Centro de Investigación Científica de Yucatán, A.C.

Posgrado en Ciencias Biológicas

EFECTO DEL AUMENTO DE TEMPERATURA Y CO_2 COMO CONSECUENCIA DEL CAMBIO CLIMÁTICO SOBRE *Capsicum chinense* Jacq.

Tesis que presenta

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Por medio de la presente, hago constar que el trabajo de tesis titulado: Efecto del aumento de temperatura y CO_2 como consecuencia del cambio climático sobre *Capsicum chínense* Jacq fue realizado en los laboratorios de la Unidad de Recursos Naturales del Centro de Investigación Científica de Yucatán, A.C. bajo la dirección de los Doctores. Roger A. A. Orellana Lanza y María Azucena Canto Aguilar, dentro de la Opción Recursos Naturales, perteneciente al Programa de Posgrado en Ciencias Biológicas de este Centro.

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LISTADO DE ABREVIATURAS

A_{max} = maximum photosynthetic activity (Máxima actividad fotosintética)

A_N = CO_2 assimilation rate (Tasa de asimilación de CO_2)

A_{sat} = light saturated photosynthetic rate (Tasa fotosintética en saturación de luz)

ATP = adenosine triphosphate (adenosín trifosfato)

C_a = atmospheric CO_2 concentration (Concentración atmosférica de CO_2)

C_i = intercellular CO_2 concentration (Concentración intercelular de CO_2)

C_i / C_a = ratio intercellular CO_2 concentration / atmospheric CO_2 concentration (Cociente de la concentración intercelular de CO_2 / concentración atmosférica de CO_2)

DASS = days after seed sowing (Días después de la siembra)

DM = dry mass (Masa seca)

DMT = diurnal maximum temperature (Temperatura máxima diurna)

DM% = dry mass proportion (Proporción de masa seca)

E = transpiration (Transpiración)

g_s = stomatal conductance (Conductancia estomática)

J_{max} = maximum electron transport rate contributing to RuBP regeneration (Tasa máxima de transporte de electrones que contribuye a la regeneración de RuBP)

l = stomatal limitation (Limitación estomática)

LAR = leaf area ratio (Área relativa de la hoja)

LWR = leaf weight ratio (Cociente del peso de la hoja)

NADPH = forma reducida del dinucleótido de nicotinamida-adenina fosfato

NAR = net assimilation rate (Tasa de asimilación neta)

PP = photoperiod (Fotoperiodo)

PPFD = photosynthetic photon flux density (Densidad de flujo de fotones para la fotosíntesis)

R_D = leaf mitochondrial respiration (Respiración mitocondrial de la hoja)

RGR = relative growth rate (Tasa de crecimiento relativo)

RGR_{DM} = relative growth rate based on dry mass (Tasa de crecimiento relativo basado en masa seca)

RGR_{LA} = relative growth rate based on leaf area (Tasa de crecimiento relativo basado en área foliar)

RH = relative humidity (Humedad relativa)

SLA = specific leaf area (Área foliar específica)

T_{air} - T_L = temperature deficit (Diferencia entre la temperatura del aire y de la hoja)

$V_{c,max}$ = CO_2 carboxylation capacity (Capacidad de carboxilación del CO_2)

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RESUMEN

Ante un inminente incremento en la concentración atmosférica de CO_2 y un aumento en la temperatura ambiental, se evaluó la respuesta biológica de un importante cultivo tropical en futuros escenarios de cambio climático. Usando tres cámaras de crecimiento ubicadas dentro de un invernadero, se realizaron dos experimentos para conocer las respuestas fisiológicas, fenológicas, el crecimiento vegetal y la calidad de los frutos en plantas de chile Habanero (*Capsicum chinense* Jacq.) ante tres temperaturas máximas diurnas y tres concentraciones atmosféricas de CO_2; en el experimento 1, diferentes temperaturas máximas diurnas (30, 35 y 40 °C) fueron establecidas en cada cámara; en el experimento 2, la temperatura máxima fue establecida a 30 °C (en las tres cámaras) y diferentes concentraciones de CO_2 (ambiental, 380; duplicado, 760; y triplicado, 1140 µmol mol^{-1}) fueron establecidas en ellas. En ambos experimentos 30 plántulas (de 40 días después de la siembra) fueron ubicadas por cámara; el crecimiento vegetal (tasa de crecimiento relativo), el intercambio de gases (tasa de asimilación de CO_2, transpiración) y el ritmo fenológico (floración y fructificación) además de otras variables fueron evaluados a lo largo del cultivo (seis meses). En el experimento de temperatura, la tasa de crecimiento relativo fue más alta en las plantas cultivadas a 40 °C que en las de 30 y 35 °C. Resultados similares se observaron en la tasa de asimilación de CO_2 y la transpiración. Por otra parte, las características analizadas de flor y fruto respondieron de forma negativa al incremento en las temperaturas máximas. De esta manera, las plantas a 35 °C florecieron seis días antes que las cultivadas a 30 °C, pero fructificaron veintisiete días después, esto debido al aborto de flores y frutos al aumentar la temperatura máxima. Por otra parte, en el experimento de CO_2 las plantas a 1140 µmol mol^{-1} florecieron y fructificaron 18 y 37 días antes respectivamente que las cultivadas en 380 µmol mol^{-1}. El número total de frutos por planta se incrementó 47 % en el tratamiento de CO_2 triplicado en comparación con las plantas cultivadas en condiciones ambientales. El tamaño de fruto también se incrementó por la disponibilidad de CO_2, mientras que el contenido total de capsaicina de los chiles en atmósferas duplicadas y triplicadas de CO_2 fueron 20 y 60 % respectivamente más altos que en condiciones ambientales. Los resultados obtenidos sugieren que niveles duplicados de CO_2 en regiones tropicales podrían promover cambios positivos en las plantas y de esta forma mitigar detrimentos causados por el incremento en la temperatura, siempre que estas no alcancen niveles extremos.

ABSTRACT

Face to imminent increase in atmospheric CO_2 concentration as well as higher temperature, we analyzed biological responses of a main tropical crop under possible futures sceneries. Using three growth chambers building inside a greenhouse, two experiments were performed to test both physiological and phenological responses as well as plant growth and quality fruits in Habanero pepper plants (*Capsicum chinense* Jacq.) under three diurnal maximum temperatures and three atmospheric CO_2 concentrations respectively. In experiment 1, different diurnal maximum temperatures (30, 35 and 40 °C) were setting in each chamber; in experiment 2, diurnal maximum temperature was set at 30 °C (in all chambers) and different atmospheric CO_2 concentrations (380, 760 and 1140 µmol mol^{-1}) were used in each. In both experiment 30 seedlings (at ca. 45 days after sowing) were placed per chamber and plant growth (rate growth relative), gas exchange (CO_2 assimilation rate, transpiration), phenological time (flowering and fruiting) and others were analyzed throughout experiments (6 months each). In temperature experiment the rate growth relative in plants at 40 °C was higher than 30 and 35 °C. Similar results were found in CO_2 assimilation rate and transpiration rate. Beside, flower and fruit traits in Habanero pepper responded negatively to higher diurnal maximum temperatures. Thus, plants at 35 °C flowered six days earlier, but fruited 27 days later than those grown at 30 °C, latter by abortion. On the other hand, CO_2 experiment the plants grown at 1140 µmol mol^{-1} CO_2 flowered 18 days earlier and fruited 37 days earlier than plants at 380 µmol mol^{-1}. Total number of pods per plant increased 47% at the highest CO_2, in comparison to plants grown at lower CO_2 conditions. Pod size was also altered by CO_2 availability and total capsaicin contents in peppers in a high CO_2 atmosphere were 20 - 60% higher than those in plants under lower concentrations. Both maximum temperature and atmospheric CO_2 concentration each had different effects on traits evaluated in Habanero pepper. The obtained results, would suggest that a doubling of atmospheric CO_2 levels in tropical-subtropical regions would promote positive changes in plants that could mitigate any deterioration caused by higher temperature, as long as this factor does not reach extremely stressful levels.

CAPÍTULO I

INTRODUCCIÓN

El concepto de cambio climático o calentamiento global se define como: "el cambio del clima en el transcurso del tiempo debido a la variabilidad natural o a las actividades humanas" (IPCC, 2007). Esta definición integra intereses ambientales, biológicos, económicos, sociales y políticos. Por tal motivo los problemas originados por el cambio climático deben ser estudiados en las diversas ramas de la ciencia.

En el aspecto biológico, las consecuencias a futuro que pueden traer consigo la contaminación ambiental, la deforestación, la emisión inmoderada de gases de efecto invernadero (GEI) y otras acciones de origen antropogénico, pueden ser lamentables para las diversas formas de vida (Thomas *et al.*, 2004).

El aumento en la concentración atmosférica de CO_2 a partir de la revolución industrial a la fecha, ha traído como consecuencia un aumento en las temperaturas promedio y máximas extremas de algunas regiones del planeta en comparación con los registros de principios del siglo XX (IPCC, 2007). De continuar con esta tendencia ascendente en la temperatura, algunos organismos vivos como las plantas podrían verse afectados considerablemente en su fenología, anatomía, morfología y fisiología (Loyd y Farqhuar, 2008; Root *et al.*, 2003), ocasionando una redistribución geográfica y en algunos casos la extinción de las mismas causando serias alteraciones de impacto ecológico, económico y social. Algunos estudios han demostrado que las respuestas de las plantas a un incremento tanto en temperatura como en CO_2 pueden ser diversas, sin embargo la mayoría coinciden que el aumento de la temperatura limita los procesos fisiológicos primarios como la fotosíntesis (Hikosaka *et al.*, 2006) y el incremento en la concentración atmosférica de CO_2 generalmente le favorece (LaDeau y Clark, 2001).

De esta manera resulta importante estudiar la respuesta biológica de las plantas ante posibles escenarios de cambio climático. En este sentido los cultivos que tienen un alto valor cultural y comercial, propios de las diferentes regiones del mundo, pueden funcionar como modelos de evaluación por sus condiciones de adaptación a condiciones edafoclimáticas específicas.

En el caso de la península de Yucatán se puede utilizar el cultivo del chile habanero (*Capsicum chinense* Jacq.) como modelo de estudio pues además de la importancia que ha adquirido internacionalmente como condimento de cocina, su aplicación no se limita al ramo alimenticio. Al industrializarse se obtiene la capsaicina, sustancia que sirve como aditivo, en gases lacrimógenos, analgésicos, agentes terapéuticos, cosméticos, recubrimientos de sistemas de riego y eléctricos (protección contra roedores) y pinturas de los barcos (agentes anticorrosivos) (Cázares-Sánchez *et al.*, 2005).

Por lo tanto, el presente trabajo tiene como finalidad contribuir con evidencia experimental sobre los efectos del incremento tanto de la temperatura como de la concentración atmosférica de CO_2 sobre la biología de un cultivo tropical anual. De esta forma nuestro estudio pretende iniciar una nueva perspectiva que debe ser considerada por los investigadores especializados en cultivos tropicales, pues consideramos que la fenología, la fisiología, el crecimiento vegetal y otras características que evaluamos no son solo el resultado de las características intrínsecas de la planta, si no que podrían estar altamente influenciadas por la interacción ambiental y en el trópico han sido poco estudiadas.

Por lo consiguiente en el presente estudio utilizando tres cámaras de crecimiento se establecieron dos experimentos, con el propósito de estudiar el efecto de la temperatura y el CO_2 sobre las respuestas fenológicas, fisiológicas, agronómicas, anatómicas y metabólicas de las plantas de chile habanero.

Por todo lo mencionado, esta tesis está organizada en seis capítulos, en el capítulo uno se presenta una serie de antecedentes que fundamentan el trabajo realizado, en los capítulos dos y tres se evaluaron diversos parámetros fisiológicos y de crecimiento ante futuros posibles escenarios de cambio climático (incremento en la temperatura máxima y en la concentración atmosférica de CO_2 respectivamente), en el capítulo cuatro se observó la respuesta fenológica del cultivo y su síndrome floral, en el capítulo cinco se evaluaron algunas características de la calidad de los frutos y en el capítulo seis se discute y se concluye de manera general.

ANTECEDENTES

El clima y el efecto invernadero

El estado del tiempo y el clima terrestre son producto de la constante y compleja interacción entre la atmósfera, los océanos, las capas de hielo y nieve, los continentes y las diversas formas de vida en el planeta. Para poder hablar con seguridad del clima, se necesita de al menos 30 años de observaciones y registros diarios de las condiciones de temperatura, lluvia, humedad, viento, nubosidad, trayectoria de huracanes y frentes fríos, etc. Mientras que el estado del tiempo, se observa en lapsos de días debido a que son las posibles variaciones en las condiciones climáticas esperadas (Conde, 2007).

La atmósfera de la Tierra está compuesta principalmente de nitrógeno y oxígeno. Contiene además pequeñas cantidades de los llamados gases de efecto invernadero (GEI): el vapor de agua, el bióxido de carbono, el metano y los óxidos de nitrógeno, entre otros. La cantidad de estos compuestos permiten que el efecto invernadero mantenga las temperaturas propicias para el desarrollo de la vida en la tierra (Conde, 2007). Sin embargo, el aumento en los últimos años de algunos de los GEI como lo son, el bióxido de carbono (CO_2), el metano (CH_4), el óxido nitroso (N_2O) y el ozono (O_3), ha sido por el efecto de las diversas acciones humanas, principalmente por el uso de combustibles fósiles y algunas actividades agrícolas. Mientras que otros gases como los halocarbonados, el hexafluoruro de azufre (SF_6), los hidrofluorocarbonos (HFC), y los perfluorocarbonos (PFC), que en la actualidad han pasado a formar parte de los gases de efecto invernadero (GEI), son exclusivamente de origen antropogénico, ya que son gases químicos artificiales provenientes de los procesos industriales (Gutiérrez, 2007 UNODC, 2007).

El efecto invernadero está íntimamente ligado con el problema del calentamiento global y el cambio climático. La idea general sobre este fenómeno es la siguiente: desde 1880 a la fecha ha habido un aumento constante del bióxido de carbono provocado por la actividad industrial y esto está provocando un efecto invernadero que conduce al calentamiento planetario. De hecho, el aumento de temperatura promedio observado desde principios del siglo 20 es de aproximadamente 0.5 °C (Mendoza, 2007).

Capítulo I

Cambio climático

Por cambio climático se entiende todo cambio del clima en el transcurso del tiempo, ya se deba a la variabilidad natural, o sea resultado de la actividad humana (IPCC, 2007).

El cambio climático es un proceso de largo plazo (puede durar hasta varios siglos) y comprende complejas interacciones entre procesos climáticos, ambientales, económicos, políticos, institucionales, sociales y tecnológicos. Esto puede tener consecuencias internacionales e intergeneracionales en el contexto de objetivos generales de la sociedad, como equidad y desarrollo sostenible. La preparación de una respuesta al cambio climático se caracteriza por la adopción de decisiones en condiciones de incertidumbre y riesgo, lo que abarca la posibilidad de cambios no lineales o irreversibles (IPCC, 2007).

La agricultura y el cambio climático

Con la gestión adecuada de la tierra para la producción de cultivos, madera y bioenergía sostenible, pueden aumentar los beneficios para la mitigación del cambio climático, esto depende de la disponibilidad de la tierra y del agua. Las mayores posibilidades biológicas para la mitigación del carbono en la atmósfera las ofrecen las regiones subtropicales y tropicales (Foley *et al.*, 2005).

Las prácticas comunes en la agricultura son, en gran medida, resultado del conocimiento ancestral sobre el clima y el campo. Hoy en día se viven anomalías que parecen ser más intensas que las experimentadas años atrás (Magaña *et al.*, 1999).

La agricultura depende grandemente de las condiciones atmosféricas, lo cual probablemente es el factor más importante en la producción. El productor no puede cambiar el clima, pero puede hacer uso del pronóstico de los factores de este. El clima afecta a la agricultura de cuatro diferentes maneras: radiación solar, temperaturas, precipitación y vientos. Indirectamente, el clima influye en la agricultura por sus efectos sobre la formación de suelos (McGregor y Nieuwolt, 1998).

En todo el mundo las cosechas agrícolas son inestables de un año a otro debido a la variabilidad climática, una ocasión puede haber excelente producción y otra escasez. En relación a esto el cambio climático inducido por el hombre es altamente relevante, debido

a que podría acarrear cambios en la naturaleza ya que los sistemas agrícolas tropicales se basan en la disponibilidad y distribución de los recursos climáticos. Dependiendo del cambio climático en la naturaleza, en algunas regiones se esperaría un incrementó en su producción agrícola, mientras que en otras se esperaría un incremento en la frecuencia y magnitud de condiciones climáticas desfavorables (Stern, 2007). En todo el planeta el clima es una limitante ambiental para la producción agrícola, determinando que tipo de actividad puede desarrollarse en una región. De esta manera el clima tiene una influencia determinante sobre la agricultura debido al control que ejerce sobre los diversos procesos (Estrella *et al.*, 2007).

La fenología: parámetro de estudio ante el cambio climático

Las plantas están sometidas a fluctuaciones del ambiente en el cual se desarrollan y éste, dependiendo de su intensidad, puede afectar el desarrollo de los organismos en mayor o menor grado. Las respuestas de los vegetales al cambio ambiental, son importantes mecanismos de adaptación para la sobrevivencia de las especies. Algunos de los mecanismos son rápidos de manifestarse y otros tardan en establecerse. De esta forma las plantas están permanentemente fluctuando en sus respuestas para adaptarse al medio (Larqué-Saavedra, 1999). En zonas tropicales, el aumento de la temperatura y la falta de agua puede afectar a los cultivos y disminuir las cosechas hasta en una tercera parte (Stern, 2007).

Entender la forma en que los factores ambientales influyen en los diversos procesos fisiológicos de las plantas (germinación, fotosíntesis, respiración, transpiración, etc.) puede resultar complejo, debido a las diferentes interacciones que puedan existir (Salisbury y Ross, 1992). Existen muy pocas regiones del planeta en donde las condiciones ambientales sean continuamente favorables para todas las funciones fisiológicas de las plantas. Lo que sí es frecuente, es que a lo largo del tiempo se produzcan cambios en el clima y por lo mismo en la disponibilidad de recursos, esto obliga a las plantas a crear mecanismos de cambio en morfología y fisiología para poder sobrevivir (Peñuelas *et al.*, 2009). Estos cambios fenológicos ocurren siempre en el momento oportuno, cuando la planta los requiere para enfrentar una situación de estrés. La existencia de tales ciclos indica que en las plantas existe un sistema que relaciona los

cambios fenológicos a los cambios ambientales. En algunos casos es la iniciación del cambio ambiental el disparador de los cambios fenológicos (Rusterholz y Erhardt, 1998).

La fenología es el estudio de secuencias temporales de eventos fisiológicos y morfológicos cíclicos y recurrentes. La fenología puede ser una herramienta de estudio en las relaciones planta-ambiente y por lo tanto puede aplicarse en estudios de manejo, adaptación y conservación. La forma más común para su evaluación es a través de análisis del crecimiento vegetal (biomasa) y la observación de los órganos reproductivos en las distintas fenofases (floración, fructificación y desarrollo vegetativo) de las plantas estudiadas (Hunt *et al.*, 2002).

El cambio climático ya está causando efecto en algunos eventos fenológicos de las plantas, la floración de ciertas especies se está presentando 2.3 días por década más temprano en la primavera (Parmesan y Yohe, 2003). Estos efectos en su gran mayoría podrían causar serios problemas, como por ejemplo abortos florales. Aloni *et al.* (2001) reportaron que al aumentar la temperatura de 28 a 32 °C en condiciones normales de CO_2 (350 ppm) en plantas de *Capsicum annuum*, se produjo un ligero incremento en el aborto floral (3 %), además disminuyó el número de frutos por planta (16.7 %) y de semillas por fruto (223 semillas). Sin embargo cuando incubaron las plantas en concentraciones elevadas de CO_2 (800 ppm) los efectos causados por el aumento en la temperatura no se reflejaron en ninguna de las variables medidas. Además observaron que ni el aumento en la temperatura y ni el aumento en la concentración de CO_2, tienen un efecto significativo sobre el número de granos de polen por antera.

