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ATRIBUTOS FUNCIONALES Y DE DEFENSA QUÍMICA FOLIAR EN TRES ESPECIES DE PLANTAS EN ETAPAS SUCESIONALES CONTRASTANTES DEL BOSQUE TROPICAL SECO DE CHAMELA, JALISCO: EFECTOS SOBRE LOS PATRONES DE HERBIVORÍA

TESIS
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RESUMEN GENERAL

La sucesión secundaria involucra cambios secuenciales a lo largo del tiempo en atributos de las comunidades, tales como la riqueza y composición de especies, densidad de plantas y biomasa, lo que permite la regeneración natural de los ecosistemas. Factores abióticos como la radiación solar, la temperatura, la humedad y la disponibilidad de agua y nutrientes afectan directamente el proceso de regeneración de los bosques secundarios, incrementando la diversidad de árboles de estadios de sucesión temprana a sucesión tardía, y afectando los atributos funcionales de las plantas. Por lo tanto, los bosques maduros y secundarios difieren en su composición de especies y en su expresión de los atributos funcionales resultado de los filtros ambientales cambiantes a lo largo del proceso de sucesión. Algunas especies de plantas tienen la capacidad para lidiar con los filtros ambientales resultado de la capacidad de aclimatación debida a la variación genética y/o plasticidad fenotípica, lo cual les ha permitido colonizar y establecerse en distintas etapas de sucesión. De tal modo, es posible que individuos de una misma especie de planta presenten cambios en atributos funcionales foliares acorde a la etapa sucesional donde se desarrollen, lo que en consecuencia podría afectar a las interacciones bióticas como la herbivoría. En la presente tesis se plantearon los siguientes objetivos: (i) Evaluar los cambios en morfología y tamaño foliar, niveles de asimetría fluctuante y porcentajes de herbivoría foliar en tres especies de plantas características y dominantes del Bosque tropical seco (BTS) de Chamela-Cuixmala, México: *Cordia elaeagnoides, Cordia alliodora* y *Achatocarpus gracilis*, en dos etapas sucesionales del bosque tropical caducifolio: bosques maduros y bosques secundarios; y ii) Comparar los cambios en atributos funcionales foliares y sus efectos sobre el daño foliar en las tres especies en ambos estadios

sucesionales. Los resultados mostraron diferencias significativas en la morfología y tamaño foliar entre bosques maduros y bosques secundarios para las tres especies de plantas analizadas, donde *C. elaeagnoides* y *A. gracilis* presentaron hojas más elongadas y de mayor área foliar total en bosques maduros, mientras que *C. alliodora* mostró hojas más delgadas y de mayor área foliar total en bosques secundarios. Ambas especies de *Cordia* exhibieron mayores porcentajes de daño foliar en bosques secundarios en comparación con bosques maduros, mientras que *A. gracilis* mostró un patrón contrario. Los mayores niveles de asimetría fluctuante (i.e. las diferencias aleatorias en la simetría perfecta de rasgos morfológicos bilaterales, generadas por la inestabilidad en el desarrollo ante un factor de estrés ambiental) foliar se presentaron en individuos del bosque maduro para las tres especies estudiadas. Estos resultados sugieren que las especies de estudio exhiben cambios en su morfología y tamaño foliar que se ajustan a las condiciones ambientales presentes en las diferentes etapas de sucesión analizadas en esta tesis. Los menores niveles de asimetría fluctuante registrados en bosques secundarios en comparación a bosques maduros para las tres especies descartan la idea de que las condiciones de mayor temperatura y menor disponibilidad de agua en bosques secundarios sean más estresantes para las especies de plantas estudiadas. Por tanto, los mayores niveles de asimetría fluctuante en bosques maduros podrían deberse al estrés producido por una mayor competencia por los recursos. Las diferencias en la disponibilidad de recursos entre bosques maduros y secundarios pudieron haber influido en los porcentajes de daño foliar reportados para las tres especies, mediante su influencia en la expresión de defensas químicas y en cambios en calidad nutricional de las plantas estudiadas. Así, encontramos que las tres especies de plantas presentaron cambios en sus atributos funcionales foliares entre bosques maduros y secundarios, en donde el área foliar específica fue mayor en bosques secundarios para las

tres especies de plantas. De forma particular, *C. elaeagnoides* y *A. gracilis* exhibieron mayor área foliar total, contenido de agua, densidad foliar, masa fresca foliar, y concentración de fenoles y flavonoides en bosques maduros. Además, en bosques maduros *C. elaeagnoides* y *C. alliodora* presentaron un mayor grosor y menores niveles de herbivoría foliar, en tanto que *A. gracilis* un mayor contenido de clorofila y daño foliar en estos sitios. Por otro lado, *Cordia alliodora* presentó mayor área foliar total, contenido de clorofila, área foliar específica y concentración de alcaloides en bosques secundarios. Finalmente, se encontró que el daño foliar covarió con el grosor foliar en las tres especies y en ambas etapas sucesionales. Las diferencias encontradas en atributos funcionales foliares entre etapas sucesionales del BTS fueron mejor explicadas al nivel intra e interespecífico por las diferencias en la historia de vida entre las tres especies estudiadas, lo que parcialmente sustenta la predicción de que las estrategias de las plantas en el uso de los recursos cambian de conservativas a adquisitivas a lo largo de la economía del espectro foliar durante la sucesión del BTSs. Estos resultados contradicen la hipótesis de la disponibilidad de recursos para bosques tropicales secos (que originalmente fue planteada para los bosques tropicales húmedos), la cual predice que plantas conservativas en bosques secundarios deberían invertir más recursos en defensas y presentar menor daño foliar que las plantas adquisitivas en bosques maduros. Estas respuestas idiosincráticas dificultan el uso de una sola hipótesis para predecir los cambios en defensa y en los niveles de daño foliar en plantas a lo largo de gradientes ambientales al nivel intra e interespecífico. Sin embargo, a pesar de ello demostramos que esclerofilia es una defensa mecánica importante en contra del daño foliar por insectos en las distintas etapas sucesionales, aunque no se descarta que cambios en la comunidad de herbívoros entre bosques maduros y secundarios hayan influido también en los niveles de daño foliar reportados en este trabajo, o que en el

caso particular de *C. alliodora*, sus hormigas simbiontes también hayan contribuido en las diferencias en los niveles de daño foliar entre bosques maduros y secundarios.

INTRODUCCIÓN GENERAL

Los bosques tropicales secos (BTSs) son ecosistemas con una marcada estacionalidad entre la temporada seca y la temporada húmeda, típicamente dominados por árboles deciduos (más del 50% de las especies) y caracterizados por tener una temperatura promedio anual >25 °C y una precipitación entre 700 y 2000 mm (Sánchez-Azofeifa et al. 2005; Stan & Sánchez-Azofeifa 2019). Los BTSs representan el 42% de los bosques tropicales del mundo (Murphy & Lugo 1986) y su extensión es de aproximadamente 1,048,700 km^2, distribuidos en tres principales regiones del mundo: (i) la de Sudamérica, que incluye el Noreste de Brasil, Sureste de Bolivia, Paraguay y Norte de Argentina (52.4%); (ii) la de Australia y el Sureste de Asia, que presentan una pequeña porción de BTSs (3.8%) y (iii) la de África, Eurasia, Centroamérica y México con un 43.8% aproximadamente (Miles et al. 2006). Particularmente, en México están presentes de manera continua en la Península de Yucatán y de manera difusa a lo largo de la costa del Pacífico (Rzedowski 1978). Los BTSs albergan una gran biodiversidad e incluyen un gran número de endemismos (Trejo & Dirzo 2000; Miles et al. 2006; Sánchez-Azofeifa et al. 2009; Stan & Sanchez-Azofeifa 2019). Sin embargo, a pesar de su gran importancia, durante las últimas décadas han sido altamente fragmentados y degradados a causa de actividades antropogénicas como la agricultura, la ganadería, la urbanización, la deforestación y el turismo, por lo que han sido considerados como uno de los ecosistemas más amenazados del mundo (Miles et al. 2006; Quesada et al. 2009). Por ejemplo, el porcentaje de deforestación de los BTSs en el Sur y Sureste de Asia es alrededor del 16%, Madagascar con 18% y más del 40% en América Latina (Olson et al. 2000; Miles et al. 2006). En 1990, se estimó que en México sólo un 27% de la cobertura original de estos bosques permanecía intacta, lo que ha generado que en la actualidad los BTSs se encuentren conformados por un mosaico compuesto por parches aislados de vegetación remanente, campos de cultivo y de ganadería, y por parches de vegetación en estado de sucesión secundaria (Quesada et al. 2009; Chazdon & Guariguata 2016; Fonseca et al. 2018).

La sucesión secundaria involucra cambios secuenciales a lo largo del tiempo en atributos de las comunidades, tales como la riqueza y composición de especies, densidad de plantas y biomasa, lo que permite la regeneración natural del ecosistema una vez que las tierras han sido abandonadas (Madeira et al. 2009; Alvarez-Añorve et al. 2012). Factores abióticos como la radiación solar, temperatura, humedad y disponibilidad de agua y nutrientes afectan directamente el proceso de regeneración de los bosques secundarios (i.e. bosques bajo regeneración natural como producto de la remoción del bosque maduro original) del BTS (Lebrija-Trejos et al. 2010, 2011; Pineda-García et al. 2013; Neves et al. 2014). Por ejemplo, algunos estudios en BTSs han demostrado que a lo largo del proceso de sucesión secundaria, la radiación solar decrece mientras la disponibilidad de agua aumenta (Lohbeck et al. 2013; Pineda-García et al. 2013), generando un gradiente hídrico que aumenta mientras la cobertura del dosel se incrementa hacia etapas de sucesión más avanzadas como la del bosque maduro (i.e. bosques con nulo o poco disturbio durante al menos los últimos 80-150 años) (Alvarez-Añorve et al. 2012; Chazdon 2014; Lohbeck et al. 2013; Pineda-García et al. 2013). Estos cambios ambientales influyen en la diversidad de especies de plantas, incrementando la diversidad de árboles de estadios de sucesión temprana hacia la etapa de sucesión tardía, y afectando los atributos funcionales foliares de las diferentes especies de plantas (Alvarez-Añorve et al. 2012; Lohbeck et al. 2013; Poorter et al. 2018), por lo que bosques maduros y secundarios difieren en la composición de especies y en la expresión de atributos funcionales que se contrastan como resultado de los filtros impuestos por las condiciones ambientales cambiantes (Alvarez-Añorve et al. 2012). Los atributos funcionales de las plantas son características medibles (i.e. morfológicas, fisiológicas y fenológicas) que están asociadas a crecimiento, sobrevivencia y reproducción, por lo que influyen en la aptitud de un organismo (Violle et al. 2007), reflejan las estrategias ecológicas de las plantas y determinan cómo éstas responden a los factores ambientales (Pérez-Harguindeguy et al. 2016).

Algunas especies de plantas tienen la capacidad para lidiar con los filtros ambientales y habitar en distintos sitios en sucesión, cuya adaptación es debida a la variación genética y/o plasticidad fenotípica (Falcão et al. 2015; Huang et al. 2009). Así, por medio de la plasticidad fenotípica, los atributos funcionales de las plantas pueden cambiar y permitir que individuos de una misma especie puedan permanecer en los bosques en regeneración mediante una especialización temporal a las condiciones ambientales cambiantes (Agrawal 2020). Asimismo, la variación de atributos funcionales entre hábitats contrastantes en individuos de una misma especie podrían ser el resultado de genotipos adaptados locamente en bosques maduros y secundarios con habilidades diferentes para dispersarse o establecerse (Read et al. 2014; Tiffin & Ross-Ibarra 2014). En cualquiera de los dos casos, ya sea por plasticidad fenotípica o por genotipos adaptados localmente, es posible que individuos de una misma especie de planta expresen un conjunto distinto de atributos funcionales foliares (i.e. síndromes foliares) dependiendo del sitio en sucesión en el que habiten (Agrawal 2020).

Frecuentemente, dentro del marco del espectro global de la economía foliar (Wright et al. 2004) han sido explicadas las variaciones en atributos funcionales foliares a través de gradientes ambientales (Wright et al. 2004; Donovan et al. 2011). Este marco describe un patrón estable de correlaciones entre atributos foliares, que resumen las estrategias ecológicas de las plantas, las cuales van de ser adquisitivas (i.e. plantas de hojas delgadas, de alto contenido de nitrógeno y capacidad fotosintética) a conservativas (i.e. atributos contrarios) en términos del uso de los recursos (Donovan et al. 2011; Díaz et al. 2016). Varios atributos funcionales foliares que cambian entre sitios en sucesión están directa o indirectamente asociados con la resistencia de las plantas ante la herbivoría, como es el caso de defensas físicas (e.g., grosor, dureza) y químicas contra la herbivoría (e.g., compuestos secundarios basados en carbono y nitrógeno) (Fonseca et al. 2018), mientras que otros atributos se relacionan tanto a la altura de las plantas como a la tasa relativa de crecimiento, como es el caso del área foliar específica (Pérez-Harguindeguy et al. 2016; De la Riva et al. 2018). Además, estos atributos funcionales podrían actuar por separado o en conjunto y reducir el daño foliar por herbívoros, dependiendo de los costos de inversión y de la disponibilidad de recursos (Poorter et al. 2004; War et al. 2012).

La hipótesis de la disponibilidad de recursos propone que plantas de rápido crecimiento en hábitats sucesionales tempranos y ricos en recursos (i.e. mayor disponibilidad de luz y recursos del suelo como el agua y nutrientes) asignan más recursos en crecimiento y en reponer tejidos perdidos por herbivoría que producir defensas (Coley et al. 1985). En caso contrario, plantas de lento crecimiento en hábitats tardíos y pobres en recursos deberían asignar más recursos en defensas basadas en carbono (e.g. fenoles y flavonoides) debido a que producir esos compuestos resulta más barato que generar nuevos tejidos (Coley 1988; Boege & Dirzo 2004). Esta hipótesis (originada de estudios en bosques tropicales húmedos y boreales) propone que plantas presentes en etapas tempranas de sucesión secundaria deberían exhibir una estrategia adquisitiva, caracterizada por la expresión de una mayor área foliar, área foliar específica y contenido de clorofila, mientras que aquellas especies de plantas presentes en bosques maduros presentarían estrategias conservativas, es decir, hojas de mayor grosor y concentración de defensas químicas (Endara & Coley 2011). Sin embargo, direcciones opuestas en el cambio de los atributos funcionales de las plantas a lo largo de gradientes sucesionales para bosques tropicales húmedos y bosques tropicales secos han sido sugeridas por estudios recientes (Lebrija-Trejos et al. 2010b; Lohbeck et al. 2013; Buzzard et al. 2016; Fonseca et al. 2018). Durante el proceso de sucesión para bosques tropicales húmedos, el principal filtro ambiental es la disponibilidad de luz (Schönbeck et al. 2015), por lo que se espera que los atributos funcionales cambien en el sentido de la hipótesis de la disponibilidad de recursos. Mientras que en BTSs, el agua es el mayor filtro ambiental, por lo que este impone estrés hídrico y fuertes restricciones en los atributos funcionales foliares (Alvarez-Añorve et al. 2012), generando que las plantas de los BTSs bajo condiciones de sucesión secundaria temprana (i.e. mayor temperatura y menor disponibilidad de agua) exhiban atributos conservativos y tolerantes a sequía, tales como una reducción en el área foliar y área foliar específica, y un incremento en densidad, grosor, contenido de materia seca (Alvarez-Añorve et al. 2012; Jimenez-Rodríguez et al. 2018) y compuestos secundarios basados en carbono (Wright & Westoby 2002), por lo que se podrían esperar menores niveles de daño por herbívoros en bosques secundarios. Así, mientras la cobertura del dosel se incrementa hacia las etapas maduras de BTSs, la humedad también aumenta, por lo que los atributos funcionales deberían cambiar a lo largo de la sucesión de conservativos a adquisitivos. De tal modo, es posible esperar que en bosques maduros las plantas presenten atributos funcionales adquisitivos relacionados con altas tasas fotosintéticas y mayor contenido de nitrógeno y fósforo (Lohbeck et a. 2013; Agrawal et al. 2020), y por lo tanto presentar mayores niveles de daño foliar. Alternativamente, los cambios en defensa química y niveles de daño foliar también pueden ser explicados con base en la hipótesis del balance carbono-nutrientes, propuesta por Bryant et al. (1983) para bosques boreales. Ésta establece que una limitación en nutrientes del suelo se traduce en una menor absorción de nutrientes en plantas, afectando negativamente en primera instancia al crecimiento y en segunda a las tasas fotosintéticas. En este sentido, plantas que crecen en ambientes con una limitada disponibilidad de recursos como las que ocurren en etapas de sucesión tardía sufren estrés por deficiencia de nutrientes debido a que los recursos no son suficientes para promover una mayor tasa de crecimiento en relación a la tasa fotosintética, por lo que crecen más lentamente, generando una acumulación de carbohidratos sintetizados en las hojas que quedan disponibles para producir defensas basadas en carbono como fenoles y flavonoides, afectando negativamente el consumo por parte de los herbívoros (Bryant et al. 1983, 1987) y reduciendo los niveles de daño foliar. En caso contrario, plantas que crecen en ambientes ricos en recursos, como las que ocurren en bosques en sucesión secundaria, presentarían inherentemente un crecimiento más estimulado sobre las tasas fotosintéticas, generando que la producción de defensas basadas en carbono decline como consecuencia de una mayor asignación de los carbohidratos sintetizados a crecimiento, lo que generaría una mayor palatabilidad para los herbívoros y un incremento en los niveles de daño foliar (Bryant et al. 1983). Así, estás plantas estarían adaptadas a ambientes ricos en recursos, promoviendo un crecimiento más rápido ante una mayor disponibilidad en nutrientes del suelo e incrementando la concentración de nutrientes en los tejidos de las plantas, lo que generaría una mayor producción de defensas basadas en nitrógeno (e.g. alcaloides, glucósidos cianogénicos) en relación a las basadas en carbono (Bryant 1983, 1987).

