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“Relación entre la fenología foliar, caracteres funcionales y herbivoría en *Quercus castanea* (Fagaceae) a escala de paisaje”

TESIS

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Presenta

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A mis papás,

*Ustedes son mi mayor motivación, ya que a lo largo de
toda mi vida siempre me han impulsado a sacar la mejor versión
de mí misma, todo lo que soy ahora se los debo a ustedes,
los quiero infinitamente.*

A mi hermana Olivia,

Eres mi mejor amiga, mi mentora y por supuesto la mejor hermana de todas.

Tú presencia en mi vida es como un tesoro invaluable, te quiero muchísimo.

CONTENIDO

CAPÍTULO I: Landscape-level variation in spring leaf phenology is driven by precipitation seasonality in the Mexican red oak *Quercus castanea*. (Ver Anexo 1)

CAPÍTULO II: Associations of insect herbivory with leaf traits and phenology along an environmental gradient in *Quercus castanea,* a Mexican red oak.

I. RESUMEN GENERAL

El estudio de la fenología foliar, en conjunto con otros rasgos foliares de naturaleza ecológica, funcional y química, hace posible determinar estrategias adaptativas e historia de vida de las especies arbóreas y su variación a lo largo de gradientes ambientales. En el presente proyecto, analizamos el efecto que tiene la disponibilidad de agua a escala de paisaje en la variación de la fenología foliar de la especie de encino *Q. castanea.* Adicionalmente, determinamos cómo afecta dicha variación fenológica y ambiental a los atributos funcionales y químicos de sus hojas. Finalmente, se determinó cómo influyen los atributos foliares y fenológicos en la herbivoría de la especie y la importancia del ambiente en esta interacción. Para ello, se seleccionaron seis sitios en la cuenca de Cuitzeo, Michoacán a través de un gradiente hídrico, que va de 909 mm a 1299 mm de precipitación anual. Se colectaron muestras de hojas de 10 árboles adultos en cada sitio y los individuos se marcaron para llevar un registro de la fenología foliar. Con las hojas se realizaron análisis de herbivoría, químicos y de calidad nutricional. Se midieron algunos caracteres funcionales de las hojas. Se obtuvieron variables de temperatura y precipitación provenientes de las estaciones meteorológicas en el estado de Michoacán, así como también de la base de datos de Worldclim. Como resultado de 2 años de muestreo, hubo diferencias significativas entre poblaciones para un mismo año y para cada población entre ambos años con respecto a su fenología foliar. La mayoría de las poblaciones tuvieron un tiempo de desarrollo foliar menor en el segundo año (2022). La variación fenológica se correlacionó positivamente con la estacionalidad de la precipitación y negativamente con las precipitaciones de los meses más cálidos. Dicha variación también está estuvo asociada significativamente con el grosor foliar. Por otro lado, la fenología de las hojas y la precipitación anual fueron fuertes predictores de la herbivoría observada, así como el tamaño de las hojas. En conclusión, tenemos que para la especie *Q. castanea* la precipitación es un fuerte influyente en las respuestas adaptativas de la especie, lo que la hace una especie con gran fortaleza para enfrentar eventos de sequía y estrés hídrico futuros.

Palabras clave: Variación fenológica, interacciones, encinos, ambiente, rasgos foliares.

II. ABSTRACT

The study of leaf phenology, together with other ecological, functional and chemical leaf traits, makes it possible to determine adaptive strategies and life history of tree species and their variation along environmental gradients. In the present project, we analyzed the effect of water availability at the landscape scale on the variation of the leaf phenology of the oak species *Q. castanea*. Additionally, we determined how this phenological and environmental variation affects the functional and chemical attributes of its leaves. Finally, we determined how leaf and phenological attributes influence herbivory of the species and the importance of the environment in this interaction. For this purpose, six sites were selected in the Cuitzeo basin, Michoacán, across a water gradient ranging from 909 mm to 1299 mm of annual precipitation. Leaf samples were collected from 10 adult trees at each site and individuals were marked to keep a record of leaf phenology. Herbivory, chemical and nutritional quality analyses were performed on the leaves. Some functional characteristics of the leaves were measured. Temperature and precipitation variables were obtained from meteorological stations in the state of Michoacán, as well as from the Worldclim database. As a result of 2 years of sampling, there were significant differences between populations for the same year and for each population between both years with respect to their leaf phenology. Most populations had a shorter leaf development time in the second year (2022). Phenological variation was positively correlated with seasonality of precipitation and negatively correlated with precipitation in warmer months. It was also significantly associated with leaf thickness. On the other hand, leaf phenology and annual precipitation were strong predictors of observed herbivory, as well as leaf size. In conclusion, we conclude that for *Q. castanea*, precipitation is a strong influencer in the adaptive responses of the species, which makes it a species with great strength to face future drought and water stress events.

III. INTRODUCCIÓN

En los últimos años, ha sido de gran relevancia el estudio de las potenciales respuestas adaptativas, tanto de especies animales como vegetales, al cambio global. Debido a esto, ha surgido una importante preocupación sobre la capacidad que tienen los árboles para enfrentarse a los cambios en los patrones climáticos (Kremer, 2007; Aitken et al., 2008) causados por actividades humanas, que están ocurriendo a un ritmo rápido sin precedentes (IPCC, 2007). El estrés hídrico es uno de los factores abióticos selectivos más importantes en la naturaleza, y se espera que sus efectos aumenten significativamente en todo el mundo en los próximos años (Allen et al., 2010; Asner et al., 2016). La sequía tiene varias implicaciones ecológicas dentro de los ecosistemas debido a que limita el crecimiento, la supervivencia y la reproducción de las plantas, además afecta las distribuciones y la evolución adaptativa de las especies (Wright et al. 2009; Ordoñez et al., 2009).

La capacidad para hacer frente a la sequía es muy variable entre individuos que están sometidos a distintas presiones bióticas o abióticas (Aranda et al., 2014). La herbivoría es un factor que regula la respuesta a la sequía, aunque no de una manera evidente, y en ocasiones con respuestas ambivalentes. Por ejemplo, se ha observado que el desarrollo de raíces finas puede verse comprometido por la defoliación y reducir a medio y largo plazo la capacidad para absorber agua y sobrevivir en condiciones de sequía, en especies de encinos europeos como *Quercus robur* y *Q. petraea* (Gieger y Thomas, 2002).

Por otro lado, las plantas tienen una gran variedad de rasgos adaptativos que les permiten tolerar diversas formas de estrés impuestas por el medio que les rodea. La teoría de inversión de recursos de Coley (1996) es la explicación más prominente de la forma en que las presiones de selección abióticas dan forma a las adaptaciones de las plantas. Dicha teoría establece que las especies que crecen bajo una limitación de recursos tenderán a invertir más en mecanismos de resistencia o tolerancia al estrés que en el crecimiento mismo de la planta, mientras que ocurre al contario en plantas que crecen en ambientes con una alta disponibilidad de recursos.

Entre los rasgos adaptativos más importantes de muchas especies de plantas se encuentra la fenología, el cual es probablemente uno de los rasgos más influidos por el clima (Bertin, 2008). La fenología vegetal se refiere a la producción periódica de las estructuras vegetativas y reproductivas como resultado de interacciones entre factores bióticos y abióticos que determinan el tiempo más adecuado para el crecimiento y la reproducción de las plantas (van Schaik et al., 1993). La variación en el momento de la formación de los brotes foliares y su apertura también dará como resultado una sincronía diferente con los herbívoros de insectos y los patógenos fúngicos, por lo que las interacciones ecológicas de una determinada especie también se verán afectadas por cambios en la fenología (Van Asch et al., 2007; Ghelardini y Santini, 2009). Por lo tanto, la fenología es un indicador prometedor de los impactos del calentamiento global a nivel de las comunidades y los ecosistemas (Badeck et al., 2004; Hernández-Calderón et al., 2013).

El conocimiento de la variación a escala local de los rasgos de la hoja y sus relaciones para una sola especie podría ser esencial para ampliar el conocimiento de los procesos ecofisiológicos desde la hoja hasta el nivel de ecosistema (Navas et al., 2010). La información cuantitativa de la mayor cantidad posible de rasgos y relaciones foliares, de naturaleza ecológica, funcional y química, en una sola especie, puede proporcionar una mejor idea de cómo las plantas se adaptan a las diferentes presiones ambientales. Los rasgos foliares tienen el potencial de vincular la ecología de comunidades y la ecología fisiológica al delinear las compensaciones (trade-offs) que definen las estrategias de historia de vida, particularmente a lo largo de los gradientes ambientales, que pueden influir en las tasas demográficas de las poblaciones (McGill et al., 2006). Por lo tanto, una mejor comprensión de la fuente de variación de los rasgos funcionales, junto con los vínculos entre la aptitud de los árboles y los rasgos funcionales, mejorará en gran medida nuestra capacidad para modelar y predecir las respuestas de los bosques en condiciones cambiantes (de Andrés et al., 2024).

Variación fenológica en respuesta al ambiente

La variación fenológica en plantas ha sido un indicador de las respuestas ecológicas al cambio climático. La capacidad de respuesta fenológica a la variación espacial del clima puede corresponder a la capacidad de una especie para rastrear el cambio climático, así como tener una mayor sensibilidad al clima, se ha relacionado con un mayor desempeño de las especies (mayor crecimiento) en el contexto del cambio climático (Cleland et al., 2012). Los datos fenológicos que se miden a lo largo de un tiempo determinado, pueden proporcionar información importante sobre las respuestas de las plantas a los cambios en las variables climáticas, como la temperatura y la precipitación (Schwartz, 2003; Chuine, 2010).

Las especies han ido cambiando algunos de sus rasgos para responder a las señales de temperatura y al aumento de la duración del día con el inicio temprano o tardío de la brotación de las yemas, equilibrando el acceso a recursos, como la luz y los nutrientes (Wright et al. 2021; Chamberlain and Wolkovich, 2023). Uno de ellos, en especies arbóreas con hábitos foliares caducifolios, es el momento de desarrollo de las hojas. Durante el periodo que prevalecen sin hojas (antes del crecimiento) las condiciones ambientales son fuertes determinantes del inicio del crecimiento, ya que existen varias limitantes para el desarrollo como las bajas temperaturas o la sequía. La caducidad de las hojas es una adaptación importante para sobrevivir a sequías severas porque ayuda a evitar la desecación (Lohbeck et al., 2015). El impacto del cambio climático global (por ejemplo, cambios en la temperatura y la precipitación) sobre la duración de la caducidad foliar y el período de crecimiento vegetativo es de suma importancia dentro de la ecología, ya que los patrones fenológicos de las plantas caducifolias siguen siendo poco comprendidos en situaciones de estrés o sequía (Guan et al., 2014).

Recientemente el estudio de la fenología de las hojas ha tomado gran relevancia, esto debido a su sensibilidad climática y a que es considerada como un referente en la respuesta de las especies a un entorno cambiante y la estacionalidad de la productividad bruta del ecosistema (Mckown et al., 2013, Wu et al., 2016, Chavana-Bryant et al., 2017, Park et al., 2024; Qian et al., 2024). A medida que se desarrollan las hojas, la temperatura del aire y la humedad relativa varían constantemente, lo que puede conducir a una variación sistemática de los rasgos de las hojas (p. ej., capacidad fotosintética, área foliar específica, contenido de nitrógeno de las hojas o contenido de materia seca de la hoja, LDMC, entre otros) (Wright et al., 2005a, Mckown et al., 2013). Reich et al. (1991) demostraron que las hojas más jóvenes generalmente tenían mayor área foliar específica y contenido de nitrógeno foliar en comparación con las hojas de mediana edad y en tres especies de hoja caduca de hoja ancha. De manera similar, Chavana-Bryant et al. (2017) demostraron que el área foliar específica y el contenido de fósforo foliar disminuyeron con el desarrollo de las hojas en 12 árboles del dosel de las tierras bajas del Amazonas. Por lo que es importante analizar en conjunto la variación fenológica foliar con rasgos foliares de la planta y así determinar el papel y la importancia de dicha variación en respuesta a variaciones ambientales en el entorno.

Las especies del género Quercus son un grupo prometedor en el que detectar la influencia de las señales climáticas y la ubicación geográfica en los patrones fenológicos en ambientes templados y con escasez de agua (Gerst et al., 2017). Algunos estudios han identificado la temperatura como un impulsor de la fenología de los encinos (Askeyev et al., 2005; Basler y Körner, 2012; Gerst et al., 2017) al encontrarse fuertemente correlacionada con la fecha de inicio de la brotación y al observarse que las temperaturas más cálidas adelantan la brotación en una variedad de especies. Sin embargo, otros estudios han informado de la importancia de la precipitación en el momento de brotación. En el 2015, Samtani, Appleby y Masiunas descubrieron que el aumento de la precipitación acumulada retrasó la brotación de dos especies de Quercus de América del Norte. Por otro lado, Gerst et al. en el 2017, compararon dos especies de encino del centro y este de América del Norte (*Q. alba* y *Q. rubra*) con dos especies occidentales (*Q. agrifolia* y *Q. lobata*) identificaron las variables de temperatura estacional como los impulsores más significativos del momento de brotación de hojas y flores para los robles occidentales, sin embargo, en el caso de las especies orientales el mayor impulsor de su variación fenológica fue la precipitación. Aunque los impulsores climáticos son los principales factores que influyen la variación fenológica foliar de las especies, también existen otros elementos no ambientales que pueden ayudar a que se desencadene dicha variación. El momento de desarrollo de las hojas también pueden determinarse por interacciones como la herbivoría o por agentes infecciosos, como la defoliación de la polilla del encino, que a su vez pueden estar influenciados por las propias variables climáticas, estimulando a los árboles a producir hojas jóvenes tempranas o tardías para evitar la sincronía con ciertos herbívoros de temporada (Visser y Holleman, 2001).

La fenología foliar es un rasgo importante de las plantas que transmite resistencia contra los folívoros en plantas anuales y caducifolias. Muchos herbívoros colonizan plantas individuales con una fenología foliar particular. En algunos casos, esto conduce a que las plantas con hojas más tempranas o más tardías acumulen más daños por herbívoros (van Asch y Visser, 2007).

Se ha demostrado que la fenología de la hoja es un determinante importante de la abundancia del minador de los encinos (Mopper y Simberloff, 1995; Pearse y Karban, 2013). Los minadores de hojas se encuentran en varias familias de Lepidóptera, Díptera, Himenóptera y Coleóptera (Connor y Taverner, 1997). Estos herbívoros suelen ser especialistas en hospedadores o oligofagos (Connor et al., 1980), y los huevos son a menudo depositados a principios de temporada por adultos que emergen de pupas en la capa de hojarasca. Su abundancia a finales de temporada puede correlacionarse con eventos fenológicos de principios de temporada (Pearse y Karban, 2013).

El género *Quercus*

La familia Fagaceae es un grupo dominante de los bosques templados en el hemisferio norte, se encuentra ampliamente distribuida en el Norte y Centro de América, Europa y Asia. En el continente americano el género se distribuye desde Canadá, EUA, México hasta llegar Colombia incluyendo Cuba (Valencia, 2004). Dentro del género se han descrito alrededor de 400 a 600 especies, siendo México uno de los países con mayor diversidad de encinos, al contar con 168 de las cuales 104 son endémicas (Valencia, 2004; Rzedowski, 2005). Las especies pueden tener distintos hábitos foliares, pudiendo ser perennifolias o caduciofolias, podemos encontrarlos en formas árboreas o arbustivas, sus flores son unisexuales y suelen ser polinizadas por el viento. La dispersión de sus semillas se da principalmente por zoocoria o por barocoria (Aldrich y Cavender-Bares, 2011). Los encinos poseen un gran valor como fuente potencial de madera, carbón y alimento; asimismo, los encinares son hábitat de una gran cantidad de plantas epífitas y de animales vertebrados e invertebrados (Valencia, 2004). Poseen una gran importancia dentro de los encosistemas, ya que tienen funciones ecológicas como el ciclaje de nutrientes y ayudan en el balance hídrico (Lewington y Streeter, 1993; Nixon, 1993). Por estas razones resulta fundamental estudiar los aspectos ecológicos del género *Quercus* en nuestro país. Pese al gran número de especies que se encuentran en México, existen pocos estudios sobre su fenología y la relación entre el clima y sus rasgos foliares (Borchert et al., 2005).

Quercus castanea como especie de estudio

Quercus castanea es una especie de encino que pertenece a la sección *Lobatae* (Valencia-Á., 2004), incluye árboles que alcanzan lo 18 m de altura y con un diámetro de tronco de 30 a 60 cm. Poseen hojas con forma obovada u oblanceolada, las venas del envés elevadas y reticuladas, tiene una coloración gris verdosa y tricomas fasciculados sésiles. La época de floración es de abril a mayo y la de fructificación es de agosto a diciembre (Vázquez, 2006). Es una especie caducifolia. Sus poblaciones se encuentran entre 1100 y 2600 m de altitud, principalmente en bosques de roble y bosques de pino-encino pero también puede encontrarse en bosques secos tropicales a altitudes más bajas. *Quercus castanea* se encuentra en áreas montañosas con climas cálidos a templados húmedos, con temperaturas medias anuales de entre 10 y 26°C (Kappelle, 2006), la temporada de lluvias en éstas área ocurre en la estación más cálida del año (Rzedowski y Huerta, 1978). Por lo tanto, *Quercus castanea* representa una especie adecuada para estudiar patrones de variación fenológica y foliar en respuesta a factores ambientales, gracias a la diversidad de ecosistemas en que podemos encontrarla. En robles tropicales como *Q. oleoides*, se ha encontrado una asociación entre los patrones fenológicos (es decir, el grado de caducidad de la hoja) y la masa foliar por área (Cavender-Bares y Ramírez-Valiente, 2017), destacando la importancia de considerar simultáneamente la fenología y otros rasgos funcionales para comprender la adaptación de las poblaciones a los gradientes ambientales dentro de las especies de roble.

IV. HIPÓTESIS

- Las poblaciones de *Q. castanea* situadas en áreas con menor disponibilidad de agua en la cuenca de Cuitzeo tenderán a ser más caducifolias, es decir, la etapa de formación y crecimiento de las hojas será más lenta en comparación con las poblaciones de menor limitación de agua y durarán más tiempo sin hojas nuevas formadas. Estas variaciones fenológicas y ambientales influirán en otros rasgos foliares (e.g. grosor foliar, área foliar, contenido de nutrientes y defensa química) y tendrá impacto también en los niveles de herbivoría.

V. OBJETIVO PRINCIPAL

- ✓ Determinar el comportamiento y la variación de algunos de atributos foliares de la especie de encino *Quercus castanea* a una escala de paisaje, tomando en cuenta poblaciones contrastantes con distintos rangos de disponibilidad de agua. Los atributos foliares estudiados serán de carácter fenológico, así como aspectos funcionales de naturaleza física, química y nutrimental. Posteriormente, se analizará la relación que existe entre la variación de dichos atributos y una de las interacciones ecológicas más importantes en los encinos como lo es la herbivoría.

OBJETIVOS ESPECÍFICOS

CAPÍTULO I

OBJETIVOS

- ✓ Caracterizar la fenología foliar de *Q. castanea* en sitios contrastantes en disponibilidad de agua durante dos años.

- ✓ Determinar la relación entre la fenología foliar y las variables ambientales de temperatura y precipitación, tanto de datos promedio de 30 años (wordlclim) como de datos de estaciones meterereológicas para cada sitio.

CAPÍTULO II

OBJETIVOS

- ✓ Comparar los niveles de herbivoría entre las poblaciones de *Q. castanea* y determinar su asociación con la fenología foliar, los caracteres funcionales foliares, de calidad nutricional y de defensa química de los individuos.

- ✓ Determinar la asociación de los niveles de herbivoría y los caracteres funcionales foliares, de calidad nutricional y de defensa química de los individuos con las variables climáticas en la distribución de *Q. castanea* en la cueca de Cuitzeo.

VI. RESULTADOS

CAPÍTULO I.

Este capítulo representa un **ARTÍCULO CIENTÍFICO** PUBLICADO en la revista internacional indexada **AoB PLANTS** con factor de impacto de **2.6**, razón por la cual el análisis de similitud de esta tesis muestra un porcentaje de 37%, dado que el mayor porcentaje de este total corresponde a la publicación de la cual mi persona, Tamara C. Ochoa Alvarez, es la primera y principal autora de este artículo, el cuál es un requisito para la obtención de grado como parte del Programa Institucional de Doctorado en Ciencias Biológicas de la UMSNH, que corresponde a:

Ochoa-Alvarez, T. C., Contreras-Negrete, G., Lara-De La Cruz, L. I., & González-Rodríguez, A. (2024). Landscape-level variation in spring leaf phenology is driven by precipitation seasonality in the Mexican red oak *Quercus castanea*. *AoB PLANTS*, plae067. DOI **https://doi.org/10.1093/aobpla/plae067.** (**Ver ANEXO 1**).