La fisiología: una herramienta ante los cambios ambientales

Por otra parte, la opinión de algunos autores es que de alguna manera el proceso de la fotosíntesis se vería beneficiado con los futuros posibles escenarios de cambio climático (González-Meler *et al.*, 2004). Sin embargo hay que enfatizar que muchos factores (agua, CO_2, luz, nutrimentos, temperatura, edad, genética, etc.) son los que influyen en la fotosíntesis vegetal. En las plantas con metabolismo C_3 debido a su punto de compensación de CO_2, la fijación de carbono depende en gran medida del incremento del CO_2 y de la luz, aunque niveles por encima de 1000 ppm de CO_2, pueden provocar cierre de estomas (Sage y Kubien, 2007). En cuanto al efecto de la temperatura, este depende

de la especie, las condiciones en las que ha crecido la planta y las condiciones ambientales imperantes durante la medición. En este campo ha sido muy estudiado el rol que juegan las hormonas ante el estrés ocasionado por la temperatura, desencadenando una serie de actividades hormonales que termina por ocasionar el cierre de los estomas (Meyers y Paterson, 2001). Un incremento normal en la temperatura influye poco en la fotólisis del H_2O o en la difusión de CO_2 en la hoja, pero influye notablemente en las reacciones bioquímicas de fijación y reducción pues no se produce con eficiencia ATP y NADPH para permitir incrementos en la fijación de CO_2, por lo que la formación de ribulosa bisfosfato se vuelve factor limitante (Sage y Kubien, 2007). De esta manera un aumento en la temperatura incrementa la tasa fotosintética hasta que comienza la desnaturalización enzimática y la destrucción de los fotosistemas. Además aumenta la perdida de CO_2 por fotorrespiración, debido al incremento en la proporción de O_2 disuelto con relación al CO_2 (Sharkey, 1988).

La fotosíntesis neta (diferencia entre lo que gana la planta por la fotosíntesis y lo que pierde por la respiración) indica el potencial de crecimiento de la planta y está relacionada estrechamente con el clima (Arellano y De Las Rivas, 2006; McGregor y Nieuwolt, 1998). La temperatura tiene un fuerte efecto sobre las plantas debido a que afecta la tasa fotosintética. Para cada especie existe un límite de tolerancia a la temperatura. La mayoría de los cultivos tropicales detienen su crecimiento cuando la temperatura se encuentra por debajo de los 15 °C o por encima de los 41 °C, y alcanzan su máximo crecimiento alrededor de los 30 y 37 °C (Sage y Kubien, 2007). Las plantas C_3 alcanzan su máxima tasa fotosintética a la mitad del rango óptimo de temperatura, pero disminuye rápidamente con temperaturas inferiores o superiores. Las plantas C_4 alcanzan la tasa máxima fotosintética con las temperaturas más altas dentro del rango, y la mantienen alta por un intervalo bastante amplio. De esta manera las plantas C_4 tienen una ventaja productiva sobre las C_3 a temperaturas por encima de los 25 °C, en contraparte las C_4 no pueden establecerse en ambientes por debajo de los 10 °C (Sage y Kubien, 2007; McGregor y Nieuwolt, 1998).

Tanto la fotosíntesis como la respiración tienen un impacto sobre la biología de las plantas, en su crecimiento, acumulación de biomasa y toma de nutrimentos, además de jugar un papel importante en el balance de carbono a nivel celular, planta y ecosistema.

Aproximadamente la mitad del CO_2 asimilado anualmente por la fotosíntesis es liberado de nuevo a la atmósfera por medio de la respiración vegetal, la cual es reducida en plantas cultivadas con altas concentraciones de CO_2 (González-Meler *et al.*, 2004).

El chile habanero

Este trabajo se realizó utilizando el cultivo de chile Habanero como modelo de estudio debido a que es una planta hortícola con un ciclo de vida relativamente corto, adaptada a las condiciones ambientales de la Península de Yucatán, con porcentajes elevados de autopolinización en ambientes cerrados, capaz de florecer y fructificar en cualquier época del año, con alto valor comercial y reconocida internacionalmente por las características de sus frutos, considerados como los más picantes del mundo.

El género *Capsicum*

Todos los tipos de chile, corresponden al género *Capsicum*, perteneciente a la familia de las Solanáceas, misma que está dividida en dos subfamilias: *Solanoideae* y *Cestroideae.* La diferencia de éstas se basa en características morfológicas, químicas y citogenéticas y en disímiles modelos de desarrollo del embrión; mientras en las *Solanoideae* el embrión está enrollado y es de un diámetro más o menos uniforme, en las *Cestroideae* el embrión es típicamente recto o ligeramente curvado (Nuez *et al.*, 1996).

Todas las especies del género, a excepción de *C. anomalum*, son originarias de América. Una hipótesis sobre el lugar y modo de evolución de una porción importante del género *Capsicum*, sugiere que se originó en un área de Bolivia sud-central con una migración posterior a los Andes y tierras bajas de la Amazonía acompañada por radiación y especiación. La hipótesis se basa en información geográfica y en el análisis electroforético en geles de almidón de la enzima glutamato oxalacetato transaminasa (Nuez *et al.*, 1996).

El proceso posterior de domesticación parece verosímil que ocurriera independientemente en varias áreas. Actualmente se cree que el complejo *annuum* fue domesticado al menos dos veces, un tipo *C. annuum* en México y un tipo *C. chinense* en la Amazonía (Pickersgill, 1969).

Capsicum chinense

Capsicum chinense Jacq., es una especie comúnmente cultivada en el Caribe, sus dos tipos de fruto más comunes son el Habanero y el Scotch Bonnet, los cuales difieren muy poco en su forma. El chile habanero del cual se dice que es el más pungente del mundo con 577,000 unidades Scoville (USc) ha sido originalmente cultivado en la península de Yucatán, México y en Belice, sus frutos al madurar pueden ser de color amarillo, naranja o rojo. El Scotch Bonnet, es cultivado extensivamente en Jamaica, usualmente maduran en amarillo, blanco, rojo o naranja, es tan picante como el habanero (Bosland y Votava, 2000).

Características botánicas del chile habanero

El chile habanero es de hábito determinado, la hoja presenta poca pubescencia, puede ser oval o lanceolada de color verde a verde intenso, de ramificación intermedia. Presenta tallo cilíndrico con pubescencia escasa o intermedia con antocianinas en el nudo de color morado claro. La posición de la flor varía de intermedia a erecta, con ausencia de pigmentación en el cáliz. La forma de la corola es de tipo acampanulada, el margen del cáliz varía de intermedio a dentado. El color de la corola es amarillo verdoso, mientras que la antera puede ser morada o azul y el filamento es blanco, amarillo o morado. El fruto carece de antocianina, con ausencia de apéndice, mientras que el arrugamiento transversal varía de intermedio a levemente corrugado, en general, carecen de cuello con base en el fruto pero presentan constricción anular del cáliz, los frutos presentan forma acampanulada y la unión con el pedicelo varia de truncado a cordado, también se observa una gran variación en el tipo de epidermis que va de lisa a semi rugosa. El color del fruto en estado intermedio puede ser verde, verde claro o morado oscuro cambiando a naranja, amarillo o rojo respectivamente en la madurez. La forma del ápice puede ser puntuada, roma o hundida (Velasco, 2003).

JUSTIFICACIÓN

Considerando que la mayoría de las investigaciones que se han desarrollado para determinar el efecto del incremento en la temperatura del aire y en la concentración de

CO_2 atmosférico sobre las plantas han sido realizadas en zonas templadas donde la temperatura máxima, el fotoperiodo, la intensidad luminosa, la humedad relativa y otros factores ambientales difieren completamente de las condiciones en el trópico. En la presente investigación se optó por utilizar una planta adaptada a las condiciones ambientales de la Península de Yucatán (características tropicales) como modelo de estudio ante posibles escenarios futuros de cambio climático, con la finalidad de contrastar los resultados obtenidos con lo reportado en estudios realizados con plantas de otras regiones climáticas y poder realizar los pronósticos adecuados para la región, además de contribuir al entendimiento de las respuestas de una planta tropical cultivable.

HIPÓTESIS

Por tratarse de un cultivo tropical con metabolismo C_3, el aumento de 30 a 35 °C en la temperatura máxima incrementará la tasa fotosintética, adelantará la floración y disminuirá el amarre de frutos y el rendimiento de las plantas de chile habanero.

El incremento duplicado y triplicado en las concentraciones de CO_2 atmosférico adelantará la floración, promoverá mayor acumulación de biomasa e incrementará la tasa de asimilación de carbono y la concentración de capsaicina en frutos.

Un escenario de máxima temperatura y máxima concentración de CO_2 adelantará el ritmo de las etapas fenológicas del cultivo y promoverá cambios en el número de estomas como respuesta de aclimatación de la planta.

OBJETIVOS

Objetivo general

Estudiar el efecto de la temperatura del aire y la concentración atmosférica de CO_2 sobre las respuestas fenológicas, fisiológicas, agronómicas, anatómicas y metabólicas del cultivo de chile habanero (*Capsicum chinense* Jacq.).

Objetivos específicos

Evaluar el efecto de la temperatura del aire sobre la tasa de asimilación de CO_2, la conductancia estomática, la transpiración, el tiempo a floración, el tiempo a fructificación, la tasa de crecimiento relativo y la producción de flores y frutos, en las etapas fenológicas del cultivo de chile habanero.

Evaluar el efecto de la concentración atmosférica de CO_2 sobre la tasa de asimilación de CO_2, la conductancia estomática, la transpiración, el tiempo a floración, el tiempo a fructificación, la tasa de crecimiento relativo y la producción de flores y frutos, en las etapas fenológicas del cultivo de chile habanero.

ESTRATEGIA EXPERIMENTAL

Para cumplir con los objetivos establecidos, usando tres cámaras de crecimiento ubicadas dentro de un invernadero, se realizaron dos experimentos. En el experimento 1, en cada cámara se estableció una temperatura máxima diurna (30, 35 y 40 °C) (Figura 1). En el experimento 2, la temperatura máxima fue establecida a 30 °C (en las tres cámaras) y en cada cámara se estableció una concentración de CO_2 (ambiental, 380; duplicado, 760; y triplicado, 1140 µmol mol^{-1}) (Figura 2). En ambos experimentos 30 plántulas fueron ubicadas por cámara; el crecimiento vegetal (área foliar, biomasa, tasa de crecimiento relativo, tasa de asimilación neta, área foliar específica, etc.), el intercambio de gases (tasa de asimilación neta, conductancia estomática, CO_2 intercelular, etc.) y el ritmo fenológico (floración y fructificación) además de otras variables (morfología de flor y fruto, rendimiento, producción, néctar, polen, contenido de capsaicina) fueron evaluados a lo largo del cultivo (seis meses), las plantas se mantuvieron todo el tiempo a capacidad de campo y se fertilizaron cada ocho días con la formula 120-120-120 (NPK; nitrógeno, fosforo y potasio).

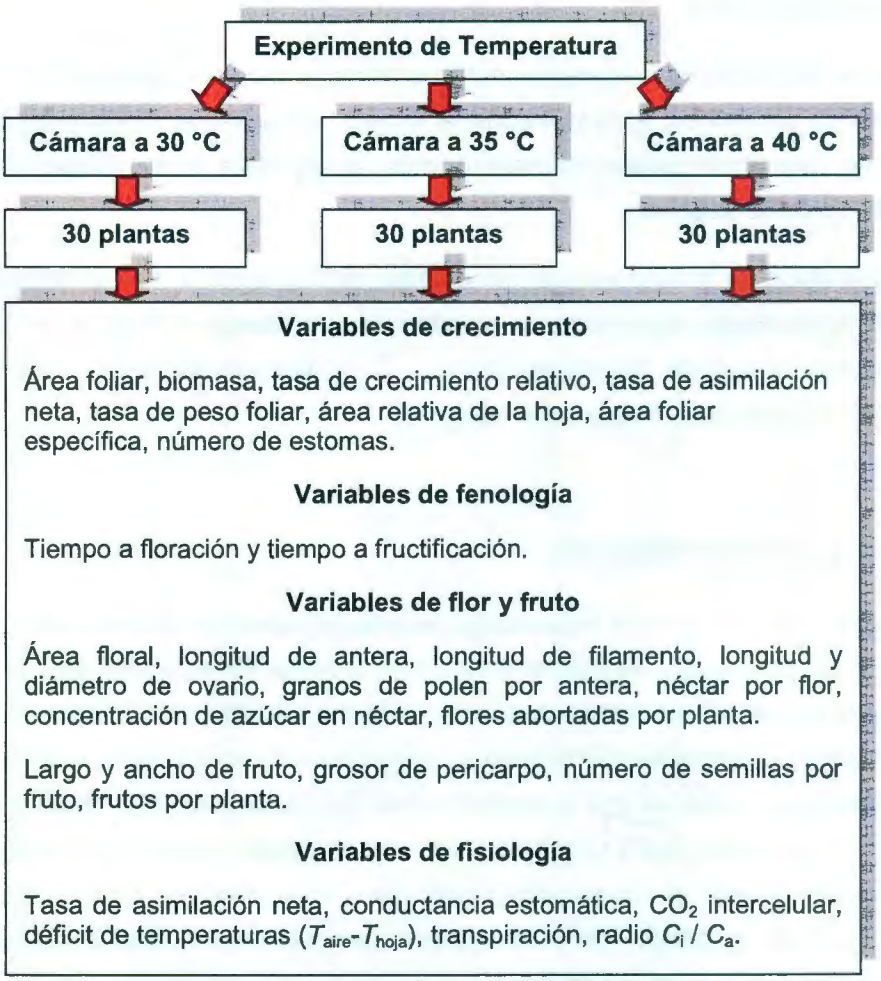

Figura 1. Diseño del experimento de temperaturas máximas diurnas.

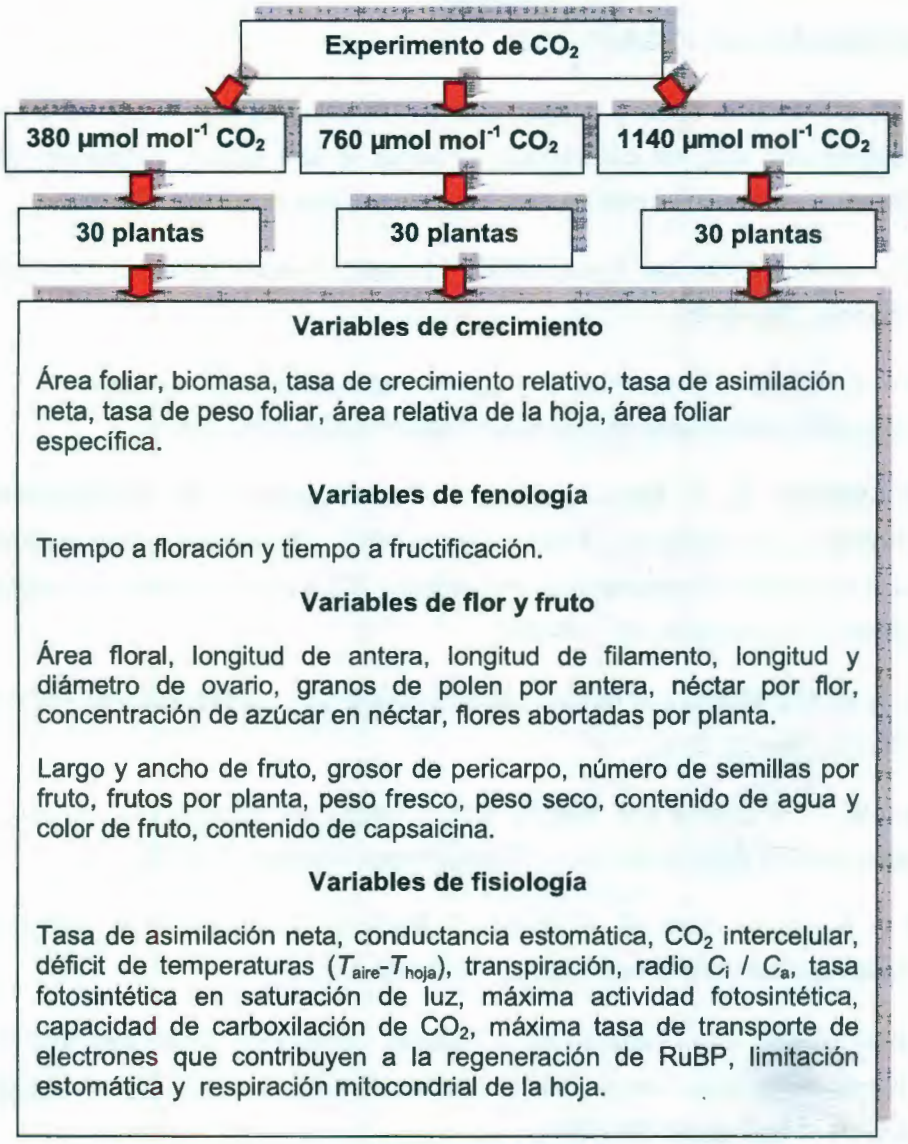

Figura 2. Diseño del experimento de concentraciones atmosféricas de CO_2.

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CAPÍTULO II

Physiological responses of Habanero pepper (*Capsicum chinense* Jacq.) to temperature variation

ABSTRACT

Due to global warming temperature is one of the main environmental factors involved and has been determined to have a direct effect on plants. However, few studies have been performed on the effect of higher temperature on tropical crops. Therefore, we performed an experiment with a tropical crop of Habanero pepper. Three growth chambers were used, with 30 plants of Habanero pepper (*Capsicum Chinense* Jacq.) each. Chambers held a diurnal maximum temperature (DMT) of 30 (chamber 1), 35 (chamber 2) and 40 °C (chamber 3); each contained peppers from juvenile stage to fruiting stage. Physiological response to variation in DMT was evaluated for each stage during five months. The results showed that dry mass proportion in leaves at 30 °C was lower when compared to the other treatments in all phenological stages. Therefore, RGR and NAR in plants at 40 °C were higher than at 30 and 35 °C. Similar results were found in CO_2 assimilation and transpiration rates. The photosynthetic apparatus of Habanero pepper plants continued assimilating CO_2 at higher DMT and plant growth at 40 °C was higher than at 30 and 35 °C. According to the foregoing results, a rise in DMT increased transpiration rate; this kept leaf temperature up to 5 °C below air temperature. Thus, we consider that the relation $T_{air} - T_L$ explains that temperature keeps healthy the major physiological processes of Habanero pepper under experimental conditions.

KEYWORDS: global warming, photosynthesis, plant growth analysis, transpiration, tropical plant.

Nota: Este capítulo será sometido a la revista "HORTSCIENCE"

INTRODUCTION

Temperature is one of the main conditions that influences plant growth and productivity. According to IPCC (2007) the global climate is predicted to increase from 2 to 4 °C during the next century as a result of augmented greenhouse gases concentration in the atmosphere. Global warming may affect plants seriously, e.g. inhibition of photosynthesis (Sage and Kubien, 2007; Hikosaka *et al.*, 2006; Salvucci and Crafts-Brandner, 2004), and variation in biomass (Poorter *et al.*, 2009; Morison and Lawlor, 1999). There are some indicators that show that plants could suffer substantial damage as a consequence of high temperature stress. Estimates range up to 17 % decrease in crop yield for each degree Celsius of temperature increase in average (Lobell and Asner, 2003). However, the crop yield is the last part of a chain of physiological events that begins with CO_2 diffusion into the leaf air space throughout the stomata and continues to CO_2 assimilation thus affecting the accumulation of biomass and its allocation to different organs that depends on both the net carbon balance and the programmed growth and expansion of them (Sharkey and Schrader, 2006). Growing organs are sinks for assimilates which interact with their sources. This interaction is critical to understanding plant responses to environmental factors (Farrar 1996; Lawlor and Keys, 1993). According to Poorter *et al.* (2009) at higher temperatures, leaf mass decreases. Yet, the response of boreal and tropical species is clearly different to that of tropical plants. Leaf mass production of tropical species is more affected for a given change in temperature (some changes in the physiological mechanism of plants has been observed). Thus, Hikosaka *et al.* (2006) suggest that growth temperature alters thermal dependence of the photosynthetic rate (temperature acclimation). In many species, the optimal temperature that maximizes the photosynthetic rate increases with rising growth temperature.

If climate warming increases the frequency of supra-optimal temperatures, it is important to understand the limitations on plant physiology, mainly over the long-term in order to be able to predict the possible effects of climate change, and adopt strategies in agricultural systems and land management to a warmer world. It is imperative to understand how temperature could affect carbon gain (Sage and Kubien 2007). Therefore, we consider important to investigate the long-term temperature response in a tropical crop. Habanero pepper (*Capsicum chinense* Jacq.) is an excellent study model because it grows to yield a

good productivity and quality in wide temperature ranges, and is a profitable crop in the tropical Caribbean and Yucatan Peninsula. It is traded in the international market, its fruits and derivatives are used worldwide as condiments, additives, and as the lachrymatory agent in pepper sprays (Cázares-Sánchez *et al.*, 2005). It is used as a fungicidal and cytotoxic agent as well (Anaya-Lopez *et al.*, 2006). Additionally, other aspects of this species has been studied in diverse fields, e.g. production of secondary metabolites (Pino *et al.*, 2006), organogenesis (Santana *et al.*, 2005), plant growth (Lannes *et al.*, 2007), genetics (Sousa and Maluf, 2003) and phenology (Jaimez *et al.*, 2000).

