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CON FENOLOGÍA INVERTIDA EN BOSQUES TROPICALES SECOS

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Presente

Me permito informar a usted que en la reunión ordinaria del Subcomité de Ecología y Manejo Integral de Ecosistemas del Posgrado en Ciencias Biológicas, celebrada el día **07 de septiembre de 2020**, se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **SÁNCHEZ LIEJA LUIS OCTAVIO**, con número de cuenta **504008821**, con la tesis titulada, "**Factores que afectan la función vegetativa y reproductiva de plantas con fenología invertida en bosques tropicales secos**", realizada bajo la dirección del **DR. MAURICIO RICARDO QUESADA AVENDAÑO**, quedando integrado de la siguiente manera:

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Sin otro particular, me es grato enviarle un cordial saludo.

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- ❖ Esta tesis y todo el esfuerzo atrás de ella se lo dedico totalmente a mis padres y a mi hermana.

A Octavio,

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A Nayeli,

**“Siempre habrá una
sombra que cobija. Alta,
alargada, inatrapable:
prueba de la existencia: de
una existencia o de toda
existencia. Siempre habrá
un árbol y su sombra. Un
árbol y una sombra es lo
que hace falta. Un buen
árbol. Una buena sombra.”**

Modificado de: Angelina Muñiz-
Huberman, 2007. La Sombra que cobija.
Universidad Nacional Autónoma de
México, Facultad de Filosofía y Letras.

Índice

Resumen	v
Abstract	vii
Introducción general	1
Objetivo general	3
Objetivos particulares	4
Estructura de la tesis	4
Referencias	6
Capítulo 1. - A field experiment to determine the effect of dry-season irrigations on vegetative and reproductive traits in the wet-deciduous tree <i>Bonellia nervosa</i> . (Artículo requisito aceptado en el <i>Journal of Tropical Ecology</i>).....	8
Capítulo 2. - Phenotypic responses to different light regimes in a wet-season deciduous tree: a test of genetic accommodation in a seasonally dry tropical forest.....	15
Discusión general	62
Conclusiones	70
Referencias	72

Resumen

Los ecosistemas estacionales se distinguen por una marcada variación en la intensidad lumínica y disponibilidad de agua que determinan el despliegue de diferentes estrategias en las plantas para lidiar con la variabilidad ambiental. Los bosques tropicales secos destacan por presentar una contrastante estacionalidad ambiental, representada por dos estaciones una de lluvias y una seca que puede durar de 3 a 6 meses. Debido a esta estacionalidad las plantas pueden expresar uno de los siguientes patrones fenológicos foliares: 1) *patrón caducifolio*, en el que las plantas pierden su follaje durante la época de secas y producen las nuevas hojas al inicio de la estación de lluvias, y 2) *patrón perennifolio*, en el que las plantas mantienen su follaje durante todo el año, usualmente en estos ambientes algunas plantas suelen estar asociadas a la vegetación riparia.

En el bosque tropical seco (BTS) de Chamela, México, existen algunas especies que despliegan un patrón foliar fenológico excepcional: durante el comienzo de la estación seca producen nuevas hojas y al inicio de la siguiente estación de lluvias pierden todo su follaje, quedando totalmente defoliadas hasta el inicio de la siguiente época seca. Este patrón fenológico invertido sugiere que las especies que lo exhiben presentan adaptaciones específicas, y posiblemente, una mayor plasticidad fenotípica que les permite producir hojas y mantener sus funciones vegetativas y reproductivas durante la estación seca, cuando las demás especies permanecen latentes y sin hojas.

La plasticidad fenotípica se refiere a la capacidad individual para producir diferentes fenotipos debido a la influencia ambiental, y ha sido propuesta como facilitadora para que ocurran cambios evolutivos. De esta manera la expresión de la plasticidad fenotípica podría facilitar la aparición de variantes fenotípicas inducidos por el ambiente, que pueden estar sujetos a la acción de la selección natural y, por lo tanto, estas nuevas características adaptativas podrían fijarse genéticamente en la población.

Desde una perspectiva ecofisiológica y evolutiva este trabajo tiene como objetivo general explorar el efecto de los principales factores ambientales en la expresión fenotípica del patrón foliar invertido y sus consecuencias en la adecuación de las plantas en el BTS de Chamela, México. Específicamente, se manipuló experimentalmente la disponibilidad de agua y la intensidad lumínica en árboles reproductivos de *Bonellia nervosa* (C. Presl) (Theophrastaceae), y se observó el efecto en caracteres vegetativos (*e.g.* patrones fenológicos, concentración de carbohidratos no-estructurales, fotosíntesis y supervivencia de hojas) y reproductivos (*e.g.* éxito reproductivo, concentración de carbohidratos no-estructurales, producción de flores y frutos).

Los resultados observados en las manipulaciones experimentales *in-situ* en *B. nervosa*, sugieren que el patrón foliar invertido en árboles reproductivos no varía al modificar la intensidad lumínica y disponibilidad de agua durante la estación seca. Estos resultados podrían sugerir que esta especie pudo haber experimentado un proceso de acomodación genética en la evolución de este patrón fenológico invertido, a través de mecanismos de plasticidad fenotípica mediados por la variabilidad ambiental. Sin embargo, las manipulaciones lumínicas e irrigaciones durante la época seca causaron múltiples respuestas plásticas en la producción de flores y frutos. Además, los resultados de las concentraciones de carbohidratos no-estructurales posterior a las manipulaciones sugieren una respuesta plástica en caracteres reproductivos, principalmente, en la asignación de recursos en frutos y semillas producidos después de las manipulaciones.

Considerando los resultados obtenidos en esta tesis se propone que el surgimiento de variantes fenotípicas, como la fenología foliar invertida en *B. nervosa*, podrían haber sido inducidos por factores ambientales en los BTS, facilitado por la plasticidad fenotípica ancestral a la heterogeneidad ambiental experimentada en un linaje a lo largo de su historia evolutiva.

Abstract

Seasonal ecosystems are distinguished primarily by light intensity and water availability variation, which determines the deployment of different strategies in plants to deal with environmental variability. Seasonally dry forests are distinguished by a contrasted environmental seasonality, represented by two seasons one expected rainy season and a dry season that could varies from 3 to 6 months. In the Neotropical seasonally tropical forest of Chamela, some species display an unusual inverted foliar pattern: during the beginning of the dry season produce new leaves, and at the beginning of the next rainy season throw away all their foliage and remains defoliated until the next dry season. The presence of this inverted phenological pattern suggests that these species exhibit specific adaptations, and possibly a greater phenotypic plasticity, which allows them to produce their leaves and maintain their physiological and reproductive functions during the dry season when other species remain dormant and leafless until the next rainy season.

Phenotypic plasticity refers to the capacity of expressing individual variation to produce different phenotypes due to environmental influence and has been proposed as a facilitator of evolutionary change. In this scenario, the expression of phenotypic plasticity facilitates the appearance of alternative phenotypes induced by the environment, which may be subject to natural selection, and therefore, eventually could be genetically fixed through genetic accommodation for a specific trait.

From an ecophysiological and evolutionary perspective this work explores: i) the effect of the main environmental factors in the phenotypic expression of this pattern and its consequences in the adaptation of the plants in the seasonally tropical forest of Chamela, Mexico.

Specifically, water availability and light intensity were experimentally manipulated in reproductive trees of *Bonellia nervosa* (C. Presl) (Theophrastaceae), and

the effect was observed on vegetative traits (e.g. phenological patterns, concentration of non-structural carbohydrates, photosynthesis and leaf survival) and reproductive traits (e.g. reproductive success, concentration of non-structural carbohydrates, flower and fruit production).

Experimental *in-situ* manipulations in *B. nervosa* showed that inverted leaf pattern in reproductive trees does not change by modifying light intensity and water availability during the dry season. This suggests that these species could have undergone a process of genetic accommodation during the evolution of this inverted phenological pattern, through mechanisms of phenotypic plasticity mediated by environmental variability. Besides, results of the non-structural carbohydrate concentrations on various tissues, suggest a plastic response in reproductive characters, mainly in the relocation of resources in fruits and seeds.

Considering the overall results of this research, it is suggested that the emergence of evolutionary novelties, such as inverted foliar phenology from the tropical seasonal forest of Chamela, is facilitated by ancestral phenotypic plasticity to the environmental heterogeneity experienced in a lineage throughout its evolutionary history.

Introducción general

Las plantas han desarrollado una variedad de mecanismos y estrategias para lidiar con la variabilidad ambiental. Principalmente, en ecosistemas estacionales la variación en la intensidad lumínica y disponibilidad de agua son factores que determinan el despliegue de diferentes estrategias fenológicas en las plantas para lidiar con la variabilidad ambiental (Borchert, 1994; Borchert et al., 2005). Por lo tanto, los diferentes tipos de patrones fenológicos pueden ser el resultado de procesos evolutivos (causas últimas), donde la selección natural es el proceso más importante, y los mecanismos biológicos (causas próximas) que se relacionan con la biología funcional que determina la operación e interacción de los elementos estructurales de estos patrones (Scholl and Pigliucci, 2014). Los bosques tropicales secos (BTSs) se distinguen por presentar una contrastada estacionalidad ambiental contrastante, representada por una estación de lluvias y una estación seca que puede llegar a variar de 3 a 6 meses (Dirzo *et al.*, 2010). La respuesta típica de las plantas a esta estacionalidad comprende esencialmente la expresión de dos patrones foliares fenológicos: 1) *plantas perennifolias*, las cuales mantienen todo el año sus hojas y usualmente están restringidas a lugares húmedos o riberas; y 2) *plantas caducifolias*, que se caracterizan por perder completamente sus hojas y permanecer en dormancia durante la estación seca, y producir nuevo follaje al inicio de la estación de lluvias (Bullock & Solís-Magallanes, 1990).

Sin embargo, en los BTSs Neotropicales algunas especies despliegan un patrón foliar inverso: al inicio de la estación seca producen nuevas hojas y al inicio de la estación de lluvias pierden su follaje quedando totalmente defoliadas hasta la reanudación de hojas en la siguiente época seca. Este patrón fenológico sugiere que las especies que lo exhiben presentan adaptaciones privativas, y posiblemente una mayor

plasticidad fenotípica, que les permiten desplegar sus hojas y mantener su fisiología vegetativa y reproductivas durante la época seca, cuando las demás especies permanecen latentes y sin hojas hasta la siguiente estación de lluvias. Además, la presencia de este patrón plantea una serie de incógnitas sobre su origen evolutivo, implicaciones ecológicas y ecosistémicas, su relación con las llamadas novedades evolutivas (Pigliucci, 2008; Levis & Pfennig, 2016), y el posible papel del ambiente en la evolución de adaptaciones a través de la plasticidad fenotípica (West-Eberhard, 2005, 2008).

La plasticidad fenotípica en plantas se refiere a la capacidad de expresar variación individual para producir diferentes fenotipos debido a la influencia ambiental, y ha sido propuesta como un mecanismo facilitador para que ocurran cambios evolutivos (Schlichting & Pigliucci, 1998; West-Eberhard, 2005). De esta manera la expresión de plasticidad fenotípica podría facilitar la aparición de variantes fenotípicas inducidas por el ambiente, sujetos a la acción de la selección natural y, por lo tanto, en última instancia fijar genéticamente nuevas características adaptativas a través de la acomodación genética (Schlichting & Pigliucci, 1998; West-Eberhard, 2005, 2008; Pigliucci *et al.*, 2006; Levis & Pfennig 2016). La acomodación genética se refiere al mecanismo de evolución en el que un nuevo fenotipo generado por una mutación o un cambio ambiental se redefine en un fenotipo adaptativo a través de cambios genéticos cuantitativos, y en algunos casos extremos, estos fenotipos pueden perder la plasticidad fenotípica ancestral, que se expresó en un tiempo evolutivo anterior, a través de la asimilación genética como una forma extrema de la acomodación genética en el que la selección causa la pérdida de la sensibilidad ambiental (*e.g.* plasticidad fenotípica) a través del tiempo evolutivo (Levis & Pfennig 2016; Levis *et al.*, 2017).

La plasticidad fenotípica que exhiben algunas especies de plantas es notable por la plasticidad necesaria para invertir su fenología foliar completamente y desplegar su

aparato fotosintético en la época de mayor estrés hídrico, en contraste a la conducta fenológica de la mayoría de las especies adaptadas a la estacionalidad climática de muchas regiones del planeta, incluyendo los trópicos (Janzen, 1970; Roupsard *et al.*, 1999). La elucidación de las fuerzas evolutivas y los factores próximos que mantienen tal patrón fenotípico han sido poco exploradas hasta ahora (Sánchez *et al.*, 2019), y es dicha elucidación lo que motiva la presente investigación de tesis.

Para realizar este trabajo de investigación se utilizó como sistema de estudio a la especie *B. nervosa* (anteriormente *Jaquinia pungens*), presente en los BTSs del pacífico mexicano, y que exhibe un patrón foliar invertido a lo largo de su distribución Neotropical (Janzen, 1970). Esta especie representa aparentemente una novedad fenológica en la familia Theophrastaceae, ya que de todas las especies que forman parte de este grupo, es la única que posiblemente expresa ese patrón fenológico invertido (Källersjö & Sthål, 2003; Ståhl, 2008; 2010). Además de esta especie en la literatura científica únicamente se ha descrito a *Faidherbia albida* (Acacia) con este patrón foliar invertido en un ecosistema estacional (Roupsard *et al.*, 1999).

Específicamente, esta tesis aborda desde una perspectiva ecofisiológica y evolutiva, el efecto de los principales factores ambientales (disponibilidad de agua y variabilidad lumínica), en la expresión de este patrón y sus consecuencias en la adecuación de plantas con fenología invertida. Asimismo, su relación con el surgimiento de variantes fenotípicas, a través de la plasticidad fenotípica producto de la variabilidad ambiental en una población local del Occidente de México.

Objetivo general

Elucidar el posible significado ecológico de la fenología invertida a través de la manipulación experimental, que simula la variación natural de factores ambientales, en la

expresión vegetativa y reproductiva de una especie arborescente prominente en los BTSs de Chamela, México.

Objetivos particulares

- Estudiar las respuestas fenológicas, fisiológicas y reproductivas –a través de experimentos *in situ*– a la manipulación de la intensidad lumínica en una especie con fenología foliar invertida.
- Explorar, a través de experimentos en campo, las consecuencias de la disponibilidad inusual de agua durante la época seca, en caracteres vegetativos y reproductivos en una especie con fenología foliar invertida en un BTS.

Estructura de la tesis

La estructura de este trabajo de investigación está conformada por una introducción general, dos capítulos principales, correspondientes a manuscritos aceptados o en preparación para ser publicados en revistas internacionales (todos en inglés), la discusión y las conclusiones generales. Específicamente, los dos capítulos principales se describen a continuación:

Capítulo 1. Experimento de campo para determinar el efecto de irrigaciones durante la época seca en caracteres vegetativos y reproductivos en el árbol deciduo en lluvias *Bonellia nevosa* (A field experiment to determine the effect of dry-season irrigations on vegetative and reproductive traits in the wet-deciduous tree *Bonellia nevosa*). (Artículo de requisito aceptado en el *Journal of Tropical Ecology*)

El capítulo versa sobre la manipulación experimental de la disponibilidad de agua durante la estación seca, cuando típicamente no hay lluvias, y sus consecuencias en los componentes vegetativos y reproductivos de *B. nevosa* (Sánchez *et al.*, 2019).

Sánchez O, Quesada M, Dirzo R, and Schlichting CD (2019). A field experiment to determine the effect of dry-season irrigation on vegetative and reproductive traits in the wet-deciduous tree *Bonellia nervosa*. *Journal of Tropical Ecology* 36, 29–35. <https://doi.org/10.1017/S0266467419000324>

Capítulo 2. Respuestas fenotípicas a diferentes regímenes de luz de un árbol decíduo en lluvias: una prueba de acomodación genética en una selva seca estacional. (Phenotypic responses to different light regimes in a wet-season deciduous tree: a test of genetic accommodation in a seasonally dry tropical forest).

(Artículo sometido a *American Journal of Botany*, status: atendiendo sugerencias y comentarios de revisores)

Este capítulo tiene como objetivo mostrar los efectos de la manipulación de la intensidad lumínica, señalada como uno de los factores importantes en la expresión fenológica en las plantas, en una especie con fenología foliar invertida, desde una perspectiva de la respuesta de la plasticidad fenotípica en caracteres fisiológicos y reproductivos, y sus implicaciones en la evolución de este patrón en un bosque tropical seco.

Finalmente, en las conclusiones de la tesis se resaltan los aspectos más notables del estudio y apunta hacia algunas de las líneas más promisorias de trabajo futuro en este campo.

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A field experiment to determine the effect of dry-season irrigation on vegetative and reproductive traits in the wet-deciduous tree *Bonellia nervosa*

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Abstract

Seasonally dry tropical forests (SDTFs) stand out by the diversity of phenological patterns used by plants to deal with dry periods. Although the predominant phenological pattern is dry deciduousness, in Mesoamerican SDTFs the heliophilous tree species *Bonellia* (formerly *Jacquinia*) *nervosa* displays an unusual inverted leaf phenology, producing and holding leaves through the dry season while becoming deciduous in the rainy season. Applying a dry season irrigation field experiment (no water, low watering, high watering), we studied the consequences of contrasting water availability from a phenological plasticity response perspective. Contrary to our expectations, our results show no effect of irrigation treatment on leaf phenology. In addition, mid-day twig water potential showed no significant differences across treatments, but reproductive phenological responses varied among treatments: canopy flowering per cent decreased gradually until the beginning of the wet season in all treatments; meanwhile canopy fruit per cent showed a significant decline under low irrigation. Finally, non-structural carbohydrate concentration (starch) was significantly higher in the high irrigation treatment. Our results showed that inverted leaf phenology remains unaffected regardless of supplemental water availability, and suggest a reallocation of non-structural carbohydrates to fruits and seeds in high-irrigation treatments. Given the current and expected increase in extreme drought events, investigations on the responses of trees of different phenologies, including those of inverted leafing such as *Bonellia nervosa*, are warranted.