A su vez, los niveles de herbivoría pueden ser distintos entre bosques maduros y secundarios como consecuencia de cambios en la comunidad de herbívoros a lo largo de la sucesión (Neves et al. 2014). Y es que las variaciones en factores abióticos y bióticos a lo largo de la sucesión secundaria pueden influir en las comunidades de insectos herbívoros por medio de cambios en la calidad de sus microhábitats y de sus recursos alimenticios, amplitud de su dieta y riesgos de depredación (Boege et al. 2019), afectando directamente la abundancia, riqueza, diversidad y composición de insectos herbívoros a lo largo de la sucesión en BTSs (Silva et al. 2012; Neves et al. 2014; Boege et al. 2019). Por ejemplo, en el BTS de Chamela-Cuixmala (México) se ha descrito que la abundancia de lepidópteros es similar entre bosques en sucesión y bosques maduros, y que la diversidad de estos herbívoros es mayor en bosques maduros que en secundarios, dando como resultado una composición de orugas distintas entre estos bosques (Boege et al. 2019), lo que podría influir en los niveles de herbivoría entre etapas sucesionales.

Aunque a nivel de comunidad es esperada una convergencia en los atributos funcionales foliares debido a que las plantas están sujetas a los mismos filtros ambientales, es posible que plantas de diferentes especies puedan presentar patrones contrastantes en la expresión de sus atributos funcionales foliares a lo largo de la sucesión secundaria como consecuencia de la forma de vida, etapa ontogenética, historia de vida, restricciones filogenéticas, entre otras (Ding et al. 2012; Letcher & Chazdon 2012; Uriarte et al. 2016). Por lo tanto, es posible esperar cambios en los atributos morfológicos y fisiológicos entre plantas de bosques maduros y bosques secundarios dependiendo de la capacidad de las especies de plantas para aclimatarse a las condiciones estresantes de mayor temperatura y sequía en bosques secundarios (Alvarez-Añorve et al. 2012).

La asimetría fluctuante (AF) se define como las diferencias aleatorias en la simetría perfecta de atributos morfológicos bilaterales, que son producidas por la inestabilidad en el desarrollo generado por el estrés ambiental o genético (Cornelissen & Stiling 2011; Cuevas-Reyes et al. 2018b; Tucić et al. 2018). Dado que la inestabilidad en el desarrollo es un auténtico indicador de estrés y refleja la incapacidad individual para mantener la homeostasis, la AF resulta ser un excelente indicador de estrés ambiental (Cuevas-Reyes et al. 2018b). Esta herramienta ha sido empleada en diferentes taxa de organismos, tales como mamíferos (Marchand et al. 2003), aves (Cuervo & Restrepo 2007), anfibios (Niemeier et al. 2019) y plantas (Cuevas-Reyes et al. 2018a, b). Para el caso de las plantas, los niveles de AF pueden variar en función de las condiciones climáticas (e.g. temperatura y precipitación), calidad del suelo (e.g disponibilidad de nutrientes, humedad, salinidad, contaminación), disponibilidad de luz, factores genéticos (e.g. hibridación, mutación) , competencia, depredación, parasitismo (Hagen et al. 2008; Telhado et al. 2010; Cornelissen & Stiling 2011; Cuevas-Reyes et al. 2013) y del daño por herbívoros (Cornelissen & Stiling 2011; Kozlov 2015; Cuevas-Reyes et al. 2018a), siendo esta última una de las principales causas de estrés en plantas (Boege et al. 2010; Cuevas-Reyes et al. 2018a).

Las plantas representan un sistema ideal para evaluar la AF debido a que éstos organismos presentan múltiples módulos repetidos, tales como las hojas, lo que permite generar varias medidas y evaluar la AF a nivel individual como producto del estrés (Sandner et al. 2019). El estrés en plantas por factores abióticos y bióticos produce inestabilidad en el desarrollo mediante alteraciones en su metabolismo (Ben-Rejeb et al. 2014), generando cascadas de señalización (Fraire-Velázquez et al. 2011), especies reactivas de oxígeno (Laloi et al. 2004), cambios hormonales que derivan en la producción de fitohormonas (e.g. ácido abscísico, salicílico, jasmónico) (Spoel et al. 2008) y en la reprogramación de la maquinaria genética para obtener una adecuada optimización y un incremento en la tolerancia para minimizar el daño biológico por estrés (Fujita et al. 2018). Sin embargo, los procesos que subyacen la inestabilidad en el desarrollo son pobremente entendidos, aunque se ha sugerido que la comunicación entre células y sus tasas de crecimiento, división y elongación se ven afectadas a nivel molecular y/o por la variación aleatoria en los procesos fisiológicos entre células (Palmer 1994; Lens et al. 2002), lo que finalmente deriva en niveles de AF. Éstos efectos serían acumulados en ambos lados (i.e. derecho e izquierdo) a lo largo del desarrollo foliar, derivando en asimetrías entre ambos lados (Lens et al. 2002). Por ejemplo, cuando un insecto herbívoro genera daño foliar, el metabolismo de la planta huésped se ve afectado y se activan rutas de señalización (e.g. aquellas relacionadas en la producción de defensas) que afectan el correcto desarrollo y homeostasis del individuo (Nabity et al. 2013; Kozlov & Zvereva 2017), generando así un incremento en AF.

La relación entre el daño foliar y la AF puede ser explicada con base en la hipótesis del estrés inducido por herbivoría (Zvereva et al. 1997) y por la hipótesis del estrés en plantas (White 1984). La primera señala que los herbívoros actúan como agentes de estrés en plantas al generar daño foliar, lo que afecta el metabolismo de las plantas, su desarrollo y homeostasis (Alves-Silva & Del-Claro 2016; Zvereva et al. 1997), y en consecuencia directa al patrón bilateral de crecimiento en las hojas, incrementando así los niveles de AF (Cuevas-Reyes et al. 2011; Cuevas-Reyes et al. 2018b). El daño foliar a su vez produce una pérdida de agua a través de los bordes dañados por los insectos, desbalances en el transporte de fluidos y/o nutrientes, reduciendo la capacidad fotosintética y propiciando la inducción de genes y más cambios en rutas metabólicas (Mound & Zapater 2003; Nabity et al. 2009; Alves-Silva & Del-Claro 2016), lo que finalmente se traduciría en mayores niveles de AF. La segunda hipótesis plantea que los insectos herbívoros perciben la AF en hojas como un indicador de calidad foliar (White 1984), en donde plantas con hojas más asimétricas serían más consumidas que plantas simétricas. Esto debido a que hojas más asimétricas tendrían una mayor calidad nutricional como producto de una mayor disponibilidad de nutrientes y a una menor concentración de defensa química (Cornelissen & Stiling 2005, 2011). Sin embargo, poco se sabe sobre cómo la inestabilidad en el desarrollo está conectada con el metabolismo de las plantas y a los cambios bioquímicos asociados a hojas asimétricas. Una mayor AF por si sola indica un mayor estrés ambiental (Maldonado-López et al. 2019), y por lo tanto una mayor susceptibilidad ante el consumo por herbívoros (Cornelissen & Stiling 2005; Maldonado-López et al. 2019). Y es que los factores abióticos pueden producir alteraciones bioquímicas y en composición química de las hojas, afectando negativamente la resistencia de las plantas y promoviendo una mayor atractividad y palatabilidad por parte los herbívoros (Cornelissen & Stiling 2005; Maldonado-López et al. 2019). El mecanismo que soporta esta hipótesis explica que bajo condiciones de estrés, las plantas incrementan los niveles de aminoácidos y decrecen la producción de metabolitos secundarios (e.g. compuestos basados en carbono como taninos y fenoles) en los tejidos foliares, lo que conlleva a un incremento la susceptibilidad ante daño foliar, y por ende, en el desempeño de los insectos herbívoros y oportunidad de sobrevivencia de la descendencia (Torrez-Terzo & Pagliosa 2007; Maldonado-López et al. 2019). En cualquiera de las dos hipótesis, niveles altos de AF han sido asociados a bajo crecimiento, sobrevivencia y reproducción en plantas (Díaz et al. 2004; Cuevas-Reyes et al. 2018a), lo que finalmente es el reflejo de la incapacidad individual para mantener la homeostasis durante el desarrollo bajo condiciones de estrés (Møller & Swaddle 1997; Cuevas-Reyes et al. 2018a).

Para entender la ecología de las plantas durante el proceso de regeneración de los BTSs, es necesario evaluar como la sucesión secundaria influye sobre los atributos funcionales foliares de las plantas, los patrones de herbivoría y los niveles de estrés mediante la asimetría fluctuante. En el presente estudio se analizaron los cambios en los atributos morfológicos y fisiológicos foliares, porcentajes de daño foliar por insectos herbívoros y niveles de asimetría fluctuante foliar en tres especies de plantas que ocurren en bosques maduros y secundarios del BTS de Chamela-Cuixmala, en México.

Estructura y objetivos del estudio. La presente tesis está compuesta por dos capítulos. En el primero se evalúo la morfología y tamaño foliar, niveles de asimetría fluctuante y daño foliar por insectos herbívoros en tres especies de plantas dominantes (*Cordia elaeagnoides, Cordia alliodora* y *Achatocarpus gracilis*) que habitan en bosques maduros y secundarios del BTS de Chamela-Cuixmala, México. También se evalúo la relación de la asimetría fluctuante con el área foliar total y los niveles de herbivoría en bosques maduros y bosques secundarios.

El segundo capítulo tuvo como objetivo el comparar los atributos físicos y químicos que están relacionados a defensa en plantas y la herbivoría en tres especies leñosas que suceden en BTSs maduros y secundarios de Chamela-Cuixmala (México): *Cordia elaeagnoides, Cordia alliodora* y *Achatocarpus gracilis*. Además, se investigaron los efectos de los atributos funcionales y de defensa en los niveles de daño foliar por herbívoros de cada etapa sucesional.

CAPÍTULO I.

Contrasting patterns of morphology, fluctuating asymmetry and leaf herbivory in three plant species of different successional stages of a tropical dry forest

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ORIGINAL ARTICLE

Contrasting patterns of morphology, fluctuating asymmetry and leaf herbivory in three plant species of different successional stages of a tropical dry forest

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Abstract

Key message **This paper shows the intraspecific plant responses in leaf morphology, herbivory and FA between mature and successional tropical dry forests, as well as the difficulty associated with understanding the relationship between FA and herbivory**

Abstract Understanding the processes that affect biotic interactions during secondary succession has implications for the maintenance of species diversity in the tropics. We studied the changes in leaf morphology, fluctuating asymmetry (FA) and herbivory in three dominant tropical dry forest plant species that occur in mature and secondary forests. We selected eight study sites: four in mature and four in successional forests. At each site, a plot of 20 × 50 m was established to characterize the vegetation structure and soil fertility. Subsequently, leaf morphology, FA and herbivory were measured in all individuals of *Cordia elaeagnoides*, *C. alliodora* and *Achatocarpus gracilis* with DBH $\geq$ 2.5 cm found in the plots. Plant abundance and plant height decreased in secondary forest, while the content of nitrates and phosphates increased in soil of secondary forests. Differences in leaf morphology between forest conditions were found for the three species. Total leaf area was higher in mature than in secondary forests for *C. elaeagnoides* and *A. gracilis*. An opposite pattern was found for *C. alliodora*. In both *Cordia* species, herbivory was higher in secondary than in mature forests. The opposite pattern was found for *A. gracilis* in secondary forests. For all the cases, FA was higher in mature forests than in secondary forests. Herbivory was positively correlated with FA in secondary forests in *C. elaeganoides* and in *A. gracilis*, whereas in *C. alliodora* herbivory was positively related with FA in mature forests. Overall, we detected changes in foliar morphology, fluctuating asymmetry and herbivory between mature and secondary forests, with a general pattern of higher FA levels in mature forests. Our findings illustrate the difficulty associated with understanding the relationship between FA and herbivory throughout the regeneration process in tropical dry forests due to the complexity of abiotic and biotic factors that can affect plant–herbivore interactions.

Keywords Environmental stress · Secondary succession · Tropical dry forests · Fluctuating asymmetry · Herbivory

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Introduction

The most threatened tropical ecosystems are the tropical dry forests (TDFs), which represent 42% of the tropical forests of the world (Trejo and Dirzo 2000). During the last decades, anthropogenic activities such as deforestation, tourism, urbanization and the conversion of mature forests to agricultural fields and livestock soils have negatively affected the TDF, generating a mosaic of fragments with natural vegetation and patches of abandoned lands under different stages of natural regeneration. Therefore, it is common to observe a high occurrence of secondary forests under different successional stages (Chazdon and Guariguata 2016).

The secondary succession process represents a feedback phenomenon between abiotic factors and vegetation characteristics. In fact, abiotic factors such as precipitation, relative humidity, temperature and soil fertility change during the successional process and can influence plant performance, population structure, biotic interactions, food webs and, therefore, the community structure (Loik and Holl 2001; Lebrija-Trejos et al. 2011). In turn, vegetation structure aspects such as plant species dominance, diversity, density and life forms may affect the abiotic factors, which in turn influence the forest regeneration process (Madeira et al. 2009; Lebrija-Trejos et al. 2010). In this way, some studies have shown that secondary forests of TDFs are characterized by a higher incidence of solar radiation and temperature and lower relative humidity and water availability in comparison with mature forests (Lohbeck et al. 2015; Pineda-García et al. 2013). Because secondary forests are hotter and drier environments that can represent situations of environmental stress for plants, it is possible to expect changes or adjustments in physiological and morphological attributes according to the ability of plant species to acclimate to such stress (Alvarez-Añorve et al. 2012; Schöb et al. 2013).

Fluctuating asymmetry (FA) describes the random differences in size or shape between two sides of a bilateral character in organisms and reflects the developmental instability caused by stressors such as pollution, extremes of temperature, solar radiation and humidity, habitat disturbance and global climate change (Cuevas-Reyes et al. 2013, 2018a; Tucić et al. 2018). Consequently, it has been considered a reliable indicator of environmental stress in different organisms such as birds (Cuervo and Restrepo 2007), mammals (Marchand et al. 2003) and amphibians (Niemeier et al. 2019). Particularly, in plants, high levels of FA have been correlated with the reduction of survival and reproduction (Díaz et al. 2004), increased urbanization (Cuevas-Reyes et al. 2013), extreme climatic conditions (Valkama and Kozlov 2001) and excesses or deficits of solar radiation, temperature and soil nutrients (Hagen et al. 2008; Cornelissen and Stiling 2011). In addition, herbivory can also be a plant stressor (Cuevas-Reyes et al. 2018b). In some cases, FA levels are positively related with herbivory, indicating more susceptibility to herbivores (plant stress hypothesis) (Cornelissen and Stiling 2005; Cuevas-Reyes et al. 2011a). However, other studies have not found this relationship (Bañuelos et al. 2004). Differences in nutritional quality and chemical defenses of plants have been suggested to explain these contrasting results (Cornelissen and Stiling 2011). On the other hand, plants can be directly stressed by herbivorous insects producing changes in leaf symmetry and increasing FA levels (herbivory-induced stress hypothesis) (Alves-Silva and Del-Claro 2016). In this way, FA in plants can increase as a result of herbivory intensity (Cuevas-Reyes et al. 2011b).

The levels of herbivory can vary between plant species and individuals (del-Val and Armesto 2010) as a result of differences in the intrinsic characteristics (e.g., genetic composition, type of growth, longevity, and leaf functional traits) (Coley 1982), temporal and spatial variation in richness and abundance of herbivores and changes in local environmental conditions, which in turn can influence the nutritional quality and chemical defenses of plants (Silva et al. 2012; Sousa-Souto et al. 2014; González-Esquivel et al. 2019). According to this idea, it is possible to expect a decrease in resource availability for plants (e.g., soil nitrogen and phosphorous) during the forest succession process, where plants that occur in early successional stages invest more resources in vegetative growth and replacement of leaf tissues lost by herbivory than in chemical defense as a result of higher photosynthetic rates and faster vegetative growth (Coley et al. 1985; González-Esquivel et al. 2019). Conversely, plants of mature forests allocate more resources for carbon-based chemical defenses because producing new leaves results in being more expensive (Silva et al. 2012).

Considering these ideas, it is possible to test the resource availability hypothesis in plants of the same species that occur in contrasting successional habitats because they might exhibit differences in their contents of chemical defenses, nutritional quality and herbivory rates (Silva et al. 2012; González-Esquivel et al. 2019). Therefore, we hypothesized that plants immersed in secondary forests will have higher levels of herbivory because they are rich in nutrients with fewer compounds associated with defense in comparison with plants of mature forests (Silva et al. 2012; González-Esquivel et al. 2019).