MATERIALS AND METHODS

Plant material

Habanero pepper seeds (Seminis®) were germinated in 200 well polystyrene trays in a peat moss substrate. After 45 days, the seedlings were transplanted to 6 Kg pots containing a soil-peat moss mix (2:1 v/v) and placed in growth chambers for the experimental phase.

Growth chambers

Three growth chambers (20 m^3 each) were placed inside a greenhouse under controlled conditions and were built with transparent glass to allow sunlight in. The temperature was controlled with a minisplit air conditioner of 5000 BTUS (LG®). The CO_2 concentration level was measured by sensors (GETelaire Ventostat 8002), and the average was ca. 380 ± 10 µmol mol^{-1} CO_2 in all chambers. One diurnal maximum temperature (DMT) was calibrated and fixed per chamber (30, 35, and 40 °C). In all chambers, relative humidity (RH), maximum photosynthetic photon flux density (PPFD), and photoperiod (PP) conditions were kept throughout the experiment (5 months) at ca. 80 ± 4%, 600 µmol m^{-2} S^{-1} (at noon) and 11 h. The latter were measured using data loggers (HOBO H08-004-02; Onset Computer Corp., Bourne, MA, USA) and Quantum sensors (LI-190SB; LI-COR, Lincoln, NE, USA) placed on top and inside of each chamber. The average night temperature was 24 ± 1 °C for all chambers.

Plant growth

Thirty healthy 45 days after seed sowing (DASS) juvenile plants were randomly selected and placed in each of the growth chambers. Furthermore, the pots were rotated weekly within the corresponding chamber to avoid edge effects. Plants traits like leaf area, dry mass (DM), dry mass proportion (DM%), relative growth rate (RGR), specific leaf area (SLA), net assimilation rate (NAR), leaf area ratio (LAR), leaf weight ratio (LWR) and stomata number (abaxial and adaxial) were determined by a series of four destructive harvests. Harvest 1 (T0) was performed at the onset of treatments (45 DASS); harvest 2 (T1) at juvenile plant stage (65 DASS); harvest 3 (T2) at flowering phenophase (110 DASS), and harvest 4 (T3) at fruiting phenophase (130 DASS). In each harvest, 5 plants per treatment were randomly selected. RGR was determined for the period including the first harvest (T0) up to the next harvest (T1, T2 and T3 respectively). Total leaf area of each plant was taken with a surface area meter (LICOR, LI-3100, Nebraska, USA). All the samples were dried in an oven at 65 °C until constant weight was reached and then dry mass (DM) was determined. Relative growth rate (RGR), leaf area ratio (LAR), specific leaf area (SLA), net assimilation rate (NAR) and leaf weight ratio (LWR) were calculated as described by Hunt *et al.* (2002). These five parameters are defined and related in the following way:

$$\mathrm{RGR} = \frac{\log \mathrm{DM2} - \log \mathrm{DM1}}{\mathrm{t2} - \mathrm{t1}}$$

$$\mathrm{LAR} = \mathrm{SLA} * \mathrm{LWR} = \frac{\mathrm{LA}}{\mathrm{DML}} * \frac{\mathrm{DML}}{\mathrm{DM}}$$

$$\mathrm{NAR} = \frac{\mathrm{RGR}}{\mathrm{LAR}}$$

Where DM is total dry mass per plant, t is time, LA is total leaf area per plant and DML is total leaf dry mass per plant.

Stomatal counts

In each phenophase (juvenile, flowering, and fruiting) 5 plants were selected, stomata number was counted per plant at three different levels (top, middle, and bottom), considering counts both abaxial and adaxial layers. Three samples per level were measured. For sampling, a glue drop of Ethyl Cyanoacrylate (Kola Loka®) was applied on a slide and over the leaf (between central leaf vein and border leaf) pressing about 30 seconds, thus obtaining a print in the slide. In a microscope (Leica BME H22) to 40X, 15 optical fields were photographed per print and stomata number was calculated per mm^2.

Gas exchange analyses

Gas exchange analyses were carried out inside each growth chamber at relevant growth conditions with a portable system infrared gas analyzer (IRGA; LICOR, LI-6400, Nebraska, USA). At 7:00, 9:30, 12:00, 14:30 and 17:00 h, fifteen fully expanded young leaves from each treatment were introduced into the gas-exchange leaf chamber of the LiCor 6400. CO_2 assimilation rate (A_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration (E) were estimated at growth chambers conditions described above. Then, intercellular CO_2 / atmospheric CO_2 (C_i / C_a) ratio and temperature deficit (T_{air} - T_L) were calculated.

Statistical analysis

A completely random design was used with 25 plants per growth chamber. Data were examined by ANOVA and treatment means were compared using Tukey's HSD test at $P < 0.05$ (Statistic Six Sigma, Release 7, StatSoft).

RESULTS

Both leaf area and dry mass of Habanero pepper plants did not exhibit significant differences in juvenile and flowering phenophase, but in fruiting phenophase leaf area and dry mass of plants grown at 40 °C DMT were lower than plants at 30 and 35 °C DMT (Figure 1 and 2 respectively).

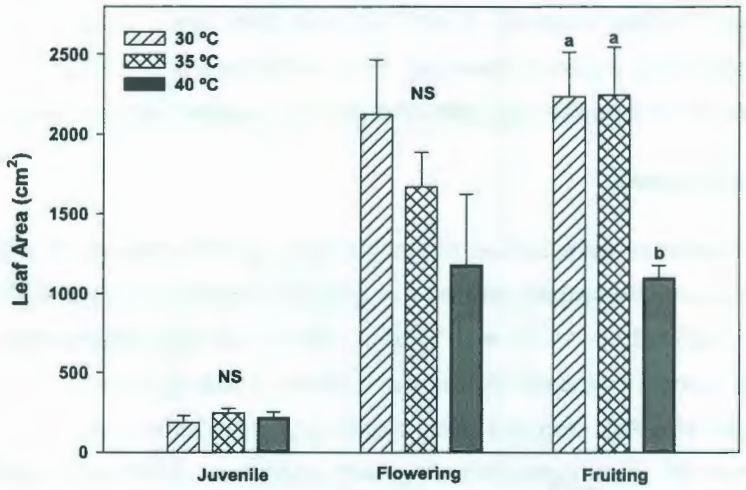

Figure 1. Leaf area of Habanero pepper plants in different phenological stages at three maximum diurnal temperatures (30, 35 and 40 °C). Data are means ± SE. Different letters in the same phenology stage represent statistical differences (Tukey, α = 0.05). NS = not significance. *n* = 5.

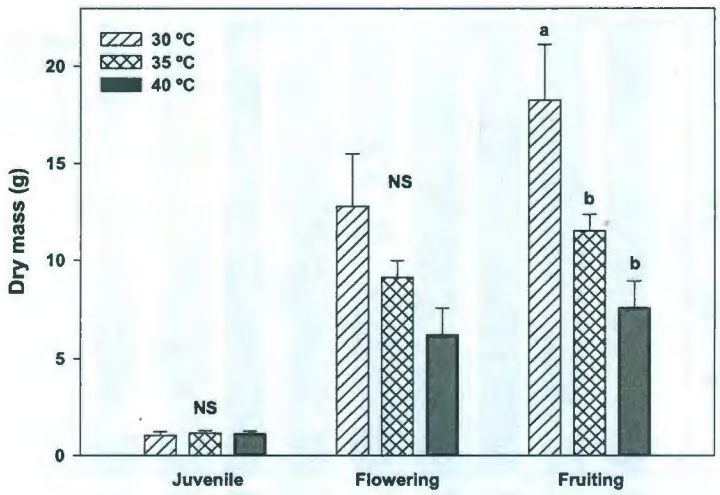

Figure 2. Dry mass of Habanero pepper plants in different phenological stages at three maximum diurnal temperatures (30, 35 and 40 °C). Data are means ± SE. Different letters in the same phenology stage represent statistical differences (Tukey, $\alpha = 0.05$). NS = not significance. $n = 5$.

The dry mass proportion per organ (DM%) changed in each phenology stage. DM% of roots in all phenological phases at 40 °C DMT was lower than at 30 and 35 °C DMT. During fruiting stage, DM% in stems was higher in plants at 40 °C DMT than in other treatments. In this sense, DM% in leaves of plants at 30 °C DMT was lower than other treatments in all phenological stages. In all treatments, DM% in roots and leaves decreased throughout phenophases. However, DM% in leaves increased from juveniles to flowering, and decreased during fruiting stage (Figure 3).

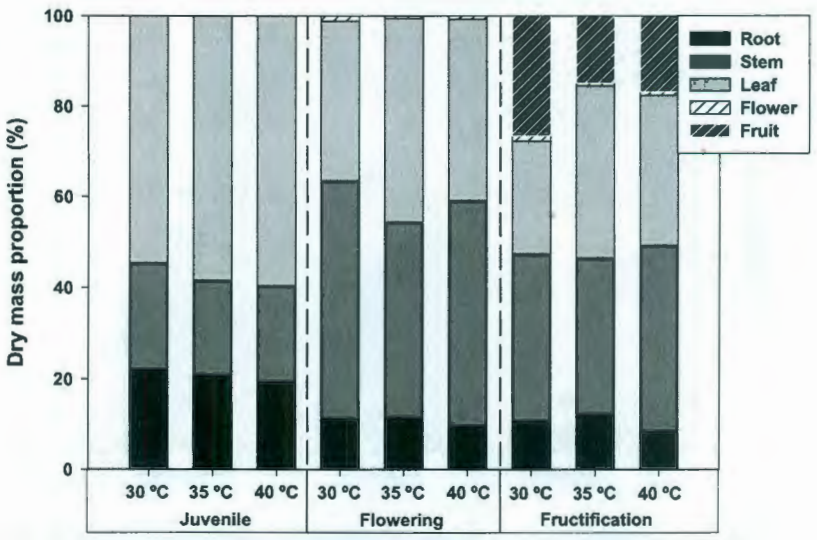

Figure 3. Average dry mass proportion of Habanero pepper plants in different phenological stages at three maximum diurnal temperatures (30, 35 and 40 °C). n = 5.

In all treatments, RGR_{DW} (based on dry mass), RGR_{LA} (based on leaf area) and NAR were not different during flowering and fruiting stages. However, in juvenile plants at 40 °C DMT RGR_{DW}, RGR_{LA} and NAR were significantly higher than 30 and 35 °C DMT (Figure 4a, 4b and 4c respectively). The LAR and the SLA did not exhibit significant differences in juveniles, as well as in flowering and fruiting stages. Both were significantly reduced when DMT was increased from 30 to 40 °C during seedling stages (Figures 4d and 4e, respectively). In contrast, the LWR was not significant in both seedling and juvenile stages; but, in flowering and fruiting stages LWR increased 30.5 and 78.2 %, respectively when DMT was increased from 30 to 35 °C (Figure 4f).

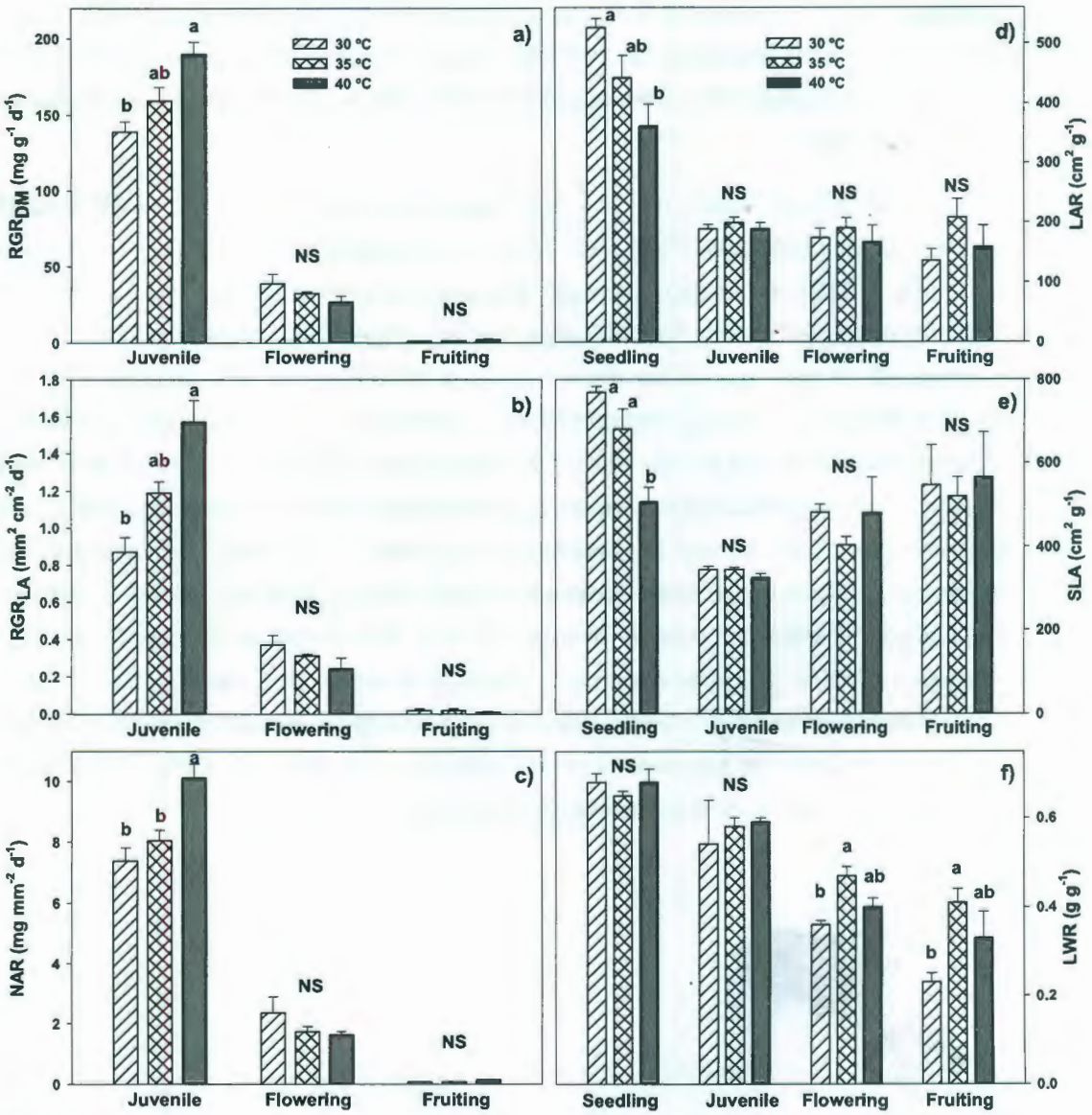

Figure 4. a) Relative Growth Rate (dry mass), b) Relative Growth Rate (leaf area), c) Net Assimilation Rate, d) Leaf Area Ratio, e) Specific Leaf Area and F) Leaf Weight Ratio of Habanero pepper plants at three maximum diurnal temperatures (30 °C, 35 °C and 40 °C) per phenological stage. Data are means ± SE. Different letters in the same phenology stage represent statistical differences (Tukey, α = 0.05). NS = not significance. *n* = 5.

Amphistomatous leaves are reported in the present study of Habanero pepper plant. However, the stomatal number in the adaxial side is lower than in the abaxial side. But, in both adaxial and abaxial sides the stomatal density decreased along phenological stages. Additionally, stomatal number decreased from the upper leaves section to bottom leaves section (Figure 5).

Plants at 40 °C DMT had a net CO_2 assimilation rate higher than those at 30 °C DMT between 12:00 and 14:30 h (Figure 6a), when photosynthetic photon flux density was at its maximum (PPFD). In addition, stomatal conductance both at 35 and 40 °C DMT was higher than in plants at 30 °C DMT along the day (Figure 6b). Intercellular CO_2 (C_i) in plants at 40 °C DMT was higher than in plants at 30 and 35 °C DMT between 9:00 and 14:30 h (Figure 6c). At noon intercellular CO_2 / atmospheric CO_2 ratio, was not different between treatments, but at 7:00 and 17:00 h the plants measured in all treatments were close to 1. The latter occurred in response to dark respiration at the start (ca. 6:40 h) and the end (ca. 17:40) of day in greenhouse conditions (Figure 6d). Transpiration rate increased in higher temperature treatments (Figure 6e) and was very similar to stomatal conductance. Temperature deficit between 12 and 14 h plants at 35 and 40 °C DMT reached 4.5 and 5 °C, respectively, ca. 2.3 and 2.8 °C higher than plants at 30 °C, e.g. at noon (with 600 µmol m^{-2} S^{-1} PPFD). Although air temperature in each chamber was at 30, 35 and 40 °C DMT the temperature in leafs was ca. 27.8, 30.5 and 35 °C, respectively. This result was significant in all treatments (Figure 6f).

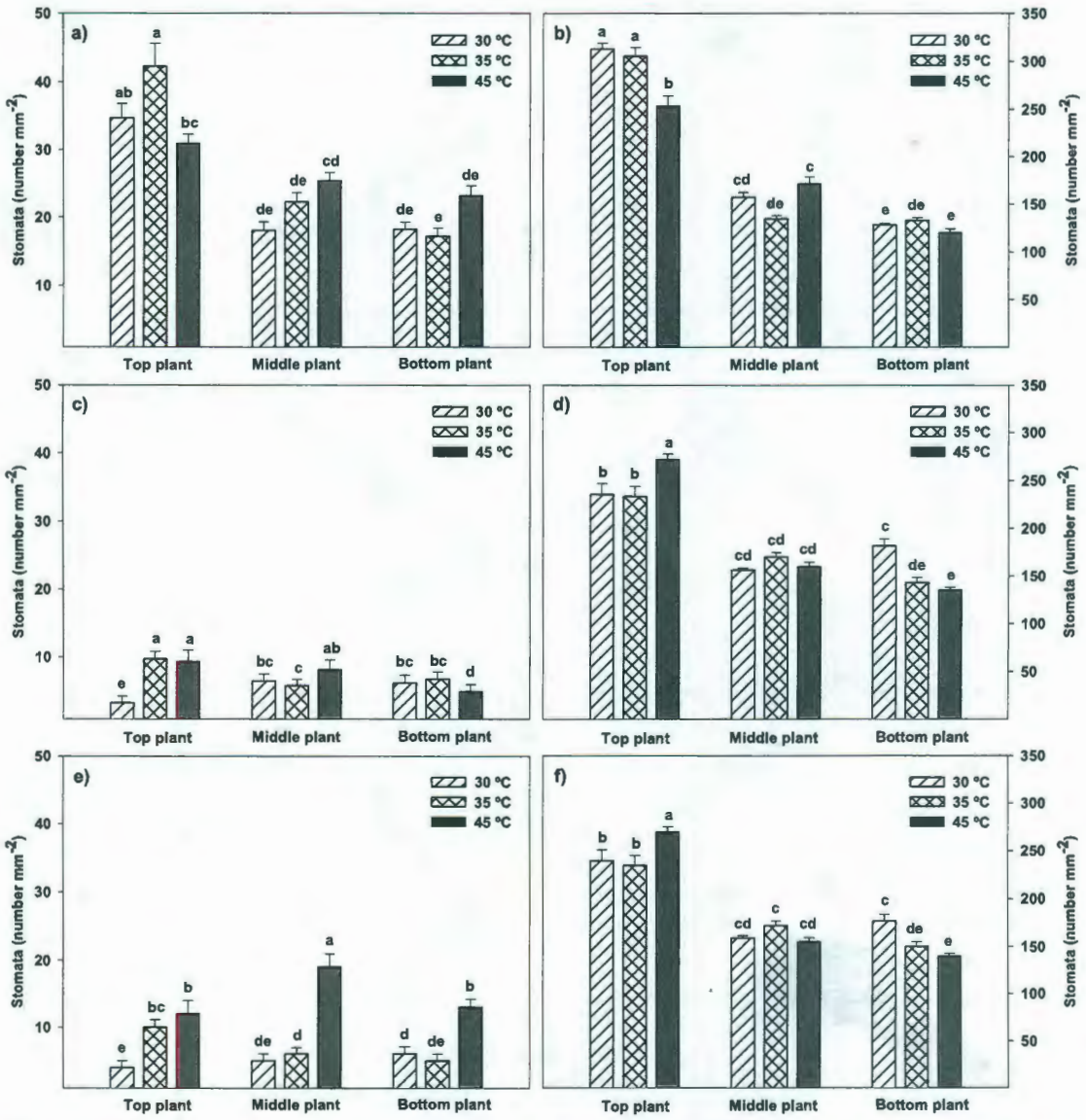

Figure 5. Stomata number (adaxial and abaxial) in three height levels in plant (top, middle, and bottom) and different phenological stages (a) Adaxial Juvenile; b) Adaxial Flowering; c) Adaxial Fruiting; d) Abaxial Juvenile; e) Abaxial Flowering and f) Abaxial Fruiting) of Habanero pepper plants at three maximum diurnal temperatures (30, 35 and 40 °C). Data are means ± SE. Different letters represent statistical difference (Tukey, $\alpha = 0.05$). $n = 5$.