Introduction

Seasonally dry tropical forests (SDTF), in contrast to wet tropical forests, alternate contrasting periods of abundant water supply and periods of drought with annual precipitation from 250 to 2000 mm and a prolonged dry season of 4–6 months (Dirzo *et al.* 2011). Because of this, precipitation seasonality becomes a key environmental factor that determines and orchestrates the biological activity of plants such as growth, leaf production and reproduction (Eamus 1999, Murphy & Lugo 1986).

SDTF trees display several physiological mechanisms to avoid water stress during drought periods (Murphy & Lugo 1986). The most evident of these mechanisms are leaf senescence and leaf fall but water stress could be likewise followed by a decline in root and leaf hydraulic conductance, stomatal closure and hormonal alterations within vascular tissues (Giraldo & Hoolbrook 2011). A variety of leaf phenology patterns have evolved in response to this seasonality, including leafless periods during the dry season or during the wet season, or year-round leaf retention (Borchert 1994a, Bullock & Solís-Magallanes 1990).

Leaf fall of deciduous species typically increases with the onset of the dry season and reduced soil water availability (Bullock & Solís-Magallanes 1990), with flowering between the mid-dry season and the end of the wet season; these are often regulated by day length (Borchert & Riviera 2001, Borchert *et al.* 2002). However, the synchronicity of leaf fall may vary both within and among species, and tree water status may be a determining factor, varying with the availability of subsoil water, and intrinsic biotic factors such as structure and lifespan of leaves, time of leaf shedding, wood density and capacity for stem water storage, and depth and density of root systems (Borchert 1998). For instance, high wood density species, usually restricted to upland sites with no access to the water table, exhibit high stem water potentials (<4 MPa) and remain dormant the entire dry season until rehydration of stem tissues (Borchert 1994a). Meanwhile, trees with residual water storage in tree trunks and access to subsoil water may flower and flush

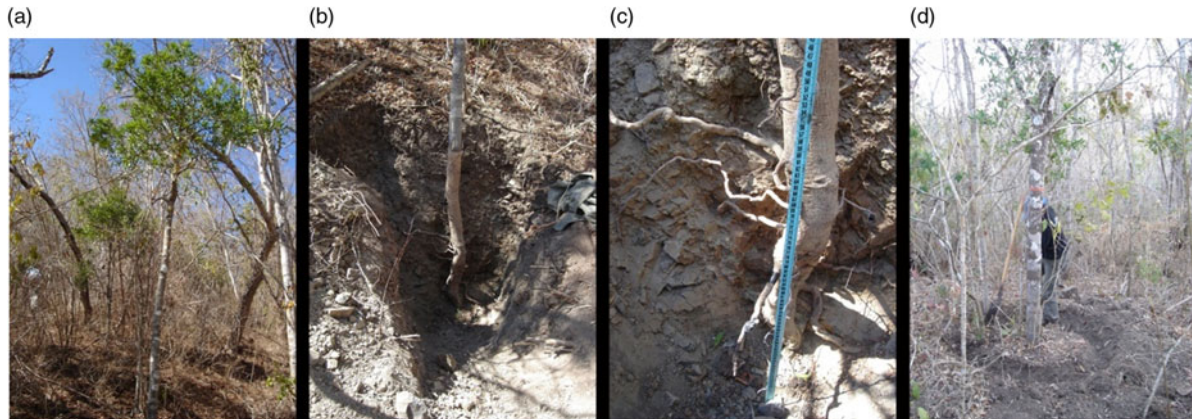


Figure 1. *Bonellia nervosa* experimental trees during the dry season in the SDTF in western Mexico: (a) adult tree with leaves during the dry season; other species are leafless; (b–c) Tap root system with lateral roots; (d) water trench around the trunk perimeter in all experimental trees.

during the dry season. Thus, seasonal water stress is likely to determine the timing of leaf shedding in SDTFs (Borchert 1994*a, b*, Bullock & Solís-Magallanes 1990, Murphy & Lugo 1986).

Phenological variation and stem water status in SDTF trees show a strong relationship that explains the common pattern of leaf production after the first rainfalls and leaf shedding during the beginning of the dry season (Méndez-Alonzo *et al.* 2012). Because soil water potential is negatively correlated with altitude, insolation and water flow accumulation (Méndez-Alonzo *et al.* 2013), deciduous species might avoid seasonal drought and respond to water pulses at the beginning of the rainy season (Borchert 1994*a, b*; Bullock & Solís-Magallanes 1990). However, the heliophilous tree species *Bonellia nervosa* (Theophrastaceae, formerly *Jacquinia pungens*) in Mesoamerican SDTFs exhibits an inverted deciduous leaf phenology, maintaining its leaves during the dry season (Bullock & Solís-Magallanes 1990, Janzen 1970): new leaves emerge at the beginning of the dry season when most of the forest canopy is leafless and are dropped about 2 weeks after the rains begin, remaining leafless and dormant throughout the rainy season (Janzen 1970). Understanding the responses of plants with different phenologies to water availability thus becomes a critical need, in light of the increasing occurrence of extreme or erratic climatic regimes (Cleland *et al.* 2007, Walther *et al.* 2002). Here, we examine the responses to water availability of the inverse-phenology tree *Bonellia nervosa*. Specifically, we study, via experimental dry-season irrigation, the consequences of unpredictable water availability for an inverted deciduous leaf phenology from a plastic-response perspective. We tested whether providing water during the dry season might trigger phenotypically plastic responses of vegetative and reproductive traits. If water is made available, we predicted that this inverted deciduous species would: (1) cease leaf activity and initiate leaf abscission, or (2) increase reproductive success in response to additional water and nutrient availability.

Methods

Study site

This work was conducted in the SDTF located within the Estación de Biología Chamela (Universidad Nacional Autónoma de México) in Jalisco, Mexico (19°29'N, 105°01'W). The region receives mean annual rainfall of 788 mm (1977–2000) concentrated (80%) between July and October, with a dry season from February to the end of

June (Bullock 1986). The dominant vegetation is tropical deciduous forest with a leafless physiognomy during the dry season, and semi-deciduous riparian forest along creeks (Lott *et al.* 1987). Although precipitation patterns are relatively constant among years, sporadic rains may occur after the end of the rainy season (December–January), important for facultative leaf flushing species (Bullock & Solís-Magallanes 1990).

Species of study

Bonellia nervosa C. Presl (Theophrastaceae) is a heliophilic understorey shrub with coriaceous, simple and alternate leaves with a needle-like tip, occurring from southern Jalisco, Mexico, to north-eastern Costa Rica (Janzen 1970, Ståhl & Källersjö 2004). Theophrastaceae species are typically evergreen, but *Bonellia nervosa* is the only known species with inverted phenology (Ståhl 2010, Ståhl & Källersjö 2004).

Experimental design

We experimentally manipulated water availability in the field for 45 reproductive trees (Figure 1a) in an upland area about 6 km² and occupying similar morpho-edaphic conditions (Cotler *et al.* 2002). To minimize ontogenetic variation within treatments we selected reproductive trees from 3–5 m height with similar trunk diameter at breast height (dbh). Experimental trees ($n = 15$ per treatment) were randomly assigned to three water irrigation treatments: low (60 mm), high (100 mm) and control. Trees in the three treatments did not initially differ for dbh ($F = 1.22$; $P = 0.30$). Irrigation treatments simulated intra-annual precipitation events observed during 1977–2007 in the Chamela SDTF (data from meteorological station at La Estación Biología de Chamela). Water volume for each tree in the low water irrigation treatment simulated the lowest monthly precipitation record (in 2005; 384 mm), and the total volume per tree for the high water irrigation treatment matched the highest monthly precipitation (in 1992; 1125 mm) (Bullock 1986, Hayden *et al.* 2010).

Irrigation set-up

Prior to irrigation we determined crown width, lateral root presence and root characteristics of two individuals of 3–5 m height and 64.9 mm and 83.8 mm dbh. After exploratory excavations into limestone (Figure 1b–c, 1.24 m depth), we found that lateral roots did not exceed crown width (2.55 m and 3.2 m, respectively), and

varied in number from 5–10 with maximum lengths of ~90 cm. The primary root was similar to a taproot, maintaining the same trunk-width observed above ground. These observations suggest that lateral roots might be involved in superficial water uptake and that primary roots might reach the water table.

In March 2008, we started the experimental irrigation with an initial standardized intensity (25 mm), simulating the mean precipitation from the first rains of the wet season that trigger vegetative activity in deciduous species (Bullock 1986). All experimental trees were irrigated with the total amount of water previously assigned by treatment. To avoid water run-off, a perimeter trench (1.5 m from the trunk base) was excavated around all experimental and control trees (Figure 1d). Low and high irrigation regimes started on the same date (21 March), with a lag time of 2 d between each watering, until the total water for each treatment was achieved: low (384 mm, April 4) and high (1125 mm, April 10). Previous to the experimental irrigations, soil moisture was assessed for each tree from five measurements (5–10 cm depth) using an Onset Soil Moisture Sensor with a 5-cm Probe Soil Moisture Smart Sensor (Onset HOBOTM S-SMC-M005).

Leaf traits

Leaf phenological response of all experimental trees was quantified monthly (March–October) using the Fournier index, based on a scale of 0–4 to determine the fraction of potential leaves in the canopy; with 0 = 0%; 1 = 1–25%; 2 = 25–50%; 3 = 50–75%; and 4 = 100% leaf canopy (for details see Fournier 1974). Monthly leaf survival was determined by monitoring a group of marked leaves on five branches of each tree; leaf survival was obtained as the number of standing leaves minus the standing leaves of the previous census for each treatment.

Whole-plant traits

Mean annual growth was estimated as the difference of trunk dbh between the beginning of irrigation (March 2008) and the end of the next dry season (May 2009). Water stress on plants was estimated for five randomly selected stems per treatment from different heights and positions of each tree. Stems were cut slantwise from every tree at the beginning and the end of the irrigation treatments (October 2008). Material was immediately placed within sealed plastic bags to avoid water loss and measured *in situ* with a Scholander pressure bomb (Model 600 Pressure Chamber Instrument, PMS Instrument Company) (Scholander *et al.* 1965).

Reproductive phenology was recorded using the same criteria as for vegetative phenology. Flowers and fruits as a percentage of canopy cover were calculated monthly, using the Fournier index. Because carbohydrate reserves decrease during the wet season when the plant is dormant (Janzen & Wilson 1974), we assumed that nutrient acquisition to produce new leaves, flowers and fruits occurred in the previous season. Thus, the effect of water manipulations on reproductive traits is expected to be measurable in the next dry season after irrigation. We randomly selected and marked five branches from each individual in all treatments and counted the total flowers produced in the dry season following irrigations. We counted the total number of fruits produced after 4 mo during the dry season, and estimated fruit-set as the proportion of the total number of fruits divided by the total number of flowers.

To determine non-structural carbohydrate (NSC) content in twigs, fruits and seeds, we randomly collected 3–5 terminal twigs and mature fruits from different heights from the canopy from five different trees in each treatment. Mean concentrations of fructose,

sucrose and glucose were determined by a modification of the Sigma® Fructose assay kit (Lara-Núñez *et al.* 2009).

Data analysis

To compare the effect of anomalous water availability during the dry season on vegetative and reproductive phenology, we quantified yearly phenological patterns for the three treatments using circular statistics. The frequency of individuals at each phenological stage within each month was calculated, frequency data were transformed to circular percentage and analyses were carried out using Oriana v.4 (Kovach 2011). We determined whether mean angles of phenological patterns differed significantly between irrigation treatments with non-parametric Watson U^2 tests that compared the mean vector lengths for each treatment with those for the pooled phenological data. The effect of irrigation on leaf longevity was analysed through a proportional hazards regression model fitted to a Weibull distribution. Annual growth and mid-day twig water potential were analysed with factorial analyses of variance with treatment and year as main effects and repeated-measures design with between-subject factors. To analyse the effect of experimental irrigation on fruit-set we conducted a general linear model (GLM) with maximum verisimilitude function and a Poisson distribution fit where individual tree variation was set as weight factor within the model test. Non-structural carbohydrate (glucose, starch and sucrose) content within fruits and seeds was analysed using a two-way ANOVA followed by Tukey tests. All data analyses with exception of phenological records were performed with JMP® 11.0.0 (SAS Institute, Inc., Cary, NC).

Results

Leaf traits

Eight months following experimental irrigation, leaf phenology showed no significant differences between irrigation treatments. Leaf canopy cover among water manipulations showed similar patterns of decline until the beginning of the wet season (Figure 2a). There were no significant leaf canopy cover differences between control and experimental irrigations or between the irrigation treatments (control vs. low: $U^2 = 0.074$, $P > 0.5$; control vs. high: $U^2 = 0.07$, $P > 0.5$; low vs. high: $U^2 = 0.043$, $P > 0.5$).

Leaf survival probability decreased during the months following experimental irrigation ($\chi^2 = 39,992$, $P < 0.0001$). Marked leaves in the low-irrigation treatment exhibited the most negative trend (April) followed by leaves from the high-irrigation treatment that showed a further decline (June) after the beginning of the wet season (Figure 2b). Leaves from control trees had higher survival after 8 mo, compared with both high- ($\chi^2 = 2,837$, $P < 0.0001$) and low- ($\chi^2 = 3,347$, $P < 0.0001$) irrigation treatments. There was also a significant difference between low- and high-irrigation treatments ($\chi^2 = 41.1$, $P < 0.0001$). During June, leaf survival decreased abruptly for all trees, however Weibull-fitted curves showed that low- and high-irrigation treatments still had lower survival than leaves on control trees during the months following irrigation (Figure 2b; dotted-lines). Finally, experimental trees from all treatments dropped all marked leaves at the onset of the rainy season (Figure 2b).

Whole-plant traits

Both control and experimental trees showed significant annual growth ($F = 3.0$, $P = 0.034$), but there were no differences among

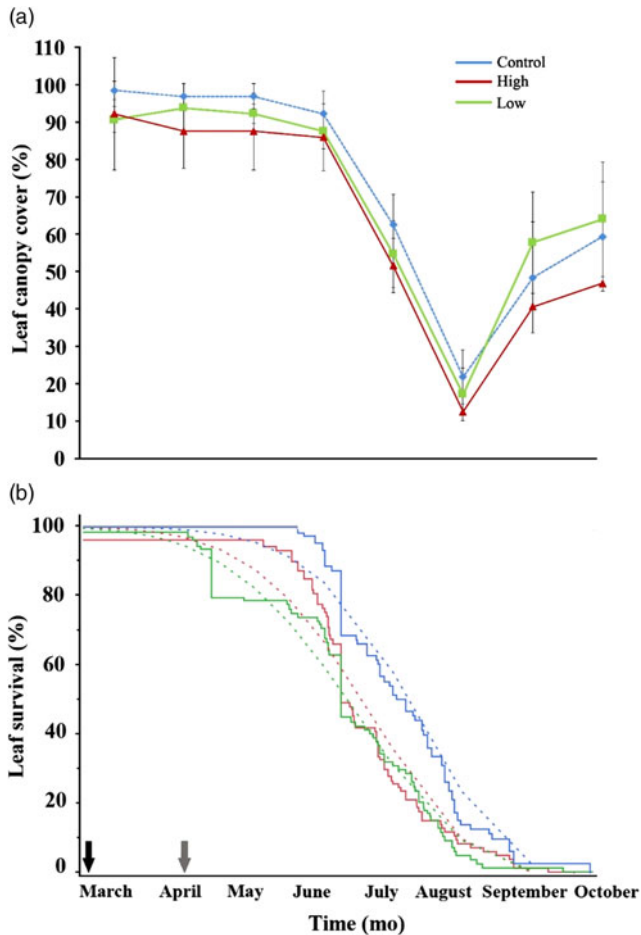


Figure 2. Vegetative responses after irrigation treatments in the inverted deciduous species *Bonellia nervosa* in the SDTF in western Mexico. (a) Monthly leaf phenological records (\pm SD); (b) Kaplan–Meier plot of survival probability on marked leaves through irrigations. Dotted line curves show data fitted to the Weibull distribution. Arrows marked the beginning (black) and end (grey) of irrigation treatments (21 March and 10 April 2008, respectively).

irrigation treatments ($F = 1.37$, $P = 0.25$) or any year \times treatment interaction ($F = 0.33$, $P = 0.71$). Contrary to expectations, mid-day twig water potential also showed no differences between the initiation and the end of irrigation ($F = 1.72$, $P = 0.17$).

After 8 mo of experimental irrigation, reproductive phenological patterns varied among treatments. Flower canopy percentage tended to decrease gradually until the wet season began, with control trees having higher percentages (Figure 3), but the Watson's U^2 paired-comparisons showed no significant differences among treatments: control vs. low ($U^2 = 0.052$, $P > 0.5$); control vs. high ($U^2 = 0.085$, $P > 0.5$); high- vs. low-irrigation ($U^2 = 0.02$, $P > 0.5$).

Fruit canopy percentage showed a significant effect of experimental irrigation, where control trees had higher fruit canopy percentage than both low ($U^2 = 0.19$, $P < 0.05$) and high ($U^2 = 0.20$, $P < 0.05$) irrigation treatments. Fruit-set was significantly lower in the low-irrigation treatment ($\chi^2 = 145.5$, $P < 0.0001$) (Figure 4).

Overall, NSC concentrations showed different patterns among different tissues from irrigated trees (Figure 5). Twigs of irrigated trees showed a lower glucose concentration compared with control trees ($F = 3.7$, $P = 0.02$), but high-irrigation trees had a significantly higher starch concentration compared with both control and low-irrigated treatments ($F = 17.9$, $P < 0.0001$; Figure 5a).

