a methodological perspective. Nonetheless, some ecological considerations were done in few of the cases.

By revision of the literature we can meet ourselves with an ample set of responses to a single environmental factor and even, the same species could be found showing a variety of responses. These ambiguities could be attributable to the universe of methodological protocols carried out by the different research groups, which make almost impossible comparison between studies or definition of general patterns for each of the studied responses. Motivated by this fact, I present in the **second chapter** of my thesis a series of trials that addressed different aspects of seeds´ biology with the aim to put on evidence different methodological “pitfalls” and propose a unified way to proceed with germination studies. Additionally, the ecological implications from each of the responses are placed in context. The principal objective was to offer a general protocol in order to assist in the consecution of more ecologically realistic experimental settings, which could in turn allow bridging the gap between laboratory and field studies with a better proxy. I aimed to response if, e.g. timing of seed collection would affect the results of germination experiments, i.e., if seeds would undergo a post-maturation stage once established (afterripening). Assess whether seed germination of epiphytic bromeliads if selectively influenced by the use of different temperature regimes such as constant or oscillating temperature and, possible dependency between water and temperature at this developmental stage. Moreover, the assessment of the importance of water availability for seed germination through a more realistic perspective by the use of different dry-wet cycles and how does it differs from the commonly used setting of subjecting plant to solutions at specific water potentials was also part of the plan.

The **third chapter** of my thesis comprises a study on the effect of temperature and CO_2 interaction on growth, morphology and biochemistry of three C_3 epiphytic bromeliads. I aimed to test if plants grown under an e[CO_2] had a better performance in terms of growth when compared to those grown under an ambient CO_2 concentration (a[CO_2]). The role of a

3°C increase in ambient temperature as a stimulator of growth responses, particularly, in plants subjected to e[CO_2] and possible function of this second as an ameliorator of heat-stress due to its higher availability in the environment was also of great interest in this work. I also monitored other typically assessed parameters in climate change studies such as variations in nitrogen content and concentration; and changes in leaf characteristics, i.e., leaf area and specific leaf area. As a final point in this chapter, I also looked for the existence of a regulatory effect of e[CO_2] in plants subjected to an increased temperature in terms of PSII efficiency.

The **fourth chapter** comprises a study on reproduction of three epiphytic bromeliads in a CO_2 and nutrient enriched future atmosphere. The enhancement of both reproductive performance and growth rates of individuals grown under an enriched atmosphere and high availability of nutrients was expected to be an obvious result. Besides, a reduced concentration of non-structural carbohydrates in leaves of plants grown under an enriched CO_2 atmosphere due to a higher investment on construction of reproductive organs, which was expected to be less pronounced in plants supplied with a high nutrient concentration. Finally, in this chapter I look for the possible buffer-effect of e[CO_2] and high nutrient availability for maintenance of life expectancy and reduction of reproductive cost, which has been typically observed after a reproductive event takes place. The latter is usually reflected in slower growth rates and low survival.

In the **fifth chapter** I do a compilation of the experimental results of the present research and drive the attention on the different pitfalls and questions that remain open to be answered and, possibly, constitute a possible starting point for future research on this topic.

Summing up, the scope of this thesis was to broaden our understanding on the actual degree of threatened faced by epiphytic bromeliads at different life stages in front of the imminent climate change. Special interest is driven on vital processes such as germination, growth and reproduction and the related internal and external changes that may be experienced as a consequence of the changing environment. Since epiphytes are highly diverse in tropical ecosystems and play an important role in it, including their capacity to offer refugees for other organisms and participation in the nutrient circulation along the forest column, a closer look at their own vulnerability to climate change may allow us to get some insights on the possible impact of it at other ecosystem levels with certain dependency on them. A better idea on the actual impact, apart from setting the bases to develop conservative strategies for this highly diverse biota may also reveal the urgency of this need.

A- Additional features

A.1- Which of the three stages (germination, growth and flowering) could represent the actually critical plant stage?... Insights

Germination and seedling establishment has been suggested to be the most vulnerable stages in the life cycle of plants because seedling´s phase is generally on a higher risk of mortality (Harper *et al.* 1997) and germination alone has generally been considered a key element in plant life history strategy (Gurvich *et al.* 2008). However, flowering has also been exposed as a critical stage of plant aspects (Bloor *et al.* 2010) and fruiting could also imposed some constrictions for populations development. Then, it seems plausible to address once again the question of which of these aspects could represent the real bottleneck of plants life history. Particularly, in a future climate change atmosphere with the simultaneous changes in CO_2 and other important abiotic factors such as water and temperature the vulnerability of each of these stages, individually or as a whole, results a subject of great interest. Seed germination responses have a direct impact on a species´ distribution and abundance since it is a key element affecting population dynamics (Godínez-Álvarez & Valiente-Banuet 1998; Valverde *et al.* 2004; Ramírez-Padilla & Valverde 2005b). Manzano and Briones (2010) expose that a diversity of environmental requirements for germination of seeds and seedling growth could determine the establishment of epiphytes in certain strata of the canopy. Additionally, some authors exposed that seeds usually respond to a combination of environmental factors that are most favorable to seedling growth and survival (De la Barrera & Nobel 2003; Ramírez-Padilla & Valverde 2005a; Simão, Socolowski & Takaki 2007). All these notions together seem to point to germination as the key step in plants population continuity. Nevertheless, I present here an assessment of different ontogenetic stages of epiphytic bromeliads in response to different environmental factors expected to be altered in a climate change scenario, with consideration of the most relevant factors at each of the stages. Understanding the impact of climate change along the different ontogenetic stages of this diverse and highly vulnerable component of tropical forest ecosystems could help us to identify changes in the forest dynamics which could, in turn, give us more proper tools to develop conservation plans for the maintenance of the diversity of this important component of tropical forest.

A.2- Nutrition

In studies with the aim to assess the effect of increased nutrient input a proper knowledge of the basic nutritional status of the plants before the experiment plays an important role, particularly, if plants coming from nurseries are planned to be used. In nurseries, plants may receive excessive amounts of nutrients. Epiphytic juvenile bromeliads grown in a nursery were monitored in order to acknowledge how long it took the plants to reduce original nutrient reserves up to an optimal basic level. The time to reach this point was approximately six months (Winkler, personal communication). However, epiphytes collected in natural populations where they experience rather intermittent water and nutrient supply over-nutrition should not be a concern. Nonetheless, one may ask how long could a plant be restricted from nutrient supply before it could cause any temporal or even permanent modification in its biochemistry, metabolism or even a change in its genetic? It would probably be important to draw attention to priming studies in order to answer this question. Priming has been described as “a mechanism that leads to a physiological state that enables plants to respond more rapidly and/or more robustly after exposure to biotic or abiotic stress” (Aranega-Bou *et al.* 2014). Individual priming can be accomplished in as fast as a matter of weeks or even hours, depending on the organism and stage. For instances, seeds of maize plants subjected to 24 hours of salinity showed higher germination rate after exposure to salinity stress at a later time (Bakht *et al.* 2011). In another study, 24-hours ´nutrients seed priming´ enhanced germination and early growth of seedlings which is usually limited in field conditions due to constrains at shoot level, in the last case (Muhammad *et al.* 2015).

Epiphytes, particularly large individuals have been found to be relatively unresponsive to increases in nutrient inputs (Laube & Zotz 2003b), however, if plants have been “deprived” of nutrition before the start of an experiment could we just confer the absence of a response to higher nutrient concentration during the course of a trial to the rather stress-tolerance behavior of these plants or to a possible change and even damage on their metabolism? Being optimistic, we could rather suggest an intensification of the response such as “extremely-slow uptake” or even no uptake at all but highly-controlled consumption of previously stored “reserves”. Bromeliads are known for luxury consumption of nutrients which is a mechanism for obtaining scarce nutrients in pulse-supplied environments (Winkler & Zotz 2009) and storage them for a later use. Thus, could a previous restriction of nutrient supply intensify this response even further making them more resistant and efficient in front of future stressful conditions? Usually, the priming effect has been reported to have a positive effect, e.g. if plants are subjected to previous stressful conditions they will showed a more effective response if this particular stress shows up at a later time compared to plants that did not undergo a priming episode. Conrath (2009) exposed the “primed” state as an increased, more efficient activation of response and enhanced resistance to challenging stress. Naturally, intra and inter-specific variability should not be excluded here or any other stressor such as water or temperature. However, limited or no response of plants grown under high nutrient supply compared to those grown under nutrient scarcity could also point to a toxic effect of high nutrient availability due to changes under previous meager conditions. In seeds of some species, a risk of seed damage and impairment of germination at high nutrient availability has been found (Roberts 1948; Ajouri, Asgedom & Becker 2004). To conclude, it could be relevant to consider that if “early” meager conditions may intensify the stress-tolerance behavior of epiphytes, the predictable much slower growth rates may have a negative impact on future species turnover and population dynamics. Hence, in the expected scenario of climatic change vulnerability of these species may reach alarming levels, with unavoidable loss of some or many of the actual existent species in epiphytic assemblages.

A.3. Plant size or age in clonal plants?

To state that the relationship between reproduction and plant size is linear could be a big fallacy as has been pointed out by many authors. Some models to prove the existence of different nature of relationship between these two parameters were already proposed over two decades ago (Klinkhamer *et al.* 1992). However, the failure would not merely remain on the relationship between reproduction and size *per se* but also in the consideration of size as a single-linear predictor of such complex life trait and, (implicitly) assume this second one as a parallel of age. Particularly, in modular organisms such plants, and even more, those with clonal comportment, with e.g. polycarpic behavior, where you may have continuous vegetative propagation, constant renewal of the "genet" by newborn shoots/"ramets", but also in those monocarpic ones, which undergo an ineludible death of the individual once it reproduced. And, here is the point, this new emerged "ramets" grows, matures and reproduces at a faster rate than the other "adults" that currently conform the "genet", even than the "adult" from which it emerged. These facts have given rise to the belief that clonal plants could be "Immortal" (de Witte & Stöcklin 2010) because there is no really enough evidence that they even become senescent (e.g. *Rhododendron ferrugineum*, (Escaravage *et al.* 1998), since the "genet" is continuously renewing.

Most of the existent demographic models aiming to explain the growth of a population use "size" as a parallel of "age" or to extrapolate it, and simulate how long would it take for a population or even an individual to reach certain age based on its actual growth rate. This last derives, inclusive, from a morphological measure of size. However, it would only carry us to the age of the actual organism ("ramet"), but not of the original individual ("genet"); or to the age of the "genet" but not of the "ramet" (or even of an organism somewhere in between) due to the clonal nature of these organisms which implies a constant renewal with faster developmental dynamics each generational event. For instances, maturity of some recently emerged shoots could be reached in a matter of three to four weeks and sizes are actually comparable to that of already existent adults in the "colony". To our eyes, these individuals would only be three to four weeks old, but a size-model and pre-existent information on growth performance may probably indicate that this individual is some decades old and that time to reach maturity and become reproductive would be also estimated to be some decades or more, but we can see it is not really the case. Consequently, there is the risk of under or over-estimating the actual age or longevity of our studied individual/population. So, we have two possibilities:

1- Take size of individuals, derived from e.g. dry mass; calculate actual growth rate, create the model with this information and obtain the age of the individual, which would correspond to the age of the genet *per se*, so we could call it instead a "genet age"? Although, one may ask what would happen if the new ramets are separated from the original genet and become physiologically independent and mature individuals? Would it be a mistake attributing to this individual the age of its "parent plant"? The fact that this recent ramet reaches a size comparable to that of an adult individual, contain its genetic material and it is maturely prepared to reproduced as long as all external conditions are given, would indicate that it is right to do so. However, some components would still be missing.

2- A second possibility would be to include additional factors in a model aiming to describe the age and growth of a population - as it is, in fact, done in more sophisticated demographic models. Take not only size, but also other features that can tell more about the life history of that particular organism (what could influence its current responses). In a relatively recent review paper by de Witte and Stöcklin (2010) it is accepted that life span in one of the least accessible demographic traits in clonal plants. The continuous renewal of the individual through vegetative propagation is assumed to affect the population's dynamics (turnover and stability). However, nowadays the longevity of clonal plants is still an issue that remains poorly understood. Recently, indirect estimations of genet age by means of size and annual shoot increments are being used. Nonetheless, these estimations are limited by the sometimes weak correlation between size and age.

Among the methods to estimate longevity of clonal plants that are not based on genet size can be mentioned: molecular divergence based on somatic mutations and cell-growth estimates (Ally, Ritland & Otto 2008) or the proportion of ramets to genets (variation due to somatic mutation vs. recombination; (Mock *et al.* 2008). Also age-based population or transition-matrix models can be useful tools to investigate life history, dynamics and individual longevity (Ehrlén & Lehtilä 2002). De Witte and Stöcklin (2010) presented a series of methods to measure life span in plants which are classified in direct and indirect methods. From the eight direct methods listed, the growth-form and phenological analysis based on annual morphological markers is used to study growth strategies, age-related patterns, size and age distribution or survival curves. A second method is the permanent plot research which involves long-lasting research efforts, but yields highly reliable age determinations (Bärlocher *et al.* 2000; Erschbamer & Winkler 2005). This last method is especially recommended for plants that may disappear from above ground for years such as geophytes (Tamm 1948; Tamm 1956; Inghe & Tamm 1985). The main disadvantage of this and the rest of direct methods presented by these authors is that only connected plant structures can be measured and attributed, with certainty, to a particular genet. Hence, the longevity of clonal plants remains systematically underestimated by any of these direct measurements.

Among the indirect estimates of age, the use of DNA fingerprinting techniques seems to facilitate precise genet identification. This modern molecular analysis renders high reliability in the identification of the entire genet. Allozymes constitutes one of the most common molecular markers that have been used (Stehlik & Holderegger 2000) and among the DNA fingerprinting techniques can be listed the use of: microsatellites (Suvanto & Latva-Karjanmaa 2005), random amplification of polymorphic DNAs (Laberge, Payette & Bousquet 2000) or amplified fragment length polymorphisms (Escaravage *et al.* 1998). Molecular markers allow the distinction of individuals, so spatially explicit sampled plant material can be with certainty taken as a genet. Once size of the genet is determined by means of this method the determination of age can be done by dividing through this size by the annual growth increment. On the other hand, the DNA fingerprinting techniques offers the possibility to develop a huge amount of markers at a great ease and low cost (Jones, Bay & Nordenhäll 1997; Mueller & Wolfenbarger 1999). Some other advantages can sum up to the use of these techniques, though ambiguities can be encountered at the moment of identifying similarities or dissimilarities among ramets (Widén, Cronberg & Widén 1994). Ambiguities are caused by a number of reasons such as mutations, contamination of samples or scoring error (Arens *et al.* 1998; Van der Hulst *et al.* 2000; Douhovnikoff & Dodd 2003). Thus, the goal would be to adopt a more rigid procedure in order to obtain more reliable results or improved these “innovative” techniques.

Another issue is that demographic models which use size- or age-structured matrix models to estimate demographic properties of long-lived plants are mostly based on ramets dynamics, but as pointed by Watkinson and Powell (1993), the use of these models can still help us to understand the population processes in clonal plants and save us the real time needed in order to follow the sequence of the long developmental processes taken for the original genet. However, some authors accept that ramet’s dynamics could certainly be used as an indirect "measure" of genet fitness, population growth and persistence (Caswell 1985; Eriksson & Jerling 1990; Weppler, Stoll & Stöcklin 2006). The apparent frequent issues of encountering negative growth rates in populations of clonal plants but unaltered ability of the population to persist can be understood by the study of the dynamics at the ramet level. For instances, a study by Eriksson (1994) estimated that clonal populations of three herbaceous species represented by 250 ramets were able to persist for a period longer than 50 years even with negative growth rates. Although, de Witte and Stöcklin (2010) pointed out in their review that the ability of population to persist for such long time periods, in spite of negative growth rate, is a direct reflection of the slow turnover rates of the genets. They also do mention of, at least, a study by Colling and Matthies (2006) which has investigated the longevity and population persistence at the level of the genet by use of matrix models and population viability analysis.

The assertion by Benzing (1990) in regard to the role of the preponderance of policarpy (sequential clonality properties) in epiphytic species as a contributing factor to fitness′ enhancement has been recently pointed out by Aarssen (2008) as a general issue in all organism endowed with clonal properties. This author stated that vegetative propagation could allow plants to gain at least a two-fold increase in their fitness by combining the sexual reproduction with the sequential and economical production of new offspring. Hence, as in many other organisms, the knowledge of clonal plants′ life spans constitutes a key element to understand their life history (Weiher *et al.* 1999), population dynamics (Harper 1977; Silvertown J. & Lovett 1993) and evolutionary fitness (Silvertown J. 1991). Moreover, the role and contribution of this clonal population in the maintenance and stability of the ecosystem they inhabit has also been addressed by some authors. Eriksson (1996, 2000), for instance, suggested that remnant population of long-lived "genets" could contribute to the stability and resilience of the ecosystem by their continuous vegetative propagation which could act as a buffer in the face of environmental variations, probably due to a physiological-genetical reinforcement through "natural priming", a by-product of historical events experienced by individuals previously conforming the genet. Increased resilience, “for instance”, is said to be due to the maintenance of similar habitat conditions created by the same population as a consequences of enhanced nutrient dynamics and recruitment of individuals after disturbance. Furthermore, this fix and preponderant capacity of clonal populations to recover and persist in their ecosystems after the introduction of disturbance could have important implications in order to regulate the vegetational component under a future climate change scenario (Guisan & Thuiller 2005).

The problem of the size-based approach in an attempt to describe the life histories and population dynamics of modular organisms is also well exemplified in an experimentation carried out with a modular organism belonging to the animal kingdom, a bryozoan species which is an invertebrate organism that comprises sessile colonies (*Watersipora subtorquata*). As Hart and Keough (2009) summarizes in their work, the fundamental assumptions that the constant renewal of the modules whether by loss or fragmentation has no more consequence than a single decrease in size is rather sketchy. Hence, by manipulating the size of the colony in a way that allow the assessment of a size, age or damage effect and the performance of both the remaining colony and the individual fragmented part of the colony they aimed to proof if the future behavior of this fragmented modular organism could, in fact, be determined by any of those variable as a single acting force. As expected, the single-state variables such as size or age do not describe the behavior of the studied species as a whole. They also found that the age of the remaining module determines the timing of the reproductive onset and fecundity. These authors concluded that knowledge of the history behind size decreases could be the most precise way to describe the population dynamics of a modular organism and put on judgment screen the existent of uniquely-size-based demographic models.

Unfortunately, the descriptions of life history and population dynamics of clonal individual’s base on size dominate the literature (Harper 1977; Kirkpatrick 1984; Hamilton, Schmid & Harper 1987; Hughes & Connell 1987; Schmidt & Zotz 2001). Apparently, size instead of ontogeny is a better simulator in clonal individuals due to two reasons: the ability of individuals to survive loss of some parts of the module which subvert/undermine the dependency between age and size, and secondly, the advantage of size for life history traits such as growth, survival and reproduction (Hughes 1984; Watkinson & White 1986; Raymundo & Maypa 2004). Hart and Keough (2009) also emphasized the bad habit of assuming that an individual´s history does not influence the future performance of an organism when modeling life history of a species based just on a size measure. Nonetheless, module fragmentation has been found to originate large differences among individuals of equivalent size (Hughes 1984).

Schmidt and Zotz (2002) simulated the annual growth of an epiphytic bromeliad (*Vriesea sanguinolenta*) by the use of different regression fits (the best fit, linear and non-linear) based on re-iteration of size-dependent annual size increments (the length of the longest leaf). Seedling size was used as a starting point up to the known maximal size of the species. They found that variations in growth response were greatly dependent on actual growing condition, which surpassed differences due to the use of the different regression models. For instances, plants growing under abundant resource availability exhibited faster development and time to reached maturity was estimated to be ca. six time faster than those grown under stressful condition (such as "El Nino" year). Thus, models attempting to simulate the growth of a population should not surpass the apparent strong influence of factors others than size. These models should also include a measure of life history which also quantifies the previous existent conditions undergone by the individual/population under study, bringing back, a “priming history”. All previous conditions experiences by the "genet" are contained in the genetic material and it will somehow influence the behavior of the remnant organism beyond of how big (in size) could it be. For instances, it may determine if once an individual reaches the reproductive size irrespective of time, a change in e.g. 1°C in temperature would be more beneficial than a 3°C change, among other changes.

Zotz (2000) brought back our attention to the importance of including size in ecophysiological studies in order to account for the intraspecific variability in responses. He based his acclaim on parallel findings on strong divergence in response of individuals varying in a few centimeters size after experimental manipulation (Schmidt & Zotz 2000). Zotz (2000) based his statement on the optimal allocation partitioning theory and stated that smaller plants should store excess nitrogen in long-lived organs such as stem for higher future productivity, whereas larger plants respond to their need to optimize their carbon gain in order to improve their current photosynthetic machinery, thus similar environmental conditions could result on different effects in conspecifics differing in size, which could cause variable effects at a physiological level. Although, type and degree of limitation could also be dependent on the ontogenetic stage we are looking at. Young individuals have different requirements and limitations than adult ones, for instances, a juvenile may divert resources to reinforce growth, which could reflect a need to improve its surface/volume relationship that could offers higher probabilities to survive drought periods. An adult individual aims to invest resources in keeping a constant photosynthetic activity instead, which allows for maintenance. This phenomenon of ontogenetic drift is, naturally, not something exclusive from epiphytes (McConnaughay & Coleman 1999; Bruhn, Leverenz & Saxe 2000), but it is particularly pronounced in this plant group (Laube & Zotz 2003b). Though, throughout this review, I attempt to call the attention to the dependency of responses not obeying to a merely transition between different maturity stage. As abovementioned, size-dependent changes could be observed between individuals inside the same ontogenetic stage. Additionally, the influence of size on the different plant responses is not only a subject of relevance in ecophysiological studies but its influence on many other plant spheres have been well-recognized.

In his review paper Zotz (2000) pointed out at the possibility that "size-effect" represents an "age effect" instead, though, he also presented a couple of experimental evidences which could counteract this view. For instances, the high variability in growth rates of an epiphytic species (Zotz 1998) ineludible predicts a rather weak correlation between plant size and age. Moreover, scarce response to nutrient availability by larger individuals compared to pronounce stimulation in smaller individuals may point at a merely size effect. However, I consider the key should be to look at the reproductive phenology, since individuals of equivalent size could have different ages and, hence, enter in the reproductive stage at different time periods. In modular organisms, it could easily happen that a population (a single colony, as well?) represents a mix of individuals belonging to different ages/time periods/generation, even though they, apparently, have the same size. Of course, Zotz (2000) does not neglect the importance of other demographic parameters such as mortality, fecundity and survival, which are key components of plants´ life history, in order to understand and make better predictions of population growth and performance. However, he continued to emphasize and suggest the importance of including size as a covariable. I do not try to argue that size does not play a role in modeling the performance of a population, but that both age and size could work together to determine the individuals/population performance (Hart & Keough 2009) as well as their reproductive behavior.

CHAPTER II

THE INFLUENCE OF COLLECTING DATE, TEMPERATURE AND MOISTURE REGIMES ON THE GERMINATION RESPONSE OF EPIPHYTIC BROMELIADS

INTRODUCTION

Vascular epiphytes account for ca. 27,000 species worldwide Zotz (2013c). Traditionally, physiological ecologists have studied larger individuals of this phylogenetically and ecologically diverse group of plants in an endeavor to understand species distributions in time and space (Zotz & Hietz 2001), but more recently there has been an increased interest in the earliest ontogenetic processes, namely germination and establishment (Fernandez, Beltramo & Caldiz 1989; field studies, e.g. : Cascante-Marín *et al.* 2008; Mondragón, 2006 #10224, laboratory studies, e.g. : Bader, Menke & Zotz 2009; Goode & Allen 2009; Manzano & Briones 2010; Tsutsumi *et al.* 2011).

Germination is arguably the most vulnerable stage of the plant life cycle and thus a key element of plant life history strategy (Harper 1977). A large proportion of published germination studies with plants in general, and epiphytes in particular, has been performed under controlled conditions in the laboratory. While allowing for strong inference by controlling all relevant ambient factors, such studies may also lead to erroneous conclusions because of oversimplified conditions or arbitrary selection of treatment differences. One way to avoid such pitfalls is to informed experimental designs by field data (e.g. on *in situ* temperature regimes, Tsutsumi *et al.* 2011), another is a general, critical revision of currently used methods (see also Baskin & Baskin 2001).

For epiphytes, the majority of germination studies have been performed with members of a single family, the Bromeliaceae, and consequently our study also focuses on this group. We touch a number of issues, from the collection of samples and seed storage to the appropriate design of experiments dealing with the influence of water supply and temperature on germination in epiphytic bromeliads.

Many authors give detailed and unambiguous information on the sampling procedure of the seeds used in experiments (e.g. Fernandez, Beltramo & Caldiz 1989; Montes-Recinas, Marquez-Guzman & Orozco-Segovia 2012), in other studies there is room for interpretation (´collected from natural populations, Bader, Menke & Zotz 2009; e.g. ´mature fruit` Pereira *et al.* 2009). Since bromeliad capsules may look “mature” weeks or even months before natural dehiscence, difference in germination success could easily stem partly or entirely from varying levels of maturity and not from difference among populations or treatment effects. For the proper interpretation of past and future studies, information on the potential of after-ripening would be essential. To this end, we investigated whether collection time (from immediately after anthesis to close to dehiscence) affects the outcome of germination experiments in three species.

For soil-rooted terrestrial plants, there is a long-standing interest in the response of the germination process to different water potentials (Ψ), both in agriculture and in basic plant science, because germination is an important bottleneck for plant recruitment in natural systems (Evans & Etherington 1990). A few studies with epiphytic bromeliads have also investigated this relationship as well (Pereira *et al.* 2009), but it can be debated whether such experiments allow relevant conclusions for the situation in nature. In contrast to soils, which provide a relatively constant environment for germinating seeds over longer periods, conditions in the epiphytic habitat are extremely volatile, in particular in the case of bark epiphytes: their seeds are either wetted by rain or fog ($\Psi \approx 0$ MPa) or, with rapid transition, exposed to very dry conditions even in moist rain forest conditions. For example, a relative air humidity (rh) of 99% at 20 °C already corresponds to a Ψ of -1.4 MPa, while rh of 90% represents a ten times lower Ψ (Nobel 2005). For this reason, some researchers (e.g. Bader, Menke & Zotz 2009) have begun to study the influence of water on the germination process in vascular epiphytes not by varying Ψ, but by alternating periods of drought and wetness. Although this approach seems much closer to the real world with its irregular rain fall, the experimental details have not been analyzed systematically. Conceivably, the outcome of such studies may strongly depend on the relative lengths of wet and dry periods, or on a possible interaction between the moisture treatment and other factors such as the temperature used. Consequently, we analyzed how variation in the duration of dry/wet cycles affected the results of germination experiment in four bromeliad species. In addition, for four other species we compared the outcome of an experiment with intermittent drought (Bader, Menke & Zotz 2009) with the germination response to varying Ψ of the solutions the seeds were immersed in.

The use of constant vs. fluctuating temperatures in experiments is a long-standing issue in germination biology (Baskin & Baskin 2001). Clearly, there are plants groups where fluctuating temperatures are *required* for germination, or are at least strongly stimulating, e.g. in many species of wetlands and floodplains (e.g. Mollard & Insausti 2011; Carta *et al.* 2013). In most studies with epiphytes, constant temperatures have been used, but this approach has apparently rarely been based on a critical evaluation of the effect of constant vs. fluctuating temperatures on germination in this group. A few researchers did apply fluctuating temperatures, but only two studies compared the outcome of such a treatment with germination under the constant, mean temperature. The results were inconsistent. While germination of the two bromeliads studied by Pinheiro and Borghetti (2003) was slightly lower and slower under fluctuating conditions, germination in four other bromeliads was significantly enhanced by varying temperatures (Pereira *et al.* 2009). Such inconsistent results are hard to interpret in an ecological context, because the maxima and minima temperatures are rarely based on relevant measurements of fluctuations in the field (but see, e.g.Pinheiro & Borghetti 2003). We compared germination under both conditions for a range of species. In addition, we studied the interactive effect of varying temperature and water supply on germination with another three species.

To conclude, laboratory experiments are simplifications and cannot capture the complexity of the natural situation, while field work (e.g. Cascante-Marín *et al.* 2008) will hardly allow the unambiguous identification and quantification of the effect of individual factors, e.g. temperature, on germination. This contribution will hopefully assist in “bridging the gap” by improving the quality of the results from controlled, *ex situ* experiments to understand the complexities *in situ* for studies with vascular epiphytes.

MATERIAL AND METHODS

All experiments were conducted with material from epiphytic Bromeliaceae collected from natural populations in Panama (Table 1), which were brought to the Plant Functional Ecology Lab at the University in Oldenburg, Germany, where the experiments were carried out. Seeds were sown in disposable Petri dishes (100x15mm) with filter paper (Machery-Nagel, 651mm, Ø 83mm), which were kept in climate cabinets (Economic Delux, Snijders Scientific, Tilburg, The Netherlands) with a light/dark period of 12/12h (PFD c. 60 µmol m^{-2} s^{-1}). If not mentioned otherwise, temperature was set to 25°C. Before starting an experiment all seed comas were clipped off and the seeds sterilized following Pickens *et al.* (2003). Coma removal can slow germination compared to intact seeds as shown for *Catopsis sessiliflora* (Wester & Zotz 2011), but the final proportion of germinated seeds seems to be unaffected. In the experiments without special water treatments seeds were watered every other day to ensure continuously moist conditions. Germination (defined as breakage of the testa by the protruding, swollen hypocotyl) was recorded daily with a dissecting microscope (Zeiss 57 50 57, Jena, Germany). Before starting experiments with intermittent water supply it was necessary to determine the amount of water evaporating from Petri dishes with and without lid. To this end, we added different amounts of water to the filter paper in open Petri dishes and weighed the dishes at 30-min intervals for 2 h. The same was done with closed Petri dishes, but in 6-h intervals over 24 hours. This procedure was repeated in all chambers at all the temperatures used in the subsequent experiments. The results of these trials allowed us to vary the absolute amount of water applied to each Petri dish at the beginning of each wet period, so that the length of the wet and dry periods were as long as planned irrespective of temperature and chamber.

Experiment 1: Seed maturity and germination response

One capsule per plant (Table 2.1, four individuals for each of three species) was collected at five different dates (November 2010 - March 2011). The first collection was done shortly after flowering (in November), the last immediately before natural dehiscence (in April). Seeds enclosed in the capsules were allowed to dry at room temperature (ca. 22°C) and were kept in paper bags until the experiments were started a few weeks after the last collection date. We have recently shown that seeds of Tillandsioideae remain viable for at least 1 year under such conditions (Zotz 2013a). For each species two replicates of every collecting date per individual with 20 seeds each were sown on filter paper in a Petri dish wetted with 2ml distilled water. The Petri dishes containing the seeds were sealed with parafilm® in order to reduce evaporation and the germination success was monitored starting three days after sowing. Germination was followed for 19 days.

Experiment 2: Germination response to oscillating and constant temperatures

Germination with a fluctuating temperature regime (15-25°C) was compared with germination at the mean constant temperature of 20°C. In the case of *Guzmania monostachia*, *Tillandsia fasciculata* and *Vriesea sanguinolenta*, 3 replicates of 20 seeds were used; in the other nine species (Table 2.1) one batch of 20 seeds each were used. Germination was followed for 22 days.

Table 2.1. Collecting sites are lowland sites with wet (San Lorenzo), moist (BCI, Barro Colorado Island) and dry tropical vegetation (Azuero) and a lower montane site (Fortuna). Plant names follow The Plant List (2013). Collecting sites are lowland sites with wet (San Lorenzo), moist (BCI, Barro Colorado Island) and dry tropical vegetation (Azuero) and a lower montane site (Fortuna). Plant names follow The Plant List (2013).

		Experiments				
Species	provenance	E1: collection time	E2: T constant vs. fluctuating	E3: dry/wet cycles	E4: dry/wet cycles vs. water potential	E5: E2 x E3
Guzmania monostachia	BCI	x	x	x		x
Tillandsia fasciculata	BCI	x	x	x		x
Vriesea sanguinolenta	BCI	x	x	x		x
Guzmania subcorymbosa	San Lorenzo		x			
Vriesea gladioliflora	San Lorenzo		x		x	
Catopsis sessiliflora	BCI		x			
Guzmania lingulata	BCI		x			
Vriesea heliconioides	BCI		x			
Tillandsia monadelpha	BCI		x			
Tillandsia bulbosa	BCI		x			
Tillandsia anceps	BCI		x			
Tillandsia subulifera	BCI		x			
Tillandsia flexuosa	Azuero			x		x
Werauhia lutheri	Fortuna				x	
Vriesea viridiflora	Fortuna				x	
Vriesea vittata	Fortuna				x	

Experiment 3: Germination response to wet-dry cycles of varying frequency

Seeds of four species (Table 2.1) were subjected to four different water treatments. Wet and dry periods made up consistently 50% of total time, but frequency of changes varied with cycles of 12/12h, 24/24h, 36/36h and 48/48 h (wet/dry). A control determined germination under continuous moisture. Each treatment was repeated in five Petri dishes containing 25 seeds each, except for *Tillandsia flexuosa* with 3 replicates of 18 seeds, related to a scarcity of seeds of this species. The experiment was terminated when seeds in each of the treatments had experienced a total of 216 h (= 9 d) of wet conditions, which is enough for all controls to reach 100% germination.

Experiment 4: Germination response to varying water potentials

Germination in seven solutions with varying osmotic potential was studied over 35 days with four species (Table 2.1). Solutions with osmotic potentials of -0.10, -0.25, -0.50, -1.0, -1.50; -2.0MPa were produced by varying the concentrations of mannitol; a control used distilled water (0 MPa). Treatments and control were replicated three times with 20 seeds each per species. Using the same seed batches, we also studied germination with different moisture regimes following the protocol described in (Bader, Menke & Zotz 2009):

D0: continuous moisture in closed Petri dishes, adding 1 ml distilled water every other day to compensate for evaporation;
D1: mild repeated drought with 2 h dryness per day;
D2: moderate repeated drought with 6 h dryness per day;
D3: severe repeated drought with 22 h dryness in 2 days.

Each treatment was replicated three times with 20 seeds each. Germination was followed for 35 days.

Experiment 5: Simultaneous variation in the water and temperature regime and germination

Seeds of four different species (five Petri dishes with 20 seeds each, Table 2.1) were subjected to three different water treatments (D0, D1 and D2 at four different temperatures (15°C, 20°C, 25°C and 32.5°C). Consistent durations of dry periods were achieved by applying different amounts of water at the beginning of each wet period and removal of the lid about two hours before the planned beginning of the subsequent dry period. Germination was followed for 35 days.

Data analysis

For statistical analysis we used R 3.0.3. (R Development Core Team 2014). There is a range of response variables that can be used to analyse germination experiments (Scott, Jones & Williams 1984). In most cases we used the germination index (GI), which is defined as:

$$GI = (\Sigma T_i N_i) / S,$$

where T_i is the number of days after sowing, N_i is the number of seeds germinated on day i, and S is the total number of seeds planted. This index reflects both the speed of germination and the final germination success. Usually, we standardized GI by setting the maximum observed value in each experiment and species to unity, which facilitates comparison across experiments. Since final germination percentages under favorable conditions were close to 100% in individual seed batches in all experiments, we interpreted lower germination success as indicative of a treatment effect and not as indicator of non-viable material, although we did not specifically test for viability after termination of an experiment. The responses of germination to varying wet/dry periods were analyzed with 1-way ANOVAs for each species separately using “lm”. Within group differences were explored with HSD tests. The effects of the combined drought and temperature treatments were studied with a three-way ANOVA. The response to constant and fluctuating temperature was analyzed with a t-test.

RESULTS

Experiment 1: Seed maturity and germination response

With the exception of the first collection date, germination success was invariably close to 100% (Fig. 2.1), and we found no significant effect of collection date on final germination percentage for any of the three species (one-way ANOVA, $P > 0.05$). Seeds from capsules collected shortly after anthesis in November 2010 did not germinate in the case of *Guzmania monostachia* and *Vriesea sanguinolenta*. Remarkably, those of *Tillandsia fasciculata* showed about 50% germination, although capsules were still greenish when collected - about 5 months before natural dehiscence and seed dispersal - capsules were still greenish when collected.

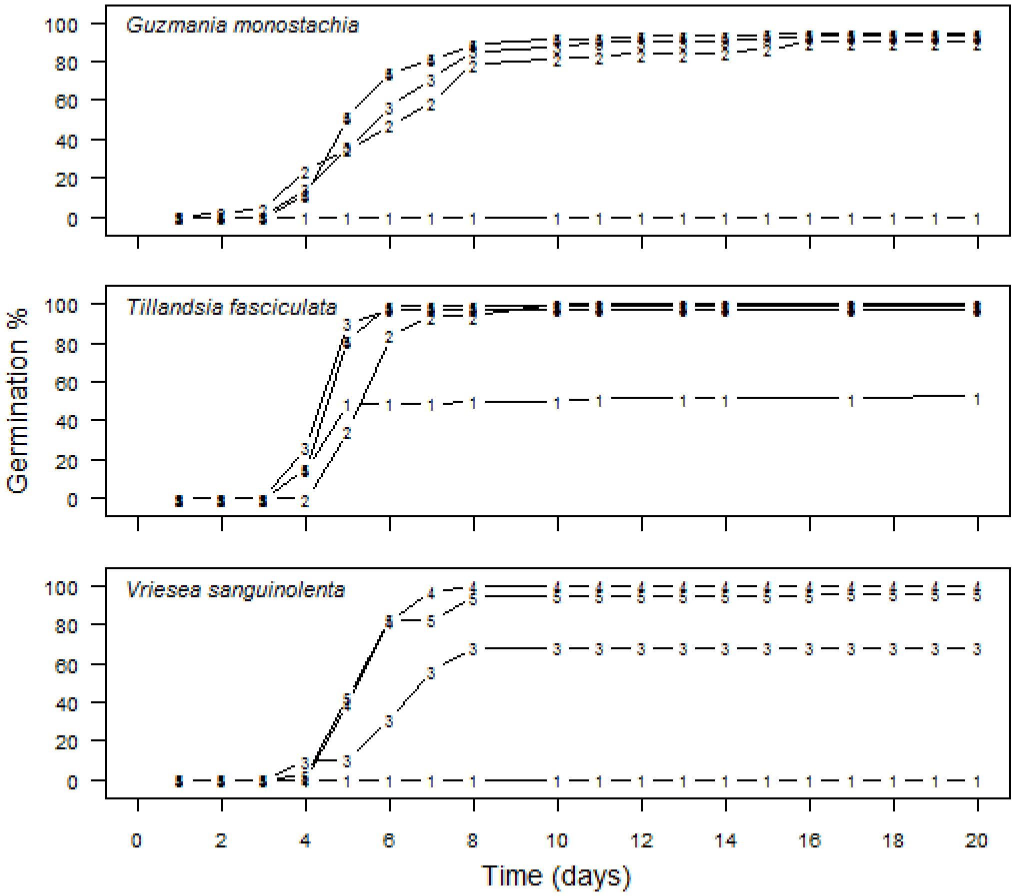


Figure 2.1 Cumulative germination of seeds collected at different stages of maturity for three bromeliad species. Data are means of four individuals. Numbers (1-5) indicate the different maturation stages: 1. immediately after anthesis (04 Nov 2010), 2. two months after anthesis (08 Jan 2011), 3. three months after anthesis (02 Feb 2011), 4. 3 $_{1/2}$ months after anthesis end (23 Feb 2011), 5. Four months after anthesis end (17 Mar 2011). For the species *Vriesea sanguinolenta*, data for the second collecting time are missing because all seed batches were attacked by a fungus.

Experiment 2: Germination response to oscillating and constant temperatures

The 12 bromeliads tested species showed no significant difference in germination response under constant and fluctuating temperature (Table 2.2, t-test, p = 0.42 for final germination percentage and p = 0.17 for GI). In three of the species, these trials were replicated three times, but again no treatment-related differences were found (*Guzmania monostachia* (t-test, p=0.08), *Tillandsia fasciculata* (t-test, p=0.51) and *Vriesea sanguinolenta* (t-test, p=0.63)).

Table 1.2. Germination success under constant and fluctuating temperature regimes in 12 epiphytic bromeliads. Results are final germination in % after 22 days or the germination index. Sample size was 1 or 3 times 20 seeds, in the latter case the data are averages.