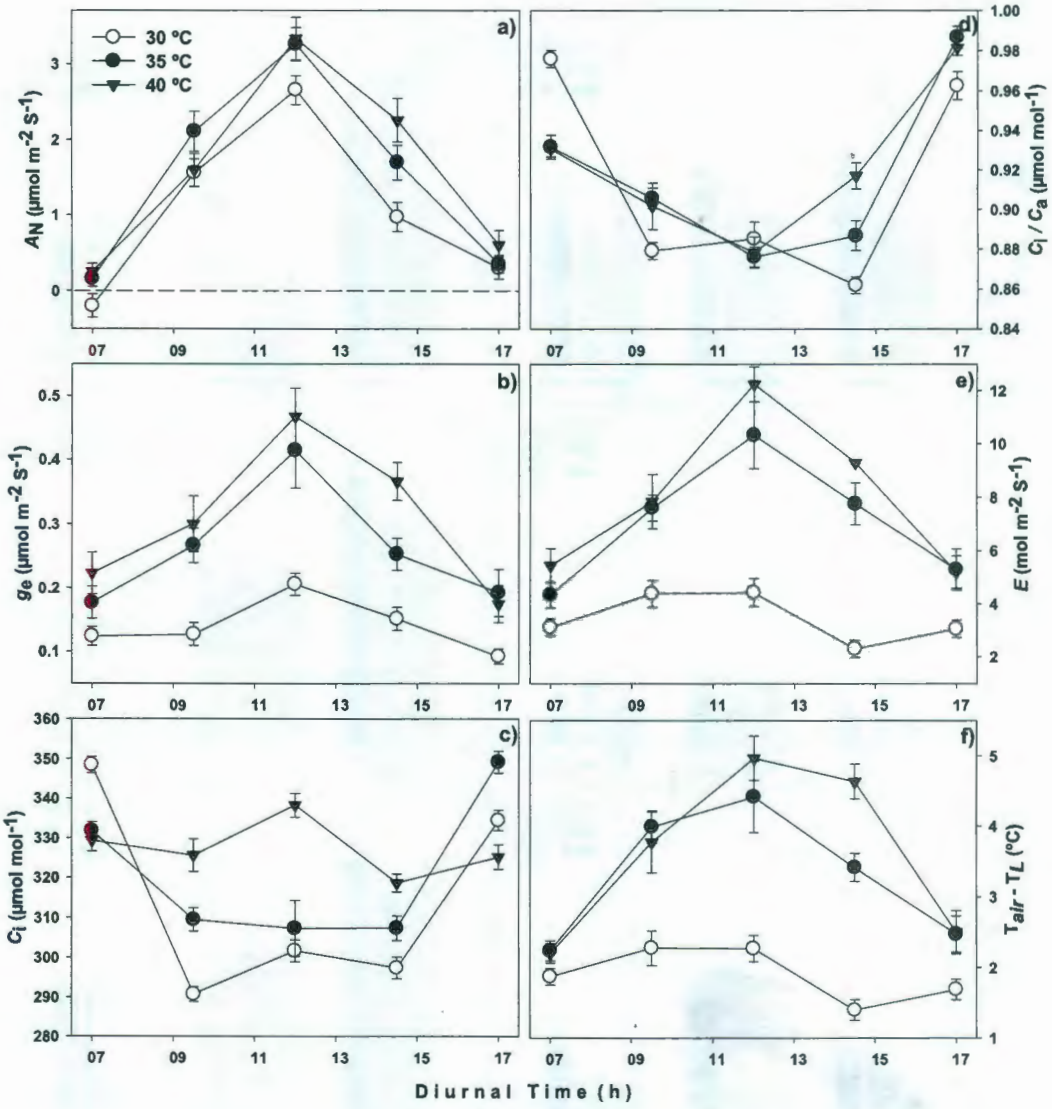

Figure 6. a) CO_2 Assimilation Rate (A_N), b) Stomatal Conductance (g_s), c) CO_2 intercellular (C_i), d) Ratio intercellular CO_2 / atmospheric CO_2 (C_i / C_a), e) Transpiration (E) and f) Temperature Deficit ($T_{air} - T_L$) of Habanero pepper plants at three maximum diurnal temperatures (30 °C, 35 °C and 40 °C). Data are means ± SE. NS = not significance. n = 15.

DISCUSSION

An increase in maximum temperature above thermal optimum should have a negative effect on physiological performance. For example, photosynthesis (Haldimann and Feller, 2005; Sharkey, 2005), respiration (Atkin *et al.*, 2006), plant growth (Hunt *et al.*, 2002) and reproductive processes such as flowering and fruit set may be especially sensitive to high temperatures (Sato *et al.*, 2006; Young *et al.*, 2004). However, in a tropical plant the thermal optimum is higher than in a temperate plant, because the former are high temperature adapted species (Larcher, 2003).

According to our results, the thermal optimum of leaf temperature in Habanero pepper should be below 35 °C. Although Nobel (1999) suggests that the increase in leaf temperature would probably be less than 1 or 2 °C. According to our results, a rise in diurnal maximum temperature increased transpiration rate with the effect that this kept leaf temperatures nearly around thermal optimum range in Habanero pepper (~35 °C) that resulted in a decreasing leaf temperature until 5 °C below the air temperature were reached. Thus, we considered the relation $T_{air} - T_L$ describes the sustenance of the healthy major physiological processes (i.e. photosynthesis and respiration), causing a rise both of stomatal conductance and CO_2 intercellular concentration, as well as a CO_2 assimilation rate higher than observed at low temperatures, as was clearly shown in juveniles plants.

According to Larcher (2003), inhibition of photosynthesis by heat stress commonly occurs on tropical and subtropical plants. However, Berry and Björkman (1980) suggest that optimal temperature for photosynthesis varies to a limited extent depending upon the conditions for growth. Furthermore, some growth parameters as leaf area and dry mass exhibited lower values at higher diurnal temperature in reproductive processes (flowering and fruiting). The latter was attributed to an increase in abortion both of flowers and fruits (see chapter IV). Marcelis *et al.* (2004), mentioned that hormonal activity have an effect on abortion of flowers and fruits in reproductive phenophases. This suggests that at elevated diurnal maximum temperature in a tropical plant like Habanero pepper does not have a negative effect on the photosynthetic apparatus, but could have a negative effect on hormonal activity, and diminish some major economic traits in tropical crops.

The purpose of whole day data measurements was to identify the photorespiration diurnal point through the increase of air temperature. According to Monteith (1977) about 30 % of the carbohydrate formed in C_3 photosynthesis is lost through photorespiration and the loss increases with temperature so that photorespiration is significantly particularly inefficient in C_3 crops in tropical climates (Long *et al.*, 2006). However, in Habanero pepper under the established temperature intervals photorespiration was never observed during diurnal measurements.

In this sense, Peñuelas and Llusia (2002) suggest that formation of some isoprenoids like monoterpenes might depend on photorespiratory activity, and that under non-photorespiratory conditions monoterpenes seem to replace photorespiration in providing protection against high temperatures. Thus, the Habanero pepper plants in elevated diurnal temperatures could be producing isoprene as plant protection against specific types of heat stress.

On the other hand, elevated temperature had no significant influence on stomata number. But, both leaf position in plant (bottom, middle, and top) as well as leaf side (abaxial and adaxial), showed significant differences in stomata number. This suggests the influence of other factors, like light intensity that could have had a major environmental effect.

Our results suggest that in a tropical plant as Habanero pepper responses are the result of physiological and metabolic events linked generally to elevated temperature regimes thus triggering a cascade of biochemical and metabolic responses supported by their gene pool. Thus, the parameters evaluated here give an idea about the health of plants, either in optimal or stress conditions.

CONCLUSION

A change in maximum diurnal temperature tends to modify a chain of biological events. Therefore, in this study we showed that an increase in air temperature raised both stomatal conductance and transpiration rate in Habanero peppers causing an increase in temperature deficit; thus, leaf temperature decreased down to 5 °C allowing higher CO_2

assimilation rate in plants at diurnal maximum air temperature (40 °C). We showed that thermal optimum range in a tropical plant crop as Habanero pepper leaf temperature is close to 35 °C; probably at 35 °C leaf temperature gas exchange through stomata is optimal in tropical species for a good performance. Unexpectedly, photorespiration was not present in our experiments. Although an increase in air temperature enhanced photosynthesis values, this was not reflected in plant production. Thus, photosynthates surplus is probably channeled to other metabolic pathways and some are assimilated and used in isoprene production instead of producing biomass. Isoprene production takes carbon directly from the Calvin cycle of photosynthesis (Affek and Yakir, 2003; Delwiche and Sharkey, 1993) and thus holds physiological process in plants, avoiding damages by photorespiration.

This research provides evidence of tropical plant responses under a global warming scenario and also opens perspectives for future studies about the influence of higher temperatures in tropical crops, which takes into account limitations and adaptations of the physiology of tropical plants. It is expected that different responses could take place in plants distributed in other parts of the world.

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CAPÍTULO III

EFFECTS OF LONG-TERM ELEVATED CO_2 CONCENTRATION ON PHYSIOLOGY OF A TROPICAL CROP (*Capsicum chinense* Jacq.)

ABSTRACT

A wealth of experiments on the effects of CO_2 enrichment in plants have frequently shown that photosynthetic stimulation and reduced stomatal aperture over short time periods (short-term) occur. Yet, experiments on the long-term effects of elevated CO_2 showed a wide range of negative feedbacks affecting plant responses to CO_2; the latter are due to photosynthetic acclimation (down-regulation of Rubisco). However, the effects of CO_2 on tropical plants are not well known. Therefore, the objective of this study was to determine the long-term elevated CO_2 exposure effects on plant growth analysis and carbon assimilation in Habanero pepper plants (*Capsicum chinense* Jacq.). Plants were grown in three growth chambers inside a greenhouse at elevated CO_2 conditions (380, 760 and 1140 µmol mol^{-1} respectively) over a long-term period (from seedling until fruiting). Only during the last phenophase plants at elevated CO_2 (760 and 1140 µmol mol^{-1}) increased both their dry mass and leaf area; but in others such traits as RGR, SLA and NAR were not significant. Besides, the dry mass proportion of fruits increased at elevated CO_2. The physiological responses were positive, because the CO_2 assimilation rate (A_N), the CO_2 carboxylation capacity ($V_{c,max}$) and the maximum electron transport rate that contributes to RuBP regeneration (J_{max}) increased and thus contributed to avoid the photosynthetic acclimation in long-term. Thus, this study shows that a tropical crop may have specific responses worth of analysis.

Keywords: elevated CO_2, long-term exposure, photosynthetic acclimation, plant growth analysis

Nota: Este capítulo será sometido a la revista "JOURNAL OF HORTICULTURAL SCIENCE AND BIOTECHNOLOGY"

INTRODUCTION

CO_2 is a key abiotic variable affecting plant growth, development and function, and has environmentally changed in the recent past and has been predicted that it will continue so in the future. One of the evident manifestations of global environmental change is the increase in atmospheric CO_2 concentration (Morison and Lawlor, 1999). According to Intergovernmental Panel on Climate Change (IPCC, 2007) atmospheric CO_2 concentration has arisen from 260 µmol mol^{-1} approximately 150 years ago to 380 µmol mol^{-1} today. This increase will continue probably into the next century; CO_2 concentration levels will double or even triple current levels. However, CO_2 has covered a much broader range of concentrations throughout geological time scales, with values estimated as high as 6000 µmol mol^{-1} during the Paleozoic (500 million years ago) and as low as 200 µmol mol^{-1} during the late Pleistocene (15000 years ago) (Berner, 1997). How do plants respond to broader ranges of CO_2 levels?

It is currently known that elevated CO_2 concentrations increase photosynthesis, dry mass production, and yield, and decrease stomatal conductance and transpiration of most plant species (Drake *et al.*, 1997; Lawlor and Mitchell, 1991). Furthermore, plants have a remarkable capacity to coordinate the growth of their organs, so that there is generally a balance in the biomass invested in whole plants (Poorter and Nagel, 2000). However, biological responses of plants to elevated CO_2 concentration depend on time exposure (short-term and long-term) and functional group (C_3, C_4 and CAM). Thus, both short-term as well as long-term responses of photosynthetic activity and productivity to increased CO_2 concentration are very different (Drake *et al.*, 1997). Frequently, the effects of CO_2 enrichment on plants showed photosynthetic stimulation and reduced stomatal aperture over short time periods (short-term). Yet, the effects of elevated CO_2 during long-term experiments showed a wide range of negative feedbacks affecting plant responses to CO_2 due to photosynthetic acclimation (down-regulation of Rubisco). The photosynthetic acclimation or down-regulation of Rubisco is a phenomenon where the initial growth stimulation provided by elevated CO_2 concentration declines with time, and in some cases, even disappears (Idso and Kimball, 1997). Photosynthetic acclimation involves a decrease in the amount of active Rubisco (Rogers and Humphries, 2000), and can be attributed to an imbalance in the source-sink relationships of plants (Arp, 1991), that leads to an accumulation of nonstructural leaf carbohydrates (Griffin *et al.*, 2000).

Generally, future projections of the effect of atmospheric CO_2 concentration on tropical plants are based on responses in temperate plants.. Long *et al.* (2006) suggests that according to projections issued by IPCC the crop yield will result generally lower in the tropics (assuming photorespiration in plants by high temperatures) and increased yields in temperate zones. However, the accuracy of these projections is uncertain, because little is known about tropical crop plants under long-term elevated CO_2 concentrations.

Therefore, the aim of this paper was to determine the long-term elevated CO_2 exposure effects on plant growth and carbon assimilation of a tropical crop (Habanero pepper, *Capsicum chinense* Jacq.). Because CO_2 exposure time might be a critical parameter in plant response, in this study plants were grown under elevated CO_2 conditions in growth chambers inside a greenhouse over a long-term period (from seedling to fruiting) and Habanero pepper was selected because it is an important tropical crop, with an international market due to its pungency (conferred by capsaicin).

MATERIALS AND METHODS

The experiment was conducted with a tropical crop *C. chinense* Jacq. (Solanaceae family) commonly grown in the Caribbean region and Yucatan Peninsula. The experimental plants were grown over 6 months (starting March 2010 until August 2010) in a greenhouse at the Centro de Investigación Científica de Yucatán (CICY), Mérida, Yucatán, México. The greenhouse has a cooling and ventilation design covering approximately 600 m^2 surface area. Inside the greenhouse three CO_2 modules (each 20 m^3; 3 m width x 3 m long x 2.2 m high), were placed and oriented along a north–south axis, each provided with the same levels of radiation. Forty-day-old plants were grown in 90 pots (one plant per pot) containing a mixture of soil and peat in a 2:1 ratio (v:v). The plants were continuously watered with 120-120-120 NPK nutrient solution.

Randomly selected plants (n=30) were placed in the first module, which was maintained at environmental CO_2 concentration level (ca. 380 $\pm$ 20 µmol mol^{-1}); another lot of 30 plants was located in the second module and kept at twice the CO_2 level (ca. 7600 $\pm$ 15 µmol mol^{-1}), whereas the remaining 30 plants were located in the third module and kept at a threefold CO_2 level (ca. 1140 $\pm$ 10 µmol mol^{-1}). The air of each module was sampled every 5 seconds by a GE Telaire Ventostat 8002 sensor, and a solenoid valve triggered by this sensor to control CO_2 supply. Additionally the CO_2 concentration was analyzed through an

infrared gas analyzer (IRGA) (LI-3600; LI-COR, NE, USA). Air probes were placed at the center of each module, 60 cm above the plants. Temperature and humidity sensors (HOBO H08-004-02; Onset Computer Corp., Bourne, MA, USA) enabled measurement of temperature and relative humidity (RH). A minisplit air conditioner of 5000 BTUS (LG®) average temperature was maintained throughout the experiment at ca. 30/25 °C (day/night) and RH at ca. 75% in each module, Quantum sensors (LI-190SB; LI-COR, NE, USA) were placed on top and inside each module to record photosynthetically active photon flux density (PPFD, average PPFD was 600 and 700 µmol m^{-2} s^{-1} during the spring and summer periods, respectively). Pots were rotated weekly to avoid edge effects and greenhouse influences that would affect the reliability of the experiment. Plant production was determined by a series of four destructive harvests performed the day the plants were placed inside their relevant module (seedling stage) (Harvest 1, T0; 40 days after sowing), juvenile stage (Harvest 2, T1; 70 days after sowing), flowering stage (Harvest 3, T2; 100 days after sowing) and fruiting stage (Harvest 4, T3; 130 days after sowing). For each harvest, 5 plants per treatment were collected. All the samples were dried in an oven at 65 °C until constant weight was reached and then the dry mass (DM) and dry mass proportion per organ (DM%) were determined. Relative growth rate (RGR), leaf area ratio (LAR), specific leaf area (SLA), net assimilation rate (NAR) and leaf weight ratio (LWR) were calculated as described by Hunt *et al.* (2002). RGR was determined for the period including first harvest (T0) until the next harvest (T1, T2, and T3). Total leaf area was measured with a surface area meter (LI-3100, LI-COR, NE, USA).

A-C_i curve determinations. Gas exchange analyses were carried out inside each greenhouse module at the relevant growth conditions. *A*-C_i determinations were conducted at 30 °C and 75 % RH with a portable system infrared gas analyzer (LI-6400, LI-COR, NE, USA). Fully expanded apical leaves were introduced into the gas-exchange leaf chamber of the LiCor 6400. The determinations were repeated in five different leaves of each treatment. The gas-exchange response to CO_2 was measured from 0 to 2000 µmol mol^{-1} CO_2. The light saturated rate of CO_2 assimilation (A_{sat}), the maximum photosynthetic rates (A_{max}), and the stomatal conductance (g_s) were estimated at a PPFD of ca. 1200 µmol m^{-2} s^{-1} using equations developed by von Caemmerer and Farquhar (1981). Estimation of the maximum carboxylation velocity of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) ($V_{c,max}$) and the maximum electron transport rate contributing to RuBP

regeneration (J_{max}) were made by fitting a maximum likelihood regression below and above inflexion of the A/C_i response using the method of Ethier and Livingston (2004). Stomatal limitation (l), which is the proportionate decrease in light-saturated net CO_2 assimilation attributable to stomata, was calculated according to Farquhar and Sharkey (1982) as $l = [(A_0-A_1)/A_0$, where A_0 is the A at C_i of 360 µmol mol^{-1} and A_1 is A at C_a of 360 µmol mol^{-1}. The leaf internal CO_2 concentration (C_i) was estimated as described by Farquhar and Sharkey (1982).

In addition, throughout the day (at ca. 7:00, 9:30, 12:00, 14:30 and 17:00 h) gas exchange analyses was performed inside each growth chamber; fifteen fully expanded young leaves from each treatment were introduced into the gas-exchange leaf chamber of the LiCor 6400 and CO_2 assimilation rate (A_N), stomatal conductance (g_s), transpiration (E), intercellular CO_2 concentration (C_i), and ratio intercellular CO_2 atmospheric CO_2 (C_i/C_a) and temperature deficit ($T_{air} - T_L$) were estimated.

RESULTS

Elevated CO_2 concentrations increased DM production up to 30 % and 57 % at 760 and 1140 µmol mol^{-1} CO_2 , respectively, during the fruiting stage (Fig. 1a). In addition, compared with the plants in 380 µmol mol^{-1} CO_2 treatment, plants at elevated CO_2 had a larger leaf area (14 and 40 % at 760 and 1140 µmol mol^{-1} respectively) in the fruiting stage (Fig 1b). Interestingly, exposure to elevated CO_2 was not significant in early stages (growth and flowering phenophases) (Fig. 1a and 1b). Moreover, plant growth analysis showed that elevated CO_2 did not significantly enhance the RGR_{DM}, RGR_{LA}, SLA, LAR, NAR, and LWR in any phenophase between treatments; however, there was a change from one stage to another (Fig. 2). Moreover, the dry mass proportion per organ (DM%) changed in each phenological stage. But, in the fruiting stage, DM% of leaves at elevated CO_2 (760 and 1140 µmol mol^{-1}) was lower than plants at 380 µmol mol^{-1}; nevertheless, DM% of reproductive organs (flower and fruits) increased in elevated CO_2 treatments (Fig. 3).

