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“DEFENSA QUÍMICA Y HERBIVORÍA: UN ESTUDIO EN *Quercus glaucooides*”

TESIS

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RESUMEN

La herbivoría es una interacción ecológica que tiene gran relevancia dentro de algunos ecosistemas naturales, debido a que afecta el crecimiento, el éxito reproductivo y la capacidad fotosintética de las plantas hospederas. Ésta se puede ver influenciada y modificada por distintos factores, ya sea de forma mecánica o química (metabolitos secundarios) aunque la herbivoría también se ve afectada por rasgos asociados con el estado nutricional de la planta y por factores abióticos como variables climáticas. En el presente estudio, quisimos analizar la relación de algunos atributos químicos, funcionales y nutrimentales de las hojas de *Quercus glaucoides*, una especie de encino blanco, con los niveles de herbivoría encontrados en 13 poblaciones de diferentes estados de México, para evaluar dicha relación también fueron tomadas en cuenta las variables ambientales de cada uno de los sitios donde fue colectado. Se tomaron 20 hojas de 10 individuos escogidos aleatoriamente de cada población, posteriormente se determinaron los niveles de herbivoría de cada una así como su área foliar y su área foliar específica (AFE), para medir su contenido de metabolitos secundarios se hicieron análisis en el laboratorio de fenoles totales, taninos totales y proantocianidinas, y para determinar la calidad de la hoja se midió el contenido de agua así como también los niveles de carbohidratos, proteínas, N, P y C. Como resultado, se observaron niveles de herbivoría en *Q. glaucoides*, en un rango de 2 a 7% en promedio por población, los cuales tuvieron una correlación positiva con la concentración de fenoles, carbohidratos y proteínas, además también tienen una fuerte relación con atributos funcionales como el AFE, sin embargo, se encontró que las variables ambientales (especialmente de precipitación) son aún más influyentes tanto en los niveles de herbivoría como en las fluctuaciones de las demás variables medidas, dado que es un árbol de ambientes más secos y áridos en comparación con otras especies de su género.

Palabras clave: Encinos, interacciones, ambiente, nutrientes, metabolitos.

ABSTRACT

Herbivory is an ecological interaction that has great relevance within some natural ecosystems, because it affects the growth, reproductive success and photosynthetic capacity of host plants. This can be influenced and modified by different factors, either mechanically or chemically (secondary metabolites) although herbivory is also affected by traits associated with the nutritional status of the plant and by abiotic factors such as climatic variables. In this study, we wanted to analyze the relationship of some chemical, functional and nutritional attributes of the leaves of *Quercus glaucoides*, a species of white oak, with herbivory levels found in 13 populations of different states of Mexico, to evaluate this relation also the environmental variables of each of the sites where it was collected were taken into account. Were taken 20 leaves from 10 randomly chosen individuals from each population, then the herbivory levels of each were determined as well as their leaf area and their specific leaf area (SLA), to measure their secondary metabolite content analyzes were made in the laboratory of total phenols, total tannins and proanthocyanidins, and to determine the quality of the leaf the water content was measured as well as the levels of carbohydrates, proteins, N, P and C. As a result, herbivory levels were observed in *Q. glaucoides*, in a range of 2 to 7% on average by population, which had a positive correlation with the concentration of phenols, carbohydrates and proteins, in addition they also have a strong relation with attributes Functional such as the SLA, however, it was found that environmental variables (especially precipitation) are even more influential both in herbivory levels and in fluctuations of the other measured variables, since it is a tree of drier and more arid environments compared to other species of its genus.

1. INTRODUCCIÓN

1.1 Herbivoría

La herbivoría es una interacción ecológica que se considera como el daño sobre cualquier órgano vegetal, ocasionado por diversos organismos (Coley y Barone, 1996). Tiene gran relevancia dentro de algunos ecosistemas naturales, como son los bosques templados y tropicales (Schoonhoven et al., 2005), debido a que juega un papel fundamental en la estructuración de las comunidades, en la regulación de los tamaños poblacionales de las plantas y en los procesos de regeneración de los mismos bosques (Weis y Berenbaum, 1989; Pugnaire y Valladares, 2007). La pérdida de área fotosintética en las plantas hospederas altera el balance de carbohidratos que a su vez interfiere con el consumo de agua y nutrientes, lo que se ve reflejado en su crecimiento, éxito reproductivo y sobrevivencia (Bañuelos y Obeso 2004, Parra-Tabla et al., 2004).

En una misma especie de planta, los niveles de herbivoría pueden verse influenciados y modificados por distintos factores, además, la intensidad del daño puede variar a distintas escalas (Castagneyrol et al., 2019). A escala del paisaje, la densidad de los herbívoros puede llegar a ser más alta en parches de hábitats donde los recursos son más abundantes, esto se debe a que la intensidad de las señales físicas y químicas hace que estos parches sean más atractivos y más probables de ser colonizados (Root, 1973; Hambäck y Englund, 2005; Andersson et al., 2013). Dentro de los parches, la herbivoría en plantas individuales está influenciada por la identidad y diversidad de sus vecinos, es decir, las diversas especies con las que coexisten dentro del mismo hábitat, las cuales alteran la apariencia física y química de la planta focal y su colonización (Finch y Collier, 2000; Castagneyrol et al., 2013; Moreira et al, 2016). Finalmente, la herbivoría está controlada por rasgos individuales de las plantas que incluyen calidad nutricional y defensas anti-herbívoro (Finch y Collier, 2000; Schoonhoven, 2005; Castagneyrol et al, 2018b).

1.2. Defensa vegetal

Las plantas han desarrollado diversos mecanismos de defensa contra los herbívoros (Ramos et al., 1998; Grotewold, 2005). La hipótesis de disponibilidad de recursos de Coley (1996) es la explicación más prominente de las presiones de selección abióticas que dan forma a las defensas de las plantas, y utiliza la tolerancia de las plantas a la herbivoría para explicar la inversión en defensa. La hipótesis establece que las especies de plantas que evolucionan bajo limitación de recursos tenderán a tener una mayor inversión en defensas generales (y energéticamente caras) contra los herbívoros, ya que la limitación de recursos hace que sea costoso para la planta reemplazar los tejidos perdidos por la herbivoría. En el caso contrario, donde las plantas crecen en ambientes con riqueza de recursos disponibles, éstos son aprovechados para el crecimiento de las mismas, ya que no es prioridad invertir en defensa dado que los daños en los tejidos foliares son fáciles de sustituir.

Así pues, se ha determinado que las defensas de las plantas pueden ser constitutivas, cuando se encuentran activas dentro de la planta todo el tiempo, o inducidas, cuando se desencadenan después de recibir un ataque (Schoonhoven et al., 2005). Las defensas constitutivas pueden ser físicas o mecánicas, incluyendo desde pelos glandulares (tricomas) y espinas, hasta compuestos químicos repelentes, antinutritivos o toxinas. Sin embargo, cuando las plantas no son atacadas por herbívoros, invertir en defensas puede resultar costoso, ya que los recursos empleados en defensas de este tipo podrían ser utilizados para otras funciones vitales como el crecimiento y la reproducción (O'Neill et al., 2010). Por lo tanto, muchas plantas invierten recursos en la producción de compuestos de defensa solo cuando perciben el ataque de los insectos herbívoros, por lo que permiten una mayor eficiencia en la utilización de recursos (Karban y Baldwin, 1997). A estas defensas se les llama inducidas.

Como acabamos de mencionar, los mecanismos de defensa se clasifican en dos tipos principales: a) defensas mecánicas, las cuales permiten a la planta resistir presiones ambientales abióticas (como el viento, lluvia, granizo, etc.), así como el ataque de algunos

herbívoros. Las principales defensas mecánicas son las espinas, los pelos o tricomas, así como la producción de hojas duras y gruesas (Swihart y Picone, 1998; Lucas et al., 2000). b) las defensas químicas, que incluyen compuestos como terpenos, taninos, fenoles, aceites, etc. (Coley y Barone, 1996; Agati et al., 2013; Ballare, 2014).