RESUMEN

En todos los sistemas naturales la disponibilidad del recurso hídrico juega un papel esencial al ser uno de los factores que determinan las estrategias de vida de las especies, así como su distribución. Estas estrategias están influenciadas por rasgos propios de las plantas como su fenología foliar y sus rasgos funcionales. En el capítulo I, se analizó cómo afecta la disponibilidad de agua a la fenología foliar de distintas poblaciones de la especie *Quercus castanea,* de igual forma se examinó el efecto sobre otros rasgos foliares como la masa foliar por área y el grosor de las hojas. Se muestrearon seis sitios dentro de la cuenca de Cuitzeo con diferentes condiciones de disponibilidad de agua. Se colectaron hojas de 10 individuos por población en 2 temporadas del año durante 2 años. La fenología foliar se monitoreó individualmente en cada árbol, en los meses de Marzo-Julio durante el año 2021 y 2022, se midieron simultáneamente condiciones de humedad del suela. Los datos de temperatura y precipitación se obtuvieron de las estaciones metereológicas de CONAGUA y de la base de datos de la plataforma de WordlClim. Como resultados observamos que la fenología foliar

varió de forma espacial y temporal significativamente. El desarrollo de las hojas fue más temprano y con una duración menor cuando se presentó una mayor acumulación de humedad en el suelo en los meses de marzo y abril. Los sitios que presentaron mayor estacionalidad de las precipitaciones y niveles de precipitación bajos para su trimestre más cálido, mostraron tiempos de desarrollo foliar más largos y crecimiento lento. El grosor de las hojas se vio afectado por los tiempos de desarrollo, ya que árboles con desarrollo lento presentaron hojas mayormente gruesas. *Quercus castanea* mostró una fenología foliar brevidecidua, que tuvo una variación significativa entre sus poblaciones como reflejo de mosaicos espacio-temporales debido a la variación ambiental y genética.

CAPÍTULO II

Associations of insect herbivory with leaf traits and phenology along an environmental gradient in *Quercus castanea*, a Mexican red oak.

Resumen

La herbivoría es una interacción ecológica de carácter antagónico que tiene gran relevancia dentro de los sistemas naturales, debido a su influencia en aspectos estructurales y reproductivos de su planta hospedera. Existen varios factores que pueden influir en el desarrollo y nivel de dicha interacción, ya que la herbivoría puede verse afectada tanto por rasgos foliares asociados a la planta como por factores abióticos como la temperatura y precipitación. En el presente estudio, se analizó la relación de algunos atributos foliares, de naturaleza fenológica, funcional, química y nutrimental, del encino *Quercus castanea* con los niveles de herbivoría encontrados en seis poblaciones a través de un gradiente de disponibilidad de agua en diferentes temporadas del año. En conjunto, se obtuvieron variables ambientales de las estaciones metereológicas cercanas a los sitios, así como de la base climatológica de WorldClim. Como resultado, se observó que lo niveles de herbivoría en *Quercus castanea* se encontraron fuertemente influenciados por la fenología de sus hojas y el tamaño de las mismas, sin embargo, la precipitación anual fue determinante al ser también uno de los mejores predictores de esta interacción.

Abstract

Herbivory is a very relevant ecological interaction within natural systems, since it affects several structural and reproductive aspects of its host plant. There are several factors that can influence the development and level of this interaction, since herbivory can be affected by both foliar traits associated with the plant and abiotic factors such as temperature and precipitation. In the present study, the relationship of some foliar attributes, of phenological, functional, chemical and nutritional nature, of *Quercus castanea* oak with the levels of herbivory found in six populations across a gradient of water availability in different seasons of the year was analyzed. Together, environmental variables were obtained from meteorological stations near the sites, as well as from the WorldClim climatological database. As a result, it was observed that herbivory levels in *Quercus castanea* were strongly

influenced by leaf phenology and leaf size; however, annual precipitation played a relevant role, as it was also one of the best predictors of this interaction.

INTRODUCTION

Herbivory is an antagonistic ecological interaction that occurs in most natural and anthropogenic ecosystems and is defined as the damage to any plant organ caused by the feeding of various organisms (Coley and Barone, 1996). This interaction is relevant due to the fundamental role it plays in the structuring of communities, the regulation of plant population sizes and the regeneration processes of the forests themselves (Weis and Berenbaum, 1989; Pugnaire and Valladares, 2007).

Interactions between plants and their insect herbivores can be strongly influenced by the prevailing climate of the region (Garibaldi et al., 2011, Pearse and Hipp, 2012). On the one hand, temperature and precipitation can affect the rate of plant development (Körner and Basler, 2010, Hänninen and Tanino, 2011), as well as leaf phenology, nutritional quality and leaf defensive compounds (Asner et al., 2009, Janecke and Smit, 2011). Several studies have determined that this variation in plants can influence herbivore resistance, as well as differences in leaf traits such as leaf thickness, leaf area or leaf toughness (Meldau et al., 2011; Blumenthal et al., 2020; Moreira et al., 2023). On the other hand, environmental factors such as temperature have a direct influence on herbivore diversity, survival rates and population size (Régnière et al., 2012). The amount of annual rainfall has been shown to affect the abundance, development and distribution of both plants and insects, and, at the same time, can affect the amount of nutrients and secondary metabolites in plants (Gutbrodt et al., 2012, Mazía et al., 2012; Leckey et al., 2014; Fyllas et al., 2022). Therefore, an increased number of studies are being conducted in an integrated manner, in which the role of the environment on the variation of plant traits and their influence on ecological interactions are analyzed simultaneously (Liu et al., 2019; De Andrés et al., 2024; Lin et al., 2024).

Variation in herbivory levels in forests is determined by individual, species, and community attributes. These attributes range from individual size and species traits to species richness and diversity, to the amount of available natural resources (Schuldt et al. 2012; Castagneyrol et al., 2017; Martini and Goodale, 2020). Within the same plant species, herbivory levels can be influenced and modified by different factors; moreover, the intensity of damage can vary at different scales (Castagneyrol et al., 2019). At the landscape scale, herbivore density can become higher in habitat patches where resources are more abundant, because the intensity of physical and chemical cues makes these patches more attractive and more likely to be colonized (Root, 1973; Hambäck and Englund, 2005; Andersson et al., 2013). Within patches, herbivory on individual plants can be influenced by the identity and diversity of their neighbors, i.e., the various species with which they coexist within the same habitat, which alter the physical and chemical appearance of the focal plant and its colonization (Finch and Collier, 2000; Castagneyrol et al., 2013; Moreira et al, 2016).

There are several hypotheses that attempt to explain insect herbivory patterns in ecosystems. The plant appearance theory (Fenny, 1976) suggests that plants, in this case specifically trees, with long-lived leaves (evergreen) are more easily attacked than trees with short-lived leaves (deciduous), because they have a more obvious appearance for prolonged periods and are therefore more easily detected by folivorous insects; therefore, these plants must invest in quantitative chemical defenses. These defenses include the production of secondary metabolites, such as tannins, since the increase or decrease of these metabolites in leaves influences interactions with herbivores and natural enemies (Massad et al., 2011).

Therefore, leaf habit is an important part of plant appearance and has been described as a significant predictor of herbivory (Castagneyrol et al. 2013; Zverev et al. 2017). However, since there is wide variation in herbivory levels among individuals within deciduous tree species and leaf phenology, including the length of the leafless period of the year also shows significant intraspecific variation, appearance theory plays an important role, because the longer the leafless period, the less exposure to possible herbivore attack.

The genus Quercus (Fagaceae) is widely distributed in temperate, tropical and subtropical forests of the northern hemisphere. There is a high biological activity among the different trophic levels associated with plants of this genus, due to their great phenotypic diversity and the heterogeneity of the ecosystems where they are found. Herbivorous insects, act as a selection force on defense compounds in oaks and, for their part, insects also suffer a selective pressure to be able to tolerate the different chemical compounds that are synthesized by oaks (Vaca-Sánchez et al., 2016). Quercus species produce chemical defenses in leaves, particularly phenolic compounds, which are effective against a wide range of phytophagous insects (Pearse and Hipp, 2009; Moctezuma et al. 2014; Abdala- Roberts et al., 2016; Moreira et al., 2018). Commonly, constitutive phenolic compounds are present in high concentrations in all oak tissues (up to 100 mg g-1 of tissue dry weight) (Pearse and Hipp, 2012; Maldonado-López et al., 2015; Moreira et al., 2017, 2018). Furthermore, the inducibility of these chemical defenses has been shown to provide an effective strategy for resistance against major insect herbivores in several Quercus species (Mizumachi et al., 2012). The main chemical groups of phenolic nature found in oaks are hydrolysable tannins, proanthocyanidins (condensed tannins), flavonoids and some simple phenolic compounds (Barbehenn et al., 2008; Barbehenn and Constabel, 2011; Moctezuma et al., 2014).

There are several studies for the genus Quercus that address herbivory from a holistic approach and include attributes of individual trees as well as environmental factors as explanatory variables of leaf damage by herbivores. For example, Lin et al. (2024), identified that the leaf habit is associated with defensive strategies along an environmental gradient by studying five species of deciduous oaks (*Q. variabilis, Q. aliena, Q. fabrei, Q. acutissima* and *Q. serrata*), observing a decrease in leaf thickness along a longitudinal gradient, with the thicker and denser leaves being present in arid areas. On the other hand, there are studies where environmental variables were not the main predictors of herbivore damage but rather the identity of the herbivores and their feeding habits (Leckey et al., 2014); however, precipitation did correlate with an increase in leaf area removed and specialized insects were the most sensitive to differences in precipitation levels. In this study, we focused on evaluating herbivory and its relationship with leaf phenological variation in a deciduous red oak, *Q. castanea*, the most abundant species in the Cuitzeo basin in Michoacán, Mexico. Simultaneously, we analyzed a series of foliar traits that may influence the amount of herbivore damage and that vary with water availability at the landscape level (Ochoa-Álvarez et al., 2024). We hypothesized that phenological variation will be associated with leaf traits of *Q. castanea* such as leaf thickness, leaf area, nutrient content, and leaf chemistry at the landscape level, and that such variation is in turn related to herbivory levels. In particular, we evaluated the following questions: i) Is there temporal (i. e., among years) and spatial (i.e., among locations) variability of *Q. castanea* leaf attributes along an environmental gradient at the landscape level? ii) What are the main variables influencing the levels of herbivory found? iii) How do leaf traits covary with each other? iiii) Which leaf traits most strongly influence variation in other traits? How do leaf attributes of *Q. castanea* vary along the environmental gradient of the Cuitzeo basin? Is there an association between leaf traits and leaf phenological variation? How is leaf herbivory associated with leaf traits, phenology and environmental variables?

Materials and methods

Study system

The study was carried out in the Cuitzeo basin, Michoacán, Mexico. In this area, populations of *Q. castanea* can be found between 2000 and 2800 m (Herrera-Arroyo et al., 2013). The basin is located between 19° 30′ and 20° 05′ N and 100° 35′ and 101° 30′ W, with an area of about 4000 km2. It is highly heterogeneous in climate, topography, and vegetation (Leal-Nares et al., 2010; Lara-De La Cruz et al., 2020).

Sampling design

In first place, a geographic and climatic gradient of the populations of *Q. castanea* in the Cuitzeo basin was made, based on the climatic data obtained from the WorldClim platform (https://www.worldclim.org/), which shows average data for 30 years (1970-2000).

Subsequently, six sites were selected with an annual precipitation range between 909 mm and 1299 mm and a mean annual temperature of 13.8 °C to 16.9 °C (**Table 1**). At each site, 10 adult Q. Castanea trees were randomly selected, marked and geo-referenced to control and make all collections on the same individuals each season.

Four times over a two-year period (spring and fall of 2021 and 2022) we collected leaf samples from each individual. Every time, at least 35 randomly selected leaves were obtained from each tree for measurements of area consumed (AC) (herbivory), leaf functional traits (leaf area (FA), leaf weight (weight), leaf thickness (thickness) and leaf mass per area (LMA)) and chemical and nutritional characterization (concentration of phenols (PheC), tannins (TanC), proanthocyanidins (PASC), non-structural carbohydrates (CarbsC), water content (% water) and total nitrogen (N), phosphorus (P) and carbon (C) contents). We also obtained the three nutrient elemental stoichiometric ratios, C:N, C:P and N:P. Phenological variation was characterized by leaf development time (DT), defined as the number of days elapsed from the first day of the the day when trees drop all their old leaves to the day when they reach full growth becoming fully developed new leaves, and ED50, which is the number of days elapsed from the first day of the current year to the day when they reach 50% of new leaf growth, using data from a previously published study (Ochoa- Alvarez et al., 2024).

Table 1. *Q. castanea* sites used for leaf collection. Precipitation and temperature data were obtained from the WORDLCLIM database (www. https://www.worldclim.org/).

Site	Lat.	Long.	Elevation (m)	Annual precipitation (mm)	Mean annual Temp. (°C)
Tamanguío (TA)	19.98°	-101.35°	2457	1299	13.8
Remolino (REM)	19.55°	-101.26°	2523	1181	14.2
Atécuaro (AT)	19.61°	-101.15°	2317	1060	15.5
San Miguel (SM)	19.62°	-101.2°	2097	1096	15.3
Umécuaro (UM)	19.62°	-101.34°	2116	945	16.9
San Nicolás (SN)	19.45°	-101.34°	2233	909	16

Herbivory levels and leaf functional traits

Fifteen randomly selected leaves of each individual (150 leaves per population and 900 in total for each collection season), were pressed and dried at 40 °C for three days. Subsequently, leaves were scanned with a flatbed scanner (ScanJet4300C). AC by insects (% herbivory, relative to the total leaf area) and the total LA were estimated using the ImageJ software (Ferreira-Rasband, 2012). Thickness was measured using a digital Vernier device. The weight was obtained with an analytical balance. The LMA was calculated by dividing the leaf area by the dry weight of the leaf.

Secondary metabolites

Total phenols. One hundred and twenty-five mg of leaf tissue were weighed and ground with liquid nitrogen. Samples were extracted with 10 mL of 80 % ethanol and centrifuged at 3000 rpm for 10 min and the supernatant was preserved. One mL of supernatant was added to 7 mL of deionized water. The blank for calibration was prepared by adding 1 mL of ethanol instead of sample and vortexing. Afterwards, 0.5 mL of the Folin and Cicalteau reagent (2.0 N, Sigma®) were added to the samples and the blanks. After 8 min, 1 mL of 20% sodium carbonate was added and the tubes were stirred again. After 1 h the absorbance was read at 760 nm in a spectrophotometer (Thermo Scientific MultiskanGO). The standard curve was created with known concentrations of tannic acid. The equation obtained by means of the calibration curve was used to make the transformation to mg g^{-1} of sample (Waterman and Mole, 1994).

Total tannins. For tannin quantification samples were extracted with 70 % aqueous acetone, allowed to stand for 1 h at room temperature with continuous stirring and centrifuged for 10 min at 3000 rpm. The acetone extract was reduced to the aqueous phase, and then frozen and lyophilized. 0.1 % ascorbic acid was used as conservative (Hagerman, 1987). The radial diffusion method was used with agarose gel added with bovine serum albumin. We punched 8 wells in each plate. Each sample was resuspended in 200 μL of 70 % acetone and 50 μL of each sample was placed in the wells. The plates were covered and placed in an incubator at 30 °C for 96 h. A digital Vernier was used to measure the diameter of the ring that formed. The area of the ring is linearly related to the number of tannins placed in the well. A calibration curve was obtained using tannic acid as a standard.

Soluble proanthocyanidins. For soluble proanthocyanidins 200 mg of each sample were grounded with liquid nitrogen, extracted with 10 mL of 70% aqueous acetone, allowed to stand for 1 h at room temperature with continuous stirring and centrifuged for 10 min at 3000 rpm. The acetone extract was reduced to the aqueous phase, and then frozen and lyophilized. 0.1 % ascorbic acid was used as conservative (Hagerman, 1987). Samples were resuspended in 50 % methanol. 0.1 mL of extract and 0.6 mL of water were added to 6 mL1-butanol/concentrated HCl (95:5, v/v). After mixing, the tubes were placed in a bath for 50 min at 95 °C. The absorbance at 555 nm was measured in a spectrophotometer (Thermo Scientific MultiskanGO). The standard curve was prepared with known concentrations of (+) catechin hydrate (Watterson and Butler, 1983).

Nutritional quality

Non-structural carbohydrates. The extraction was performed from leaves previously dried in on oven at 40 ° C and then grinded. Two-hundred mg of grinded sample were placed in an Eppendorf tube and 1.5 mL of ethanol at 80% were added. Tubes were centrifuged for 4 min at 10,000 rpm and the supernatant was recovered and diluted to a volume of 4 mL. Then, one mL of this extract was combined with 1 mL of water in a test tube and with 50 µL of 80% phenol and the mix was vortexed for 5 seconds. Then, 2 mL of 80% H2SO4 were added, and the solution was vortexed again for 20 s. The samples were read at 487 nm in a spectrophotometer (Thermo Scientific MultiskanGO). The content of simple sugars was calculated from a standard glucose curve (Marquis et al., 1997).

Water content. The fresh weight of 10 leaves of each individual was recorded. Subsequently, the leaves were dried in an oven at 60 ° C for 72 h and the weight was recorded again. Water content was calculated as (fresh weight – dry weight)/fresh weight (Marquis et al., 1997).

Total nitrogen, phosphorus and carbon. These analyses were performed in only three individuals per population because relatively low within-population variance was observed in these initial trials. N and P were determined by subjecting the samples to acid digestion in a mixture of concentrated H2SO4 and K2SO4 with CuSO4 as a catalyst. Digested samples were analyzed colorimetrically with a Bran-Luebbe Auto Analyzer 3 (Norderstedt, Germany). Nitrogen was determined by the micro-Kjeldahl method (Bremmer, 1996) and P by the molybdate colorimetric method following ascorbic acid reduction (Murphy and Riley, 1962). Leaf C content was determined by combustion and coulometric detection (Huffman,1977) in a total carbon analyzer UIC model CM5012 (Chicago, USA). Nutrient concentration was expressed on a dry mass basis. From the values of N, P and C content the following ratios were calculated, C:N, C:P and N:P since these are important indicators of nutritional quality of the leaves.

Meteorological and soil moisture data

For each collection site we downloaded 19 climatic variables from the WorldClim data base (https://www.worldclim.org/). From the data matrix we excluded highly correlated variables (|r| > 0.8), resulting in the following set: Annual Mean Temperature, Isothermality, Mean

Temperature of Driest Quarter, Mean Temperature of Warmest Quarter, Annual Precipitation, Precipitation Seasonality, Precipitation of Driest Quarter and Precipitation of Warmest Quarter. In addition, we maintained a record of soil moisture for each individual in all populations, for the years 2021 and 2022. The recording was carried out every two weeks from March to August for both years. Soil moisture was measured only during this period because these are the months of leaf development for *Q. castanea*. Subsequently, we used data of soil moisture for the months of March, April and May, since it has been observed that soil humidity in the spring is determinant for leaf phenology patterns in this species (Ochoa-Alvarez et al., 2024). Soil moisture was measured near the base of the trunk, using a Delta-T soil moisture device (Cambridge, UK).

Statistical analysis

The pattern of variation of leaf attributes in *Q. castanea* populations was explored with principal components analysis (PCA), obtaining a PCA including data of all seasons. A two-way ANOSIM test was performed with the PCA results considering two explanatory factors: season and population, to determine the importance of both on the multivariate variation pattern of the measured traits.

In addition, network analyses were used to summarize bivariate relationships between traits, herbivory and environmental variables. The analysis was performed with the igraph package (Csardi and Nepusz, 2006) in R (R Core Team, 2013). Networks describing significant correlations between all measured variables were constructed. First, correlation matrices were constructed for each of the stations with the mean values per individual of all measured variables. Subsequently, the graph_from_adjacency_matrix function was applied, which uses the “undirected” mode and the absolute values of the pairwise Pearson correlation coefficients between variables as the adjacency matrix (Blumenthal et al., 2020). The importance of variables within the networks was measured using the sum of the r- values for each significant correlation per variable.