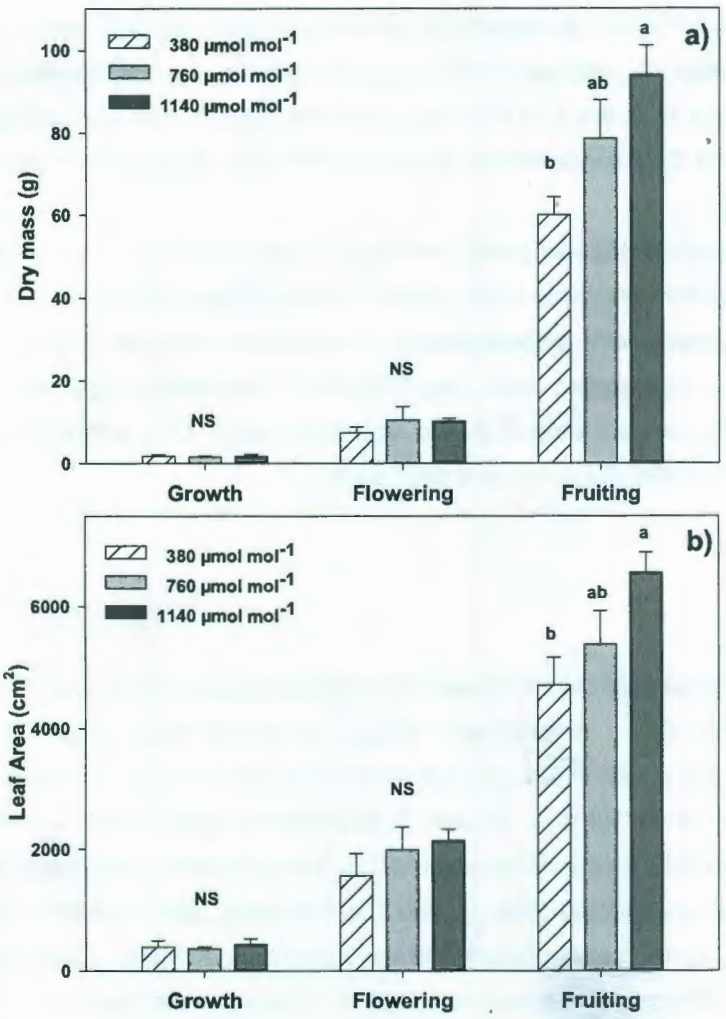

Figure 1. a) Dry mass and b) Leaf area of Habanero pepper plants in different phenology stages at three atmospheric CO_2 concentrations (380, 760 and 1140 µmol mol^{-1}). Data are means ± SE. Different letters in the same phenological stage represent statistical differences (Tukey, $\alpha = 0.05$); NS = not significance. $n = 5$.

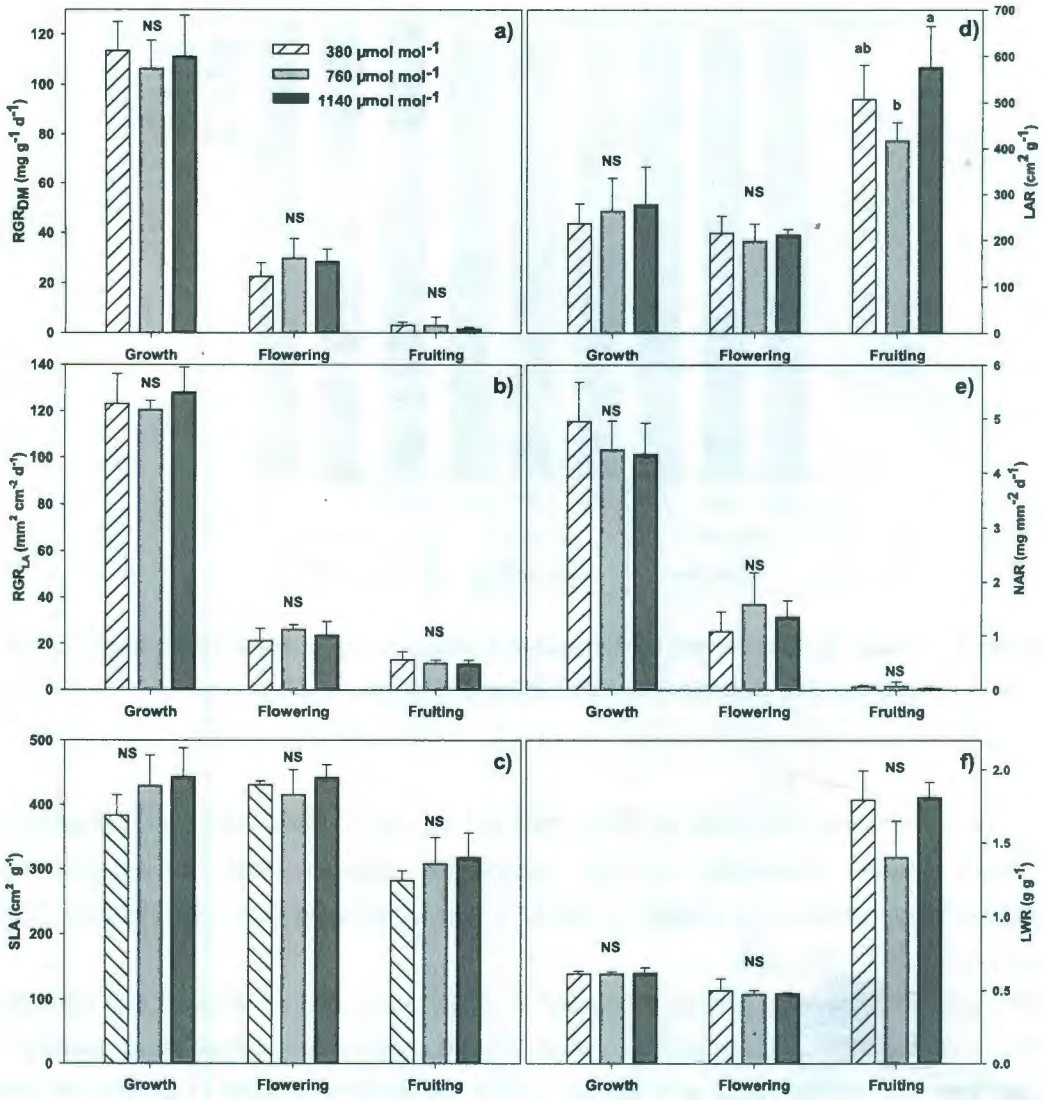

Figure 2. a) Relative Growth Rate (dry mass), b) Relative Growth Rate (leaf area), c) Specific Leaf Area, d) Leaf Area Ratio, e) Net Assimilation Rate and f) Leaf Weight Ratio in Habanero pepper plants at three atmospheric CO_2 concentrations (380, 760 and 1140 µmol mol^{-1}). Data are means ± SE. Different letters in the same phenological stage represent statistical differences (Tukey, α = 0.05); NS = not significance. n = 5.

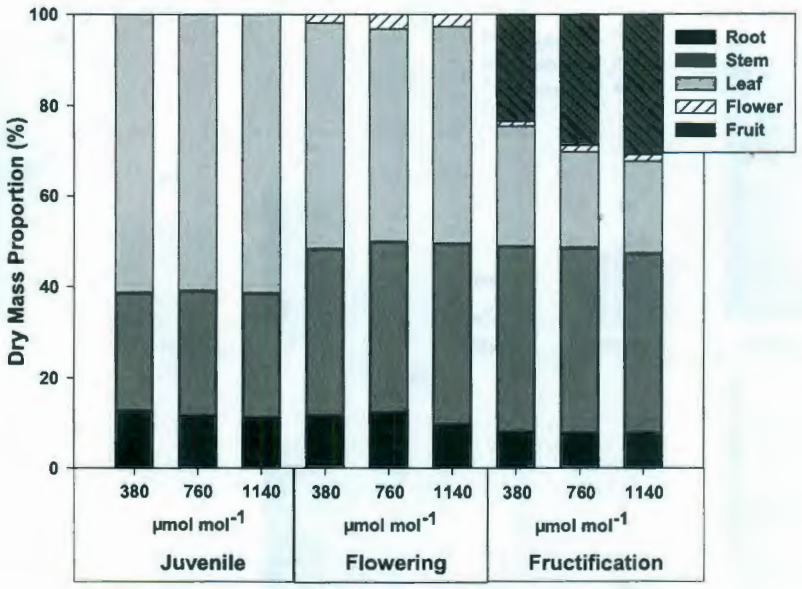

Figure 3. Average Dry Mass Proportion of Habanero pepper plants in different phenological stages at three atmospheric CO_2 concentrations (380, 760 and 1140 µmol mol^{-1}). n = 5.

On the other hand, from 100 to 2800 µmol m^{-2} s^{-1} the A–PPFD curve measurements showed greater adaptability to CO_2 assimilation rate in order to increase the photosynthetic photon flux density in plants grown at elevated CO_2 (i.e. 760 and 1140 µmol m^{-2} s^{-1} CO_2) (Fig. 4a).

The A–C_i curve determinations conducted in *C. chinense* leaves showed that elevated CO_2 increased carboxylation activity, which was reflected by an increase in the maximum photosynthetic activity (A_{max}) and light saturated photosynthetic rates (A_{sat}) (Fig. 4b and Table 1). Similarly, plants grown under 1140 µmol mol^{-1} CO_2 had a higher CO_2 carboxylation capacity ($V_{c,max}$) and a maximum electron transport rate contributing to RuBP regeneration (J_{max}) (Table 1). Exposure to elevated CO_2 diminishes stomatal conductance (g_s), but enhances the intercellular CO_2 relative to the ambient CO_2 concentration (C_i/C_a) (Table 1). In addition, elevated CO_2 increased stomatal limitation (l), and modified leaf mitochondrial respiration (R_D).

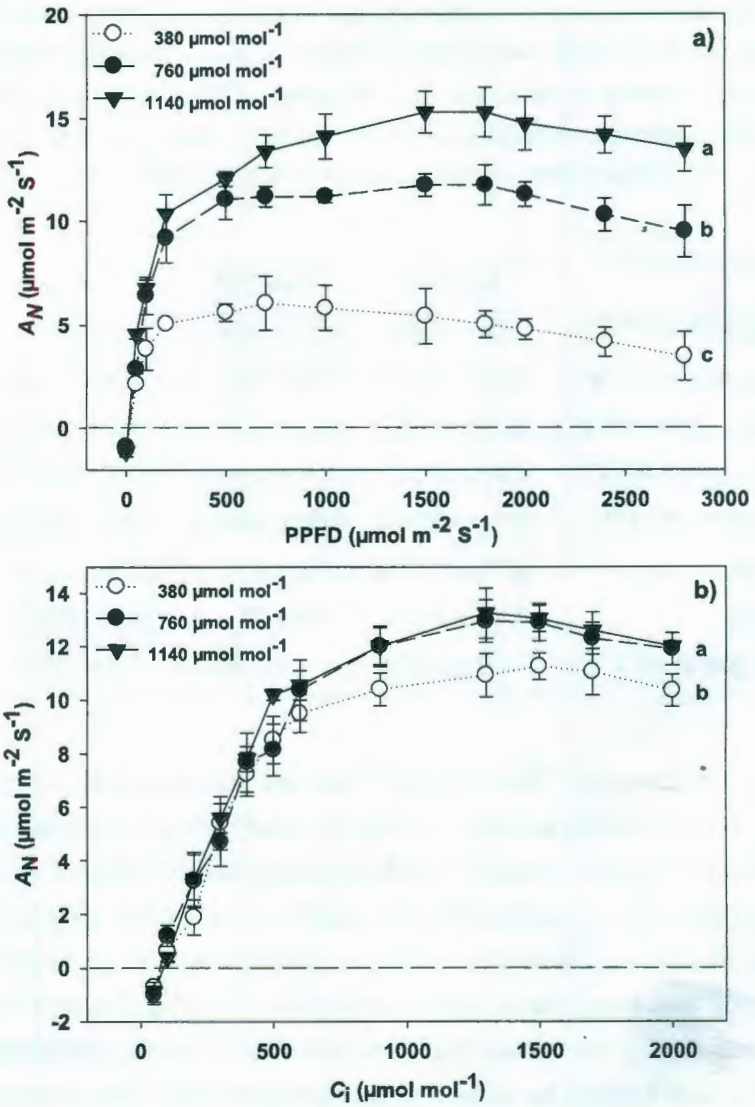

Figure 4. The response of photosynthetic CO_2 uptake rate to PPFD (a) and C_i (b) in Habanero pepper plants at three atmospheric CO_2 concentrations. Open circles correspond to plants grown under ambient CO_2 (ca. 380 µmol mol^{-1}), closed circles to those grown in twofold CO_2 concentration (760 µmol mol^{-1}) and closed triangles to those grown in threefold CO_2 concentration (1140 µmol mol^{-1}). Data are means ± SE. The different letters indicate significant differences (Tukey, $\alpha = 0.05$) among treatments. $n = 5$.

Table 1. Long-term (5 months) elevated CO_2 exposure (ambient CO_2 vs. twofold and threefold CO_2) effect in terms of saturating maximum photosynthetic rate (A_{sat}), maximum photosynthetic rate (A_{max}), maximum velocity of RuBP carboxylation by Rubisco ($V_{c,max}$), maximum capacity of RuBP regeneration (J_{max}), stomatal conductance (g_s), intercellular CO_2 relative to the ambient CO_2 concentration (C_i/C_a), stomatal limitation (l) and dark respiration (R_D) in Habanero pepper plants. Data are means ± SE. Different letters within same row indicate statistical differences (Tukey, α = 0.05). n = 5.

	Ambient	Twofold	Threefold
A_{sat} (µmol m^2 s^{-1})	5.6 ± 0.40 c	10.3 ± 0.20 b	12.0 ± 0.40 a
A_{max} (µmol m^2 s^{-1})	12.5 ± 0.50 b	13.5 ± 1.50 a	13.7 ± 1.40 a
$V_{c,max}$ (µmol m^2 s^{-1})	22.9 ± 6.40 b	23.2 ± 5.90 ab	25.2 ± 0.80 a
J_{max} (µmol m^2 s^{-1})	43.7 ± 2.01 b	46.8 ± 0.50 a	48.4 ± 5.27 a
g (µmol m^2 s^{-1})	0.11 ± 0.01 a	0.08 ± 0.05 b	0.07 ± 0.01 b
C_i/C_a	0.70 ± 0.11 a	0.78 ± 0.09 a	0.78 ± 0.09 a
l (%)	8.60 ± 2.87 b	15 ± 10 b	44.5 ± 9.50 a
R_D (µmol m^2 s^{-1})	0.9 ± 0.39 b	1.1 ± 1.18 ab	1.5 ± 0.41 a

The plants at 1140 µmol mol^{-1} CO_2 increased their net CO_2 assimilation rate above 380 and 760 µmol mol^{-1} from 9:30 to 14:30 h, when the maximum photosynthetic photon flux density (PPFD) occurred; then, except for early-morning measurements (at 7:00 h), the A_N in plants at 380 µmol mol^{-1} was lower than in those at elevated CO_2 treatments (Fig. 5a) throughout day. Furthermore, during all diurnal courses the stomatal conductance in plants at 1140 µmol mol^{-1} was lower than in plants at 380 µmol mol^{-1} (Fig. 5b). With the exception of noon and end of day, the transpiration rate was similar among treatments (Fig. 5c). Intercellular CO_2 was different for all treatments during the day, in that elevated CO_2 was higher than in the treatment with the lowest values (Fig. 5d). The intercellular CO_2 / atmospheric CO_2 ratio was not different among plants at elevated CO_2 treatments (Fig. 5e) and the temperature deficit never exceeded 0.5 °C among treatments during the day (Fig. 5f).

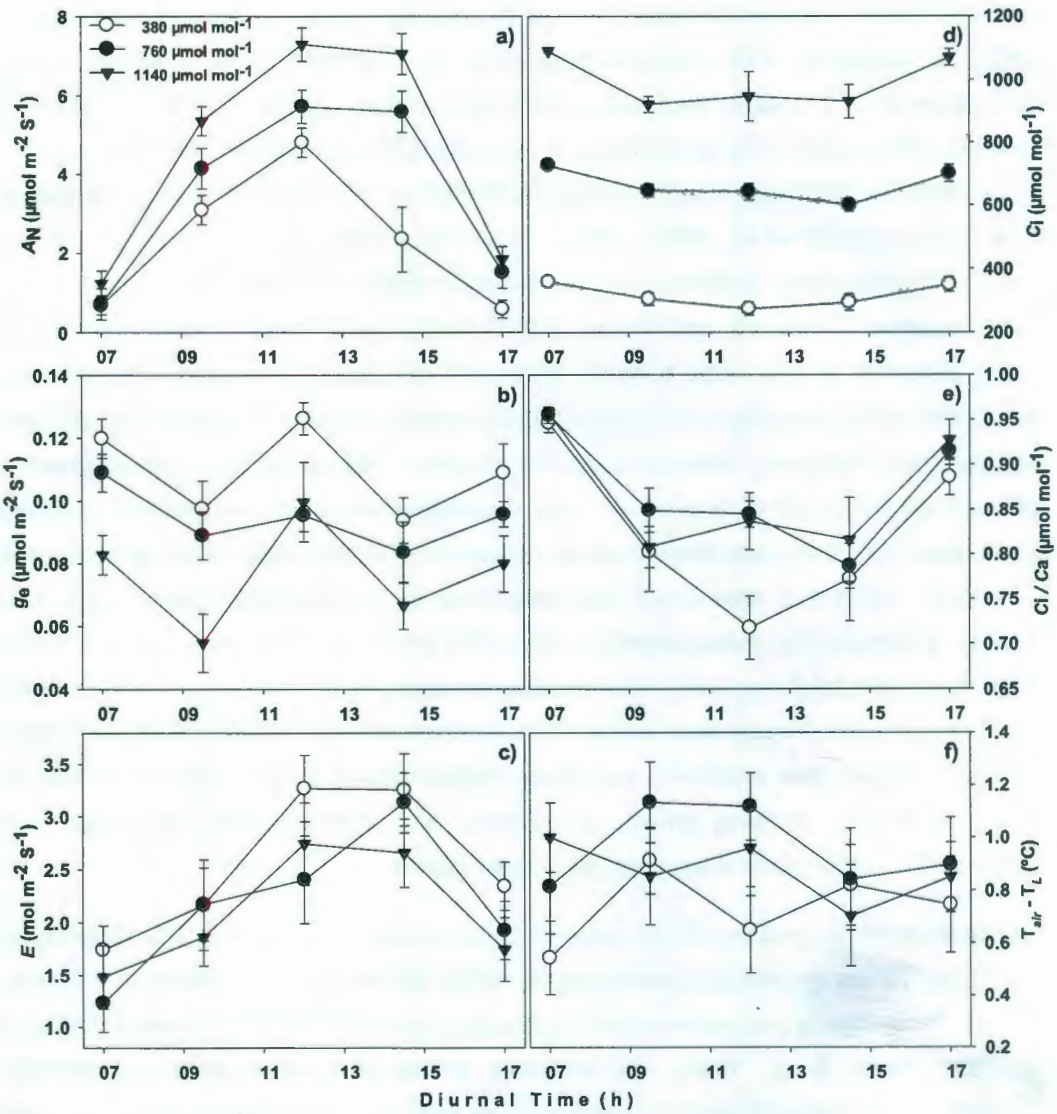

Figure 5. a) CO_2 Assimilation Rate (A_N), b) Stomatal Conductance (g_s), c) Transpiration (E), d) CO_2 intercellular (CI), e) Ratio intercellular CO_2 / atmospheric CO_2 (C_i / C_a) and f) Temperature Deficit ($T_{air} - T_L$) in Habanero pepper plants at three atmospheric CO_2 concentrations (380, 760 and 1140 µmol mol^{-1}). Data are means ± SE. n = 15.

DISCUSSION

Both short-term (minutes to hours) as well as long-term (days to weeks) responses of plants to increased CO_2 concentrations are very different (Drake *et al.*, 1997). Consequently, in long-term responses it is expected that prolonged growth of plants at increased atmospheric CO_2 concentrations will enhance their biomass. This prediction is in agreement with most experimental results performed at optimal growth conditions (Davey *et al.*, 2004; Lindroth *et al.*, 1998). Nevertheless, some studies suggest that initial growth stimulation provided by elevated CO_2 concentration decline with time and in some cases, even disappear (Ainsworth and Rogers, 2007; Grulke *et al.*, 1990; Tissue and Oechel, 1987). However, in this study both dry mass and leaf area in elevated CO_2 increased significantly only during the fruiting stage (80 days after beginning of treatments). We think that higher dry mass was observed due to an increase in fruit production in plants grown at elevated CO_2 (760 and 1140 µmol mol^{-1}) as a positive consequence of long-term exposure at elevated CO_2. DM% was evident as an increase in fruit proportion of plants at elevated CO_2; thus, higher leaf area could have been due to the fact that bigger leaves were needed to produce the photosynthates sink for the production of surplus fruits. However, RGR, SLA, LAR, NAR, and LWR differences were not significant among treatments. In this way, some studies (Poorter and Navas, 2003; Poorter and Perez-Soba, 2002; Centritto *et al.*, 1999) suggest that a problem with these measurements is that the effect of CO_2 on RGR and other underlying growth components are relatively small and often time-dependent, occurring only at early stages of plant growth.