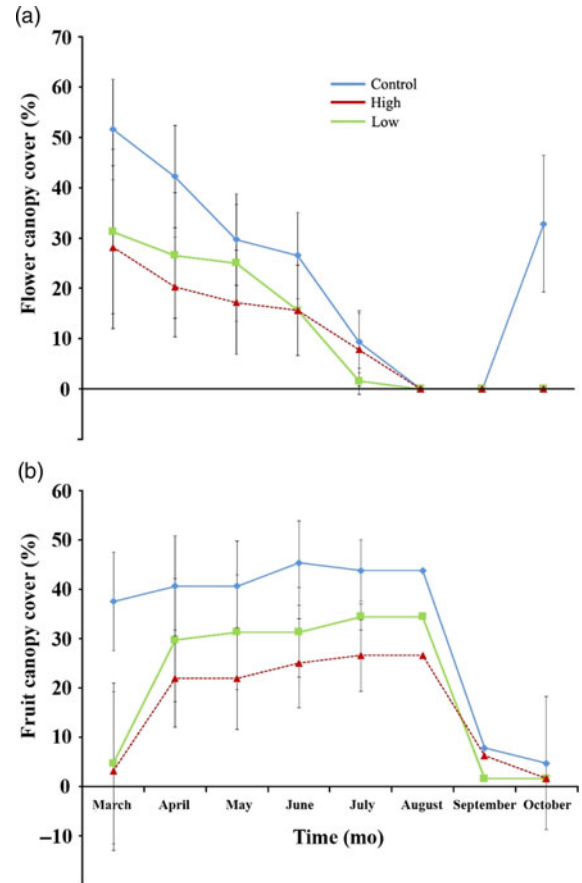


Figure 3. Flower and fruit canopy percentage (%) in *Bonellia nervosa* reproductive trees under experimental water irrigations during the dry season: (a) flowers and (b) fruits. Each point represents the mean value observed for that month (\pm SD).

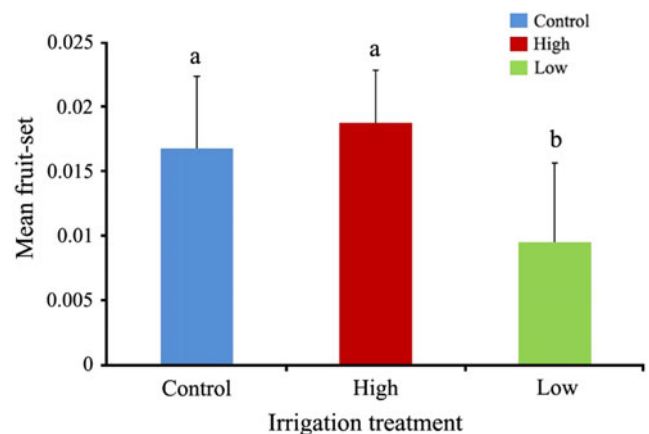


Figure 4. Mean fruit-set (\pm SE) in *Bonellia nervosa* adult trees after 1 year of irrigation treatments in the SDTF in Chamela, western Mexico.

There were no differences in sucrose content between treatments ($F = 1.46$, $P = 0.24$). Glucose concentration of fruits and seeds showed different trends (Figure 5c): while there were no differences for fruits ($F = 2.33$, $P = 0.10$), seeds showed higher concentration in both irrigation treatments ($F = 23.5$, $P < 0.0001$). Starch concentration within fruits and seeds was higher in the low and high irrigation treatments ($F = 5.43$, $P = 0.007$; and $F = 10.32$, $P = 0.0002$, respectively; Figure 5b) compared with controls.

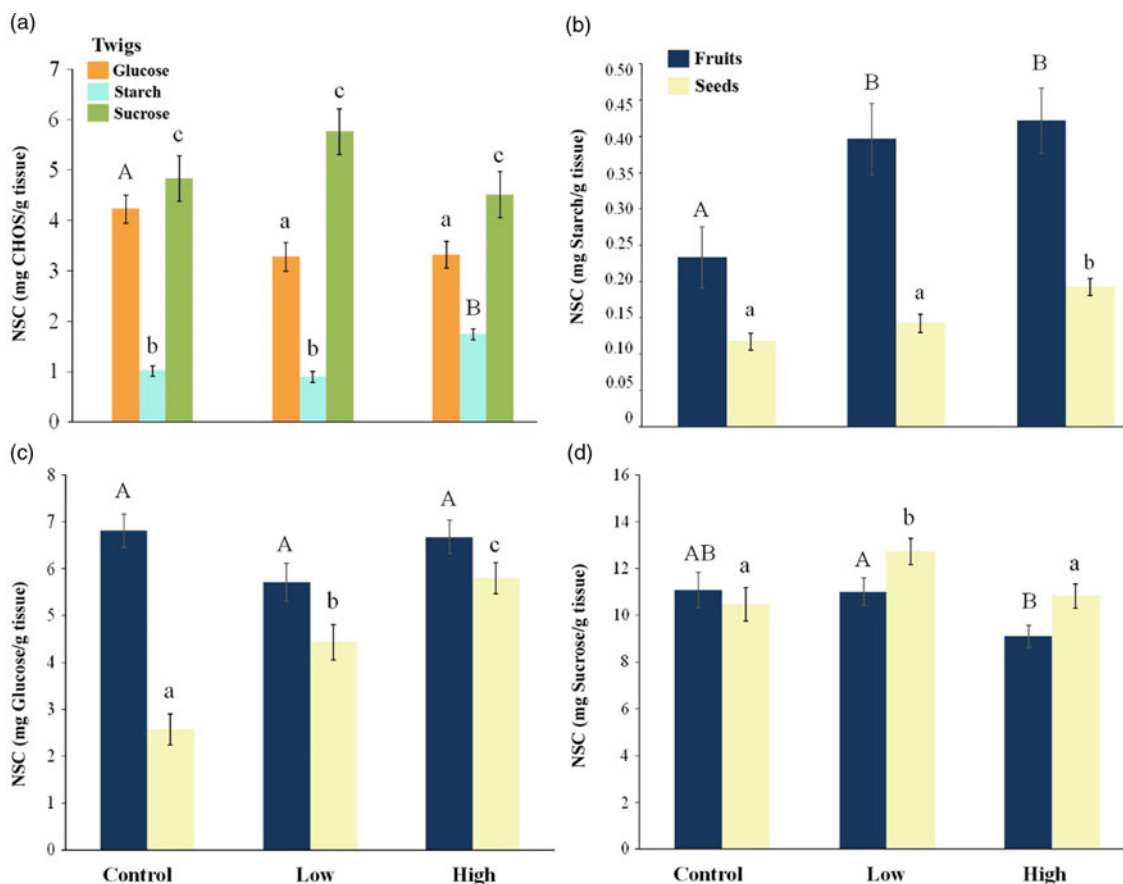


Figure 5. Mean concentration of non-structural carbohydrates (NSC) in *Bonellia nervosa* tissues (± SE) (twigs, fruits and seeds) after experimental irrigation during the dry season in the SDTF in western Mexico. (a) Differences in twigs NSC content. (Capital vs. lowercase letters show significant differences among treatments). Fruit and seed (b–d) differences are marked with capital and lowercase letters, respectively.

Sucrose concentration decreased in fruits from high irrigation trees ($F = 4.21$, $P = 0.021$), while seeds from low irrigation trees had a higher concentration than control and high-irrigation treatments ($F = 4.18$, $P = 0.022$; Figure 5d).

Discussion

We predicted that experimental irrigation of *Bonellia nervosa* during its typical dry season should alter its leafing phenology or perhaps increase its reproductive output. Overall, our results supported neither of these predictions. Perhaps most surprisingly for this species with inverted phenology, leaf phenology was unaffected, and although some whole-plant traits (e.g. fruit and seed composition) did show differences across treatments, there was no increase in reproductive output relative to the control as a result of watering.

Leaf phenology

Experimental irrigations during the dry season did not alter the normal occurrence of leaf drop following the first rains of the wet season. Contrary to our expectations, we did not find a significant difference between leaf abscission among irrigation treatments (Figure 2b). We expected that precipitation, as a key environmental factor in vegetative activity, should affect inverse vegetative activity in *Bonellia nervosa*. However, our results suggest that leaf abscission in this species may be under genetic

control rather than governed by environmental factors (Mojica *et al.* 2016), despite evidence that many phenological responses of deciduous species in SDTFs are driven by water availability or photoperiod (Borchert 1994a, b; Bullock & Solís-Magallanes 1990).

Sánchez *et al.* (in preparation) have also found that inverted leaf phenology in *Bonellia nervosa* remains unaltered after experimental shading, suggesting that the inverse phenological behaviour has no plasticity (i.e. is genetically fixed). This species also presents no phenological plasticity to environmental light variability in Costa Rican populations (Chaves & Avalos 2008). Together, these findings on immutable leaf phenology support the idea that inverted leaf phenology may have been genetically accommodated in this species.

Whole-plant traits

Our findings integrating off-season water availability and whole-plant traits do not explain the benefits of inverted phenology with activity during drought conditions. The effect of dry-season irrigation on inverted leaf phenological species has not previously been described, but our findings are consistent with other research that found little effect of irrigation on leaf longevity, stem water status or storage (Myers *et al.* 1998), nor differences in subsequent relative growth rate after one-time experimental irrigation of four (non-inverted) deciduous species in the SDTF of western Mexico (Hayden *et al.* 2010).

These results could be explained through four different lines of reasoning: (1) primary roots are storing water or (2) directly accessing the water-table, or (3) unusual water availability during the dry season produces plastic responses to reallocate nutrients to reproductive traits. In general, deciduous trees with no access to soil or stem water storage in the dry season are not in a state of dormancy, but rather in a drought-induced rest period whereby they remain inactive until rehydration (Borchert 1994b). In contrast to most dry season deciduous trees, *Bonellia nervosa* exhibits a root system composed of long deep roots that could potentially store water or reach the groundwater table (Figure 1b, c). In *Faidherbia (Acacia) albida*, an inverse phenology species in semi-arid Africa, water uptake comes from roots distributed to a depth of 7 m, probably extending to the permanent water-table (Roupsard *et al.* 1999). Such a mechanism may explain water status during the dry season in *Bonellia nervosa* and the lack of any effects of irrigation treatments on mid-day stem water potentials (Appendix 1).

Typically, SDTF tree roots have no access to the groundwater table and soil water reserves are depleted early in the dry season in dense tree stands (Borchert 1994a). However, *Bonellia nervosa* roots could act as water reservoirs during the dry season for reproduction and vegetative growth. Flower and fruit phenology are restricted to the utilization of residual water, allowing rehydration of stem tissues and the subsequent flowering and flushing (Borchert 1994b). Typically, *Bonellia nervosa* flowers at the same time as it flushes new leaves during the shift between wet and dry season, suggesting that reactivating phenology does not require the first rains, but rather utilizes stored water within stem wood or the root system. We lack quantification of soil water oxygen ($\delta^{18}\text{O}$ and $\delta^{16}\text{O}$) or carbon ($\delta^{13}\text{C}$) isotopic composition that could identify specific patterns of water-relations and potential evapotranspiration (Fardusi *et al.* 2016).

Janzen & Wilson (1974) demonstrated that carbohydrate allocation among roots, stems and twigs of *Bonellia nervosa* occurs during the dry season, depleting one-half of the stored reserves while the tree is leafless during the rainy season. Our results suggest that some non-structural carbohydrate reallocation occurred within fruits and seeds following dry-season irrigations, although irrigation had no quantitative effects on flower and fruit production. It is possible that water from the root system is being redistributed to reproductive components. Although the evolutionary pathways of inverted phenology are unclear, the occurrence of different adaptive strategies exhibited by *Bonellia nervosa* such as physiological plasticity, efficient internal cycling, conservative utilization of soil nutrients and water use efficiency, are probably necessary to endure drought conditions in SDTFs.

Conclusions

This study provides a first experimental examination of mechanisms underlying an exceptional inverted leaf phenological pattern during the dry season in SDTF, evaluating phenotypic plasticity in response to supplying water out of phase. Although our findings suggest that phenology is not affected by experimental irrigation, mean fruit-set in trees from high-irrigation seem to produce more fruits than low treatment, and non-structural carbohydrates are reallocated among twigs, fruits and seeds. The reason for these contradictory results is unclear now, but these differences can be explained in part by the plasticity of hydraulic redistribution of water from deep layers by roots (where deep soil nutrients are mobilized and taken up by plants; McCulley *et al.* 2004), and by

the physiological plasticity of reproductive traits (Valladares *et al.* 2007). In addition, our results could indicate the adaptive mechanisms used by plants in seasonal environments to reallocate nutrients from senescent tissues to the production of new leaves, flowers or fruits, when water supply is limited (Lal *et al.* 2001), specifically reallocation of non-structural carbohydrates to seeds may occur in high-irrigation treatments.

The overall results of this study do not delineate the occurrence of inverted leaf phenology of *Bonellia nervosa*. However, our findings provide the first attempt to elucidate water uptake, nutrient allocation and phenotypic plasticity response to unusual water availability from a species with an exceptional inverted leaf phenological pattern during the dry season, when drought conditions and leaf deciduousness are mostly the norm for SDTF trees.

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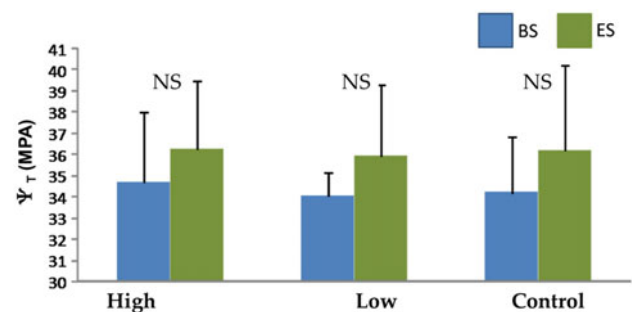
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Appendix 1

Mean mid-day twig water potentials of *Bonellia nervosa* trees in the SDTF in western Mexico (15 individuals per treatment) in response to three watering treatments. Measurements (\pm SE) were made at the beginning (BS) and end of the dry season (ES).



1 **Phenotypic responses to different light regimes in a wet-season deciduous tree:**

2 **A test of genetic accommodation in a seasonally dry tropical forest**

3

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17 Phenotypic responses to different light regimes in a wet-season deciduous tree in a
18 seasonally dry tropical forest

1 **PREMISE OF THE STUDY:** In seasonally dry tropical forests, the norm is for plants
2 to drop their leaves during the dry season. However, *Bonellia nervosa* displays an unusual
3 leaf phenology: it produces and maintains its leaves during the dry season and drops them
4 during the wet season, whereby environmental perturbation leads to developmental
5 reorganization for production and genetic accommodation on inverted leaf phenology.

6 **METHODS:** We set experimental light-manipulations (0%, 35%, 75% shading) in 30
7 reproductive trees in the seasonally dry tropical forest of Chamela, Mexico. We followed
8 vegetative and reproductive traits related to plant performance during 3-dry seasons.
9 Because of the non-linearity of environmental data, all analyses were carried out via
10 General Additive Models (GAMs) and analysis of variance.

11 **KEY RESULTS:** Experimental manipulations suggested genetic accommodation as
12 inverted leaf phenology exhibits no plasticity despite shading. While vegetative traits
13 showed negative responses under extreme shading, mostly expected for a heliophilous
14 species but leaf phenology remains unperturbed. However, reproductive traits showed
15 multiple plastic responses: fruit production increased under 30 % shading, however,
16 control and under 75% shading decreased; fruit-set decreased in control and 75%
17 treatments but seed and fruit weight and nutrient content increased, in turn leading to
18 enhanced radicle growth.

19 **CONCLUSIONS:** Our results suggest that *B. nervosa* responded in multiple ways to
20 light heterogeneity with facultative plasticity on whole-plant performance and post-
21 germination traits where inverted phenology has likely undergo genetic accommodation.

22 **KEYWORDS** leaf phenology; inverted leaf phenology; wet-deciduous; seasonally dry
23 tropical forest; Chamela, *Bonellia nervosa*.

24

1 Environmental factors have been recognized as evolutionary drivers of novel phenotypic
2 variants through the expression of phenotypic plasticity on preexisting phenotypes
3 (Pigliucci, 2005; West-Eberhard, 2005; Tawfik, 2010; Hallgrímsson et al., 2012; Palmer,
4 2012; Schlichting and Wund, 2014). Phenotypic plasticity refers to the capacity of
5 expressing individual variation to produce different phenotypes (physiology, gene
6 expression, trait development, phenotypic integration, behavior or rate of activity) due to
7 environmental influence, and has been proposed as a facilitator of evolutionary change
8 (Schlichting and Pigliucci, 1998; West-Eberhard, 2005; Schlichting and Wund, 2014;
9 Palacio-López et al., 2015). Phenotypic plasticity expression is expected to facilitate the
10 appearance of environmentally induced novel phenotypes that, following natural
11 selection, may eventually lead to genetically-fixed novel traits (West-Eberhard, 2003).
12 Later quantitative genetic changes cause the immediate adaptative adjustment of the
13 phenotype (genetic accommodation) (Schlichting and Pigliucci, 1998; West-Eberhard,
14 2005; Pigliucci et al., 2006; Levis and Pfennig, 2016). Further, selection might favor
15 environmentally induced phenotypes to lose their environmental sensitivity –the absence
16 of variation on that trait- over evolutionary time (genetic assimilation), even when the
17 population experiences the ancestral environment (West-Eberhard, 2005; Levis and
18 Pfennig, 2016). A representative example is provided by van Hinsberg (1998) who
19 selected the plant *Plantago lanceolata* L. (Plantaginaceae) for long and short leaf lengths
20 under two light regimes—low and high red:far red ratios. He showed that seeds from
21 artificial selection lines exposed to high red:far red ratios had larger seeds and had lower
22 dormancy than those selected for long leaves in low red:far red ratios. These results
23 suggest that large genotypic variation in the germination responses to ambient light
24 conditions within selection lines, have the potential to evolve in response to natural
25 selection (van Hinsberg, 1998).