Finally, the relationship between FA and herbivory suggests that FA may also serve as an indicator of plant susceptibility to herbivory, where more asymmetric leaves have higher nutritional quality than symmetric leaves, making them more susceptible to herbivory and implying that leaf morphology and FA can also be used as an indicator of plant quality (Lempa et al. 2000; Cornelissen et al. 2003, Cornelissen and Stiling 2005). However, herbivory itself can also act as a stressor and directly contribute to increase the level of FA (Zvereva et al. 1997; Møller and Shykoff 1999; Maldonado-López et al. 2019). Consequently, environmental factors such as temperature, humidity and incidence of light can affect both leaf morphology and plant quality, which in turn, might influence the herbivory levels (Cunningham et al. 1999; Givnish 1987; Cuevas-Reyes et al. 2011a, b). Thus, based on these ideas and considering that secondary forests are hotter and drier habitats, we hypothesized that secondary forests present stressful conditions to plants that will be expressed in changes in leaf morphology, higher levels of foliar FA, and a higher preference of herbivores for asymmetric leaves that could be reflected in higher levels of herbivory.

In the present study, we tested this hypothesis using geometric morphometric techniques to analyze leaf size and shape, fluctuating asymmetry and leaf herbivory in three dominant plant species in mature and secondary forests of a tropical dry forest in Chamela-Cuixmala, Mexico. Specifically, we addressed the following questions: (1) do leaf morphology and size of *C. elaeagnoides*, *C. alliodora* and *A. gracilis* change in function of successional TDFs stages? (i.e., mature and successional TDFs); (2) do levels of FA differ between the two forest conditions and between plant species*?* (3) Are herbivory levels different between mature and secondary forests in the three plant species? (4) Is FA related with total leaf area and levels of herbivory in both forest conditions?

Materials and methods

Study area

This study was conducted at the Chamela-Cuixmala Biosphere Reserve on the Pacific coast of Jalisco, Mexico (19° 22′–19° 35′ N, 104° 56′–105° 03′ W) that covers an extension of 16,000 ha, and in outside areas of abandoned agricultural fields, hereafter called secondary forests (García-Oliva et al. 2002; Sánchez-Azofeifa et al. 2009).

We selected eight study sites using Google Earth high-resolution imagery (http://earth.google.com) and classified satellite images (Avila-Cabadilla et al. 2012); four sites of mature forests localized within the Chamela-Cuixmala Biosphere Reserve and four secondary forests outside the Biosphere reserve. Mature forests have remained intact in the last 60 years, while secondary forests were cattle pastures and have between 3 and 8 years of abandonment (Avila-Cabadilla et al. 2012). In general, the land use history of the secondary forests in the study sites was the following: (1) most of the original vegetation was removed through slash and burn; (2) lands were used for agriculture between 2 and 5 years and then converted to cattle ranching by burning the vegetation periodically; and (3) secondary succession occurred because of the land abandonment by farmers (Jimenez-Rodríguez et al. 2018).

Because topographic variables can affect the composition of the plant community, our study sites were located at an average elevation of 143 m with slopes averaging 10° (range 15°), mainly oriented to the south and southeast (Balvanera et al. 2002). We characterized each forest condition by measuring soil properties and vegetation structure (Jimenez-Rodríguez et al. 2018).

Vegetation measurements

To characterize the vegetation structure of mature and secondary forests, in each study site, all woody plants with diameter at breast height (DBH) $\geq$ 2.5 cm were marked and identified at the species level and then the following parameters were measured: (1) number of primary and secondary branches up to 1.30 m, (2) DBH, (3) plant height, (4) total number of individuals, (5) plant species richness and (6) basal area (Jimenez-Rodríguez et al. 2018).

Soil properties

Fifteen soil samples (0–10 cm depth) were randomly collected from each study site using a bucket auger and mixed into a single soil sample. This sample was divided into three subsamples that were used to obtain the following measurements: (1) total content of C, N and P, (2) soil humidity, pH, inorganic N and P, and (3) enzymatic activity of three enzymes, phosphatase, beta-glucosidase and *N*-acetylglucosaminidase. Before soil collection, all the aboveground materials were carefully removed. All soil samples were stored in sealed plastic bags and transported to the laboratory in a cooler (Jimenez-Rodríguez et al. 2018).

For soil moisture determination (H), a subsample (100 g) was oven-dried at 75 °C to constant weight, using the gravimetric method to adjust for water content when expressing nutrient concentration on the basis of dry soil mass. Carbon forms analyzed in all samples were determined in a total carbon analyzer (UIC model CM5012, Chicago, USA), while the N and P forms analyzed were determined colorimetrically in a Bran–Luebbe AutoAnalyzer 3 (Norderstedt, Germany). Prior to the total soil nutrient analyses, soil samples were dried and ground with a pestle and mortar. Total carbon (Ct) was determined by combustion and colorimetric detection (Huffman 1977). Total organic carbon (Cti) was calculated as the difference between TC and inorganic carbon. For total N (Nt) and total P (Pt) determination, samples were acid digested at 360 °C. Soil N (N) was determined by the macro-Kjeldahl method (Bremner 1996), while soil P

(P) was determined by the molybdate colorimetric method following ascorbic acid reduction (Murphy and Riley 1962).

Available, dissolved and microbial nutrient forms were extracted from field moist soil samples. Available inorganic N (NH_4^+ and NO_3^-) was extracted from 10 g of fresh soil subsamples with 2 M KCl, followed by filtration through a Whatman No. 1 paper filter and determined colorimetrically by the phenol-hypochlorite method. The PO_4 content was determined by extraction with 0.5 M $NaHCO_3$ at pH 8.5 according to Hedley sequential P fractionation (Tiessen and Moir 1993). Enzyme activity was analyzed colorimetrically and expressed as micromoles of product formed per gram dry weight of soil per hour.

Study species

Cordia elaeagnoides A. DC. It is a deciduous tree that can grow up to 20 m, distributed exclusively on the Pacific coast of Mexico, including the states of Sinaloa, Jalisco and Oaxaca. It has been shown that herbivores such as *Coptocycla leprosa* (Chrysomelidae) are associated with *C. elaeagnoides* (Trager and Bruna 2006).

Cordia alliodora (Ruíz and Pav.) Oken. It iss a deciduous tree growing up to 25 m. In Mexico, it is distributed along the Gulf of Mexico and Pacific coast from Sinaloa to Chiapas (Pennington and Sarukhán 2005). Herbivorous insects attacking *C. alliodora* belong to Saturniidae, Chrysomelidae and Tingidae (Trager and Bruna 2006).

Achatocarpus gracilis H. Walter. It iss a deciduous shrub typical of mature forests with a low frequency in secondary forests (Boege et al. 2019). To our knowledge, the herbivore community associated with *A. gracilis* has not been documented, but during the study, we observed at least four species of Lepidoptera of different families responsible for most of the apparent herbivory.

Sampling design

Sampling was conducted at the end of the rainy season, after the peak of herbivore activity and the leaves were completely expanded. In each study site, a transect of 20 × 50 m (0.1 ha) was established. On each transect, all individuals of the three species with diameter at breast height (DBH) ≥ to 2.5 cm were selected, and then marked and georeferenced to avoid sampling the same individual twice (*C. elaeagnoides*: $N = 67$, 33 individuals of mature and 34 of secondary forests; *C. alliodora*: $N = 68$, 34 in mature and 34 for secondary forests; and *A. gracilis*: $N = 106$, 55 in mature and 51 in secondary forest). Plants sampled from mature forest ranged in DBH as follows: *C. elaeagnoides* varied from 7.8 to 10.2 cm, *C. alliodora* from 8.5 to 11.4 cm and *A. gracilis* from 3.8 to 6.4 cm, while in secondary forests the DBH ranged in *C. elaeagnoides* from 7.6. to 11.6 cm, *C. alliodora* from 8.1 to 12.2 cm and *A. gracilis* from 3.7 to 6.9 cm. For each individual, three branches were randomly chosen of each tree canopy strata (i.e., high, middle and low) (Cuevas-Reyes et al. 2011b), and then we selected 50 leaves in total: 25 undamaged leaves to determine the patterns of leaf morphology and fluctuating asymmetry using geometric morphometric techniques and 25 randomly selected leaves to evaluate herbivory levels using ImageJ software (Cuevas-Reyes et al. 2018a).

Morphometric analysis of leaves

To evaluate the differences in leaf morphology and size of the three plant species between forest conditions (mature forest vs secondary forest), a digital image was obtained of each undamaged leaf (25 leaves in total per individual) and then 32 anatomical marks (i.e., landmarks) were placed on each image. These landmarks represent the leaf shape and correspond to homologous loci, which are unambiguous and repeatable in all the leaves (Cuevas-Reyes et al. 2018a). For each leaf image, the coordinates (x and y) of the 32 landmarks were recorded using a TpsDig program (Rohlf 2015). We performed a Procrustes superimposition analysis using the Integrated Morphometric Package (IMP series: http://www.canisius.edu/~sheets/morphsoft.html) to configure the landmark coordinates. After that, the average configuration of all leaves was considered as reference to eliminate the leaf size effect (Bookstein 1991). The shape variables (Procrustes distances) were calculated by a superimposition coordinates analysis (Cuevas-Reyes et al. 2011b). Finally, for each plant species separately, a principal components analysis was applied to determine the differences in leaf morphology between mature and secondary forests (Cuevas-Reyes et al. 2018a).

Fluctuating asymmetry measurements

Fluctuating asymmetry was measured in 25 fully expanded and intact mature leaves of each tree sampled in the two forest conditions. For this, we obtained a digital image of each leaf and measured the distance from the right side (RW) and the left side (LW) from the leaf border to the midrib at the midpoint of the leaf corresponding to its widest part, using the program ImageJ 1.51j87 (https://imagej.nih.gov/ij/) (Cuevas-Reyes et al. 2018b). FA was calculated as the absolute value of the difference between the distances from the midrib to the left and right borders of the leaf ($|Ai - Bi|$), divided by the average distance ($Ai + Bi/2$), to correct for the fact that asymmetry may be size dependent (Cornelissen and Stiling 2005). A value of FA was obtained for each tree from the average of the 25 leaves measured. Besides, a subsample of 30 leaves was measured again to control the measurement error in FA, and then we calculated the significance of FA relative to measurement error using a two-way mixed-model ANOVA (Palmer and Strobeck 2003; Cuevas-Reyes et al. 2018b). The significance of the interaction (individual × leaf × side) showed that the variation in FA between sides was greater than expected by measurement error ($F = 102.4 = 26$; $P < 0.0001$).

Because there are three types of asymmetry (i.e., fluctuating asymmetry, directional asymmetry and antisymmetry) (Palmer and Strobeck 2003), it is important to discriminate between them (Cuevas-Reyes et al. 2018b). FA calculates the variance of random differences between the two sides of a bilateral trait distributed with a mean value of zero. Directional asymmetry is defined as the differences between the two sides that are distributed about a mean that is significantly greater or less than zero. Finally, antisymmetry is the absence of symmetry and is characterized by presenting a bimodal or platykurtic distribution of the differences between two sides about a mean of zero (Palmer and Strobeck 1992). Therefore, FA determines small random variation from the optimal development of a trait with bilateral symmetry, whereas directional asymmetry and antisymmetry are unsuitable descriptors of developmental instability, since they are developmentally controlled and are probably adaptive (Palmer and Strobeck 1986).

To test if our data presented only FA and no other type of symmetry, a Student's *t* test was performed to discard directional asymmetry, considering if the average value of the differences of the right side minus the left (L–R) differed from zero. Additionally, to discard antisymmetry, a Lilliefors' normality test was used, considering the distribution of the differences of L–R (Alves-Silva and Del-Claro 2016). Since we did not find a significant deviation from zero ($t = 1.6$; $P > 0.05$) in the average value of the L–R differences, and the distribution of L–R differences showed a normal distribution ($P > 0.05$), the values were considered a reliable measure of FA.

Measurement of herbivory

The herbivory by insects was assessed in 25 leaves of each tree sampled in the two forest conditions, from digital images that were taken for each leaf previously. To calculate the percentage of herbivory, we first estimated the total leaf area and leaf area removed by herbivores using the program ImageJ 1.51j87 (https://imagej.nih.gov/ij/). Then, herbivory data were transformed as square root arcsine and then the normal distribution was confirmed in all cases (Cuevas-Reyes et al. 2018b).

Statistics analyses

We performed a one-way permutation test based on Monte Carlo resampling separately to determine the differences between mature and secondary forests in vegetation structure (species richness, plant abundance, basal area, plant height) and soil properties (humidity, pH, C, N, P, C:N, C:P, N:P, NH_4, NO_3, PO_4).

To determine the effects of forest condition (mature forest vs secondary forest) on total leaf area of each plant species, we conducted a one-way ANOVA test for each plant species separately, where forest condition was considered as the explanatory variable and the total leaf area as the response variable (SAS 2000). We conducted a Student's *t* test to compare the mean of foliar FA levels between mature and secondary forests for each plant species, respectively. We used a two-way ANOVA test to determine the effects of forest condition and plant species on herbivory. The model considered forest condition and plant species as explanatory variables, and herbivory was used as response variable. A posteriori tests of LSMeans were performed to compare means when ANOVA tests presented significant differences ($P < 0.05$) (SAS 2000). Finally, to assess the relationship between total leaf area, FA levels and herbivory, a Spearman's rank correlation was used within each forest condition for each plant species separately. Because these tests involved multiple comparisons, a Bonferroni correction was applied to adjust *P* values.

Results

We found that plant abundance and plant height were higher in mature than in secondary forests. We did not find differences in the other vegetation measurements analyzed (Table 1). In the same way, only nitrates and phosphate content of the soil was different between forest conditions (Table 1).

Based on coordinate superimposition analysis, we found differences between mature forests and secondary forests in the leaf shape of *C. elaeagnoides*. Leaves of mature forests were more elongated and thinner than leaves of secondary forests (Fig. 1a). This result is supported by the principal components analysis, where the PC1 and PC2 accounted for 57.61 and 14.37% of the total variation, respectively, and separated two different groups (Fig. 2a). Similarly, in *C. alliodora*, leaves of mature forests were more elongated and wider than leaves of secondary forests (Fig. 1b). This result was confirmed by the principal components analysis, where the PC1 accounted for 49.76% and PC2 explained the 17.57% of variation, separating two distinct groups of leaves (Fig. 2b). Finally, in the case of *A. gracilis*, leaves of mature forests were wider at the base in comparison with leaves of secondary forests (Fig. 1c). The principal components analysis separated leaves into two groups: leaves from mature forest and leaves of secondary forest, where PC1 explained 62.76% and PC2 13.13% of variation (Fig. 2c).

Table 1 Comparison of vegetation attributes and soil properties between mature and secondary forests

Attributes	Mature forests	Secondary forests	Z	*P* <
Vegetation				
Species richness	83 ± 12	62 ± 42	0.94	n.s.
Plant abundance	477 ± 83	304 ± 28	1.8	0.04
Basal area	19,641.2 ± 4687	11,003.5 ± 2056.1	2.9	0.04
Plant height	3.8 ± 0.3	3.6 ± 0.5	0.9	n.s.
Soils				
Humidity (%)	16.6 ± 8.8	19.1 ± 9.4	− 0.4	n.s.
pH	6.6 ± 0.5	6.8 ± 0.3	− 0.5	n.s.
Carbon (mg/g)	18.2 ± 6.3	20.3 ± 6.9	− 0.5	n.s.
Nitrogen (mg/g)	1.2 ± 0.6	1.7 ± 0.5	− 0.9	n.s.
Phosphorous (mg/g)	0.27 ± 0.26	0.34 ± 0.21	− 0.4	n.s.
C:N ratio	15.5 ± 2.7	12.9 ± 2.9	1.2	n.s.
C:P ratio	106.2 ± 57.0	76.4 ± 44.4	0.9	n.s.
N:P ratio	6.7 ± 3.3	5.7 ± 1.9	0.6	n.s.
Ammonium (NH_4) (μg/g)	15.9 ± 8.6	4.1 ± 5.6	1.9	n.s.
Nitrate (NO_3) (μg/g)	8.0 ± 9.3	11.9 ± 5.6	1.5	0.04
Phosphate (PO_4) (μg/g)	23.9 ± 8.5	53.4 ± 15.2	1.9	0.03

Comparisons were made using one-way permutation tests based on Monte Carlo resampling. The means and the SE are shown

Total leaf area of *C. elaeagnoides* differed significantly between forest conditions ($F = 7.3$; *d.f.* = 1; $P < 0.007$). In mature forests, the mean of total leaf area was 95.0 cm^2 ± 1.67 SE and in secondary forests 88.1 cm^2 ± 1.9 SE (Fig. 3a). We found significant differences in leaf size of *C. alliodora* between the two successional stages ($F = 51.8$; *d.f.* = 1; $P< 0.0001$). For this species, the leaf size was smaller in mature forests (35.3 cm^2 ± 0.6 cm^2 SE) than in secondary forests (41.9 cm^2 ± 0.7 cm^2 SE). For *A. gracilis*, the total leaf area was different between the forest conditions ($F = 98.3$; *d.f.* = 1; $P< 0.0001$), being higher in mature forest (6.0 cm^2 ± 0.08 cm^2 SE) than in secondary forests (4.9 cm^2 ± 0.06 cm^2 SE) (see Fig. 3a).

The herbivory differed significantly between forest conditions ($F = 7.4$; *d.f.* = 1; $P < 0.006$) and between plant species ($F = 12.1$; *d.f.* = 2; $P < 0.0001$). In both *Cordia* species, the herbivory was higher in secondary forests in comparison with mature forests. In contrast, herbivory was higher in mature than in secodary forests for *A. gracilis*. *Cordia alliodora* had higher herbivory levels than *A. gracilis* and *C. elaeagnoides* (Fig. 3b).