To evaluate the hypothesis of an effect of precipitation seasonality and water availability on plant traits and foliar herbivory, generalized linear mixed models were fitted to account for the nested nature of the sampling design. The models included variables of water availability and the sampling period as fixed effects, in three different nested combinations: 1. including only the main effect of the indicator of water availability (we call these models “gradient”); including water availability variables and the sampling season (or year for phenological variables, which only present data for each year and not by season), but without including the interaction of water availability and season (“both” models) and; 3. including the interaction between water availability variables and the sampling season (“interaction” models). Since 11 indicators of water availability were available (**Table S9**), 33 different models were fitted for each response variable, as well as a null model with no predictors. All the models included individual trees as a random effect. Population (site) was at first included also as a random effect, but as its associated variance was almost negligible and caused problems during model fitting for several response variables, it was excluded. A gamma probability distribution was set for all the models to account for patterns in the variance of the residuals, except for the model for AC, which was modeled using a Tweedie distribution to account for a spike in zero values. All the models were fitted using the glmmTMB function from the homonymous package (Brooks et al., 2017). All the models were visually diagnosed to evaluate model assumptions (Zuur and Ieno, 2016), using the check.model function in the performance package (Lüdecke et al., 2021). Finally, to identify the model that best predicts each response we used the corrected Akaike Information Criterium (AICc) to contrast the 34 fitted models for each response variable (Anderson, 2008). All the models with a $\Delta AIC < 2$ were considered equally likely and are reported, although only the predictions for the best models $\Delta AIC = 0$) are presented. All the procedures on model fitting were performed in R v4.3.2 (Team, 2023).

Results

Principal components analysis and ANOSIM test

The first two components of the PCA explained 24.8 and 16.7% of the total variance (**Fig. 1**), respectively. The most evident pattern corresponded to a separation by season, with most of the samples collected in seasons 1 and 2 (year 2021) occurring towards the right part of the plot, while samples from seasons 3 and 4 (year 2022) were in the left part. Within this main groups, some segregation by populations was also evident. Samples of 2021 were in general characterized by higher concentrations of N and P, tannins (TanC), water percentage and larger ED50 values. In contrast, samples of 2022 presented higher C:N and C:P ratios and higher LMA.

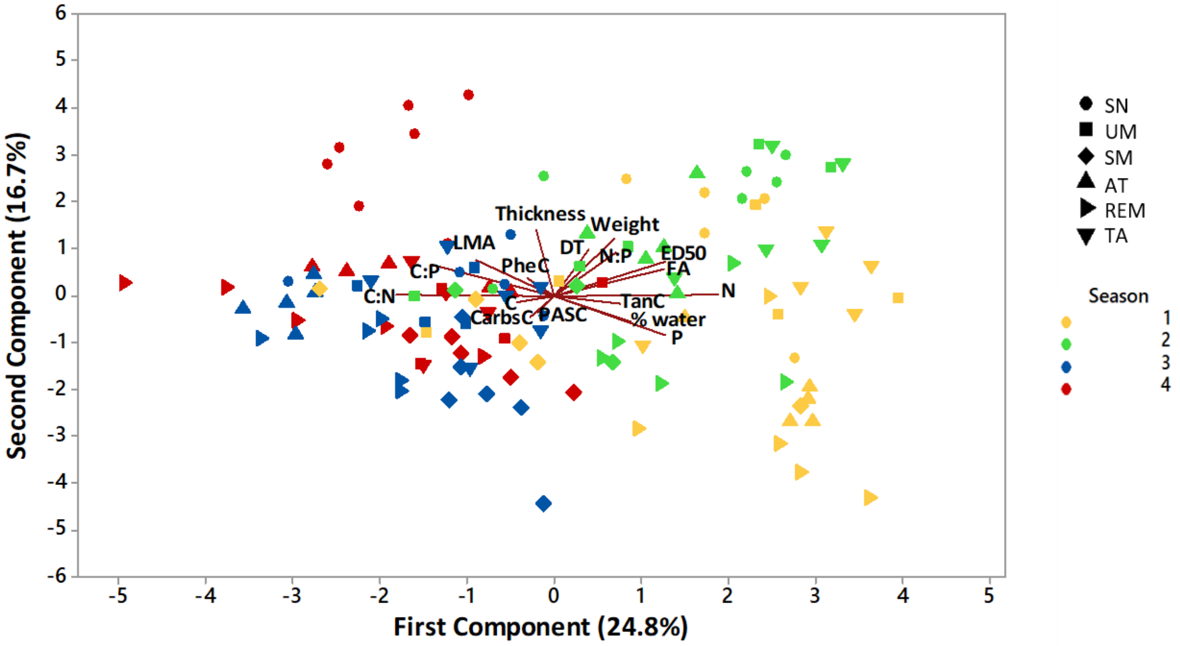

Figure 1. Principal component analysis (PCA) for six populations of *Q. castanea* according to their foliar attributes (functional, chemical and nutritional) (mean value per individual), all seasons.

The results of the two-way ANOSIM (**Table 2**) confirmed a significant effect of season on the leaf attributes multivariate pattern of *Q. castanea* individuals (ANOSIM R = 0.46; P =

0.0001) and an additional significant effect of the population factor (ANOSIM R = 0.35; P = 0.0001).

Table 2. Two-way ANOSIM results for principal component analysis (PCA) of all seasons, separated by factors: season and population.

	SEASON FACTOR	**GROUP FACTOR (POPULATION)**
Permutation N	9999	9999
R	0.46	0.35
P value	0.0001*	0.0001*

Network analysis of pairwise correlations among traits

Some of the variables most strongly interconnected with other variables, as described by node sizes (summed R^2 values), were similar in the same seasons sampled in different years (**Fig. 2**; **Tables S5-S8**). For season 1 (July - August 2021), LMA ($\sum R^2 = 4.45$) is one of the strongest nodes, which is repeated in season 3 (July - August 2022) LMA ($\sum R^2 = 2.63$). The consistently weak nodes for the mentioned seasons were pheC ($\sum R^2 = 0.37, 1.58$; respectively for the two seasons) and PAS ($\sum R^2 = 1.41, 1.36$; respectively).

In season 2 (November - December 2021), ED50 ($\sum R^2 = 3.43$), leaf thickness ($\sum R^2 = 1.98$) and leaf weight ($\sum R^2 = 1.79$) were the nodes with the highest strength, which was also observed in season 4 (November - December 2022). The consistently weak nodes for the aforementioned seasons were soil moisture in May ($\sum R^2 = 0.37, 1.49$; respectively), pheC ($\sum R^2 = 0.81, 0.92$; respectively) and tanC ($\sum R^2 = 0.54, 0.45$; respectively).

The % water formed a large node with multiple significant connections only in season 3 ($\sum R^2 = 5.99$). This is contrary to season 1, where it was one of the weakest nodes with a $\sum R^2$ of 0.60. Some variables, such as ED50, LMA and AC consistently showed several significant connections in two or more seasons.

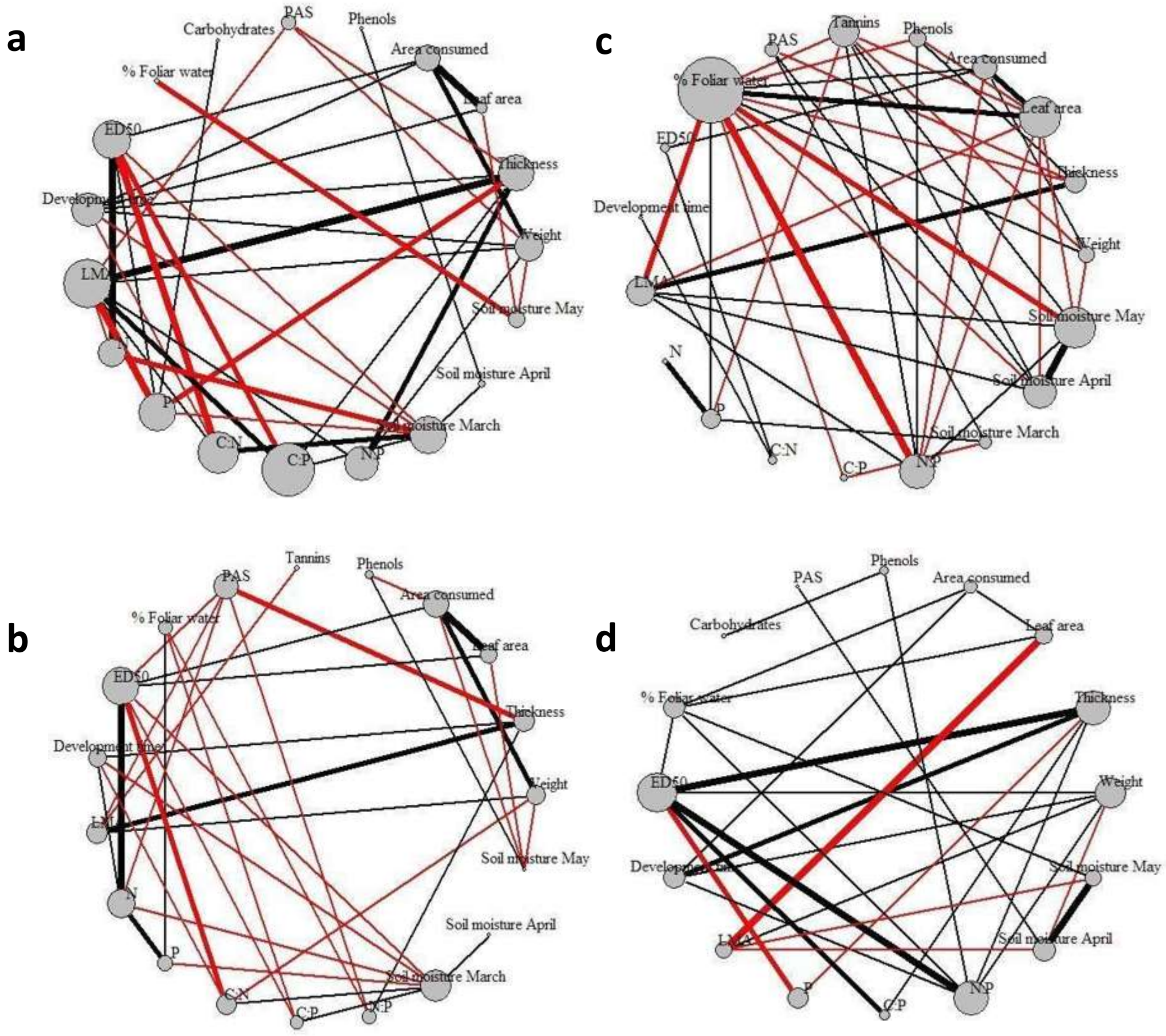

Figure 2. Network plots based on the correlation matrices trait correlation matrices for *Q. castanea* seasons: **a)** Season 1, **b)** Season 1, **c)** Season 3 and **4)** Season 4. The size of each circle indicates the sum of the pairwise correlation coefficients for each trait. Black lines represent positive correlations. Red lines represent negative correlations. The thickness of the line indicates the significance of the correlation (thin: $0.05 < p < 0.01$; intermediate: $0.01 < p < 0.001$; thick: $p < 0.001$). For details, see Tables in the supplementary material.

Generalized mixed models

Table 3 shows the models that presented the best fit to the data after using the Akaike information criterion (ΔAIC < 2) as well as the predictors that explain the variation observed in the herbivory, functional, phenological, chemical and nutritional traits of *Quercus* *castanea* leaves. For area consumed, the best predictor was the long-term annual rainfall (WorldClim).

Table 3. Best predictor models for each of the variables. Models are shown models were selected using the criterion of deltaAIC < 2.

Response variable	**Predictor**	**Type_model**	**AICc**	**delta_AICc**
AC	Annual_Rainfall_WClim	Interaction	516.8	0
AC	Warm_quarter_Rainfall_WClim	Interaction	517.1	0.3
FA	May_Rainfall_interpol	Interaction	1498	0
Thickness	Annual_Rainfall_interpol	Interaction	-880.2	0
Thickness	Jun_Rainfall_interpol	Interaction	-878.3	2
Weight	May_Rainfall_interpol	Interaction	-452.5	0
LMA	May_Rainfall_interpol	Both	2083	0
LMA	Annual_Rainfall_interpol	Both	2084.5	1.4
PheC	Annual_Rainfall_interpol	Interaction	1782.9	0
TanC	Cold_quarter_Rainfall_WClim	Interaction	2515.4	0
PASC	Annual_Rainfall_interpol	Interaction	1395.2	0
PASC	Nov_Rainfall_interpol	Interaction	1396.7	1.5
CarbsC	Jul_Rainfall_interpol	Interaction	930.6	0
% wáter	May_Rainfall_interpol	Interaction	1379.6	0
DT	Warm_quarter_Rainfall_WClim	Interaction	1110.9	0
DT	Annual_Rainfall_WClim	Interaction	1112.5	1.6
ED50	Warm_quarter_Rainfall_WClim	Interaction	1160.9	0
ED50	Annual_Rainfall_WClim	Interaction	1161.1	0.1
ED50	Cold_quarter_Rainfall_WClim	Interaction	1162.4	1.4
C	Nov_Rainfall_interpol	Gradient	1069.5	0
C	Annual_Rainfall_interpol	Both	1071	1.4
C	Jul_Rainfall_interpol	Gradient	1071.1	1.6
C	May_Rainfall_interpol	Both	1071.5	2
C:N	Jun_Rainfall_interpol	Both	801.3	0
C:N	Nov_Rainfall_interpol	Both	802.2	0.9
C:N	SM_May	Interaction	803.3	2
C:P	Annual_Rainfall_interpol	Both	1490.3	0
C:P	SM_May	Interaction	1492.3	2
N	Jun_Rainfall_interpol	Both	531.1	0
N	Nov_Rainfall_interpol	Both	532.7	1.5
N:P	Warm_quarter_Rainfall_WClim	Interaction	523.8	0
N:P	Annual_Rainfall_interpol	Interaction	524.4	0.6
P	Annual_Rainfall_interpol	Both	-40.6	0
P	SM_May	Interaction	-38.8	1.8

For the functional and chemical leaf traits, in most cases the best predictors were interpolated values of rainfall in May and July, followed by the interpolated annual rainfall and the long-term average of rainfall of the coldest quarter from WorldClim (**Fig. 3**). In these cases, the best models were "interaction", except for the case of LMA, in which the best model was the "both" model.

For phenological variables, the best predictors were the long-term averages of rainfall of the warmest quarter and annual rainfall, both form WorldClim (**Fig. 4**). The best model included the interaction between the water availability variables and the year (because phenology can be evaluated only once per year). Finally, for the nutritional variables, the best predictors are interpolated annual rainfall and June rainfall followed by November rainfall for C and warm trimester rainfall for N:P (**Fig. 5**). For these variables the best model was "both". Only for N:P the best model did include the interaction.

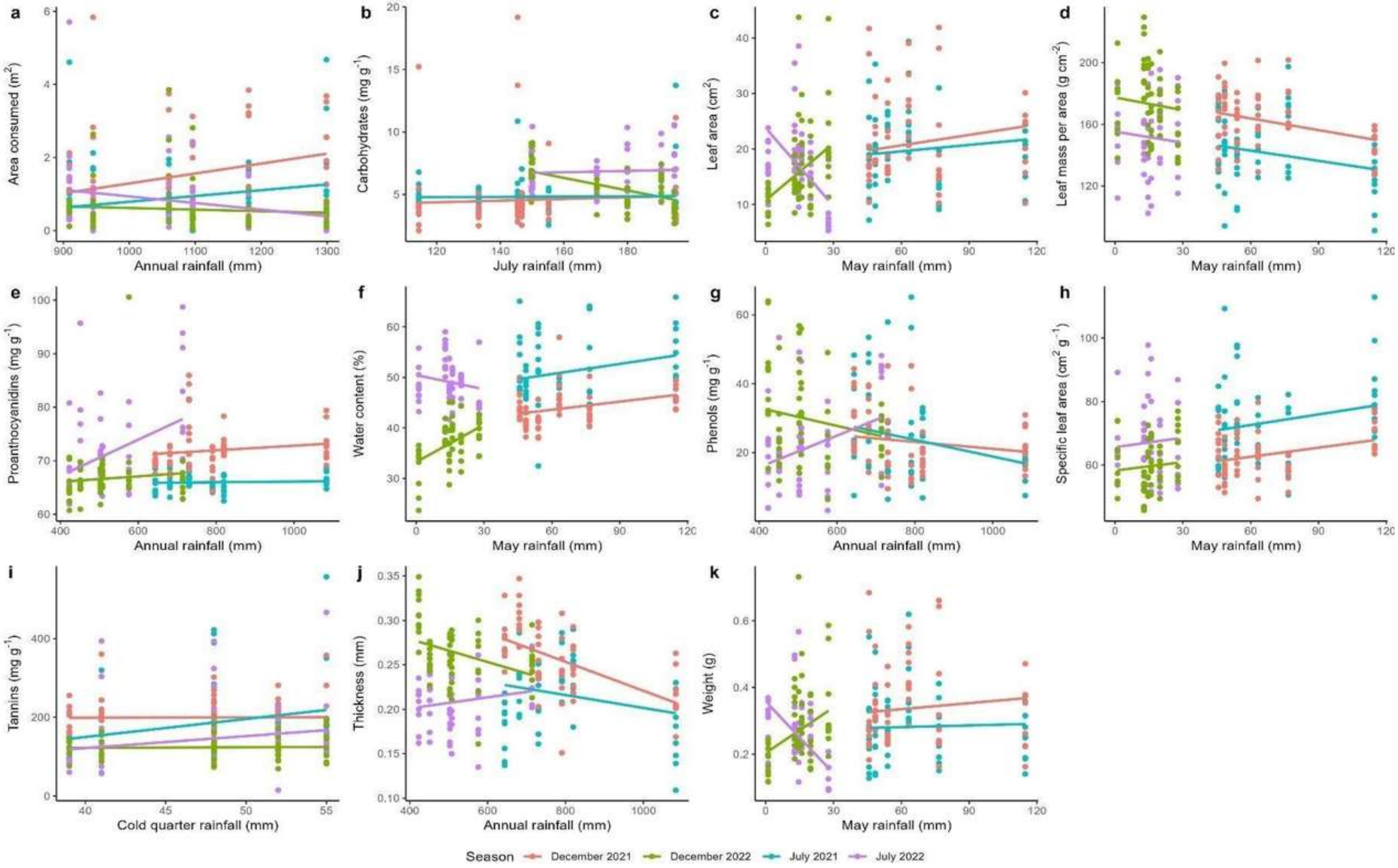

Figure 3. Predictive models for area consumed (herbivory), functional and chemical variables.