	Final germination (%)		Germination index		
Species	fluctuating	constant	fluctuating	constant	n
Guzmania monostachia	90	97	1833	2344	3 x 20
Tillandsia fasciculata	97	97	1707	2072	3 x 20
Vriesea sanguinolenta	94	98	1890	2264	3 x 20
Guzmania subcorymbosa	85	89	1493	1792	1 x 20
Vriesea gladioliflora	100	100	1095	1694	1 x 20
Catopsis sessiliflora	100	100	1905	1630	1 x 20
Guzmania lingulata	100	93	1558	1293	1 x 20
Vriesea heliconioides	95	79	1708	1164	1 x 20
Tillandsia monadelpha	95	100	1588	1750	1 x 20
Tillandsia bulbosa	90	90	1485	1405	1 x 20
Tillandsia anceps	90	100	1615	2340	1 x 20
Tillandsia subulifera	90	90	1845	2365	1 x 20

Experiment 3: Germination response to wet-dry cycles of varying frequency

Germination was invariably fastest in the controls and slowest in the treatment with the most frequent dry periods (12h / 12h), with intermediate responses of the other drought treated samples (Appedix 1). A different picture emerged when relating the germination index to the periods of moisture, i.e. hydrotime (Fig. 2.2). Although the 12h/12h treatment continued to impose a significant effect on germination (ANOVAs, HSD tests < 0.05, with one exception; *Guzmania monostachia*), the effects of the other treatments were basically indistiguishable statistically, both among each other and from the control (ANOVAs, HSD tests > 0.05).

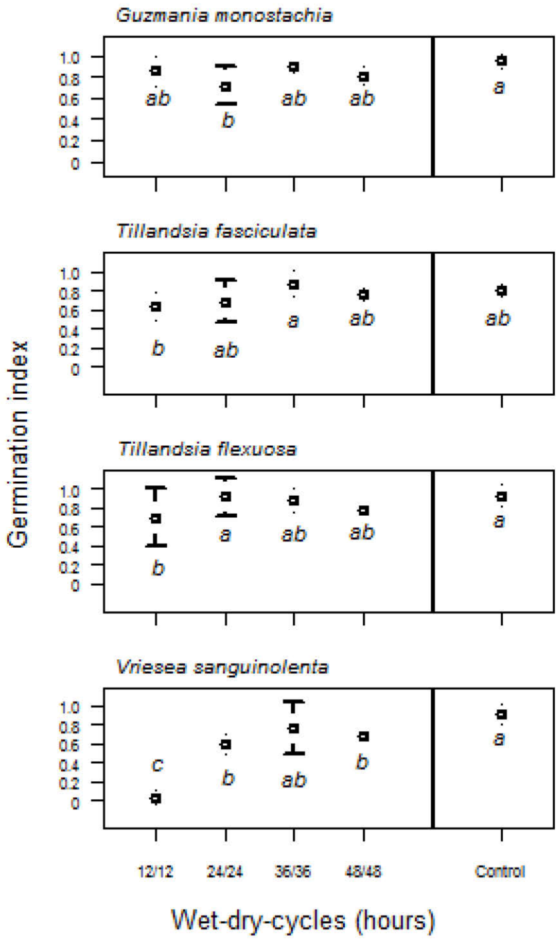


Figure 1.2. Standardized germination indices as a function of different wet-dry-periods in four bromeliad species (left panels) and a control (constantly wet, right panels). Data are means ± SD, sample size was 5 x 25 seeds, except for *Tillandsia flexuosa* with 3 x 18 seeds. Significant differences (ANOVA, HSD, $p < 0.05$) are indicated by different letters. Detailed germination kinetics are shown in Appendix 1.

Experiment 4: Germination response to varying water potentials

The germination index followed a logistic relationship with varying water potential (Fig. 2.3). Remarkably, two of the four species still germinated, if at low percentage, at water potentials < -1.5 MPa. Aligning these response curves with the GIs of the four drought treatments (D0-D3) yielded an inconsistent quantitative pattern. For example, the germination response under the driest wet/dry treatment in *V. gladioliflora* was comparable to that with a water potential of -1.7 MPa, while the effect of the same treatment on *V. viridiflora* was equivalent to that of an osmoticum of only -0.8 MPa.

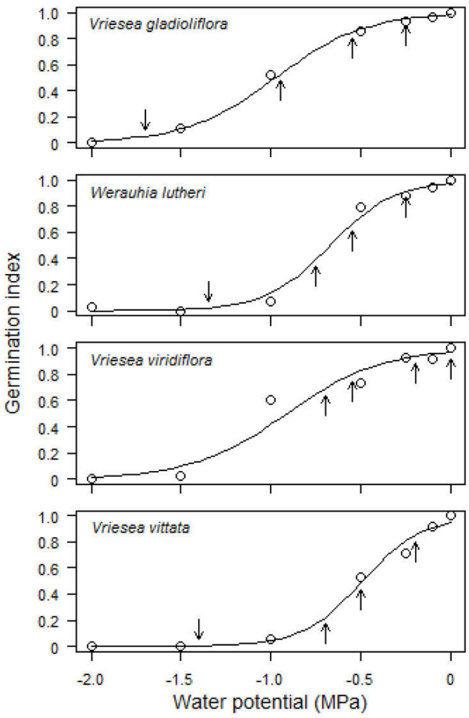


Figure 2.3. Germination responses to different water potentials in four epiphytic bromeliads. Open symbols are the mean germination indices at different water potentials (0 to -2.0 MPa), lines show logistic regressions fitted to these data, and arrows indicate the mean germination indices of the four different treatments with intermittent drought (D0 – D3). Detailed germination kinetics are shown in Appendix 2.

Experiment 5: Simultaneous variation in the water and temperature regime and germination

Temperature, water regime and species affected germination (Table 2.3, Fig. 2.4). Germination success of all four studied lowland species varied consistently with temperature – it increased from 15 °C to 25 °C with no further increase or even a decrease at 32.5 °C. Much less consistent was the response to intermittent drought.

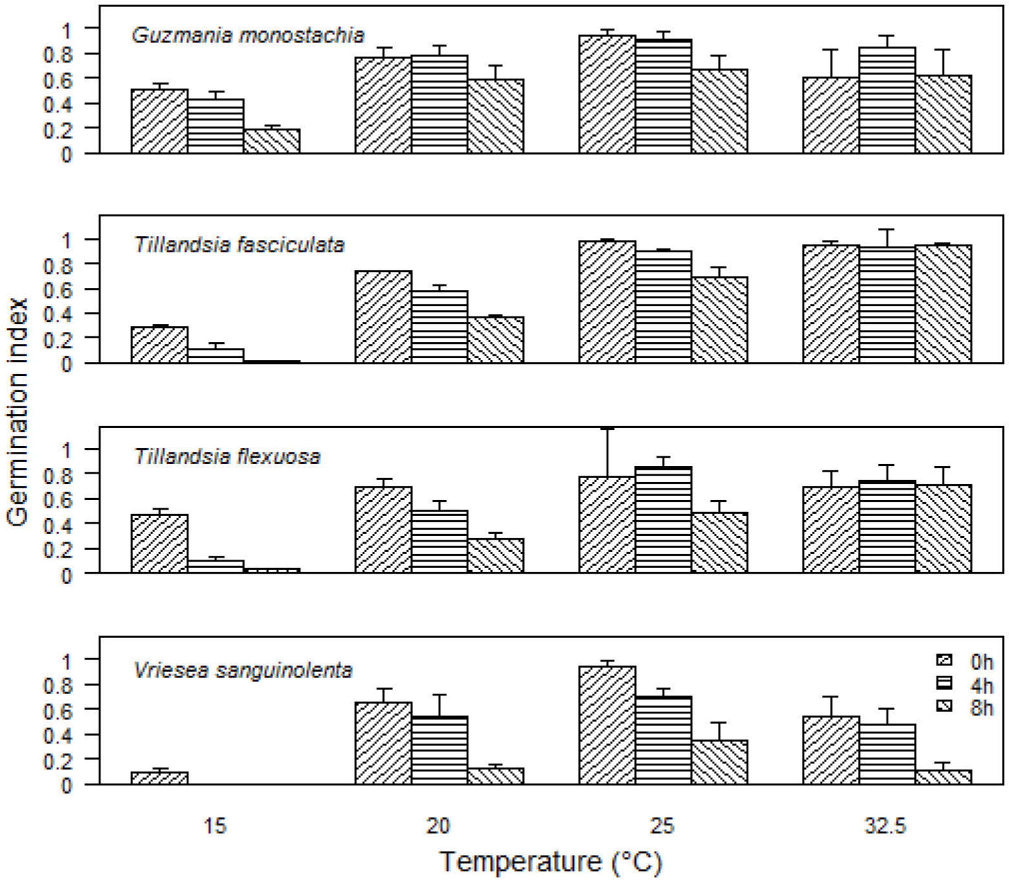


Figure 2.4. Germination indices in response to different water and temperature treatments for four epiphytic bromeliads. Water treatments: continuous moisture, 4 hours dry period and 8 hours dry periods. Temperature: 15°C, 20°C, 25°C and 32.5°C. Data are means ± SE. Detailed germination kinetics are shown in Appendix 3A & 3B

A negative effect of longer drought on germination was strongest at low temperatures (-63% to -100%), intermediate at 20 and 25 °C (-24% to -81%), while at 32.5 °C a reduction was only found in *V. sanguinolenta* (-80%, Fig. 2.4).

Table 2.3. Results of a three-way ANOVA on the effects of water, temperature and species on the standardized germination index for the species *Guzmania monostachia*, *Tillandsia fasciculata*, *Tillandsia flexuosa* and *Vriesea sanguinolenta*

Factor	d.f	F	P-value
Temperature (T)	3	367.5	< 0.001
Water (W)	2	158.8	< 0.001
Species (S)	3	81.4	< 0.001
T x W	6	11.1	< 0.001
T x S	9	15.8	< 0.001
W x S	6	6.9	< 0.001
T x W x S	18	3.5	< 0.001
Error	191		

DISCUSSION

Only a decade ago Baskin and Baskin (2001) concluded in a general review that "not much is known about the germination ecophysiology of epiphyte seeds". This situation has improved considerably by now with a number of recent publications on the subject (e.g. Cota-Sanchez & Abreu 2007; Toledo-Aceves & Wolf 2008; Bader, Menke & Zotz 2009; Goode & Allen 2009; Manzano & Briones 2010; Valencia-Díaz *et al.* 2010; Montes-Recinas, Marquez-Guzman & Orozco-Segovia 2012; Sosa-Luría *et al.* 2012). Overall, however, our understanding of this aspect of epiphyte ecology is still rather sketchy and highly biased – the majority of studies have focused on bromeliads. This contribution addresses methodological issues in an effort to ensure ecologically meaningful germination studies with bromeliads and epiphytes in general.

A first consideration in any germination study is to be sure about the level of maturity of the used material (Baskin & Baskin 2001). Unless seeds are collected from naturally opened fruit, "maturity" is not unambiguously defined. In our experience, fruit may look "mature" long before natural opening. Information on afterripening in epiphytes is scarce (Fernandez, Beltramo & Caldiz 1989; Schwallier, Bhoopalan & Blackman 2011). In *Tillandsia recurvata*, immature seeds germinated at relatively high percentage, but lost viability much faster than mature seeds (Fernandez, Beltramo & Caldiz 1989). The advantage of collecting capsules before dehiscence are obvious: 1) during collecting trips material from species of different phenologies and conspecifics of varying developmental state can be collected together and 2) as discussed by Ruiz *et al.* (2008) seeds inside unopened capsules are in sterile conditions, which makes sterilization procedures unnecessary. The results of our germination trials with immature seeds (Fig. 2.1) suggest that it is safe to collect material before natural dehiscence and obtain meaningful results.

In nature, fluctuating rather than constant temperatures are the rule. Although some authorities therefore advocate the invariable use of alternating temperature regimes (Baskin & Baskin 2001), it is far from clear that arbitrarily set temperature variation yields results that allow better, i.e. less ambiguous interpretation in an ecological context. Undoubtedly, experimental designs informed by data of field conditions are preferable, but in the absence of such data we see no reason to promote fluctuating temperatures in studies with epiphytic bromeliads. This statement is based on the inconsistent results in published studies and our own results. For instance, some studies have shown that bromeliad species have elevated germination percentages at alternating temperatures (Pereira *et al.* 2009), while others showed the opposite pattern (Pinheiro & Borghetti 2003). The bromeliad *Pitcairnia albiflos* even failed to germinate in an alternating temperature regime (Pereira *et al.* 2010). Our results with 12 species of epiphytic bromeliads (Table 2.2) are in line with these previous observations. There was no consistent difference between germination in constant and fluctuating temperature conditions.

Two recent papers have used wet/dry cycles to study the effect of intermittent water supply on germination in epiphytes (Bader, Menke & Zotz 2009; Wagner, Bogusch & Zotz 2013). The ecological realism of this new approach is certainly much higher than the use of different osmotica, but considering their frequent use in germination studies (Baskin & Baskin 2001) a comparison of the effects of both approaches is desirable. Although there was a consistent, and unsurprising, reduction in germination response with increasing drought (Fig. 2.3), either produced by shorter periods of wetness or lower water potential, a quantitative comparison revealed substantial interspecific variation in that relationship: different osmotica are clearly not a simple proxy for the impact of intermittent drought of different severity.

There are many other conceivable variations of wet/dry cycles in nature. In another experiment, we explored how the length of wet and dry periods affected gemination, while keeping the total duration of wet and dry periods constant with 216h each in c. 18 days. Compared to the controls, the delay in germination was inversely related to the length of the wet/dry cycles (Appendix 1). However, calculating the germination index for periods of hydration only (i.e. the hydrotime,Black, Bewley & Halmer 2006), revealed very similar kinetics in most treatments (Fig. 2.2). Apparently, the germination process is only temporarily arrested during dry periods, and continues immediately after remoistening. A substantial reduction in the germination index by very rapid fluctuations of wet and dry periods (12 h / 12h) was only observed in one species, *V. sanguinolenta*. This is remarkable, because this tank bromeliad tends to occupy more exposed, and hence drier, microsites than two of the other studied species (*G. monostachia* and *T. fasciculata*, Zotz 1997), which were hardly affected.

The response to intermittent water supply depends on temperature as well (Fig. 2.4). Although the expected reduction in germination index with increasing duration of intermittent drought was found at most temperatures, GI did not show this trend in three of four species at the highest temperature. Moreover, the proportional reduction varied substantially among lower temperatures. Hence, results of such experiments may be quantitatively, and even qualitatively, misleading when experimental conditions are not based on the typical situation in the field. Unfortunately, we are largely ignorant of the temperatures that germinating seeds of epiphytic plants experience in nature. Records from climate stations may be used as a first approximation, but conditions, particularly on exposed branches, are likely to deviate considerably from such standardized measurements.

To conclude, we present the results of a series of germination experiments with epiphytic bromeliads. We show that it is save to collect capsules for experiments before natural dehiscence. The application of fluctuating temperatures is not imperative. The effects of different water potentials and intermittent drought on germination are not quantitatively comparable among species, and we advocate the use of the latter because of their greater ecological realism. However, the impact of intermittent drought on germination depends on temperature. Hence, data on *in situ* temperatures during germination are needed to design experiments in such a way as to ascertain unambiguous interpretation of the results in an ecological context.

CHAPTER III

THE EFFECT OF CO_2 AND TEMPERATURE ON THE RELATIVE GROWTH RATE OF EPIPHYTIC BROMELIADS

INTRODUCTION

Global climate has been markedly changing since the pre-industrial era due to anthropogenic activities that lead to increased greenhouse gas emissions, for instances, carbon dioxide (CO_2), and there is an expectation that the concentration of this gas will be at about twice of current level by the end of the century (IPCC 2014; Dlugokencky & Tans 2015), 720 – 1000 vs 400 ppm, Dlugokencky & Tans 2015). Since plants use CO_2 as a primary substrate in photosynthesis (Körner & Bader 2010), increases on the concentration of this gas may be expected to have direct and important effects on vegetation. Hence, there is a growing concern about the extent of the impact that it could have on global biodiversity (Lovejoy & Hannah 2005), mostly, when it comes to tropical ecosystems - as the rain forest - because they support the highest biodiversity worldwide (Zotz & Bader 2009).

A group of plants found in the canopies of tropical forests are epiphytes which account for ca. 9% of global plant diversity (Zotz 2013b) and can represent up to 50% of the total vascular flora present in a single locality (Kelly *et al.* 2004). Epiphytes use trees just as structural support and take water and nutrient directly from the atmosphere. Due to this tight coupling to atmospheric inputs, epiphytes have been called "particularly vulnerable" to global change (Lugo & Scatena 1992; Benzing 1998). However, in spite of the importance of this plant group, studies addressing impact of climate change on epiphytes are rather scarce.

The available evidence from laboratory (Granados & Körner 2002; Hättenschwiler *et al.* 2002; Monteiro, Zotz & Körner 2009; Zotz *et al.* 2010), growth chambers), greenhouse (Körner & Arnone 1992), field studies (Ziska *et al.* 1991; Lovelock, Kyllo & Winter 1996; Würth, Winter & Körner 1998a; Würth, Winter & Körner 1998b) and horticultural settings (Li, Liang & Hew 2002) carried out with tropical plants have shown alterations in biomass distribution, morphology, physiology and biochemistry due to growth in an elevated CO_2 atmosphere. Responses are varied among the different studies and even species-specific patterns have become evident (Bazzaz & Carlson 1984; Reekie & Bazzaz 1989; Würth, Winter & Körner 1998a), which make intends to define general response patterns almost impossible.

Relative growth rates, for instances, have been reported to be stimulated in plants grown under an elevated CO_2 environment. Non-saturation of photosynthetic rates at the current CO_2 level, at least in C_3 plants, and a CO_2-enriched atmosphere (Bunce 2004) have been suggested to induce the observed enhancements in growth rates of plants (Monteiro, Zotz & Körner 2009). However, the association between observed stimulation of photosynthesis and faster growth is far from straightforward (Körner 2000). For instance, Schmidt, Stuntz and Zotz (2001) found that the net CO_2 uptake of the epiphyte species *V. sanguinolenta* grown under field conditions increased by almost 50% in larger individuals while RGR decreased by more than 30%. Similarly, terrestrial herbaceous plants had faster growth rates under elevated CO_2 with no evidence of an increased photosynthetic rate per unit leaf area (Curtis 1996).

In those plants that have shown enhanced growth but low carbon uptake at high CO_2, the stimulated effect on growth was explained by changes in biomass allocation, as enhanced leaf ratios (DeLucia, Sasek & Strain 1985; Wulff, Winter & Körner 1998; Laube & Zotz 2003). Laube and Zotz (2003) also suggested that the differences in allocation patterns and plant structure (storage of assimilates and nutrients, changes in leaf mass ratio) must elucidate the abovementioned mismatches. In the present chapter I focus on the morphological and biochemical traits that may be associated with changes in the relative growth rate of epiphytic bromeliads such as leaf area (LA), specific leaf area (SLA), concentration of non-structural carbohydrates and nitrogen.

Accumulation of total non-structural (TNC) in leaves is one of the most consistent plant responses to elevated CO_2 (Würth, Winter & Körner 1998b). Several studies support the notion that this TNC response may even persist while other responses could be expected to disappear (Körner & Miglietta 1994). However, there is a believe that the initial growth acceleration seen in enrichment studies as a results of carbohydrates accumulation is not indefinitely maintained due to a limitation by nitrogen (N) availability (Loehle 1995). Nonetheless, Nijs I., T. and Impens (1995) found that relative effect of elevated CO_2 hardly depends on leaf N status. Since the species mechanistic approached they carried out showed that the stimulatory effect of elevated CO_2 was sustained under low N concentration.

Decreases in N after a long-term exposure to CO_2 have been partly accompanied by a reduction in SLA in some species (Campbell, Allen & Bowes 1990; Rowland-Bamford *et al.* 1991). More specifically, there is an indication of a strong positive correlation between SLA and N concentration for different data sets (Lambers, Chapin III & Pons 2008). Additionally, reductions in SLA are suggested to be partly due to accumulation of TNC and, considered the most obvious adjustment upon prolonged exposure to elevated CO_2 (Lambers, Chapin III & Pons 2008). Contrary, Leaf area (LA) has been observed to increase under an enriched CO_2 environment, e.g., (Hättenschwiler & Körner 1996) found higher LA of plant grown at elevated CO_2, though this increase resulted from more leaves rather than from larger leaves.

Together with increases in atmospheric CO_2 concentration are expected changes in global temperature patterns. Mean annual temperatures in tropical latitudes are expected to increase by ca. 3°C by the end of the current century (Solomon *et al.* 2007). Even though, it could appear to be insignificant when compare to temperature seasonal variations seen in temperate areas and, even day/night ranges in the same tropics, it may still have strong effects on the vegetation inhabiting these ecosystems. Climatic conditions in the tropics are more or less uniform along the year, hence tropical plants have been said to be more narrowly adapted to the existing conditions (Janzen 1967). Therefore, effects on plants physiology and growth are to be expected.

The relative effect of elevated CO_2 concentration would be expected to be enhanced by increased temperatures (Hogan, Smith & Ziska 1991; Rawson 1992). Gifford (1989) affirmed that increased yields under an enriched CO_2 atmosphere appeared to be further promoted at warm environments rather than at cool ones. Additionally, Idso *et al.* (1987) observed that high temperature increased the growth-enhancing effect of elevated CO_2. The growth modification factor calculated for the five species tested in that study was larger in plants grown at elevated CO_2 level (1.56) than in plants grown at ambient level (1.30) when mean air temperature was increased by 3°C.

On the other hand, high temperature could affect carbon assimilation of fast growing sinks by reducing carbohydrates, which caused instead an up-regulation of photosynthesis under an enriched CO_2 atmosphere (Reddy, Rasineni & Raghavendra 2010). Würth, Winter and Körner (1998b) further supported the idea that high temperatures result in generally diminished leaf total NSC concentration in the tropics as compare to cooler climatic zones. With increased temperature also increases plant respiration, which in turn produce respiratory carbon losses. Nonetheless, elevated CO_2 has also been seen to have a positive effect in front of high temperatures, e.g. it can reduce respiration (Ziska *et al.* 1991). Hence, an enriched CO_2 atmosphere has been suggested to enhance plant tolerance to environmental stresses, as that caused by extreme temperatures and photoinhibition (Hogan, Smith & Ziska 1991).

Chlorophyll fluorescence may be used to assess the effects of both low and high temperatures. Moreover, it could also give a measure of photosynthetic efficiency of photosystem II, which is the reaction centre of photosynthetic activity. Damage to the photosystem II is usually considered as the first signal of stress in a leaf. There is a well-established empirical relationship between the ratio of electron transport and CO_2 assimilation (Maxwell & Johnson 2000). Maize leaves subjected to low temperature experienced a higher ratio of electron transport in relation to that of CO_2 assimilation, which may indicate the use of electron transport in processes other than photosynthesis, e.g. production of active oxygen species in order to protect from the low temperature stress (Fryer *et al.* 1998). This phenomenon could also take place under a warmer environment if a continuous increase in respiration would take place. An e[CO_2] may help plants to compensate for respiratory carbon loss and, consequently, values of fluorescence could be expected to be in the typical optimal value reported for many species of ca. 0.83 (Björkman & Demming 1987; Johnson *et al.* 1993). Contrary, plants in the warmer environment, but ambient CO_2 could have more difficulties in dealing with stress of increased temperature and would have lower values of fluorescence.

Based on all previous observations, I conducted the present study to assess the interactive effect of CO_2 and temperature on growth, morphology, biochemistry and biophysics of three C_3 bromeliad species in a context of climate change. I expected to find that: 1) Plants grown under elevated CO_2 concentration (e[CO_2]) were going to have higher relative growth rates (RGR) than the ones subjected to ambient (a[CO_2]), 2) An increase in 3°C in ambient temperature was going to cause a more pronounced stimulation of RGR when plants grew at a CO_2 enriched atmosphere due to an amelioration of heat-stress by a higher availability of this gas, 3) Plants grown under an e[CO_2] were going to acclimate after a time, 4) Lower N concentration in leaves of plants growing under e[CO_2], 5) Plants grown at e[CO_2] and high temperature were going to exhibit higher values (inside the normal range) of PSII efficiency than plants growing under high temperature and a[CO_2] as a consequences of reduced photorespiratory losses by e[CO_2], 6) LA of plants grown at e[CO_2] was going to be larger – related to enhanced RGR - even at high temperature, 7) Decreased SLA in plant grown at e[CO_2] level, 8) Increase in foliar total NSC concentration of plants grown at e[CO_2] due to growth acclimation (sink demands ceases).

MATERIAL AND METHODS

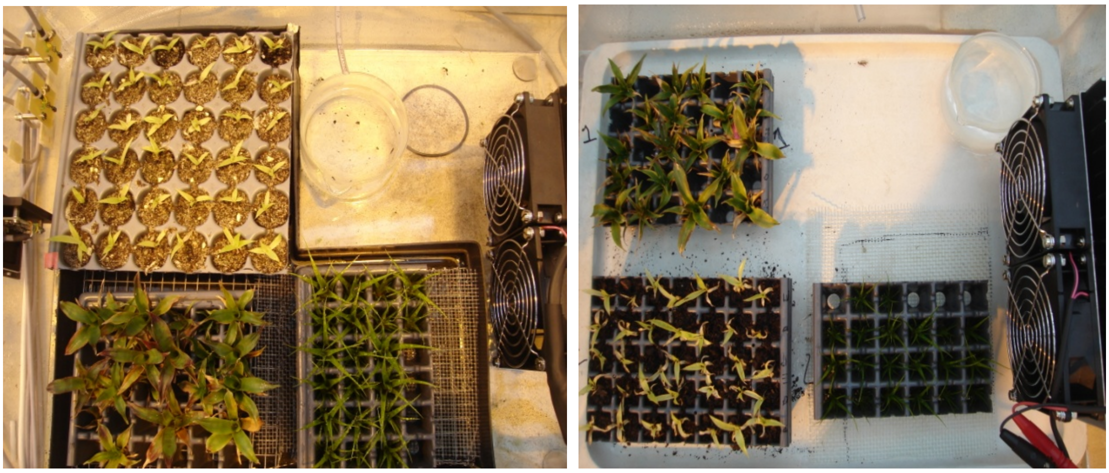
Plant material

Juveniles from three C_3 species of Bromeliaceae were obtained from a commercial nursery (Corn. Bak B.V., Asseldelft, Holland): *Guzmania wittmackii* (André) André ex Mez, *Tillandsia dyeriana* André, *Vriesea duvaliana* E. Morren.

All plants were kept in the greenhouse at Oldenburg University for three months before starting the experiments. During this time they were only irrigated with rain water three times a week to allow high nutrients levels to decrease. The conditions in the greenhouse were: 25°C/22°C day/night temperature, relative humidity of 75%/48% day/night and a photoperiod of 12/12 h light/dark, with natural sunlight being supplemented with artificial light (Metal halid lamps, 400W, master HPI-T plus; Philips, The Netherlands) to achieve a photosynthetic photon flux density (PFD) of at least 150 µmol m^{-2} s^{-1} at the level of the plants.

Experimental design

A long-term growth experiment was carried out with the three species in four custom-built growth chambers at Oldenburg University. Each consisted of a plexiglas box (50cm x 40 cm x 21cm, length x width x height) with controlled CO_2 concentration $[CO_2]$ and temperature (Fig.3.1). Both $[CO_2]$ and temperature in the chambers were continuously monitored and regulated via a feedback mechanism by a pulse valve for pure CO_2 entry and a thermoelectric element for heating and cooling.



A B

Figure 3.1. Picture of plants boxes in the chamber within a box with water (A) and without box with water (B).

Our experimental design combined two CO_2 levels (ambient= ca. 400ppm (a[CO_2]) and elevated=800ppm (e[CO_2]), set values) and two different temperatures (low=27°C/22°C and high=30°C/25°C, day/night set values). Air with a[CO_2] was taken from the outside of the greenhouse. During winter the incoming air was humidified using two gas-wash bottles before entering the chambers. Elevated [CO_2] was obtained by connecting a CO_2 bottle to the control valve of the experimental chambers.

The actual [CO_2] inside the chambers deviated from the setpoints averaging, respectively 487.5 ± 49.3 $\mu l\ l^{-1}$ (mean ± SD of 28331 measurements at the a[CO_2], probably due to the closeness to the houses and highway and/or the concentration outside the greenhouse during the calibration time of the chambers) taken every 10 minutes and 765 ± 21 $\mu l\ l^{-1}$ at the e[CO_2] treatments. We routinely cross-checked the validity of the CO_2 readings with a calibrated commercial device (Li-cor 6400, DETAILS) by sucking air with a plastic tube from inside the chambers through a small hole. A detailed comparison of these measurements is given in Table 3.1.

Table 3.1. Comparison of $[CO_2]$ means registered by the chambers and by gas analyzer systems as well as corrected mean.

Set value (ppm)	Instrument	Mean $[CO_2]$ (ppm)	SD $[CO_2]$ (ppm)	Corrected mean	n
400	All Chambers	487.5	49.3	449.3	36
	Gas analyzer	450.4	40.2		36
800	All Chambers	755	29	742	36
	Gas analyzer	745.2	20.3		36
400	Chamber1	487.5	37	449.3	18
	Gas analyzer	440	27.3		18
800	Chamber1	763.4	8	751	36
	Gas analyzer	754.4	8.3		36
400	Chamber2	472.1	50.2	432.4	36
	Gas analyzer	450	39.2		36
800	Chamber2	754.2	45	741	18
	Gas analyzer	735	35.3		18
400	Chamber3	461	51.5	420.1	18
	Gas analyzer	413	22		18
800	Chamber3	742	13.2	727.5	36
	Gas analyzer	734.1	12.2		36
400	Chamber4	522	39	487	36
	Gas analyzer	480	37		36
800	Chamber4	776	28.2	765	18
	Gas analyzer	759.44	16.4		18

All chambers made a calibration process at midnight by using the $[CO_2]$ inside the greenhouse as a reference point which ranges from 390ppm to 502ppm. In general, chambers were more stable at controlling the $e[CO_2]$ than the ambient one.

Temperature means measured by chambers system were: 27.41°C ± 0.62, n=) / 22.8°C (± 1.42, n=), 30.1°C (± 0.35, n=) / 25.8°C (±1.20, n=). Humidity inside chambers was not controlled, but measured by humidity sensors every 10 minutes. Humidity per set temperatures were: 91% (± 8, n= at 27°C), 92.98% (± 7.06, n= at 22°C), 94.82% (± 5.5, n= at 30°C), 91.06% (± 8.91, n= at 25°C). PFD reaching plants inside the chambers was 158µmol m^{-2} s^{-1} (±42.74, n=6). PFD was measure six times along the study, three times during the darker winter days and three times during the summer days.

The position of the plant groups inside the chambers were rotated every other week to ensure even exposure of each species to any of the chamber sides (see picture 1). Samples were also changed between chambers every four months (setting the conditions of the new chamber to those of the previous), with the aim of avoiding any chamber effects. Each chamber had one [CO_2] every four months and one temperature every two months. It means that every two months samples were rotated to the second chambers with the same [CO_2] (ambient or elevated) and just the temperature was changed (low or high).

Plants were watered four times a week and fertilized just once a week with a spray bottle. The nutrient solution contained NPK in the proportions 8-8-6 (Wuxal Universal Dünger) diluted from which I used 1.4ml per each liter of water. In the case of tanks plants, they were both irrigated with a spray bottle and the tanks were completely filled, with the help of a pipette when needed.

At the beginning of the experiment 10 individuals from each species were sampled to determine fresh (FW) and dry weights (DW), leaf area (LA), nitrogen (N) concentration and total NSC (soluble sugars and starch). Additionally, I measured chlorophyll fluorescence signals (Fv/Fm) in 15-20 minutes dark-adapted healthy leaves with the use of a mini-pump, as an indicator of stress level. This measure was carried out along the experiment: in initial samples, after six months of treatment and at the end of the experiment (after one year). Fresh weights (FWs) were determined for the initial samples one day after watering in order to minimize the difference in water content between treatments at the time of weighing. When there was still water in the tank of the plants it was removed and dried with tissue paper.

Leaf areas (LAs) were estimated from digital photographs by using the program Photoshop. After determination of FW and LA plants were dried in an oven for four days at 60°C to determine plant DW. Before drying plants were microwaved for 1.5 min to stop any further enzymatic activity, required for total NSC determination. Soluble carbohydrates (glucose, sucrose, fructose, maltose) were extracted from ground plant tissue (20mg) in water for 30 min at 80 °C and phases separated by centrifugation. Starch in the pellet after extraction of soluble carbohydrates was broken down to glucose using α-amylase and amyloglucosidase (Roche, Germany) in acetate buffer (pH 5,0: Na-Acetat x 3H2O (100mM) + 2.94g CaCl2x2H2O in 800ml of water) for 2 h at 30°C and phases separated by centrifugation as well. Finally, the different fractions of non-structural carbohydrates were analyzed from dilutions of end-supernatant by HPLC (ICS-3000, Dionex Corp., Sunnyvale, CA).

Nitrogen (N) content was determined from 2-3mg of dry-ground plant mass using a CHN-O Element Analyzer (Thermo Electron Corporation, Delft, the Netherlands).

Growth was calculated as the RGR in mg g^{-1} d^{-1} after Evans (1972):

$$\text{RGR}= 100 \times (\ln(M_{t2}) - \ln(M_{t1}))/\Delta t,$$

where M= biomass (g) and t= time (days). Initial biomass corresponds to the dry mass of individuals harvested at the beginning of the experiment and for the rest of the individuals in every treatment it was estimated from the FW of each individual using an empirically established relationship (linear regression, r^2= 0·9, n= 10) of FW and DW. This relationship was calculated using the samples taken at the beginning of the experiment and after every harvesting time (every 6 months over one year).

I selected 20 individuals from each of the three species above-mentioned and assigned them to each treatment combination.

Statistical analyses

All statistical analyses were conducted using R 3.0.3 (R Development Core Team 2014). Survival data was analyzed with a logistic regression (glm), using a quasibinomial correction of the underdispersed data. This analysis was only applied to the results of the firsts six months of treatments for the species *Guzmania wittmackii*. RGR was analyzed using an Anova type II (lm) for different treatment periods: a- First half of the treatment (first six months), b- Second half of the treatment (last six months) and, c- From the beginning of the experiment until the end of it (over 12 months). In case of a significant interaction effect; I applied a *post-hoc* test (lsmeans). For all morphological (LA and SLA), biochemical (N concentration and N content, NSC) and physical-chemical chlorophyll fluorescence variables, confidence intervals of the initial set of samples (just for leaves) of each species are shown in the corresponding graph. Additionally, I evaluated the change on each of these variables between treatment periods (a and b) looking at the effect of CO_2 and temperature by using the model: lm(Y ~ time*CO_2*Temperature) and differences between treatments groups among treatment periods by using the model: lm(Y ~ CO_2*Temperature. In all cases we did a model simplification using the Akaike information criterion (AIC) and the Anova (with Chisq test) comparison in order to obtain the simplest model to describe our data following the principle of parsimony. Statistics for the morphological and biochemical data are shown in Table 3. In the case of the species *G. wittmackii*, I only analyzed the effect of temperature at e[CO_2] for both time periods due to the high mortality of individuals in the treatment of 400ppm and 30°C. It is only taken into account at the first half of the treatment for RGR analysis.

RESULTS

Survival

The three species tested responded quite differently to the treatment. While *G. wittmackii* had 90% mortality at a high temperature and ambient CO_2 (a[CO_2]) already after six months, the other two species had low mortality going from 0% in *V. duvaliana* up to a maximum of 10% in *T. dyeriana* (Fig.3.2).

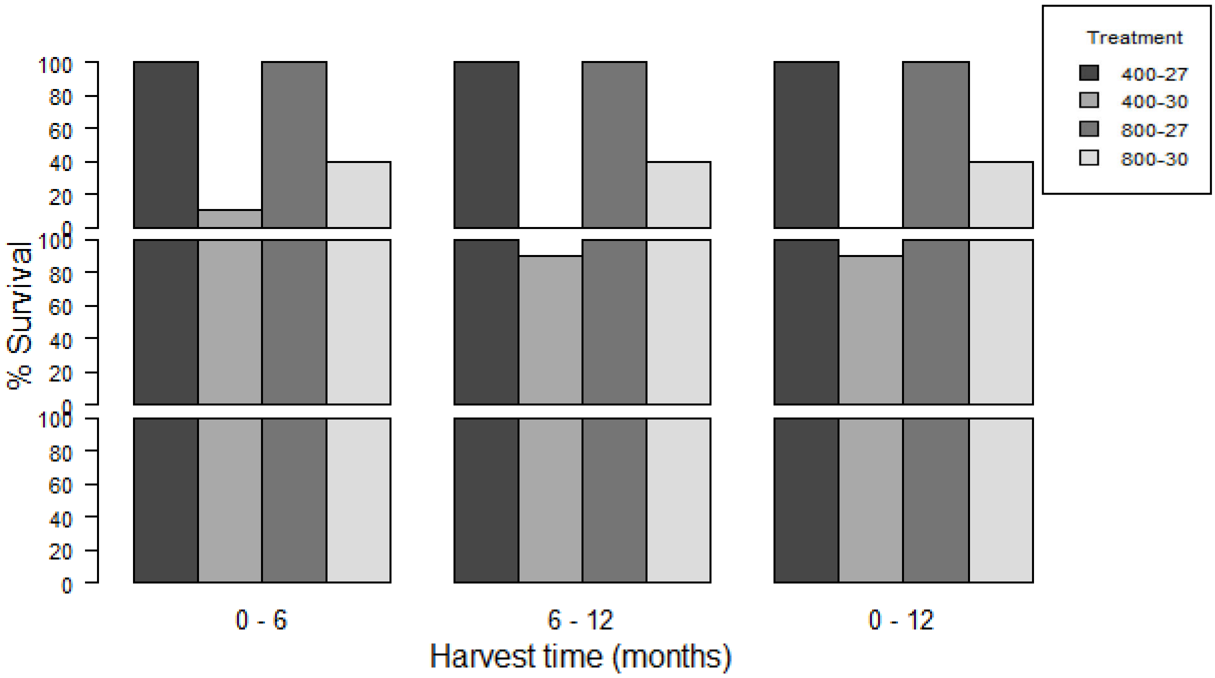


Figure 3.2. Survival percentage for three Bromeliaceae species in response to CO_2 and temperature interaction. First panel: *Guzmania wittmackii*, second panel: *Tillandsia dyeriana*, third panel: *Vriesea duvaliana*. Sample size (n) equal to: 0-6: all 20 but 400-30 (2) & 800-30 (8) for *G. wittmackii*; 6-12: all 10 but 400-30 (0) & 800-30 (4) for *G. wittmackii* & 400-30 (9) for *T. dyeriana*; 0-12: all 10 but 400-30 (0) & 800-30 (4) for *G. wittmackii* & 400-30 (9) for *T. dyeriana*.

Survival rate of the species *G. wittmackii* was significantly affected by the interactive effect of CO_2 and temperature (Table 3.2, $P < 0.05$).

Table 3.2. Results of a logistic regression on the effect of CO2 and temperature on survival percentage after six months for the species Guzmania wittmackii

Coefficients	Estimate	Std. Error	t value	P (>|t|)
Intercept	2.9	1	2.8	0.0056
CO_2(800ppm)	-1.6	1.2	-1.4	0.17
Temperature(T, 30°C)	-5.1	1.3	-3.9	< 0.001
CO_2:T	3.6	1.5	2.4	0.018

***Sample size (n) equal to 20 for 400-27 & 800-27, 2 for 400-30 & 8 for 800-30.**

Relative growth rate (RGR)

Relative growth rate from the three tested species changed significantly between treatment periods (first half and second half, $P < 0.001$, Fig. 3.3, Table 3.3). Mean RGRs along species and treatments were between -0.0233mg g^{-1} d^{-1} (lower limit) and 7.55mg g^{-1} d^{-1} (upper limit).

Table 3.3. Results of a two-way Anova on the effect of Harvest time, CO_2 and temperature interaction on RGR over one year for three Bromeliaceae species

Species	Factor	df	F	P value
G. wittmackii	Harvest Time (HT)	2	8.53	< 0.001
	CO_2	1	8.99	0.003
	Temperature (T)	1	14.73	< 0.001
	Error	92		
T. dyeriana	Harvest Time (HT)	2	24.11	< 0.001
	CO_2	1	2.88	0.09
	Temperature (T)	1	8.63	0.004
	Error	153		
V. duvaliana	Harvest Time (HT)	2	125.07	< 0.001
	CO_2	1	49.65	< 0.001
	Temperature (T)	1	21.83	< 0.001
	HTxCO_2	2	5.68	0.004
	HTxT	2	9.74	< 0.001
	CO_2xT	1	13.48	< 0.001
	HTxCO_2xT	2	10.51	< 0.001
	Error	148		

******G. wittmackii*** **(n= 98),** ***T. dyeriana*** **(n= 158) &** ***V. duvaliana*** **(n=160)**

For the species *V. duvaliana*, the significant effects of CO_2 and temperature on RGR were dependent on time ($P < 0.001$) whereas for the other two species the significant effects of CO_2 ($P < 0.003$, *G. wittmackii*) and temperature ($P < 0.001$, *G. wittmackii*; $P < 0.05$, *T. dyeriana*) were independent of time ($P > 0.05$). *Guzmania wittmackii* and *T. dyeriana* had a better performance at the low temperature (27°C) treatments whereas the species *V. duvaliana*, had the highest growth mean (1.6 mg g^{-1} d^{-1} ± 2.3, mean ± SD, n=10) when grown at 30°C and a[CO_2], though it was just the case at the second half of the treatment (Fig. 3.3). The effects of CO_2 and temperature were not significant at that treatment period ($P > 0.05$).