1 In addition, plants that exhibit phenotypic plasticity to new conditions might respond not
2 only by adjusting their phenotypes (Bradshaw, 1965; Schlichting, 1986; Sultan and
3 Bazzaz, 1993; Nicotra et al., 1997, 2008; Nicotra, 1999; Sultan, 2000; Griffith et al.,
4 2005), but also by altering those of their offspring, through changes in the quantity and
5 quality of resource provisioning for leaves, fruits and seeds, with consequences on post-
6 germination traits under new environmental conditions (Sultan, 2000; Donohue et al.,
7 2010).

8 Plant phenology involves complex interactions of physiological, morphological and
9 behavioral traits to environmental variability (Price et al., 1988; Singer and Parmesan,
10 2010; Morin et al., 2009; Forrest and Miller-Rushing, 2010), and may incorporate novel
11 developmental variants that shape life-history trade-offs (Ims, 1990; Forrest and Miller-
12 Rushing, 2010). Thus different types of phenological patterns might be the result of
13 evolutionary processes (ultimate causes), where natural selection is the most important
14 process, and biological mechanisms (proximate causes) that concern to the functional
15 biology which determines the operation and interaction of structural elements (Scholl and
16 Pigliucci, 2014). In seasonally dry tropical forests (SDTF), trees express two major
17 functional types - deciduous and evergreen - that evolved in response to environmental
18 seasonality (Bullock and Solís-Magallanes, 1990; Borchert et al., 2005; Singh and
19 Kushwaha, 2005). Rainfall and daylength seasonality are likely to drive leaf phenological
20 patterns in tropical and arid ecosystems (Borchert, 1994; Borchert et al., 2004; Jolly and
21 Running, 2004). Further, ultimate factors can be responsible for optimal phenological
22 response underlying trade-offs related to the life-history evolution of plants, leading to
23 relative benefits of reaching reproductive maturity earlier in the season, growing larger
24 before reproducing or benefit insect pollinators (Forrest et al., 2010).

1 In SDTFs of Mesoamerica, the norm is for plants to drop their leaves during the dry
2 season. However, *Bonellia nervosa* C. Presl (Theophrastaceae), (Janzen, 1970), become
3 leafless during the wet season and produce and extend their new leaves at the dry-wet
4 transition, with leaves retained throughout the entire dry season, at the time when the
5 majority of the species (dry-deciduous) are leafless (Janzen, 1970; Bullock and Solís-
6 Magallanes, 1990). Inverted phenological patterns in Neotropical dry forests (Janzen,
7 1970) challenge the usual physiological mechanisms in seasonal environments (Wickens
8 1969; 1983; Roupsard et al., 1999). Leaf flushing during the dry season forces plants to
9 deal with extreme temperatures, high-intensity sunlight, low or null water supply and
10 atypical daylengths (Rivera et al., 2002; Kutschera and Niklas, 2009; Forrest and Miller-
11 Rushing, 2010;). Among environmental cues, light heterogeneity is critical in
12 determining the optimum physiological mechanisms displayed by plants to survive under
13 contrasting light conditions (Valladares, 2003; Valladares and Niinemets, 2008). Typical
14 responses of shaded plants result from their individual ability to alter their physiology and
15 morphology (Schlichting, 1986; Bradshaw, 2006). Thus, inverted species should deploy
16 physiological mechanisms expected to be close to that of species located towards
17 minimum shade tolerance, to achieve optimum light-harvesting strategies and
18 reproductive success.

19 In this work, we aim to study, via experimental light manipulations, the consequences of
20 expressing inverted phenology, as a phenotypical variant induced by environmental cues,
21 in terms of vegetative and reproductive shade tolerance traits: leaf traits (e.g., leaf
22 turnover, leaf area, inclination angle) and whole-plant traits (e.g., growth rate, fruit-set,
23 seed size and seedling establishment, carbohydrate storage, or survival) (Ackerly et al.,
24 2000; Valladares and Niinemets 2008; Donohue et al., 2010). We asked, first, if this
25 phenological habit can be explained by the current phenotypic plasticity in shade-

1 tolerance plant traits, and to what extent do different shading regimes affect the
2 phenotypic responses of vegetative traits (leaf phenology and leaf physiology traits).
3 Second, if there is such plasticity, are there any detectable developmental adjustments
4 regarding reproductive traits and post-germination performance? Third, if no plasticity or
5 variation is observed in leaf phenological pattern might suggest genetic (leaf phenology)
6 accommodation on that trait. To accomplish this we examine vegetative and reproductive
7 traits in response to different light regimes using *in situ* manipulations in the inverted-
8 deciduousness tree *B. nervosa* (Theophrastaceae), in a SDTF from Western Mexico.
9 Specifically, we measured vegetative plastic responses to light variability (leaf survival,
10 annual growth, photosynthetic rates, mid-day twig water potential), and reproductive
11 responses as measured of plant fitness (flowers and fruit phenology, fruit-set, fruit and
12 seed weight, non-structural carbohydrate allocation to seeds, and seed radicle elongation).
13 We predicted that responses of vegetative and reproductive traits are affected by unusual
14 shading during the dry season when the canopy is absent and that exposure to dry-season
15 shading can reveal the phenotypic plastic responses of plant traits with proximate
16 implications in the evolution of inverted leaf phenology. Thus, phenotypic plasticity in
17 different combination of independently varying traits can suggest an adaptative
18 adjustment of phenotypes to environmental change. For instance, asynchrony in
19 reproductive phenology because of lower photosynthetic rates that triggers a reduction on
20 non-structural carbohydrates assigned to reproduction (fruits and seeds), affecting post-
21 germination development. Further, we predicted that inverted leaf phenology might
22 remain unaltered after light manipulations as an indicator of genetic accommodation to
23 light variability, suggesting that this unusual pattern might be a result of the ancestral
24 response to environmental light heterogeneity. In this scenario, selection can actively

1 eliminate phenological plasticity, causing the favored phenotype (inverted leaf
2 phenology) to be fixed in the population.

3 **MATERIALS AND METHODS**

4

5 **Study site**

6 We conducted our study in the SDTF located within the Estación de Biología Chamela
7 (Universidad Nacional Autónoma de México) in Jalisco, Mexico (19° 29' N, 105° 01' W)
8 during the dry seasons of 2007 to 2010. Annual rainfall averages 788 mm (1977-2000)
9 and is concentrated (80%) between July and October; the dry season extends from
10 February to the end of June (Bullock, 1986). Rainfall is rare after the end of the rainy
11 season in December-January (Bullock and Solís-Magallanes, 1990). The dominant
12 vegetation is the tropical deciduous forest with an entirely leafless physiognomy during
13 the dry season, with semi-deciduous riparian forest along creeks (Lott, 1985; Lott et al.,
14 1987). Leaf flushing is synchronous among most deciduous species after the beginning
15 of the rainy season, except for *B. nervosa* (Theophrastaceae), *Coccoloba liebmannii*
16 (Polygonaceae) and *Forchhammeria pallida* (Capparaceae), which are reported to
17 produce new leaves early in the dry season and maintain those new leaves throughout the
18 drought (Bullock and Solís-Magallanes, 1990).

19

20 **Species of study**

21 *Bonellia nervosa* (Theophrastaceae; basionym: *Jacquinia nervosa*) (Ståhl and Källersjö,
22 2004; Ståhl 2010), is a 3-5 m tall heliophilic understory tree occurring from southern
23 Jalisco, Mexico, to northeastern Costa Rica (Janzen, 1970; Källersjö and Ståhl, 2003;
24 Ståhl, 2008). Among the typically evergreen *Bonellia* species, *B. nervosa* is the only
25 deciduous species and likely, the only with this unusual phenology within the seven

1 genera recognized in the Theophrastaceae family (Ståhl, 2010). Its closest relative is the
2 evergreen *Bonellia frutescens* (formerly *J. frutescens*) restricted to northern South
3 America (Källersjö and Sthål, 2003). In the STDF of Chamela is the only representative
4 species of the Theophrastaceae family (Lott, 1985). *B. nervosa's* leaves are coriaceous,
5 simple and alternate with a needle-like tip, but new leaves emerge at the beginning of the
6 dry season when most of the forest canopy is leafless, and leaves are shed about two
7 weeks after the rains begin; it remains deciduous and dormant throughout the rainy season
8 (Janzen, 1983). Vegetative growth and reproduction take place at the beginning of the dry
9 season and practically all branch elongation occurs at the same time that leaves are
10 produced (Janzen, 1970). Carbohydrate allocation to roots, stems and twigs occurs during
11 the dry season, decreasing gradually to one-half of the stored reserves when the tree is
12 leafless during the rainy season (Janzen and Wilson, 1974).

13

14 **Experimental Design**

15 To manipulate light, we selected 45 reproductive trees and individually mesh-screened
16 30 from the base of the trunk to the top of the canopy and 15 remained uncovered as
17 control trees (for details see Appendix S1). To minimize ontogenetic variation within
18 treatments we selected reproductive trees 3–5 m tall with similar diameter at breast height
19 (DBH). Trees in the three treatments showed non-significant DBH differences ($F_{2, 45} =$
20 1.54 , $P = 0.24$). Experimental trees were randomly assigned to one of three light
21 manipulation treatments ($N=15/\text{treatment}$): 0%, 30%, and 75% shading (Fig.1). These
22 treatments simulated the seasonal changes observed in the canopy cover, known to affect
23 photosynthetically active radiation availability in SDTF (Lemos-Filho et al., 2010). To
24 minimized soil variation effects experimental trees were distributed within an area about
25 6 km^2 with uniform topographic and edaphic conditions (Cotler et al., 2002).

1

2 ***Shading enclosures microclimate*** -To characterize microclimate within the enclosures
3 and control trees (and thus assess the effectiveness of manipulations and intra
4 microclimate variation) we randomly attached two Data loggers (HOBO[®] data loggers
5 model U12, Bourne, Massachusetts, USA) per treatment. Data loggers were placed at 1.5
6 m above ground, protected within an open plastic cage and set to record hourly measures
7 of air temperature, air relative humidity, light incidence and soil temperature (at 5 cm
8 depth) for 33 days (for details see Appendix S1).

9

10 ***Leaf trait responses*** - Phenological leaf patterns were recorded in all trees through
11 monthly observations during twenty-eight months (October 2007-February 2010). Data
12 were collected during the last week of each month, with a 30 ± 3 -day interval between
13 each recording date. Percentage of leaf canopy cover (calculation of standing leaves
14 covering the whole-tree canopy) was calculated using a quantitative phenological index,
15 *P* (for details see Fournier, 1974 and Appendix S1).

16 Mean leaf survival was estimated monthly for individually marked branches, as the
17 number of standing leaves minus the standing leaves of the last census for each treatment
18 (1989 total leaves).

19 We recorded maximum photosynthetic rate (A_{\max}) on 5-10 fully exposed mature leaves
20 from 10 to 15 individuals per treatment, avoiding leaves with herbivore or pathogen
21 damage or senescent tissue. To minimize variation, we selected the fourth leaf following
22 a phyllotaxis approach. All A_{\max} measurements were recorded between 0900 and 1200 h
23 during cloudless days using a portable gas exchange system (LiCor 6400, LI-COR,
24 Lincoln, Nebraska, USA), with leaves clamped inside the chamber at constant leaf
25 temperature (26° C).

1 Light response curves were calculated for a different set of leaves (3-5/ind), selected from
2 5 to 7 trees per treatment. Leaves were located and selected between 1 and 1.5 m,
3 following the same timing and age criteria as above. The resulting adaptive light response
4 curves provide information about typical acclimation light curves and quantum efficiency
5 of photosynthesis, that are related to rates of maximum carbon assimilation by sun-
6 acclimated plants. Since the photosynthetic apparatus responds almost immediately to
7 light, we started all measurements with a leaf equilibrated to complete darkness, spending
8 2 or 5 minutes at each light value, and increasing in steps of $200 \mu\text{mol} \cdot \text{mol}^{-1}$ to 1600
9 $\mu\text{mol} \cdot \text{mol}^{-1}$. In some cases, collected data were recalculated to correct for leaf area
10 inside the gas chamber using the software provided by the gas chamber system (for details
11 see Appendix S1).

12

13 ***Whole-plant interrelated traits' responses to light heterogeneity*** - Mean annual growth
14 was estimated for every treatment as the difference in DBH between the beginning
15 (January) and the end of the dry season (June).

16 To calculate hydric stress on plants, we calculated the mid-day twig water potential for 5
17 stems randomly selected from different heights and positions and slantwise-cut from
18 every tree for each treatment during the middle of the dry season (April, 2009). Collected
19 material was placed in sealed plastic bags and immediately measured with a Scholander
20 pressure bomb (Model 600, Pressure Chamber Instrument, PMS Instrument Company,
21 Albany, Oregon, USA) (Scholander et al., 1965).

22 Reproductive responses, i.e., percentage of flowers and fruits presence on canopy cover
23 (mean % of standing flowers or fruits on the whole-tree canopy), were calculated through
24 monthly observations using the quantitative phenological index described above. To
25 determine the effect of shading regimes on reproductive traits, we randomly selected and

1 marked 5 branches from each individual in all treatments and counted the total flowers
2 produced during two consecutive years. Before initial flowering, all exclusions were half
3 opened (late November) during ~ 3 weeks to permit full flower development and the
4 entrance of winged pollinators.

5 Individual tree fruit production was estimated by counting the total number of fruits
6 produced after 4 months at the end of the dry season. Fruit-set was estimated as the total
7 number of fruits over the total number of flowers per individual. All fruits were collected
8 during the second study-year to determine weight and nutrient contents (for details see
9 Appendix S1). We assumed that, because carbohydrate reserves decrease during the wet
10 season when the plant is dormant (Janzen and Wilson, 1974), nutrient acquisition to new
11 leaves, flowers and fruits likely occurred in the previous season (Janzen, 1970). Thus, the
12 effect of light manipulations should be observable in the growing season after the
13 experiment was initiated.

14 To determine soluble carbohydrates content on branches, fruits and seeds, we randomly
15 selected 3 different branches at the same height and 3 to 5 fruits from 5 different trees
16 from each treatment (for details see Appendix S1). The mean content of fructose, sucrose
17 and glucose were determined using a modified Sigma[®] fructose assay kit (Lara-Núñez et
18 al., 2009). Before germination, seeds from all light treatments were subjected to 100%
19 mechanical scarification using a Dremel[®] MultiPro[®] (Model 395-0/15, Robert Bosch
20 Tool Corporation, Mt. Prospect, Illinois, USA). Scarified seeds were germinated within
21 plastic Petri dishes with Agar-Agar as growth medium (CAS 9002-18-0, Research
22 Organics, Cleveland, Ohio, USA), sealed and kept in a growth chamber at $24 \pm 2^\circ\text{C}$ with
23 a 12:12 photoperiod (Percival Model I-35 LL, Percival Mfg. Co., Boone, Iowa, USA)
24 during 6 days.

1 To determine the effect of light manipulations on subsequent seed performance we
2 measured the mean radicle growth of seeds from each treatment using digital calipers
3 (S500-160, Mitutoyo America Corporation, Aurora, Illinois, USA) at intervals of 6 days
4 over a 36-day period. These intervals cover the growth sequence of primary root
5 establishment, the appearance of secondary roots, the emergence of cotyledon and the
6 emergence of first leaves. All measurements were conducted within a vertical laminar
7 flow cabinet to avoid pathogens or bacterial contamination within growth medium and
8 seeds. Finally, radicle growth was calculated as the increase in root length for 6-day
9 intervals until true leaves were produced (36 days total).

10

11 **Statistical Analyses**

12 All analyses were carried out via General Additive Models (GAMs) and analysis of
13 variance using R version 3.2.0 (R Core Team, 2017) with RStudio interface
14 version 0.99.879, or through Generalized Linear Models using JMP® 11.0.0 (SAS
15 Institute, Inc.). Before each analysis, data were tested for normality and homogeneity of
16 variance using the Kolmogorov–Smirnov and Levene tests or the Shapiro–Wilk tests,
17 depending on whether the X-factor had two levels and samples were greater than 2000,
18 or the sample size was less than or equal to 2000, respectively. When criteria of normality
19 and homoscedasticity were not met, we used non-parametric procedures.

20 Generalized linear mixed models (GLMM) were used to test for differences in
21 microclimate conditions among light exclusion treatments (between-subject factor) and
22 time (within-subject factor), and their interaction was set as fixed effect. We used the
23 restricted maximum likelihood method to separate the variance of fixed effects from that
24 of the random effect. Relative humidity and light data were arcsine-square-root and log-
25 transformed before analyses, respectively. To evaluate the effect of light manipulations

1 on phenological patterns we conducted circular statistics, vegetative and reproductive
2 phenological data were transformed to circular percentages scale ranging from 0-100.
3 Differences in mean angles of phenological patterns between light exclusion treatments
4 were tested with a Watson-William F-Test analysis using Oriana version 4 (Kovach,
5 2011). The effect of light exclusion on leaf longevity was analyzed through a Proportional
6 Hazards Regression Model by a recapture (Rcapture) function to compare light treatments
7 using an R platform.