1.3. Factores de variación en los niveles de herbivoría al nivel individual

a) Metabolitos secundarios

Como parte de su metabolismo, las plantas acumulan moléculas con funciones especializadas gracias a las cuales puede obtener energía y nutrientes para realizar sus funciones básicas de supervivencia (i. e. fotosíntesis, glicólisis, síntesis de aminoácidos) (Pichersky y Gang 2000). De tales procesos también se derivan otras moléculas que no cumplen necesariamente una función metabólica básica pero si una importante función ecológica: los llamados metabolitos secundarios. Los cuáles no son necesarios para la supervivencia misma de la planta, sin embargo, le proporcionan a ésta una alta plasticidad fenotípica en respuesta a una fuerte presión selectiva, como es la herbivoría (Neilson, 2013). La concentración de dichos compuestos químicos en la planta puede presentar variación dependiendo de la edad de las hojas o de la arquitectura vascular (Shelton, 2004; Brenes-Arguedas y Coley, 2005). De la misma manera, las condiciones del ambiente como cambios en la fertilidad del suelo, disponibilidad de nutrientes y CO₂, entre otros, claramente alteran la dinámica de la planta: crecimiento, distribución, calidad nutricional y las concentraciones de metabolitos secundarios (Coley et al., 2002; Forkner et al., 2004; Barbehenn y Constabel, 2011).

Una gran concentración de fenoles en las plantas, específicamente los taninos condensados e hidrolizables, así como los flavonoides, reducen la digestibilidad de las plantas al unir las enzimas digestivas y alterar los tejidos digestivos de los herbívoros mediante la producción de especies reactivas de oxígeno (Barbehenn et al. 2009; Barbehenn y Constabel, 2011), afectando el desempeño de los herbívoros. Sin embargo,

es importante tener en cuenta que los metabolitos secundarios pueden tener menores efectos sobre los herbívoros que otros rasgos defensivos de las plantas (Carmona et al., 2011). Esto se debe a la capacidad de algunos herbívoros para superar las defensas químicas, a través de la desintoxicación, la secreción o la degradación de toxinas (Karban y Agrawal, 2002; Després et al., 2007). También puede ocurrir una respuesta de alimentación compensatoria de los herbívoros a las defensas de las plantas (Barbehenn et al., 2009; Damestoy et al., 2018) mediante la cual los herbívoros aumentan su consumo para compensar la ingestión de hojas de peor calidad (altamente defendidas).

En otra instancia, los metabolitos secundarios no siempre son utilizados para la defensa de las plantas, cabe mencionar que algunos grupos de compuestos fenólicos (por ejemplo, los flavonoides) están involucrados en la foto-protección (Ballare, 2014) y pueden ser inducidos por el aumento de la radiación solar en elevaciones más altas. Con el fin de reducir el daño oxidativo y la penetración de la luz UV en las capas de células fotosintéticas, las plantas pueden acumular flavonoides y fenilpropanoides en la epidermis de la hoja tanto como en los tejidos esponjosos del mesófilo (Mazza et al., 2000; Agati et al., 2013).

Por otra parte, el impacto de los compuestos secundarios sobre los insectos herbívoros debe estudiarse en conjunto con la variación de nutrientes de la planta, factor relevante para el crecimiento del insecto (Forkner et al., 2004; Agrawal, 2007; Loranger et al., 2012) (ver la siguiente sección).

b) Calidad nutricional de la hoja

En los últimos años, se han realizado diversos estudios en los que se asume implícitamente una relación causal entre el porcentaje de daño que reciben las plantas por herbivoría y la cantidad de recursos que éstas asignan a la defensa (Rasmann et al., 2014). Sin embargo, cabe resaltar que la herbivoría no es meramente influenciada por la capacidad de resistencia de las plantas, sino que también se debe a rasgos asociados con su estado nutricional (Karban, 1992; Agrawal, 2007). Los principales factores que determinan la calidad nutricional de las hojas, haciéndolas atractivas para los insectos, son

el contenido de carbohidratos, nitrógeno, fósforo, proteínas y agua en los tejidos (Schultz et al., 1982; Erickson et al., 2004). Ahora bien, las cantidades de nutrientes que las plantas contienen son generalmente bajas con respecto a las necesidades nutrimentales de los insectos (Milanović et al., 2014; Wetzel et al., 2016), por lo que dichos nutrientes pueden ser factores limitantes para los herbívoros. Por ejemplo, el fósforo y el nitrógeno son macronutrientes que se encuentran típicamente en bajas concentraciones en muchos taxones de plantas. En consecuencia, la concentración de estos macronutrientes a menudo está fuertemente correlacionada de forma positiva con los niveles de herbivoría de la hoja (Mattson, 1980; Forkner y Hunter, 2000; Huberty y Denno, 2006; Jones et al., 2008). En algunos casos la concentración de estos nutrientes es un mejor predictor de los niveles de herbivoría que la concentración de metabolitos secundarios (Loranger et al., 2012).

c) Factores ambientales

Un número considerable de estudios han revelado que la herbivoría también se encuentra influenciada, ya sea directa o indirectamente, por factores abióticos como son las variables climáticas (Masters et al., 1998; Bale et al., 2002). Además de las condiciones climáticas, también influyen fuertemente en los rasgos defensivos de las plantas la distribución geográfica (Pearse y Hipp, 2012; Abdala-Roberts et al., 2016). Por ejemplo, se ha demostrado que las especies de plantas que crecen en latitudes más bajas con frecuencia experimentan mayores cantidades de herbivoría que sus contrapartes en latitudes más altas (Schemske et al., 2009; Marquis et al., 2012; Lim et al., 2015; Zhang et al., 2016). Por lo tanto, se espera que las especies de plantas hayan evolucionado niveles más altos de defensas anti-herbívoros en latitudes más bajas, donde la presión de los herbívoros es mayor (Rasmann y Agrawal, 2011; Pearse y Hipp, 2012; Moreira et al., 2014, Abdala-Roberts et al., 2016; Moreira et al., 2017). Esto sugiere que las concentraciones de nutrientes en las hojas, otra aproximación de la calidad de la planta a los herbívoros, también podrían asociarse con los gradientes latitudinales en la herbivoría y exhibir clinas (Marquis et al. 2012). Sin embargo, también se ha determinado que este tipo de

tendencias pueden ser muy variables en fuerza y dirección (Moles y Ollerton, 2016). Además, el efecto de los factores abióticos puede no variar de forma latitudinal o mostrar tendencias latitudinales opuestas a las de la herbivoría, lo que puede llevar a tendencias latitudinales inesperadas en la inversión en defensa de las plantas (Abdala-Roberts et al. 2016, Stevens et al. 2016; Moreira, 2017).

1.4. El género *Quercus* y el estudio de la defensa química en plantas

Los bosques templados del hemisferio norte están dominados por la familia Fagaceae, la familia de los encinos, cuyos representantes están ampliamente distribuidos en el Norte y Centro de América, Europa y Asia. El género *Quercus* (Fagaceae) se encuentra en casi todos los bosques templados del hemisferio norte así como en algunas regiones tropicales y subtropicales del mismo, incluyendo el sureste de Asia y nororiente de África. En América, el género se distribuye desde Canadá hasta Colombia incluyendo Cuba (Valencia, 2004). El género *Quercus* cuenta con aproximadamente de 400 a 500 especies, la mitad de las cuales existen en el Nuevo Mundo, especialmente concentradas en México y Centroamérica, sobre todo en las regiones montañosas (Rzedowski, 2005). Las especies de dicho género pueden ser perennifolias o caducifolias, su forma de crecimiento suele ser arbórea o arbustiva, sus flores son unisexuales y suelen ser polinizadas por el viento. La dispersión de las 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). En el aspecto ecológico, los encinos son considerados comunidades vegetales muy características que, junto con los pinares, constituyen la mayor parte de la cubierta vegetal en los bosques templados México. Poseen una gran importancia ya que proveen funciones ecológicas como el ciclaje de nutrientes y el balance hídrico (Lewington y Streeter, 1993; Nixon, 1993).