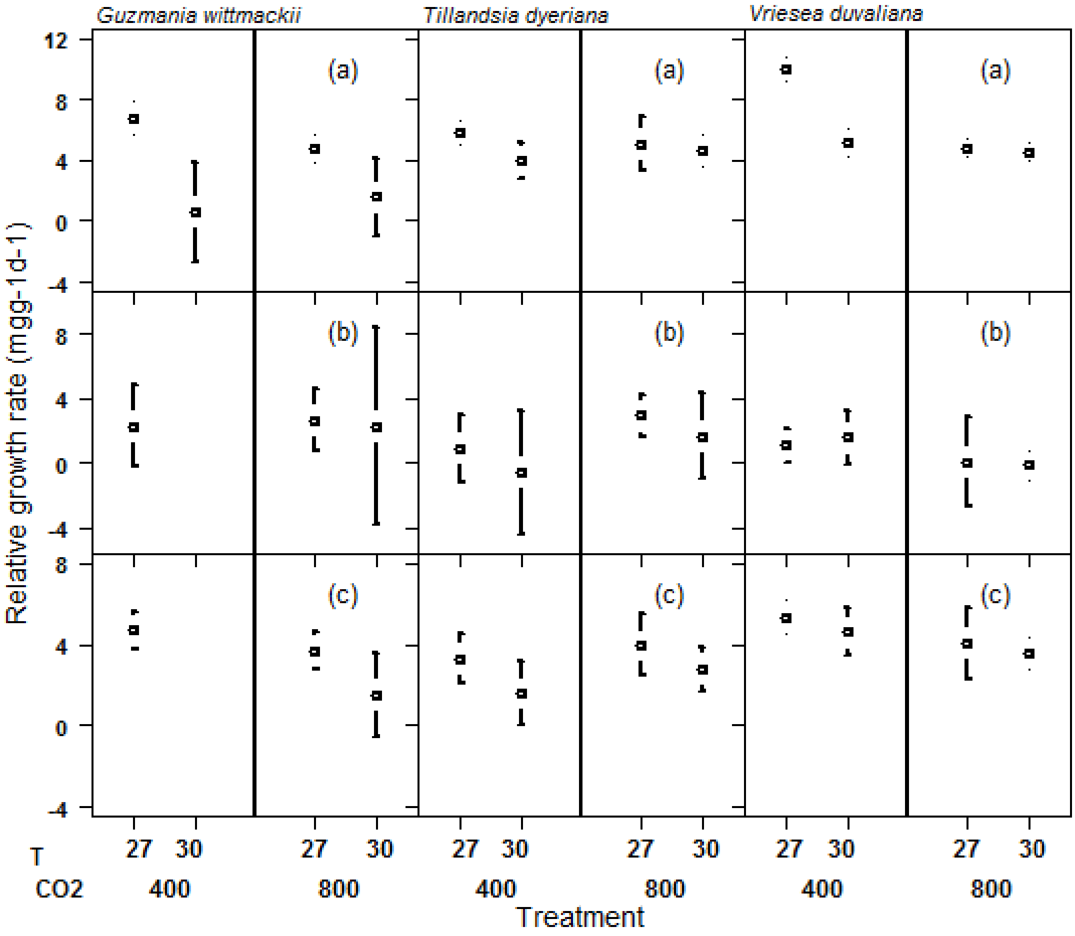


Figure 3.3. Relative growth rate (mg g-1 d-1) for three Bromeliaceae species in response to the combined effect of CO_2 and temperature (T °C) along three different harvesting times. (a). After six months (n= 10), (b). Between the sixth and the 12th month (n= 10), (c). From time 0 until the 12th month (n= 10). Data is shown as means and 95% confidence interval. For the species Exceptions for sample size (n) are in *G. wiitmackii*: 400-30 (2), 800-27 (9), 800-30 (4) in (a) & 400-30 (2), 800-27 (9), 800-30 (4) in (b); in *T. dyeriana*: 400-30 (9) in (b) & 400-30 (8) in (b); in *V. duvaliana*: 400-30 (8) in (b).

As a generality, in the first half of the treatment all species had the highest growth means at the low temperature when CO_2 was low (6.8 mg g^{-1} d^{-1} ± 2.3 (*G. wittmackii*); 5.8 mg g^{-1} d^{-1} ± 1.8 (*T. dyeriana*); 10 mg g^{-1} d^{-1} ± 1.7 (*V. duvaliana*), mean ± SD, n=20). Nonetheless, in the second half of the study the two former species had the highest growth means when grown at low temperature but elevated CO_2 (e[CO_2]) (2.7 mg g^{-1} d^{-1} ± 2.7, *G. wittmackii*; 2.9 mg g^{-1} d^{-1} ± 1.8, *T. dyeriana*). The described effects were significant only for the former species and for *V. duvaliana* just in the first half of the treatment (Table 3.4).

Table 3.4. Results of a two-way Anova on the effects of CO2 and temperature interaction on RGR at the first half of the treatment for three Bromeliaceae species

Species	Factor	df	F	P value
	CO_2	1	6.08	0.017
G. wittmackii	Temperature (T)	1	21.14	< 0.001
	Error	46		
	CO_2	1	0.006	0.94
T. dyeriana	Temperature (T)	1	3.84	0.05
	Error	77		
	CO_2	1	71.61	< 0.001
V. duvaliana	Temperature (T)	1	54.97	< 0.001
	CO_2xT	1	45.63	< 0.001
	Error	76		

*** Sample size (n) equal to 20 except at 400-30 (2) & 800-30 (8) for *G. wittmackii*.**

In the second half of the treatment, the effect of CO_2 on RGR of the species *V. duvaliana* was strongly dependent on temperature (Table 3.5, $P < 0.001$) while for *G. wittmackii* the significant effects of CO_2 ($P < 0.05$) and temperature ($P < 0.001$) were independent. At the second half of the treatment, all three species grew rather slow and reductions in RGR were relatively similar for both CO_2 treatments.

Table 3.5. Results of a two-way Anova on the effects of CO_2 and temperature interaction on RGR at the second half of the treatment for three Bromeliaceae species

Species	Factor	df	F	P value
G. wittmackii	CO_2*	1	0.047	0.083
	Error	12		
T. dyeriana	CO_2	1	3.75	0.06
	Temperature (T)	1	1.56	0.22
	Error	36		
V. duvaliana	CO_2	1	3.18	0.08
	Temperature (T)	1	0.03	0.86
	Error	37		

***The effect of the factor was analyzed in a single Anova due to the 2nd missing treatment. Sample size (n) equal to 10 except at 800-30 (4) for *G. wittmackii* & 400-30 (9) for *T. dyeriana*.**

When looking at the global results over one year of treatment, I could also notice that the species *G. wittmackii* and *T. dyeriana* grew better at the low temperature but e[CO_2] similar than at the second half of the treatments. However, the species *V. duvaliana* had the same performance than in the first half of the treatment. In *Guzmania* and *Tillandsia*, the effect of temperature on RGR was significant (Table 3.6, $P < 0.05$).

Table 3.6. Results of a two-way Anova on the effects of CO_2 and temperature interaction on RGR over one year for three Bromeliaceae species

Species	Factor	df	F	*P* value
Guzmania wittmackii	CO_2	1	3.06	0.094
	Temperature (T)	1	7.79	0.01
	Error	21		
Tillandsia deyeriana	CO_2	1	2.47	0.125
	Temperature (T)	1	5.67	0.022
	Error	36		
Vriesea duvaliana	CO_2	1	3.18	0.082
	Temperature (T)	1	0.03	0.861
	Error	37		

***Sample size (n) equal to 10 except at 400-30 for *T. dyeriana*. See Appendix 4 for RGR means per treatment over a year**

Leaf Area (LA) and Specific leaf area (SLA)

We observed a significant reduction in LA and SLA from the first to the second half of the treatment in most of the cases for the three species tested (*G. wiittmakii* (SLA, $P = 0.001$, see appendix 5), *T. dyeriana* (SLA, $P < 0.001$) and *V. duvaliana* (LA, SLA $P < 0.001$)).

Although, *T. dyeriana* was the only case in which we could notice instead a significant increase in leaf area ($P < 0.001$) from six to twelve months of treatment (Fig. 3.4). The observed responses in these two parameters were determined by a temperature effect in most of the cases (Fig.3.4 and Fig.3.5).

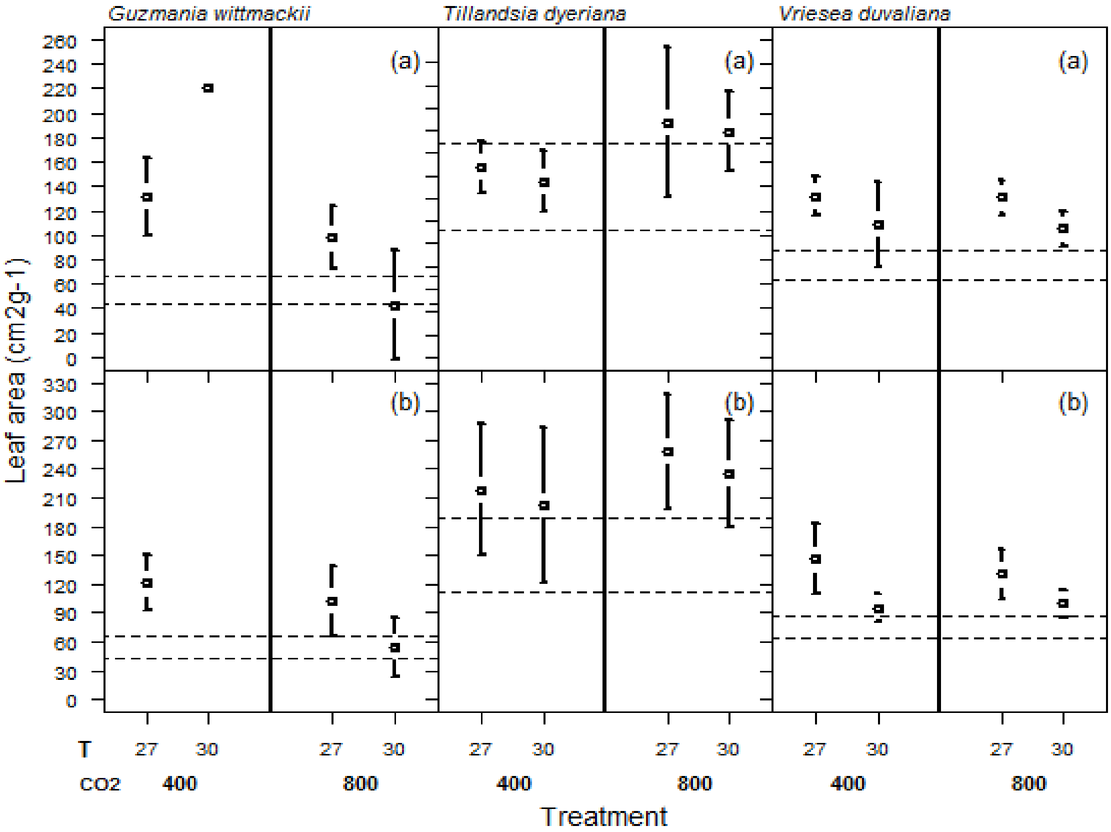


Figure 3.4. Leaf area at different temperatures (T °C) and CO_2 (ppm) treatments at two different time periods ((a) first half year, (b) second half year) for three Bromeliaceae species. The dashed lines represent the 95% confidence interval of leaf area for initial samples (n=10). Values for the different treatments are shown as means and confidence interval (n=10 in (a) first half year and n=5 in (b) second half year). Exceptions for sample size (n) are in *G. wiitmackii*: 400-30 (2), 800-27 (9), 800-30 (4) in (a) & 400-30 (2), 800-27 (9), 800-30 (4) in (b); in *T. dyeriana*: 400-30 (9) in (b) & 400-30 (8) in (b); in *V. duvaliana*: 400-30 (8) in (b).

For example, leaves of the species *G. wittmackii* and *V. duvaliana* were significantly smaller when grown a high temperature than at the lower one at both treatment times for the former species ($P < 0.05$) and, only at the first half for the species *Vriesea* (Table 3.7, $P < 005$). For SLA, we found that it was significantly higher in plants grown at the high temperature treatments for the species *G. wittmackii* at the first treatment half (Table 3.7, $P < 0.05$) and *T. dyeriana* at the second treatment half ($P < 0.05$).

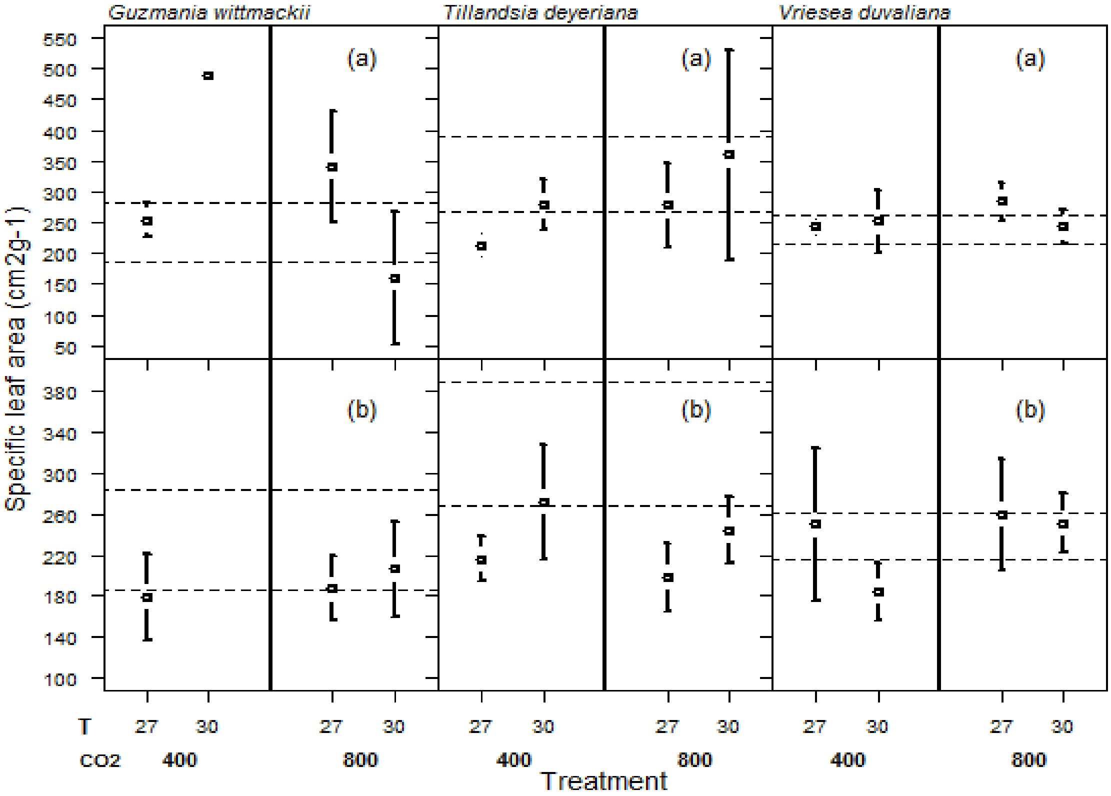


Figure 3.5. Specific leaf area at different temperatures (T °C) and CO_2 (ppm) treatments at two different time periods ((a) first half year, (b) second half year) for three Bromeliaceae species. The dashed lines represent the 95% confidence interval of leaf area for initial samples (n=10=). Values for the different treatments are shown as means and confidence interval (n=10 in (a) and n=5 in (b) second half year). Exceptions for sample size (n) are in *G. wiitmackii*: 400-30 (2), 800-27 (9), 800-30 (4) in (a) & 400-30 (2), 800-27 (9), 800-30 (4) in (b); in *T. dyeriana*: 400-30 (9) in (b) & 400-30 (8) in (b); in *V. duvaliana*: 400-30 (8) in (b).

A CO_2 effect could only be observed on LA of the species *T. dyeriana* at the first treatment half ($P < 0.05$) and on SLA of *V. duvaliana* at the second treatment half ($P < 0.05$). The species *T. dyeriana* had larger leaves and higher SLA when plants were grown at elevated CO_2.

Table 3.7. Results of a two-way-Anova for Leaf area (LA) and specific leaf area (SLA) of three Bromeliaceae species (n= 5 for all except where indicated*)

Species	Time	Variable	Effect	Df	F value	*P*
Guzmania wittmackii	6 months	LA	CO_2	1	11.4	< 0.01
400-30 (n= 1)*			Temperature	1	0.9	> 0.05
800-30 (n=4)			CO_2 x T	1	9.7	< 0.01
			Error	20		
		SLA	CO_2	1	0.61	> 0.05
			Temperature	1	3.22	0.087
			CO_2 x T	1	17.41	< 0.001
			Error	20		
	12 months	LA	Temperature	1	6.2	< 0.05
800-30 (n=4)			Error	7		
		SLA	CO_2	1	11.51	< 0.01
			Error	12		
Tillandsia dyeriana	6 months	LA	CO_2	1	5.37	< 0.05
			Error	37		
		SLA	CO_2	1	2.98	< 0.1
			Temperature	1	3.05	0.08
			Error	36		
	12 months	LA	CO_2	1	0.03	> 0.05
400-27 (n=6)			Temperature	1	1.8	> 0.05
400-30 (n=6)			Error	17		
800-27 (n=8)						
800-30 (n=7)		SLA	CO_2	1	3.55	> 0.1
			Temperature	1	9.96	< 0.01
			Error	17		
Vriesea duvaliana	6 months	LA	CO_2	1	6.87	> 0.05
400-27 (n=7)			Error	38		
400-30 (n=7)						
800-27 (n=6)		SLA	CO_2	1	1.12	> 0.05
			Temperature	1	1.14	> 0.05
			CO2 x T	1	2.9	0.1
			Error	36		
	12 months	LA	CO_2	1	0.42	> 0.05
			Temperature	1	2.29	> 0.05
			Error	17		
		SLA	CO_2	1	6.3	< 0.05
			Error	18		

Plant biochemistry

Comparison of the two treatment periods showed a significant reduction in nitrogen (N) concentration and content together with an increased total non-structural carbohydrate (NSC) concentration in most of the cases for the three species under study by the second half of the treatment (Fig. 3.7 & Fig.3.6, respectively). Differences between time periods were highly significant in N concentration for *G. wittmackii*, *T. dyeriana* and *V. duvaliana* ($P < 0.001$, see appendix 6), in N content only for *T. dyeriana* ($P < 0.001$) and in NSC concentration for *T. dyeriana* and *V. duvaliana* ($P < 0.001$).

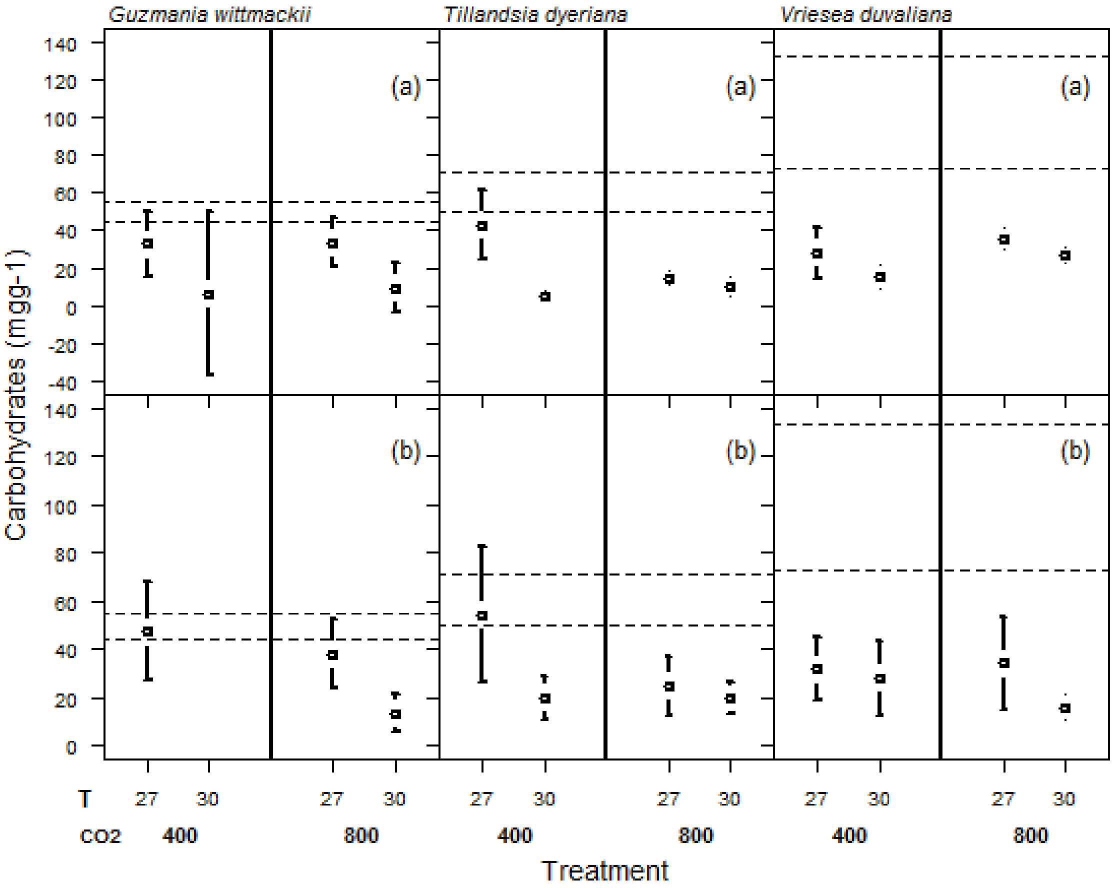


Figure 3.6. Total carbohydrates content at different temperatures (T °C) and CO_2 (ppm) treatments at two different time periods ((a) first half year, (b) second half year) for three Bromeliaceae species. The dashed lines represent the 95% confidence interval of carbohydrates content of initial samples. Values for the different treatments are shown as means and confidence interval (n=10 in initial samples, in (a) and (b) n=5, except for *G. wittmackii* at 400-30 (n=1) and 800-30 (n=4)).

When looking at the four combined treatments at each time period I found significantly higher N concentration at the high temperature treatments by the second time period for the three species (*G. wittmakii* (Table. 3.8, $P < 0.05$), *T. dyeriana* ($P < 0.05$), and *V. duvaliana* ($P < 0.001$)), but at the first time period, means were significantly higher just in *G. wittmakii* ($P <$

0.001) and *T. dyeriana* ($P < 0.05$). For the last species, it was also found a highly significant effect of CO_2 at the first half of the treatment ($P < 0.001$), with a higher N concentration in plants grown at the 800ppm, particularly at the 30°C treatment.

A significant reduction in N content per dry mass was found in the species *G. wittmakii* and *T. dyeriana* at the second half of the treatment ($P < 0.05$), particularly, in *G. wittmakii* individuals treated with e[CO_2] and 30°C. Additionally, in *G. wittmakii*, plants grown at the a[CO_2] and 27°C had consistently higher N content at both treatment times while in *T. dyeriana*, I observed that plants grown at the e[CO_2] treatments had a higher N content ($P <$ 0.05, at first half).

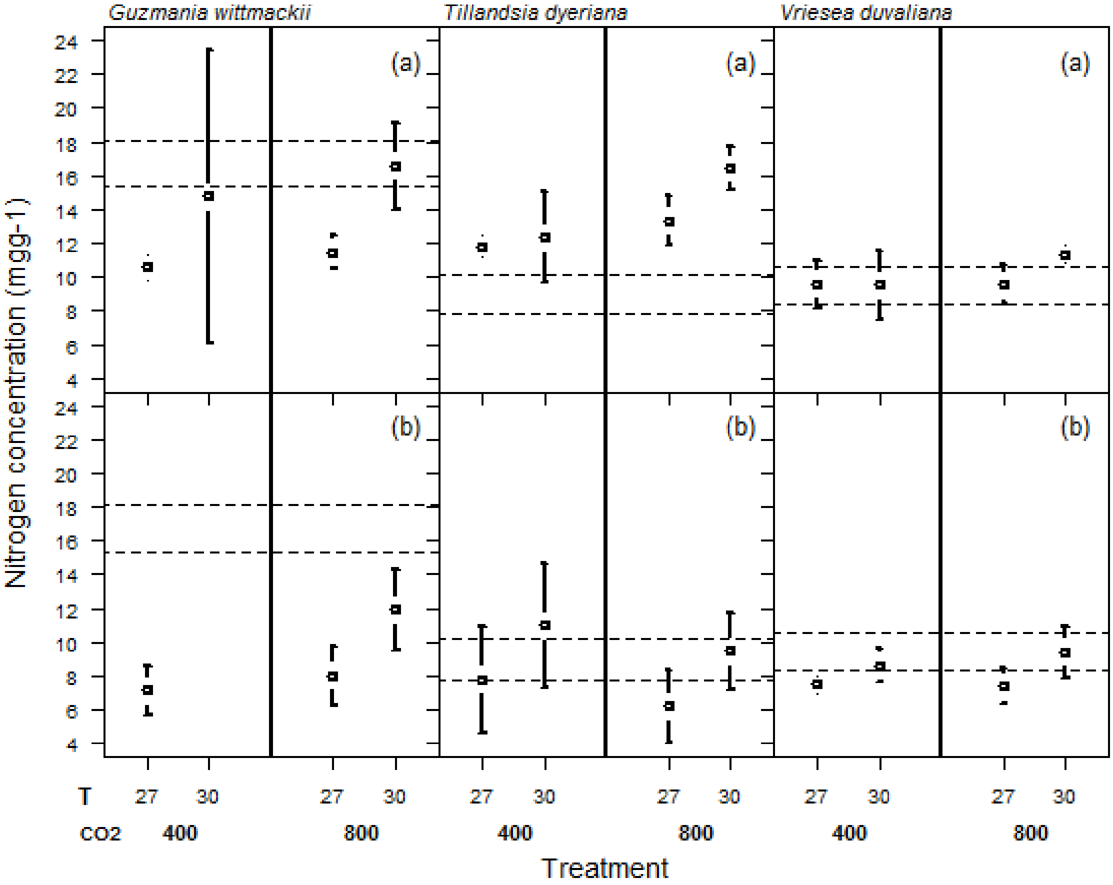


Figure 3.7. Nitrogen concentration at different temperatures (T °C) and CO_2 (ppm) treatments at two different time periods ((a) first half year, (b) second half year) for three Bromeliaceae species. The dashed lines represent the 95% confidence interval of nitrogen concentration for initial samples. Values for the different treatments are shown as means and confidence interval (n=10 in (a) and initial time and n=5 in (b) second half year).

Concentration of NSC was significantly determined by the interactive effect of CO_2 and temperature ($P < 0.001$) in the species *T. dyeriana* (Fig. 3.6), plants grown at 400ppm and 27°C had the highest concentration means at both treatment times (first half: $P < 0.001$ and second half: $P < 0.05$). In the other two species, *G. wittmackii* and *V. duvaliana*, NSC concentration was significantly influenced by temperature at both time periods ($P < 0.05$, in the three studied cases), plants grown at the lower temperature had the higher concentration means. For *Vriesea duvaliana*, NSC concentration was even higher in plants grown at a CO_2 enriched atmosphere during the first treatment half ($P < 0.05$).

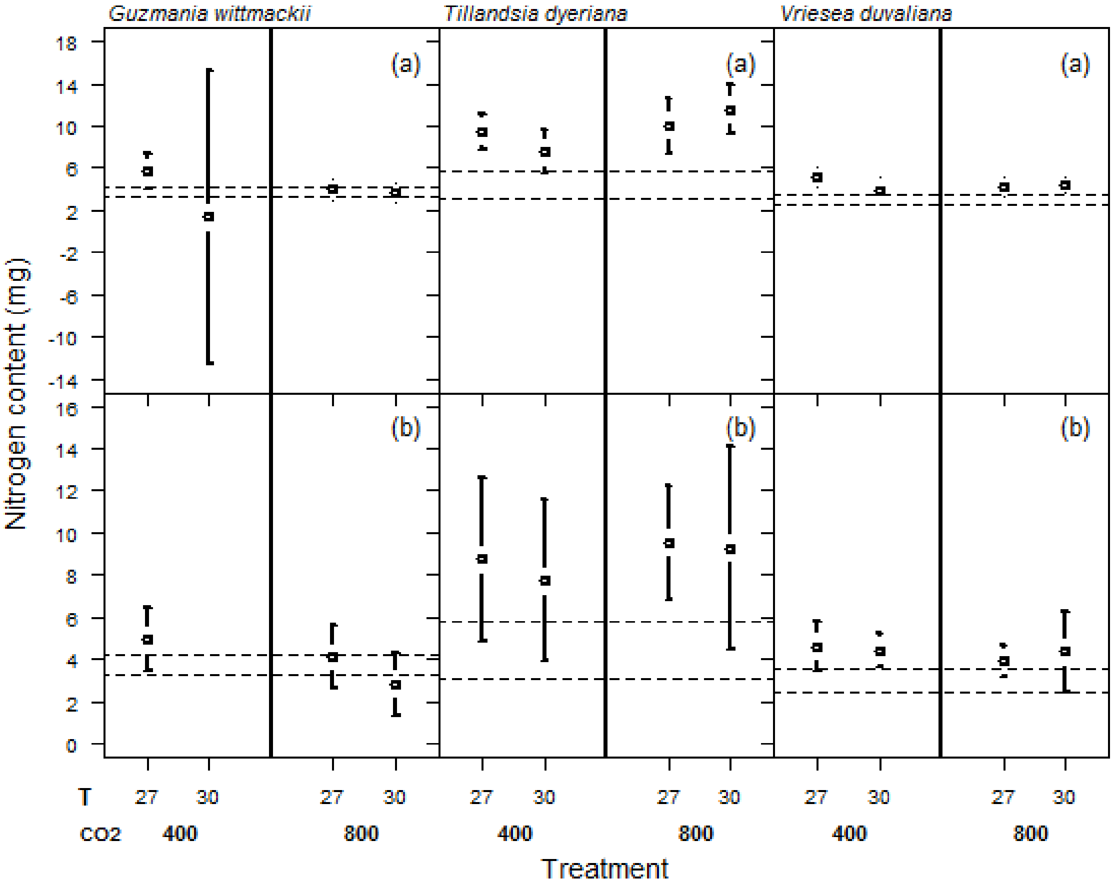


Figure 3.8. Nitrogen content at different temperatures (T °C) and CO_2 (ppm) treatments at two different time periods ((a) first half year, (b) second half year) for three Bromeliaceae species. The dashed lines represent the 95% confidence interval of nitrogen content for initial samples. Values for the different treatments are shown as means and confidence interval (n=10 in (a) and initial time and n=5 in (b) second half year, see more detail in Table 3.8).

At the beginning of the experiment, glucose constituted the highest fraction of total NSC carbohydrates in leaves of the three species tested (33.1% (± 10.87; *G. wittmackii*), 41.89% (± 12.71.; *T. dyeriana*), 39.07% (± 12.50; *V. duvaliana*), though in leaves of *G. witmackii* there

Table 3.8. Results for leaves biochemistry (N concentration and content and, carbohydrates concentration) of three Bromeliaceae species.

Species	Time	Variable	Effect	Df	F value	*P*
Guzmania wittmackii	6 months	N concentration	Temperature	1	16	< 0.01
			Error	7		
		N content	CO_2	1	3.4	0.08
			Error	22		
		[Carbohydrates]	Temperature	1	33.4	< 0.001
			Error	14		
	12 months	N concentration	Temperature	1	15.4	< 0.01
			Error	7		
		N content	Temperature	1	2.37	> 0.05
			Error	7		
		[Carbohydrates]	Temperature	1	15.1	< 0.01
			Error	7		
Tillandsia dyeriana	6 months	N concentration	CO_2	1	12.9	< 0.001
			Temperature	1	5.3	< 0.05
			Error	36		
		N content	CO_2	1	5.34	< 0.05
			Temperature	1	0.1	> 0.05
			CO_2 x T	1	3.04	0.09
			Error	35		
		[Carbohydrates]	CO_2	1	10.71	< 0.01
			Temperature	1	42.9	< 0.001
			CO_2 x T	1	24.7	< 0.001
			Error	17		
	12 months	N concentration	Temperature	1		
			Error	18		
		N content	CO_2	1	0.73	> 0.05
			Temperature	1	0.23	> 0.05
			Error	17		
		[Carbohydrates]	CO_2	1	6.4	< 0.05
			Temperature	1	11.3	< 0.01
			CO_2x T	1	5.9	< 0.05
			Error	16		

Continuation Table 3.8. Results for leaves biochemistry (N concentration and content and, carbohydrates concentration) of three Bromeliaceae species.

Species	Time	Variable	Effect	Df	F value	*P*

Vriesea duvaliana	6 months	N concentration	CO_2	1	1.95	> 0.05
			Temperature	1	1.88	> 0.05
			Error	37		
		N content	CO_2	1	0.14	> 0.05
			Temperature	1	1.7	> 0.05
			Error	37		
		[Carbohydrates]	CO_2	1	9.5	< 0.01
			Temperature	1	12.8	< 0.01
			Error	17		
	12 months	N concentration	Temperature	1	16.7	< 0.001
			Error	18		
		N content	CO_2	1	0.73	> 0.05
			Temperature	1	0.09	> 0.05
			Error	17		
		[Carbohydrates]	CO_2	1	0.91	> 0.05
			Temperature	1	4.69	< 0.05
			CO_2 x T	1	2.16	> 0.05
			Error	17		

***Sample size n= 10 for initial samples and for the rest of the treatment conditions and periods except for: *G. wittmackii* at 400-30 (2), 800-27 (4) after 6 months &400-27 (5), 400-30 (1), 800-27 (5), 800-30 (4) after 12 months; *T. deyeriana* at 800-30 (9) after 6 months & all treatments after 12 months with n = 5; *V. duvaliana* all treatments after 12 months with n = 5.**

was still a higher concentration of sucrose (38.70% ± 10.25). Fructose and starch also represented a high fraction of the total in the species *T. dyeriana* (starch, 38.19% ± 14.60) and *V. duvaliana* (fructose, 27.20% ± 8.48 and starch, 25.19% ± 20.29) whereas maltose comprises the lowest fraction of the total in the three species with means ranging from 0.26% to 0.85% (± s.e.). From all soluble sugars maltose was found in low concentration over the whole experiment, but it reached higher means in the low temperature (0.30%-1.10%)) and a[CO_2] (0.30%-2.10%) treatments. Glucose, fructose and sucrose constituted a high proportion of the total NSC fraction in plants grown at an enriched CO_2 atmosphere, they accounted for 13%-46% (± 3-5%), 8%-30% (2± .5-7%) and 4%- 68% (± 1-3%.), respectively. The last two soluble sugars were also highly concentrated at the high temperature treatments constituting 12%-30% (± 3.80-6.80%) and 13%-68% (± 15-3%) of the total fraction. Finally, glucose together with starch was especially concentrated at the low temperature treatments conforming 20%-46% (± 5.77-5.08%) and 14%-82% (± 8-9%) of total NSC, respectively.

There was an obvious reduction in fluorescence potential after six and 12 months in most of the treatments for the three studied species (Fig. 3.9). However, values were still closer to the optimum usually reported for non-stressed plants which ranges between 0.7 and 0.80 (see Maxwell & Johson 2000). Fluorescence values of the species *T. dyeriana* and *V. duvaliana* were significantly higher at the e[CO_2] treatments at both time periods (Table 3.9, $P < 0.001$ and $P < 0.05$, respectively). For *T. dyeriana*, fluorescence means were slightly higher in this same CO_2 treatment when temperature was 3°C higher. Nonetheless, for the species *V. duvaliana*, fluorescence means were slightly lower in this same treatment already after six months, though after the second half plants grown under 30°C and 800ppm had the highest fluorescence mean ($P < 0.05$). The species *G. wittmackii* was strongly affected by temperature at both time periods. Plants grown at 30°C had fluorescence means below 0.7 already after six months of treatment and they were significantly lower than that of plants grown at 27°C ($P < 0.001$). At the second half of the treatment *G. wittmackii* had a higher photochemical efficiency in plants grown at 800ppm and high temperature comparable to that shown by plants growing at 400ppm and 27°C.

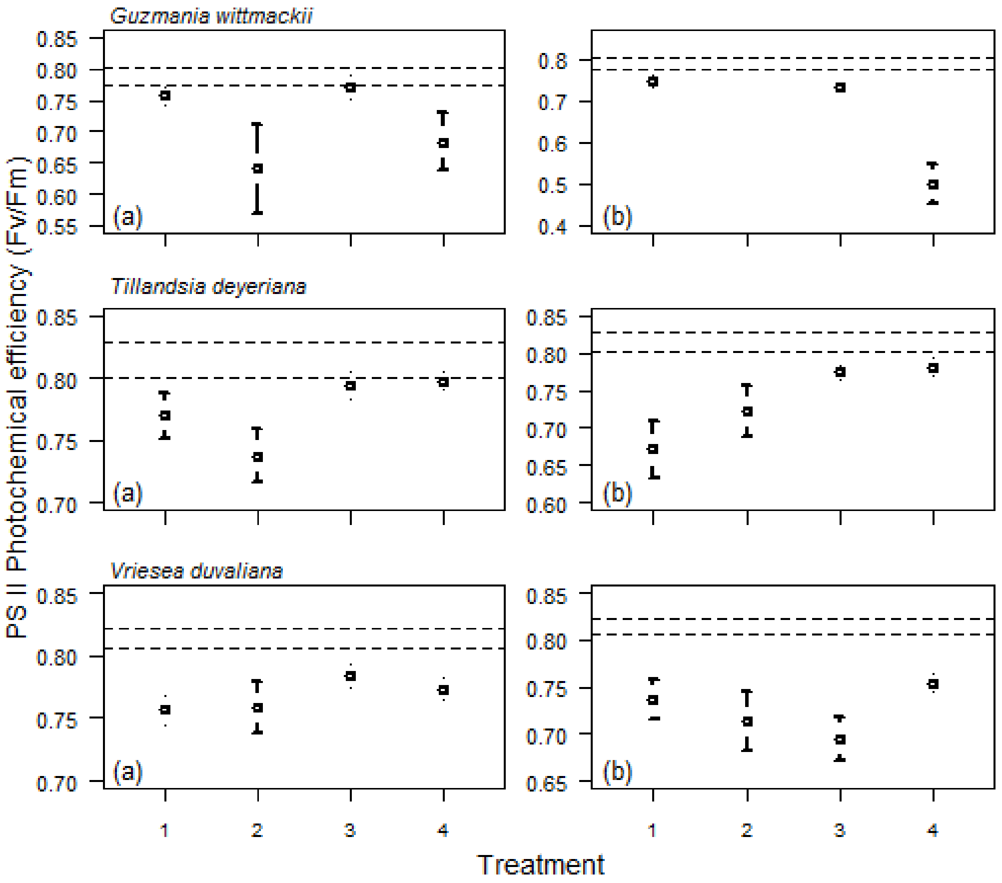


Figure 3.9. Chlorophyll fluorescence of three Bromeliaceae species in response to four different combined CO_2 and temperature treatments: 1- 400ppm-27°C, 2- 400ppm-30°C, 3-800ppm-27°C, 4-800ppm-27°C. Letters in parenthesis indicates: (a) fluorescence measured after six months of treatment and (b) fluorescence measured after twelve months of treatment. The dashed lines correspond to the 95% confidence interval of Fv/Fm measured at the beginning of the experiment. Data for the different treatments are shown as means and confidence interval. Sample size (n) equal

to 10 for initial samples and for the different treatments at the different time periods was also 10 except for *G. wittmackii* at 400-30 (2), 800-27 (5) & 800-30 (8) after 6 months & 800-30 (4) after 12 months. For *T. duvaliana* n= 9 at 400-27 & 400-30 after 12 months and for *V. duvaliana* n=9 at 800-27 after 6 & 12 months.