On the other hand, ,photosynthetic rates of plants exposed to elevated CO_2 were higher than those values registered in plants grown under ambient CO_2 conditions in Habanero pepper. These results suggest a larger CO_2 fixation rate at the whole plant level (Davey *et al.*, 2006; Norby *et al.*, 1999). The analyses of stomatal conductance (g_s), stomatal limitation (l), and intercellular CO_2 relative to the ambient CO_2 concentration (C_i/C_a) data reveal that after saturation levels of intercellular CO_2 (C_i) have been reached a likely partial stomatal closure could be involved in the decrease of the stomatal conductance. The A–C_i curve parameters (A_{sat}, A_{max}, $V_{c,max}$, and J_{max}) suggest that non stomatal processes also could have contributed to the increase in Rubisco activity. Such results imply that these plants did not show photosynthetic acclimation. Then, the reduction in the magnitude of

the stimulation of net CO_2 uptake (A_N) following long-term growth at elevated CO_2 has been termed photosynthetic acclimation and has been largely attributed to a reduction in the amount of active Rubisco (down-regulation of Rubisco) caused by an imbalance in the source-sink relationships of plants (Arp, 1991), which leads to an accumulation of leaf carbohydrates (there is a link between the production of carbohydrates and reduction in production of Rubisco protein) (Ainsworth and Rogers, 2007; Griffin *et al.*, 2000; Rogers and Humphries, 2000; Sicher and Bunce, 1997). However, more detailed studies are necessary to increase the understanding of the processes that lead to the onset of acclimation (Griffin *et al.*, 2000; Rogers and Humphries, 2000). Furthermore, the *A*–PPFD curve revealed that plants grown at elevated CO_2 have better responses to other environmental factors. These suggest that Habanero pepper plants under field conditions (in the tropic PPFD at ca. 1800 μmol m^2 s^{-1}) in a future scenario of CO_2 concentration (at least duplicated according to theoretical projections) would enhance its photosynthetic response and consequently would exhibit an increase in desired traits like fruit production.

The fact that Habanero pepper plants at elevated CO_2 had a larger DM production suggests that those plants were capable of avoiding photosynthetic acclimation due to their larger C sink strength, which in turn might have enabled photosynthetic enhancement. The ability to increase sink strength suggests that the demand for recently fixed C was sufficient to balance the enhanced carbohydrate supply under elevated CO_2 conditions (Aranjuelo *et al.*, 2009).

Calculated respiration suggests carbohydrate respiration as one of the main processes contributing to increase C sink strength. The increase in respiration shown here is consistent with the widely reported increase in leaf soluble carbohydrates during long-term growth at elevated CO_2 concentration (Moore *et al.*, 1999). Azcon-Bieto and Osmond (1983) showed that increase in leaf nonstructural carbohydrate content produced by manipulation of photosynthesis during the photoperiod increased subsequent dark respiration rates. Therefore, a similar response might be expected when elevated CO_2 concentration increases photosynthesis and leaf nonstructural carbohydrate content during the photoperiod.

CONCLUSION

Although in recent years the effects of elevated atmospheric CO_2 in plants have been intensively studied, knowledge on such effects in crop plants is still underway. Therefore, in this research we analyzed plant responses under possible future scenarios of atmospheric CO_2 concentration increase in a regional economically important crop. Based on our results we conclude that the long-term effects of growth in elevated CO_2 in Habanero pepper plants, both DM and LA over time showed a better response than in early stage. Thus, to avoid the photosynthetic acclimation (down regulation of Rubisco) in plants at elevated CO_2 concentration, fruit production increased (see DM% or also chapter IV and V), causing a strong sink by fruit production, avoiding nonstructural carbohydrates excess in leaves. Thus the CO_2 assimilation rate (A_N), the CO_2 carboxylation capacity ($V_{c,max}$), and the maximum electron transport rate that contribute to RuBP regeneration (J_{max}) were higher (compared with ambient CO_2); then down regulation of Rubisco was avoided and the source-sink relation was balanced.

On the other hand, the fact that plants decreased stomatal conductance when intercellular CO_2 was saturated, and thus lowered their transpiration rate, suggest a better hydric drive. Therefore, this study opens perspectives to others experiments with altered CO_2 levels where tropical crops are better represented in research programmes, because it was shown that its physiological responses could be different if compared to plant species in other areas of the globe.

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CAPÍTULO IV

CHANGES IN FLOWERING AND FRUITING OF HABANERO PEPPER IN RESPONSE TO HIGHER TEMPERATURE AND CO_2

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ABSTRACT

Global climate change is likely to increase maximum temperatures and atmospheric CO_2 concentrations. High temperatures can alter plant reproductive phenology, resulting in earlier or later flowering. High atmospheric CO_2 levels have a positive fertilizing effect in plants, producing higher biomass and flower number. The objectives of this research work were to quantify the effects of higher maximum temperature (Experiment 1) and atmospheric CO_2 concentration (Experiment 2) on reproductive phenology in the tropical crop Habanero pepper (*Capsicum chinense* Jacq.). Three growth chambers were used in each experiment. In Experiment 1, different diurnal maximum temperatures (30, 35 and 40 °C) were used in each chamber. In Experiment 2, diurnal maximum temperature was set at 30 °C and different atmospheric CO_2 concentrations (380, 760 and 1140 µmol mol^{-1}) were used in each chamber. Relative humidity, maximum photosynthetic photon flux density (PPFD) and photoperiod were the same in all chambers of both experiments. Variables were flowering days, fruiting days, flower production, flower abortion, fruit production and flower morphology traits. A maximum temperature increase from 30 to 35 °C caused flowering to be 6 days earlier. But, at 35 °C, fruiting was 27 days later than at 30 °C. When comparing plants grown at 380 µmol mol^{-1} CO_2, those grown at 1140 µmol mol^{-1} flowered 18 days earlier and fruited 37 days earlier. Both maximum temperature and CO_2 concentration had significant effects on flower abortion and fruit production. Overall, Habanero pepper responded negatively to higher diurnal maximum temperatures and positively to higher CO2 concentrations.

INTRODUCTION

It is expected that by the late 21st Century, CO_2 concentration levels will double or even triple current levels (380µmol mol^{-1}), and air temperature will rise at least 2 °C. Indeed, it is quite probable that most regions on earth are already experiencing higher than average maximum temperatures, more hot days and a higher heat index [16]. Agriculture is largely subjected to atmospheric conditions [20], and any change under these conditions will modify planting dates and perhaps force the relocation of certain crops [16].

Higher temperatures adversely affect crop productivity and yield [23, 25, 28, 36]. Increases in temperature can have various effects on plants, including alterations in phenological stages [16, 27, 30], particularly in reproductive phenology [32]. For instance, higher temperatures can cause both earlier and later flowering [14, 30], and hinder fertilization, which can consequently inhibit fruit set [10]. In contrast, increases in atmospheric CO_2 concentrations generally have a fertilizing effect, increasing biomass and leaf area [4, 22], causing earlier flowering and raise flower count per plant [11, 12]. Nonetheless, any difference in phenological stage timing can cause pollinator desynchronization, consequently lowering the number of fruit yield and reducing seed quantity per fruit [5].

A small number of studies have been done on the effects of temperature and CO_2 concentration with *Capsicum annuum* (Solanaceae) as a model [10, 26]. To our knowledge, no previous studies had been undertaken that assess these effects using a regional variety of *C. chinense* known as Habanero pepper. This variety is an interesting model because it is adapted to hot tropical zones, can flower in any season, has a short life cycle and can grow under different environmental conditions. It is also commercially valuable and its fruit and derivatives are used worldwide as condiments, cosmetics additives and as a lachrymatory agent in pepper sprays [7]. Although Habanero pepper flowers can be self-pollinated, cross-pollination is needed to increase the number and quality of fruits and especially seed production [6]. In pepper plants, nectar functions as a pollinator attractor, and therefore any variation in nectar characteristics (e.g. sugar quantity and nectar volume) can potentially increase attractiveness to pollinators, consequently enhancing cross pollination and improving fruit quality and crop yield [29]. Any variability in nectar traits is important to document since it can clearly affect crop success. In addition, fruit quality in pepper plants depends heavily on increased flower morphology variation produced by

changes in environmental conditions [2]. The aim of the study was to evaluate the effect of increased temperature and atmospheric CO_2 concentration on flowering and fruiting, as well as on flower, fruit morphology, and fruit yield under greenhouse conditions in an effort to understand plant responses in this type of crops.

MATERIALS AND METHODS

Two experiments were conducted from January 2009 to August 2010 in the Scientific Research Center of Yucatán, México. Each experiment used three 9 m^2 growth chambers designed specifically for the experiment and located in a greenhouse under controlled conditions. Each growth chamber was built of transparent glass to allow sunlight in, and equipped with a 5000 BTU (LG®) air conditioner to control temperature and a solenoid valve triggered by a GE Telaire® Ventostat 8002 sensor to control CO_2 supply. Dataloggers (HOBO H08-004-02; Onset Computer Corp., Bourne, MA, USA) were placed on the top of plants in each chamber to monitor air temperature, air humidity, and light environment. Also, quantum sensors (LICOR, Li-190; Nebraska, USA) and thermometers of maximum-minimum were used to measure horizontal and vertical gradients of PAR (photosynthetically active radiation) and temperature respectively.

To evaluate the effect of temperature increases on plant (temperature experiment), growth chambers were maintained under similar conditions with 82 ± 4% relative humidity, 700 µmol $m^{-2}s^{-1}$ maximum photosynthetic photon flux density (PPFD), and 11 h of natural daylight (photoperiod). Air temperature was the only factor that differed among chambers. A fixed maximum diurnal temperature was set in each chamber and the nocturnal temperature was left to natural variation (24 ± 1 °C). In chamber one, the maximum diurnal temperature was maintained at 30 °C, which has been considered as a non-stress temperature for plants in tropical environments; in chamber two, maximum diurnal temperature was set at 35 °C, simulating an air temperature increase of five degrees; and chamber three was set at 40 °C, simulating an air temperature increase of 10 °C. In the second experiment (CO_2 experiment), relative humidity, maximum photosynthetic photon flux density, photoperiod and nocturnal temperature were maintained similar to the temperature experiment, but diurnal maximum temperature was set at 30 °C in the three

growth chambers (to avoid temperature stress). In addition, different atmospheric CO_2 concentrations were used among chamber. Chamber one was maintained at 380 µmol mol^{-1}, which corresponds to the mean world CO_2 atmospheric level reported until 2007 (IPCC, 2007); in chamber two, the atmospheric CO_2 level was twofold concentrated (760 µmol mol^{-1} CO_2); and in chamber three, the CO_2 concentration was threefold (1140 µmol mol^{-1}).

Habanero pepper (Kukulcan, Seminis®) seeds were planted in polystyrene well trays in a peat moss substrate and watered every three days in a nursery. After germination, 150 seedlings (45 days old) were randomly selected from a pool of 400 seedlings and transplanted to individual pots containing a 2:1 mixture of soil and peat moss. In each experiment, seedlings were randomly distributed in the growth chambers (25 seedlings per chamber, i.e., 75 plants per experiment) and they were watered every 3-4 days and fertilized bi-weekly with 120:120:120 NPK mixture. Environmental effects in the chambers were controlled for by randomly changing plant location every 20 days.

In both experiments, flowering stage was considered to have reached when more than half (50%+1) of the plants in each growth chamber exhibited flowers. Flowering stage was considered as having ended and fruiting stage begun when more than half (50%+1) of the plants in each growth chamber exhibited fruits.

During the flowering stage in each experiment, five plants per chamber were randomly chosen and flower morphology described for ten flowers per plant. For each flower, measurements were taken of anther, filament, style, and pistil length, ovary length and width, number of anthers, flower area, nectar volume and number of pollen grains per anther. Flower area, the petal area in a fully open flower, was measured with a surface area meter (LICOR, LI-3100; Nebraska, USA). Nectar was extracted from each flower's nectary by carefully inserting a 0.5 µL volume microcapillary tube (Drummond®), and volume estimated by measuring nectar column length inside the tube. Nectar total soluble solids in sucrose equivalents were measured with a refractometer (0-100% °Bx) and the results used to estimate total nectar sugar concentration (mg cm^{-3}) [35]. Pollen grains per anther were estimated by placing the anther of each sampled flower in an Eppendorf tube inside a plastic bag containing silica gel and leaving the tubes uncapped for approximately 72 h. When the anthers had completely opened and the pollen grains had been released,

200 µL $tube^{-1}$ of an alcohol/liquid soap mixture (95:5 v/v) were added. Tubes were then agitated for 2 min in a vortex to ensure homogeneous pollen grain distribution in the solution. Using a graduated micropipette, 20 µL of this solution were extracted and placed on the surface of a Neubauer chamber and the grains counted using an optical microscope (10X). All the identifiable pollen grains in sixteen 1 mm^2 sub-quadrants of the chamber were counted and pollen grain density per mm^3 estimated [13].

In each experiment, flower production (flowers $plant^{-1}$) was calculated by making weekly flower counts beginning at first appearance of flowers; fruit production (fruits $plant^{-1}$) was calculated by weekly fruit counts starting at first appearance of fruit. Mature fruits were harvested weekly, fruit length, width and pericarp thickness measured, and seed number per fruit counted. Data were analyzed using ANOVA and significance differences between the means calculated with a Tukey test. All statistical analyses were run with the Statistica 7 package (Statsoft®).

RESULTS

In the temperature experiment, the flowering stage was reached six days earlier in plants at 35 °C than plants at 30 °C, but fruiting was delayed by 27 days (Fig. 1A and 1B). Plants at 30 °C treatment were the first to go through the fruiting stage (Fig. 1B). Flower abortion in plants both 35 °C as well as at 40 °C increased sharply (Fig. 2A). Thus, flowering and fruiting in plants were substantially reduced (Fig. 1A and 1B). Contrastingly, plants in 1140 µmol mol^{-1} CO_2 chamber began to flower 18 days earlier (Fig. 3A) and produced fruits 37 days earlier (Fig. 3B). Flower abortion was also significantly reduced in plants from the chamber at 380 µmol mol^{-1} to plants at the 1140 µmol mol^{-1} CO_2 chamber (P = 0.04, F = 3, df = 2) (Fig. 2D). No differences were observed in flowering times between the 760 µmol mol^{-1} and 1140 µmol mol^{-1} CO_2 treatments (Fig. 3A), although onset of fruiting at 760 µmol mol^{-1} was later than at 1140 µmol mol^{-1} (Fig. 3B).

Fruit number per plant decreased with higher temperatures (P = 0.041, F = 3.45, df = 2) and increased with higher CO_2 concentration (P = 0.033, F = 1.13, df = 2). As the temperature increased, the fruit number decreased from 72 fruits per plant at 30 °C treatment to 35 fruits at 35°C and 15 fruits at 40 °C (Fig. 2B). In contrast, fruit number

increased from 61 fruits per plant at 380 µmol mol^{-1} to 94 fruits at 760 µmol mol^{-1} treatment and up to 115 fruits at 1140 µmol mol^{-1} CO_2 (Fig. 2E).

Overall, flower traits remained unchanged under the three temperature treatments. Exceptions were pistil length, which was longer in plants maintained at 40 °C than at 30 °C and 35 °C (P = 0.004, F = 8, df = 2), and filament length, which was longer in plants at 35 °C than at 45 °C and 30 °C (P = 0.005, F = 5, df = 2) (Table 1). Changes in CO_2 concentration led to changes in flower morphology, with flower area increasing along the increasing CO_2 concentration gradient (P = 0.001, F = 15, df = 2) (Table 1). Pollen grain number per anther did not differ in either temperature or CO_2 experiments (Table 1).

Nectar volume was unaffected by temperature, but nectar sugar concentration decreased as temperature increased between chambers (P = 0.0013, F = 12, df = 2) (Table 2). Higher CO_2 concentration raised flower nectar volume (P = 0.006, F = 7, df = 2) (Table 2), but lowered nectar sugar concentration (P = 0.0001, F = 15, df = 2) (Table 2).

Fruit length and seed number were unaffected by temperature, although fruit width (P = 0.02, F = 4, df = 2) and pericarp thickness (P = 0.001, F = 8, df = 2) decreased with higher temperature (Table 3). Fruit morphology differed only minimally between the 380 µmol mol^{-1} and 760 µmol mol^{-1} CO_2 concentrations, but plants in the 1140 µmol mol^{-1} CO_2 concentration led to increased fruit width (P = 0.05, F = 2.9, df = 2) and pericarp thickness (P = 0.05, F = 3.3, df = 2). Seed number also increased with CO_2 concentration (P = 0.03, F = 3.8, df = 2) (Table 3).

DISCUSSION

Results suggest that high temperature and high atmospheric CO_2 concentration have an effect on Habanero pepper biology, a species adapted to warm and tropical conditions. The present study contributed to understanding plant responses to changes in atmospheric conditions. Most studies evaluating plant response to climate change have been done in temperate regions where maximum temperature, photoperiod, light intensity and other environmental conditions differ from those in the tropics. To date, different studies of plant phenology under higher maximum temperature and CO_2 concentrations

have produced highly variable and inconsistent results, most likely as a result of different temperatures and CO_2 concentrations used. Although, experiments made under greenhouse chamber conditions may be limited for predicting plant responses to overall climate changes, the results presented herein are valuable because they highlight how air temperature and CO_2 increase can change plant phenology, flower, and fruit traits in a tropical crop.

Interestingly, plants responded differently in temperature and CO_2 experiments. With an increase in maximum temperature from 30 to 35 °C, flowering onset was at least 6 days earlier than normal. This is in agreement with a number of previous studies. Hovenden *et al.* [14] reported earlier flowering in grasses and weeds with an average 2 °C increase in temperature, while Crepinsek *et al.* [8] observed flowering to begin from 3 to 7 days earlier in *Junglans regia* L. with a 0.9 °C rise in temperature. In a meta-analysis covering almost 30 years and including 542 plant species a high positive correlation between increased temperature and earlier flowering (2.5 days decade^{-1}) was found [21].

Increased temperature had a contrary effect on fruiting, in that higher temperatures raised floral abortion and consequently diminished fruit production. In a previous study a similar response in *C. annuum* was reported, in which flower abortion was enhanced with temperature increase, resulting in fewer fruits per plant [3]. In other studies with *C. annuum*, fruit production decreased at higher temperatures, and fruit abortion was probably due to hormonal imbalance [9, 10]. High temperature induces abortion of the reproductive organs in *C. annuum* due to decreased transport of auxin (IAA) and increased ethylene biosynthesis (ACC) [15]. Plants of *Solanum lycopersicum* exposed to temperatures above 38 °C exhibited higher levels of gibberellines and auxins [17]. This may explain why in the present study the *C. chinense* plants in the 40 °C treatment could not even attain 50% flowering or fruiting.

Increase in atmospheric CO_2 clearly affected flower and fruit phenology in the *C. chinense* plants in the CO_2 experiment. Flowering was much earlier, flower abortion much lower and fruit production higher as CO_2 concentration increased. These results coincide with those of Ward and Strain [34], who reported that a 75% rise in CO_2 concentration resulted in earlier flowering by five days in *Arabidopsis thaliana*. When CO_2 concentration was increased from 350 to 700 µmol mol^{-1} plants of *Vicia faba* they exhibited a longer flowering period and the flowers per fruit ratio was 25% higher, suggesting that additional photosynthetic

assimilates generated by greater atmospheric CO_2 availability are utilized to begin flowering earlier and are used for flower maintenance [24]. In contrast, no differences in flowering time (number of days to flowering) or number of flowers per plant in *Tropaeolum majus* were reported by Lake and Hughes [18] when increasing CO_2 concentrations from 380 µmol mol^{-1} to 750 µmol mol^{-1}. In the present study, the 1140 µmol mol^{-1} CO_2 level also led to higher seed number per fruit and increases in morphological measurements such as fruit length and width, plus pericarp thickness. Many of these changes are probably associated with photosynthetic assimilates production in response to CO_2 levels. We have observed that Habanero pepper plants at 1140 µmol mol^{-1} CO_2 increased their CO_2 assimilation rate two-fold with respect to values obtained for plants at 380 µmol mol^{-1} CO_2, as estimated by infrared gas analyzer (IRGA) (Garruña-Hernández R unpublished). For instance, when the carbon-assimilate source decreases, flower and fruit abortion in pepper increase linearly [19]. Competition for photosynthetic assimilates among fruits under a CO_2-limited concentration atmosphere, can be strong as the growing fruit process may demand up to 50% of the carbon assimilated by photosynthesis and thus may limit fruit morphology and increase fruit abortion [1, 2]. However, environments with CO_2-enriched atmosphere may reduce flower and fruit abortion in pepper; mainly because competition among fruits can be avoided by a surplus supply of photosynthetic assimilates.