Los bosques de encinos representan zonas con una alta actividad biológica entre los diferentes niveles tróficos asociados a estos; siendo los insectos una fuerza de selección sobre los compuestos de defensa y donde los mismos insectos también sufren una presión selectiva para tolerar los distintos compuestos que son sintetizados por los encinos (Vaca-Sánchez et al., 2016). Las especies de *Quercus* producen defensas químicas en las hojas, particularmente compuestos fenólicos, que son efectivos contra una amplia gama de insectos fitófagos (Pearse y Hipp, 2009; Moctezuma et al. 2015; Abdala-Roberts et al., 2016; Moreira et al., 2018). Comúnmente, los compuestos fenólicos constitutivos están presentes en altas concentraciones en todos los tejidos de los encinos (hasta 100 mg g⁻¹ de peso seco del tejido) (Pearse y Hipp, 2012; Maldonado-López et al., 2015; Moreira et al., 2017, 2018). Además, se ha demostrado que la inducibilidad de estas defensas químicas proporciona una estrategia eficaz para la resistencia contra los principales herbívoros de insectos en varias especies de *Quercus* (Mizumachi et al., 2012). Los principales grupos químicos de naturaleza fenólica que se encuentran en los encinos son los taninos hidrolizables, las proantocianidinas (taninos condensados), los flavonoides y algunos compuestos fenólicos simples (Salminen et al., 2004; Yarnes et al., 2006; Barbehenn et al., 2006a, 2008; Barbehenn y Constabel, 2011; Moctezuma et al., 2014).

1.5. *Quercus glaucoides* como especie de estudio

Quercus glaucoides es una especie que se distribuye en el centro y sur de México. Es un árbol que mide de 3 a 13 m de altura y su tronco posee un diámetro de 17 a 50 cm. Su corteza tiene un color de gris a gris oscuro y es escamosa. Las hojas tienen una forma oblanceolada y miden de 3.7 a 15.5 cm de largo por 1.5 a 7.0 cm de ancho, su margen es ondulado con 3 a 6 dientes a cada lado, el haz tiene un color verde grisáceo y opaco y su envés es glauco; su fruto es anual, solitario o en grupos de tres; la bellota es ovoide de 12 a 18 mm de largo.

Quercus glaucoides, ha sido catalogada dentro de la subsección *Glaucoideae* de acuerdo con sus características taxonómicas (Nixon y Muller, 1992). Las especies de esta subsección se caracterizan por ocupar hábitats que son generalmente áridos, además de

poseer características embrionarias que los hacen distintivos de otros grupos, que se encuentran relacionadas al síndrome de germinación, donde el eje embrionario se deposita más profundamente en el suelo, lo cual puede ser una adaptación a la sequía o a los incendios (Nixon, 2002). La especie se encuentra en las montañas de dos de las principales provincias fisiográficas de México, la Faja Volcánica Trans-mexicana (FVTM), y la Sierra Madre del Sur (SMS). Su distribución altitudinal va desde los 1000 hasta los 2200 msnm, en sitios por lo general cálidos y secos, ocasionalmente húmedos; sobre suelos calizos, pedregosos con roca basáltica, arcillosos, generalmente con poco humus (Valencia, 2004).

De acuerdo con lo anterior, *Q. glaucoides* frecuentemente puede encontrarse en las zonas de transición de bosque templado hacia el bosque tropical caducifolio. Este último tipo de vegetación corresponde a una selva de menos de 15 m de altura donde los árboles pierden su follaje en la época seca; la cual se caracteriza por climas semisecos y cálidos con temperatura media anual superior a los 20 °C, precipitación media anual de entre 500 a 1200 mm y temporada seca larga y marcada (Miranda y Hernández, 1963). En estas zonas de transición es donde *Q. glaucoides* forma comunidades bajas, desarrollándose en un intervalo de temperatura media anual entre 12 a 29 °C, con vegetación asociada que incluye a *Opuntia* sp. (Cactaceae), *Acacia* sp. (Fabaceae) y *Brahea* sp. (Aracaceae), entre otras (Rzedowski, 2005).

2. HIPÓTESIS

- Los niveles de herbivoría son diferenciales entre las poblaciones de *Q. glaucoides* y se asocian con caracteres funcionales foliares, de calidad nutricional y de defensa química de los individuos.
- Las variables climáticas a lo largo de la distribución de *Q. glaucoides* tendrán influencia sobre la herbivoría, ya sea directamente a través de su efecto en las poblaciones de insectos o indirectamente debido a su impacto sobre los caracteres funcionales, de calidad nutricional y de defensa química de las plantas.

3. OBJETIVOS

- Comparar los niveles de herbivoría entre las poblaciones de *Q. glaucoides* y determinar su asociación con 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. glaucoides*.

4. ARTICLE

Geographic variation of herbivory intensity in *Quercus glaucoides*: the role of leaf traits, nutritional quality, chemical defense and climatic factors.

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ABSTRACT

Differences in herbivory intensity among individuals and populations within plant species has been related to variation in leaf traits, nutritional quality, and chemical defense; as well as to environmental differences across localities. However, the relative importance of these factors in determining herbivory levels, as well as their multiple interrelationships, are not yet clear. In this study, we evaluated geographic variation in herbivory along the distribution of *Quercus glaucoides*, a Mexican white oak associated to warm and dry habitats. In total, we sampled 13 populations encompassing the distribution of the species in central and southern Mexico. In each of the 130 individuals collected, we measured leaf traits (leaf area, leaf dry mass, specific leaf area), nutritional quality (nonstructural carbohydrates, proteins and water content, as well as total leaf C, N and P in a subsample of individuals), secondary metabolites (total phenols, total tannins and soluble proanthocyanidins). Percentage area removed by folivorous insects showed positive associations with leaf area, specific leaf area, and the concentration of total phenols, nonstructural carbohydrates and proteins. At the population level, herbivory showed a positive association with precipitation seasonality and precipitation of the driest quarter. Precipitation seasonality was also a significant predictor of leaf area and total phenols concentration, while the concentration of nonstructural carbohydrates was positively related to latitude and precipitation of the warmest quarter, and protein content was positively related to longitude and negatively to annual precipitation. Our data suggest complex interactions among herbivory levels, plant traits (leaf traits, nutritional quality and chemical defense) and climate variables.

Key words: resource availability hypothesis, apparency hypothesis, Mexico, temperate forest, tropical dry forest.

INTRODUCTION

Herbivory is an antagonistic interaction between plants and animals that is greatly relevant in all terrestrial ecosystems, particularly in temperate and tropical forests (Schoonhoven et al., 2005). Herbivory is usually deleterious for plants since it can affect their growth and reproductive success (Bañuelos and Obeso 2004, Pastur et al., 2004). Herbivory can be influenced and modified by various factors; in addition, herbivore damage patterns can vary at different scales (Castagneyrol et al., 2019). For example, at the landscape scale, the density of herbivores may be higher in patches of habitat where resources are more abundant since the physical and chemical cues that attract herbivores are more intense in these patches and therefore, are more likely to be colonized (Andersson et al., 2013; Hamback and Englund, 2005; Root, 1973). Within patches, herbivory on individual plants can be influenced by the identity and diversity of the neighboring plant community (Castagneyrol et al., 2013; Finch and Collier, 2000; Moreira et al., 2016) as well as by features of individual plants, including nutritional quality and anti-herbivore defenses (Castagneyrol et al., 2018b; Finch and Collier, 2000; Schoonhoven, 2005).