DISCUSSION

Epiphytes live in stressful environments governed by a diverse range of microclimatic conditions (Benzing 1990), intermittent water supply and low nutrient availability (Benzing 2000). All these features together with the rather instability of the growing substrate in which they are found could contribute to the frequently observed slow growth rates and limit their survival under natural conditions. Schmidt and Zotz (2002) also reported high mortality percentages in medium size individuals - of similar size to the ones used in the present study – of the bromeliad species *V. sanguinolenta* between 24 and 35%, in a lowland forest in Panama, possibly related to the higher evaporative demand during that particularly growing year.

The significantly higher mortality that I observed in the species *G. wittmackii* of up to 90% at the 400ppm and 30°C treatment and, simultaneously higher survival of individuals grown at the same temperature but e[CO_2], could also indicate a higher evaporative demand with an only 3°C increase in temperature. This increase evaporative loss could have possibly overcame the biological limits of this species, which could not be ameliorated at the a[CO_2] whilst in an enriched CO_2 scenario it could. Kriedemann, Sward and Downton (1976) reported a similar result than us for neotropical tree species, avocado and papaya plants grown at high temperature and a[CO_2] showed accelerated senescence or even died but they succeeded in e[CO_2], though the temperatures they used were 7°C to 10°C higher than the ones I used and their e[CO_2] treatment had a higher level (1200-1300ppm) as well.

Mean RGRs along species and treatments over one year were in accordance with the ranges found in nature and the reported for laboratory studies with epiphyte species. For instance, Laube and Zotz (2003) reported mean RGR of medium-size individuals – from the same size than ours – grown *in situ* between 1.56 - 4.7 mg g^{-1} d^{-1} and Monteiro, Zotz and Körner (2009) obtained an overall mean across treatments of 2.8 mg g^{-1} d^{-1} in plants grown in growth chambers. Additionally, Schmidt and Zotz (2002) found a mean RGR of 1.8 mg g^{-1} d^{-1} for the species *V. sanguinolenta* in *in situ* conditions against a mean of 6.9 mg g^{-1} d^{-1} in a greenhouse setting for the same species. They attributed the higher means in the controlled greenhouse environment to the higher availability of resources.

In this study, I ensured adequate water and nutrient supply to the plants; hence, the slightly higher growth means that I obtained in my study in comparison to the observed in nature could be attributed to the conditions of the study as well. Though, I had an additional axe of variation since I subjected the plants to different CO_2 treatments in order to test if there was a stimulating effect of e[CO_2] on RGR compare to a[CO_2].

Unexpectedly all species grew better at the a[CO_2] than at the elevated one in the first half of the treatment. The growth-enhancing effect of e[CO_2] was perceivable just at the second half of the treatment, at least for two of the species (*G. wittmackii* and *T. dyeriana*). Regardless the much higher growth means of *G. wittmackii* and *T. dyeriana* in this treatment period at e[CO_2] compared to the means at the a[CO_2], they were still much lower than the observed in the first treatment half. Monteiro, Zotz and Körner (2009) also found a low stimulation of just 5.5% of e[CO_2] (560ppm) compared to pre-industrial [CO_2] on RGR of six epiphyte species; they considered the influence of other variables on CO_2 responses, as light and nutrient availability or even excess watering (in case of negative response in the species *Tillandsia fasciculata*) as the possible cause for the observed response.

On the other hand, the results from Hättenschwiler and Körner (1996) on the effects of CO_2 on three understory slow-growing species did show a significant effect with a twice higher biomass in plants grown at doubled CO_2 (420 ppm) compared to pre-industrial (280 ppm), but no further stimulation at 560ppm. Granados and Körner (2002) also found a non-linear increase in biomass in response to CO_2 enrichment in tropical climbing plants. They used three CO_2 concentrations (280ppm, 420ppm, 560ppm and 700ppm) and found that biomass stimulation was almost +50% between 280 and 420ppm whilst it was just +23% from 420 to 560ppm and the response leveled off at 700ppm.

Hättenschwiler and Körner (1996) based on these non-linear responses suggested that stimulating effect of [CO_2] is reaching its maximum or even, that it has already been reached at the current [CO_2] and, concluded saying that the responses in a future CO_2 scenario may be smaller in the understory species that they used in their study. In my study, the actual [CO_2] to which the plants were subjected (487.5 ± 49.3 ppm and 755 ± 29ppm) are between the ranges used in the two previously described studies. The a[CO_2] treatment is already in the no-response interval from the first study and in the lower-response interval from the second study whilst the eCO_2 treatment is already over the maximum limit used in the second study. Certainly, it could make comparisons of these studies a bit confusing together with the fact that the species are different. Nonetheless, if we only take into account the general responses to the range of [CO_2], we can take two important issues: 1- the current [CO_2] may be already saturating for some plants species and, 2- Acclimation after prolonged exposure to e[CO_2] could also be a plausible consequence in some species. Epiphyte species tested in this study showed a higher respond to a[CO_2] instead and, the usually reported stimulatory effect of e[CO2] took much longer to be perceived, though, the respond was still low This last occurrence just show that respond of epiphyte to the upcoming increases in atmospheric [CO_2] may take place at a very slow rate due to the conservative nature of these species, whereby a possible acclimation respond may be seen much later in time. A study by Monteiro, Zotz and Körner (2009) could further support this notion since the epiphyte species they tested also showed such a low overall response to the e[CO_2] of just 560ppm.

Plants living in rather stressful habitats may develop a series of strategies that allow them to survive in these environments. For instances, Loehle (1995) made the observation that some plants inhabiting impoverished environments could not respond to enrichment whether of nutrients or water and called this a risk-averse growth strategy. This could be very similar to the stress-tolerance strategy seen, for example, in desert cacti and agave species, which can survive prolonged dry periods as seedlings (Jordan & Nobel 1979), as well in epiphytic bromeliad species whose germination response depended on duration rather than on frequency of wet periods (Correa & Zotz 2014). Thus, species avoid responding or show a slow respond even though conditions are suitable. As referring to CO_2, Loehle (1995) predicted that species that display a risk-averse strategy will respond little or not at all to CO_2.

The epiphyte species we tested seem to perform a risk-averse strategy since they showed a CO_2 stimulation just at the second half of the treatment, but the response was low; hence it emphasizes the high conservatism of epiphytic bromeliads in front of environmental changes mostly due to their rather stress-tolerance behavior. Growth responses over the 12 months of treatments showed comparable growth means at both CO_2 levels, at least for the species *T. dyeriana* and *V. duvaliana*, hence we could expect that in an enriched CO_2 atmosphere RGR of epiphyte species experience a brief and delayed stimulation of e[CO_2] and a slow return to the growth rates similar to those of plants growing at the current CO_2 level such as it has been found in other studies (Wong 1993; Fonseca, Den Hertog & Stulen 1996).

I also expected an enhancement of e[CO_2] effect on RGR when species were grown at a high temperature but it was not the case for any of the species. They grew better at the low temperature treatments at both [CO_2] and treatment periods. An increase in just 3°C in tropical temperatures would already result critical for these epiphyte species in a future climate change scenario and, an increase in CO_2 seems not to improve the plant tolerance to heat-stress as suggested for some authors (Gifford 1989; Rawson 1992). At higher temperatures increase the evaporative demand of the plant, a transpiration increase which causes water losses. Thus, the effect of high temperature on plants is directly or indirectly related to water stress and reaffirm the importance of water for these species as a limiting factor.

Temperature affects a range of enzymatically catalyzed and membrane-associated processes in the plant and, it is a major factor affecting plant distribution (Lambers, Chapin III & Pons 2008). For instances, obeying to the predicted changes in the global temperature patterns and [CO_2] the competitive balance and ecological distributions of C_3 and C_4 species is expected to change (Hogan, Smith & Ziska 1991). At high temperatures the oxygenase properties of Rubisco increase more than the carboxylase one, which is in partly due to reduced solubility of CO_2 compared to that of O_2. This would cause photorespiration to become more important. Thus, responding to the day-carbon-fixation mechanism of C_3 plants, their performance is expected to be negatively affected with a temperature increase. But, CO_2 uptake stimulation may be enhanced by an enriched CO_2 atmosphere under higher temperatures (Morison & Lawlor 1999), which could in turn generate higher growth means (Monteiro, Zotz & Körner 2009). Nevertheless, the three C_3 species that I tested in my study were neither stimulated by e[CO_2] nor by higher temperature as was observed for several crop species grown at 33/28°C and higher and e[CO_2] when compared to plants grown at 23/18°C (growth modification factor 1.29 vs. 1.49). Additionally, shoot dry weight of a C_3 crop species (cotton) was significantly enhanced by CO_2 enrichment only at higher temperature (Rawson 1992).

Variation in growth rate with temperature is also associated with changes in plant carbon balance. Usually, there is a reduction in carbon reserves due to increased respiration under a warmer environment. These carbon losses through plant respiration may set a competition between this process and continuous vegetative development. Hence, a negative impact on growth rates due to increased respiration at the higher temperature may be expected. In my study, particularly the species *G. wittmackii* suffered a significant decrease in its RGR already at the first half of the treatment at the warmer growing conditions, which caused a high mortality of the individuals. Growth reductions and increased mortality may have been determined by parallel and significant reductions in NSC concentration in leaves of plants grown at 30°C. Nonetheless, an e[CO_2] did seem to have confer tolerance to individuals of the species *G. wittmackii* grown at the warmer environment in comparison to those grown at the ambient environment. However, this positive effect was not so pronounced as expected.

On the other side, nitrogen (N) concentration was also higher in plants grown at the high temperature treatments, especially in the species *G. wittmackii*. This may be related to a high concentration of N-based proteins or enzymes such as those involved in the photosynthetic and respiratory processes. Rubisco is the enzyme in charge of carboxylation and oxygentation activities and, this enzyme may account for more than 60% of the total N present in leaves of plants. Hence, in the present study, the higher concentration of N found in leaves of plants grown in the high temperature treatments may correspond to the dominant presence of Rubisco.

In spite of the low respond to e[CO_2] at the bigger scale of growth, at a finer scale such as the photochemical level, respond of epiphytes is conspicuous. Epiphytic bromeliads grown at the e[CO_2] treatment and high temperature had higher values of photochemical efficiency than those plants grown under high temperature but a[CO_2]. Photochemical efficiency of unstressed plants is usually close to 0.83 (Maxwell & Johnson 2000), here plants grown at high temperature under the elevated CO_2 environment had fluorescence values between 0.7 and 0.8 whereas those at most of the other treatments had much lower values. Hence, an e[CO_2] could be advantageous for fighting against the stress generated by future warmer atmosphere at the scale of cells reactions by preventing a significant oxidative damage. Furthermore, species-specificity was also clearly reflected in the fluorescence, e.g., the species *G. wittmackii* showed to be the most sensitive species to a 3°C increased in temperature with much higher values of fluorescence at the low temperature treatments instead, at both [CO_2]s. Estimated field temperatures experienced by this species are between 20.74°C ± 0.3 (mean minimum ± sd, n= 12, temperature estimation done following method by Müller, Albach and Zotz (2017)) and 30.42°C ± 0.51 (mean maximum ± sd, n= 12). The mean maximum temperature of ca. 30°C experienced by this species in field conditions, which is similar to the high temperature treatment (+3°C) at the present study, suggest that this species should do relatively well at that treatment, however, it was not the case. Certainly, this species was previously growing in greenhouse conditions were temperature may have been set at a constant of ca. 25°C during the growing period similar to the other two species tested in this study. *Guzmania wittmackii* could have undergone a stronger priming effect, which decreased its resistant to higher temperature in relationship to the other two species.

Field temperature estimations for the three studied species correspond to just one location in South America (Colombia) and this species has a larger distributional range that may differ a bit in climatic conditions. Information on the provenance of the species is missing. But, the behavior of *G. wittmackii* may be telling that some of the strongly intrinsic features of plant species remain in spite of the changing environment or, at least, it could take a more extended period in order to be transformed or eradicated. Epiphytes seem to be species with especially slow turnover rate, in particular, the epiphytic bromeliads.

Finally, it is important to do some comments on the functioning of the custom-built chambers used to carry out the experiment of the present study. As it was shown in Table 3.1, there were certain discrepancies between the values set and the actual values of [CO_2]. But, there were also some discrepancies in the set temperatures and some errors that occurred along the whole time frame of the study. For instances, in a couple of occasions plants were subjected for days or a week to very low temperatures because of an error in the temperature sensors. During the experimental period temperature inside the chambers reached much higher values than the maximum desired at the present study. All this together may have caused some noise in the data. However, apparent extreme endurance or even high "stress-plasticity" of epiphytes to survive through different stress conditions seems to have, partly, left aside any deviation of responses from the relatively usual expectation. For instances the conservatism, already seen at the level of seeds by Müller, Albach and Zotz (2017) was still remarkable in the young adult plants tested here.

CONCLUDING REMARKS

Epiphytic bromeliads resulted very sensitive to an increase of just 3°C in temperature and showed a better performance at the low temperature treatments at both CO_2 concentrations. However, the performance was, unexpectedly, much better at the ambient CO_2 concentration.

The stimulatory effect of an enriched CO_2 atmosphere was perceivable only from the 6th month on, but the respond was still rather low. Hence, epiphytic bromeliads seem to benefit little from an enriched CO_2 environment. Nonetheless, the pronounced species-specificity of the responses in front of the two factors tested was noticeable, which may have important implications in biodiversity of epiphyte in a future climate change scenario.

Carbohydrates and N concentrations may be negatively influenced by growth under the future warmer scenario due to increased respiratory activity. This may significantly compromise growth and development of these plant species with consequent repercussions on population dynamics.

CHAPTER IV.

EFFECT OF CO_2 AND NUTRIENT CONCENTRATION ON REPRODUCTION OF EPIPHYTIC BROMELIADS

INTRODUCTION

Flowering phenology in tropical plants has been associated for more than three decades with seasonal patterns in climate or the availability of pollinators (Frankie, Baker & Opler 1974; Opler, Frankie & Baker 1976; Stiles 1977; Auspurger 1981; Ackerman & Montalvo 1985; Rathcke & Lacey 1985; Van Schaik, Terborgh & Wright 1993; Wright 1996; Borchert *et al.* 2005). Among the environmental factors often considered as main modulators of flowering phenology are temperature, photoperiod and moisture (Rathcke & Lacey 1985; Wright & Van Schaik 1994; Prasad, Allen & Boote 2005). The expectation of a future CO_2 enriched atmosphere should be also of great concern when it comes to flowering phenology since increases in the concentration of this gas may also influence the global patterns of temperature and moisture (Solomon *et al.* 2007). Variations in carbon availability itself could also have direct effects on phenological patterns since increases in atmospheric CO_2 concentration has the potential to alter the strength, timing and direction of plant biomass allocation patterns (Hovende *et al.* 2008). Additionally, changes in plant productivity (Navas *et al.* 1995) and metabolic processes such water-use-efficiency (Jackson *et al.* 1994) due to increases in CO_2 concentration ($[CO_2]$) lead to consider the possibility of future changes in reproductive output of plant species (Hovende *et al.* 2008).

For instance, carbon availability influenced the rate of flower development in a herbaceous (*Sinapsis alba*) species and even altered its photoperiodic requirement for flowering (Bodson *et al.* 1977). Advancement of spring leaf and flowering initiation in middle and higher latitudes was related to a possible effect of anthropogenic climate change and associated raise of temperatures (Badeck *et al.* 2004). These changes parallel the advanced drawdown of atmospheric CO_2 in spring/summer. Elevated CO_2 concentration ($e[CO_2]$) was also found to induce earlier flowering in a tree species and the percentage of flowering individuals was increased over the whole flowering period (Grünzweig & Körner 2003). Jablonski, Wang and Curtis (2002) carried out a meta-analysis with data of 79 crop and wild species to study the effect of e[CO_2] on reproduction and found that CO_2 enrichment caused increases in number of flowers, fruits and seeds in all species tested. However, the degree of responsiveness to elevated CO_2 was still dependent on the species and functional groups. On the other hand, Hovende *et al.* (2008) observed no response of e[CO_2] on flowering commencement in temperate grassland species and concluded it was unlikely that a future enriched atmosphere could have any influence on flowering times. These authors pointed out that the poor nutrient availability dominating in the study area may have limited the capacity of the studied species to benefit from the enriched CO_2 environment; which confirms earlier suggestions by Körner (2003; 2006). It has been previously observed that growth under an enriched CO_2 atmosphere may increase nutritional demands of plant individuals (Tuba & Lichtenthaler 2007). Particularly, decreases in nitrogen concentration have been usually observed after long-term exposure to e[CO_2] (Lincoln, Couvet & Sionit 1986; Osbrink, Trumble & Wagner 1987; Fajer 1989; Johnson & Lincoln 1990; Lindroth, Arteel & Kinneay 1995).

Tropical forests grow on relatively nutrient-poor soils, partly consequence of highly washed substrates (Nye & Greenland 1960). Epiphytes are common dwellers of these forests canopies which are also known to be rather oligotrophic habitats (Benzing 1973). Hence, epiphytes have always been reported as limited by low nutrient availability *in situ* and macronutrients as nitrogen and/or phosphorus pointed out to be as the most limiting (Zotz 2004; Zotz & Asshoff 2010). In order to achieve an "efficient" nutrient economy and survive in these impoverish environments epiphytes display a variety of physiological, morphological and reproductive strategies. For instances, inherently slow growth rates (Schmidt & Zotz 2001), mono- and polycarpy (Benzing & Renfrow 1971), presence of trichomes (Benzing & Burt; Benzing 1973), particularly abundant in plants with atmospheric strategy, and exploitation of secondary nutrient sources as those accumulated in the water-nutrient impoundments called tanks hold by some bromeliad species (Benzing 1970; Benzing & Burt 1970). Temperature increased as a consequence of global warming could lead to higher decomposition and mineralization rates, which may in turn increased nutrient availability in the environment (Nadelhoffer *et al.* 1991; Silver 1998; Lewis, Malhi & Phillips 2004; Conant *et al.* 2011). Findings by Lasso and Ackerman (2013) suggests that a continuous increased in nutrient availability enhanced reproductive output of the most abundant vascular epiphyte found in a tropical mountain forest located in Puerto Rico. A higher number of seeds, which may potentially develop into seedlings, was suggested to have a positive effect on epiphytic populations growth as long as the availability of colonization sites is ensured (Ackerman, Sabat & Zimmerman 1996).

Due to their localization in the forest column, along the nutrients fluxes (through the stems) epiphytes have been suggested to play an important role in the storage and movements of mineral elements among the forest ecosystem (Nadkarni & Primack 1989). Hence, direct impact of climate change on epiphytic flora may also have indirect consequence on the rest of forest components; which may cause important changes in ecosystem dynamics. However, a higher epiphytic load may also have negative effects on their hosts since branches occupied by them run the risk of breakage, which is in detriment of the hosts lifetime. Consequences derived from climate change may still varied depending on the species, forest types and other attributes. For instances, Cascante Marín *et al.* (2008) found that establishment of epiphytes in a premontane forest was limited by seed dispersal. Moreover, long term-exposure to e[CO_2] in crop species increased leaf temperature due to decrease transpiration cooling caused by partial stomatal closure, which in turn may explain the hastening of flowering for the same species in different studies (Sionit, Strain & Flint 1987; Allen *et al.* 1988; Baker *et al.* 1989). However, this same temperature effect has been mentioned to be responsible of reduced seed set at e[CO_2] (Matsui *et al.* 1997; Prasad, Allen & Boote 2005).

If climate change should have similar, negative impact on tropical species in regard to reproductive and physiological attributes important changes in population dynamics could also be expected to take place (Cernusak *et al.* 2013). Wright and Calderón (2006) already observed enhanced reproductive performance of plant species in a Panamanian tropical forest over almost two decades. If these changes are related to the variations in [CO_2] that have taken place during the past decades a continuous allocation of carbon to reproduction could be foreseen to parallel future increases in [CO_2] (Cernusak *et al.* 2013). Nevertheless, carbon seems not to be the currency for reproduction since reproductive structure may be able of producing by their own the carbon needed to sustain their building requirements (Ashman 1994; Aschan & Pfanz 2003). Observations of the physiological mechanisms and the characteristics of reproductive organs of an epiphytic bromeliad suggest that reproductive structures of these plants may also be relatively autonomous in terms of carbon (Zotz & Richter 2006). This point out to a different factor such a nutrient availability as the limiting resource for successful reproductive performance and there are some evidence that lead to nitrogen or phosphorous as crucial elements for reproduction (Benzing & Davidson 1979; Reekie & Bazzaz 1987; Zotz 1999; Zotz & Richter 2006; Lasso & Ackerman 2013).

Epiphytes has been found to invest a huge amount of resources in reproductive effort with a final biomass of reproductive structures reaching up to 30% (Benzing & Davidson 1979; Zotz 1999) or more (38 ± 5%, mean ± s.e., Zotz & Richter 2006) of the total plant biomass.

There is a common assertion that flowering and vegetative growth may compete for resources (Nitsch 1971; Larcher 1980). Usually, plants experienced a pronounced decrease in growth, survival and even in subsequent reproduction after reproductive events has taken placed, which has been referred as cost of reproduction (Obeso 2002). Both the low nutrient availability characteristic of canopy soils and the great investment of resources on reproduction carried out by epiphytes may result in a significant cost of reproduction for this flora. For instances, Schmidt and Zotz (2002) observed that reduced growth rates in the orchid *Aspasia principisa* coincided with increased reproductive potential. In addition, Zotz, Laube and Schmidt (2005) found that the tank epiphytic bromeliad *V. sanguinolenta* experienced a decrease in plant size after reproduction, it also suffered and increased mortality and previously reproductive individuals still standing in the subsequent year were unable to reproduce. Some individuals of this bromeliad species develop a new shoot short before death, which may account for the final reductions in size (ca. -30%, Schmidt & Zotz 2002). This polycarpic nature of this and many other epiphytic species has been pointed out as a good strategy of nutrient economization since nutrients still remaining in post-fruiting shoots could be allocated to recently developed shoots. However, diverting of some of the resources actually destined to sexual reproduction onto vegetative replication may also contribute to increased fitness of the species (Benzing 1990) and with it the maintenance of genes in the population.

More recently, Aarssen (2008) stated that the clonal comportment of many plant species could allow plants to gain, at least, a two-fold increase in their fitness by combining the sexual reproduction with the sequential and economical production of new offspring by vegetative replication. If an e[CO_2] may contribute to increase vegetative propagation as reported for some plant species (Kriedemann, Sward & Downton 1976), recently developed shoots may ensure growth maintenance as well. However, the smaller size of new shoots may traduce in negative growth rates. Additionally, changes in leaf area and specific leaf area could also be expected. But, plants grown under an e[CO_2] experienced a stimulation of growth rates as long as nutrients are not limited. Increases in leaves carbohydrates may also traduced in increased leaf thickness (Lambers, Chapin III & Pons 2008) and, increased nutrient supply may contribute to vegetative development (Siband 1981), ensure a vigorous growth of plant individuals (Poorter, Remkes & Lambers 1990; Frink. C. R., Waggoner & Ausubel 1999; YY *et al.* 2014) as well as a more vigorous foliage development such it has been observed in different plant species (Valverde, Pisanty & Rincón 1997; Tian *et al.* 2016). However, the degree of stimulation of higher resource availability may depend on the species and morphotype.

Epiphyte are generally slow-growing species (Schmidt & Zotz 2002) and, the atmospheric morphotypes have been found to be relatively unresponsive to increased nutrient availability with especially low maturation rates (Benzing & Renfrow 1971). Contrary, tank epiphytes are supposed to reach maturity in half the period of atmospheric ones due to the possibility to exploit a continuous nutrient supply conferred by the presence of the tank structure. Nevertheless, large individuals are usually less responsive to increased nutrient supply. Thus, it remains to be seen which would be the direction of the response in the epiphytic species as the ones used in the following study. Finally, it has been observed that the amount of nutrients in vegetative structures may also be reduced due to increased allocation to reproduction, but nutrients still presence in fruit stalks once seeds have been shed may be re-invested in production of offshoots in the polycarpic species. Hence, changes in nutrient concentration in vegetative and reproductive parts along the whole cycle may also be expected.

In regard to seed quality there are both positive and negative findings in respond to e[CO_2] as well as some neutral observations. For instances, Thomas (2001) found not effect of e[CO_2] on seed non-structural carbohydrates at any stage, which led to suggest that seeds once set are already well-buffered and respond to increased availability of CO_2 may be limited. Contrary, other authors did find increased seed quality (Allen *et al.* 1988; Wu *et al.* 1997) and other reported increased seed number (Bhattacharya *et al.* 1985; Ahmed, Hall & Madore 1993) in respond to e[CO_2]. The former trait (seed quality) has been described as composed of three different aspects, i.e., individual seed size, composition or nutritional quality and germinability (Prasad, Allen and Boote (2005). However, most of the existent studies focuses just on seed size leaving the other two aspects rather understudied, as was also highlighted by the same authors. In epiphyte plants, nutritional quality of seeds has been the most studied of these aspects as part of resource allocation studies (Reekie & Bazzaz 1987; Zotz 1999; Zotz & Richter 2006), in response to nutrient availability (Fenner 1986; Lasso & Ackerman 2013) and, in response to nutrient and water availability simultaneously (Benzing & Davidson 1979) whereas the other two domains remain rather understudied. As a general issue, effects of e[CO_2] on any aspect of the reproductive biology of epiphytic flora have not been addressed so far.

Finally, reproductive output has been usually related to plant size (Harper 1967; Schaffer & Gadgil 1975; Klinkhamer, Meelies & de Jong 1990; Sterns 1992). Larger individuals may have significantly higher reproductive performance than small-sized individuals (Sletvold 2002). Nonetheless, a study by Klinkhamer *et al.* (1992) pointed out to a rather non-linear relationship between reproductive output and plant size. Furthermore, the initiation of reproduction and intensity may also be dependent on the type of reproductive dynamic performed by the specific species, whether it is a monocarpic or a polycarpic species. For instances, in facultative biennals species with monocarpy initiation of reproduction may be determined by size rather than age (Lacey 1986). In regard to reproductive output, optimal allocation models have shown that in the absence of intensified reproductive output with plant size, the optimal life-history could be annual or polycarpic, but never monocarpic perennial (Pugliese 1988; Iwasa & Cohen 1989). Epiphytic species have both types monocarpic and polycarpic species and description of the reproductive behavior of each type have been well explained by Benzing (1990). However, I would add up that in the polycarpic epiphytic bromeliads used in the present study age rather than size may play a more relevant role in determining the final reproductive output. Additionally, a better knowledge of the life history of plants is mandatory in order to explain their reproductive behavior. Especially in the future enriched atmosphere, thus wrong interpretations could be avoided.

In spite of the sphere of consequences that a future enriched atmosphere may have on vegetation such it has been exposed above and, the evident scarcity of information in regard to the impact on such particular group such as are epiphytes, they remain rather unattended. The high diversity of these plants, the dependency of other organisms on them plus their participation in nutrient cycling along the forest, among other functions in the ecosystem sum up to the reasons that justify the relevance to study and evaluate the intrinsic vulnerability of epiphytic plants in a future enriched atmosphere. Particularly, to assess their ability to survive and maintain its genes in the community in a climate change context could be crucial in order to have some insights on the degree of their vulnerability and, it may allow taking some actions for their conservation if needed. Hence, I was particularly interest on how the simultaneous effect of a future CO_2 and nutrient enriched atmosphere may affect the reproduction of this astonishing plant group. Specifically, I hypothesized the following issues: (1) Higher reproductive output and quality in plants grown at high nutrient concentration and e[CO_2]; (2) Higher growth rates under e[CO_2] and high nutrient availability; (3) Non-structural carbohydrates (NSC) of reproductively active individuals will be reduced in vegetative organs (not tested, insufficient data). Most production intended to construction of reproductive organs; (4) Reproductive plants grown under high nutrient availability will experience continuous growth stimulation and higher survival after reproduction due to compensation of reproductive cost by higher nutrient availability.

MATERIAL AND METHODS

I set up a multifactorial experiment with three bromeliad species: *Catopsis nutans* (Sw.) Griseb (C_3), *Tillandsia bulbosa* Hook (CAM) and *Tillandsia subulifera* Mez (CAM), collected from natural population in the tropical lowland forest in Panama in February 2012. The collection criterion of species individuals was reproductive maturity (with adult size individuals that start the flowering process). Plants were kept in the greenhouse at Oldenburg University since September 2012 until the beginning of the experiments.

In the greenhouse the plants were irrigated twice a week with rain water and once a week with a nutrient (8:8:6 (N:P:K), Wuxal Universal Dünger) solution for the first five months, after it, they were only watered in order to deplete the nutrient storage. Nine individuals of the species *C. nutans* flowered, so we waited until fruit production (from April to July 2013) for the collection of the seeds to be used in a germination experiment. The conditions in the greenhouse were: 25°C/22°C day/night temperature, relative humidity of 75%/48% day/night and a photoperiod of 12/12 h light/dark, with natural sunlight being supplemented with artificial light (Metal halid lamps, 400W, master HPI-T plus; Philips, The Netherlands) to achieve a photosynthetic photon flux density (PFD) of 150-180 μmol m^{-2} s^{-1} at the level of the plants.

Experiment setup

A set of 60 individuals for *C. nutans*, 72 individuals for *T. bulbosa* and 72 individuals for *T. subulifera* were equally distributed in 6 different treatments consisting of two CO_2 concentrations (ambient= 350ppm (a[CO_2] and elevated= 700ppm (e[CO_2]) and three nutrient levels (NPK1 > NPK2 > NPK3, the latter are abbreviations for nitrogen, phosphorous and potassium, the macro-components in the fertilizer mixture). The NPK1 (high concentration treatment) or stock nutrient solution contained 0.21g NH_4NO_3, 0.20g K_2SO_4, 0.35g $NaH_2PO_4 x 2H_2O$, 0.71g $CaCl_2 x 2H_2O$, 0.42g $MgSO_4 x 7H_2O$, 5ml Fe-EDTA and 10ml microelements (8.4g NaCl, 0.38g $MnSO_4xH_2O$, 0.39g $ZnSO_4x7H_2O$, 0.19g (NH4)6$MO_7O_2$4, 0.3g H_3BO3, 0.025g, $CuSO_4x5H_2O$ in one liter of water. From this solution were originated the other two through dilution to 1% (NPK2) and 10% (NPK3). This stock solution was prepared following the description by Zotz, Schmidt and Mikona (2011).

Climatic conditions in both chambers were: Temperature (25°/21°C, dark/night), humidity (80%) and photon flux density (PFD (197-215µmol m^{-2} s^{-1} measured at different positions in all four quadrants of the chambers at the level of the plants). Fertilization treatments were started two weeks after the setting of the plants inside the chambers. Plants were fertilized with the prepared NPK solution once a week and water every day with a weekend break, where plants were not given water or nutrients.

Plant traits (fresh weight and leaf area) of every individual: *Catopsis sessiliflora* (6), *Tillandsia subulifera* (10) and *Tillandsia bulbosa* (5) from each treatment were measured at the beginning of the experiment, after six and twelve months and reproductive development were monitored along the whole experiment. Fresh weights (FW, in grams) were determined one day after watering in order to minimize the difference in water content at the time of weighing. Remainder water in the tank of the plants was removed and dried with a tissue paper. Leaf area (LA) was estimated from leaf pictures by using the measuring tools in Adobe Photoshop CS 5 extended.

Sample plants (FW and LA) were microwaved for 1.30 min to stop any further enzymatic activity, required for carbohydrate determination. Later, samples were dried in an oven for four days at 70°C to determine biomass. Non-structural carbohydrates (NSC; glucose, sucrose, fructose and maltose) were extracted from grounded leaves tissue (20mg) in water for 30 min at 80 °C and phases separated by centrifugation. Starch content in the pelleted material after extraction of soluble carbohydrates was broken down to glucose using α-amylase and amyloglucosidase (Roche, Germany) in acetate buffer (pH 5,0: Na-Acetat x $3H_2O$ (100Mm) + 2.94g $CaCl_2x2H_2O$ in 800ml of water) for 2 h at 30°C and phases separated by centrifugation as well. Finally, NSC and starch were determined from dilutions of end-supernatant by HPLC (ICS-3000, Dionex Corp., Sunnyvale, CA).

Nitrogen content was determined from 2-3mg of dry-ground plant mass using a CHN-O Element Analyzer (Thermo Electron Corporation, Delft, the Netherlands). Mineral nutrient concentration (P, K, Ca, Mg) of the same oven-dried and milled samples (5mg) were determined by atomic absorption photometer. Digestion was made in screw-cap tubes using 200 µl HNO_3 and left in a water bath for 6 hours at 95°C, then 30µl of H_2O_2 were added and samples left in water bath for other 2 hours at 56°C. Afterwards, 770µl of water were added and lecture with the spectrophotometer were made. Both nutrient and carbohydrate contents found in the different plant components were estimated by multiplication of nutrient concentration by the biomass of each organ.

Relative growth rate (RGR) was calculated in milligrams per gram per day after a modified form of Evans´ (1972) equation:

$$RGR= (\ln(M_{t+\Delta t}) - \ln(M_t))/\Delta t,$$

where M= biomass (g) and t= time (days). Initial biomass correspond to the dry mass (DW) of individuals harvested at the beginning of the experiment and for the rest of the individuals in every treatment it was estimated from the FW of each individual using an empirically established relationship (linear regression, $r^2= 0{\cdot}9$) of FW with DW (= biomass). This relationship was calculated using the samples taken at the beginning of the experiment. I used different equations for each species: *C. nutans*: DW = 0.19x + 0.22 (R^2 = 0.77, DW:FW), *Tillandsia bulbosa*: DW= 0.026x + (-3.23) (R^2 = 0.87, DW:LL, 350-NPK2) and DW = 0.24x + 0.18 (R^2 = 0.75, DW:FW, rest of treatments), *Tillandsia subulifera*: DW = 0.15x + 0.55 (R^2 = 0.55, DW:FW).

Reproductive output was measure as: emergence of flower buds (qualitative), number, weight and quality of seeds (including commas-hairs and seed walls). Quality of seeds was measured in terms of nutrients and carbohydrate concentration. Additionally, chlorophyll fluorescence of every individual was measured in order to evaluate plant health. A last harvesting of individuals was done at the end of the experiment of all individuals still remaining in the single treatments.

The individuals that flowered before the experiment onset were allocated to each of the treatments as follows: two individuals to the NPK1 treatment at both CO_2 concentrations, one to the NPK2 and NPK3 treatments at both CO_2 concentrations, except to the 700-NPK3 (two individuals). The other two species included in the study did not flowered during the stay at the greenhouse but their reproductive performance during the course of the treatment was either inconspicuous or incomplete as well.

Statistical analysis

Relative growth rate (RGR) data for the three studied species were analyzed with a type II Anova using lineal models (lm) with CO_2 and nutrient supply as explanatory variables (interaction plus individual effect). Since *C. nutans* was the single species who successfully completely the reproductive cycle with seed production we decided to carry out thorough statistical analyses only on the data from this species in order to explain the RGR and reproductive response.

Many individuals developed vegetative buds after reproduction or before drying out completely. It has been reported that the nutritional status of old senescence leaves is passed to the new born leaves. Hence, in cases where the mother plant died, we assumed that the remaining buds inherit if not all, at least most of the nutritional status of the mother plant. This assumption allowed us to qualitatively relate RGR response to the concentration of different nutritional elements (N, P, K, Ca, Mg) in leaves of plant individuals of the species *C. nutans*, and study how much of the changes we observed in RGR was explained by leaves non-structural carbohydrates (NSC) concentration and content and SLA with a type II Anova using lm (only NSC and SLA).

Survival of reproductive and non-reproductive individuals, vegetative propagation and number of reproductive individuals (presence/absence response variable) were analyzed with a logistic regression (glm), using a quasibinomial correction in cases where the data were over or under dispersed. In order to assess the reproductive output of individuals we use an Anova type II (lm) with number of seeds and seed weight as response variables with CO_2, nutrient concentration, maturity status at the beginning of the experiment (adult or bud), reproduction before experiment onset and time to flower (just in *C. nutans*) as explanatory variables in two separate models. Additionally, we evaluated the differences in the quality of seeds in terms of nutrients (N, P, K, Ca, Mg) and total non-structural carbohydrates in response to CO_2 and nutrient concentration with an Anova type II (lm) as well.

I also evaluated differences in leaves NSC concentration and content in response to the different combinations of CO_2 and nutrient supply in all individuals after one year of treatment with a type II Anova using lm. Differences in leaves NSC concentration and content between reproductive and non-reproductive individuals were not assessed due only few individuals survived until the end of the experiment.

Some individuals developed vegetative buds after reproduction but I did not monitored the date at which a new bud showed up. Hence I decided to evaluate growth performance after reproduction of the species *C. nutans* in response to CO_2 and nutrient availability by use of two response variables “Post-Survival of reproductive individuals” and “Presence of vegetative propagation” (both binomial variables). The latter variable includes those individuals that developed new buds after sexual reproduction and those that simply dried out without performing sexual reproduction but left a leaf bud as representor until experiment conclusion. The former variable includes all individuals that experienced a reproductive event during the course of the experiment whether they remained as individuals or represented for a recently formed leaf bud. We also performed a logistic regression (glm) to analysis of this data.

In case of significant differences, we applied a post hoc test (lsmeans). We applied logarithmic and square root transformation when the response variable showed strong deviation from a normal distribution. Furthermore, we did model simplification following the principle of parsimony by means of AKAIKE Criterion (AIC) and anova comparison. In some cases, AIC value recommended to keep certain model whereas graphs inspection (QQNorm and histogram) suggested the contrary. In those cases, we gave more weight to the graphical analysis.

In some cases, the vegetative bud developed during the course of the experiment performed sexual reproduction, which shows that the species *C. nutans* has a rapid maturation rate. At the end of the two treatment periods we had three different categories of individuals: recently developed buds attached to a living part of the mother plant, reproductive mother plants with and without buds and reproductive and non-reproductive single buds (with dead mother plant still attached). For the statistical analysis, if an individual performed sexual reproduction whether the original reproductive individual continue alive or just a vegetative bud at the end of every period, we still took into account the reproductive event of that specific individual since it could also help to explain any variation in the response.

RESULTS

Reproductive success

A few individuals of *C. nutans*, developed flowers just three weeks after the experiment onset in all treatments but at the higher nutrient supply at both CO_2 concentrations. The rest of the flowering events started three and a half months later. The early flowering by some of the individuals may be determined by an overall effect of the interaction between nutrient supply and maturity status (MS) of the individuals at the beginning of the experiment (Table 4.1, Logistic regression, $P < 0.01$).

In general, higher number of adult individuals (30%) flowered short after the beginning of the experiment in comparison to young individuals or new ramets (20%) and those individuals were subjected to the lowest nutrient concentration at both CO_2 treatments ($P > 0.05$, Tukey´s HSD test, not included). The interaction between CO_2 and MS on the early flowering was not significant, though marginal (Logistic regression, $P = 0.06$), though only a slightly higher number of individuals (30%) flowered at the ambient CO_2 concentration (a[CO_2]) and 20% of these individuals were adults. However, no significant differences between groups were found in any of the cases (Tukey´s HSD test, $P > 0.05$, not included).

Table 4.1. Results of a logistic regression on the effect of CO_2, nutrient and maturity status on early flowering of the species *Catopsis nutans*

Species	Response variable	Factor	df	LR Chisq	*P* value
C. nutans	*Early flowering	[CO_2]	1	0.15	> 0.05
		[Nutrients]	2	2.41	> 0.05
		Maturity Status (MS)	1	0.2	> 0.05
		[CO_2] x MS	1	3.44	0.06
		[Nutrients] x MS	2	11.52	< 0.01

***Not significant differences between groups in *Post hoc* test (results are not shown). Sample size (n) for Anova: 2 (350-NPK1 & 700-NPK1), 8 (350-NPK2), 6 (350-NPK3 & 700-NPK3), 3 (700-NPK2).**

After one year of treatment, the number of reproductively successful individuals of *C. nutans* was significantly influenced by the interactive effect of [CO_2], MS and nutrient concentration (Logistic regression, $P < 0.05$, both, Table 4.2). For instances, a higher number of individuals flowered at the a[CO_2] and ca. 62% of those individuals were adults (Tukey´s HSD test, *not included*). Even further, the highest number of reproductive individuals was found at the lowest nutrient concentration and ca. 70% (700 ppm) to ca. 80% (350 ppm) of these individuals were adults. Nonetheless, a couple of buds that emerged during the course of the experiment performed sexual reproduction just a few months later (data not shown).

In the case of *T. subulifera*, plants grown at the elevated CO_2 concentration (e[CO_2]) performed sexual reproduction earlier than plants grown at the a[CO_2] (5 vs. 8 months after treatment onset). However, the higher number of reproductive individuals was found at the a[CO_2] than at the e[CO_2], particularly, at the higher nutrient concentration but it did not significantly differed from the rest of the treatments (Tukey´s HSD test, not included, $P > 0.05$). Additionally, the occurrence of a consecutive reproductive event in those individuals that flowered before the experiment onset was slightly more common in individuals subjected to a[CO_2] than in those grown at an e[CO_2] (50 to 70% vs. 30%, Three-way-Anova, P = 0.067).