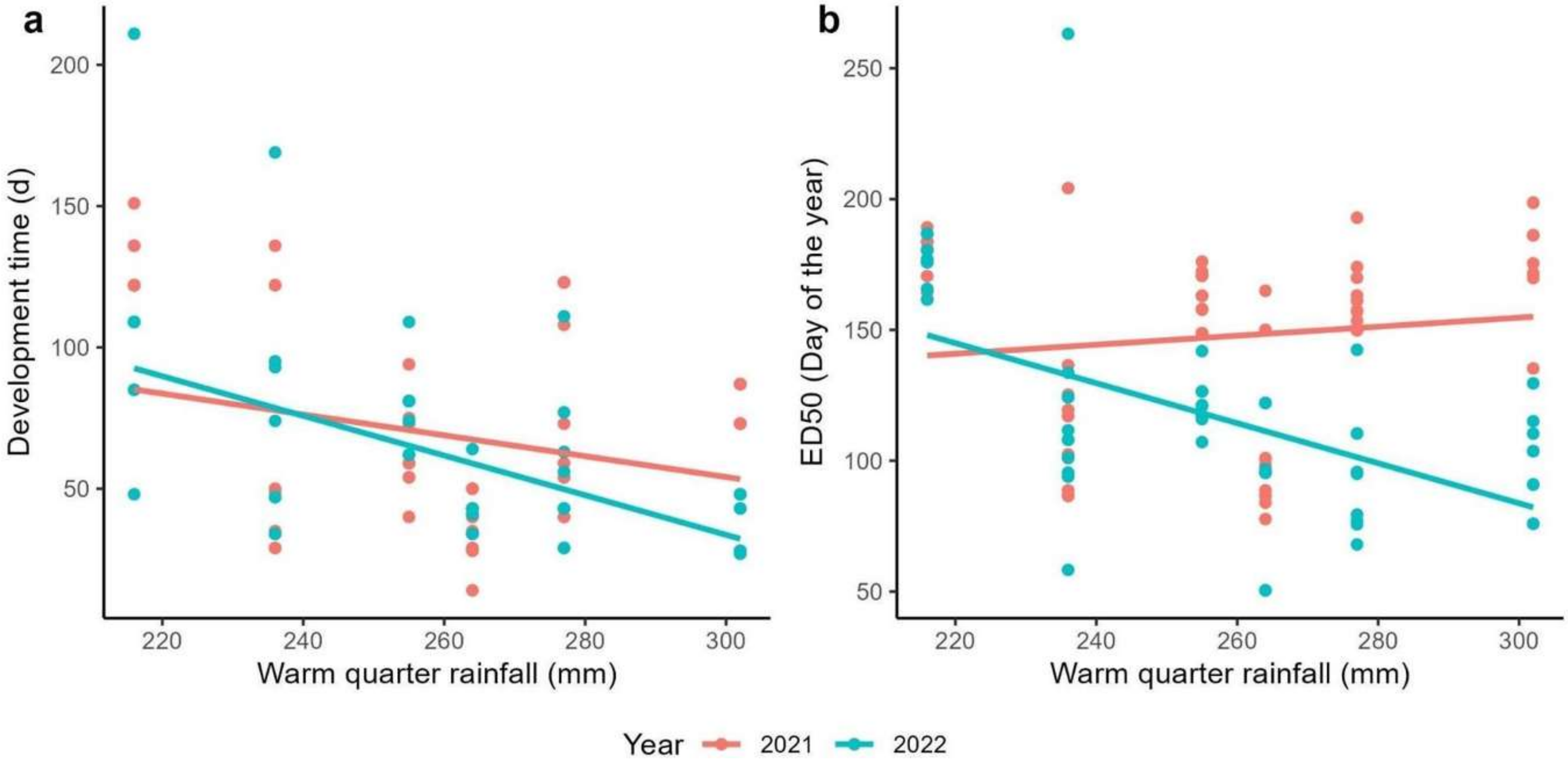

Figure 4. Predictive models for phenological variables.

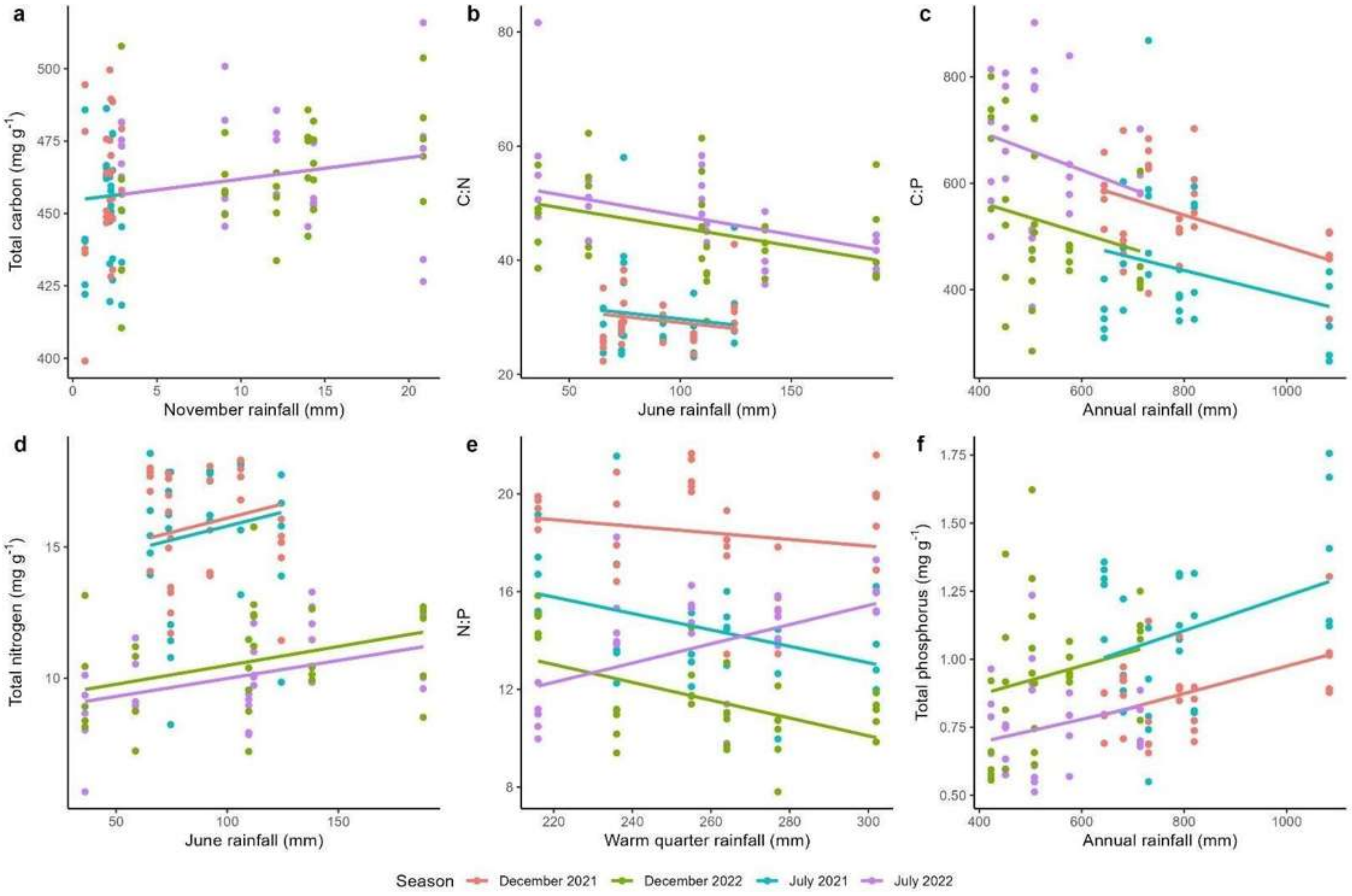

Figure 5. Predictive models for nutritional variables.

Discussion

Many oak species are characterized by their wide distribution across different habitats, making them useful model trees for establishing the influence of biotic and abiotic factors on ecological interactions at large scales (Gratani et al., 2003; Romero et al., 2009; Leckey et al., 2014). In the present study, it has been possible to determine the role played by the environment and the deciduous habit of *Q. castanea* on its functional, chemical and nutritional traits, as well as in its interactions with herbivorous insects.

In our study system, we found average herbivory percentages between 4.6% and 7% across seasons, which are relatively low with respect to other deciduous oak species. Mediavilla et al. (2018) reported that, in the Iberian Peninsula, *Q. pyrenaica* and *Q. faginea*, both deciduous species, exhibited herbivory percentages between 10% and 15%. On the other hand, for *Q. ilex* (an evergreen species), a lower leaf area lost to herbivory of between 1.2% and 4.1% was observed (Ruiz-Carbayo et al., 2020). It has been considered that an important consequence of a deciduous habit may be a reduction in herbivory rates (Blumenthal et al., 2020). Herbivory could be an unrecognized factor that weighs in favor of a deciduous habit compared to an evergreen habit with continuously maintained leaves (Karban and Baldwin, 2007). However, the finding of a diversity of herbivory levels in evergreen and deciduous oaks indicates that leaf habit cannot be the only determinant to understand herbivory levels in these trees (Moreira et al., 2016), which shows the complexity that exists between several factors that intervene in this interaction.

On the other hand, there was no pattern between herbivory observed on young leaves with respect to more mature leaves, since by 2021 young leaves showed greater area consumed, but this was not consistent in 2022, when mature leaves received more attack from herbivores.

There are studies that support that chemical defenses against herbivores in younger leaves are low compared to mature leaves (Donaldson et al., 2006; Ochoa-López et al., 2015; Mediavilla et al., 2018), however, other authors suggest that appearance could be an attractant for insects (Castagneyrol et al., 2013; Moreira et al., 2017) with mature leaves being more detectable and therefore more attacked. In consequence to these contrasting responses, some researchers have pointed out (Goodger et al., 2006; Ochoa-López et al., 2015) that defensive strategies may vary at different growth stages but that this may depend on the environmental pressures present in each case.

In the absence of clear patterns in research that has focused on determining the best predictors of plant-herbivore interaction, our work sought to evaluate multiple factors, spatially and temporally, as a function of a large number of leaf traits in *Q. castanea* in order to have a better understanding of the complexity behind this interaction.

Our generalized mixed model analyses showed that, for most of the response variables, the best model included the interaction between the water availability variables and the sampling season, except for the nutritional variables, for which the interaction was not significant. Particularly, the best environmental predictor (the one that appears for several response variables) is the interpolated annual rainfall, followed by several water availability variables, particularly at the end of the dry season and the beginning of the rainy season: precipitation in May, June, or July or precipitation of the warmest quarter. Our results are in agreement with previous studies concluding that variations in leaf traits in plants arise from ecological strategies in response to environmental pressures (Westoby et al., 2002; Ackerly et al., 2006; Deirdre Loughnan and Williams, 2019). These studies have suggested that there is an abiotic component on plant ecological strategies, mediated directly or indirectly through effects on resource availability or enemy pressure. In oaks, climatic variables have been found to play a key role in shaping patterns of intraspecific variation in leaf traits (Abdala-Roberts et al., 2018), such that covariation in leaf traits and available resource use has been recorded (Pearse and Hipp, 2012).

Recently, a work by de Andrés and collaborators (2024) highlighted the variation of morphological traits of two deciduous oak species (*Q. faginea* and *Q. humilis*) modulated by environmental variables such as water availability, both species showed a high sensitivity to drought and more conservative strategy responses to water use, such as a reduction in leaf area and an increase in LMA (leaf mass per area) under drier conditions.

In our study, herbivory was strongly influenced positively by annual rainfall and precipitation of the warmest quarter of the year. Our findings contrast with the results of other studies, such as that of Loughnan and Williams (2019), which found a negative relationship between insect herbivory abundance and mean summer precipitation. On the contrary, Leckey et al. (2014) found a positive correlation between precipitation and herbivore damage on leaves of four oak species, as the amount of rainfall can affect the intensity of herbivory by leafminers and gall-forming insects. Consequently, sites with abundant rainfall could enhance herbivore activity and thus facilitate the growth of their populations, which could result in increased herbivory pressure (Wang et al., 2016).

Additionally, the network plots showed a consistent association of the area consumed by herbivores with phenological variables in several seasons. As Pearse and Karban (2013) point out, it is not clear which aspect of leaf phenology (timing of leaf formation, speed of leaf expansion, or leaf retention) is the best predictor of significant associations of tree species with herbivory. While there are several studies that support the timing of leaf formation (Diaz et al., 2007; Blumenthal et al., 2020) or the amount of time deciduous trees retain leaves (Karban, 2007; Pearse and Karban, 2013) as strong influencers of responses to herbivory pressures, the speed of leaf expansion has received less attention. Our work shows evidence that one of the most relevant phenological aspects predicting herbivory, at least for the oak species *Q. castanea*, is the speed of leaf expansion. Our results clearly and consistently show that in both years of sampling, there is a positive and significant correlation between the area consumed and the development time of the leaves and their ED50, that is, trees with a slow leaf growth presented greater amounts of area consumed by herbivores. It has been suggested that the speed of leaf expansion can be considered an escape strategy from herbivores (Aide, 1993). This is because gall-forming insects that oviposit their eggs early in the rainy season and throughout the rainy season prefer slow-growing leaves because they can derive greater benefits from them. Growing leaves have high levels of N, which is a great attraction for insects. The longer the leaves are growing, the greater the amount of N that can be used with respect to fast-growing leaves, since, being fully developed leaves, they lower their N levels and lose nutritional quality for herbivores. Thus, when the larvae of these insects emerge, they will begin to feed on plant tissue, causing greater damage in trees where there was a higher incidence of oviposition.

Herbivory levels were also associated with foliar traits. In all four seasons, a positive association of AC was found with leaf area and leaf weight. In one of the seasons, we found a positive association of AC with %water, agrees with other studies where it has been determined how leaf water content correlates with the performance of many herbivorous species (Agrawal, 2004; Johnson, 2011; González-Carrera et al., 2024).

Conclusions

In conclusion, we can determine how the best predictors of herbivory for our species are the phenology and leaf size traits on the one hand, but we must also recognize the importance of abiotic factors such as precipitation, which directly influenced the area consumed and indirectly on the foliar traits, previously mentioned, related to herbivore consumption. Thus, we were able to gain a better understanding of the possible factors driving variation in plant-herbivore interactions at temporal and spatial levels. It is critical for future studies on herbivory issues to consider environmental variables in conjunction with a wide variety of tree traits, rather than separately, in order to gain a comprehensive view of plant responses to different selective pressures and a better understanding of the drivers of this interaction. Over time, a great diversity of traits has been shown to correlate with a plant's resistance or susceptibility to herbivory. It is increasingly recognized that a plant's defense may depend on the effects of these traits acting in concert (Agrawal and Fishbein, 2006; Agrawal, 2007; Carmona et al., 2011).

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SUPPORTING INFORMATION

Table S1. Results of significant correlations for six populations of *Q. castanea* between their foliar attributes (functional, chemical and nutritional) and their soil moisture content in March, April and May 2021 (mean value per individual). Season 1.

Variable(1)	**Variable(2)**	**Pearson**	**p-value**
Area consumed	Weight	0.63	0.000210
Area consumed	Foliar area	0.76	0.000001
PAS	Weight	-0.44	0.014086
PAS	Thickness	-0.49	0.006160
ED50	Area consumed	0.49	0.005844
Development time	Weight	0.38	0.038123
Development time	Thickness	0.47	0.008956
Development time	Foliar area	0.37	0.042963
Development time	Area consumed	0.56	0.001189
LMA	Weight	0.42	0.022312
LMA	Thickness	0.75	0.000002
LMA	PAS	-0.48	0.006704
N	ED50	0.74	0.000003
N	LMA	-0.52	0.003552
P	Thickness	-0.59	0.000567
P	Carbohydrates	0.36	0.049729
P	ED50	0.52	0.003520
P	LMA	-0.73	0.000006
C:N	ED50	-0.71	0.000010
C:N	Development time	-0.38	0.039863
C:N	LMA	0.44	0.014261
C:P	Thickness	0.45	0.011541
C:P	ED50	-0.59	0.000533
C:P	LMA	0.64	0.000161
N:P	Weight	0.38	0.038739
N:P	Thickness	0.61	0.000382
N:P	Development time	0.48	0.006613
SM March	ED50	-0.431	0.017294
SM March	Development time	-0.397	0.029989
SM March	N	-0.610	0.000341
SM March	P	-0.487	0.006300
SM March	C:N	0.625	0.000225
SM March	C:P	0.543	0.001930
SM April	Phenols	0.374	0.041548
SM April	SM March	0.371	0.043516
SM May	Weight	-0.468	0.009100
SM May	Foliar area	-0.505	0.004426

SM May	% foliar water	-0.604	0.000406

Table S2. Results of significant correlations for six populations of *Q. castanea* between their foliar attributes (functional, chemical and nutritional) and their soil moisture content in March, April and May 2021 (mean value per individual). Season 2.

Variable(1)	Variable(2)	Pearson	p-value
Area consumed	Weight	0.600	0.000463
Area consumed	Foliar area	0.688	0.000027
Phenols	Area consumed	-0.384	0.036325
PAS	Thickness	-0.582	0.000734
ED50	Foliar area	0.434	0.016436
ED50	Area consumed	0.381	0.037613
ED50	PAS	-0.400	0.028327
Development time	Thickness	0.435	0.016275
LMA	Weight	0.393	0.031547
LMA	Thickness	0.582	0.000734
LMA	Tannins	-0.541	0.002006
LMA	PAS	-0.437	0.015745
N	PAS	-0.427	0.018544
N	ED50	0.694	0.000021
N	Development time	0.421	0.020658
P	% foliar water	0.461	0.010257
P	N	0.606	0.000390
C:N	Weight	-0.368	0.045397
C:N	ED50	-0.648	0.000109
C:N	Development time	-0.476	0.007835
C:P	% foliar water	-0.392	0.032299
C:P	ED50	-0.438	0.015501
N:P	Thickness	0.384	0.035955
N:P	PAS	-0.445	0.013774
N:P	% foliar water	-0.503	0.004628
SM March	ED50	-0.431	0.017294
SM March	Development time	-0.397	0.029989
SM March	N	-0.473	0.008369
SM March	P	-0.405	0.026493
SM March	C:N	0.411	0.024061
SM March	C:P	0.376	0.040423
SM April	SM March	0.371	0.043516
SM May	Weight	-0.422	0.020201
SM May	Foliar area	-0.461	0.010325
SM May	Area consumed	-0.367	0.046148
SM May	Phenols	0.422	0.020144

Table S3. Results of significant correlations for six populations of *Q. castanea* between their foliar attributes (functional, chemical and nutritional) and their soil moisture content in March, April and May 2022 (mean value per individual). Season 3.

Variable(1)	Variable(2)	Pearson	p-value
Area consumed	Weight	0.406	0.025812
Area consumed	Foliar area	0.574	0.000910
Phenols	Thickness	0.431	0.017372
Phenols	Foliar area	-0.401	0.027986
Tannins	Weight	-0.392	0.032309
Tannins	Foliar area	-0.377	0.039898
PAS	Thickness	-0.499	0.004964
% foliar wáter	Weight	0.380	0.038174
% foliar wáter	Thickness	-0.473	0.008319
% foliar wáter	Foliar area	0.604	0.000414
% foliar wáter	Area consumed	0.498	0.005144
% foliar wáter	Phenols	-0.381	0.037999
% foliar wáter	Tannins	-0.391	0.032657
ED50	Area consumed	0.456	0.011256
LMA	Thickness	0.553	0.001543
LMA	Foliar area	-0.486	0.006430
LMA	% foliar water	-0.629	0.000194
P	Tannins	-0.394	0.031313
P	% foliar water	0.392	0.032016
C:N	ED50	0.456	0.011310
C:N	Development time	0.404	0.026626
C:P	% foliar water	-0.389	0.033401
N:P	Foliar area	-0.443	0.014157
N:P	Area consumed	-0.386	0.035120
N:P	Phenols	0.364	0.048145
N:P	Tannins	0.383	0.036465
N:P	% foliar water	-0.675	0.000043
N:P	LMA	0.452	0.012209
SM March	PAS	0.434	0.016509
SM March	P	0.428	0.018436
SM March	C:P	-0.395	0.030828
SM April	Foliar area	-0.448	0.013034
SM April	Tannins	0.409	0.024637
SM April	PAS	0.430	0.017712
SM April	% foliar water	-0.530	0.002593
SM April	LMA	0.506	0.004335
SM May	Weight	-0.365	0.047035
SM May	Foliar area	-0.500	0.004910
SM May	Tannins	0.547	0.001750
SM May	% foliar water	-0.650	0.000103
SM May	LMA	0.489	0.006132
SM May	N:P	0.547	0.001759
SM May	SM April	0.719	0.000008

Table S4. Results of significant correlations for six populations of *Q. castanea* between their foliar attributes (functional, chemical and nutritional) and their soil moisture content in March, April and May 2022 (mean value per individual). Season 4.

Variable(1)	**Variable(2)**	**Pearson**	**p-value**
Area cosumed	Foliar area	0.506	0.004345
Carbohydrates	Phenols	0.452	0.012099
% foliar wáter	Foliar area	0.420	0.021009
% foliar wáter	Area cosumed	0.393	0.031737
ED50	Weight	0.506	0.004295
ED50	Thickness	0.676	0.000042
ED50	% foliar water	0.371	0.043732
Development time	Weight	0.492	0.005772
Development time	Thickness	0.596	0.000510
Development time	Area cosumed	0.379	0.038867
LMA	Weight	0.450	0.012556
LMA	Foliar area	-0.655	0.000086
P	Thickness	-0.425	0.019117
P	ED50	-0.605	0.000400
C:P	Thickness	0.420	0.020997
C:P	ED50	0.615	0.000301
N:P	Weight	0.406	0.026143
N:P	Thickness	0.505	0.004422
N:P	Phenols	0.467	0.009198
N:P	% foliar water	0.399	0.028741
N:P	ED50	0.817	0.000000
N:P	Development time	0.538	0.002162
SM April	Weight	-0.391	0.032538
SM April	PAS	0.379	0.039111
SM April	LMA	-0.542	0.001990
SM May	% foliar water	0.384	0.036138
SM May	LMA	-0.389	0.033506
SM May	SM April	0.719	0.000008

Table S5. Result of the sum of significant correlations (r value) for each foliar attribute and soil moisture content in the months of March, April and May 2021 for the six populations of *Q. castanea*.