In recent years, there have been various studies that assume a causal relation between the amount of damage that a plant receives from herbivores and the investment of resources that are allocated to defense (Rasmann et al., 2014). However, herbivory levels are not merely influenced by plant resistance but are also related to traits associated with nutritional status (Karban, 1992; Agrawal, 2007). The amount of nutrients that are found in plant tissues (such as nitrogen, phosphorus, proteins and carbohydrates) are generally low with respect to the nutritional needs of insects (Milanović et al., 2014; Wetzal et al., 2016); so these nutrients may be limiting factors for herbivores and in some cases their concentration is an accurate predictor of herbivory levels (Loranger et al., 2012). Also, it is important to note that secondary metabolites may have lower effects on the performance of herbivores than other defensive features of the plants (Carmona et al., 2011) such as thorns, hairs or trichomes, as well as leaf hardness and firmness, which have various morphophysiological basis and ecological functions (Lucas et al., 2000). This

is due to the ability of some herbivores to overcome chemical defenses, through detoxification, secretion, or the degradation of toxins (Karban and Agrawal, 2002; Despres et al., 2007). On the other hand, secondary metabolites are not always used for defense by plants; for example, some groups of phenolic compounds (as flavonoids) are involved in photo-protection (Ballare, 2014) and can be induced by the increase of solar radiation at higher elevations. Therefore, in order to reduce oxidative damage and the penetration of UV light into the layers of photosynthetic cells, plants may accumulate flavonoids and phenylpropanoids on the epidermis of the leaf as well as in the spongy tissues of the mesophyll (Mazza et al., 2000; Agati et al., 2013). In turn, the thickness, hardness or hairiness of the leaves are structural characteristics which have been reported to affect negatively the performance of chewing herbivores (Clissold et al., 2009; Caldwell et al., 2016). Therefore, it is necessary to take into account the chemical and structural features of the leaves to better understand the mechanisms linked to the performance of herbivores that feed on plants.

Other studies have shown that herbivory is also influenced, either directly or indirectly, by environmental factors such as climate variables and soil characteristics (Masters et al., 1998; Bale et al., 2002). It has been shown that species of plants that grow at lower latitudes often experience greater amounts of herbivory than their counterparts at higher latitudes (Schemske et al., 2009; Marquis et al., 2012; Lim et al., 2015; Zhang et al., 2016). Therefore, it is expected that plant species have evolved higher levels of anti-herbivore defenses at lower latitudes, where the pressure of herbivores is higher (Rasman and Agrawal, 2011; Pearse and Hipp, 2012; Moreira et al., 2014; Abdala-Roberts et al., 2016; Moreira et al., 2017), suggesting that nutrient concentration in leaves (another aspect of the quality of the plant to herbivores), in addition to chemical defenses, could be associated with latitudinal gradients in herbivory and exhibit clinal patterns of variation (Marquis et al., 2012). However, it has also been found that these patterns can be very variable in strength and direction (Moles and Ollerton, 2016), probably because the relevant environmental factors may vary in a non-latitudinal way or show opposing latitudinal trends (Abdala-Roberts et al., 2016; Stevens et al., 2016;

Moreira, 2017). Therefore, to fully understand the geographic variability in herbivory intensity, it is necessary to take into account the mechanical, chemical, and nutritional features of the plants (at the interspecific and intraspecific levels) as well as environmental factors (Pellissier et al, 2016).

The oaks (genus *Quercus*) constitute a major element in the temperate forests of Mexico and have a great importance since they provide ecosystem services such as nutrient cycling and water balance (Lewington and Streeter, 1993; Nixon, 1993). Oaks forests also represent zones with a high biological activity among the different associated trophic levels and have been studied for decades in the context of the ecology of insect-plant interactions and the theory of plant defense (Feeny, 1976; Schultz and Baldwin, 1982). Most studies on the secondary chemistry of oaks have reported that the predominant groups are phenolic compounds, mainly hydrolysable tannins, proanthocyanidins (condensed tannins), flavonoids and some simple phenolic compounds (Salminen et al., 2004; Yarnes et al., 2006; Barbehenn et al., 2006a, 2008; Barbehenn and Constabel, 2011; Moctezuma et al., 2014) that are probably used by the oaks as broad-spectrum defense mechanisms against herbivores and regulate herbivory levels (Forkner et al., 2004; Barbehenn and Constabel, 2011; Maldonado-López et al., 2015).

In this study, we used *Quercus glaucoides* (Fig. 1) as a model system to assess some of the possible mechanisms that underly geographic variation in herbivory levels. This species has some ecological particularities that differentiate it from most of the Mexican oak species. It usually occurs in small and discrete monospecific stands in exposed slopes with rocky soils in warm and dry areas in the transition between temperate forests and tropical dry forests (Miranda y Hernández, 1963). Therefore, it is an adequate system to test for the effect of individual plant characteristics (leaf traits, nutritional quality, concentration of secondary metabolites) on herbivory, minimizing the effect of the variable, complex and diverse tree communities where most Mexican oak species are found. Also, its distributional range encompasses a gradient from 16.5 to 20.5° N latitudinally, 95.97 to 102.07° W longitudinally, and from 1127 to 2042 m in elevation (Valencia, 2004, Table 1), mainly in the morphotectonic provinces of the Transmexican

Volcanic Belt, the Balsas Depression and the Sierra Madre del Sur (Fig. 1). Along this distribution, there is significant variation in climatic variables, with mean annual temperature ranging from 17.09 to 22.15 °C and annual precipitation from 482 to 1336 mm (Table 1). The specific questions addressed were 1) Do herbivory levels in this oak species depend on variation in leaf traits, nutritional quality and chemical defense among individual trees? 2) Are herbivory levels, leaf traits, nutritional quality and chemical defense associated to geographical and climatic gradients along the distribution of the species?

Materials and methods

Sampling design

Throughout the geographical distribution of the species in Mexico, we selected thirteen populations located at the states of Querétaro, Michoacán, Guanajuato, Puebla and Oaxaca, which cover an altitudinal gradient between 1100-2000 m and represent the ranges of temperature and precipitation experienced by the species (Table 1, Figure 1C). In each population, 10 individuals were located and at least 35 randomly selected leaves were collected from each one for herbivory and chemical measurements. Twenty of these leaves were used to estimate herbivory levels, leaf area, dry leaf mass and specific leaf area for each individual, while the remaining were preserved at -20° C in liquid nitrogen until the chemical and nutrient content analyses were performed. All collections were performed at the end of the rainy season in the first week of November of 2017.

Herbivory patterns and leaf functional traits

Twenty randomly selected leaves of each individual (100 leaves per population and 1300 in total) were scanned using a flatbed scanner (ScanJet4300C). Leaf area consumed by insects (as a proportion of the total leaf area) was estimated using the ImageJ software

(Ferreira-Rasband, 2012). For each leaf we also determined the dry mass with an analytical balance and the specific leaf area (leaf area/dry mass) was calculated.

Secondary metabolites

Total phenols. One hundred and twenty-five mg of a sample of each leaf were weighed and ground with liquid nitrogen. The 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 minutes, 1 mL of 20% sodium carbonate was added and the tubes were stirred again. After 1 hour 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 amount 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 mL 1-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 an 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).

Proteins. The samples were extracted as described above for non-structural carbohydrates. Separately, the Bradford reagent was prepared by dissolving 100 mg of Blue Comassie G in 50 mL of 96% ethanol and then adding 100 mL of H₃PO₄ and distilled water to a final volume of 1 L. To determine the protein concentration, 100 mL of sample

extract was added with 5 mL of the Bradford reagent and the absorbance was measured at 595 nm in a spectrophotometer (Thermo Scientific MultiskanGO). The calibration standard was prepared with bovine serum albumin.

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 hrs 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 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.

Climatic variables

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 (Bio1),

temperature seasonality (Bio4), temperature annual range (Bio7), mean temperature of the driest quarter (Bio9), annual precipitation (Bio12), precipitation seasonality (Bio15), precipitation of the driest quarter (Bio17), precipitation of the warmest quarter.

Statistical analysis

The significance of the differences among populations for herbivory (proportion of leaf area removed) and leaf trait, nutritional quality and secondary compound variables were evaluated through one-way analyses of variance (ANOVA) using individual tree values. Before the analyses, all variables were checked for normality and transformed accordingly if necessary. In this way, proportion of leaf area removed was square root arcsine transformed, and leaf area, leaf dry mass, specific leaf area, and concentration of total phenols, total tannins, proanthocyanidins and total P were natural-log transformed.

To assess the relationship of herbivory levels with the various predictor variables, we performed three different stepwise multiple regression analyses. In the first one we included all predictor variables for which we had data for ten individuals per population (i.e. all variables except total leaf C, N, P and their ratios). In the second analysis, we included all predictor variables but only for the three individuals per population for which we had the complete data set. Finally, in the third analysis we used average values per population to assess if climate and geographic (i.e. the latitude, longitude and elevation of the populations) variables have an effect on herbivory levels or on the functional, nutritional and chemical traits of *Q. glaucoides*. In all cases, model selection was performed using the Akaike information criterion (AICc). Analyses were performed with the JMP 8 software (SAS Institute).