For all plant species analyzed, FA was higher in mature forests than in secondary forests: *C. elaeagnoides* ($t_{1402} = 24.5$; $P< 0.01$); *C. alliodora* ($t_{1669} = 30.8$; $P< 0.001$); *A. gracilis* ($t_{2585} = 18.2$; $P< 0.02$) (Fig. 4). In the case of *C. elaeganoides,* after a Bonferroni correction, Spearman's rank correlation revealed that herbivory was positively correlated with FA in secondary forests (Table 2a), whereas in *C. alliodora* herbivory was positively related with FA in mature forests (Table 2b). A positive relationship between herbivory and FA in *A. gracilis* was found in secondary forest (Table 2c).

Discussion

The process of ecological succession not only implies sequential changes in species richness, composition and abundance, but also variations in abiotic factors (Lebrija-Trejos et al. 2010). For example, previous studies in TDF have found that the vegetation canopy openness varies from 5.3 to 18.7% in mature forest and from 5.9 to 32% for secondary forests, producing differences in solar radiation, temperature and availability of water between the two conditions, with the secondary forests being hotter and drier (Maza-Villalobos et al. 2011; Alvarez-Añorve et al. 2012). In our study, we found that abundance and basal area of plants were higher in mature than in secondary forests. These results suggest that secondary forests are hotter and drier as a result of differences in solar radiation and temperature associated with the vegetation structure (Jimenez-Rodríguez et al. 2018). Differences in these environmental conditions along forest succession impose physiological demands to plants, resulting in adjustment in some traits such as leaf morphology, symmetry and leaf size according to both the degree of tolerance of individuals to environmental stress and phenotypic plasticity (Cuevas-Reyes et al. 2011b; Alvarez-Añorve et al. 2012). This is in accord with our results, because all plant species studied presented differences in leaf morphology and size between mature and secondary forests. For example, *C.*

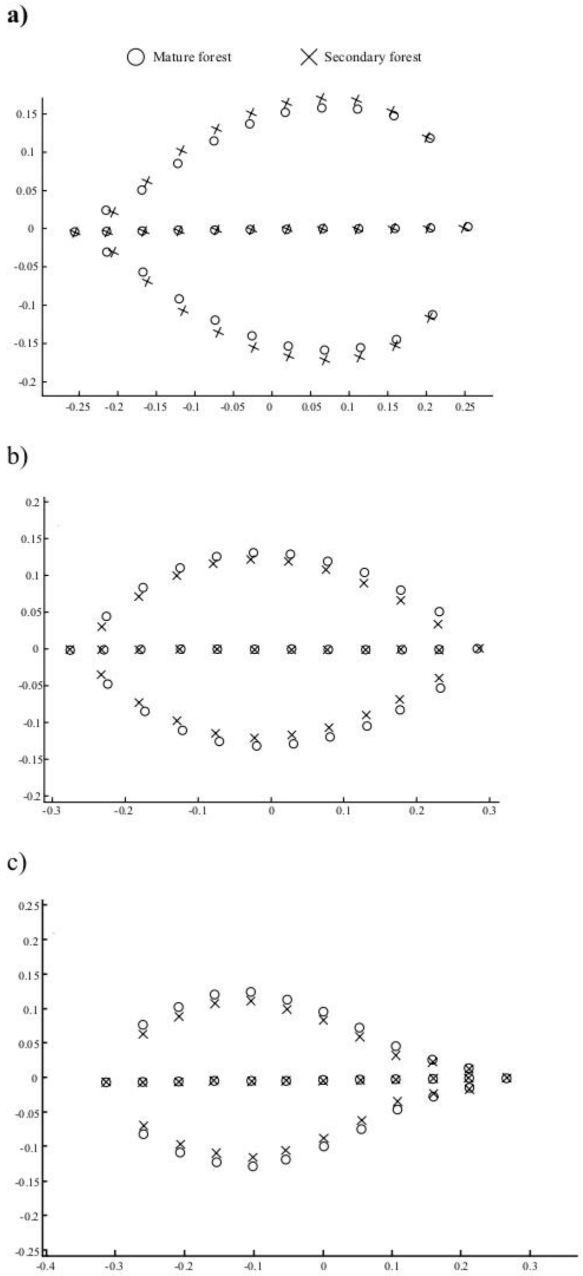

Fig. 1 Leaf morphological variation between mature (black circles) and secondary forests (black crosses) for each plant species: mean of coordinates of landmark configuration of leaves. *C. elaeagnoides* (**a**), *C. alliodora* (**b**) and *A. gracilis* (**c**)

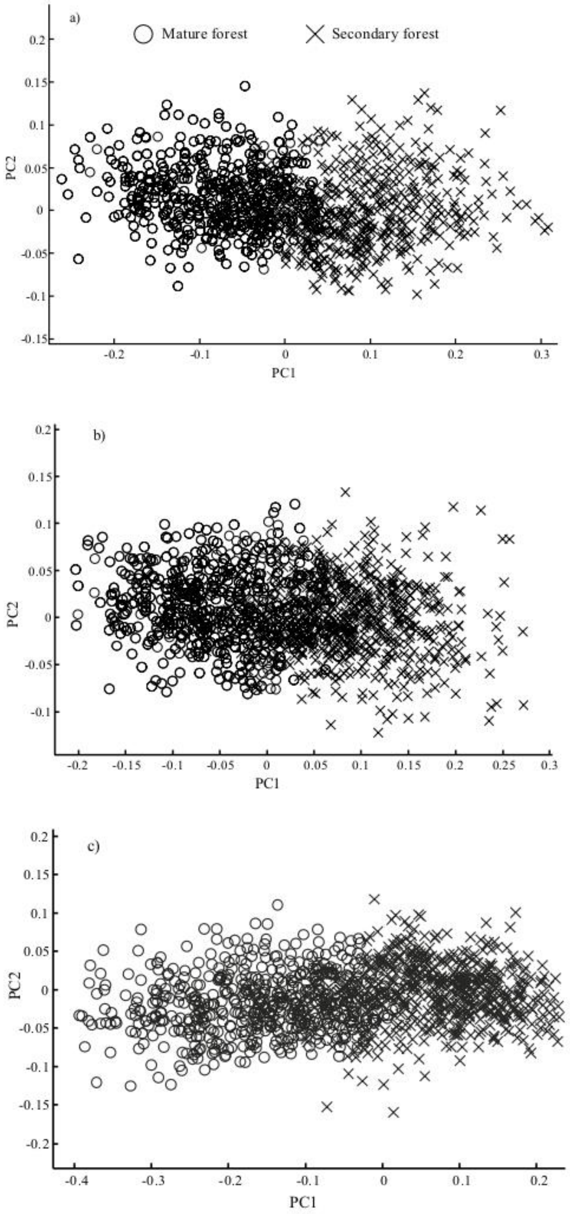

Fig. 2 Differences in leaf shape morphology between mature and secondary forests for each plant species according to canonical variate analysis. Black circles: mature forest. Black crosses: secondary forest. *Cordia elaeagnoides* (**a**), *Cordia alliodora* (**b**) and *A. gracilis* (**c**)

elaeagnoides and *A. gracilis* had smaller leaves in secondary than in mature forests, which can be the result of morphological and physiological adjustment to avoid water loss in hotter and drier sites such as secondary forests (Alvarez-Añorve et al. 2012; Pineda-García et al. 2013). In contrast, *C. alliodora* had larger leaves in secondary than in mature forest, supporting the hypothesis of resources availability (Coley et al. 1985), which predicts that fast-growing plant species such as *C. alliodora* develop larger leaves in sites with more availability of resources (e.g., light incidence, N and P) (Cole and Ewel 2006). Therefore, an increase of light

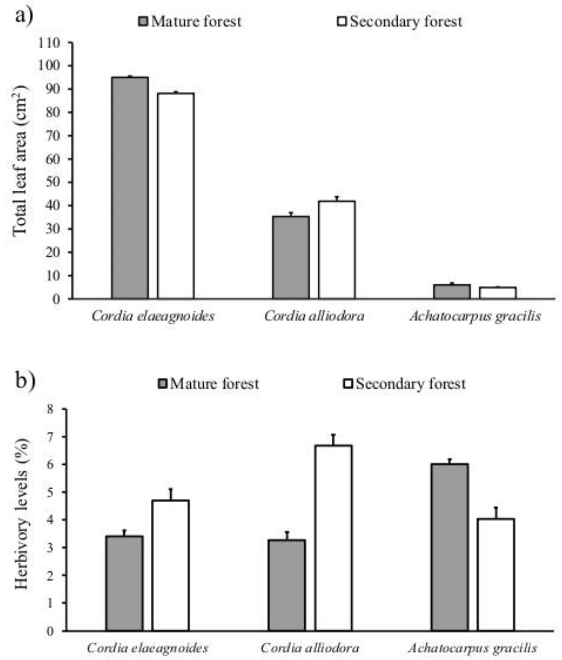

Fig. 3 Comparison of total leaf area between mature and intermediate successional forests for each plant species (**a**), differences in herbivory by insects between mature and secondary forests and between plant species (**b**)

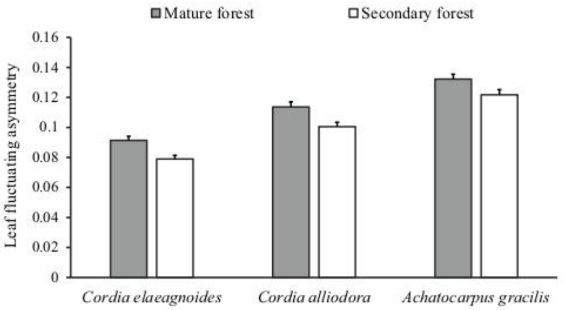

Fig. 4 Differences in leaf fluctuating asymmetry between mature and secondary forests for each plant species

incidence in secondary forests can be associated with high photosynthetic rates to absorb nutrients quickly, grow fast and produce large leaves (Lohbeck et al. 2015).

Fluctuating asymmetry is one of the main indicators used to evaluate environmental stress in plants (Cuevas-Reyes et al. 2018a). However, contrary to our prediction, based on the idea that secondary forests are hotter and drier sites and therefore present stressful conditions to plants, our results showed that in the three plant species analyzed, the FA levels were highest in mature forest in comparison with secondary forests. Thus, it is possible that the environmental conditions such as high radiation incidence and extremes of temperature and humidity of secondary forests do not act as stressor agents for the plant species studied. In fact, plant species of TDFs have optimal strategies for dealing with drought (e.g., a better water use efficiency) and are adapted to high temperatures (Pineda-García et al. 2013; Lohbeck et al. 2015). On the other hand, the vegetation of mature forest has higher density and larger sized trees that can promote greater dispute for light and soil nutrients. Thus, it is probable that resource limitations, especially light and soil nutrients, may be the stressor agents responsible for the highest FA observed in plant species of mature TDF (Alvarez-Añorve et al. 2012).

Moreover, another important result in this study is the fact that herbivory levels were higher in the mature forests, and in both forest conditions there was a significant relationship between herbivory and FA levels. A similar correlation has been shown in other ecological studies (Zvereva et al., 1997; Cornelissen et al. 2003), suggesting that FA also may serve as an indicator of plant susceptibility to herbivory (plant stress hypothesis) (Cornelissen and Stiling 2011), but also can indicate that herbivory itself acts as a plant stressor and directly increases the level of FA (herbivory-induced stress hypothesis) (Zvereva et al. 1997; Møller and Shykoff 1999).

Unfortunately, in our study, the positive correlation between foliar FA and herbivory is not enough to distinguish between both hypotheses, because the FA measurements were made after herbivory had occurred. Hence, for future studies, it is necessary to include analyses of nutritional quality and chemical defense in leaves undamaged by herbivory throughout the successional stages of tropical dry forest. Particularly, it is possible that individuals of *C. elaeagnoides* and *A. gracilis* experience more favorable developmental conditions in mature than in secondary forests considering that they had more elongated, wider and larger leaves in mature forests. As a result of these morphological differences, we hypothesize that in plants from the mature forests defense chemical compounds should be lower and foliar nutritional quality should be higher and, as a consequence, herbivorous insects respond with higher consumption rates in mature forest. This idea is supported by studies that have proposed that the variation in plant chemistry is a possible cause for these differences (Lempa et al. 2000; Bañuelos et al. 2004; Cornelissen et al. 2003; Cuevas-Reyes et al. 2011a, b, 2018a).

Herbivory is an antagonistic interaction that negatively affects the survival, growth and reproduction of plants (López-Carretero et al. 2014). However, plants have evolved physical, nutritional and chemical defenses to prevent the loss of plant tissue by herbivores (Coley et al. 1985). Previous studies have demonstrated that herbivory levels vary along successional gradients due to variations

Table 2 Spearman's correlation coefficients of total leaf area, herbivory and fluctuating asymmetry (**$P < 0.05$)

	Total leaf area	Herbivory	Fluctuating asymmetry	
(a) *Cordia elaeagnoides*				
Total leaf area (cm^2)	–	− 0.33	0.001	Mature forest
Herbivory (%)	− 0.01	–	− 0.03	
Fluctuating asymmetry	0.14	0.46**	–	
Secondary forest				
(b) *Cordia alliodora*				
Total leaf area (cm^2)	–	− 0.09	− 0.05	Mature forest
Herbivory (%)	− 0.17	–	0.36**	
Fluctuating asymmetry	− 0.08	0.10	–	
Secondary forest				
(c) *Achatocarpus gracilis*				
Total leaf area (cm^2)	–	− 0.22	− 0.03	Mature forest
Herbivory (%)	0.06	–	0.02	
Fluctuating asymmetry	0.04	0.35**	–	
Secondary forest				

C. elaeagnoides (a), *C. alliodora* (b) and *A. gracilis* (c) in mature and secondary forests

in herbivory abundance, chemical defenses of plants and high temperatures that interfere with the plant defensive system and physiological processes (Brown et al. 1987; Bach and Tabashnik 1990; Silva et al. 2012; González-Esquivel et al. 2019). For example, plant species of secondary forests tend to be more consumed as a result of higher abundance of herbivore insects and lower chemical defenses. In this way, the resource availability hypothesis proposes that plant chemical defense varies with the successional position of species (Coley et al. 1985). Because plant species of mature forests are characterized by slow growth in low light environments, they produce more chemical defenses because it is less costly to produce them than to replace lost tissues by herbivory. Therefore, these species are well defended against herbivores and other factors that cause plant damage. In contrast, plant species of secondary forests growing in habitats of high-light availability (e.g., gaps) invest more resources in replacing lost tissues by herbivory than in chemical defense. This idea is in agreement with our results, since both species of *Cordia* had higher herbivory levels in secondary than in mature forests. Contrary to this, *A. gracilis* had higher levels of herbivory in mature forest condition. This result could be due to differences in leaf nutritional quality between individuals of mature and secondary forests (bottom-up effects), which can be influenced by differences in soil fertility (Cuevas-Reyes et al. 2004). In our study, the content of nitrates and phosphates was higher in soils of secondary forests. Nitrates are often limited and represent an important structural component of proteins, nucleic acids and many secondary metabolites of plants, while an increase of availability of phosphates is associated with a high nutritional quality of plants (Patterson et al. 2010). Therefore, to explain our results, we invoke the soil fertility hypothesis that proposes that low soil fertility, indicated by low contents of phosphorous, has been associated with higher herbivore incidence considering that plant species that grow under conditions of infertile soils tend to have lower rates of growth and accumulate higher concentrations of secondary metabolites such as oils, phenols, alkaloids and terpenoids affecting the herbivory levels (Fernandes and Price 1991; Fernandes et al. 1994; Cuevas-Reyes et al. 2004).

In conclusion, our study shows changes in morphological leaf traits along regeneration processes and their effects on herbivory patterns in plant species of tropical dry forests. We detected differences in foliar morphology, fluctuating asymmetry and herbivory between mature and secondary forests. The general pattern showed higher levels of FA in mature forests. Our findings illustrate the difficulty associated with understanding the relationship between FA and herbivory throughout the regeneration process of tropical dry forests due to the complexity of abiotic and biotic factors involved that affect plant–herbivore interaction.

Author contribution statement PCR, JAP and YML proposed the ideas and design of the study. LDAC and MAA provided information of the biotic and abiotic characteristics of the study sites. JAP contributed to the sampling and estimates of total leaf area, herbivory and fluctuating asymmetry. JAP, PCR and YML wrote the manuscript. JAP, MF, MLF and AGR performed the statistical analysis and helped discuss the manuscript. All authors participated in the review of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors mentioned above declare that there is no conflict of interest associated with this manuscript. The work represents an original research carried out by the authors. All authors agree with the contents of the manuscript and its submission to the journal.

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CAPÍTULO II.

Contrasting successional stages lead to intra- and inter-specific differences in leaf functional traits and herbivory levels in a Mexican tropical dry forest

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ORIGINAL PAPER

Contrasting successional stages lead to intra- and interspecific differences in leaf functional traits and herbivory levels in a Mexican tropical dry forest

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Abstract

We analyzed leaf functional traits, chemical defenses and their effects on herbivory in three plant species that occur at two contrasting successional stages: mature and secondary forests in Mexico. In each successional stage, 15 individuals of *Cordia elaeagnoides*, *C. alliodora* and *Achatocarpus gracilis* were selected to analyze physical and chemical defenses and herbivory. Chlorophyll content, leaf thickness, leaf water content and leaf fresh mass were higher in mature forests, whereas SLA was higher in secondary forests, partially supporting the prediction that leaf traits vary from conservative to acquisitive along the leaf economics spectrum during succession. Content of secondary compounds did not show a consistent pattern among successional stages, but a relatively consistent trend was observed for *C. elaeagnoides* and *A. gracilis*, which exhibited higher levels of phenols and flavonoids in mature forests. Herbivory was higher in secondary than in mature forests for *Cordia* species, but the opposite pattern was found for *A. gracilis*. These results contradict the predictions of the resource availability hypothesis for tropical dry plant species, which state that resource-conservative plants in secondary forests invest more in chemical defenses and have less herbivore damage than resource-acquisitive plants in mature forests. Such idiosyncratic responses make it difficult to use a single hypothesis to predict plant defense and herbivory variations along environmental gradients at both intra- and interspecific levels. Considering the current intensity of human disturbance in forests, further studies are necessary to evaluate the impacts of changes in leaf functional traits on plant fitness during succession in the tropics.