Table 4.2. Results of a logistic regression on the effect of CO_2, nutrient, maturity status and time to flower on reproductive success of the species *Catopsis nutans* and *Tillandsia subulifera* as sexual reproduction and vegetative propagation

Species	Response variable	Factor	df	LR Chisq	*P* value
C. nutans	Nr. Reproductive Individuals	[CO_2]	1	2.58	> 0.05
		[Nutrients]	2	8.9	< 0.05
		Maturity Status (MS)	1	4.31	< 0.05
		[CO_2] x MS	1	4.43	< 0.05
T. subulifera	Nr. Reproductive Individuals	[CO_2]	1	0.14	> 0.05
		[Nutrients]	2	2.51	> 0.05
		Pre-reproduction (PR)	1	1.28	> 0.05
		[CO_2] x PR	1	3.35	0.067
C. nutans	Vegetative propagation	[CO_2]	1	0.5	> 0.05
		[Nutrients]	2	23.5	< 0.001
		Maturity Status (MS)	1	0.12	> 0.05
		[CO_2] x Nutrients	2	3.62	> 0.05
		[CO_2] x MS	1	7.14	< 0.01
		[Nutrients] x MS	2	11.44	< 0.01
T. subulifera	Vegetative propagation	[CO_2]	1	0.61	> 0.05
		[Nutrients]	2	0.91	> 0.05
		Pre-Reproduction (PR)	1	7.23	< 0.01
		[CO_2] x Nutrients	2	6.8	< 0.05
		[CO_2] x PR	1	4.33	< 0.05
		[Nutrients] x PR	2	10.7	< 0.01

*** Sample size (n) equal to: 2 (350-NPK1 & 700-NPK1), 8 (350-NPK2), 6 (350-NPK3 & 700-NPK3), 3 (700-NPK2) for reproductive output *C. nutans* and, 2 (350-NPK1 & 700-NPK1), 8 (350-NPK2), 6 (350-NPK3 & 700-NPK3), 3 (700-NPK2), for vegetative propagation *of C. nutans*. For *T. subulifera*: a) n for reproductive output: 9 (350-NPK1), 5 (350-NPK2 & 700-NPK2), 6 (350-NPK3 & 700-NPK1), 5 (700-NPK2) and, b) n for vegetative propagation: 6 (350-NPK1), 5 (350-NPK2 & 700-NPK2), 8 (350-NPK3), 7 (700-NPK1), 4 (700-NPK3). *Post hoc* for Vegetative propagation in Appendix 5.**

Reproductive output

Plant individuals of the *C. nutans* grown at the a[CO_2] had a higher (5648) total number of seeds than plants grown with e[CO_2] (3150). Almost twice of the individuals (63%) with the a[CO_2] were adults whereas at the e[CO_2] the amount of adults and ramets was almost equivalent (55% vs. 45%, respectively). Nevertheless, at both CO_2 concentrations the observed sexual reproduction events were performed in a higher proportion for adult individuals (ca. 65% at 350ppm and 73% at 700ppm). Different than expected, the amount of reproductively active adult individuals in respect to all individuals present at each CO_2 environment was slightly higher at the a [CO_2] compared to the e[CO_2] (37% vs. 27%).

The plants grown at a[CO_2] with intermediate nutrient concentration (NPK2) produced the higher total number of seeds, while the individuals growing with high nutrient (NPK1) concentration produced the lowest one (3247 vs. 264). Plants with e[CO_2] and, highest nutrient concentration also produced the lowest total number of seeds, compared with the other two nutrient concentrations at this CO_2 treatment (-51% and -75% the number in NPK2 and NPK3, respectively). Only 20% of the individuals with high nutrient concentration at both CO_2 environments were adults and just about 30% of these individuals were able to performed sexual reproduction. Meanwhile, plants grown under low nutrient concentration were constituted by 50% and 30% adult individuals at ambient and e[CO_2], respectively, and the reproductive performance was shown by ca. 80% to 60% of these individuals at each of the concentrations.

Maximal number of seeds was produced by individuals grown at the lowest nutrient at both ambient (143) and e[CO_2] (142) whereas individuals grown at the high nutrient concentration reach a maximum number of seeds that was ca. 10% lower at both CO_2 treatments (129). Fruits produced by *C. nutans* can be divided in: 1- 0-50 seeds, 2- 50-100 seeds and 3- 100-150 seeds. Individuals grown at the lowest nutrient concentration at both CO_2 atmospheres produced a higher number of fruits which contained 100 to 150 seeds (350-NPK3: 118 ± 16 and 700-NPK3: 128 ± 10, n= 11 for both treatments). Thirty six of the fruits produced by the individuals grown at the a[CO_2] contained between 100 and 150 seeds and 24 of these fruits were produced by individuals grown at the intermediate (113 ± 8, n= 24). Furthermore, the number of individuals that produced capsules containing less than 100 seeds was more common in plants subjected to CO_2 enrichment (not tested).

Seed quality

Mean seed number

Seed production by individuals of *C. nutans* had only marginal differences in response to the interactive effect of CO_2 and nutrient concentration (Fig. 4.1). Plant individuals grown at the lower nutrient concentration and e[CO_2] produced the highest mean number of seeds per individual (ca. 105 ± 28) followed by the plants grown at the high nutrient concentration but a[CO_2] t (98 ± 42). Contrary, plants grown at the higher nutrient concentration but e[CO_2] had the second lowest mean seed production (80 ± 14).

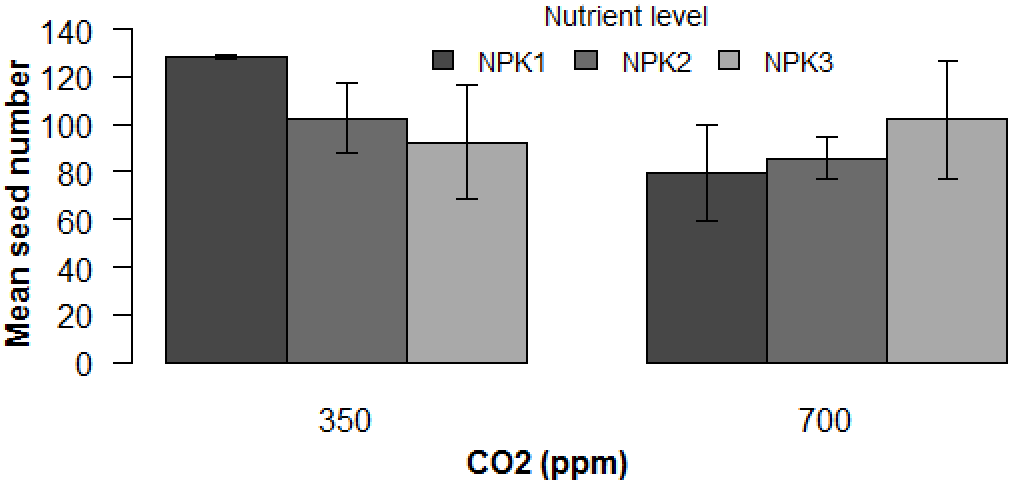


Figure 4.1. Mean seed number of the species *Catopsis nutans* in response to CO_2 and nutrient concentration. Magnitude of nutrient levels is: NPK1 > NPK2 > NPK3. Values are shown as mean ± se. Sample size (n) equal to 2 (350-NPK1 & 700-NPK1), 8 (350-NPK2), 3 (700-NPK2), 6 (350-NPK3 & 700-NPK3).

Mean seed weight

Plants grown at the a[CO_2] produced ca. 50% heavier seeds (Fig. 4.2, 0.08g ± 0.05, n=70) than the ones grown at the e[CO_2] (0.06g ± 0.03, n=45), though influenced of CO_2 on seed weight was only marginal (Four-way Anova, $F = 3.11$, $P = 0.09$, Table 4.3). Moreover, weight of the seed produced by plants subjected to the highest nutrient concentration did not significantly differ from the plants grown at the lowest concentration. Fruits of plants produced by individuals of this species could also be separated in four weight classes: 0-0.1g (0.046g ± 0.03, n= 56), 0.1-0.2g (0.15g ± 0.03, n=56), 0.2-0.3g (0.24g ± 0.04, n=6) and > 0.3g (0.41g ± 0.11, n=5). Regarding weight of seed loads, they could also be separately in four weight classes: 1) > 0.2 (0.27 ± 0.04, n= 3), 2) 0.1 (0.11 ± 0.02, n= 29), 3) < 0.1 (0.05 ± 0.027, n= 81), 4) < 0.01 (0.01 ± 0.001, n= 2).

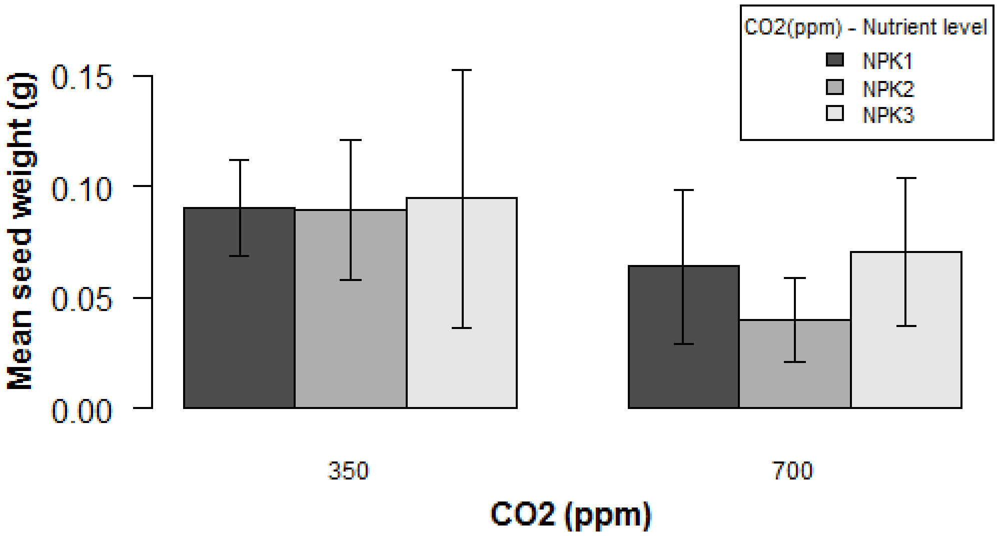


Figure 4.2. Mean seed weight of the species *Catopsis nutans* in response to CO_2 and nutrient concentration. Values are shown as mean ± se. Sample size (n) equal to 2 (350-NPK1 & 700-NPK1), 8 (350-NPK2), 3 (700-NPK2), 6 (350-NPK3 & 700-NPK3).

Finally, mean number of seeds and weight did not significantly differ between individuals that flowered three weeks after the experiment onset and those that flower after this period ($p > 0.05$). Maturity status of the individual at the beginning of the experiment did not significantly affect the reproductive output of the individuals either (Four-way Anova, $F = 0.21$, $P > 0.05$, Table, 4.3).

Table 4.3. Results of a Four-way Anova on the effect of CO_2, nutrient, maturity status and time to flower on reproductive output (mean seed weight and number) of the species *Catopsis nutans*

Species	Response variable	Factor	df	F	*P* value
Catopsis nutans	Mean Seed weight (gr.)	$[CO_2]$	1	3.11	0.09
		[Nutrients]	2	0.13	> 0.05
		Maturity Status	1	0.21	> 0.05
		Time to Flower	1	0.3	> 0.05
		Error	18		
	Mean seed Number	$[CO_2]$	1	1.57	> 0.05
		[Nutrient]	2	0.24	> 0.05
		Maturity Status	1	1.85	> 0.05
		Time to flower	1	0.02	>0.05
		$[CO_2]$ x [Nutrients]	2	3.2	0.07
		Error	16		

***Sample size (n) equal to 2 (350-NPK1 & 700-NPK1), 8 (350-NPK2), 3 (700-NPK2), 6 (350-NPK3 & 700-NPK3)**

Seed quality (biochemistry)

Carbohydrate concentration and content

Neither concentration nor content of total non-structural carbohydrates (NSC) in seeds of *C. nutans* were not significantly affected by CO_2 or nutrient availability (Three-way-Anovas, *P* > 0.05; Table 4.4). However, there were still perceivable differences between treatments (Fig. 4.3 & Fig. 4.4). For instances, total NSC concentration and content of individuals grown at $e[CO_2]$ were lower than in seeds produced by individuals grown at the $a[CO_2]$ (-45% and -14%). Although, whereas total NSC content was higher in plants grown under low nutrient supply (3.8 ± 5 mg, n=6) concentration was higher in seeds of plants grown under a high nutrient supply (38 ± 12, n= 2).

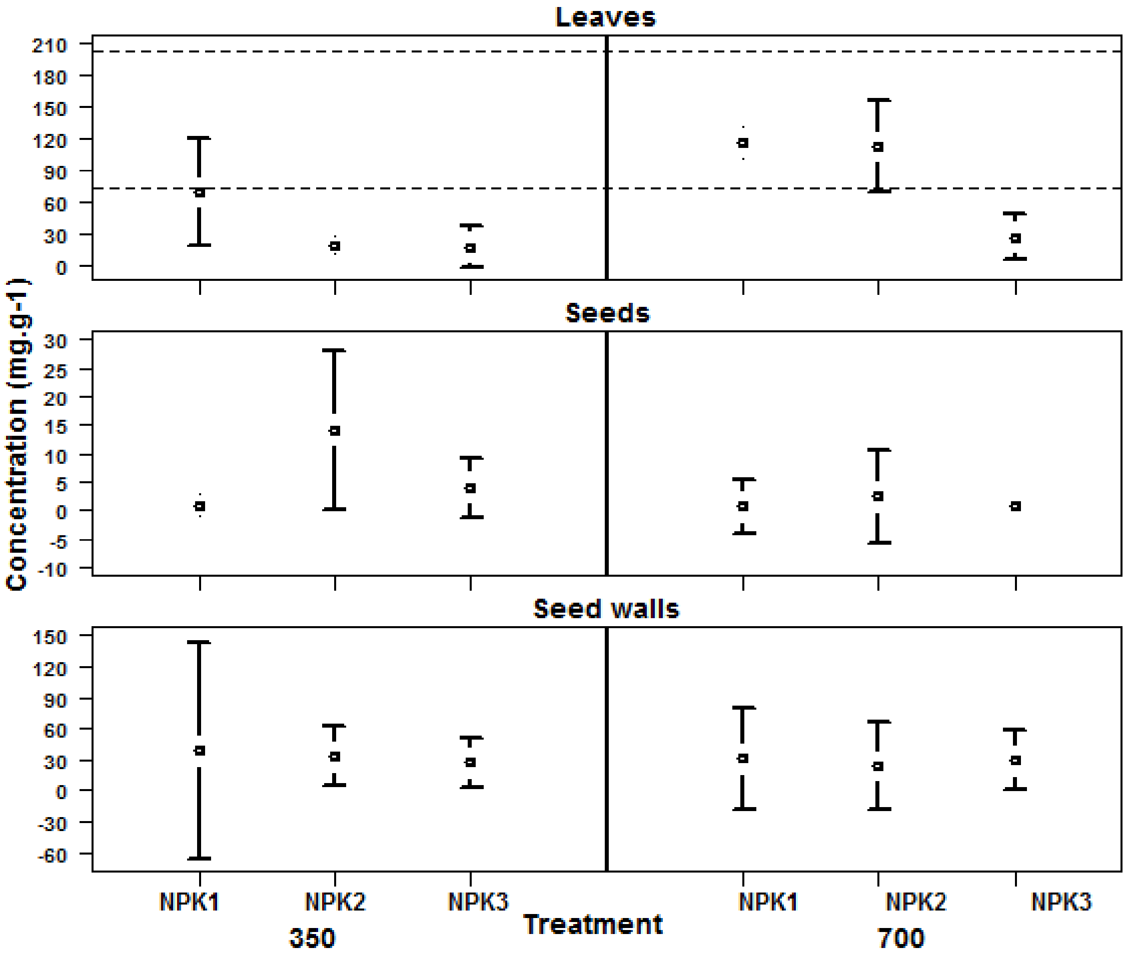


Figure 4.3. Carbohydrate concentration in different plant compartments of the species *Catopsis nutans* in response to different nutrient concentrations under ambient and elevated CO_2 concentration. Nutrient concentration: NPK1= High, NPK2= Medium, NPK3= Low, 350= ambient CO_2 (ppm), 700= elevated CO_2 (ppm). The dash lines in the upper graph corresponds to the carbohydrate concentration in initial samples at the beginning of the experiment (n=7, CI=0.95). Values are means ± CI (n for leaves equal to, n for seeds & seed walls equal to 2 (350-NPK1 & 700-NPK1), 6 (350-NPK2 & 350-NPK3), 3 (700-NPK3), 4 (700-NPK3)).

Maturity status of individuals at the beginning of the experiment had a significant effect on carbohydrates concentration (Three-way-Anova, $P < 0.05$), though it did not influence the content of carbohydrates (Three-way-Anova, $F= 3.4$, $P = 0.08$). For instances, seeds with the highest carbohydrate concentration and content where mostly produced by adult individuals (treatments: 350-NPK1 and 350-NPK3 treatments for content and 350-NPK1 and 700-NPK1 for concentration) compared to the amount produced by young adults (Appendix 6).

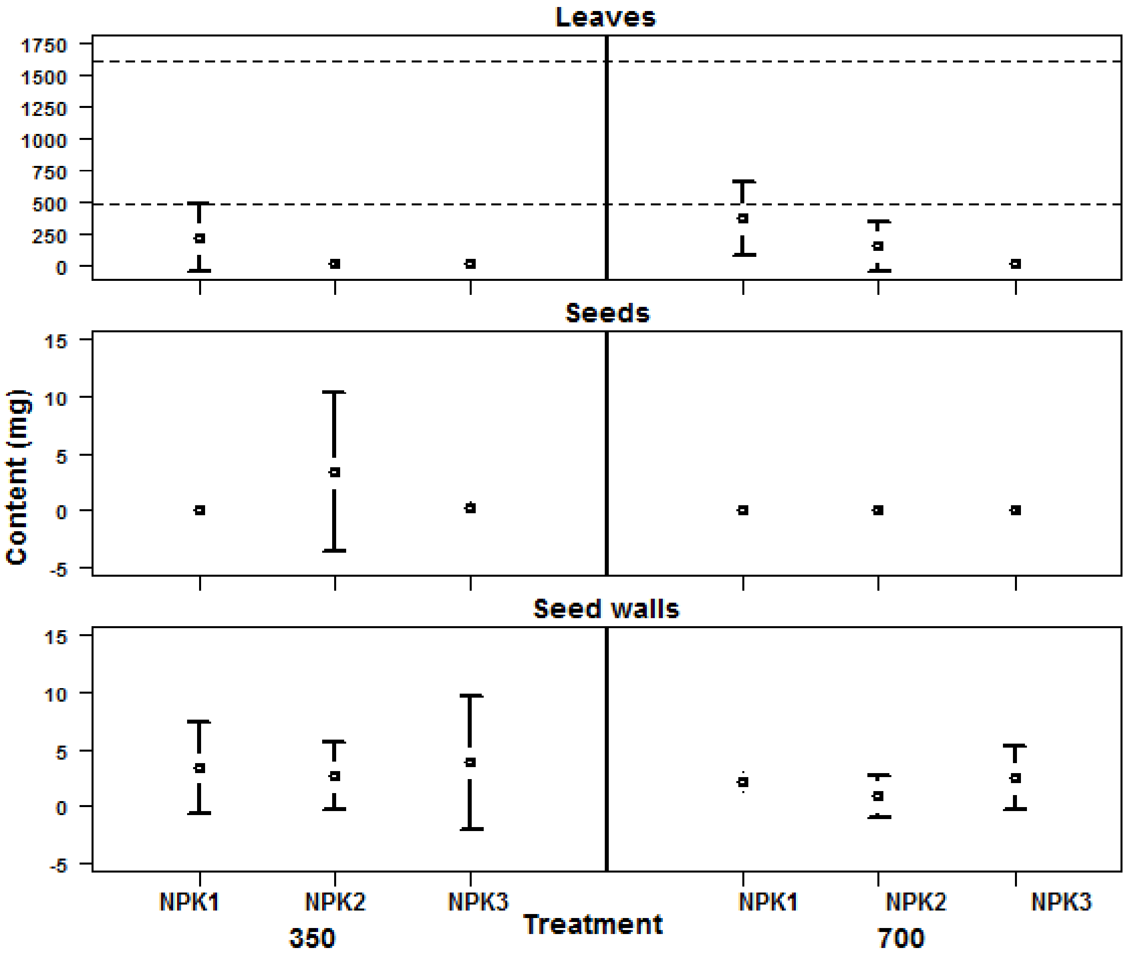


Figure 4.4. Carbohydrate content in different plant compartments of the species *Catopsis nutans* in response to different nutrient concentrations under ambient and elevated CO_2 concentration. Nutrient concentration: NPK1= High, NPK2= Medium, NPK3= Low, 350= ambient CO_2 (ppm), 700= elevated CO_2 (ppm). The dash lines in the upper graph corresponds to the carbohydrate content in leaves of initial samples at the beginning of the experiment (n=7, CI=0.95). Values are means ± CI (n for leaves equal to, n for seeds & seed walls equal to 2 (350-NPK1 & 700-NPK1), 6 (350-NPK2 & 350-NPK3), 3 (700-NPK3), 4 (700-NPK3)).

At the a[CO_2] circa 80% of the total NSC fraction for both concentration and content was given by starch whereas saccharose and frutose as seconds in abundance conformed just between 13% and 12% and 6% and 8%, respectively.

Table 4.4. Results of a Three-way Anova on the effect of CO2, nutrient concentration and seed maturity on carbohydrate concentration and content just in seeds of the species *Catopsis nutans*

Response Variable	Factor	d.f.	*F*	*P* value
[Carbohydrates]	[CO_2]	1	0.005	> 0.05
	[Nutrients]	2	0.5	> 0.05
	Maturity Status (MS)	1	6.55	< 0.05
	Error	17		
Carbohydrates content	[CO_2]	1	0.31	> 0.05
	[Nutrients]	2	1.33	> 0.05
	Maturity Status (MS)	1	3.4	0.083
	Error	17		

***Sample sizes (n) equal to: 2 (350-NPK1 & 700-NPK1), 6 (350-NPK2 & 350-NPK3), 3 (700-NPK3), 4 (700-NPK3)**

Nutrient concentration and content

Concentration of nitrogen (N), potassium (K) and calcium (Ca) was higher in seeds of plants grown at the ambient CO_2 concentration (+74%, 62%, 6%, respectively, Fig. 4.5 & 4.6) whereas phosphorus (P) and magnesium (Mg) were in slightly higher concentration in seeds of plants grown at e[CO_2] (24%, 4%, respectively, Appendix 7). Parallel, content of each nutritional element per seed mass was always higher in seeds of plants grown at the ambient than at the e[CO_2] (N: +43%, P: +40%, K: +66%, Ca: 58%, Mg: 44%). Phosphorus, potassium and calcium were at a higher amount and concentration in seeds of plants subjected to the higher nutrient supply than the rest of the nutritional elements.

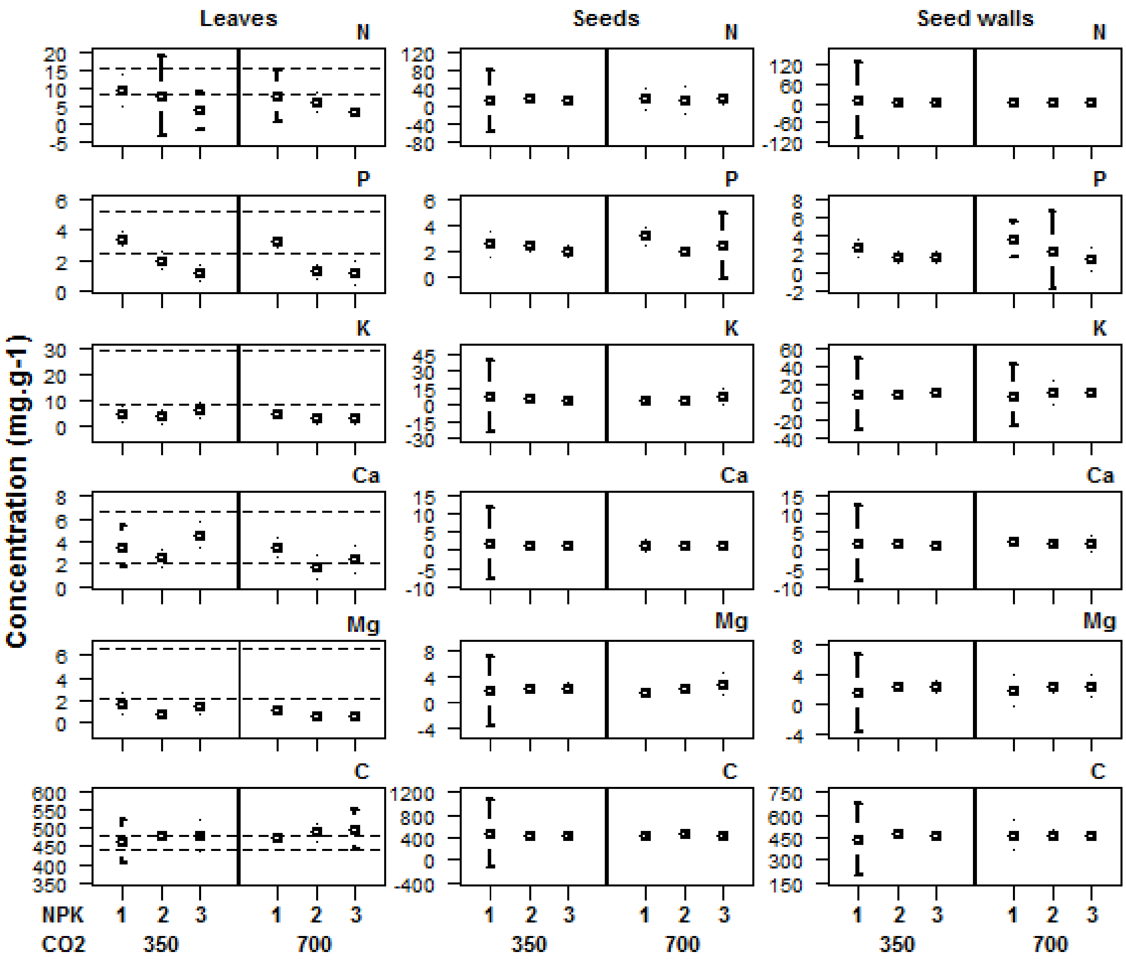


Figure 4.5. Nutrient concentration in leaves and fruit parts of the species *C. nutans* in response to different nutrient concentrations under ambient and elevated CO_2 concentration. Nutrient concentration: NPK1 = High, NPK2= Medium, NPK3= Low, 350= ambient CO2 (ppm), 700= elevated CO2 (ppm). The dash lines in each graphs from the left panel correspond to the nutrient concentration at the beginning of the experiment (n=7, CI=0.95). Capital letters at the upper left side of each graph correspond to the abbreviation of each nutrient. Values are means ± sd (n for leaves equal to 5 except treatment 700-NPK2 with n=4, n for seeds & seed walls: 2 (350-NPK1 & 700-NPK1), 5 (350-NPK2 & 350-NPK3), 4 (700-NPK3).

In terms of content, all nutritional elements were in higher amount at the a[CO_2] when nutrient concentration was high except for N and Mg, which were in higher amount at the intermediate nutrient concentration under the same CO_2 treatment. Nonetheless, only nitrogen concentration was significantly affected by the interaction between [CO_2], nutrients and maturity status (MS) of individuals at the beginning of the experiment (Three-way Anova, [N]: $F = 8.8$, $P < 0.05$, n= 2-6, Appendix 7).

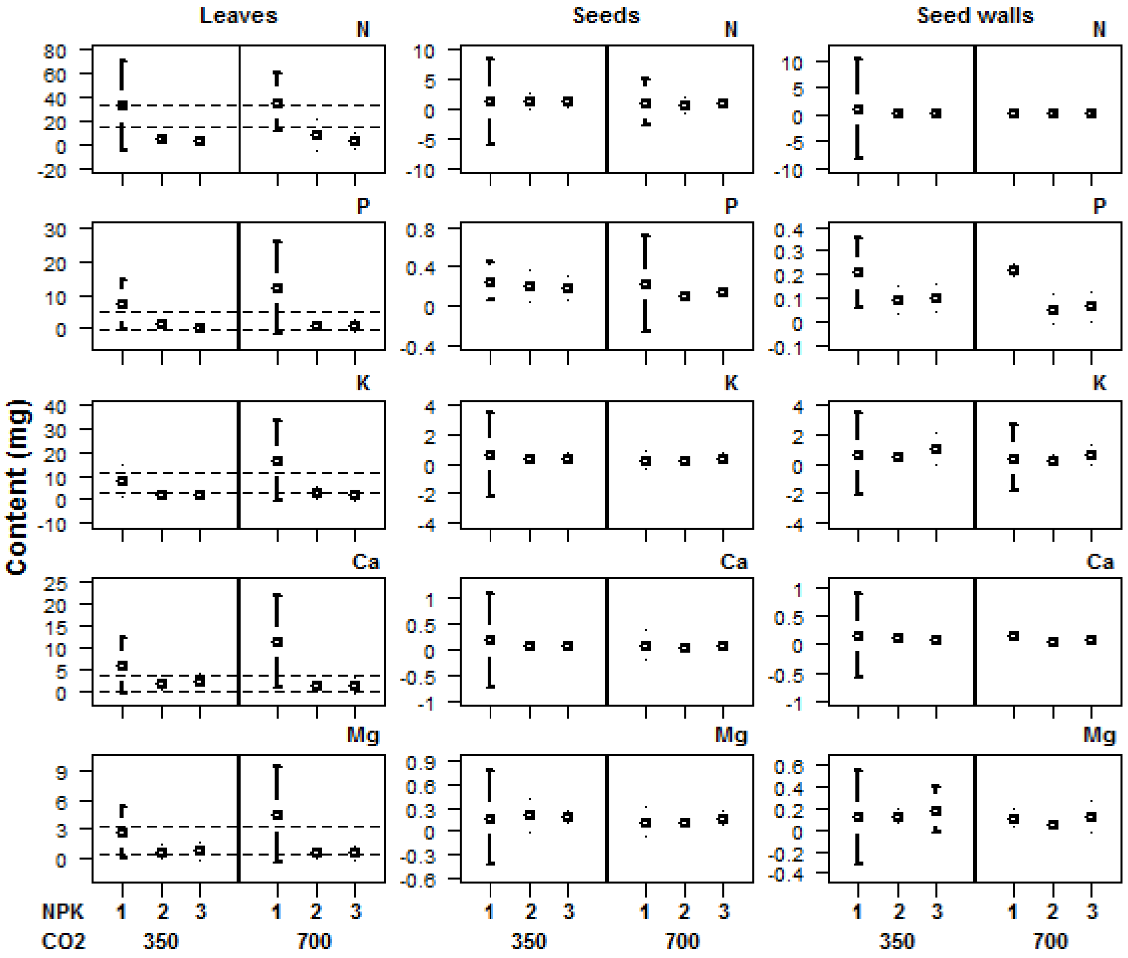


Figure 4.6. Nutrient content in leaves and fruit parts compartments of the species *Catopsis nutans* in response to different nutrient concentrations under ambient and elevated CO_2 concentration. Nutrient concentration: NPK1= High, NPK2= Medium, NPK3= Low, 350= ambient CO_2 (ppm), 700= elevated CO_2 (ppm). The dash lines in each graphs from the left panel correspond to the nutrient concentration at the beginning of the experiment (n=7, CI=0.95). Capital letters at the upper left side of each graph correspond to the abbreviation of each nutrient. Values are means ± sd (n for leaves equal to 5 except treatment 700-NPK2 with n=4, n for seeds & seed walls: 2 (350-NPK1 & 700-NPK1), 5 (350-NPK2 & 350-NPK3), 4 (700-NPK3).

Nitrogen content was affected by the interactive effect of [CO_2] and MS (Three-way-Anova, *F*= 5.3, $P < 0.05$) and nutrients and MS (Three-way-Anova, *F*= 5.71, $P < 0.05$) in the case of content. Even though, seeds with the highest nutritional value were produced in the treatments only or mostly consisting of adult individuals comparison of nutritional quality of seeds produced by adults and ramets did not differ between each other (no statistically analyzed). (Second highest concentration of Mg, K and N is at the low nutrient concentration treatments but under e[CO_2])

Relative growth rate (RGR)

Relative growth rates of the three studied species were differentially affected by CO_2 and nutrient concentration at the different treatment periods (Fig. 4.7). Over a year, growth rates of *C. nutans* were not significantly affected by nutrient concentration, though marginal marginally affected by nutrient concentration (Table 4.5, Two-way Anova, $F = 7.69$, $P = 0.05$). Contrary, *T. bulbosa* was strongly dependent on nutrient availability ($P < 0.001$). Plants of *C. nutans* grown at the high NPK treatments at each CO_2 growing environment had higher growth means (a[CO_2] = 2.24mg.g^{-1}.d^{-1} ± 1.97 and e[CO_2] = 2.98mg.g^{-1}.d^{-1} ± 1.71) than plants grown at the medium and low nutrient concentrations (-0.0063mg.g^{-1}.d^{-1} vs. 1.26 mg.g^{-1}.d^{-1}, mean of NPK2 and NPK3 treatments at a[CO_2] and e[CO_2], Appendix 8, Tukey´s HSD test, $P \leq 0.1$,).

Growth rates of *T. bulbosa* was strongly dependent on nutrient availability, effect of [CO_2] on RGR of the species *T. bulbosa* was strongly dependent on nutrient availability (Table 4.5, Two-way Anova $F = 89.8$, $P < 0.001$). Individuals of this species subjected to e[CO_2] were significantly enhanced by high nutrient concentration compared to those of plants grown at the rest of the combined treatments (Tukey´s HSD, $p < 0.001$, Appendix 8). At the high nutrient concentration and e[CO_2] individuals had a mean growth rate of 13mg.g^{-1}.d^{-1} compared to too low (0.4 mg.g^{-1}.d^{-1}) or even negative mean rates for the other treatments.

At this treatment half, only *T. bulbosa* was positively influenced by e[CO_2] (Two-way Anova, $F = 89.8$, $P < 0.001$). Whereas at all nutrient treatments at the a[CO_2] environment plant individuals had a significant reduction of their growth rates (-3.36mg.g^{-1}.d^{-1} (> 50%), overall mean per CO_2 level) in plants subjected to CO_2 enrichment the reduction at the high NPK treatment was just ca. 50% of that observed at a[CO_2] (Tukey´s HSD, $P < 0.05$, Appendix 8). Nonetheless, positive effect of CO_2 was even higher at the medium and low nutrient concentration treatments (NPK2 and NPK3, Fig. 4.7). In *T. subulifera*, plants with a high nutrient solution and e[CO_2] had higher growth means (1.61 mgg-1d-1 ± 1.92), though, in this and the other two treatment periods the effects of CO_2 and nutrient concentration on RGR of this species were not significant (Table 4.5, $P > 0.05$).

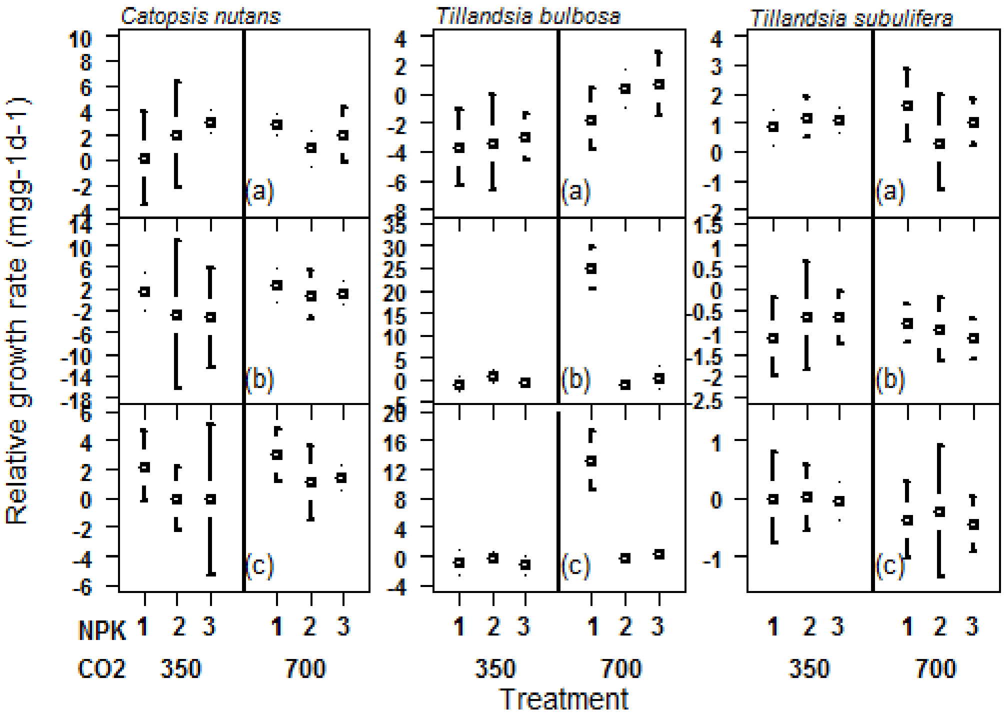


Figure 4.7. Relative growth rate of three Bromeliaceae species in response to different CO_2 and nutrient concentrations. NPK corresponds to the nutrient supply: 1= 100%, 2= 10%, 3 = 1%) and CO_2 concentration (Low: 350ppm and high: 700ppm) in three different time periods ((a)-. First 6 months (*C. nutans*: n= 7 -10, *T. bulbosa*: n= 8-11, *T. subulifera*: n= 8-12), (b)-. Last 6 months (*C. nutans*: 3 -6, *T. bulbosa*: n=3-9, *T. subulifera*: n=5-9), (c)-. Along 12 months (*C. nutans*: n= 3 -6, *T. bulbosa*: n=3-9, *T. subulifera*: n= 5-9).

In the second half of the treatment, CO_2 and nutrient concentration has significant effect on the RGR of the species *C. nutans* and *T. bulbosa* (Table 4.5, Two-way Anova, $F = 8.4$, $P < 0.05$ and $P < 0.001$, respectively). The growth of the species *T. bulbosa* was once again strongly favored by high nutrient concentration under an enriched CO_2 atmosphere than at any of the other treatments (Appendix 8, Tukey´s HSD, $P < 0.001$). At high nutrient concentration and a[CO_2] growth mean of *T. bulbosa* showed a significant reduction of -1 mg.g^{-1}.d^{-1} compared to a gain of 24mg.g^{-1}.d^{-1} at the e[CO_2]. The species *C. nutans* had significantly higher growth means when plant individuals were supplied with a high nutrient concentration at both CO_2 levels (e[CO_2] = 2.58mg.g^{-1}.d^{-1} vs. a[CO_2] = 1.46mg.g^{-1}.d^{-1}) whereas an intermediate and low nutrient supply resulted in growth means that were 65% to 85% lower in plants grown at the e[CO_2] treatment but at the ambient concentration growth means were even negative ($P < 0.05$).

Table 4.5. Results of two-way Anova on the effect of CO$_2$ and nutrient interaction on RGR for three Bromeliaceae species

Species	Harvest time	Factor	df	F	*P* value
Catopsis nutans	Over a year	[Nutrient]	2	3.38	0.05
	(n= 3-6)*	Error	25		
	First half (0-6 months)	[CO$_2$]	1	0.06	0.81
	(n= 7-10)	[Nutrient]	2	0.64	0.52
		[CO$_2$] x [Nutrient]	2	1.46	0.24
		Error	46		
	Second half (6-12 months)*	[CO$_2$]	1	5.05	< 0.05
	(n= 3-6)	[Nutrient]	2	4.73	< 0.05
		[CO$_2$] x [Nutrient]	2	8.4	< 0.05
		Error	12		
		[Nutrient]	2	0.49	0.61
Tillandsia bulbosa	Over a year	[CO$_2$]	1	89.8	< 0.001
	(n= 8-11)	[Nutrient]	2	120.5	< 0.001
		[CO$_2$] x [Nutrient]	2	125.2	< 0.001
		Error	29		
	First half (0-6 months)	[CO$_2$]	1	16.64	< 0.001
	(n= 3-9)	Error	53		
	Second half (6-12 months)	[CO$_2$]	1	56.34	< 0.001
	(n= 3-9)	[Nutrient]	2	141	< 0.001
		[CO$_2$] x [Nutrient]	2	164.6	< 0.001
		Error	29		
Tillandsia subulifera	Over a year	[CO$_2$]	1	1.8	> 0.05
	(n= 5-9)	Error	38		
	First half (0-6 months)	[CO$_2$]	1	0.021	> 0.05
	(n= 8-12)	Error	65		
	Second half (6-12 months)	[CO$_2$]	1	0.9	> 0.05
	(n= 5-9)	[Nutrient]	2	0.1	> 0.05
		Error	36		

***Sample size varied across treatment conditions and period for the three species tested.**

Survival

Surprisingly, high nutrient concentration had the strongest negative effect on survival of the species *T. bulbosa* and *T. subulifera* at both CO_2 concentrations, over a year and at the second half of the treatment (Fig. 4.8). Survival was as low as 25% for the former species at both CO_2 environments and between 40% (350ppm) to 50% (700ppm) for the second species at both treatment periods. However, the effect of nutrient concentration on survival was only significant for the species *T. bulbosa* at both second half (Table 4.6, $F = 8.06$, $P < 0.05$) and one year of treatment ($F = 6.61$, $P < 0.05$). For instances, at the e[CO_2], survival of individuals of this species grown at the high nutrient concentration was three times lower compared to that of the individuals grown at the intermediate nutrient concentration at the second half (Appendix 9, *t.ratio* = -2.5, $P < 0.05$).