Season 1

Variable	**$\sum R^2$**
C:P	4.77
LMA	4.45
C:N	3.78
ED50	3.48
Soil moisture March	3.46
P	3.42
Thickness	3.36
N:P	3.10
Development time	3.04
Weight	2.71
N	2.59
Area consumed	2.44
Soil moisture May	1.58
PAS	1.41
Leaf área	1.13
Soil moisture April	0.75
% Foliar wáter	0.60
Phenols	0.37
Carbohydrates	0.36

Table S6. Result of the sum of significant correlations (r value) for each foliar attribute and soil moisture content in the months of March, April and May 2021 for the six populations of *Q. castanea*.

Season 2

Variable	**$\sum R^2$**
ED50	3.43
Soil moisture March	2.86
N	2.62
Area consumed	2.42
PAS	2.29
Thickness	1.98
LMA	1.95
C:N	1.90
Weight	1.79
Development time	1.73
Leaf área	1.58
P	1.47
% Foliar wáter	1.36
N:P	1.33
C:P	1.21
Phenols	0.81
Tannins	0.54
Soil moisture April	0.37
Soil moisture May	0.37

Table S7. Result of the sum of significant correlations (r value) for each foliar attribute and soil moisture content in the months of March, April and May 2022 for the six populations of *Q. castanea*.

Season 3

Variable	$\sum \mathbf{R}^2$
% Foliar wáter	5.99
Leaf área	3.83
Soil moisture May	3.82
N:P	3.25
Soil moisture April	3.04
Tannins	2.89
LMA	2.63
Area consumed	2.32
Thickness	1.96
P	1.80
Phenols	1.58
Weight	1.54
PAS	1.36
Soil moisture March	1.26
ED50	0.91
C:N	0.86
C:P	0.78
N	0.58
Development time	0.40

Table S8. Result of the sum of significant correlations (r value) for each foliar attribute and soil moisture content in the months of March, April and May 2022 for the six populations of *Q. castanea*.

Season 4

Variable	**$\sum R^2$**
ED50	3.59
Thickness	3.15
N:P	3.13
Weight	2.77
Soil moisture April	2.03
Development time	2.00
% Foliar water	1.97
P	1.88
Leaf área	1.58
LMA	1.49
Soil moisture May	1.49
Area consumed	1.28
C:P	1.03
Phenols	0.92
N	0.82
Tannins	0.46
Carbohydrates	0.45
PAS	0.38

Table S9. Indicators used in mixed models to describe water availability.

Water availability indicator	Description
SM_May	Soil moisture, with 10 measurements per site (one per tree) and repeated for the years 2021 and 2022 in May (beginning of the rainy season).
CRA	Soil moisture holding capacity, which was taken per tree (one measurement).
May_Rainfall_interpol	Precipitation data interpolated for the month of May from CONAGUA's network of meteorological stations. One data per site and per year.
Jun_Rainfall_interpol	Precipitation data interpolated for the month of June from CONAGUA's network of meteorological stations.
Jul_Rainfall_interpol	Precipitation data interpolated for the month of July from CONAGUA's network of meteorological stations.
Nov_Rainfall_interpol	Precipitation data interpolated for the month of November from CONAGUA's network of meteorological stations.
Dic_Rainfall_interpol	Precipitation data interpolated for the month of December from CONAGUA's network of meteorological stations.
Annual_Rainfall_interpol	Precipitation data interpolated for the year 2021 and 2022 from CONAGUA's network of meteorological stations.

Annual_Rainfall_WClim	Annual precipitation. Data from WorldClim.
Warm_quarter_Rainfall_WClim	Precipitation in the warmest quarter. Data from WorldClim.
Cold_quarter_Rainfall_WClim	Precipitation in the coldest quarter. Data from WorldClim.

VII. DISCUSIÓN GENERAL

Las respuestas de las plantas hacia la variabilidad ambiental han sido ampliamente estudiadas desde finales del siglo pasado hasta la actualidad. Crecientemente, este tipo de estudios ha tomado mayor relevancia debido a que los cambios en el entorno natural cada vez se desarrollan de una manera más abrupta y a una mayor velocidad. Por lo que es necesario facilitar la predicción de estas respuestas a través de investigaciones que se centren en estudiar las limitaciones ecológicas y evolutivas de las plantas a los cambios globales a través de sus rasgos y la sinergia entre ellos (Wright et al., 2004, Blumenthal et al., 2020). En el presente trabajo, se buscó determinar cuál es la influencia de la disponibilidad de agua dentro de los sistemas naturales sobre la variación potencialmente adaptativa en la especie de encino *Q. castanea* para seis sitios contrastantes en distintas épocas del año, centrándose principalmente en la fenología foliar, así como en sus características funcionales, químicas y nutrimentales. Posteriormente, se infirió cómo esta variación foliar y ambiental puede modular las relaciones entre *Q. castanea* y enemigos naturales como los insectos herbívoros.

Las especies del género *Quercus* a menudo han sido consideradas como especies modelo, por lo que han sido foco de investigaciones que buscan establecer el grado de influencia de las señales climáticas sobre especies arbóreas (Gerts et al., 2017). Gracias a su amplia distribución geográfica, el género está representado en hábitats contrastantes en ambientes templados y/o con escasez de agua. Dichas diferencias ambientales se asocian con historias naturales distintas que pueden conducir a una variación en las estrategias ecológicas de las especies (Abdala-Roberts et al., 2018). Los encinos están adaptados para tener un uso eficiente de los recursos y puede haber cierta plasticidad ecológica y distintos hábitos foliares entre especies a lo largo de la temporada de crecimiento, respondiendo a cambios de precipitación y temperatura (Armstrong-Herniman y Greenwood, 2021)

A lo largo de esta investigación se destaca el papel adaptativo de la fenología de las hojas en los árboles de *Q. castanea*, ya que al ser un rasgo fuertemente correlacionado con condiciones ambientales que favorecen el crecimiento o en su defecto pueden impedirlo, tiene implicaciones en su desarrollo estructural a nivel individual, así como en sus relaciones con otras especies dentro de los ecosistemas (Coyle et al., 2010; Denny et al., 2014; Cole y Sheldon, 2017). A pesar de que México posee una gran diversidad del género, nuestro trabajo es uno de los pocos estudios en nuestro país que abordan el tema de la fenología y rasgos foliares en una especie de encino con hábito foliar deciduo.

Nuestros resultados en el primer capítulo indican que la fenología foliar observada dentro de la cuenca de Cuitzeo para *Q. castanea* tuvo una variación significativa entre poblaciones, de igual manera, esta variación también fue significativamente diferente en ambos años reportados (2021-2022). Para los dos años, el ED50 y el DT mostraron diferencias significativas entre las poblaciones, posiblemente asociadas a la diversidad ambiental de los sitios muestreados (Mendoza et al. 2011). Pese a la cercanía geográfica entre las poblaciones, existe un evidente contraste de hábitats, algunos caracterizados por bosques templados y otros por bosques tropicales secos, lo que influye en una variación fenológica a nivel espacial.

Asimismo, encontramos diferencias en los valores de ED50 para tres poblaciones entre años y en el DT para una población. Lo que demuestra la importancia de la temporalidad en los patrones fenológicos observados. La variación a nivel temporal se puede encontrar asociada a factores ambientales fluctuantes como los son la temperatura y la precipitación. En este caso, determinamos que dos de las variables más significativas con las que se relaciona la variación observada en la fenología foliar primaveral es la precipitación otoñal del año anterior (octubre) y la humedad del suelo en la primavera del mismo año (marzo o abril). La disponibilidad de agua como resultado de las lluvias otoñales, sugieren un posible efecto retardado. Además, para ambos años, observamos que el momento y la intensidad de las primeras lluvias primaverales son muy importantes para dar paso a los primeros brotes y posteriormente al desarrollo foliar (Aguilar-Romero et al., 2017).

De modo similar, se identificaron asociaciones adicionales entre los parámetros fenológicos y los valores medios a largo plazo de dos variables bioclimáticas, PS (estacionalidad de la precipitación) y PWQ (precipitación del trimestre más cálido). Por lo tanto, estos patrones resaltan la gran importancia de la disponibilidad de agua durante el período más seco del año para los patrones fenológicos de *Q. castanea*. A pesar de que se tomaron en cuenta variables ambientales de temperatura, para este estudio no mostraron influencia, sugiriendo que *Q. castanea* tiene un comportamiento distinto a las especies templadas de encino, que toman como principal señal ambiental la temperatura para el inicio de la brotación de hojas (Basler y Körner, 2012; Wright et al. 2021; Meger et al. 2024), y mostrando una mayor similitud con especies tropicales que tienen como principal impulsor climático para el desarrollo de sus hojas a la precipitación (Borchert et al., 2005; Samtani, Appleby y Masiunas, 2015). Por otro lado, uno de los rasgos foliares con mayor correlación tanto con el ED50 y el DT en ambos años fue el grosor de las hojas (LT), mostrando hojas más gruesas en individuos cuyo ED50 y DT fueron mayores, es decir que el crecimiento lento está asociado con un mayor grosor de las hojas. Algunos estudios han demostrado que las hojas gruesas de los árboles tienen una mayor eficiencia fotosintética que ayuda a compensar la inversión de carbono necesaria para la construcción de las hojas (Niinemets, 1999; Niinemets, 2001).

En el segundo capítulo, nuestros resultados permitieron inferir nuevamente la importancia que tienen los factores ambientales en la variación de los rasgos foliares y primordialmente en la interacción de la herbivoría. A pesar de haber encontrado bajos niveles de herbivoría en los individuos, probablemente en relación con un hábito foliar caducifolio (Blumenthal et al., 2020), se determinó que la herbivoría se vio fuertemente influenciada de forma positiva por las precipitaciones anuales y la precipitación en el trimestre más cálido del año. En los lugares con precipitaciones más abundantes podría observarse una mayor actividad de los herbívoros y facilitar así el crecimiento de sus poblaciones, lo que podría resultar en una mayor presión herbívora hacia los árboles (Leckey et al., 2014; Wang et al., 2016). Asimismo, la precipitación anual también fue el mejor predictor ambiental para la mayoría de los rasgos foliares que medimos, lo que sugiere evidencia de un componente abiótico sobre las estrategias ecológicas de la especie de encino estudiada (Ackerly et al., 2006; Loughnan y Williams, 2018). En trabajos previos, se ha observado que las variables climáticas desempeñan un papel clave en la conformación de patrones de variación intraespecífica en los rasgos foliares del género *Quercus*, además se ha observado que dichos rasgos pueden covariar en función de los recursos disponibles (Pearse y Hipp, 2012; Abdala-

Roberts et al., 2018). Sin embargo, no solo influye un factor ambiental sobre la herbivoría encontrada, los resultados indican que las variables fenológicas y las variables funcionales como el área foliar y el peso se correlacionan positivamente con el área consumida por herbívoros.

En primer lugar, los árboles que presentaron una velocidad de crecimiento lenta tuvieron mayores cantidades de área consumida por los herbívoros en sus hojas respecto a aquellos con un tiempo de desarrollo más corto y, por tanto, más rápido. Por otro lado, los niveles de herbivoría se correlacionaron positivamente con el área foliar y el peso de la hoja, siendo las hojas más grandes y pesadas más atractivas para el consumo. Estudios recientes han determinado que la percepción de los insectos hacia las hojas, es fundamental a la hora de la elección del alimento (González-Carrera et al., 2024), así pues, hojas más grandes son más perceptibles y atractivas para los herbívoros, por lo que llegan a ser mayormente consumidas, el tamaño también llega a ser un referente sobre la calidad de la hoja. Cada vez más se reconoce que la defensa de una planta contra los herbívoros puede depender de los efectos de una variedad de rasgos foliares actuando de forma concertada (Agrawal y Fishbein, 2006; Agrawal, 2007; Carmona et al., 2010).

VIII. CONCLUSIONES

- Las poblaciones de *Q. castanea* encontradas en sitios con menor disponibilidad de agua y con una estacionalidad de precipitación más marcada en la cuenca de Cuitzeo tienen un crecimiento foliar más lento en comparación con las poblaciones menos estacionales.
- La fenología foliar difirió entre sitios, tanto para 2021 como para 2022, lo que significa que la variación está respondiendo a cambios ambientales. Los encinos de Michoacán parecen ajustar su fenología de acuerdo a la precipitación y acumulación de agua en el ambiente, un comportamiento que se ha reportado en especies forestales estacionalmente secas.
- Algunos rasgos foliares específicos de la planta, como el grosor foliar, juegan un papel crucial en la fenología de los encinos en latitudes tropicales.
- Los mejores predictores de la herbivoría para nuestra especie son la fenología y el área foliar por otro lado, también se encontraron factores abióticos con fuerza predictora como la precipitación. Esta influyó directamente en el área consumida y en algunos rasgos foliares relacionados con el consumo de herbívoros como el AF**.**

IX. BIBLIOGRAFÍA COMPLEMENTARIA

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X.ANEXO 1. Corresponde al CAPÍTULO I de este documento de tesis en donde se incluye el artículo publicado como requisito para la obtención de grado como parte del Programa Institucional de Doctorado en Ciencias Biológicas de la UMSNH, que corresponde a: Ochoa-Alvarez, T. C., Contreras-Negrete, G., Lara-De La Cruz, L. I., & González-Rodríguez, A. (2024). Landscape-level variation in spring leaf phenology is driven by precipitation seasonality in the Mexican red oak *Quercus castanea*. *AoB PLANTS*, plae067. DOI https://doi.org/10.1093/aobpla/plae067

Landscape-level variation in spring leaf phenology is driven by precipitation seasonality in the Mexican red oak *Quercus castanea*.

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ABSTRACT

Water availability is one of the essential factors that determine the distribution of plant species, as well as their ecological strategies. The study of leaf phenology, in conjunction with other leaf traits of an ecological nature, such as functional traits, makes it possible to determine the life history strategies of plant species and their variation along environmental gradients, which in turn influences the demographic rates of populations. In the present study, we analyzed the effect of water availability at the landscape scale on spring leaf phenology and foliar traits such as leaf mass per area (LMA) and leaf thickness (LT) in the oak species *Quercus castanea* from a tropical latitude in central-western Mexico. Six sites were selected in the Cuitzeo basin, Michoacán, across a water availability gradient, ranging from 900 mm to 1299 mm of mean annual precipitation. Leaf samples were collected from 10 adult trees at each site and LT and LMA were estimated. Leaf phenology was monitored for each tree every two weeks between March and July for two consecutive years, 2021 and 2022, alongside soil moisture measurements.

Temperature and precipitation variables for the two study years were obtained from meteorological stations and long-term bioclimatic variables from the Worldclim database. Significant spatial and temporal variation in leaf phenology was observed. Earlier leaf development and shorter development times were observed with increased soil moisture in March and April, and with higher precipitation in October of the previous year. Also, sites with long-term higher precipitation seasonality and with lower precipitation of the warmest quarter showedlonger development times. A positive association between development times and leaf thickness was also observed. In Conclusion, *Quercus castanea* shows a brevideciduous leaf phenology but with significant variation among populations, reflecting spatiotemporal mosaics of environmental and genetic variation and in covariation with leaf functional traits such as leaf thickness.

Key words: Adaptation, Cuitzeo basin, environmental heterogeneity, phenotypic plasticity, leaf functional traits.

ABBREVIATIONS

MAT	Mean annual temperature.
MTDQ	Mean temperature of the driest quarter.
MTWQ	Mean temperature of the warmest quarter.
AP	Annual precipitation.
PS	Precipitation seasonality.
PDQ	Precipitation of the driest quarter.
PWQ	Precipitation of the warmest quarter.
LT	Dry leaf thickness.
LMA	Leaf mass per area.
DOY	Day of the year.
ED50	Represents the number of days that elapse from the beginning of the year until the day on which the tree reaches 50% of its leaf development.
DT	Development time.

INTRODUCTION

Plant phenology refers to the periodic production of vegetative and reproductive structures; particularly, to the timing of initiation and the duration of growth and reproductive cycles (Schwartz, 2003). The analysis of leaf phenology has taken great relevance in recent years, since it is considered an essential adaptive trait in plants (Cleland et al., 2007; Gerst et al., 2017; Armstrong and Greenwood, 2021). That is, through the course of their evolution, plants have optimized the period of the year that is most favorable for leaf burst and leaf expansion under different environments. Such adjustment allows plants to meet their metabolic requirements while avoiding damage due to unfavorable environmental conditions, as extreme temperatures, or periods of drought (Singh et al., 2017). Therefore, leaf phenology plays a fundamental role in mediating carbon balance and herbivory levels (Polgar and Primack, 2011; Cleland et al., 2012; Gallinat et al., 2015).

Different environmental cues are related to leaf phenology patterns in plants, varying according to ecosystem type. In temperate deciduous forests, the increase in temperature during the spring triggers the sprouting of leaves of most tree species (Chuine and Régnire, 2017). When temperature decreases, as it does when autumn starts, the process of senescence and leaf fall begins (Borchert et al., 2005). Another important factor linked to leaf phenology is photoperiod (Schaber and Badeck, 2003; Samtani, Appleby and Masiunas, 2015). This is because a minimum daily exposure to sunlight is essential to accomplish plant biological functions and, therefore, the time of leaf growth is usually associated to daylength. In a future climate, photoperiod could be important in limiting the timing of spring phenology when increasingly warmer climatic conditions accelerate development (earlier bud break). These changes in phenology will tend to be observed mostly in temperate latitudes where there is less adaptation to high temperatures with respect to tropical latitudes (Basler and Körner, 2012; Dewan et al., 2020; Faticov et al., 2020; Koenig et al., 2021).

In contrast, in other biomes which also experience a marked environmental seasonality, such as tropical deciduous forests, leaf phenology is mainly determined by the temporal variation in precipitation, while temperature variation is of less importance (Armstrong and Greenwood, 2021). However, in tropical regions with large seasonal variation in precipitation, phenology cannot be predicted from climatic data alone. This is because the onset of leaf growth is mainly determined by seasonal variation in tree water status, day length and old leaf fall (Borchert, 1994, 2004). The growing season in these forests is not significantly reduced even during prolonged periods of low rainfall, since water tends to remain stored in the soil, protecting trees against seasonal drought (Borchert et al., 2005).

The genus *Quercus*, with more than 500 extant species, is one of the most important groups of woody plants in the northern hemisphere and often a dominant component of very valuable and diverse ecosystems, such as forests and shrubland communities (Hipp et al., 2018; Cavender-Bares, 2016). In general, compared to other forests, oak forests are characterized by greater species diversity, stratification, litter production and soil fertility (Bargali et al., 2015). *Quercus* species have been the basis of numerous studies seeking to explain how climatic cues influence phenological patterns. Research has focused mainly on the influence of temperature and precipitation patterns on leaf phenology of species in temperate zones (Morin et al., 2009; Vitasse et al., 2009; Basler and Körner, 2012). For example, higher temperatures can induce early bud burst and leaf flushing in Californian oaks such as *Q. lobata* and *Q. agrifolia*, whereas eastern oaks as *Q. rubra* and *Q. alba* are more responsive to spatial and temporal variations in precipitation, because the deep tap roots of *Q. agrifolia* and *Q. lobata* may protect them from temporal variation in precipitation (Gerst et al., 2017). However, in another study with five Californian oak species, *Q. agrifolia*, *Q. kelloggii*, *Q. douglasii*, *Q. garryana* and *Q. lobata*, precipitation in the winter season was found to be the main climatic driver of spring leaf phenology (Armstrong-Herniman and Greenwood, 2021). Studies in Europe have focused on analyzing the influence that temperature increase resulting from global change has had as the main driver of phenological changes in oaks on that continent (Morin et al, 2010; Dantec et al, 2014; Caignard et al, 2017; Journé et al, 2021). For example, the number of dry spells, the number of frost days in the autumn and the spring, and the number of hot days in the autumn were associated with longer canopy duration in *Q. robur* and *Q. petraea*, with consequences for reproductive output (Journé et al., 2021).

Studies of leaf phenology in oak species at tropical latitudes are scarcer and have shown contrasting responses. For example, significant correlations were found of the timing and duration of leaf development with temperature and soil water potential for *Q. magnoliifolia*, but for *Q. resinosa* the duration of leaf development was negatively correlated with precipitation, with both species growing at different positions of the same altitudinal gradient (Hernández-Calderón et al. 2013). These results therefore indicate different drivers for leaf phenology in these two partially coexisting oak species and suggest that temperature, precipitation and soil water availability are relevant to understand leaf phenology of oaks in tropical regions.