8 The effect of light exclusion on annual growth was compared during two consecutive yrs
9 using a Two-Way Mixed-Design Anova test. Treatment and year were main effects and
10 annual growth was the dependent variable; differences between shade treatments were
11 compared using a contrast T-test. The effects of experiments on predawn soil moisture
12 were evaluated with a Wilcoxon/ Kruskal-Wallis Test with a best Hsu's MCB
13 comparison. To estimate the effect of shading on mid-day twig water potentials we
14 conducted a GLM procedure with a maximum likelihood model where individual
15 variation was set as weight factor within the model, with a contrast analysis to test for
16 differences among treatment. Maximum photosynthetic rate across treatments was
17 analyzed using a one-way analysis of variance (ANOVA), repeated-measures design. A
18 multiple comparison procedure using a Tukey's HSD test was conducted to compare
19 differences among experimental treatments. To estimate the effect of light manipulations
20 on the light curve response of leaves to different photosynthetic active radiations (PAR),
21 we carried out a linear mixed-effects model fit by maximum likelihood with random
22 effects.

23 Fruit-set and total flowers and fruits analyses, non-structural carbohydrates and seeds
24 performance were carried out via GAMs or through GLM where individual variation
25 (maternal effect) was set as weight factor within the model.

1 To test the effect of light shading treatments on relocation of non-structural
2 carbohydrates, within fruits and seeds we used a two-way Anova test followed by a T-
3 test. Finally, to test the effect of light treatments on fresh weight of fruits and radicle
4 growth we conducted a one-way ANOVA test using a contrast T-test; seeds weight was
5 evaluated with a Kruskal-Wallis test. To reduced individual effects in both analyses,
6 individual variation was set as weight factor within each ANOVA model using JMP®
7 11.0.0 (SAS Institute, Inc.).

8

9 **RESULTS**

10

11 **Shading enclosure microclimate**

12 Daily patterns of microclimatic conditions were similar among light exclusion treatments,
13 except for light incidence ($F_{1, 2323} = 5.74, P < 0.0001$). As expected, 75% shading
14 showed greater amplitude of variation among treatments ($F_{2, 64} = 57.3, P < 0.0001$), and
15 shading differences among treatments depended on the hour of the day. Overall, 75%
16 shading showed no-significant effects on air temperature, air relative humidity, and soil
17 temperature (Appendix S2), although air relative humidity showed less variation among
18 control and shading treatments ($F_{2, 62} = 1.54, P = 0.21$). Thus, manipulations essentially
19 only affected light availability. In addition, predawn soil measurements showed no
20 significant effect of shading treatments ($X^2_{12} = 0.24, P = 0.88$); suggesting that
21 experimental manipulations did not affect soil water supply (cf. overall treatments mean
22 7.4 ± 0.53 bar). Overall, these results are consistent with the stable meteorological
23 conditions during the dry season in the STDF of Chamela (Bullock, 1986).

24

1 **Leaf traits responses**

2 After 2.6 yrs of experimental manipulation, phenological patterns of vegetative traits did
3 not differ among control and 30% shading ($F_{1, 464} = 2.82, P = 0.09$) and 75% shading
4 ($F_{1, 464} = 1.64, P = 0.2$) treatments, while trees under 30% shade showed no significant
5 difference with respect to 75% shaded trees ($F_{1, 464} = 0.71, P = 0.67$). Leaf production
6 within all light manipulations followed an invariable inverse pattern concerning rain
7 (dropping all leaves) and drought (leafing new leaves) seasonality (Fig. 2A).

8 Our results showed that shaded trees drop their leaves sooner compared to control trees:
9 75% shading showed the largest reduction, compared to 30% shading ($X^2 = -8.1, P <$
10 0.0001) and control ($X^2 = -13.23, P < 0.0001$). There was also a significant difference
11 between 30% shading and control treatment (30% survival less for control trees; $X^2 =$
12 $2.19, P = 0.028$). During late April, leaf survival decreased for all trees, with monthly
13 censuses showing a more negative trend under shading. A sharp decrease of survival
14 probability occurred for all treatments, with 75% shading showing the lowest survival,
15 during July, which overlapped the first rains and the emergence of canopy and lateral
16 shade from surrounding trees. Finally, from August to September all experimental trees
17 dropped all their leaves period (Fig. 2B).

18 Maximum rate of photosynthesis was affected by treatment ($F_{1, 34} = 7.84, P < 0.0001$).
19 Individuals under 75% shade exhibited a significant decrease compared with controls ($F_{1,}$
20 $2 = 5.82, P = 0.007$), while trees under 30% shade showed no significant difference
21 concerning controls or 75% shaded trees. Light curve responses to PAR were also
22 significantly different (Appendix S3). Linear mixed-effects model fit by maximum
23 likelihood differentiate three distinct photosynthetic curve responses to progressively
24 increasing radiations of $200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ($F_{4, 307} = 361.6, P < 0.0001$). Overall, leaves
25 from the control treatment showed more efficient adaptive response curves to high

1 radiation compared to 30% and 75% shading leaves: control vs. 30% shade-leaves ($t =$
2 4.69, $df = 27$, $P = 0.00006$); control vs. 75% shade-leaves ($t = 7.87$, $df = 27$, $P < 0.00001$);
3 30% shade-leaves vs. 75% shade-leaves ($t = 3.07$, $df = 27$, $P = 0.004$). This response
4 implies that leaves from control treatments showed typical sun-acclimation light curves
5 with a higher net CO_2 uptake (5-times and 3-times more A_{max}), than leaves from 75% and
6 30% shading treatments, however leaves from all treatments showed similar light
7 saturation points ($\approx 300 \text{ PAR}_i$).

8

9 **Whole-plant effects**

10 The 75% shaded treatment had significantly lower annual growth compared to 30% ($F_{1,}$
11 $2 = 9.00$, $P < 0.05$), followed by the control group with the highest growth ($F_{1, 9} = 19.74$,
12 $P < 0.01$). Mean growth in control and experimental groups did not vary across years ($F_{1,}$
13 $2 = 1.96$, $P = 0.15$), and there was no significant treatment*time interaction ($F_{1, 4} = 0.48$,
14 $P = 0.74$) (Appendix S4).

15 Mid-day stem water potential measurements showed significant differences ($F_{2, 32} =$
16 16.09 , $P < 0.0001$), with 75% shading trees suffering less water stressed than control (X^2
17 $= 17.22$, $P = 0.00003$) and 30% trees ($X^2 = 17.65$, $P = 0.00002$). Meanwhile, control and
18 30% shading trees did not differ ($X^2 = 0.13$, $P = 0.71$) (Appendix S5).

19 Both flowers and fruit production showed differences among treatments. Control and
20 30% shading had 2-times higher percentages of standing flowers and fruits on canopy (at
21 wet-dry transition and dry season, respectively) than the 75% treatment (Fig. 3). Although
22 flower canopy cover showed a decrease throughout the experimental period in all
23 treatments (Fig. 3A), this was more pronounced in the 75% shading treatment during the
24 three following dry seasons ($F_{3, 464} = 8.614$, $P = 0.003$). Control and 30% shading
25 showed a non-significant difference in the percentage of flowers canopy cover during the

1 same period of time ($F_{3, 864} = 3.77, P = 0.053$); no differences were found in standing
2 flowers on canopy cover between 30% and 75% shading treatments ($F_{3, 897} = 22.40, P$
3 $= 2.58$). Standing fruits percentage on canopy cover showed a significant effect of light
4 manipulation, where 75% shading had a negative impact up to three consecutive dry
5 seasons, compared with 30% shading and controls ($F_{3, 864} = 8.51, P = 0.003$). Standing
6 fruits on canopy cover decreased in trees under shading during both the dry and rainy
7 seasons, 30% and 75% shading produced less fruit compared with control trees that
8 presented a slightly increase during the second year. No significant differences were
9 detected between 30% and control treatments, but a constant decrease of fruit canopy
10 cover during the following dry seasons was observed regardless of shading ($F_{3, 864} =$
11 $2.12, P = 0.091$). For 75% shading trees this constant fruit canopy cover decrease was
12 observed as well, with significant differences persisting in all dry seasons (Fig. 3B).

13 Total fruit production showed a trend to decrease with shading (Appendix S6). Fruit
14 production per plant was different among treatments and years ($F_{1, 44} = 4.79, P = 0.003$),
15 there was a significant effect of light treatment on fruit production across years ($F_{1, 44}$
16 $= 6.49, P = 0.002$), with a significant interaction of year*treatment ($F_{1, 44} = 4.76, P =$
17 0.009). Trees from 75% shading and control showed fewer fruits after one year of shading
18 whereas 30% of shaded trees had similar fruit production in both years. Overall, flower
19 and fruit production during both years were significantly different from control and 30%
20 shaded trees. Finally, no effect on fruit-set was detected comparing both years ($F_{2, 82}$
21 $= 0.78, P = 0.37$) (Appendix S6).

22 Fruit fresh weight was affected by light manipulations ($F_{1, 104} = 7.62, P < 0.0001$). Fruits
23 under 75% shading showed 1-times and 1.2-times more biomass when compared with
24 30% shade and control trees, respectively ($t = 1.99; df = 59, P = 0.05$). We also found a
25 significant effect of shading on seed fresh weight ($X^2 = 165.63, df = 2, P < 0.0001$), with

1 seeds from 75% shading trees heavier compared with seeds from 30% and control
2 treatments ($|d| = 2.21$, $\alpha=0.05$). Thus plants from 75% shading not only have similar
3 number of seeds as control trees (Control = 252, 30% = 349; 75% = 247 total seeds) but
4 allocate ~30% more biomass than control and 30% shading trees.

5 Fruits from 30% shading had a higher glucose concentration than 75% and control
6 treatments (F3, 14 = 4.51, P = 0.015), but seeds from 75% treatment showed a higher
7 glucose content than control and 30% shading (F3, 14 = 4.32, P = 0.018). Fruits (F3, 14
8 = 6.0, P = 0.004) and seeds (F3, 14 = 5.48, P = 0.006) from 75% treatment had higher
9 starch content (see Table 1). Finally, seeds from 75% treatment had higher sucrose
10 content than seeds from control trees (F3, 14 = 7.72, P = 0.001). Seed radicle growth
11 showed significant differences among treatments (F3, 1349 = 605.97, P = 0.0001), and
12 after 12-days, seedlings from 30% and 75% treatments showed a higher radicle growth
13 than those of controls (F3, 1349 = 13, 39, P = 0.0001) (Fig. 4). Growth differences lasted
14 for at least 30 days (control vs 75%: F1, 2 = 17, 42, P = 0.0001 and 30% vs control, F1,
15 2 = 21, 47, P = 0.0001). Finally, 30% and 75% shading treatment radicle growth remained
16 similar to the end of the trial (F1, 2 = 0.31, P = 0.057).

17

18 **DISCUSSION**

19 Our results suggest that *B. nervosa* displays phenotypic plasticity on a selected group of
20 traits to adjust to environmental heterogeneity (leaf lifespan, reproductive traits and non-
21 structural carbohydrate within tissues), and genetic accommodation as inverted leaf
22 phenology exhibits no plasticity despite extreme shaded conditions, suggesting that
23 selection can actively eliminate phenological plasticity, inverted phenology to be fixed in
24 the population. While reproductive traits responded in multiple ways for whole-plant
25 development and flower and fruit production. Besides, the effects of environmental

1 variability are extended to the next generation, in terms of increase post-germination
2 nutrient allocation and radicle growth, suggesting an immediate adaptative adjustment of
3 the phenotype.

4

5 **Shading enclosures microclimate**

6 Our environmental measurements confirmed that the targeted factor, light, was
7 effectively manipulated with essentially no effects on other variables. We thus have
8 minimized possible confounding effects - the differences we uncovered are largely related
9 to shading effects.

10

11 **Leaf traits associated with light-harvesting**

12 Our 2.5 yr observations confirmed a permanent, genetically fixed inverted leaf phenology
13 and the immediate adaptative adjustment of leaf traits (genetic accommodation) of *B.*
14 *nervosa*, despite its heliophilous behavior and unexpected shading during the time of
15 totally canopy absence. This is in contrast to what has been shown in some deciduous
16 species (e.g., *Shorea siamensis*, Dipterocarpaceae) that flush new leaves during dry
17 periods in the Asian monsoon forest - those patterns are dependent on distance to subsoil
18 water reserves and tree size (Elliot et al., 2006).

19 Leaves under extreme shading showed an early expression of senescence but inverted leaf
20 phenology from all experimental trees remained unaltered during our study. Such a
21 decrease in leaf survival could have been caused by the emerging canopy at that time,
22 corresponding to the onset of rains (early July), resulting in an extreme unnatural shading
23 for *Bonellia* plants. Furthermore, species of the family Theophrastaceae are assumed to
24 exhibit an evergreen phenology and only *B. nervosa* has been reported to be deciduous
25 (Sthål, 2010). This unusual phenology is reported to be maintained in *B. nervosa*

1 populations in the dry forest of Santa Rosa National Park, Costa Rica (Chaves and Avalos,
2 2006, 2008). Our results on the none plasticity in leaf phenology after light manipulation
3 together with the study of Chaves and Avalos (2008), which reported that this species
4 maintained their leaves through the entire dry season despite day length variation, might
5 suggest that inverted leaf phenology it's expressed along the species Neotropical
6 distribution.

7 Various studies have demonstrated a direct effect of light on leaf lifespan and the
8 advancement of senescence (Biswal and Biswal, 1999; Vincent, 2006). Overall, when
9 light intensities are either higher or lower than an optimal level, senescence is accelerated.
10 For instance, light intensities may be regarded as environmental photosynthetic stressors
11 leading to different physiological and biochemical consequences to the plants (Niinemets
12 and Valladares, 2004). However, evidence on seedlings of evergreen species shown
13 longer leaf lifespans under low light due to a slower aging rhythm, as a result of a slower
14 photosynthetic metabolism (Vincent, 2006). Thus, our results on leaf survival decrease
15 show the opposite pattern and could be explained by light incidence variation caused by
16 enclosures affecting the induction of leaf flushing as previously reported by declining day
17 length in *B. nervosa* populations at Santa Rosa, Costa Rica (Chaves and Avalos, 2008),
18 however, inverted leaf phenology is conserved unaltered in both populations despite
19 natural light variation or light manipulations. Overall, leaves from control treatment
20 reached a higher maximum photosynthetic rate, which suggest a better use of water and
21 nutrients and a greater capacity for carbon fixation (Mooney and Gulmon, 1979).
22 However, the similar light saturation points of all treatment leaves, could suggest an
23 adaptive physiological constrain that remain genetically fixed in *B. nervosa* populations
24 despite environmental change.

1 Our results on soil and stem water potential suggest that plants under experimental
2 manipulations maintain stable water relationships. Predawn soil measurements suggest
3 that plants under 1 MPa are not limited by soil water supply, even during the day, which
4 allows them to maintain physiological activity. Further, experimental trees deployed the
5 typical water stress pattern despite experimental manipulations necessary to tolerate and
6 maintain physiological activity during drought periods, previously reported for species
7 with xerophytic characteristics where water deficit in leaves and xylem usually reaches
8 60-70% of their gross fresh weight (Bhaskar and Ackerly, 2006).

9 The results on maximum photosynthetic rate and leaf survival exhibited by leaves from
10 experimental trees suggested that leaf emergence, leaf longevity and maximum
11 photosynthetic capacity are adapted to high light intensity during the dry season, which
12 permits reproduction, transport of nutrients and water to upper leaves. This is consistent
13 with previous studies that have elucidated the principal factors determining tree
14 phenology in STDF (Reich, 1995; Singh and Kushwaha, 2005). For instance, Piao et al.
15 (2019) reviewed the key factors that control the variation and changes in plant phenology,
16 including temperature, photoperiod, nutrient and water availability, and the
17 interconnections among different phenological events. *B. nervosa* seems to be a
18 simultaneous-leaf emergence species with some self-shading that decreases
19 photosynthesis and growth; a likely explanation is that leaf longevity tends to become
20 shorter in insolated, compared to shaded environments where photoperiod and
21 temperature decreased (Kikuzawa and Ackerly, 1999; Kikuzawa, 2003).

22

23 **Whole-plant traits variation under light manipulations**

24 Our findings on whole-plant responses showed the adaptive nature of inverse phenology
25 in *B. nervosa*. As predicted, extreme light shading affected total flower and fruit

1 production during the dry season (Fig. 3). Our results can be explained by the allelic
2 individual variation in some photoreceptor genes as phytochrome C, which has an
3 important role in modulating seedling growth and flowering time in natural populations
4 of *Arabidopsis thaliana* that are major agents of natural variation in plant flowering and
5 growth response (Balasubramanian et al 2006). Additionally, Bullock and Solis-
6 Magallanes (1990) recorded the natural variation in phenological activity for the SDF
7 species of Chamela and found distinctive reproductive phenological patterns followed by
8 predominant environmental cues such as precipitation seasonality, daylength and canopy
9 dynamics. However, it is still largely unclear how environmental factors such as
10 temperature and photoperiod interact in determining plant phenological events (Piao et
11 al., 2019).