RESULTS

Most of the analyzed variables (herbivory, leaf functional traits, secondary chemistry and nutritional quality traits) showed significant differences among *Q. glaucooides* populations (Tables 2 and 3), except for total carbon, total leaf nitrogen and the C:N ratio (Table 3). Mean percentage of leaf area removed in the populations varied between 2.02 and 7.05%, with populations Matatlán and San Nicolás Obispo showing the lower and higher value, respectively (Table S1). Leaf area varied between 14.27 and 41.24 cm², leaf dry mass between 0.19 and 0.42 g, and specific leaf area between 69.86 and 119.32 cm² g⁻¹. Regarding the concentration of secondary compounds, we found 52.03-93.7 mg g⁻¹ of total phenols, 25.33-57.16 mg g⁻¹ of total tannins and 0.03-0.14 mg g⁻¹ of soluble proanthocyanidins. With respect to nutritional quality variables, water content varied between 38.47 and 44.34%, the content of non-structural carbohydrates between 60.09 and 100.12 mg g⁻¹ and protein content between 29.83 and 31.07 mg g⁻¹. Also, total phosphorous was between 0.68 and 2.14 mg g⁻¹, total nitrogen between 15.83-21.23 mg g⁻¹, and total carbon between 391.89 and 427.74 mg g⁻¹. Finally, the C:N, C:P and N:P ratios were between 19.57-20.08, 177.64-597.75 and 9.06-27.65, respectively (Table S3).

Stepwise multiple regression analyses indicated that herbivory at the individual level was positively related to leaf area, specific leaf area, and the concentration of total phenols, nonstructural carbohydrates and proteins (Table 4). In the case of the analysis also including total leaf C, N and P and their ratios, but for only three individuals per population, the only significant variables were leaf area, specific leaf area and the concentration of nonstructural carbohydrates. Finally, at the population level, herbivory showed a positive association with precipitation seasonality and precipitation of the driest quarter. Interestingly, all the predictor variables that significantly contributed to explain herbivory levels at the individual level also showed associations with geographic and climate variables (Table 5). Leaf area and the concentration of total phenols were positively correlated with precipitation seasonality, while the concentration of total nonstructural carbohydrates was positively correlated with latitude and the precipitation

of the warmest quarter and protein content was positively correlated with longitude and negatively with annual precipitation (Table 5).

DISCUSSION

The results of this study indicate significant geographic variation in the levels of herbivory and some functional, chemical and nutritional variables among the studied populations of *Q. glaucoides*. Per population, average levels of herbivory were between 2.02 and 7.05 %, which are similar to those found in previous studies of oak species such as *Q. affinis*, *Q. castanea*, *Q. dentata*, *Q. deserticola*, *Q. laurina*, *Q. oleoides*, *Q. robur*, and *Q. variabilis*, that have reported damage levels ranging from 2.2% to 16.7% (Masashi Kitamura et al., 2007; Moctezuma et al., 2014; Maldonado-López et al., 2015; Wang et al., 2016; Cuevas-Reyes et al., 2017; Vaca- Sánchez et al., 2018; Castagneyrol et al., 2019).

We aimed at establishing the relationship of herbivory levels with functional, chemical and nutritional variables across the geographical and environmental gradient of the distribution of *Q. glaucoides*. A large number of studies have shown that herbivory levels are influenced by several factors, which have to do with the chemical defense of the plant (Abdala-Roberts et al. 2016, Castagneyrol et al., 2018b), with the concentration of nutrients (Rivas-Ubach et al., 2014; Maldonado-López et al., 2015) and abiotic factors (Pellissier et al., 2016). In this study, multiple regression analyses indicated that all those factors significantly influenced herbivory intensity in *Q. glaucoides* at the individual and population levels. However, disentangling multiple interactions and causation relationships among factors may be a difficult task.

At the individual level, we found a significant positive relationship of herbivory with leaf area and specific leaf area, indicating that individuals with larger and thinner leaves are more attractive to herbivores in this oak species (Stiegel et al., 2017). Several studies have also found variable contributions of these leaf traits in explaining variation in insect herbivory (Garibaldi et al. 2011; Castaygnerol et al. 2019; Lynn and Fridley, 2019).

For example, in *Nothofagus pumilio* herbivory was strongly correlated positively with leaf size and weakly correlated negatively with specific leaf area (Garibaldi et al. 2011), while in *Q. robur* a weak positive correlation of herbivory with specific leaf area and a stronger negative correlation with leaf dry mass were found, while the effect of leaf size per se was not evaluated (Castaygnerol et al. 2019). These results confirm the importance of leaf traits in mediating insect-plant interactions, but the identification of the traits that consistently show the highest relevance requires more research using comparable methodologies.

In this study we also evaluated the association between herbivory levels and plant quality, measured in terms of nutritional quality (water content, total nonstructural carbohydrates, protein concentration and total C, N and P content) and chemical defense (total tannins, total phenols and soluble proanthocyanidins). It is known that insects rely on a complex system of decision cues for selecting the plants they forage on (Andersson et al., 2013) and generally select nutritionally rich leaves for their diet (Bruyn et al., 2002, Agrawal, 2004). N content is the most commonly measured indicator of plant nutritional quality in herbivory studies, since it is considered to be a limiting factor for most plant and insect populations (Butler et al., 2012; Mendes and Cornelissen, 2017). However, some studies have shown the importance of carbohydrates and the proportion between carbohydrates and proteins for the correct development of insects (Roeder and Behmer, 2014). In this study, we found a positive association of herbivory levels with carbohydrate and protein content in *Q. glaucooides*, suggesting the importance of both aspects of food quality for insect choice. In contrast, herbivory did not show an association with the concentration of total tannins and soluble proanthocyanidins and only a weak positive association with the concentration of total phenols. These results are in line with the literature asserting that plant secondary compounds (particularly tannins) can have a variety of effects on herbivorous insects, ranging from positive to negative depending on their concentration, the insect species (generalist vs specialist) and the interaction with other plant variables such as the amount of water and protein (Bernays, 1981; Smilanich et al., 2016).

At the population level, we found evidence of climate-associated trends in herbivory levels in *Q. glaucoides*, but geographical variables were not significant in the multiple regression model (Table 3). Several studies have tested the hypothesis of a general northwards decrease in herbivory intensity at both the plant-community and intraspecific levels, based on the premise that herbivory pressure should be higher at lower latitudes due to lower climatic stress on insect populations (Garibaldi et al., 2011; Moreira et al., 2015; Abdala-Roberts et al., 2016). This expectation has been confirmed in some studies (Lim et al., 2015; Moreira et al. 2015), but the inverse pattern has also been observed (Adams and Zhang, 2009; Moreira et al. 2015), as well as a lack of latitudinal pattern (Andrew et al., 2005, Moles et al., 2011). Our results indicate that the climatic variables influencing geographic variation in herbivory levels in *Q. glaucoides* are precipitation seasonality and precipitation of the driest quarter. As previously mentioned, this oak species is usually found in warm and dry areas in the transition between pine-oak forests and tropical dry forests. In these sites, precipitation seasonality is known to be the most important climate factor influencing the whole community phenology and dynamics (Sánchez-Azofeifa et al., 2013). However, it is interesting to consider whether the associations of herbivory levels with these climate variables in *Q. glaucoides* are explained by the direct effect of the environment on insect populations, indirectly through the mediation of plant traits, or both (e.g. Garibaldi et al., 2011). It could be hypothesized that more seasonal sites are harsher for insect populations than sites with more constant temperature and precipitation throughout the year, but these effects are expected to be stronger during the dry season than during the rainy season, that is when the samples were taken for this study. And on the contrary, we found higher herbivory levels in more seasonal sites. Notably, phenol concentration and leaf area also showed a positive correlation with precipitation seasonality (Table 4). These results are congruent with the resource availability hypothesis, which predicts that plants growing in more resource-poor environments will have higher amounts of chemical defenses (Coley et al., 1985; Endara and Coley, 2011). In our case, it can be assumed that sites with more marked dry and wet periods (i.e. more seasonal) are more resource-limited (since there is water limitation

during a longer period of the year) than less seasonal sites. Possibly through a compensation response to higher phenol concentration, insects have to eat larger amounts of tissue to acquire enough nutrients, as has been observed in several studies (Lazarević et al., 2002, Barbehenn et al., 2009)