Increased temperatures can lead not only to changes in the ovary size, but also in the number of seeds per fruit [2]. Contrastingly, results in this work showed that temperature increases had no effect on seed number per fruit, flower morphology or ovary size, although it did lead to higher flower abortion rates and lower numbers of fruit. This is to be expected since plants tend to preserve flower structure uniformity, even under high stress conditions [18]. In the CO_2 experiment, by contrast, increases in CO_2 had a positive effect on seed number per fruit and caused flower morphology traits to augment in size, as reported previously in wild plants [33]. However, in *Arabidopsis thaliana* higher CO_2 levels resulted in a 60% decline in seed number per fruit [34], suggesting that the effect of CO_2 level may vary between plant species.

Temperature had no effect on nectar volume but lowered nectar sugar concentration, while higher CO_2 levels increased nectar volume and lowered nectar sugar concentration. This result is similar to that in Lake and Hughes [18], but differs from other studies of nectar production under high CO_2 levels. In studies of *Vicia faba* [24] and other five annual plants [31],

no increases in nectar volume or sugar concentration were observed at higher CO_2 levels. Nonetheless, the present results showed that under optimum conditions sugar concentration was diluted as nectar volume increased, but under stress conditions (e.g. 40 °C MT) sugar concentration dropped dramatically as probably sugar molecules became too expensive to produce by the plant.

CONCLUSIONS

In an effort to isolate the effects of each environmental factor in the present study, maximum temperature and atmospheric CO_2 concentration were evaluated separately. Each had different effects on reproductive traits in Habanero pepper, raising the question: Which factor (temperature or CO_2) would have a greater effect under a climate change scenario? Answering this question will prove very challenging since many other variables are involved. The present results, however, would suggest that a doubling of atmospheric CO_2 levels in tropical-subtropical regions would promote positive changes in plants that could mitigate any deterioration caused by higher temperature, as long as this factor does not reach extremely stressful levels. Under greenhouse conditions, the present results are promising since maximum temperature can be controlled, preventing any detrimental effects, while high CO_2 levels can be applied to accelerate phenological stages, increase the number of flowers and fruit, and consequently improve fruit yields.

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TABLES

Table1. Flower morphology traits in Habenero pepper (*Capsicum chinense*) at three maximum temperatures (Experiment 1) and three atmospheric CO_2 concentrations (Experiment 2). Values are means with standard errors. Within experiments different letters in the same row represent statistical difference (Tukey, $\alpha = 0.05$). $n = 50$.

	Experiment 1			Experiment 2		
	Maximum temperature (°C)			CO_2 concentration (µmol mol^{-1})		
	30	35	40	380	760	1140
Flower area (cm^2)	0.9 ± 0.05	1 ± 0.03	1 ± 0.02	0.86 ± 0.03b	1.09 ± 0.03a	1.08 ± 0.03a
Anther length (mm)	2.0 ± 0.02	2.01 ± 0.01	2.13 ± 0.03	1.99 ± 0.03b	2.13 ± 0.03a	2.15 ± 0.03a
Filament length (mm)	2.5 ± 0.1b	2.78 ± 0.06a	2.65 ± 0.1ab	2.42 ± 0.07b	2.78 ± 0.06a	2.85 ± 0.05a
Pistil length (mm)	3.46 ± 0.06ab	3.26 ± 0.1b	3.66 ± 0.1a	3.36 ± 0.09	3.36 ± 0.05	3.45 ± 0.07
Ovary length (mm)	2.28 ± 0.05	2.38 ± 0.05	2.16 ± 0.06	2.21 ± 0.04b	2.57 ± 0.06a	2.47 ± 0.07a
Ovary diameter (mm)	1.94 ± 0.03	2.02 ± 0.03	1.95 ± 0.02	1.89 ± 0.04b	2.07 ± 0.04a	1.98 ± 0.03ab
Pollen grains anther^{-1} (n)	14602 ± 1230	13621 ± 950	16400 ± 1162	9027 ± 973	10968 ± 877	10583 ± 158

Table 2. Nectar volume and sugar concentration in Habanero pepper (*Capsicum chinense*) plants at three maximum temperatures (Experiment 1) and three atmospheric CO_2 concentrations (Experiment 2). Values are means with standard errors. Within experiments different letters in the same row represent statistical difference (Tukey, $\alpha = 0.05$). $n = 50$.

	Experiment 1			Experiment 2		
	Maximum temperature (°C)			CO_2 concentration (µmol mol^{-1})		
	30	35	40	380	760	1140
Nectar Flower^{-1} (µL)	1.79 ± 0.22	2.02 ± 0.24	1.57 ± 0.29	0.45 ± 0.05b	0.63 ± 0.05b	1.26 ± 0.13a
Total sugar concentration (g cm^{-3})	23 ± 1.90a	17 ± 0.63ab	12 ± 1.07b	57.7 ± 4.6a	68.8 ± 2.6a	43.2 ± 1.8b

Table 3. Fruit morphology traits in Habanero pepper (*Capsicum chinense*) at three maximum temperatures (Experiment 1) and three atmospheric CO_2 concentrations (Experiment 2). Values are means with standard errors. Within experiments different letters in the same row represent statistical difference (Tukey, $\alpha = 0.05$). $n = 100$.

	Experiment 1			Experiment 2		
	Maximum temperature (°C)			CO_2 concentration (µmol mol^{-1})		
	30	35	40	380	760	1140
Fruit length (mm)	25.00 ± 0.93	22.00 ± 1.21	24.80 ± 1.41	30.10 ± 0.61b	31.13 ± 0.62b	34.00 ± 0.65 a
Fruit width (mm)	20.08 ± 0.69ab	23.10 ± 1.73a	17.17 ± 1.69b	25.44 ± 0.73b	27.00 ± 0.50ab	29.17 ± 0.46a
Pericarp thickness (mm)	1.66 ± 0.07a	1.41 ± 0.06ab	1.34 ± 0.05b	2.12 ± 0.080b	2.10 ± 0.046b	2.45 ± 0.095a
Seed $fruit^{-1}$ (n)	19.85 ± 3.1	15.25 ± 1.52	17.12 ± 2.37	25.2 ± 2.35b	28.9 ± 1.64b	40.6 ± 3.4a

FIGURE CAPTIONS

Figure 1. Flowering time (A) and fruiting time (B) of Habanero pepper plants at three maximum temperatures, 30 °C (open circles) 35 °C (closed circles), 40 °C (closed triangles). Semi-solid line indicates 50% of individuals with flowers (A) or fruit (B). Values are means with standard errors. An ANOVA was run and $P = 0.05$ is considered significant (*) and $P \leq 0.01$ is considered significant higher (**). $n = 25$.

Figure 2. Flowers aborted per plant, fruit number per plant and ratio of aborted flowers per fruit in Habanero pepper plants at three maximum temperatures (A, B and C) and three atmospheric CO_2 concentrations (D, E and F). Values are means with standard errors; Different letters in the same parameter represent statistical difference (Tukey, $\alpha = 0.05$). $n = 25$.

Figure 3. Flowering time (A) and fruiting time (B) of Habanero pepper plants at three atmospheric CO_2 concentrations: 380 µmol mol^{-1} (open circles); 760 µmol mol^{-1} (closed circles); 1140 µmol mol^{-1} (closed triangles). Semi-solid line indicates 50% of individuals with flowers (A) or fruit (B). Values are means with standard errors. An ANOVA was run and $P = 0.05$ is considered significant (*) and $P = 0.01$ is considered significant higher (**). $n = 25$.

FIGURES

FIGURE 1

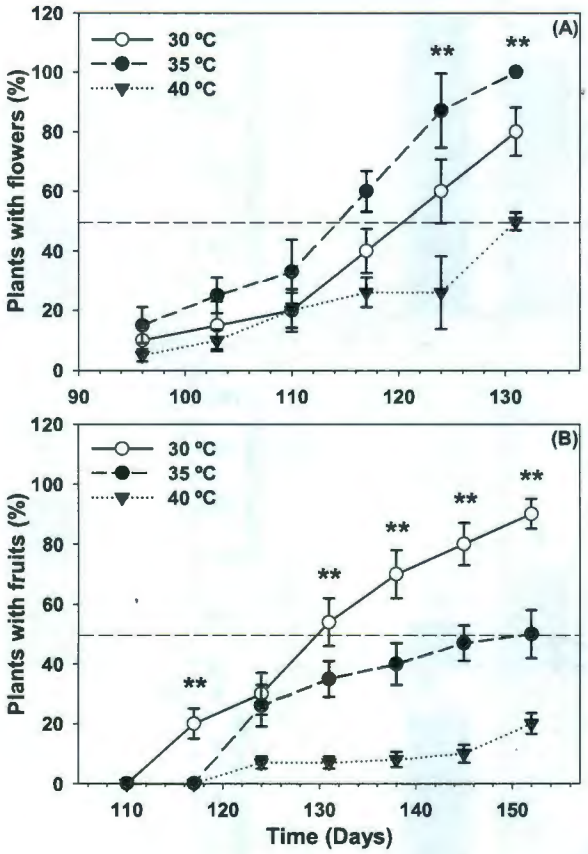


FIGURE 2

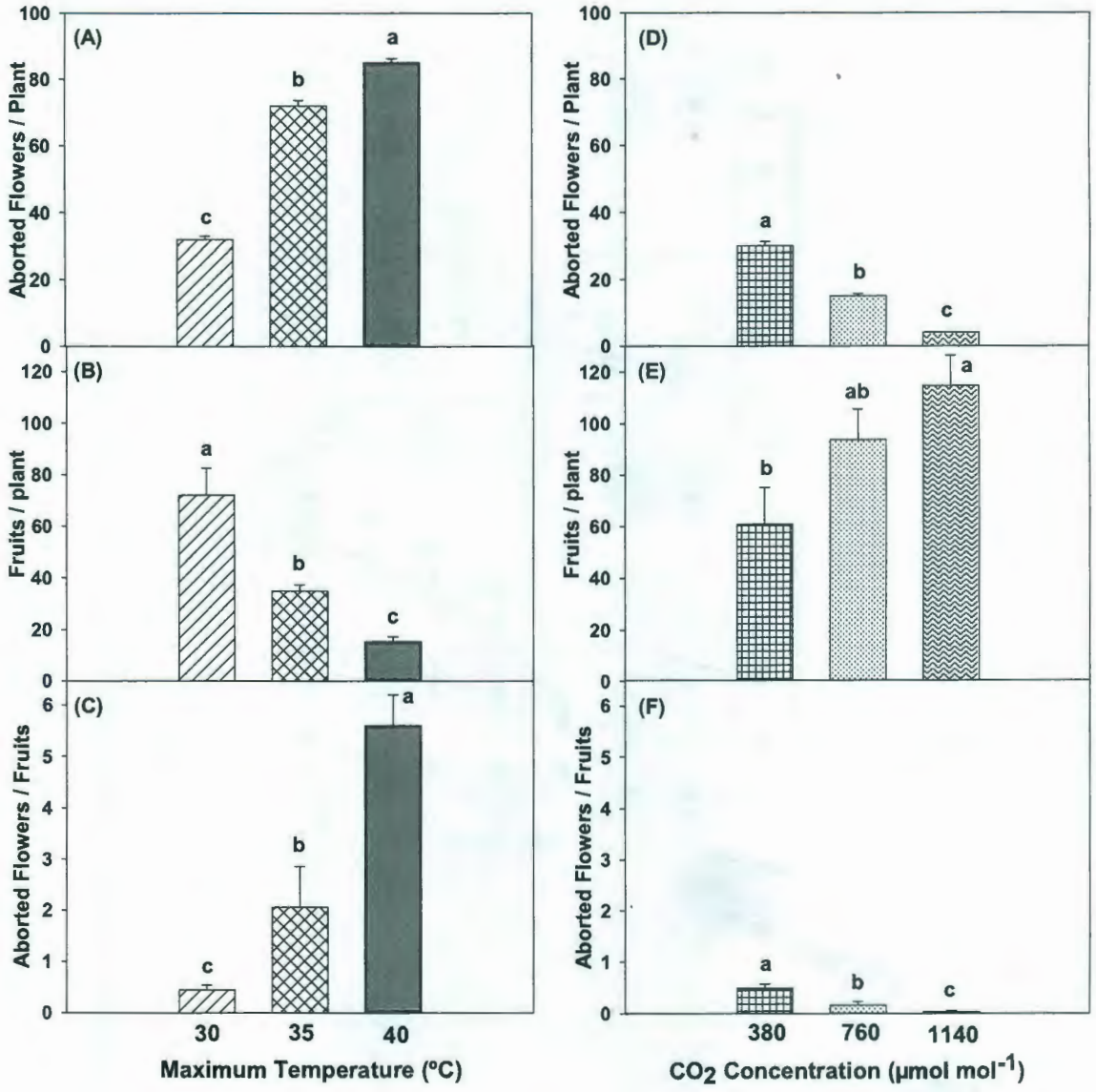
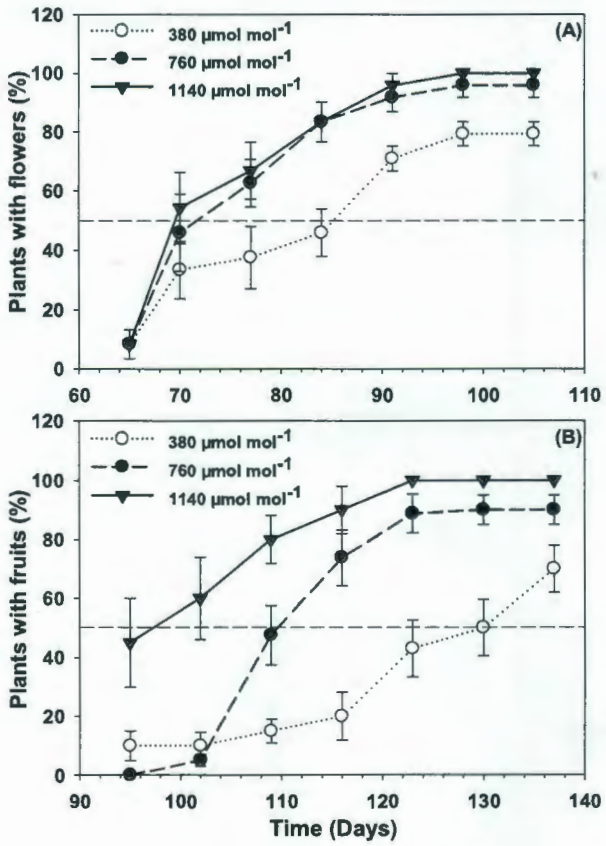

(A)
(B)
(C)
(D)
(E)
(F)
Aborted Flowers / Plant
Fruits / plant
Aborted Flowers / Fruits
a
b
c
ab
30
35
40
380
760
1140
Maximum Temperature (°C)
CO2 Concentration (µmol mol-1)

FIGURE 3


380 µmol mol-1
760 µmol mol-1
1140 µmol mol-1
(A)
Plants with flowers (%)
120
100
80
60
40
20
0
60
70
80
90
100
110
(B)
Plants with fruits (%)
90
100
110
120
130
140
Time (Days)

CAPÍTULO V

Enrichment of the CO_2 atmosphere increases capsaicinoids content in Habanero peppers (*Capsicum chinense* Jacq.)

Running title: Effects of CO_2 concentration on Habanero peppers

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ABSTRACT

BACKGROUND: The effects of the increase of atmospheric CO_2 on agricultural productivity have been mainly analysed through its impact on biomass yield, and little attention has been directed to quality traits, such as nutritional or organoleptic attributes. In here, plants of hot Habanero pepper (*Capsicum chinense* Jacq.) were grown in growth chambers under three different CO_2 levels; 380 (normal atmospheric value), 760 and 1140 µmol mol^{-1}, and their effects on pod yield, size, colour and pungency, were monitored.

RESULTS: Total number of pods per plant increased by 88.5% at the highest CO_2, in comparison to plants grown at normal CO_2 conditions. Pod size and yield per plant also increased when plants were grown at the highest CO_2 concentration (partial pressure). Furthermore, total capsaicinoids contents in ripe peppers under a high CO_2 atmosphere were 27% higher than those from plants under lower concentrations, but it was not the case for immature pods.

CONCLUSION: These data suggest that the increase of atmospheric CO_2 could modify specific routes of secondary metabolism as well as others desirable traits, thus affecting the quality of *Capsicum* pepper products.

Keywords: capsaicinoids; carbon dioxide; climate change; peppers; pungency

INTRODUCTION

The increase of CO_2 concentration in the atmosphere has raised major concerns among the general public, since it is considered one of the causes of the climate change.[1] It has been estimated that CO_2 in the atmosphere has increased from 280 to up 400 µmol mol^{-1} since the beginning of the industrial revolution.[1] Moreover, CO_2 is increasing at a rate of 2 µmol mol^{-1} per year, due mainly to the combustion of fossil fuels.[1] At this pace, CO_2 could reach 560 µmol mol^{-1} by the year 2035, which would induce a 2°C increase in global temperature.[2] The increase of both atmospheric CO_2 and temperature will affect agricultural production, as has been well documented.[1,2] However, most studies concerning the increase of atmospheric CO_2 on agricultural production refer to biomass yield, which is directly related to the efficiency of carbon fixation.[1,2] There are only few

others that take into account agricultural traits, like quality of the products. Quality, being defined by organoleptic and nutritional attributes, would have a direct an impact on revenues from commodity agricultural products.[3]

Hot peppers represent one of the main spices traded in international markets. The typical burning sensation of peppers, known as pungency, is due to the presence of capsaicinoids, acid amides derived from phenylalanine.[4,5] Capsaicinoids are synthesized in the placenta of hot peppers and its formation is affected by several environmental factors, including temperature, water status and nutrient availability.[6,7] Being a nitrogenous compound, nitrate fertilization also affects capsaicinoids accumulation.[8] In peppers, this effect is directly related to the amount of nitrate that reaches the placenta.[9] Since carbon availability has a direct effect on the accumulation of nitrogenous metabolites in plants,[10] CO_2 variations would modify the accumulation of capsaicinoids in peppers. We are interested in the effect that CO_2 variation may have on pepper production. Since pungency is the main quality trait of hot peppers, in here we have addressed the effects that culturing Habanero peppers plants under three different CO_2 concentrations exert on capsaicinoid accumulation. Our results suggest that CO_2 availability not just increased net pod productivity, but also their capsaicinoids content. Other quality indicators, such as pod size and colour, were also evaluated.

MATERIALS AND METHODS

Plant material

Habanero pepper seeds (*Capsicum chinense* Jacq.) of orange variety (Seminis seeds, México) were germinated and sown in Sphagnum peat moss (Premier Mexico). After 45 days in the nursery, seedlings were transplanted to pots containing a 2:1 (v:v) mixture of soil and peat moss and placed in sealed growth chambers, under the CO_2 treatments described below, for further development.

Construction of the growth chambers

Growth chambers (19.8 m^3) were made of Plexiglas, sealing tightly all unions. Temperature in the chambers was maintained between 27 and 30 °C using a 5000 BTU air conditioner unit (LG Electronics, Seoul). Plants were naturally illuminated during 11 hours per day. Maximum photosynthetically active photon flux density at noon was 600 µmol m^{-2}

s^{-1} (Quantum sensor LI-190SB; LI-COR, Lincoln, NE, USA). CO_2 levels in the chambers were maintained at the specified concentrations using sensors (GE Tel aire Vento stat 8002) programed to automatically release the gas from a canister (99.8% purity, Infra SA, Mexico), it decreased 2% of the pre-set value (see treatments).

CO_2 treatments and fruit sampling

Plants were cultivated under three different CO_2 treatments: 380 µmol mol^{-1} (T1, control), which correspond to the average CO_2 atmospheric concentration, 760 µmol mol^{-1} (T2) and 1140 µmol mol^{-1} (T3). Plants (25 per treatment) were kept for five months under these conditions, collecting pods every week. Pods were collected at two developmental stages: prior to ripening (green, 32 days post-anthesis or DPA), or once ripen (orange, 40 DPA). The number of pods harvested, as well as the total pod biomass, was individually registered for each plant.