12 Our results on structural carbohydrates content in seeds from experimental light
13 manipulations supported the seasonal variation in carbohydrate storage across different
14 plant tissues (Newell et al., 2002). Contrary to our expectations, content of structural
15 carbohydrates differ under exposure to intense shading (see Table 1). Lal et al. (2001)
16 have found that 51% of N and 53% of P were relocated from the senescing leaves within
17 the hot-dry period when soil water is limited. This suggested an adaptive strategy of the
18 species growing on the SDF, with nutrient-poor soils, as is the case of Chamela, where P
19 is the most limiting resource (Cotler et al., 2002). For instance, Kim et al. (2015)
20 suggested that current-year photosynthetic production is important for *Erythronium*
21 *japonicum* subsequent reproduction. Their results show that plants under early shading
22 place priority on current-year photosynthate allocation to reproduction, while the
23 subsequent low photosynthetic production reduced seed-set in quantity and quality, which
24 could result in a decrease in the germination rate. Overall, our results on foliar non-
25 structural carbohydrates indicated a substantial amount of nutrient resorption before

1 senescence and a tight nutrient budgeting. Newell et al. (2002) found that some species
2 have maximum concentrations in total non-structural carbohydrates (NSC) when the
3 canopies were fully expanded. However, species as *Cecropia*, *Urera* and *Luehea*
4 continued to increase their NSC concentrations at the beginning of the dry season when
5 trees start dropping their senescent leaves despite their full drought-deciduous phenology.
6 For instance, Lloret et al. (2018) measured total NSC and soluble sugars (SS) in roots and
7 stems during drought and after a rain pulse in plants exhibiting leaf loss and in
8 undefoliated ones. Their results suggested that plant carbon economy and drought
9 responses of co-occurring woody species were species-specific where C stocks,
10 diminished in plants affected by prolonged drought and did not increase after a pulse of
11 seasonal rain. Our results suggest the existence of compensatory plasticity whereby fruits
12 and seeds from 30% and 75% shading have more non-structural carbohydrates/g tissue at
13 their disposal for growth and development of emerging plants. These strategies might
14 ensure tree survival and the maintenance of reproduction within the populations. For
15 instance, the mechanism by which plants reabsorb non-structural carbohydrates from
16 leaves and differentially relocate it to reproductive structures, such as fruits and seed
17 embryos, could guarantee the survival of both the new seedlings and adult trees. Donohue
18 et al. (2010) provided evidence that variation in germination can be associated with
19 previous plasticity to environmental variability, with consequences in local adaptation to
20 novel environmental conditions by new sapling phenotypes. Differences in germination
21 time observed in our study could suggest an automatic adaptative nutrient relocation
22 plasticity to environmental change within reproductive traits with transgenerational
23 consequences (West-Eberhard, 2003), as seed germination will influence not only
24 seedling survival but also the phenotypic expression of post-germination traits suitable
25 for selection action on those traits (Franklin and Ågren, 2002; Donohue et al. 2010;

1 Ruban, 2015). In sum, seeds from both shading treatments increased their radicles 2.2-
2 times more rapidly than controls, suggesting more concentration and use-efficiency of
3 resources by the embryos.

4 In conclusion, our results suggest that inverted leaf phenology fitness-related traits vary
5 in plastic responses to light heterogeneity, specifically reproductive traits and
6 carbohydrate storage showed the most variability to light manipulation, suggesting an
7 automatic consequence of multidimensional adaptative plasticity to environmental
8 change (West-Eberhard, 2003), meanwhile inverted leaf phenology within measured
9 phenological patterns showed no alteration on that trait, suggesting that phenotypes have
10 lost their environmental sensitivity (Levis and Pfennig, 2016). As we have shown, *B.*
11 *nervosa* exhibits a unique inverted leaf phenology within the STDF of Chamela with a
12 completely fixed phenology (genetic accommodation for that trait) despite unexpected
13 shading during the dry season. Beyond the ecophysiological approach undertaken in this
14 study, further phylogenetic analysis is needed to advance our understanding of the
15 evolution of such a phenotypic variant as inverted plant phenology.

16

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8

9 **AUTHOR CONTRIBUTIONS**

10 O.S., M.Q. and R.D. designed the study; O.S. carried out the experiments; O.S. and
11 C.D.S. analyzed the data; O.S., R.D., M.Q. and C.D.S. wrote and revised the manuscript.

12

13 **SUPPORTING INFORMATION**

14 Additional Supporting Information may be found online in the supporting information
15 tab for this article.

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1 **TABLE 1.** Two-way ANOVA summary for the effect of light shading treatments on three
 2 non-structural carbohydrate concentrations of *B. nervosa* in stems, fruits and seeds.

Non-Structural Carbohydrate	Tree Component	Source	SS	df	Mean	F	P
Glucose	Branch	Treatment	2	3143.69	1571.8	2.73	0.076
		Error	40	22960.3	574.01		
		Total	42	26104			
	Fruit	Treatment	2	27.06	13.53	4.51	0.015
		Error	49	146.72	2.99		
		Total	51	173.79			
	Seed	Treatment	2	13.54	6.77	4.32	0.018
		Error	52	81.41	1.56		
		Total	54	94.95			
Starch	Branch	Treatment	2	1803.55	901.77	12.24	< 0.0001
		Error	44	3241.32	73.66		
		Total	46	5044.88			
	Fruit	Treatment	2	0.38	0.19	6	0.0045
		Error	51	1.65	0.03		
		Total	53	2.04			
	Seed	Treatment	2	0.02	0.01	5.48	0.0069
		Error	52	0.12	0.002		
		Total	54	0.15			
Sucrose	Branch	Treatment	2	10.15	5.07	1.46	0.24
		Error	53	183.88	3.46		
		Total	55	194.03			
	Fruit	Treatment	2	10.69	5.34	1.08	0.34
		Error	42	207.41	4.93		
		Total	44	218.10			
	Seed	Treatment	2	76.25	38.12	8.33	< 0.001
		Error	39	178.50	4.57		
		Total	41	254.76			

3

1 **APPENDICES**

2

3 **Appendix S1. Experimental Design**

4

5 ***Shading enclosures microclimate*** - Microclimatic data recording started on 24 August
6 2010 and ended 31d later on 25 September 2010 (Fig. 2). Predawn soil measurements
7 within enclosures were conducted in 4 selected randomly trees from each treatment
8 during the middle of dry season (early April). To measure soil-water potential within
9 enclosures 5 records from 5-10 cm depth were done following a left to right circular
10 direction taking the trunk as center using an Onset Soil Moisture Sensor with a 5 cm Probe
11 Soil Moisture Smart Sensors (Onset HOBO™ S-SMC-M005, Bourne, Massachusetts,
12 USA).

13

14 ***Shading Enclosures*** - Enclosures consisted of a double hoop made of wire rod (1/4")
15 attached with electro galvanized wire (4.06 mm) for each individual (2.5 - 4.5 m canopy
16 diameter). Then a black greenhouses shade cloth (polyethylene, HDPE, material) was
17 attached with galvanized wire to the metallic ring perimeter. A second black shade cloth
18 (3-6 m length) was attached from the wider part of the net to the metallic ring. In some
19 cases, a second section of shade cloth was attached with a black high-strength polyester
20 embroidery thread to achieve full canopy enclosure. To rise enclosures, four double waxy
21 strings were attached to equidistant points along the perimeter of the metallic ring. The
22 two strings located behind the lengthwise edges were lifted over the canopy (1-2 m) using
23 neighboring tree forks as a pulley. Then, two opposite strings were lifted forward to
24 surround the complete tree. Finally, the edges of the shade cloth were attached with
25 galvanized wire to assure complete enclosure.

1 **Fournier index** - This index is based on five cover categories (1: 0 %; 2: 1-25 %; 3: 26-
2 50 %; 4: 51-75 % and 5: 75-100 %) and calculated as:

3
$$P(t) = \sum_{i=0}^n \left(\frac{x_i}{n(C_m)} \right) 100$$

4 where x_i = observed category i at time t , n = number of observations at time t , and C_m =
5 maximum category assume at time t . This index can be used, with the same principle, for
6 leaves, flowers or fruit occurrence on tree canopy cover. Mean leaf/flower/fruit
7 phenological status P for each treatment at a given time (t) was calculated as the total sum
8 of the observed categories (x_i) divided by the sum of expected categories from the
9 observed individuals, which assumes that all trees exhibit a full coverage (Category 4) of
10 the phenological characteristic at that moment.

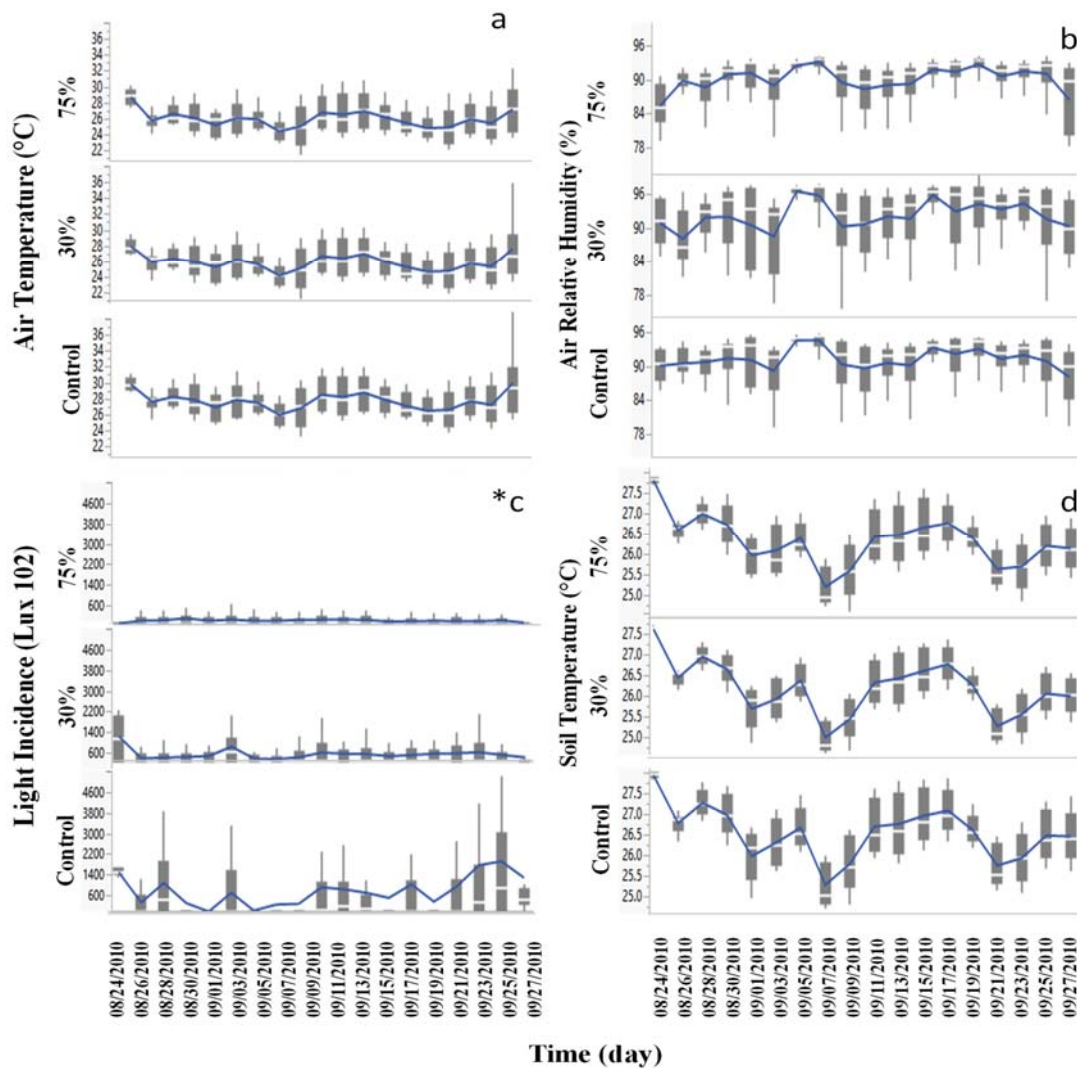
11

12 **Light response curves**- Recalculated leaf area was obtained by marking the outside leaf
13 portion clamped in the chamber and collecting the measured leaf, long and length of the
14 inside portion was digitized to obtain the total area. To calculate the correct area of the
15 leaves, digital photographs were analyzed using the image analyzer software, SigmaScan
16 Pro 5, (Image Analysis Software, SPSS Inc. Chicago, Illinois, USA).

17

18 **Carbohydrates Concentration**- Branch tissue, fruit pulp and seed powder were extracted
19 with heated 80% ethanol in a Polytron homogenizer using 700 ml per 0.1g of tissue,
20 centrifugated at 14000 g for 15 min, and the supernatant was saved. The pellet was
21 washed with boiled water and centrifugated as above. Supernatants were combined and
22 ethanol was evaporated at 60°C during 24 hrs, the final pellet was resuspended in 500 µl
23 H₂O and then used to the analysis of soluble sugars.

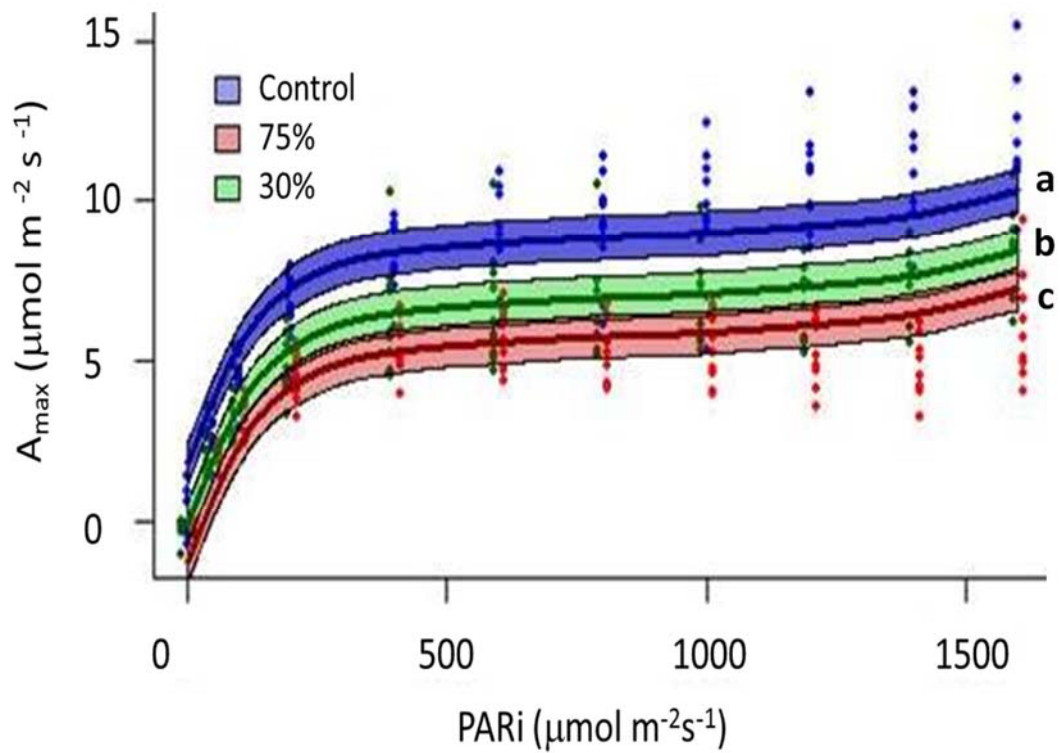
1 **Appendix S2. Figure 1.** Microclimatic variability observed under the experimental light
 2 manipulations (August-September). Hourly records of a) air temperature, b) air relative
 3 humidity, c) light incidence and d) soil temperature. Solid-line indicates the mean for
 4 each treatment during the observation time. Box-plots correspond to the microclimatic
 5 variation observed for each day (+/- SD). * indicates significant differences among
 6 treatments for that microclimate variable.



7

1 **Appendix S3. Figure 3.** Effect of shading on mean light curve respond (A_{\max}) to different
2 photosynthetic active radiations (PARi). A set of 3-5 leaves per individual was selected
3 from 5 to 7 trees for each treatment. Solid lines and shaded regions (SD +/-) are indicated
4 in different colors following a linear adjustment through a Proportional Hazards
5 Regression Model for each treatment.

6



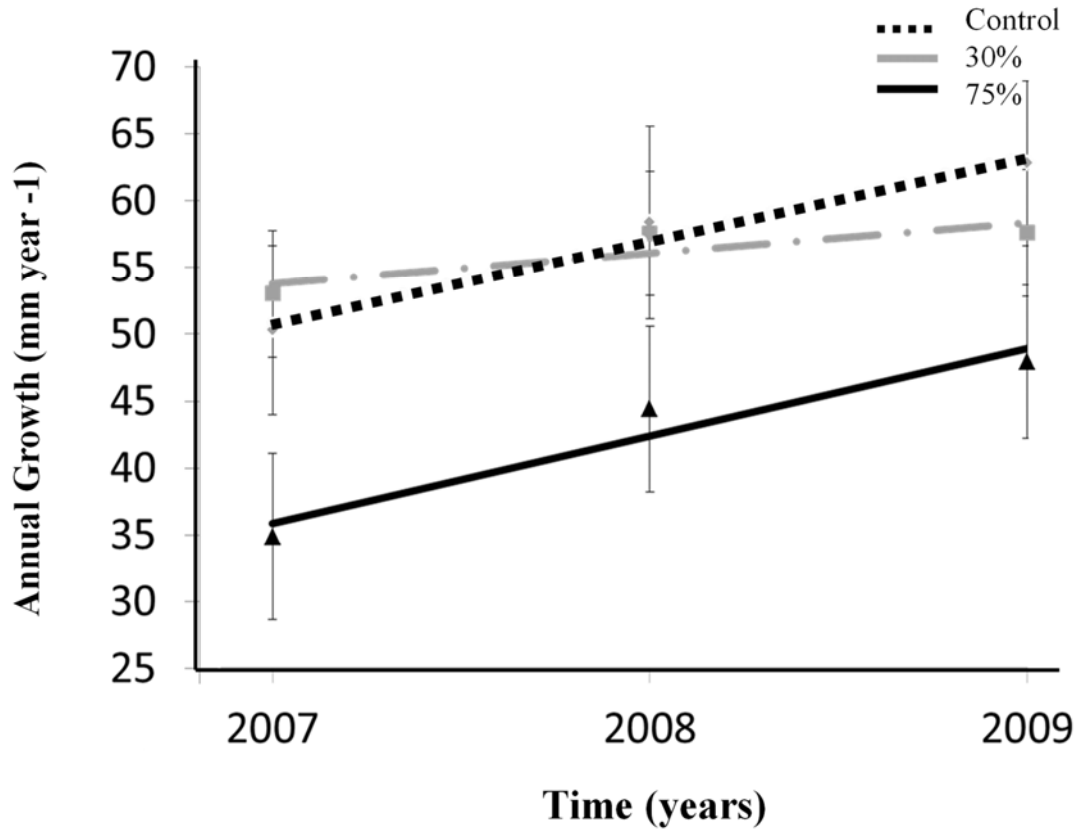
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8

1 **Appendix S4. Figure 4.** Effect of light shading on the mean annual growth of *B. nervosa*
2 during the dry season (+/- SD).