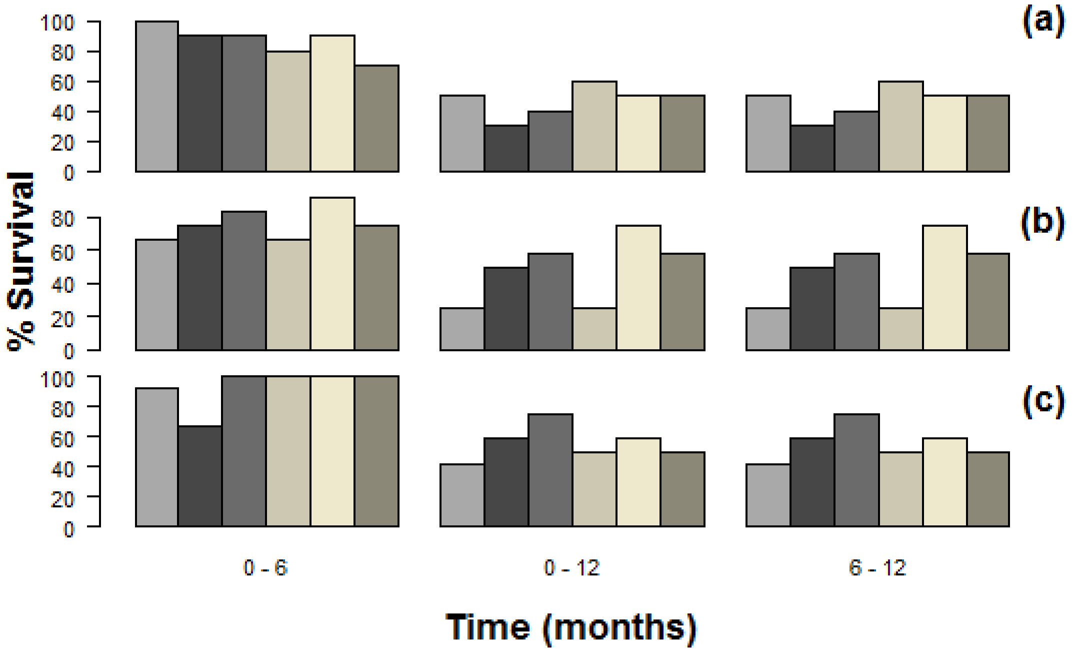


Figure 4.8. Survival of three Bromeliaceae species in response to different CO_2 and nutrient concentrations at three different time periods. The different letters in parenthesis correspond to the species: (a) *Catopsis nutans* (n= 10), (b) *Tillandsia bulbosa* (n= 12), (c) *Tillandsia subulifera* (n= 12). Magnitude of nutrient levels is: NPK1 > NPK2 > NPK3.

In the first treatment half, *T. subulifera*´s survival was significantly affected by CO_2 (Table 4.2, F = 22.04, $P < 0.001$) and nutrient concentration (Table, 4.6, F = 19.41, $P < 0.001$). Survival of individuals grown at the a[CO_2] and intermediate nutrient concentration was 25% lower than plants subjected to high nutrient concentration and a[CO_2] and ca. 33% compared to that of plants grown at the rest of the treatments (Tukey´s HSD, Appendix 9). Nonetheless, after assessing for significant differences among these groups, we could not find real significance.

In this same treatment half, the tank species *C. nutans* had a maximal survival under high nutrient concentration and a[CO_2]. Survival was reduced in 20% when individuals were subjected to the same nutrient concentration but e[CO_2]. *Catopsis nutans* had a maximum survival of 60% when the species grew with high nutrient concentration and e[CO_2]. Nevertheless, the observed differences were not significant at any treatment period (Tukey´s HSD, $P > 0.005$, Appendix 9).

Table 4.6. Results of a logistic regression on the effect of CO_2 and nutrient on survival percentage at different time periods for three Bromeliaceae species

Species	Harvest time	Factor	df	LR Chisq	*P* value
Catopsis nutans	Over a year	[CO_2]	1	1.04	0.31
	First half (0-6 months)	[CO_2]	1	2.32	0.13
	Second half (6-12 months)	[CO_2]	1	1.07	0.3
Tillandsia bulbosa	Over a year	[Nutrient]	2	6.61	< 0.05
	First half (0-6 months)	[Nutrient]	2	1.9	0.4
	Second half (6-12 months)	[Nutrient]	2	8.06	< 0.05
Tillandsia subulifera	Over a year	[Nutrient]	2	1.78	> 0.05
	First half (0-6 months)	[CO_2] [Nutrient]	1 2	22.04 19.41	< 0.001 < 0.001
	Second half (6-12 months)	[Nutrient]	2	1.78	> 0.05

***Sample size (n) equal to 10 for *Catopsis nutans* and n= 12 for the *Tillandsia* species**

Survival after reproduction

Most of the individuals of the species *C. nutans* that performed sexual reproduction at the first six months of the treatment survived until the end of the treatment when grown at an e[CO_2], particularly when nutrient concentration was low (75%). However, these results were not significantly determined either by CO_2 (Table 4.2, Two-way-Anova, *LR Chisq*= 3.2, $p > 0.05$, both) or nutrient concentration (Two-way-Anova, *LR Chisq*= 3.2, $P > 0.05$, both). After twelve months, vegetative propagation in plant individuals under low nutrient concentration was paired to sexual reproduction in 100% of the cases at both CO_2 environments (data not shown). Furthermore, at the e[CO_2] treatments mostly adult individuals propagated vegetatively, particularly at the low nutrient concentration 70% of the vegetative propagation events were performed by adult individuals. Nonetheless, whereas the overall effect of these interactive effects of CO_2 and nutrient supply with maturity status (MS) were significant (Table 4.2*,* Three-way-Anova, CO_2*MS: *LR*= 7.14, $P < 0.01$, Nutrients*MS: *F*= 11.44, $P < 0.01$) I was unable to find significant differences between the different treatment groups (Tukey´s HSD test, $P > 0.05$, Appendix 5).

Meanwhile, at the e[CO_2] and high nutrient supply only one individual presented vegetative propagation and this single event did not match sexual reproduction. The number of individuals that performed vegetative reproduction at the rest of the treatments ranges between three and four.

Vegetative propagation in *T. subulifera* was significantly affected by the interactive effects of CO_2 and nutrient supply (Three-way-Anova, *F*= 6.8, $P < 0.05$), CO_2 and pre-reproductive status (PR: reproduction before experiment onset, Three-way-Anova, *F*= 4.33, $P < 0.05$) and by nutrient supply and PR (Three-way-Anova, *F*= 10.7, $P < 0.01$). At the lowest nutrient supply and ambient CO_2 and in those subjected to high nutrient concentration but e[CO_2] a higher number of plant individuals performed vegetative propagation (58% and 67%, respectively), though, it was not significantly different than the degree of propagation observed in the rest of the treatments (Tukey.HSD test, $P > 0.05$). Additionally, vegetative propagation in those individuals that had already flowered before the experimental onset was more common in those individuals grown at an a[CO_2] atmosphere than in those grown at an e[CO_2] (Tukey.HSD test, $P < 0.05$).

Additionally, individuals from the species *T. subulifera* that performed sexual reproduction before the experiment onset showed a significantly different behavior in their vegetative propagation response when exposed to low nutrient concentration which depended on the CO_2

growing environment ($P < 0.05$), whereas a higher number of individuals grown at the a[CO_2] performed vegetative propagation at the e[CO_2] just few individuals did it ($P < 0.05$).

Plants morphology

Leaf area (LA) and specific leaf area (SLA)

Leaf area of *C. nutans* was mostly explained by nutrient concentration (Fig. 4.9, Table 4.7, Two-way-Anova, $F = 9.42$, $P < 0.05$). Individuals grown at the highest nutrient concentration developed leaves 200% broader than individuals growing at the other two nutrient concentrations (Tukey´s HSD, $P < 0.05$, mean including both CO_2 treatments, Appendix 10). Additionally, individuals subjected to high nutrient concentration had ca. 250% and ca. 290% broader leaves than individuals grown at a medium and low nutrient concentration under a[CO_2] (Tukey´s HSD, $P < 0.05$, Appendix 10), respectively. Similar, individuals grown at a high nutrient concentration and e[CO_2] had leaves 172% and 215% broader than plants supplied with medium and low nutrient concentration (Tukey´s HSD, $P < 0.005$, Appendix 10). Plants grown under high nutrient concentration and e[CO_2] were ca. 50% broader than leaves of individuals grown at the same nutrient concentration but a[CO_2] treatment after one year (Tukey´s HSD, $P < 0.05$, 376 cm^2 ± 210 vs. 252cm^2 ± 151, respectively).

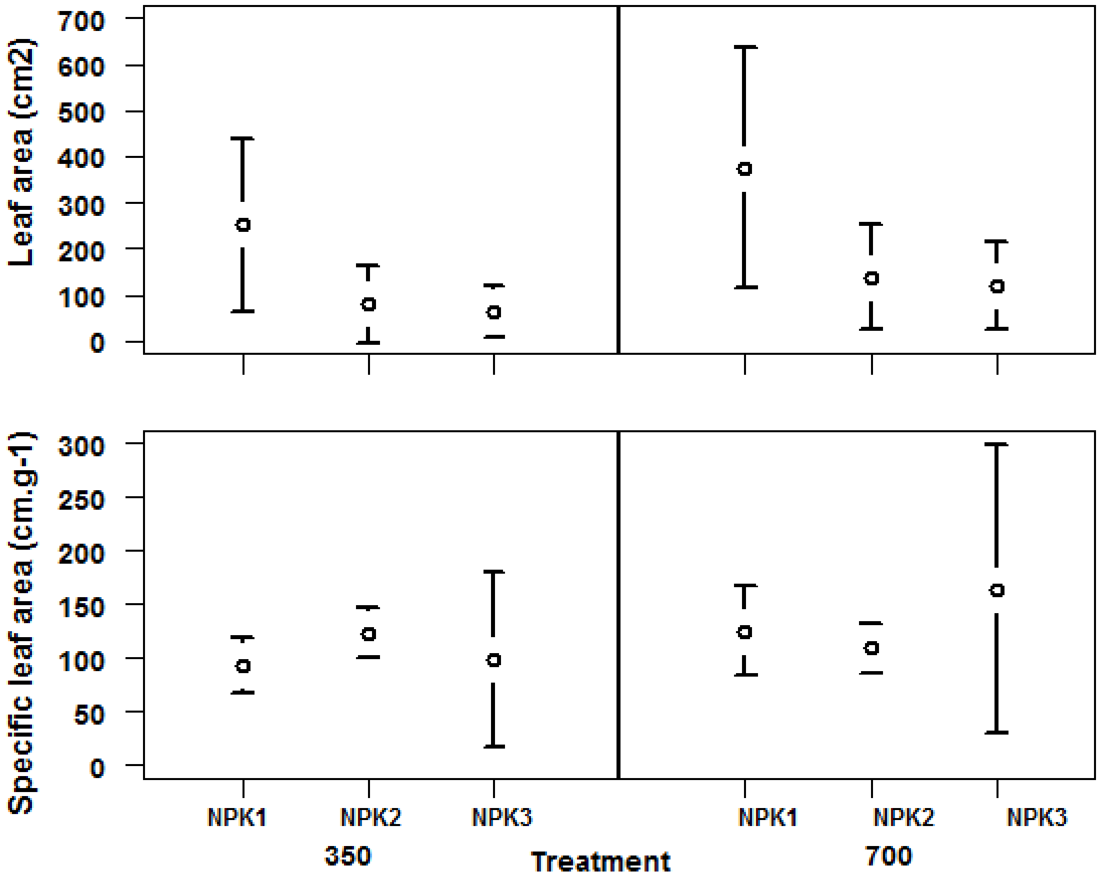


Figure 4.9. Leaf Area and Specific leaf area in response to different nutrient concentrations under ambient (350ppm) and elevated (700) CO_2 concentration after one year of treatment. Magnitude of nutrient concentrations: NPK1 > NPK2 > NPK3, values are shown as means ± standard error. Sample size (n) equal to 5 except for the treatments 350-NPK2 and 350-NPK3 with n= 3.

Individuals grown at the e[CO_2] had on average 29% higher SLA than those individuals grown at the a[CO_2] but the effect was not significant (Table 4.7, Two-way Anova, $F = 1.77$, $P > 0.05$). For example, plant individuals grown at the high nutrient concentration had leaves with a SLA 5% and 22% higher than that of plants grown at the medium and low nutrient concentration, respectively (Fig. 4.9). This same pattern was maintained inside each CO_2 treatment with differences ranging between 24% to 6% at the ambient CO_2 treatment and between 14% and 23% under CO_2 enrichment. Furthermore, plant individuals grown at the e[CO_2] and high nutrient concentration had significantly higher SLA (+35%) than plants grown at the same nutrient concentration but a[CO_2].

Table 4.7. Results of two-way Anova on the effect of CO_2 and nutrient interaction on morphological traits in the species *Catopsis nutans*

Species	Response variable	Factor	df	F	*P* value
C. nutans	Leaf Area (LA)	[Nutrients]	2	9.42	< 0.05
		[CO_2]	1	2.9	0.1
		Error	22		
	Specific leaf area (SLA)	[Nutrients]	2	0.66	> 0.05
		[CO_2]	1	1.77	> 0.05
		Error	22		

***Sample size (n) equal to 5 except for the treatments 350-NPK2 and 350-NPK3 with n= 3**

Plant biochemistry

Nutrients concentration and content in leaves

Concentration and content of most nutritional elements (N, P, K, Ca, Mg) in leaves of the species *C. nutans* also experienced pronounced decreases, which were significantly explained by the influenced of CO_2 and nutrient supply in most of the cases (Fig. 4.5 – 4.6, Table 4.8 , Two-way Anova). Content and concentration of N, P, K, Ca, Mg was lower at the low and intermediate nutrient supply, especially with e[CO_2] after one year of treatment (Appendix 11).

Plant individuals grown under high nutrient supply presented the highest content of N, P, K, Ca and Mg (Tukey´s HSD, $P < 0.05$ and $P < 0.001$, Appendix 11). Similarly, content and concentration of all elements but one (N) were approximately twice higher in plants grown under e[CO_2] than in plants grown at ambient CO_2. But, nitrogen (N) concentration and content in plants grown at the high nutrient concentration differ just in ca. -17% and ca. +10% , respectively, in plants grown at e[CO_2] compared to a[CO_2] (Tukey´s HSD, $P > 0.05$, Appendix 11).

Table 4.8. Results of a Two-way Anova on the effect of CO_2, nutrient concentration and seed maturity on nutrient concentration and content in leaves of the species *Catopsis nutans*

Nutrientes	**Factor**	**d.f.**	***F***	***P* value**
Nitrogen content*	[CO_2]	2	29.39	< 0.001
	[Nutrients]	2	39.39	< 0.001
	[CO_2] x [Nutrients]	2	1.04	> 0.05
	Error	18		
Nitrogen concentration*	[CO_2]	2	13.81	< 0.001
	[Nutrients]	2	4.39	< 0.05
	Error	20		
Phosphorus content*	[CO_2]	2	3.2	0.055
	[Nutrients]	2	18.81	< 0.001
	[CO_2] x [Nutrients]	2	0.64	> 0.05
	Error	29		
Phosphorus concentration*	[CO_2]	2	25.71	< 0.001
	[Nutrients]	2	21.28	< 0.001
	[CO_2] x [Nutrients]	2	0.53	> 0.05
	Error	29		
Potassium content	[CO_2]	2	3.62	< 0.05
	[Nutrients]	2	11.41	< 0.001
	[CO_2] x [Nutrients]	2	1.36	> 0.05
	Error	29		
Potassium concentration	[CO_2]	2	14.28	< 0.001
	[Nutrients]	2	2.06	> 0.05
	[CO_2] x [Nutrients]	2	1.95	> 0.05
	Error	29		

Tabla 4.8. Continuity

Nutrientes	**Factor**	**d.f.**	***F***	***P* value**
Calcium content	[CO_2]	2	0.49	> 0.05
	[Nutrients]	2	10.22	< 0.001
	[CO_2] x [Nutrients]	2	2.07	> 0.05
	Error	29		
Calcium concentration	[CO_2]	2	3.33	< 0.05
	[Nutrients]	2	4.22	< 0.05
	[CO_2] x [Nutrients]	2	2.27	> 0.05
	Error	29		
Magnesium concentration	[CO_2]	2	18.27	< 0.001
	[Nutrients]	2	4.14	< 0.05
	Error	31		
Magnesium content	[CO_2]	2	1.19	> 0.05
	[Nutrients]	2	7.95	< 0.01
	Error	31		

***Sample size (n) was equal to 7 for initial samples, 3 for nitrogen of leaves at the different treatments and 5 for the rest of the nutrients at each of the treatments.**

Carbohydrates concentration and content in leaves

After one year concentration and of non-structural carbohydrate (NSC) in leaves of *C. nutans* significantly decreased in all treatments with CO_2 nutrient concentration (Fig. 4.3, Table 4.9, Two-way Anova, concentration: $F = 16.7$, $P < 0.05$) and as by [CO_2] ($F = 5.74$, $P < 0.05$). Whereas NSC content was only significantly influenced by nutrient supply (Fig. 4.5, $F = 13.07$, $P < 0.001$) after one year. In leaves of plants grown at high nutrient supply reductions in NSC content oscillated between 83% and 70% at ambient and e[CO_2], respectively. Carbohydrates content in leaves of plants grown at low nutrient supply decreased in 99% at both CO_2 treatments (Fig. 4.4). Carbohydrates content in leaves of plants grown at e[CO_2] was reduced in 85% whereas that in leaves of plant individuals grown at the a[CO_2] suffered a reduction of 93%. Total NSC content was 74% higher in leaves of plants grown at high nutrient concentration and e[CO_2] than plants grown with high nutrient supply but a[CO_2] (369 mg vs. 212 mg, Tukey´s HSD, $P < 0.001$, Appendix 12).

Fructose constituted 86% of leaves total NSC fraction at the beginning of the experiment whereas starch constituted the highest fraction (65% to 77%, data not shown) but not in the plants with the treatment 350-NPK2. In plants grown at 350-NPK2, fructose was still the highest fraction of total NSC (45.08%). Glucose was found as the second component in abundance in leaves of plants after one year (11% to 21% of total fraction).

Plant individuals with high nutrient concentration had lower reduction in NSC concentration than individuals grown at the other two nutrient levels at both [CO_2] (-39% in NPK1 vs. -57% and -85% in NPK2 and NPK3, respectively, Fig. 4.3, Appendix 12). Reduction in NSC concentration in leaves was also lower in plants grown at an enriched [CO_2] than under a[CO_2] (-44% vs. -76%). Concentration of NSC in plants grown under high nutrient supply and e[CO_2] was 67% higher compared to plants grown at the same nutrient concentration but a[CO_2] (117 mg g^{-1} vs. 70 mg g^{-1}).

Table 4.9. Results of a two-way Anova on the effect of CO_2 and nutrient on carbohydrates concentration and content in leaves of the species *Catopsis nutans*

Species	Response variable	Factor	df	F	*P* value
C. nutans	[Carbohydrate]	[Nutrients]	4	16.7	< 0.001
		[CO_2]	1	5.74	< 0.05
		Error	30		
	Carbohydrate content	[Nutrients]	4	13.07	< 0.001
		[CO_2]	1	0.35	> 0.05
		Error	30		

***Sample size (n) equal to 7 for initial samples and n= 5 for all treatments but 700-NPK3 with n= 4**

DISCUSSION

Reproductive success

From the three studied species only *C. nutans* completed successfully its reproductive cycle with the production of flowers that developed into viable fruits, constituted by an average load of healthy seeds. Individuals of this species are characterized by the formation of water-nutrient impoundments called tanks that allows them to count on a relatively continuous supply of all resources needed for their growth and survival. The other two species in the study follow the atmospheric strategy, which is characterized by succulent leaves covered with a dense lawyer of trichomes that serves as water storage and assist in water and nutrient uptake, respectively. Plants with atmospheric strategy have been found to mature at a slower rate than tank and terrestrial plants. Apparently first flowering may occur just four years after germination, while many tank forms could achieve the same state of maturity in half the time (Benzing 1973). This may explain the general low reproductive response of the species *T. bulbosa* from which only one isolated individual achieved to produce flowers and, the limitation of *T. subulifera* to produced mature fruits with well-developed and viable seeds during the time of the experiment. These two species started to produce flowers between three and a half to eight months after the experiment onset, which left a range of four to nine months for fruits development and seed-filling, apparently, this time was not enough to become mature. Hence, the observed results more than highlighting the ineffectiveness of each of the treatments to induce a pronounced response on these species reinforce previous findings of the slow developmental rate that characterizes these morphotypes. These results also show that the species *C. nutans* has a very fast developmental rate contrary to the other species and, generalizations addressed in previous studies such that tank species grow very slowly even under near optimal conditions (Hietz, Ausserer & Schindler 2002; Schmidt & Zotz 2002) should be considered carefully.

One support for the relatively low maturation rate of reproductive organs from the two *Tillandsia* species are the extremely low carbohydrates concentrations that I found in a small set of capsules of the species *T. subulifera* (data not shown). If reproductive structures in epiphytes, as suggested by Zotz and Richter (2006), should fix by their own the carbon needed for their development and seed-feeling, assimilation rates may be extremely low or nutrients allocation could also occur at a very slow rate, mostly in the atmospheric species. It seems to be that in spite of high resources availability atmospheric species will continue to show a rather conservative comportment in their development. However, in this species an e[CO_2] did induce an earlier reproductive response compared to plants grown at the a[CO_2], but at the end the number of reproductive individuals was still higher at the a[CO_2]. Elevated [CO_2] has been reported to induce early flowering in some terrestrial species (Rogers *et al.* 1984; Ellis *et al.* 1995). The increases in leaf temperature observed in crop species after prolonged growth under an e[CO_2] concentration (Valle *et al.* 1985; Prasad *et al.* 2002) was suggested as the possible cause of flowering stimulation in those plant species grown under these enriched conditions. The increases in temperature may be due to the reduced stomatal opening, frequently observed under this condition, which limit the gas exchange and, consequently induces an accumulation of energy that could lead to this reported increase.

In regard to reproductive success of *C. nutans* I found that a few individuals of this species experienced sexual reproduction in a period as short as three weeks after the experiment started. Plants started to receive the nutritional supplement just two weeks after the experiment onset whereas the CO_2 was applied since the beginning of the trial. Hence, one may assume that the earlier flowering of those individuals just one week after plants were given the nutrient solution could indicate that the effect of this factor was determinant to activate the reproductive response in those individuals. Nonetheless, other individuals in the trial did not show such response; additionally, the existence of a significant influence of the interactive effect between nutrient supply and maturity status of the individuals before the experiment onset on the early flowering response, indicate that nutrient concentration alone is not responsible for such response. A slightly higher number of those individuals that performed early flowering were adults which confer a higher probability to reproduce compare to “young” ramets. However, the number of adult individuals and “young” ramets in each of the treatments was equivalent and I would have expected that all adults in every treatment would have shown the same response, hence, this result still point out to a different factor that may be responsible of such response.

Another plausible issue is that a high number of the adult individuals comprising the highest nutrient concentration treatments had already undergone, at least, one flowering event before the experimental onset (data not shown), which could have reduced their possibility to reproduce the subsequent year (during my trial). This inability to reproduce in subsequent years has been already reported by Zotz, Laube and Schmidt (2005) under field conditions for other epiphytic species. This apparent difficulty of epiphytic plants to reproduce in subsequent years may also explain the generally low reproductive success of plants grown at the high nutrient supply at both CO_2 concentrations, even after one year of exposure to this high resource availability at the present study. Those treatments were constituted by adult individuals that have already flowered and “young” ramets in “reproductive size”. It seems to indicate that a high nutrient supply fail to increase the probabilities of experiencing a subsequent reproductive event, at least for this species and, “young” ramets may have needed more time to accumulate the resources for reproductive performance to take place. Benzing and Davidson (1979) suggested that those asexual ramets produced by parent shoots grown under scarce nutrient availability may require more time to get mature (over three years).

Certainly, those ramets were produced during the nutrient-deprived storage time at the greenhouse, previous to the experiment.

This left the question open in regard to the higher sexual reproductive performance by ramets subjected to the lowest nutrient concentration at both CO_2 treatments. In this case, I would suggest that the higher response shown by the ramets in these treatments compared to lower or no response of the ramets grown in the high nutrient treatments, that age of the individuals and not size would be a key element to pay attention to. At the beginning of the experiment, there was a mixed of individuals of different ages. During the time in the field after collection and the storage period in the greenhouse, some of the plants used in this study experienced different processes such reproduction and mortality due to senescence or desiccation. After this, some of the plants develop one o more ramets which also went through any of these processes in subsequent time or were on their way to become mature individuals. These mixed of ontogenetic stages or "genetic ages" could be the main responsible of the diverse responses observed in this study. Successfully reproductive adults and ramets in the low nutrient treatment may have been at a maturity age at which just a low amount of resources was needed to pass into the reproductive stage. Opposite, the majority of adults and ramets in the higher resources treatment may have needed more time to reach this life phase or accumulate the optimal resources load. In addition, those that had already undergone a reproductive event may have needed more time to recover from the previous reproductive effort. Epiphytes are known to allocate a huge amount of resources to reproduction (Benzing & Davidson 1979; Zotz 1999), which results in a high reproductive cost (usually observed in these species) where successive growth and survival is highly compromised (Ackerman & Montalvo 1990; Zotz 1998; Schmidt & Zotz 2002) and, probabilities to reproduce successively remains limited as mentioned above.

The previous explanation is also valid for the stronger reproductive response in plants subjected to the a[CO_2] rather than at the e[CO_2], which was contrary to what I had hypothesized that would occur. A higher number of adult individuals were present in the a[CO_2] than at the e[CO_2]. Moreover, the number of individuals that had already flowered before the experiment onset was higher at the e[CO_2]. Similar than observed for high nutrient concentration, an e[CO_2] seems to be also unable to enhance the probabilities of these plant species to experience a successive reproductive event. Finally, the fact that some ramets that emerged during the course of the treatment also produced flowers that developed into viable fruits may set back the explanation about age of individuals limiting the sexual performance of "young" ramets. At least that we look at age as the "genet" age, which would imply that the new and rapidly developed individual would have the same or similar comportment than its own progenitor since life history of previous generations would be contained in its genetic information. The ability of these recently developed ramets to reproduce during the course of the experiment may also point out at a possible stimulatory effect of the treatment, but this occurred in so few isolated cases along the different treatments that these arguments lose strength. Nonetheless, it may be important to keep this in mind in future studies with this group of plants in order to find out the more plausible explanation for it.

Reproductive output

Total seed number

Unexpectedly but deducible from the previous results, reproductive output was also higher at the a[CO_2] than at the e[CO_2]. In terms of total number of seeds, plants grown at the a[CO_2] produced ca. 80% more seeds than those at the e[CO_2]. Also different than I had hypothesized, plants grown at the high nutrient concentration produced the lowest total number of seeds at both [CO_2]. Both results also seem to be explained by the higher number of adult individuals that presented sexual reproduction compared to young ramets at the a[CO_2] and low nutrient concentration treatments. There are many studies that supports the notion of a dependency of reproduction on size or age of the individual (Law 1983; Lacey 1986; Kapela & Lasker 1999; Childs *et al.* 2003; Burd *et al.* 2006), for instance, the larger the individual plant the larger would be its reproductive output. In this study not only a high number of adult became reproductively active, but also a high number of ramets with similar adult size or even some with much smaller vegetative bodies than the “adult” ones. As previously stated, it would not only mean that the age of the genet and not the size would be determinant, but it would also decline the commonly held view of a linear relationship between plant size and reproductive output. Besides, it supports the contrary notion of a presumably and rather non-linear relationship between plant size and reproductive output. Such suggestion is supported on a study dated from ca. two decades ago where the data of three different species was modeled in order to figure out the nature of this relationship, which revealed stronger non-linear relationships between these two “attributes” instead (Klinkhamer *et al.* 1992).

Individuals of the species *C. nutans* also produced the highest number of seeds per capsule at the low nutrient concentration treatments; however, this maximal number was not much higher than that contained in a capsule of plants grown at the high nutrient treatments. The biggest different was in the class of the fruit, in respect to seed number, that was commonly produced by plant individuals at each of the treatments. I found that this species produced three classes of fruits characterized by different seed loads. Plants grown at the lower resources treatments produced a higher number of seeds belonging to the highest class (3er: 100-150 seeds) whereas those grown at the higher resources treatments produced more fruits belonging to the lowest class (1st: 0-50 seeds). Similar seed load ranges were also reported for vigorous adults of the epiphytic species *T. circinnata* grown under high resource availability in a single season (60-140 seeds each). All species aim for a common goal such as to increase their fitness or their probability to pass their genes into the next generation. Hence, higher fitness could be directly achieved by increases in the number of offspring, in this case, a higher number of seeds. In this study growth under low resources availability seems to sustain the probabilities of this plant species to keep their chances to perpetuate until the next generation at an adequate level. However, it would be a more common expectation that high resource availability would enhance the fitness of this or any species. For instances, plants grown near pastures and arable fields (where nutrient availability is high) produced twice the number of seed than plants grown in poorest soils (Valverde & Silvertown 1995). Epiphytic individuals of the species *T. circinnata* grown in nutrient-stressed sites had in average 108 ± 43 seeds, whereas those grown in more fertile sites had twice to five times the average seed load found in poorest sites (Benzing & Davidson 1979).

Epiphytes dwell the canopy of rain forest which are rather poor environments where access to water and nutritional elements is limited. Hence, they have developed a series of strategies that allows them to survive under these conditions. The results of the present study may be telling us that epiphytes are already so evolved and adapted to inhabit these poor environments that higher resource availability would not represent a relevant change for them. Thus, growth under a future enriched environment may not cause big changes in the population dynamics of these species. Nonetheless, based on the results from the field study by Benzing and Davidson (1979), my findings could represent just an isolated case. In addition, a field study by Lasso and Ackerman (2013) did found a higher reproductive success and output in an epiphytic bromeliad in fertilized plots of a tropical forest, but the interactive effect with an e[CO_2] was not evaluated in that study. A better understanding of the nature of the interaction between nutrient concentration and e[CO_2] would be needed in order to elucidate the causes of these results. Growth of plants under an e[CO_2] may be limited since a higher photosynthetic activity by increased availability of the carbon substrate could increase nutritional demands (Tuba & Lichtenthaler 2007) and, low nutrient availability could make it more difficult to sustain the increased demands.

Plants grown under low nutrient supply could have also become more efficient in their physiological activity than those under high nutrient supply, which could be a consequence of a "unintentional" priming possibly undergone by those plant individuals during storage conditions prior to the beginning of the study. During the stay in the greenhouse plants were restricted from nutrient supply for a period of ca. six months, whereby, it might have induced a change in the physiology, such as modification of use and uptake of nutrients, in individuals of this species. Priming of other plants species have been carried out in periods as shorts as few days or weeks, hence a period of six months may have caused a significant change from which I was not aware before the experimental onset. A determination of nutrients concentration in plant individuals from another epiphytic species grown in a nursery showed that excess nutrient content experienced by these plants during those growth conditions may be reduced to basic label in a period of six months (Winkler. U., personal communication). However, there are not such measures in plant epiphytic species collected from natural populations that may allow us to draw conclusions on this fact. Undoubtedly, it is still arguable that a six month restriction of nutrient supply could be somehow deleterious causing, e.g. inability for nutrient uptake or, even beneficial, by increased of nutrient-used-efficiency under further stressful conditions. This last could be translated into extremely low or null nutrient uptake and maximal slowdown of stored reserves. Such process could cause that a high nutrient input could result toxic for these plants and counter effects such reduced performance is achieved instead. To exemplify, increased availability of iron may have resulted toxic and triggered a signal for primary root growth arrest at a gene level in a terrestrial species (Ward *et al.* 2008).

A less complicated explanation could still point out to age, the higher presence of older individuals ("adults") in these low resources treatments that could have had a longer time to accumulate all resources needed to fulfill the nutritional requirements for fruit production or, developed a more efficient strategy in order to show this more pronounced reproductive response. Nonetheless, plant individuals at all treatments experienced an intense and relatively similar reductions in the concentration of all nutritional elements after one year of treatment, which may indicate a similar reproductive effort in all reproductive plants irrespective of the treatment.

Seed quality

Mean seed number

In terms of average seed number, the combination of high availability of one resource (e.g. CO_2) and lower availability of the other one (e.g. nutrients) induced the higher mean seed load per fruit whereas a higher availability of both resources resulted in the lowest mean seed loads. Nonetheless, the observed differences were not significantly explained by any of the factors and the maturity status did not explain either these results. In this species seed load ranges seems to be relatively standards in spite of the conditions. Nevertheless, the variability is high, which is in line with the abovementioned findings in regard to the different seed loads classes defined for this species.

Mean seed weight

Mean weight of whole seed set per capsule was only marginally higher at the a[CO_2], but it may be mostly due to the higher number of seeds found at these treatments than to an actual increase in the mass of each single seed. If the slightly higher seed weight at the a[CO_2] is just a consequence of an increased number of seeds, then individual seeds must still be very light. In general, it would be more advantageous to produce lighter seeds but a higher number instead of, heavier seeds but a lower number in order to increase the probabilities of colonization success and continuity of the species. These results assemble those observations between large and small population of some grassland species. Large populations of grassland species were also found to produce a higher number of seeds than small populations, but these seeds were smaller. Apparently, habitat quality did not explain these differences but other factors such as pollinators' visits and quantity and quality of pollen may have been responsible (Kéry, Matthies & Spillmann 2000). Populations of plants with intramorph incompatibility with a morph frequency away from 50% may also experience reduced fruit and seed set (Byers & Meagher 1992). Since *C. nutans* is a self-compatible and "wind-pollinated" species lower quality and quantity of pollen of plants at the e[CO_2] may be a more plausible explanation. Presumably, an increased competition for resources between vegetative growth and reproduction under an e[CO_2] may have caused a negative impact on quality of reproductive organs that could not be supported even on a high nutrient availability. Hence, CO_2 enrichment seems to have a doubtful contribution to an enhanced fitness in this species.

Seed quality (biochemical composition)

Carbohydrates and nutrient concentration and content

Finally, the expectancy of increased seed quality in plants grown under e[CO_2] was not fulfilled, higher amount and concentration of carbohydrates and nutrients was rather found in seeds of plants grown at a[CO_2]. However, at least in terms of content the higher values of nutrients and non-structural carbohydrates found in seeds of plants grown under a[CO_2] may mostly be due to the higher number of seeds that contribute to the pool rather than to an increased quality *per se*, which is confirmed by the absence of pronounced differences in terms of concentration. An e[CO_2] seems not to represent a great advantage for seed quality as it has been found in some studies with terrestrial species (Biswas & Hileman 1985; Behboudian & Tod 1995). Apparently, plants grown under the actual CO_2 atmosphere do quite well in producing well equipped seeds with good chances to survive once entered into the highly vulnerable seedling stage and, a higher nutrient availability intensify this response. Some authors have also reported decreases or even no change in seeds mineral and carbohydrates constitution in commercial crop species grown under e[CO_2] (Poorter & Grodzinski 1984; Wu *et al.* 1997; Dijkstra *et al.* 1999; Thomas *et al.* 2003).

Growth under e[CO_2] is suggested to increase nutrients demands and, nitrogen and phosphorus are expected to be the ones more susceptible to variations since they participate in a series of physiological activity (Tuba & Lichtenthaler 2007). Similarly, formation of reproductive structures also requires a considerable amount of essential nutrients. Hence, plants grown under this enriched condition should have made a big effort in compensation mechanisms to distribute the available resources the most efficient way for both processes to take place successfully. A higher availability of nutrients in the environment may partly compensate for the increased nutritional demand (Pleijel *et al.* 1999; Kimball *et al.* 2001) without further negative consequences on the final product of reproduction. Although, in my study, plants grown under e[CO_2] could not increase plants´ efficiency to allocate higher load of nutrients to produce seeds of higher quality, even when plants were grown at the highest nutrient concentration. In some legumes species a maximization of nitrogen uptake, for instances, is achieved by an enhanced root system that present a symbiotic relationship with N_2 fixating bacteria (Bottomley & Rogers 1993), which greatly contribute to support the current plant demands for both vegetative and reproductive processes.

In the epiphytic bromeliads in my study the formation of a tank by the overlap of leaves in the rosette may, in principle, confer plants a higher and continuous availability of nutrients, which could result in richer seeds but it was not actually the case. If excess nutrient should be absorbed they must be accumulated in other plant structures such as stems (judging by the also lower amount in leaves). This result could also suggest that expected raise in [CO_2] may represent a challenge for epiphytic plants during their reproductive stage and biological processes may not be well compensated. Maturity status of individuals allocated to each of the treatments at the beginning of the treatment could have also influenced the observed results. Plants grown at the ambient environment were mostly adults, which seem to allocate a higher fraction of their nutrients to seed production that consequently results in seeds of higher content and concentration of soluble sugars and essential nutrients. Plants grown at the a[CO_2] did experience a slightly higher decrease in leaves nitrogen compared to plants grown at the e[CO_2], which could support the idea of higher investment of nutrients in reproduction by plants grown under the a[CO_2]. Although, the quality of seeds produced by young and adult individuals did not show such pronounced differences in most of the cases.

In this study, a higher nutrient availability did cause increase quality of seeds in plants subjected to a[CO_2]. Higher nutrient availability also increase quality of seeds in a lowland epiphytic bromeliad (Lasso & Ackerman 2013), however, quality was measured as seed viability in terms of the proportion of ovules that actually developed into seed. Additionally, the most abundant elements in seeds of plants grown under a[CO_2] were nitrogen, potassium and phosphorus in decreasing order. Similarly, Zotz and Richter (2006) also reported high amount of N, P and K in a tank epiphytic bromeliad but phosphorus was in higher amount than the other two. Moreover, the content of the different elements in seeds of the tank bromeliad tested in this study (*C. nutans*) were considerably lower than that reported in the study by those authors. The content of the different nutrients were in a range located under the lower limit reported by Zotz and Richter (2006). In respect to carbohydrates, the most abundant fraction was also starch, which comprised more than 50% of total carbohydrates contained in seeds of the epiphytic species in their study. Moreover, total non-structural carbohydrates ranges from 2 to 17mg.g^{-1} while in my study total non-structural carbohydrates ranged from very close to cero to a maximum of 17mg g^{-1} along the treatments. However, the highest concentration and content were found at the intermediate nutrient concentration treatment.

Relative growth rate (RGR)

In regard to RGR both the C_3 (*C. nutans*) and CAM (*T. bulbosa* and *T. subulifera*) species behaved relatively similar in most of the cases. As we expected, nutrient concentration had a positive and, in some cases, quite strong influence on the RGR of the three studied species. A high nutrient concentration enhanced even more the positive effects of e[CO_2] on RGR. For instance, individual plants grown under e[CO_2] and high nutrient concentration had the highest growth means and this effect could be observed in all treatment periods in most of the studied cases. However, the species *C. nutans* showed more positive and slightly faster growth means than the CAM species (with few exceptions), which coincides with reports in the literature of C_3 species expected to have higher response to [eCO_2] due to the carbon fixating mechanism (Monteiro, Zotz & Körner 2009). Zotz and Asshoff (2010) also reported consistently lower stimulation of growth rate in CAM epiphytic species irrespective of the [CO_2] whereas C_3 species experienced about 40% stimulation in their growth rates under an e[CO_2]. Nonetheless, when plants were given a much higher nutrient supply they observed a significantly higher stimulation in growth rates of the CAM species as well. For instances, the species *T. fasciculata* achieved a 300% increased of biomass with a final growth rate of 7mg g^{-1}. Similarly, in the present study the CAM *Tillansioid* species *T. bulbosa* grown under e[CO_2] underwent a pronounced increased in its growth rates after one year but just when it was given a high nutrient supply, which was almost twice of that reported for *T. fasciculata*. However, at the rest of the treatments growth rates of *T. bulbosa* were seemly slow.