Keywords Functional traits · Resource availability hypothesis · Herbivory · Secondary metabolites · Successional stages

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Introduction

Through evolutionary history, plants have developed the ability to acclimate to different environmental conditions, frequently resulting in changes in leaf functional traits across different habitats (Falcão et al. 2015; Poorter et al. 2018). During forest secondary succession, colonization by pioneer plant species changes local conditions (Connell and Slatyer, 1977), in a feedback process that creates environmental filters limiting the establishment of late species (Lebrija-Trejos et al. 2010a, b; Letcher et al. 2015). In this phase, secondary forests (i.e., forests in regeneration through natural processes after clearance of the original mature forest) are very frequent and dominant in the landscape (Chazdon 2014). As succession progresses to mature forests (i.e., forest that has suffered little or null disturbance during the last 80–150 years) (Chazdon 2014), light availability decreases due to higher vegetation density and canopy coverage, with a consequent decrease in temperature and increase in air and soil humidity (Lohbeck et al. 2015; Maza-Villalobos et al. 2011; Pineda-García et al. 2013). Thus, secondary and mature forests usually have different species composition with contrasting functional traits because of the filtering process imposed by changing environmental conditions (Alvarez-Añorve et al. 2012).

However, some plants species are capable of bypassing this filter and occupy multiple successional habitats. This acclimation can occur through natural genetic variation and/or phenotypic plasticity (Falcão et al. 2015; Huang et al. 2009). Plant functional traits can change quickly due to phenotypic plasticity, allowing temporary specialization to changing environmental conditions (Agrawal 2020) and favoring those individuals from the same species that remain in a regenerating forest. On the other hand, within-species trait variation among different habitats could also arise through contrasting dispersal or establishment abilities of locally adapted genotypes (Read et al. 2014; Tiffin and Ross-Ibarra 2014) in mature versus secondary forests. In any case, it is expected that individuals from the same plant species will exhibit a different set of functional leaf traits (i.e., leaf syndromes; Agrawal 2020) according to the successional habitat where they occur.

The variations on leaf functional traits across environmental gradients are frequently explained within the framework of the worldwide leaf economics spectrum (WLES; Wright et al. 2004; Donovan et al. 2011). The WLES describes a consistent pattern of correlations among leaf traits that summarizes plant ecological strategies ranging from acquisitive (i.e., plants with thin leaves and reduced self-shading of chloroplasts resulting in high nitrogen content and photosynthetic capacity) to conservative in terms of resource use (i.e., the opposite traits) (Díaz et al. 2016; Donovan et al. 2011). Some of the leaf functional traits that differ between successional habitats are directly or indirectly involved in plant resistance to herbivory, such as physical (e.g., thickness, toughness) and chemical (e.g., carbon and nitrogen-based secondary compounds) defenses against herbivores, while other functional traits such as specific leaf area (SLA) are related to plant height and the relative growth rate (Pérez-Harguindeguy et al. 2016; De la Riva et al. 2018). According to the resource availability hypothesis (RAH; Coley et al. 1985), fast-growing plants in resource-rich, early successional habitats (i.e., high availability of light, water and soil nutrients) invest more resources in growth and replacement of lost tissues by herbivory than in producing defenses. On the other hand, slow-growing plants in late, resource-poor environments invest more in carbon-based defenses (e.g., phenols and flavonoids), because the production of these compounds is less expensive than generating new tissues (Boege and Dirzo 2004; Coley 1988). Under this hypothesis, originally derived from studies in tropical wet and boreal forests (Coley et al. 1985), plants that occur in early stages of secondary succession would present acquisitive strategies (e.g., higher leaf area, specific leaf area and chlorophyll content), whereas those present in mature forests would exhibit conservative strategies (e.g., higher leaf thickness and chemical defense production (Endara and Coley 2011).

However, recent studies suggested opposite directions of change in plant functional traits along successional gradients for tropical wet and dry forests (Buzzard et al. 2016; Fonseca et al. 2018; Lebrija-Trejos et al. 2010b; Lohbeck et al. 2013). In tropical wet forests, where the main environmental filter during succession is light availability (Guariguata and Ostertag, 2001; Schönbeck et al. 2015), functional traits are expected to change as predicted by the RAH. On the other hand, in tropical dry forests (TDFs), water stress imposes stronger constraints on leaf functional traits. Thus, TDF plants in hot and dry conditions of early secondary succession would present traits to tolerate drought (i.e., conservative traits), by reducing the total and the specific leaf area and increasing density, thickness, dry matter content (Alvarez-Añorve et al. 2012; Jimenez-Rodríguez et al. 2018) and carbon-based defenses (Wright and Westoby 2002) (see Fig. 1). As the canopy closes, humidity increases in mature TDFs (Lebrija-Trejos et al. 2011), selecting for drought-avoidance, acquisitive functional traits related to high photosynthetic rates and nitrogen and phosphorus contents (Agrawal 2020; Lohbeck et al. 2013).

Although a convergence on plant functional traits is expected at the community level due to environmental filtering, different plant species may exhibit contrasting patterns of trait change along succession, influenced by growth form, ontogenetic stage, life history strategies and phylogenetic

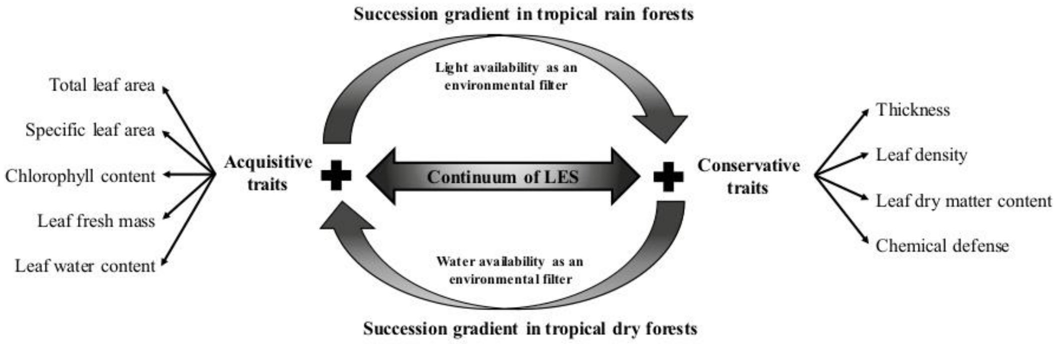

Fig. 1 The conceptual framework of the leaf economic spectrum (LES) and the expected changes in leaf functional traits throughout succession progress in tropical rainforest (TRF) and tropical dry forest (TDF) plant species in accordance with resource availability hypothesis (RAH) (Coley et al. 1985). For TRF, leaf traits change from acquisitive to conservative, while for TDF the foliar attributes change in the opposite sense from conservative to acquisitive

constraints, among others (Ding et al. 2012; Letcher and Chazdon 2012; Uriarte et al. 2016). Some studies have reported a phylogenetic signal for functional traits during community assembling, with closely related species with similar traits clustering according to the successional stage (Ding et al. 2012; Maza-Villalobos et al. 2020; Muscarella et al. 2016). The aim of the present study was to compare leaf physical and chemical traits putatively related to plant defense and herbivory of three woody species that occur in both mature and secondary TDFs in Chamela-Cuixmala, in Mexico: the congeneric trees *Cordia elaeagnoides* and *C. alliodora*, and the shrub *Achatocarpus gracilis*. We also investigated the effects of leaf traits, including chemical defenses, on herbivory levels in each successional stage. We asked the following questions: (i) Do leaf functional traits and herbivory vary between individuals from the same species in mature and secondary forests? (ii) Are the patterns of leaf traits and herbivory variation between stages consistent across different species? and (iii) Do variations in leaf functional traits affect herbivory levels? We expect a change from conservative to acquisitive leaf traits and an increase in herbivory from secondary to mature forests for all three species as a consequence of environmental filtering, regardless of phylogenetic relatedness or life history differences.

Materials and methods

Study area

The study was conducted in the Pacific coast region at the southwest of Jalisco state, Mexico, in the Chamela-Cuixmala Biosphere Reserve (19°22′–19°35′N, 104°56′–105°03′W) and surrounding areas (see Supplementary Material, Fig. S1). This region has a warm, subhumid tropical climate, with mean annual temperature of 24.6 °C. The mean annual rainfall is 788 mm and occurs mostly (80%) during the rainy season (July to October) (García-Oliva et al. 2002). The predominant vegetation is tropical dry-deciduous forest (Rzedowski 1978). Surrounding the reserve, many areas are under secondary succession after land abandonment by peasants, who previously cleared the TDFs using the slash-and-burn technique in order to grow crops and establish pastures for livestock (Sánchez-Azofeifa et al. 2009).

Study sites

Eight permanent TFD study sites were selected: four inside the Chamela-Cuixmala Biosphere Reserve, without anthropogenic alterations in at least the last 60 years (mature forests), and four sites outside the reserve (secondary forests) that had between 3 and 8 years of abandonment in the sampling period (2018) and were previously used as cattle pastures (Avila-Cabadilla et al. 2012). Early succession sites had a similar history of management which consisted in the elimination of native vegetation by slash-and-burn. Afterward, the cleared areas were used for agriculture and livestock from 3 to 5 years. Finally, the study sites were abandoned by peasants, and the secondary succession process started (Jimenez-Rodríguez et al. 2018). We performed a spatial autocorrelation analysis using the Moran's I coefficient, which measures the degree of correlation between the values of a particular variable as a function of spatial location (Deléglise et al. 2011; Fu et al. 2011). Thus, this coefficient indicates whether the data has a scattered, clustered or random spatial distribution pattern. The Moran's values range from − 1 to 1, where values close to 1 and -1 indicate high positive and negative spatial autocorrelation, respectively, and values close to 0 represent a random spatial distribution (i.e., no spatial relationship) (Fu et al. 2014). The values of Moran's I coefficient can be visualized in a scatterplot where the x-axis is represented by the original variable studied, the y-axis represents the spatially lagged variable and the slope of the linear fit is the coefficient value (Bonada et al. 2011). For this analysis, a distance-weight matrix with the variables of the x- and y-coordinates was used as the east and north coordinates, as well as a distance band estimated automatically from the inverse of the distance using the GeoDA 1.8 software (Fu et al. 2011, 2014).

The values of the Moran's I coefficient were close to zero for all leaf functional traits (TLA = − 0.22, CC = − 0.18, LT = − 0.23, LWC = − 0.19, SLA = 0.0.28, LD = − 0.29, LDMC = − 0.14, LFM = − 0.16), secondary metabolites (phenols = − 0.27, flavonoids = − 0.21, alkaloids = − 0.19) and the PLAR (PLAR = − 0.019) for the three studied species. This result indicates a random spatial distribution of our data and confirms that all analyzed study sites can be considered independent data points in all statistical analyzes.

Because the plant community composition and leaf traits could be affected by topographic variables, the eight TDF study sites were at 143 m.a.s.l. and predominantly orientated to the south and southeast (Balvanera et al. 2002), with slopes of 10° on average. In general, mature forests were taller and presented a higher number of individuals, branches and strata than secondary forest (Supplementary Material, Fig. S2). Additionally, a higher level of soil humidity was observed in mature forests in comparison to secondary forests (Supplementary Material, Fig. S3). Conversely, the soils of secondary forests had a higher concentration of NH_4^+, NO_3 and PO_4 than soils of mature forests (Supplementary Material, Fig. S4). A full characterization of successional differences in forest structure and soil traits is given in Supplementary Material.

Study species

We selected three plant species that are deciduous woody plants very abundant in both successional and mature forests: *Cordia elaeagnoides* A. DC. (Boraginaceae), *C. alliodora* (Ruíz and Pav.) Oken and *Achatocarpus gracilis* H. Walter (Achatocarpaceae). These plant species have similar vegetative phenological patterns, shedding their leaves during the dry season, and with massive leaf flushing in the beginning of the rainy season (Cuevas-Reyes et al. 2004; Frankie et al. 1974; Rzedowski 1978).

Cordia elaeagnoides A. DC. (Boraginaceae) is a deciduous tree distributed along the Pacific coast of Mexico (i.e., from Sonora to Chiapas) and through the Yucatán peninsula (POWO, 2019). It grows up to 20 m and its leaves are simple and arranged in spiral. Leaf shape is ovate-elliptical, with acuminate apex, entire margins and the base is slightly attenuated (Pennington and Sarukhán 2005).

Cordia alliodora (Ruíz and Pav.) Oken (Boraginaceae) is a deciduous tree distributed throughout the Neotropics, from Mexico to Argentina (POWO, 2019). It grows up to 25 m and its leaves are simple and arranged in spiral. The shape of its leaves is ovate-lanceolate, elliptical or oblong, sometimes oblanceolate, with acute or acuminate apex, entire margins and the base is acute or sometimes obtuse (Pennington and Sarukhán 2005).

Achatocarpus gracilis H. Walter (Achatocarpaceae) is a deciduous shrub distributed along the Pacific Coast of Mexico, from Sonora to Oaxaca (POWO, 2019). It grows up to 6 m and its leaves are alternate and glabrous. Leaf shape is obovate-lanceolate, with obtuse or flattened apex, entire margins and cuneate to slightly attenuated base.

Leaf functional traits

In each successional stage, 15 adult plants for each of the three plant species were marked and georeferenced (N = 30 individuals per species in total) (see Table S1 in the Supplementary Material). To analyze leaf functional traits, in each marked individual we randomly collected five mature, fully expanded leaves, without damage by herbivores from each canopy strata (upper, middle and lower; 15 leaves per individual, 225 leaves per successional stage) at the beginning of the growing season (July and August). In the laboratory, we analyzed traits associated with (1) water use: leaf water content (LWC, leaf fresh mass—leaf dry mass / total leaf area); (2) traits associated with light acquisition: relative chlorophyll content (CC), assessed by the SPAD-502 Plus Meter (Konica Minolta Sensing Europe); SLA (leaf area/dry mass); and (3) traits associated with nutrient use (i.e., differential allocation of acquired resources for plant survival and growth functions; Aerts and Chapin 2000), and conservation (i.e., when plants prioritize survival over growth, resulting in the preservation of well-protected tissues): leaf area (LA) was obtained with the ImageJ program (Rasband 2006) from digitalized images; leaf thickness (LT) was obtained using a Vernier caliper; leaf density (LD, dry mass/leaf area × leaf thickness); leaf fresh mass (LFM, leaf fresh mass/leaf area); leaf dry matter content (LDMC, leaf dry mass/leaf fresh mass). To obtain the leaf fresh mass, the leaves were weighed after sampling, then oven-dried at 70 °C for 72 h and weighed again to obtain leaf dry mass (Jimenez-Rodríguez et al. 2018; Pérez-Harguindeguy et al. 2016).

Plant chemical defense traits

To analyze leaf secondary compounds, we arbitrarily selected other 10 fully expanded, intact leaves from each canopy strata (upper, middle and lower; 30 leaves per individual, 450 leaves per successional stage) at the beginning of the growing season (July and August) from the same individuals sampled for the functional traits analyses. These leaves were collected and transported to the laboratory in liquid nitrogen and kept under—80 °C until analyses of phenols, flavonoids and alkaloids (Maldonado-López et al. 2014). Phenols are an extensive group of carbon-based compounds that have functions associated with cell protection against chain reactions of free radicals produced by some chemical reactions and UV radiation, as well as defense against pathogens and herbivorous insects (Marchiosi et al. 2020). Similarly, flavonoids are secondary metabolites derived from phenols that act in various physiological and ecological functions, protecting against biotic and abiotic stressors such as UV radiations, pathogens and herbivorous insects (Rausher 2006; War et al. 2012). Finally, alkaloids are a diverse array of nitrogen-based secondary metabolites, which principal function is to defend against pathogens and herbivorous insects (Matsuura and Fett-Neto, 2015).

To determine the total content of soluble phenolics, a modified Folin–Ciocalteu technique was used (Pascual-Alvarado et al. 2008). Three samples of fresh leaf tissue (each one of 150 mg) were weighed from each individual, and every sample was grounded in liquid nitrogen. Ten milliliters of ethanol at 80% was added to each sample and centrifuged at 3000 rpm for 10 min. Then, 1 mL of supernatant was taken and mixed with 7 mL of deionized water. Subsequently, 0.5 mL of 2 N Folin–Ciocalteu reagent was added, and after 8 min, 1 mL of Na_2CO_3 at 20% was added. Finally, after 1 h of incubation in complete darkness, we measured the absorbance at 760 nm on an UV–Vis Genesys 10S spectrophotometer. The concentration of total phenolics was calculated from a standard curve of tannic acid (Maldonado-López et al. 2014).