3

4

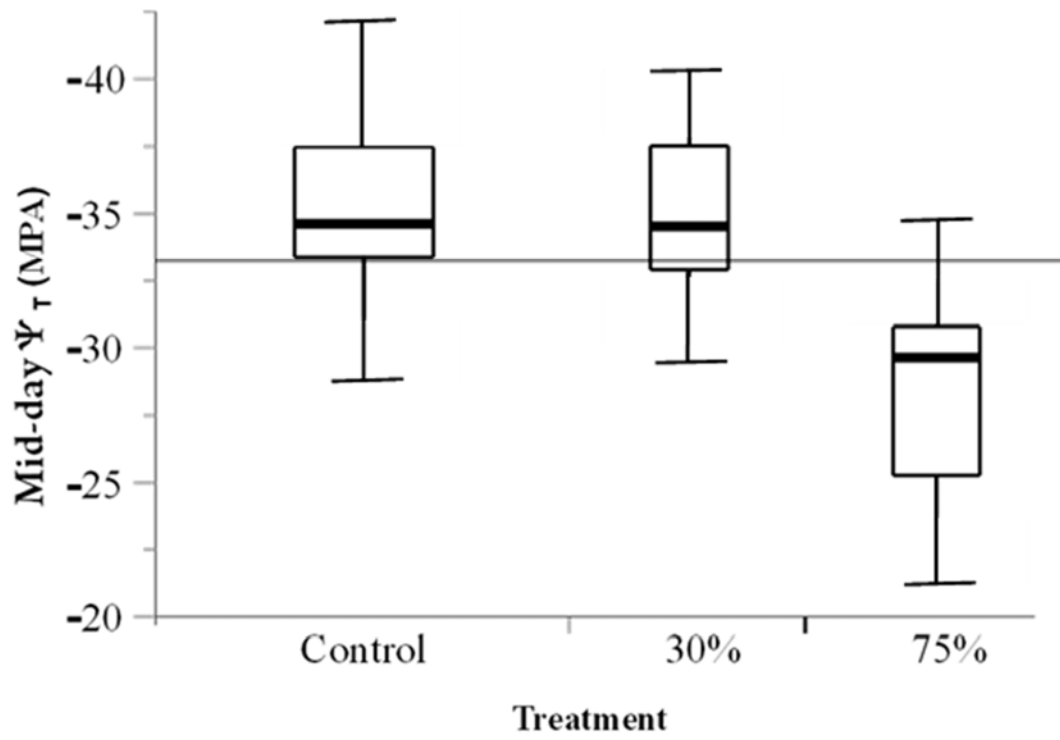


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6

1 **Appendix S5. Figure 5.** Mean mid-day twig water potential of *B. nervosa* trees within
2 shading treatments (+/- SD).

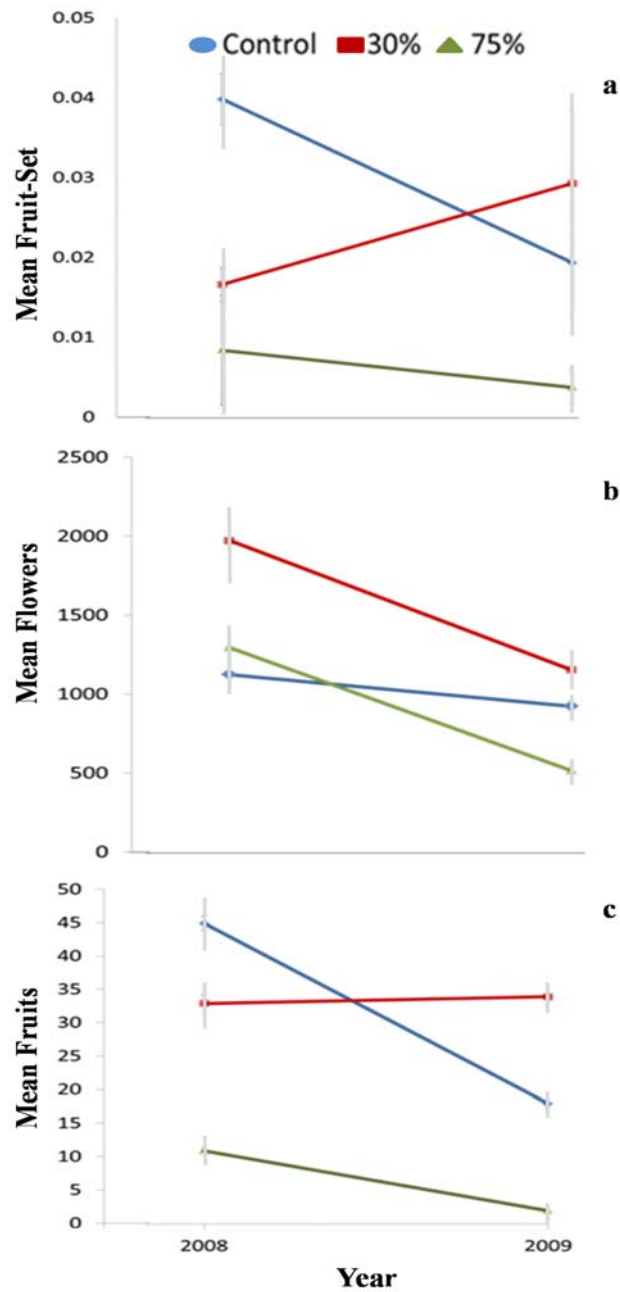
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5

1 **Appendix S6. Figure 6.** Reproductive plastic responses in *B. nervosa* reproductive trees
2 under experimental light manipulations. Lines represent the mean reaction norm of
3 reproductive traits measured: a) fruit-set, b) flowers and c) leaves, respectively. Each
4 point represents the mean value of the trait for that year (\pm SD).



5

1 **FIGURE LEGENDS**

2

3 **FIGURE 1.** Experimental light manipulations under natural conditions using
4 reproductive trees of *B. nervosa* in Chamela's seasonally dry forest. Each treatment
5 consisted of artificial enclosures: A) control, B) 30% shading, and C) 75% shading.

6

7 **FIGURE 2.** Vegetative phenological variation recorded in 45 experimental trees during
8 twenty-eight months. Extent of seasons: wet season (July to October) and dry season
9 (February to the end of June). A) Leaf canopy cover recorded following Fournier's Index
10 (see details in text). Squares, control; diamonds, 30% shading; triangles, 75%. Greenline
11 indicates leaf phenology of deciduous dry species in Chamela's seasonally dry forest. B)
12 Effect of shading on mean leaf survival during the dry season. Leaf survival represents
13 the total standing leaves minus the total standing leaves of the previous census for each
14 treatment. Green: control, purple: 30% and magenta: 75% shading. Box size indicates
15 +/-SD.

16

17 **FIGURE 3.** Reproductive phenological variation under experimental shading during
18 twenty-eight months. Phenological canopy cover following Fournier's Index: A) flowers
19 and B) fruits (+/- SD). Squares, control; diamonds 30% shading; triangles, 75% shading.

20

21 **FIGURE 4.** Effect of light variation on radicle growth in newly emerged seedlings of *B.*
22 *nervosa* trees during 36 days. Different letters indicate statistically significant differences
23 ($P \leq 0.05$).

24

1 **FIGURE 1.**

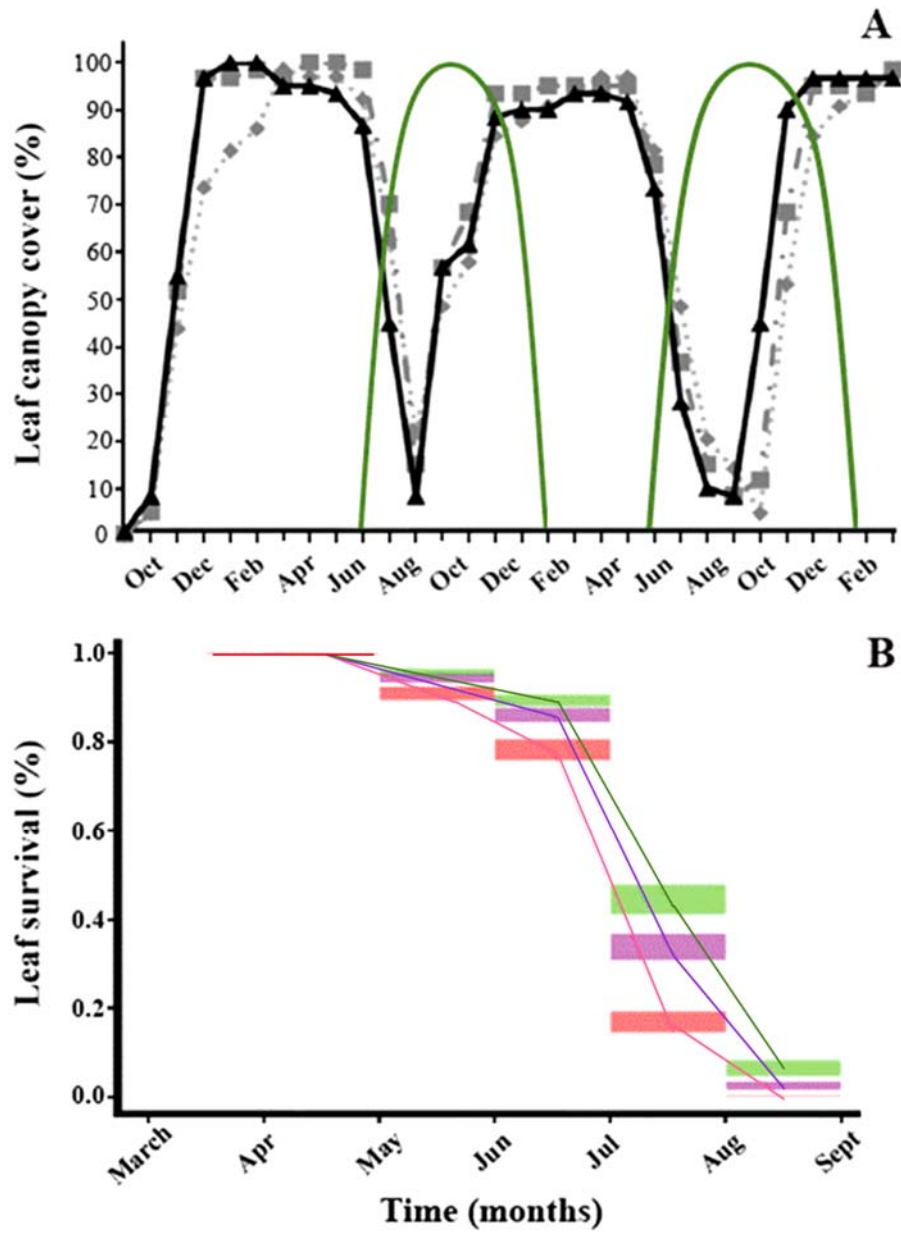


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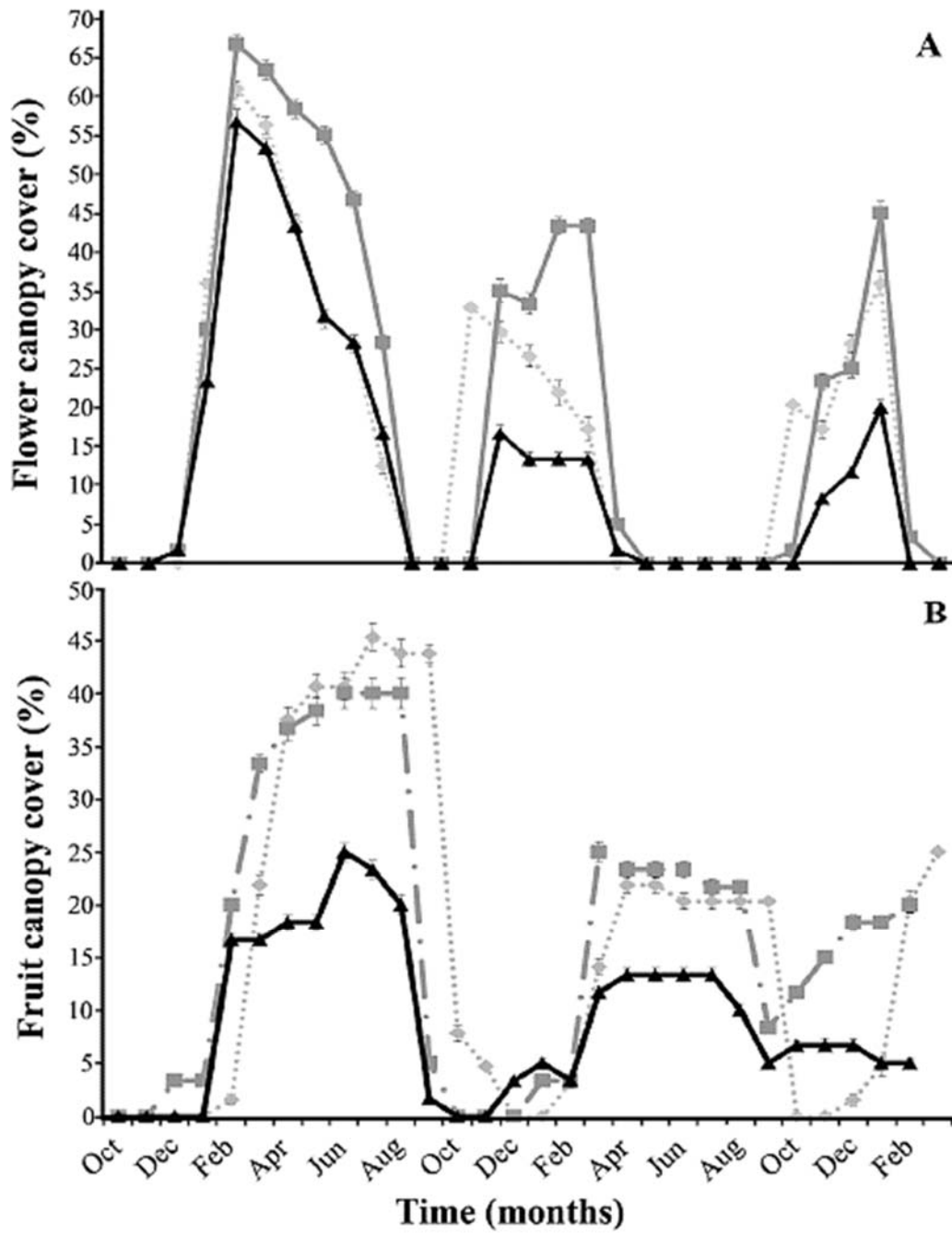
1 FIGURE 2.



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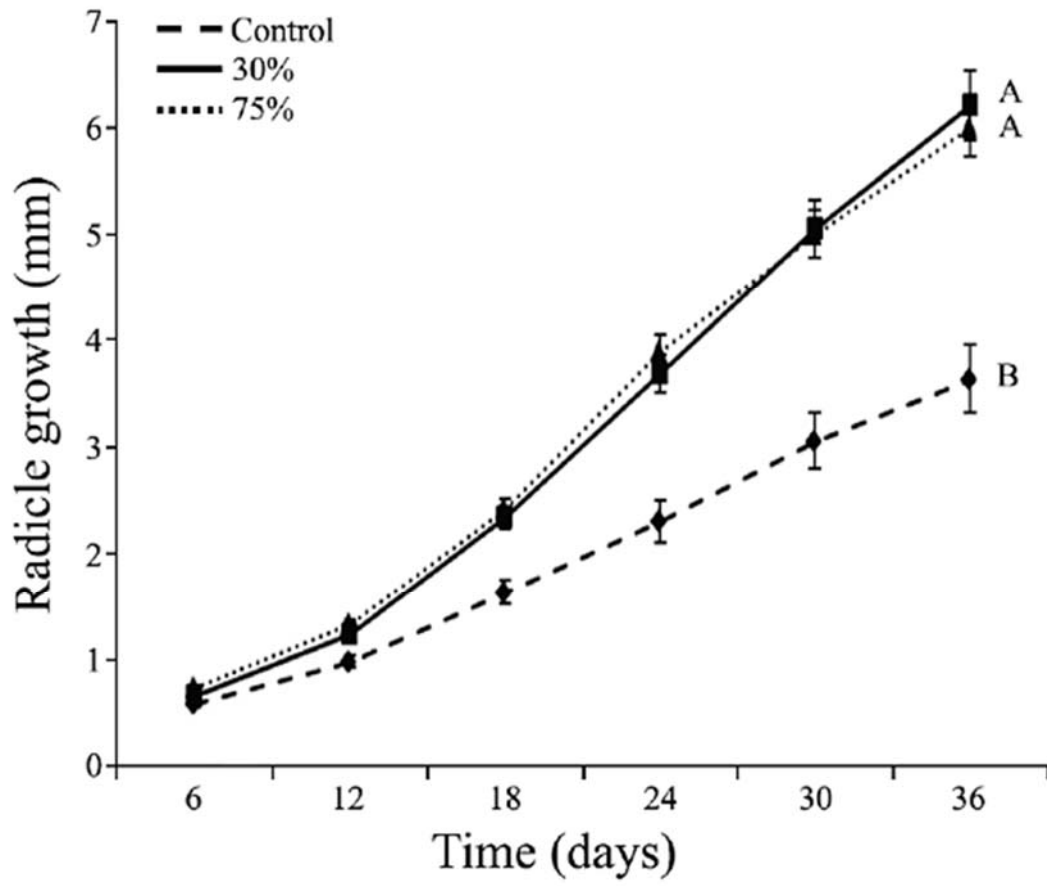
1 FIGURE 3.