The other CAM species in this study, *T. subulifera* was not as successful as its congener in any of the treatment, even in the high resources treatments. Relative growth rates of this species were significantly lower with values much lower than zero and even negative. Zotz and Asshoff (2010) also reported growth rates for this same species that were slightly below zero but in plant individuals grown under water stress. During the present experiment, all species were ensured an adequate water supply; hence, the significantly slow or negative growth rates of this species must be due to a different reason. After six months of treatment most individuals of the species *Tillandsia subulifera* had experienced sexual reproduction, which could have compromised the allocation dynamics of those individuals prior and posterior to the reproductive event. As it has been mention before, vegetative growth and reproductive performance are believed to compete for resources (Nitsch 1971; Larcher 1980; Zotz & Richter 2006). In some annual species biomass allocation to reproduction has been seen to increase gradually (Struick 1965; Bell, Hiatt & Niles 1979; Chiariello & Roughgarden

1984). Hence, the investment of organic and inorganic material in formation of reproductive structures might have caused the decreases in growth rates during the second half and by the end of the treatment in this species. These reductions in growth rates once reproduction has taken place have also been called ´reproductive cost´ (Obeso 2002). In other epiphytic bromeliads reduced growth rates in large individuals coincided with increased potential for reproduction (Schmidt & Zotz 2002).

The species *T. bulbosa* was not so successful in reproductive performance since only one individual achieve to produce flower, which did not even develop into a mature fruit. Nonetheless, this species was still unable to experience significant increases in its relative growth rates and these were certainly slow. These slow RGRs may be still related to the preparation of the plants individual for the reproductive phase. Probably, plants allocated most of recently absorbed resources or, displaced available reserves to reproduction, which unavoidable had negative consequences on vegetative growth. The construction of reproductive structures could constitute a major investment and may represent more than 30% of the total plant biomass (Zotz & Richter 2006). But, *T. bulbosa* and *T. subulifera* are atmospheric species, which are usually found in the most exposed areas of the canopy where resources are scarcer and are rather considered oligotrophic habitats (Benzing & Davidson 1979). Slow biomass production implies a slower absorption of the necessary nutrients to grow and reach maturity. A higher availability of resources seems not to alter the rather conservative nature of the biological processes of this epiphytic type. Benzing (1973) already mentioned the unresponsiveness of atmospheric epiphytes to increased nutrient concentration; specifically water and light are considered to limit the capacity for growth even in the presence of nutrients and moisture supplements. Hence, the isolated cases of pronounced increased growth rates in *T. bulbosa* under high resource availability observed in this and another study (Benzing and Davidson 1979) must be taken as exceptional cases and should not downplay the usual comportment of resource economization/conservatism performed by species with this strategy type.

In the C_3 species *C. nutans*, negative and relatively close to zero growth rates were also observed but these were not as lower as those reported for the other two species, particularly, those of *T. subulifera*. Apart from the benefit conferred by the performance of C_3 metabolism, *C. nutans* count on the tank impoundments, which give the possibility to benefit from a continuous nutrient supply. This continuous availability of resources may allow for a compensation of the increase nutrient demand for formation of reproductive structures, but it could also give the possibility to sustain relatively decent growth rates, which ensure maintenance of plant individuals.

An e[CO_2] has been reported to counteract the negative effect of different environmental stressors. Nonetheless, in cases where nutrient availability is scarce a positive effect of [CO_2] has not been apparent. In order to make use of the available carbon reserves for plant metabolism plant needs to have an adequate nutrient status; in the contrary case carbon stimulation could not be triggered (Tuba & Lichtenthaler 2007). In our study, plants grown in the treatments with the lower nutrient availabilities had the poorest growth performance with a significant reduction in their growth rates which indicate a substantial loss of biomass during the course of the treatment. However, decreases in RGR of the different species were still lower at the e[CO_2]. Hence, in future climate change scenario an e[CO_2] could still stimulate growth rate of species inhabiting impoverished environments, such those dwelled by epiphyte species. Growth stimulation may not be so pronounced but it could still reduced mortality of individuals.

Mean growth rates of the three studied species were generally low and comparable to those of other epiphytic species grown under extreme water stress (Zotz & Asshoff 2010), but also similar to those of large reproductive individuals of another tank species (*Vriesea sanguinolenta*) reported by Laube and Zotz (2003). In this last study, large individuals were also relatively unresponsive to increase nutrient supply compared to small individuals. Hence, the results of my study are in line with previous observation.

Survival

Survival of the species *C. nutans* was compromised by growth under an e[CO_2] even in spite of a high nutrient supply, but just at the first treatment half. The slightly lower survival of individuals under e[CO_2] may indicate a possible stress response by increase physiological activity due to a higher availability of substrate. This increased activity could have caused the faster senescent of some of the individuals, particularly some of those that have already undergone sexual reproduction. By the end of the first treatment half, most individuals have already performed sexual reproduction, which could have also decreased the availability of reserves for future growth of some of the individuals. As previously mention, investment in reproduction is huge in epiphytes plants (Benzing & Davidson 1979; Benzing & Ott 1981; Zotz 1999). This, together with previous observations of relatively low responsiveness of large epiphytic individuals of another tank species to increased nutrient availability (Laube & Zotz 2003) may have represented a great challenge for individuals of *C. nutans* to sustain the current growth and survive. Possibly, increased physiological activity of some of the individuals by higher availability of CO_2 may have led to use up the remaining nutrient reserves due to inability to take up the immediate available resources on demand and, this response may have been intensified by lower nutrient availability. Nonetheless, at the second half and by the end of the treatment the effect of an enriched resources environment was rather positive. This may indicate that those few individuals that suffered mortality at the first half could have been unable to support the increased stimulation of activity by higher resource availability, but after a time, some of the more plastic individuals plants seemed to have acclimated to the richer environment, which favored a higher survival compared to ambient conditions.

Survival of the two *Tillandsia* species was significantly lower than that observed for the species *C. nutans.* An increased nutrient supply has a pronounced negative effect on these species survival. Both of them are atmospheric species usually found in the most exposed areas of the canopy, which are characteristic oligotrophy environments (Benzing & Davidson 1979). Baraloto, Goldberg and Bonal (2005) found that seedling of neotropical trees with rather slow-grow had a higher survival when resources were limiting and suggested that it may be due to a more plastic assimilation rate. This comprises a trade-off with faster growth when resources are less limiting. Atmospheric species has been reported to have rather slow growth and maturation rate and, apparently, unresponsive to increase nutrient supply (Benzing & Renfrow 1971; Benzing 1973; Benzing & Davidson 1979), with some exception. Here, at least the species *T. bulbosa* showed the highest growth rate under high resources availability, although, just few individuals were responsible of such increase whereas a great number did not survive until the end of the treatment. The presumably slow assimilation rate in this species may have caused the death of the great majority because already stored reserves could have been insufficient to maintain growth stimulation under an enriched atmosphere. The other atmospheric species, *Tillandsia subulifera* did have a relatively high investment of resources in reproduction; nonetheless, this species was unable to complete fruit maturation by the end of the treatment. This is one more support of the relatively slow assimilation and developmental rate showed by these morphotypes as well as presume limitation to its survival.

Survival after reproduction

In most of the cases, high survival in the species *C. nutans* was related to the presence of leaf offshoots produced by individuals once reproduction has taken place and individuals grown at the lowest nutrient concentration were more prone to developed new ramets. Benzing and Davidson (1979) also observed that, particularly, in plant individuals grown in unusual sterile sites or with scarce resource availability vegetative propagation proceeded sexual reproduction. Similarly, in the present study leaf buds production was paired to sexual reproduction 100% of the cases under low resources availability at both CO_2 concentrations. In a study with a crop species fertilization with nitrogen and phosphorous resulted in rather extra biomass through increased tillering (Siband 1981). Moreover, an enriched atmosphere has already been observed to stimulate axillary shoot development in guava trees (Kriedemann, Sward & Downton 1976). In my study, e[CO_2] also intensified the degree of vegetative propagation, especially under scarce nutrient availability. Vegetative propagation may be less “expensive” than sexual reproduction and, in epiphytes a suitable growth habit together with production of leaf buds represent a mean of resource economization (Benzing 1973), but plants still requires a decent amount of resources in order to succeed with this process. Obviously, low resource availability in the environment could imply certain limitation, which may have led plants to become more efficient in translocation of resources from other structures in order to fulfill the needs. In the case of adult plants, resources might have proceed from the remaining nutrient in fruits stalk once seed feeling was over (Benzing & Renfrow 1971) and, ramets that have not undergone a reproductive event (that do not currently hold reproductive structures) may take directly from the stem. Zotz and Richter (2006) found that large reproductive plants allocated a huge amount of non-structural carbohydrates to stems, which may help for successive vegetative development. Probably, non-reproductive individuals could undergo similar carbon allocation dynamics under stress conditions in order to ensure successive development. Only higher nutrient concentrations in stem were reported for these individuals by the same authors. But, these individuals could also count on a more efficient uptake capacity in order to absorbed resources from the few available in the environment. This ability of most epiphytes to replicate vegetatively or their polycarpic nature could result quite helpful in increasing species fitness or probability to preserve their genes for later generations (Benzing 1990). However, in studying this aspect reproductive phenology under climate change scenario one should be careful at the moment to interpret the results. If an e[CO_2] causes stimulation of vegetative propagation, the recently developed shoots may mature at a faster rate. Since new shoots may become mature in less than one year (Benzing & Renfrow 1971) this could lead to asynchrony in flowering display in nature. Hence, it could be mistaken as a change in timing of flowering along the seasons, but it is just a consequence of a mix of individuals with different ages and independent reproductive rhythm. Asynchrony of flowering could have an important impact in ecosystem dynamics, since animals that use to pollinate these plants may keep active for a longer period of time or even change their preference. Rathcke and Lacey (1985) based on the independency of the different units or ramets of clonal plant species in terms of resource assimilation and allocation, predicted that increased autonomy and environmental sampling would influence the onset and duration of flowering and fruiting within and individuals together with possible variation in the temporal germination patterns of offspring.

Plant morphology

Leaf area (LA) and specific leaf area (SLA)

In spite of the high survival under low nutrient availability relative growth rates of standing individuals of the species *C. nutans* were rather low. These low rates could be reflected in the very low leaf areas presented by plants grown under these treatments. Tilman (1988) stated that species that are better adapted to limited soil resources should optimize their biomass allocation to roots for a more efficient exploitation of the limited resource. However, in tank epiphytic bromeliads participation of roots in resource acquisition is still unclear. Apparently, some authors have measured uptakes rates in adult individuals of *Guzmania monostachia* (S. Reisinger, A. Richter, W. Wanek, P. Hietz, and A. Reich, unpubl. Res) that were comparable to those measured in leaves of the same plant species, but other authors were unable to measure uptake by roots (Nadkarni & Primack 1989; Winkler & Zotz 2009). Benzing (2000) have even suggested that roots in these bromeliads´ type may possible serve just as holdfast structures and, Zotz (2016) suggested that the role of roots may not have a significant role under natural conditions. Hence, the reduction in LA observed in the present study in plants grown under low resource availability may not be the consequence of an increase allocation to roots but a different reason may determine this behavior.

Possibly, plants allocated a higher amount of resources to storage reserves in order to continue growth when conditions should improve, which could be confirmed by the observed decrease in SLA. Reduced SLA has been related to increase carbohydrate concentration in leaves, which in turn could explain the increased thickness observed by some authors under stress conditions (Lambers, Chapin III & Pons 2008). Nonetheless, another possible explanation is related to the maturity status of the individuals. In the low nutrient concentration treatment most individuals were adults and, most of them performed sexual reproduction. Additionally, most of these individuals developed offshoots, which together with sexual reproduction may have implied a significant divertion of resources from leaves construction to each of these processes. Epiphytic individuals of larger size grown under field conditions where nutrient availability is scarce (Benzing 1990; Zotz & Hietz 2001) have been reported to grow relatively slow (Schmidt & Zotz 2002) and, large individuals of the epiphytic bromeliad *Vriesea sanguinolenta* also grown under natural conditions were equally reported to have much more lower SLA than smaller individuals. Conversely, in the present study an increased nutrient availability led to increase LA and SLA, especially when plants grew under an e[CO_2]. In a different study with other three epiphytic bromeliads an increase nutrient availability showed no effect on SLA or LAR of any of the tested species (Zotz & Asshoff 2010). But in studies with crop species, at least, a positive effect of e[CO_2] on leaf area and leaf specific weight has been reported (Allen *et al.* 1990; Vu *et al.* 2001; Prasad, Allen & Boote 2005). However, in the present study higher LA and SLA were probably due to an increased number of leaves per plant than to an increased thickness or even more, to the production of one o more new ramets per plant that may have summed up to the total LA of the mother plant.

As it has been mentioned before, the species *C. nutans* constitutes rosettes that developed a tank-like structure, which is created by the juxtaposition of the leaves at the base of the rosette. Tank serves as storages of water, organic matter rests and, even living organism inhabits these compartments offering plants a continuous resources reserve. The reduced leaf areas presented by individuals grown at the lower nutrient concentrations created a wider distant between leaves in the rosette, which limited the amount of water these plants could catch and store during each watering event. Hence, this poorly developed tank may have forced plants to absorb water and nutrients immediately after the resource is supplied. Sometimes, individuals held one or more leaves that were partly or completely dry, which once in contact with water could easily get rotten and cause the complete loss of that leaves or even death of the whole individual and consequent reductions of foliar mass.

Plant biochemistry

Nutrients concentration and content in leaves

There is a suggestion that the amount of nutrient present in plants is correlated to the availability of them in their surrounding habitat (Gerloff & Krombholz 1966). This suggestion has been also observed in epiphytes, whose leaves nutritional levels may correlates that of their hosts trees (Cardelús & Mack 2010). Similarly, in the present study nutrient concentration in leaves of plant from the species *C. nutans* were generally lower at the lower nutrient concentration treatments and, values were even lower in leaves of plants subjected to an e[CO_2]. As previously mentioned, growth under an e[CO_2] increases nutrient demands (Tuba & Lichtenthaler 2007); hence, plants grown under low availability of each of the nutritional elements may have had higher limitation in keeping a high nutrient balance when grown at e[CO_2]. Nonetheless, the lowest average concentration of each of the elements was similar to those reported for the tank species *V. sanguinolenta* (Zotz & Richter 2006) and, slightly higher than those reported for other eight tank epiphytes (Benzing 1973) both studies with plants collected from natural populations. For instances, lowest mean concentration of the most relevant elements nitrogen (N), phosphorus (P) and potassium (K) in leaves of the eight tank species at the second study were five times, twenty three times and circa three times lower than those found in leaves of *C. nutans* in the present study, respectively. At the upper limit, mean concentrations were also much higher in leaves of *C. nutans* at the present study. Nitrogen was ca. seven times higher in leaves of *Catopsis* (9.18 mgg^{-1} (aCO_2-NPK1) vs. 1.36 mgg^{-1}), P was 38 times higher (3.45 mgg^{-1} (aCO_2-NPK1) vs. 0.091 mgg^{-1}) and K was ca. three times higher (6.09 mgg^{-1} (aCO_2-NPK3 vs. 1.94 mgg^{-1}). In respect to the first field study mentioned above, mean concentrations of each of the main elements listed before in leaves of reproductive plants of *V. sanguinolenta* were near or under the lower limit than those found in leaves of *C. nutans*. In the field P seems to be the most limiting resource and, there are even some suggestions that P could be the most important and most limiting resource for reproductive success (Benzing & Davidson 1979; Zotz & Richter 2006; Lasso & Ackerman 2013). In my study, reproduction was not limited but rather vegetative development once reproduction took place. Particularly, leaf area of those individuals grown under low nutrient availability was the most affected plant trait. Magnesium (Mg) deficiency, for instances, play an important role in chlorophyll formation, photosynthetic carbon fixation, photoassimilate phloem loading and partitioning and, it may also reduce leaf growth (Lemoine *et al.* 2013). However, mean concentrations of Mg in leaves of *C. nutans* were from the 0.68 mgg^{-1} (e[CO_2]-NPK3) to 1.72 mgg^{-1} (a[CO_2]-NPK1) whereas in field collected plants were between 0.11 and 0.24 mgg^{-1}. Still, concentrations were circa six to seven times more than those reported for field collected plants. Hence, it seems to be that a future CO_2 enriched atmosphere may represent a great challenge for performance and development of epiphyte plants in natural conditions, particularly; survival after reproduction may be highly compromise.

In respect to nitrogen, several studies have shown that plants grown under CO_2 enrichment tend to contain less nitrogen than those grown under ambient conditions (Lincoln, Couvet & Sionit 1986; Osbrink, Trumble & Wagner 1987; Fajer 1989; Johnson & Lincoln 1990; Lindroth, Arteel & Kinneay 1995). Additionally, Benzing and Renfrow (1971) found that concentration of N, P and K were most of the time higher in pre-fruiting shoots than in post-fruiting ones in the atmospheric species *T. circinnata*. But the amount of the other nutritional elements (calcium, sodium and magnesium) experienced few or no variation at all in response to age of individual. Most plant individuals of *C. nutans* subjected to the lower nutrient concentration treatments at both CO_2 concentrations were mostly adult individuals in post-fruiting stage, which may explained the observed results. These lower nutrient concentrations in post-fruiting shoots may be due to the contribution of older individuals to the mineral nutrition of attached younger shoots. Zotz and Richter (2006) also found reduced amounts of most nutritional elements in vegetative tissues of reproductive plants of the species *V. sanguinolenta* compared to non-reproductive ones. Phosphorus, calcium and magnesium were particularly reduced at all vegetative compartments. In *T. circinnata* it was found that amount of N needed for reproduction to take place should be over 0.25% to 0.30% and adequate amount of other nutritional elements may also be needed (Benzing & Renfrow 1971). Concentration of nutritional elements found at the present study may also indicate that plants individuals of *C. nutans* may have experienced active storage of reserves.

Finally, epiphytic tank species grown under adequate or high nutrient availability could develop broader leaves that contribute to the formation of a more compact tank structure where water and rest of organic matter can be easily intercepted and storage. This could constitute an additional and longer lasting nutritional source for individuals grown under this condition that helps to optimize biomass production and metabolic and developmental processes. On the other hand, plants grown under low nutrient availability may have some limitation in their performance, since development of narrower leaves could limit the formation of a compact tank structure due to a higher space among the leaves. Thus, the nutritional and moisture reserve may be smaller. The need of all plants for minerals is intimately linked to the production of biomass, growth rates and it is particularly important in environments of low fertility. They must accumulate specific amounts depending on the species, each essential element in order to perform at an optimum. However, vascular epiphytes are usually limited by low nutrient availability in the field (Zotz 2004; Cardelús & Mack 2010; Zotz & Asshoff 2010).

Carbohydrates concentration and content in leaves

The total non-structural carbohydrates concentration in leaves of the species *C. nutans* experiencedd a pronounced reduction at all treatments, which may indicate an active carbon translocation to growing sinks. Reductions were more pronounced at the low nutrient concentration treatments (ca. 100%) and the a[CO_2] (ca. 93%). In those treatments a higher number of individuals experienced sexual reproduction and vegetative propagation. In the tank species *V. sanguinolenta* reproductive structure have been found to represent more than 30% of total plants biomass. However, based on the green color of the different reproductive structures (fruit stalks, bracts and immature capsules) of epiphytes there is the possibility that these organs may be partly self-supporting in terms of carbon (Zotz & Richter 2006). Current photosynthetic activity of reproductive structures in other plant species has been found to account for up to 60% of the carbon needed for their own construction (Chapin, Schulze & Mooney 1990; Aschan & Pfanz 2003). Since I measured the total carbohydrates fraction contained in the whole leaves mass of both maternal plants and ramets, it seems to indicate that a higher amount of carbon was actually invested in reproductive effort rather than in vegetative development. Nonetheless, a significant amount may have also been transferred to the stem as it has previously observed for another epiphytic species (Zotz & Richter 2006), but it is not possible to be sure of this since I did not measure NSC in stems.

There are some observations that plants grown under stressful conditions, particularly, water stress may experience an increment in leaves carbohydrates concentration (Sala & Hoch 2009; Müller *et al.* 2011; Sala, Woodfruff & Meinzer 2012). Apparently, stored carbohydrates in the vacuole may contribute to osmotic adjustment (Wullschleger, Tschaplinski & Norby 2002). Metabolic processes such a respiration may also consume the available carbon reserves (Sala, Woodfruff & Meinzer 2012). Changes in storage may occur just as a consequence of an imbalance between carbon input via photosynthesis and carbon use through respiratory activity (Sala, Woodfruff & Meinzer 2012). Nonetheless, in the present study temperature was kept at an optimal level (25°C) and, an adequate water supply was also ensured. However, based on the significant decreases in carbohydrate concentration at the different treatments plants seem to have suffered an extreme reduction in their photosynthetic activity, which may have been traduced in negligible increases in carbon inputs. Moreover, nocturnal respiration may have still taken place and these carbon losses may have not been compensated by the presumably extremely low photosynthetic rates. Nevertheless, I do not count on measures of the respiratory activity in order to confirm this argument.

Initial carbohydrates reserves were possibly on constant demand by different plant processes at the same time, which could have caused the observed decreases. Sala, Woodfruff and Meinzer (2012) attempted to prove that carbon may be actively allocated to storage in trees, which may compete with processes such growth and metabolism. These carbon reserves could be a source for later and continuous growth in these slow-growing species. Individuals of *C. nutans* used in this study seems to have also actively allocated a considerable part of recently fixed carbon to storage reserve due to stressful conditions in the time previous to the experiment start. In other epiphytic species resumed growth activity after a stressful condition such as that imposed by drought stress, is apparently based on existent carbon reserves (Zotz 1999). Chapin, Schulze and Mooney (1990) also indicated a most probable role of stored carbon in the recovery after tissues loss rather than a relevant contribution to reproduction. Nevertheless, significant reduction of fructose in leaves of *Catopsis* by the end of the treatment may indicate a direct divertion of this monosaccharide to flowers and fruits construction. The relevant presence of fructose as part of flowers nectar is quite known (Baker & Baker 1975; 1983).

The significant reduction in leaves NSC of plants at all treatments may also be a consequence of the very low concentrations of potassium (K) found in leaves of plants at all treatments. Potassium plays an important role in the loading and long-distant transport of sugar in the phloem (Lemoine *et al.* 2013). Hence, deficiencies of this nutrient could possibly cause some impediments in the circulation of sugars along the plant. Finally, there are several reports of increased carbohydrates concentration in crops species after exposure to e[CO_2] (Jolliffe & Ehret 1985; Allen *et al.* 1988; Ahmed, Hall & Madore 1993; Vu *et al.* 2001). Similarly, plants of *Catopsis* grown under a high nutrient supply and CO_2 enrichment were able to keep a higher proportion of NSC (+36%) than plants grown at the same nutrient concentration but a[CO_2]. This indicates that CO_2 enrichment may buffer carbon reductions derived from actual metabolic or developmental demands.

RELEVANT FINDINGS OF PRESENT CHAPTER

Regardless the intrinsically rapid dynamics that some epiphytic species such as C. *nutans* may have, the response to external factors continue to be very conservative. In spite of the enhancement effect that e[CO_2] may have caused on growth and survival, this effect was not so pronounced. Particularly, reproductive output resulted even much lower in plants grown at the resource enriched atmosphere (e[CO_2] and high nutrient supply) compared to plants grown in the poorer resource environment. However, this result may be mostly explained by the higher amount of adult individuals that were present at the latter treatment. Since individuals along the treatments were similar in sizes and even individuals with smaller size than usual for an adult individual were able to reproduce successfully, this result suggests that age rather than size of the individual and their associated life history may have played a more relevant role in explaining the final output. Hence, in these epiphytic species the relationship between size and reproductive output may be non-linear, differently to what it has been suggested in the literature.

Individuals grown at the a[CO_2] contained between 100 and 150 seeds whereas at the a[CO_2] individuals produced fruits containing less than 100 seeds in average.

Vegetative propagation was also more common in individuals grown at the lower resource availability, which is in agreement with previously findings by Benzing (1973).

At the biochemical level (non-structural carbohydrates concentration and essential nutrients) the tested species did not show either such a pronounced response to e[CO_2] and nutrient. Concentration of essential nutrients was slightly higher at the e[CO_2] and high nutrient supply. However, nitrogen was the element most affected by growth under e[CO_2]. This could have important implications in population growth in a future climate change scenario.

In the present study, plants could have maximized their nutrient-use-efficiency since plants seem to have almost exclusively used their initial storage reserved for growth, survival and reproduction and not apparent uptake may have taken place or, probably, just at a extremely low rate. Hence, growth in a future enriched atmosphere may be quite compromised with important consequences in species turnover. In this case, an ineludible lost of epiphytic diversity may occur.

Mature individuals of the studied epiphytic bromeliads may have actually allocated a significant amount of carbon to storage at the expense of growth since their relative growth rates were pretty slow since the beginning of the trial. Furthermore, a significant amount of this storage carbon may have then been invested in reproduction, which traduced in a high cost of reproduction such lower leaf area and, consequent low growth rates and survival after the reproductive event. This effect was particularly pronounced in individuals grown at the low nutrient supply. Meanwhile, individuals grown at the higher resource supplied environment had a slightly higher growth rate, though they died short after reproduction. Regardless of the observations, it is not possible to generalize on this happening due to the inequality of young vs. older adults present at the different treatments. To close, it is yet no clear if an enriched resource atmosphere could contribute to reductions in cost of reproduction in epiphytic bromeliads.

CHAPTER V

SYNTHESIS

Since bromeliads may constitute an important fraction of the biomass present in tropical forests and their role in these ecosystems seems to be of high relevance. Direction of future effects of climate change on this important component becomes a subject of concern. However, not only the biology of this plant group is relatively understudied when compared to other plant groups but also the possible effects of the imminent climate change on the epiphytic flora has barely been studied. This situation motivated the current thesis in which I decided to study the impact of climate change along different ontogenetic stages of epiphytic bromeliads. By looking at the effect of climate change on epiphyte we could not only have some insights on the actual vulnerability of this plant group but also indirectly infer about the possible changes at other levels of the ecosystem, such as their hosts, nutrient cycling and pollinators' dynamics under the future climate change scenario. Particularly, I focused on germination, growth and reproduction of epiphytic bromeliads in response to changes in temperature, water and nutrient concentration in combination to changes in the concentration of carbon dioxide ([CO_2]). The study of each biological process was divided into three different segments.

The **second chapter** of the present work dealt with the germination of 16 epiphytic bromeliads in response to water and temperature as single and interactive factors and the influence of seed maturity on germination success. This first data chapter was thought as a methodological approach to offer a unified way to study the germination of epiphytic plants and, consequently, facilitate future research and interpretation of results on this topic. The **third chapter** focused on the response of young individuals to a simulated increase of 3°C in temperature, which is the expected increase for tropical areas, and its interaction with elevated CO_2 concentration (e[CO_2]). One of my main objectives was to evaluate if e[CO_2] would enhance tolerance of epiphytes to the presumably heat-stress in this future warmer environment, which may keep constant and even traduce in higher relative growth rates (RGR). Additionally, I expected that after long-term exposure to e[CO_2] individuals would experience acclimation of their growth response. In the **fourth chapter**, I aimed to study the reproduction of mature individuals in response to nutrient availability and e[CO_2]. I was mainly expecting that plants grown under high resource availability (high nutrient supply and e[CO_2]) would show the more pronounced reproductive investment, measured as number of seeds. Additionally, I hypothesized that plants grown in this same rich environment would experience a lower reproductive cost than those subjected to the ambient conditions. This last aspect may play an important role in the maintenance of natural population of epiphytes.

As generality along the three studies included in the present thesis, I found that epiphytic bromeliad species unsurprisingly showed rather idiosyncratic behavior at all ontogenetic stages in respond to each of the factors tested, with species-specific responses as a rule. However, there was a consistent trend towards conservatism of responses in front of environmental changes along the different studies. In spite of the high resource availability, epiphytes continue to show a stress-tolerance behavior, that is, plant individuals did not show an enhanced response neither once the resource was available nor even when it was abundant. Even, the species *C. nutans* with its very rapid developmental dynamics did not experience such a pronounce stimulation on its response to a resource enriched environment. Thus, this result just shows that regardless the intrinsically rapid dynamics that may present some epiphytic species, the response to external factors continue to be very conservative. It was previously stated that some species with so called risk-adverse strategy (Loehle 1995) will show little or no response to e[CO_2]. Based on the results of the different chapters, epiphytes may fit into this category but not only with regards to carbon availability but also to any other factor. For instances, in the **second chapter**, expectedly, the duration of watering rather than its frequency resulted more important for epiphytes before they could start the germination process. Similarly, in the **third chapter** the three species showed an increased growth response to e[CO_2] just after half year of exposure to this treatment, but the response was still low. In the **fourth chapter**, none of the three studied species showed a consistent and strong positive effect to increased availability of resources along the different treatment periods in terms of survival or growth. In addition, also in contrast to expectation reproductive output was even much lower in plants grown at the high nutrient supply and e[CO_2] than in those grown under ambient conditions and low nutrient availability. Nevertheless, in the last two chapters, it was possible to see that e[CO_2] could still counteract some of the negative effect of increased temperature and low nutrient availability, respectively, at least in regard to growth rates of the different studied species. However, this effect was not so pronounced, which support the consideration of epiphytic plants being in a highly vulnerable position in front of the future change scenario.

Particularly, when it comes to increased temperature, epiphytes resulted highly sensitive with extreme reduction in their growth rates accompanied by a very high mortality, at least in one of the species. The repeated finding of highly species-specific responses in epiphytic bromeliads and the ample spectrum of these responses, i.e., the high tolerance of some species and very low tolerance of others indicate that epiphytic communities in a future warmer environment may be missing some of the species currently present in their assemblages. Due to the important contribution of epiphytes, particularly, of epiphytic bromeliads to the ecosystem, impact on the whole may result of relevant magnitude. The high sensitivity to increased temperature, even when just 3°C, point indirectly out to the commonly held view of water being the most limiting factor for epiphytes growth and survival. Increased temperature raised evaporative demand and tissue water loss becomes an important obstacle for growth. A study by Müller, Albach and Zotz (2017) showed that germination of epiphytic bromeliads may not be negatively affected by the future increase in 3°C in tropical temperatures. Predicted mean annual temperature is among the optima thermal niches breadths of most species. However, these authors suggest that due to the high niche conservatism that characterizes the family Bromeliaceae – as we have observed along this study -, a possible negative effect should not be dismissed. Moreover, the existence of species-specific responses in that study and the present one still set epiphytes on a highly vulnerable position in front of a 3°C increase in tropical temperatures. It was already proposed by Janzen (1967) that tropical species may suffer more from any small changes in environmental factors due to their coexistence in such relatively uniform conditions defining tropical ecosystem. The species that were used in the two growth experiments performed C_3 and CAM metabolism. Species featuring C_3 metabolism are supposed to be highly favored by increased carbon availability in the atmosphere whereas CAM species due to their carbon fixating mechanism would barely benefit. Nonetheless, there are different reports of increased vegetative development in CAM terrestrial species subjected to a[CO_2] (Poorter 1993; Nobel & Israel 1994; North, Moore & Nobel 1995; Zhu & Goldstein 1997; Drennan & Nobel 2000). Even for epiphytic plants there is a study that reports comparable growth rates of C_3 and CAM epiphytic species (Monteiro, Zotz & Körner 2009). Hence, the low responses to increased concentration of this gas observed in the present studies may be rather surprising and it only emphasizes the conservative nature of epiphytes in front of the environmental changes.

An important component to take into account at the moment to run a study with epiphytes is age of individuals besides size. Due to their clonal nature, life history information saved in their genetic material may still influence their actual response in front of the different growth environments and cause high variability in the results. In the fourth chapter, the observed differences between the different nutrient and CO_2 treatment may be mostly an artifact caused by the different age of individuals tested in the study. For instances, at the lower nutrient concentration and ambient CO_2 concentration (a[CO_2]) there was a higher proportion of young adults. Nonetheless, some of the young adults with similar and lower size than the older adults were also able to perform sexual reproduction. This leads to confirm the notion of a rather non-linear relationship between reproductive output and size of individuals. If an e[CO_2] or an increased nutrient availability may have determined the earlier response of some of the younger individuals (accelerate their maturation at a smaller size) is still to be determined since the number of individuals showing this behavior was rather few and somehow stochastic.

The epiphytic bromeliad species tested in the present study show to be very efficient in front of environmental stress and even more their characteristic stress-tolerance was strongly exemplified. For instances, in the fourth chapter a higher number of individuals grown at a[CO_2] and lower nutrient availability propagated vegetatively and the frequency of occurrence per individual along the experiment was also higher in comparison to individuals grown at the higher nutrient and carbon availability. Vegetative propagation has been referred to as a strategy of nutrient economization, particularly under scarcity of resources (Benzing 1973). But this polycarpic nature of epiphytes that favors the asexual replication of individuals, may also constitute a strategy to increase the chances of the species to pass its genes into the next generation (Benzing 1990); such as it has been previously suggested for species with clonal nature in general (Aarssen 2008). Even in spite of the observed differences in the behavior between young and old epiphytic adults in front of environmental changes. The ability of adult plants to perform an efficient strategy in order to ensure the longevity of their genes in the population, such as the generation of leaf offshoots, remarks once again the adaptability of these plants to the stochastic nature of canopies´ habitats defined by the highly intermittent availability of resources (Zotz & Hietz 2001). Additionally, the polycarpic nature of these plants and the mix of developmental stages of individuals derived from the same rosette could cause some confusion at the moment of interpreting phenological patterns of epiphytes, especially in front of environmental changes. For instances, polycarpy may cause that more than one ramet attached to the same mother plant could flower simultaneously. These ramets may be at different maturity stages or their developmental dynamic may have been shortened in some of the existent new generations, which could have directly heritage the chronological age of their immediate ancestor**.** Hence, interpretation of results from a study aimed to assess the effect of e[CO_2] or nutrient availability on phenological patterns of epiphytes may be done with high care since changes in the developmental dynamics or flowering display of individuals may only reflect the natural vegetative and phenological dynamics of these plants and not a variation in response to the novel environmental conditions.

In regard to reproductive cost in a future CO_2-enriched atmosphere results of these studies are not so conclusive. Plant growth after reproduction was enhanced in plants grown at the e[CO_2] treatments, particularly when nutrient concentrations were high. However, these treatments were constituted by a very low number of individuals and few of them survived until the end of the experiment. The higher growth rate may also be a consequence of the more conspicuous reproductive organ (floral stalk) developed by these individuals, which had up to two axis. Additionally, individuals grown in the low resource availability treatment were in majority individuals belonging to a different age category (old adults), which apart from having a higher intrinsic capacity to reproduce compared to younger adults may have also done such a pronounced investment in reproduction that it may have compromised future growth. Moreover, e[CO_2] may act as stress for this plants obeying to the high mortality in spite of increased nutrient availability. Growth under e[CO_2] has been reported to increase nutrient demands, particularly, nitrogen (N) has been reported to decrease after long-term exposure to e[CO_2]. One of the possible explanations is the dilution of nitrogen by increased accumulation of non-structural carbohydrates. Taub & Wang (unpublished) distinguish between biomass dilution and functional dilution. The former occurs when the total increase in total biomass under e[CO_2] relative to growth under a[CO_2] surpasses the increase in N. Functional dilution based on the functional balance concept which sets N tissue concentration as dependent on the relative activities of shoots and roots. However, this last one may not be completely applied to epiphytic bromeliads due to the still uncertain functionality of their roots beyond holdfast. Furthermore, high nutrient concentration may rather result toxic for this slow growing species such as it has been reported for other plant species (Ward *et al.* 2008).

After long-term exposure to e[CO_2] many plants have shown increased carbohydrate concentration in leaves (Lambers, Chapin III & Pons 2008). It usually occurs when sinks ceased growth and demand no more assimilates due to acclimation to the e[CO_2]. Specifically, accumulation of soluble hexoses has been suggested to act as osmotica in order to deal with water-stress and there is some evidence of its occurrence in terrestrial species (Wullschleger *et al.* 2002). However, this may not be a plausible strategy for epiphytes since the water is usually unavailable in the canopies substrates. Hence, epiphytes may confront water stress derived from low water availability or increased temperature by different means. In the present study, unexpectedly, plants grown under e[CO_2] had lower carbohydrate concentration and this response was slightly more pronounced in plants subjected to high temperature. Apparently, e[CO_2] did not represent such an advantage for these plants, especially, in reducing the increased respiratory losses of carbon under a warmer environment. However, this response was also species-specific since, at least, one species was able to survive in the warmer environment when CO_2 was the double whereas at the ambient treatment it experienced 100% mortality already after six month. It is important to remind that carbohydrates concentration did not increase along the treatment, but species grown at a[CO_2] were more able to keep a higher balance concentration of the initial reserves available. This shows one more that epiphytes seem to be well-adapted to their actual growing conditions in the field. In regard to different nutrient supplies along the treatment, I observed that an increased availability of it did confer a benefit over plants grown at the lower availability. For instances, the observed significant decreases of initial carbohydrates concentration were less pronounced in plants grown at the highest nutrient concentration compared to the rest of the treatments. These results consistently indicate that at the biochemical level epiphytes seems not to benefit in such a higher proportion as expected from e[CO_2] atmosphere. Furthermore, these strongly "testify" for the conservative nature of epiphytes.

Nitrogen (N) concentration was found in significantly higher proportion in plants grown under high temperature and e[CO_2]. However, N content was lower in plants grown in the warmer environment. As mentioned above N concentration has usually been found to decrease under e[CO_2], possibly due to dilution of N by increased concentration of carbohydrates and, decreases has been reported to be more pronounced in presence of higher levels of other factors such as temperature (Yin 2002). Higher N concentration under increased temperature is often related to higher presence of N based enzymes and proteins involved in the protection against oxidative damage (Heat-shock enzymes) by reactive oxygen species (Ougham & Howarth 1988). However, even when I observed detrimental effects of high temperature in plants subjected to a 3°C increase in temperature results are too vague as to affirm the occurrence of oxidation in the tested species if, i.e., more pronounced variations and much more higher temperatures in nature are taken into account. But, there is a subtle indication that plants grown at the enriched environments are apparently – to a certain extent – more efficient in dealing with increased temperatures. In regard to the lower N content it may just be a reflection of biomass loss due to, i.e., leaves desiccation after water loss through increased evapotranspiration. A low nutrient availability also has negative consequences on N concentration and other nutritional elements, particularly when plants were grown under e[CO_2]. However, effects of e[CO_2] on N continue to be more pronounced since all nutritional elements except N were found in ca. twice the amount in comparison to the a[CO_2]. This result indicates that N may certainly be the factor limiting plants growth and development under a future enriched panorama. If N may also represent the actual limiting factor for reproduction in epiphytic plants could not be deduced from the results of the current study. In the literature, there are consistent references to phosphorus (P) as the limiting factor for reproduction in epiphytic bromeliads (Zotz & Richter 2006; Benner J., Vitousek & Ostertag 2010; Lasso & Ackerman 2013). However, there exist some suggestions about a possible co-limitation by P and N (Zotz & Asshoff 2010) or that it could even be N as single, the factor limiting reproduction (Wanek & Zotz 2011). Nevertheless, identifying the limiting factor for reproduction is beyond the scope of this study, though, I just wanted to do a short mention on the actual status of this notion.