Interestingly, the other two significant variables accounting for variation in herbivory levels, i.e. the concentration of nonstructural carbohydrates and proteins, showed different and contrasting climatic and geographic associations. Nonstructural carbohydrates increased latitudinally and with the precipitation of the warmest quarter and protein concentration increased from west to east and decreased with annual precipitation (Table 4). Higher carbohydrate concentrations in sites with a higher amount of rain during the summer (the warmest quarter in this part of Mexico) makes sense because it means there is more water availability to sustain photosynthetic functions during the growing season. However, geographical trends of both proteins and carbohydrates are more difficult to explain and may be accounted for by unmeasured factors such as soil characteristics (Lynn and Fridley, 2019). Nevertheless, our study highlights the complex patterns of variation and interactions among herbivory levels, leaf traits, nutritional quality, secondary metabolite concentration and climate factors. Future studies should consider other aspects of the local environment of tree populations, as it has been demonstrated that, for example, the characterization of soil nutrient content is a highly relevant factor in explaining herbivory levels (Lynn and Fridley, 2019).

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Table 1. Site, State and geographic coordinates of the locations in which *Quercus glaucooides* samples were collected.

Site	State	Longitude (°W)	Latitude (°N)	Altitude (m)	Annual Precipitation (mm)	Annual Mean Temperature (°C)
Tequisquiapan	Querétaro	-99.89	20.52	2042	482	17.9
Tarimoro	Guanajuato	-100.73	20.29	1930	648	18.2
San Bartolo de Coro	Michoacán	-100.88	19.89	1904	738	17.5
San Nicolás Obispo	Michoacán	-101.30	19.65	1961	825	17.1
Zitzio	Michoacán	-100.91	19.51	1127	930	22.6
Jicalán viejo	Michoacán	-102.07	19.29	1366	929	21.5
Cuernavaca	Morelos	-99.27	18.95	1860	1141	19.6
Izucar de Matamoros	Puebla	-98.41	18.51	1449	797	22.1
Teotitlán	Puebla	-97.05	18.19	1878	1336	17.5
San Juan Tonaltepec	Oaxaca	-96.95	17.56	1846	699	17.5
Tlazoyaltepec	Oaxaca	-96.84	17.07	1708	675	21
Matatlán	Oaxaca	-96.36	16.83	1884	632	19.1
El camarón	Oaxaca	-95.97	16.54	1133	877	20.1

Table 2. Results of the one-way ANOVAs for the comparison of percentage of leaf area removed and leaf trait, nutritional quality and chemical defense variables among 13 populations of *Q. glaucoides* in Mexico.

Variable	<i>F</i>_{12,128}	<i>P</i>
Percentage of foliar area removed	2.5852	0.0045
Leaf area	11.928	<0.0001
Leaf dry mass	7.2299	<0.0001
Specific leaf area	3.8851	<0.0001
Total phenols	4.069	<0.0001
Total tannins	8.8416	<0.0001
Soluble proanthocyanidins	7.9723	<0.0001
Water content	1.9296	0.038
Nonstructural carbohydrates	6.3007	<0.0001
Proteins	7.4576	<0.0001

Table 3. Results of the one-way ANOVAs for the comparison of total C, N, P and their ratios among 13 populations of *Q. glaucoides* in Mexico.

Variable	$F_{12,35}$	P
Leaf total C	0.6885	0.75
Leaf total N	2.1065	0.06
Leaf total P	8.779	<0.0001
C:N	1.0445	0.44
C:P	6.2297	<0.0001
N:P	6.7817	<0.0001

Table 4. Results of stepwise multiple regression analyses for herbivory as a function of: A) leaf trait, nutritional quality and secondary metabolite concentration for all individuals (excluding leaf total C, N and P; B) all predictor variables, including total C, N, P and their ratios for three individuals per population, and C) geographic and climate variables of the sampled localities.

Data set	Estimate	Standard error	T	P
A) Nine predictor variables, all individuals				
Intercept	-1.042	0.318	-3.28	0.0014
Leaf area	0.049	0.015	3.23	0.0016
Specific leaf area	0.078	0.031	2.48	0.0146
Total phenols	0.042	0.021	2.00	0.0473
Nonstructural carbohydrates	0.005	0.002	2.94	0.0040
Proteins	0.019	0.008	2.44	0.0161
B) Sixteen predictor variables, three individuals per population				
Intercept	-0.855	0.224	-3.82	0.0006
Leaf area	0.0654	0.027	2.39	0.0227
Specific leaf area	0.165	0.055	2.98	0.0054
Nonstructural carbohydrates	0.009	0.003	2.81	0.0085
C) Population average				
Intercept	-11.198	3.067	-3.65	0.0045
Precipitation Seasonality	0.146	0.029	5.02	0.0005
Precipitation of the driest quarter	0.069	0.023	2.91	0.0155

Table 5. Results of stepwise multiple regression analyses for the effect of geographic and climate factors on leaf trait, nutritional quality and secondary metabolite variables that significantly influenced herbivory levels in *Q. glaucoides*.

Response variable	Estimate	Standard error	T	P
Leaf area				
Intercept	-49.134	23.562	-2.09	0.0611
Precipitation seasonality	0.722	0.235	3.07	0.0107
Total phenols				
Intercept	-5.371	4.516	-1.19	0.259
Precipitation seasonality	0.134	0.045	2.97	0.0127
Nonstructural carbohydrates				
Intercept	-7.458	7.943	-0.94	0.37
Latitude	1.0105	0.374	2.70	0.0222
Precipitation of the warmest quarter	0.0202	0.008	2.46	0.0338
Proteins				
Intercept	53.752	5.526	9.73	<0.0001
Longitude	0.222	0.055	4.01	0.0025
Annual precipitation	-0.001	0.0005	-2.37	0.0393

SUPPLEMENTARY MATERIAL

Table S1. Mean and standard error of each population for functional variables.

Sites	Foliar area	% herbivory	Foliar weight (grs)	Specific foliar area
San Nicolás Obispo	41.24 (5.6)	7.05 (0.9)	0.42 (0.02)	106.84 (14.7)
San Bartolomé de Coro	21.86 (3.5)	6.34 (1.8)	0.25 (0.04)	98.99 (10.8)
Zitzio	29.36 (3.2)	4.16 (1.4)	0.35 (0.04)	86.43 (4.5)
Jicalan viejo	35.13 (11.09)	6.29 (3.2)	0.41 (0.05)	93.88 (14.4)
Tequisquiapan	20.76 (2.5)	4.19 (1.1)	0.27 (0.03)	77.69 (2.02)
Tarimoro	28.79 (3.5)	5.83 (0.9)	0.39 (0.05)	75.72 (3.5)
Matatlan	14.27 (0.7)	2.02 (0.4)	0.20 (0.01)	69.86 (2.2)
El camarón	15.20 (2.6)	4.56 (1.6)	0.19 (0.03)	87.33 (8)
Tlazoyaltepec	15.61 (1.1)	2.41 (0.4)	0.20 (0.01)	80.04 (4.1)
San Juan Tonaltepec	16.57 (1.3)	5.06 (1)	0.19 (0.01)	90.69 (5.29)
Teotitlan	14.99 (1.4)	4.98 (1.2)	0.19 (0.02)	80.61 (6)
Izucar de Matamoros	14.32 (1.4)	5.27 (0.5)	0.21 (0.01)	73.89 (2)
Cuernavaca	34.80 (2.2)	6.58 (1.1)	0.33 (0.03)	119.32 (16)

Table S2. Mean and standard error of each population for chemical variables.