2

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1 FIGURE 4.



2

Discusión general

Las manipulaciones experimentales de los factores ambientales agua y luz durante la época seca en el árbol con fenología foliar invertida, *Bonellia nervosa*, mostraron diferentes patrones de respuesta dependiendo el factor ambiental que se manipuló y las variables vegetativas y reproductivas que se midieron.

El experimento de irrigación llevado a cabo durante la época seca mostró que la fenología foliar en *B. nervosa* permanece inalterada, a pesar de que se esperaba que la disponibilidad de agua durante la época seca afectara la producción de hojas y la abscisión de hojas, especialmente en una especie cuya actividad de crecimiento vegetativo ocurre durante la estación de mayor estrés hídrico (Borchert, 1994a, b; Bullock & Solís-Magallanes, 1990). Los resultados de la fenología foliar sugieren que la abscisión foliar en *B. nervosa* podría estar bajo control genético en lugar de factores hídricos. Este resultado es consistente con otros estudios que manipularon la disponibilidad de agua en especies caducifolias, donde se encontró un menor efecto de las irrigaciones en la longevidad foliar, almacenamiento de agua en tallos (Myers *et al.* 1998), o el nulo efecto en la tasa de crecimiento subsecuente a la irrigación experimental durante la época seca en un bosque tropical estacional de México (Hayden *et al.* 2010). Por el contrario, características relacionadas con el desempeño vegetativo (*e.g.* composición de carbohidratos no-estructurales en semillas y frutos) mostraron diferencias significativas entre los tratamientos de irrigación. Los resultados en la concentración de carbohidratos no-estructurales sugieren que podría existir una reorganización de estos compuestos en frutos y semillas posterior a las irrigaciones experimentales, sin embargo, no se encontró evidencia de algún efecto en la producción de flores y frutos totales. Igualmente, no se encontró ningún efecto significativo en el

estrés hídrico al medio día después de los tratamientos de irrigación. Estos resultados pueden ser explicados a través de cuatro distintas líneas de razonamiento: 1) la raíz principal está almacenando agua y nutrientes o 2) tiene acceso directo al manto freático, o 3) la disponibilidad de agua durante la época seca produce respuestas plásticas de reorganización de nutrientes, principalmente, en caracteres reproductivos.

Al contrario de la mayoría de los árboles deciduos en el bosque seco, *B. nervosa* exhibe un sistema radicular compuesto de una raíz principal profunda y gruesa que podría almacenar agua o alcanzar el manto freático (O. Sánchez obs. pers.). *Faidherbia albida* (Acacia), una de las pocas especies con fenología invertida que se ha estudiado, adquiere el agua a través de una raíz de más de 7 metros de profundidad que probablemente alcanza el manto freático (Roupsar *et al.* 1999). Este mecanismo que despliega *F. albida* podría explicar el estado hídrico de *B. nervosa* durante la época seca y la falta de efecto en el potencial hídrico en ramas durante el medio día posterior a los tratamientos de irrigación. Además, la fenología de flores y frutos en los BTS está restringida por el uso de agua residual, que permite la rehidratación de tallos y la subsecuente floración y fructificación (Borchert, 1994b). En el caso de *B. nervosa* podría estar ocurriendo este mismo patrón, donde la raíz podría actuar como reservorio de agua y reservas de almidón durante la época seca cuando ocurre la reproducción y crecimiento vegetativo, sugiriendo que la reactivación fenológica no responde inmediatamente a la presencia de las primeras lluvias en esta especie en comparación con especies caducifolias típicas (Bullock & Solís-Magallanes, 1990). Janzen & Wilson (1974) demostraron que *B. nervosa* (anteriormente *Jacquinia pungens*) pierde cerca de la mitad de sus carbohidratos almacenados en raíces, ramas y tallos cuando permanece defoliada durante la época de lluvias. Los resultados en la concentración de carbohidratos no-estructurales entre los tratamientos de irrigación sugieren la existencia

de un patrón de reorganización de carbohidratos en frutos y semillas, observados anteriormente en especies caducifolias en BTS (Lal *et al.*, 2001).

Por otra parte, el experimento de manipulación de la intensidad lumínica mostró que *B. nervosa*, responde con marcada plasticidad en un grupo de caracteres fisiológicos y reproductivos que le permite enfrentar la variabilidad lumínica. Además, el efecto de esta plasticidad se extiende a la siguiente generación, específicamente, en características pre y post-germinativas como son la concentración de azúcares no-estructurales y el crecimiento radicular en semillas, sugiriendo un ajuste inmediato de los fenotipos a la variabilidad ambiental (West-Eberhard, 2005). Sin embargo, la falta de plasticidad en el patrón fenológico foliar invertido en los tratamientos de sombra, sugiere que esta especie podría haber experimentado un proceso de acomodación genética en la producción foliar para lidiar con la heterogeneidad ambiental.

Los resultados fenológicos sugieren que la fenología foliar invertida en *B. nervosa* está posiblemente fija genéticamente (acomodación genética) en la población, al no presentar ningún efecto negativo a pesar de su comportamiento heliófilo y de la repentina modificación lumínica durante la época seca. Esto sugiere, que al no existir evidencia de plasticidad en la fenología foliar de los individuos experimentales, la selección pudo eliminar la sensibilidad ambiental ancestral que presentaban los individuos caducifolios novedosos con respecto al patrón normal, favoreciendo aquellos individuos con un patrón foliar invertido y, por lo tanto, fijando genéticamente este carácter adaptativo. Este resultado es contrastante con otros estudios en especies deciduas (*e.g.*, *Shorea siamesis*) que producen nuevas hojas durante periodos secos donde no existe agua disponible. Sin embargo, los patrones foliares observados en esta especie dependen de la distancia relativa a las reservas de agua en el manto freático en los bosques monzónicos de Asia (Elliot *et al.*, 2006). En el caso de *B. nervosa*, Sánchez

et al. (2019) mostraron que esta especie no altera su fenología foliar a pesar de la disponibilidad de agua durante la época seca. Por el contrario, los resultados de la nula plasticidad en la fenología foliar de *B. nervosa*, después de las manipulaciones lumínicas, sugieren que el patrón fenológico foliar invertido se conserva, y probablemente, se mantiene a lo largo de su distribución geográfica considerando los resultados de obtenidos por Chaves & Avalos (2008), quienes reportaron que esta especie mantiene sus hojas durante toda la época seca a pesar de la variación lumínica diaria. Varios estudios han demostrado un efecto directo en la supervivencia de las hojas y en el avance de la senescencia (Biswal & Biswal, 1999). En general, cuando la intensidad lumínica varía (alta o baja) la senescencia de hojas se acelera, por lo tanto, los resultados observados en este estudio en la disminución de la supervivencia de hojas en los diferentes tratamientos, pueden ser explicados por la inducción natural en la senescencia foliar al alterar la duración del día por efecto de los tratamientos de sombra aplicados (Vincent, 2006).

Los resultados de las tasas fotosintéticas máximas sugieren que *B. nervosa* alcanzó adaptaciones eficaces para sobrevivir durante la época seca cuando no existe un dosel y la radiación solar es alta. Esto es consistente con estudios previos que han estudiado los principales factores y adaptaciones que determinan la fenología de árboles en los BTS (Reich, 1995; Singh & Kushwaha, 2005). En el caso de *B. nervosa*, parece que esta especie produce simultáneamente sus hojas produciendo auto-sombra lo que podría inhibir su capacidad fotosintética; además, algunos estudios han mostrado que la longevidad de las hojas tiende a ser corta en lugares insolados comparados con ambientes sombreados (Kikuzawa & Ackerly, 1999; Kikuzawa, 2003).

Los resultados del desempeño reproductivos muestran diferentes respuestas plásticas en *B. nervosa*, al experimentar diferentes intensidades lumínicas. Por ejemplo, los

tratamientos control y 75% de sombra mostraron una menor producción de frutos; sin embargo, el tratamiento de 30% mostró una mayor producción. La relación de frutos por flor (fruit-set) disminuyó en los tratamientos control y 75% sombra. Por el contrario, el peso de frutos y semillas y contenido de carbohidratos no-estructurales aumentó, ocasionando un mayor crecimiento radicular. Estos resultados son consistentes con la variación observada en la producción de flores y el crecimiento de plántulas y su relación con la variación de algunos genes fotorreceptores que codifican al fitocromo C, que tiene un importante papel en el crecimiento y floración natural en poblaciones de *Arabidopsis thaliana* (Balasubramanian *et al.*, 2006). La variación individual de estos alelos fotorreceptores podría explicar la variación en la fenología de flores y frutos observada en este estudio, así como la reportada por Bullock & Solís-Magallanes (1990) en la comunidad de plantas en el BTS de Chamela, donde registraron la actividad fenológica y su variación encontrando varios patrones reproductivos que respondían a señales ambientales como las precipitaciones, duración del día y la dinámica del dosel.

La variación de la precipitación anual reportada para la estación de Biología Chamela podría explicar la variación encontrada en el crecimiento y respuesta reproductiva de *B. nervosa*. Janzen & Wilson (1974) sugieren que esta especie almacena nutrientes y agua asimilados durante la estación de lluvias previo a la formación de nuevas hojas, flores y frutos en la subsiguiente época seca. La estación seca en el BTS de Chamela inicia entre noviembre y diciembre con algunas precipitaciones esporádicas (cabañuelas) al inicio del siguiente año (Bullock & Solís-Magallanes, 1990), por lo tanto, los datos de crecimiento y la respuesta reproductiva podrían depender de la precipitación media anual (PMA) del año anterior a las mediciones y observaciones realizadas. Durante el periodo de estudio los años con mayor PMA fueron el año 2006 (1059.42 mm) y 2007

(1032.12 mm). Para estos años el crecimiento no mostró un patrón evidente sobre una posible relación entre la variación interanual del crecimiento y la precipitación anual. Por ejemplo, el tratamiento 30% de sombra mostró un menor crecimiento en el año 2009 (650 mm), a pesar que el año anterior la PMA fue mayor (2008, 955.5 mm); por el contrario, el tratamiento control y 75% de sombra mostraron un crecimiento constante a pesar de la disminución de la PMA durante los dos últimos años de estudio (2008, 955.5 mm; 2009, 650 mm). Asimismo, la respuesta reproductiva muestra diferentes patrones con respecto a la PMA. Por ejemplo, el tratamiento control y 75% de sombra mostraron una tendencia de menor producción de flores y frutos que corresponde a la disminución de la PMA durante los años de estudio, sin embargo, el tratamiento 30% de sombra mostró una mayor producción de frutos y un aumento del fruit-set a pesar de la disminución de la PMA durante esos mismos años (2007, 1032,12 mm; 2008, 955.5 mm). Esto podría sugerir que las diferencias observadas en el crecimiento y respuesta reproductiva en *B. nervosa* son consecuencia de la manipulación de la intensidad lumínica y no de la variación de la precipitación del BTS de Chamela.

Los resultados del contenido de carbohidratos no-estructurales en semillas respaldan la variación estacional de almacenamiento de carbohidratos en diferentes tejidos (Newell *et. al.*, 2002). Por ejemplo, Kim *et al.* (2015) sugieren que la producción de carbohidratos durante una estación es importante en la subsecuente reproducción de *Erythonium japonicum*. Sus resultados muestran que las plantas que experimentan un sombreado temprano de forma natural tienden a priorizar la redistribución de fotosintetatos producidos en esa misma estación para la reproducción, mientras que, el subsecuente decremento en la tasa fotosintética provoca la reducción del total y calidad de semillas, lo que puede resultar en la reducción de las tasas de germinación. Lal *et al.*, (2001) encontraron diferentes patrones en la reabsorción de nutrientes en los BTS,

comparando diferentes especies entre los periodos calientes y secos cuando la disponibilidad de agua es limitada. Sus resultados indican que el 51% del N y el 53% del P son redistribuidos desde las hojas senescentes. Además, encontraron una gran variación en la estrategia en que las distintas especies redistribuyen eficientemente los nutrientes. Esto podría sugerir la existencia de una estrategia adaptativa de las especies presentes en los BTS que se caracterizan por tener suelos pobres en nutrientes, como es el caso del BTS de Chamela (Cotler *et al.*, 2002). Por ejemplo, la proporción de C asignado a raíces es más alta en sitios con estaciones secas prolongadas, lo que sugiere estrategias específicas de la asignación de recursos en las plantas ante un incremento del estrés hídrico, por lo cual asignan más energía a la biomasa subterránea comparado con sitios con precipitaciones medias anuales altas donde la concentración de C en el suelo disminuye (Campo *et al.*, 2016).

Los resultados de la concentración de carbohidratos no-estructurales sugieren la existencia de plasticidad compensatoria en la que frutos y semillas de los tratamientos de 30% y 75% de sombra, presentan mayor concentración de carbohidratos no-estructurales/g de tejido, que potencialmente, podría ser asignado para el crecimiento y desarrollo de las plántulas. Este mecanismo observado en el que las plantas redistribuyen carbohidratos no-estructurales desde las hojas a diferentes estructuras reproductivas, tales como frutos y embriones, puede garantizar la supervivencia de plántulas y árboles adultos, aumentando el número de semillas, la probabilidad de germinación y el número de plántulas. Donohue *et al.*, (2010) señala que la variación en la germinación puede estar asociada con la plasticidad ambiental previa, y podría manifestarse en la adaptación local a nuevas condiciones ambientales de los nuevos fenotipos de plántulas. Los diferentes tiempos en germinación que se observaron en esta tesis podrían sugerir una posible redistribución de nutrientes en los caracteres

reproductivos con consecuencias transgeneracionales, donde la germinación de semillas puede influenciar no solo la supervivencia de las plántulas, pero también la expresión fenotípica de características post-germinativas (Franklin & Ågren, 2002; Donohue *et al.*, 2010; Ruban, 2015). En síntesis, las semillas provenientes de ambos tratamientos de sombra incrementaron sus radículas 2.2 veces más rápido que las semillas de los controles, esto podría sugerir una mayor concentración de nutrientes y probablemente un uso eficiente de los recursos por parte del embrión, sin embargo, esto necesita ser comprobado por estudios específicos en la utilización de recursos por embriones provenientes de ambientes lumínicos contrastantes.

Aunque esta tesis no realizó un estudio comparativo entre linajes u otras poblaciones, datos publicados de poblaciones de *B. nervosa* en Santa Rosa, Costa Rica, muestran un alta sincronidad en la producción de hojas y senescencia foliar a pesar la variabilidad lumínica durante la época seca, sugiriendo que esta población podría haber perdido igualmente la plasticidad fenotípica en esos caracteres vegetativos (Chaves & Avalos, 2006; Sánchez *et. al.*, 2019). Los resultados independientes de las manipulaciones experimentales muestran que la fenología foliar invertida en *B. nervosa* permanece inalterada, esto sugiere que la fenología foliar no presenta plasticidad a pesar de la manipulación de los principales factores ambientales que determinan la fenología vegetativa en BTS (disponibilidad de agua e intensidad lumínica). Lo que podría sugerir que la fenología foliar invertida en *B. nervosa* exhibe acomodación genética, en la cual un fenotipo muestra múltiples adaptaciones inmediatas para mantener su funcionalidad y, por lo tanto, no muestra actualmente plasticidad fenotípica a ese factor ambiental ancestral dentro de la población (Levis & Pfennig, 2016).

Conclusiones

Este trabajo de investigación confirma que los bosques tropicales secos albergan una gran diversidad de adaptaciones para lidiar con la variabilidad ambiental. Principalmente, la senescencia de hojas y los patrones fenológicos de las plantas son afectados por la variación lumínica y disponibilidad de agua, dando como resultado diversas estrategias para lidiar con esta variabilidad. Dentro de la diversidad de adaptaciones la fenología foliar invertida en *B. nervosa*, destaca sobre los patrones fenológicos conocidos, por lo tanto, esta tesis es uno de los pocos estudios que han explorado los mecanismos ecofisiológicos y evolutivos de esta inusual especie en ecosistemas tropicales estacionales secos.

Los resultados de las manipulaciones de factores ambientales muestran que el patrón foliar invertido en árboles reproductivos no varía al modificarse la disponibilidad de agua (Capítulo 1) e intensidad lumínica (Capítulo 2) durante la estación seca. Lo cual podría sugerir que esta especie pudo haber experimentado un proceso de acomodación genética a través de mecanismos de plasticidad fenotípica mediados por la variabilidad ambiental. Además, los resultados de las concentraciones de carbohidratos no-estructurales en frutos y semillas de *B. nervosa* muestran una respuesta plástica en la asignación de recursos para la reproducción, sugiriendo una respuesta automática adaptativa a cambios ambientales (West-Eberhard, 2003). Considerando los resultados obtenidos en esta tesis se sugiere que el surgimiento de variantes fenotípicas, como la fenología foliar invertida, podría ser facilitado por la plasticidad fenotípica a la heterogeneidad ambiental experimentada a lo largo de su historia evolutiva.

Más allá del enfoque ecofisiológico de esta tesis, se alienta a la realización de más investigaciones que abarquen el análisis filogenético de *B. nervosa* y otras especies (de

familias distintas), que aparentemente comparten el mismo patrón fenológico invertido, a lo largo de sus distribuciones geográficas, así como trabajos, que exploren la posible relación gen-ambiente detrás de la expresión de este patrón inusual y la posible existencia de otras interacciones bióticas relevantes de estas especies como: micorrizas, insectos, aves y mamíferos, entre otros organismos.

Finalmente, estudios sobre este novedoso sistema – *fenología foliar invertida* - podrían resultar relevantes si se considera que los modelos climáticos proyectan mayores eventos de sequías en el mundo por efectos del calentamiento global, lo que pone de manifiesto la relevancia de investigar y conocer el origen, funcionamiento e implicaciones ecológicas y evolutivas del patrón foliar invertido no solo en los bosques tropicales secos de México.

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