To determine the level of flavonoids, we used the technique described by Wu and Ng (2008) with modifications (Tan et al. 2014). Three samples of 150 g of fresh leaf tissue were weighed for each individual, and each sample was grounded in liquid nitrogen. Five milliliters of aqueous acetone at 70% was added and shook for one hour at room temperature. Then, samples were centrifuged at 3000 rpm for 10 min and the supernatant was transferred to a new test tube. This procedure was repeated three times for each sample, to perform a total of three extractions for each sample. Subsequently, an aliquot of 0.5 mL was placed in a new test tube and added 0.3 mL of $NaNO_2$ at 5%. Later, 0.3 mL of $AlCl_3$ at 10% was added, and after 6 min, 0.8 ml of NaOH at 4% was added and mixed. Finally, absorbance was measured at 510 nm on an UV–Vis Genesys 10S spectrophotometer. The flavonoid content was calculated using a quercetin standard curve.

The total content of alkaloids was determined using the technique described by Somit et al. (2013) with modifications. Three samples of 150 g of dry leaf tissue were weighed for each individual, and each sample was grounded in liquid nitrogen. Five milliliters of acetic acid (CH_3COOH) at 20% in ethanol was added to each sample and macerated for 10 h. After that, the solutions were filtered with Whatman paper number 1 and the liquid phase obtained was placed in a water bath at 60 °C for one hour. Subsequently, ammonium hydroxide (NH_4OH) was added to each sample, and the samples were reposed for 12 h until the precipitate formation. After this time, a second filtration was performed with a Whatman paper number 1 which was previously weighed. After the filtrations, the liquid medium was discarded and each filter paper with its respective precipitate was dried in an oven and weighed again. The difference between the initial and final weights was used to calculate the alkaloid content for each sample.

Leaf herbivory

To evaluate leaf herbivory, we first removed three branches from three canopy strata (upper, middle and lower) for each individual, and then we randomly collected 25 leaves from each branch (Cuevas-Reyes et al. 2018) at the end of the growing season (September, October) (Jimenez-Rodríguez et al. 2018). Leaf herbivory (i.e., the removal of the leaf blade) by chewing insects was obtained from digitalized images of the collected leaves. We used the software ImageJ 1.51j87 to convert each digitalized image to black and white and measure the leaf area damaged by herbivorous insects (i.e., leaf area after herbivory). Then, we reconstructed each leaf damaged by delineating the margin and filling the leaf holes to obtain the total leaf area (i.e., leaf area before herbivory) (Cuevas-Reyes et al. 2013). The percentage of leaf area removed (PLAR) was calculated using the following formula: [(removed area / total area) × 100]. For statistical analyzes, we averaged PLAR for each individual. All values are given as mean ± standard error.

Statistical analyses

To deal with the nested design of our sampling (i.e., four study sites in each of the two forest successional stages), mixed-effect models were carried to test the effects of successional stage (mature vs secondary forest), plant species (*C. elaeagnoides* vs *C. alliodora* vs *A. gracilis*), and the interaction between successional stage × plant species on leaf functional traits (total leaf area, chlorophyll content, leaf thickness, leaf water content, specific leaf area, leaf density, leaf dry matter content and leaf fresh mass), plant secondary compounds (phenols, flavonoids and alkaloids), and the PLAR. Study sites were nested within the successional stage as random factor, and the successional stage, plant species and their interaction were considered as fixed factors (explanatory variables). We constructed a separate model for each of the 12 response variables (i.e., leaf functional traits, secondary compounds and PLAR). Finally, a Tukey post hoc test was performed to evaluate the differences on leaf functional trait and PLAR among successional stages for each plant species. Most variables were log-transformed to meet the normality and homoscedasticity of the residuals. The normality and the homoscedasticity were tested using a Kolmogorov–Smirnov and a Levene test, respectively, for all leaf functional traits, phenols, flavonoids, alkaloids and PLAR (Silva et al. 2015).

We evaluated the arrangement of the three plant species at the two successional stages according to their leaf traits using a principal component analysis (PCA). All individuals of three species and the covariation of all leaf traits and chemical compounds were considered. We obtained a PCA biplot with the two major axes of variation in which the individuals of each species per successional stage are positioned, allowing the detection of the covariation of leaf traits (Pineda-García et al. 2011).

We performed mixed-effect analyses of covariance (ANCOVA tests) to determine whether functional attributes and secondary compounds covariate with PLAR in both successional stages for each plant species separately (eleven mixed ANCOVA analyses were carried for each plant species) (Baskett and Schemske 2018). Successional stages, leaf traits and the interaction between successional stage × leaf traits were considered as independent variables, study site as a random factor and the PLAR was considered as response variable (Baskett and Schemske 2018). All statistical analyses were performed using JMP statistical software version 15.1.0 (SAS Institute Inc., Cary, NC, USA).

Results

We found differences in CC, LT, LWC, SLA, LFM and the PLAR by herbivorous insects between mature and secondary forests (Table 1). Individuals from mature forests had greater CC, LT, LWC and LFM than individuals of secondary forests. The opposite was observed for SLA and the PLAR (Table 1). However, for all leaf functional traits analyzed, a significant interaction between successional stage and plant species was detected, indicating that the effects of forest succession vary between the plant species (Table 1). Particularly, for *C. elaeagnoides* and *A. gracilis*, LWC, LFM, and total phenol and flavonoid concentrations were higher in mature than in secondary forests, but no significant variation was detected for *C. alliodora* (Fig. 2d, h, i and j). Similarly, TLA was higher in mature forests for *C. elaeagnoides* and *A. gracilis*, but the opposite was observed for *C. alliodora* (Fig. 2a). In mature forests, CC was higher in *A. gracilis*, whereas this leaf trait was higher in secondary forests for *C. alliodora* (Fig. 2b). LT and LDMC were higher in mature forests for *C. elaeagnoides* and *C. alliodora* (Fig. 2c, g). In contrast, LT was higher in secondary forests for *A. gracilis* (Fig. 2c). For the three plant species, SLA was higher in secondary forests. LD was higher in mature forests for *C. elaeagnoides* and *A. gracilis* in mature forests (Fig. 2f). *Cordia alliodora* exhibited variations in total alkaloid concentrations, which levels were higher in secondary forests (Fig. 2k).

The PCA of functional traits showed a clear separation of individuals from the secondary and mature forests, regardless of plant species (Fig. 3). Individuals from secondary forests were mostly positioned at the upper-left diagonal of the PCA biplot, associated mostly to SLA, although individuals of *C. elaeagnoides* were slightly to the right and affected by TLA, FL and AL. Individuals from mature forests were mostly positioned at the bottom-right side of the biplot. Individuals of *C. elaeagnoides* and *C. alliodora* were associated predominantly with LDMC and LT, and separated from *A. gracilis*, which was affected by LD, CC, LFM, LWC and PH (Fig. 3).

The PLAR differed significantly among mature (3.2 mean ± 0.3 SE) and secondary forests (6.1 ± 0.2). However, this pattern was not totally consistent for all the studied plant species. In *C. elaeagnoides* and *C. alliodora*, the PLAR was higher for individuals from secondary than mature forests (Fig. 2l). On the other hand, higher herbivory levels were observed for *A. gracilis* in mature forests (Fig. 2l). The results of mixed-effects ANCOVA for each species showed that PLAR only significantly covaried with LT for the three plants species in both successional stages (Table 2), with negative relationships in all cases (Fig. 4a–c).

Discussion

In general, our results indicated that the differences in leaf functional traits and PLAR among successional stages varied according to the plant species. First, TLA, LWC, LD and LFM values were higher in mature than in secondary forests for *C. elaeagnoides* and *A. gracilis*, whereas SLA was higher in secondary forests for the three plant species analyzed. These findings only partially support the prediction that, during TDFs succession, leaf traits would change from conservative to acquisitive along the leaf economics spectrum (Buzzard et al. 2016; Lohbeck et al. 2013). Second, we did not corroborate the predictions of the RAH for TDFs since *C. elaeagnoides* and *A. gracilis* had mainly lower concentrations of chemical defenses (flavonoids and phenolic content) and higher values of SLA in secondary forests. Third, we detected a lower concentration of phenols and flavonoids, as well as higher values of SLA and PLAR only for C. *elaeagnoides* individuals growing in secondary forests. This fact is in line with a tolerance strategy, in which the plant reduces the effects of tissues loss to herbivorous insects on its fitness through compensatory growth, resulting in the production of new leaves (Krimmel and Pearse 2016;

Table 1 Summary of the results of each mixed model testing the effects of successional stage (mature vs secondary forests), plant species (*Cordia elaeagnoides* vs *C. alliodora* vs *Achatocarpus gracilis*) and their interaction (successional stage × plant species) on leaf functional traits, secondary compounds and the percentage of leaf area removed

Response variable	Explanatory variable	numDF	denDF	*F*	*P*	R^2	Random effect variance
TLA (cm^2)	Successional stage	1	88	2.9	0.15	0.94	0.03
	Species	2	87	664.8	<0.0001*		
	Successional stage × Species	2	86	12.7	<0.0001*		
CC (SPAD units)	Successional stage	1	88	15.8	<0.007*	0.83	0.02
	Species	2	87	12.4	<0.0001*		
	Successional stage × Species	2	86	40.3	<0.0001*		
LT (mm)	Successional stage	1	88	19.4	<0.01*	0.89	0.01
	Species	2	87	58.1	<0.0001*		
	Successional stage × Species	2	86	21.1	<0.0001*		
LWC (g·cm^{-2})	Successional stage	1	88	67.4	<0.0008*	0.82	0.05
	Species	2	87	49.5	<0.0001*		
	Successional stage × Species	2	86	23.1	<0.0001*		
SLA (cm^2·g^{-1})	Successional stage	1	88	181.7	<0.007*	0.81	0.04
	Species	2	87	12.4	<0.0001*		
	Successional stage × Species	2	86	9.7	<0.0004*		
LD (g·cm^{-3})	Successional stage	1	88	8.4	0.07	0.83	0.01
	Species	2	87	1.412	0.25		
	Successional stage × Species	2	86	35.6	<0.0001*		
LDMC (mg·g^{-1})	Successional stage	1	88	12.5	0.05	0.90	0.05
	Species	2	87	38.9	<0.0001*		
	Successional stage × Species	2	86	6.2	0.003*		
LFM (g·cm^{-2})	Successional stage	1	88	155.9	<0.0003*	0.87	0.02
	Species	2	87	44.9	<0.0001*		
	Successional stage × Species	2	86	25.1	<0.0001*		
Phenols (mg·g^{-1})	Successional stage	1	88	5.1	0.07	0.82	0.10
	Species	2	87	40.936	<0.0001*		
	Successional stage × Species	2	86	5.2	<0.007*		
Flavonoids (mg·g^{-1})	Successional stage	1	88	5.2	0.06	0.88	0.11
	Species	2	87	36.2	<0.0001*		
	Successional stage × Species	2	86	6.6	<0.002*		
Alkaloids (mg·g^{-1})	Successional stage	1	88	0.38	0.56	0.80	0.01
	Species	2	87	5.4	<0.006*		
	Successional stage × Species	2	86	5.0	<0.009*		
PLAR (%)	Successional stage	1	88	11.7	<0.03*	0.89	0.07
	Species	2	87	0.79	0.45		
	Successional stage × Species	2	86	21.0	<0.0001*		

Total leaf area (TLA), chlorophyll content (CC), leaf thickness (LT), leaf water content (LWC), specific leaf area (SLA), leaf density (LD), leaf dry matter content (LDMC), leaf fresh mass (LFM) and percentage of leaf area removed (PLAR). R^2 indicates the strength of overall model. Random effect variance is the proportion of variance explained by study sites, which were considered as a random effect in each model

*Significant values

Gianoli and Salgado-Luarte 2017). There was also a lack of consistency in leaf functional traits values (except for SLA) and their effects on herbivory for the three species among successional stages. The PCA showed different functional strategies for each plant species, and we discuss these idiosyncratic differences in functional traits among successional stages at the intra- and interspecific level, considering differences in life history among the three plant species.

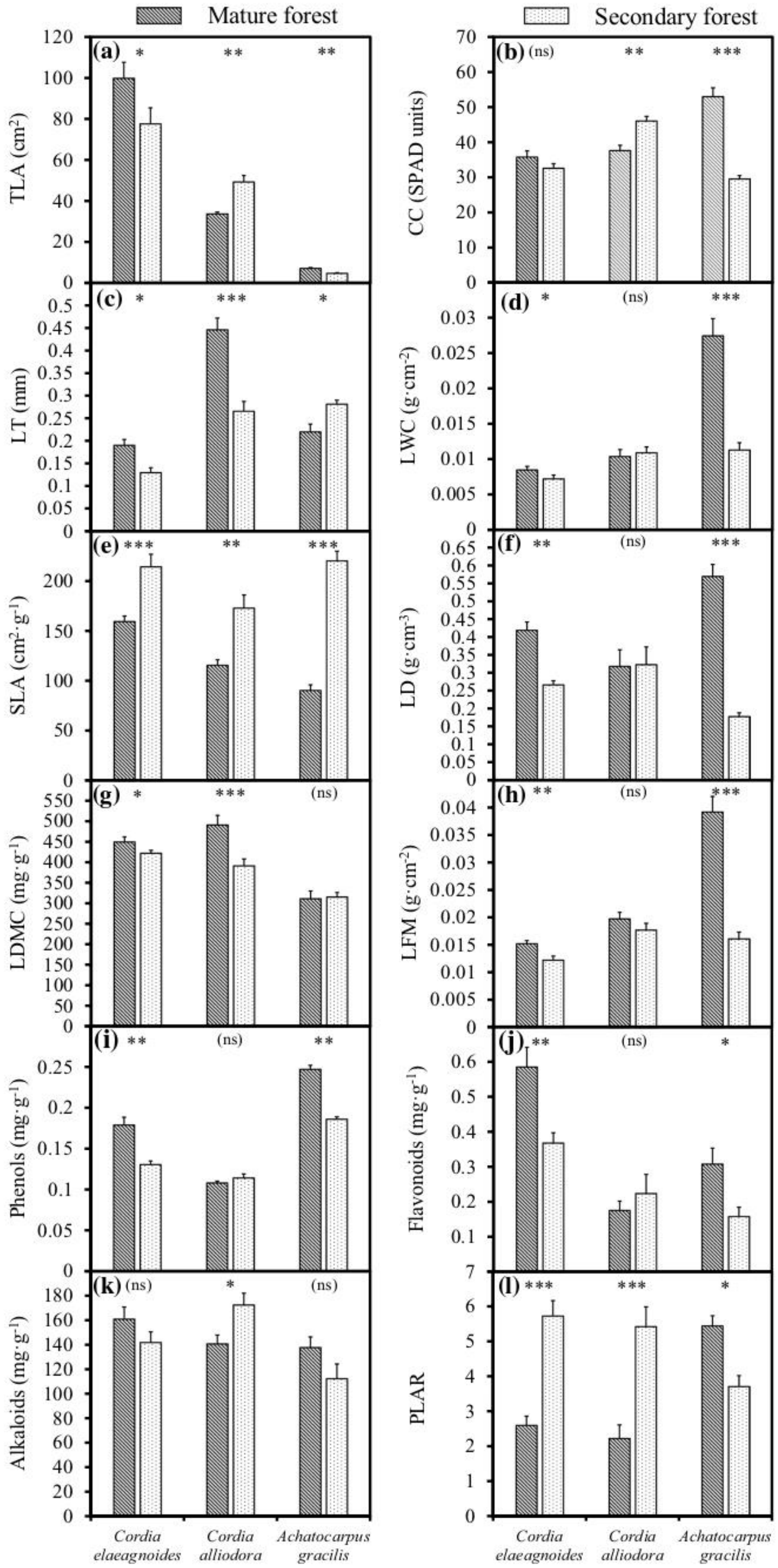

Fig. 2 Leaf functional traits, secondary compounds and percentage of leaf area removed (PLAR) for three plant species in secondary and mature forests. **a** Total leaf area (TLA), **b** chlorophyll content (CC), **c** leaf thickness (LT), **d** leaf water content (LWC), **e** specific leaf area (SLA), **f** leaf density (LD), **g** leaf dry matter content (LDMC), **h** leaf fresh mass (LFM), **i** phenols, **j** flavonoids, **k** alkaloids and **l** percentage of leaf area removed (PLAR). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and (ns) nonsignificant

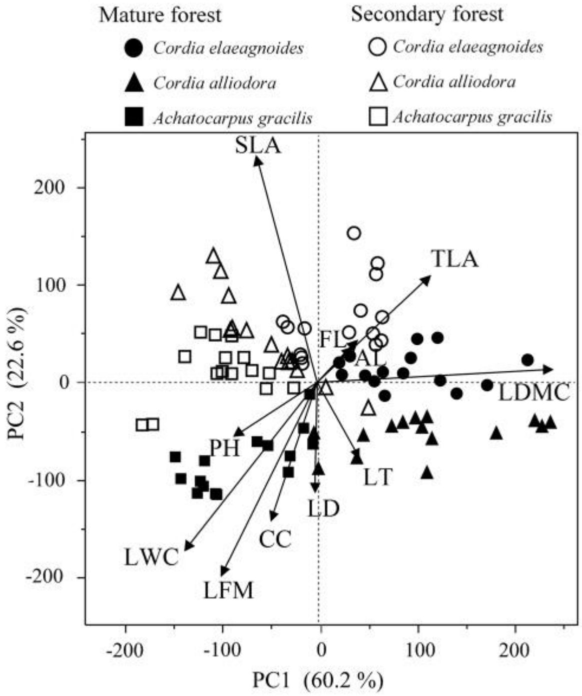

Fig. 3 Results of principal component analysis of leaf functional traits and secondary compounds on individuals of three plant species from two forest successional stages. Filled symbols represent individuals from mature forests, and open symbols represent those from secondary forests. TLA = total leaf area, CC = chlorophyll content, LT = leaf thickness, LWC = leaf water content, SLA = specific leaf area, LD = leaf density, LDMC = leaf dry matter content, LFM = leaf fresh mass, PH = phenols, FL = flavonoids, AL = alkaloids

Table 2 Results of the mixed-effects ANCOVA testing the effects of successional stage (mature vs secondary forests), leaf thickness and their interaction (successional stage × leaf trait) on the percentage of leaf area removed (PLAR). Only models in which the leaf trait covaried significantly with PLAR are shown

Variable response	Explanatory variable	$df_{num,den}$	*F*	*P*
(a) *Cordia elaeagnoides*				
PLAR	Successional stages	1, 28	9.89	0.0256*
	LT	1, 29	19.91	0.0002*
	Successional stages × LT	1, 29	3.39	0.0777
(b) *Cordia alliodora*				
PLAR	Successional stages	1, 28	0.03	0.0350*
	LT	1, 29	37.62	0.0001*
	Successional stages × LT	1, 29	2.23	0.1491
(c) *Achatocarpus gracilis*				
PLAR	Successional stages	1, 28	2.49	0.0177*
	LT	1, 29	14.90	0.0008*
	Successional stages × LT	1, 29	0.02	0.8832

*Significant values

Successional variations in functional traits

We observed significant differences in some leaf functional traits among successional stages for the three studied species, which may be related to phenotypic plasticity or genetic variation and allow these species to co-occur under contrasting environmental conditions. Most studies tested the conservative–acquisitive spectrum in TDFs and the RAH hypothesis along environmental gradients at the community level, sometimes with mixed results and only partial corroboration (Endara and Coley 2011; Fonseca et al. 2018; Lohbeck et al. 2013). At the intraspecific level, a review of common garden experiments by Hahn and Maron (2016) provided evidence for the lack of applicability of the RAH to predict changes in plant traits related to herbivory defense. In addition, a meta-analysis by Endara and Coley (2011) indicates that, when the effects of phenotypic plasticity are accounted for in studies that compared two or more species from different sites, little support to the RAH was observed. According to Hahn and Maron (2016), there are eight different hypotheses to explain the differences in plant defense: four predicts lower levels of defense in high-resource environments, three predicts the same response for low-resource environments and one predicts mixed results. Thus, it is possible that the counter-balancing influences of the different mechanisms proposed in each hypothesis would generate no clear patterns along environmental gradients (Endara and Coley 2011).