Epiphytes are known by luxury uptake of nutritional elements (Winkler & Zotz 2010), but they seem to use the acquired resources at an extremely slow rate, which characterizes their adaptation to the highly stressful environments they inhabit. Similarly, a prolonged period of forced-starvation, that is, artificially imposed after collection from the field, may slow-down even more this uptake of nutrients. This may be inferred from the behavior shown by plants at the present study, which were subjected to nutrient starvation for a period of six month before the experiment started. Based on the pronounced decrease of initial nutrients concentration along the treatment, even in plants grown under high nutrient availability, which concentrations means did not surpass those from initial samples, it could be speculated that plants in the present study either did not take up nutrients during the treatment term or they became so conservatively efficient by just absorbing the exact amount needed to immediately compensate the current use. This could be supported by the still higher amount of nutrients present in leaves of plants grown in the environments with higher nutrient availability compared to those under low availability. Epiphytic bromeliads grown in a nursery under excessive nutritional conditions needed about six months in order to sink their nutrient reserves to basic levels (a level comparable to field-grown plants?, Winkler, personal communication). Field-grown epiphytes are subjected to intense nutrient scarcity, hence, based on the previous observation field collected plants would have been expected to experience a complete depletion of nutritional elements in a shorter time period. However, they seem to have maximized their nutrient use instead since initial concentration of each nutritional element were much higher than other field collected epiphytic species (Benzing 1973) and, individuals seems to have manage to growth, survive and reproduce by almost exclusive use of this previous reserves. If the optimization of nutrient use is just a temporal change or rather represent a change already fixed at a physiological or even genetic level could not be ensured. But, luxury consumption seems to be even more pronounced under laboratory and greenhouse conditions, where these plants are given an adequate or even abundant resource supply. Furthermore, this finding does emphasize once again the extremely conservative nature of epiphytes at the biochemical level. Finally, it seems plausible that a maximization of nutrient use efficient under a climate change scenario could not only decrease future growth rates but also extend the time needed for species turnover, which consequently traduce in loss of diversity of epiphytes communities. Jump and Peñuelas (2005) already pointed out that effects of simultaneous habitat fragmentation and climate change could cause a decrease of the genetic diversity of slow-growing species with large generational time such as trees (Parmesan 2006) and, epiphytes are well-known for their inherently slow-growing and developmental dynamics.

Similar to observations in other plant groups, leaf area (LA) of epiphytic species were also reduced in plants grown at the low resource or “stressful” conditions, i.e., low nutrient supply and high temperature. Reduced amount of magnesium (Mg) has usually been related to reduced leaf tissue (Lemoine *et al.* 2013) and, in this study levels of magnesium in leaves experienced a reduction along the treatment (see Chapter IV), which may partly explain the observed reduction in the photosynthetic organ. In the case of temperature, reduced LA could be the product of reduced surface/volume ratio due to increased evaporative demand. Species under high levels of light or temperature usually show a reduction in leaf surfaces, but an increase of volume (succulence) in order to counteract the water losses by increased evaporative demands. An enriched CO_2 atmosphere is apparently unable to confer any advantage to plants to counteract the negative effects of increased temperature on LA. Additionally, individuals grown at the higher temperature developed a series of vegetative buds along the study, which may partly explain the reduction in LA and, at the same time the reduced specific leaf area (SLA). The new buds had smaller size but the number of leaves was higher, which may account for the increased weight of leaves traduced in reduced SLA. Decreased LA in plants grown under low nutrient availability may also be explained by higher number of adult individuals in these treatments, which performed sexual reproduction. A higher amount of resources may have been directed to construction of reproductive structures rather than more leaves. Similarly, in these low nutrient treatments the production of leaf offshoots was more common, which could have also contributed to the smaller LAs and reduced SLAs.

In the second chapter, as part of the methodological protocol I also found that maturity of seeds did not represent a high impediment for germination. Collecting seeds even four months before natural dehiscence led to relatively high germination percentages in all of the species tested. A similar observation was already reported in the literature about three decades ago for a *tillandsioid* epiphytic species (Fernández, Beltramo & Caldiz 1989). It seems that these plants are able to produced well-equipped seeds from the beginning of the fructification. Probably as strategy to ensure the germination in case dispersal is set into course by a sudden environmental change. Another important observation from the germination studies was on the importance of water for epiphytic species. When it comes to germination, as previously mentioned, the duration and not its frequency was more crucial for this plant group since a lower number of seed germinated when grown under these conditions. But, I also observed that water scarcity becomes more important when plants are grown under temperatures as low as 20°C and 15°C. However, this aspect may mostly indicate that those two are suboptimal temperatures for the activation of the enzymes involved in the germination process, at least, for these species. Finally, I found that in studying the germination of epiphytic species the use of dry-wet cycles of specific time extension is only qualitatively similar to the use of water potential. Species in nature are met with intermittent or long lasting rain events, which extend in scale of hours, days or weeks. Hence, the simulation of different dry-wet cycles in an hours-scale may be a more realistic proxy to assess the water relationships in germination of epiphytic species and, allowed better extrapolation from laboratory to field conditions. Certainly, this procedure may be applicable to species others than epiphytes.

Taking together results from **chapter III** and **chapter IV**, it becomes evident that stressors such as temperature and nutrient availability may play a more relevant role in limiting plant performance than changes in the concentration of CO_2 *per se*. Even further, a doubling of current $[CO_2]$ may not immediately counteract the negative effects of those environmental stressors. A possible buffer effect of CO_2 on growth, development, morphology and biochemistry of epiphytic bromeliads may take a longer time period in order to be perceived. It recalls on the risk-adverse-strategy which states that species of impoverished habitats, such those inhabit by epiphytes, may show little or not respond to enriched environmental conditions. And, regarding to CO_2, Loehle (1995) predicted that species that present a risk-adverse-strategy will respond little or not at all to $e[CO_2]$.

OUTLOOK

1- Results showed that before starting an experiment with polycarpic epiphytic bromeliads it is necessary to take into account not only the size of individuals but also their age since due to their clonal nature, life history of individuals may influence the responses. Even more, in studies dealing with reproduction of the polycarpic bromeliads it would be highly recommended to grow the species from seeds in the lab, at least, that you can ensure that all plants belong to the same generation. By this mean, you ensure that you are aware about all possible biological processes that may have taken place along the course of individuals´ development. Seeds may be easily primed and the time needed to endure a certain trait in them, such as, drought tolerance can be reached in a period as short as 24 hours. Thus, it may ensure that once individuals reach maturity any variability observed comes from any other source less different generational time.

2- An interesting follow-up experiment may be to assess the germinability of the seeds produced by individuals at the different treatments in order to look for any possible differences depending on the growth environment of mother plants.

3- Epiphytes are usually said to be inherently slow-growing species, however, due to the rapid dynamics showed by the tank species *C. nutans* in this study it would be interesting to assess developmental dynamics in a bigger set of tank epiphytic species in order to avoid generalizations. Part of the previous assertion is based on the developmental dynamics of the species *V. sanguinolenta*, which individuals reaches much bigger sizes than *Catopsis* species, hence, one may take into that the time needed to develop a bigger body should be certainly longer. Nonetheless, species such as those holding atmospheric strategy, which usually have small bodies, are known for developing at a much slower rate than tank species, which may obey to the inability to storage nutrients such tank species do. It would still be interesting to assess if there are other tank species, which may develop at a slower rate and what makes them different from each other. Additionally, assessment of a big set of tank and atmospheric bromeliads may be reconsidered in order to update our knowledge on the actual differences in their developmental dynamics.

4- It may be interesting planning nutrient-starvation priming studies with epiphytic bromeliads by subjecting them to different levels of nutrient scarcity and evaluate in deep the changes that may take place in their physiology, such as posterior nutrient uptake and use, that is, nutrient-use-efficiency. Possible effects in photosynthetic responses that may affect after-growth could also be included.

Since N may be limiting growth of epiphytes under e[CO_2] it could be interesting to look in depth at the nutrient uptake mechanisms of epiphytes, particularly, if roots may have an important role in uptake rates since in terrestrial species decreases of N concentration after long-term exposure to e[CO_2] may be caused by different reasons beyond the carbon dilution hypothesis. Most of those other hypotheses are related to the ability and structure of root system and, interaction of it with the surrounding soil. This may be specially addressed when experiments to test the effects of nutrient availability are planned.

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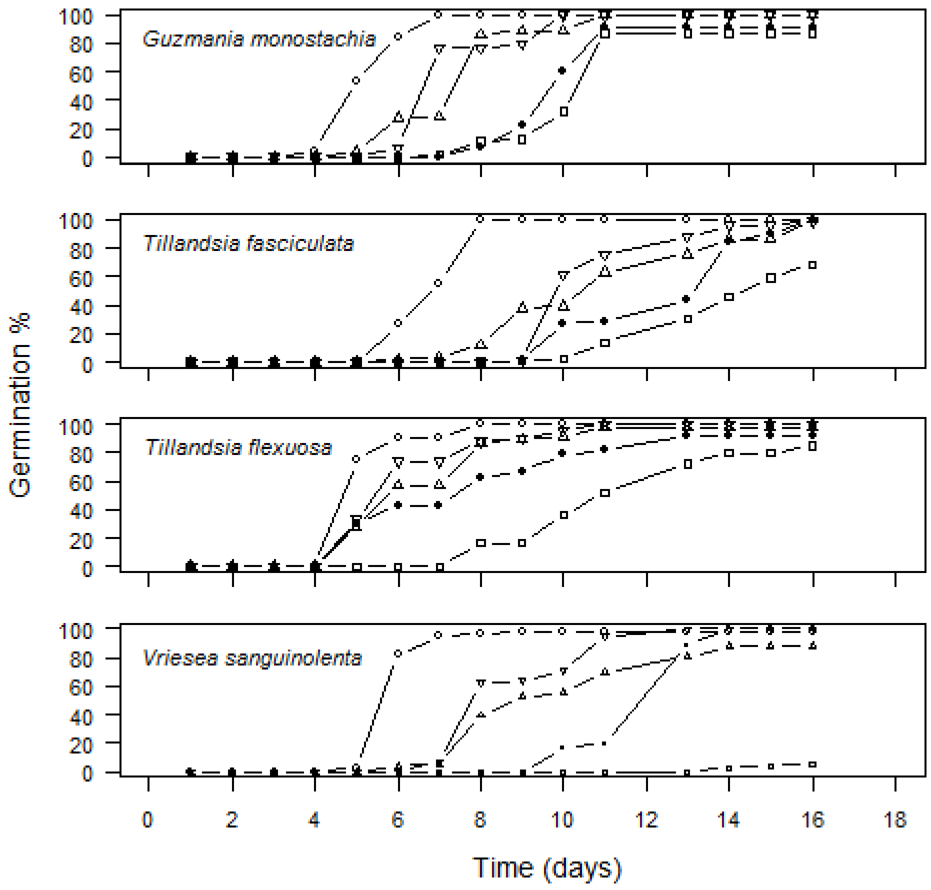
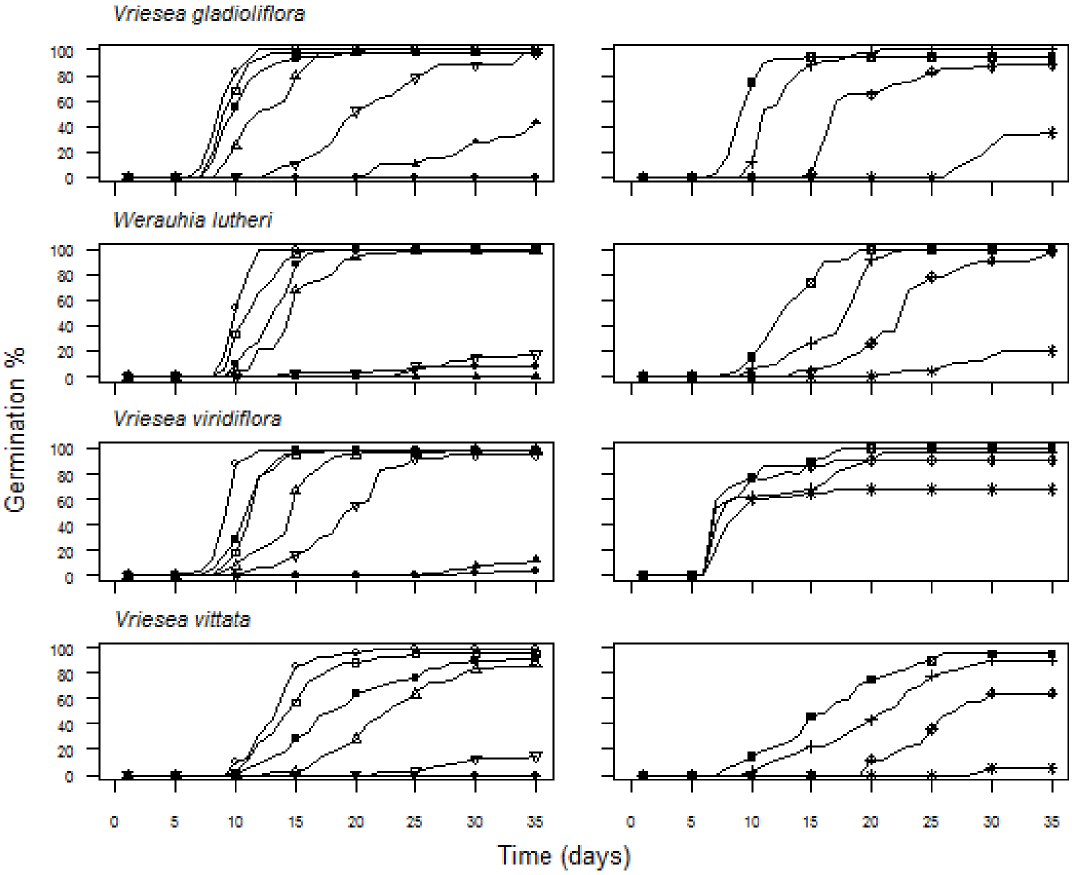
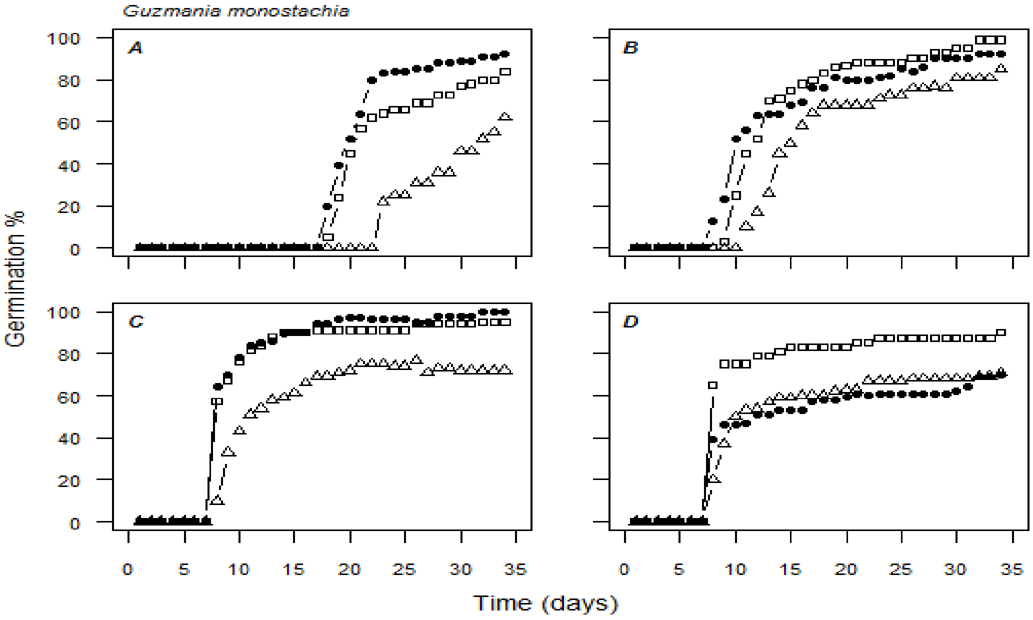
APPENDICES

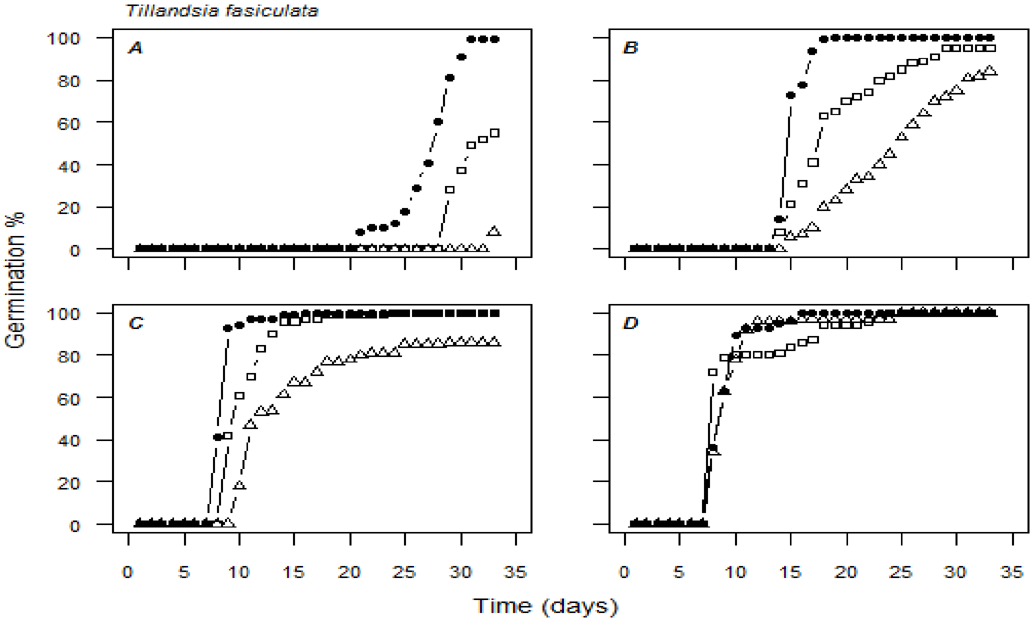


Appendix 1. Cumulative germination in response to different wet-dry-periods for the species: A. *Guzmania monostachia*, B. *Tillandsia fasciculata*, C. *Tillandsia flexuosa*, D. *Vriesea sanguinolenta*. Symbols in lines represent the different dry/wet periods in hours: Line with filled circles: control treatment (continuous moisture), line with filled squares: 12/12, line with solid circle: 24/24, filled triangle point-up: 36/36, filled triangle point-down: 48/48.

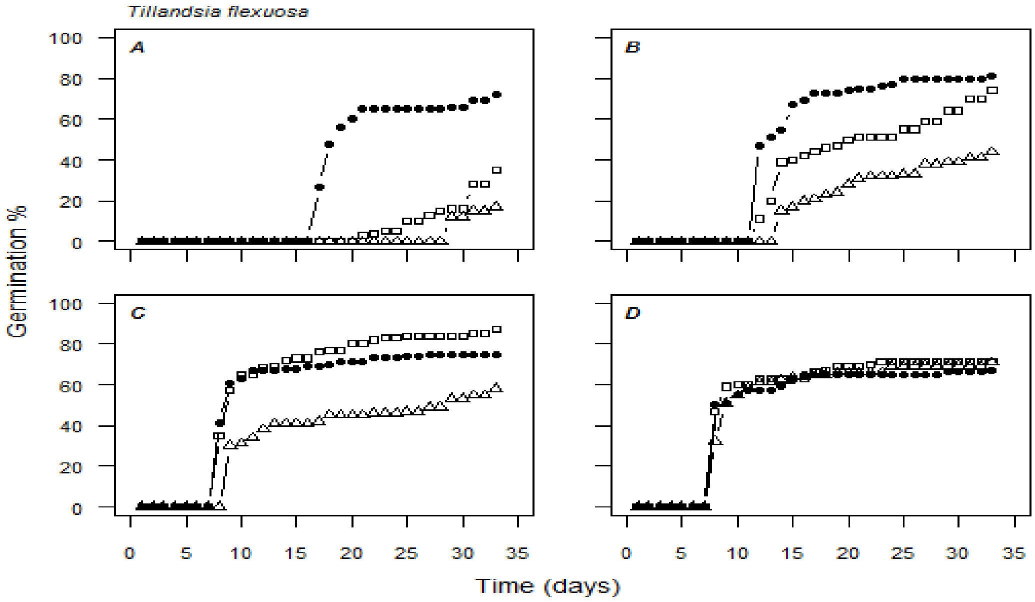


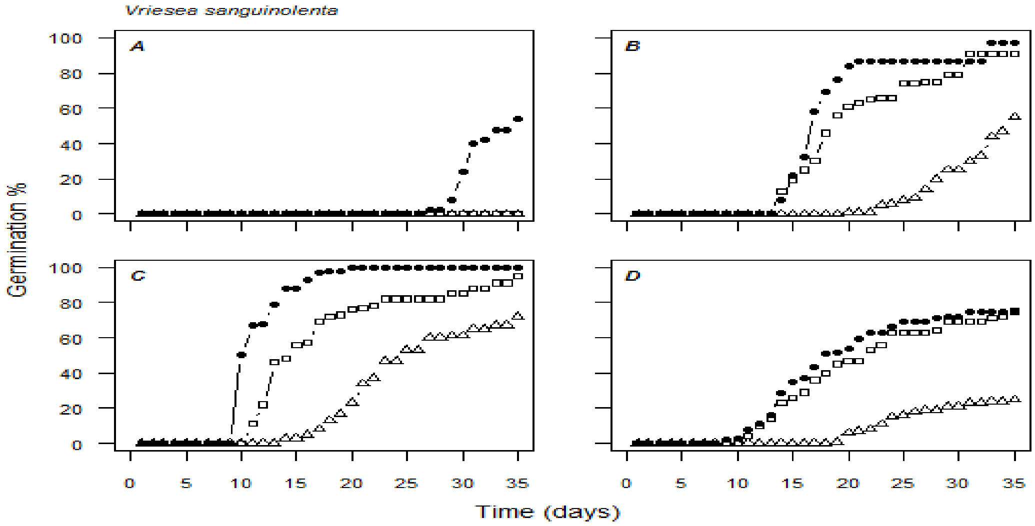
Appendix 2. Cumulative germination response of four bromeliads at different water potentials (left side) and different treatments with intermittent drought (right side). Water potentials were 0 (filled circle), -0.10 (filled square), -0.25 (solid square), -0.50 (filled triangle point-up), -1.0 (filled triangle point-down), -1.50 (solid triangle), and -2.0MPa (solid circle). The different drought treatments are D0 (square cross), D1 (plus), D2 (diamond plus) and D3 (star).





Appendix 3.A. Cumulative germination in response to different temperature and water combined treatments for the species *Guzmania monostachia*, *Tillandsia fasciculata*, *Tillandsia flexuosa*, and *Vriesea sanguinolenta*. A: 15°C, B: 20°C, C: 25C, D: 32.5°C. Symbols in lines represent the different water treatments: solid circles: continuous moisture, filled squares: 2 hours dry period, triangle point-up: 6 hours dry period.





Appendix 3.B. Cumulative germination in response to different temperature and water combined treatments for the species *Guzmania monostachia*, *Tillandsia fasciculata*, *Tillandsia flexuosa*, and *Vriesea sanguinolenta*. A: 15°C, B: 20°C, C: 25C, D: 32.5°C. Symbols in lines represent the different water treatments: solid circles: continuous moisture, filled squares: 2 hours dry period, triangle point-up: 6 hours dry period.

Appendix 4. Growth means of three Bromeliaceae species in response to different combinations of CO_2 and temperature

Guzmania wittmackii

Treatment (ppm - °C)	400-27	400-30	800-27	800-30
RGR (mgg-1d-1), t0-t6	6.79	0.62	4.74	1.58
RGR (mgg-1d-1), t6-t12	2.29	NA	2.68	2.29
RGR (mgg-1d-1), t0-t12	4.73	NA	3.70	1.54

Tillandsia dyeriana

Treatment (ppm - °C)	400-27	400-30	800-27	800-30
RGR (mgg-1d-1), t0-t6	5.82	3.96	5.08	4.61
RGR (mgg-1d-1), t6-t12	0.94	-0.55	2.96	1.70G
RGR (mgg-1d-1), t0-t12	3.31	1.62	3.98	2.83

Vriesea duvaliana

Treatment (ppm - °C)	400-27	400-30	800-27	800-30
RGR (mgg-1d-1), t0-t6	10.40	5.14	4.78	4.55
RGR (mgg-1d-1), t6-t12	1.10	1.61	0.11	-0.14
RGR (mgg-1d-1), t6-t12	5.37	5.20	4.74	4.31

Appendix 5. Results of a Post hoc test (HSD Tukey-test) on the effect of CO_2 and nutrient on vegetative propagation of the species *Catopsis nutans* and *Tillandsia subulifera*

Species	Response variable	Contrast	df	t.ratio	*P* value
C. nutans	Vegetative propagation	350-NPK2-Adult vs. 700-NPK1-Bud	NA	3.1	0.0812
		700-NPK2-Adult vs. 700-NPK1-Bud	NA	3.1	0.0897
		350-NPK3-Adult vs. 700-NPK1-Bud	NA	3.31	0.0441
		700-NPK3-Adult vs. 700-NPK1-Bud	NA	3.3	0.0457
		700-NPK1-Bud vs. 350-NPK3-Bud	NA	-3.2	0.06
T. subulifera	Vegetative propagation	Non-significance of any group			

Appendix 6. Results of a Two-way Anova on the effect of CO_2 and nutrient concentration on carbohydrate concentration and content in seeds and capsules of the species *Catopsis nutans*

Species	Response variable	Factor	df	F	*P* value
Capsules	Carbohydrate concentration	[CO_2]	1	12.94	< 0.05
		[Nutrients]	2	5.6	< 0.05
		Error	19		
Seeds	Carbohydrate concentration	[CO_2]	1	0.2	> 0.05
		[Nutrients]	2	0.2	> 0.05
		Error	18		
Capsules	Carbohydrate content	[CO_2]	1	14.1	< 0.05
		[Nutrients]	2	3.09	0.069
		Error	19		
Seeds	Carbohydrate content	[CO_2]	1	1.16	> 0.05
		Error	20		

Appendix 7. Results of a Three-way Anova on the effect of CO_2, nutrient concentration and seed maturity on nutrient concentration and content just in seeds of the species *Catopsis nutans*

Nutrients	Factor	d.f.	*F*	*P* value
[Nitrogen]	[CO_2]	1	1.17	> 0.05
	[Nutrients]	2	0.43	> 0.05
	Maturity Status (MS)	1	3.5	0.09
	[CO_2] x Nutrients	2	2.17	> 0.05
	[CO_2] x MS	1	10.75	< 0.01
	[Nutrients] x MS	1	12.5	< 0.01
	[CO_2] x Nutrients x MS	1	8.8	< 0.05
	Error	12		
Nitrogen Content	[CO_2]	1	4.2	0.06
	[Nutrients]	2	0.7	> 0.05
	Maturity Status (MS)	1	1.4	> 0.05
	[CO_2] x Nutrients	2	1.46	> 0.05
	[CO_2] x MS	1	5.3	0.04
	[Nutrients] x MS	1	5.71	0.03
	[CO_2] x Nutrients x MS	1	3.13	> 0.05
	Error	12		
[P]	[CO_2]	1	0.03	> 0.05
	[Nutrients]	2	0.005	> 0.05
	Maturity Status (MS)	1	0.02	> 0.05
	Error	17		
Phosphorous Content	[CO_2]	1	2.8	> 0.05
	[Nutrients]	2	1.1	> 0.05
	Maturity Status (MS)	1	0.03	> 0.05
	Error	17		
[K]	[CO_2]	1	0.07	> 0.05
	[Nutrients]	2	0.1	> 0.05
	Maturity Status (MS)	1	0.7	> 0.05
	[CO_2] x Nutrients	2	2.12	> 0.05
	Error	15		
K Content	[CO_2]	1	1.61	> 0.05
	[Nutrients]	2	0.6	> 0.05
	Maturity Status (MS)	1	0.03	> 0.05
	Error	17		

Appendix 7: Continuity

Nutrientes	Factor	d.f.	*F*	*P* value
[Ca]	[CO_2]	1	0.003	> 0.05
	[Nutrients]	2	0.6	> 0.05
	Maturity Status (MS)	1	0.13	> 0.05
	[CO_2] x Nutrients	2	0.52	> 0.05
	[CO_2] x MS	1	0.75	> 0.05
	Error	14		
Ca Content	[CO_2]	1	3.5	0.09
	[Nutrients]	2	3.8	0.05
	Maturity Status (MS)	1	0.6	> 0.05
	[CO_2] x Nutrients	2	0.91	> 0.05
	[CO_2] x MS	1	0.87	> 0.05
	[Nutrients] x MS	1	1.5	> 0.05
	[CO_2] x Nutrients x MS	1	4.2	0.063
	Error	12		
[Mg]	[CO_2]	1	0.58	> 0.05
	[Nutrients]	2	2.5	> 0.05
	Maturity Status (MS)	1	0.1	> 0.05
	[CO_2] x Nutrients	2	0.34	> 0.05
	[Nutrients] x MS	1	0.2	> 0.05
	Error	14		
Mg Content	[CO_2]	1	1.5	> 0.05
	[Nutrients]	2	0.2	> 0.05
	Maturity Status (MS)	1	0.3	> 0.05
	Error	17		

Appendix 8. Results of a Post hoc (Tukey HSD test) on the effect of Time, CO_2 and nutrient interaction on RGR for three Bromeliaceae species

Species		Contrast	df	t.ratio	*P* value
Catopsis nutans	Over a year	NPK1 - NPK2	25	2.21	0.09
		NPK1 - NPK3	25	2.2	0.09
		NPK2 - NPK3	25	-0.07	0.99
	Second half (6-12 months)	350-NPK1 vs. 350-NPK2	12	4.6	< 0.05
		700-NPK1 vs. 350-NPK2	12	4.7	< 0.05
		350-NPK2 vs. 700-NPK2	12	-4.66	< 0.05
		350-NPK2 vs. 700-NPK3	12	-3.14	0.07
Tillandsia bulbosa	Over a year	350-NPK1 vs. 700-NPK1	29	-18.2	< 0.0001
		700-NPK1 vs. 350-NPK2	29	19.9	< 0.0001
		700-NPK1 vs. 700-NPK2	29	21.2	< 0.0001
		700-NPK1 vs. 350-NPK3	29	22	< 0.0001
		700-NPK1 vs. 700-NPK3	29	19.7	< 0.0001
		350-NPK3 vs. 700-NPK3	29	-3.03	0.052
	Second half (6-12 months)	350-NPK1 vs. 700-NPK1	29	-19.5	< 0.0001
		700-NPK1 vs. 350-NPK2	29	20.91	< 0.0001
		700-NPK1 vs. 700-NPK2	29	23.8	< 0.0001
		700-NPK1 vs. 350-NPK3	29	22.54	< 0.0001
		700-NPK1 vs. 700-NPK3	29	21.74	< 0.0001

Appendix 9. Results of a Post hoc test (HSD Tukey-test) on the effect of CO_2 and nutrient on survival percentage at different time periods for three Bromeliaceae species

Species	Harvest time	Contrast	df	t.ratio	*P* value
Tillandsia bulbosa	0-12 months	NPK1 vs. NPK2	NA	-2.3	0.057*
		NPK1 vs. NPK3	NA	-2.04	0.1
		NPK2 vs. NPK3	NA	0.3	0.95
	6-12 months	NPK1 vs. NPK2	NA	-2.5	0.03
		NPK1 vs. NPK3	NA	-2.2	0.06
		NPK2 vs. NPK3	NA	0.28	0.95
Tillandsia subulifera	First half (0-6 months)	350-NPK1 vs. 350-NPK2	NA	2.37	0.1668
		700-NPK1 vs. 700-NPK2	NA	2.37	0.1668

Appendix 4. Results of a Post hoc test (HSD Tukey-test) on the effect of CO_2 and nutrient on leaf area of the species *Catopsis nutans*

Species	Contrast	df	t.ratio	*P* value
C. nutans	350-NPK1 vs. 350-NPK2	22	3.55	0.02
	350-NPK1 vs. 350-NPK3	22	3.83	0.01
	350-NPK2 vs. 700-NK1	22	3.62	0.02
	350-NPK3 vs. 700-NPK1	22	3.83	0.01
	700-NPK1 vs. 700-NPK2	22	3.55	0.02
	700-NPK1 vs. 700-NPK3	22	3.83	0.01

Appendix 5. Results of a Post hoc test (HSD Tukey-test) on the effect of CO_2, nutrient concentration and maturity status on nutrient concentration and content in leaves of the species *Catopsis nutans*

Species	Contrast	df	t.ratio	*P* value
Catopsis nutans	Initial vs. 350-NPK2	18	4.82	0.006
	Initial vs. 350-NPK3	18	5.73	0.0009
N Content	Initial vs. 700-NPK3	18	6.6	0.0002
	350-NPK1 vs. 350-NPK2	18	4.78	0.006
	350-NPK1 vs. 700-NPK2	18	3.47	0.0813
	350-NPK1 vs. 350-NPK3	18	5.55	0.0013
	350-NPK1 vs. 700-NPK3	18	6.3	0.0003
	700-NPK1 vs. 350-NPK2	18	5.16	0.0028
	700-NPK1 vs. 700-NPK2	18	3.85	0.0393
	700-NPK1 vs. 350-NPK3	18	5.93	0.0006
	700-NPK1 vs. 700-NPK3	18	6.66	0.0001
[N]	Initial vs. 350-NPK3	20	4.43	0.0102
	Initial vs. 700-NPK3	20	5.11	0.0024
P Content	350-NPK1 vs. 350-NPK3	29	4.02	0.0162
	350-NPK1 vs. 700-NPK3	29	3.66	0.0381
	700-NPK1 vs. 700-NPK2	29	3.29	0.0876
	700-NPK1 vs. 350-NPK3	29	4.95	0.0015
	700-NPK1 vs. 700-NPK3	29	4.6	0.0038
[P]	Initial vs. 350-NPK2	29	4.1	0.0132
	Initial vs. 700-NPK2	29	5.34	0.0005
	Initial vs. 350-NPK3	29	5.95	0.0001
	Initial vs. 700-NPK3	29	5.97	0.0001
	350-NPK1 vs. 700-NPK2	29	4.2	0.0104
	350-NPK1 vs. 350-NPK3	29	4.7	0.0031
	350-NPK1 vs. 700-NPK3	29	4.7	0.0029
	700-NPK1 vs. 700-NPK2	29	3.71	0.0342
	700-NPK1 vs. 350-NPK3	29	4.15	0.012
	700-NPK1 vs. 700-NPK3	29	4.2	0.011
K Content	700-NPK1 vs. 350-NPK2	29	3.8	0.028
	700-NPK1 vs. 700-NPK2	29	3.51	0.0539
	700-NPK1 vs. 350-NPK3	29	3.81	0.027
	700-NPK1 vs. 700-NPK3	29	4.2	0.0112

Appendix 6. Results of a Post hoc test (HSD Tukey-test) on the effect of CO_2 and nutrient on carbohydrate concentration and content in leaves of the species *Catopsis nutans*

Species	Response variable	Contrast	df	t.ratio	*P* value
C. nutans	[Carbohydrate]*	350-NPK1 vs. 350-NPK3	30	5.33	< 0.001
		350-NPK2 vs. 700-NPK1	30	-4.81	< 0.05
		350-NPK2 vs. 700-NPK2	30	-4.44	< 0.05
		350-NPK2 vs. Init., Init.	30	-5.15	< 0.05
		350-NPK2 vs. 350-NPK3	30	3.92	< 0.05
		350-NPK3 vs. 700-NPK1	30	-5.63	< 0.001
		350-NPK3 vs. 700-NPK2	30	-5.91	< 0.001
		350-NPK3 vs. Init. Init.	30	-6.83	< 0.0001
		700-NPK1 vs. 700-NPK3	30	5.33	< 0.001
		700-NPK2 vs. 700-NPK3	30	3.92	< 0.05
		700-NPK3 vs. Init. Init.	30	-4.62	< 0.05
	Carbohydrates content	350-NPK1 vs. Init. Init.	30	-4.83	< 0.05
		350-NPK2 vs. Int. Init.	30	-5.8	< 0.001
		350-NPK2 vs. Init. Init.	30	-4.34	< 0.05
		350-NPK3 vs. Init. Init.	30	-6.45	< 0.001
		700-NPK1 vs. Init. Init.	30	-4.33	< 0.05
		700-NPK2 vs. Init. Init.	30	-4.19	< 0.05
		700-NPK2 vs. Init. Init.	30	-5.05	< 0.05
		700-NPK3 vs. Init. Init.	30	-5.73	< 0.001

CURRICULUM VITAE

PERSONAL INFORMATION

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EDUCATION

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Sep. 2009	**Licenciada in Biology**
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Feb. - Sep. 2008	**Samples identification** in Herbarium Botanical Garden for the thesis in Caracas, Venezuela
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April 2003 - Marz 2004	**Laboratory of Aquatics Plants Ecology** Universidad de Venezuela */Research intern/student in practice*
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May 2005 – Sep. 2005	**Laboratory of Soil Ecology (IVIC)** */Research intern/student in practice* Altos de Pipe, Caracas, Venezuela
Sep. 2009- Jan. 2010	*/Scientific Assistance* **Instituto de Medicina tropical**, Caracas, Venezuela */Scientific staff, research assistant*
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PUBLICATIONS

Correa & Zotz. 2014. The influence of collecting date, temperature and moisture regimes on the germination of epiphytic bromeliads. *Seed Science Research,* 24: 353-363.

CONFERENCE CONTRIBUTIONS

2015 Correa, S & Zotz, G. Effect of CO_2 and temperature on relative growth rate of tropical epiphyte plants.
Oral presentation at the *Annual Conference of the German Ecological Society For Tropical Ecology*. Zürich, Switzerland.

2011 Correa, S. & Zotz, G. Germination of epiphytic bromeliads: The controlling factors
Poster at the *41st Annual Meeting of the Ecological Society of Germany, Austria and Switzerland*. Oldenburg, Germany

2009 Correa, S. & Casanova, N. "Diversity and structure of vascular epiphyte communities in a lowland wet forest located in the "Zona Protectora del Macizo de Nirgua, Venezuela?"
Oral presentation at the *Venezuelan National Congress Of Ecology*. Falcón, Venezuela.

COURSES

Experimental Design and Data Analysis (advanced statistics using R, as listener, Universität Bremen, 2013).

Meta-analysis in Biology and Environmental Sciences. Göteborgs Universitet (Prof. Jessica Gurevitch and Prof. Julia Koricheva, SLC Tjärnö, Sweden, August 2016)

AUTHORS´ CONTRIBUTION

Chapters 2 is already published.

I develop the general concept of the project: “The study of the vulnerability of epiphytes in front of the future climate change scenario along different ontogenetic stages”. The contributions of the authors to every of the chapters were as followed:

Chapter 2 – Correa, Siouxsie & Zotz, Gerhard. The influence of collecting date, temperature and moisture regimes on the germination response of epiphytic bromeliads

The concept for this chapter as a methodological paper was conceived by Gerhard Zotz. Adaptation of the concept on/to an ecological perspective as well as climate change context was conceived by me and Gerhard Zotz. I did the experiments of seed maturity and wet-dry-cycle and, the experiments of water potential, temperature and water interaction and, temperature regimes (constant vs. fluctuating) were carried out by bachelor students that were part of our working group (Wiebcke Bogusch and Lars Volkmer) and by one of our technicians (Ingeborg Eden). The data preparation and germination response analysis were done by me. The statistics were carried out by both Gerhard Zotz and me. The text was written initially by myself and adapted by Gerhard Zotz for final published version. Literature search was conducted by both authors and all figures were done by myself except one (Figure Water potential vs. Wet-dry-cycles, created by Gerhard Zotz)

Chapter 3 – Correa, Siouxsie & Zotz , Gerhard. The concept for this chapter was conceived by myself. The experimental design was conceived by me with helpful contributions from Gerhard Zotz. I monitored the experiments, collected the data, did the analyses and literature search. I wrote the text and revised it with contributions from Gerhard Zotz

Chapter 4 – Correa, Siouxsie & Zotz, Gerhard. The concept for this chapter was conceived by myself. I created the experimental design with some suggestions from Gerhard Zotz. I monitored the experiment, collected the data, did the analyses and literature search. I wrote the text and revised it with contributions from Gerhard Zotz

Als Betreuer der Arbeit bestätige ich die Richtigkeit der Autorenbeiträge zu den aufgeführten Kapiteln bzw. deren Manuskripten oder Veröffentlichungen.

Prof. Dr. Gerhard Zotz

Hiermit bestätige ich die Richtigkeit der Autorenbeiträge zu den aufgeführten Kapiteln bzw. deren Manuskripten oder Veröffentlichungen.

Siouxsie Correa

Allgemeine Erklärungen

Es wird der akademische Grad eines Doktors der Naturwissenschaften (Dr. rer. nat.) angestrebt.

Die Dissertation „Vascular epiphytes as bioindicators of climate change and their own vulnerability“

- wurde von mir selbstständig verfasst und es wurden nur die angegebenen Hilfsmittel benutzt.
- liegt oder lag weder in Teilen noch in ihrer Gesamtheit einer anderen wissenschaftlichen Hochschule zur Begutachtung in einem Promotionsverfahren vor.
- wurde nach den Leitlinien guter wissenschaftlicher Praxis der Carl von Ossietzky Universität Oldenburg angefertigt.
- wurde ohne Inanspruchnahme kommerzieller Vermittlungs- oder Beratungsdienste (Promotionsberatung) angefertigt.

Teile der Arbeit wurden bereits veröffentlicht (Kapitel 2, siehe „Authors’ contributions“).

Ich bestätige hiermit die Richtigkeit der allgemeinen Erklärungen.

Siouxsie Correa