In this study, we focused on leaf phenology variation of the Mexican red oak *Q. castanea* at the landscape level in the Cuitzeo basin, Michoacán. From casual observations, the species is considered deciduous or brevideciduous (Aguilar-Romero et al., 2017; Kaproth et al., 2023) but there are no formal studies in this regard. The basin has an area of approximately 4000 km^2 and is highly heterogeneous topographically and climatically, with an increase in precipitation and a decrease in temperature from north to south and with elevation (Mendoza et al., 2006). Among approximately 13 oak species that occur in this basin, *Q. castanea* is the most abundant and widely distributed, and it can be found in diverse communities such as oak forests, pine-oak forests and subtropical scrublands (Aguilar-Romero et al., 2016; Lara-De la Cruz et al., 2020). Previous studies identified a significant association between leaf thickness (a trait indicating the degree of sclerophylly) of *Q. castanea* populations in the basin and precipitation seasonality (Lara-De La Cruz et al., 2020). In tropical oaks such as *Q. oleoides*, an association between phenological patterns (i.e. degree of leaf deciduousness) and leaf mass per area has been found (Cavender-Bares and Ramírez-Valiente, 2017), highlighting the importance of simultaneously considering phenology and other functional traits for understanding population adaptation to environmental gradients within oak species.

Here, we hypothesized that *Q. castanea* populations located in areas with higher precipitation seasonality in the Cuitzeo basin will be more deciduous than those in sites with lower seasonality. To test the hypothesis, we determined foliar phenology patterns of *Q. castanea* at six sites along the climatic gradient in the basin over a two-year period. Then, we assessed the influence of climatic and environmental variables by obtaining data of temperature and precipitation as well as soil moisture. In particular, we evaluated the following questions: i) Is there temporal (i. e. between years) and spatial (i. e. among sites) variability in leaf phenology patterns of *Q. castanea* across the climatic gradient? ii) What are the main environmental variables that influence leaf phenology variation? iii) How does variation in leaf functional traits (leaf thickness and leaf mass per area) relate to leaf phenology?

MATERIALS AND METHODS

Study system

The study was carried out in the Cuitzeo basin, Michoacán. In this area, populations of *Q. castanea* can be found between 2000 and 2800 meters above sea level (Herrera-Arroyo et al., 2013). The basin is located between 19° 30′ 0′′ and 20° 0′ 0′′ latitude N and 100° 45′ 0′′ and 101° 30′ 0′′ longitude W, with an area of about 4000 km^2. It is highly heterogeneous in climate, topography and vegetation (Leal-Nares et al., 2010; Lara-De La Cruz et al., 2020).

Sampling design

Six sites with populations of *Quercus castanea* were selected across the geographic and climatic gradient of the Cuitzeo basin. According to WorldClim (https://www.worldclim.org/) data for the 1970-2000 period, annual precipitation in the six sites ranges from 909 mm to 1299 mm and mean annual temperature from 13.8 °C to 16.9 °C (**Table 1**, **Figure 1**). In each of the six sites chosen, 10 adult *Q. castanea* trees with diameters at breast height between 30 and 80 cm were randomly selected (maintaining a minimum distance of at least 20 m between individuals) and marked in order to keep a record of their leaf phenology during the years 2021 and 2022.

Leaf phenology

Since previous studies indicated that the period of highest litterfall production in *Q. castanea* in the Cuitzeo basin occurs between February and March (Chávez-Vergara et al., 2015), monitoring was conducted every two weeks in the period from March to July of 2021 and 2022. During visits, previously marked individuals were monitored, for which photographs were taken of the branches and crowns of the trees, to later determine the stage of leaf development of each tree. Five leaf development stages were determined and assigned a numerical value for subsequent analysis. The stages were as follows: foliage of the previous year = 0, dormant buds = 1, bud burst = 2, leaf unfolding = 3, and developed leaves = 4 (**Figure 2**).

Meteorological and soil moisture data

Temperature (minimum, mean and maximum) and rainfall monthly data were obtained from 40 weather stations of the National Water Commission (Comisión Nacional del Agua) (CONAGUA, 2020) located in the northern part of Michoacán state, near the study sites (**Figure S1**) for 2021 and 2022. To obtain specific data for the six study sites, we performed regionalized spatial interpolation of precipitation and temperature from weather stations with the R package RegRAIN (version 0.1.0) (Alzate-Velásquez et al., 2017). This package incorporates multiple linear regression, spline and inverse distance weighting interpolations. For calculations the program uses a digital elevation model, as well as climate data. In our case, a digital elevation model for Michoacán state, with resolution of 15 m, was obtained from the National Institute of Statistics and Geography (Instituto Nacional de Estadística y Geografía, INEGI). Raster maps were obtained from the interpolations and monthly minimum, mean and maximum temperature, and precipitation, were obtained for the six study sites using ArcGIS ver. 10.3.

In addition, we used WordlClim data, which describe average temperature and precipitation data for a 30-year period (1970-2000). We downloaded the 19 bioclimatic variables for each monitored site (https://www.worldclim.org/). Subsequently, the climatic variables that were redundant (with correlation higher than 0.7) were eliminated, leaving a total of eight variables with which the regression analyses with the phenological variables were carried out. The variables used were the following: mean annual temperature (MAT); isothermality (defined as mean diurnal range of the temperature divided by the temperature annual range and multiplied by 100); mean temperature of the driest quarter (MTDQ); mean temperature of the warmest quarter (MTWQ); annual precipitation (AP); precipitation seasonality(PS), which measures the deviation from a uniform distribution of rainfall throughout the year, with small values indicating little or no seasonal variation in precipitation; Walsh and Lawler, 1981); precipitation of the driest quarter (PDQ); and precipitation of the warmest quarter (PWQ). Besides, during each visit to the study sites, we measured soil moisture near the base of the trunk of each monitored individual at a depth of aproximately 15 cm, using the soil moisture Delta-T Device (Cambridge, UK) sensor.

Leaf functional traits

For each monitored *Q. castanea* individual, we measured dry leaf thickness (LT) and leaf mass per area (LMA). For this purpose, 15 mature autumn leaves were collected in November from each monitored tree during the two study years. Leaves were pressed and dried at 40°C for three days. Leaf thickness was then measured using a digital Vernier device. The weight of each of the 15 leaves collected for each tree was obtained on an analytical balance and leaf area was measured in the ImageJ program using digitialized images. LMA was obtained by dividing the dry mass (weight) by the leaf area.

Data analysis

To analyze leaf phenology patterns of each monitored tree during 2021 and 2022, dose–response relationships were modeled using the “drc” library in R (Ritz et al., 2015). In these analyses, the independent variable was the day of the year (DOY) of each phenological observation, considering as day 1 January first, and the dependent variable was the leaf development stage of each individual. Data obtained for both years were separately analyzed. First, a four-parameter log-logistic model (i. e., lower limit, slope, ED50, upper limit,) was tested. Then, based on this model, we tested the best fitting model (i. e., L.L.2, 3, 4 or 5 parameters, linear, quadratic, cubic, and Weibull 1 or 2) which was selected according to the Akaike Information Criterion (AIC) with the "mselec" function. From these models, for each individual tree we estimated ED50 (effective dose-response at 50%, which in our case represents the number of days that elapse from the beginning of the year until the day on which the tree reaches 50% of its leaf development, DOY). Additionally, we calculated development time (DT), as the number of days elapsed between stage 1 (dormant buds) and stage 4 (fully expanded leaves).

Non-parametric Kruskall-Wallis tests were performed to determine if there were differences in ED50 and DT between populations within a single year and between years for each population, using values for each individual tree.

To identify environmental variables influencing leaf phenology variation, we used data of the eight selected bioclimatic variables obtained from the Worldclim database and performed regression analysis with each of the phenological variables (ED50 and DT). Similarly, we used the monthly averages of maximum, minimum and mean temperature as well as monthly rainfall for the years 2021 and 2022. Finally, soil moisture data registered during each monitoring date was also used as an independent variable

To determine the association between leaf functional traits and phenological variables, linear regression analyses were performed with data of LT and LMA obtained for each year and corresponding values of ED50 and DT.

RESULTS

Spatial and temporal variation in leaf phenology

There were significant differences among populations within years and for populations between years for ED50 and DT (**Table 2, Figure 3**). In 2021, the population average DOY for ED50 varied between 104 and 179, corresponding to San Miguel and Tamanguío, respectively. In 2022, the DOY for ED50 varied between 95 and 177, with San Miguel and Remolino showing the first value and San Nicolás showing the later value. Between years, the ED50 for Atécuaro, Remolino and Tamanguío were significantly different, with 2022 showing an earlier DOY in the three cases.

For the year 2021, the shortest DT was observed in San Miguel (37 days), and the longer in San Nicolás (128 days). In 2022, Tamanguío showed the shortest development time (38 days) and San Nicolás the longest (108). Differences between years were only significant in the case of Tamanguío, with a decrease from 74 days in 2021 to 38 days in 2022.

As expected from the observed interannual variation in ED50 and DT, individual tree values of these two variables were significantly correlated ($P < 0.01$) between years 2021 and 2022, but with low or moderate R^2 (0.15 for ED50 and 0.41 for DT) (**Figure S2**).

Correlations between phenological and environmental variables

In Figure 4 we show only the correlations that were significant between population mean phenological variables for 2021 and 2022 and the long-term bioclimatic variables and the precipitation variables from 2021 and 2022 derived from meteorological stations. DT of 2021 was positively correlated with the average PS for 1970-2000 ($R^2 = 0.76$; $P = 0.02$) (**Figure 4 a**), and DT of 2022 was negatively correlated with average PWQ for 1970-2000 ($R^2 =0.79$; $P = 0.02$) (**Figure 4 b**). Additionally, DT of 2022 was negatively correlated with the precipitation observed in October 2021 in the sites ($R^2 = 0.73$; $P = 0.03$) (**Figure 4 c**), showing that the amount of rainfall at the end of the rainy season of the previous year may influence leaf phenology of the following year.

Using values for individual trees, we found that the ED50 and DT of 2021 were negatively correlated with soil moisture in March of the same year ($R^2 = 0.29$; $P < 0.0001$ and $R^2 = 0.22$; $P = 0.0002$, respectively) (**Figure 5 a, b**). In 2022, DT was negatively correlated with soil moisture in April of the same year ($R^2 = 0.13$; $P = 0.0053$) (**Figure 5 c**).

Phenological stage with respect to soil humidity

In order to determine the soil moisture at the time when bud burst occurs, we plotted the average monthly soil moisture by population for the two study years with the values corresponding to the phenological stage observed on each date. In 2021 (**Figure S3**), the change from stage 1 to stage 2 in all the populations occurred when soil humidity was between 10 and 20 %. The values of soil humidity for all monitored dates ranged between 4 and 40 %. In 2022 (**Figure S4**), in San Nicolás and Atécuaro, bud burst occurred when soil humidity was between 20% and 35%, while in Umécuaro, El Remolino, San Miguel and Tamanguío bud burst occurred with 5-10% of soil humidity.

Relation of functional variables with leaf phenology

In 2021, with individual tree data, we observed a positive correlation between DT and LT ($R^2 = 0.13$; $P = 0.005$) (**Figure 6**) and in 2022 both ED50 ($R^2 = 0.29$; $P < 0.0001$) and DT ($R^2 = 0.29$; $P < 0.0001$) were correlated with LT. We did not find correlations between phenological variables and LMA.

DISCUSSION

The results of our study clearly indicate that there is significant spatial and temporal variation in the spring leaf phenology of *Q. castanea* populations in the Cuitzeo basin, despite the short geographic distance among sampling sites, but probably associated to the considerable environmental heterogeneity in the area (Mendoza et al. 2011; Maldonado-López et. al, 2015). In a previous study in the Cuitzeo basin, water use strategies were compared among nine oak species, including *Q. castanea*, along an aridity gradient (Aguilar-Romero et al. 2017). The study found that species occurring in the more arid parts of the basin tend to be more deciduous than species in less arid areas. *Q. castanea* was characterized as a brevideciduous species with comparatively intermediate values of xylem resistance to embolism and an intermediate position in the aridity gradient (Aguilar-Romero et al., 2017). Our data from this study also confirm that *Q. castanea* shows a brevideciduous leaf phenology, as defined by Singh and Kushwaha (2005a; 2016). According to this classification, species with periods of less than 4 months without leaves are considered brevideciduous and those with more than 4 months without leaves are considered deciduous.

We showed that the average difference in ED50 between the population with the earlier leaf development and the one with the later development was on average 78.5 days in the two study years, and that the duration of the period elapsed between the dormant buds and fully expanded leaves varied from 37 to 128 days. Also, from one year to the next, three populations differed in ED50, with an earlier DOY by an average of 62 days in 2022 in comparison to 2021. DT was also significantly shorter in one population in 2022, with a reduction from 74 to 38 days. We found that two of the most significant variables to which the observed variation in spring leaf phenology was related to were fall precipitation of the previous year (October) and soil moisture in the spring of the same year (March or April). Interestingly, the month of October was unusually rainy in 2021, with an average precipitation of 128.7 mm across the six sites and a range between 92.3 and 160.8 mm, in comparison to 15.7 mm (8.9-21.4 mm) in 2020 and 38 mm (24.3-45.8 mm) in 2022 (Supplementary Material 1). Based on the significant correlation between DT in 2022 and precipitation in October of 2021, we consider that the phenological advancement observed in 2022 was related to the increased water availability resulting from these exceptional fall rains, suggesting the existence of lagged effects. Besides this, for both years, we observed that the timing and intensity of the first spring rains are very important for triggering leaf development through their influence on soil humidity.

Additional associations were identified between phenological parameters and the long-term mean values of two bioclimatic variables, PS and PWQ. For our six study sites, PS ranged from 95 to 100, indicating that the overall rainfall regime is markedly seasonal with a long dry season (Walsh and Lawler, 1981). Thus, all these patterns point to the critical importance of water availability during the driest period of the year for the phenological patterns of *Q. castanea*, similar to what occurs in seasonally dry tropical forest trees (Borchert et. al., 2005) occurring at lower elevations in the state of Michoacán and much of western Mexico.

Several provenance tests, common garden experiments, and more recently genomic studies, have identified high heritability values and signatures of local adaptation in the leaf phenological variation patterns among populations of various tree species (Vitasse et al. 2009; Albert et al. 2011; Papper and Ackerly, 2021; Wright et al, 2021; Meger et al. 2024). In *Q. castanea* such studies are still lacking, but our results suggest that the phenological variation we observed is reflecting spatiotemporal mosaics of environmental and genetic variation (Blonder et al., 2023). The correlations between DT and long-term averages of PS and PWQ could have arisen as a result of differential selection pressures at each population, given high environmental heterogeneity despite short geographic distances, and resulting in adaptive variation; but this hypothesis requires formal evaluation. On the other hand, the interannual phenological differences and the moderate correlation of individual ED50 and DT values between years point out to a role for plastic responses in *Q. castanea* trees adjustment to the prevailing conditions during the initiation of leaf development and also of lagged effects of previous months, or even years.

Our study is also useful to understand the evolution of the genus *Quercus* in the Mexican territory. As oaks migrated and diversified into the Mexican landscapes, they adapted mostly to moisture gradients (Hipp et al. 2018), with temperature, and particularly minimum temperatures, imposing less significant selection pressures in this process. Also, oaks in Mexico and Mesoamerica exhibited a doubling in transitions among leaf habitat states (i. e. deciduousness, brevideciduousness and evergreeness) in comparison to USA counterparts, suggesting the evolutionary importance of this trait, together with other ecophysiological and functional responses, in the adaptation to various levels of drought stress (Hipp et al. 2018; Kaproth et al. 2023). Concurrently, in our results, at the intraspecific level, temperature variables did not contribute to explain phenological differences among *Q. castanea* populations, with only precipitation and soil humidity variables being significant, while in oaks from higher latitudes, temperature variables have been recurrently found to be important for explaining phenological variation among populations (Papper and Ackerly, 2021; Journé et al. 2021; Wright et al. 2021; Meger et al. 2024).

In general, we observed that the percentage of soil moisture required to start leaf development was 15%, revealing that the accumulated water in the soil is an environmental cue that initiates leaf burst. However, in some populations, the soil moisture did not reach this percentage and despite this, bud burst occurred. This means that some trees are making use of their water reserves or accessing deep underground water. From studies that have been conducted on *Quercus* species, it is known that these trees use deep roots to efficiently absorb available water to survive severe droughts (Gieger and Thomas, 2002; Suseela and Tharayil, 2020).

Q. castanea is probably not only using the environmental signal of the onset of the rainy season, but also seems to be making use of other factors that indicate the adequate time for leaf development. Other cues or drivers that influence leaf phenology may be irradiance (Saleska et al., 2007) and photoperiod (Basler and Körner, 2012), although these may vary in response to environmental constraints (Tang et al., 2016). In addition to abiotic cues, there are also biological factors such as competition, herbivory, resource limitation, and genetics, which control phenology (Wolkovich et al. 2014).

On the other hand, we observed that some foliar functional traits, such as leaf thickness, are associated to the phenological variation of *Q. castanea*. In particular, thicker leaves were associated to longer development times and later ED50. Leaf thickness exhibits associations with various anatomical and physiological features of the leaves and its functional significance has been discussed based on large scale surveys across many tree species from all major biomes (Niinemets, 1999; Niinemets, 2001). In general, LMA is positively related to LT, and also to leaf density, but the latter two are not necessarily correlated, suggesting independent control over these two variables through different mechanisms. Higher photosynthetic efficiency has been observed as leaf thickness increases, resulting in shorter "leaf pay-back" times, that is, the time required to assimilate the amount of carbon necessary for leaf construction (Niinemets, 2001). It is possible that in our study system, the higher deciduousness and thicker leaves observed in *Q. castanea* individuals from sites with more seasonal precipitation arises from a trade-off between shorter canopy duration because of the water availability restrictions imposed by a more marked dry season, and the need of a higher photosynthetic efficiency to compensate carbon investment in this comparatively shorter period. However, this hypothesis would require assessment, together with alternative hypothesis (i. e. thicker leaves may be more resistant to herbivory or to other stress factors).

In conclusion, we observed that the populations of *Q. castanea* found in sites with lower water availability and with a more marked seasonality of precipitation in the Cuitzeo basin have a slower leaf growth compared to the less seasonal populations. Leaf phenology differed between sites, both for 2021 and 2022, which means that such variation is responding to environmental changes. The oaks of Michoacán seem to adjust their phenology according to precipitation and water accumulation in the environment, a behavior similar to that of seasonally dry forest species. We conclude that, in addition to environmental signals, plant-specific foliar traits, such as LT, play a crucial role in the phenology of oaks at tropical latitudes.

DATA AVAILABILITY STATEMENT

Data are available in a repository and can be accessed via a DOI link. The data underlying this article are available in the Harvard Dataverse Repository, at https://doi.org/10.7910/DVN/ENHAMD.

ACKNOWLEDGEMENTS

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TABLES AND FIGURES

Table 1. *Quercus castanea* sites used for phenological monitoring and collection. Precipitation and temperature data were retrieved from the WORDLCLIM (www. https://www.worldclim.org/) database.

Site (Abbreviation)	Latitude	Longitude	Elevation (masl)	Annual Precipitation (mm)	Mean annual temperature (°C)
Tamanguío (TA)	19.98°	-101.35°	2457	1299	13.8
Remolino (REM)	19.55°	-101.26°	2523	1181	14.2
Atécuaro (AT)	19.61°	-101.15°	2317	1060	15.5
San Miguel (SM)	19.62°	-101.2°	2097	1096	15.3
Umécuaro (UM)	19.62°	-101.34°	2116	945	16.9
San Nicolás (SN)	19.45°	-101.34°	2233	909	16

Table 2. Differences in mean phenological variables (ED50 and development time) among monitored sites and between years 2021 and 2022. Values highlighted in black correspond to those that showed significant differences between years according to a Kruskal-Wallis test. Different letters indicate significant differences between populations in the same year. The numbers in parentheses correspond to the standard error. The abbreviations correspond to the names of the populations as shown in Table 1.