Sites	Phenols mg/g	Proanthocyanidins	Tannins (mg/g)
San Nicolás Obispo	9.64 (1.2)	0.05 (0.01)	33.26 (3.4)
San Bartolomé de Coro	8.91 (1)	0.14 (0.02)	30.13 (2.5)
Zitzio	7.28 (0.9)	0.06 (0.01)	25.33 (1.8)
Jicalan viejo	10.34 (0.7)	0.05 (0.005)	33.71 (7.4)
Tequisquiapan	5.42 (0.6)	0.06 (0.006)	63.96 (4)
Tarimoro	9.59 (0.6)	0.05 (0.06)	57.16 (5.2)
Matatlan	8.36 (0.6)	0.05 (0.006)	45.38 (2.1)
El camarón	6.03 (0.7)	0.05 (0.08)	45.96 (3.4)
Tlazoyaltepec	6.45 (0.4)	0.05 (0.05)	48.36 (2.2)
San Juan Tonaltepec	6.50 (0.6)	0.03 (0.06)	41.97 (2.5)
Teotitlan	9.67 (1)	0.03 (0.004)	46.06 (3.9)
Izucar de Matamoros	7.77 (0.2)	0.03 (0.007)	32.59 (1.8)
Cuernavaca	7.64 (0.7)	0.03 (0.004)	42.05 (2.4)

Table S3. Mean and standard error of each population for nutrient variables.

Sites	Concentration of water %	Carbohydrates mg/g	Proteins mg/g	PT mg/g	NT mg/g	CT mg/g	C:N	C:P	N:P
San Nicolás Obispo	41.93 (0.9)	14.83 (1.02)	29.83 (0.15)	0.83 (0.04)	17.32 (1.6)	406.47 (4.5)	23.87 (2.1)	491.82 (35.3)	21.06 (2.9)
Bartolomé de Coro	43.39 (0.9)	18.19 (0.7)	30.78 (0.3)	0.85 (0.1)	17.82 (1.2)	404.49 (7.6)	22.92 (1.7)	494.91 (67)	21.54 (2.3)
Zitzio	40.13 (1.7)	15.30 (0.8)	31.59 (0.3)	0.68 (0.02)	18.56 (0.4)	404.61 (24.6)	21.76 (0.9)	597.75 (53.6)	27.35 (1.4)
Jicalán viejo	41.70 (1.5)	15.21 (1.04)	29.88 (0.3)	0.97 (0.2)	19.08 (0.6)	408.81 (12.5)	21.47 (0.8)	446.63 (64)	20.78 (3.2)
Tequisquiapan	38.93 (1.3)	17.42 (0.4)	31.35 (0.2)	1.45 (0.1)	19.21 (0.3)	407.03 (19.6)	21.17 (0.7)	281.54 (7.4)	13.35 (0.8)
Tarimoro	38.08 (2)	17.86 (0.7)	30.62 (0.2)	0.79 (0.05)	16.01 (2.04)	391.89 (15.9)	25.08 (4.7)	495.15 (1.6)	20.48 (3.9)
Matatlan	39.95 (0.5)	14.34 (1)	31.62 (0.3)	1.13 (0.18)	17.40 (1.4)	403.18 (16.3)	23.48 (1.9)	363.83 (30.4)	15.72 (1.9)
El camarón	38.47 (0.9)	14.34 (1.1)	31.97 (0.3)	0.96 (0.1)	15.83 (2)	403.89 (19.5)	26.26 (4)	429.43 (56)	16.38 (0.2)
Tlazoyaltepec	43.69 (1.8)	16.05 (0.8)	30.74 (0.2)	2.14 (0.15)	19.14 (0.7)	376.48 (11)	19.77 (1.3)	177.64 (12.6)	9.06 (0.9)
San Juan Tonaltepec	42.30 (1)	12.70 (1.4)	30.75 (0.3)	1.21 (0.13)	20.94 (0.6)	408.73 (27.3)	19.57 (1.5)	346.34 (50)	17.59 (1.6)
Teotitlán	41.29 (1)	19.51 (0.4)	30.38 (0.1)	1.76 (0.15)	21.23 (0.3)	427.74 (23.9)	20.19 (1.4)	247.44 (29.1)	12.25 (1.1)
Izucar de Matamoros	39.95 (0.5)	19.12 (1.3)	31.14 (0.2)	0.93 (0.1)	19.41 (0.9)	408.40 (5.3)	21.17 (1.3)	451.83 (54)	21.15 (1.6)
Cuernavaca	44.34 (2.6)	17.92 (2)	30.33 (0.2)	1.42 (0.2)	20.89 (0.6)	426.52 (10.6)	20.42 (0.3)	315.53 (34)	15.40 (2)

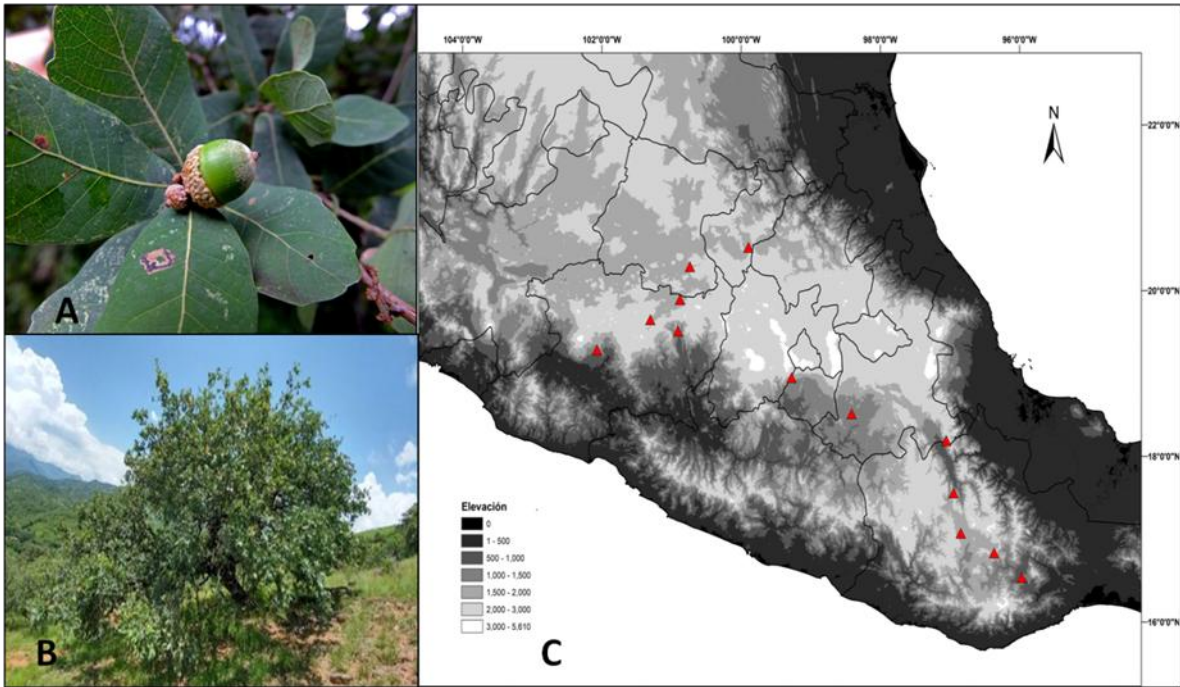


Figure 1. *Quercus glaucoides* A) Leaves and fruit B) growth form C) populations sampled, indicated with red triangles.

5. DISCUSIÓN

La herbivoría ha sido ampliamente estudiada a lo largo del tiempo. Actualmente, sabemos que para poder entender todas las causas y consecuencias de los patrones de herbivoría, hay que estudiar meticulosamente una gran variedad de aspectos en las plantas, que tienen que ver tanto con su morfofisiología como con su contexto ecológico. Dentro de este estudio, se consideraron algunos de estos aspectos, por lo que tomamos en cuenta tanto variables morfológicas y funcionales como químicas, nutrimentales y ambientales de la especie bajo estudio, *Quercus glaucoides*.