Some studies have already reported different patterns of phenotypic plasticity response to environmental conditions among species with different strategies (Grassein et al. 2010; Huang et al. 2009). Although our field study cannot clearly separate the effects of plasticity from genetic variation, a better understanding of the intra- and interspecific differences in plant functional traits along succession can be achieved by the analyses of each species life history. For example, *C. elaeagnoides* has thick but low-density stems, with thin vessel walls and xylem with high capacity to store water, indicating a low level of drought tolerance (Méndez-Alonso et al. 2012, 2013; Pineda-García et al. 2013). This is because species with high capability to store water in xylem, such as *C. elaeagnoides*, are highly susceptible to xylem embolism or cavitation at drier sites (Méndez-Alonzo et al. 2013). Pineda-García et al. (2013) demonstrated that, to deal with this, saplings of this species can survive at drier sites as secondary forests by reducing the leaf area to prevent water loss by transpiration, a conservative strategy that allows them to prevent hydraulic failure. Therefore, it is reasonable to think that trees of *C. elaeagnoides* in secondary forests reduce the leaf area, maintaining the xylem capacity to provide water

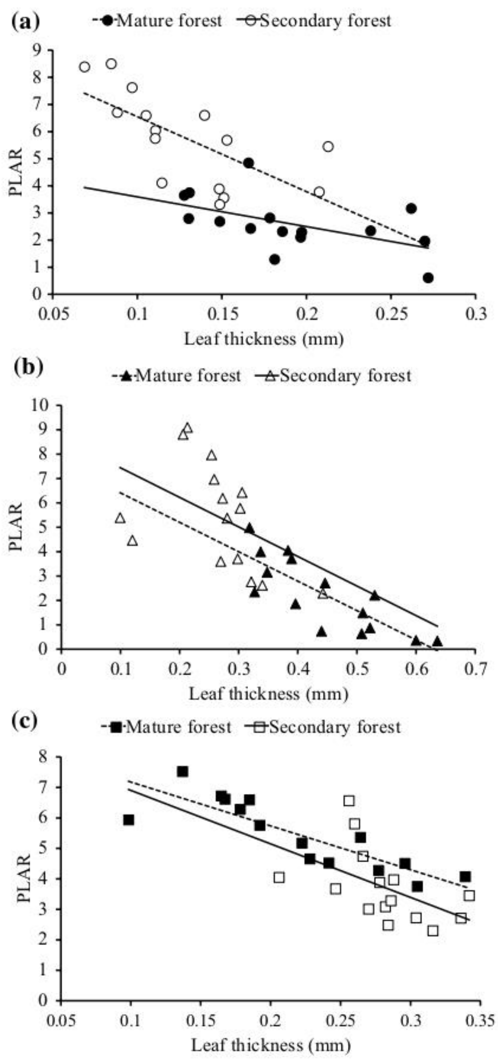

Fig. 4 Relationships between PLAR and leaf thickness for **a** *C. elaeagnoides* (mature forests: $R^2=0.28$, $P=0.03$; secondary forests: $R^2=0.45$, $P=0.005$); **b** *C. alliodora* (mature forests: $R^2=0.65$, $P=0.0003$; secondary forests: $R^2=0.21$, $P=0.04$); and **c** *A. gracilis* (mature forests: $R^2=0.73$, $P=0.0001$; secondary forests: $R^2=0.25$, $P=0.04$)

during short droughts (i.e., between 7 and 30 days) that occur during the rainy season in Chamela, maintaining the vital functions and surviving at those sites exposed to more desiccation (Pineda-García et al. 2013).

Although belonging to the same genus, the trends in functional traits observed for *C. alliodora* were different from *C. elaeagnoides*, with higher leaf area, SLA and chlorophyll content in individuals from secondary forest. Unlike *C. elaeagnoides*, this species has thin and high-density stems, thick vessel walls and low xylem water content (Méndez-Alonso et al. 2012, 2013). Pineda-García et al. (2013) stated that plant species with these traits exhibit high resistance to xylem embolism, allowing them to establish in secondary forests. Thus, these intrinsic characteristics and the increment in the mentioned leaf traits in secondary forests allow this species to grow fast in environments with high-resource availability (e.g., light and soil nutrients) due to low vegetation density and increased photosynthetic capacity, production of larger leaves, and fast nutrient absorption and growth (Coley et al. 1985; Lohbeck et al. 2013, 2015).

Achatocarpus gracilis exhibited differences among forest conditions for most of the leaf functional traits analyzed. The results obtained in this study agree with those reported by Alvarez-Añorve et al. (2012), who observed that plants in secondary forests tend to cope with the scarce water availability by developing leaves with low total area, but with high thickness. Individuals of *A. gracilis* showed the same pattern, besides presenting leaves with lower water content and fresh leaf mass in secondary forests to diminish water loss. Such changes may be a consequence of a lower water availability in this successional stage, which was observed for *C. elaeagnoides*. On the other hand, the increase in leaf area, chlorophyll content, leaf water content and fresh leaf mass, and the decrease in leaf thickness in mature forests, likely constitute an acclimation that confers *A. gracilis* a better acquisition of light in these sites light-limited habitats (Alvarez-Añorve et al. 2012; Poorter et al. 2009).

In general, the level of phenols and flavonoids was higher in mature forests for *C. elaeagnoides* and *A. gracilis*. These findings contradict our predictions for TDF plant species based on the RAH: that resource-conservative plants in secondary forests would invest more in chemical defenses and, therefore, would be less damaged by herbivores than resource-acquisitive plants in mature forests. On the other hand, our results partially corroborate other studies carried out in TDFs showing that water stress imposes restrictions on functional attributes associated with water loss in some early successional species, reducing the total leaf area and increasing leaf thickness and dry matter content (Alvarez-Añorve et al. 2012; Jimenez-Rodríguez et al. 2018).

Alternatively, the trends observed in the present study may be explained by the carbon-nutrient balance hypothesis, proposed by Bryant et al. (1983). According to this hypothesis, plants growing slowly in nutrient-limited (i.e., a high C/N ratio), highly competitive late successional stages, would accumulate carbohydrates that are directed to carbon-based defenses such as phenols and flavonoids (Bryant et al. 1983, 1987). Although we did not directly assess plant growth rates in the present study, the higher SLA and soil concentration of NH_4^+, NO_3 and PO_4 obtained for secondary forests suggest that plants are growing faster in this successional stage, where the C/N ration has been reportedly lower compared to mature forests (Huante et al. 1995; Poorter et al. 2018). In our study, the C/N ratio was slightly lower for secondary forests, although not statistically significant (see Figure S4 in Supplementary Materials). Under these conditions, carbohydrates would be used to growth, decreasing the level of carbon-based defenses. A concomitant investment in nitrogen-based defenses, such as alkaloids, would be expected in secondary forests (Bryant et al. 1983), but it was only observed for *C. alliodora*. Thus, as already indicated in previous studies (Hamilton et al. 2001; Endara and Coley 2011; Hahn and Maron 2016), it is unlikely that generalizations on habitat-driven differences in plant chemical defenses can be made at the intra- and interspecific levels based on a single hypothesis, especially under field conditions.

Functional traits and herbivory

Overall, the effects of successional stage on PLAR did not show a consistent pattern, since *C. elaeagnoides* and *C. alliodora* had higher PLAR in individuals from secondary than mature forests, and the opposite was observed for *A. gracilis*. Our results indicated that differences in the PLAR among successional stages were caused by the changes in leaf thickness, which was the only trait that significantly covaried (negatively) with PLAR in the mixed-effect models. LT is an indicator of leaf sclerophylly (Cuevas-Reyes et al. 2011; Lobregat et al. 2018) and is positively related to leaf dry mass (Niinemets 2001). Therefore, the positive association that we observed between LT and LDMC in the PCA for individuals of both *Cordia* species from mature forests may constitute a biomechanical resistance against herbivore damage (Lohbeck et al. 2014; Poorter et al. 2009). These traits are usually associated with leaf toughness because the volume of leaf tissue is occupied by both small cells with thick walls and by small intracellular spaces (Poorter et al. 2018), providing resistance to herbivory (Lohbeck et al. 2014; Poorter et al. 2018). Some studies have found that herbivore attack on host plants was mediated by soil nutrient availability (Cuevas-Reyes et al. 2004; Pires and Price 2000). Thus, the higher sclerophylly levels of *Cordia* species observed in late successional plants may be explained by a limitation on soil nutrients in these habitats, which in turn, decreased herbivory levels in comparison with those individuals from early successional stages. In case of *A. gracilis*, its increment in LT in secondary forest as a result of less water availability was reflected in the less PLAR.

On the other hand, some of the observed variation on the PLAR among secondary and mature forests may be related to differences in the herbivore community. In general, inconsistent patterns have been reported on insect herbivore diversity along successional gradients in tropical dry forests. Some studies reported no successional differences (Poorter et al. 2004; Boege et al. 2019), greater species richness of insect herbivores in late (Neves et al. 2014; Macedo-Reis et al. 2016) and early stages of succession (González-Esquivel et al. 2019; Silva et al. 2012, 2015). Also, the absence of a positive relationship between the herbivore diversity and the herbivory levels seems to be a common phenomenon along successional gradients (Campos et al. 2006; Ernest 1989; Silva et al. 2012; Varanda and Pais 2006) and can be explained in two different ways: (i) herbivore diversity is more influenced by climatic variables (i.e., temperature, air humidity, wind speed and insolation), plant community composition and structure, and complex multitrophic interactions (i.e., interspecific competition, predation, parasitism), whereas consumption rates depend more on plant quality (i.e., chemical defenses and leaf nutritional quality) and leaf palatability (physical defenses) (Basset et al. 2001; Denno and Perfect 1994; Didham and Springate 2003; Ødegaard 2003; Stiling and Moon 2005; Strong et al. 1984). Although we did not directly assess insect diversity in the present study, the lack of relationship between herbivore insect diversity and herbivory levels reported by previous studies (Rossetti et al. 2017; Silva et al. 2012) suggests that differences on PLAR levels detected for the three species considered here are most likely associated with plant defensive traits.

We conclude that leaf functional traits differences among TDF successional stages were better explained at intra- and interspecific level due to differences in life history between the three plant species analyzed. Despite the observed idiosyncrasies in leaf functional trait variations, we found partial support to the prediction that during TDFs succession, plant resource use strategy changes from conservative to acquisitive along the leaf economics spectrum. However, our findings contradicted the predictions of the RAH for TDFs, because two plants species in secondary forests did not have higher concentrations of chemical defenses and had a higher SLA and herbivory levels. In general, idiosyncratic responses of each plant species make it difficult to use a single hypothesis to predict plant defense and herbivory differences along environmental gradients at both intra- and interspecific levels simultaneously. Despite that, we showed that mechanical defenses, such as leaf sclerophylly, are important deterrents of herbivory levels in different successional stages, although this pattern should be confirmed using a larger set of plant species.

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Author's contributions PCR, LDAC, JSAP and MYAA contributed to the ideas and design research of this study. JSAP and MSVS collected the field data. RRC, KO and YML contributed to the design and direction of chemical methods to analyze foliar chemical defense. JSAP, MSVS and MF carried out statistical analysis. JSAP, PCR, KO, MMES and YML wrote the manuscript. All authors discussed the results and reviewed the manuscript.

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Data availability All data obtained in this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest No potential conflict of interest was reported by the authors.

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DISCUSIÓN GENERAL

En este estudio se encontró que individuos de las tres especies analizadas (i.e. *C. elaeagnoides*, *C. alliodora* y *A. gracilis*) presentaron diferencias en la morfología, tamaño y la asimetría fluctuante foliar entre bosques maduros y bosques secundarios. Particularmente, *C. elaeagnoides* y *A. gracilis* mostraron hojas de menor tamaño en bosques secundarios, cuya reducción podría ser consecuencia de ajustes en su morfología y fisiología para evitar la pérdida de agua en estos sitios que son más calientes y secos en comparación con bosques maduros (Alvarez-Añorve et al. 2012; Pineda-García et al. 2013). Contrariamente, *C. alliodora* presentó hojas más grandes en el bosque secundario como resultado de una mayor disponibilidad de luz en estos sitios, lo que produciría altas tasas fotosintéticas para obtener nutrientes rápidamente, crecer más rápido y generar hojas más grandes (Lohbeck et al. 2015). Esto sustenta la hipótesis de la disponibilidad de nutrientes (Coley et al. 1985), donde plantas de rápido crecimiento tienden a desarrollar hojas de mayor tamaño en bosques con una mayor disponibilidad de recursos como luz, nitrógeno y fósforo (Cole & Ewel 2006).

Contrario a lo esperado, los niveles de AF resultaron ser mayores en bosques maduros para las tres especies de plantas, descartando la idea de que las condiciones del bosque secundario (más calientes y secos) sean más estresantes para las especies de planas en estudio. Esto puede ser debido a que las plantas del bosque tropical seco están adaptadas a altas temperaturas y presentan estrategias para optimizar los recursos (e.g. mejor eficiencia en el uso del agua) y hacer frente a la sequía (Pineda-García et al. 2013; Lohbeck et al. 2015). De manera que, los mayores niveles de AF para las tres especies de plantas en bosques maduros podrían deberse a una mayor competencia por recursos (i.e. luz y recursos del suelo) al haber árboles más grandes y mayor densidad de estos, lo que se vería reflejado en una menor disponibilidad de recursos e incrementos en los niveles de estrés (Alvarez-Añorve et al. 2012). Además, se encontró que los niveles de AF se relacionaron positivamente con los niveles de herbivoría encontrados en individuos de bosques secundarios para *C. elaeagnoides* y *A. gracilis*, indicando que la AF podría ser un indicador de la susceptibilidad de las plantas ante la herbivoría (i.e. Hipótesis del estrés en plantas) (Cornelissen & Stiling 2011). Una explicación alternativa sobre la relación positiva entre los niveles de AF y herbivoría es que la herbivoría representa un agente que provoca estrés directamente en las plantas y por lo tanto, los niveles de AF se incrementan (i.e. Hipótesis del estrés inducido por herbivoría) (Møller & Shykoff 1999). Sin embargo, los cambios en morfología foliar para estas especies indican que las condiciones del bosque maduro serían más favorables al presentar hojas más largas, anchas y elongadas, aunque también podrían estar asociados a cambios en defensa química y calidad nutricional (Cuevas-Reyes et al. 2011a; Cuevas-Reyes et al. 2018a), lo que podría afectar también los niveles de daño foliar. Las relaciones positivas entre la AF y el daño foliar, ya sea porque los insectos previamente fueron atraídos por hojas más asimétricas (White 1984) o porque el daño generado por los insectos haya provocado mayor AF (Zvereva et al. 1997), nos indican que detrás de los niveles de AF pudieran haber diferencias en calidad nutricional o defensas químicas de las hojas, indicando el grado de susceptibilidad de las plantas y de palatabilidad por parte de los insectos herbívoros. Se destaca la relevancia de evaluar la AF en plantas a fin de determinar el grado de estrés presente y de susceptibilidad ante el ataque de insectos herbívoros.