Site	ED50 (doy)		Development time (number of days)	
	2021	2022	2021	2022
TA	**179 (7.2) ^{a}**	**100 (19.7) ^{ab}**	**74 (4.9) ^{b}**	**38 (4.9) ^{c}**
REM	**163 (6.9) ^{a}**	**95 (18.6) ^{b}**	80 (9.6) ^{b}	56 (9.6) ^{bc}
AT	**163 (6.9) ^{a}**	**124 (18.6) ^{ab}**	64 (4.6) ^{bc}	77 (4.6) ^{ab}
SM	104 (6.9) ^{b}	95 (18.6) ^{b}	37 (3.2) ^{c}	42 (3.2) ^{bc}
UM	117 (6.9) ^{b}	120 (18.6) ^{ab}	67 (13.4) ^{bc}	72 (13.4) ^{abc}
SN	175 (6.9) ^{a}	177 (18.6) ^{a}	128 (9.4) ^{a}	108 (9.4) ^{a}

doy, day of the Year

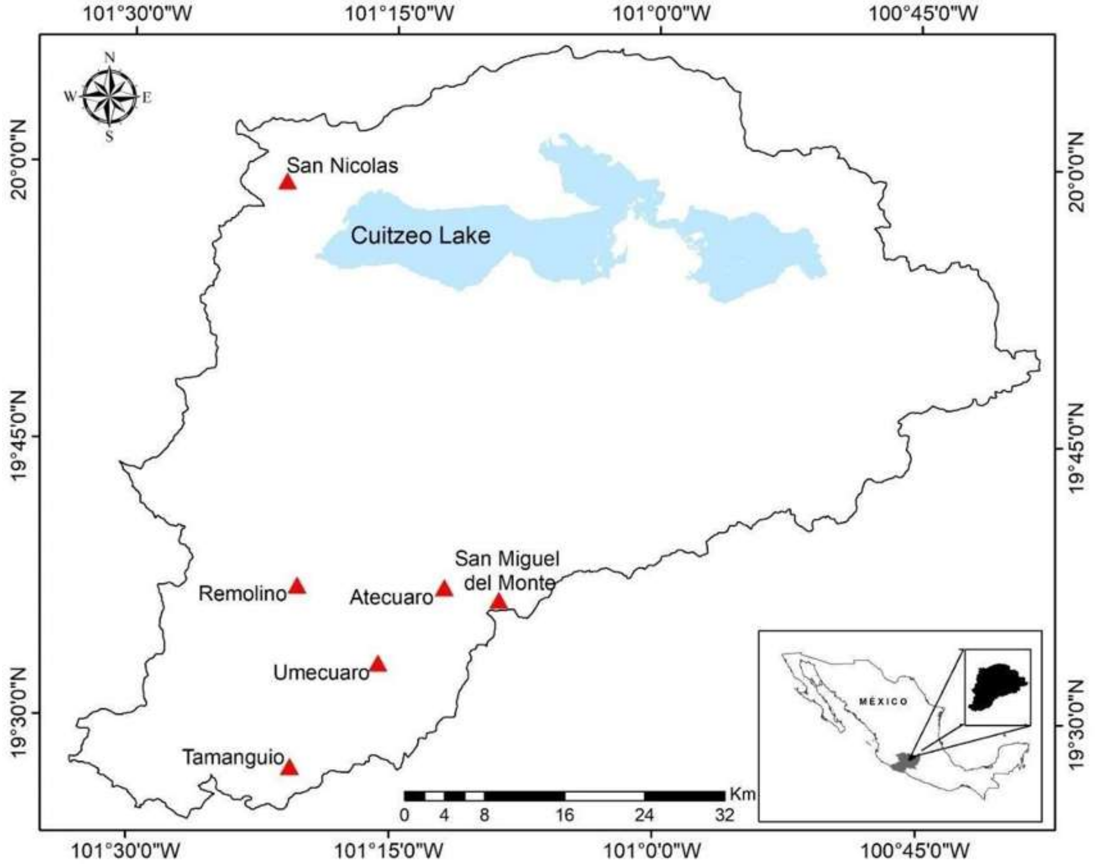

Figure 1. Map of *Quercus castanea* populations studied in the Cuitzeo basin.

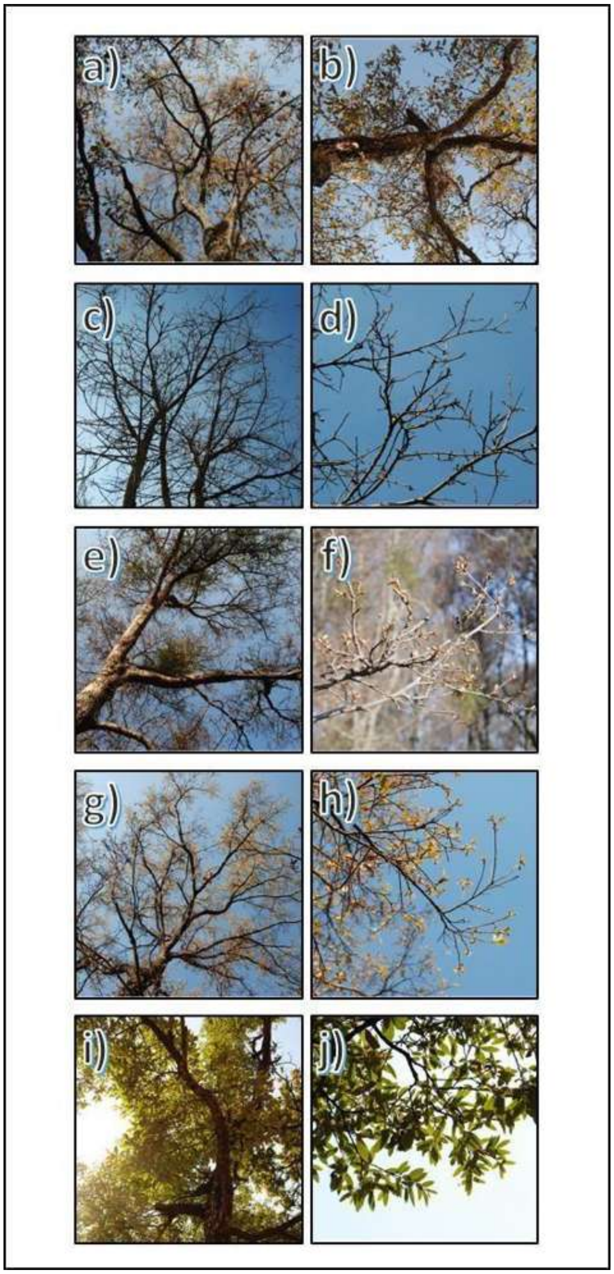

Figure 2. Stages of leaf phenology of *Quercus castanea.* **a)** Foliage of the previous year (tree crown); **b)** Foliage of the previous year (branch); **c)** dormant buds (tree crown); **d)** dormant buds (branch); **e)** bud burst (tree crown); **f)** bud burst (branch); **g)** leaf unfolding (tree crown); **h)** leaf unfolding (branch); **i)** developed leaves (tree crown) and **j)** developed leaves (branch).

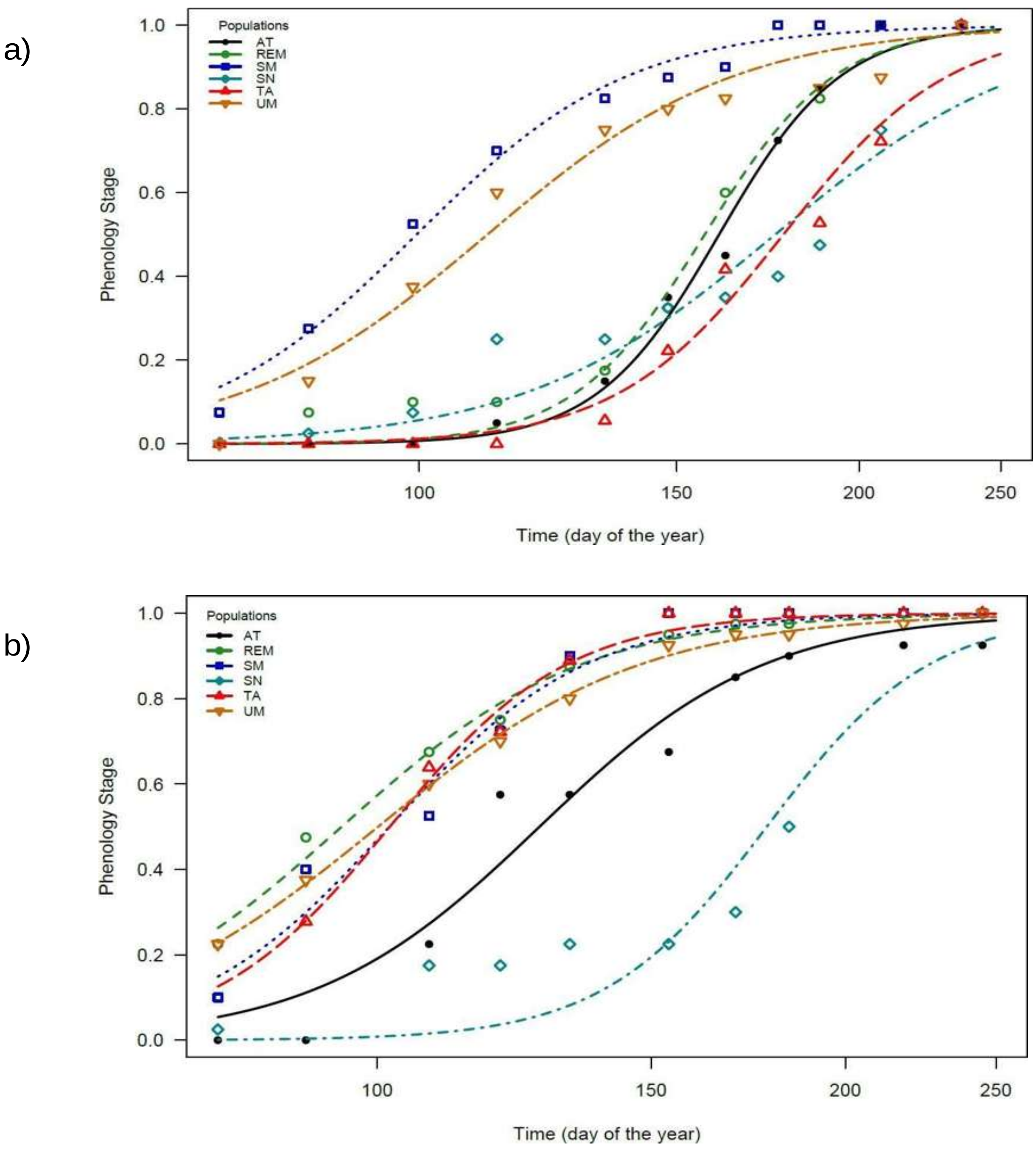

Figure 3. Log-logistic models for leaf development of *Q. castanea* in six sites of the Cuitzeo basin in a) 2021 and b) 2022. See Table 1 for site abbreviations.

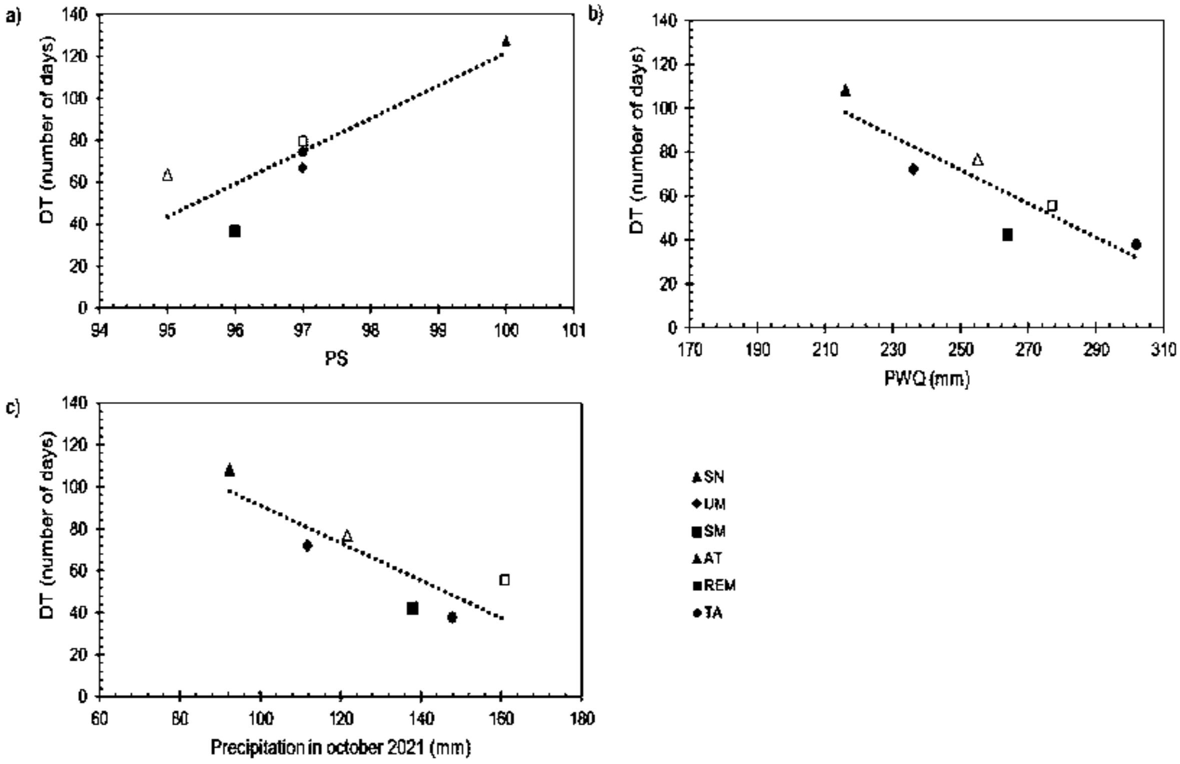

Figure 4. Linear regression analysess for significant relationships between mean population values of leaf phenology variables (ED50 and development time, DT) and environmental variables. a) DT in 2021 and PS ($R^2 = 0.76$; $P = 0.02$). b) DT of 2022 and PWQ ($R^2 = 0.79$; $P = 0.02$). c) DT of 2022 in and precipitation in October 2021 ($R^2 = 0.73$; $P = 0.03$).

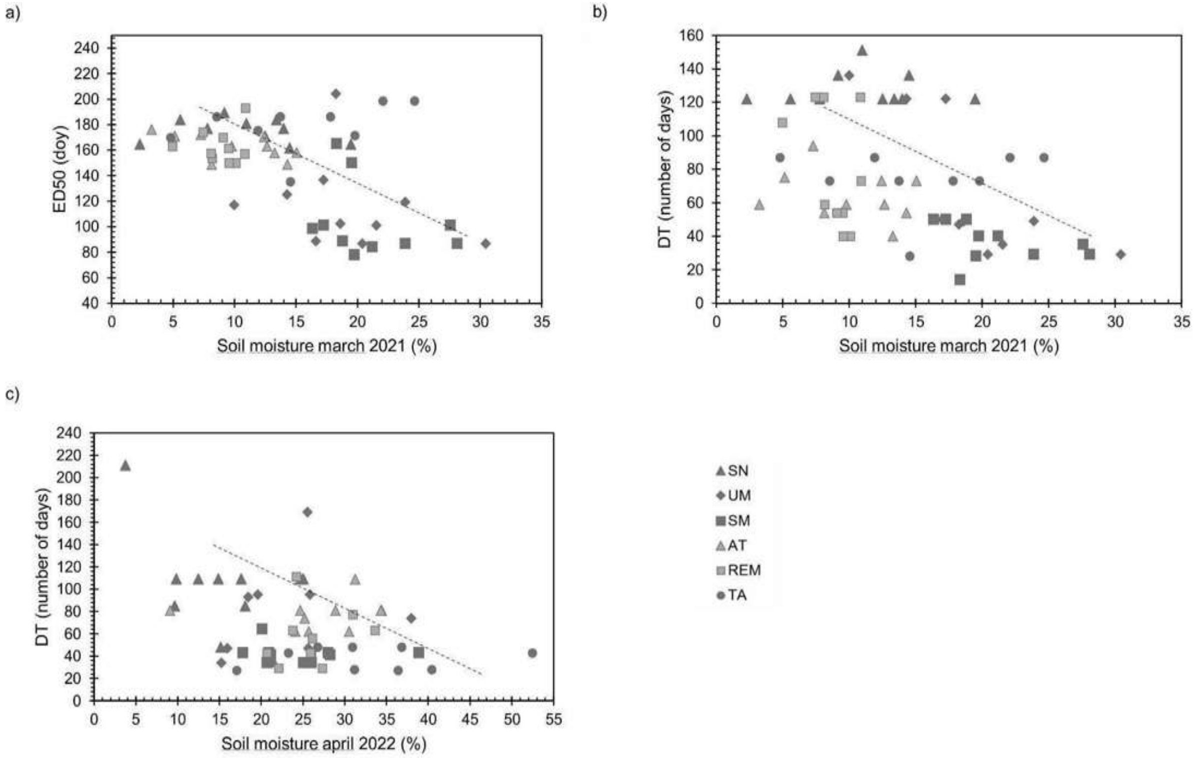

Figure 5. Linear regression analysis for significant relationships between soil moisture by date monitored and phenological variables of individual trees. a) ED50 of 2021 and soil moisture in March 2021 ($R^2 = 0.29$; $P < 0.0001$). b) DT in 2021 and soil moisture in March 2021 ($R^2 = 0.22$; $P = 0.0002$). c) DT in 2022 and soil moisture in April 2022 ($R^2 = 0.13$; $P = 0.0053$).

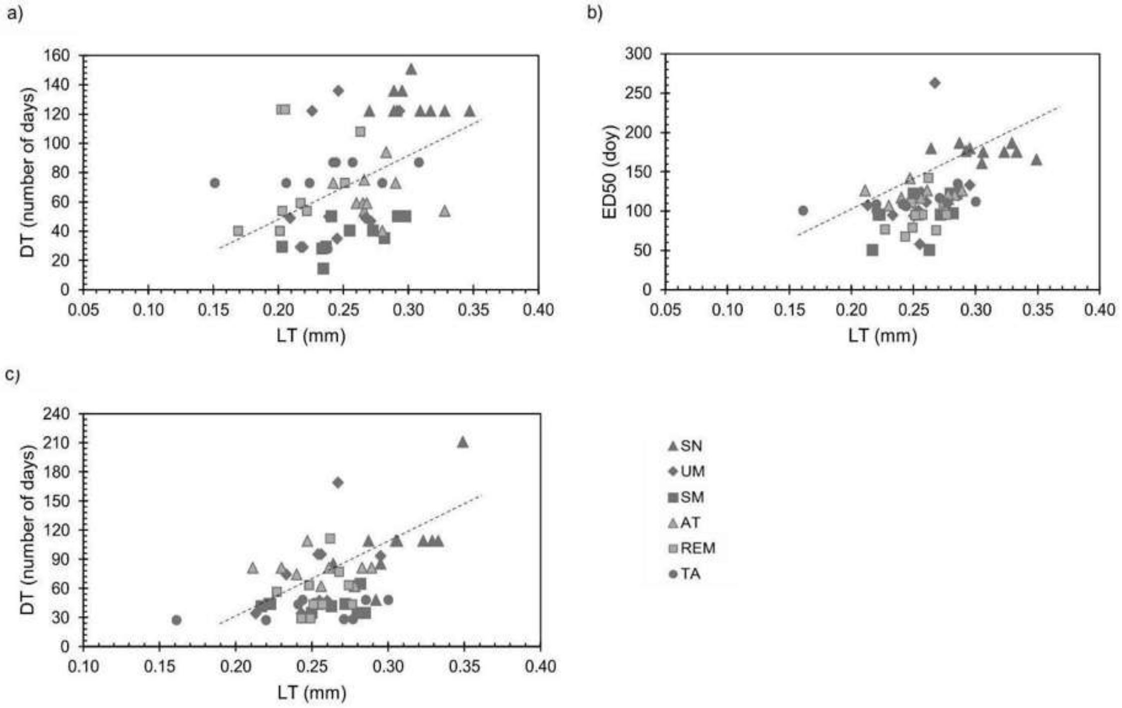

Figure 6. Linear regression analysis for significant relationships between leaf functional traits (LT in mature fall leaves) and phenological variables of individual trees. a) DT in 2021 ($R^2 = 0.13$; $P = 0.005$). b) ED50 in 2022 ($R^2 = 0.29$; $P = 0.0001$). c) DT in 2022 ($R^2 = 0.29$; $P = 0.0001$)

SUPPORTING INFORMATION

Tables 1S. Interpolated data for 2021 from CONAGUA meteorological stations. a) Monthly Precipitation and b) Average monthly temperatures.

a)