Es bien conocido que las especies de *Quercus* producen compuestos químicos, particularmente compuestos fenólicos, que son efectivos contra una amplia gama de insectos fitófagos (Feeny, 1970; Roslin y Salminen, 2008; Pearse y Hipp, 2009; Abdala-Roberts et al., 2016; Moreira et al., 2018). Por estas razones los encinos han sido ampliamente utilizados como un grupo modelo para evaluar patrones globales de rasgos defensivos. Como resultado, la relación entre los rasgos defensivos putativos de los encinos y su vulnerabilidad real a la herbivoría por insectos es bien entendida (Hipp y Pearse, 2012), ya que las defensas contra los herbívoros se han comprobado en diversos grados en los encinos (Forkner et al., 2004; Karban, 2007; Kitamura et al., 2007).

Nuestros resultados indicaron que hay diferencias significativas entre las poblaciones estudiadas de *Q. glaucoides*, al evaluar las distintas variables consideradas. En primer lugar, hubo variación en los niveles de herbivoría entre las poblaciones, presentando porcentajes que coinciden con los observados en otras especies de encinos (Maldonado-López et al., 2015; Cuevas-Reyes et al., 2017; Moctezuma et al., 2014; Castagneyrol et al., 2019; Wang et al., 2016; Masashi Kitamura et al., 2007; Vaca- Sánchez et al., 2018). Cabe resaltar que las poblaciones de *Q. glaucoides* eran pequeñas (reducido número de individuos) y generalmente se encontraban coexistiendo con pocas especies, lo cual pudo haber tenido un efecto sobre la intensidad de la herbivoría. El tema del aislamiento sobre la herbivoría ha sido estudiado a lo largo de los años, y varios estudios han determinado que la riqueza de especies y la densidad de varios insectos que atacan a

las plantas son notoriamente menores en poblaciones aisladas que se encuentran en comunidades con baja diversidad de especies (como en islas) que en la situación contraria (Blondel y Pradel, 1990; Dapporto y Dennis, 2009; Hausdorf y Hennig, 2005).

Recientemente, Moreira et al. (2019) estudiaron dichos efectos sobre la herbivoría en *Q. ilex* así como también en su defensa química. Se encontró que las poblaciones aisladas (insulares) de encinos exhibieron una herbivoría más baja por insectos masticadores de hojas que sus contrapartes continentales. Este mismo tipo de efecto podría contribuir a explicar los resultados obtenidos en este estudio, ya que de acuerdo con nuestras observaciones cualitativas hubo un mayor porcentaje de herbivoría en las poblaciones menos aisladas y que se encontraban en comunidades de plantas más diversas. Sin embargo, es necesario poner a prueba formalmente esta hipótesis, cuantificando la diversidad de la comunidad en cada sitio donde se encuentra *Q. glaucoides* y evaluando su grado de aislamiento mediante herramientas de análisis espacial. Por otro lado, el grado de aislamiento y la diversidad de las comunidades también podrían seguir los patrones espaciales y ambientales descritos en este trabajo.

En segunda instancia, las concentraciones de metabolitos secundarios y de nutrimentos también difirieron entre poblaciones y algunos de ellos, como los fenoles, los carbohidratos, las proteínas y el contenido de agua, se asociaron positivamente con los porcentajes de herbivoría. Existe una gran cantidad de trabajos acerca de la importante relación que hay entre los compuestos químicos de las hojas, en este caso metabolitos secundarios, y la herbivoría, donde se habla de la capacidad que tienen los insectos para detectar, por medio de señales químicas, las plantas más viables para su forma de alimentación (Andersson et al., 2013). Por lo que se supone que las plantas con mayores niveles de dichos metabolitos son aptas para alejar a los insectos y disminuir el daño sobre sus hojas (Herms y Mattson, 1992, Forkner et al., 2004). En *Q. glaucoides* observamos algo contrario a esta suposición, ya que las plantas con mayores concentraciones de fenoles fueron las que presentaron mayor daño, pero esto coincide también con otros estudios, donde dicho resultado se interpreta como una respuesta de alimentación compensatoria (Lazarević et al., 2002, Barbehenn et al., 2009), donde los insectos herbívoros consumen

mayor cantidad de hojas (que no tienen sus estándares nutrimentales) para contrarrestar los efectos de la baja calidad de la hoja. Los otros metabolitos medidos, tales como taninos totales y proantocianidinas, no tuvieron ninguna relación con la herbivoría.

Por otro lado, muchos rasgos foliares que se han implicado en la defensa de la herbivoría también se han implicado en la resistencia a la sequía, la radiación ultravioleta (UV) o la tolerancia al calor en las plantas (Pearse y Hipp, 2012). Por ejemplo, los flavonoides (una clase de compuestos fenólicos) protegen el maíz de la radiación UV (Stapleton y Walbot, 1994). De manera similar, los tricomas protegen las porciones fotosintéticamente activas de las hojas de *Q. ilex* de la radiación UV (Skaltsa et al., 1994). Los tricomas también pueden reducir la pérdida de agua y contribuir a la tolerancia a la sequía (Espigares y Peco, 1995). Lo que podría explicar su presencia en las hojas pero no necesariamente su papel como mecanismo de defensa.

Por otra parte, las hojas que presentaron mejores estándares de calidad, al tener altas concentraciones de carbohidratos, proteínas y agua, fueron las más dañadas, ya que los herbívoros generalmente seleccionan hojas nutricionalmente ricas para su dieta (Scheirs et al., 2002, Agrawal, 2004).

A nivel de las poblaciones, los patrones de herbivoría en *Q. glaucooides* tuvieron asociaciones significativas con varios factores, pero sin duda el que tuvo una mayor influencia, tanto con la herbivoría como con otras variables medidas, fue la estacionalidad de la precipitación. En este caso, encontramos que las poblaciones con mayor estacionalidad tuvieron los porcentajes más altos de herbivoría en sus hojas. Una posible explicación para una mayor inversión defensiva en áreas menos estacionales sería si hubiera una mayor presión de herbívoros en áreas menos estacionales, ya que la teoría de defensa óptima predice que las plantas que están expuestas a alta presión de herbívoros deberían invertir más en defensas (Rhoades y Cates, 1976; Sello, 2003). De hecho, se ha demostrado que la estacionalidad de la retención de las hojas afecta fuertemente la presión de los herbívoros en algunos sistemas, incluidos los encinos (Karban, 2007).

Nuestros resultados sugieren que la estacionalidad de la precipitación fue el predictor más consistente de los rasgos de *Q. glaucoides* y los índices de defensa en nuestro conjunto de datos, así como de los patrones mismos de herbivoría. Por otro lado, los metabolitos secundarios analizados no tuvieron relación como mecanismos de defensa en sí; sin embargo, dado que ya se sabe que tienen diferentes funciones dentro de las hojas, habría que hacer un análisis más robusto y considerar otro tipo de variables, como podría ser el índice de radiación solar de cada población, por ejemplo, para así poder determinar si su función, en este caso dentro de *Q. glaucoides*, tiene más asociación con la foto-protección en las hojas de los rayos UV y no meramente con la defensa. Así como ya se había mencionado anteriormente, para estudios próximos también se debería analizar el papel que tiene el efecto insular en cualquier estudio relacionado con la herbivoría, ya que es un factor determinante en todo sistema de estudio, también es importante tomar en cuenta la estratificación de los árboles, dado que la susceptibilidad a la herbivoría cambia según el estrato estudiado.

6. CONCLUSIONES

- La especie *Quercus glaucoides* presenta bajos niveles de herbivoría los cuales no están relacionados con las concentraciones de metabolitos secundarios ni con la calidad de sus nutrientes, sin embargo, la variación que existe entre las poblaciones depende de algunos atributos funcionales y sobre todo de variables ambientales relacionadas con la precipitación, dado que es una árbol de ambientes más secos y áridos en comparación con otras especies de su género.
- Los patrones de variación geográfica en los niveles de herbivoría, rasgos foliares, calidad nutricional y defensa química son congruentes con la hipótesis de disponibilidad de recursos. Sin embargo, los estudios futuros deberían considerar otros aspectos del entorno local de las poblaciones de árboles, ya que se ha demostrado que, por ejemplo, la caracterización del contenido de nutrientes del suelo es un factor muy relevante en términos de la hipótesis de disponibilidad de recursos (Lynn y Fridley, 2019).

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