La herbivoría afecta negativamente a las plantas, y se ha visto que esta interacción antagónica varía a lo largo de gradientes sucesionales como consecuencia de las diferencias en la abundancia de herbívoros y en las defensas de las plantas (Silva et al. 2012; González-Esquivel et al. 2019). Para el caso de nuestras especies en estudio, las diferencias encontradas en los porcentajes de daño foliar podrían deberse a factores bottom-up como cambios en defensas químicas y a calidad nutricional producidos por diferencias en la disponibilidad de recursos entre bosques maduros y secundarios, y a factores top-down, tales como diferencias en las abundancias relativas, riqueza, diversidad y composición de los insectos herbívoros entre bosques maduros y secundarios del BTS de Chamela, que ya se han sido reportadas previamente (Boege et al. 2019). Así, los mayores porcentajes de daño foliar encontrados en el bosque secundario para ambas especies de *Cordia* podrían deberse a una menor inversión en defensas químicas como producto de una mayor disponibilidad de nutrientes en estos sitios (Coley et al. 1985), y para el caso particular de C. *alliodora*, es posible que otros factores no evaluados en el presente trabajo también hayan influenciado en estos resultados, tales como el mecanismo de defensa indirecta que posee esta especie con sus hormigas simbiontes (Pringle et al. 2011). En tanto que *A. gracilis* mostró mayor daño foliar en bosques maduros, lo que podría ser consecuencia de cambios en calidad nutricional entre individuos de bosques maduros y secundarios, propiciados por diferencias en la fertilidad del suelo (Cuevas-Reyes et al. 2004), o por una mayor riqueza de herbívoros especialistas presentes en bosques maduros (Villa-Galaviz et al. 2012).

Por otro lado, se encontró que los atributos funcionales foliares y los porcentajes de daño foliar fueron diferentes entre las etapas sucesionales, dependiendo de la especie de planta. Así, se encontró que en boques maduros los valores de área foliar total, contenido de agua foliar, densidad foliar y masa fresca foliar fueron mayores en comparación a los bosques secundarios para *C. elaeagnoides* y *A. gracilis*, mientras que el área foliar específica fue mayor para las tres especies en bosque secundarios. Estos resultados parcialmente soportan la predicción de que los atributos foliares cambian de conservativos a adquisitivos a lo largo de la economía del espectro foliar durante la sucesión de los bosques tropicales secos (Lohbeck et al. 2013; Buzzard et al. 2016). Además, *C. elaeagnoides* y *A. gracilis* presentaron bajas concentraciones de defensas químicas (fenoles y flavonoides) y mayor área foliar específica en bosques secundarios, por lo que no es sustentada la predicción de la hipótesis de la disponibilidad de recursos para BTSs. Esta inconsistencia en los atributos funcionales, con excepción del área foliar específica, refleja diferentes estrategias para cada especie de planta, por lo que estas diferencias en atributos funcionales foliares son discutidas considerando la historia de vida de cada una de las tres especies de plantas analizadas.

Previos estudios han reportado patrones distintos en la plasticidad fenotípica como respuesta a las condiciones ambientales entre especies con estrategias distintas (Grassein et al. 2010; Huang et al. 2009). Si bien este estudio de campo no permite distinguir entre los efectos de la plasticidad de la variación genética, una mejor comprensión de las diferencias intra e interespecíficas en atributos funcionales de las plantas a lo largo de la sucesión es obtenida mediante el análisis de la historia de vida de cada especie. Se ha descrito que *C. elaeagnoides* presenta un bajo nivel de tolerancia a sequía al tener tallos delgados y de baja densidad, con vasos de paredes delgadas y un xilema con alta capacidad para almacenar agua (Méndez-Alonso et al. 2012; 2013; Pineda-García et al. 2013). Esta alta capacidad para almacenar agua en xilema convierte a las plantas como *C. elaeagnoides* en altamente susceptibles a cavitación o embolismo del xilema en sitios más secos (Méndez-Alonso et al. 2013). Para tratar con ello, se ha documentado que individuos juveniles de *C. elaeagnoides* reducen el área foliar para evitar la pérdida de agua por transpiración y sobrevivir en sitios más secos como bosques secundarios, una estrategia conservativa que los previenen de fallas hidráulicas (Pineda-García et al. 2013). En este sentido, es lógico pensar que árboles de *C. elaeagnoides* reducen el área foliar para mantener la capacidad del xilema y proveer de agua en sequías cortas (i.e. entre 7 y 30 días) que suceden en la estación de lluvias en Chamela, y así sobrevivir y mantener las funciones vitales en estos sitios más secos (Pineda-García et al. 2013).

En relación a *C. alliodora,* los cambios observados en atributos funcionales fueron distintos al de *C. elaeagnoides*, al presentar una mayor área foliar, área foliar específica y contenido de clorofila en individuos del bosque secundario. A pesar de que ambas especies pertenecen al mismo género, *C. alliodora* a diferencia de *C. elaeagnoides*, presenta tallos delgados y de alta densidad, con paredes gruesas de los vasos y bajo contenido de agua en el xilema (Méndez-Alonso et al. 2012; 2013), lo que le permite tener una alta resistencia a embolismo del xilema, permitiéndole establecerse en bosques secundarios (Pineda-García et al. 2013). Estas características intrínsecas de *C. alliodora*, acompañadas de un incremento en los atributos mencionados en condiciones del bosque secundario, le permiten crecer rápido en sitios con alta disponibilidad de luz y nutrientes del suelo, como consecuencia de una baja densidad en la vegetación y un incremento en la capacidad fotosintética, hojas más grandes, y un crecimiento y absorción de nutrientes más rápido (Coley et al. 1985; Lohbeck et al. 2013, 2015).

Con respecto a *A. gracilis*, esta especie mostró diferencias para casi todos los atributos foliares analizados entre ambas condiciones de bosque, los cuales coinciden con los reportados previamente en Alvarez-Añorve et al. (2012), donde se reportó que las plantas en condiciones de bosque secundario tienden a desarrollar hojas más chicas y más gruesas para afrontar la escasa disponibilidad de agua. Estos resultados indican que individuos de *A. gracilis* exhibieron el mismo patrón, acompañados también de un menor contenido de agua y de masa fresca foliar en bosques secundarios para reducir la pérdida de agua. En caso contrario, un decremento en grosor foliar y un incremento en área foliar total, contenido de clorofila, contenido de agua foliar y masa fresca foliar en bosques maduros, posiblemente sean parte de una aclimatación que permite a *A. gracilis* tener una mejor adquisición de la luz en esos ambientes con menor disponibilidad de este recurso (Alvarez-Añorve et al. 2012; Poorter et al. 2009).

Por otro lado, se encontró una mayor concentración de fenoles y flavonoides en el bosque maduro para *C. elaeagnoides* y *A. gracilis*, cuyos resultados contrastan la predicción basada en la hipótesis de la disponibilidad de recursos para plantas de los BTSs (i.e. plantas bosques secundarios serían conservadoras de los recursos e invertirían más recursos en la producción de defensas químicas y ser menos consumidas por herbívoros que las plantas presentes en bosques maduros, quienes mostrarían estrategias adquisitivas) (Coley et al. 1985). En consecuencia, los resultados pueden ser explicados alternativamente por la hipótesis del balance carbono-nutrientes (Bryant et al. 1983), la cual predice que plantas crecen lentamente en sitios limitados en nutrientes (i.e. con alta proporción C/N), son altamente competitivas en etapas sucesionales tardías, tendrían a acumular carbohidratos que serían asignados a defensas basadas en carbono (e.g. fenoles y flavonoides) (Bryant et al. 1983, 1987). Por el contrario, una mayor área foliar específica y concentración de NO_3^- y PO_4^{3-} en sitios de bosque secundario, sugiere que las plantas crecen más rápido en tal etapa sucesional, en las cuales se ha reportado que la proporción de C/N es inferior en comparación a bosques maduros (Huante et al. 1985; Poorter et al. 2018). Así, bajo condiciones de bosque secundario, los carbohidratos obtenidos serían empleados para crecer, produciendo una reducción en la concentración de defensas basadas en carbono. Por otro lado, una inversión en defensas basadas en nitrógeno tales como los alcaloides son de esperarse en bosques secundarios acorde a la hipótesis del balance carbono-nutrientes (Bryant et al. 1983), sin embargo, esto solo ocurrió en *C. alliodora*. Esto es debido a que una mayor disponibilidad de nutrientes en suelo promueve una mayor concentración de los mismos en los tejidos vegetales, resultando relativamente más barato producir defensas basadas en nitrógeno que en carbono (Bryant et al. 1983). Una mayor disponibilidad de nutrientes en suelo promueve un crecimiento más rápido, propiciando que los carbohidratos sintetizados sean mayormente asignados para esta función más que la producción de defensas basadas en carbono, por lo que las defensas basadas en nitrógeno toman una mayor relevancia. Como ya ha sido indicado en estudios previos (Hamilton et al. 2001; Endara & Coley 2011; Hahn & Maron 2016), es poco recomendado realizar generalizaciones o predicciones para explicar las diferencias en defensas químicas de las plantas a nivel intra e interespecífico basados en una única hipótesis, especialmente bajo condiciones naturales, ya que los factores limitantes o filtros ambientales pueden ser distintos entre ecosistemas y/o especies, generando que las predicciones puedan ser distintas en función del tipo de ecosistema o especie de planta.

Los porcentajes de daño foliar no presentaron un patrón general entre las etapas sucesionales debido a que ambas especies de *Cordia* presentaron mayores porcentajes de daño foliar en bosques secundarios, mientras que *A. gracilis* mostró lo contrario. Estos resultados sugieren que los cambios en los porcentajes de daño foliar pueden deberse a las diferencias en grosor foliar, cuyo atributo covarió negativamente con la herbivoría. El grosor foliar estuvo asociado positivamente con el contenido de materia seca foliar (Niinemets 2001) y es un indicador de esclerofilia (Cuevas-Reyes et al. 2011b; Lobregat et al. 2018). En este sentido, la asociación positiva entre el grosor foliar y contenido de materia seca, relacionada a individuos de las especies de *Cordia* del bosque maduro, puede constituir una resistencia biomecánica contra el ataque de insectos herbívoros (Poorter et al. 2009; Lohbeck et al. 2014). Estas hojas más esclerófilas de ambas especies de *Cordia* en condiciones de bosque maduro podrían ser el resultado de una limitación en los nutrientes del suelo, lo que su vez generó un decremento en los niveles de daño foliar con relación a los individuos presentes en bosques secundarios. Complementario a esto, es muy probable que no solo los atributos funcionales hayan influenciado en los niveles de herbivoría de *C. elaeagnoides*, sino que también los cambios en la comunidad de herbívoros asociada a esta especie, y es que de forma general se ha reportado que las comunidades de herbívoros son distintas entre bosques maduros y secundarios del BTS (Villa-Galaviz et al. 2012; Boege et al. 2019), factor que también pudo haber contribuido en las diferencias en los niveles de herbivoría para esta especie. Adicionalmente, las diferencias en los porcentajes de daño foliar entre bosques maduros y secundarios para *C. alliodora*, pudieron también haber sido influenciadas por cambios en un mecanismo de defensa indirecta que posee esta especie y que no fue evaluado en el presente trabajo. Y es que *C. alliodora* presenta un mutualismo simbiótico, principalmente con hormigas de la especie *Azteca pitiieri* en la región de Chamela (Pringle et al. 2011). En esta asociación, la planta produce estructuras especializadas en los nodos de las ramas, denominados domacios, en los cuales las hormigas se establecen y brindan protección a su planta hospedera, generando así una relación simbiótica con *C. alliodora* (Pringle & Dirzo 2011). En este sentido, estudios previos han demostrado que las hormigas mutualistas de *C. alliodora* ejercen un efecto positivo en sus plantas hospederas al influir negativamente en los niveles de herbivoría (Pringle et al. 2011) y en la diversidad de los insectos herbívoros de los árboles en que habitan (Pringle & Gordon 2013). A nivel regional se ha descrito que el daño foliar es menor cuando el tamaño de la colonia de hormigas es mayor (Pringle et al. 2011; Pringle & Gordon 2013), y que aquellos árboles de *C. alliodora* que poseen una mayor densidad de hormigas tienden a exhibir una menor abundancia y riqueza de herbívoros, y por ende menor daño foliar (Pringle & Gordon 2013). En otro estudio a nivel regional se determinó que en sitios de menor precipitación, árboles de *C. alliodora* tuvieron colonias más grandes de hormigas como resultado de una mayor inversión por parte de las plantas en sus hormigas, como respuesta a una mayor limitación del agua y riesgo de herbivoría (Pringle et al. 2013), sugiriendo que la intensidad del mutualismo es mayor cuando los recursos son más limitados. Si bien en el presente trabajo no fue evaluado el tamaño de las colonias de hormigas, es probable que individuos de *C. alliodora* que habitan en el bosque maduro posean un mayor número de hormigas en comparación a los del bosque secundario. Como ya discutimos anteriormente, una menor disponibilidad del agua en bosques secundarios no representa un factor limitante para árboles de *C. alliodora* desde que esta especie es resistente a sequía y posee una alta resistencia a embolismo (Pineda-García et al. 2013), por lo que partiendo de esto y de los antecedentes mencionados, es probable que la mayor limitación en los nutrientes del suelo en bosques maduros pudieran estar promoviendo un mayor tamaño de las colonia de hormigas en árboles que crecen en estos bosques en comparación a los del bosque secundario. Esto traería consigo una mayor intensidad en el mutualismo de *C. alliodora* con sus hormigas en el bosque maduro y estaría influyendo en conjunto con los atributos funcionales, en los menores niveles de herbivoría reportados para esta especie en esta condición de bosque. De ser cierta esta predicción, es también probable que esta incrementada intensidad de mutualismo que planteamos para bosques maduros haya influenciado en la menor riqueza de lepidópteros reportada para individuos de *C. alliodora* que habitan en estos bosques en comparación a los que crecen en bosques en sucesión secundaria (Villa-Galaviz et al. 2012). Esto es concordante, ya que Villa-Galaviz et al. (2012) reportó una menor riqueza de herbívoros en individuos de *C. alliodora* presentes bosques maduros en comparación con aquellos en sucesión secundaria, la cual pudiera ser afectada por la mayor densidad de hormigas que planteamos para los bosques maduros, y que esto a su vez y en conjunto con los atributos funcionales, estén determinando los menores niveles de herbivoría encontrados para *C. alliodora* en bosques maduros en el presente trabajo. Sin embargo, esto requiere investigación futura y detallada, a fin de determinar si el tamaño de las colonias de hormigas realmente cambia a lo largo de la sucesión secundaria y si esto afecta los patrones de herbivoría.

Respecto a *A. gracilis*, un mayor grosor foliar en bosques secundarios es el reflejo de una menor disponibilidad de agua, lo que resultó en una menor herbivoría. Sin embargo, las diferencias en el daño foliar entre bosques maduros y secundarios podrían estar asociadas a cambios en la comunidad de herbívoros. Aunque en el presente trabajo no evaluamos la diversidad de insectos, la falta de relación entre los niveles de herbivoría y diversidad de insectos en estudios realizados (Rosseti et al. 2017; Silva et al. 2012), apunta a que los cambios en los porcentajes de daño foliar para las especies analizadas en este estudio, probablemente estén más relacionados con atributos de defensa en plantas. Sin embargo, no descartamos que la identidad y/o riqueza de herbívoros hayan influido en nuestros resultados a la par de los atributos funcionales, y es que en un estudio previo (Villa-Galaviz et al. 2012) se encontró que la riqueza de orugas es mayor en bosques maduros que en secundarios para *A. gracilis*, lo que podría sugerirnos que esta mayor riqueza en bosques maduros pudiera haber favorecido el mayor porcentaje de daño foliar evaluado en estas condiciones.

En conclusión, el presente estudio demostró como el proceso de sucesión secundaria implica cambios en los atributos morfológicos y fisiológicos que ejercen efectos sobre los patrones de herbivoría en tres especies del bosque tropical seco al detectar diferencias en morfología foliar, asimetría fluctuante, atributos funcionales foliares, y porcentajes de daño foliar entre individuos de bosques maduros y secundarios del BTS de Chamela-Cuixmala. Las diferencias encontradas en atributos funcionales foliares entre etapas sucesionales del BTS fueron mejor explicadas al nivel intra e interespecífico por las diferencias en la historia de vida entre las tres especies estudiadas. Estas diferencias idiosincráticas en los cambios en atributos foliares sustentan parcialmente la predicción de que la estrategia de las plantas en uso de los recursos cambia de conservativas a adquisitivas a lo largo de la economía del espectro foliar durante la sucesión de BTSs. Por otro lado, los resultados contradicen la hipótesis de la disponibilidad de recursos para BTSs debido a que dos especies de plantas no presentaron mayores concentraciones de defensa química en bosques secundarios. Estas respuestas idiosincráticas por parte de cada una de las especies analizadas dificultan la aplicación de una sola hipótesis para predecir diferencias en defensa y herbivoría a lo largo de gradientes ambientales al nivel de intra e interespecíficos simultáneamente. Sin embargo, se muestra como defensas mecánicas como la esclerofilia, pueden contrarrestar la herbivoría en diferentes etapas sucesionales, aunque no se descarta que cambios en la comunidad de herbívoros entre bosques maduros y secundarios hayan influido también en los niveles de herbivoría reportados en este trabajo, o que en el caso particular de *C. alliodora*, sus hormigas simbiontes también hayan contribuido en las diferencias en los niveles de herbivoría reportados para bosques maduros y secundarios.

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