Site	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21
San Nicolás	0.0	0.0	2.6	1.4	48.5	65.3	133.2	225.5	109.9	92.3	0.7	1.6
Umécuaro	0.0	0.0	6.4	0.0	76.6	124.3	146.8	235.4	115.6	111.6	2.3	0.7
San Miguel	0.0	0.0	5.3	0.0	45.7	74.5	145.5	221.2	97.2	137.9	2.4	0.6
Atécuaro	0.0	0.0	6.5	0.0	54.0	73.6	114.4	210.4	61.0	121.6	2.2	0.0
El Remolino	2.2	0.0	5.0	0.9	114.8	92.2	195.2	344.8	164.2	160.8	2.0	1.3
Tamanguío	0.0	0.0	5.4	0.0	63.3	106.0	155.2	229.7	80.2	147.7	2.9	0.2

b)

Site	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21
San Nicolás	13.8	14.9	17.8	18.4	18.9	17.7	17.6	17.4	17.0	17.1	14.5	14.2
Umécuaro	15.1	16.0	18.9	19.2	19.9	18.0	18.0	18.0	18.0	18.1	15.2	15.0
San Miguel	14.2	15.1	17.9	17.9	18.8	17.1	17.2	17.1	16.9	17.1	14.4	14.3
Atécuaro	14.2	15.1	17.9	18.0	18.8	17.0	17.1	17.1	16.9	17.1	14.4	14.3
El Remolino	11.9	12.7	15.2	15.4	15.7	14.5	14.6	14.8	14.6	15.1	12.4	12.3
Tamanguío	14.0	14.7	17.3	17.6	18.1	16.7	16.7	16.8	16.9	17.1	14.2	14.1

Tables 2S. Interpolated data for 2022 from CONAGUA meteorological stations. a) Monthly Precipitation and b) Average monthly temperatures

a)

Site	Jan-22	Feb-22	Mar-22	Apr-22	May-22	Jun-22	Jul-22	Aug-22	Sep-22	Oct-22	Nov-22	Dec-22
San Nicolás	0.0	0.0	0.0	0.0	12.8	36.0	190.6	95.7	60.8	24.3	2.9	0.0
Umécuaro	6.6	1.7	1.7	27.0	19.8	188.2	179.9	132.8	81.4	45.8	20.9	8.2
San Miguel	0.7	0.0	1.3	0.0	14.6	111.9	150.1	91.7	81.1	34.2	14.3	3.2
Atécuaro	3.9	0.0	0.6	9.4	27.8	109.7	149.8	79.4	78.0	28.6	14.0	6.7
El Remolino	0.9	0.0	0.0	0.0	1.1	58.7	194.7	80.3	65.1	41.4	9.0	0.0
Tamanguío	2.8	0.7	0.3	4.6	16.0	138.1	170.4	98.0	75.5	54.0	12.1	3.5

b)

Site	Jan-22	Feb-22	Mar-22	Apr-22	May-22	Jun-22	Jul-22	Aug-22	Sep-22	Oct-22	Nov-22	Dec-22
San Nicolás	13.8	14.4	16.1	18.3	19.9	18.3	17.8	17.3	17.1	17.0	16.0	13.7
Umécuaro	14.8	15.5	17.3	19.3	21.2	19.2	18.5	18.4	18.1	17.8	16.6	13.3
San Miguel	14.1	14.7	16.4	18.2	20.0	17.9	17.5	17.3	17.2	17.1	16.0	13.3
Atécuaro	14.1	14.7	16.5	18.3	20.1	18.0	17.4	17.3	17.2	17.0	15.9	12.8
El Remolino	11.8	12.2	13.7	15.3	16.8	15.4	15.3	14.9	15.3	15.1	13.8	10.9
Tamanguío	13.5	14.0	15.7	17.5	19.5	18.0	17.6	17.5	17.2	16.7	15.5	12.8

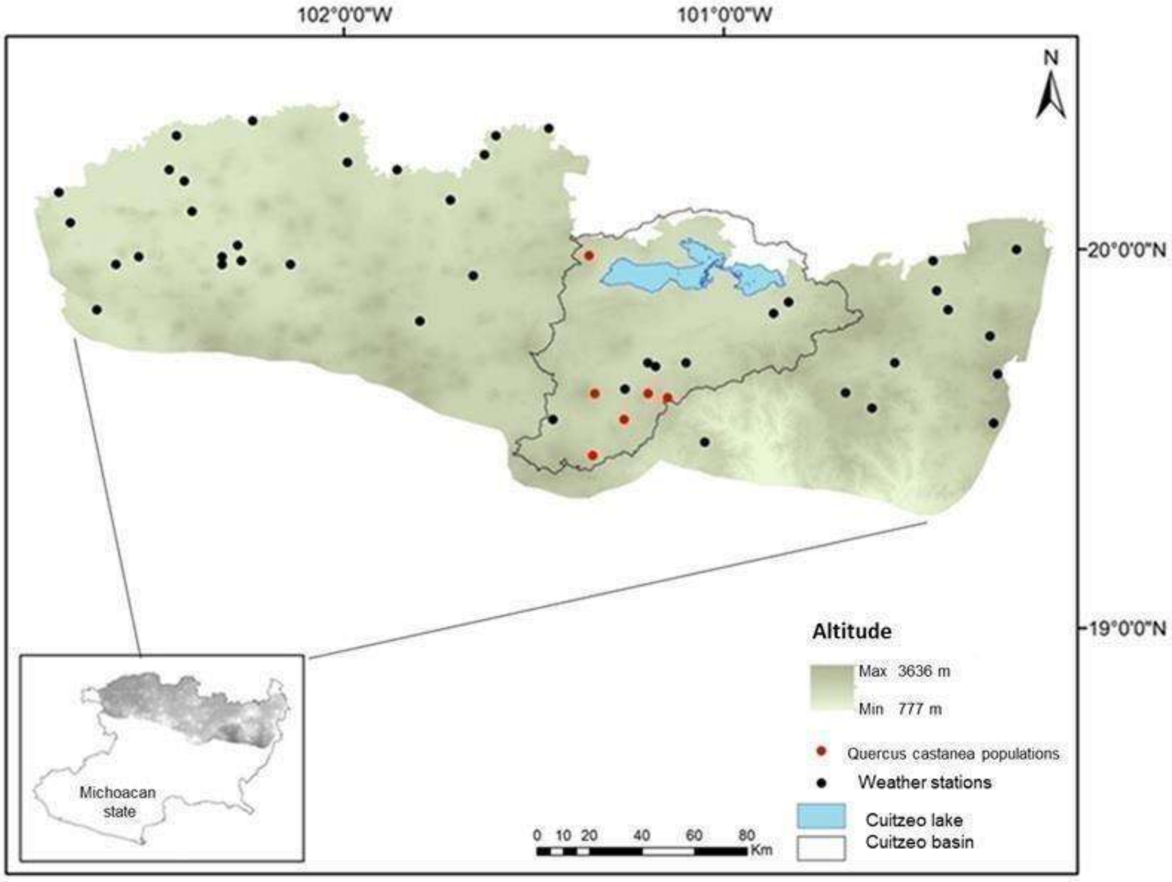

Figure S1. Weather stations in the state of Michoacán near the Cuitzeo basin.

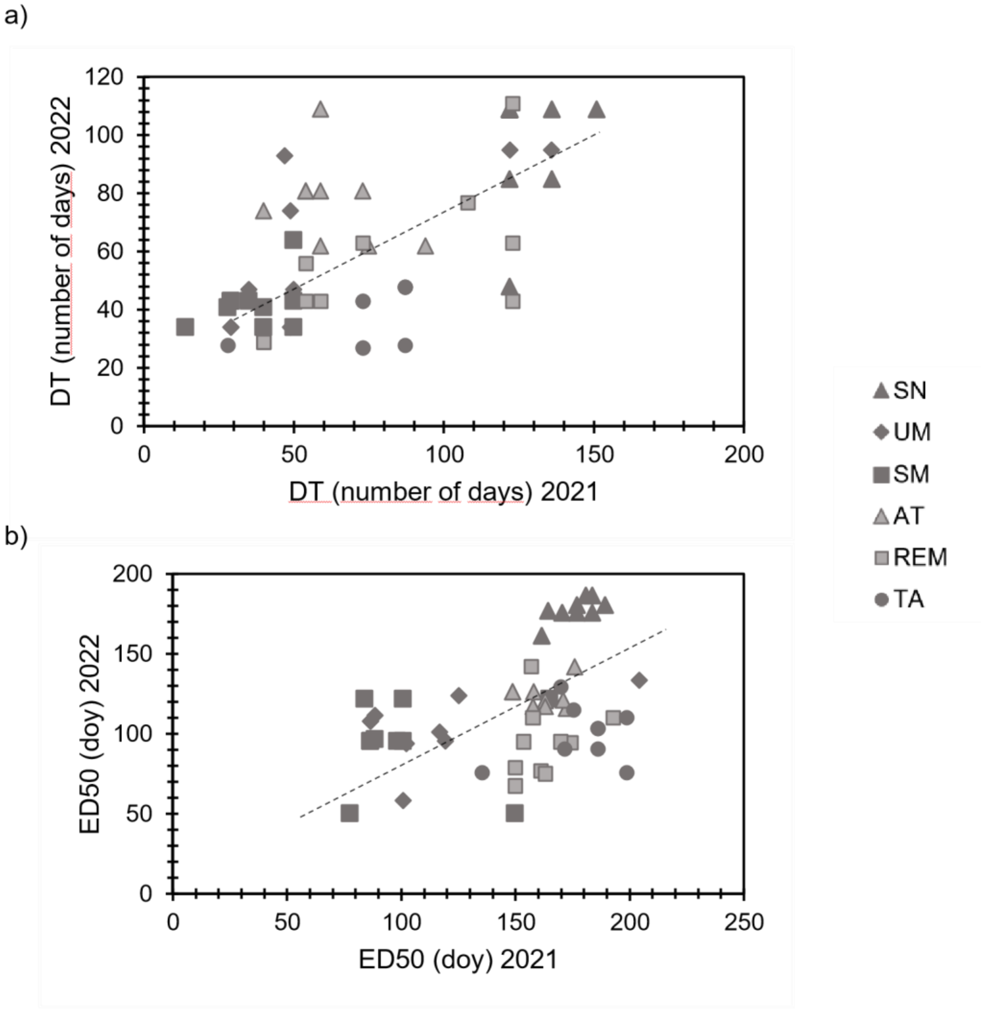

Figure S2. Linear regression analysis for significant relationships between DT in 2021 and 2022 and ED50 in 2021 and 2022 of individual trees. a) DT in 2021 and 2022 ($R^2 = 0.41$; $P < 0.0001$). b) ED50 in 2021 and 2022 ($R^2 = 0.15$; $P = 0.0028$).

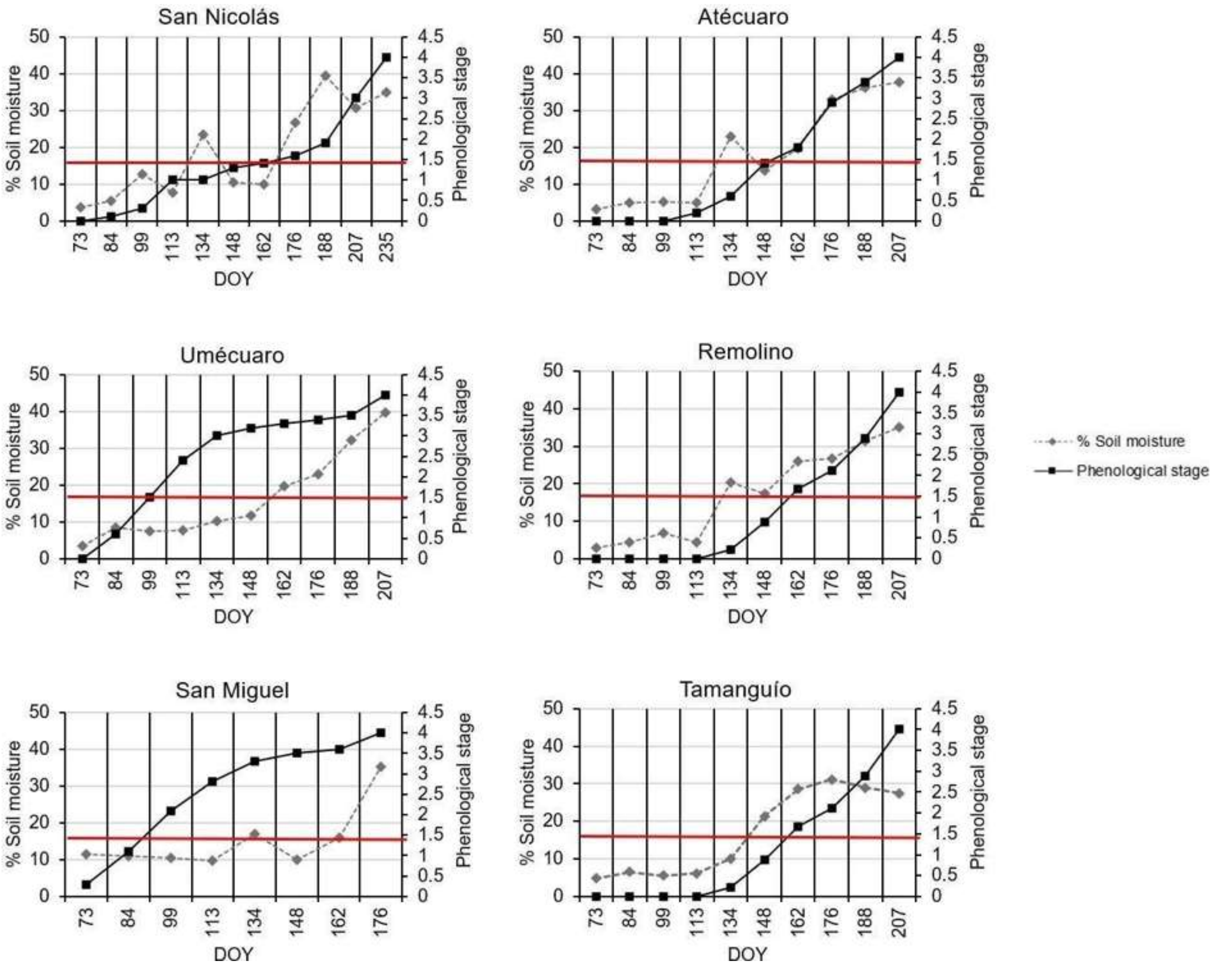

Figure S3. Phenological stages corresponding to 6 populations of *Quercus castanea* with their soil moisture. Dates in 2021.

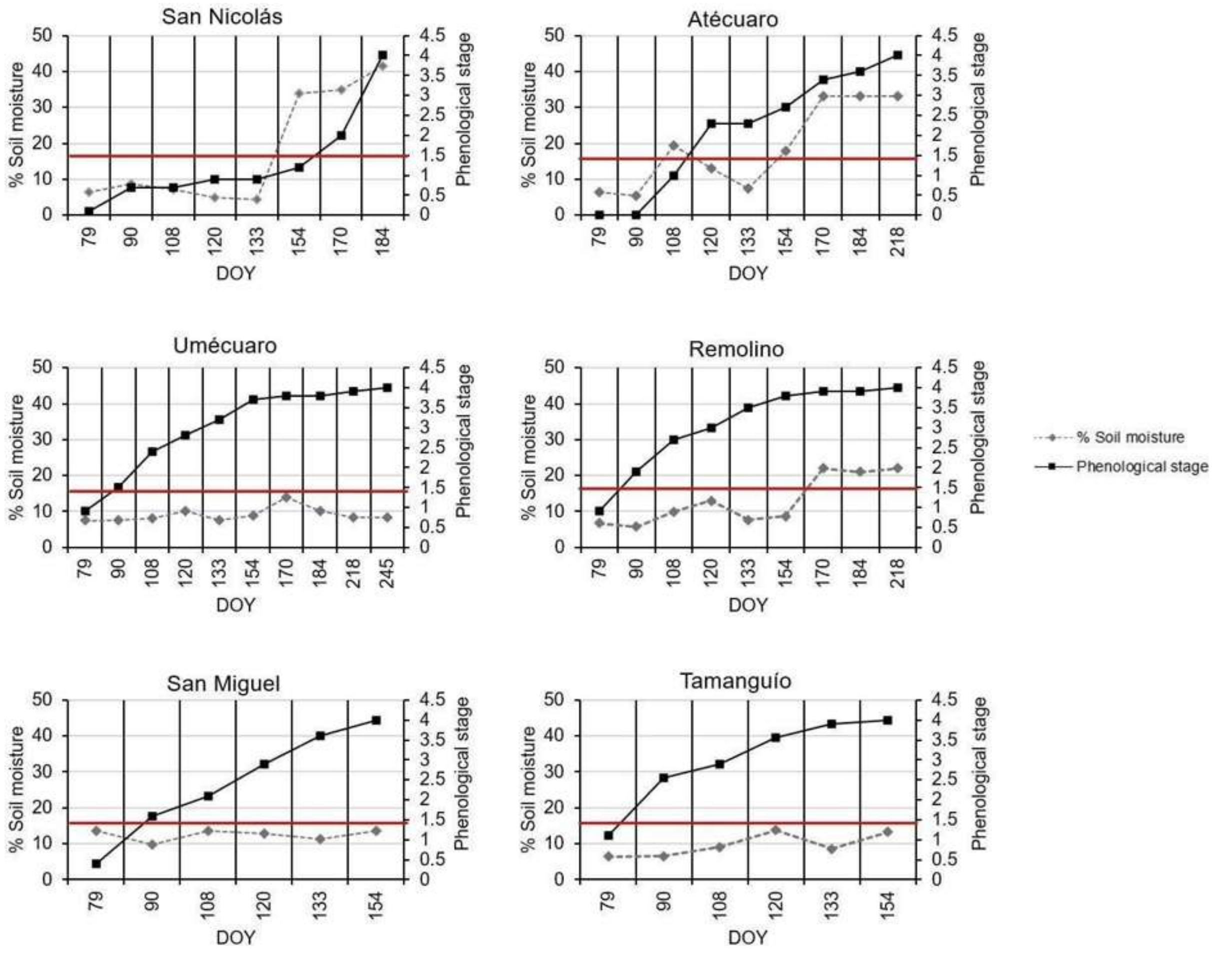

Figure S4. Phenological stages corresponding to 6 populations of *Quercus castanea* with their soil moisture. Dates in 2022.

Tamara Citlali Ochoa Alvarez

Relación entre la fenología foliar, caracteres funcionales y herbivoría en Quercus castanea (Fagacea

Universidad Michoacana de San Nicolás de Hidalgo

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Formato de Declaración de Originalidad y Uso de Inteligencia Artificial

Coordinación General de Estudios de Posgrado
Universidad Michoacana de San Nicolás de Hidalgo

A quien corresponda,

Por este medio, quien abajo firma, bajo protesta de decir verdad, declara lo siguiente:

- Que presenta para revisión de originalidad el manuscrito cuyos detalles se especifican abajo.
- Que todas las fuentes consultadas para la elaboración del manuscrito están debidamente identificadas dentro del cuerpo del texto, e incluidas en la lista de referencias.
- Que, en caso de haber usado un sistema de inteligencia artificial, en cualquier etapa del desarrollo de su trabajo, lo ha especificado en la tabla que se encuentra en este documento.
- Que conoce la normativa de la Universidad Michoacana de San Nicolás de Hidalgo, en particular los Incisos IX y XII del artículo 85, y los artículos 88 y 101 del Estatuto Universitario de la UMSNH, además del transitorio tercero del Reglamento General para los Estudios de Posgrado de la UMSNH.

Datos del manuscrito que se presenta a revisión		
Programa educativo	Programa Institucional de Doctorado En Ciencias Biológicas	
Título del trabajo	Relación entre la fenología foliar, caracteres funcionales y herbivoría en *Quercus castanea* (Fagaceae) a escala de paisaje.	
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Uso de Inteligencia Artificial		
Rubro	**Uso (sí/no)**	**Descripción**
Asistencia en la redacción	NO	

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Uso de Inteligencia Artificial

Rubro	Uso (sí/no)	Descripción
Traducción al español	NO	
Traducción a otra lengua	NO	
Revisión y corrección de estilo	NO	
Análisis de datos	NO	
Búsqueda y organización de información	NO	
Formateo de las referencias bibliográficas	NO	
Generación de contenido multimedia	NO	
Otro	NO	

Datos del solicitante

Nombre y firma	Tamara Citlali Ochoa Alvarez [redacted]
Lugar y fecha	20 de febrero del 2025