Pepper production and quality traits

Pod production was determined as the total number of pods harvested per plant, total biomass formed (estimated both as fresh and dry weight), and the number of seeds set per fruit. Water content of pods was estimated as the difference between dry and fresh mass. Dry mass was obtained by freeze-drying entire pods. Quality traits included pod size (length and external diameter or width), pericarp thickness and colour at ripening, and pungency evaluated as the content of capsaicinoids. Pericarp colour was established according to the Royal Horticultural Society charts for plant colour identification, which include almost 900 different shades, mainly referred to flowers and fruits.[11]

Analysis of capsaicinoid contents

After harvest, the pedicel was removed and the complete pod was lyophilized and pulverized. A sample of 100 mg of this powder was extracted overnight with 10 mL acetone at room temperature with gentle shaking. The debris was separated by filtration and the supernatant was evaporated to dryness. The residue was dissolved in 1 ml of methanol and a 5 □l aliquot was chromatographed on silica plates, using a chloroform: ciclohexane: acetic acid (70: 20: 10) mixture as the mobile phase. Capsaicinoids, reported as capsaicin equivalents, were quantified by *in situ* densitometry in a CS-930 densitometer equipped with a DR 2 data collector (Shimadzu, Kyoto, Japan) after thin layer

chromatographic separation, as previously described.[12] Data are expressed as contents (in mg g^{-1} DW) or pungency (Scoville Heat Units; SHU). One SHU equals 0.015 mg g^{-1} DW of capsaicinoids.[12]

Statistical analysis

Data were examined by analysis of variance (ANOVA) and treatment means were compared using the Tukey's honestly significant difference (HSD) test at $P < 0.05$ (Statistic Six Sigma, Release 7, Stat Soft).

RESULTS

As a general trend, increasing CO_2 concentration in the chamber´s atmosphere positively affected both pod yield and quality traits. In this work, productivity was estimated as the number of pods per plant, total biomass production (Table 1) and seeds set per pod (Table 2). Quality was monitored as pod individual size and mass, colour at maturity and pungency levels (Tables 2, 3, 4 and 5).

Habanero pepper plants increased pod yields at high CO_2 atmospheres (T2 and T3), which resulted in a higher biomass production (Tables 1 and 2). In average, the number of pods harvested from T2 and T3 plants surpassed by 33 and 54 those from T1 plants respectively (Table 1). Besides, in comparison to plants cultured under average CO_2 concentration (T1), pod dimensions (length, width and pericarp thickness) in plants exposed at elevated CO_2 (T3) were increased (Table 2). Furthermore, ripe pods from T3 plants were also heavier than those from T1 plants, estimated as fresh (by 28%) and dry (by 35%) weight (Table 3). A similar result was observed for immature pods (Table 3). It should be noticed that statistical differences in water content of pods were found among the treatments in the ripe, but not in the unripe pods. However, such difference was lower than 1% (Table 3), suggesting that pods from plants grown under a CO_2-enriched atmosphere could have increased their dimensions (Table 2), without enlarging their dry matter content per unit (Table 3). Most dry biomass of pepper pods can be accounted for their content of cellulose, lignin and pectin.[13] Therefore, an increase in dry matter content in the pods may reduce their palatability, making the product less appealing to consumers.

In peppers, a uniform colour, true to type of cultivar, is a valuable trait.[14] Exposure to elevated CO_2 did not change the colour of the ripe pericarp, since most of the pods were in the orange and strong orange group, with some of them included the brilliant orange and strong orangish yellow group, despite the treatment (Table 4). However, the main

desirable trait for habanero pepper's consumers is pungency, which is directly related to capsaicinoids contents.[15] Therefore, they were evaluated in pods at two developmental stages (Table 5). Although no clear effect from the different CO_2 treatments were observed on capsaicinoids contents in green pods, after ripening, it increased between 15 and 26% in pods from T2 and T3 plants, compared to T1 (Table 5).

DISCUSSION

Most of the work about the effects of the increase of CO_2 levels on agriculturally important plants has mainly focused on biomass production, which results from primary metabolism (carbon fixation and respiration).[16-18] Little is known about these effects on quality attributes, like organoleptic traits, which are frequently related to secondary metabolites. In this study, we analyzed the effect of increasing CO_2 levels on Habanero pepper pungency and other pod quality indicators. We included two development stages; since CO_2 effects may differ drastically at different developmental phases.[19-21] The chosen attributes were pod size, colour and pungency, which correspond to the main management traits for the Habanero pepper.[11,15]

The pods' size from plants under a two-fold increase of the average CO_2 levels (T2) was not affected (Table 2). However, when CO_2 was tripled (T3), length, width and pericarp thickness were higher than those from plant cultured under average levels (T1; Table 2). Pepper pods may take upto 50% of the newly fixed carbon during development.[22,23] We have observed that T3 Habanero pepper plants increased their CO_2 assimilation rate two-fold with respect to values obtained for T1 plants, as estimated by infrared gas analyzer (IRGA). Moreover, transpiration rate in T3 plants was reduced in *ca.* 30% when compared to T1 plants, as a consequence of a higher intercellular CO_2 content, which caused a lowered stomata conductance (submitted data). This carbon surplus may have reduced photosynthate competition among fruits and other tissues. It also may be accounted for the larger number of pods produced per plant, since an excess of carbohydrates may reduce flower abortion.[24,25] Colour of mature peppers was placed in the orange and strong orange groups, showing little variations when CO_2 was increased. This suggests that carotenoids, which are responsible for pepper's colour, are more sensitive to other environmental factors, such as light or temperature, rather than CO_2 availability.[26] In addition, capsaicinoids contents when CO_2 was doubled and tripled increased between 15 and

26%, which may point to a positive CO_2 effect on pungency through the maturation of the fruit. In this way, at least part of the additional CO_2 fixed when the plant was placed in an atmosphere, enriched could be channeled to secondary metabolites.

Thus, our data suggest that Habanero pepper plants cultivated in an increased CO_2 atmosphere could direct part of the carbon excess to pods, resulting not just in a bigger fruits, but also affecting their phytochemical composition as the increase in capsaicinoid levels show. The fact that an increased CO_2 concentration had a positive effect on pungency suggests that, at least part of the additional fixed CO_2, could have been directed to the synthesis of secondary metabolites.

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TABLES

Table 1. Effect of long-term CO_2 exposure on the number of pods produced per plant and yield (grams per plant) of Habanero peppers plants. The different letters indicate significant difference (α=0.05) between CO_2 treatments as determined by the Tukey test. Each value represents the mean ± SE. *n* = 25 plants.

[CO_2] (μmol mol^{-1})	**Production** (pods plant^{-1})	**Yield** (g plant^{-1})
380 (T1)	61 ± 14 b	280 ± 65 c
760 (T2)	94 ± 12 ab	454 ± 57 b
1140 (T3)	115 ± 12 a	677 ± 69 a

Table 2. Effect of long-term CO_2 exposure on pod size and number of seeds per pod of Habanero peppers. The different letters indicate significant difference (α=0.05) between CO_2 treatments as determined by the Tukey test. Each value represents the mean + SE. *n* = 300 pods by treatment.

[CO_2] (μmol mol^{-1})	**Length** (mm)	**Width** (mm)	**Pericarp thickness** (mm)	**Seeds pod^{-1}**
380 (T1)	30.10 ± 0.61 b	25.44 ± 0.73 b	2.12 ± 0.08 b	25.2 ± 2.35 b
760 (T2)	31.13 ± 0.62 b	27.00 ± 0.50 ab	2.10 ± 0.05 b	28.9 ± 1.64 b
1140 (T3)	34.00 ± 0.65 a	29.17 ± 0.46 a	2.45 ± 0.09 a	40.6 ± 3.4 a

Table 3. Effect of long-term elevated CO_2 exposure on weight (fresh mass and dry mass) and water content in two different developmental stages (immature and ripe) of Habanero peppers fruits. The different letters indicate significant difference (α=0.05) between CO_2 treatments as determined by the Tukey test. Each value represents the mean ± SE. *n* = 100 pods by treatment.

	Immature pods			Ripe pods		
[CO_2] ($\mu mol\ mol^{-1}$)	Fresh mass (g)	Dry mass (g)	Water content (%)	Fresh mass (g)	Dry mass (g)	Water content (%)
380 (T1)	3.78 ± 0.18 b	0.50 ± 0.02 b	86.76 ± 0.33 a	4.58 ± 0.15 b	0.62 ± 0.02 b	86.27 ± 0.15 a
760 (T2)	4.92 ± 0.15 a	0.63 ± 0.02 a	87.12 ± 0.24 a	4.83 ± 0.34 b	0.67 ± 0.04 b	86.13 ± 0.11 ab
1140 (T3)	5.28 ± 0.21 a	0.69 ± 0.03 a	87.18 ± 0.23 a	5.89 ± 0.16 a	0.84 ± 0.02 a	85.74 ± 0.13 b

Table 4. Effect of long-term elevated CO_2 exposure (ambient CO_2 vs. duplicated and triplicated CO_2) on color of Habanero peppers ripe pods. Based on the tables of Royal Horticultural society (5ta edition).[11] FAN I Groups (yellow, yellow-orange, orange, orange-red, red). *n* = 100 pods by treatment.

Colour classification	CO_2 Treatments (µmol mol^{-1})		
	380 (T1)	760 (T2)	1140 (T3)
	Pods		
25A Strong Orange	33	28	4
25B Strong Orange	13	11	21
24A Strong Orange	8	5	17
N25B Orange	8	11	-
N25C Orange	28	39	42
N25D Orange	4	-	3
24B Strong Orangish Yellow	6	-	13
25C Brilliant Orange	-	6	-

Table 5. Effect of long-term elevated CO_2 exposure on capsaicinoids content and pungency in two different developmental stages (immature and ripe pods) of Habanero peppers fruits. From both immature and ripe pods, three pods from 5 different plants of each treatment were collected (15 units) and each unit was analysed as triplicates (n=45). The different letters indicate significant difference (α=0.05) between CO_2 treatments as determined by the Tukey test. Each value represents the mean ± SE. Scoville Heat units (SHU) = 0.015 mg g^{-1} DW (0.0015 % based in DW). DW=Dry weight.

[CO_2]	Immature		Ripe	
(µmol mol^{-1})	mg g^{-1} DW	SHU	mg g^{-1} DW	SHU
380 (T1)	15.17 ± 0.67 ab	227550 ab	12.54 ± 0.40 b	188100 b
760 (T2)	15.88 ± 1.03 a	238200 a	15.90 ± 0.65 a	238500 a
1140 (T3)	13.35 ± 0.53 b	200250 b	14.4 ± 0.46 a	216000 a

CAPÍTULO VI

DISCUSIÓN GENERAL

De acuerdo a las proyecciones que existen sobre los efectos del cambio climático, son varios los factores ambientales que se verían afectados (temperatura, concentración atmosférica de CO_2, precipitación pluvial, etc.) y para poder comprender los efectos que estos tendrían sobre las plantas sería ideal recrear los posibles escenarios futuros con la interacción de todas las variables ambientales involucradas. Sin embargo, resultaría imposible de realizar pues el número de experimentos se multiplicaría cada vez que una variable ambiental se sumara al escenario deseado, esto implicaría contar con decenas de cámaras de crecimiento y un presupuesto ilimitado. En este sentido, los experimentos donde se evalúan las variables ambientales de manera individual como es el caso de este trabajo donde se evalúo primero el efecto de la temperatura (Capítulos 2 y 4) y posteriormente el efecto del CO_2 atmosférico (Capítulos 3, 4 y 5) sobre el cultivo de chile Habanero, han demostrado ser una buena alternativa proporcionando información interesante, además por ser una sola variable ambiental por controlar los datos obtenidos suelen ser más confiables.

La mayoría de los trabajos realizados sobre el efecto de condiciones ambientales en plantas solo evalúan una fracción del ciclo de vida de los individuos monitoreados. En esta investigación el hecho de trabajar con una hortaliza como modelo de estudio nos permitió evaluar el ciclo completo del cultivo bajo las condiciones ambientales a las que estaban sometidas las plantas, y así observar respuestas muy específicas en cada una de sus etapas fenológicas. Un ejemplo de ello fue lo que sucedió en el paso transitivo de floración a fructificación en donde se observó que un aumento de 5 °C en la temperatura máxima diurna aceleró la floración, sin embargo al analizar la siguiente fenofase las plantas que adelantaron la floración (sometidas a 35 °C) incrementaron la cantidad de flores abortadas retrasando la fructificación en comparación con las plantas a 30 °C (Capítulo IV, Figura 1).

El incremento tanto de la temperatura del aire como del CO_2 ambiental tiene efectos sobre la fisiología de las plantas, en este caso sobre el cultivo de chile Habanero. El aumento de temperaturas por encima de su óptimo térmico se esperaría que se reflejara en las plantas

cultivadas de manera negativa (Atkin *et al.*, 2006; Sato *et al.*, 2006; Haldimann y Feller, 2005; Sharkey, 2005; Young *et al.*, 2004; Hunt *et al.*, 2002). Sin embargo, en los cultivos tropicales el rango óptimo térmico es mayor que en las plantas de ambiente templado, debido a su adaptación en términos ecológicos y evolutivos en ambientes más calurosos (Larcher, 2003; Hogan *et al.*, 1991).

Los resultados obtenidos en el presente trabajo han demostrado que el óptimo térmico en un cultivo tropical como el chile Habanero debe estar por debajo de los 35 °C de temperatura de la hoja. Berry and Björkman (1980) sugieren que el óptimo térmico para la fotosíntesis depende de las condiciones de crecimiento de las plantas. En este estudio ha quedado claro que las hojas *C. chinense* tienen la capacidad de adaptar sus procesos fisiológicos (incrementar transpiración, conductancia estomática y carbono intercelular) para contrarrestar los posibles daños ocasionados por elevadas temperaturas del aire y así evitar la fotorrespiración y continuar con la fotosíntesis, una variable fundamental fue la diferencia entre la temperaturas de la hoja y la del aire (T_{aire} - T_{hoja}) (Capítulo II, Figura 6). Sin embargo, a pesar de todas las adaptaciones fisiológicas realizadas por las plantas a nivel fenológico se observan repercusiones, al aumentar la temperatura máxima se incrementan los abortos de flores y se alteran los ritmos fenológicos (Capítulo IV, Figuras 1 y 2), esto es debido a un desajuste hormonal (Erickson and Markhart 2002 y 2001). A pesar de ello la morfología tanto de flores como de frutos no se ve afectada (Capítulo IV, Cuadros 1 y 3).

Por otra parte, el efecto del aumento en la concentración atmosférica de CO_2 generalmente se espera que al inicio de los tratamientos incremente la tasa fotosintética al igual que algunos parámetros de crecimiento como la biomasa y el área foliar (Davey *et al.*, 2004) y que con el paso del tiempo estos parámetros se reduzcan (Grulke *et al.*, 1990), debido a una aclimatación (disminución en la regulación de Rubisco) por parte de la planta (Griffin *et al.*, 2000). Sin embargo los resultados demostraron que las plantas de chile Habanero después de 80 días de tratamiento en atmósferas de CO_2 elevado incrementaron tanto la biomasa como el área foliar, además de mantener los niveles fisiológicos (A_N, C_i, $V_{c,max}$, J_{max}) más altos que las plantas en condiciones normales de CO_2 (Capítulo III, Cuadro 1, Figuras 1 y 3). Adicionalmente, el aumento en el CO_2 disminuyó el aborto de flores e incrementó la producción de frutos considerablemente adelantando las etapas fenológicas (Capítulo IV, Figuras 2 y 3). De esta manera la demanda de

fotosintatos por los frutos evitó la acumulación de carbohidratos no estructurales en las hojas manteniendo en constante activación la maquinaria fotosintética y evitando así su aclimatación fotosintética (disminución en la regulación de Rubisco). Además el aumento de CO_2 favoreció el alargamiento tanto del filamento como de las anteras (estambres) e incrementó el área floral (Capítulo IV, Cuadro 1), lo cual podría ocasionar algunas alteraciones en la interacción planta-polinizador en situaciones de campo, o en ambientes cerrados como invernadero se podría incrementar la autopolinización de la flor debido a que el alargamiento sufrido por los estambres permitiría que los granos de polen estuvieran más cerca del estigma. Adicionalmente se incrementó la producción de frutos por planta, el peso y el tamaño de frutos (Capítulo V, Cuadros 1, 2 y 3). Posiblemente esto ocurrió debido a que los frutos contaban con suficiente fuente de carbono (fotosintatos) para evitar la competencia entre ellos. También es posible que la cantidad de carbono disponible por la planta pudiera haber sido utilizada en rutas de metabolismo secundario y por lo cual los frutos de las plantas expuestas al aumento de CO_2 incrementaron los niveles de capsaicina (Capítulo V, Cuadro 5), lo que conllevaría a un aumento en el interés comercial del cultivo de chile Habanero debido a los múltiples usos que actualmente se le dan a la capsaicina.

CONCLUSIONES

- El incremento en la temperatura del aire aumentó la conductancia estomática y la transpiración de las plantas causando una disminución de hasta 5 °C en el déficit de temperaturas (T_{aire} - T_{hoja}), ocasionando un aumento en la tasa de asimilación, evitando así la fotorrespiración. Además, las temperaturas elevadas disminuyeron la producción de frutos, aumentando el aborto de flores y retrasando los ritmos fenológicos, lo que sugiere que los fotosintatos producidos al aumentar la tasa de asimilación podrían haber sido designados a otras rutas metabólicas y en lugar de ser usados en la producción de biomasa se destinen a la producción de isoprenos para evitar daños por fotorrespiración (Affek and Yakir, 2003; Delwiche and Sharkey, 1993).

- El aumento en la concentración atmosférica de CO_2 a largo plazo incrementó la biomasa y el área foliar, adelantó la fenología reproductiva, modificó la morfología floral alargando los estambres e incrementó el tamaño y la producción de frutos. A nivel fisiológico aumentó la tasa de asimilación, el carbono intercelular y la limitación estomática, disminuyendo la conductancia estomática y evitando la aclimatación fotosintética. A nivel metabólico incrementó la concentración de capsaicina en frutos.

- Con base en los resultados obtenidos en las plantas sometidas a experimentación se podría sugerir que las respuestas observadas fueron consecuencia de una cadena de eventos fisiológicos que bajo un régimen de temperaturas máximas diurnas y concentraciones de CO_2 elevadas se reflejaron en la fenología, la producción de flores, la calidad de frutos, el crecimiento y el desarrollo de la planta, muy probablemente respaldada por sus características genéticas.

- La utilización de plantas de chile Habanero resultó ser un excelente modelo de estudio, su ciclo de vida permitió evaluar cada una de sus etapas fenológicas, la morfología y textura de sus hojas facilitó la evaluación del intercambio de gases sin inconvenientes y sus respuestas agronómicas como el crecimiento vegetativo y la producción de frutos respondieron de forma muy marcada entre cada uno de los tratamientos ambientales a los que fueron sometidas, lo cual nos facilitó la obtención de conclusiones.

PERSPECTIVAS

Los resultados obtenidos en este trabajo abrieron varias perspectivas a desarrollar en plantas tropicales, algunas de las cuales se mencionan a continuación:

1) A pesar de la vasta información que existe sobre las respuestas fisiológicas de las plantas ante futuros posibles escenarios de CO_2 y temperatura a nivel mundial, muy pocos de ellos se han realizado en plantas tropicales, por tal motivo se debería considerar este tipo de plantas como modelo de estudio para realizar

2) Investigaciones al mismo nivel que en plantas procedentes de zonas templadas. Por lo tanto sería conveniente realizar experimentos similares a los que aquí presentamos pero incrementando las variables a nivel de isótopos, utilizando isótopos estables (^{13}C) se podría establecer con mayor precisión la distribución del carbono a nivel de organismo.
3) Por otra parte, se deberían plantear dentro de la misma cámara de crecimiento al menos alguno de los escenarios futuros más probables interaccionando las variables ambientales concentración de CO_2 X temperatura, con la finalidad de comparar con los resultados obtenidos al evaluar las variables aisladas.
4) Otra de las proyecciones de cara al calentamiento global es la restricción en el régimen hídrico, por lo tanto sería de gran interés realizar experimentos manipulando esta condición ambiental y de ser posible en interacción con los otros factores ya estudiados.
5) El estudio de la interacción planta-polinizador posterior a un cambio en la morfología de la flor ocasionado por el aumento del CO_2 atmosférico debería ser un tema prioritario de abordar en futuras investigaciones.
6) Finalmente, a pesar de la complejidad sería trascendental establecer experimentos a nivel de campo manipulando las concentraciones de CO_2, para poder descartar los efectos enmascarados de otros factores ambientales (intensidad luminosa, fotoperiodo, HR) inherentes a las cámaras de crecimiento o invernaderos.

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